

# Phenylethanoid Glycosides from Seeds of *Paulownia coreana*\*<sup>1</sup>

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## ABSTRACT

Seeds of *Paulownia coreana* were collected, extracted with acetone-H<sub>2</sub>O (7 : 3, v/v), concentrated under reduced pressure and successively fractionated with *n*-hexane, methylene chloride, ethyl acetate and water on a separatory funnel. The H<sub>2</sub>O soluble fraction was chromatographed on a Sephadex LH-20 column using aqueous methanol and ethanol-hexane as washing solvents. Two isomeric phenylethanoid glycosides, verbascoside (1) and isoverbascoside (2), and one epimeric phenylethanoid glycoside, campneoside II (3), were isolated and their structures were elucidated on the basis of chemical and spectroscopical data.

*Keywords* : *Paulownia coreana*, seeds, water soluble fraction, phenylethanoid glycosides, epimeric isomer

## 1. INTRODUCTION

*Paulownia coreana* is a useful medicinal hardwood indigenous to Korea and has been used in traditional medicinal formulations for the treatment of symptoms such as bronchitis, cough, phlegm, hemorrhoid, asthma, high blood pressure and bacteriologic diarrhea (Jiangsu New Medical College, 1977; Kim, 1996). The H<sub>2</sub>O soluble fraction of the seeds, when applied daily to the scalp, was reported to promote healthy hair growth and to turn grey hair into black (Jiang, 2003). Its wood also is widely used in the wood industry for building construction, furniture making and musical instruments (Ayan *et al.*, 2003).

However, phenolic extractives of the seeds have not been comprehensively investigated, though the medicinal activities of the seeds are well known. Our previous study on the ethyl acetate soluble fraction of *P. coreana* seeds led to the isolation of 6 phenolic compounds: caffeic acid, naringenin, apigenin, luteolin, kaempferol and quercetin (Si, *et al.*, 2006).

Phenylethanoid glycosides such as verbascoside, isoverbascoside and campneoside I have been isolated in EtOH and MeOH extracts of *P. tomentosa* wood (Ota *et al.*, 1993). Campneoside I and II have been isolated in MeOH extracts of *P. tomentosa* wood (Jang, 1992).

In the present paper, we report the isolation and identification of three phenylethanoid gly-

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cosides from the aqueous soluble fraction of *P. coreana* seeds.

## 2. MATERIALS and METHODS

### 2.1. General

Melting points were determined on an Electro Thermal 9100 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. NMR spectra were recorded in CD<sub>3</sub>OD using a Bruker Avance DPX 400 spectrometer. Positive FAB-MS and MALDI-TOF-MS were done with a Micromass Autospec M363 spectrometer and a Model Voyager-DE STR mass spectrometer, respectively. TLC analysis were performed on 25 DC-Plastik-folien Cellulose F (Merk) plates and developed with *t*-BuOH-HOAc-H<sub>2</sub>O (3 : 1 : 1, v/v/v, solvent A) and HOAc-H<sub>2</sub>O (3 : 47, v/v, solvent B). Visualization was done by illuminating ultraviolet light (UV, 254 and 365 nm) and using spraying reagent of 1% ethanolic FeCl<sub>3</sub> followed by heating. Two dimensional TLC was also tried to verify the purification of the isolated compounds.

### 2.2. Extraction and Isolation

Seeds of *P. coreana* were collected from the experimental forest of Kangwon National University in October, 2002 and the air-dried and ground seeds (5 kg) were extracted in acetone-H<sub>2</sub>O (7 : 3, v/v) for more than one week at room temperature. After filtration and concentration under reduced pressure, the aqueous residue was sequentially fractionated with *n*-hexane, methylene chloride, ethyl acetate and water. Each fraction was then freeze-dried to a brown powder. A portion of the resulting H<sub>2</sub>O soluble fraction (38 g) was applied to a column on Sephadex LH-20 and eluted with MeOH-H<sub>2</sub>O

(3 : 1, v/v) to get four fractions and labeled PCFW-1 (2.4 g), PCFW-2 (33.6 g), PCFW-3 (1.8 g) and PCFW-4 (149 mg). PCFW-4 was identified as compound **2** (149 mg) and PCFW-3 was further chromatographed using aqueous MeOH (1 : 1, 1 : 4, v/v) to give compound **1** (280 mg), compound **2** (142 mg) and compound **3** (35 mg).

