Two New Cerebrosides from the Aerial Parts of *Tithonia diversifolia*

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Two new cerebrosides, (2*R*)-*N*-{(1*S*,2*S*,3*R*,8*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide (**1**) and (2*R*)-*N*-{(1*S*,2*R*,8*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide (**2**), were isolated from the aerial parts of *Tithonia diversifolia* (HEMSL.) A. Gray. Their structures were determined on the basis of spectroscopic analysis (IR, HR-ESI-MS, and 1D-, and 2D-NMR).

Instruction. – *Tithonia diversifolia* (HEMSL.) A. GRAY (Compositae) is known as Mexican Sunflower, tree marigold, shrub sunflower or Japanese sunflower, sepeleba, *pua renga*, *kava-kava*, and *matala*, natives to Mexico and Central America, and it has also been introduced to other countries to serve as ornamental and green manure to prevent soil erosion [1]. Presently, field investigations showed that *T. diversifolia* had been currently distributed in 53 counties of Yunnan Province, China. *T. diversifolia* is known to be used in folk medicine to treat various illnesses including malaria [2][3], inflammation [4], diabetes [5], haematomas, dissolving lumps [1], as well as bacterial and parasitic infections [6]. Meanwhile, the cytotoxic, anti-amoebic, and spasmolytic activities [7][8] have been described. Phytochemical investigations of *T. diversifolia* revealed the presence of sesquiterpenes [9][10] and chromones [11] as major constituents, although flavonoids [10], quininic acid, diterpenes, anthraquinones, ceramide [12], and furocoumarin have also been isolated. Among these isolates, tagitinin C was the major sesquiterpene lactone and showed gastroprotective [13] and anti-human-glioblastoma activities [14][15]. Chlorogenic acids from *Tithonia diversifolia* exhibited better anti-inflammatory effects than indomethacin and its sesquiterpene lactones [16].

To find biologically active substances, the aerial parts of *T. diversifolia* were phytochemically investigated to afford two new cerebrosides, (2*R*)-*N*-{(1*S*,2*S*,3*R*,8*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide (**1**) and (2*R*)-*N*-{(1*S*,2*R*,8*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide (**2**). Here, we report the isolation and structure elucidation of these two new cerebrosides (*Fig. 1*).

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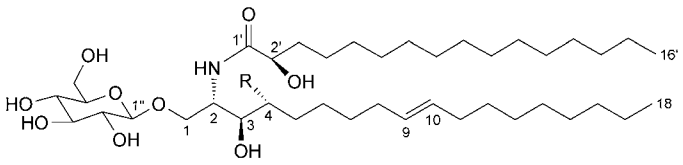
[1]) The first two authors contributed equally to this work.



