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# Enzymatic resolution of 2-phenyl-1-propanol by enantioselective hydrolysis of its ester having a bulky group in an acyl moiety

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**Abstract**

Enzymatic resolution of 2-phenyl-1-propanol, enantiomeric ratios of which were recently improved up to 31 and 41 with lipase PS and PPL, respectively, by transesterification using vinyl 3-phenylpropanoate, was more excellently attained by PPL-catalyzed hydrolysis of its ester of 3-phenylpropanoic acid in 107 of $E$ value. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* 2-Phenyl-1-propanol; Enzymatic resolution; Enantioselective hydrolysis; Enantioselectivity

## 1. Introduction

The kinetic resolution of racemic alcohol by lipase-catalyzed solvolysis and transesterification is known as a powerful approach to get optically active alcohol [1–6]. Many organic chemists first screen the commercially available enzymes, mainly lipases, to resolve a racemic alcohol [7]. In the case of a racemic secondary alcohol, there are many examples of good enantioselective resolution ($E > 20$ [8]) in different appropriate reaction conditions of solvent [9], temperature [10,11], additives [5], and so on. On the other hand, the resolution of a racemic primary alcohol is usually difficult. In 1990s, the change of carboxylic acid moiety from acetate to longer chain carboxylate was reported to afford good enantioselectivity for resolution of several primary alcohols [12–19]. However, it was difficult to attain highly enantioselective resolution of 2-phenyl-1-propanol by lipase-catalyzed reaction ($E < 13$) [20,21]. Therefore, the resolution of 2-phenyl-1-propanol has been a challenge for synthetic chemists. Recently, we have succeeded in the resolution of 2-phenyl-1-propanol by lipase-catalyzed transesterification with vinyl esters having an aromatic ring in acyl moiety [1]. In the new method, the enantioselectivity increased to 31 of $E$ value with lipase PS and 41 of one with PPL by transesterification using vinyl 3-phenylpropanoate. The success encouraged us to study the enzymatic hydrolysis

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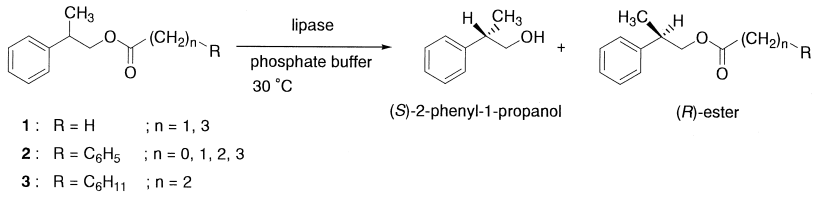



Scheme 1. Enzymatic resolution of 2-phenyl-1-propanol esters with lipases.

of a racemic 2-phenyl-1-propanol ester of alkanoic acid having a bulky group, benzene or cyclohexane ring, at the ω site, as shown in Scheme 1.

## 2. Materials and methods

### *2.1. Esters*

The esters of **1** ($n = 3$), **2** ($n = 0$, 1, 2, and 3), and **3** ($n = 2$) were prepared by condensation of the corresponding carboxylic acid and racemic 2-phenyl-1-propanol in the presence of dicyclohexylcarbodiimide with catalytic amounts of 4-dimethylaminopyridine in dichloromethane at room temperature. Ester **1** ($n = 1$) was synthesized by acetylation using acetic anhydride and pyridine in dichloromethane. All the esters were fully characterized by their $^1$H NMR, $^{13}$C NMR and IR spectral data.

$^1$H and $^{13}$C NMR spectra, with tetramethylsilane as an internal standard, were obtained with a JEOL JNM-LA500 spectrometer and chemical shifts are expressed in ppm. Multiplicity is designated as: s, singlet; d, doublet; t, triplet; quart, quartet; quint, quintet; sext, sextet; m, multiplet.

IR data of neat liquid film samples were recorded with a JASCO FT/IR-8000 spectrometer.

Ester **2** ($n = 0$), $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.98 (d, $J = 7.3$ Hz, 2H), 7.54–7.21 (m, 8H), 4.43 (dd, $J = 10.8$, 6.8 Hz, 1H), 4.38 (dd, $J = 10.8$, 7.3 Hz, 1H), 3.25 (sext, $J = 7.0$ Hz, 1H), 1.40 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 166.48, 143.21, 132.86, 130.36, 130.35, 129.55, 128.53, 128.33, 127.35, 126.72, 69.88, 39.12, 18.05; IR (neat, cm$^{-1}$) 2969, 1719, 1453, 1273, 1113, 712.

