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Original Article

Synthesis and antioxidant activities of flavonoids derivatives, troxerutin and 3', 4', 7-triacetoxyethoxyquercetin

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ABSTRACT

Syntheses of two very important derivatives of quercetin, troxerutin and 3', 4', 7-triacetoxyethoxyquercetin were described. The latter was synthesized by highly selective esterification reaction in first time. The compounds were characterized by NMR, IR and Mass spectroscopy. Additionally, the antioxidant activities of the compounds were tested by means of improved pyrogallol autoxidation method. This was the first in using this method to test the antioxidant activities of these two compounds *in vitro*. The optimum system of pyorgallol autoxidation spectrophotometry was investigated and established according to the reaction rules. The assay indicated that these compounds showed noticeable antioxidant activities, and compound **2** was much more effective as a free radical scavenger than the compound **1** vitamin C was used as a reference material.

1. Introduction

Flavonoids, such as flavones and flavonols, are plant polyphenolic compounds, which are found in many foods, especially in fruits and vegetables [1]. As the most common natural flavonoid, quercetin and its derivatives are widely present in nuts, beverages and Chinese herbal medicine. Given their broad range of pharmacological effects and biological activities, such as efficient anti-oxidant and scavenging oxygen free radicals injury [2,3], quercetin and its derivatives not only are very efficient drugs for reducing the blood pressure [4], but also appear to be active in many diseases related to aging such as vasodilatation [5,6], cardiovascular [7], neurodegenerative [8] diseases and cancer [9], especially ovarian cancer, breast cancer and leukemia [10,11]. Furthermore, an enormous number of scientific studies indicate that they possess antimicrobial activity [12], anti-inflammatory and analgesic properties [13]. Nevertheless, only a small percentage of the ingested quercetin can be absorbed by the human body, as previously reported [14]. Thus, arduous efforts should be made to increase their solubility and slow down their metabolism by chemical modification of the natural compound on the premise of maintaining the ability to regenerate the original molecules [15].

Troxerutin (trihydroxy-ethylrutoside) possesses extensive pharmacological activities. Its mechanism is probably related to the inhibition of platelet aggregation and the prevention of thrombosis [16]. Therefore, it is used as a treatment against the 5-hydroxytryptamine, bradykinin-induced vascular injury and increases the healing of capillary endothelial defects. Moreover, it can also reduce capillary permeability and prevent edemas caused by increasing vascular permeability [17,18]. Thus, two quercetin derivatives were designed and synthesized by using rutin as a substrate, expecting to obtain more active derivatives. In addition, there are several methods for determining the superoxide scavenging activities of foods, including cytochrome c reduction, electron spin resonance (ESR), chemiluminescence [19], and so on. All of these methods require special and expensive instruments or biological agents. Yet, pyrogallol (1,2,3-trihydroxybenzene) can autoxidize in alkaline solutions to produce $^{\bullet}O_2^-$ anion radicals and colored intermediate products, quinones, which are easily detected by a spectrophotometer. The antioxidant can inhibit the autoxidation of pyrogallol. Thus, it can inhibit the colored intermediate products. So the absorbance reflects the generation of both oxidation products and superoxide radicals ($^{\bullet}O_2^-$). The improved pyrogallol method is a reliable and cheap superoxide-scavenging assay suitable for many types of flavonoids. So the scavenging activities of the compounds were evaluated by means of an improved pyrogallol autoxidation method. And what is more, in previous reports, flavonoids played a remarkable role in antioxidant activities mainly by means of chelating with variable valence metal ion or scavenging oxygen free radicals [20]. The first site involved the complex formation process was the 3-hydroxy-4-carbonyl in the original structure [21].

