Title
Lipase-catalyzed Asymmetric Transesterification of 3-Hydroxydithiopentanoate in an Organic Solvent
(Commemoration Issue Dedicated to Professor Shigeo Tanimoto On the Occasion of His Retirement)

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Citation

Issue Date
1992-10-30

URL
http://hdl.handle.net/2433/77461

Type
Departmental Bulletin Paper

Textversion
publisher
Kyoto University
Lipase-catalyzed Asymmetric Transesterification of 3-Hydroxydithiopentanoate in an Organic Solvent

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Received July 27, 1992

Chiral 3-hydroxydithiopentanoate has been prepared via resolution of racemic compound with a lipase in an organic solvent.

KEY WORDS: Asymmetric synthesis / Biocatalyst / Lipase / Enantioselective / Transesterification

The application of biocatalysts in selective transformations of synthetic substrates is a useful method to provide chiral building blocks of organic synthesis4-5. Especially, chiral 3-hydroxyalkanoates have been used as versatile chiral building blocks because of the facility to prepare these compounds asymmetrically by yeast reduction6-8 or catalytic hydrogenolysis9. On the other hand, their sulfur analogs, chiral 3-hydroxydithioalkanoates have not received so much attention probably because of difficulty in preparing chiral derivatives. However, dithioalkanoates are known to have quite interesting properties and are receiving increased interest in asymmetric syntheses as useful chirons10,11. In the present paper, we would like to report kinetic resolution of ethyl 3-hydroxydithiopentanoate by using lipase-catalyzed transesterification in an organic solvent. The use of organic solvents in biocatalytic reactions has several advantages: 1) easy separation of biocatalyst from the product, 2) increase in solubility of nonpolar substrates, 3) protection of enzymes from inhibition by a substrate under high concentrations, 4) to keep the reaction system free from water-dependent side reactions.

Methyl 3-hydroxydithiopentanoate (1), which is not soluble to water and unstable under aerobic conditions, is a suitable substrate to exploit the reaction in organic solvents. We, therefore, investigated several lipases for their catalytic activity in transesterification of 1 with isopropenyl acetate as an acylating reagent as well as the solvent.

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Lipase-catalyzed Transesterification

Results

Racemic 1 was prepared by the reaction shown in Scheme 1; methyl dithioacetate was prepared by the reaction of methyl magnesium bromide with carbon disulfide, which was followed by methylation with methyl iodide\textsuperscript{12).} The reaction of methyl dithioacetate with propionaldehyde in the presence of sodium hydride gave 1 in 9.9\% overall yield. A lipase to be screened was added to a solution of 1 in isopropenyl acetate and the reaction was followed by HPLC. The results are listed in Table 1. Four lipases exerted the activities in transesterification and were used for further studies.

The enantioselectivity of lipase-catalyzed reaction was determined as follows. When the starting material was consumed about 50\%, the reaction was quenched and the amount of the starting material unreacted as well as the enantiomeric excess (e.e.) in 1 and the corresponding acetate (2) were measured. The e.e. of the acetate was determined with \textsuperscript{1}H NMR analysis using (+)-Eu(hfc)\textsubscript{3} as a chiral shift reagent. The e.e. of 1 was determined after converting it into the corresponding acetate chemically with AcCl/pyridine in benzene. The absolute configuration of the chiral 1 was determined by converting it into the corresponding carboxylic ester (3) as shown in Scheme 2. The results of lipase-catalyzed reactions are summarized in Table 2.

\begin{scheme}
1) CS\textsubscript{2}/THF
2) Mel

\begin{equation}
\text{CH}_3\text{MgBr} \quad \text{CS}_2/\text{THF} \quad \text{Me} \quad \text{SMe} \quad \text{CH}_3\text{CHO}/\text{THF} \quad \text{NaH} \quad \text{OH} \quad \text{S} \quad \text{Me} \quad \text{SMe} \quad 1
\end{equation}
\end{scheme}

