ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 4 (1998) 53–60

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Enantioselectivity of *Candida antarctica* lipase for some synthetic substrates including aliphatic secondary alcohols

Takehiko Ohtani [b], Hiroki Nakatsukasa [a], Makoto Kamezawa [b], Hojun Tachibana [b], Yoshinobu Naoshima [a,*]

[a] *Faculty of Informatics, Okayama University of Science, 1-1 Ridai-cho, Okayama 700, Japan*
[b] *Konan Chemical Industry Co., Ltd., 5-21 Nakagawa-cho, Takatsuki-shi, Osaka 569, Japan*

Received 28 April 1997; revised 30 June 1997; accepted 3 July 1997

Abstract

Candida antarctica lipase (Novozym 435) showed good to high enantioselectivity in the acylation of aliphatic and aromatic secondary alcohols ($E = 38–166$), and higher enantioselectivity in the hydrolysis of their corresponding acetates than other lipases tested, including lipases AY and MY (*Candida* sp.) and lipases PS, AK and LIP (*Pseudomonas* sp.).

Keywords: Lipases; *Candida antarctica*; Enantioselectivity; Hydrolysis; Esterification; Secondary alcohols

1. Introduction

Lipases have been widely used as an asymmetric catalyst for the preparation of enantiomerically pure organic compounds [1,2]. The enantioselectivity of lipases is known to be usually high in the kinetic resolution of racemic alcohols and esters, but their reactivity and enantioselectivity for various synthetic substrates are not always sufficient [3,4].

In the course of the chemo–enzymatic synthesis of an optically active natural product, (4*E*,15*E*)-docosa-4,15-dien-1-yn-3-ol possessing chiral 4-en-1-yn-3-ol skeleton, we found lipase Novozym 435 (*Candida Antarctica*, Novo Nordisk Bioindustrial A/S, Denmark) to be the more effective catalyst, compared with *Pseudomonas* sp. lipases PS, AK and LIP, for the enantioselective transformation of the racemic enyne alcohol [5]. This finding gave us the opportunity to further study the enantioselectivity of Novozym 435 for some synthetic substrates (see Fig. 1), as part of our efforts to enhance the utility of lipases in organic synthesis [3,6]. The present synthetic substrates can be useful as chiral intermediates for the asymmetric synthesis of optically active natural products.

2. Experimental

2.1. Materials

Each of the acetates **1a–12a** was prepared by treating corresponding alcohols **1–12** with acetic anhydride in the presence of 4-pyrrolidinopyri-

* Corresponding author. Tel.: (81-86) 256-8003; Fax: (81-86) 256-8006.

1381-1177/98/$19.00
PII S1381-1177(97)00021-0

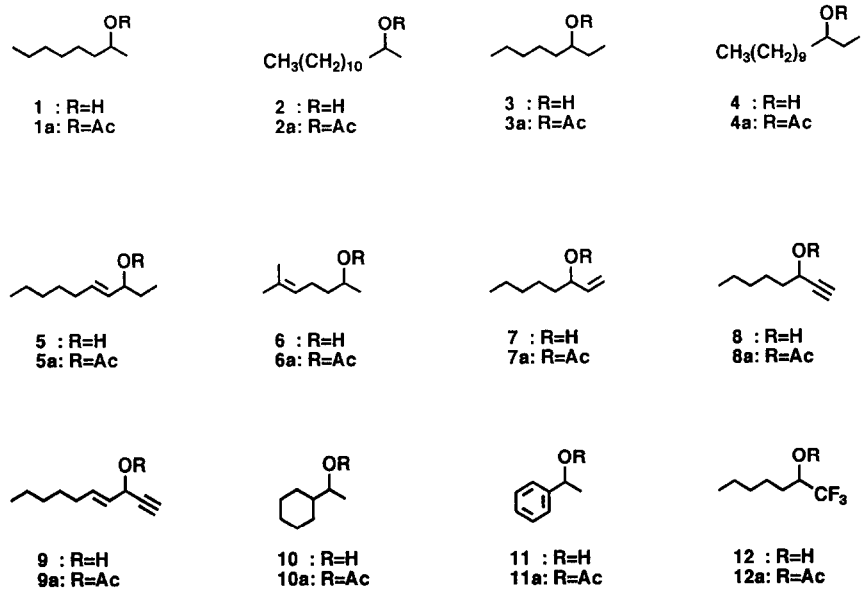


Fig. 1. Synthetic substrates used in lipase-catalyzed biotransformations.

