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ORIGINAL PAPER

NMR studies of daidzein and puerarin: active anti-oxidants in traditional Chinese medicine

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Abstract

Radix puerariae—a popular traditional Chinese medicine—is used for the treatment of diarrhea, acute dysentery, deafness, and cardiovascular diseases. It can also be used as an effective antioxidant and has been tested as an anticancer drug. Daidzein and puerarin are its main active compounds. The present contribution was focused on experimental and theoretical studies of the ^{1}H and ^{13}C NMR chemical shifts and nuclear magnetic shielding parameters of daidzein and puerarin. Experimental data were gained by exploring standard one-dimensional spectra and a set of two-dimensional measurements: COSY, HSQC, and HMBC. The theoretical gauge independent atomic orbital density functional theory supporting studies, were performed to determine shielding constants and chemical shifts in vacuum and methanol-*d4* solvent. The correlation between experimental and theoretical data was fairly good, especially when the DFT/PBE0 approach was used. The molecular properties of daidzein and puerine related to antiradical activity were studied in the context of a single-step hydrogen atom transfer mechanism and its correlation with ^{13}C NMR chemical shifts.

Keywords Radix puerariae · Daidzein · Puerarin · NMR measurements · Density functional theory · Chemical shifts · Nuclear magnetic shielding

Introduction

In China, traditional Chinese medicine (TCM) based on natural products plays an important role, and accounts for approximately 40% of total consumption of pharmaceuticals. However, the active components of plants and their pharmacological mechanisms are still not well recognized and understood. Chinese scholars believe that the curative effect of some herbal medicines is closely related to the antioxidative activity found in flavonoids, saponins, polysaccharides, organic acids, terpenes, etc. [1].

Radix puerariae, belonging to the families of fabaceae or leguminosae, has been used traditionally for the treatment of diarrhea, acute dysentery, deafness, and cardiovascular diseases. Since 1950, interest in its phytochemistry and pharmacological activity has increased significantly [2, 3]. Over 70 chemical constituents of radix puerariae have been identified. This collection includes daidzein (Fig. 1a) and puerarin (Fig. 1b), daidzin, genistein, genistin, and formononetin. Puerarin—the first identified major isoflavone—constitutes the greatest part [1.88–2.55% (w/w)] of this plant [4]. Puerarin, daidzin, and daidzein have been reported to exhibit estrogenic, antiestrogenic, and anti-inflammatory activities [5,

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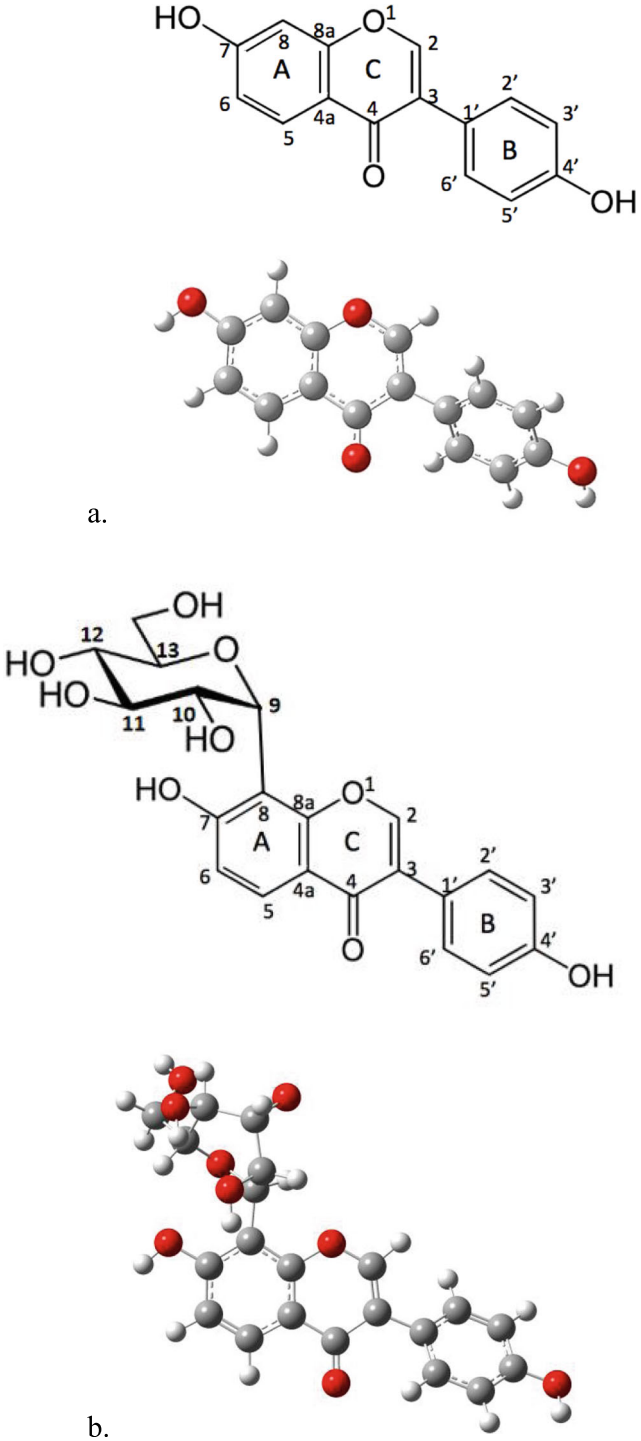


