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Glycocerebroside bearing a novel long-chain base from *Sagina japonica* (Caryophyllaceae)

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ABSTRACT

A new glycocerebroside (**1**), along with one reported one (**2**), was isolated from the ethanol extract of *Sagina japonica* (Caryophyllaceae) and was fully characterized. The structures of two compounds were identified as (2S, 3S, 4R, 8E)-1-(β-D-glucopyranosyl-3, 4-dihydroxy-2-[(R)-2′- hydroxypalmitoyl]amino-8-heptadecaene (**1**) and (2S, 3R, 8E)-1-(β-D-glucopyranosyl-3-hydroxy-2-[(R)-2′-hydroxypalmitoyl]amino-8-octadecaene (**2**) by using spectroscopic methods (^{1}H, ^{13}C, and 2D NMR, MS) and chemical degradation.

1. Introduction

Sphingolipids are compounds with a sphingoid base backbone, an amide linked nonpolar aliphatic "tail", and a polar head group. There are more than 70 different sphingoid base backbones that vary in alkyl chain lengths (14–22 carbon atoms), degree of unsaturation and position of the double bonds, the presence or absence of a hydroxyl group at position 4, and branching of the alkyl chain. The amino group of the sphingoid base is usually substituted with a long-chain fatty acid. The fatty acids vary in chain length (14–30 carbon atoms), degree of unsaturation, and the presence or absence of a hydroxyl group on the carbon atom. Subsequent addition of a double bond at the 4, 5 carbon–carbon bond of the sphingonine backbone results in the formation of ceramide (N-acyl-sphingosine). Structurally versatile sphingolipids are formed when polar head groups are added at position 1 of ceramide [1,2].

Sphingolipids are constituents of the cytoplasmic membrane of eukaryotic cells, and they are known to function as receptors for certain bioactive compounds and are thus thought to play an important role in the transportation of cations [3,4]. It has also been reported that sphingolipids have other physiological activities such as, activation of certain enzymes [5–7], antimicrobial and antitumor activity [8,9], anti-ulcerogenic activity [10], and nerve growth factor-like activity [11]. A distinct and novel chemical ecological activity was found by Kawai and Ikeda [12–14] and Kawai et al. [15], who have demonstrated that the sphingolipids from *Schizophyllum commune* and other fungi stimulate fruiting body formation of *S. commune*. Moreover, the 8*E*-double bond of the sphingoid has been proposed as one key element determining the activity. Kawai et al. found deeply that some plant cerebrosides also show a hormone-like activity toward fruiting body formation in a mushroom *S. commune*. A survey of sphingolipids from various sources, certain cerebrosides in wheat grain were found to be active, and Whe II was determined to be a major active compound [16].

As part of our current interest in bioactive substances from Caryophyllaceae [17,18], we have chemically analyzed a fraction previously isolated from the whole plant *Sagina japonica*. We now describe the isolation and structural determination of two glycocerebrosides (**1**) and (**2**) from *S. japonica* (Fig. 3).

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2. Results and discussion

The EtOH extract of the whole plant of *S. japonica* was subjected to silica gel column chromatography (CC), using $CHCl_3$ and MeOH as eluents. The chloroform-soluble eluent was separated by normal-phase followed by reversed-phase CC to give compounds **1** and **2**. The following is the structural elucidation of new compound **1**.

Compound **1** was obtained as white amorphous powder and was negative to ninhydrin but positive after hydrolysis with concentrated hydrochloric acid [19]. $[\alpha]_D^{24}+10.1°$ (c 0.26, MeOH). The molecular formula of $C_{39}H_{75}NO_{10}$ for 1 was determined by positive high resolution FAB-MS at *m/z* 718.5473 $[M+H]^+$ (calcd. 718.5480). In the positive FAB-MS, compound **1** exhibited significant fragment peaks at *m/z* 718 $[M+H]^+$, 556 $[M-162(\text{glucosyl})]^+$, 538 $[M-162-H_2O]^+$. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl at 3400 cm^{-1}, glycosidic (C–O) at 1080 and 1030 cm^{-1}, a secondary amide at 1536 and 1650 cm^{-1}, and long aliphatic chains at 2965, 2928, 2860 (C–H), 1475, 1309, 1120, 965 (*trans* C C), and 720 $[(CH_2)_n]$ cm^{-1}. The ^1H and ^{13}C NMR spectral data of **1** indicated the presence of a sugar, an amide, and long-chain aliphatic moieties, strongly suggesting the glycolipid nature of the molecule (Table 1).

