Chemoenzymatic epoxidation of citronellol catalyzed by lipases
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A B S T R A C T
The chemoenzymatic epoxidation of a terpene alcohol, citronellol, is reported. Some experimental conditions, such as the use of lipases from different sources, oxidizing agents (H₂O₂ or urea–hydrogen peroxide, UHP), reaction time, acyl donor type (C₆–C₁₆), temperature (15–40 °C) and the influence of organic media, were evaluated. In most cases, citronellol oxide 2 or the ester citronellol oxide 3 were obtained. Depending on the reaction conditions, high yields of products 2 or 3 were obtained (>99%). CAL-B was the most effective catalyst in this reaction. For epoxide 2, the highest yields of 80% and 77% were obtained at 20 °C and 25 °C, respectively, using UHP as an oxidizing agent and octanoic acid as an acyl donor. The organic medium appears to be one of the most important parameters in the reaction. Using chloroform or dichloromethane, product 2 was obtained at a >99% yield after 24 h. When different mixtures consisting of varied organic solvents and an imidazolium-based ionic liquid (IL) were used, the results were dependent on both the solvent and IL counter-ion (18–75%).

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1. Introduction
Citronellol, also known as dihydrogeraniol, is a natural acyclic monoterpenoid. It is a constituent of the essential oils of plants, such as (+)-citronellol present in citronella oils [1] and (−)-citronellol in rose and geranium (Pelargonium sp.) oils [2]. Monoterpenes can be classified into two major groups: the monoterpenic hydrocarbons and oxygenated monoterpenes. The latter group includes alcohols, aldehydes, ketones, ethers and carboxylic acids [2,3].

The oxidation of terpenes has important industrial applications because their epoxides are used as starting materials for the synthesis of commercially important fragrances and flavoring materials [4]. Epoxides can undergo various chemical reactions with a variety of nucleophiles and are readily converted into diols, amino-alcohols and ethers. An important recent application is the use of chiral epoxides as intermediates for the production of chiral pharmaceuticals [5].

Among the many processes available for the preparation of epoxides, one of the most commonly used methods is the formation of peroxo acids (also called peracids or peroxide acids). These are prepared from the corresponding anhydride or acid by using hydrogen peroxide. In this reaction, an extra oxygen atom is added between the carbonyl group and its hydrogen, thereby transforming these compounds into acid half-esters of hydrogen peroxide [6,7].

One the most powerful oxidizing peroxo acids is peroxo trifluoroacetic acid, but the most commonly used peroxo acid is meta-chloro perbenzoic acid (m-CPBA) [6]. In the early 1990s, Björkling et al. described the use of enzymes in the perhydrolysis of carboxylic acids in the presence of hydrogen peroxide. Hydrogen peroxide (H₂O₂) reacts with the acyl-enzyme complex, formed by the fatty acid and the hydroxyl group of the amino acid serine in the active site, to produce the corresponding percarboxylic acid [8].

In addition, the use of hydrogen peroxide in complex with urea, termed urea–hydrogen peroxide (UHP), has produced good results. UHP is a dry form of hydrogen peroxide, which has the potential to release hydrogen peroxide in a controlled manner; this avoids the presence of water in the reaction while minimizing undesirable reactions of the oxidized products [9].

