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Asymmetric Hydrolysis of (*dl*)-1-Acyloxy-2-halo-1-phenylethanes with Lipases

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Asymmetric hydrolysis of (*dl*)-1-acyloxy-2-halo-1-phenylethanes by lipoprotein lipase Amano P from *Pseudomonas fluorescens* and the lipase from *Chromobacterium viscosum* afforded the optically active (*R*) residual substrates and (*S*)-2-halo-1-hydroxy-1-phenylethanes in 100% enantiomeric excess (*e.e.*). The length of acyl residues from acetyl to octanoyl in the substrates did not influence the enantioselectivity.

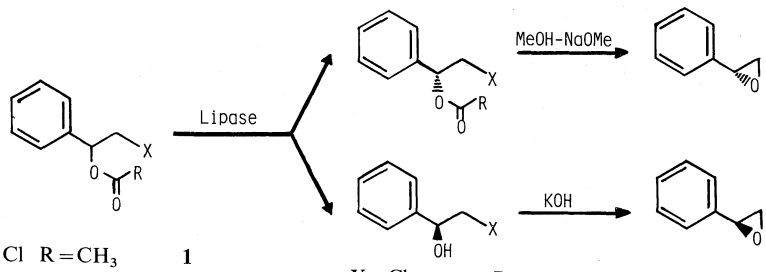
Both enantiomers of optically active styrene oxides were synthesized from the enzymatic products.

Chiral 2-halo-1-hydroxy-1-phenylethanes are versatile intermediates for the syntheses of natural compounds[1] and new drugs,[2] since they are easily converted to optically active styrene oxides or 1,2-dihydroxy-1-phenylethanes.[3] Several methods for the syntheses of chiral 2-halo-1-hydroxy-1-phenylethanes have been described in recent years, such as the microbial,[4～7] enzymatic,[8] and organic synthetic[9] methods. Microbial reduction of phenacylchloride gave optically pure (*R*)-2-chloro-1-hydroxy-1-phenylethane.[5] The rest of the methods, however, did not afford optically pure products. In this paper, we describe the enzymatic enantioselective synthesis of 2-halo-1-hydroxy-1-phenylethanes by lipases.

Lipases hydrolyze triglycerides as their natural substrates. The substrate specificities are relatively wide and some unnatural compounds are also recognized as the substrates but there are not many unnatural substrates hydrolyzed by lipases highly enantioselectively. Asymmetric hydrolysis of (*dl*)-1,2-diacetoxy-3-bromopropane and (*dl*)-1,2-diacetoxy-1-phenylethane by a lipase afforded the residual substrates in 77% *e.e.* and 73% *e.e.*, respectively.[10] Lipases worked more stereoselectively on (*dl*)-1,2-diacetoxy-3-chloropropane and (*dl*)-1-acetoxy-2,3-dichloropropane[11] to give both residual substrates in 90% *e.e.* On the other hand, (*dl*)-5-acyloxymethyl-3-alkyl-2-oxazolidinone[12,13] and (*dl*)-2-acyloxy-3-chloropropyl *p*-toluenesulfonate[14] were hydrolyzed by a lipase with complete enantioselectivity. Therefore, an exact fitness between lipases and the unnatural substrates is indispensable for the syntheses of optically pure compounds.

Among the 25 commercially available enzymes studied for this paper, lipoprotein lipase Amano P from *Pseudomonas fluorescens* (L.P.L. Amano P) and the lipase from *Chromobacterium viscosum* (Lipase Toyo) hydrolyzed (*dl*)-1-acyloxy-2-halo-1-phenylethanes (**1**～**6**) enantioselectively and gave (*S*)-2-halo-1-hydroxy-1-phenylethanes (**7**, **8**) and (*R*) residual substrates (**1**～**4**) in 100% *e.e.*

Racemic **7** and **8** were synthesized by the reduction of the corresponding phenacyl halides with $NaBH_4$ and were acylated using acyl chloride and triethylamine. The compounds (**1**～**6**) thus obtained were used for the substrates of enzymatic hydrolysis. Enzymes which hydrolyzed about 50% of (*dl*)-1-acetoxy-2-chloro-1-phenylethane (**1**) were selected out of the 25 commercially available hydroly-