Seven hydroxyl groups in the original structure of **1** was determined by acetylation with acetic anhydride/pyridine at room temperature to give its peracetate derivative **1a**, which showed a molecular ion peak at *m/z* 1011 $[M]^+$ in its EI-MS, consistent with the composition $C_{53}H_{89}NO_{17}$ for **1a**. The existence of a fragment ion peak at *m/z* 681 $[M+H-331$ (tetraacetyl hexose)$]^+$ confirmed hexose as the sugar residue. Meanwhile, the EI-MS data of **1a** also displayed the diagnostic fragments of the sugar moiety at *m/z* 331 (base peak), 271, 229, 211, 169, and 109, due to an acetylated glucopyranoside [20]. Thereby indicating the presence of seven hydroxyl groups in the original structure of **1**.

On methanolysis [21,22], compound **1** yielded a fatty acid methyl ester, a mixture of α- and β-anomers of methyl glucoside, and an LCB (Fig. 1). The methyl ester **1b** was identified as methyl 2′-hydroxypalmitate by the help of GC–MS analysis, with a molecular ion peak at *m/z* 286, corresponding to the composition $C_{17}H_{34}O_3$. Comparison of the optical rotation data ($[\alpha]_D^{24}-1.3°$) with those reported in the literature [23,24] led us to propose that the relative stereochemistry at C-2′ of the fatty acid methyl ester was *R*. That the optical rotation of the methyl glucoside, $[\alpha]_D^{24}+76.8°$ (determined on the methanolysis product from 1), was close to that of the authentic sample, $[\alpha]_D^{25}+77.3°$ [25], indicated that glucose was present as its D-isomer.

Table 1
^1H and ^{13}C nuclear magnetic resonance (NMR) data for compound **1** and **2** in pyridine-d5. For abbreviation see Table 1.

Atom no.	1 δ ^1H (*J* in Hz)	δ ^{13}C (ppm)	2 δ ^1H (*J* in Hz)	δ ^{13}C (ppm)
Long-chain base				
1	4.76 (*m*), 4.16 (*m*)	70.07 *t*	4.68 (*dd*, 3.77, 9.47), 4.17 (*m*)	70.28 *t*
2	5.08 (*br. s*)	54.81 *d*	4.64 (*m*)	54.58 *d*
3	4.11 (*m*)	72.95 *d*	4.59 (*m*)	72.55 *d*
4	4.09 (*m*)	72.00 *d*	1.85 (*m*)	35.67 *d*
5	2.20 (*m*)	35.87 *t*	1.13–1.61 (*m*)	25.83 *d*
6	1.90 (*m*)	26.67 *t*	1.13–1.61 (*m*)	29.54–30.26 *t*
7	2.08 (*m*)	32.38 *t*	1.85 (*m*)	32.15 *t*
8	5.55 (*br. s*)	132.76 *d*	5.50 (*br. s*)	130.72 *d*
9	5.55 (*br. s*)	131.42 *d*	5.50 (*br. s*)	130.72 *d*
10	2.08 (*m*)	32.38 *t*	2.06 (*m*)	32.15 *t*
11	1.14–1.62(*m*)	26.08 *t*	1.13–1.61 (*m*)	29.54–30.26 *t*
12–15	1.14–1.62(*m*)	29.82–30.47 *t*	1.13–1.61 (*m*)	29.54–30.26 *t*
16	1.14–1.62(*m*)	23.15 *t*	1.13–1.61 (*m*)	29.54–30.26 *t*
17	0.93 (*t*, 6.71)	14.48 *q*	1.13–1.61 (*m*)	22.94 *t*
18			0.90 (*t*, 6.48)	14.26 *q*
NH	8.34 (*d*, 8.76)		8.21 (*d*, 8.71)	
N-acyl moiety				
1′		176.27 *s*		175.57 *s*
2′	4.68 (*m*)	72.69 *d*	4.58 (*m*)	71.77 *d*
3′	2.12 (*m*)	33.14 *t*	1,85 (*m*)	34.82 *t*
4′	1.14–1.62(*m*)	23.15 *t*	1.76 (*m*), 1,85 (*m*)	29.54–30.26 *t*
4′–13′				
5′–13′	1.14–1.62(*m*)	29.82–30.47 *t*	1.13–1.61 (*m*)	29.54–30.26 *t*
14′	1.77 (*m*)	25.95 *t*	1.13–1.61 (*m*)	32.98 *t*
15′	1.14–1.62(*m*)	23.15 *t*	1.13–1.61 (*m*)	22.94 *t*
16′-CH_3	0.93 (*t*, 6.71)	14.48 *q*	0.90 (*t*, 6.48)	14.26 *q*
Sugar moiety				
1″	4.89 (*d*, 7.67)	105.29 *d*	4.85(*d*, 8.35)	105.57 *d*
2″	4.01 (*dd*, 7.81, 8.30)	75.24 *d*	4.13 (*dd*, 7.97, 8.51)	75.12 *d*
3″	4.18 (*m*)	78.52 *d*	4.16 (*m*)	78.52 *d*
4″	4.10 (*m*)	71.80 *d*	4.15 (*m*)	71.48 *d*
5″	3.90 (*m*)	78.52 *d*	3.98 (*m*)	78.52 *d*
6″	4.45 (*d*, 11.43), 4.29 (*d*, 11.41)	63.01 *t*	4.50 (*br. d*, 11.74), 4.33 (*br. d*, 11.03)	62.86 *t*

