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# Apigenin Derivatives of *Paulownia coreana* Uyeki Leaves*1

Chuan-Ling Si*2, Jin-Kyu Kim*2, Dong-Joo Kwon*2, and Young-Soo Bae*2†

## ABSTRACT

The leaves of *Paulownia coreana* Uyeki were extracted with acetone-$H_2O$ (7:3, v/v), concentrated under reduced pressure and fractionated successively with *n*-hexane, methylene chloride and ethyl acetate, leaving residual water soluble fraction. A portion of the resulting aqueous soluble powder was chromatographed on a Sephadex LH-20 column using aqueous methanol and ethanol-hexane as washing solvents. Three apigenin derivatives were isolated and identified as apigenin-7-O-$\beta$-D-glucpyranoside, apigenin-7-O-$\beta$-D-glucuronopyranoside and apigenin-7-O-[$\beta$-D-glucuronopyranosyl(1→2)-O-$\beta$-D-glucuronopyranoside] by spectroscopic methods including NMR and FAB-MS.

*Keywords* : *Paulownia coreana* Uyeki, column chromatography, apigenin derivatives, apigenin-7-O-$\beta$-D-glucupyranoside, apigenin-7-O-$\beta$-D-glucuronopyranoside, apigenin-7-O-[$\beta$-D-glucuronopyranosyl (1→2)-O-$\beta$-D-glucuronopyranoside]

## 1. INTRODUCTION

*Paulownia coreana* Uyeki (Scrophulariaceae) is one of useful medicinal hardwood tree species widely grown in Korea. The wood has multiple uses as construction and woodworking materials. The tree has been also used in medicines to treat bronchitis, cough, phlegm, carbuncle, traumatic bleeding, hemorrhoid, gonorrhea, erysipelas, asthma, high blood pressure, upper respiratory tract infection, bronchopneumonia, tonsillitis, bacteriologic diarrhea, enteritis, conjunctivitis, parotitis and swelling (Jiangsu New Medical College, 1977). Some studies on the chemical compositions of flowers (Oh *et al.*, 2000), leaves (Si *et al.*, 2005), and young plant (Damtoft and Jensen, 1993) of *P. coreana* have been reported. However, the detailed chemical composition of the species is still research topic to be investigated and this is the first report of the isolation on apigenin derivatives from *Paulownia.*

## 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 before grinding.

*1 Received on November 03, 2005; accepted on December 14, 2005.
*2 College of Forest Sciences, Kangwon National University, Chunchon 200-701, Korea
† Corresponding author : Young-Soo Bae (bae@kangwon.ac.kr)

## 2.2. Equipments

Chromatographic column was packed with Sephadex LH-20 and MeOH-$H_2O$ (1:1, 1:3, 1:5, v/v) and EtOH-hexane (4:1, 3:1, 2:1, v/v) as eluting solvents. Eluents were collected using a Gilson FC 204 fraction collector.

TLC was performed on precoated cellulose plates (Merk 25 DC-Plastik-folien Cellulose F) 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) and by spraying 1% $FeCl_3$ (in EtOH) or vanillin-HCl-ethanol (60:0.15:6, w/v/v) followed by heating. Two dimensional TLC was also performed to verify the purification of the isolated compounds.

$^1H$ and $^{13}C$-NMR spectra were obtained using a Bruker Avance DPX 400 MHz NMR spectrometer to elucidate the structures of the isolated compounds. For the determination of the molecular weight, positive FAB-MS was recorded using α-monothioglycerol matrix with a Micromass Autospec M363 spectrometer.

## 2.3. Extraction and Fractionation

The ground leaves (5.0 kg) were extracted with aceton-water (7:3, v/v) three times at room temperature. Then the extractive were combined together, filtered and evaporated under reduced pressure. The mixture was sequentially partitioned with *n*-hexane, $CH_2Cl_2$ and EtOAc using a separatory funnel. Each fraction was concentrated and freeze dried to yield *n*-hexane soluble (29.0 g), $CH_2Cl_2$ soluble (6.6 g), EtOAc soluble (11.6 g) and water soluble powder (246.0 g).

