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The Structure of Forsythiaside isolated from *Forsythia suspensa*[1)]

Sansei Nishibe,* Kazuko Okabe, Hiroki Tsukamoto, Akiyo Sakushima, and Sueo Hisada

Faculty of Pharmaceutical Sciences, Higashi Nippon Gakuen University, Ishikari-Tobetsu, Hokkaido, 061-02, Japan

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A new caffeoyl glycoside of 3,4-dihydroxy-β-phenethyl alcohol, designated as forsythiaside (**1**), was isolated from the fruits of *Forsythia suspensa* V_AHL (Oleaceae).

The structure of **1** is proposed to be 3,4-dihydroxy-β-phenethyl-*O*-α-L-rhamnopyranosyl-(1→6)-4-*O*-caffeoyl-β-D-glucopyranoside on the basis of analysis of the carbon-13 nuclear magnetic resonance spectrum.

Keywords——*Forsythia suspensa*; Oleaceae; new caffeoyl glycoside of 3,4-dihydroxy-β-phenethyl alcohol; forsythiaside; 3,4-dihydroxy-β-phenethyl-*O*-α-L-rhamnopyranosyl-(1→6)-4-*O*-caffeoyl-β-D-glucopyranoside; ^{13}C-NMR spectra

We have already reported the isolation of triterpenoids, lignans and flavonoid from the fruits of *Forsythia suspensa* V_AHL (Oleaceae).[2,3)]

As a continuation of our investigation on the constituents, we isolated a new caffeoyl glycoside of 3,4-dihydroxy-β-phenethyl alcohol, designated as forsythiaside (**1**).

This paper describes the spectroscopic analysis of the structure of **1**, based on carbon-13 nuclear magnetic resonance (^{13}C-NMR).

The extraction and separation were carried out as described in Experimental.

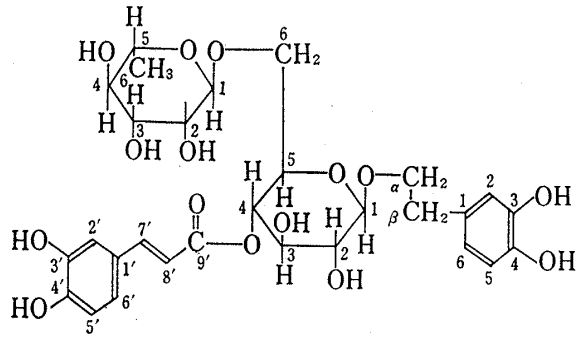

Chart 1

The glycoside **1** was isolated as a pale yellowish powder, $C_{29}H_{36}O_{15}\cdot 2H_2O$, mp 144—150°C (uncorr.), $[\alpha]_D^{20}$ −18.6° (EtOH), whose molecular weight was confirmed by the observation of *m/z* 647 ($M^+ + {}^{23}Na$) on field desorption mass spectrometry (FD–MS).

The ultraviolet (UV) spectrum of **1** showed absorption maxima at 216, 245 sh, 290, 302 and 332 nm, which gave bathochromic shifts to 300.5 and 379 nm on addition of base, indicating the presence of free phenolic hydroxyl groups.

The infrared (IR) spectrum of **1** suggested the presence of a conjugated ester (1700 cm^{-1}) and an aromatic ring (1600 cm^{-1}).

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** exhibited signals at δ 5.26 (1H, br s), due to the methine proton of a carbon bearing the ester group and at δ 6.30 (1H, d, *J*=15 Hz) and 7.56 (1H, d, *J*=15 Hz), due to the double bond protons of a conjugated ester.

The ^{1}H-NMR spectrum of acetate of **1** showed the presence of five alcoholic acetoxyl and four phenolic acetoxyl groups.

The alkaline treatment of **1** followed by acid hydrolysis gave caffeic acid and 3,4-dihydroxy-β-phenethyl alcohol, which were identified by comparison with authentic samples by gas chromatography (GC) and thin-layer chromatography (TLC).

The presence of D-glucose and L-rhamnose in the hydrolyzate was detected in a ratio of 1:1 by GC.

