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The influence of organic solvent and ionic liquids on the selective formation of 2-(2-ethylhexyl)-3-phenyl-1,2-oxaziridine mediated by lipases†

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This paper describes the influence of the addition of ionic liquids (ILs) based on [BMIm][X], where [X] = SCN, Cl, BF_4, and PF_6, on the chemo-enzymatic oxidation of *N*-benzyliden-2-ethylhexylamine to form the corresponding *E*- and *Z*-isomers of oxaziridines mediated by *Pseudomonas sp.* (PSL) and *Candida antarctica* (CAL-B) lipases in various organic solvents at room temperature (25 °C) with urea hydrogen peroxide (UHP). The results showed that the use of different organic solvents in the presence of ILs, critically changes the conversions (5–99%) and the isomeric ratio *E*:*Z*, (50–100% *E*-isomer) of the products formed. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: ionic liquids; lipases; organic solvents; oxaziridines

INTRODUCTION

Oxaziridines are useful compounds, which have received considerable attention, for example, as aprotic oxidizing reagents,[1–4] and these compounds may be used as chiral auxiliaries in the epoxidation of terpenes.[5] Several methods have been employed for the oxidation of imines to the corresponding oxaziridines. The best-known of these methods is the MCPA (*m*-chloroperbenzoic acid oxidation method,[6] and other methods described in the literature include oxidation with oxone, urea hydrogen peroxide (UHP), or using a trichoroloacetonitrile system.[7–9] However, most of these methods produce by-products, which complicates the product purification. These oxidants are, in general, very expensive and relatively toxic for common use in organic synthesis.[10,11]

The production of *N*-alkyloxaziridines via a chemo-enzymatic method using lipases has been carried out with a series of Schiff Bases. This method is particularly interesting because the products can be prepared under mild conditions (room temperature and neutral pH) in lower reaction time, and they can be easily purified.[12] In this case, the peroxyacid is generated *in situ* by lipases and carboxylic acids, in a first step and then the peroxyacid[13,14] is used in the oxidation of C = N bonds of the imines to obtain the corresponding *N*-alkyloxaziridines.[12] The same method can be applied to alkene epoxidation,[13–16] sulfide oxidation to form sulfoxides,[17] and Baeyer–Villiger oxidation.[18] The use of lipases in this process means that the enzyme can be reused many times, decreasing the costs of the process, and they do not require the use of co-factors and special handling.[12–16]

Recently, ionic liquids (ILs) have received increasing attention as solvents due to their properties and applications in biocatalytic processes.[19,22] The link between ILs and green chemistry is clearly related to the properties of these solvents, however, aspects related to their degradability and aquatic toxicity are still not clear.[23] Studies are currently being carried out to investigate these properties.[24–26] ILs in enzymatic reactions can act as a reservoir for the substrate and products, and in many cases this decreases the substrate and product inhibition by water.[21,22,27] The choice of organic solvents in enzymatic reactions is very important, because enzymes are very sensitive to the medium employed, due to their selectivity and activity which may be affected by medium polarity.[28,29] Therefore, many recent publications have described the use of lipases in the presence of ILs as co-solvents, including their properties in biocatalytic processes.[30–34]

Herein, two commercial lipases – from *Pseudomonas sp.* (PSL) and *Candida antarctica* (CAL-B) were employed in the chemo-enzymatic oxidation of *N*-benzyliden-2-ethylhexylamine (**1**) with octanoic acid as the acyl donor, to obtain the corresponding *E*- and *Z*-isomers of oxaziridines (**2**) and (**3**). The conversion and selectivity of the products were analyzed as a function of the reaction medium employed, such as the use of pure organic solvents or in mixtures with ILs from a series of 1-butyl-3-methyl imidazolium-[BMIm][X], where X = BF_4, PF_6, SCN, and Cl (Scheme 1).

