ENANTIOSELECTIVE ACYLATION OF 3-BENZYLOXY-1,2-PROPANEDIOL IN SUPERCRITICAL CARBON DIOXIDE WITH DIFFERENT LIPASES

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Enantioselective acylation of 3-benzyloxy-1,2-propanediol was studied in supercritical carbon dioxide using different lipases (porcine pancrease lipase (PPL), Lipase AK "Amano", Lipase PS "Amano", *Trichoderma reesei* RUT-C30, *Thermoascus thermophilus* (NRRL5208), *Talaromices emersonii* (NRLL3221)). Vinyl acetate was used as acyl donor. Using the studied lipases monoacetate and diacetate derivatives of the substrate were formed in different amounts during the reactions. Application of Lipase AK Amano enzyme led to the highest conversion (84.7 %) and the highest enantiomer selectivity for the diacetate compound ($ee_{diacetate}$ = 71.6 %).

INTRODUCTION

Nowadays application of enzyme catalyzed reactions are widely used in pharmaceutical and food industries. Conventionally enzymatic reactions are performed in aqueous solution. Since Randolph et al. [1], Hammond et al. [2] and Nakamura et al. [3], who described in the mid 1980's that enzyme are stable and active in supercritical solvents, there is a growing number of studies on the applications of supercritical fluids, especially carbon dioxide, as a solvent of different types of enzyme catalytic reactions. Supercritical carbon dioxide provides environmentally benign reaction media; its low critical temperature makes it an ideal solvent for heat sensitive substances. By changing the pressure and the temperature of carbon dioxide the separation of the reaction mixture becomes possible, and the final product is free from solvent residuals.

Due to the great stability of lipases under supercritical conditions most of research works are focused on lipase catalysed esterification, transesterification and hydrolyses/alcoholyses reactions. In case of biologically active substances the enantiomer selectivities of the applied enzymes are also important, since several times only one of the two enantiomers shows the required biological effect. The other one represents 50 % impurity or responsible for the side effects. A number of studies proved that lipases were also able to hold their enantioselectivity under high pressure [4-6].

Chiral glycerol derivatives are important C_3 intermediates of several biologically active subszances such as phospholipids [7], phospholipase A_2 inhibitors [8], PAF (platelet activating factors) [9].
In this study enantioselective acylation of 3-benzyloxy-1,2-propanediol was investigated in supercritical carbon dioxide in the presence of commercial lipases (porcine pancrease lipase (PPL), Lipase AK "Amano", Lipase PS "Amano") and lipases from thermophilic filamentous fungi [10] (*Trichoderma reesei* RUT-C30, *Thermoascus thermophilus* (NRRL5208), *Talaromices emersonii* (NRLL3221)). Vinyl acetate was used as acyl donor.

I-MATERIALS AND METHODS

1.1 Matherials

3-benzyloxy-1,2-propanediol was prepared in our laboratory. The used CO_2 was 99.5 %(w/w) pure and supplied from Messer Griesheim Hungaria Ltd. (Budapest).
Lipase AK and Lipase PS were obtained from Amano Europe, PPL was purchased from Sigma. Vinyl acetate was product of Aldrich, other analytical grade reagents were obtained from Reanal Ltd. (Budapest).

1.2 Methods

Enzyme catalytic reactions were performed in a thermostated, 5 ml volume tube-reactor (Figure 1.).

