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Lipase-mediated epoxidation of α-pinene

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Abstract

This work describes the lipase-mediated synthesis of α-pinene oxide at ambient temperature. The immobilized lipase from *Candida antarctica* (Novozyme 435) is used to generate peroxyoctanoic acid directly from octanoic acid and hydrogen peroxide. The peroxy acid formed is then applied for in situ oxidation of α-pinene. High conversion of α-pinene to α-pinene oxide (approximately 70%) was achieved when using a two-phase system of toluene and water. Various parameters affecting the conversion of α-pinene to α-pinene oxide were studied.

Keywords: α-Pinene; *Candida antarctica*; Octanoic acid

1. Introduction

Monoterpenes are widely distributed in nature and they are mainly found in essential oils. Their antimicrobial and antifungal activity has been well known for many years now. Monoterpene epoxides and/or their corresponding diols are often used as intermediates for the synthesis of fragrances, flavors and biologically active compounds. Generally, they are synthesized chemically using various metal catalysts under extreme oxidizing conditions [1]. The past decade it has been shown that it is possible to synthesize these compounds under extremely mild oxidizing conditions [2–4]: an immobilized lipase can be used to generate peroxycarboxylic acid from carboxylic acid and hydrogen peroxide; the peroxy acid formed can then be applied for epoxidation of alkenes (Scheme 1).

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2. Experimental

2.1. Materials

Lipase from *Candida antarctica* (Novozyme 435) was used immobilized on a polyacrylate resin. 30% Hydrogen peroxide (percentage given as wt.% H_2O_2 in water) and all chemicals (α-pinene, α-pinene oxide, octanoic acid and toluene) were of analytical grade.

2.2. Oxidation reactions

α-Pinene (10 mmol, 2 M) and octanoic acid (10 mmol, 2 M) were dissolved in toluene (5 ml) and immobilized *Candida antarctica* lipase (100 mg) was added. The reaction was initiated with H_2O_2 (15 mmol, 3 M), which was gradually added in the reaction mixture under magnetic stirring at ambient temperature. Aliquots from the organic phase were withdrawn at different time intervals for further analysis.

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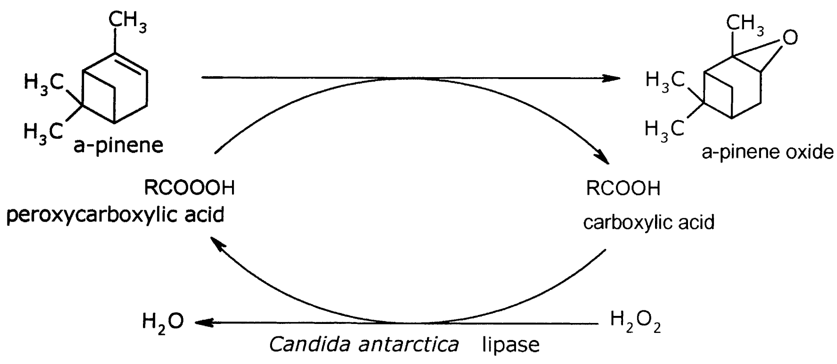

Scheme 1. Synthesis of α-pinene oxide by lipase-catalyzed formation of peroxycarboxylic acid.

2.3. Analytical methods

Sample analysis was performed by gas chromatography (GC Shimadzu 17A equipped with a flame ionization detector). The column used was α-DEX 120 (Supelco), 30 m × 0.25 mm × 0.25 μm. The temperature of the column was 130 °C for 5 min and then it was increased to 200 °C by 20 °C/min. The carrier gas used was helium (He) and the flow 1.1 ml/min. The injection and detection temperatures were set to 250 and 300 °C, respectively and the split mode was 1/100. The identity of the product (α-pinene oxide) was made by comparison with an authentic sample. For the analysis of the results, the appropriate standard curves were used.

3. Results

3.1. Effect of H_2O_2 rate addition

One of the most important parameters affecting the conversion of α-pinene to α-pinene oxide is the hydrogen peroxide addition rate. As it is shown in Fig. 1, highest conversions of α-pinene are obtained when the hydrogen peroxide was added in the reaction mixture over 1.5 h. It seems though that α-pinene oxide is quite unstable in water, and as a result, it is hydrolyzed and by-products are formed. When the H_2O_2 is added over 6 h, the conversion of α-pinene to α-pinene oxide proceeds slower and the conversion after 7 h reaches approximately 30%.

