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Preparation of an (−)-ephedrine intermediate through asymmetric reduction of 1-phenyl-1,2-propanedione by anaerobically pre-treated baker's yeast

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Abstract

The influence of using an anaerobically pre-treated baker's yeast on the reduction of (*R*)-1-hydroxy-1-phenyl-2-propanone (**2**) and (*S*)-2-hydroxy-1-phenyl-1-propanone (**4**) was investigated in comparison with non-pre-treated baker's yeast reduction (control experiments). We observed that there is no significant difference between the anaerobically pre-treated yeast and the control experiment on the reduction rates of **2**. On the other hand, the rate of reduction of **4** mediated by the anaerobically pre-treated yeast is much slower than the aerobic experiment. To improve the regioselectivity of reduction of 1-phenyl-1,2-propanedione (**1**), a baker's yeast suspension was pre-treated with nitrogen (60 min) followed by oxygen (20 min), to give **2** in 28–31% of yields (96% e.e.) and **3** in 42–62% (>99% e.e.) after 75–90 min of reaction.

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Keywords: Baker's yeast; (*R*)-1-hydroxy-1-phenyl-2-propanone; Regioselectivity; Ephedrine analogs

1. Introduction

(*R*)-1-hydroxy-1-phenyl-2-propanone (**2**) (L-phenyl acetyl carbinol (L-PAC)) is a versatile chiral intermediate that has been used in the manufacture of the pharmaceuticals (−)-ephedrine and (−)-pseudoephedrine [1]. Usually, L-PAC is produced through biological methods. As such, strains of *Saccharomyces cerevisiae* and *Candida utilis* are frequently employed to mediate the acyloin condensation of benzaldehyde and pyruvic acid [2,3].

Another alternative for the biological production of **2** is the baker's yeast mediated reduction of diketone **1**, which affords a mixture of optically active **2**, **4** and (1*R*,2*S*)-1-phenyl-1,2-propanediol (**3**) (Scheme 1) [4]. Some of these products may be preferentially obtained depending on the method in which this biotransformation is performed. For instance, it was reported that benzoyn **4** is obtained as the main product, when (1) the reaction was carried out in a phosphate buffer solution at pH 5.0 [5]; (2) baker's yeast was submitted to a thermal pre-treatment at 53 °C and 3-buten-2-one (methyl vinyl ketone (MVK)) addition, as an enzyme inhibitor [6]; (3) microencapsulated yeast cells were used in organic solvents [7]. In addition, when the yeast reduction was performed at 5 °C for 9 h a mixture of **2–4** was delivered [4], whereas only compound **3** was isolated at 27–30 °C [4,8,9].

The previously reports [4,6,10] show α-hydroxy ketones **2** and **4** as intermediates in the baker's yeast reduction of **1–3** (Scheme 1). One or more enzymes must be involved in each step. Nakamura et al. [6] applied enzyme inhibitors and thermally pre-treated yeast to improve the regioselectivity of the reduction of **1** affording a product mixture of **4** and **2** (94:6, respectively). The addition of MVK suppressed formation of **3** due to inhibition of enzymes that act on the steps II and IV, and the thermal pre-treatment denatured responsible enzymes for step I [10]. Then, the aim of this work was to improve the regioselectivity of baker's yeast reduction of **1** in order to obtain acyloin **2** using anaerobically pre-treated cells.

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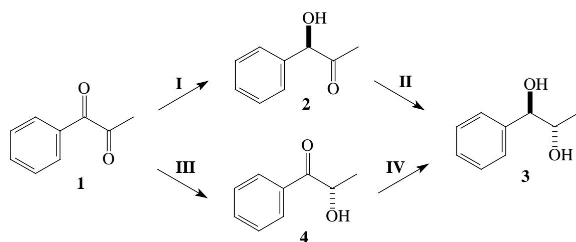

Scheme 1.

2. Experimental

Gas chromatographic (GC) analyses were performed on an Agilent 6890/HP 5973 mass selective (MS) detector system, with a HP-5MS column (cross-linked 5% phenylmethylsiloxane, 0.25 mm i.d., 30 m length). Enantiomeric excesses were determined by GC analysis using a chiral column (stationary phase: heptakis-(2,6-methyl-3-pentyl)-β-cyclodextrine). Thin layer chromatography were performed with silica gel 60 GF_{254} and HF_{254} (Merck). Commercially available dry baker's yeast (N.V. Algist–Bruggeman S.A.) was used in this work. ^{1}H NMR and ^{13}C NMR spectra were performed on a Varian Gemini 300P nuclear magnetic resonance spectrometer, using deutered chloroform as solvent and trimethylsilane as internal standard. IR spectra were performed on a Bomem MB Series Hartmann and Braun infrared spectrometer, using film on NaCl cells.

