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Article

New Phenylethanoid Glycosides from the Fruits of *Forsythia Suspense* (Thunb.) Vahl

Fu Nan Wang [1,2], Zhi Qiang Ma [3], Ying Liu [4], Ying Zhi Guo [2] and Zhong Wei Gu [5,*]

[1] Graduate School, Peking Union Medical College, Beijing 100005, P.R.China; E-mail: fnwang9966@yahoo.com.cn (F-N.W.)

[2] National Research Institute for Family Planning, Beijing 100081, P.R.China

[3] Harbin Pharm. Group Co., Ltd., Second Chinese Medicine Factory, Harbin,150078, P.R.China; E-mail: mazhiqiangsapo@tom.com (Z-Q.M.)

[4] Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China; E-mail: liuying@imm.ac.cn (Y. L.)

[5] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, P.R.China

* Author to whom correspondence should be addressed; E-mail: zwgu@scu.edu.cn; Fax: +86-28-85410653.

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Abstract: Forsythosides H-J (**1-3**), three new caffeoyl phenylethanoid glycosides (CPGs), were isolated from the fruits of *Forsythia suspense* (Thunb.) Vahl., together with six known phenylethanoid glycosides: Forsythoside A (**4**), Forsythoside F (**5**), Forsythoside E (**6**), 2-(3,4-dihydroxyphenyl)ethyl-*β*-D-glucopyranoside (**7**), phenethyl alcohol *β*-D-xylo-pyranosyl-(1→6)-*β*-D-glucopyranoside (**8**) and calceolarioside B (**9**). Their structures were determined by spectroscopic and chemical methods.

Keywords: Caffeoyl phenylethanoid glycosides; *Forsythia suspense* (Thunb.) Vahl.; Forsythosides H-J.

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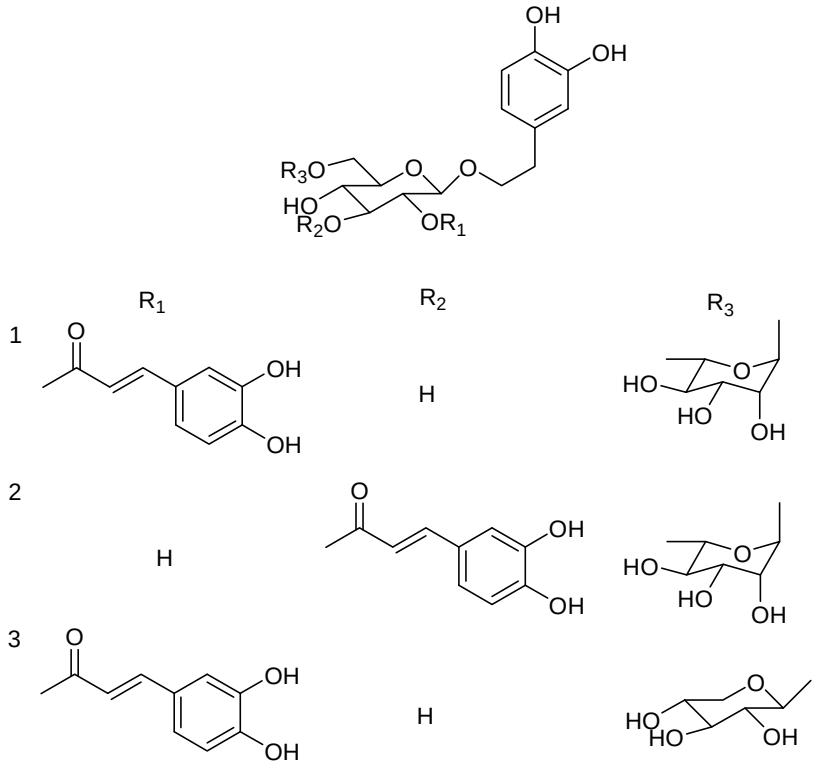
1. Introduction

Forsythia suspense (Thunb.) Vahl. is widely distributed in China, Korea and Japan. The fruits of this plant, known as "Lianqiao" (Chinese), have been used as a Chinese traditional medicine to treat inflammation, pyrexia, ulcer, gonorrhea and erysipelas [1]. A number of chemical constituents with diverse structures, including phenylethanoid glycosides [2-11], lignans [12-14] and flavonoids [2,15] have been reported from species of this genus. The interesting chemical, pharmacological, and clinical significance of *Forsythia suspense* (Thunb.) Vahl. prompted us to carry out the current project, which has led to the isolation of three new caffeoyl phenylethanoid glycosides **1**-**3** and six known compounds.

