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The Use of Microorganisms in Organic Synthesis. IV. Microbiological Asymmetric Reduction of Methyl 3-Phenyl 2-Oxobutyrate

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The synthesis of optically active α-hydroxy β-methyl esters V by means of microbiological reduction of the corresponding α-keto β-methyl esters IV was carried out. Methyl 3-phenyl-2-oxobutyrate **3** was found to be reduced by a variety of yeasts to the α-hydroxy β-methyl esters (**7b** and **8a**) with (2*R*,3*S*)- and (2*R*,3*R*)-configurations, respectively, and by carrying out screening experiments, yeasts which give products with high optical purity were actually found.

Keywords——α-keto β-methyl ester; α-hydroxy β-methyl ester; asymmetric reduction; microbiological reduction; yeast

In the course of our synthetic studies on chiral synthons useful for the synthesis of optically active natural products, we reported[1] that α-methyl β-keto ester I was successfully reduced to optically active α-methyl β-hydroxy esters II and III having a β-C_3–OH group by means of selected yeasts. In particular, the *syn*[2]-isomers II (β-C_3–OH) which are difficult to obtain by other means were produced with high optical purity. The isomers (IIc′ and IIIc′) having an α-C_3-hydroxyl group were obtained only when Ic was reduced with *Kloeckera saturnus*.

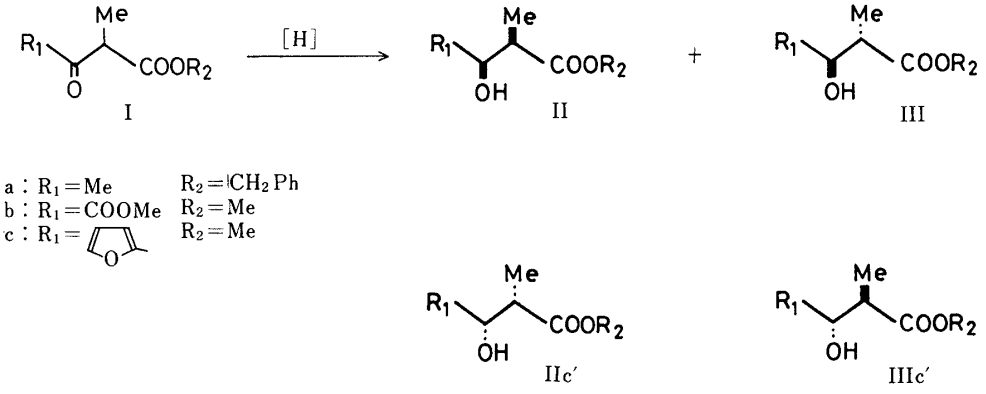


Chart 1

We then examined whether the α-keto β-methyl ester IV could be reduced microbiologically to the optically active α-hydroxy β-methyl ester V. If reduction of α-keto ester by yeasts proceeds smoothly as in the case of β-keto ester, the optically active methyl *syn*-(2*S*, 3*R*)-indolmycenate **1**[3] which is reported to be an important intermediate for the synthesis of indolmycin **2**[3] will be produced from the corresponding ketone.

As a model experiment, we first examined reduction of methyl 3-phenyl-2-oxobutyrate **3** using various yeasts. The substrate **3** was synthesized from acetophenone as shown in Chart 3.

