PERGAMON

Phytochemistry 51 (1999) 147–151

PHYTOCHEMISTRY

# Isoflavone glycosides from the flowers of *Pueraria thunbergiana*

Hee-Juhn Park[a], Jong-Hee Park[b], Jeon-Ok Moon[b], Kyung-Tae Lee[c], Won-Tae Jung[d], Sei-Ryang Oh[e], Hyeong-Kyu Lee[e,*]

[a]*Department of Botanical Resources, Sangji University, Wonju 220-702, South Korea*
[b]*College of Pharmacy, Pusan National University, Keumjong-ku, Pusan 609-735, South Korea*
[c]*College of Pharmacy, Kyung-Hee University, Dongdaemun-ku, Seoul 130-701, South Korea*
[d]*Central Research Institute, Il-Yang Co., Yongin 449-900, South Korea*
[e]*Biomaterial Research Division, Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon 305-333, South Korea*

Received in revised form 10 November 1998

**Abstract**

Two new isoflavone glycosides together with glycitein, tectoridin and glycitin, were isolated from the flowers of *Pueraria thunbergiana*. These structures were determined as 4′,7-dihydroxy-6-methoxyisoflavone 7-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside and 4′,5,7-trihydroxy-6-methoxyisoflavone 7-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside. 

*Keywords:* *Pueraria thunbergiana*; Leguminosae; Flowers; Isoflavone glycosides; $^{13}$C NMR

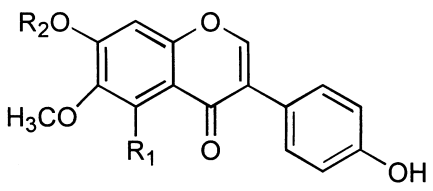
## 1. Introduction

In oriental herbal medicine, the roots of *Pueraria thunbergiana* have been widely used as an antipyretic and analgesic in the treatment of colds (Kim, 1996). In contrast, the flowers of this species have been used to treat diabetes mellitus and lingering intoxication. From the flowers, kakkalide (irisolidone-7-*O*-*β*-D-xylopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside) together with irisolidone, genistein and daidzein were isolated (Kurihara and Kikuchi, 1973, 1975, 1976) and 4′,6-dihydroxy-7-methoxyisoflavone was reported as a new compound from the same plant material (Kubo, Sasaki, Namba, Naruto, & Nishimura, 1975). In addition, pueraria glycosides-1, -2, -3 and -6 and puerarol were isolated from *Pueraria* root (Namgoong, Lee, & Kim, 1994) and puetuberosanol was isolated from *P. tuberosa* (Khan, Agrawal, & Kapil, 1996). The inhibitory effects of several isoflavonoids from *Puerariae radix* on mouse lymphocyte proliferation in vitro are reported (Oshima, Okuyama, Takahashi, Takizawa, & Shibata, 1988).

In our recent research to screen superoxide dismutase mimicking crude drugs from a number of Korean medicinal plants, the flowers of *P. thunbergiana* were selected for investigation. We report the isolation and structural elucidation of two new isoflavone glycosides together with three known compounds (Fig. 1). The structures of these compounds were deduced by 2D-NMR techniques.

## 2. Results and discussion

Silica gel column chromatography of the ethyl acetate fraction of the methanolic extract of the flowers of *P. thunbergiana* led to the isolation of four subfractions. Recrystallization of subfractions 1, 2 and 3 from meth-




| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | H | H |
| **2** | OH | Glc |
| **3** | H | Glc |
| **4** | H | Glc$^6$-Xyl |
| **5** | OH | Glc$^6$-Xyl |

Fig. 1. Structures of compounds isolated from *Pueraria thunbergiana*.

* Corresponding author. Tel.: +82-42-860-4413; fax: +82-42-860-4608; e-mail: hykylee@mail.kribb.re.kr.

