ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 16 (2001) 169–173

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Enzymatic synthesis of chiral menthyl methacrylate monomer by pseudomonas cepacia lipase catalysed resolution of (±)-menthol

Vilas Athawale*, Narendra Manjrekar, Manoj Athawale

Department of Chemistry, University of Mumbai, Vidyanagari, Santacruz(E), Mumbai-400098, India

Received 16 July 2001; received in revised form 13 September 2001; accepted 13 September 2001

Abstract

Optically active methacrylate ester of (−)-menthol was synthesised by enantioselective transesterification of (±)-menthol using pseudomonas cepacia lipase (Lipase-PS) as a biocatalyst. Effect of change in various reaction parameters such as amount of catalyst, type of acyl donor, solvent and temperature on the conversion as well as enantioselectivity was studied. Methyl methacrylate, vinyl methacrylate and 2,3-butanedione mono-oxime methacrylate were used as acylating agents. Oxime methacrylate gave better conversion as compared to other acyl donors used in the present study. Diisopropylether (DIPE) was found to be the most suitable solvent. (−)-Menthyl methacrylate was obtained with 96% yield and 98% enantiomeric excess.

Keywords: Chiral; Lipase; (−)-Menthol oxime metshacrylate; Transesterification

---

1. Introduction

Optically active synthetic polymers are of current interest due to their use as chiral stationary phases in HPLC for separation of mixtures of enantiomers, chiral recognition properties and their potential applications for asymmetric synthesis [1]. (−)-Menthol is a component of peppermint oil and is produced on industrial scale by optical resolution of (±)-menthol. (−)-Menthol and its esters are more important from industrial point of view than (±)-menthol. (−)-Menthol, because of its cooling and refreshing effects, is an important fragrance and flavour compound that is used largely in cosmetics, toothpaste, chewing gums, cigarettes, sweets and medicines. It also possesses the characteristic peppermint odour, which is lacking in the other isomer. The esters of (−)-menthol can be expected to have interesting characteristic such as fragrance, which is emitted as the ester bond is hydrolysed. The chiral menthyl methacrylate may be co-polymerised with other monomers to obtain synthetic polymers, which would exhibit the property of sustained release perfume, as a result of hydrolysis of the menthyl ester to menthol in the presence of moisture. The chiral menthol esters have been used in the asymmetric synthesis [2].

Acrylic monomers are highly versatile building blocks. In general, they are synthesised by esterification or transesterification processes using chemical catalysts [3,4]. In order to overcome the drawbacks reported in the use of chemical catalysts, which usually lead to the formation of undesirable by-products and to make the process ecofriendly, we thought of exploring the use of lipase as a catalyst.

The lipase catalysed reactions, in organic solvents, under nearly anhydrous conditions offer advantages

---

* Corresponding author. Tel.: +91-22-652-79-57-61x567; fax: +91-22-652-85-47.
E-mail address: vilasda@yahoo.com (V. Athawale).

1381-1177/01/$ – see front matter
PII: S1381-1177(01)00060-1

such as better overall yields and the suppression of the undesired side reactions like hydrolysis of labile groups by water [5]. Various activated esters like enol esters [6] and acid anhydrides [7] were used as acyl donors for irreversible enzymatic acylation. However, these methods suffer from some drawbacks, like the generation of the toxic side products thereby inhibiting the formation of the required product. On the contrary, oxime esters have been shown to act as irreversible acylating agents [8], wherein the leaving group, an oxime, owing to its low nucleophilicity suppresses the reversible reaction.

2. Experimental

All the solvents used were purchased from Aldrich and dried over a molecular sieve (4 Å). (±)-Menthol (**1**) and (−)-menthol (**2**) were purchased from Aldrich. Lipase-PS, (specific activity 35 units/mg) was obtained from Amano Pharmaceutical (Japan). Porcine pancreatic lipase (activity 44 units/mg) and candida rugosa lipase (activity 665 units/mg) were purchased from Sigma (USA). 2,3-butane dione mono-oxime methacrylate (**3**) was synthesised by reaction of methacryloyl chloride with 2,3-butane dione mono-oxime (**4**) in presence of triethylamine as a base [9]. The progress of the reactions was monitored using a Hewlett-Packard 5890 gas chromatograph equipped with a DB23 column (see Scheme 1).

In a typical experiment, (−)-menthol (0.001 mol) and **3** (0.001 mol) were dissolved in 20 ml solvent in a 50 ml screw-capped vial followed by the addition of lipase. The reaction mixture was stirred at 500 rpm. The progress of the reaction was monitored by removing the aliquots of reaction mixture and subsequent analysis with a Hewlett-Packard 5890 gas chromatograph. (±)-Enantiomers of menthol and their respective esters were analysed on CP Cyclodex β236M(0.25 mm × 50 m: Chrompack, Middelberg, The Netherlands). The absence of any undesired competing chemical acyl transfer reaction was verified by a controlled experiment in the absence of enzyme.

