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NEW LIGNAN FROM THE FLOWERS OF *Forsythia koreana*

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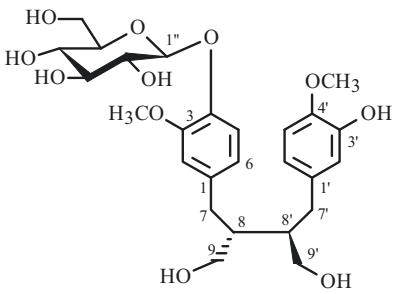
*A new lignan (**1**) was isolated from the flowers of* Forsythia koreana *along with one known lignan, 4,4′-di*-O-β-*D-glucosylpinoresinol (**2**). The molecular structures were determined using spectral methods. These compounds were isolated from* F. koreana *flowers for the first time.*

Keywords: flower, *Forsythia koreana*, koreanaside C, lignan, pinoresinol 4,4′-di-*O*-*β*-D-glucopyranoside.

Forsythia koreana (Oleaceae), a perennial shrub, is widely distributed in China and Korea. It grows up to 1–3 m high and has oblong and ovate-lanceolate leaves [1]. The fruits of *F. koreana* (Forsythiae fructus), which are known in Korea as "Yeon-kyo," are used in oriental medicine as an antipyretic medication and a detoxification agent [2]. They also exhibited inhibition effects on inflammatory and asthmatic symptoms [3, 4]. Previous studies isolated numerous phenylethanoids and lignans from Forsythiae fructus [5, 6]. However, phytochemicals present in the flowers of *F. koreana* have not been well studied. Here, we describe one new lignan of *F. koreana* flowers.

Dried *F. koreana* flowers were extracted in aqueous MeOH, and the concentrates were successively partitioned into EtOAc, *n*-BuOH, and aqueous fractions by polarity according to [7, 8]. Repeated SiO_2 column chromatography of the *n*-BuOH and H_2O fractions yielded two lignans. Their chemical structures were determined on the basis of NMR, IR, and FAB-MS data. The known lignan, compound **2**, was identified as pinoresinol 4,4′-di-*O*-*β*-D-glucoside by comparison with previously reported data [9].



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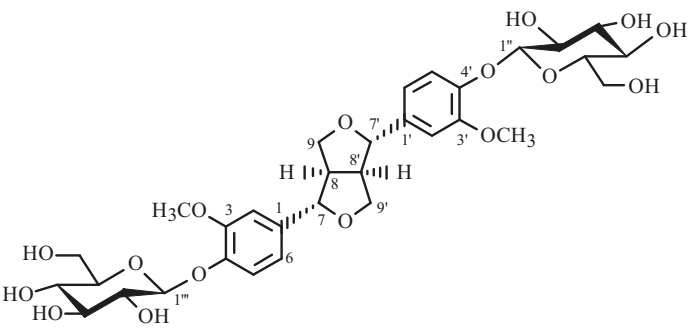

2

Compound **1** was isolated as a white amorphous powder, with mp of 83–84°C and an $[\alpha]_D$ value of –47.8°. The molecular weight was determined to be 524 from the molecular ion peak *m/z* 547 $[M + Na]^+$ in positive FAB/MS, and the molecular formula was determined to be $C_{26}H_{36}O_{11}$ according to the highly-resolved molecular ion peak *m/z* 547.2151 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_{11}Na$, 547.2155) in positive HR-FAB/MS. IR absorbance bands of hydroxyl (3455 cm^{-1}) and aromatics (1625, 1615, 1525 cm^{-1}) were detected. The ^1H NMR signals of six olefine methines [δ 6.59 (1H, dd, J = 8.0, 2.0 Hz,