A portion of resulting aqueous soluble powder (25.7 g) was chromatographed on a Sephadex LH-20 column eluted with methanol-water (1:1, v/v) to give 14 main fractions labelled as PCLW 1~14. PCLW 4 was reapplied on a column for further purification with MeOH-$H_2O$ (3:1, v/v) affording 3 subfractions as PCLW 41~43. When treated with MeOH, some crystalline material was precipitated in PCLW 42. Compound 1 (20 mg) and 2 (277 mg) were isolated by further purification. PCLW 2 was also rechromatographed on a Sephadex LH-20 with MeOH-$H_2O$ (1:1, 1:3, 1:5, v/v) and EtOH-Hexane (4:1, 3:1, 2:1, v/v) as eluting solvents to give compound 3 (369 mg).

### 2.3.1. Compound 1

$R_f$: 0.51 (solvent A) and 0.59 (solvent B).

FAB-MS: Caculated for $C_{21}H_{20}O_{10}$ 432, Found $[M+H]^+$ *m/z* 433, $[M+H\text{-glucose}]^+$ *m/z* 271.

$^1H$-NMR (400 MHz, $\delta$, DMSO-$d_6$): 3.1~3.6 (5H, *m*, H-2″, 3″, 4″, 5″, 6″), 5.0 (1H, *d*, *J*=7.1 Hz, H-1″), 6.2 (1H, *d*, *J*=1.9 Hz, H-6), 6.6 (1H, *d*, *J*=1.9 Hz, H-8), 6.6 (1H, s, H-3), 6.8 (2H, *d*, *J*=8.5 Hz, H-3′, 5′), 7.7 (1H, *d*, *J*=8.5 Hz, H-2′, 6′).

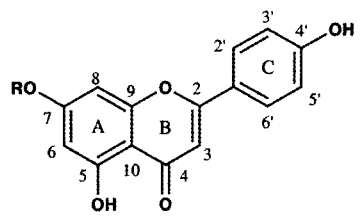
$^{13}C$-NMR (100 MHz, $\delta$, DMSO-$d_6$): Table 1.

### 2.3.2. Compound 2

$R_f$: 0.51 (solvent A) and 0.05 (solvent B).

FAB-MS: Caculated for $C_{21}H_{18}O_{11}$ 446, Found $[M+H]^+$ *m/z* 447, $[M+H\text{-GluA}]^+$ *m/z* 271.

$^1H$-NMR (400 MHz, $\delta$, DMSO-$d_6$): 3.6 (3H, m, H-2″, 3″, 4″), 3.9 (1H, *d*, *J*=8.4 Hz, H-5″),




Compound 1: R=β-D-Glucose
Compound 2: R=β-D-GluA
Compound 3: R=β-D-GluA(2→1)β-D-GluA

Fig. 1. Apigenin derivatives from *P. coreana* Uyeki leaves (GluA=glucuronic acid).

Table 1. $^{13}$C-NMR (100 MHz) spectral data of compounds 1, 2 and 3 in DMSO-$d_6$

| C | δ | | | |
|---|---|---|---|---|
| | apigenin (Si *et al.*, 2005) | 1 | 2 | 3 |
| Aglycone | | | | |
| 2 | 164.6 | 164.6 | 164.7 | 164.9 |
| 3 | 103.7 | 103.1 | 103.3 | 103.0 |
| 4 | 182.6 | 182.2 | 182.2 | 182.3 |
| 5 | 162.0 | 161.3 | 161.7 | 160.6 |
| 6 | 99.6 | 99.9 | 99.9 | 100.4 |
| 7 | 165.0 | 163.2 | 163.3 | 162.9 |
| 8 | 94.7 | 94.9 | 94.4 | 95.7 |
| 9 | 158.2 | 157.3 | 157.6 | 157.1 |
| 10 | 104.4 | 105.7 | 105.6 | 105.7 |
| 1′ | 122.0 | 121.8 | 121.8 | 121.0 |
| 2′ | 129.3 | 129.7 | 129.1 | 129.0 |
| 3′ | 116.8 | 116.3 | 116.7 | 116.3 |
| 4′ | 162.3 | 162.1 | 162.8 | 161.4 |
| 5′ | 116.8 | 116.3 | 116.7 | 116.3 |
| 6′ | 129.3 | 128.8 | 129.1 | 129.0 |
| Sugars | | | | |
| 1″ | | 100.0 | 99.5 | 98.0 |
| 2″ | | 74.3 | 73.9 | 80.1 |
| 3″ | | 77.8 | 75.5 | 74.7 |
| 4″ | | 72.2 | 70.1 | 72.0 |
| 5″ | | 76.7 | 76.6 | 76.0 |
| 6″ | | 63.4 | 173.4 | 172.9 |
| 1‴ | | | | 102.6 |
| 2‴ | | | | 74.0 |
| 3‴ | | | | 74.8 |
| 4‴ | | | | 71.5 |
| 5‴ | | | | 75.6 |
| 6‴ | | | | 173.6 |

