Bioreduction of α-methyleneketones

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Dedicated to Prof. R. A. Abramovitch in honor of his 70th birthday
(received 01 May 01; accepted 09 Oct 01; published on the web 17 Oct 01)

Abstract
Bioreduction of methyleneketones was carried out with Rhizopus arrhizus, Pseudomonas fluorescens and immobilized Saccharomyces cerevisae. The α-substituted enones were enantioselectively reduced to saturated ketones with good to excellent ee, depending of microorganism. With the S. cerevisae good ee (12-93%) was obtained while excellent ee (99 %) was achieved with P. fluorescens.

Keywords: Bioreduction, methyleneketones, acrylophenones

Introduction
The growing interest in asymmetric synthesis has promoted great developments in biotransformations in organic synthesis applied for the synthesis of chiral compounds.1 Baker’s yeast has been widely used, mainly for the reduction of the carbonyl groups of pro-chiral ketones, producing alcohols with high enantiomeric purity.2 Reduction of a C=C bond has also been achieved, although it is mostly frequently conjugated with a carbonyl group.3 Less attention has been paid to the bioreduction of α-methyleneketones and most studies were carried out with baker’s yeast.4 Chiral α-methyleneketones are structural moieties in a large class of compounds, such as drugs and pheromones5 and, in many cases bioactivity is dependent upon the
configuration of the α-carbon. Herein we wish to report chemo- and enantioselective bioreduction studies of α-methylene ketones using as catalyst Saccharomyces cerevisae (baker’s yeast), Rhizopus arrhizus and Pseudomonas fluorescens. The α-substituted enones are reduced to the saturated ketones with good to excellent ee, depending on the microorganisms.

Results and Discussion

Synthetic routes to α-methylene carbonyl compounds have received considerable attention due to the importance of this class of compounds as useful intermediates. A number of methods have been described and reviewed. We have tested different methods for a direct methylene transfer, the most successful, efficient and general method was the direct Mannich α-methylenation introduced by Kim. Treatment of the appropriate ketone with 37% aqueous formaldehyde solution in the presence of morpholine in refluxing acetic acid directly furnished the desired unsaturated ketone in good yield (see Table 1).

Baker’s yeast reduction of 2-methyl-1-phenylprop-2-en-1-one 2a was fast and gave iso-propyl phenyl ketone 3a in reasonable yields. In order to facilitate the isolation of the product from the broth we employed yeast immobilized (IMBY) onto montmorillonite K10. Using short periods (6 h) of incubation only the ketone 3a (nearly 50% yield) and the starting acrylophenone 2a were isolated. After 12 h of reaction, 3a was isolated in 76% yield and traces of the alcohol 3-methyl-1-phenyl-2-propanol. With long periods, reduction of the ketone to the alcohol increased very little. It is known that aryl methyl ketones are reduced to (S)-alcohols in modest yield with approximately 70% ee. Treatment of the acrylophenone 2b with IMBY gave, after 12 h, a 67% yield of (2R)-1-phenyl-2-methylbutan-1-one 3b in 93% ee. The absolute configuration was determined comparing the [α]D –24.0 with the published value. Reduction of methyl 3-benzoylbut-3-enoate 2c with IMBY for 12 h gave a good yield (59%) of methyl (-)-(3S)-3-benzoyl-3-methylbutanoate 3c with poor enantioselective 12% ee. A satisfactory result was obtained with 2e which gave 3e in 65% yield with 85% ee. Attempts to reduce acid 2d were not successful since products were not isolated from the reaction with IMBY.

In order to find other active microorganisms with the above substrates we chose the fungus Rhizopus arrhizus and the bacterium Pseudomonas fluorescens. The biotransformations were conducted with a resting cell mass/substrate ratio of 30/1 in a phosphate buffer solution (PBS) at pH 7 at 30 °C and 110 rpm. In Table 2 we present the results. Reduction of substrates 2a-e with R. arrhizus gave the same reduction products with yields similar to those obtained with IMBY but the ee are poor. Better results were achieved with P. fluorescens, giving higher yields and
enantioselectivities than with IMBY and *R. arrhizus*. The enzyme enoate reductase present in *P. fluorescens* gave, with all substrates, ee not less than 99% for the reduction of the C=C.

