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Effect of additives on the bioreduction of 2-chloro-1-phenyl-2-propen-1-one by baker's yeast

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Abstract

The reduction of 2-chloro-1-phenyl-2-propen-1-one (**2**) by baker's yeast (*Saccharomyces cerevisiae*) was performed under various reaction conditions, such as the addition of allyl alcohol, sucrose, thermal pre-treatment of the cells and use of a citrate buffer at pH 4.0. 2-Chloro-1-phenyl-1-propan-1-one (**3**) was produced after 2 h in a poor enantiomeric excess (ee) while after a longer period of fermentation, the compounds 1*R*,2*R*- and 1*R*,2*S*-2-chloro-1-phenylpropan-1-ol (**4**) were obtained at 80–90 and 28–63% ee.

Keywords: α-Chloropropiophenone; Enantio-selective bioreduction; *Saccharomyces cerevisiae*; Allyl alchool; α-Methylene ketone

1. Introduction

The α-, β-unsaturated ketones have been investigated as potential substrates for biocatalytic reductions that can lead to the introduction of one, two or three new chiral centres into an achiral structure [1–7]. The baker's yeast reduction of α-methylene ketones is dependent on the substituents and in general can give products with reduced C=C bonds and to a lesser extent, with reduced C=O bonds [5]. The main advantages of using biotransformations mediated by baker's yeast in syntheses are the mild reaction conditions required, such as room temperature, close to neutral pH and a stereo-selective low expense process.

In this work, we present the results of the baker's yeast reduction of 2-chloro-1-phenyl-2-propen-1-one (**2**) in order to obtain chiral products with both reduced C=C and C=O bonds. Since the reduction of the C=C bond should occur first [5], one can expect the production of **3** as an intermediate. It is known that the baker's yeast reduction of **3** is a powerful tool for obtaining chiral halohydrines with high enantiomeric purity [8,9]. These halohydrins can be converted into chiral epoxides, which are important chiral building blocks for the synthesis of biologically active molecules [10,11].

2. Experimental

The ^{1}H NMR spectra were recorded on an INOVA-500 spectrometer with $CDCl_3$ as solvent and TMS as internal standard. Gas chromatography analyses were performed using a QP 5000-SHIMADZU gas chromatograph/mass spectrometer and helium as carrier gas. A 30 m × 0.25 mm (i.d.) capillary column of fused silica SIMPLICITY 1 SUPELCO^{TM} was used and the chiral column employed in the determination of enantiomeric excess (ee) was a

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30 m × 0.25 mm (i.d.) BETA-DEX SUPELCO™ column. Column chromatography was carried out using silica gel flash (Merck). TLC was carried out on plates prepared with HF_{254} and GF_{254} silica gel (Merck). The IR spectra were recorded on a BOMEM MB SERIES Hartmann & Braun spectrometer. Optical rotations were measured on a Carl Zeiss Polamat A. Commercially available chemicals and solvents were used with no further purification. The commercially available dry baker's yeast Suprema, Akmaya Sanay ve Ticaret A. S. was used in this work.

2.1. 2-Chloro-1-phenyl-2-propen-1-one (**2**)

A solution of 37% aqueous formaldehyde (20 ml) was added to a mixture of ω-chloroacetophenone (7.725 g, 50 mmol) and morpholine (2.2 ml, 25 mmol) in 90 ml of glacial acetic acid heated under reflux. After 5 h, aqueous NaOH (10%) was added to the cold reaction mixture until neutral pH and the products were extracted with ethyl acetate. The organic layer was successively washed with aqueous HCl (10%), saturated sodium bicarbonate solution, water and brine, and dried over anhydrous magnesium sulfate. Evaporation of the solvent resulted in 7.0 g of an oil, which after purification by silica gel column chromatography (1:3 ethyl acetate/hexane), yielded 4.2 g (50.4%) of **2** as a yellow oil. IR (film): 1680, 1600, 1450 cm^{-1}, ^1H NMR: δ 6.07 (s, 1H), 6.28 (s, 1H), 7.48 (m, 5H); MS: *m/z* (%) 166 (M^+, 15), 105 (100), 77 (71), 51 (45).

