

Microbial Metabolism. Part 6.¹⁾ Metabolites of 3- and 7-Hydroxyflavones

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Fermentation of 3-hydroxyflavone (1) with *Beauveria bassiana* (ATCC 13144) yielded 3,4'-dihydroxyflavone (3), flavone 3-O- β -D-4-O-methylglucopyranoside (4) and two minor metabolites. 7-Hydroxyflavone (2) was transformed by *Nocardia* species (NRRL 5646) to 7-methoxyflavone (5) whilst *Aspergillus alliaceus* (ATCC 10060) converted it to 4',7-dihydroxyflavone (6). Flavone 7-O- β -D-4-O-methylglucopyranoside (7) and 4'-hydroxyflavone 7-O- β -D-4-O-methylglucopyranoside (8) were the metabolic products of 7-hydroxyflavone (2) when fermented with *Beauveria bassiana* (ATCC 7159). One of the minor metabolites of 3-hydroxyflavone (1) was tentatively assigned a β' -chalcanol structure (9). Compounds 4, 7 and 8 are reported as new compounds. Structure elucidation of the metabolites was based on spectroscopic data.

Key words flavonoid; microbial metabolism; *Beauveria bassiana*; *Aspergillus alliaceus*; *Nocardia*

Flavonoids and their glycosides form a large group of polyphenolic compounds which are widely distributed in plants.^{2,3)} Animals depend on plants for flavonoids as they are unable to biosynthesize them.⁴⁾ The exception was the detection of flavonoids in butterfly wings with the likely source being the plant food of the larva.⁵⁾ Flavonoids add colour,⁶⁾ flavour and processing characteristics to many foods (fruits and vegetables) and drinks (tea, wine).⁷⁾ So far, over 4000 flavonoid derivatives have been identified and numerous beneficial health effects including, anti-inflammatory,^{8–10)} antiviral¹¹⁾ and anti-cancer^{8,12,13)} properties have been reported. Some epidemiological studies have also demonstrated that the intake of flavonoids reduced the risk of cardiovascular diseases.^{8,14–16)} Most of the pharmacological activities could be attributed to their ability to inhibit certain enzymes and to their oxygen free radical scavenging and iron chelating capabilities.¹⁷⁾ The antioxidant properties are suggested to be due to the number and arrangement of their phenolic hydroxyl groups.¹⁸⁾ It has been shown that 3-hydroxy-, 7-hydroxy- and 5-hydroxyflavones have hypochlorite scavenging activity with 3-hydroxyflavone showing the greatest effect.¹⁹⁾ Depending on the concentration and the reaction conditions, flavonoids could act as antioxidants as well as prooxidants.¹⁷⁾ Toxicity of some flavonoids has been attributed to their prooxidant behavior. Hence, careful examination of flavonoids for their behavior in varying reaction conditions has to be carried out before being considered for therapeutic uses.¹⁷⁾ Although *in vitro* experiments have shown that flavonoids possess a wide range of biological activities, their overall function *in vivo* has to be clarified. It has been observed that only aglycones and flavonoid glucosides are absorbed in the small intestine.¹⁸⁾ They are then metabolized rapidly to methylated, glucuronidated or sulphated metabolites in the jejunal and ileal parts of the small intestine. After metabolism in the intestine, the flavonoids are further metabolized in the liver to yield various conjugated forms. The reactions include methylation, sulphation and glucuronidation. Conjugation is essentially a detoxification process in which the compounds are made to eliminate by way of bile and urine by increasing their hydrophilic character.²⁰⁾ The unabsorbed flavonoids undergo further metabolism by the bacterial en-

zymes in the colon.²¹⁾ Using animal models, human trials and *in vitro* fermentation experiments, it was shown that the intestinal microorganisms are greatly responsible for catabolism and scission of the flavonoid. Scission of the flavonoid structure depends on their hydroxylation pattern.²¹⁾ Absence of hydroxyl groups in the B-ring for example, prevents ring scission. *In vitro* metabolism studies of flavonoids using rat liver microsomes showed that the B-ring is the main structural moiety that undergoes biotransformation. B-rings with a single hydroxyl group at C-4' or none at all get hydroxylated by microsomal cytP₄₅₀ enzymes.²¹⁾ It has been demonstrated that the bioavailability of flavonoids in general is low due to limited absorption and rapid elimination. For example, the peak plasma concentrations of citrus flavonoids was less than 1 μ M/l.²⁰⁾ It is also known that they are rapidly and extensively bio-transformed into metabolites which do not always have the same biological activities of the parent compounds.²²⁾ As seen in *in vitro* antioxidant experiments, most circulating flavonoids are actually the metabolites rather than the original compounds indicating that the active compounds may not be the dietary flavonoids.^{22,23)} Thus, to ascertain the role of flavonoids as biologically active compounds it is essential to know the chemical nature and the bioavailability of the absorbed forms. Being predictive models for mammalian drug metabolism, microorganisms are used to establish the metabolic fate of biologically active compounds.^{24–26)} This procedure gives significant amounts of metabolites for complete structure elucidations and for further pharmacological evaluations. Reports on the microbial transformation of flavonoids are relatively few²⁷⁾ and thus, we initiated a program to investigate the microbial metabolism of some selected flavonoids. Here we report the transformation of 3-hydroxyflavone (1) by *Beauveria bassiana* (ATCC 13144) and 7-hydroxyflavone (2) by *Nocardia* species, *Aspergillus alliaceus* (ATCC 10060) and *Beauveria bassiana* (ATCC 7159).

