Stereochemical Control in Microbial Reduction. XXV. Additives Controlling Diastereoselectivity in a Microbial Reduction of Ethyl 2-Methyl-3-oxobutanoate

Yasushi Kawai,* Kousuke Takanobe, and Atsuyoshi Ohno

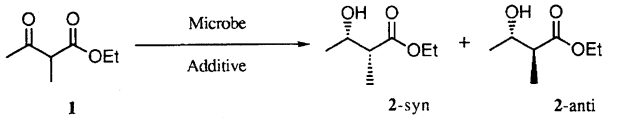
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611

(Received July 12, 1994)

Stereoselectivity of microbe-catalyzed reduction of ethyl 2-methyl-3-oxobutanoate to optically active 3-hydroxy-2-methylbutanoate can be controlled by the addition of methyl vinyl ketone to the reaction system. The reduction by *Geotrichum candidum*, *Endmyces magnusii*, and *Mucor javanicus* with an appropriate additive affords the corresponding *anti*-(2*S*,3*S*)-hydroxy ester selectively, whereas the reduction by bakers' yeast and *Candida tropicalis* with an additive gives the corresponding *syn*-(2*R*,3*S*)-hydroxy ester selectively.

Stereoselective synthesis of alkyl 3-hydroxy-2-methylbutanoate is an interesting subject in asymmetric organic syntheses, because the ester contains two chiral centers as well as two functional groups that are readily convertible into other functions. A number of researchers have reported the synthesis of this compound by the use of a biocatalyst.[1—6] Microbial reduction of the corresponding keto ester is one of the most popular methods for obtaining this compound.[1—6] Although the enantioselectivity of these reactions is mostly excellent, the diastereoselectivity, *syn*/*anti* ratio,[7] is unsatisfactory.[1—6] In previous papers, we reported several methods for improving and/or controlling the diastereoselectivity of microbial reduction. Modification of the substrate is one of the tricks; a structural change in the ester moiety affects diastereoselectivity of the bakers' yeast reduction appareciably.[8,9] The reduction of octyl[8] and neopentyl[9] esters of 2-methyl-3-oxobutanoic acid affords the corresponding *syn*-(2*R*,3*S*)-hydroxy ester, selectively. Modification of reaction conditions is another technique: The use of an organic medium for the reduction mediated by a microbe is helpful for obtaining a satisfactory result. The reduction mediated by immobilized *Geotrichum candidum* in an organic solvent in the presence of cyclopetanol as an additive gives ethyl *syn*-(2*R*,3*S*)-3-hydroxy-2-methylbutanoate selectively.[10] The use of an isolated enzyme is also a recommended method for stereoselective syntheses of optically pure alkyl 3-hydroxy-2-methylbutanoates. One of the β-keto ester reductases from bakers' yeast, which has been abbreviated as **L-1**, affords ethyl *syn*-(2*R*,3*S*)-3-hydroxy-2-methylbutanoate,[11,12] whereas one of the β-keto ester reductases from *Geotrichum candidum* gives the *anti*-(2*S*,3*S*)-hydroxy ester, another stereoisomer, selectively.[13]

Recently, we developed a valuable method of stereoselective reduction with bakers' yeast: the introduction of methyl vinyl ketone (MVK) to the reaction system results in great improvement of diastereoselectivity, giving the *syn*-(2*R*,3*S*)-hydroxy esters selectively.[14—16] Although the method is quite useful because of its facility in operation, preparation of the other stereoisomers of this compound has not yet been completed. We therefore extended the method for reductions mediated by a microbe other than bakers' yeast, and found that the reduction by mold with an appropriate additive affords the corresponding *anti*-(2*S*,3*S*)-hydroxy ester selectively, whereas the reduction by yeast with an additive gives the corresponding *syn*-(2*R*,3*S*)-hydroxy ester selectively. This report will describe details in the preparation of ethyl *anti*-(2*S*,3*S*)- and *syn*-(2*R*,3*S*)-3-hydroxy-2-methylbutanoate in excellent stereoselectivities. (Eq. 1).



1 2-syn 2-anti

(1)

Experimental

Instruments. NMR spectra were recorded on a Varian VXR-200 and a JEOL JNM-GX 400 spectrometers in $CDCl_3$ solutions with tetramethylsilane (TMS) as an internal reference. Gas chromatograms were recorded on a Shimadzu GC-14A (PEG-20M Bonded, 25 m) and GC-9A (Chiraldex G-TA, 20 m, 70 °C) gas chromatographs.

Materials. Organic reagents and solvents were purchased from Nacalai Tesque, Inc. and Aldrich Chemical Co.