1 R = OH
2 R = H

Fig. 1. *Structures of compounds* **1** *and* **2**

Results and Discussion. – Compound **1** was obtained as white powder. Its molecular formula was determined as $C_{40}H_{77}NO_{10}$ by its HR-ESI-MS (*m/z* 732.5595 ($[M+H]^+$, $C_{40}H_{78}NO_{10}^+$; calc. 732.5620)) and ^{13}C-NMR data analysis. The IR spectrum of **1** evidenced the presence of OH (3385 cm^{-1}), amide (1635 and 1540 cm^{-1}), olefine (1698 cm^{-1}), sp^3-C–H (2920 and 2850 cm^{-1}), C–H (and 1384 cm^{-1}), and C–O (1079 cm^{-1}) groups. The NMR spectra of **1** (*Table*) exhibited signals of an amide N–H (δ(H) 8.56 (*d*, *J* = 9.0, 1 H)) and a C=O group (δ(C) 175.6), and of several H-atoms (δ(H) 1.20–1.30 (*m*, 36 H) and 0.84 (*t*, 6 H)), indicating that **1** might belong to the sphingolipid class. The ^{1}H-NMR spectrum of **1** revealed the presence of a (*E*)-C=C bond with a signal at δ(H) 5.49 (*dt*, *J* = 15.0, 6.0, H–C(9)[2]) and 5.46 (*dt*, *J* = 15.0, 6.0, H–C(10)), while the 15.0-Hz coupling constant indicated an (*E*)-geometry. This was confirmed by the following HSQC correlations: H–C(9)/C(9) (δ(C) 130.6) and H–C(10/C(10) (δ(C) 130.8). In addition, signals at δ(H) 4.93 (*d*, *J* = 7.8, 1 H), 4.16–4.21 (*m*, 2 H), 3.83–3.85 (*m*, 1 H), 4.33 (*dd*, *J* = 5.4, 12.0, 1 H), and 4.47 (*d*, *J* = 2.4, 12.0, 1 H) confirmed the presence of the glucopyranose moiety. The ^{13}C-NMR signals at δ(C) 105.6, 75.1, 78.5, 71.4, 78.4, and 62.5 also suggested that the sugar in **1** was a glucopyranose. The glucopyranose moiety was determined to have the β-configuration, supported by the coupling constant of the glucose anomeric H-atom (δ(H) 4.93, *J* = 7.8). The ^{13}C-NMR signals at δ(C) 70.4 (C(1)), 75.8 (C(3)), 72.4 (C(4)), and 72.4 (C(2′)) indicated the presence of four more oxygenated C-atoms. This was confirmed by the following HSQC correlations: δ(H) 4.50 (*dd*, *J* = 4.2, 10.8, 1 H–C(1)), 4.69 (*dd*, *J* = 6.6, 10.8, 1 H–C(1))/δ(C) 70.4; δ(H) 4.27 (*dd*, *J* = 4.8, 7.2, H–C(3))/δ(C) 75.8; δ(H) 4.16–4.21 (*m*, H–C(4))/δ(C) 72.4, and δ(H) 4.56 (*dd*, *J* = 3.6, 7.8, H–C(2′))/δ(C) 72.4. Furthermore, the H-atom signal at δ(H) 5.26–5.30 (*m*, H–C(2)) and the C-atom signals at δ(C) 70.4 (C(1)), 51.4(C(2)), 75.8 (C(3)), and 72.4 (C(4)) in the NMR spectra of **1** were in good agreement with those of a reported cerebroside with a 1,3,4-trihydroxy long-chain base [17]. This was confirmed by the following ^{1}H,^{1}H-COSY correlations (*Fig. 2*): δ(H) 4.69 (H_b–C(1))/H–C(2), H–C(2)/4.27 (H–C(3)), 4.27/H–C(4) and HMBC correlations (*Fig. 2*): δ(H) 4.69 (H_b–C(1)) and 4.50 (H_a–C(1)) with δ(C) 51.4 (C(2) and 75.8 (C(3)); 5.28 (H–C(2)) with 70.4 (C(1)) and 72.4 (C(4)); and 4.27 (H–C(3)) with 70.4 (C(1)). According to the MS data, the number of C-atoms in the lipid's base and lipid's amide were determined to be 18 (fragment ions at *m/z* 316.2829 ($[M+NH_3]^+$)) and 16 (fragment ions at *m/z* 272.2595 ($[M+NH_3]^+$)), respectively (*Fig. 3*). Meanwhile, the position of the C=C bond was confirmed by the

[2]) Atom numbering as shown in *Fig. 1*. For systematic names, see the *Exper. Part.*

