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The Use of Microorganisms in Organic Synthesis. II. Microbiological Asymmetric Reduction of 2-Methyl-3-oxosuccinates

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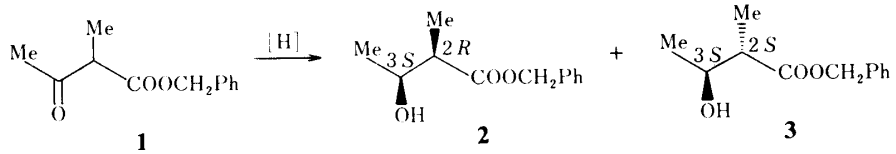
In order to synthesize four optically active methyl 2-methylmalates (**10**—**13**), microbiological asymmetric reduction of the corresponding dimethyl 2-methyl-3-oxosuccinate (**9**) was carried out. The β-keto diester **9** was found to be reduced by fermenting baker's yeast (*Saccharomyces cerevisiae*) and *Candida albicans* to afford a mixture of the (2*R*, 3*R*)-isomer **10** and the (2*S*, 3*R*)-isomer **11**. Although the optical purity of **10** produced by *Candida albicans* was reasonably high (95% *e.e.*), optical yields of other products were unexpectedly low. However, identification of the four possible isomers **14**—**17** was found to be easily carried out by means of nuclear magnetic resonance spectroscopy.

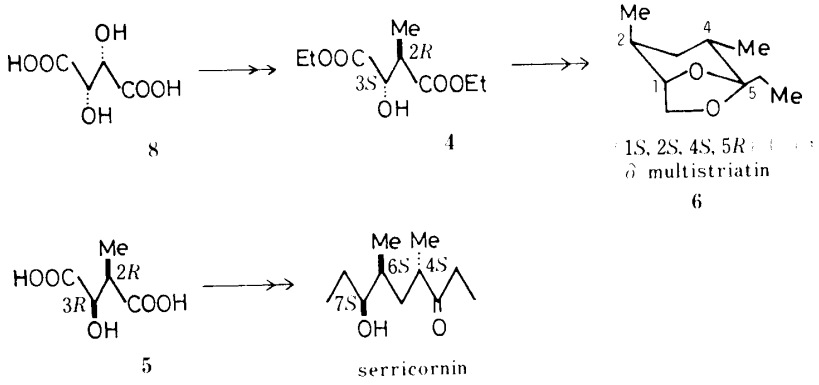
Keywords——α-methyl-β-keto ester; methylmalate; asymmetric reduction; microbiological reduction; yeast; methyl 2-methylmalate

In the previous paper,[1] we reported that the α-methyl-β-keto ester **1** was reduced by a variety of yeasts to the optically active α-methyl-β-hydroxy esters **2** and **3** with (2*R*, 3*S*)- and (2*S*, 3*S*)-configuration, respectively, and we also established a facile and practical nuclear magnetic resonance (NMR) method for the determination of the absolute configuration and the optical purity of the reduction products.



1 **2** **3**

Meanwhile, optically active methylmalic acid derivatives (**4** and **5**) have been reported to be useful in the synthesis of optically active insect pheromones[2] as shown in Chart 1. Namely,



