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Chemoenzymatic Deracemization of Chiral Secondary Alcohols: Process Optimization for Production of (*R*)-1-Indanol and (*R*)-1-Phenylethanol

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Abstract:

A process for preparing chiral secondary alcohols by chemoenzymatic deracemization was optimized. First, the transesterification process of 1-indanol and 1-phenylethanol with vinyl acetate as the acyl donor was optimized using lipase Novozym 435 as biocatalyst. The effects of acyl donors, substrate concentration, solvent type, and enzyme amount on activity and enantioselectivity of the said transesterification were investigated. Second,on the basis of the optimized conditions, an efficient biocatalytic resolution system was established with high selectivity, where volumetric productivity of the reaction against (*R*)-1-indanol and (*R*)-1-phenylethanol reached 529 and 198 g L^{-1} d^{-1}, respectively, during reuse of the enzyme in repeated-batch transesterification reactions. After 10 batches of the reaction, the enzyme still remained stable. Finally, (*R*)-1-indanol and (*R*)-1-phenylethanol were obtained in 95% and 97% ee and in 67% and 71% isolated yields from the corresponding resolution mixture, through an in situ Mitsunobu inversion of the unreacted alcohols followed by chemical hydrolysis of (*R*)-acetates.

1. Introduction

Bearing specific functional groups, chiral alcohols are important intermediates for the synthesis of many chiral medicines and also are widely used in the preparation of hormones, flavors, fragrances, liquid crystals and chiral auxiliaries. Chiral alcohols can be synthesized by chemical or biological approaches. The technology of biocatalysis has drawn broad attention for its high efficiency, mild reaction condition, outstanding stereospecificity, and so on.[1–3] This method normally refers to kinetic resolution of racemic alcohols or asymmetric reduction of prochiral ketones. Generally, optically pure chiral alcohols can be obtained by biocatalyzed asymmetric reduction in 100% yield in theory. However, only very few successful cases of biocatalytic asymmetric reductions have been reported in industrialized applications, because the reaction catalyzed by reductase demands cofactors, which are often too expensive. Other reasons also exist, such as low substrate concentration and low catalysis efficiency resulting from low solubility of the hydrophobic substrate, and the toxicity of ketones.[2] To solve such problems, lipase-catalyzed kinetic resolution has already been studied for the synthesis of chiral alcohols for over 2 decades; its disadvantages and bottlenecks lie in a theoretical yield of 50% and difficulty in separation of the reactant and the product. Dynamic kinetic resolution (DKR), especially the combined use of enzymes and transition metal catalysis, provides a convenient route to resolution of secondary alcohols.[4,5] Using metal catalysts for the in situ racemization of enzymatically unreactive enantiomers in the enzymatic resolution of racemic substrates overcomes the limitation of the maximum 50% yield in the traditional kinetic resolution. As an example, enzymatic resolution of secondary alcohols, e.g., 1-phenylethanol and 1-indanol, coupled with ruthenium-catalyzed racemization employing catalyst **1** resulted in full transformation of the racemic alcohols to enantiomerically pure acetate in yields of 80% and 77%.[6] However, the reactions needed a large amount of enzyme (i.e., 30 mg enzyme/1 mmol scale) as a result of the activity loss/inhibition in the presence of chemical reagents. In addition, at least three column extractions are required to purify complex **1**.[7] Using catalyst **2**, dynamic kinetic resolution of 1-phenylethanol also obtained enantiomerically pure acetate in 83% selectivity at 91% conversion,[7] but the reactions needed TEMPO as a co-catalyst, which was expensive and led to formation of a side product.

As an alternative approach, a method combining chemical and enzymatic approaches has been developed, by which on the basis of classic enzymatic resolution, chiral alcohols are directly converted into carboxylic esters of opposite configuration by addition of some chemical reagents, without separation of the product from the substrate, and as a result optically pure product may be obtained in a theoretical yield of 100%.[8–10] In addition, the activity of enzyme can be fully kept by this two-step one-pot method.

