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NEW NEOLIGNAN GLYCOSIDES AND A NEW CEREBROSIDE FROM *Symplocos caudata*

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A phytochemical investigation of the roots of Symplocos caudata *Wall (Symplocaceae) resulted in the isolation and characterization of two optical isomers of a neolignan glycoside (**1**) and a new cerebroside (**2**). Their structures were elucidated as (7*R*,8*S*)-erythro-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-O-4′-neolignan-4-O-β-D-glucopyranoside, (7*S*,8*R*)-erythro-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-O-4′-neolignan-4-O-β-D-glucopyranoside (**1**), and 1-O-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*,12*E*)-2-N-[(2′*R*)-2′-hydroxyheptacosanoyl]-8,12-docosadiene-1,3,4-triol (**2**), respectively, on the basis of spectroscopic data (1D and 2D NMR, MS and CD).*

Keywords: *Symplocos caudata*, Symplocaceae, lignan, isomers of neolignan, cerebroside.

Symplocos caudata Wall, commonly called "Shan Fan" in China, is a herbal drug grown in mountainous areas in southwestern China. The roots of this plant have been traditionally used to treat jaundice, dysentery, and profuse uterine bleeding by local citizens [1]. However, studies on the bioactive constituents of *S. caudata* have rarely been carried out, and there is only one previous report regarding the isolation of seven phenolics and *β*-daucosterol, glucose, sucrose, and inositol [2]. In our investigation of the bioactive compounds from the roots of *S. caudata*, four optical isomers of a neolignan glycoside, two lignan lactone glycosides, and two phenylpropanoid glycosides were isolated [3]. As part of our continuing study, this report describes the isolation and structural elucidation of two optical isomers of a new neolignan glycoside (**1**) and a new cerebroside (**2**) from the same plant part. Their structures were determined as (7*R*,8*S*)-erythro-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside, (7*S*,8*R*)-erythro-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside (**1**), and 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*,12*E*)-2-*N*-[(2′*R*)-2′-hydroxyheptacosanoyl]-8,12-docosadiene-1,3,4-triol (**2**) on the basis of 1D NMR, 2D NMR, MS, and CD spectrometric data.

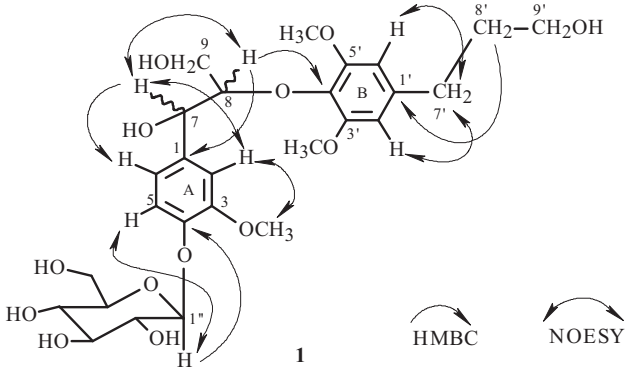

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Fig. 1. The structure and key HMBC and NOESY correlations of compound **1**.

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TABLE 1. ^{1}H and ^{13}C NMR Data, HMBC, ^{1}H–^{1}H COSY, NOESY of **1** and ^{1}H NMR Data of **1a** (δ, ppm, J/Hz)

