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ScienceDirect
J. of Supercritical Fluids 41 (2007) 386–390
THE JOURNAL OF Supercritical Fluids
www.elsevier.com/locate/supflu

Enzymatic synthesis of benzoin in supercritical carbon dioxide

Nuray Celebi [a], Nuray Yildiz [b,*], Ayhan S. Demir [c], Ayla Calimli [b]

[a] *Baskent University, Faculty of Engineering, 06530 Ankara, Turkey*
[b] *Ankara University, Faculty of Engineering, Department of Chemical Engineering, 06100 Tandogan, Ankara, Turkey*
[c] *Middle East Technical University, Faculty of Arts and Sciences, Department of Chemistry, 06531 Ankara, Turkey*

Received 21 March 2006; received in revised form 6 November 2006; accepted 15 December 2006

Abstract

Enantioselective enzymatic hydrolysis of benzoyl-benzoin catalyzed by *Candida cylindracea* (CCL) lipase was carried out in $SCCO_2$. It was found that CCL lipase enantioselectively hydrolyzed the (*R*)-benzoin. The enantiomeric excess of product (ee_p) was maximized at 35 °C near the critical region 90 bar and obtained 61.3% at a 50% conversion. CCL did not catalyze the reaction in an atmospherical condition.

Keywords: Supercritical carbondioxide; *Candida cylindracea* (CCL); Enantiomeric excess (ee); Benzoin; Enantioselectivity

1. Introduction

Supercritical fluids (SCF) are those compounds that exist at a temperature and pressure that are above their corresponding critical values. Their physical properties make them very attractive for biocatalytic processes. They exhibit low surface tension and viscosity and high diffusivity that is comparable to gases. On the other hand, they show liquid like density, which promotes the enhanced solubility of solutes compared to the solubility of gases. The most important characteristic is that the solubility of solutes can be manipulated by changes in pressure and temperature, especially near the critical point [1]. While the supercritical fluids have been used mainly for extractions, the application of this medium to other processes such as chromatography, polymer coating, encapsulation of nanoparticles, chemical and enzymatic reactions have been extensively explored [2].

Today, enzymes are widely used in pharmacology food, and detergent industries. Therefore, the number of publications of enzymatic organic synthesis increased rapidly over the last few decades [3]. Enzymes are used as chiral catalysts because of their high selectivity. The biological effect of chiral compounds is dependent on one enantiomer, to where it is important to produce in an enantiomerically pure form. The resolution of enantiomers by enantioselective synthesis with enzymes has been exploited extensively for 20 years. Special interest in the use of lipases as synthetic chiral catalyst has increased rapidly. Because of their ability to catalyze reactions, in organic as well as in aqueous systems, lipases lead to the enantioselective synthesis of a wide variety of compounds via hydrolysis, esterification, and transesterification [4].

The first report on the enzyme catalyzed reactions in supercritical fluids was published in 1985 [5]. Manipulating the physical properties of the solvent by changing the pressure or temperature distinguishes supercritical fluid from conventional solvents [2]. Carbon dioxide is especially favored among fluids, because it is non-toxic, nonflammable, inexpensive, and safe, also in which its critical temperature and pressure are suitable for thermally unstable compounds. The enzymes employed in most of the work involving supercritical fluids, and more specifically, carbon dioxide, are lipases [6]. Advantages of using supercritical fluids in enzymatic reactions are as follows: (i) increasing the rate of mass transfer, (ii) simple separation of the product and (iii) environmentally benign reaction media [7]. $SCCO_2$ can replace the commonly used nonpolar organic solvents in organic synthesis [3]. With respect to enantioselectivity, most of the literature investigated enzymatic reactions that had shown high enantioselectivity in conventional organic media. An advantage of using supercritical fluid for enantioselectivity has not yet been clarified [8].

Generally only one enantiomer of chiral compounds has the desired biological activity. The biologically inactive enantiomer may cause unwanted side effects. Chiral α-hydroxy ketones especially benzoins are important structural units in many biologically active compounds and they are versatile

* Corresponding author. Tel.: +90 312 2126725; fax: +90 312 2121546.
E-mail address: nyildiz@eng.ankara.edu.tr (N. Yildiz).

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doi:10.1016/j.supflu.2006.12.007

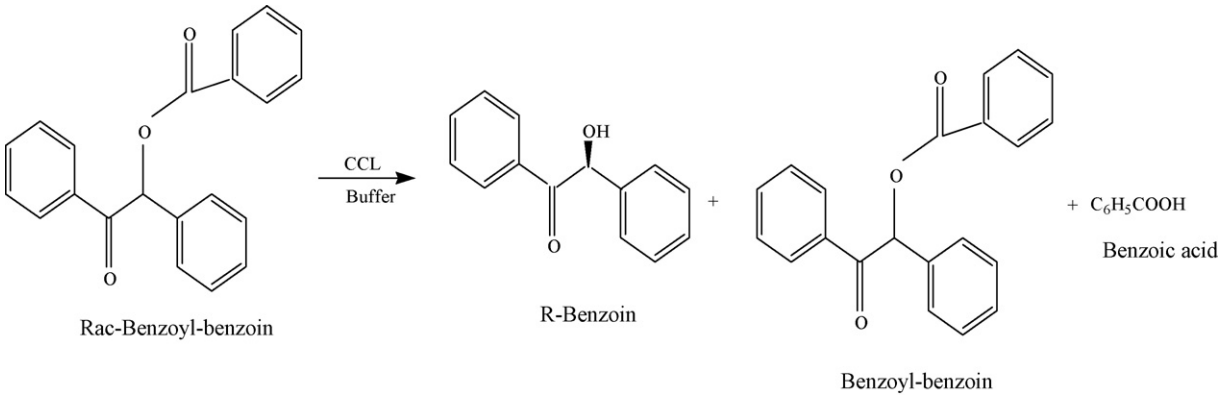

Fig. 1. Reaction scheme of the enantioselective hydrolysis of benzoyl-benzoin.

