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Journal of Molecular Catalysis B: Enzymatic 10 (2000) 531–534

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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# Enantio-reversal in *Candida rugosa* lipase-catalyzed esterification of 3-hydroxybutyric acid

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Received 18 November 1999; received in revised form 22 February 2000; accepted 22 February 2000

**Abstract**

*Candida rugosa* lipase (CRL)-catalyzed esterification of racemic 3-hydroxybutyric acid with different nucleophilic alcohols was studied. The alcohol chain length was found to have a profound role in the enantioselectivity of the reaction. As compared to the esterification with 1-butanol, use of longer alcohols surprisingly led to an enantio-reversal. Under optimized condition, the (*R*)-ester with 95–98% enantiomeric excess (ee) was obtained when the esterification was carried out with 1-hexanol and 1-octanol in the presence of freshly activated molecular sieve 4 Å. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Enantio-reversal; *Candida rugosa* lipase; Nucleophilic alcohols; 3-Hydroxybutyric acid

## 1. Introduction

Despite the vast repertoire of chiral molecules in nature, a majority of them does not fulfil the requirement for the synthesis of various target bioactive compounds. Hence, there is a growing demand for versatile unnatural chirons in organic synthesis. The desirable attributes of these chirons are the presence of varied functionality and availability in both antipodal forms. Biocatalytic transformation is very appealing in this pursuit especially for small substrates. 3-Hydroxybutyric acid (**1**) and its esters are prominent members in the above category and have been extensively used in the syntheses of several classes of natural products [1,2] and many therapeutic agents [3,4]. Especially its ethyl ester has been exploited extensively for the syntheses of compounds with diverse structural features viz. macrolides, spiroketal pheromones, antibiotics, etc.

In view of the above importance, several methodologies have been attempted for the preparation of **1** or its esters in enantiomerically pure or enriched forms. Most of these strategies were based on the asymmetric reduction of ethyl acetoacetate (EAA) using chemical [5] and biological agents [6,7]. The major drawbacks of the chemical process are the use of high temperature and pressure for hydrogenation as well as handling and preparation of pyrophoric Ni catalyst used in this method. In the biocatalytic

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PII: S1381-1177(00)00095-3

methods, baker's yeast mediated reduction of EAA has been studied [6,7] in great detail. However, in spite of its simplicity, this often leads to the product in poor chemical yield and varying optical purities (70–97% enantiomeric excess (ee)) [7]. Use of other microorganisms [8–10] was also less successful. The (*R*)-antipode of the ethyl ester of **1** is best obtained by thermal depolymerization [11] of polyhydroxybutyrate (PHB).

In view of the above shortcomings, we felt that a lipase-catalyzed esterification of commercially available (±)-**1** would be a useful alternative for its enantioselective preparation. Indeed, esterification of **1** with 1-butanol in the presence of *Candida rugosa* lipase (CRL) efficiently produced [12] the ester (*S*)-**1a** in 42% yield and > 97% ee. However, the ee of the unreacted acid ((*R*)-**1**) was poor even under optimized condition. Hence, we studied the effect of various aliphatic alcohols on the course of the reaction, which surprisingly altered the enantioselectivity of the lipase.

## 2. Experimental

Commercial grade *C. rugosa* lipase (CRL, type VII, 952 units/mg of solid, Sigma) and (±)-3-hydroxybutyric acid (Fluka) were used without purification. The alcohols viz. 1-hexanol, 1-octanol (Fluka) and the reaction media viz. diisopropyl ether, toluene, hexane, dichloromethane, THF, benzene (E. Merck, AnalR) were desiccated at 25°C for 72 h over freshly activated 4A° molecular sieve (Linde) prior to use. Column and TLC grade silica gel (Acme, India) were used for column chromatography and preparative TLC, respectively. The IR spectra were scanned with a Perkin-Elmer spectrophotometer model 837. The $^1H$ NMR spectra were recorded in $CDCl_3$ with a Bruker AC-200 (200 MHz) instrument. The optical rotations were measured with a Jasco DIP 360 polarimeter. The GLC analyses were carried out using a Shimadzu GC-7A chromatograph fitted with stainless steel column and flame ionization detector. For normal GLC analyses, 2 mt. × 0.5 mm column was used with $N_2$ flow rate 40 ml/min, while for capillary GLC the same were 50 mt. × 0.25 mm (split ratio 1:100) and 2 ml/min He, respectively.