Fig. 1 Chemical schemes and structures of **a** daidzein and **b** puerarin (daizein-8-C-glucoside)

6]. Studies indicate that the cardioprotective effects of puerarin against ischemia and reperfusion injury are mediated by opening the calcium-activated potassium channels and activating protein kinase C—both effects may be due to an antioxidative activity [7]. Also genistein, being an isoflavone, possesses antioxidative and pharmacologic activities against cardiocerebrovascular diseases; its activity is based on scavenging of oxygen free radicals [8, 9].

To date, several pharmacological studies on puerarin, daidzin have been performed, applying liquid chromatography (LC), joint liquid chromatography with mass spectrometry (LC–MS), and liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) to provide chemical properties of investigated compounds [10–12]. NMR chemical shifts of daidzein and puerarin have also been measured [13–15]; however, magnetic shielding has never been reported for either compound. The present work reports experimental ^{1}H and ^{13}C NMR chemical shifts of daidzein and puerarin dissolved in methanol-d_4. For the same solutions, we also completed measurements of magnetic shielding using an original method from our laboratory [16]. By considering features of ^{13}C NMR spectroscopy, chemical shifts can be used as promising descriptors for quantitative structure–activity relationships [17].

Daidzein [7-hydroxy-3-(4-hydroxyphenyl) chromen-4-one] and puerarin (the 8-C-glucoside of daidzein) are isoflavones [18, 19]. Molecular properties related to their antioxidative activity are presented. The ^{1}H and ^{13}C NMR measurements of nuclear magnetic shielding and chemical shifts were determined, and supplementary theoretical studies allowed for its careful analysis. Theoretical studies include molecular and corresponding radical structures, atomic charge distribution, electron spin density distribution for radicals, and O–H bond dissociation energies (BDE).

Methods

Experimental

The NMR spectra of daidzein and puerarin were obtained at 298.2 K using a Bruker AVANCE III HD 500 MHz spectrometer operating at 500.20 and 125.79 MHz for ^{1}H and ^{13}C nuclei, respectively, with the 5 mm CPPBBO BB probehead and 5 mm o.d. glass tubes (528PP, Wilmad, https://www.wilmad-labglass.com). Liquid CD_3OD was used as the reference of shielding and lock solvent at the same time. ^{1}H and ^{13}C shielding values were determined relative to the CD_3^- signal of pure methanol-d_4. All ^{1}H and ^{13}C shielding values were obtained on the basis of observed absolute resonance frequencies of studied samples. The ^{1}H and ^{13}C NMR chemical shifts were measured relative to pure liquid TMS used as external reference standards [20, 21]. The assignment of chemical shifts to individual atoms was completed on the above basis and additionally using the variety of two-dimensional(2D: ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) spectra, shown as Figs. S1–S10 in the Supporting Materials.