5.1 (1H, *d*, *J*=6.0 Hz, H-1″), 6.5 (1H, *d*, *J*=2.0 Hz, H-6), 6.6 (1H, *d*, *J*=2.0 Hz, H-8), 6.8 (1H, s, H-3), 7.0 (2H, *d*, *J*=8.3 Hz, H-3′, 5′), 7.8 (1H, *d*, *J*=8.3 Hz, H-2′, 6′).

$^{13}$C-NMR (100 MHz, δ, DMSO-$d_6$): Table 1.

#### 2.3.3. Compound 3

$R_f$: 0.19 (solvent A) and 0.18 (solvent B).

FAB-MS: Caculated for $C_{27}H_{26}O_{17}$ 622, Found $[M+H]^+$ *m/z* 623, $[M+H\text{-}GluA]^+$ *m/z* 447, $[M+H\text{-}GluA]^+$ *m/z* 271.

$^1$H-NMR (400 MHz, δ, DMSO-$d_6$): 3.2~3.8 (8H, m, H-2″, 3″, 4″, 5″, 2‴, 3‴, 4‴, 5‴), 4.7 (1H, *d*, *J*=7.7 Hz, H-1‴), 5.3 (1H, *d*, *J*=7.3 Hz, H-1″), 6.6 (1H, *d*, *J*=2.0 Hz, H-6), 6.8 (1H, *d*, *J*=2.0 Hz, H-8), 7.0 (1H, s, H-3), 7.0 (2H, *d*, *J*=8.3 Hz, H-3′, 5′), 8.0 (1H, *d*, *J*=8.3 Hz, H-2′, 6′).

$^{13}$C-NMR (100 MHz, δ, DMSO-$d_6$): Table 1.

## 3. RESULTS and DISCUSSION

### 3.1. Apigenin-7-O-β-D-glucupyranoside

Compound 1 was isolated as yellow powder and gave a dark brown spot on a cellulose plate when was visualized with $FeCl_3$-EtOH (1:99, W/V) $R_f$ values were 0.63 (solvent A) and 0.68 (solvent B). It gave a $[M+H]^+$ ion at *m/z* 433 in the positive FAB-MS spectrum, which was consistent with the molecular formula $C_{21}H_{20}O_{10}$. Fragment at *m/z* 257 was observed for aglycon. In the $^1$H-NMR spectrum, the typical flavonoid glucoside signals were observed. An AA′BB′ system constituted by two doublets (*J*=8.5 Hz) at δ 7.7 and δ 6.8 attributable to H-2′,6′ and H-3′,5′ due to a *para*-substituted B ring. The *meta* coupling of H-6 and H-8 on phloroglucinol A ring presented at δ 6.2 (*d*, *J*=1.9 Hz) and δ 6.6 (*d*, *J*=1.9 Hz) respectively. The singlet of aromatic 3-H of heterocyclic C ring was observed at δ 6.6. The presence of a β-configuration glucose was evidenced by a one-proton doublet (*J*=7.1 Hz) at δ 5.0, together with 5 protons between δ 3.1~3.6. (Kamerling *et al.*, 1972; Andary *et al.*, 1982). Consequently, compound 1 was hypothesized as apigenin glycoside. In $^{13}$C-NMR spectrum, the sugar moiety was easily identified as β-D-glucose and its linkage to the 7-OH of apigenin was established on the basis of the typical glycosylation chemical shifts experienced by the

aglycone, particularly the ipso carbon (C-7) upfielded 2 ppm, *ortho* (C-6 and C-8) and *para* carbons (C-10) downfielded 0.3 ppm and 1.3 ppm respectively when compared with apigenin (Agrawal, 1989). In the HMBC spectrum, long range correlations were observed between the anomeric proton signals ( $\delta$ 5.0) of glucose and C-7 of the aglycon (163.2 ppm) demonstrating glucosylation at the C-7 of apigenin. Therefore, compound 1 was elucidated as apigenin-7-O-$\beta$-D-glucopyranoside and the literature data supported the result (Harbon and Mabry, 1982).

