Chem. Pharm. Bull. 33(11)4657—4661(1985)

Microbial Reduction of 2-Phenylpropionic Acid, 2-Benzyloxypropionic Acid and 2-(2-Furfuryl)propionic Acid

YOSHIKO TSUDA,* KEN-ICHI KAWAI and SHOICHI NAKAJIMA

Faculty of Pharmaceutical Sciences, Hoshi University, Ebara, Shinagawa-ku, Tokyo 142, Japan

(Received January 28, 1985)

Racemic 2-phenylpropionic acid ((±)-**1**) and 2-benzyloxypropionic acid ((±)-**4**) were subjected to microbial reduction and simultaneous resolution with several molds, *i.e.*, *Malus* and *Prunus* strains of *Glomerella cingulata*, *Gloeosporium olivarum* and *Gloeosporium laeticolor*, yielding (*R*)-2-phenylpropanol ((*R*)-**2**) and (*S*)-2-benzyloxypropanol ((*S*)-**5**), and leaving (*R*)-2-phenylpropionic acid ((*R*)-**1**) and (*R*)-2-benzyloxypropionic acid ((*R*)-**4**), respectively. The microbial reduction of racemic 2-(2-furfuryl)propionic acid ((±)-**8**) gave optically inactive (±)-2-(2-furfuryl)propanol ((±)-**9**).

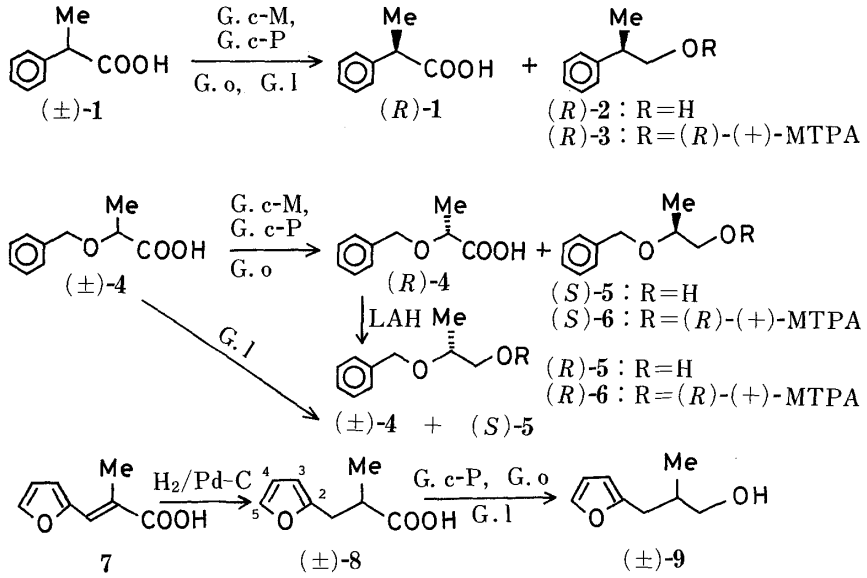
Keywords——asymmetric reduction; 2-benzyloxypropionic acid; 2-(2-furfuryl)propionic acid; microbial reduction; microbial resolution; 2-phenylpropionic acid

The authors reported in the preceding papers[1,2] that microbial reduction of various racemic 2-alkyl-2-aryloxyacetic acids with *Glomerella cingulata* (*Malus* strain) and *Gloeosporium olivarum* yielded optically active 2-alkyl-2-aryloxyethanols, and, at the same time, as a result of the microbial resolution that occurs simultaneously, optically active 2-alkyl- 2-aryloxyacetic acids. This microbial technique of preparing compounds with a chiral center at the position adjacent to a carboxyl or a carbinol group would be useful in synthetic chemistry, if it could be applied generally for the preparation of propionic acids substituted by various functional groups.

We now report the successful microbial reduction and resolution of propionic acids substituted at the 2-position by a phenyl or benzyloxy group, *i.e.*, 2-phenylpropionic acid ((±)-**1**) and 2-benzyloxypropionic acid ((±)-**4**), producing optically active (*R*)-2-phenylpropanol ((*R*)-**2**) and (*S*)-2-benzyloxypropanol ((*S*)-**5**), and leaving (*R*)-2-phenylpropionic acid ((*R*)-**1**) and (*R*)-2-benzyloxypropionic acid ((*R*)-**4**), respectively. 2-(2-Furfuryl)-propionic acid ((±)-**8**) gave optically inactive 2-(2-furfuryl)propanol ((±-**9**) in this microbial reduction.

