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REDUCTION BY BAKERS' YEAST IN BENZENE[1]

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Abstract: Reduction of α-keto esters by non-immobilized bakers' yeast in an organic solvent is reported. When benzene is employed as the solvent, the reduction tends to afford the corresponding (*R*)-hydroxy ester predominantly.

Reduction of various carbonyl compounds by bakers' yeast (*Saccharomyces cerevisiae*) have widely been employed in organic syntheses.[2,3] In general, the reduction by a microbe is carried out in an aqueous medium, and the method is unable to be applied to the reduction of water-susceptible or insoluble substrates such as α-keto esters. Thus, a method to use bakers' yeast in organic solvents is worth to be explored. The procedure to isolate the product from a non-aqueous medium is much easier than that from an aqueous medium, which adds another benefit to the reaction in organic solvents.

Some hydrolytic enzymes such as lipases and proteases are known to retain their activity in organic solvents.[4] However, the dehydrogenases and reductases are different from hydrolytic enzymes in the sense that they require the assistance of stoichiometric amount of NAD-coenzyme, or the coenzyme in the microbe has to be reproduced in order to reduce the substrates catalytically. However, the reproduction of the consumed coenzyme cannot be convinced in organic solvents because organic solvents often cause a serious damage on the hydrophobic cell membranes of a microbe. Previously, we reported that bakers' yeast immobilized in polyurethane reduces α-keto esters into the corresponding α-hydroxy esters in hexane.[5,6] The reports were followed by a couple of claims from other laboratories for the reduction by a protected microbe in organic solvents.[7,8]

In this paper, we wish to report that dry bakers' yeast (DBY)[9] reduces α-keto esters in organic media without immobilization. So far as we understand, this is the first example of microbial reduction in an organic solvent without protection. The trick to the success stems from the amount of water added to the system: no reduction proceeds in an absolutely dry benzene. However, the addition of a drop of water to the system is

enough to promote the reduction. Usually, 0.2 - 1.2 equivalent amounts of water (mL H_2O/g DBY) was employed. The amount of water should not exceed 1.2 equivalents, because the presence of excess water again suppresses the reduction dramatically. The best result was obtained when 0.4 equivalent amounts of water was added to the system.

Slightly better results were obtained when an aqueous buffer solution (0.1 M AcOH/AcONa, pH 5) in 0.6 - 0.8 equivalent amounts was used in place of pure water. In order to increase the contacting surface area, it is recommended to use well pulverized DBY.

In a typical run, ethyl 2-oxoheptanoate (**1c**, 172 mg, 1 mmol) was added to a suspension of pulverized DBY[11] (10 g) in dry benzene (50 mL). An aqueous buffer solution (pH 5, 8 mL) was added to the system and the mixture was stirred for 24 h at 30 °C. After filtration, the filtrate was evaporated *in vacuo*. The residual oil was subjected to preparative GLC to isolate ethyl 2-hydroxyheptanoate in 26% yield. The results are summarized in Table 1.

The characteristic of the reduction by DBY in benzene is that the stereoselectivity differs from that exerted in the aqueous system: in the reduction of ethyl 2-oxoheptanoate (**1c**), (*S*)-hydroxy ester ((*S*)-**2c**, 92% e.e.) was obtained from the aqueous system, whereas the antipode ((*R*)-**2c**, 86% e.e.) was obtained from the benzene solution. The same result was afforded in the reduction of ethyl 3-methyl-2-oxobutanoate (**1b**), where the reduction in water gave the (*R*)-isomer in 19% e.e., whereas the selectivity increased up to 90% e.e. when the reaction was run in benzene. Although the product from the reduction of ethyl pyruvate (**1a**) in benzene is predominated by the (*S*)-isomer, the selectivity decreases to 73% e.e. from that of 93% e.e. in water. Thus, there is no doubt that the reduction by DBY in benzene tends to afford the (*R*)-predominant product.

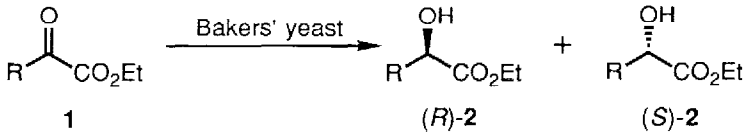
When the amount of water (or buffer) added to the system is taken into consideration, it is quite conceivable that a microbe can keep its activity in organic media if the cell-membrane and/or the enzyme(s) packed in the cell can hold its original structure by the aid of a water-layer (or layers); the fact which has neither been tested nor claimed.

Although hexane is another candidate as the solvent, the stereochemical shift observed in this solvent is less satisfactory than that in benzene.

It was found that the production of (*S*)-**2c** by the reduction of **1c** in water stems from the enantioselective decomposition of (*R*)-**2c** instead of the (*S*)-preferred reduction. When racemic **2c** was added to an aqueous DBY, stereoselective consumption of the substrate to produce hexanol took place, and less reactive (*S*)-**2c** was isolated unreacted together with hexanol, the reaction product from the (*R*)-isomer. The formation of hexanol was suppressed in hexane and enantioselective consumption of **2c** was observed in benzene.[12] The result suggests that the reduction of **1c** by DBY itself produces the (*R*)-isomer preferentially. However, since the (*R*)-isomer is consumed by DBY in water more rapidly than the (*S*)-isomer *via* the oxidation-reduction and decarboxylation, we obtain the product dominant in the (*S*)-isomer in low chemical yield from the aqueous reaction system. On the other hand, no stereoselective consumption of **2b** in aqueous medium is observed. Therefore, more preferential formation of the (*R*)-isomer, or more precisely, the suppression of the formation of the (*S*)-isomer, in benzene seems to be a characteristic property associated to the reduction of **1b**. Similar observation was reported for the reductions of α-keto esters by immobilized bakers' yeast in aqueous and organic media.[6]

The research to apply the present method to other compounds and to elucidate the mechanism to stressing the

biocatalyst upon the contact with an organic solvent so as to cause a shift of stereoselectivity of the reduction.



1 (*R*)-2 (*S*)-2

a R = CH_3
b $(CH_3)_2CH$
c C_5H_{11}

Table 1. Stereoselectivity in the Reduction of α-Keto Esters with Dry Bakers' Yeast in Various Solvents

Subst.[a]	R	DBY,g	Solvent[b]	Addendum[c]	Yield,%[d]	E.e.,%[e]
1a	CH_3	5	water	——	79	93 (*S*)
		10	benzene	water	55	81 (*S*)
		10	benzene	buffer	56	73 (*S*)
1b	$(CH_3)_2CH$	5	water	——	63	19 (*R*)
		10	benzene	water	39	87 (*R*)
		10	benzene	buffer	49	90 (*R*)
1c	C_5H_{11}	5	water	——	9	92 (*S*)
		5	hexane	water	30	37 (*R*)
		10	benzene	water	18	84 (*R*)
		10	benzene	buffer	26	86 (*R*)

[a] 1 mmol. [b] 50 mL. [c] 8mL. The buffer employed was 0.1 M AcOH/AcONa, pH 5. [d] Chemical yield was determined on GLC. [e] Enantiomer excess was calculated from GLC of their corresponding (*R*)-MTPA esters. Absolute configurations were determined as reported previously.[5]

References and Notes

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9. In order to preserve the reproducibility, we employed DBY instead of raw bakers' yeast.[10]
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12. Chemical hydrolysis of the ester took place slowly.

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