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Highly regioselective propanoylation of dihydroxybenzenes mediated by *Candida antarctica* lipase B in organic solvents

Toshifumi Miyazawa,* Manabu Hamada, Ryohei Morimoto, Takashi Murashima and Takashi Yamada

Department of Chemistry, Faculty of Science and Engineering, Konan University, Higashinada-ku, Kobe 658-8501, Japan

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Abstract—*Candida antarctica* lipase B (CAL-B) was found to be a highly active biocatalyst for the direct acylation of the phenolic hydroxyls of substituted hydroquinones and resorcinols with vinyl propanoate as an acyl donor. The acylation reactions took place generally in a very regioselective manner. Especially in the case of 4-substituted resorcinols, the hydroxyl remote from the substituent was regiospecifically acylated to afford only the 1-*O*-propanoylated resorcinols.
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The high potential of lipases (triacylglycerol hydrolases, E.C. 3.1.1.3) as biocatalysts for organic synthesis has been well documented.[1] Lipases from a variety of sources have been utilized for preparing homochiral compounds related to pharmaceuticals and agrochemicals through resolution or desymmetrization. Besides the stereoselective nature of lipases, their regioselective properties have also been exploited for the preparation of selectively protected derivatives of compounds containing multi-hydroxyl groups such as carbohydrates and steroids. Thus, the lipase-catalyzed acylation or deacylation procedure has proven to be much superior to the standard chemical methodology which often requires a series of tedious steps for such transformations. These enzymatic acyl-transfer approaches can protect or deprotect a hydroxyl in the presence of several others under optimized reaction conditions. Compared to such studies on alcoholic hydroxyls, much fewer examples have been accumulated on phenolic hydroxyls. Further attention should be paid to the exploitation of lipase's ability to discriminate between hydroxyls of this type, which usually show only a small difference in reactivity toward conventional chemical reagents. Although several papers have dealt with the lipase-catalyzed deacylation of peracetylated polyhydroxybenzenes[2] and flavonoids[3] via transesterification with an alcohol such as 1-butanol,[4] the lipase-catalyzed direct acylation of phenolic hydroxyls have rarely been reported. This is probably because a phenolic hydroxyl is generally far less nucleophilic than an alcoholic hydroxyl and/or phenolic compounds are known to inhibit some enzymes. Nicolosi and co-workers reported the lipase-catalyzed regioselective protection of hydroxy groups in aromatic dihydroxyaldehydes and ketones:[5,6] the hydroxyl other than the one at position *ortho* to the carbonyl was selectively acylated employing *Burkholderia* (*Pseudomonas*) *cepacia* lipase and vinyl acetate in cyclohexane–*t*-amyl alcohol (9:1). They attributed the high regioselectivity observed to the chelation of the carbonyl with the *o*-hydroxy group. Stimulated by this work, we have started to examine the regioselectivity in the lipase-catalyzed acylation of the simplest members of polyphenols, that is, dihydroxybenzenes (hydroquinones and resorcinols) carrying substituents other than the carbonyl. The preparation of regioselectively protected derivatives of polyphenolic compounds by direct acylation procedure seems to be an extremely challenging task.

Initially, several hydroquinones bearing different substituents including the acetyl group on the benzene ring (**1**) were subjected to acylation with vinyl propanoate (3 M equiv) in the presence of immobilized *B. cepacia* lipase (Amano lipase PS) on Celite in the same mixed solvent system as mentioned above at 45 °C (Scheme 1). Hydroquinone **1** can undergo enzymatic acylation through two pathways to form either the 4-*O*-propanoyl

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* Corresponding author. Tel.: +81 78 431 4341; fax: +81 78 435 2539; e-mail: miyazawa@konan-u.ac.jp

