

Bio-transformation of Prochiral Ketones to pharmaceutically used Chiral Alcohols by Baker's Yeast

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ABSTRACT: Novel biotransformation of prochiral ketones to bring about stereo selective synthesis of chiral alcohols by means of reduction of 2-heptanone, 4-chloro acetophenone, using Baker's Yeast (*Saccharomyces cerevisiae*) as the source of plant enzyme. After optimization of the reaction conditions, products will be synthesized, isolated and purified by chromatographic techniques and characterized on the basis of spectral analysis viz. IR spectral data.

KEYWORDS: Biotransformation, prochiral ketones, chiral alcohols, Baker's Yeast.

I. INTRODUCTION

Biotransformation (1) is the process whereby a substance is changed from one chemical to another (transformed) by a chemical reaction within the body. Chemical reactions catalyzed by biocatalysts which are complete cells like microorganisms or by enzyme preparations isolated from living organisms biomass are termed biotransformation. An organic compound is modified by simple chemically defined reactions, catalyzed by enzymes present in complete cell or isolated cells, into a product that is recoverable.

Some chiral alcohols are useful starting materials in the synthesis of various pharmaceuticals and chemicals. These can be synthesized by the reduction of their respective ketones using biotransformation processes (bioreduction). Various chemical methods like reduction using NaBH_4 , LiAlH_4 , etc. are available for the same, but factors like cost and environmental toxicity limits its use. Thus, biotransformation is used as a cheap and eco-friendly means of reduction.

Demand for enantiopure chiral compounds continues to rise, primarily for use in pharmaceuticals but also in three other sectors: flavor and aroma chemicals, agricultural chemicals and specialty materials. Biotransformation has a number of advantages when compared to the corresponding chemical methods. Biocatalysts are known to possess some interesting and advantageous features i.e. high efficiency, mild environmental friendly operation conditions, versatility and last but not least have high selectivity (chemo, regio, and stereoselectivity). The selectivity and particularly the stereochemical preference are observed when biocatalyst act on their substrates. Besides this economically, some biotransformation can be cheaper and more direct than their chemical analogues and the conversion normally proceeds under conditions that are regarded as ecologically acceptable. Because of these reasons the use of enzymes for biotransformation of man-made organic compounds has been used for more than hundred years where in whole cells, organelles or isolated enzymes were employed.

II. RELATED WORK

The Baker's Yeast (2-5) is a common micro-organism that can be used for reduction of carbonyl compounds since it is economical and more easily available. When whole microbial cells, such as baker's yeast, are used as the catalyst for the asymmetric reduction of carbonyl compounds, two enzyme systems are mainly involved in the production reaction. One is the enzyme catalyzing the asymmetric reduction of prochiral carbonyl compounds to chiral alcohols, i.e.

carbonyl reductases (6-8). The other is a cofactor regeneration system, which supplies NADH or NADPH through the oxidation of the energy source, such as carbohydrates and alcohols. The use of immobilized Baker's yeast (ImBY) as biocatalyst has several advantages such as removal of the biocatalyst from the reaction mixture is easy and its repeated use is also possible.

III. EXPERIMENTAL

In a one litre round bottom flask, equipped with a magnetic stirrer (UTS 2MLH make) 200 ml water, 5 gm fresh BY and 4gm glucose were placed and the suspension for 30 minutes. The dicarbonyl compound was separately dissolved in minimum quantity of absolute alcohol and the ethanolic solution was poured gradually into Baker's Yeast suspension. The resulting solution was magnetically stirred for suitable period. The suspension changes its colour from orange to yellow during the course of reaction.(9-11)

After the completion of the reaction the resulting mixture was filtered. The water was removed from the solution by distillation. The residue was then extracted repeatedly with diethyl ether. The ether layer was allowed to evaporate. After evaporation product was isolated, purified and characterized by combined application chromatographic techniques and spectroscopy.

IV. RESULTS AND DISCUSSION

The actual reducing agent in this system is NADH (Nicotinamide Adenine Dinucleotide Hydride). NADH donates hydride (H-) to aldehydes and ketones and thereby reduces them. The electron lone pair on nitrogen atom of NADH pushes out the hydride ion that is added to a carbonyl group of another molecule to cause its reduction. The process is completed by addition of proton to the carbonyl oxygen. The scheme of the reduction is depicted in the following Fig.

