

Full Paper

Cerebrosides from the Roots of *Serratula chinensis*

Tiejun Ling ^{1,*}, Tao Xia ¹, Xiaochun Wan ¹, Daxiang Li ¹ and Xiaoyi Wei ²

¹ Key Laboratory of Tea Biochemistry and Biotechnology of Ministry of Education and Ministry of Agriculture, Anhui Agricultural University, Hefei 230036, P.R. China; E-mails: xiatao62@126.com (Tao Xia); xcwan@ahau.edu.cn (Xiaochun Wan); dxli@ahau.edu.cn (Daxiang Li)

² South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, P.R. China; E-mail: wxy@scbg.ac.cn

* Author to whom correspondence should be addressed; e-mail: lingtj@ahau.edu.cn

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Abstract: A new cerebroside, 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,8*E*)-2-[(2'*R*)-2-hydroxy-palmitoylamino]-8-octadecene-1,3-diol, along with aralia cerebroside and 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2-hydroxybehenoylamino]-8-octadecene-1,3,4-triol were isolated from the roots of *Serratula chinensis* S. Moore. The structure of the new cerebroside was established by spectroscopic and chemical means. Occurrence of cerebrosides in *Serratula* is reported here for the first time.

Keywords: *Serratula chinensis*, Compositae, cerebrosides.

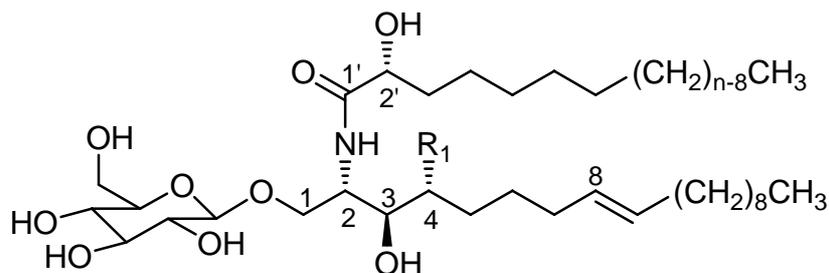
Introduction

Serratula chinensis S. Moore (Compositae) is a perennial herbaceous plant growing mainly in South China [1]. Its roots have long been used as a folk medicine in China for treatment of pharyngitis and morbilli [2]. During the course of our continuing investigation of bioactive natural products found in folk medicinal plants from the southern part of mainland China, we have investigated the chemical constituents of *S. chinensis* roots and have reported in previous papers [3,4] the isolation of seven ecdysteroids and a mixture of five ceramides from that source. This paper deals with the isolation and structure elucidation of some cerebrosides, a novel class of constituents for this plant.

Results and Discussion

The EtOH extract of the powdered dry roots of *S. chinensis* was successively fractionated with petroleum ether, CHCl₃ and *n*-BuOH. The CHCl₃ fraction was separated by a combination of silica gel, Sephadex LH-20, and RP-18 silica gel column chromatography (CC) to yield compounds **1**, **2**, and **3** (Figure 1).

Figure 1. Structures of compounds **1-3**.



- 1** R₁ = H, n = 16
- 2** R₁ = OH, n = 16
- 3** R₁ = OH, n = 22

Compound **1** was isolated as a white amorphous powder, $[\alpha]_D^{25} + 8.8$ (*c* 0.17, MeOH). Its positive ESI-MS showed a $[M + K]^+$ peak at *m/z* 754, a $[M + Na]^+$ peak at *m/z* 738 and a $[M + H]^+$ peak at *m/z* 716, all in accordance with the molecular formula C₄₀H₇₇NO₉. The IR spectrum showed strong absorption bands for hydroxyl (3430 cm⁻¹), amide (1646 and 1540 cm⁻¹) and (CH₂)_n (721 cm⁻¹) groups. The ¹H- and ¹³C-NMR spectra of **1** (Table 1) indicated the presence of a β-D-glucopyranosyl moiety (δ_H 4.86, 1H, d, *J* = 7.6 Hz, anomeric proton; δ_C 105.6, 75.1, 78.6, 71.7, 78.5, and 62.8), an amide linkage (δ_H 8.40, 1H, d, *J* = 9.2 Hz; δ_C 175.7), an amidomethine (δ_H 4.69, δ_C 54.6), an oxygenated methylene (δ_H 4.70 and 4.18; δ_C 70.4), two oxygenated methines (δ_H 4.16 and 4.59; δ_C 71.3 and 72.5), and two long chain aliphatic moieties. The above structural features indicated a dihydrosphingosine type cerebroside [5]. Methanolysis of **1** afforded a fatty acid methyl ester (FAME) and a long chain base (LCB) [6]. The FAME was identified as methyl 2-hydroxypalmitate by GC-MS analysis. The dihydrosphingosine moiety of **1** was derived as 2-amino-octadecene-1,3-diol by analysis of the ¹H-¹H COSY of **1** and the positive ESI-MS of the LCB. The absolute configuration of C-2' was determined to be *R* form from the specific rotation of the FAME [7]. The 2*S*,3*R* stereochemistry was determined by comparison of the ¹³C-NMR chemical shifts of C-2 and C-3 with those of plakosides C and D [5,6]. In order to determine the position of the double bond in the dihydrosphingosine moiety, the KMnO₄ oxidation was performed on the LCB [4]. The oxidation afforded *n*-decanoic acid which was determined by GC-MS analysis. This allowed the location of the double bond at C-8. The *trans* (*E*) configuration of the double bond in **1** was indicated by the olefinic proton signals which appeared as two double triplets (*J* = 14.4, 5.6 Hz) at δ 5.36 and 5.33 in CD₃OD. This was supported by two carbon signals at δ 33.2/33.1 for the carbons next to the double bond in the ¹³C-NMR spectrum [8-10], which were assigned by the aid of ¹H-¹H COSY and HMQC. In conclusion, **1** was established to be 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,8*E*)-2-[(2'*R*)-2-hydroxypalmitoylamino]-8-octadecene-1,3-diol.

