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Biotransformation of benzaldehyde to L-phenylacetylcarbinol (L-PAC) by *Torulaspora delbrueckii* and conversion to ephedrine by microwave radiation

Vilas B Shukla,[1] Virendra R Madyar,[2] Bhushan M Khadilkar[2] and Pushpa R Kulkarni[1]*

[1]Food & Fermentation Technology Division, University Dept of Chemical Technology, University of Mumbai (UDCT), Nathalal Parekh Marg, Matunga, Mumbai - 400 019, India

[2]Organic Chemistry Division, University Dept of Chemical Technology, University of Mumbai (UDCT), Nathalal Parekh Marg, Matunga, Mumbai - 400 019, India

Abstract: In a 5 dm^3 stirred tank reactor, bioconversion of 30 g benzaldehyde by cells of *Torulaspora delbrueckii* yielded 22.9 g of pure L-phenylacetylcarbinol (L-PAC). Facile functional group transformation of 4.5 g of L-PAC to 2-(methylimino)-1-phenyl-1-propanol by exposure to microwave irradiation for 9 min resulted in 2.48 g of product. Conversion of 4.8 g of 2-(methylimino)-1-phenyl-1-propanol to 3.11 g of ephedrine was achieved by exposure to microwaves in a reaction time of 10 min. The identity of all the products was confirmed by ^{1}H NMR and FT-IR analysis.

Keywords: benzaldehyde; biotransformation; ephedrine; L-phenylacetylcarbinol; imine formation; microwave irradiation; reduction; *Torulaspora delbrueckii*

INTRODUCTION

Ephedrine is an important drug used as a decongestant and anti-asthmatic. L-Ephedrine is obtained from dried plants of various species of the genus *Ephedra* by initial treatment with alkali, followed by extraction with organic solvent. Extraction, purification and isolation of these drugs is time-consuming, costly and complicated by the presence of undesired by-products. L-Phenylacetylcarbinol (L-PAC; **B**) which is a precursor for ephedrine is produced by biotransformation of benzaldehyde (**A**) using yeast cultures. The chemical conversion of L-PAC to ephedrine has proved to be more advantageous than the extraction route. L-PAC could be converted by a chemical reductive amination with methylamine to optically pure L-ephedrine. The use of microwave irradiation for chemical synthesis is of increasing importance,[4] since it provides a simple alternative to classical chemical routes with rapid reactions yielding high conversion and selectivity. The present work was undertaken to explore the possibility of conducting the synthesis using microwaves as an alternative to these routine chemical synthetic reactions. A two-step simple synthetic reaction was carried out in a homogeneous reaction medium under exposure to microwaves. A homogeneous reaction medium ensures better thermal homogeneity under microwave heating and facilitates scale-up of the reaction. The procedure is superior to methods involving complex hydrogenation[5] procedures and those involving reduction of protected cyanohydrins.[6] The L-PAC required for ephedrine synthesis was produced by bioconversion of benzaldehyde using a yeast isolate identified as *Torulaspora delbrueckii* and which is capable of producing L-PAC from benzaldehyde.[7]

MATERIALS

Microbial media components were obtained from Hi-Media Ltd, Mumbai, methylamine and sodium borohydride (AR grade) from SD Fine Chemicals Ltd, Mumbai and methanol and diethylether from Merck India Ltd, Mumbai. For GC analysis standard benzaldehyde and benzyl alcohol (Sigma Chemicals Co, St Louis, USA) were used while L-PAC and PAC-diol were obtained by purification of the biotransformation broth as described previously.[8] For biotransformation of benzaldehyde to L-PAC a yeast isolate from molasses, identified as *T delbrueckii*, was used. The maintenance medium[9] for the yeast

* Correspondence to: Pushpa R Kulkarni, Food & Fermentation Technology Division, University Dept of Chemical Technology, University of Mumbai (UDCT), Nathalal Parekh Marg, Matunga, Mumbai - 400 019, India
E-mail: rekha@foodbio.udct.ernet.in
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comprised ($g\,dm^{-3}$): glucose 20, peptone 10, yeast extract 10, and agar 20, at pH 5.5, while the growth medium comprised ($g\,dm^{-3}$): glucose 30, peptone 20, and yeast extract 10, at pH 5.5. The biotransformation medium comprised ($g\,dm^{-3}$): glucose 30, and peptone 20, at pH 4.5. The maintenance medium, growth medium and biotransformation medium were used only for biological reactions. For chemical reactions using microwave irradiation a modified IFB Neutron kitchen oven (760 W output and 2450 MHz frequency) was used.

