Chem. Pharm. Bull.
33(5)1955—1960(1985)

Microbial Reduction and Resolution of Herbicidal 2-Alkyl-2-aryloxyacetic Acids by *Gloeosporium olivarum*

YOSHIKO TSUDA,* KEN-ICHI KAWAI and SHOICHI NAKAJIMA

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

(Received August 22, 1984)

When a mold, *Gloeosporium olivarum*, was fermented with (±)-2-alkyl-2-aryloxyacetic acids (**1**—**8**), an enantioselective microbial reduction as well as microbial resolution took place, giving rise to (*S*)-2-alkyl-2-aryloxyethanols (**1a**—**8a**) and (*R*)-2-alkyl-2-aryloxyacetic acids (**1b**—**8b**). The configurations of the chiral 2-alkyl-2-aryloxyethanols and 2-alkyl-2-aryloxyacetic acids were consistent with those of the corresponding products formed by *Glomerella cingulata*, with the sole exception of **8b**. The (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of the (*S*)-2-alkyl-2-aryloxyethanols showed larger lanthanide-induced shifts for the methoxy groups (**1a**, **4a**—**8a**) or an aromatic proton (**2a**) in the nuclear magnetic resonance (NMR) spectra than the esters of the corresponding (*R*)-2-alkyl-2-aryloxyethanols. This result can be applied generally for the determination of the absolute configuration of 2-alkyl-2-aryloxyethanols.

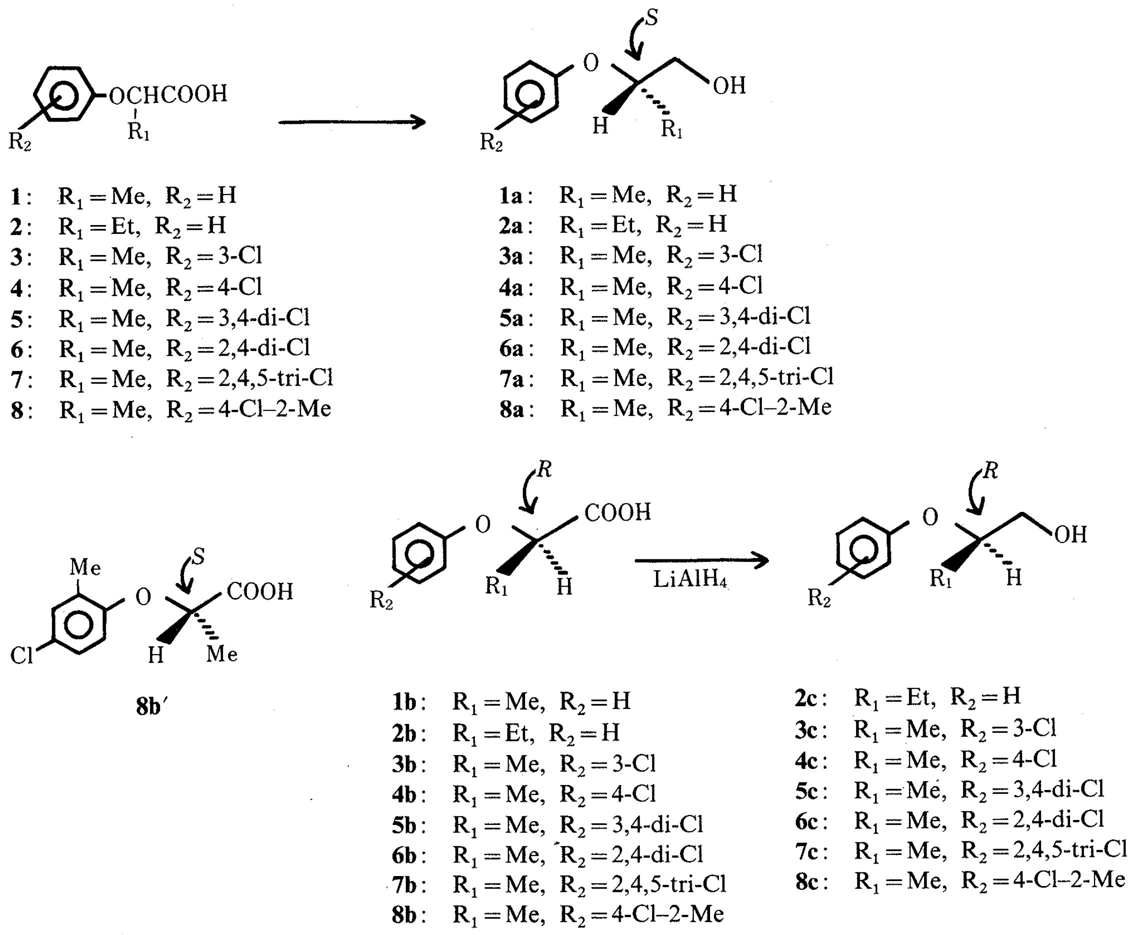
Keywords——2-alkyl-2-aryloxyacetic acid; 2-alkyl-2-aryloxyethanol; herbicide; microbial reduction; microbial resolution

