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ARTICLE

Candida antarctica lipase B-catalyzed regioselective deacylation of dihydroxybenzenes acylated at both phenolic hydroxy groups

Toshifumi Miyazawa, Manabu Hamada, and Ryohei Morimoto

Abstract: *Candida antarctica* lipase B proved to be highly active in the deacylation of substituted hydroquinones and resorcinols acylated at both phenolic hydroxy groups. The deacylation reactions were much faster than the corresponding direct acylations of these dihydroxybenzenes catalyzed by the same lipase. More importantly, they took place generally in a markedly regioselective manner: the acyloxy group remote from the substituent was preferentially cleaved. The main or exclusive products obtained were the regioisomers of those produced through the direct acylation of the dihydroxybenzenes. In the case of alkyl-substituted hydroquinone derivatives, the regioselectivity increased with an increase in the bulk of the substituent. In the case of 4-substituted diacylated resorcinols, the 3-*O*-monoacyl derivatives were obtained generally as the sole products. Quite interestingly, some secondary alcohols proved to act as better acyl acceptors than the corresponding primary alcohols in these enzymatic deacylations.

Key words: Candida antarctica lipase B, hydroquinones, resorcinols, regioselective deacylation, regioselectivity, secondary alcohols.

Résumé : Il a été démontré que la lipase B de *Candida antarctica* possède une activité très élevée pour effectuer la désacylation d'hydroquinones substituées et de résorcinols acylés aux deux positions phénoliques. Les réactions de désacylation ont été beaucoup plus rapides que les acylations directes correspondantes de ces dihydroxybenzènes catalysées par la même lipase. Fait plus important, elles avaient lieu généralement de manière généralement régiosélective : le groupement acyloxy opposé au substituant était clivé de manière préférentielle. Les produits principaux ou exclusifs obtenus ont été les régioisomères produits par l'acylation directe des dihydroxybenzènes. Dans le cas de dérivés alkylés de l'hydroquinone, la régiosélectivité allait croissant en fonction de l'augmentation de la taille du substituant. Dans le cas des résorcinols diacylés substitués en position 4, les dérivés 3-*O*-monoacylés ont généralement été les seuls produits obtenus. Il est intéressant de noter que dans ces désacylations enzymatiques, certains alcools secondaires se révélés de meilleurs accepteurs d'acyles que les alcools primaires correspondants. [Traduit par la Rédaction]

Mots-clés : lipase B de *Candida antarctica*, hydroquinones, résorcinols, désacylation régioselective, régioselectivité, alcools secondaires.

Introduction

The use of enzymes for organic synthesis has become an area attracting more and more attention for organic and bioorganic chemists. Unlike most conventional chemical catalysts, enzymes are environmentally benign, since they are completely degradable. Among them, hydrolases and especially lipases (triacylglycerol hydrolases, EC 3.1.1.3) have been recognized as the most attractive biocatalysts due to their high stability and activity toward a broad range of substrates.[1] Besides, they act under mild conditions and without added cofactors. Moreover, since they are available from a variety of sources, especially bacteria and fungi, there must be a fair chance of finding an enzyme suitable for a transformation of interest in terms of catalytic activity and (or) selectivity. They have been employed mainly for the preparation of homochiral compounds related to pharmaceuticals and agrochemicals through kinetic resolution of racemic compounds or desymmetrization of prochiral precursors by hydrolysis, esterification, or transesterification. Besides the stereoselective capabilities of lipases, their regioselective properties have also been exploited for the preparation of compounds that are not easily obtainable by chemical methodologies. For example, the lipase-catalyzed acylation or deacylation procedure has been applied to the synthesis of selectively protected derivatives of polyhydroxy compounds such as carbohydrates.[2] These enzymatic acyl-transfer approaches are more straightforward than the conventional chemical ones because they can recognize a particular hydroxy group in the presence of several others within the same molecule under optimized reaction conditions. Compared to such studies on alcoholic hydroxy groups, there have been much less studies on phenolic hydroxy groups. The ability of lipases to discriminate between hydroxy groups of this type should deserve further attention because of its importance in organic synthesis. Polyphenolic compounds occur widely in nature and many of their analogs exhibit a variety of biological activities.[3] Their activities can be modulated by regioselective modification of the hydroxy groups. It can also alter their properties such as stability, solubility, and bioavailability. Parmar and co-workers have reported on the lipase-catalyzed deacylation of peracetylated polyhydroxy acetophenones and related aromatic ketones[4] to obtain selectively acylated aromatic ketones as starting material for the synthesis of biologically active polyphenolic compounds. When these peracetylated polyhydroxy aromatic ketones were subjected to transesterification with 1-butanol as an acyl acceptor in the presence of

