

Tetrahedron Vol. 51, No. 3, pp. 687-694, 1995 Copyright © 1995 Elsevier Science Ltd Printed in Great Britain. All rights reserved 0040-4020/95 \$9.50+0.00

0040-4020(94)00970-8

Mechanistic Study for Stereochemical Control of Microbial Reduction of α -Keto Esters in an Organic Solvent¹

Kaoru Nakamura,* Shin-ichi Kondo, Nobuyoshi Nakajima[†], and Atsuyoshi Ohno

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

¹Department of Nutritional Science, Faculty of Health and Welfare, Okayama Prefectural University, Soja, Okayama 719-11, Japan

Abstract: To elucidate the mechanism for stereochemical control of yeast reduction of α -keto esters in organic media, seven enzymes responsible for the reduction have been isolated from bakers' yeast and kinetic parameters for enzymatic reductions have been measured. In yeast reduction of ethyl 3-methyl-2oxobutanoate (1f), enantiomeric excess in the produced (R)-hydroxy ester increases when an organic solvent is used as the reaction medium in place of water. Difference in K_m of the enzymes contributes largely to the stereochemistry of reduction by whole yeast cell. Four enzymes contribute to catalytic reduction of 1f. K_m of the (R)-producing enzyme has been found to be the lowest among those of four enzymes. Stereochemical course of the reduction shifts toward the (R)-product by lowering the substrate concentrations, because the (R)-producing enzyme is most active among the enzymes under diluted conditions. In yeast reduction of ethyl 2-oxohexanoate (1d), the corresponding (S)-hydroxy ester is obtained in water, whereas the antipode is given in benzene. Five enzymes participate to the reduction of 1d and the (R)-producing enzymes have smaller K_m s than those of the (S)-producing enzymes. When the reaction is run in benzene, however, the produced α -hydroxy ester does not undergo further decomposition. The inhibition of enzymatic decomposition in an organic solvent is also accounted for by low concentration of α -hydroxy ester in aqueous phase surrounding the bakers' yeast.

Microbial reductions have widely been used to synthesize chiral alcohols because of their easiness in treatment and mild reaction conditions.²⁻⁴ Unfortunately, however, enantioselectivities of microbial reductions are not usually satisfactory, and it is necessary to improve low enantioselectivity of yeast reduction. Until now, several methods have been developed for this purpose. Among them, methods to control stereochemistry of yeast reduction without changing microbes have been reported, *i.e.*, modification of substrate,⁵⁻⁹ addition of an inorganic salt,¹⁰ addition of an inhibitor to the specific enzyme,¹¹⁻¹⁵ and thermal treatment.^{16,17} To explain mechanism of stereochemical control from the viewpoint of enzyme chemistry, enzymes that catalyze yeast reduction of β -keto esters have been isolated from bakers' yeast and purified.^{15,18}

Recently, we reported that the use of an organic solvent affects largely the stereochemistry of yeast reduction of α -keto esters (Scheme 1).¹⁹⁻²² For example, ethyl 2-oxoheptanoate (1e) was reduced to (S)-ethyl 2-hydroxyheptanoate ((S)-2e) in 92% e.e. and to the antipode, (R)-2e, in 86% e.e. by the reaction in water and benzene, respectively.

The same tendency was observed in the reduction of 1f. Here, although both reactions in water and in benzene afford the same isomer, (R)-products, enantioselectivity increases from 19% e.e. to 90% e.e. by changing the medium from water to benzene. Enantioselectivity of the reduction shifts to (R)-side in nonpolar organic solvent such as benzene or hexane compared to that in water.^{21,22)}