2. Results and Discussion

Repeated column chromatography of the extract of *Forsythia suspense* (Thunb.) Vahl. yielded three new caffeoyl phenylethanoid glycosides designated as Forsythosides H-J (**1**-**3**, Figure 1), together with six known phenylethanoid glycosides. These known compounds were identified as Forsythoside A (**4**) [10], Forsythoside F (**5**) [12], Forsythoside E (**6**) [6], 2-(3,4-dihydroxyphenyl)ethyl*β*-D-glucopyranoside (**7**) [17], phenethyl alcohol *β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside **(8)** [18] and calceolarioside B (**9**) [19] by comparison of their spectroscopic data (UV, IR, ESIMS, ^{1}H- and ^{13}C-NMR) with that reported in the literature.

Figure 1. Structures of Forsythoside H (**1**), Forsythoside I (**2**) and Forsythoside J (**3**).



Forsythoside H (**1**) was obtained as a brown amorphous powder. The presence of hydroxyl (3,339 cm^{-1}) and carbonyl (1,694 cm^{-1}) groups were evident in its IR spectrum. The negative mode ESIMS of **1** gave a quasi-molecular ion peak at *m/z* 623 $[M-H]^-$. Its molecular formula, $C_{29}H_{36}O_{15}$, was established by HRESIMS 623.1960 (calcd. for $C_{29}H_{35}O_{15}$: 623.1976), corresponding to twelve degrees of unsaturation. The ^{1}H-NMR spectrum revealed the presence of two sets of ABX systems [δ 6.54 (br.s), δ 6.55 (d, J = 8.4 Hz) and δ 6.41 (dd, J = 8.4, 1.8Hz) for the 3,4-dihydroxyphenylethyl moiety; and δ 7.06 (br.s), δ 6.76 (d, J = 7.2 Hz) and δ 7.01 (br.d, J = 7.2 Hz) for the caffeoyl moiety], two trans-olefinic protons [δ 6.27 and 7.49 (each d, J = 16.2 Hz)], together with two anomeric protons at δ 4.49 (d, J = 8.4 Hz) for β-glucose, and δ 4.60 (br.s) for α-rhamnose.

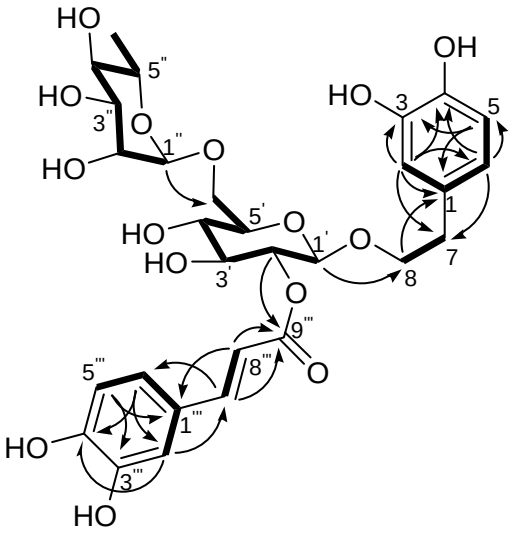
Table 1. NMR Data for Compounds **1-4** [a].