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porcine pancreatic lipase or *Candida cylindracea* (*rugosa*) lipase in organic solvents, it was found that deacylation took place predominantly at positions other than *ortho* to the ketonic group, generally the acetoxy group at the *para* position being preferentially cleaved over the one at the *meta* position. The authors concluded that the carbonyl group attached to the benzene ring plays an important role in the recognition of acetoxy groups in the polyphenolic peracetates, and they postulated the formation of a transient Schiff's base-type complex with the lysine residue in the active site of porcine pancreatic lipase.[4d] They have also reported on the lipase-catalyzed chemo- and regioselective deacylation of peracetylated aromatic acid esters and ketones[4d] and peracetylated enolic forms of polyphenolic benzyl phenyl ketones.[5] In connection with these studies, Nicolosi and co-workers have reported on the regioselectivity observed in the *Pseudomonas cepacia* lipase catalyzed deacylation with 1-butanol of peracetylated flavonoids that contain a carbonyl group attached to the benzene ring.[6] It is worth while to examine how substituents other than the carbonyl can affect the regioselectivity in the lipase-catalyzed deacylation of peracylated polyphenols. In this regard, Klibanov and co-workers have investigated the lipase-catalyzed deacylation with 1-butanol of octylhydroquinone butanoylated at both phenolic hydroxy groups, and they even found the reversion of the regioselectivity of *Pseudomonas cepacia* lipase upon a change from toluene to acetonitrile as the reaction medium.[7]

These studies have prompted us to investigate the lipase-catalyzed deacylation of diacylated dihydroxybenzenes, i.e., hydroquinones and resorcinols, carrying several substituents other than the carbonyl. We found that in these deacylations, *Candida antarctica* lipase B (CAL-B)[8] was more active than other lipases so far employed and moreover highly regioselective and that some secondary alcohols acted as better acyl acceptors than the corresponding primary alcohols.[9] The present paper reports the results of our investigation in detail.

Materials and methods

All of the hydroquinones and resorcinols used in this study, except ethyl-, isopropyl-, and fluorohydroquinoes[10] and 4-*t*-butylresorcinol, were purchased from Tokyo Chemical Industry Co. or Aldrich. All of the alcohols were commercially available and employed after drying over molecular sieves prior to use. CAL-B was supplied by Boehringer Mannheim (BioCatalytics) as an immobilized form (chirazyme L-2), which had a specific activity of 3.2 U mg^{-1} lyophilized powder with tributyrin at 25 °C. All organic solvents were distilled following standard protocols and dried over molecular sieves prior to use.

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Varian Unity 500 spectrometer using DMSO-d_6 as a solvent with TMS as an internal standard. The liquid chromatograph employed was a Shimadzu LC-10AD instrument, equipped with a Rheodyne 7125 sample injector and a Shimadzu SPD-10A variable-wavelength UV monitor. A Shimadzu C-R8A data processor was used for data acquisition and processing. TLC was run on Merck precoated silica gel plates and Wakogel C-300 was used for column chromatography.

The NMR data for all of the monopropanoates of substituted hydroquinones and resorcinols used in this study except the 3-*O*-propanoate (**6c**) of 4-*t*-butylresorcinol are available elsewhere.[11]

Preparation of 4-*t*-butylresorcinol (8c)

This substituted resorcinol was prepared through the reaction of resorcinol with *t*-butanol in the presence of concentrated sulfuric acid in glacial acetic acid according to the literature method.[12] Oil. ^{1}H NMR δ: 1.28 (9H, s, $C(CH_3)_3$), 6.10 (1H, dd, J = 8.5 and 2.5 Hz, H-6), 6.24 (1H, d, J = 2.5 Hz, H-2), 6.86 (1H, d, J = 8.5 Hz, H-5), 8.90 (1H, s, OH), 9.07 (1H, s, OH).

Preparation of the di-*O*-propanoyl derivatives of substituted hydroquinones and resorcinols

The 1,4-di-*O*-propanoates (**1**) of substituted hydroquinones and 1,3-di-*O*-propanates (**5**) of 4-substituted resorcinols were prepared by the reaction of each hydroquinone or resorcinol with propanoyl chloride in pyridine. The synthetic procedure and the NMR data for all of the dipropanoyl compounds except **5c** were reported before.[11]

1,3-Di-O-propanoyl-4-t-butylresorcinol (5c)

Oil. ^{1}H NMR δ: 1.12 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.16 (3H, t, J = 7.5 Hz, CH_2CH_3), 1.29 (9H, s, $C(CH_3)_3$), 2.58 (2H, q, J = 7.5 Hz, CH_2), 2.64 (2H, q, J = 7.5 Hz, CH_2), 6.90 (1H, d, J = 2.5 Hz, H-2), 6.96 (1H, dd, J = 8.5 and 2.5 Hz, H-6), 7.39 (1H, d, J = 8.5 Hz, H-5). Anal. calcd. for $C_{16}H_{22}O_4$: C 69.04, H, 7.97; found: C 68.85, H 8.05.

