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STRUCTURES OF RENGYOSIDES A, B AND C, THREE GLUCOSIDES OF *FORSYTHIA SUSPENSA* FRUITS

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Key Word Index-Forsythia *suspensa;* Oleaceae; rengyoside A; rengyoside B; rengyoside C; cyclohexylethyl glucosides.

Abstract-Three new glucosides, rengyosides A, B and C, having as aglycones the reduced forms of phenylethanol, were isolated from Forsythia suspensa fruits. Chemical and spectroscopic studies established the structures of these natural products to be 2-(1,4-dihydroxycyclohexyl)ethyl β-D-glucopyranoside, 2-(1-hydroxy-4-ketocyclohexyl)ethyl β-D-glucopyranoside and 2-(1,4-dihydroxycyclohexyl)ethyl β-D-6-*O*-[2-(4-hydroxyphenyl)acetyl]glucopyranoside, respectively. Salidroside, a possible biogenetic precursor of these glucosides, was also isolated.

INTRODUCTION

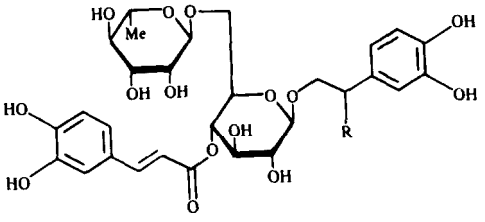
The crude drug 'rengyo' (the air-dried fruits of Forsythia *suspensa* Vahl.) is used in Oriental medicine as an anti-inflammatory, a diuretic and an antidote. The crude drug exhibits antibacterial activity due to the presence of a group of phenol glucoside esters, forsythosides A (1, R=H) and C (1, R=OH) [1–4]. Recently, this material was also shown to contain, in addition to cornoside (2), a series of unique natural alcohols, rengyol (3), isorengyol (4), rengyoxide (5) and rengyolone (6), having an unprecedented hydroxylated cyclohexylethane system which may be formed from a phenylpropanoid system [5, 6]. The isolation of these and related compounds from other sources has been reported [7-93.

In this paper we report the isolation of three possible metabolic intermediates, rengyosides A (7), B (8) and C (9), of compounds 3–6. To the best of our knowledge this is the first characterization of such glycosides having the saturated C_6–C_2 carbon skeleton.

RESULTS AND DISCUSSION

The methanol extract of *F. suspensa* fruits was partitioned against ethyl acetate and water. The water-soluble portion was extracted further with butanol to give butanol solubles which were then fractionated by a combination of silica gel, Sephadex LH-20 and Toyopearl HW-40 chromatography to afford rengyosides A (7), B (8) and C (9), along with salidroside (10) of known constitution [10].

Rengyoside A (7) was assigned the molecular formula $C_{14}H_{26}O_8$ from the ion peak at m/z 344 $[M+Na-H]^+$ in its field desorption mass spectrum. Its ^1H NMR spectrum exhibited signals at δ 1.67 and 3.79 (each 2H, *t*, *J* = 8 Hz) and δ 3.55 (1H, m, $W_{1/2}$ = 20 Hz) attributable to a



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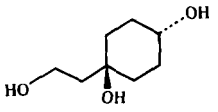

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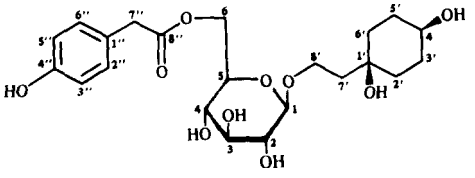

