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Secoiridoid constituents from the fruits of *Ligustrum lucidum*

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Abstract

Two secoiridoid glucosides, lucidumosides A and B, as well as six known glucosides, oleoside dimethyl ester, ligustroside, oleuropein, nuezhenide, isonuezhenide, and neonuezhenide, were isolated from the fruits of *Ligustrum lucidum*. Their structures were elucidated by spectroscopic methods. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Ligustrum lucidum*; Oleaceae; Secoiridoid glucoside; Lucidumoside A; Lucidumoside B

1. Introduction

The fruits of *Ligustrum lucidum* Ait. (Oleaceae) are known as Nuzhenzi and they are commonly used for their tonic effects in Chinese medicine (Li et al., 1994). Previous studies have found volatile components, triterpenes, flavonoids, secoiridoid glucosides, and phenolic compounds from this plant, and constituents such as phenylethanoids, monoterpenes, and secoiridoid glucosides from other species of the genus *Ligustrum* (Inouye and Nishioka, 1972; Garibodi et al., 1986; He et al., 1992, 1994; Tian et al., 1996; Fukuda et al., 1996). Screening studies in our laboratory have found that the ethanol extract of the fruits of *L. lucidum* showed significant inhibitory effects on free radical induced hemolysis of red blood cells. This paper describes the isolation and structure elucidation of six known glucosides, oleoside dimethyl ester (**1**), ligustroside (**2**), oleuropein (**3**), nuezhenide (**4**), isonuezhenide (**5**), and neonuezhenide (**6**) and two new secoiridoid glucosides, named lucidumosides A (**7**) and B (**8**) from the active fraction of the fruits of *L. lucidum* (Scheme 1).

2. Results and discussion

The six known structures, oleoside dimethyl ester (**1**), ligustroside (**2**), oleuropein (**3**), nuezhenide (**4**), isonuezhenide (**5**) and neonuezhenide (**6**), were identified by comparing their ^{1}H and ^{13}C NMR and MS data with those reported in literature (Kikuchi and Yamakuchi, 1985; Garibodi et al., 1986; Fukuyama et al., 1987).

Compound **7** was obtained as a colorless powder. The molecular formula was determined to be $C_{25}H_{34}O_{12}$ ($[M+H]^+$ 527.5455, calc. 527.5457) by positive HRFABMS. The IR spectrum showed the presence of hydroxyl groups (3400 cm^{-1}) and two carbonyl groups (1700 and 1630 cm^{-1}). The ^{1}H and ^{13}C NMR signals of **7** were assigned by using a 2D NMR experiment including ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectral data. In the ^{1}H NMR spectrum, the characteristic signals due to an olefinic proton (H-3) appeared at δ 7.48 as a singlet, an acetalcarbinol proton (H-1) as a doublet at δ 5.42, two methine groups, two methylene groups, one methyl group, and one methoxyl group, were assigned as the secoiridoidic aglycone moiety. The doublet at δ 4.69 was attributed to the anomeric proton of the glucose moiety. In addition, the characteristic aromatic protons of A_2B_2 spin systems at δ 7.06 and δ 6.72 showed with J values of 8.8 Hz together with two methylene groups indicated the presence of a *para*-substituted phenylethyl group (Table 1). The HMBC experiment of **7** showed two ^3J interactions between H-1″ (δ 4.18) and C-7 (δ 174.1) as

