



Contents lists available at ScienceDirect

Tetrahedron: Asymmetry

journal homepage: www.elsevier.com/locate/tetasy

Enantioselective enzymatic resolution of racemic alcohols by lipases in green organic solvents

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ARTICLE INFO

Article history:

Received 18 January 2017

Revised 27 January 2017

Accepted 8 February 2017

Available online xxxx

ABSTRACT

The effects of two eco-friendly solvents, 2-methyltetrahydrofuran (MeTHF) and cyclopentyl methyl ether (CPME), on the enzyme activity and enantioselectivity of Novozym 435, *Candida rugosa* lipase (CRL), Porcine pancreas lipase (PPL), Lipase AK, Lipase PS, and Lipozyme, a series of commercial lipases, in the enantioselective transesterifications of racemic menthol, racemic sulcatol and racemic α -cyclogeraniol were studied. Vinyl acetate was chosen as the acyl donor and the reactions were carried out at water activity 0.06. The activity of lipases in CPME was similar to that observed in other largely employed organic solvents [toluene and *tert*-butyl methyl ether (MTBE)], and was slightly lower in MeTHF. However, for most of the lipases tested, the enantioselectivity was higher in the eco-friendly solvents. Lipase AK exhibited a high enantioselectivity ($E = 232$) for the resolution of racemic menthol but the reaction rate was low. Lipase formulation (the enzyme was frozen and lyophilized in potassium phosphate buffer without and with 5% (w/v) of sucrose, D-mannitol, or methoxy poly(ethylene glycol)) was tested with this lipase in order to improve its activity, which increased up to 4.5 times, compared to the untreated enzyme. CALB was found to be a useful biocatalyst for the resolution of racemic sulcatol, where high activity and enantioselectivity were obtained ($E \geq 1000$). For the resolution of the racemic primary alcohol α -cyclogeraniol, most of the lipases tested were active but not enantioselective, except lipase PS which displayed a moderate enantioselectivity ($E = 19$). The effect of the presence of a low percentage of two ionic liquids (ILs) 1-Butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][TFSI]) (5% (v/v)) and 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]) (1% (v/v)) in the medium was also investigated. Only in the case of CRL the ILs slightly increased the enantioselectivity from $E = 91$ to $E = 103$ and $E = 120$ for [BMIM][TFSI] and [BMIM][BF₄], respectively. However, in all cases ILs caused a decrease of enzyme activity.

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1. Introduction

Among industrial enzymes, lipases are one of the most important for the preparations of enantiomerically pure compounds.^{1,2} They have a remarkable ability to catalyze a variety of reactions in organic solvents such as esterification, transesterification, and aminolysis with high activity, chemo-, regio-, and enantioselectivity for various synthetic substrates.^{3–5} Moreover, they can operate in mild and simple reaction conditions. The use of organic solvents as reaction media offer many advantages such as the possibility to solubilize water-insoluble substrates, to facilitate the recovery of reaction products removing the biocatalysts by simple filtration, and the possibility to increase the enzyme thermal stability.^{6,7} Furthermore, organic solvents for lipase catalysis can markedly

affect enzyme enantioselectivity, thus making the selection of solvent of crucial importance.^{6,8}

A prerequisite for the application of biocatalysts on the large scale is the sustainability and low environmental impact, reducing the detrimental effects of the developed industrial process and of the chemicals used on the environment. From this perspective, the use of solvents derived from renewable resources (bio-solvents) and environmentally friendly (green) solvents is a necessity.^{9,10} Recently, solvents such as 2-methyltetrahydrofuran (MeTHF), a biomass-derived solvent, and cyclopentyl methyl ether (CPME) have been reported as promising media for biocatalysis reactions due to their favorable characteristics. MeTHF (boiling point 80 °C) and CPME (boiling point 106 °C) may replace the commonly used THF with the advantage of a reduced solvent evaporation during the reaction. Moreover, because of the limited miscibility in water (4.1% and 1.1% w/w for MeTHF and CPME, respectively, at 23 °C), these solvents can be used in two-phase