EXPERIMENTAL

Step 1: Biotransformation of benzaldehyde to L-phenylacetylcarbinol (L-PAC) using *T delbrueckii* cellmass

A: Maintenance and growth of T delbrueckii

To maintain *T delbrueckii* one loop full of yeast suspension was streaked on the agar slants of the maintenance medium. The slants were incubated at 30 °C for 24 h and stored at 2–8 °C until further transfer or when used for incubating the cultures.

One cm^3 of 24-h-old suspension of the organism containing 10^6 yeast cells was inoculated into 9 cm^3 of growth medium and incubated on a rotary shaker at 30 (±2 °C) at 240 rpm for 24 h. The culture obtained was inoculated into 100 cm^3 of growth medium and incubated for 24 h under the same conditions. The 220 cm^3 of culture pooled from two flasks after 24 h incubation was inoculated into 5 dm^3 of sterile growth medium in a 7 dm^3 laboratory-scale fermenter (Chemap AG[10]). Air was sparged through a pipe sparger placed below the bottom disc turbine (DT) impeller and the pitched blade down flow turbine (PTD) used as an upper impeller at 250 rpm with a gas flow rate of 5 $dm^3\ min^{-1}$. After 24 h growth, the medium was centrifuged at 17000 × *g* and 15 °C for 15 min (Beckman centrifuge model J2-MC).

B: Biotransformation using T delbrueckii

The cell mass obtained as described above was aseptically inoculated into 5 dm^3 of biotransformation medium in the fermenter using a peristaltic pump. The reaction conditions described previously for this biotransformation were used.[10] The gas flow rate was 1.5 $dm^3\ min^{-1}$. Impellers used in the study were disc turbine (DT) as a lower impeller and pitched blade down flow turbine (PTD) as an upper impeller at a speed of 250 rpm. After the yeast had been adapted for 1 h in the biotransformation medium, 0.6% (w/v) of benzaldehyde and 0.6% (v/v) of acetaldehyde (30–35%) were added aseptically and the reaction continued for 2 h. The experiment was repeated three times and the average of each datum point determined.

C: Analysis of biotransformation products

Analysis of the products of the biotransformation was carried out by GC using a Chemito-8510 GC with FID and Oracle-1 computing integrator. A 4 m long 5% OV-17 column was used. The flow rates of the N_2 gas, H_2 gas and air were 18, 20, and 200 $dm^3\ min^{-1}$ respectively. The temperature programming used was: column temp 75 °C for 3 min, then heating at 10 °C min^{-1} up to 250 °C and holding for 5 min. The injector temperature was 250 °C and the detector temperature was 265 °C. The amount of sample injected was 2 mm^3. The retention times of benzaldehyde, benzyl alcohol, L-PAC and PAC-diol were 12.1 min, 13.9 min, 17.8 min and 19.5 min respectively. The purification of L-PAC was done by the method described earlier.[8]

Step 2: Conversion of L-phenylacetylcarbinol (L-PAC) (B) to 2-(methylimino)-1-phenyl-1-propanol (C)

L-PAC (obtained as above; 0.03 moles, 4.5 g) was placed in a 100 cm^3 round-bottom flask containing 10 cm^3 of ethanol, cooled in crushed ice and the pH adjusted to 4, by dropwise addition of conc HCl. Three cm^3 of a 40% (v/v) solution of methylamine was added dropwise with constant stirring. The reaction mixture was brought to ambient temperature (30 ± 2 °C) and was irradiated for 3 min at 50% power in a modified domestic microwave oven.[11] The reaction was further continued for 6 min (two cycles of 3 min at 50% power) with addition of 3 cm^3 40% methylamine solution during each irradiation cycle. After exposure to the microwaves, the reaction mixture was cooled in crushed ice with 10 cm^3 of added water. The pH of the reaction mixture was adjusted to 4 and the reaction mixture was washed with ether (25 cm^3 × 3) to collect unreacted L-PAC. The aqueous layer was neutralized with $NaHCO_3$ and the pH value adjusted to between 7 and 8. The aqueous layer was extracted with ether (25 cm^3 × 3) and the combined ether layers were washed again with 15 cm^3 of cold water. The ether layer was dried by passing through anhydrous sodium sulphate; ether was removed in a rotavac to obtain the product (**C**) as a yellow oil. This oil was further purified by silica gel (60–120 mesh) column chromatography using ethyl acetate and toluene (6:4) as eluent.