#### 2.2.1. Compound 1

Yellow amorphous powder;  $[\alpha]_D^{20} -71.8^\circ$  (*c*, 0.0025 in MeOH); *R<sub>f</sub>*: 0.63 (solvent A) and 0.68 (solvent B); Melting point: 135~136°C; Positive FAB-MS:  $[M+H]^+$  at *m/z* 625,  $[M+Na]^+$  at *m/z* 647; <sup>1</sup>H and <sup>13</sup>C-NMR: See Table 1 and 2.

#### 2.2.2. Compound 2

Yellow amorphous powder;  $[\alpha]_D^{20} -32.5^\circ$  (*c*, 0.0025 in MeOH); *R<sub>f</sub>*: 0.43 (solvent A) and 0.39 (solvent B); Melting point: 134~135°C; Positive FAB-MS:  $[M+H]^+$  at *m/z* 625,  $[M+Na]^+$  at *m/z* 647; <sup>1</sup>H and <sup>13</sup>C-NMR: See Table 1 and 2.

#### 2.2.3. Compound 3

Yellow amorphous powder;  $[\alpha]_D^{20} -61.6^\circ$  (*c*, 0.0025 in MeOH); *R<sub>f</sub>*: 0.72 (solvent A) and 0.69 (solvent B); Melting point: 157~159°C; MALDI-TOF-MS:  $[M+Na]^+$  at *m/z* 663,  $[M+K]^+$  at *m/z* 679,  $[2M+Na]^+$  at *m/z* 1303,  $[2M+K]^+$  at *m/z* 1319; <sup>1</sup>H and <sup>13</sup>C-NMR: See Table 1 and 2.

## 3. RESULTS and DISCUSSION

Two isomeric phenylethanoid glycosides, verbascoside (**1**) and isoverbascoside (**2**), as shown in Fig. 1, were isolated as yellowish powder which showed physical and spectral data identical to those which we previously reported (Si *et al.*, 2006).

Table 1. <sup>1</sup>H-NMR chemical shifts of compounds (1)~(3) in CD<sub>3</sub>OD

Position	1	2	3
Aglycone			
2	6.70 ( <i>d</i> , 1.8)	6.68 ( <i>d</i> , 1.9)	6.85 ( <i>d</i> , 2.0)
5	6.68 ( <i>d</i> , 8.2)	6.64 ( <i>d</i> , 8.1)	6.77 ( <i>d</i> , 8.1)
6	6.56 ( <i>dd</i> , 8.2&1.8)	6.53 ( <i>dd</i> , 8.1&1.9)	6.56 ( <i>dd</i> , 8.1&2.0)
7	2.79 ( <i>m</i> )	2.78 ( <i>m</i> )	4.75 ( <i>m</i> )
8	3.72( <i>m</i> ), 4.04 ( <i>m</i> )	3.72( <i>m</i> ), 3.96 ( <i>m</i> )	3.95 ( <i>m</i> ), 3.60 ( <i>m</i> ) / 3.83 ( <i>m</i> ), 3.71 ( <i>m</i> )
Caffeoyl			
2''	7.06 ( <i>d</i> , 1.9)	7.04 ( <i>d</i> , 2.0)	7.06 ( <i>d</i> , 1.2)
5''	6.78 ( <i>d</i> , 8.2)	6.77 ( <i>d</i> , 8.2)	6.80 ( <i>d</i> , 8.3)
6''	6.95 ( <i>dd</i> , 8.2&1.9)	6.68 ( <i>dd</i> , 2.0&8.2)	6.96 ( <i>dd</i> , 8.3&1.2)
7''	7.59 ( <i>d</i> , 15.8)	7.56 ( <i>d</i> , 15.9)	7.60 ( <i>d</i> , 15.8)
8''	6.28 ( <i>d</i> , 15.8)	6.29 ( <i>d</i> , 15.9)	6.28 ( <i>d</i> , 15.8)
Glucose			
1'	4.38 ( <i>d</i> , 7.8)	4.33 ( <i>d</i> , 7.9)	4.43 ( <i>d</i> , 7.8)/4.41 ( <i>d</i> , 7.8)
2'	3.39 ( <i>m</i> )	3.35 ( <i>m</i> )	3.42 ( <i>m</i> )
3'	3.81 ( <i>m</i> )	3.53 ( <i>m</i> )	3.82 ( <i>m</i> )
4'	4.93 ( <i>m</i> )	3.41 ( <i>m</i> )	4.92 ( <i>m</i> )
5'	3.54 ( <i>m</i> )	3.55 ( <i>m</i> )	3.54 ( <i>m</i> )
6'	3.52 ( <i>m</i> ), 3.62 ( <i>m</i> )	4.35 ( <i>m</i> ), 4.50 ( <i>m</i> )	3.52 ( <i>m</i> ), 3.61 ( <i>m</i> )
Rhamnose			
1'''	5.19 ( <i>d</i> , 1.4)	5.18 ( <i>d</i> , 1.3)	5.22 ( <i>s</i> )/5.20 ( <i>s</i> )
2'''	3.92 ( <i>m</i> )	3.94 ( <i>m</i> )	3.96 ( <i>m</i> )
3'''	3.59 ( <i>m</i> )	3.69 ( <i>m</i> )	3.60 ( <i>m</i> )
4'''	3.29 ( <i>m</i> )	3.39 ( <i>m</i> )	3.31 ( <i>m</i> )
5'''	3.57 ( <i>m</i> )	4.00 ( <i>m</i> )	3.56 ( <i>m</i> )
6'''	1.09 ( <i>d</i> , 6.0)	1.25 ( <i>d</i> , 6.0)	1.07 ( <i>d</i> , 6.0)