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reaction systems, which favors product recovery.^{11–14} Furthermore, MeTHF is considered a green solvent because (i) it can be produced from renewable sources such as furfural or levulinic acid, in accordance with the 7th principle of Green Chemistry, and (ii) it is environmentally degraded in air and by sunlight.¹⁵ Antonucci et al. reported the toxicological response of MeTHF in rats, resulting in a permitted daily exposure in humans of 6.2 mg/day, making it an appropriate green solvent for pharmaceutical and chemical purposes.¹⁶ Compared to the classical ether solvents CPME has proven to be quite useful as a process solvent in numerous organic synthesis because it is free from drawbacks like low boiling point, easy peroxide (formation, and solubility in water, thus resulting in inefficient recovery.¹⁷

In spite of the interesting features of these environmentally friendly solvents, to the best of our knowledge, there is a lack of systematic studies on their influence on the enantioselectivity of some frequently used commercial lipases. In the present study, we investigated the enzymatic kinetic resolution of some industrially interesting racemic alcohols in the above mentioned green solvents, carried out with some widely used lipases, comparing the outcome with that previously obtained using the most commonly used organic solvents.

In particular, we studied three model substrates that possess different structural features regarding the alcohol functional group: (i) a cyclic and sterically hindered secondary alcohol; (ii) a linear secondary alcohol and (iii) a primary alcohol possessing a sterically hindered stereocentre in the α -position. More specifically we singled out the cyclic secondary alcohol (\pm)-menthol, the linear secondary alcohol (\pm)-sulcatol and the primary alcohol (\pm)- α -cyclogeraniol (Fig. 1).

(–)-Menthol is one of the most important flavor compounds and it is used extensively as a food additive. Racemic menthol is a cheap commodity produced by Haarman and Raimer process but the desired organoleptic properties are related only to (1*R*,3*R*,4*S*) isomer. Therefore a number of resolution processes, including one based on lipase-mediated esterification, have been developed.¹⁸ In spite of this, a study on the use of environmentally friendly solvents in such a process is still lacking.

Sulcatol has been used as model compound for a number of studies on enzyme-catalysis¹⁹ and its selection as a substrate for the present research can afford experimental data that can be compared with those previously obtained.

α -Cyclogeraniol (namely (2,6,6-trimethylcyclohex-2-enyl)methanol) is a relevant chiral building block that has been employed for the synthesis of carotenoids and carotenoid-deriving natural products.^{20,21} To date, only one enzymatic resolution procedure for the preparation of enantioenriched α -cyclogeraniol has been described.²² We have already investigated the lipase-mediated resolution of primary alcohols possessing a stereocentre in the α -position.^{23,24} Since the esterification of the latter substrates is highly dependent both on the enzyme used and on the experimental conditions, we decided to investigate the influence of the solvent on the enantioselectivity of the α -cyclogeraniol resolution.

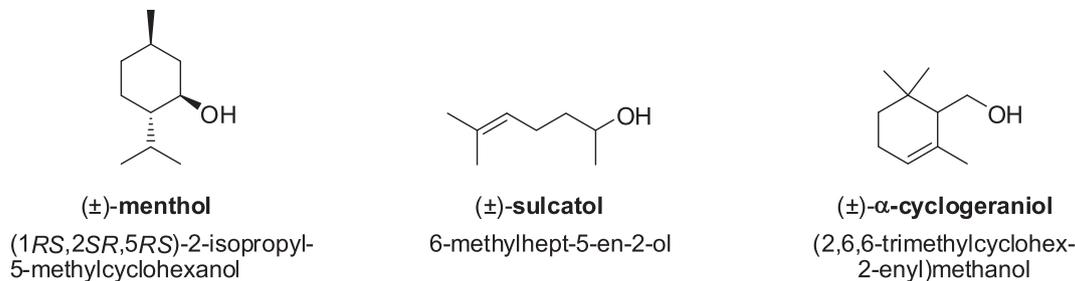


Figure 1. The three model substrates investigated. Menthol, sulcatol and α -cyclogeraniol.