Results and Discussion

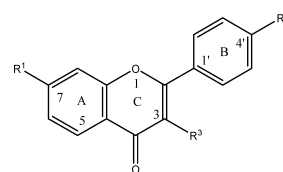
Initial screening of compounds 1 and 2 was carried out with forty organisms using the standard two stage procedure.²⁴⁾ Several organisms showed the capability of transforming the flavonoids. However, *B. bassiana* (ATCC

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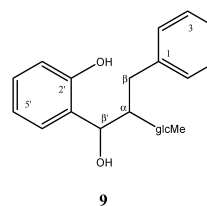
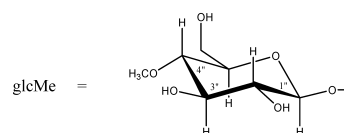
13144) was selected for scale-up studies of compound **1** as it gave a number of metabolites compared to other organisms. Flavonol (**1**) was converted to 3,4'-dihydroxyflavone (**3**), flavone 3-*O*- β -D-4-*O*-methylglucopyranoside (**4**) and two other minor metabolites, whilst 7-hydroxyflavone (**2**) was metabolized to 7-methoxyflavone (**5**) and 4',7-dihydroxyflavone (**6**) by *Nocardia* species (NRRL 5646) and *A. alliaceus* (ATCC 10060), respectively. Transformation of flavone (**2**) to flavone 7-*O*- β -D-4-*O*-methylglucopyranoside (**7**) and 4'-hydroxyflavone 7-*O*- β -D-4-*O*-methylglucopyranoside (**8**) was observed when fermented with *B. bassiana* (ATCC 7159). None of the extracts showed detectable amounts of substrates **1** and **2**.

Metabolite **3**, (5 mg, 1.25% yield) isolated as a faint yellow solid, was shown by high resolution electrospray ionization mass spectrometric (HR-ESI-MS) data to have a molecular formula $C_{15}H_{10}O_4$ corresponding to a monooxygenated product of flavonol (**1**). IR absorption bands at 3308, 2924, 1602 and 1653 cm^{-1} suggested the presence of $-\text{OH}$, $-\text{C}-\text{H}$, $-\text{C}=\text{C}$ and $-\text{C}=\text{O}$ groups, respectively. The ^1H -NMR spectrum differed from that of starting compound (**1**) in the reduction of aromatic protons from nine to eight resulting in the appearance of two doublets at δ 8.09 and 6.93 in an AA'BB' system characteristic of a 1,4-disubstituted benzene moiety showing *p*-hydroxylation. The ^{13}C -NMR spectrum revealed a highly deshielded carbon at δ 159.9 corresponding to C-4'. Long-range heteronuclear multiple correlation (HMBC) confirmed this assignment. The metabolite **3** was thus characterized as the known 3,4'-dihydroxyflavone.

