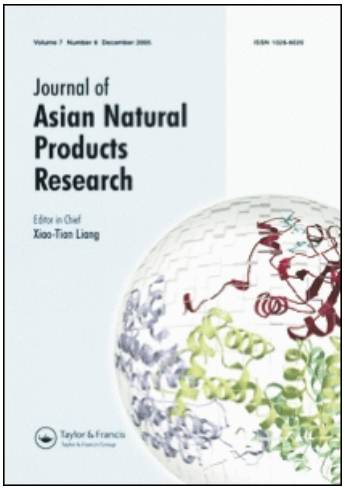

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Syntheses of R-β-rutinosides by rutin-degrading reaction

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Rutinose and five R-β-rutinosides were obtained by means of rutin-degrading reaction in water or aqueous alcohol (ROH, R = methyl, ethyl, propyl, isopropyl, and benzyl) with rutin-degrading enzyme as catalyst and rutin as starting material in 84–94% yields, of which methyl-β-rutinoside, propyl-β-rutinoside, isopropyl-β-rutinoside, and benzyl-β-rutinoside are firstly reported in this paper. Based on spectral analysis, the structures of all products were elucidated.

Keywords: rutinoside; rutin-degrading reaction; rutinose; rutin

1. Introduction

Natural rutinose (α-L-rhamnopyranosyl-(1 → 6)-α/β-D-glucopyranosyl) extensively exists in many plants in a pattern of glycosides, of which most are flavonoid glycosides [1], but few were found in free form. Up to now, all the chemical syntheses of rutinose from flavonoid started from natural rutinoside with acid- or enzyme-catalyzed hydrolysis reaction [2–12]. To the best of our knowledge, the report on hydrocarbonyl-β-rutinoside preparation from rutin is not found.

Some studies revealed that there were flavonol-3-heterodisaccharide glycosidase [11] and rutin-degrading enzyme (RDE) [8,10,13,14] in *Fagopyrum* plants, which can decompose rutin into quercetin and rutinose in water solution [13–15], and have higher selectivity of substrates. Our previous research also showed that RDE in the seed of *Fagopyrum tataricum* (L.) Gaertn had not only very high activity, but also strong resistance against acid, base, heat, as well as high concentrated ethanol [16]. In our continuing study on the degradation product of rutin by RDE, ethyl rutinoside was found when aqueous ethanol was used as reaction solvent. This result is in agreement with that reported by Yasuda [17]. Based on the evocation of the above study, we further examined rutin degradation reactions occurring in other alcohol solvents instead of ethanol. Finally, rutinose (**1**) and five R-β-rutinosides [R = methyl (**2**), ethyl (**3**), propyl (**4**), isopropyl (**5**), and benzyl (**6**)], which have potential application value as food additives, cosmetics additives, and pharmaceuticals, were prepared in high yield, of which **2**, **4**, **5**, and **6** as new compounds are reported for the first time in this paper, and meanwhile this report discloses the first one-step reaction of rutin with alcohols in the presence of RDE and provides a direct, convenient, and green biosyntheses of hydrocarbonyl-β-rutinoside from rutin (Scheme 1).

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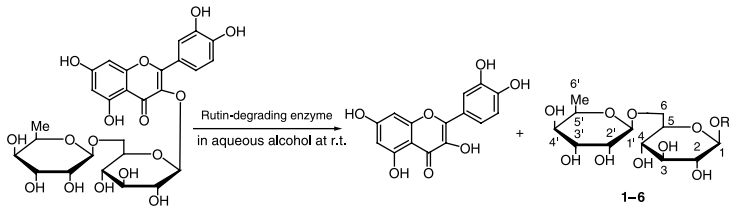

Compounds	R	Yield (%)	Compounds	R	Yield (%)
1	H	92	**4**	$(CH_3)_2CH$	91
2	CH_3	90	**5**	$CH_3CH_2CH_2$	94
3	CH_3CH_2	84	**6**	$C_6H_5CH_2$	88

Scheme 1. Preparation of R-β-rutinosides by rutin-degrading reaction.

