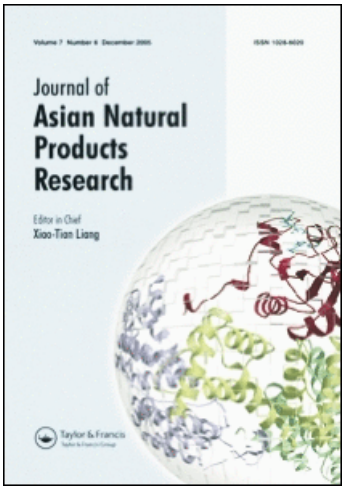

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Studies on the phenylethanoid glycosides with anti-complement activity from *Paulownia tomentosa* var. *tomentosa* wood

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Four epimeric phenylethanoid glycosides, including a new one, *R*,*S*-β-ethoxy-β-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl(1 → 3)-β-D-(6-*O*-*E*-caffeoyl)-glucopyranoside named isoilicifolioside A (**1**), and three known compounds, ilicifolioside A (**2**), campneoside II (**3**), and isocampneoside II (**4**), were isolated from *Paulownia tomentosa* var. *tomentosa* wood. The structures of the four compounds were elucidated by the interpretation of 1D and 2D NMR and MS spectra. This is the first report of the chemical profile of this tree. Compounds **1**–**4** exhibited excellent anti-complement activity with IC_{50} values less than 74 μM, compared with tiliroside (IC_{50} = 104 μM) and rosmarinic acid (IC_{50} = 182 μM) that were used as positive controls.

Keywords: *Paulownia tomentosa* var. *tomentosa*; epimeric phenylethanoid glycoside; isoilicifolioside A; anti-complementary activity

1. Introduction

The human complement system plays an important role in the host defense system against foreign invasive organisms such as viruses, bacteria, and fungi, as well as an external wound. Its effects are normally beneficial to the host, but it can also cause adverse effects depending on the site, extent, and duration of complement activation [1]. The activation of the system may lead to pathologic reactions in a variety of inflammatory and degenerative diseases such as multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, dermatological disease, rheumatoid arthritis, and gout. Therefore, the modulation of complement activity is important and there is a need to develop anti-complementary agents from various sources such as plants [2,3].

Paulownia tomentosa var. *tomentosa* (Scrophulariaceae), a deciduous tree indigenous to China, is widely distributed in eastern Asia and used in traditional medicine to treat cough, bronchitis, phlegm, haemorrhoid, asthma, high blood pressure, and bacterial diarrhea [4–6]. A phytochemical investigation on other species of *Paulownia* led to the isolation of *C*-geranyl compounds, phenylethanoid glycosides, lapachol-type naphthoquinones, iridoids, and lignans [7–15]. Nevertheless, no information about chemical composition and bioactivity of *P. tomentosa* var. *tomentosa* has been reported. Thus, as a part of a project aimed at discovering bioactive

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and structurally novel compounds from natural sources, the secondary metabolites of *P. tomentosa* var. *tomentosa* wood and their anti-complement activity were studied.

