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Phenylpropanoid Glycosides of *Paulownia coreana* Uyeki Leaves*1

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ABSTRACT

The leaves of *Paulownia coreana* Uyeki were collected, extracted with acetone-H_2O (7:3, v/v), concentrated under reduced pressure and successively fractionated using *n*-hexane, methylene chloride, ethyl acetate and water on a separatory funnel. A portion of the ethyl acetate soluble powder was chromatographed on a Sephadex LH-20 column using aqueous methanol and ethanol-hexane as washing solvents. Two isomeric phenylpropanoid glycosides were isolated and elucidated as verbascoside and isoverbascoside by NMR and MS spectrometers.

Keywords : *paulownia coreana* uyeki, column chromatography, phenylpropanoid glycosides, verbascoside, isoverbascoside

1. INTRODUCTION

Paulownia coreana Uyeki (Scrophulariaceae) is one of useful medicinal hardwood species grown in Korea and has been widely used for construction, furniture making, musical instruments and handicrafts. It has been also used in medicines for a long time. Its leaves, seeds and stem have some pharmacological effectiveness on bronchitis, cough, phlegm, carbuncle and traumatic bleeding. In case of its bark also can relieve hemorrhoid, gonorrhea and erysipelas and the seed is effective on asthma and high blood pressure, especially. The flower is useful to relieve upper respiratory tract infection, bronchopneumonia, tonsillitis, bacteriologic diarrhea, enteritis, conjunctivitis, parotitis and swelling (Ji and Kim, 1996; Jiang, 2003; Jiangsu New Medical College, 1977). Several researchers have isolated iridoids (Damtoft and Jensen, 1993), sesquiterpenes, lactones and quinones (Oh *et al.*, 2000). We also have isolated some flavonoids and phenolic acids (Si *et al.*, 2005) in previous studies. However, it has never been reported on the detailed chemical constituents of the species. This study includes the isolation and structure elucidation work of isomeric phenylpropanoid glycosides from the ethyl acetate soluble fraction of the leaves.

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2. MATERIALS and METHODS

2.1. Plant Materials

The leaves of *P. coreana* were collected in the campus forest, Kangwon National University in September 2002, air-dried for 2 weeks at room temperature and the leaves were grounded to fine powder to be extracted.

2.2. Extraction and Fractionation

The ground leaves (5.02 kg) were extracted with acetone-water (7:3, v/v) three times at room temperature, then the solvent was decanted, filtered and evaporated. The combined extractives were successfully partitioned with *n*-hexane, methylene chloride (CH_2Cl_2) and ethyl acetate (EtOAc) using a separatory funnel. Each fraction was concentrated and freeze dried to get powder.

2.3. Column Chromatography

A column was packed with Sephadex LH-20 using methanol-water (5:1, 3:1, 2:1, 1:1, 1:3, 1:5, v/v) and ethanol (EtOH)-hexane (4:1, 3:1, 2:1, v/v) for elution. Eluents were collected using a Gilson FC 204 fraction collector. The columns were washed with acetone:water (1:1, v/v) when the eluents were colorless.

2.4. Thin Layer Chromatography

TLC was performed on 25 DC-Plastik-folien Cellulose F (Merk) plates and developed with *t*-butanol-acetic acid-water (3:1:1, v/v/v, TBA (solvent A)) or acetic acid-water (3:47, v/v, 6% HOAc (solvent B)). Visualization was done by illuminating ultraviolet light (UV, 254 and 365 nm), by spraying 1% $FeCl_3$, and by heating. Two dimensional TLC was also tried to verify the purification of the isolated compounds.

2.5. Instrumentation

To determine the structures of the isolated compounds, the peak of ^{1}H-NMR, ^{13}C-NMR and two dimensional NMR such as COSY, HMBC and DEPT were obtained using a Bruker A-vance DPX 400 MHz NMR spectrometer. For the determination of molecular weights of the isolated compounds, FAB-MS was performed using a Micromass Autospec M363 spectrometer at the Central Laboratory of Kangwon National University. CD_3OD was used as NMR solvent with TMS as an internal standard.