The use of enzymes in the chemoenzymatic epoxidation of double bonds has been widely used. The simplicity of the process, the efficiency at normal temperature and pressure as well as the reusability of the enzymes has been shown to be of significant advantage [10]. The use of immobilized enzymes, e.g., lipase from Candida antarctica B immobilized on a macroporous support (CAL-B or Novozyme 435), in the chemoenzymatic epoxidation of olefins has been recently reported [10–14]. This lipase has been highlighted because of its outstanding efficiency in the catalysis of the perhydrolysis of octanoic acid, displaying good stability and the possibility of reuse [15]. Warwel and Klass [16] have successfully reused CAL-B 15 up to times without significant loss of activity in the self-epoxidation of oleic acid by slowly adding 60% hydrogen peroxide to the reaction system. Subsequently, the first use of CAL-B for the self-epoxidation of oleic acid with 30% hydrogen peroxide was reported; the fresh enzyme displayed an approximately
50% loss in activity [17]. Skouridou et al. used this lipase to catalyze the formation of peroxy octanoic acid from the corresponding carboxylic acid and hydrogen peroxide in toluene. The peroxy carboxylic acid formed was used in situ for the oxidation of α-pinene to the corresponding epoxide. During the process, varied amounts of hydrogen peroxide were tested over five reaction cycles. The activity of the lipase decreased by 60–90% after five cycles, depending on the amount of hydrogen peroxide used [18]. This lipase was also used for chemoenzymatic epoxidation of the methyl esters of sunflower oil in a biphasic system of CH₂Cl₂/H₂O with 30% (v/v) aqueous hydrogen peroxide. In some cases, the epoxide yields were higher than 99% [19].

In this paper, the chemoenzymatic epoxidation of citronellol under different conditions is reported. Several experimental parameters were evaluated in this study to optimize the process, including the use of lipases from different sources, reaction times, oxidizing agents, different organic solvents, acyl donor chain lengths and temperatures (Scheme 1).

2. Experimental

2.1. Materials

Citronellol 95% was purchased from Acros Organics. Acetonitrile (99.5%), chloroform (99.8%), ethanol (99.5%) and t-butanol (99%) were obtained from Vetec; dichloromethane (99.5%), methanol (99.5%), n-hexane (98.5%) were obtained from Synth; t-butyl methyl ether (MTBE, 99.9%) was obtained from Tedia; ethyl ether (98%) was obtained from Chemis; and ethylacetate (99.8%) was obtained from Grupo Química. Ionic Liquids (ILs) 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄] (97%), 1-butyl-3-methyl-imidazolium chloride ([BMIM][Cl] (99%), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF₆](96%) and 1-butyl-3-methylimidazolium thiocyanate ([BMIM][SCN] (97%) were purchased from Fluka. Octanoic (C₈; 99.5%), dodecanolic (C₁₂; 98%) and hexadecanoic (C₁₆; 98%) acids were obtained from Vetec. Hexanoic (C₆; 99.5%), 2-bromopentanoic (99%), 2-bromohexanoic (97%), 2-bromohexadecanoic (97%) and 2-ethyhexanoic (99%) acids were obtained from Sigma–Aldrich, and dodecanol (C₁₂; 98%) and tetradecanoic (C₁₄; 98%) acids were obtained from Fluka. Urea–hydrogen peroxide (UHP; percentage given as 30% in H₂O₂) was supplied by Sigma–Aldrich, and 30% aqueous hydrogen peroxide (percentage given as wt% H₂O₂ in water) was obtained from Vetec. Lipases from C. antarctica B (CAL-B, Novozym 435, 10,000 PU/g) immobilized on a polycarbonate resin; Rhizomucor miehei (Lipzyme RM IM, 5–6BAU/g) and Mucor miehei (Lipzyme IM, 5–6BAU/g) were donated by Novozymes. Lipases from Burkholderia cepacia (PS-C Amano I, 1638 U/g; PS Amano SD, 30,000 U/g; PS Amano, 30,000 U/g; PS Amano IM, 500 U/g; PS-C Amano II, 1000 U/g; Rhizopus oryzae (RZ 15, 150 U/g)); Candida rugosa (AY Amano 30, 30,000 U/g); Pseudomonas fluorescens (AK, 25,000 U/g); Aspergillus niger (A Amano 12, 120,000 U/g) and Mucor javanicus (M Amano 10, 10,000 U/g) were donated by Amano Pharmaceuticals.