0031-9422/99/$ - see front matter 
PII: S0031-9422(98)00729-8

anol yielded compounds **1**, **2** and **3**, respectively. Successive separation of subfraction 4 by RP-18 reverse-phase column chromatography led to the isolation of compounds **4** and **5**. In their IR spectra, compounds **1**–**5** each showed the characteristic absorption bands of an $\alpha,\beta$-unsaturated ketone (near 1635 $cm^{-1}$) and hydroxyl group (near 3400 $cm^{-1}$); compounds **2**–**5** showed strong glycoside functions at 1100–1000 $cm^{-1}$, in contrast to weak bands in **1**. From the above, we deduced that the structural skeleton as isoflavone for **1** and isoflavone glycosides for **2**–**5**.

In the $^1$H NMR spectrum of **1**, the characteristic isoflavone signal for H-2 was observed at $\delta$ 8.19 (1H, *s*) along with aromatic proton singlets at δ 6.91 (1H, s) and $\delta$ 7.43 (1H, s), indicating that the A-ring was substituted at C-6 and C-7. The two aromatic hydrogen peaks at $\delta$ 7.37 (2H, d, $J=8.7$ Hz) and $\delta$ 6.79 (2H, d, $J=8.7$ Hz) could be attributed to a 4-hydroxyphenyl moiety (B-ring). Since the methoxyl peak ($\delta_H$ 3.88) could be confirmed by the interpretation of HMBC and NOESY spectra (data not shown), compound **1** was identified as 4′,6,7-trihydroxyisoflavone-6-methylether (glycitein) (Main, Gestetner, Kirson, Birk, & Bondi, 1973). The results of $^1$H and $^{13}$C NMR assignments are shown in Tables 1 and 2.

In the $^1$H NMR spectrum of **2**, a pair of 2H signals at $\delta$ 7.40 (d, $J=8.4$ Hz) and $\delta$ 6.83 (d, $J=8.4$ Hz) indicated that the B-ring was substituted at C-4′, as in compound **1**. The characteristic H-2 peak was observed at $\delta$ 8.43 (1H, s) together with only one aromatic hydrogen peak at $\delta$ 6.88 (1H, s) indicating that three substituents were linked to the A-ring. The anomeric proton of the sugar at $\delta$ 5.09 (1H, d, $J=7.5$ Hz) indicated that the sugar was $\beta$-linked to the aglycone. The position of aromatic hydrogen ($\delta_H$ 6.88) and methoxyl group ($\delta_H$ 3.78) could be assigned at C-8 and C-6, respectively, by interpretation of NOESY (Fig. 2) and HMBC spectra (Table 3). Thus, compound **2** was confirmed as 4′,5,7-trihydroxy-6-methoxyisoflavone-7-*O*-$\beta$-D-glucopyranoside (tectoridin) (Lee, Woo, Woo, & Kim, 1989).

In the $^1$H NMR spectrum of **3**, the characteristic H-2 signal of an isoflavone was exhibited at δ 8.37 (1H, s) and the 4-hydroxyphenyl nature of the B-ring was deduced from the signals at $\delta$ 7.41 (2H, d, $J=8.6$ Hz, H-2′, 6′) and $\delta$ 6.82 (2H, d, $J=8.6$ Hz, H-3′,5′). The *J* value (7.4 Hz) of the anomeric proton at $\delta$ 5.17 indicated that the sugar was in the $\beta$-configuration. Hydrolysis of **3** gave **1** as the aglycone and D-glucose. In the NOESY spectrum of **3**, the position of the sugar was deduced to be at C-7 from the correlation between the anomeric proton of D-glc ($\delta$ 5.17) and H-8 ($\delta$ 7.32) (Fig. 2). Likewise, the methoxyl group ($\delta_H$ 3.88) could be positioned at C-6 by the fact that H-5 ($\delta$ 7.48) was correlated with the methoxyl proton (Table 3). Thus, compound **3** was identified as 4′,6,7-trihydroxyisoflvone-6-methylether-7-*O*-glucopyranoside (glycitin) (Main et al., 1973).

