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ACETYLATION OF PHENOLS IN ORGANIC SOLVENT CATALYZED BY A LIPASE FROM CHROMOBACTERIUM VISCOSUM

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Abstract: Lipase from *Chromobacterium viscosum*, adsorbed on an inert support, was employed as catalyst for the esterification of monohydric phenols in organic solvent, with vinyl acetate as acyl donor. The effect of aromatic ring substitution on the initial rate of transesterification was investigated.

INTRODUCTION

In the last years the use of the enzymic catalysis in organic media has known an extraordinary growth.[1] From these studies it appears that lipases and proteases can be used successfully as catalysts for the acylation of alcohols, in which esters act as acyl donors.[2] In contrast, no information is available until now on the analogous enzyme-catalyzed reaction of phenols,

$$\text{RO-Acyl} + \text{Ar-OH} \rightleftharpoons \text{ROH} + \text{Ar-OAcyl} \quad (1)$$

possibly because the equilibrium 1 is shifted toward the reactants.

In the case of alcohols, to overcome drawbacks due to the reversible nature of the transesterification reaction (long reaction times and large excess of esters to attain an acceptable conversion), vinyl or isopropenyl esters have been proposed as the acylating agent to obtain an irreversible acylation,[3]

$$R\text{-}OH + CH_2{=}CH\text{-}OAcyl \rightleftharpoons R\text{-}OAcyl + [CH_2{=}CH\text{-}OH] \rightleftharpoons CH_3CHO \quad (2)$$

$$R\text{-}OH + CH_2{=}C(CH_3)\text{-}OAcyl \rightleftharpoons R\text{-}OAcyl + [CH_2{=}C(CH_3)\text{-}OH] \rightleftharpoons CH_3COCH_3 \quad (3)$$

$$R\text{-}OH + (R'\text{-}CO)_2O \rightleftharpoons R\text{-}OCO\text{-}R' + R'\text{-}COOH \quad (4)$$

because the enol formed is immediately and irreversibly converted into acetaldehyde or acetone (eqs 2 and 3). A complete shift of the reaction toward the products can also be obtained with anhydrides[4] (eq 4), since in this case the reaction in opposite direction is thermodinamically unfavored.

Consideration of these data prompted us to examine the possibility of extending to phenols the irreversible acylation procedure and in the present paper we wish to describe the results obtained.

RESULTS AND DISCUSSION

Preliminarily, phenol was subjected to transacetylation in cyclohexane using acetic anhydride or vinyl acetate as acylating agent. In the experimental conditions adopted, acetic anhydride gave a substantial percentage of non-catalyzed acetylation and was therefore discarded as acetyl donor. Conversely, with vinyl acetate no reaction occurred in the absence of the enzyme and this ester could be conveniently used in the continuation of the work. Of the six commercial lipases screened as catalyst (lipases from *Chromobacterium viscosum*, *Candida cylindracea*, *Aspergillus niger*, *Mucor javanicus*, *Rhizopus javanicus*, and porcine pancreas), only that from *C. viscosum* was effective in the

transesterification of vinyl acetate with phenol. This enzyme, adsorbed on Hyflo Super Cel, was used to catalyze the acetylation of a series of substituted monohydric phenols. Since many of them are sparingly soluble in cyclohexane, more polar solvents (acetone, *tert*-amyl alcohol, and dichloromethane) were considered and found to be unsuitable. Finally, a 95:5 mixture of cyclohexane and tetrahydrofuran was routinely used as solvent.

Table 1. Initial rate of transesterification of vinyl acetate with monohydric phenols[a]

Substituent	Initial rate (μmol/h)	$\log P_{oct}$[b]
H	1.18	
ortho		
OMe	no reaction	
Ph	no reaction	
i-Pr	no reaction	
Me	0.03	
Cl	0.13	
F	0.61	
meta		
OMe	0.90	
Me	1.03	
F	1.08	
Cl	1.09	
para		
CHO	0.98	1.35
i-Pr	1.04	
Br	1.72	2.65
Ph	1.92	
OPh	2.02	
Cl	2.56	2.40
Me	2.71	1.94
F	3.19	1.79
OMe	10.67	1.39

[a]Substrate (40 μmol), vinyl acetate (200 μmol), immobilized *Chromobacterium viscosum* lipase (20 mg) in 2 mL of a 95/5 cyclohexane/THF mixture, 40 °C, 300 rpm.

