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Journal of Molecular Catalysis B: Enzymatic 8 (2000) 285–288

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Letter

Asymmetric bioreduction of a bisaryl ketone to its corresponding (*S*)-bisaryl alcohol, by the yeast *Rhodotorula pilimanae* ATCC 32762

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Received 4 January 1999; received in revised form 14 July 1999; accepted 14 October 1999

Abstract

The screening of 310 microbial strains yielded eight as suitable biocatalysts for the asymmetric bioreduction of a highly hindered bisaryl ketone to its corresponding alcohols. The production of both enantiomers with elevated optical purity (ee > 96%) was achieved by different microorganisms. When scaling up the asymmetric bioreduction process in laboratory bioreactors (23 l scale), the production of preparative amounts (1.5 g) of the (*S*) enantiomer with elevated optically purity (ee > 96%) was achieved when employing the yeast *Rhodotorula pilimanae* ATCC 32762. Achieving this asymmetric bioreduction with enantiocomplementarity in employing such a hindered substrate is remarkable and highlights the potential of such biological approach.

Keywords: Bioreduction; Bioconversion; Biotransformation; Biocatalysis; Fermentation

1. Introduction

The evaluation of the pharmacokinetic properties of single enantiomers of pharmaceutical drugs has demonstrated that in many instances only one of the enantiomers is pharmaceutically active [1,2]. The need for efficient tools to assist chemists in their asymmetric syntheses is increasing. Novel methodologies and chiral reagents are being rapidly developed to meet this demand [3]. Chiral alcohols are key intermediates in many chiral syntheses and their production through the asymmetric reductions of prochiral ketones employing biocatalysis is becoming a valuable method [4,5].

A phosphodiesterase 4 inhibitor (**3**) targeted for the treatment of asthma [6,7] has one chiral center which renders its chemical synthesis challenging (Fig. 1). As a potential route to the chiral synthesis, we investigated the asymmetric bioreduction of the diaryl ketone precursor (**1**) to the corresponding chiral alcohol (**2**) as outlined in Fig. 1. This communication presents the

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1381-1177/00/$ - see front matter
PII: S1381-1177(99)00103-4

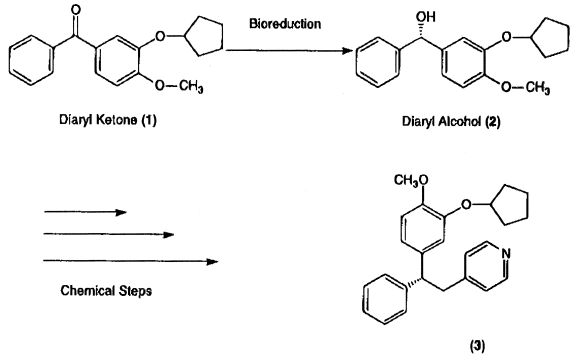

Fig. 1. Asymmetric reduction of a bisayl ketone to its corresponding bisayl alcohol.

screening effort that led to the identification of several microbial cultures capable of producing both enantiomer with elevated optical purity, as well as the process for the production of preparative amounts of optically pure alcohol.

2. Preparation of alcohol and ketone

For analytical purposes, an authentic racemic sample of the diaryl alcohol was prepared as follows. Isovanillin (100 g, 0.65 mol) and potassium carbonate (138.2 g, 1.12 mol) were added to dimethylsulfoxide (600 ml) portionwise at room temperature. The mixture was heated to 60°C and cyclopentyl bromide (112 ml, 1.12 mol) was added over 30 min at 60°C. The mixture was aged at 60°C for 14 h. The mixture was cooled to room temperature and water (600 ml) was added in one portion. The solution was stirred for 30 min. The mixture was extracted twice with toluene (800 ml, 400 ml). The combined organic layers were washed with a dilute HCl aqueous solution (0.2 N, 0.8 l) and with water (twice 0.6 l). The organic layer was concentrated to 1 l. To the above toluene solution, phenylmagnesium bromide solution in ether (230 ml, 3 M) was added at −10°C and the mixture was aged for 30 min below 0°C. Aqueous HCl solution (1 N, 700 ml) and ethyl acetate (700 ml) were added successively and the layers were separated. The organic layer was washed with water (twice 500 ml) and concentrated to dryness. Crystallization of the crude alcohol in 1:6 ethyl acetate/hexanes mixture (1.4 l) gave 167.8 g of a white solid (87% yield).

