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Enzymatic resolution of *cis*- and *trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetate derivatives

Nobuko Shimizu,[a,*] Hiroyuki Akita[b] and Takeshi Kawamata[a]

[a]*Institute for Advanced Medical Research, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*

[b]*Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan*

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Abstract—Extensive screenings using commercially available enzymes were performed in relation to the asymmetric hydrolysis reactions of the (±)-*cis*- (**1**, **3** and **5**) and (±)-*trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates (**7**, **9** and **11**). The combinations of enzymes from plants and *cis*-equatorial compounds as well as the combinations of lipases from microorganisms and *trans*-axial-5-methoxycarbonyl acetate showed high enantiomeric ratio in asymmetric hydrolysis. However, the enantiomeric ratio of the combinations of lipases from microorganisms and *trans*-axial isomers except *trans*-axial-5-methoxycarbonyl derivative was less satisfactory. High *E* values (>200) were observed when β-amylase from *wheat* and lipase from *Candida cylindracea* were used for the (±)-*cis*-acetate **1** and the (±)-*trans*-isomer **7**, respectively, to obtain the corresponding chiral products with high enantiomeric ratios (>99:1).

1. Introduction

Enzyme-catalyzed organic synthesis has been widely used and is a most effective method for the synthesis of enantiomerically pure compounds. In particular, enantioselective hydrolysis with hydrolase-containing lipases has proved broadly useful because it is efficient, can be carried out on a large scale, and can also be applied to a broad range of substrates. Additionally, hydrolytic enzymes are environmentally friendly catalysts that are commercially available at low cost, and furthermore, they do not require expensive and unstable coenzyme systems.

Optically active bicyclic compounds have valuable pharmacological effects and are also useful building blocks for synthesis of various pharmacologically important natural products, such as sesquiterpenoids, diterpenoids and steroids.[1]

We have reported the enantioselective reduction of bicyclic ketones with microorganisms (yeast).[2] We then turned our attention to enzyme-catalyzed asymmetric hydrolysis in view of the brief treatment and large scale involved.[3] Herein, we describe investigations which were carried out to find suitable combinations of commercial enzymes and *cis*- and *trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetate derivatives (5-COOMe, 5-Me and 5-H) for efficient hydrolytic kinetic resolution (Scheme 1).

2. Results

In preliminary experiments, it was found that 1:1 mixtures of two racemates [(±)-**1** and (±)-**2**], [(±)-**3** and (±)-**4**], [(±)-**5** and (±)-**6**], [(±)-**7** and (±)-**8**], [(±)-**9** and (±)-**10**] and [(±)-**11** and (±)-**12**] were well separated by high-performance liquid-chromatographic (HPLC) analysis with two kinds of chiral columns (Chiralcel OD-H and ChiralPAk As). The results were shown in Table 1. Thus, chemical yields and enantiomeric purities of the reaction products could be determined by using the above method. First, screening of the asymmetric hydrolyses of (±)-*cis*- and (±)-*trans* octahydronaphthyl acetate derivatives using 27 kinds of enzymes was carried out, and the enzymes with *E* value[4] of >10 were selected. Next, we performed a quantitative analysis using 5 mg of substrate by the selective enzymes (Tables 2 and 4) and we then carried out preparative experiments using a substantial amount (ca. 100 mg) of the substrate (Tables 3 and 5).

* Corresponding author.

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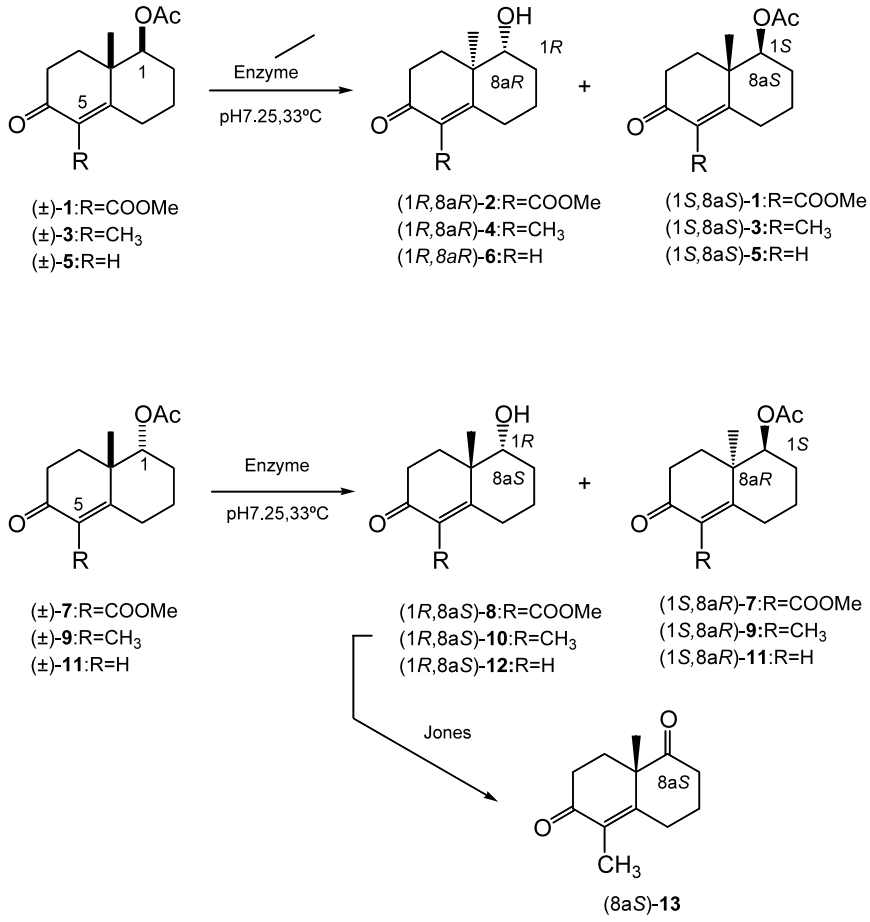

Scheme 1.

