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Neolignan glycosides from *Symplocos caudata*

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Abstract

A phytochemical investigation of the roots of *Symplocos caudata* Wall (Symplocaceae) resulted in isolation and characterization of four optical isomers of a neolignan glycoside (**1**–**4**), a lignan lactone glycoside (**5**), a phenylpropanoid glycoside (**6**), as well as two known compounds (**7**, **8**). Their structures were elucidated as (7*S*,8*S*)-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**1**), (7*R*,8*R*)-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**2**), (7*R*,8*S*)-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**3**), (7*S*,8*R*)-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**4**), 8*R*,8′*R*-matairesinol-4-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside (**5**), 1-*O*-[β-D-xylopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenyl-phenol (**6**), matairesinoside (**7**), and (*R*)-1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol (**8**) on the basis of spectroscopic data (1D and 2D NMR, MS and CD) and chemical evidence.

Keywords: *Symplocos caudata*; Symplocaceae; Lignan; Phenylpropanoid; Isomers of neolignan

1. Introduction

The plants of the genus *Symplocos* (Symplocaceae) are widely distributed in tropical and subtropical areas in Asia, Oceania and America. The roots, barks, or leaves of many *Symplocos* plants have been used as traditional herbal medicines for treatment of diarrhoea, dysentery, menorrhagia, uterine disorders (Ali et al., 1990), as well as malaria, nephritis and snake bite (Li et al., 2003). Previous phytochemical studies on this genus have yielded many kinds of chemicals, such as triterpenoids, flavonoids, lignans, phenols, steroids, alkaloids, and iridoids (Huo et al., 2007). Recently, much attention has been paid to *Symplocos* species due to their diverse biological activities, particularly anti-HIV (Ishida et al., 2001), inhibition of phosphodiesterase I (Ahmad et al., 2003, 2004; Abbasi et al., 2004; Choudhary et al., 2004), and antitumor applications (Li et al., 2003; Tang et al., 2004).

Symplocos caudata Wall, commonly called "Shan Fan" in China, is a herbal drug grown in mountainous areas of southwestern China. The roots of this plant have been traditionally used to treat jaundice, dysentery, and profuse uterine bleeding by local citizens (Jiangsu College of New Medicine, 1977). However, the study on the bioactive constituents of *S. caudata* was rarely carried out and there was only one previous report regarding the isolation of seven phenolics, β-daucosterol, glucose, sucrose, and inositol from the roots of *S. caudata* (Jiang et al., 2005). In our further investigation of the bioactive compounds from the roots of *S. caudata*, four compounds, which were optical isomers of a neolignan glycoside, were isolated and their structures were determined as (7*S*,8*S*)-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopy-

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ranoside (**1**), (7*R*,8*R*)-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**2**), (7*R*,8*S*)-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**3**) and (7*S*,8*R*)-*erythro*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside (**4**) (Fig. 1) on the basis of analysis of 1D NMR, 2D NMR, HRFABMS, CD spectroscopic data and other chemical evidence. Among them, glycosides **1**–**3** were new compounds. Glycoside **4** had been isolated from *Lonicera gracilipe*, but its configuration was reported incorrectly (Matsuda and Kikuchi, 1996a). A new lignan lactone glycoside (**5**), and a new phenylpropanoid glycoside (**6**) (Fig. 1) were also obtained from this plant together with other two known compounds, a lignan lactone glucoside, matairesinoside (**7**) and a phenylpropanoid glucoside, (*R*)-1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol (**8**). The present paper describes isolation of compounds **1**–**8** and the structural characterization of six new compounds **1**–**6**.

2. Results and discussion

The *n*-BuOH part of EtOH extract of *S. caudata* was subjected to Diaion HP-20, silica gel, Sephadex LH-20 column chromatography and preparative HPLC to give compounds **1**–**8**. The structures of new compounds were elucidated on the basis of spectroscopic data (1D and 2D NMR, MS and CD) and chemical evidence.

The molecular formula of compound **1** was established as $C_{26}H_{36}O_{12}$ based on negative HRFABMS (*m*/*z* 539.2132 $[M-H]^-$, calcd. for $C_{26}H_{35}O_{12}$, 539.2134). In its