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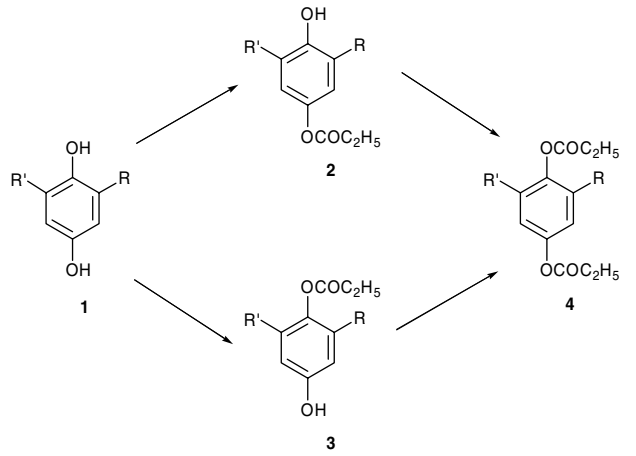
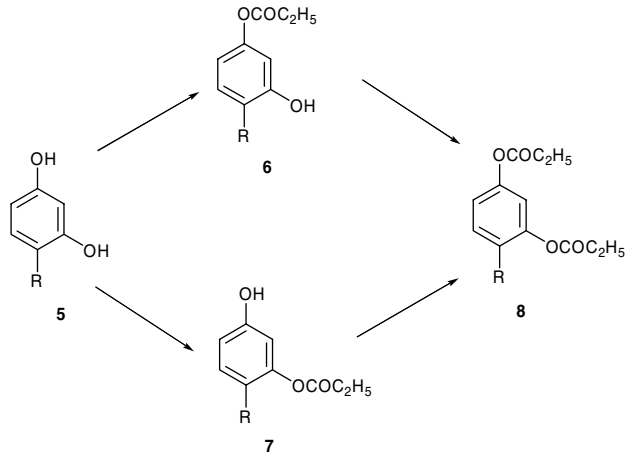


Scheme 1. CAL-B-catalyzed regioselective acylation of substituted hydroquinones (**1**). R: a, Me; b, Me; c, Et; d, *i*-Pr; e, *t*-Bu; f, MeO; g, Ac; h, F; i, Cl; j, Br. R′: a and c–j, H; b, Me.



Scheme 2. CAL-B-catalyzed regioselective acylation of 4-substituted resorcinols (**5**). R: a, Me; b, Et; c, $(CH_3)_3CCH_2C(CH_3)_2$–; d, Bn; e, Cl; f, Br.

derivative (**2**) or the 1-*O*-propanoyl derivative (**3**), and finally to afford the 1,4-di-*O*-propanoyl derivative (**4**).[7] The results obtained after 3 days of incubation are shown in Table 1. The phenolic hydroxyls of all the hydroquinones (**1**) examined managed to be acylated, though the reactions were rather slow. The reaction with acetylhydroquinone (**1g**) was the slowest among those with the substituted hydroquinones examined, and besides the expected 4-*O*-propanoyl derivative (**2g**) the isomeric 1-*O*-propanoyl derivative (**3g**) was also produced in a small amount, indicating the incompleteness of this lipase's regioselectivity even toward the aromatic dihydroxyketone. However, it exhibited some regioselectivity toward the hydroquinones carrying substituents other than the carbonyl: the hydroxyl remote from the substituent was preferentially acylated. In the case of methylhydroquinone (**1a**) bearing the smallest substituent, the regioselectivity was very poor. Accordingly, lipases from microbial and pancreatic sources[8] were screened for the acylation of **1a** with vinyl propanoate at 45 °C in diisopropyl ether.[9] Of the enzymes tested, *Candida antarctica* lipase B (CAL-B) proved to be the best in terms of both activity and regioselectivity. The screening of lipases was also conducted employing 4-ethylresorcinol (**5b**) and vinyl propanoate (Scheme 2). In general, the acylations of **5b** were slower than those of **1a**.

Table 1. *B. cepacia* lipase-catalyzed propanoylation of substituted hydroquinones (**1**) with vinyl propanoate[a]

Substrate	R	R′	Yield (%)		
			2	**3**	**4**
1a	CH_3	H	41.3	26.9	30.4
1b	CH_3	CH_3	78.8	3.5	0
1e	$(CH_3)_3C$	H	73.2	1.9	1.2
1f	CH_3O	H	41.1	15.6	12.9
1g	CH_3CO	H	21.7	5.8	0

[a] Reactions were conducted using **1** (0.1 mmol), vinyl propanoate (0.3 mmol), and *B. cepacia* lipase immobilized on Celite (40 mg) in 240 μl of cyclohexane–*t*-amyl alcohol (9:1, v/v) at 45 °C for 3 days.

While the regioselectivity was very low with *B. cepacia* lipase,[10] the acylation catalyzed by CAL-B proceeded regiospecifically: the 1-*O*-propanoyl derivative (**6b**) was obtained as the sole product.[11] These results indicate that CAL-B[12] was an enzyme of choice for the direct acylation of phenolic hydroxyls. Accordingly, further studies were conducted using this lipase. Although a large number of examples have already been accumulated on the effect of organic solvents on lipase-catalyzed reactions,[13] it is still difficult to predict the solvent effect, especially on regioselectivity. Screening experiments for an appropriate solvent revealed that CAL-B was most active with a tolerable regioselectivity in diisopropyl ether.