2.2. General procedure for baker's yeast reduction of 2-chloro-1-phenyl-2-propen-1-one (**2**)

Distilled water (250 ml) and commercial dry baker's yeast (20–100 g) were added to a 500 ml three-necked flask. This mixture was stirred for 15 min at 30°C. The additive allyl alcohol and/or sucrose was eventually added according to Table 1. Subsequently, 1.20–4.62 mmol of **2**, dissolved in 1 ml of ethyl alcohol, were added, and the stirring was continued at 30°C. After 2 h, samples were withdrawn from the reaction mixture, extracted with ethyl acetate and analyzed by GC/MS, showing only the formation of **3**. After 120–200 h, celite (100 g) and ethyl acetate (50 ml) were added to the reaction mixture with constant stirring. Subsequently, the organic layer was separated, dried with magnesium sulfate and the solvent evaporated to obtain a mixture of *syn*- and *anti*-**4** isomers that was analyzed by GC/MS to determine the *syn*/*anti* ratio, followed by the diastereomeric separation procedure.

Thermal pre-treatment of baker's yeast: a mixture of baker's yeast-water was pre-heated at 50°C for 30 min. Subsequently, the general procedure for baker's yeast reduction of **2** was followed (Table 1, entry 5).

Baker's yeast reduction of **2** in citrate buffer at pH 4: 10 ml of aqueous citric acid (1 M) was added to a mixture of baker's yeast-water followed by the addition of aqueous NaOH (1 M) to maintain the reaction mixture at pH 4. Subsequently, the general procedure

Table 1
Baker's yeast reduction of 2-chloro-1-phenyl-2-propen-1-one (**2**) at 30°C

Entry	Dry baker's yeast (g)[a]	**2** (mmol)	Sucrose (g)	Allyl alcohol (ml)	**3**[b] Enantiomeric ratio	**4**[c] (Isomer)			
						ee (%) *syn* (1*R*,2*R*)	ee (%) *anti* (1*R*,2*S*)	*Syn*/*anti* Ratio	Yield (%)
1	100	4.62	50	–	1.20	80	50	0.96	51
2	100	4.62	–	–	1.42	86	43	0.80	78
3	100	6.00	50	1.00	0.83	88	48	1.12	56
4	100	6.00	–	1.00	0.89	90	63	1.28	34
5[d]	40	1.80	–	0.40	0.62	–	–	–	–
6[e]	20	1.20	20	–	1.02	88	28	0.45	60

[a] In 250 ml of water.
[b] After 2 h.
[c] After 120–200 h.
[d] Baker's yeast pre-treated by 30 min at 50°C.
[e] Citrate buffer pH 4.0.

for baker's yeast reduction of **2** was followed (Table 1, entry 6).

2.3. 2-Chloro-1-phenyl-1-propanone (*3*)

The GC/MS data of compound **3** were identical to an authentic sample obtained by reduction of **2** (1 mmol) dissolved in CH_2Cl_2(10 ml) with H_2 Pd/C at 1 atm and 25°C for 30 min. The stereomeric ratio (see Table 1) was obtained by GC/MS using a chiral column.

2.4. Syn- and anti-2-chloro-1-phenylpropan-1-ol (*4*)

The *syn*- and *anti*-**4** isomers were separated by preparative TLC, developed at −20°C, using petrol ether/ethyl ether (90:10) as eluent. The ee of each *syn*- and *anti*-**4** isomers (see Table 1) were obtained by GC/MS using a chiral column.

