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Preparation of optically active amines from oximes: Yeast catalyzed selective reduction

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ABSTRACT

A variety of yeast cultures from NCIM (National Collection of Industrial Microorganisms), NCL, Pune, India were screened for oxime reductases activity using 4-methyl-2-pentanone oxime as model substrate. Yeast cells were grown on MGYP medium separated from the broth and used as biocatalyst. The selectivity of the yeast in the presence of other reducible groups was also studied; thus diacetyl monoxime, which had a reducible keto group, demonstrated that the reaction was chemoselective and dimethyl glyoxime, having two oxime groups, though toxic for most yeast showed the reaction was regioselective. The reaction on acetophenone oxime and benzoin oxime exhibited that in general aromatic groups tended to facilitate the reduction reaction. A sample product analysis for each substrate indicated the product was optically active.

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KEYWORDS

Oxime;
Biocatalysis;
Optical rotation;
Yeast;
Reduction.

INTRODUCTION

Chiral amines are important compounds in organic synthesis, as they form key intermediates in drugs and agrochemicals. New methods of synthesis of enantiomerically pure amines are a constant challenge. Commonly used biochemical method for preparing enantiopure amines involves resolution of racemic amides by hydrolysis, transesterifiction reactions by lipases[1-3]. A number of inorganic reagents have been used for chemical reduction of oximes to their corresponding amines[4-6]. Traditionally these reactions are carried out by hydrogenation using Pd/C catalysts[7]. Chemical methods using inorganic reagents generally involve hazardous conditions of high temperatures, pressures etc. and are not selective, other functional groups susceptible to reduction such as ketones and double bonds are reduced. Abiraj and Gowda (2003) reported a Zinc/ammonium formate system for selective reduction of oxime to amine at reflux temperatures[8]. Biocatalytic routes are steadily being preferred due to the mild reaction conditions and being environmentally safe, region, chemo and enantioselective.

Bakers' yeasts have been widely used for the reduction of carbonyl compounds for preparation of important chiral alcohols[9,10]. Reports on reduction of compounds with N-O bonds such as nitro alkenes, nitroarenes[11], nitrosoarenes[12] and N-oxides[13] are

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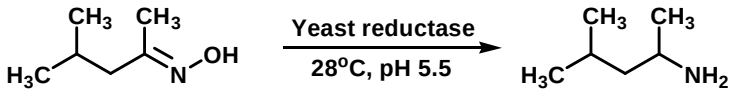


4-methyl-2-pentanone oxime (+/-)-1,3-dimethylbutylamine

Figure 1 : Bioreduction of 4-methyl-2-pentanone oxime to 1, 3-dimethylbutylamine

also to be found in literature. Reduction of prochiral oximes to enantiomerically pure amines is often an important reaction step in multistep synthesis of drugs, agrochemicals and chiral auxiliaries, where carbonyl derivatives are transformed to amines via oximes. Except for report on Bakers' yeast by Gibbs & Barnes[14], this area of research has remained vastly unexplored[14]. The aim of this project was to screen cultures from different yeast genus for asymmetric reduction of oxime to amine. A compound often contains other functional groups that could interfere with selectivity of the reactive group; hence besides the enantioselectivity of the catalyst, we investigated the chemoselective and regioselective nature of the reaction, the effect of aromatic and electron withdrawing substituents.

EXPERIMENTAL

Cultures

Forty-eight different yeast cultures were obtained from NCIM, NCL, Pune, India, a few representatives from different yeast families were chosen for their reductive potential. Baker's yeast was purchased from AB Mauri India Pvt. Ltd.

Chemicals

4-methyl-2-pentanone oxime and acetophenone oxime was prepared chemically, by condesation of the ketone with hydroxylamine in sodium hydroxide[15a]. The other chemicals were obtained as follows- benzoin oxime -Loba Chemie, Mumbai, diacetyl monoxime - Thomas baker, dimethyl glyoxime - Merck India and standard 1, 3-dimethyl butylamine-Acros Organics, Belgium. Standard amines of benzoin oxime and dimethyl glyoxime were prepared chemically in the laboratory[5].

Biocatalyst

The cells were grown in medium composition g L^{-1} of malt extract 3g, glucose 10g, yeast extract 3g, peptone 5g at 220 rpm and 28°C for 24 h. The cells were separated from fermentation broth by centrifugation washed with potassium phosphate (0.1mM, pH 5.5) buffer. The whole cell preparation was used as biocatalyst in reduction reaction.