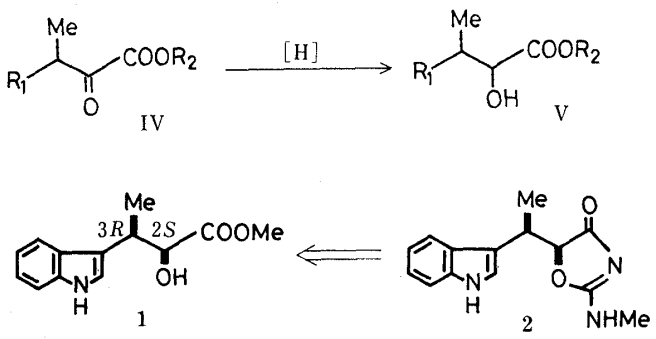


Chart 2

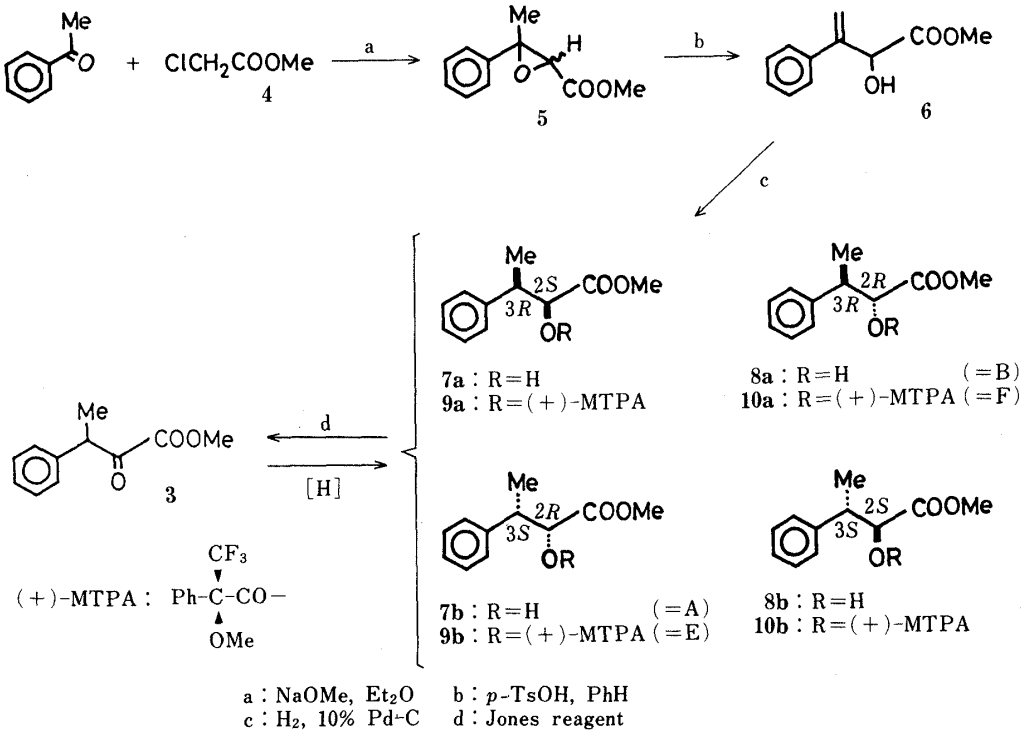


Chart 2

Darzens condensation of acetophenone and methyl chloroacetate **4** in the presence of sodium methoxide in ether gave the glycidic ester **5**, which was treated with a catalytic amount of *p*-TsOH in benzene to afford the α-hydroxy ester **6** in an overall 66% yield from acetophenone. The presence of the allylic alcohol moiety in **6** was confirmed by the presence of a two-proton doublet at δ 5.47 (2H, $J=4$ Hz; exo-olefinic protons), a one-proton doublet at δ 5.06 ($J_{H,OH}=5.3$ Hz; proton adjacent to the hydroxyl group), and a one-proton doublet at δ 3.27 ($J_{H,OH}=5.3$ Hz; C_2–OH) in the nuclear magnetic resonance (NMR) spectrum as well as a characteristic band due to the hydroxyl group at 3520 cm^{-1} in the infrared (IR) spectrum. Hydrogenation of **6** in the presence of 10% palladium–charcoal in MeOH afforded a mixture of two racemates, *syn*-isomer **7** and *anti*[2]-isomer **8**, in 85% yield. The Jones oxidation of the crude mixture in acetone provided the desired α-keto β-methyl ester **3** in 75% yield. A mixture of **7** and **8** was then treated with (+)-α-methoxy-α-trifluoromethylphenylacetic acid chloride ((+)-MTPACl[4]) in pyridine. The 400 MHz NMR spectra of the (+)-MTPA esters revealed four distinctly separated singlets (δ 3.657, 3.677, 3.712, and 3.758) due to ester methyls of the four possible diastereoisomers in the absence of any shift reagent, as shown in Fig. 1. These results show that the structures of the four possible diastereoisomers obtainable by the