Table 1. HPLC analysis of six pairs of racemates, (±)-**1** and (±)-**2**, (±)-**3** and (±)-**4**, (±)-**5** and (±)-**6**, (±)-**7** and (±)-**8**, (±)-**9** and (±)-**10** and (±)-**11** and (±)-**12** using chiral columns

Entry	Substrate	Analytical conditions	Retention time (min)
1	(±)-**1** (±)-**2**	Column (Chiralcel OD-H), detector (UV 230 nm), flow rate (0.5 ml/min), solvent (*iso*-propanol (16.8%)/EtOH (4%)/*n*-hexane)	46, 53, 77, 93
2	(±)-**3** (±)-**4**	Column (Chiralcel OD-H), detector (UV 230 nm), flow rate (0.5 ml/min), solvent (0–24 min; *iso*-propanol (5%)/*n*-hexane, 25–95 min; *iso*-propanol (2.1%)/*n*-hexane)	22, 26, 66, 70
3	(±)-**5** (±)-**6**	Column (Chiralcel OD-H), detector (UV 230 nm), flow rate (0.5 ml/min), solvent (0–35 min; *iso*-propanol (2.4%)/*n*-hexane, 35–70 min; *iso*-propanol (3.5%) *n*-hexane)	29, 34, 62, 68
4	(±)-**7** (±)-**8**	Column (Chiralcel OD-H), detector (UV 230 nm), flow rate (0.4 ml/min), solvent (EtOH (2%)/*n*-hexane) Column (ChiralPAK As), detector (UV 230 nm), flow rate (0.5 ml/min), solvent (EtOH (2.4%)/*n*-hexane)	58, 65 113, 122
5	(±)-**9** (±)-**10**	Column (Chiralcel OD-H), detector (UV 230 nm), flow rate (0.5 ml/min), solvent (*iso*-propanol (2.9%)/*n*-hexane)	18, 23, 42, 46
6	(±)-**11** (±)-**12**	column (Chiralcel OD-H), detector (UV 230 nm), flow rate (0.5 ml/min), solvent (*iso*-propanol (4.1%)/*n*-hexane)/hexane)	22, 28, 45, 53

2.1. Enantioselective hydrolysis of (±)-*cis*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetate derivatives 1, 3 and 5

The first enzyme screenings were performed for the enantioselective hydrolysis of (±)-*cis*-8a-methyl-6-*oxo*-octahydronaphthyl acetates **1**,[5] **3**[6] and **5**.[7] Nine enzymes were found with E values of >10 in the hydrolysis of the 5-methoxycarbonyl derivative (±)-**1**, whilst ten were found with E >10 for the 4-methyl derivative (±)-**3**, and five enzymes had E >10 for substrate (±)-**5**. Next, quantitative hydrolysis of *cis*-8a-methyl-octahydro-

Table 2. Quantitative enzymatic hydrolysis of *cis*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates (±)-**1**, (±)-**3** and (±)-**5**

Entry	Substrate (5 mg)	Enzyme (5–15 mg)	Time (h)	Alcohol			Recovered acetate		C^*	E^*
				ee (%)	Yield (%)		ee (%)	Yield (%)		
1	(±)-**1**	β-AmW	7	(1*R*,8a*R*)-**2**	93	50	(1*S*,8a*S*)-**1**	98	48	51	127
2	(±)-**1**	LWG	24	(1*R*,8a*R*)-**2**	86	46	(1*S*,8a*S*)-**1**	74	48	46	29
3	(±)-**1**	β-AmSP	37	(1*R*,8a*R*)-**2**	98	33	(1*S*,8a*S*)-**1**	57	66	37	176
4	(±)-**1**	LMJ	200	(1*R*,8a*R*)-**2**	87	12	(1*S*,8a*S*)-**1**	23	55	21	18
5	(±)-**1**	LRN	200	(1*R*,8a*R*)-**2**	95	14	(1*S*,8a*S*)-**1**	18	60	16	47
6	(±)-**3**	β-AmW	53	(1*R*,8a*R*)-**4**	98	21	(1*S*,8a*S*)-**3**	28	78	22	130
7	(±)-**3**	LWG	3	(1*R*,8a*R*)-**4**	98	44	(1*S*,8a*S*)-**3**	77	56	44	>200
8	(±)-**3**	α-AmB	36	(1*R*,8a*R*)-**4**	88	10	(1*S*,8a*S*)-**3**	9	88	9	17
9	(±)-**3**	LAN	28	(1*R*,8a*R*)-**4**	79	11	(1*S*,8a*S*)-**3**	17	47	18	10
10	(±)-**3**	PHP	108	(1*R*,8a*R*)-**4**	>99	3	(1*S*,8a*S*)-**3**	1	88	1	>200
11	(±)-**5**	β-AmW	3	(1*R*,8a*R*)-**6**	99	21	(1*S*,8a*S*)-**5**	29	71	23	>200
12	(±)-**5**	LWG	3	(1*R*,8a*R*)-**6**	78	42	(1*S*,8a*S*)-**5**	87	38	53	23
13	(±)-**5**	α-AmB	25	(1*R*,8a*R*)-**6**	89	31	(1*S*,8a*S*)-**5**	44	56	33	27
14	(±)-**5**	α-AmBS	40	(1*R*,8a*R*)-**6**	98	22	(1*S*,8a*S*)-**5**	27	98	22	129
15	(±)-**5**	LMJ	47	(1*R*,8a*R*)-**6**	82	11	(1*S*,8a*S*)-**5**	10	70	11	11

* See Ref. 4.
β-AmW: β-Amylase from *wheat*.
LWG: Lipase from *wheat germ*.
β-AmSP: β-Amylase type I-B from *sweet potato*.
LMJ: Lipase from *Mucor javanicus*.
LRN: Lipase from *Rizopus niveus*.
α-AmB: β-Amylase type VIII-A from *barley*.
LAN: Lipase from *Aspergillus niger*.
PHP: Pancreatin from *hog pancreas*.
α-AmBS: α-Amylase from *Bacillus subtilis*.

