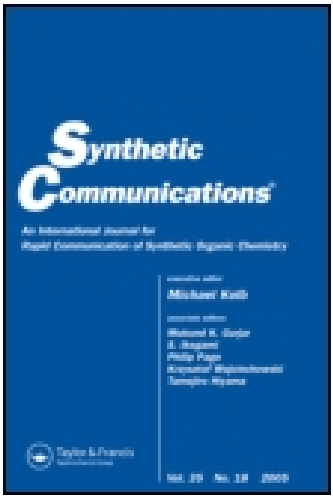

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

β-Ketoester Reduction by Baker's Yeast Immobilized in Calcium Alginate: An Examination of pH Effects on Enantiospecificity

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Published online: 24 Sep 2006.

To cite this article: U. T. Bhalerao , Y. Chandraprakash , R. L. Babu & N. W. Fadnavis (1993) β-Ketoester Reduction by Baker's Yeast Immobilized in Calcium Alginate: An Examination of pH Effects on Enantiospecificity, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 23:9, 1201-1208, DOI: 10.1080/00397919308011204

To link to this article: http://dx.doi.org/10.1080/00397919308011204

SYNTHETIC COMMUNICATIONS, 23(9), 1201-1208 (1993)

β-KETOESTER REDUCTION BY BAKER'S YEAST IMMOBILIZED IN CALCIUM ALGINATE: AN EXAMINATION OF pH EFFECTS ON ENANTIOSPECIFICITY

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Abstract: Enantiospecificity of the reduction process and product yields in the reduction of ethyl acetoacetate and ethyl benzoylacetate by baker's yeast immobilized in calcium aliginate beads depend strongly on the pH of the medium, and under optimum conditions products with high yields (80-85%) and high optical purity (e.e. 90-99%) can be obtained.

Enantiospecific reduction of β-ketoesters by fermenting baker's yeast using glucose as energy source in tap water is now a standard methodology for the synthesis of chiral β-hydroxy acid esters[1]. When the process is not completely enantioselective, modification of the experimental conditions are made[2]. For example, use of organic media[3] or an additive[4], change of energy source[5], immobilization technique[6] etc., have been found to influence the stereochemistry and the optical purity of the product. Baker's yeast possesses several alcohol dehydro-

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IICT Communication No. 3131

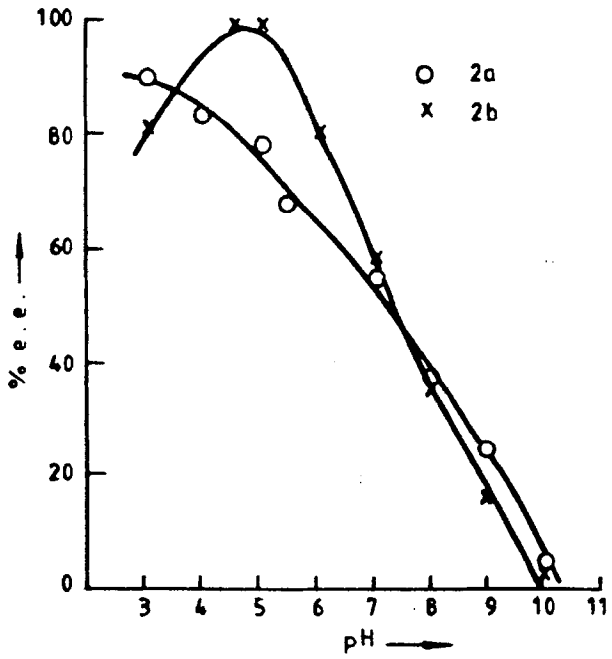

FIG 1

pH Effect on the Enantiospecificity of Reduction of Ethyl acetoacetate 1a and Ethyl benzoylacetate 1b by Baker's Yeast Immobilized in Calcium Alginate[9]

genases and at least two alcohol dehydrogenases with opposing stereospecificity which are capable of reducing β-ketoesters to -(R) and -(S) alcohols. The pH optima for pro-(R) and pro-(S) enzymes were found to be 6.1 and 6.9 respectively. Further, for reduction of ethyl 3-oxobutyrate the Km values were found to be 17 mM and 0.9 mM for the pro-(R) and pro-(S) enzymes respectively[7]. This prompted us to study the influence of pH of the reaction medium on the enantiospecificity of the reduction process. We visualized that pH of the

medium should be playing a vital role in determining the enantiospecificity of reduction since each alcohol dehydrogenase present in baker's yeast would have its own pH-activity profile. Here we present the results of our investigations on the effect of pH on the enantiospecificity of reduction of ethyl acetoacetate **1a** and ethyl benzoylacetate **1b** to ethyl (S)-3-hydroxybutanoate **2a** and ethyl (S)-(-)-3-hydroxy-3-phenylpropionate **2b** respectively, mediated by baker's yeast immobilized in calcium alginate beads.

