A Phenylethanoid Glycoside and Other Constituents from the Fruits of *Forsythia koreana*

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From the methanol extract of the fruits of *Forsythia koreana* two new compounds, one phenylethanoid glycoside and one pyrrolidine alkaloid, have been isolated together with six known compounds. The structures of the isolated compounds were elucidated on the basis of 1D and 2D NMR spectroscopic methods and comparison with literature data.

Key words: Forsythia koreana, Phenylethanoid, Pyrrolidine Alkaloid

Introduction

The Oleaceae is a family of medium size with 25 genera and about 600 species. With regard to chemical constituents, it has been quite extensively investigated. It is mainly characterized by the presence of iridoid glucosides and phenylethanoid derivatives in the form of esters and glycosides of tyrosol (*p*-hydroxyphenyl-ethanol) and in particular the closely related dopaol (3,4-dihydroxyphenyl-ethanol). Coumarins and lignan glucosides are also common in the family, but they appear to have a more limited distribution [1 – 2].

The fruits of *Forsythia koreana* Nakai (Oleaceae) are known to have diuretic and damp-heat clearing actions, and have been used for the treatment of dysuria, edema, urinary tract infection, and retention of fluid in oriental traditional medicine [3]. This plant is known to possess antibacterial, antiinflammatory, and diuretic activities [4]. However, the mechanisms of action of this plant are still unknown.

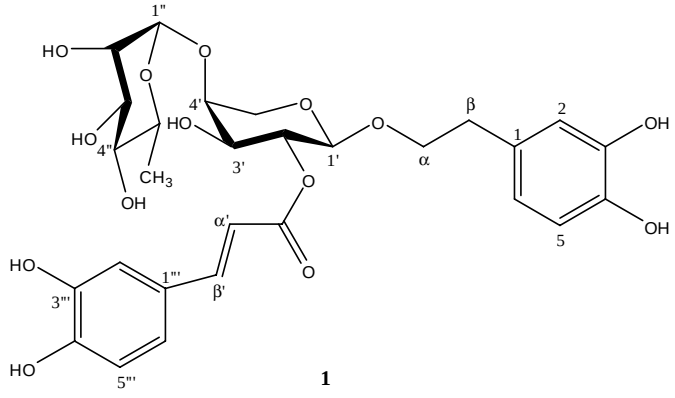
We describe here the isolation and structure elucidation of two new compounds from the methanol extract of the dried fruits of *F. koreana*, β-(3,4-dihydroxyphenyl)ethyl-*O*-α-rhamnopyranosyl-(1→4)-2-*O*-(*E*)-caffeoyl-α-arabinopyranoside (**1**) and (2*R*,3*S*)-3-hydroxy-2-hydroxypropylpyrrolidine (**2**) together with six known compounds (**3** – **8**).

Results and Discussion

The air-dried fruits of *F. koreana* (2 kg) were extracted with hot methanol. The methanol extract was successively portioned with hexane, EtOAc and *n*-BuOH. The resulting *n*-BuOH extract was applied to column chromatography using polyamide, silica gel and Sephadex LH-20 to obtain eight compounds (**1** – **8**), of which six were previously known. The known compounds exhibited physical and spectroscopic data identical to values reported in the literature: matairesinol (**4**) [5], arctigenin (**5**) [6 – 7], (+)-pinoresinol-4′-β-D-glucopyranoside (**6**) [7 – 8], tropine (**7**) [9], and nortropine (**8**) [9]. Two new compounds (Fig. 1), β-(3,4-dihydroxyphenyl)ethyl-*O*-α-rhamnopyranosyl-(1→4)-2-*O*-(*E*)-caffeoyl-α-arabinopyranoside (**1**) and (2*R*,3*S*)-3-hydroxy-2-hydroxypropylpyrrolidine (**2**) were identified based on chemical and spectroscopic evidence. Among the six known isolated compounds, **3**, **7** and **8** are reported here for the first time in the genus *Forsythia*.

