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Phenolic Compounds from the Inner Bark of *Paulownia coreana*[*1]

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ABSTRACT

Paulownia coreana inner bark was collected, extracted in 70% acetone, concentrated under reduced pressure and sequentially fractionated using *n*-hexane, CH_2Cl_2, EtOAc and H_2O, then freeze dried to give brown powders. A portion of the EtOAc soluble powder was chromatographed on a Sephadex LH-20 column using a serious of aqueous methanol and ethanol-hexane mixture as eluting solvents. Two phenolic acid, *p*-courmaric acid and caffeic acid, two isomeric phenylethanoid glycosides, verbascoside and isoverbascoside, and one epimeric phenylpropanoid glycoside, cistanoside F, were isolated and their structures were elucidated by spectroscopic analysis such as NMR and MS.

Keywords : *Paulownia coreana*, inner bark, EtOAc soluble fraction, phenylpropanoid glycosides, epimer

1. INTRODUCTION

Paulownia coreana Uyeki (Scrophyklariaceae) is a deciduous tree indigenous to the Korean peninsula, especially in Ullungdo of South Korea and has been cultivated together with *P. tomentosa* throughout the sightseeing places and villages as a bee tree or ornament species. Its wood is widely used as a material for construction, furniture, musical instrument or handicrafts (Kim, 1996; Ayan *et al.*, 2003). The tree has also been used as a traditional medicinal plant or a long time to treat symptoms such as bronchitis, cough, phlegm, hemorrhoid, asthma, high blood pressure and bacteriologic diarrhea (Jiangsu New Medical College, 1977).

We have previously investigated the chemical composition of *P. coreana* leaves (Si *et al.*, 2005; Si *et al.*, 2006a) and seeds (Si *et al.*, 2006b). In this paper, we report the isolation procedures and structure determination of phenolic compounds from the inner bark of *P. coreana*.

2. MATERIALS and METHODS

2.1. Plant Materials

Paulownia coreana tree samples were obtained in the campus forest of Kangwon Nation-

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al University, Korea in September 2002 and the bark was stripped from the freshly fallen tree to separate into outer and inner bark.

2.2. Extraction and Fractionation

The inner bark (5 kg) was air-dried, ground and extracted in acetone-H_2O (7:3, v/v) for several days at ambient temperature. The extract was decanted, filtered and evaporated under the reduced vacuum to give an aqueous residue which was then freeze-dried to a brown powder. The powder was successfully partitioned with *n*-hexane, CH_2Cl_2 and EtOAc in a separatory funnel to give 24 g of EtOAc soluble material.

2.3. Column Chromatography

An open column was packed with Sephadex LH-20 using MeOH-H_2O (3:1, 1:1, 1:3, v/v) and EtOH-hexane (4:1, 3:1, 2:1, v/v) mixture. Eluents were collected using a Gilson FC 204 fraction collector. The columns were washed with acetone-H_2O (1:1, v/v) when the eluents were almost colorless.

2.4. Thin Layer Chromatography

TLC analysis were performed on 25 DC-Plastik-folien Cellulose F (Merk) plates and developed with *t*-BuOH-HOAc-H_2O (3:1:1, v/v/v, (solvent A)) or HOAc-H_2O (3:47, v/v, (solvent B)). Visualization was done by illuminating ultraviolet light (254 and 365 nm) or by spraying 1% ethanolic $FeCl_3$ solution followed by heating.

2.5. Instrumentation

The ^1H NMR, ^{13}C NMR, DEPT and correlation NMR spectra such as HMBC, HMQC and TOCSY were recorded in CD_3OD with TMS as an internal standard using a Bruker Avance DPX 400 spectrometer at the operating frequency of 400 MHz (^1H) and 100 MHz (^{13}C). EI and positive FAB mass spectroscopy were performed with a Micromass Autospec M363 Spectrometer and MALDI-TOF mass spectroscopy were done on a Model Voyager-DE STR spectrometer. Melting points were determined on an Electro Thermal 9100 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter in MeOH.