Syn-**4** isomer: ^{1}H NMR δ 1.38 (d, $J = 6.6$ Hz, 3H), 2.80 (d, $J = 3.3$ Hz, 1H), 4.22 (dq, $J = 6.6$, 7.7 Hz, 1H) 4.6 (dd, $J = 3.3$, 7.7 Hz, 1H), 7.4 (m, 5H). *Anti*-**4** isomer: ^{1}H NMR δ 1.38 (d, $J = 6.6$ Hz, 3H), 2.44 (d, $J = 3.3$ Hz, 1H), 4.30 (dq, $J = 3.6$, 6.6 Hz, 1H), 4.94(dd, $J = 3.3$, 3.6 Hz, 1H), 7.40 (m, 5H); MS: *m/z* (%) 170 (M^+, 1), 107 (100), 79 (80).

2.5. Determination of the absolute configuration of syn- and anti-2-chloro-1-phenylpropan-1-ol (*4*)

The absolute configurations of *syn*- and *anti*-**4** isomers were established by derivation of each isomer into the corresponding *cis*- and *trans*-**5** epoxides, following the general procedure. Compound **4** (1.00 g, 5.86 mmol) was added to a 50 ml of aqueous NaOH (2N) and the resulting mixture stirred for 4 h at 25°C. The reaction mixture was extracted with ethyl ether, dried under anhydrous magnesium sulfate and the solvent evaporated, giving 0.47 g of epoxide (3.52 mmol, 60%). *Cis*-**5** epoxide: a colorless oil, ^{1}H NMR δ 1.08 (d, $J = 5.1$ Hz, 3H), 3.33 (dq, $J = 4.0$, 5.1 Hz, 1H), 4.06 (d, $J = 4.0$ Hz, 1H), 7.30 (m, 5H); $[\alpha]_D^{20}$ −12.3 (0.6 $CHCl_3$), {lit. [15]: $[\alpha]_D^{20}$ + 43.7 (1.43 $CHCl_3$) for the isomer 1*S*, 2*R*}. *Trans*-**5** epoxide a colorless oil, δ 1.45 (d, $J = 5.1$ Hz, 3H), 3.03 (dq, $J = 2.2$, 5.1 Hz, 1H), 3.57(d, $J = 2.2$ Hz, 1H), 7.33 (m, 5H); $[\alpha]_D^{20}$ +21.0 (0.6 $CHCl_3$), {lit. [15]: $[\alpha]_D^{20}$ +48.5 (0.94 $CHCl_3$) for the isomer 1*R*, 2*R*}.

2.6. Determination of the stereo-isomer compositions of 2-chloro-1-phenylpropan-1-ol (*4*)

The following set of equations was used for the calculation of the stereo-isomers composition of **4** as stated in Table 2, from each entry data of Table 1:

$$\mathrm{ee}_{1R,2R}(\%) = \left[\frac{(1R,2R - 1S,2S)}{(1R,2R + 1S,2S)}\right]$$

$$\mathrm{ee}_{1R,2S}(\%) = \left[\frac{(1R,2S - 1S,2R)}{(1R,2S + 1S,2R)}\right]$$

$$syn/anti\,\mathrm{ratio} = \frac{(1R,2R + 1S,2S)}{(1R,2S + 1S,2R)}$$

$$100 = 1R,2R + 1S,2S + 1R,2S + 1S,2R$$

Table 2
Composition of stereo-isomers of 2-chloro-1-phenylpropan-1-ol (**4**) in the baker's yeast reduction of 2-chloro-1-phenyl-2-propen-1-one (**2**) at 30°C

Entry	Dry baker's yeast (g)[a]	**2** (mmol)	Sucrose (g)	Allyl alcohol (ml)	1*R*,2*R* (%)	1*S*,2*R* (%)	1*R*,2*S* (%)	1*S*,2*S* (%)	Ratio $\frac{1R,2R}{1S,2R}$	Ratio $\frac{1R,2S}{1S,2S}$
1	100	4.62	50	–	44	13	38	5	3.4	7.6
2	100	4.62	–	–	41	16	40	3	2.7	13
3	100	6.00	50	1.00	50	12	35	3	4.2	12
4	100	6.00	–	1.00	53	8	36	3	6.6	12
5[b]	40	1.80	–	0.40	–	–	–	–	–	–
6[c]	20	1.20	20	–	29	25	44	2	1.2	22

[a] In 250 ml of water; (b) after 2 h; (c) after 120–200 h.
[b] Baker's yeast pre-treated for 30 min at 50°C.
[c] Citrate buffer pH 4.0.

