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Letter

Lipase-catalyzed enantioselective deacetylation of *ortho*-substituted phenyl acetates with 1-butanol in organic solvents

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Abstract

Lipase from *Candida antarctica* catalyzed enantioselective deacetylation of *ortho*-substituted phenyl acetates with 1-butanol in organic solvents. The enantioselectivity of the lipase-catalyzed reaction depended on the nature of the substrates and the solvents employed; a significant inversion on the enantioselectivity was observed when 1,4-dioxane was used as solvent instead for cyclohexane. This work is the first example of solvent-mediated inversion of the enantioselectivity of a lipase-catalyzed deacetylation in organic solvents. © 1999 Elsevier Science B.V. All rights reserved.

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Lipases in organic solvents have been used as effective catalysts [1]. A variety of racemic alcohols have been resolved into the corresponding enantiomers by lipase-catalyzed (trans)esterification in organic solvents [1]. Recently, we have reported that enantioselectivity of the lipase-catalyzed transesterification of α-methylbenzyl alcohols strongly depended on the substituent on the aryl group; the effect of *ortho*-substituents on the aromatic ring on the reactivity and selectivity of the lipase reactivity was completely different from those of *para*- and *meta*-substituents [2].

Many ferroelectric liquid crystals consist of phenols [3–7], and the phenols have an asymmetric carbon remote from the benzene ring. Substituted phenols which have an asymmetric carbon directly attached to the benzene ring are thought to be new materials for ferroelectric liquid crystals [4,8].

In the present study, we investigated the lipase-catalyzed deacetylation of *ortho*-substituted phenyl acetates with 1-butanol in organic solvents (Scheme 1). Kinetic resolution of phenyl esters have been scarcely investigated [9–12]. We selected cyclohexane and 1,4-dioxane as solvents: the former is a hydrophobic solvent (log $P = 3.2$ [13]) while the latter is hydrophilic (log $P = -1.1$). It is well known that the hydrophobicity of organic solvents can

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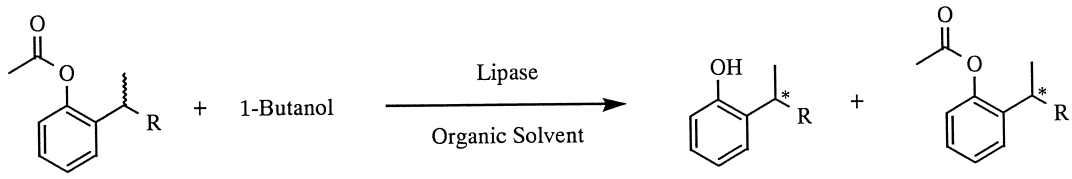


(±)-**1a**: R = CH_2CH_3,
(±)-**2a**: R = $CH_2(CH_2)_2CH_3$
(±)-**3a**: R = $CH_2(CH_2)_3CH_3$
(±)-**4a**: R = $CH_2(CH_2)_4CH_3$
(±)-**5a**: R = $CH_2(CH_2)_6CH_3$

Scheme 1. Lipase-catalyzed enantioselective deacetylation with 1-butanol in organic solvents.

