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Lignan and Neolignan Glycosides from *Ulmus davidiana* var. *japonica*

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Four lignan xylosides and two neolignan glycosides were isolated from the stem and root barks of *Ulmus davidiana* var. *japonica*. Their structures were identified as lyoniside, nudiposide, 5'-methoxyisolariciresinol-9'-*O*-β-D-xylopyranoside, isolariciresinol-9'-*O*-β-D-xylopyranoside, rel-*trans*-dihydrodehydroconiferyl alcohol 4'-*O*-α-L-rhamnopyranoside and icariside E3 by comparison of their spectral data with those reported in the literatures, respectively.

Key words: *Ulmus davidiana* var. *japonica*, Ulmaceae, Lignan xylosides, Neolignan glycosides, Icariside E3

INTRODUCTION

In our previous works on stem and root barks of *Ulmus davidiana* Planch var. *japonica* Nakai (Ulmaceae), we reported five new neuroprotective triterpene esters (Lee and Kim, 2001). To isolate other bioactive compounds from this plant, further work on the *n*-BuOH fraction resulted in the isolation of four lignan xylosides and two neolignan glycosides. These compounds were identified as lyoniside (**1**), nudiposide (**2**), 5'-methoxyisolariciresinol-9'-*O*-β-D-xylopyranoside (**3**), isolariciresinol-9'-*O*-β-D-xylopyranoside (**4**), rel-*trans*-dihydrodehydroconiferyl alcohol 4'-*O*-α-L-rhamnopyranoside (**5**) and icariside E3 (**6**) by comparison of its spectral data with those reported in the literatures, respectively.

MATERIALS AND METHODS

General experimental procedure

IR spectra were obtained on a Perkin-Elmer 1710 spectrometer. The NMR spectra were taken on either a JEOL GSX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a JEOL LA 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer. HRFABMS were taken on a JMS-SX 102A spectrometer (JEOL, Japan) and optical rotation on a JASCO DIP-1000 polarimeter. Column chromatography was performed over Si gel 60 (Merck, 230-400 mesh) and Sephadex LH-20 (Phamacia, Sweden).

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Plant materials

The stem and root barks of *U. davidiana* var. *japonica* were purchased from Kyungdong Oriental Herbal Market, Seoul, Korea and identified by Dr. Dae S. Han, an emeritus professor of the College of Pharmacy, Seoul National University. A voucher specimen has been deposited in the Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University.

Extraction and isolation

The dried stem and root barks of *U. davidiana* var. *japonica* (10 kg) were cut into pieces and extracted three times with 80% MeOH in an ultrasonic apparatus. Removal of the solvent *in vacuo* yielded a methanolic extract (936 g). This methanolic extract was then suspended in distilled water and partitioned with CH_2Cl_2. The water fraction was partitioned with *n*-BuOH.

Column chromatography of *n*-BuOH fraction over XAD resin using H_2O-MeOH mixture gave five fractions (fr.1~fr.5). Following silica gel column chromatography of fr.3 with a solvent mixture of $CHCl_3$ and MeOH (20:1) yielded ten fractions (fr.3-1~fr.3-10). Among these fractions, column chromatography of fr.3-2 over Sephadex LH-20 (MeOH) gave four fractions (fr.3-2-1~fr.3-2-4) and

continuous column chromatography of fr.3-2-2 over RP-18 using H_2O-MeOH mixture gave seven fractions (fr.3-2-2-1~fr.3-2-2-7). Compounds **1** and **2** were isolated from fr.3-2-2-1 by semipreparative HPLC on RP_{18} eluted with AcCN-MeOH-H_2O (10:10:80). Compond **4** was also purified from fr.3-2-2-3 by the above method. Compound **5** was obtained from fr.3-2-2-4. Compound **3** was purified from fr.3-2-1 by semipreparative HPLC on RP_{18} eluted with AcCN-MeOH-H_2O (10:10:80).

