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# Lipase-Catalysed Acylation of Alcohols by Fatty Acid Anhydrides. Evaluation of the Selectivity Based on Kinetic Measurements.

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Abstract : A method based on the analysis of kinetic measurements is described for evaluating the enantioselectivity of the lipase-catalysed acylation of alcohols by fatty acid anhydrides in cases where enantiomer separation is not feasible.

In a previous paper, we reported that the acylation of a mixture of two stereoisomeric alcohols, geraniol and nerol, by fatty acid anhydrides catalysed by crude porcine pancreatic lipase (PPL) could be described by a pseudo first-order kinetics up to a high conversion ratio of the more reactive isomer<sup>1</sup>.

A full kinetic study (to be published), based on the analysis of a Bi-Bi Ping-Pong process<sup>2</sup>, provided these findings with mathematical support. Equations for the rate of the transformation of both competitive alcohols were drawn up using the same formalism as that by Michaelis-Menten, and at the concentrations used, the transformation of both substrates was expected to have pseudo first-order kinetics. A recent similar study of non-competitive lipase-catalysed of geraniol leads to identical conclusion<sup>3</sup>.

In the case of the PPL-catalysed acylation of geraniol (B) and nerol (C) by butyric anhydride (A), gas chromatography (GC) was performed to separate alcohols B and C and their respective esters Q and R, and it was thus possible to quantify at any time the four components of the reactional mixture.

In cases where it is not possible to separate alcohols B and C and/or esters Q and R by performing achiral GC analysis, the experimental values obtained are the sums [S]=[B]+[C] and [T]=[Q]+[R]. This is always the case with non-derivatized enantiomeric mixtures. The aim of the present study was to show how it is possible to evaluate the selectivity of the acylating process by performing a kinetic analysis of the values of [T], paying special attention to the case of enantiomeric mixtures.

# Theoretical development.

The pseudo first-order kinetics of each transformation implies the following relationships :  $\ln [B]/[B_0] = k_B t$  and  $\ln[C]/[C_0] = k_C t$ , where [B\_0] and [C\_0] are the initial concentrations of the substrates, t the time and  $k_B$  and  $k_C$  the pseudo first-order constants associated with the two alcohols. The ratio  $k_B/k_C$  is the selectivity factor E, used by Sih<sup>4</sup>. Hence, [T] can be expressed by the equation :  $[T] = [Q]+[R] = [B_0] (1 - e^{kBt}) + [C_0] (1 - e^{kCt})$  which can be rewritten as

Eq. 1: 
$$[T] = [B_0] (1 - e^{kBt}) + [C_0] (1 - e^{kBt/E})$$

The overall rate of ester formation (vT) is the sum of the two partial rates vB and vC and can be expressed by the simple equation : vT = kB[B] + kC[C].

At the beginning of the reaction, vT is expressed by :

Eq. 2 : 
$$v_{T0} = k_B[B_0] + k_B/E[C_0]$$

which is simplified in the case of enantiomeric mixtures where  $[B_0]=[C_0]$ .

This initial rate can be determined from the tangent at the origin of the curve describing the ester formation vs time. For a given value of E, after determining vT0 from experimental data, one can calculate from Eq. 2 the corresponding value for kB and use it in Equation 1. Using arbitrary values of t, one can then draw theoretical curves which have the same tangent at the origin with a slope kB+kC. By comparing these curves with the experimental plots, one can estimate the value of E.

#### Checking the kinetic model.

This model was checked by analysing two reactions. The first was the previously described acylation of geraniol-nerol mixture and the second, the acylation of  $(\pm)$ -2-hexanol. Acylation of geraniol-nerol mixture.

The experimental data previously obtained 1 were artificially modified by summing the quantities of esters. These new values were plotted vs time as shown in Figure 1.



Figure 1 : Acylation of geraniol-nerol mixture ([B0]= 64 mM; [C0]= 50 mM) by butyric anhydride ([A0]= 114 mM) in ether; PPL: 50 mg; Reactional volume : 10 ml; 20° Plots: experimental values; Graphs: theoretical curves from Eq. 1 with E= 1(a), 2(b), 5(c), 10(d), 20(e) and  $\infty$  (f).





Figure 2 : Acylation of ( $\pm$ )-2-hexanol ([B<sub>0</sub>]= [C<sub>0</sub>]= 50 mM) by butyric anhydride ([A<sub>0</sub>]= 110mM); PPL : 250 mg in ether; reactional volume : 10 ml; 20°. Plots : experimental values. Graphs : theoretical curves from Eq.1 (E = 20 (a), 50 (b) and  $\infty$ (c)).



The initial rate vT0 was evaluated at 0.25 mM.mn<sup>-1</sup> for 1 g of PPL per liter. The values of kB were obtained by Eq. 2 for E = 1 (non-selective process), 2, 5, 10, 20 and  $\infty$  (fully selective process with kC=0) and then introduced into Eq. 1. Using for [B0] and [C0] the values 64 and 50 mM used to calculate Eq. 1, along with several values of t, yielded the theoretical progress curves shown in Figure 1. The experimental plots showed a fit between the theoretical curves corrresponding to to E=5 and E=20. Hence the experimental value of E was evaluated to be close to that obtained (E=8) by measuring the proportions of the four species present in the reactional mixture<sup>1</sup>.

PPL has been reported to catalyse highly enantioselective acylations of secondary alcohols such as  $(\pm)$ -2-octanol<sup>5</sup> or sculatol<sup>6</sup>. In addition, lipase from *Mucor miehei* has been found to catalyse the enantioselective acylation of  $(\pm)$ -2-hexanol using octanoic acid as acyl donor<sup>7</sup>.



