Lipase-Mediated Formation of Peroxyoctanoic Acid Used in Catalytic Epoxidation of α-Pinene from Turpentine Oil

Wijayati N., Kusoro Siadi, Hanny Wijaya, and Maggy Thenawijjaja Suhartono

***Abstract*—This work describes the lipase-mediated synthesis of α -pinene oxide at ambient temperature. The immobilized lipase from *Pseudomonas aeruginosa* 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 78%) was achieved when using 0,1 g enzim lipase, 6 mmol H_2O_2, dan 5 mmol octanoic acid. Various parameters affecting the conversion of α -pinene to α -pinene oxide were studied**

***Index Terms*—α–Pinene, *P. aeruginosa*, octanoic acid.**

I. Introduction

Turpentine is generally produced in places having vast tracts of pine. Turpentine oils are mobile liquids, usually non-colored or slightly colored, with characteristic pleasant odor. The boiling point of the oils varies between 154-170 ℃, and melting point between -60 and 50 ℃. The density varies between 0.854-0.868 g/mL. The oils are not soluble in water, but are soluble in alcohols, ethers carbon bisulfite and in other oils.3 Highly pure α- and β-pinene can be obatined by fractional distillation of turpentine oil. Chemically, turpentine is a mixture of cyclic monoterpene hydrocarbons, $C_{10}H_{I6}$, such as α-pinene, camphene, β-pinene and 3-carene. Alpha-Pinene is the main constituent of turpentine oil [1], [2]. The chemical reactivity of turpentine varies with its composition but generally is that characteristic of α-pinene.

Monoterpenes are widely distributed in nature and they are mainly found in essential oils. Their antimicrobial and antifungal activity had been well known for many years now. Biotechnology has of very good excellent the potential to generate these products through biotransformation using microorganisms and their enzymatic systems [3]-[7]. Other advantages of biotransformation include the fact that a single stage in such a process can encompass a series of steps in chemical synthesis and results in the formation of the desired products. In chemical industry, selective oxidation of pinene with some catalysts gives many compounds for perfumery, such as artificial odorants. An important oxidation product is verbenone, along with pinene oxide, verbenol and verbenyl hydroperoxide [8]-[10].

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 [11]. During recent years the use of lipases in organic chemical processing has been studied extensively and technologies for production and application of lipases have been developed. As a consequence, the lipases are now recognized as efficient and useful catalysts for modification of fats and oils by acidolysis of the triglycerides substrate and for synthesis or hydrolysis of carboxylic acid esters [9], [11]. These reactions often exhibit a high region-and stereo-selectivity which may be exploited for synthesis of optically active compound. Forthermore the lipases offer unique benefits due to the mild reaction conditions employed in the lipase-catalysed reaction.

Lipases (EC 3.1.1.3) belong to a class of enzymes called hyrolases and are members of a family of enzymes, which in biological systems, mainly hydrolyse carboxylic esters in the form of triacyl glycerol esters (fats). In vivo, enzymes mostly perform their catalytic processes in aqueous media [1], [2], [3], [6], [12].

The activity of lipases towards peroxy-compounds is not yet a subject of much attention. So far, scientist reports the capability of certain lipased to catalyse perhydrolysis (lysis by hydrogen peroxide) of carboxylic acid esters, forming peroxy-carboxilic acids in aqueous hydrogen peroxide solutions, and stereospecific lipase-catalysed synthesis of various peroxycarboxylic acids in hexane using immobilized C. antartica lipase have been reported. In continuation of our application of enzymes in organic synthesis, we have found that immobilized lipases can be applied for generating peroxycarboxylic acid in a suitable organic solvent directly from the parent carboxylic acid [12]. Futhermore, the peroxy acids formed under these very mild reaction conditions can be apllied concomitantly for epoxidation of alkenes. In this fashion epoxidation of α-pinene can be carried out using octanoic acid, acetic acid and proponoic acid in catalytic amounts.

During the last 30 years the scope of biocatalysis has been expanding due to the advances in several technological fields. Diverse techniques as structural enzyme improvement (*e.g*. protein engineering, direct evolution), engineering approaches (*e.g*. ionic liquids, supercritical fluids) and physical stabilization (*e.g*. immobilization, CLEAS) have been developed, which in combination are powerful tools to improve biotransformation and to synthesize new products

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[13], [14]. In this study, relevant results of the epoxidation of α-pinene and peroxycarboxylic acids using lipase from p. aeruginosa was presented.

II. Procedure

A. Materials

Lipase from *Pseudomonas aeruginosa* was used immobilized. Hydrogen peroxide 35% (percentage given as wt.% H_2O_2 in water) and all chemicals (α -pinene, Na_2SO_4, octanoic acid and toluene) were of analytical grade.

B. Oxidation Reactions

Alpha-pinene (10 mmol) and octanoic acid (10 mmol) were dissolved in toluene (5 ml) and immobilized *Pseudomonas aeruginosa* lipase (100 mg) was added. The reaction was initiated with H_2O_2 (12 mmol), 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.