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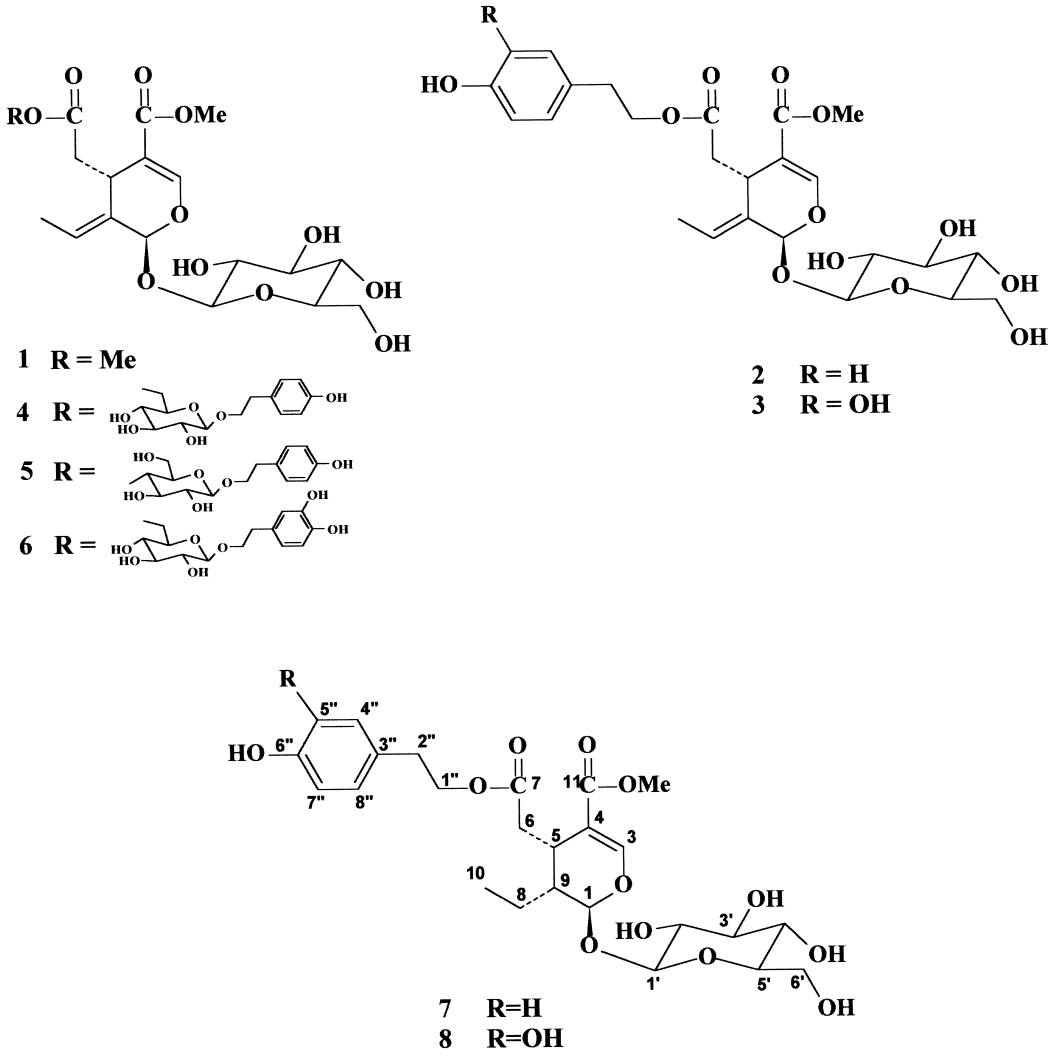

Scheme 1.

well as protons of a methoxyl group (δ 3.64) and C-11 (δ 168.6). The phenylethyl group in compound **7** should be conjugated at C-7. Analysis of the ^{1}H and ^{13}C NMR spectra indicated the aglycone moieties of compounds **7** and **2** were different. The H-8 signals appeared at δ 1.60 and δ 1.75 as two multiplets in compound **7**, whereas the signal at δ 6.08 was a quartet in compound **2**. Futhermore, the ^{1}H NMR spectrum of compound **7** showed its H-9 signal as a multiplet at δ 2.10 and the H-10 signal as a triplet at δ 1.17. This suggested that the bond between C-8 and C-9 in compound **7** was a single bond, which is consistent with the evidence observed in the ^{13}C NMR spectrum (Table 2). In order to define the stereochemistry of compound **7**, a NOESY experiment was performed. The NOESY spectrum of **7** exhibited a NOE enhancement between H-1 (δ 5.42) and H-10 (δ 1.17) as well as H-5 (δ 3.27) and H-9 (δ 2.10) in Fig. 1. This indicates that H-5 and H-9 are on the β-face while H-1 is on the α-face as depicted in compound **7**. Thus **7** was lucidumoside A.

Compound **8** was obtained as a colorless powder. Its molecular formula was established as $C_{25}H_{34}O_{13}$ ($[M+H]^{+}$ 543.5447, calc. 543.5451) by positive HRFABMS. The IR spectrum showed the presence of hydroxyl groups (3400 cm^{-1}) and two carbonyl groups (1700 and 1630 cm^{-1}). The ^{1}H and ^{13}C NMR chemical shift assignments were made by means of ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra. Analysis of ^{1}H and ^{13}C NMR indicated that the spectral data of compound **8** were similar to those of compound **7** except for the appearance of a set of signals as the aromatic protons with ABX system at δ 6.76 (^{1}H, *s*), δ 6.75 (^{1}H, *d*, $J=8.6$ Hz) and δ 6.57 (^{1}H, *d*, $J=8.6$ Hz). By comparison of the ^{13}C NMR spectrum of compound **8** with those of compound **7**, the chemical shift assignable to C-5″ of 8 shifted downfield at δ 146.8, *ortho* C-4″ and C-6″ shifted upfield at δ 117.0 and δ 144.8, respectively. This information indicated that the phenyl ring in phenylethyl moiety in compound **8** was 3,4-substituted. In HMBC experiments of **8**, characteristic correlation peaks were