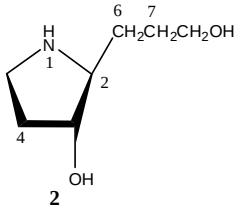
The molecular formula of compound **1** was determined as $C_{28}H_{34}O_{14}$ by positive-ion HR-FABMS, exhibiting a molecular ion peak at *m/z* = 595.2021 (calcd. 595.2026 for $C_{28}H_{35}O_{14}$, $[M+H]^+$). The ^{1}H NMR spectrum of **1** (Table 1) showed two anomeric protons at δ_H = 4.63 (d, *J* = 1.5 Hz, H-1″ of rhamnose) and 4.35 (d, *J* = 6.6 Hz, H-1′ of arabinose), indicating the presence of two sugar units in **1**. The corresponding anomeric carbon resonances were observed at δ_C = 101.1 and 103.3, respectively, in the ^{13}C NMR spectrum. The low *J* values of the anomeric protons indicated α-orientations for both rhamnopyranosyl and arabinopyranosyl moieties.

Acid hydrolysis of **1** gave rhamnose and arabinose which were identified by TLC, confirming these deductions. The ^{1}H NMR spectrum also displayed pro-



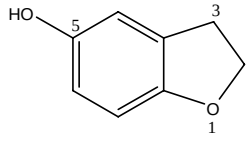
1

β-(3,4-Dihydroxyphenyl)ethyl-*O*-*α*-rhamnopyranosyl-(1➛4)-2-*O*-(*E*)-caffeoyl-*α*-arabinopyranoside



2

(2R,3*S*)-3-Hydroxy-2-hydroxypropylpyrrolidine



3

2,3-Dihydrobenzo[*b*]furan-5-ol

Fig. 1. Compounds **1** – **3** isolated from the fruits of *Forsythia koreana* Nakai.

ton signals characteristic of an *E*-caffeoyl group (three aromatic protons resonating at δ = 7.10, 6.98 and 6.82 as an ABX system and two *trans* olefinic protons as an AB system at δ = 7.62 and 6.33, J = 15.9 Hz) and a 3,4-dihydroxyphenylethanol moiety (three aromatic protons at δ = 6.73, 6.71 and 6.59 as an ABX system, and signals of the side chain of the aglycone moiety as a double doublet at δ = 2.81 due to a β-methylene group which are mutually coupled with two non-equivalent protons of α-methylene at δ = 4.01 and 3.74).

Although some of the remaining sugar proton signals (Table 1) overlapped in the ^{1}H NMR spectrum, the corresponding NMR signals were well dispersed and assigned by an HSQC experiment in which all protons were unambiguously correlated with those of the corresponding carbon atoms. The ^{13}C NMR data and DEPT experiments (Table 1) showed clearly the presence of one methyl, three methylene, seventeen methine and seven quaternary carbon resonances. The linkage of the acyl group to the arabinosyl moiety was deduced from the downfield shifted signal of H-2′ at δ = 4.05 in the ^{1}H NMR spectrum. This was confirmed by the signal of C-2′ shifted downfield to δ = 74.5 in the ^{13}C NMR spectrum, and the HMBC correlation between H-2′ at δ = 4.05 and the carbonyl carbon of the acyl moiety at δ = 167.3. Additionally, the attachment of the rhamnosyl moiety to C-4′ of arabinose was deduced from the downfield shift of C-4′ of arabinose to δ = 74.6 and was confirmed by the HMBC correlation between H-1″ of rhamnose at δ = 4.63 and C-4′ of arabinose at δ = 74.6. It was also proved by the HMBC spectrum, that the α-arabinosyl moiety was the core sugar, showing correlation between the anomeric proton H-1′ at δ = 4.35 and C-α of the aglycone moiety at δ = 70.8. Consequently, the structure of compound **1** was elucidated to be β-(3,4-dihydroxyphenyl)ethyl-*O*-α-rhamnopyranosyl-(1→4)-2-*O*-(*E*)-caffeoyl-α-arabinopyranoside.