Table 3. Preparative hydrolysis of *cis*-1,2,3,4,5,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates (±)-**1**, (±)-**3** and (±)-**5**

Entry	Substrate (mg)	Enzyme	Time (h)	Alcohol			Recovered acetate		*C**	*E**
				ee (%)	Yield (%)		ee (%)	Yield (%)		
1	(±)-**1** (100)	β-AmW	18	(1*R*,8a*R*)-**2**	98	40	(1*S*,8a*S*)-**1**	>99	46	50	>200
2	(±)-**3** (100)	LWG	4	(1*R*,8a*R*)-**4**	92	51	(1*S*,8a*S*)-**3**	>99	44	52	180
3	(±)-**5** (100)	β-AmW	12	(1*R*,8a*R*)-**6**	94	50	(1*S*,8a*S*)-**5**	97	41	51	136

* See Ref. 4.

naphthyl acetates using the selective enzymes (E >10) was performed. The results are shown in Table 2. The enzyme β-AmW[8] was found to be the most effective for enantioselective hydrolysis of (±)-**1** with respect to the reaction time and E values (Table 2, entry 1). A preparative experiment on (±)-**1** using β-AmW was performed to give the (−)-**2** (40% yield, 98% ee) and the recovered acetate (+)-**1** (46% yield, >99% ee) (Table 3, entry 1). Thus, the combination of (±)-**1** and β-AmW showed high enantiomeric ratio (E >200).

The lipase LWG was found to be the most effective for the enantioselective hydrolysis of 5-methyl derivative (±)-**3** with respect to the reaction time and E values (Table 2, entry 7). A preparative experiment on (±)-**3** using the LWG was performed to give (−)-**4** (51% yield, 92% ee) and the recovered acetate (+)-**3** (44% yield, >99% ee) (Table 3, entry 2). Thus, the combination of (±)-**3** and LWG showed high enantiomeric ratio (E= 180).

The enzyme β-AmW was found to be the most effective for enantioselective hydrolysis of (±)-**5** with respect to the reaction time and E values (Table 2, entry 11). A preparative experiment on (±)-**5** using β-AmW was performed to give the *cis*-alcohol (−)-**6** (50% yield, 94% ee) and the recovered acetate (+)-**5** (41% yield, 97% ee) (Table 3, entry 3). Thus, the combination of (±)-**5** and β-AmW showed high enantiomeric ratio (E=136).

The absolute configuration of the *cis*-alcohol (−)-**2** was found to be 1*R*,8a*R*, because the negative sign of the rotation of alcohol **2** ($[\alpha]_D$ −139 ($CHCl_3$)) was opposite to that of the authentic sample of (1*S*,8a*S*)-**2** ($[\alpha]_D$ +105 ($CHCl_3$));[2a,c] accordingly, that of the recovered acetate **1** was assigned as 1*S*,8a*S*. The absolute configurations of the *cis*-alcohols (−)-**4** ($[\alpha]_D$ −147 ($CHCl_3$)) and (−)-**6** ($[\alpha]_D$ −166 ($CHCl_3$)) were established to be 1*R*,8a*R* on comparison with the rotation of the authentic samples (1*S*,8a*S*)-**4** ($[\alpha]_D$ +162 ($CHCl_3$));[1a] and (1*R*,8a*R*)-**6** ($[\alpha]_D^{25}$ −196 (c=1.4, $CHCl_3$));[1d] respectively.

2.2. Enantioselsective hydrolysis of (±)-*trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates 7, 9 and 11

The first enzyme screenings were performed for the enantioselective hydrolysis of (±)-*trans*-8a-methyl-6-oxo-octahydronaphthyl acetates **7**,[9] **9**[10] and **11**.[10] The number of enzymes with E values of >10 was eight in the hydrolysis of the 5-methoxycarbonyl derivative (±)-**7**, seven for the 5-methyl derivative (±)-**9** and four for the substrate (±)-**11**, respectively.

Next, quantitative hydrolysis of *trans*-8a-methylnaphthyl acetates using the selected enzymes (E >10) was performed. The results are shown in Table 4. The lipases LMJ, LCCOF and LCCMY were selected as suitable catalysts for (±)-**7** (Table 4, entries 2, 3 and 4). The reaction rate using LCCOF was a little faster (39 h) than those of the others (43 h). A preparative experiment on (±)-**7** using lipase LCCOF was performed to give the optically active alcohol (+)-**8** (49% yield, 99% ee) and the recovered acetate (−)-**7** (46% yield, 98% ee, Table 5, entry 1). The combination of (±)-**7** and lipase LCOF showed high enantiomeric ratio (E >200).

The lipases LCCOF and LCR were proved to be suitable catalysts for the hydrolytic kinetic resolution of (±)-**9** (Table 4, entries 5 and 6). The reaction rate using LCCOF (56 h) was faster than that using LCR (207 h). A preparative experiment on (±)-**9** using LCCOF was performed to give the optically active alcohol (+)-**10** (73% yield, 30% ee) and the recovered acetate (−)-**9** (22% yield, >99% ee) (Table 5, entry 2). In this reaction, the combination of the compound (±)-**9** and lipase LCCOF showed low enantiomeric ratio (E=8), though the enantiomeric excess of the recovered acetate (−)-**9** was high.

