STRUCTURE OF FORSYTHOSIDE B, AN ANTIBACTERIAL PRINCIPLE OF FORSYTHIA KOREANA STEMS[1]

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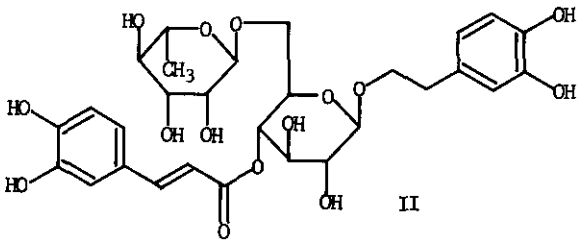
Abstract -- A new glycoside, forsythoside B, exhibiting antibacterial activity, has been isolated from Forsythia koreana stems. Chemical and spectroscopic studies have established the structure of forsythoside B as shown in formula I.

Forsythia koreana Nakai 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 had formerly been attributed to a lignan-glycoside, phillyrin.[2] However, we have recently disclosed that the principal antibacterial constituent of the crude drug from F. suspensa Vahl was a new glycoside, forsythoside A (II), which occurred also in the leaves of the same plant in a high content.[3]



II

Continuing our chemical and pharmacological studies on the crude drug, we have found that contrary to the case of F. suspensa leaves,[3] F. koreana leaves contained no forsythoside A (II), but instead, acteoside (III)[4] in a high concentration. Furthermore, stems of the latter plant were revealed to contain a new antibacterial glycoside, forsythoside B, together with acteoside (III). This report deals with the structure determination of the new substance, forsythoside B.

Forsythoside B (I), $[\alpha]_D$ -93.0° (MeOH), was a somewhat air-sensitive amorphous substance exhibiting the following spectroscopic properties: UV λ_{max} nm (ε): 291 (10680), 333 (14400); ^{1}H NMR (CD_3OD) δ: 1.09 (3H doublet, J 6.0 Hz), 2.78 (2H triplet, J 7.5 Hz), 4.36 (1H doublet, J 8.0 Hz), 4.91 (1H doublet, J 2.5 Hz), 5.16 (1H doublet, J 1.5 Hz), 6.26 (1H doublet, J 16.0 Hz), 6.45-7.16 (6H multiplet), 7.58 (1H doublet, J 16.0 Hz); FD-MS: m/e 795 ($M + K^+$), 779 ($M + Na^+$).

Treatment of forsythoside B with methyl iodide and potassium carbonate in acetone yielded the air-stable tetramethyl ether (IV), $[\alpha]_D$ -91.2° (MeOH). The molecular ion peak at m/e 812 in the mass spectrum of the ether (IV) confirmed the molecular weight and suggested the elemental composition of $C_{38}H_{52}O_{19}$. The ^{1}H NMR spectrum of the ether (IV) (CD_3OD-acetone-d_6) indicated the presence of a secondary methyl group (δ 1.11, 3H doublet, J 6.0 Hz), a benzylic methylene group (δ 2.88, 2H triplet, J 7.5 Hz), four methoxyl groups (δ 3.79, 3.83, 3.87, 3.87, 3H singlet each), three anomeric methine groups (δ 4.40, 1H doublet, J 8.0 Hz; 4.92, 1H doublet, J 2.5 Hz; 5.20, 1H doublet, J 1.5 Hz) and a trans-disubstituted conjugated enone system (δ 6.42, 1H doublet, J 16.0 Hz, 7.68, 1H doublet, J 16.0 Hz), in addition to six aromatic hydrogens (δ 6.75-7.30). These characteristics resembled well those of forsythoside A (II),[3] indicating that these two substances

have some similarity in structure.

Alkaline hydrolysis of the ether (IV) with methanolic potassium hydroxide afforded caffeic acid dimethyl ether and the deacyl derivative (V), $[\alpha]_D$ -44.2° (MeOH); UV λ_{max} nm (ε): 278 (2400); ^{1}H NMR (CD_3OD) δ: 1.15 (3H doublet, J 6.5 Hz), 2.78 (2H triplet, J 7.0 Hz), 3.69 (3H singlet), 3.72 (3H singlet), 4.20 (1H doublet, J 8.0 Hz), 4.90 (1H doulbet, J 2.5 Hz), 5.06 (1H doublet, J 1.5 Hz), 6.65—6.85 (3H multiplet); FD-MS: m/e 623 (M^++1). Further hydrolysis of the deacyl derivative (V) with ethanolic sulfuric acid yielded 3,4-dimethoxyphenylethanol, D-glucose, L-rhamnose and D-apiose.