Using immobilized CAL-B[14] as the biocatalyst in diisopropyl ether the product distribution of a number of hydroquinones (**1**) carrying different substituents on the benzene ring was examined in the acylation with vinyl propanoate.[15] The time-course of the propanoylation of methoxyhydroquinone (**1f**) was followed through the quantification of the products by HPLC analysis (Fig. 1). The starting hydroquinone was consumed abruptly during the first 6 h, then decreased gradually,

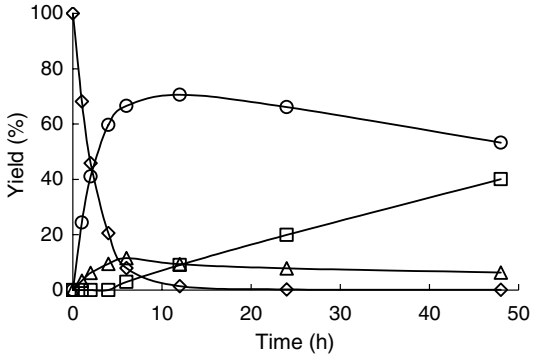


Figure 1. Reaction profile in the CAL-B-catalyzed acylation of methoxyhydroquinone (**1f**) with vinyl propanoate in diisopropyl ether. Symbols: diamond, **1f**; circle, **2f**; triangle, **3f**; square, **4f**.

Table 2. CAL-B-catalyzed propanoylation of substituted hydroquinones (**1**) with vinyl propanoate[a]

Substrate	R	R′	Yield (%)		
			2	**3**	**4**
1a	CH_3	H	51.4	17.5	17.2
1c	CH_3CH_2	H	73.2	8.4	11.5
1d	$(CH_3)_2CH$	H	75.0	0	16.0
1e	$(CH_3)_3C$	H	93.3	0	0
1f	CH_3O	H	70.7	10.5	18.6
1h	F	H	39.0	33.0	19.0
1i	Cl	H	40.9	16.4	4.7
1j	Br	H	47.0	22.1	7.7

[a] Reactions were conducted using **1** (0.1 mmol), vinyl propanoate (0.3 mmol), and CAL-B (40 mg) in diisopropyl ether (240 μl) at 45 °C for 1 day.

Table 3. CAL-B-catalyzed propanoylation of substituted resorcinols (**5**) with vinyl propanoate[a]

Substrate	R	Yield (%) of **6**[b]
5a	CH_3	82.2
5b	CH_3CH_2	37.0
5c	$(CH_3)_3CCH_2C(CH_3)_2$	23.1
5d	$C_6H_5CH_2$	36.7
5e	Cl	74.1
5f	Br	69.0

[a] Reactions were conducted using **5** (0.1 mmol), vinyl propanoate (0.3 mmol), and CAL-B (40 mg) in diisopropyl ether (240 μl) at 45 °C for 3 days.

[b] The 3-*O*-propanoate (**7**) and 1,3-di-*O*-propanoate (**8**) were not detected in all the cases.

and finally disappeared after 24 h. The amount of the 4-*O*-acylated product (**2f**) increased steeply at first, then reached a maximum after ca. 12 h and then turned to a gradual decrease. Thus, the product distribution was dependent largely on the reaction time. Accordingly, the results obtained after 1 day of incubation at 45 °C are compiled in Table 2. In all the cases, the 4-*O*-propanoyl derivative (**2**) was obtained as the main product, and the 1-*O*-propanoyl derivative (**3**) and/or the 1,4-di-*O*-propanoyl derivative (**4**) were also produced in various amounts depending on the substituents. When the substituent was changed from a methyl group (in **1a**) to an ethyl group (in **1c**), the proportion of the 4-*O*-propanoyl derivative in the reaction products increased to a considerable extent. With isopropylhydroquinone (**1d**) the formation of the 1-*O*-propanoyl derivative (**3d**) was not observed, and the 4-*O*-propanoyl derivative (**2e**) was produced as the exclusive product with *t*-butylhydroquinone (**1e**). Thus, the selectivity for the propanoylation of the hydroxyl remote from the substituent increases with the increase in the bulk of the substituent, that is, methyl to isopropyl/*t*-butyl. This implies that the steric bulk of the substituent must be one of the contributory factors for the observed regioselectivity. The behavior of **1f** resembles that of **1c**, which also suggests the importance of steric requirement in activity and regioselectivity. With fluorohydroquinone (**1h**) the regioselectivity deteriorated to a great extent, though its activity toward the acylation was as high as those of the alkyl-substituted hydroquinones. The other halogen substituents (in **1i** and **1j**) reduced the activity of hydroquinone, with a moderate regioselectivity. The effect of halogen substituents on the activity and regioselectivity seems rather complicated, indicating that besides the steric effect other factors, such as the electronic effect, must be taken into consideration.

