Tetrahedron Letters, Vol.31, No.34, pp 4875-4878, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

STEREOSELECTIVE LIPASE-CATALYSED ACYLATION OF TERPENIC ALLYLIC ALCOHOLS BY FATTY ACID ANHYDRIDES.

Jean-Dominique Fourneron*[1], Mélissa Chiche[1] and Gérard Piéroni[2].
[1] Centre de Spectroscopie Moléculaire. Faculté de St. Jérôme. Avenue Escadrille Normandie-Niemen.
13397 Marseille Cedex 13. France.
[2] Centre de Biochimie et de Biologie Moléculaire. 31 chemin Joseph Aiguier.
13402 Marseille Cedex 9. France.

Abstract: Lipase from pancreatic powder preferentially catalyses the acylation of the E isomer of terpenic allylic alcohols using fatty acid anhydrides as the acylating agent. The course of the reaction can be described by a first-order equation.

Thanks to the pioneering work of Klibanov (1) and Sih (2), the use of lipases in organic media for separating enantiomeric alcohols by selective acylation is now a routine process. The number of commercially available lipases is increasing and rapid screening makes it possible for chemists to find the most appropriate enzyme for the specific substrates to be separated.

In a recent publication, Bianchi et al.(3) described an acylation process using acid anhydrides as acylating agent. The possibility of using these reagents was predictable from the results of Yamamoto et al.(4) who were able to acylate simple alcohols (methanol or butanol) enantiotopically with a prochiral cyclic anhydride. Another recent publication by Uemura et al.(5) describes the regioselective acylation by hexanoic anhydride of sugar moieties of nucleosides.

One great advantage in using anhydrides is the simplicity of the process. Assuming that a two-step mechanism is involved in these reactions, (acylation of the enzyme followed by deacylation by the alcohol), the first step seems to be greatly facilitated when an anhydride is used instead of other acylating agents such as trichloroethyl esters(3).

In a recent biochemical study, we have shown that anhydrides are very suitable substrates for lipases and esterases (6). As an example, the presence of a low percentage of butyric anhydride in tributyrin increases 3 to 4 fold the lipase activity, as measured from the protons released.

Due to the high catalytic activity of lipases in hydrolysing anhydrides, another advantage of these acylating agents is probably the fast dehydration of the medium. In some cases it has been shown that decreasing the water content of the lipase powder and solvent strongly increases the selectivity of the process (7).

Most of the investigations in this area are aimed at racemate resolution. In this note, we report preliminary data on the separation of terpenic allylic alcohols resulting from lipase-catalysed stereoselective acylation.

Results.

The isomeric alcohols used in this study were geraniol/nerol (G/N), 2EZ-6E farnesol (F) and racemic 2EZ-phytol (P). G and N were commercial products and were used in equimolar mixture, the mixture of the two 6E isomers of F (E/Z: 67/33) was a gift from Roure-Bertrand-Dupont and P (E/Z: 67/33) was the commercial product. Four lipases were tested : *PPL* from hog pancreas (Fluka), bacterial lipases from *Pseudomonas fluorescens* (*PF*) (Fluka), *Candida cylindracea* (*CC*) (Sigma) and *Mucor miehi (MM)* (Lipozyme, NOVO Industry). Butyric, hexanoic and octanoic anhydrides were used. Acetic anhydride gave poor yield and selectivity. Reactions were performed in four solvents: ether, hexane, toluene and dichloromethane. In analytical trials, mixtures of alcohols (1 mmol), stoichiometric amounts of anhydride and 50 mg of enzymatic powder in 10 ml of solvent were incubated at room temperature under magnetic stirring. Aliquots (40 µl) were taken at regular intervals and diluted in hexane (1 ml). Analyses were performed by GPC equipped with capillary column (SuperOX, 0.32 mm, 30 m, He as carrier gas, 2 ml/mn, FID as detector, injection "on column", temperature: 70° C to 250° C at 10°/mn). Peaks were integrated using a Shimadzu CR1B integrator. Response factors of alcohols and esters were determined as 1.

When working with *PPL*, the best solvent was found to be ether. In hexane, the selectivity was the same but reactions were slower. Toluene and dichloromethane were found to be unsuitable. In ether, lipases *CC* and *MM* catalysed acylation of the three couples of isomeric alcohols studied, but no selectivity was observed. By contrast, *PPL* and *PF* preferentially catalysed the acylation of the E isomer of the three substrates. Only a small percentage of non-selective acylation was observed in the absence of lipase (9). No catalysis by lipase occurred when an acid was used as acylating agent, which contrasts with Iwai's findings in aqueous medium (8). The following study was carried out with *PPL*.

Chromatographic analysis was performed to quantify ester formation *vs* time. The kinetics fitted a first-order reaction. The pseudo first-order rate constants (k_E and k_Z) for the formation of E and Z esters were calculated using the classical equations $\ln(A_E/A_{E0}) = k_E * t$ and $\ln(A_Z/A_{Z0}) = k_Z * t$, respectively where A_E and A_Z were the concentration of alcohols at a given time (t), A_{E0}, A_{Z0}, the initial concentration of E and Z alcohols The ratio $k_E/k_Z = \ln(A_E/A_{E0})/\ln(A_Z/A_{Z0})$ is thus equal to the factor $E = V_E K_Z / V_Z K_E$, where V_E, V_Z, K_E and K_Z were the maximal velocities and Michaelis constants for E and Z isomers respectively: this factor was defined by Sih (2). The results of a run performed with G/N (1/1) and hexanoic anhydride are shown in table 1.

