STRUCTURES OF FORSYTHOSIDE C AND D, ANTIBACTERIAL PRINCIPLES OF FORSYTHIA SUSPENSA FRUITS[1]

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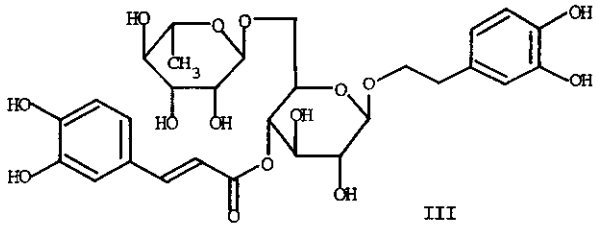
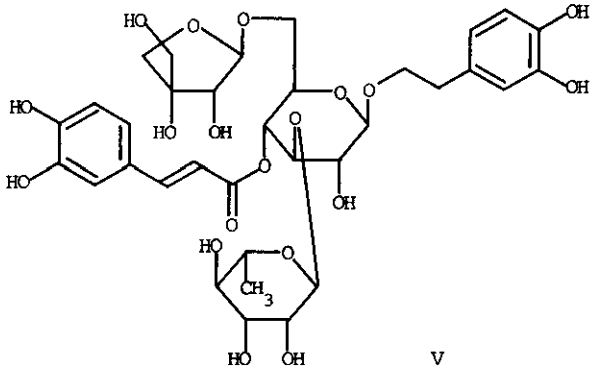
Abstract —— New glycosides, forsythoside C and forsythoside D, which exhibit antibacterial activity, have been isolated from Forsythia suspensa fruits. Degradative and spectroscopic studies have established their structures as represented by formulas I and II, respectively.

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, and we have recently disclosed that the principal antibacterial constituent of the crude drug originating from the fruits of F. suspensa was a glycoside, forsythoside A (III), which occurred also in the leaves of the same plant.[2] It has also been revealed that in the leaves of F. koreana Nakai no forsythoside A (III) was detected, but instead, the 3-rhamnosyl isomer acteoside (IV) was identified as the principal antibacterial constituent and, in addition, another glucoside, forsythoside B (V) was found in the stems.[3]

In a continuation of our chemical and pharmacological studies on the crude drug, we have isolated two new glycosides, forsythoside C: $C_{29}H_{36}O_{16}$, $[\alpha]_D$ -21.0° (MeOH), and forsythoside D: $C_{20}H_{30}O_{13}$, $[\alpha]_D$ -30.5° (MeOH), along with rutinose: $C_{12}H_{22}O_{10}$, $[\alpha]_D$ -1.6° (MeOH), D-glucose: $C_6H_{12}O_6$, m.p. 146°C, $[\alpha]_D$ +51.0° (MeOH), and L-rhamnose: $C_6H_{12}O_5$, $[\alpha]_D$ +5.6° (MeOH) from a methanol extract of the fruits of F. suspensa. This report is concerned with the structure determination of these two new glycosides forsythoside C and forsythoside D.



III



V

Forsythoside C (I) was an air sensitive amorphous substance, exhibiting ion peaks at m/e 663 ($M+Na^+$) and 679 ($M+K^+$) in its FAB-mass spectrum. Its UV (CH_3OH) and ^1H NMR (CD_3OD) spectra indicated the presence of a secondary methyl group (δ 1.19, 3H broad doublet, J 6 Hz), two anomeric methine groups (δ 4.39, 1H doublet, J 8 Hz; 4.61, 1H broad doublet, J 1.5

Hz), a trans-α,β-disubstituted conjugated enone system (λ_{max} 331 nm; δ 6.27, 1H doublet, J 16 Hz; 7.57, 1H doublet, J 16 Hz), and two trisubstituted phenyl groups (λ_{max} 220, 233, 250, 290 nm; δ 6.60-7.20, 6H multiplet). These structural characteristics were similar to those of forsythoside A (III),[2] suggesting that this glycoside was also a caffeic acid ester of rhamnosylglucose.