Since only a few mold strains have been found that are capable of reducing carboxylic acids to alcohols,[1-5] we have investigated the reduction with *Glomerella cingulata* (*Prunus* strain) and *Gloeosporium laeticolor*, as a follow-up to our previous work on the molds *Glomerella cingulata* (*Malus* strain) and *Gloeosporium olivarum*.[1,2] All the molds proved to be effective for the microbial reduction of carboxylic acids to alcohols.

When the racemic 2-phenylpropionic acid ((±)-**1**) was fermented with any of the above four molds, 2-phenylpropanol ((*R*)-**2**) of *R*-configuration was produced. This result is apparently in conflict with our previous data[1,2] on the microbial reduction of 2-alkyl-2-aryloxyacetic acids, which gave not (*R*)- but (*S*)-alcohols. However, if the reaction is considered in terms of the actual stereochemical bulkiness around the asymmetric carbon, the results are consistent with each other. The carboxylic acid that remained in the culture broth was optically active 2-phenylpropionic acid ((*R*)-**1**) of *R*-configuration with all the molds. The absolute configuration and the optical purity of this acid ((*R*)-**1**) from each mold were



G.c-M: *Glomerella cingulata* (*Malus* spp.)
G.c-P: *Glomerella cingulata* (*Prunus* spp.)
G.o: *Gloeosporium olivarum*
G.l: *Gloeosporium laeticolor*

Chart 1

determined by comparing the optical rotation with that reported in the literature.[6]

Though the value of the specific rotation of (*R*)-2-phenylpropanol is described in the literature,[7] the enantiomeric purity was not high. Thus, the optical purity of the alcohol ((*R*)-**2**) obtained by the microbial reduction with each mold was determined by deriving it into the (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester ((*R*)-**3**), and measuring the relative intensities of the methoxyl 100 MHz proton nuclear magnetic resonance (^{1}H-NMR) signals of the (*R*,*R*)- and (*R*,*S*)-diastereomers in the presence of Sievers' lanthanide shift reagent, Eu(fod)$_3$. This reagent was found to cause a larger downfield shift of the methoxyl signals of the (*R*,*R*)- rather than the (*R*,*S*)-diastereomer. This result is in parallel with the result for (*R*,*R*)- and (*R*,*S*)-diastereomers of the (*R*)-(+)-MTPA esters of 2-alkyl-2-aryloxyethanols[1,2] on the basis of the stereochemical bulkiness around the chiral carbon.

The incubation times, yields, values of specific rotation and optical purities of the products from the fermentation of (±)-2-phenylpropionic acid ((±)-**1**) with the microorganisms are shown in Table I. In view of the microbial resolution which takes place as the result of the microbial reduction, the (*S*)-acid is expected to be left when the (*R*)-alcohol is produced. The fact that this was not the case in our experiment might be due to a greater rate of degradation of the (*S*)-acid than the (*R*)-acid during fermentation.

2-Benzyloxypropionic acid ((±)-**4**) was also subjected to microbial reduction, providing 2-benzyloxypropanol ((*S*)-**5**) of *S*-configuration with all four strains. This result is in parallel with our previous results[1,2] on the microbial reduction of (±)-2-alkyl-2-aryloxyacetic acids to (*S*)-2-alkyl-2-aryloxyethanols. The culture broth after fermentation contained 2-benzyloxypropionic acid ((*R*)-**4**) of *R*-configuration in the cases of fermentation with *Malus* and *Prunus* strains of *Glomerella cingulata* and *Gloeosporium olivarum*, but with *Gloeosporium laeticolor*, the acid obtained was optically inactive.