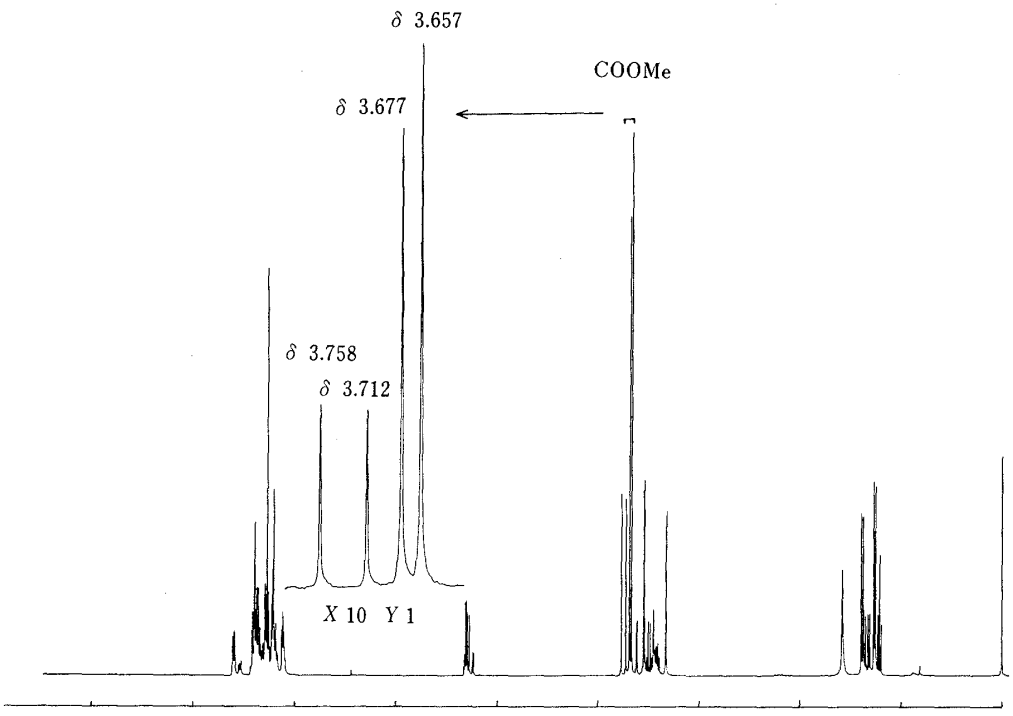
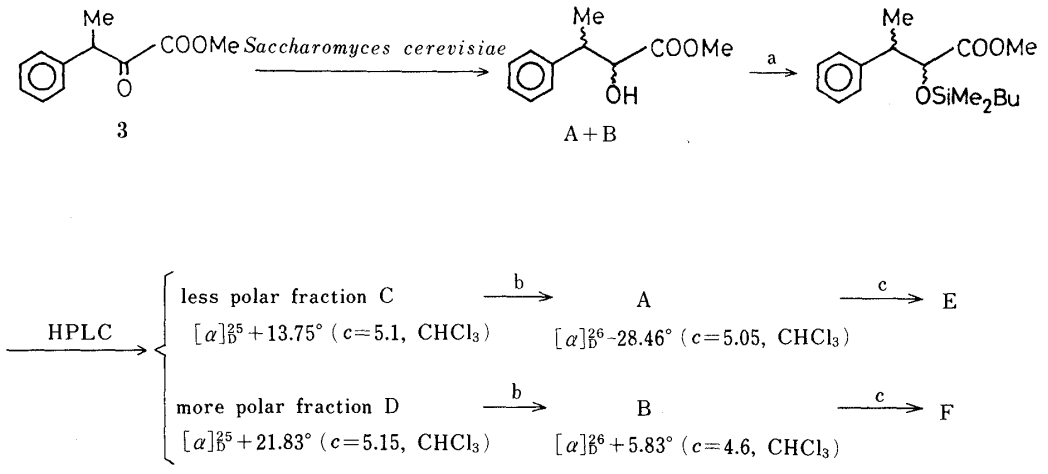


Fig. 1

microbiological reduction of **3** can be differentiated by converting them to the (+)-MTPA esters and by measuring the 400 MHz NMR spectra.

Reduction of **3** with fermenting baker's yeast (*Saccharomyces cerevisiae*) was then undertaken. An inseparable mixture of α-hydroxy β-methyl esters (A and B) was produced in 62% yield, the ratio being 37 : 63 in favour of B. The mixture was converted to the silyl ethers, which could be separated by high pressure liquid chromatography into the less polar fraction C ($[\alpha]_D^{25}$ +13.75° (c=5.1, $CHCl_3$)) and the more polar fraction D ($[\alpha]_D^{25}$ +21.83° (c=5.15, $CHCl_3$)). Both silyl ethers (C and D) were treated with tetrabutylammonium fluoride ($Bu_4N^+F^-$) to afford the optically active α-hydroxy β-methyl esters A; $[\alpha]_D^{26}$ −28.46° (c=5.05, $CHCl_3$) and B; $[\alpha]_D^{26}$ +5.83° (c=4.6, $CHCl_3$), respectively, as shown in Chart 4.