2.6. Isolation of Compounds

A portion of ethyl acetate soluble powder (10.7 g) was applied on a Sephadex LH-20 column using methanol-water (3:1, v/v) as an eluent and seven main fractions were collected and labeled as PCLE 1～7.

Fraction PCLE 3 was reapplied on a column for further purification with MeOH-H_2O (1:1, 1:2, 1:4, 1:6, 1:2, v/v) and EtOH-Hexane (2:1, 4:1, 3:1, 4:3, v/v) as eluting solvents to give verbascoside (Ⅰ) and isoverbascoside (Ⅱ).

2.6.1. Compound (Ⅰ) (Verbascoside)

R_f: 0.63 (solvent A) and 0.68 (solvent B).

FAB-MS: Calculated for $C_{29}H_{36}O_{15}$ 624, Found m/z $[M+H]^+$ 625.

^{1}H-NMR and ^{13}C-NMR: Table 1. HMBC correlations: H-2→C-3/C-4/C-6/C-7, H-5→C-1/C-3/C-4, H-6→C-2/C-4, H-7→C-1/C-6/C-2/C-8, H-8→C-7, H-1′→C-8, H-2′→C-1′/C-3′, H-3′→C-2′/C-4′, H-4′→C-3′/C-5′/C-6′, H-5′→C-4′, H-6′→C-4′/C-5′/C-8″/C-9″, H-2″→C-3″/C-4″/C-6″/C-7″, H-5″→C-1″/C-3″/C-4″, H-6″→C-2″/C-4″/ C-7″, H-7″→C-1″/C-2″/C-6″/C-9″, H-8″→C-1″/ C-9″, H-1‴→C-3′/C-3‴/C-5‴, H-2‴→C-3‴/C-4‴, H-3‴→4‴, H-4‴→C-3‴/C-5‴, H-5‴→C-4‴, H-6‴→C-4‴/C-5‴.

Table 1. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data of compounds I and II in CD_3OD

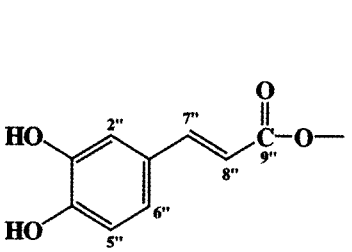
Position	δ_C		δ_H (multi. and J_{HH} (Hz))	
	I	II	I	II
Aglycone				
1	131.50	131.43	6.70 (*d*, 1.8)	6.68 (*d*, 1.9)
2	117.15	117.12		
3	146.15	146.15		
4	144.69	144.68		
5	116.35	116.40	6.68 (*d*, 8.2)	6.64 (*d*, 8.1)
6	121.31	121.31	6.56 (*dd*, 8.2, 1.8)	6.53 (*dd*, 8.1, 1.9)
7a,b	36.59	36.71	2.79 (*m*)	2.78 (*m*)
8a,b	72.29	72.38	3.72 (*m*), 4.04 (*m*)	3.72 (*m*), 3.96 (*m*)
Caffeyl				
1″	127.68	127.72		
2″	115.26	115.13	7.06 (*d*, 1.9)	7.04 (*d*, 2.0)
3″	146.85	146.80		
4″	149.82	149.65		
5″	116.56	116.58	6.78 (*d*, 8.2)	6.77 (*d*, 8.2)
6″	123.27	123.20	6.95 (*dd*, 8.2, 1.9)	6.68 (*dd*, 2.0, 8.2)
7″	148.06	147.29	7.59 (*d*, 15.8)	7.56 (*d*, 15.9)
8″	114.72	114.87	6.28 (d, 15.8)	6.29 (*d*, 15.9)
9″	168.34	169.18		
Glucose				
1′	104.22	104.41	4.38 (*d*, 7.8)	4.33 (*d*, 7.9)
2′	76.22	75.72	3.39 (*m*)	3.35 (*m*)
3′	81.70	83.99	3.81 (*m*)	3.53 (*m*)
4′	70.60	70.42	4.93 (*m*)	3.41 (*m*)
5′	76.04	75.43	3.54 (*m*)	3.55 (*m*)
6′a,b	62.38	64.66	3.52 (*m*), 3.62 (*m*)	4.35 (*m*), 4.50 (*m*)
Rhamnose				
1‴	103.07	102.74	5.19 (*d*, 1.4)	5.18 (*d*, 1.3)
2‴	72.37	72.45	3.92 (*m*)	3.94 (*m*)
3‴	72.07	70.29	3.59 (m)	3.69 (*m*)
4‴	73.81	74.03	3.29 (*m*),	3.39 (*m*)
5‴	70.45	70.07	3.57 (*m*)	4.00 (*m*)
6‴	18.49	17.91	1.09 (d, 6.0)	1.25 (d, 6.0)

