



## Enzymatic resolution of ( $\pm$ )-6-(5-chloropyridin-2-yl)-7-vinyloxy-carbonyloxy-6,7-dihydro[5H]pyrrolo[3,4-b]pyrazin-5-one. Synthesis of (+)-zopiclone

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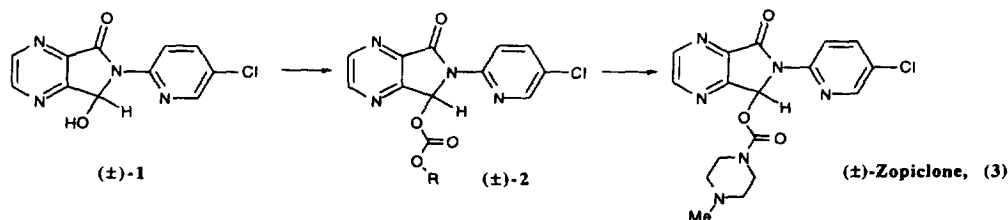
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**Abstract:** The lipase from *Candida antarctica* (CAL) catalyzes the resolution of a precursor of zopiclone through hydrolysis and transcarbonation processes. © 1997 Elsevier Science Ltd

The ( $\pm$ )-zopiclone **3**, is an hypnotic agent of the cyclopyrrolone class possessing a pharmaceutical profile of high efficacy and low toxicity similar to that of the benzodiazepines.<sup>1</sup> Until now, zopiclone has been commercialized as a racemic mixture, however, recent studies have confirmed that the (+)-enantiomer is significantly more active and less toxic than the (-)-enantiomer.<sup>2,3</sup>

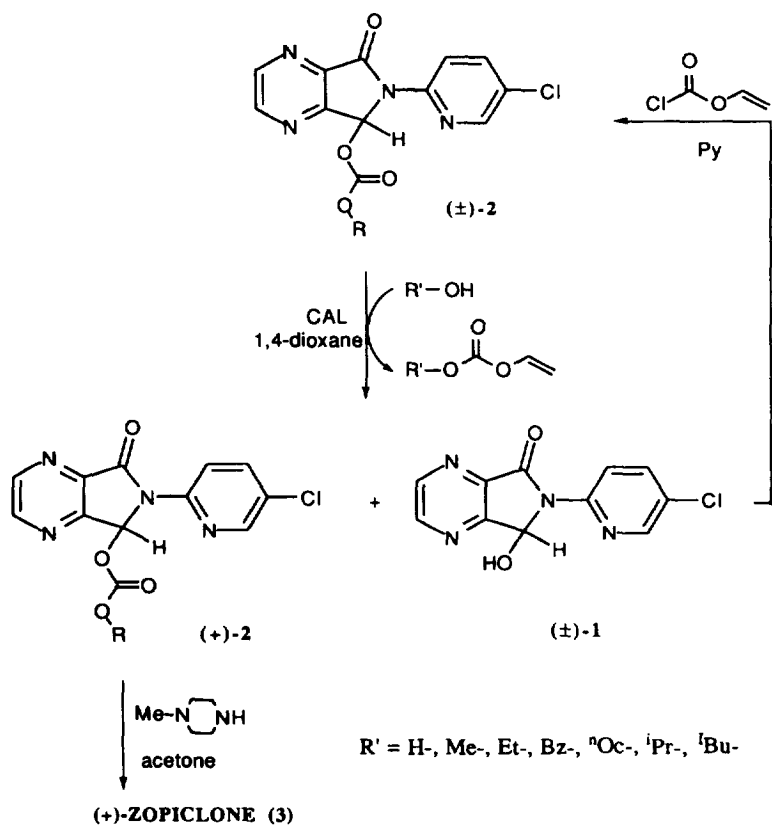
The preparative enantioseparation of ( $\pm$ )-zopiclone has been carried out by formation of the diastomeric salts with (+)- or (-)-malic acid<sup>2</sup> or with (+)-*O,O'*-dibenzoyltartaric acid<sup>3</sup> and selective crystallization of the mixture. Now we report a chemo-enzymatic method for the preparation of enantiopure (+)-zopiclone, which offers some interesting advantages, especially in relation to its application on an industrial scale.<sup>4</sup>

As it is indicated in the Scheme 1, the two final steps in the synthesis of ( $\pm$ )-zopiclone are the carbonation of the hydroxyl group in the compound ( $\pm$ )-**1** and the subsequent reaction of the carbonate ( $\pm$ )-**2** with *N*-methylpiperazine.<sup>5</sup> With this in mind, and taking into account that some carbonates are suitable substrates in lipase catalyzed reactions,<sup>6</sup> we thought it of interest to try the resolution of the precursors ( $\pm$ )-**1** and ( $\pm$ )-**2** through enzymatic carbonation, hydrolysis or transcarbonation processes. First, we examined the potential of some lipases (*Candida cylindracea*, *Pseudomonas cepacia*, *Aspergillus niger* and *Candida antarctica*) to catalyze the carbonation of ( $\pm$ )-**1** for the preparation of the enantiopure carbonate **2**. We studied this process, at room temperature and at 60°C, with four different carbonates: diallyl, benzyl vinyl, phenyl vinyl carbonate and acetone *O*-[(vinyloxy)carbonyl]oxime. However, in all the cases, compound ( $\pm$ )-**1** is recovered unaltered.



