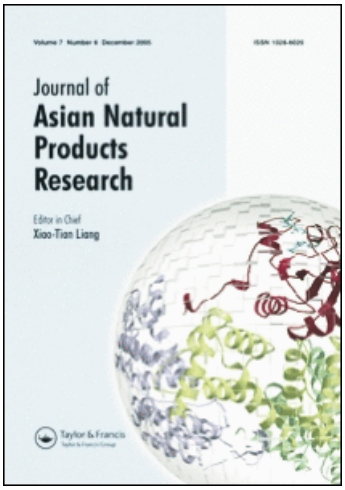

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ORIGINAL ARTICLE

Two new dimeric secoiridoid glycosides from the fruits of *Ligustrum lucidum*

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Two new secoiridoid glycosides, ligusides A and B (**1** and **2**), as well as seven known compounds (**3**–**9**), were isolated from the fruits of *Ligustrum lucidum*. Their structures were elucidated on the basis of spectroscopic and chemical analysis.

Keywords: *Ligustrum lucidum*; Oleaceae; secoiridoid glycoside; ligusides A and B

1. Introduction

Ligustrum lucidum Ait. (Oleaceae), a well-known medicinal plant, is distributed widely in China and Southeast Asia. The fruits of this plant were used comprehensively as a tonic agent for liver and kidney protection in the traditional Chinese medicine. The aqueous extract of the fruits showed significant antioxidant [1], antiviral [2], anticancer [3], and antisenile [4] activities. Previous phytochemical research of the fruits afforded plenty of oleoside-type secoiridoid glycosides in the water-soluble fraction [5,6]. In our previous work [7], 15 triterpenes were isolated from the ethanol extract of the fruits of *L. lucidum*. In continuation of our chemical investigation on this plant, two new secoiridoid glycosides, ligusides A (**1**) and B (**2**), together with seven known compounds, 8(*E*)-nuezhenide (**3**) [8], Gl3 (**4**) [9], 1‴-*O*-β-D-glucosylformoside (**5**) [10], oleuropeinic acid (**6**) [11], oleoside dimethyl ester (**7**) [12], 8(*Z*)-nuezhenide A (**8**) [13], and oleonuezhenide (**9**) [14] (Figure 1), were isolated. In this paper, we report the isolation and structural determination of the two new secoiridoid glycosides.

2. Results and discussion

Compound **1** was obtained as an amorphous powder. Its molecular formula was determined as $C_{48}H_{64}O_{27}$ by HR-ESI-MS at *m/z* 1095.3525 $[M + Na]^+$. The UV spectrum showed absorption maxima at 236 nm. The IR spectrum of **1** showed the presence of hydroxyl (3413 cm^{-1}), carbonyl (1701 cm^{-1}), and olefinic bond (1631 cm^{-1}). The presence of D-glucopyranosyl was confirmed by acid hydrolysis of **1** with 1 M HCl, followed by the GC analysis comparing with authentic samples. The ^1H NMR spectrum of **1** exhibited signals for two oleoside 11-methyl ester moieties [H-3 at δ_H 7.56 and 7.52 (each 1H, s), H-8 at δ_H 6.18 and 6.14 (each 1H, q, $J = 7.0$ Hz), H-1 at δ_H 6.02 and 5.96 (each 1H, s), H-1′ at δ_H 4.82