X=Cl R=CH_3 **1**
n-C_3H_7 **2**
$(CH_3)_2CH$ **3**
n-C_5H_{11} **4**
n-C_7H_{15} **5**

X=Br R=CH_3 **6**

X=Cl **7**
Br **8**

FIG. 1. Synthesis of Optically Active 2-Halo-1-hydroxy-1-phenylethanes and Styrene Oxides.

TABLE I. ENZYMATIC ASYMMETRIC HYDROLYSIS OF **1**

Enzyme (g)	Incubation period (hr)	Residual substrate **1**			Alcohol **7**		
		Yield[a] (%)	$[\alpha]_D^{25}$ [b]	*e.e.*[d] (%)	Yield[a] (%)	$[\alpha]_D^{25}$ [c]	*e.e.*[e] (%)
L.P.L. Amano P (0.2)	18	24	−78.5° (*R*)	100	29	+53.3° (*S*)	100
Lipase Toyo (0.2)	16	20	−80.0° (*R*)	100	25	+50.4° (*S*)	100
Acylase No. A-2156 (0.3)	40	41	−57.4° (*R*)	72	29	+32.9° (*S*)	59
Acylase No. A-8376 (0.02)	68	37	+38.4° (*S*)	48	26	−27.7° (*R*)	50

[a] Chemical yield after silica gel purification.
[b] $c=2$, acetone.
[c] $c=2$, cyclohexane.
[d] Measured by NMR.
[e] Measured by HPLC.
Reaction: substrate 1.0 g in 50 mM phosphate buffer (pH 7.0, 100 ml), 34°C.

tic enzymes. The hydrolysis of **1** (1 g) by the enzymes (200 mg) thus selected was done in 100 ml of phosphate buffer (50 mM, pH 7.0) at 34°C with the controlled addition of 1 N sodium hydroxide solution to keep the pH at 7.0. In the case of L.P.L. Amano P or Lipase Toyo, the hydrolysis reaction completely stopped when a half molar equivalent of sodium hydroxide to the substrate was consumed. The products were extracted with dichloromethane and purified by silica gel column chromatography. As shown in Table I, L.P.L. Amano P and Lipase Toyo hydrolyzed **1** enantioselectively to give the residual substrate (*R*)-**1** and (*S*)-2-chloro-1-hydroxy-1-phenylethane (**7**) in 100% *e.e.*, but partial asymmetric hydrolysis of **1** was observed with acylase No. A-2156 (Sigma), and (*R*)-**1** in 72% *e.e.* and (*S*)-**7** in 59% *e.e.* were produced. The three enzymes mentioned above specifically hydrolyzed the (*S*)-enantiomer, but it is interesting to note that acylase No. A-8376 catalyzed the hydrolysis of (*R*)-**1** to give (*R*)-**7** in 50% *e.e.* The *e.e.* of **1** and **7** were measured by ^{1}H-NMR in the presence of Eu(hfc)$_3$ and HPLC using a chiral column, respectively. The specific rotation of (*S*)-**7** produced by L.P.L. Amano P was $[\alpha]_D^{25}$ +53.3° ($c=2.0$, cyclohexane), while

TABLE II. EFFECT OF ACYL GROUP IN SUBSTRATES (**2**~**5**) ON ASYMMETRIC HYDROLYSIS BY L.P.L. AMANO P

Substrate (R=)	Incubation period (hr)	Residual substrate			Alcohol **7**		
		Yield[a] (%)	$[\alpha]_D^{25}$ [b]	*e.e.*[d] (%)	Yield[a] (%)	$[\alpha]_D^{25}$ [c]	*e.e.*[e] (%)
n-C_3H_7	20	43	−68.6° (*R*)	100	30	+55.7° (*S*)	100
$(CH_3)_2CH$	52	—	−80.0° (*R*)	100	12	+54.4° (*S*)	100
n-C_5H_{11}	30	40	−61.8° (*R*)	100	22	+55.4° (*S*)	100
n-C_7H_{15}	62	33	−46.3° (*R*)	—	20	+54.1° (*S*)	100