General procedure for the CAL-B-catalyzed deacylation of the di-*O*-propanoyl derivatives of substituted hydroquinones and resorcinols in an analytical scale

A solution of a substituted 1,4-di-*O*-propanoylhydroquinone (**1**) or a 4-substituted 1,3-di-*O*-propanoylresorcinol (**5**) (0.1 mmol) and 2-propanol (24 μL, 0.3 mmol) in anhydrous diisopropyl ether (240 μL) was stirred with CAL-B (40 mg) at 45 °C in a thermostated incubator. After a certain period of time, the reaction mixture was filtered through a glass filter and evaporated to dryness under reduced pressure. The residue was subjected to NMR analysis to determine the conversion and product distribution, as the ^{1}H NMR spectra of the starting dipropanoate, both isomeric monopropanoates, and the parent diol are available with each hydroquinone or resorcinol. In the reactions of substituted 1,4-di-*O*-propanoylhydroquinones (**1a–1h**), the proton signals used for the purpose were different from compound to compound. The $ArCH_3$ proton signals together with the $COCH_2CH_3$ signals were utilized with **1a**. The proton signals appearing as a singlet were employed in the following cases: **1d**, $C(CH_3)_3$; **1e**, OCH_3. The proton signals (H-5, H-3, or H-6) in the aromatic region were used with **1b**, **1c**, and **1f-h**. In the reactions of 4-substituted 1,3-di-*O*-propanoylresorcinols (**5a–5g**), the proton signals (H-5, H-2, or H-6) in the aromatic region were mainly employed for the purpose. The whole content of the reaction mixture was used up for one analysis, and several discrete reaction mixtures were used at different reaction times.

HPLC monitoring of the deacylation of 1,4-di-*O*-propanoylmethoxyhydroquinone (1e)

A solution of **1e** (45 mg, 0.18 mmol) and an alcohol (0.54 mmol) in diisopropyl ether (450 μL) was stirred with CAL-B (75 mg) at 45 °C in a 1-mL vial. Aliquots (approximately 10 μL) of the reaction mixture were withdrawn at frequent intervals, diluted with diethyl ether, and filtered through a PTFE membrane filter. After evaporation of ether, the residue was dissolved in acetonitrile (1 mL) and subjected to HPLC analysis under the following conditions: column, Ascentis RP-Amide (4.6 mm i.d. × 250 mm); mobile phase, 34% aqueous acetonitrile containing H_3PO_4 (0.01 M); flow rate, 1.0 mL min^{-1}; column temperature, 30 °C; detection, UV at 280 nm. The details of the analytical procedure are available elsewhere.[11]

Preparation of mono-*O*-propanoyl derivatives through CAL-B-catalyzed gram-scale deacylations

3-O-propanoyl-4-t-butylresorcinol (6c)

1,3-Di-*O*-propanoyl-4-*t*-butylresorcinol (**5c**) (1.11 g, 4.0 mmol) was dissolved in anhydrous diisopropyl ether (10 mL) followed by the addition of 2-propanol (1.0 mL, 132 mmol) and then CAL-B (1.6 g). The reaction mixture was stirred at 45 °C in an incubator. The reaction was stopped after 90 min by filtrating the enzyme powder, which was washed with diethyl ether. Evaporation of the solvent in vacuo from the combined filtrate and the washing af-

forded a pale yellow oil, which was subjected to column chromatography on silica gel using hexane – ethyl acetate (10:1, *v*/*v*) as an eluent. Thus, a small amount of the unreacted diester (**5c**) eluted first and further elution afforded the 3-*O*-acyl derivative (**6c**) (830 mg, 93%) as a colorless oil, which was characterized by the following NMR and analytical data.

3-O-propanoyl-4-t-butylresorcinol (6c)[13]

Oil. ^{1}H NMR δ: 1.15 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.23 (9H, s, $C(CH_3)_3$), 2.61 (2H, q, *J* = 7.5 Hz, CH_2), 6.40 (1H, d, *J* = 2.5 Hz, H-2), 6.58 (1H, dd, *J* = 8.5 and 2.5 Hz, H-6), 7.14 (1H, d, *J* = 8.5 Hz, H-5), 9.44 (1H, s, OH). Anal. calcd. for $C_{13}H_{18}O_3$: C 70.24, H 8.16; found: C 70.47, H 8.31.

1-O-propanoyl-t-butylhydroquinone (2d)

A solution of 1,4-di-*O*-propanoyl-*t*-butylhydroquinone (**1d**) (1.25 g, 4.5 mmol) and 2-propanol (1.1 mL, 14.4 mmol) in anhydrous diisopropyl ether (11 mL) was stirred with CAL-B (1.8 g) at 45 °C. After 90 min, the enzyme was filtered and washed with diethyl ether. Evaporation of the solvent in vacuo afforded a pale yellow oil from which the sole product (**2d**) (950 mg, 95%) was isolated by column chromatography on silica gel using hexane – ethyl acetate (10:1, *v*/*v*). It was characterized by NMR data as reported before.[11]

1-O-propanoylmethoxyhydroquinone (2e)

A solution of 1,4-di-*O*-propanoylmethoxyhydroquinone (**1e**) (1.14 g, 4.5 mmol) and 2-propanol (1.1 mL, 14.4 mmol) in anhydrous diisopropyl ether (11 mL) was stirred with CAL-B (1.8 g) at 45 °C. After 20 min, the enzyme was filtered and washed with diethyl ether. The pale yellow oil obtained through evaporation of the solvent was subjected to column chromatography on silica gel using hexane – ethyl acetate (10:1, *v*/*v*). Thus, after the unreacted diester (**1e**) (340 mg) was recovered, the 1-*O*-acyl derivative (**2e**) (410 mg, 46%) and the 4-*O*-acyl derivative (**3e**) (55 mg, 6%) were obtained as colorless oils. They were characterized by NMR data as reported before.[11]