For chemical reactions, compounds were introduced into the reaction flask via a hypodermic syringe. Reactions under anhydrous conditions were carried out under Ar using freshly dried solvents. The organic extracts were dried over anhydrous $Na_2SO_4$.

### 2.1. Esterification of 3-hydroxybutyric acid (±)-1

A mixture of (±)-**1** (2 mmol), the corresponding alcohol (6 mmol) and CRL (0.5 g) in toluene (20 ml) was magnetically stirred at room temperature for different periods of time. The reaction mixture was diluted with ethyl acetate and the insoluble enzyme removed by filtration. After concentrating under reduced pressure, the resultant product mixture was chromatographed over silica gel (0–20% EtOAc/hexane) to isolate the individual components.

### 2.2. Hexyl 3-hydroxybutyrate (R)-1b

Yield: 0.147 g (39%); $[\alpha]_D^{22}$ −31.0 (c 2.1, $CHCl_3$); IR: 3440, 1730 $cm^{-1}$; PMR: $\delta$ 0.93 (dist. t, 3H), 1.16 (d, $J = 6$ Hz, 3H), 1.3–1.6 (m, 8H), 2.3 (br. s, $D_2O$ exchangeable, 1H), 2.52 (d, $J = 6$ Hz, 2H), 4.03 (t, $J = 6$ Hz, 2H), 4.1–4.4 (m, 1H).

### 2.3. Octyl 3-hydroxybutyrate (R)-1c

Yield: 0.125 g (29%); $[\alpha]_D^{22}$ −29.3 (c 1.7, $CHCl_3$); IR: 3380, 1730 $cm^{-1}$; PMR: $\delta$ 0.9 (dist. t, 3H), 1.1 (d, $J = 6$ Hz, 3H), 1.3–1.6 (m, 12H), 2.41 (br. s, $D_2O$ exchangeable, 1H), 2.5 (d, $J = 6$ Hz, 2H), 4.0 (t, $J = 6$ Hz, 2H), 4.1–4.3 (m, 1H).

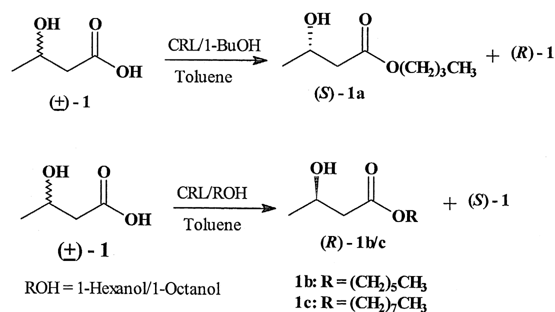
### *2.4. Preparation of (R)-1,3-butanediol from (R)-1b*

To a stirred suspension of lithium aluminium hydride (LAH) (0.114 g, 3.0 mmol) in ether (20 ml), the ester (***R***)-**1b** (2.0 mmol) was added. After stirring for 12 h, the reaction was quenched with aqueous saturated $Na_2SO_4$, the precipitate filtered, the residue washed with EtOAc and the combined organic extracts concentrated in vacuo. The residue was purified by preparative TLC (5% MeOH/$CHCl_3$). Yield: 0.126 g (70%); $[\alpha]_D^{22}$ −30.28 (c 1.2, EtOH), (lit. [13] $[\alpha]_D^{22}$ −31.0 (c 1.5, EtOH)); IR: 3480, 1060 $cm^{-1}$; PMR: $\delta$ 1.15 (d, $J = 6$ Hz, 3H), 1.4–1.6 (m, 2H), 2.7 (br. s, $D_2O$ exchangeable, 2H), 3.6–3.9 (m, 3H).

## 3. Results and discussion

For the present study, CRL-catalyzed esterification of (±)-**1** was carried out using two aliphatic alcohols viz. 1-hexanol and 1-octanol. Since all lipase-catalyzed reactions involve two substrates, the nucleophile (water, alcohol, etc.) and an acyl donor which must bind in close proximity to the active site of the enzyme, in principle, the enantioselectivity might be governed by both reaction partners. In fact, the effect of acyl chain length [14] on the enantioselectivity of several such reactions has been studied. However, information related to the role of the nucleophiles on enantio-discrimination in the resolution of acids/esters is rather scarce. Recently, a reversal of enantio-selectivity in the esterification of 2-methylalkanoic acids with alcohols of different chain lengths has been reported [15].