dine in dry CH_2Cl_2. (*E*)-4-decen-3-ol (**5**) was synthesized as previously described [3]. (*E*)-4-decen-1-yn-3-ol (**9**) was prepared by the Grignard reaction of (*E*)-2-octenal [3] with ethynylmagnesium bromide. 1,1,1-Trifluoro-2-heptanol (**12**) was prepared by treatment of trifluoroacetic acid with pentylmagnesium bromide and subsequent reduction with $LiAlH_4$ in dry diethyl ether. All other racemic alcohols were purchased from Tokyo Kasei Kogyo or Aldrich. Lipase Novozym 435 was supplied from Novo Nordisk AS. Lipases PS (*Pseudomonas cepacia*, 30000 U/g), AK (*Pseudomonas fluorescens*), and AY (*Candida rugosa*) were obtained from Amano. Lipase MY (*Candida cylindracea*, 30000 U/g) was from Meito Sangyo and lipase LIP (*Pseudomonas aeruginosa*, 1000 U/g) from Toyobo. All reaction products were fully characterized by their IR and ^{1}H-NMR spectral data.

IR spectra were determined with a Fourier transform Perkin–Elmer IR spectrometer. ^{1}H-NMR spectra were obtained with a Fourier transform Hitachi R-1500 (60 MHz) spectrometer or a Bruker AMX-R400 spectrometer for $CDCl_3$ solutions with Me_4Si as an internal standard. Gas chromatography was carried out on a Hitachi G-5000 gas chromatograph equipped with different capillary columns (GL Sciences), using He as the carrier gas. Optical rotations were measured by a Horiba SEPA-200 high sensitivity polarimeter.

2.2. Determination of enantiomeric purity

The enantiomeric purity of the chiral alcohols **1–4**, **6–8**, **10** and **12** was determined by GLC analysis of their diastereomeric propionates prepared by treating each of their alcohols with (*S*)-2-acetoxypropionyl chloride [3,7] [1]. The diastereomeric esters of (±)-**1**-(±)-**4**, (±)-**6**-(±)-**8**, and (±)-**10** were each separated into two equal peaks of t_R 11.3 and 11.5 min for (±)-**1** (column, TC-1 30 m × 0.25 mm; column temperature, 110–230°C (5°C/min)), t_R 14.1

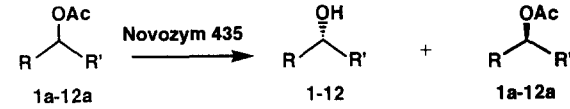
[1] An excess of the acid chloride was added to dilute solutions of the alcohols in a mixture of diethyl ether and triethylamine, and the mixture was allowed to stir at room temperature overnight. Further, there was no trace of the alcohol starting material in GLC analysis of the ethereal solutions of diastereomeric esters, ensuring that the reaction had not been submitted to diastereoselective kinetic resolution.