Next, the CAL-B-catalyzed propanoylation of a number of resorcinols (**5**) carrying alkyl, aralkyl, or halogen substituents on the benzene ring was examined under the same reaction conditions as above. The results obtained after 3 days of incubation at 45 °C are shown in Table 3. The reactions were retarded largely compared with those of hydroquinones having the same or similar substituents. Quite interestingly, however, the acylation proceeded regiospecifically independent of the substituent, yielding the 1-*O*-propanoyl derivative (**6**) as the exclusive product and none of the 3-*O*-propanoyl derivative (**7**) nor the 1,4-di-*O*-propanoyl derivative (8). The steric factor of substituents in the substituted resorcinols had a much larger effect on its activity than that in the substituted hydroquinones. With 4-methylresorcinol (**5a**) the acylation proceeded rather smoothly among the resorcinols examined. It was retarded in the presence of bulkier substituents. This is probably because of the less accessibility of the lipase even toward the hydroxyl at position 1, to say nothing of the hydroxyl at position 3. The acylation of halogen-substituted resorcinols (**5e** and **5f**) proceeded at the rate between those of **5a** and the other alkyl-substituted resorcinols.

With the purpose of comparison some typical non-enzymatic procedures were also tried for the acylation of phenolic hydroxyls. When 4-ethylresorcinol (**5b**) was heated in propanoic anhydride at 100 °C for 30 min, the product distribution was as follows: **6b**, 16.8%; **7b**, 16.8%; **8b**, 32.9%. In the presence of a few drops of sulfuric acid the acylation with propanoic anhydride was accelerated to a great extent. The reaction at 25 °C yielded 20.0% of **6b**, 0% of **7b**, and 80.0% of **8b** after 5 min.[16] On the other hand, the reaction of **5b** with propanoyl chloride (1 M equiv) in toluene in the presence of pyridine (1 M equiv) as catalyst at 50 °C gave 57.0% of **6b**, 12.2% of **7b**, and 30.8% of **8b** after 30 min. Thus, the acylations by the conventional methods were rather faster than the CAL-B-catalyzed acylation, though the difference in the reaction conditions makes a direct comparison difficult. However, it is certain that these chemical procedures were much inferior in terms of regioselectivity, though a preference for the 1-*O*-propanoyl derivative (**6**) was observed to one degree or another in some cases.

In conclusion, CAL-B is a highly active biocatalyst for the direct acylation of the phenolic hydroxyls of hydroquinones and resorcinols. The acylation reactions take place generally in a very regioselective manner; in particular, they are regiospecific in the case of resorcinols. The main or, in some cases, exclusive products obtained through the direct acylation of those dihydroxybenzenes are the regioisomers of those obtained through the CAL-B-catalyzed deacylation of dihydroxybenzenes acylated at both phenolic hydroxyls.[4] This should be

of significant importance from a synthetic standpoint, because either regioisomer of monoacylated derivatives of dihydroxybenzenes can easily be obtained by choosing either acylation or deacylation mediated by the single biocatalyst which is easily available.