8 **4** 1S, 2S, 4S, 5R δ multistriatin **6**

5 serricornin **7**

Chart 1

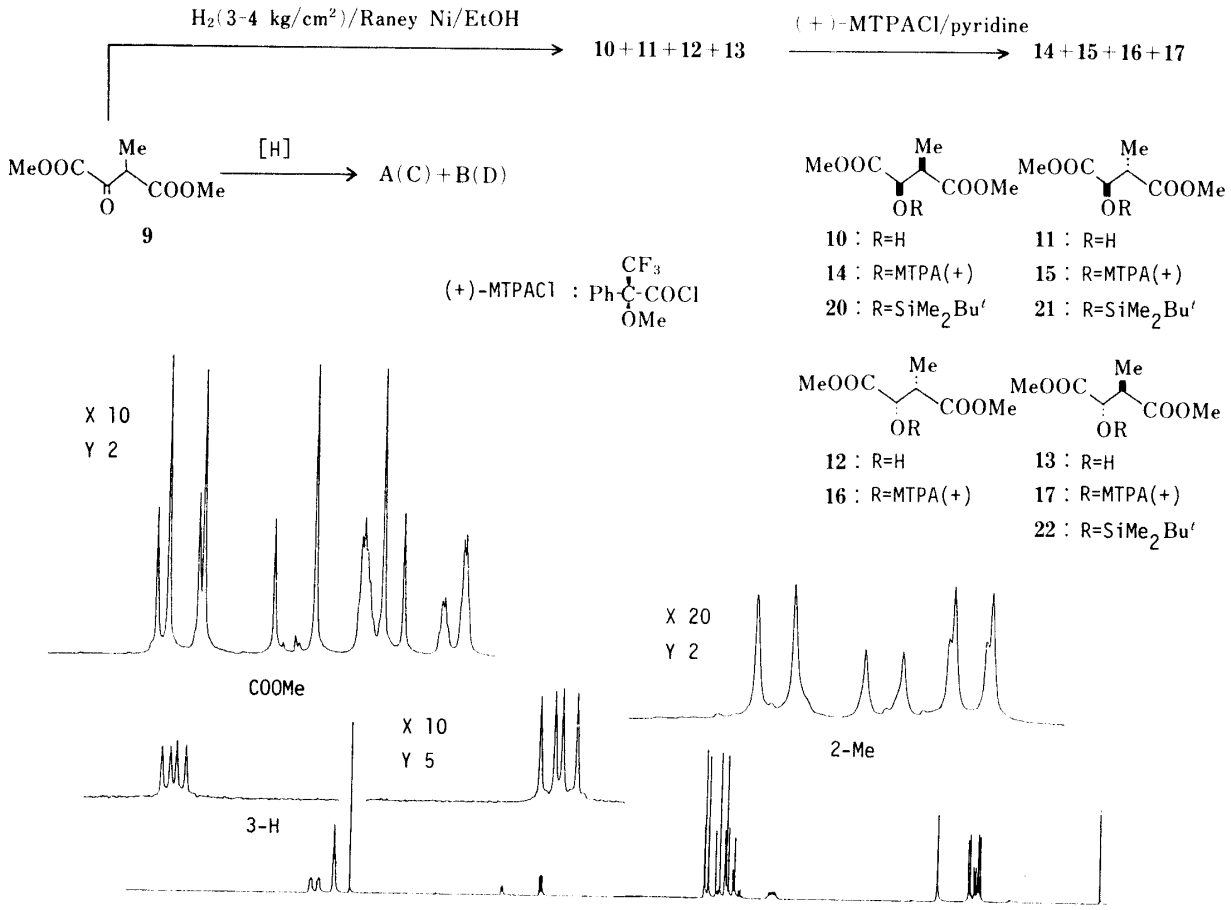
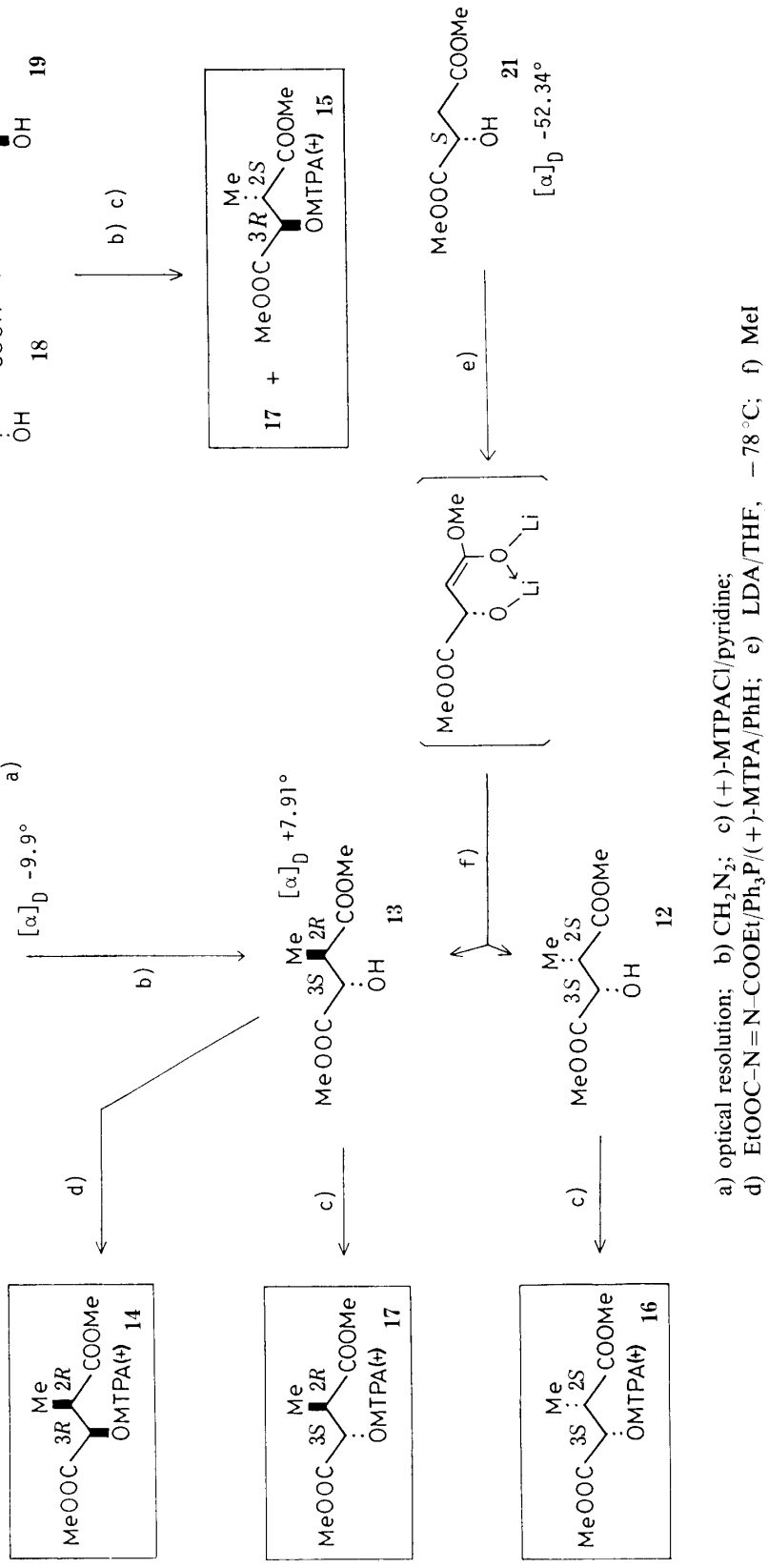