Column chromatography of fr.2 over silica gel with a solvent gradient of acetone in $CHCl_3$ yielded fifteen fractions (fr.2-1~fr.2-15). Among these fractions, column chromatography of fr.2-12 over Sephadex LH-20 (MeOH) gave five fractions (fr.2-12-1~fr.2-12-5) and continuous column chromatography of fr.2-12-1 over RP-18 using H_2O-MeOH mixture gave five fractions (fr.2-12-1-1~fr.2-12-1-5). Compound **6** was purified from fr.2-12-1-3 using Sephadex LH-20 (MeOH) column chromatography.

Compound **1** (lyoniside): $[\alpha]_D$+43.0° (c=0.8, MeOH), UV (MeOH) λ_{max}; 215, 279 nm, Positive FABMS (m-NBA): 575 $[M+Na]^+$, 552 $[M]^+$, 419 [M+H-xylose]$^+$, ^{1}H-NMR (300 MHz, CD_3OD, δ) 6.54 (1H, s, H-2), 6.40 (2H, s, H-2', 6'), 4.35 (1H, d, *J*=6.6 Hz, H-7'), 4.19 (1H, d, *J*=7.6 Hz, H-1''), 3.83 (1H, m, H-9b''), 3.83 (3H, s, OCH_3), 3.81 (1H, m, H-5a''), 3.72 (6H, s, 2 × OCH_3), 3.60 (2H, m, H-9), 3.46 (1H, m, H-4''), 3.41 (1H, m, H-9a'), 3.32 (3H, OCH_3), 3.29 (1H, m. H-3''), 3.22 (1H, m, H-5a''), 3.17 (1H, m, H-2''), 2.65 (1H, m, H-7), 2.30 (1H, m, H-8'), 1.68 (1H, m, H-8). ^{13}C NMR (75 MHz, CD_3OD) see the Table I.

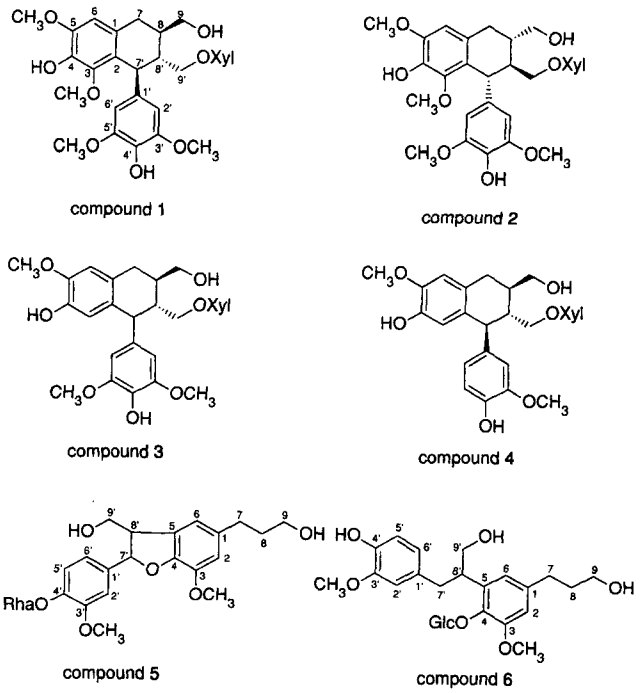

Fig. 1. Chemical structures of compounds **1-6**

Compound **2** (nudiposide): $[\alpha]_D$ -21.0° (c=0.4, MeOH), UV (MeOH) λ_{max}; 223, 281 nm, Positive FABMS (m-NBA): 575 $[M+Na]^+$, 552 $[M]^+$, 419 [M+H-xylose]$^+$, ^{1}H-NMR (300 MHz, CD_3OD, δ) 6.54 (1H, s, H-2), 6.38 (2H, s, H-2', 6'), 4.20 (1H, d, *J*=7.3 Hz, H-7'), 4.07 (1H, d, *J*=7.3 Hz, H-1''), 3.85 (1H, m, H-5a''), 3.83 (3H, s, OCH_3), 3.81 (1H, m, H-9b''), 3.72 (6H, s, 2 × OCH_3), 3.62 (2H, m, H-9), 3.58 (1H, m, H-9a'), 3.48 (1H, m, H-4''), 3.32 (3H, OCH_3), 3.28 (1H, m. H-3''), 3.19 (1H, m, H-5a''), 3.15 (1H, m, H-2''), 2.68 (1H, m, H-7), 2.03 (1H, m, H-8'), 1.69 (1H, m, H-8). ^{13}C NMR (75 MHz, CD_3OD) see the Table I.

