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A novel 8,4′-oxyneolignan diglycoside from *Ligusticum sinensis*

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Abstract

A novel 8,4′-oxyneolignan diglycoside, named ligusinenoside D (**1**), was isolated from the rhizomes of *Ligusticum sinensis*, together with five known analogues **2**–**6**. The absolute configurations of **1** and **2** were elucidated by means of enzymatic hydrolysis and spectroscopic data.

Keywords: *Ligusticum sinensis* Oliv.; 8,4′-Oxyneolignan diglycoside; Ligusinenoside D

The rhizome of *Ligusticum sinensis* Oliv. (Umbelliferae) is known as *Gaoben* in traditional Chinese medicines, which is used for the treatment of headache, rheumatic arthralgia, pain in the abdomen and diarrhea [1]. Previous studies revealed numbers of phthalides [2], coumarins [3], terpenoids [4], phenylpropanoids [5] and glycosides [6] from the *Ligusticum* genus plants. In our ongoing study to find active natural products, extensive chemical studies have been carried out on the *n*-BuOH extract of *L. sinensis*, and obtained six 8,4′-oxyneolignan glycosides, including a new diglycoside, named ligusinenoside D (**1**) and five known analogues (**2**–**6**).

The BuOH-soluble part (280 g) of 95% EtOH extract from rhizomes of *L. sinensis* (10 kg) was separated over macroporous resin column chromatography (CC) (i.d. 10 cm × 80 cm) and eluted with EtOH/H_2O gradient systems (0, 10%, 30%, 50%, 75%, 95%) to give fractions A–F. Fr. C (30% EtOH fraction, 15 g) was subsequently subjected to repeated silica gel, ODS, Sephadex LH-20, and semi-preparative HPLC, yielding compounds **1** (11 mg), **2** (13 mg), **3** (12 mg), **4** (9 mg), **5** (28 mg) and **6** (7 mg).

Compound **1** (Fig. 1) was isolated as white amorphous powders with negative optical rotation ($[\alpha]_D^{21}$ −55.0, *c* 0.26, MeOH), had a molecular formula of $C_{31}H_{42}O_{16}$ derived from its *quasi*-molecular ion peak at *m/z* 693.2351 ($[M+Na]^+$, calcd. for $C_{31}H_{42}O_{16}Na$, 693.2371) by HR-ESI-MS spectrum. The enzymatic hydrolysis yielded D-apiose and D-glucose, which were confirmed by GC comparison with the authentic samples. The IR spectrum exhibited absorption bands for hydroxy group (3406 cm^{-1}) and aromatic ring (1603, 1510 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) were nearly identical to those of compound **2** [6]. The ^1H NMR (500 MHz, CD_3OD) signals for the aglycone moiety were attributed to two 1,3,4-trisubstituted phenyl groups at δ 7.01 (br s, 2H), 6.90 (d, 1H, *J* = 8.2 Hz), 6.86 (br d, 1H,

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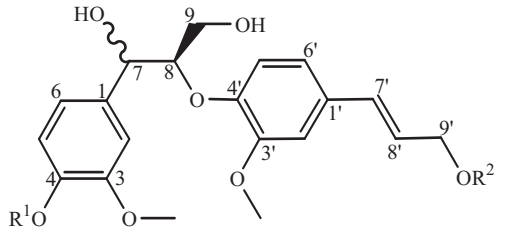

	R^1	R^2	
1	H	Api(1→6)Glc	7*S*
2	H	Api(1→6)Glc	7*R*
3	Glc	H	7*S*
4	Glc	H	7*R*
5	H	Glc	7*R*
6	Glc	Glc	7*R*

Fig. 1. Chemical structures of **1**–**6**.

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) and HMBC data for **1** (CD_3OD, δ in ppm, J in Hz).