Computations

All calculations were performed within the density functional theory (DFT) [22], applying B3LYP [23–25], M06-2X [26], and PBE0 [27] functionals with standard aug-cc-pVTZ [28] and 6-311G(2d, 2p) atomic basis sets [29]. The structure of molecules was optimized for every combination of functional/basis set, and frequency calculations additionally confirmed the proper determination of equilibrium geometries. Enthalpy values were calculated for normal conditions. The resulting structures were used for further studies. The gauge independent atomic orbital (GIAO) method was used for carrying out magnetic shielding parameters [30–32], and theoretical chemical shifts were determined by using the corresponding calculations for tetramethylsilane (TMS) (Table 1). The polarized continuum model (PCM) was applied to represent solvent [33]. All calculations were performed utilizing Gaussian16 code [34]. Electron spin density plots were produced by applying the GausView6 program [35].

Results and discussion

Experimental and theoretical NMR studies

We used the same experimental and theoretical procedures in our present study for daidzein and puerarin as in a previous report [36]. We performed a complete set of ^{1}H and ^{13}C frequency measurements for chemicals dissolved in methanol-d_4. According to Equations (1) and (2) [16], the shielding isotropic parameters of protons and carbon nuclei (σ_H and σ_C) were determined as follows:

$$\sigma_H = 1-0.153506104(\nu_H/\nu_D)\left(1-\sigma^*_D\right) \quad (1)$$

$$\sigma_C = 1-0.610389782(\nu_C/\nu_D)\left(1-\sigma^*_D\right) \quad (2)$$

where ν_H, ν_C, and ν_D are the resonance frequencies of protons, carbons, and deuterons, respectively. The resonance frequency of $-CD_3^-$ deuterons in methanol-d_4 was ν_D = 76.7839977 MHz, and the deuteron shielding constant was σ_D^* = 0.000029593 [16]. Note that the application of Eqs. 1 and 2 requires the use of real values of shielding, not expressed in ppm.

Table 1 Calculated ^{13}C and ^{1}H shielding of tetramethylsilane (TMS) applying different theoretical approaches (in ppm)

Methodology	Isolated molecule		In methanol-d_4	
	^{13}C	^{1}H	^{13}C	^{1}H
B3LYP/aug-cc-pVTZ	184.50	31.74	185.45	31.73
B3LYP/6-311G(2d,2p)	183.80	31.82	184.29	31.81
M06-2X/aug-cc-pVTZ	188.81	31.83	189.40	31.82
M06-2X/6-311G(2d,2p)	188.33	31.92	188.66	31.90
PBE0/aug-cc-pVTZ	189.12	31.57	190.06	31.55
PBE0/6-311G(2d,2p)	188.42	31.66	188.94	31.64

The analogous characterization was performed theoretically. A comparison of new experimental and calculated results is shown in Table 2. Here, we present experimental and calculated magnetic shielding parameters of daidzein. The theoretical results are given for an isolated molecule (a molecule in vacuum) and for the same molecule dissolved in methanol-d_4. According to root mean square (RMS) values, the correlation between calculated and experimental shielding parameters in methanol-d_4 is better than similar calculations for isolated molecules. For example, the RMS value in methanol-d_4 (7.41) calculated by the PBE0/aug-cc-pVTZ approach is lower than the value 7.63 for the molecule in vacuum, and values used B3LYP/aug-cc-pVTZ and M06-2X/aug-cc-pVTZ are 7.54 and 8.77 in methanol-d_4, which are also lower than values (7.58 and 9.10) in vacuum. For different functionals within the same basis set, the PBE0 functional is better comparing to B3LYP and M06-2X in methanol-d_4.