2.2. General procedure for the chemoenzymatic epoxidation of citronellol

In a typical reaction, 0.36 mL (2 mmol) of citronellol, 0.24 mL (2 mmol) of octanoic acid, 470 mg (5 mmol) of urea–hydrogen peroxide (UHP) and 2001 U of lipase were added to 10 mL of acetonitrile in a 125 mL Erlenmeyer flask. The reaction was agitated on a rotatory shaker (Cerontal MO, 150 rpm) at 25°C for 24 h. Aliquots were withdrawn at specified intervals, and the lipase and UHP were then filtered. The UHP was separated from the lipase by successive washes with water. Finally, the biocatalyst was washed five times with the organic solvent. Acetonitrile was concentrated on a rotary evaporator under reduced pressure (2 Torr) at 50°C to obtain the crude reaction products in the form of colorless oil. The reactions were performed in triplicate. Product formation was monitored by 1H nuclear magnetic resonance on a Varian EM360L spectrometer (400 MHz) using CDC₃ as the solvent. The conversion into citronellol oxide 2 was determined through a comparison of the relative area of the triplet centered at 2.70 ppm, which is related to the hydrogen of the oxirane ring, with that of the triplet at 5.06 ppm, which is characteristic of the hydrogen of the double bond of citronellol. In most studies, the 1H NMR spectra also presented a triplet centered at 4.13 ppm; this peak was assigned to the methylene hydrogens of the ester citronellol oxide 3. The reaction mixture obtained was purified using a small silica column and eluted with a mixture of hexane:ethylacetate (90:10, v/v). The product was isolated as pure UHP at an 85% yield. These values were compared with those reported in the literature [20,21].

Citronellol oxide 2. 1H NMR (CDCl₃): δ 3.70 (m, 2H), 2.70 (t, 1H), 2.02 (s, 1H), 1.50 (m, 2H), 1.40 (m, 3H), 1.30 (s, 3H), 1.27 (s, 3H), 0.98 (d, 3H). The reaction samples were also analyzed by gas chromatography (Aglient Technology 7820 A) using a flame ionization detector. The separation was performed on a polarity column (Shimadzu CBP-5M25, 25 m) with a column temperature program from 80 to 250°C (10°C/min). The injector and detector were set at 280°C and 290°C, respectively. The flow rate of the carrier hydrogen gas was 7 mL/min, resulting in an analysis time of 15 min. The retention time was 5.14 min and 13.95 min for epoxy 2 and ester citronellol oxide 3, respectively. Yields were calculated using the peak areas.

In studies related to the use of different lipases, the conditions of reaction time, oxidant agent, acyl donor, temperature and solvent effects were specified for each experiment. Control experiments were also conducted without lipases or UHP under similar reaction conditions; no product was obtained.

3. Results and discussion

As the aim of this report is the preparation of high yields of citronellol oxide 2 or the ester, citronellol oxide 3, by chemoenzymatic epoxidation of citronellol, several experimental parameters were evaluated.

3.1. Screening of enzymes

In the first approach, 12 different commercially available enzymes were screened for their efficiency in the chemoenzymatic epoxidation of citronellol, using the reaction conditions described in this study (Section 2.2). Octanoic acid was selected as the acyl donor and acetonitrile as the organic solvent because these were successfully used in the chemoenzymatic epoxidation of (+)-3-carene [10].

Citronellol oxides 2 or 3 were not detected when lipases M Amano 10, PS Amano IM, Lipoyzme IM and Amano 12 were used. With lipases PS-C Amano 1, F-AP15 and AY Amano 30, only product 3 was detected with a low yield of less than 5%. Using lipases PS Amano SD, PS Amano, AK, PS-C Amano II and Lipoyzme RM IM, products 2 and 3 were both formed, but yields were <10%. The highest conversions to 2 and 3 were achieved in reactions catalyzed by CAL-B and Amano 1, which gave 70% of product 2 and 30% of product 3 and 26% of product 2 and 3% of product 3, respectively, after 24 h of reaction. Considering the above results, CAL-B was selected for the following studies.