Bioreduction

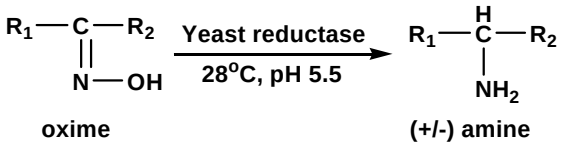
In a typical bioreduction reaction, 5mL phosphate buffer pH 5.5, 1g wet weight cells were mixed and 500mg glucose, 0.1g L^{-1} $MgSO_4$, 35mM substrate was added (acetophenone oxime and benzoin oxime which were insoluble in water were dissolved in minimal ethanol before addition to reaction mixture); a blank (without substrate) was also prepared. The reaction mixture was incubated at 220 rpm and 28°C for 24 h. Initially forty-eight yeast cultures were screened for bioreduction of a simple oxime i.e. 4-methyl-2-pentanone oxime. Selected cultures were used further for bioreduction of other oximes having varied substituents (Figure 1). A larger scale reaction at 100mL scale carried out using Bakers' yeast, for each oxime; the product was used for recording optical rotation and IR spectra.

Product isolation

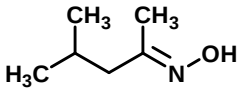
To separate the unreacted oxime from the amine, the reaction mixture was acidified to pH 2 with 3N HCL and the cells centrifuged off. The unreacted oxime was extracted twice with equal volume of ethyl acetate, dried with Na_2SO_4 and solvent removed on rota vapor. The amine salt in aqueous part was precipitated by saturation with NaCl and MeOH and keeping in the cold overnight. The precipitate was collected by repeated washing with MeOH. The optical rotations of these salts were determined. For IR spectra amine was made free, by basifying with 3N NaOH and extracting in hexane.

Analysis

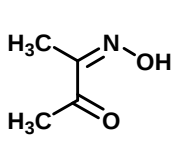
Initial screening of culture was done using 4-methyl-2-pentanone oxime, as substrate and the reaction monitored by TLC, solvent system pet. ether - ethyl acetate (8:2). Assay of product was done by Spectrophotometric method[16]. Optical rotations $[\alpha]_D$ of all amines in its hydrochloride salt form were taken on



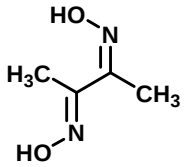
General reaction of oxime reduction to amine



4-methyl-2-pentanone oxime (MPO)

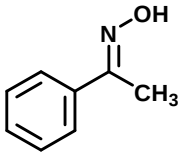


Diacetyl monoxime (DAMO)

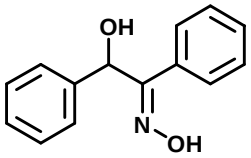


Dimethyl glyoxime (DMG)

Aliphatic oximes



Acetophenone oxime (APO)



Benzoin oxime (BNO)

Aromatic oximes

Figure 2 : Bioreduction of oximes by yeast

JASCO P1020 polarimeter. IR spectra of the products (discussed in results and discussion section) were carried out on Perkin Elmer Spectrum One, FT-IR spectrometer.

RESULTS AND DISCUSSION

The reduction of prochiral oxime to chiral amine are carried out under mild conditions with fermenting yeasts, as they are dependent on nicotinamide cofactors (e.g. NADPH). Leeds & Kirst (1988) have reported a mild single step reduction of oxime to amine using $TiCl_3$ as catalyst and $NaBH_3CN$ as hydride source[17]. However, the method besides not being enantioselective is applicable only for substrates wherein the intermediate imine is stable towards hydrolysis to corresponding ketones. No reversible reaction of oxime to ketone was observed when yeast was the catalyst. Chemoselective reduction of oxime to amine by Zinc/ammonium formate system has also been investigated[8]

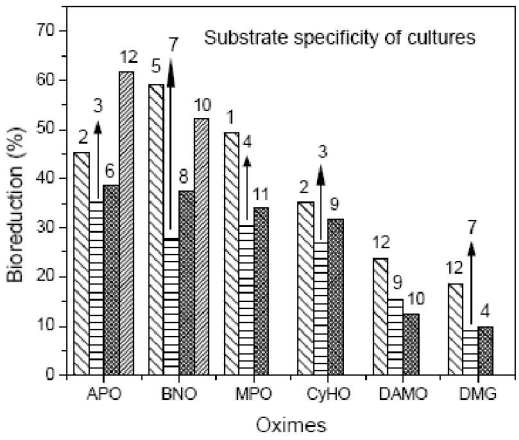


Figure 3 : Substrate specificity profile of different cultures: Column heading indicates. Entry number of cultures as in TABLE 2, entry no. 2, 3, 6, &12; BNO: 5, 7, 8, 10; MPO: 1, 4, 11; CyHO: 2, 3, 9; DAMO: 12, 9, 10; DMG: 12, 7, 4

but the product formed is not optically active.