Compound **3** was isolated as a yellowish amorphous powder and gave a dark brown color on a TLC plate with ethanolic FeCl<sub>3</sub>, suggesting the presence of phenolic hydroxyl group in the molecule (Imakura *et al.*, 1985). R<sub>f</sub> values were 0.72 (solvent A) and 0.69 (solvent B).

The MALDI-TOF mass spectrum of compound **3** gave *m/z* 663 for [M+Na]<sup>+</sup> ions, *m/z* 679 for [M+K]<sup>+</sup> ions, *m/z* 1303 for [2M+Na]<sup>+</sup> ions and *m/z* 1319 for [2M+K]<sup>+</sup> ions which were consistent with the molecular weight 640 and molecular formula C<sub>29</sub>H<sub>36</sub>O<sub>16</sub>.

The <sup>1</sup>H-NMR spectrum of compound **3** showed the presence of two overlapping secondary methyl groups of rhamnose [δ 1.07 (6H, *d*, *J*=6.0 Hz)], anomeric protons of β-D-glucose

moiety [δ 4.43 (1H, *d*, *J*=7.8 Hz), δ 4.41 (1H, *d*, *J*=7.8 Hz)], anomeric protons of α-L-rhamnose moiety [δ 5.22 (1H, *s*), δ 5.20 (1H, *s*)], one pair of overlapped *trans* olefinic protons as AB system signals [δ 7.60 (2H, *d*, *J*=15.8 Hz), δ 6.28 (2H, *d*, *J*=15.8 Hz)], two pairs of overlapped ABX type proton signals [δ 6.85 (2H, *d*, *J*=2.0 Hz), δ 6.56 (2H, *dd*, *J*=8.1 and 2.0 Hz), δ 6.77 (2H, *d*, *J*=8.1 Hz)] and another two pairs of overlapped ABX system proton peaks [δ 7.06 (2H, *d*, *J*=1.2 Hz), δ 6.96 (2H, *dd*, *J*=8.3 and 1.2 Hz), δ 6.80 (2H, *d*, *J*=8.3 Hz)]. The above spectroscopic data suggested that compound **3** might be a pair of epimers of a phenylethanoid disaccharide glycoside.

