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Entomopathogenic Filamentous Fungi as Biocatalysts in Glycosylation of Methylflavonoids

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Abstract: Flavonoids are known for their numerous biological activities; however, their pharmacological application is limited by poor bioavailability. Glycosides are usually more stable and more soluble in water and in this form, flavonoids are present in nature. Likewise, the presence of the methyl group in the flavonoid skeleton results in facilitated absorption and greater bioavailability. Entomopathogenic filamentous fungi are effective in the biotransformation of flavonoids; they are known especially for efficient glycosylation. In the current study we used strains of *Beauveria bassiana* KCH J1.5 and *Isaria fumosorosea* KCH J2 to biotransform flavonoids with a single methyl group. 2'-Hydroxy-5'-methylchalcone was biotransformed by both strains into 2'-hydroxy-5'-methylchalcone 3-O-β-D-(4''-O-methyl)-glucopyranoside. In the culture of *B. bassiana* KCH J1.5 four products were obtained from 6-methylflavanone: 4'-hydroxy-6-methylflavanone 3'-O-β-D-(4''-O-methyl)-glucopyranoside; 4'-hydroxyflavanone 6-methylene-O-β-D-(4''-O-methyl)-glucopyranoside; 6-hydroxymethylflavanone 3'-O-β-D-(4''-O-methyl)-glucopyranoside and 4'-hydroxy-6-hydroxymethylflavanone 3'-O-β-D-(4''-O-methyl)-glucopyranoside. Biotransformation with *I. fumosorosea* KCH J2 as a biocatalyst resulted in the formation of 6-methylflavanone 4'-O-β-D-(4''-O-methyl)-glucopyranoside and 2-phenyl-6-methylchromane 4-O-β-D-(4''-O-methyl)-glucopyranoside. All of these flavonoids can be used in biological activity tests and can be useful in studies concerning structure—bioactivity relationships.

Keywords: flavonoids; biotransformations; glycosylation; methylchalcone; methylflavanone; O-methylglucosides; *Beauveria bassiana*; *Isaria fumosorosea*

1. Introduction

Flavonoid compounds are plant secondary metabolites engaged in various plants' interactions with the environment [1]. They are able to exert a wide range of biological activities: antioxidant, anti-inflammatory, antiallergic, antimicrobial, antiplatelet, anticancer and neuroprotective [1–3]. Their occurrence in a plant based diet is ubiquitous [4]. However, pharmacological application of flavonoid aglycons is limited by their poor bioavailability [5,6]. In nature, flavonoid derivatives—products of glycosylation, methylation, prenylation, acetylation and polymerization—are very common [7] and the most abundant are glucosides. Addition of sugar moiety (or moieties) to flavonoid aglycone is catalyzed by uridine diphosphate (UDP) glycosyltransferases (UGTs) [8,9]. Flavonoids glycosylation results in the modulation of their physicochemical and biological properties, most of all by improving their aqueous solubility and facilitating their intracellular and intercellular transportation. Nonetheless, the impact of the glycosylation on the biological properties of flavonoids is complicated, because it depends on the structure of the flavonoid core, the position of the sugar attachment and the number of sugar moieties [10–16].

Many flavonoid glycosides with potential biological activity occur in plants in small amounts and therefore their extraction is difficult. Chemical synthesis is inefficient in the production of flavonoid glycosides, because of laborious procedures necessary to achieve regio- and stereo-selectivity, and low isolated yields due to drastic reaction conditions resulting in the decomposition of aglycones. However, flavonoid glycosides, both well-known and novel, can be obtained by microbial glycosylation of already existing flavonoid aglycons. Enzymatic glycosylation allows to overcome these obstacles. It can be carried out by a few different methods: whole-cell biotransformation, in vivo total biosynthesis and in vitro enzymatic reaction [11,12,17–20]. Among these methods biotransformation is promising, because it offers an eco-friendly, one-pot and one-step process [21].

Methylation at the ring position of flavonoids leads to derivatives with increased metabolic stability due to insusceptibility to glucuronic acid or sulfate conjugation. Methylation also leads to greatly improved intestinal absorption, and bioavailability [1,7,22]. Methylated flavonoids are widely present in plants, but still less abundant than unmethylated flavonoid aglycons and flavonoid glycosides. Among two types of methylation in flavonoids, O-methylation and C-methylation, the second one is less common and has been found in limited plant species, such as *Cleistocalyx operculatus* [23–25], *Picea neoveitchii* [26], *Corymbia torelliana* [27,28] and *Campomanesia xanthocarpa* [29], and occurs especially at C-6 of the flavonoid skeleton.

There is some evidence of biological activity of C-methylated flavonoids isolated from plants. From the twigs and leaves of *P. neoveitchii* Mast, two C-methylated flavonoids were isolated: 5,7-dihydroxy-3-methoxy-6-methylflavone 8,4'-di-O- β -D-glucopyranoside and 4',5,7,8-tetrahydroxy-3-methoxy-6-methylflavone 8-O- β -D-glucopyranoside. The first one exhibited moderate antifungal activity against several plant pathogens (*Pyricularia grisea* (Cooke) Sacc., *Sclerotium rocsii* Sacc. and *Alternaria mali* Roberts). The second one showed a significant inhibitory effect against *S. rocsii*. Both compounds exhibited potent cytotoxicity against *Spodoptera litura* Fabricius [26].

Methylated chalcones from *C. operculatus* also showed biological activity. (E)-2',4,4'-Trihydroxy-6'-methoxy-3',5'-dimethylchalcone, 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone, 2',4'-dihydroxy-3'-methyl-6'-methoxychalcone and 2,2',4'-trihydroxy-6'-methoxy-3',5'-dimethylchalcone displayed notable inhibitory activity on the viral neuraminidases from two influenza viral strains, H1N1 and H9N2. The first one exhibited strong inhibition against the neuraminidases from novel influenza H1N1 (WT) and oseltamivir-resistant novel H1N1 (H274Y mutant) expressed in 293T cells. Kinetic studies have indicated that tested chalcones acted as noncompetitive inhibitors to neuraminidases which are responsible for enabling viruses to leave infected cells by decomposition of their cell membranes [25].

In studies conducted on oocytes of *Xenopus laevis* with expressed human recombinant GABA_A receptors 6-methylflavanone acted as a positive allosteric modulator. GABA_A receptors are the main inhibitory neurotransmitters in the central nervous system and are classified under six subfamilies of protein subunits: α , β , γ , δ , ϵ and θ . Anxiolysis and sedation are considered to be mediated via GABA_A receptors (α_2 and α_1 , respectively). The tested compound was much more effective at $\alpha_2\beta_2\gamma_{2L}$ than at $\alpha_1\beta_2\gamma_{2L}$ and $\alpha_1\beta_2$ GABA_A receptors. This may be quite a unique type of selectivity, significant in seeking new targeted drugs, acting as GABA_A receptors agonists and producing certain sedative effects without the unwanted side effects of benzodiazepines [30].