Moreover, with the intent to verify if commonly adopted strategies useful to improve enzyme activity in organic solvents can also be exploited with these eco-friendly solvents,^{25,26} the role of the enzyme formulation^{27,28} and the addition of ionic liquids (ILs)^{29–32} were also evaluated. Thanks to a low vapor pressure, ILs have attracted significant attention as “green solvents” in biocatalysis.^{33,34} In fact, the use of lipases in ionic liquids as solvent or (co)-solvent has presented many advantages, such as high conversion rates, high enantioselectivity and better enzyme stability.³⁵ Moreover, ionic liquids exhibit excellent physical characteristics (melting point, polarity, and miscibility with water or organic solvents) including the ability to dissolve polar and nonpolar organic, inorganic, and polymeric compounds.³⁶

2. Results and discussion

2.1. Rates of lipase catalyzed transesterification of racemic alcohols in organic solvents

In a first screening, lipases were tested in the transesterification of racemic alcohols to see their efficiency with the two green solvents (MeTHF and CPME) against two other conventional solvents (toluene and MTBE) for comparison. The conversion of racemic alcohols was calculated after 24 h as shown in Table 1. It was observed that the conversion degree is dependent on the lipase and on the organic solvent.

Among all the lipases screened for transesterification with (\pm)-menthol, CRL was found to be the best in terms of conversion, where high values were observed in shorter times; however, the enantioselectivity was low. Lipase AK exhibited the best enantioselectivity but with low activity and reaction rate. CALB, lipase PS, lipozyme, and PPL were inactive, with no product being detected even after 72 h reaction. On the other hand, CRL and lipase AK were more active in CPME, toluene, and MTBE compared to MeTHF. CALB, CRL, and lipase AK showed a higher reaction rate with (\pm)-sulcatol leading to a higher conversion. Lipozyme and lipase PS showed moderate activity and PPL very low activity. For the resolution of the primary alcohol all lipases (except PPL) were active in all solvents tested, and the highest reaction rate was displayed by CRL.

2.2. Enantioselectivity of lipase catalyzed transesterification of racemic alcohols in organic solvents

The use of organic solvents as media for reactions catalyzed by lipases is a well-established procedure. Moreover, it is known that by modifying solvent composition it is possible to modulate lipases activity and enantioselectivity.^{6,27,28} However, no data have been reported on the activity and enantioselectivity of lipases in MeTHF and CPME with respect to other organic solvents. For a comparison, in this study we selected toluene and MTBE, two solvents that are extensively used, for the resolution of racemic menthol, sulcatol,

Table 1

Substrate conversion, initial rate and enantiomeric ratio (*E*) of lipase catalyzed transesterification reaction with (±)-menthol, (±)-sulcatol or (±)-α-cyclogeraniol as substrate and MeTHF, CPME, toluene or MTBE as reaction media^a

Substrate	Lipase	<i>c</i> (%) ^b ; initial rate (nmol h ⁻¹ mg ⁻¹); <i>E</i> ^c			
		MeTHF	CPME	Toluene	MTBE
(±)-menthol	CRL ^d	35; 36; 91	47; 144; 119	41; 146; 58	53; 158; 101
	Lipase AK ^e	5; 2; 121	9; 3; 232	8; 2; 103	7; 4; 106
	CALB ^f	0.4	0.5	0.4	0.7
	Lipase PS ^e	0.5	0.9	1.4	1.1
	Lipozyme ^e	1.3	1.7	1.2	1.4
	PPL ^d	0.1	0.2	0.3	0.2
(±)-sulcatol	CRL ^d	51; 160; 4	84; 350; 5	99; 340; 6	92; 344; 8
	Lipase AK ^e	38; 40; 28	51; 40; 35	53; 55; 21	56; 40; 26
	CALB ^f	66; 1690; 222	70; 1490; 422	83; 1800; 58	79; 1700; >1000
	Lipase PS ^e	11; 7; 37	26; 20; 25	31; 19; 46	22; 16; 12
	Lipozyme ^e	13; 10; 49	20; 15; 58	20; 12; 43	22; 16; 61
	PPL ^d	6; 1.6; 25	12; 2; 15	7; 1.8; 21	12; 3; 19
(±)-α-cyclogeraniol	CRL ^d	96; 161; 1	97; 496; 1	99; 632; 1	86; 484; 1
	Lipase AK ^e	29; 6; 6	27; 10; 11	25; 10; 9	16; 10; 6
	CALB ^f	32; 14; 8	46; 50; 12	38; 90; 6	29; 34; 7
	Lipase PS ^e	18; 4; 11	23; 10; 18	19; 5; 7	16; 10; 19
	Lipozyme ^e	7; 1.5; 1	9; 5; 2	8; 5; 1	78; 5; 1
	PPL ^d	1; 0.4; 1	2.5; 0.6; 2	8; 0.8; 1	4; 1.2; 2