$\begin{array}{c} \bigcirc H \\ R \\ \hline CO_2Et \end{array} \xrightarrow{\text{bakers' yeast}} R \\ \hline O \\ in water \\ (S)-2 \\ \hline 1 \\ \hline \end{array} \xrightarrow{\text{bakers' yeast}} R \\ \hline O \\ O \\ O \\ CO_2Et \\ \hline \text{in benzene} \\ R \\ \hline O \\ O \\ CO_2Et \\ \hline \end{array} \xrightarrow{\text{bakers' yeast}} R \\ \hline O \\ O \\ O \\ CO_2Et \\ \hline O \\ O \\$							
		water			benzene		
	R	Yield (%)	E.e. (%)	Config.	Yield (%)	E.e. (%)	Config.
a	CH ₃	79	93	S	56	73	S
b	C_2H_5	44	78	S	39	13	R
c	C_3H_7	23	76	S	44	63	R
d	C ₄ H ₉	5	99	S	54	86	R
e	$C_{5}H_{11}$	9	92	S	26	86	R
f	(CH ₃) ₂ CH	63	19	R	49	90	R

We proposed that concentration of α -keto ester in yeast cell plays an important role for the stereochemistry of reduction in an organic solvent system.²² Since the substrate is more soluble in benzene than in water, the substrate concentration in the aqueous phase around yeast cell becomes small when an organic solvent is employed as the bulk reaction medium. Improvement of enantioselectivity by using a diluted substrate concentration in water has been reported for yeast reduction of ethyl acetoacetate.²³ Thus, substrate concentration is one of the major factors that affect stereochemistry of microbial reductions. Mechanism for enhancement of enantioselectivity as a function of substrate concentration has been investigated with the wholecell system,²⁴ whereas the mechanism has not been reported for an enzymatic system. Enzymatic studies are necessary to elucidate the effect of substrate concentration on enantioselectivity of the reduction. In this paper, we report the mechanism of solvent effect on stereochemistry of the yeast reduction on the basis of kinetic parameters of α -keto ester reductases from bakers' yeast.

In addition, it has been found that α -hydroxy esters produced by the yeast reduction is decomposed enzymatically in water (Scheme 2) and this asymmetric decomposition does not take place during the reduction in an organic solvent such as benzene.²² However, mechanistic feature of this phenomenon has not been elucidated yet. This enzymatic decomposition is inhibited in the presence of butanesulfonyl fluoride.²⁵ The present paper will also report the mechanism of asymmetric decomposition of α -hydroxy ester catalyzed by bakers' yeast.

Scheme 2



Further decomposition

Scheme 1

Michaelis-Menten Constant

When plural enzymes contribute to a reaction, difference in K_m of the enzyme affects largely the rate of reaction catalyzed by each enzyme. For simplicity, a two-enzyme system is considered here. Suppose that *R*-enzyme and *S*-enzyme produce the (*R*)- and (*S*)-products, respectively. Let K_m and V_{max} of each enzyme be fixed at $K_{m,R} = 0.1 \text{ mM}$ and $V_{max,R} = V_{max,S} = 0.1 \text{ mM s}^{-1}$ with a variable $K_{m,S}$. Then, enantioselectivities in the product at 99% conversion are calculated with several $K_{m,S}$ and initial substrate concentrations ([S]_{in} as plotted in Fig.1.

The enantioselectivity of reaction depends on initial substrate concentration. When it is low, stereoselectivity of the reduction shifts toward the (R)-product in all cases. The highest enantiomeric excess is seen in the system of $K_{m,R} \ll K_{m,S}$ with diluted initial substrate concentrations. The result indicates that enantioselectivity of the reaction in preference of the (R)-product is improved when initial substrate concentration is kept low and K_m s of the enzymes differ largely.

Kinetic Parameters for the Reduction by YKERs

As reported previously, seven enzymes contribute to the reduction of α -keto esters (yeast keto ester reductases, YKERs).²⁶ One of these (designated as YKER-III) is identified to be yeast alcohol dehydrogenase, which depends on NADH and catalyzes ethanol-acetaldehyde oxidoreduction in yeast cell. YKER-I, -VI, and -VII correspond to L-enzyme-1, L-enzyme-2, and D-enzyme-2 for β -keto esters, respectively, purified and reported previously.¹⁵ The other three reductases (YKER-II, -IV, and -V) are reductases specific to α -keto esters. Enantioselectivities of YKERs for the reduction of six α -keto esters were determined. The results are summarized in Table 1.



