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STEREOCHEMICAL CONTROL OF MICROBIAL REDUCTION. 2.[1] REDUCTION OF β-KETO ESTERS BY IMMOBILIZED BAKERS' YEAST

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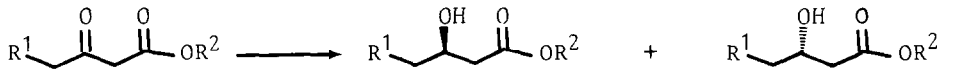
Ketones in β-keto esters are reduced asymmetrically by immobilized bakers' yeast. The configuration and the enantiomer excess of the products are dramatically changed by the entrapment of yeast cells in dense polyurethane matrices.

Chiral β-hydroxy esters are versatile chiral synthons in organic synthesis especially in the preparation of natural products.[2-7] A commonly used method for the preparation of chiral β-hydroxy esters belongs to the use of bakers' yeast (BY)[8-11] as a chiral catalyst to reduce β-keto esters asymmetrically because BY is a cheap and easily obtainable *reagent*. BY, however, does not always afford β-hydroxy esters of desirable configuration in satisfactorily high enantiomer excess (e.e.). Improvement of this defect has so far been attempted either by seach for various microorganisms[12-14] or by some modification of substrates.[1,15-19] These may, of course, be effective in many cases, but are rather troublesome and not always sufficiently effective. Now, we would like to report a new and powerful method for stereochemical control of microbial transformation; the immobilization of BY can cause a definite change of configuration and e.e. of the reduction products *i.e.* the asymmetric yield in the reduction of β-keto esters by BY was controlled by immobilization. In addition, the troublesome isolation of the products from the muddy reaction mixture with BY was circumvented by the method; immobilized cells were easily separated from the reaction mixture by rapid filtration.

The reduction of ethyl 4-chloroacetoacetate (1a) by BY (Red Star) was recently reported to give ethyl (*S*)-4-chloro-3-hydroxybutanoate (2a) by Sih and his coworkers,[16,20] and they described that e.e. of the alcohol depends on the concentration of the substrate. A similar phenomenon has been reported in the reduction of ethyl acetoacetate.[12] These results are indicative of the participation of plural enzymes in the reduction and the facts allured us into the application of cell immobilization technique to the stereochemical control of microbial transformations. A general procedure employed here is as follows. One mmol of a substrate was added to a bioreactor containing 4 g of BY (either



1a-d 2a-d 3a-d

a; R^1 = Cl, R^2 = Et c; R^1 = Me, R^2 = Me

b; R^1 = Cl, R^2 = Me d; R^1 = H , R^2 = Et

Table 1. Reduction of β-Keto Esters by Immobilized Bakers' Yeast

Substrate	Concentration (mM)	Method of Immobilization[a]	Product[b]	Sign of Rotation	e.e.[c]
1a	10	None	3a	+	42
	20	None	3a	+	27
	50	None	3a	+	15
	20	Alg	3a	+	16
	20	Car	3a	+	11
	10	PAA	3a	+	3
	10	PU	2a	-	82
	50	PU	2a	-	82
1b	10	None	3b	+	31
	20	None	3b	+	12
	20	Alg	3b	+	10
	20	PU	2b	-	90
1c	20	None	2c	-	5
	20	Alg	2c	-	17
	20	PU	2c	-	86
1d	20	None	3d	+	>98
	20	Alg	3d	+	92
	20	PU	3d	+	60

[a] None: free BY was used. Alg: BY entrapped in calcium alginate (BY/sodium alginate = 10/1 (W/W)). Car: BY entrapped in carrageenan[22] BY/carrageenan = 4/1 (W/W)). PAA: BY entrapped in polyacrylamide[23] BY/acrylamide = 1/1 (W/W)). PU: BY entrapped in polyurethane (PU6)[24] (BY/PU = 1/1 (W/W)). [b] Chemical yields of the products were between 60 and 80 %. [c] Enantiomeric ratios were determined by NMR (400 MHz)(for **a** and **b**) or by GLC (OV-1701, 25 m, 200°C) (for **c** and **d**) analyses of their (+)-α-methoxy-α-trifluoromethylphenylacetates (MTPA).

free or entrapped in a matrix indicated) in varing amount of water (total volume of 20 to 100 ml including the immobilized bioreductant), and reacted at 30°C with gentle shaking of the reactor. After completion of the reduction (within one day), the mixture was separated by filtration and the immobilized cells were washed with water, if necessary, with pressing, and further with ether. The filtrate was extracted with two portions of ether, and the extract was dried and concentrated. After purification of the alcohol product by preparative GLC, $[\alpha]_D$ values of the alcohol were routinely measured. Determination of e.e. was made either by GLC or by NMR analysis on MTPA ester of the alcohol.[21]