The lipase LCCMY was selected as a catalyst for (±)-**11** (Table 4, entry 7). A preparative experiment on (±)-**11** using lipase LCCMY was performed to give (+)-**12** (48% yield, 93% ee) and the recovered acetate (−)-**11** (42% yield, 83% ee) (Table 5, entry 3). In this reaction, the enantiomeric excess of the optically active alcohol (+)-**12** was high, and the combination of the compound **12** and LCCMY showed moderate enantiomeric ratio (E=72).

The absolute configuration of the *trans*-alcohol (+)-**8** was found to be 1*R*,8a*S*, because the positive sign of the optical rotation of **8** ($[\alpha]_D$ +84 ($CHCl_3$)) was opposite to that of the authentic sample (1*S*,8a*R*)-**8** ($[\alpha]_D$ −77 ($CHCl_3$));[2a,c] accordingly, the absolute configuration of the recovered acetate **7** was 1*S*,8a*R*. The sign of the optical rotation in diketone (+)-**13** ($[\alpha]_D$ +18

Table 4. Quantitative enzymatic hydrolysis of *trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates (±)-**7**, (±)-**9** and (±)-**11**

Entry	Substrate (5 mg)	Enzyme (5–10 mg)	Time (h)	Alcohol			Recovered acetate		*C**	*E**
				ee (%)	Yield (%)		ee (%)	Yield (%)		
1	(±)-**7**	β-AmSP	72	(1*R*,8a*S*)-**8**	90	14	(1*S*,8a*R*)-**7**	26	62	22	25
2	(±)-**7**	LMJ	43	(1*R*,8a*S*)-**8**	90	30	(1*S*,8a*R*)-**7**	84	39	48	50
3	(±)-**7**	LCCOF	39	(1*R*,8a*S*)-**8**	92	21	(1*S*,8a*R*)-**7**	32	55	50	33
4	(±)-**7**	LCCMY	43	(1*R*,8a*S*)-**8**	86	40	(1*S*,8a*R*)-**7**	87	38	50	38
5	(±)-**9**	LCCOF	56	(1*R*,8a*S*)-**10**	73	39	(1*S*,8a*R*)-**9**	60	43	45	12
6	(±)-**9**	LCR	207	(1*R*,8a*S*)-**10**	82	19	(1*S*,8a*R*)-**9**	19	64	19	12
7	(±)-**11**	LCCMY	48	(1*R*,8a*S*)-**12**	18	80	(1*S*,8a*R*)-**11**	>99	2	85	7

* See Ref. 4.
LCCOF: Lipase from *Candida cylindracea*.
LCCMY: Lipase from *Candida cylindracea*.
LCR: Lipase from *Candida rugosa*.

Table 5. Preparative hydrolysis of *trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates (±)-**7**, (±)-**9** and (±)-**11**

Entry	Substrate (mg)	Enzyme	Time (h)	Alcohol			Recovered acetate		C*	E*
					ee (%)	Yield (%)		ee (%)	Yield (%)		
1	(±)-**7** (100)	LCCOF	65	(1*R*,8a*S*)-**8**	99	49	(1*S*,8a*R*)-**7**	98	46	50	>200
2	(±)-**9** (60)	LCCOF	42	(1*R*,8a*S*)-**10**	30	73	(1*S*,8a*R*)-**9**	>99	22	77	8
3	(±)-**11** (78)	LCCMY	54	(1*R*,8a*S*)-**12**	93	48	(1*S*,8a*R*)-**11**	83	42	47	72

* See Ref. 4.

(MeOH), corresponding to 30% ee) obtained by Jones' oxidation of the hydrolysed product (+)-**10** was the same as that of the authentic sample (8a*S*)-**13** ($[\alpha]_D$ +79 (MeOH), corresponding to >99% ee).[11] The absolute configuration of the *trans*-alcohol (+)-**10** was thus established as 1*R*,8a*S*. Accordingly, that of the recovered acetates **9** was 1*S*,8a*R*. The absolute configuration of the recovered acetate (−)-**11** ($[\alpha]_D$ −81 ($CHCl_3$)) was the same as that of an authentic sample of (1*S*,8a*R*)-**11** ($[\alpha]_D$ −93 ($CHCl_3$));[12] accordingly, the *trans*-alcohol **12** was assigned to have 1*R*,8a*S* configuration.

3. Discussion

High enantioselectivities (E 136∼>200) were observed when both *cis*-isomers (±)-**1**, (±)-**3** and (±)-**5**, with equatorial substituents at the C-1 position, and the 5-methoxycarbonyl isomer (±)-**7**, with an axial substituent at C-1, were subjected to asymmetric hydrolysis using enzymes from plants and using lipase from microorganisms, respectively. Moderate enantioselectivities (E 8∼72) were observed when *trans*-isomers (±)-**9** and (±)-**11** with axial substituents at the C-1 position were hydrolyzed using lipase from *Candida* sp. From the reactivities of the six substrates towards the enzymatic hydrolysis process, it is important to point out that the reaction of the (1*R*)-enantiomer proceeded faster than that of the (1*S*)-enantiomer in all cases. These results are in accordance with Kazlauskas' rule[13] for resolution of secondary alcohols. Namely, a lipase distinguishes the two enantiomers on the basis of the size of the substituents, large (L) and medium (M), at the alcohol bearing the stereocenter [L-CH(OH)-M]. The more reactive enantiomer involves the (*R*) absolute configuration when the Cahn–Ingold–Prelog priority of the L group is higher than that of the M group and H is behind the plane (Fig. 1). In the case of (±)-*cis*-5-methoxycarbonyl-8a-methyl-6-oxo-octahydronaphthyl acetate **1**, the L substituent is the cyclohexenone bearing methyl and methoxycarbonyl groups and the M substituent is only a methylene group. The great difference in the size of the substituents can well explain the very high enantioselectivity observed in practice. The stereochemical preference of enzymes observed during the above-described kinetic resolution is in line with Kazlauskas' rule, namely, derivative **2** possessing 1*R*-configuration was preferentially formed. So far as the size of the 5-substituent is concerned, the E value for the *cis*-isomers increased in proportion to the size of the substituent, in the order (±)-**5**, (±)-**3** and (±)-**1**.

