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Regio- and enantioselective reduction of methyleneketoesters mediated by *Saccharomyces cerevisiae*

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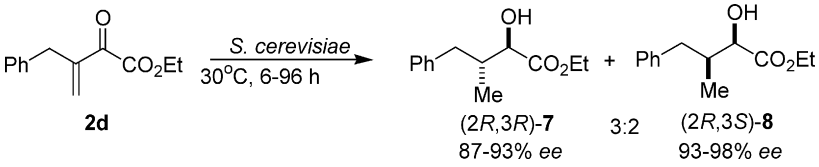
Abstract

Methyleneketoesters were readily prepared in high yields by performing a direct α-methylenation of the corresponding ketoesters using a previously described protocol. Reactions of ethyl 2-methylene-3-oxo-3-arylpropanoates **2a–c** catalyzed by *S. cerevisiae* were performed with good conversions to give reductions of the C=C, C=O or both, depending on the reaction conditions and on the substitution of the aryl moiety. Reaction of 3-methylene-2-oxo-4-phenylbutyrate **2d** was carried out with free yeast cells and with yeast cells immobilized with calcium alginate, in which the major products resulted from C=C and C=O bond reduction.



2d

Keywords: Methylenation; Ketoesters; *Saccharomyces cerevisiae*; Biocatalysis

1. Introduction

Currently, much emphasis is being given to obtaining enantiomerically pure compounds in good yields under environmentally friendly conditions [1]. As a result, biocatalysis with either enzymes (crude or isolated) or whole cells has blossomed over the past few years as a viable alternative to costly or difficult chemistry in the manufacture of chiral compounds [2–6]. Biocatalysts display wide substrate acceptance and can catalyze chemo-, regio- and stereoselective transformations of organic compounds under very mild, environmentally safe reaction conditions [7,8].

The α,β-unsaturated ketones and ketoesters have been investigated as potential substrates for biocatalytic reductions that can lead to the introduction of one, two, or three new chiral centers into an prochiral structure [9–11]. The syntheses and further transformations of α-methyleneketoesters have been explored to a limited extent [12,13], which encouraged us to develop efficient methods to prepare these somewhat overlooked but important molecules [14]. Reduction of α-methyleneketones and aldehydes by *Saccharomyces cerevisiae* depends on the substituents and, in general, can give products with reduced C=C bonds and to a lesser extent, with reduced C=O bonds [15–18]. Recently, we reported our attempts to set up stereoselective reductions of a large number of acyclic α-methyleneketones using the yeasts *S. cerevisiae* [19,20] and *Pichia stipitis* [21]. Herein, we describe our results on the reduction of methyleneketoesters mediated by *S. cerevisiae*. The main advantage of using biotransformations mediated by *S. cerevisiae* in syntheses is the mild reaction conditions required, such as room temperature and close to neutral pH as well as having a less expensive stereoselective process with a simple work-up.

2. Experimental

2.1. General

The IR spectra were recorded on a BOMEM MB SERIES Hartmann & Braun spectrometer. The ^{1}H NMR and ^{13}C

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NMR were recorded on a VARIAN-INOVA spectrometer. Mass spectra were recorded on a Shimadzu GC-MS-QP 5000 gas chromatograph–mass spectrometer equipped with a 30 m × 0.25 mm × 0.25 μm capillary column of fused silica, SUPELCO SIMPLICITY 1TM. The chiral-phase CG analyses were performed using a 25 m × 0.25 mm × 0.25 μm Hydrodex-β-3P chiral column from CHROMPACK. An injector temperature of 200 °C, detector temperature of 250 °C and hydrogen as carrier gas were used. HPLC analyses were carried out using a SHIMADZU LC-20AT Prominence instrument and a Chiralcel OJ-H chiral column from Daicel. HRMS were obtained on a Fison VG Autoespec. Optical rotations were measured using a Carl Weiss POLAMAT A polarimeter. Ethyl benzoylacetate (97%), ethyl *p*-nitrobenzoylacetate (98%), *p*-methoxybenzoylacetate (97%) and ethyl 2-oxo-4-phenylbutanoate were purchased from Aldrich or Fluka. Lyophilized *Sacharomyces cerevisiae* was purchased from Emulzint LDA (Belgium) and stored in a refrigerator. All other chemicals were at least analytical grade.