## 3.2. Apigenin-7-O-$\beta$-D-glucuronopyranoside

Compound 2 was obtained as yellow crystal from MeOH giving a dark brown spot on a cellulose TLC when sprayed with the detecting reagent of 1% $FeCl_3$. The $^1H$ and $^{13}C$-NMR spectral data of compound 2 were very similar to those of compound 1 with the exception that the sugar's carboxyl carbon typically resonance at 173.4 ppm indicating a glucuronic acid moiety instead of a glucose unit attached to 7-OH of aglycone.

This structure is also corroborated by its positive FAB-MS spectrum where, besides the quasimolecular peak at *m/z* 447 for $[M+H]^+$, consistent with the molecular formula $C_{21}H_{18}O_{11}$, the peak due to the loss of glucuronic acid $[M+H-176]^+$, at 271 *m/z* was evident. Thus the structure was established as apigenin-7-O-$\beta$-D-glucuronopyranoside in correspondence with an authentic sample reported by other researchers (Hase *et al.*, 1995; Roseiro *et al.*, 2005).

## 3.3. apigenin-7-O-[$\beta$-D-glucuronopyranosyl(1→2)-O-$\beta$-D-glucuronopyranoside]

Compound 3 was isolated as pale yellowish

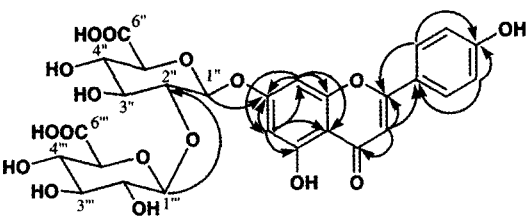



Fig. 2. Selected HMBC correlations observed in compound 3 (H→C).

powder and gave a yellow spot on a cellulose plate when was visualized with vanillin-hydrochloric acid-ethanol. In the positive FAB-MS spectrum three characteristic peaks could be identified, with *m/z* 623 for $[M+H]^+$ ions indicating the molecular weight 622 and two other peaks at *m/z* 447 and 271 ascribing to successfully loss of one and two glucuronic acids respectively (Qu *et al.*, 2001). On the comparison of $^1H$ and $^{13}C$-NMR spectrum with those of compound 2, one additional $\beta$-D-glucuronic acid was observed in compound 3. Careful examination of the $^{13}C$-NMR sprectrum of 3 showed that the signal assigned to C-2″ shifted downfield by approximately 6 ppm with upfield shift of the adjacent carbons (C-1″ and C-3″), confirming the attachment of a second glucuronic acid moiety to apigenin-7-O-glucuronopyranoside at C-2″. In the HMBC spectrum (Fig. 2), the anomeric proton signals of the first and second glucuronic acid respectively showed long range correlations with $^{13}C$-NMR signals at 162.9 ppm (C-7) and 81.1 ppm (C-2″) demonstrating apigenin-7-O-[$\beta$-D-glucuronopyranosyl (1→2)-O-$\beta$-D-glucuronopyranoside] linkage structure which is well coincided with the report (Stochmal *et al*, 2001; Yoshida *et al.*, 1993).

# 4. CONCLUSIONS

Three apigenin derivatives were isolated by column chromatography using Sephadex LH-20 from the aqueous soluble fraction of *P. coreana*

leaves, and elucidated as apigenin-7-O-$\beta$-D-glucopyranoside (20 mg), apigenin-7-O-$\beta$-D-glucuronopyranoside (277 mg) and apigenin-7-O-[$\beta$-D-glucuronopyranosyl(1→2)-O-$\beta$-D-glucurono pyranoside] (369 mg) by $^{1}H$ and $^{13}C$-NMR and FAB-MS spectroscopy. This is the first report on the apigenin derivatives from *Paulownia* family.

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