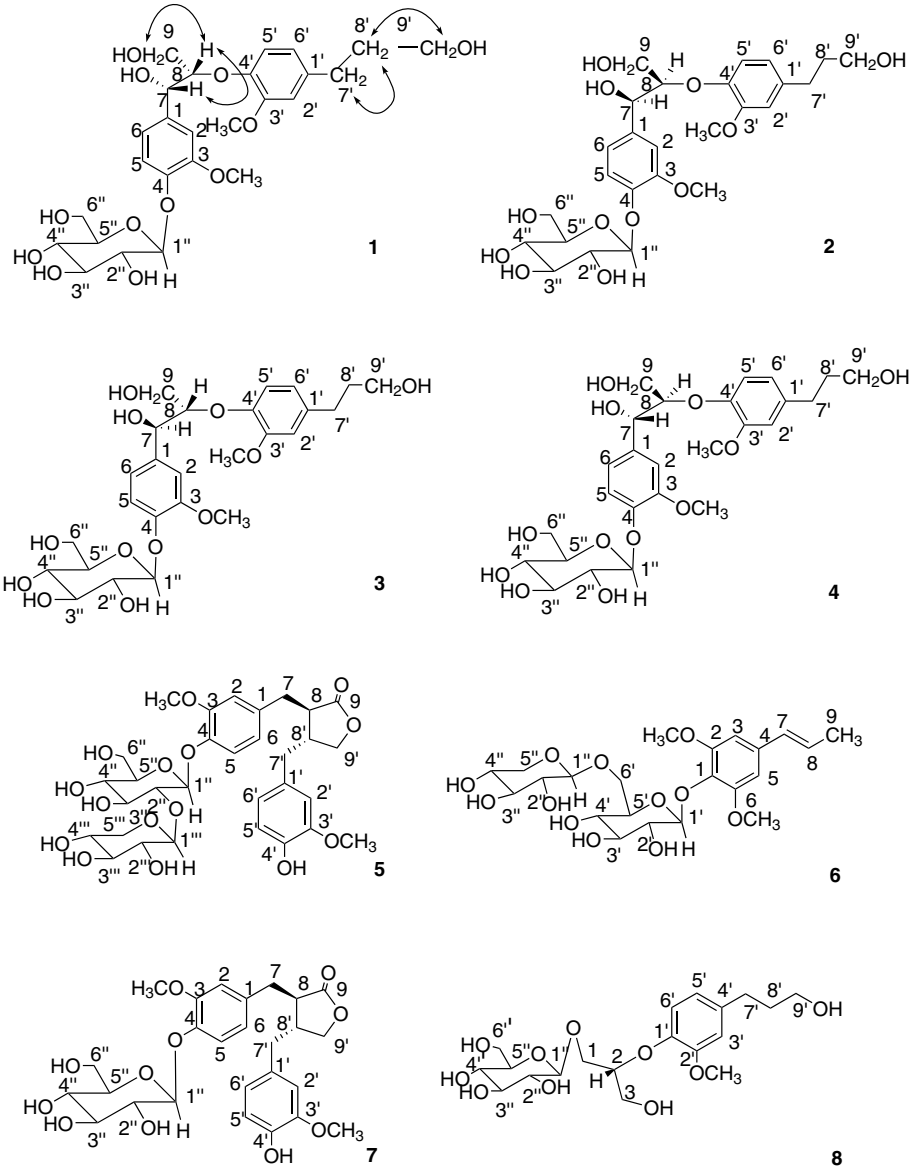

Fig. 1. Structures of **1-8** and ^1H ^1H COSY of **1**.

^{1}H NMR spectrum, two sets of ABX proton signals at δ 7.04 (1H, *d*, $J = 1.5$ Hz), 6.99 (1H, *d*, $J = 8.5$ Hz), 6.85 (1H, *dd*, $J = 8.5$, 1.5 Hz) and 6.78 (1H, *d*, $J = 1.5$ Hz), 6.92 (1H, *d*, $J = 8.5$ Hz), 6.64 (1H, *dd*, $J = 8.5$, 1.5 Hz) attributed to two 1,3,4-trisubstituted benzene rings, two methoxyl group protons at δ 3.72 (3H, s) and 3.74 (3H, s), and a glucopyranosyl anomeric proton at δ 4.86 (1H, *d*, $J = 7.0$ Hz) were observed. The proton signals at δ 4.75 (1H, *d*, $J = 4.5$ Hz), 4.19 (1H, *m*), 3.21 (1H, *m*), 3.58 (1H, *m*) and 3.39 (2H, *m*), 1.67 (2H, *m*), 2.51 (2H, *t*, $J = 7.5$ Hz) established the occurrence of an 1,2,3-propanetriol moiety and an 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 for **1** (Tables 1 and 2). The HMBC correlation peaks of H-8 and C-4′, CH_3O and C-3, CH_3O and C-3′, and the anomeric proton resonance of glucose H-1″ to C-4 indicated that the compound **1** was a 3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside.

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 and *erythro* form, respectively (Braga et al., 1984). So **1** was hydrolyzed with snailase to prepare its aglycone. After hydrolysis, its aglycone **1a** and D-glucose were obtained. In the ^{1}H NMR spectra of **1a** in $CDCl_3$, a large coupling constant $J_{7,8} = 8.1$ Hz was observed, thus the relative configuration of C-7 and C-8 of **1a** and **1** was determined to be in the *threo*-form. The absolute configurations at C-7 and C-8 of **1** and **1a** were established on the basis of the CD spectroscopic evidence. Both the CD spectra of **1** and **1a** showed positive Cotton effects at about 235 nm (Figs. 2 and 3), indicating that **1** and **1a** had the 7*S*, 8*S*-configuration according to the study of related system (Arnoldi and Merlini, 1985). Based on the above evidence, the structure of **1** was definitely determined to be (7*S*,8*S*)-*threo*-7,9,9′-trihydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyranoside as shown in Fig. 1.