Table. *^{1}H- and ^{13}C-NMR Data* (C_5D_5N) *for Compounds* **1** *and* **2**. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Postion	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.50 (*dd*, *J* = 4.2, 10.8, 1 H), 4.69 (*dd*, *J* = 6.6, 10.8, 1 H)	70.4	4.50 (*dd*, *J* = 4.8, 10.8, 1 H), 4.72 (*dd*, *J* = 6.6, 10.8, 1 H)	70.4
2	5.26–5.30 (*m*, 1 H)	51.4	4.68–4.74 (*m*, 1 H)	54.5
3	4.27 (*dd*, *J* = 4.8,7.2, 1 H)	75.8	4.18 (*dd*, *J* = 4.8, 7.2, 1 H)	71.2
4	4.16–4.21 (*m*, 1 H)	72.4	1.86–1.94 (*m*, 1 H), 2.18–2.28 (*m*, 1 H)	33.9
5	1.85–1.90 (*m*, 1 H), 2.22–2.27 (*m*, 1 H)	33.8	1.86–1.94 (*m*, 2 H)	27.5
6	1.71–1.76 (*m*, 1 H), 1.95–1.99 (*m*, 1 H)	26.0	1.90–2.00 (*m*, 2 H)	26.1
7	1.20–1.30 (*m*, 2 H)	29.5–30.3	1.20–1.30 (*m*, 2 H)	29.5–30.3
8	1.95–2.03 (*m*, 2 H)	33.0	1.97–2.05 (*m*, 2 H)	33.0
9	5.49 (*dt*, *J* = 6.0, 15.0, 1 H)	130.6	5.46 (*dt*, *J* = 5.4, 14.4, 1 H)	130.6
10	5.46 (*dt*, *J* = 6.0, 15.0, 1 H)	130.8	5.44 (*dt*, *J* = 5.4, 14.4, 1 H)	130.2
11	2.07–2.13 (*m*, 2 H)	33.3	2.06–2.12 (*m*, 2 H)	33.0
12–16	1.20–1.30 (*m*, 10 H)	29.5–30.3	1.20–1.30 (*m*, 10 H)	29.5–30.3
17	1.20–1.30 (*m*, 2 H)	22.9	1.20–1.30 (*m*, 2 H)	22.9
18	0.84 (*t*, *J* = 6.6, 3 H)	14.3	0.84 (*t*, *J* = 6.6, 3 H)	14.3
1′	–	175.6	–	175.5
2′	4.56 (*dd*, *J* = 3.6, 7.8, 1 H)	72.4	4.60 (*dd*, *J* = 3.6, 7.8, 1 H)	72.4
3′	1.94–2.00 (*m*, 1 H), 2.14–2.19 (*m*, 1 H)	35.5	1.94–2.00 (*m*, 1 H), 2.14–2.19 (*m*, 1 H)	35.6
4′	1.80–1.90 (*m*, 2 H)	25.8	1.75–1.83 (*m*, 2 H)	25.8
5′–14′	1.20–1.30 (*m*, 20 H)	29.5–30.3	1.20–1.30 (*m*, 20 H)	29.5–30.3
15′	1.20–1.30 (*m*, 2 H)	22.9	1.20–1.30 (*m*, 2 H)	22.9
16′	0.84 (*t*, *J* = 6.6, 3 H)	14.3	0.84 (*t*, *J* = 6.6, 3 H)	14.3
NH	8.56 (*d*, *J* = 9.0, 1 H)		8.43 (*d*, *J* = 8.4, 1 H)	
1″	4.93 (*d*, *J* = 7.8, 1 H)	105.6	4.90 (*d*, *J* = 7.8, 1 H)	105.7
2″	3.99 (*dd*, *J* = 7.8, 7.8, 1 H)	75.1	4.02 (*dd*, *J* = 7.8, 7.8, 1 H)	75.1
3″	4.16–4.21 (*m*, 1 H)	78.5	4.19–4.22 (*m*, 1 H)	78.6
4″	4.16–4.21 (*m*, 1 H)	71.4	4.16–4.21 (*m*, 1 H)	71.6
5″	3.83–3.85 (*m*, 1 H)	78.4	3.94–4.02 (*m*, 1 H)	78.5
6″	4.33 (*dd*, *J* = 5.4, 12.0, 1 H), 4.47 (*dd*, *J* = 2.4, 12.0, 1 H)	62.5	4.35 (*dd*, *J* = 5.4, 12.0, 1 H), 4.50 (*dd*, *J* = 2.4, 12.0, 1 H)	62.7

NMR spectrum and ESI-MS/MS fragment analysis (fragment ions at *m*/*z* 133.1013 and 107.0849; *Fig. 3*). The location of the glucopyranose moiety was fixed at C(1) by the observed HMBC correlation from H–C(1″) to C(1) (δ(C) 70.4), and the H-atom signals at δ(H) 4.50 (H–C(1)) and 4.69 (H–C(1)) showed correlations with the glucose anomeric C-atom signal at δ(C) 105.6. The chemical shifts of the C-atom signals of C_2–C_4 of glucosphingolipids are especially suitable for determination of the absolute configuration of the phytosphingosine moiety [18][19]. Based on the literature and the ^{13}C-NMR spectral data, the relative configuration of C(2) (δ(C) 51.4)), C(3) (δ(C) 75.8), and C(4) (δ(C) 72.4) were determined as (2*S*), (3*S*), and (4*R*), respectively. It is