Various examples of microbial reduction of ketones into alcohols have been reported,[1] and many of them represent excellent methods for obtaining chiral alcohols with good optical purities.[2] However, only a few instances[3-5] have so far been reported of the reduction by molds of carboxylic acids to alcohols. Our previous paper[3] described the mold reduction of 2,4-dichlorophenoxyacetic acid and other related herbicidal compounds to the corresponding alcohols.

It is well-known that, in microbial reduction, the enantioselectivity of the microorganism is influenced by the bulkiness of the groups adjacent to the position which is attacked by the microbial enzymes.[6] As we reported in a short communication,[7] we obtained chiral (*S*)-2-methyl-2-aryloxyethanols by the microbial reduction with *Glomerella cingulata* of racemic (±)-2-methyl-2-aryloxyacetic acids. In the present paper, we report the similar microbial reductions of racemic (±)-2-alkyl-2-aryloxyacetic acids (**1**—**8**) with another mold, *Gloeosporium olivarum*.

The microbial reduction of racemic 2-alkyl-2-aryloxyacetic acids (**1**—**8**) to optically active 2-alkyl-2-aryloxyethanols (**1a**—**8a**) took place satisfactorily. At the same time, optically active 2-alkyl-2-aryloxyacetic acids (**1b**—**8b**) were isolated from the culture broth as a result of the enantioselective reduction of the substrates by the mold. The absolute configurations of these chiral acids were determined by comparing their optical rotations with those of the acids described in the literature.[8-14] It was found that all the 2-alkyl-2-aryloxyacetic acids (**1b**—**8b**) have (*R*)-configurations at their asymmetric carbons. These results are in agreement with those we reported[7] on the reduction of the same compounds by *Glomerella cingulata*,[7] with the sole exception of 2-methyl-2-(4-chloro-2-methyl)phenoxyacetic acid (**8**), for which the *Glomerella cingulata* left the (*S*)-acid instead of the (*R*)-acids.

The absolute configurations of the reduction products (**1a**—**8a**) were determined by measurement of the optical rotations. Since there are no reports on either the preparation or



1: R_1 = Me, R_2 = H
2: R_1 = Et, R_2 = H
3: R_1 = Me, R_2 = 3-Cl
4: R_1 = Me, R_2 = 4-Cl
5: R_1 = Me, R_2 = 3,4-di-Cl
6: R_1 = Me, R_2 = 2,4-di-Cl
7: R_1 = Me, R_2 = 2,4,5-tri-Cl
8: R_1 = Me, R_2 = 4-Cl–2-Me

1a: R_1 = Me, R_2 = H
2a: R_1 = Et, R_2 = H
3a: R_1 = Me, R_2 = 3-Cl
4a: R_1 = Me, R_2 = 4-Cl
5a: R_1 = Me, R_2 = 3,4-di-Cl
6a: R_1 = Me, R_2 = 2,4-di-Cl
7a: R_1 = Me, R_2 = 2,4,5-tri-Cl
8a: R_1 = Me, R_2 = 4-Cl–2-Me

8b′

1b: R_1 = Me, R_2 = H
2b: R_1 = Et, R_2 = H
3b: R_1 = Me, R_2 = 3-Cl
4b: R_1 = Me, R_2 = 4-Cl
5b: R_1 = Me, R_2 = 3,4-di-Cl
6b: R_1 = Me, R_2 = 2,4-di-Cl
7b: R_1 = Me, R_2 = 2,4,5-tri-Cl
8b: R_1 = Me, R_2 = 4-Cl–2-Me

2c: R_1 = Et, R_2 = H
3c: R_1 = Me, R_2 = 3-Cl
4c: R_1 = Me, R_2 = 4-Cl
5c: R_1 = Me, R_2 = 3,4-di-Cl
6c: R_1 = Me, R_2 = 2,4-di-Cl
7c: R_1 = Me, R_2 = 2,4,5-tri-Cl
8c: R_1 = Me, R_2 = 4-Cl–2-Me