3. Results and discussion

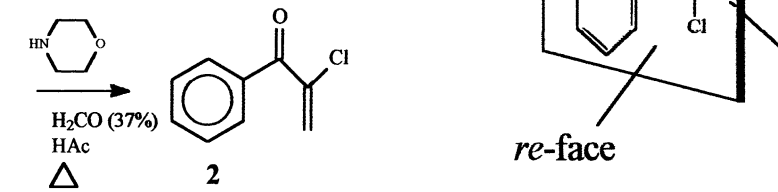
Compound **2** was prepared from **1** according to the reference [12] by the Mannich reaction (Scheme 1).

Compound **2** was biotransformed by baker's yeast under diverse conditions, as shown in Table 1. Compound **3** was obtained in the course of 2 h, and the enantiomeric ratio of **3** was determined by chiral GC/MS analysis of a sample withdrawn from the reaction mixture. After a longer period, compound **3** was subsequently biotransformed into *syn*- and *anti*-**4** isomers (Scheme 2).

The absolute configurations of *syn*- and *anti*-**4** isomers were established by conversion of each isomer into the respective *cis*- and *trans*-**5** epoxides, and the optical rotations of these epoxides were compared with the data previously published (Scheme 3) [13–15]. Thus, the major enantiomers of *syn*- and *anti*-**4** have 1*R*,2*R* and 1*R*,2*S* configurations, respectively.

While the stereo-selectivity of the C=C bond reduction of compound **2** was poor, the stereo-selectivity of the C=O bond reduction was reasonable. Attempts to improve the stereo-selectivities of these reductions gave interesting results. Thermal pre-treatment of the cells and the addition of allyl alcohol or methyl vinyl ketone have been used extensively in bioreductions mediated by baker's yeast as potent additives that can influence the enantio-selectivity [16–20]. In fact the bioreductions of **2** with the addition of allyl alcohol and thermal pre-treatment of the cells, gave **3** containing an excess of the enantiomer with the opposite configuration to that obtained in excess when the bioreduction was performed without additives. Table 1 shows that the enantiomeric ratios change from 1.42 to 0.62 (entries 1–5). When the bioreduction of **2** was performed in a citrate buffer at pH 4, racemic **3** was obtained (Table 1, entry 6).

In the bioreduction of 3, the hydride transfer to the C=O bond occurs selectively to the *si*-face following



Scheme 3.

Scheme 1.

