SECOIRIDOID GLUCOSIDES FROM THE LEAVES OF *Ligustrum sinense*

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TÓM TẮT

CÁC HỢP CHẤT SECOIRIDOID GLUCOSIDE TỪ LÁ LOÀI RÂM TRUNG QUỐC *Ligustrum sinense*

*Năm hợp chất secoiridoid glucoside bao gồm ligujaponoside A (**1**), ligujaponoside B (**2**), ligsutroside (**3**), oleuropein (**4**), và ligustaloside A dimethyl acetal (**5**) đã được phân lập từ dịch chiết methanol của phần lá loài râm Trung Quốc Ligustrum sinense. Cấu trúc của các chất này được xác định dựa trên phân tích các dữ kiện phổ khối lượng, phổ cộng hưởng từ hạt nhân một chiều và hai chiều, và kết hợp so sánh với các dữ liệu phổ đã công bố.*

***Từ khóa**: Loài râm Trung Quốc, secoiridoid glucoside.*

1. INTRODUCTION

Ligustrum sinense L. is a common shade-tolerant evergreen shrub with an expansive non-native global range found invading riparian forests throughout the southeastern United States [1, 2]. In the flora of Taiwan, *L. sinense* is one of four species represented by the genus *Ligustrum*. It was often used as herbal tea in traditional Chinese medicine for over 1,000 years [3, 4]. Recently, several research has shown that this species increased the white blood cell count and was of value when used to prevent bone marrow loss in cancer chemotherapy patients. Besides, it can potentially in treating AIDS [4-6]. The plant has been reported to hold several pharmacological and medicinal properties, such as anti-oxidant and anti-tumor activity, and a remedy to treat pain, hepatitis, and inflammation [7, 8]. The constituents of this species have been shown to contain phenolic glycosides, flavonoid glycosides, and lignans [9-13]. However, in Vietnam, the phytochemical study of this plant has yet to be studied. This research reported the isolation and structure elucidation of five secoiridoid glucosides from the methanol extract of the leaves of *L. sinense* species.

2. MATERIALS AND METHODS

2.1. Plants materials

The leaves of *Ligustrum sinense* L. were collected at Vinh Phuc, Vietnam, in September 2021 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (NCCT-LSL01) was deposited at the Institute of Ecology and Biological Resources, VAST.

2.2. Experimental equipment

All NMR spectra, including ^{1}H-NMR (600 MHz), ^{13}C (150 MHz), HSQC, and HMBC, were recorded on a Bruker AM600 FT-NMR spectrometer, and TMS was used as an internal standard. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Column chromatography (CC) was performed using silica gel (Kieselgel 60, 70-230 mesh, and 230-400 mesh, Merck) or RP-18 resins (30-50 μm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) was done using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck). HPLC was carried out via an Agilent 1100 HPLC system using a J'sphere H-80 column (250x20 mm) at a flow rate of 3.0 ml/min, and a DAD detector.

2.3. Extraction and separation

The dried leaves of *Ligustrum sinense* L. (5 kg) were well grinded and sonicated with hot MeOH 80% three times and then concentrated under reduced pressure to give MeOH extract (500g). This extract was suspended in 2 liters of water and successively extracted with *n*-hexane, EtOAc to provide the corresponding *n*-hexane (LH, 67.65g), EtOAc (LE, 53.9g), and H_2O (LW, 380g) extracts after removal of the solvents in *vacuo.* The aqueous layer was chromatographed on a Diaion column and eluted with MeOH-H_2O with a stepwise increase of MeOH (MeOH:H_2O – 25:75 → 100:0, v/v) to obtain four fractions (LW1→4).

The fraction LW1 was subjected to a silica gel column and eluted with a CH_2Cl_2-MeOH stepwise gradient to give four fractions (LW1A→1D). The fraction LW1B was applied to a silica gel column eluting with CH_2Cl_2-MeOH (8:1, v/v) to give two fractions ($LW1B_1$, $LW1B_2$). The fraction LW1C was purified in an RP-18 column using MeOH-H_2O (1:2, v/v) as eluent to obtain eight fractions ($LW1C_{1\to8}$). The fraction $LW1C_8$ was separated by preparative HPLC to give ligujaponoside A (**1**, 4.6mg) and ligujaponoside B (**2**, 30mg).