A literature survey indicated a single report on asymmetric reduction of oximes by a biocatalyst, Bakers' yeast[14] for reducing 2-butanone oxime and its derivatives to its corresponding amine. The investigators restricted their study to simple oximes, used high concentration 7.5g of catalyst for conversion of 3mM substrate, and report a chemical yield 17 %. In our experiments, we have used just 1g wet yeast cells in 5mL phosphate buffer and substrate concentration of 35mM and achieved considerably better conversions in most cases. Compounds having multiple functional groups can often jeopardize the selectivity of catalysts; we therefore applied the reaction to oximes having varying substituents (Figure 2). Thus, diacetyl monoxime and dimethyl glyoxime were used to study chemoselective and regioselective properties of the catalyst respectively. While acetophenone oxime and benzoin oxime were studied for effect of aromatic substituents on the reaction.

Screening of yeast genus

Forty-eight yeasts of different species were screened for reductase activity (TABLE 1) on the reaction of 4-methyl-2-pentanone oxime to 1,3-dimethyl butyl amine (Figure 1), as it was simple aliphatic compound. Eleven of these yeast cultures including Bakers' yeast gave positive results and these were used study other oximes.

Candida genus is prominent yeast which has with a wide variety of cultures, thus a maximum of twenty

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TABLE 1 : Screening of yeast genus for reduction 4-methyl-2-pentanone oximes to 1, 3-dimethylbutylamine

Entry No.	Cultures	No. of Yeast screened	Oxime reducing cultures
1	*Arthroascus javanensis*	1	+1
2	*Candida sp.*	20	+5
3	*Klyuveromyces marxianus*	2	+1
4	*Metchnikowia sp.*	3	-
5	*Pachyosolan tannophilus*	2	+1
6	*Pichia sp.*	3	+1
7	*Rhodotorula sp.*	3	-
8	*Saccharomyces cerevisiae*	9	+2
9	*Saccharomyces fibulogera*	1	-
10	*Schizosaccharomyces pombe*	1	-
11	*Sporobolomyces salmonicola*	1	-
12	*Yerrowia lipolytica*	1	-
13	*Zygosachharomyces rouxii*	1	-
14	Baker's yeast		+
	Total	48	11

yeasts (Entry 2) from this family were selected; of which only five cultures namely *C. apicola, C. bombi, C. brumptii, C. catenula* and *C. lambica* showed oxime reductase activity. *Saccharomyces* (Entry 8), the first yeast genus to be identified, is routinely used in chemical and food industry but of the nine strains studied only two along with Bakers yeast gave positive reaction.

Some of yeasts, having lesser variety of strains, which successfully catalyzed these reactions were, *Arthroascus javanensis, Klyuveromyces marxianus, Pachyosolan tannophilus and Pichia farinosa,* [Entries 1, 3, 5, 6].

Application of selected yeast to different oximes

The eleven yeast cultures, which reduced 4-methyl-2-pentanone oxime to 1, 3- dimethyl butylamine (Figure 1), were tested for reduction of oxime having different substituents as stated earlier. Figure 2 depicts the oximes studied and the results obtained by each of the selected yeast are summarized in Table 2. The percentage conversion of oxime to amine, were calculated from the standard graph of amines as described in earlier work[16]. The biotransformation for all oximes was below 50 % this could be attributed to selective reaction of only one isomer of the E-Z mixture of the oxime. The reaction with Bakers' yeast showed asymmetric reduction towards the (R) enantiomer in all cases

TABLE 2 : % Conversion different oximes to corresponding amines by selected yeast

Entry no.	Cultures	NCIM no.	Aliphatic oximes			Aromatic oximes	
			MPO	DAM	DMG	APO	BNO
1	*A. javanensis*	3435	49.30	7.72	-	32.59	28.41
2	*C. apicola*	3367	25.34	10.64	-	45.26	44.60
3	*C. bombi*	3531	13.24	11.54	-	35.20	26.81
4	*C. brumptii*	3402	30.44	4.06	9.85	4.19	7.79
5	*C. catenula*	3337	48.55	-	-	34.16	59.04
6	*C. lambica*	3532	5.06	-	-	38.65	33.20
7	*K. marxianus*	3232	10.98	-	9.10	22.65	27.63
8	*P. farinose*	3461	30.02	8.67	-	20.09	37.43
9	*P. tannophilus*	3445	6.97	15.40	-	5.80	15.88
10	*S. cerevisiae*	3494	22.52	12.42	-	46.86	52.12
11	*S. cerevisiae*	3570	33.96	6.98	-	22.08	21.44
12	*Baker's yeast*		33.44	23.71	18.62	61.73	18.19

(TABLE 3).