The $^1$H NMR spectrum of **4** was similar to that of **3**. However, two sugar moieties were evident from the detection of anomeric proton signals at δ 5.11 (H-1 of D-glc) and $\delta$ 4.16 (H-1 of D-xyl). On acid hydrolysis, **4** gave **1** as the aglycone and D-glucose and D-xylose. Spectral data for the aglycone were identical to **1**. The anomeric proton of D-glc ($\delta$ 5.11) in the HMBC spectrum was correlated to C-7 ($\delta_C$ 151.4) and the anomeric proton

Table 1
$^1$H-NMR data for compounds **1**–**5** from *P. thunbergiana* (300 MHz, DMSO-$d_6$)

| Position | **1** | **2** | **3** | **4** | **5** |
|---|---|---|---|---|---|
| 2 | 8.19 (s) | 8.43 (s) | 8.37 (s) | 8.32 (s) | 8.36 (s) |
| 5 | 7.43 (s) | – | 7.48 (s) | 7.48 (s) | – |
| 8 | 6.91 (s) | 6.88 (s) | 7.32 (s) | 7.39 (s) | 6.93 (s) |
| 2′,6′ | 7.37 (d, 8.7[a]) | 7.40 (d, 8.4) | 7.41 (d, 8.6) | 7.41 (d, 8.4) | 7.39 (d, 7.2) |
| 3′,5′ | 6.79 (d, 8.7) | 6.83 (d, 8.4) | 6.82 (d, 8.6) | 6.82 (d, 8.4) | 6.82 (d, 7.2) |
| Glc-1 | | 5.09 (d, 7.5) | 5.17 (d, 7.4) | 5.11 (d, 6.9) | 5.02 (d, 7.2) |
| 2 | | 3.34 (m) | 3.30 (m) | 3.36 (m) | 3.33 (m) |
| 3 | | 3.34 (m) | 3.30 (m) | 3.32 (m) | 3.32 (m) |
| 4 | | 3.20 (m) | 3.17 (m) | 3.20 (m) | 3.20 (m) |
| 5 | | 3.45 (m) | 3.46 (m) | 3.63 (m) | 3.63 (m) |
| 6-a | | 3.49 (m) | 3.43 (m) | 3.63 (m) | 3.63 (m) |
| 6-b | | 3.69 (m) | 3.70 (m) | 3.90 (m) | 3.93 (m) |
| Xyl-1 | | | | 4.18 (d, 7.2) | 4.19 (d, 7.2) |
| 2 | | | | 3.01 (m) | 3.00 (m) |
| 3 | | | | 3.09 (m) | 3.08 (m) |
| 4 | | | | 3.31 (m) | 3.28 (m) |
| 5-a | | | | 2.98 (m) | 2.96 (m) |
| 5-b | | | | 3.71 (m) | 3.70 (m) |
| 6-OMe | 3.88 (s) | 3.78 (s) | 3.88 (s) | 3.88 (s) | 3.76 (s) |

[a] Coupling constant in Hz.

Table 2
$^{13}$C-NMR spectral data of **1**–**5** from *P. thunbergiana* (75.5 MHz, DMSO-$d_6$)