As can be seen from **Fig. 1**, pH of the reaction medium has a strong influence on the optical purity of the product alcohols and best enantiospecificity is observed for reactions conducted at low pH. At optimum pH of 4.5 **2b** is obtained from reduction of ethyl benzoylacetate in 80-85% isolated yield and with an e.e as high as 99%. For ethyl acetoacetate, the enantiospecificity of the product **2a** is somewhat low[8] (e.e. 90%) but again product recovery is excellent (80-85%). As pH increases both optical purity of the product and product recovery decreases.

Many factors can contribute to the effect of pH on the enantiospecificity of the reduction process, and 3 of them are major. Firstly, the concentration of the pro-(R) enzyme could be less than that of pro-(S) enzyme. Heidlas and co-workers actually report isolation of 5.8 units of pro-(R) enzyme as compared to 1.6 units of pro-(S) enzyme from 7.5 g of crude extract of baker's yeast[7] indicating that this is not so[10]. However, the pH-activity profiles for the two competing

enzymes could be such that the pro-(S) enzyme which has a pH optimum at 6.9 is sufficiently active at low pH while the pro-(R) enzyme with pH optimum at pH 6.1 has very little activity at this pH, so that the effective concentration of active pro-(S) enzyme is larger than the pro-(R) enzyme. As the pH of the medium increases, the pro-(R) enzyme also becomes active and starts competing for the substrate causing a decrease in enantiospecificity. Secondly, the velocity of the reduction process (k_{cat}) for the pro-(R) enzyme could be slower than that for pro-(S) enzyme under comparable reaction conditions. This could account for predominance of (S)-enantiomer at all pH values. Thirdly, since the Km for the two enzymes are different (17 mM and 0.9 mM respectively for reduction of ethyl acetoacetate by pro-(R) and pro-(S) enzymes), a low concentration of the substrate would cause most of the substrate to bind to the pro-(S) enzyme preferentially. Other factors such as differences in product inhibition constants etc. may also play some role. These arguments hold good even if there are different alcohol dehydrogenases acting on the same substrate under a given set of reaction conditions. The highest enantiospecificity that can be achieved finally depends upon the intrinsic properties of the enzyme itself; conformation of the active site, the binding of the substrate and its orientation etc. Hydrolysis of the ester function by the esterases of baker's yeast[11] and further decomposition of the products apparently cause a decrease in the yield of the hydroxy esters at higher pH values. Calcium alginate as the immobilization matrix

is very useful in avoiding messy emulsions formed during workup when free cells are used.

Our results thus demonstrate the importance of maintaining pH of the medium during baker's yeast reduction. By simply carrying out the reduction at a low pH it is possible to get product of very high optical purity and yields with consistent results, and the methodology can adopted as a general procedure for microbial reductions of a variety of substrates.

Experimental

In a typical experiment commercial dried baker's yeast (Eagle brand, Bombay, India; 7.5 g) was immobilized in calcium alginate (100 ml, 2% sodium alginate)[12] and the beads were suspended in citrate-phosphate-borate buffer (200 mL, 0.05 M) of appropriate pH containing glucose (10 g) and stirred with magnetic bead at room temperature. The substrate (400 mg in 3 mL ethanol) was added slowly over 48 hrs and the reactants were stirred for another 24 hrs. No extra glucose was added and pH of the solution was maintained at a fixed value by addition of 10% ammonia solution. After complete reduction of the ketone the supernatent was decanted and extracted with chloroform. The beads were also washed with chloroform and combined organic extracts were evaporated. The residue was passed through a column of neutral alumina to get the pure product. The ^{1}H NMR and IR spectra of the products were identical with those reported in literature[13]. Optical

purity of the product was determined by the ^{19}F NMR its Mosher ester (400 MHz, $CDCl_3$). 2b e.e. >99% $[\alpha]_D^{25}$ - 41.8 (c 1.3 $CHCl_3$); (lit[11] $[\alpha]_D^{25}$ - 25.8 (c 1.3 $CHCl_3$).

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8 The substrates used for assay of the activity of the two enzymes were different and thus it is not possible to say whether the activity in units term really represents the relative concentrations of the two enzymes.

9 The results in fig. 1 are reported as average of 3 determinations using commercial baker's yeast of different batches. The enantiospecificity is reproducible within ± 1%. The yield of the products were 80-85% for reactions at pH 3 to 5 and decreased gradually from 75% to 40% as the pH increased from 6 to 8. At pH 9 to 10 the yield was only 10 to 15%.

10 The product 2a is obtained with e.e. 90% at optimum pH of 3 as compared to **2b** which is obtained with e.e. $>$ 99% under optimum conditions. It has been observed by several workers earlier that baker's yeast shows a better enantiospecifity toward compounds possessing phenyl groups[1b,1c].

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(Received in UK 5 November 1992)