Microbial Hydroxylation and Methylation of Genistein by *Streptomycetes*

Mohammed Hosny[†] and John P. N. Rosazza*

Division of Medicinal and Natural Products Chemistry, Center for Biocatalysis and Bioprocessing, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52242

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Streptomyces griseus (ATCC 10137) and *Streptomyces catenulae* (ATCC 23893) were used to convert genistein—5,7,4′-trihydroxyisoflavone (**1**)—a major isoflavone found in soybeans, into five metabolites, four of which were previously unknown. These were 5,7,3′,4′-tetrahydroxy-8-methylisoflavone (**3**), 5,7,3′,4′-tetrahydroxy-8-methylisoflavanone (**4**), 5,7,4′-trihydroxy-3′-methoxyisoflavone (**5**), 5,7,8,3′,4′-pentahydroxyisoflavone (**7**), and the known compound, 5,7-dihydroxy-3′,4′-dimethoxyisoflavone (**6**). Identities of these metabolites were established by HRFABMS and 1D and 2D NMR correlation spectroscopy.

Dietary soybeans have been widely acclaimed for a variety of favorable physiological roles in human health. Genistein (5,7,4′-trihydroxyisoflavone, **1**) has been described as a cancer chemoprotectant,[1] an inhibitor of tyrosine-specific protein kinases,[2] and an antioxidant,[3] in addition to its estrogenic,[4] antifungal,[5] antibacterial,[6] and antihemolytic[3] activities. Several isoflavones have been identified in urine collected from human subjects on soy diets. These included dihydrodaidzein, *O*-desmethylangolensin, glycitein, 6′-hydroxy-*O*-desmethylangolensin, dihyrogenistein, dehydro-*O*-desmethylangolensin,[7−12] and equol.[13] Microorganisms and their enzymes have been widely used to catalyze biotransformations of flavonoids;[14−19] however, few microbial transformation studies have been conducted using genistein as substrate,[18,20] Two bacterial strains isolated from "tempe", a traditional Indonesian soybean-derived food, transformed genistein to 5,6,7,4′-tetrahydroxyisoflavone and 5,7,8,4′-tetrahydroxyisoflavone.[20] Surprisingly, biotransformation of genistein by cytochrome P-450 minus mutants of *Streptomyces griseus* yielded chlorinated metabolites, 8-chlorogenistein and 6,8-dichlorogenistein.[18] Genistein, a well-known component of soybean meal is an inducer of a soluble, cytochrome P-450 enzyme system in *S. griseus*. [21] When wild-type *S. griseus* strains were grown in soybean-meal medium, genistein was gradually consumed.[22] However, no genistein metabolites could be observed in culture extracts.

Streptomycetes are well-known for the enormous chemical and biochemical diversity they display in the biosynthesis of numerous structural classes of antibiotics.[23] They also catalyze many oxidative, reductive, and conjugative biotransformations of natural and synthetic compounds. These include aromatic and aliphatic hydroxylation, *O*-and *N*-dealkylation, *N*-oxidation, C−C scission, glycosidation, deglycosidation, methylation, and halogenation reactions.[18,23,24]

This paper describes microbiological transformations of genistein catalyzed by whole growing cultures of two *Streptomycetes* strains. We were interested in preparing rare or novel isoflavones from genistein as an abundant prototype structure available from soybean processing and in determining the metabolic fate of genistein in *S. griseus* strains. Screening experiments revealed that *S. griseus* (ATCC 10137 and ATCC 13273) and *S. catenulae* (ATCC 23893) transformed genistein into metabolites. We report the production, isolation, and characterization of four isoflavones and one isoflavanone produced by these microorganisms.