2.2. General procedure for α-methylenation

The reactions were carried out under a nitrogen atmosphere. The ketoester (1.0 mmol) and a freshly prepared solution of morpholine (0.3 mmol) in glacial acetic acid (5.0 mL) were mixed in a 25 mL double-necked round bottoned flask with a coiled reflux condenser. Molecular sieves (4Å) and *p*-formaldehyde (9.0 mmol) were then added and the mixture was stirred and heated to 70 °C for 4 h. The reaction mixture was cooled to room temperature and quenched with solid $NaHCO_3$ followed by repeated (3 times) extractions with an equal volume of ethyl acetate. The combined organic extracts were washed with brine, water and dried with anhydrous $MgSO_4$ followed by evaporation of the solvent under reduced pressure.

2.2.1. Ethyl 2-benzoylacrylate (2a)

Purification by silica gel column chromatograpy (hexane–ethyl acetate, 9:1) yielded a colorless oil (82% yield); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.87-7.84 (m, 2H), 7.61-7.56 (m, 1H), 7.46 (t, *J* = 7.8 Hz, 2H), 6.69 (s, 1H), 6.06 (s, 1H), 4.22 (q, *J* = 7.2 Hz, 2H), 1.19 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 193.0, 164.3, 141.5, 136.3, 133.5, 131.3, 129.4, 128.5, 61.5, 14.0; IR (film, cm^{-1}): 3060, 2982, 1731, 1682, 1238, 1025, 937, 735; MS (*m/z*, % rel.): 51 (40), 77 (89), 78 (5), 105 (100), 106 (7), 130 (4), 158 (3), 175 (2), 204 (M‡, 2).

2.2.2. Ethyl 2-(4-methoxybenzoyl)acrylate (2b)

Purification by silica gel column chromatograpy (hexane–ethyl acetate, 9:1) gave a pale yellow oil (80% yield); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.87-7.84 (m, 2H), 6.96-6.92 (m, 2H), 6,65 (s, 1H), 5.99 (s, 1H), 4.23 (q, *J* = 7.2 Hz, 2H), 3.88 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 191.6, 164.3, 163.9, 141.5, 131.8, 130.9, 130.3, 113.7, 61.5, 55.6, 14; IR (film, cm^{-1}): 2982, 2944, 1725, 1667, 1600, 1316, 1182; MS (*m/z*, % rel.): 63 (11), 77 (26), 92 (14), 107 (8), 135 (100), 160 (2), 160 (2), 189 (2), 234 (M‡, 8); HRMS calcd for $C_{13}H_{14}O_4$: 234.08921, found: 234.06699.

2.2.3. Ethyl 2-(4-nitrobenzoyl)acrylate (2c)

Attempts of purification by silica gel column chromatography or distillation promoted decomposition of the product. This crude product was tested in the biotransformations. MS (*m/z*, % rel.): 50 (47), 76 (53), 89 (2), 104 (42), 120 (16), 134 (2), 150 (100), 175 (7), 203 (14), 220 (12), 232 (1), 249 (M‡, 2).

2.2.4. Ethyl 3-benzyl-2-oxobut-3-enoate (2d)

Purification by silica gel column chromatography (hexane–ethyl acetate, 9:1) yielded a pale yellow oil (87% yield); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.33-7.17 (m, 5H), 6.22 (s, 1H), 5,97 (s,1H), 4.35 (q, *J* = 7.2 Hz, 2H), 3.64 (s, 2H), 1.36 (t, *J* = 7.2 Hz), 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 188.0, 163.9, 144.4, 137.6, 133.1, 129.2, 128.6, 126.6, 62.1, 35.7, 14.0; IR (film, cm^{-1}): 3029, 2983, 2940, 1738, 1683, 1280, 1037, 702; MS (*m/z*, % rel.): 44 (2), 51 (6), 65 (9), 78 (4), 91 (41), 117 (102), 129 (3), 145 (56), 172 (2), 189 (9), 200 (5), 218 (12).

2.3. General procedure for the biotransformations with Baker's yeast free cells

The methylene ketoester (0.4 mmol) was added to a mixture of dry Baker's yeast (Emulzint®, 1 g) and glucose (1 g) in water (50 mL) at 30 °C. The resulting mixture was maintained at 30 °C in a shaker at 170 rpm. Samples were withdrawn from the reaction mixture at appropriate intervals and analyzed by the GC–MS technique. Subsequently, at the end of the reactions, Celite®, ethyl acetate (50 mL) and brine (50 mL) were added to the reaction mixture, and stirring was continued for 30 min. The cells–Celite mixture was filtered off and the filtrate extracted three times with ethyl acetate (15 mL). The organic layer was washed with brine, dried over magnesium sulfate and the solvent was evaporated. The crude oil was purified by silica gel column chromatography.