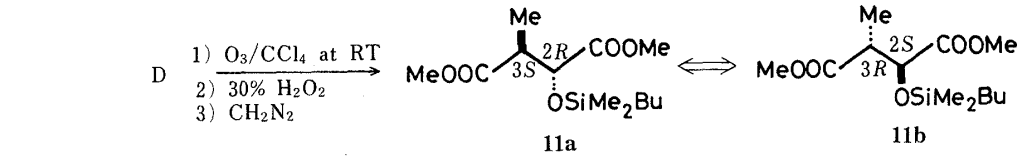


a : *tert*-$BuMe_2SiCl$, imidazole in DMF b : $Bu_4N^+F^-$ c : (+)-MTPACl, pyridine

Chart 4

In order to determine the stereostructure and optical purity of A and B, the α-hydroxy esters were converted to the corresponding (+)-MTPA esters (E and F) by treatment with (+)-MTPACl in pyridine and the 400 MHz NMR spectra were measured. From the small peaks due to isomers present in E and F, the optical purities of E and F were calculated to be 91 and 47% e.e., respectively.

The absolute structures of the reduction products A and B were determined as follows. The silyl ether D was subjected to ozonolysis in CH_2Cl_2 under dry ice-acetone cooling, and subsequent treatment with 30% H_2O_2 and esterification with CH_2N_2 gave the silyl diester **11a** [$[\alpha]_D^{24}$ +15.75° (c=0.8, $CHCl_3$; corresponds to 47% e.e.)], as shown in Chart 5. The spectral data (400 MHz NMR and IR) of **11a** were identical with those of the authentic (2*S*, 3*R*)-silyl diester **11b**[1d] ($[\alpha]_D^{27}$ −48° (c=4.25, $CHCl_3$)), but the sign of $[\alpha]_D$ was opposite to that of **11b**. Consequently, the absolute configuration of D and thence those of B and F are 2*R*, 3*R* (therefore B=**8a**, F=**10a**). Also, since the relative configuration of C_2 and C_3 in B was determined as *anti*, A should have 2,3-*syn* configuration. The absolute structure of A was determined by the following chemical correlation. The (2*R*, 3*R*)-α-hydroxy ester **8a** (=B) was converted to the corresponding *syn*-(+)-MTPA ester **9a** by treatment with (+)-α-methoxy-α-trifluoromethylphenylacetic acid ((+)-MTPA[4]) in the presence of diethyl azodicarboxylate (EtOOC–N=N–COOEt) and triphenylphosphine (Ph_3P) (Mitsunobu's method[5]).



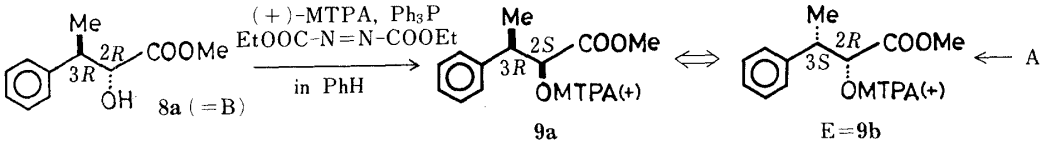


Chart 5

The (+)-MTPA ester **9a** derived from B and the (+)-MTPA ester E derived from A were found to give different NMR spectra although both of them have 2,3-*syn* relative configuration. Therefore, E must be an enantiomer of **9a** and since **9a** has (2*S*, 3*R*)-structure, E should be assigned as the (2*R*, 3*S*)-(+)-MTPA ester **9b**.

Thus, the three signals at δ 3.657, 3.758, and 3.712 could be ascribed to **10a** (2*R*, 3*R*), **9a** (2*S*, 3*R*), and **9b** (2*R*, 3*S*), respectively, by direct comparison with those of the authentic specimens obtained above. Therefore, the remaining unassigned signal at δ 3.677 (see Fig. 1) should be due to the (2*S*, 3*S*)-isomer **10b**. Now that all peaks due to the ester methyls of the diastereoisomers **9a**, **9b**, **10a**, and **10b** could be assigned, we carried out a screening experiment using sixteen different yeasts. In each experiment, about 50 mg of **3** was used as a substrate and the same cultivation method as described in the previous paper[1b,c,e] was employed. The progress of the reduction was monitored by thin-layer chromatography (TLC) inspection. After completion of the reduction, the products were directly treated with (+)-MTPACl in pyridine and the resulting crude (+)-MTPA esters were purified by means of preparative thin-layer chromatography. The 400 MHz spectra of these (+)-MTPA esters were measured and