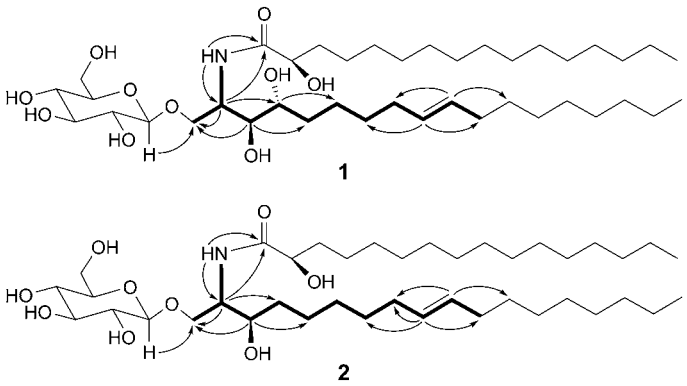


Fig. 2. *$^1H,^1H$-COSY* (—) *and key HMBC* (H → C) *correlations of compounds* **1** *and* **2**

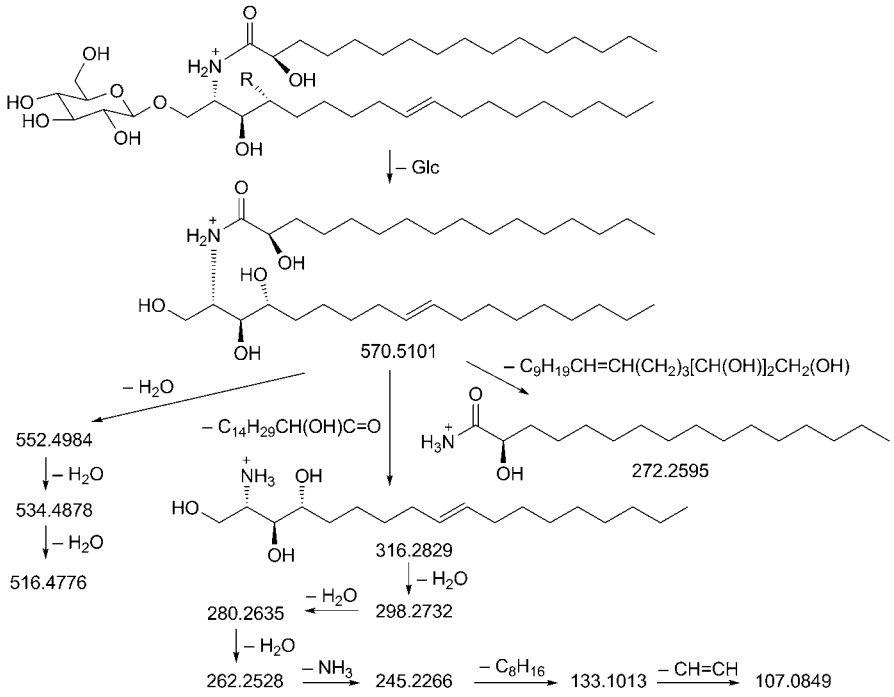


Fig. 3. *Key HR-ESI-MS/MS fragment ions of compound* **1**

reported that the absolute configuration of C(2′) in all cerebrosides isolated from natural plants is (*R*). The chemical shift of C(2′) (δ(C) 72.4) is very similar to those cerebrosides which have the same configuration [18][19]. Thus, based on the above evidences, compound **1** was determined as (2*R*)-*N*-{(1*S*,2*S*,3*R*,8*E*)-1-[(*β*-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide.

Compound **2** was obtained as a white powder, whose molecular formula $C_{40}H_{77}NO_9$ was inferred from the positive-ion HR-ESI-MS (*m/z* 716.5660 ($[M+H]^+$, $C_{40}H_{78}NO_9^+$; calc. 716.5673)). The IR, NMR, and mass spectra were similar to those of compound **1**, which indicated that compound **2** had a glucosylceramide structure. The main difference, compared with compound **1**, was the absence of HO–C(4) in compound **2**. The length of the chain and the location of the C=C bond were the same as in **1**. The C-atom chemical shifts at δ(C) 54.5 (C(2)), 71.2 (C(3)), and 72.4 (C(2′)) indicated the (2*S*), (3*S*), and (2′*R*) configurations, respectively, in agreement with those reported cerebrosides [20]. Accordingly, compound **2** was determined as (2*R*)-*N*-{(1*S*,2*R*,8*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide.

