New Quinoid Glycosides from *Forsythia suspensa*

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Three novel compounds, suspenolic acid (**1**), forsythenside A (**2**), and forsythenside B (**3**), have been isolated from the fruits of *Forsythia suspensa*. Their structures were elucidated by spectral methods and chemical reactions.

Extensive chemical studies on the constituents of *Forsythia* species have been reported, and many compounds, including caffeoyl glycosides, cyclohexylethane derivatives, flavonoids, iridoid glycosides, lignans, and triterpenes, have been isolated.[1−5] In the case of *Forsythia suspensa* (Thunb.) Vahl. (Oleaceae), a plant used in traditional Chinese medicine as an antiinflammatory and antibacterial agent, a number of compounds such as forsythiaside, oleanolic acid, phillygenin, rengyol, and rutin have been identified.[6−9] However, no specific therapeutically useful compounds from this plant have been found. As part of a chemosystematic study and search for biologically active substances from traditional Chinese medicines, we have reinvestigated the chemical constituents of *F. suspensa*. The EtOAc- and *n*-BuOH-soluble fractions of an 70% EtOH extract were fractionated by Si gel column chromatography, respectively, to afford a number of compounds. This paper describes the isolation and structure elucidation of three new compounds, suspenolic acid (**1**), forsythenside A (**2**), and forsythenside B (**3**), along with six known compounds, all of which were obtained from this species for the first time.

Suspenolic acid (**1**) was obtained as an amorphous solid, $[\alpha]^{20}_D$ 4.7° (*c* 0.10, $CHCl_3$). Elemental analysis and a quasi-molecular ion $[M + H]^+$ occurring in the FABMS at *m*/*z* 199 indicated a molecular formula of $C_{10}H_{14}O_4$. Analysis of the IR spectrum of **1** suggested that it contained an ester carbonyl group (1734 cm^{-1}) and a carboxylic group (1691 cm^{-1}). This was also supported by fragment ions at *m*/*z* 138 $[M - AcOH]^+$, *m*/*z* 93 $[M - AcOH - COOH]^+$, and *m*/*z* 43 $[CH_3CO]^+$ in the CIMS. The ^{1}H-NMR spectrum of **1** (Table 1) exhibited signals for an acetyl methyl group (δ 2.07 s), an oxymethine group (δ 4.99 m), and a trisubstituted olefinic proton (δ 5.70 s). The olefinic proton signal occurred as singlet at lowfield, suggesting that no proton was affixed to the adjacent carbon. The ^{13}C-NMR spectrum of **1** (Table 1) showed an acetyl methyl, an ester carbonyl, a carboxylic acid carbon, an oxymethine, an olefinic methine, an olefinic quaternary carbon, and four methylene signals. The above information implied that compound **1** contains an exocyclic double bond in its structure. Furthermore, the resonances at δ 5.70 s (H-2) and the carbon signals at δ 113.5 (C-2), 163.2 (C-3), and 170.9 (C-1) provided evidence for the presence of an α,β-unsaturated carboxylic acid. In the ^{1}H−^{1}H COSY NMR spectrum, the H-6 signal coupled with the H-5 and H-7 resonances, which, in turn, were correlated with the H-4 and H-8 signals, respectively. The correlations indicated an acetoxy group on a cyclohexane ring in the para position to the double bond. The configuration of the acetoxy group at the C-6 position was assigned to be equatorial, based on the half-height width, $W_{1/2}$ 18.7 Hz, of the multiplet signals for C-6 methine hydrogen.[4] The signals corresponding to H-4 and H-8 were determined by NOE-difference experiments in which enhancements between H-8 and H-2 confirmed the signals of H-8. This further demonstrated that the carboxylic acid unit was in a cis orientation with C-4. The H-4 proton signals occurred at lower field (δ 2.74, 3.15) and were due to the deshielding effect of the carboxylic acid unit. The homonuclear ^{1}H−^{1}H COSY and heteronuclear ^{1}H−^{13}C COSY NMR spectra permitted the complete assignment of all protons and carbons, as shown in Table 1. The above evidence led to the establishment of the structure of suspenolic acid as **1**.

