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Lipase-Catalyzed Transesterification of Aryl-Substituted Alkanols in an Organic Solvent

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Lipase-catalyzed transesterifications of aryl-substituted alcohols with vinyl acetate in organic solvents have been investigated. $V_{\max}$ of (*R*)-1-phenylethanol is much larger than its (*S*)-counterpart, although their K_ms are similar each other. It is proposed that a lipase from *Pseudomonas cepacia* can form a complex easily with each enantiomer, although the complex with the (*S*)-enantiomer is abortive. Enantioselectivity for the *ortho*-substituted 1-phenylethanol is smaller than those for the others. A three-dimensional model for the active domain of the lipase has been proposed to explain the enantioselectivity and substrate selectivity of the lipase.

Lipases in organic solvents have been used widely by organic chemists as effective catalysts. They catalyze esterification, transesterification, lactonization, and even aminolysis.[1—5] Many racemic alcohols, carboxylic acids, and esters have been resolved into their corresponding enantiomers by lipase-catalyzed transesterifications.[6] However, there have been little information on the substrate selectivity and stereoselectivity of a lipase in an organic solvent. Thus, organic chemists have had no straightforward recipe to find the most suitable lipase and reaction conditions for obtaining the best result. The selectivity of a lipase in an organic solvent is a function of the structure of the substrate. Several reports have contributed to understanding the effects of carbon-chain length of the substrate on catalytic efficiency and enantioselectivity.[7—17] Furthermore, since a lipase-catalyzed reaction involves two substrates, an alcohol and an acyl donor, the variation in the structure of alcohol influences structure-dependence of enantioselectivity for acylating agent,[18—23] and vice versa.[19,24—28] Nevertheless, there have been few studies to discover the effects of substrate structure from a kinetic viewpoint.[7—11]

To understand the effect of substrate structure on lipase-catalyzed transesterification in organic solvents, we studied the reactions of substrates with various carbon-chain length.[29] When ω-phenyl-1-alkanols and ω-cyclohexyl-1-alkanols are used as substrates for transesterification with vinyl acetate, the reactivity of the lipase for these substrates depends on the carbon-chain length. Benzyl alcohol is the most reactive and 2-phenylethanol is the least reactive among the ω-phenyl-1-alkanols studied. On the other hand, cyclohexylmethanol is not a special substrate and behaves similarly to other ω-cyclohexyl-1-alkanols.

It has been reported that 1-phenylethanol, a chiral analog of benzyl alcohol, can be resolved by a lipase-catalyzed (trans)esterification with high enantioselectivity.[30—37] Catalytic efficiency of a lipase for the (*R*)-enantiomer is much higher than that for the corresponding (*S*)-enantiomer; in most cases, the *E*-value[38] is more than 100. On the basis of abnormal enantioselectivity exerted by some lipases for 1-phenylethanol[30—37] and of the finding of hydrophobic regions lined with phenyl rings at the vicinity of catalytic sites of lipases from *Candida rugosa*[39,40] and *Rhizomucor miehei*,[41] we proposed that a lipase from *Pseudomonas cepacia* provides a pocket that has special affinity to an aromatic moiety of a substrate (anchor effect) and the location of the pocket is quite close to the catalytic site.[29]

In this paper, we wish to discuss the substrate selectivity and stereoselectivity of a lipase from *Pseudomonas cepacia* on the bases of kinetic parameters for a series of substrates. Although it is well-known that 1-phenylethanol itself can be resolved into enantiomers with high enantioselectivities, little is known on the effects of aromatic substituents in a substrate on catalytic efficiency and enantioselectivity of a lipase.[42—44] Thus, 1-phenylethanol and its analogs or derivatives were reacted to gain understanding of the effects of aryl substituents as well as the effects of carbon-chain length on the substrate selectivity and stereoselectivity of a lipase from *Pseudomonas cepacia*. The substrates used in the reaction are portrayed in Scheme 1.

Results

Kinetics, analyzed as pseudo-first-order in alcohol, were followed by gas chromatography, observing the increase in concentration of produced acetic esters. Cyclohexane and vinyl acetate were used as an organic solvent and an acyl donor, respectively.

Racemic alcohol of a constant concentration (500 mM : 1 M = 1 mol dm^{-3}) was used. Nevertheless, the catalytic effi-

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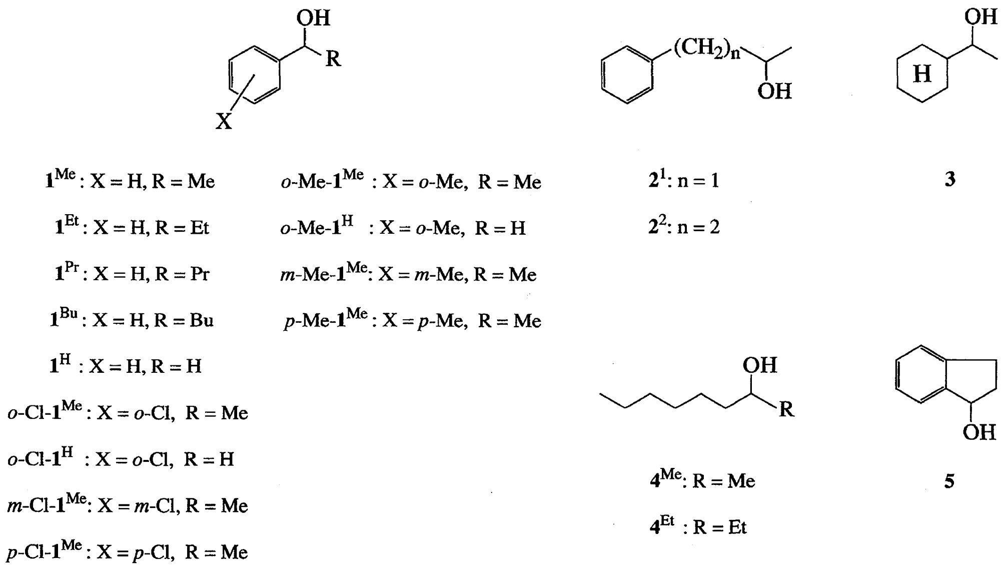


Scheme 1.

ciency of the lipase can be measured independently for each enantiomer by using an appropriate chiral column. The results are summarized in Table 1. Kinetic parameters for the lipase-catalyzed transesterifications with certain substrates are listed in Tables 2 and 3. K_m and V_{max} of the reaction were calculated by a Hanes–Hoolf plot.[45] Here, the value of V_{max} is normalized as V_{max}/mg lipase.