2.3.1. Ethyl 2-methyl-3-oxo-3-phenylpropanoate (3a)

Purification using silica gel column chromatography (hexane–ethyl acetate, 6:1) gave racemic compound **3a** as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.92 (d, *J* = 7.2 Hz, 2H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.50-7.45 (m, 2H), 4.37 (q, *J* = 6.9 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 1.50 (d, *J* = 7.2 Hz, 3H), 1.71 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 195.9, 170.9, 135.9, 133.4, 128.7, 128.6, 61.3, 48.4, 13.9, 13.7; IR (film, cm^{-1}): 2985, 2941, 1737, 1687, 1450, 1218, 1033, 702; MS (*m/z*, % rel.): 51 (24), 77 (48), 78 (2), 105 (100), 106 (7), 133 (1), 161 (1), 206 (M‡, 1).

2.3.2. (2R,3S)-Ethyl 3-hydroxy-2-methyl-3-phenylpropanoate (4a)

Purification using silica gel column chromatography (hexane–ethyl acetate, 9:1) yielded compound **4a** as a pale yellow oil. Enantiomeric excesses were determined by chiral HPLC using 15% isopropanol-hexane, flow rate = 1 mL min^{-1} and λ = 220 nm, [(2*S*,3*R*) = 6.53 min, (2*R*,3*S*) = 8.32 min]. ^{1}H

NMR (300 MHz, $CDCl_3$): δ (ppm) 7.34-7.28 (m, 5H), 4.74 (dd, J = 8.4 Hz, J = 4.5 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.02 (d, J = 4.5 Hz, 3H), 2.85-2.75 (m, 1H), 1.26 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 175.8; 141.6, 128.5, 127.9, 126.6, 76.4, 60.7, 47.1, 14.5, 14.1; IR (film, cm^{-1}): 3463, 3031, 2980, 1733, 1250, 1025, 767; MS (*m/z*, % rel.): 56 (42), 74 (100), 79 (49), 102 (84), 107 (65), 133 (1), 145 (3), 161 (2), 190 (2), 208 (M‡ , 1); $[\alpha]_D^{25}$: - 48,7 (*c* 1.0, $CHCl_3$), lit.[28] $[\alpha]_D^{23}$: - 42,3 (*c* 2.5, $CHCl_3$).

2.3.3. (2S,3S)-Ethyl 3-(4-methoxyphenyl)-2-methyl-3-oxopropanoate (5a)

Purification using silica gel column chromatography (hexane–ethyl acetate, 6:1) gave compound **5a** as a pale yellow oil. Enantiomeric excesses were determined by chiral HPLC using 5% isopropanol–hexane, flow rate = 0.6 mL min^{-1} and λ = 220 nm, [(2*R*,3*R*) = 22.87 min, (2*R*,3*S*) = 24.75 min]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.35-7.24 (m, 5H), 5.0 (m, 1H), 4.10 (q, J = 6.9 Hz, 2H), 2.98 (d, J = 3.3 Hz, 1H), 2.77 (qd, J = 7.2 Hz, J = 3.9 Hz, 1H), 1.6 (s, 1H), 1.21 (t, J = 6.9 Hz, 3 H), 1.13 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 175.9, 141.4, 128.2, 127.5, 126.0, 60.7, 46.4, 14.1, 10.5, 14.8; IR (film, cm^{-1}): 3488, 3031, 2982, 1731, 1454, 1374, 1253, 770, 701; MS (*m/z*, % rel.): 51 (49), 77 (100), 79 (58), 102 (82), 107 (42), 133 (1), 145 (5), 190 (2); $[\alpha]_D^{25}$: - 25,4 (c 1.0, $CHCl_3$,), lit.[28] $[\alpha]_D^{23}$: - 15,7 (*c* 2.1, $CHCl_3$).