Ester **2** ($n = 1$), $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.29–7.14 (m, 10H), 4.21 (dd, $J = 10.7$, 6.8 Hz, 1H), 4.14 (dd, $J = 10.7$, 7.3 Hz, 1H), 3.56 (s, 2H), 3.06 (sext, $J = 7.0$ Hz, 1H), 1.24 (d, $J = 7.3$ Hz, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 171.4, 143.07, 143.03, 143.02, 129.27, 129.26, 128.51, 128.45, 127.31, 127.30, 127.0, 126.64, 126.63, 69.70, 41.45, 38.90, 17.90; IR (neat, cm$^{-1}$) 2969, 1734, 1495, 1455, 1260, 1156, 1007.

Ester **2** ($n = 2$), $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.31–7.13 (m, 10H), 4.19 (dd, $J = 10.7$, 6.8 Hz, 1H), 4.13 (dd, $J = 10.7$, 7.3 Hz, 1H), 3.06 (sext, $J = 7.0$ Hz, 1H), 2.88 (t, $J = 7.8$ Hz, 2H), 2.58 (t, $J = 7.8$ Hz, 2H), 1.26 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 172.76, 143.16, 140.49, 128.48, 128.46, 128.24, 127.28, 126.67, 126.22, 69.37, 38.93, 35.85, 30.91, 17.98; IR (neat, cm$^{-1}$) 2969, 1732, 1495, 1455, 1163, 1022.

Ester **2** ($n = 3$), $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.31–7.11 (m, 10H), 4.21 (dd, $J = 10.7$, 6.8 Hz, 1H), 4.13 (dd, $J = 10.7$, 7.1 Hz, 1H), 3.09 (sext, $J = 7.1$ Hz, 1H), 2.58 (t, $J = 7.6$ Hz, 2H), 2.27 (t, $J = 7.3$ Hz, 2H), 1.89 (quint, $J = 7.6$ Hz, 2H), 1.29 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 173.37, 143.22, 141.39, 128.49, 128.36, 127.29, 126.68, 125.95, 69.21, 38.99, 35.06, 33.59, 26.47, 18.13; IR (neat, cm$^{-1}$) 2965, 1734, 1495, 1455, 1171, 1144, 1022.

Ester **3** ($n = 2$), $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 7.32–7.20 (m, 5H), 4.20 (dd, $J = 10.8$, 7.3 Hz, 1H), 4.12 (dd, $J = 10.7$, 7.3 Hz, 1H), 3.09 (sext, $J = 7.0$ Hz, 1H), 2.26 (t, $J = 7.8$ Hz, 2H), 1.65 (bm, 5H), 1.46 (quart, $J = 7.5$ Hz,

2H), 1.30 (d, $J = 7.4$ Hz, 3H), 1.16 (m, 4H), 0.85 (m, 2H); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 174.06, 143.28, 128.47, 128.46, 127.31, 126.65, 126.64, 69.16, 39.00, 37.16, 32.95, 32.33, 31.90, 26.55, 26.22, 18.03; IR (neat, $cm^{-1}$) 2926, 2120, 1740, 1495, 1453, 1252, 1127.

### 2.2. Enzymes

Lipase OF from *Candida rugosa* was obtained from Meito Sangyo (Japan). Lipase AK from *Pseudomonas fluorescence* and lipase PS from *P. cepacia* were obtained from Amano, CHIRAZYME (L-2, lyo) from *C. antarctica* was obtained from Roche, KK. PPL from porcine pancreas was purchased from Sigma.

### 2.3. Enzymatic hydrolysis reaction

All reactions were carried out in 30-ml glass vials. Enzymes were suspended in phosphate buffer solution (0.1 M, pH 7.0, 5 ml) at 30°C. Esters (0.5 mmol) in acetonitrile (0.1 ml) were added and the mixture was stirred vigorously at this temperature. The progress of the hydrolytic reaction was monitored by HPLC. The reaction was quenched by addition of acetone. After centrifuging (3000 rpm, 5 min), organic solvent was removed from the supernatant under the reduced pressure. Water layer was extracted with ethyl acetate and the organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated under the reduced pressure and the resultant alcohol and remaining ester were separated through a silica gel column.

### 2.4. Analytical condition and retention time

The enantiomers of 2-phenyl-1-propanol and esters **1** ($n = 1$) and **2** ($n = 0$ and 1) were separated by HPLC (Garyver, JASCO, Japan ) with a Daicel Chiralcel OB-H column (Daicel, Japan) under the following conditions: mobile phase, *n*-hexane/2-propanol (9/1); column temperature, 30°C; detection, 254 nm; flow rate, 0.5 ml/min. Retention times of each *R* and *S* of the alcohol and esters **1** ($n = 1$) and **2** ($n = 0$ and 1) were 10.5 and 9.9, 10.9 and 11.6, 10.6 and 11.2, and 15.7 and 16.9 min, respectively.