Assignments were made by distortionless enhancement by polarization transfer (DEPT) and heteronuclear multiple quantum coherence (HMQC) analysis.

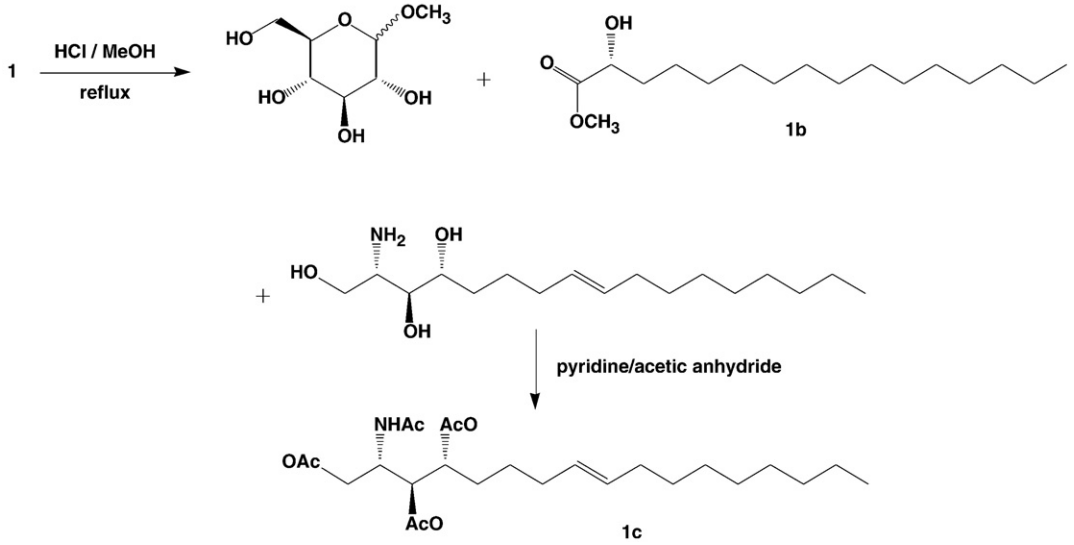

Fig. 1. Hydrolysis of compound **1**.

The sugar moiety was further indicated as glucose by NMR spectrum. In the ^{1}H NMR spectrum of **1** an anomeric signal indicative of the sugar moiety was observed at δ 4.89, and the coupling constant (d, $J = 7.67$ Hz) of this signal suggested the β-configuration of a glycosidic linkage. The six oxygenated carbon signals at δ 105.29 (CH), 78.52 (CH), 78.52 (CH), 75.24 (CH), 71.80 (CH), and 63.01 (CH_2) in the ^{13}C NMR spectrum also supported the presence of the β-g1ucopyranoside moiety in **1** by comparison of the observed and reported chemical shifts [26]. In addition, from the heteronuclear multiple bond correlation spectrum, the correlation between H-1″ [δ 4.89 (1H, d)] and C-l [δ 70.07 (CH_2)] suggested that the β-D-glucose was attached to the C-1 position of the LCB.