EXPERIMENTAL

All chemicals are commercially available and were used without purification. All solvents were analytical grade and were dried

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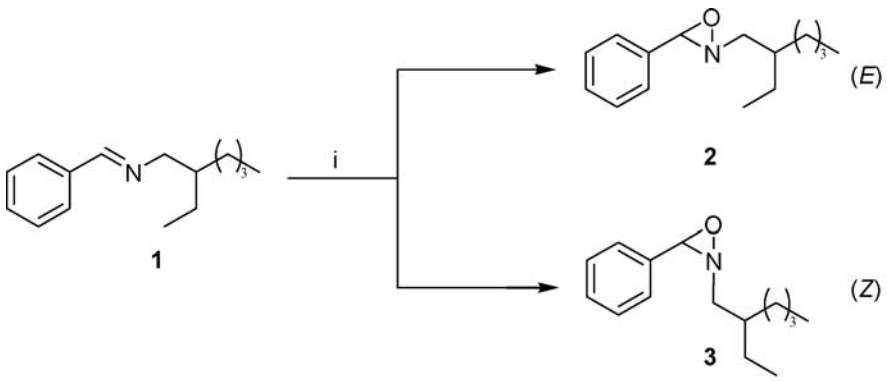

Scheme 1. Chemo-enzymatic formation of *N*-alkyloxaziridines **2** e **3** mediated by lipases

by storing over activated 3 A° molecular sieves before use. The chemo-enzymatic reaction for the preparation of 2-(2-ethylhexyl)-3-phenyl-1,2-oxaziridines **2** and **3** was carried out using 0.4 mmol of *N*-benzyliden-2-ethylhexylamine **1** (prepared and characterized as previously described),[12] 1.0 mmol of the acyl donor (octanoic acid-Aldrich 98%), 25 mg of the two lipases (CAL-B lipase – 10 000 PLU/g and PSL lipase – 30 000 U/g) and 0.4 mmol of UHP(18% – Acros) at room temperature, 150 rpm in 10 ml of different organic solvents or 1 ml of different ILs from a series of imidazolium-based ILs [BMIm][X] (Acros 98%), and 9 ml to organic solvents. The crude product mixture of **2** and **3** was separate and purified using a small column of SiO_2. The solvent was evaporated under reduced pressure at 40 °C to give the pure product separately as light yellow oil (*E*-isomer) and colorless oil (*Z*-isomer).

The reaction progress and the isomeric ratio were measured by GC on a Shimadzu GC14B instrument equipped with a Shimadzu CBP-5 column using H_2 as the carrier gas, with the flame ionization detector set at 280 °C, an injector set at 270 °C, and a column temperature range of 70–250 °C (10 °C/min). The results are expressed as a medium of two values and the estimated errors were <5%.

The characterization of the products was performed by spectroscopic analysis on a Perkin-Elmer FTIR 1600 (range 4000–400 cm^{-1}). ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, with a Varian 400 Mercury Plus spectrometer, using $CDCl_3$ as solvent and TMS as the internal standard.

The spectral data are: **2**-(*E*)-isomer: yellow oil: ^{1}H NMR ($CDCl_3$): δ_H 7.41–7.37 (5H, m, Ph), 4.47 (1H, s), 2.86–2.75 (2H, m), 1.71 (1H, m), 1.36–1.26 (8H, m), 0.89 (3H, s), 0.87 (3H, s); ^{13}C NMR ($CDCl_3$): δ_C 130,2, 130,1, 128,7, 128,5, 128,0, 127,8, 80,9, 65,6, 39,5, 34,3, 31,8, 29,0, 25,0, 14,3, 11,0. IR (ZnSe window): cm^{-1} 2924, 1633, 1549, 1459, 1370, 1270, 731.

3-(*Z*)-isomer: colorless oil: ^{1}H NMR ($CDCl_3$): δ_H 7.41–7.37 (5H, m, Ph), 5.25 (1H, s), 2.84–2.73 (2H, m), 1.70 (1H, m), 1.39–1.26 (8H, m), 0.89 (3H, s), 0.87 (3H, s); ^{13}C NMR ($CDCl_3$): δ_C 133.1, 130.2, 128.7, 128.0, 127.8, 127.5, 79.9, 63.4, 42.8, 39.5, 34.2, 24.4, 22.8, 14.1, 11.0. IR (ZnSe window): cm^{-1} 2962, 1653,1423, 1375, 1297, 756.