Compound **2** was obtained as an amorphous powder, whose molecular formula, $C_{26}H_{36}O_{12}$, was confirmed by the HRFABMS (*m*/*z*: 539.2143 $[M-H]^-$). Its ^{1}H NMR

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**4** (DMSO-d_6) and their aglycones **1a**–**4a** ($CDCl_3$)

Position	1	1a	2	2a	3	3a	4	4a
1								
2	7.04 *d* (1.5)	6.75–7.04	7.03 *d* (1.5)	6.75–7.04	7.04 *d* (1.5)	6.74–6.97	7.05 *d* (1.5)	6.74–6.97
3								
4								
5	6.99 *d* (8.5)	6.75–7.04	6.99 *d* (8.5)	6.75–7.04	7.00 *d* (8.5)	6.74–6.97	7.00 *d* (8.5)	6.74–6.97
6	6.85 *dd* (8.5, 1.5)	6.75–7.04	6.85 *dd* (8.5, 1.5)	6.75–7.04	6.87 *br d* (8.5)	6.74–6.97	6.86 *dd* (8.5, 1.5)	6.74–6.97
7	4.75 *d* (4.5)	4.95 *d* (8.1)	4.75 *d* (4.5)	4.95 *d* (7.8)	4.75 *d* (5.0)	4.96 *d* (4.8)	4.75 *d* (5.0)	4.96 *d* (4.5)
8	4.19 *m*	3.96 *m*	4.19 *m*	3.96 *m*	4.22 *m*	3.96 *m*	4.24 *m*	3.96 *m*
9a	3.21 *m*	3.46 *dd* (12.6, 3.9)	3.21 *m*	3.46 *dd* (12.6, 3.9)	3.21 *m*	3.62 *dd* (12.6, 3.3)	3.21 *m*	3.62 *dd* (12.3.,, 3.3)
9b	3.58 *m*	3.62 *dd* (12.6, 3.3)	3.57 *m*	3.62 *dd* (12.6, 3.3)	3.60 *m*	4.11 *m*	3.61 *m*	4.11 *m*
1′								
2′	6.78 *d* (1.5)	6.75–7.04	6.78 *d* (1.5)	6.75–7.04	6.73 *d* (1.5)	6.74–6.97	6.74 *d* (1.5)	6.74–6.97
3′								
4′								
5′	6.92 *d* (8.5)	6.75–7.04	6.91 *d* (8.5)	6.75–7.04	6.87*d* (8.5)	6.74–6.97	6.89 *d* (8.5)	6.74–6.97
6′	6.64 *dd* (8.5, 1.5)	6.75–7.04	6.64 *dd* (8.5, 1.5)	6.75–7.04	6.62 *dd* (8.5, 1.5)	6.74–6.97	6.62 *dd* (8.5, 1.5)	6.74–6.97
7′	2.51 *t* (8.0, 7.5)	2.68 *t* (8.1, 7.2)	2.51 *t* (8.0, 7.5)	2.68 *t* (7.8, 7.5)	2.50 *t* (8.0, 7.5)	2.68 *t* (7.8, 7.5)	2.51 *t* (8.0, 7.5)	2.69 *t* (7.8,7.5)
8′	1.67 *m*	1.88 *m*	1.67 *m*	1.88 *m*	1.67 *m*	1.89 *m*	1.67 *m*	1.89 *m*
9′	3.39 *m*	3.69 *t* (6.3)	3.39 *m*	3.69 *t* (6.3)	3.39 *m*	3.69 *t* (6.3)	3.39 *m*	3.69 *t* (6.3)
3-OCH_3	3.72 *s*	3.89	3.72 *s*	3.89	3.69 *s*	3.89	3.69 *s*	3.89
3′-OCH_3	3.74 *s*	3.91	3.74 *s*	3.91	3.72 *s*	3.91	3.72 *s*	3.91
1″	4.86 *d* (7.0)		4.85 *d* (7.0)		4.84 *d* (7.0)		4.85 *d* (7.0)	
2″	3.23		3.23		3.23		3.23	
3″	3.24		3.24		3.24		3.24	
4″	3.14		3.14		3.14		3.14	
5″	3.26		3.26		3.26		3.26	
6″a	3.43		3.43		3.43		3.43	
6″b	3.64 *br d* (11.5)		3.64 *br d* (11.5)		3.62 *br d* (11.5)		3.64 *br d* (11.5)	

Table 2
^{13}C NMR spectroscopic data of compounds **1–4** (DMSO-d_6) and their aglycones **1a–4a** ($CDCl_3$)

Position	1	2	3	4	1a	2a	3a	4a
1	135.76	135.77	136.13	136.18	131.67	131.67	131.91	131.92
2	111.19	111.20	111.86	111.96	112.53	112.53	112.53	112.53
3	148.28	148.28	148.32	148.43	145.79	145.79	145.24	145.24
4	145.44	145.46	145.52	145.59	146.79	146.79	146.76	146.76
5	114.48	114.53	114.71	114.77	109.53	109.53	108.74	108.74
6	118.68	118.65	119.21	119.28	121.25	121.22	121.19	121.26
7	70.66	70.72	71.49	71.56	74.17	74.16	72.88	72.86
8	84.47	84.51	83.83	83.97	89.93	89.89	87.74	87.82
9	59.95	59.96	59.88	59.99	61.21	61.20	60.87	60.90
1′	135.25	135.25	135.13	135.26	138.45	138.43	138.40	138.42
2′	112.73	112.73	112.94	113.06	114.45	114.46	114.38	114.39
3′	149.51	149.51	149.56	149.68	151.29	151.26	151.59	151.61
4′	146.14	146.15	145.84	145.94	145.72	145.71	145.10	145.10
5′	115.90	115.96	116.20	116.35	120.44	120.43	119.16	119.16
6′	120.13	120.12	120.03	120.14	121.49	121.48	121.45	121.45
7′	31.28	31.27	31.12	31.22	32.03	32.03	32.03	32.03
8′	34.49	34.48	34.30	34.40	34.41	34.40	34.38	34.39
9′	60.16	60.14	60.05	60.15	62.32	62.31	62.33	62.34
3-OCH_3	55.44	55.43	55.55	55.65	56.15	56.14	56.14	56.15
3′-OCH_3	55.56	55.56	55.58	55.69	56.07	56.06	56.05	56.05
1″	100.04	100.13	100.29	100.31				
2″	73.24	73.23	73.20	73.30				
3″	76.97	76.96	76.90	77.00				
4″	69.63	69.63	69.63	69.74				
5″	76.87	76.86	76.79	76.90				
6″	60.60	60.62	60.60	60.70				