Compound **4** (20 mg, 5% yield) was obtained as an amorphous white solid with a molecular formula, $C_{22}H_{22}O_8$, as determined by HR-ESI-MS data. The IR spectrum showed strong absorption bands at 3374 (OH), 2924 (C-H), 1615 (br) cm^{-1} (C=C) and (C=O). Distortionless enhancement by polarization transfer (DEPT) spectra indicated that the 19 signals observed in the ^{13}C -NMR comprised one primary, one secondary, eleven tertiary and six quaternary carbons. ^1H - and ^{13}C -NMR spectra showed the characteristic pattern of flavonol (**1**) with a 4-*O*-methyl- β -D-glucopyranosyl moiety attached to the 3-hydroxyl. Resonances at δ 4.68 (1H, d, $J=8.4\text{ Hz}$, H-1''), 3.51 (1H, dd, $J=8.4\text{ Hz}$, H-2''), 3.62 (1H, dd, $J=9.0\text{ Hz}$, H-3''), 3.02 (1H, dd, $J=9.0\text{ Hz}$, H-4''), 3.46 (3H, s, OMe-4''), 3.08 (1H, br, m, H-5'') 3.34, 3.45 (2H, m, H-6'') in the ^1H -NMR spectrum revealed the presence of the 4-*O*-methyl- β -D-glucopyranosyl moiety. Further evidence came from the ^{13}C -NMR spectrum which showed signals at δ 74.1, 75.8, 77.2 and 78.9 for hydroxymethine carbons, signals at δ 60.8 for an *O*-methyl carbon, at δ 62.3 for a hydroxymethylene carbon and at δ 105.7 for the anomeric carbon. The 3-*O*-glucosylation was deduced from the chemical shifts of C-3 (δ 138.0) and C-2 (δ 159.1). The β -configuration of the sugar unit was consistent with the coupling constant of the anomeric proton (δ 4.68, d, $J=8.4\text{ Hz}$).²⁸⁾ The sugar part was further confirmed by comparing the ^{13}C resonances with those reported for the corresponding carbons in a similar 4-*O*-methylglucopyranoside.²⁹⁾ The remaining signals of the NMR spectra confirmed the structure of the unchanged aglycone moiety. The assignment of signals of compound **4** was based on ^1H - ^1H correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), HMBC spectra. Consequently, metabolite **4** was identified as the new com-



	R ¹	R ²	R ³
1	H	H	OH
2	OH	H	H
3	H	OH	OH
4	H	H	glcMe
5	OMe	H	H
6	OH	OH	H
7	glcMe	H	H
8	glcMe	OH	H



pound, flavone 3-*O*- β -D-4'-*O*-methylglucopyranoside.

One of the minor metabolites (**9**) (3 mg, 0.75% yield) of flavonol (**1**) had a molecular formula of $C_{22}H_{28}O_8$ by HR-ESI-MS. This was an increase of six mass units as compared to **4**. It was reflected in the ^1H -NMR spectrum, where additional peaks were observed at δ 4.17 (1H), 3.50 (1H), 3.44 (1H) and 2.20 (2H). The IR spectrum showed a broad absorption at 3319 cm^{-1} indicating the presence of hydroxyl group(s). The absence of a peak due to carbonyl in the ^{13}C -NMR spectrum indicated the absence of a carbonyl group in the molecule. The NMR spectra of compound **9** however, showed close resemblance to those of **4** with respect to the A and B rings and the sugar moiety. NMR data also suggested 3-*O*-glucosylation as in **4**. However, the disappearance of peaks due to C-2 (δ 159.1), C-3 (δ 138.0) and C-4 (δ 175.9) in **4** and the appearance of peaks due to a methylene group (δ 30.0) and carbons linked to oxygen at δ 76.7 and 69.7 in the ^{13}C -NMR spectrum together with the observation of proton signals at δ 4.17, 3.50, 3.44, 2.2 in the ^1H -NMR spectrum, suggested a new β' -chalcanol structure for **9**.

Metabolite **5** (57.2 3% yield) with a molecular formula of $C_{16}H_{12}O_3$ as determined by HR-ESI-MS, ^{13}C -NMR and DEPT data, was isolated as a white solid. Strong absorption peaks at 2925 (C-H), 1633 (br) cm^{-1} (C=C) and (C=O) were observed in the IR spectrum. The NMR spectra of **5** resembled those of **2** in all aspects except that the hydroxyl group at C-7 has been methylated. Analysis of high-resolution NMR spectra and a comparison with reported data³¹⁾ confirmed that **5** was 7-methoxyflavone.

HR-ESI-MS, ^{13}C -NMR and DEPT data suggested a molecular formula $C_{15}H_{10}O_4$ for metabolite **6** (123 mg, 10% yield). The additional 16 mass units indicated that it was a

monohydroxylated derivative of flavone **2**. The IR spectrum showed absorption bands at 3190 (OH) and 1630 (br) (C=C, C=O) cm^{-1} . The NMR data of **6** closely resembled those of **2**. The metabolite however, displayed a *para*-substituted B ring [δ : 7.87 (2H, d, $J=8.4$ Hz), 6.90 (2H, d, $J=8.4$ Hz)]. Comparison with literature data³¹⁾ confirmed that compound **6** was 4',7-dihydroxyflavone.