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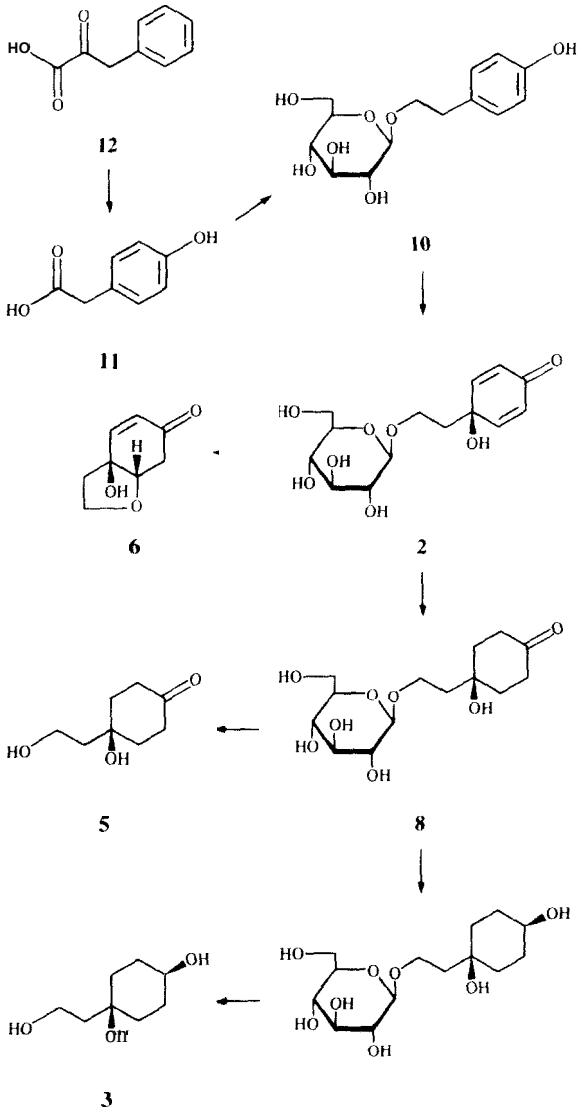

hydroxyethyl group and a hydroxymethylene group flanked by two methylene groups, respectively. Further, the signal at S4.24 (1H, *d*, *J* = 7 Hz) was assigned to an anomeric hydrogen of a sugar. Acetylation of 7 with acetic anhydride in pyridine afforded a pentaacetate. The mass spectrum of the product gave fragment ion peaks at ***m/z*** 331, corresponding to an oxonium ion of a tetra-acetylhexose, and ***m/z*** 200 and 141, attributable to the monoacetylated aglycone moiety. Enzymatic hydrolysis of 7 with crude hesperidinase in citrate-phosphate buffer (pH 4.1) at 40" yielded the aglycone and glucose, the former was identified to rengyol(3) based on its ^{1}H NMR spectrum and chromatographic behaviour [5,6]. The configuration of the glycosidic linkage of 7 was assigned to be β from the chemical shift and coupling constant of the anomeric hydrogen signal as mentioned above. Consequently, the structure of rengyoside A was established as that represented by formula 7.

Rengyoside B (8) exhibited ion peaks at ***m/z*** 358 [M + K-H] $^+$ and 359 [M + K] $^+$ in its field desorption mass spectrum corresponding to the molecular formula $C_{14}H_{24}O_8$. It showed absorption bands of hydroxyl groups (3300 cm $^{-1}$) and a carbonyl group (1700 cm $^{-1}$) in its IR spectrum. The ^{1}H NMR spectrum displayed a signal assignable to an anomeric hydrogen of a glucosyl group at 64.25 (1H, *d*, *J* = 7 Hz), which was considered to have an axial configuration from the chemical shift and coupling constant. Acetylation of 8 with acetic anhydride in pyridine gave a tetraacetate. The mass spectrum of this ester showed ion peaks at *m/z* 331. 169 and 107, compatible to the fragment ions of glucose tetraacetate, together with peaks at *m/z* 157, 141 and 127, attributable to the aglycone moiety. Enzymatic hydrolysis of this glucoside (8) with crude hesperidinase yielded the aglycone and glucose, the former was identified as rengyoxide (5) by TLC and spectral data [5]. Thus the structure of rengyoside B was concluded to be that represented by formula 8