B. bassiana is an entomopathogenic filamentous fungi that affects a broad range of arthropods. It has been used as a biological control agent of various arthropod pests for many years [31–33]. The pathogenesis and virulence of *Beauveria* isolates is connected with the production of toxicogenic metabolites, antioxidant enzymes and primarily extracellular cuticle-degrading enzymes such as chitinases, lipases, and proteases [33,34]. Since *B. bassiana* genome sequence is already known [35] there is a lot of interest in recognition of genes involved in its pathogenesis and virulence [32,36]. Enzymatic systems of *B. bassiana*, that are responsible for a cuticle-degradation are also applied to the biotransformations. *B. bassiana* has the ability to transform a broad range of substrates such as cyclic and aliphatic ketones, flavonoids and steroids [37]. This fungus is capable of performing reactions such as hydroxylation, glycosylation or deglycosylation, acetylation and reduction [37]. Most of the

enzymes responsible for these reactions have not been described until now [37]. Likewise *I. fumosorosea* serves as a biological control agent and alternative to chemical pesticides [38]. Its secondary metabolites exhibit a great variety of biological activities [39]. This genus was used as a biocatalyst by our team in previous studies of flavonoid biotransformations [12–15].

The current studies are a continuation of our previous work with 6-methylflavone as the biotransformation substrate [12]. In the present study we synthesized two flavonoid compounds with methyl group at C-6 of flavanone skeleton (C-5' in the case of chalcone) and afterwards biotransformed them with entomopathogenic filamentous fungi strains of *B. bassiana* KCH J1.5 and *I. fumosorosea* KCH J2 as biocatalysts. As a result of 2'-hydroxy-5'-methylchalcone biotransformation in both cultures one main product 2'-hydroxy-5'-methylchalcone 3-O- β -D-(4''-O-methyl)-glucopyranoside was obtained. 6-Methylflavanone was efficiently biotransformed by both used biocatalysts. Each strain formed different biotransformation products. Six flavanones 4''-O-methylglucopyranosides were obtained. All biotransformation products have not been previously described in the scientific literature. The biotransformation products may be utilized in further studies as potentially biologically active compounds and can be useful in investigations concerning structure—bioactivity relationships.

2. Results and Discussion

The following results are a continuation of studies focused on biotransformations of flavonoid compounds with a methyl group at C-6 in cultures of entomopathogenic filamentous fungi. In the previous paper about biotransformation of 6-methylflavone by the strain *I. fumosorosea* KCH J2, two new products were obtained: 6-methylflavone 8-O- β -D-(4''-O-methyl)-glucopyranoside (6.6% yield) and 6-methylflavone 4'-O- β -D-(4''-O-methyl)-glucopyranoside (13.2% yield) [12].

In the present paper two flavonoid substrates (chalcone and flavanone), that are analogs of the 6-methylflavone, were utilized. Two strains of the entomopathogenic filamentous fungi *I. fumosorosea* KCH J2 and *B. bassiana* KCH J1.5, isolated from the environment and characterized previously [12,37] were used as biocatalysts. Experiments performed in a semi-preparative scale enabled us to determine the chemical structures of biotransformation products and their isolated yields unambiguously. Hereby, seven biotransformation products that have not yet been described in the scientific literature were obtained: 2'-hydroxy-5'-methylchalcone 3-O- β -D-(4''-O-methyl)-glucopyranoside (**3a**), 4'-hydroxy-6-methylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4a**), 4'-hydroxyflavanone 6-methylene-O- β -D-(4''-O-methyl)-glucopyranoside (**4b**), 6-hydroxymethylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4c**), 4'-hydroxy-6-hydroxymethylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4d**), 6-methylflavanone 4'-O- β -D-(4''-O-methyl)-glucopyranoside (**4e**) and 2-phenyl-6-methylchromane 4-O- β -D-(4''-O-methyl)-glucopyranoside (**4f**).

The substrates for biotransformation were obtained by a two-step synthesis (Scheme 1).

Scheme 1. Synthesis of 2'-hydroxy-5'-methylchalcone (**3**) and 6-methylflavanone (**4**).

2.1. Biotransformations of 2'-hydroxy-5'-methylchalcone (**3**)

In the experiment 2'-hydroxy-5'-methylchalcone (**3**) was synthesized in a Claisen–Schmidt condensation reaction between 2'-hydroxy-5'-methylacetophenone (**1**) and benzaldehyde (**2**) with a 50.1% yield. As a result of its 10-day biotransformation, in the *B. bassiana* KCH J1.5 culture one main product 2'-hydroxy-5'-methylchalcone 3-O- β -D-(4''-O-methyl)-glucopyranoside (**3a**) with a 29.5% yield (Scheme 2) was isolated and purified by means of the preparative Thin Layer Chromatography

(TLC) method with chloroform and methanol (9:1 *v/v*) as eluents. The same product was obtained using *I. fumosorosea* KCH J2 as a biocatalyst.

Scheme 2. Microbial transformation of 2'-hydroxy-5'-methylchalcone (**3**) in *B. bassiana* KCH J1.5 culture.

The product **3a** was analyzed by NMR spectroscopy that allowed to establish its chemical structure (Tables 1 and 2, Scheme 3).

The presence of a glucose unit in the compound **3a** was confirmed by five characteristic carbon signals observed in the region from $\delta = 80.5$ ppm to $\delta = 62.3$ ppm in the Carbon-13 Nuclear Magnetic Resonance (^{13}C -NMR) spectrum, as well as proton signals of δH ranging from $\delta = 3.92$ ppm to $\delta = 3.19$ ppm in the Proton Nuclear Magnetic Resonance (^1H -NMR) spectrum. Moreover, the attachment of a sugar unit to substrate **3** was confirmed by a one-proton doublet from the proton at the anomeric carbon atom at $\delta = 5.03$ ppm in the ^1H -NMR spectrum. The β -configuration of the glucose unit was proved by the coupling constant ($J = 7.8$ Hz) for the anomeric proton. A three-proton singlet at $\delta = 3.57$ ppm in the ^1H -NMR spectrum and the corresponding signal at $\delta = 60.6$ ppm in the ^{13}C -NMR spectrum evidences that one of the hydroxyl groups of sugar unit has been methylated. *O*-methylation occurred in the C-4'' hydroxyl group of the glucose unit. It was detected in the Heteronuclear Multiple Bond Coherence (HMBC) spectrum, where the proton signal due to $-\text{OCH}_3$ was correlated with the signal of C-4'' (about $\delta = 80$ ppm) in the glucose unit. Sugar moiety was attached to C-3, because in the HMBC spectrum signal due to the proton at hemiacetal carbon atom ($\delta = 5.03$ ppm) was correlated with the C-3 signal ($\delta = 159.2$ ppm), which was shifted from $\delta = 129.2$ ppm, indicating the attachment of an electronegative atom. Additionally, protons in the ^1H -NMR spectrum at C-2 and C-6 became non-equivalent, and the proton at C-3 disappeared. Shifted signals can be observed: one multiplet from the proton at C-2 ($\delta = 7.61$ ppm), which became isolated in the correlation spectrum, one doublet of doublets of doublets from the proton at C-4 ($\delta = 7.16$ ppm) and one doublet from the proton at C-6 ($\delta = 7.47$ ppm). Signal from proton at C-5 was slightly shifted and merged with signal from the proton at C-4'. Signals from one proton of the hydroxyl group at C-2' and three protons of the methyl group at C-5 remained intact.

