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Regioselective Reduction of Nitro Ketones by *Rhizopus arrhizus*

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Biocatalytic regioselective reduction of 2-, 3-, 4-nitro acetophenone, 3-nitro-4-methyl acetophenone and 3-nitro propiophenone was accomplished using *Rhizopus arrhizus* NCIM 878 to yield corresponding (S) -nitro alcohols in good yield and ee (70-87 %). Here we report, *Rhizopus arrhizus* has a great potential to bring selective reduction of carbonyl group of nitro ketones. However, the biotransformation is very sensitive to the effect of substituent group along with the position of nitro group and steric effect.

Key Words: Biotransformation, *Rhizopus arrhizus*, Nitro ketones, Regioselective reduction.

INTRODUCTION

The growing interest in asymmetric synthesis has promoted great developments in biotransformation applied for the synthesis of chiral compounds in organic chemistry. Use of microorganisms *i.e.* whole cell system which is economical and operationally simple is emerging as a practical alternative in the area of biotechnology. The importance of chiral technology in the production of pharmaceutical is highly practical and clean method to access enantiomerically pure alcohols containing the nitro group. Nitro alcohols are important building blocks in academic organic synthesis as well as in pharmaceutical industry due to their antifungal activity[1]. In addition, this class of compounds is precursor of the corresponding amines by reduction[2,3]. Classical method for preparing nitroalkanols involved base catalyzed addition of nitro alkane to carbonyl compounds[4]. Recently, several improved procedures have developed for synthesis of chiral nitro alcohols. Chirality has been conveniently introduced into substrate containing this functionality through biocatalytic transformation or by chemical method employing chiral reagents[5-7]. However, from a synthetic standpoint, among various practical strategies currently available for assembling nitroalcohols, biocatalytic transformations using whole cells have been shown to be extremely stereoselective in the reduction of nitro ketones *viz.* fungus *Aspergilus niger*[8], Baker's yeast[9], or Liophilised cells of the bacterium *Comonas testosterone* DSM 1455[10].

The fungus *Rhizopus arrhizus* has been recently exploited for reduction of variety of carbonyl compounds[11]. As an extension of our work on bioreduction[12], it was interesting to evaluate the potential of *Rhizopus arrhizus* to bring about microbial reduction of nitro ketones. The importance of the organic nitro alcohols mentioned

above led us to extend this study to the bioreduction of acetophenone and 2-, 3-, 4-nitro acetophenone as well as 3-nitropropiophenone using the selected strain of fungus *Rhizopus arrhizus* NCIM 878 to yield corresponding (S)-nitro alcohols with good yield and enantiomeric excess (ee). In order to evaluate the effect of substituent group along with 3-position of nitro group, we have chosen 3-nitro-4-methyl acetophenone but this compound failed to undergo microbial reduction.

EXPERIMENTAL

Chemical synthesis of substrates: (i) The substrate acetophenone and its 2-, 3-, 4-nitro derivatives **1(a-d)** were obtained from Lanchester, (ii) The substrate 3-nitro propiophenone **1(e)** and 3-nitro-4-methyl acetophenone **1(f)** were synthesized by nitration of propiophenone and 4-methyl acetophenone, respectively. The products were purified and characterized by physical and spectral analysis.

Culture and analytical methods: The fungus *Rhizopus arrhizus* NCIM 878 was 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.

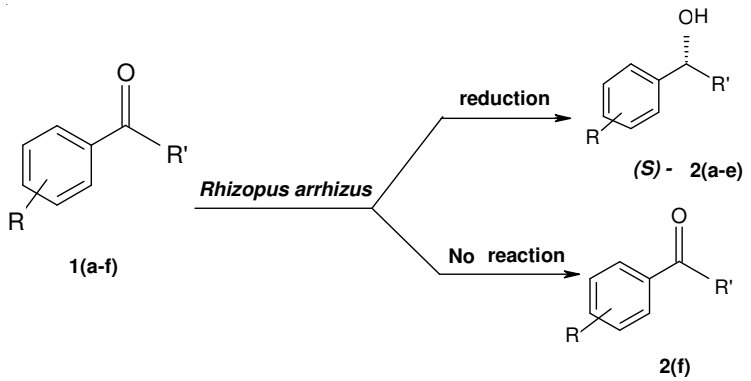
Preparation of microbial culture: The *Rhizopus arrhizus* NCIM 878 from PDA slants were inoculated in sterilized Czapak dox medium[13] (150mL) and allowed to grow under static conditions at 25 ºC for 72 h.

General procedure for biotransformation: The substrates **1(a-f)** (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 and organism control were also run simultaneously. 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, ^1H NMR spectroscopy and optical rotations.

RESULTS AND DISCUSSION

Initially, using acetophenone **1(a)** as a model substrate, various reaction conditions were optimized so as to get maximum yield and enantioselectivity. In order to optimize the time for the microbial reduction, experiments were carried out using *R. arrhizus* NCIM 878 for varying incubation periods (24 h-14 d). The time course of bioreduction revealed that the best results were found for 7 d. Therefore the

substrates **1(a-f)** were incubated with *Rhizopus arrhizus* for the same time interval (**Scheme-I**). The incubation process was terminated after the optimized time interval. The mycelial mass was separated by filtration and the filtrate was extracted as discussed in experimental procedure. The metabolites obtained were purified by preparative TLC and characterized by IR, NMR and optical rotations. The results of the study are summarized in Table-1.