The fractions LW3 and LW4 were combined, and the residue (LW5) was chromatographed on a silica gel column and eluted with increasing polarity of CH_2Cl_2-MeOH mixture to yield four fractions LW5A→D. The fraction LW5C was continuously subjected to a silica gel using CH_2Cl_2-MeOH (7:1, v/v) as eluent to give six fractions ($LW5C_{1\to6}$). The fraction $LW5C_4$ was purified by preparative HPLC to give ligustroside (**3**, 3.3mg). The fractions $LW5C_6$ and $LW1B_2$ were combined, and the residue was then separated on a silica gel RP-18 column using MeOH/H_2O (1/1.5, v/v) as eluent to give four fractions LW_5C_6A→D. The fraction LW_5C_6B was then purified by preparative HPLC to give oleuropein (**4**, 14.9mg) and ligustaloside A dimethyl acetal (**5**, 13mg).

Ligujaponoide A (**1**): pale yellow amorphous powder; $[\alpha]_D^{25}$ –15.8 (*c* 0.1, MeOH); ESI-MS *m/z* 557 $[M+H]^+$, $C_{25}H_{32}O_{14}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1;

Ligujaponoside B (**2**): pale yellow amorphous powder; $[\alpha]_D^{25}$ –25.2 (*c* 0.1, MeOH), ESI-MS *m/z* 573 $[M+H]^+$, $C_{25}H_{32}O_{15}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1;

Ligustroside (**3**): pale yellow amorphous powder; $[\alpha]_D^{20}$ –51.1 (*c* 0.1, MeOH), ESI-MS *m/z* 525 $[M+H]^+$, $C_{25}H_{32}O_{12}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1;

Oleuropein (**4**): colorless amorphous powder; $[\alpha]_D^{26}$ –68.3 (*c* 0.67, MeOH), ESI-MS *m/z* 539 $[M-H]^-$, $C_{25}H_{32}O_{13}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2;

Ligustaloside A dimethyl acetal (**5**): white amorphous powder; $[\alpha]_D^{26}$ –23.5 (*c* 0.1, MeOH), ESI-MS *m/z* 603 $[M+H]^+$, $C_{27}H_{38}O_{15}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2.

