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Lipase-catalyzed synthesis of 4-methoxy cinnamoyl glycerol

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ABSTRACT

Cinnamoyl esters are used as organic ultraviolet (UV) filters in sunscreens and cosmetic formulations. To avoid any possible harmful effects from chemically synthesized product, the enzymatic synthesis appears to be an excellent way to satisfy the present consumer demand for natural products. Enzymatic esterification of 4-methoxy cinnamic acid (4MCA) with glycerol was carried out in organic solvents using immobilized lipase B from *Candida antarctica*, in which the maximum conversion of 34% was found in isooctane at 70 °C after 24 h with 12% of enantiomeric excess. If the reaction continued for longer times (48 h) it leads to the formation of 16% of diester along with 56% of monoester. The results of enzymatic esterification were compared with reported chemical esterification and the present method was found to be superior in terms of conversion yields and priority to the formation of monoester. Synthesized monoester product was isolated and characterized by spectroscopic techniques.

1. Introduction

Octyl methoxycinnamate is commonly used as an UV filters in sunscreen and cosmetic formulations to protect skin damage by solar radiation [1]. The unsaturated C=C bond in cinnamic acid along with methoxy group in para position through aromatic ring allows for a continuous conjugated π-system throughout the molecule which is responsible for the absorbance of UV light. In addition to the cinnamoyl esters those are mainly used as UV filters some esters of ferulic acid are also reported [2,3]. These esters are mainly lipophilic in nature due to the presence of alkyl chain in ester functionality. An ideal sunscreen should be more hydrophilic, adherent to the skin and impermeable to systemic circulation to avoid toxicity [4,5]. To prepare a sunscreen with hydrophilic character longer alkyl chain alcohols may be replaced by glycerol in esterification reaction to make it more hydrophilic.

Glycerol is an important by-product generated in transesterification of vegetable oils for the production of biodiesel [6,7]. The increasing production of biodiesel from vegetable oils [8,9] has led to a drastic surplus of glycerol in the chemical markets. Glycerol production averages more than 350,000 tons per year in the United States and it has tripled to approximately 600,000 tons per year within the past 10 years in Europe. One concern regarding biodiesel as well as soap and fatty acid production is how to utilize this by-product [10,11]. Thus, producing value-added products from glycerol would be beneficial by considering the current enormous supply of glycerol.

Recently the synthesis of 4-methoxy cinnamoyl glycerol (4MCG) was reported by chemical esterification reaction in toluene using *p*-toluenesulfonic acid catalyst [12]. In the present study, we report the synthesis of same ester for the first time using enzyme catalyst. Lipases are broadly used as a biocatalyst in the preparation of pharmaceuticals [13], biosurfactants [14], cosmetics, flavors [15], foods [16], perfumery and other organic synthetic materials [17]. Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are characterized by their ability to catalyze the hydrolysis and the reverse reaction of esterification. Lipase B from *Candida antarctica* is one of the most widely used lipase, which is commercialized both in free and immobilized form by diverse commercial suppliers. Consequently, in the present study, immobilized *Candida antactica* lipase B was employed as the biocatalyst for synthesis of 4MCG by esterification of 4MCA with glycerol (Fig. 1).

2. Materials and methods

2.1. Materials

C. antarctica lipase B (Novozym 435, immobilized on acrylic resin) and 2-methyl-2-butanol were purchased from Sigma–Aldrich (Steinheim, Germany). All other solvents used for esterification reaction were obtained commercially and were of analytical grade. Glycerol and methanol (HPLC grade) were purchased from Sisco Research Laboratory, Mumbai, India. 4MCA was prepared by Knoevenagel condensation between anisaldehyde and malonic acid [18]. Chemicals and solvents used for preparation of 4MCA were purchased from local supplier.