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Experimental Part

General. Silica gel *GF$_{254}$* (SiO_2, 100–200 mesh; *Luyou company of Yantai*), *RP-C18* (43–60 μm, *Merck*). TLC: Silica gel *60 RP-18 F254* (*Merck*) and *MCI* gel (*Mitsubishi chemical corporation*). Optical rotations: *Perkin-Elmer* polarimeter (serial No. 9903). IR Spectra: *Bruker Vector 22* spectrometer; KBr pellet; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-600* spectrometer at 600 (^1H) and 150 MHz (^{13}C); δ in ppm rel. to the solvent peaks δ(H) 7.22 and δ(C) 135.5 for pyridine, *J* in Hz. HR-ESI-MS: *Varian MAT-212* mass spectrometer and a *Agilent Technologies 6538 UHD* accurate-mass Q-TOF LC/MS spectrometer (*Agilent Technologies*, MA, USA); in *m/z*.

Plant Material. Aerial parts of *Tithonia diversifolia* (HEMSL.) A. GRAY (Compositae), collected in Mengzi, Yunnan Province, China in September, 2007 were identified by *W.-S. C.* A voucher specimen (No. TD20070927) was deposited with the Department of Pharmacognosy, Second Military Medical University, Shanghai, China.

Extraction and Isolation. The aerial parts of *T. diversifolia* (21 kg) were percolated with 80% EtOH (3 × 200 l, total amount 600 l) at r.t., and the EtOH was removed under reduced pressure to give a residue (2.48 kg), The residue was suspended with H_2O (21 l) and then extracted with petroleum ether (3 × 21 l), AcOEt (4 × 21 l), and BuOH (3 × 21 l) to afford 128.0 g of AcOEt extract.

The AcOEt extract (80 g) was subjected to CC (SiO_2 (100–200 mesh, 110 × 18 cm); CH_2Cl_2/MeOH 30:1, 15:1, 10:1, 5:1, 3:1, 2:1, and MeOH) to yield seven fractions, *Frs. 1–7. Fr. 3* was subjected to CC (*MCI* gel (20 × 5 cm); MeOH/H_2O 4:1, 1:1, 1:4, and MeOH) to give four fractions, *Frs. 3.1–3.4*. Compounds **1** (76.3 mg) and **2** (34.8 mg) were purified by CC (*ODS* gel (15 × 2.5 cm); MeOH/H_2O 9:1, 19:1) from *Fr. 3.4*.

*(2*R*)-*N*-{(1*S*,2*S*,3*R*,8*E*)-1-[(β-*D*-Glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide* (**1**). White powder. $[\alpha]_D^{20} = +9.1$ ($c = 0.35$, MeOH). IR (KBr): 3385, 2920, 2850, 1716, 1698, 1635, 1540, 1467, 1384, 1254, 1079. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS: 733.79 ($[M+H]^+$), 755.66 ($[M+Na]^+$), 767.37 ($[M+Cl]^-$). HR-ESI-MS/MS: 732.5595 ($[M+H]^+$), 754.5413 ($[M+Na]^+$), 570.5101 ($[M-Glu+H]^+$), 552.4984, 534.4878, 516.4776, 344.2806, 316.2829, 298.2732, 280.2635, 272.2595, 262.2528, 245.2266, 133.1013, 107.0849.

*(2*R*)-*N*-{(1*S*,2*R*,8*E*)-1-[(β-*D*-Glucopyranosyloxy)methyl]-2-hydroxyheptadec-8-en-1-yl}-2-hydroxyhexadecanamide* (**2**). White powder. $[\alpha]_D^{20} = +9$ ($c = 0.3$, MeOH). IR (KBr): 3395, 2917, 2849, 1650, 1541, 1470, 1384, 1251, 1072. ^1H- and ^{13}C-NMR: see the *Table*. ESI-MS: 752.33($[M+Cl]^-$). HR-ESI-MS/MS: 716.5660 ($[M+H]^+$), 738.5480 ($[M+Na]^+$), 554.5139 ($[M-Glu+H]^+$), 536.5020, 518.4934, 300.2893, 282.2794, 264.2684, 247.2416, 135.1165, 109.1011.

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