Lipase amount was (d) 50 or (e) 20 or (f) 10 mg.

^a Conversion, initial rate and *E* are in normal, italic and bold character, respectively.

^b Conversion values after 24 h of reactions.

^c All the lipases tested transformed preferentially (–)-menthol, (*R*)-α-cyclogeraniol and (*R*)-sulcatol, except CRL that had enantioselectivity for (*S*)-sulcatol. *E* was calculated at 20% conversion according to the formula (1) and (2) using the enantiomeric excess of product in the case of racemic menthol and sulcatol and the enantiomeric excess of substrate in the case of the primary alcohol.

and α-cyclogeraniol at low water activity, using lipases as biocatalysts.

The data of Table 1 show that although CRL exhibited with (±)-menthol the highest activity in MTBE, a relatively good activity was observed in CPME, which was similar to that found in toluene, while a moderate activity was obtained in MeTHF. However, the enantioselectivity of CRL in both CPME and MeTHF, was similar to that observed in MTBE, but higher than that in toluene. Lipase AK showed the highest enantioselectivity in the kinetic resolution of (±)-menthol in CPME (*E* = 232). However, this enzyme had a lower activity than CRL in all the solvents tested.

It can also be seen from Table 1 that with (±)-sulcatol in MeTHF and CPME the lipases tested had similar activity and enantioselectivity to that observed with toluene and MTBE. Interestingly, in the two green solvents CALB had an enantioselectivity significantly higher than that observed in toluene.

The enantioselectivity of most lipases with (±)-α-cyclogeraniol was low in all solvents except moderate values in the case of lipase PS in CPME and MTBE *E* = 18 and *E* = 19, respectively (Table 1). However, these lipases seem very active with this primary alcohol where high conversions were obtained except with lipozyme and PPL.

2.3. Effects of lipase formulation and ionic liquids on the performance of lipases

Numerous studies have shown that lipase behavior may be greatly altered by the formulation.^{28,37,38} Therefore we adopted this strategy to increase the transesterification activity of lipases in CPME. In particular, in the present study it has been shown that Lipase AK has high enantioselectivity in the kinetic resolution of (±)-menthol, that is a prerequisite for the scaling up of this kinetic resolution. Nevertheless, the low catalytic activity of the enzyme might be a drawback. Therefore, in order to improve the performance of the catalyst, the influence of the enzyme formulation on the reaction rate and enantioselectivity was evaluated. Table 2

indicates that enzyme dissolution in buffer at pH 8, followed by lyophilization, increased 3.6 times the initial rates of the transesterification reaction in CPME, compared to the non-treated commercial enzyme. The increase was slightly higher (up to 4.5-fold) when lipase AK was lyophilized in the presence of MeOPEG or sucrose or D-mannitol. The increase of activity could be due both to the “pH-memory” effect,^{7,39} being the enzyme lyophilized from a solution at the optimal pH for activity⁴⁰ and to the lyoprotectant effect of the additives, particularly MeOPEG, as previously reported for lipases.³⁷ Beyond these effects, the fact that with the additives the enzyme molecules are more dispersed in the lyophilized sample than in the commercial untreated powder, also contributes to the increase of catalytic activity. Thus, besides formulation with additives, from the perspective of further improving the catalytic activity of lipase AK in organic solvents, enzyme immobilization on suitable supports might be a valid approach.⁴¹ However, lyophilization in the presence of this latter additive caused a plunge of the enantioselectivity of lipase AK. A decrease of enantioselectivity was also observed with lipase AK lyophilized with the sugars sucrose and D-mannitol, even though high values (102 and 100) were still obtained.

