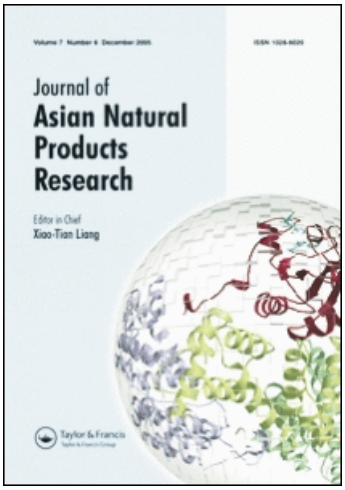

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Studies on chemical constituents from *Ilex pubescens*

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Two new phenolic glycosides, ilexpubsides A and B, along with four known lignan glycosides were isolated from the roots of *Ilex pubescens*. By spectral evidence, the structures of the new compounds were elucidated as 4-*O*-β-D-[6′-*O*-(4″-*O*-β-D-glucopyranosylvanilloyl)glucopyranosyl] vanillic acid (**1**) and syringinic 6′-*O*-β-D-xylopyranoside (**2**). The known compounds were identified to be liriodendrin (**3**), (-)-olivil (**4**), tortoside A (**5**) and (+)-cyclo-olivil (**6**). All compounds were first isolated from *Ilex pubescens*.

Keywords: *Ilex pubescens*; Ilexpubside A; Ilexpubside B; Lignan glycoside

1. Introduction

'Mao-dong-qing', the roots of *Ilex pubescens*, is widely used for the treatment of cardiovascular diseases, hypercholesteremia etc. In a preliminary screening test for the antioxidant and antivirus action of several crude drugs, this plant showed significant activity. For the purpose of looking for the active principle from this plant, the chemical constituents of Mao-dong-qing were investigated. Six compounds have been isolated from the 70% EtOH extract of the roots of *Ilex pubescens*. Based on the spectral analysis, the structures of new compounds were elucidated as 4-*O*-β-D-[6′-*O*-(4″-*O*-β-D-glucopyranosylvanilloyl)glucopyranosyl] vanillic acid (**1**) and syringinic 6′-*O*-β-D-xylopyranoside (**2**), named ilexpubsides A and B. The known ones were identified as liriodendrin (**3**) [1], (-)-olivil (**4**) [2], tortoside A (**5**) [3] and (+)-cyclo-olivil (**6**) [4].

2. Results and discussion

Compound **1** was obtained as a white amorphous solid, $[\alpha]_D^{22} - 70.4$ (MeOH) and its molecular formula was determined as $C_{28}H_{34}O_{17}$ by HRFAB-MS at *m/z* 665.1701 $[M + Na]^+$ and 665 $[M + Na]^+$ in the FAB-MS. In the UV spectrum, **1** showed absorption

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bands at 226 (4.23), 268 (4.02) and 298 (3.95) nm, and showed absorptions at 3423 (OH), 1716 (conj. COOR), 1684 (conj. COOH), 1603, 1512 cm^{-1} (aromatic ring) in the IR spectrum. The ^{1}H and ^{13}C NMR spectra of **1** with the aid of HMQC and HMBC revealed the presence of two 1, 3, 4-trisubstituted benzene rings, two methoxyl groups and two glucosyl residues (table 1). In addition, acid hydrolysis of **1** only gave glucose as a sugar residue. Two anomeric protons at δ 5.12 (1H, d, $J = 9.0$ Hz) and 5.04 (1H, d, $J = 9.0$ Hz) in the ^{1}H NMR spectrum indicated that the anomeric protons of the glucose moieties were confirmed to be β-configuration. The ^{13}C NMR spectrum of **1** showed two signals due to carbonyl carbons at δ 165.8 and 167.7, indicating **1** may be a glucoside of a benzoic acid derivative. These spectral data were similar to those of known compound, 4-*O*-β-D-(6′-*O*-vanilloylglucopyranosyl) vanillic acid, except for the presence of a glucosyl residue instead of a hydroxyl group at C-4″ of the known compound [5].