3.2. Influence of reaction time

The rates of formation of products 2 and 3 were then evaluated. Fig. 1 shows the 1H NMR spectra, in the 2.4–5.3 ppm region, for citronellol samples withdrawn and analyzed after 2, 4, 6, 8 and 10 h of reaction. After 2 h of reaction, 21% conversion of epoxide 2 was obtained. After 10 h of reaction, the peak at 5.5 ppm, which corresponds to the double bond of the alcohol, was no longer observed. Instead, a triplet centered at 4.13 ppm was detected and attributed to the methylene hydrogens corresponding to the octyl ester of citronellol oxide 3. This compound may be a by-product of the reaction of citronellol oxide 2 and octanoic acid, which is regenerated from peroxy octanoic acid in the presence of lipase (Scheme 1). As previously mentioned, products 2 and 3 were obtained at 70 and 30% yields, respectively, after 24 h of reaction (results not shown in Fig. 1). These data show that depending on the reaction time, both products can be obtained. Because the main focus was to obtain the highest yields of product 2 or 3, the reaction time of 24 h was selected for subsequent experiments.

3.3. Effects of type and amount of oxidizing agent

The type and amount of oxidizing agent has been reported to influence the formation of peroxy acid in the first step of the reaction [9,15–17]. To better understand the influence of the agent with respect to urea–hydrogen peroxide (UHP), different amounts (0–10 mmol) of UHP were used in the chemoenzymatic epoxidation of citronellol and evaluated after 24 h of reaction. Fig. 2 shows that an increase in the amount of UHP from 0.5 to 5 mmol resulted in an increase in the conversion to products 2 (10–76%) and 3 (14–33%).
Interestingly, when 0.5 mmol of UHP was used, octyl citronellate was also obtained in addition to products 2 and 3. These results were not surprising because UHP is necessary for the formation of peroxo acid, which is used in the epoxidation reaction. In the absence of UHP, octyl citronellate was obtained as sole product of the reaction.

At concentrations of UHP greater than 5 mmol, no significant changes in the yields were observed for either product. Similar experiments using 30% \( \text{H}_2\text{O}_2 \) (v/v) as oxidizing agent were conducted under the same reaction conditions. The results were similar to those obtained with UHP; after 24 h, both products 2 and 3 were obtained in yields of 69% and 30%, respectively. Under the same experimental conditions, products 2 or 3 were not detected in the absence of UHP or 30% \( \text{H}_2\text{O}_2 \). Studies suggest that the use of UHP presents some advantages over aqueous \( \text{H}_2\text{O}_2 \) [9]; therefore, 5 mmol of UHP was selected for use in subsequent experiments.

### 3.4. Influence of the amount and type of acyl donor

The effect of acyl donor chain length (C6–C16) on the epoxidation of citronellol with UHP was then investigated. In the first step of reactions catalyzed by lipases, a tetrahedral intermediate acyl-enzyme was formed. Thus, the primary factor affecting the affinity between acid and enzyme is the chain length of the fatty acids. Using the induced-fit model for enzyme action, Faber explained this in terms of the binding energy, which is released when a substrate binds to an active site [22]. The shape and physico-chemical properties of the scissile fatty acid binding sites are also important in understanding the molecular basis of substrate specificity [23].

Fig. 3 shows that by using linear alkyl carboxylic acids, the epoxide 2 yield increased from 41 to 71% as the length of the alkyl chain of the carboxylic acid increased. The highest yield (71%) was achieved when octanoic acid was the acyl donor. Similar results have been reported for the chemoenzymatic epoxidation of

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**Scheme 1.** Chemoenzymatic epoxidation of citronellol.

**Fig. 1.** Influence of time on the chemoenzymatic epoxidation of citronellol. Reaction conditions: citronellol (2 mmol), octanoic acid (2 mmol), UHP (5 mmol), CAL-B (200 U), acetonitrile (10 mL), room temperature, 150 rpm.