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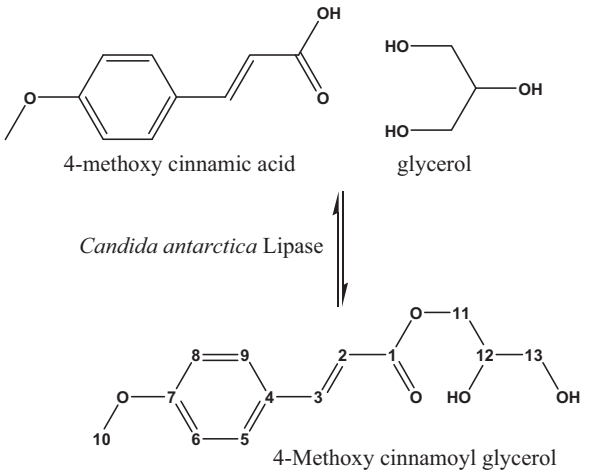

Fig. 1. Lipase catalyzed esterification of 4-methoxy cinnamic acid with glycerol.

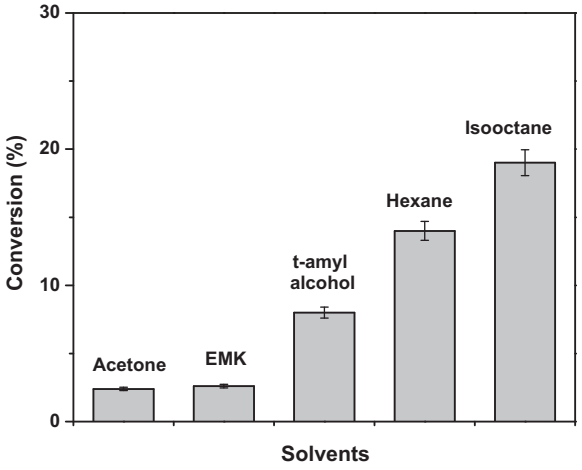

Fig. 2. Effect of solvent on conversion of 4MCA to 4-methoxy cinnamoyl glycerol after 24 h at 60 °C.

2.2. Enzymatic esterification

Lipase catalyzed esterification was carried out in 100-ml round bottom flask by using 5 mmol of dry glycerol (dried by heating it in porcelain dish up to 180 °C with constant stirring with glass rod, cooled to room temperature then kept in desiccator), 1 mmol of 4MCA and 250 mg lipase in 18 ml of solvent. The reaction mixture was kept in temperature-controlled oil bath over a magnetic stirrer for 24 h. After completion, the reaction mixture was dissolved in methanol and enzyme removed by filtration. The filtrate was concentrated on rotary evaporator at reduced pressure. Concentrated reaction mass was dissolved in methanol for HPLC analysis and conversions were calculated on basis of acid.

To separate the desired product, concentrated residue was dissolved in deionized water to remove un-reacted 4MCA which is insoluble in aqueous medium. Residue washed with cold deionized water and the filtrate was extracted with diethyl ether, dried over sodium sulfate and concentrated on rotary evaporator. The product was also separated by silica gel column chromatography using mixed ethyl acetate hexane solvent system. The purified monoester product was characterized by FTIR and NMR spectroscopy.

2.3. Analysis

The quantification of esterification reaction was carried out using HPLC analysis. The HPLC system consisted of an XTerra RP C_{18} column (4.6 mm × 250 mm; Waters) eluted with methanol as the mobile phase at a flow rate of 1 ml/min using UV detection at 254 nm.

Infrared spectra were recorded as KBr pellets on a Nicolet Nexus-870 FTIR spectrometer. PerkinElmer's Spectrum Software Version 5.3 was used to generate the FTIR spectrum. NMR spectra were operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C using Bruker AC NMR spectrometer.

3. Results and discussion

The ester product, 4MCG was separated by dissolving the crude reaction mass in deionized water to remove the un-reacted acid as residue. Filtrate containing un-reacted glycerol and the product the latter was extracted with diethyl ether. The product was also purified by silica gel column chromatography; it has been carried out by subjecting the concentrated reaction mass to silica gel (60–120 mesh, eluent 50–60% ethyl acetate in hexane) column chromatography to afford the ester product.