In the HMBC spectrum of **1**, long-range couplings were observed between H-2″ (δ 7.45) and C-3″ (δ 149.3), C-7″ (δ 165.8); H-5″ (δ 7.16) and C-3″ (δ 149.3), C-4″ (δ 151.4); H-6″ (δ 7.50) and C-4″ (δ 151.4), C-7″ (δ 165.8), and the methoxy proton (δ 3.79) and C-3″

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1** and **2** (δ in ppm, J in Hz).

	1		***2***	
Position	δ_H	δ_C	δ_H	δ_C
1	–	123.7	–	132.7
2	7.46 d (1.8)	114.9	6.71 s	104.4
3	–	149.1	–	152.7
4	–	150.6	–	133.7
5	7.19 d (8.4)	113.4	–	152.7
6	7.53 dd (1.8, 8.4)	123.3	6.71 s	104.4
7	–	167.7	6.44 d (15.5)	128.5
8			6.33 dt (15.5, 5.5)	130.2
9			4.08 bt (5.5)	61.5
OCH_3	3.80 s	56.4 (at 3)	3.76 s	56.3 (at 3 and 5)
1′	5.12 d (9.0)	99.8	4.88 d (7.0)	102.50
2′	3.04 ~ 3.19 m	73.8	2.98 ~ 3.19 m	74.02
3′		77.6		76.12
4′		70.6		69.66
5′		74.6		76.33
6′	4.16 dd (7.0, 12.0) 4.60 dd (2.0, 12.0)	64.7	3.51 dd (5.5, 11.0) 3.82 dd (1.8, 11.0)	68.05
1″	–	124.9	4.05 d (8.0)	103.55
2″	7.45 d (2.0)	115.0	2.98 ~ 3.19–m	73.26
3″	–	149.3		76.33
4″	–	151.4		69.51
5″	7.16 d (8.5)	113.4	2.89 dd (2.0, 11.0) 3.62 dd (5.0, 11.0)	65.41
6″	7.50 dd (2.0, 8.5)	123.6		
7″	–	165.8		
OCH_3	3.79	56.2 (at 3″)		
1	5.04 d (7.0)	100.2		
2‴	3.04 ~ 3.19–m	73.7		
3‴′		77.4		
4‴		70.1		
5‴		77.3		
6‴	3.49 dd (5.0, 12.0) 3.64 dd (1.8, 12.0)	61.2		

Compounds **1** and **2** were measured in DMSO-d6. All of the signals were assigned with the assistance of the HMQC and HMBC data.

(δ 149.3), suggesting the presence of a vanillic acid residue in **1**. Likewise, the presence of another vanillic acid residue was established by the HMBC experiment (figure 1).

The anomeric proton (H-1′) showed long-range correlation with C-4 of the vanillic acid, H-6′ showed long-range correlation with C-7″ of another vanillic acid, so the two vanillic acids were linked through this glucosyl residue. And the anomeric proton (H-1‴) of the terminal glucose showed long-range correlation with C-4″ of the interior vanillic acid. From the above evidence, compound **1** was determined to be 4-*O*-β-D-[6′-*O*-(4″-*O*-β-D-glucopyranosyl vanilloyl)glucopyranosyl] vanillic acid, named ilexpubside A.