| Position | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 2 | 152.1 (CH) | 154.6 (CH) | 153.2 (CH) | 152.9 (CH) | 154.5 (CH) |
| 3 | 123.0 (C) | 122.1 (C) | 123.3 (C) | 123.1 (C) | 122.1 (C) |
| 4 | 174.3 (C) | 180.8 (C) | 174.6 (C) | 174.4 (C) | 180.7 (C) |
| 5 | 104.7 (C) | 152.9 (C) | 105.0 (C) | 104.8 (CH) | 153.1 (C) |
| 6 | 146.8 (C) | 132.5 (C) | 147.7 (C) | 147.4 (C) | 132.6 (C) |
| 7 | 152.8 (C) | 156.6 (C) | 152.7 (C) | 151.4 (C) | 156.4 (C) |
| 8 | 102.7 (CH) | 94.0 (CH) | 103.0 (CH) | 103.6 (CH) | 93.9 (CH) |
| 9 | 151.7 (C) | 152.4 (C) | 151.7 (C) | 151.3 (C) | 152.6 (C) |
| 10 | 116.2 (C) | 106.5 (C) | 118.0 (C) | 117.9 (C) | 106.7 (C) |
| 1′ | 122.7 (C) | 121.0 (C) | 122.8 (C) | 122.6 (C) | 121.1 (C) |
| 2′, 6′ | 129.9 (CH) | 130.1 (CH) | 130.3 (CH) | 130.0 (CH) | 130.1 (CH) |
| 3′, 5′ | 115.0 (CH) | 115.1 (CH) | 115.2 (CH) | 115.0 (CH) | 115.1 (CH) |
| 4′ | 157.0 (C) | 157.5 (C) | 157.6 (C) | 157.2 (C) | 157.5 (C) |
| Glc-1 | | 100.2 (CH) | 99.8 (CH) | 99.7 (CH) | 100.2 (CH) |
| 2 | | 73.1 (CH) | 73.2 (CH) | 73.0 (CH) | 73.1 (CH) |
| 3 | | 76.7 (CH) | 76.9 (CH) | 76.7 (CH) | 76.6 (CH) |
| 4 | | 69.7 (CH) | 69.8 (CH) | 69.7 (CH) | 69.8 (CH) |
| 5 | | 77.3 (CH) | 77.4 (CH) | 75.7 (CH) | 75.8 (CH) |
| 6 | | 60.7 ($CH_2$) | 60.8 ($CH_2$) | 68.7 ($CH_2$) | 68.6 ($CH_2$) |
| Xyl-1 | | | | 104.2 (CH) | 104.1 (CH) |
| 2 | | | | 73.4 (CH) | 73.4 (CH) |
| 3 | | | | 76.5 (CH) | 76.5 (CH) |
| 4 | | | | 69.4 (CH) | 69.4 (CH) |
| 5 | | | | 65.6 ($CH_3$) | 65.6 (CH) |
| 6-OMe | | | | 55.9 ($CH_3$) | 60.2 ($CH_3$) |

Each carbon in the parentheses was characterized by DEPT.

signals of D-glc ($\delta$ 5.11) and D-xyl ($\delta$ 4.18) in the NOESY spectrum were correlated to H-8 ($\delta$ 7.39) and H-6 of D-glc ($\delta$ 3.63, 3.90), respectively, (Table 3 and Fig. 2). The linkage patterns of the two sugars were in the $\beta$-configuration, from the $J$ values of the anomeric protons (D-glc, $J=6.9$ Hz; D-xyl, $J=7.2$ Hz). Thus, compound **4** was identified as 4′,6,7-trihydroxyisoflavone-6-methylether-7-*O*-$\beta$-D-xylopyranosyl-(1→6)-$\beta$-D-glucopyranoside. This compound is reported for the first time from a natural source.

From consideration of the HMBC and NOESY spectra of **5** (Table 3 and Fig. 2), the anomeric proton of D-glc ($\delta_H$ 5.02) was correlated to H-8 of the aglycone ($\delta_H$ 6.93) and the anomeric proton of D-xyl ($\delta_H$ 4.19) with H-6 and C-6 of D-glc. Therefore, compound **5** was identified as 4′,5,7-trihydroxy-6-methoxyisoflavone-7-*O*-$\beta$-D-xylopyranosyl-(1→6)-$\beta$-D-glucopyranoside. This compound is also reported for the first time from a natural source.

Although previous workers have isolated many isoflavonoids from the flowers of *P. thunbergiana*, compounds **1**–**5** have not been reported before from this plant source.

## 3. Experimental

M.p.'s are uncorr. IR were recorded in KBr pellets. EI-MS were recorded at 70 eV. NMR, HMBC and NOESY were taken on Brucker DRX-300 or Brucker DRX-600 spectrometers with TMS as standard.

### 3.1. Plant material

Flowers of *P. thunbergiana* were collected at Chiak mountain, Kangwon Province, Korea, in September 1997 and the species was identified by Professor J.H. Park (Department of Pharmacy, Pusan National University, Pusan, Korea). A voucher specimen is deposited in the herbarium of Life Science and Natural Resources, Sangji University, Wonju, Korea.