2.1. 1-phenyl-1,2-propanedione **1**

Compound **1** was prepared from propiophenone which was nitrosated by Slater's method [11] followed by acidic hydrolysis. Then, **1** was extracted with ethyl ether, and the organic phase was evaporated and the residue subjected to a preparative thin layer chromatography using hexane, affording **1** in 42–60% isolated yields for two steps (nitrosation and hydrolysis). IR (NaCl), ν (cm^{-1}): 3063, 2924, 2851, 1713, 1672, 1593, 1450, 1155, 900, 697. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.52 (s, 3H), 7.49 (m, 2H), 7.64 (m, 1H), 8.01 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.3, 128.8, 130.31, 131.7, 134.6, 191.4, 200.5.

2.2. (R)-1-hydroxy-1-phenyl-2-propanone **2**

To a stirred suspension of freeze-dried baker's yeast (4 g) in 50 ml of a potassium phosphate buffer solution (pH 3.0, 0.5 mol l^{-1}), kept at 30 °C for 60 min under nitrogen atmosphere, followed by oxygen bubbling for 20 min, a solution of diketone **1** (0.25 g, 1.69 mmol) in ethanol (1 ml) was added. After 75–90 min, ethyl acetate (300 ml) was added to reaction mixture to extract the products. The organic phase was evaporated and the residue subjected to a preparative thin layer chromatography using hexane-ethyl acetate (9:1), affording **2** in 28–31% isolated yields, and 96% e.e. IR (NaCl), ν (cm^{-1}): 3454, 3067, 3036, 2923, 2856, 1713, 1491, 1450, 1357, 1223, 1176, 1089, 1064, 749, 698. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H), 3.88 (b, 1H), 5.09 (s, 1H), 7.34 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.3, 80.1, 127.2, 128.6, 128.9, 137.8, 206.8.

2.3. (1R,2S)-1-phenyl-1,2-propanodiol **3**

The same procedure as above was followed and after 300 min, ethyl acetate (300 ml) was added to reaction mixture to extract the products. The organic phase was evaporated and the residue subjected to a preparative thin layer chromatography using hexane-ethyl acetate (9:1), affording **3** in 74–97% isolated yields, and >99% e.e. IR (NaCl), ν (cm^{-1}): 3387, 3067, 3030, 2978, 2931, 1494, 1452, 1259, 1078. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (d, J = 6.2 Hz, 3H), 2.63 (b, 1H), 3.16 (b, 1H), 3.86 (dq, J = 4.0, 6.2 Hz, 1H), 4.54 (d, J = 4.0 Hz, 1H), 7.22 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.0, 71.3, 77.4, 126.4, 127.5, 128.1, 140.1.

2.4. (S)-2-hydroxy-1-phenyl-1-propanone **4** [6]

To a stirred suspension of freeze-dried baker's yeast (4 g) in 50 ml of a potassium phosphate buffer solution (pH 7.0, 0.05 mol l^{-1}), kept at 53 °C for 60 min, MVK (150 mg, 2.14 mmol) was added. The reaction mixture was stirred for 30 min at 30 °C, and then a solution of diketone **1** (0.12 g, 0.81 mmol) in ethanol (3 ml) was added, the reaction was kept stirring at 30 °C. After 300 min, ethyl acetate (300 ml) was added to reaction mixture to extract the products. The organic phase was evaporated and the residue subjected to a preparative thin layer chromatography using hexane-ethyl acetate (9:1), affording **4** in 45–68% isolated yields, and 96% e.e. IR (NaCl), ν (cm^{-1}): 3480, 2990, 2928, 2856, 1687, 1450, 1367, 1259, 1135, 1069, 1012, 965, 898. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (d, J = 7.0 Hz, 3H), 3.81 (d, J = 6.2 Hz, 1H), 5.17 (dq, J = 6.2, 7.0 Hz, 1H), 7.51 (m, 2H), 7.63 (m, 1H), 7.93 (m, 2H). ^{1}H NMR (300 MHz, $CDCl_3/D_2O$): δ 1.46 (d, J = 7.0 Hz, 3H), 5.16 (q, J = 7.0 Hz, 1H), 7.51 (m, 2H), 7.63 (m, 1H), 7.93 (m, 2H) ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.8, 69.7, 129.0, 129.2, 133.6, 134.3, 202.5.