Table 1
^{1}H NMR spectral data for compounds **2**, **7** and **8** in CD_3OD[a]

Proton	**2**	**7**	**8**
Aglycone			
1	5.90 (1H, *s*)	5.42 (1H, *d*, 6.0)	5.44 (1H, *d*, 6.1)
3	7.51 (1H, *s*)	7.48 (1H, *s*)	7.46 (1H, *s*)
5	4.12 (1H, *m*)	3.27 (1H, *m*)	3.26 (1H, *m*)
6	2.45 (1H, *dd*, 9.2, 14.1)	2.47 (1H, *dd*, 9.0, 13.2)	2.45 (1H, *dd*, 8.8, 14.2)
	2.80 (1H, *dd*, 4.4, 14.1)	2.65 (1H, *dd*, 3.1, 13.2	2.61 (1H, *dd*, 3.3, 14.2)
8	6.08 (1H, *q*, 7.1)	1.75 (1H, *m*)	1.71 (1H, *m*)
		1.60 (1H, *m*)	1.62 (1H, *m*)
9		2.10 (1H, *m*)	2.09 (1H, *m*)
10	1.65 (3H, *d*, 7.1)	1.17 (3H, *t*, 6.0)	1.16 (3H, *t*, 6.0)
OMe	3.72 (3H, *s*)	3.64 (3H, *s*)	3.61 (3H, *s*)
Glucose			
1′	4.81 (1H, *d*, 7.7)	4.69 (1H, *d*, 7.8)	4.66 (1H, *d*, 7.8)
Phenylethyl			
1″	4.21 (2H, *t*, 7.0)	4.18 (2H, *m*)	4.06 (2H, *m*)
2″	2.82 (2H, *t*, 7.0)	2.83 (2H, *t*, 6.0)	2.86 (2H, *t*, 6.0)
4″	7.05 (1H, *d*, 8.7)	7.06 (1H, *d*, 8.8)	6.76 (1H, *s*)
5″	6.73 (1H, *d*, 8.7)	6.72 (1H, *d*, 8.8)	
7″	6.73 (1H, *d*, 8.7)	6.72 (1H, *d*, 8.8)	6.75 (1H, *d*, 8.6)
8″	7.05 (1H, *d*, 8.7)	7.06 (1H, *d*, 8.8)	6.57 (1H, *d*, 8.6)

[a] Coupling constants (*J* values in Hz) are shown in parentheses.

Table 2
^{13}C NMR spectral data of compounds **2**, **7** and **8** in CD_3OD

Carbon	**2**	**7**	**8**
Aglycone			
1	94.9	98.4	98.8
3	155.0	154.0	154.1
4	109.1	110.4	110.7
5	31.5	30.8	30.9
6	41.1	36.6	38.4
7	173.0	174.1	174.2
8	124.8	35.0	35.1
9	130.0	37.4	37.8
10	13.6	14.3	14.3
11	168.4	168.6	168.9
OMe	51.9	51.8	51.7
Glucose			
1′	100.5	100.6	100.9
2′	74.4	74.7	74.6
3′	77.6	77.9	78.1
4′	71.2	71.5	71.8
5′	78.5	78.3	78.3
6′	62.5	62.8	62.7
Phenylethyl			
1″	66.7	66.9	67.0
2″	34.9	35.1	36.0
3″	129.7	129.9	131.4
4″	130.8	130.8	117.0
5″	116.1	116.2	146.8
6″	156.7	156.9	144.8
7″	116.1	116.2	116.4
8″	130.8	130.8	121.8

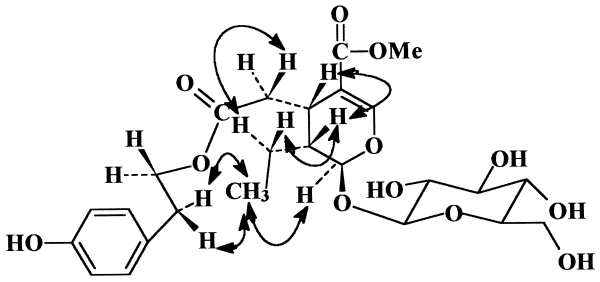

Fig. 1. NOESY experiment of compound **7**.

observed between H-1″ (δ 4.06) and C-7 (δ 174.2) as well as protons of a methoxyl group (δ 3.61) and C-11 (δ 168.9). The 3,4-dihydroxyl phenylethyl group should be connected to the aglycone at C-7. The result of NOESY experiment of compound **8** are identical with those of **7**, suggesting that H-5 and H-9 are on the β-face while H-1 is on the α-face as compound **8**. The structure of compound **8** was characterized as lucidumoside B.