No.	δ_C	δ_H	HMBC	No.	δ_C	δ_H	HMBC
1	134.5		H-5, 7, 8	8′	125.6	6.22 (dt, 1H, 15.9, 6.2)	H-9′
2	112.3	7.01 (br s, 1H)	H-6, 7	9′	71.5	4.46 (dd, 1H, 13.4, 6.6)	H-7′, 8′, 1″
3	149.2		H-2, 5, 3-OCH_3			4.28 (dd, 1H, 13.3, 6.8)	
4	147.5		H-2, 5, 6	3′-OCH_3	57.0	3.79 (s, 3H)	
5	116.1	6.72 (d, 1H, 8.1)		1″	103.7	4.34 (d, 1H, 7.8)	H-9′, 2″
6	121.5	6.83 (dd, 1H, 8.1, 1.7)	H-2, 7	2″	75.6	3.21 (dd, 1H, 8.8, 7.8)	H-3″
7	74.5	4.82 (d, 1H, 5.8)	H-2, 6, 8, 9	3″	78.5	3.34 (m, 1H)	H-2″, 4″
8	86.6	4.36 (dt, 1H, 7.3, 5.7)	H-7, 9	4″	72.2	3.28 (t, 1H, 9.0)	H-3″, 5″, 6″
9	62.7	3.84 (dd, 1H, 12.1, 6.8)	H-7, 8	5″	77.4	3.39 (m, 1H)	H-4″, 6″
		3.80 (dd, 1H, 12.2, 6.3)		6″	69.2	3.99 (dd, 1H, 11.4, 1.7)	H-4″, 1‴
3-OCH_3	56.8	3.79 (s, 3H)				3.61 (dd, 1H, 11.2, 6.1)	
1′	133.2		H-2′, 5′, 7′, 8′		111.5	5.03 (d, 1H, 2.6)	H-2‴, 4‴, 6″
2′	111.9	7.01 (br s, 1H)	H-6′, 7′	1‴	78.5	3.91 (d, 1H, 2.5)	H-4‴, 5‴
3′	152.3		H-2′, 5′, 3′-OCH_3	2‴	81.0		H-1‴, 4‴, 5‴
4′	149.6		H-8, 2′, 5′, 6′	3‴	75.5	3.98 (d, 1H, 9.7)	H-1‴, 5‴
5′	119.2	6.90 (d, 1H, 8.2)		4‴		3.76 (d, 1H, 9.7)	
6′	121.5	6.86 (br d, 1H, 8.2)	H-2′, 7′		66.0	3.57 (s, 2H)	H-2‴, 4‴
7′	134.3	6.58 (d, 1H, 15.9)	H-2′, 6′	5‴			

J = 8.2 Hz), 6.83(dd, 1H, J = 8.1, 1.7 Hz) and 6.72 (d, 1H, J = 8.1 Hz), one 1-ol-2(*E*)-propenyl moiety at δ 6.58 (d, 1H, J = 15.9 Hz), 6.22 (dt, 1H, J = 15.9, 6.2 Hz), 4.46 (dd, 1H, J = 13.4, 6.6 Hz) and 4.28 (dd, 1H, J = 13.3, 6.8 Hz), two oxygenated methines at δ 4.82 (d, 1H, J = 5.8 HZ) and 4.36 (dt, 1H, J = 7.3, 5.7 Hz), one oxygen-bearing methylene at δ 3.84 (dd, 1H, J = 11.9, 6.8 Hz) and 3.80 (dd, 1H, J = 12.2, 6.3 Hz), and two methoxy groups at δ 3.79 (s, 6H), demonstrating a citrusin A-like 8,4′-oxyneolignan diglycoside [6]. Moreover, the ^{13}C NMR (125 MHz, CD_3OD) signals at δ 111.5 (C), 81.0 (C), 78.5 (CH), 75.5 (CH), 66.0 (CH_2) and a +5.8 ppm downfield shift at C-6 of glucose, revealed a β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl moiety. In the HMBC spectrum, significant correlations of 3-OCH_3/C-3, 3′-OCH_3/C-3′, H-1″/C-9′, H-9′/C-1″, H-6″/C-1‴ and H-1‴/C-6″ were observed (Fig. 2), confirming the connectivities of two methoxyls with C-3 and C-3′, a β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy group at C-9′ of the aglycone, respectively. By enzymatic hydrolysis, *erythro*-form aglycone was obtained (400 MHz, acetone-d_6, $J_{7,8}$ = 5.4 Hz) [7]. A negative chirality appearing around 250–300 nm (c 1.0 g/L, MeOH, θ: −360,000 (281 nm), −160,000 (271 nm), 200,000 (265 nm), 250,000 (257 nm)) in the CD spectrum of the aglycone consolidated the absolute configuration of 8*R*. Therefore, compound **1**, named ligusinenoside D was elucidated to be (7*S*, 8*R*)-9′-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3,3′-dimethoxy-8,4′-oxyneolign-7′-ene-4,7,9-triol.