The ^{1}H NMR data (Table 1) of **1** revealed the presence of two terminal methyls at δ 0.93 (6H, t, $J = 6.71$ Hz), methylene protons at δ 1.14–1.62 (m), an amide proton signal at δ 8.34 (d, $J = 8.76$ Hz), an anomeric proton at δ 4.89 (d, $J = 7.67$ Hz), and carbinol protons appearing as multiplets between δ 3.90 and 4.68. A signal appearing at δ 5.08 (m, H-2) was assigned as a methine proton vicinal to the nitrogen atom, clearly suggesting a cerebroside containing a 2-hydroxy fatty acid [21,23]. The ^{13}C NMR spectrum of **1** exhibited carbon signals at δ 176.27 (carbonyl carbon), 54.81 (CHNH, C-2), 29.82–30.47 (methylene carbons), 14.48 (two terminal methyls, C-17 and C-16′). One olefinic carbon signal observed at δ 132.76 (CH) and 131.42 (CH) suggested that **1** possessed one double bond. In the ^{1}H–^{1}H homonuclear correlation spectroscopy spectrum, some key correlations were observed (Fig. 2). These correlations have thus unambiguously assigned the position of the one double bond at C-8. The analysis was further supported by HMBC spectrum of **1** (Fig. 2). The geometry of the C-8/C-9 alkene bond was determined to be E by the ^{13}C NMR chemical shift of the methylene carbon C-7 (δ 32.38) and C-10 (32.38) vicinal to the olefinic carbon [23,27]. It is thus clear that **1** possesses a novel sphingoid moiety with (8E) geometry, 2-amino-1, 3, 4-trihydroxy-8-heptadecaene. In addition, treatment of the methanolysis product of **1** with acetic anhydride/pyridine at 70 °C afforded production of a triacetyl LCB **1c**, which we suggest is 2-acetoamino-1, 3, 4-triacetoxy-8-heptadecaene on the bases of the molecular ion at m/z 469 and observed key EI-MS fragments of **1c**, 469 $[M]^+$, 349 $[M - 2 \times HOAc]^+$, 152 $[CH_3(CH_2)_7CH = CHCH_2]^+$, 144 $[AcOCH_2CHNHAc + H]^+$, 102 $[144 - Ac + l]$, 84 $[144 - HOAc]^+$ [28]. All of the above spectral evidence further supported that **1** is a cerebroside composed of a (8E)-2-amino-l, 3, 4-trihydroxy-8-heptadecaene, (2R)-2-hydroxy fatty acid, and β-D-glucopyranose.

The relative stereochemistry at C-2, C-3, and C-4 in **1** was presumed as 2S, 3S, and 4R which was shown to be same as that of aralia cerebrosides **1** [28]. On the basis of the above evidence, the structure of **1** was therefore established as (2S, 3S, 4R, 8E)-1-(β-D-glucopyranosyl)-3, 4-dihydroxyl-2-[(R)-2′-hydroxyl palmitoyl] amino-8-heptadecaene (Fig. 3).

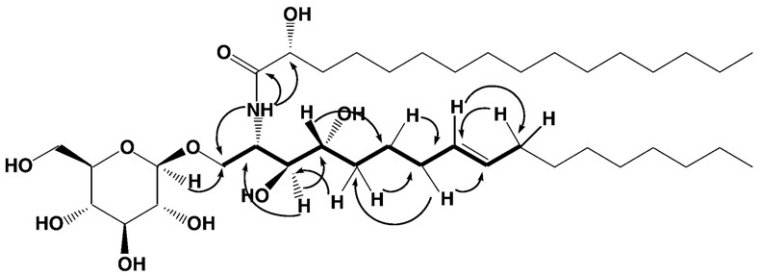

Fig. 2. Key HMBC, and H–H COSY correlations of compound **1**.