Position	**1** (DMSO-d_6)					**1a** ($CDCl_3$)
	δ_C	δ_H (mult.)	HMBC	^{1}H–^{1}H COSY	NOESY	δ_H (mult.)
1	136.2					
2	111.2	6.98 (d, J = 2.5)	C-1, 3, 4, 6,7		H-8, 3-OCH_3	6.97 (d, J = 1.8)
3	148.4					
4	145.3					
5	114.6	7.01 (d, J = 9.5)	C-1, 3, 4, 6	H-6	H-6, 1″	6.86 (d, J = 8.1)
6	119.1	6.82 (dd, J = 9.5, 2.5)	C-2, 4, 5, 7	H-5	H-5, 8	6.75 (dd, J = 8.1, 1.8)
7	71.78/71.85	4.82 (d, J = 4.5)	C-1, 2, 6, 8, 9	H-8	H-2, 6, 8	5.00 (d, J = 3.9)
8	85.94/86.00	4.05 (m)	C-1, 4′, 7	H-7, 9a, 9b	H-2, 6, 7	3.91 (m)
9a	59.6	3.34 (m)		H-8, 9b	H-9b	3.47 (dd, J = 12.0, 2.7)
9b		3.67 (m)	C-7, 8	H-8, 9a	H-9a	4.11 (m)
1′	137.7					
2′	105.6	6.48 (s)	C-1′, 3′, 4′, 6′, 7′		H-7′, 3′-OCH_3	6.48 (s)
3′	152.5					
4′	133.20/133.28					
5′	152.5					
6′	105.6	6.48 (s)	C-1′, 2′, 4′, 5′, 7′		H-7′, 5′-OCH_3	6.48 (s)
7′	32.0	2.53 (t, J = 8.0, 7.5)	C-1′, 2′, 6′, 8′, 9′	H-8′	H-2′, 6′	2.70 (t, J = 7.8, 7.5)
8′	34.3	1.70 (m)	C-1′, 7′, 9′	H-7′, 9′	H-9′	1.89 (m)
9′	60.2	3.40 (m)	C-7′, 8′	H-8′	H-8′	3.71 (t, J = 6.3)
3-OCH_3	55.6	3.74 (s)	C-3		H-2	3.90
3′-OCH_3	55.9	3.72 (s)	C-3′		H-2′	3.87
5′-OCH_3	55.9	3.72 (s)	C-5′		H-6′	3.87
1″	100.1	4.86 (d, J = 7.0)	C-4, 3″	H-2″	H-5, 3″, 5″	
2″	73.2	3.23	C-1″, 3″, 4″	H-1″, 3″	H-4″	
3″	77.0	3.24	C-1″, 2″, 4″, 5″	H-2″, 4″	H-1″	
4″	69.6	3.15	C-3″, 5″	H-3″, 5″	H-2″	
5″	76.9	3.24	C-1″, 3″, 4″, 6″	H-4″, 6″a	H-1″	
6″a	60.6	3.43	C-5″	H-5″, 6″b	H-6″b	
6″b		3.64	C-4″	H-6″a	H-6″a	

The molecular formula of compound **1** (Fig. 1) was established as $C_{27}H_{38}O_{13}$ based on negative HR-FAB-MS (*m*/*z* 569.2236 $[M - H]^-$, calcd for $C_{27}H_{37}O_{13}$, 569.2239). In its ^{1}H NMR (500 MHz) spectrum, one set of ABX proton signals at δ 6.98 (1H, d, J = 2.5 Hz), 7.01 (1H, d, J = 9.5 Hz), and 6.82 (1H, dd, J = 9.5, 2.5 Hz) attributed to one 1,3,4-trisubstituted benzene ring, two equivalent aromatic protons at δ 6.48 (2H, s), three methoxyl group protons at δ 3.74 (3H, s) and 3.72 (6H, s), and a *β*-glucopyranosyl anomeric proton at δ 4.86 (1H, d, J = 7.0 Hz) was observed. The proton signals at δ 4.82 (1H, d, J = 4.5 Hz), 4.05 (1H, m), 3.34 (1H, m), 3.67 (1H, m), 3.40 (2H, m), 1.70 (2H, m), and 2.53 (2H, t, J = 8.0, 7.5 Hz) revealed the presence of the 1,2,3-propanetriol moiety and the 1-propanol moiety. The above evidence suggested the presence of two C_6-C_3 units arising both from a neolignan and a glucose moiety, which was supported by analysis of the ^{13}C NMR, COSY, and HMBC spectra. One- and two-dimensional NMR techniques (DEPT, COSY, HMQC, and HMBC) permitted assignments of all the ^{1}H and ^{13}C NMR signals (125 MHz) for **1** (Table 1). The HMBC correlation peaks of H-8 and C-4′, CH_3O and C-3, and CH_3O and C-3′/C-5′, and the anomeric proton resonance of glucose H-1″ to C-4 indicated that compound **1** was a 3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside. Though **1** only gave one spot on TLC and gave one peak in HPLC, the ^{13}C NMR spectral data of **1** (Table 1) showing two sets of chemical shifts for C-7 (δ 71.78/71.85), C-8 (δ 85.94/86.00), and C-4′ (δ 133.20/133.28) indicated that **1** existed as epimers at the C-7 and C-8 like 7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside isolated from *S. caudata* [3].

