# SYNTHESIS AND CHARACTERIZATION OF NOVEL UNNATURAL DI(8-DAIDZEINYL)METHANE

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A novel unnatural biflavonoid analog, di(8-daidzeinyl)methane, was synthesized using the C–C coupling reaction of daidzein and formaldehyde catalyzed by aliphatic amine in DMF, and its structure was characterized by ESI-MS, NMR, and FT-IR. Meanwhile, the effect parameters (catalyst, catalyst loading, temperature, and molar ratio of the substrates) were investigated and the optimized reaction conditions obtained using 9 mol % triethylamine as the catalyst, with the reaction run at 80°C for 20 h.

Keywords: daidzein, flavonoid, coupling reaction, synthesis.

Flavonoids generally refer to a broad group of natural products possessing a C6–C3–C6 carbon framework, or more specifically a phenylbenzopyran skeleton. Biflavonoids, one of the subclasses of flavonoids, are flavonoid dimers having a C–C or C–O–C linkage between monomers. Several natural biflavonoids, such as ginkgetin and ochnaflavone isolated from natural plants, were found to be inhibitors of group II secretory phospholipase  $A_2$ . Moreover, ginkgetin was found to exert inhibitory activity against COX-2-mediated PGE<sub>2</sub> production by inhibiting of COX-2 expression [1, 2]. Recently, some biflavonoids were prepared and found to have cytotoxic, antibacterial, and anticancer activities [3, 4]. Bidaidzeins are very interesting targets for synthesis since daidzein (4',7-dihydroxyisoflavone), which is mainly present in leguminous plants, was previously found to have antioxidant, antiallergic, anti-inflammatory, antimicrobial, and anticancer activities [5–8]. Therefore, efficient synthetic pathways for obtaining biflavonoid derivatives of daidzein have potential application value.

The impressive biological activities of biflavonoids are well known, and many of their derivatives are found in commercial products or in nature. The synthesis of novel biflavonoids has attracted considerable attention due to their increasing applications in medicinal fields in recent years. Some methods for synthesizing biflavonoids have been developed. Al-Maharik's group reported the synthesis of C–C linked biflavones by the reaction of 2-bromomethyl-7,4'-dimethoxyisoflavone with ethyl cyanoacetate anion or tetraethylammonium cyanide by Pd-catalyzed ethoxycarbonylation [9]. Zheng's group synthesized gem-diuoromethylenated biflavonoids *via* Suzuki coupling between 6-iodinated flavones and 3'-boronate flavone [10]. Sawyer and Frlan et al. prepared C–O–C linked biflavones *via* intermolecular Ullmann ether syntheses using 6-bromo flavone analogs and 7-hydroxyflavone analogs as key intermediates [11, 12]. However, these developments suffer from complicated starting materials, multistep procedures, metal salts as the catalyst, etc. Zhang's group synthesized a kind of biflavone derivatives, which included two flavones coupled with the  $CH_2$  group [13, 14]. However, the compounds were obtained by a two-step reaction. The first step (hydroxymethylation) had no regioselectivity when strong base and acid are used as catalysts.



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C atom	$\delta_{\rm C}  4$	δ <sub>C</sub> 1	Δδ	$\delta_{\rm H}$ 4	$\delta_{\rm H}$ 1	Δδ
2	153.1	153.2	-0.1	8.31	8.29	0.02
3	123.1	123.0	0.1			
4	175.6	175.1	0.5			
5	124.8	127.7	-2.9	7.83	7.98	-0.15
6	114.6	115.6	-1.0	6.88	6.95	-0.07
7	160.7	163.0	-2.3			
8	113.8	102.5	11.3	_	6.87	_
9	156.2	157.9	-1.6			
10	117.0	117.1	-0.1			
1′	123.4	123.9	-0.5			
2', 6'	130.5	130.5	0.0	7.37	7.39	-0.02
3', 5'	115.4	115.4	0.0	6.78	6.83	-0.05
4′	157.5	157.6	-0.1			
CH <sub>2</sub>	17.4	_	_	4.28	_	_
7-0H				10.55	10.82	-0.27
4'-OH				9.51	9.56	-0.05

TABLE 1. <sup>1</sup>H and <sup>13</sup>C NMR Data of **1** and **4** (DMSO-d<sub>6</sub>,  $\delta$ , ppm)



a. Triethylamine, 80°C, DMF; b. HCHO, triethylamine; c. 1, triethylamine

Scheme 1

Daidzein is one of the most important flavonoids, while  $CH_2$  bridged bidaidzein has not been reported. Herein, we present a one-pot method for direct synthesis of the novel unnatural biflavonoid analog, di(8-daidzeinyl)methane using daidzein and formaldehyde catalyzed by aliphatic amine under very mild conditions. This method offers a new, convenient approach to the synthesis of the unnatural biflavonoid analog in good yield, as shown in Scheme 1. It is especially worth mentioning that this reaction has high regioselectivity and only di(8-daidzeinyl)methane was obtained.