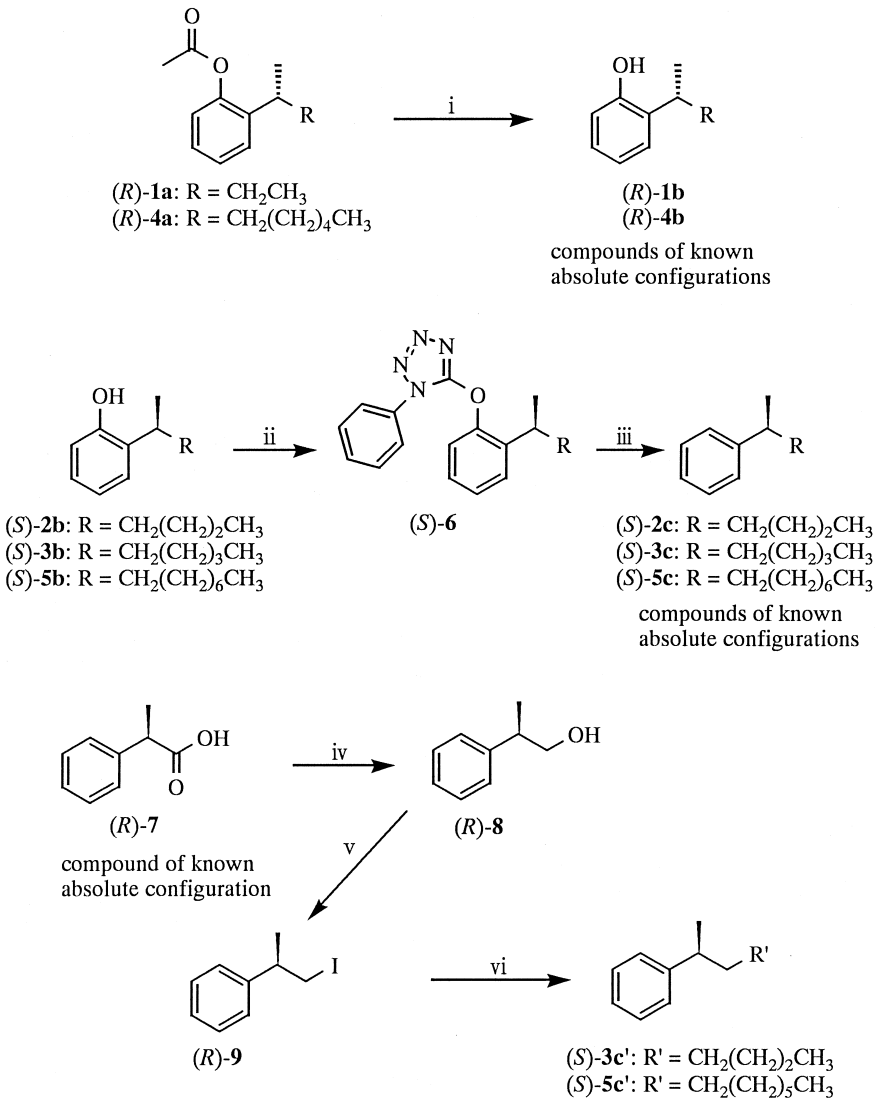
influence the enantioselectivity of lipases-catalyzed reaction [14].

All the *ortho*-substituted phenyl acetates were synthesized and gave satisfactory results in IR spectroscopy, NMR spectroscopy, and elemental analyses.

As a typical run, 100 mg of a lipase was placed in a vial and 2 ml of a 1,4-dioxane solution containing 60 μmol (±)-**2a** and 180 μmol 1-butanol. Then the resulting suspension was stirred magnetically at 35°C for 0.5 h. The reaction was quenched by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel column using hexane-acetone (15:1 (v/v)) as an eluent. An aliquot of the combined fractions containing the phenol produced was analyzed on HPLC (Daicel, Chiralcel OD-H column, hexane:2-propanol = 100:1 (v/v)) to determine the ee of the phenol. The ee of the unreacted substrate was determined after hydrolysis (NaOH, MeOH) to the corresponding phenol.