Rengyoside C (9) was purified and characterized as its pentaacetate whose molecular formula, $C_{32}H_{42}O_{15}$, was assigned from an ion peak at ***m/z*** 667 [M + H] $^+$ in its field desorption mass spectrum. The ^{1}H NMR spectrum of the

acetate exhibited A_2B_2 type signals at 67.02 (1H, *d*, J = 9 Hz) and 7.26 (2H, *d*, J = 9 Hz), corresponding to a *p*-substituted phenol moiety, and a signal at δ 3.64 (2H, s) for an isolated methylene group next to a phenyl and a carbonyl group. Further, the hydrogen signals of another methylene group at δ 3.65 (2H, *t*, J = 7 Hz), a carbinyl methine group at 64.66 (1H, *m*, $W_{1/2}$ = 20 Hz) and an axial anomeric methine group at 64.45 (1H, *d*, J = 7 Hz) were similar to the corresponding peaks of rengyoside A pentaacetate (63.69, 4.70 and 4.49, respectively). Thus, it was assumed that rengyoside C was an ester of rengyoside A and a carboxylic acid having a p-substituted phenol structure. Alkaline hydrolysis of rengyoside C pentaacetate with aqueous potassium carbonate afforded rengyoside A (7) and p-hydroxyphenylacetic acid (**11**), the products being identified by TLC behaviour and ^{1}H NMR spectra. Further it was considered that the ester group in rengyoside C was located at C-6 of the glucose moiety, since only the chemical shifts of H-6 hydrogens of the acetate of 9 shifted slightly but distinctly to lower-field by 0.02 ppm from those of the acetate of 7.

In order to confirm the location of the ester group, esterification at C-6 of the glucose moiety of 7 to form 9 was attempted. There are several methods available for the introduction of an acyl group exclusively to the C-6 position of a glucose moiety, e.g. through a bromide [11] or by the stannylation reaction [12]. The 6-*O*-*p*-hydroxyphenylacetylglucose derivative has been obtained in good yield (72%) on reaction of phenyl β-D-glucopyranoside and p-hydroxyphenylacetic acid (**11**) with oxalyldiimidazole [13]. However, various experiments to obtain 9 by direct condensation of rengyoside A (7) and 11 did not work and gave complete recovery of the starting materials. Enzymatic transglucosylation with phenyl glucoside and rengyol (3) also failed if the substrate carried a *p*-hydroxyphenylacetyl group at C-6 of the glucose [14]. The successful instance where the ester 9 was obtained from 7 and **11** was by the method which used oxalyldiimidazole in DMF at 150°, though the yield was low (12%). These results indicated the existence of a severe steric interaction between rengyol and the phenylacetyl groups in rengyoside C (9). This may be the reason why 9 was very easily hydrolysed even during the isolation and purification procedures. It is also noteworthy that such a steric interaction is not appreciable if the rengyol moiety is exchanged with a phenoxyl group. Thus length rather than the apparent bulkiness seems to be more important. The synthetic rengyoside C (9) exhibited in its ^{1}H NMR spectrum signals at 64.02 and 4.25 (each 1H, *d*, J = 11 Hz), compatible to the esterified C-6 methylene group of the glucose moiety. Further, this product was acetylated with acetic anhydride and pyridine to afford the corresponding pentaacetate identical to the acetylation product of natural rengyoside C (TLC and spectral characteristics).

Salidroside (10) was identified by comparing its TLC behaviour and ^{1}H NMR spectrum with those of an authentic sample prepared from acetobromoglucose and p-hydroxyphenylethanol [15]. The co-existence of forsythosides (**1**), cornoside (2) and rengyol (3) in the same plant material indicates that the C_6–C_2 parts of these congeners are related biosynthetically. This view became more certain with the characterization of salidroside (10) which stood as a direct precursor of 2. The dienone system can be easily constructed by simple oxygenation of the phenol group, and the successive reduction products of 2 corresponding to rengyosides 8 and 7 [16]. This means that the aromatic and the aliphatic C_6–C_2 moieties lie in a single metabolic route.