It has been reported that the lipase-catalyzed (trans)-esterification proceeds by a ping-pong mechanism.[46] Since initial step of the overall reaction is acylation of a lipase with vinyl acetate, the initial rate depends on the concentration of vinyl acetate. Since a constant concentration of vinyl acetate (500 mM) was used throughout this study, the observed K_m and V_{max} are not absolute but apparent parameters. Furthermore, since a lipase is not soluble in an organic solvent, the reaction system is heterogeneous, and the rate of lipase-catalyzed (trans)esterification depends on the efficiency of stirring. Therefore, different apparatus may produce different results. For these two reasons, it should be noted that kinetic parameters herein reported are valuable only when they are discussed as relative magnitudes.

Table 1. Relative Reactivities of Lipase-Catalyzed Transesterification for Various Substrates

Substrate	Conversion/%[a]		*E*-value
	R	*S*	
$\mathbf{1}^{Me}$	62.6	0	—
o-Cl-$\mathbf{1}^{Me}$	24.0[b]	0.520[b]	53
o-Me-$\mathbf{1}^{Me}$	37.3[b]	9.33[b]	4.8
m-Cl-$\mathbf{1}^{Me}$	48.7	0.211	317
m-Me-$\mathbf{1}^{Me}$	50.3	0.119	590
p-Cl-$\mathbf{1}^{Me}$	46.7	0	—
p-Me-$\mathbf{1}^{Me}$	48.5	0	—
$\mathbf{1}^{Et}$	13.5	0	—
$\mathbf{1}^{Pr}$	1.24	0	—
$\mathbf{1}^{Bu}$	1.39	0	—
$\mathbf{2}^{1}$	21.3	0	—
$\mathbf{2}^{2}$	21.0	0	—
3	27.8	0.425	77
$\mathbf{4}^{Me}$	30.9	6.94	5.1
$\mathbf{4}^{Et}$	33.2	5.47	7.2
5	ca. 100	0	—

a) After 1 h. b) After 24 h.

Discussion

Three-Dimensional Model of Active Domain. Catalytic efficiency of the lipase is defined as the amount of substrate reacted (%) after a constant reaction time. Table 1 shows that (*R*)-enantiomers react faster than the corresponding (*S*)-enantiomers in all the substrates studied, and the *E*-values are extremely high in general. However, it is noteworthy that the reactivities of $\mathbf{1}^{Pr}$ and $\mathbf{1}^{Bu}$ are too low to be assigned large *E*-values for these substrates without ambiguity. It is also recognized that the unsatisfactory *E*-value stems from surprisingly high reactivity of the (*S*)-enantiomer relative to the (*S*)-enantiomers of the other substrates.

The reactivities of (*R*)-*m*- and -*p*-Cl-$\mathbf{1}^{Me}$ and (*R*)-*m*- and -*p*-Me-$\mathbf{1}^{Me}$ are about 80% of that of (*R*)-$\mathbf{1}^{Me}$ irrespective of the electronic property and position of the substituent. The reactivities of *ortho*-substituted substrates, on the other hand, seem to be lower than those for the others except for that of (*R*)-**5**. In enzymatic reactions, it is not uncommon that a substituent at the *ortho*-position of the substrate interferes with the reaction. In other words, the extremely increased reactivity observed for (*R*)-**5** is unique.

Factors that affect the *E*-value as well as that provide unusual reactivity to the *ortho*-substituted substrates will be discussed later in detail on the bases of kinetic parameters.

Generally, two "pockets" of different size are assigned for the active domain of a lipase to explain the substrate selectivity and enantioselectivity.[10,39,47,48] However, the fact that reactivities of the (*S*)-enantiomers are not parallel to those of the corresponding (*R*)-isomers suggests that the traditional

Table 2. Kinetic Parameters for the Lipase-Catalyzed Transesterification of 1-Phenylethanol and Its Derivatives

Substrate	K_m/mM		V_{max}/μM s^{-1} mg^{-1}lipase		$10^6 V_{max}/K_m$/s^{-1} mg^{-1}lipase	
	R	*S*	*R*	*S*	*R*	*S*
$\mathbf{1}^{Me}$	49.4± 3.6	154±12	4.48 ±0.10	0.0400 ±0.0017	90.6 ±6.3	0.260 ±0.018
o-Cl-$\mathbf{1}^{Me}$	467 ± 7	889±21	0.193±0.002	0.00830±0.00017	0.413±0.004	0.00934±0.00010
m-Cl-$\mathbf{1}^{Me}$	44.3± 1.9	237±14	3.77 ±0.04	0.0452 ±0.0016	85.1 ±3.6	0.191 ±0.009
p-Cl-$\mathbf{1}^{Me}$	31.4± 2.8	124±11	3.16 ±0.06	0.00970±0.00041	101 ±9	0.0785 ±0.0064
o-Me-$\mathbf{1}^{Me}$	354 ±32	497±12	0.322±0.019	0.0817 ±0.0016	0.912±0.062	0.164 ±0.003
m-Me-$\mathbf{1}^{Me}$	84.2± 6.8	501±17	3.07 ±0.11	0.0304 ±0.0009	36.5 ±2.7	0.0606 ±0.0011
p-Me-$\mathbf{1}^{Me}$	63.3± 6.6	258±30	4.29 ±0.16	0.0268 ±0.0019	67.8 ±6.7	0.104 ±0.010