Compound **3** ((+)-5'-methoxyisolariciresinol-9'-O-β-D-xylopyranoside): $[\alpha]_D$ +34.7° (c=0.4, DMSO), UV (MeOH) λ_{max}; 211, 283 nm, Positive FABMS (m-NBA): 545 $[M+Na]^+$, 522 $[M]^+$, 389 [M+H-xylose]$^+$, ^{1}H-NMR (300 MHz, CD_3OD, δ) 6.59(1H, s, H-2), 6.41 (2H, s, H-2', 5'), 6.04 (1H, s, H-5), 4.04 (1H, d, *J*=6.8 Hz, H-7'), 3.89 (1H, d, *J*=7.5 Hz, H-1''), 3.85 (1H, m, H-9b'), 3.72 (3H, s, OCH_3), 3.67 (6H, s, OCH_3), 3.65 (1H, m, H-5b''), 3.57 (1H, m, H-9b), 3.48 (1H, m, H-9a), 3.29 (1H, m, H-4''), 3.05 (1H, m, H-3''), 3.02 (1H, m, H-2''), 2.98 (1H, m,

Table I. ^{13}C NMR data of compounds **1~4** (Solvent: CD_3OD, *: DMSO-d_6)

	1	2	3*	4
1	130.1	130.1	127.3	134.6
2	107.8	107.7	112.1	113.2
3	147.6	147.5	145.9	148.0
4	138.9	138.9	144.4	146.7
5	148.6	148.6	116.5	118.2
6	126.4	126.3	132.8	130.0
7	33.9	34.0	33.0	24.5
8	40.5	40.6	37.8	41.5
9	66.0	66.0	62.9	66.2
1'	139.4	139.6	136.1	139.5
2'	106.9	106.9	107.0	115.0
3'	148.9	148.6	148.2	150.0
4'	134.4	134.4	133.9	146.0
5'	148.9	148.6	148.2	116.8
6'	106.9	106.9	107.0	124.0
7'	43.0	43.3	44.2	46.4
8'	46.7	46.8	46.6	49.3
9'	71.0	71.0	67.5	71.1
OCH_3	60.0	59.9	56.3	57.2
OCH_3	56.8	56.8	56.3	57.2
	56.8	56.8	55.8	
	56.6	56.6		
1''	105.5	105.0	105.0	105.5
2''	74.9	74.9	73.7	75.7
3''	78.0	78.0	76.9	78.8
4''	71.2	71.2	69.9	72.1
5''	66.9	67.0	66.0	67.8

H-5a''), 2.95 (1H, m, H-9a'), 2.72 (2H, m, H-7), 1.89 (1H, m, H-8), 1.70 (1H, m, H-8'). ^{13}C NMR (75 MHz, CD_3OD) see the Table I.