Compound **2** was obtained as a white amorphous solid, $[\alpha]_D^{22} - 60.4$ (MeOH) and its molecular formula was determined as $C_{22}H_{32}O_{13}$ by HRFAB-MS at *m/z* 527.1741 $[M + Na]^+$ and 527 $[M + Na]^+$ in the FAB-MS. In the UV spectrum, **2** showed absorption bands at 226 (4.26), 268 (4.08) nm, and it showed absorptions at 3485 (OH), 1716 (conj. COOR), 1633, 1589 (aromatic ring), 1000–1100 cm^{-1} in the IR spectrum. The ^1H NMR spectrum of **2** indicated the presence of *trans*-olefinic protons at δ 6.44 (d, $J = 15.5$ Hz) and 6.31 (dt, $J = 15.5$, 5.5 Hz). The ^{13}C NMR spectrum suggested that the sugar residue of **2** was composed of glucose and xylose by comparing the data with those of standard monosaccharides. In addition, acid hydrolysis of **2** gave glucose and xylose. Two anomeric protons at δ 4.88 (1H, d, $J = 7.0$ Hz) and 4.05 (1H, d, $J = 8.0$ Hz) in the ^1H NMR spectrum indicated that the anomeric protons of the glucose and xylose were confirmed to have β-configuration. The proton signals at δ 3.74 and carbon signals at δ 56.3 in the ^1H NMR and ^{13}C NMR spectra indicated that the two methoxyl groups should be on symmetrical positions in the phenyl ring. In the HMBC of **2** (figure 2), anomeric proton of glucose residue showed long-range correlation with the carbon C-4, suggesting that the glucose was linked to the aglycon moiety at C-4. The linkage between the xylose and glucose was assigned to be a $1 \rightarrow 6$ *O*-glycosidic linkage based on the HMBC. Thus, compound **2** was determined to be syringinic 6′-*O*-β-D-xylopyranoside, named ilexpubside B.

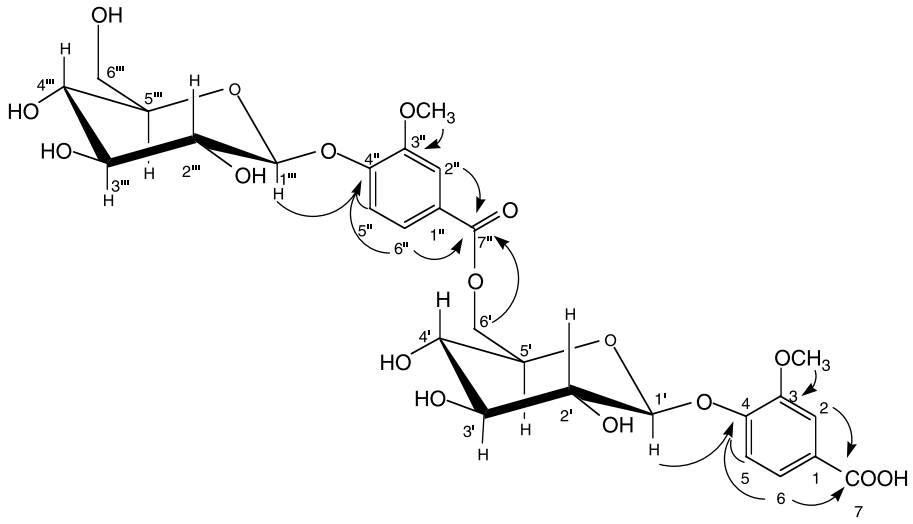

Figure 1. Key HMBC correlations of compound **1**.