The absolute configurations of the preferred enantiomers were determined by optical rotation measurements (Scheme 2). The results of the case in which CAL as a lipase and 1,4-dioxane as an organic solvent were used are described below. The unreacted esters of (±)-**1a** and (±)-**4a** were converted to the corresponding phenols ((*R*)-**1b** and (*R*)-**4b**) by hydrolysis (NaOH, MeOH). The absolute configurations of (*R*)-**1b** and (*R*)-**4b** were established by comparing their optical rotations to known rotations in the literature. (*R*)-**1b**, 48.3% ee: $[\alpha]_D^{26}$ −10.9 (*c* 3.73, benzene) [lit. [15], $[\alpha]_D$ −1.05 ± 0.01 (*c* 13.36, benzene, 7.18% ee, (*R*))]; (*R*)-**4b**, 74.9% ee: $[\alpha]_D^{22}$ −0.79 (*c* 5.21, EtOH) [lit. [16], $[\alpha]_D^{20}$ +0.80 (*c* 2.0, EtOH, 94.8 ± 7.8% ee, (*S*))]. The phenols produced from (±)-**2a**, (±)-**3a** and (±)-**5a**, (*S*)-**2b**, (*S*)-**3b** and (*S*)-**5b** were converted to the corresponding phenyltetrazolyl ethers followed by catalytic hydrogenolysis over 10% palladium on charcoal to give the corresponding 2-phenylalkanes ((*S*)-**2c**, (*S*)-**3c** and (*S*)-**5c**) [17]. The absolute configuration of (*S*)-**2c** was established by comparing the optical rotation to known rotation in the literature. (*S*)-**2c**, 45.9% ee: $[\alpha]_D^{22}$ +11.61 (*c* 5.11, heptane) [lit. [18], $[\alpha]_D^{25}$ −23.10 (*c* 2.445, heptane (*R*)]. Because the preparations of optically active 2-phenylheptane and 2-phenyldecane have not been reported, we prepared (*S*)-2-phenylheptane ((*S*)-**3c**′) and (*S*)-2-phenyldecane ((*S*)-**5c**′) from (*R*)-2-phenylpropanoic acid ((*R*)-**7**) which is commercially available according to the method described in Scheme 2 [19,20]. (*S*)-**3c**′, $[\alpha]_D^{28}$ +22.25 (*c* 2.08, benzene), (*S*)-**5c**′, $[\alpha]_D^{27}$ +21.06 (*c* 4.63, benzene). The absolute configuration of (*S*)-**3c** and (*S*)-**5c** was established by comparing their optical rotation to the rotations of (*S*)-**3c**′ and (*S*)-**5c**′. (*S*)-**3c**, 25.8% ee: $[\alpha]_D^{26}$ +6.21 (*c* 6.78, benzene), (*S*)-**5c**, 47.0% ee: $[\alpha]_D^{23}$ +9.83 (*c* 5.47, benzene).

Five commercially available lipases (Amano PS from *Pseudomonas cepacia*, Amano AK



Scheme 2. Determination of absolute configurations of preferred enantiomers. Reagents and conditions: (i) 1 M NaOH, MeOH; (ii) *t*-BuOK, DMF; 5-chloro-1-phenyl-1*h*-tetazole; (iii) H_2, 10% Pd-C, THF; (iv) $LiAlH_4$, EtO_2; (v) I_2, imidazole, Ph_3P, CH_2Cl_2; (vi) *n*-butylmagnesium bromide or *n*-heptylmagnesium bromide, 5 mol % $Cu(OAc)_2$, THF.

from *Pseudomonas* sp., Amano AY from *Candida cylindracea*, pocine pancreatic lipase (PPL) and Novozym® 435 from *C. antarctica*) were tested for their selective deacetylation ability of (±)-**1a** in 1,4-dioxane. Amano PS and Amano AK showed low enantioselectivity ($E < 3$ [21]). Amano AY and PPL had little activity of deacetylation. Novozym® 435 (CAL) showed the highest enantioselectivity ($E = 6.3$). Therefore, CAL was selected for further study.