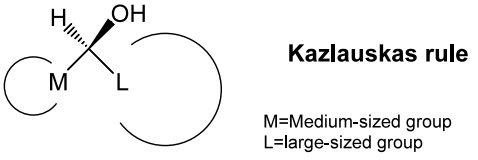

Figure 1.

The poorer enantioselectivity in the hydrolysis of (±)-*trans*-8a-methyl-6-*oxo*-octahydronaphthyl acetates **9** and **11** could not be explained using the same empirical rule, because both M and L substituents for (±)-**3** and (±)-**9** as well as (±)-**5** and (±)-**11** are the same. A predictive active site model for lipase YS from *Pseudomonas* sp. toward the racemic secondary alcohol having pseudo-axial configuration is proposed.[14] At present, it is not known why the enzymatic hydrolysis of substrates possessing axial substituents was difficult. We will investigate further in relation to the stereostructure and enantioselectivity for substrates having an axial substituent at the C-1-position. The hydrolysis of compounds with the equatorial stereocenter at the C-1-position proceeded faster than that of compounds having an axial substituent at C-1. The high E value of 5-methoxycarbonyl isomer (±)-**7** with an axial substituent at C-1 was high (>200) as well as that of the *cis*-isomer (±)-**1** with a 5-methoxycarbonyl group. The 5-methoxycarbonyl group in the octahydronaphthyl acetate might have a stabilizing effect over the stereostructure of the substrate fitting into the active site as suggested by Lemke.[15]

4. Conclusion

We were able to show that (±)-*cis*- **1**, **3** and **5** and (±)-*trans*-octahydronaphthyl acetates **7**, **9** and **11** can be resolved using the enzymes selected by screening, giving the corresponding enantiomers in good yield and high enantiomeric excess. *cis*- and *trans*-Octahydronaphthyl acetate (±)-**1** and (±)-**7**, bearing a 5-carbomethoxy group were successfully resolved with very high enantioselectivities (E >200) by using amylase β-AmW from *wheat* and lipase LCCOF from *Candida cylindracea*, respectively.

5. Experimental

5.1. General

Melting points were measured with a Kofler micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on a JEOL JNM-GSX (500 Hz) spectrometer were taken as 5–10% (w/v) solutions in $CDCl_3$ with Me_4Si as an internal reference. Infrared (IR) spectra (KBr) were measured on a JASCO FT/IR 410 spectrophotometer. Mass spectra were obtained on a JEOL JMS-700H fast atom bombardment and JEOL JMS D-300 and JEOL JMS-AX505HA mass spectrometer. Optical rotations were measured with a JASCO DIP-360 polarimeter in a $CHCl_3$ solution. The HPLC system was composed of a Shimadzu LC 10AD flow system, a Soma S-310A UV detector and Shimadzu chromatopac CR-4A data analysis system.

5.2. HPLC analysis of pairs of racemates, (±)-1 and (±)-2, (±)-3 and (±)-4, (±)-5 and (±)-6, (±)-7 and (±)-8, (±)-9 and (±)-10 and (±)-11 and (±)-12

Two 1:1 mixtures of six racemates give well separated peaks as shown in Table 1.

5.3. Screening for enantioselective hydrolysis of (±)-*cis*- and *trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates 1, 3, 5, 7, 9 and 11

A mixture of substrate and enzyme in phosphate buffer (2 ml, pH 7.25) was shaken at 33°C for a suitable time under TLC monitoring. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent gave a crude product. The reaction mixture was subjected to a silica gel short column (ca. 3 g) using *n*-hexane–ethyl acetate (1:1, 10 ml) as the eluent to afford the products, which were analyzed by HPLC under the analytical condition, as shown in Table 1.

5.4. General procedure for the quantitative analysis of enantioselective hydrolysis of (±)-*cis*- and *trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetates 1, 3, 5, 7, 9 and 11

A mixture of substrate (5 mg) and enzyme (5–15 mg) in phosphate buffer (2–4 ml, pH 7.25) was shaken at 33°C for a suitable time under TLC monitoring. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was subjected to a silica gel (ca. 5 g) short column using *n*-hexane–ethyl acetate (1:1, 30 ml) as the eluent to afford the reaction product. Thus obtained reaction product was separately analyzed by HPLC and the results are shown in Tables 2 and 4.

