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Baker's Yeast Mediated Bioreduction. A New Procedure Using Ethanol as an Energy Source

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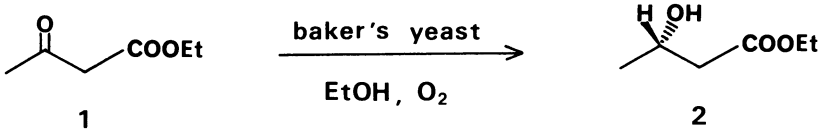
A practical procedure using ethanol as an energy source was developed on the baker's yeast mediated bioreduction of prochiral ketones.

The baker's yeast mediated bioreduction of prochiral ketones has been recognized as a useful technique for synthetic organic chemists,[1] and is usually performed in an aqueous solution of glucose or sucrose. It is widely accepted that the sugar is essential as an energy source for undergoing the continuous reduction, as NADPH, which is an actual reducing agent, can be efficiently regenerated from $NADP^+$ through the hexose monophosphate pathway for glucose oxidation in the yeast cell.[2] However, we recently found that not only the metabolism of glucose but the oxidative pathway of ethanol, which was formed from glucose, yielded the energy of this bioreduction.[3] Now, we would like to report the new procedure using ethanol instead of the sugar as an energy source.



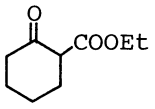
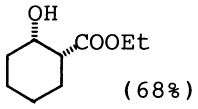
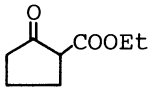
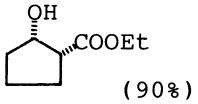
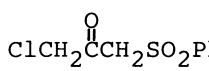
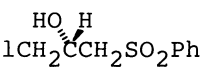
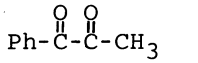
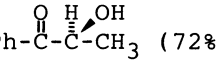
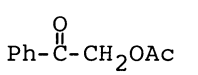
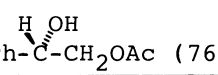
1 2

The yeast mediated reduction of ethyl acetoacetate (1)[4] was examined in an aqueous solution of ethanol. A suspension of 2.8 g of pressed baker's yeast (Oriental Yeast Co.) in 50 ml of an aqueous solution of 0.50 g (77 mM) of 1 and 0.58 ml (200 mM) of ethanol was shaken at 30 °C under the atmosphere and the reaction was monitored by GLC analyses. The reduction of 1 proceeded at the similar rate comparing with the original procedure using glucose,[5] and the ethanol was continuously consumed. After 10 h, the ordinary work-up afforded (S)-(+)-ethyl 3-hydroxybutanoate (2) in 67% yield; $[\alpha]_D^{23}$ 41.6 (c 1.00, $CHCl_3$). The enantiomeric excess (ee) was determined by HPLC analysis of its MTPA ester[4] to be 95%. Yield and ee are comparable to the results obtained by the original procedure.[4] On the other hand, the bioreduction did not proceed under nitrogen atmosphere. It is assumed that NADPH would be regenerated through the oxidative pathway of ethanol[6] in the presence of oxygen. The investigation on the mechanism in the yeast cell is now in progress.

Several prochiral ketones were also reduced by this procedure (Table 1). The configuration of each chiral secondary alcohol obtained was the same as that reported for the product reduced by the original procedure.[7-11]

As the amount of sugar used in the original procedure is quite excess, the metabolism of the sugar is troublesomely accompanied by a vigorous foam of carbon dioxide and ill-smelling by-products. So, the new procedure should be entried as a practical one for the yeast mediated reduction.

Table 1. Yeast Mediated Reduction using EtOH

Substrate	Product (yield)	$[\alpha]_D^{23}$ a) / ob)
	 (68%)	+25.87 (+25.28)
	 (90%)	+14.99 (+13.72)
	 (75%)	+13.60 (+13.06)
	 (72%)	-39.40 (-38.79)
	 (76%)	+56.03 (+55.68)

a) Measured in $CHCl_3$.

b) The $[\alpha]_D^{23}$ of the chiral alcohols prepared by the original procedures using sugar were measured in our hand and are shown in parenthesis.

References

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2) G.M. Whitesides and C.-H. Wong, Angew. Chem., Int. Ed. Engl., 24, 617(1985); H. Yamada and S. Shimizu, ibid., 27, 622 (1988).

3) The details will be reported in near future.

4) T. Sugai, M. Fujita, and K. Mori, Nippon Kagakukai Shi, 1983, 1315; B. Wipf, E. Kupfer, R. Bertazzi, and H. G. W. Leuenberger, Helv. Chim. Acta, 66, 485 (1983); D. Seebach, M.A. Sutter, R.H. Weber, and M.F. Züger, Org. Synth., 63, 1 (1985).

5) When a suspension of 2.8 g of pressed baker's yeast in 50 ml of an aqueous solution of 0.50 g (77 mM) of 1 and 5.40 g (600 mM) of glucose was conducted in the same manner, the substrate disappeared after 8 h.

6) The regeneration system of NADPH using ethanol and two pure enzymes (alcohol dehydrogenase/aldehyde dehydrogenase) had been already reported by Cohen and Whitesides. (R.P. Chamber, J.R. Ford, J.H. Allender, W.H. Baricos, and W. Cohen, Enzyme Eng., 2, 195 (1973); C.-H. Wong and G.M. Whitesides, J. Org. Chem., 47, 2816 (1982)) We assume that the similar system would be active in the baker's yeast cell.

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