2. Results and discussion

Crude RDE (cRDE) solution was prepared from the seed of *F. tataricum* (L.) Gaertn according to Xu's method [18] and used for rutin degradation reaction without further purification. Rutinose **1** and rutinosides **2**–**6** were prepared at room temperature by rutin degradation reaction in water or aqueous alcohols in the presence of cRDE. All compounds **1**–**6** were separated as yellow gum from the reaction solution by Sephadex LH-20 column chromatography. The α-naphthol tests for compounds **1**–**6** all revealed purple, showing that compounds **1**–**6** belong to saccharide. The HR-MSs of **2**, **4**, **5**, and **6** showed the $[M + Na]^+$ ion peaks at *m/z* 363.1255, 391.1572, 391.1582, and 439.1569, respectively, corresponding to $C_{13}H_{24}O_{10}Na$, $C_{15}H_{28}O_{10}Na$, $C_{15}H_{28}O_{10}Na$, and $C_{19}H_{28}O_{10}Na$. And the molecular formulas of **1** ($C_{12}H_{22}O_{10}$) and **3** ($C_{14}H_{26}O_{10}$) were derived from the combination of NMR and FAB-MS. The ^{13}C NMR spectra (Table 1) of compounds **1**–**6** revealed a similarity. All signals of sugar carbons of **1**–**6** were in the range of δ 67–104, except that the methyl carbon of rhamnosyl was in the range of 16.4–19.4. As for **2**–**5**, the signals of carbons in R group appeared in the range of 9.9–70.0. However, for **6**, there were three signals in the range of δ 131–139 assigned to aromatic carbons in benzyl group. In addition, compared with $\delta_{H\text{-}1}$ and $J_{H1,H2}$ in ^1H NMR spectra of **1**–**6** recorded in D_2O, it was found that H-1 of compounds **2**–**6** were all linked to C-1 by axial bond observed at δ 4.0–4.5 ($J = 7.6$–10.8 Hz). But compound **1** was confirmed to be the mixture of α- and β-rutinosides by $\delta_{H\text{-}1}$ and $J_{H1,H2}$ coupling constant, in which α-rutinoside ratio was 40% based on $\delta_{\alpha\text{-}H\text{-}1}$ 4.99 (d, $J = 4.8$ Hz, 0.4H) and β-rutinoside ratio was 60% based on $\delta_{\beta\text{-}H\text{-}1}$ 4.40 (d, $J = 10.8$ Hz, 0.6H). Moreover, the structure characteristics of **1**–**6** were also supported by their IR and FAB-MS spectra.

Our previous study found that the activity and heat resistance of RDE greatly reduced after cRDE was purified, therefore cRDE was directly used for the rutin degradation reaction in this paper. In order to know whether there is β-rutinoside synthesis enzyme in cRDE, or RDE has both the catalytic function of rutin degradation and the synthesis activity of hydrocarbonyl-β-rutinoside from rutinose, we further examined the reaction of rutinose instead of rutin as starting material in methanol–water solution. The result showed that methyl-β-rutinoside (**2**) was not found in mixed solution.

Table 1. ^{13}C NMR spectral data of compounds **1–6** in D_2O (400 MHz).