Table 3. Kinetic Parameters for the Lipase-Catalyzed Transesterification of Benzyl Alcohol and Its *ortho*-Substituted Derivatives

Substrate	K_m/mM	V_{max}/μM s^{-1} mg^{-1}lipase	V_{max}/K_m/s^{-1} mg^{-1}lipase
$\mathbf{1}^{H}$	4.82±1.09	56.8±12.3	0.0118 ±0.0008
o-Cl-$\mathbf{1}^{H}$	6.45±0.93	38.5± 5.4	0.00596±0.00017
o-Me-$\mathbf{1}^{H}$	4.67±1.20	75.9±18.6	0.0163 ±0.0013

interpretation, which is based on the metaphysical size of the pockets, is insufficient for understanding the phenomenon. For example, in a comparison of the reactivity of (*S*)-$\mathbf{1}^{Me}$ to that of (*S*)-*o*-Me-$\mathbf{1}^{Me}$ (or of (*S*)-*o*-Cl-$\mathbf{1}^{Me}$), the traditional idea must tolerate a controversy that the small pocket cannot accommodate a phenyl ring but does an *o*-tolyl (and even an *o*-chlorophenyl) group. The small pocket can accept alkyl groups from the ethyl in (*R*)-$\mathbf{1}^{Et}$ to the hexyls in (*S*)-$\mathbf{4}^{Me,Et}$. The *E*s-value^49) of a phenyl ring is not much different from those of hexyl and cyclohexyl groups. This fact suggests limitation of the idea that the substrate selectivity of a lipase depends only on the bulkiness of the substrate. In our previous paper, we proposed an anchor effect,^29) by which the aromatic moiety of a substrate can find an appropriate position to be trapped into a pocket at the vicinity of the catalytic site. In addition, it should be noted that there has been no report on lipase-catalyzed (trans)esterification of tertiary alcohols, which might suggest that one of the substituents at the α-carbon of an alcohol must be a hydrogen.

A revised active site model to explain the relative reactivities of substrates is proposed as shown in Scheme 2, where the circle represents a small pocket that is on the front side. In addition to the large and small pockets so far proposed, we add a third pocket, which is the smallest and can accept a hydrogen only without steric strain. Ser-OAc denotes the acetylated serine residue, and we assume that the large pocket is the site which exerts an anchor effect to keep the aromatic moiety. High reactivity of (*R*)-**5** is taken into consideration for drawing relative positions of the catalytic serine residue and the pockets for aromatic ring and hydrogen.

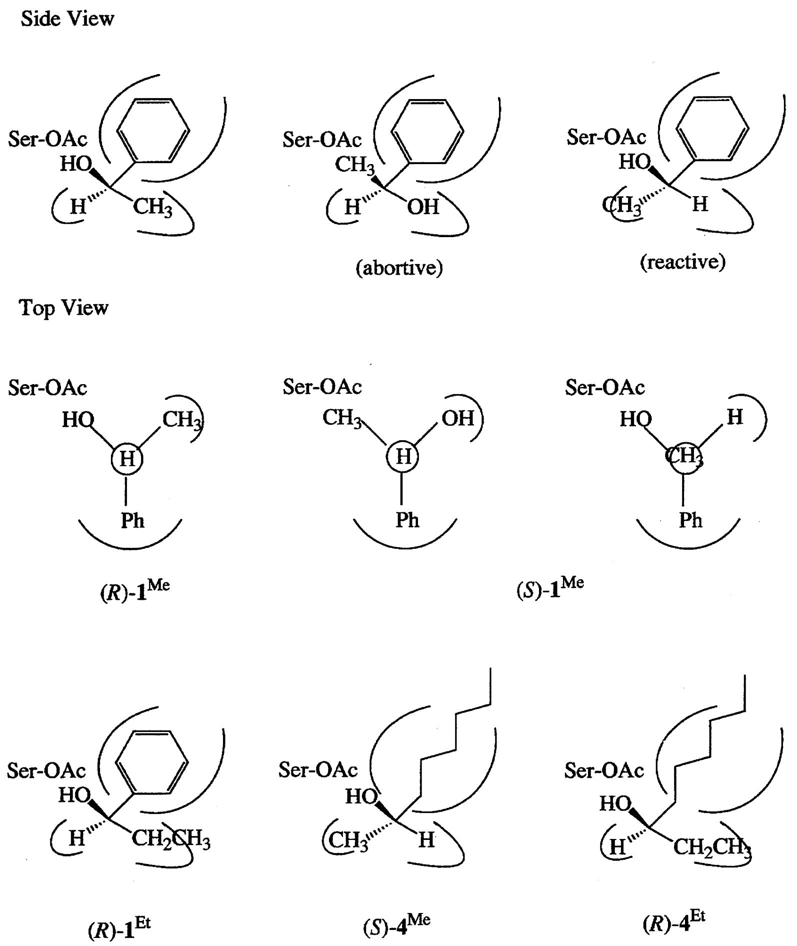
Since the phenyl group of (*R*)-$\mathbf{1}^{Me}$ fits well in the large pocket with the methyl group at the medium-sized pocket, this substrate can enjoy the catalysis of lipase more easily than (*S*)-$\mathbf{1}^{Me}$. When (*S*)-$\mathbf{1}^{Me}$ is incorporated into the lipase, on the other hand, its phenyl group will be set in the large pocket to be stabilized by the anchor effect in a similar manner to that of (*R*)-$\mathbf{1}^{Me}$. Then, the hydroxyl group of (*S*)-$\mathbf{1}^{Me}$ has to be located in the medium-sized pocket, which is an inappropriate position for the reaction with the acetylated serine residue. In order to set the hydroxyl group appropriately for the reaction, the α-methyl group is forced to come into the small pocket designed for incorporating a hydrogen only.