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H-6′), 6.71 (1H, d, J = 2.0 Hz, H-2′), 6.86 (1H, d, J = 8.0 Hz, H-5′), 6.63 (1H, dd, J = 8.0, 2.0, H-6), 6.72 (1H, d, J = 2.0, H-2), 7.19 (1H, d, J = 8.0, H-5)] were attributed to two 1,2,4-trisubstituted benzene rings. Proton signals were also observed for two oxygenated methylenes (δ 3.71, overlapped, H-9b, 9′b; 3.81, overlapped, H-9a, 9′a), two methoxyls (δ 3.40 s; 3.43 s), two methylenes (δ 2.74, overlapped, H-7, 7′), and two methines (δ 2.12, m, H-8, 8′). The proton signals of a hemiacetal (δ 5.28, d, J = 6.4 Hz, H-1″), an oxygenated methylene (δ 4.08, dd, J = 12.0, 5.2 Hz, H-6″b; 4.27, dd, J = 12.0 Hz, 1.2, H-6″a), and four oxygenated methines (3.53, overlapped, H-5″; 3.65, overlapped, H-2″; 3.69, overlapped, H-4″; 3.73, overlapped, H-3″) were attributed to a hexose. The large coupling constant of the anomer proton signal (J = 6.4 Hz) confirmed that both the anomer proton (H-1″) and the next proton (H-2″) were in axial positions. The above-mentioned proton signals indicated that compound **1** was an enterodiol-type lignan monoglycoside. The ^{13}C NMR data showed 26 peaks comprising those of one hexose and two methoxyls, confirming that compound **1** is a lignan monoglycoside with two methoxy groups and one hexose. The carbon chemical shifts revealed the hexose to be *β*-glucopyranose. Four oxygenated olefine quaternaries (δ 146.9, C-4; 149.2, C-4′; 150.2, C-3; 150.7, C-3′), two olefine quaternaries (δ 133.7, C-1; 137.0, C-1′), six olefine methines (δ 114.2, C-2; 114.9, C-2′; 116.9, C-5; 117.2, C-5′; 122.7, C-6; 123.1, C-6′), two oxygenated methylenes (δ 61.7, C-9′; 61.9, C-9), two methoxyls (δ_C 56.6, C-OCH$_3$ × 2), two methines (δ 44.9, C-8′; 45.1, C-8), and two methylenes (δ 36.4, C-7, 7′) were attributed to the aglycone moiety. The gHMBC correlation between the anomer proton (δ 5.28, H-1″) and oxygenated olefine quaternary carbon (δ 146.9, C-4) designated the β-glucose to be at C-4. Two methoxy protons signals (δ 3.43; 3.40) correlated with the oxygenated olefine quaternary carbons (δ 149.2, C-4′; 150.2, C-3), indicating two methoxyls to be at C-3 and C-4′. The absolute stereostructures of chiral carbons were determined to be 8*R* and 8′*R* from the negative Cotton effect at 239 nm (Δε –2.3) and the positive Cotton effect at 284 nm (Δε +1.3) in the CD spectrum [10]. Taken together, the chemical structure of compound **1** was determined to be a (8*R*,8′*R*)-3′-hydroxy-3,4′-dimethoxyenterodiol 4-*O*-*β*-D-glucopyranoside, which is a new lignan that we named koreanaside C.

EXPERIMENTAL

General. Experiments were performed as described previously [11]. Circular dichroism (CD) spectra were obtained with a Chirascan Plus instrument (Applied Photophysics, Surrey, UK).

Plant Material. *F. koreana* flowers were collected from Kyunghee University Campus, Yong-In, Korea in April 2015 and their identity was confirmed by Prof. D. K. Kim, Woossuk University, Jeonju, Korea. A voucher specimen was lodged with the Natural Product Chemistry Laboratory, Kyunghee University.

