

Effects of Additives in the Reduction Using Bakers' Yeast Cell-Free Extract

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By addition of diisopropyl fluorophosphate the undesirable hydrolysis of α -acetoxy ketones or alcohols was completely inhibited in the asymmetric reduction using bakers' yeast cell-free extract, but hardly inhibited in the reduction using the whole cell suspension. The control of stereoselectivity by addition of metal chlorides was also conducted more effectively in the cell-free extract.

The asymmetric reduction of various carbonyl compounds by bakers' yeast has been investigated to obtain chiral alcohols because of simplicity and high stereoselectivity of the reduction.¹ The reduction shows rather wide substrate specificity, although it does not always afford alcohols with a desired configuration in satisfactory enantiomeric excess (ee). Nakamura *et al.* reported that the introduction of a third reagent into the reduction system changed the stereoselectivity to afford a product of the desired configuration in high ee.² Recently, we have reported that the asymmetric reduction of 1-acetoxy-2-alkanones (**1**) to (S)-1-acetoxy-2-alkanols (**2**) by use of the bakers' yeast cell-free extract can be carried out efficiently with regeneration of NADPH and inhibition of the hydrolysis of substrate **1** and product **2** by addition of diisopropyl fluorophosphate (DFP).³

Here we would like to report novel features of additives in the reduction using bakers' yeast cell-free extract.

As shown in the scheme, the reduction of α -acetoxy ketones **1** using bakers' yeast is accompanied by the hydrolysis of substrate **1** and product **2**, which produced diol **3** in 10 - 30% yields with 50 - 90% ee.^{4,5} To prevent the hydrolysis, the bakers' yeast cell-free extract was found to be highly effective as shown in Table 1. The serine protease inhibitor (DFP) added to the cell-free extract with the intention to protect the α -acetoxy ketone reducing enzyme from hydrolysis, turned out to inhibit the hydrolysis of **1** and **2**.

The hydrolysis in the cell-free extract was completely inhibited by addition of 0.5 mM DFP but that in the whole cell suspension was hardly inhibited. These facts suggest that the hydrolysis takes place enzymatically. The hydrolytic enzymes concerned in the whole cell seem to be protected by the cell wall and membrane against the inhibitor. The cell-free extract itself, without the inhibitor, afforded hydrolyzed product **3** in lower yields of 8 - 15%, as compared with 19 - 31% for the whole cell suspension. This diminished hydrolytic activity may be attributed to the loss of hydrolytic enzymes during the preparation of the cell-free extract.

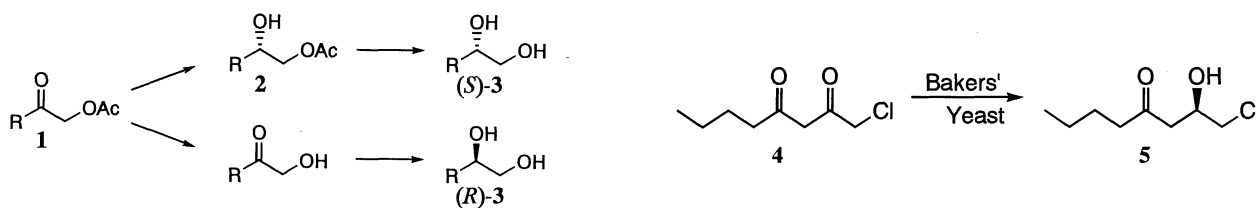


Table 1. Asymmetric Reduction of α -Acetoxy Ketones (**1**) to α -Acetoxy Alcohols (**2**) using Bakers' Yeast Suspension and Its Cell-Free Extract

substrate	cell-free extract ^a , yield/% ^b				whole cell suspension ^c , yield/% ^b			
	0.5 mM DFP ^d		none		0.5 mM DFP ^d		none	
	2	3	2	3	2	3	2	3
<i>n</i> -C ₅ H ₁₁ COCH ₂ OAc	83 (99, <i>S</i>)	0	65 (99, <i>S</i>)	15	55 (99, <i>S</i>)	28	51 (99, <i>S</i>)	31
C ₆ H ₅ COCH ₂ OAc	73 (96, <i>S</i>)	0	77 (95, <i>S</i>)	8	75 (84, <i>S</i>)	17	79 (84, <i>S</i>)	19

^a Substrate 1 mmol, glucose 3 mmol, bakers' yeast cell-free extract 60 ml (30 g of pressed yeast), 30 °C.

