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Microbial Transformation of Prochiral Ketones Using *Rhizopus arrhizus*

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The various species of *Rhizopus arrhizus* have been used for microbial transformations of *p*-chloro, *p*-methyl and *p*-methoxy propiophenone to synthesize corresponding enantiomerically pure (S) alcohols in 70-90 % ee. In a screening of various species of *Rhizopus arrhizus* for enantioselective reduction of propiophenone derivatives, *Rhizopus arrhizus* NCIM-878 showed high activity for reduction of above prochiral ketones and it efficiently hydrolyzes the racemic acetate of *p*-methoxy propiophenone to corresponding (R) alcohol.

Key Words: Biotransformations, Transformation, Prochiral ketones, *Rhizopus arrhizus*, Bioreduction, Carbinols.

INTRODUCTION

The past few years have witnessed significant development in the field of biocatalytic reduction of carbonyl compounds. Stereoselective reduction of prochiral ketones to the corresponding alcohols using biocatalysts have attracted much attention from the viewpoint of green chemistry. Due to simplicity, stereospecificity and high efficiency of these processes make them attractive alternative to existing methods in asymmetric catalysis for obtaining highly functionalized chiral alcohols in enantiomerically pure form. Among different approaches asymmetric reduction of prochiral ketones into chiral non-recemic secondary alcohols and stereoselective hydrolysis of recemic acetates are the main routes in the synthesis of homochiral carbinols. The alkyl aryl carbinols are versatile intermediates in the synthesis of agrochemicals, pharmaceuticals, fine chemicals, pheromones, flavours, fragrances and can also acts as chiral auxiliaries in asymmetric synthesis of chiral molecules[1]. There are various reports of using fungi as biotransformating agent[2-4] and *Rhizopus arrhizus* has the versatility for reduction of variety of carbonyl compounds and hydrolysis of acetates[5]. From the literature survey on microbial reduction of aryl alkyl ketones by *Rhizopus arrhizus*, it was observed that the enantioselectivity increases with increase in number of carbon atoms in alkyl chain and electronic effect of the substituents in the aromatic ring plays an important role in biotransformation reactions. Over the recent few years, many scientists have used the products of enantioselective bioreduction of propiophenone derivatives as starting material for the synthesis of wide variety of optically active compounds. This leads us to study the preparation

of enantiomerically pure alcohols using *Rhizopus arrhizus via* microbial reduction of propiophenone derivatives. Herein, we report enantioselective bioreduction of propiophenone and its analogues using various strains of *Rhizopus arrhizus* to obtain the corresponding (S)-aryl propanols and stereoselective hydrolysis of recemic acetate of *p*-methoxy propiophenone to corresponding (R)-aryl propanol with good yield and ee.

EXPERIMENTAL

Chemical synthesis of substrates: (1) The substrate propiophenone **2(a)** was obtained from Lancaster while *p*-chloro propiophenone, *p*-methyl propiophenone and *p*-methoxy propiophenone **2(b-d)** have been synthesized by using chlorobenzene, toluene and anisole with polyphosphoric acid (PPA) and propionic acid by using conventional as well as microwave methods[6,7]. (2) The corresponding recemic acetate **4(d)** was obtained by chemical reduction of **2(d)** using $NaBH_4/CH_3OH$ followed by acetylation using Ac_2O/pyridine. (3) (R)-(+) α-Methoxy-α-trifluro methyl phenyl acetic acid [(R)-MTPA] was the product of Lancaster. The metabolites were purified and characterized by IR, NMR.

Cultures and analytical methods: The various *Rhizopus* species, *R. arrhizus* NCIM 877, *R. arrhizus* NCIM 878, *R. arrhizus* NCIM 879, *R. arrhizus* NCIM 997, *R. arrhizus* NCIM 1009, were obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory Pune, India.

IR spectra were recorded on Perkin-Elmer FTIR spectrometer. The NMR spectra were recorded in $CDCl_3$ with TMS internal reference standard on varian-Gemini 200 NMR spectrometer. Optical rotations were measured on Jasco digital polarimeter.

Preparation of microbial culture: The modified Czapek Dox medium[8] was prepared by mixing $FeSO_4$ (10 mg), $MgSO_4$ (250 mg), KCl (0.5 g), KH_2PO_4 (0.95 g), $NaNO_3$ (2 g), yeast extract (0.5 g), cornsteep liquor (5 g) and glucose (8 g) in distilled water (1 L). After adjusting the pH to 4.5-4.8, the medium was sterilized, the fungus from PDA slants were incubated in the above medium (150 mL) and allowed to grow under static conditions at 25 ºC for 72 h.