5.5. Enantioselective hydrolysis of (±)-*cis*-1,2,3,4,6,7,8,8a-octahydro-5-methoxycarbonyl-8a-methyl-6-*oxo*-naphthyl acetate, 1

A mixture of (±)-**1** (100 mg) and β-amylase (100 mg) in phosphate buffer (200 ml, pH 7.25) was shaken at 33°C for 18 h. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent gave a crude product, which was subjected to preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford (1*R*,8a*R*)-**2** (34 mg, 40% yield, $t_R=77$ min (99%), $t_R=93$ min (1%), 98% ee) and (1*S*,8a*S*)-**1** (46 mg, 46% yield, $t_R=46$ min (>99%), $t_R=53$ min (<0%), >99% ee). (1*R*,8a*R*)-**2**: colorless prism, mp 151–152°C. $[\alpha]_D^{32}$ −139 ($c=1.19$, $CHCl_3$). IR (KBr) cm^{-1}: 3460, 1719, 1672, 1618. ^{1}H NMR ($CDCl_3$) δ: 1.24 (s, 3H), 3.50 (dd, 1H, $J=4$, 8 Hz), 3.81 (s, 3H). HRMS m/z: 238.1191 (calcd for $C_{13}H_{18}O_4$: 238.1205). EIMS m/z: 238 (M^+), 206, 178, 163, 150. (1*S*,8a*S*)-**1**: colorless prism, mp 119–123°C. $[\alpha]_D^{32}$ +103 ($c=0.99$, $CHCl_3$). IR (KBr) cm^{-1}: 1719, 1671, 1620. ^{1}H NMR ($CDCl_3$) δ: 1.32 (s, 3H), 2.09 (s, 3H), 3.82 (s, 3H), 4.72 (dd, 1H, $J=4$, 8 Hz). HRMS m/z: 280.1307 (calcd for $C_{15}H_{20}O_5$: 280.1303). EIMS m/z: 280 (M^+), 238, 206, 188, 178, 150.

5.6. Enantioselective hydrolysis of (±)-*cis*-1,2,3,4,6,7,8,8a-octahydro-5,8a-dimethyl-6-*oxo*-naphthyl acetate, 3

A mixture of (±)-**3** (101 mg) and lipase (101 mg) in phosphate buffer (40 ml, pH 7.25) was shaken at 33°C for 6.5 h. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent afforded a crude product, which was subjected to preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford (1*R*,8a*R*)-**4** (42 mg, 51% yield, $t_R=66$ min (96%), $t_R=70$ min (4%), 92% ee) and (1*S*,8a*S*)-**3** (44 mg, 44% yield, $t_R=22$ min (>99%), $t_R=26$ min (<0%), >99% ee). (1*R*,8a*R*)-**4**: colorless oil. $[\alpha]_D^{28}$ −147 ($c=1.0$, $CHCl_3$). IR (KBr) cm^{-1}: 3398, 1640, 1602. ^{1}H NMR ($CDCl_3$) δ: 1.18 (s, 3H), 1.78 (d, 3H, $J=1$ Hz), 3.42 (dd, 1H, $J=5$, 11 Hz). FAB HRMS m/z: 195.1390 (calcd for (M^++H) $C_{12}H_{19}O_2$: 195.1385). FAB MS m/z: 195 (M^+). (1*S*,8a*S*)-**3**: colorless oil. $[\alpha]_D^{28}$ +95 ($c=1.0$, $CHCl_3$). IR (KBr) cm^{-1}: 1735, 1668, 1615. ^{1}H NMR ($CDCl_3$) δ: 1.25 (s, 3H), 1.79 (d, 3H, $J=1$ Hz), 2.08 (s, 3H), 4.65 (dd, 1H, $J=4$, 12 Hz). HRMS m/z: 236.1409 (calcd for $C_{14}H_{20}O_3$: 236.1412). EI-MS m/z: 236 (M^+), 194, 138, 123.

5.7. Enantioselective hydrolysis of (±)-*cis*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetate, 5

A mixture of (±)-**5** (100 mg) and β-amylase (100 mg) in phosphate buffer (40 ml, pH 7.25) was shaken at 33°C for 12 h. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent afforded a crude product, which was subjected to

preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford (1*R*,8a*R*)-**6** (42 mg, 50% yield, $t_R = 62$ min (97%), $t_R = 68$ min (3%), 94% ee) and (1*S*,8a*S*)-**5** (41 mg, 41% yield, $t_R = 29$ min (98.5%), $t_R = 34$ min (1.5%), 97% ee). (1*R*,8a*R*)-**6**: colorless oil. $[\alpha]_D^{28}$ −166 ($c = 0.8$, $CHCl_3$). IR (KBr) cm^{-1}: 3430, 1682 (sh), 1658, 1618. ^1H NMR ($CDCl_3$) δ: 1.21 (d, 3H, $J = 0.5$ Hz), 3.44 (dd, 1H, $J = 5$, 11 Hz), 5.80 (s, 3H). FAB HRMS m/z: 181.1229 (calcd for $(M^+ + H)$ $C_{11}H_{17}O_2$: 181.1228). FAB MS m/z: 181 (M^+). (1*S*,8a*S*)-**5**: colorless prism, mp 56–58°C. $[\alpha]_D^{28}$ +102 ($c = 1.0$, $CHCl_3$). IR (KBr) cm^{-1}: 1730, 1682, 1618. ^1H NMR ($CDCl_3$) δ: 1.28 (s, 3H), 2.08 (d, 3H, $J = 0.4$ Hz), 4.65 (dd, 1H, $J = 4$, 12 Hz), 5.81 (d, 1H, $J = 2$ Hz). HRMS m/z: 222.1247 (calcd for $C_{13}H_{18}O_3$: 222.1256). EIMS m/z: 222 (M^+), 180, 124, 109.

