Asymmetric Reduction of α-Keto Esters and α-Diketones with a Bakers' Yeast Keto Ester Reductase

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Optically pure α-hydroxy esters and α-hydroxy ketones have been synthesized by the reduction of the corresponding ketones with a keto ester reductase isolated from bakers' yeast (YKER-I). The reduction of α-keto esters affords the corresponding (*S*)- or (*R*)-hydroxy esters selectively, where the stereochemical course depends on the chain length of the alkyl substituent on the carbonyl group. An α-keto short alkanoic ester affords the corresponding (*S*)-hydroxy ester, whereas a long alkanoate yields the corresponding (*R*)-hydroxy ester. The reduction of α-diketones affords the corresponding (*S*)-2-hydroxy ketones regio- and stereoselectively.

Optically pure α-hydroxy carbonyl compounds have been used widely as chiral building blocks because of the ease of their transformation into other functional groups: e.g., *vic*-diols,[1] α-amino ketones,[2] halo esters,[3] glycols,[4] epoxides,[5] and other functional groups. A number of methods for the synthesis of α-hydroxy carbonyl compounds have been reported. These include hydrogenation of enol ethers[6] and ketones,[7] oxidation of enolates,[8] dihydroxylation of enol ethers,[9] microbial reduction of keto esters[10,11] and diketones,[12—15] and others. It is well known that biocatalysts are very efficient *reagents* to obtain such chiral compounds. Among them, bakers' yeast has widely been used for the synthesis of chiral compounds because of its availability and ease of operation.[16,17] However, the stereoselectivity and regioselectivity associated with the reduction of an artificial substrate by a whole yeast cell is not always satisfactory. For example, the bakers' yeast reduction of α-keto esters affords α-hydroxy esters with unsatisfactory stereoselectivity.[10,11] The reduction of α-diketones affords a mixture of regioisomers of ketols together with the corresponding diol.[12—15] No efficient system has yet been developed by biocatalytic approaches for this purpose.[18] We recently elucidated that, in the whole-cell reduction of α-keto esters, unsatisfactory enantioselectivity is the result of the simultaneous action of several enzymes that have opposite stereochemistry toward the same substrate.[19,20] It is conceivable that the unsatisfactory regioselectivity in the reduction of α-diketones is analogous to this situation. In such a case, the use of an isolated enzyme is recommended for obtaining a pure enantiomer. Recently, we reported the isolation of several keto ester reductases from microbes.[19,21—23] Among them, YKER-I[24] (Yeast Keto Ester Reductase-I) is the most useful enzyme in the field of organic synthesis because of its excellent stereoselectivity and easy availability.[20,25—29] In this paper, we would like to report the preparation of optically pure α-hydroxy carbonyl compounds by means of YKER-I.

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Results and Discussion

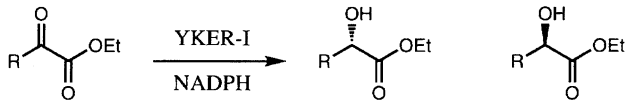
Reduction of α-Keto Esters. A series of α-keto esters was subjected to the reduction with YKER-I (Eq. 1) and the results are listed in Table 1. The substrates[11] and the reductase[21] were prepared according to the literature. D-Glucose-6-phosphate/D-glucose-6-phosphate dehydrogenase couple was employed to regenerate NADPH from $NADP^+$. In all cases the reduction proceeded with a satisfactory stereoselectivity to give the corresponding α-hydroxy esters, whereas the chemical yields are moderate to low. It is well known that α-keto esters are too labile to exist in water even under neutral conditions to form α-keto acids. For example, the half-life of **1b** is about 5 h under the reaction conditions. The resulting α-keto acid is not a substrate for YKER-I. Thus the enzymatic reduction has to compete with the spontaneous hydrolysis of a substrate. In practice, there exists a correlation between the relative activity of the

Table 1. Asymmetric Reduction of α-Keto Esters **1** with YKER-I

	R	Relative activity[a]/%	Yield/%	ee/%	Configuration
a	Me	100	35	98	*S*
b	Et	30	2.5	82	*S*
c	Pr	13	4.1	>99	*R*
d	Bu	18	22	>99	*R*
e	Pentyl	21	35	>99	*R*

a) Determined spectrophotometrically.

enzyme and chemical yield, as shown in Table 1. The chemical yield would be much improved if a large amount of the enzyme was used. In order to stabilize the substrate kinetically, the corresponding *t*-butyl ester was employed as the substrate. Although the chemical yield was improved appreciably by this procedure, the stereoselectivity decreased to a level unsatisfactory for synthetic purposes in every substrate (data not shown).