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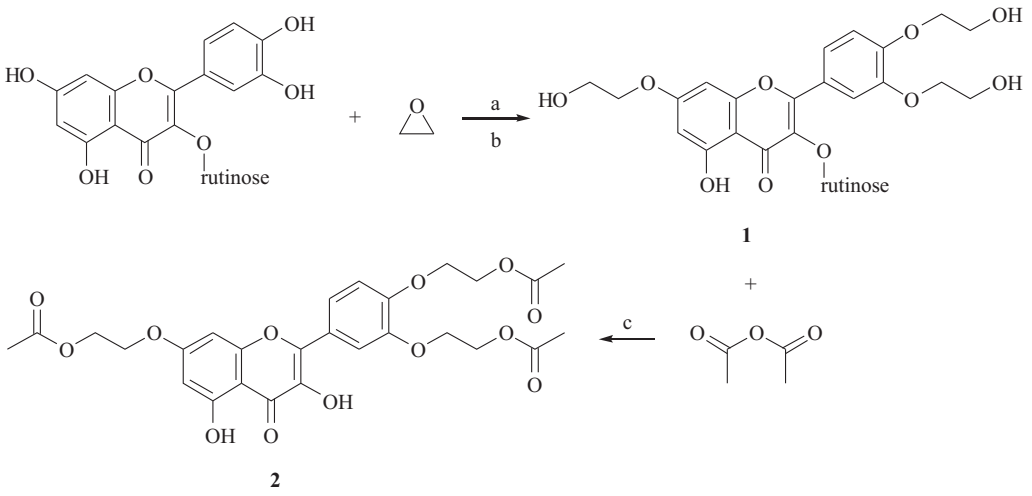

Scheme 1. Condition and regent: (a) NaOH; (b) H_2O; (c) H_2SO_4.

2. Experimental

Ethylene oxide (6.60 g, 150 mmol) and NaOH (0.28 g, 7 mmol) were added to a solution of rutin (12.21 g, 20 mmol) in H_2O (100 mL) at room temperature. After stirring at 75 °C for 6 h, the pH value of the mixture was adjusted to 4.0 by concentrated hydrochloric acid. Then, the yellow solid was formed. The crude product was collected by filtration and washed with cold water. It was repeatedly recrystallized by methyl alcohol and the yellow powder, compound **1**, was obtained. The purity was 99.84% tested by HPLC (CH_3OH/H_2O = 8:1; v/v) equipment (Shimadzu).

Troxerutin (1.00 g, 1.35 mmol) was dissolved in acetic anhydride (20.00 mL) in a 250 mL round-bottomed flask and the solution was stirred for half an hour at room temperature. H_2SO_4 (0.40 mL, 50%) was added dropwise into the reaction system on the stir condition. After completion of the addition, the resulting solution was stirred at RT for 2 h followed by keeping it at RT for 5 h. An additional 150 mL of ultrapure water was added and the resulting mixture was stirred at RT for 2 h until the faint yellow deposition separated out. Extract the deposited material and wash them with ultrapure water several times to obtain the faint yellow crystals. The filter cake was dried in a vacuum oven to give 0.57 g (57%, m) of the desired compound **2** (Scheme 1).

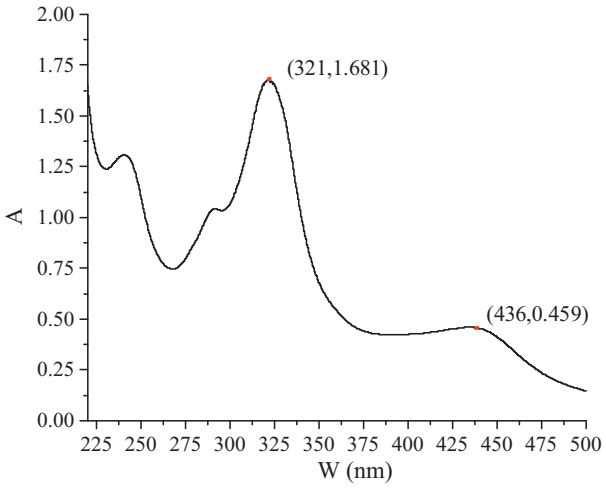

Fig. 1. Absorption spectrum of pyrogallol autoxidation at different wavelengths. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The pyrogallol autoxidation system was established according to previous studies [22,23]. A pyrogallol solution (60 mmol/L, in 1 mol/L HCl, 0.10 mL) was thoroughly mixed with Tris–HCl (physiological pH = 7.4, 5.00 mL) buffer containing 1 mmol/L Na_2EDTA. Additionally, ultrapure water (4.90 mL) was added slowly into the system. After the mixture was vigorously shaken, the absorbance of the mixture was measured by UV–vis at RT (Fig. 1). The procedure was repeated, using different concentration solutions of three drugs, 4×10^{-6} mol/L, 8×10^{-6} mol/L, 12×10^{-6} mol/L, 16×10^{-6} mol/L, 20×10^{-6} mol/L. The protocol of velocity determination of pyrogallol autoxidation was shown in Table 1.