**Fig. 2.** Influence of UHP on the chemoenzymatic epoxidation of citronellol for the formation of epoxide (■) and epoxide-ester (●). Reaction conditions as in Fig. 1 except for UHP (0.1–10 mmol), 24 h of reaction.
3-carene and α-pinene and the synthesis of N-alkyloxaziridines mediated by CAL-B [10,18,24]. Consequently, the effects of different amounts of octanoic acid (in mmol) were evaluated (Fig. 4).

Fig. 4 shows that yields of products 2 and 3 depend on the amount of octanoic acid used; product 2 yields increase with increasing amounts of octanoic acid (up to 0.5 mmol) and then remains almost constant in the range of 70–74%. Using 0.10 or 0.25 mmol of octanoic acid, the yields of products 2 and 3 were 8 or 0% and 8 or 17%, respectively. These results highlight the importance of using a specific amount of the acyl donor to produce the corresponding peroxo acid for use in chemoenzymatic epoxidation. These data are also consistent with previous reports on the synthesis of α-pinene oxide [18].

Next, 2-bromoalkyl acids and 2-ethylhexanoic acid were evaluated with respect to their ability to produce the corresponding peroxo acids. When 2-bromopentanoic acid, 2-bromohexanoic acid and 2-bromohexadecanoic acid were used, only a modest conversion (20–34%) to the ester citronellyl was observed, regardless of the alkyl chain length. Products 2 and/or 3 were not detected, thereby indicating no formation of peroxo acids in the presence of these acids. The reaction with 2-ethylhexanoic acid produced no detectable product.

Fig. 4. Influence of the amount of octanoic acid on the chemoenzymatic epoxidation of citronellol. Epoxide (■) and ester–epoxide (●). Reaction conditions as described in Fig. 1 but varying the amount of octanoic acid (0.1–2 mmol), 24 h of reaction.

3.5. Influence of temperature

Another key factor that affects the rate of a reaction catalyzed by an enzyme is the temperature. Temperature can influence the activity, selectivity and stability of the biocatalyst as well as the reaction equilibrium [26].

In order to evaluate this effect, the influence of temperature on the chemoenzymatic epoxidation of citronellol was studied in the 15–40 °C range (Fig. 5). In this temperature range, both products 2 and 3 were detected. The highest yields of 80% and 77% for epoxide 2 were obtained at 20 °C and 25 °C, respectively. In the 30–40 °C range, yields of product 2 decreased (71–63%), and yields of product 3 increased (23–37%). It is probable that at higher temperatures, some UHP decomposition occurs and, thus, the formation of the corresponding peroxo acid also decreases.

3.6. Effects of the organic medium

Next, the effect of the organic medium was evaluated. Enzyme activity is strongly affected by the choice of organic solvent [27–29]. log P (logarithm of the partition coefficient of the solvent for the standard octanol/water two-phase system) is the most useful parameter to classify the solvents for bio-catalytic reactions. In general, the use of solvents with a log P > 4.00 (non-polar) gives better reaction rates. Solvents with a log P between 2.00 and 4.00 are moderately effective as polar solvents, whereas those with a log P < 2.00 are often ineffective [27].

Recently, the use of lipases in ionic liquids (ILs) has demonstrated many advantages, such as high yields, high enantioselectivity, better enzyme stability, and better recyclability. ILs in enzymatic reactions can act as reservoirs for the substrate and products; in many cases, this decreases the substrate and product inhibition by water. ILs can also form strong hydrogen bonds, which stabilize enzyme–substrate interactions [30–32].

