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# A NEW CEREBROGALACTOSIDE FROM *Juglans mandshurica*

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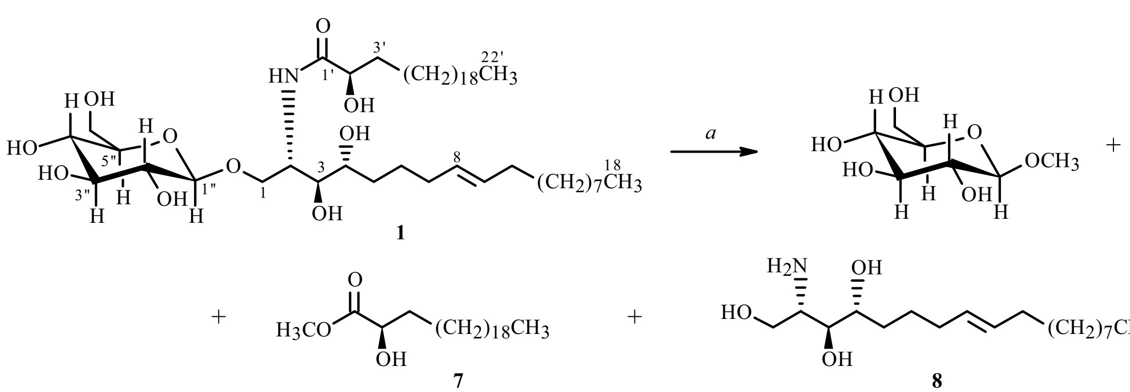
*A new cerebrogalactoside, Juglans cerebroside A (**1**), together with five known compounds, quercetin-3-*O-*β-D-galactopyranoside (**2**), myricetin-3-*O-β-*D-galactopyranoside (**3**), 2″E-quercetin-3-*O-β-*D-(6‴′-*O*-[3″-(4‴-hydroxyphenyl) propylene acyl]) glucopyranoside (**4**), gallic acid (**5**), and 2-methyl-1-hexadecanol (**6**) were isolated from the leaves of* Juglans mandshurica *Maxim. The structures of these compounds were determined by 1D, 2D NMR, and MS techniques.*

**Keywords**: *Juglans mandshurica* Maxim., cerebrogalactoside, flavonoid.

Plants of the genus *Juglans* (Juglandaceae) contain mainly naphthoquinones, flavonoids, diaryl heptanes, terpenoids, and organic acids. Some of them are used in traditional Chinese medicine for the treatment of lithiasis in the urinary system, chronic bronchitis, dermatitis, cancer, desinsection, and so on [1, 2].

*Juglans mandshurica* Maxim., a species of the *Juglans* genus, is widespread in Northeast China, North China, Hebei, and other regions. It has long been used in folk medicine in China, and modern pharmaceutical investigations have revealed its antibacterial, anti-inflammatory, and anticancer effects. In our study on the subject, we herein report one new compound (**1**) and five (**2–6**) known compounds isolated from the leaves of the title plant. All of these known compounds were isolated from *J. mandshurica* Maxim. for the first time.



*a*. 1N HCl, aq. MeOH

Compound **1** was isolated as a white amorphous powder and was assigned the molecular formula $C_{46}H_{89}NO_{10}$ by its HR-ESI-MS (838.63350 $[M + Na]^+$, calcd 838.6379 $[M + Na]^+$). IR bands at 3351 and 1623 suggested the presence of hydroxyl and amide groups. It showed one amide group at δ 7.53 (1H, d, J = 9.6 Hz) in its $^1$H NMR spectrum and at δ 173.7 in its $^{13}$C NMR spectrum (Table 1). One glucopyranosyl moiety was indicated by comparing the $^{13}$C NMR data (δ 103.4, 73.4, 76.5, 70.1, 76.8 and 61.0) and a doublet (J = 7.6 Hz) at δ 4.15 in the PMR spectrum with those reported in the literature [3].

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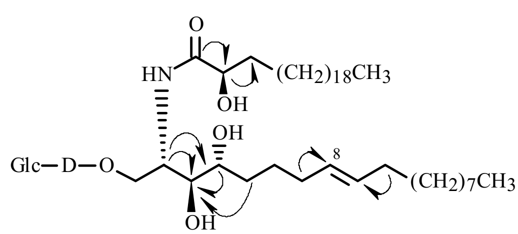
TABLE 1. PMR and $^{13}$C NMR Spectra of **1** (DMSO-$d_6$, δ, ppm, J/Hz)