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

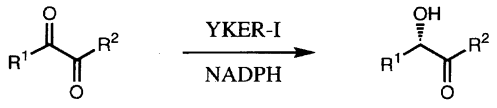
In a series of reductions of α-keto acid derivatives so far reported, the enzyme always affords a single stereoisomer selectively: e.g., L-lactate dehydrogenase,[30] D-lactate dehydrogenase,[31] hydroxyisocaproate dehydrogenase,[32] and glycerol dehydrogenase.[33] Contrary, it is interesting to note that the stereochemistry of the reduction with YKER-I changes from *S*- to *R*-preference by elongating the chain length of a substrate. We reported previously that, in a series of the reduction of β-keto esters with YKER-I, the elongation of carbon chain in a substituent on the carbonyl group much decreases the reactivity of this enzyme.[26] Both reactivity and selectivity reported herein suggest, although details are yet unclarified, that the pocket of YKER-I has a shape that can accommodate a methyl group adjacent to a carbonyl group to be reduced most appropriately, but it is stressed when an alkyl group larger than a methyl group is forced to come in.

Reduction of α-Diketones. α-Diketones **3** were prepared according to the literature and were subjected to the reduction with YKER-I (Eq. 2). The results are listed in Table 2. D-Glucose/D-glucose dehydrogenase couple was employed to regenerate NADPH from $NADP^+$.[26] When the substituent R^1 is a methyl group, both stereoselectivity and regioselectivity are excellent for all the substrates, **3a—f**. However, the elongation of carbon chain in the substituent up to three decreases stereoselectivity, regioselectivity, and chemical yield, indicating that the acetyl group is reduced preferentially with this enzyme.

Table 2. Asymmetric Reduction of α-Diketones **3** with YKER-I

	R^1	R^2	Yield/%	ee/%	Configuration
a	Me	Pr	92	>99	*S*
b	Me	Pentyl	90	>99	*S*
c	Me	Ph	92[a]	>95	*S*
d	Me	2-Me-C_6H_4	67	97	*S*
e	Me	4-Me-C_6H_4	39	>98	*S*
f	Me	4-MeO-C_6H_4	36	>99	*S*
g	Et	Ph	26[b]	96	—[c]
h	Pr	Ph	10[d]	30	—[c]

a) 1-Hydroxy isomer was obtained in 2% yield. b) 1-Hydroxy isomer was obtained in 15% yield. c) Not determined. d) 1-Hydroxy isomer was also obtained in 7% yield.



3 (*S*)-**4** (2)

Absolute Configuration. The absolute configurations of the α-hydroxy esters **2a—e** were determined by GLC (Chiraldex G-TA): the procedures were the same as those reported in previous papers.[11,20] The absolute configurations of **4a** and **4b** were determined to be *S* by comparing its retention times with those of the corresponding authentic samples prepared from (*S*)-lactonitrile. The absolute configuration of **4b** was also determined to be *S* by comparing the optical rotation with that reported.[12] The absolute configuration of **4c** was determined to be *S* by comparing the ^1H NMR data of the corresponding (*R*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) ester with those reported.[13] The absolute configurations of **4d—f** were determined to be *S* by comparing their retention times with those of the corresponding authentic samples prepared from ethyl (*S*)-lactate.

Experimental

Instruments. UV-spectra were obtained on a Hitachi U-3210 spectrophotometer. ^1H NMR spectra were recorded on a Varian VXR-200 NMR spectrometer. Gas chromatographic data were recorded on a Shimadzu GC-14B (OV-1701, 25 m) and GC-9A (Chiraldex G-TA, CP-Cyclodextrin-B-236-M12) gas chromatographs with a Shimadzu CR-6A Chromatopac.