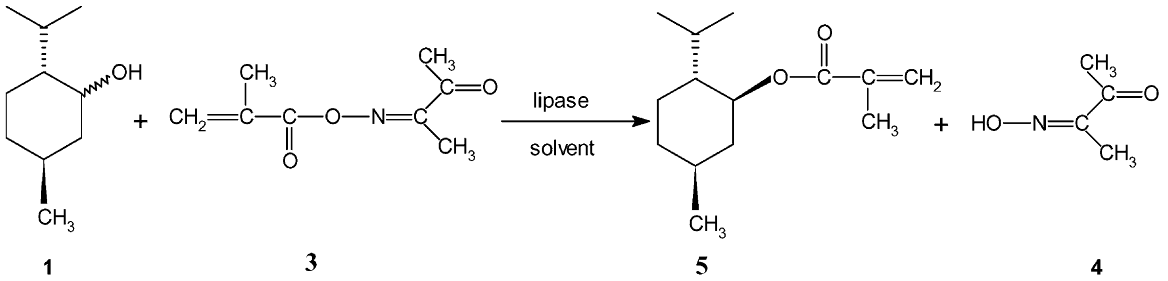
3. Results and discussion

Lipases are very selective towards various substrates. Therefore, it was necessary to find out the most suitable lipase, which would catalyse the transesterification of (−)-menthol (**2**) to (−)-menthyl methacrylate (**5**). Three different lipases, namely Lipase-PS, PPL and CRL were tested for their catalytic activity, using oxime methacrylate as an acylating agent and DIPE as a solvent at 30 °C. The highest conversion (98.7%) of **2** to **5** was obtained with Lipase-PS, as against 92.1% and 89.9% conversion with CRL and PPL respectively. Therefore, it was decided to use Lipase-PS as a bio-catalyst for the detailed study of various reaction parameters.

Lipase catalysed reactions are very much influenced by the amount of lipase, the type of acyl donor, the solvents used in the reaction and the reaction temperature. To begin with, we examined the effects of the above factors on the transesterification of (−)-menthol with **3**.

3.1. Effect of amount of catalyst

For the bio-catalytic process to be competitive, when compared to the corresponding chemical



Scheme 1. Lipase catalysed transesterification of (±)-menthol with oxime methacrylate.

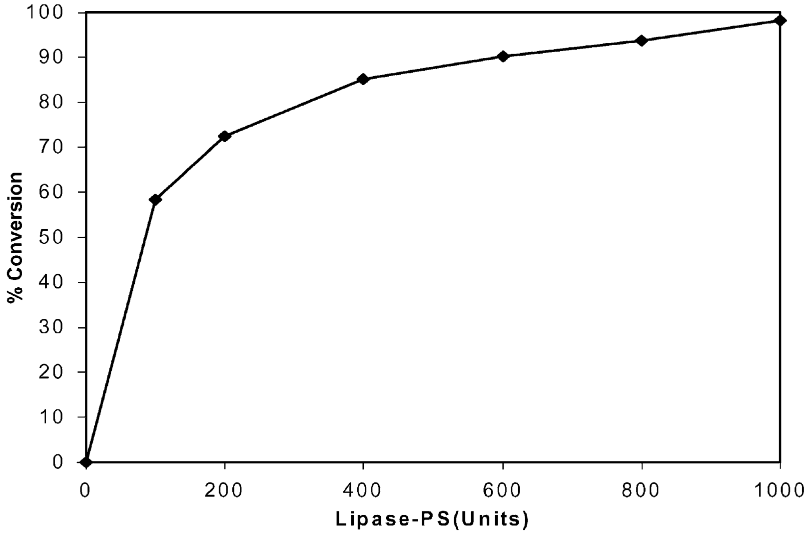


Fig. 1. Effect of amount of enzyme on the transesterification of (−)-menthol with oxime methacrylate. Reaction was performed at 30 °C in DIPE at a 1:1 mole ratio of (−)-menthol (0.001 mol) to oxime methacrylate (0.001 mol) for 24 h.

process, the amount of lipase used becomes an important economic factor. With this in mind we felt it worthwhile to study the effect of amount of lipase on the conversion of (−)-menthol (**2**) to (−)-menthyl methacrylate (**5**). The amount of enzyme used is a crucial economical factor for successful industrial applications. The percentage conversion of **2** to **5** increased from 58.4 to 98.1%, when the enzyme load was increased from 100 to 1000 units (Fig. 1). The increase was rather slow after 400 units. Thus, it is possible to decide the optimum enzyme amount required for making the reaction commercially viable.