[a] Chemical yield after silica gel purification.
[b] $c=1$, acetone.
[c] $c=1$, cyclohaxane.
[d] Measured by NMR.
[e] Measured by HPLC.
Reaction: substrate 1.0 g, L.P.L. Amano P 200 mg in 50 mM phosphate buffer (pH 7.0, 100 ml), 34°C.

TABLE III. ENZYMATIC ASYMMETRIC HYDROLYSIS OF **6**

Enzyme	Incubation period (hr)	Residual substrate **6**			Alcohol **8**		
		Yield[a] (%)	$[\alpha]_D^{25}$ [b]	*e.e.*[d] (%)	Yield[a] (%)	$[\alpha]_D^{25}$ [c]	*e.e.*[e] (%)
L.P.L. Amano P	45	24	−62.8° (*R*)	94	11	+51.3° (*S*)	99.8
Lipase Toyo	45	22	−60.0° (*R*)	90	11	+49.5° (*S*)	99.0

[a] Chemical yield after silica gel purification.
[b] $c=1$, acetone.
[c] $c=1$, $CHCl_3$.
[d] Measured by NMR.
[e] Measured by HPLC.
Reaction: substrate 1.0 g, enzyme 0.2 g in 50 mM phosphate buffer (pH 7.0, 100 ml), 34°C.

Imuta *et al.* reported $[\alpha]_D^{25}$ −48.1° ($c=1.73$, cyclohexane) for (*R*)-**7**.[5] The same authors acylated (*R*)-**7** and got (*R*)-**1** in $[\alpha]_D^{25}$ −53.8° ($c=5.06$, acetone),[5] but the specific rotation of (*R*)-**1** resolved by Lipase Toyo reached $[\alpha]_D^{25}$ −80.0° ($c=2.0$, acetone).

The substrate specificity of L.P.L. Amano P was examined using racemic substrates (**2**~**5**) having various acyl groups. The results in Table II indicate that the length of the acyl group did not affect the enantioselectivity, and the product (*S*)-**7** was obtained in 100% *e.e.* The residual substrates were also optically pure. As the acyl group became longer, the hydrolysis proceeded more slowly. Among the substrates studied, acetylated substrate **1** was hydrolyzed most rapidly by L.P.L. Amano P.

As shown in Table III, (*dl*)-1-acetoxy-2-bromo-1-phenylethane (**6**) was hydrolyzed enantioselectively by L.P.L. Amano P and Lipase Toyo to produce (*S*)-2-bromo-1-hydroxy-1-phenylethane (**8**). The apparent reaction, however, did not stop when a half molar equivalent of 1 N sodium hydroxide to the substrate was consumed. It was not because of the poor enantioselectively of the enzyme on **6**, but because of the unstability of **8**, part of which was spontaneously converted into styrene oxide in accordance with the addition of sodium hydroxide. Specific rotations for (*R*)-**6** and (*R*)-**8** reported were $[\alpha]_D^{25}$ −54.1° ($c=2.81$, acetone) and $[\alpha]_D^{25}$ −39° ($c=8.00$, chloroform),[5] respectively. In our results the residual substrate (*R*)-**6** and the product (*S*)-**8**

showed the specific rotation of $[\alpha]_D^{25}$ $-62.8°$ ($c=1.0$, acetone) and $[\alpha]_D^{25}$ $+51.3°$ ($c=1.0$, chloroform), respectively.