In the last decade, numerous studies have focused on the use of ionic liquids (ILs) as green media for lipases. In addition, ILs have also been suggested as additives, in biocatalyzed reactions, for improving the performance of enzymes.^{30,42–44} Herein, the effect of the presence of a low percentage of ionic liquid in the green solvents was also investigated. In particular, we selected the two ionic liquids [BMIM][TFSI] and [BMIM][BF₄]. Analogously to organic solvents, these ILs do not dissolve lipases. Therefore, their use does not interfere with some of the advantages of using organic solvents (e.g., easy recovery of the products and increase of the enzyme thermal stability).^{45,46} The use of [BMIM][TFSI] (1%) and [BMIM][BF₄] (5%) caused a decrease of the initial reaction rates for CRL and lipase AK in transesterification of (±)-menthol (Table 3). However, the enantioselectivity of CRL was slightly improved in the MeTHF with [BMIM][TFSI] and [BMIM][BF₄], and in CPME with [BMIM][BF₄]. Instead, Lipase AK showed a decrease of

Table 2
Transesterification activity of lipase AK lyophilized with different additives in transesterification of (\pm)-menthol

Catalyst	CPME			
	ee_p (%)	C (%)	E^a	ν (nmol h ⁻¹ mg ⁻¹)
Lipase AK ^{b,e}	98.9	23.0	232	3
Lipase AK lyophilized without additives ^{b,f}	93.3	44.1	64	11
Lipase AK + Saccharose ^{c,f}	95.1	46.3	102	13
Lipase AK + MeOPEG ^{c,f}	87.2	43.3	29	13
Lipase AK + D-Mannitol ^{d,f}	97.0	31.1	100	7
Lipase AK + MeOPEG ^{d,f}	91.0	29.6	31	7

Lipase amount was (^{b,c}) 20 or (^d) 10 mg.

Lipase/additive ratio (w/w) was (^c) 4/1 or (^d) 2/1.

Reactions time was (^c) 120 or (^f) 72 h.

^a In all cases the enantioselectivity was for (–)-menthol.

Table 3
Effect of ionic liquids on the activity and enantioselectivity of lipases in the transesterification of (\pm)-menthol

Lipase	Medium	Solvent							
		MeTHF				CPME			
		ee_p (%)	c (%)	E^a	ν (nmol h ⁻¹ mg ⁻¹)	ee_p (%)	c (%)	E^a	ν (nmol h ⁻¹ mg ⁻¹)
CRL ^b	Without Ionic Liquid	97.3	19.2	91	36	97.1	36.5	119	144
	[BMIM][TFSI] (1%)	97.6	17.8	103	42	97.0	34.0	108	134
	[BMIM][BF ₄] (5%)	98.1	11.9	120	34	97.7	27.3	121	76
Lipase AK ^c	Without Ionic Liquid	98.0	16.7	121	2	98.9	23.0	232	3
	[BMIM][TFSI] (1%)	97.4	11.9	87	1.5	99.1	16.6	254	2
	[BMIM][BF ₄] (5%)	95.3	10.1	46	1.5	98.3	16.0	138	1.5

Reactions time was (^b) 4 or (^c) 120 h.

^a In all cases the enantioselectivity was for (–)-menthol.

enantioselectivity in the presence of ionic liquids except in CPME with [BMIM][TFSI]. These results differ from those reported by other research groups with other lipases. In fact, Itoh et al.^{47,48} found that the addition of small amounts of different ILs in organic solvents, caused a remarkable enhancement of enantioselectivity of lipase PS-catalyzed acetylation of 1-phenylethanol, using vinyl acetate as an acyl donor. Filice et al.⁴⁹ showed that *Rhizomucor miehei* lipase activity was enhanced, maintaining a high regioselectivity, by addition of ILs in the hydrolysis of hexa-O-acetyl lactal.