2. Results and discussion

Isoilicifolioside A (**1**) was obtained as a pale yellow amorphous powder. 2D TLC and HPLC data of **1** identified it to be a single compound. The MALDI-TOFMS displayed a pseudo-molecular ion peak at *m/z* 691.2205 $[M + Na]^+$, indicating the molecular formula of $C_{31}H_{40}O_{16}$. The presence of phenolic hydroxyl group in **1** was recognized from grey–green color with 1% ethanolic $FeCl_3$ solution on TLC [16]. Its FTIR absorption revealed the presence of hydroxyl group (3400 cm^{-1}), conjugated ester (1705 cm^{-1}), carbonyl functionality (1695 cm^{-1}), and aromatic ring (1620 and 1605 cm^{-1}). The ^{1}H NMR spectrum of **1** presented two characteristic anomeric protons of β-glucose at δ_H 4.34 and 4.40 (1H each, d, $J = 8.1$ Hz), two anomeric protons of α-rhamnose at δ_H 5.15 and 5.18 (1H each, d, $J = 1.5$ Hz), and two overlapping secondary methyl groups of α-rhamnose at δ_H 1.24 (6H, d, $J = 6.2$ Hz) [17]. Multiplets irritating between δ_H 3.35 and 4.45 were attributable to rest protons of sugar moieties. The aromatic aglycone residue showed two pairs of partially overlapped ABX proton signals centered between δ_H 6.62 and 6.78. The overlapping signals (δ_H 3.44/3.43 as multiplets and δ_H 1.19/1.18 as triplets with coupling constants of $J = 7.0$ Hz) were ascribable to ethoxyl functionalities connecting to β(7). A pair of overlapping *trans*-olefin protons at δ_H 6.28 and 7.63 as doublets with a distinctive coupling constant of $J = 16.0$ Hz, together with two pairs of overlapped ABX system proton signals for the catechol ring between δ_H 6.73 and 7.02, corresponded to a pair of overlapped *E*-caffeoyl moieties. A combination of the above spectroscopic data and further column chromatography separation, methylation, and HPLC analysis (two adjacent peaks in HPLC) indicated that compound **1** existed as epimers, though it only gave one spot on TLC [18]. In addition, these ^{1}H NMR spectral evidences were almost the same as those of *R*,*S*-β-ethoxy-β-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl(1 → 3)-β-D-(4-*O*-*E*-caffeoyl)-glucopyranoside (ilicifolioside A, **2**, Table 1) except for the upfield shift at H-4 (δ_H 3.42) and downfield shift at H-6 (δ_H 4.36 and 4.45) of glucose, which indicated that the *trans*-caffeoyl group was attached to C-6 (δ_C 64.7) of glucose [19]. The TOCSY experiment clearly distinguished the protons of one epimer from those protons belonging to the other epimer [8]. The ^{1}H NMR spectral data of **1** were identical with those for **4** (isocampneoside II), with the only difference that the H-β(7) in aglycone of epimeric compound **1** was slightly upfielded to δ_H 4.45/4.44 by about 0.3 ppm because of the neighboring ethyl group [8]. The $[\alpha]_D^{20}$ of compound **1** was −63 (*c* 0.4, MeOH), while $[\alpha]_D^{20}$ −39.4 (*c* 0.025, MeOH) was reported for **4** [8]. In the ^{13}C NMR spectrum, the duplicated signals of **1** also resemble those of **2** except for the downfield chemical shifts at C-3 (δ_C 84.2 and 84.4) and C-6 (δ_C 64.7) of glucose by 2.4–2.9 ppm. These evidences substantially concluded that the *E*-caffeoyl unit was connected to glucose C-6 [20,21].

The HMBC experiment was performed to establish the location of the functional groups and the full structure of **1**. The caffeoyl unit was located at glucose C-6 due to the long-range correlation between glucose H-6 and the quaternary carbon in the caffeoyl unit. Further, cross-peaks confirmed the binding sites assignment and thus established **1** as *R*,*S*-β-ethoxy-β-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl(1 → 3)-β-D-(6-*O*-*E*-caffeoyl)-glucopyranoside (Figure 1), which was an isomer of ilicifolioside A (**2**) named as isoilicifolioside A.

The known epimeric phenylethanoid glycosides **2**–**4** were determined by the spectroscopic analysis (1D and 2D NMR, and MALDI-TOFMS) and a comparison with the literature data [8,18,22].

Compounds **1**–**4** isolated from *P. tomentosa* var. *tomentosa* were assayed for their anti-complement activities on the complement

Table 1. ^{1}H (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) NMR spectral data for **1** and **2**.