Low enantioselectivity of the lipase for $\mathbf{4}^{Me}$ can also be explained with this model: Since the large pocket is provided for accepting an aromatic moiety, the hexyl group is not affected by the anchor effect, and it is not bound tightly in the large pocket of the enzyme. Consequently, the α-methyl group of (*S*)-$\mathbf{4}^{Me}$ can experience a reasonable freedom in the small pocket. Thus, (*S*)-$\mathbf{4}^{Me}$ is a better substrate for the lipase than (*S*)-$\mathbf{1}^{Me}$. However, since $\mathbf{4}^{Me}$ has no phenyl group and cannot enjoy the anchor effect, even its (*R*)-enantiomer is harder to incorporate into the catalytic domain than (*R*)-$\mathbf{1}^{Me}$, and the overall reactivity of (*R*)-$\mathbf{4}^{Me}$ is smaller than that of (*R*)-$\mathbf{1}^{Me}$.

The reactivities of (*R*)-$\mathbf{1}^{Et,Pr,Bu}$ are smaller than those of (*R*)-$\mathbf{4}^{Me,Et}$. Because the aromatic rings of (*R*)-$\mathbf{1}^{Et,Pr,Bu}$ are again fixed tightly in the pocket, the position of the alkyl groups are completely restricted and they cannot avoid steric interference from the amino acid residues constructing the small pocket. On the contrary, according to the looseness of bonding, (*S*)-$\mathbf{4}^{Me,Et}$ remain small reactivities.

Effects of an *Ortho*-Substituent. When a substituent is introduced into an aromatic ring, both electronic and steric effects have to be taken into account. Therefore, we selected methyl and chloro substituents for perturbing the molecule of $\mathbf{1}^{Me}$. These two substituents have similar van der Waals radii^50) but their electronic properties are opposite.

On inspection of Table 2, it is recognized that a high enantioselectivity of the lipase can be accounted for mainly by a large difference in V_{max} between the (*R*)- and (*S*)-enantiomers. The difference in K_m between the (*R*)- and (*S*)-

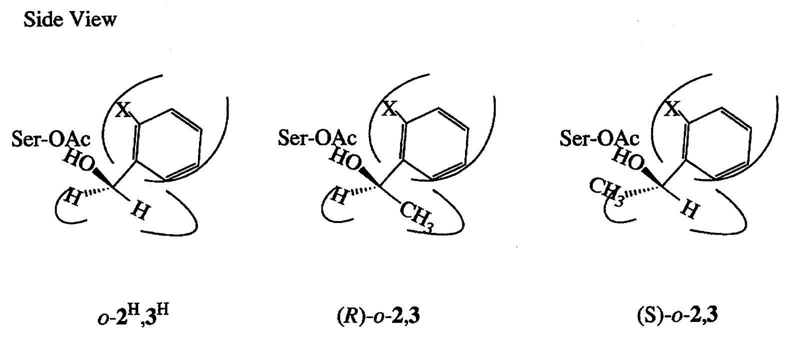


Scheme 2.

enantiomers of $\mathbf{1}^{\mathrm{Me}}$ is small, which predicts that the (*S*)-enantiomer can form a complex with the enzyme as easily as the (*R*)-enantiomer does.

The observation is consistent with our proposal on the shape of the active domain of the lipase: The (*S*)-enantiomer also has its phenyl group stored in the large pocket easily, or the formation of the complex is as easy as that for the (*R*)-enantiomer. However, in this complex, the position of the hydroxyl group of the (*S*)-enantiomer is inappropriate and the reaction does not proceed further. Since only those that are incorporated into the domain at a large sacrifice of steric fitness as illustrated in Scheme 2 can undergo further reaction, the V_{max} for (*S*)-$\mathbf{1}^{\mathrm{Me}}$ appears very small. In other words, in this case, the real figure of the reaction may be understood by imaging a large K_{m} for the *reactive* ES complex.

The most striking features seen in Table 2 are (a) an extremely large K_{m} for the *ortho*-substituted substrates and (b) small V_{max}s for *o*-Cl-$\mathbf{1}^{\mathrm{Me}}$ and (*S*)-*p*-Cl-$\mathbf{1}^{\mathrm{Me}}$. *Ortho*-substituted benzyl alcohols, *o*-Cl-$\mathbf{1}^{\mathrm{H}}$ and *o*-Me-$\mathbf{1}^{\mathrm{H}}$, do not have the abnormality, as seen in Table 3. As mentioned above, since benzyl alcohol and its *ortho*-substituted derivatives are replaced by two hydrogens at the α-position and these hydrogens are stored respectively in the small and medium-sized pockets, steric strain is not important for trapping and transforming these substrates even though the substrate is twisted for the incorporation (Scheme 3). Molecular orbital



Scheme 3.

