A Simple Synthesis of 7,4′-Dihydroxy-6-methoxyisoflavone, Glycitein, the Third Soybean Isoflavone

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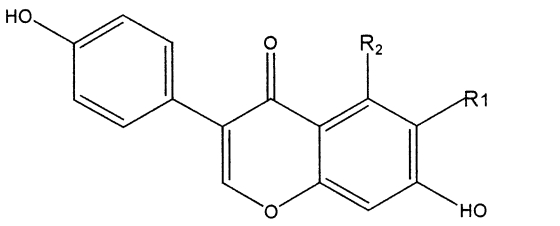
4-Methoxyresorcinol (**3**) was synthesized as the precursor for glycitein (**6**) synthesis by the oxidation of 3-hydroxy-4-methoxybenzaldehyde (**1**) to the aryl formate with H_2O_2 and a catalytic amount of SeO_2. Glycitein (**6**) was synthesized by cyclization of 2,4,4′-trihydroxy-5-methoxydeoxybenzoin (**5**) with *N*,*N*-dimethylformamide, boron trifluoride diethyl ether, and methanesulfonyl chloride in a microwave oven.

The soy isoflavones, daidzein, genistein, and glycitein (**6**) (Figure 1), and their glucosides are associated with important health protective properties including cancer prevention, plasma cholesterol lowering when associated with soy protein, and reduction of postmenopausal bone loss.[1] Daidzein and genistein were isolated and identified from soybean flour by Walter,[2] and glycitein (**6**) was first isolated from a concentrated ethanol extract of defatted soybeans by Naim et al.[3] Although numerous studies have evaluated the biological activities of daidzein and genistein, the biological activity of glycitein (**6**) only recently was reported by Song et al.[4] They showed that glycitein has weak estrogenic activity comparable to that of daidzein and genistein. On an equal mole basis, glycitein (**6**) actually has a stronger estrogenic response than genistein and daidzein in the mice uterine enlargement assay. Zhang et al.[5] reported that glycitein (**6**) was more bioavailable than daidzein in humans. Therefore, although glycitein accounts for only 5−10% of the total isoflavones in soybeans, its biological activities and potential health effects cannot be neglected. Soy germ contains approximately 10 times the concentration of isoflavones compared to the rest of the soybean seed. Soy germ or hypocotyls contain significant concentrations of the glycitein forms that have lead to development of nutraceutical supplements derived from soy germ. However, the ratio of genistein/daidzein/glycitein in germ is 1:5:4, in contrast to the ratio in soybean seeds, 5:4:1.[6]

To evaluate the biological activities of these isoflavones, a stable and inexpensive source of these phytochemicals is desirable. Isolation of isoflavones from soybean products is time-consuming and produces low yields. Chemical synthesis is a practical way to produce these compounds in large, high-purity quantities. A simple, high-yield synthesis approach using conventional microwave ovens to produce genistein and daidzein was reported by Chang et al.[7] and modified by Song et al.[6] Chemical synthesis of glycitein (**6**) was reported in 1995 by Nógrádi and Szöllösy[8] using an oxidative rearrangement of the fully protected corresponding chalcone by TI $(NO_3)_3$ in methanol (MeOH) followed by deprotection and ring closure. However, their procedure to synthesize glycitein (**6**) involved multistep protection and deprotection, making the synthetic process complicated and hard to perform for the nonorganic chemist. Because of the structural similarity between daidzein, genistein, and glycitein (**6**), we proposed that glycitein could be synthesized by using a pathway similar to the microwave-mediated synthesis of daidzein and genistein,[7] if the appropriate reagents were available. Since one of the required starting reagents is not commercially available for glycitein (**6**) synthesis, in contrast to those required for genistein and daidzein, synthesis of the missing glycitein precursor was necessary and is reported here along with the synthesis of glycitein (**6**). Although others have reported synthesis of 4-methoxyresorcinol (**3**),[9] no one has reported glycitein (**6**) synthesis using the cyclization method of Chang et al.[7]



Isoflavone	R1	R2
Daidzein	H	H
Genistein	H	OH
Glycitein	OCH_3	H

Figure 1. Structures of soybean isoflavone aglucons genistein, daidzein, and glycitein.

4-Methoxyresorcinol (**3**) is the required intermediate to synthesize glycitein (**6**) using the microwave-mediated synthesis of Chang et al.[7] (Figure 2). It was prepared by the oxidation of 3-hydroxy-4-methoxybenzaldehyde (**1**) to the aryl formate (**2**) with H_2O_2 and a catalytic amount of SeO_2 in methylene chloride (CH_2Cl_2). Treatment of **2** with K_2CO_3 in MeOH at room temperature yielded **3**. Guzman et al.[9] reported a 75% yield over a 12 h oxidation reaction in *tert*-butyl alcohol for **3**. They provided no other spectral information to compare with our data. Our reaction scale was about 10 times larger than that of Guzman et al.[9] Hauer et al.[10] report NMR spectra similar to our data and report **3** as an oil.

