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1-Phenyl-1-Propanol Obtained by Reduction of Propiophenone with *Nocardia corallina* B-276

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Abstract: Currently the production of flavoring agents, agrochemicals and pharmaceutical products has become important, through the development of environmentally friendly biotechnological processes to obtain enantiomerically pure compounds by enzymes or microorganisms. 1-phenyl-1-prophanol is an important chiral reagent used as a flavoring, in perfumery and precursor of chiral complex compounds in organic chemistry, with a high commercial value, so in this investigation enantioselective bioreduction of propiophenone to 1-phenyl-1-propanol had been studied with whole cells of *Nocardia corallina* B-276 in phosphate buffer at pH 7.0 with a substrate:wet cells ratio (m/m) 1:500; compounds were characterized by IR and ^{1}H NMR and ^{13}C NMR, analytical methods were developed by GC to determine percentage conversion and by HPLC to determine enantiomeric ratio. According to the results it was observed that the bioreduction of propiophenone to 1-phenyl-1-propanol with *Nocardia corallina* is pH dependent; the optimum pH of the supernatant of the liquid fermentation for the conversion of the ketone to the *R*-enantiomer is at pH 5.67 in 72 h.

Key words: *Biotransformation, 1-pheny-1-propanol, enantioselectivity, active compounds, Nocardia corallina.*

INTRODUCTION

Enantiomerically pure chemicals are important building blocks for the production of flavoring agents, agrochemicals and pharmaceutical products[1] because chirality is the key factor in the effectiveness of these

compounds[1]. An alternative for their preparation, that in recent years had become very important, is the use of biotransformation processes [1,2].

Between the most important characteristics of bioconversions are the chemo-, regio-, and stereoelectivity of the reaction, for the production of enantiomerically pure compounds[3]. Biocatalytic reactions can be performed by the use of isolated enzymes or whole cells. In general, whole-cell biocatalysts exhibit, in comparison to isolated enzymes, an increased and lower production costs. Furthermore, in the case of oxidoreductases like alcohol dehydrogenases (ADH) that need cofactors (NADPH), whole-cell biocatalysts offer the possibility of using the intracellular cofactor regeneration system. Isolated enzymes on the other side demand the supplementary addition of costly cofactors and cofactor regeneration enzymes[4].

1-phenyl-1-propanol is an important chiral reagent for the build of chiral complex compounds in organic chemistry, with a high commercial value for example $183.4 per one milliliter of the *S* enantiomer and $201.60 per one milliliter of *R* enantiomer[5] because it has a balsamic, floral fragrance with a sweet, honey-like taste is used as food additive permitted for direct addition to food for human consumption, also has been used in perfumery[6].

Recent United States and European Community regulations labelled as 'natural' the flavor products prepared by enzymatic or microbial processes, allowing them to be included in products for human consumption, especially processed foods. These regulations have stimulated the research and development of new biotechnological processes for the production of these valuable compounds, specially the food industry shows a strong interest in inexpensive processes for the production of 'natural' products[7].

The aim of this work was to study the enantioselective bioreduction of propiophenone to 1-pheny-1-propanol **(Figure 1)** with whole cells of *Nocardia corallina* B-276 in phosphate buffer at pH 7.0.

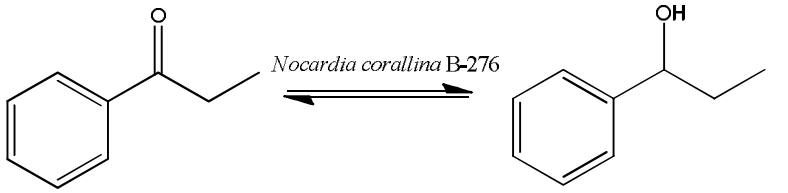

Fig.1: Biotransformation of propiophenone with *Nocardia corallina* B-276.