Results and Discussion

Initial screening revealed that two *S. griseus* (ATCC 13273, ATCC 10137) and one *S. catenulae* (ATCC 23893) strains gave mixtures of metabolites when genistein was used as substrate. *S. griseus* (ATCC 10137) and *S. catenulae* (ATCC 23893) were selected for preparative scale reactions because they reproducibly formed all of the genistein metabolites observed in screening. Five metabolites were isolated and characterized from preparative scale microbial transformations of genistein by these organisms. Metabolites **3**, **4**, **5**, and **6** were obtained from *S. griseus*, and metabolites **3** and **7** were obtained from *S. catenulae*. Metabolites **3**, and **5**−**7** were identified as isoflavones[25,26] based upon UV spectral absorbances with λ_{max} 263−272 nm and by a typical lowfield singlet signal for H-2 between 8.2 and 8.4 ppm in their ^{1}H NMR spectra. Metabolite **4** was determined to be an isoflavanone by UV (λ_{max} 286, 325 sh nm)[25,26] and by characteristic ^{1}H NMR, H-3, and H-2 proton resonances. All metabolites contained A-ring 5,7-hydroxyl groups based on positive $AlCl_3$ and NaOAc-induced UV spectral shifts.[25,26] Metabolites **3**, **4**, and **7** contained *ortho*-dihydroxy groups based on UV shifts when $AlCl_3$ was added.[25,26] Retro Diels−Alder MS fragmentations were useful in confirming structural changes in ring A or B.

HRFABMS of **3** gave *m/z* 301.0717 $[M + H]^+$ for $C_{16}H_{13}O_6$ (HRFABMS) for a metabolite containing one additional oxygen atom plus a methyl group versus **1**. A 3H singlet at 2.14 ppm in the ^{1}H NMR spectrum confirmed the presence of a new methyl group in **1**. The absence of signals for H-6 and H-8 of **1** and the appearance of a new, lone singlet at 6.48 ppm in **3**, suggested that the methyl group was on C-8.[27−30] The ^{13}C NMR spectrum showed a new quaternary carbon signal at 104.84 ppm corresponding to C-8, while that at 98.95 ppm (C-8 of **1**) was absent. Further, C-6 of **3** was shifted downfield 2.28 ppm versus that of **1**. The HMBC NMR spectrum of **3** showed that H-6 (6.48 ppm) was coupled to carbons C-5, C-7, C-8, and C-10 at 155.59, 163.09, 104.84, and 104.00 ppm, respectively. Also, H-2 (8.32 ppm) was coupled to C-1′, C-3, C-4, and C-9 at 121.49, 123.80, 180.27, and 158.81 ppm, respectively. A two-bond coupling between the 3H singlet at 2.14 ppm and C-8 (104.84 ppm) established the methyl group at C-8. The

* To whom correspondence should be addressed. Tel.: (319) 335-4902. Fax: (319) 335−4901. E-mail: john-rosazza@uiowa.edu.
[†] Present address: Al-Azhar University, Faculty of Pharmacy, Department of Pharmacognosy, Cairo, Egypt.

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additional oxygen atom was assigned to C-3′ by a combination of UV and NMR spectral evidence. A second UV band at 272 nm for **3** (vs none in **1**) suggested a 3′,4′-*ortho*-dihydroxy system for ring B.[25,26] Bathochromic shifts observed upon addition of NaOAc + H_3BO_3 and $AlCl_3$/$AlCl_3$ + HCl further supported a ring-B catechol system. The B-ring catechol was confirmed by ^{1}H NMR, which showed *meta*-coupling between signals for H-2′ and H-6′ (J = 2.5 Hz) and *ortho*-coupling between H-6′ and H-5′ (J = 8.3 Hz). The 145.76 and 145.20 ppm chemical shifts for C-3′ and C-4′ also supported the 3′,4′-catechol arrangement for ring B.[31] Thus, metabolite **3** was 5,7,3′,4′-tetrahydroxy-8-methylisoflavone.

HRFABMS of **4** gave *m*/*z* 303.0874 $[M + H]^+$ for $C_{16}H_{15}O_6$ for a dihydro-analogue of **3**. The ^{1}H, ^{13}C, and HMBC NMR spectra showed that rings A and B were very similar for **3** and **4**. The major difference was that the singlet for H-2 in **3** at 8.32 ppm was absent in **4**, indicating that the 2,3-olefinic double bond had been reduced in **4**. Metabolite **4** showed an ABX system for H-2a, H-2b, and H-3 characteristic of C-ring protons of an isoflavanone.[25,26] The UV (MeOH) absorption at 282 nm also supported an isoflavanone structure. Thus, **4** was identified as 5,7,3′,4′-tetrahydroxy-8-methylisoflavanone. Although **4** exhibits a positive specific rotation (+ 9.3°), the absolute stereochemistry at C-3 remains unknown.