2.3.4. (2R,3R)-Ethyl 3-benzyl-2-hydroxybutanoate (7)

Purification using silica gel column chromatography (hexane–ethyl acetate, 6:1) gave compound **7** as a pale yellow oil. Enantiomeric excesses were determined by chiral HPLC using 15% isopropanol–hexane, flow rate = 0.4 mL min^{-1} and λ = 220 nm, [(2*S*,3*S*) = 25.35 min, (2*R*,3*R*) = 14.68 min]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.30-7.26 (m, 2H), 7.21-7.14 (m, 3H), 4.22-4.14 (m, 2H), 4.10 (dd, J = 5.1 Hz, J = 3.3 Hz, 1H), 2.86 (d, J = 5.1 Hz, 1H), 2.69 (dd, J = 13.5 Hz, J = 6 Hz, 1H), 2.48 (dd, J = 13.8 Hz, J = 9 Hz, 1H), 2.32-2.30 (m, 1H), 1.31 (t, J = 7.5 Hz, 3H), 0.98 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 174.7, 140.2, 129.2, 128.2, 126.0, 74.1, 61.7, 39.3, 37.4, 16.0, 14.3; IR (film, cm^{-1}): 3513, 2977, 2934, 1729, 1496, 1219, 1142, 1062, 701; MS (*m/z*, % rel.): 57 (12), 76 (60), 91 (100), 104 (93), 119 (2), 131 (4), 147 (1), 204 (1), 222 (M‡ , 1); $[\alpha]_D^{25}$: - 38,75 (c 1.0, $CHCl_3$).

2.3.5. (2R,3S)-Ethyl 3-benzyl-2-hydroxybutanoate (8)

Purification using silica gel column chromatography (hexane–ethyl acetate, 6:1) gave compound **8** as a pale yellow oil. Enantiomeric excesses were determined by chiral GC using the following method: 60S to 180 °C (5 °C min^{-1}), flow rate of H_2 = 0.8 mL min^{-1}. [(2*S*,3*R*) = 27.75 min, (2*R*,3*S*) = 28.26 min]. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.32-7.18 (m, 5H), 4.31-4.15 (m, 2H), 4.06 (dd, J = 5.1 Hz, J = 2.4 Hz, 1H), 2.81 (dd, J = 13.5 Hz, J = 8.1Hz, 1H), 2.77 (d, J = 5.4 Hz, 1H), 2.59 (dd, J = 13.2 Hz, J = 7.5 Hz, 1H), 2.29-2.16 (m, 1H), 1.28 (t, J = 6.9 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 175.3, 140.3, 129.2, 128.3, 126.1, 72.0, 61.7, 39.5, 38.8, 14.2, 13.2; IR (film, cm^{-1}): 3509, 2965, 2934, 1728, 1453, 1254, 1217, 1133, 742; MS (*m/z*, % rel.): 57 (14), 76 (48), 91 (100), 104 (74), 119 (4), 131 (14), 147 (1), 176 (1), 204 (1), 222 (M‡ , 1); $[\alpha]_D^{25}$: - 20,5 (c 1.0, $CHCl_3$).

2.3.6. (R)-Ethyl 3-benzyl-2-hydroxybut-3-enoate (6)

Purification by using silica gel column chromatography (hexane–ethyl acetate, 6:1) yielded compound **6** as a pale yellow oil. Hydrogenation of **6** (0.220 g, 1 mmol) in ethyl acetate (10 mL) catalyzed by Pd/C (5%) at room temperature and under 1 atm of H_2 for 24 h, followed by filtration though Celite and evaporation of the solvent gave a mixture of (2*R*,3*R*)-**7** and (2*R*,3*S*)-**8** (96% yield, dr 60:40), which was then separated by silica gel column chromatography (hexanes–ethyl acetate, 6:1). The enantiomeric excess of (*R*)-**6** was determined from the chiral HPLC chromatograms of the corresponding reduced derivatives. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.31-7.17 (m, 5H), 5.27 (s, 1H); 4.97 (d, J = 1.2 Hz, 1H), 4.56 (d, J = 5.7 Hz, 1H), 4.20-3.98 (m, 2H), 3.50 (d, J = 15.6 Hz, 1H), 3.40 (d, J = 15.3 Hz, 1H), 3.15 (d, J = 5.4 Hz, 1H), 1.24 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 173.4, 145.6, 138.3, 129.3, 128.3, 126.3, 73.6, 62.1, 38.4, 14.0; IR (film, cm^{-1}): 3498, 3029, 2983, 2910, 1734, 1260, 1206, 1090, 1023, 701; MS (*m/z*, %rel.): 41 (5), 65(7), 77 (5), 91 (41), 115 (17), 129 (92), 147 (100), 156 (1), 174 (6), 202 (1), 220 (M‡ , 2); HRMS calcd. for $C_{13}H_{16}O_3$: [M‡] = 220,10995 found. [M‡] = 220.10994; $[\alpha]_D^{25}$: - 15,5 (c 1.0, $CHCl_3$).