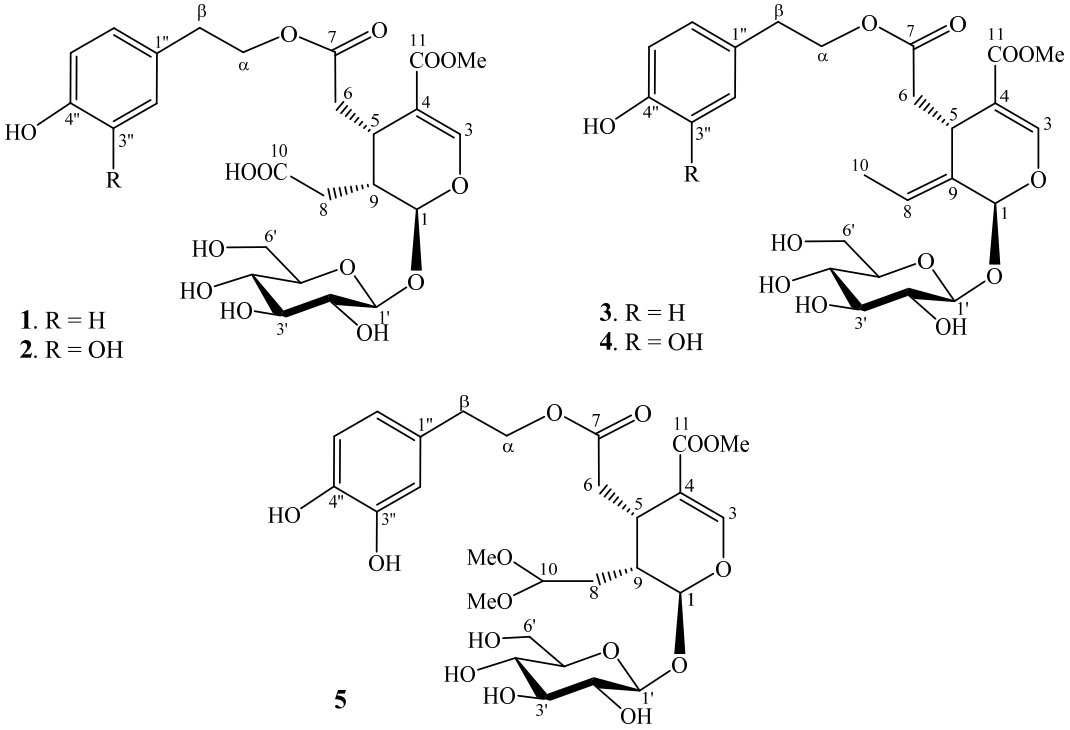


Figure 1. Chemical structure of compounds 1-5

3. RESULTS AND DISCUSSIONS

Compound **1** was obtained as a pale yellow amorphous powder. Its molecular formula was predicted to be $C_{25}H_{32}O_{14}$ based on a quasi-molecular ion peak at *m/z* 555.17 $[M-H]^-$ in the ESI-MS and ^{13}C-NMR data.

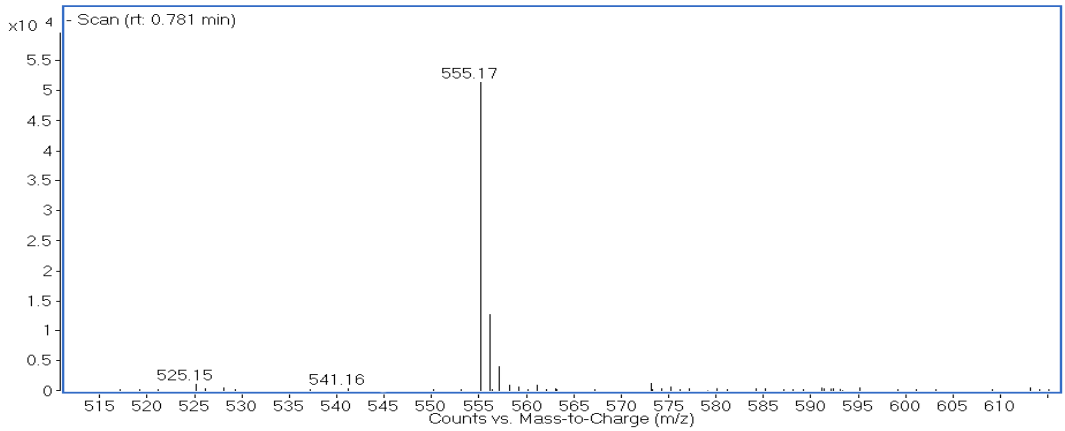


*Figure 2. ESI-MS of compound **1***

The ^{1}H-NMR spectrum of **1** showed signals for one olefinic proton at δ_H 7.49 (1H, s, H-3), two acetalcarbinol protons at δ_H 5.44 (1H, d, J = 7.8 Hz, H-1) and δ_H 4.70 (1H, d, J = 7.8 Hz), one methoxy group at δ_H 3.68 (3H, s), and two sets of one methylene at δ_H 1.61 and 1.82, suggesting the presence of a secoiridoid skeleton [14]. In addition, the characteristic aromatic protons of A_2B_2 spin systems at δ_H 6.74 (2H, d, J = 8.4 Hz) and 7.08 (2H, d, J = 8.4 Hz), along with two methylene groups at δ_H 4.21 (2H, m) and 2.85 (2H, t, J = 7.2 Hz) indicated the presence of a *p*-hydroxy phenylethyl group.