In the technology transfer process of biocatalysis from laboratory scale to industrialization level, activity, selectivity

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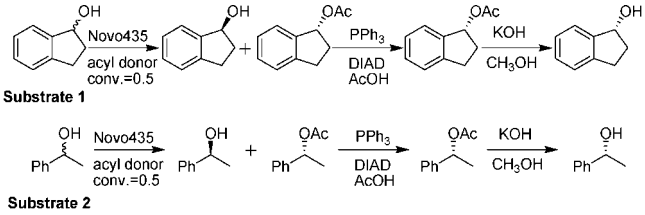
Scheme 1. **Preparation of chiral secondary alcohols by chemoenzymatic deracemization**



and stability of an enzyme all have to be taken into consideration, and thus selection of a biocatalyst with high activity and establishment of a biocatalytic system with high efficiency and in adaptation to the former are of special importance. In addition, for evaluation of the biocatalyst used in a specific process, not only performance specifications of the biocatalyst but those of the process are essential, including turnover number, stability of the biocatalyst, and volumetric productivity of the reactor. Although the resolution-inversion routes shown in Scheme 1 have been reported previously in the literature,[8–10] they were investigated only from the aspect of synthetic chemistry and were not optimized systematically in respect to reaction engineering principles. In this study, the enzymatic resolution process via transesterification of two typical aryl secondary alcohols, i.e., 1-indanol (substrate **1**) and 1-phenylethanol (substrate **2**), was optimized, through which the effects of some key parameters such as acyl donors, solvents, reaction temperature, substrate concentration, and enzyme amount on activity and enantioselectivity of the catalyst were investigated in detail in order to achieve higher efficiency. Stability of the biocatalyst in repeated use was also examined. As a result, chiral alcohols of single configuration were obtained by a Mitsunobu inversion of the reaction mixture followed by a saponification reaction (see Scheme 1). The purpose of this study is to develop a highly efficient chemoenzymatic process for producing valuable chiral alcohols. The results will provide some beneficial references for industrial preparation of chiral alcohols by biocatalysis.

2. Results and Discussion

Effect of Acyl Donor and Substrate Concentration on Resolution Reaction. The application of carboxylic acid as an acyl donor for an ester formation reaction in an organic solvent system is limited by its acidity and water formation in the reaction.[11] In contrast, in the case of an enol ester as an acyl donor, the lipase-catalyzed transesterification with a secondary alcohol can take place more easily, affording the corresponding ester of the chiral alcohol and vinyl alcohol. The latter can escape from the reaction system in the form of an aldehyde after isomerization, therefore making the reaction irreversible. For this reason, the effect of two enol esters as acyl donors on the activity of the enzyme in the resolution of substrates **1** and **2** was investigated in this study. Table 1 indicates that in the case of 1-indanol the effect of the two acyl donors on the enzyme activity is minor, whereas in the case of 1-phenylethanol, the effect is more significant: using vinyl propionate as the acyl donor brings higher enzyme activity than using vinyl acetate. Investigation of the enzyme enantioselectivity revealed

Table 1. **Effect of acyl donor on enzyme activity**

	initial rate (mM· min^{-1}·g $enzyme^{-1}$)	
substrate	vinyl acetate	vinyl propionate
1-phenylethanol	80.0	125.0
1-indanol	482.5	432.5

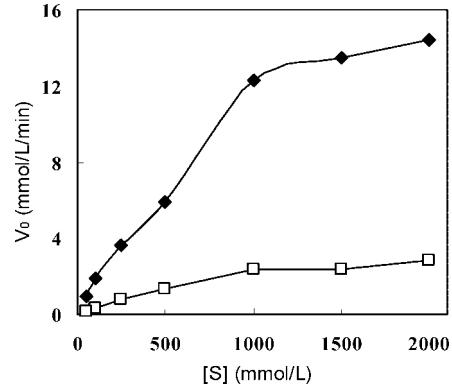


Figure 1. **Effect of substrate concentration on the initial reaction rate: (◆) 1-indanol and (□) 1-phenylethanol.**

that in the case of 1-indanol, vinyl acetate gave a higher ee value than vinyl propionate, whereas a minor effect was shown in the case of 1-phenylethanol. Because a relatively higher ee value is preferred in the Mitsunobu inversion, we chose vinyl acetate as the acyl donor in following experiments.

Effect of substrate concentration on the enzyme activity was investigated. As shown in Figure 1, the initial rate increases linearly with the substrate concentration until the latter reaches 1 mol/L, and further increase of substrate concentration brings a slow increase in the initial rate, which keeps continuously increasing even when substrate concentration tops 2 mol/L. This effect of substrate concentration is far beyond that of using carboxylic acid as the acyl donor.[11]

The effect of temperature on the enzymatic activity was also investigated, indicating that the initial rates for substrates **1** and **2** at 50 °C were 2.3- and 1.6-fold, respectively, that at 30 °C, while the effect on enantioselectivity of substrates **1** and **2** is minor. Therefore we chose 50 °C as the temperature optimum in the following experiments.