In terms of the possible staggered conformers with intramolecular hydrogen bonding of the benzylic hydroxyl and aryloxyl groups, the large and small J values for H-7 and H-8 of 8-*O*-4′ neolignan diastereoisomers correspond to the *threo* form (6.0–8.6 Hz) and *erythro* form (2.7–5.0 Hz), respectively [3, 4]. So **1** was hydrolyzed with snailase to prepare its aglycone.

TABLE 2. ^{1}H and ^{13}C NMR Data, HMBC and ^{1}H–^{1}H COSY Correlations of **2** (C_5D_5N, δ, ppm, J/Hz)

Position	δ_C	δ_H (mult.)	HMBC	^{1}H–^{1}H COSY
1b	70.4	4.71 (dd, J = 10.5, 6.5)	C-2, 3	H-1a, 2
1a		4.51 (dd, J = 10.5, 4.5)		H-1b, 2
2	51.7	5.29 (m)		H-1a, 1b, 3, NH
3	75.8	4.28 (m)	C-1, 2, 4, 5	H-2, 4
4	72.4	4.19 (m)	C-2, 3, 6	H-3, 5
5	33.9	2.27, 1.90 (m)		H-4, 6
6	26.8	2.01, 1.76 (m)		H-5, 7
7	27.9	2.21		H-6, 8
8	130.4	5.41	C-10	H-7, 9
9	130.2	5.54	C-7	H-8, 10
10	27.5	2.09, 2.05 (m)		H-9, 11
11	33.3	2.17, 2.12 (m)		H-10, 12
12	130.6	5.45	C-14	H-11, 13
13	130.8	5.53	C-11	H-12, 14
14	33.0	1.97		
15–21	22.9–32.1			
22	14.3			
1′	175.6			
2′	72.4	4.57 (dd, J = 8.0, 3.5)	C-1′, 3′, 4′	H-3′
3′	35.5	2.17, 1.96 (m)	C-2′, 4′	H-2′, 4′
4′	25.8	1.73, 1.67 (m)	C-3′	H-3′
CH_2	22.9–32.1	1.22–1.38		
CH_3	14.3	0.84 (t, J = 7.0, 6.0)		
NH		8.57 (d, J = 9.0)	C-1′, 2	H-2
1″	105.6	4.94 (d, J = 8.0)	C-1, 2″, 3″	H-2″
2″	75.1	4.00 (br.t, J = 8, 8.5)	C-1″, 3″, 4″	H-1″, 3″
3″	78.4	4.18 (m)	C-2″, 4″, 5″	H-2″, 4″
4″	71.4	4.20 (m)	C-2″, 3″, 5″, 6″	H-3″, 5″
5″	78.5	3.84 (m)	C-4″	H-4″, 6″a, 6″b
6″a	62.5	4.48 (dd, J = 12.0, 2.0)	C-4″, 5″	H-5″, 6″b
6″b		4.33 (dd, J = 12.0, 5.0)	C-4″, 5′	H-5″, 6″a

After hydrolysis, its aglycone **1a** and D-glucose were obtained. In the ^{1}H NMR spectra of **1a** in $CDCl_3$, a small coupling constant $J_{7,8}$ = 3.9 Hz was observed; thus, the relative configuration of C-7 and C-8 of **1a** and **1** was determined to be in the *erythro* form. The absolute configurations at C-7 and C-8 of 8-*O*-4′-neolignan were usually established on the basis of CD spectroscopic evidence; the positive Cotton effects at 210–240 nm indicated a 7*S*,8*S*-configuration, whereas the negative CD effects at 210–240 nm justified a 7*R*,8*R*-configuration, according to the study of related systems [5]. Since almost no CD absorption was observed at the above range, compound **1** was definitely determined to be a mixture of the two optical isomers, (7*R*,8*S*)-*erythro*-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside and (7*S*,8*R*)-*erythro*-7,9,9′-trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-*β*-D-glucopyranoside, in a ratio of 1:1. The ratio herein is consistent with that obtained from ^{13}C NMR.