General Procedure for Cultivation of Microbes. *Geotrichum candidum* (IFO 4597): In a 1 L of 0.1 M (1 M=1 mol dm^{-3}) potassium phosphate buffer at pH 6.2, 30 g of glycerol, 10 g of yeast extract, and 5 g of polypeptone

were dissolved. The solution was sterilized for 20 min at 121 °C in an autoclave, then the microbe was cultivated at 27 °C for 2 d. The mixture was filtered on a filter paper.

***Endmyces magnusii* (IFO 4600):** Composition of a culture medium and the procedure for cultivation were the same as that for *G. candidum*.

***Mucor javanicus* (IAM 6101):** In a 1 L of deionized and distilled water, 33.4 g of glucose, 10 g of KNO_3, 2.5 g of $MgSO_4 \cdot 7H_2O$, 5.0 g of KH_2PO_4, 0.25 mg of $FeSO_4 \cdot 7H_2O$, and 2.5 mg of $ZnSO_4 \cdot 7H_2O$ were dissolved. The pH of the solution was kept at 4.5. The solution was sterilized for 20 min at 121 °C in an autoclave, then the microbe was cultivated at 27 °C for 3d. The mixture was filtered on a filter paper.

***Candida tropicalis* (IAM 6052):** In a 1 L of deionized and distilled water, 20 g of glucose, 1 g of corn steep liquor, 1 g of $MgSO_4 \cdot 7H_2O$, 5 g of $NH_4H_2PO_4$, 2.5 g of KH_2PO_4, and 1 mg of $FeCl_3$ were dissolved. The pH of the solution was kept at 6.2. The solution was sterilized for 20 min at 121 °C in an autoclave, then the microbe was cultivated at 27 °C for 2d. The mixture was filtered on a filter paper.

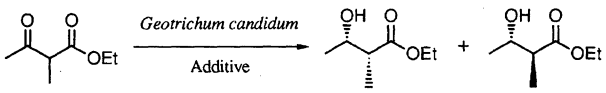
Reduction of Ethyl 2-Methyl-3-oxobutanoate with a Microbe. In general, a suspension of 2.5 g of microbe in 22.5 mL of water was preincubated for 1 h at 30 °C in the presence or absence of 0.75 mmol of an appropriate additive, then, 0.50 mmol of ethyl 2-methyl-3-oxobutanoate (**1**) was added to this reduction system. The mixture was shaken at 100 rpm for 24 h at 30 °C. Hyflo Super-Cel and ethyl acetate were added, and the mixture was filtered. The precipitates were washed with ethyl acetate. The combined washings and filtrate were washed with water and brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (5/1) used as an eluent, giving the hydroxy ester **2**. The chemical yields are listed in Table 2.

Determination of Enantiomeric and Diastereomeric Excesses. Diastereomeric excesses (d.e.) in the product **2**, were determined with GLC equipped with a capillary column PEG 20 M (Bonded, 0.25 mm×25 m, 110 °C).[17] Enantiomeric excesses (e.e.) in the product **2** were determined with GLC equipped with a capillary column Chiraldex G-TA (0.25 mm×20 m, 70 °C). The absolute configuration of the isomer corresponding to each peak of ethyl 3-hydroxy-2-methylbutanoate obtained from the reduction of **1** by $NaBH_4$ (composed of all four isomers) was determined by comparing its retention time with those of the authentic samples prepared by methylation of racemic and ethyl (*S*)-3-hydroxybutanoate, and by the reduction of **1** with bakers' yeast.[3] The e.e. and d.e. values are listed in Tables 1 and 2.

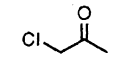
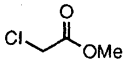
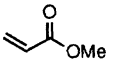
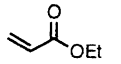
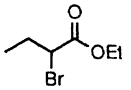
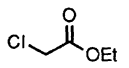
Results and Discussion

When bakers' yeast is incubated with MVK before it is employed for the reduction of **1**, *syn*-(2*R*,3*S*)-hydroxy ester, *syn*-**2**, is obtained selectively.[14] The improvement of stereoselectivity was accounted for by the difference in inhibition constant, K_i, toward MVK between two enzymes that participate to the reduction with different stereoselectivity.[16] The method for improving the stereoselectivity of the reduction by bakers' yeast might be applicable effectively to the reduction with other microbes, provided the microbe contains plural enzymes participating the reduction. We therefore investigated the effect of an additive on diastereoselectivity of microbial reduction. Previous research has reported that *Geotrichum candidum* is able to reduce various β-keto esters.[5,18—23] Because organic chemists can cultivate this mold easily, it was employed as the typical microbe for investigation.