Effect of Organic Solvents. Six kinds of organic solvents were used to investigate their effect on the transesterification reaction. The results are shown in Table 2.

Transesterification does not occur in a strongly polar solvent like DMSO, whereas it occurs quickly in hydrophobic solvents (e.g. *n*-hexane, diisopropyl ether, *n*-heptane, and isooctane) with similar enzymatic activity, which is significantly higher than that in the middle-polarity solvent vinyl acetate. The enantioselectivity (*E* value) of the enzyme differs largely among different solvents and substrates. In the case of 1-phenylethanol, the *E* value is significantly higher than that with 1-indanol at a substrate concentration of 100 mM. Among various solvents employed, diisopropyl ether (DIPE) gave relatively high activity and selectivity towards 1-indanol. By comparison of enzyme activity and selectivity against substrates **1** and **2** in different

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Table 2. **Effect of solvents on transesterification of 1-indanol and 1-phenylethanol with vinyl acetate catalyzed by lipase Novozym 435**

	1-indanol		1-phenylethanol	
solvent	initial rate[a]	E^b	initial rate[a]	E^b
n-hexane	1738	3	618	300
diisopropyl ether	**1445**	**55**	**338**	**>1000**
n-heptane	1558	28	633	300
isooctane	1363	32	510	500
vinyl acetate	483	182	80	>1000
DMSO	0	/	0	/

[a] Calculated as mM·min^{-1}·g enzyme^{-1}. [b] The reactions were carried out in a 2-mL volume containing 0.1 M of (±)-**1** or (±)-**2** and 4 mg of Novozym 435 at 50 °C. The enantioselectivity (*E* value) was calculated according to ref 12 by ee_s and ee_p values measured at 25 h of reaction.

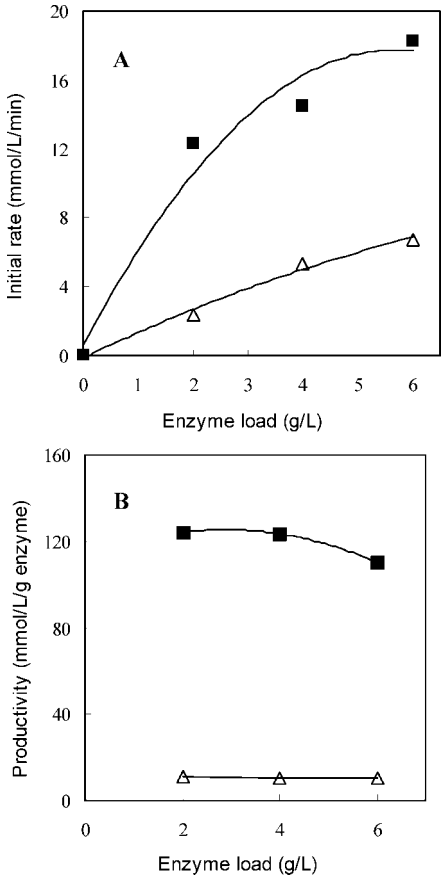

Figure 2. **Initial rate (A) and productivity (B) of the enzymatic transesterification of 1-indanol (■) and 1-phenylethanol (△) with vinyl acetate with different enzyme loads.**

solvents, DIPE was chosen as an appropriate solvent in following experiments.

Effect of Enzyme Load. Using DIPE as the solvent, the effects of the enzyme load on the initial rate of the transesterification and on the enzyme productivity were then studied. It is shown in Figure 2 that initial rates of enzymatic transesterification for substrates **1** and **2** increased along with the enzyme amount, and the augment decreased gradually. Productivity of the enzyme catalyzing transesterification of substrate **1** decreased along with the enzyme amount, whereas in the case of substrate **2** it changed little within the range of enzyme amount examined. Taking into consideration both the catalysis