<table>
<thead>
<tr>
<th>Lipase (origin)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amano AY (Candida rugosa)</td>
<td>+</td>
</tr>
<tr>
<td>Amano P (PCL) (Pseudomonas cepacia)</td>
<td>+</td>
</tr>
<tr>
<td>CCL (Candida rugosa)</td>
<td>+</td>
</tr>
<tr>
<td>Toyobo Type A (TA) (Pseudomonas sp.)</td>
<td>+</td>
</tr>
<tr>
<td>Amano A (Aspergillus niger)</td>
<td>−</td>
</tr>
<tr>
<td>Amano AK (Pseudomonas sp.)</td>
<td>−</td>
</tr>
<tr>
<td>Amano CE (Humicola lanuginosa)</td>
<td>−</td>
</tr>
<tr>
<td>Amano D-10 (Rhizopus delamari)</td>
<td>−</td>
</tr>
<tr>
<td>Amano F-API5 (Rhizopus javanicus)</td>
<td>−</td>
</tr>
<tr>
<td>Amano G (Penicillium sp.)</td>
<td>−</td>
</tr>
<tr>
<td>Amano GC (Geotrichum candidum)</td>
<td>−</td>
</tr>
<tr>
<td>Amano L (Candida lipolytica)</td>
<td>−</td>
</tr>
<tr>
<td>Amano M (Mucor javanicus)</td>
<td>−</td>
</tr>
<tr>
<td>Amano N (Rhizopus niveus)</td>
<td>−</td>
</tr>
<tr>
<td>Amano R (Penicillium roqueforti)</td>
<td>−</td>
</tr>
<tr>
<td>PLE (Porcine liver)</td>
<td>−</td>
</tr>
<tr>
<td>PPL (Porcine pancreas)</td>
<td>−</td>
</tr>
</tbody>
</table>

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Scheme 2

\[
\begin{align*}
\text{Lipase from} & \quad \text{Pseudomonas} \\
\text{MeOH} \quad \text{CuO/CuCl}_2 \\
\text{(-)-(S)-methyl 3-hydroxypentanoate}
\end{align*}
\]

Table 2. Resolution of 1 with lipase

<table>
<thead>
<tr>
<th>Lipase</th>
<th>Temp./°C</th>
<th>Time/h</th>
<th>Conv./%</th>
<th>Yield/%</th>
<th>([\alpha]D)</th>
<th>e.e./%(Config.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>PCL</td>
<td>25</td>
<td>191</td>
<td>49</td>
<td>48</td>
<td>45</td>
<td>+18.9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>60</td>
<td>46</td>
<td>44</td>
<td>43</td>
<td>+18.9</td>
</tr>
<tr>
<td>TA</td>
<td>25</td>
<td>20</td>
<td>49</td>
<td>51</td>
<td>49</td>
<td>+14.5</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>6</td>
<td>54</td>
<td>42</td>
<td>44</td>
<td>+16.6</td>
</tr>
<tr>
<td>AY</td>
<td>25</td>
<td>17</td>
<td>47</td>
<td>51</td>
<td>45</td>
<td>-6.2</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>68</td>
<td>42</td>
<td>41</td>
<td>40</td>
<td>-3.9</td>
</tr>
<tr>
<td>CCL</td>
<td>25</td>
<td>400</td>
<td>43</td>
<td>45</td>
<td>44</td>
<td>-5.2</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>315</td>
<td>45</td>
<td>44</td>
<td>37</td>
<td>-4.2</td>
</tr>
</tbody>
</table>

a) 100×[2]/([1]+[2]). b) Chemical yield, 100×[1 or 2]/([1] at 0 h).

Discussion

Four lipases exerted the activity in transesterification and, among those, Amano P (PCL) and Toyobo Type A (TA) are originated from Pseudomonas, and Amano AY (AY) and CCL are produced from Candida rugosa. Therefore, it is concluded that the lipases originated from those two species only can exert activity in transesterification. Furthermore, both lipases from Pseudomonas (PCL and TA) react with the (R)-enantiomer of 1 selectively and the other two lipases from Candida react preferentially with the (S)-enantiomer of 1 as shown in Scheme 3, although the selectivity of the latter enzymes are relatively low.