3.2. Effect of H_2O_2 concentration

The concentration of the hydrogen peroxide was found to be an important parameter on the epoxide synthesis. High conversion of α-pinene (∼65%) is achieved when 12 M of H_2O_2 is used. If lower concentrations of H_2O_2 are used, the conversions are lower but remain stable after 24 h (by-products are formed after 24 h when 12 M of H_2O_2 was added in the reaction mixture over 3 h) Fig. 2.

3.3. Effect of octanoic acid concentration

As it can be seen from Fig. 3, when high concentrations of octanoic acid are used (>2 M), low conversions of alkene to epoxide are achieved, probably due to an inhibitory effect of the fatty acid on the catalytic action of lipase. Highest epoxide formation is observed when lower concentrations of octanoic acid

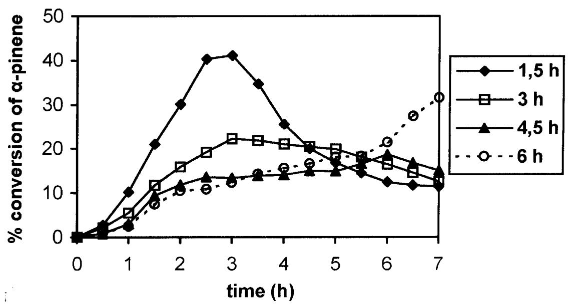

Fig. 1. Effect of H_2O_2 rate addition on the conversion of α-pinene.

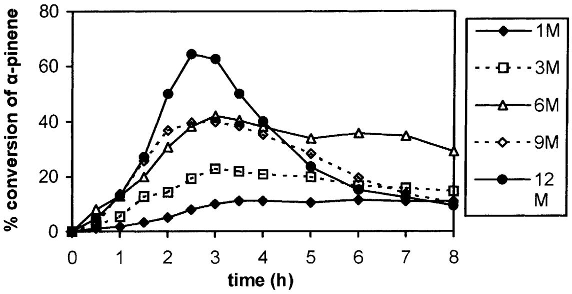

Fig. 2. Effect of H_2O_2 concentration (addition over 3 h).

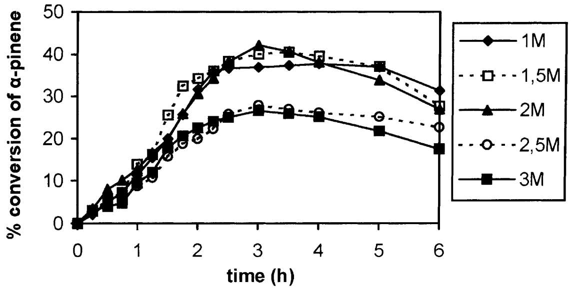

Fig. 3. Effect of octanoic acid concentration (addition of 6 M H_2O_2 over 3 h).

are used (<2 M); the conversion of α-pinene reaches 40 and 42% when 1.5 and 2 M of hydrogen peroxide is used, respectively. The initial velocities calculated are: 11.43, 12.59, 13.44, 9.03 and 8.85 mM/h for 1, 1.5, 2, 2.5 and 3 M of octanoic acid, respectively.

3.4. Effect of enzyme concentration

The effect of the concentration of the enzyme in the reaction mixture on the synthesis of α-pinene oxide was studied. As it can be seen in Table 1, when the concentration of the lipase increases, the amount of α-pinene oxide formed also increases. Highest conversions of the alkene are observed after 3 h of enzymatic reaction, when all of the hydrogen peroxide has been added in the reaction mixture. After 24 h though, the concentration of α-pinene oxide in the reaction mixture decreases, probably due to instability problems of the product in the reaction system (by-products are formed).

Table 1
Effect of enzyme concentration (addition of 6 M H_2O_2 over 3 h)

Enzyme concentration (mg/ml solvent)	α-Pinene oxide (mM) synthesized after		
	80 min	180 min	24 h
10	147.6	498	178.6
20	256.2	741.1	270.1
30	283.2	725.2	320.1
40	288.4	1226.8	844.4

4. Conclusions

The lipase-mediated synthesis of α-pinene oxide under mild conditions depends on various factors such as the concentration and the rate of addition of H_2O_2 on the reaction system as well as the concentration of fatty acid used and the concentration of the immobilized lipase. Further work is in progress in our laboratory in order to investigate factors affecting the lipase as well as α-pinene oxide stability in the reaction system.

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