Scheme 3. Key Correlation Spectroscopy (COSY), (on the left) and HMBC (on the right) correlations for the structure elucidation of product **3a**.

Table 1. ^1H -NMR chemical shifts δ (ppm) and coupling constants J (Hz) of 2'-hydroxy-5'-methylchalcone (**3**) in Chloroform- d , and product of its biotransformation **3a** in Acetone- d_6 , 600 MHz (Supplementary Materials).

Proton	Compound	
	3	3a
H-2	7.68 (m)	7.61 (m)
H-3	7.45 (dd) $J = 4.8, 1.8$	-
H-4	7.45 (dd) $J = 4.8, 1.8$	7.16 (ddd) $J = 8.1, 2.3, 0.6$
H-5	7.45 (dd) $J = 4.8, 1.8$	7.40 (dd) $J = 13.0, 5.0$
H-6	7.68 (m)	7.47 (d) $J = 7.7$
H- α	7.68 (m)	8.03 (d) $J_{\alpha, \beta} = 15.5$
H- β	7.92 (d) $J_{\beta, \alpha} = 15.5$	7.87 (d) $J_{\beta, \alpha} = 15.5$
H-2'	-	-
H-3'	6.94 (d) $J = 8.4$	6.89 (d) $J = 8.4$
H-4'	7.32 (dd) $J = 8.4, 2.1$	7.40 (dd) $J = 13.0, 5.0$
H-5'	-	-
H-6'	7.68 (m)	8.12 (d) $J = 1.3$
H-1''	-	5.03 (d) $J = 7.8$
H-2''	-	3.52 (ddd) $J = 15.1, 9.1, 5.1$
H-3''	-	3.66 (m)
H-4''	-	3.19 (m)
H-5''	-	3.61 (ddd) $J = 9.7, 5.8, 2.2$
H-6''	-	3.92 (ddd) $J = 11.8, 5.6, 2.1$ 3.73 (dt) $J = 12.1, 6.2$
C4''-OCH ₃	-	3.57 (s)
C2'-OH	12.63 (s)	12.67 (s)
C5'-CH ₃	2.36 (s)	2.35 (s)

Table 2. ^{13}C -NMR chemical shifts δ (ppm) of 2'-hydroxy-5'-methylchalcone (**3**) in Chloroform-d, and product of its biotransformation **3a** in Acetone-d₆, 151 MHz (Supplementary Materials).

Carbon	Compound	
	3	3a
C-1	134.8	137.1
C-2	128.8	116.7
C-3	129.2	159.2
C-4	131.0	120.2
C-5	129.2	130.8
C-6	128.8	124.3
C- α	120.4	122.0
C- β	145.4	145.6
C-1'	119.8	120.5
C-2'	161.7	162.5
C-3'	118.5	118.7
C-4'	137.7	138.5
C-5'	128.1	129.3
C-6'	129.5	131.1
C-1''	-	101.7
C-2''	-	75.0
C-3''	-	78.1
C-4''	-	80.5
C-5''	-	77.2
C-6''	-	62.3
C4''-OCH ₃	-	60.6
C = O	193.8	194.9
C5'-CH ₃	20.8	20.4

Glycosylation of chalcones is rarely reported. Among reported microbial transformations of the chalcones into their glycosides, there are known reactions with xanthohumol as a substrate. These biotransformations led to the production of: xanthohumol 4'-O- β -D-glucopyranoside in the cultures of *Penicillium chrysogenum* 6933 [40], *Absidia coerulea* AM93 and *Rhizopus nigricans* UPF701 [41], xanthohumol 4,4'-O- β -diglucopyranoside in the culture of *P. chrysogenum* 6933 [40], xanthohumol 4'-O- β -D-(4''-O-methyl)-glucopyranoside in the cultures of *B. bassiana* AM278 [42] and *B. bassiana* AM446 [41], and isoxanthohumol 7-O- β -D-glucopyranoside in the cultures of *Cunninghamella elegans* 6992 [40] and *Mortierella mutabilis* AM404 [41]. Several studies with enzymes of the glycosyltransferase family led to the formation of the chalcone glycosides at three sites: 2', 4' and less often 4 [43]. However, there is no evidence of the glycosylation at C-3 of the chalcone skeleton and also no reports of 2'-hydroxy-5'-methylchalcone (**3**) biotransformations.

2.2. Biotransformations of 6-methylflavanone (**4**) in the culture of *B. bassiana* KCH J1.5

6-Methylflavanone (**4**) was synthesized from 2'-hydroxy-5'-methylchalcone (**3**) with 62.3% yield and utilized in 10-day biotransformation in the culture of *B. bassiana* KCH J1.5. Four biotransformation products were obtained and isolated: 4'-hydroxy-6-methylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4a**) with 6.9% yield, 4'-hydroxyflavanone 6-methylene-O- β -D-(4''-O-methyl)-glucopyranoside (**4b**) with 7.9%

yield, 6-hydroxymethylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4c**) with 3.4% yield and 4'-hydroxy-6-hydroxymethylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4d**) with 8.0% yield (Scheme 4).

Scheme 4. Microbial transformation of 6-methylflavanone (**4**) in *B. bassiana* KCH J1.5 culture.

The products **4a–4d** were analyzed by NMR spectroscopy which allowed for the establishment of their chemical structures (Tables 3 and 4, Schemes 5–8).

The presence of a glucose unit in compounds **4a–4d** was established by five characteristic carbon signals observed in the region from about $\delta = 80.0$ ppm to about $\delta = 62.0$ ppm in the ^{13}C -NMR spectra, as well as proton signals of δH ranging from about $\delta = 3.9$ ppm to $\delta = 3.1$ ppm in the ^1H -NMR spectra. Additionally, the attachment of a sugar unit to substrate **4** was confirmed by a one-proton doublet from the proton at the anomeric carbon atom in the ^1H -NMR spectrum of **4a** at $\delta = 4.79$ ppm, **4b** at $\delta = 4.36$ ppm, **4c** at $\delta = 4.98$ ppm and **4d** at $\delta = 4.82$ ppm. The β -configuration of the glucose unit was proved by the coupling constant (about $J = 7.9$ Hz) for the anomeric proton. A three-proton singlet at about $\delta = 3.6$ ppm in the ^1H -NMR spectra and the corresponding signal at about $\delta = 60.6$ ppm in the ^{13}C -NMR spectra of **4a–4d** evidences that one of the hydroxyl groups of sugar unit has been methylated. O-methylation occurred in all products at the C-4'' hydroxyl group of the glucose unit. It was detected in the HMBC spectra, that the proton signals due to $-\text{OCH}_3$, were correlated with the signals of C-4'' (about $\delta = 80$ ppm) in the glucose units.