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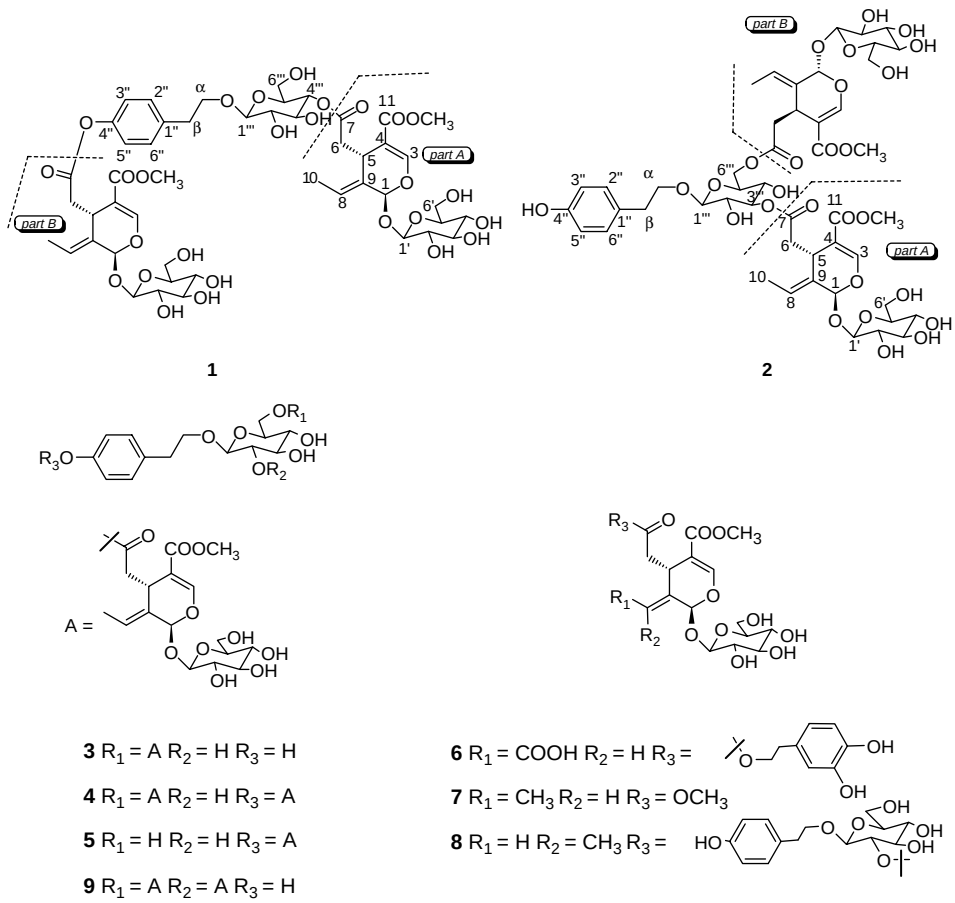

Figure 1. Chemical structures of compounds **1**–**9**.

(1H, d, $J = 7.9$ Hz) and 4.80 (1H, d, $J = 8.1$ Hz), OMe at δ_H 3.73 and 3.71 (each 3H, s), H_3-10 at δ_H 1.76 (6H, d, $J = 7.0$ Hz)] [9], a *p*-hydroxyphenethoxyl moiety [δ_H 7.29 (2H, d, $J = 8.5$ Hz), 6.99 (2H, d, $J = 8.5$ Hz), 4.08 (1H, m), 3.77 (1H, m), 2.93 (2H, m)], as well as an additional anomeric proton [δ_H 4.33 (1H, d, $J = 7.7$ Hz, H-1‴)]. The coupling constant of the anomeric proton indicated the presence of β-configuration of the D-glucose unit. In accordance with the above findings, the ^{13}C NMR and DEPT spectra of **1** showed carbon signals corresponding to two secoiridoid glucoside units, together with the resonances of a *p*-hydroxyphenethoxyl and an additional glucose moiety. With the aid of the 2D NMR (^{1}H–^{1}H COSY, HSQC, and HMBC) spectra, all the ^{1}H and ^{13}C NMR signals of **1** were assigned and are shown in Table 1. The ^{1}H and ^{13}C NMR spectral data of **1** were closely similar to those of Gl3 (**4**) [9], except for the signals arising from the additional glucose unit, which indicated that an oleoside 11-methyl ester unit could be attached to different positions of the glucose. The HMBC correlation between H-4‴ (δ_H 4.67) and C-7 (δ_C 172.4, in part A) suggested that the oleoside 11-methyl ester unit (part A) was esterified with the hydroxyl group at C-4‴ of an additional glucose moiety. In addition, the HMBC correlation between H-1‴ and C-α suggested that the additional glucose

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (CD_3OD, J in Hz)[a,b].