^b The yields for **2** include the yields (~10%) of 1,2-migrated acetates. The enantiomeric excesses and configuration of **2** are indicated in parentheses. ^c Substrate 1 mmol, glucose 3 mmol, bakers' yeast 30 g, 30 °C.

^d 0.5 mmol dm⁻³ diisopropyl fluorophosphate.

Table 2. Asymmetric Reduction of 1-Chloro-2,4-octanedione (**4**) to 1-Chloro-2-hydroxy-4-octanone (**5**) Using Bakers' Yeast and Its Cell-Free Extract with Metal Chlorides

additive	cell-free extract ^a				dry yeast ^b				dry yeast ^b in swelling				pressed yeast			
	concn /M ^c	yield /%	ee ^d /%	R/S ^e	concn /M ^c	yield /%	ee ^d /%	R/S ^e	concn /M ^c	yield /%	ee ^d /%	R/S ^e	concn /M ^c	yield /%	ee ^d /%	R/S ^e
none	--	63	72	R	--	63	7	R	--	58	7	R	--	61	5	R
MgCl ₂	0.25	40	86	R	2	40	87	R	2	47	50	R	2	42	38	R
SrCl ₂	0.25	49	89	R	2	46	89	R	2	42	53	R	2	45	39	R
MnCl ₂	0.25	50	90	R	2	56	83	R	2	---	---	--	2	---	---	--

^a Substrate 1 mmol, glucose 3 mmol, bakers' yeast cell-free extract 60 ml (30 g of pressed yeast), 35 °C, 18 h.

^b Substrate 1 mmol, dry yeast 15 g, tap water 60 ml, glucose, 30 °C, 18 h. ^c M = mol dm⁻³.

^d Determined by using 200 MHz ¹H NMR spectra of MTPA esters.

^e Determined by comparing the [α]_D values of dechlorinated derivatives.

The control of stereoselectivity in the bakers' yeast reduction is also effectively conducted by use of the cell-free extract. As shown in Table 2, in the bakers' yeast reduction of 1-chloro-2,4-octanedione (**4**) to 1-chloro-2-hydroxy-4-octanone (**5**),⁶ enhancement of the enantiomeric excess to 86-90 % ee was achieved by using only 0.25 M metal chlorides in the cell-free extract as compared with the 2 M concentration in the whole cell suspension. It is likely that the concentration of metal chlorides in the interior of yeast cell was near to 0.25 M even in the 2 M metal chlorides solutions. It is also likely that the salts penetrate into the cells of dry yeast more effectively than those of the dry yeast after swelling or the pressed yeast.

Further studies on the effect and function of additives are currently under investigation.

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References and Notes

- 1 S. Servi, *Synthesis*, **1**, 1990; B. I. Glänzer, *Chem. Rev.*, **91**, 49 (1991); L. Poppe and L. Novak, "Selective Biocatalysis," VCH, Weinheim, Chapter 5 (1992).
- 2 K. Nakamura, K. Inoue, K. Ushio, S. Oka, and A. Ohno, *Chem. Lett.*, **1989**, 875; K. Nakamura, Y. Kawai, S. Oka, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **62**, 875 (1989); K. Nakamura, Y. Kawai, and A. Ohno, *Tetrahedron Lett.*, **30**, 2245 (1989).
- 3 K. Ishihara, T. Sakai, S. Tsuboi, and M. Utaka, *Tetrahedron Lett.*, **35**, 4569 (1994).
- 4 A. Manzocchi, A. Fiecchi, and E. Santaniello, *J. Org. Chem.*, **53**, 4405 (1988).
- 5 M. Utaka, T. Sakai, and S. Tsuboi, *J. Synth. Org. Chem. Jpn.*, **50**, 647 (1991).
- 6 To be reported in due course.