Metabolite **7** (186.9 mg, 21% yield) showed a molecular mass of 414 comprising of a 7-hydroxyflavone (m/z 238) and an *O*-methylglucopyranosyl moiety (m/z 178). The exact molecular mass was consistent with a molecular formula of $\text{C}_{22}\text{H}_{22}\text{O}_8$. The ^1H -NMR spectrum showed that the aromatic protons had nearly the same chemical shifts and J values as recorded for 7-hydroxyflavone (**2**). Similarly, the ^{13}C -NMR data were nearly identical to that of flavone **2**, confirming that the flavone nucleus had not been transformed. Five of the resonances in the ^{13}C -NMR spectrum were in the region for aliphatic oxygenated carbons (δ 60–80) and were consistent with the presence of a glucosyl moiety. The methine carbon resonating at δ 100.3 showed a one-bond correlation (HMQC) with the anomeric proton at δ 5.14 which in turn had a three-bond correlation (HMBC) with the methine carbon at δ 162.3 (C-7). The latter showed two-bond correlation to H-6 (δ 7.12). These data suggested *O*-glucosylation at C-7 of the flavone. HMBC correlations together with an observed downfield shift for C-4" (δ 79.5) in the ^{13}C -NMR spectrum indicated the presence of an *O*-methyl group at C-4". The *O*-methyl group resonated at δ 60.4 in the ^{13}C -NMR spectrum and at δ 3.46 in the ^1H -NMR spectrum. The large coupling constant ($J=7.6$ Hz) between the (anomeric) proton H-1" and H-2" indicated a *trans*-diaxial relationship establishing a β -glycosidic linkage. Large coupling constants, $J_{2''/3''}$, $J_{3''/4''}$, $J_{4''/5''}$, indicated the *trans*-diaxial relationships between each of the consecutive pairs of protons. The collective spectroscopic data suggested that compound **7** represent a methylglucoside of **2**. Thus, **7** was identified as the new compound, flavone 7-*O*- β -D-4-*O*-methylglucopyranoside.

Compound **8** (64.8 mg, 7% yield), the most polar metabolite of **2** was assigned the molecular formula, $\text{C}_{22}\text{H}_{22}\text{O}_9$, as determined from HR-ESI-MS. NMR data of **7** were similar to those of **2**, except in the region of B-ring protons which showed *para*-substitution [δ 7.97 (2H, d, $J=8.8$ Hz), 6.92 (2H, d, $J=8.8$ Hz)]. The mass spectrum showed an additional oxygen indicating that compound **8** was a monohydroxy derivative of metabolite **7**. Correlations observed in the HMBC spectrum permitted the assignment of the hydroxyl group at C-4'. Thus, the structure of compound **8** was established as the new compound, 4'-hydroxyflavone 7-*O*- β -D-4-*O*-methylglucopyranoside.

It is unclear whether the 4-*O*-methylglucopyranosyl moiety in the metabolites, **4**, **7** and **8** originated from the medium or the fungus.

Conclusion

In the present study, four metabolites of 3-hydroxyflavone (**1**) produced by *B. bassiana* (ATCC 13144) and four of 7-hydroxyflavone (**2**), converted by *Nocardia* species (NRRL 5646), *A. alliaceus* (ATCC 10060) and *B. bassiana* (ATCC 7159) were isolated. Whilst all the products of flavone **2** were characterized, only two metabolites of compound **1** were identified. The other two were isolated in quantities insuffi-

cient for full structure elucidations. However, one was assigned a tentative structure (**9**) using available spectroscopic data. The transformations observed in this study are the results of functionalization (Phase I) and conjugation (Phase II) reactions. As detected in previous transformation experiments with most microorganisms,²⁷⁾ we observed hydroxylation of the B-ring at C-4' in metabolites, **3**, **6** and **8**. Relatively poor yields of the three metabolites could be due to decreased binding of flavones **1** and **2** to the enzymes responsible for hydroxylation.²⁷⁾ Both flavones yielded the conjugated products, **4**, **5**, **7**, **8** and **9**. The formation of hydroxylation and conjugation products in this study are paralleled in mammals^{32–34)} indicating the use of microbes to mimic mammalian metabolism. Microbes usually form glucosides rather than glucuronides as occurs in mammals.³⁵⁾ As reported, conjugation are the most common final step reactions in mammalian metabolism of intact flavonoids.²⁰⁾ Metabolite **9** is a cleavage product of flavone **1**.³⁶⁾ Previous work on microbial transformation of flavonoids also reported some cleavage products.³²⁾ Cleavage of flavonoids in mammals has been shown to be brought about by the colonic microflora.²⁰⁾ The metabolites obtained are in sufficient quantities for biological as well as further chemical studies. Compounds **4**, **7** and **8** are described as new compounds

Experimental

General Experimental Procedures UV spectra were run on a Hewlett Packard 8452A diode array spectrometer. IR spectra were recorded in CHCl_3 using an ATI Mattson Genesis series FTIR spectrophotometer. ^1H - and ^{13}C -NMR were recorded in CDCl_3 on Varian Mercury 400 and Varian Unity Inova 600 spectrometers. HR-ESI-MS data were obtained using a Bruker GeoApex 3.0.