Table 1. NMR Data for Suspenolic Acid (**1**) (δ values, in $CDCl_3$)

position	δ_C	δ_H
1	170.9	
2	113.5	5.70 s
3	163.2	
4	25.6	2.74, 3.15 m
5	31.4	1.74, 1.94 m
6	70.3	4.99 m
7	32.1	1.76, 1.95 m
8	33.9	2.25, 2.44 m
9	170.5	
10	21.3	2.07 s

Forsythenside A (**2**) and forsythenside B (**3**) were found to be very unstable in air. Therefore, the structural determinations of **2** and **3** were based mainly on their tetraacetate (**2A**) and pentaacetate (**3A**), respectively. Forsythenside A tetraacetate (**2A**) was obtained from the purification of forsythenside A (**2**) after acetylation. Compound **2A**, an amorphous solid, showed a molecular formula of $C_{30}H_{34}O_{14}$ from elemental analysis and its FABMS data. The IR absorption bands of **2A** showed the presence of hydroxyl (3431 cm^{-1}) and carbonyl (1666 cm^{-1}) groups. The ^{1}H-NMR spectrum (Table 2) of **2A** displayed aromatic signals at δ 7.03 (2H, d, J = 8.5 Hz) and 6.67 (2H, d, J = 8.5 Hz), which demonstrated a 1,4-disubstituted aromatic ring. The ^{1}H-NMR spectrum of **2A** also exhibited a singlet due to two benzylic protons at δ 3.52 (2H). The above information, together with the observation of an ester carbonyl

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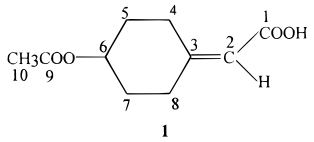
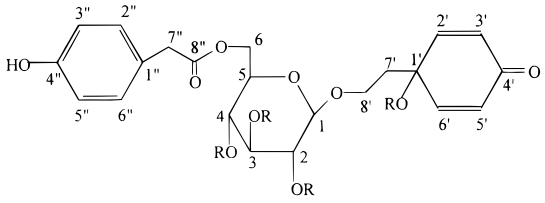
Table 2. NMR Data for **2A** and **3A** (in CD_3OD)

	2A		**3A**	
position	δ_C	δ_H	δ_C	δ_H
1	101.3	4.52 d (8.0)	101.5	4.59 d (8.0)
2	72.7	4.78 dd (9.5,8.0)	72.7	4.78 dd (9.5,8.0)
3	74.3	5.16 t (9.5)	74.3	5.17 t (9.5)
4	69.9	4.91 t (9.5)	69.6	4.96 t (9.5)
5	73.0	3.76 m	72.8	3.76 m
6	63.2	4.15 dd (10.8,4.4)	63.1	4.16 dd (12.4,3.8)
		4.17 dd (10.8,3.8)		4.18 dd (12.4,2.3).
1′	76.8		76.8	
2′, 6′	151.2	6.92 dd (10.0,2.8)	151.1	6.95 dd (10.0,2.8)
3′, 5′	129.0	6.12 dd (10.0,2.1)	129.5	6.13 dd (10.0,2.0)
4′	187.4		187.3	
7′	40.5	1.91 m	40.5	1.91 m
8′	65.0	3.77, 4.17 m	65.0	3.77,4.14 m
1″	126.1		75.1	
2″, 6″	131.5	7.03 d (8.5)	149.7	7.13 dd (10.0,2.8)
3″, 5″	116.3	6.67 d (8.5)	129.1	6.19 dd (10.0,1.6)
4″	157.6		187.0	
7″	40.9	3.52 s	44.7	2.89,2.84 d (15.1)
8″	173.3		169.0	
O-***CO***CH_3	171.0		171.0	
	171.1		171.0	
	171.2		171.1	
	171.6		171.2	
			171.6	
COO***CH₃***	20.5	1.91	20.5	1.85
	20.7	1.94	20.6	1.90
	20.9	1.96	20.7	1.96
	21.1	1.99	21.0	1.99
			21.1	2.04

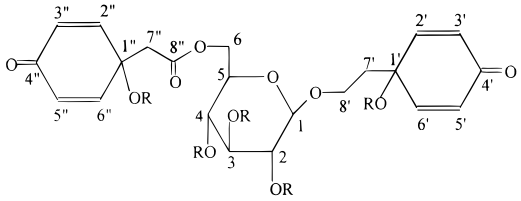
signal at δ 173.3, a methylene signal at 40.9, methine signals at δ 116.3 and 131.5 (each 2C), and quaternary aromatic carbon signals at δ 157.6 and 126.1 (as established by the ^{13}C-NMR and DEPT spectra), indicated the presence of a *p*-hydroxyphenylacetoxyl unit in the molecule. The characteristic chemical shift of the carbonyl resonance (δ 187.4) in the ^{13}C-NMR spectrum, along with four olefinic carbons [δ 151.2 (2C), 129.0-(2C)], two methylene carbons (δ 40.5, 65.0), and a quaternary carbon (δ 76.8), demonstrated that compound **2A** had a partial structure constituted by 4-acetoxy-4-hydroxyethyl-2,5-cyclohexadienone. Seven sugar protons [δ 3.76 (m, H-5), 4.15 (dd, J = 10.8, 4.4 Hz, H-6a), 4.17 (dd, J = 10.8, 3.8 Hz, H-6b), 4.52 (d, J = 8.0 Hz, H-1), 4.78 (dd, J = 9.5, 8.0 Hz, H-2), 4.91 (t, J = 9.5 Hz, H-4), 5.16 (t, J = 9.5 Hz, H-3)] were observed in the ^{1}H-NMR spectrum of **2A**. Using ^{1}H−^{1}H COSY NMR experiments, the assignments of these seven sugar protons could be made. In addition, the anomeric carbon signal at δ 101.3, the methine signals at δ 72.7, 74.3, 69.9, and 73.0; and the methylene signal at δ 63.2 in the ^{13}C-NMR spectrum indicated that the monosaccharide unit was glucose, which was also established by comparison on TLC with the standard sugar after hydrolysis. The configuration of the anomeric proton of the glucose was proposed as β on the basis of the coupling constant (8.0 Hz) of the ^{1}H-NMR signal at δ 4.52. The respective positions of the substituents were determined using long-range heteronuclear correlations observed by HMBC. The correlations showed three-bond coupling from H-1 to C-8′ and H-6 to C-8″ which indicated that the oxymethylene C-8′ and carboxylic C-8″ were attached to the glucose C-1 and C-6, respectively. Moreover, the spectral data of **2A**, except for the *p*-hydroxyphenylacetoxyl unit, were very similar to those of the pentaacetate of the known compound cornside.[4,10] The structure for forthyenside A, therefore, was assigned as **2**.



