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Asymmetric reduction of 3-chloropropiophenone to (*S*)-3-chloro-1-phenylpropanol using immobilized *Saccharomyces cerevisiae* CGMCC 2266 cells

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ABSTRACT

(*S*)-3-Chloro-1-phenylpropanol is an important chiral precursor for numerous antidepressants such as tomoxetine. A high enantiomeric excess (e.e.) of (*S*)-3-chloro-1-phenylpropanol can be achieved by asymmetric reduction of 3-chloropropiophenone using *Saccharomyces cerevisiae* CGMCC 2266 cells immobilized in calcium alginate. Thermal pretreatment of the immobilized cells at 50 °C for 30 min resulted in high enantioselectivity (99% e.e.) and good percent conversion (80%). The effects of various conditions on the reduction reaction were investigated. The optimal conditions were found to be as follows: sodium alginate concentration, 2%; bead diameter, 2 mm; temperature, 30 °C; re-culture time, 24 h; and batch addition of the substrate. After reusing these three times, the immobilized cells retained approximately 60% of their original catalytic activity with their enantioselectivity intact.

1. Introduction

The search for novel methods to synthesize chiral drugs is a focus area in contemporary organic synthesis due to a worldwide increase in the demand for enantiomerically pure forms of chiral drugs. Enzymes can catalyze reactions with remarkable chemo-, regio-, and stereoselectivity under mild conditions of pH and temperature. Consequently, the number of biocatalysts used in the production of chiral compounds has rapidly increased during the last few decades [1].

Chiral alcohols with additional functional groups are very important intermediates in the synthesis of enantiomerically pure pharmaceuticals and other important chemicals [2]. For example, (*S*)-3-chloro-1-phenylpropanol and (*R*)-3-chloro-1-phenylpropanol are building blocks of (*S*)-fluoxetine (**3**), (*R*)-tomoxetine (**4**), and nisoxetine (**5**), which are prescription drugs used in the treatment of major depressive disorders [3–7]. Unlike chemical tricyclic antidepressants such as imipramine, (*R*)-tomoxetine and (*S*)-fluoxetine have been shown to specifically inhibit norepinephrine and selective serotonin uptake in humans at doses that are clinically well tolerated. These compounds are also reported to be relatively weak ligands for α-1, α-2, and β-adrenergic receptors [8,9]. These receptors are believed to be responsible for the undesirable side effects associated with antidepressants. Clearly, an enantioselective preparation of (*R*)-tomoxetine and (*S*)-fluoxetine would be desirable. Based on these observations, we initiated studies on the reduction of 3-chloropropiophenone (**1**) because (*S*)-3-chloro-1-phenylpropanol (**2**) is a simple precursor of popular serotonin/norepinephrine reuptake inhibitors [3]. The reduction of 3-chloropropiophenone (**1**) is an economical method for obtaining optically active (*S*)-3-chloro-1-phenylpropanol (**2**) because 3-chloropropiophenone is easily synthesized and cheap (Fig. 1).

The conversion of a ketone to its corresponding optically active alcohol is one of the most common redox reactions in organic chemistry. The vast majority of dehydrogenases and reductases used for ketone reduction and alcohol oxidation require nicotinamide cofactors such as NADH and NADPH [10]. In fermentative processes, internal cofactor regeneration occurs in whole cells; as a result, the addition of cheap glucose is sufficient to drive the reaction [1,11–13]. In comparison to the application of isolated enzymes,

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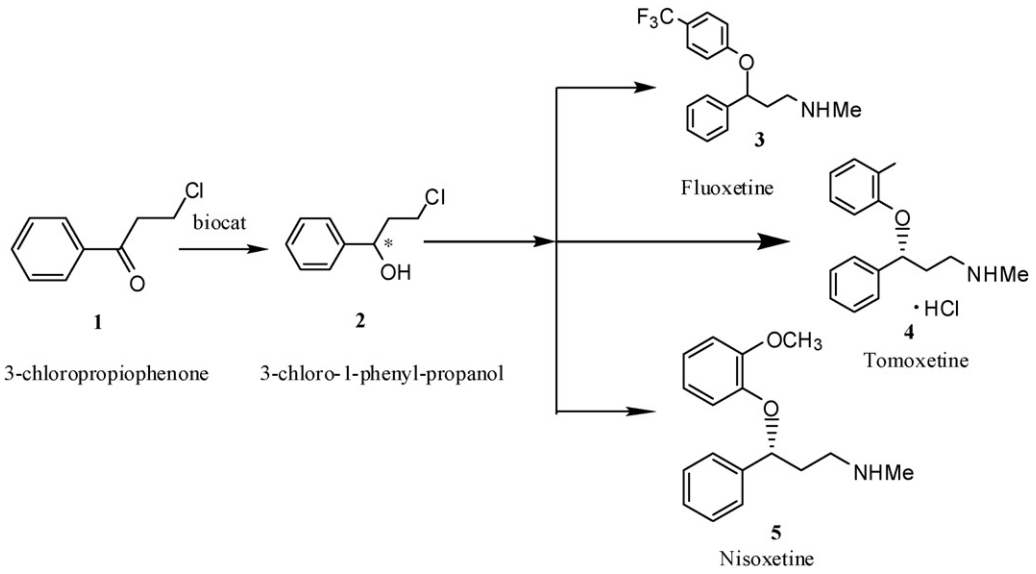