Substrates 3-Hydroxyflavone (**1**) and 7-hydroxyflavone (**2**) were obtained from Aldrich Co. (Milwaukee, Wisconsin).

Organisms and Metabolism Microorganisms (37) from the collection of the National Center for Natural Products Research, University of Mississippi, were used in the preliminary screening experiments to identify organisms capable of metabolizing **1** and **2**. Medium α ²⁴⁾ consisting of dextrose, 20 g; NaCl, 5 g; K_2HPO_4 , 5 g; bacto-peptone (Difco Labs, Detroit, MI, U.S.A.), 5 g and yeast extract (Difco Labs), 5 g per liter of distilled water was used in all fermentation experiments based on a two-stage procedure.²⁴⁾ Initial screening was performed in 125 ml Erlenmeyer flasks (125 ml) containing 25 ml medium- α . Compounds **1** and **2** were added separately in dimethylformamide (0.5 mg/ml) solution to 24 h old stage II cultures. Incubation was carried out at room temperature for a period of 14 d on a rotary shaker (New Brunswick Model G10-21) at 100 rpm. Monitoring of the samples was carried out at 7-d intervals, using precoated Si gel 60 F_{254} TLC plates (E. Merck) with EtOAc–hexane (3 : 2) as the solvent system. Spots on TLC were visualized by UV light (254, 365 nm) and also with the help of the spray reagent, *p*-anisaldehyde. Five 21 flasks, each containing 100 mg of substrate dispersed in 500 ml of medium- α were used for preparative scale fermentation of 3-hydroxyflavone (**1**) with *B. bassiana*. Similarly, 100 mg of 7-hydroxyflavone (**2**) in each of five 21 flasks containing 500 ml of the same medium were used with each of *Nocardia* species, *A. alliaceus* and *B. bassiana*. The combined culture filtrates were extracted with EtOAc/(Me)₂CHOH–EtOAc (2 : 9) and the solvents were evaporated. The residues obtained were subjected to partition or column chromatography. Wherever needed, further purification was carried out by preparative thin layer (Silica gel 60 F_{254}) chromatography. Substrate and culture controls were run simultaneously with the above experiments.²⁴⁾

Microbial Transformation of 3-Hydroxyflavone (**1**) by *B. bassiana*

The combined culture filtrates were extracted with EtOAc and the solvent was evaporated to dryness. The resulting brown solid was column chromatographed over silica gel (Si gel 230–400 mesh; E. Merck, 30 g, column diameter: 20 mm.) with CHCl_3 gradually enriched with MeOH. Purification of the metabolites was by repeated column and preparative layer chromatography (EtOAc–hexane, 3 : 2). Four compounds were isolated. Only structures of metabolites **3** (5 mg) and **4** (20 mg) were fully characterized as the other two compounds were in quantities insufficient for full structure elucidation.

However, one of the minor compounds was assigned a tentative chalconol structure (**9**) (2 mg).

3,4'-Dihydroxyflavone (**3**) was obtained as a pale yellow solid (5 mg, 1.25% yield). *R_f* 0.72 [MeOH-CH₂Cl₂ (1:19)]; ¹H-NMR 600 MHz (CDCl₃) δ: 8.09 (2H, d, *J*=8.4 Hz, H-2', 6'), 8.08 (d, *J*=7.2 Hz, H-5), 7.76 (br dd, *J*=7.8, 7.2 Hz, H-7), 7.71 (br d, *J*=7.8 Hz, H-8), 7.43 (1H, br dd, *J*=7.8, 7.2 Hz, H-6), 6.93 (2H, d, *J*=8.4 Hz, H-3'—H-5'). ¹³C-NMR 150 MHz (CDCl₃, CD₃OD): δ 173.2 (C-4), 159.9 (C-4'), 155.1 (C-9), 146.8 (C-2), 138.5 (C-3), 134.1 (C-7), 130.5 (C-1'), 130.2 (C-2', -6'), 125.4 (C-5), 125.1 (C-6), 118.9 (C-8), 116.1 (C-3'). UV λ_{max} (MeOH) nm (log ε): 208 (3.67), 232 (3.49), 250 (3.44), 314 (3.19), 358 (3.49); IR ν_{max} (CHCl₃) cm⁻¹: 3308, 2924, 1653, 1602, 1558, 1484, 1280, 1178, 750. HR-ESI-MS [M+H]⁺: (*m/z*) 255.0596 (Calcd for C₁₅H₁₀O₄+H: 255.0659).