Since these four components were obtained by mild acid hydrolysis of the deacyl derivative (V), they were obviously linked through glycoside bonds. The linkages of these components were then examined by means of the ^{13}C NMR spectroscopy (Table I). Since the ^{13}C NMR resonances originating from the rhamnosyl group and the apiosyl group showed no low field shifts due to O-alkyl substitution except those at the anomeric positions, these two groups were thought to bind to the other part of the molecule only at the C-1 positions. Hence the rhamnosyl group and the apiosyl group should link to the glucose moiety in the deacyl derivative (V), and consequently, *3,4-dimethoxyphenylethanol is attached to the C-1 position of the glucose moiety.* The stereochemistry of the glycoside linkage at C-1 of the glucose moiety was easily assigned to be β from the chemical shifts (δ 4.36, 4.40 and 4.20) and the large coupling constant (J 8.0 Hz) of the anomeric hydrogen signals in the ^{1}H NMR spectra of forsythoside B and its derivatives (IV and V) together with the ^{13}C-^{1}H coupling constant of 158 Hz for the anomeric center.[3,5] Similarly, the configuration of the glycoside linkage at C-1 of the rhamnosyl group was ascribed to be α from the chemical shifts (δ 5.16, 5.20 and 5.06) of the anomeric hydrogen signals in the ^{1}H NMR spectra of forsythoside B and its derivatives (IV and V) as well as the ^{13}C-^{1}H coupling constant of 168 Hz for the anomeric center.[3,5]

The data shown in Table I further indicated that the ^{13}C NMR resonances of C-3 and C-6 of the glucose moiety in the deacyl derivative (V) exhibited appreciable low field shifts. These effects were attributed to the O-alkyl substitutions at these positions, and the chemical shifts agreed with those of the C-3 resonance of the deacyl derivative of acteoside tetramethyl ether (VI), α-rhamnosyl(1→3)-β-glucoside,[3,4] and of the C-6 resonance of the deacyl derivative of forsythoside A tetramethyl ether (VII), α-rhamnosyl(1→6)-β-glucoside,[3] respectively.

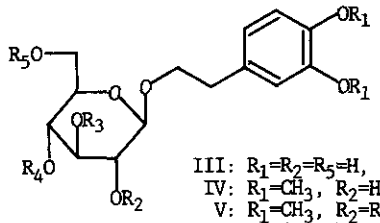
Characteristic low field shifts of the ^{13}C resonances of C-3 and C-5 in the glucose moiety caused by removal of the caffeoyl group from the ether (IV) to the deacyl derivative (V) established the location of the acyl group at C-4 in the glucose moiety.[6] *The data so far* obtained demonstrated that the structure of forsythoside B should be either the 6-apiosyl derivative of acteoside or the 3-apiosyl derivative of forsythoside A, and selection was made by the following experiments.

Partial hydrolysis of the ether (IV) with ethanolic sulfuric acid gave the deapiosyl derivative, $[\alpha]_D$ -71.8° (MeOH), ^{1}H NMR (acetone-d_6) δ: 1.12 (3H doublet, J 6.0 Hz), 3.76 (3H singlet), 3.79 (3H singlet), 3.86 (3H singlet), 3.88 (3H singlet), 4.44 (1H doublet, J 8.0 Hz), 4.90 (1H triplet, J 8.5 Hz), 5.30 (1H doublet, J 1.5 Hz), 6.44 (1H doublet, J 16.0 Hz), 7.66 (1H doublet, J 16.0 Hz); MS: m/e 680 (M^+), and the deapiosylderhamnosyl derivative, $[\alpha]_D$ -21.0° (MeOH), ^{1}H NMR ($CDCl_3$) δ: 2.90 (2H triplet, J 8.0 Hz), 3.84 (3H singlet), 3.86 (3H singlet), 3.91 (6H singlet), 4.37 (1H doublet, J 8.0 Hz), 4.96 (1H triplet, J 8.5 Hz), 6.32 (1H doublet, J 16.0 Hz), 7.69 (1H doublet, J 16.0 Hz); MS: m/e 534 (M^+). These hydrolysis products were found to be identical with acteoside tetramethyl ether (VIII) and its derhamnosyl derivative (IX),[3] establishing the location of the rhamnosyl group at C-3, and in consequence, that of the apiosyl group at C-6.

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

		I	IV	V	VI	VII	platycodin D
glucose moiety	C-1	103.9	103.9	104.2	104.0	104.4	
	C-2	73.7	73.7	74.0	73.7	74.9	
	C-3	79.6	80.1	83.5	83.5	78.3	
	C-4	70.3	70.8	70.0	69.6	71.6	
	C-5	74.3	74.4	76.9	78.0	77.0	
	C-6	68.2	68.3	68.6	62.4	68.2	
rhamnose moiety	C-1	102.9	102.9	102.8	102.3	102.3	
	C-2	72.3	72.4	72.6	72.4	72.6	
	C-3	72.3	72.4	72.5	72.3	72.1	
	C-4	75.5	75.6	75.3	75.2	73.9	
	C-5	70.2	70.2	69.8	69.6	69.6	
	C-6	19.0	18.8	18.6	18.4	18.6	
apiose moiety	C-1	111.0	111.0	111.0			111.2
	C-2	77.7	77.8	77.7			77.9
	C-3	80.3	80.3	80.4			80.0
	C-4	75.0	75.0	74.9			75.0
	C-5	65.3	65.3	65.3			65.7

* δ in pyridine-d_5
Discrimination of three moieties was made by PRFT measurements on the ether (IV)