2.6.2. Compound (II) (Isoverbascoside)

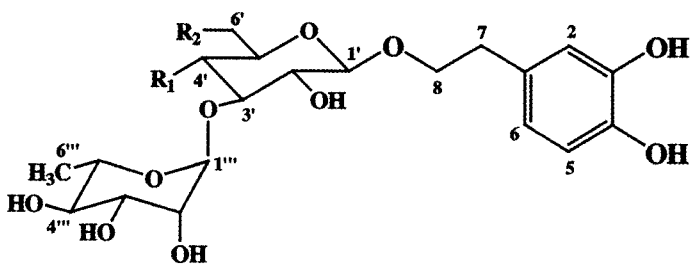
R_f: 0.43 (solvent A) and 0.39 (solvent B).

FAB-MS: Calculated for $C_{29}H_{36}O_{15}$ 624, Found m/z $[M+H]^+$ 625.

^{1}H-NMR and ^{13}C-NMR: Table 1. HMBC correlations: H-2→C-3/C-4/C-6/C-7, H-5→C-1/C-3/C-4, H-6→C-2/C-4, H-7→C-1/C-6/C-2/C-8, H-8→C-7, H-1′→C-8, H-2′→C-1′/C-3′, H-3′→C-5′/C-4′, H-4′→C-3′/C-5′/C-6′, H-5′→C-4′, H-6′→C-8″/C-9″, H-2″→C-3″/C-4″/C-6″/C-7″, H-5″→C-1″/C-3″/C-4″, H-6″→C-2″/C-4″/C-7″, H-7″→C-1″/C-2″/C-6″/C-9″, H-8″→C-1″/C-9″, H-1‴→C-3′/C-3‴/C-5‴, H-2‴→C-3‴/C-4‴, H-3‴→4‴, H-4‴→C-3‴/C-5‴, H-5‴→C-4‴, H-6‴→C-4‴/C-5‴.



(caffeyl)



R_1=caffeyl, R_2=OH : verbascoside (I)
R_1=OH, R_2=caffeyl : isoverbascoside (II)

Fig. 1. Phenylpropanoid glycosides from *P. coreana* Uyeki leaves.