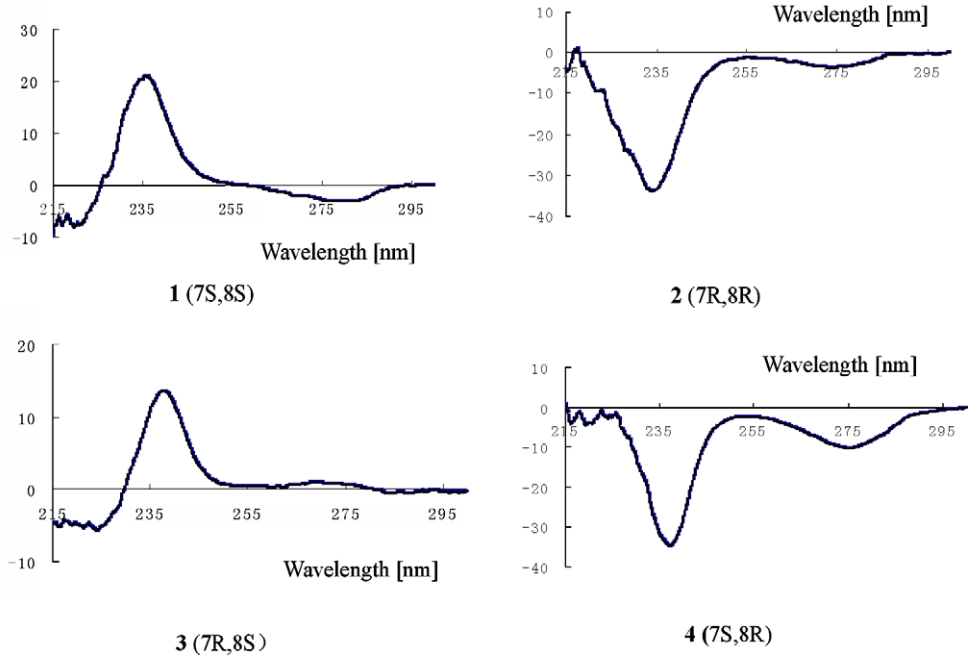

Fig. 2. CD spectra of **1–4**.

and ^{13}C NMR spectroscopic data were identical with those of **1** (Tables 1 and 2), suggesting that the overall structure of **2** was the same as that of **1**. On hydrolysis with snailase, **2** gave D-glucose and an aglycone (**2a**). The large $J_{7,8}$ coupling constant (7.8 Hz) in the ^1H NMR of **2a** suggested a relative-*threo* configuration. The negative CD effects at 234 and 235 nm of **2** and **2a**, respectively (Figs. 2 and 3), justified a 7*R*,8*R*-configuration as shown in Fig. 1.

Compound **3**, an amorphous powder, exhibited a quasi-molecular ion peak at *m/z* 539.2144 $[M-H]^-$ in negative HRFABMS analysis, corresponding to a molecular formula of $C_{26}H_{36}O_{12}$. Its ^1H NMR and ^{13}C NMR spectroscopic

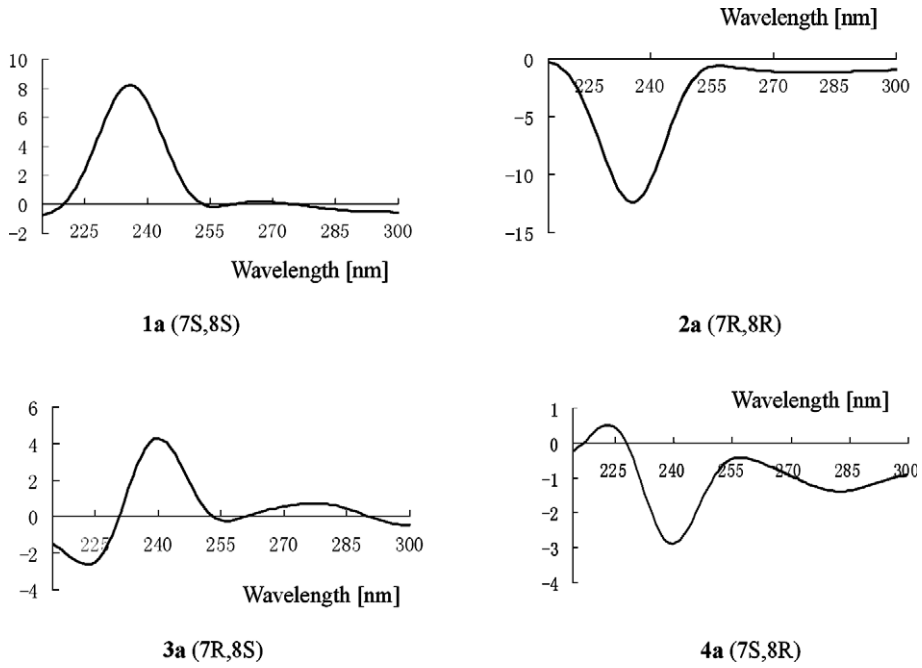

Fig. 3. CD spectra of **1a–4a**.