2.5. General procedure for reduction of substrates **1**, **2** and **4** by baker's yeast (control experiments)

Typical procedure: to a stirred suspension of freeze-dried baker's yeast (4 g) in 50 ml of a potassium phosphate buffer solution (pH 3.0, 0.5 mol l^{-1}), kept at 30 °C, a solution of the substrate (1.69 mmol) in ethanol (1 ml) was added. Samples (1 ml) were withdrawn from the reaction mixture at intervals of 30 min and extracted with ethyl acetate (6 ml). After solvent evaporation, 1 ml of a standard solution of 2-fluoro-1,1′-biphenyl (0.1 mg/ml, used as internal standard for the calibration of the molar detector response factor of

each compound) was added and the resulting solution was analyzed by GC–MS.

*2.6. General procedure for reduction of substrates **1, 2** and **4** by anaerobically pre-treated baker's yeast*

A suspension produced by 4 g of dry baker's yeast in 50 ml of a potassium phosphate buffer solution (pH 3.0, 0.5 mol l^{-1}) was stirred for 30 min at 30 °C under nitrogen atmosphere. A solution of the substrate (1.69 mmol) in ethanol (1 ml) was added, and then the same procedure as above was followed.

3. Results and discussion

Recently [12], we showed that the decomposition rates of the intermediaries **2** and **4** decreased significantly when the baker's yeast reduction of **1** was conducted at pH 3.0, and the ratio of **2**/**4** increased when a suspension of baker's yeast in a potassium phosphate buffer solution (pH 3.0, 0.5 mol l^{-1}) was pre-treated for 20 min under nitrogen atmosphere at 30 °C, giving a reaction profile shown in Fig. 1.

In order to investigate the influence of the anaerobic pre-treatment on the steps II and IV (Scheme 1), α-hydroxy ketones **2** and **4** were subjected, in parallel, to reduction mediated by anaerobically pre-treated baker's yeast in comparison with non-pre-treated baker's yeast reduction (control experiments). These biotransformations were monitored by GC–MS affording the reactions profiles presented in Fig. 2. We observe that there is no significant difference between the anaerobically pre-treated yeast and the control on the reduction rates of **2**. On the other hand, the reduction of **4** mediated by the anaerobically pre-treated yeast is much slower than the control. Since the ratio of **2/4** increase when **1** is reduced by the pre-treated yeast [12], we can assume that the ratio of step rates I/III is higher for the anaerobically pre-treated yeast than aerobic condition.

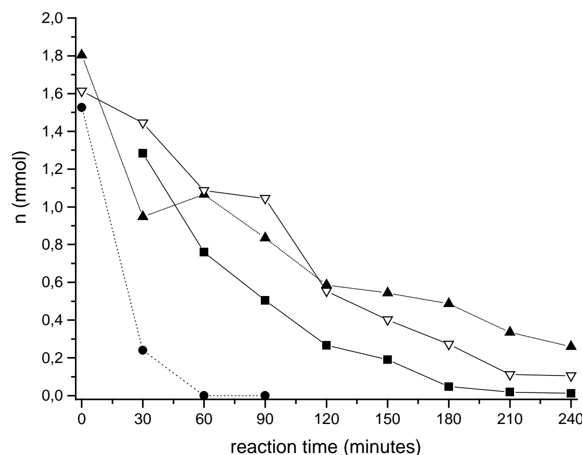

Fig. 2. Consumption of (*R*)-1-hydroxy-1-phenyl-2-propanone (**2**) (▲) and (*S*)-2-hydroxy-1-phenyl-1-propanone (**4**) (▽) mediated by anaerobically pre-treated baker's yeast. Consumption of **2** (■) and **4** (●) mediated by baker's yeast (control experiments).