no.	**1**		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		129.1		129.5		129.2		129.2
2	6.54 br.s	115.4	6.61 d (1.8)	115.5	6.55 d (1.2)	115.4	6.62 br.s	115.5
3		144.9		144.7		144.9		144.9
4		143.5		143.5		143.5		143.5
5	6.55 d (8.4)	116.2	6.63 d (7.8)	116.3	6.54 d (7.8)	116.2	6.63 d (7.8)	116.3
6	6.41 dd (1.8, 8.4)	119.6	6.48 dd (1.8, 7.8)	119.5	6.41 dd (1.2, 7.8)	119.6	6.49 dd (1.8, 7.8)	119.5
7	2.56 m	35.1	2.69 m	35.1	2.56 m	35.0	2.67 m	35.1
8	3.76 m	69.8	3.82 m	70.2	3.78 m	69.8	3.83 m	70.3
	3.54 m		3.62 m		3.53 m		3.61 m	
1′	4.49 d (8.4)	100.2	4.34 d (7.8)	102.7	4.74 d (7.8)	100.1	4.31 d (7.8)	102.9
2′	4.64 t (8.4)	73.4	3.18 dd (7.8, 9.0)	71.4	4.65 t (7.8)	73.3	3.41 dd (7.8, 9.0)	73.0
3′	3.42 m	74.1	4.88 t (9.0)	77.5	3.42 m	74.1	3.10 m	73.5
4′	3.44 m	70.7	3.28 dd (9.0, 10.2)	68.1	3.22 dd (9.0, 9.6)	70.0	4.66 t (9.6)	71.0
5′	3.38 m	75.5	3.42 m	75.1	3.39 m	75.7	3.45 m	73.9
6′	3.84 br.d (10.2)	66.6	3.82 br.d (9.6)	66.5	3.96 br.d (11.4)	65.7	3.53 br.d (13.0)	66.1
	3.48 m		3.49 m		3.58 dd (5.4, 11.4)		3.33 dd (7.5, 13.0)	
1″	4.60 br.s	100.7	4.59 br.s	100.7	4.20 d (7.2)	104.0	4.50 br.s	100.5
2″	3.63 m	70.5	3.62 m	70.6	2.98 dd (7.2, 8.4)	73.3	3.59 m	70.6
3″	3.45 m	70.3	3.62 m	70.4	3.09 dd (8.4, 9.0)	76.6	3.36 dd (9.0, 10.2)	70.6
4″	3.19 t (9.6)	72.0	3.44 m	71.9	3.28 m	69.6	3.57 t (9.0)	71.9
5″	3.46 m	68.4	3.48 m	68.4	3.70 m	68.2	3.34 m	68.4
					3.02 m			
6″	1.14 d (6.6)	18.0	1.13 d (6.6)	17.9			1.05 d (6.0)	17.8
1‴		125.5		125.6		125.5		125.4
2‴	7.06 br.s	114.8	7.04 d (1.8)	114.9	7.06 br.s	114.8	7.05 br.s	114.8
3‴		145.6		145.5		145.6		145.6
4‴		148.5		148.2		148.5		148.6
5‴	6.76 d (7.2)	115.8	6.75 d (8.4)	115.7	6.76 d (7.8)	115.8	6.76 d (8.4)	115.8
6‴	7.01 br.d (7.2)	121.3	7.01 dd (1.8, 8.4)	121.3	7.01 d (7.8)	121.3	7.01 br.d (8.4)	121.4
7‴	7.49 d (16.2)	145.2	7.47 d (15.6)	144.9	7.49 d (15.6)	145.1	7.50 d (15.6)	145.6
8‴	6.27 d (16.2)	114.2	6.26 d (15.6)	114.7	6.27 d (15.6)	114.2	6.25 d (15.6)	113.6
9‴		165.7		166.1		165.7		165.9

[a] NMR data were measured in DMSO-d_6 for **1-4** at 400 MHz for ^{1}H-NMR and at 100 MHz for ^{13}C-NMR. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H-^{1}H COSY, HSQC, HMBC and phase sensitive ^{1}H-^{1}H COSY experiments.