Chart 1

the isolation of chiral 2-alkyl-2-aryloxyethanols, except (*R*)-(−)-2-methyl-2-phenoxyethanol (**1a**),[15] we prepared the chiral (*R*)-2-alkyl-2-aryloxyethanols (**2c**—**8c**) for comparison of the optical rotations by reducing the chiral (*R*)-2-alkyl-2-aryloxyacetic acids (**1b**—**8b**) with lithium aluminum hydride. All the alcohols (**1a**—**8a**) obtained by the microbial reduction with *Gloeosporium olivarum* were found to have the (*S*)-configuration, as in the case of *Glomerella cingulata*.[7] The period of incubation, the absolute configurations of the reduction products and the remaining acids, the yields of the products, and their optical purities are shown in Table I.

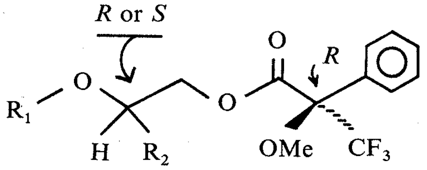
It is interesting that *Glomerella cingulata* gives the (*S*)-(+)-alcohol (**8a**) and the (*S*)-(−)-acid (**8b′**), while *Gloeosporium olivarum* gives the (*S*)-(+)-alcohol (**8a**) and the (*R*)-(+)-acid (**8b**). It is likely that *Glomerella cingulata* decomposes the (*R*)-(+)-acid (**8b**) enantioselectively, simultaneously with the reduction of the (*S*)-(−)-acid (**8b′**).

The optical purities of (*S*)-2-alkyl-2-aryloxyethanols (**1a**—**8a**) were determined by Mosher's method.[16] They were esterified with (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chloride in the presence of pyridine. Most of the (*R*)-(+)-MTPA esters (**1d**, **2d**, **4d**—**8d**) prepared were examined by 100 MHz nuclear magnetic resonance (NMR) spectroscopy in the presence of a lanthanide shift reagent, Eu(fod)$_3$. As an example, the (*R*)-(+)-MTPA ester (**8d**) of 2-methyl-2-(4-chloro-2-methylphenoxy)ethanol (**8a**) showed clearly separated signals due to the methoxy groups for the diastereomeric isomers. In one case, namely the MTPA ester (**2d**) of 2-ethyl-2-phenoxyethanol (**2a**), the signals for one of the aromatic protons, but not the methoxy signals, were clearly separated. For the (*R*)-(+)-MTPA ester (**3d**) of 2-methyl-2-(3-chlorophenoxy)ethanol (**3a**), we

TABLE I. Asymmetric Reduction and Resolution of 2-Alkyl-2-aryloxyacetic Acids by *Gloeosporium Olivarum*

Substrate	Product	Incubation (d)	Yield (%)	Absolute configuration	Optical purity
1	**1a**	14	8.2	*S*	44
	1b		14.8	*R*	35
2	**2a**	11	63.9	*S*	36
	2b		33.0	*R*	35
3	**3a**	21	47.7	*S*	82
	3b		24.4	*R*	20
4	**4a**	14	22.4	*S*	38
	4b		39.8	*R*	34
5	**5a**	25	15.8	*S*	80
	5b		69.8	*R*	19
6	**6a**	12	36.4	*S*	72
	6b		54.9	*R*	16
7	**7a**	32	33.3	*S*	70
	7b		50.4	*R*	11
8	**8a**	24	5.2	*S*	99
	8b		57.3	*R*	16

TABLE II. Lanthanide-Induced Shift of the Methoxy Group Signal or an Aromatic Proton Signal of Diastereomeric (*R*)-(+)-MTPA Esters[a]



Compound No.	R_1	R_2	Optimum molar ratio $\frac{\text{Eu(fod)}_3}{\text{substrate}}$	Difference in lanthanide-induced shifts δ (ppm)	Configuration of alcohols with a larger value of shift difference
1d	Phenyl	Me	0.83	0.07	*S*(+)[8]
2d	Phenyl	Et	1.63	0.18	*S*(−)[9]
4d	4-Cl-Phenyl	Me	3.20	0.11	*S*(+)[11]
5d	3,4-Di-Cl-phenyl	Me	1.82	0.12	*S*(+)[12]
6d	2,4-Di-Cl-phenyl	Me	2.23	0.25	*S*(+)[12]
7d	2,4,5-Tri-Cl-phenyl	Me	0.90	0.12	*S*(+)[13]
8d	4-Cl–2-Me-phenyl	Me	3.90	0.33	*S*(+)[14]

a) Lanthanide-induced shift was observed at the methoxy group in **1d** and **4d**—**8d**, and at an aromatic proton in **2d**.

used 400 MHz NMR spectroscopy and obtained well separated methoxy signals without adding the shift reagent. Thus, through integration of the signals either of the methoxy protons or an aromatic proton, the *R*–*S* ratios or the optical purities of the product alcohols (**1a**—**8a**) could be determined. The assignment of the signal of the particular aromatic proton described above was impossible in the case of **2d** because of the complex overlapping of the protons of the two benzene nuclei.