HRFABMS of **5** gave *m*/*z* 301.0718 $[M + H]^+$ for $C_{16}H_{13}O_6$ for a compound containing an additional CH_3O- moiety versus **1**. Characteristic UV absorptions indicated A-ring hydroxyl groups at positions 5 and 7.[25,26] The ^{1}H NMR spectrum of **5** was almost identical to that of **1** for rings A and C, with methine signals for H-2 and *meta*-coupled doublets at 6.74 and 6.41 ppm for H-8 and H-6, respectively. A 3H-methoxy singlet was evident at 3.82 ppm. The H-3′ and H-4′ substitution pattern for ring B was evident by an ABX pattern for protons at positions 2′, 5′, and 6′. The relative positions of the 3′-methoxy and 4′-hydroxy were confirmed by NOE NMR spectroscopy. Irradiation of the methoxy signal at 3.82 ppm caused an 18% increase in H-2′ at 7.32 ppm. Conversely, irradiation of the H-2 signal enhanced the methoxy signal. Therefore, **5** was 5,7,4′-trihydroxy-3′-methoxyisoflavone.

HRFABMS of **6** gave *m*/*z* 315.0879 $[M + H]^+$ for $C_{17}H_{15}O_6$ for a structure containing one more methyl group than **5**. ^{1}H and ^{13}C NMR spectra of **6** showed *meta*-coupled proton signals at 6.59 ppm (99.70) and 6.88 ppm (97.48) for H-6 and H-8, respectively, and an H-2 singlet at 8.34 ppm. Thus, rings A and C are essentially the same as with **5**. Signals for two methoxy groups were observed at 3.90 ppm (55.86) and 3.84 ppm (55.98). The aromatic proton pattern for ring B clearly indicated that two methoxy groups were at positions 3′ and 4′ with *ortho*-coupled doublets at 6.97 ppm (J = 8.3 Hz, H-5′) and 7.18 ppm (J = 8.3,2.5 Hz, H-6′) and a *meta*-coupled doublet at 7.39 ppm (J = 2.5 Hz, H-2′). The ^{1}H NMR and MS data were comparable for 5,7-dihydroxy-3′,4′-dimethoxyisoflavone, 3′-O-methylpratensein, previously isolated from *Bolusanthus speciosus*.[32]

HRFABMS of **7** gave *m*/*z* 303.0509 $[M + H]^+$ for $C_{15}H_{11}O_6$ by a dihydroxy metabolite of **1**. The methanolic UV spectral band at 271 nm underwent shifts in the presence of NaOAc, NaOMe, and $AlCl_3$, indicating C-5 and C-7 hydroxyl groups. The presence of only one, unsplit proton signal at 6.46 ppm, indicated either a 5,6,7- or 5,7,8-trihydroxy A-ring structure. The B ring contained a 3′,4′-catechol moiety as suggested by analogy of the signals for H-2′, H-5′, and H-6′ with metabolites **3** and **4**. Irradiation of the C-5 hydroxyl proton singlet at 12.65 ppm produced a small enhancement of the 6.46 ppm signal for H-6. A C-8 hydroxy is consistent with the observed chemical shift of 98.79 ppm.[31] As with **3** and **4**, the B ring contained the 3′,4′-catechol structure. Accordingly, **7** was identified as 5,7,8, 3′,4′-pentahydroxy-8-methylisoflavone.

All the metabolites identified in this study contain an *ortho*-dihydroxy ring B, as well as different patterns of methylation. Methyl groups are located on phenolic oxygen atoms at positions C-3′ and C-4′, and on C-8 in metabolites **3** and **4**. The structures of the metabolites strongly indicate that a pathway for biotransformation of genistein by *S. catenulae* and *S. griseus* requires initial 3′-hydroxylation to form the unisolated catechol **2**, followed by subsequent additional methylation and reduction to form **3** and **4**, methylation to form **5** and **6**, and further hydroxylation to form a second catechol **7** (see Scheme 1). Soybean isoflavones are known inducers of a soluble cytochrome P450 system in *S. griseus*.[21] It now appears that genistein is both an inducer of a cytochrome P450 hydroxylase and a substrate that undergoes catechol formation. It is interesting that the methylation of catechols produced by initial hydroxylation by genistein-containing cultures is similar to a mammalian, Phase-II conjugation-type metabolic reaction.[33] Based upon our earlier work with genistein using cytochrome P450 minus mutants, which chlorinate, (but do not hydroxylate) genistein, it appears that genistein may induce both a P450 hydroxylase system as well as a transmethylation system capable of *O*- and *C*-methylation reactions.[34] We have purified and characterized an O-methyltransferase from *S. griseus*, and the description of the enzyme and its properties is the subject of another communication.