The ^{13}C NMR analysis and DEPT measurements of **2** (Table 2) revealed the presence of five methylene and two methine carbon atoms. This result and the positive-ion HR-FABMS of **2** which exhibited the molecular ion peak at *m/z* = 146.1173 (calcd. 146.1181 for $C_7H_{16}NO_2$, $[M+H]^+$) established that the molecular formula was $C_7H_{15}NO_2$.

The ^{1}H NMR data (Table 2), together with HSQC and HMBC experiments, defined the complete connectivity of the carbon and hydrogen atoms in **2**. From these NMR data, the relatively down-field methine carbon at δ = 65.6 (C-2) and the methylene carbon at δ =

Table 1. ^{1}H and ^{13}C NMR data and HMBC correlations of compound **1** (600 MHz, CD_3OD).

Position	δ_C	DEPT	δ_H	HMBC (C→H)
Aglycone				
1	130.2	C		H-α, H-β, H-5
2	113.5	CH	6.73 d (2.1)	H-6
3	145	C		H-5
4	143.5	C		H-2, H-6
5	115.2	CH	6.71 d (8.0)	
6	120.2	CH	6.59 dd (2.1/8.0)	H-β, H-2
α	70.8	CH_2	4.01 m, 3.74 *m*	H-1′
β	35.5	CH_2	2.81 t (6.9)	H-2, H-6
Arabinose				
1′	103.3	CH	4.35 d (6.6)	H-α, H-5′
2′	74.5	CH	4.05 dd (6.6/10.1)	H-1′, H-4′
3′	72.7	CH	3.52[a]	H-1′, H-5′
4′	74.6	CH	3.92[a]	H-1″, H-2′
5′	66.4	CH_2	3.84 dd (2.8/11.5)	H-1′
Rhamnose				
1″	101.1	CH	4.63 d (1.5)	H-4′, H-3″
2″	71.1	CH	3.91 dd (1.5/3.5)	
3″	71.3	CH	3.65 dd (3.5/ 9.7)	H-1″
4″	73.5	CH	3.91[a]	H-2″, H-6″
5″	68.9	CH	3.52[a]	
6″	16.9	CH_3	1.23 d (6.1)	
Caffeoyl				
1‴	126.5	C		H-α′, H-β′, H-5‴
2‴	115.4	CH	7.10 d (1.8)	
3‴	145.6	C		H-5‴
4‴	146.5	C		H-2‴, H-5‴, H-6‴
5‴	116	CH	6.82 d (8.1)	
6‴	122	CH	6.98 dd (1.8/8.1)	H-2‴, H-β′
α′	114	CH	6.33 d (15.9)	H-β′
β′	148.6	CH	7.62 d (15.9)	H-2‴, H-6‴
C=O	167.2	C		H-2′, H-α′, H-β′

[a] Overlapping signals.

38.3 (C-5) indicated that these must be bonded to the nitrogen atom of the heterocyclic ring. The downfield methine carbon at δ = 81.0 (C-3) was attributed to the hydroxymethine carbon. Additionally, four methylene carbons at δ = 34.7, 29.9, 31.5 and 64.9 were assigned to C-4, C-6, C-7 and C-8 bearing an OH group, respectively.

In the HMBC spectrum of **2**, the long range correlation between H-3 at δ_H =3.84 and both C-5 at δ_C = 38.3 and C-6 at δ_C = 29.9 and *vice versa* supported the site of hydroxylation of the pyrrolidine ring.

The relatively large coupling constant of the pyrrolidine ring H-2 and H-3 protons ($J_{2,3}$ = 7.1 Hz) suggested that this proton pair is in the *trans* configuration.