The enantiomeric excesses (ee's) of the remaining esters, **1** ($n = 3$), **2** ($n = 2$ and 3), and **3** were determined by analyzing the ratio of the resultant alcohol by HPLC after hydrolyzing the corresponding esters by 0.1 M NaOH in MeOH. The absolute configuration of the preferentially obtained alcohol was established by comparing its retention time on HPLC with the retention time of a purchased authentic sample.

The enantiomeric ratio, *E* value [8], was calculated using the equation described in the literature.

## 3. Results and discussion

We tried to improve the enantioselectivity of 2-phenyl-1-propanol by lipase-catalyzed hydrolysis of its ester based on the following concepts: both introduction of a bulky group, benzene or cyclohexane ring, into ω site and change of chain length in acyl moiety will vary a conformation of substrate-binding site of lipase, resulting in improving the enantioselectivity of this ester. Benzene and cyclohexane rings are larger groups than straight chain alkyl ones [22,23] and are expected to exert larger steric effect on a change of conformation and benzene ring may also have $\pi$–$\pi$ or CH–$\pi$ interactions (electronic interactions) between amino acid residues in the active site [24,25]. These steric effects and electronic interactions might lead to the induction of favorable conformational change of the active site in an enzyme.

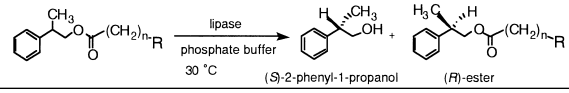
In five lipases used, lipase OF displayed no enantioselectivity ($E = 1.1$–$1.7$) with esters **2** and **3** as with ester **1**. Judging from no improvement in *E*, introduction of a bulky group and change of methylene length may have no effect on conformational change or may not exert

desirable conformational change in ester-recognition site.

The results using the other four lipases toward esters **1**, **2**, and **3** are summarized in Table 1. These results show that esters **2** and **3** are hydrolyzed more selectively than ester **1** in most cases. In consequence, it was concluded that introduction of a bulky group induced some desirable conformational change. However, hydrolysis of ester **2** ($n = 0$ and 1) needed relatively a longer reaction time.

With lipase AK and PS, though $E$ values of ester **2** ($n = 1$) were as low as those of ester **1** ($E = 1.1$–2), esters **2** ($n = 0$, 2, and 3) were hydrolyzed more selectively ($E = 3.4$–12). There may exist a slight effect on conformational change. CHIRAZYME-catalyzed hydrolysis of esters **2** and **3** displayed $E$ values twice

Table 1
Enzymatic hydrolysis of **1**, **2**, and **3** with four lipases




| Lipase | Ester | Time (h) | Conversion (%) | $ee_s$ (%) | $ee_p$ (%)[a] | $E$[b] |
|---|---|---|---|---|---|---|
| Lipase AK | **1** ($n = 1$) | 1 | 36 | 10 | 18 | 1.6 |
| | ($n = 3$) | 1 | 43 | 2.7 | 3.5 | 1.1 |
| | **2** ($n = 0$) | 30 | 25 | 24 | 71 | 7.6 |
| | ($n = 1$) | 44 | 9 | 23 | 2 | 1.6 |
| | ($n = 2$) | 2 | 52 | 51 | 48 | 4.6 |
| | ($n = 2$)[c] | 2.5 | 43 | 55 | 72 | 11 |
| | ($n = 3$) | 1 | 27 | 18 | 49 | 3.4 |
| | **3** ($n = 2$) | 14 | 26 | 35 | 57 | 5.0 |
| Lipase PS | **1** ($n = 1$) | 2 | 49 | 23 | 24 | 2 |
| | ($n = 3$) | 2 | 79 | 28 | 7.5 | 1.4 |
| | **2** ($n = 0$) | 51 | 27 | 28 | 76 | 9.6 |
| | ($n = 1$) | 72 | 5 | 6 | 1 | 1.1 |
| | ($n = 2$) | 2 | 52 | 63 | 57 | 6.8 |
| | ($n = 2$)[c] | 6 | 46 | 62 | 73 | 12 |
| | ($n = 3$) | 0.5 | 27 | 25 | 66 | 6.3 |
| | **3** ($n = 2$) | 10 | 22 | 23 | 81 | 12 |
| CHIRAZYME | **1** ($n = 1$) | 0.5 | 38 | 49 | 80 | 14 |
| | ($n = 3$) | 0.5 | 65 | 79 | 43 | 5.7 |
| | **2** ($n = 0$) | 24 | 39 | 58 | 88 | 29 |
| | ($n = 1$) | 0.5 | 50 | 85 | 83 | 28 |
| | ($n = 2$) | 2 | 21 | 24 | 90 | 24 |
| | ($n = 2$)[c] | 24 | 42 | 62 | 85 | 23 |
| | ($n = 3$) | 1 | 24 | 28 | 91 | 30 |
| | **3** ($n = 2$) | 1 | 20 | 22 | 88 | 20 |
| PPL | **1** ($n = 1$) | 2 | 35 | 40 | 76 | 11 |
| | ($n = 3$) | 2 | 46 | 74 | 67 | 11 |
| | **2** ($n = 0$) | 30 | 0[d] | – | – | – |
| | ($n = 1$) | 96 | 0[d] | – | – | – |
| | ($n = 2$) | 2.5 | 35 | 53 | 97 | 107 |
| | ($n = 2$)[c] | 24 | 50 | 55 | 55 | 5.8 |
| | ($n = 3$) | 3 | 18 | 20 | 90 | 24 |
| | **3** ($n = 2$) | 1 | 26 | 30 | 85 | 17 |