2.3.7. Preparation of MPA-esters of alcohols 7 and 8 for absolute configuration determination

A solution of hydroxyester **7** or **8** (30 mg, 0.14 mmol) in dry dichloromethane (5 mL) was treated with triethylamine (23.4 mL, 0.17 mmol) and (*R*)-MPA-Cl or (*S*)-MPA-Cl (52 mg, 0.28 mmol) and stirred at 25 °C for 12 h. Evaporation of the solvent and purification by silica gel column chromatography (hexanes–ethyl acetate, 14:1) gave the MPA-ester derivatives that were characterized by ^{1}H NMR spectroscopy.

(*R*)-MPA-**7** ester (79% yield): ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.52-7.50 (m, 2H), 7.41-7.35 (m, 3H), 7.28-7.24 (m, 2H), 7.19 (m, 1H), 7.12-7.10 (m, 2H), 4.98 (d, J = 4 Hz, 1H), 4.87 (s, 1H), 3.99 (m, 2H), 3.51 (s, 3H), 2.76 (dd, J = 12.5 Hz, J = 4.5 Hz, 1H), 2.47 (dd, J = 12.5 Hz, J = 9 Hz, 1H), 2.44 (m, 1H), 1.10 (t, J = 7 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H).

(*S*)-MPA-**7** ester (60% yield): ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.53-7.51 (m, 2H), 7.42-7.35 (m, 3H), 7.24 (d, J = 7.5 Hz, 2H), 7.19 (m, 1H), 7.02 (d, J = 7.0 Hz, 2H), 4.96 (s, 1H), 4.94 (d, J = 4.5 Hz, 1H), 4.18 (q, J = 7.0 Hz, 2H), 3.59 (s, 3H), 2.66 (dd, J = 12 Hz, J = 4.5 Hz, 1H), 2.34 (m, 1H), 2.32 (dd, J = 12 Hz, J = 9.5 Hz, 1H), 1.26 (t, J = 7.0 Hz, 3H), 0.69 (d, J = 6.5 Hz, 3H).

(*R*)-MPA-**8** ester (70% yield): ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.55-7.54 (m, 2H), 7.42-7.39 (m, 2H); 7.37 (m, 1H), 7.30-7.17 (m, 3H), 6.93 (m, 2H), 4.91 (s, 1H) 4.86 (d, J = 3Hz), 4.07 (m, 2H), 3.56 (s, 3H), 2.56 (dd, J = 13.5 Hz, J = 8.5 Hz, 1H), 2.50 (dd, J = 13.5 Hz, J = 7.5 Hz, 1H), 2.40 (m, 1H), 1.11 (t, J = 7.0 Hz, 3H), 0.95 (d, J = 7.0 Hz, 3H).

(*S*)-MPA-**8** ester (55% yield): ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.56-7.55 (m, 2H), 7.47-7.43 (m, 3H), 7.15-7.13 (m, 3H), 6.62-6.65 (m, 2H), 4.96 (s, 1H), 4.80 (d, J = 3 Hz, 1H), 4.18 (m,

2H), 3.49 (s, 3H), 2.32 (m, 1H), 2.24 (m, 2H), 1.22 (t, $J = 7$ Hz, 3H), 0.90 (d, $J = 7$ Hz, 3H).

2.4. Immobilization of Baker's yeast with sodium alginate

To a suspension of *Saccharomyces cerevisiae* cells (2 g) in water (28.8 mL) was added a 2% solution of sodium alginate (0.56 g). The suspension was slowly extruded into a 0.2 mol L^{-1} $CaCl_2$ solution (200 mL) by using a syringe (connected to a 1 mm diameter orifice needle) and the spheres formed were stirred in the same solution for 20 min. After this, the spheres were filtered and rinsed with water (3 × 10 mL).