Aliphatic oximes

4-methyl-2-pentanone oxime

This compound had only oxime as functional group; it was therefore used for screening yeast cultures. The product 1, 3-methylbutylamine was tested at first by Riminis' test for primary amine[15b]. The characterization was confirmed by comparing the IR spectra of substrate and product (NIST Chem. WebBook). In the product, broad OH stretching band disappears and minor $-NH_2-$ was observed, bands at 1378, 1464 and 1652 cm^{-1} were as per the standard spectrum. The $[\alpha]_D$ of the compound was determined to be (+) 5.41 in 0.5 % MeOH (TABLE 3, entry 1). The reported $[\alpha]_D$ for the neat S- enantiomer being (-) 11.2[18].

Diacetyl monoxime

Though the compound had another reducible C = O group, the C = N-OH group was selectively reduced to the corresponding amine. Kreuz et al.[10] report enantioselective bioreduction of (E)-1-phenyl-1,2-alkanedione 2-(o-methyloxime), where the keto group was reduced in preference to oxime group[10]. Our conclusion was corroborated by the IR spectrums of the substrate and product. The expected stretching bands[15c] keto C = O (1725-1705cm^{-1}) and the oxime C = N (1660-1590cm^{-1}) in the substrate merged to show a broad band at 1648 cm^{-1}. In the product, 2-amino-3-butanone amine only a single sharp band at

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TABLE 3 : Optical rotation of amines obtained from Bakers' yeast catalyzed reactions

No.	Reaction: Oxime → Amine.HCl	Observed $[\alpha]^{25}_D$ % in MeOH		Reported $[\alpha]_D$ Conc.		Ref.
1	4-methyl-2-pentanoneoxime→1,3-dimethylbutylamine	+5.41°	0.5	-11.2°	neat	[18]
2	Diacetyl monoxime → 2- amino-3-butanone	+4.19°	0.5	*N.F	-	-
3	Dimethyl glyoxime → 2-amino-3-butanone oxime	+5.60°	0.3	*N.F	-	-
4	Acetophenone oxime → R (+) methyl benzyl- amine	+10.19°	0.9	+38°	neat	[19]
5	Benzoin oxime → 2 amino-1,2-dipheny- ethanol	+7.17°	0.7	+7.7°	0.6% EtOH	[20]

N.F.: not found

1735cm^{-1} due to keto group was observed. However the electron withdrawing effect of the C = O group apparently has detrimental effect on the reductase activity (TABLE 2). The key factor being that the reaction is chemoselective in nature. The $[\alpha]_D$ of the product was determined to be (+)5.41 in 0.5% MeOH (TABLE 3, entry 2).

Dimethyl glyoxime

The compound had two oxime groups; it was therefore selected to study the regioselectivity of the catalyst; only one oxime group was reduced. This was again concluded from the IR spectra of the substrate and product. The two C = N stretching bands due to the two oxime in the substrate, are not observed at all (SDBS –NO = 495), this could be attributed to some hindrance of the groups; but in the product the presence of a single C = N stretching band at 1648cm^{-1} is clearly visible. The compound was toxic for all cultures, only low activities were observed for *Candida brumptii, Kluyveromyces marxianus,* and Bakers' yeast (TABLE 2, entries 4, 7 & 12). The $[\alpha]_D$ was determined to be +5.60 in 0.3% MeOH (TABLE 3, entry 4). The importance of the study was reaction is regio and enantio selective in nature. Efforts to increase yield are ongoing.

Aromatic oximes

Acetophenone oxime

The aromatic ring of the compound seems to facilitate the reduction as the yields were enhanced by almost all yeast (TABLE 2). Gibbs et al.[14] in their experiments did not succeed in the reduction of acetophenone oxime and its derivatives by Bakers' yeast; they attributed this to the insolubility of the substrate[14]. We dissolved the compound in minimal amount of ethanol and added it to the reaction mixture; all the selected yeasts achieved positive results. The $[\alpha]_D$ was (+) 10.19 in 0.9 % MeOH (TABLE 3, entry 4) the rotation for neat R- (+) methyl benzyl- amine is (+) 38[19].

Benzoin oxime

All the selected yeasts were able to reduce the compound to its amine. The product, 2-amino-1, 2-diphenyethanol was optically pure, the $[\alpha]_D$ = (+) 7.17 (TABLE 3, entry 5) was comparable to the one in literature[20]. The effect of different substituents on reaction is depicted in figure 3, profiles of yeasts showing best conversion for the substrates examined. Substrates having phenyl group acetophenone oxime and benzoin oxime in the molecule enhanced the reduction reaction, whilst electron-withdrawing substituents had detrimental effect on the reaction, as compared to the simple aliphatic oxime 4-methyl-2-pentanone oxime.

ABBREVIATIONS

APO- acetophenone oxime
BNO-benzoin oxime
CyHO –cyclohexanone oxime
DAM-diacetylmonooxime
DMG-dimethylglyoxime
MPO- 4-methyl -2-pentanone oxime
NIST-National Institute of Standard Technology
SDBS- Standard Data Base System

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