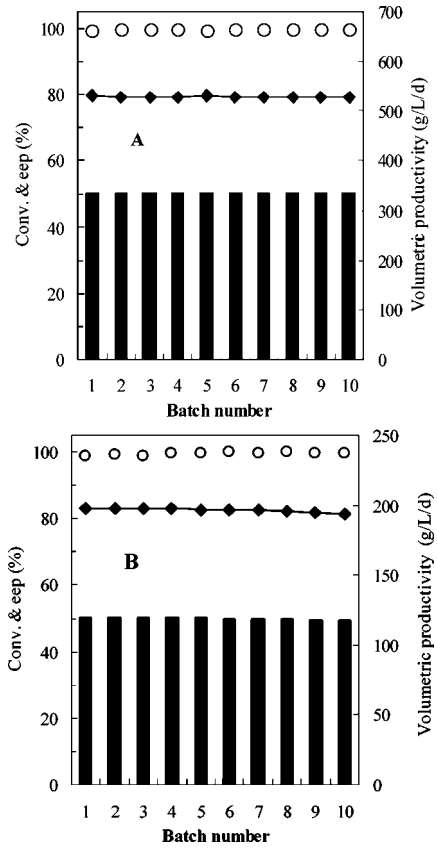

Figure 3. **Repeated batch reactions of enzymatic transesterification of 1-indanol (A) and 1-phenylethanol (B): (solid bar) conversion, (◆) volumetric productivity; (○) ee_p.**

efficiency and catalyst cost, an enzyme load of 6 g/L was adopted in the following repeated-batch reactions.

Operation Stability of the Enzyme. It is shown in Figure 3 that the enzyme remains stable with high activity, enantioselectivity, and stability after 10 batches of transesterification of substrates **1** and **2** at a high substrate concentration of 1 mol/L. After 10 batches of reaction, the conversions for both of these two alcohols were kept at ca. 49.5−50.2%, with ee values above 99% for the unreacted (*S*)-alcohols. Generally, volumetric productivity for biocatalytic reaction on industrial scale[13] ranges from 100 to 1000 g·L^{-1}·d^{-1}. Straathof[14] mentioned that among 134 bioconversions at the industrial level, the average of volumetric productivity for fine chemicals reached 372 g·L^{-1}·d^{-1} (or 15.5 g·L^{-1}·h^{-1}). In this work, the volumetric productivity for enzymatic transesterification of 1-indanol and 1-phenylethanol using merely 6 g of enzyme per liter were 529 and 198 g·L^{-1}·d^{-1} ,respectively, which are approaching or beyond the average value for industrial practice.

After filtering off the enzyme and evaporating the solvent contained in the transesterification mixture collected and combined from 10 batch reactions, the residue was treated by chemical inversion and saponification, followed by purification with silica gel column. Consequently, (*R*)-1-indanol was obtained in 67% isolated yield and 95% ee and (*R*)-1-phenylethanol was obtained in 71% isolated yield and 97% ee.

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In conclusion, the biocatalytic reaction system was carefully optimized for optical resolution of 1-indanol and 1-phenylethanol, resulting in the following optimal conditions: vinyl acetate as acyl donor, DIPE as solvent, initial concentration of alcohol 1 mol/L, enzyme load 6 g/L, and reaction temperature 50 °C. Under these optimized conditions, operational stability of the enzyme for substrates **1** and **2** was examined with 10 batch reactions. After combining the reaction mixture of 10 batches and evaporation of the organic solvent, followed by in situ Mitsunobu inversion of the unreacted alcohols and saponification of the esters, a single enantiomer of each alcohol was obtained. The volumetric productivities of enzymatic transesterification for 1-indanol and 1-phenylethanol were as high as 529 and 198 $g \cdot L^{-1} \cdot d^{-1}$ respectively. This study suggests that chemoenzymatic deracemization has industrial level efficiency and is potentially applicable for the efficient preparation of chiral secondary alcohols in industry. It will also be a reference for the production of other valuable chiral alcohols with high efficiency by biocatalysis.

3. Experimental Section

Enzyme and Reagents. This work was conducted with lipase (Novozym 435) procured from Novozymes Co. (Denmark). 1-Indanol (98% purity) and 1-phenylethanol (97% purity) were purchased from Alfa-Aesar. Other chemicals were from local reagent suppliers and used directly without any further treatment.

Typical Procedure for Transesterification Catalyzed by Novozym 435. Racemic alcohol **1** (1-indanol) or **2** (1-phenylethanol) was added to vinyl acetate (the molar ratio of substrate to acyl donor was 1:5) in a 10-mL test tube (A), and 2 mL of solvent and 4 mg of enzyme were added into another 10-mL test tube (B). After both test tubes were preheated to the required temperature, the contents of test tube A was poured into tube B, and the latter was shaken at the required temperature for an appropriate time. Samples were taken at certain times followed by dilution with ethanol of equal volume for detection of the degree of conversion by gas chromatography or dilution with hexane and isopropyl alcohol (98:2) of 10 times volume for measurement of ee_s and ee_p by HPLC.

