ABSTRACT

Silybin is major component of silymarin isolated from seeds of the milk thistle (*Silybum marianum*). This compound is widely used in human medicine against liver disorders and as a protectant against a number of hepatotoxins. It also exhibits other interesting activities as anticancer and chemoprotective, dermatoprotective and also hypocholesterolemic effects. Natural silybin is a nearly equimolar mixture of two diastereoisomers, silybin A and silybin B, whose analytical separation is quite feasible, but preparative separation is extremely complicated. The aim of this work was to find suitable method leading to separation of both silybin diastereoisomers. A library of hydrolases (lipases, esterases and proteases) was tested for their diastereoisomeric discrimination of the selective alcoholysis of 23-*O*-acetylsilybins. Novozym 435 (lipase B from *Candida antarctica* immobilized on acrylic resin) proved to be the most suitable enzyme for the preparative production of both optically pure silybin A and B by enzymatic hydrolysis. Under the optimized conditions, silybin A was obtained in 42 % yield and 97 % purity while silybin B was obtained in 67 % yield and 99 % purity.

Covalent modifications of Novozym 435 (acetylation, succinylation, and hydroxyethylamidation), which should lead to improvement of diastereoselectivity, were tested, but they have not resulted in increased diastereoselectivity. However, acetylated Novozym 435 exhibited similar activity and selectivity as the unmodified enzyme.

The galactosides of silybin were prepared and the ability of β-D-galactosidase to discriminate both diastereoisomers was tested. Although the prepared substrate was hydrolysed very rapidly and with high conversion, the enzyme failed to show any diastereopreference.

A new and easy way to prepare selectively protected derivatives of silybin was found. Using lipase AK and peracetylated silybin as a substrate, two products were obtained - 3,5,20,23-tetra-*O*-acetyl-silybin and 3,20,23-tri-*O*-acetyl-silybin. Their conventional preparation is not feasible and they can be used as starting materials in further chemical synthesis.

An important task for the future is to prepare new derivatives of silybin starting from its optically pure stereoisomers and determine their biological activity, focusing on the possibility to use them subsequently in the medicine.

Diploma thesis is written in Czech.