	δ (DEPT)					
Carbon	**1**	**2**	**3**	**4**	**5**	**6**
C-1	96.0 (CH)	103.5 (CH)	102.0 (CH)	101.0 (CH)	102.4 (CH)	104.1 (CH)
C-2	72.7 (CH)	72.2 (CH)	72.1 (CH)	74.3 (CH)	73.3 (CH)	75.8 (CH)
C-3	75.7 (CH)	75.9 (CH)	75.8 (CH)	75.9 (CH)	76.0 (CH)	78.4 (CH)
C-4	70.2 (CH)	69.9 (CH)	69.8 (CH)	70.2 (CH)	70.3 (CH)	72.3 (CH)
C-5	74.8 (CH)	75.0 (CH)	74.8 (CH)	75.0 (CH)	74.9 (CH)	74.8 (CH)
C-6	68.7 (CH_2)	67.2 (CH_2)	67.0 (CH_2)	67.2 (CH_2)	67.1 (CH_2)	69.4 (CH_2)
C-1′	100.6 (CH)	100.9 (CH)	100.7 (CH)	100.8 (CH)	100.8 (CH)	103.9 (CH)
C-2′	71.4 (CH)	70.2 (CH)	70.1 (CH)	70.4 (CH)	70.4 (CH)	72.7 (CH)
C-3′	72.1 (CH)	70.4 (CH)	70.2 (CH)	70.7 (CH)	70.7 (CH)	74.5 (CH)
C-4′	74.1 (CH)	73.2 (CH)	73.1 (CH)	72.3 (CH)	72.6 (CH)	77.5 (CH)
C-5′	70.0 (CH)	68.8 (CH)	68.7 (CH)	68.8 (CH)	68.8 (CH)	71.4 (CH)
C-6′	16.7 (CH_3)	16.9 (CH_3)	16.7 (CH)	16.9 (CH)	16.9 (CH_3)	19.4 (CH_3)
α-C		57.4 (CH_2)	66.3 (CH_2)	70.0 (CH)	70.1 (CH_2)	72.9 (CH_2)
β-C			14.4 (CH_3)	22.7 (CH_3)	22.5 (CH_2)	
γ-C					9.9 (CH_3)	
C-1″						139.3 (C)
C-2″, C-6″						131.2 (CH)
C-3″, C-5″						131.6 (CH)
C-4″						131.4 (CH)

This revealed that all R-rutinosides **2–6** obtained in this paper were directly formed in the process of rutin degradation, but not converted from rutin hydrolysis product, rutinose. But why were the configurations of all R-rutinosides β-type? The reason is probably that there is an effect of neighboring group participation between 2-OH and C-1 on backbone of glucose in rutin molecule.

Based on the analysis of reaction rates of all rutinosides formation by thin layer chromatography, it was found that the forming rates of ethyl rutinoside and benzyl rutinoside were obviously slower than that of others. We think that this is resulted from that high concentration of ethanol can resist the activity of RDE [14], and that the formation of benzyl rutinoside mainly occurred on the two-phase interface because the solubility of benzyl alcohol in water is very low.

Although there exist some differences among forming rates of all five rutinosides, all products were obtained in high yield. This result showed that the preparation of R-β-rutinosides by rutin-degrading reaction is generally feasible either considering the type, size, or steric hindrance of R in alcohol or the water solubilities of alcohols.

3. Experimental

3.1 General experimental procedure

Optical rotations were measured using a Jasco DIP-360 digital polarimeter. UV spectra were recorded on PE LAM-BDA17 UV/vis photometer (H_2O, concentration about 10^{-4} mol/l). IR spectra were obtained on Bio-Rad WIN-IR spectrophotometer. FAB-MS were taken on VG ZAB-HS mass spectrometer. The ^1H and ^{13}C NMR spectra were carried out on a BRUKER-AM-400 spectrometer (^1H 400 MHz and ^{13}C 125 MHz). Samples were run in D_2O and referenced to TMS. *J* values are given in Hertz. *F. tataricum* (L.) Gaertn was breeded by Prof. Yan Chai in College of Agronomy in Northwest A&F University, Yangling, China, and the flour of *F. tataricum* (L.) Gaertn was friendly offered by him. Rutin (purity 98%) was purchased from Shanghai Chemical Reagent Corporation, Shanghai, China, and all alcohols as reaction

reagents were analytically pure grade and used without further purification.

3.2 Preparation of rutin-degrading enzyme

About 50 g flour from the seed of *F. tataricum* (L.) Gaertn was added in 400 ml (0.02 mol/l, pH 5.0) acetic acid buffer and stirred for 2 h at 4°C. The suspension solution was centrifugated at 4°C with 3000 r/min speed for 30 min. The upper clear solution was collected, and $(NH_4)_2SO_4$ was added to the solution to 80% degree of saturation. The solution stood for 12 h at 4°C, and then was centrifugated for 30 min at 4°C with 10,000 r/min speed. The deposit was collected, dissolved in 100 ml (0.02 mol/l, pH 5.0) acetic acid buffer, and loaded in a dialysis bag (27 mm long, 8000–14,400 Mw). The dialysis bag was immersed in 4°C distilled water to dialysis for 10 h, meanwhile dialysate was refreshed in every 2 h. Finally, the cRDE solution in the dialysis bag was transferred into a 200 ml conical flask and preserved at 4°C to be directly used in the rutin degradation reaction.