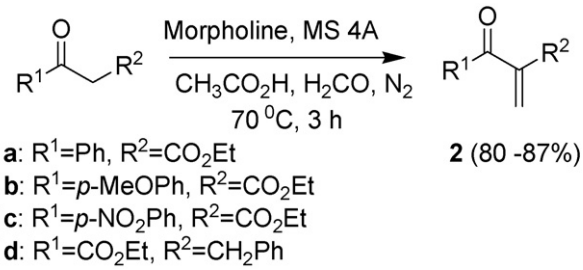
2.5. General procedure for the biotransformations with Baker's yeast immobilized with alginate

The methylene ketoester (0.4 mmol) was added to a mixture of immobilized Baker's yeast (prepared as above from 1 g of the dry Baker's yeast) and glucose (1 g) in water (50 mL) at 30 °C. The resulting mixture was maintained in a shaker at 30 °C. Samples were withdrawn from the reaction mixture at appropriate intervals and analyzed by the GC–MS technique. Subsequently, at the end of the reactions, the mixture was filtered and the immobilized biocatalyst was rinsed with ethyl acetate (2 × 10 mL) and the aqueous phase extracted with ethyl acetate (3 × 15 mL). The combined organic extracts were then washed with brine, dried over magnesium sulfate and the solvent was evaporated. The crude oil was purified by silica gel column chromatography as described in 2.3.1.

3. Results and discussion

The methyleneketoesters were readily prepared by performing a direct α-methylenation of the corresponding ketoesters using a protocol previously described by us [22] (Scheme 1). Attempts to purify compound **2c** led to decomposition, so the crude substrate was used without further purification.

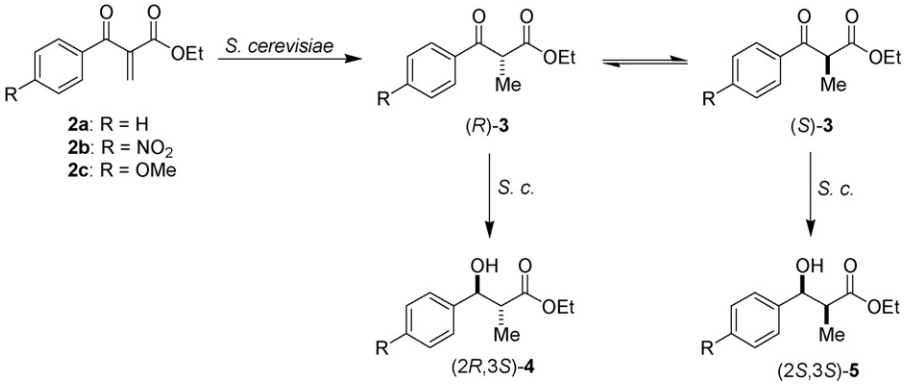
The reductions of the α-methylene-β-ketoesters **2a**–**c** by Baker's yeast were performed by stirring the reaction mixture in a shaker at 30 °C in the presence of glucose (Scheme 2). After appropriate intervals, samples were withdrawn from the



Scheme 1.

reaction mixture and analyzed by GC–MS in order to determine the relative rate of reduction. Enantiomeric excesses of the products were calculated by comparing the chiral-GC and chiral-HPLC chromatograms with those from racemic samples. Results are summarized in Table 1. There was a significant difference between the reduction rates of C=C and C=O that were mainly dependent on the substrate and pH of the reaction medium. Good results for substrate **2a** were observed when the reactions were performed in water. After 96 h all the starting material was converted into the products, leading to the preparation of the alcohols (2*R*,3*S*)-**4a** and (2*S*,3*S*)-**5a** in 86% *ee* and 97% *ee*, respectively. It was also observed that longer reaction times led to a decrease of the enantiomeric excess of the products. It is worth mentioning that when the same reaction was performed in pH 4.74 buffer no alcohol formation was observed, but after 168 h the starting material was totally converted into racemic ketoester **3a**.

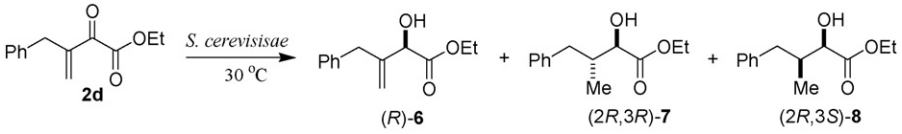
Our previous results [17] on the Baker's yeast reduction of α-methyleneketones **2** (R^1 = alkyl, Ph; R^2 = alkyl) showed that high enantioselectivity (*ee* 88 to >99%) is obtained in the C=C bond reduction only when R^1 = Me, and this process is faster than the C=O bond reduction. In the present work, we can rationalize the results obtained with **2a**–**b** as presented in Scheme 2. The production of racemic β-ketoester **3a** is due to C=C bond reduction of **2a** in low *ee* and/or followed by a racemization of **3a** by keto–enol tautomerism. In a second step, the reduction of the C=O bond of **3** is performed in high enantioselectivity giving (2*R*,3*S*)-**4** and (2*S*,3*S*)-**5**. Hydride transfer to the C=O bond occurs at the *re*-face, irrespective of the configuration of the adjacent methyl-bearing carbon, as



Scheme 2.