Fig. 1

diethyl (2*R*, 3*S*)-2-methylmalate (**4**) was converted to optically active (−)-*δ*-multistriatin (**6**), the sex attractant of the European population of *Scolytus multistriatus*[3] and (2*R*, 3*R*)-2-methylmalic acid (**5**) led to optically active serricornin (**7**), the sex pheromone of the female cigarette beetle *Lasioderma serricorne* F.[4] In these pheromone syntheses, however, the optically active intermediate **4** was synthesized from D-(−)-tartaric acid (**8**) through many steps[3] and **5** was prepared by a process involving a rather tedious optical resolution.[4] In the hope of obtaining these compounds more conveniently, we examined the microbiological asymmetric reduction of 2-methyl-3-oxosuccinate.

Reduction of dimethyl 2-methyl-3-oxosuccinate (**9**), prepared by ester condensation of methyl propionate with dimethyl oxalate with fermenting baker's yeast (*Saccharomyces cerevisiae*) produced an inseparable mixture of dimethyl 2-methylmalates (A and B) in 57% yield. In order to examine whether the above mentioned NMR technique for the determination of the absolute structure and optical purity of reduction products is applicable in the present system, a mixture of the four possible stereoisomers (**10**—**13**) was initially prepared. When **9** was subjected to catalytic reduction with Raney Ni (W-7), a mixture of four alcohols was obtained, and was treated with (+)-MTPACl ((+)-α-methoxy-α-trifluoromethylphenylacetyl chloride)[5] to give the corresponding (+)-MTPA esters (**14**—**17**). In the 400 MHz spectra of the above mixture of (+)-MTPA esters, four doublets due to four types of secondary methyl groups, eight singlets due to eight types of ester methyl groups, and four doublets due to C_3–H were observed as distinctly separated signals in the absence of any shift reagent, as shown in Fig. 1.