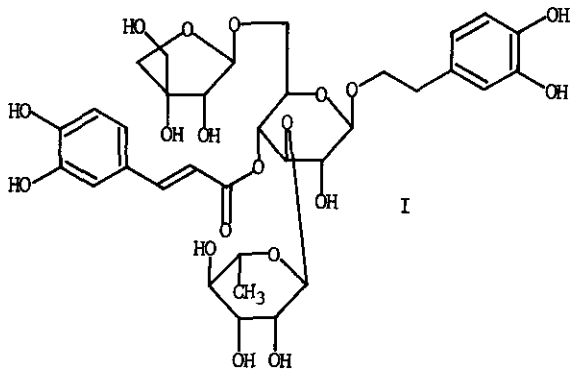


III: R_1=R_2=R_5=H, R_3=rhamnosyl, R_4=caffeoyl
IV: R_1=CH_3, R_2=H, R_3=rhamnosyl, R_4=O,O-dimethylcaffeoyl, R_5=apiosyl
V: R_1=CH_3, R_2=R_4=H, R_3=rhamnosyl, R_5=apiosyl
VI: R_1=CH_3, R_2=R_4=R_5=H, R_3=rhamnosyl
VII: R_1=CH_3, R_2=R_3=R_4=H, R_5=rhamnosyl
VIII: R_1=CH_3, R_2=R_5=H, R_3=rhamnosyl, R_4=O,O-dimethylcaffeoyl
IX: R_1=CH_3, R_2=R_3=R_5=H, R_4=O,O-dimethylcaffeoyl
X: R_1=R_2=R_4=CH_3, R_3=2,3,4-tri-O-methylrhamnosyl, R_5=2,3,5-tri-O-methylapiosyl
XI: R_1=R_2=R_4=R_5=CH_3, R_3=2,3,4-tri-O-methylrhamnosyl

Those transformations also confirmed the stereochemical features of the glycoside linkages of the glucose moiety and the rhamnose moiety as well as the location of the caffeoyl group.

The configuration of the anomeric center of the apiosyl group was next examined. Although the coupling constant of 2.5 Hz for the signals due to the anomeric hydrogens of the apiosyl groups in the ^1H NMR spectra of forsythoside B (I) and its derivatives (IV and V) was not informative in discrimination of the configurations at C-1 in apiosides, the chemical shifts (δ 4.91 for I, 4.92 for IV and 4.90 for V) and the ^{13}C-^1H coupling constant of 168 Hz were consistent with the values for β-apiosides such as methyl 2,3,5-tri-O-methyl-β-apioside (δ 4.97, doublet, J 2 Hz[7]) and onjisaponin F and G (^{13}C-^1H J 171 Hz[8]). The ^{13}C NMR resonances of the apiosyl group also coincided with the corresponding resonances of platycodin-D having a β-apiosyl part structure[9] (Table I) and, more reliably, the chemical shift of the anomeric carbon (δ 111.0) indicated that the glycoside linkage at C-1 was trans to the adjacent hydroxyl group at C-2.[10] The difference of the molecular rotations of -182° between the ether (IV), $[M]_D$ -741°, and acteoside tetramethyl ether (VIII), $[M]_D$ -559°, also agreed with the differences for β-D-apiosides (-205° for Mi-saponin A and Mi-saponin B,[11] and -107° for platycodin-D_3 and its deapiosyl counterpart[9]), verifying the β-configuration of the glycoside linkage of the apiosyl group in forsythoside B. Further, methylation of deacyl derivative (V) by Hakomori's method afforded the

decamethyl ether (X), $[\alpha]_D$ -52.9° (MeOH), ^{1}H NMR ($CDCl_3$) δ: 1.26 (3H doublet, J 6.0 Hz), 4.23 (1H doublet, J 8.0 Hz), 5.07 (1H doublet, J 2.5 Hz), 5.27 (1H doublet, J 1.5 Hz), 3.33, 3.39, 3.41, 3.42, 3.44, 3.45, 3.49, 3.53, 3.82, 3.83 (3H singlet each); MS: m/e 735 (M^++1). Methanolysis of the decamethyl ether (X) with methanolic hydrochloric acid furnished methyl 2,3,5-tri-O-methyl-β-D-apioside,[7] $[\alpha]_D$ -63.3° (MeOH), ^{1}H NMR ($CDCl_3$) δ: 3.35 (3H singlet), 3.37 (3H singlet), 3.41 (3H singlet), 3.46 (3H singlet), 4.93 (1H doublet, J 2.5 Hz), and the deapiosyl counterpart which, after complete methylation, was identified as permethyldeacylacteoside (XI),[4] $[\alpha]_D$ -44.0° (MeOH). The molecular rotation difference of -135° between the decamethyl ether (X), $[M]_D$ -388°, and permethyldeacylacteoside (XI), $[M]_D$ -253°, rigorously established the β-configuration of the apioside linkage (cf. methyl 2,3,5-tri-O-methyl-α-D-apioside: $[M]_D$ +239°; methyl 2,3,5-tri-O-methyl-β-D-apioside: $[M]_D$ -163°[7]).



I

Accumulated data have thus established the structure of forsythoside B as represented by formula I, which is the 6-β-D-apiosyl derivaitve of acteoside (III).

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

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