Stereoselective benzylic hydroxylation of ethylbenzene and propylbenzene using the mycelia of *Aspergillus flavus* MTCC-1783 and MTCC-1884

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Abstract: The aim of this study was to provide syntheses of optically pure (*R*)-1-phenylethanol and (*R*)-1-phenylpropanol from ethylbenzene and propylbenzene, respectively, using the fungal mycelia of new fungal species, namely *Aspergillus flavus* MTCC-1783 and *Aspergillus flavus* MTCC-1884, as catalysts. The mycelia of *A. flavus* MTCC- 1783 and *A. flavus* MTCC-1884 were prepared by growing the fungal strains in liquid culture medium containing ethylmethylketone as the sole carbon source. The mycelia were suspended in potassium phosphate buffer pH 7.0. The suspensions of mycelia were used for the transformations of ethylbenzene and propylbenzene. Ethylbenzene and propylbenzene were converted to (*R*)-1-phenylethanol and (*R*)-1-phenylpropanol, in 100% and 99% ee, respectively. The mycelia of *A. flavus* MTCC-1783 and *A. flavus* MTCC-1884 can be used for the preparation of (*R*)-1-phenylethanol and (*R*)-1-phenylpropanol in 100% and 99% ee, respectively, from ethylbenzene and propylbenzene, respectively. The studies report convenient methods for the syntheses of optically pure isomers, (*R*)-1-phenylethanol and (*R*)-1-phenylpropanol, which are important chiral building blocks in the preparations of fine chemicals and pharmaceuticals. The reactions are ecofriendly, occur at 30 °C, and the time required was 24 h.

Key words: Aspergillus flavus, benzylic hydroxylation, biocatalyst, (*R*)-1-phenylethanol, (*R*)-1-phenylpropanol.

Résumé : On a réalisé la synthèse des (*R*)-1-phényléthanol et (*R*)-1-phénylpropanol, à partir respectivement de l'éthylbenzène et du propylbenzène, en présence de mycélium fongiques provenant de nouvelles espèces fongiques, soit l'*Aspergillus flavus* MTCC-1783 et l'*Aspergillus flavus* MTCC-1884, utilisées comme catalyseurs. Les mycélium d'*A. flavus* MTCC-1783 et d'*A. flavus* MTCC-1884 ont été préparés en procédant à la culture des souches fongiques dans des milieux de culture liquide ne contenant que de l'éthylméthylcétone comme source de carbone. Les mycélium ont été suspendus dans un tampon de sulfate de potassium, de pH 7,0. On a ensuite utilisé les suspensions de mycélium pour les transformations de l'éthylbenzène et du propylbenzène. L'éthylbenzène et le propylbenzène ont été convertis en (*R*)-1-phényléthanol et en (*R*)-(1)-phénylpropanol avec des excès énantiomères respectivement de 100 % et 99 %. En conclusion, les mycélium d'*A. flavus* MTCC-1783 et d'*A. flavus* MTCC-1884 peuvent être utilisés dans la préparation de (*R*)-1-phényléthanol et (*R*)-(1)-phénylpropanol avec des excès énantiomères respectivement de 100 % et 99 %, à partir respectivement l'éthylbenzène et du propylbenzène. Les études décrites dans ce travail rapportent la mise au point de méthodes commodes de synthèses d'isomères optiquement purs de (*R*)-1-phényléthanol et de (*R*)-(1)-phénylpropanol qui sont des synthons chiraux importants dans les préparations de produits chimiques fins et pharmaceutiques. Les réactions sont écologiques; elles se produisent à 30 °C et les temps requis sont de l'ordre de 24 heures.

Mots-clés : Aspergillus flavus, hydroxylation benzylique, biocatalyseur, (*R*)-1-phényléthanol, (*R*)-(1)-phénylpropanol.

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Introduction

Chiral alcohols are important intermediates and are structural elements in the syntheses of biologically active compounds and natural products.[1–5] The optically active 1-phenylethanol is used as a chiral building block and synthetic intermediate in fine chemicals and in pharmaceutical industries.[6–8] (*R*)-1-Phenylethanol is widely used as a fragrance in the cosmetic industry because it has a mild floral odour. Other applications include use as a salvatochromic dye,[9] use as an opthalic preservative, and use as an inhibitor of cholesterol intestinal absorption.[10] β-Aminoalcohols have been used for the syntheses of 1-phenylethanol-2-[(2-phenyl-1-alkylethyl)amino]ethanol derivatives, a new important class of antidiabetic agents.[11] A large genus of mushroom flies has been reported to be attracted to 1-phenylethanol in field tests.[12] Thus, secondary alcohols act as pheromones also. However, the chemical methods for their syntheses are not