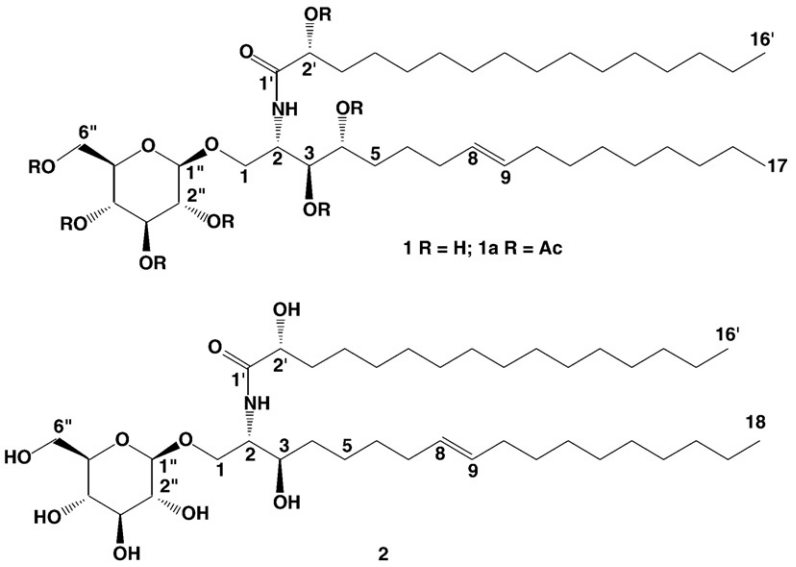

Fig. 3. The chemical structures of compound **1**, **1a**, and **2**.

Compound **2** obtained as white amorphous powder was also negative to ninhydrin but positive after hydrolysis with concentrated hydrochloric acid [19]. The HR-FAB-MS data, ^{1}H and ^{13}C NMR (Table 1) data, and IR absorptions indicated that compound **2** was one known gylcosphingolipid as (2S, 3R, 8E)-1-(β-D-glucopyranosyl-3-hydroxy-2-[(R)-2′-hydroxypalmitoyl]amino-8-octadecaene (2) (Fig. 3), which were previously obtained from the Roots of *Serratula chinensis* [29,30].

Sphingolipids are ubiquitous membrane constituents of animals, plants, and also lower forms of life, the principal components of which are the LCBs or sphingoid bases. In nature, the most widely occurring LCB is D-*erythro*-4(*E*)-sphingenine containing even carbon atoms, whereas LCBs containing odd carbon atoms are less common in higher plants [31,32]. We have demonstrated in *S. japonica* a previously unrecognized cerebroside containing a novel LCB, (8*E*)-heptadecaene. The cerebrosides containing odd-numbered LCBs have been found in marine animals, such as starfish [33], sea cucumber [34], sponge [35], and coral [36]. From the viewpoint of comparative biochemistry, these glycocerebrosides will be of considerable interest to elucidate fully their distribution and also to investigate the physiological significance of the odd-numbered LCBs, as well as the biosynthesis pathway.

Both of these two compounds possess the acid amide linkage and 8*E*-double bond, which are essential for the achievement of high hormone-like activity [16]. Moreover, an acyl moiety with a carbon chain of fewer than 24 units and/or a 2-hydroxy group increases such activity [14]. To confirm such a relationship between structure and activity and get further insight, it is necessary to carry out further experiments of hormone-like activity towards higher fungi shows necessary.

3. Experimental part

3.1. Chromatographic and instrumental methods

Melting points were obtained on an XRC-1 apparatus (Sichuan University, Sichuan, China) and the thermometer was uncorrected. Optical rotations were taken on a Horiba SEPA-300 automatic polarimeter (Horiba, Tokyo, Japan). The nuclear magnetic resonance (NMR; ^{1}H, ^{13}C, and two-dimensional NMR) spectra were acquired on Bruker AM 400 spectrometer (Karlsruhe, Germany); tetramethylsilane was used as an internal standard, and coupling constants were represented in Hertz. Mass spectra were measured with a VG Autospec3000 mass spectrometer (VG, Manchester, England). Infrared (IR) spectra were obtained in KBr pellets on a Bio-Rad FTS-135 IR spectrophotometer (Bio-Rad, Richmond, CA). Gas chromatography mass spectrometry (GC–MS) was performed with a Finnigan 4510 GC–MS spectrometer (San Jose, CA) employing the electron impact (EI) mode (ionizing potential 70 eV) and a capillary column (30 mm × 0.25 mm) packed with 5% phenyl/95% methylsilicone on 5% phenyl-dimethylsilicone (HP-5) (Hewlett-Packard, Palo Alto, CA). Helium was used as carrier gas; column temperature 160–240 °C (rate of temperature increase: 5 °C/min).

