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Preparation of shikonin by hydrolyzing ester derivatives using basic anion ion exchange resin as solid catalyst

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ABSTRACT

An efficient catalytic hydrolysis approach was developed to prepare shikonin from its ester derivatives with anion exchange resins as solid catalyst. The performance of seven anion exchange resins including D290, D296, D261, D280, 201×7, D301-G and D301-R has been evaluated. The research results indicate that D290 resin is the most appropriate for preparation of shikonin. The reaction conditions such as reaction temperature, reaction time, catalyst amount and catalyst reuse have been studied. The maximum yield of shikonin reached 93.74 ± 2.99% under optimal conditions of hydrolysis temperature 20 °C, hydrolysis and desorption time both 30 min and resin amount 10% (w/v). HPLC chromatograms of samples, obtained after the new catalytic process, demonstrated that conversion of shikonin ester derivatives into shikonin performed well.

1. Introduction

Shikonin, a naphthoquinone, is an active compound isolated from the root of the species of Boraginaceae family as known as *Lithospermum erythrorhizon*, *Alkanna tinctoria*, *Arnebia euchroma*, and *Macrotomia Euchroma* (Assimopoulou et al., 2008; Papageorgiou et al., 1999). In China, *A. euchroma* and *L. erythrorhizon* are the two most well developed resources of shikonin and widely used in several systems of medicine. The roots of Boraginaceae family are known to be used for treatment of burns, anal ulcers, hemorrhoids, infected crusts, bedsores, external wounds and oozing dermatitis. Besides pharmacological applications, shikonin and its ester derivatives are also considered as natural colorants in the printing, textile, food and cosmetic fields in the east and Europe (Huang et al., 2007; Papageorgiou et al., 1999). In recent times, multiple pharmacological actions have been attributed to shikonin, e.g. anti-inflammatory (Kourounakis et al., 2002), anti-bacterial (Shen et al., 2002), anti-fungal (Sasaki et al., 2002), antitumor (Yang et al., 2009), anti-viral (Chen et al., 2003), antithrombotic (Papageorgiou et al., 1999) and antioxidant (Assimopoulou et al., 2004) activities.

Shikonin ester derivatives (SED), mainly esters from the side chain hydroxyl group, have been identified in the roots of several plants of the Boraginaceae family, especially *A. tinctoria*, *L. erythrorhizon*, *A. euchroma* etc. The SED are as follows deoxyshikonin (DS), isobutylshikonin (IBS), β,β-dimethylacrylshikonin (DAS), 2-methyl-n-butyrylshikonin (MBS), isovalerylshikonin (IVS), acetylshikonin (AS), β-acetoxyisovalerylshikonin (ATS) and so on. Different herbs contain specific and different SED, and SED were found to be the most abundant in *A. euchroma* species (Hu et al., 2006). The chemical structures of main SED are given in Table 1.

Traditional research on SED converting into shikonin is about hydrolysis preparation. The homogeneous base catalysts, such as sodium hydroxide and potassium hydroxide are generally used for esters hydrolysis in industry (Lu et al., 2004). However, the aqueous catalysts cannot be recovered after use and have to be neutralized at the end of the hydrolysis reaction, which is usually economically undesirable and may result in secondary pollution. Such drawbacks could be minimized by the use of non-corrosive, environmentally sound, easily recoverable and cost effective heterogeneous catalysts dos (Reis et al., 2005; Oliveira et al., 2008; Sakai et al., 2009).

Ion exchange resins are well-known solid catalysts that possesses high acidity or basicity with both gelular and macroporous structures. They have been applied in wastewater treatment (Wawrzkiewicz and Hubicki, 2009), biodiesel production (Shibasaki-Kitakawa et al., 2007; Özbay et al., 2008), production of high-purity product (Fernandez-Olmo et al., 2007), performing esterification, transesterification and adsorption activities (Reis et al., 2005; Harmer and Sun, 2001). As for the anion

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** Corresponding author at: Box 332, Northeast Forestry University, Hexing Road 26, Harbin, Heilongjiang Province, China. Tel.: +86 451 82191517; fax: +86 451 82102082.
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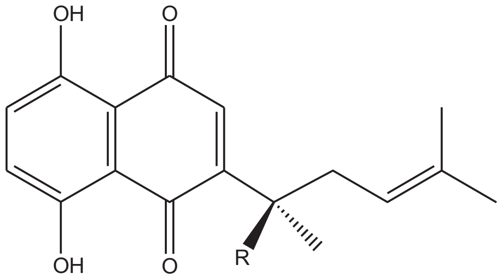
Table 1
Characteristic shikonin and SED found in roots of *A. euchroma*.