RESULTS AND DISCUSSION

Firstly, the influence of the ILs 1-butyl-3-methylimidazolium tetrafluorborate [BMIm][BF_4] and 1-butyl-3-methylimidazolium hexafluorphosphate [BMIm][PF_6] in mixtures with dichloromethane (DCM) was evaluated using CAL-B as the biocatalyst. The use of ILs is an alternative tool which can sometimes increase or decrease the conversion and the selectivity of products.[21,22,35] Herein, the amount of ILs was progressively increased from 0 to 90% v/v, and the results are given in Fig. 1. In a previous study using pure DCM,[12] a moderate conversion of 60% into the product was achieved in 3 h of reaction forming isomers **2** and **3** with an *E*/*Z* ratio of 65:35. The conversions were measured by gas chromatography (GC) and the values are expressed as the sum of the *E*-(**2**) and *Z*-(**3**)-isomers in the mixture.

The results showed a dependence on the anion nature and content of the IL in the conversion into **2** and **3**. With the use of DCM:[BMIm][PF_6], the highest values were reached with an organic solvent:IL mixtures of 90/10 and 80/20 v/v, these being 72 and 76%, respectively. With the use of the organic solvent:IL mixture of 70/30 v/v, the conversion was 62%, this being similar to that obtained with pure organic solvent. However, a decrease in the degree of conversion to 27% was observed using an organic solvent:IL mixture of 10/90 v/v.

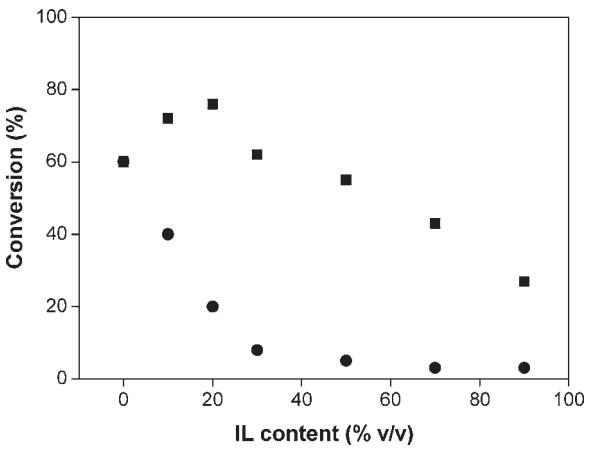

Figure 1. Effects of the content of ionic liquids as co-solvents on the chemo-enzymatic oxidation of *N*-benzyliden-2-ethylhexylamine (**1**), (■) reaction in [BMIm][PF_6], and (●) [BMIm][BF_4]. Reaction conditions: (**1**) (0.4 mmol), octanoic acid (1 mmol), UHP (0.4 mmol), in dichloromethane (reaction media 10 ml), CAL-B (25 mg), r.t., 3 h and 150 rpm

A possible reason for this result could be that the [BMIm][PF_6] might interact with charged groups of the enzyme, either at the active site or at its periphery, causing changes in the enzyme structure. In addition, a high concentration of IL causes not only high ionic strength of the reaction medium, which might inactivate the enzyme, but also a high viscosity of the reaction mixture, which limits the diffusion of substrates and products from the active site of the enzyme, resulting in a drop in enzymatic reaction rate.[19,20,35–39]

The study on the influence of [BMIm][BF_4] content showed a decrease in the conversion degrees of *N*-alkyloxaziridines with an increase in the IL content. With the use of IL contents of 30 and 50%, the conversion degrees were only 8 and 5%, respectively. However, using higher IL contents, the conversions were even lower (3%). These results probably reflect the nature of [BMIm][BF_4], which is particularly hydrophilic, and they are in agreement with the findings of Zhao *et al*. who observed that ILs based on BF_4 did not dissociate into ions in the absence of water, and thus a considerable decrease in the enzyme activity and in the conversion into the product was observed.[40] With the use of both ILs the *E*:*Z* ratio was 65:35.

Considering the above results, an organic solvent:[BMIm][PF_6] mixture of 90/10 v/v was selected for the subsequent experiments.

The conversion into oxaziridines **2** and **3** as a function of time was then investigated with PSL and CAL-B using acetonitrile (ACN) or DCM as organic solvents and the IL [BMIm][PF_6]. The values for the total conversion into **2** and **3**, using both lipases, are given in Fig. 2.

Using PSL, the total degree of conversion into products was 18% in pure ACN and 45% when an ACN:IL mixture of 10% v/v was used. A small increase was observed with the use of DCM, and the conversion degrees increased from 25 to 30% using pure organic solvent or in a mixture with the IL, both in 12 h of reaction.