Based on the HMBC spectrum, a sugar moiety was attached to C-3' in the case of compound **4a**, due to the proton at the anomeric carbon atom ($\delta = 4.79$ ppm) being coupled with the C-3' signal ($\delta = 146.1$ ppm), which was shifted from $\delta = 129.5$ ppm, indicating the attachment of an electronegative atom. Moreover, protons in the ^1H -NMR spectrum at C-2' and C-6' became non-equivalent and protons at C-3' and C-4' were not present, suggesting substitution. The C-4' signal at the ^{13}C -NMR spectrum ($\delta = 149.0$ ppm), which was shifted from $\delta = 129.3$ ppm, indicates the attachment of an electronegative oxygen atom from a hydroxyl group. In the ^1H -NMR spectrum shifted signals of flavanone ring B can be observed: one multiplet from the proton at C-2' ($\delta = 7.39$ ppm), one doublet from the proton at C-5' ($\delta = 6.91$ ppm), and one doublet of triplets at C-6' ($\delta = 7.16$ ppm). In the COSY spectrum protons at C-5' and at C-6' correlate, and the proton at C-2' does not couple with any proton, which also confirms substitution at C-3' and C-4'. In the HMBC spectrum, a strong cross-peak between C-3' and H-2' and between C-3' and H-5', while no peak between C-3' and H-6', can be observed. At the same time a strong cross-peak between C-4' and H-2' (and H-5' and H-6') can be seen. Cross-peak by three chemical bonds occurs less likely than by two chemical bonds in HMBC experiments. Therefore, we assigned the C-3' signal as having cross-peaks by one, and two chemical bonds, and the C-4' signal as having cross-peaks by one and two chemical bonds [44–46]. Chemical shifts of the other signals in the ^1H -NMR and ^{13}C -NMR spectra have only slightly changed. The signal from the three protons of the methyl group at C-6 remained at the same position as in the ^1H -NMR spectrum of substrate **4** which indicates the presence of the methyl group in product **4a**.

Table 3. ^1H -NMR chemical shifts δ (ppm) and coupling constants J (Hz) of 6-methylflavanone (**4**) and products of its biotransformation **4a–4f** in Acetone- d_6 , 600 MHz (Supplementary Materials).

Proton	Compound						
	4	4a	4b	4c	4d	4e	4f
H-2	5.59 (dd) $J_{2,3ax} = 13.0$, $J_{2,3eq} = 2.8$	5.46 (dd) $J_{2,3ax} = 12.8$, $J_{2,3eq} = 2.9$	5.52 (dd) $J_{2,3ax} = 13.0$, $J_{2,3eq} = 2.8$	5.61 (dd) $J_{2,3ax} = 12.5$, $J_{2,3eq} = 3.0$	5.48 (dd) $J_{2,3ax} = 12.8$, $J_{2,3eq} = 2.9$	5.53 (dd) $J_{2,3ax} = 12.9$, $J_{2,3eq} = 2.9$	5.36 (dd) $J = 12.1$, $J = 1.9$
H-3 _{ax}	3.11 (dd) $J_{3ax,2} = 13.0$, $J_{3ax,3eq} = 16.7$	3.11 (dd) $J_{3ax,2} = 12.8$, $J_{3ax,3eq} = 16.7$	3.16 (dt) $J = 8.3$, $J_{3ax,3eq} = 16.8$	3.13 (dd) $J_{3ax,2} = 12.5$, $J_{3ax,3eq} = 16.8$	3.15 (dd) $J_{3ax,2} = 12.9$, $J_{3ax,3eq} = 16.8$	3.12 (dd) $J_{3ax,2} = 12.9$, $J_{3ax,3eq} = 16.7$	2.43 (dt) $J = 14.1$, $J = 2.2$
H-3 _{eq}	2.85 (d) $J_{3eq,2} = 2.9$	2.78 (dd) $J_{3eq,2} = 2.9$, $J_{3eq,3ax} = 16.8$	2.79 (dd) $J_{3eq,2} = 2.9$, $J_{3eq,3ax} = 16.8$	2.90 (d) $J = 3.2$	2.80 (dd) $J_{3eq,2} = 2.9$, $J_{3eq,3ax} = 16.7$	2.79 (dd) $J_{3eq,2} = 2.9$, $J_{3eq,3ax} = 16.8$	2.07 (ddd) $J = 6.0$, $J = 3.6$, $J = 1.2$
H-4	-	-	-	-	-	-	4.92 (t) $J = 2.8$
H-5	7.63 (s)	7.61 (d) $J_{5,7} = 2.3$	7.83 (d) $J_{5,7} = 2.2$	7.82 (d) $J_{5,7} = 2.1$	7.82 (d) $J_{5,7} = 2.3$	7.62 (d) $J_{5,7} = 1.5$	7.22 (d) $J_{5,7} = 1.9$
H-6	-	-	-	-	-	-	-
H-7	7.40 (dt) $J = 10.9$, $J = 4.7$	7.39 (m)	7.61 (dd) $J_{7,8} = 8.5$, $J_{7,5} = 2.2$	7.58 (dd) $J_{7,8} = 8.5$, $J_{7,5} = 2.3$	7.56 (dd) $J_{7,8} = 8.5$, $J_{7,5} = 2.1$	7.39 (m)	7.08 (dd) $J_{7,8} = 8.3$, $J_{7,5} = 1.9$
H-8	6.98 (d) $J_{8,7} = 8.4$	6.96 (d) $J_{8,7} = 8.4$	7.02 (d) $J_{8,7} = 8.6$	7.07 (dd) $J_{8,7} = 8.3$, $J = 1.7$	7.02 (d) $J_{8,7} = 8.5$	6.95 (d) $J_{8,7} = 8.4$	6.80 (d) $J_{8,7} = 8.3$
H-2'	7.58 (d) $J_{2',3'} = 7.6$	7.39 (m)	7.41 (m)	7.29 (m)	7.41 (dd) $J = 4.5$, $J = 2.1$	7.49 (m)	7.50 (d) $J = 7.2$
H-3'	7.45 (t) $J = 7.5$	-	6.90 (m)	-	-	7.12 (m)	7.40 (t) $J = 7.6$
H-4'	7.40 (dt) $J = 10.9$, $J = 4.7$	-	-	7.07 (dd) $J = 8.3$, $J = 1.7$	-	-	7.33 (m)
H-5'	7.45 (t) $J = 7.5$	6.91 (d) $J = 8.2$	6.90 (m)	7.35 (t) $J = 8.0$	6.92 (d) $J = 8.2$	7.12 (m)	7.40 (t) $J = 7.6$
H-6'	7.58 (d) $J = 7.6$	7.16 (dt) $J = 2.0$, $J = 8.2$	7.41 (m)	7.21 (d) $J = 7.7$	7.17 (dd) $J_{6',5'} = 8.3$, $J = 1.9$	7.49 (m)	7.50 (d) $J = 7.2$
H-1''	-	4.79 (d) $J = 7.9$	4.36 (d) $J = 7.8$	4.98 (d) $J = 7.8$	4.82 (d) $J = 7.9$	4.97 (d) $J = 7.8$	4.49 (d) $J = 7.8$
H-2''	-	3.50 (t) $J = 9.4$	3.26 (m)	3.48 (m)	3.50 (t) $J = 8.2$	3.48 (ddd) $J = 9.8$, $J = 5.0$, $J = 2.0$	3.30 (m)
H-3''	-	3.63 (m)	3.50 (m)	3.62 (ddd) $J = 9.1$, $J = 7.6$, $J = 4.2$	3.63 (d) $J = 6.6$	3.63 (t) $J = 9.0$	3.52 (m)
H-4''	-	3.22 (m)	3.10 (m)	3.21 (m)	3.21 (m)	3.21 (dd) $J = 9.6$, $J = 9.1$	3.12 (dd) $J = 9.6$, $J = 9.0$
H-5''	-	3.45 (ddd) $J = 9.8$, $J = 4.9$, $J = 1.9$	3.26 (m)	3.48 (m)	3.46 (m)	3.48 (ddd) $J = 9.8$, $J = 5.0$, $J = 2.0$	3.30 (m)
H-6''	-	3.85 (dt) $J = 8.5$, $J = 4.4$, 3.69 (m)	3.82 (d) $J = 10.0$, 3.66 (m)	3.84 (dd) $J = 9.3$, $J = 5.2$, 3.68 (m)	3.85 (d) $J = 10.6$, 3.68 (m)	3.83 (d) $J = 12.2$, 3.69 (m)	3.89 (m) 3.71 (dt) $J = 11.4$, $J = 5.8$
-CH ₂ -	-	-	4.86 (dd) $J = 11.8$, $J = 2.6$, 4.61 (dt) $J = 11.7$, $J = 2.7$	4.61 (d) $J = 5.2$	4.61 (m)	-	-
C6-CH ₃	2.32 (s)	2.31 (s)	-	-	-	2.31 (s)	2.27 (s)
C4''-OCH ₃	-	3.55 (s)	3.53 (s)	3.56 (s)	3.55 (s)	3.56 (s)	3.54 (s)