Repeated-Batch Reaction. Into a 100-mL Erlenmeyer flask with vinyl acetate (4.60 mL, 50 mmol) dissolved in 4.18 mL of DIPE was added racemic alcohol **1** (1.34 g, 10 mmol) or **2** (1.22 mL, 10 mmol). After these reactants were preheated to 50 °C, 60 mg of carrier-bound lipase (Novozym 435) was added. The mixture was magnetically stirred at 50 °C for 3 h (for alcohol **1**) or 10 h (for alcohol **2**). The reaction was stopped by filtering off the solid enzyme. After washing the enzyme with DIPE (10 mL × 2), fresh substrate was added into the flask for the next reaction, and in total 10 batches of reactions were carried out.

Typical Procedure for Mitsunobu Inversion and Saponification Reaction. After filtering off the enzyme and evaporating the solvent in transesterification mixture, the residue was transferred to a 250-mL flask to which were added AcOH (0.78 g, 13 mmol), PPh_3 (3.40 g, 13 mmol), and 50 mL of diethyl ether. The reaction mixture was immediately cooled to 0 °C, and a solution of diisopropyl azodicarboxylate (DIAD) (2.63 g, 13 mmol) with 50 mL of diethyl ether was added dropwise, under vigorous magnetic stirring during 1 h. The mixture was allowed to warm to room temperature and stirred for 24 h.

To the same flask was added 15 mL of a solution of KOH in methanol (10% w/v), and the reaction mixture was stirred at 50 °C for 2 h. After the saponification was completed (as determined by GC), to this mixture was added 150 mL of demineralized water. The pH was adjusted with hydrochloric acid to 7.0, followed by extraction with dichloromethane (150 mL × 3) and washing of the organic phase with saturated sodium chloride solution (20 mL × 2), and the collected organic phase was then dehydrated overnight with anhydrous sodium sulfate. Concentration of the organic phase followed by silica gel column chromatographic purification of the residue using hexane and ethyl acetate (5:1, v/v) gave only (*R*)-1-indanol and (*R*)-1-phenylethanol. (*R*)-1-Indanol: ^{1}H NMR (CD_3OD, 500 MH_Z) 1.89–1.91 (m, 1H), 2.38–2.40 (m, 1H), 2.74–2.78 (m, 1H), 2.98–3.02 (m, 1H), 5.14 (t, $J = 6.21$, 1H), 7.17–7.38 (m, 4H); MS (EI^+) *m*/*z* 135 (MH^+). (*R*)-1-Phenylethanol: ^{1}H NMR (CD_3OD, 500 MH_Z) 1.43 (d, $J = 6.51$ H_Z, 1H), 4.81 (dd, $J_1 = 12.95$ H_Z, $J_2 = 6.47$ H_Z, 1H), 7.20–7.37 (m, 5H); MS (EI^+) *m*/*z* 123 (MH^+). Qualitative determinations of alcohols were done via TLC analysis, purity and yield by GC, and ee by HPLC.

Analysis. Gas chromatography was used for the determination of reaction conversion (Shimadzu GC-14C, SE-54 quartz capillary column, injector 280 °C, detector 350 °C, column 150 °C, splitting ratio 1:50). The retention times for 1-indanol, 1-phenylethanol, 1-indanyl acetate and 1-phenylethyl acetate were 4.7, 2.9, 7.4 and 3.9 min, respectively. HPLC (Shimadzu) and chiralcel OD-H column (Daicel, Japan) were used for determination of the enantiomeric excess of remaining substrate alcohols and product acetates (hexane/*i*-PrOH 98:2 (v/v), flow rate 1.0 mL/min, UV detector wavelength 212 nm). The retention times (all in minutes) for the alcohols and their esters are as follows: 1-indanol, $t_{(S)} = 17.0$, $t_{(R)} = 20.0$; 1-phenylethanol, $t_{(S)} = 12.9$, $t_{(R)} = 16.3$; 1-indanyl acetate, $t_{(S)} = 5.0$, $t_{(R)} = 6.6$; 1-phenylethyl acetate, $t_{(S)} = 4.9$, $t_{(R)} = 6.2$.

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