data were similar to those of **1** except for a little difference in chemical shifts of C-2, 6, 7 and 8 ($\Delta\delta$ 0.5–0.9). Based on analysis of the 2D NMR spectra, the assignments of all the proton and carbon signals were achieved (Tables 1 and 2), and the structure of **3** was established as the same as that of **1**. Accordingly, compound **3** was deduced to be diastereisomer of **1**. In the ^{1}H NMR spectrum of its aglycone (**3a**), obtained by enzymatic hydrolysis of **3**, a small coupling constant of H-7 ($J = 4.8$ Hz), was observed. This was different from that of **1a**, and indicated that the relative configuration of C-7 and C-8 of **3** was in the *erythro*-form. Furthermore, the positive Cotton effect at 238 and 240 nm in the CD spectra of **3** and **3a**, respectively, (Figs. 2 and 3) determined the absolute configuration of compound **3** to be 7*R*,8*S* as shown in Fig. 1.

Compound **4** was obtained as an amorphous powder, whose molecular formula was confirmed to be $C_{26}H_{36}O_{12}$ by the TOFMS. Its ^{1}H NMR and ^{13}C NMR spectroscopic data were in good agreement with those of **3** (Tables 1 and 2). The ^{1}H NMR spectrum of the aglycone (**4a**), obtained by enzymatic hydrolysis of **4**, established a small coupling constant of $J_{7,8}$ ($J = 4.5$ Hz) (Table 1). Therefore, C-7 and C-8 of compound **4** was determined to be in the *erythro*-configuration. In addition, the CD spectra of **4** and **4a** gave a negative Cotton effect at 237 and 240 nm, respectively (Figs. 2 and 3). So, the absolute configuration of **4** was determined to be 7*S*,8*R* as shown in Fig. 1.

Compound **5**, white amorphous powder, exhibited a HRFABMS quasimolecular ion peak at *m*/*z* 651.2290 $[M-H]^-$ (calcd. 651.2294) corresponding to a molecular formula of $C_{31}H_{40}O_{15}$. Its ^{1}H NMR and ^{13}C NMR data were very similar to those of matairesinoside (**7**) (Abe and Yamauchi, 1986), except for a set of additional signals assignable to a β-D-xylose moiety in **5** (Agrawal, 1989). Downfield shift of C-2″ (δ 81.38) of glucose in **5** suggested the linkage point of the xylose was at C-2″ of glucose, which was further confirmed by the cross-peak between the anomeric proton H-1‴ of xylose and C-2″ of glucose in the HMBC experiment. Thus, the structure of compound **5** was deduced to be matairesinol-4-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside. The different chemical shifts of the two protons of H-9′ at δ 3.85 and 4.08 indicated that the two benzyl group at C-8 and C-8′ of lignan lactone **5** should adopt the *trans*-configuration (Lopes et al., 1983). The absolute configuration of C-8 and C-8′ in **5** was established as 8*R*,8′*R* according to the negative Cotton effects at 231 and 278 nm found in its CD spectrum (Ren and Yang, 2002). Therefore, compound **5** was concluded to be 8*R*,8′*R*-matairesinol-4-*O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-glucopyranoside as shown in Fig. 1.

Compound **6** was obtained as a white amorphous powder, whose molecular formula, $C_{22}H_{32}O_{12}$, was deduced by the HRFABMS (*m*/*z*: 487.1823 $[M-H]^-$, calcd. for $C_{22}H_{31}O_{12}$, 487.1821). The ^{1}H NMR spectrum of **6** exhibited one singlet of two aromatic protons at δ 6.63 (2H, *s*), indicating the presence of one symmetrical tetrasubstituted benzene moiety. The proton signals at δ 6.31 (1H, *d*, $J = 16.0$ Hz), 6.23 (1H, *dq*, $J = 16.0$, 6.5 Hz) and 1.81 (3H, *d*, $J = 6.5$ Hz) suggested the presence of a propenyl group in a *trans*-configuration, which was further confirmed by the correlations in the COSY and HMBC spectra. In the ^{1}H NMR spectrum of **6**, two anomeric proton signals at δ 4.86 (1H, *d*, $J = 7.0$ Hz) and 4.04 (1H, *d*, $J = 7.0$ Hz), and one singlet of two aromatic methoxy

protons at δ 3.75 (6H, *s*, OMe × 2), were also observed. The above evidence and the ^{13}C NMR spectrum indicated that compound **6** was a phenylpropanoid with a hexose moiety and a pentose moiety. By comparing the ^{13}C NMR spectroscopic data of the sugar moiety of **6** with those in the literature, it was found that the ^{13}C NMR data of sugars of **6** was identical to those of the sugar moiety of 7-hydroxy-4′-methoxy-flavonol-7-*O*-β-D-xylopyranosyl (1 → 6)-*O*-β-D-glucopyranoside (Agrawal, 1989). This suggested that the sugar moiety of **6** was β-D-xylopyranosyl (1 → 6)-*O*-β-D-glucopyranosyl, which was further confirmed by the HMBC cross-peak between the anomeric proton of xylose H-1′′ and C-6′ of glucose. The HMBC correlation between H-1′ of glucose and C-1 provided key evidence for attaching the glucosyl moiety to C-1 of aglycone. In the HMBC spectrum, the correlation peaks of H-8 and C-4, H-7 and C-3, 5, H-3, 5 and C-1, 7, CH_3O- and C-2, 6 indicated that the propenyl group was linked at C-4 of the benzene ring and the two methoxyl groups were located at the C-2 and C-6 of the benzene ring, respectively. Therefore, compound **6** was established as 1-[*O*-β-D-xylopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl]-2,6-dimethoxy-4-propenyl-phenol.