Materials. NADPH was purchased from Kojin Co., Ltd. D-Glucose dehydrogenase (from *Bacillus megaterium*) and D-glucose-6-phosphate dehydrogenase (from *Bacillus stearothermophilus*) were purchased from Sigma Chemical Co. Organic reagents and solvents were purchased from Nacalai Tesque Inc., Wako Pure Chemical Ind. Ltd., and Aldrich Chemical Co. α-Keto esters **1a—e**[11] and α-diketone **3b**[12] were prepared according to the literature, respectively. α-Diketones **3a** and **3c** were purchased from Aldrich Chemical Co. YKER-I was isolated from the cells of bakers' yeast (Oriental Yeast Co., Ltd.) and purified as described previously.[21]

Enzyme Assay. A 50 μL (0.05 units) of the solution of YKER-I was added to 3.00 mL of a solution of 0.10 M (1 M = 1 mol dm^{-3}) potassium phosphate buffer (pH 7.0) containing a substrate **1** (3 mM) and NADPH (0.09 mM). The reaction rate was determined spectrophotometrically at 30 °C by following the decrease in absorbance of NADPH at 340 nm. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the oxidation of 1 μmol of NADPH with **1a** per minute at 30 °C under the conditions employed.

General Procedure for Enzymatic Reduction of 1. In a glass reaction vessel were placed 25 mL of buffer solution (0.01 M, Tris-HCl, pH 7.0) containing 1.5 unit of enzyme, 0.5 mmol of **1**, 5 mg (6 μmol) of NADPH, 150 mg (0.5 mmol) of D-glucose-6-phosphate monosodium salt, and 0.5 mg of glucose-6-phosphate dehydrogenase. The reaction vessel was shielded from light. The reaction mixture was stirred magnetically for 24 h at 30 °C. The mixture was purified by column chromatography on silica gel with hexane/ethyl acetate (5/1) used as an eluent, giving the product **2**. The spectral data of the products were identical with those reported.[11] The stereoselectivity and absolute configuration of the α-hydroxy esters were determined by GLC (Chiraldex G-TA); the

procedures were the same as those reported in previous papers.[11,20] Chemical yields and e.e.s are summarized in Table 1.

Preparation of 1-Aryl-1,2-alkanedione. A slight modification of the reported method was adopted for the synthesis of these compounds.[34] Potassium permanganase (3.17 g, 20.0 mmol) was added slowly to a stirred 20 mL acetic anhydride solution containing 3.78 mmol of 1-aryl-1-alkene at −10 °C and the reaction mixture was stirred for 3 h below 0 °C. Then, 15 mL of ethyl acetate/hexane (2/1) which was cooled to 0 °C was added to the reaction vessel. The organic layer was washed with ice-cooled aqueous solution which had previously been prepared by mixing 100 g of $NaHSO_3$ and 0.5 L of water, and 0.5 L of saturated brine until the color of potassium permanganase disappeared. The organic portion was evaporated under reduced pressure to obtain a yellow oil. The oil was dissolved in 3 mL of pyridine, and 5 mL of water was added with stirring to hydrolyze the remaining acetic anhydride. The solution was stirred at room temperature overnight and 40 mL of ether was added. Then the organic phase was washed with 20 mL of 2 M hydrochloric acid, 20 mL of saturated sodium carbonate, and 10 mL of brine. The solvent was removed under reduced pressure and the residue was subjected to silica gel column chromatography with an eluent of hexane/ether (3/1) mixture to give 1-aryl-1,2-alkanedione.

1-(2-Methylphenyl)-1,2-propanedione (3d): 52% yield; ^{1}H NMR($CDCl_3$, TMS) δ = 2.53 (3H, *s*), 2.55 (3H, *s*), 7.24—7.34 (2H, *m*), 7.43—7.52 (1H, *m*), and 7.58—7.64 (1H, *m*); IR (neat) 1715 and 1672 cm^{-1}. Found: C, 73.86; H, 6.22%. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21%.

1-(4-Methylphenyl)-1,2-propanedione (3e): 43% yield; ^{1}H NMR($CDCl_3$, TMS) δ = 2.43 (3H, *s*), 2.51 (3H, *s*), 7.25—7.34 (2H, *m*), and 7.87—7.97 (2H, *m*); IR (neat) 1711 and 1671 cm^{-1}. Found: C, 74.32; H, 6.43%. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21%.