The positive ESI-MS of **3** showed a $[M + Na]^+$ peak at m/z 838, consistent with the composition $C_{46}H_{89}NO_{10}$. The IR spectrum of **3** was similar to that of **1**. The 1H - and ^{13}C -NMR data of **3** (Table 1) were essentially identical with those of poke-weed cerebrosides [6], indicating a cerebroside comprised of monounsaturated (2*S*,3*S*,4*R*)-phytosphingosine, (2*R*)-2-hydroxy fatty acid and β -D-glucopyranose moieties. GC-MS analysis of the FAME obtained from the methanolysis of **3** indicated that the chain length of the fatty acid moiety was C_{22} , and the positive ESI-MS data of the LCB from the methanolysis showed that the chain length of the phytosphingosine moiety was C_{18} . The double bond was located at C-8, as seen in **1**, by the $KMnO_4$ oxidation method. The presence of the two double triplets ($J = 14.8, 4.8$ Hz) at δ 5.35 and 5.32 in the 1H -NMR spectrum of **3** (CD_3OD) and the chemical shifts of the carbons next to the double bond (δ 33.4, 33.1) in the ^{13}C -NMR spectrum also indicated a *trans* double bond in the phytosphingosine moiety. Thus, compound **3** was identified as 1-*O*- β -D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2-hydroxybehenoylamino]-8-octadecene-1,3,4-triol [11]. Cerebroside **2** was identified as aralia cerebroside {1-*O*- β -D-glucopyranosyl-(2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2-hydroxypalmitoylamino]-8-octadecene-1,3,4-triol} by the methods described for **1** and **3**, and by direct comparison of its spectral data with the reported literature values [12].

Conclusions

The present study provides the first report on the presence of cerebrosides in *Serratula* spp., in addition to the identification of the new cerebroside **1**. Cerebrosides have a wide range of biological functions, all potentially related to the amphipathic nature of the molecule [13]. The broad bioactivity spectrum of cerebrosides suggests the further potential utilization of *S. chinensis* roots as a valuable crude drug.

Experimental

General

Optical rotations were measured with a Perkin Elmer 343 spectropolarimeter. The IR spectra were taken in KBr on a WQF-410 FT-IR spectrophotometer. The 1H -NMR (400 MHz), ^{13}C -NMR (100 MHz), and 2D-NMR spectra were recorded on a Bruker DRX-400 instrument. Chemical shifts were expressed in ppm (δ) with TMS as an internal standard. The positive ESI-MS data were obtained with a MDS SCIEX API 2000 LC/MS/MS system by direct inlet using MeOH as solvent. The GC-MS analyses were performed with a Shimadzu QP-5000 instrument [GC conditions: DB-1 capillary column (30 m \times 0.25 mm); column temperature, 60 \rightarrow 260 $^\circ$ C for **1-2**, and 40 \rightarrow 260 $^\circ$ C for **3**; rate of temperature increase, 10 $^\circ$ C/min; injector temperature, 270 $^\circ$ C; He at 15 mL/min]. Silica gel 60 (200-300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, People's Republic of China), Develosil ODS (5 μ m, Nomura Chemical Co. Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography (CC). TLC was performed on precoated plates (Kieselgel 60GF₂₅₄, Merck) with detection effected by exposure to iodine vapor.

