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Lipase catalyzed synthesis of cinnamyl acetate via transesterification in non-aqueous medium

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ABSTRACT

Cinnamyl acetate is used as flavor and fragrance ingredient in food and cosmetic industries. This work focuses on the synthesis of cinnamyl acetate via lipase catalyzed transesterification of cinnamyl alcohol with vinyl acetate in non-aqueous medium. Among the different immobilized lipases employed, Novozym 435 was found to be the best catalyst in toluene as solvent. The effects of various parameters were studied systematically. With a mole ratio of 1:2 of cinnamyl alcohol to vinyl acetate and 10 mg catalyst, 96% conversion was obtained in 1 h at 40 °C. The ternary complex mechanism with inhibition by cinnamyl alcohol was found to fit the data well. The kinetics of the reaction was studied by using non-linear regression analysis. Enzymatic synthesis of cinnamyl acetate is an efficient process vis-à-vis chemical catalysis.

1. Introduction

Organic esters are extensively used as solvents and plasticizers in chemical, pharmaceutical, flavor, fragrance, cosmetic, food and beverage industries. They are also used as lubricants in high precision machinery (mechanical and automobile) industries. Traditionally esters are synthesized by a chemical reaction of an alcohol with an organic acid in the presence of strong acids such as sulfuric acid, *p*-toluene sulfuric acid and phosphoric acid as homogenous catalysts [1,2]. These processes are fraught with many drawbacks such as high temperature or pressure requirement, use of hazardous chemicals, longer reaction times, low conversions, corrosion-proof costly equipment and costly downstream processes. Although several new heterogeneous catalysts have been developed in recent years to improve the yield and selectivity of the reaction, they also share the common drawbacks like requirement of high temperature and harsh conditions [1–3]. Different strategies such as modification of reaction conditions, development of reusable catalysts [2,3] and use of biocatalysts [4–10] have been adopted to address these problems and make them green and environmentally benign processes.

Lipase catalyzed esterification and transesterification reactions are among the most promising alternatives to traditional chemical methods. Lipases are inherently superior to chemical catalysis, due to their broad substrate specificity; they have good ability to recognize regio and stereo selectivity; need no co-factors; work under mild reaction conditions; and require low energy input [11,12]. Most of the organic substances are poorly soluble in aqueous media that limit their accessibility to enzymes in organic synthesis. As compared to conventional aqueous medium, non-aqueous medium leads to more enzyme activity and stability, increased solubility of hydrophobic compounds, ease of recovery of enzyme and product, prevention of the microbial contamination, and increase in the reaction rate by shifting the equilibrium towards product formation [6–10,13,14]. During past few years, numerous reactions such as hydrolysis [15], esterification [16,17], transesterification [18], amidation [19], epoxidation [20–23], Baeyer–Villiger oxidation [24] and Michael addition [25] catalyzed by immobilized lipases have been conducted in non-aqueous media.

Flavor and fragrance chemicals are used to enhance the taste and odor of particular compounds in food, pharmaceutical and cosmetic industries. Global market for these compounds was valued at around $14.87 bn in 2009 [26]; most of these compounds are produced by chemical methods like organic synthesis and carry a tag of being “artificial”. Some are isolated by extraction from natural sources but are too expensive. Increasing demands by consumers in recent years for clean processes have forced these industries to search for alternative processes. Biotransformation is one of the alternatives for selective synthesis of desired

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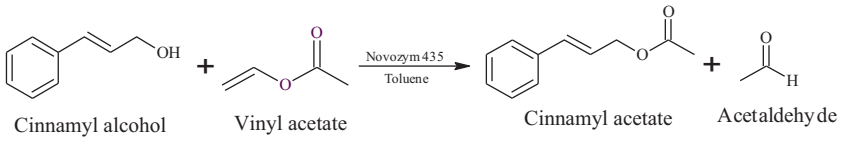

Scheme 1. Lipase catalyzed transesterification of cinnamyl alcohol with vinyl acetate.

compounds, in which microbial cells or enzymes are used as catalysts. In general biocatalysts and especially enzymes are non-toxic, easily biodegradable and have no harmful effect on health. Flavor and fragrance compounds produced by enzymatic route can be considered as being close to 'natural' and can potentially satisfy the requirements of food and cosmetic industries [27]. Cinnamyl acetate, a phenyl propanoid class of compound, naturally occurs in fresh bark of cinnamon and is an important ingredient used in the synthesis of flavors, fragrances and fine chemicals. Worldwide use of cinnamyl acetate is about 100 metric ton per annum [28]. Cinnamyl acetate can be synthesized by direct esterification of cinnamyl alcohol with acetic anhydride in presence of 85–98% *p*-toluenesulfonic acid which is more corrosive in nature [29]. Another process is the reaction between cinnamyl bromide and sodium acetate in the presence of tetra-butyl ammonium bromide as a phase transfer catalyst at high temperatures [30]. There is dearth of information on the synthesis of cinnamyl acetate using lipase as catalyst.

In enzymatic reactions, several factors have significant influence on enzyme activity and reaction rate. These factors are the type of enzyme (microbial origin, specificity), type of support and immobilization technique employed reaction medium, enzyme loading, substrate concentrations and operating conditions, mainly temperature and speed of agitation. The present work highlights the effects of these parameters on the lipase catalyzed transesterification of cinnamyl alcohol with vinyl acetate to make cinnamyl acetate in non-aqueous medium. The study was carried out by systematically varying one parameter at a time. The reaction mechanism and kinetics are also studied. Scheme 1 represents the reaction.