3. RESULTS and DISCUSSION

Compound (I) was isolated as a yellowish powder and gave a dark brown spot on a cellulose plate when was visualized with 1% $FeCl_3$. R_f valuses were 0.63 (solvent A) and 0.68 (solvent B). The FAB-MS gave m/z 624 for $[M+H]^+$ ions, which was consistent with the molecular formula $C_{29}H_{36}O_{15}$. In the ^{1}H-NMR spectrum, the six aromatic protons exhibited two sets of ABX protons, one at δ 6.70 (*d*, *J*=1.8 Hz), δ 6.68 (*d*, *J*=8.2 Hz) and δ 6.56 (*dd*, *J*=8.2 Hz and 1.8 Hz) belongs to the aglycone of 3,4-dihydroxyphenylethyl skeleton for H-2, H-5 and H-6 respectively, and the other at δ 7.06 (*d*, *J*=1.9 Hz), δ 6.95 (*dd*, *J*=8.2 Hz and 1.9 Hz) and δ 6.78 (*d*, *J*=8.2 Hz) was to the caffeyl group for H-2″, H-6″ and H-5″ respectively. The signals at δ 7.59 (*d*, *J*=15.8 Hz) and δ 6.28 (*d*, *J*=15.8 Hz) indicated trans olefinic protons of AB type attributable to H-7″ and H-8″ of the caffeyl group (Ternai and Markham, 1976). A multiplet signal at δ 2.79 was assigned to the α-CH_2 (H-7a,b). However, the two protons of the β-CH_2 (H-8a,b) at δ 3.72 and δ 4.04 were non-equivalent. The anomeric proton at δ 4.38 originated from the glucose and its coupling constant (7.8 Hz) indicated the β-configuration (Kamerling, 1972). The α-linked rhamnose was characterized for the anomeric proton resonating at δ 5.19 with coupling constant of 1.4 Hz, and the methyl protons typically appeared at δ 1.01 (*J*=6.0 Hz) (Mabry, 1970).

The ^{13}C-NMR signals (Table 1) corroborated the presence of β-D-glucose, α-L-rhmnose, caffeyl and 3,4-dihydroxyphenylethyl moeity in compound (I). In the HMBC spectrum, the anomeric proton signals of glucose and rhamnose showed long range correlations with ^{13}C-NMR signals at 72.29 ppm (C-8) and 81.70 ppm (C-3′) demonstrating glucosylation at the C-8 of aglycone and rhamnosylation at C-3′ of glucose. ^{1}H signal at δ 3.18 (H-4′) correlating with carbon signal at 168.34 ppm (C-9″) indicated the linkage of caffeyl and glucose at C-9″ and C-4′.

The ^{13}C and DEPT (45, 90 and 135°) NMR showed the presence of 29 carbons including 7 quaternary carbons, 18 methine, 3 methylene and 1 methyl group.

According to the above data, compound (I) was elucidated as β-(3,4-dihydroxyphenyl)-ethyl-O-α-L-rhmnopyranosyl(1″→3′)-β-D-(4′-O-caffeyl)-glucopyranoside which is well coincided with the literature (Andary *et al.*, 1982; Imakura *et al.*, 1985; Jia *et al.*, 1991; Owen, *et al.*, 2003).

Compound (II) was also obtained as a yellowish powder which R_f values were 0.43 (solvent A) and 0.39 (solvent B), lower than those of verbascoside. The FAB-MS afforded

m/z 624 for $[M+H]^+$ ions, same as compounds (Ⅰ). The NMR data confirmed that compounds (Ⅱ) is identical to compounds (Ⅰ), besides the only difference that the caffeyl group was attached to glucose C-6′ instead of C-4′ (normal chemical shift for H-4′ and C-4′, downfield shifts of signals assigned to H-6′a,b and C-6′) which also could be confirmed by its HMBC spectrum.

Consequently, compound (Ⅱ) was determined as β-(3,4-dihydroxyphenyl)-ethyl-O-α-L-rhmnopyranosyl(1‴→3′)-β-D-(6′-O-caffeyl)-glucopyranoside in correspondence with an authentic sample reported by the other researchers (Liu and Jia, 1991; Jia *et al.*, 1992; Owen, *et al.*, 2003).

4. CONCLUSIONS

From the ethyl acetate soluble fraction of *P. coreana* leaves, two phenylpropanoid glycosides were isolated by column chromatography using Sephadex LH-20 and elucidated as verbascoside (Ⅰ) (902 mg) and isoverbascoside (Ⅱ) (321 mg) by ^{1}H and ^{13}C-NMR and FAB-MS spectroscopy. This is the first time isolation of phenylpropanoid glycosides from this species. Verbascoside was one of the main constituents of the ethyl acetate soluble fraction.

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