Compound **4** ((+)-isolariciresinol-9'-*O*-β-D-xylopyranoside): $[\alpha]_D$ +39.2° (c=0.5, MeOH), UV (MeOH) λ_{max}; 223, 281 nm, Positive FABMS (m-NBA): 515 $[M+Na]^+$, 492 $[M]^+$, 358 [M-xylose]$^+$, ^{1}H-NMR (300 MHz, CD_3OD, d) 6.73 (1H, d, *J*=8.0 Hz, H-5'), 6.70 (1H, d, *J*=2.0 Hz, H-2'), 6.63 (1H, s, H-2), 6.57 (1H, dd, *J*=2.0, 8.0 Hz, H-6'), 6.16 (1H, s, H-5), 3.98 (1H, d, *J*=7.5 Hz, H-1''), 3.81 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.78 (1H, d, *J*=7.1 Hz, H-7'), 3.75 (2H, m, H-9), 3.72 (1H, m, H-5b''), 3.61 (2H, m, H-9''), 3.43 (1H, m, H-4''), 3.23 (1H, m, H-3''), 3.14 (1H, m, H-2''), 3.01 (1H, m, H-5a''), 2.95 (1H, m, H-9a'), 2.80 (2H, m, H-7), 1.97 (1H, m, H-8), 1.93 (1H, m, H-8'). ^{13}C NMR (75 MHz, CD_3OD) see the Table I.

Compound **5** (rel-*trans*-dihydrodehydroconiferyl alcohol 4'-*O*-α-L-rhamnopyranoside): $[\alpha]_D$ -21.0° (c=0.4, MeOH), UV (MeOH) λ_{max}; 263, 291 nm, Positive FABMS (m-NBA): 529 $[M+Na]^+$, 506 $[M]^+$, 360 [M-glucose]$^+$, ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (75 MHz, CD_3OD) see the Table II.

Compound **6** (icariside E_3): $[\alpha]_D$ -61.0° (c=1.0, MeOH), UV (MeOH) λ_{max}; 215, 278 nm, Positive FABMS (m-NBA): 548 $[M+H+Na]^+$, 363 [M+H-rhamnose]$^+$, ^{1}H-NMR (300 MHz, CD_3OD) and ^{13}C-NMR (75 MHz, CD_3OD) see the Table II.

RESULTS AND DISCUSSION

Compounds **1** ($[\alpha]_D$+25.4°) and **2** ($[\alpha]_D$ 60.0°) were isolated in low amounts by reverse-phase HPLC and identified as lyoniside [(+)-lyoniresinol 3α-*O*-β-D-xylopyranoside] and nudiposide [(-)-lyoniresinol 3α-*O*-β-D-xylopyranoside], respectively. Their ^{13}C NMR spectra and optical rotations were in excellent accordance with the literature data (Inoshiri *et al.*, 1987; Smite *et al.*, 1995). The ^{1}H NMR spectra of **1** and **2** differed only with respect to the signal for the anomeric proton and for some alicyclic protons (Inoshiri *et al.*, 1987). Complete assignments of the spectra were made with ^{1}H-^{1}H COSY and ^{13}C-^{1}H HETCOR techniques. Compounds **1** and **2** have been found in several species previously (Inoshiri *et al.*, 1987; Smite *et al.*, 1995) but were isolated for the first time from Ulmaceae.

Compounds **3** and **4** were identified as (+)-5'-methoxyisolariciresinol-9'-O-β-D-xylopyranoside and (+)-iso-

Table II. ^{1}H and ^{13}C NMR data of compounds **5** and **6** (solvent: CD_3OD)

	5		**6**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	137.5	-	140.3
2	6.62, brs	114.6	6.70, s	111.7
3	-	145.6	-	143.6
4	-	147.9	-	153.1
5	-	130.1	-	138.5
6	6.63, brs	119.6	6.70, s	120.3
7	2.61, t, 7.8 Hz	33.3	2.63, t, 6.5 Hz	33.1
8	1.80, m	36.2	1.81, m	35.5
9	3.55, t, 6.5 Hz	62.7	3.56, t, 6.4 Hz	62.2
1'	-	139.2	-	133.3
2'	6.93, d, 1.9 Hz	111.7	6.55, d, 8.1 Hz	113.0
3'	-	147.0	-	145.3
4'	-	145.6	-	148.4
5'	6.98, d, 8.3 Hz	118.4	6.54, d, 1.7 Hz	115.6
6'	6.81, dd, 1.9, 8.3 Hz	120.0	6.46, dd, 1.7, 8.1 Hz	122.6
7'	5.43, d, 8.0 Hz	89.0	2.97, dd, 5.1, 13.6 Hz	39.2
	-		2.69, dd, 9.5, 13.6 Hz	
8'	3.44, m	56.0	3.95, m	42.7
9'	3.75, m	65.5	3.76, m	67.1
OCH_3	3.78, s	56.9	3.68, s	56.2
	3.84, s	57.2	3.78, s	56.3
1''	5.25, d, 1.5 Hz	101.8	4.60, d, 7.3 Hz	105.6
2''	4.06, m	72.7	3.42, m	75.9
3''	3.88, m	72.5	3.38, m	78.0
4''	3.75, m	74.3	3.12, m	71.2
5''	3.78, m	71.3	3.63, m	77.8
6''	1.20, d, 6.3 Hz	18.4	3.76, m	62.5

