The Structure of Forsythiaside isolated from *Forsythia suspensa*\(^1\)

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

The structure of 1 is proposed to be 3,4-dihydroxy-\(\beta\)-phenethyl-\(O\)-\(\alpha\)-L-rhamnopyranosyl-(1→6)-4-O-caffeoyl-\(\beta\)-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-\(\beta\)-phenethyl alcohol; forsythiaside; 3,4-dihydroxy-\(\beta\)-phenethyl-\(O\)-\(\alpha\)-L-rhamnopyranosyl-(1→6)-4-O-caffeoyl-\(\beta\)-D-glucopyranoside; \(^{13}\)C-NMR spectra

We have already reported the isolation of triterpenoids, lignans and flavonoid from the fruits of *Forsythia suspensa* Vahl (Oleaceae).\(^{2,3}\)

As a continuation of our investigation on the constituents, we isolated a new caffeoyl glycoside of 3,4-dihydroxy-\(\beta\)-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.

The glycoside 1 was isolated as a pale yellowish powder, C\(_{29}\)H\(_{30}\)O\(_{15}\)·2H\(_2\)O, 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 \(\delta\) 5.26 (1H, br s), due to the methine proton of a carbon bearing the ester group and at \(\delta\) 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-\(\beta\)-phenethyl alcohol, which were identified by comparison with authentic samples by gas chromatography (GC) and thin-layer chromatography (TLC).

The presence of \(\beta\)-glucose and \(\alpha\)-rhamnose in the hydrolyzate was detected in a ratio of 1:1 by GC.
These data suggest that I 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 ramosioides* (Gesneriaceae),⁵ and to myricoside (3,4-dihydroxy-β-phenethyl-O-β-D-apiosyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside) isolated from *Clerodendrum myricoides* (Verbenaceae).⁶

**Table I. **¹³C-NMR Chemical Shifts¹

<table>
<thead>
<tr>
<th>3,4-Dihydroxy-β-phenethyl alcohol</th>
<th>Rutinose moiety</th>
<th>Caffeine moiety</th>
<th>Chlorogenic acid</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Glc-1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C-1</td>
<td>131.3</td>
<td>104.4</td>
<td>127.6</td>
</tr>
<tr>
<td>C-2</td>
<td>116.3</td>
<td>104.5</td>
<td>127.6</td>
</tr>
<tr>
<td>C-3</td>
<td>116.2</td>
<td>75.1</td>
<td>115.2</td>
</tr>
<tr>
<td>C-4</td>
<td>144.0</td>
<td>75.5</td>
<td>115.1</td>
</tr>
<tr>
<td>C-5</td>
<td>144.7</td>
<td>77.9</td>
<td>116.6</td>
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<tr>
<td>C-6</td>
<td>117.1</td>
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<td>Rham-1</td>
<td>76.6</td>
<td>123.0</td>
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<td>114.7</td>
<td>128.5</td>
</tr>
</tbody>
</table>
| a) The spectra were taken with a JNM-FX 60 spectrometer (15.00 MHz) in CD3OD with TMS as an internal reference, using microcrystals.

The ¹³C-NMR spectrum of I 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 ¹³C-NMR data and their assignments.

The chemical shifts of the rutinose moiety of I 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-CH₂OH group was reported to be ca. +7.6 ppm.⁷

The calculated chemical shift of the C-α carbon of I 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 I 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 I is proposed to be 3,4-dihydroxy-β-phenethyl-O-α-L-rhamnopyranosyl-(1→6)-4-O-caffeoyl-β-D-glucopyranoside (Chart 1).

I shows high inhibitory activity against cyclic adenosine monophosphate (cAMP)-phosphodiesterase in vitro (IC₅₀ 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; ¹H-NMR, Hitachi R-40 with tetramethylsilane (δ = 0) as an internal reference; ¹³C-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, N₂ (20 ml/min). Precoated thin-layer chromatography plates, silica gel 60F₂₅₄ (Merck), were used for TLC and preparative
TLC. The spots were detected by spraying the plates with dil. FeCl₃ soln.
Silica gel (100 mesh, Mallinkrodt) 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 CHCl₃-MeOH gradient.
The fractions were monitored by TLC developed with the upper layer of CH₃COCH₃-AcOEt-HCOOH-H₂O-C₂H₆ (4: 3: 1: 1: 2).
The fractions showing a TLC spot at Rf 0.20, which gave a greenish-blue color with dil. FeCl₃ soln.,
were concentrated to afford crude 1.