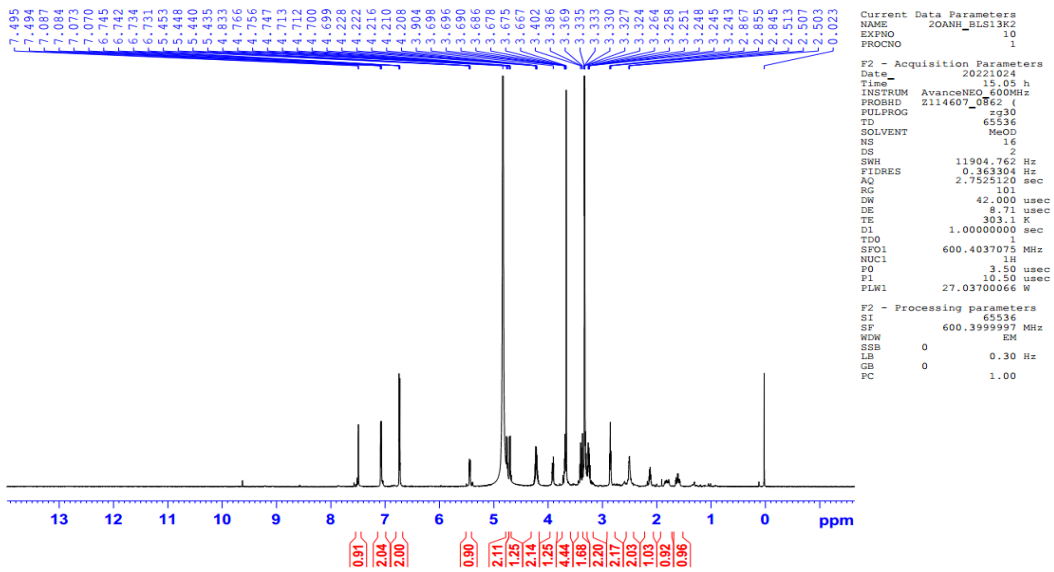


*Figure 3. ^{1}H-NMR spectrum of compound **1***

The ^{13}C-NMR and HSQC spectrum of **2** showed the signals of 25 carbons, including three carbonyl groups at δ_C 174.4 (x2), 168.9, six aromatic carbons at δ_C 130.0, 131.0, 116.3, 157.1, 116.3, 131.0, two olefinic carbons at δ_C 154.1, 110.6, one methoxy group at δ_C 51.7, seven sp^3-hybridized carbons, and six carbons of a sugar moiety. The signals of D-glucopyranoside were proved based on the appearance of the characteristic signals on the ^{1}H-NMR spectrum at δ_H 4.70 (1H, d, J = 7.8 Hz), hydroxymethylene at δ_H 3.69 (dd, J = 5.4, 12.0 Hz) and 3.90 (d, J = 12.0 Hz), and the ^{13}C-NMR spectra at δ_C 100.6, 78.4, 77.9, 74.8, 71.6, and 62.8 ppm. The β-configuration of the anomeric proton of D-glucose was determined from the large coupling constant J = 7.8 Hz of H-1' (δ_H 4.70).