Table 4. ^{13}C -NMR chemical shifts δ (ppm) of 6-methylflavanone (**4**) and products of its biotransformation **4a–4f** in Acetone- d_6 , 151 MHz (Supplementary Materials).

Carbon	Compound						
	4	4a	4b	4c	4d	4e	4f
C-2	80.3	80.0	80.4	80.0	80.1	80.0	73.8
C-3	45.1	44.9	44.8	44.9	44.7	44.9	37.5
C-4	191.9	192.3	192.3	191.9	192.3	192.2	70.2
C-4a	121.6	121.6	121.5	121.5	121.5	121.5	120.5
C-5	127.0	126.9	126.7	125.2	125.2	126.9	132.7
C-6	131.6	131.5	132.3	136.7	136.5	131.5	129.6
C-7	137.7	137.7	136.7	135.6	135.5	137.8	131.5
C-8	118.8	118.8	118.8	117.1	118.8	118.7	117.5
C-8a	160.5	160.6	162.0	161.4	161.5	160.5	154.1
C-1'	140.5	131.8	131.0	141.9	131.6	133.9	142.7
C-2'	127.3	117.9	129.0	115.4	117.7	128.7	127.1
C-3'	129.5	146.1	116.2	158.9	146.1	117.3	129.2
C-4'	129.3	149.0	158.7	118.8	149.0	158.8	128.6
C-5'	129.5	116.8	116.2	130.5	116.8	117.3	129.2
C-6'	127.3	123.3	129.0	120.8	123.3	128.7	127.1
C-1''	-	104.2	103.0	101.5	104.0	101.6	101.4
C-2''	-	75.0	75.2	75.0	74.9	74.9	75.3
C-3''	-	77.5	78.1	78.1	77.5	77.9	78.2
C-4''	-	80.1	80.5	80.1	80.2	80.1	80.6
C-5''	-	77.3	76.9	77.0	77.2	77.0	76.9
C-6''	-	62.0	62.4	62.1	62.0	62.0	62.6
C6-CH ₂ -OH	-	-	70.8	63.9	63.9	-	-
C-4''-OCH ₃	-	60.6	60.5	60.5	60.6	60.5	60.5
C6-CH ₃	20.4	20.4	-	-	-	20.4	20.5

Scheme 5. Key COSY (on the left) and HMBC (on the right) correlations for the structure elucidation of product **4a**.

In the case of compound **4b**, the sugar moiety was attached to the hydroxyl group—previously formed by microbial oxidation of the methyl group—because in the HMBC spectrum the proton at the anomeric carbon atom ($\delta = 4.78$ ppm) was coupled with the C-6-CH₂- signal ($\delta = 70.3$ ppm). Moreover, the signal from the three protons of the methyl group at C-6 present in the ^1H -NMR spectrum ($\delta = 2.32$ ppm) of substrate **4** disappeared and two signals appeared from protons at 4.86 ppm and

4.61 ppm (unequal protons from methylene group), which confirms attachment of the glucose unit to the methylene bridge at C-6. In the ^1H -NMR spectrum, proton signals of the flavanone ring A became only slightly shifted because of it. Moreover, signals from protons of the ring B became shifted, indicating another substitution in ring B. Two multiplets from the protons at C-2' and C-6' ($\delta = 7.41$ ppm) and at C-3' and C-5' ($\delta = 6.90$ ppm) were observed and the signal from proton at C-4' was missing which indicates a substitution at C-4'. In the ^{13}C -NMR spectrum C-4' signal ($\delta = 158.7$ ppm), which was shifted from $\delta = 129.3$ ppm, indicates the attachment of a hydroxyl group. Chemical shifts of the other signals in the ^1H -NMR and ^{13}C -NMR spectra have only slightly changed that signalize that the flavanone skeleton remained intact.

Scheme 6. Key COSY (on the left) and HMBC (on the right) correlations for the structure elucidation of product **4b**.

Based on the HMBC spectrum, in compound **4c**, a sugar moiety was attached at C-3', due to the proton at the anomeric carbon atom ($\delta = 4.98$ ppm) being coupled with the C-3' signal ($\delta = 158.9$ ppm), which was shifted from $\delta = 129.5$ ppm, and points out the attachment of an electronegative atom. Moreover, protons in the ^1H -NMR spectrum at C-2' and C-6' became non-equivalent. In the ^1H -NMR spectrum shifted proton signals of ring B can be observed: multiplet from proton at C-2' ($\delta = 7.29$ ppm), triplet from proton at C-5' ($\delta = 7.35$ ppm) and doublet from proton at C-6' ($\delta = 7.21$ ppm), and signal from proton at C-4' that overlapped with the signal from proton at C-8 ($\delta = 7.07$ ppm). In the COSY spectrum proton at C-2' became isolated, confirming substitution at C-3'. Moreover, the signal from the three protons of the methyl group at C-6 present in the ^1H -NMR spectrum ($\delta = 2.32$ ppm) of substrate **4** disappeared. The presence of another moiety—a hydroxymethyl group at C-6 can be noticed in the ^{13}C -NMR spectrum, because the signal from C-6 was shifted from $\delta = 131.6$ ppm to $\delta = 136.7$ ppm. It was also confirmed by the appearance of a signal from two protons at 4.61 ppm [47] and slightly shifted signals from protons of ring A: doublet from the proton at C-5 ($\delta = 7.82$ ppm), doublet of doublets from the proton at C-7 ($\delta = 7.58$ ppm) and doublet of doublets from the proton at C-8 ($\delta = 7.07$ ppm). Chemical shifts of the other signals in the ^1H -NMR and ^{13}C -NMR spectra have only slightly changed which indicates that the flavanone skeleton remained unaffected.