Table 1. Effect of Additive on the Diastereoselectivity of *Geotrichum candidum* Reduction[a]



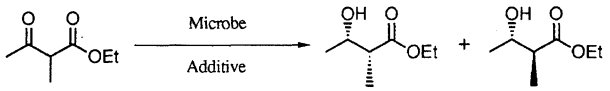
1 2-syn 2-anti

Additive[b]	*syn*/*anti* Ratio	Conversion[c]/%
None	44/56	100
	3/97	96
	3/97	3.3
	7/93	50
	10/90	79
	11/89	84
	19/81	98
	21/79	6.3

a) *G. candidum*, 2.5 g; Water 22.5 ml; Substrate, 0.50 mmol; Additive, 0.75 mmol. b) Preincubated for 1 h in the presence of an additive at 30 °C. c) Determined on GLC after 24 h.

Effect of Additive on Diastereoselectivity. Reduction of **1** by *G. candidum* without an additive affords the corresponding *anti*-hydroxy ester, *anti*-**2**, in 12% d.e. (Table 1). For the purpose of screening a reagent effective for improving diastereoselectivity of the reduction with *G. candidum*, various organic and inorganic compounds were added to a reaction mixture on preincubation at 30 °C for 1 h, and **1** was then added to this preincubated system. Diastereoselectivities associated with the reductions in the presence of effectual additive are summarized in Table 1. Certain alkylating reagents are effective for improving the *anti*-selectivity of the reduction. α-Halo carbonyl compounds are particularly effective, whereas α-halo esters retard the reaction drastically. α,β-Unsaturated carbonyl compounds also improve diastereoselectivity in the *anti*-product. MVK is again a useful additive for improving the stereoselectivity for the present reaction. Chloroacetone (CA) and

Table 2. Effect of Additive on the Diastereoselectivity of Microbial Reduction[a)]



1 2-syn 2-anti

Microbe	Additive[b)]	*syn*/*anti*	e.e / %		yield / %
			(2*R*,3*S*)/-*syn*	(2*S*,3*S*)/-*anti*	
Geotrichum candidum	None	47/53	>99	95	62
	MVK	6/94	>99	95	43
	CA	4/96	>99	95	52
Endmyces magnusii	None	31/69	>99	90	72
	MVK	9/91	>99	92	58
	CA	6/94	—	80	46
Mucor javanicus	None	25/75	>99	>99	39
	MVK	10/90	>99	>99	30
Bakers' yeast[c)]	None	87/13	>95	>95	75
	MVK	96/ 4	>95	>95	72
Candida tropicalis	None	81/19	>99	99	58
	MVK	86/14	>99	93	40

a) Microbe, 2.5 g; Water, 22.5 ml; Substrate, 0.50 mmol. b) Preincubated for 1 h in the presence of an additive at 30 °C. c) Data from Ref. 16.

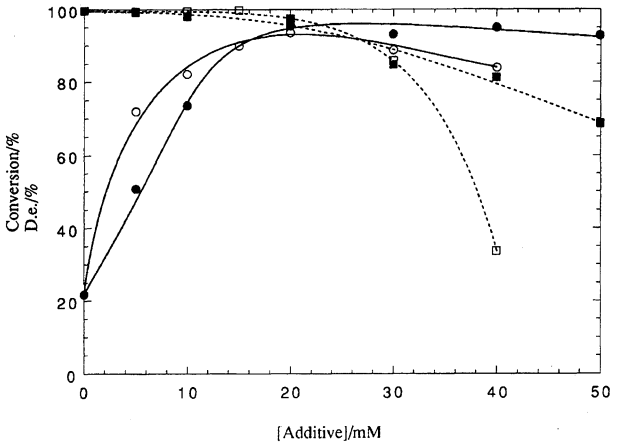


Fig. 1. Effect of MVK (open symbols) and CA(closed symbols) on diastereoselectivity (circles) and reactivity (squares) of the reduction of ethyl 2-methyl-3-oxobutanoate **1** by *G. candidum*.

MVK were employed as additives for further studies, because of their excellent selectivity and reactivity.

In Fig. 1, diastereoselectivity in the product is shown as a function of the concentration of additive. The diastereoselectivity in the *anti*-product increases with an increase in the concentration of additive; the best result was obtained with a concentration of 20 mM for both additives, but the efficiency of reduction decreases drastically after this concentration. For the reduction by bakers' yeast, mechanism for the participation of MVK has been elucidated at enzyme level.[16)] Similar mechanism might be operating here for *G. candidum*.