and 14.3 min for (±)-**2** (column, TC-1 30 m × 0.25 mm; column temperature, 150–230 °C (5°C/min)), t_R 8.6 and 8.9 min for (±)-**3** (column, TC-WAX 30 m × 0.25 mm; column temperature, 120°C), t_R 13.5 and 13.7 min for (±)-**4** (column, TC-1 30 m × 0.25 mm; column temperature, 150–230°C (5°C/min)), t_R 13.4 and 13.6 min for (+)-**6** (column, TC-1 30 m × 0.25 mm; column temperature, 100–230°C (5°C/min)), t_R 4.8 and 5.0 min for (±)-**7** (column, TC-WAX 30 m × 0.25 mm; column temperature, 140°C), t_R 10.5 and 10.7 min for (±)-**8** [column, TC-1 30 m × 0.25 mm; column temperature, 110–230°C (5°C/min)], t_R 14.3 and 14.5 min for (±)-**10** (column, TC-1 30 m × 0.25 mm; column temperature, 100–230°C (5°C/min)), and t_R 12.7 and 12.9 min for (±)-**12** (column, TC-1 30 m × 0.25 mm; column temperature, 70–230°C (5°C/min)). The enantiomeric excesses of the chiral alcohols **5** and **9** were determined on HPLC analysis of the corresponding 3,5-dinitrophenylurethane derivatives prepared by treatment with 3,5-dinitrophenyl isocyanate using a Sumichiral OA 2100I 4.0 × 250 mm column [3]. For (±)-**5**: t_R 25.8 and 29.6 min (eluent, *n*-hexane-1,2-dichloroethane–EtOH (80:10:0.4); flow rate, 1.0 ml/min; detection, 254 nm). For (±)-**9**: t_R 48.7 and 57.1 min (eluent, *n*-hexane-1,2-dichloroethane–EtOH (80:10:0.4); flow rate, 1.0 ml/min; detection, 254 nm). The ee of the chiral alcohol **11** was evaluated after HPLC using a Chiralcel OB 4.6 × 250 mm column as previously described [10]. For (±)-**11**: t_R 9.8 and 12.8 min (*n*-hexane-2-propanol (9:1); flow rate, 1.0 ml/min; detection, 254 nm). The enantiomeric purity of the chiral acetates **1a–12a** was based on that of their corresponding alcohols **1–12**; these chiral acetates were each converted into the respective alcohols by alkaline hydrolysis, the enantiomeric excesses of the alcohols being determined as described above. Each absolute configuration of the chiral alcohols **1–8** and **10–12** was determined by comparison of their optical rotation values with those reported previously [3,6,9,10]. The configuration of the chiral alcohol **9** was estimated by $LiAlH_4$–$CoCl_2$ reduction to yield (*R*)-(−)-3-decanol [3], and by comparing the optical rotation value.

2.3. Enantioselective hydrolysis with Novozym 435

A mixture of each acetate (0.5 g), lipase Novozym (0.2 g) and a 0.1 M phosphate buffer (15 ml, pH 7.0) was stirred for the required period. After filtration through Celite, each filtrate was extracted with diethyl ether and the extract was washed with brine, dried over Na_2SO_4, and concentrated. Purification by column chromatography of each product gave chiral alcohols and acetates, which were characterized on the basis of their spectral data. According to this procedure, the following alcohol products were obtained: (*R*)-**1**, $[\alpha]_D^{20}$ −15.89°

Table 1
Enzymatic hydrolysis of **1a–12a** with lipase Novozym 435

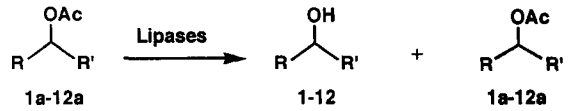


R'= CH_3, C_2H_5, $CH{=}CH_2$, $C{\equiv}CH$, CF_3

Acetate	Time (h)	Conversion (%) [a]	Yield (%) [b]/ee (%)		*E* [c]
			alcohol	acetate	
1a	0.5	39	32/ > 99.5	60/78.7	> 300
2a	0.5	30	26/ > 99.5	66/45.3	> 300
3a	2	27	26/99	60/43.8	285
4a	1	28	28/99.3	68/42.5	> 300
5a	1.5	43	38/98.7	50/82.5	> 300
6a	0.5	37	34/99	58/48	> 300
7a	0.5	39	36/97.2	56/79	176
8a	0.5	43	38/99	42/88.6	> 300
9a	1	43	40/99.3	54/95.7	> 300
10a	0.5	35	34/98.2	56/63.5	220
11a	0.5	46	30/99.3	48/90	> 300
12a	2	45	45/ > 99.5	41/99	> 300

[a] All reaction products were analyzed by capillary GLC. Conversions (%) were determined on the basis of the peak areas of substrates and products.
[b] The % yield refers to isolated chiral alcohol and acetate.
[c] *E* value was calculated according to Chen et al. [8].