Table 1
Reduction of **2a–c** with *S. cerevisiae* in water

Entry	R	pH	Time (h)	%Composition (% *ee*)		
				3	(2*R*,3*S*)-**4**	(2*S*,3*S*)-**5**
1	H	7.0	24	12	28 (90)	15 (98)
2	H	7.0	48	15	43 (85)	30 (98)
3	H	7.0	72	18	44 (84)	31 (98)
4	H	7.0	96	21	45 (86)	31 (97)
5	H	7.0	120	20	45 (84)	32 (94)
9	NO_2	7.0	12	–	–	–
6	OMe	7.0	16	31	0 (0)	0 (0)
7	OMe	7.0	96	73	0 (0)	0 (0)
8	OMe	7.0	120	97	0 (0)	0 (0)
10	H	pH 4.7	96	31	0 (0)	0 (0)
11	H	pH 4.7	168	100	0 (0)	0 (0)
13	NO_2	pH 4.7	12	–	–	–
12	OMe	pH 4.7	48	24	0 (0)	0 (0)
14	OMe	pH 4.7	96	32	0(0)	0 (0)



Scheme 3.

has been observed previously in the Baker's yeast reduction of aliphatic [23] and aromatic ketones [16,24,25]. Also, *syn*-**5** prevails over its *anti*-**4** diastereoisomer, as has been observed in the reduction of various racemic α-substituted-β-ketoesters mediated by Baker's yeast [26].

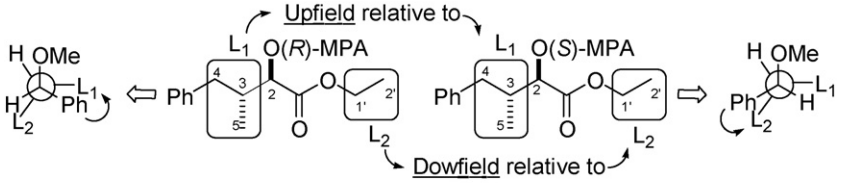
Similar results were obtained when compound **2c** was used as substrate. After 120 h reaction in water a high chemoselectivity was observed and compound **3c** was obtained exclusively with 97% conversion. The C=C bond reduction happens in the same way as with **2a**, but the subsequent reduction of the C=O bond of **3b** does not occur due to a deactivation caused by the methoxy group bound to the aromatic ring. This deactivation effect has been observed with various examples of Baker's yeast reduction [27]. When substrate **2b** was used, however, a fast decomposition of the products was observed (retro-aldol reaction). The absolute stereochemistry of alcohols **4a** and **5a** were determined by comparing the $[\alpha]_D$ values of the products obtained with those from the literature [28].

Similar to substrate **2a**, the bioreduction of the β-methylene-α-ketoester **2d** mediated by *Saccharomyces cerevisiae* led to the preparation of the corresponding β-methyl-α-hydroxyesters **7** and **8** in good enantiomeric excess (Scheme 3). Results are shown in Table 2. For example, when the reaction was performed in water (pH 7.0) after 24 h it was observed that 88% of **2d** was converted into products and compounds (2*R*,3*R*)-**7** and (2*R*,3*S*)-**8** were obtained in 92% *ee* and 98% *ee*, respectively. At pH 4.7, as expected the reduction yield of the C=C bond increased and the alcohols **7** and **8** were isolated. In that aspect **2d** has different behavior when compared with the reduction of **2a–b** (Table 1, entries 1–8). In this compound, the C=O bonds are activated for reduction by the ethoxycarbonyl group. Therefore, the reduction rate of the C=O bond is enhanced and competes

Table 2
Reduction of **2d** with free and immobilized *S. cerevisiae*

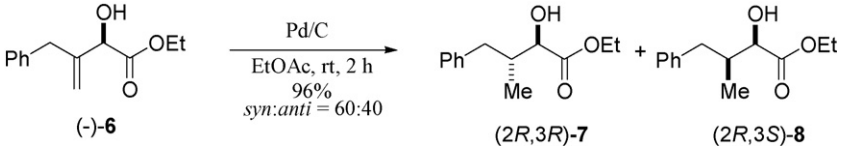
Entry	Reaction medium	Time (h)	%Composition (% *ee*)		
			(*R*)-**6**	(2*R*,3*S*)-**7**	(2*R*,3*R*)-**8**
1	H_2O	6	18 (20)	30 (93)	21 (98)
2	H_2O	24	14 (35)	44 (92)	42 (98)
3	H_2O	48	12 (33)	44 (90)	44 (95)
4	pH 4.7	24	43 (29)	19 (88)	38 (96)
5	pH 4.7	48	33 (19)	27 (89)	40 (95)
5	pH 4.7	96	32 (18)	28 (87)	40 (93)
6	H_2O[a]	48	13 (32)	37 (84)	19 (93)
7	H_2O[a]	96	8 (30)	39 (82)	22 (91)
8	pH 4.7[a]	96	6 (29)	37 (80)	19 (88)