These data suggest that **1** bears a marked structural resemblance to acteoside (3,4-dihydroxy-β-phenethyl-*O*-α-L-rhamnopyranosyl (1→3)-4-*O*-caffeoyl-β-D-glucopyranoside) isolated from *Syringa vulgaris* (Oleaceae)[4] and *Conandron ramoidioides* (Gesneriaceae),[5] and to myricoside (3,4-dihydroxy-β-phenethyl-*O*-β-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside) isolated from *Clerodendrum myricoides* (Verbenaceae).[6]

Table I. ^{13}C-NMR Chemical Shifts[a]

3,4-Dihydroxy-β-phenethyl alcohol			Rutinose moiety			Caffeate moiety		
	1	3,4-Dihydroxy-β-phenethyl alcohol		**1**	Rutin		**1**	Chlorogenic acid
C-1	131.3	132.2	Glc-1	104.4	104.5	C-1′	127.6	127.6
C-2	116.3	116.2	Glc-2	75.1	75.5	C-2′	115.2	115.1
C-3	146.0	146.0	Glc-3	75.8	77.9	C-3′	146.7	146.6
C-4	144.6	144.7	Glc-4	75.1	71.1	C-4′	149.7	149.3
C-5	117.1	116.8	Glc-5	74.7	76.9	C-5′	116.5	116.4
C-6	121.3	121.0	Glc-6	67.6	68.3	C-6′	123.0	122.8
C-α	72.2	64.1	Rham-1	102.2	102.1	C-7′	147.5	146.9
C-β	36.7	39.6	Rham-2	72.0	71.8	C-8′	114.7	115.1
			Rham-3	72.2	72.0	C-9′	168.2	168.5
			Rham-4	73.9	73.7			
			Rham-5	69.8	69.4			
			Rham-6	18.0	17·6			

a) The spectra were taken with a JNM-FX 60 spectrometer (15.00 MHz) in CD_3OD with TMS as an internal reference, using micro cells.

The ^{13}C-NMR spectrum of **1** was correlated with those of known compounds, *i.e.*, 3,4-dihydroxy-β-phenethyl alcohol, rutin bearing the rutinose moiety and chlorogenic acid bearing the caffeate moiety.

Table I presents the ^{13}C-NMR data and their assignments.

The chemical shifts of the rutinose moiety of **1** relative to those of rutin appear downfield by 4.0 ppm at the C-4 carbon of glucose (Glc-4) and upfield by 2.2 ppm at both the C-3 and C-5 carbons of glucose (Glc-3 and Glc-5), due to the attachment of the caffeate moiety at Glc-4.

The glycosidation shift on the α-carbon of the R-CH_2OH group was reported to be *ca.* +7.6 ppm.[7]

The calculated chemical shift of the C-α carbon of **1** from that of 3,4-dihydroxy-β-phenethyl alcohol at 64.1 ppm was 71.7 ppm. The observed chemical shift of C-α carbon of **1** at 72.2 ppm suggested the linkage of the 3,4-dihydroxy-β-phenethyl moiety to the C-1 carbon of glucose (Glc-1).

Consequently, the structure of **1** is proposed to be 3,4-dihydroxy-β-phenethyl-*O*-α-L-rhamnopyranosyl-(1→6)-4-*O*-caffeoyl-β-D-glucopyranoside (Chart 1).

1 shows high inhibitory activity against cyclic adenosine monophosphate (cAMP)-phosphodiesterase *in vitro* (IC_{50} 11×10^{-5} mol/l).[8]

Experimental

The following instruments were used: melting point, Yanagimoto micro-melting point apparatus; optical rotation value, Union Giken PM-201; UV spectra, Shimadzu UV-210; IR spectra, Shimadzu IR-400; ^{1}H-NMR, Hitachi R-40 with tetramethylsilane ($\delta=0$) as an internal reference; ^{13}C-NMR spectra, JEOL JNM-FX 60, equipped with a JEC-980 computer; FD-MS, JEOL JMS-DX 300; MS, Hitachi RMU-7L; GC, Shimadzu GC-6AM.

The conditions for GC were as follows: glass column (3 mm×1 m), 1.5% OV-1 on Shimalite-W (80—100 mesh); column temp., 150—200°C (3°C/min); injection and detector temp., 230°C; carrier gas, N_2 (20 ml/min).

Precoated thin-layer chromatography plates, silica gel 60_{F-254} (Merck), were used for TLC and preparative

TLC. The spots were detected by spraying the plates with dil. $FeCl_3$ soln.

Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br s, broad singlet: sh, shoulder.

Isolation——Fruits of *Forsythia suspensa* VAHL (500 g) were crushed and extracted with hot water. The extract was cooled, and the precipitate was filtered off. The filtrate was lyophilized to give a powder. The powder was extracted with MeOH. The MeOH extractives were subjected to column chromatography over silica gel with a $CHCl_3$–MeOH gradient.