### 3.2. Extraction and isolation

Dried flowers (4.8 kg) were pulverized and extracted 3× with MeOH under reflux. The MeOH extract was filtered and the sol. conc. under red. pres. to give a viscous residue (680 g). This was suspended in $H_2O$ and partitioned with $CHCl_3$ (115 g), EtOAc (64 g) and *n*-BuOH (210 g), successively. A part of the EtOAc fr. (20 g) was subjected to CC on silica gel (Merck, Art. No. 7734) using $CHCl_3$–MeOH–$H_2O$ (7:3:1) to give 4 frs. Recrystallization of the frs 1–3 from MeOH yielded compounds **1** (38 mg), **2** (30 mg) and **3** (106 mg), respectively. Compound **4** (40 mg) and **5** (25 mg) were isolated from fr. 4 by RP-18 reverse phase CC (YMC GEL ODS-A, 5 × 50

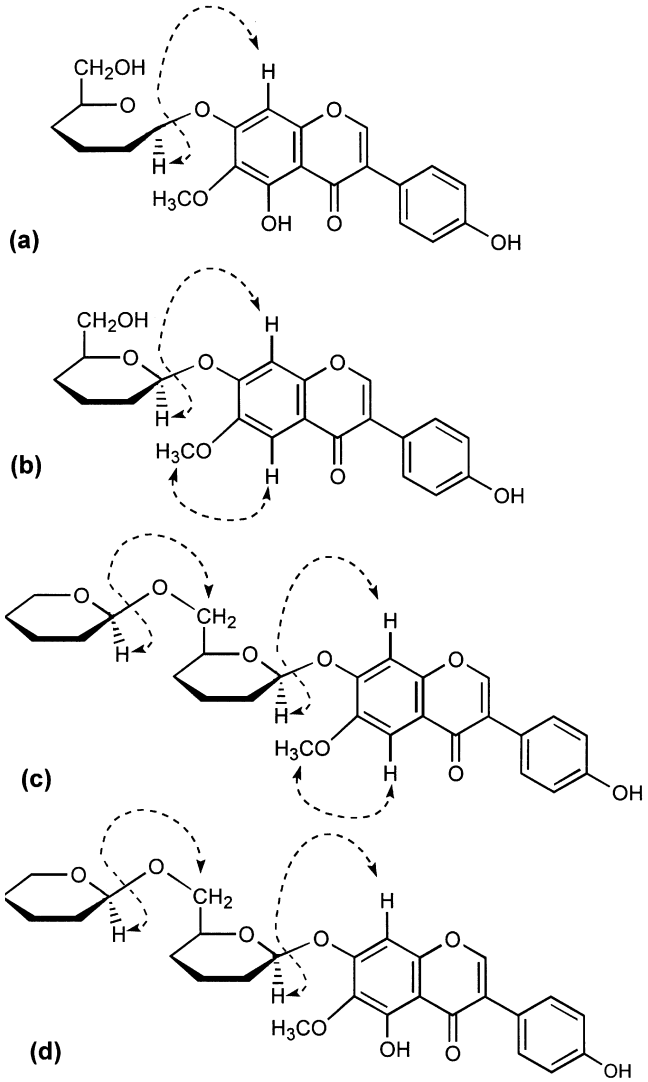



Fig. 2. NOE correlations of compounds 2(a), **3**(b), **4**(c) and **5**(d).

cm, MeOH–$H_2O$, 2:3) and each was identified as a single compound by HPLC (CAPCELL PAK C18, 4 × 250 mm, Shiseido, MeOH–$H_2O$, 1:1).