Fig. 1. Effects of initial substrate concentration and Michaelis-Menten constant on enantioselectivity of reaction.

Reduction of ethyl pyruvate (1a) with YKER-I affords (S)-2a in an excellent e.e., whereas the reduction of 1d with the same enzyme gave (R)-2d in 90% e.e. Stereoselectivity changes from S-preference to R-

preference by elongating the chain length of substrate. YKER-I does not catalyze the reduction of 1f. YKER-II and -III are effective to the reduction of 1a only. YKER-IV is excellent in enantioselectivity to afford the (R)-hydroxy esters in all reductions so far studied. On the other hand, YKER-V affords the (S)-hydroxy esters in all the substrates used here. It is interesting to note that, although YKER-VI affords the (S)-product in the reduction of 1a and 1f, the same enzyme afforded (R)-product in the reduction of 1c, 1d, and 1e. YKER-VII results in the formation of (S)-product in the reduction of 1b-1e, whereas the enzyme affords the (R)-product from 1f.

Enzyme	E.e. (%) [Configuration]						
	1a	1b	1c	1 d	1e	1f	
YKER-I	98.2 <i>[S</i>]	71.0[S]	90.3 [R]	90.1 [<i>R</i>]	99.1 [<i>R</i>]		
YKER-II	98.2[S]					<u> </u>	
YKER-III	97.9 [S]						
YKER-IV	>99 [<i>R</i>]	>99 [R]	96.5 [R]	88.3 [R]	>99 [R]	>99 [<i>R</i>]	
YKER-V	93.5 [S]	83.0 [<i>S</i>]	59.7 [S]	31.4 [S]	8.3 [S]	76.8[S]	
YKER-VI	94.5 [S]	17.6[S]	87.8 [<i>R</i>]	93.6 [<i>R</i>]	94.9 [<i>R</i>]	91.8[S]	
YKER-VII		94.5 [S]	43.8 [S]	83.9 [S]	94.4 [S]	67.8[R]	

Table 1. Enantioselectivities of YKERs for the Reduction of α -Keto Esters

High enantiomeric purities in the (R)-hydroxy esters at yeast reduction of α -keto esters (1b-1f) in benzene predicts quite reasonably that the R-enzyme(s) has smaller K_m than the S-enzyme(s). To prove this hypothesis, K_m s and k_{cal} s of seven YKERs with three α -keto esters were measured. Results are summarized in Table 2.

Enzyme	$K_{\rm m}$ (mM) / $k_{\rm cat}$ (s ⁻¹) / $V_{\rm max}$ (U kg ⁻¹ yeast) ^{a)}					
	1a	1 d	1f			
YKER-I	4.73 / 4.91 / 121	8.40 / 1.53 / 37.7				
YKER-II	135 /29.2 / 918					
YKER-III	590 /28 /10043					
YKER-IV	0.434 / 1.66 / 15	0.142/ 4.59/ 41	0.265 / 8.12 / 73			
YKERV	5.06 /30.5 / 712	5.72 / 27.8 / 649	79.4 / 37.0 / 864			
YKER-VI	3.92 / 0.426/ 360	1.03 / 2.10/1774	44.0 / 0.67/ 566			
YKER-VII	b)	27.3 /127 / 501	93.6 /121 / 477			

Table 2. Kinetic Parameters of YKERs for the Reduction of α -Keto Esters

a) V_{max} s were estimated from chromatogram in enzyme purification. b) K_{m} was too large to determine (over 300 mM).