Biotransformation studies: Various species of *Rhizopus arrhizus* were inoculated in autoclaved (15 psi for 20 min) 500 mL cotton plugged conical flask containing 150 mL of Czapak-dox medium. The substrates **2(a-d)** (100 mg each in 1 mL of 95 % ethanol) were added to 72 h grown culture and incubated on rotary shaker for different time intervals. The substrate control and organism control were run simultaneously.

Microbial reduction of 2(a-d): At the end of biotransformation, the mycelial mass was filtered from the culture medium. The filtrate was extracted with chloroform, washed with water and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue obtained was isolated and purified by preparative TLC. The mycelial mass was washed with acetone (3 × 50 mL) and acetone was removed. An oily residue obtained was then taken into water, extracted with ethyl acetate and dried over anhydrous Na_2SO_4 and evaporated to give an oily mycelial

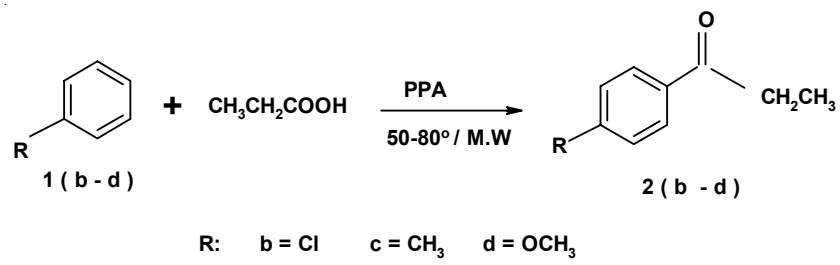
extract. Controlled experiments were also extracted in similar way. The transformed products were purified by preparative TLC (silica gel GF-254) and characterized by IR, ^{1}H NMR spectroscopy and optical rotations.

Enzymatic hydrolysis of recemic acetate of 4(d): The recemic acetate was incubated with *Rhizopus arrhizus* NCIM 878 at different time interval (24 to 96 h) and worked up as usual. The corresponding (R) alcohol and (S)-acetate was isolated from the filtrate and mycelia respectively and purified by preparative TLC and was characterized by spectral methods.

MTPA chloride and its corresponding esters of compounds **3(a-d)** were synthesized according to literature procedure[9].

RESULTS AND DISCUSSION

In recent years, polyphosphoric acid (PPA) has been widely used in synthetic chemistry for cyclization, dehydration, acylation, alkylation, *etc*. The derivatives of propiophenone like *p*-chloro, *p*-methyl and *p*-methoxy propiophenone **2(b-d)** [**Scheme-I**] which are used as important drug intermediates, have been synthesized with moderate to good yield by acylation using chlorobenzene, toluene and anisole with PPA and propionic acid by using conventional and microwave methods.

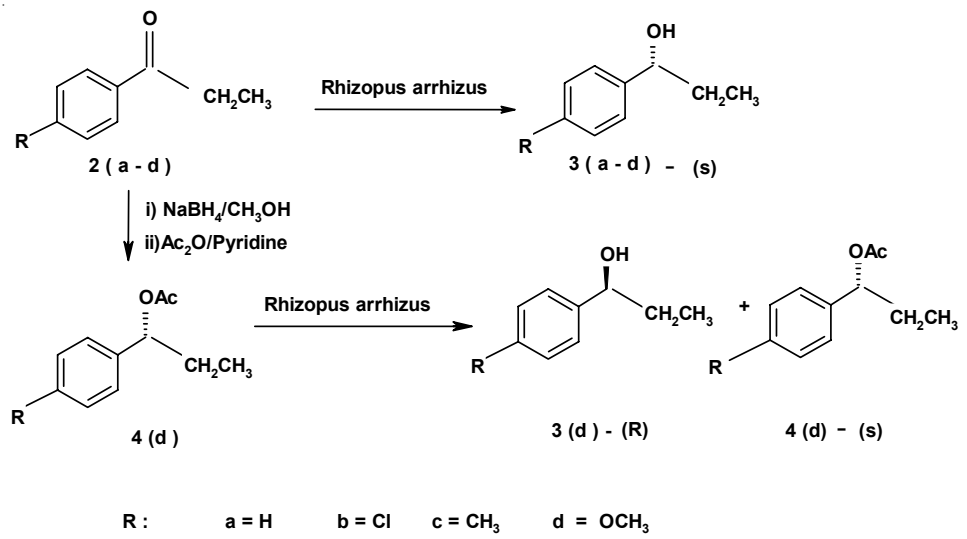


Scheme-I

The prepared prochiral ketones were then subjected to microbial transformation by various strains of *R. arrhizus* at different time intervals (**Scheme-II**).