The absolute configuration and the optical purity of (*R*)-2-benzyloxypropionic acid ((*R*)-**4**) obtained by *Prunus* strain of *Glomerella cingulata* were determined by comparison of the value of specific rotation with that given in the literature.[8] The acid produced by *Malus* strain of *Glomerella cingulata* or *Gloeosporium olivarum* could not be purified easily due to

TABLE I. Asymmetric Reduction and Resolution of 2-Phenylpropionic Acid, 2-Benzyloxypropionic Acid and 2-(2-Furfuryl)propionic Acid

Substrate	Microorganism	Incubation (d)	Products	Yield (%)	$[\alpha]_D^{20}$ [a]	Optical purities
(±)-**1**	G.c-M	21	(*R*)-**1**	22	−33.2 (C)	43[b]
			(*R*)-**2**	15	+4.1 (B)	30[c]
	G.c-P	18	(*R*)-**1**	59	−32.9 (C)	43[b]
			(*R*)-**2**	22	+2.5 (B)	16[c]
	G.o	20	(*R*)-**1**	34	−10.2 (C)	13[b]
			(*R*)-**2**	5	+3.8 (B)	26[c]
	G.l	22	(*R*)-**1**	39	−19.5 (C)	26[b]
			(*R*)-**2**	2	+1.5 (B)	12[c]
(±)-**4**	G.c-M	12	(*R*)-**4**	11		57[d]
			(*S*)-**5**	63	+21.2 (C)	54[c]
	G.c-P	10	(*R*)-**4**	26	+32.5 (E)	35[b]
			(*S*)-**5**	66	+9.5 (C)	24[c]
	G.o	20	(*R*)-**4**	33		66[d]
			(*S*)-**5**	31	+14.5 (C)	37[c]
	G.l	14	(±)-**4**	3	0	0
			(*S*)-**5**	74	+7.8 (C)	20[c]
(±)-**8**	G.c-P	18	(±)-**9**	36	0	0
	G.o	18	(±)-**9**	38	0	0
	G.l	18	(±)-**9**	32	0	0

a) Solvents: B, benzene; C, $CHCl_3$; E, EtOH. Concentrations: (*R*)-**1** and (*S*)-**5**, $c=2.5$; (*R*)-**2** and (*R*)-**4**, $c=3.3$. *b*) Determined by comparison of $[\alpha]_D^{20}$ with the literature value. *c*) Determined by the (*R*)-(+)-MTPA ester method. *d*) Determined by the (*R*)-(+)-MTPA ester method with the corresponding alcohol derived by reduction with $LiAlH_4$.

metabolic contamination, so the acid was reduced to the corresponding alcohol ((*R*)-**5**) by treatment with lithium aluminum hydride and purified by distillation.

The (*R*)-(+)-MTPA ester ((*R*)-**6**) of the alcohol ((*R*)-**5**) and that ((*S*)-**6**) of (*S*)-**5** were prepared, and the optical purities of (*R*)-**6** (hence (*R*)-**5**), and (*S*)-**6** (hence (*S*)-**5**) were determined, as described above in the case of the (*R*)-(+)-MTPA ester ((*R*)-**3**) and or the alcohol ((*R*)-**2**). The incubation times, yields, values of specific rotation and the optical purities of the products from the fermentation of (±)-2-benzyloxypropionic acid ((±)-**4**) with each microorganism are shown in Table I.

2-(2-Furfuryl)propionic acid ((±)-**8**) was prepared by catalytic hydrogenation of 2-(2-furfurylidene)propionic acid (**7**) in the presence of 10% palladium charcoal, and was subjected to microbial reduction with *Glomerella cingulata* (*Prunus*), *Gloeosporium olivarum*, or *Gloeosporium laeticolor*. In all cases, the reduction product, 2-(2-furfuryl)propanol ((±)-**9**) was optically inactive, and the acid (**8**) that remained in the culture broth after fermentation was racemic.

Experimental

Infrared (IR) spectra were determined on a Hitachi model 215 spectrometer. ^{1}H-NMR spectra were recorded at 100 MHz with tetramethylsilane as an internal standard on a JEOL FX-100 spectrometer. Optical rotations were measured on a JASCO DIP-101 automatic polarimeter with a cell of 10 cm light path length. The mass spectra (MS) were obtained on a JEOL JMS-D 300 instrument.

