Note

Stereoselective Reduction of \( \alpha \)- and \( \beta \)-Keto Esters with Aerobic Thermophiles, Bacillus Strains

Kohji Ishihara, Keisuke Iwai, Hitomi Yamaguchi, Nobuyoshi Nakajima, Kaoru Nakamura, and Toshihisa Ohshima

Department of Chemistry, Kyoto University of Education, Fushimi-ku, Fukakusa, Kyoto 612, Japan
*Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-11, Japan
**Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

Received June 10, 1996

The first example of stereoselective reduction with aerobic thermophiles is reported. Various \( \alpha \)- and \( \beta \)-keto esters were reduced stereoselectively to the corresponding alcohols by the aerobic thermophiles, Bacillus strains. In particular, the reduction of ethyl 3-methyl-2-oxobutanate with B. steatorrhophilus DSM 297 gave the corresponding (R)-alcohol with high yield in excellent enantioselectivity (>99% e.e.). The conversions of keto esters to the corresponding hydroxy esters with Bacillus strains were increased by introduction of glycerol in the reaction mixture as an additive.

Key words: stereoselective reduction; keto esters; aerobic thermophile; Bacillus steatorrhophilus

Microbial conversion has been studied widely for preparation of various chiral compounds. For example, baker’s yeast has been used often for the reduction of pro-chiral ketones to obtain optically active alcohols. The alcohols are used as useful chiral building blocks for the synthesis of various biologically active compounds. Until now, the reductions of various carbonyl compounds with an anaerobic thermophile, Thermoanaerobacter brockii DSM 1457 (Thermoanaerobium brockii) has been the only one reported. The commercial TBADH (the alcohol dehydrogenase from T. brockii) has high thermostability and high stereospecificity toward various ketones. Therefore, the enzyme is available for organic syntheses. However, a little information has been known about the reduction of carbonyl compounds using other thermophiles as biocatalysts. In this paper, we would like to report the stereoselective reduction of \( \alpha \)- and \( \beta \)-keto esters with aerobic thermophilic bacteria.

The bacteria (Bacillus strains) were incubated in the medium (200 ml) for 48 h at 55°C aerobically with vigorous shaking in baffled 500-ml flasks. The wet cells were collected by centrifugation at 5000 \( \times g \) for 15 min. Saline (20 ml) and the substrate (0.1 mmol) were added to the collected wet cells (0.2 g) and the reaction mixture was incubated at 37°C for 20 h, aerobically shaking. The conversion ratio (%) and enantioselectivity (e.e. %) of the product were measured by gas chromatography (HR-20M capillary column. 0.25 mm \( \times \) 20 m, 100°C or 150°C, and Chiraldex G-TA optically capillary column. 0.25 mm \( \times \) 30 m, 110°C) analysis.

Twenty-six selected Bacillus strains were tested for the reducing activity of \( \alpha \)- and \( \beta \)-keto esters. The results of the conversion of \( \beta \)-ketoester (1a-d) and \( \alpha \)-keto ester (3a-c) to the corresponding alcohols with ten Bacillus strains are summarized in Table 1.

It was found that various keto esters were converted to the corresponding alcohols with Bacillus strains. In the conventional method (without additive), the substrates (1a-d, 3a-c) were reduced to the corresponding alcohol (2a-d, 4a-c) with a low conversion ratio (as shown in parentheses in Table 1), while these ratio were increased by introduction of glycerol in the reaction mixture as an additive. The addition of glycerol increased only the ratio in almost cases tested, however introduction of other additives (citric acid, malic acid, glucose, 2,3-butanediol, 2-propanol, methanol, ethanol, and 1-butanol) did not increased the ratio in the reaction (under 5 mm of substrate and 250 mm of additives). The addition of 50-fold the molar quantity of glycerol against the substrate gave the satisfactory results.

In the stereochimistry of products, the reduction of ethyl 2-methyl-3-oxobutanate (1d) by three Bacillus strains (DSM 297, 730, and 2027) gave the corresponding \( \beta \)-hydroxy alcohol (2d) in high diastereoselectivity (syn/anti = 80-89/11-20) compared with a baker’s yeast reduction of the same substrate. Further, the enantioselectivity of syn-(2R,3S)- and anti-(2S,3S)-2d reduced by Bacillus strains were >99% e.e. and >98% e.e., respectively.

Many useful methods for the reduction with bakers’ yeast have been reported. Among them, we found that the addition of a third reagent to the reaction system changed the stereoselectivi-

\[
\text{Scheme}
\]

1a: \( R_1 = \text{Cl}, R_2 = \text{H}, R_3 = \text{Et} \)
1b: \( R_1 = \text{Me}, R_2 = \text{H}, R_3 = \text{Me} \)
1c: \( R_1 = \text{Me}, R_2 = \text{H}, R_3 = \text{Et} \)
1d: \( R_1 = \text{Me}, R_2 = \text{Me}, R_3 = \text{Et} \)
2a: \( R_4 = \text{Me}, R_5 = \text{Et} \)
2b: \( R_4 = \text{Me}, R_5 = \text{Et} \)
3a: \( R_4 = \text{Me}, R_5 = \text{Et} \)
3b: \( R_4 = \text{Me}, R_5 = \text{Et} \)
3c: \( R_4 = \text{Ph}, R_5 = \text{Et} \)

\( ^1 \) To whom correspondence should be addressed.