*3.2.1. Compound **1***

Pale yellowish powder (MeOH), m.p. 170–180°C, UV (MeOH): $\lambda_{max}$ (log ε) 211.4 (4.29), 231.6 (4.18), 260.2 (4.30), 319.0 (3.94). IR $\nu_{max}$ (KBr) $cm^{-1}$: 3472 (OH), 1635 (α,β-unsaturated ketone), 1520, 1419, 1373 (aromatic C═C), 1232, 1207, 1178. MS (70 eV, rel. int.) *m/z*: 284.1 (100) $[C_{16}H_{12}O_5]^+$, 166.0 (30) $[C_8H_6O_4]^+$. 118.0 (11) $[C_8H_6O]^+$, $^1$H NMR (300 MHz, DMSO-$d_6$) Table 1; $^{13}$C NMR (75.5 MHz, DMSO-$d_6$): Table 2.

*3.2.2. Compound **2***

Colourless needles (MeOH), m.p. > 300°C, UV (MeOH): $\lambda_{max}$ (log ε) 209.4 (4.39), 266.2 (4.46). IR $\nu_{max}$ (KBr) $cm^{-1}$: 3472 (OH), 1635 (α,β-unsaturated ketone), 1517, 1498, 1469, 1433 (aromatic C═C), 1100–1000 (glycoside). FAB-MS: *m/z* 463 $[M+H]^+$. $^1$H NMR (300 MHz, DMSO-$d_6$) Table 1; $^{13}$C NMR (75.5 MHz) Table 2.

*3.2.3. Compound **3***

White powder (MeOH), m.p. 210°C, UV (MeOH): $\lambda_{max}$ (log ε) 208.0 (4.32), 262.6 (4.29), 320.2 (3.74). IR $\nu_{max}$ (KBr) $cm^{-1}$: 3472 (OH), 1635 (α,β-unsaturated ketone), 1516, 1498, 1469, 1433 (aromatic C═C), 1274, 1218 (C–O), 1100–1000 (glycoside). FAB-MS: *m/z* 447 $[M+H]^+$. $^1$H NMR (300 MHz, DMSO-$d_6$) Table 1; $^{13}$C NMR (75.5 MHz) Table 2.

*3.2.4. Compound **4***

Colourless needles (MeOH), m.p. > 300°C, $[\alpha]_D^{25}$ −54.3° (DMSO; *c* 0.35). IR $\nu_{max}$ (KBr) $cm^{-1}$: 3459 (OH), 1635 (α,β-unsaturated ketone), 1517, 1498, 1474 (aromatic C–H), 1273, 1218 (C–O), 1100–1000 (glycoside). FAB-MS: *m/z* 579 $[M+H]^+$, UV (MeOH): $\lambda_{max}$ 261, 320; (+NaOMe) $\lambda_{max}$ 287; (+$AlCl_3$) $\lambda_{max}$ 261, 320; (+$AlCl_3$/HCl) $\lambda_{max}$ 228, 261, 320; (+NaOAc) $\lambda_{max}$ 263, 317; (+NaOAc/HBr) $\lambda_{max}$ 262, 320. $^1$H NMR (300 MHz, DMSO-$d_6$) Table 1; $^{13}$C NMR (75.5 MHz) Table 2.

*3.2.5. Compound **5***

Pale yellowish powder (MeOH), m.p. > 300°C, $[\alpha]_D^{25}$ −43.3 (DMSO; *c* 0.15), FAB-MS: *m/z* 595 $[M+H]^+$. IR $\nu_{max}$ (KBr) $cm^{-1}$: 3470 (OH), 1635 (α,β-unsaturated ketone), 1516, 1496, 1469 (aromatic C═C), 1274, 1218 (C–O), 1100–1000 (glycoside). UV (MeOH): $\lambda_{max}$ 273, 336; (+NaOMe) $\lambda_{max}$ 273; (+$AlCl_3$) $\lambda_{max}$ 276; (+$AlCl_3$/HCl) $\lambda_{max}$ 276, 390; (+NaOAc) $\lambda_{max}$ 268; (+NaOAc/HBr) $\lambda_{max}$ 267. $^1$H NMR (300 MHz, DMSO-$d_6$) Table 1; $^{13}$C NMR (75.5 MHz) Table 2.