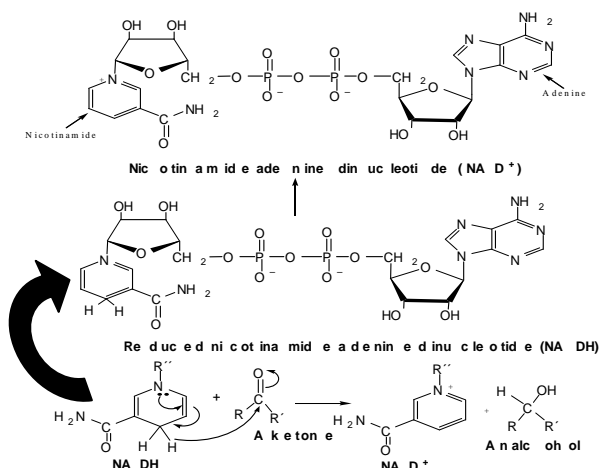


Fig. Depicting biological pathway for reduction of carbonyl group by NADH

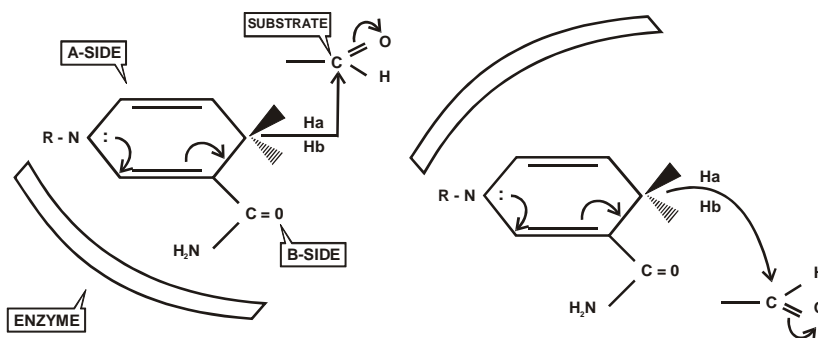
The amount of NADH in the yeast cell is limited to a very low level. In order to allow the reduction continuously, it is therefore, necessary to activate another biological pathway to reduce NAD⁺ in to NADH. Yeast contains some saccharides in the cell, which reduces, NAD⁺ to NADH via pentose phosphate pathway. The addition of glucose to reaction mixture also activates the pentose-phosphate pathway simultaneously feeding the yeast cells while results in an increased concentration of NADH and this ultimately ensure an increase in the enantiomeric excess of the product.

Mechanism: In an enzyme-catalysed addition to a carbonyl compound, only one of the enantiomers is formed. The enzyme can block one face of the carbonyl compound so that it can not be attacked, or it can position the nucleophile so that it is able to attack the carbonyl group from only one side of the molecule.

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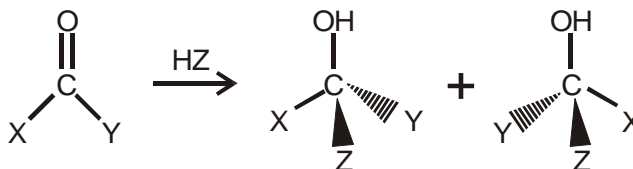


The enzyme blocks the B-side of the coenzyme, So the substrate binds to the A- side.

The enzyme blocks the A-side of the coenzyme, So the substrate binds to the B- side.

An enzyme has a specific binding site for the coenzyme, and when it binds the coenzyme, the enzyme blocks one of the coenzymes sides. If the enzyme blocks the B-side of NADH, the substrate will bind to the A-side and the Ha hydride ion will be transferred to the substrate, If the enzyme blocks the A-side of the coenzyme, the substrate will have to bind to the B-side and the Hb hydride ion will be transferred currenty, 156 dehydrogenases are known to transfer Ha, and 21 are known to transfer Hb.

But in chemical method their is no blockage and they forms equal amounts of the two enantiomers.



A Pair of enantiomers

The characterization of products was carried by IR spectra which were recorded on Bruker ATR Spectrophotometer.

The results of above reductions are summarized in Table 1.

Table: 1 Characterization table for reduction of prochiral carbonyl compounds.

Name of substrate	IR data (cm ⁻¹)	Product Identified
2-Heptanone	2960,2870 (methyl stretching) 2925 ,2855 (methylene stretching) 1200-1030 (C-O stretching) 3500-3000 (-OH group stretching)	2-hydroxy heptane

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4-Chloro Acetophenone	2960,2870 (methyl stretching) 3099-3069 (Ar-H stretching) 3070 (C-H stretching) 1200-1030 (C-O stretching) 3500-3000 (-OH group stretching)	1-(4-chloro phenyl) ethanol
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V. CONCLUSION

Result outcome of the present work is an extremely efficient, easy and cost effective method to synthesize commercially valuable products of biological interest by eco-friendly means.

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