Known compounds **7**, **8** were identified as matairesinoside (**7**) and (*R*)-1-*O*-(β-D-glucopyranosyl)-2-[2-methoxy-4-(ω-hydroxypropyl)-phenoxyl]-propan-3-ol (**8**) (Abe and Yamauchi, 1986; Matsuda and Kikuchi, 1996b), respectively, on the basis of their mass, CD and NMR spectroscopic analysis.

3. Concluding remarks

To our knowledge, it is very rare to simultaneously obtain four optical isomers of a 8-*O*-4′ neolignan from same plant and this paper is the first report on the isolation and structure elucidation of four optical isomers of 8-*O*-4′ neolignan from *S. caudata*. While a 7*S*,8*R*-configuration glycoside (**4**) had been initially reported from *Lonicera gracilipe* (Matsuda and Kikuchi, 1996a) after detailed analysis of the data in the literature, we found that its CD spectra showed a positive Cotton effect at 239 nm. In fact, comparision of its CD data with that of the pure stereomer *erythro*-(1*R*,2*S*)-2-(2-hydroxy-phenoxyl)-1-phenylpropan-1-ol synthesized by Arnoldi and Merlini (Arnoldi and Merlini, 1985) indicated a 7*R*,8*S* but not 7*S*,8*R*-configuration. So we think the compound isolated from *Lonicera gracilipe* should be the same as glycoside **3** but not **4** of this paper. In addition, it is also noted that *S. caudata* is rich in lignan derivatives according to our phytochemical investigation and the data published in the literature. However, no 8-*O*-4′ type neolignan has been isolated from the other species of genus *Symplocos*, so the 8-*O*-4′ type neolignans might be characteristic chemical markers of *S. caudata* and be of great chemotaxonomic importance in the genus *Symplocos*.

4. Experimental

4.1. General

Optical rotations were measured on a Perkin–Elmer 243B digital polarimeter. The CD spectra were measured on a JASCO J-810 spectropolarimeter. The ^1H, ^{13}C NMR, as well as 2D NMR spectra were taken on a Bruker Avance DRX 500 NMR spectrometer using TMS as internal standard. HRFABMS 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 reversed phase) and monitored by UV detector at 254 nm.

4.2. Plant material

The roots of *S. caudata* were collected in January 2004 from Sichuan Province (China), and identified by Prof. Hubiao Chen (School of Pharmaceutical Sciences, Peking University Health Science Center). A voucher specimen (DNM2007-01) was deposited at the Herbarium of the School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing, China.

4.3. Extraction and isolation

4.3.1. Isolation of compounds *1–8*

Air-dried roots (7.5 kg) of *S. caudata* were extracted with 95% EtOH. After evaporation of the solvent under reduced pressure, the residue was suspended in H_2O and extracted successively with petrol ether, EtOAc and *n*-BuOH. The *n*-BuOH extract (140 g) was subjected to cc on Diaion HP-20 and eluted with H_2O, H_2O–EtOH (9:1, v/v); H_2O–EtOH (7:3, v/v), H_2O–EtOH (1:1, v/v) and H_2O–EtOH (3:7, v/v), successively. The fraction eluted with H_2O–EtOH (1:9, v/v) was subjected to silica gel cc [$CHCl_3$–MeOH–H_2O (7:1:0.1)] to give three fractions (fractions A–C). Fraction B (3.5 g) was further applied to a preparative HPLC [CH_3CN–H_2O (1:9)], to give 13 fractions (Fr1-13). Fraction 7 was purified by preparative HPLC [CH_3CN–H_2O (7:93)] to give **8** (33 mg). Fraction 10 was subjected to chromatography on a Sephadex LH-20 (H_2O), and then further purified by preparative HPLC [CH_3CN–MeOH–H_2O (9:6:85)] to give **3** (9 mg) and **4** (22 mg). Fraction 12 was further purified by preparative HPLC [CH_3CN–H_2O (1:9)] to give **1** (56 mg) and **2** (38 mg).

The fraction eluted with 30% EtOH was subjected to silica gel column chromatography [$CHCl_3$–MeOH–H_2O (8:2:0.2) to give four fractions (fractions A–D). Fraction A was purified on a Rp-18 [CH_3CN–H_2O (17:83)] cc to give **7** (30 mg). Fraction C (9.6 g) was chromatographed on a Sephadex LH-20 (H_2O), to give 11 fractions (Fr1-11).

Fraction 7 was subjected to a Sephadex LH-20 chromatography (H_2O), to give **5** (180 mg). Fraction 11 was recrystallized from MeOH to give **6** (8 mg).