Next, authentic (+)-MTPA esters (**14**—**17**) were prepared as shown in Chart 2 in order to clarify the relationship between chemical shift and stereostructure.



a) optical resolution; b) CH_2N_2; c) (+)-MTPACl/pyridine;
d) EtOOC–N=N–COOEt/Ph_3P/(+)-MTPA/PhH; e) LDA/THF, −78 °C; f) MeI

Chart 2

Esterification of the known (2*R*, 3*S*)-dicarboxylic acid **18**[6] ($[\alpha]_D$ −9.9° (*c*=3, H_2O)) obtained by optical resolution of the corresponding racemate (**18** and **19**) gave the (2*R*, 3*S*)-isomer **13** ($[\alpha]_D$ +7.91° (*c*=4.9, $CHCl_3$)). Treatment of **13** with (+)-MTPACl gave the authentic (2*R*, 3*S*)-(+)-MTPA ester **17**, and when **13** was treated with (+)-MTPA ((+)-α-methoxy-α-trifluoromethylphenylacetic acid)[5] in the presence of diethyl azodicarboxylate (EtOOC–N=N–COOEt) and triphenylphosphine (Ph_3P) (Mitsunobu's method)[7] the (2*R*, 3*R*)-(+)-MTPA ester **14** was obtained. Alternatively, the same **13** and the (2*S*, 3*S*)-isomer **12** could be prepared by the methylation of dimethyl (−)-malate (**21**) according to the method of Seebach *et al.*[8] as shown in Chart 2. In this case, **13** should be obtained as the main product.[9] A mixture of the resulting hydroxy esters **12** and **13** was converted in the usual way to a mixture of the authentic (2*S*, 3*S*)-(+)-MTPA ester **16** and **17**. The 400 MHz NMR spectra of **14**, **17** and a mixture of **16** and **17** were then measured. Comparison of two doublets (centered at δ 5.795 and δ 5.425) in the C_3–H region of the mixture of **16** and **17** with the signal of the authentic **17** (δ 5.425) shows that the doublet appearing at lower field (δ 5.795) should be that of **16**.

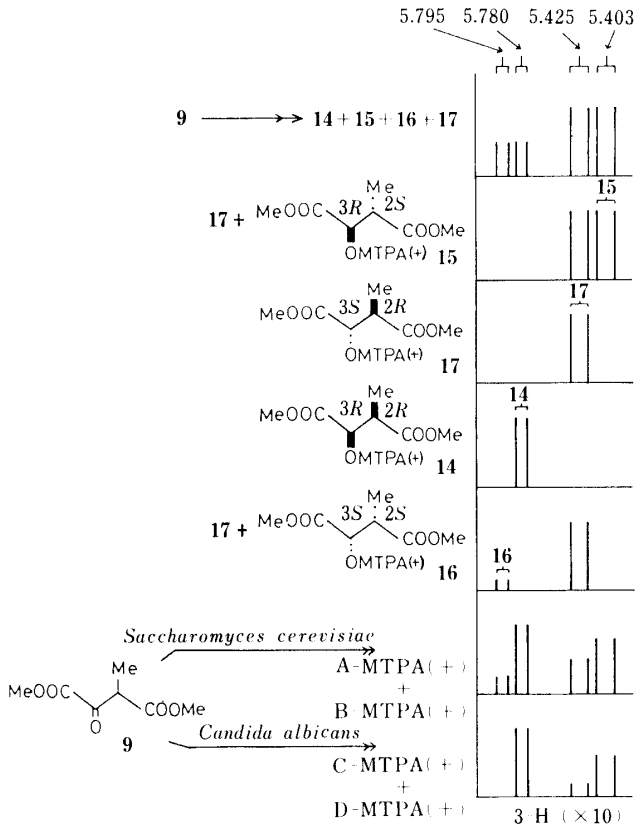


Chart 3

Three doublets of the four unidentified doublets due to C_3–H were thus rigorously assigned. Consequently, the remaining doublet centered at 5.403 should be assigned to C_3–H of the (2*S*, 3*R*)-(+)-MTPA ester **15**. In fact, doublets due to C_3–H of racemic *anti*[10]-(+)-MTPA ester (**15** and **17**) appear at 5.403 as well as at 5.425.

