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Highly Efficient Enzymatic Asymmetric Reduction by Use of Regenerating NADPH in Bakers' Yeast Cell-Free Extract

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Abstract: Bakers' yeast cell-free extract was found to reduce 1-acetoxy-2-alkanones to (*S*)-1-acetoxy-2-alkanols in 59 – 88% yield and 95 – >99% ee by use of a catalytic amount of NADPH with glucose as a hydride source and without addition of any enzyme for the cofactor regeneration.

Bakers' yeast reduction is known as a practical synthetic method for preparing various chiral intermediates,[1] as typically demonstrated by the asymmetric reduction of ethyl 3-oxobutanoate to (*S*)-3-hydroxybutanoate.[2] This reduction, however, seems to need improvement in comparison with abiological catalytic asymmetric hydrogenation using BINAP-Ru(II) complexes.[3,4] Then, development of novel methods for synthesizing useful chiral synthons by the aid of bakers' yeast is awaited in recent years.

Herein we wish to report a novel and efficient performance of bakers' yeast that the yeast cell-free extract reduces 1-acetoxy-2-alkanones (**1**) to (*S*)-1-acetoxy-2-alkanols (**2**) enantioselectively as the sole product in 95 – >99% ee and 59 – 88% yield[5] (Table 1).

Table 1. Asymmetric Reduction of 1-Acetoxy-2-alkanones (**1**) to 1-Acetoxy-2-alkanols (**2**) with Regenerating NADPH by Use of Bakers' Yeast Cell-Free Extract [a]

$RCOCH_2OAc$ (1)		$RCHOHCH_2OAc$ (2)				
	R	time(h)	% yield[b]	% ee[c]	R/S[d]	$[\alpha]_D$(c, $CHCl_3$)
a	CH_3	6	59	> 99	*S*	+20.0 (1.65)
b	C_2H_5	8	62	> 99	*S*	+11.3 (1.67)
c	*n*-C_3H_7	8	78	99	*S*	+7.34 (1.88)
d	*n*-C_4H_9	9	82	95	*S*	+7.08 (1.55)
e	*n*-C_5H_{11}	10	83	99	*S*	+6.74 (1.78)
f	*n*-C_6H_{13}	10	88	> 99	*S*	+4.89 (1.62)
g	*n*-C_7H_{15}	14	83	99	*S*	+3.94 (1.88)

a) To a mixture of the substrate (**1**) (1 mmol) and NADPH (10 μmol) was added glucose (3 mmol) and cell-free extract (60 ml, 30g of yeast) with stirring at 35 °C. b) The yields include the yields of 1,2-migrated products, (*S*)-2-acetoxy-1-alkanols (5-15%). We confirmed that 2-acetoxy-1-pentanol thus obtained was identical with 1-acetoxy-2-pentanol (**2c**) with respect to configuration and enantiomeric excess. c) Determined by using 500MHz NMR spectra of MTPA esters. d) Determined by use of $[\alpha]_D$ values.

The products, (*S*)-alcohols, possess the antipodal configuration to (*R*)-1,2-alkanediols obtained from the conventional yeast reduction of 1-hydroxy-2-alkanones.[6] By use of the fermenting bakers' yeast, **1**

was reduced to (*S*)-**2** in high purity of 95 – >99% ee with somewhat low (41 – 65%) yield, inevitably accompanied by (*S*)-1,2-alkanediols as byproduct in low (53 – 89%) ee with 7 – 34% yield.[7] Thus, use of the cell-free extract has realized significant improvements. The high efficiency of the cell-free extract is derived from 1) an NADPH regenerating system using glucose as a hydride source and 2) inhibition of hydrolysis of the acetates **1** and **2**.

Enzyme-catalyzed asymmetric reductions coupled with an enzymatic NAD(P)H regenerating system are increasingly attracting interest in view of preparative organic chemistry.[8] In general, both of enzymes for reduction of substrate and for regeneration of the cofactor were obtained commercially or isolated from microbes in the laboratory. In the present method, the cell-free extract contains both enzymes and requires no enzyme for the cofactor regeneration. This is the unprecedented enzyme-catalyzed asymmetric reduction using bakers' yeast as enzyme source. To the best of authors' knowledge, such an NADPH regenerating system using glucose has never been reported.

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References and Notes

1. Servi, S. *Synthesis* **1990**, 1 – 25. Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49 – 97.
2. Seebach, D.; Sutter, M. A.; Weber, R. H.; Züger, M. F. *Org. Synth.* **1985**, *63*, 1 – 9.
3. Noyori, R.; Takaya, H. *Acc. Chem. Res.* **1990**, *23*, 345 – 350.
4. Harada, K.; Munegumi, T. In *Comprehensive Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 8 (Reduction), pp 139 – 158. Nishizawa, M.; Noyori, R. *ibid.*, pp 159 – 182.
5. In 60 ml of 0.1 M morpholinoethanesulfonate buffer (pH 6.0) containing 0.5 mM diisopropyl fluorophosphate, 30g of pressed bakers' yeast was suspended and homogenized with 60 ml of glass beads (0.5 mm in diameter) using a Vibrogen cell mill at 4 °C. The homogenate was centrifuged at 12,000 rpm for 30 min at 4 °C. The supernatant (60 ml) was collected and used without further purification. Activity of the cell-free extract was determined to be 15 units / 60 ml at 25 °C for the reduction of 1-acetoxy-2-heptanone (**1e**) by following the decrease in absorbance at 340 nm for NADPH. One unit is defined as the amount of enzyme required to convert 1 mmol of substrate per min.
6. Levene, P. A.; Walti, A. *Org. Synth. Coll. Vol. II*, **1943**, 545 – 547. Barry, J.; Kagan, H. S. *Synthesis* **1981**, 453 – 454. See our paper cited in ref 7.
7. Utaka, M.; Sakai, T.; Tsuboi, S. *J. Synth. Org. Chem., Jpn.* **1991**, *7*, 647 – 656. A similar feature of bakers' yeast reduction was reported for 2-acetoxy-1-phenylethanone. See Manzocchi, A.; Fiecchi, A.; Santaniello, E. *J. Org. Chem.* **1988**, *53*, 4405 – 4407.
8. Whitesides, G. M.; Wong, C. -H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617 – 718.

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