Different organic solvents were studied for the lipase-catalyzed synthesis of 4MCG at 60 °C (Fig. 2). Despite better solubility of substrates in polar solvents like acetone, ethyl methyl ketone (EMK), acetonitrile and 1, 4 dioxane the resulting conversion was either very low or no conversion was observed except in 2-methyl-2-butanol. However, better conversion was resulted in non-polar solvents. Comparing the percentage conversion in hexane and isooctane, better conversion was found in isooctane (19%) than hexane (14%). Similar results on enzymatic esterification of 4MCA with 2-ethyl hexanol and glycerol with fatty acid were reported i.e. better conversion was achieved in isooctane than hexane using *C. antarctica* lipase B [19] and *Candida rugosa* lipase [20], respectively. Higher conversion in isooctane than hexane can be explained on the basis of hydrophobicity. Water immiscible solvents would not entrain water and consequently shifts the equilibrium towards ester synthesis in a greater extent. Polarity of the organic solvent, generally measured by log *P* (logarithum of partition coefficient of organic solvent in water and 1-octanol), affects enzymatic synthesis, the term log *P* is generally used to describe the hydrophobicity of organic solvents [21]. Considering the partition coefficient (log *P*) of solvents these result were due to higher hydrophobicity of isooctane (4.5) than hexane (3.5).

C. antarctica lipase, immobilized on acrylic resin is well known heat tolerant enzyme with maximum activity at high temperatures [2]. To study the effect of temperature on conversion of esterification reaction, reactions were carried out at 60, 70 and 80 °C (Fig. 3). The conversion increased with an increase in the temperature from 60 to 70 °C; however, there is almost same conversion at 70 and 80 °C.

To get maximum conversion, esterification reaction was carried out at longer time under the similar reaction conditions in isooctane at 70 °C. It was observed that, the formation of monoester was increased to 56.6% after 48 h. However, increased reaction time resulted in the formation of 16.5% diester. Holser [22] studied the esterification of 4MCA with glycerol using *p*-toluenesulfonic acid catalyst in toluene, where they got 35.8% of monoester along with the formation of 16.7% of diester. Consequently the present method of enzymatic esterification was found superior to the chemical method not only in terms of conversion yields but also priority to the formation of monoester (Table 1).

Mono-acylation of prochiral molecule glycerol may lead to the formation of enantiomers. Kato et al. [23] described the asymmetric transesterification of glycerol with benzoates using enzyme catalyst. Batovska et al. [24] reported enzymatic mono-esterification of glycerol with different acid anhydrides with varying enantiomeric

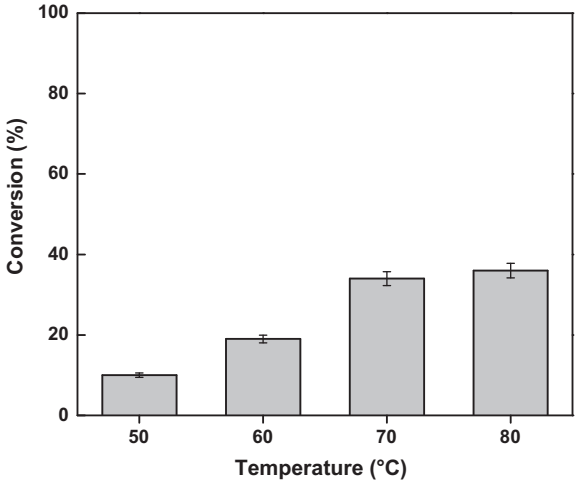

Fig. 3. Effect of reaction temperature on conversion of 4MCA to 4-methoxy cinnamoyl glycerol after 24 h in iso-octane.

