The enantioselective β-keto ester reductions by *Saccharomyces cerevisiae*

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Abstract: The enantioselective yeast reduction of aromatic β-keto esters, by use of potassium dihydrogen phosphate, calcium phosphate (monobasic), magnesium sulfate and ammonium tartrate (diammonium salt) (10:1:1:50) in water at pH 7 as a buffer for 72–120 h with 45–90 % conversion to the corresponding aromatic β-hydroxy esters was achieved by means of *Saccharomyces cerevisiae*.

Keywords: bioreductions, yeast-catalyzed reductions, aromatic β-keto esters, *Saccharomyces cerevisiae*, enantioselective reduction.

INTRODUCTION

Biotransformations by baker’s yeast in aqueous media are currently receiving significant attention as synthetic tools for accomplishing efficient organic manipulations.1 Optically active 3-hydroxybutanoate moieties serve as valuable chiral building blocks for the synthesis of natural products.2 The methodology of *S. cerevisiae* reduction has been applied to other dicarbonyl moieties, such as β-keto esters,2f α-keto ester,3 α-keto phosphates,4 α-diketones,5 and certain ketones.2f,g The reductions frequently show a high degree of stereoselectivity. Recently, the enantio-, regio-, and chemoselectivity in the reduction of several symmetrical and nonsymmetrical para-substituted benzil derivatives was investigated under aerobic fermenting conditions via *S. cerevisiae*6 in the presence of potassium phosphate buffer (pH 7, 0.01 M) in aqueous media with copious production of carbon dioxide. It is generally assumed that the stereoselectivity of the yeast reduction of acyclic ketones will be governed by the predictions of the Prelog rule, depending upon the relative size of the groups R_S and R_L, as they are recognized by the oxidoreductase in the yeast (Scheme 1).6,7

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RESULTS AND DISCUSSION

The two faces of the pre-formed carbonyl group of the substrate are enantiotopic. Hence, by controlling the addition so that it occurs mainly from one face, the reaction gives almost exclusively single enantiomers of 1–5. Both the enzyme and NADH are single enantiomers and they cooperate by binding to the substrate (to some extent pyruvic acid prototype) in specific way, as shown in the adopted model system (Scheme 1), so that the hydride is transferred to one enantiotopic face of the ketone. However, Scheme 1 can not be taken to imply the absolute configuration of 2–5. Even though the chiral reduction of achiral ethyl acetoacetate and 3-oxo-3-phenylpropanoic acid ethyl ester into a chiral (S)-(+)6 and (S)-(−)-13,10 were previously reported, to the best of our knowledge the reduction of aromatic β-keto esters, such as 2–5 (Scheme 1 and Fig. 1), has not been reported. Synthesis of compound 4 in 96.7 % yield has recently been published. Unfortunately, the analysis was by chiral GC-HPLC and, hence, no optical rotation data was reported.10a

To improve the yield of β-hydroxy esters, the reaction conditions have previously been varied e.g., using free cells in organic media,11 introducing a sulphenyl group at the α-position of the ester,2 using non-fermenting baker’s yeast media,12 etc. While initially the reduction procedure did not afford satisfactory results, the use of potassium dihydrogen phosphate, calcium phosphate (monobasic), magnesium sulfate and ammonium tartrate (diammonium salt) (10:1:1:50) in tap water pH 7 as the buffer for 72–120 h proved more promising. The chemoselectivity was confirmed before and after reduction

Scheme 1. Reagents and conditions: i, aerobic fermenting, S. cerevisiae, potassium phosphate buffer (pH 7, 0.01 M), H2O, for 72–120 h; potassium dihydrogen phosphate, calcium phosphate (monobasic), magnesium sulfate and ammonium tartrate (diammonium salt) (10:1:1:50).
with yeast by means of the IR spectra of 2, 3 and 5. The characteristic symmetrical and strong asymmetrical absorption bands due to the NO2 group in the region between 1390–1260 and 1660–1500 cm\(^{-1}\), respectively, were observed in all spectra. The yields of the bioreduction for compounds 2, 3 and 5, having an NO2 electron-withdrawing group, were rather higher than that of 4 having a Br substituent group.