Conversion (X) was defined here as moles of monoterpene converted per 100 moles of monoterpene feed. The selectivities to oxidation product α-pinene oxide (S) was defined as moles of these products formed per 100 moles of α-pinene converted.

C. Analytical Methods

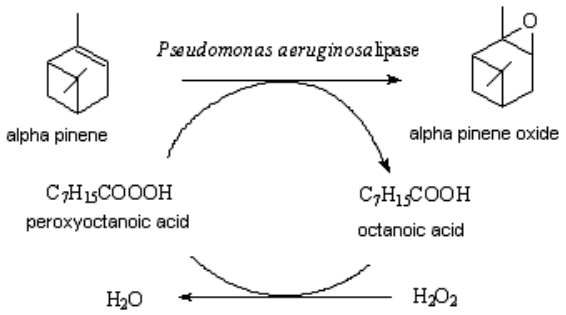
Sample analysis was performed by gas chromatography (GC-2014 Shimadzu equipped with a flame ionization detector). The column used was Rtx(R)-1 Croscond 100% dimethyl Polysiloxane. The temperature of the column was 120oC for 5 min and then it was increased to 180oC by 2oC/min. The carrier gas used was helium (He) and the flow 0,4 μL/min. The injection and detection temperatures were set to 250 and 250°C, respectively and the split mode was 1/100.

The identity of the produc (α-pinene oxide) was made by comparison with an authentic sample or by GC-MS instrument in a HP 6980 gas chromatograph with a 30 m fused silica non-polar OB-1 capillary column. GC-MS instrument (Agilent GC/MSD (7890A/ 5975C). The column used was DB-5MS 27m × 0.25mm × 0.25um B-5, Mass range: 41-500amu, Carrier gas flow rate: 1ml/min, Injector temperature: 260oC; Temperature program: 50oC (2min) - 260oC (5min), heating rate 5oC/min; Flow: 1 mL/min; split ratio: 20. Note: 3uL of sample TP diluted with 1mL of methanol for GCMS analysis.

III. Result and Discussion

During recent years the use of lipase in organic chemical processing has been studied extensively and the technologies for production and application of lipases have been highly developed. As a consequence, the lipases are now recognized as efficient and useful catalysts for modification of fats and oils by acidolysis of triglycerides and for synthesis or hydrolysis of carboxylic ucid esters. In these reactions the lipases often exhibit a high regio- and stereo-selectivity which may be exploited for synthesis of optically active compounds. Furthermore the lipases offer unique benefits due to the mild reaction conditions employed in lipase-catalysed reactions [2], [3].

In continuation of our work on application of enzymes in organic synthesis, we have found that immobilized lipased can be applied for generating peroxycarboxylic acids in suitable organic solven directly from the parent carboxylic acid and dimethyldioxirane. Furthermore, the peroxy acids formed under these very mild reaction conditions can be applied concomitantly for epoxidation of α-pinene. In this fashion epoxidation of α-pinene can be carried out using fatty acid in catalytic amounts (scheme 1).



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

As indicated above, the smooth lipase-catalysed formation of peroxycarboxilic acids lends it self to lipase-catalysed synthesis of epoxide from α-pinene and peroxycarboxilic acids in the presence of catalytic amounts of fatty acids (scheme 1). The reaction can be perfomed simply by adding oxone as a 12 mmol to suspention of immobilised lipase in a solution octanoic acid and α-pinene in an organic solvent. In case of liquid alkenes the conversion was easily carried out simply by dispersing the immobilised lipase in the alkene and gradually adding fatty acid.

In comparison of convertion to the highly acidic conditions usually applied for in situ generation of peroxyoctanoic acid [7] the present method provides a very mild and simple alternative. Moreever, the method provides for lipase-mediated epoxidation of alkenes represents a safe and cost-effective apoxidation amenable for large-scale organic chemical manufacture of even sensitive apoxides [2]. Finally, lipase-catalysed synthetis of peroxycarboxylic acids may prove usable in any other oxidation involving the use of peroxycarboxylic acids in organic solvents.

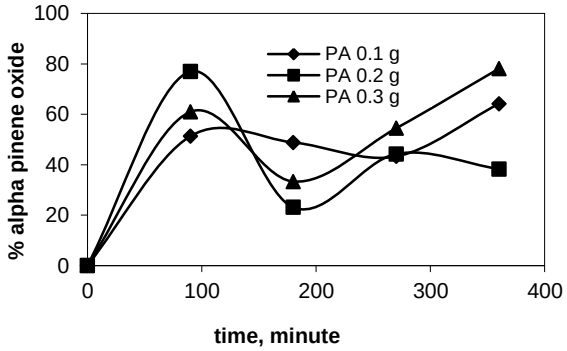


Fig. 1. Effect of enzyme concentration.

A. 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 Fig. 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 4,5 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).

B. Effect of mmol H_2O_2

The mmol of the hydrogen peroxide was found to be an important parameter on the epoxide synthesis. High conversion of α-pinene (~78%) is achieved when 6 mmol of H_2O_2 is used. If uper mmol of H_2O_2 are used, the conversions are lower. The reusability of lipase was studied in four reactions cyclus and was found to depend on the mmol of the hydrogen peroxide used (see Fig. 2).