Since a relationship between the chemical shift and stereostructure of the four (+)-MTPA esters (**14**—**17**) was thus established, the 400 MHz NMR spectrum of (+)-MTPA esters of A and B produced by the reduction of the β-keto ester **9** with fermenting baker's yeast was measured. Based on the chemical shifts due to C_3–H shown in Chart 3, A and B were found to be (2*R*, 3*R*)-**14** and (2*S*, 3*R*)-**15**, respectively. The optical purities of these

compounds were calculated on the basis of the ratio of intensities due to C_3–H in the 400 MHz NMR spectra. The *syn*[10]-isomer A and the *anti*-isomer B were found to have 65 and 20% *e.e.*, respectively, as the (+)-MTPA esters. The ratio of A/B was found to be 53/47. When *Candida albicans* was used, reduction products (C and D) were obtained in 22% yield and were found to have the same absolute configurations as A and B (C-(+)-MTPA, (2*R*, 3*R*), optical purity 95% *e.e.*; D-(+)-MTPA, (2*S*, 3*R*), optical purity 58% *e.e.*). The ratio of *syn*-C/*anti*-D was found to be 64/36. It should be emphasized that the optical purity of the *syn* isomer C was significantly improved by the use of this microorganism.

The β-keto diester **9** was thus proved to be reduced by fermenting baker's yeast and *Candida albicans* to afford a mixture of the (2*R*, 3*R*)-isomer **10** and the (2*S*, 3*R*)-isomer **11**. Although the optical purity of **10** produced by *Candida albicans* is reasonably high (95% *e.e.*), the optical yields of other products are unexpectedly low. This result, combined with the observation that the separation of diastereomeric *syn*-**10** and *anti*-**11** isomers is rather difficult[11] suggests that the present method is not suitable for the production of the desired optically active alcohol esters **10**—**13**. However, identification of the four possible isomers **14**—**17** was again found to be easily achievable by NMR spectroscopy. This technique was effectively used in the determination of the stereostructures of the reduction products in other systems after conversion to these isomers, as will be reported in the subsequent paper.

Experimental

Infrared (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 (Senshu Pack N 50-5 column (Nucleosil, 5 μm, 8 i.d. × 300 mm)). $[\alpha]_D$ was measured on a Perkin-Elmer model 241 MC polarimeter.

Synthesis of Dimethyl 2-Methyl-3-oxosuccinate (9)——A mixture of methyl propionate (4.4 g, 0.05 mol) and lithium diisopropylamide (LDA; 0.055 mol) in dry tetrahydrofuran (THF, 30 ml) was stirred under an argon atmosphere for 30 min at −78 °C, and then a solution of dimethyl oxalate (5.9 g, 0.05 mol) in dry THF (20 ml) was added. The mixture was stirred for 25 min at −78 °C and then for 1 h at room temperature under an argon atmosphere. After addition of H_2O and acidification with AcOH, the reaction mixture was extracted with ether. The ether extract was washed with sat. $NaHCO_3$ aq. and sat. NaCl aq., then dried over $MgSO_4$ and concentrated to give an oil. Distillation of the oily product provided a colorless oil **9** (bp 81—88 °C (3 mmHg), 4.352 g; 50% yield). *Anal.* High-resolution MS. Calcd for $C_7H_{10}O_5$ (M^+; *m/e*): 174.053. Found: 174.052. IR ν_{max} cm^{-1}: 1732, 1745 (sh). NMR δ: 1.426 (3H, d, J=7.2 Hz; 2-Me), 3.738, 3.898 (each 3H, s; COOMe), 4.135 (1H, q, J=7.2 Hz; 2-H).

Asymmetric Reduction of 9 with Baker's Yeast (*Saccharomyces cerevisiae*)——i) A suspension of baker's yeast (available from Oriental Yeast Co., Ltd., 20 g), sucrose (20 g) and the substrate **9** (1 g) in H_2O (100 ml) was shaken at 30 °C for 5 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 (**10**+**11**; 513 mg, 51% yield) as a homogeneous oil from the *n*-hexane–ethyl acetate (10 : 1) eluate. **10**+**11**: *Anal.* High-resolution MS. Calcd for $C_7H_{12}O_4$ (M^++1; *m/e*): 177.076. Found: 177.074. 3,5-Dinitrobenzoates of **10** and **11**: *Anal.* High-resolution MS. Calcd for $C_{14}H_{14}N_2O_{10}$ (M^++1; *m/e*): 371.072. Found: 371.071.