General structure	R	Name	Abbreviations	Naphthoquinone content (mg/g)[a]
	OH	Shikonin	S	1.52
	$OCOCH_3$	Acetylshikonin	AS	19.7
	H	Deoxyshikonin	DS	0.262
	$OCOCH_2C(CH_3)_2OCOCH_3$	β-Acetoxyisovalerylshikonin	ATS	6.50
	$OCOCH(CH_3)_2$	Isobutylshikonin	IBS	4.78
	$OCOCH{=}C(CH_3)_2$	β,β-Dimethlyacrylshikonin	DAS	12.0
	$OCOCH(CH_3)CH_2CH_3$	2-Methyl-n-butyrylshikonin	MBS	11.7
	$OCOCH_2CH(CH_3)_2$	Isovaerylshikonin	IVS	14.0

[a] Hu et al.

exchange resins, their catalytic role in ester hydrolysis has been well demonstrated (Miyazaki et al., 1996; Samelson and Hammett, 1956). Besides catalytic property, these anion exchange polymeric resins are found to preferably create ion exchange in many organic substances through electrostatic interaction and other particular interactions, implying that the organic substance formed during ester hydrolysis could be probably captured by the resins. However, little is known about natural products hydrolytic preparation and simultaneous ion exchange enrichment by these anion exchange resins.

In the present study, we compared catalytic activities of various OH-type anion exchange resins and investigated reaction temperature, reaction time, catalyst amount and catalyst reuse on the hydrolysis reaction. An efficient method was developed for the conversion of SED into shikonin by anion exchange resins as hydrolytic catalyst. This research will provide opportunities for a better understanding of the efficient extraction and for the development of separation in natural products field.

2. Experimental

2.1. Reagents and materials

Shikonin standard was purchased from China's national institute for the control of pharmaceutical and biological products (Beijing, China). Appropriate amount of standard was dissolved in methanol to yield the stock solution at concentration of 0.4182 mg/ml. Methanol of chromatographic grade was purchased from J & K Chemical Ltd. (Beijing, China). All the other reagents obtained from Beijing Chemical Reagents Co. (Beijing, China) were of analytical grade. Deionized water was purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA). Dried roots of *A. euchroma* were purchased from San Keshu Trading (Heilongjiang, China) and identified by Prof. Shao-quan Nie from Key Laboratory of Forest Plant Ecology, Northeast Forestry University. Dried roots were powdered into a homogeneous size and then sieved (60–80 mesh). The water content of roots powder was 5.4%.

2.2. Ion-exchange resins

Ion-exchange resins including D290, D296, D261 and D280 were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China), 201 × 7, D301-G and D301-R were purchased from Chemical Plant of Nankai University (Tianjin, China). Their physical and chemical properties are listed in Table 2. Each ion exchange resin tested was supplied as chloride form. The resin was washed with ethanol for 2 h before use, removing the monomers and porogenic agents trapped inside the pores during the synthesis process. To convert Cl-type to OH-type, the resins were pre-treated by 1 M HCl and 1 M NaOH solutions successively to displace chloride ions with hydroxyl ions, then washed with deionized water until neutral pH (7–7.5) and finally followed by ethanol. To determine moisture content of resins, three samples of each kind of ion exchange resins were weighed, then placed in a drying oven, and dried at 105 ± 2 °C until the mass did not change. The results of moisture content are shown in Table 2.

2.3. Preparation of crude extracts of *A. euchroma*

200 g roots powder was extracted twice with 2 l of 80% ethanol (v/v) in a Waring blender (Philips, Guangdong, China) for 4 min. The extracts were purified by membrane filtration and then transferred to a rotary evaporator and concentrated under vacuum to dryness. The content of shikonin in extracts was 0.24%, 80% ethanol (v/v) was added to get sample solutions at the concentration of shikonin 6.049 μg/ml. The obtained crude extracts of *A. euchroma* were stored in low temperature and dark condition.

Table 2
Physical and chemical properties of ion-exchange resins used.

Resins	Character[a]	Ionic form as shipped	Crosslinking level[a]	Functional groups[a]	Exchange capacity[a] (mequiv./g)	Particle size[a] (mm)	Moisture content (%)
D296	MR[b]	OH	High	$-N^+(CH_3)_3$	≥3.6	0.315–1.25	62.49 ± 1.37
D290	MR	OH	High	$-N^+(CH_3)_3$	≥3.3	0.315–1.25	70.69 ± 1.52
D280	MR	OH	High	$-N^+C_5H_4CH_3$	≥3.0	0.315–1.25	59.94 ± 1.30
D261	MR	OH	High	$-N^+(CH_3)_3$	≥3.6	0.315–1.25	58.20 ± 1.28
201 × 7	G[c]	OH	High	$-N^+(CH_3)_3$	≥3.6	0.315–1.25	60.31 ± 1.33
D301R	MR	OH	Low	$-N(CH_3)_2$	≥4.8	0.315–1.25	61.25 ± 1.34
D301G	MR	OH	Low	$-N(CH_3)_2$	≥4.2	0.315–1.25	60.94 ± 1.34

[a] Parameters in the table provided by manufacturer of the resins.
[b] Macroreticular structure.
[c] Gel type polymer.