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Study on Hydrolysis of Icariin Glycoside to Anhydroicaritin

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ABSTRACT. *In order to optimize the preparation process of icariin, the method of hydrolyzing icariin was used to prepare icariin. The effects of acid type, solvent concentration, reaction temperature and reaction time on the yield were investigated. The optimum preparation conditions were obtained, under the action of concentrated sulfuric acid, 90% ethanol was used as solvent, and the reaction temperature was 80℃ for 3 hours.*

KEYWORDS: *icariin glycoside, hydrolysis, anhydroicaritin*

1. Introduction

Anhydroicaritin is a flavonoid compound, also known as dehydrated icariin, which mainly exists in *Epimedium*, a perennial plant of Berberidaceae [1]. The studies have shown that anhydroicaritin has estrogen-like, immune regulation, anti-inflammatory, promoting myocardial cell regeneration, promoting bone protection, promoting nerve cell differentiation, anti-liver injury, anti-cancer and reducing blood sugar and other effects [2, 3], Moreover, anhydroicaritin was not found to have significant toxic and side effects on normal cells. It is concluded that anhydroicaritin has high safety and wide application prospects.

Because the content of anhydroicaritin in plants is low [4, 5], at present, its source is mainly synthesized by chemical synthesis method. The combination of enzymatic hydrolysis, acidolysis and enzymatic hydrolysis of icariin glycoside can prepare anhydroicaritin [6, 7]. In order to further study and develop anhydroicaritin, the reaction conditions of hydrolysis of icariin glycoside to anhydroicaritin were studied, the preparation method was optimized and the yield was increased. It will provide reference and guarantee for the follow-up study of anhydroicaritin.

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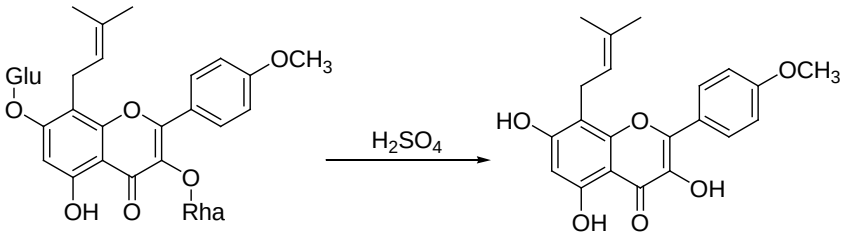
2. Experiments

2.1 Reagents and Instruments

Concentrated sulphuric acid, concentrated hydrochloric acid, phosphoric acid, acetic acid, icariin glycoside, etc. The reagents or drugs used are AR or CP grade on the market; magnetic stirrer, ultraviolet spectrometer, rotary evaporator, X-4 digital display microscopic melting point detector, Bruker nuclear magnetic resonance instrument, etc.

2.2 Hydrolysis Principle

Icariin glycoside bond is first protonated, then broken into cationic intermediates of aglycone and sugar, which are solvated to hydrolyze cationic carbon ion, then dehydrogenated to form sugar molecule, so that Icariin can be hydrolyzed into anhydroicaritin.



Icariin Glycoside **Anhydroicaritin**

Figure. 1 Hydrolysis principle of icariin

2.2 Hydrolysis Method

1.48 mmol icariin glycoside was added to 100 mL reaction flask, 90% ethanol 50 mL and 2.96 mmol concentrated sulfuric acid were added to stir the reaction for 3 hours at 80 C. After cooling, the reaction solution was added to 100 mL distilled water. The pH of the reaction solution was adjusted by sodium hydroxide for about 5.0 minutes, and then it was placed for 30 minutes, filtered, washed, dried filter residue and separated by column chromatography. Anhydroicaritin) was obtained as yellow solid with a yield of 40.5%. The melting point is 224-226 C, ^{1}H NMR(400 MHz,DMSO-d_6) ,δ: 12.37(s,1H, OH), 10.75(s, 1H, OH), 9.47(s, 1H, OH), 8.14(d, 2H, *J*=8.0 Hz, PhH), 7.12(d, 2H, *J*=8.0 Hz, PhH), 6. 30(s, 1H, PhH), 5.17(t, 1H, *J*=4.0 Hz, C=CH), 3.85(s, 3H, OCH_3), 3. 43(d, 2H, *J*=4.0 Hz, CH_2), 1.75(s, 3H, CH_3), 1.63(s, 3H, CH_3), and the NMR data are consistent with those in reference [8].

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3. Results and discussion

3.1 Effect of acid type on response

In order to investigate the effect of acid types on the reaction, as shown in Table 1, four acids with different intensities were selected and 1.48 mmol icariin glycoside, 50 mL absolute ethanol and 2.96 mmol acid were stirred in the reaction bottle for 1 hour at 80℃. The reaction was carried out according to the method of "1.2.2".

Table 1 Effect of acid type on reaction yield

Acid type	H_2SO_4	HCl	H_3PO_4	CH_3COOH
Yield /%	23.2	18.7	12.5	41

As can be seen from Table 1, the yield of concentrated sulfuric acid hydrolysis is the highest, reaching 23.2%, followed by concentrated hydrochloric acid and phosphoric acid, hydrolyzed by acetic acid, the yield is only 4.1%. The results show that acidity has an important influence on the reaction, and the yield increases with the increase of acidity.

