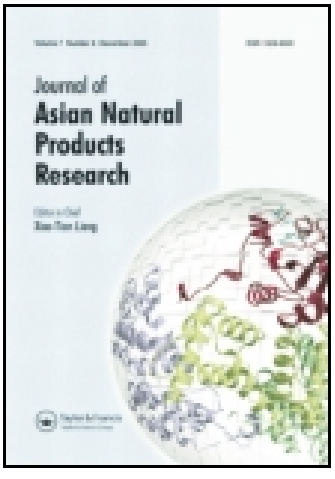

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New synthetic method of 8-oxocoptisine starting from natural quaternary coptisine as anti-ulcerative colitis agent

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New synthetic method of 8-oxocoptisine starting from natural quaternary coptisine as anti-ulcerative colitis agent

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Quaternary coptisine (**1**), a natural bioactive quaternary protoberberine alkaloid (QPA), was treated with potassium ferricyanide in aqueous solution of 5 N sodium hydroxide leading to the acquisition of 8-oxocoptisine (**2**) with much higher yield than reported in the literature. This is the first report of the oxidation of a natural QPA by applying potassium ferricyanide as an oxidant. 8-Oxocoptisine showed significant anti-ulcerative colitis efficacy *in vitro* with EC_{50} value being 8.12×10^{-8} M.

Keywords: 8-oxocoptisine; quaternary coptisine; potassium ferricyanide; anti-ulcerative colitis

1. Introduction

Ulcerative colitis (UC) is a kind of chronic nonspecific inflammatory bowel disease and has been identified as a nasty disease by World Health Organization [1]. It has been reported that the clinical incidence of UC is 1.2–20.2 cases in 100,000 people each year in China, with much higher rate occurred in North Europe and North America, and the etiology of UC has not been clear [2]. Up to now, only several drugs are used to treat UC clinically, such as preparations from aminosalicylic acid, glucocorticoids, and immunosuppressive agents [1]. Realistically, many problems abound nowadays, such as limited efficacy, serious side effect, and relapse after treatment, among others, leading to no complete cure to UC. Therefore, significantly effective anti-UC drugs are urgently needed. Recent investigations have shown that the pathogenesis of UC is, in large part, closely related to the abnormality of key downstream transcription factor X-box-binding protein 1 (*xbp*1) that associates with noncontrollable endoplasmic reticulum stress response within intestinal epithelial cells [3,4]. In other words, the variation of the coding region and the expressive abnormalities of *xbp*1 are often characteristic of UC patients. Therefore, it is speculated that *xbp*1 may be the potential new drug targets of treating UC. However, there has been no claim as yet about the development of anti-UC drugs targeting at *xbp*1 during recent research at home and abroad in the literature. This paper involves an investigation on anti-UC activity of 8-oxocoptisine (**2**) based on *xbp*1 pharmacological model *in vitro*, which was obtained for the first time in considerable yield from natural quaternary coptisine (**1**) as the starting material by treatment with potassium ferricyanide as an oxidant and exhibited significantly

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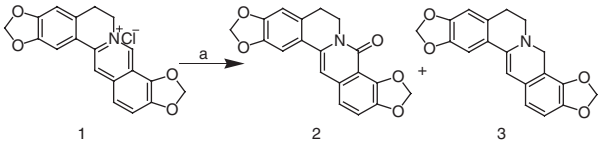

Scheme 1. Synthesis of 8-oxocoptisine. Reagents and conditions: (a) $K_3Fe(CN)_6$, 5 N NaOH in water, reflux.

potent efficacy with EC_{50} value reaching the level of 10^{-8} M.