Acid hydrolysis of **1** yielded D-glucose and L-rhamnose in a ratio of 1:1 according to GC analysis of the trimethylsilyl-L-cysteine derivatives of the component monosaccharides, compared with the trimethylsilyl-L-cysteine derivatives of sugar standards. The NMR spectra of **1** were similar to those of the co-occurring Forsythoside A (**4**), with the only difference being in the position of the caffeoyl ester units, i.e. **1** is a positional isomer of **4**. Comparison of the ^{13}C-NMR spectral data of **1** with those of **4**, showed the chemical shifts of C-1′, C-2′ and C-3′ were changed by -2.7, +0.4 and +0.6 ppm, respectively (Table 1), indicating that the caffeoyl residue is located at C-2. The ^{1}H-NMR spectrum was in agreement with this, in particular, the low field position of H-2′ of the glucopyranosyl group (δ 4.64) showed that this was the point of acylation. Analysis of the HMQC and ^{1}H-^{1}H COSY spectra of **1** led to the unambiguous assignment of proton and carbon signals in the NMR spectra. In the HMBC spectrum, two- and three-bond correlations (Figure 2, arrows) from H-1′ to C-8 and from H-1′′ to C-6′, together with chemical shift values of these protons and carbons, revealed the connection among the 3,4-dihydroxyphenylethyl and the two sugar moieties of **1** was identical to that of Forsythoside A (**4**). Meanwhile, the location of the caffeoyl unit in **1** was indicated unequivocally by HMBC correlation from H-2′ to C-9′′′. Accordingly, the structure of **1** was determined as 2-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→6)-2-*O*-trans-caffeoyl-β-D-glucopyranoside, and was named Forsythoside H.

Figure 2. Main ^{1}H-^{1}H COSY (thick lines) and HMBC (arrows from proton to carbon) correlations of Forsythoside H (**1**).



Forsythoside I (**2**) was obtained as a brown amorphous powder, and its spectroscopic data (Table 1 and Experimental Section) indicated that it is another isomer of Forsythoside A (**4**) with a different connectivity between the caffeoyl and glucopyranosy moieties. Acid hydrolysis of **2** released D-glucose and L-rhamnose, identified by GC analysis. In the ^{13}C-NMR spectrum of **2**, a characteristic resonance at δ_C 77.5 ppm indicated a (9′′′→3′) connection between the caffeoyl moiety and glucopyranosy moiety [8]. The NMR data assignments (Table 1) and structure of **2** were established by 2D NMR experiments. Thus, compound **2** was determined to be 2-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→6)-3-*O*-trans-caffeoyl-β-D-glucopyranoside, named as Forsythoside I.

Forsythoside J (**3**) was obtained as a brown amorphous powder. It showed a quasi-molecular ion peak at *m/z* 609 $[M-H]^-$. The ^1H- and ^{13}C-NMR spectroscopic data indicated that compound **3** is a caffeoyl phenylethanoid glycoside with two sugar moieties. The chemical shifts of compound **3** were almost the same as those of **1**. However, a pair of proton signals attributed to a *β*-xylopyranosyl unit replaced those of the outer *α*-rhamnopyranosyl of **1**. Acid hydrolysis of **3** produced D-glucose and D-xylose in a ratio of 1:1 by GC analysis of the trimethylsilyl-L-cysteine derivatives of the component monosaccharides. These data demonstrated that **3** is an analogue of **1** with an outer *β*-xylopyranosyl unit. Unambiguous assignments of the NMR data of **3** (Table 1) were accomplished from the 2D NMR spectra. In the HMBC spectrum long-range correlations of H-1″ to C-6′ indicated that the *β*-xylopyranosyl moiety of **3** was located at C-6′. Therefore, **3** was established as 2-(3,4-dihydroxyphenyl)-ethyl-*O*-*β*-D-xylopyranosyl-(1→6)-2-*O*-trans-caffeoyl-*β*-D-glucopyranoside, and it was named Forsythoside J.

3. Experimental

3.1. General

IR spectra were recorded as KBr disks on Shimadzu FTIR-8700 (Shimadzu Co. Japan). 1D and 2D-NMR spectra were obtained at 400 MHz for ^1H and at 100 MHz for ^{13}C, respectively, on a Bruker AV400 spectrometer in DMSO-d_6 with TMS as references. ESIMS data were measured with a Q-Trap LC/MS/MS (Turbo Ionspray source) spectrometer. HRESIMS data were measured on an AccuToFCS JMS-T100CS spectrometer. GC data were measured on a Perkin Elmer Autosystem XL Gas Chromatograph instrument. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Preparative HPLC separation (Agilent 1100) was carried out on a reversed-phase column using a differential refractometer detector. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 5% H_2SO_4 in 95% EtOH, followed by heating**.**

3.2. Plant Material

The fruits of *Forsythia suspense* (Thunb.) Vahl. were collected at Shanxi Province, People's Republic of China, in September 2006. The plant identification was verified by Professor Qi-shi Sun (Shenyang Pharmaceutical University). A voucher specimen was deposited in the Herbarium of Shool of Traditional Chinese Medicines of Shenyang Pharmaceutical University, China.