We have commented above that aryl alkyl ketones are reduced by baker’s yeast in low yields although the study of the reduction of γ-ketoesters 1c and 1e was not systematically consummated. In contrast, it is well known that α- and β-ketoesters are very suitable substrates for biocatalysts. The ketoester 1e was not metabolized by IMBY since the p-methoxy group is a strong electron donor that inactivates the substrate. Reaction of the γ-ketoester 1c with IMBY gave, after several days, two main compounds, lactone 4 and γ-ketoacid 1d. Considering that the amount of 1d is dependent on reaction time, we carried out the reaction of 1d as substrate with IMBY. After 20 h the (−)-(S)-5-phenyl-4,5-dihydrofuran-2(3H)-one 4 was isolated in 70% yield and 94% ee. This lactone was obtained in low yield by bioreduction of ethyl 3-benzoylpropionate with free BY after 7 days of incubation. Further studies of bioreduction of γ-ketoacids are in development and will be published soon.

In summary, IMBY is a good biocatalyst for enantioselective reduction of the C=C bond of α-methyleneketones to saturated ketones. Excellent ee was obtained with the bacterium *P. fluorescens*.

\[
\begin{align*}
\text{H}_2\text{CO/\HOAc} & \quad \text{morpholine} \\
\text{1} & \quad \text{2}
\end{align*}
\]

a. \( R = H; R^1 = \text{CH}_3 \)  
\( d. \ R = H; R^1 = \text{CH}_2\text{CO}_2\text{H} \)

b. \( R = H; R^1 = \text{CH}_2\text{CH}_3 \)  
\( e. \ R = \text{OCH}_3; R^1 = \text{CH}_2\text{CO}_2\text{CH}_3 \)

c. \( R = H; R^1 = \text{CH}_2\text{CO}_2\text{CH}_3 \)

\[
\begin{align*}
\text{2a-e} & \quad \text{biocatalyst} \\
\text{3a-e}
\end{align*}
\]
Table 1. \(\alpha\)-Methylation of representative ketones

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Time, h</th>
<th>Yield (%)</th>
<th>Ref.</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>1a</td>
<td>(2a)(^a)</td>
<td>8</td>
<td>82</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>2b</td>
<td>12</td>
<td>84</td>
<td>18</td>
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<tr>
<td>3</td>
<td>1c</td>
<td>2c</td>
<td>6</td>
<td>91</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>2d</td>
<td>6</td>
<td>62</td>
<td>---</td>
</tr>
<tr>
<td>5</td>
<td>1e</td>
<td>2e</td>
<td>6</td>
<td>71</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) All Products exhibited satisfactory spectral properties (\(^1\)H-NMR, \(^13\)C-NMR, IR, MS) fully in accord with known or expected values.

\(^b\) References for the known compounds.

Table 2. Reduction of \(\alpha\)-methyleneketones with microorganisms

<table>
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<tr>
<th>Entry</th>
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<th>Biocatalyst</th>
<th>Time h</th>
<th>Yield %</th>
<th>ee</th>
<th>Ref.(^a)</th>
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</thead>
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<td>P. fluorescens</td>
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<td>90</td>
<td>---</td>
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</tr>
<tr>
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<td>3b</td>
<td>IMBY</td>
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<td>67</td>
<td>93</td>
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<td>14</td>
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<td>69</td>
<td>25</td>
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<td>P. fluorescens</td>
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<td>73</td>
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<td>3e</td>
<td>IMBY</td>
<td>17</td>
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<td>56</td>
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<td>P. fluorescens</td>
<td>24</td>
<td>69</td>
<td>99</td>
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</tbody>
</table>

\(^a\) References for the known compounds
Experimental Section

**General Procedures.** The IR spectra were recorded on a Hartmann & Braun BOMEM MB SERIES spectrometer. The $^1$H-NMR and $^{13}$C-NMR were recorded on a VARIAN-INOV A spectrometer. Mass spectra were recorded on a SHIMADZU GC/MS - QP 5000 gas chromatograph/mass spectrometer and with helium as carrier gas. A 30m X 0.25 mm I.D. SUPELCO SIMPLICITYTM capillary column was used and the chiral column employed in the determination of enantiomeric excess ($ee$) was a 25m X 0.25 mm I.D. CHIRASIL-DEX from CHROMPACK. An injector temperature of 230 °C and a detector temperature of 280 °C, with the column at 50 °C for 3 min; then using a rate of 20 °C/min. up to 280 °C, with a pressure of 100 kPa and gas flow of 80 ml/min. Optical rotations were measured using a Carl Weiss POLAMAT A polarimeter. CD spectra were recorded on a JASCO-J720 spectropolarimeter at 25 °C. Preparative column chromatography was carried out using silica gel 60 (Merck). Commercially available chemicals and solvents were used without further purification.