5.8. Enantioselective hydrolysis of (±)-*trans*-1,2,3,4,6,7,8,8a-octahydro-5-methoxycarbonyl-8a-methyl-6-*oxo*-naphthyl acetate, 7

A mixture of (±)-**7** (100 mg) and lipase (100 mg) in phosphate buffer (40 ml, pH 7.25) was shaken at 33°C for 65 h. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent afforded a crude product, which was subjected to preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford (1*R*,8a*S*)-**8** (44 mg, 49% yield, $t_R = 122$ min (99%), $t_R = 113$ min (1%), 98% ee) and (1*S*,8a*R*)-**7** (46 mg, 46% yield, $t_R =$ 58 min (99%), $t_R = 65$ min (1%), 98% ee). (1*R*,8a*S*)-**8**: colorless prism, mp 146–149°C. $[\alpha]_D^{30}$ +84 ($c = 1.1$, $CHCl_3$). IR (KBr) cm^{-1}: 3522, 1732, 1662, 1618. ^1H NMR ($CDCl_3$) δ: 1.20 (s, 3H), 3.60 (s, 1H), 3.74 (s, 3H). Anal. calcd for $C_{13}H_{18}O_4$: C, 65.53; H, 7.62. Found: C, 65.27; H, 7.65%. (1*S*,8a*R*)-**7**: colorless oil. $[\alpha]_D^{30}$ −100 ($c = 0.98$, $CHCl_3$). IR (KBr) cm^{-1}: 1730, 1665, 1628. ^1H NMR ($CDCl_3$) δ: 1.36 (s, 3H), 2.08 (s, 3H), 3.82 (s, 3H), 4.87 (dd, 1H, $J = 3$, 3 Hz). HRMS m/z: 280.1329 (calcd for $C_{15}H_{20}O_5$: 280.1311). EIMS m/z: 280 (M^+), 238, 206, 150.

5.9. Enantioselective hydrolysis of (±)-*trans*-1,2,3,4,6,7,8,8a-octahydro-5,8a-dimethyl-6-*oxo*-naphthyl acetate, 9

A mixture of (±)-**9** (78 mg) and lipase (78 mg) in phosphate buffer (80 ml, pH 7.25) was shaken at 33°C for 42 h. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent afforded a crude product, which was subjected to preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford (1*R*,8a*S*)-**10** (47 mg, 73% yield, $t_R = 42$ min (65%), $t_R = 46$ min (35%), 30% ee) and (1*S*,8a*R*)-**9** (17 mg, 22% yield, $t_R = 23$ min (>99%), $t_R = 18$ min (<0%), >99% ee). (1*R*,8a*S*)-**10**: colorless prism, mp 108–109°C. $[\alpha]_D^{30}$ +39 ($c = 1.9$, $CHCl_3$). IR (KBr) cm^{-1}: 3419, 1641, 1605. ^1H NMR ($CDCl_3$) δ: 1.23 (d, 3H, $J = 1.5$ Hz), 1.79 (d, 3H, $J = 1.5$ Hz), 3.60 (bs, 1H). Anal. calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found C, 74.11; H, 9.39%. (1*S*,8a*R*)-**9**: colorless prism, mp 68–70°C. $[\alpha]_D^{30}$ −79 ($c = 1.4$, $CHCl_3$). IR (KBr) cm^{-1}: 1727, 1665, 1656, 1610. ^1H NMR ($CDCl_3$) δ: 1.29 (s, 3H), 1.80 (d, 3H, $J = 1.5$ Hz), 2.05 (s, 3H), 4.80 (dd, 1H, $J = 2$, 3 Hz). Anal. calcd for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 71.21; H, 8.57%.

5.10. Enantioselective hydrolysis of (±)-*trans*-1,2,3,4,6,7,8,8a-octahydro-8a-methyl-6-*oxo*-naphthyl acetate, 11

A mixture of (±)-**11** (60 mg) and lipase (100 mg) in phosphate buffer (80 ml, pH 7.25) was shaken at 33°C for 54 h. The reaction mixture was extracted with ethyl acetate and the organic solution was dried over anhydrous $MgSO_4$. Evaporation of the organic solvent afforded a crude product, which was subjected to preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford (1*R*,8a*S*)-**12** (23 mg, 48% yield, $t_R = 53$ min (96.5%), $t_R = 45$ min (3.5%), 93% ee) and (1*S*,8a*R*)-**11** (25 mg, 42% yield, $t_R = 28$ min (91.5%), $t_R = 22$ min (8.5%), 83% ee). (1*R*,8a*S*)-**12**: colorless prism, mp 84–86°C. $[\alpha]_D^{27}$ +93 ($c = 1.6$, $CHCl_3$). IR (KBr) cm^{-1}: 3434, 1659 (sh), 1645, 1608. ^1H NMR ($CDCl_3$) δ: 1.25 (d, 3H, $J = 0.5$ Hz), 3.66 (bs, 1H), 5.87 (d, 1H, $J = 1.5$ Hz). Anal. calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.11; H, 8.99%. (1*S*,8a*R*)-**11**: colorless prism, mp 47–50°C. $[\alpha]_D^{27}$ −81 ($c = 1.1$, $CHCl_3$). IR (KBr) cm^{-1}: 1731, 1666, 1617. ^1H NMR ($CDCl_3$) δ: 1.32 (s, 3H), 2.06 (s, 3H), 4.85 (bs, 3H), 5.89 (d, 1H, $J = 1.5$ Hz). Anal. calcd for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.07; H, 8.20%.