The five known analogues were identified as ligusinenoside C (**2**), alaschanioside A (**3**), citrusin A (**4**), hyuganoside IIIb (**5**) and ligusinenoside B (**6**) [6,8,9].

Ligusinenoside C (**2**) was previously deduced to be *threo*-form relative configuration. By enzymatic hydrolysis, *threo*-form aglycone was obtained ($J_{7,8}$ = 5.8 Hz) [7]. A negative chirality appearing at 250–300 nm (c 2.3 g/L, MeOH, θ: −60,000 (281 nm), −30,000 (275 nm), 60,000 (267 nm), 90,000 (257 nm)) in the CD spectrum of the aglycone demonstrated the absolute configuration of 8*R*. Therefore, the absolute configuration of ligusinenoside C (**2**) was further clarified to be (7*R*, 8*R*)-9′-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3,3′-dimethoxy-8,4′-oxyneolign-7′-ene-4,7, 9-triol.

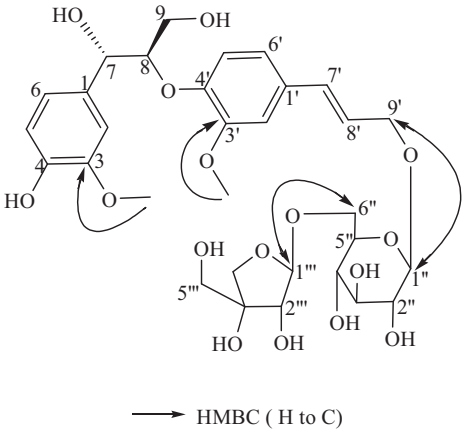

Fig. 2. Structure and key HMBC correlations of **1**.

Enzymatic hydrolysis of **1**–**2**. Each compound (5 mg) was dissolved in acetate buffer (pH 3.8, 2 mL) and incubated for 15 h at 40 °C with 27 mg of hesperidinase (H8137 (EC 3.2.1.40, Sigma)). Then, the extraction mixture was extracted by the same volume of AcOEt. The aglycone was analyzed by ^{1}H NMR. The water soln. was subjected to CC (Sephadex LH-20, MeOH/H_2O 10:1) to afford a sugar fraction. The sugar fraction and standard D-glucose and D-apiose (Sigma, USA) were each treated with Lcysteine methyl ester hydrochloride (2 mg) in pyridine (1 mL) at 60 °C for 1 h. Then the soln. was treated with *N*,*O*-bis(trimethylsilyl)trifluoro-acetamide (0.02 mL) at 60 °C for 1 h. Subsequently, the supernatant was subjected to GC analysis (Supelco, 230 °C, flow rate 15 mL/min). D-Glucose (standard: t_R 24.1 min; **1**: t_R 24.2 min) and D-apiose (standard: t_R 14.3 min; **1**: t_R 14.2 min) were detected in the sugar fractions from **1**.

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