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convenient and ees are generally low.[13] On the other hand, biocatalytic methods are convenient and enantiomeric excesses are high.[14]

Several biocatalytic methods to synthesize enantiomeric pure secondary alcohols have been developed during the recent past owing to the increasing demand for these valuable compounds.[5] Stereoselective reduction of ketones[14] and enantioselective oxidation of racemic secondary alcohols have been studied for the preparations of pure enantiomeric secondary alcohols. Although the hydroxylation of nonactivated centres in hydrocarbons is one of the most useful biotransformations, so far it has been used mainly for the hydroxylation of steroids and terpeniods.[3,13] The biotransformation of ethylbenzene to 1-phenylethanol has rarely been studied.[15–20] The conversion of ethylbenzene and a number of its para-substituted derivatives to the corresponding optically active 1-phenylethanols with enantiomeric excesses varying between 5% and 40% using the fungus *Mortierella isabellina* have been reported.[15] The fungi *Cunninghamella echinulata* var. *elegans* and *Heminthosporium* were also capable of performing some of these transformations.[15] The hydroxylation of ethylbenzene almost exclusively at the secondary carbon atom giving 1-phenylethanols in the ratio 2:1 of the R and S isomers using cytochrome P450camp has been reported.[16] Seventeen fungi and two yeast species that could hydroxylate ethylbenzene and propylbenzene to 1-phenylethanol and 1-phenylpropanol, respectively, have been reported.[17] One potent strain, *Fusarium moniliforme*, oxidizes ethylbenzene and propylbenzene to the corresponding benzylic alcohols with enantiomeric excesses of 98% in the (R) (+) form.[17] The involvement of cytochrome P450 in this transformation has been demonstrated.[18] Szaleniec et al.[19] reported the oxidation of ethylbenzene to (*S*)-(–)-1-phenylethanol by the denitrifying bacterium *Azoarcus* species strain EbN1. A nobel Mo–Fe–S enzyme, anaerobic ethylbenzene dehydrogenase, has been isolated and characterized.[20] Keeping in mind the potential for the biotransformation of ethylbenzene to optically pure isomers of 1-phenylethanol, we initiated a search for the fungal strains that could carry out these useful transformations. In this communication, we report two fungal strains, namely *Aspergillus flavus* MTCC-1783 and *Aspergillus flavus* MTCC-1884, which transform ethylbenzene to (*R*)-1-phenylethanol and propylbenzene to (*R*)-1-phenylpropanol in 100% and 99% ee, respectively.

Materials and methods

Chemicals

Methylbenzene, ethylbenzene, propylbenzene, ethylmethylketone, racemic (±)-1-phenylethanol, (*R*)-1-phenylethanol, racemic (±)-1-phenylpropanol, and (*R*)-1-phenylpropanol were purchased from Sigma-Aldrich Chemicals Private Limited, New Delhi, India. All other chemicals were purchased either from S.D. Fine-chem Ltd., Mumbai, India, or from Qualigens Chemicals, Mumbai, India, and were used without further purification.

Fungal strains

Both fungal strains were procured from the Microbial Type Culture Collection Centre and Gene Bank, Institute of Microbial Technology, Chandigarh, India, and were maintained on Bennett's agar medium,[17] which consisted of 1.5% (*w*/*v*) glucose, 0.5% peptone, 0.2% yeast extract, 0.2% Ehlrich's beef extract, and 1.5% agar in tap water.

Preparation of mycelia

The microorganisms were cultivated in 100 mL of basal medium I (BM I) containing 1 mL of unsterilized ethylmethylketone (*v*/*v*) in 250 mL Erlenmeyer flasks at 30 °C on a rotary shaker at 150 rpm for 3 days. BM I contained 10 g of $NaNO_3$, 2 g of NH_4Cl, 2 g of KH_2PO_4, 3 g of K_2HPO_4, 2 g of NaCl, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.5 g of yeast extract, and 2 mL of metal solution having pH 7.0 in 1 L of deionized water. The metals solution consisted of 400 mg of $MnCl_2{\cdot}2H_2O$, 350 mg of $FeCl_2{\cdot}4H_2O$, 200 mg of $ZnCl_2$, 20 mg of $CoCl_2$, 20 mg of $CuCl_2{\cdot}H_2O$, 10 mg of $Na_2MoO_4{\cdot}2H_2O$, 10 mg of $Na_2B_4O_7{\cdot}10H_2O$, and 2 mL of concentrated HCl in 100 mL of deionized water. The mycelia were collected by filtration on ordinary filter papers, washed twice with 30 mL of 25 mmol/L potassium phosphate buffer (KPB, pH 7.0), and were used fresh.