Esterification of (±)-**1** with 1-hexanol in toluene gave the ester (*R*)-**1b** and the unreacted acid (*S*)-**1** in 24 and 72% isolated yields, respectively (Scheme 1). Interestingly, in contrast to butyrylation [12], esterification with 1-hexanol proceeded preferentially with (*R*)-**1** albeit with poor enantioselectivity (Table 1). The poor result can possibly be ascribed to the slowness of the reaction caused by (i) the longer chain length of the chosen alcohol and (ii) the water produced in situ, which restricts the approach of the substrate molecules to the enzyme. Hence, the reaction was carried out in the presence of freshly activated molecular sieve powder 4 Å. This not only improved the yield, but also gave (*R*)-**1b** and (*S*)-**1** with > 96% and 75% ee, respectively.




Scheme 1.

The optical purities of (*R*)-**1b** and (*S*)-**1** (as its methyl ester) were determined by their $^1H$ NMR spectra in presence of Eu(hfc)$_3$. The stereochemical outcome of the reaction was easily deduced by direct by comparison of the $[\alpha]_D$-value of the resolved acid **1** with our previous results [12]. In addition, different (*S*)-esters of **1** are known [16] to have positive specific rotation. Since our product (*R*)-**1b** was levorotatory, it must possess (*R*)-configuration. For further confirmation, the reaction products viz. (*S*)-**1** and (*R*)-**1b** were individually reduced with LAH to afford the enantiomers of butane-1,3-diol. Correlation of their $[\alpha]_D$-values with those reported [13] established their configurations unambiguously.

Considering that water is a detrimental factor in this reaction, the esterification was also carried out with the lipase predried for 4 h under vacuum (entry 3, Table 1). However, very little conversion was observed possibly due to the extreme water susceptibility of CRL. Clearly, the reaction was not totally stereoselective as is evident from the % ee of the resolved acid.

Table 1
Effect of alcohols on CRL-catalyzed esterification of **1**

| Entry | Alcohol | Condition | Time (h) | Yield (%) | | ee (%) | |
|---|---|---|---|---|---|---|---|
| | | | | Ester | Acid | Ester | Acid |
| 1 | 1-Hexanol | No mol. sieve | 24 | 12 | 72 | 12.0 | 10.0 |
| 2 | 1-Hexanol | mol. sieve | 24 | 39 | 56 | 96.1 | 75.0 |
| 3 | 1-Hexanol | mol. sieve, dried lipase | 24 | 10 | 84 | 8.0 | n.d. |
| 4 | 1-Hexanol | mol. sieve | 17 | 31 | 60 | 98.0 | 60.0 |
| 5 | 1-Hexanol | mol. sieve, 1.0 eq. alcohol | 24 | 24 | 70 | 95.9 | 57.0 |
| 6 | 1-Octanol | mol. sieve | 24 | 29 | 64 | 93.0 | 69.2 |

Hence, we carried out the reaction for a shorter period (17 h) (entry 4, Table 1), when a marginal improvement in the % ee was observed for the ester (*R*)-**1b**. Use of a stoichiometric quantity of the alcohol (1.0 eq) had little effect on the enantioselectivity of the reaction (entry 5, Table 1). Introduction of an additional amount of CRL (0.05 g/ml of solvent) neither improved the yield of (*R*)-**1b** nor its % ee even after 48 h. In our previous study [12], toluene was found to be the best solvent for the reaction. Hence, the present study was mainly conducted in this solvent. Subsequently, the effect of some other solvents was also checked (data not shown) and no improvement was noticed.

In order to find the generality of the protocol, the reaction was carried out with 1-octanol, which produced similar results (entry 6, Table 1). Expectedly, the higher chain length of the alcohol slowed down the reaction. Furthermore, the higher boiling point of the nucleophile led to difficulties in the isolation of the ester (*R*)-**1c**. The regenerated enzyme after washing with hexane retained almost all of its initial activity even after five cycles. This feature could be useful for preparative scale reactions and was employed for the preparation of (*R*)-**1b** on a multi-gram scale.

In conclusion, we have developed a simple route for the preparation of the versatile chiron, (*R*)-3-hydroxybutyrate via a CRL-catalyzed esterification of the commercially available acid **1**. This method in conjunction with the complementary protocol developed earlier from our laboratory [12] can lead to both antipodes in high enantiomeric purity. The present method also constitutes a novel protocol for tailoring lipase specificity by the substrate chemistry.

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