3. Results and discussion

Compound **1**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.50 (s, 1 H, 5-OH), 7.83 (s, 1H, 2′-ArH), 7.70 (d, 1H, J = 8.46 Hz, 6′-ArH), 7.13–7.10 (d, 1H, J = 8.73 Hz, 5′-ArH), 6.72 (s, 1H, 8-ArH), 6.36 (s, 1H, 6-ArH), 4.95–4.89 (m, 3H, $CH_2CH_2O^*H$), 4.10–4.04 (m, 6H, $OC^*H_2CH_2OH$), 3.73–3.28 (m, 6H, $OCH_2C^*H_2OH$), 0.96–0.93 (m, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.0 (3′,4′,7-O-$CH_2C^*H_2OH$), 70.63 (3′,4′,7 -O-$^*CH_2CH_2OH$), 98.8 (6-ArH), 105.5 (10-ArH), 113.3 (2′-ArH), 114.9 (5′-ArH), 122.8 (6′-ArH), 123.0 (1′-ArH), 134.1 (3-C), 148.0 (3′-ArH), 151.4 (4′-ArH), 156.9 (2-C), 157.0 (5-ArH), 161.3 (9-C), 165.1 (7-ArH), 177.9 (4-C). mp 185.2–187.0 °C. The ^{13}C NMR data agrees well with previous studies [24].

Compound **2**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.38 (s, 1H, 5-OH), 9.71 (s, 1H, 3-OH), 7.86–7.88 (d, 1H, J = 8.52 Hz, 2′-ArH), 7.81 (d, 1H, J = 7.20 Hz, 6′-ArH), 7.17–7.20 (d, 1H, J = 8.70 Hz, 5′-ArH), 6.80 (s, 1H, 8-ArH), 6.37 (s, 1H, 6-ArH), 4.02–4.34 (m, 12H, $OC_2{}^*H_4O \times 3$), 2.04 (s, 9H, COC^*H_3). ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.05($^*CH_3CO$), 62.58–63.04 (3′, 4′, 7-$OCH_2{}^*CH_2- \times 3$), 67.10–68.02 (3′, 4′, 7-$O^*CH_2CH_2- \times 3$), 93.22 (C-8), 98.25 (C-6), 104.71 (C-10), 114.51 (C-2′), 115.10 (C-5′), 122.97 (C-6′), 124.45 (C-1′),

Table 1
Sample preparation for the elimination experiment of $^\bullet O_2^-$ of compounds.

No.	Tris–HCl[a] (mL)	Ultrapure water/mL	Samples (mL)			Pyrogallol (mL)
			1	**2**	VC	
1	5	4.9	0	0	0	0.1
2	5	4.8	0.1	0	0	0.1
3	5	4.8	0	0.1	0	0.1
4	5	4.8	0	0	0.1	0.1