Table 2
Enzymatic hydrolysis of **1a–12a** with lipases PS, AK, LIP, AY and MY



1a-12a **1-12** **1a-12a**

R'= CH_3, C_2H_5, $CH=CH_2$, $C\equiv CH$, CF_3

Acetate	Lipase	Time (h)	Conversion (%)	Alcohol/acetate yield (%) [b]/ee (%)	Configuration	E [c]
1a	PS	20	33	30/81	*R*	13
				40/32	*S*	
	AK	27	29	24/76	*R*	10
				64/34	*S*	
	AY	72	37	30/8.7	*S*	1.2
				46/7.2	*R*	
	MY	120	37	32/12.8	*S*	1.4
				46/9.4	*R*	
2a	PS	19	32	26/96	*R*	73
				52/41	*S*	
	AK	38	34	30/82	*R*	18
				54/57	*S*	
	LIP	25	38	32/63.4	*R*	7.1
				54/47.5	*S*	
	AY	50	33	28/5.7	*S*	1.1
				60/2.8	*R*	
	MY	80	38	34/7	*S*	1.2
				54/3	*R*	
3a	PS	54	32	29/81	*R*	15
				64/45	*S*	
	AK	103	38	22/83	*R*	16
				42/40.4	*S*	
	LIP	107	36	30/64.5	*R*	6.8
				46/42	*S*	
	AY	150	32	24/3.7	*S*	1.1
				50/1.0	*R*	
4a	PS	49	46	35/80	*R*	12
				42/32	*S*	
5a	PS	8	33	30/97	*R*	113
				58/54	*S*	
	AK	6	35	28/98.2	*R*	198
				54/58	*S*	
	LIP	20	35	28/86.5	*R*	26
				42/62.5	*S*	
6a	PS	41	32	29/94	*R*	42
				45/36	*S*	
	AK	82	29	22/94	*R*	50
				52/48	*S*	
	LIP	49	31	25/48	*R*	3.7
				50/26	*S*	

[a] All reaction products were analyzed by capillary GLC. Conversions (%) were determined on the basis of the peak areas of substrates and products.
[b] The yield (%) refers to isolated chiral alcohol and acetate.
[c] E value was calculated according to Chen et al. [8].

Table 2b (continued)

Acetate	Lipase	Time (h)	Conversion (%)	Alcohol/acetate yield (%) [b]/ee (%)	Configuration	E [c]
	AY	35	33	28/46.5	*S*	3.6
				50/28.5	*R*	
	MY	60	37	28/37.2	*S*	2.8
				59/28	*R*	
7a	PS	17	35	29/56	*S*	4.8
				54/34	*R*	
	AK	34	27	24/47	*S*	3.4
				48/25	*R*	
	LIP	54	30	25/53	*S*	4.2
				52/32	*R*	
	MY	80	39	28/5.3	*R*	1.1
				52/2.5	*S*	
8a	PS	10	32	27/21	*S*	1.7
				58/14	*R*	
	AK	15	37	25/10	*S*	1.3
				34/7.5	*R*	
	LIP	54	35	32/89	*S*	31
				58/57	*R*	
	AY	22	39	32/18	*R*	1.5
				56/9.5	*S*	
	MY	28	38	28/20	*R*	1.7
				56/12.5	*S*	
9a	PS	2.5	28	28/56	*S*	4.3
				70/21	*R*	
	AK	6	38	34/72	*S*	9.8
				60/48	*R*	
	LIP	4	36	30/90	*S*	33
				58/57	*R*	
10a	PS	24	31	28/ > 99.5	*R*	> 300
				58/50.4	*S*	
	AK	31	30	26/ > 99.5	*R*	> 300
				64/50	*S*	
	LIP	29	32	30/99	*R*	> 300
				66/55	*S*	
	AY	120	33	28/66	*R*	7
				56/37	*S*	
	MY	120	32	26/62	*R*	6
				60/35	*S*	
11a	PS	3.5	33	32/97	*R*	122
				64/60.4	*S*	
	AK	3	30	28/99	*R*	> 300
				70/49	*S*	
	AY	91	38	32/51	*R*	4.5
				52/40	*S*	
	MY	96	37	28/39	*R*	3.1
				52/34	*S*	
12a	PS	3	34	30/37	*R*	2.6
				50/20	*S*	
	AY	20	45	34/97	*R*	243
				48/95	*S*	