1



2 R=H
2A R=Ac



3 R=H
3A R=Ac

Forsythenside B pentaacetate (**3A**), an amorphous solid, was determined to have a molecular formula of $C_{32}H_{36}O_{16}$ on the basis of its molecular ion peak in the FABMS at *m/z* 699 $[M + Na]^+$ and by elemental analysis. The ^{1}H-NMR spectrum (Table 2) of **3A** exhibited signals for five acetyl groups, seven sugar protons, and eight olefinic protons. Two doublets at δ 2.89, 2.84 (J = 15.1 Hz) were ascribed to the C-7″ protons. Compounds **3A** and **2A** were found to represent the same cornside tetraacetate partial structure by comparing their NMR spectra.[4,10] The observed differences were that the signals at δ 6.67 (2H, d, J = 8.5 Hz) and 7.03 (2H, d, J = 8.5 Hz) in the ^{1}H-NMR spectrum of **2A** were shifted to δ 6.19 (2H, each dd, J = 10.0, 1.6 Hz) and δ 7.13 (2H, each dd, J = 10.0, 2.8 Hz), respectively, as in **3A**; and the signals of the *p*-hydroxyphenylacetoxyl unit of compound **2A** in the ^{13}C-NMR spectrum at δ 126.1, 116.3 (2C), 131.5 (2C), and 157.6 were replaced in **3A** by the signals at δ 75.1, 149.7 (2C), 129.1 (2C), and 187.0, respectively. This suggested that the *p*-hydroxyphenylacetoxyl of **2A** was changed into 4-carboxylic acid methyl-4-acetoxy-2,5-cyclohexadienone unit[11] in **3A**. After acid hydrolysis of **3A**, β-glucose was identified on TLC with a reference sample. Thus, the structure of forsythenside B was elucidated as **3**. The coexistence of suspenolic acid and forsythiasides A and B in the same plant material indicated that the C_6−C_2 parts of these congeners are related biosynthetically. A hypothetical transformation from compound **3** to **1** was suggested in Figure 1 on the basis of the possible biogenesis of rengyol.[8]

Besides the three new compounds **1**−**3,** other isolated compounds from the plant were identified as adoxosidic acid,[12] caffeic acid,[13] daucosterol,[14] *p*-hydroxyphenyl acetic acid,[8] succinic acid,[15] and vanillic acid[15,16] on the basis of spectral evidence and comparison of physical data with literature values.

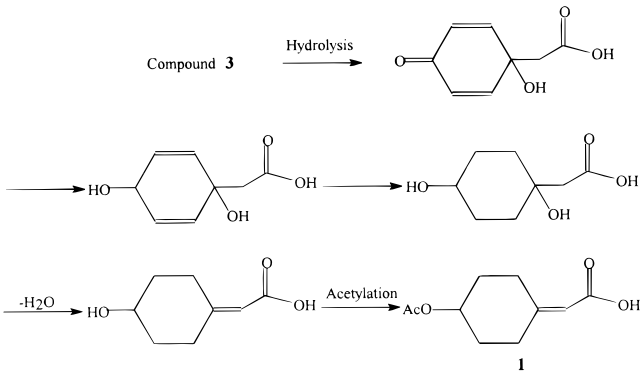


Figure 1.