Results and discussion

Initially, 2-substituted 1,4-di-*O*-propanoylhydroquinones (**1**) were subjected to deacylation with alcohols as acyl acceptors in the presence of CAL-B. We have recently reported on the CAL-B-mediated direct acylation of phenolic hydroxy groups of substituted hydroquinones and resorcinols, which takes place generally in a markedly regioselective manner.[11] This lipase has not so far been employed for such a purpose with success. As is shown in Scheme 1, the deacylated hydroquinones (**1**) can undergo enzymatic transesterification through two pathways to form either 1-*O*-propanoylhydroquinones (**2**) or 4-*O*-propanoylhydroquinones (**3**) and finally to afford the parent hydroquinones (**4**). We found that in these deacylation reactions, secondary alcohols such as 2-propanol acted as better acyl acceptors than the corresponding primary alcohols, which will later be described in detail. Accordingly, the CAL-B-catalyzed deacylation of **1** carrying different substituents on the benzene ring was examined using 2-propanol (3 molar equiv.) in diisopropyl ether[14] at 45 °C. As is usual with this type of reaction, the product distribution can be time-dependent. Figure 1 depicts the time-course of the deacylation of the methylhydroquinone derivative (**1a**) as a typical example. The yield of the 1-*O*-acyl derivative (**2a**) increased at first in inverse proportion to the decrease of **1a** and then reached a plateau after approximately 60 min, while that of the 4-*O*-acyl derivative (**3a**) increased slightly at first, then reached a maximum after approximately 15 min, and then turned to a decrease and that of the parent hydroquinone (**4a**) exhibited a slight but steady increase during that time. The initial rates of formation of **2a** and **3a** were 7.7×10^{-6} and 3.8×10^{-7} mol h^{-1} (mg of lipase preparation)$^{-1}$, respectively: the former was approximately 20 times faster than the latter.[15] The product distributions at three different times with the substrates bearing alkyl substituents (**1a–1d**) are compiled in Table 1. There remained (almost) no starting diacylated hydroquinones after 60 min in all cases. Thus, CAL-B was found to be active enough toward the deacylation of these compounds and its activity seemed to be much higher than those of other lipases so far employed.[16] Moreover, CAL-B showed a high regioselectivity in the deacylation of these compounds bearing no carbonyl group: the acyloxy group remote from the substituent R was preferentially deacylated to afford the 1-*O*-acyl derivatives (**2a–2d**) as major products. The steric demand of the R group seems to be greatly responsible for the regioselectivity observed. Thus, with methyl- and ethylhydroquinone derivatives (**1a** and **1b**), the isomeric 4-*O*-acyl derivatives (**3a** and **3b**) and the parent hydroquinones (**4a** and **4b**) were also produced in small amounts. On the other hand, with the substrates bearing larger substituents, regioselectivity became stricter: with the isopropylhydroquinone derivative (**1c**), a small amount of the parent hydroquinone (**4c**) was detected, while with the *t*-butylhydroquinone derivative (**1d**), the 1-*O*-acyl derivative (**2d**) was the sole product. The time-course of the deacylation of the methoxyhydroquinone derivative (**1e**) is shown in Fig. 2 and the product distribution in Table 1. The difference in the initial rates of formation of the two isomeric monoacyl derivatives was smaller than that observed with alkyl-substituted hydroquinones: 2.8×10^{-6} and 7.1×10^{-7} mol h^{-1} (mg of lipase preparation)$^{-1}$ with the 1-*O*-acyl derivative (**2e**) and the 4-*O*-acyl derivative (**3e**), respectively. The time-course suggests that in the deacylation of **1e**, the pathway 1→2 in Fig. 1 is faster than the pathway 1→3, the pathway 3→4 is as fast as the pathway 1→2, and the pathway 2→4 is rather slow. Thus, **3e** is further deacylated quickly to afford the parent hydroquinone (**4e**) and never accumulates, while **2e** re-

Scheme 1. CAL-B-catalyzed regioselective deacylation of substituted 1,4-di-*O*-propanoylhydroquinones (**1**). R: a, Me; b, Et; c, *i*-Pr; d, *t*-Bu; e, MeO; f, F; g, Cl; h, Br.

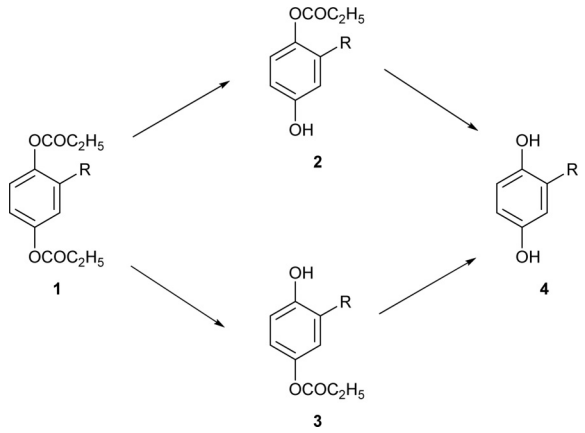


Fig. 1. Reaction profile of the CAL-B-catalyzed deacylation of 1,4-di-*O*-propanoylmethylhydroquinone (**1a**) with 2-propanol. Diamonds, **1a**; circles, **2a**; triangles, **3a**; squares, **4a**.