($c = 1.29$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3346, 2929, 2859, 1464, 1376, 1070, 939; ^{1}H-NMR δ: 0.88 (3H, t, $J = 7$ Hz), 1.19 (3H, d, $J = 6$ Hz), 1.30–1.44 (10H, m), 1.61 (1H, broad s), 3.80 (1H, m). (*R*)-**2**, $[\alpha]_D^{20}$ −8.69° ($c = 1.61$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3334, 2925, 2854, 1468, 1375, 1119, 930; ^{1}H-NMR δ: 0.85 (3H, t, $J = 7$ Hz), 1.19 (3H, d, $J = 6$ Hz), 1.21–1.55 (21H, m), 3.78 (1H, m). (*R*)-**3**, $[\alpha]_D^{20}$ −9.62° ($c = 1.08$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3353, 2931, 2860, 1461, 1379, 1114, 934; ^{1}H-NMR δ: 0.91 (3H, t, $J = 7$ Hz), 0.95 (3H, t, $J = 7$ Hz), 1.28–1.59 (11H, m), 3.53 (1H, m). (*R*)-**4**, $[\alpha]_D^{20}$ −7.48° ($c = 3.34$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3351, 2928, 2857, 1464, 1378, 1115, 964; ^{1}H-NMR δ: 0.89 (3H, t, $J = 7$ Hz), 0.93 (3H, t, $J = 7$ Hz), 1.19–1.52 (21H, m), 3.51 (1H, m). (*R*)-**5**, $[\alpha]_D^{20}$ −5.76° ($c = 5.69$, *n*-pentane); The IR and ^{1}H-NMR spectra of (*R*)-**5** were identical with those of (*R*)-**5** reported previously [3]. (*R*)-**6**, $[\alpha]_D^{20}$ −14.78° ($c = 2.11$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3350, 2968, 2926, 1674, 1451, 1376, 1128, 953; ^{1}H-NMR δ: 1.18 (3H, d, $J = 6$ Hz), 1.48 (2H, m), 1.62 (3H, s), 1.69 (3H, s), 1.86 (1H, broad s), 2.06 (2H, m), 3.80 (1H, m), 5.13 (1H, t, $J = 7$ Hz). (*S*)-**7**, $[\alpha]_D^{20}$ +7.16° ($c =$ 1.38, *n*-pentane); the IR and ^{1}H-NMR spectra were identical with those of (*S*)-**7** reported previously [3]. (*S*)-**8**, $[\alpha]_D^{20}$ −20.96° ($c = 1.34$, *n*-pentane); the IR and ^{1}H-NMR spectra were identical with those of (*S*)-**8** reported previously [3]. (*S*)-**9**, $[\alpha]_D^{20}$ +29.59° ($c = 2.79$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3312, 2928, 2873, 2118, 1671, 1467, 1272, 1135, 971, 926; ^{1}H-NMR δ: 0.86 (3H, t, $J = 7$ Hz), 1.18–1.40 (6H, m), 2.02–2.12 (3H, m), 2.56 (1H, d, $J = 2$ Hz), 4.83 (1H, d, $J = 6$ Hz), 5.58 (1H, dd, $J = 6$, 16 Hz), 5.85–5.90 (1H, m). (*R*)-**10**, $[\alpha]_D^{20}$ −6.08° ($c = 2.02$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3361, 2926, 2853, 1450, 1374, 1129, 1063, 939, 893; ^{1}H-NMR δ: 0.92–1.02 (2H, m), 1.15, (3H, d, $J = 6$ Hz), 1.18–1.30 (4H, m), 1.58–1.88 (6H, m), 3.54 (1H, quintet, $J = 6$ Hz). (*R*)-**11**, $[\alpha]_D^{20}$ +57.57° ($c = 2.11$, $CHCl_3$); the IR and ^{1}H-NMR spectra were identical with those of authentic racemic **11**. (*S*)-**12**, $[\alpha]_D^{20}$ −32.38° ($c = 2.49$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3393, 2967, 2869, 1464, 1280, 1171, 1141, 938, 850, 696; ^{1}H-NMR δ: 0.90 (3H, t, $J = 7$ Hz), 1.22–1.78 (8H, m), 2.93 (1H, d, $J = 6$ Hz), 3.89 (1H, m).