Experimental Section

General Experimental Procedures. Melting points were determined with a XT4−100X micromelting point apparatus and are uncorrected. Specific rotations were obtained on a Perkin−Elmer 241 polarimeter. IR spectra were recorded on a Perkin−Elmer 683 spectrophotometer. NMR spectra were run on a Bruker AM-500 spectrometer. EIMS and FABMS were recorded on a ZAB-2F instrument. Elemental analysis was performed on a MOD1106 elemental analyzer. Si gel (HaiYang 180−200 mesh, produced by Qing Dao Hai Yang Chemical Group Co., Qing Dao, People's Republic of China) was used for column chromatography. Precoated Si gel plates (GF_{254}) were used for analytical and preparative TLC.

Plant Material. The fruits of *F. suspensa* were collected in Beijing, People's Republic of China, in July 1995. The plant material was identified by Professor Ruo-Yun Chen, and a voucher specimen has been deposited in the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation. The fruits of *F. suspensa* (10 kg) were extracted with hot 70% EtOH (30 L) three times, and the solutions were combined and concentrated *in vacuo*. A suspension of the EtOH extract in H_2O was extracted with EtOAc and *n*-BuOH six times. The combined EtOAc extracts (180 g) were chromatographed over Si gel (180−200 mesh) eluted with a petroleum ether−Me_2CO gradient solvent system. Fractions with similar R_f values by TLC were evaporated and combined to give 30 fractions. Each fraction was subjected to repeated column chromatography over Si gel eluted with petroleum ether−Me_2CO or $CHCl_3$−EtOAc to give caffeic acid (12 mg), *p*-hydroxyphenyl acetic acid (160 mg), succinic acid (30 mg), vanillic acid (35 mg), and **1** (8 mg). The combined *n*-BuOH extracts (400 g) were chromatographed on a Si gel (180−200 mesh) column eluted with a $CHCl_3$−MeOH gradient, starting with $CHCl_3$, to obtain adoxosidic acid (20 mg), daucosterol (200 mg), **2** (16 mg), and **3** (15 mg). Because compounds **2** and **3** were very unstable in air, their structures, therefore, were not characterized in their native form but were immediately acetylated as described below. Forsythenside A (**2**) in pyridine containing Ac_2O was stirred at room temperature for 5 h. The mixture was diluted with H_2O, and the solution was extracted with EtOAc. The organic phase was dried (Na_2SO_4) and concentrated and then purified by preparative TLC using $CHCl_3$−Et_2O (1:1) as solvent to afford pure forsythenside A tetraacetate (**2A**, 9 mg). Forsythenside B (**3**) was acetylated and purified using the same method as **2** to afford forsythenside B pentaacete (**3A**, 8 mg).

Suspenolic acid (1): amorphous solid, mp 74−76 °C; $[\alpha]^{20}_D$ +4.7° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 2947, 2852, 1734, 1691, 1649, 1246, 1038 cm^{-1}; ^{13}C- and ^1H-NMR data, see Table 1; FABMS *m/z* 199 $[M + H]^+$; CIMS *m/z* 138 $[M - AcOH]^+$ (50), 121 (10), 93 (60), 79 (20), 66 (10), 53 (8), 43 (100); *anal.* C 60.65, H 7.01, calcd for $C_{10}H_{14}O_4$, C 60.61, H 7.07.

Forsythenside A tetraacetate (2A): amorphous solid; $[\alpha]^{20}_D$ −18.1° (*c* 0.10, CH_3OH); IR (KBr) ν_{max} 3431, 2928, 1753, 1666, 1371, 1223, 1040, 737 cm^{-1}; ^{13}C- and ^1H-NMR data, see Table 2; FABMS *m/z* 619 $[M + H]^+$; *anal.* C 58.24, H 5.53, calcd for $C_{30}H_{34}O_{14}$, C 58.25, H 5.50.

Forsythenside B pentaacetate(3A): amorphous solid; $[\alpha]^{20}_D$ −14.2° (*c* 0.10, CH_3OH); ^{13}C- and ^1H-NMR data, see Table 2; FABMS *m/z* 699 $[M + Na]^+$; *anal.* C 56.80, H 5.33, calcd for $C_{32}H_{36}O_{16}$, C 56.71, H 5.39.

Acid hydrolysis of 2A and 3A. Compound **2A** (2 mg) was refluxed with 3% H_2SO_4 (1 mL) for 3 h. The reaction mixture was neutralized with 5% NaOH, then filtered. The filtrate showed the presence of *p*-hydroxyphenylacetic acid [identified by TLC comparison using $CHCl_3$−EtOAc−AcOH (1:1:0.1), detection with 0.05% bromophenol blue reagent] and glucose [identified by TLC comparison using $CHCl_3$-MeOH−H_2O (7:3:0.3), detection with anisaldehyde−H_2SO_4 reagent]. The same method was also used for the hydrolysis of **3A**, and glucose was detected from the hydrolysis solutions.

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