Preparation of (±)-2-(2-Furfuryl)propionic Acid ((±)-8)——2-(2-Furfurylidene)propionic acid (**7**), mp 119 °C, was synthesized as described in the literature.[9] This compound (9.1 g) was dissolved in MeOH (2.4 l), and hydrogen was passed into the solution in the presence of 10% Pd–C (3.0 g) at room temperature, until one molar equivalent of hydrogen was absorbed. The mixture was filtered, the filtrate was evaporated, and the residual liquid was distilled *in*

vacuo to give 2-(2-furfuryl)propionic acid ((±)-**8**); yield 7.9 g, bp 40—45 °C (4 mmHg). IR ν_{max}^{film} cm^{-1}: 2950, 2650, 1700, 1595, 1505, 1455, 1410, 1380, 1335, 1280, 1230, 1172, 1142, 1008, 930, 733. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, d, J=6.8 Hz, CH_3), 2.80 (3H, m, CH_2 and CH), 6.06 (1H, dd, J=3.2, 0.8 Hz, furan-3-H), 6.27 (1H, dd, J=3.2, 1.8 Hz, furan-4H), 7.31 (1H, dd, J=1.8, 0.8 Hz, furan-5H), 7.60 (1H, br s, OH). MS *m/z*: 154 (M^+).

Preparation of (±)-2-Benzyloxypropionic Acid ((±)-4)——Ethyl 2-bromopropionate (14.8 g) was added to a solution of Na (1.0 g) in benzyl alcohol (4.3 g) and the mixture was refluxed under stirring for 5 h, then cooled and filtered. The filtrate was distilled to give ethyl 2-benzyloxypropionate (4.5 g), bp 109—120 °C (9 mmHg) (lit.[10] bp 105—106 °C (1.5 mmHg)). This ester was hydrolyzed by heating with 60% KOH (5.0 g) for 2 h, and the acid ((±)-**4**) was obtained by acidification with 10% HCl followed by extraction with ether, drying of the extract over anhydrous Na_2SO_4, and removal of the ether; yield 2.5 g, bp 120—128 °C (1 mmHg). IR ν_{max}^{film} cm^{-1}: 3030, 2990, 1722, 1600, 1495, 1450, 1370, 1270, 1118, 1060, 1020, 910, 820, 740, 690. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, d, J=8.0 Hz, CH_3), 4.04 (1H, q, J=8.0 Hz, CH), 4.44 (1H, d, J=12.0 Hz, OCH-H), 4.70 (1H, d, J=12.0 Hz, OCH-H), 7.31 (5H, s, aromatic protons), 7.38 (1H, s, OH). MS *m/z*: 180 (M^+).

Preparation of (*R*)-(+)-MTPA Ester ((*R*,*S*)-3) of Racemic 2-Phenylpropanol ((*R*,*S*)-2)——Pyridine (0.3 ml) was added to a mixture of the alcohol ((*R*,*S*)-**2**) (20 mg) and (*R*)-(+)-MTPA chloride (52 mg), and the mixture was stirred for 26 h at room temperature. After addition of water, the reaction mixture was extracted with ether, and the extract was washed with a small amount of saturated aq. NaCl solution, dried over anhydrous Na_2SO_4 and evaporated to give an oil, which was subjected to preparative thin-layer chromatography (TLC) on silica gel (20 cm × 20 cm), with $CHCl_3$ as the eluting solvent, to provide pure (*R*)-(+)-MTPA ester ((*R*,*S*-3)) as an oil; yield 24 mg. MS (CI) *m/z*: 353 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, d, J=7.1 Hz, CH_3), 3.17 (1H, m, CH), 3.40 (3H, s, OMe), 4.39 (2H, m, CH_2), 7.21—7.35 (10H, m, aromatic protons). ^{1}H-NMR (10 mg in $CDCl_3$ in the presence of 17.5 mg of Eu $(fod)_3$): (*R*)-OMe 6.06 and (*S*)-OMe 5.92.