building blocks in asymmetric synthesis [9]. It is important to obtain enantiopure benzoin from racemic benzoyl-benzoin. In this work, the enzymatic synthesis of chiral benzoin in high enantiomeric excess from racemic benzoyl benzoin was investigated (Fig. 1). In the next step we will apply the results to the unsymmetrical benzoins to develop a method for enantioselective synthesis of unsymmetrical benzoins. Because there is no convenient method for the enantioselective synthesis of unsymmetrical benzoins. It is the first study to synthesize benzoin in $SCCO_2$ media.

2. Experimental

2.1. Materials

Porcine pancreatic lipase (PPL) was purchased from Merck (60 U/mg). Lipase basic kit was supplied from Fluka. It contained *Pseudomonas cepacia* lipase (40 U/mg), *Candida cylindracea* lipase (CCL) (2.8 U/mg), *Rhizopus niveus* lipase (1.7 U/mg), *Aspergillus* lipase (0.94 U/mg), *Mucor miehei* lipase (1.4 U/mg), *Hog pancreas* lipase (23.9 U/mg), *Candida antarctica* lipase (2.7 U/mg), *Pseudomonas florescens* lipase (36 U/mg), *Rhizopus arrhizus* lipase (2.2 U/mg). Other chemicals were obtained from Merck.

2.2. Methods

2.2.1. Starting material preparation

Racemic benzoyl-benzoin was synthesized according to the literature [10]. One thousand and fifty miligrams benzil and 481 μL benzaldehyde were dissolved in 3 mL dimethyl formamide (DMF) and 66 mg KCN was added to this solution. Then, benzoyl-benzoin formation was monitored with thin layer chromatography (TLC) analysis. Benzoyl-benzoin was isolated from the impurities with preparative column chromatography on silica gel 60 (mesh size 40–63 μm).

2.2.2. Analysis

TLC was carried out on aluminum sheets precoated with silica gel $60F_{254}$ (Merck) and the spots were visualized with UV light ($\lambda = 254$ nm). Enantiomeric excess was determined by HPLC analysis. The column was applied from Daicel industries type Chiralcel OD (0,46 cm × 25 cm). The compounds were detected with a UV-detector at 254 nm. The column was operated at room temperature. The mobile phase was *n*-hexane/2-propanol (9:1 v/v). The flow rate was set to 0.8 mL/min, and the injection volume was 10 μL.

2.2.3. Screening of lipases

In a screening test, 10 commercially available lipases were studied. The aim for this screening was to obtain benzoin with high enantiomeric excess. It was determined that four of them, *Porcine pancreas*, *C. antarctica*, *Aspergillus*, and *C. cylindracea* catalyzed the reaction and the others, *P. florescens, Hog pacreas, M. miehei*, *R. niveus*, *P. cepacia*, and *R. arrhizus* could not produce the benzoin. In the experiments, 10 mg benzoyl-benzoin was put into a 0.6 mL DMSO, and 10 mg lipase enzyme was added to 4 mL potassium phosphate buffer (50 mM, pH = 7). The enzyme and substrate solution were mixed in $SCCO_2$ at 35 °C and 90 bar afterwards. As shown in Table 1, the best result was achieved with the CCL lipase. After 90 min, a 50% conversion with an enantiomeric excess of 61.3% was accomplished for CCL. In a comparable experiment a long reaction time of approx. 12 h was obtained with only 14.0% enantiomeric excess of benzoin with *P. pancreas* lipase. Enantiomeric excess values for benzoin with *Aspergillus* and *C. antarctica* were 29.5% with a 4 h reaction time and 33.1% at 50% conversion with a 9 h reaction time, respectively, at the same temperature and pressure. Therefore, *P. pancreas*, *Aspergillus* and *C. antarctica* were not suitable for the synthesis of benzoin in $SCCO_2$. The (*S*)-enantiomer reacted faster than the (*R*)-enantiomer, affording (*S*)-benzoin with PPL and

Table 1
Screening of lipases for enantioselective hydrolysis of benzoyl-benzoin in $SCCO_2$ at 90 bar, 35 °C

Enzyme	Conversion (%)	ee_p (%)
Porcine pancreas	50	14.0 (*S*)
Candida antarctica	50	33.1 (*S*)
Aspergillus	50	29.5 (*R*)
Candida cylindracea	50	61.3 (*R*)

ee_p = (moles of major enantiomer − moles of other enantiomer/total moles of both enantiomers) × 100.