The relative configuration at the stereogenic centers of the pyrrolidine ring was also corroborated by the NOESY spectrum of **2** following the NOE correlations between H-3 and C-6 (CH_2) protons. Furthermore, a NOE between H-2 and H-4α was observed. These definite NOE correlations indicate that H-3 and C-6 (CH_2) are on the same side of the pyrrolidine ring, and H-2 and the hydroxyl group at C-3 are on the opposite side of the ring. The optical rotation of **2** was −43.7°, similar to that of (2*R*,3*S*)-3-hydroxy-2-hydroxymethylpyrrolidine [10]. Therefore the absolute configuration of **2** was assumed to be (2*R*,3*S*). Hence, alkaloid **2** was determined to be (2*R*,3*S*)-3-hydroxy-2-hydroxypropylpyrrolidine (Fig. 1).

Table 2. ^{1}H and ^{13}C NMR data of compounds **2** and **3** (600 MHz, CD_3OD).

Position	δ_C	DEPT	δ_H
Compound **2**			
2	65.6	CH	3.31 ddd (4.6/6.4/7.1)
3	81	CH	3.84 ddd (4.4/7.1/8.1)
4	34.7	CH_2	1.97 m, H-4α
			1.75 m, H-4β
5	38.3	CH_2	2.90 m, H-5α
			3.01 m, H-5β
6	29.9	CH_2	1.68 m
7	31.5	CH_2	1.60 m
8	64.9	CH_2	3.76 m
Compound **3**			
2	63.5	CH_2	3.69 t (7.2)
3	38.4	CH_2	2.67 t (7.2)
4	120.2	CH	6.67 d (1.8)
5	144.9	C	
6	116	CH	6.53 dd (1.8/7.8)
7	115.2	CH	6.70 d (7.8)
3a	130.6	C	
7a	143.4	C	

Although compound **3** has been chemically synthesized [11], this is the first report of its natural occurrence. The molecular formula of **3** was determined to be $C_8H_8O_2$ by positive-ion HR-FABMS which exhibited a molecular ion peak at *m/z* = 137.0595 (calcd. 137.0602 for $C_8H_9O_2$, $[M+H]^+$). The ^{1}H NMR spectrum of **3** (Table 2) showed signals at δ_H = 2.67 (t, *J* = 7.2 Hz) and 3.69 (t, *J* = 7.2 Hz), assigned to the 3-methylene and the 2-oxymethylene groups, respectively, corresponding to the furan ring of the dihydrobenzofuran. These proton signals were correlated with the carbon signals at δ_C = 38.4 (C-3) and 63.5 (C-2) in the HSQC experiment and indicate the presence of a 2,3-dihydrobenzofuran unit. The ^{1}H NMR spectrum also showed the presence of three aromatic protons at δ_H = 6.70 (d, *J* = 7.8 Hz), 6.67 (d, *J* = 1.8 Hz) and 6.53 (dd, *J* = 7.8/1.8 Hz), assigned to H-7, H-4 and H-6, respectively. The ^{13}C NMR spectrum and DEPT measurements of **3** (Table 2) revealed in addition to the two methylene groups of the furan ring two me-

thine carbons at δ_C = 116.0 (C-6) and 120.2 (C-4) and three quaternary carbons at δ_C = 130.9 (C-3a), 143.4 (C-7a) and 144.9 (C-5). In the HMBC spectrum of **3**, the correlation peaks H-2/C-7a, H-2/C-3a, H-3/C-4, H-6/C-4, H-4/C-6 and H-7/C-5 also supported the substitution on the benzene ring. Therefore, the structure of **3** was established as 2,3-dihydrobenzo[*b*]furan-5-ol (Fig. 1).

Experimental Section

General experimental procedures

For column chromatography (CC), silica gel 60, 0.040 – 0.063 mm (Merck), polyamide 6, 50 – 160 μm (Fluka) and Sephadex LH-20 (Pharmacia) were used. TLC analysis was carried out using silica gel 60 F_{254} plates (Merck); chromatograms were visualized under UV light at 240 and 366 nm and sprayed with anisaldehyde reagent (Aldrich). UV spectra were recorded on an X-ma 2000 UV/vis spectrophotometer. IR spectra were obtained on a Bruker IFS 113v instrument. Optical rotations were measured on a Jasco P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were obtained using a Varian Unity Inova 600 spectrometer in [D_4]-MeOH. The chemical shifts are given in δ (ppm) relative to Me_4Si. HR-FABMS spectra were recorded on a Jeol JMS-SX 102A spectrometer.

