ABSTRACT

Analysis of anthraquinone secondary metabolites produced by *Geosmithia* spp.

Zdena Křesinová

Geosmithia species are little known fungal symbionts of bark beetles. Secondary metabolites from lilac colored species *G. lavendula* (strain MK 1008) and nine other *Geosmithia* species were investigated in order to elucidate their structures and quantify their production during submerged cultivation. Five hydroxylated anthraquinones (HAs) were isolated from culture media during submerged cultivation of the fungi and three of them were identified using NMR and MS techniques as 1,3,6,8-tetrahydroxyanthraquinone (**1**), rhodolamprometrin (1-acetyl-2,4,5,7-tetrahydroxyanthraquinone; **2**), and 1-acetyl-2,4,5,7,8-pentahydroxyanthraquinone (**3**).

Preparation, quantification and identification of HAs in fungal samples involved a SPE step, semi-preparative HPLC/UV and UPLC/UV methods. For optimization of analytical methods, separation qualities of two types of reversed phase sub-2-micron particle sized columns and one 5-micron particle sized column were tested. The most efficient Sheild RP C18 column filled with 1.7 μm particles was then used for quantification of HAs production during the cultivation period. Calibration curves for metabolites 2 and 3 (representing the majority of produced metabolites) were determined in the range from 1.95 to 1000 g mL^{-1} and exhibited correlation coefficients 0.999. Limits of detection and quantification, accuracy, precision and reproducibility of retention times were also determined.

Compounds **2** and **3** were further tested for their biological activities using a growth inhibition assay with G+/- bacteria *Staphylococcus aureus* and *Bacillus subtilis* and by an anti-inflammatory activity test with cyclooxygenase-2.