Optically active styrene oxide was synthesized from optically pure (*R*)-**1** or (*S*)-**7** produced by L.P.L. Amano P. The compound (*S*)-**7** was stirred in chloroform and water (1 : 1) with potassium hydroxide at room temperature. The product, (*S*)-styrene oxide, purified by distillation showed $[\alpha]_D^{25}$ $+25.0°$ (($c=0.78$, chloroform), reported $[\alpha]_D^{25}$ $+24.6°$ ($c=1.37$, chloroform))[3] in 78% yield. From (*R*)-**1**, (*R*)-styrene oxide ($[\alpha]_D^{25}$ $-21.4°$ ($c=1.3$, chloroform)) was obtained in 66% yield using sodium methoxide in methanol.[3]

In conclusion (*dl*)-1-acyloxy-2-halo-1-phenylethanes were hydrolyzed enantioselectively by L.P.L. Amano P and Lipase Toyo, and optically pure (*S*)-2-halo-1-hydroxy-1-phenylethanes and (*R*)-residual substrates were obtained. The enzymatic asymmetric hydrolysis offers a convenient synthetic method for optically active styrene oxides of both enantiomers.

EXPERIMENTAL

NMR spectrometry was done on a Varian EM-390 spectrometer (90 MHz) with TMS as the internal standard. Mass spectra were recorded with a Shimadzu GCMS-QP1000. Gas chromatography was done on a Hitachi 163 with a column (5% Silicone OV-17, 2 m × 0.3 cm, 1.2 kg/cm^3 of N_2) at 135°C except compounds **4** and **5** which were analyzed at 180°C. Optical rotations were measured with a PM-101 automatic digital polarimeter (Union Giken Co.).

Synthesis of 1-acyloxy-2-chloro-1-phenylethanes.

Preparation of 2-chloro-1-hydroxy-1-phenylethane (**7**). Sodium borohydride (1.9 g, 50 mmol) was added to a stirred suspension of phenacyl chloride (15.5 g, 100 mmol) in MeOH (50 ml) over 0.5 hr at 0°C. The reaction mixture was allowed to warm up to room temperature for 0.5 hr. The solution was neutralized by 1 N HCl and MeOH was evaporated. After extraction with $CHCl_3$ (50 ml × 2), the organic layer was washed twice with the same volume of water, dried over Na_2SO_4, and concentrated *in vacuo* to give an oily product. The crude product was distilled at 80°C (3 mmHg) to give a colorless oil (11.6 g, 75% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.85 (1H, s, OH), 3.62 (2H, dd, $J=$ 4.5, 7.5 Hz, $-CH_2-$), 4.82 (1H, dd, $J=4.5$, 7.5 Hz, –CH–), 7.33 (5H, s, Ar-H). MS *m/z* (EI): 158, 156 (M^+), 107 (M^+-CH_2Cl), *m/z* (CI): 141, 139 ($(M-H_2O)H^+$).