Scheme 7. Key COSY (on the left) and HMBC (on the right) correlations for the structure elucidation of product **4c**.

Based on the HMBC spectrum, a sugar moiety was attached to C-3' in the case of compound **4d**, due to the proton at the anomeric carbon atom ($\delta = 4.81$ ppm) being coupled with the C-3' signal ($\delta = 146.1$ ppm), which was shifted from $\delta = 129.5$ ppm. Additionally, protons in the ^1H -NMR spectrum at C-2' and C-6' became non-equivalent. Shifted signals can be observed: one doublet of doublets

from the proton at C-2' ($\delta = 7.41$ ppm), one doublet of triplets at C-6' ($\delta = 7.17$ ppm), and one doublet from the proton at C-5' ($\delta = 6.92$ ppm). In the ^{13}C -NMR spectrum signal from C-4' ($\delta = 149.0$ ppm) and signal from C-6 ($\delta = 136.5$ ppm) was also shifted compared with the signals from substrate **4** $\delta = 129.3$ ppm and $\delta = 131.6$ ppm, respectively. These changes indicate the introduction of a hydroxyl group at C-4' and hydroxymethyl group at C-6. Methylglucosyl moiety was attached to C-3' and hydroxyl moiety to C-4' same as in product **4b**, which was confirmed by cross-peaks in the HMBC spectrum [44–46]. Signals from the protons of ring A were only slightly changed, but exchange of a methyl group on the hydroxymethyl group at C-6 was confirmed by the disappearance of the signal from the three protons of the methyl moiety that was present in the ^1H -NMR spectrum ($\delta = 2.32$ ppm) of substrate **4** and the appearance of a signal from two protons at 4.61 ppm [47].

Scheme 8. Key COSY (on the left) and HMBC (on the right) correlations for the structure elucidation of product **4d**.

Glycosylations of flavanones performed by *B. bassiana* strains have already been described in the scientific literature. Isoxanthohumol and 8-prenylnaringenin were transformed by *B. bassiana* AM278 into their 7-*O*- β -D-(4''-*O*-methyl)-glucosides [48]. Other flavanones, like pinocembrin, naringenin, eriodictyol and hesperetin were glycosylated and 4''-*O*-methyl-glycosylated at C-7 in the cultures of *B. bassiana* AM278. Moreover, hesperetin biotransformation resulted in additional products glycosylated and 4''-*O*-methyl-glycosylated at C-3' [49]. The strain *B. bassiana* KCH J1.5 used in the currently presented studies showed a different regioselectivity of glycosylation. Interestingly *B. bassiana* KCH J1.5 was also able to hydroxylate 6-methyl moiety in products **4b–4d**. Presence of the four biotransformation products with different moieties attached indicates the involvement of a few enzymes in the transformation of 6-methylflavanone (**4**) in the culture of *B. bassiana* KCH J1.5. In the case of product **4b** (4'-hydroxyflavanone 6-methylene-*O*- β -D-(4''-*O*-methyl)-glucopyranoside) hydroxylation took place at C-4' and C-6-CH₃, followed by attachment of the glucose unit to hydroxymethyl group (-CH₂-OH) at C-6. The presumable course of microbial transformation of 6-methylflavanones is shown in the Scheme 9.

Scheme 9. The probable course of transformation of 6-methylflavanones.

2.3. Biotransformations of 6-methylflavanone (**4**) in the culture of *I. fumosorosea* KCH J2

6-Methylflavanone (**4**) was also utilized in a 10-day biotransformation in the culture of *I. fumosorosea* KCH J2 and gave two biotransformation products: 6-methylflavanone 4'-*O*- β -D-(4''-*O*-methyl)-glucopyranoside (**4e**) with 22.1% yield and 2-phenyl-6-methylchromane 4-*O*- β -D-(4''-*O*-methyl)-glucopyranoside (**4f**) with 23.3% yield (Scheme 10).

Scheme 10. Microbial transformation of 6-methylflavanone (**4**) in *I. fumosorosea* KCH J2 culture.

The products **4e** and **4f** were analyzed by ^1H -NMR and ^{13}C -NMR spectroscopy which allowed for the establishment of their chemical structures (Tables 3 and 4, Schemes 11 and 12).

Scheme 11. Key COSY (on the left) and HMBC (on the right) correlations for the structure elucidation of product **4e**.

Scheme 12. Key COSY (on the left) and HMBC (on the right) correlations for the structure elucidation of product **4f**.

The presence of a glucose unit in compounds **4e** and **4f** was confirmed by five characteristic carbon signals observed in the region from about $\delta = 80.0$ ppm, to about $\delta = 62.0$ ppm, in the ^{13}C -NMR spectra, as well as proton signals of δH ranging from about $\delta = 3.9$ ppm, to $\delta = 3.1$ ppm in the ^1H -NMR spectra. Additionally, the attachment of a sugar unit to substrate **4** was confirmed by a one-proton doublet at the anomeric carbon atom at $\delta = 4.49$ ppm in the ^1H -NMR spectrum of **4e**, $\delta = 4.97$ ppm in the ^1H -NMR spectrum of **4f**. The β -configuration of the glucose unit was proved for **4e** and **4f** by the coupling constant ($J = 7.8$ Hz) for the anomeric proton. A three-proton singlet at about $\delta = 3.6$ ppm in the ^1H -NMR spectra and the corresponding signal at $\delta = 60.5$ ppm in the ^{13}C -NMR spectra of **4e** and **4f** evidences that one of the hydroxyl groups of sugar unit has been methylated. *O*-methylation occurred at the C-4'' hydroxyl group of the glucose unit. It was detected in the HMBC spectrum, where the proton signal due to $-\text{OCH}_3$ was correlated with the signal of C-4'' (about $\delta = 80$ ppm) in the glucose unit.

Based on the HMBC spectrum, a sugar moiety was attached to C-4' in the case of compound **4e**, due to the proton at the anomeric carbon atom ($\delta = 4.78$ ppm) being coupled with the C-4' signal ($\delta = 158.8$ ppm), which was shifted from $\delta = 129.3$ ppm. In the ^1H -NMR spectrum, multiplets from protons at C-2' and C-6' ($\delta = 7.49$ ppm) and from protons at C-3' and C-5' ($\delta = 7.12$ ppm) can be observed, which is characteristic of an AA'BB' coupling system, signaling the presence of flavanone ring B substitution at C-4'. Chemical shifts of the other signals in the ^1H -NMR and ^{13}C -NMR spectra have only slightly changed, which indicates that the flavanone skeleton remained intact. The signal from the three protons of the methyl group at C-6 remained at the same position as in the ^1H -NMR spectrum of substrate **4**, which means that the methyl group in product **4e** was present.