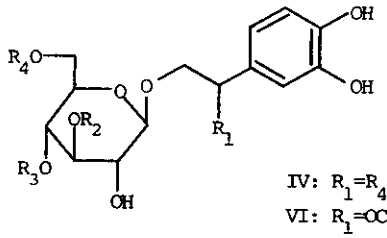
On mild alkaline hydrolysis with 2% sodium hydroxide in 50% aqueous methanol at room temperature followed by neutralization with Amberlite IR 120 (H^+ form), forsythoside C yielded methyl caffeoate: $C_{10}H_{10}O_4$, m.p. 150°C; ^{1}H NMR (CD_3OD) δ: 3.74 (3H singlet), 6.23 (1H doublet, J 16 Hz), 6.60-7.10 (3H multiplet), 7.52 (1H doublet, J 16 Hz); MS m/e: 194 (M^+), and decaffeoyl-forsythoside C: $C_{20}H_{30}O_{13}$, $[\alpha]_D$ -35.9° (MeOH); ^{1}H NMR (CD_3OD) δ: 1.14 (3H broad doublet, J 6 Hz), 4.30 (1H doublet, J 8 Hz), 4.72 (1H doublet, J 1.5 Hz), 6.55-6.90 (3H multiplet); FD-MS m/e: 501 ($M+Na^+$), 517 ($M+K^+$). The deacylated product was identical to forsythoside D (II) and hence, the structure determination of the two glycosides could be done with either of these compounds.

Forsythoside D (II) was hydrolysed enzymatically with crude hesperidinase in citrate-phosphate buffer (pH 4.0) to yield glucose, rhamnose and the phenolic aglycon: $C_8H_{10}O_4$, m.p. 116°C, $[\alpha]_D$ +7° (MeOH); ^{1}H NMR (CD_3OD) δ: 3.54 (2H doublet, J 7 Hz), 4.51 (1H triplet, J 7 Hz), 6.50-6.90 (3H multiplet); MS m/e: 170(M^+), these spectral data being in accord with those of 3',4'-dihydroxy-phenylethane-1,2-diol.[4]

The component units were then reassembled to formulate the original structure of forsythoside C. The ^{13}C NMR resonances (pyridine-d_5) of C-6 (δ 67.3 broad triplet) of the glucose moiety in forsythoside C exhibited the characteristic low field shift because of alkyl substitution attributable to the binding of rhamnosyl group at this position. Further, the ^{1}H and ^{13}C NMR resonances of the rhamnosyl group (δ 1.19, 3H broad doublet, J 6 Hz; 4.61, 1H doublet, J 1.5 Hz; δ 102.0, doublet (C-1); 72.2, doublet (C-2); 72.2, doublet (C-3); 73.6, doublet (C-4); 69.5, doublet (C-5); 18.3, quartet (C-6)) demonstrated the α-configuration of the glycosidic linkage.[2,6] The two sugar units so bound constitute rutinose. The phenolic aglycon was thus linked to the C-1 position of the glucose moiety. Stereochemistry of the glycosidic bond was determined to be β by the higher field chemical shift (δ 4.39) and a large diaxial coupling constant (J 8 Hz) of the anomeric hydrogen signal in the ^{1}H NMR spectrum.[2,5]

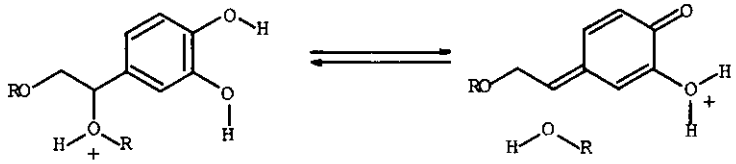
Although ^{13}C NMR spectra have proven to be of high diagnostic value in locating an acyl group in the glucose moiety,[2,3,6] forsythoside C exhibited two sets of ^{13}C NMR signals for the glucose moiety (e.g. C-1 appeared at δ 105.0 and 104.3, totaling one carbon equivalent), because, as will be mentioned later, the glycoside was a mixture of two epimers at the benzylic hydroxyl group, causing the ambiguity in the individual assignments. Attempts to separate the diastereoisomers were unsuccessful because of the instability of the hydroxyl group at the benzylic position. Standing of a methanol solution of forsythoside C at room temperature for several days led to a slow substitution reaction to yield almost quantitatively the corresponding methyl ether (VI): $C_{30}H_{38}O_{16}$, $[\alpha]_D$ -19.1° (MeOH); ^{1}H NMR (CD_3OD) δ: 1.18 and 1.19 (total 3H broad doublets, J 6 Hz), 3.21 and 3.23 (total 3H singlets), 4.35 (1H broad doublet, J-7 Hz), 4.39 (1H broad doublet, J 8 Hz), 4.61 (1H broad doublet, J 1.5 Hz), 4.82 (1H broad triplet, J 9 Hz). The ether (VI) was found to be an almost equimolar mixture of epimers with respect to the benzylic position, exhibiting the duplicate signals of the methoxyl group in the ^{1}H NMR spectrum. Interestingly, the hydrogens of the fairly distant methyl group in the rhamnose moiety also appeared as a pair of doublets at δ 1.18 and 1.19 and most of the other hydrogens gave broadened signals in its ^{1}H NMR spectrum.