**α-Methylenenation of benzophenones and γ-ketoesters and acids.**

The mixture of a ketone (10 mmol) and morpholine (5 mmol) in 20 mL of glacial acetic acid was heated under reflux. To this refluxing mixture a 37% aqueous formaldehyde solution (5 mL) was added dropwise over several hours (6-24 h). After completion of the reaction, acetic acid was stripped off under reduced pressure and the residue was diluted with ethyl acetate. The organic layer was washed successively with 10% aqueous hydrochloric acid, saturated sodium bicarbonate solution, water and brine, and then dried over anhydrous magnesium sulfate. The solvent was evaporated. The crude oil was purified by silica gel column chromatography.

**Methyl 3-benzoylbut-3-enoate (2c).**

Oil, 91% yield; IR (film) ν 2955, 1740, 1687, 1659, 1599, 1449, 1203 cm$^{-1}$; $^1$H-NMR (300 MHz, CDCl$_3$) δ 3.56 (2H, s), 3.69 (3H, s), 5.79 (1H, s), 6.00 (1H, s), 7.45 (2H, t, $J = 7.3$), 7.53 (1H, t, $J = 7.0$), 7.79 (2H, d, $J = 7.0$). $^{13}$C-NMR (75 MHz, CDCl$_3$) δ 37.83, 52.02, 128.19, 129.65, 132.32, 137.19, 141.18, 171.41, 196.99; MS $m/e$ 204(M$^+$, 5), 172(14), 145(8), 117(5), 105(100), 77(61), 59(8). Anal. Calcd for C$_{12}$H$_{12}$O$_3$: C, 70.57; H, 5.92. Found: C, 70.53; H, 6.02.
3-Benzoylbut-3-enoic acid (2d).
Pale yellow oil, 62% yield; IR (film) \( \nu \) 3062, 1712, 1657, 1597, 1449, 1233 cm\(^{-1}\); \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \( \delta \) 3.56 (2H, s), 5.82 (1H, s), 6.04 (1H, s), 7.27 (1H, s), 7.44 (2H, t, \( J = 7.7 \)), 7.54 (1H, t, \( J = 6.2 \)), 7.78 (2H, d, \( J = 5.1 \)); \(^1\)C-NMR (75 MHz, CDCl\(_3\)) \( \delta \) 37.89, 58.41, 128.22, 129.70, 129.75, 132.45, 136.98, 140.63, 175.73, 197.18; MS \( m/e \) 190 (M+, 3), 147 (3), 131 (3), 105 (100), 77 (48), 51 (26). Anal. Calcd for C\(_{11}\)H\(_{10}\)O\(_3\): C, 69.46; H, 5.30. Found: C, 69.40; H, 5.34.

Methyl 3-(4-methoxybenzoyl)but-3-enoate (2e). Pale oil, 71% yield; IR (film) \( \nu \) 2954, 2925, 1736, 1650, 1602, 1461, 1257, 1160 cm\(^{-1}\); \(^1\)H-NMR (300 MHz, CDCl\(_3\)) \( \delta \) 3.55 (2H, s), 3.67 (3H, s), 3.87 (3H, s), 5.72 (1H, s), 5.91 (1H, s), 6.94 (2H, d, \( J = 7.0 \)), 7.85 (2H, d, \( J = 7.9 \)); \(^1\)C-NMR (75 MHz, CDCl\(_3\)) \( \delta \) 38.33, 52.11, 55.57, 113.74, 127.30, 132.41, 141.54; 163.55, 171.80, 196.05; MS \( m/e \) 234 (M+, 15), 203 (12), 175 (11), 135 (100), 107 (33), 92 (52), 77 (85), 64 (52), 50.28. Anal. Calcd. for C\(_{13}\)H\(_{14}\)O\(_4\): C, 66.67; H, 5.98. Found: C, 66.35; H, 6.04.