1-(4-Methoxyphenyl)-1,2-propanedione (3f): 11% yield; ^{1}H NMR($CDCl_3$, TMS) δ = 2.51 (3H, *s*), 3.89 (3H, *s*), 6.92—7.00 (2H, *m*), and 7.97—8.06 (2H, *m*); IR (KBr) 1709, 1651, 1265, and 1026 cm^{-1}. Found: C, 67.28; H, 5.66%. Calcd for $C_{10}H_{10}O_3$: C, 67.41; H, 5.66%.

1-Phenyl-1,2-butanedione (3g): 41% yield; ^{1}H NMR ($CDCl_3$, TMS) δ = 1.20 (3H, t, *J* = 7.3 Hz), 2.92 (2H, *q*, *J* = 7.3 Hz), 7.45—7.56 (2H, *m*), 7.59—7.69 (1H, *m*), and 7.95—8.02 (2H, *m*); IR (neat) 1713 and 1672 cm^{-1}. Found: C, 74.05; H, 6.22%. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21%.

1-Phenyl-1,2-pentanedione (3h): 50% yield; ^{1}H NMR ($CDCl_3$, TMS) δ = 1.01 (3H, *t*, *J* = 7.4 Hz), 1.74 (2H, 6, *J* = 7.3 Hz), 2.86 (2H, *t*, *J* = 7.3 Hz), 7.43—7.56 (2H, *m*), 7.59—7.68 (1H, *m*), and 7.95—8.02 (2H, *m*); IR (neat) 1711 and 1674 cm^{-1}. Found: C, 75.16; H, 6.82%. Calcd for $C_{11}H_{12}O_2$: C, 74.98; H, 6.86%.

General Procedure for Enzymatic Reduction of 3. In a glass reaction vessel were placed 50 mL of β-hydroxy-4-morpholinepropanesulfonate (MOPSO) buffer solution (0.05 M, pH 7.0) containing 1.5 unit of enzyme, 0.5 mmol of substrate **3**, 5 mg (6 μmol) of NADPH, 900 mg (5.0 mmol) of D-glucose, 0.5 mg of glucose dehydrogenase. The reaction vessel was shielded from light. The reaction mixture was stirred magnetically for 12 h at 30 °C. The mixture was purified by column chromatography on silica gel with hexane/ethyl acetate (5/1) used as an eluent, giving the product **4**. Chemical yields and e.e.s are summarized in Table 2. The spectral data of **4a—4c**, **4e**, and **4f** were identical with those reported.[12,13,18,35]

1-(2-Methylphenyl)-2-hydroxy-1-propanone (4d): ^{1}H NMR ($CDCl_3$, TMS) δ = 1.32 (3H, *d*, *J* = 7.0 Hz), 2.50 (3H, *s*), 3.85 (1H, *d*, *J* = 5.6 Hz), 5.06 (1H, *q*, *d*, *J* = 7.0, 5.6 Hz), and 7.24—7.57 (4H, *m*); IR (neat) 3461 and 1690 cm^{-1}. Found: C, 73.15; H, 7.53%. Calcd for $C_{10}H_{12}O_2$: C, 73.15; H, 7.37%.

1-Phenyl-2-hydroxy-1-butanone (4g): ^{1}H NMR ($CDCl_3$, TMS), δ = 0.94 (3H, *t*, *J* = 7.4 Hz), 1.62 (1H, *m*), 1.93 (1H, *m*), 3.70 (1H, *br*), 5.01—5.13 (1H, *m*), and 7.27—7.99 (5H, *m*).

1-Phenyl-2-hydroxy-1-pentanone (4h): ^{1}H NMR ($CDCl_3$, TMS) δ = 0.92 (3H, *t*, *J* = 7.0 Hz), 1.30—1.93 (4H, *m*), 3.68 (1H, *br*), 5.01—5.14 (1H, *m*), and 7.25—7.94 (5H, *m*).

Preparation of (*S*)-Lactonitrile. Lipase (Novo SP435, 500 mg, 23100 unit; standard: triglyceride) was added to a stirred 25 mL benzene solution containing 2.50 g of racemic lactonitrile (35.2 mmol) and 22.0 g of vinyl acetate (256 mmol). The reaction mixture was stirred for 3.5 h at room temperature. The solution was filtered and the filtrate was concentrated under reduced pressure to give a mixture of the unreacted alcohol and acetate. The mixture was subjected to silica gel column chromatography with an eluent of dichloromethane/ethyl acetate (5/1) to give 1.20 g of the alcohol (16.9 mmol) in 48.0% yield. E.e. in the alcohol was determined by GLC (Chiraldex G-TA, 0.25 mm×30 m, 115 °C) to be 40%. Absolute configuration of lactonitrile was determined by comparison of the sign of its optical rotation with that reported.[36] Since the optical rotation of (*R*)-lactonitrile was reported to be +8.2 (*c* = 5, $CHCl_3$), the absolute configuration of lactonitrile thus obtained, $[\alpha]_D^{24}$ = −11.7 (*c* = 5.3, $CHCl_3$), has been established to be *S*.