4-Methoxyresorcinol (**3**) was refluxed with 4-hydroxyphenylacetic acid (**4**) and boron trifluoride etherate (BF_3·Et_2O) to furnish compound **5**, 2,4,4′-trihydroxy-5-methoxydeoxybenzoin (TMD). Cyclization of TMD was achieved by placing a mixture of TMD, *N*,*N*-dimethylformamide (DMF), and BF_3·Et_2O under medium microwave energy for 21 s before adding methane sulfonyl chloride

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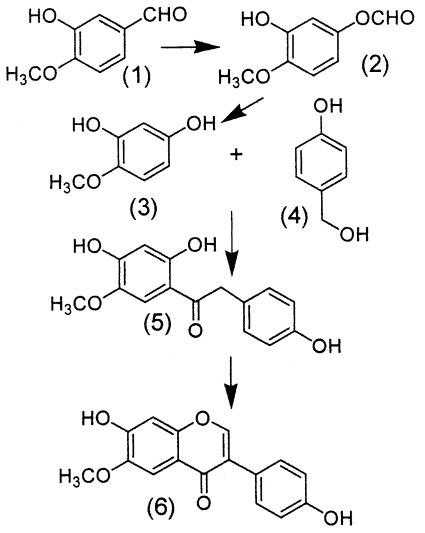

Figure 2. Reaction scheme for synthesis of 4-methoxyresorcinol followed by cyclization to glycitein.

and heating it for an additional 70 s under medium microwave energy. Pale yellow crystals of glycitein (**6**) were obtained after recrystallization from 80% MeOH. The UV spectra, HPLC, mass spectrum, and ^{1}H NMR conformed to those reported for authentic glycitein.[3,4,8,11] The melting point for our synthesized glycitein (337−339 °C) compared well with the melting point reported for glycitein synthesized by Nógrádi and Szöllösy[8] (337−339 °C) and for glycitein isolated from soy germ by Song et al.[4] (337−339 °C).

The yield of glycitein (**6**) by this microwave-mediated method was 26%, which compared well with the 36% yield reported by Chang et al.[7] for genistein but lower than the 55% yield of Nogradi and Szollosy.[7] We routinely produce glycitein in a one-pot synthesis style similar to Balasubramanian and Nair,[12] although they did not report an attempt to produce glycitein. We isolated and evaluated the intermediates for the purposes of this paper to confirm we were producing the correct compounds. However, for routine work, this is not necessary. The one-pot synthesis method can be routinely scaled up to produce gram quantities of glycitein for biological studies.

Experimental Section

General Experimental Procedures. All chemicals used in the synthesis were purchased from Sigma Chemical Company (St. Louis, MO). A Kenmore U88-332 (1350 W) microwave oven was used in the cyclization step. The identification and purity of glycitein were confirmed by using HPLC, UV spectral analysis, melting point, mass spectrum, and NMR. Glycitein was analyzed by using HPLC on a Beckman System Gold chromatography system including a Model 507 autosampler, Model 126 dual pumps, a Model 168 photodiode array detector, and an IBM 486 computer with Beckman Gold System HPLC data processing software (version 8, 1993) according to Murphy et al.[11] UV spectral analysis was performed according to Mabry et al.[13] using a Beckman DU 7400 spectrophotometer. The melting point of glycitein was measured with a Perkin-Elmer 7 series differential scanning calorimeter (DSC) (Perkin-Elmer Inc., Norwalk, CT). Mass spectral analysis, using chemical ionization, was performed on a Finnigan Model TSQ-700 mass spectrometer (Finnigan Inc., Piscataway, NJ). ^{1}H NMR was performed in deuterated DMSO on a Bruker DRX 400 MHZ for **5** and a Varian VXR 300 MHz for **3** and **6**.