[a] The concentration of Tris–HCl is 50 mmol/L

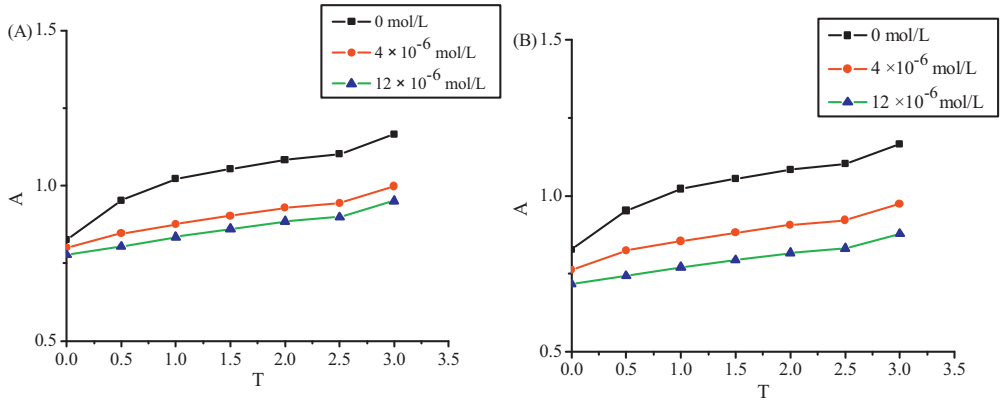

Fig. 2. Effect of compound **1**(A) and **2**(B) concentration on their $^{\bullet}O_2^-$ elimination activity.

Table 2
$^{\bullet}O_2^-$ elimination rate of compounds (%).

Compounds	Concentration ($\times 10^{-6}$ mol/L)				
	4	8	12	16	20
1	14.28	15.79	18.35	25.47	32.84
2	16.32	18.18	24.63	31.07	40.16
VC	15.07	21.66	29.81	38.11	55.37

137.18 (C-3), 146.66 (C-3′), 148.13 (C-2), 150.54 (C-4′), 156.52 (C-9), 160.90 (C-5), 164.30 (C-7), 170.73–170.77 (CH_3*CO × 3), 176.59 (C = O). m.p.139.2–140.5 °C. IR (Bruker Tensor 27, KBr, cm^{-1}): *ν*3299, 1737, 1650, 1379, 1259. ESI-MS: *m*/*z* 561.5 (M + 1). Elemental Anal. (Perkin Elmer PE 2400 II CHNS/O): calcd. for $C_{27}H_{28}O_{13}$ (560.50): C, 57.85; H, 5.03; O, 37.11. Found: C, 57.90; H, 5.0; O, 37.1.

As shown in Fig. 1, two bands, 321 nm and 436 nm, were obtained in our experimental conditions. In earlier studies [25], 420 nm was selected as the monitoring wavelength for $^{\bullet}O_2^-$ generation. However, the UV spectra showed that 321 nm was much more sensitive than 436 nm. In this study, 321 nm was therefore regarded as the best monitoring wavelength for detecting concentrations of $^{\bullet}O_2^-$. The ratio of ΔA/min was in the specified range. The absorbance of the solutions at 321 nm was measured up to 5 min. The oxidation rate of pyrogallol for samples was calculated as the slope of the absorbance line (ΔA_1). The value of A_{321nm} was measured against the Tris–HCl buffer every 30 s for 5 min. The $^{\bullet}O_2^-$ scavenging ability was calculated as:

Scavenging (%) = $[(A_0 - A) \div A_0] \times 100\%$

Here, A_0 was the absorbance of the reaction mixture without trolox, and *A* was that with trolox.

The results of effect of compound **1** (A) and **2** (B) concentration on its $^{\bullet}O_2^-$ elimination activity were shown in Fig. 2. The results suggested that the antioxidant activity of the compound **2** was more sensitive in the same concentration than the compound **1**. Effect of compounds concentration on their $^{\bullet}O_2^-$ elimination activities was investigated and the results showed in Table 2. The scavenging rate was easily figured out. The antioxidant activity of compound **2** made up 81.46% of the activity of VC, and compound **1** was 66.69% in our experimental conditions.

4. Conclusion

In recent years, the studies focusing on the flavonoids and their bioactivities have been increasing gradually [26]. Our chemical structure modification and activity test of flavonoids are of great importance and necessity. The structure was modified *via* highly selective esterification reaction from the reaction mechanism of synthesis of compounds. The glycosyl in 3-*O*-rutinose of compound **1** was substituted by 3-OH in compound **2**. Only three acetoxyethoxy groups in 7, 3', 4' were prepared by acylation on the strict controlled reaction condition. The results indicated that compound **1** and **2** were all showed antioxidant activity. However, the compound **2** was much more effective in inhibiting the antioxidant of pyrogallol than the compound **1**. A possible reason was that chemical structure modification enhanced its complex ability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.01.016.

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