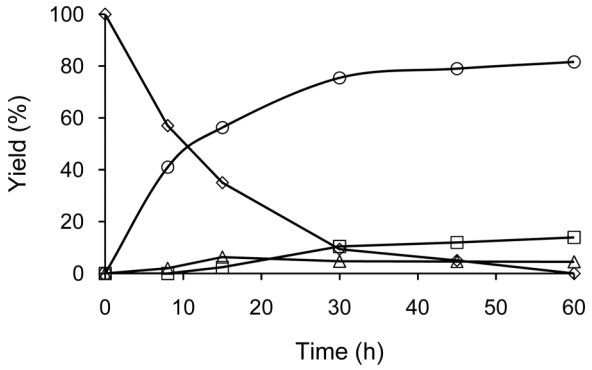
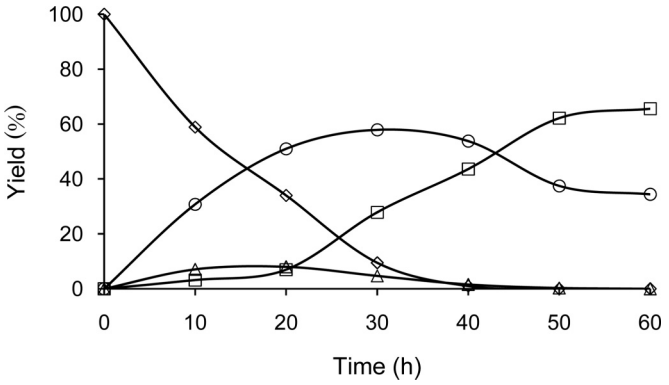


Table 1. CAL-B-catalyzed deacylation of substituted 1,4-di-*O*-propanoylhydroquinones (**1**) with 2-propanol.

		Yield (%)								
		15 min			30 min			60 min		
Substrate	R	**2**	**3**	**4**	**2**	**3**	**4**	**2**	**3**	**4**
1a	CH_3	56	6	2	76	5	10	82	4	14
1b	CH_3CH_2	64	6	4	86	4	5	88	2	10
1c	$(CH_3)_2CH$	86	0	0	91	0	5	94	0	6
1d	$(CH_3)_3C$	71	0	0	93	0	0	97	0	0
1e	CH_3O	40	8	3	58	5	28	34	0	66
1f	F	39	23	33	15	0	85	21	0	79
1g	Cl	35	8	54	22	8	69	12	0	87
1h	Br	50	18	21	22	3	71	8	0	86

Note: Reaction conditions: 0.1 mmol of **1**, 0.3 mmol of 2-propanol, and 40 mg of CAL-B in 240 μL of anhydrous diisopropyl ether at 45 °C.

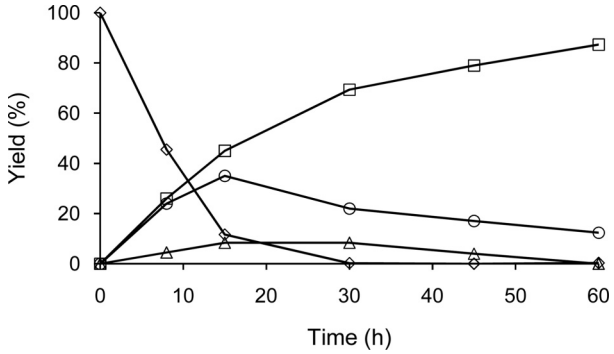
Fig. 2. Reaction profile of the CAL-B-catalyzed deacylation of 1,4-di-*O*-propanoylmethoxyhydroquinone (**1e**) with 2-propanol. Diamonds, **1e**; circles, **2e**; triangles, **3e**; squares, **4e**.



mains in a certain amount even after 60 min. The behavior of **1e** also implies the importance of steric requirement in regioselectivity, which resembles that of the ethylhydroquinone derivative (**1b**) in the initial stage of the reaction. The results with the halogen-substituted hydroquinone derivatives (**1f–1h**) are also included in Table 1 and the time-course with the chlorohydroquinone derivative (**1g**) is shown in Fig. 3. The yield of the 1-*O*-acyl derivative (**2g**) increased gradually to reach a maximum at around 15 min and then turned to a decrease, while even at this point, the parent hydroquinone (**4g**) became the major product, increasing steadily afterwards. The difference in the initial rates of formation of the two isomeric monoacyl derivatives was smaller. The effect of halogen substituents on the reactivity and regioselectivity in the deacylation of **1f–1h** seems rather complicated, indicating that besides the steric effect, other factors, such as the electronic effect, must be taken into consideration.