Preparation of 1-acetoxy-2-chloro-1-phenylethane (**1**). Acetyl chloride (6.0 g, 77 mmol) was added over 1 hr to a solution of **7** (11.6 g, 74 mmol) and Et_3N (7.8 g, 77 mmol) in CH_2Cl_2 (200 ml) at 0°C. The mixture was stirred at room temperature for 2 hr and then washed with water (100 ml × 2). The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo* to give an oily product. The crude product was distilled at 93°C (2 mmHg) and pure **1** was obtained as a colorless oil (10.7 g, 73% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.13 (3H, s, $-CH_3$), 3.73 (2H, dd, $J=$ 6.0, 7.2 Hz, $-CH_2-$), 5.93 (1H, dd, $J=6.0$, 7.2 Hz, –CH–), 7.34 (5H, s, Ar-H). MS *m/z* (EI): 162 (M^+-HCl), 141, 139 (M^+-OAc), 43 (Ac), *m/z* (CI) 201, 199 (MH^+), 141, 139 ($(M-AcOH)H^+$). The other 1-acyloxy-2-chloro-1-phenylethanes (**2**~**5**) were synthesized in the same manner from **7**. 1-Butyryloxy-2-chloro-1-phenylethane (**2**): bp 107°C (2 mmHg), yield 75%, ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, t, $J=7.5$ Hz, $-CH_3$), 1.69 (2H, m, $J=7.5$ Hz, $-CH_2-CH_2-CH_3$), 2.37 (2H, t, $J=7.5$ Hz, $-CH_2-CH_2-CH_3$), 3.70 (2H, dd, $J=5.4$, 6.9 Hz, $-CH_2-Cl$), 5.98 (1H, dd, $J=5.4$, 6.9 Hz, –CH–), 7.33 (5H, s, Ar-H). MS *m/z* (EI): 190 (M^+-HCl), 141, 139 ($M^+-OCOC_3H_7$), 71 (COC_3H_7), *m/z* (CI): 229, 227 (MH^+), 141, 139 ($(M-C_3H_7COOH)H^+$). 1-Isobutyryloxy-2-chloro-1-phenylethane (**3**): bp 110°C (2 mmHg), yield 78%, ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, d, $J=7.1$ Hz, $-CH_3$), 1.23 (3H, d, $J=7.1$ Hz, $-CH_3$), 2.65 (1H, m, $J=7.1$ Hz, $-CH-(CH_3)_2$), 3.73 (2H, dd, $J=5.4$, 6.6 Hz, $-CH_2-Cl$), 5.93 (1H, dd, $J=5.4$, 6.6 Hz, –CH–CH_2–), 7.33 (5H, s, Ar-H). MS *m/z* (EI): 190 (M^+-HCl), 141, 139 ($CM^+-OCOCH(CH_3)_2$), 71 ($COCH(CH_3)_2$), *m/z* (CI): 229, 227 (MH^+), 141, 139 ($(M^+-(CH_3)_2CHCOOH)H^+$). 1-Hexanoyloxy-2-chloro-1-phenylethane (**4**): bp 120°C (2 mmHg), yield 77%, ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, $-CH_3$), 1.10~1.80 (6H, m, $-(CH_2)_3-CH_3$), 2.40 (2H, t, $J=7.5$ Hz, $-CH_2-(CH_2)_2-CH_3$), 3.73 (2H, dd, $J=5.7$, 6.9 Hz, $-CH_2-Cl$), 5.93 (1H, dd, $J=5.7$, 6.9 Hz, –CH–CH_2–), 7.33 (5H, s, Ar-H). MS *m/z* (EI): 218 (M^+-HCl), 141, 139 ($M-OCOC_5H_{11}$), 99 (COC_5H_{11}), *m/z* (CI): 257, 255 (MH^+), 219 ($(M-HCl)H^+$), 141, 139 ($(M-C_5H_{11}COOH)H^+$). 1-Octanoyloxy-2-chloro-1-phenylethane (**5**): yield 85%, ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, $-CH_3$), 1.03~1.90 (10H, m, $-(CH_2)_5-CH_3$), 2.37 (2H, t, $J=7.2$ Hz, $-CH_2-(CH_2)_5-CH_3$), 3.70 (2H, dd, $J=5.4$, 6.9 Hz, $-CH_2-Cl$), 5.90 (1H, dd, $J=5.4$, 6.9 Hz, –CH–CH_2–Cl), 7.30 (5H, s, Ar-H). MS *m/z* (EI): 246 (M^+-HCl), 141, 139 ($M^+-OCOC_7H_{15}$), 127 (COC_7H_{15}), *m/z* (CI): 285, 283 (MH^+), 247 ($(M-HCl)H^+$), 141, 139 ($(M-C_7H_{15}COOH)H^+$).

Synthesis of 1-acetoxy-2-bromo-1-phenylethane (**6**).

Preparation of 2-bromo-1-hydroxy-1-phenylethane (**8**). Phenacyl bromide was reduced as in the synthesis of **7**. Compound **8**: bp 86°C (0.5 mmHg), yield 35%, ^{1}H-NMR ($CDCl_3$) δ: 3.23 (1H, s, –OH), 3.43 (2H, dd, $J=5.4$, 8.7 Hz, $-CH_2-$), 4.73 (1H, dd, $J=5.4$, 8.7 Hz, –CH–), 7.23 (5H, s, Ar-H). MS *m/z* (EI): 202, 200 (M^+), 107 (M^+-CH_2Br), *m/z* (CI): 185, 183 ($(M-H_2O)H^+$).