Similar reactions with lipases PS (0.2 g), AK (0.2 g) and LIP (0.5 g) were carried out in a mixture of acetone (6 ml) and phosphate buffer (9 ml, pH: 7.5–8.0). Lipases AY (0.2 g)- and MY (0.2 g)-catalyzed hydrolyses were run in a phosphate buffer (15 ml, pH 7.0).

2.4. Enantioselective acylation with Novozym 435

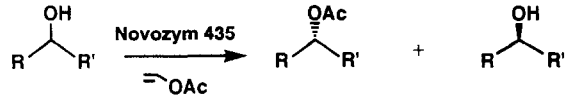
Each of racemic alcohols **2**, **6**–**10** and **12** (0.5 g) was treated with vinyl acetate (0.64 g–1.02 g) in dry diethyl ether (15 ml) in the presence of Novozym 435 (0.2 g). The mixture was stirred for the required period. After filtration through Celite, the filtrate was worked up as described above. Purification by column chromatography yielded chiral acetates and alcohols, which were characterized on the basis of their spectral data. According to this procedure, the following acetate products were obtained. (*R*)-**2a**, $[\alpha]_D^{20}$ −3.83° ($c = 6.58$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 2927, 2856, 1741, 1466, 1372, 1274, 1126, 1023, 952; ^{1}H-NMR δ: 0.88 (3H, t, $J = 7$ Hz), 1.19 (3H, d, $J = 6$ Hz), 2.20 (3H, s), 4.88 (1H, m). (*R*)-**6a**, $[\alpha]_D^{20}$ −11.37° ($c = 2.37$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 2975, 2932, 1740, 1450, 1374, 1243, 1132, 1020, 957; ^{1}H-NMR δ: 1.21 (3H, d, $J = 6$ Hz), 1.58 (3H, s), 1.68 (3H, s), 2.03 (3H, s), 4.88 (1H, m), 5.08 (1H, t, $J = 6$ Hz). (*S*)-**7a**, $[\alpha]_D^{20}$ −2.73° ($c =$ 0.95, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3087, 2934, 2862, 1742, 1648, 1372, 1241, 1021, 930; ^{1}H-NMR δ: 0.88 (3H, t, $J = 6$ Hz), 2.06 (3H, s), 5.16 (1H, m), 5.20–5.25 (2H, m), 5.73–5.81 (1H, m). (*S*)-**8a**, $[\alpha]_D^{20}$ −54.46° ($c = 6.66$, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3294, 2933, 2863, 2123, 1746, 1373, 1236, 1023, 893; ^{1}H-NMR δ: 0.88 (3H, t, $J = 7$ Hz), 2.07 (3H, s), 2.44 (1H, d, $J = 2.2$ Hz), 5.35 (1H, dt, $J = 2.2$,

