The Use of Glycosidase from *Bupleurum aureum* Fisch. in Identification of Flavonol-3-Biosides

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Flavonol glycosides are numerous and widespread class of phenolic compounds. More than 200 aglycones and about 500 their O- and C-glycosides are known (Zaprometov, 1993). Therefore, it is a necessary to identify them in various physiological and biochemical studies. The sugars are commonly attached to the C-3 hydroxyl groups of the flavonols and rarely to the C-7 hydroxyl groups. Thus flavonol-3-glycosides are most distributed compounds and flavonol 3,7-diglycosides follow them. To identify aglycones and carbohydrate moieties flavonol glycosides are hydrolyzed by treatment with mineral acids. Rate of hydrolysis depends mainly on sugar nature, effect of aglycone nature is expressed less.

Enzymes removing disaccharides from flavonol diglycosides without splitting glycoside bonds are important for identification of them. The glycosidase possessing strong substrate specificity towards flavonol 3-biosides was isolated from *Bupleurum aureum* Fisch. The flavonol 3-rutinosides are its preferential substrates. Bupleurum glycosidase does not attack flavon 7-rutinoside and flavonol 3-monoglycosides (Zhanaeva et al., 1980). The use of *Bupleurum* glycosidase would serve as an additional method for identification of flavonol 3-biosides.

The purpose of this work was to study a possibility of application of *Bupleurum* glycosidase to establish flavonol glycosides structure using as example flowers of *Paeonia albiflora* - the plant containing flavonol glycosides which differ in aglycones and carbohydrate moieties.

It was found that the ethanol extract of *Paeonia* flowers contained six flavonol glycosides. *Bupleurum* enzyme hydrolysis of the crude ethanol extract gave three new flavonoid compounds: kaempferol, quercetin, which were found by co-chromatography with standard markers and UV spectral analyses and the unknown compound. The third compound was similar to one of six flavonol glycosides present in ethanol extract. Therefore, enzyme hydrolysis of the crude extract already allowed to conclude that at least three of six flavonol glycosides are flavonol 3-biosides. The flavonol glycosides I, II, III, IV, V and VI were isolated with column and paper chromatography. Enzyme hydrolysis of I gave quercetin, II and III - kaempferol, IV - glycoside V. *Bupleurum* glycosidase did not attack glycosides V and VI. Flavonol glycosides of flowers of *Paeonia albiflora* were identified as I - quercetin-3-glucosyarabinoside, II - kaempferol-glucosylarabinoside, III - kaempferol-3-diglucoside, IV - kaempferol-3-diglucosyl-7-glucosylglucuronide, V - kaempferol-7-glucosylglucuronide and VI - kaempferol-3-glucoside.

This procedure would be helpful for identification of flavonol 3-biosides due to their capability to undergo degradation yielding carbohydrate moieties as disaccharides. On the basis of the results presented in this communication, we propose that *Bupleurum* glycosidase could be used as additional tool for establishment of the structure of flavonol-3-biosides since it is able to remove carbohydrate moiety only from C-3 position of flavonol glycosides as disaccharides but not monosaccharides.