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

**Properties of Forsythiaside (I)**—A pale yellowish powder, mp 144—150°C (uncorr.), [α]D^20 = -18.6° (c ≈
0.8 in EtOH).
UV λ_max (log e): 216 (4.29), 245 (4.05) sh, 290 (4.09), 302 (4.08), 332 (4.17).
IR υ_max (cm⁻¹): 3600—3100 (OH), 1700 (conjugated CO), 1600 (C=O), Anal. Calcd for C₉H₉O₄: 2H₂O: C, 52.72; H, 6.10. Found: C, 52.39; H, 6.60. FD-MS m/z: 647 (M^+ + Na^+, 100%), 455 (647—24% Na, 24%), 335 (647—24% Na, double cation, 14%), 1^H-NMR (in CDCl₃): δ: 1.23 (3H, d, J = 6 Hz,
rhamnose-CH₂), 2.80 (2H, t, J = 7 Hz, Ar-CH₂), 4.35 (1H, br s, COOCH₂—COCH₂), 5.26 (1H, br s, COOCH₂—COCH₂), 6.30 (1H, d, J = 15 Hz, Ar—CH=CH—). 6.93—7.10
(6H, m, arom.H), 7.56 (1H, d, J = 15 Hz, Ar—CH=CH—).

**Acetate of Forsythiaside (I)**—1 was acetylated with acetic anhydride—pyridine in the usual way. The
acetic acetate was purified by preparative TLC to give the acetate as an amorphous powder. MS m/z: 1002
(M^+·3H₂O·C₂H₅OH). UV λ_max (log e): 220 (4.11) sh, 283 (4.05). IR υ_max (cm⁻¹): 1750 (CO), 1640, 1500
(C=O). 1^H-NMR (in CDCl₃): 1.23 (3H, d, J = 6 Hz, thannose-CH₂), 1.91, 1.93, 2.07 (15H, each s, alcoholic
CH₂CO), 2.27 (12H, s, phenolic CH₂CO), 2.75—2.95 (2H, m, Ar—CH₂), 6.30 (1H, d, J = 15 Hz, Ar—CH=CH—)
6.93—7.40 (6H, m, arom.H), 7.56 (1H, d, J = 15 Hz, Ar—CH=CH—).

**Alkaline Treatment of 1 followed by Acid Hydrolysis**—1 (10 mg) in 2% NaOH soln. was kept overnight
under N₂ at room temperature. The reaction mixture was acidified with dil. HCl soln. and extracted with
Et₂O. The Et₂O 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 CH₃COCH₃—
AcOEt—HCOOH—H₂O—C₂H₆ (4: 3: 1: 1: 2)].

Theaq. layer was extracted with n-BuOH. The n-BuOH layer was washed and evaporated to dryness.
The residue in 1% H₂SO₄ soln. was heated on a water bath for 1 h, then cooled. The mixture was extracted
with Et₂O. The Et₂O 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 CHCl₃—
AcOEt (1: 1) and tₖ 5.79 min for the TMS ether on GC].

Theaq. layer was neutralized with BaCO₃ and the precipitate was filtered off. The filtrate was evaporated
to dryness. The residue was examined by GC to identify rhamnose (tₖ: 4.02, 5.09 for TMS ether and
D-glucose (tₖ: 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.

**Acknowledgement** We are grateful to Dr. K. Ina of Tokyo College of Pharmacy for FD-MS measurement
and to Mr. K. Oda of our faculty for 13C-NMR measurement.

**References and Notes**

1) Part V in the series "Studies on the Chinese Crude Drug "Forsythiae Fructus"." For Part IV see M.
8) Private communication from Dr. T. Nikaido, Faculty of Pharmaceutical Sciences, Toho University.