5.11. Jones' oxidation of (1*R*,8a*S*)-10

Jones' reagent (two drops) was added to a stirred solution of the hydrolysed product (1*R*,8a*S*)-**10** (18 mg) in acetone (2 ml) and the mixture was cooled in an ice-salt bath for 30 min. After the addition of isopropyl alcohol and sodium hydrogen carbonate, the reaction mixture was filtered. The filtrate was evaporated to give a crude product, which was subjected to preparative TLC [silica gel, 20×20 cm; developing solvent, hexane–ethyl acetate (1:1)] to afford the (8a*S*)-diketone **13** [16 mg, 88% yield from (1*R*,8a*S*)-**10**]. (8a*S*)-diketone $[\alpha]_D^{30}$ +18 ($c = 0.8$, MeOH). ^1H NMR ($CDCl_3$) δ: 1.18 (s, 3H), 1.78 (d, 1H, $J = 1$ Hz). The NMR data of (8a*S*)-**13** were identical with those the reported (8a*S*)-**13**.[11]

References

1. (a) Duthcer, J. S.; Macmillan, J. G.; Heathcock, C. H. *J. Org. Chem.* **1976**, *41*, 2663–2669; (b) Welch, S. C.; Rao, A. S. C. P. *J. Org. Chem.* **1978**, *43*, 1957–1961; (c) Magee, T. V.; Bornmann, W. G.; Isaacs, R. C. A.; Danishefsky, S. J. *J. Org. Chem.* **1992**, *57*, 3274–3276; (d) Grieco, P. A.; Collins, J. L.; Moher, E. D.; Fleck, T. J.; Gross, R. S. *J. Am. Chem. Soc.* **1993**, *115*, 6078–6093; (e) Yeo, S.-K.; Hata, N.; Seki, M.; Kanematsu, K. *Tetrahedron* **1995**, *51*, 3499–3506; (f) Danishefsky, S. J.; Masters, J. J.; Young, W. B.; Link, J. T.; Snyder, L. B.; Magee, T. V.; Jung, D. K.; Isaacs, R. C. A.; Bornmann, W. G.; Alaimo, C. A.;

Coburn, C. A.; Di Grandi, M. J. *J. Am. Chem. Soc.* **1996**, *118*, 2843–2859; (g) Cheung, W. S.; Wong, H. N. C. *Tetrahedron* **1999**, *55*, 11001–11016.

2. (a) Inayama, S.; Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1986**, *34*, 2660–2663; (b) Inayama, S.; Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1987**, *35*, 429–432; (c) Inayama, S.; Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y. *Chem. Pharm. Bull.* **1989**, *37*, 712–717; (d) Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1989**, *37*, 1023–1027; (e) Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1989**, *37*, 2561–2563; (f) Shimizu, N.; Ohkura, T.; Akita, H.; Oishi, T.; Iitaka, Y.; Inayama, S. *Chem. Pharm. Bull.* **1991**, *39*, 2973–2979; (g) Saito, N.; Kawaguchi, H.; Ohtsuka, Y.; Shimizu, N.; Inayama, S. *Chem. Pharm. Bull.* **1991**, *39*, 3085–3087; (h) Shimizu, N.; Akita, H.; Oishi, T.; Inayama, S. *Chem. Pharm. Bull.* **1994**, *42*, 1160–1162.
3. Shimizu, N.; Akita, H.; Kawamata, T. *Chem. Pharm. Bull.* **1996**, *44*, 665–669.
4. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Shi, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
5. The conversion of (±)-3,4,8,8a-tetrahydro-8a-methyl-5-methoxycarbonyl-1,6(2*H*,7*H*)-naphthalenedione[2a,c] to the *trans*-acetate (±)-**1** was successfully achieved by the sequential reaction of reduction with $NaBH_4$ in MeOH followed by separation using column chromatography and acetylation with Ac_2O/pyridine.
6. The conversion of (±)-3,4,8,8a-tetrahydro-5,8a-dimethyl-1,6(2*H*,7*H*)-naphthalenedione[1a] to the *trans*-acetate (±)-**3** was successfully achieved by the sequential of reduction with $NaBH_4$ in MeOH and acetylation with Ac_2O/pyridine.
7. The conversion of (±)-3,4,8,8a-tetrahydro-8a-methyl-1,6(2*H*,7*H*)-naphthalenedione to the *trans*-acetate (±)-**5** was successfully achieved by the sequential of reduction with $NaBH_4$ in MeOH followed by separation using column chromatography and acetylation with Ac_2O/pyridine.
8. Details of the enantioselective hydrolysis of *cis*-acetates by β-amylase will be reported in the near future.
9. The conversion of (±)-3,4,8,8a-tetrahydro-8a-methyl-5-metoxycarbonyl-1,6(2*H*,7*H*)-naphthalenedione to the *trans*-acetate (±)-**7** was successfully achieved by the sequential reduction with L-selectride in THF at −78°C, oxidation with MnO_2 in CH_2Cl_2 followed by separation using column chromatography and acetylation with Ac_2O/pyridine.
10. Shimizu, T.; Hiranuma, S.; Nakata, T. *Tetrahedron Lett.* **1996**, *37*, 6145–6148.
11. (a) Eder, U.; Sauer, G.; Wiechert, R. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 496–497; (b) Hiroi, K.; Yamada, S. *Chem. Pharm. Bull.* **1975**, *23*, 1103–1109; (c) Uma, P.; Swaminathan, S.; Rajagopalan, K. *Tetrahedron Lett.* **1984**, *25*, 5825–5828.
12. Fuhshuku, K.; Funa, N.; Akeboshi, T.; Ohta, H.; Hosomi, H.; Ohba, S.; Sugai, T. *J. Org. Chem.* **2000**, *65*, 129–135.
13. (a) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656–2665; (b) Cygler, M.; Grochulski, P.; Kazlauskas, R. J.; Schrag, J. D.; Bouthillier, F.; Rubin, B.; Serreqi, A. N.; Gupta, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 3180–3186.
14. (a) Naemura, K.; Fukuda, R.; Konishi, M.; Hirose, K.; Tobe, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1253–1256; (b) Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385–2394; (c) Naemura, K.; Murata, M.; Tanaka, R.; Yano, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1996**, *7*, 3285–3294.
15. Lemke, K.; Lemke, M.; Theil, F. *J. Org. Chem.* **1997**, *62*, 6268–6273.