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Regioselective acylation of 1,6-anhydro-β-D-glucopyranose catalysed by lipases

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***Abstract** : Pseudomonas fluorescens lipase (Amano) was found to be highly regioselective (85%) in the catalysed transesterification of 1,6-anhydro-β-D-glucopyranose using vinyl acetate as an acyl donor and solvent.*

Carbohydrates constitute a very powerfull source of natural chirons but the presence of several hydroxy functions precludes their use as synthons in the native form. With this respect a great deal of strategies for selective protection has been developped. Among the methods proposed, enzymes take a particularly important place as those biocatalysors are able to catalyse organic reactions stereoselectively and regioselectively. An excellent review on this topic[1] reveals that lipases can be used with success to acylate regioselectively the hydroxy functions in positions 6 and 1 of the furanoses and of the pyranoses. Nevertheless, only few papers deal with the ability of the lipases to operate selectively in the 2, 3, and 4 positions when the 1- and 6-hydroxy groups are already protected or absent. Thus, good results were obtained with L-galacto- and manno-pyranoside[2] and with 4,6-O-benzylidene-α- and β- D- glucopyranoside[3].

The aim of this work is to present some results about the regioselectivity of the transesterification reaction of 1,6-anhydro-β-D-glucopyranose (glucosan) catalysed by lipases. The choice of this model was supported by the following considerations :

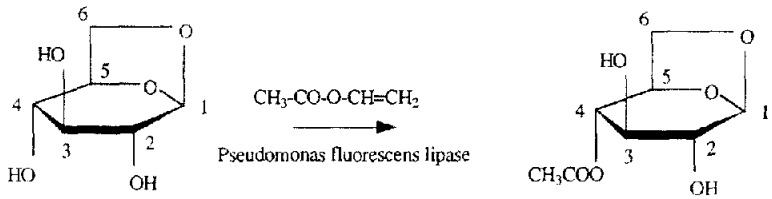
- the conformational rigidity of this kind of compound and by way of fact the existence of the β-D-form only.
- the relative simplicity of synthesis[4, 5].
- the possibility to prepare the stereoisomers of glucosan in order to study the stereoselectivity as a function of axial/equatorial positions in connexion with steric hindrance, and finally to check if the lipases known for their regioselectivity towards the hydroxyl functions of glycerol (Mucor miehi, Rhizopus arrhizus, Porcine pancreatic lipase) would exhibit a similar trend in the presence of such models. A particularly important point would be to determine the conditions for the possible isomerisation of the corresponding mono- and

diesters (if yes, catalysed or spontaneous?) in order to compare the behaviour of these compounds to the glycerol esters' one[6,7].

- Furthermore enzymatic reactions involving 1,6-anhydro-β-pyranoses have not been widely studied up to now and only the lipases which catalyse the hydrolysis of the triesters of the 1,6-anhydro-β-D-glucopyranose[8,9] and of the -galactopyranose (galactosan)[10] are reported in the litterature. The transesterification reactions using vinyl esters are of great interest since they avoid the reverse reaction, so the selectivity of the enzymes is often quite different comparatively to the ester's hydrolysis.

RESULTS AND DISCUSSION

In order to check the regioselectivity in the transesterification reaction, we tried the behaviour of the five common lipases, namely Candida cylindracea (CCL), Mucor miehi (MML), porcine pancreatic lipase (PPL) Pseudomonas fluorescens (PSL) and Rhizopus arrhizus (RAL).



The reactions were carried out at room temperature using vinyl acetate as an acyl donor and solvent. The course of the reaction was monitored by TLC and proton, carbon 13 NMR spectroscopy. The structure of the esters synthesized was determined by comparison with the spectra of glucosan[11,12] and literature data[8,9]. A verification of the attributions was performed by means of the two dimensional NMR spectroscopy experiments (COSY and C-H correlation, see figure 1).The yields and the relative percentages of the different esters obtained are summarized in the table 1. The best conversions (99 %) were obtained with CCL and PSL lipases and in the conditions used the esterification occured preferentially on the position 4.

Table 1 : *Lipase catalysed transesterification of glucosan with vinyl acetate as an acyl donor after 15 days of incubation; PSL* : results after 7 days of reaction.*

Lipase	Yield	monoacetate %			diacetate %			triacetate %
	%	2	3	4	2,3	2,4	3,4	
CCL	99	0	0	22	0	53	20	5
MML	28	0	0	80	0	0	20	0
PPL	20	0	0	80	0	0	20	0
PSL	99	0	0	62	0	24	14	0
PSL*	95	0	0	85	0	9	6	0
RAL	31	0	0	76	0	0	24	0