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As the direct enzymatic carbonation of the alcohol ( $\pm$ )-1 was unsuccessful, we studied the enzymatic hydrolysis of ( $\pm$ )-2, (Scheme 2, when R=vinyl and R'=H). The CAL catalyzes this process in an aqueous buffer solution or in organic solvent. The best enantioselectivity and the higher rate is obtained when the reaction is carried out in 1,4-dioxane as solvent;<sup>7</sup> under these conditions, the enzymatic hydrolysis shows an  $E>100$ .<sup>8</sup> The conversion of the reaction must be 50% in order to recover the enantiomerically pure substrate that has the correct configuration for the preparation of the (+)-zopiclone. A 48% of conversion is reached in three days but the reaction become slower near the 50% at 30°C. The 50% conversion can be achieved after six days reaction. Nevertheless, under these conditions, the enzymatic hydrolysis is completely enantioselective and, even after longer incubation times, the conversion never overpasses 50%. When the reaction was carried out in other solvents as THF, diisopropyl ether or *tert*-butyl methyl ether lower conversions are obtained. On the other hand, in an aqueous buffer solution (0.1M phosphate buffer, pH=7), the reaction rate is similar to that carried out in 1,4-dioxane but it shows lower enantioselectivity ( $E=15$ ).



Scheme 2.

When other carbonates ( $\pm$ )-2 (R=phenyl, ethyl, 2,2,2-trichloroethyl or benzyl) are subjected to the CAL catalyzed hydrolysis, in all the cases less than 10% of conversion after ten days of reaction is achieved.

Finally, we study the transcarnation reaction of ( $\pm$ )-2 using different alcohols as nucleophiles (Scheme 2); these enzymatic reactions are carried out under the same conditions as the hydrolysis<sup>7</sup> (CAL as catalyst and dioxane as solvent) and again, only the vinylcarbonate ( $\pm$ )-2a is a suitable substrate for the enzyme. The enantioselectivity of the enzymatic transcarnation is also dramatically

high ( $E > 100$ ); 50% of conversion is achieved after six days at 30°C or two days at 60°C, after longer incubation times this conversion is not overpassed. The results obtained using different alcohols: benzylic alcohol, methanol, ethanol, octanol, isopropanol or isobutanol do not show significant differences. The rate and enantioselectivity of this enzymatic process seem not to be dependent on the nucleophile.

In both processes, hydrolysis and transcarbonatation, the product of the enzymatic reaction is the alcohol **1**. This alcohol suffers a spontaneous racemization in the reaction medium which allows the recycling of the compound (see Scheme 2). This means that, although the maximum yield in a kinetic resolution is a 50%, the overall efficiency of this enzymatic process is 100%. The racemization of the non desired enantiomer can be very advantageous for the large-scale preparation of the drug.

Finally, according with the usual procedure,<sup>5</sup> the enantiomerically pure carbonate (+)-**2** is easily converted into the (+)-zopiclone **1**<sup>9</sup> by treatment with *N*-methylpiperazine. No racemization is observed in this last process.

### Acknowledgements

Financial support of this work by the II Plan Regional de Investigación del Principado de Asturias, (Project PA-TDI94-01). We also thank Novo Nordisk Co. for the generous gift of the lipase.

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7. The reaction mixture containing the carbonate ( $\pm$ )-6-(5-chloropyridin-2-yl)-7-vinyloxycarbonyloxy-6,7-dihydro[5H]pyrrolo[3,4-*b*]pyrazin-5-one ( $\pm$ )-**2a** (0.45 mmol), H<sub>2</sub>O or alcohol (1.25 mmol), CAL (150 mg) and dry 1,4-dioxane (5 ml) is incubated on a rotatory shaker (250 rpm) until no progress of the reaction is detected (six days at 30°C or two days or at 60°C). The progress of the reaction is monitored by TLC using the solvent system ethyl acetate–hexane 1:1 as eluent and aliquots taken at different times are analyzed by <sup>1</sup>H-NMR. At the indicated time the reaction is stopped by filtration. The residue is chromatographed on a silica gel column using the same eluent as for TLC and the products ( $\pm$ )-**1** and (+)-**2** are separated. The racemization of the product **1** and the enantiomeric excess of the substrate are determined by <sup>1</sup>H-NMR analysis in the presence of Eu(hfc)<sub>3</sub>. (+)-**2**:  $[\alpha]_D^{25} = +143$  (c=0.4, HCCl<sub>3</sub>).
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(Received in UK 27 January 1997)