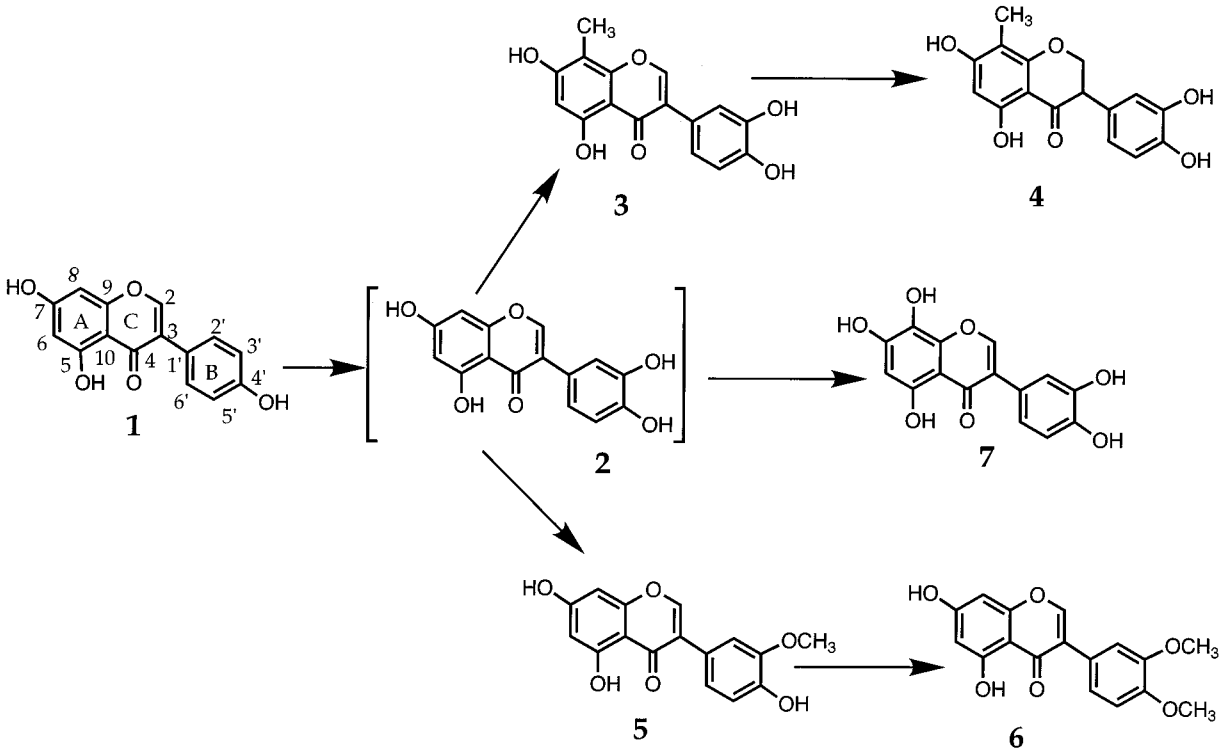
Experimental Section

Instrumentation. Thin-layer chromatography (TLC) was carried out on precoated Si gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by fluorescence quenching under 254-nm UV light and by spraying with Pauly's reagent[35] ($NaNO_2$ 0.5%, sulfanilic acid 0.5% in HCl 2%, and NaOH 5% in 50% ethanol). Equal volumes of $NaNO_2$ and sulfanilic acid solutions were mixed immediately prior to use and sprayed onto plates, followed by NaOH and warming with a heat gun for 3 min to turn isoflavone compounds burnt-orange. Flash column chromatography was performed using J. T. Baker glassware with 40 mm Si gel (Baker) and Sepralyte C_{18}, 40 mm as the stationary phase. UV spectra were determined on a Hitachi 340 spectrophotometer. IR spectra were obtained using a Nicolet 205 FT-IR spectrometer (Nicolet Instruments, Madison, WI) connected to a Hewlett−Packard ColorPro plotter. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker NM 360 spectrometer (Bruker Instruments, Billerica, MA), operating at 360.134 and 90.56 MHz, respectively. All NMR spectra were obtained in pyridine-d_5 using TMS as internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (J) in Hertz COSY and HMBC experiments were carried out using a Bruker AMX-600 high field spectrometer equipped with an IBM Aspect-2000 processor. NOE NMR experiments were performed using the spectral subtraction technique (NOEDS). The sample for NOE measurements was previously degassed by bubbling argon through the solution for 40 min. HRFABMS spectra were taken on a Fisons VG-ZAB-HF reversed-geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.).