| C atom | $\delta_C$ | $\delta_H$ | C atom | $\delta_C$ | $\delta_H$ |
|---|---|---|---|---|---|
| 1 | 68.9 | 3.81 (1H, m); 3.65 (1H, m) | 2′-OH | | 5.57 (1H, d, J = 5.2) |
| 2 | 49.8 | 4.08 (1H, m) | 3′ | 34.3 | 1.58 (1H, m); 1.23–1.29 (1H, br.s) |
| 3 | 74.1 | 3.38 (1H, m) | 4′ | 24.4 | 1.49 (1H, m) |
| 3-OH | | 4.92 (1H, d, J =3.6) | 5′–19′ | 28.6–29.1 | 1.23–1.29 (br.s) |
| 4 | 70.5 | 3.36 (1H, m) | 20′ | 31.3 | 1.23–1.29 (br.s) |
| 4-OH | | 4.88 (1H, t, J =2.0) | 21′ | 22.1 | 1.23–1.29 (br.s) |
| 5 | 32.3 | 1.93 (1H, m); 1.49 (1H, m) | 22′ | 13.9 | 0.86 (3H, t, J = 6.8) |
| 6 | 25.5 | 1.49 (2H, m) | 1″ | 103.4 | 4.15 (1H, d, J = 7.6) |
| 7 | 31.7 | 1.93 (2H, m) | 2″ | 73.4 | 2.94 (1H, m) |
| 8 | 129.7 | 5.36 (1H,dt, J =15.5, 6.5) | 2″-OH | | 4.75 (1H, d, J = 9.6) |
| 9 | 130.2 | 5.35 (1H, dt, J =15.5, 6.5) | 3″ | 76.5 | 3.14 (1H, m) |
| 10 | 32.0 | 1.93 (2H, m) | 3″-OH | | 3.31 (1H, m) |
| 11–15 | 28.6–29.1 | 1.23–1.29 (br.s) | 4″ | 70.1 | 3.07 (1H, m) |
| 16 | 31.3 | 1.23–1.29 (br.s) | 4″-OH | | 3.28 (1H, m) |
| 17 | 22.1 | 1.23–1.29 (br.s) | 5″ | 76.8 | 3.11 (1H, m) |
| 18 | 13.9 | 0.86 (3H, t, J = 6.8) | 6″ | 61.0 | 3.68 (1H, m); 3.43 (1H, m) |
| 1′ | 173.7 | – | 6″-OH | | 4.50 (1H, t, J = 6.0) |
| 2′ | 73.4 | 3.86 (1H, m) | NH | | 7.53 (1H, d, J = 9.6) |




**1**

Fig. 1. Key correlations observed for analysis of the HMBC spectrum of compound **1**.

The PMR exhibited the presence of seven hydroxyl groups at 4.75 (1H, d, J = 9.6 Hz), 3.31 (1H, m), 3.28 (1H, m), 4.50 (1H, t, J = 6.0 Hz), δ 5.57 (1H, d, J = 5.2 Hz), 4.92 (1H, d, J = 3.6 Hz), and 4.88 (1H, t, J = 2.0 Hz), where the first four data indicated the presence of a glucopyranosyl moiety, too. Furthermore, a six-proton triplet (J = 6.8 Hz) at δ 0.86 and one pair of olefinic proton signals at δ 5.36 and 5.35 (Table 1) demonstrated that compound **1** possessed two aliphatic chains containing one double bond, suggesting that it was probably a cerebroside [4]. This deduction was reinforced by analysis of the $^{13}$C NMR spectrum (Table 1) as well as by methanolysis of **1**, which liberated the anticipated methyl *β*-D-glucose and two aliphatic molecules (**7** and **8**), all identified by EI mass spectroscopies. The presence of the 1-*O*-glucopyranosyl, 2-amino and 3,4,2′-trihydroxy groups as well as the 8,9-double bond in the main chain was elucidated by analysis of the PMR and $^{13}$C NMR spectroscopic data of **1**, which was assigned unambiguously by extensive 2D NMR techniques (Table 1) (Fig. 1). There are 23 carbons in aliphatic molecule **7**. This was proven by the EI mass spectra of **7**, giving the quasimolecular ion at 370 $[M + H]^+$. Thus, the aliphatic molecule **8** has 18 carbons, which was calculated along with the molecular formula $C_{46}H_{89}NO_{10}$ of **1**. Regarding the stereochemistry, the formulated absolute configuration of compound **1** was based on the carbon chemical shifts at δ 7.53 (1H, d, J = 9.5 Hz), 68.9 (C-1), 49.8 (C-2), 74.1 (C-3), 70.5 (C-4), 173.7 (C-1′), and 73.4 (C-2′), which happened to be fairly close to those previously reported for (2*S*, 3*R*, 4*S*, 2′*R*) sphingosine moieties [3]. In conclusion, compound **1** is assigned as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*R*,4*S*,8*E*)-2-(2′*R*-hydroxyheneicosenoylamino)-8-octadecene-1,3,4-triol, a hitherto undescribed cerebroside. We have named compound **1** Juglans cerebroside A.