Preparation of (*R*)-(+)-MTPA Ester ((*R*,*S*)-6) of Racemic 2-Benzyloxypropanol ((*R*,*S*)-5)——The (*R*)-(+)-MTPA ester ((*R*,*S*)-**6**) was prepared from (*R*,*S*)-**5** (21 mg) in the same way as in the case of the preparation of the (*R*,*S*)-**3** from (*R*,*S*)-**2**. The pure ester ((*R*,*S*)-**6**) obtained was an oil; yield 18 mg. MS *m/z*: 382 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, d, J=6.4 Hz, CH_3), 3.54 (3H, s, OMe), 3.82 (1H, m, CH), 4.33 (2H, m, CH_2), 4.51 (2H, s, benzylic H), 7.15—7.50 (10H, m, aromatic protons). ^{1}H-NMR (8 mg in $CDCl_3$ in the presence of 24.0 mg of Eu$(fod)_3$): (*S*)-OMe 4.75 and (*R*)-OMe 4.79.

Microbial Reduction and Resolution of (±)-2-Phenylpropionic Acid ((±)-1), (±)-2-Benzyloxypropionic Acid ((±)-4) and (±)-2-(2-Furfuryl)propionic Acid ((±)-8)——a) General Fermentation Procedures: The microorganisms, *i.e.*, *Malus* and *Prunus* strains of *Glomerella cingulata* and *Gloeosporium laeticolor* were provided by Dr. Y. Oonuma of Yamagata Horticultural Experiment Station. *Gloeosporium olivarum* was a gift from Prof. Dr. T. Tani of Kagawa University, who isolated the mold from an infected natural olive.

Fermentation in a jar fermenter was not applicable, because the air-bubbling into the culture solution which was necessary for the growth of the molds was found to suppress the microbial reduction. Thus, the stationary culture method was adopted. The cultivation period was adjusted as necessary for growing the molds.

A substrate (3 g) was added to the culture broth containing peptone (60 g), KH_2PO_4 (6 g), $MgSO_4 \cdot 7H_2O$ (2.4 g), sucrose (300 g) and water (6 l), and the whole mixture was kept in 20 Roux bottles after adjustment of the pH to 5.5. The Roux bottles were stoppered with cotton, autoclaved, cooled, and inoculated with a microorganism. Culture was carried out at 27 °C for the periods designated in Table I. Longer incubation periods resulted in a decrease of the optical purity of the produced alcohols, though the yields increased. After removal of the mycelium by filtration, the culture filtrate was alkalized to pH 10, and shaken with ether (2 l). The ethereal extract was washed with water and dried over anhydrous Na_2SO_4. The crude product obtained after evaporation of the solvent was purified by column chromatography on silica gel, using $CHCl_3$ as the eluting solvent, followed by distillation *in vacuo*. (*R*)-2-Phenylpropanol ((*R*)-**2**), (*S*)-2-benzyloxypropanol ((*S*)-**5**) and (±)-2-(2-furfuryl)propanol ((±)-**9**) thus obtained each gave a single spot on pre-coated Silica Gel 60 F254 TLC plates (Merck Co., Ltd.).

The culture filtrate after ethereal extraction at pH 10 was acidified to pH 2, and shaken with ether (2 l). The ethereal extract was washed with water and dried over anhydrous Na_2SO_4. The crude product obtained after evaporation of the solvent was purified by silica gel column chromatography, using $CHCl_3$ as the eluting solvent, followed by distillation *in vacuo*. The (*R*)-2-phenylpropionic acid ((*R*)-**1**) from various mold strains, and the (*R*)-2-benzyloxypropionic acid ((*R*)-**4**) from *Prunus* strain of *Glomerella cingulata* were thus obtained in a pure state, and showed single spots on pre-coated Silica Gel 60 F254 TLC plates. However, the (*R*)-2-benzyloxypropionic acid ((*R*)-**4**) obtained by microbial resolution with *Glomerella cingulata* (*Malus*) or *Gloeosporium olivarum* was not easy to purify by this procedure, so it was derived into the corresponding alcohol by reaction with lithium aluminum hydride and then purified by distillation as described below.

b) (*R*)-2-Phenylpropionic Acid ((*R*)-**1**): The compound ((*R*)-**1**) obtained by fermentation showed bp 85—90 °C (3 mmHg). IR ν_{max}^{film} cm^{-1}: 3000, 2600, 1700, 1600, 1500, 1450, 1400, 1290, 1230, 1050, 940, 850, 700. ^{1}H-NMR ($CDCl_3$) δ: 1.51 (3H, d, J=7.3 Hz, CH_3), 3.73 (1H, q, J=7.3 Hz, CH), 7.30 (5H, s, aromatic protons), 9.56 (1H, br s, OH). MS *m/z*: 150 (M^+). The optical purities were calculated on the basis of $[\alpha]_D$ +76.9° in $CHCl_3$ for the authentic pure (*S*)-isomer,[6] and are listed in Table I.