\(^1\)H-NMR spectroscopy of compounds 1–5, in contrast to their precursor starting β-keto esters, clearly showed that a hydroxyl group and a hydrogen atom at C-3 giving a doublet of doublets were present in the molecules (Table I).

These data, together with the absence of ketone C=O stretching vibration bands and the presence of O–H stretching vibrations in the IR spectra, suggested the presence of a hydroxyl functional group in the molecules 1–6.

**TABLE I.** 500 MHz \(^1\)H-NMR (CDCl\(_3\)): Chemical shifts and coupling constants of C-3 protons in compounds 1–5

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(S)-(−)-1</th>
<th>(+)-2</th>
<th>(+)-3</th>
<th>(+)-4</th>
<th>(+)-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ/ppm</td>
<td>5.32</td>
<td>5.25</td>
<td>5.26</td>
<td>5.28</td>
<td>5.37</td>
</tr>
<tr>
<td>J/Hz</td>
<td>dd, J 3.8,</td>
<td>dd, J 3.8,</td>
<td>dd, J 4.0,</td>
<td>q, J 4.4,</td>
<td>dd, J 4.1,</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>4.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.2</td>
</tr>
</tbody>
</table>

In a recent study, Stewart and coworkers\(^{13}\) systematically analyzed almost 18 individual reductases present in \(S. \text{cerevisiae}\) and showed that the cell systems are complicated by opposing enantio preferences for these different enzymes. Thus simple explanation of the conversion yields and ee is not facile. Hence, at this stage no clarification is available for the reversal of the stereoselectivity in the reduction of 2–5 compared to 1, see Table I. While several yeast reductases have been isolated and studied,\(^{14–16}\) and systematic investigations of their properties have commenced, numerous putative reductases are still awaiting full characterization.\(^{17}\)
A typical average of yeast/substrate ratio could be ca. 1200–1800 g/mol (ca. 6–10 w/w). In the case of ethyl 3-oxobutanoate, this ratio could be lowered to 417 g/mol (ca. 3.2 w/w).

### TABLE II. Preparative conversions with *Saccharomyces cerevisiae* in aqueous fermentation medium

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield/%</th>
<th>ee %</th>
<th>${[\alpha]_D}^{25}$ (c,solvent)</th>
<th>Time/h</th>
<th>Amount of subs. used/g</th>
<th>Conc. g/L</th>
<th>Yeast/Subs. w/w (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59.4</td>
<td>90</td>
<td>$-23$ (0.2, diethyl ether)</td>
<td>240</td>
<td>1</td>
<td>73</td>
<td>15 (2880)</td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>–</td>
<td>$+31$ (1.2, CHCl$_3$)</td>
<td>73</td>
<td>1</td>
<td>107</td>
<td>15 (2778)</td>
</tr>
<tr>
<td>3</td>
<td>93</td>
<td>–</td>
<td>$+32$ (1, CHCl$_3$)</td>
<td>72</td>
<td>1.5</td>
<td>77</td>
<td>6.67 (1852)</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>–</td>
<td>$+22$ (0.5, diethyl ether)</td>
<td>96</td>
<td>1.5</td>
<td>77</td>
<td>6.67 (1818)</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>–</td>
<td>$+34$ (1, diethyl ether)</td>
<td>72</td>
<td>1.5</td>
<td>77</td>
<td>6.67 (1887)</td>
</tr>
<tr>
<td>6</td>
<td>95</td>
<td>90</td>
<td>$+37$ (1.5, diethyl ether)</td>
<td>72</td>
<td>2.5</td>
<td>70</td>
<td>3.2 (417)</td>
</tr>
</tbody>
</table>

When substrates or products are more toxic, this ratio becomes higher (for example, see Table II, entries 1, 2). Approximately, 5 g of β-keto esters could be reduced by 50–70 g of baker’s yeast in an overall volume of 500 mL by the current method.