To reproduce stereochemistry of yeast reduction in benzene from the activity of each enzyme, total unit of

each enzyme should be elucidated. However, since the amount of denatured enzyme during purification is not the same for all, it is difficult to elucidate it exactly. Therefore, we employed estimated total unit (V_{max}) for each substrate, which is a parameter calculated on the basis of activity of total enzyme isolated after purification. The values are listed in Table 2. Yeast reduction of 1a in water affords (S)-2a in 93% e.e., whereas 73% e.e. was observed in benzene. The stereochemistry of reduction shifts toward (R)-side in benzene. As seen in Table 1, since all enzymes except for YKER-IV afford (S)-2a on reduction of 1a, YKER-IV is the sole enzyme responsible to the production of (R)-2a. Since K_m of YKER-IV against 1a is one to two orders of magnitude smaller (0.43 mM) than those of the other enzymes, contribution of YKER-IV becomes more important in benzene than in water because of the effect of substrate concentration. However, since V_{max} of YKER-IV for this substrate is much smaller than those of the others, yeast reduction results in the production of (S)-2a even in benzene: e.e. decreases form 93% (water) to 73% (benzene) showing the (R)-shift. Yeast reduction of 1d gives (S)- and (R)-2d in water and benzene, respectively. Enzymatically, YKER-V and -VII yield (S)-2d, and YKER-I, -IV, and -VI afford (R)-2d. Kinetic study reveals that YKER-IV and -VI have small K_m (0.14 mM and 1.03 mM, respectively). Then, the reduction of 1d in benzene is catalyzed mainly by YKER-IV and -VI affording (R)-2d, whereas the reduction in water is also contributed by YKER-V and -VII. Although stereoselectivity of the reduction in benzene is, thus, explainable quantitatively (e.e. of yeast reduction = 86% (R) and that of YKER-IV = 88% (R)) on the basis of enzyme activity, that in water can be reproduced only qualitatively: e.e. of yeast reduction (99% (S)) exceeds that of enzymatic reduction (31% (S) and 84% (S) for YKER-V ($K_m = 5.7 \text{ mM}$) and -VII ($K_m = 27 \text{ mM}$), respectively). The phenomenon can be accounted for by asymmetric decomposition of the (R)-product, which will be discussed later. Enantioselectivity of yeast reduction of 1f is better in benzene (90% (R)) than that in water (19% (R)). Both YKER-IV and -VII give (R)-2f, whereas YKER-V and -VI afford (S)-2f. Kinetic study reveals that K_m of YKER-IV is the smallest among those of the contributing enzymes. Then, the reduction in benzene is mainly contributed by YKER-IV resulting in the formation of (R)-2f in an excellent e.e. Yeast reduction of 1f affords (R)-2f in water although the e.e. is low. The observation stems from the fact that 2f is a stable compound, unlikely other hydroxy esters, under the reaction conditions. Thus, the e.e. observed here represents true enantioselectivity of the reduction, and, therefore, **If** might be the best substrate for the discussion of enantioselectivity in the reduction.

As was discussed above, YKER-IV has extremely high enantioselectivity in reduction of α -keto esters and the reduction affords the corresponding (R)-hydroxy ester. Furthermore, YKER-IV has the smallest K_m . These characteristics of YKER-IV might explain stereoselectivity of yeast reduction of α -keto esters in a dilute substrate concentration, or in an organic solvent, as shown in Scheme 1 despite the fact that V_{max} of YKER-IV for **1f** is smaller than those of other enzymes. Indeed, the rate of each enzyme-catalyzed reduction of **1f** was calculated by Michaelis-Menten equation from K_m s and estimated V_{max} s, and ratios of the rates of reduction of **1f** were found to be YKER-IV : -V : -VI : -VII = 85.7 : 5.4 : 6.4 : 2.5 and 14.2 : 34.3 : 34.9 : 16.6 at 0.16 mM and 20 mM of substrate concentrations, respectively. The result confirms that YKER-IV plays a major part in the reduction by whole yeast cell under dilute substrate concentrations.