The optical purifies of (*R*)-2-alkyl-2-aryloxyacetic acids (**1b**—**8b**) were determined by calculation from their $[\alpha]_D$ values compared with the values given for authentic pure samples in the literature.[8-14]

The difference in the lanthanide-induced shift, at the optimum shift reagent concentrations, for seven tested alcohols (**1d**, **2d**, **4d**—**8d**) are listed in Table II. As shown in the last column of this table, and in accord with the result obtained with *Glomerella cingulata*,[7] the configurations of the alcohols which showed larger downfield shifts were all *S*. This conclusion may be useful in determining the absolute configurations of 2-aryloxyalkanols in general. As shown in Table I, (*S*)-(+)-2-methyl-2-(4-chloro-2-methylphenoxy)ethanol (**8a**) can be obtained in an almost pure state, and (*S*)-(+)-2-methyl-2-(3-chlorophenoxy)ethanol (**3a**) and (*S*)-(+)-2-methyl-2-(3,4-dichlorophenoxy)ethanol (**5a**) can be prepared in more than 80% optical purity without any further enantioenrichment process (recrystallization, *etc.*).

Compounds **3**—**8** are well-known herbicides under the commercial names of CP, 4-CPP, 3,4-DP, Dichlorprop, Silvex and MCPP. The herbicidal activity is undoubtedly due to one of the chiral isomers,[17] but chemical resolution is tedious, and the separation of the chiral isomers has never been realized industrially. Nevertheless, in order to avoid possible hazards arising from residual toxicity, it is desirable to use chiral isomers instead of racemates. From this view-point, the microbial resolution of these herbicides has possible commercial significance, though it is evident that the culture conditions require optimization to improve the yields not only optically but also chemically.

Experimental

The infrared (IR) spectra were measured on a Hitachi model 215 spectrometer. NMR spectra were recorded, with tetramethylsilane as an internal standard, on 100 MHz JEOL FX-100 and 400 MHz JEOL FX-400 spectrometers. Optical rotations were measured on a JASCO DIP-101 automatic polarimeter with a cell of 10 cm light path length. Gas-liquid chromatographic (GLC) analyses were performed on a Shimadzu GC-4CM PF instrument with a flame ionization detector and a glass column (2 m × 3 mm) packed with 2% OV-1 on Chromosorb W, at a nitrogen pressure of 1 kg/cm^2. The mass spectra (MS) were obtained on a JEOL JMS-D 300 instrument.

General Procedure for Microbial Reduction and Resolution of (±)-2-Alkyl-2-aryloxyacetic Acids (1—8)——The microorganism, *Gloeosporium olivarum* Almeida, was provided by Prof. Dr. T. Tani of Kagawa University, who isolated the mold from infected natural olives in Kagawa Prefecture.

The substrates, (±)-2-alkyl-2-aryloxyacetic acids (**1**—**8**) in amounts of up to 4.5 g, were added to the culture broth containing peptone (90 g), KH_2PO_4 (9 g), $MgSO_4 \cdot 7H_2O$ (3.6 g), sucrose (450 g) and water (9 l), and the whole mixture was poured into 30 Roux bottles after adjusting the pH to 5.5. The Roux bottles were stoppered with cotton, autoclaved, cooled, and inoculated with *Gloeosporium olivarum*. Stationary culture was carried out at 27 °C for the periods designated in Table I. After removal of the mycelium by filtration, the culture filtrate was alkalized to pH 10 and shaken with ether (3 l). The ethereal extract was purified by passing it through a column of Silica gel (Kanto Kagaku Co., 100 mesh) in $CHCl_3$. The (*S*)-2-alkyl-2-aryloxyethanols (**1a**—**8a**) thus obtained each showed a single spot in thin-layer chromatography (TLC) ($CHCl_3$) on pre-coated Silica gel 60 F_{254} chromatographic plates (Merck).