Step 3: Reduction of 2-(methylimino)-1-phenyl-1-propanol (C) to 2-(methylamino)-1-phenyl-1-propanol (D) (ephedrine)

The imine (2-(methylimine)-1-phenyl-1-propanol; 0.03 moles, 4.89 g) was placed in a round-bottom flask containing 10 cm^3 of ethanol. To this solution $NaBH_4$ (0.09 moles, 3.24 g) was added in increments of 0.02 moles for each microwave irradiation of 2 min at 50% power. The total reaction time under microwave exposure was 10 min (2 min × five cycles of 50% power). After exposure to the microwaves, the reaction mixture was cooled in ice and quenched by adding 10 cm^3 of ice-cold water and some pieces of ice. This solution was then extracted with ether (25 cm^3 × 3). The combined ether layers were washed twice with

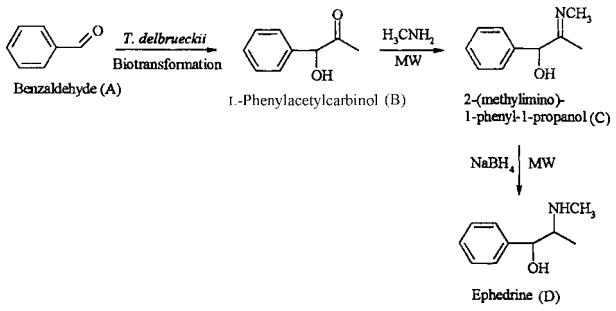

Figure 1. Scheme for ephedrine synthesis.

15 cm^3 of cold water and dried by passing through anhydrous sodium sulphate. The ether layer was removed in a rotavac to give the oil containing product and unreacted imine. The mixture was separated by column chromatography using silica gel (60–120 mesh) and ethyl acetate–toluene (8:2) as eluent. The isolated product obtained after elution of the column was recrystallized in hot ethanol and dried to give ephedrine. The biotransformation process using T *delbrueckii* and the chemical process using microwave irradiation is shown in Fig 1. Characterization of (**B**), (**C**) and (**D**) was carried out using a Jasco-300 E Spectrophotometer FT-IR as well as an Perkin-Elmer IR Spectrophotometer (model 783) and expressed in terms of wave number (cm^{-1}). The ^1H NMR spectra were recorded in $CDCl_3$ using a Bruker ACP-300 NMR. The chemical shifts are given in parts per million using tetramethyl silane as internal standard.

RESULTS AND DISCUSSION

The biotransformation of benzaldehyde to L-PAC by *T delbrueckii* yielded 458 mg of L-PAC, 216 mg of benzyl alcohol, 2 mg of PAC-diol and 4.5 mg of unreacted benzaldehyde per 100 cm^3 of the biotransformation medium. The results of the biotransformation were found to be reproducible in the range of ±5%. The yield of L-PAC after the purification was 19.5 g, ie. 47%.

Step (2) of the reaction deals with condensation of a keto group in L-PAC with that of the amino group of methylamine to give an imine with loss of water molecule. Synthesis of imine[12,13] has been achieved by using several reagents such as Brønsted acids and Lewis acids such as $AlCl_3$, $ZnCl_2$, $TiCl_4$, or molecular sieves and alumina etc. Verma *et al*[14] carried out clay-catalyzed synthesis of imines in solvent-less reaction conditions under microwave irradiation and achieved high yields of imines. In the present work, a homogeneous reaction medium such as ethanol was used for carrying out condensation between L-PAC and methyl amine under acidic conditions. It was observed that by using microwaves this imine formation step was fast and clean, giving satisfactory yields. The reaction was repeated three times and was found to be reproducible in the range of ±2%. Since the yield of **C** reached 55% in about 9 min, beyond which time only a marginal improvement in yield was observed, the optimized time for microwave irradiation for this conversion was taken as 9 min (Fig 2). On purification by column chromatography the yield of **C** was 2.48 g.