3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded using SHIMADZU UV-3100PC spectrophotometer. IR

absorption spectra were obtained with an IR-450 instrument as a film on KBr disk. FABMS were recorded on VG Autospec 3000 system, and ESIMS on Finnigan TSQ 7000. ^{1}H and ^{13}C spectra were obtained with Bruker 400 instrument operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C, respectively. Chemical shifts are reported in parts per million on the δ scale with TMS as the internal standard, and coupling constants are in Hertz. Column chromatographic separations were performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd, China), Chromatorex ODS (Fuji Silysia Chemical Ltd, Japan), D-101 (Tianjin Agricultural Chemical Co. Ltd, China), and Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd). TLC separations were performed on precoated Si gel 60 F_{254} plates (0.2 mm thick, Merck) with $CHCl_3$–MeOH (10:1, 9:1 and 8:2, v/v) and RP-18 F_{254s} (0.2 mm thick, Merck) with MeOH–H_2O (8:2, 7:3, 6:4 and 6:5, v/v), and spots were detected by UV illumination and by spraying 10% ethanolic H_2SO_4 reagent.

3.2. Plant material

Dry fruits of *L. lucidum* was bought from a Chinese medicine shop in Hong Kong. The voucher specimen was deposited in the Museum of Institute of Chinese Medicine, Chinese University of Hong Kong.

3.3. Extraction and isolation

The dried fruits (2.30 kg) of *L. lucidum* were extracted with EtOH (5 l×3). The EtOH extract was concentrated in vacuo to give a residue (775.3 g). The residue was suspended in H_2O (50 l), and divided into aqueous and insoluble parts. The aqueous layer was absorbed on D101 (2 kg) and then eluted with H_2O (20 l), 60% EtOH aqueous (6 l) and EtOH (6 l), to afford three fractions A (170.1 g), B (63.1 g), and C (1.8 g). Fraction B (28.4 g) was applied to a silica gel column (2 kg) eluted with $CHCl_3$–MeOH (8:2) and then the silica gel in the column was divided equally into 18 fractions, each fraction was eluted with MeOH (300 ml) and concentrated. The 18 fractions were combined into six fractions, frs. 1–6, based on silica TLC ($CHCl_3$–MeOH, 8:2) results. Fr. 2 (0.90 g) was purified by Sephadex LH-20 eluting with EtOH–H_2O (6:4) and then by silica gel eluting with $CHCl_3$–MeOH to give oleoside dimethyl ester (**1**) (5.1 mg), lucidumoside A (**7**) (7.8 mg), and lucidumoside B (**8**) (6.5 mg). Fr. 3 (1.08 g) was purified by silica gel ($CHCl_3$–MeOH (9:1) as eluent) and then by ODS [MeOH–H_2O (7:3)] to afford ligustroside (**2**) (190.0 mg) and oleuropein (**3**) (6.5 mg). Fr. 5 (2.1 g) was purified by Sephadex LH-20 [EtOH–H_2O (6:4)], silica gel [$CHCl_3$–MeOH (8:2) and ODS [MeOH–H_2O (6:5)] to give nuezhenide (**4**) (9.1 mg) and isonuezhenide (**5**) (13.2 mg). Fr. 6 (1.5 g) was chromatographed on Sephadex LH-20 [MeOH–H_2O (6:4)], and purified by silica gel [$CHCl_3$–MeOH (8:2)] and RP-18 [MeOH–H_2O (6:5)] to yield neonuezhenide (**6**) (20.1 mg).

3.4. Lucidumoside A (7)

Colorless powder, $[\alpha]_D^{15}$ −90°C (*c* 0.41, MeOH); UV (EtOH) λ_{max} (log ε) 238 (4.00), 279 (3.37) nm; IR (KBr) λ_{max} 3400, 1700, 1630, 1510, 1080, 920, 830 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2); ESIMS *m/z* 525 $[M-H]^-$, 405 $[M-121]^-$, 363 $[M-glc]^-$; HRFAB-MS *m/z*: found 527.5455 $[M+H]^+$ ($C_{25}H_{35}O_{12}$, calc. 527.5457).

3.5. Lucidumoside B (8)

Colorless powder, $[\alpha]_D^{15}$ −103°C (*c* 0.21, MeOH); UV (EtOH) λ_{max} (log ε) 239 (3.91), 280 (3.40) nm; IR (KBr) λ_{max} 3400, 2950, 1700, 1630, 1520, 1070 cm^{-1}; for ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2). ESI-MS *m/z*: 541 $[M-H]^-$, 379 $[M-glc]^-$; HRFAB-MS *m/z*: found 543.5447 $[M+H]^+$ ($C_{25}H_{35}O_{13}$, calc. 543.5451).

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