Scheme-I

TABLE-1
BIOCATALYTIC REDUCTION OF NITROKETONES MEDIATED BY *Rhizopus arrhizus*

Subs.	Product	R	R'	ee (%)	Yield (%)	Confi.
1a	**2a**	H	CH_3	70	35	(S)
1b	**2b**	2-Nitro	CH_3	75	60	(S)
1c	**2c**	3-Nitro	CH_3	82	75	(S)
1d	**2d**	4-Nitro	CH_3	77	75	(S)
1e	**2e**	3-Nitro	C_2H_5	87	80	(S)
1f	**2f**	3-Nitro-4-methyl	CH_3	–	–	–

In view of this results, we report here, the *R. arrhizus* NCIM 878 is a versatile biocatalyst and has a great potential for selective reduction of carbonyl group in presence of nitro group. Among acetophenone and 2-, 3-, 4-nitro acetophenone the best results for the bioreduction were obtained when *R. arrhizus* was used with 3- nitro acetophenone **1(c)** to yield (S)-3-nitro phenyl ethanol with 75 % conversion and 82 % ee. In order to explore the comparative effect of presence of CH_3 group on side chain and on aromatic ring, we have extended this methodology purposely for bioreduction of 3-nitro propiophenone **1(e)** and 3-nitro-4-methyl acetophenone **1(f)**. It was found that 3-nitro propiophenone **1(e)** gave (S)-1-(3-nitro phenyl) propan-1-ol in 87 % ee while 3-nitro-4-methyl acetophenone **1(f)** failed to undergo microbial reduction. When we compare the results obtained for bioreduction of 3-nitro aceto-phenone **1(c)** and 3-nitro propiophenone **1(e)**, it was found that, increase in length of alkyl chain led to increase in enantioselectivity giving the product alcohol in 87 % ee.

However, in the second comparison for the results obtained in bioreduction of 3-nitro acetophenone **1(c)** and 3-nitro-4-methyl acetophenone **1(f)**, 3-nitro acetophenone gave best results for bioreduction but 3-nitro-4-methyl acetophenone **1(f)** doesn't undergo microbial reduction. This might be attributed to +I effect of methyl group present on phenyl ring and thus inductive effect of substituent groups on phenyl ring may affect the activity of the molecule itself.

This explanation is supported by its spectral data. IR data of **2(a-e)** showed a broad hydroxy band at 3400-3450 cm^{-1} suggesting the reduction of carbonyl group which was absent in IR spectrum of **2(f)**. Also, the metabolites **2(a-f)** were tested for 2,4-DNP. It was observed that, the metabolites **2(a-e)** gave a negative test to 2,4-DNP but **2(f)** gave positive test for 2,4-DNP indicating a dark orange coloured spot of keto group. This clearly indicates that the bioreduction of 3-nitro-4-methyl-acetophenone is not possible by *Rhizopus arrhizus*.

In conclusion, the study demonstrated the applicability of the simple microbial whole cell system of *R. arrhizus* NCIM 878 for the biotransformation of structurally different nitro ketones. The reactivity and the enantioselectivity were governed mainly by the steric factors of the groups flanking the reaction site and + I effect of the substituent group.

Herein, the spectral data of the selected optically active alcohols (**2c,2e**) reported:

2c) 1-(3-Nitro-phenyl)-ethanol: $[\alpha]_D^{25}$- 26.5 (c 0. 65 $CHCl_3$), IR (cm^{-1}): 3368 (-OH), 1519, 1089, 855, 699; ^{1}H NMR (δ): 1.5 (d, 3H, *J* = 6.4 Hz-CH_3), 1.87-1.99 (bs,1H, -OH, D_2O exchangeable), 5.05 (q, 1H) 7.55 (d, 2H), 8.2 (d, 2H); Elemental analysis ($C_8H_9O_3N$) Calcd.: C, 57.48; H, 5.38;N, 8.38; Found: C, 57.40;H, 5.30; N, 8.35 %.

2e) 1-(3-Nitro-phenyl)-propan-1-ol: $[\alpha]_D^{25}$- 15.2 (c O.82 $CHCl_3$), IR (ν_{max}, cm^{-1}): 3436 (-OH), 1556, 1423, 934, 708; ^{1}H NMR (δ): 0.87 (t, 3H, -CH_3), 1.6 (m, 2H, -CH_2), 5.1 (bs, 1H, -OH, D_2O exchangeable), 4.2 (t,1H, -CH), 7.2-7.5 (m, Ar-H); Elemental analysis ($C_9H_{11}NO_3$) Calcd. C, 54.66; H, 6.07; N, 7.73; Found: C, 54.50; H, 6.0; N, 7.55 %.

2f) 3-Nitro-4-methyl-acetophenone: IR (ν_{max}, cm^{-1}): 1692 (conj. C=O),1190, 760 cm^{-1}; ^{1}H NMR (δ): 2.65 (s, 3H -CH_3), 2.68 (s, Ar-CH_3), 7.5-8.5 (m, Ar-H); Elemental analysis ($C_9H_9O_3N$) Calcd: C, 60.33; H, 5.02; N, 7.82, Found: C, 60.30, H, 5.0, N, 7.80 %.

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