Preparation of 1-acetoxy-2-bromo-1-phenylethane (**6**). Compound **8** was acetylated as mentioned above. Compound **6**: bp 108°C (3 mmHg), yield 46%, ^{1}H-NMR ($CDCl_3$) δ: 2.1 (3H, s, $-CH_3$), 3.6 (2H, dd, J=4.8, 7.5 Hz, $-CH_2-$), 5.93 (1H, dd, J=4.8, 7.5 Hz, –CH–), 7.33 (5H, s, Ar-H). MS *m/z* (EI): 185, 183 (M^+-OAc), 162 (M^+-HBr), 43 (Ac), *m/z* (CI): 185, 183 ($(M-AcOH)H^+$), 163 ($(M-HBr)H^+$).

Screening of hydrolytic enzymes. Twenty-five commercially available hydrolytic enzymes including 23 lipases and 2 acylases were used for screening for the asymmetric hydrolysis of **1**. The enzymes with good enantioselectivities were lipoprotein lipase Amano P from *Pseudomonas fluorescens* (L.P.L. Amano P; Amano Pharmaceutical Co.), the lipase from *Chromobacterium viscosum* (Toyo Jozo Co., Ltd.), the acylase No. A-8376 from porcine kidney (Sigma Chemical Co.), and the acylase No. A-2156 from *Aspergillus* species (Sigma Chemical Co.). The incubation mixtures consisted of enzyme (5 mg) and substrate **1** (25 mg) in 2 ml of Na_2HPO_4–KH_2PO_4 buffer (200 mM, pH 7.0). Incubations were done with shaking at 30°C for 1 to 4 days. The reactions were followed by GLC and the enzymes which hydrolyzed about 50% of substrate **1** were selected for further studies.

Asymmetric hydrolysis of substrates **1**~**6**. The enzyme selected (0.2 g) and the substrate (1.0 g) were incubated in 100 ml of Na_2HPO_4–KH_2PO_4 buffer (50 mM, pH 7.0) with stirring at 34°C. In the course of the reaction the pH was kept at 7.0 by controlled addition of 1 N NaOH solution. The reaction was monitored by the amount of consumption of NaOH solution. When a half molar equivalent of NaOH to the substrate was consumed, the incubation mixture was extracted with CH_2Cl_2 (150 ml × 3) and the organic layer was evaporated to dryness under reduced pressure. The residual oil was chromatographed on a silica gel column (30 cm × 2.4 cm) using toluene as an eluent. The fractions of the residual substrate or product were collected and concentrated *in vacuo* to give pure compounds. The data are summarized in Tables I, II, and III.

Measurement of enantiomeric excess. The enantiomeric excess of the product, **7** or **8**, was measured by HPLC using chiralcel OB (Daicel Chemical Industries, Ltd.) as a column (hexane–isopropanol = 30 : 1, 1 ml/min for **7** and 0.5 ml/min for **8**, detection: UV at 220 nm). The retention time of each compound was as follows: (*R*)-**7**, 12 min; (*S*)-**7**, 15 min; (*R*)-**8**, 28 min; (*S*)-**8**, 33 min. Measurements of the enantiomeric excess of the residual substrates, **1**~**6**, were done with ^{1}H-NMR. The racemic **1** (10 mg) and $Eu(hfc)_3$ (about 50 mg) were dissolved into $CDCl_3$ (400 μl) and ^{1}H-NMR spectra were measured. The signal of the methyl protons in the acetyl group showed two singlets ((*R*)-**1** δ: 2.85 ppm, (*S*)-**2** δ: 2.95 ppm), whereas the residual substrate **1** gave a singlet in the same condition. Similar ^{1}H-NMR spectra were obtained for the methylene or methine protons next to the carbonyl group in acyl substituents of racemic **2**~**4**. Racemic **5** gave too complicated a ^{1}H-NMR spectrum to measure the enantiomeric excess.

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