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Lipase-catalyzed transesterification of primary terpene alcohols with vinyl esters in organic media

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Abstract

Several microbial lipases were screened for their ability to catalyze the synthesis of citronellyl and geranyl esters in organic media using vinyl esters as acylating agents. Based on the results, an immobilized lipase, SP 435 from *Candida antarctica* was chosen for the study of the effect of reaction parameters on terpenyl acetate synthesis. This enzyme at 10% (w/w reactants) gave yields of 97.7 and 98.6% for geranyl and citronellyl acetate, respectively. Time course studies showed optimum yields could be obtained after 8 and 16 h incubation, respectively. For both terpenyl acetates, solvents with log $P > 3.2$ gave highest yields and a temperature range between 30–50°C seemed suitable for the synthesis of these primary terpenyl acetates. With some solvents, up to 100% yield of geranyl acetate was obtained. In these solvents, SP 435 showed preference for geraniol. © 1998 Elsevier Science B.V.

Keywords: Candida antarctica; Lipase; Organic media; Transesterification; Terpene; Vinyl esters

1. Introduction

The use of enzymes, in particular lipases, in organic rather than aqueous media has been the object of numerous studies. Several advantages such as the shift in thermodynamic equilibria in favor of synthesis over hydrolysis, increased solubility of nonpolar substrates, elimination of hydrolytic side reactions, ease of enzyme and product recovery, increased enzyme thermostability and elimination of microbial contamination due to the low water activity of organic solvents have been suggested [1].

Lipases have successfully been used as biocatalysts for the production of flavor esters by direct esterification [2–6] and transesterification reactions [7–12], using different enzymes and substrates.

Vinyl esters were chosen as acyl donors because they have previously been shown to be useful for lipase-catalyzed preparation of enantiomerically pure compounds [13]. The vinyl alcohol freed from the transesterification reaction tautomerizes to acetaldehyde [14]. This makes the process irreversible [14]. More specifically, vinyl esters have been reported as acylating agents for the enzymatic synthesis of menthyl acetate using lipase PS from *Pseudomonas sp*. [15].

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In this paper we have chosen to study lipase-catalyzed transesterification of citronellyl and geranyl esters with vinyl esters as acyl donors using lipases SP 435 and SP 382, from *Candida antarctica*; IM 60 and IM 20, from *Rhizomucor miehei*; PS-30, from *Pseudomonas sp*. and AY-30 from *Candida rugosa*. The purposes of this research were to screen selected microbial lipases and to study the effect of acyl donor chain length on the synthesis of primary terpenyl esters using suitable lipases. Reaction parameters such as enzyme load, time course, reaction media and reaction temperature with vinyl acetate as the acyl donor and SP 435 as the catalyzing lipase were also investigated.

2. Materials and methods

2.1. Reagents

Non-specific lipases PS-30 (36,000 U/g) from *Pseudomonas sp*. and AY-30 (33,000 U/g) from *Candida rugosa* were obtained from Amano International Enzyme Co. (Troy, VA). Nonspecific lipases, SP 435 (7000 Propyl Laurate Units/g, PLU/g), cloned into *A. oryzae* and SP 382 (40 Batch Interesterification Units/g, BIU/g), immobilized on acrylic resin, both from *Candida antarctica*, and *sn*-1,3 specific lipases IM 60 (5–6 Batch Acidolysis Units Novo/g, BAUN/g) cloned into *A. oryzae* and IM 20 (24 BIU/g), immobilized on macroporous anion exchange resin from *Rhizomucor miehei*, were obtained from Novo Nordisk Biochem North America (Franklinton, NC). DL-Citronellol (95% pure) and geraniol (98% pure) were obtained from Sigma Chemical Co. (St. Louis, MO). Vinyl acetate (99% pure) and propionate (98% pure) were obtained from Aldrich Chemical Co. (Milwaukee, WI), whereas vinyl butyrate (99% pure) was obtained from Fluka Chemical Corp. (Ronkonkoma, NY). *n*-Hexane and all other solvents (HPLC grade) were purchased from Fisher Scientific (Norcross, GA).

