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Original article

Controlled acid hydrolysis and kinetics of flavone *C*-glycosides from trollflowers

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ABSTRACT

Acid hydrolysis mechanisms of orientin-2″-*O*-galactopyranoside (OGA), orientin and other flavone *C*-glycosides in the trollflowers (*Trollius chinensis* Bunge) were studied in this report for the first time. Hydrolysis parameters including temperature, acidity, solvent and reaction time were comprehensively investigated. OGA could be hydrolyzed to orientin, followed by an isomerization to isoorientin *via* a reversible Wessely–Moser rearrangement reaction under stronger acidic conditions. A first-order kinetic model fitted the hydrolysis process of OGA well. Under the optimal hydrolysis conditions of 80 °C, 1.0 mol/L H^+ and 7 h reaction time, about 77% OGA was transformed to orientin with no detectable isoorientin. These results could be helpful for better understanding of the acid hydrolysis kinetics of flavone *C*-glycosides, as well as the preparation of these valuable components under controlled acid hydrolysis conditions.

1. Introduction

Flavone *C*-glycosides, orientin (luteolin-8-glucoside) and vitexin for example, are functional ingredients existing in some food and pharmaceutical products such as hawthorn [1], bamboo leaf extracts [2] and trollflowers [1,3–6]. Flavone *C*-glycosides are composed of flavone aglycones and glycosides connected with C–C bonds, which are chemically stable and resistant to normal acid hydrolysis, different from flavone *O*-glycosides [7]. Under strong acidic conditions, isomerization of flavone *C*-glycosides could occur, known as Wessely–Moser rearrangement [8,9]. However, detailed investigations of isomerization kinetics of flavone *C*-glycosides remain obscure. During our investigations of functional ingredients of the flowers of *Trollius chinensis* Bunge, orientin-2″-*O*-galactopyranoside (OGA), orientin, vitexin and their ester derivatives were isolated and identified [10]. Moreover, some refined fractions (TC-1 to TC-3) of this herbal extract and their acid hydrolysates also possessed antioxidant capacities [11]. These flavone *C*-glycosides were known to be responsible for multiple pharmacological functions of trollflowers including antiviral, antimicrobial and anti-inflammation activities [5,12,13]. Therefore, innovative separation technologies are necessary to obtain these ingredients for in-depth food and pharmaceutical applications. The objective of the present study was to investigate the preparation of these flavone *C*-glycosides and its mechanisms under acidic conditions. The TC-1 fraction, which contains mainly OGA, orientin and vitexin, was selected as a model sample to evaluate the kinetic parameters of OGA in detail. This is the first investigation on the acid hydrolysis kinetics of flavone *C*-glycosides.

2. Experimental

2.1. Chemicals and reagents

OGA, orientin and vitexin standards were purified and identified by their ESIMS, NMR, and UV adsorption spectra in the authors' lab [10]. The purities of the three compounds were all higher than 98.0% as determined by HPLC. Isoorientin (JZ20120912B) and isovitexin (JZ20130321B) standards were purchased from Nanjing Jingzhu Bio-technology Co., Ltd. (Nanjing, Jiangsu, China). Three flavone *C*-glycosides' fractions, TC-1 to TC-3, were prepared from *T. chinensis* as

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reported before and the qualitative and quantitative analyses of OGA and other analytes were conducted using the HPLC method [11].

2.2. Acid hydrolysis of samples

: For acid hydrolysis of OGA, orientin, isoorientin, vitexin, isovitexin standards (1.0 mg for each one) and TC-1 fraction (5.0 mg), each sample was dissolved in 2 mol/L hydrochloric acid (5.0 mL) and the mixture was allowed to stir for 6 h. The reaction temperature was set at 80 °C. The reaction solutions were neutralized using 4 mol/L NaOH at set time and then analyzed by HPLC. For the kinetic analysis of acid hydrolysis process of the TC-1 fraction, each sample (50 mg) was dissolved in 10.0 mL 1.0 mol/L sulfuric acid (dissolved with 20% ethanol) in a glass tube, and hydrolyzed at 80 °C. Sample solutions (0.4 mL) at set time (0–24 h) were taken out, neutralized immediately using 4 mol/L NaOH to pH 6–7, diluted with methanol to 1.0 mL followed by HPLC analysis. Further hydrolysis tests on TC-1 were conducted to evaluate the effects of different hydrolysis parameters, including substrate concentration (3.0–20.0 mg/mL of TC-1), solvent concentration (10%–50% of ethanol), acidity (0.5–3.0 mol/L of H^+), temperature (60, 70 and 80 °C) and reaction time (0–24 h). Sample solutions were then prepared as mentioned above followed by HPLC analysis.