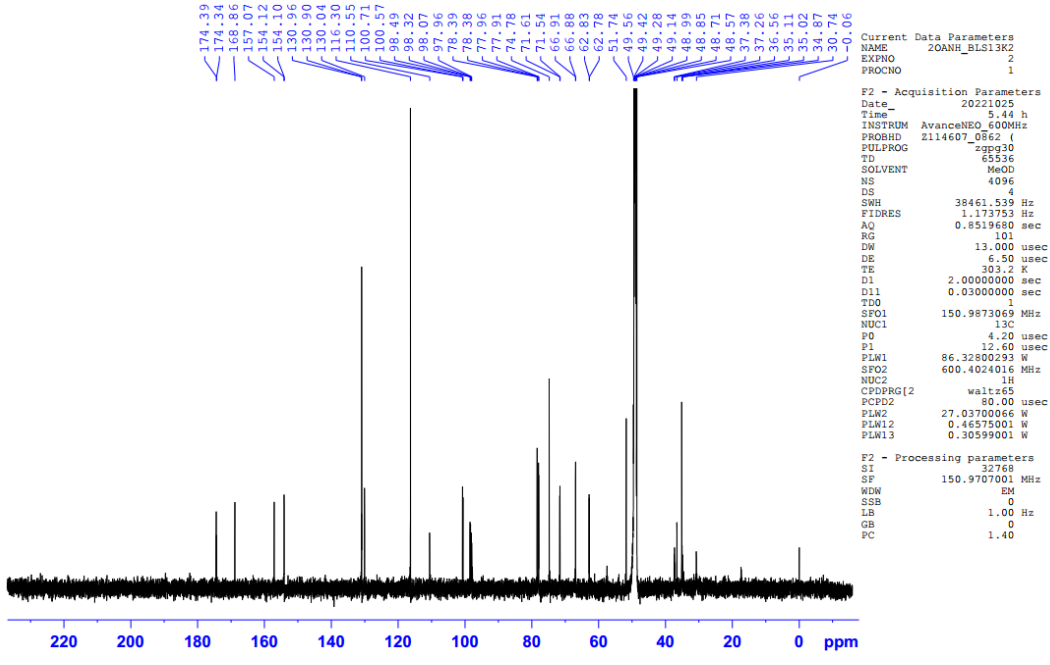


*Figure 4. ^{13}C-NMR spectrum of compound **1***

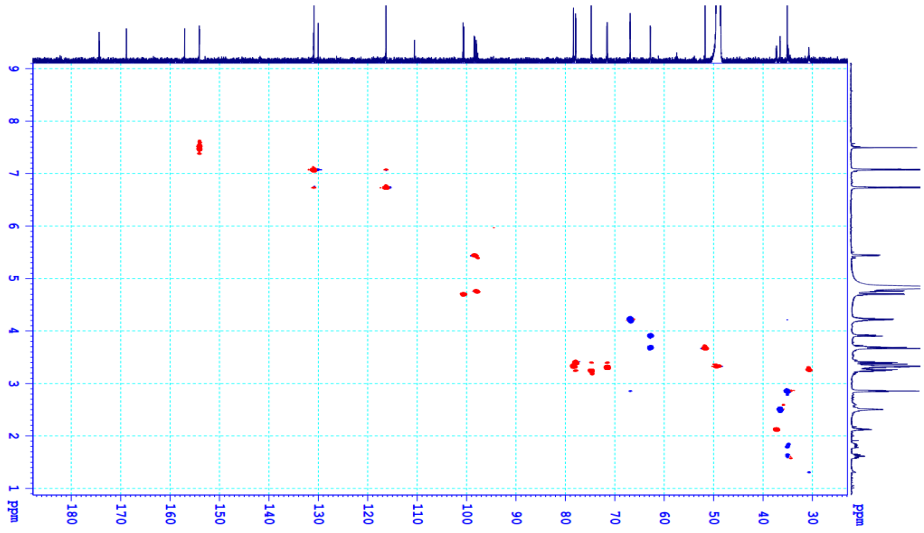


*Figure 5. HSQC spectrum of compound **1***

In the HMBC spectrum, it found HMBC correlations between H-3 (δ_H 7.49) and C-1 (δ_C 98.3)/C-4 (δ_C 110.6)/C-11 (δ_C 168.9), and also between a methoxy group (δ_H 3.68) and a carbonyl carbon (δ_C 168.9, C-11). It lets suggestion that the double bond should be located at C-3/C-4 and the methyl ester group at C-11. The HMBC experiment of **1** also showed the interactions between H-α (δ_H 4.21) and C-7 (δ_C 174.4), so the phenylethyl group should be attached to C-7. The HMBC correlations from H-9 (δ_H 2.13) to the carbonyl carbon at δ_C 174.4 suggested that a carbonyl group was located at C-10. Furthermore, the HMBC correlations between H-1 (δ_H 5.44) and C-1′ (δ_C 100.6) and between H-1′ (δ_H 4.70) and C-1 (δ_C 98.3) determined the location of the D-glucose at C-1 of aglycone secoiridoid (Figure 7).

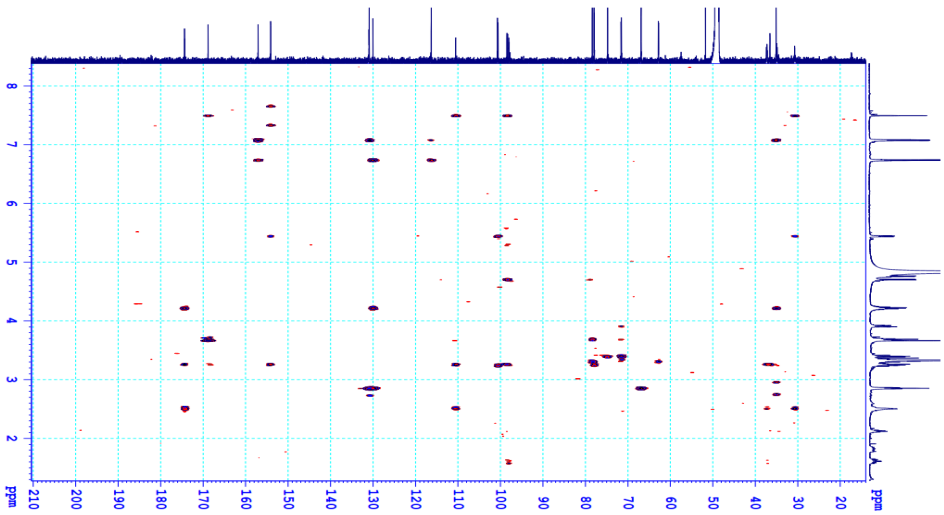


*Figure 6. HMBC spectrum of compound **1***

Based on the above evidence and literature, the structure of **1** was identified as ligujaponoside A, a secoiridoid glucoside previously isolated from the fruits of *Ligustrum japonicum* [2].