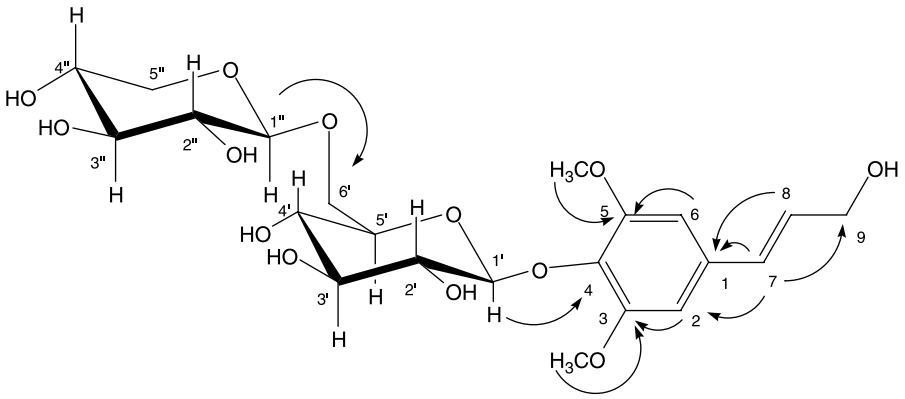

Figure 2. Key HMBC correlations of compound **2**.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained on a Shimadzu UV-260 spectrophotometer. IR spectra were taken in KBr on a Perkin–Elmer 683 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with an Inova-500 spectrometer, operating at 500.1 MHz (^{1}H) and 125.65 MHz (^{13}C) using TMS as an internal standard. ESI-MS and FAB-MS were measured on Agillent 1100 Series LC/MSD Trap and Autospec-Ultima ETOF, respectively. Column chromatography was performed using silica gel (Qingdao Haiyang Chemical Group Co., China). TLC was conducted on Si-gel GF_{254} (Qingdao Haiyang Chemical Group Co., China) and monitored at UV 254 nm. ODS were purchased from YMC Co. Ltd. Japan.

3.2 Plant material

The roots of *Ilex pubescens* were collected from Henan province of China in September 2004 and identified by Mr. Chengbo Jiang, Maodongqing Technique and Develop Company, Henan. A voucher specimen has been deposited in the Institute of Materia Medica, Chinese Academy of Medical Sciences.

3.3 Extraction and isolation

The roots of *Ilex pubescens* (6.0 kg) were extracted three times with 70% EtOH under reflux. The combined 70% EtOH extract was concentrated to give a residue (550 g), which was roughly separated by passing it over porous polymer gel column eluding with water, 30%, 60% EtOH continuously. The 30% EtOH fraction (128 g) was chromatographed over a silica gel column (2500 g) using a mixture of CH_3Cl/MeOH/H_2O (8:2:0.1) to give **1** (50 mg) and **3** (1.32 g). Fractions 3 and 5 were combined and subjected to a column chromatography on silica gel eluted with CH_3Cl/MeOH (39:1) as eluent to obtain **4** (628 mg). Fractions 6 and 17 were combined and subjected to a silica gel column chromatography eluted with CH_3Cl/MeOH (19:1) to give **5** (15 mg) and **6** (19 mg). Fractions 46 and 47 were combined and subjected to a column chromatography on silica gel eluted with CH_3Cl/MeOH (5:1) to obtain **2** (20 mg).

3.4 Characterization of compounds

Ilexpubside A **(1)** white amorphous solid; $[\alpha]_D^{22}$: − 70.4 (*c* 0.5, MeOH); UVλ_{max}^{MeOH}(nm)(log ε): 226 (4.23), 268 (4.02), and 298 (3.95); IRν_{max}^{KBr} (cm^{-1}): 3423, 1716, 1684, 1603, 1512; ^{1}H and ^{13}C NMR spectra data (see table 1); HRFAB-MS *m/z*: 665.1701$[M + Na]^+$ (calcd for $C_{28}H_{34}O_{17}Na$, 665.1693), FAB-MS *m/z*: 665$[M + Na]^+$.

Ilexpubside B **(2)** white amorphous solid; $[\alpha]_D^{22}$: − 60.4 (*c* 0.3, MeOH); UVλ_{max}^{MeOH}(nm)(log ε): 226 (4.26), 268 (4.08); IRν_{max}^{KBr}(cm^{-1}): 3485, 1716, 1633, 1589, 1000–1100; ^{1}H and ^{13}C NMR spectra data (see table 1); HRFAB-MS *m/z*: 527.1741 $[M + Na]^+$ (calcd for $C_{22}H_{32}O_{13}Na$, 527.1741), FAB-MS *m/z*: 527 $[M + Na]^+$.