3.3. Extraction and Isolation.

The fruits of *Forsythia suspense* (Thunb.) Vahl. (4.0 kg) were extracted with 85% EtOH under reflux. After concentration *in vacuo*, the crude EtOH extract (1.8 kg) was suspended in water and partitioned successively with petroleum ether, ethyl acetate (EtOAc), and *n*-butanol. The *n*-butanol-soluble part (182.5 g) was subjected to normal silica gel column chromatography, eluting with a gradient of increasing MeOH (0-50%) in $CHCl_3$, afford seven fractions A-G. Fraction C (36.2 g) was subjected to column chromatography, using $CHCl_3$-MeOH-H_2O as the eluting solvent, to afford six

subfractions C_1-C_6. Subfraction C_2 (164.3 mg) and C_3 (150.6 mg) were separately purified by reversed-phase preparative HPLC, using MeOH-H_2O (25:75 and 30:70) as the mobile phases, respectively, to afford **1** (9.5 mg), **2** (10.2 mg), **3** (12.3 mg), and **4** (16.1 mg) and **5** (13.6 mg). Subfraction C_6 (185.6 mg) was further separated by silica gel column chromatography, using EtOAc-MeOH-H_2O as the eluting solvent, and then purified by reversed-phase preparative HPLC, using MeOH-H_2O (45:65) as the mobile phases, to yield **6** (14.2 mg), **7** (14.2 mg), **8** (8.9 mg) and **9** (32.5 mg).

Forsythoside H (**1**): a brown amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3,339, 2,941, 1,694, 1,601, 1,518; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; HRESIMS *m/z* 623.1960 (calcd. for $C_{29}H_{35}O_{15}$: 623.1976).

Forsythoside I (**2**): a brown amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3,361, 2,933, 1,692, 1,601, 1,519; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; HRESIMS *m/z* 623.1962 (calcd. for $C_{29}H_{35}O_{15}$: 623.1976).

Forsythoside J (**3**): a brown amorphous powder; IR (KBr) ν_{max} cm^{-1}: 3,340, 2,933, 1,693, 1,597, 1,499; ^{1}H-NMR and ^{13}C-NMR data, see Table 1; HRESIMS *m/z* 609.1813 (calcd, for $C_{28}H_{33}O_{15}$: 609.1813).

3.4. Acid Hydrolysis of **1**, **2** *and* **3**

Each glycoside (5 mg) was refluxed in 2 N HCl for 3 h at 80 °C. The reaction mixture was extracted with $CHCl_3$ (3 × 5 mL) and the aqueous phase was neutralized with 1 N NaOH and dried using a stream of N_2. The residue were separately subjected to CC over silica gel with MeCN-H_2O (9:1) as the eluent to yield D-glucose and L-rhamnose from **1** and **2**, and D-glucose and D-xylose from **3**, respectively [20]. The sugar residue was then dissolved in pyridine (1 mL) and L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was left at 60 °C for 2 h and evaporated under a N_2 stream and dried *in vacuo*. The residue was trimethylsilylated with *N*-trimethylsilylimidazole (0.2 mL) at 60 °C for 1 h. The mixture was partitioned between *n*-hexane and H_2O (3 × 1 mL), and the *n*-hexane extract was subjected to GC analysis to identify the sugars. Capillary column DB-5 (30 m × 0.25 mm × 0.25 μm); detection FID; detector temperature 280 °C; injection temperature 250 °C; the initial column temperature was 100 °C, and the temperature was gradually raised to 280 °C at the rate of 10 °C/min and maintained for 5 min; carrier N_2 gas. Retention times for D-glucose, D-xylose, and L-rhamnose were 19.6, 17.6, and 18.4 min, respectively.

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Sample Availability: Available from the authors.