Microbial oxidation of α-pinene to (+)-α-terpineol by Candida tropicalis

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α-Pinene has been subjected to microbial oxidation by the yeast Candida tropicalis MTCC 230. An overall reaction yield of 77% has been obtained for the biotransformation product [(R)*-21,1, e=1.5, CHCl3] in 96 hr, the structure of which has been determined by spectral methods.

Microorganisms and their enzymes are widely used as a class of biocatalytic reagents and hence have received considerable attention for the past few years. Reactions through biocatalytic pathway are preferable since these can be carried out under the mildest reaction conditions and hence can be used to overcome the difficulties encountered by the traditional chemical methods. Several microorganisms, like fungi, yeasts and bacteria have been extensively used for the transformations of terpene derivatives. It has been previously reported that the yeast Candida tropicalis can synthesize long chain α,ω-dicarboxylic acids from the corresponding normal alkanes, which has been found to occur via the β-oxidation pathway. The present study deals with the microbial oxidation of a bicyclic monoterpene hydrocarbon, α-pinene (1), by the yeast Candida tropicalis MTCC 230 to (+)-α-terpineol (2), a potent flavor compound (Scheme I).

The product was characterized by TLC, FTIR, 1H NMR, 13C NMR and GC-MS spectroscopy.

Materials and Methods

Organism and cultivation condition

The microorganism Candida tropicalis MTCC 230 was purchased from the Institute of Microbial Technology (Chandigarh, India). The microorganism was maintained on 2% agar slants at 30°C. The composition of the medium was (w/v) 0.3% malt extract, 0.3% yeast extract, 0.5% peptone and 1.0% glucose in distilled water. The pH of the medium was adjusted to 7.0 by the addition of dilute NaOH and the broth was sterilized at 15 psi at 121°C for 15 min. The sterile broth was inoculated with a loop of microbial cells into a 100 mL medium contained in a 250 mL Erlenmeyer flask and incubated under shaking conditions at 30°C for 3 days. To the resulting culture broth 0.5% (w/v) of α-pinene was added as substrate and was incubated at 30°C under continuous shaking for 4 days.

Extraction and identification of biotransformation product

To isolate the biotransformation product the medium (containing the treated substrate and yeast cells) was centrifuged at 6000 rpm for 15 min at 30°C to remove the yeast cells. The supernatant was extracted with ethyl acetate (3×15 mL). The combined organic extract was washed with distilled water (3×20 mL) and dried over anhy. Na2SO4. The solvent was removed under reduced pressure (10 mm of Hg) and the crude viscous residue was charged onto a silica gel (60-120 mesh) column. Elution was carried out with a mixture of analytical grade n-hexane and diethyl ether (97:3, v/v) with a flow rate of 3.0 mL/min. After removal of solvent under reduced pressure (10 mm of Hg, at 30°C), the column chromatographed fractions were subjected to TLC analysis on glass plates with a 0.2 mm layer of silica gel G, using a solvent system of n-hexane and diethyl ether (7:3, v/v). Spots were visualized by iodine absorption (Rf of α-pinene 0.93; Rf of biocconversion product 0.57). The optical rotation of the purified α-terpineol was measured in chloroform. The starting material α-pinene (1) remained unaffected in the blank run where the experimental set-up was without the microorganism.
Table I—Characterization of 1 and 2

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<th>1</th>
<th>2</th>
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<tr>
<td>$[\alpha]_D^{25}$</td>
<td>+21.1 (c = 1.5 CHCl₃)</td>
<td>3380, 2925, 2364, 1643, 1447, 1124</td>
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<td>FTIR (NaCl) cm⁻¹</td>
<td>2920, 2875, 1428, 1375</td>
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<td>$^1$H-NMR (CDCl₃, TMS) δ ppm</td>
<td>5.17 (t, 1H, J=3.0 Hz), 2.32(m, 1H), 1.92(m, 2H), 1.64(s,3H), 1.25(s,3H), 0.82(s,3H)</td>
<td>5.35 (t, 1H, J=3.0 Hz), 4.66 (bs, 1H), 2.00 (m, 1H), 1.70 (s, 3H), 1.62 (d, 2H, J=4.5 Hz), 1.40 (m, 2H), 1.19 (m, 2H), 1.15 (s, 3H), 1.14 (s, 3H)</td>
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<tr>
<td>$^{13}$C-NMR (CDCl₃, TMS) δ ppm</td>
<td>110.93 (C), 120.51 (CH), 72.71 (C), 44.97 (CH), 30.98 (CH₂), 27.29 (CH), 26.96 (CH₃), 26.29 (CH₃)</td>
<td>154(M⁺, 5.8), 136(48), 121(100), 107(19), 93(58), 69(8)</td>
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<td>GC-MS (m/z)</td>
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Results and Discussion

Identification of the bioconversion product. The bioconversion product of α-pinene was subjected to FTIR, $^1$H and $^{13}$C NMR, and GC-MS spectra. The spectral data of bioconversion product, α-pinene (1), was in good agreement with that of (+)-α-terpineol (2), which are given in Table 1.

Influence of substrate concentration. During the study, the optimum α-pinene concentration, at which maximum yield of (+)-α-terpineol was obtained, was determined. The concentration of α-pinene in the medium was varied between 0.1 and 2.0 g/L. From Figure 1 it is observed that with increasing concentration of α-pinene the percent molar conversion of α-pinene to (+)-α-terpineol steadily increases, reaching a maximum at 0.5 g/L, and then declines. At the optimum α-pinene concentration the molar percentage yield of (+)-α-terpineol was found to be 77% (0.43 g). The decrease in percent molar conversion with increasing concentration of α-pinene may be attributed to the adverse effect of α-pinene on growth of Candida tropicalis MTCC230.

Time course of biotransformation of α-pinene. The time course of biotransformation of α-pinene by Candida tropicalis MTCC 230 is shown in Figure 2. The molar percentage conversion of α-pinene to (+)-α-terpineol was found to maximize in four days (96 hr) after which it remained constant, indicating that the microorganism Candida tropicalis MTCC 230 did not metabolize (+)-α-terpineol any more.

The present biotransformation by the yeast Candida tropicalis MTCC 230, therefore offers a simple, inexpensive and efficient method to obtain aroma compound (+)-α-terpineol in good yield.
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References