### EXPERIMENTAL

#### Preparation of (S)-(−)-3-hydroxy-3-phenylpropanoic acid ethyl ester (I, C$_{11}$H$_{14}$O$_3$)

A typical procedure

To a 250 mL 3-neck round bottom flask was added 20 g (60 mmol) sucrose, 150 mL warm H$_2$O and 0.25 g potassium dihydrogen phosphate, 0.025 g calcium phosphate (monobasic), 0.025 g magnesium sulfate and 1.25 g ammonium tartrate (diammonium salt). The resulting mixture was stirred at room temperature for several minutes to produce a homogeneous solution, whereupon 10 g active dry yeast was added. After the mixture had been stirred for 20 min at 35 °C, 1 g (5.2 mmol) of 3-oxo-3-phenylpropanoic acid ethyl ester was added dropwise over 2 h through a syringe to the mixture. The evolved carbon dioxide was bubbled through a bent glass tube into a saturated aqueous solution of Ba(OH)$_2$ in a 200 mL Erlenmeyer flask. The reaction mixture was stirred vigorously for 120 h at 35 °C. The progress of the reaction was monitored by TLC (the solvent system used was ligroin: petroleum ether 3:1 (v:v)). After 120 h, 10 g sucrose and 5 g yeast (dissolved in 30 mL of water) were added to the fermenting solution. The reaction mixture was allowed to stir for an additional 120 h at the same temperature. After this time, the yeast cells were removed by filtration through 15 g of celite filter aid. The yeast cells were washed with 2x25 mL water. The filtrate was saturated with NaCl and then extracted with 5x20 mL portions of diethyl ether. The combined organic layers were dried over MgSO$_4$ and the solvent evaporated under reduced pressure to leave a residue of the crude product, which was purified on a silica gel column using 1:3 (v:v) of EtOAc:ligroin as the eluent. The chromatography afforded 0.6 g (3.08 mmol, 59.4 %), $[\alpha]_D^{25} = -26^0$ cm$^2$ g$^{-1}$ (c = 0.2, EtOEt), (lit., $-25.8^0$ cm$^2$ g$^{-1}$) ee = 90 %. IR (neat): 3400 (O–H), 2950 (C–H), 1710 (C–O), 1450 (C–C), 1370 (CH$_3$), 1260 (C–O) cm$^{-1}$; $^1$H-NMR (500 MHz (CDCl$_3$)): 1.26 (3H, t, J = 7.4 Hz, CH$_3$), 2.73 (2H, dd, J = 3.0, 4.0 Hz, CH$_2$CO), 3.2 (1H, s, OH), 4.15 (2H, q, J = 7.1 Hz, CH$_2$CH$_3$), 5.32 (1H, dd, J = 3.8, 4.8, CHOH), 7.3 (5H, m, Ar).

#### Preparation of (+)-3-hydroxy-3-(3-nitrophenyl)propanoic acid ethyl ester (2, C$_{11}$H$_{13}$NO$_5$)

A similar procedure as used for 1 was employed, except instead of stirring for 240 h, the solution was stirred for 60 h. The crude product was purified on a silica gel column, using 13:1 (v:v) of EtOAc:ligroin as the eluent. The chromatography afforded 1.6 g (6.06 mmol, 90 %) m.p. = 57 °C (reported in the literature as 58 °C), $[\alpha]_D^{25} = +31^0$ cm$^2$ g$^{-1}$ (c = 1.2, CHCl$_3$), ee = (the absolute con-
Preparation of \((\pm)-3\text{-hydroxy-3-(4-nitrophenyl)propanoic acid ethyl ester (3, C}_11\text{H}_2_3\text{O}_5\text{N})\)

A similar procedure as used for 1 was employed, except instead of stirring for 240 h, the solution was stirred for 120 h. The crude product was purified on a silica gel column, using 1:1 (v/v) of CH\(_2\)Cl\(_2\) : ligroin as the eluent. The chromatography afforded 0.6 g (40 %), \(\delta\) (ppm) 500 MHz (CDCl\(_3\)): 1.16 (3H, t, \(J = 7.0\) Hz, CH\(_3\)), 2.7 (2H, dd, \(J = 6.3, 7.65\) Hz, CH\(_2\)CO), 3.72 (1H, s, CH\(_2\)), 5.25 (1H, dd, \(J = 3.8, 4.0\), CHOH), 7.62 (1H, t, \(J = 7.5\) Hz, Ar), 7.72 (1H, t, \(J = 7.5\) Hz, Ar), 7.94 (2H, d, \(J = 8.1\) Hz, Ar) ppm.