Flavone 3-*O*-β-D-4-*O*-methylglucopyranoside (**4**) was isolated as a light yellow solid (20 mg, 5% yield). *R_f* 0.34 [MeOH-CHCl₃ (1:9)]; ¹H-NMR 600 MHz (CDCl₃) δ: 8.24 (br d, *J*=8.4 Hz, H-5), 8.08 (2H, d, *J*=7.8 Hz, H-2', 6'), 7.73 (br dd, *J*=8.4, 7.2 Hz, H-7), 7.56 (br d, *J*=8.4 Hz, H-8), 7.52 (3H, m, H-3'—H-5'), 7.44 (1H, br d, *J*=8.2 Hz, H-6), 4.68 (1H, d, *J*=8.4 Hz, H-1"), 3.62 (1H, dd, *J*=9.0 Hz, H-3") 3.51 (1H, dd, *J*=8.4 Hz, H-2"), 3.46 (3H, s, OMe-4"), 3.45 (1H, m, H-6"), 3.34 (1H, m, H-6"), 3.08 (1H, br m, H-5"), 3.02 (1H, dd, *J*=9.0 Hz, H-4"). ¹³C-NMR 150 MHz (CDCl₃, CD₃OD): δ 175.9 (C-4), 159.1 (C-2), 155.8 (C-9), 138.0 (C-3), 134.6 (C-7), 131.5 (C-1'), 129.6 (C-2'), 128.3 (C-3', -4', -5'), 126.2 (C-5), 125.6 (C-6), 123.7 (C-10), 118.4 (C-7), 105.7 (C-1"), 78.9 (C-4"), 77.2 (C-3"), 75.8 (C-5"), 74.1 (C-2"), 62.3 (C-6"), 60.8 (OMe). UV λ_{max} (MeOH) nm (log ε): 214 (2.15), 244.0 (4.23), 300 (4.19); IR ν_{max} (CHCl₃) cm⁻¹: 3374, 2924, 1615, 1469, 1397, 1240, 1077, 762. HR-ESI-MS [M+H]⁺: (*m/z*) 415.1364 (Calcd for C₂₂H₂₂O₈+H: 415.1394).

Chalcone 3-*O*-β-D-4-*O*-methylglucopyranoside (**9**) was obtained as a white solid (3 mg, 0.75% yield). *R_f* 0.12 [MeOH-CHCl₃ (1:9)]; ¹H-NMR 600 MHz (CDCl₃) δ: 8.17 (br d, *J*=8.4 Hz, H-2'), 8.04 (2H, d, *J*=7.8 Hz, H-2, 6), 7.69 (br dd, *J*=8.4 Hz, H-4'), 7.53 (br d, *J*=8.4 Hz, H-5'), 7.45 (3H, m, H-3, -4, -5), 7.40 (1H, m, H-3'), 4.69 (1H, d, *J*=7.8 Hz, H-1"), 4.17 (1H, m, H-β'), 3.56 (1H, m, H-3"), 3.50 (1H, m, H-2"), 3.44 (1H, m, H-6"), 3.04 (1H, br m, H-5"), 2.20 (2H, m, H, β). ¹³C-NMR 150 MHz (CDCl₃, CD₃OD): δ 159.1 (C-6'), 134.6 (C-4'), 131.5 (C-1), 129.5 (C-2), 128.3 (C-3, -4, -5), 126.0 (C-2'), 125.6 (C-3'), 123.6 (C-1'), 118.4 (C-5'), 105.2 (C-1"), 76.7 (C-3", 4"), 76.3 (C-5"), 74.0, 69.7 (C-2"), 62.0 (C-6"), 59.0 (OMe), 30.0. UV λ_{max} (MeOH) nm (log ε): 210 (4.07), 228 (4.05), 278 (3.33); IR ν_{max} (CHCl₃) cm⁻¹: 3319, 2926, 1629, 1451, 1385, 1092, 771, 649. HR-ESI-MS [M+H]⁺: (*m/z*) 421.2331 (Calcd for C₂₂H₂₈O₈+H: 421.1863).