	1				**2**			
	δ_H		δ_C		δ_H		δ_C	
No.	Part A	Part B	Part A	Part B	Part A	Part B	Part A	Part B
1	5.96 (s)	6.02 (s)	95.5	95.4	5.92 (s)	5.96 (s)	95.1	95.6
3	7.52 (s)	7.56 (s)	155.3	155.4	7.52 (s)	7.53 (s)	155.2	155.3
4	–	–	109.3	109.3	–	–	109.3	109.4
5	4.02 (dd, J = 4.0, 9.8)	4.11	31.6	31.8	4.01	4.01	31.6	31.8
6	2.57 (dd, J = 9.8, 14.9)	2.73 (dd, J = 9.2, 14.4)	40.7	41.0	2.74	2.74	40.7	41.3
	2.85 (dd, J = 4.0, 14.9)	2.96				2.50		
7	–	–	172.4	171.7	–	–	172.8	172.9
8	6.18 (q, J = 7.0)	6.14 (q, J = 7.0)	125.4	125.1	6.09 (q, J = 7.1)	6.12 (q, J = 7.1)	124.9	125.1
9	–	–	130.4	130.6	–	–	130.5	130.5
10	1.76 (d, J = 7.0)	1.76 (d, J = 7.0)	13.8	13.8	1.72 (dd, J = 1.4, 7.1)	1.77 (dd, J = 1.5, 7.1)	13.7	13.8
11	–	–	168.7	168.7	–	–	168.9	168.9
OCH_3	3.71 (s)	3.73 (s)	52.0	52.0	3.69 (s)	3.73 (s)	52.0	52.0
1′	4.82 (d, J = 7.9)	4.80 (d, J = 8.1)	101.2	101.0	4.81 (d, J = 7.8)	4.81 (d, J = 7.8)	100.8	100.9
2′	3.33	3.33	74.6	74.7	3.31	3.31	74.7	74.7
3′	3.33	3.33	78.3	78.3	3.41	3.41	77.9	77.9
4′	3.33	3.33	71.2	71.4	3.27	3.27	71.5	71.6
5′	3.40	3.40	77.9	77.9	3.33	3.33	78.4	78.4
6′	3.86 (t, J = 13.2)	3.86 (t, J = 13.2)	62.4	62.5	3.88 (dd, J = 2.1, 12.1)	3.90 (dd, J = 2.0, 12.2)	62.8	62.8
	3.69 (d, J = 11.4)	3.69 (d, J = 11.4)			3.66	3.66		
α	3.77			71.6	3.96			72.3
	4.08				3.72			
β	2.93			36.5	2.84			36.4
1″	–			137.9	–			130.6
2″, 6″	7.29 (d, J = 8.5)			131.0	7.04 (d, J = 8.5)			130.9
3″, 5″	6.99 (d, J = 8.5)			122.5	6.68 (d, J = 8.5)			116.2
4″	–			150.5	–			156.8
1‴	4.33 (d, J = 7.7)			104.2	4.38 (d, J = 7.8)			104.3
2‴	3.26			75.1	3.32			73.1

Table 1 – *continued*

	1				2			
	δ_H		δ_C		δ_H		δ_C	
No.	Part A	Part B	Part A	Part B	Part A	Part B	Part A	Part B
3‴	3.51 (dd, J = 7.2, 7.7)			75.8	4.90			78.9
4‴	4.67 (dd, J = 7.2, 7.8)			72.9	3.43			69.8
5‴	3.40			75.6	3.53			74.9
6‴	3.58			62.6	4.23 (dd, J = 5.4, 11.9)			64.6
	3.44 (dd, J = 6.4, 9.5)				4.31 (dd, J = 2.1, 11.8)			

Notes: [a] Assignments were established by DEPT, ^{1}H–^{1}H COSY, HSQC, and HMBC experiments.
[b] Overlapped signals are reported without designating multiplicity.

moiety was substituted by the *p*-hydroxyphenethoxyl unit at C-1‴. Therefore, the structure of **1** was established and named as liguside A.

Compound **2** was obtained as an amorphous powder. Its molecular formula was determined to be $C_{48}H_{64}O_{27}$ by HR-ESI-MS at *m/z* 1071.3547 $[M - H]^-$. Acid hydrolysis of **2** also yielded D-glucose, which was identified by the GC analysis. The ^{1}H and ^{13}C NMR spectra of **2** showed signals corresponding to two oleoside 11-methyl ester units, together with the resonances of a *p*-hydroxyphenethoxyl moiety and an additional glucose moiety as observed in **1**. The combined analysis of 1D and 2D NMR spectra led to the assignment of all the ^{1}H and ^{13}C NMR spectral signals of **2** (Table 1). The ^{1}H and ^{13}C NMR data of **2** were closely similar to those of oleonuezhenide (**9**) [14]. The main differences were the signals assigned to the additional glucose unit. In the HMBC spectrum of **2**, the correlations between H-3‴ (δ_H 4.90) and C-7 (δ_C 172.8, in part A), as well as between H-6‴ (δ_H 4.23 and 4.31) and C-7 (δ_C 172.9, in part B), suggested that the two oleoside 11-methyl ester units were substituted at the C-3‴ and C-6‴ positions of the glucose, respectively. Thus, the structure of **2** was assigned and named as liguside B.

3. Experimental

3.1 General experimental procedures

Melting points were recorded on an XT-4 micromelting point apparatus without correction. Optical rotation values were measured on a JASCO P-1020 digital polarimeter with a 0.01 dm length cell. IR spectra (KBr) were recorded on a JASCO FT/IR-480 plus Fourier transform infrared spectrometer. UV spectra were obtained on a JASCO V-550 UV/vis spectrophotometer with a 0.1 dm length cell. ^{1}H (400 or 500 MHz), ^{13}C (100 or 125 MHz), and 2D NMR spectra were recorded on Bruker AV-400 or AV-500 spectrometers. HR-ESI-MS

spectra were measured using an Agilent 6210 LC/MSD TOF mass spectrometer. Preparative HPLC was carried out on a Varian instrument equipped with UV detectors (Varian, Palo Alto, CA, USA) and a reversed-phase C_{18} column (5 μm, 20 mm × 250 mm; Cosmosil, Houston, TX, USA). Column chromatographies were carried out using silica gel (200–400 mesh; Qingdao Haiyang Chemical Group Corporation, Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC analysis was performed on precoated silica gel GF_{254} plates (Yantai Chemical Industrial Institute, Yantai, China).