The acylation of  $(\pm)$ -2-hexanol (100 mM), catalysed by PPL (250 mg), was performed with butyric anhydride (100 mM) in ether. The composition of the mixture ([S] and [T]) was monitored by GC and the

formation of both esters is shown in Figure 2. At this stage, one could see i) that the reaction was 25 fold slower than that of geraniol, hence the Km values of the two enantiomers of 2-hexanol were presumably larger than 200 mM, value found for geraniol. At the concentrations used (50 mM), low in comparison with this latter Km, a pseudo first-order kinetics was therefore to be expected, and ii) that the reaction stopped at a 50% conversion level which suggested that we were dealing with an enantioselective process.

Theoretical curves, which are shown in Figure 2, were obtained from Eq. 1, using for VTO the value 0.01 mM.mn<sup>-1</sup>for 1 g of PPL per liter, for E, the following arbitrary values: 20 (curve a), 50 (curve b) and  $\infty$  (curve c), and for [B0] and [C0], 50 mM. From the fit of curve c with experimental plots, the factor E was evaluated as being greater than 50 : the reaction therefore appeared to be highly enantioselective. This selectivity was fully confirmed by GC analysis of (-)-camphanyl ester of unreacted alcohols and alcohols resulting from ester saponification.

# Application of the kinetic model.

This new method for evaluating the selectivity was applied to two reactions performed with racemic mixtures of  $(\pm)$ -citronellol and  $(\pm)$ -3-hexanol.

#### Acylation of $(\pm)$ -citronellol.

Klibanov has described the remarkably enantioselective acylation of this terpene by pig liver esterase<sup>8</sup>. The bacterial lipase from *Pseudomonas sp.* has been reported to be non-selective in the acylation of this substrate<sup>9</sup>. Because of the structural analogy between citronellol and geraniol, it seemed to be of interest to check whether PPL was able to selectively catalyse the acylation of this substrate.



Figure 3 : Acylation of  $(\pm)$ -citronellol  $([B_0] = [C_0] = 70 \text{ mM})$  by butyric anhydride  $([A_0] = 180 \text{ mM})$ ; PPL : 50 mg in ether; reactional volume : 10 ml; 20°. Plots : experimental values. Graphs : theoretical curves from Eq. 1 with E = 1 (a), 2 (b) and 4 (c).

## Acylation of $(\pm)$ -3-hexanol.

In the case of this substrate, the asymmetry is located one atom farther away than in the case of 2-hexanol. Working with  $(\pm)$ -3-octanol Klibanov<sup>5</sup> observed a loss of selectivity in comparison with its 2-isomer. Acylation of  $(\pm)$ -3-hexanol was performed as with the 2-isomer.





The ester formation was monitored as previously and plotted vs time (Figure 3). Since the initial acylation rates of geraniol and cironellol by butyric anhydride were of the same magnitude, one might assume that the Michaelis' parameters of citronellol were of the same magnitude as those of geraniol, and pseudo first-order kinetics were to be expected. The initial rate was 0.136 mM.mn<sup>-1</sup>for 1 g of PPL per liter. Using equations 1 and 2, theoretical curves a, b, c were obtained for E = 1, 2and 4, respectively. It turned out that the best fit was obtained for E = 1. The acylation of  $(\pm)$ -cironellol catalysed by PPL was therefore not enantioselective.



The rate (0.0056 mM.mn<sup>-1</sup> for 1 g of PPL per liter) was lower and our previous considerations about the values of the parameters were again assumed to hold true.

Experimental ester formation values and the theoretical curves obtained with E = 15 (a) and  $\infty$  (b) are given in Figure 4. It emerged that the reaction was more enantioselective than in the case described by Klibanov with  $(\pm)$ -3-octanol, where the E value works out at 8 based on Sih's equation 10.

Figure 4 : Acylation of ( $\pm$ )-3-hexanol ([B0]= [C0]= 50 mM) by butyric anhydride ([A0]= 110 mM); PPL : 250 mg in ether; reactional volume : 10 ml; 20°. Plots : experimental values. Graphs : theoretical curves from Eq. 1 with E = 15 (a) and  $\infty$  (b).

## Discussion and conclusion.

The present study shows how it is possible by performing kinetic measurements to evaluate the selectivity of a lipase-catalysed acylation by acid anhydrides of a mixture of two alcohols, in the case where the two alcohols and the two esters cannot be analysed separately.

The accuracy of this method depends on three main factors: i) the stability of the enzyme during the reaction, ii) the accuracy of the experimental values and iii) the relevance of a pseudo first-order equation rate for describing the kinetic process.

The stability of the enzyme is assessed by recycling the biocatalist in hydrolytic reactions where no loss of activity is observed<sup>1</sup> or through its activity in a further acylation reaction. The accuracy of the experimental values was evaluated at 5% (values of error bars in Figures 1-4) on the basis of several repetitions of the same experimentation. In our hands pseudo first-order kinetics were always observed with diluted solutions (50-100 mM) of (S)-(-)-citronellol or primary or optically pure secondary alcohols Failures can occur with this method in cases where a reversible reaction occurs, or when the ester formed

Failures can occur with this method in cases where a reversible reaction occurs, or when the ester formed interferes in the process as an inhibitor or competitive acyl donor. Using butyric anhydride, we have not observed any interference due to released butyric acid or butyric esters.

The accuracy of this method is not comparable with direct analysis of the enantiomeric species present in the reaction mixture, but the present method requires only a conventional GC (or HPLC) apparatus and careful experimentation.

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