3.2. Effect of solvent

The nature and polarity of organic solvents have profound effects on the enzymatic transformations. The polarity of the organic solvents can be quantitatively measured by the value of log P, the logarithm of the partition coefficient of a given solvent between water and 1-octanol. Organic solvents with log P values <2.0 are generally not considered good for biocatalysis [10]. In the present study, various organic solvents were examined for their suitability for lipase-catalysed transesterification of **2** (Table 1). The conversion rate

Table 1
Effect of solvents on the transesterification[a]

Solvent	log P value	(−)-menthyl methacrylate from (−)-menthol[b] conversion (%)	(−)-menthylmethacrylate from (±)-menthol[c] conversion(%)	ee%
DIPE	2.0	97.2	48.5	98.2
n-Hexane	3.5	93.2	47.9	97.5
Ether	0.85	94.7	48.0	97.8
Cyclohexane	3.2	73.4	35.7	96.4
Toluene	2.5	61.3	29.4	96.1
$CHCl_3$	2.0	18.9	8.2	68.5

[a] Reactions were performed at 30 °C for 24 h using 1000 units of Lipase-PS.

[b] At a 1:1 mole ratio of (−)-menthol (0.001 mol) to oxime methacrylate (0.001 mol).

[c] At a 2:1 mole ratio of (±)-menthol (0.001 mol) to oxime methacrylate (0.0005 mol).

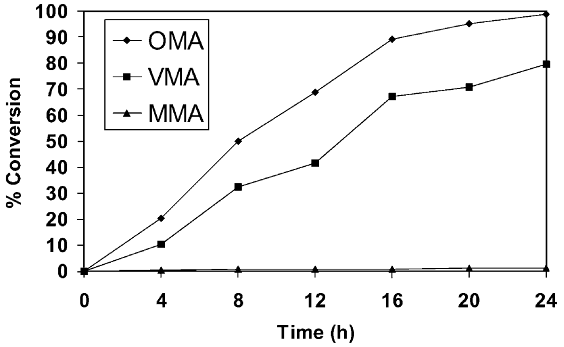


Fig. 2. Effect of acyl donors on the transesterification of (−)-menthol. Reaction was performed at 30 °C in DIPE at 1:1 mole ratio of (−)-menthol (0.001 mol) to (1) methyl methacrylate, (2) vinyl methacrylate and (3) oxime methacrylate using 1000 units of Lipase-PS.

of **2** to **5** was faster in DIPE among all the solvents studied. It was observed (Fig. 2, graph line corresponding to OMA) that initially the rate of conversion was faster (89.3% in 16 h), however, continuing the reaction further for 8 h showed only 9.4% increase in the conversion (total 98.7%).

After these initial reactions, in order to study the effect of solvents on conversion as well as enantioselectivity, we synthesised chiral menthyl methacrylate by stereoselective resolution of (±)-menthol (**1**) using Lipase-PS as a catalyst in various solvents (Table 1). In all the solvents used in the present study, the (-)-enantiomer of **1** reacted selectively to form **5**. Chloroform ($\log P = 2.0$) gave 8.2% conversion. Eventhough, the $\log P$ values of diethyl ether and DIPE were low (0.85 and 2.0 respectively), the percentage conversion was approximately 48% and enantiomeric excess (ee %) values were as high as 98%. Hexane as a reaction medium showed similar results as that of diethyl ether and DIPE. Cyclohexane and toluene gave low conversion but good ee % values. From the above results, it can be concluded that the $\log P$ value is not the only decisive parameter for the conversion and enantioselectivity.

3.3. *Effect of acyl donor*

Type of acyl donors plays an important role in the reaction kinetics when enzymes are used as catalyst. Three acyl donors viz. methyl methacrylate, vinyl methacrylate and oxime methacrylate have been used in the present work to study their effect on the conversion of (−)-menthol to (−)-menthyl methacrylate. All the reactions were carried out at 30 °C using DIPE as a solvent and 1000 units of Lipase-PS as a catalyst. Among the acyl donors used, the rate of conversion was the fastest in the case of oxime methacrylate and the slowest for methyl methacrylate (Fig. 2). This difference in the rate of conversion may be because of the following reasons. Since methanol is a poor-leaving group as compared to vinyl and oxime group, the rate of conversion was the slowest with methyl methacrylate. Further, it has been proved that in case of the vinyl esters as acyl donors, the acetaldehyde formed as a by-product during transesterification had detrimental effect on the enzyme activity, whereas, in the case of oxime esters, the oxime being a weak nucleophile does not take part in the reversible reaction, there by improving the conversion rate [8].