3.2 Plant material

The fruits of *L. lucidum* were collected in Nanjing City, Jiangsu Province of China, in October 2006, and were authenticated by Prof. Min-Jian Qin (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China). A voucher specimen (No. 20060517) was deposited in the herbarium of the China Pharmaceutical University, Nanjing.

3.3 Extraction and isolation

Dried and roughly powdered fruits of *L. lucidum* (40 kg) were refluxed with 70% EtOH (3 × 40 liters, 1 h each). The EtOH extract was concentrated *in vacuo* to yield a crude extract (3.2 kg), which was redissolved in water and partitioned successively by petroleum ether, EtOAc, and *n*-BuOH, respectively. The *n*-BuOH layer was concentrated under vacuum to afford a residue (1.2 kg), which was subjected to a macroporous resin D101 (3 kg) column, and eluted with EtOH–H_2O (0:100, 15:85, 35:65, 55:45, 95:5). The 35% EtOH extract (200 g) was separated by silica gel column chromatography (200–300 mesh, 1.5 kg) using gradient mixtures of $CHCl_3$–MeOH–H_2O (90:10:0.1–50:50:5) as eluants to afford 11 fractions (Fr. 1–11). Fr. 7 (10 g) was subjected to an ODS column using gradient mixtures of MeOH–H_2O (28:72, 30:70, 35:65, 40:60, 50:50, and 70:30, each 1000 ml) as eluants to yield 10 subfractions. Compound **3** (2 g) was obtained upon recrystallization in MeOH–H_2O (1:1) from subfraction 7-3 (4 g). Subfraction 7-5 (1 g) was subjected to a preparative HPLC (MeOH–H_2O, 35:65) to give **1** (10 mg), **4** (40 mg), **5** (300 mg), **6** (12 mg), **7** (5 mg), and **9** (10 mg), respectively. Subfraction 7-8 (2.3 g) was purified by a preparative HPLC (MeOH–H_2O, 45:55) to afford **2** (11 mg). Fraction 8 (12 g) was also subjected to a preparative HPLC (MeOH–H_2O, 30:70) to yield **8** (9 mg).

3.3.1 Compound **1**

An amorphous powder, mp 130–131°C; $[\alpha]_D^{20}$ − 169.5 ($c = 0.2$, MeOH); UV (MeOH) λ_{max}: 236 nm; IR (KBr) ν_{max}: 3413, 1701, 1631, 1516, 1075, 1019 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS: *m/z* 1095.3525 $[M + Na]^+$ (calcd for $C_{48}H_{64}O_{27}Na$, 1095.3527).

3.3.2 Compound **2**

An amorphous powder, mp 128–130°C; $[\alpha]_D^{20}$ − 100.5 ($c = 0.2$, MeOH); UV (MeOH) λ_{max}: 231 nm; IR (KBr) ν_{max}: 3424, 2853, 2922, 1722, 1633, 1519, 1444, 1310, 1182, 1079, 1043 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table 1; HR-ESI-MS: *m/z* 1071.3547 $[M - H]^-$ (calcd for $C_{48}H_{63}O_{27}$, 1071.3562).

3.4 Acid hydrolysis and GC analysis of 1 and 2

Compounds **1** and **2** (each 1 mg) were refluxed with 1 M HCl in 60% EtOH (1 ml) at 95°C for 2 h, respectively. Each reaction mixture was evaporated with a stream of N_2 to yield a residue, which was then dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was concentrated and dried

by N_2, and treated with pyridine (1 ml) and L-cysteine methyl ester hydrochloride (3 mg), followed by heating at 60°C for 2 h, and then concentrated to dryness with N_2. The residue was added to *N*-(trimethylsilyl) imidazole (0.2 ml) and kept at 60°C for 1 h. Subsequently, the solution was diluted with H_2O (1 ml) and extracted with cyclohexane (2 ml). The supernatant was subjected to GC analysis, [Shimadzu GCMS-QP2010 plus gas chromatograph/-mass spectrometer; column: HP-1701 (0.25 mm × 30 m), detector: FID, column temperature: 200–250°C (5°C/min), detector temperature: 280°C, injector temperature: 250°C, carrier gas: N_2]. The standard D-glucose and L-glucose were subjected to the same reaction and GC analysis under the above conditions [retention times (min): 32.289 (D-glucose), 34.874 (L-glucose)]. For **1**–**2**, D-glucose was detected.

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