The substitution reaction could be reversed by treating the methyl ether (VI) with Amberlite IR 120 (H^+ form) in water at room temperature for a few days. The optical rotation of the regenerated forsythoside C, $[\alpha]_D$ -20.5° (MeOH), was almost the same as that of the original

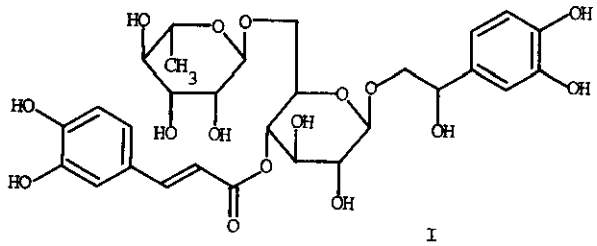


IV: R_1=R_4=H, R_2=α-L-rhamnosyl, R_3=caffeoyl
VI: R_1=OCH , R_2=H, R_3=caffeoyl, R_4=α-L-rhamnosyl
VII: R_1=OCH , R_2=R_3=H, R_4=α-L-rhamnosyl

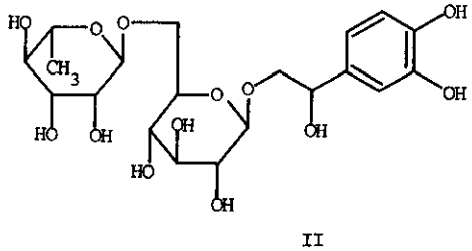
material, undoubtedly suggesting that complete equilibration at this asymmetric center had already occurred, if not in the plant tissues, then during the isolation procedures. The weak optical rotation observed for the aglycon should therefore be taken as the result of equilibration between the two diastereoisomeric glycosides, and the sign did not necessarily reflect the stereochemistry of the original natural glycoside. Similar substitution reactions were also observed with the forsythoside D and the aglycon 3',4'-dihydroxyphenylethane-1,2-diol to yield the corresponding methyl ethers, forsythoside D methyl ether (VII): $C_{21}H_{32}O_{13}$, $[\alpha]_D$ -26.9° (MeOH); ^{1}H NMR (CD_3OD) δ: 1.24 (3H broad doublet, J 6 Hz), 3.21 and 3.22 (total 3H singlets), 4.30 (1H broad doublet, J 8 Hz), 4.70 (1H broad doublet, J 1.5 Hz), 6.50-6.85 (3H multiplet), and 2-[3',4'-dihydroxyphenyl]-2-methoxyethanol: $C_9H_{12}O_4$, ^{1}H NMR (CD_3OD) δ: 3.23 (3H singlet), 3.52 (1H double doublet, J 5 and 13 Hz), 3.60 (1H double doublet, J 8 and 13 Hz), 4.10 (1H double doublet, J 5 and 8 Hz). These ready interconversions of substituents at the benzylic position may be explained by the presence of the following protonated species.



Although the location of the caffeoyl group in forsythoside C was inconclusive from analysis of the ^{13}C NMR spectrum because of the instability of the molecule, as mentioned above, its location at C-4 in the glucose moiety of forsythoside C seemed to be the most probable by analogy with other constituents (III, IV and V), and further, none of the spectral data were found inconsistent with this possibility. The suggested structure I corresponded to the hydroxyl derivative of forsythoside A (III), and hence direct interconversions were attempted. As has already been mentioned, the ready elimination of the benzylic hydroxyl group of forsythoside C suggested the presence of the para-quinonoid type intermediate, and this type of functional group may easily be reduced by hydride type reagents. In fact, reduction of forsythoside C or its methyl ether (VI) with sodium borohydride in weakly acidic aqueous butanol yielded a product which



I



II

was found to be identical with forsythoside A (III).[2]

Accumulated data have thus rigorously established the structure of forsythoside C and forsythoside D as those represented by formulas I and II, respectively.[7] It is also worthy to note that F. suspensa contains 6-rhamnosylglucosides, while F. koreana yields 3-rhamnosylglucosides.[2,3]

Forsythoside C (I) and forsythoside D (II) exhibited antibacterial activity against Staphylococcus aureus at a concentration less than 2 mM.

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NOTES AND REFERENCES

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4) The compound was identified by direct comparison with an authentic sample.
5) L. D. Hall, Tetrahedron Letters, 1964, 1457; D. E. Dorman and J. D. Roberts, J. Amer. Chem. Soc., 1970, 92, 1355.
6) K. Yoshimoto, Y. Itatani and Y. Tsuda, Chem. Pharm. Bull., 1980, 28, 2065.
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