Next, 4-substituted 1,3-di-*O*-propanoylresorcinols (**5**) were subjected to deacylation with 2-propanol in the presence of CAL-B under the same reaction conditions as above (Scheme 2). The product distributions at three different times are compiled in Table 2. Compared to the deacylation of 2-substituted 1,4-di-*O*-propanoylhydroquinones (**1**), regioselectivity observed was high enough in almost all cases. With the resorcinol derivatives carrying the alkyl or aralkyl substituents (**5b–5e**), the 3-*O*-acyl derivatives (**6b–6e**) were obtained as the sole products of deacylation even after 60 min of incubation, at the end of which 90%–100% conversions were reached. Thus, CAL-B showed a complete regiospecificity in the deacylation of these compounds bearing no carbonyl group. The steric bulk of the substituent seems to be responsible for the observed regiospecificity, for the resorcinol derivative (**5a**) carrying the smallest methyl group exhibited a deteriorated regioselectivity. The deacylation of the resorcinol

Fig. 3. Reaction profile of the CAL-B-catalyzed deacylation of 1,4-di-*O*-propanoylchlorohydroquinone (**1g**) with 2-propanol. Diamonds, **1g**; circles, **2g**; triangles, **3g**; squares, **4g**.



Scheme 2. CAL-B-catalyzed regioselective deacylation of 4-substituted 1,3-di-*O*-propanoylresorcinols (**5**). R: a, Me; b, Et; c, *t*-Bu; d, $(CH_3)_3CCH_2C(CH_3)_2$; e, Bn; f, Cl; g, Br.

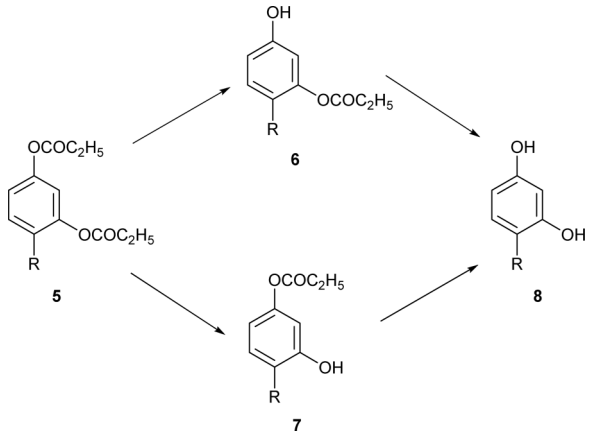


Table 2. CAL-B-catalyzed deacylation of 4-substituted 1,3-di-*O*-propanoylresorcinols (**5**) with 2-propanol.

		Yield (%)								
		15 min			30 min			60 min		
Substrate	R	**6**	**7**	**8**	**6**	**7**	**8**	**6**	**7**	**8**
5a	CH_3	72	7	7	82	4	9	80	3	17
5b	CH_3CH_2	92	0	0	96	0	0	100	0	0
5b[a]								92	2	5
5b[b]								31	3	0
5c	$(CH_3)_3C$	49	0	0	82	0	0	90	0	0
5d	$(CH_3)_3CCH_2C(CH_3)_2$	54	0	0	92	0	0	93	0	0
5e	$C_6H_5CH_2$	87	0	0	96	0	0	100	0	0
5f	Cl	88	0	12	84	0	16	76	0	24
5g	Br	92	0	8	91	0	9	88	0	12

Note: Reaction conditions: 0.1 mmol of **5**, 0.3 mmol of 2-propanol, and 40 mg of CAL-B in 240 μL of diisopropyl ether at 45 °C.

[a]In acetonitrile.

[b]In toluene.

derivatives carrying halogen substituents (**5f** and **5g**) proceeded more smoothly, reaching completion after 15 min. Although the 3-*O*-acyl derivatives (**6f** and **6g**) were the major products (approximately 90% yield after 15 min), the parent resorcinols (**8f** and **8g**) were also produced, while the isomeric 1-*O*-acyl derivatives (**7f** and **7g**) were not detected. The yield of the 3-*O*-acyl derivatives decreased gradually, while that of the parent resorcinols increased in inverse proportion. These results on the CAL-B-catalyzed deacylation of the 4-substituted resorcinol derivatives indicate

that of the two acyloxy groups, the one remote from the substituent R and hence sterically less hindered was preferentially deacylated. Although a large number of experimental data have already been accumulated on the effect of organic solvents on lipase-catalyzed reactions,[17] it is still difficult to predict the solvent effect, especially on regioselectivity. When the solvent was changed from diisopropyl ether to a more polar solvent, acetonitrile, regioselectivity was diminished to some extent, as shown in the case of **5b** in Table 2. In a more hydrophobic solvent, toluene, both reactivity and regioselectivity, especially the former, remarkably deteriorated. In any case, however, the reversal of regioselectivity was never observed unlike in the *Pseudomonas cepacia* lipase-catalyzed deacylation of the hydroquinone derivative mentioned above.[7]