Table 4. Enantiomeric Exccesses and Optical Rotations of the Alcohols

Substrate	Enantiomeric exccesses/%
	Optical rotations
(*S*)-$\mathbf{1}^{\mathrm{Et}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 100 °C)
	$[\alpha]^{25}_{\mathrm{D}}-59^\circ$ (c 0.130, $CHCl_3$) (lit,[54] $[\alpha]_{\mathrm{D}}-46.7^\circ$ (c 0.0409, $CHCl_3$), 95% ee (*S*))
(*S*)-$\mathbf{1}^{\mathrm{Pr}}$	31.8 (Chiraldex G-TA, 105 °C)
	$[\alpha]^{16}_{\mathrm{D}}-13.5^\circ$ (c 1.65, C_6H_6) (lit,[56] $[\alpha]_{\mathrm{D}}+40.0^\circ$ (c 4.39, C_6H_6), 92% ee (*R*))
(*S*)-$\mathbf{1}^{\mathrm{Bu}}$	25.2 (Chiraldex G-TA, 110 °C)
	$[\alpha]^{24}_{\mathrm{D}}-8.62^\circ$ (c 1.08, C_6H_6) (lit,[56] $[\alpha]_{\mathrm{D}}+30.1^\circ$ (c 3.01, C_6H_6), 96% ee (*R*))
(*R*)-*o*-Cl-$\mathbf{1}^{\mathrm{Me}}$	99.4 (CP-Cyclodextrin-B-236-M-19, 142 °C)
	$[\alpha]^{16}_{\mathrm{D}}65.7^\circ$ (c 0.625, $CHCl_3$) (lit,[52] $[\alpha]^{20}_{\mathrm{D}}+41^\circ$ (c 1.00, $CHCl_3$), 67% ee (*R*))
(*S*)-*o*-Cl-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 142 °C)
	$[\alpha]^{16}_{\mathrm{D}}-64.5^\circ$ (c 0.545, $CHCl_3$) (lit,[52] $[\alpha]^{20}_{\mathrm{D}}+41^\circ$ (c 1.00, $CHCl_3$), 67% ee (*R*))
(*R*)-*m*-Cl-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 142 °C)
	$[\alpha]^{21}_{\mathrm{D}}+44^\circ$ (c 0.220, $CHCl_3$) (lit,[53] $[\alpha]^{20}_{\mathrm{D}}+36.7^\circ$ (c 1.00, $CHCl_3$), 84.6% ee (*R*))
(*S*)-*m*-Cl-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 142 °C)
	$[\alpha]^{29}_{\mathrm{D}}-41^\circ$ (c 0.36, $CHCl_3$) (lit,[53] $[\alpha]^{20}_{\mathrm{D}}+36.7^\circ$ (c 1.00, $CHCl_3$), 84.6% ee (*R*))
(*R*)-*p*-Cl-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 120 °C)
	$[\alpha]^{16}_{\mathrm{D}}+51.3^\circ$ (c 0.595, Et_2O) (lit,[54] $[\alpha]_{\mathrm{D}}-48.9^\circ$ (c 0.0613, Et_2O), 94% ee (*S*))
(*S*)-*p*-Cl-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 120 °C)
	$[\alpha]^{16}_{\mathrm{D}}-52.4^\circ$ (c 0.555, Et_2O) (lit,[54] $[\alpha]_{\mathrm{D}}-48.9^\circ$ (c 0.0613, Et_2O), 94% ee (*S*))
(*R*)-*o*-Me-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 130 °C)
	$[\alpha]^{16}_{\mathrm{D}}+70^\circ$ (c 0.340, EtOH) (lit,[54] $[\alpha]^{20}_{\mathrm{D}}-14^\circ$ (c 0.400, EtOH), 25% ee (*S*))
(*S*)-*o*-Me-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (CP-Cyclodextrin-B-236-M-19, 130 °C)
	$[\alpha]^{18}_{\mathrm{D}}-75.2^\circ$ (c 0.270, EtOH) (lit,[54] $[\alpha]^{20}_{\mathrm{D}}-14^\circ$ (c 0.400, EtOH), 25% ee (*S*))
(*R*)-*m*-Me-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (Chiraldex G-TA, 115 °C)
	$[\alpha]^{21}_{\mathrm{D}}+40.4^\circ$ (c 0.530, EtOH) (lit,[55] $[\alpha]^{22}_{\mathrm{D}}+23.4^\circ$ (c 1.00, EtOH), 55% ee (*R*))
(*S*)-*m*-Me-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (Chiraldex G-TA, 115 °C)
	$[\alpha]^{25}_{\mathrm{D}}-41.9^\circ$ (c 0.500, EtOH) (lit,[55] $[\alpha]^{22}_{\mathrm{D}}+23.4^\circ$ (c 1.00, EtOH), 55% ee (*R*))
(*R*)-*p*-Me-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (Chiraldex G-TA, 95 °C)
	$[\alpha]^{22}_{\mathrm{D}}57.7^\circ$ (c 0.36, $CHCl_3$) (lit,[54] $[\alpha]_{\mathrm{D}}-54.1^\circ$ (c 0.0532, $CHCl_3$), 96% ee (*S*))
(*S*)-*p*-Me-$\mathbf{1}^{\mathrm{Me}}$	>99.9 (Chiraldex G-TA, 95 °C)
	$[\alpha]^{22}_{\mathrm{D}}-57.3^\circ$ (c 0.190, $CHCl_3$) (lit,[54] $[\alpha]_{\mathrm{D}}-54.1^\circ$ (c 0.0532, $CHCl_3$), 96% ee (*S*))
(*S*)-$\mathbf{2}^{1}$	>99.9 (Chiraldex G-TA, 93 °C)
	$[\alpha]^{15}_{\mathrm{D}}+39.7^\circ$ (c 0.515, $CHCl_3$) (lit,[52] $[\alpha]^{20}_{\mathrm{D}}-8^\circ$ (c 1.00, $CHCl_3$), 24% ee (*R*))
(*S*)-$\mathbf{2}^{2}$	>99.9 (Chiraldex G-TA, 102 °C)
	$[\alpha]^{22}_{\mathrm{D}}+22^\circ$ (c 0.380, C_6H_6) (lit,[57] $[\alpha]_{\mathrm{D}}+3.08^\circ$ (c 4.70, C_6H_6), (*S*))
(*R*)-**3**	98.7 (Chiraldex G-TA, 100 °C)
	$[\alpha]^{26}_{\mathrm{D}}-8.1^\circ$ (c 0.480, Et_2O) (lit,[58] $[\alpha]^{20}_{\mathrm{D}}-7.79^\circ$ (c 3.10, Et_2O), 96% ee (*R*))
(*S*)-**3**	>99.9 (Chiraldex G-TA, 100 °C)
	$[\alpha]^{26}_{\mathrm{D}}-7.7^\circ$ (c 0.220, Et_2O) (lit,[58] $[\alpha]^{20}_{\mathrm{D}}-7.79^\circ$ (c 3.10, Et_2O), 96% ee (*R*))
(*S*)-$\mathbf{4}^{\mathrm{Et}}$	79.5 (Chiraldex G-TA, 100 °C)
	$[\alpha]^{22}_{\mathrm{D}}+7.11^\circ$ (c 0.980, $CHCl_3$) (lit,[54] $[\alpha]^{25}_{\mathrm{D}}+7.8^\circ$ (c 2.90, $CHCl_3$), 81% ee (*S*))
(*S*)-**5**	>99.9 (Chiraldex G-TA, 110 °C)
	$[\alpha]^{24}_{\mathrm{D}}+31.7^\circ$ (c 2.09, $CHCl_3$) (lit,[52] $[\alpha]^{20}_{\mathrm{D}}-26^\circ$ (c 1.00, $CHCl_3$), 96% ee (*R*)).