The lipase-catalyzed transesterification of ethyl 3-hydroxybutanoate (4), the oxygen version of 1, in an organic solvent has been reported. It is interesting to
compare the reactivity and stereochemistry of 1 with those of 4. The reaction of 4 proceeds in the order; CCL > PCL > PPL. However, PCL is much more active than CCL in the reaction with the dithioester, while PPL exerts no activity on it. Although PCL reacts with the (R)-enantiomer of both alcohols, CCL prefers the different enantiomer of 1 from that of 4. It seems plausible that the cavity of the enzyme at the catalytic site is smaller in CCL than that in PCL and the bulky dithocarboxyl group is not accepted at the correct position of cavity in CCL. It should be noted that not only the change in stereochemistry but also tremendous retardation of the reaction is seen in the reaction of 1 with CCL.

Thus, the present reaction is worth to be employed for synthesizing chiral building blocks. Namely the reaction with PCL exerts a high enantioselectivity (E = 90) in the transesterification, which indicates that resolution of 1 proceeds smoothly and selectively.

**Experimental**

**Instruments**

$^1$H NMR spectra were recorded on a Varian VXR-200 (200 MHz) in CDCl$_3$ with Me$_4$Si as an internal reference. Gas chromatography was recorded on a Shimazu GL 14 A gas chromatograph equipped with an OV 1701 capillary column (0.25 mm $\times$ 25 m) for analytical purpose, whereas for preparative separation, a Varian Aerograph Model 920 gas chromatograph was employed. HPLC was performed with a Hitach 655 Liquid Chromatography (pump) and IRICA 852-III spectrometer, using a 4.6 $\times$ 150 mm Wacosil 5 SIL column. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

**Materials**

Organic reagents were purchased from Nacalai Tesque Co. and Aldrich Chemical Co. unless otherwise indicated. Solvents and commercially available starting materials were generally used without additional purification unless otherwise indicated. Pyridine and benzene were refluxed on calcium hydride for 1 day and distilled before the use.

**Preparation of Methyl 3-hydroxydithiopentanoate (1). Methyl dithioacetate**

A stirred mixture of methyl magnesium bromide (prepared from 0.6 mol of magnesium ribbon and 0.46 mol of methyl bromide) in 150 ml of tetrahydrofuran (THF) was cooled to $-10^\circ$C and a THF solution of carbon disulfide (0.46 mol) was
added dropwise to the mixture. The resulted mixture was stirred at 60°C for 1 h, then the mixture was cooled to 15°C and a THF solution of methyl iodide (0.46 mol) was added dropwise. The whole mixture was stirred for 1 h. Ice-cold water (100 ml) and ether were added to the mixture successively and the organic materials were extracted. The ethereal solution was washed with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was distilled yielding 28.8 g (0.27 mol, 58.6%) of methyl dithioacetate: bp 44°-45°C / 22 mm Hg. 1H NMR (CDCl₃) δ 2.61 (s, 3 H), 2.86 (s, 3 H).

Methyl 3-hydroxydithiopentanoate (1).

A THF solution of methyl dithioacetate (0.19 mol) and propionaldehyde (0.19 mol) was added gradually to a cooled (−20 °C) mixture of sodium hydride (0.23 mol) in THF (30 ml). The reaction mixture was stirred for additional 30 min at the same temperature. Ice-cold water was added to the mixture and the mixture was acidified by diluted hydrochloric acid (pH 1). The organic materials were extracted with ether and the ethereal solution was washed with water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was distilled yielding 6.4 g (33 mmol, 17.3%) of methyl 3-hydroxydithiopentanoate (1): bp 85°-93°C/5 mm Hg. 1H NMR (CDCl₃) δ 0.98 (t, 3H, J=7.4 Hz), 1.50-1.65 (m, 2H), 2.64 (s, 3H), 2.87 (d, 1H, J=3.8 Hz), 3.00-3.26 (m, 2H), 3.97-4.14 (m, 1H).

Recemic methyl 3-acetoxydithiopentanoate (rac-2).