TABLE I

Entry	Microorganism	Substrate **3** (mg)	Chemical yield (%) as (+)-MTPA ester	*syn*/*anti*	Optical yield (% e.e.)
1	*Endomycopsis fibligera*	53	72	48/52	*syn* **7b**: 92 *anti* **8a**: 78
2	*Hansenula anomala*	57	44	43/57	*syn* **7b**: 79 *anti* **8a**: 94
3	*Hansenula anomala* NI-7572	52	44	40/60	*syn* **7b**: 83 *anti* **8a**: 87
4	*Kloeckera saturnus*	53	46	18/82	*syn* **7b**: 35 *anti* **8a**: 8
5	*Lipomyces starkeyi*	48	15	85/15	*syn* **7b**: >99 *anti* **8b**: 58
6	*Pichia farinosa*	50	40	15/85	*syn* **7b**: 68 *anti* **8a**: 30
7	*Pichia membranaefaciens*	52	51	53/47	*syn* **7b**: 64 *anti* **8a**: 50
8	*Rhodotorula glutinis*	54	46	68/32	*syn* **7b**: 58 *anti* **8a**: 10
9	*Saccharomyces acidifaciens*	51	14	44/56	*syn* **7b**: 76 *anti* **8a**: 91
10	*Saccharomyces delbrueckii*	52	63	28/72	*syn* **7b**: 90 *anti* **8a**: 43
11	*Saccharomyces fermentati*	49	70	28/72	*syn* **7b**: 89 *anti* **8a**: 40
12	*Candida albicans*	52	26	17/83	*syn* **7b**: >99 *anti* **8a**: 31

the absolute configuration, the optical purity (% e.e.) and the ratio of *syn*/*anti* isomers were determined. Selected data are shown in Table I.

These results show that the α-keto β-methyl ester **3** is reduced by a variety of yeasts to give α-hydroxy β-methyl esters having (2*R*, 3*S*)-, (2*R*, 3*R*)-configurations. Only when *Lipomyces starkeyi* (entry 5) was used, the products having (2*R*, 3*S*)-, and (2*S*, 3*S*)-configurations were obtained. In some cases, reduction products with high optical purity were obtained (entries 1, 2, 3, 10, 11, and 12). Unfortunately, the desired optically active (2*S*, 3*R*)-α-hydroxy ester **7a** having the same absolute configuration as the important intermediate **1** for indolmycin **2** was not produced. However, it should be emphasized that the (2*R*, 3*R*) isomer **8a** produced in high optical yields (91—94% e.e.) with *Hasenula anomala* (entry 2) and *Saccharomyces acidifaciens* (entry 9), is convertible to the (2*S*, 3*R*) isomer **7a** by applying Mitsunobu's method. An application of the present method to the synthesis of natural products is in progress.

Experimental

All melting points were measured with a Kofler micro melting point apparatus and are uncorrected. IR spectra (CCl_4) were measured on a JASCO A-3 spectrophotometer. NMR spectra were measured either on a JEOL FX-60 spectrometer or a JEOL FX-400 instrument. Spectra were taken as 5—10% w/v solutions in $CDCl_3$ with Me_4Si as an internal reference. Gas chromatography-mass spectroscopy (GC-MS) was carried out on a Hitachi RMU-6M mass spectrometer and high-resolution mass spectra were taken with a Hitachi M-80 GC-MS spectrometer. High pressure liquid chromatography (HPLC) was carried out on a JASCO TRI ROTAR with a Senshu Pack N 50-5 (Nucleosil, 5 mμ, 8 i.d. × 300 mm) column. $[\alpha]_D$ was measured on a Perkin–Elmer model 241 MC polarimeter.

Synthesis of Four Kinds of Methyl 2-Hydroxy-3-phenyl Butyrates (7a, 7b, 8a, and 8b)——i) Freshly prepared solid NaOMe [prepared from Na (6.9 g) in absolute MeOH (100 ml)] was added to a solution of acetophenone (12 g, 0.1 mol) and chloroacetate (32.6 g, 0.3 mol) in dry ether (300 ml) under an argon atmosphere at −20 °C, and the reaction mixture was stirred for 30 min, then for a further 2 h at room temperature. After the addition of aqueous AcOH [AcOH (10 ml)–H_2O (150 ml)], the reaction mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., then dried over $MgSO_4$. Removal of the solvent gave a crude Darzens condensation product (**5**), which was used without further purification.