References and notes

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4. We also have recently reported the highly regioselective deacylation of resorcinols and hydroquinones acylated at both phenolic hydroxyls mediated by *Candida antarctica* lipase B: Miyazawa, T.; Hamada, M.; Morimoto, R.; Murashima, T.; Yamada, T. *Tetrahedron Lett.* **2007**, *48*, 8334.
5. Nicolosi, G.; Piattelli, M.; Sanfilippo, C. *Tetrahedron* **1993**, *49*, 3143.
6. They also reported the formation of two derivatives protected only at the A ring, that is, 5-*O*-acetylcatechin and 7-*O*-acetylcatechin, in the reaction of (+)-catechin [(2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol] with vinyl acetate in the presence of supported *B. cepacia* lipase in acetonitrile: Lambusta, D.; Nicolosi, G.; Patti, A.; Piattelli, M. *Synthesis* **1993**, 1155.
7. The authentic samples of isomeric monoesters of each dihydroxybenzene were prepared by enzymatic methods. For example, both the monopropanoates of *t*-butylhydroquinone (**1e**) were prepared as follows: 4-*O*-Propanoyl-*t*-butylhdyroquinone (**2e**) was prepared as the main product of the lipase-catalyzed direct acylation of **1e** (0.5 mmol) as described in Ref. 15 and purified by preparative TLC on silica gel using hexane–ethyl acetate (2:1, v/v) as a developing solvent. On the other hand, the isomer of **2e**, that is, 1-*O*-propanoyl-*t*-butylhydroquinone (**3e**), was prepared as the main product of the lipase-catalyzed deacylation of 1,4-di-*O*-propanoyl-*t*-butylhydroquinone (**4e**) (1.5 mmol) with 2-propanol (4.5 mmol) in diisopropyl ether at 45 °C and purified in the same manner as above. The structure of these isomeric monoesters was unambiguously determined by ^{1}H NMR (500 MHz), ^{13}C NMR, and 2D NMR (HMQC, HMBC). Thus, for example, cross-peaks of the proton of 1-OH with the carbons at C-1, C-2, and C-6 appeared in the HMBC spectrum of **2e**. Selected data for **2e**: oil; ^{1}H NMR (DMSO-d_6) δ 1.11 (3H, t, $J = 7.5$ Hz, CH_3), 1.32 (9H, s, $C(CH_3)_3$), 2.53 (2H, q, $J = 7.5$ Hz, CH_2), 6.73-6.75 (1H, distorted dd, $J = 8.5$ and 2.0 Hz, H-5), 6.75-6.77 (1H, distorted d, $J = 8.5$ Hz, H-6), 6.80 (1H, d, $J = 2.0$ Hz, H-3), 9.39 (1H, s, OH). For **3e**: oil; ^{1}H NMR (DMSO-d_6) δ 1.14 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 1.24 (9H, s, $C(CH_3)_3$), 2.58 (2H, q, $J = 7.5$ Hz, CH_2CH_3), 6.58 (1H, dd, $J = 3.0$ and 8.5 Hz, H-5), 6.74 (1H, d, $J = 3.0$ Hz, H-3), 6.77 (1H, d, $J = 8.5$ Hz, H-6), 9.29 (1H, s, OH). On the other hand, the authentic samples of the dipropanoates of dihydroxybenzenes were prepared by the reaction of each dihydroxybenzene with propanoyl chloride in pyridine. Selected data for **4e**: oil; ^{1}H NMR (DMSO-d_6) δ 1.13 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 1.17 (3H, t, $J = 7.5$ Hz, CH_2CH_3), 1.28 (9H, s, $C(CH_3)_3$), 2.59 (2H, q, $J = 7.5$ Hz, CH_2), 2.65 (2H, q, $J = 7.5$ Hz, CH_2), 7.01 (1H, dd, $J = 9.0$ and 2.5 Hz, H-5), 7.06 (1H, d, $J = 2.5$ Hz, H-3), 7.07 (1H, d, $J = 9.0$ Hz, H-6).
8. The lipases tested include those from *Candida antarctica* B, *Alcaligenes* sp., *Achromobacter* sp., porcine pancreas, and *Chromobacterium viscosum*. All the lipases were employed in the immobilized form.
9. This solvent was employed 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.
10. The product distribution after 3 days was as follows: **6b**, 24.0%; **7b**, 20.0%; **8b**, 7.3%.
11. For the product distribution after 3 days, see Table 3.
12. This lipase was found to be highly regioselective as well as active in the deacylation of resorcinol and hydroquinone derivatives: see Ref. 4.
13. *Enzymatic Reactions in Organic Media*; Koskinen, A. M. P., Klibanov, A. M., Eds.; Blackie A&P: Glasgow, 1996.
14. Boehringer Mannheim Chirazyme L-2, which had a specific activity of 3.2 U/mg lyophilized powder with tributyrin at 25 °C.
15. Typical experimental procedure: A solution of a dihydric phenol (0.1 mmol) and vinyl propanoate (0.3 mmol) in anhydrous diisopropyl ether (240 μl) was stirred with an immobilized lipase preparation (40 mg) 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 residual oil was dissolved in DMSO-d_6 and subjected to ^{1}H NMR (500 MHz) analysis for the quantification of the reaction products. The proton signals 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.
16. Under the same reaction conditions 4-*t*-octylresorcinol (**5c**) afforded 46.4% of **6c**, 0% of **7c**, and 53.6% of **8c**.