Initially using (**2a**) as model substrate, microbe selection was optimized so as to get maximum yield and enantioselectivity. During screening, among the various *Rhizopus* species, *R. arrhizus* NCIM 877, *R. arrhizus* NCIM 878, *R. arrhizus* NCIM 879, *R. arrhizus* NCIM 997, *R. arrhizus* NCIM 1009, only two microorganisms, *R. arrhizus* NCIM 877 and *R. arrhizus* NCIM 879 (entries 1,3) gave 50-55 % conversion. Other strains *R. arrhizus* NCIM 878, *R. arrhizus* NCIM 997, *R. arrhizus* NCIM 1009 (entries 2, 4, 5) gave 70-80 % conversion while it was excellent with *Rhizopus arrhizus* NCIM 878. (Table-1).

For further optimization of the protocol in terms of conversion and optical purity, microbial transformation of propiophenone and its *p*-chloro, *p*-methyl and *p*-methoxy derivatives was carried out with three strains of *Rhizopus arrhizus*, *i.e.*,



Scheme-II

TABLE-1
SCREENING OF VARIOUS STRAINS OF *Rhizopus arrhizus* FOR MICROBIAL TRANSFORMATION OF PROPIOPHENONE (**2a**)

Entry	Organism	Conversion (%)
1	*R. arrhizus* NCIM 877	52
2	*R. arrhizus* NCIM 878	80
3	*R. arrhizus* NCIM 879	55
4	*R. arrhizus* NCIM 997	70
5	*R. arrhizus* NCIM 1009	74

R. arrhizus NCIM 878, *R. arrhizus* NCIM 997, *R. arrhizus* NCIM 1009 for the enantiomeric preparation of corresponding (S)-alcohols (Table-2). Among them, as *R. arrhizus* NCIM 878, gave best conversion and ee, was selected for further hydrolysis of recemic acetate of *p*-methoxy propiophenone to give corresponding (R) alcohol.

In general *R. arrhizus* 878 was the best microorganism furnishing the alcohols **3(a-d)** with quantitative enantioselectivity. The *para* substitution in the phenyl ring improved the enantioselectivity of the reduction as compared to unsubstituted substrate (**2a**) except (**2d**). Indeed the *p*-chloro propiophenone (**2b**) was reduced with high enantioselectivity possibly due to -I effect of the substituent. The minor role of inductive effect was also manifested in the reduction of *p*-methyl propiophenone (**2c**) which showed marked improved in ee. However poor results obtained with (**2d**) containing alkoxy group. In this case possibly the + M effect of the substituent outweights their -I effect leading to above result. This observation agrees with proposal that the hydride transfer from NADH or NADPH to the ketone carbonyl carbon is mediated by a dehydrogenase rather than by radical mechanism.

TABLE-2
BIOREDUCTION OF PROPIOPHENONE WITH SUBSTITUENTS IN THE BENZENE RING MEDIATED BY SELECTED STRAINS OF *Rhizopus arrhizus*

Substrate	Product	Organism	Yield (%)	% ee	Configuration
2a	**3a**	*R. arrhizus* NCIM 878	55	80	S
2a	**3a**	*R. arrhizus* NCIM 997	48	72	S
2a	**3a**	*R. arrhizus* NCIM 1009	50	75	S
2b	**3b**	*R. arrhizus* NCIM 878	75	88	S
2b	**3b**	*R. arrhizus* NCIM 997	65	80	S
2b	**3b**	*R. arrhizus* NCIM 1009	72	83	S
2c	**3c**	*R. arrhizus* NCIM 878	70	80	S
2c	**3c**	*R. arrhizus* NCIM 997	62	73	S
2c	**3c**	*R. arrhizus* NCIM 1009	67	70	S
2d	**3d**	*R. arrhizus* NCIM 878	60	45	S
2d	**3d**	*R. arrhizus* NCIM 997	48	35	S
2d	**3d**	*R. arrhizus* NCIM 1009	52	42	S

The strain *R. arrhizus* NCIM 1009 was also very effective with **2(a-d)** with 75-85 % ee except alkoxy substituent in phenyl ring. The enantioselectivity obtained with *R. arrhizus* NCIM 997 was comparatively poor.