3. Conclusions

Herein, the eco-friendly solvents MeTHF and CPME were used for the first time as reaction media for the lipase-catalyzed kinetic resolution of (\pm)-menthol, (\pm)-sulcatol and (\pm)- α -cyclogeraniol. Lipase AK was found to be the best catalyst for the resolution of racemic menthol in terms of enantioselectivity. The reaction rate, which was low, could be improved in CPME, at least 2.5-fold by lipase formulation. CALB should be the best choice for the resolution of racemic sulcatol in MeTHF and CPME, thanks to its excellent enantioselectivity and reaction rate. However, it has to be emphasized that although the increase of activity obtained is valid only for the exact formulations utilized, at an industrial level the goal is to develop biocatalytic processes always with formulated and immobilized enzymes that can facilitate enzyme reuse and improve their properties (activity, stability, selectivity). In fact, just for lipases, the catalytic activity depends on the conformation of a α -helix fragment, named “lid”, which regulates the entrance to the catalytic active site (e.g., moving from a “close” to an “open” position). Thus, the formulation and immobilization method are crucial to favor and maintain the open position, which has been correlated to the activation of lipases.⁵⁰

In the resolution of the primary alcohol, the lipases tested showed a low or, at most, moderate enantioselectivity, as in the case of lipase PS in CPME and MTBE. A very high reaction rate was observed with CRL. The presence of a low percentage of ILs in the reaction medium increased slightly the enantioselectivity but decreased the reaction rate. The results highlight that both CPME and MeTHF can be considered as excellent and greener substitutes of toluene and MTBE. We also observed that CPME gave better results compared to MeTHF, both in terms of enzyme catalytic activity and of enantioselectivity. It should be mentioned that, as already shown, the enantioselectivity of a given enzyme can change, sometimes dramatically, as a function of the nature of the solvents and of the substrates.⁸ The results obtained in this study strongly encourage the use of green solvents as media for the kinetic resolution of secondary alcohols, especially in the perspective to develop sustainable large scale industrial processes.

4. Experimental

4.1. Materials and reagents

Novozym 435 (immobilized lipase from *Candida antarctica* B) was donated by Novozymes, CRL (lipase from *Candida rugosa*) and PPL (lipase from porcine pancreas) were purchased from Sigma, Lipase AK (lipase from *Pseudomonas fluorescens*), Lipase PS (lipase from *Burkholderia cepacia*), Lipozyme IM from Novo. (\pm)-Sulcatol (purity > 99%), (\pm)-menthol, vinyl acetate (>99%), vinyl laurate, toluene, *tert*-butyl methyl ether (MTBE), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][TFSI], $\geq 98\%$), and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄], $\geq 98.5\%$) were purchased from Sigma–Aldrich. Cyclopentyl methyl ether (>99%) and 2-methyltetrahydrofuran (99%) were purchased from Alfa Aesar. Racemic α -cyclogeraniol ((2,6,6-

trimethylcyclohex-2-enyl)methanol) was prepared by LiAlH_4 reduction of ethyl α -cyclogeraniate.²³ (–)-menthol, (S)-sulcatol were purchased from Sigma–Aldrich; (–)-(S)- α -cyclogeraniol $\{[\alpha]_D^{20} = -106.5$ (c 2.1, EtOH), 95% ee) was prepared by resolution of the racemic alcohol.²² Other chemicals were from commercial sources and were of the highest purity available.