Step (3) involves reduction of the imine, ie carbon–nitrogen double bond to give ephedrine. Many metal hydrides have been reported to carry out such a reduction.[15] Varma and Dahiya[16] have reported imine reduction with $NaBH_4$ supported on montmorillonite K-10 clay under microwave irradiation in solvent-less conditions with high yields of reduced product. In the present work the reduction of imine was studied using $NaBH_4$ in ethanol. The optimal ratio of imine to $NaBH_4$ was 1:3 for reduction of imine (**C**) to ephedrine (**D**). The reaction was repeated three times and found to be reproducible in the range of ±2%. In the case of the reduction of imine to ephedrine the optimum isolated yield of 64%, ie 3.11 g, was obtained in 10 min of microwave irradiation. The time for microwave irradiation was not increased further as development of a brown coloration in the reaction mixture resulted in the quality of the product being affected (Fig 3).

The yields of the product and the characterization by FT-IR and ^1H NMR for the products obtained in the biotransformation and chemical transformation are summarized in Table 1.

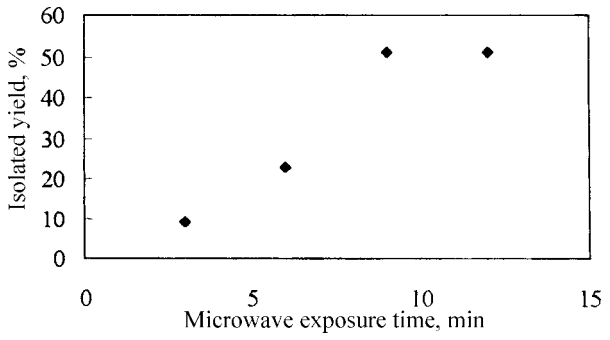

Figure 2. Optimization of L-phenylacetylcarbinol (L-PAC) to 2-(methylimino)-1-phenyl-1-propanol under microwave irradiation (Step 2).

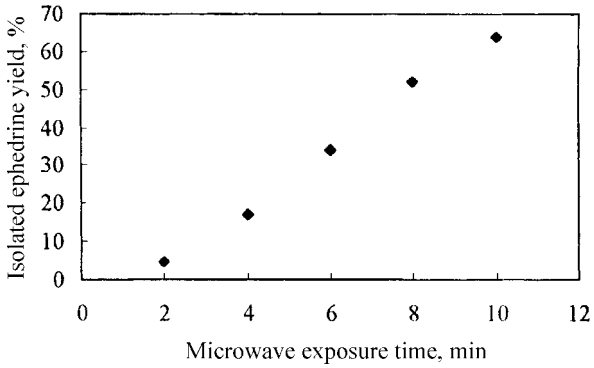

Figure 3. Optimization for reduction of 2-(methylimino)-1-phenyl-1-propanol to 2-(methylamino)-1-phenyl-1-propanol (ephedrine) under microwave exposure (Step 3).

Table 1. Isolated yield percent with its FT-IR and ^{1}H NMR characterization of products obtained in biotransformation and chemical conversion

Product	*Isolated yield, %*	*^{1}H NMR (300 MHz, $CDCl_3$) δ*	*FT-IR (KBr), ν cm^{-1}*
L-Phenylacetylcarbinol (L-PAC) (**B**)	47	2.1 (s, 3H, CH_3), 4.4 (broad s, 1H, OH), 5.1 (s, 1H, CH), 7.4–7.5 (m, 5H, Ar)	3458 (O—H), 3030 (C—H aromatic), 2925 (C—H aliphatic), 1730 (C=O), 1597 (C=C aromatic), 749,697 (monosubstituted benzene)
2-(Methylimino)-1-phenyl-1-propanol (**C**)	55	0.9–1 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 4.5 (broad s, 1H, OH), 4.8 (s, 1H, CH), 7.4–7.5 (m, 5H, Ar)	3357 (O—H) 1644 (C=N)
2-(Methylamino)-1-phenyl-1-propanol (ephedrine) (**D**)	64	0.9–1 (d, 3H, CH_3), 2.3 (s, 3H, CH_3), 2.9–3 (m, 1H, CH), 4.4 (broad s, 1H, OH), 4.8–4.9 (d, 1H, CH), 7.2–7.6 (m, 5H, Ar and 1H, NH)	3433 (O—H), 3048 (C—H aromatic), 2925 (C—H aliphatic), 1636 (N—H bending)

CONCLUSION

T delbrueckii biomass biotransformed benzaldehyde to L-PAC, giving a yield of 458 mg of L-PAC per 100 cm^3 of biotransformation medium. In a rapid two-step process using microwave irradiation, the L-PAC was readily converted to ephedrine. The two steps could be completed within 19 min under microwave irradiation. In conclusion a unique combination of biotransformation and microwave assistance has been reported here for the first time for the synthesis of ephedrine.

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