The rate of microbial reduction were *R. arrhizus* NCIM 878 > *R. arrhizus* NCIM 1009 > *R. arrhizus* NCIM 997 although no correlation between conversion and % ee was obtained. However the hydrolytic rates were specially high with all the microorganisms containing *p*-alkoxy phenyl moieties, which may partly explain the lack of enantioselectivity. Also the given protocol can provide good enantioselectivity for propiophenone and its *p*-chloro and *p*-methyl analogues, only *p*-methoxy propiophenone appeared promising for the enantioselective preparation of (R)-aryl propanol and hence was chosen for further hydrolysis of its recemic acetate. We have carried out the hydrolysis of recemic acetate of (2d) to corresponding (R) alcohol with reasonable yield and good enantioselectivity using selected species of *R. arrhizus* NCIM 878. On the basis of ^{1}H NMR study of MTPA ester, the alcohol was assigned the (R) configuration[10]. The ^{1}H NMR data of the esters of the alcohols obtained from microbial reduction of **2(a-d)** showed a prominent signal for the (S) enantiomer and were assigned (S) configuration. All the transformed products gave satisfactory spectral analysis. Herein, the spectral data of corresponding optically active alcohols reported:

3a: Colourless liquid; IR: 3380 cm^{-1} ν(-OH), ^{1}H NMR: $CDCl_3$: δ 0.8 (t, *J* = 6.2 Hz, 3H, CH_2-CH_3, 1.6-1.7 (m, 2H, CH_2-CH_3), 2.23 (bs, 1H, D_2O exchangeable), 4.46 (t, *J* = 6 Hz 1H), 7.0-7.5 (m, 5 Ar- H).

3b: Colourless liquid, IR: 3416 cm^{-1} ν(-OH), ^{1}H NMR: $CDCl_3$: δ 0. 91 (t, *J* = 6 Hz, 3H, CH_2-CH_3), 1.7-1.8 (m, 2H, CH_2-CH_3), 2.36 (bs, 1H, D_2O exchangeable), 4.59 (t, *J* = 6 Hz, 1H), 7.2-7.5 (m, Ar-H).

3c: Colourless liquid, IR: 3392 cm^{-1} ν(-OH), ^{1}H NMR: $CDCl_3$: δ 0. 96 (t, *J* = 6 Hz, 3H, CH_2-CH_3), 1.6-1.7 (m, 2H, CH_2-CH_3), 2.39 (s, 3H, Ar-CH_3), 4.52 (t, *J* = 6 Hz) 1H) 4.85 (s, 1H), 7.2-7.5 (m, Ar-H).

3d: Colourless liquid, IR: 3392 cm^{-1} ν(-OH), ^1H NMR: $CDCl_3$: δ 0.8939 (t, *J* = 6 Hz, 3H, CH_2-CH_3), 1.70-1.79 (m, 2H, CH_2-CH_3), 3.80 (s, 3H, Ar- OCH_3), 4.50 (t, *J* = 6 Hz, 1H), 4.80 (s, 7.2-7.5 (m, Ar-H) 1H), 7.2-7.5 (m, Ar-H).

In conclusion, among various strains of *Rhizopus arrhizus*, biotransformation using *R. arrhizus* NCIM 878, provides an inexpensive, operationally simple method for asymmetric reduction of propiophenone and its analogues as well as asymmetric hydrolysis of recemic acetate of *p*-methoxy propiophenone. The application of this methodology in stereoselective reduction of prochiral ketones has shown wide substrate specificity, good yield and enantioselectivity in ecofriendly environment.

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REFERENCES

1. K. Faber, Biotransformation in Organic Chemistry, Springer-Verlag, Berlin (2000).
2. J.S. Yadav, G.S.K.K. Reddy, G. Sabitha, A.D. Krishna, A.R. Prasad, H.-U.-R. Rahaman, K.V. Rao and A.B. Rao, *Tetrahedron Asymm.*, **18**, 717 (2007).
3. P. Soni and U.C. Banerjee, *Biotechnol. J.*, **1**, 80 (2005).
4. B. Jarosz and A. Siewnski, *J. Basic Microbiol.*, **36**, 245 (2007).
5. J. Augusto, R. Rodrigues, P.J.S. Moran, G.J.A. Conceicao and L.C. Fardelone, *Food Technol. Biotechnol.*, **42**, 295 (2004).
6. V.B. Naber and N.A. Kudav, *Indian J. Chem.*, **14B**, 807 (1976).
7 D.A. Learmonth, *Synth. Commun.*, **32**, 2757 (2002).
8. B.R. Prema and P.K. Bhattacharya, *J. Appl. Microbiol.*, **10**, 524 (1962).
9. J.A. Dale and H.S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
10. S.M. Brown, S.G. Davies and J.A.A de Sousa, *Tetrahedron Asymm.*, **4**, 813 (1993).

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