Plant material

Fruits of *Forsythia koreana* Nakai were purchased from the GyungDong Herbal drug market and identified by faculty members of the Department of Forest Products, Kookmin University. A voucher specimen (YKP04-624) has been kept in the herbarium of the Department of Forest Products, Kookmin University, Seoul, Korea.

Extraction and isolation

The dried powdered fruits (2 kg) were extracted with hot MeOH (8 L). The combined extracts were concentrated under reduced pressure to yield a dark gum (43 g) which was suspended in water and partitioned with hexane, EtOAc and *n*-BuOH.

The lyophilized *n*-BuOH phase (15 g) was fractionated over polyamide CC eluting with increasing amounts of MeOH in EtOH (20 – 100 %) to afford 6 fractions.

Fraction 1 was further applied to polyamide CC, eluted with H_2O-EtOH (5 : 1) to give **4** (35 mg) and **5** (43 mg). Purification of fraction 2 by Sephadex LH-20 CC (MeOH) furnished **7** (80 mg) and **8** (22 mg). Fraction 3 was applied to silica gel CC eluting with CH_2Cl_2-MeOH (3 : 1) to afford **3** (41 mg). Fraction 4 was separated by silica gel CC (CH_2Cl_2-MeOH-H_2O, 4.5 : 1 : 0.1) to give **2** (22 mg). Fraction 5 was chromatographed over silica gel eluting with CH_2Cl_2-MeOH (1 : 1) to give **6** (28 mg). Repeated chromatography of fraction 6 on a Sephadex LH-20 column (MeOH) yielded **1** (52 mg).

*Acid hydrolysis of compound **1***

A solution of compound **1** (10 mg) in 2M HCl (2 mL) was refluxed for 3 h. The reaction mixture was diluted with H_2O and then extracted with EtOAc. The aqueous phase was neutralized with $BaCO_3$ and evaporated to dryness. The residue was identified by comparison with authentic samples of rhamnose and arabinose by TLC on silica gel with pyridine-EtOAc-AcOH-H_2O (36 : 36 : 7 : 21) and aniline phthalate as spray reagent.

*β-(3,4-Dihydroxyphenyl)ethyl-O-α-rhamnopyranosyl-(1→4)-2-O-(E)-caffeoyl-α-arabinopyranoside (**1**)*

Amorphous pale yellow solid. $[\alpha]_D$ = −48° (*c* = 0.5, MeOH). – UV (MeOH): λ_{max} = 226, 249(sh), 292, 324 nm. – IR (KBr): ν = 3415 (OH), 1690 (C=O), 1625 (C=C), 1605, 1520, 1457 (aromatic rings) cm^{-1}. – ^{1}H and ^{13}C NMR spectra: see Table 1.

*(2R,3S)-3Hydroxy-2-hydroxypropylpyrrolidine (**2**)*

Amorphous white solid. $[\alpha]_D$ = −43.7° (*c* = 1.5, MeOH). – IR (KBr): ν = 3480 (OH), 3350 (NH), 2905 (C–H), 1650 (C–C), 1120 (C–O) cm^{-1}. – ^{1}H and ^{13}C NMR spectra: see Table 2.

*2,3-Dihydrobenzo[b]furan-5-ol (**3**)*

Amorphous brown solid. UV (MeOH): λ = 290 nm. – IR (KBr): ν = 3358 (OH), 1632, 1532, 1509, 1462 (aromatic ring), 1109 (C–O) cm^{-1}. – ^{1}H and ^{13}C NMR spectra: see Table 2.

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