ii) Imidazole (372 mg) and *tert*-butyldimethylchlorosilane (823 mg) were added to a solution of the baker's yeast reduction products (**10**+**11**; 800 mg) in dimethylformamide (DMF, 5 ml) and the reaction mixture was stirred for 17 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 (45 g). Elution with *n*-hexane–ethyl acetate (10 : 1) afforded 1.257 g of silyl ethers (**20**+**21**). The silyl ethers were subjected to HPLC with *n*-hexane–ethyl acetate (50 : 1) as a solvent system (flow rate; 3 ml/min), yielding the less polar fraction **20** (89 mg) and the more polar fraction **21** (427 mg). **20**: *Anal.* High-resolution MS. Calcd for $C_{13}H_{26}O_5Si$ (M^++1; *m/e*): 291.163. Found: 291.165. $[\alpha]_D^{24}$ +16.34° (*c*=5.35, $CHCl_3$; corresponding to 65% *e.e.*). IR ν_{max} cm^{-1}: 1735, 1750 (sh), 1760 (sh), 400 MHz NMR δ: 0.022, 0.083 (each 3H, s; $SiMe_2$), 0.880 (9H, s; *tert*-Bu), 1.151 (3H, d, J=7.1 Hz; 2-Me), 2.948 (1H, qq, J=4.2, 7.1 Hz; 2-H), 3.693, 3.735 (each 3H, s; COOMe), 4.675 (1H, d, J=4.2 Hz; 3-H). **21**: *Anal.* High-resolution MS. Calcd for $C_{13}H_{26}O_5Si$ (M^++1; *m/e*):

291.163. Found: 291.163. $[\alpha]_D^{24}$ +5.56° (c=5.25, $CHCl_3$; corresponding to 20% *e.e.*). IR ν_{max} cm^{-1}: 1740. 400 MHz NMR δ: 0.051, 0.070 (each 3H, s; $SiMe_2$), 0.878 (9H, s; *tert*-Bu), 1.151 (3H, d, J=7.1 Hz; 2-Me), 2.938 (1H, qq, J=6.3, 7.1 Hz; 2-H), 3.679, 3.736 (each 3H, s; COOMe), 4.372 (1H, d, J=6.3 Hz; 3-H).

iii) A mixture of **21** (213 mg) and $Bu_4N^+F^-\cdot 3H_2O$ (600 mg) in THF (10 ml) was stirred for 2.5 h at room temperature and the reaction mixture was extracted with ether after the addition of H_2O. The ether extract was washed with sat. NaCl aq., then dried over $MgSO_4$ and evaporated to give an oil, which was chromatographed on silica gel (8 g) to afford *anti*-**11** (32 mg) as a homogeneous oil from the *n*-hexane–ethyl acetate (10 : 1) eluate. $[\alpha]_D^{28.5}$ −0.625° (c=3.2, $CHCl_3$; corresponding to 20% *e.e.*). Spectral data (IR and NMR) of *anti*-**11** were identical with those of authentic (2*R*, 3*S*)-**13** described later in this paper.

Preparation of Four Alcohols (10—13) and Their (+)-MTPA Esters (14—17)——i) A solution of **9** (660 mg) in EtOH (20 ml) was catalytically hydrogenated in the presence of Raney Ni (W-7; prepared from Raney alloy, 2 g) under a hydrogen atmosphere (3—4 kg/cm^2) with shaking for 2 h. The filtrate was evaporated to give an oily product (**10**+**11**+**12**+**13**; 598 mg). The ratio of *syn*-(**10**+**12**)/*anti*-(**11**+**13**) was found to be 1/2 by comparison of integral intensities due to secondary methyl groups (δ: 1.207 and 1.304, respectively **10**+**12** and **11**+**13**) in the 60 MHz NMR spectra.

ii) Pyridine (0.5 ml) was added to a mixture of the four alcohols (80 mg) and (+)-MTPACl (156 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., then dried over $MgSO_4$. Removal of the solvent gave an oil, which was chromatographed on silica gel (20 g) to afford the (+)-MTPA esters (**14**+**15**+**16**+**17**; 140 mg) as a homogeneous oil from the *n*-hexane–ethyl acetate (9 : 1) eluate. *Anal.* High-resolution MS. Calcd for $C_{17}H_{19}F_3O_7$ (M^+; *m/e*): 392.108. Found: 392.111. **14**+**16**: 400 MHz NMR δ: 1.175, 1.215 (each 3H, d, J=7.2 Hz; 2-Me), 3.582, 3.708, 3.781, 3.821 (each 3H, s; COOMe), 5.780 (1H, d, J=3.7 Hz; 3-H), 5.795 (1H, d, J=3.4 Hz; 3-H). **15**+**17**: 400 MHz NMR δ: 1.172, 1.268 (each 3H, d, J=7.2 Hz; 2-Me), 3.602, 3.668, 3.777, 3.810 (each 3H, s; COOMe), 5.403, 5.425 (each 1H, d, J=5.6 Hz; 3-H).

