The application of hydrolases for the reactions of transesterification in organic solvents

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The aim of this work was to develop new biocatalytic methods for synthesis of selected esthers, that are important building blocks in organic chemistry and substrates for the synthesis of biologically active compounds. A special attention was focused on the enzymatic transesterification reactions. This research was expanded on the enzymatic hydrolysis and esterification.

A new enzymatic method for synthesis of ethyl 2-methylene-3-phenyl-3-hydroxypropionate was developed, and successfully used for the production of target compound with high enantioselectivity.

The next studies on enzymatic kinetic resolution of racemic *trans*-2-phenylocyclopropane-1-carboxylic acid revealed that the concentration of cosolvent is crucial for enantioselectivity. The developed method enabled to obtain the target ethyl esther with very good yield and enantioselectivity value 150.

A special attention was focused on the synthesis of acetoacetic acid esthers. An effective biocatalytic method for the synthesis of this class of compounds was developed. This method enables to obtain the respective products with high yields and does not require using low pressure nor high temperature. The transesterification reaction of ethyl acetoacetate was found to be efficiently catalysed by a mixture of enzymes. Systematic studies confirmed that kinetic effect of carefully selected mixture of enzymes is much higher than for a single enzyme. The group of substrates studied was expanded on keto-esters substrate, possesing the carbonyl group in alpha, gamma, delta, epsilon positions. Furthermore, it was revealed than *tert*-butyl acetoacetate is also a convenient substrate for the transesterification with alcohols.

Since *tert*-butyl esters are not substrates for enzymatic reactions, a special structure of the active site of enzyme is required to hydrolyze *tert*-butyl esthers. A new mechanism, explaining the reactivity of *tert*-butyl acetoacetate and other *tert*-butyl keto-esters in enzymatic transesterification, was proposed.