2.6. Isolation of the Compounds

A portion of EtOAc soluble powder (20.5 g) was chromatographed on a Sephadex LH-20 eluting with MeOH-H_2O (3:1, v/v) to give four main fractions and labeled as E-1 (187 mg), E-2 (19.2 g), E-3 (142 mg) and E-4 (437 mg), respectively. Fraction E-3 was elucidated as compound II (142 mg). Fraction E-1 was re-treated on a column for further purification with MeOH-H_2O (1:1, v/v) to isolate 26 mg of compound I. Fraction E-2 was also applied on a column with MeOH-H_2O (1:1, 1:3, v/v) and EtOH-hexane (4:1, 3:1, 2:1, v/v) to give compound III (85 mg), compound IV (1.2 g) and compound V (45 mg).

2.6.1. Compound I

Yellow amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f : 0.88 (solvent A) and 0.41 (solvent B); EI MS: Found $[M]^+$ at *m/z* 164, $[M\text{-}OH]^+$ at *m/z* 147 and $[M\text{-}COOH]^+$ at *m/z* 119.

^1H NMR (400 MHz, δ, CD_3OD): 6.23 (1 H, *d*, J=16.0 Hz, H-8), 6.81 (2 H, *d*, J=8.6 Hz, H-3,5), 7.45 (2 H, *d*, J=8.6 Hz, H-2,6), 7.61 (1 H, *d*, J=16.0 Hz, H-7).

^{13}C NMR (100 MHz, δ, CD_3OD): 115.55 (C-8), 116.78 (C-3,5), 127.20 (C-1), 131.07 (C-2,6), 146.66 (C-7), 161.11 (C-4), 171.03 (C-9).

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

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

2.6.2. Compound II

Yellow amorphous powder; $FeCl_3$ test: Positive (dark brown); R_f : 0.75 (solvent A) and 0.31 (solvent B); EI MS: Found $[M]^+$ at *m/z* 180, $[M\text{-}OH]^+$ at *m/z* 163 and $[M\text{-}COOH]^+$ at *m/z* 135.

^{1}H NMR (400 MHz, δ, CD_3OD): 6.22 (1H, *d*, J=15.9 Hz, H-8), 6.78 (1H, *d*, J=8.2 Hz, H-5), 6.94 (1H, *dd*, J=2.0 Hz and J=8.2 Hz, H-6), 7.04 (1H, *d*, J=2.0 Hz, H-2), 7.53 (1H, *d*, J=15.9 Hz, H-7).

^{13}C NMR (100 MHz, δ, CD_3OD): 115.10 (C-8), 115.65 (C-5), 116.52 (C-2), 122.89 (C-6), 127.84 (C-1), 146.82 (C-3), 147.01 (C-7), 149.47 (C-4), 171.17 (C-9).

2.6.3. Compound III

Yellow amorphous powder; $[\alpha]_D^{20}$ −71.8° (*c*, 0.0025 in MeOH); $FeCl_3$ test: Positive (dark brown); R_f: 0.63 (solvent A) and 0.68 (solvent B); Melting point: 135∼136°C; Positive FAB MS: $[M+H]^+$ at *m/z* 625 and $[M+Na]^+$ at *m/z* 647; ^{1}H and ^{13}C NMR: See Table 1.

Table 2. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound V in CD_3OD

Position		δ_C		δ_H (multi. and J_{HH} (Hz))	
		α-form	β-form	α-form	β-form
Caffeoyl	1	126.65	126.69		
	2	114.21	114.21	7.07 (1 H, *d*, 2.0)	7.06 (1 H, *d*, 2.0)
	3	145.82	145.82		
	4	148.74	148.78		
	5	115.51	115.51	6.79 (1 H, *d*, 8.1)	6.79 (1 H, *d*, 8.1)
	6	122.24	122.24	6.97 (1 H, *dd*, 8.1&2.0)	6.96 (1 H, *dd*, 2.0&8.1)
	7	146.90	147.01	7.60 (1 H, *d*, 15.9)	7.60 (1 H, *d*, 15.9)
	8	113.70	113.85	6.29 (1 H, *d*, 15.9)	6.28 (1 H, *d*, 15.9)
	9	167.42	167.33		
Glucose	1′	93.04	97.16	5.13 (1 H, *d*, 3.6)	4.56 (1 H, *d*, 7.9)
	2′	75.22	76.38	3.56 (1 H, *m*)	3.38 (1 H, *m*)
	3′	78.21	80.73	4.06 (1 H, *m*)	3.81 (1 H, *m*)
	4′	69.70	69.80	4.94 (1 H, *m*)	4.93 (1 H, *m*)
	5′	70.23	73.71	4.02 (1 H, *m*)	3.58 (1 H, *m*)
	6′a,b	61.37	61.50	3.18 (1 H, *m*), 3.49 (1 H, *m*)	3.18 (1 H, *m*), 3.49 (1 H, *m*)
Rhamnose	1″	102.03	102.12	5.20 (1 H, *d*, 1.6)	5.15 (1 H, *d*, 1.6)
	2″	71.32	71.32	3.94 (1 H, *m*)	3.94 (1 H, *m*)
	3″	71.06	71.06	3.61 (1 H, *m*)	3.61 (1 H, *m*)
	4″	72.78	72.82	3.32 (1 H, *m*)	3.31 (1 H, *m*)
	5″	69.38	69.70	3.58 (1 H, *m*))	3.57 (1 H, *m*)
	6″	17.48	17.48	1.10 (3 H, *d*, 6.0)	1.10 (3 H, *d*, 6.0)