Enzymatic Decomposition of α -Hydroxy Esters

During yeast reduction in water, certain α -hydroxy esters (2c, 2d, and 2e), the products, are decomposed under the catalysis of bakers' yeast.^{19,22} Thus, the (*R*)- α -hydroxy esters are decomposed preferentially remaining the antipodes unaffected. The asymmetric decomposition is one of the tricks for stereochemical control of yeast reduction of α -keto esters in water. For example, when racemic 2e was incubated with bakers' yeast in water, the *R*-isomer disappeared after 2 h and only the *S*-counterpart was recovered.²² On the other hand, the enzymatic decomposition of (*R*)-2e was not observed when the reaction was run in benzene under the same conditions as those for the reduction in benzene. Suppress of asymmetric decomposition in benzene is also accounted for by the decrease in substrate concentration in the aqueous phase. Apparent K_m for the decomposition was determined to be 2.2 mM by measuring initial rate of the decomposition of 2e catalyzed by whole bakers' yeast cell in water. Partition coefficient of 2e between water and benzene ([2e]_{benzene} / [2e]_{aq}) was determined to be 2.8 × 10², then the maximum concentration of 2e in aqueous phase during the reaction in benzene was calculated to be 7.1×10^{-2} mM. Since the concentration of 2e in the aqueous phase is much smaller than K_m for the decomposition, the rate of decomposition of the produced α -hydroxy esters is dramatically depressed in benzene.

The concept of stereochemical control by changing substrate concentration may be applicable to all multienzyme systems when K_m of the corresponding highly enantioselective enzyme is smaller than those of the others. We recently reported that enantioselectivity of the reduction of ketopantolactone by bakers' yeast was improved by diluting concentration of the substrate.^{27,28} The observation is again understandable from the viewpoint of stereochemistry as functions of substrate concentration and kinetic parameter.

EXPERIMENTAL SECTION

Instruments

¹H NMR spectra were recorded at 200 MHz on a Varian VXR-200 Fourier transform NMR spectrometer. Gas chromatographic data were recorded on a Shimadzu GC-14A and Yanaco G-2800 gas chromatographs with a Shimadzu CR-6A Chromatopac. Isolations of materials with gas chromatography were performed with a Varian Aerograph Model 920 gas chromatograph.

Materials

Ethyl pyruvate and ethyl lactate were purchased from Nacalai Tesuqe Co., Ltd. Ethyl 3-methyl-2-oxobutanoate and (R)-(+)- α -methoxy- α -(trifluoromethyl)phenylacetic acid were purchased from Aldrich Chemical Co., Inc. Other α -keto and α -hydroxy esters were prepared according to the literature.¹⁹ NADH and NADPH were purchased from Kojin Co., Ltd.

Preparation of YKERs

All YKERs were isolated from cells of bakers' yeast (Oriental Yeast Co., Ltd.) and purified as described previously.²⁴

Determination of K_m and k_{cat} for Each YKERs

A 50 μ l aliquot of enzyme solution was added to 2.95 ml of 0.10 M potassium phosphate buffer (pH 7.0) containing NAD(P)H (0.1 mM) and an appropriate α -keto ester. Since the activity of an enzyme changes from a substrate to another, the amount of enzyme was controlled appropriately depending on the substrate in order to obtain reliable kinetics. Initial rate of reduction by YKER was followed spectrophotometrically at 30 °C by following the decrease in absorbance of NAD(P)H at 340 nm. K_m and k_{cat} of YKER were calculated from initial rates of reaction with appropriate range of substrate concentrations. At least five different concentrations were employed for obtaining an [S]/v-[S] plot.

Determination of Enantioselectivity in the Reaction with YKER

An enzyme solution was added to a solution (2.0 ml) containing NAD(P)H (0.04 mmol) and an α -keto ester (0.04 mmol), and the mixture was incubated at 30 °C. The reaction was monitored by GLC (PEG 20M, 20 m, 100–120 °C). After the starting material disappeared, the product was extracted with ether (2 × 5 ml). Combined ether layer was washed with brine, and dried over anhydrous magnesium sulfate, then the solvent was evaporated under reduced pressure. Enantiomeric excesses in α -hydroxy esters, except for ethyl lactate, were measured by GLC equipped with a chiral stationary phase (Tokyo Kasei Co., Ltd., G-TA, 30 m, 100 °C), whereas that in ethyl lactate was determined by capillary GLC (PEG 20M, 25 m, 170 °C) of the corresponding MTPA ester derivative.²⁹

Determination of Apparent K_m for Decomposition of α -Hydroxy Ester by Whole Yeast Cell