	(**1**) (α/β)[a]		Ilicifolioside A (**2**) (α/β)[a,b]	
Position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
Aglycone				
1	131.4/131.6, qC		131.5/131.9, qC	
2	115.2/115.3, CH	6.76/6.78, d (2.0)	115.0/115.1, CH	6.78/6.76, d (2.0)
3	146.1/146.2, qC		146.4/146.5, qC	
4	146.4/146.3, qC		146.3, qC	
5	116.2/116.3, CH	6.67/6.66, d (8.0)	116.3, CH	6.73, d (8.0)
6	119.9/119.8, CH	6.63/6.62, dd (8.0, 2.0)	119.8/119.9, CH	6.68/6.65, dd (8.0, 2.0)
α(8)	74.9/75.4, CH	3.98, 3.81/3.79, 3.62, m	74.6/75.2, CH	3.98, 3.82/3.70, 3.62, m
β(7)	81.8/82.6, CH_2	4.45/4.44, m	81.7/82.5, CH_2	4.45, m
Ethoxyl				
OCH_2	65.2/65.3, CH_2	3.44/3.43, m	65.1/65.2, CH_2	3.42, m
CH_3	15.5/15.6, CH_3	1.19/1.18, t (7.0)	15.5/15.6, CH_3	1.18/1.16, t (6.8)
Caffeoyl				
1	127.9, qC		127.7, qC	
2	115.0, CH	7.02/7.01, d (2.0)	115.3, CH	7.04, d (2.0)
3	146.7, qC		146.8, qC	
4	149.6, qC		149.8, qC	
5	116.6, CH	6.73, d (8.0)	116.6, CH	6.75, d (8.0)
6	123.2, CH	6.88, dd (2.0, 8.0)	123.3, CH	6.94, dd (2.0, 8.0)
α(8)	114.8, CH_2	6.28, d (16.0)	114.7, CH_2	6.25, d (16.0)
β(7)	147.8, CH_2	7.63, d (16.0)	148.1, CH_2	7.57, d (16.0)
CO	169.2/169.3, qC		168.3, qC	
Glc				
1	104.2/104.8, CH	4.34/4.40, d (8.1)	104.0/104.6, CH	4.37/4.42, d (8.0)
2	75.7/76.0, CH	3.35, m	76.1/76.4, CH	3.45, m
3	84.2/84.4, CH	3.55, m	81.4/81.5, CH	3.82/3.80, t (9.6)
4	70.4/70.3, CH	3.42, t (9.5)	70.5/70.6, CH	4.91, t (9.6)
5	75.4/75.5, CH_2	3.54, m	76.0/76.1, CH_2	3.53.m
6	64.7, CH	4.36, 4.45, m	62.4, CH	3.51, 3.52, m
Rha				
1	102.5/102.6, CH	5.15/5.18, d (1.5)	102.9/103.0, CH	5.18/5.21, d (1.7)
2	72.4, CH	3.96, m	72.4, CH	3.94, m
3	72.1, CH	3.42, m	72.1, CH	3.59, m
4	73.9, CH	3.43, m	73.8, CH	3.31, m
5	70.1, CH	3.96, m	70.4, CH	3.56, m
6	17.9, CH_3	1.24, d (6.2)	18.5, CH_3	1.08, d (6.0)

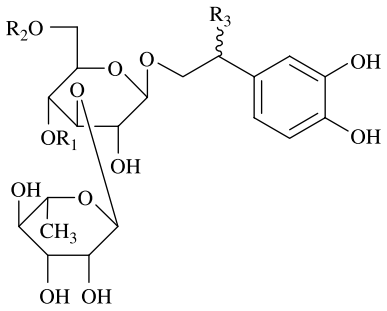
[a] Multiplicities deduced by DEPT experiments.
[b] From Wu *et al.* [18]

system of classical pathway (CP) *in vitro* and the results were summarized in Table 2. Isoilicifolioside A (**1**), ilicifolioside A (**2**), campneoside II (**3**), and isocampneoside II (**4**) exhibited strong anti-complement activity with IC_{50} values of 63, 74, 58, and 67 μM, while for tiliroside and rosmarinic acid 104 and 182 μM, respectively, which were used as positive controls. These facts suggested that the four epimeric phenylethanoid glycosides could be used as anti-complement agents.

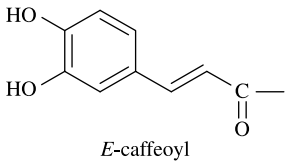
3. Experimental

3.1 General experimental procedures

Melting points were measured with an Electrothermal 9100 apparatus and are uncorrected. Optical rotations were determined on a JASCO



1 R_1=H, R_2=*E*-caffeoyl, R_3=OEt
2 R_1=*E*-caffeoyl R_2=H R_3=OEt
3 R_1=*E*-caffeoyl R_2=H R_3=OH
4 R_1=H, R_2=*E*-caffeoyl, R_3=OH



E-caffeoyl

Figure 1. Structures of compounds **1**–**4**.

DIP-1000 digital polarimeter in MeOH. The IR spectra were obtained on a Perkin-Elmer BX FTIR spectrometer in a KBr disk. The UV spectra were measured with a Jenway 6405 spectrophotometer in MeOH. The ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in CD_3OD with TMS as an internal standard using a Bruker Avance DPX 400 spectrometer. The MALDI-TOFMS spectra were performed with a Model Voyager-DE STR spectrometer. Sephadex LH-20 (Sigma, St Louis, MO, USA) was used for column chromatography. The TLC analysis was carried out on DC-Plastikfolien Cellulose F (Merck, Darmstadt, Germany) plates and developed with *t*-BuOH–HOAc–H_2O (3:1:1, v/v/v) and HOAc–H_2O (3:47, v/v). The TLC spot visualization was performed by illuminating UV light (254 and 365 nm) or by spraying 1% $FeCl_3$ (in ethanol) solution followed by heating. The separation was also performed through preparative HPLC on a reverse-phase μ-Bondapak C_{18} column (7.8 × 300 nm, Waters, Milford, MA, USA). The preparative HPLC

Table 2. Inhibitory effects of isolated phenylethanoid glycosides by the complementary system of classical pathway *in vitro*.