Compound **2** was obtained as a pale yellow amorphous powder. The molecular formula of **2** was deduced as $C_{25}H_{32}O_{15}$ based on ESI-MS (*m/z*: 573, $[M+H]^+$) and in consistent with ^{13}C-NMR data. Comparison of the ^{1}H-NMR and ^{13}C-NMR data of **1** and **2** were similar except for the signals belonging to the phenylethyl moiety. The presence of an ABX spin system at δ_H 6.69 (d, J = 1.8 Hz, H-2"), 6.71 (d, J = 8.4 Hz, H-5"), and 6.57 (d, J = 8.4 Hz, H-6") in **2** instead of A_2B_2 spin systems at δ_H 6.74 (2H, d, J = 8.4 Hz) and 7.08 (2H, d, J = 8.4 Hz) in **1**. These implied that compound **2** has an additional hydroxy group at phenylethyl moiety. From the above spectral findings and comparison with the NMR data of ligujaponoside B, a compound also previously isolated from the fruits of *Ligustrum japonicum* [2], the structure of compound **2** was established.

Compound **3** was isolated as a pale yellow amorphous powder. Its molecular formula was predicted to be $C_{25}H_{32}O_{12}$ based on a quasi-molecular ion peak at *m/z* 525 $[M+H]^+$ in the ESI-MS and ^{13}C-NMR data. The ^{1}H-NMR and ^{13}C-NMR spectra data

*Table 1. The ^{1}H- and ^{13}C-NMR data of compounds **1-3** and reference compounds*

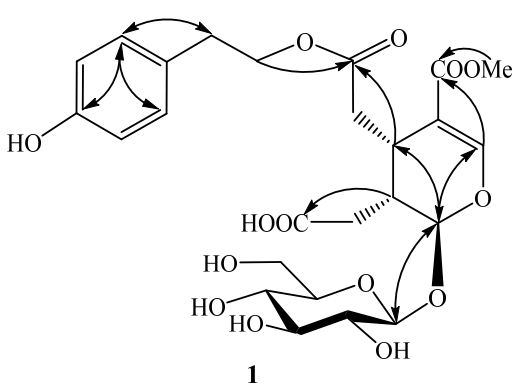
C	1		2		3	
	δ_C[a,b]	δ_H[a,c] (mult., J = Hz)	δ_C[a,b]	δ_H[a,c] (mult., J = Hz)	δ_C[a,b]	δ_H[a,c] (mult., J = Hz)
1	98.3	5.44 (d, 7.8)	98.3	5.44 (d, 7.8)	95.2	5.94 (s)
3	154.1	7.49 (s)	154.1	7.49 (s)	155.1	7.53 (s)
4	110.6	-	110.5	-	109.4	-
5	30.7	3.27 (m)	30.7	3.26 (m)	31.8	3.99 (dd, 4.8, 9.0)
6	36.6	2.50 (dd, 6.0, 2.4)	36.6	2.51 (dd, 6.0, 2.4)	41.2	2.46 (dd, 9.0, 14.4) 2.72 (dd, 4.8, 14.4)
7	174.4	-	174.3	-	173.2	-
8	34.9	1.61 (m) / 1.82 (m)	34.9	1.61 (m) / 1.82 (m)	124.9	6.10 (q, 7.2)
9	37.3	2.13 (dd, 6.6, 12.6)	37.3	2.12 (dd, 6.6, 12.6)	130.1	-
10	174.4	-	174.4	-	13.5	1.67 (dd, 1.2, 7.2)
11	168.9	-	168.9	-	168.7	-
11-OMe	51.7	3.68 (s)	51.8	3.69 (s)	51.9	3.73 (s)
1-*O*-Glc						
1′	100.6	4.70 (d, 7.8)	100.5	4.71 (d, 7.8)	100.9	4.83*
2′	74.8	3.24 (m)	74.7	3.25 (m)	74.8	3.32 (m)
3′	77.9	3.40 (t, 8.4)	77.8	3.40 (t, 9.0)	78.0	3.44 (t, 8.4)
4′	71.6	3.32 (m)	71.5	3.32 (m)	71.5	3.33 (m)
5′	78.4	3.33 (m)	78.3	3.32 (m)	78.5	3.35 (m)
6′	62.8	3.69 (dd, 5.4, 12.0) 3.90 (d, 12.0)	62.8	3.69 (dd, 5.4, 12.0) 3.90 (d, 12.0)	62.8	3.69 (dd, 5.4, 12.0) 3.89 (d, 12.0)
1″	130.0	-	130.8	-	130.1	-
2″	131.0	7.08 (d, 8.4)	116.4	6.69 (d, 1.8)	131.0	7.07 (d, 8.4)
3″	116.3	6.74 (d, 8.4)	146.2	-	116.3	6.74 (d, 8.4)
4″	157.1	-	144.9	-	157.1	-
5″	116.3	6.74 (d, 8.4)	117.0	6.71 (d, 8.4)	116.3	6.74 (d, 8.4)
6″	131.0	7.08 (d, 8.4)	121.2	6.57 (d, 8.4)	131.0	7.07 (d, 8.4)
α	66.9	4.21 (m)	66.9	4.21 (m)	66.9	4.12 (dt, 7.2, 10.8) 4.24 (dt, 7.2, 10.8)
β	35.1	2.85 (t, 7.2)	35.3	2.79 (t, 7.2)	35.2	2.84 (t, 7.2)