2.2. Transesterification procedure

Ester synthesis was performed in screw capped test tubes in duplicate as previously described by Yee et al. [10] in which 0.1 M of terpene alcohol and in this case 0.1 M vinyl ester were added to 2 ml hexane followed by 10% (w/w of reactants) of appropriate enzyme. Samples were incubated in an orbital water bath shaker at 30°C, 200 rpm for 24 h (unless otherwise stated), along with their respective controls (samples with no enzymes).

2.3. Extraction and experimental analysis

After the incubation period, samples were removed and 100 μl of reaction product was diluted with 1 ml hexane containing 1.746 mg ethyl caproate (Aldrich Chemical Co., Milwaukee, WI) as internal standard. Samples were then passed through an anhydrous sodium sulfate (Na_2SO_4) column to remove residual water and enzyme. For solvent-free samples, reaction products were dissolved in 2 ml hexane after incubation but prior to analysis.

Product analysis was performed by injecting a 1 μl aliquot in a splitless mode into a gas liquid chromatograph (GLC) (Hewlett-Packard HP 5890 Series II, Avondale, PA) equipped with a flame-ionization detector. A DB-5 fused silica capillary column (30 m $\times$ 0.32 mm i.d., film thickness 1 μm, J&W Scientific, Folsom, CA) heated isothermally at 170°C was used to separate and identify terpene esters. Injector and detector temperatures were set at 250 and 260°C, respectively. Helium was the carrier gas, with a total flow rate of 5 ml/min. Synthetic yields of terpene esters were determined from the amount of product formed, using peak area integrated by an on-line computer.

3. Results and discussion

3.1. Enzyme screening

Table 1 illustrates the biocatalytic ability of six lipases on the synthesis of citronellyl and

Table 1
Effect of different lipases on reaction yields of geraniol and citronellyl acetate[a]

Lipase	Yield (%)	
	geraniol acetate	citronellyl acetate
PS-30[b]	85.0	81.2
AY-30[b]	21.5	23.4
SP 435	97.1	99.5
SP 382	96.2	98.8
IM 60	94.5	68.9
IM 20	64.9	69.7

[a]Reactions were performed in duplicate in 2 ml *n*-hexane with 0.1 M terpene alcohol, 0.1 M vinyl acetate and 10% (w/w of reactants) of lipase. Yields are expressed as % molar conversion after 24 h incubation.
[b] Non-immobilized enzymes.

geranyl acetate. Overall, the lipases screened showed slightly greater substrate preference towards geraniol than citronellol. Lipase AY-30 performed poorly compared to other lipases tested, while *Candida antarctica* lipases, SP 435 and 382 gave the best yields for both acetates. Previously, Claon and Akoh [6] reported highest yields with *C. antarctica* lipases for the synthesis of geranyl and citronellyl esters by direct esterification using acetic acid as the acyl donor. The structure of geraniol and citronellol are shown in Fig. 1.

3.2. Effect of acyl donor chain length

Results of the effect of acyl donor chain length on lipases PS-30 and SP 435 ability to catalyze the syntheses of primary terpenyl acetates, propionates and butyrates are shown in Table 2. Yields of up to 100% were observed for citronellyl butyrate with lipase SP 435. Lowest yields were obtained for citronellyl propionate with both enzymes. Yields did not seem to greatly improve with increase in acyl chain length, as we have observed earlier [9,11]. Overall, lipase SP 435 gave the best yields for primary terpenyl acetates.

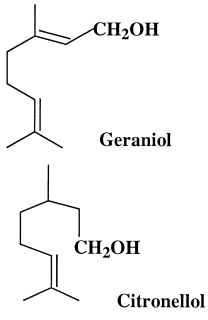

Fig. 1. Structure of terpene alcohols used for the transesterification reaction.