2. Materials and methods

All enzymes and chemicals were received from reputed companies. Novozym 435 (lipase B from *Candida antarctica*, supported on a macroporous acrylic resin and enzyme activity 7000 PLU/g. PLU is propyl laurate unit, based on reaction between propyl alcohol and lauric acid). Lipozyme RM-IM (lipase from *Rhizomucor miehei*, supported on a macroporous anion exchange resin and enzyme activity 30 U/g, based on tristearin assay), Lipozyme TL IM (lipase from *Thermomyces lanuginosus*, supported on porous silica granulates and enzyme activity 175 IU/g, IU is international unit, based on tributyrin assay) were received as gift samples from Novo Nordisk, Denmark. Amano AYS (*Candida rugosa* lipase and enzyme activity 240 U/g, based on tributyrin assay) was received from Amano Pharmaceuticals, Japan. Vinyl acetate, toluene, 1,4-dioxane, tetrahydrofuran, *t*-butanol and other analytical reagents were purchased from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India. Cinnamyl alcohol was purchased from Sisco Chemicals Pvt. Ltd., Mumbai. All chemicals and enzymes were used without any further modification.

2.1. Experimental setup

The experimental set-up consisted of a 3 cm i.d. mechanically agitated glass reactor of 50 cm^3 capacity, equipped with four baffles and a six-bladed turbine impeller. The entire reactor assembly was immersed in a thermostatic water bath, which was maintained at the desired temperature with an accuracy of ±1 °C. A typical reaction mixture consisted of 0.01 mol cinnamyl alcohol and 0.02 mol vinyl acetate diluted to 15 cm^3 with toluene as a solvent. The reaction mixture was agitated at 40 °C for 15 min at a speed of 200 rpm and then 10 mg of enzyme was added to initiate the reaction. Clear liquid samples free from the catalyst particles were withdrawn periodically from the reaction mass and analyzed by gas chromatography.

2.2. Analysis

Analysis of liquid samples was carried out by GC (Chemito 8610) equipped with flame ionization detector using 4 m × 3.8 mm stainless steel column packed with 10% SE-30 stationary phase. Nitrogen was used as carrier gas at a flow rate of 1 $cm^3\ min^{-1}$. The temperature program was as follow: 50 °C for 1 min; 5 °C/min up to 160 °C; 20 °C/min up to 275 °C; then steady temperature for 5 min. The injector and detector temperatures were both kept at 290 °C. Undecane was used as internal standard and percentage conversion was calculated based on area under curve of limiting reactant as follows:

$$\text{Conversion}\,(\%) = \frac{[(A_0/I_0) - (A/I)]}{A_0/I_0} \times 100$$

whereas A_0, A = area under curve of limiting reactant at time $t = 0$ and $t = t$ min, I_0, I = area under curve of internal standard at time $t = 0$ and $t = t$ min. Formation of cinnamyl acetate was confirmed by GC–MS (Clarus 500 GC/MS, PerkinElmer, USA).

3. Results and discussion

3.1. Effect of lipases from different microbial origin

Lipases from different microbial origin such as lipase B from *C. antarctica*, *R. miehei* lipase, *T. lanuginosus* lipase and *C. rugosa* lipase were used under similar reaction condition (Fig. 1). Lipase B from *C. antarctica* (Novozym 435) gave a conversion of 96% in 1 h, which was the maximum as compared to other lipases. *R. miehei* lipase and *T. lanuginosus* lipase gave only 6 and 4% conversion, respectively. These two lipases are suitable for esterification/interesterification of high molecular weight fatty acids and their ester derivatives [31]. *C. rugosa* lipase gave less than 2% conversion; it shows that this enzyme may be deactivated by acetaldehyde, a co-product of reaction which forms the Schiff base with lysine residue of enzyme [32]. Thus, *C. antarctica* lipase B was chosen as the best catalyst for this system and used in further experiments.

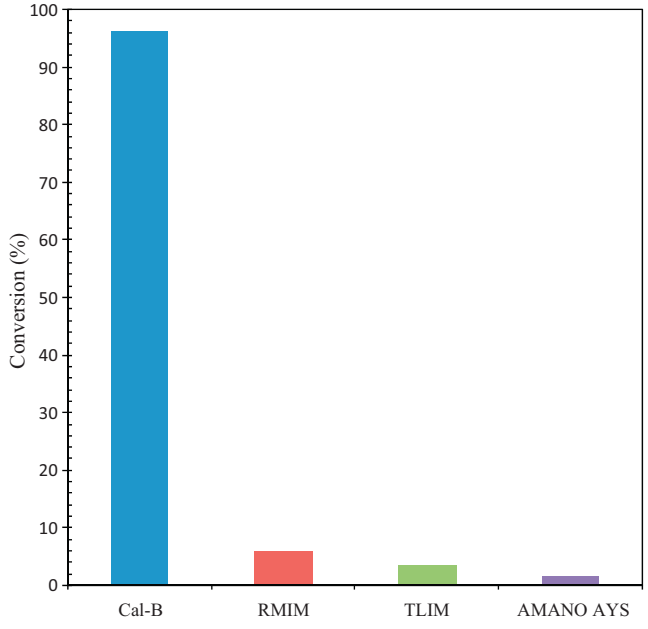

Fig. 1. Effect of different lipase in transesterification of cinnamyl alcohol with vinyl acetate. (Reaction conditions: cinnamyl alcohol, 0.01 mol; vinyl acetate, 0.02 mol; solvent up to 15 cm^3; speed of agitation, 200 rpm; catalyst loading, 4.67 U/ml; temperature, 40 °C, Cal-B (lipase B from *Candida antarctica*), RMIM (*Rhizomucor miehei* lipase), TLIM (*Thermomyces lanuginosus* lipase), AMANO AYS (*Candida rugosa* lipase)).