*Recorded in [a]CD_3OD, [b]150 MHz, [c]600 MHz,*overlap signals*

also indicated the presence of a secoiridoid glucoside moiety. A comparison between 1D NMR data **3** and **1** found quite similar except for signals belonging to the aglycone moiety. In particular, the addition of proton olefinic at δ_H 6.10 (q, J = 7.2 Hz) and the corresponding carbons at δ_C 124.9 and 130.1 in compound **3** suggested the double bond at C-8/C-9. Moreover, the absence of carbonyl carbon at C-10 in compound **3** and the appearance of a methyl group at δ_H 1.67 (dd, J = 1.2, 7.2 Hz) and δ_C 13.5 in compound **3** implied the replacement of the carboxylic acid at C-10 in **1** to the methyl group in **3**. Besides, the NMR data of **3** were in good agreement with those reported (table 1) [15]. From the above evidence, the structure of **3** was elucidated as ligustroside.

Compound **4** was isolated as a colorless amorphous powder. It gave a molecular formula of $C_{25}H_{32}O_{13}$ at m/z 539 $[M-H]^-$ in the ESI-MS and ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **3** except for signals belonging to phenol moiety (1,3,5-trisubstituted benzene ring). The above evidence implied that the structure of **4** was identical to **3** except for an additional hydroxy group at the phenol moiety. Moreover, an ABX coupled system [δ_H 6.69 (d, J = 1.8 Hz), δ_H 6.71 (d, J = 7.8 Hz), δ_H 6.57 (dd, J = 1.8, 7.8 Hz)] in the ^{1}H-NMR spectrum of **4** differed from an A_2B_2 coupled system [δ_H 6.74 (2H, d, J = 8.4 Hz), δ_H 7.07 (2H, d, J = 8.4 Hz)] in the ^{1}H-NMR spectrum of **3**, suggesting that the additional hydroxy group substituted at C-3 in **4** instead of a proton in **3**. From the data mentioned above, compound **4** was elucidated as oleuropein, a secoiridoid glucoside previously isolated from the fruits of *Ligustrum lucidum* **[15]**.

