STRUCTURE OF FORSYTHOSIDE A, AN ANTIBACTERIAL PRINCIPLE OF *FORSYTHIA SUSPENSA* LEAVES[1]

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Abstract — A new glycoside, forsythoside A, exhibiting antibacterial activity, has been isolated from *Frosythia suspensa* leaves. Degradative and spectroscopic studies have established the structure of forsythoside A as shown in formula I.

Forsythia suspensa Vahl (Oleaceae) is an important original plant of the crude drug "rengyo" (Forsythiae Fructus) which has been used for antiinflammatory, diuretic, drainage and antidotal purposes in Oriental medicine. The crude drug has also been known to exhibit antibacterial activity which has hitherto been attributed to a lignan-glycoside, phillyrin.[2] Quite recently *d*-pinoresinol was assigned as the phosphodiesterase inhibiting component of the crude drug.[3]

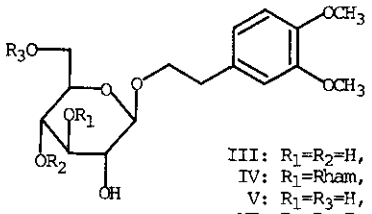
During our chemical and pharmacological studies on the crude drug, antibacterial activity against *Staphylococcus aureus* was found in the methanol extract. A survey indicated that the principal activity was observed only in a fraction where phillyrin was not detected. However, that fraction was a mixture of substances which were tedious to separate, but we found that the leaves of the same plant contained the same substance in a fairly large quantity and the proportion of the accompanying substances was rather small. Thus, when the methanol extract of the leaves was diluted with water and extracted successively with ethyl acetate and butanol, the antibacterial principle was effectively concentrated in the butanol fraction from which a new glycoside, forsythoside A, was isolated as the principal antibacterial constituent.

Forsythoside A (I), $[\alpha]_D$ -23.2° (MeOH) was a somewhat air-sensitive amorphous substance exhibiting the following spectroscopic properties: UV λ_{max} nm (ε): 291 (11,520) and 332 (14,500); ^{1}H NMR (CD_3OD) δ: 1.20 (3H doublet, *J* 6.0 Hz), 2.79 (2H triplet, *J* 7.5 Hz), 4.35 (1H doublet, *J* 8.0 Hz), 4.63 (1H doublet, *J* 1.5 Hz), 6.28 (1H doublet, *J* 16.0 Hz), 6.49 - 7.06 (6H broad) and 7.58 (1H doublet, *J* 16.0 Hz); FD-MS: *m/e* 647 $(M + Na)^+$.

Treatment of forsythoside A with methyl iodide and anhydrous potassium carbonate in acetone yielded the air-stable tetramethyl ether (II), $[\alpha]_D$ -37.3° (MeOH). The heaviest ion peak at *m/e* 680 in the mass spectrum confirmed the molecular weight, suggesting the elemental composition of $C_{33}H_{44}O_{15}$ for the ether (II). The ^{1}H NMR spectra of the ether (II) (acetone-d_6) indicated the presence of a secondary methyl group (δ 1.19, 3H doublet, *J* 6.0 Hz), a benzylic methylene group (δ 2.88, 2H triplet, *J* 7.5 Hz), four methoxyl groups (δ 3.77, 3.81, 3.87, 3.90, all 3H singlet), two anomeric methine groups (δ 4.44, 1H doublet, *J* 8.0 Hz, 4.73, 1H doublet, *J* 1.5 Hz), a *trans*-disubstituted conjugated enone system (δ 6.46, 1H doublet, *J* 16.0 Hz, 7.68, 1H doublet, *J* 16.0 Hz), in addition to six aromatic hydrogens (δ 6.80 - 7.40).