calculations on $\mathbf{1}^{\mathrm{Me}}$, *o*-Cl-$\mathbf{1}^{\mathrm{Me}}$, and *o*-Me-$\mathbf{1}^{\mathrm{Me}}$ by the aid of the MNDO93 2.3/U-2.3 program predict that the hydroxyl and phenyl moieties in $\mathbf{1}^{\mathrm{Me}}$ occupy the same plane, but those in *ortho*-substituted substrates are twisted by about 60 °. Thus, it is noteworthy that not only the trapping mode but also the conformation of the molecule is affected by the *ortho*-substituent. The fact that (*R*)-**5** is most reactive among all the substrates so far studied suggests that as small an *ortho*-substituent as a methylene group does not interfere with the reaction, but a methyl group is already large enough to perturb the reactivity.

On the other hand, when one of the hydrogens at the α-position is replaced by a methyl group, twisting the aromatic ring in the large pocket results in a large steric perturbation in the medium-sized (for the (*R*)-enantiomer) or small (for the (*S*)-enantiomer) pocket. Since the methyl groups of (*R*)- and (*S*)-enantiomers are trapped in different pockets, the amplitudes of perturbation are different for different enantiomers.

There is no unambiguous explanation for the retardation of reaction rates by *ortho*-chloro substituents. One possibility is that a polar substituent such as a chlorine atom may disturb forces, e.g., hydrogen bonding, that exist in the large and hydrophobic pocket. The disturbance may induce a conformational change of the enzyme so that the electronic effect of the substituent can affect the overall reaction rate. Such a disturbance is induced by the (*S*)-enantiomers only, because the conformation of the enzyme is already distorted by incorporating a methyl group into the small pocket when the (*S*)-enantiomer is the substrate. It is not surprising for an enzymatic reaction that conformational disorder results in the change of the rate-determining step. Thus, a chemical reaction process may constitute a part of the rate-determining step by conformational distortion.

Experimental

Instrumental. Gas chromatograms were recorded on a Shimadzu GC-14B with OV 1701 Bonded capillary column, 30 m×0.25 mm, a Shimadzu GC-14B with CP-Cyclodextrin-B-236-M-19 capillary column, 25 m×0.25 mm, a Shimadzu GC-14B with OV 101 Bonded capillary column, 17 m×0.25 mm, and a Shimadzu GC-9A with Chiraldex G-TA capillary column, 30 m×0.25 mm gas chromatographs. Optical rotation was measured with a Perkin–Elmer 241 polarimeter. ^{1}H NMR were recorded on a Varian VXR-200 and a JEOL EX-400 spectrometers. IR spectra were obtained from a JASCO FT/IR-5300 and a Shimadzu FT/IR-8100 spectrometers. Elemental analyses were done with a Yanako MT-3 Elemental Analyzer.

Materials. Lipases were supplied by Novo Nordik Co., Ltd (SP 435) and Amano Pharmaceutical Co., Ltd (Amano PS). Vinyl acetate, (*R*)- and (*S*)- as well as racemic 1-phenylethanols ($\mathbf{1}^{\mathrm{Me}}$), racemic 1-phenyl-2-propanol ($\mathbf{1}^{\mathrm{Et}}$), (*R*)-, (*S*)-, and racemic 2-octanols ($\mathbf{4}^{\mathrm{Me}}$), racemic 3-nonanol ($\mathbf{4}^{\mathrm{Et}}$), racemic 1-indanol (**5**), ketones as the precursors of the other alcohols and cyclohexane as the solvent were obtained from certain commercial sources and used without further purification. However, since *p*-methylacetophenone, from which racemic 1-(*p*-tolyl)ethanol was prepared, was contaminated by small amounts of its *o*- and *m*-isomers, it was purified according to the procedure in the literature.[51] Racemic alcohols that were not obtained from commercial sources were obtained by $NaBH_4$ reduction of the corresponding ketones and used as the authentic samples for gas chromatography.

All the alcohols and esters gave satisfactory results in ^{1}H NMR and IR spectroscopies and elemental analyses.

Preparation of Optically Active Alcohols. As a typical run, 1 g of lipase from Novo Nordik was added to 100 ml of a hexane solution containing 32 mmol of racemic 1-(*p*-tolyl)ethanol (*p*-Me-$\mathbf{1}^{\mathrm{Me}}$) and 41 mmol of vinyl acetate. The mixture was stirred at 35 °C for 11 h. The reaction was quenched by filtration and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column using hexane–ethyl acetate (3 : 1) as an eluent. The combined fractions containing the alcohol were distilled to give (*S*)-*p*-Me-$\mathbf{1}^{\mathrm{Me}}$ in 25% yield. The combined fractions containing the ester were concentrated under reduced pressure to give the acetic ester of (*R*)-*p*-Me-$\mathbf{1}^{\mathrm{Me}}$.