ii) A mixture of the above crude **5** and *p*-TsOH (1 g) in dry benzene (200 ml) was refluxed for 30 min. After the addition of H_2O, the reaction mixture was extracted with ether and the ether extract was washed with sat. $NaHCO_3$ aq. and sat. NaCl aq., then dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (300 g) to give α-hydroxy esters **6** (12.65 g, 66% yield from acetophenone) as a homogeneous oil from the *n*-hexane–ethyl acetate (4 : 1) eluate. IR ν_{max} cm^{-1}: 1735, 3520. NMR δ: 5.47 (2H, d, J=4 Hz; exo-methylene), 5.06 (1H, d, J=5.3 Hz, 2-H), 3.71 (3H, s; COOMe), 3.27 (1H, d, J=5.3 Hz; 2-OH).

iii) A solution of **6** (22.581 g) in MeOH (170 ml) was hydrogenated at ordinary temperature and pressure over

10% Pd–C (5 g). After hydrogen absorption had ceased, the catalyst was filtered off and the filtrate was evaporated to give a residue, which was chromatographed on silica gel (250 g) to provide a mixture (19.41 g, 85% yield) of *syn*-racemate (**7a** and **7b**) and *anti*-racemate (**8a** and **8b**) as a homogeneous oil from the *n*-hexane–ethyl acetate (4 : 1) eluate. A part of the above mixture was converted to the corresponding 3,5-dinitrobenzoates, which were recrystallized from MeOH to give pale yellow prisms. mp 115.5—116.5 °C. *Anal.* Calcd for $C_{18}H_{16}N_2O_8$: C, 55.67; H, 4.15; N, 7.21. Found: C, 55.62; H, 4.15; N, 7.08. **7a**+**7b**+**8a**+**8b**; IR ν_{max} cm^{-1}: 1740, 3530. *syn* (**7a** and **7b**); NMR δ: 1.29 (3H, d, J=7.1 Hz; 3-Me), 3.76 (3H, s; COOMe). *anti* (**8a** and **8b**); NMR δ: 1.44 (3H, d, J=7.1 Hz; 3-Me), 3.70 (3H, s; COOMe). The ratio of *syn*/*anti* was found to be *ca.* 1/2 by NMR analysis.

Preparation of Methyl 3-Phenyl 2-Oxobutyrate 3——A solution of the four isomers (**7a**+**7b**+**8a**+**8b**; 7.810 g) in acetone (20 ml) was oxidized with Jones reagent (20 ml) under stirring for 5 h at 0 °C. The reaction mixture was treated with iso-PrOH (2 ml) and extracted with ether after excess water had been added. The ether extract was washed with sat. $NaHCO_3$ aq. and sat. NaCl aq., then dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (200 g) to afford the desired α-keto ester **3** (5.768 g, 75% yield) along with unchanged starting material (645 mg, 8% recovery) from the *n*-hexane–ethyl acetate (9 : 1—4 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{11}H_{12}O_3$ (M^+, *m/e*): 192.079. Found: 192.078. IR ν_{max} cm^{-1}: 1730, 1740. NMR δ: 1.47 (3H, d, J=7 Hz; 3-Me), 3.73 (3H, s; COOMe), 4.51 (1H, q, J=7 Hz; 2-H).

Preparation of the Four (+)-MTPA Esters (9a, 9b, 10a, and 10b)——Pyridine (0.5 ml) was added to a mixture of the four α-hydroxy esters (**7a**+**7b**+**8a**+**8b**; 72 mg) and (+)-MTPACl (110 mg), and the reaction mixture was stirred for 12 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (30 g) to afford four (+)-MTPA esters (**9a**+**9b**+**10a**+**10b**; 84 mg, 55% yield) along with unchanged starting material (33 mg, 45% recovery) from the *n*-hexane–ethyl acetate (19 : 1—3 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{21}H_{21}F_3O_5$ (M^+, *m/e*): 410.134. Found: 410.133. *syn* (**9a** and **9b**); NMR δ: 1.315, 1.356 (each 3H, d, J=7.1 Hz; 3-Me), 3.712, 3.758 (each 3H, s; COOMe). *anti* (**10a** and **10b**); NMR δ: 1.259, 1.380 (each 3H, d, J=7.3 Hz; 3-Me), 3.657, 3.677 (each 3H, s; COOMe).