lariciresinol-9′-*O*-β-D-xylopyranoside by comparison with previously reported spectral data (Vecchietti *et al.*, 1979; Yoshinari *et al.*, 1989; Achenbach *et al.*, 1992; Yahara *et al.*, 1992; Cortez *et al.*, 1998), respectively. Complete assignments of the ^{1}H and ^{13}C NMR spectra were made with ^{1}H-^{1}H COSY, ^{13}C-^{1}H HETCOR and HMBC spectra, apparently for the first time.

Compound **5** was isolated as a viscous oil, $[\alpha]_D$ 51.8°. Its molecular formula was deduced as $C_{26}H_{34}O_{10}$ by FABMS (*m/z*, 529 $[M+Na]^+$) and by carbon counts in the ^{13}C NMR spectrum. And then, the fragment ion peak at *m/z* 360 $[M-146]^+$ in the FABMS suggested the presence of rhamnosyl moiety in this compound and this was supported by two characteristic rhamnopyranosyl doublets at δ_H 5.25 (1H, *J*=1.5 Hz) and δ_H 1.20 (3H, *J*=6.3 Hz). The ^{1}H- and ^{13}C NMR showed the presence of 1,3,4-trisubstituted and 1,3,4,5-tetrasubstituted benzene rings, two methoxyl and two primary alcohols, and the ^{1}H-^{1}H COSY NMR spectrum suggested the connectivity of CH_2-CH_2-CH_2O- and OCH-CH-CH_2O-. From the above spectral data, **5** was suggested as the rhamnoside of benzofuran type neolignan. The linkage position of rhamnose to dihydrodehydroconiferyl alcohol was clarified as C-4′ by HMBC spectrum. The relative stereochemistry of C-7 aryl and C-8 hydroxymethyl substituents was proposed *trans* by comparison with previously reported ^{1}H NMR spectral data (Miyase *et al.*, 1988; Yoshinari *et al.*, 1989; Kouno *et al.*, 1991; Shen *et al.*, 1998). From all these data and HMBC correlations, **5** was identified as rel-*trans*-dihydrodehydroconiferyl alcohol 4′-*O*-α-L-rhamnopyranoside.

Compound **6** was obtained as colorless syrup, and its molecular formula was deduced as $C_{26}H_{36}O_{11}$ from FABMS (548 $[M+H+Na]^+$) and carbon counts in ^{13}C NMR spectrum. The presence of two benzene rings, two methoxyl groups and a glucose moiety was clarified by its ^{1}H- and ^{13}C-NMR spectra, and six extra aliphatic carbons were also suggested by the ^{13}C-NMR spectrum. These findings indicated that **6** is also a lignan. The ^{1}H-NMR spectrum of **6** indicated that one of the two benzen rings is 1,3,4-trisubstituted and the other is 1,3,4,5-tetrasubstituted, and the ^{1}H-^{1}H COSY spectrum suggested the connectivity of CH_2-CH-CH_2O- and CH_2-CH_2-CH_2O- moieties. The linkage position of glucose was elucidated by HMBC spectrum as C-4. All these data and the HMBC correlations demonstrated the structure as shown in Fig. 1. This compound was previously isolated from *Epimedium grandiflorum* and named icariside E_3 (Kouno *et al.*, 1991).

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