Substrate. Genistein was obtained from Indofine Chemical Company, Inc. (Somerville, NJ). The purity of substrate was determined by TLC, UV, and ^{1}H NMR spectra.

Microorganisms. Cultures of *S. griseus* (ATCC 10137), *S. griseus* (ATCC 13273), and *S. catenulae* (ATTC 23893) were

Scheme 1



maintained on Sabouraud maltose agar slants and stored in a refrigerator at 4 °C prior to use.

Screening Procedure. Cultures were grown according to a two-stage procedure in 25 mL of soybean meal−glucose medium held in stainless steel-capped, 125-mL Delong culture flasks. The soybean meal−glucose medium contained (in g/L) 20 dextrose, 5 yeast extract, 5 soybean meal, 5 NaCl, and 5 K_2HPO_4 in distilled H_2O and adjusted to pH 7.0 with 6 N HCl before being autoclaved at 121 °C for 15 min. Cultures were incubated with shaking at 250 rpm at 28 °C on a New Brunswick Scientific, Innova 5000 Gyrotory tier shaker. A 10% inoculum from 72-h-old stage I cultures was used to initiate stage II cultures, which were incubated for 24 h before receiving 5 mg of **1** in 0.5 mL of *N*,*N*-dimethylformamide as substrate and incubated as before. Substrate controls consisted of sterile medium incubated under the same conditions but without microorganism. Substrate-containing cultures were generally sampled by removing 3 mL of the entire culture at 24, 72, and 144 h after addition of substrate. After determination of pH, they were acidified to pH 2.0 with 6 N HCl and extracted with equal volumes of (EtOAc−*n*-BuOH 9:1). The organic layers were separated by centrifugation for 1 min in a desk-top centrifuge and used for analysis, where 30−40-mL samples were spotted on TLC plates developed with $CHCl_3$−MeOH−H_2O (80:20:2).

Preparative Biotransformations of 1. *S. griseus* (ATCC 10137) and *S. catenulae* (ATTC 23893) were selected for preparative biotransformation reactions. For 24-h-old stage II cultures, a total of 200 mg of **1** was distributed among 20 125-mL DeLong flasks for *S. griseus*, and 180 mg of **1** was distributed among 18 125-mL DeLong flasks for *S. catenulae.* Substrate-containing cultures were incubated for 144 h for *S. griseus* and 72 h for *S. catenulae*, at which time the cultures were separately harvested and centrifuged at 7000 × *g* for 10 min (Sorvall RC-5 Superspeed Refrigerated centrifuge). The supernatants were adjusted to pH 2.0 with 6 N HCl, and each was extracted with EtOAc−*n*-BuOH (9: 1, 3 × 500 mL). The organic extracts were washed with distilled H_2O, dried over anhydrous $NaSO_4$, and concentrated in vacuo to yield viscous residues from *S. griseus* (137 mg) and *S. catenulae* (97 mg).

Isolation of Metabolites. The extracts were separately subjected to Si gel flash column chromatography (1.5 × 50 cm) eluted with CH_2Cl_2−MeOH (90:10→60:40) to yield fractions **A**, (46 mg), **B** (43 mg), and **C** (30 mg) from *S. griseus* and two fractions, **D** (50 mg) and **E** (32 mg) from *S. catenulae.* Successive flash column chromatography of each fraction was conducted over reversed-phase [Sepralyte C_{18}] Si gel, eluted with (MeCN−H_2O−HCO_2H (50:50:1), using column pressures of 0.28 kg/cm^2, at a flow rate of 2 mL/min, while 5- mL fractions were collected. Final sample purifications were done by Sephadex LH-20 column chromatography eluted with MeOH to afford metabolites **3** (7 mg), **4** (9 mg) **5** (6 mg), and **6** (9 mg) from *S. griseus*, and **3** (7 mg) and **7** (9 mg) from *S. catenulae.*