3.3 General procedure for synthesis of R-β-rutinosides

Rutin (0.1 g) was dissolved in 100 ml water or aqueous alcohol. The cRDE solution (10 ml) prepared as the method was to add dropwise to the solution of rutin under stirring. The resulting solution was stirred for 12 h at room temperature, and then the mixed solution was filtered. The filtrate was collected and concentrated to 3.0–5.0 ml under reduced pressure. The concentrated solution was sequentially subjected to Sephadex LH-20 column chromatography using distilled water as eluent. Fractions were detected by thin layer chromatography with $CHCl_3$—CH_3OH—H_2O (6:3.5:0.5, v/v/v) as developing solvent and with 5% sulfuric acid in EtOH as developer. The fractions containing saccharide were combined and evaporated to dryness to give expected rutinoside as yellow gum.

3.3.1 Rutinose (**1**)

Water was used as reaction solvent. Yield: 49 mg (92%), yellow gum, perfectly soluble in water, and soluble in methanol and ethanol, respectively; $[\alpha]_D^{25} = -0.5$ ($c = 0.00834$, H_2O); IR ν_{max}^{KBr} (cm^{-1}): 3385 (br, —OH), 1051, 1134 (C—O). The ^{13}C NMR spectral data were shown in Table 1. ^1H NMR (D_2O, TMS) δ: 4.99 (0.4H, d, $J = 4.8$ Hz, α-H-1), 4.40 (0.6H, d, $J = 10.8$ Hz, β-H-1), 4.59 (1H, br s, H-1′), 1.06 (3H, d, $J = 8.0$ Hz, H-6′), 3.0–4.0 (10H, m, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′). FAB-MS *m/z*: 349.2 $[M + Na]^+$, 333.2 $[M + Li]^+$.

3.3.2 Methyl α-L-rhamnopyranosyl-(1 → 6)-β-D-glucoside (**2**)

A 95% w/w aqueous solution of methanol was used. Yield: 50 mg (90%), yellow gum, perfectly soluble in water, and soluble in methanol and ethanol, respectively; $[\alpha]_D^{25} = -34.1$ ($c = 0.02730$, H_2O); IR ν_{max}^{KBr} (cm^{-1}): 3399 (br, —OH), 1048, 1127 (C—O). The ^{13}C NMR spectral data were shown in Table 1. ^1H NMR (D_2O, TMS) δ: 4.62 (1H, br, H-1′), 4.14 (1H, d, $J = 8.8$ Hz, H-1), 3.12 (3H, s, OCH_3), 1.07 (3H, d, $J = 4.8$ Hz, H-6′), 3.0–4.0 (10H, m, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′). FAB-MS *m/z*: 363.1 $[M + Na]^+$, 347.3 $[M + Li]^+$. HR-MS *m/z* 363.1255 $[M + Na]^+$ (calcd for $C_{13}H_{24}O_{10}Na$, 363.1267).

3.3.3 Ethyl α-L-rhamnopyranosyl-(1 → 6)-β-D-glucoside (**3**)

A 95% w/w aqueous solution of ethanol was used. Yield: 1.49 mg (84%), yellow gum, perfectly soluble in water, and soluble in methanol and ethanol, respectively; $[\alpha]_D^{25} = -39.6$ ($c = 0.00998$, H_2O); IR ν_{max}^{KBr} (cm^{-1}): 3404 (br, —OH), 2977, 2926 (CH_3,CH_2), 1040, 1064 (C—O), 1384 (CH_3). The ^{13}C NMR spectral data were shown in Table 1. ^1H NMR (D_2O, TMS) δ: 4.62 (1H, d, $J = 9.2$ Hz, H-1′), 4.24 (1H, d, $J = 10.8$ Hz, H-1), 1.08 (3H, d, $J = 7.6$ Hz, H-6′), 1.01 (3H, t, $J = 10.0$ Hz, CH_2CH_3), 3.0–4.0 (12H, m, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′, CH_2CH_3). FAB-MS *m/z*: 377.2 $[M + Na]^+$, 361.2 $[M + Li]^+$.