The culture filtrate after the ethereal extraction at pH 10 was acidified to pH 2 and shaken with ether (3 l). The ethereal extract was purified by silica gel column chromatography with $CHCl_3$ as the eluting solvent. The purified (*R*)-2-alkyl-2-aryloxyacetic acids (**1b**—**8b**) thus obtained each showed a single spot in TLC ($CHCl_3$) on pre-coated Silica gel 60 F_{254} chromatographic plates.

(*S*)-(+)-2-Methyl-2-phenoxyethanol (1a) and (*R*)-(+)-2-Methyl-2-phenoxyacetic Acid (1b)——Compound **1a**, obtained in a yield of as much as 0.32 g, showed a single peak on GLC analysis ($t_R = 3.3$ min; column temp. 100 °C isothermal). $[\alpha]_D^{20}$ +13.7° ($c = 0.9$, MeOH). IR ν_{max}^{film} cm^{-1}: 3350, 2940, 1600, 1590, 1488, 1240, 1049, 933, 752. NMR ($CDCl_3$) δ: 1.23 (3H, d, $J = 6.3$ Hz, CH_3), 2.70 (1H, s, OH), 3.69 (2H, d, $J = 5.4$ Hz, CH_2), 4.46 (1H, sextet like, CH), 6.86—7.35 (5H, m, ArH). The (*R*)-(+)-MTPA ester (**1d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 23.25 mg of $Eu(fod)_3$) signals due to (*S*)-OMe protons (72%) ($\delta = 6.01$) and (*R*)-OMe protons (28%) ($\delta = 5.94$); 44% ee. The optical purity was also determined by calculation from the value (−30°) of $[\alpha]_D$ for the (*R*)-(−)-isomer given in the literature,[15] 46% ee. MS *m/z*: 152.0815 (M^+), Calcd for $C_9H_{12}O_2$, 152.0794.

Compound **1b**, yield 0.63 g, showed $[\alpha]_D^{21}$ +13.9° ($c = 4.2$, abs. EtOH). The optical purity was determined as 35% by calculation from the value (+40°) given in the literature.[8] MS *m/z*: 166 (M^+).

(*S*)-(−)-2-Ethyl-2-phenoxyethanol (2a) and (*R*)-(+)-2-Ethyl-2-phenoxyacetic Acid (2b)——Compound **2a**, obtained in a yield of as much as 2.7 g, showed a single peak on GLC analysis ($t_R = 4.0$ min; column temp. 150 °C isothermal). $[\alpha]_D^{21}$ −3.2° ($c = 2.6$, MeOH). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3610, 3450, 2975, 2950, 2885, 1595, 1490, 1237, 1048, 690.

NMR ($CDCl_3$) δ: 0.97 (3H, t, CH_3), 1.67 (2H, m, CH_2), 1.92 (1H, s, OH), 3.76 (2H, m, CH_2), 4.30 (1H, m, CH), 6.98—7.28 (5H, m, ArH). The (*R*)-(+)-MTPA ester (**2d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 44.23 mg of Eu(fod)$_3$) signals due to (*S*)-aromatic protons (68%) (δ=7.63) and (*R*)-aromatic protons (32%) (δ=7.81); 36%ee. MS *m*/*z*: 166 (M^+).

Compound **2b**, yield 1.5 g, showed $[\alpha]_D^{20}$ +22.0° (*c*=2.5, acetone). The optical purity was determined as 35% by calculation from the value (−65.5°) of $[\alpha]_D$ given in the literature.[9] MS *m*/*z*: 180 (M^+).

(*S*)-(+)-2-Methyl-2-(3-chlorophenoxy)ethanol (3a) and (*R*)-(+)-2-Methyl-2-(3-chlorophenoxy)acetic Acid (3b)——Compound **3a**, obtained in a yield of as much as 2.0 g, showed a single peak on GLC analysis (t_R=4.5 min; column temp. 150 °C isothermal). $[\alpha]_D^{20}$ +27.0° (*c*=2.9, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 2940, 1592, 1495, 1379, 1240, 1229, 1051, 948. NMR ($CDCl_3$) δ: 1.27 (3H, d, *J*=6.1 Hz, CH_3), 3.71 (1H, OH), 3.72 (2H, d, *J*=5.4 Hz, CH_2). The *J*-value between CH and CH_2 was determined after deuteration of the adjacent hydroxyl. δ: 4.47 (1H, m, CH), 6.90—7.28 (4H, m, ArH). The (*R*)-(+)-MTPA ester (**3d**) showed NMR (400 MHz, $CDCl_3$) signals due to (*S*)-OMe protons (91%) (δ=3.52) and (*R*)-OMe protons (9%) (δ=3.50); 82%ee. MS *m*/*z*: 186.0455 (M^+), Calcd for $C_9H_{11}{}^{35}ClO_2$, 186.0462 and 188.0446 (M^++2), Calcd for $C_9H_{11}{}^{37}ClO_2$, 188.0474.

