Bioreduction of α-methyleneketones

Moacir de Mancilha, Roseli De Conti, Paulo J. S. Moran, and J. Augusto R. Rodrigues*

Universidade Estadual de Campinas, Instituto de Química, 13083-970 Campinas-SP, Brazil
E-mail: jaugusto@iqm.unicamp.br

Dedicated to Prof. R. A. Abramovitch in honor of his 70th birthday

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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 pheromones[5] and, in many cases bioactivity is dependent upon the

configuration of the α-carbon. the. [6] Herein we wish to report chemo- and enantioselective bioreduction studies of α-methyleneketones 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 the importance of this class of compounds as useful intermediates.[7] A number of methods have been described and reviewed.[8] We have tested different methods for a direct methylene transfer;[9] the most successful, efficient and general method was the direct Mannich α-methylenation introduced by Kim.[10] 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).

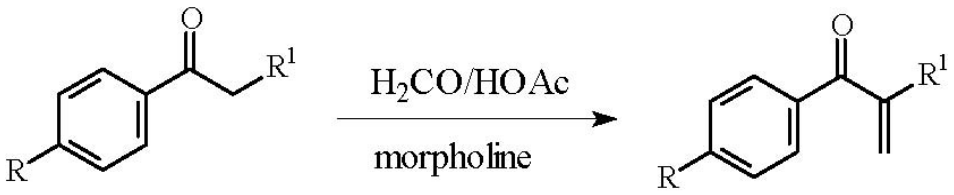
Baker's yeast reduction of 2-methyl-1-phenylprop-2-en-1-one **2a** was fast and gave *iso*-propyl phenyl ketone **3a** in reasonable yields. In order to facilitate the isolation of the product from the broth we employed yeast immobilized (IMBY) onto montmorillonite K10.[11] Using short periods (6 h) 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*.[12] Treatment of the acrylophenone **2b** with IMBY gave, after 12 h, a 67% yield of (2*R*)-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.[13] 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*.[14] 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 ^{0}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.

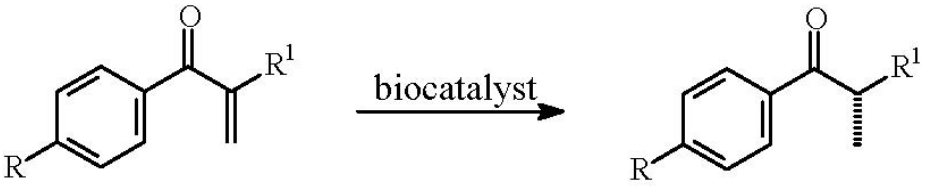
We have commented above that aryl alkyl ketones are reduced by baker's yeast in low yields although the study of the reduction of γ-ketoesters **1c** and **1e** was not systematically consummated. In contrast, it is well known that α- and β-ketoesters are very suitable substrats for biocatalysts.[2b-c] The ketoester **1e** was not metabolized by IMBY since the *p*-methoxy group is a strong electron donor that inactives the substrate.[15] 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(3*H*)-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.16 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. fluoresncens*.

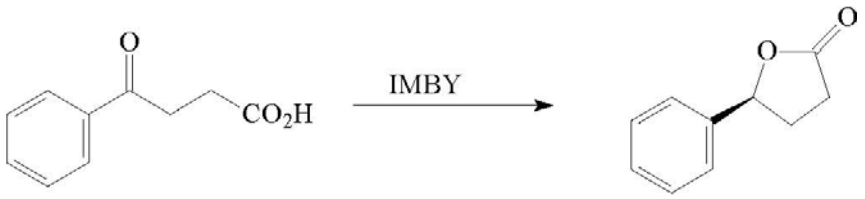


1 **2**

a. R = H; $R^1 = CH_3$ **d.** R = H; $R^1 = CH_2CO_2H$
b. R = H; $R^1 = CH_2CH_3$ **e.** $R = OCH_3$; $R^1 = CH_2CO_2CH_3$
c. R = H; $R^1 = CH_2CO_2CH_3$



2a-e **3a-e**



1d **4**

Table 1. α-Methylenation of representative ketones[a]

Entry	Substrate	Product	Time, h	Yield (%)	Ref.[b]
1	**1a**	(**2a**)[a]	8	82	17
2	**1b**	**2b**	12	84	18
3	**1c**	**2c**	6	91	19
4	**1d**	**2d**	6	62	---
5	**1e**	**2e**	6	71	---

[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 α-methyleneketones with microorganisms

Entry	Product	Biocatalyst	Time h	Yield %	*ee*	Ref[a].
1	**3a**	IMBY	12	76	---	20
		R. arryzus	24	65	---	
		P.fluorecens	24	90	---	
2	**3b**	IMBY	12	67	93	13
		R. arryzus	24	76	43	
		P.fluorecens	24	79	99	
3	**3c**	IMBY	12	59	12	14
		R. arryzus	24	69	25	
		P.fluorecens	24	73	99	
4	**3d**	IMBY	17			
5	**3e**	IMBY	17	65	85	
		R. arryzus	24	56	35	---
		P.fluorecens	24	69	99	

[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-INOVA 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 ^{0}C and a detector temperature of 280 ^{0}C, with the column at 50 ^{0}C for 3 min; then using a rate of 20 0C/min. up to 280 ^{0}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 ^{0}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).[14]
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) ν 3062, 1712, 1657, 1597, 1449, 1233 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 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); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 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) ν 2954, 2925, 1736, 1650, 1602, 1461, 1257, 1160 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 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* = 79.2); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 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 ^{0}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 ^{0}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 ^{0}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 ^{0}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 ^{0}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 ^{0}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}; ^{1}H-NMR (300 MHz, $CDCl_3$) 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). $[\alpha]^{25}_D$ -6.0(c 0.01, $CHCl_3$).

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}. ^{1}H-NMR (300 MHz, $CDCl_3$) 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). $[\alpha]^{25}_D$ -15(c 0.55, $CHCl_3$). HRMS calcd. for $C_{13}H_{16}O_4$: 236,2637; found *m/z* 236,2629 (M^+).

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