As shown in Table 1, the lipase exhibits low enantioselectivities in cyclohexane. The configuration of the preferably deacetylated enantiomer changed between $R = C_5$ and $R = C_6$. Such a reversal of configuration has been previously observed in crude pancreatic extract-catalyzed esterifications [22]. The reaction rates were decreased with increase in the length of substituent R. The poor selectivities in cyclohexane are largely improved using 1,4-dioxane as a

Table 1
CAL-catalyzed deacetylation with 1-butanol in organic solvents

Substrate	Solvent	Reaction time (h)	Conversion (%)	*E*
(±)-**1a**	Cyclohexane	5.5	23.7	3.7 (*S*)[a]
(±)-**2a**	Cyclohexane	6	18.2	2.0 (*S*)
(±)-**3a**	Cyclohexane	6.5	19.9	1.5 (*S*)
(±)-**4a**	Cyclohexane	24	23.6	1.2 (*R*)
(±)-**5a**	Cyclohexane	42	28.2	2.1 (*R*)
(±)-**1a**	1,4-dioxane	1	15.5	6.3 (*S*)
(±)-**2a**	1,4-dioxane	0.5	19.9	16 (*S*)
(±)-**3a**	1,4-dioxane	0.5	16.5	13 (*S*)
(±)-**4a**	1,4-dioxane	0.6	10.0	8.7 (*S*)
(±)-**5a**	1,4-dioxane	4	22.6	4.5 (*S*)

[a]The enantiomer preferentially deacetylated is designated in parenthesis.

solvent. The resolution of (±)-**2a** showed the highest selectivity ($E = 16$). Kazlauskas et al. reported enzyme-aided kinetic resolution of *ortho*-substituted phenol with an asymmetric phosphorus atom in its substituent, of which structure is highly similar to those of compounds of ours, and the reaction shown low enantioselectivity ($E < 5$) [12]. It is interesting that when (±)-**4a** or (±)-**5a** was used as a substrate, the lipase preferred different enantiomers in between two solvents, cyclohexane and 1,4-dioxane.

The selectivity of lipase for (±)-**5a** was inverted according to the change in solvent. The inversion was also observed in the deacetylation of (±)-**4a**. The solvent-induced inversion of enantioselectivity of a protease was first reported by Klibanov et al. in 1992 [23] and they also reported the reversal of prochiral selectivity of a protease [24]. Concerning lipases, Hirose et al. [25] reported the inversion of prochiral selectivity while Ueji et al. [26] reported the inversion of the enantioselectivity of esterification. The present study is the first example which shows solvent dependent inversion of enantioselectivity of the lipase-catalyzed deacetylation in organic solvents.

Generally, it is thought that a lipase has a large pocket and a small pocket on its active site [27]. When a secondary alcohol is incorporated into the active center, the large pocket accommodates the large substituent at the stereocenter of the alcohol while the small pocket accommodates the small substituent. However, since the structure of (±)-**5a** is very different from that of the secondary alcohol, the two substituents (methyl and *n*-octyl groups) of (±)-**5a** will not be incorporated into the pockets of CAL. Instead, they will be located in the area other than the two pockets in the active site. The location of the substituents may be more loosely limited than that of the secondary alcohol. Therefore, the two substituents can move freely in the active site according to the change in environment at the site induced by the variation in solvent hydrophobicity [23,24] or by the difference in the degree of incorporation of solvent molecules [28]. This movement is thought to be attributed to the inversion of enantioselectivity.

In the deacetylation of (±)-**4a** and (±)-**5a**, the lipase changed the preferred enantiomer by changing the solvent from 1,4-dioxane to cyclohexane. Although the lipase prefers the (*S*)-isomer of (±)-**1a**, (±)-**2a** and (±)-**3a** in cyclohexane, the selectivity observed in the solvent is smaller than those in 1,4-dioxane. Thus the lipase prefers the (*S*)-isomer in 1,4-dioxane and its preference for (*S*)-isomer tends to shift to that for (*R*)-isomer in cyclohexane.

Finally, we also conducted deacetylation of (±)-**2a** catalyzed by CAL in the preparative scale (1.0 g, 4.5 mmol) in 1,4-dioxane and obtained (*R*)-2-(1-methylpentyl)phenol, the ester of which was an unfavorable enantiomer to the lipase, in a 24% overall yield and with an ee of $> 99.9\%$ ($[\alpha]_D^{25}$-2.33 (*c* 4.28, EtOH)).

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