4.3.2. HPLC analysis of compounds *1–4*

HPLC analysis of four isomers **1–4** were performed on a YMC Rp-18 4.6 × 250 mm column with 5 μm particle size, using CH_3CN–H_2O (11:89, v/v) as eluent. The flow rate was 1.0 ml/min with detection at 204 nm. The retention times of isomers **1–4** were shown at $t_{R1} = 44.91$ min, $t_{R2} = 42.07$ min, $t_{R3} = 36.68$ min and $t_{R4} = 38.03$ min, respectively.

4.3.3. Enzymatic preparation of aglycones *1a–4a* from *1–4*

Compounds **1–4** (4.0 mg, 4.0 mg, 1.8 mg and 2.0 mg, respectively) were treated with 10.0 mg, 10.0 mg, 4.5 mg, 5.0 mg of snailase (Protoplasts Productivity: 50%, Beijing Biotech Biochemistry Technical Co.) in citric acid buffer solution (pH 4.5, 5.0 ml), respectively. The mixture was stirred at 40 °C for 7 h, and then extracted with an equal amount of EtOAc (×4). The EtOAc layer was evaporated under reduced pressure to give the aglycones **1a-4a**. **1a**: $[\alpha]_D^{25}$ + 10.0 (*c* 0.20, MeOH); For CD, ^1H and ^{13}C NMR spectroscopic data, see Fig. 3, Tables 1 and 2. **2a**: $[\alpha]_D^{25}$ −38.9 (*c* 0.24, MeOH); For CD, ^1H and ^{13}C NMR spectroscopic data, see Fig. 3, Tables 1 and 2. **3a**: $[\alpha]_D^{25}$ 0.0 (*c* 0.06, MeOH); For CD, ^1H and ^{13}C NMR spectroscopic data, see Fig. 3, Tables 1 and 2. **4a**: $[\alpha]_D^{25}$ −19.0 (*c* 0.07, MeOH); For CD, ^1H and ^{13}C NMR spectroscopic data, see Fig. 3, Tables 1 and 2.

4.4. Physical data of new compounds

4.4.1. (7S, 8S)-threo-7,9,9′-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan-4- O-β-D-glucopyranoside (*1*)

White amorphous powder, $[\alpha]_D^{25}$ +20.0 (*c* 0.40, MeOH); CD (MeOH) nm: 235 (+20.6), 276 (−3.0); UV (MeOH) λ_{max} nm: 275, 225; IR (KBr) ν_{max} cm^{-1}: 3383, 2925, 2855, 1599, 1512, 1463, 1420, 1265, 1224, 1135, 1075, 1030; HRFABMS *m/z* 539.2132 $[M-H]^-$ (calcd. for $C_{26}H_{35}O_{12}$, 539.2134). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.2. (7R,8R)-threo-7,9,9′-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan-4-O-β-D-glucopyranoside (*2*)

White amorphous powder, $[\alpha]_D^{25}$ −72.0 (*c* 0.50, MeOH); CD (MeOH) nm: 234 (−33.6), 275 (−3.5); UV (MeOH) λ_{max} nm: 275, 225; IR (KBr) ν_{max} cm^{-1}: 3395, 2924, 2854, 1601, 1512, 1458, 1420, 1264, 1223, 1076, 1030; HRFABMS *m/z* 539.2143 $[M-H]^-$ (calcd. for $C_{26}H_{35}O_{12}$, 539.2134). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.3. (7R,8S)-erythro-7,9,9′-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan-4-O-β-D-glucopyranoside (*3*)

White amorphous powder, $[\alpha]_D^{25}$ −28.6 (*c* 0.14, MeOH); CD (MeOH) nm: 238 (+13.4), 275 (+0.72); UV (MeOH) λ_{max} nm: 275, 225; IR (KBr) ν_{max} cm^{-1}: 3383, 2930, 2854, 1598, 1511, 1461, 1420, 1266, 1224, 1156, 1133, 1075, 1031; HRFABMS *m/z* 539.2144 $[M-H]^-$ (calcd. for $C_{26}H_{35}O_{12}$, 539.2134). For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.4. (7S, 8R)-erythro-7,9,9′-trihydroxy-3,3′-dimethoxy-8-O-4′-neolignan-4-O-β-D-glucopyranoside (*4*)

White amorphous powder, $[\alpha]_D^{25}$ −23.1 (*c* 0.78, MeOH); CD (MeOH) nm: 237 (−34.2), 275 (−10.2); UV (MeOH) λ_{max} nm: 275, 225; IR (KBr) ν_{max} cm^{-1}: 3361, 2936, 2877, 1611, 1528, 1446, 1351, 1266, 1220, 1157, 1132, 1075, 1048; TOF-MS: *m/z* 563 $[M+Na]^+$. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.4.5. 8R, 8′R-matairesinol 4-O-β-D-xylopyranosyl-(1→2)-O-β-D-glucopy- ranoside (*5*)

