

ENZYMATIC RESOLUTION OF A RACEMIC MIXTURE BY ACYLATION IN IONIC LIQUIDS

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INTRODUCTION

The synthesis of enantiomerically pure compounds is becoming increasingly important in the pharmaceutical and the fine chemicals industries. The use of enzymes as enantioselective catalysts in kinetic resolutions has now become a common method to obtain pure enantiomers. The applicability of this approach has been greatly expanded by replacing the conventional aqueous solutions with nonconventional solvents, not only organic media but also supercritical fluids (sc-fluids) and ionic liquids. Ionic liquids offer a new approach for catalyst recycling/reutilization when combined with a sc-fluid [1], [2]: e.g. the enzyme remains in the ionic liquid and reaction products can be extracted with sc-CO₂.

Biocatalysis is a convenient method for the kinetic resolution of alcohols. There are many reports in the literature on the resolution of secondary alcohols in ionic liquids [3], [4], [5-8]. Of these, only a few refer to aliphatic alcohols, in particular of longer alkyl chain lengths [3], [5], [8]. The use of enol-esters as acylating agents has the advantage of being irreversible but the aldehyde formed can decrease enzyme activity. This problem has been circumvented by using succinic anhydride instead [3], which additionally led to higher reaction rates and allowed facilitated separation of the target molecule. Succinic anhydride has since been used for the enzymatic resolution of a wide range of substrates [9]. Recently it has been shown that the resolution of aryl alcohols with succinic anhydride is feasible in 1-*n*-butyl-3-methylimidazolium hexafluorophosphate ([C₄mim][PF₆]) using an immobilized lipase [4].

Here we report on the kinetic resolution of (*R,S*)-2-butanol via transesterification with succinic anhydride in [C₄mim][PF₆], catalyzed by two different lipases at fixed water activity (*a_w*).

MATERIALS AND METHODS

Immobilized *Candida antarctica* lipase B (Novozym 435) was a gift from Novo Nordisk Bioindustrial, Spain. *Pseudomonas cepacia* lipase (lipase PS) was from Amano, Japan. (*R,S*)-2-butanol (> 98 % purity) was from Fluka. [C₄mim][PF₆] (> 98 % purity) was from Solchemar, Portugal, and was used without further purification. Reactions were performed in plastic vials (reaction volume up to 1 cm³) placed in a constant temperature (35 °C) orbital shaker set for 450 rpm. The ionic liquid was added to succinic anhydride (400 mM), followed by addition of the amount of water required to reach *a_w* = 0.1 [5], enzyme and, finally, (*R,S*)-2-butanol (400 mM) to start the reaction. Tridecane (3 mM) was used as external standard for GC analysis. Water concentration was measured by Karl-Fisher titration. Reaction conversion was measured by GC analysis performed with a Trace 2000 Series

Unicam gas chromatograph equipped with a 30 m x 0.32 mm i.d. fused silica capillary column coated with a 0.25 μm thickness film of 20 % 2,3-dimethyl-6-*tert*-butyldimethylsilyl)- β -cyclodextrin dissolved in BGB-15 from BGB Analytik AG.

RESULTS AND DISCUSSION

Both enzymes readily catalyzed the hydrolysis of succinic anhydride that was then followed by the reaction between succinic acid and (*R,S*)-2-butanol. The products obtained depended strongly on the enzyme used. Although reaction progress was slower with lipase PS, this enzyme was more selective for the envisaged application. As shown in Figure 1, lipase PS promoted the formation of mostly hemiesters, whereas Novozym 435 led to the formation of hemiesters and diesters in similar concentrations.

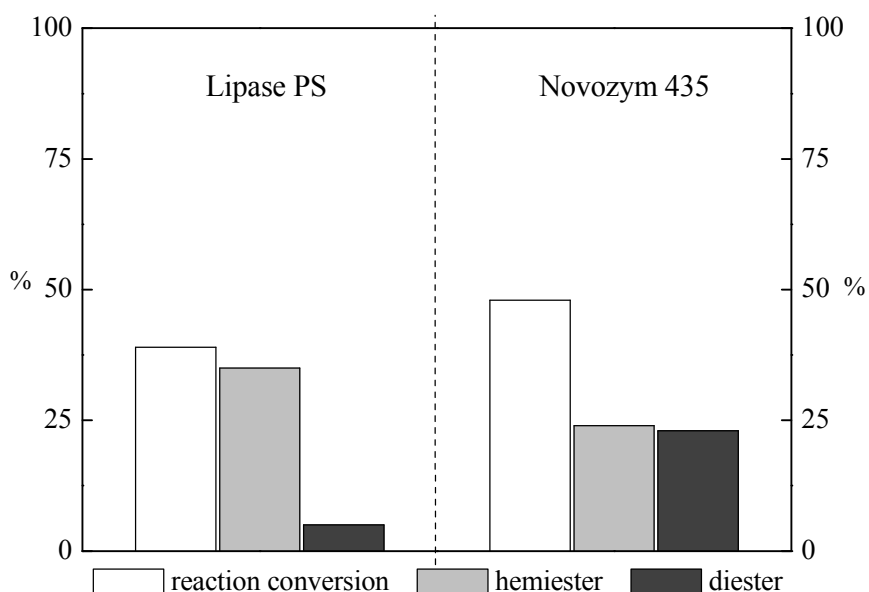


Figure 1 – Reaction conversion and selectivity after a reaction time of 8 days.

REFERENCES :

- [1] van Rantwijk, F., Madeira Lua, R., Sheldon, R. A., Trends Biotechnol., Vol. 21, **2003**, p. 131.
- [2] Garcia, S., Lourenço, N. M. T., Lousa, D., Sequeira, A. F., Mimoso, P., Cabral, J. M. S., Afonso, C. A. M., Barreiros, S., Green Chem, Vol. 6, **2004**, p. 466.
- [3] Bianchi, D., Cesti, P., Battistel, E., J. Org. Chem., Vol. 53, **1988**, p. 5531
- [4] Rasalkar, M. S., Potdar, M. K., Salunkhe, M. M., J. Mol. Catalysis B: Enzymatic, Vol. 27, **2004**, p. 267.
- [5] Reetz, M. T., Wiesenhöfer, W., Franciò, G., Leitner, W., Adv. Synth. Catal. Vol. 345, **2003**, p. 717
- [6] Gutman, A. L., Brenner, D., Boltanski, A., Tetrahedron: Asymmetry, Vol. 4, 1993, p. 839
- [7] Yamano, T., Kikumoto, F., Yamamoto, S., Miwa, K., Kawada, M., Ito, T., Ikemoto, T., Tomimatsu, K., Mizuno, Y., Chem. Lett. **2000**, p.448
- [8] Matsuda, T., Watanabe, K., Harada, T., Nakamura, K., Arita Y., Misumi, Y., Shinichiro, I., Ikariya, T., Chem. Commun., **2004**, p.2286

[9] Sorgedraeger, M. J., Malpique, R., van Rantwijk, F., Sheldon, R. A., *Tetrahedron: Asymmetry*, Vol. 15, **2004**, p. 1295

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