3. Results and discussion

3.1. Acid hydrolysis of the TC-1 fraction and reference compounds

The TC-1 fraction had been extracted previously from troll-flowers using macroporous resin, which was mainly composed of OGA, orientin and vitexin [11]. During our investigation of the antioxidant properties of TC-1 and its acid hydrolysates, OGA and other ingredients were observed to convert to some hydrolysates under acidic conditions. To further investigate these phenomena, hydrolysis of TC-1 was conducted at 80 °C using 2 mol/L HCl, and the hydrolysates at set time intervals were analyzed using the HPLC method. By comparing the retention time of the hydrolysates with that of the standards, the transformation of OGA and other flavone *C*-glycosides could be readily monitored as shown in Fig. 1. OGA (**1**) could be hydrolyzed to orientin (**2**) slowly and disappeared after 4 h. Orientin (**2**) isomerized to isoorientin (**4**) after 6 h of hydrolysis, and finally reached equilibrium with a ratio of about 1:0.8 of **2**:**4** after 24 h. Vitexin (**3**) could also undergo a similar isomerization to isovitexin (**5**) and reached equilibrium (1:0.5 of **3**:**5**) after 24 h under the same hydrolysis conditions. These transformations could also take place when using the OGA, orientin, isoorientin, vitexin or isovitexin standards, respectively (data not shown).