The biflavonoid analog di(8-daidzeinyl)methane (4) was synthesized by the reaction of daidzein (1) and 37% formaldehyde solution catalyzed by triethylamine in DMF (Scheme 1) and characterized by ESI-MS, FT-IR, <sup>1</sup>H NMR, and <sup>13</sup>C NMR. In the mass spectrum, the ion peak at m/z 519  $[M - H]^-$  indicated the formation of a daidzein dimer, the biflavonoid analog di(8-daidzeinyl)methane (4). The FT-IR spectrum of compound 4 showed a characteristik peak at 1420 cm<sup>-1</sup>, which could be attributed to the CH<sub>2</sub> group. Table 1 shows the NMR data of compound 4 and daidzein 1. In the <sup>13</sup>C NMR spectrum of compound 4, the chemical shifts of C-8 on the A-ring appeared at 113.8 ppm, shifted 11.3 ppm downfield compared with that of daidzein, and the signals of C-5, C-7, and C-9 were shifted 2.9, 2.3, 1.6 ppm upfield, respectively, indicating that the dimer featured a C8-CH<sub>2</sub>-C8 structure skeleton. The chemical shifts for carbons on the B-ring and C-ring do not visibly change. In the <sup>1</sup>H NMR spectrum of compound 4, the absence of a singlet at 6.87 ppm and the presence of a signal at 4.28 ppm confirmed the formation of a C8–CH<sub>2</sub>–C8 linked dimer.

Factors such as catalyst, temperature, molar ratio of substrates, and reaction time have important effects on the coupling reactions. We initially conducted a brief catalyst screening by running the reaction of daidzein and formaldehyde solution (molar ratio1:1) at 80°C for 20 h in the presence of different aliphatic amines, including ethylamine, diethylamine, propylamine, isopropamide, dipropylamine, *n*-butylamine, and di(*n*-butyl)amine. The result showed that the coupling reaction performed smoothly in the presence of 9 mol% triethylamine, affording the coupling product in high yield with easy purification. Decreasing the catalyst loading led to lower yields even with prolonged reaction time, and increasing the catalyst loading to 12 mol% or 20 mol% increased the reaction rate but did not improve the yield significantly. This is tentatively attributed to the rapid formation of a large amount of intermediate **3** (8-hydroxymethyldaidzein), which consumed the starting material and led to insufficient daidzein to form the dimer. The intermediate **3** was separated and characterized (white powder, <sup>1</sup>H NMR (DMSO-d<sub>6</sub> + D<sub>2</sub>O,  $\delta$ , ppm): 8.00 (1H, s, H-2), 7.58 (1H, d, J = 8.6 Hz, H-5), 7.33 (2H, d, J = 7.7 Hz, H-2', 6'), 6.78 (2H, d, J = 7.7 Hz, H-3', 5'), 6.35 (1H, d, J = 8.6 Hz, H-6), 4.69 (2H, s, CH<sub>2</sub>OH). ESI-MS *m*/z 283 [M – H]<sup>-</sup>. The reaction process was seen to go through the formation of intermediate **3**, which was in agreement with the report of Zhang et al. [13] and He et al. [14].

Running the coupling reaction at different temperatures (0°C, 20, 50, 80, 110, 140, 165°C) proved the reaction was sensitive to reaction temperature, and 80°C was the optimal one. Temperatures lower than 80°C resulted in slow reaction rates and low yields, while higher temperatures (> 80°C) generated complicated side reactions and decomposition of the product. The effect of the molar ratio of daidzein and 37% formaldehyde solution on the reaction was also investigated, and 1.5:1 proved to be the optimum ratio (yield 82.1%). Initially, increasing the amount of formaldehyde solution improved the yield of di(8-daidzeinyl)methane, while a molar ratio greater than daidzein–formaldehyde 1.5:1 resulted in low yields of di(8-daidzeinyl)methane and the major formation of 8-hydroxymethyldaidzein.

#### **EXPERIMENTAL**

**General Procedures**. Daidzein was purchased from the Shanxi Huike Botanical Development Company. All other materials were of analytical reagent grade purity. FT-IR spectra were recorded using an IR-200 Fourier transform spectrometer in KBr pellets. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer operating at 400 MHz using DMSO-d<sub>6</sub> as solvent at room temperature ( $20 \pm 2^{\circ}$ C). The chemical shifts were expressed in ppm with reference to TMS, and coupling constants (J) in Hz. Mass spectra were obtained on a Bruker Esquire 3000 instrument from ESI-MS measurements at negative mode using methanol as solvent.

**Di(8-daidzeinyl)methane (4)**. Daidzein (1) (0.5 g, 2.0 mmol) was dissolved in DMF (10 mL), and then triethylamine (0.8 mL, 0.4 mmol) and 37% formaldehyde solution (**2**, 0.16 mL, 2.0 mmol) were added. The mixture was heated at 80°C until reaction completion after about 20 h. After cooling, the solvent was evaporated *in vacuo*, and a solid residue was obtained. Recrystallization of the residue from methanol afforded the biflavonoid analog di(8-daidzeinyl)methane (**4**). White powder, FT-IR (KBr, v, cm<sup>-1</sup>): 3475 (OH), 1624 (C=O), 1420 (CH<sub>2</sub>). For <sup>1</sup>H and <sup>13</sup>C NMR spectral data, see Table 1. ESI-MS *m/z* 519  $[M - H]^{-}$ .

In conclusion, we present here a simple protocol for the synthesis of a novel unnatural biflavonoid analog di(8-daidzeinyl)methane using the triethylamine-catalyzed coupling reaction of daidzein and formaldehyde solution. Compound **4** was obtained with a yield of 82.1% when running the reaction of daidzein and formaldehyde (molar ratio 1.5:1) using 9 mol % triethylamine at 80°C for 20 h. Studies on the synthesis of other daidzein dimer derivatives will be reported in due course.

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