c) (*R*)-2-Phenylpropanol ((*R*)-**2**): The compound ((*R*)-**2**) obtained by fermentation showed bp 40—60 °C (4 mmHg). IR ν_{max}^{film} cm^{-1}: 3400, 3000, 2800, 1600, 1500, 1430, 1380, 1040, 890, 760, 700. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, d, J=7.1 Hz, CH_3), 1.60 (1H, s, OH), 2.95 (1H, m, CH), 3.70 (2H, d, J=6.6 Hz, CH_2), 7.28—7.35 (5H, m, aromatic protons). MS *m/z*: 136 (M^+). The (*R*)-(+)-MTRA ester ((*R*)-**3**) was prepared in the same way as in the case of the preparation of (*R,S*)-**3** from (*R,S*)-**2**. The pure ester ((*R*)-**3**) was an oil; yield 24 mg. MS (CI) *m/z*: 353 (MH^+). ^{1}H-NMR ($CDCl_3$) δ: 3.40 (3H, s, OMe). ^{1}H-NMR (10 mg in $CDCl_3$ in the presence of 25.4 mg of Eu (fod)$_3$): (*R*)-OMe 6.32 and (*S*)-OMe 6.16. The optical purities of the compounds ((*R*)-**2**) from the various molds were calculated based on the integrated areas of the (*R*)-OMe *vs.* (*S*)-OMe signals in the ^{1}H-NMR spectra of (*R*)-**3** in the presence of Eu (fod)$_3$, and the results are listed in Table I.

d) (*R*)-2-Benzyloxypropionic Acid ((*R*)-**4**): The compound ((*R*)-**4**) obtained by fermentation showed bp 50—60 °C (3 mmHg). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, d, J=8.0 Hz, CH_3), 4.06 (1H, q, J=8.0 Hz, CH), 4.44 (1H, d, J=12.0 Hz, benzylic H), 4.70 (1H, d, J=12.0 Hz, benzylic H), 7.31 (5H, s, aromatic protons), 7.38 (1H, s, OH). MS *m/z*: 180 (M^+). The optical purity of (*R*)-**4** from the fermentation with *Glomerella cingulata* (*Prunus*) was calculated on the basis of the value of $[\alpha]_D$ −92° in EtOH for authentic pure optical (*S*)-isomer,[8] and the results are listed in Table I. The same acid ((*R*)-**4**) from *Glomerella cingulata* (*Malus*) or *Gloeosporium olivarum* was further derived into (*R*)-**5** then (*R*)-**6** for determination of the optical purity, as mentioned below.

e) (*S*)-2-Benzyloxypropanol ((*S*)-**5**): The compound ((*S*)-**5**) obtained by fermentation showed bp 50—60 °C (6 mmHg). IR ν_{max}^{film} cm^{-1}: 3350, 2875, 1500, 1450, 1370, 1340, 1200, 1140, 1040. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, d, J=6.1 Hz, CH_3), 1.62 (1H, s, OH), 3.61 (3H, m, CH_2 and CH), 4.47 (1H, d, J=11.5 Hz, benzylic H), 4.67 (1H, d, J=11.5 Hz, benzylic H), 7.34 (5H, s, aromatic protons). MS *m/z*: 166 (M^+). The (*R*)-(+)-MTPA ester ((*S*)-**6**) was prepared from (*S*)-**5** (20 mg) in the same way as in the case of the preparation of the (*R,S*)-**3** from (*R,S*)-**2**. The pure ester ((*S*)-**6**) was obtained as an oil; yield 23 mg. MS *m/z*: 382 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 3.54 (3H, s, OMe). ^{1}H-NMR (10 mg in $CDCl_3$ in the presence of 25.3 mg of Eu(fod)$_3$): (*S*)-OMe 4.71 and (*R*)-OMe 4.76. The optical purities of the compounds ((*S*)-**6**) from various molds were calculated from the integrated areas of the (*S*)-OMe *vs.* (*R*)-OMe signals in the ^{1}H-NMR spectra in the presence of Eu(fod)$_3$, and the results are listed in Table I.