As shown in analytical scale reactions mentioned above, CAL-B proved to be a very active biocatalyst for the deacylation of substituted dihydroxybenzenes acylated at both phenolic hydroxy groups, and the reaction was much faster than the direct acylation of the corresponding dihydroxybenzenes.[18] Interestingly, the main products obtained by these two different enzymatic procedures were complementary. In the case of 4-substituted diacylated resorcinols, the 3-*O*-acyl derivatives were the main products. Accordingly, a gram-scale preparation of the 3-*O*-acyl derivative was carried out via the CAL-B-catalyzed deacylation of 1,3-di-*O*-propanoyl-4-*t*-butylresorcinol (**5c**) with 2-propanol in diisopropyl ether. The aimed-at compound (**6c**), which was the exclusive product in this case, was obtained in a 93% isolated yield after 90 min of incubation. In the case of substituted hydroquinone derivatives, the situations were somewhat different and complicated. With the diacylated hydroquinones carrying alkyl substituents, the regioselectivity toward the acyloxy group remote from the substituent increased with the increase in the bulk of the substituent, affording the 1-*O*-acyl derivatives as the main products. A gram-scale preparation of the 1-*O*-acyl derivative was carried out via the deacylation of 1,4-di-*O*-propanoyl-*t*-butylhydroquinone (**1d**) with 2-propanol in diisopropyl ether. The aimed-at compound (**2d**), which was the exclusive product in this case, was obtained in a 95% isolated yield after 90 min. The regioselectivity was not very high with the methoxy- and halogen-substituted hydroquinone derivatives. Consequently, it is extremely important in these cases to determine the conversion at which the aimed-at compound, the 1-*O*-acyl derivative, is produced in the highest yield, while the formation of the isomeric 4-*O*-acyl derivative and the parent hydroquinone is kept suppressed. We tried to prepare the 1-*O*-acyl derivative through a gram-scale deacylation of 1,4-di-*O*-propanoylmethoxyhydroquinone (**1e**) with 2-propanol in diisopropyl ether, as the progress of deacylation was easily monitored by HPLC analysis. When the reaction was stopped after 20 min, the aimed-at compound (**2e**) was obtained in a 46% isolated yield and the starting di-*O*-propanoyl derivative was recovered in a nearly quantitative yield.

During the course of investigation, we found that 2-propanol acted as a better acyl acceptor than primary alcohols such as 1-propanol or 1-butanol usually used in the enzymatic alcoholysis.[4–7] As typical examples, Table 3 shows the comparison between 1-propanol and 2-propanol in the deacylation of some 4-substituted 1,3-di-*O*-propanoylresorcinols (**5b**, **5d**, and **5e**). The yields of the sole products, the 3-*O*-acyl derivatives (**6**), were examined at different times. With either substrate, the secondary alcohol proved to be superior as the acyl acceptor to the primary alcohol at all times. The difference in rate of alcoholysis with the two alcohols was more conspicuous in the initial stage of the reaction. This is probably ascribable to the substrate inhibition by the primary alcohol, which is much stronger than that by the secondary counterpart.[19] Stimulated by these unexpected results, we intended to investigate extensively the ability of alcohols as acyl acceptors in the CAL-B-catalyzed deacylation of resorcinol and hydroquinone derivatives. Table 4 summarizes the effect of various alcohols on the deacylation of **5b**, **5,** and **5e**. Irrespective of the substrates and the alcoholic acyl acceptors employed, the 3-*O*-acyl derivative (**6**) was obtained as the exclusive product. From the comparison of the yields of **6b**, it is obvious that all of the secondary alcohols examined acted as better acyl acceptors than the isomeric primary alcohols (entries 1 and 2, entries 3 and 4, entries 5–7, and entries 8–10). By contrast, the tertiary alcohols were far pooler acyl acceptors (entries 11 and 12).[20] The superiority of the secondary alcohols to the primary counterparts was further confirmed with the other two substrates **5d** and **5e**. Table 5 summarizes the results obtained with the methoxyhydroquinone derivative (**1e**). As expected, 2-propanol and 3-pentanol acted as better acyl acceptors than their respective primary counterparts (entries 1 and 2 and entries 3 and 4), as judged by the conversion after 20 min. On the other hand, the ability of 4-heptanol (entry 6) bearing two *n*-propyl groups was as low as that of 1,5-dimethyl-3-pentanol (entry 7) having two isopropyl groups and it was much lower than that of the isomeric primary alcohol. The fact derived from the results shown in Tables 4 and 5 that secondary alcohols bearing at least one methyl or ethyl group acted as better acyl acceptors may be rationalized by taking into consideration that alcohols must be accommodated in the alcohol binding site of the enzyme in order for the hydroxy group to reach the reaction center, as in the enzymatic kinetic resolution of secondary alcohols through enantioselective acylation where CAL-B is known to be an excellent enantioselective biocatalyst.[8] In contrast, this lipase has no measurable activity on reactions with tertiary alcohols or secondary alcohols bearing two large or bulky groups. Such a salient substituent size limitation has been explained by the existence in the lipase's alcohol binding site of the stereospecificity pocket,[21] which practically can accept a group shorter than the *n*-propyl group.

