

Production of Allyl Phenyl Carbinol (APC) by Biotransformation using *Rhizopus arrhizus*

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ABSTRACT

The objective of the present work was to study the stereoselective synthesis of homo-allylic alcohol using stereoselective hydrolysis by *Rhizopus arrhizus* and also to study the stereoselective synthesis of allyl phenyl carbinol (APC) or 1-Phenyl-3-butene-1-ol by combination of chemical synthesis and biotransformation from cheap raw materials such as benzaldehyde and allyl bromide. Stereospecific synthesis of allyl phenyl carbinol (APC) was achieved by acetate hydrolysis by *R. arrhizus* giving R (+) enantiomer. Maximum enantiomeric excess of APC was obtained at 16 h where as maximum yield of it was obtained at 48 h of biotransformation.

Key words: Allyl Phenyl Carbinol, Biotransformation, *Rhizopus arrhizus*

INTRODUCTION

Asymmetric hydrolysis of racemic esters or esterification of racemic alcohols are the main routes for the synthesis of chiral carbinols. Use of R enantiomer of allyl phenyl carbinol (APC) or 1-Phenyl-3-butene-1-ol for preparation of valuable intermediates for drugs and agro-chemicals (Hosomi 1988), and for preparation of substituted cyclophosphamide analogues for treatment of leukaemia of the cells resistant to cyclophosphamide (Borch and Canute 1989, 1991) is well reported. Chemical synthesis of R enantiomer of APC is described by Keck *et al.* (1993) and Mathre *et al.* (1993). Synthesis of S enantiomer has also been reported (Barrett *et al.* (1986), Hoffman & Herold (1991)). However biotransformation is known to offer several advantages over the traditional chemical synthesis like reaction specificity, regioselectivity, stereospecificity and requirements of mild conditions. Enantioselective hydrolysis of racemic acetate of APC by crude pig liver acetone powder has been successful in giving 56-72 % optical purity (Basavarajah *et al.* 1990). The mould *Rhizopus arrhizus* has proved its versatility for reduction of a variety of carbonyl compounds and hydrolysis of acetates (Patil *et al.* 1993, 1996, Salvi *et al.* 1995a, b). However, stereospecific synthesis of APC using microbial

biotransformation has not been reported so far. Therefore preliminary attempts were made to use the acetate hydrolysis activity of *Rhizopus arrhizus* for yielding stereospecific APC with a view to avoiding harsh experimental conditions and use of costly raw materials. For this, first chemical synthesis of the substrate was achieved and then it was subjected to biotransformation.

MATERIALS AND METHODS

Micro-organism *Rhizopus arrhizus* (NCIM 997) was obtained from National Collection of Industrial Micro-organisms, National Chemical Laboratory, Pune. The culture was maintained on PDA (Hi-Media) slants.

Chemical synthesis of (±) 1-phenyl-3-butene-1-ol : This was synthesised from benzaldehyde, allyl bromide, using activated zinc by Christian and Luche's method (1985). The purified compound was confirmed on the basis of FT-IR spectrum of the compound.

Chemical synthesis of (±) 4-acetoxy-4-phenyl-1-butene (A) : This was obtained by acetylation (Furniss *et al.* 1989) of (±) 1-phenyl-3-butene-1-ol and confirmed on the basis of FT-IR.

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Biotransformation studies : 10^6 spores of fungus *R. arrhizus* were inoculated into 150 ml (500 ml conical flask) of sterilised modified Czapek Dox medium (M/s. Hi-Media Ltd. Mumbai) (Prema and Bhattacharya 1962). The organism was allowed to grow on a rotary shaker at 180 rpm and $30 \pm 2^\circ\text{C}$ for 72 h. The substrate viz. (\pm) 4-acetoxy 4-phenyl -1-butene (A) (100 mg) was dissolved in 1ml of 95% ethanol and added into flasks containing grown *R. arrhizus*. The reaction was allowed to take place on a rotary shaker for different periods. A substrate control (with substrate but without micro-organism) and organism control (with organism but without substrate) were also run simultaneously. The complete scheme is shown in the Fig.1.

Analysis of products : At the end of biotransformation, the mycelial mass was filtered from the culture medium. The filtrate was extracted with chloroform (M/s. S. D. Fine Chemicals Ltd. Mumbai) (3 x 100ml) washed with water (2 x 20ml) and dried over anhydrous Na_2SO_4 . The solvent was removed under reduced pressure and the residue obtained was monitored on TLC (Silica gel- G, M/s. Merck India, Mumbai) using hexane : diethyl ether : glacial acetic acid (80:20:2 v/v) as a solvent system. The control flasks were also extracted similarly. The product were purified by preparative TLC. using 5% ethyl acetate in pet ether (60-80). The compounds purified as above were tested for purity using GC and NMR as well as subjected to Chiral HPLC as follows. ^1H NMR Spectra were recorded on Varian EM-360 60MHz using CDCl_3 solvent and chemical shifts were given in parts per millions from Tetramethyl silane as internal standard. IR Spectra were recorded on Perkin-Elmer IR Spectrophotometer model 783. GC analysis was carried out on a Shimadzu Gas Chromatograph GC-16A with FID detector. A column 3% OV-17 (80-100 mesh) was used with nitrogen flow at 40ml/min. Initial temperature was 90°C and final temperature was 240°C , rate of heating used was $4^\circ\text{C}/\text{min}$. Retention time of 1-phenyl-3-butene-1-

ol, was 9.17 min. The chiral analysis of purified 1-phenyl 3-butene obtained was done on Jasco - PU 980 HPLC with UV 975 as detector (220 nm). Column used was Chirex - 3001 (ss) having dimension 4.67×25 cm. Mobile phase used was 0.2% isopropyl alcohol and 2% dichloro ethylene in n- Hexane. Flow rate of the solvent system was 1.0 ml/min. Amount of sample injected used was 20 μl .