Extraction of *F. koreana* Flowers and Isolation of Lignans. *F. koreana* flowers were dried in the shade for 3 days and stored at 4°C until used for extraction. The dried flowers of *F. koreana* (962 g) were extracted with 80% aqueous MeOH (33 L × 2) at room temperature for 24 h. The concentrated extracts were poured into H_2O (500 mL) and successively extracted with ethyl acetate (EtOAc, 500 mL × 5) and *n*-butanol (*n*-BuOH, 500 mL × 4). Separation of each layer and concentration under reduced pressure resulted in an EtOAc fraction (45 g, FKE), *n*-BuOH fraction (110 g, FKB), and aqueous fraction (213 g, FKH). Fraction FKB (110 g) was subjected to silica gel (SiO_2) column chromatography (CC) (∅ 11 × 15 cm) and eluted with $CHCl_3$–MeOH–H_2O (30:3:1→20:3:1→10:3:1, 43 L of each) → EtOAc-*n*-BuOH–H_2O (8:10:2, 45 L) by examining the obtained fractions using TLC, yielding 15 fractions (FKB-1–FKB-15). Fraction FKB-6 [elution volume/total volume (Ve/Vt) 0.413–0.486, 3.1 g] was subjected to SiO_2 CC (∅ 5 × 17 cm) and eluted with $CHCl_3$–MeOH–H_2O (24:6:2, 3.9 L), giving 16 fractions (FKB-6-1–FKB-6-16). Fraction FKB-6-12 (Ve/Vt 0.382–0.547, 1.6 g) was subjected to ODS CC (∅ 4 × 5 cm) and eluted with MeOH–H_2O (1:3, 2 L) to give 13 fractions (FKB-6-12-1–FKB-6-12-13), including purified compound **1** [FKB-6-12-9, 55.6 mg, Ve/Vt 0.215–0.600, TLC (Kieselgel 60 F_{254}) R_f 0.51, $CHCl_3$–MeOH–H_2O (7:3:1), (RP-18 F_{254S}) 0.55, acetone–H_2O (1:3)]. Fraction FKH (213 g) was subjected to Diaion HP-20 CC (∅ 12 × 15 cm) and eluted with 100% H_2O (30 L) → 100% MeOH (13 L), yielding six fractions (FKH-1–FKH-6). Fraction FKH-6 (Ve/Vt 0.815–1.000, 30.0 g) was subjected to SiO_2 CC (∅ 11 × 15 cm) and eluted with $CHCl_3$–MeOH–H_2O (10:3:1→8:3:1→7:3:1, 35 L of each) to give 15 fractions (FKH-6-1–FKH-6-15). Fraction FKH-6-8 (Ve/Vt 0.271–0.486, 816 mg) was subjected to ODS CC (∅ 3 × 5 cm) and eluted with MeOH–H_2O (2:3→2:1, 800 mL of both) to give 13 fractions (FKH-6-8-1–FKH-6-8-13). Fraction FKH-6-8-3 (Ve/Vt 0.043–0.077, 325 mg) was subjected to Sephadex LH-20 CC (∅ 2 × 60 cm) and eluted with 100% MeOH (1 L) to give 10 fractions (FKH-6-8-3-1–FKH-6-8-3-10). Fraction FKH-6-8-3-6 (Ve/Vt 0.500–0.515, 119 mg) was subjected to SiO_2 CC (∅ 2.5 × 15 cm) and eluted with EtOAc–*n*-BuOH–H_2O (12:3:1→8:3:1, 1.6 L of both) to give 15 fractions (FKH-6-8-3-6-1–FKH-6-8-3-6-15), including purified compound **2** [Fr. FKH-6-8-3-6-13, 14.5 mg, Ve/Vt 0.500–0.646, TLC (Kieselgel 60 F_{254}) R_f 0.35, EtOAc–*n*-BuOH–H_2O (6:3:1), (RP-18 F_{254S}) 0.72, acetone–MeOH–H_2O (1:1:2)].

Koreanaside C (1). White amorphous powder (MeOH), mp 83–84°C, $[\alpha]_D$ –47.8° (*c* 0.5, MeOH). HR FAB/MS *m/z* 547.2151 $[M + Na]^+$ (cacld for $C_{26}H_{36}O_{11}Na$, 547.2155). IR (KBr, ν, cm^{-1}): 3455, 1625, 1615, 1525. ^1H NMR (400 MHz, pyridine-d_5, δ, J/Hz): 2.12 (2H, m, H-8, 8′), 2.74 (4H, overlapped, H-7, 7′), 3.40 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.53 (1H, overlapped, H-5″), 3.65 (1H, overlapped, H-2″), 3.69 (1H, overlapped, H-4″), 3.71 (2H, overlapped, H-9b, 9′b), 3.73 (1H, overlapped, H-3″), 3.81 (2H, overlapped, H-9a, 9′a), 4.08 (1H, dd, J = 12.0, 5.2, H-6″b), 4.27 (1H, dd, J = 12.0, 1.2, H-6″a), 5.28 (1H, d, J = 6.4, H-1″), 6.59 (1H, dd, J = 8.0, 2.0, H-6′), 6.63 (1H, dd, J = 8.0, 2.0, H-6), 6.71 (1H, d, J = 2.0, H-2′), 6.72 (1H, d, J = 2.0, H-2), 6.86 (1H, d, J = 8.0, H-5′), 7.19 (1H, d, J = 8.0, H-5). ^{13}C NMR (100 MHz, pyridine-d_5, δ, ppm): 36.4 (C-7′), 36.4 (C-7), 44.9 (C-8′), 45.1 (C-8), 56.6 (C-$OCH_3 \times 2$), 61.7 (C-9′), 61.9 (C-9), 63.0 (C-6″), 71.9 (C-4″), 75.5 (C-2″), 79.0 (C-3″), 79.3 (C-5″), 103.4 (C-1″), 114.2 (C-2), 114.9 (C-2′), 116.9 (C-5), 117.2 (C-5′), 122.7 (C-6), 123.1 (C-6′), 133.7 (C-1), 137.0 (C-1′), 146.9 (C-4), 149.2 (C-4′), 150.2 (C-3), 150.7 (C-3′).

Pinoresinol-4,4′-di-*O*-*β*-D-glucopyranoside (2). Colorless needles (MeOH), mp 219–220°C, $[\alpha]_D$ –76.4° (*c* 1.0, MeOH). FAB-MS *m/z* 683.2 $[M + H]^+$. IR (KBr, ν, cm^{-1}): 3460, 3450, 1597, 1514, 1460.

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