Compound **5** was obtained as a white amorphous powder. The molecular formula of **5** was established as $C_{27}H_{38}O_{15}$ on the basis of ESI-MS (m/z: 603, $[M+H]^+$) consistent with ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectrum of compound **5** were close to those of **2**, except for replacing two methoxy groups to the carboxylic group at C-10 of the secoiridoid aglycone moiety compared to **2**. The two methoxy groups were clearly observed at δ_H 3.33 (6H, s) in the ^{1}H-NMR spectrum and the corresponding δ_C 54.0, 54.1 in the ^{13}C-NMR spectrum of compound **5**. The location of the two methoxy groups was reconfirmed by the analysis of 2D experiments (HSQC and HMBC). Clear HMBC correlations between protons of the two methoxy groups δ_H 3.33 to C-10, as shown in figure 2, confirmed these methoxy groups located at C-10 of the secoiridoid moiety. Therefore, compound **5** was characterized as ligustaloside A dimethyl acetal, a compound previously reported from the leaves of *Ligustrum ovalifolium* [16]. Its NMR data agreed with those reported data [16].



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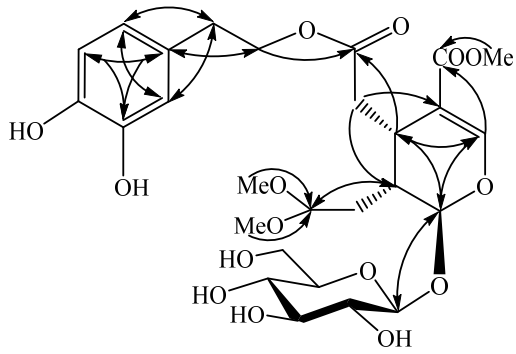

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Figure 7. The key HMBC correlations of compounds 1 and 5

Table 2. The ^{1}H- and ^{13}C-NMR data of compounds 4-5 and reference compounds

C	**4**		**5**	
	δ_C[a,b]	δ_H[a,c] (mult., *J* = Hz)	δ_C[a,b]	δ_H[a,c] (mult., *J* = Hz)
1	95.2	5.93 (s)	98.4	5.43 (d, 7.8)
3	155.1	7.53 (s)	154.1	7.50 (s)
	109.4	-	110.6	-
5	31.8	3.99 (dd, 4.8, 9.0)	30.9	3.25 (m)
6	41.3	2.46 (dd, 9.0, 14.4) 2.72 (dd, 4.8, 14.4)	36.6	2.51 (m)
7	173.2	-	174.4	-
8	124.9	6.10 (q, 7.2)	31.0	1.62 (dt, 6.0, 14.4) 1.83 (dt, 6.0, 14.4)
9	130.8	-	37.0	2.05 (m)
10	13.5	1.68 (dd, 1.2, 7.2)	104.6	4.63 (t, 6.0)
10-OMe			54.0	3.33 (s)
10-OMe			54.1	3.33 (s)
11	168.7	-	168.8	-
11-OMe	51.9	3.73 (s)	51.8	3.68 (s)
1-*O*-Glc				
1′	100.9	4.83*	100.7	4.71 (d, 7.8)
2′	74.8	3.33 (m)	74.8	3.26 (m)
3′	77.9	3.44 (t, 8.4)	78.0	3.40 (t, 9.0)
4′	71.5	3.34 (m)	71.6	3.33 (m)
5′	78.4	3.35 (m)	78.4	3.34 (m)
6′	62.7	3.70 (dd, 5.4, 12.0) 3.90 (dd, 1.8, 12.0)	62.9	3.70 (dd, 5.4, 12.0) 3.92 (dd, 1.8, 12.0)
1″	130.5	-	130.8	-
2″	116.5	6.69 (d, 1.8)	116.4	6.69 (d, 2.4)
3″	146.2	-	146.3	-
4″	144.9	-	144.9	-
5″	117.1	6.71 (d, 7.8)	117.0	6.71 (d, 7.8)
6″	121.3	6.57 (dd, 1.8, 7.8)	121.2	6.57 (dd, 2.4, 7.8)
α	66.9	4.13 (dt, 7.2, 10.8) 4.23 (dt, 7.2, 10.8)	66.9	4.21 (m)
β	35.4	2.78 (t, 7.2)	35.3	2.80 (t, 6.6)

*Recorded in [a] CD_3OD, [b] 150 MHz, [c] 600 MHz, *overlap signals*

4. CONCLUSION

Five secoiridoid glucosides including ligujaponoside A (**1**), ligujaponoside B (**2**), ligsutroside (**3**), oleuropein (**4**), and ligustaloside A dimethyl acetal (**5**) were isolated from the methanol extract of the leaves of *Ligustrum sinense* using combined chromatographic methods.

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