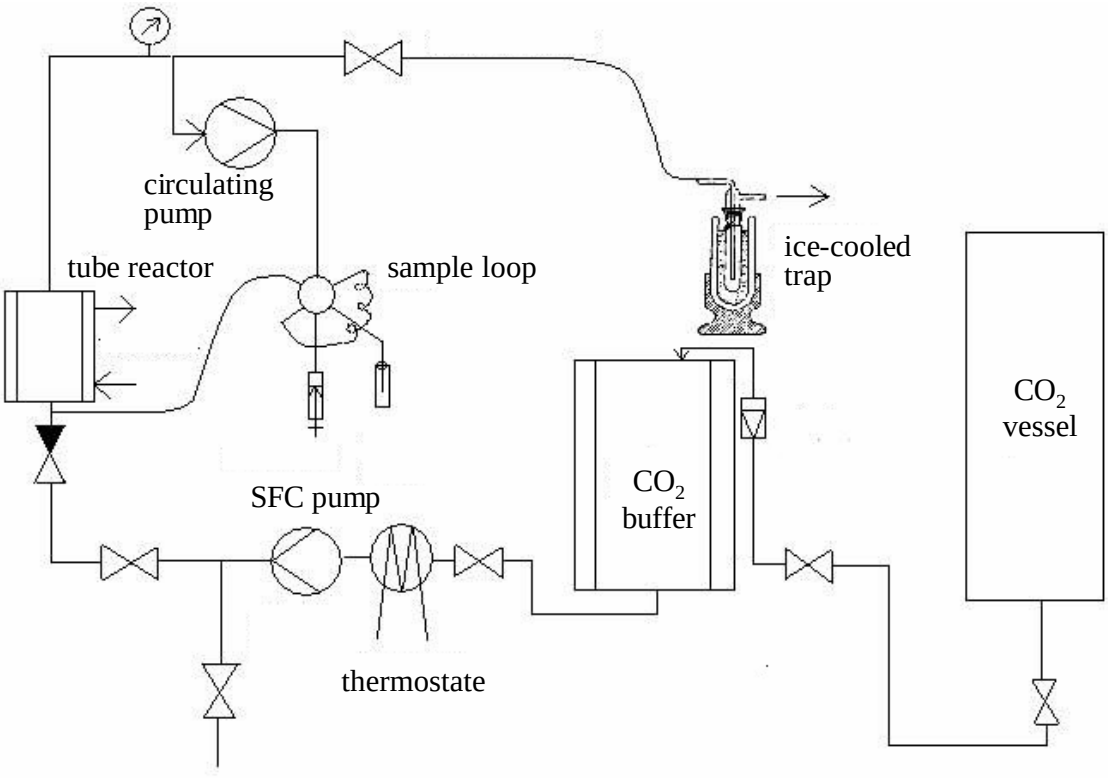


Figure 1: Laboratory scale equipment for enzymatic reaction in supercritical carbon dioxide.

In all experiments raceme 3-benzyloxy-1,2-propanediol (rac-**1,** 0.3 g) was evaporated from CH_2Cl_2 (20 ml) onto Ersorb-4 (zeolite type adsorbent, Erdokémia-ker Ltd., Budpast) and the

obtained dry material was filled into the tube reactor. Over this layer enzyme (commercial enzyme: 0.25 g, lipases from filamentous fungi: 0.50 g) and vinyl acetate (2.5 ml) were added. After the filling of the system with CO_2 by SFC 300 pump (Carlo Erba), the CO2 was circulated through the reactor at 120 bar and 38 °C at for 4 hours. Removal of the reaction mixture was carried out by releasing the CO_2 into a dry ice-cooled trap. The trap, and the support retained in the reactor were washed with acetone. The collected acetone solutions were evaporated in vacuum and the residue was analyzed by GC.

1.3 Analyses

GC analyses of the samples were performed by an AGILENT 4890D gas chromatograph at the following parameters:

Carrier gas:	hydrogen
Injector temperature:	200 °C
FID detector temperature:	250 °C
Temperature program:	40 – 230 °C, 12 min
Linear flow rate:	6 ml/min, 25 °C
Column:	0,25 µm phenyl-methyl-silica (30 m x 0.32 mm)
Split:	1:5

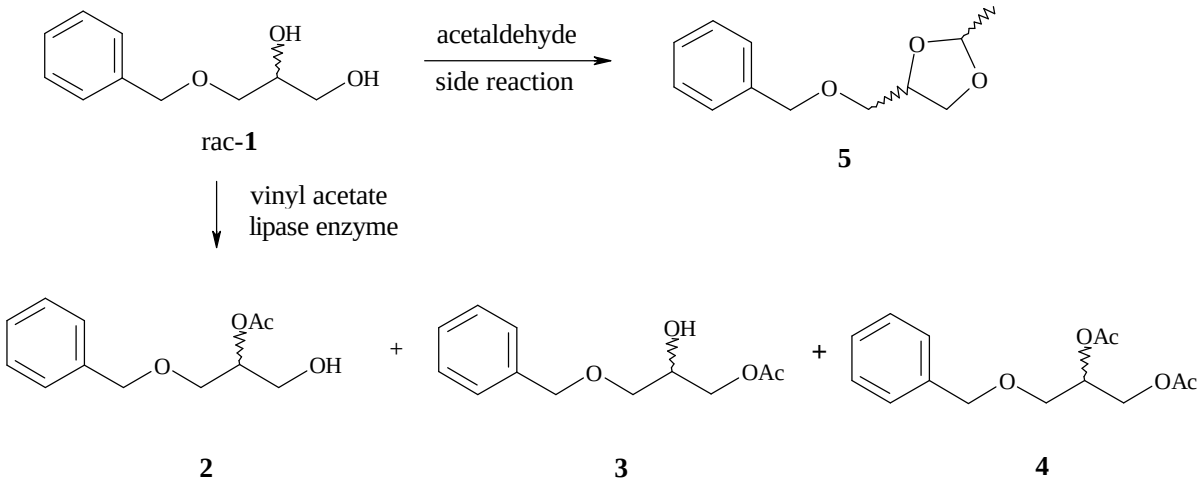
Chiral analysis:

Carrier gas:	hydrogen (3 bar)
Analysis temperature:	160 °C
Column:	FSOT (12 m x 0,01 mm)
Stationary phase:	35 % 6-tercbutyl-dimethylsilil-2,3-methyl-β-cyclodextrin
Split:	1:300

II-RESULTS AND DISCUSSION

The mechanism of the acylation reaction is given in Scheme 1. Using the studied lipases monoacetates (**2,3**) and diacetate (**4**) derivatives of the substrate were formed in different amounts during the reactions. Due to the presence of acetaldehyde formed from vinyl acetate, acetale isomers (**5**) of the substrate were also formed.