It is easy to predict that hydrolysis of 2 yields a dienone alcohol which will cyclize spontaneously to rengyolone (6) since the dienone acetate, hallerone, yields 6 as the sole product on hydrolysis [7]. Compound 6 could then be transformed into rengyoxide (5) and rengyol(3) by a series of biological processes. However, a more plausible biogenetic route to 5 and 3 is that the dienone system of 2 is reduced successively to form 8 and 7 which are then hydrolysed to form 5 and 3 respectively. The characterization of rengyosides B (8) and A (7) support the latter course, although the possibility that the glucosides are derived from 5 and 3 cannot be ruled out. The characterization of p-hydroxyphenylacetic acid (**11**) as the ester, rengyoside C (9) is also noteworthy because it links *p*-hydroxyphenylethanol, found in salidroside (**10**), to the presumed starting compound, phenylpyruvic acid (12).

EXPERIMENTAL

Extraction *and isolation.* The crude drug (the air-dried fruits of Forsythia *suspensa*) *(500 g)* was extracted x 3 with MeOH (2 l) for 56 hr at room temp. Removal of the solvent from the combined MeOH soln under red. pres. gave the MeOH extract (50 g). The extract was dissolved in EtOAc (300 ml) and partitioned with H_2O (100 ml). The H_2O phase was re-extracted with *n*-BuOH (100 ml) and removal of the solvent from the organic phase under reduced pressure provided the *n*-BuOH extract (9.4 g) which was subjected repeatedly to chromatography on silica gel with CH_2Cl_2–MeOH systems, on Sephadex LH-20 with MeOH and on Toyopearl HW-40 with MeOH to furnish fractions containing rengyosides A (7) (11 mg), B (8) (1.2 mg) and C (9) (18 mg), along with salidroside (10) (7 mg). Final purification of rengyoside B (8) was accomplished by HPLC (Toyo Soda LS-410K, 7.5 mm i.d. x 30 cm; H_2O; 0.5 ml/min; RI detection). Rengyoside C (9) was purified by acetylation with Ac_2O–pyridine (1:2) followed by chromatography on silica gel (CH_2Cl_2–MeOH 97: 3) to give rengyoside C pentaacetate.

Rengyoside A (7). Amorphous solid; $[\alpha]_D^{23}$ – 11.0° (MeOH; c 0.18); ^{1}H NMR (CD_3OD): δ 1.2-2.0 (8H, H-2′, H-3′. H-S and H-6′), 1.67 (2H, *t*, J = 8 Hz, H-7′), 3.4-4.1 (6H, H-2, H-3, H-4, H-5 and H-6), 3.55 (1H, *m*, $W_{1/2}$ = 20 Hz, H-4′), 3.79 (2H, *t*, J = 8 Hz, H-8′), 4.24 (1H, *d*, J = 7 Hz, H-l); FDMS *m/z*: 344 $[M + Na - H]^+$.

Acetylation of rengyoside A. A soln of 7 (2 mg) and Ac_2O in pyridine was allowed to stand at room temp. for 24 hr. The crude product was chromatographed on silica gel to give the pentaacetate as a colourless oil; ^{1}H NMR ($CDCl_3$): 61.68 (2H, *t*, J = 8 Hz, H-7′), 2.00, 2.01, 2.02, 2.04, 2.08 (each 3H, s, OAc), 3.6-3.8 (1H, m, H-5), 3.69 (2H, *t*, J = 8 Hz, H-8′), 4.16 (1H, *dd*, J = 12, 2 Hz, H-6), 4.23 (1H, dd, J= 12, 5 Hz, H-6), 4.49 (1H, *d*, J = 7 Hz, H-l), 4.70 (1H, m, $W_{1/2}$ = 20 Hz, H-4′); EIMS m/z (rel. int.): 331 (22), 200 (4), 169 (74), 141 (12), 107 (54), 43 (100).