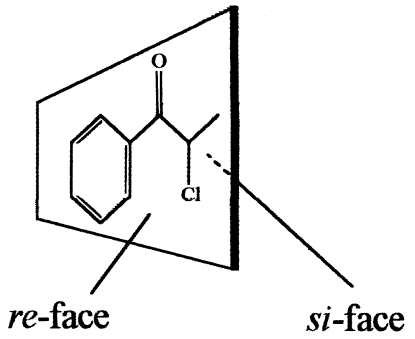
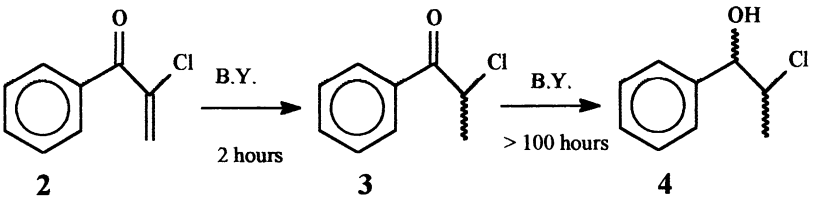
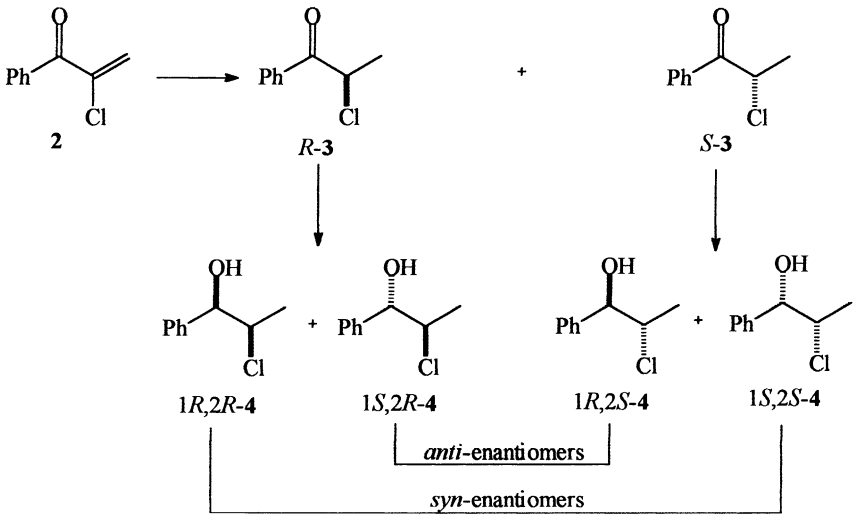


Fig. 1. The *re*-face and *si*-face of 2-chloro-1-phenyl-1-propan-1-one.



Scheme 2.



Scheme 4.

the Prelog rule, considering that the phenyl group is larger than the chloroethyl group (Fig. 1) [21]. It is interesting that this hydride transfer is independent of the configuration of the adjacent chloro-bearing carbon, as has been observed in the baker's yeast reduction of aliphatic [22] and aromatic ketones [9,23]. The influence of the additives was also observed in the subsequent bioreduction of **3**. It is important to know how the first chiral center influences the creation of the second chiral center in these bioreductions.

For a better understanding of the data shown in Table 1, a set of equations was derived from ee (%) of 1*R*,2*R*-**4**, ee (%) of 1*R*,2*S*-**4** and *syn*/*anti* ratio of **4**, which give us the composition of each stereo-isomer of **4** (see Table 2). The 1*R*,2*R*-**4**/1*S*,2*R*-**4** and 1*R*,2*S*-**4**/1*S*,2*S*-**4** ratios are related to the enantiomeric discrimination of C=O bond faces in the bioreduction of *R*- and *S*-**3** isomers, respectively (Scheme 4).

In general, the hydride was preferentially transferred to the *si*-face of both isomers, but for the isomer *S*-**3** this preference was remarkable, especially when the experiments were run without the addition of sucrose and also with the addition of allyl alcohol (Table 2, entries 2 and 4). The addition of allyl alcohol also increased the preference for the hydride transfer to the *si*-face of the *S*-**3** isomer, even when the experiment was performed with the addition of sucrose (Table 2, entry 3). The preference for hydride transfer to the *si*-face of the *S*-**3** isomer was highest when the experiment was performed at pH 4, while for the *R*-**3** isomer those was practically no preference (Table 2, entry 6).

4. Conclusion

In conclusion, while the use of allyl alcohol as an additive has little influence on the stereo-selectivity of the baker's yeast reduction of the C=C bond of **2**, it greatly influences the subsequent bioreduction of the C=O bond of **3**. The discrimination of the C=O faces of **3** to the hydride transfer is higher for the *S*-**3** than for the *R*-**3** isomer, hydride transfer to the *si*-face prevailing in both cases.

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