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Stereospecificity of pig liver esterase in the hydrolysis of racemic esters derived from 1,8-cineole

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INTRODUCTION

The efficient synthesis of biologically active natural compounds as well as non natural ones frequently requires chiral syntons. Enzymes, as chiral catalysts, are widely used for this purpose since it is usually difficult to carry out highly enantioselective transformations using chemical methods. Esterases, such as pig liver esterase (PLE), a serine hydrolase, can hydrolize a wide range of substrates with high stereoselectivity. Additionally, its stability, low cost, and the fact of not requiring co-enzyme justify the wide use of PLE. This enzyme has been used for the hydrolysis of different esters, mainly *meso* and *prochiral* diesters (Tamm, 1992).

On the other hand, when using achiral reagents traditional synthesis produce racemic mixtures and enzymatic resolution is frequently a very convenient solution to obtain homochiral products.

1,8-cineole (**1**) or eucalyptol is a bicyclic monoterpene ether widespread in the plant kingdom (Fig. 1). It is the main constituent of a number of essential oils particularly those produced by several species of the genus *Eucaliptus*. Cineole is a common ingredient of pharmaceutical preparations because of its direct expectorant action. Owing to its pleasant and distinctive aroma it is also used in lotions and cosmetics. Although it is widely spread in essential oils, only a few natural derivatives of cineole (**1**) are known. The secondary alcohols **2a/2b** and **3a/3b**, and the primary alcohol **9** (Fig. 2) were detected in urine of brushtail possum *Trichosurus volpecula* fed with a diet containing 0.5% 1,8-cineole (Boyle *et al.*, 2000). The corresponding acetates **4a,b-5a,b** were detected as odor principles of *Alpine galanga* Willd. rhizomes (Kubota *et al.*, 1999). (+)-Enantiomers of both acetate **4a** and alcohol **2a** possess a more vivid and sweeter aroma than their corresponding (-)-antipodes. The *exo*-alcohol **2b** is a component of the sauvignon grape variety while the *endo* alcohol **2a** is a component of the aroma of ginger flowers.

We have previously reported the first method of direct functionalization of 1,8-cineole, which led to 5-keto and 5,8-diketocineole in good yields (Boggiato *et al.*, 1986). From these ketones we obtained a series of racemic di- and polyfunctionalized derivatives. We selected the racemates of acetates **5a** and **6a,** and the diacetate **6b** with the purpose of studying the specificity of PLE and its utility for preparing pure enantiomers. This procedure constitutes what is denominated 'green chemistry', since chemical reagents are not used.

METHODOLOGY

For each compound the enzymatic reaction was carried out using a PLE suspension (2,530 units,

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Aldrich) in buffer phosphate solution (pH 7.00, 15 ml) , to which the substrate (4.71 mmol) was added in a single portion. The mixture was incubated at 37°C. Aliquotes were taken at different times during 0-48 hours. Each sample was saturated with sodium chloride followed by extraction of the organic compounds with methylene chloride. The reaction was followed by TLC and by gas chromatography coupled to mass spectrometry (GC-MS) using a Hewlett Packard 6890 gas chromatograph coupled to a selective mass detector HP 5973. The identification of the products was carried out on the basis of their retention times, and by comparison with mass spectra of authentic samples.

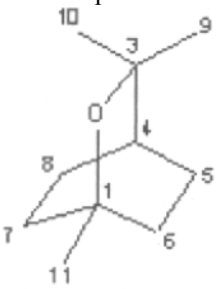

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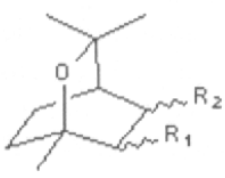
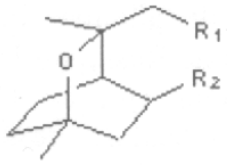
Figure 1. Structure of 1,8-cineole





2a R_1 = *endo* OH, R_2 = H
2b R_1 = *exo* OH, R_2 = H
3a R_1 = H, R_2 = *endo* OH
3b R_1 = H, R_2 = *exo* OH .
4a R_1 = *endo* OAc, R_2 = H
4b R_1 = *exo* OAc, R_2 = H
5a R_1 = H, R_2 = *endo* OAc
5b R_1 = H, R_2 = *exo* OAc

6a R_1 = OAc, R_2 = H
6b R_1 = OAc, R_2 = OAc
7a R_1 = OAc, R_2 = *endo*OH
7b R_1 = OH, R_2 = OAc
8 R_1 = OH, R_2 = *endo*OH
9 R_1= OH, R_2 = H

Figure 2. Structures of 1,8-cineole derivatives

RESULTS AND DISCUSSION

The incubation at 37°C of the secondary acetate **5a** with PLE showed after twelve hours two products in an approximate 1:1 relationship. They were identified by GC/MS as the ester **5a** and alcohol **3a**. The data reported in the literature indicated they correspond to (*S*)-ester and (*R*)-alcohol respectively (Luzzio and Duveau, 2002). On the contrary, under the same conditions PLE didn't show selectivity for the acetate **6a**, which was completely hydrolized after 30 minutes incubation, the racemate of the corresponding primary alcohol **9** being obtained. On the other hand, the racemate of diacetate **6b** analysed after 3 hours incubation with PLE gave a 1:1 mixture of the hydroxyacetate **7b** and diol **8**, remaining approximately 60% of unaffected diacetate. At present, chirality of **7b** and **8** is being investigated.

CONCLUSIONS

Our results indicate that the enzyme is effective for the enantioselective hydrolysis of the racemate of **5a**. However, it doesn't discriminate the racemate of the primary acetate **6a**. The results of the hydrolysis of the diacetate **6b** are in aggreement with those obtained on hydrolysis of the respective monoesters, being noticed an important negative kinetic effect of the ester group at C-5 on the hydrolysis of the ester residue at C-9. On the other hand, this method represents an easy access to **7b** which is difficult to obtain by acetylation of the corresponding alcohol and also for obtaining pure enantiomers of **3a**.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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