A solution of sodium hydroxide (10%; 13 ml) was added to 30 ml of a methanol solution containing about 17 mmol of the crude ester obtained above. The mixture was stirred at room temperature for 12 h, then 1 M hydrochloric acid was added to the mixture. The mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was separated and the solvent was evaporated under reduced pressure.

Thus the obtained alcohol was again used for the lipase-catalyzed esterification: 1 g of the lipase was added to 100 ml of a hexane solution containing about 13 mmol of (*R*)-*p*-Me-$\mathbf{1}^{\mathrm{Me}}$ and 15 mmol of vinyl acetate. The mixture was stirred at 35 °C for 2 h. Thus obtained optically pure *p*-Me-$\mathbf{1}^{\mathrm{Me}}$ was converted into the corresponding acetic ester and its enantiomeric excess was measured gas chromatographically.

Enantiomeric excesses (ee) and optical rotations of the alcohols are summarized in Table 4. The column used for measuring ee (%) and analysis temperature (°C) are indicated in parentheses. Absolute configurations of the alcohols were identified by comparison of the optical rotations with those of the corresponding authentic samples reported.

Transesterification. Lipase PS from Amano Pharmaceutical (10 mg) was placed in a vial and 2 ml of a cyclohexane solution containing 400 μmol racemic alcohol and 1 mmol vinyl acetate. Then, the resulting suspension was stirred magnetically at 395 rpm at 35 °C. After 1 h, an aliquot was withdrawn and analyzed on gas chromatography to determine the ee's of unreacted alcohol and the produced ester.

E-values were calculated according to the literature method.[38] When ee of the unreacted alcohol could not be determined with gas chromatography, the alcohol was separated from the ester with a short silica-gel column using hexane–ethyl acetate (3 : 1 in most cases) as an eluent, and was converted into the corresponding acetic ester with acetyl chloride. Conversely, when the ee of the product ester could not be identified with gas chromatography, the ester was separated from the alcohol with the similar procedure mentioned above, and hydrolyzed to the corresponding alcohol. Absolute configurations of the unreacted alcohols were identified by comparison of retention times with those of the corresponding authentic samples.

Kinetics for Transesterification. A lipase was placed in a vial, and 2 ml of a cyclohexane solution containing 1 mmol vinyl acetate, an alcohol, and a higher hydrocarbon (dodecane, tridecane, tetradecane, pentadecane) as an internal standard for gas chromatography were added to the vial. Then, the resulting suspension was stirred magnetically at 395 rpm at 35 °C. Periodically, samples were withdrawn from the vial and analyzed gas chromatographically. Initial rate was measured by following the increase in the concentration of the product. Kinetics were followed up to about 15% conversion of the substrate and 5 to 7 data points were usually collected in a run. Initial concentration of alcohol was changed from 25 to 700 mM. The Hanes–Hoolf ($[S]_0/v_0$ vs. $[S]_0$) plot[45] was used for calculating K_m and V_{max}.

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References

1) A. Zaks and A. M. Klibanov, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 3192 (1985).

2) C. S. Chen and C. J. Sih, *Angew. Chem., Int. Ed. Engl.*, **28**, 695 (1989).

3) J. S. Dordick, *Enzyme Microb. Technol.*, **11**, 194 (1989).

4) A. M. Klibanov, *Acc. Chem. Res.*, **23**, 114 (1990).

5) W. Boland, C. Frössl, and M. Lorenz, *Synthesis*, **1991**, 1049.

6) K. Faber and S. Riva, *Synthesis*, **1992**, 895.

7) K. Takahashi, T. Yoshimoto, A. Ajima, Y. Tamaura, and Y. Inada, *Enzyme*, **32**, 235 (1984).

8) T. Nishio, K. Takahashi, T. Suzuki, T. Yoshimoto, Y. Kodera, A. Matsushima, Y. Saito, and Y. Inada, *J. Biotechnol.*, **8**, 39 (1988).

9) S. Parida and J. S. Dordick, *J. Am. Chem. Soc.*, **113**, 2253 (1991).

10) S. Parida and J. S. Dordick, *J. Org. Chem.*, **58**, 3238 (1993).

11) Y. Kodera, H. Nishimura, A. Matsushima, M. Hiroto, and Y. Inada, *J. Am. Oil Chem. Soc.*, **71**, 335 (1994).

12) I. L. Gatfield, *Lebsom. u. -Technol.*, **19**, 87 (1986); *Chem. Abstr.*, **105**, 277 (1986).

13) T. Nishio and M. Kamimura, *Agric. Biol. Chem.*, **52**, 2933 (1988).

14) E. H. Hölmberg, P. Szmulik, T. Norin, and K. Hult, *Biocatalysis*, **2**, 217 (1989).

15) M. J. Hills, I. Kiewitt, and K. D. Mukherjee, *Biochim. Biophys. Acta*, **1042**, 237 (1990).

16) G. B. Oguntimein, H. Erdmann, and R. D. Schmid, *Biotechnol. Lett.*, **15**, 175 (1993).

17) R. P. Hof and R. M. Kellogg, *Tetrahedron*: *Asymmetry*, **5**, 565 (1994).

18) P. Berglund, M. Holmquist, E. Hedenström, K. Hult, and H. E. Högberg, *Tetrahedron*: *Asymmetry*, **4**, 1869 (1993).

19) P. Y. Chen, S. H. Wu, and K. T. Wang, *Biotechnol. Lett.*, **15**, 181 (1993).