Quaternary coptisine (**1**) is a natural quaternary protoberberine alkaloid (QPA) isolated previously from some *Coptis* and *Corydalis* species belonging to the plant families of Ranunculaceae and Papaveraceae [5,6], respectively. It has been demonstrated that quaternary coptisine exhibits some kinds of biological and pharmacological activities, including the anti-fungal and cardioprotective properties, inhibition of monoamine oxidase of type A, and so on [7–12]. 8-Oxocoptisine (**2**) was initially isolated from *Fumaria indica* as a natural protoberberine alkaloid with amide moiety being one of its structural features [13,14]. Wu et al. have reported that the multidrug resistance (MDR) inhibition activity of **2** is similar to verapamil, which is an active inhibitor of the *p*-glycol protein-induced MDR [15,16]. However, too low natural yield (0.004%) makes the obtaining of **2** from natural resources very arduous [5]. Therefore, ever more increasing interest has been focused on developing convenient synthetic route to get the artificial 8-oxocoptisine and its derivatives. The preparation of **2** through total synthesis has been reported [17]. However, the approach was uneconomical and tedious due to the reality of multistep and low yield (only 3% for six steps). A complicated synthetic method has also been reported [18]. Lamentedly, the raw materials were noncommercially available. Reimann et al. have used isoquinoline derivative as the starting material to synthesize **2** with a total yield of only 10% being observed for six steps [19]. Dostál et al. have converted quaternary coptisine to compound **2** during their NMR investigation on related compounds with DMSO-d_6 as a solvent, but the conversion needed a very long duration of reaction and underwent the preparation of 8-hydroxy-7,8-dihydrocoptisine as an intermediate product [20]. Recently, Cui et al. have reported the synthesis of **2** by reacting **1** with 50% KOH solution, and also with poor yield being obtained [21]. Moreover, this method resulted in a significant portion of dihydrocoptisine (**3**) when repeated by us, which is very difficult to separate from **2**. Based on the reality mentioned above, this paper focused on searching for efficient synthetic route of **2** (Scheme 1) starting from natural quaternary coptisine as complementary contents for the above-mentioned anti-UC agent.

2. Results and discussion

It has been confirmed that quaternary coptisine (**1**) yields one oxidative product (8-oxocoptisine (**2**)) and one reductive product (dihydrocoptisine (**3**)) as a result of reaction with unknown mechanism when reacted only with 50% KOH solution. An idea was thus proposed based on the common sense of organic chemistry that whether an oxidant can be added to make the reactant predominantly or completely transformed to the oxidative product (**2**). In the process of searching for such an oxidizing agent, we found that potassium ferricyanide is very suitable. Potassium ferricyanide has ideal oxidizing properties under alkaline conditions.

Table 1. Reaction conditions and results.

Entry	Potassium ferricyanide:**1** (mol/mol)	**2**:**3**	Total yield (%)
1	0:1	1:1	52.3
2	2:1	5:1	68.5
3	4:1	20:1	72.1
4	5:1	1:0	73.0

In our work, the treatment of **1** with potassium ferricyanide has been conducted with several ratios of reactants being investigated under alkaline condition and the results are depicted in Table 1. Potassium ferricyanide was dissolved in the solution of 5 N NaOH in water and **1** was added to give a suspension. The reaction of the mixture was carried out under reflux conditions leading to products **2** and **3** being obtained (Scheme 1) in most cases. It was substantiated that the relative amount of potassium ferricyanide had significant effect on the resultant proportion of **2** and **3**, as well as the total yield. As shown in Table 1, a ratio of 5:1 of potassium ferricyanide/**1** (mol/mol) was found to be optimal for the preparation of **2**, with **1** completely being transformed to **2** under this condition. When **1** was more than one-fifth of potassium ferricyanide by mole in amount added to the reaction system, compound **3** would always be obtained along with **2** at different ratios. The structures of the products were settled based on their physical and spectroscopic (^{1}H and ^{13}C NMR, ESI-MS, and IR) properties, although the detailed reaction mechanism was not entirely clear.

8-Oxocoptisine (**2**) was evaluated for its selective activation effect on *xbp*1 *in vitro* with a blank group (nontransfection) as control 1 and a group transfected with pGL3-p*xbp*1 plasmid (promoter −330 to +60) as control 2, and the result is shown in Figure 1. No positive control was used in this paper because 8-oxocoptisine (**2**) was the first reported *xbp*1 activator. The result indicated that **2** had certain transcription-activating effect on *xbp*1. Further experiment confirmed that **2** exhibited a significant anti-UC efficacy *in vitro* with its EC_{50} value being 8.12×10^{-8} M (Figure 2).