Liriodendrin **(3)** white amorphous solid; ESI-MS *m/z* 765 $[M + Na]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.03 (2H, m, H-8 and H-8′), 3.04 ∼ 3.19 (8H, m, glc-H-2 ∼ 5 and glc-H-2′ ∼ 5′), 3.40 (2H, dd, J = 11.5, 6.0 Hz, glc-H-6α and glc-H-6′α), 3.59 (2H, dd, J = 11.5, 5.5 Hz, glc-H-6β and glc-H-6′β), 3.75 (12H, s, 4 × OCH_3), 3.83 (2H, dd, J = 3.0, 9.0 Hz, H-9α and H-9′α), 4.21 (2H, m, H-9β and H-9′β), 4.67 (2H, d, J = 3.0 Hz, H-7 and H-7′), 4.89 (2H, d, J = 10.0 Hz, glc-H-1 and glc-H-1′), 6.65 (4H, s, H-2 and H-2′, H-6 and H-6′).

(-)-Olivil **(4)** white amorphous solid; ESI-MS *m/z* 399 $[M + Na]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.31 (1H, m, H-8), 3.02 (2H, ABq, J = 14.0 Hz, H-7′), 3.59, 3.75 (2H, ABq, J = 10.0 Hz, H-9′), 3.76 (1H, dd, J = 11.0, 6.0 Hz, H-9α), 3.89 (1H, dd, J = 11.0, 5.5 Hz, H-9β), 3.81 (6H, s, 2 × OCH_3), 4.71 (1H, d, J = 8.0 Hz, H-7), 6.71 ∼ 6.76 (3H, overlap, H-5, H-5′, H-6), 6.88 (1H, dd, J = 8.0, 1.5 Hz, H-6′), 6.95 (1H, d, J = 1.5 Hz, H-2), 7.13 (1H, d, J = 1.5 Hz, H-2′).

Tortoside A **(5)** white amorphous solid; ESI-MS *m/z* 603 $[M + Na]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.02 ∼ 3.19 (6H, m, glc-H-2′ ∼ 5′ 和 H-8, H-4′), 3.31 (2H, m, H-8′ and H-9′(), 3.40 (1H, dd, J = 11.5, 6.0, glc-H-6′α), 3.59 (1H, dd, J = 11.5, 5.5 Hz, glc-H-6′β), 3.74 (6H, s, 2 × OCH_3), 3.75 (6H, s, 2 × OCH_3), 3.80 (1H, dd, J = 4.0, 7.0 Hz, H-9α), 4.16 (2H, dd, J = 7.0, 12.5 Hz, H-9β and H-9′β), 4.26 (1H, d, J = 5.0 Hz, H-7), 4.67 (1H, d, J = 4 Hz, H-7′), 4.89 (1H, d, J = 10.5 Hz, glc-H-1′), 6.59 (2H, d, J = 1.4 Hz, H-2 and H-6), 6,65 (2H, d, J = 1.4 Hz, H-2′ and H-6′).

(+)-Cyclo-olivil **(6)** white amorphous solid; ESI-MS *m/z* 399 $[M + Na]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.45 (1H, d, J = 16.5 Hz, H-1α), 3.11 (1H, d, J = 16.5 Hz, H-1β), 1.90 (1H, d, J = 12.0 Hz, H-3), 3.70 (6H, s, 2 × OCH_3), 3.86 (1H, d, J = 12.0 Hz, H-4), 6.05 (1H, s, H-5), 6.52 ∼ 6.54 (2H, m, H-6′ and H-2′), 6.65 (1H, s, H-8), 6.70 (1H, d, J = 8.0 Hz, H-5′); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 40.0 (C-1), 43.1 (C-4), 45.5 (C-3), 55.5 (3′-OCH_3), δ 55.6 (7-OCH_3), 58.9 (C-3a), 67.9 (C-2a), 72.9 (C-2), 112.3 (C-2′), 113.4 (C-8), 115.3 (C-5′), 116.1 (C-5), 121.7 (C-6′), 125.1 (C-9), 132.0 (C-10), 136.9 (C-1′), 143.8 (C-6), 144.7 (C-4′), 145.7 (C-7), 147.3 (C-3′).

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