Enzymatic hydrolysis of rengyoside A. Crude hesperidinase (1 mg) was added to a soln of 7 (2 mg) in citrate-phosphate buffer (pH 4.1, 1 ml) and the mixture was incubated at 40° overnight followed by concn *in vacuo*. The residue was then subjected to chromatography on silica gel (CH_2Cl_2–MeOH 97: 3) affording rengyol (3) as an amorphous solid; ^{1}HNMR (CD_3OD): 61.66 (2H, *t*, J = 7 Hz, H-7), 3.71 (2H, *t*, J = 7 Hz, H-8), 3.51 (1H, m, H-4); EIMS m/z (rel. int.): 142 $[M - H_2O]^+$ (3), 115 $[M - C_2H_5O]^+$ (35), 98 (100); TLC: R_f = 0.63 (silica gel/CH_2Cl_2–MeOH 3: 1). Glucose; TLC: R_f 0.32 (silica gel/MeOH–$CHCl_3$–HCO_2H 10:24:1).

Rengyoside B (**8**). Amorphous solid; $[\alpha]_D^{23} - 10.4°$ (EtOH: *c* 0.28); ^{1}H NMR (CD_3OD): δ1.90 (2H, *t*, $J = 7$ Hz, H-7′), 2.5–2.9 (4H, H-3′ and H-5′), 3.80 (2H, *t*, $J = 7$ Hz, H-8′), 4.25 (1H, *d*, $J = 7$ Hz, H-1); IR ν_{max}^{nujol} cm^{-1}: 3300 (OH), 1700 (C=O); FDMS *m/z*: 358 $[M + K - H]^+$, 359 $[M + K]^+$

Acetylation of rengyoside B. **8** (1 mg) was acetylated with Ac_2O (0.1 ml) in pyridine (0.2 ml) to give the tetraacetate. ^{1}H NMR ($CDCl_3$): δ1.86 (2H, *t*, $J = 7$ Hz, H-7′), 2.01, 2.03, 2.06, 2.08 (each 3H, *s*, OAc), 4.16 (1H, *br d*, $J = 12$ Hz, H-6), 4.23 (1H, *dd*, $J = 12$, 5 Hz, H-6), 4.50 (1H, *d*, $J = 7$ Hz, H-1); EIMS *m/z* (rel. int.): 331 (13), 169 (77), 157 (8), 141 (18), 123 (16), 107 (36), 43 (100).

Enzymatic hydrolysis of rengyoside B. **8** (0.5 mg) was hydrolysed in the same manner as **7** to give rengyoxide (**5**) as an amorphous solid, IR ν_{max}^{nujol} cm^{-1}; 3400 (OH), 1700 (C=O); EIMS *m/z* (rel. int.): 158 $[M]^+$ (1), 140 $[M - H_2O]^+$ (2), 113 $[M - C_2H_5O]^+$ (3), 18 (100); TLC: R_f 0.53 (silica gel/CH_2Cl_2–MeOH 4:1). Glucose; TLC: R_f 0.32 (silica gel/MeOH-$CHCl_3$-HCO_2H 10:24:1).

Rengyoside C pentaacetate. Amorphous solid; $[\alpha]_D^{23} - 17.1°$ (CH_2Cl_2; *c* 0.04); ^{1}H NMR ($CDCl_3$): δ2.00, 2.00, 2.03, 2.04, 2.28 (each 3H, *s*, OAc), 3.5–3.8 (1H, *m*, H-5), 3.64 (2H, *s*, H-7″), 3.65 (2H, *t*, $J = 7$ Hz, H-8′), 4.19 (1H, *br d*, $J = 13$ Hz, H-6), 4.25 (1H, *br d*, $J = 13$ Hz, H-6), 4.45 (1H, *d*, $J = 7$ Hz, H-1), 4.66 (1H, *m*, $W_{1/2} = 20$ Hz, H-4′), 7.02 (2H, *d*, $J = 9$ Hz, H-3″ and H-5″), 7.26 (2H, *d*, $J = 9$ Hz, H-2″ and H-6″); FDMS *m/z*: 667 $[M + H]^+$, 666 $[M]^+$.