Compound **3b**, yield 1.10 g, showed $[\alpha]_D^{20}$ +10.7° (*c*=2.3, acetone). The optical purity was determined as 20% by calculation from the value (−54.5°) given in the literature.[10] MS *m*/*z*: 200.0225 (M^+), Calcd for $C_9H_9{}^{35}ClO_3$, 200.0212.

(*S*)-(+)-2-Methyl-2-(4-chlorophenoxy)ethanol (4a) and (*R*)-(+)-2-Methyl-2-(4-chlorophenoxy)acetic Acid (4b)——Compound **4a**, obtained in a yield of as much as 0.94 g, showed a single peak on GLC analysis (t_R=5.1 min; column temp. 150 °C isothermal). $[\alpha]_D^{20}$ +3.4° (*c*=2.5, acetone). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 3350, 2930, 1720, 1590, 1485, 1452, 1379, 1232, 1045. NMR ($CDCl_3$) δ: 1.24 (3H, d, *J*=6.1 Hz, CH_3), 3.71 (2H, d, *J*=4.8 Hz, CH_2), 3.72 (1H, OH). The *J*-value between CH and CH_2 was determined after deuteration of the adjacent hydroxyl. δ: 4.43 (1H, m, CH), 6.84 (2H, d, *J*=9.0 Hz, aromatic-2,6-H), 7.22 (2H, d, *J*=9.0 Hz, aromatic-3,5-H). The MTPA ester (**4d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 80.80 mg of Eu(fod)$_3$) signals due to (*S*)-OMe protons (69%) (δ=6.16); and (*R*)-OMe protons (31%) (δ=6.05); 38%ee. MS *m*/*z*: 186.0454 (M^+), Calcd for $C_9H_{11}{}^{35}ClO_2$, 186.0461.

Compound **4b**, yield 1.79 g, showed $[\alpha]_D^{20}$ +19.5° (*c*=2.8, acetone). The optical purity was determined as 34% by calculation from the value (+57.4°) of $[\alpha]_D$ given in the literature.[11] MS *m*/*z*: 200.0254 (M^+), Calcd for $C_9H_9{}^{35}ClO_3$, 200.0267.

(*S*)-(+)-2-Methyl-2-(3,4-dichlorophenoxy)ethanol (5a) and (*R*)-(+)-2-Methyl-2-(3,4-dichlorophenoxy)acetic Acid (5b)——Compound **5a**, obtained in a yield of as much as 0.67 g, showed a single peak on GLC analysis (t_R=4.2 min; column temp. 170 °C isothermal). $[\alpha]_D^{20}$ +13.7° (*c*=3.8, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 2940, 2860, 1592, 1470, 1260, 1042. NMR ($CDCl_3$) δ: 1.26 (3H, d, *J*=6.5 Hz, CH_3), 3.73 (2H, d, *J*=6.0 Hz, CH_2), 3.74 (1H, OH). The *J*-value between CH and CH_2 was determined after deuteration of the adjacent hydroxyl group. δ: 4.44 (1H, m, CH), 6.77 (1H, dd, *J*=3.5, 9.0 Hz, aromatic-6-H), 7.03 (1H, d, *J*=3.5 Hz, aromatic-2H), 7.31 (1H, d, *J*=9.0 Hz, aromatic-5-H). The (*R*)-(+)-MTPA ester (**5d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 43.32 mg of Eu(fod)$_3$) signals due to (*S*)-OMe protons (90%) (δ=6.13) and (*R*)-OMe protons (10%) (δ=6.01); 80%ee. MS *m*/*z*: 220.0074 (M^+), Calcd for $C_9H_{10}{}^{35}Cl_2O_2$, 220.0090 and 222.0034 (M^++2). Calcd for $C_9H_{10}{}^{35}Cl^{37}ClO_2$, 222.0039.

Compound **5b**, yield 3.14 g, showed $[\alpha]_D^{20}$ +10.3° (*c*=2.6, acetone). The optical purity was determined as 19% by calculation from the value (+54.8°) given in the literature.[12] MS *m*/*z*: 234 (M^+), 236 (M^++2, 65% intensity of M^+) and 238 (M^++4, 11% intensity of M^+).