Comparison of <sup>13</sup>C-NMR spectrum of com-

Phenylethanoid Glycosides from Seeds of *Paulownia coreana*Table 2.  $^{13}\text{C}$ -NMR chemical shifts of compounds (1)~(3) in  $\text{CD}_3\text{OD}$ 

Position	1	2	3
Aglycone			
1	131.50	131.43	133.61/133.87
2	117.15	117.12	114.65/114.67
3	146.15	146.15	146.12/146.03
4	144.69	144.68	146.31/146.26
5	116.35	116.40	116.21/116.17
6	121.31	121.31	119.00/119.11
7	36.59	36.71	73.57/74.21
8	72.29	72.38	76.18/76.69
Caffeoyl			
1''	127.68	127.72	127.66
2''	115.26	115.13	115.24
3''	146.85	146.80	146.85
4''	149.82	149.65	149.82
5''	116.56	116.58	116.54
6''	123.27	123.20	123.28
7''	148.06	147.29	148.08
8''	114.72	114.87	114.79
9''	168.34	169.18	168.30/168.29
Glucose			
1'	104.22	104.41	104.60/104.06
2'	76.22	75.72	76.12/76.43
3'	81.70	83.69	81.31/81.43
4'	70.60	70.42	70.45
5'	76.04	75.43	76.07/76.09
6'	62.38	64.66	62.32
Rhamnose			
1'''	103.07	102.74	102.94/103.00
2'''	72.37	72.45	72.37
3'''	72.07	70.29	72.06
4'''	73.81	74.03	73.79
5'''	70.45	70.07	70.38
6'''	18.49	17.91	18.48

pound **3** with those of verbascoside (compound **1**) revealed the downfield shifts of C-7 (approximately -37 ppm) and C-8 (approximately -4 ppm), indicating that the substituent was attached to C-7.

The DEPT spectrum indicated 29 carbons, including 7 quaternary, 19 methines, 2 methylenes and 1 methyl carbons in each epimeric isomer, and corroborated C-7 was a methine carbon.

Long range H→C couplings (HMBC, Fig. 2)

were observed between H-1' and C-8, H-4' and C-9'', as well as H-1''' and C-3' in each epimeric moiety, which substantiated attachment positions of compound **3**.

Further physical and spectral comparison with published literature (Kitagawa *et al.*, 1984; Imakura *et al.*, 1985; Miyase *et al.*, 1996; Han *et al.*, 2004; Wu *et al.*, 2004) indicated compound **3** is *R,S*-7-(3',4'-dihydroxyphenyl)-ethyl-*O*- $\alpha$ -L-rhamnopyranosyl(1→3)- $\beta$ -D-(4-*O*-caf-

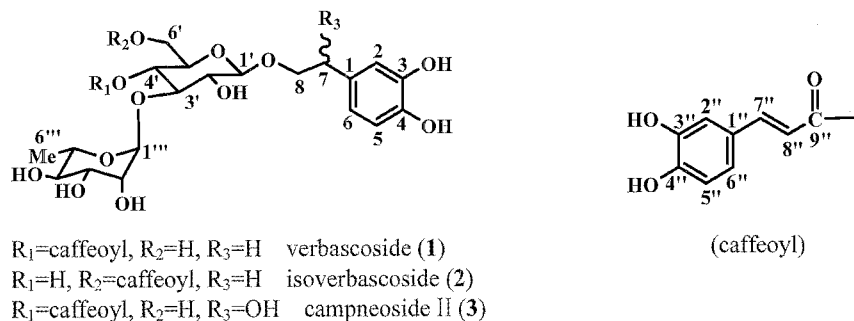


Fig. 1. Phenylethanoid glycosides identified from the seeds of *P. coreana*.

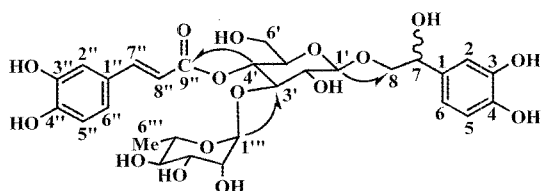


Fig. 2. Selected HMBC correlations observed in compound 3.

feoyl)-glucopyranoside, with trivial name campneoside II. The relative ratio of the epimers was deduced to be *ca* 4 : 3. This is the first report of its occurrence in this species although it has been previously isolated from *P. tomentosa* (Jang, 1992).

## 4. CONCLUSIONS

From the aqueous soluble fraction of *P. coreana* seeds, two isomeric and one epimeric phenylethanoid glycosides were isolated by column chromatography using Sephadex LH-20 and elucidated as verbascoside (1) (280 mg), isoverbascoside (2) (291 mg) and campneoside II (3) (35 mg) by 1D, 2D-NMR, positive FAB mass and MALDI-TOF mass spectroscopy.

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