Alkaline hydrolysis of the ether (II) afforded caffeic acid dimethyl ether,[4] and the deacyl derivative (III), $[\alpha]_D$ -41.3° (MeOH); UV λ_{max} nm (ε): 277 (2,360); ^{1}H NMR (CD_3OD) δ: 1.26 (3H doublet, *J* 6.0 Hz), 2.88 (2H triplet, *J* 7.5 Hz), 3.77 (3H singlet), 3.80 (3H singlet), 4.32 (1H doublet,

J 8.0 Hz), 4.78 (1H doublet, J 1.5 Hz), 6.80 - 6.96 (3H broad); MS: m/e 490 (M^+), 371, 182, 166, 165, 164, 151. Further hydrolysis of the deacyl derivative (III) with ethanolic sulfuric acid yielded 3,4-dimethoxyphenylethanol[5] together with D-glucose and L-rhamnose.



III: R_1=R_2=H, R_3=Rham
IV: R_1=Rham, R_2=Caff, R_3=H
V: R_1=R_3=H, R_2=Caff
VI: R_1=R_2=R_3=H

Rham: rhamnosyl
Caff: O,O-dimethylcaffeoyl

Since these three components were obtained by mild acid hydrolysis of the deacyl derivative (III), 3,4-dimethoxyphenylethanol, glucose and rhamnose were obviously linked through glycoside linkages. The structure of the deacyl derivative (III) was examined by the ^{13}C NMR spectra of the deacyl derivative (III) (Table I). Thus, the rhamnose moiety was attached to the other part of the molecule only at C-1 since its ^{13}C NMR resonances showed no low field shift due to alkyl substitution except at the anomeric position. Hence this group should bind to the glucose moiety in the deacyl derivative (III) and consequently 3,4-dimethoxyphenylethanol was linked to the C-1 position of the glucose moiety. The data in Table I further indicated that the rhamnosyl group is attached to C-6 position of the glucose moiety since the C-6 resonance of the latter exhibited a characteristic low field shift due to alkyl substitution.

Table I. Carbon-13 shieldings of sugar carbons of forsythoside A and related substances*

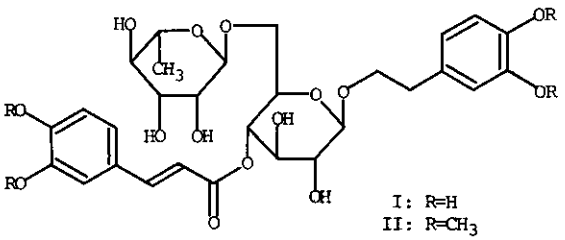
compound	glucose moiety†						rhamnose moiety†					
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
I	104.4	74.9	75.6	71.8	74.6	67.4	102.3	72.4	72.3	73.7	69.6	18.5
II	104.2	74.8	75.6	71.7	74.5	67.5	102.2	72.3	72.3	73.6	69.6	18.4
III	104.4	74.9	78.3	71.6	77.0	68.2	102.3	72.6	72.1	73.9	69.6	18.6
IV	104.0	73.7	80.3	70.3	76.1	62.1	102.9	72.4	72.4	75.7	70.1	19.0
rutin	104.7	75.8	78.3	71.5	77.1	68.5	102.3	72.3	72.2	74.0	69.6	17.9

* Chemical shifts from internal TMS in pyridine-d_5
† Discrimination of two moieties was made by PRFT measurements on the deacyl derivative (III)

The glycosidic linkage at C-1 of the glucose moiety was easily assigned to be β from the chemical shifts (δ 4.35 for I, 4.44 for II, 4.32 for III) and the large coupling constant (J 8.0 Hz) of the anomeric hydrogen signals in the ^1H NMR spectra as well as from the ^{13}C-^1H coupling constant of J 155 Hz for the anomeric center.[6]

The configuration at the anomeric position of the rhamnosyl group was next examined. Although the coupling constant of J 1.5 Hz of the signals for the anomeric hydrogens of the rhamnosyl group in the ^1H NMR spectra of forsythoside A and its derivative (II and III) was not informative in discrimination of the configurations at C-1 in rhamnosides, their chemical shifts (δ 4.63 for I, 4.73 for II, 4.78 for III) suggested the β configuration.[7] On the other hand, the ^{13}C-^1H coupling constant of J 168 Hz for the anomeric center indicated the anomeric hydrogen to be in the equatorial configuration[6] and agreed with the value for α-methyl rhamnoside (J 171.1 Hz).[8] In agreement with this, the ^{13}C resonances of the rhamnose moiety coincided with those of α-rhamnosides such as α-methyl rhamnoside, acteoside tetramethyl ether (IV)[9] and rutin (Table I). In order to confirm the configuration at this center, the molecular rotations of appropriate derivatives of forsythoside A