(*S*)-(+)-2-Methyl-2-(2,4-dichlorophenoxy)ethanol (6a) and (*R*)-(+)-2-Methyl-2-(2,4-dichlorophenoxy)acetic Acid (6b)——Compound **6a**, obtained in a yield of as much as 1.54 g, showed a single peak on GLC analysis (t_R=6.6 min; column temp. 150 °C isothermal). $[\alpha]_D^{20}$ +7.0° (*c*=2.5, acetone). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600, 2930, 2850, 1720, 1540, 1472, 1250. NMR ($CDCl_3$) δ: 1.30 (3H, d, *J*=6.1 Hz, CH_3), 2.30 (1H, OH), 3.75 (2H, d, *J*=4.8 Hz, CH_2), 4.44 (1H, m, CH), 6.91 (1H, d, *J*=9.0 Hz, aromatic-6-H), 7.16 (1H, dd, *J*=3.5, 9.0 Hz, aromatic-5-H), 7.36 (1H, d, *J*=3.5 Hz, aromatic-3-H). The MTPA ester (**6d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 53.14 mg of Eu(fod)$_3$) signals due to (*S*)-OMe protons (86%) (δ=6.57) and (*R*)-OMe protons (14%) (δ=6.32); 72%ee. MS *m*/*z*: 220.0044 (M^+), Calcd for $C_9H_{10}{}^{35}Cl_2O_2$, 220.0032 and 222.0024 (M^++2), Calcd for $C_9H_{10}{}^{35}Cl^{37}ClO_2$, 222.0021.

Compound **6b**, yield 2.47 g, showed $[\alpha]_D^{20}$ +5.8° (*c*=2.5, acetone). The optical purity was determined as 16% by calculation from the value (35.2°) given in the literature.[12] MS *m*/*z*: 233.9839 (M^+), Calcd for $C_9H_8{}^{35}Cl_2O_3$, 233.9829 and 235. 9856 (M^++2), Calcd for $C_9H_8{}^{35}Cl^{37}ClO_3$, 235.9891.

(*S*)-(+)-2-Methyl-2-(2,4,5-trichlorophenoxy)ethanol (7a) and (*R*)-(+)-2-Methyl-2-(2,4,5-trichlorophenoxy)acetic Acid (7b)——Compound **7a**, obtained in a yield of as much as 1.42 g, showed a single peak on GLC analysis (t_R=2.6 min; column temp. 200 °C isothermal). $[\alpha]_D^{15}$ +14.6° (*c*=2.1, acetone). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 2940, 2850, 1460, 1420, 1200, 920. NMR ($CDCl_3$) δ: 1.33 (3H, d, *J*=6.1 Hz, CH_3), 2.10 (1H, OH), 3.75 (2H, d, *J*=4.9 Hz, CH_2), 4.46 (1H, m, CH), 7.09 (1H, s, aromatic-6-H), 7.46 (1H, s, aromatic-3-H). The (*R*)-(+)-MTPA ester (**7d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 36.80 mg of Eu(fod)$_3$) signals due to (*S*)-OMe protons (85%) (δ=6.24) and (*R*)-OMe protons (15%) (δ=6.12); 70%ee. MS *m*/*z*: 254 (M^+), 256 (M^++2, 98% intensity of M^+) and 258 (M^++4, 30% intensity of M^+).

Compound **7b**, yield 2.27 g, showed $[\alpha]_D^{15}$ +5.5° (*c*=2.6, acetone). The optical purity was determined as 11% by calculation from the value (+48.9 °) given in the literature.[13] MS m/z: 268 (M^+).

(*S*)-(+)-2-Methyl-2-(4-chloro-2-methylphenoxy)ethanol (8a) and (*R*)-(+)-2-Methyl-2-(4-chloro-2-methylphenoxy)acetic Acid (8b)——Compound **8a**, obtained in a yield of as much as 0.22 g, showed a single peak on GLC analysis (t_R=5.0 min; column temp. 150 °C isothermal). $[\alpha]_D^{20}$ +20.5 ° (c=0.9, $CHCl_3$). IR $\nu_{\max}^{CCl_4}$ cm^{-1}: 3700, 3400, 1600, 1480, 1240, 1048. NMR ($CDCl_3$) δ: 1.25 (3H, d, J=7.9 Hz, CH_3), 2.08 (1H, OH), 2.19 (3H, s, CH_3), 3.74 (2H, d, J=6.0 Hz, CH_2), 4.43 (1H, m, CH), 6.83—7.12 (3H, m, ArH). The (*R*)-(+)-MTPA ester (**8d**) showed NMR (100 MHz, 10 mg in $CDCl_3$ in the presence of 97.3 mg of Eu(fod)$_3$) signals due to (*S*)-OMe protons (99.5%) (δ=6.45) and (*R*)-OMe protons (0.5%) (δ=6.12); 99% ee. MS m/z: 200.0594 (M^+), Calcd for $C_{10}H_{13}{}^{35}ClO_2$, 200.0584 and 202.0572 (M^++2), Calcd for $C_{10}H_{13}{}^{37}ClO_2$, 202.0571.