The bisaryl ketone was prepared from the bisaryl alcohol as follows. The alcohol (167 g, 0.56 mol) was dissolved in 10:1 tetrahydrofuran/water (660 ml) and $RuCl_3$ (2.1 g, 1.8 mol%) was added at room temperature. The mixture was then warmed to 50°C and *t*-butyl hydrogen peroxide (300 ml, 70% in water) was added dropwise (at this point the mixture should be at reflux). Upon completion, the mixture was cooled to room temperature and washed with Na_2SO_3 (1.2 l, 2 N) followed by water (twice 600 ml). The organic layer was concentrated to dryness and the resulting solid recrystallized from 3:1 hexanes/ethyl acetate (2 l) at 0°C to give 142.2 g of a white solid (72% yield).

3. Analytical

A Rainin HPLC system (Rainin, Woburn, MA) equipped with a Zorbax RX-C8 column (4.6 mm × 25 cm) (Mac-Mod Analytical, Chadds Ford, PA), was employed for the separation of the bisaryl ketone and its corresponding alcohol. Separation was achieved by isocratic elution, employing a mobile phase, comprised of acetonitrile and acidified water (0.1% phosphoric acid) [50:50 v/v] at a flow rate of 1.0 ml/min. Detection was performed at 210 nm and at 22°C, with the ketone and the alcohol eluting after 12.4 and 9.3 min, respectively. The optical purity of the produced alcohol was evaluated on a similar HPLC system, employing a Chiracel OJ (4.6 mm × 25 cm) column and a mobile phase made of ethanol/hexane (15:85; v/v) delivered at a flow rate of 0.6 ml/min. Detection was performed at 210 nm with the (*R*) and (*S*) alcohols eluting after 10.2 and 11.8 min, respectively (absolute configuration deter-

mined by conversion to final product; J. Lee, J. Lynch, W.-B. Choi, unpublished results).

4. Microbial screening

Microorganisms obtained from the Merck Microbial Resources Culture Collection (Merck, Rahway, NJ) were preserved on Sabouraud dextrose (Difco, Detroit, MI), YME [8] or nutrient agar (Difco) slants at 4°C for yeasts, fungi, and bacteria, respectively. Erlenmeyer flasks (250 ml) containing 25 ml of the appropriate medium were inoculated with a loopfull of cells and incubated on an orbital shaker (220 rpm) at 28°C. After 48 h, of cultivation, 25 mg of bisaryl ketone dissolved in 1 ml of ethanol were added to each flask (1 g/l final concentration) to initiate the bioconversion. The flasks were returned to the same incubation conditions as described above. The presence of the desired product was detected after 48 h (post substrate addition) by HPLC analysis of the supernatant diluted (1:5) with acetonitrile.

A total of 310 microorganisms (53 bacteria, 113 yeasts and 144 fungi) were evaluated for their ability to bioreduce the bisaryl ketone to its corresponding alcohol employing this procedure. Analyses of the extracts indicated that eight strains were able to catalyze the desired reaction (Table 1). After evaluating the optical purity of the alcohol produced by these eight microbial strains, it was found that either enantiomer could be produced with elevated enantiomeric excess (Table 1).

Table 1
Microbial strains catalyzing the asymmetric bioreduction of the diarylketone

Microbial strain	Config.	% e.e.	% conv.
Aspergillus nidulans MF 121	(*S*)	96	7.6
A. nidulans MF 145	(*S*)	86	30.0
Bullera tsugae MY 1669	(*S*)	93	11.0
Hansenula holstii MY 1538	(*R*)	100	3.0
Mucor sulfa MF 37	(*S*)	95	4.0
Rhodotorula pilimanae ATCC 32762	(*S*)	96	10.0
Saccharomyces cerevisiae MY 1690	(*R*)	13	3.0
Unidentified fungus HB 920750	(*S*)	54	4.0