Preparation of Racemic *anti*-(+)-MTPA Esters (15 and 17)——Racemic *anti*-methylmalic acid (**18**+**19**, prepared by Izumi's method[6] from diethyl 2-methyl-3-oxosuccinate) was converted into dimethyl esters (**11**+**13**) by treatment with a solution of CH_2N_2 in ether. Pyridine (0.5 ml) was added to a mixture of racemic *anti*-dimethyl esters (**11**+**13**; 60 mg) and (+)-MTPACl (117 mg), and the mixture was stirred for 12 h at room temperature, then worked up and purified in the same way as in the case of preparation of the four (+)-MPTA esters (**14**+**15**+**16**+**17**) to provide the racemic *anti*-(+)-MTPA esters (**15**+**17**; 12.5 mg) as a homogeneous oil. 400 MHz NMR δ: 1.172, 1.268 (each 3H, d, J=7.2 Hz; 2-Me), 3.602, 3.668, 3.777, 3.810 (each 3H, s; COOMe), 5.403, 5.425 (each 1H, d, J=5.6 Hz; 3-H).

Preparation of (2*R*, 3*S*)-Dimethyl Ester 13, Its (+)-MTPA Ester 17 and *tert*-Butyldimethylsilyl Ester 22——i) Racemic *anti*-methylmalic acid (**18**+**19**) was subjected to optical resolution with brucine by Izumi's method[6] to afford (2*R*, 3*S*)-methylmalic acid (**18**; $[\alpha]_D^{26}$ −9.9° (c=3, H_2O)). Treatment of **18** (687 mg) with a solution of CH_2N_2 in ether gave an oil, which was chromatographed on silica gel (13 g) to provide the (2*R*, 3*S*)-dimethyl ester **13** (338 mg) as a homogeneous oil from the *n*-hexane–ethyl acetate (10 : 1) eluate. $[\alpha]_D^{26.5}$ +7.91° (c=4.9, $CHCl_3$). NMR (60 MHz) spectra of **13** were identical with those of an authentic sample **13** prepared by Seebach *et al.*[8]

ii) Pyridine (0.5 ml) was added to a mixture of **13** (32 mg) and (+)-MTPACl (54 mg), and the reaction mixture was stirred for 48 h at room temperature, then worked up in the same way as in the case of preparation of the racemic *anti* mixture (**15** and **17**) to give an oily product, which was subjected to preparative thin layer chromatography [prep. TLC; silica gel, 20 cm × 20 cm; solvent, *n*-hexane–ethyl acetate (3 : 1)] to provide to (2*R*, 3*S*)-(+)-MTPA ester (**17**; 32 mg) as a homogeneous oil. 400 MHz NMR δ: 1.268 (3H, d, J=7.2 Hz; 2-Me), 3.668, 3.777 (each 3H, s; COOMe), 3.182 (1H, qq, J=5.6, 7.2 Hz; 2-H), 5.425 (1H, d, J=5.6 Hz; 3-H).

iii) A solution of **13** (70 mg), imidazole (32 mg) and *tert*-butyldimethylchlorosilane (72 mg) in DMF (0.8 ml) was stirred for 24 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of silylation of baker's yeast reduction products (**10** and **11**) to afford the (2*R*, 3*S*)-silyl ether **22** (43 mg) as a homogeneous oil. Spectra data (IR and NMR) were identical with those of the more polar fraction **21** derived from baker's yeast reduction products of **9**. $[\alpha]_D^{27}$ −48.00° (c=4.25, $CHCl_3$).

Preparation of (2*R*, 3*R*)-(+)-MTPA Ester 14——A mixture of **13** (23 mg), (+)-MTPA (39 mg), EtOOC–N=N–COOEt (27 mg) and Ph_3P (41 mg) in dry benzene (3 ml) was stirred under an argon atmosphere for 120 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 evaporated to give an oil, which was purified in the same way as in the case of preparation of **17** to provide the (2*R*, 3*R*)-(+)-MTPA ester (11 mg) as a homogeneous oil. 400 MHz NMR δ: 1.175 (3H, d, J=7.2 Hz; 2-Me), 3.142 (1H, qq, J=3.7, 7.2 Hz; 2-H), 3.582, 3.821 (each 3H, s; COOMe), 5.780 (1H, d, J=3.7 Hz; 3-H).