Plant material

Roots of *S. chinensis* were collected in Lechang County, Guangdong Province, China, in the autumn of 2001, and identified by Prof. Zexian Li, South China Botanical Garden, Chinese Academy of Sciences. A voucher sample (No. 621633) was deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences.

Extraction and isolation

Ground dry roots of *S. chinensis* (8 kg) were extracted with 95% EtOH by percolation at room temperature. The EtOH percolate was concentrated *in vacuo* to a syrup (500 g). This syrup was suspended in H₂O and the aqueous suspension was successively extracted three times each with petroleum ether, CHCl₃, and *n*-BuOH. The CHCl₃ extract, on concentration, yielded a brown syrup (15 g). This syrup was subjected to CC on silica gel, eluting with CHCl₃-MeOH mixtures of increasing polarities (from 98:2 to 9:1), to obtain three fractions A-C. Fraction B, obtained on elution with 96:4 CHCl₃-MeOH was further subjected to silica gel CC, using 92:8 CHCl₃-MeOH as eluant, to afford two subfractions B1 and B2. Subfraction B2 was separated by CC on Sephadex LH-20 with MeOH as eluant, followed by CC over RP-18 silica gel eluting with 9:1 MeOH-H₂O, to yield **1** (20 mg), **2** (18 mg), and **3** (8 mg).

Cerebroside 1

White amorphous powder. $[\alpha]_D^{25} + 8.8$ (*c* 0.17, MeOH). IR (KBr) cm⁻¹: 3430, 2921, 2852, 1646, 1540, 1467, 1278, 1164, 1078, 721. ¹H- and ¹³C-NMR data, see Table 1. Positive ESI-MS *m/z*: 754 [M + K]⁺, 738 [M + Na]⁺, 716 [M + H]⁺, 554 [M - Hexose + H]⁺.

Methanolysis of 1

Compound **1** (2.0 mg) was refluxed with 0.9 N HCl in 82 % aqueous MeOH (15 mL) for 18 h. The resulting solution was extracted three times with *n*-hexane. The *n*-hexane solution was dried over anhydrous Na₂SO₄ and then concentrated to yield the FAME 2*R*-hydroxypalmitic acid methyl ester (0.6 mg), as a white amorphous powder, $[\alpha]_D^{25} -4.2$ (*c* 0.01, CHCl₃), GC-MS: GC *t*_R 13.13 min, EI-MS *m/z*: 286 [M]⁺ (4), 268 [M - H₂O]⁺ (0.2), 227 [M - CH₃OCO]⁺ (9), 182 [M - CH₃OCO - CH₂OHCH₂]⁺ (0.4), 159 (3), 145 (2), 127 [C₉H₁₉]⁺ (5), 125 (3), 111 (7), 97 (20), 90 [CH₃OC(OH)=CHOH]⁺ (19), 83 (26), 69 (30), 57 (85). The H₂O layer, after evaporation of MeOH, was adjusted to pH 9 with aqueous ammonia and extracted with Et₂O. The Et₂O layer was dried over anhydrous Na₂SO₄ and evaporated to yield the LCB 2-aminooctadec-8-ene-1,3-diol (0.7 mg); positive ESI-MS *m/z*: 300 [M + H]⁺, 282 [M - H₂O + H]⁺.

Oxidation of the LCB from 1

The LCB resulting from methanolysis of **1** (0.3 mg) was dissolved in a mixture of 10 % H₂SO₄ and acetone (5 mL each). KMnO₄ (50 mg) was added and the reaction mixture was stirred overnight at room temperature. The reaction was then quenched with aqueous Na₂S₂O₃ (5%). The reaction mixture, after removal of acetone, was extracted with Et₂O. The Et₂O layer was dried over anhydrous Na₂SO₄,

and concentrated, to give *n*-decanoic acid, GC-MS: GC t_R 12.34 min, EI-MS m/z 172 [M]⁺ (4), 155 [M - OH]⁺ (0.5), 143 (3), 129 (27), 73 (70), 60 [CO₂H₂]⁺ (98), 55 (100).

Table 1. ¹H- and ¹³C-NMR Data of **1** and **3** (in pyridine-*d*₅).