Compound **2** was isolated as a white amorphous powder. HR-FAB-MS furnished the molecular formula $C_{55}H_{105}NO_{10}$, with four degrees of unsaturation. The ^{1}H NMR data (500 MHz) of **2** indicated the presence of an amide linkage (δ 8.57, d, 1H, J = 9.0 Hz; δ_C 175.6), a broad signal centered at δ 1.22–1.38 (methylene protons), a triplet at δ 0.84 (two terminal methyl groups), a *β*-glucopyranosyl anomeric proton at δ 4.94 (1H, d, J = 8.0 Hz), and two oxymethylene protons at δ 4.51 (dd, J = 10.5, 4.5 Hz) and δ 4.71 (dd, J = 10.5, 6.5 Hz), suggesting it to be a cerebroside [6]. In addition, the ^{1}H and ^{13}C NMR data (125 MHz) of **2** exhibited resonances for three oxymethine protons at δ_H/δ_C 4.28/75.8, 4.19/72.4, and 4.57/72.4, and four olefinic protons at δ_H/δ_C 5.41/130.4, 5.54/130.2, 5.45/130.6, and 5.53/130.8.

In the HMBC spectrum, the oxymethine proton at δ 4.57 and the amine proton at δ 8.57 showed correlations with the carbonyl carbon at δ 175.6, indicating that a hydroxyl group was located at C-2′. The fatty acid moiety was identified as 2-hydroxyheptacosanoate by the fragment ion at 582 $[963 - C_{26}H_{53}O]^+$ and 536 $[963 - C_{27}H_{53}O_2 - H_2O]^+$ in FAB-MS.

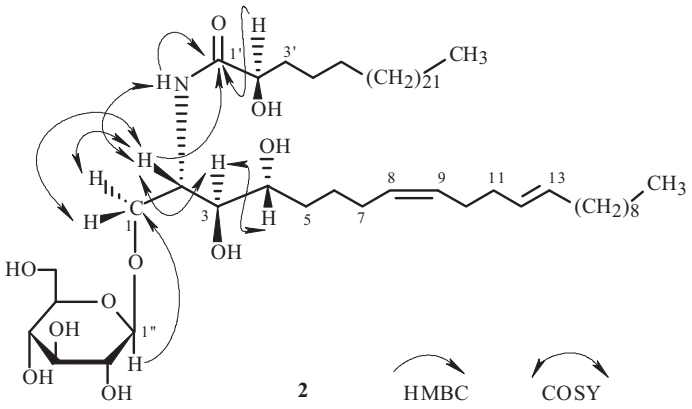

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Fig. 2. The structure and key ^{1}H–^{1}H COSY and HMBC of **2**.

The presence of a 1-*O*-*β*-D-glucopyranosyl-3,4-dihydroxy unsaturated C_{22} long-chain base (LCB) was deduced from the ^{1}H–^{1}H COSY, HMBC, and MS data. The COSY spectrum of **2** showed correlations between the NH signal at δ 8.57 and H-2 at δ 5.29, H-2 and H-1 at δ 4.51 and 4.71, H-2 and H-3 at δ 4.28, and H-3 and H-4 at δ 4.19. Furthermore, the HMBC correlation of H-1″ of glucose with the C-1 at δ 70.4 indicated the attachment of glucose to the C-1 hydroxyl group. The fragment ions at 684 $[963 - C_{19}H_{35}O]^{+}$ and 654 $[963 - C_{20}H_{37}O_2]^{+}$ supported the finding that the LCB moiety possessed 22 carbons containing two double bonds. A careful analysis of the HMBC and ^{1}H–^{1}H COSY spectra enabled us to locate the double bonds in the LCB residue at C-8 and C-12 (Fig. 2 and Table 2). The *trans* (*E*) configuration of the double bond at C-12 was evidenced by the chemical shifts of the methylene carbon adjacent to the olefinic carbon (C-11 signal at δ 33.3 and C-14 signal at δ 33.0), and the cis (*Z*) configuration of the double bond at C-8 was evidenced by the chemical shifts of the methylene carbon (C-7 signal at δ 27.9 and C-10 signal at δ 27.5), which were observed at δ ~ 27 in (*Z*)- isomers and at δ ~ 33 in (*E*)-isomers [7].

The chemical shift of H-2 signal and the ^{13}C chemical shifts of C-1~C-4, C-1′, and C-2′ of sphingosine are especially suitable for determination of the absolute stereochemistry of the phytosphingosine moiety [8]. The chemical shift of H-2 (δ 5.29) and the carbon chemical shifts at δ 70.4 (C-1), 51.7 (C-2), 75.8 (C-3), 72.4 (C-4), 175.6 (C-1′), and 72.4 (C-2′) in **2** were virtually identical with those of the reported data of other (2*S*,3*S*,4*R*,2′*R*)-phytosphingosine moieties. In conclusion, the structure of **2** was determined as 1-*O*-*β*-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*,12*E*)-2-*N*-[(2′*R*)-2′-hydroxy-heptacosanoyl]-8,12-docosadiene-1,3,4-triol.