Microbial Transformation of 7-Hydroxyflavone (2**) by *Nocardia* Species (NRRL 5646)** The combined culture medium including the mycelium was extracted with EtOAc/(Me)₂CHOH-EtOAc (2:9). The solvents were evaporated to dryness and the resulting brown solid was subjected to flash chromatography (Biotage Horizon) over silica gel (KP-sil™ 60 Å cartridge) with CHCl₃ enriched with MeOH as the eluent. A single metabolite (**5**) (57.2 mg) was obtained.

7-Methoxyflavone (**5**) was isolated as a white solid (57.2 mg, 3% yield). *R_f* 0.64 [MeOH-CHCl₃ (1:19)]; ¹H-NMR 400 MHz (DMSO-*d*₆) δ: 8.05 (2H, d, *J*=7.2 Hz, H-2', 6'), 7.90 (1H, d, *J*=8.4 Hz, H-5), 7.55 (3H, m, H-3'—H-5'), 7.27 (1H, d, *J*=2.4 Hz, H-8), 7.03 (1H, dd, *J*=8.4, 2.4 Hz, H-6), 6.91 (1H, s, H-3), 3.89 (3H, s, -OMe). ¹³C-NMR 150 MHz (DMSO-*d*₆): δ 177.1 (C-4), 164.5 (C-7), 163.0 (C-2), 158.1 (C-9), 132.3 (C-4'), 131.8 (C-1'), 129.8 (C-3'), 126.9 (5), 126.9 (C-2'), 117.8 (C-10), 115.4 (C-6), 107.4 (C-3), 101.6 (C-8), 56.8 (OMe). UV λ_{max} (MeOH) nm (log ε): 212 (4.11), 252 (3.95), 306 (4.03). IR ν_{max} (KBr) cm⁻¹: 3386, 2925, 2853, 1633, 1451, 1439, 1376, 1357, 1248, 772. HR-ESI-MS [M+H]⁺: (*m/z*) 253.0864 (Calcd for C₁₆H₁₂O₃+H: 253.0865).

Microbial Transformation of 7-Hydroxyflavone (2**) by *A. alliaceus* (ATCC 10060)** The EtOAc/(Me)₂CHOH-EtOAc (2:9) extract of the combined culture media and the EtOAc extract of the MeOH extract of the mycelium showed a similar TLC profile. Partition chromatography (Saki High HPCPC: LLB-M) however, was effected on the extracts using hexane-EtOAc-(Me)₂C=O-MeOH-H₂O (3:2:2:2:2) as the solvent system. One metabolite (**6**) (123.5 mg) was isolated and characterized.

4',7-Dihydroxyflavone (**6**) was obtained as a light yellow solid (123.5 mg, 10% yield). *R_f* 0.2 [EtOAc-Hexane (3:2)]; ¹H-NMR 400 MHz (DMSO-*d*₆) δ: 7.87 (2H, d, *J*=8.4 Hz, H-2', 6'), 7.84 (1H, d, *J*=8.8 Hz, H-5), 6.94 (1H, br s, H-8), 6.90 (2H, d, *J*=8.4 Hz, H-3'—H-5'), 6.88 (1H, d, *J*=8.8 Hz, H-6), 6.67 (1H, s, H-3), ¹³C-NMR 150 MHz (DMSO-*d*₆): δ 177.0 (C-4), 163.4 (C-7), 163.2 (C-2), 161.4 (C-4'), 158.1 (C-9), 128.8 (C-2', 6'), 127.1 (C-5), 122.5 (C-1'), 116.8 (C-10), 116.6 (C-3', 5'), 115.5 (C-6), 105.1 (C-3), 103.1 (C-8). UV λ_{max} (MeOH) nm (log ε): 216 (4.31), 232 (4.28), 254 (4.05), 3.08

(4.38). IR ν_{max} (KBr) cm⁻¹: 3190, 2360, 2336, 1630, 1602, 1554, 1450, 1383, 1273, 1231, 1178, 827, 665. HR-ESI-MS [M+H]⁺: (*m/z*) 255.0619 (Calcd for [C₁₅H₁₀O₄+H]: 255.0658).

Microbial Transformation of 7-Hydroxyflavone (2**) by *B. bassiana*** The EtOAc/(Me)₂CHOH-EtOAc (2:9) extract of the combined fermentation broth when evaporated yielded a brown solid. It was subjected to column chromatography (Alumina-neutral: 50—200 μÅ: Acrose Organic) using CHCl₃ gradually enriched with MeOH. One compound (**7**) (186.9 mg) was isolated and identified.