Table 2
Effect of acyl donor chain length on lipase-catalyzed synthesis of terpene esters[a]

Acyl donor	Lipase	Yield (%)	
		geraniol ester	citronellol ester
Vinyl acetate	PS-30	78.2	80.2
	SP 435	96.3	95.8
Vinyl propionate	PS-30	87.6	77.4
	SP 435	99.3	87.4
Vinyl butyrate	PS-30	85.0	89.9
	SP 435	91.5	100.0

[a]Reactions were performed in duplicate in 2 ml *n*-hexane with 0.1 M terpene alcohol and 0.1 M vinyl acetate, propionate and butyrate, respectively. 10% (w/w reactants) of lipase PS-30 and SP 435 were added. Yields are expressed as % molar conversion after 24 h incubation.

3.3. Enzyme load

The effect of enzyme load on terpenyl acetates is shown in Fig. 2. Yields as high as 92.4 and 89.9% were observed for geranyl and cit-

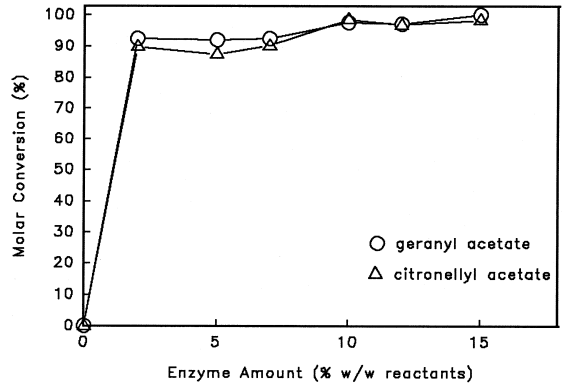

Fig. 2. Effect of enzyme load on the synthesis of citronellyl and geranyl acetate. Samples were prepared by adding 0.1 M terpene alcohol and 0.1 M vinyl acetate to 2 ml *n*-hexane and incubated at 30°C with 0–15% (w/w of reactants or 0–50 PLU) of SP 435 lipase for 24 h.

ronellyl acetate, respectively, at 2% added SP 435 enzyme (0.0010 g, approximately 7 PLU). This experimental parameter was assayed at a fixed time of 24 h so that any improvement in yield will be attributed to the enzyme amount. At 10% (34 PLU) enzyme, yields of 97.7 and 98.6% were observed after which yields did not increase much more. However, it is note worthy that at 15% (50 PLU) the yield for both geranyl and citronellyl acetate reached 100% after 24 h incubation. Although higher yields were obtained at a higher enzyme load, there seemed to be no economic or practical advantage of using such amounts since acceptable yields were observed at low enzyme load of 2% (only an increase of 7.6–10.1% or 1.4–2.3% in yield going from 2 to 15% and from 10 to 15%, of enzyme for both esters, respectively). We recommend an enzyme load of 2–10% for this reaction. The results for citronellyl esters are total conversion and no attempt was made to check for enentioselectivity of the SP 435 lipase for either the D- or L-isomer.

3.4. Time course

The time course is used to monitor reaction progress and to possibly minimize process cost. After 1 h incubation with SP 435 lipase at 10% (w/w of reactants), yields of 80.5 and 69.3% were observed for geranyl and citronellyl acetate, respectively (Fig. 3). Yields for both esters increased with time. Optimum yields were achieved after 8 and 16 h reaction for geranyl and citronellyl acetate (99.9 and 98.1%, respectively). We stopped assay at 24 h because high and acceptable yields of both esters have been obtained. Equilibrium may have been reached at 24 h incubation. The remaining reaction parameters were assayed at these incubation periods.

3.5. Effect of reaction media

Although it has been proven that enzymes work well in organic solvents [1,16], the type of enzyme and substrate used are also important.