2.6.4. Compound IV

Yellow amorphous powder; $[\alpha]_D^{20} -32.5^{\circ}$ (*c*, 0.0025 in MeOH); $FeCl_3$ test: Positive (dark brown); R_f: 0.43 (solvent A) and 0.39 (solvent B); Melting point: 134~135°C; Positive FAB MS: $[M+H]^+$ at *m/z* 625 and $[M+Na]^+$ at *m/z* 647; ^{1}H and ^{13}C NMR: See Table 1.

2.6.5. Compound V

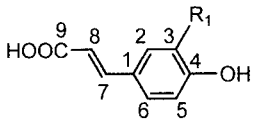
Yellow amorphous powder; $[\alpha]_D^{20} -79.6^{\circ}$ (*c*, 0.0025 in MeOH); $FeCl_3$ test: Positive (dark brown); R_f: 0.60 (solvent A) and 0.73 (solvent B); Melting point: 129~130°C; Positive FAB MS: $[M+H]^+$ at *m/z* 489 and $[M+Na]^+$ at *m/z* 511; MALDI-TOF-MS: $[M+Na]^+$ at *m/z* 511, $[M+K]^+$ at *m/z* 527, $[2M+Na]^+$ at *m/z* 999 and $[2M+K]^+$ at *m/z* 1015; ^{1}H and ^{13}C NMR: See Table 2.

3. RESULTS and DISCUSSION

Two phenolic acids, *p*-coumaric acid (I) and caffeic acid (II) and two isomeric phenylethanoid glycosides, verbascoside (III) and isoverbascoside (IV), as shown in Scheme 1, were isolated as yellowish powders which showed physical and spectral data virtually identical to those we had reported before (Si *et al.*, 2005; Si *et al.*, 2006a; Si *et al.*, 2006b).

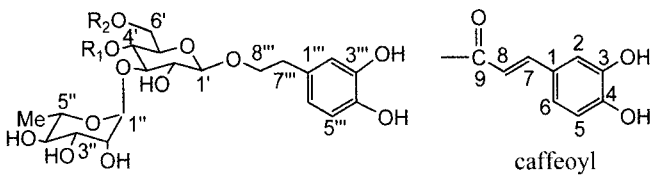
Compound V was isolated as a yellowish amorphous powder and exhibited dark brown color on a TLC plate when spread with 1% $FeCl_3$, suggesting the presence of phenolic hydroxyl groups in the molecule (Imakura *et al.*, 1985). Its R_f values were 0.60 and 0.73 when it was treated with solvent A and B, respectively.

The ^{1}H MNR spectrum of compound V



R_1=H : *p*-courmaric acid (**I**)

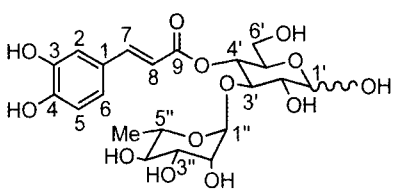
R_1=OH : caffeic acid (II)



caffeoyl

R_1=caffeoyl, R_2=H : verbascoside (III)

R_1=H, R_2=caffeoyl : isoverbascoside (IV)



cistanoside F (V)

Scheme 1. Structures of phenolic compounds of *P. coreana* inner bark.