\( ^{**} \) Present address: Department of Biological Science and Technology, Faculty of Engineering, Tokushima University, Minami-Jyosanjima, Tokushima 770, Japan.
Table I. The Conversion (%) of Substrates to the Corresponding Alcohols by *Bacillus* Strains with Glycerol

<table>
<thead>
<tr>
<th>Bacillus strains</th>
<th>DSM No.</th>
<th><em>β</em>-Keto esters</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1a</td>
<td>1b</td>
<td>1c</td>
<td>1d</td>
<td>3a</td>
<td>3b</td>
<td>3c</td>
<td>3d</td>
<td></td>
</tr>
<tr>
<td><em>B. steaerothermophilus</em></td>
<td>297</td>
<td>23</td>
<td>20</td>
<td>8</td>
<td>8</td>
<td>29</td>
<td>13</td>
<td>73</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><em>B. steaerothermophilus</em></td>
<td>457</td>
<td>34</td>
<td>69</td>
<td>32</td>
<td>8</td>
<td>7</td>
<td>13</td>
<td>32</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><em>B. smithii</em></td>
<td>460</td>
<td>22</td>
<td>00</td>
<td>14</td>
<td>9</td>
<td>11</td>
<td>1</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>B. sphaericus</em></td>
<td>461</td>
<td>9</td>
<td>0</td>
<td>14</td>
<td>9</td>
<td>8</td>
<td>28</td>
<td>78</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><em>B. thermocatenulatus</em></td>
<td>465</td>
<td>19</td>
<td>13</td>
<td>21</td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>466</td>
<td>42</td>
<td>11</td>
<td>19</td>
<td>2</td>
<td>39</td>
<td>2</td>
<td>6</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>494</td>
<td>13</td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>14</td>
<td>50</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>730</td>
<td>38</td>
<td>11</td>
<td>20</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>1550</td>
<td>7</td>
<td>0</td>
<td>17</td>
<td>4</td>
<td>80</td>
<td>14</td>
<td>57</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>2027</td>
<td>14</td>
<td>4</td>
<td>11</td>
<td>6</td>
<td>40</td>
<td>39</td>
<td>34</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

- The conversion ratios of the substrates to the corresponding alcohols were measured by GLC with a capillary column HR-20M (0.25 mm x 25 m).
- The substrates (5 mm), glycerol (250 mm), and saline (20 ml) were added to the wet cells (0.2 g) and the reaction mixture were incubated at 37°C for 20 h.
- The conversion ratios without glycerol are shown in parentheses.

Table II. Reductions of *β*-Keto Esters (1d) and *α*-Keto Esters (3b) by *Bacillus* Strains

<table>
<thead>
<tr>
<th>Ethyl 2-methyl-3-oxobutanoate (1d)</th>
<th>Ethyl 3-methyl-2-oxobutanoate (3b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus strains</strong></td>
<td><strong>DSM No.</strong></td>
</tr>
<tr>
<td><em>B. steaerothermophilus</em></td>
<td>297</td>
</tr>
<tr>
<td><em>B. sphaericus</em></td>
<td>461</td>
</tr>
<tr>
<td><em>B. thermocatenulatus</em></td>
<td>730</td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>1550</td>
</tr>
<tr>
<td><em>B. stearothermophilus</em></td>
<td>2027</td>
</tr>
</tbody>
</table>

- Saline (20 ml), the substrates (5 mm), and glycerol (250 mm) were added to the wet cells (0.2 g) and the reaction mixtures were incubated at 37°C for 20 h.
- Isolated yields.
- The ratios of syn/anti were measured by GLC with a capillary column HR-20M (0.25 mm x 25 m).
- The e.e. (%) and configuration were measured with GLC with an optically active capillary column ChiralDEX G-TA (0.25 mm x 20 m).

The conversion ratio of the substrates to the corresponding alcohols were measured by GLC with a capillary column HR-20M (0.25 mm x 25 m). The substrates (5 mm), glycerol (250 mm), and saline (20 ml) were added to the wet cells (0.2 g) and the reaction mixture were incubated at 37°C for 20 h. The conversion ratios without glycerol are shown in parentheses.

Further, the reduction of ethyl 3-methyl-2-oxobutanoate (3b) by four *Bacillus* strains (DSM 297, 461, 466, and 1550) also gave the corresponding *α*-hydroxy ester (4b) in high enantiopurity as shown in Table II.

In particular, the reduction of 3b with *B. stearothermophilus* DSM 297 afforded (R)-4b in high chemical yield (82%) with excellent stereoselectivity (>99 e.e.). These results indicated that the reduction of keto esters with *Bacillus* strains would be available for a tool for organic asymmetric syntheses.

This work is the first example of the stereoselective reduction of carbonyl compounds by aerobic thermophiles.

The mechanism for the improvement of conversion ratio by addition of glycerol is not clear. It seems that the increase of reduced nicotinamide-adenine dinucleotide (NADH or NADPH) due to the oxidative degradation of glycerol in the cells of these aerobic thermophiles would accelerate the stereoselective reduction of α- and β-keto esters to the corresponding optically pure alcohols. Further detailed studies including purification of the enzymes which contribute to the reduction system are now in progress in our laboratory.

**References and Notes**

7. A synthetic culture medium containing 1 liter of water, bactopeptone (15 g), yeast extract (2 g), meat extract (2 g), KH₂PO₄ (2 g), KH₂PO₄ (2 g), MgSO₄, 7H₂O (0.1 g), adjusted to pH 7.2 with 1 N-KOH.