Flavone 7-*O*-β-D-4-*O*-methylglucopyranoside (**7**) was isolated as a white solid (186.9 mg, 21% yield). *R_f* 0.34 [MeOH-CHCl₃ (1:9)]; ¹H-NMR 400 MHz (DMSO-*d*₆) δ: 8.05 (2H, d, *J*=7.2 Hz, H-2', 6'), 7.95 (1H, d, *J*=8.4 Hz, H-5), 7.55 (3H, m, H-3'—H-5'), 7.37 (1H, s, H-8), 7.12 (1H, br d, *J*=8.4 Hz, H-6), 6.94 (1H, s, H-3), 5.55 (1H, d, *J*=8.4 Hz, OH-2"), 5.34 (1H, d, *J*=4.8 Hz, OH-3"), 5.14 (1H, d, *J*=7.6 Hz, H-1"), 4.78 (1H, m, OH-6"), 3.67 (2H, m, H-6"), 3.52 (1H, br d, *J*=8.4 Hz, H-5"), 3.46 (3H, s, OMe-4"), 3.44 (1H, m, H-3") 3.32 (1H, dd, *J*=8.4 Hz, H-2"), 3.07 (1H, dd, *J*=8.4 Hz, H-4"). ¹³C-NMR 150 MHz (DMSO-*d*₆): δ 177.2 (C-4), 163.1 (C-2), 162.3 (C-7), 157.8 (C-9), 132.4 (C-4'), 131.8 (C-1'), 129.8 (C-5'), 126.9 (C-2'), 118.7 (C-10), 116.2 (C-6), 107.5 (C-3), 104.4 (C-8), 100.3 (C-1"), 79.5 (C-4"), 76.8 (C-3"), 76.4 (C-5"), 74.0 (C-2"), 60.8 (C-6"), 60.4 (OMe). UV λ_{max} (MeOH) nm (log ε): 214 (4.27), 252 (4.24), 308 (4.35). IR ν_{max} (CHCl₃) cm⁻¹: 3429, 2905, 1447, 1372, 1086, 773, 650. HR-ESI-MS [M-H]⁺: (*m/z*) 413.1267 (Calcd for C₂₂H₂₂O₈-H: 413.1236).

A fraction of the above extract when subjected to flash chromatography (Biotage Horizon) over silica gel (KP-sil™ 60 Å cartridge) with MeOH-CHCl₃ (1:9) as the solvent system yielded the metabolite **8** (64.8 mg).

4'-Hydroxyflavone 7-*O*-β-D-4-*O*-methylglucopyranoside (**8**) was isolated as a white solid (64.8 mg, 7% yield). *R_f* 0.16 [MeOH-CHCl₃ (3:2)]; ¹H-NMR 400 MHz (DMSO-*d*₆) δ: 7.97 (2H, d, *J*=8.8 Hz, H-2', 6'), 7.92 (1H, d, *J*=8.8 Hz, H-5), 7.33 (1H, d, *J*=2.4 Hz, H-8), 7.10 (1H, dd, *J*=8.8, 2.4 Hz, H-6), 6.92 (2H, d, *J*=8.8 Hz, H-3', H-5'), 6.77 (1H, s, H-3), 5.50 (1H, d, *J*=4.8 Hz, OH-2"), 5.30 (1H, d, *J*=5.2 Hz, OH-3"), 5.12 (1H, d, *J*=8.0 Hz, H-1"), 4.75 (1H, m, OH-6"), 3.65 (2H, m, H-6"), 3.50 (1H, *J*=7.6 Hz, H-5"), 3.45 (3H, s, OMe-4"), 3.43 (1H, m, H-3"), 3.28 (1H, *J*=8.0 Hz, H-2"), 3.05 (1H, *J*=8.8 Hz, H-4"). ¹³C-NMR 150 MHz (DMSO-*d*₆): δ 177.0 (C-4), 163.6 (C-2), 162.1 (C-7), 161.5 (C-4'), 157.7 (C-9), 128.9 (C-2', 6'), 126.9 (C-5), 122.3 (C-1'), 118.7 (C-10), 116.6 (C-3', 5'), 116.0 (C-6), 105.3 (C-3), 104.3 (C-8), 100.3 (C-1"), 79.5 (C-4"), 76.8 (C-3"), 76.4 (C-5"), 74.0 (C-2"), 60.8 (C-6"), 60.4 (OMe). UV λ_{max} (MeOH) nm (log ε): 212 (4.11), 230 (4.02), 254 (3.79), 326 (4.08). IR ν_{max} (KBr) cm⁻¹: 3426, 2921, 1628, 1564, 1509, 1447, 1383, 1251, 1138, 639. HR-ESI-MS [M-H]⁺: (*m/z*) 429.1212 (Calc for C₂₂H₂₂O₉-H: 429.1185).

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