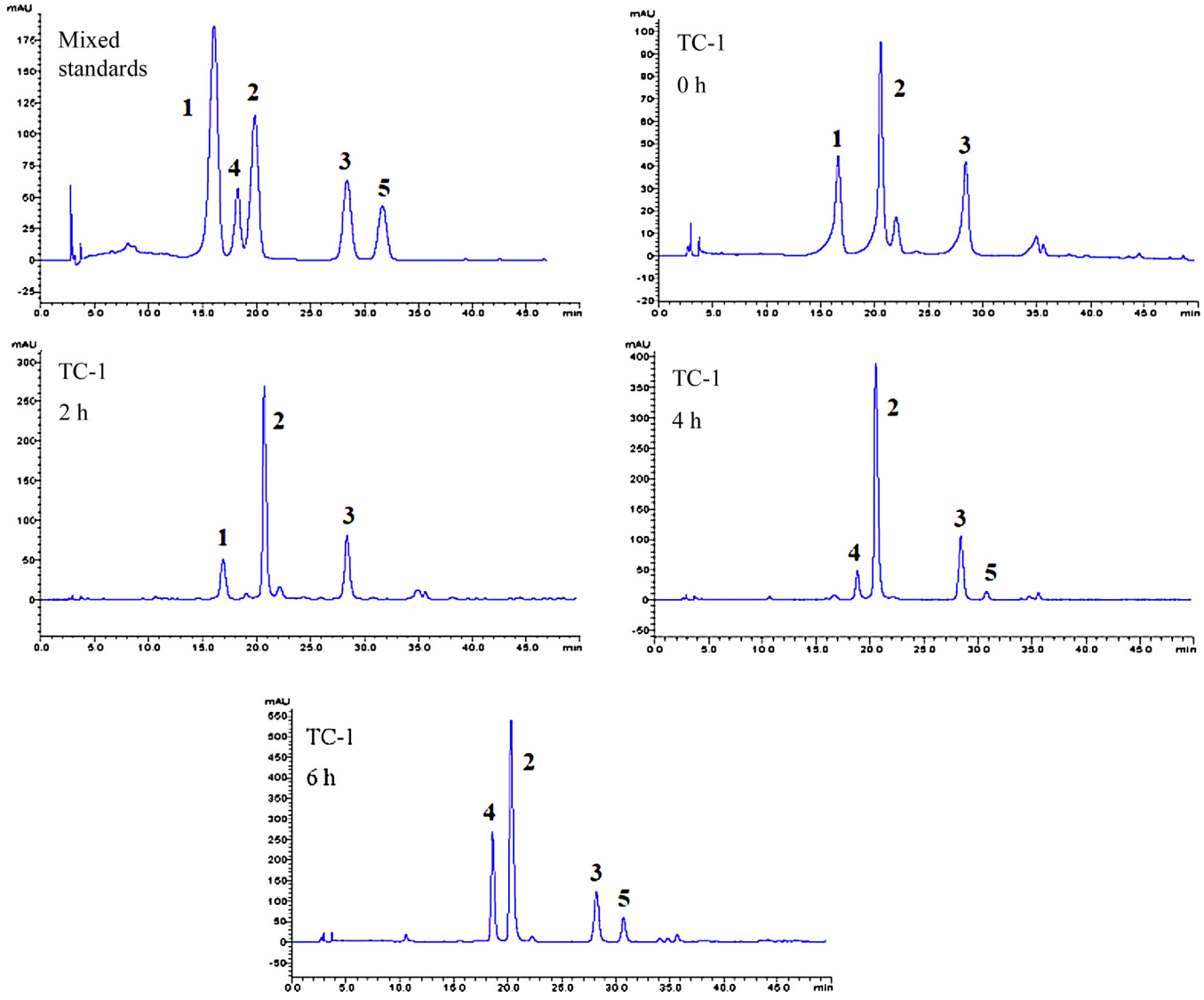

Fig. 1. HPLC chromatograms of mixed standards and TC-1 hydrolysates at 0, 2, 4 and 6 h monitor at 340 nm. (**1**) OGA, (**2**) orientin, (**3**) vitexin, (**4**) isoorientin, (**5**) isovitexin.

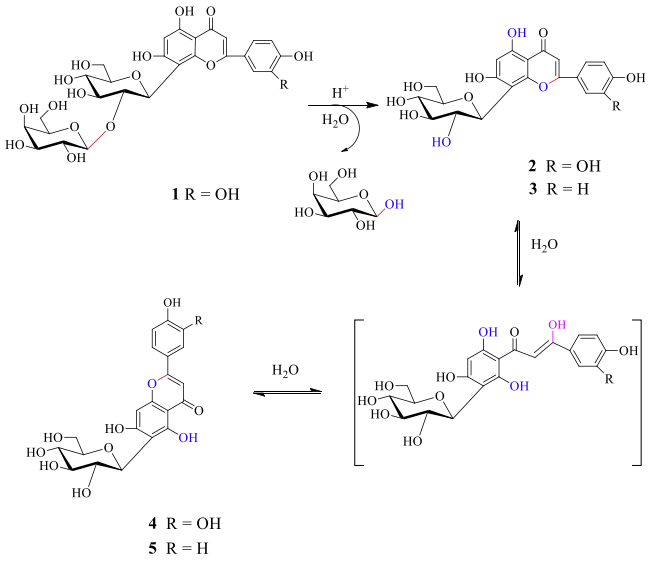

Fig. 2. Acid hydrolysis reaction of OGA (**1**), orientin (**2**) and vitexin (**3**) in TC-1 fraction. OGA could be hydrolyzed to orientin (**2**); orientin (**2**) and vitexin (**3**) could be hydrolyzed to isoorientin (**4**) or isovetexin (**5**), respectively *via* a chaltone intermediate; **4**/**5** could be hydrolyzed to **2**/**3** either and finally reach in equilibrium.