To sum up, due to the rich natural resources of quaternary coptisine (**1**), the method described here is convenient and economic in practice for the preparation of **2**, with considerable yield being obtained. Moreover, this method can be expected to expand for oxidation of other QPAs, such as quaternary berberine. However, our preliminary investigation showed that the yield of oxidative product is not as good as oxidizing quaternary coptisine in the case of quaternary berberine under the same condition (data not shown), and searching

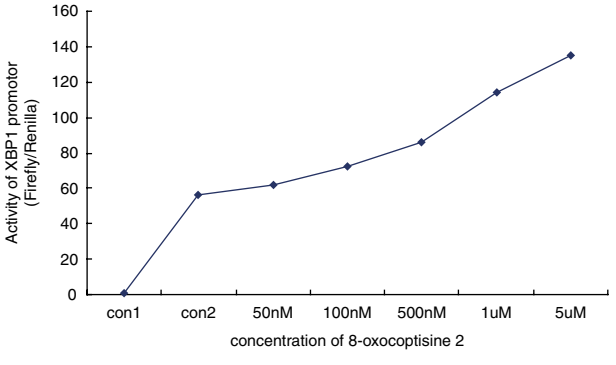

Figure 1. Activity of **2** as *xbp*1 activator.

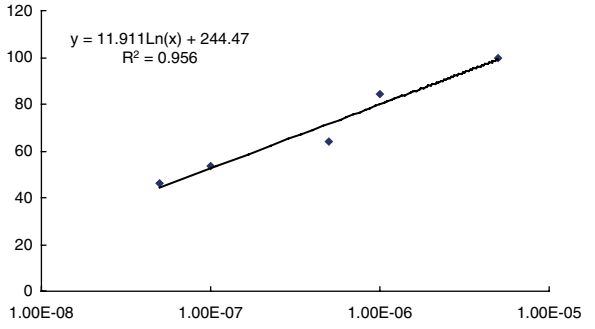

Figure 2. Experiment of EC_{50} value of **2** (8.12×10^{-8} mol/l).

for optimal procedure is in progress in our laboratory. Based on the above-mentioned advantages of this procedure, exploitation of **2** can be considered for its known, or possibly unknown, biological properties.

3. Experimental

3.1 General experimental procedures

IR spectrum was obtained on a Nicolet 5700 spectrometer (Thermo Electron, Madison, WI, USA). ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Mercury-300 NMR spectrometer or a Varian Mercury-400 NMR spectrometer (Varian, Inc., Palo Alto, CA, USA), respectively, and reported with TMS as the internal standard and DMSO-d_6 and pyridine-d_5, respectively, as solvents. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. ESI-MS was obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer (Santa Clara, CA, USA). TLC analyses were carried out on glass plates with silica gel GF_{254} being precoated (Qingdao Marine Chemical, Inc., Qingdao, China). Starting material was isolated and purified by our group from natural resources of *Coptis chinensis* and other plants with the purity of quaternary coptisine being determined to be over 99% by HPLC procedure (data not shown). The structure of quaternary coptisine was confirmed on the basis of chemical and spectroscopic evidences. All reagents and solvents were reagent grade.