were determined. Thus, treatment of the ether (II) with ethanolic sulfuric acid yielded the desrhamnosyl derivative (V), $[\alpha]_D$ -14.0° (MeOH); ^{1}H NMR ($CDCl_3$) δ: 2.91 (2H triplet, *J* 7.5 Hz), 3.85 (3H singlet), 3.87 (3H singlet), 3.91 (6H singlet), 4.37 (1H doublet, *J* 8.0 Hz), 4.97 (1H triplet, *J* 8.5 Hz), 6.32 (1H doublet, *J* 16.0 Hz), 6.70 - 7.18 (6H broad), 7.68 (1H doublet, *J* 16.0 Hz); MS: *m/e* 534 (M^+), which was found identical with the desrhamnosyl derivative of acteoside tetramethyl ether (IV),[9] confirming the joint feature of 3,4-dimethoxyphenylethanol moiety and glucose moiety including the configuration of the glucoside linkage. Hydrolysis of the desrhamnosyl derivative (V) with methanolic potassium hydroxide yielded 3,4-dimethoxyphenylethyl glucoside (VI), $[\alpha]_D$ -20.1° (MeOH), ^{1}H NMR ($CDCl_3$) δ: 2.86 (2H triplet, *J* 7.5 Hz), 3.78 (6H singlet), 4.36 (1H broad), 6.70 (3H broad); MS: *m/e* 344 (M^+). The molecular rotation difference between the ether (II) and the desrhamnosyl derivative (V) ($\Delta[M]_D$ -179°) as well as that between the deacyl derivative (III) and the phenylethyl glucoside (VI) ($\Delta[M]_D$ -133°) (Table II) demonstrated the α-rhamnoside structure.[10] On the basis of the above evidence, the rhamnoside linkage was concluded to be α. The discrepancy with the suggestion derived from the ^{1}H NMR data may be explained by some unusual reason such as an anisotropic shielding effect from a benzene ring.



I: R=H
II: R=CH_3

Table II. Molecular optical rotation difference of forsythoside A derivatives

compound	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
II	-37.3°	-254°	} -179°*
V	-14.0°	- 75°	
III	-41.3°	-202°	} -133°*
VI	-20.1°	- 69°	

* α-Methyl rhamnoside: $[M]_D$ -111°, β-methyl rhamnoside: $[M]_D$ +170°

The transformations described above also established the location of the caffeoyl group at C-4 of the glucose moiety which was further substantiated by the finding that the ^{13}C resonances of C-3 and C-5 suffered from characteristic low field shifts of 2.7 and 2.5 ppm, respectively, by removal of the caffeoyl group from the ether (II) to the deacyl derivative (III).[11]

Accumulated data have established the structure of forsythoside A (I) which is a positional isomer of acteoside[9] with respect to the linkage of the rhamnosyl group.

Forsythoside A (I) exhibited the antibacterial activity against *Staphylococcus aureus* at a concentration less than 2 mM.

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4) M.p. 182°, ^{1}H NMR ($CDCl_3$) δ: 3.91 (6H singlet), 6.35 (1H doublet, *J* 16.0 Hz), 6.80 - 7.20 (3H

broad), 7.80 (1H doublet, J 16.0 Hz); MS: m/e 208, 191, these data being consistent with those of the methylation product of caffeic acid with dimethyl sulfate and sodium hydroxide. The compound was identified by direct comparison with an authentic sample.
5) ^{1}H NMR ($CDCl_3$) δ: 2.77 (2H triplet, J 7.5 Hz), 3.81 (2H triplet, J 7.5 Hz), 3.84 (6H singlet), 6.73 (3H broad); MS: m/e 182, 151, these data being in accord with those of the reduction product of homoveratric acid with lithium aluminum hydride. The compound was identified by direct comparison with an authentic sample.
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