It is well known that flavone *O*-glycosides can be hydrolyzed to the corresponding aglycones and sugar moieties under acidic conditions. Flavone *C*-glycosides, however, are chemically stable and resistant to normal acid hydrolysis. Under higher temperatures and acidity, isomerizations of flavone *C*-glycosides could occur following a reversible Wessely–Moser rearrangement reaction [8,9]. Accordingly, the terminal *O*-glucosyl fragment of OGA (**1**) could be hydrolyzed to produce orientin (**2**) initially, as depicted in Fig. 2. Under stronger acid conditions or longer time as shown in Fig. 1, orientin and vitexin could then isomerize to corresponding isoorientin or isovitexin, and reach equilibrium finally. This process was also confirmed by the acid hydrolysis of isovitexin and other flavone *C*-glycosides as mentioned above. It should be noted that other derivatives of flavone *C*-glycoside ester, including 6‴-(3-hydroxy-3-methylglutaroyl)-2″-*O*-*β*-D-galactopyranosyl orientin, 7-methoxyl-2″-*O*-(2‴-methylbutyryl) orientin, 7-methoxyl-2″-*O*-(3‴,4‴-dimethoxybenzoyl) vitexin *etc.* [10] could be hydrolyzed to orientin or vitexin in a similar way, as observed in the TC-2 fraction [11].

Since the hydrolysis process of flavone *C*-glycosides was not sufficiently investigated before, the kinetics of the OGA acid hydrolysis was further analyzed using the TC-1 fraction as a model sample. It was also of concern to investigate whether these reactions could be carried out under proper control, so that the isomerizations between flavone *C*-glycosides were inhibited to maintain their natural characters as expected.

Table 1
Regression equations and rate constants for the acid hydrolysis of OGA in TC-1 fraction at different temperature and acidity.

Temperature (°C)	H^+ (mol/L)	Regression equation	R^2	k (h^{-1})
60	1.5	$y = -0.0425x - 0.3897$	0.9764	0.0978
	2.0	$y = -0.0307x - 0.4076$	0.9760	0.0707
70	1.0	$y = -0.0462x - 0.3976$	0.9809	0.1063
	1.5	$y = -0.0780x - 0.3625$	0.9916	0.1796
	2.0	$y = -0.1105x - 0.4037$	0.9897	0.2544
80	1.0	$y = -0.1699x - 0.4077$	0.9905	0.3912
	1.5	$y = -0.2729x - 0.3925$	0.9897	0.6284

3.2. Optimal process and kinetic of acid hydrolysis of TC-1 fraction

As for the acid hydrolysis of TC-1, some reaction conditions were investigated initially. The concentration of TC-1 was set at 5 mg/mL in this case. The addition of organic solvents, such as methanol or ethanol (10%–50%), was necessary to improve the solubility of the substrates, and 20% ethanol was found to be optimal. Sulfuric acid was applied instead of the volatile hydrochloride acid. Suitable acidities (0.5–3.0 mol/L) and temperatures (60–80 °C) were then investigated to achieve satisfied reaction rate and product purity. Finally, the reaction rate was measured in detail under three different acidities (1.0, 1.5 and 2.0 mol/L of H^+) and temperatures (60, 70 and 80 °C) (Table 1).

Under these circumstances, OGA was hydrolyzed to orientin smoothly in 7 h. Orientin would not isomerize to isoorientin during the 7 h hydrolysis period unless at 80 °C and with 1.5 mol/L H^+, in which case isoorientin concentration accounted for 5% of the mixture and 18% of the OGA remained. As our aim was to minimize the isomerization of orientin in this survey, no longer reaction time or stronger hydrolysis condition was conducted in the following tests.

To quantitatively measure acid hydrolysis kinetics of OGA in TC-1, concentrations of OGA in different hydrolysates were determined using the HPLC method. As shown in Fig. 3(A), the hydrolysis process was affected significantly by the reaction temperature. Reaction rate was rather slow at 60 °C, faster at 70 °C, and quite rapid at 80 °C. Acidity could also affect the reaction rate to some content.