5. Production at the preparative scale

One yeast strain, *Rhodotorula pilimanae* ATCC 32762 that produced the (*S*) enantiomer with ee > 96%, was selected for study of the production of preparative amounts of chiral bisaryl alcohol. The scale-up of this asymmetric bioreduction process was performed in 23 l fermentors (B. Braun, Allentown, PA). A volume of 16 l of production medium (Sabouraud dextrose) and 16 ml of P2000 antifoam agent (Poly Glycol, Dow Chemical, Midland, MI) were sterilized in situ at 121°C for 25 min. A volume of 500 ml of a 24 h old second stage seed prepared in shake flask was used to inoculate the bioreactor. The bioreactor was operated at 28°C with an agitation set at a minimum of 220 rpm, an aeration of air at 6 l/min, and a back pressure of 0.6 bar. Dissolved oxygen tension was maintained at 30% of initial saturation by computer controlled ramping of the agitation and by manually increasing the airflow. The bioreduction was initiated 50 h after inoculation by the addition of 16 g of bisaryl ketone (final concentration 1 g/l) dissolved in 640 ml of ethanol.

Fig. 2 shows that a maximum bisaryl alcohol concentration of about 100 mg/l was achieved 48 h after the addition of the bisaryl ketone. No further increases in this titer were observed after that time, and a yield of about 10% was therefore achieved in this preparative scale experiment.

The whole broth (16 l) containing about 1.65 g of alcohol was extracted with one volume (16 l) of ethyl acetate. The organic layer was vacuum concentrated 27-fold to yield 600 ml of liquors containing 1.44 g of alcohol. This liquor was subsequently chromatographed on a silica gel column using 15:85 ethyl acetate/hexanes mixture. Fractions containing the alcohol were combined and concentrated to dryness. The re-

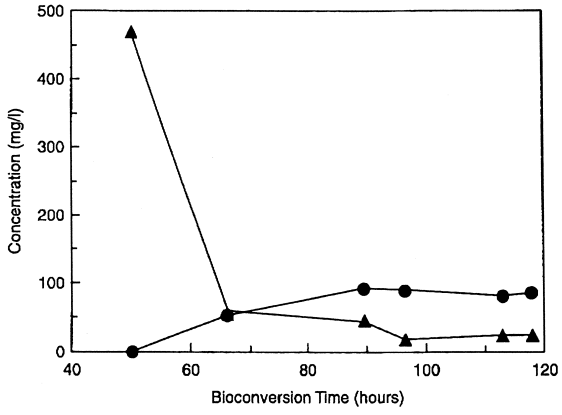

Fig. 2. Time course for the asymmetric bioreduction of a bisayl ketone to its corresponding (*S*) alcohol by the yeast *R. pilimanae* ATCC 32762. Experiments were performed at the 23 l scale as described in the text. Triangles and circles represent the bisaryl ketone and bisaryl alcohol, respectively.

sulting solid was crystallized from hexanes to give 0.92 g of a white solid. The authenticity of the desired product was confirmed by proton NMR (Brucker 300). The assignments for the bisaryl alcohol are: ppm in $CDCl_3$; 7.4–7.2 (m, 5H's, aromatic); 6.9–6.7 (m, 3H's, aromatic); 5.8 (d, $J = 3$ Hz, 1 H); 4.7 (m, 1 H); 3.8 (s, 3 H); 2.2 (d, $J = 3$ Hz, 1 H); 1.9–1.5 (m, 8 H).

The enantiomeric excess of the purified (*S*)-bisaryl alcohol was evaluated to be greater than 96% by normal phase chiral HPLC.

6. Conclusion

In conclusion, we report here a method for the preparation of optically pure (ee > 96%) (*S*)-bisaryl alcohol by asymmetric bioreduction of its ketone precursor, employing the yeast *R. pilimanae* ATCC 32762. Enantiocomplementarity of this approach was also demonstrated and production of preparative amounts of the (*R*)-bisaryl alcohol using *Hansenula holstii* MY 1538 should be straightforward. The low yields achieved in this bioreduction may be due to either partial microbial degradation of the ketone, or to a low recovery caused by strong adsorption to the surface of the microbial membrane. Possible process improvements may be achieved by the use of organic co-solvents or of emulsifying agents, as well as through the optimization of classical microbial fermentation parameters such as environmental conditions and cultivation medium. Achieving this asymmetric bioreduction with enantiocomplementarity in employing such a hindered substrate is remarkable and highlights the potential of such an approach.

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