Based on the HMBC spectrum, in the case of product **4f**, glycosylation occurred at C-4, due to the proton at the anomeric carbon atom ($\delta = 4.49$ ppm) being coupled with the C-4 signal ($\delta = 70.2$ ppm), which was shifted from $\delta = 191.9$ ppm, indicating reduction of the carbonyl group at C-4. Furthermore, in the ^1H -NMR spectrum, a triplet at $\delta = 4.92$ ppm appeared from one-proton at C-4. Moreover, only slightly shifted signals from all ten protons from substrate **4** were visible, confirming the inability of another position of substitution. A doublet of doublets at $\delta = 5.36$ ppm from one proton at C-2 became shifted from $\delta = 5.59$ ppm, the same as the signals from the pseudo-axial and pseudo-equatorial protons at C-3, respectively ($\delta = 2.43$ ppm shifted from $\delta = 3.11$ ppm and $\delta = 2.07$ ppm shifted from $\delta = 2.85$ ppm). In the COSY spectrum there was a correlation between proton 3_{ax} and 3_{eq} with a new signal from the proton at C-4. In the HMBC spectrum correlations between the proton at C-5 and the carbon at C-4, and between the pseudo-axial proton at C-3 and the carbon at C-4, and between the proton at C-1'' and the carbon at C-4 were also present, which confirms that reduction and glycosylation occurred at C-4. The signal from the three protons of the methyl group at C-6 remained at the same position as in the ^1H -NMR spectrum of substrate **4** which indicates the presence of the methyl group in product **4f**.

The first product of the biotransformations presented above—6-methylflavanone 4'-O- β -D-(4''-O-methyl)-glucopyranoside (**4e**)—is analogous to the 6-methoxyflavanone 4'-O- β -D-(4''-O-methyl)-glucopyranoside that was obtained in the microbial transformations of 6-methoxyflavanone by the same strain, *I. fumosorosea* KCH J2 [15]. Glycosylation at C-4' also occurred in the biotransformation of naringenin in the culture of *I. fumosorosea* ACCC 37814, performed by Dou et al. [16]. More unusual, is the second biotransformation product—2-phenyl-6-methylchromane 4-O- β -D-(4''-O-methyl)-glucopyranoside (**4f**). We assume that attachment of the glycosyl moiety at C-4 was preceded by the carbonyl group reduction, which was previously observed as a result of 2'-methoxyflavanone and 3'-methoxyflavanone biotransformations [15]. However, subsequent glycosylation at the same position has never been observed before. In each biotransformation product a methyl moiety at C-6 was retained, indicating that *I. fumosorosea* KCH J2 was not able to remove this moiety, unlike *B. bassiana* KCH J1.5.

3. Materials and Methods

3.1. Substrates

The substrates for biotransformations were obtained by two-step synthesis (Scheme 1). The first step was Claisen–Schmidt condensation between 2'-hydroxy-5'-methylacetophenone (**1**) and benzaldehyde (**2**) (purchased from Sigma-Aldrich (St. Louis, MO, USA)) dissolved in methanol, under alkaline conditions at high temperature, according to the method described previously [50–53]. 2'-Hydroxy-5'-methylchalcone (**3**) was obtained with a 50.1% yield and was used as the biotransformation substrate, and also as substrate in the second step of the synthesis. 6-Methylflavanone (**4**) was obtained by cyclization of 2'-hydroxy-5'-methylchalcone (**3**) in the presence of sodium acetate [54,55] with 62.3% yield.

The physical data, including the retention time t_R (min), the optical rotation $[\alpha]_D^{20}$, and concentration c (M), as well as NMR spectral data of the resulting compounds **3** and **4** are presented below, and in Tables 1–4, and in the Supplementary Materials.

3.1.1. 2'-Hydroxy-5'-methylchalcone (3)

$C_{16}H_{14}O_2$, $M_w = 238.28$ $[M + H]^+$ 239.1054; 1H -NMR, see Table 1, ^{13}C -NMR, see Table 2.

3.1.2. 6-Methylflavanone (4)

$C_{16}H_{14}O_2$, $M_w = 238.28$, $[M + H]^+$ 239.1086; $[\alpha]_D^{20} = 0$ ($c = 0.65$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

3.2. Microorganisms

The studies were carried out using two strains of entomopathogenic filamentous fungi *I. fumosorosea* KCH J2 and *B. bassiana* KCH J1.5, that were collected from the Department of Chemistry of Wrocław University of Environmental and Life Sciences, Poland. The description of material collection, propagation of structures of the fungi, and genetic identification have already been described in our previous papers [12,37]. The microorganisms were maintained on potato slants at 4°C and subcultured before use in the experiments.

3.3. Analysis

The course of the biotransformation was assessed by chromatographic methods (TLC, HPLC). TLC analysis was carried out using TLC Silica gel 60/Kieselguhr F254 (0.2 mm thick) plates (Merck, Darmstadt, Germany). The developing system was a mixture of chloroform and methanol (9:1 *v/v*). The products were observed (without additional visualization) under the ultraviolet lamp at the two wavelengths 254 nm and 365 nm.

HPLC analyses were performed on a Dionex Ultimate 3000 instrument (Thermo Fisher Scientific, Waltham, MA, USA) with a diode array detector using an analytical octadecyl silica (ODS) 2 column (4.6 × 250 mm, Waters, Milford, MA, USA) and pre-column. The gradient program was as follows: initial conditions—32.5% B in A, 4 min—40% B in A, 8 min—40% B in A, 10 min—45% B in A, 15 min—95% B in A, 18 min—95% B in A, 19 min—32.5% B in A, 23 min—32.5% B in A. The flow rate was 1 mL/min, the injection volume was 5 µL, and detection wavelength 280 nm.

Separation of the products obtained by the scale-up biotransformation was attained using 500 and 1000 µm preparative TLC silica gel plates (Analtech, Gehrden, Germany). After elution of the compounds from the adsorbent on TLC plates with chloroform and methanol (9:1 *v/v*) as eluents, compounds were extracted from the selected gel fractions using 20 mL ethyl acetate 3 times. The extracts from a single fraction were combined and ethyl acetate was evaporated under reduced pressure.

NMR analyses (1H -NMR, ^{13}C -NMR, COSY, Heteronuclear Multiple Quantum Correlation (HSQC), HMBC) were performed using a DRX Avance™ 600 MHz NMR spectrometer (Bruker, Billerica, MA, USA). The prepared samples were dissolved in deuterated acetone, except 3 dissolved in deuterated chloroform.

Optical rotation was measured using digital polarimeter P-2000-Na (ABL & E-JASCO, Kraków, Poland).