[a] In all cases *S*-alcohol was obtained preferentially.
[b] Calculated from ees.
[c] Reaction was carried out at 4°C.
[d] PPL-catalyzed hydrolysis did not proceed at all.

as that of ester **1**. Methylene length in acyl moiety did not affect the enantioselectivity with CHIRAZYME. This result suggests that the effect on conformational change due to a bulky group in acyl portion overcame the effect due to the length of methylene chain.

On the other hand, PPL-catalyzed hydrolysis afforded dramatic improvement of the enantioselectively. The $E$ value of ester **2** ($n = 2$) increased to 107, which is the best value so far described in the literature for the resolution of 2-phenyl-1-propanol. This observation suggests that both introduction of benzene ring and change of methylene length in acyl moiety play an important role into the change of conformation, resulting in the improvement of stereoselectivity of the ester.

In order to improve the enantioselectivity of ester **2** ($n = 2$), lipase-catalyzed hydrolysis was carried out at 4°C, since the advantage of low temperature for obtaining higher enantioselectivity was reported [10,11]. The $E$ value observed dropped from 107 to 5.8 with PPL. With CHIRAZYME, the effect of lowered temperature on enantioselectivity was not observed ($E$ = 24 at 30°C and 23 at 4°C). With lipase AK and lipase PS, ester **2** ($n = 2$) was more selectively hydrolyzed (from 4.6 to 11 and from 6.8 to 12 of $E$ values, respectively) at 4°C. These results suggest that the effect of lowered temperature on enantioselectivity depends on lipases.

The displacement of benzene ring to cyclohexane ring (ester **2** ($n = 2$) to ester **3** ($n = 2$)) gave rise to the following results. Though the displacement did not affect the enantioselectivity with lipase AK and CHIRAZYME, it led to slight improvement of $E$ value of 6.8 up to 12 with lipase PS. However, the enantioselectivity with PPL decreased the $E$ value from 107 to 17.

In the previous paper, we reported the lipase PS-catalyzed transesterification of 2-phenyl-1-propanol with vinyl 3-phenylpropanoate in cyclohexane [1], corresponding to the lipase PS-catalyzed hydrolysis of ester **2** ($n = 2$) in this paper. In the transesterification, the enantiomeric ratio ($E$ value) was improved from 3 (vinyl acetate) to 18 (vinyl 3-phenylpropanoate) at 30°C. However, the larger change of $E$ value was not observed in the enantioselective hydrolysis, as the enantiomeric ratio changed from 2 (ester **1** ($n = 1$)) to 6.8 (ester **2** ($n = 2$)). We discussed the direct and indirect effects of a bulky group in an acyl moiety on the lipase-catalyzed transesterification [1]. In the lipase-catalyzed hydrolysis, the same effects might also be considered. The difference in the change of $E$ values between enantioselective hydrolysis and transesterification could be mainly caused by the difference in the environment of solvent.

In this paper we described the effectiveness of the acyl moiety having benzene and cyclohexane rings on enzymatic hydrolysis of 2-phenyl-1-propanol ester. We are investigating further applications by introducing the bulky group into an acyl moiety in ester for enzymatic resolution of other racemic alcohol by enantioselective solvolysis and transesterification.

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