3.2 Effect of solvent concentration on response

The effect of solvent concentration (ethanol concentration) on the reaction was investigated. As shown in Table 2, 1.48 mmol icariin glycoside, 50 mL solvent and 2.96 mmol concentrated sulfuric acid were mixed in the reaction bottle at 80℃ for 1 h and operated according to the method "1.2.2".

Table 2 Effect of solvent concentration on reaction yield

Concentration	100%	90%	80%	70%
Yield /%	32.7	36.8	35.6	22.1

Table 2 shows that with the concentration of ethanol from 100% to 70%, the yield increases first and then decreases. When the concentration is 90%, the yield reaches the maximum of 36.8%. The results showed that the concentration of solvent affected the reaction process and yield.

3.3 Effect of temperature on response

Next, the effect of reaction temperature on the yield was investigated. As shown in Table 3, 1.48 mmol icariin glycoside, 50 mL 90% ethanol and 2.96 mmol concentrated sulfuric acid were mixed in the reaction bottle at different temperatures for 1 hour and then operated according to the method of "1.2.2".

ISSN 2616-5767 Vol. 2, Issue 4: 7-11, DOI: 10.25236/AJETS.2019.020402

Table 3 Effect of reaction temperature on yield

Temperature /℃	60	70	80	90
Yield /%	20.2	28.0	36.8	35.7

From Table 3, it can be seen that the yield increases from 20.2% to 36.8% with the reaction temperature from 60℃ to 80℃, and decreases to 35.7% with the further increase of temperature to 100℃. From the reaction tracking point of view, more impurities are generated at 100℃, which affects the reaction yield.

The three-point localization experiment was carried out in 10 groups, and the record data were shown in Table 2. It can be concluded from the table, the estimated value of X axis coordinate system and the Y axis and the target actual value of X axis coordinates and the Y axis deviation was less than 2cm.

3.4 Effect of time on response

Finally, the effect of reaction time on the yield was investigated. As shown in Table 4, 1.48 mmol icariin glycoside, 50 mL 90% ethanol and 2.96 mmol concentrated sulfuric acid were mixed in the reaction bottle at 80℃, and the reaction was carried out according to the method "1.2.2".

Table 4 Effect of reaction time on yield

Time/h	1	2	3	4
Yield /%	24.3	36.8	40.5	35.9

Table 4 shows that the reaction time has a direct impact on the yield. When the reaction time increases from 1 h to 3 h, the yield reaches 40.5%. With the further increase of reaction time, the yield decreases and by-products increase.

4. Conclusion

Anhydroicaritin was prepared by acid hydrolysis of icariin glycoside. The type of acid, concentration of solvent, reaction temperature and reaction time had significant effects on the yield. It was found that under the action of concentrated sulfuric acid, 90% ethanol was used as solvent, and the reaction time was 3 hours at 80℃, which was the optimum process for the preparation of anhydroicaritin. This method provides a reference for the development and comprehensive utilization of anhydroicaritin.

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References

[1] H.P. Ma, X.R. He, Y. Yang, et al. The genus Epimedium: an ethnopharmacological and phytochemical review. Journal of Ethnopharmacology, vol.134, no.3, p.519-541.
[2] Z.G. Zheng, X. Zhang, Y.P. Zhou, et al. Anhydroicaritin, a SREBPs inhibitor, inhibits RANKL-induced osteoclastic differentiation and improves diabetic osteoporosis in STZ-induced mice. European Journal of Pharmacology, vol.809, p.156-162.
[3] M.L. Nan, S.C. Li, Y.W. Zhao, et al. Preparation method and pharmacological activity of anhydroicaritin. Chinese Journal of Experimental Traditional Medical Formulae, vol.21, no.7, p.227-231.
[4] J. Huang, L. Yuan, X. Wang, et al. Icaritin and its glycosides enhance osteoblastic, but suppress osteoclastic, differentiation and activity in vitro. Life Sciences, vol.81, no.10, p.832-840.
[5] S. Li, M.H. Pan, C.S. Lai, et al. Isolation and syntheses of polymethoxyflavones and hydroxylated polymethoxyflavones as inhibitors of HL-60 cell lines. Bioorganic and Medicinal Chemistry, vol.15, no.10, p.3381-3389.
[6] J.Y. Zhu, X.L. Li. Study on acid hydrolysis condition of icariin glycoside. Studies of Trace Elements and Health, vol.27, no.3, p.49-51.
[7] Y.P. Li, X.H. Zhang, H. Peng, et al. Effects of anhydroicaritin and 2"-hydroxy-3"-en- anhydroicaritin on the proliferation and differentiation of MC3T3-E1 osteoblasts. Natural Product Communications, vol.7, no.11, p.1461-1464.
[8] V.S. Nguyen, Shi L, S.C. Wang, et al. Synthesis of icaritin and β-anhydroicaritin Mannich base derivatives and their cytotoxic activities on three human cancer cell lines. Anti-Cancer Agents in Medicinal Chemistry, vol.17, no.1, p.137-142.