Bakers' yeast (200 mg) was added to a solution of rac-2e (0.6 – 7.0 mM, 8 points) in water. The reaction mixture was stirred at 30 °C. An aliquot (400 µl) was withdrawn from the reaction mixture at each 5 min and added to a 300 µl of ethereal solution of tetradecane (3 mM). The mixture was agitated vigorously to extract the remaining 2e. Concentration of 2e was determined by GLC (OV 330, 1m, 120 °C) with tetradecane as an internal standard. Initial rate of reaction was calculated from the decrease in concentration of 2e. The apparent $K_{\rm m}$ was determined by curve fitting for initial concentration-initial reaction rate relationship. The $K_{\rm m}$ and $V_{\rm max}$ were, thus, calculated to be 2.2 mM and 7.2 mmol min⁻¹ (mg yeast)⁻¹, respectively.

Determination of Partition Coefficient of 2f between Water and Benzene

A benzene solution (1 ml) containing tetradecane (3 mM) and 2e (10.35 mM) was added to distilled water (30 ml). The biphasic mixture was stirred for 1 h at 30 °C. The concentration of 2e in benzene was determined by the same method as described above. Partition coefficient of 2e between water and benzene was calculated to be $(2.8 \pm 0.2) \times 10^2$ from the concentrations of 2e in each solvent.

Simulation of Enantioselectivity

Suppose a hypothetical reduction system composed of *n* enzymes. Each enzyme, enzyme *i*, has specific K_m , K_m^i and V_{max} , V_{max}^i toward a substrate. Then, the concentration of the product given by enzyme *i* at reaction time *t*, $[P]_i^i$ is calculated by Eq. 1.

$$[P]_{i}^{i} = \frac{V_{\max}^{i}[S]_{i}}{K_{m}^{i} + [S]_{i}}$$
(1)

, where $[S]_t$ represents the concentration of the substrate at a time interval of t. Let e_i be a parameter to indicate stereoselectivity associated with enzyme i. We can normalize e_i so that e_i assumes a value between -1 (complete S-selectivity) and +1 (complete R-selectivity) (Eq. 2).

$$-1 \le e_i \le +1 \tag{2}$$

Then, the concentrations of the R- and S-products at the final stage of the reaction in participation of all responsible enzymes, $[R]_f$ and $[S]_f$, respectively, are obtained by Eqs. 3 and 4.

$$[\mathbf{R}]_{r} = \int_{0}^{\infty} \sum_{i=1}^{n} \left\{ \frac{(1+e_{i})[P]_{i}^{i}}{2} \right\} dt$$

$$(3)$$

$$= \int_{0}^{\infty} \left[((1-e_{i})[P]^{i}) \right] dt$$

$$(4)$$

$$[S]_{r} = \int_{0}^{\infty} \sum_{i=1}^{n} \left\{ \frac{(1-e_{i})[P]_{i}^{i}}{2} \right\} dt$$
(4)

The infinity in reaction time may be substituted by an appropriate reaction time T, where the reaction is 99% completed. Furthermore, the integration may be substituted by summation experimentally affording Eqs. 5 and

6, provided the number of experimental points for t is large enough.

$$[\mathbf{R}]_{f} = \sum_{i=0}^{T} \sum_{i=1}^{n} \frac{(1+e_{i})[P]_{i}^{i}}{2}$$
(5)
$$[\mathbf{S}]_{f} = \sum_{i=0}^{T} \sum_{i=1}^{n} \frac{(1-e_{i})[P]_{i}^{i}}{2}$$
(6)

Finally, stereoselectivity in overall reaction by a whole-cell system, expressed on the basis of the *R*-product, is obtained by Eq. 7.

e.e. =
$$\frac{[R]_f - [S]_f}{[R]_f + [S]_f} \times 100$$
 (%) (7)

The stereoselectivities of hypothetical reduction systems have been calculated from Eq. 7 based on assumed values for K_m^i and V_{max}^i with $e_i = +1$ and -1 for *R*- and *S*-enzymes, respectively. The results are depicted in Fig. 1.