Stereoselective Reduction with a Microbe. As described above, we expect that the addition of an additive to a microbial transformation system affects the stereochemical outcome. We, therefore, extended the method to other microbial reductions. Since susceptibility of the reduction to an additive depends on a microbe, concentration of the additive has to be optimized for each microbe. Thus elucidated optimum concentrations of MVK or CA are 20, 25, 10, 83, and 25 mM for *G. candidum*, *Endmyces magnusii*, *Mucor javanicus*, bakers' yeast, and *Candida tropicalis*, respectively. The results are summarized in Table 2. The absolute configurations and enantiomeric excesses in products were determined on a chiral capillary GC-column (Chiraldex G-TA), cf. Experimental section. *G. candidum*, *E. magnusii*, and *M. javanicus* afford the *syn*-product preferentially, whereas bakers' yeast and *C. tropicalis* prefers to afford the *anti*-product in the presence of the additive. It is interesting to note that the microbes in the former group are molds and those in the latter are yeasts.

We have thus elucidated that the introduction of an additive to a microbial reduction improves the diastereoselectivity in great extent, and affords satisfactory results in the preparation of *anti*-(2*S*,3*S*)- and *syn*-(2*R*,3*S*)-hydroxy esters by the use of various microbes. We believe that the present technique is applicable to other microbiological transformations, provided unsatisfactory results stem from the operation of plural enzymes. The detailed mechanism for the present reduction at enzyme level will be reported elsewhere.

The present work was partially supported by a Grant-in-Aid for Scientific Research No. 06740549 from the Ministry of Education, Science and Culture.

References

1) R. W. Hoffman, W. Ladner, K. Steinbach, W. Massa, R. Schmidt, and G. Snatzke, *Chem. Ber.*, **114**, 2786 (1981).
2) H. Akita, A. Furuichi, H. Koshiji, K. Horikoshi, and T. Oishi, *Chem. Pharm. Bull.*, **31**, 4376 (1983).
3) G. Fráter, U. Müller, and W. Günther, *Tetrahedron*, **40**, 1269 (1984).
4) T. Itoh, Y. Yonekawa, T. Sato, and T. Fujisawa, *Tetrahedron Lett.*, **27**, 5405 (1986).
5) D. Buisson, C. Sanner, M. Larcheveque, and R. Azerad, *Tetrahedron Lett.*, **28**, 3939 (1987).
6) W.-R. Shieh and C. J. Sih, *Tetrahedron: Asymmetry*, **4**, 1259 (1993).
7) For simplicity of expression, we use the terms *syn* and *anti* for *erythro* and *threo*, respectively.
8) K. Nakamura, T. Miyai, K. Nozaki, K. Ushio, S. Oka, and A. Ohno, *Tetrahedron Lett.*, **27**, 3155 (1986).
9) K. Nakamura, T. Miyai, A. Nagar, S. Oka, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **62**, 1179 (1989).
10) K. Nakamura, S. Takano, and A. Ohno, *Tetrahedron Lett.*, **34**, 6087 (1993).
11) K. Nakamura, Y. Kawai, T. Miyai, S. Honda, N. Nakajima, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **64**, 1467 (1991).
12) Y. Kawai, M. Tsujimoto, S. Kondo, K. Takanobe, K. Nakamura, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **67**, 524 (1994).
13) Y. Kawai, K. Takanobe, M. Tsujimoto, and A. Ohno, *Tetrahedron Lett.*, **35**, 147 (1994).
14) K. Nakamura, Y. Kawai, T. Miyai, and A. Ohno, *Tetrahedron Lett.*, **31**, 3631 (1990).
15) K. Nakamura, Y. Kawai, and A. Ohno, *Tetrahedron Lett.*, **32**, 2927 (1991).
16) Y. Kawai, S. Kondo, M. Tsujimoto, K. Nakamura, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **67**, 2244 (1994).
17) K. Nakamura, T. Miyai, A. Nagar, B. R. Babu, T. Ando, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **63**, 298 (1990).
18) B. Wipf, E. Kupfer, R. Bertazzi, and H. G. W. Leuenberger, *Helv. Chim. Acta*, **66**, 485 (1983).
19) R. Bernardi, R. Cardillo, and D. Ghiringhelli, *J. Chem. Soc., Chem. Commun.*, **1984**, 460.
20) D. Buisson and R. Azerad, *Tetrahedron Lett.*, **27**, 2631 (1986).
21) D. Buisson, S. Henrot, M. Larcheveque, and R. Azerad, *Tetrahedron Lett.*, **28**, 5033 (1987).
22) D. Buisson, R. Azerad, C. Sanner, and M. Larcheveque, *Biocatalysis*, **3**, 85 (1990).
23) D. Buisson, R. Azerad, C. Sannere, and M. Larcheveque, *Tetrahedron: Asymmetry*, **2**, 987 (1991).