7 Hz). (*S*)-**9a**, $[\alpha]_D^{20}$ +46.75° (*c* = 2.25, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 3295, 2959, 2930, 2860, 2127, 1743, 1372, 1229, 1016, 972; ^{1}H-NMR δ: 0.89 (3H, t, *J* = 7 Hz), 2.13 (3H, s), 2.57 (1H, d, *J* = 2 Hz), 5.55 (1H, dd, *J* = 6, 16 Hz), 5.83 (1H, d, *J* = 6 Hz), 5.98–6.06 (1H, m). (*R*)-**10a**, $[\alpha]_D^{20}$ +3.58° (*c* = 3.55, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 2981, 2931, 1737, 1451, 1372, 1247, 1133, 1059, 951, 892; ^{1}H-NMR δ; 0.92–1.02 (2H, m), 1.15 (3H, d, *J* = 6.5 Hz), 2.03 (3H, s), 4.72 (1H, quintet, *J* = 6.5 Hz). (*S*)-**12a**, $[\alpha]_D^{20}$ +6.31° (*c* = 1.44, *n*-pentane); IR ν_{max} (neat) (cm^{-1}): 2962, 2937, 2867, 1763, 1462, 1376, 1285, 1223, 1179, 1113, 1084, 954, 901, 860, 699; ^{1}H-NMR δ: 0.89 (3H, t, *J* = 6 Hz), 1.29–1.43 (6H, m), 2.15 (3H, s), 5.30 (1H, m).

3. Results and discussion

Table 1 shows the enantioselectivity of Novozym 435 in the enzyme-mediated hydrolysis of the acetates of 12 secondary alcohols, **1a–12a**. Lipase Novozym displayed an excellent enantioselectivity for all the acetates examined, the corresponding chiral secondary alcohols **1–12** with an enantiomeric purity of 97–99.5% ee being obtained. Because the enantioselectivity of lipase PS had been higher in an acetone–water solvent system than in an aqueous system [6], the present lipase PS-catalyzed hydrolysis was carried out in the organic solvent system as shown in Table 2. However, the enantioselectivity of lipase PS in the solvent system for all the substrates, except **10a**, was much lower than that of Novozym 435 in an aqueous system. The enantioselectivity of lipase AK in an acetone–water system was similar to that of lipase PS. Although the enantioselectivity of lipase LIP in acetone–water was somewhat different from that of lipases PS and AK for some substrates, such as **6a** (LIP, *E* = 3.7; PS and AK, *E* = 42 and 50) and **9a** (LIP, *E* = 33; PS and AK, *E* = 4.3 and 9.8), LIP showed lower enantioselectivities for all the

Table 3
Enzymatic acylation of some aliphatic secondary alcohols with lipase Novozym 435



R'= CH_3, $CH{=}CH_2$, $C{\equiv}CH$, CF_3

Alcohol	Time	Conversion (%) [a]	Yield (%) [b]/ee (%)		*E* [c]
			acetate	alcohol	
2	15 min	47	46/96.6	52/85.5	159
6	10 min	46	44/96.7	48/70	128
7	30 min	37	30/93.4	56/45.8	46
8	1 h	50	48/88.7	44/76	38
9	30 min	33	30/95	58/42.8	59
10	20 min	43	40/97.7	52/63.7	166
12	12 h	40	26/94.7	55/51.8	61

[a] All reaction products were analyzed by capillary GLC. Conversions (%) were determined on the basis of the peak areas of substrates and products.
[b] The yield (%) refers to isolated chiral alcohol and acetate.
[c] *E* value was calculated according to Chen et al. [8].

acetates than Novozym 435. *Pseudomonas* lipases PS, AK and LIP each exhibited relatively high enantioselectivity for the substrate **10a** (*E* > 300). Since the hydrolysis with *Candida* sp. lipases AY and MY, unlike similar reactions with *Pseudomonas* sp. lipases PS, AK and LIP, hardly proceeded in an acetone–water system, the former hydrolysis was performed in an aqueous system. As summarized in Table 2, the enantioselectivity of both the *Candida* lipases was generally lower than that of *Pseudomonas* sp. lipases and much lower than that of lipase Novozym, for most of the present substrates; *Candida* lipase AY (*E* = 243) gave higher selectivity only for the trifluoromethyl acetate **12a** than *Pseudomonas* lipase PS (*E* = 2.6).