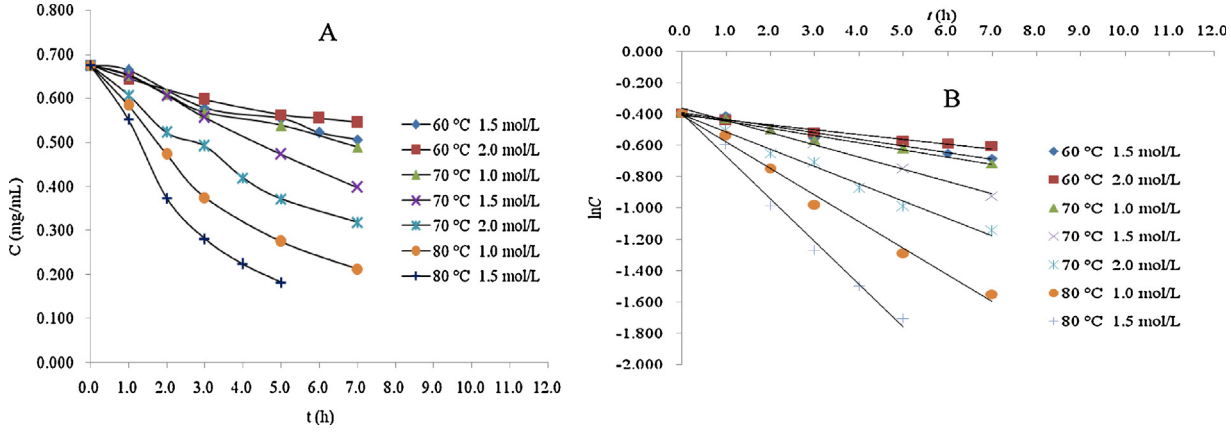

Fig. 3. Contents of OGA in TC-1 fraction during different acid hydrolysis processes (A) and simulated first-order reaction kinetics for OGA at different temperatures and acidities (B).

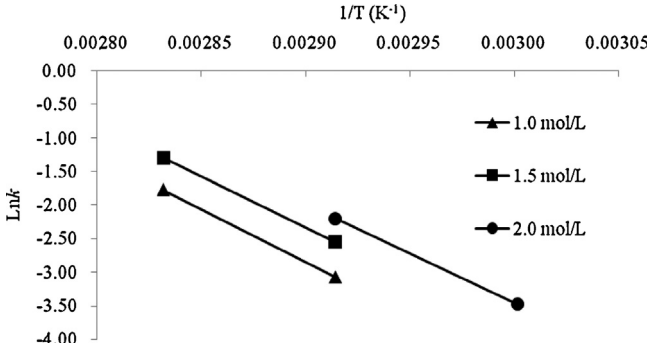

Fig. 4. Arrhenius plot of OGA in TC-1 fraction at different temperatures and rate constants.

The reaction follows first-order reaction kinetics and a plot of ln *C vs. t* produces a straight line according to Eq. (1):

$$\ln C = -kt + \ln C_0 \quad (1)$$

in which C (mg/ml) is the concentration of OGA, k the rate constant, t the reaction time, C_0 the initial concentration of OGA in the reaction system [12]. In Fig. 3(B) and Table 1, the reaction fitted first-order reaction model, with R^2 values greater than 0.95.

Furthermore, the temperature dependency of the rate constant k can be described by the Arrhenius equation (2):

$$\ln k = \frac{-E}{RT} + \ln A \quad (2)$$

in which k is the rate constant, E the activation energy of the reaction, T the temperature (in Kelvin), A the pre-exponential factor, and R the universal gas constant [14]. The Arrhenius plot of ln k *vs.* $1/T$ was thus obtained according to the data shown in Table 1 (Fig. 4). Activation energy E was extracted from the slope of the Arrhenius plot as 126.4 ± 3.7 kJ/mol. Curves of different acid concentrations at three different temperatures were in parallel, indicating the independency of E on the acidity and validating the accuracy of the results.

Finally, the optimal hydrolysis conditions were set at 80 °C, 1.0 mol/L H^+ and 7 h, and about 77% OGA could be converted to orientin with no detectable isoorientin in this case. Preparations of sample hydrolysates were thus carried out on the basis of the conditions optimized above. As a result, TC-1h to TC-3h was successively obtained from TC-1 to TC-3, respectively. The process ran with satisfaction. For example, by comparison with that of TC-1, the content of orientin in TC-1 h (671.3 μg/mg) increased 1.38-fold with little OGA left.