Asymmetric Reduction of 3 with Baker's Yeast (*Saccharomyces cerevisiae*)——i) A suspension of baker's yeast (from Oriental Yeast Co., Ltd., 20 g), sucrose (20 g) and substrate **3** (1 g) in H_2O (120 ml) was shaken at 30 °C for 48 h. The reaction mixture was filtered with the aid of celite and the filtrate was extracted with ether. The ether extract was washed with sat. NaCl aq., then dried over $MgSO_4$. Removal of the solvent gave an oily product, which was chromatographed on silica gel (40 g) to give the reduction products (A and B; 627 mg, 62% yield) along with unchanged starting material **3** (39 mg, 4% recovery) from the *n*-hexane–ethyl acetate (9 : 1) eluate.

ii) Imidazole (264 mg) and *tert*-butyl dimethylchlorosilane (585 mg) were added to a solution of the reduction products (A and B; 627 mg) in dimethylformamide (DMF, 4 ml), and the reaction mixture was stirred for 48 h at room temperature. After the addition of H_2O, the reaction mixture was extracted with ether. The ether extract was washed with sat. NaCl aq., then dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (40 g). Elution with *n*-hexane–ethyl acetate (19 : 1) afforded 848 mg (85% yield) of silyl ethers (C and D). This mixture was subjected to HPLC with *n*-hexane–ethyl acetate (200 : 1) as a solvent system (flow rate; 3 ml/min), yielding the less polar fraction C (222 mg) and the more polar fraction D (451 mg). In this case, the ratio of C/D was found to be 37/63 by HPLC analysis. C: MS: *m/e*: 309 (M^++1). $[\alpha]_D^{25}$ +13.75° (*c*=5.1, $CHCl_3$). 400 MHz NMR δ: −0.239, −0.118 (each 3H, s; $SiMe_2Bu$), 0.836 (9H, s; $SiMe_2Bu$), 1.301 (3H, d, J=7.1 Hz; 3-Me), 3.227 (1H, qq, J=7.1, 5 Hz; 3-H), 3.626 (3H, s; COOMe), 4.227 (1H, d, J=5 Hz; 2-H). D: MS: *m/e*: 309 (M^++1). $[\alpha]_D^{25}$ +21.83° (*c*=5.15, $CHCl_3$). 400 MHz NMR δ: −0.184, −0.110 (each 3H, s; $SiMe_2Bu$), 0.796 (9H, s; $SiMe_2Bu$), 1.278 (3H, d, J=7.1 Hz; 3-Me), 3.127 (1H, qq, J=7.1, 6.8 Hz; 3-H), 3.648 (3H, s; COOMe), 4.205 (1H, d, J=6.8 Hz; 2-H).

iii) A mixture of C (200 mg) and $Bu_4N^+F^-\cdot 3H_2O$ (600 mg) in tetrahydrofuran (THF, 10 ml) was stirred for 1.5 h at room temperature and the reaction mixture was washed with sat. NaCl aq., then dried over $MgSO_4$ and concentrated to give an oil, which was chromatographed on silica gel (15 g) to afford A (118 mg, 93% yield) as a homogeneous oil from the *n*-hexane–ethyl acetate (3 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{11}H_{14}O_3$ (M^+, *m/e*): 194.094. Found: 194.094. $[\alpha]_D^{26}$ −28.46° (*c*=5.05, $CHCl_3$). IR ν_{max} cm^{-1}: 1735, 3525. 400 MHz NMR δ: 1.290 (3H, d, J=7.1 Hz; 3-Me), 2.816 (1H, d, J=5.4 Hz; 2-OH), 3.224 (1H, qq, J=7.1, 3.8 Hz; 3-H), 3.745 (3H, s; COOMe), 4.320 (1H, dd, J=3.8, 5.4 Hz; 2-H). A mixture of D (200 mg) and $Bu_4N^+F^-\cdot 3H_2O$ (600 mg) in THF (10 ml) was stirred for 1.5 h at room temperature and the reaction mixture was worked up in the same way as in the case of C to provide B (100 mg, 79% yield) as a homogeneous oil from the *n*-hexane–ethyl acetate (3 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{11}H_{14}O_3$ (M^+, *m/e*): 194.094. Found: 195.095. A part of B (49 mg) was converted to the 3,5-dinitrobenzoate, which was recrystallized from MeOH to afford colorless prisms. mp 114—115 °C. *Anal.* Calcd for $C_{18}H_{16}N_2O_8$: C, 55.67; H, 4.15; N, 7.21. Found: C, 55.65; H, 4.22; N, 7.04. B; $[\alpha]_D^{26}$ +5.83° (*c*=4.6, $CHCl_3$). IR ν_{max} cm^{-1}: 1735, 3525. 400 MHz NMR δ: 1.448 (3H, d, J=7.1 Hz; 3-Me), 2.557 (1H, d, J=6.8 Hz; 2-OH), 3.261 (1H, qq, J=7.1, 3.9 Hz; 3-H), 3.703 (3H, s; COOMe), 4.339 (1H, dd, J=3.9, 6.8 Hz; 2-H).