Yields of the products formed in the acylation reaction are shown in Figure 2. The commercial enzymes (PPL, Lipase PS "Amano" and Lipase AK "Amano") catalyse mainly the formation of the monoacetate-1 derivative, but in case of lipases from thermophilic fungi the amounts of the monoacete-1 and diacetate are comparable. Monoacetate-2 derivative was also detectable from all reaction mixtures, but the amounts of that were lower then 5 mol% in all cases.



Scheme 1: Acylation of 3-benzyloxipropane-1,2-diol in the presence of lipase enzyme using vinyl acetate as acyl donor.

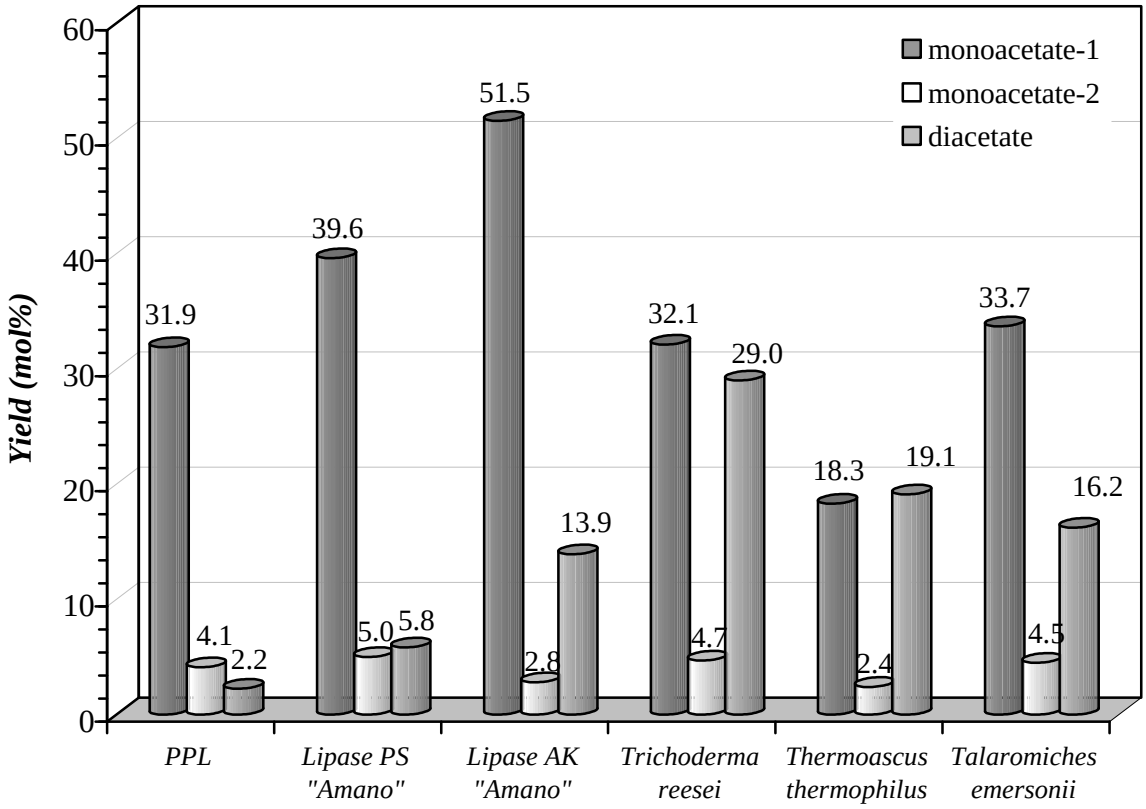


Figure 2: Yield of the acyl derivatives.

Conversion and enantiomeric excess values for the diacetate product ($ee_{diacetate}$) are summarized in Table 1. Using fungi lipases and Lipase AK the fractional conversion is above 80 %, but in case of fungi lipases the diacetate compound formed with low enantiomer selectivity ($ee_{diacetate}$ = 25 %).

Lipase AK and Lipase PS enzymes proved to be more selective for the production of the diacyl derivative with the highest degree of enantiomer selectivity ($ee_{diacetate}$ > 70 %).

From the tested six enzymes application of Lipase AK provides the acylation of rac-**1** with a high conversion and remarkable yield in enantioselective way.

Table 1: Conversion and enantiomeric excess of the diacetate derivative obtained during the acylation of rac-**1** with vinyl acetate in supercritical carbon dioxide using different lipases.

Enzyme	**Conversion (%)**	**$ee_{diacetate}$ (%)**
PPL	50.1	45.1
Lipase PS "Amano"	66.5	73.6
Lipase AK "Amano"	84.7	71.6
Trichoderma reesei	84.6	25.0
Thermoascus thermophilus	83.6	21.2
Talaromiches emersonii	80.6	19.2

CONCLUSION

Enantioselective acylation of 3-benzyloxy-1,2-propanediol was studied in supercritical carbon dioxide using commercial lipases, and prepared lipases from thermophil filamentous fungi. Amoung the enzymes investigated, Lipase AK "Amano" proved to be the most selective for providing optically active **4** product with the highest reaction conversion.

ACKNOWLEDGEMENT

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