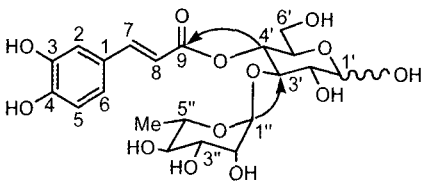
showed the presence of two β-D-glucose anomeric protons [δ 5.13 (1 H, *d*, J=3.6 Hz), δ 4.56 (1 H, *d*, J=7.9 Hz)], two α-L-rhamnose anomeric protons [δ 5.20 (1 H, *d*, J=1.5 Hz), δ 5.15 (1 H, *d*, J=1.6 Hz)], two overlapping secondary methyl groups of rhamnose [δ 1.10 (6 H, *d*, J=6.1 Hz)], one pair of overlapped *trans* olefinic protons as AB system signals [δ 7.60 (2 H, *d*, J=15.9 Hz), δ 6.29 (2 H, *d*, J=15.9 Hz)] and two pairs of overlapped ABX type proton signals [δ 7.07 (2 H, *d*, J=2.0 Hz), δ 6.79 (2 H, *dd*, J=8.1 and 2.0 Hz), δ 6.97 (2 H, *d*, J=8.1 Hz)]. The above spectroscopic data suggested that compound V is an epimer of a phenylpropanoid disaccharide glycoside. In addition, one doublet at δ 5.13 (1 H, *d*, J=3.6 Hz, H-1′, α-form) was assigned to the H-1′ and from the coupling constant J=3.6 Hz confirmed to be α-form glucose proton by the coupling constant J=3.6 Hz, while the β-form glucose proton gave a signal at δ 4.56 (1 H, *d*, J=7.9 Hz, H-1′, β-form).

In the ^{13}C-NMR spectrum of compound V, the peaks resonated at 94.04 ppm and 98.16 ppm were characterized for C-1′ on α and β-form glucose residue, respectively (Harbone and Mabry, 1982).

In the HMBC spectrum, correlations were observed between H-1″ and C-3′, together with H-4′ and C-9 in each diastereomer, which confirmed the proper structure linkage, as shown in Scheme 2.

The DEPT (45, 90 and 135°) spectrum suggested 21 carbons, including 4 quaternary carbons, 15 methines, 1 methylene and 1 methyl group in each epimeric of compound V.

Compound V existed in solution as an epimer at C-1′ of the glucose (α- and β-form, relative



Scheme 2. HMBC correlations observed in compound V.

ratio calculated 7:9 owing to integration of anomeric proton H-1′ of glucose) moiety and the duplicated signals due to the epimeric glucose was observed in the ^{1}H and ^{13}C-NMR spectra. Owing to the unequal intensities of α and β-form, together with the aid of 2D-NMR spectrum such as HMBC and TOCSY, other signals arising from each anomer could be completely and unambiguous allocated, as shown in Table 2.

The positive FAB-MS of compound V indicated $[M+H]^+$ ions at *m/z* 489, $[M+Na]^+$ ions at *m/z* 511, and the MALDI-TOF-MS gave *m/z* 511 for $[M+Na]^+$ ions, *m/z* 527 for $[M+K]^+$ ions, *m/z* 999 for $[2M+Na]^+$ ions and *m/z* 1015 for $[2M+K]^+$ ions, which were consistent with the molecular weight 488 and molecular formula $C_{21}H_{28}O_{13}$.

On the basis of the above evidences and the comparison of previously reported data (Kobayashi *et al.*, 1985; Otsuka *et al.*, 1992; Nishibe *et al.*, 1993; Li *et al.*, 2000; Han *et al.*, 2004; Wu *et al.*, 2004), compound V was determined as α-L-rhamnopyranosyl(1→3)-*O*-(4-*O*-caffeoyl)-D-glucopyranoside, a phenylpropanoid glycoside with trivial a name, cistanoside F. and this is the first time report in *Paulownia* species.

4. CONCLUSIONS

Two phenolic acids, ρ-courmaric acid and caffeic acid, two isomeric phenylpropanoid glycosides, verbascoside and isoverbascoside, and one epimeric phenylpropanoid glycoside, cistanoside F were purified from the EtOAc fraction of *P. coreana* inner bark by column chromatography using Sephadex LH-20 and the chemical structures were elucidated by NMR and MS spectroscopy. To our knowledge, this is the first time isolation of an epimeric phenylpropanoid glycoside, cistanoside F, from *Paulownia* species.

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