f) 2-(2-Furfuryl)propanol ((±)-**9**): The compound ((±)-**9**) obtained by fermentation showed bp 60—80 °C (5 mmHg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600—3350, 2930, 2870, 1580, 1550, 1450, 1380, 1245, 1215, 1145, 1030, 1010, 740. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, d, J=6.6 Hz, CH_3), 1.60 (1H, br s, OH), 2.02 (1H, m, CH), 2.64 (2H, m, furfurylicH), 3.49 (2H, d, J=5.9 Hz, CH_2OH), 6.01 (1H, dd, J=0.8, 3.2 Hz, furan-3-H), 6.28 (1H, dd, J=1.9, 3.2 Hz, furan-4-H), 7.29 (1H, dd, J=0.8, 1.9 Hz, furan-5-H). MS *m/z*: 140 (M^+).

Chemical Reduction of (*R*)-2-Benzyloxypropionic Acid ((*R*)-4) into (*R*)-2-Benzyloxypropanol ((*R*)-5)——A solution of (*R*)-**4** (400 mg) [obtained by microbial resolution with *Glomerella cingulata* (*Malus*) or *Gloeosporium olivarum*] in dry ether (6 ml) was added to a suspension of $LiAlH_4$ (60 mg) in dry ether (6 ml), and the mixture was stirred for 2 h at reflux tempetature, then cooled. Water was added and the whole was shaken with ether. The ethereal layer was washed with a small amount of saturated aq. NaCl solution, and dried over anhydrous Na_2SO_4, then the solvent was removed. The residual liquid was purified by distillation *in vacuo*; bp 50—60 °C (6 mmHg). The compound ((*R*)-**5**) thus prepared showed the same IR and ^{1}H-NMR spectra as the corresponding compound ((*S*)-**5**) obtained directly by fermentation. This compound ((*R*)-**5**) was derived into its (*R*)-(+)-MTPA ester ((*R*)-**6**) as in the case of the preparation of (*R,S*)-**3** from (*R,S*)-**2**, in order to determine the optical purity of (*R*)-**5** (hence (*R*)-**4**). The optical purities thus determined of (*R*)-**4** obtained with *Glomerella cingulata* (*Malus*) and *Gloeosporium olivarum* are shown in Table I.

Acknowledgement The authors wish to thank Prof. Dr. T. Tani of Kagawa University for providing *Gloeosporium olivarum*, and Dr. Y. Oonuma of Yamagata Horticultural Experimental Station for providing *Malus* and *Prunus* strains of *Glomerella cingulata* and *Gloeosporium laeticolor*. ^{1}H-NMR spectra were measured by Mrs. M. Yuyama of this university.

References

1) Y. Tsuda, K. Kawai and S. Nakajima, *Agric. Biol. Chem.*, **48**, 1373 (1984).
2) Y. Tsuda, K. Kawai and S. Nakajima, *Chem. Pharm. Bull.*, **33**, 1955 (1985).
3) S. Nakajima, N. Naito and T. Tani, *Chem. Pharm. Bull.*, **21**, 671 (1973).
4) A. M. Amici, A. Minghetti and C. Spalla, *Biochim. Appl.* (Italia), **12**, 50 (1965).
5) R. Howe, R. H. Moore, B. S. Rau and A. H. Wood, Brit. Patent 1183850 (1967) [*Chem. Abstr.*, **73**, 33905 (1970)].
6) S. D. Bakshi and E. E. Turner, *J. Chem. Soc.*, **1961**, 171.
7) P. A. Lovene, R. E. Marker and A. Rothen, *J. Biol. Chem.*, **100**, 589 (1933).
8) H. Ott, A. J. Frey and A. Hofmann, *Tetrahedron*, **19**, 1675 (1963).
9) K. Mislow, R. E. O'Brien and Schaefer, *J. Am. Chem. Soc.*, **84**, 1940 (1962).
10) K. Thinius and K. Friese, *Plaste Kautsch.*, **7**, 3 (1960).