[a] Baker's yeast immobilized in sodium alginate.



	(*R*)-MPA ester	(*S*)-MPA ester	$\Delta\delta L^{RS}$
H_{4a} (ppm)	2.76	2.66	+ 0.10
H_{4b} (ppm)	2.47	2.32	+ 0.15
H_3 (ppm)	2.44	2.34	+ 0.10
H_5 (ppm)	0.89	0.69	+ 0.20
$H_{1'}$ (ppm)	3.99	4.18	- 0.19
$H_{2'}$ (ppm)	1.10	1.26	- 0.16

Scheme 4.



Scheme 5.

with the reduction of the C=C bond, giving the product (*R*)-**6** in low *ee*, and (2*R*,3*S*)-**7** and (2*R*,3*R*)-**8** in high *ee*. The reduction of substrate **2d** was also tested using the biocatalyst immobilized with sodium alginate [29]. Despite the easier work-up, both the conversion of starting material and the enantiomeric excesses of the products were observe to decrease when immobilized Baker's yeast was used.

The absolute configuration of the chiral alcohols (−)-**7** and (−)-**8** were determined by ^{1}H NMR. α-Methoxy-α-phenylacetic acid (MPA) is one of the most frequently used auxiliary reagent for the assignment of the absolute configuration of secondary alcohols by NMR. In the most representative conformer of the ester formed has the methoxy group of MPA, the carbonyl of the ester and C(H) proton of the alcohol are in the same plane. These groups are arranged in such way that in the (*R*)-MPA ester, substituent L_1 is shielded by the phenyl ring while L_2 is unaffected, whereas, in the (*S*)-MPA ester, substituent L_2 is the shielded group and L_1 unaffected [30]. Thus, the chiral alcohols (−)-**7** and (−)-**8** were initially derivatized with the two stereoisomers of α-methoxy-α-phenylacetylchloride (MPA-Cl) and the assignment of the ^{1}H NMR signals of the resulting diastereoisomeric esters clearly showed different chemical shifts, as shown for the MPA-esters of compound **7** (Scheme 4). This effect allowed us to calculate the parameters $\Delta\delta^{RS}L_1$ ($\delta L_1(R) - \delta L_1(S)$) and $\Delta\delta^{RS}L_2$ ($\delta L_2(R) - \delta L_2(S)$) for these derivatives, which represent the differences between the ^{1}H NMR signs for the substituents L_1 and L_2, respectively [31] (Scheme 4). Correlation of these parameters with the data reported in the literature [31,32] led us to deduce the absolute configuration as being (2*R*,3*R*) for compound (−)-**7**. By applying the same protocol, the absolute configuration of compound (−)-**8** was found to be (2*R*,3*S*).

Absolute stereochemistry of the allylic alcohol (−)-**6** was determined by derivatization. Thus, a palladium catalyzed hydrogenation of (−)-**6** led to a mixture of alcohols (2*R*,3*R*)-**7** and (2*R*,3*S*)-**8** (Scheme 5). Since the Pd/C catalyzed hydrogenation does not affect the stereogenic center of (−)-**6**, the absolute stereochemistry of this compound was attributed as being (*R*).

4. Conclusion

In conclusion, the Baker's yeast reduction of the α-methyleneketoesters led to the preparation of chiral methyl hydroxyesters with high enantiomeric excesses. Reduction of ethyl 2-methylene-3-oxo-3-arylpropanoates **2a–c** by *S. cerevisiae* gave good conversion of the C=C followed, in some cases by C=O reduction, depending on the reaction conditions and on the substitution of the aryl moiety. Reaction of 3-methylene-2-oxo-4-phenylbutyrate **2d** was carried out with free yeast and yeast immobilized with calcium alginate. The major products resulted from C=O reduction followed by C=C reduction due to the C=O activation by the ethyl carboxylate group.

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