Position	1		3	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	4.70 m	70.4	4.71 dd (10.4, 6.8)	70.4
1b	4.18 m		4.51 dd (10.4, 4.0)	
2	4.69 m	54.6	5.30 m	51.7
3	4.16 m	71.3	4.29 m	76.0
4	2.10 m	34.9	4.20 m	72.5
5	1.78 m	25.9	2.10 m	34.0
6	1.27-1.60	29.7-30.5	1.70 m	25.9
7 and 10	2.10 m	33.2	2.10 m	33.4
		33.1		33.1
8 and 9	5.47 m ^{a)}	130.8	5.48 m ^{b)}	130.9
				130.7
11-15	1.27-1.60	29.7-30.5	1.23-1.32	29.7-30.1
16	1.27-1.60	32.3	1.23-1.32	32.2
17	1.27-1.60	23.1	1.23-1.32	23.0
18	0.88 t (6.8)	14.4	0.86 t (6.8)	14.4
NH	8.40 d (9.2)		8.58 d (9.2)	
1'		175.7		175.8
2'	4.59 dd (6.8, 3.6)	72.5	4.58 dd (7.6, 3.2)	72.5
3'	2.10 m	35.7	2.10 m	35.6
4'	2.10 m, 1.78 m	26.3	2.10 m, 1.70 m	26.9
5'-(n - 3)'	1.27-1.60	29.7-30.5	1.23-1.32	29.7-30.1
(n - 2)'	1.27-1.60	32.3	1.23-1.32	32.2
(n - 1)'	1.27-1.60	23.1	1.23-1.32	23.0
n'	0.88 t (6.8)	14.4	0.86 t (6.8)	14.4
1''	4.86 d (7.6)	105.6	4.94 d (7.6)	105.5
2''	3.98 t (8.0)	75.1	4.00 t (8.0)	75.2
3''	4.18 m	78.6	4.20 m	78.6
4''	4.18 m	71.7	4.20 m	71.6
5''	3.87 m	78.5	3.87 m	78.5
6''a	4.48 br d (11.6)	62.8	4.48 dd (11.6, 2.0)	62.7
6''b	4.30 dd (11.6, 5.6)		4.30 dd (11.6, 6.8)	

a): δ 5.36 and 5.33 (each dt, $J = 14.4, 5.6$ Hz) when measured in CD₃OD.
b): δ 5.35 and 5.32 (each dt, $J = 14.8, 4.8$ Hz) when measured in CD₃OD.
n = 16 for **1**; n = 22 for **3**.

Cerebroside 3

White amorphous powder; $[\alpha]_D^{25} + 9.4$ (*c* 0.11, MeOH); IR (KBr) cm⁻¹: 3400, 2923, 2854, 1722, 1643, 1537, 1466, 1381, 1261, 1167, 1078, 720; ¹H- and ¹³C-NMR data, see Table 1; positive ESI-MS m/z : 854 [M + K]⁺, 838 [M + Na]⁺, 816 [M + H]⁺, 654 [M - hexose + H]⁺.

Methanolysis of **3**

Methanolysis of **3** (2.0 mg), performed by the same method as described for **1**, also yielded a FAME (0.6 mg) and a LCB (0.5 mg). The FAME, 2-hydroxybehenic acid methyl ester, was a white amorphous powder; $[\alpha]_D^{25} - 3.7$ (c 0.01, CHCl_3); GC-MS: GC t_R 27.03 min; EI-MS m/z 370 $[\text{M}]^+$ (6), 352 $[\text{M} - \text{H}_2\text{O}]^+$ (0.5), 311 $[\text{M} - \text{CH}_3\text{OCO}]^+$ (7), 266 $[\text{M} - \text{CH}_3\text{OCO} - \text{CH}_2\text{OHCH}_2]^+$ (1), 159 (2), 145 (3), 127 $[\text{C}_9\text{H}_{19}]^+$ (8), 125 (5), 111 (13), 97 (28), 90 $[\text{CH}_3\text{OC}(\text{OH})=\text{CHOH}]^+$ (25), 83 (30), 69 (31), 57 (88). The LCB, 2-amino-octadec-8-ene-1,3-diol, had the following ESI-MS: m/z 316 $[\text{M} + \text{H}]^+$, 298 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$, 280 $[\text{M} - 2\text{H}_2\text{O} + \text{H}]^+$, 262 $[\text{M} - 3\text{H}_2\text{O} + \text{H}]^+$.

Oxidation of the LCB from **3**

The LCB from **3** (0.3 mg) was oxidized with KMnO_4 by the same method as described for that from **1** to give *n*-decanoic acid, GC-MS: GC t_R 14.07 min, EI-MS m/z 172 $[\text{M}]^+$ (5), 155 $[\text{M} - \text{OH}]^+$ (0.1), 143 (5), 129 (34), 115 (10), 101 (5), 73 (72), 60 $[\text{CO}_2\text{H}_2]^+$ (100), 55 (55).

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Sample Availability: Samples of compound **1** are available from the authors.

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