[b]P is the partition coefficient in an octanol-water two-phase system.

The results of these experiments, summarized in table 1, allow the following considerations to be made. The rate of the reaction, which is affected little, if any, by substituents in *meta* to the phenolic hydroxyl, is reduced or nullified by an *ortho* substituent. Most of the groups in *para* position, independently from their electron-attracting or -donating power and, consequently, from their pK_a, enhance the reaction rate. A rough correlation was observed between the hydrophobic character of a specific substrate (as measured by its $\log P_{oct}$, where P is the partition coefficient in a standard octanol-water system[4]) and the initial rate of the transesterification reaction. The only exception of

p-hydroxybenzaldeyde is perhaps related to the strong intermolecular association by means of hydrogen bonds.

In summary, lipase from *C. viscosum* in organic medium catalyzes efficiently, under mild experimental conditions, the irreversible transesterification of phenols using vinyl acetate as the acetyl donor.

EXPERIMENTAL

General methods

Gas-chromatography was carried out in the following conditions: HP-1 methylsilicone capillary column 25 m x 0.2 mm i.d., helium as gas carrier, flow 0.40 ml/min, split 1:50, injector temperature 250 °C, flame ionization detector.

Chemicals

All the chemicals were analytical grade and their purity was checked by gc and/or tlc. Tetrahydrofuran was distilled over $LiAlH_4$ and stored over 4-Å molecular sieves. The reference samples of acetates were prepared from the corrisponding phenols by acetylation with acetic anhydride and pyridine according to the conventional procedure. The physical properties of all the products were in agreement with those reported in the literature. Lipases from *Candida cylindracea* and porcine pancreas were purchased from Sigma Chemical Co. *Chromobacterium viscosum* lipase was obtained from FinnSugar Biochemicals. Lipases from *Aspergillus niger* (AP-6), *Rhizopus javanicus* (FAP-15) and *Mucor javanicus* (M-10) were a gift from Amano International Enzyme Co.

Treament of phenol with acetic anhydride or vinyl acetate in the absence of enzyme

A solution of phenol (9.5 mg, 0.1 mmol) and acetic anhydride (51 mg,

0.5 mmol) in cyclohexane (5 mL) was kept at 40 °C for 2 h. Gas-chromatographic analysis of the reaction mixture revealed the presence of significant amounts of phenyl acetate (ca. 25%). In a parallel experiment in which acetic anhydride had been replaced by an equimolar amount of vinyl acetate non-catalyzed acetylation did not occur.

Preparation of the immobilized enzyme

Hyflo Super Cel (10 g) was added to a solution of *Chromobacterium viscosum* lipase (3 g) in 0.1N phosphate buffer (10 mL). The slurry, spread on a Petri dish, was left to dry at room temperature (24 h) and then taken overnight at reduced pressure. The adsorbed enzyme had a water content less than 2% as determined by the K. Fisher method. Its catalytic activity in the esterification of phenol was about 5-fold higher that of the enzyme "straight from the bottle".

Enzyme-catalyzed acetylation of phenol

In a typical experiment the adsorbed *Chromobacterium viscosum* lipase (20 mg) was added to a solution of substrate (40 μmol) and vinyl acetate (200 μmol) in 2 mL of a mixture of cyclohexane-tetrahydrofuran (95/5 vol/vol). The mixture was incubated at 40 °C under continuous shaking (300 rpm). Aliquots were taken at regular time intervals and, after removal of the catalyst by centrifugation, analyzed by gas-chromatography. Identity of the products was based on comparison with authentic specimens.

Stability of the adsorbed enzyme

The adsorbed enzyme recovered after a 24 h acetylation run (phenol as the substrate) retained about 82% of the initial activity.

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