Table 1: Composition of reaction mixture as a function of time using G/N (1/1), 0.1 M; hexanoic anhydride: 0.1 M; 50 mg of *PPL* in ether (10 ml) at 20° C

Time (mn)	A_Z[a]	A_E[a]	E_Z[a]	E_E[a]	k_E[b]	k_Z[b]	k_E/k_Z
00	50	50					
15	48.5	44	1.5	6	91	23	4
30	48	36	2	14	100	13	8
60	47	25	3	25	110	11	10
90	46	17	4	33	120	8	15
120	45	13	5	37	110	8	14
180	43	6	7	44	110	8	14
300	39	2	11	48	110	8	14

[a])A_Z, A_E, E_Z, E_E = molar percentage of alcohols (A) and esters (E) , Z and E . [b]) $10^{-4} mn^{-1}$

The results obtained under the same conditions with the three isomeric alcohols and the three anhydrides after a 2 h reaction time are given in table 2. Values of k_E and k_Z were obtained with incubation times of 30, 60, 90 and 120 mn and averaged.

Table 2: Yield, selectivity and pseudo first-order constant of the acylation of G/N, F and P with butyric, hexanoic and octanoic anhydrides after a 2 h incubation

n°	Substrate[a]	Anhyd.	E:yield[b]	Z:yield[b]	ie[c]	k_E[d]	k_Z[d]	k_E/k_Z
1	G/N	C4	85	16	68	160	15	11
2	G/N	C6	66	7	80	90	7	13
3	G/N	C8	72	7	82	107	7	15
4	F	C4	64	17	77	86	16	5.5
5	F	C6	67	22	72	93	21	4.5
6	F	C8	65	21	73	89	20	4.5
7	P	C4	67	22	73	93	21	4.5
8	P	C6	54	17	86	66	16	4

[a]) G/N = 1; F: E/Z = 2; P: E/Z = 2. [b]) calculated from E and Z isomers present in the initial mixture.
[c]) ie: isomeric excess: (E-Z/E+Z)*100 (analogous to enantiomeric excess ee (2)). [d]) $10^{-4}mn^{-1}$. Experimental conditions: Alcohol (E+Z): 1mmol; anhydride: 1mmol; ether: 10 cm^3; *PPL*: 50 mg; 120 mn; room temperature.

The reaction was performed on the 20 mmol scale with F mixture and hexanoic anhydride in ether (50 ml) and 1 g of *PPL*. After long reaction times (6 to 12 h) silica-gel chromatography was performed to obtain a mixture of esters and a mixture of alcohols containing 99% pure unreacted Z isomer with a 40 to 45% yield based on the Z isomer present in the initial mixture. Experiments conducted with shorter reaction times (2 to 3 h) yielded after silica-gel chromatography less pure residual Z alcohol than before and a mixture of esters enriched in E isomer (88%). The latter mixture was saponified and the alcohols recovered subjected to a new acylation step leading to 98% pure E ester and 50 to 60% overall yield. All lipase hydrolytic activity (10) of the biocatalyst recuperated by filtration after a 12 h reaction time was recovered as was to be expected from the constancy of the k_E and k_Z values *vs* time (table 1).

Discussion and conclusion.

The present results show that lipases catalyse stereospecific acylation of terpenic alcohols by aliphatic anhydrides. The selectivity of the reaction for a given alcohol depends only slightly on the nature of the anhydride.

In enantioselective processes involving secondary alcohols, the enzyme is able to recognize structural dissymmetry located three atoms away from the bond to be created and the best results were obtained (1,3) when the two substituents had very different sizes. With the allylic alcohols used in this study, this dissymmetry was located five atoms away and yet the enzyme displayed a relatively high selectivity. These results may contribute to establishing the molecular geometry of the active centre of the lipase.

It is surprising that under our conditions where both substrates, anhydride and alcohols, were in stoichiometric amounts, pseudo first-order kinetics was observed. A possible explanation is that the transfer of the acyl group from the acyl-enzyme to the alcohol be the rate limiting step, and since studies in progress indicate that the Km values for G and FE

range around 150 mM, as compared to 100 mM used in the present study, first-order kinetics might be expected on the basis of the Michaelis-Menten model.

References.

1- B. Cambou and A.M. Klibanov, *J. Am. Chem. Soc.*, 1984,**106**, 2687 and *Biotechnol. Bioeng.*, 1984, **26**, 1449.

2- C. S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Amer. Chem. Soc.*, 1982, **104**, 7294 and C. S. Chen and C. J. Sih, *Angew. Chem. Int. Ed. Engl.*, 1989, **28**, 695, and ref. cited.

3- D. Bianchi, P. Cesti and E. Battistel, *J. Org. Chem.*, 1988, **53**, 5531.

4- K. Yamamoto, T. Nishioka, J. Oda and Y. Yamamoto, *Tetrahedron Letters*,1988, **29**, 1717.

5- A. Uemura, K. Nozaki, J. Yamashita and M. Yasumoto , *Tetrahedron Letters*, 1989, **30**, 3817.

6- G. Piéroni and J. D. Fourneron, accepted for publication to *Eur. J. Biochem.*

7- T. M. Stokes and A. C. Oehlschlager, *Tetrahedron Letters*, 1987, **28**, 2091.

8- M. Iwai, S. Okumura and Y. Tsujisaka, *Agric. Biol. Chem.*, 1980, **44**, 2731 and S. Okumura, M. Iwai and Y. Tsujisaka, *Biochim. Biophys. Acta* , 1979, **575**, 156.

9- We have recently observed that a small percentage of non-catalysed acylation occurs in the injector of the GPC apparatus. This reaction is not reproducible but the maximum percentage we have observed is smaller than 2% of the initial alcohol. This artefact mainly disturbed the initial values.

10- Hydrolytic activity refers to the number of microequivalents of protons released from tributyrin per minute.

(Received in France 30 March 1990)