Thus, solvents with different polarities, such as hexane (log P = 3.50), chloroform (log P = 2.00), t-butanol (log P = 1.45), t-buty1 methyl ether-MTBE (log P = 1.43), dichloromethane (log P = 0.93), ethyl ether (log P = 0.85), ethylacetate (log P = 0.68), ethanol (log P = −0.24), acetonitrile (log P = −0.33) and methanol
Table 1
The effect of organic medium on the chemoenzymatic epoxidation of citronellol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>log P</th>
<th>Conversion [%]</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>8 h</td>
</tr>
<tr>
<td>1</td>
<td>Hexane</td>
<td>3.50</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>2.00</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>t-Butanol</td>
<td>1.45</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>t-Butyl methyl ether</td>
<td>1.43</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Dichloromethane</td>
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<td>68</td>
</tr>
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<td>6</td>
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<td>23</td>
</tr>
<tr>
<td>7</td>
<td>Ethylacetate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.68</td>
<td>19</td>
</tr>
<tr>
<td>8</td>
<td>Ethyl ether</td>
<td>0.85</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Ethanol</td>
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<td>4</td>
</tr>
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<td>10</td>
<td>Acetonitrile</td>
<td>-0.32</td>
<td>85</td>
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<tr>
<td>11</td>
<td>Methanol</td>
<td>-0.76</td>
<td>0</td>
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<tr>
<td>12</td>
<td>Hexane/[BMIm][BF&lt;sub&gt;4&lt;/sub&gt;]&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>5</td>
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<tr>
<td>13</td>
<td>Hexane/[BMIm][Cl]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>Hexane/[BMIm][PF&lt;sub&gt;6&lt;/sub&gt;]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>Chloroform/[BMIm][PF&lt;sub&gt;6&lt;/sub&gt;]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>0</td>
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<tr>
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<td>MTBE/[BMIm][Cl]&lt;sup&gt;b&lt;/sup&gt;</td>
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</tr>
<tr>
<td>17</td>
<td>MTBE/[BMIm][PF&lt;sub&gt;6&lt;/sub&gt;]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>31</td>
</tr>
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</table>

<sup>a</sup> Reaction conditions as in Fig. 1, but varying the organic media.

<sup>b</sup> Determined by 1H NMR (400 MHz, CDCl<sub>3</sub>).

<sup>c</sup> From Ref. [27].

<sup>d</sup> Without acyl donor.

<sup>e</sup> Solvent/[ILs] (9:1, v/v).

(log P = −0.76), were screened. Mixtures of n-hexane, t-butyl methyl ether or chloroform:ionic liquids (9:1, v/v) from a series of 1-butyl-3-methyl imidazolium-[BMIm][X], where X = BF<sub>4</sub><sup>−</sup>, PF<sub>6</sub><sup>−</sup>, SCN<sup>−</sup>, or Cl<sup>−</sup>, were also used to evaluate the effect of the organic medium on the reaction.

The degree of conversion into epoxide 2 or epoxy-ester 3 was dependent on the polarity of the organic solvent and reaction time (Table 1). Using n-hexane or chloroform, products 2 and 3 were obtained at yields of 19 or 38% and 45 or 19%, respectively, after 8 h of reaction time (Table 1, entries 1 and 2). Using t-butanol, MTBE, ethyl ether or methanol produced no detectable end product under these reaction conditions, regardless of reaction time (Table 1, entries 3, 4, 8 and 11). Using ethylacetate, with or without the acyl donor, and ethanol resulted in the formation of only epoxide 2 in yields of 23, 19, and 4%, respectively (Table 1, entries 6, 7 and 9). Dichloromethane or acetonitrile also produced the single product, epoxide 2, in yields of 68 and 85%, respectively, after 8 h of reaction time. However, using acetonitrile for 24 h of reaction time resulted in the formation of both products 2 and 3, with 75 and 25% degrees of conversion, respectively (Table 1, entries 5 and 10). Interestingly, when dichloromethane or chloroform was used for 24 h of reaction time, almost quantitative (>99%) amounts of epoxide 2 were obtained, suggesting the high selectivity of the process.

However, these data show no linear relationship between the degree of conversion and solvent polarity (expressed as log P values). Similar results were recently reported for the regio-selective acylation of D-ribonol-1,4-lactone, catalyzed by lipases [33].

The results obtained using mixtures of organic solvent and ILs were quite interesting, and in most cases, the results were also dependent on both the organic solvent and the ionic liquid counter anion.