Considering that compounds **2** and **4** are barely separable by silica gel chromatography [6], the improvement on the regioselectivity of the reduction of **1** could afford compound **2** avoiding **4**. Thus, a pre-treatment consisting of bubbling nitrogen followed by oxygen was developed, in order to favor the formation of **2** and to improve selectively the rate of consumption of **4**. After some experiments, the best results were obtained with a pre-treatment consisting in bubbling nitrogen for 60 min, followed by oxygen for 20 min in the yeast suspension, before addition of diketone **1**. Fig. 3 shows a backlog of **2**, due to its major production from **1**, while **4** is obtained in minor quantity and rapidly converted to **3**. Using this procedure, we isolated **2** in 28–31% of yields

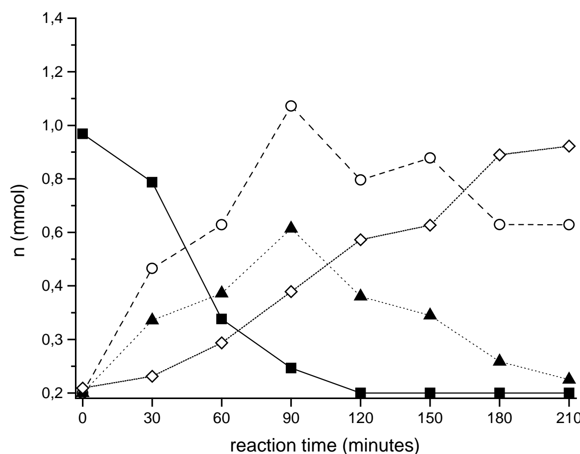

Fig. 1. Anaerobic process. Conversion of 1-phenyl-1,2-propanedione (**1**) (■) to (*R*)-1-hydroxy-1-phenyl-2-propanone (**2**) (○), (1*R*,2*S*)-1-phenyl-1,2-propanediol (**3**) (◇) and (*S*)-2-hydroxy-1-phenyl-1-propanone (**4**) (▲) mediated by anaerobically pre-treated baker's yeast.

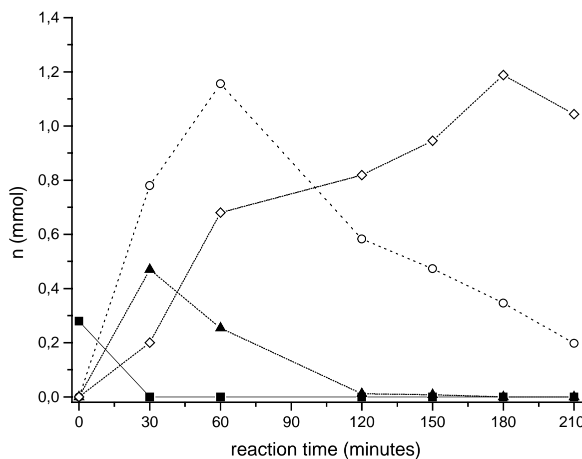

Fig. 3. Conversion of 1-phenyl-1,2-propanedione (**1**) (■) to (*R*)-1-hydroxy-1-phenyl-2-propanone (**2**) (○), (1*R*,2*S*)-1-phenyl-1,2-propanediol (**3**) (◇) and (*S*)-2-hydroxy-1-phenyl-1-propanone (**4**) (▲) mediated by pre-treated baker's yeast with nitrogen (60 min) followed by oxygen (20 min).

(96% e.e.) and **3** in 42–62% (>99% e.e.) after 75–90 min of reaction.

Although 1.69 mmol of **1** was initially added to the cells suspension, Fig. 3 shows that only part of this amount was present in the first sample withdrawn from the reaction mixture. This observation may be an indication that compound **1** was rapidly absorbed by the yeast cells and after its reduction, the products were released to the reaction medium.

4. Conclusions

We concluded that there is no significant difference between the anaerobically pre-treated yeast and the control (non-pre-treated) on the reduction rates of **2**, but, on the other hand, the reduction of **4** mediated by the anaerobically pre-treated yeast is much slower than the aerobic experiment. Since the ratio of **2/4** increase when **1** is reduced by the pre-treated yeast [12], we can assume that the ratio of step rates I/III is higher for the anaerobically pre-treated yeast than for the control. The optimum result avoiding the formation of **4**, was obtained with a pre-treatment consisting in bubbling nitrogen for 60 min, followed by oxygen for 20 min in the yeast suspension, affording **2** in 28–31% of yields (96% e.e.) and **3** in 42–62% (>99% e.e.) after 75–90 min of reaction.

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