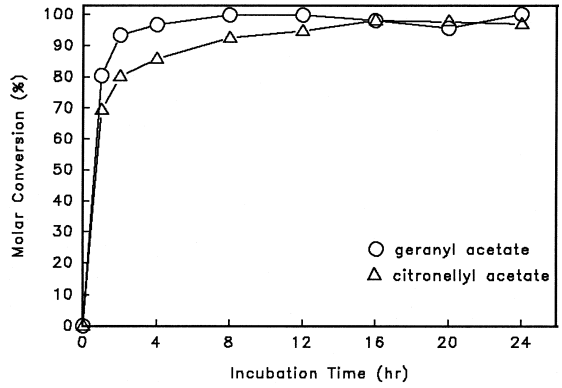

Fig. 3. Time course of SP 435-catalyzed synthesis of citronellyl and geranyl acetate in *n*-hexane. Samples were analyzed after 1, 2, 4, 8, 12, 16, 20 and 24 h incubation. The enzyme load was 10% (w/w of reactants or 34 PLU).

The log *P* value, the partition coefficient between water and octanol, has been shown to be a good indicator of solvent polarity since it is believed that hydrophobic solvents are the best media for enzymatic synthesis [17,18]. Generally, solvents with log *P*-values < 2 show little biocatalytic activity, while those with log $P > 4$ allow high biocatalytic activity.

Table 3 shows the effect of various organic solvents on lipase SP 435-catalyzed synthesis of citronellyl and geranyl acetates. Solvents with log $P > 3.2$ showed highest yields, while chloroform (log $P = 2.0$) performed very poorly. In the case of geranyl acetate, cyclohexane, hex-

Table 3
Effect of organic solvents on lipase SP 435-catalyzed synthesis of geraniol and citronellyl acetate

Solvent[a]	Log *P* value[b]	Molar conversion (%)	
		geranyl acetate	citronellyl acetate
No solvent	—	99.9	72.4
Iso-octane	4.5	100.0	85.5
Heptane	4.0	100.0	89.1
n-Hexane	3.5	100.0	94.5
Cyclohexane	3.2	100.0	89.3
Pentane	3.0	78.1	66.2
Toluene	2.5	89.7	80.6
Benzene	2.0	90.1	81.3
Chloroform	2.0	11.5	16.4

[a] Solvents were dried over molecular sieve 4 Å. Amount of lipase was 10% (w/w of reactants or 34 PLU).
[b] Source: Ref. [17].

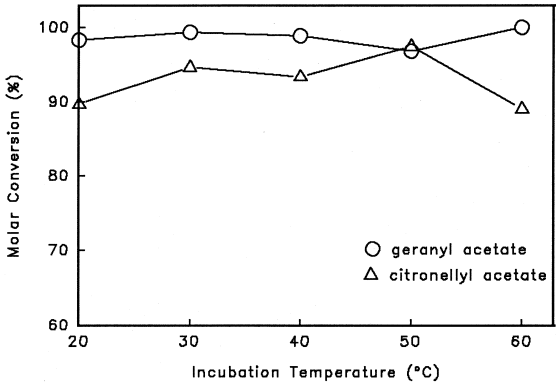

Fig. 4. Effect of temperature on lipase SP 435-catalyzed synthesis of citronellyl and geranyl acetate in *n*-hexane. Temperatures ranged from 20–60°C and the enzyme amount was 10% (w/w of reactants or 34 PLU).

ane, heptane and *iso*-octane gave values of 100%. Benzene (log $P = 2.0$, 90.1%) and no solvent (99.9%) also gave high yields. For citronellyl acetate, hexane (log $P = 3.5$) was the most suitable reaction medium with a yield of 94.5%.

3.6. Effect of temperature

Synthesis of terpenyl acetates by lipase SP 435 (10% w/w of reactants) at various temperatures are shown in Fig. 4. In general a wide temperature range was observed for the SP 435 lipase for both ester syntheses. For geraniol acetate all temperatures gave good results, with a 100% yield at 60°C. In the case of citronellyl acetate, 30–50°C seemed suitable, although a slight decrease was observed at 60°C.

4. Conclusion

Our results have demonstrated that vinyl esters are excellent acyl donors for the enzymatic synthesis of primary terpene esters. Very high yields were obtained with lipase SP 435 as the biocatalyst.

Acknowledgements

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