Biotransformation reactions

The biotransformation reactions were performed using the reported method.[17] Wet mycelia (0.1 g) were suspended in 2 mL of 25 mmol/L KPB in test tubes (17 mm in diameter and 150 mm in height), and 200 μmol of ethylbenzene (21 μL) was added. The test tubes were closed with stoppers and incubated at 30 °C in a reciprocal shaker at 200 rpm. After 24 h, the reaction solutions were acidified by the addition of 0.2 mL of 6 N HCl. The products formed in the reaction solution were extracted thrice using 2.0 mL of *n*-hexane each time. Biotransformations of propylbenzene and methylbenzene were also studied using the above method. The extracts were analysed for 1-phenylethanol, 1-phenylpropanol, and benzylalcohol by using HPLC as mentioned in the following. In one typical scale-up experiment, 7.5 g of the mycelia of *A. flavus* MTCC-1884 suspended in 100 mL of 25 mmol/L KPB and 1.5 mL of ethylbenzene were reacted for 24 h at 25 °C and then extracted with 30 mL of *n*-hexane; 20 μL of the extract was subjected to HPLC analysis. In another experiment, 14 g of the mycelia of *A. flavus* MTCC-1783 suspended in 100 mL of KPB and 3.0 mL of ethylbenzene at 25 °C was reacted for 24 h, then extracted with 30 mL of *n*-hexane, and then 20 μL of the extract was subjected to HPLC analysis. Similar experiments were performed with propylbenzene.

HPLC analysis

HPLC analyses were done using a Waters HPLC (model 600E) and a Spherisorb C_{18} 5 UV (4.5 mm × 250 mm) column. The eluent phase was a methanol–water mixture in a 1:1 (*v*/*v*) ratio at 1 mL/min. The *n*-hexane extracts of the products (20 μL) were injected and the detections were made using a Waters UV detector (model 2487) at 254 nm.

Spectroscopic analysis

The identifications of the biotransformation products were done by IR and ^1H and ^{13}C NMR spectroscopic techniques.

^1H NMR

The ^1H NMR spectra of the biotransformation products

were recorded using a Jeol AL 300 MHz spectrometer in $CDCl_3$ using TMS as the internal standard reference at the Department of Chemistry, Banaras Hindu University, Varanasi, India.

^{13}C NMR

The ^{13}C NMR spectra of the biotransformation products were recorded on a Jeol-ECX 500 Hz spectrophotometer in $CDCl_3$ using TMS as the internal standard reference at the Department of Chemistry, Indian Institute of Technology, Kanpur, India. The chemical shift values were reported in ppm.

IR

The IR spectra of the biotransformation products were recorded on a Bruker IR-VERTEX Flash 70 spectrometer in the range of 400–4000 cm^{-1} at the Department of Chemistry, Indian Institute of Technology, Kanpur, India.

GC–MS

Products were analysed by GC–MS using an HP GC–MS model No. 5975C-inert MSDS with triple axis detector using helium gas (5 mL/min) as the mobile phase at the Department of Chemistry, Indian Institute of Technology, Kanpur, India.

Determination of ee

The ees were determined using a Chiralcel OD column (4.6 mm × 250 mm) manufactured by Daicel Chiral Technologies Pvt. Ltd. (Japan) using a 90:10 (*v*/*v*) mixture of *n*-hexane and isopropylalcohol as the eluent phase at a flow rate of 0.5 mL/min at the Department of Chemistry, Indian Institute of Technology, Kanpur, India.

Results and discusion

The results of the identification of the biotransformation products, (*R*)-1-phenylethanol and (*R*)-1-phenylpropanol, using the suspensions of fungal mycelia of the strains *A. flavus* MTCC-1783 and *A. flavus* MTCC-1884 are given in the Supplementary data. The fungal mycelia of *A. flavus* MTCC-1783 converted ethylbenzene to (*R*)-1-phenylethanol in 100% enantiomeric excess and propylbenzene to (*R*)-1-phenylpropanol in 99% enantiomeric excess. The reaction times were 24 h and no ethylbenzene and propylbenzene were detected at the end of transformation reactions showing that ethylbenzene and propylbenzene were fully transformed to their corresponding biotransformation products. Methylbenzene was also transformed to benzylalcohol by these two fungal strains showing that hydroxylations occur at benzylic carbon centre. In typical scale up experiments as mentioned in the materials and methods section (Biotransformation reactions) 1.5 mL of ethylbenzene and 3.0 mL of propylbenzene were fully converted to (*R*)-1-phenylethanol and (*R*)-1-phenylpropanol, respectively, showing the feasibility of the method on laboratory scale.

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/v2012-034.

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