4-Methoxyresorcinol (3). 3-Hydroxy-4-methoxybenzaldehyde (**1**) (0.05 mol, 7.603 g) was added to a mixture of CH_2Cl_2 (100 mL), 0.11 mol of H_2O_2 (13 mL), and SeO_2 (3.96 mmol, 0.44 g). The reaction mixture was stirred at room temperature in a water bath for 16 h. The mixture was filtered through Whatman No. 42 paper, and the filtrate was washed with 100 mL of water. The organic layer was separated and washed with 100 mL of 10% $NaHSO_3$, followed by 100 mL of 10% Na_2CO_3, and dried over anhydrous Na_2SO_4. The residue obtained after evaporation of the solvents was dissolved in 30 mL of MeOH. Then, 25 mL of a 14% solution of K_2CO_3 in water was added. The mixture was stirred at room temperature for 1 h, and the solvent was removed using a rotary evaporator. To the residue, 50 mL of water was added and extracted with ethyl ether (2 × 60 mL). The aqueous layer was saturated with NaCl and extracted again with ethyl ether (2 × 40 mL). The combined ether layers were washed with 200 mL of saturated NaCl and dried over anhydrous Na_2SO_4. The ether was removed by evaporation to give 0.034 mol (4.7818 g) (68%) of 4-methoxyresorcinol (**3**) as a clear, yellow oil: M^+ 140 [EI 140(96%), 125(100%), 97(57%), 73(20%), 61(25%), 45(21%)]; ^{1}H NMR (d_6-DMSO, 300 MHz) δ 3.67 (s, 3H, OCH_3); 6.17 (d, J = 8.5 Hz, 1H, 6-H); 6.35 (s, 1H, 2-H); 6.67 (d, J = 8.6 Hz, 1H, 5-H); 8.80 (s, 1H, OH); 8.77 (s, 1H, OH).

2,4,4′-Trihydroxy-5-methoxydeoxybenzoin (TMD) (5). 4-Methoxyresorcinol (**3**) (0.0157 mol, 2.1975 g) was added to a mixture containing 4-hydroxyphenylacetic acid (**4**) (0.0148 mol, 2.2521 g) and BF_3·Et_2O (4.5 mL). The reaction mixture was refluxed for 10 min and cooled, and 60 mL of water was added; the aqueous layer was extracted with ethyl ether (3 × 50 mL). The combined ether layers were washed with saturated aqueous sodium acetate (30 mL) and saturated $NaHCO_3$ (15 mL), respectively. The layers were separated, and the ether layer was dried with anhydrous Na_2SO_4. Removal of ether by evaporation gave 0.01135 mol (3.1106 g) (77%) of a dark yellow oil of 2,4,4′-trihydroxy-5-methoxydeoxybenzoin (**5**), $C_{15}O_5H_{14}$ (MW 274): MS spectrum showed M^+ 274; ^{1}H NMR (d_6-DMSO, 400 MHz) δ 3.75 (s, 3H, OCH_3); 4.15 (s, 2H, $-CH_2$); 6.30 (s, 1H, 5-H); 6.68 (d, J = 11.2 Hz, 2H, 2′,6′-H); 7.07 (d, J = 11.2 Hz, 2H, 3′,5′-H); 7.39 (s, 1H, 8-H).

Glycitein (6). TMD (**5**) (1.55 mmol, 0.4247 g) was dissolved in 8 mL of DMF in a 500 mL glass beaker. BF_3·Et_2O (4 mL) was added, and a vigorous exothermic reaction occurred. The reaction mixture was heated in microwave oven for 21 s using 40% energy. Then 4 mL of methanesulfonyl chloride was added to the beaker, and the mixture was heated for 70 s in the microwave oven using 40% energy. Addition of cold water (400 mL) led to the formation of a dark yellow precipitate. The supernatant and precipitate were extracted separately with ethyl ether (3 × 60 mL), the ethyl ether fractions were combined, and the ether fraction was dried with anhydrous Na_2SO_4. Evaporation of ether afforded 7,4′-dihydroxy-6-methoxyisoflavone (glycitein) (**6**) as pale yellow crystals. Glycitein (**6**) was recrystallized from 80% methanol, yielding 0.381 mmol (0.1082 g) (26%), $C_{16}O_5H_{12}$ (MW 284): MS spectrum showed M^+ 284 [EI 284 (100%), 283(42%), 167 (11%), 166(24%); ^{1}H NMR (d_6-DMSO, 300 MHz) δ 3.86 (s, 3H, OCH_3); 6.78 (d, J = 8.7 Hz, 2H, 3′,5′-H); 6.93 (s, 1H, 8-H), 7.36 (d, J = 8.4 Hz, 2H, 2′,6′-H); 7.42 (s, 1H, 5-H), 8.27 (s, 1H, 2-H); mp 337−339 °C, uncorrected; λ_{max} (methanol) 257, 319 nm; λ_{max} (sodium methoxide) 259, 344 nm; λ_{max} ($AlCl_3$) 257, 317 nm; λ_{max} ($AlCl_3$−HCl) 257, 317 nm; λ_{max} (sodium acetate) 255, 346 nm; λ_{max} (sodium acetate−H_3BO_3) 255, 320 nm.

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