REFERENCES AND NOTE

- 1. Stereochemical Control in Microbial Reductions. Part 26.
- 2. Servi, S. Synthesis 1990, 1.
- 3. Csuk, R; Glänzer, B. I. Chem. Rev. 1991, 91, 49.
- 4. Mori, K. Tetrahedron 1989, 45, 3233.
- 5. Zhou, B.; Gopalan, A. S.; VanMiddlesworth, F.; Shieh, W.-R.; Sih, C. J. J. Am. Chem. Soc. 1983, 105, 5925.
- 6. Hirama, M.; Shimizu, M.; Iwashita, M. J. Chem. Soc., Chem. Commun., 1983, 599.
- 7. Nakamura, K.; Ushio, K.; Oka, S.; Ohno, A. Tetrahedron Lett. 1984, 25, 3979.
- 8. Fujisawa, T.; Itoh, T.; Sato, T. Tetrahedron Lett. 1984, 25, 5083.
- 9. Nakamura, K; Miyai, T.; Nagar, A.; Oka, S.; Ohno, A. Bull. Chem. Soc. Jpn. 1989, 62, 1179.
- 10. Nakamura, K; Kawai, Y.; Oka, S.; Ohno, A. Tetrahedron Lett. 1989, 30, 2245.
- 11. Nakamura, K; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. Chem. Lett. 1987, 679.
- 12. Nakamura, K; Kawai, Y.; Oka, S.; Ohno, A. Bull. Chem. Soc. Jpn. 1989, 62, 875.
- 13. Nakamura, K; Kawai, Y.; Ohno, A. Tetrahedron Lett. 1990, 31, 267.
- 14. Nakamura, K; Kawai, Y.; Miyai, T.; Ohno, A. Tetrahedron Lett. 1990, 31, 3631.
- 15. Nakamura, K; Kawai, Y.; Nakajima, N.; Ohno, A. J. Org. Chem. 1991, 56, 4778.
- 16. Nakamura, K; Kawai, Y.; Ohno, A. Tetrahedron Lett. 1991, 32, 2927.
- 17. Kawai, Y.; Kondo, S.; Tsujimoto, M.; Nakamura, K.; Ohno, A. Bull. Chem. Soc. Jpn. 1994, 67, 2244.
- 18. Shieh, W.-R.; Gopalan, A. S.; Sih, C. J. J. Am. Chem. Soc. 1985, 107, 2993.
- 19. Nakamura, K.; Inoue, K.; Ushio, K.; Oka, S.; Ohno, A. J. Org. Chem. 1988, 53, 2589.
- 20. Nakamura, K.; Miyai, T.; Inoue, K.; Kawasaki, S.; Oka, S.; Ohno, A. Biocatalysis 1990, 3, 17.
- 21. Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. Tetrahedron Lett. 1991, 32, 7075.
- 22. Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. Bull. Chem. Soc. Jpn. 1993, 66, 2738.
- 23. For example, Wipf, B.; Kupfer, E.; Bertazzi, R.; Leuenberger, H. G. W. Helv. Chim. Acta 1983, 66, 485.
- Chen, C.-S.; Zhou, B.-N.; Girdaukas, G.; Shieh, W.-R.; VanMiddlesworth, F.; Gopalan, A. S.; Sih, C. J. Bioorg. Chem., 1984, 12, 98.
- 25. Ushio, K.; Yamauchi, S.; Masuda, K. Biotechnol. Lett. 1991, 13, 495.
- 26. Nakamura, K.; Kondo, S.; Kawai, Y.; Nakajima, N.; Ohno, A. Biosci. Biotech. Biochem. in press.
- 27. Nakamura, K.; Kondo, S.; Kawai, Y.; Ohno, A. Tetrahedron: Asymmetry 1993, 4, 1253.
- 28. Nakamura, K.; Kondo, S.; Ohno, A. Bioorg. Med. Chem. 1994, 2, 433.
- 29. Dale, J. A.; Dull, D. L.; Mosher, H. S. J. Org. Chem. 1969, 34, 2543.

(Received in Japan 3 October 1994; accepted 28 October 1994)