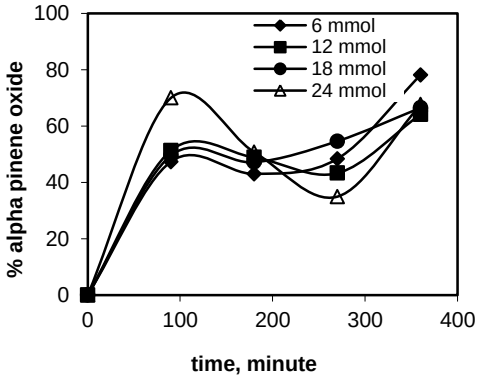


Fig. 2. Effect of mmol H_2O_2.

C. Effect of mmol Octanoic Acid

As it can be seen from Fig. 3, when high mmol of octanoic acid are used (>5 mmol), 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 mmol of octanoic acid are used (5 mmol); the conversion of α-pinene reaches 73% when 12 mmol of hydrogen peroxide is used, respectively.

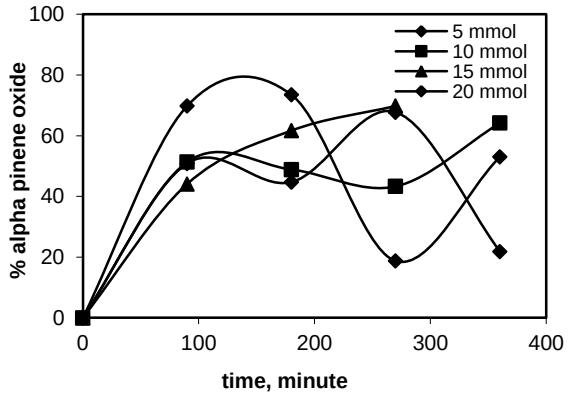


Fig. 3. Effect of mmol octanoic acid.

D. Effect of Fatty Acid

As can be seen from Fig. 4, if 5 mmol of octanoic acid are used, produced high conversions of alkene to epoxide. It is probably due to an inhibitory effect of the fatty acid on the catalytic action of lipase. Highest epoxide formation is observed when lower mmol of 5 mmol octanoic acid are used.

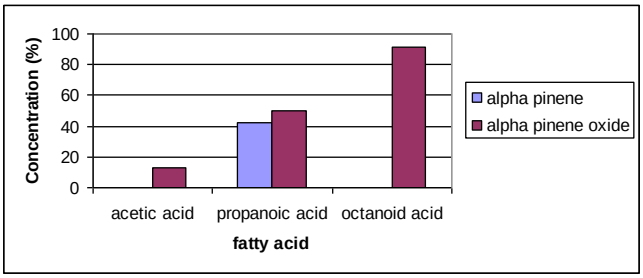


Fig. 4. Effect of fatty acid upon formation of α-pinene oxide.

The lipase-catalysed synthetis of peroxycarboxylic acid was thus performed in two-phase system where the immobilized enzyme efficiently catalytised the reaction on the water-solvent interphase.

The IR spectrum (Fig. 5.) shows the results of α-pinene biotransformation reaction using lipase from Pseudomonas aeruginosa. The peak at 1710 cm^{-1} region, indicating the presence of carbonyl compounds. IR-spectrum of absorption peaks, of which 1280 cm^{-1}, 937cm^{-1} and 727cm^{-1} were the three characteristics of epoxide uptake. This suggests that the biotransformation of α-pinene reaction using lipase from *Pseudomonas aeruginosa* can produce epoxide compound.

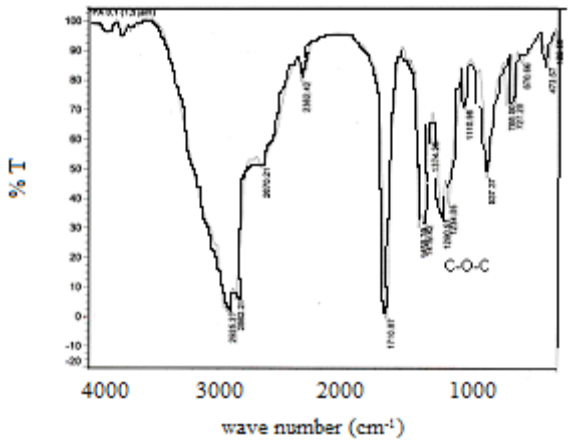


Fig. 5. IR Spectrum of α-pinene oxide.

In comparison of convertion to the highly acidic conditions usually applied for in situ generation of peroxyoctanoic acid [7] the present method provides a very mild and simple alternative. Moreever, the method provides for lipase-mediated epoxidation of alkenes represents a safe and cost-effective apoxidation amenable for large-scale organic chemical manufacture of even sensitive epoxides [2]. Finally, lipase-catalysed synthetis of peroxycarboxylic acids may prove usable in any other oxidation involving the use of peroxycarboxylic acids in organic solvents.

IV. Conclusion

The lipase-mediated synthesis of α-pinene oxide under mild conditions depends on various factors such as the mmol of H_2O_2 on the reaction system as well as the mmol 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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