Genistein, 5,7,4′-trihydroxyisoflavone (1): UV (MeOH) λ_{max} (log ϵ) 238 (4.12), 276 (4.48), + NaOMe 296, + $AlCl_3$ 276, + HCl 278, + NaOAc 279 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 8.32 (1H, s, H-2), 6.23 (1H, d, J = 1.9 Hz, H-6), 6.82 (2H, dd, J = 8.0, 1.9 Hz, H-8), 7.35 (2H, dd, J = 8.0, 1.9 Hz, H-2′, H-6′), 6.93 (1H, d, J = 8.0 Hz, H-5′), 9.10 (1H, brs, HO-4′), 12.44 (1H, br s, HO-5); ^{13}C NMR (pyridine-d_5, 90.56 MHz) δ 154.20 (C-2), 124.14 (C-3), 180.19 (C-4),163.08 (C-5),100.10 (C-6), 165.00 (C-7), 98.95 (C-8), 159.20 (C-9), 106.18 (C-10), 123.16 (C-1′), 130.86 (C-2′, C-6′), 115.73 (C-3′, C-5′), 158.26 (C-4′); FABMS, m/z 271 [M + H]$^+$, (calcd for $C_{15}H_{11}O_5$).

5,7,3′,4′-Tetrahydroxy-8-methylisoflavone (3): yellow, amorphous powder (7 mg); $[\alpha]^{20}_D$ +7.9° (c 0.69 pyridine); UV (MeOH) λ_{max} (log ϵ) 238 (4.12), 272 (4.32), + NaOMe 289, + $AlCl_3$ 279, + HCl 278, + NaOAc 283 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 8.32 (1H, s, H-2), 6.48 (1H, s, H-6), 7.18 (1H, d, J = 2.5 Hz, H-2′), 6.97 (1H, d, J = 8.3 Hz, H-5′), 7.30 (1H, dd, J = 8.3, 2.5 Hz, H-6′), 2.14 (3H, s, Me-8) 12.65 (1H, brs, HO-5); ^{13}C NMR (pyridine-d_5, 90.56 MHz) δ 153.42 (C-2), 123.80 (C-3), 180.27 (C-4), 155.59 (C-5), 102.38 (C-6), 163.09 (C-7), 104.84 (C-8), 158.81 (C-9), 104.00 (C-10), 121.49 (C-1′), 115.78 (C-2′), 146.76 (C-3′), 145.20 (C-4′), 114.64 (C-5′), 119.12 (C-6′), 21.18 (Me-8); HRFABMS m/z 301.0717 [M + H]$^+$, (calcd for $C_{16}H_{13}O_6$ 301.0715).

5,7,3′,4′-Tetrahydroxy-8-methylisoflavone (4): yellow, amorphous powder (9 mg); $[\alpha]^{20}_D$ +9.3° (c 0.59 pyridine); UV (MeOH) λ_{max} (log ϵ) 243 (4.29), 282 (4.56), + NaOMe 298, + $AlCl_3$ 289, + HCl 292, + NaOAc 299 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 4.52 (1H, dd, J = 5.7, 11.5 Hz, H-2a), 4.73 (1H, dd, J = 4.0, 11.5 Hz, H-2b), 4.28 (1H, t, J = 4.0 Hz, H-3), 6.51 (1H, s, H-6), 6.95 (1H, d, J = 2.5, Hz, H-2′), 6.78 (1H, d, J = 8.0 Hz, H-5′), 7.11 (1H, dd, J = 2.5, 8.0 Hz, H-6′), 2.18 (3H, s, Me-8), 12.18 (1H, br s, HO-5); ^{13}C NMR (pyridine-d_5,

90.56 MHz) δ 71.85 (C-2), 47.59 (C-3), 194.82 (C-4), 163.87 (C-5), 96.48 (C-6), 164.25 (C-7), 108.12 (C-8), 160.77 (C-9), 103.90 (C-10), 127.25 (C-1′), 111.60 (C-2′), 148.96 (C-3′), 148.28 (C-4′), 111.15 (C-5′), 120.34 (C-6′), 21.10 (Me-8); HRFABMS, *m/z* 303.0874 $[M + H]^+$, (calcd for $C_{16}H_{15}O_6$, 303.0868).