Alkaline hydrolysis of rengyoside C pentaacetate. K_2CO_3 (5 mg) was added to a soln of rengyoside C pentaacetate (2 mg) in H_2O (0.5 ml) and the mixture was kept stirred at room temp. for 2 hr. The reaction mixture was concd *in vacuo* and the residue was subjected to CC on silica gel to afford rengyoside A (**7**) as an amorphous solid; ^{1}H NMR (CD_3OD): δ1.66 (2H, *t*, $J = 7$ Hz, H-7′), 3.50 (1H, *m*, H-4′), 3.76 (2H, *t*, $J = 7$ Hz, H-8′), 4.24 (1H, *d*, $J = 7$ Hz, H-1); TLC: R_f 0.73 (silica gel/CH_2Cl_2–MeOH 4:1) and *p*-hydroxyphenylacetic acid (**11**) as a colourless powder; ^{1}H NMR ($CDCl_3$): δ3.66 (2H, *s*, H-7), 6.93 (2H, *d*, $J = 10$ Hz, H-3 and H-5), 7.22 (2H, *d*, $J = 10$ Hz, H-2 and H-6), 7.2 (1H, *m*, OH); EIMS *m/z* (rel. int.): 153 $[M + H]^+$ (29), 107 $[M - CO_2H]^+$ (100); TLC: R_f 0.75 (silica gel/CH_2Cl_2 – MeOH 4:1).

Salidroside (**10**). Amorphous powder; $[\alpha]_D^{23} - 27.7°$ (H_2O; *c* 0.13); ^{1}H NMR (CD_3OD): δ2.81 (2H, *t*, $J = 7$ Hz, H-7′), 3.71 (2H, *t*, $J = 7$ Hz, H-8′), 4.26 (1H, *d*, $J = 7$ Hz, H-1), 6.68 (2H, *d*, $J = 8$ Hz, H-3 and H-5), 7.01 (2H, *d*, $J = 8$ Hz, H-2 and H-6); FDMS *m/z*: 300 $[M]^+$. These data were identical with those of synthetic salidroside [15].

Condensation of rengyoside A (**7**) *and* p-*hydroxyphenylacetic acid* (**11**). A mixture of **11** (304 mg) and oxalyldiimidazole (380 mg) suspensed in DMF (5 ml) at 80°, was kept stirred for 2 hr. Then **7** (128 mg) in DMF (5 ml) was added to the mixture and the stirring continued for 7 hr at 150°. The reaction mixture was concentrated *in vacuo* and the residue subjected repeatedly to silica gel chromatography. Elution with CH_2Cl_2–*iso*-PrOH (2:1) afforded a colourless powder, rengyoside C (**9**) (35 mg), $[\alpha]_D^{23} - 21.9°$ (*iso*-PrOH; *c* 0.06); ^{1}H NMR (CD_3OD): δ1.70 (2H, *t*, $J = 7$ Hz, H-7′), 3.67 (2H, *s*, H-7″), 4.02 (1H, *d*, $J = 11$ Hz, H-6), 4.25 (1H, *d*, $J = 11$ Hz, H-6), 4.26 (1H, *d*, $J = 7$ Hz, H-1), 6.73 (1H, *d*, $J = 9$ Hz, H-3″ and H-5″), 7.02 (1H, *d*, $J = 9$ Hz, H-2″ and H-6″); FDMS *m/z*: 457 $[M + H]^+$.

The synthetic **9** (2 mg) was acetylated with Ac_2O (0.2 ml) in pyridine (0.4 ml) to give the pentaacetate as an amorphous solid, $[\alpha]_D^{23} - 18.0°$ (CH_2Cl_2; *c* 0.08); ^{1}H NMR ($CDCl_3$): δ1.70 (2H, *t*, $J = 7$ Hz, H-7′), 2.01, 2.01, 2.03, 2.04, 2.29 (each 3H, *s*, OAc), 3.64 (2H, *s*, H-7″), 3.68 (3H, *m*, H-8′ and H-5), 4.66 (1H, *m*, $W_{1/2} = 20$ Hz, H-4′), 7.03 (1H, *d*, $J = 9$ Hz, H-2″ and H-6″), 7.25 (1H, *d*, $J = 9$ Hz, H-3″ and H-5″); FDMS *m/z*: 667 $[M + H]^+$. These data were identical to those of the acetate of natural rengyoside C.

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