Sample		IC_{50} (μM)[a]
Phenylethanoid glycosides	Isoilicifolioside A (**1**)	63
	Ilicifolioside A (**2**)	74
	Campneoside II (**3**)	58
	Isocampneoside II (**4**)	67
Positive controls	Tiliroside	104
	Rosmarinic acid	182

[a] Data were expressed as the mean of three independent replicates.

spectra were carried out with a Jasco PU-1580 pump, using a LG-1580-04 gradient, DG-1580-54 degasser, and UV-2075 plus detector (Jasco, Tokyo, Japan). The mobile phases were 0.05% H_3PO_4 (solvent A) and MeOH (solvent B), with a flow rate of 3.0 ml/min. Gradient started from 95 to 50% of solvent A for 60 min. The sample volume was 0.1 ml and the eluate was observed at 320 nm.

3.2 Plant material

The plant material was obtained from the campus forest of Kangwon National University (KNU), Korea in October 2004 and authenticated by Prof. Wan-Keun Park, School of Forest, KNU. A voucher specimen (voucher no. 20040108) has been deposited at the herbarium of Laboratory of Natural Products, KNU.

3.3 Extraction and isolation

The air-dried and finely ground *P. tomentosa* var. *tomentosa* wood (5.0 kg) was extracted three times with 70% acetone at room temperature and concentrated *in vacuo* to give an aqueous residue, then stepwisely fractionated and finally freeze-dried to give *n*-hexane (6.1 g, yield 0.12%), CH_2Cl_2 (5.5 g, yield 0.11%), EtOAc (50.5 g, yield 1.01%), and H_2O-soluble (264.5 g, yield 5.29%) fractions.

A portion of the resulting H_2O-soluble fraction (36 g) was subjected to passage over a Sephadex LH-20 open column eluting with MeOH–H_2O (1:1, v/v) to provide seven major fractions labeled as H_1–H_7 according to TLC. Fraction H_2 was also submitted to Sephadex LH-20 column chromatography (CC) using MeOH–H_2O (1:3, v/v) to obtain five subfractions, and repeated purification of the second (H_{22}) and the fourth (H_{24}) subfractions on Sephadex LH-20 CC with MeOH–H_2O (1:5 and 1:7, v/v) and hexane–CH_2Cl_2 (3:1 and 1:1, v/v) forwarded **1** (8 mg) and **2** (15 mg), respectively. Fraction H_4 was rechromatographed over a Sephadex LH-20 column with MeOH–H_2O (1:2, v/v) to obtain three subfractions and further separation of the second one (H_{42}) with MeOH–H_2O (1:4 and 1:6, v/v) and hexane–CH_2Cl_2 (3:1 and 1:1, v/v) to yield **3** (179 mg) and **4** (98 mg).

3.4 Anti-complement assay

Anti-complement properties of the isolated epimeric phenylethanoid glycosides were evaluated by a method adopted from Yamada *et al.* [23]. A diluted solution of normal human serum (complement serum, 80 μl) was mixed with a gelatin veronal buffer (GVB^{2+}, 80 μl) without or with samples. The mixture was preincubated at 37°C for 30 min, followed by the addition of sensitized erythrocyte (sheep red blood cells, 40 μl). After incubation under the same conditions, the mixture was centrifuged (4°C, 1500 rpm) and the absorbance of the supernatant (100 μl) was measured at 450 nm by a UV spectrometer (Libra S32, Biochrom, Cambridge, UK). Each sample was dissolved in DMSO as a negative control, while tiliroside and rosmarinic acid were used as positive controls. The anti-complement activity was determined as a mean of three independent trials and expressed as the 50% inhibitory concentration (IC_{50}) values from complement-dependent hemolysis of the control [24,25].

3.4.1 Isoilicifolioside A (1)

Pale yellow amorphous powder, m.p. 124–126°C; $[\alpha]_D^{20}$ −63 (*c* 0.4, MeOH); IR (KBr) ν_{max} 3400, 1705, 1695, 1620, 1605 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 220, 234 (sh), 290, 327; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; MALDI-TOFMS *m/z* 691.2205 (calcd for $C_{31}H_{40}O_{16}Na$, 691.2209).

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