3.2. Materials

Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Ltd., Qingdao, China) and Sephadex LH-20 gel (25–100 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). Reversed-phase chromatography was carried out on LiChroprep® RP-8 (40–63 μm) (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was carried out on plates precoated with Merck RP-18 and silica gel (Qingdao Marine Chemical Ltd., China), and detection was achieved by spraying with 10% H_2SO_4 followed by heating, and accompanied with cycloperptidic TLC detection method [19]. All solvents were distilled before use. The whole plants of *S. japonica* were collected in Songming county of Yunnan province, China, in September 2005. It was identified by Prof. M. J. Qin, and a voucher specimen was preserved in the Herbarium of Kunming Institute of Botany (CAS).

The dried whole plants of *S. japonica* (20 kg) were extracted 3 times with 95% EtOH under reflux (3 × 100 L) for 3, 2 and 1 h, respectively. After evaporation of the combined extracts, the residue was suspended in H_2O and then extracted with petroleum ether (60–90 °C), AcOEt, and BuOH. The AcOEt extract (620.0 g) was decolored on Diaion HP 20 eluting with a gradient H_2O/MeOH 0:1 → 1:0, The 90% MeOH elute (200.0 g) was subsequently subjected to CC (silica gel, $CHCl_3$/MeOH 50:1 → 5:1), and resubmitted to CC (silica gel, $CHCl_3$/MeOH 15:1 → 9:1) to give glycocerebroside **1** (21.0 mg) and glycocerebroside **2** (28.0 mg).

(2S, 3S, 4R, 8E)-1-(β-D-glucopyranosyl-3, 4-dihydroxyl-2-[(R)-2′-hydroxylpalmitoyl]amino-8-heptadecaene (**1**). White amorphous powder (methanol); $[\alpha]_D^{24} + 10.1°$ (c 0.26, MeOH); IR (KBr) ν_{max} 3400 (OH), 2930, 2845 (C–H), 1650 (C O), 1536 (NH), 1475, 1320, 1075 (C–O), 965 (*trans* C C), 720 $[(CH_2)_n]$ cm^{-1}; ^1H and ^{13}C NMR spectra are given in Table 1; positive fast atom bombardment-mass spectrometry (FAB-MS) *m/z* (relative intensities, %) 718 $[M+H]^+$ (77.5), 700 $[M-H_2O]^+$ (5.1), 556 $[M+H-162]^+$ (100), 538 $[M+H-H_2O]^+$ (47.0), 284 (16.5), 133 (89.0); positive high resolution FAB-MS *m/z* 718.5473 $[M+H]^+$ (calcd. for $C_{40}H_{77}NO_{10}$, 718.5480).

3.3. Acetylation of **1**

The method of acetylation was the same as Refs. [21,22]. The residue obtained was subjected to do EI-MS measurement. EI-MS (70 eV) *m/z* (relative intensities,%): 1011 $[M]^+$ (4.8), 951 $[M-HOAc]^+$ (7.5), 891 $[M-2\times HOAc]^+$ (3.3), 681 $[M+H-Glc(OAc)_4$, glucose = Glc$]^+$ (0.9), 390 $[H_2N^+=CHCH_2OGlc(OAc)_4]$ (23.1), 331 $[Glc(OAc)_4]^+$ (100.0), 271 (32.8).

3.4. Methanolysis of **1**

The method of methanolysis was the same as Refs. [21,22]. The t_R of methyl (2R)-2-hydroxypalmitate (**1b**) was 12.6 min; white solid. $[\alpha]_D^{24} - 1.3$ (c 0.04, *n*-hexane) [23]; EI-MS (70 eV) *m/z* (relative intensities, %) 286 $[M]^+$ (4.5), 254 $[M-CH_3OH]^+$ (5.3), 227 $[M-CH_3COO]^+$ (3.0). 2-Acetoamino-1, 3-diacetoxy-4-methoxyl-8-heptadecaene (**1c**) was white solid. EI-MS (70 eV) *m/z* (relative intensities, %) 469 $[M]^+$ (5.2), 410 $[M-Ac]^+$ (4.5), 392 $[M-Ac-H_2O]^+$ (5.8), 349 $[M-2\times HOAc]^+$ (6.9). 1-O-Methyl-D-glucopyranoside was obtained as described in Ref. [24]. $[\alpha]_D^{24} + 76.8°$ (c 0.08, methanol), [literature (24) $[\alpha]_D^{25} + 77.3°$]; FAB$^+$-MS *m/z* 194 $[M]^+$.