4. Conclusions

Transformation mechanisms of flavone *C*-glycosides of *T. chinensis* were studied under acidic conditions. OGA could be hydrolyzed to orientin quantitatively, followed by an isomerizing to isoorientin via the Wessely–Moser rearrangement reaction under stronger acidic conditions. Acid hydrolysis kinetics of OGA in TC-1 fitted first-order model. At optimal conditions set at 80 °C, 1.0 mol/L H^+ and 7 h, about 77% OGA was hydrolyzed to orientin with no detectable isoorientin. The results could be helpful for better understanding of the acid hydrolysis kinetics of flavone glycosides and the preparation of these valuable ingredients under controlled acid hydrolysis conditions.

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References

[1] Chinese Pharmacopeia Committee, Chinese Pharmacopeia (Part I), China Medical Science and Technology Press, Beijing, 2010, p. 372 (Hawthorn leave extract); 828 (Trollflower throat lozenge).
[2] Y. Zhang, B. Bao, B. Lu, et al., Determination of flavone *C*-glucosides in antioxidant of bamboo leaves (AOB) fortified foods by reversed phase high-performance liquid chromatography with ultraviolet diode arraydetection, J. Chromatogr. A 1065 (2005) 177–185.
[3] R.F. Wang, X.W. Yang, C.M. Ma, Trolliside, a new compound from the flowers of *Trollius chinensis*, J. Asian Nat. Prod. Res. 6 (2004) 139–144.
[4] (a) J.H. Zou, J.S. Yang, L. Zhou, Acylated flavone *C*-glycosides from *Trollius Ledebouri*, J. Nat. Prod. 67 (2004) 664–666;
(b) J.H. Zou, J.S. Yang, Y.S. Dong, L. Zhou, G. Lin, Flavone *C*-glycosides from flowers of *Trollius ledebouri*, Phytochemistry 66 (2005) 1121–1125.
[5] S.Q. Cai, R. Wang, X. Yang, et al., Antiviral flavonoid-type *C*-glycosides from the flowers of *Trollius chinensis*, Chem. Biodivers. 3 (2006) 343–348.
[6] X.Q. Li, Z.L. Xiong, X.X. Ying, et al., A rapid ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometric method for the qualitative and quantitative analysis of the constituents of the flower of *Trollius ledibouri* Reichb, Anal. Chim. Acta 580 (2006) 170–180.
[7] Y. Zhang, X. Tie, B. Bao, X. Wu, Y. Zhang, Metabolism of flavone *C*-glucosides and *p*-coumaric acid from antioxidant of bamboo leaves (AOB) in rats, Br. J. Nutr. 97 (2007) 484–494.
[8] N. Krafczyk, M.A. Glomb, Characterization of phenolic compounds in rooibos tea, J. Agric. Food Chem. 56 (2008) 3368–3376.
[9] A.K. Verma, R. Pratap, Chemistry of biologically important flavones, Tetrahedron 68 (2012) 8523–8538.
[10] J.Y. Liu, J.Y. Feng, S.Y. Li, et al., Flavone *C*-glycosides from the flowers of *Trollius chinensis* and their anti-complementary activity, J. Asian Nat. Prod. Res. 15 (2013) 325–331.
[11] Y. Sun, H.Y. Yuan, L.L. Hao, et al., Enrichment and antioxidant properties of flavone *C*-glycosides from trollflowers using macroporous resin, Food Chem. 141 (2013) 533–541.
[12] Y.L. Li, S.C. Ma, Y.T. Yang, S.M. Ye, P.P. But, Antiviral activities of flavonoids and organic acid from *Trollius chinensis* Bunge, J. Ethnopharmacol. 79 (2002) 365–368.
[13] X.A. Wu, Y.M. Zhao, N.J. Yu, Flavone C-glycosides from *Trollius ledebouri* reichb, J. Asian Nat. Prod. Res. 7 (2005) 1–4.
[14] C.N. Sun, M.M. Shen, L.L. Deng, J.Q. Mo, B.W. Zhou, Kinetics of ring-opening polymerization of octamethylcyclotetrasiloxane in microemulsion, Chin. Chem. Lett. 25 (2014) 621–626.