Molecular formulas of products were confirmed by UPLC-qTOF-MS/MS analysis. Identification of compounds was performed as described previously, with slight modifications [56], on the Acquity ultra-performance liquid chromatography (UPLC) system, coupled with a quadrupole-time of flight (q-TOF) MS instrument (UPLC/Synapt q-TOF MS, Waters Corp., Milford, MA, USA), with an electrospray ionization (ESI) source. The separation was achieved on the Cadenza CD-C18 UP (75 mm × 2.0 mm i.d., 3 µm) column (Imtakt, Kyoto, Japan). The mobile phase was a mixture of 0.1% aqueous formic acid *v/v* (A) and acetonitrile (B). The gradient program was as follows: initial conditions—1% B in A, 12 min—25% B in A, 19 min—100% B, 20 min—1% B in A. The flow rate was 0.45 mL/min, and the injection volume was 5 µL. The column was operated at 30°C. The major operating parameters for the q-TOF MS were set as follows: capillary voltage 2.0 kV, cone voltage 40 V, cone gas flow of 11 L/h, collision energy 28–30 eV, source temperature 100 °C, desolvation temperature

250 °C, collision gas, argon; desolvation gas (nitrogen) flow rate, 600 L/h; data acquisition range, m/z 100–2000 Da; ionization mode, negative and positive. The data were collected with Mass-Lynx V 4.1 software. The runs were monitored at a wavelength of 254 nm.

3.4. Screening Procedure

Experiments were carried out using Sabouraud medium (10 g aminobac, 30 g glucose, 1 L distilled water). The microorganism was transferred to a 300 mL Erlenmeyer flask with 100 mL medium. Pre-incubation was carried out on a rotary shaker (140 rpm) at 25 °C for 72 h. The screening was also performed in 300 mL Erlenmeyer flasks with 100 mL of Sabouraud liquid medium. The pre-grown culture (0.5 mL) was transferred to an Erlenmeyer flask, and after 72 h incubation, 10 mg of substrate **3** or **4**, dissolved in 0.5 mL of dimethyl sulfoxide, was added. The molar concentrations of substrates **3** and **4** were 0.42 mM. The biotransformation was run under the same conditions as pre-incubation. After 3, 6 and 9 days of substrate incubation samples were collected and extracted once with 30 mL of ethyl acetate. The extracts were dried with anhydrous magnesium sulfate for 5 min, concentrated in vacuo and analyzed by TLC and HPLC methods. Stability of the substrate was evaluated under identical conditions, without using a biocatalyst. Control cultivation with no substrate has also been performed.

3.5. The Semi-Preparative Biotransformations

The semi-preparative biotransformations were performed in 2 L flasks with 500 mL of the Sabouraud medium (10 g aminobac, 30 g glucose, 1 L distilled water) each. The pre-incubation culture (1 mL) was transferred to the flask and incubated for 72 h same as during the screening procedure. Afterwards, 50 mg of substrate **3** or **4**, dissolved in 2.5 mL of dimethyl sulfoxide, was added and the cultures were shaken on a rotary shaker for 10 days. The molar concentrations of substrates **3** and **4** were 0.42 mM. After the confirmation of complete substrate conversion (or lack of further substrate conversion) metabolites were extracted 2 times using each time 300 mL of ethyl acetate. The combined extracts were dried with anhydrous magnesium sulfate for 5 min and then concentrated using a rotary evaporator. Biotransformation products were separated using preparative TLC plates and analyzed by NMR and UPLC-qTOF-MS/MS.

The physical data, including the retention time t_R (min), the optical rotation $[\alpha]_D^{20}$, and concentration c (M), as well as NMR spectral data of the resulting compounds **3a**, **4a–4f** are presented below, and in Tables 1–4, and in the Supplementary Materials.

3.5.1. 2'-Hydroxy-5'-methylchalcone 3-O- β -D-(4''-O-methyl)-glucopyranoside (**3a**)

$C_{23}H_{26}O_8$, $M_w = 430.45$, $[M - H]^-$ 429.1574; 1H -NMR, see Table 1, ^{13}C -NMR, see Table 2.

3.5.2. 4'-Hydroxy-6-methylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4a**)

$C_{23}H_{26}O_9$, $M_w = 446.45$, $[M - H]^-$ 445.1463; $[\alpha]_D^{20} = -10.5$ ($c = 0.325$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

3.5.3. 4'-Hydroxyflavanone 6-methylene-O- β -D-(4''-O-methyl)-glucopyranoside (**4b**)

$C_{22}H_{24}O_9$, $M_w = 446.45$, $[M - H]^-$ 445.1549; $[\alpha]_D^{20} = -6.1$ ($c = 0.365$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

3.5.4. 6-Hydroxymethylflavanone 3'-O- β -D-(4''-O-methyl)-glucopyranoside (**4c**)

$C_{22}H_{24}O_9$, $M_w = 446.45$, $[M - H]^-$ 445.1506; $[\alpha]_D^{20} = 9.1$ ($c = 0.175$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

3.5.5. 4'-Hydroxy-6-hydroxymethylflavanone 3'-O-β-D-(4''-O-methyl)-glucopyranoside (4d)

$C_{22}H_{24}O_{10}$, $M_w = 462.45$, $[M - H]^-$ 461.1418; $[\alpha]_D^{20} = 54.3$ ($c = 0.390$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

3.5.6. 6-Methylflavanone 4'-O-β-D-(4''-O-methyl)-glucopyranoside (4e)

$C_{23}H_{26}O_8$, $M_w = 430.45$, $[M - H]^-$ 429.1532; $[\alpha]_D^{20} = -50.3$ ($c = 0.950$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

3.5.7. 2-Phenyl-6-methylchromane 4-O-β-D-(4''-O-methyl)-glucopyranoside (4f)

$C_{23}H_{28}O_7$, $M_w = 416.46$, $[M - H]^-$ 415.1776 (adduct $[M + 46 - H]^-$ $[M + HCOO]^-$ 461.1814 [57]); $[\alpha]_D^{20} = -25.3$ ($c = 0.390$, acetone); 1H -NMR, see Table 3, ^{13}C -NMR, see Table 4.

4. Conclusions

In this paper we present the results of the glycosylation of a flavanone and chalcone with methyl moiety in entomopathogenic filamentous fungi cultures. The strain *B. bassiana* KCH J1.5 was able to hydroxylate methyl moiety at C-6 of 6-methylflavanone and also to attach to the glycosyl moiety at this site. These microbial transformations have not yet been described in the scientific literature. Moreover, *B. bassiana* KCH J1.5 glycosylated 2'-hydroxyl-5'-methylchalcone at C-3 and 6-methylflavanone at C-3'. The *B. bassiana* KCH J1.5 enzymatic system also catalyzed hydroxylation of the flavanone skeleton at C-4'. The second utilized strain—*I. fumosorosea* KCH J2—glycosylated 2'-hydroxyl-5'-methylchalcone at C-3 and 6-methylflavanone at C-4' and C-4. It can be assumed that attachment of the glycosyl moiety at C-4 was preceded by the carbonyl group reduction. This strain was not able to hydroxylate the methyl moiety. All biotransformation products have not been previously described in the scientific literature and may be used in studies assessing their biological activity and bioavailability.

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