3.4. *Effect of temperature*

It is well known that the stereoselectivity of the enzymes is highly temperature sensitive and the experimental observations [11] support the same. Enzymes are known to show maximum activity at ambient conditions. Therefore, we chose to study the transesterification of (−)-menthol with oxime methacrylate at 25, 30 and 35 °C for 24 h. The rate constants, k for the conversion of (−)-menthol to (−)-menthyl methacrylate were determined. The results indicated that the reaction followed first order kinetics. It is clear from Table 2, that there was a 2.5-fold rise in the rate of conversion, when the reaction temperature was increased from 25 to 30 °C. However, further

Table 2
Rate constants for the transesterification of (−)-menthol to (−)-menthyl methacrylate at various temperatures[a,b]

Temperature (°C)	Rate constant (k) × 10^{-3} min^{-1}
25	1.45
30[b]	3.62
35	1.75

[a] Reactions were performed in DIPE at a 1:1 mole ratio of (−)-menthol (0.001 mol) to oxime methacrylate (0.001 mol.) using 1000 units of Lipase-PS for 24 h.

[b] $\Delta E = 14.12$ kJmol^{-1}; $\Delta G = 88.4$ kJmol^{-1}; $\Delta H = 11.6$ kJmol^{-1}; $\Delta S = -253.5$ kJmol^{-1}.

Table 3
Effect of temperature on the transesterification of (±)-menthol[a]

Temperature (°C)	ee (%)	Conversion (%) of (±)-menthol to (−)-menthyl methacrylate
20	99.3	33.8
30	98.4	48.1
40	95.9	43.5
50	93.4	40.1

[a] Reactions were performed in DIPE at a 2:1 mole ratio of (±)-menthol (0.001 mol) to oxime methacrylate (0.0005 mol) using 1000 units of Lipase-PS for 24 h.

increase in the reaction temperature to 35 °C showed 50% drop in the conversion rate as compared to that at 30 °C. This could be because of the deactivation of the lipase at 35 °C. Though, the above method gives good conversion (98% conversion at 30 °C), yet it may not be an economically viable process.

Keeping this in mind, we thought it worthwhile to study the effect of temperature on conversion as well as enantioselectivity in the transesterification of (±)-menthol with oxime methacrylate (Table 3). The conversion of **1** to **5** increased from 67.7 to 96.3% with increase in the temperature from 20 to 30 °C. However, increasing the temperature above 30 °C resulted in a sharp decrease in the conversion. The results also showed that there was a decrease in the stereoselectivity with the increase in the reaction temperature. This could be attributed to the deactivation of the lipase at higher temperature. Therefore, 30 °C is the most suitable reaction temperature for good conversion and enantioselectivity. The above results show that the synthesis of chiral menthyl methacrylate from (±)-menthol would be ideal to make the process commercially viable.

Based on the present study, the optimum reaction conditions for the synthesis of chiral menthyl methacrylate were found to be 1000 units of Lipase-PS in DIPE as a solvent at 30 °C.

4. Conclusions

The present work is a comprehensive study on the reaction parameters influencing the enzymatic synthesis of chiral menthyl methacrylate. The amount of bio-catalyst, nature of solvent as well as reaction temperatures were found to have profound effects on both, conversion as well as enantioselectivity. Oxime methacrylate was the best acylating agent and DIPE was an ideal solvent. The reaction conditions reported in the present study are mild and 'clean' as compared to the chemical method for the synthesis of chiral monomers. The newly synthesised (−)-menthyl methacrylate could then be polymerised for its potential use as a sustained release perfume. The optimised reaction conditions suggested in this work with suitable modifications can be extended for viable commercial production of monomeric esters of different flavors as well.

References

[1] Y. Okamoto, S. Honda, I. Okamoto, H. Yuki, J. Am. Chem. Soc. 103 (1981) 6971.
[2] H. Walborsky, F. Impastato, L. Barash, J. Am. Chem. Soc. 81 (1959) 1514.
[3] P. Benneville, L. Luskin, H. Sims, J. Org. Chem. 23 (1958) 1355.
[4] F. Merger, H. Hutmacher, W. Schwarz, G. Nestler, M. Szucsanyi, R. Mueller-Mall R, US Patent 4 777 265 (1988).
[5] C. Wong, Y. Wang, S. Chen, K. Liu, Tetrahedron Lett. 15 (1989) 1917.
[6] B. Maillard, M. Degueil-Castaing, B. Jeso, S. Drouillard, Tetrahedron Lett. 28 (1987) 953.
[7] P. Cesti, E. Battistel, D. Bianchi, J. Org. Chem. 53 (1988) 5531.
[8] G. Sudesh Kumar, A. Ghogare, J. Chem. Soc., Chem. Commun. 20 (1989) 1533.
[9] V. Athawale, N. Manjrekar, J. Mol. Catal. B: Enzymatic 10 (2000) 551.
[10] D. Elkins, L. Hansch, Chem. Rev. 71 (1971) 525.
[11] R. Phillips, Enzyme Microb. Technol. 14 (1992) 417.