Acetyl chloride (7.5 mmol) and pyridine (0.6 ml) were added successively to the solution of 1 (0.75 mmol) in dry benzene. After stirring for 1 h, the reaction mixture was filtered and the benzene was removed from the filtrate in vacuo giving 2. 1H NMR (CDCl₃) δ 0.94 (t, 3H, J=7.4 Hz), 1.60-1.75 (m, 2H), 2.03 (s, 3H), 2.62 (s, 3H), 3.15-3.40 (m, 2H), 5.28-5.42 (m, 1H).

Screening of lipases.

Isopropenyl acetate (1 ml) and 1 (0.36 mmol) were added to a lipase (60 mg) and the mixture was stirred under an argon atmosphere. At appreciate time intervals, 1 μl each of the reaction mixture was taken up and dissolved in 4 ml of dichloromethane and the solution was filtered through a short silica-gel column (Bond Elut). The filtrate was subjected to HPLC. Results are summarized in Table 1.

Transesterification with 1 under lipase-catalysis: general procedure.

Isopropenyl acetate (1 ml) and 1 (0.36 mmol) were added to a lipase (60 mg) and the mixture was stirred under an argon atmosphere. The reaction was monitored as described above. When the conversion reached about 50%, the reaction mixture was transferred onto Bond Elute and was filtered by using dichloromethane (10 ml) as an eluent in order to remove the lipase. The filtrate was concentrated in vacuo and a part of the residue was subjected to 1H NMR analysis in order to determine the amount of converted starting material. The sample used for the NMR anglysis was evaporated in vacuo and the combined residue was subjected to column
Lipase-catalyzed Transesterification chromatography on silica gel using dichloromethane as an eluent yielding 1 and 2, respectively. In order to determine the e.e. in the product, 17 mg of 2 and 34 mg (0.3 equivalent) of \((+)-\text{Eu(hfc)}_3\) were added to 0.6 ml of CDCl\(_3\) and analyzed by \(^1\)H NMR spectroscopy. The e.e. of 2 was determined by comparing the areas of signals from acetoxy-methyl protons in each enantiomer. Chemical shifts of products from the (S)-acetate appeared at higher field than those from the (R)-enantiomer. E.e. of 1 was determined after converting it into the corresponding acetate chemically with AcCl/pyridine.

**Absolute configuration of chiral 1.**

Absolute configuration of 1 was determined according to Scheme 2. Isopropenyl acetate (4 ml), 1 (1.4 mmol), and PCL (240 mg) were mixed and stirred under an argon atmosphere for 60 h. The reaction mixture was filtered through Bond Elut and washed with dichloromethane. The combined filtrate was subjected to a column chromatography as described above affording 1 (0.64 mmol, 46%, \([\alpha]_{D}^{20} = +18.1^\circ (c, 0.94 \text{ CHCl}_3)) and 2 (0.60 mmol, 43%, \([\alpha]_{D}^{20} = -71.9^\circ (c, 0.83 \text{ CHCl}_3))

A solution of thus prepared (+)-1 (0.59 mmol) in methanol (8 ml) was added dropwise to a mixture of copper(II) oxide (1.8 mmol) and copper(II) chloride (1.8 mmol) in methanol (8 ml). Water (0.1 ml) was added to the mixture and the resulted mixture was stirred for 12 h. The mixture was filtered and the filtrate was evaporated in vacuo and the residue was subjected to a preparative gas chromatography (PEG, 1.5 m, 120°C) yielding methyl 3-hydroxybutanoate (0.17 mmol, 22%, \([\alpha]_{D}^{20} = -29.4^\circ (c, 0.87 \text{ CHCl}_3))

Since the optical rotation of (R)-methyl 3-hydroxydithiopentanoate was reported to be −36.9°, the absolute configuration of 1 obtained from the lipase reaction (unreacted starting material) was established as S.

**References**

1) On leaf from Department of Applied Chemistry, Osaka Institute of Technology, Asahi-ku, Osaka 353, Japan.
14) $E = \frac{\ln(1-c)(1-\text{ee}(S))}{\ln(1-c)(1+\text{ee}(S))}$, where $c$=conversion, ee(S)=e.e. of the starting material. C-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.*, 104, 7294 (1982).