General Procedure for Tetrahydropyranylation of Alcohols. Alcohols and 3,4-dihydro-2*H*-pyran were treated in the same manner as previously reported to give the corresponding THP ethers.[37]

(*S*)-2-(2-Tetrahydropyranyloxy)propanenitrile: 96% yield.

Ethyl (*S*)-2-(1-Tetrahydropyranyloxy)propionate: 89% yield.

Preparation of (*S*)-2-Hydroxy-3-alkanone. A solution of alkyl magnesium bromide was prepared from 1.19 g of magnesium (49.0 mmol), 48.6 mmol of corresponding alkyl bromide and 60 mL of dry ether. To the solution of the Grignard reagent which was cooled in an ice bath, a mixture of 6.28 g (*S*)-2-(2-tetrahydropyranyloxy)propanenitrile (40.5 mmol) and 7 mL dry ether was added slowly with stirring. After being kept at room temperature for 2 h, the mixture was hydrolyzed by adding 70 mL of 2 M hydrochloric acid and stirred overnight at room temperature. The ether layer was separated and the aqueous layer was extracted twice with 20 mL of ether. The ether solution was washed with saturated aqueous sodium hydrogen carbonate and brine. The solution was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue was subjected to column chromatography on silica gel with an eluent of hexane/ether (3/1) mixture to give the product.

(*S*)-2-Hydroxy-3-hexanone (4a): 23% yield. GC data (Chiraldex G-TA, 0.25 mm×20 m, 100 °C, He: 2 mL min^{-1}; *R*: 5.8 min, *S*: 6.4 min).

(*S*)-2-Hydroxy-3-octanone (4b): 18% yield. GC data (Chiraldex G-TA, 0.25 mm×30 m, 100 °C, He: 2 mL min^{-1}; *R*: 11.2 min, *S*: 11.9 min).

Preparation of (*S*)-1-Aryl-2-hydroxy-1-propanone. A solution of butyllithium in hexane (6.9 mL, 11.7 mmol) was added to 50 mL dry THF solution containing aryl halide (11.7 mmol) at −90 °C and the reaction mixture was stirred for 2 h. Then ethyl (*S*)-2-(1-tetrahydropyranyloxy)propanoate (2.37 g, 11.7 mmol) was added to the mixture and the solution was stirred for 2 h at −75 °C. The solution was washed with 0.1 M ammonium chloride solution and saturated brine successively. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue was added to 30 mL of ethanol so-

lution containing 100 mg of pyridinium *p*-toluenesulfonate and the resulting solution was stirred for 2 h at 60 °C. Then the solvent was evaporated under reduced pressure and the residue was subjected to silica gel column chromatography with an eluent of hexane/ethyl acetate (3/1) to obtain the product.

(*S*)-2-Hydroxy-1-(2-methylphenyl)-1-propanone (4d): 45% yield. $[\alpha]_D^{24} = -8.9$ ($c = 2.01$, EtOH). GC data (Chiraldex G-TA, 0.25 mm×30 m, 130 °C, He: 2 mL min^{-1}; *R*: 13.6 min, *S*: 14.9 min).

(*S*)-2-Hydroxy-1-(4-methylphenyl)-1-propanone (4e): 54% yield. GC data (CP-Cyclodextrin-B-236-M12, 0.25 mm×25 m, 135 °C, He: 2 mL min^{-1}; *R*: 14.2 min, *S*: 14.8 min).

(*S*)- 2- Hydroxy- 1- (4- methoxyphenyl)- 1- propanone (4f): 4.1% yield. GC data (CP-Cyclodextrin-B-236-M12, 0.25 mm×25 m, 150 °C, He: 2 mL min^{-1}; *R*: 20.8 min, *S*: 21.6 min).

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