RESULTS AND DISCUSSION

Initially substrate used for biotransformation was chemically synthesised and confirmed on the basis of the FT-IR spectrum of substrate. As the previous reports on biotransformation of different substrates by acetate hydrolysis activity by the same organism have suggested at least 48 hrs for more enantioselective synthesis of products (Patil *et al.* 1993, 1996, Salvi *et al* 1995^b) in this biotransformation the level of products was determined at various time intervals after 16h. The extracts from supernatant were subjected to TLC. The extract showed presence of both 1-phenyl-3-butene-1-ol and unreacted substrate. Purification of extract gave product obtained by acetate hydrolysis reaction. The yields and enantiomeric excess of the product and yield and specific optical rotation of unreacted substrate are as shown in the Table 1. The maximum yield of R (+) was observed at 16 h of biotransformation whereas the maximum yield was at 48h of biotransformation. Beyond 48hs both the yield and enantiomeric excess decreased. This is the first report on biotransformation of (\pm) 4-acetoxy-4-phenyl-1-butene synthesised from benzaldehyde and allyl bromide to R (+) 1-phenyl-3-butene-1-ol using *Rhizopus arrhizus*. The ^1H NMR of the product so obtained gave peaks at frequency (δ ppm) - 7.3 - 7.7 m (-Ar), 5.7 - 6.3 m (-CH), 5.3 - 5.5 s (-CH₂), 4.7- 4.9 s (-CH-OH), 2.2 - 2.5 t (=CH₂), 2.6 - 2.7 s (-OH) confirming identity of 1-phenyl-3-butene-1-ol.

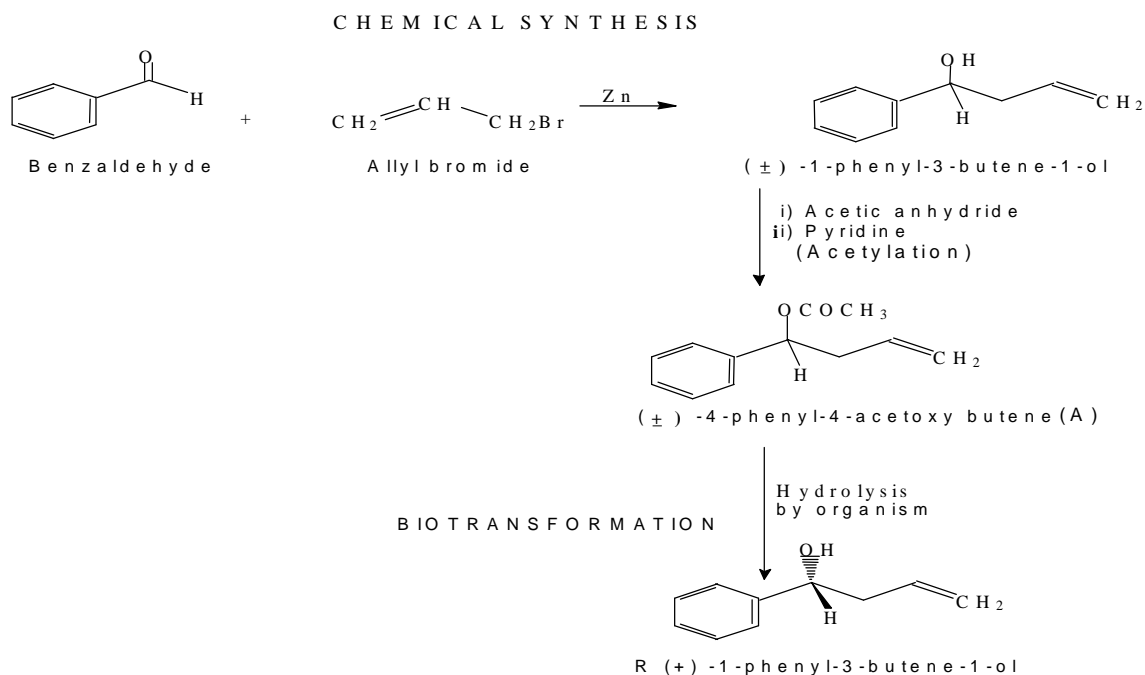


Figure 1 - Scheme for chemical synthesis and biotransformation of 4-phenyl-4-acetoxy butene

Table 1 - The enantiomeric excess and yield of 1-phenyl-3-butene-1-ol obtained by acetate hydrolysis and specific optical rotation and yield of unreacted substrate of biotransformation

Time of biotransformation	Enantiomeric excess* of R (+) 1-phenyl-3-butene-1-ol obtained by acetate hydrolysis by biotransformation	Specific optical rotation $[\alpha]_D^{27}$ in Benzene (0.6 c) and yield of unreacted substrate (4-acetoxy-4-phenyl-1-butene)
16 hrs	71.13 (47.2)	-57.61 (15.5)
24 hrs	67.27 (47.9)	-64.06 (13.1)
48 hrs	56.13 (49.1)	-65.24 (8.6)
72hrs	54.63 (43.4)	-65.52 (7.6)

* - Obtained by Chiral HPLC analysis. Figures in the paranthesis indicate yield/100 mg of substrate

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