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Effect of Allyl Alcohol on Reduction of β-Keto Esters by Bakers' Yeast[1)†]

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Stereochemical course of the reduction of β-keto esters by bakers' yeast was controlled by treating the reduction systems with allyl alcohol.

Optically active 3-hydroxy alkanoic acid derivatives are useful chiral building blocks for the synthesis of biologically active compounds.[2)] Nowadays, bakers' yeast is often used as a reducing *reagent* in converting 3-oxoalkanoic acid derivatives to the corresponding 3-hydroxy derivatives,[3)] because bakers' yeast is cheap and easily obtainable. However, the reduction with bakers' yeast does not always afford alcohols with desired configurations in satisfactory enantiomer excess (ee). For example, although the reduction of ethyl 3-oxobutanoate by bakers' yeast gave relatively good result (97-70% ee),[4)] application of this system to ethyl 3-oxopentanoate afforded unsatisfactory results; the corresponding *R*-D-alcohol[5)] was obtained in only 40% ee.[6)] Hence, some methods for controlling stereochemical course in yeast reduction are required. To control the stereochemistry of reduction, chemists have developed several methods; search of suitable microbes[7)] or search of suitable modified substrates[8)] for the reduction. Recently, we reported that the microbial reduction could be controlled by immobilization of microbes.[9)] We now wish to report a new method for stereochemical control in microbial reduction. The concept and strategy employed here is following. Assuming that the low ee obtained in a microbial reduction originates from a multi-dehydrogenase system, i.e. each enzyme in this system reduces substrates enantioselectively but in opposite directions, we may be able to control the stereochemical course of the reduction by inhibiting enzymes that give one stereoisomer specifically while preserving the activity of other enzymes that give the other stereoisomer. We found that allyl alcohol has such an effect.

The reduction of methyl 3-oxopentanoate with bakers' yeast gave the corresponding D-alcohol in 59% ee (substrate concentration = 50 mM),[10,11)] while treatment of the same system with 1.0 g/l of allyl alcohol gave the same product in 96% ee. The selectivity in the stereochemical course to D-alcohols is thus promoted by treatment of the cell system with allyl alcohol. Table 1 shows

† This paper is dedicated to the late Professor Ryozo Goto, Kyoto University.

the results.

Increase in the amount of added allyl alcohol results in the increase in the amount of D-alcohol in all β-keto esters so far studied.

We suppose that this effect is due to a difference in inhibition to L- and D-isomer-producing-enzymes. Allyl alcohol may inhibit the L-isomer-producing-enzyme more efficiently than the D-isomer-producing-enzyme. This effect is also applicable to the reduction of 4-chloro-3-oxobutanoate (2) and ethyl 3-oxo-

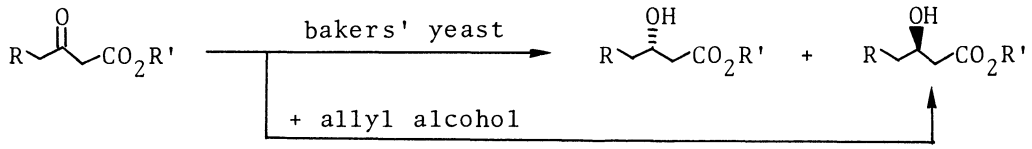

Table 1. Reduction of β-Keto Esters by Bakers' Yeast[a)]

Substrate	Allyl alcohol, g/l	Product[b)] Configuration	ee/%	Chemical yield/%[f)]
Cl, O, CO_2Et	0	D	43[c)]	62
	0.5	D	73	54
	1.0	D	82	44
	2.0	D	85	42
Me, O, CO_2Me	0	D	59[d)]	54 (86)
	0.5	D	89	54
	1.0	D	96	56
Me, O, CO_2Et	0	L	77[d)]	66 (90)
	1.0	L	40	68

a) The reaction conditions are described in the text.

b) The absolute configuration was determined by the sign of optical rotation of the alcohols.

c) The ee was determined by HPLC analysis (Cosmosil 5SL, hexane : ethyl ether = 20 : 1) of the corresponding MTPA ester.[10)]

d) The ee was determined by GLC analysis (OV 1701, 25 m, 180 °C) of the corresponding MTPA ester.

e) Isolated yields by preparative gas chromatography. Yields in parentheses are gas chromatographic yields caluculated with an internal standard (PEG, 1.5 m 80-130 °C).

butanoate (3). In the former case, the selectivity is enhanced from 43% ee to 85% ee by treatment with allyl alcohol. The reduction of 3 without treatment with allyl alcohol afforded (L)-3-hydroxybutanoate in 77% ee while treatment with allyl alcohol gave the L-alcohol in 40% ee. Although the selectivity decreased in the latter case, the trend that the increase of the relative yield of the D-alcohol to L-alcohol was also observed on this treatment.

An effect of allyl alcohol to microbial reduction was reported previously, where allyl alcohol enhances the reduction by *Shizosaccharomyces pombe*.[12] The difference between this and our systems may depend on physiological conditions of microbes used. In the former system, the substrate and allyl alcohol was added to a growing culture of the microbe, and they assumed that the allyl alcohol may act as an inducer of a suitable reductase. In our case, resting cells were used and reductase was not produced under the conditions. Consequently, the allyl alcohol inhibited a specific enzyme. The effect of allyl alcohol in the former case (*S. pombe*) is also explainable with the idea of inhibition. The allyl alcohol inhibits a certain dehydrogenase which is not related to the reduction of the substrate, resulting in the increased reducing power by supplying an enough amount of reduced coenzymes to the desired dehydrogenase, then activating the reduction.

The precise mechanism of the effect of allyl alcohol on the yeast reduction of β-keto esters is not clear yet. However, we believe that the method cited above is a convenient and useful device in the reduction of β-keto esters to D-alcohols by bakers' yeast.

For a typical experiment, to a suspension of bakers' yeast (2 g) in 20 ml of water was added an appropriate amount of allyl alcohol and the resulting suspension was stirred at 30 °C for 30 min. Then a substrate (1 mmol) and glucose (1 g) was added and stirring was continued at the same temperature. After 1 day, Hyflo-super cel and ethyl acetate was added and the mixture was filtered. The celite was washed with ethyl acetate and combined filtrates were extracted with ethyl acetate. The organic portion was washed with water and dried with anhydrous sodium sulfate, concentrated and subjected to preparative gas chromatography (PEG, 1 m, 130-170 °C), giving the corresponding alcohol.

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11) Differences in ee's between the present result and reported values (Ref. 6, ee = 40% and Ref. 9, ee = 5%) might be due to the difference in the strain of bakers' yeast (Ref. 6) and the effect of substrate and glucose concentration. The effect of substrate concentration to ee has been reported (Sih et al. in Ref. 8 and Wipf et al. in Ref. 3). The effect of glucose concentration has been investigated in our laboratory and will be reported elsewhere.

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