3.5. (2S, 3R, 8E)-1-(β-D-glucopyranosyl-3-hydroxy-2-[(R)-2′-hydroxypalmitoyl]amino-8-octadecaene (**2**)

White amorphous powder. mp 191–194 °C; $[\alpha]_D^{24} + 8.6°$ (c 0.12, methanol); IR (KBr) ν_{max}: 3425 (OH), 2960, 1645, 1540, 1070, 720 cm^{-1}; FAB$^+$-MS *m/z* (relative intensities,%) 716 $[M+H]^+$ (62.0), 554 $[M+H-162]^+$ (100), 387 (8.1), 282 (10.2); HR-FAB$^+$-MS *m/z* 716.5687 $[M+H]^+$ ($C_{40}H_{76}NO_9$, calcd. 716.5680). The NMR (Table 1) and IR spectra of **2** were identical with those reported in the literature [29,30].

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References

[1] Sang SM, Kikuzaki H, Lapsley K, Rosen RT, Nakatani N, Ho CT. Sphingolipid and other constituents from almond nuts (*Prunus amygdalus* Batsch). J Agric Food Chem 2002;50:4709–12.
[2] Vesper H, Schmelz EM, Nilolova-Karakashian MN, Dillehay DL, Lynch DV, Merrill Jr AH. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. J Nutr 1999;129:1239–50.
[3] Hakomori S. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. Annu Rev Biochem 1981;50:733–64.
[4] Karlsson KA. In: Chapman D, editor. Biological membranes, vol. 4. London: Academic press; 1982. p. 1–74.
[5] Igarashi M, Suzuki K. Effect of exogenous lipids on activities of the rat brain cholesterol ester hydrolase localized in the myelin sheath[1]. J Neurochem 1976;27:859–66.
[6] Tsujita Y, Endo A. Purification and characterization of the two molecular forms of membrane acid protease from *Aspergillus oryzae*. Eur J Biochem 1978;84:347–53.
[7] Clapshaw PA, Seifert W. Effects of detergents, proteins, and lipids on 2′:3′-cyclic-nucleotide 3'-phosphodiesterase activity. J Neurochem 1980;35:164–9.
[8] Carter GT, Rinehart Jr KL. Aplidiasphingosine, an antimicrobial and antitumor terpenoid from an Aplidium species (marine tunicate). J Am Chem Soc 1978;100:7441–2.
[9] Cateni F, Zilic J, Zacchigna M, Procida G. Cerebrosides with antiproliferative activity from *Euphorbia peplis* L. Fitoterapia 2010;81:97–103.
[10] Okuyama E, Yamazaki M. The principles of tetragonia tetragonoides having anti-ulcerogenic activity. II. Isolation and structure of cerebrosides. Chem Pharm Bull 1983;31:2209–19.
[11] Tsuji S, Arita M, Nagai Y. GQ1b, a bioactive ganglioside that exhibits novel nerve growth factor (NGF)-like activities in the two neuroblastoma cell lines. J Biochem 1983;94:303–6.
[12] Kawai G, Ikeda Y. Fruiting-inducing activity of cerebrosides observed with *Schizophyllum commune*. Biochim Biophys Acta 1982;719:612–8.
[13] Kawai G, Ikeda Y. Chemistry and functional moiety of a fruiting-inducing cerebroside in *Schizophyllum commune*. Biochim Biophys Acta 1983;754:243–8.
[14] Kawai G, Ikeda Y. Structure of biologically active and inactive cerebrosides prepared from *Schizophyllum commune*. J Lipid Res 1985;26:338–43.
[15] Kawai G, Ikeda Y, Tubaki K. Fruiting of *Schizophyllum commune* induced by certain ceramides and cerebrosides from *Penicillium funiculosum*. Agric Biol Chem 1985;49:2137–46.
[16] Kawai G, Ohnishi M, Fujino Y, Ikeda Y. Stimulatory effect of certain plant sphingolipids on fruiting of *Schizophyllum commune*. J Biol Chem 1986;261:779–84.
[17] Jia AQ, Tan NH, Zhou J. Two new minor cyclopeptides from *Sagina japonica* (Caryophyllaceae). Fitoterapia 2009;80:192–5.
[18] Jia AQ, Tan NH, Zhao YX, Li N, Zhou J. Two new cyclopeptides from *Arenaria oreophila* (Caryophyllaceae). Helv Chim Acta 2003;86:756–8.
[19] Zhou J, Tan NH. Application of a new TLC chemical method for detection of cyclopeptides in plants. Chin Sci Bull 2000;45:1825.
[20] Biemann K, DeJongh DC, Schnoes HK. Application of mass spectrometry to structure problems. Acetates of pentoses and hexoses. J Am Chem Soc 1963;85:1763–8.
[21] Natori T, Morita M, Akimoto K, Koezuka Y. Agelasphins, novel antitumor and immunostimulatory cerebrosides from the marine sponge *Agelas mauritianus*. Tetrahedron 1994;50:2771–84.
[22] Gao JM, Dong ZJ, Liu JK. A new ceramides from the basidiomycete *Russula cyanoxantha*. Lipids 2001;36:175–80.
[23] Shibuya H, Kawashima K, Sakagami M, Kawanishi H, Shimomura M, Ohashi K, et al. Sphingolipids and glycerolipids. I. Chemical structures and ionophoretic activities of soyacerebrosides I and II from soybean. Chem Pharm Bull 1990;38:2933–8.
[24] Duran R, Zubia E, Ortega MJ, Naranjo S, Salva J. Phallusides, new glucosphingolipids from the ascidian *Phallusia fumigate*. Tetrahedron 1998;54:14597–602.
[25] Jin W, Rinehart KL, Jares-Erijman ER. Ophidiacerebrosides: cytotoxic glycosphingolipids containing a novel sphingosine from a sea star. J Org Chem 1994;59:144–7.
[26] Walker TE, London RE, Whaley TW, Barker R, Matwiyoff NA. Carbon-13 nuclear magnetic resonance spectroscopy of [1–13C] enriched monosaccharides. Signal assignments and orientational dependence of