Preparation of \((\pm)-3\text{-}(4\text{-bromophenyl})-3\text{-hydroxypropanoic acid ethyl ester (4, C}_11\text{H}_2_3\text{O}_3\text{Br})\)

A similar procedure as used for 1 was employed, except instead of stirring for 240 h, the solution was stirred for 120 h. The crude product was purified on a silica gel column, using EtOEt, \(\delta\) (ppm) 500 MHz (CDCl\(_3\)): 1.5, \(\epsilon = 41.7\) – 43.7° cm\(^2\) g\(^{-1}\), lit \(\epsilon = 41.7\) – 43.7° cm\(^2\) g\(^{-1}\), with 2CH\(_3\), 4.17 (2H, q, \(J = 5.9, 7.1\) Hz, CH\(_2\)CH\(_2\)), 5.28 (1H, dd, \(J = 4.4, 5.0\) Hz, CHOH), 7.55 (2H, t, \(J = 7.7\) Hz, Ar), 7.7 (2H, t, \(J = 8.1\) Hz, Ar).

Preparation of \((\pm)-3\text{-}(3,5\text{-dinitrophenyl})-3\text{-hydroxypropanoic acid ethyl ester (5, C}_11\text{H}_2_3\text{N}_2\text{O}_3\))

A similar procedure as used for 1 was employed, except instead of stirring for 240 h, the solution was stirred for 72 h. The crude product was purified on a silica gel column using petroleum ether:CH\(_2\)Cl\(_2\) (1:10) to afford 0.9 g (59 %), \(\delta\) (ppm) 500 MHz (CDCl\(_3\)): 1.26 (3H, t, \(J = 7.1\) Hz, CH\(_3\)), 2.7 (2H, dd, \(J = 2.6, 3.7\) Hz, CH\(_2\)CO), 3.7 (1H, s, O–H), 4.1 (2H, q, \(J = 7.1\) Hz, CH\(_2\)CH\(_2\)), 5.2 (1H, dd, \(J = 4.1, 5.2\) Hz, CHOH), 8.16 (2H, t, \(J = 6.0\) Hz, Ar), 8.52 (1H, q, \(J = 1.1, 6.6\) Hz, Ar).

Preparation of \((\pm)-3\text{-hydroxybutanoic acid ethyl ester (6, C}_6\text{H}_1_2\text{O}_3\))

A similar procedure as used for 1 was employed, except instead of stirring for 120 h, the solution was stirred for 48 h. The yeast cells were washed with 2 × 25 mL portions of 25 % acetic acid. The filtrate solution was saturated with NaCl and then extracted with 5 × 20 mL portions of diethyl ether. The combined organic layers were dried (MgSO\(_4\)) and the solvent evaporated under reduced pressure to leave a residue of the crude product. The crude product was purified on a silica gel column, using petroleum ether:CH\(_2\)Cl\(_2\) (5:1) to afford 1.7 g (95 %), \(\delta\) (ppm) 500 MHz (CDCl\(_3\)): 1.26 (3H, t, \(J = 7.1\) Hz, CH\(_3\)), 2.7 (2H, q, \(J = 7.2\) Hz, CH\(_2\)CH\(_2\)), 2.4 (2H, q, \(J = 7.1\) Hz, CH\(_2\)CH\(_2\)), 3.3 (1H, s, OH), 4.2 (3H, m, CHOH and OCH\(_2\)CH\(_2\)).
IZVOD

ENANTIOSELEKTIVNE REDUKCIJE β-KETO-ESTARA POMOĆU Saccharomyces cerevisiae

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Enantioselективна редукција ароматичних β-кето-естара, у подршку хидрогенизације, монобазног калијума, фосфата и диаммонијум-тартара (10:1:1:50) у води као пуферу pH 7, током 72–120 h уз конверзију од 45–90 % у одговарајуће ароматичне β-хидрокси-естере, постигнута је применом квасца Saccharomyces cerevisiae.

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REFERENCES