Compound **8b**, yield 2.60 g, showed $[\alpha]_D^{20}$ +4.8 ° (c=2.8, acetone). The optical purity was determined as 16% by calculation from the value (+29.3 °) of $[\alpha]_D$ given in the literature.[14] MS m/z: 214.0417 (M^+), Calcd for $C_{10}H_{11}{}^{35}ClO_3$, 214.0437.

Chemical Reduction of (*R*)-2-Alkyl-2-aryloxyacetic Acids (2b—8b) to (*R*)-2-Alkyl-2-aryloxyethanols (2c—8c)——Each of the (*R*)-2-alkyl-2-aryloxyacetic acids (**2b**—**8b**) (100 mg) obtained by microbial resolution was dissolved in dry ether (4 ml), and the solution was added to a suspension of $LiAlH_4$ (50 mg) in dry ether (5 ml). The mixture was stirred for 1.5 h at reflux temperature. The mixture was 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 anhyd. Na_2SO_4, then the solvent was evaporated off. The compounds (**2c**—**8c**) thus obtained showed the same IR and NMR spectra, and t_R values on GLC as the corresponding compounds (*S*)-2-alkyl-2-aryloxyethanols (**2a**—**8a**) obtained by microbial reduction. Yields: **2c**; 77 mg, **3c**; 74 mg, **4c**; 73 mg, **5c**; 81 mg, **6c**; 70 mg, **7c**; 75 mg, **8c**; 74 mg. $[\alpha]_D^{20}$: **2c**; +4.0 (c=2.5, MeOH), **3c**; −7.0 (c=3.5, $CHCl_3$), **4c**; −3.1 (c=1.2, acetone), **5c**; −3.3 (c=3.0, $CHCl_3$), **6c**; −1.0 (c=4.2, acetone), **7c**; −2.3 (c=4.5, acetone), **8c**; −4.3 (c=2.4, $CHCl_3$).

Acknowledgement The authors wish to thank Prof. Dr. T. Tani of Kagawa University for providing the strain of *Gloeosporium olivarum*.

References

1) K. Kieslich, "Microbial Transformations," by Georg Thieme Publishers, Stuttgart, 1976, pp. 633—665.
2) V. E. Althouse, D. M. Feigl, W. A. Sanderson and H. S. Mosher, *J. Am. Chem. Soc.*, **88**, 3595 (1966).
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) V. Prelog, *Industrie Chimique Belge*, **27**, 1309 (1962).
7) Y. Tauda, K. Kawai and S. Nakajima, *Agric. Biol. Chem.*, **48**, 1373 (1984).
8) W. J. Gottstein and L. C. Cheney, *J. Org. Chem.*, **30**, 2062 (1965).
9) M. Matell, *Arkiv Kemi*, **6**, 251 (1953).
10) A. Fredga, A. M. Weider and C. Gronwall, *Arkiv Kemi*, **17**, 265 (1961).
11) M. Matell, *Arkiv Kemi*, **7**, 437 (1954).
12) M. Matell, *Arkiv Kemi*, **4**, 473 (1952).
13) M. S. Smith, R. L. Wain and F. Wightman, *Ann. Appl. Biology*, **39**, 295 (1952).
14) M. Matell, *Arkiv Kemi*, **6**, 365 (1953).
15) W. Frommer, H. Kubin and E. Rauenbusch, (Farbenfabriken Bayer), Ger. Patent 1157237 (1961); [*Chem. Abstr.*, **60**, 15102 (1964)].
16) *a*) J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, **34**, 2543 (1969); *b*) J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, **95**, 512 (1973).
17) G. Hoerlein, H. Nestler, H. Koecher and P. Langelueddeke (Hoechst A.-G.), Ger. Offen. 2748658 (1979); [*Chem. Abstr.*, **91**, 193054*f* (1979)].