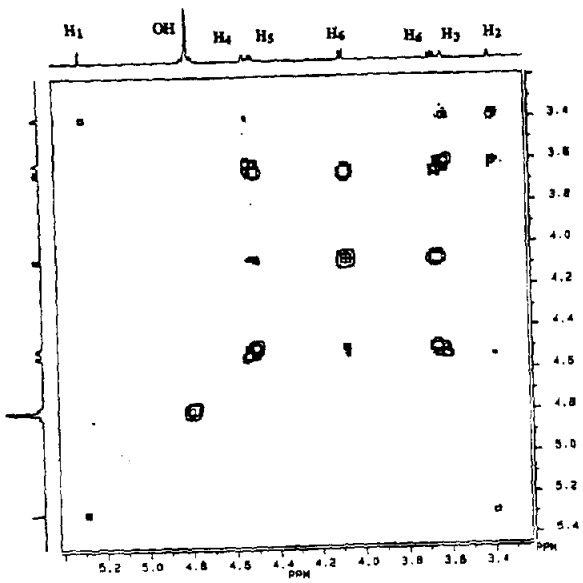
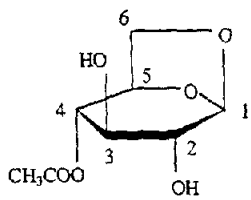


Figure 1: *Assignement of the ^1H proton NMR spectra of the 4-monoacetate of glucosane (COSY experiment - solvent : $CDCl_3$ - 500 MHz Bruker spectrometer).*



Both good selectivity and yield are obtained with PSL lipase and it should be pointed out that those results obtained after 15 days (100 % conversion) are obviously not the best ones. A kinetic study of this reaction has shown that for 95 % conversion the mixture contained 85 % of the 4-monoacetate, only 9 % of the -2,4-diacetate and small amounts of the -3,4-diacetate (see figure 2).

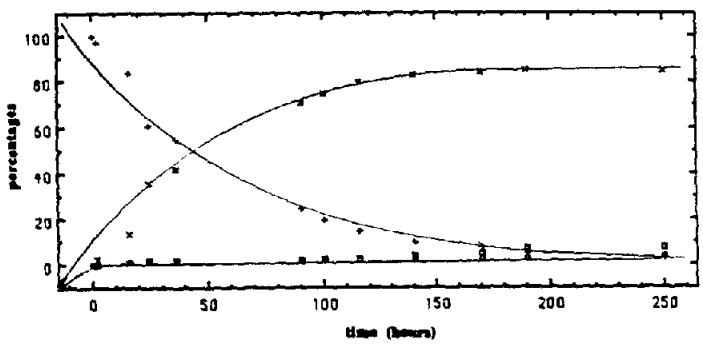


Figure 2 : *Kinetic study of the acylation of glucosan.. The measurements were performed by means of the integration of the proton NMR spectra* (□ : 2,4-diacetate, o :3,4-diacetate, x: 4-monoacetate, +:glucosane)

These results are not in accordance with those obtained in the hydrolysis reaction of the corresponding acetate[9]. In our case PPL lipase gives only a very poor conversion (20%) even after several days of incubation

without any change in the composition of the media while the hydrolysis is nearly complete after 24h[8,9]. Furthermore, when the hydrolysis is carried out in the presence of small quantities of methanol the PPL lipase exerts an opposite regioselectivity towards the position 2. This enzyme becomes selective for the 4 position when the solvent is a mixture of water/methanol (1:1). Another difference lies in the stability of the esters synthesized according to the media. When using the triacetate in the hydrolysis, some acyl group migrations were observed and the perbutyrates had to be employed to avoid this undesirable phenomenon. In the transesterification reaction conducted in an organic solvent, no isomerisation seems to occur. Thus, a solution of the pure 4-monoacetate prepared from the PSL transesterification was incubated in an organic solvent ($CHCl_3$) in the presence of the PSL lipase for several weeks without any modification.

The lipase mediated hydrolysis of the triesters of galactosan follows a similar behaviour as the enzymes used act more rapidly on the position 2[10]. In that case also, the better yield and selectivity obtained with the tributyrates instead of the triacetates were probably due to the acyl migration occuring in the water with the latter.

In that work, we have shown that the transesterification of vinyl acetate with glucosan mediated by PSL is a highly regioselective reaction permitting the synthesis of the 4-monoacetate in good yields. Work is in progress in the laboratory in that direction with the other isomers of glucosan and longer chains for the acyl donor in order to synthesize new detergents from sugar derivatives.

EXPERIMENTAL : In a typical experiment 100 mg of glucosane was introduced into a flask with 0,5 ml of vinyl acetate and 100 mg of the lyophilized lipase preparation. The mixture was stirred and the course of the reaction was monitored by means of TLC and ^1H NMR spectroscopy. At the end of the reaction the lipase was filtered, the excess of vinyl acetate eliminated under vacuum and the products of the reaction were separated over a silica gel column (eluent : ethyl acetate/petroleum ether : 3/1). In that conditions the monoester was easily separated from the diesters which were obtained as a mixture.

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