In the reduction of **1a** by BY (Oriental), **3a** was obtained in 42 % e.e. at a low substrate concentration (10 mM) and in 15 % e.e. at a high substrate concentration (50 mM). The difference in configulation of the presently obtained alcohol from that reported before[16] might be resulted from the difference in the strain and in physiological state of BY used. When BY was immobilized with alginate,[22] carrageenan,[23] or polyacrylamide,[24] **3a** was obtained in 16, 11, and 3 % e.e., respectively. To our much interest, BY entrapped in polyurethane (PU)[25] afforded **2a** in 82 % e.e. Methyl 4-chloroacetoacetate was also subjected to the reduction. BY entrapped in PU gave **2b** in 90 % e.e. (again (-)-alcohol), whereas free BY resulted in the formation of **3b** in 31 % e.e. at 10 mM of the substrate concentration. Furthermore, **2c** was obtained in good e.e. of 86 % upon the reduction of **1c** with PU-entrapped BY (Table 1). In the reduction of **1d**, e.e. of the product **3d** was lowered toward the (-)-alcohol by PU entrapment. Thus, it was shown that the immobilization of cells in thick PU matrix commonly shifts the stereoselectivity of the reduction with yeast toward the direction to give preferentially β-hydroxy esters with (-)-rotation irrespective of substrate keto esters so far investigated. Significantly, the e.e. of each alcohol was unaffected by the substrate concentration in the reduction by PU-entrapped BY, whereas the e.e. value was susceptible to the concentration in the reduction by free BY, *i.e.*, a constant e.e. value was obtained in the reduction with PU-entrapped cells for 10 mM and 50 mM of **1a**. The results are those expected for reactions with a controlled steady-state concentration of the substrate.

Although the precise mechanism of such dramatic change in the e.e. and configuration of the products by cell entrapment is to be elucidated, it was clearly demonstrated that the stereochemistry of the yeast reduction can be controlled by immobilization. In the case of the reduction of β-keto esters with a short chain alcohol, if one need to obtain a D-(-)-alcohol[2-4] one would generally be recommended to use BY (or possibly other microbial cells) entrapped in a thick PU matrix.

Applications of the immobilized microbial system to other substrates are now under further investigation.

References

1. For the preceding paper in this series, see: Nakamura, K.; Ushio, K.; Oka, S.; Ohno, A.; Yasui, S., *Tetrahedron Lett.*, 25, 3979 (1984).
2. Fráter, G., *Helv. Chim. Acta*, 62, 2829 (1979).
3. Mori, K.; Ikunaka, M., *Tetrahedron*, 40, 3471 (1984).
4. Hirama, M.; Uei, M., *J. Am. Chem. Soc.*, 104, 4251 (1982).
5. Kitahara, T.; Mori, K., *Tetrahedron Lett.*, 26, 451 (1985).
6. Mori, K.; Tanida, K., *Tetrahedron*, 37, 3221, (1981).
7. Georg, G. I., *Tetrahedron Lett.*, 25, 3779 (1984).
8. Lemieux, R. U.; Giguere, J., *Can. J. Chem.*, 29, 678 (1951).
9. Ridley, D. D.; Stralow, M., *J. Chem. Soc., Chem. Commun.*, 400 (1975).
10. Deol, D. S.; Ridley, D. D.; Simpson, G. W., *Aust. J. Chem.*, 29, 2459 (1976).
11. Seebach, D.; Renaud, P.; Schweizer, B.; Züger, M. F.; Brenne, M.-J., *Helv. Chim. Acta*, 67, 1843 (1984).
12. Wipf, B.; Kupfer, E.; Bertazzi, R.; Lenenberger, H. G. W., *Helv. Chim. Acta*, 66, 485 (1983).
13. Seebach, D.; Züger, M. F.; Giovannini, F.; Sonnleitner, B.; Fiechter, A., *Angew. Chem. Int. Ed. Engl.*, 23, 151 (1984).
14. Bernardi, R.; Cardillo, R.; Ghiringhell, D. *J. Chem. Soc., Chem. Commun.*, 460 (1984).
15. Hoffman, R. W.; Helbig, W.; Ladner, W., *Tetrahedron Lett.*, 23, 3479 (1982).
16. Zhou, B.-N.; Gopalan, A. S.; Van Middlesworth, F.; Shieh, W. R.; Sih, C. J., *J. Am. Chem. Soc.*, 105, 5925 (1983).
17. Hirama, M.; Shimizu, M.; Iwashita, M. J., *J. Chem. Soc., Chem. Commun.*, 599 (1983).
18. Fujisawa, T.; Itoh, T.; Sato, T., *Tetrahedron Lett.*, 25, 5083 (1984).
19. Fuganti, C.; Grassellei, P.; Casati, P.; Carmeno, M., *Tetrahedron Lett.*, 26 101 (1985).
20. Chen, C.-S.; Zhou, B.-N.; Girdaukas, G.; Shieh, W.-R.; Van Middlesworth, F.; Gopalan, A. S.; Sih, C. J., *Bioorg. Chem.*, 12, 98 (1984).
21. Dale, J. A.; Dull. D. L.; Mosher, H. S., *J. Org. Chem.*, 34, 2543 (1969).
22. Kierstan, M.; Bucke, C., *Biotechnol. Bioeng.*, 19, 387 (1977).
23. Sato, T.; Nishida, Y.; Tosa, T.; Chibata, I., *Biochim. Biophys. Acta*, 570, 179 (1979).
24. Chibata, I.; Tosa, T.; Sato, T., *Appl. Microbiol.*, 27, 878 (1974).
25. Fukushima, K.; Nagai, T.; Fujita, K.; Tanaka, A.; Fukui, S., *Biotechnol. Bioeng.*, 20, 1465 (1978). The urethane prepolymer used in this study was kindly supplied by Dr. A. Tanaka of Department of Industrial Chemistry, Faculty of Engineering, Kyoto University.

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