With the use of pure organic solvent or in mixture with the ILs, the selectivity of the products formed was approximately 65:35 for the isomers *E*:*Z*.

A similar behavior was observed when CAL-B was used as the catalyst, where the conversion degrees showed an increase with the addition of 10% v/v [BMIm][PF_6]. With the use of this lipase, the conversion values were of 81 and 97% using ACN pure or in mixture with [BMIm][PF_6] in 3 h of reaction. Using DCM pure or in mixture with IL, the conversion values were of 60 and 83%, respectively, also in 3 h of reaction.

A maximum conversion of >99% was reached in pure ACN or in mixture with [BMIm][PF_6], in 12 h of reaction. With the use of pure DCM or DCM:IL mixture, the conversion values were of 63 and 91%, respectively (Fig. 2 B).

The selectivity of the products formed was the same as that obtained using PSL (*E*:*Z* ratio of 65:35).

These data showed that, in the presence of PSL or CAL-B as biocatalysts and with the use of 10% v/v [BMIm][PF_6], an increase in the conversion degrees of oxaziridines **2** and **3** was obtained both in mixtures with ACN or DCM. However, no improvement in the selectivity of the *E*:*Z* ratio was observed.

Considering that organic solvents strongly influence the reaction rate and selectivity, especially in relation to the oxaziridine formation,[12] five organic solvents (*n*-hexane log *P* 3.5, DCM log *P* 0.93, ethyl ether log *P* 0.85, ethanol log *P* −0.24, and ACN log *P* 0.33[41]) were selected to evaluate the influence of different anions in a series of imidazolium-based ILs ([BMIm][X])

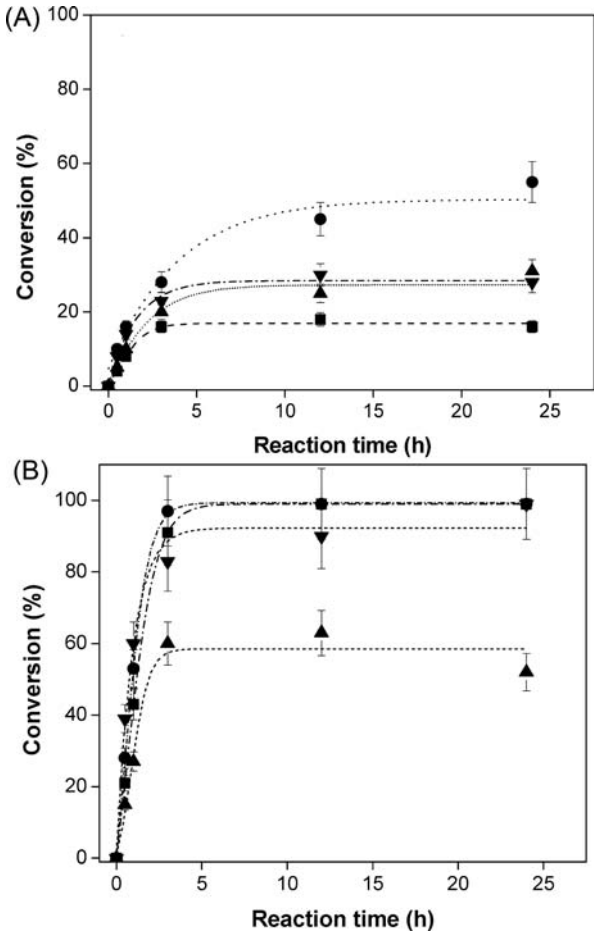

Figure 2. [BMIm][PF_6] effects on the chemo-enzymatic formation of *N*-alkyloxaziridines **2** and **3** using PSL (A) and CAL-B (B) as the biocatalyst as a function of time carried out in DCM/IL 10% v/v (▼), pure DCM (▲), ACN/IL 10% v/v (●) and pure ACN (■). Reaction conditions as described in Fig. 1

where X = BF_4, PF_6, SCN, and Cl. The degree of conversion into the mixture of **2** and **3** were determined by GC in 12 h reaction. The results obtained as a function of the type of pure organic solvent or in mixtures with imidazolium IL are described in Fig. 3. The organic solvents were ordered according log *P* values.[41]

The results presented in Fig. 3 are in agreement with recent publications in the literature.[19,20,42–49] It is well described that ILs, in many cases, can change the tridimensional structure of the enzymes due to polar interactions through the presence of different anions. In particularly, in this study, the degrees of conversion into the oxaziridines **2** and **3** ranged from 5 to 99%, depending on the organic solvent and the nature of the IL anion.

