Abstract

Natural flavonoids and flavonolignans feature beneficial properties for living organisms such as antioxidant and hepatoprotective effects, anticancer, chemoprotective, dermatoprotective and hypocholesterolemic activities. Their metabolism in mammals is complex, the exact structure of their metabolites still remains partly unclear and the standards are usually not commercially available. Hence, this project focused on the preparation of potential and defined biotransformation Phase II sulfated metabolites of silymarin flavonolignans: silybin, 2,3-dehydrosilybin, isosilybin, silychristin, silydianin and flavonoids quercetin, taxifolin, rutin and isoquercitrin. Pure sulfated derivatives were prepared using aryl sulfotransferase from *Desulfitobacterium hafniense* and aryl sulfotransferase from rat liver.

Using heterologously expressed PAPS (3´-phosphoadenosine-5´-phosophosulfate) - independent arylsulfotransferase from *Desulfitobacterium hafniense* and cheap *p*-nitrophenyl sulfate as sulfate donor, sulfated flavonolignans and flavonoids were obtained in high yields. Silymarin flavonolignans afforded exclusively monosulfates at the position C-20 (C-19 in the case of silychristin), except 2,3-dehydrosilybin that yielded also the 7,20-*O*-disulfated derivative. Isoquercitrin and rutin were selectively sulfated at C-4′ position of the catechol moiety. Taxifolin was sulfated at the C-4′ position as well, however, a minor amount of the C-3′ isomer was also formed. Sulfation of quercetin proceeded preferentially at the C-3′ position, but a lower proportion of the C-4′ isomer was isolated as well.

On the contrary, recombinant mammalian PAPS-dependent aryl sulfotransferase was less efficient and had a narrower substrate specificity. The enzyme from rat liver catalyzed only sulfation of silybin B (at C-20), quercetin and taxifolin (at C-3′) as evidenced from isolated products. Silybin A and the quercetin glycosides (rutin and isoquercitrin) remained intact.

The sulfated products prepared by both aryl sulfotransferases were fully characterized by HRMS and NMR methods. The sulfated metabolites can be used for *in vitro* evaluation of biological activities and as authentic standards for metabolic studies *in vivo*.