Fig. 1. Synthesis of *R*-(-)-tomoxetine, fluoxetine, and nisoxetine from 3-chloro-1-phenyl-propanol.

the use of whole cells has distinct characteristics. Whole cells contain enzymes that are generally more stable since they are present in their natural environment. There are reports on several carbonyl reductases that reduce ketones to optically active alcohols in microorganisms [14–16]. Michio and Yoshinori [17,18] reported the production of optically active (*S*)-3-chloro-1-phenylpropanol by several microorganisms. Although these microorganisms could produce optically active (*S*)-3-chloro-1-phenylpropanol of high enantiomeric purity, the conversion was very low. Bakers' yeast (*Saccharomyces cerevisiae*) is a popular biocatalyst in organic synthesis and has been shown to catalyze C–C bond formation and dissociation, oxidation, hydrolysis, and a variety of reduction reactions [19]. It is widely used for the asymmetric reduction of prochiral ketones because such reductions are easy to perform, and the cells are inexpensive and readily available [20]. In some cases, several reductases present in a single cell are involved in the reduction of a particular ketone. Due to the different stereospecificities of these enzymes, the desired alcohol has low optical purity [21,22]. Moreover, many of the *S. cerevisiae*-mediated methods used for the asymmetric reduction of ketones are unsuitable for the large-scale production of chiral alcohol. Some recent studies on the use of immobilized cells for the enantioselective reduction of ketones have attempted to overcome these drawbacks [23–25]. The use of immobilized microbial cells in organic synthesis is considered to be both technically and economically advantageous [26–28].

In this paper, we report the use of the *S. cerevisiae* CGMCC 2266 strain (accession number: CGMCC 2266, China General Microbiological Culture Collection Center (CGMCC)) as a novel biocatalyst for the production of (*S*)-3-chloro-1-phenylpropanol from the prochiral compound 3-chloropropiophenone. The biocatalyzed reduction reaction was carried out with thermally pretreated immobilized cells to achieve high enantioselectivity. We determined the effects of different immobilization methods, such as crosslinking with glutaraldehyde–gelatin and entrapment in an agar or Ca-alginate gel, on the reduction of 3-chloropropiophenone by immobilized *S. cerevisiae* CGMCC 2266 cells. We also investigated other factors that influenced the percent conversion and enantiomeric excess (e.e.) of 3-chloro-1-phenylpropanol.

2. Experimental

2.1. Chemical materials

3-Chloropropiophenone, (*R*)-3-chloro-1-phenylpropanol, and (*S*)-3-chloro-1-phenylpropanol were purchased from Aldrich Chemical Co., Inc. All other chemicals were of analytical grade and obtained commercially.

2.2. Strain and culture conditions

The microorganism used in this study was *S. cerevisiae* CGMCC 2266, which was obtained from Zhejiang University of Technology, Hangzhou, Zhejiang Province, PR China. The strain was maintained at 4 °C on MYPG solid medium. The composition of 1 L of MYPG medium was as follows: wort (10 g), yeast extract (3 g), peptone (5 g), glucose (10 g), and agar (20 g). The liquid medium for the growth of strains contained glucose (30 g/L), yeast extract (3 g/L), $(NH_4)_2SO_4$ (5 g/L), $MgSO_4{\cdot}7H_2O$ (0.5 g/L), $K_2HPO_3{\cdot}3H_2O$ (1 g/L), and KH_2PO_3 (1 g/L).

To prepare the seed culture, the strain was inoculated in sterilized liquid medium (100 mL) in a 500-mL shake flask that was shaken (160 rpm) at 30 °C for 24 h. The seed culture (10 mL) was added to the liquid medium (100 mL), which was shaken at 30 °C for one day. The cells were collected by centrifugation and washed with distilled water.

2.3. Immobilization of S. cerevisiae CGMCC 2266 cells

2.3.1. Immobilization in crosslinked glutaraldehyde–gelatin beads

The cells were immobilized in crosslinked glutaraldehyde–gelatin beads as follows. Gelatin (8 g) was dissolved in distilled water (36 mL) by warming to 60 °C. *S. cerevisiae* CGMCC 2266 cell suspension (10 mL) was mixed with the gelatin solution at 40 °C, and the gelatin concentration was adjusted to 10% (w/v). After the microbial cells were well dispersed, glutaraldehyde (25%, 1 mL) was added with stirring, and the solution was poured into a petri plate and allowed to dry overnight at room temperature. After sieving, immobilized microbial granules of particle size 3 mm were obtained.