Preparation of a Mixture of (2*R*, 3*S*)-(+)-MTPA Ester 17 and (2*S*, 3*S*)-(+)-MTPA Ester 16——A mixture of (2*R*, 3*S*)-**13** and (2*S*, 3*S*)-**12** was prepared from dimethyl (*S*)-(−)-malate ($[\alpha]_D^{24}$ −52.34° (c=5, $CHCl_3$)) by Seebach's method.[8] (+)-MTPACl (69 mg) and then pyridine (0.5 ml) were added to a mixture (40 mg) of **13** and **12** and the whole was stirred for 24 h at room temperature. The reaction mixture was worked up and purified in the

same way as in the case of preparation of **17** to afford a mixture (24 mg) of the (2*R*, 3*S*)-(+)-MTPA ester **17** and (2*S*, 3*S*)-(+)-MTPA ester **16** as a homogeneous oil. **16**: 400 MHz NMR δ: 1.215 (3H, d, J=7.2 Hz; 2-Me), 3.708, 3.781 (each 3H, s; COOMe), 5.795 (1H, d, J=3.4 Hz; 3-H).

(+)-MTPA Esterification of Baker's Yeast Reduction Products (10 and 11)——Pyridine (0.5 ml) was added to a mixture of baker's yeast reduction products (**10**+**11**; 31 mg) and (+)-MTPACl (54 mg), and the mixture was stirred for 48 h at room temperature. The reaction mixture was worked up and purified in the same way as in the case of preparation of **17** to provide the (+)-MTPA esters (**14**+**15**; 38 mg) as a homogeneous oil. The ratio of *syn*-**14**/*anti*-**15** and hence that of *syn*-**10**/*anti*-**11** was found to be 53/47 by a comparison of integral intensity of C_3–H signals in the 400 MHz NMR spectra. The optical purity of *syn*-**14** and hence that of *syn*-**10** was found to be 65% *e.e.* and that of *anti*-**15** and hence that of *anti*-**11** was calculated as 20% *e.e.*

Asymmetric Reduction of 9 with *Candida albicans*[12]——i) An Erlenmeyer flask (500 ml) containing 75 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) was inoculated with *Candida albicans* and the yeast was cultured at 30 °C for 3 d with continuous shaking. Then 75 mg of the substrate **9** was added, and culture was continued for a further 3 d under the same conditions. The asymmetric reduction of **9** (finally 300 mg of **9**) was carried out four times on the same scale.

ii) The reaction mixture was worked up and purified in the same way as in the case of baker's yeast reduction of **9** to provide the reduction products (**10**+**11**; 68 mg, 22% yield). Pyridine (0.5 ml) was added to a mixture of *Candida albicans* reduction products (68 mg) and (+)-MTPACl (116 mg), and the mixture was stirred for 12 h at room temperature, then worked up and purified in the same way as in the case of preparation of **17** to afford the (+)-MTPA esters (**14**+**15**; 30 mg) as a homogeneous oil. The ratio of *syn*-**14**/*anti*-**15** and hence that of *syn*-**10**/*anti*-**11** was found to be 64/36 from the 400 MHz NMR spectra. The optical purity of *syn*-**14** and hence that of *syn*-**10** was found to be 95% *e.e.* and that of *anti*-**15** and hence that of *anti*-**11** was calculated as 58% *e.e.*

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References and Notes

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9) The fact that the same **13** was obtained by two different methods clearly showed that **13** should have the (2*R*, 3*S*) structure.
10) The terms *syn* and *anti* are used according to S. Masamune, Sk. A. Ali, D. L. Snitman, and D. S. Garvey, *Angew. Chem. Int. Ed. Engl.*, **19**, 557 (1980).
11) The *tert*-butyldimethysilyl ethers (**20** and **21**) of the corresponding *syn*- and *anti*-alcohols can be separated by HPLC, but the deprotection by *n*-$Bu_4N^+F^-$ followed by purification by silica gel chromatography caused a substantial loss of the product.
12) K. Horikoshi, A. Furuichi, H. Koshiji, H. Akita, and T. Oishi, *Agric. Biol. Chem.*, **47**, 435 (1983).