geminal and vicinal carbon–carbon and carbon–hydrogen spin–spin coupling constants. J Am Chem Soc 1976;98:5807–13.
[27] Yi JH, Luo YG, Li BG, Zhang GL. Phytoecdysteroids and glycoceramides from *Eriophyton wallchii*. Steroids 2004;69:809–15.
[28] Kang SS, Kim JS, Xu YN, Kim YH. Isolation of a new cerebroside from the root bark of *Aralia elata*. J Nat Prod 1999;62:1059–60.
[29] Ling TJ, Xia T, Wan XC, Li DX, Wei XY. Cerebrosides from the roots of *Serratula chinensis*. Molecules 2006;11:677–83.
[30] Mori K, Kinsho T. Synthesis of wheat grain cerebroside with fruiting-inducing effect on *Schizophyllum commune*. Liebigs Ann Chem 1991;12: 1309–15.
[31] Lin YP, Yan J, Qiu MH. Novel imine from *Hemsleya macrocarpa* var. *calavata*. Lipids 2006;41:97–9.
[32] Ahmad I, Anis I, Fatima I, Malik A, Khan S, Afza N, et al. Two new protease-inhibiting glycosphingolipids from *Buddleja crispa*. Chem Biodiver 2007;4:917–24.
[33] Higuchi R, Jhou JX, Inukai K, Komori T. Biologically active glycosides from asteroidea, XXVIII. Glycosphingolipids from the starfish *Asterias amurensis versicolor* Sladen, 1. Isolation and structure of six new cerebrosides, asteriacerebrosides A-F, and two known cerebrosides, astrocerebroside A and acanthacerebroside C. Liebigs Ann Cheml 1991;8:745–52.
[34] Yamada K, Sasaki K, Harada Y, Isobe R, Higuchi R. Constituents of Holothuroidea, 12.[1)] Isolation and structure of glucocerebrosides from the sea cucumber *Holothuria pervicax*. Chem Pharm Bull 2002;50: 1467–70.
[35] Kawatake S, Nakamura K, Inagaki M, Higuchi R. Isolation and structure determination of six glucocerebrosides from the starfish *Luidia maculate*. Chem Pharm Bull 2002;50:1091–6.
[36] Shin J, Seo Y. Isolation of new ceramides from the gorgonian Acabaria undulate. J Nat Prod 1995;58:948–53.