**Immobilization of baker’s yeast.** The immobilization of the yeast was obtained by adding fresh baker’s yeast (20 g) to a suspension of montmorillonite (20 g) in water at 30 °C and then gently shaking the resulting suspension for 2 h. After vacuum filtration, the immobilized baker’s yeast (IMBY) was kept in a refrigerator.

**General procedure for the bioreductions with IMBY**
To a water suspension (100 mL) of IMBY was added commercial sugar (10 g) and CaCl\(_2\) (1.5 g) under mechanic stirring at 30 °C. After 30 min was added 1 mmol of substrate dissolved in ethanol (1 mL). At the end of the reaction (determined by extraction of 1 mL samples with ethyl acetate and analysis in a CG/MS) ethyl acetate (50 mL) was added and the mixture was stirred for a further 1 h at 30 °C. The reaction mixture was filtered under reduced pressure, the medium was extracted with ethyl acetate (3 x 20 mL), the organic layer was dried over magnesium sulphate and filtered through a celite column. The solvent was evaporated under reduced pressure and the residue was purified on chromatographic plates and eluted with ethyl acetate:hexane (1:10). The purified product was dissolved in ethyl acetate (10 mg/ml) and analyzed by GC/MS.

**General procedure for the bioreductions with Rhizopus arrhizus and Pseudomonas fluorescens**
The microorganisms were reactivated in nutrient broth (NB-nutrient broth-Difco) (50 mL) for 24 h at 30 °C. The cells were transfereed to an erlenmeyer (2 L) in NB (1 L) and the
microorganisms were incubated in a shaker at 100 rpm and 30 °C for 17 h. After that, the cells were centrifuged for 30 min. at 300 rpm and then transferred to erlenmeyers (250 mL) containing pH 7 PBS. (100 mL). The substrates (50 mg) were dissolved in ethanol (1 mL) and added to the cell medium in a cell/substrate ratio of 30:1. The mixture was incubated at 30 °C and 100 rpm for 48 h. The reaction mixture was then extracted with ethyl acetate (3 x 20 mL), the organic layer dried over magnesium sulphate and filtered through a celite column. The solvent was evaporated under reduced pressure and the residue purified on chromatographic plates and eluted with ethyl acetate:hexane (1:10). The purified product was dissolved in ethyl acetate (10 mg/ml) and analyzed by GC/MS.

**Methyl (-)-4-phenyl-3-methyl-4-oxo-butanoate (3c).** Pale yellow oil, 73% yield, 99% ee; IR (film) ν 2953, 2923, 1738, 1687, 1597, 1464, 1347, 1174 cm⁻¹; ¹H-NMR (300 MHz, CDCl₃) δ 0.99 (3H, d, J = 7.0), 2.35-2.37 (1H, m), 2.97 (2H, dd, J = 8.0), 3.65 (3H, s), 7.48 (2H, t, J = 7.7), 7.59 (1H, t, J = 7.1), 7.99 (2H, d, J = 7.0); MS m/e 206(M⁺, 2), 175(4), 132(2), 105(100), 77(37), 51(17). [α]²⁵ D -6.0 (c 0.01, CHCl₃).

**Methyl (-)-4-(4-metoxyphenyl)-3-methyl-4-oxobutanoate (3e).** Pale yellow oil, 69% yield, 99% ee; IR (film) ν 2927, 2853, 1738, 1679, 1600, 1461, 1379, 1244, 1170 cm⁻¹. ¹H-NMR (300 MHz, CDCl₃) δ 0.87 (1H, d, J = 7.0), 2.38-2.42 (1H, m), 2.96 (2H, dd, J = 7.9), 3.65 (3H, s), 3.88 (3H, s), 6.95 (2H, d, J = 8.8), 7.98 (2H, d, J = 9.2); MS m/e 236(M⁺, 2), 205(3), 135(100), 107(7), 92(9), 77(14). [α]²⁵ D -15 (c 0.55, CHCl₃). HRMS calcd. for C₁₃H₁₆O₄: 236.2637; found m/z 236.2629 (M⁺).

**Acknowledgements**

The authors acknowledge and are grateful for grants from FAPESP (Processo 1998/16181-2) and a scholarship from CNPq for M. de M.

**References**