3.3.4 *Isopropyl α-L-rhamnopyranosyl-(1 → 6)-β-D-glucoside (**4**)*

A 95% w/w aqueous solution of isopropanol was used. Yield: 1.55 mg (91%), yellow gum, perfectly soluble in water, and soluble in methanol and ethanol, respectively; $[\alpha]_D^{25} = -11.1$ ($c = 0.01324$, H_2O); IR ν_{max}^{KBr} (cm^{-1}): 3401 (br, —OH), 2979, 2922 (CH_3, CH_2), 1044, 1064 (C–O), 1383, 1379 (CH_3). The ^{13}C NMR spectral data were shown in Table 1. ^1H NMR (D_2O, TMS) δ: 4.62 (1H, d, $J = 3.6$ Hz, H-1′), 4.40 (1H, d, $J = 8.0$ Hz, H-1), 1.07 (3H, d, $J = 6.4$ Hz, H-6′), 1.03 (6H, d, $J = 6.4$ Hz, $CH(CH_3)_2$), 3.0–4.0 (11H, m, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′, $CH(CH_3)_2$). FAB-MS *m/z*: 391.5 $[M + Na]^+$, 375.4 $[M + Li]^+$. HR-MS *m/z* 391.1572 $[M + Na]^+$ (calcd for $C_{15}H_{28}O_{10}Na$, 391.1580).

3.3.5 n-*Propyl α-L-rhamnopyranosyl-(1 → 6)-β-D-glucoside (**5**)*

A 95% w/w aqueous solution of *n*-propanol was used. Yield: 57 mg (94%), yellow gum, perfectly soluble in water, and soluble in methanol and ethanol, respectively; $[\alpha]_D^{25} = -30.0$ ($c = 0.03162$, H_2O); IR ν_{max}^{KBr} (cm^{-1}): 3404 (br, —OH), 2985, 2931 (CH_3,CH_2), 1041, 1058 (C–O), 1380 (CH_3). The ^{13}C NMR spectral data were shown in Table 1. ^1H NMR (D_2O, TMS) δ: 4.97 (1H, d, $J = 3.6$ Hz, H-1′), 4.20 (1H, d, $J = 8.0$ Hz, H-1), 1.39 (2H, m, $CH_2CH_2CH_3$), 1.05 (3H, d, $J = 5.6$ Hz, H-6′), 0.67 (3H, t, $J = 7.6$ Hz, $CH_2CH_2CH_3$), 3.0–4.0 (12H, m, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′, $CH_2CH_2CH_3$). FAB-MS *m/z*: 391.4 $[M + Na]^+$, 375.3 $[M + Li]^+$. HR-MS *m/z* 391.1582 $[M + Na]^+$ (calcd for $C_{15}H_{28}O_{10}Na$, 391.1580).

3.3.6 *Benzyl-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucoside (**6**)*

The mixed solution of benzyl alcohol and water was used. Yield: 60 mg (88%), yellow gum, perfectly soluble in water, and soluble in methanol and ethanol, respectively; $[\alpha]_D^{25} = -43.25$ ($c = 0.02528$, H_2O). IR ν_{max}^{KBr} (cm^{-1}): 3396 (br, —OH), 3087, 3066, 3036 (Ar-H), 1607, 1499 (aromatic ring), 1043, 1062 (C—O). UV $\lambda_{max}^{H_2O}$ (nm): 213, 257. The ^{13}C NMR spectral data were shown in Table 1. ^1H NMR (D_2O, TMS) δ: 7.44 (m, 5H, Ar-H), 4.89 (1H, d, $J = 12.0$ Hz, $CH_2C_6H_5$), 4.82 (1H, d, $J = 3.9$ Hz, H-1′), 4.75 (1H, d, $J = 12.0$ Hz, $CH_2C_6H_5$), 4.51 (1H, d, $J = 7.6$ Hz, H-1), 1.30 (3H, d, $J = 5.1$ Hz, H-6′), 3.0–4.0 (10H, m, H-2, H-3, H-4, H-5, H-6, H-2′, H-3′, H-4′, H-5′). FAB-MS *m/z*: 439.3 $[M + Na]^+$, 423.4 $[M + Li]^+$. HR-MS *m/z* 439.1569 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_{10}Na$, 439.1580).

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