(+)-MTPA Esterification of A——Pyridine (0.5 ml) was added to a mixture of A (51 mg) and (+)-MTPACl (99 mg), and the reaction mixture was stirred for 18 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of the preparation of the four (+)-MTPA esters to provide E (97 mg, 91%

yield) as a homogeneous oil. 400 MHz NMR δ: 1.315 (3H, d, J=7.1 Hz; 3-Me), 3.712 (3H, s; COOMe). The optical purity of E and thence that of **9b** was found to be 91% e.e.

(+)-MTPA Esterification of B——Pyridine (0.5 ml) was added to a mixture of B (49 mg) and (+)-MTPACl (95 mg), and the reaction mixture was stirred for 48 h at room temperature, then worked up and purified in the same way as in the case of the preparation of the four (+)-MTPA esters to afford F (92 mg, 89% yield) as a homogeneous oil. 400 MHz NMR δ: 1.259 (3H, d, J=7.3 Hz; 3-Me), 3.657 (3H, s; COOMe). The optical purity of F and thence that of **10a** was found to be 47% e.e.

Conversion of D into Dimethyl (2*R*, 3*S*)-3-Methyl-2-(*tert*-butyldimethylsilyloxy)succinate (11a) by Ozonolysis——Ozone was passed through a solution of D (508 mg) in CCl_4 (10 ml) for 6 h at room temperature, then 30% H_2O_2 aq. (2 ml) was added to the ozonolyzed product and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was then extracted with ether after the addition of H_2O, and the extract was washed with sat. NaCl aq., and dried over $MgSO_4$. Removal of the solvent gave a residue, which was treated with a solution of CH_2N_2 in ether to provide an oily product. It was chromatographed on silica gel (35 g) to afford **11a** (8 mg) as a homogeneous oil from the *n*-hexane–ethyl acetate (100 : 1) eluate. $[\alpha]_D^{24}$ +15.75° (c=0.8, $CHCl_3$). The spectral data (400 MHz NMR and IR) of **11a** were identical with those of an authentic specimen of **11b**.[1d]

Preparation of (2*S*, 3*R*)-(+)-MTPA Ester (9a) from (2*R*, 3*R*)-α-Hydroxy Ester (B=8a) by Mitsunobu's Method——A mixture of B (38 mg), (+)-MTPA (69 mg), EtOOC–N=N–COOEt (51 mg) and Ph_3P (77 mg) in dry benzene (3 ml) was stirred under an argon atmosphere for 65 h at room temperature. After the addition of H_2O, the reaction mixture was washed with sat. NaCl aq., dried over $MgSO_4$ and evaporated to give an oil, which was subjected to preparative thin-layer chromatography [prep. TLC, 20 cm × 20 cm; solvent, *n*-hexane–ethyl acetate (3 : 1)] to provide the (2*S*, 3*R*)-(+)-MTPA ester (**9a**, 15 mg, 19% yield) as a homogeneous oil. 400 MHz NMR δ: 1.356 (3H, d, J=7.1 Hz; 3-Me), 3.758 (3H, s; COOMe).

Screening Experiment with Various Yeasts——Microorganisms described in the previous paper[1b] were used to reduce the starting material **3**. Erlenmeyer flasks (500 ml) containing 100 ml of culture medium comprising 5% glucose, 0.1% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.05% urea, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2 \cdot 2H_2O$, 0.1% yeast extract, a trace of mineral solution (0.1% $FeSO_4 \cdot 7H_2O$, 0.1% $MnCl_2 \cdot 4H_2O$, 0.1% $ZnSO_4 \cdot 7H_2O$; 0.2 ml per 100 ml of culture medium) and tap water (pH 7.0) were inoculated with microorganisms and cultured at 30 °C for 3 d with continuous shaking. Then substrate (*ca.* 50 mg of **3**) was added to the 100 ml of seed culture and the whole was incubated for a further 3 d under the same conditions. The reaction mixtures were separately worked up in the same way as in the case of baker's yeast reduction to give crude reduction products. Pyridine (0.5 ml) was added to a mixture of each reduction product and (+)-MTPACl (*ca.* 90 mg), and the reaction mixture was stirred for 48 h at room temperature. The reaction mixtures were worked up and purified in the same way as in the case of A to afford (+)-MTPA esters as shown in Table I. The results are summarized in Table I.

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