3.2 Procedure for the synthesis of compound 2

Potassium ferricyanide (2.2 g, 6.68 mmol) was dissolved in the solution of 5 N NaOH in water (15 ml). To the suspension was added slowly 500 mg of **1** (1.41 mmol). When the addition was completed, the mixture was refluxed for 10 h and then allowed to cool down to room temperature. The precipitate was filtered and washed with water and dried in high vacuum to give pure 8-oxocoptisine (**2**) (344 mg, 73.0%). 8-Oxocoptisine (**2**): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.86 (t, $J = 5.4$ Hz, 2H, NCH_2CH_2), 4.09 (t, $J = 5.4$ Hz, 2H, NCH_2CH_2), 6.07 (s, 2H, OCH_2O), 6.19 (s, 2H, OCH_2O), 6.92 (s, 1H, ArH), 7.11 (s, 1H, ArH), 7.15 (d, $J = 8.1$ Hz, 1H, ArH), 7.34 (d, $J = 8.1$ Hz, 1H, ArH), 7.47 (s, 1H, —CH═); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.2, 148.0, 147.0, 146.1, 145.7, 134.9, 131.6, 129.9, 123.2, 119.4, 114.0, 109.8, 107.9, 104.8, 102.1, 101.7, 101.4, 38.9, 27.7; IR (cm^{-1}): υ_{max}: 3047, 2904, 2787, 1655, 1596, 1483, 1389, 1270, 1228, 1101, 1044, 941 [21].

On the case of other ratio of reactants as shown in Table 1, both 8-oxocoptisine (**2**) and dihydrocoptisine (**3**) were obtained as a mixture after the same procedure as described above. It is very difficult to separate **3** from **2**. The following spectro-

scopic data of dihydrocoptisine (**3**) were recorded with pure compound prepared by reduction of starting material (quaternary coptisine **1**) with sodium borohydride ($NaBH_4$) and potassium carbonate (K_2CO_3), which were used to determine the existence of dihydrocoptisine (**3**) in the resultant of the reaction. Dihydrocoptisine (**3**): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.78 (t, $J = 5.7$ Hz, 2H, $NCH_2C\underline{H}_2$), 3.04 (t, $J = 5.7$ Hz, 2H, $NC\underline{H}_2CH_2$), 4.15 (s, 2H, NCH_2), 5.96 (s, 2H, $\overline{O}CH_2O$), 5.99 (s, 2H, OCH_2O), 6.08 (s, 1H, ArH), 6.46 (d, $J = 7.8$ Hz, 1H, ArH), 6.68 (d, $J = 7.8$ Hz, 1H, ArH), 6.74 (s, 1H, ArH), 7.28 (s, 1H, ArH). Considering the unsteadiness of dihydroprotoberberine alkaloid in DMSO-d_6, the ^{1}H and ^{13}C NMR spectra of **3** were further recorded with pyridine-d_5 as solvent. ^{1}H NMR (400 MHz, pyridine-d_5) δ: 2.73 (t, $J = 6.0$ Hz, 2H, $NCH_2C\underline{H}_2$), 2.93 (t, $J = 6.0$ Hz, 2H, $NC\underline{H}_2CH_2$), 4.27 (s, 2H, NCH_2Ar), 5.93 (s, $\overline{2}H$, OCH_2O), 5.96 (s, 2H, OCH_2O), 6.27 (s, 1H, ArH), 6.63 (s, 1H, ArH), 6.69 (d, $J = 8.0$ Hz, 1H, ArH), 6.80 (d, $J = 8.0$ Hz, 1H, ArH), 744 (s, 1H, ArH). ^{13}C NMR (100 MHz, pyridine-d_5) δ: 29.9, 49.0, 49.1, 97.3, 101.5, 101.6, 104.4, 107.6, 108.3, 110.6, 116.4, 125.1, 129.2, 130.4, 141.8, 143.2, 145.7, 147.3, 147.9. ESI-MS (*m*/*z*): 321.3 $[M]^+$.

3.3 pGL3-pxbp1 Transcription-activating effect and EC_{50} determination

The IEC-6 cells in vigorous growth period were seeded in 48-well culture plates with cell numbers in each well being 5×10^4. The cells were uniformly dispersed in the wells and cultured at 37°C in cell incubator containing 5% CO_2 under humidified condition. When the cells grew to 70–80% confluence, the pGL3-p*xbp*1 plasmid was transfected to each well following a standard protocol (0.6 μg/well). After 4 h, compound **2** (1×10^{-5} mol/l) was added to transfected cells ($n = 3$) and co-cultured for 48 h. The luciferase activity was detected using dual luciferase report gene detection reagent kit (Promega, Madison, WI, USA) on experimental sample, and the EC_{50} value was determined.

Acknowledgments

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