METHODS

Compounds **1** and **2** were purchased from Aldrich and they were characterized by infrared spectra, recorded on a Perkin-Elmer Paragon 1600 FT, as liquid films; Hydrogen and Carbon nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), were recorded on a Varian DMX 600 MHz instrument, in $CDCl_3$ using tetramethylsilane as internal reference; and by TLC on silica gel 60 GF_{254} (Merck). HPLC analysis was performed on an Agilent 1100 liquid chromatograph, equipped with a diode array detector and a Chiralcel OD (L x ID: 25.0 x 0.46 cm) column; the mobile phase was hexane-isopropyl alcohol (97:3), with a flow rate of 1.0 mL min^{-1}, λ 220 nm and at 25ºC. Absolute configuration of **2** was assigned according to literature[8]. The GC analysis was performed

on a Hewlett-Packard HP 6890 gas chromatograph, equipped with a flame ionization detector, a Supelcowax^{TM}-10 column (30 m × 0.25 mm x 0.25µm), at 180°C, N_2 as carrier gas, at 0.8 mL min^{-1}.

Nocardia corallina B-276 (ATCC 31338) was grown at 28-30°C on agar plates (15 g L^{-1} agar; 3 g L^{-1} beef extract; 5 g L^{-1} peptone L^{-1}) for 96 h.[5] Sterile liquid medium was inoculated and incubated in an orbital shaker for 72-96 h, the broth composition was: 10 g L^{-1} glucose and 10 g L^{-1} yeast, and the pH adjusted to 8.5 (± 0.5) and incubated at 28-30°C on an orbital shaker at 150 rpm. The cells were collected by centrifugation at 4500 rpm for 15 min. Cells were washed twice with potassium phosphate buffer (0.1 mol L^{-1}, pH 7.0).

The cells were incubated in 50 mL phosphate buffer, 0.1 mol L^{-1}, pH 7.0, for 30 min. at 28-30°C on an orbital shaker (150 rpm), then propiophenone was added to the whole cells, with a substrate:cells ratio (m/m) 1:500, using 0.6 % (v/v) of *N*,*N*-dimethylformamide. The mixture was shacked under the same conditions, samples were analyzed at different times. The sample was centrifuged at 4500 rpm for 15 min and was extracted with ethyl acetate (3 × 15 mL), organic layer was concentrated to dryness.[5] It was obtained a yellow oil and the data of IR and NMR were in full accordance with the literature values for alcohol.[9, 10] The product was dissolved in 0.5 mL of isopropyl alcohol HPLC grade, and analyzed by GC to determine the conversion extent, $t_{R(ketone)}$ = 4.38 min and $t_{R(alcohol)}$ = 5.58 min; then was analyzed by HPLC to determine the enantiomeric ratio $t_{R(R\text{-}alcohol)}$ = 12.7 min and $t_{R(S\text{-}alcohol)}$ = 14.9 min.

RESULTS

Two experiments were performed for the biotransformation of **1** using the harvested cells of *Nocardia corallina* B-276 (figure 1) suspended in a phosphate buffer solution. The first with the supernatant liquid culture medium at pH of 5.67 (L1) and the other at pH 5.36 (L2), with a 1:500 ratio of substrate:wet cells. The results are shown in table 1.

Table 1: Results of the biotransformation of propiophenone to 1-phenyl-1-propanol

EXP	pH of the liquid culture medium before biotransformation	Time (h)	ketone/alcohol	Enantiomeric ratio *R*/*S*
L1	5.67	72	20/80	73/27
L1	5.67	96	32/68	72/28
L2	5.36	72	27/73	88/12
L2	5.36	96	41/59	45/55

Ratio substrate:wet cells= 1:500

According to the results it was observed that the reduction is slightly higher at pH 5.67 than at 5.36 and the maximum reduction of propiophenone was achieved at 72 h **(Table 1)**, with a ratio of 20/80. Is important to notice that *Nocardia corallina* catalyze the enantioselective reduction of propiophenone to the "*R*" enantiomer, at 5.67 h the enantiomeric ratio *R*/*S* was 73/27 and at pH 5.36 was 88/12. After some time *N. corallina* begins to oxidize the alcohol to the ketone, it was observed that at 96 h the oxidation of the alcohol is slower at pH 5.67 than at pH 5.36.

CONCLUSIONS

The bioreduction of propiophenone to 1-phenyl-1-propanol with *Nocardia corallina* is pH dependent; the optimum pH of the supernatant of the liquid fermentation for the conversion of the ketone to the *R*-enantiomer is at pH 5.67 in 72 h.

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