*3.2.6. Acid hydrolysis of **3** and **4***

These two compounds were hydrolyzed in 5% $H_2SO_4$ in MeOH–$H_2O$ (1:1) under reflux for 3 h, respectively. The reaction mixt. was then partitioned against EtOAc, the EtOAc fr. conc. under red. pres. and recrystallized from MeOH to give the same aglycone, which was identified as **1** by direct comparisons of spectral data with those of **1**. The aq. layers were neutralized with $NH_4OH$ and evap. in vacuo and the resulting residues applied to a TLC plate and developed with EtOAc–MeOH –$H_2O$–OHAc (13:6:3:3). The $R_f$ value of the sugar obtained from **3** was identical to that of D-glucose and those obtained from **4** were identical to those of D-glucose and D-xylose by co-TLC of standard sugars.

*3.2.7. Acid hydrolysis of **2** and **5***

Compounds **2** and **5** gave the same aglycone after hydrolysis. The MS showed the $[M]^+$ at *m/z* 300.1 (100%) $[C_{16}H_{12}O_6]^+$, and the RDA fragment ion *m/z* 182.0 (28%) $[C_8H_6O_5]^+$, 118 (9%) $[C_8H_6O]^+$. The sugar from **2** was identified as D-glc and those from **5** as D-Glc and D-xyl, by TLC analysis.

Table 3
HMBC correlation of **2**, **3**, **4** and **5** (600 MHz, DMSO-$d_6$)

| Carbon | **2** | **3** | **4** | **5** |
|---|---|---|---|---|
| 2 | – | – | – | – |
| 3 | H-2, H-2′+6′ | H-2, H-2′+6′ | H-2, H-2′+6′ | H-2, H-2′+6′ |
| 4 | H-2, | H-2, H-5, H-8 | H-2, H-5, H-8 | H-2 |
| 5 | H-8 | H-8 | H-8 | – |
| 6 | H-8, H-6-OMe | H-5, H-8, H-6-OMe | H-5, H-8, H-6-OMe | H-8, H-6-OMe |
| 7 | H-1″ | H-1″ | H-1″ | H-1″ |
| 8 | H-5 | H-5 | H-5 | H-5 |
| 9 | H-2, H-8 | H-2, H-5, H-8 | H-2, H-5, H-8 | H-2, H-8 |
| 10 | H-2, H-8 | H-2, H-5, H-8 | H-2, H-5, H-8 | H-2, H-8 |
| 1′ | H-2, H-3′+5′ | H-2, H-3′+5′ | H-2, H-3′+5′ | H-2, H-3′+5′ |
| 2′, 6′ | H-3′+5′ | H-3′+5′ | H-3′+5′ | H-3′+5′ |
| 3′, 5′ | H-2′+6′ | H-2′+6′ | H-2′+6′ | H-2′+6′ |
| 4′ | H-3′+5′, H-2′+6′ | H-3′+5′, H-2′+6′ | H-3′+5′, H-2′+6′ | H-3′+5′, H-2′+6′ |
| Glc-1″ | – | – | – | H-2″ |
| 2″ | – | – | – | H-3″ |
| 3″ | – | – | – | – |
| 4″ | H-5″ | H-5″ | H-5″ | H-6″ (δ3.93) |
| 5″ | H-6″ (δ3.69) | H-6″ (δ3.70) | H-6″ (δ3.90) | H-6″ (δ3.93) |
| 6″ | – | – | H-1‴ | H-1‴ |
| Xyl-1‴ | | | H-5‴, H-6‴ | H-5‴, H-6‴ |
| 2‴ | | | H-3‴ | H-3‴ |
| 3‴ | | | H-5‴ | H-4‴, H-5‴ |
| 4‴ | | | H-5‴ | H-3‴, H-5‴ |
| 5‴ | | | H-1‴, H-4‴ | H-1‴, H-4‴ |
| 6-OMe | – | – | – | – |

## Acknowledgements

This research was supported in part by a grant (1996) from the Korea Research Foundation.

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