Using ethyl ether in a mixture with ILs, the conversion degrees increased as compared with the use of the pure organic solvent (conversion of 26%). Using mixtures of the different ILs the conversion degrees were around 91% in 12 h of reaction, except for [SCN] (17%). Surprisingly, the selectivity was dependent on the nature of the IL anion. Using the imidazolium ILs with [SCN], [Cl], [BF_4], and [PF_6] as anions, the selectivity values were 100, 80, 60 and 50% in relation to the *E*-isomer, respectively.

When *n*-hexane was used pure or in mixtures with ILs, the opposite effect was observed as compared with ethyl ether, that is, the conversion degrees decreased in the presence of the ILs.

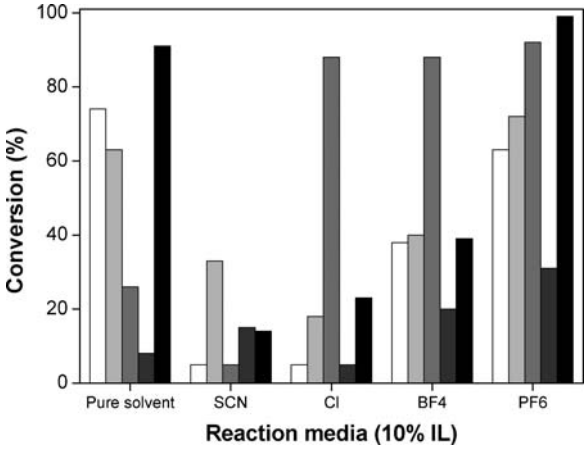

Figure 3. Effect of ionic liquids on the chemo-enzymatic formation of 2-(2-ethylhexyl)-3-phenyl-1,2-oxaziridines using CAL-B as the biocatalyst. Reactions carried out in *n*-hexane (□), dichloromethane (▨), ethyl ether (▩), ethanol (■), and acetonitrile (■) pure and or in mixtures with [BMIm][SCN], [BMIm][Cl], [BMIm][BF_4], [BMIm][PF_6] 9:1 v/v in 12 h of reaction

Using pure *n*-hexane the conversion was 73% and the selectivity towards the *E*-isomer was 65%. Using [BMIm][PF_6] in a mixture with this solvent, the conversion was 64% in 12 h of reaction. Using *n*-hexane in a mixture with [BMIm][PF_6] and [BMIm][BF_4], the selectivity was 80%, and in a mixture with [BMIm][Cl] and [BMIm][SCN] it was 100%, in relation to the *E*-isomer.

A similar behavior was observed using ACN or DCM in a mixture with [BMIm][PF_6] with conversion degrees of >99 and 70%, respectively. The selectivity was the same, that is, 70% towards the *E*-isomer.

Using ethanol as a solvent in mixtures with ILs, a moderate effect was observed. The conversion degrees were 15, 5, 20, and 30% using [BMIm][SCN], [BMIm][Cl], [BMIm][BF_4], and [BMIm][PF_6], respectively, in 12 h of reaction. These values were higher than that obtained using pure ethanol (8% of conversion in 2 h reaction). However, the selectivity was of 100% towards the *E*-isomer, being much better than that reached with pure ethanol (70%).

These results clearly demonstrated that the use of different organic solvents in mixtures with ILs is a useful alternative to increase the conversion into products and also selectivity towards the formation of the *E*-isomer. However, no correlation between the conversion degrees and log *P* values was observed, probably to the fact that is a two-step reaction.[12]

Another advantage of this method is the use of mild conditions (room temperature and neutral pH) and the absence of strong oxidants such as Oxone or peracetic acid.

CONCLUSIONS

We investigated the chemo-enzymatic system for oxidation of *N*-benzyliden-2-ethylhexylamine mediated by CAL-B and PSL using five different organic solvents in mixtures with imidazolium ILs as the co-solvents. The conversion degrees and *E*:*Z* ratios were strongly dependent on the organic solvents and the IL type and content. No oxidation product was detected in the control experiments, that is, in the absence of lipases.

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