EXPERIMENTAL

General Procedures. Optical rotations were measured on a Perkin–Elmer 243B digital polarimeter. The CD spectra were measured on a JASCO J-810 spectropolarimeter. The ^{1}H, ^{13}C NMR, as well as 2D NMR spectra were taken on a Bruker Avance DRX 500 NMR spectrometer using TMS as internal standard. HR-FAB-MS were performed on a Bruker Apex II FI-ICR mass spectrometer. Diaion HP-20 (Mitsubishi Chemical Co.), Sephadex LH-20 (Pharmacia Co.), and silica gel 200–300 mesh (Qingdao Marine Chemical Factory, China) were used for column chromatography. Preparative HPLC was performed on a Waters-600 apparatus using a YMC prepacked column (ODS, 10 × 250 mm, for the reverse phase).

Plant Material. The roots of *S. caudata* were collected in January 2004 from Sichuan Province in China and identified by Prof. Hubiao Chen (School of Pharmaceutical Sciences, Peking University Health Science Center). A voucher specimen (No. DNM2007-01) was deposited at the Herbarium of the School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China.

Extraction and Isolation. Air-dried roots (7.5 kg) of *S. caudata* were extracted with 95% ethanol. After evaporation of the solvent under reduced pressure, the residue was suspended in water and extracted with petroleum ether, ethyl acetate, and *n*-butanol successively. The *n*-butanol extract (140 g) was subjected to column chromatography on Diaion HP-20 and eluted with water and 10%, 30%, 50% and 70% ethanol successively. The fraction eluted with 10% ethanol was chromatographed on a silica gel column [chloroform–methanol–water (7:1:0.1)] to give three fractions (fractions A–C). Fraction A (3.8 g) was further chromatographed on a preparative HPLC column [acetonitrile–water (1:9)] to give thirteen fractions (Fr1-13). Fraction

2 was purified on a Sephadex LH-20 column (water) to give compound **1** (93 mg). The ethyl acetate extract (65 g) was subjected to column chromatography on silica gel [petroleum ether–acetone (9:1)], to give ten fractions (Fr1-10). Fraction 10 was further chromatographed on a silica gel column [chloroform–methanol (92:8→9:1)] to give compound **2** (42 mg).

Enzymatic Preparation of 1a. Compound **1** (4.0 mg) was treated with snailase (Protoplasts productivity 50%, Beijing Biotech Biochemistry Technical Co.) in citric acid buffer solution (pH 4.5, 5.0 mL). The mixture was stirred at 40°C for 7 h and then extracted with an equal amount of ethyl acetate (× 4). The ethyl acetate layer was evaporated under reduced pressure to give the aglycone **1a**.

(7*R*,8*S*)-*erythro*-7,9,9′-Trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside and (7*S*,8*R*)-*erythro*-7,9,9′-Trihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (1). White amorphous powder, $[\alpha]_D^{25}$ –32.0° (*c* 1.00, MeOH). UV (MeOH, λ_{max}, nm): 274, 225. HR-FAB-MS: *m*/*z* 569.2236 (calcd 569.2239 for $C_{27}H_{37}O_{13}$) $[M - H]^-$. For ^1H and ^{13}C NMR, HMBC, ^1H–^1H COSY, and NOESY data, see Table 1.

1-*O*-β-D-Glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*,12*E*)-2-*N*-[(2′*R*)-2′-hydroxyheptacosanoyl]-8,12-docosadiene-1,3,4-triol (2). White amorphous powder. HR-FAB-MS: *m*/*z* 940.7805 (calcd 940.7817 for $C_{55}H_{106}NO_{10}$) $[M + H]^+$. FAB-MS: *m*/*z* 963 $[M + Na + 1]^+$, 684 $[963 - C_{19}H_{35}O]^+$, 654 $[963 - C_{20}H_{37}O_2]^+$, 582 $[963 - C_{26}H_{53}O]^+$, and 536 $[963 - C_{27}H_{53}O_2 - H_2O]^+$. For ^1H and ^{13}C NMR, HMBC, and ^1H–^1H COSY data, see Table 2.

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