In the presence of ionic liquids, citronellol was not totally consumed. When mixtures of chloroform/[BMIm][X] were used, where X = BF<sub>4</sub><sup>−</sup>, SCN<sup>−</sup>, or Cl<sup>−</sup> (9:1, v/v), no conversion to products 2 or 3 was observed (results not shown). However, using the more hydrophobic ionic liquid [BMIm][PF<sub>6</sub>], epoxide 2 and epoxy-ester 3 were obtained in moderate yields of 33 and 24%, respectively, after 24 h of reaction time (Table 1, entry 15). In this case, a negative effect was observed for these ILs in comparison with the use of pure organic solvents, where citronellol oxide 2 was selectively obtained with a 99% degree of conversion (Table 1, entry 2).

Using mixtures of MTBE/[BMIm][X], where X = BF<sub>4</sub><sup>−</sup> or SCN<sup>−</sup> (9:1, v/v), no detectable amounts of products 2 or 3 were formed. These results are similar to those obtained with the pure organic solvent (results not shown in Table 1). However, with the use of MTBE/[BMIm][X], where X = Cl<sup>−</sup> or PF<sub>6</sub><sup>−</sup> (9:1, v/v), and depending on the reaction time, both products showed moderate degrees of conversion (31–53%) (Table 1, entries 16 and 17). Using these mixtures, the presence of ILs had a positive effect on the overall reaction. Interestingly, these conditions favored the formation of product 3 over product 2.

Using mixtures of n-hexane/[BMIm][X], where X = BF<sub>4</sub><sup>−</sup>, Cl<sup>−</sup>, or PF<sub>6</sub><sup>−</sup> (9:1, v/v), the results were, in some cases, better than those obtained in the presence of a pure organic solvent (Table 1, entry 1). When a mixture of n-hexane/[BMIm][SCN] was used, no detectable amounts of products 2 or 3 were formed, showing a strong and negative influence of this IL on the chemoenzymatic epoxidation of citronellol (results not shown). Using the mixture of n-hexane/[BMIm][BF<sub>4</sub>], products 2 and 3 were detected in moderate amounts, depending on the reaction time. After 24 h of reaction, the degrees of conversion were 31 and 24%, respectively. Better results were achieved when mixtures of n-hexane/[BMIm][Cl] and n-hexane/[BMIm][PF<sub>6</sub>] were used, suggesting the influence of the anionic nature of the ILs (Table 1, entries 13 and 14). Using these mixtures, the epoxy-ester 3 was formed in higher yields (69 and 75%, respectively) than those obtained using a pure organic solvent (42%). This ionic liquid is considered more apolar and, thus, more suitable for use in enzymatic catalysis [30,31]. Recently, the epoxidation of methyl oleate supported by imidazolium-based ionic liquids was described; the best results were also achieved when [BMIm][PF<sub>6</sub>] and/or [BMIm][NTf<sub>2</sub>] were used [14]. In other work, the beneficial influence of coordinating hydrogen bond-donating ionic liquids on epoxidation and on Baeyer–Villiger oxidation was described [34].

4. Conclusions

The chemoenzymatic epoxidation of citronellol was performed under mild conditions. CAL-B was the most effective catalyst, and the reaction was dependent on reaction time, oxidant agent, temperature and acyl donor; products 2 and/or 3 were produced in moderate to good amounts. The electronic and steric effects of different acyl donors were also evaluated. The organic medium had the greatest influence on the reaction. Using chloroform or dichloromethane, >99% of product 2 was obtained after 24 h. With the use of mixtures of an organic solvent and an imidazolium-based ionic liquid, the results were dependent on both the solvent and IL counter ion used. Using [BMIm][PF<sub>6</sub>] in mixtures with n-hexane or MTBE demonstrated better results than those obtained in pure organic solvents. In summary, the data reported herein demonstrate that the organic medium is one of the most important experimental parameters in the chemoenzymatic epoxidation of citronellol. Bio-catalysis in ionic liquids is an exciting, burgeoning area of research, and therefore, future investigations should be conducted on different epoxidation reactions.

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References