4.2. Lipase catalyzed transesterification of racemic alcohols

For all reactions tested, all reagents, solvents, and lipases, before use, were separately equilibrated for at least 24 h at water activity (a_w) value of 0.06 in sealed vessels in the presence of LiBr saturated salt solution at 25 °C.⁵¹ The reaction mixture was prepared by adding 1 mL of organic solvent (MeTHF, CPME, toluene, or MTBE) containing racemic alcohols (\pm)-menthol (32 mM), (\pm)-sulcatol (35 mM) or (\pm)- α -cyclogeraniol (32 mM) and vinyl acetate (325 mM) to 10–50 mg of lipases in a 3 mL vial. Then the reaction mixture was shaken at 150 rpm at 25 °C and at scheduled times a sample from the supernatant was withdrawn and analyzed by GC. When the effect of ionic liquids was tested, the reaction was carried out by adding to the organic solvent 5% or 1% (v:v) of [BMIM][TFSI] or [BMIM][BF₄], respectively. Initial reaction rates ($\text{nmol h}^{-1} \text{mg}^{-1}$) were calculated only from conversion values lower than 15%. In this study, the amount of lipases refers to crude powder unless otherwise specified.

Absolute configuration of the transformed enantiomers was assigned by comparison of the chiral chromatographic analyses of the various lipase catalyzed reaction with literature data that reported studies with the same substrate and lipase.^{8,22,52,53}

4.3. Formulation of lipase AK

Lipase AK formulations were prepared freezing (at –80 °C) and lyophilizing enzyme solutions obtained by dissolving 20 mg of the commercial enzyme powder in 1 mL of 20 mM potassium phosphate buffer (pH 8) or the same buffer containing 5% (w/v) of additive (sucrose, D-mannitol, or methoxy poly(ethylene glycol)).

4.4. Chiral GC analysis

The enantiomeric purity and conversion were determined by a gas chromatograph (Agilent Technologies 6850) equipped with a hydrogen flame ionization detector (FID) and a chiral capillary column DMEPentilBETACDX (25 m \times 0.25 mm \times 0.15 μm) for GC analysis of (\pm)-sulcatol and a MEGA-DEX DAC Beta column (25 m \times 0.25 mm \times 0.25 μm) for analysis of (\pm)-menthol and (\pm)- α -cyclogeraniol. The temperature of the injector and the detector was 250 °C. Nitrogen was used as carrier gas. The temperature programs were: 80°(2 min)-2°/min-110°-15°/min-150°(2 min) for separating sulcatol enantiomers and 80°(2 min)-5°/min-150°(2 min) for separating menthol enantiomers and α -cyclogeraniol enantiomers. The following retention times were (in min): (S)-sulcatol 5.44; (R)-sulcatol 5.79; (S)-sulcatyl acetate 6.68; (R)-sulcatyl acetate 8.33; (+)-menthyl acetate 8.63; (–)-menthyl acetate 8.83; (–)-menthol 9.27; (+)-menthol 9.62; (R)- α -cyclogeraniol 7.26; (S)- α -cyclogeraniol 7.37; (R) and (S)- α -cyclogeraniol acetate 9.21.

The enantioselectivity value (E) was calculated from the conversion (c) and the enantiomeric excess of the product (ee_p) or of the remaining substrate (ee_s), based on the following equations:

$$E = \frac{\ln[1 - c(1 + ee_p)]}{\ln[1 - c(1 - ee_p)]} \quad (1)$$

$$E = \frac{\ln[(1 - c)(1 - ee_s)]}{\ln[(1 - c)(1 + ee_s)]} \quad (2)$$

Where

$$c = 1 - \frac{A + B}{A_0 + B_0} \quad (3)$$

$$ee_s = \frac{B - A}{A + B} \quad (4)$$

$$ee_p = \frac{P - Q}{P + Q} \quad (5)$$

where A_0 and B_0 represent the initial enantiomers concentration of substrate, A and B represent the substrate enantiomers concentrations after a certain time of reaction, P and Q represent the product enantiomers concentrations after a certain time of reaction,⁵⁴ in a reaction where A is the faster reacting enantiomer that produces P .

Acknowledgements

Francesco Secundo acknowledges the funding of COST Action CM1206 EXIL “Exchange on Ionic Liquids”, for supporting participation at meetings on ionic liquids and allowing discussion of the results presented in this work.

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