The fractions were monitored by TLC developed with the upper layer of $CH_3COC_2H_5$–AcOEt–HCOOH–H_2O–C_6H_6 (4: 3: 1: 1: 2).

The fractions showing a TLC spot at *Rf* 0.20, which gave a greenish-blue color with dil. $FeCl_3$ soln., were concentrated to afford crude **1**.

Purification was achieved by repeated re-chromatography over silica gel followed by preparative TLC. Recrystallization from MeOH gave 16.7 mg of **1**.

Properties of Forsythiaside (1)——A pale yellowish powder, mp 144—150°C (uncorr.), $[\alpha]_D^{20}$ −18.6° (c= 0.8 in EtOH). UV λ_{max}^{EtOH} nm (log ε): 216 (4.29), 245 (4.05) sh, 290 (4.09), 302 (4.08), 332 (4.17). UV $\lambda_{max}^{EtOH+NaOH}$ nm: 300.5, 379. IR ν_{max}^{KBr} cm^{-1}: 3600—3100 (OH), 1700 (conjugated CO), 1600 (C=C). *Anal.* Calcd for $C_{29}H_{36}O_{15}\cdot 2H_2O$: C, 52.72; H, 6.10. Found: C, 52.39; H, 6.00. FD–MS m/z: 647 ($M^+ + {}^{23}Na$, 100%), 485 (647−caf., 24%), 335 (647+^{23}Na, double cation, 16%). ^{1}H-NMR (in CD_3OD) δ: 1.23 (3H, d, J=6 Hz, rhamnose-CH_3), 2.80 (2H, t, J=7 Hz, Ar–CH_2–), 4.35 (1H, d, J=8 Hz, glucose-anomeric H), 4.63 (1H, s, rhamnose-anomeric H), 5.26 (1H, br s, –COOCH–COOCH<), 6.30 (1H, d, J=15 Hz, Ar–C<u>H</u>=CH–), 6.53—7.10 (6H, m, arom.H), 7.56 (1H, d, J=15 Hz, Ar–CH=C<u>H</u>–).

Acetate of Forsythiaside (1)——**1** was acetylated with acetic anhydride–pyridine in the usual way. The crude acetate was purified by preparative TLC to give the acetate as an amorphous powder. MS m/z: 1002 (M^+, $C_{47}H_{54}O_{24}$). UV λ_{max}^{EtOH} nm (log ε): 220 (4.11) sh, 283 (4.05). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (CO), 1640, 1500 (C=C). ^{1}H-NMR (in $CDCl_3$) δ: 1.23 (3H, d, J=6 Hz, rhamnose-CH_3), 1.91, 1.93, 2.07 (15H, each s, alcoholic CH_3CO), 2.27 (12H, s, phenolic CH_3CO), 2.75—2.95 (2H, m, Ar–CH_2–), 6.30 (1H, d, J=15 Hz, Ar–C<u>H</u>=CH–), 6.93—7.40 (6H, m, arom.H), 7.56 (1H, d, J=15 Hz, Ar–CH=C<u>H</u>–).

Alkaline Treatment of 1 followed by Acid Hydrolysis——**1** (10 mg) in 2% NaOH soln. was kept overnight under N_2 at room temperature. The reaction mixture was acidified with dil. HCl soln. and extracted with Et_2O. The Et_2O soln. was washed and evaporated to dryness. Caffeic acid in the residue was identified by comparison with an authentic sample [*Rf* 0.84 on TLC developed with the upper layer of $CH_3COC_2H_5$–AcOEt– HCOOH–H_2O–C_6H_6 (4: 3: 1: 1: 2)].

The aq. layer was extracted with *n*-BuOH. The *n*-BuOH layer was washed and evaporated to dryness. The residue in 1% H_2SO_4 soln. was heated on a water bath for 1 h, then cooled. The mixture was extracted with Et_2O. The Et_2O layer was washed and evaporated to dryness. 3,4-Dihydroxy-β-phenethyl alcohol in the residue was identified by comparison with an authentic sample [*Rf* 0.31 on TLC developed with $CHCl_3$–AcOEt (1: 1) and t_R 5.79 min for the TMS ether on GC].

The aq. layer was neutralized with $BaCO_3$ and the precipitate was filtered off. The filtrate was evaporated to dryness. The residue was examined by GC to identify L-rhamnose (t_R: 4.02, 5.09 for TMS ether) and D-glucose (t_R: 10.29, 13.84 for TMS ether) in a ratio of 1: 1 in comparison with the products obtained from rutin by the same procedure.

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References and Notes

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