The enantioselectivity of lipase Novozym 435 was also examined in the enzyme-catalyzed acylation of 7 secondary alcohols **2**, **6–10** and **12** containing saturated and unsaturated synthetic substrates (Table 3). Good to high enantioselectivity was observed in the acylation (*E* = 38–166), but the enantioselectivity of Novozym 435 in the acylation with vinyl acetate, as an acyl

donor [2], was poorer than that in the hydrolysis as described above. Although the reasons for the lower enantioselectivity in the acylation with Novozym 435, compared with the enantioselectivity in the hydrolysis with the lipase, are not clear, it is a reasonable explanation to assume that this phenomenon is attributable to a conformational change of the lipase enzyme induced by a particular interaction between Novozym and the organic solvent used in acylation, or to the choice of solvent, that is, hydrophobicity or water solubility of the particular solvent [11].

We have recently described that lipase PS-catalyzed hydrolysis gave higher enantioselectivity in an organic solvent system than in an aqueous system [6] and that lipase PS showed an enhanced enantioselectivity for some unsaturated substrates [3]. The lipase, however, had poor enantioselectivity for alkyl vinyl and alkyl ethynyl carbinols such as **7** and **8**, even when the catalytic reaction was carried out in organic solvent systems. It is clear that lipase Novozym 435, which is distinct from the other lipases tested, is applicable to a variety of aliphatic and aromatic alcohols including compounds **1–12** and particularly shows an excellent enantioselectivity for synthetic organic substrates possessing vinyl or ethynyl functions such as secondary alcohols **7–9**; the other lipases (e.g. PS, LIP and AY) were an efficient catalyst only for limited substrates (Table 2). Our results are comparable with those of Novozym-mediated biotransformations undertaken by others [11,12] and the lipase Novozym-catalyzed hydrolysis can be expected to facilitate the enantioselective synthesis of optically active natural products such as pheromones and antibiotics [5,13,14]. Work on the effect of unsaturation in substrate molecules and of organic solvents on the enantioselectivity of Novozym 435 is under way.

[2] Norin et al. have carried out lipase Novozym-mediated transesterification with several secondary alcohols, including **1** and **10**, in the presence of *S*–ethyl thiooctanoate as acyl donor [9].

References

[1] J.R. Whitaker, P.E. Sonnet (Eds.), Biocatalysts in Agricultural Biotechnology, ACS Symposium Series 389, American Chemical Society, Washington, DC, 1989.
[2] C.-H. Wong, G.M. Whitesides, Enzymes in Synthetic Organic Chemistry, Pergamon, Oxford, 1994.
[3] M. Kamezawa, T. Raku, H. Tachibana, T. Ohtani, Y. Naoshima, Biosci. Biotech. Biochem. 59 (1995) 549.
[4] J.J. Lalonde, C. Govardhan, N. Khalaf, A.G. Martinz, K. Visuri, A.L. Margolin, J. Am. Chem. Soc. 117 (1995) 6845.
[5] T. Ohtani, K. Kikuchi, M. Kamezawa, H. Hamatani, H. Tachibana, T. Totani, Y. Naoshima, J. Chem. Soc. Perkin Trans. 1 (1996) 961.
[6] Y. Naoshima, M. Kamezawa, H. Tachibana, Y. Munakata, T. Fujita, K. Kihara, T. Raku, J. Chem. Soc. Perkin Trans. 1 (1993) 557.
[7] K.N. Slessor, G.G.S. King, D.R. Miller, M.L. Winston, T.L. Cutforth, J. Chem. Ecol. 11 (1985) 1659.
[8] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294.
[9] C. Orrenius, N. Ohrner, D. Rotticci, A. Mattson, K. Hult, T. Norin, Tetrahedron Asymmetry 6 (1995) 1217.
[10] Y. Naoshima, Y. Akakabe, Phytochemistry 30 (1991) 3595.
[11] C. Orrenius, T. Norin, K. Hult, G. Carrea, Tetrahedron Asymmetry 6 (1995) 3023.
[12] C. Chamorro, R.G. Muniz, S. Conde, Tetrahedron Asymmetry 6 (1995) 2343.
[13] M. Kamezawa, T. Raku, H. Tachibana, T. Ohtani, Y. Naoshima, Biosci. Biotech. Biochem. 58 (1994) 598.
[14] A. Sharma, S. Sankaranarayanan, S. Chattopadhyay, J. Org. Chem. 61 (1996) 1814.