In conclusion, CAL-B proved to be highly active in the deacylation of substituted hydroquinones and resorcinols acylated at

Table 3. CAL-B-catalyzed deacylation of 4-substituted 1,3-di-*O*-propanoylresorcinols (**5**) with 1-propanol or 2-propanol at different times.

		Yield (%) of **6**		
Substrate	Alcohol	15 min	30 min	60 min
5b	$CH_3CH_2CH_2OH$	56	69	76
	$CH_3CH(OH)CH_3$	92	96	100
5d	$CH_3CH_2CH_2OH$	30	57	78
	$CH_3CH(OH)CH_3$	54	92	93
5e	$CH_3CH_2CH_2OH$	60	88	94
	$CH_3CH(OH)CH_3$	87	96	100

Note: Reaction conditions: 0.05 mmol of **5**, 0.15 mmol of an alcohol, and 25 mg of CAL-B in 240 μL of diisopropyl ether at 45 °C.

Table 4. CAL-B-catalyzed deacylation of 4-substituted 1,3-di-*O*-propanoylresorcinols (**5**) with various alcohols.

		Yield (%) of **6**			
Entry	Alcohol	**5b**, 30 min	**5b**, 60 min	**5d**, 60 min	**5e**, 60 min
1	$CH_3CH_2CH_2OH$	69	76	78	94
2	$CH_3CH(OH)CH_3$	96	100	93	100
3	$CH_3CH_2CH_2CH_2OH$		64	83	91
4	$CH_3CH(OH)CH_2CH_3$		100	91	100
5	$CH_3CH_2CH_2CH_2CH_2OH$	64	77	79	86
6	$CH_3CH_2CH_2CH(OH)CH_3$	92			
7	$CH_3CH_2CH(OH)CH_2CH_3$	100	100	88	94
8	$CH_3CH_2CH_2CH_2CH_2CH_2OH$	70			
9	$CH_3CH_2CH_2CH_2CH(OH)CH_3$	83			
10	$CH_3CH_2CH_2CH(OH)CH_2CH_3$	83			
11	$(CH_3)_3COH$		54	47	36
12	$CH_3CH_2C(CH_3)_2OH$		50	46	38

Note: Reaction conditions: 0.05 mmol of **5**, 0.15 mmol of an alcohol, and 25 mg of CAL-B in 240 μL of diisopropyl ether at 45 °C.

Table 5. CAL-B-catalyzed deacylation of 2-methoxy-1,4-di-*O*-propanoylhydroquinone (**1e**) with various alcohols.

Entry	Alcohol	Yield (%) 2e	3e	4e	Conversion (%)
1	$CH_3CH_2CH_2OH$	32	8	3	43
2	$CH_3CH(OH)CH_3$	51	8	7	66
3	$CH_3CH_2CH_2CH_2CH_2OH$	27	8	3	38
4	$CH_3CH_2CH(OH)CH_2CH_3$	40	12	12	64
5	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2OH$	30	9	3	42
6	$CH_3CH_2CH_2CH(OH)CH_2CH_2CH_3$	17	7	3	27
7	$(CH_3)_2CHCH(OH)CH(CH_3)_2$	17	5	2	24

Note: Reaction conditions: 0.18 mmol of **1e**, 0.54 mmol of an alcohol, and 75 mg of CAL-B in 450 μL of diisopropyl ether at 45 °C for 20 min.

both phenolic hydroxy groups. Contrary to expectation, some secondary alcohols were found to act as better acyl acceptors than the corresponding primary alcohols in these enzymatic deacylations. The deacylation reactions were much faster than the corresponding direct acylations of these dihydroxybenzenes catalyzed by the same lipase. More importantly, they took place generally in a markedly regioselective manner: the acyloxy group remote from the substituent was preferentially deacylated. The main or, in some cases, exclusive products obtained through enzymatic deacylation were the regioisomers of those produced through the direct acylation of the dihydroxybenzenes. This should be of significant importance from a synthetic standpoint because either regioisomer of monoacyl derivatives of dihydroxybenzenes can easily be obtained by choosing either acylation or deacylation mediated by the easily available single biocatalyst. In the case of alkyl-substituted hydroquinone derivatives, the regioselectivity increased with the increase in the bulk of the substituent. In the case of 4-substituted diacylated resorcinols, the 3-*O*-acyl derivatives were obtained generally as the sole products.

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(13) For comparison, we tried to prepare the authentic sample of the isomeric 1-*O*-propanoyl derivative (**7c**) by the direct acylation with vinyl propanoate of 4-*t*-butylresorcinol (**8c**) in the presence of CAL-B according to the general procedure reported before (see reference 11), but no reaction occurred after 3 days in this case.

(14) We employed this solvent in the lipase-catalyzed highly enantioselective acylation of 2-aryloxy-1-propanols: Miyazawa, T.; Yukawa, T.; Koshiba, T.; Sakamoto, H.; Ueji, S.; Yanagihara, R.; Yamada, T. *Tetrahedron: Asymmetry* **2001**, *12*, 1595. doi:10.1016/S0957-4166(01)00258-0. It was employed also in the CAL-B-catalyzed direct acylation of dihydroxybenzenes (see reference 11).

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