20) T. Miyazawa, S. Kurita, S. Ueji, T. Yamada, and S. Kuwata, *Biotechnol. Lett.*, **14**, 941 (1992).

21) H. E. Högberg and K. Hult, *Biocatalysis*, **5**, 289 (1992).

22) K. H. Engel, *Tetrahedron*: *Asymmetry*, **2**, 165 (1991).

23) S. H. Pan, T. Kawamoto, T. Fukui, K. Sonomoto, and A. Tanaka, *Appl. Microbiol. Biotechnol.*, **34**, 47 (1990).

24) P. E. Sconnet and M. W. Baillargeon, *J. Chem. Ecol.*, **13**, 1279 (1987).

25) H. Stamatis, A. Xenakis, and F. N. Kolisis, *Biotechnol. Lett.*, **15**, 471 (1993).

26) T. M. Stokes and A. C. Oehlschlager, *Tetrahedron Lett.*, **28**, 2091 (1987).

27) P. E. Sconnet, *J. Org. Chem.*, **52**, 3477 (1987).

28) S. Koshiro, K. Sonomoto, A. Tanaka, and S. Fukui, *J. Biotechnol.*, **2**, 47 (1985).

29) K. Nakamura, M. Kawasaki, and A. Ohno, *Bull. Chem. Soc. Jpn.*, **67**, 3053 (1994).

30) A. J. M. Janssen, A. J. H. Klunder, and B. Zwanenberg, *Tetrahedron*, **47**, 7645 (1991).

31) B. Morgan, A. C. Oehlschlager, and T. M. Stokes, *Tetrahedron*, **47**, 1611 (1991).

32) A. L. Gutman, D. Brenner, and A. Boltanski, *Tetrahedron*: *Asymmetry*, **4**, 839 (1993).

33) B. Morgan, A. C. Oehlschlager, and T. M. Stokes, *J. Org. Chem.*, **57**, 3231 (1992).

34) D. Bianchi, P. Cesti, and E. Battistel, *J. Org. Chem.*, **53**, 5531 (1988).

35) K. Laumen and M. P. Schneider, *J. Chem. Soc.*, *Chem. Commun.*, **1988**, 598.

36) K. Laumen, D. Breitgoff, and M. P. Schneider, *J. Chem. Soc.*, *Chem. Commun.*, **1988**, 1459.

37) K. Hult and T. Norin, *Pure Appl. Chem.*, **64**, 1129 (1992).

38) C. S. Chen, Y. Fujimoto, G. Girdaukas, and C. J. Sih, *J. Am. Chem. Soc.*, **104**, 7294 (1982).

39) M. Cygler, P. Grochuski, R. J. Kazlauskas, J. D. Schrag, F. Bouthillier, B. Rubin, A. N. Serreqi, and A. K. Gupta, *J. Am. Chem. Soc.*, **116**, 3180 (1994).

40) S. Longhi, F. Fusetti, R. Grundori, M. Lotti, M. Vanoni, and L. Alberghina, *Biochim. Biophys. Acta*, **1131**, 227 (1992).

41) L. Brady, A. M. Brzozowski, Z. S. Derewenda, E. Dodson, G. Dodoson, S. Tolly, J. P. Turkenburg, L. Christiansen, B. H. Jensen, L. Norskov, L. Thimand, and U. Menge, *Nature*, **343**, 767 (1990).

42) G. Nicolosi, M. Piattelli, and C. Sanfilippo, *Tetrahedron*, **48**, 2477 (1992).

43) K. Naemura, R. Fukuda, M. Konishi, K. Hirose, and Y. Tobe, *J. Chem. Soc.*, *Perkin Trans. 1*, **1994**, 1253.

44) T. Miyazawa, S. Kurita, S. Ueji, T. Yamada, and S. Kuwata, *J. Chem. Soc.*, *Perkin Trans. 1*, **1992**, 2253.

45) I. H. Segel, "Enzyme Kinetics," John Wiley & Sons, New York (1975), p. 211.

46) S. Mitsuda and S. Nabeshima, *Recl. Trav. Chim. Pays-Bas*, **110**, 151 (1991).

47) R. J. Kazlauskas, A. N. E. Weissfloch, A. T. Rappaport, and L. A. Cuccia, *J. Org. Chem.*, **56**, 2656 (1991).

48) M. J. Kim and H. Cho, *J. Chem. Soc.*, *Chem. Commun.*, **1992**, 1411.

49) R. W. Taft, Jr., *J. Am. Chem. Soc.*, **74**, 3120 (1952).

50) M. S. Newman, "Steric Effects in Organic Chemistry," John Wiley & Sons, New York (1956), p. 601.

51) D. D. Perrin and W. L. F. Armarego, "Purification of Laboratory Chemicals," 3rd ed, Pergamon Press, Oxford (1988), p. 220.

52) E. F. J. de Vries, J. Brussee, C. G. Kruse, and A. van der Gen, *Tetrahedron*: *Asymmetry*, **5**, 377 (1994).

53) T. Hayashi and Y. Matsumoto, *Tetrahedron*: *Asymmetry*, **2**, 601 (1991).

54) M. B. Carter, B. S. Chiøtt, A. Gutiérrez, and S. L. Buchwald, *J. Am. Chem. Soc.*, **116**, 11667 (1994).

55) S. T. Pickard and H. E. Smith, *J. Am. Chem. Soc.*, **112**, 5741 (1990).

56) S. Niwa and K. Soai, *J. Chem. Soc.*, *Perkin Trans. 1*, **1991**, 2717.

57) R. Fornasier, F. Reniero, P. Scrimin, and U. Tonellato, *J. Org. Chem.*, **50**, 3209 (1985).

58) Y. Umezawa and T. Hayashi, *J. Am. Chem. Soc.*, **113**, 9887 (1991).