White amorphous powder, $[\alpha]_D^{25}$ +60.0 (*c* 0.80, MeOH); UV (MeOH) λ_{max} nm: 278, 220; CD (MeOH) nm: 231 (−17.6), 278 (−2.3); IR (KBr) ν_{max} cm^{-1}: 3406, 2920, 1760, 1599, 1515, 1458, 1424, 1383, 1271, 1232, 1161, 1125, 1074, 1038; HRFABMS (negative mode): *m/z* 651.2290 $[M-H]^-$ (calcd. for $C_{31}H_{39}O_{15}$, 651.2294); FAB-MS (negative mode): *m/z* 519 $[M-132-H]^-$, 357 $[M-132-162-H]^-$; ^1H NMR (DMSO-d_6) δ 2.40 (1H, *m*, H-8′), 2.45 (1H, *dd*, *J* = 8.0, 5.5 Hz, H-7′a), 2.56 (1H, *dd*, *J* = 8.0, 5.5 Hz, H-7′b), 2.72 (1H, *m*, H-8), 2.78 (2H, *m*, H-7), 2.98 (1H, *m*, H-2‴), 2.99 (1H, *br. d*, *J* = 11.5 Hz, H-5‴a), 3.12 (1H, *m*, H-3‴), 3.21 (2H, *m*, H-4″, H-4‴), 3.32 (1H, *m*, H-5″), 3.42 (1H, *br. d*, *J* = 10.5 Hz, H-6″a), 3.47 (1H, *m*, H-3″), 3.48 (1H, *m*, H-2″), 3.56 (1H, *dd*, *J* = 11.5, 5.5 Hz, H-5‴b), 3.65 (1H, *dd*, *J* = 10.5, 5.0 Hz, H-6″b), 3.70 (6H, *s*, 3-OCH_3, 3′-OCH_3), 3.85 (1H, *t*, *J* = 8.5, 9.0 Hz, H-9′a), 4.08 (1H, *t*, *J* = 8.0 Hz, H-9′b), 4.51 (1H, *d*, *J* = 7.0 Hz, H-1‴), 4.99 (1H, *d*, *J* = 7.0 Hz, H-1″), 6.48 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6′), 6.63 (1H, *d*, *J* = 1.5 Hz, H-2′), 6.64 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6), 6.65 (1H, *d*, *J* = 8.0 Hz, H-5′), 6.75 (1H, *d*, *J* = 1.5 Hz, H-2), 6.95 (1H, *d*, *J* = 8.0 Hz, H-5), 8.76 (1H, *s*, 4′-OH); ^{13}C NMR (DMSO-d_6) δ 33.35 (C-7), 36.82 (C-7′), 40.76 (C-8′), 45.52 (C-8), 55.52 (3-OCH_3), 55.91 (3′-OCH_3), 60.47 (C-6″), 65.64 (C-5‴), 69.32 (C-4″), 69.51 (C-4‴), 70.66 (C-9′), 74.27 (C-2‴ 75.79 (C-3‴), 76.17 (C-3″), 76.71 (C-5″), 81.38 (C-2″), 98.58 (C-1″), 104.30 (C-1‴), 112.69 (C-2′), 114.14 (C-2), 114.75 (C-5), 115.39 (C-5′), 120.76 (C-6′), 121.35 (C-6), 129.52 (C-1′), 131.76 (C-1), 144.92 (C-4′), 145.19 (C-4), 147.49 (C-3′), 148.58 (C-3), 178.47 (C-9).

4.4.6. 1-[O-β-D-xylopyranosyl-(1→6)-O-β-D-glucopyranosyl]-2,6- dimethoxy-4-propenyl-phenol (*6*)

White amorphous powder, $[\alpha]_D^{25}$ −180 (*c* 0.40, MeOH); UV (MeOH) λ_{max} nm: 261, 217; IR (KBr) ν_{max} cm^{-1}: 3415, 2924, 2853, 1587, 1506, 1461, 1418, 1383, 1340, 1241, 1128, 1094, 1043; HRFABMS (negative mode): *m/z* 487.1823 $[M-H]^-$ (calcd for $C_{22}H_{31}O_{12}$, 487.1821); ^1H NMR (DMSO-d_6) δ 1.81 (3H, *d*, *J* = 6.5 Hz, H-9), 2.87 (1H, *m*,

H-2″), 2.89 (1H, *br. d*, $J = 11.5$ Hz, H-5′a), 2.99 (1H, *m*, H-3″), 3.18 (3H, *m*, H-2', H-4′, H-5′), 3.21 (1H, *m*, H-3′), 3.22 (1H, *m*, H-4″), 3.51 (1H, *dd*, $J = 11.5$, 5.5 Hz, H-6′a), 3.60 (1H, *dd*, $J = 11.5$, 5.5 Hz, H-5″), 3.75 (6H, *s*, 2-OCH_3, 6-OCH_3), 3.81 (1H, *br. d*, $J = 11.5$ Hz, H-6′b), 4.04 (1H, *d*, $J = 7.5$ Hz, H-1″), 4.86 (1H, *d*, $J = 7.0$ Hz, H-1′), 6.23 (1H, *dq*, $J = 16.0$, 6.5 Hz, H-8), 6.31 (1H, *d*, $J = 16.0$ Hz, H-7), 6.63 (2H, *s*, H-3, 5); ^{13}C NMR (DMSO-d_6) δ 18.17 (C-9), 56.32 (2-OCH_3, 6-OCH_3), 65.43 (C-5″), 68.04 (C-6′), 69.51 (C-4″), 69.64 (C-4′), 73.28 (C-2″), 74.02 (C-2′), 76.12 (C-3′), 76.37 (C-5′, C-3″), 102.52 (C-1′), 103.55 (C-1″), 103.97 (C-3, C-5), 124.92 (C-8), 130.77 (C-7), 133.26 (C-4), 133.42 (C-1), 152.67 (C-2, C-6).

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