## EXPERIMENTAL

**General Comments**. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 983G spectrometer. NMR spectra were measured in DMSO-$d_6$ on a Bruker AM-500 spectrometer using TMS as an internal standard. NMR experiments included the HMQC and HMBC pulse sequences. Coupling constants (J values) are given in Hz. An Autospec-Ultima ETOF spectrometer was used to record the ESI-MS and HR-ESI-MS. Column chromatography was performed on silica gel H (10–40 μm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People′s Republic of China) and Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ, U.S.A.)

**Plant Material and Extraction and Isolation**. The leaves of *J. mandshurica* Maxim. were collected in the outskirts of Jiaohe City, Jilin Province of the People′s Republic of China, in August 2008 and authenticated by Prof. Ming-Lu Deng of Changchun University of Chinese Medicine. The leaves (4.5 kg) of *J. mandshurica* Maxim. were shade-dried, ground, and extracted with refluxing 95% EtOH successively (45 L, 2 h, 2 times). The EtOH extract was evaporated *in vacuo* to yield a semisolid (1400 g), 600 g of which was suspended in $H_2O$ (3000 mL) and partitioned successively with petroleum ether (3 × 3 L), $CHCl_3$ (3 × 3 L), EtOAc (3 × 3 L), and *n*-BuOH (3 × 3 L) to yield 185 g, 23 g, 42 g, and 102 g, respectively. The $CHCl_3$ extracts (18 g) were column chromatographed over silica gel using petroleum ether and EtOAc step gradient as eluents. The petroleum ether and EtOAc (9:1, 8:2) eluates were purified individually by repeated column chromatography over silica gel to yield **6** (8 mg). The EtOAc extract (35 g) was subjected to column chromatography over silica gel eluted with $CHCl_3$–MeOH (100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 1:1) and MeOH to yield fractions 1–8. Fraction 5 (5.65 g) was purified successively with MeOH over Sephadex LH-20 to afford **1** (11 mg), **2** (14 mg), **3** (21 mg), **4** (10 mg), and **5** (17 mg).

**Methanolysis of Compound 1**. A solution of **1** (9.2 mg) in a mixture of MeOH (2 mL), water (0.2 mL), and 12 N HCl (0.2 mL) was refluxed for 7 h [5]. The reaction mixture was immediately cooled and dried by a stream of $N_2$, then subjected to gel filtration over Sephadex LH-20 with MeOH, which afforded the fatty acid methyl ester **7**, long-chain base **8**, and methyl glucopyranoside.

**Juglans Cerebroside A (1)**. $C_{46}H_{89}NO_{10}$, white amorphous powder (MeOH), mp 221–223°C (MeOH). IR (KBr, ν, $cm^{-1}$): 3351, 2955, 2920, 2851, 1623, 1538, 1468, 1371, 1083, 1037, 963 and 721. HR-ESI-MS *m/z* 838.63350 $[M + Na]^+$ (calcd 838.6379 $[M + Na]^+$). Table 1 lists the PMR and $^{13}C$ NMR spectrum.

**Quercetin-3-*O*-*β*-D-galactopyranoside (2)**. Yellow powder (MeOH), mp 182–184°C. ESI-MS *m/z:* 463 $[M – H]^–$ [6].

**Myricetin-3-*O*-*β*-D-galactopyranoside (3)**. Yellow powder (MeOH), mp 193–194°C. ESI-MS *m/z*: 479 $[M – H]^–$ [7].

**2″*E*-Quercetin-3-*O*-*β*-D-(6″″-*O*-[3″-(4‴-hydroxyphenyl)propylene acyl]) glucopyranoside (4)**. Yellow powder (MeOH). The physicochemical constants and spectral (NMR and MS) data of **4** indicate that it is identical to 2″*E*-quercetin-3-*O*-*β*-D-(6″″-*O*-[3″-(4‴-hydroxyphenyl)propylene acyl]) glucopyranoside [8].

**Gallic Acid (5)**. Colorless crystalline needles (MeOH). ESI-MS *m/z:* 169 $[M – H]^–$ [9].

**2-Methyl-1-hexadecanol (6)**. White amorphous powder. EI-MS (70 eV), *m/z* ($I_{rel}$, %): 256 ($M^+$, 2), 238 (5), 125 (10), 111 (21), 97 (32), 83 (43), 69 (54), 57 (100), 43 (90), 29 (20).

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