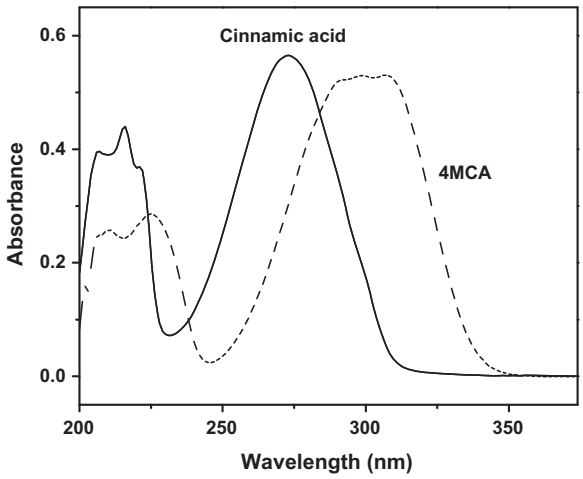

Fig. 4. UV spectra of cinnamic acid and 4-methoxy cinnamic acid in methanol.

excess using lipase (Chirazyme L-2) from *C. antarctica*. In the present study, the isolated product was found to optically active with enantiomeric excess of 12% which was determined by Chiradex (4.6 mm × 250 mm) HPLC column (4.6 mm × 250 mm, 95% acetonitrile in water, flow rate 0.8 ml/min).

To observe the typical difference in acid and ester carbonyl stretching frequency, infrared spectra of 4MCA and 4MCG were recorded. A strong absorption was observed in 3200–3600 cm^{-1} region for hydroxyl groups and a pair of bands at 1172 and 1116 cm^{-1} consistent with the C–O stretching mode. In addition, carbonyl frequency of ester was observed at 1700 cm^{-1} which was typically shifted from 1686 cm^{-1} (4MCA).

The synthesized product was also characterized by NMR spectroscopy in deuterated chloroform ($CDCl_3$) solvent and spectra were recorded using Bruker AC NMR spectrometer.

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.68 (d, 1H, *J* = 16.0), 7.48 (d, 2H, *J* = 8.8), 6.91 (d, 2H, *J* = 8.8), 6.33 (d, 1H, *J* = 16), 4.31 (ddd, 2H, *J* = 11.6, 4.4), 4.00 (t, 1H, *J* = 4.4), 3.84 (s, 3H), 3.73 (dd, 1H, *J* = 11.6, 4.0), 3.65 (dd, 1H, *J* = 11.6, 5.6).

^{13}C NMR ($CDCl_3$, 100 MHz): 167.8 C1, 161.6 C7, 145.6 C3, 129.9 C5, 129.9 C9, 126.8 C4, 116.1 C2, 114.4 C6, 114.3 C8, 70.4 C12, 65.2 C11, 63.2 C13, 55.3 C10.

The UV spectra (Fig. 4) of cinnamic acid and 4MCA were recorded on UV–vis double beam spectrophotometer (UV-1601, Shimadzu, Japan). The UV absorption of cinnamic acid shows up to 315 nm whereas in case of 4MCA it is extended up to 350 nm. An ideal sunscreen is one which can absorb a wide spectrum of UV radiation [25], 4MCA absorbed at longer wavelength than cinnamic acid. The synthesized monoesters have shown the same absorption characteristic as they are having the same UV absorptive moiety. Considering the UV absorbance capacity and increased hydrophilic nature of 4MCG [22], it turns to potential for use in personal care products as UV filters.

Table 1
Comparison of chemical and enzymatic esterification on the formation of mono and diester.

Esterification	Time (h)	Monoester (%)	Diester (%)
Chemical method[a]	2	20.0	–
	8	35.8	16.7
Enzymatic method[b]	24	34.3 ± 1.6	nd
	48	56.6 ± 2.7	16.5 ± 0.6

[a] Ref. [22].
[b] 5 mmol of dry glycerol, 1 mmol of 4MCA, 250 mg lipase in 18 ml of isooctane at 70 °C.
nd = Not detected.

4. Conclusion

Enzymatic esterification of 4MCA with glycerol was successfully carried out using *C. antarctica* lipase. Maximum conversion of 34% as a monoester was achieved after 24 h in isooctane at 70 °C. Almost same conversions were obtained at 70 and 80 °C. Prolonged reaction time (>30–36 h) leads to the formation diester along with monoester. Since present method is superior to chemical method in terms of conversion yield and priority for the monoester formation, it can be applied for the production of 4MCG.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2011.07.002.

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