5,7,4′-Trihydroxy-3′-methoxyisoflavone (5): yellow, amorphous powder (6 mg), $[\alpha]^{25}_D$ +6.8° (*c* 0.83 pyridine); UV (MeOH) λ_{max} (log ϵ) 267 (4.27) + NaOMe 281, + $AlCl_3$ 278, + HCl 276, + NaOAc 280 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 8.21 (1H, s, H-2), 6.41 (1H, d, J = 2.0 Hz, H-6), 6.74 (1H, d, J = 2.0 Hz, H-8), 7.32 (1H, d, J = 2.2 Hz, H-2′), 6.98 (1H, d, J = 8.5 Hz, H-4′), 7.12 (1H, dd, J = 8.5, 2.2 Hz, H-6′), 3.82 (3H, s, MeO-3′), 9.26 (1H, br s, HO-7), 12.83 (1H, br s, HO-5); ^{13}C NMR (pyridine-d_5, 90.56 MHz) δ 154.52 (C-2), 124.86 (C-3), 179.30 (C-4), 163.10 (C-5), 98.55 (C-6), 164.80 (C-7), 97.53 (C-8), 155.48 (C-9), 105.81 (C-10), 123.18 (C-1′), 114.13 (C-2′), 148.95 (C-3′), 150.62 (C-4′), 111.50 (C-5′), 119.32 (C-6′), 55.85 (MeO-3′); HRFABMS *m/z* 301.0718 $[M + H]^+$, (calcd for $C_{16}H_{13}O_6$, 301.0718).

5,7-Dihydroxy-3′,4′-dimethoxyisoflavone (6): yellow, amorphous powder (9 mg); $[\alpha]^{20}_D$ +13.9° (*c* 0.75 pyridine); UV (MeOH) λ_{max} (log ϵ) 263 (4.40), + NaOMe 282, + $AlCl_3$ 276, + HCl 278, + NaOAc 279, + H_3BO_3 272 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 8.34 (1H, s, H-2), 6.59 (1H, d, J = 2.5 Hz, H-6), 6.88 (1H, d, J = 2.5 Hz, H-8), 7.39 (1H, d, J = 2.5 Hz, H-2′), 6.97 (1H, d, J = 8.3 Hz, H-5′), 7.18 (1H, dd, J = 8.3, 2.5 Hz, H-6′), 3.90 (3H, s, MeO-3′), 3.84 (3H, s, MeO-4′), 9.59 (1H, br s, HO-7), 12.52 (1H, br s, HO-5); ^{13}C NMR (pyridine-d_5, 90.56 MHz) δ 152.75 (C-2), 124.23 (C-3), 178.40 (C-4), 162.19 (C-5), 99.70 (C-6), 163.50 (C-7), 97.48 (C-8), 155.90 (C-9), 105.79 (C-10), 124.36 (C-1′), 113.10 (C-2′), 148.95 (C-3′), 147.36 (C-4′), 110.91 (C-5′), 122.12 (C-6′), 55.86 (MeO-3′), 55.98 (MeO-4′); HRFABMS *m/z* 315.0879 $[M + H]^+$, (calcd for $C_{17}H_{15}O_6$, 315.0868).

5,7,8,3′,4′-Pentahydroxy-8-methylisoflavone (7): yellow, amorphous powder (9 mg); $[\alpha]^{20}_D$ +11.5° (*c* 0.52 pyridine); UV (MeOH) λ_{max} (log ϵ) 235 (4.18), 271 (4.63), + NaOMe 293, + $AlCl_3$ 279, + HCl 281, + NaOAc 284 nm; ^{1}H NMR (pyridine-d_5, 600 MHz) δ 8.28 (1H, s, H-2), 6.46 (1H, s, H-6), 6.87 (1H, d, J = 2.2 Hz, H-2′), 6.98 (1H, d, J = 8.0 Hz, H-5′), 7.26 (1H, dd, J = 8.0, 2.2 Hz, H-6′), 12.65 (1H, br s, HO-5); ^{13}C NMR (pyridine-d_5, 90.56 MHz) δ 152.37 (C-2), 121.95 (C-3), 179.12 (C-4), 154.89 (C-5), 98.79 (C-6), 163.24 (C-7), 133.40 (C-8), 159.36 (C-9),103.72 (C-10),121.56 (C-1′), 116.21 (C-2′), 144.82 (C-3′), 144.18 (C-4′), 114.28 (C-5′), 119.45 (C-6′); HRFABMS *m/z* 303.0509 $[M + H]^+$, (calcd for $C_{15}H_{11}O_7$, 303.0504).

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