The ^{13}C and ^{1}H chemical shifts of daidzein determined experimentally and theoretically are presented in Table 3. Comparing the correlation between experimental and calculated data, PBE0 is again better than the other two functionals. RMS values for B3LYP and PBE0 with the same basis set are also better in methanol-d_4 than for the isolated molecule. The RMS value of chemical shift in methanol-d_4 calculated in the PBE0/aug-cc-pVTZ approach is 7.51, which is lower than the value of 7.69 for the molecule in vacuum. Using B3LYP/aug-cc-pVTZ approach, values are 7.63 in methanol-d_4 and 7.82 in vacuum. For chemical shifts, the basis set 6-311G(2d,2p) is better than aug-cc-pVTZ. RMS values are 7.38 for the PBE0/6-311G(2d,2p) approach in methanol-d_4 and 7.51 for PBE0/aug-cc-pVTZ, and B3LYP and M06-2X methods present same conclusion, i.e., that the basis set 6-311G(2d,2p) is better than aug-cc-pVTZ both in methanol-d_4 and in vacuum. Obviously, the differences of experimental and theoretical ^{1}H chemical shifts are small (almost within ±1 ppm), showing high consistency between experimental and calculated results. The correlation between experimental and theoretical chemical shifts (δ) for daidzein is shown in Fig. 2b. This correlation looks better than the previous plot shown for shielding in Fig. 2a. In particular, the plot crosses the origin of coordinates precisely in Fig. 2b.

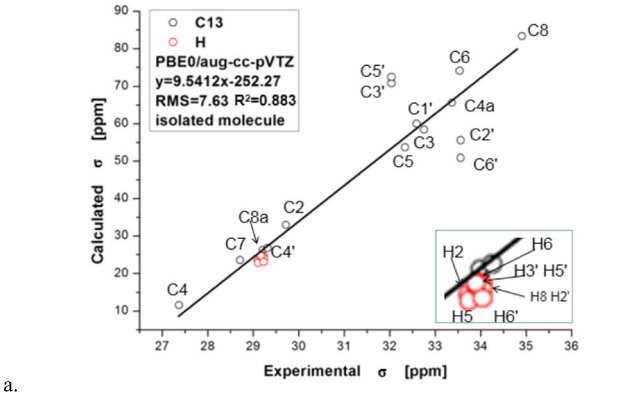
Tables 4 and 5 present the ^{13}C and ^{1}H shielding values and chemical shifts of puerarin determined experimentally and theoretically in the two phases as before. The differences in ^{13}C shielding constants for atoms C4, C6, C8, C3' and C5' are much higher in our list (from −16.07 ppm to 40.84 ppm for PBE0/aug-cc-pVTZ method in vacuum)—the correlation line is shown in Fig. 3a. However, ^{1}H shielding changes are similar to those of daidzein between −4.48 ppm [H(6)] and

Table 2 ^{13}C and ^{1}H shielding values of daidzein determined theoretically and experimentally (in ppm). *RMS* Root mean square

	Theoretical												Experimental
	Isolated molecule						In methanol-d_4						
	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	This work
C(2)	26.90	27.27	17.93	19.01	32.92	33.65	23.86	24.55	14.43	15.90	29.85	30.86	29.72
C(3)	51.15	51.83	43.90	44.63	58.38	59.46	51.43	52.36	44.51	45.42	58.80	60.05	32.76
C(4)	4.74	5.52	−6.60	−4.37	11.51	12.66	1.74	3.16	10.33	7.50	8.83	10.49	27.37
C(4a)	58.59	59.51	50.71	51.83	65.58	66.94	59.74	60.70	52.07	53.20	66.85	68.22	33.37
C(5)	48.62	48.83	37.16	38.27	53.70	54.36	49.96	50.00	38.42	39.32	55.06	55.55	32.34
C(6)	68.58	68.97	59.86	60.66	74.12	74.87	65.91	66.28	56.83	57.65	71.44	72.16	33.54
C(7)	16.36	16.68	7.01	8.31	23.55	24.48	14.92	15.27	5.27	6.58	22.00	22.96	28.71
C(8)	77.89	78.11	69.58	70.32	83.39	83.93	77.66	77.91	69.42	70.17	83.12	83.67	34.91
C(8a)	18.66	19.67	10.44	12.14	26.23	27.72	18.40	19.45	10.20	11.94	25.98	27.50	29.21
C(1′)	52.96	53.04	44.32	44.82	60.02	60.60	52.59	52.96	43.95	44.68	59.83	60.64	32.59
C(2′)	50.09	50.68	39.24	40.24	55.56	56.55	48.32	49.08	37.33	38.50	53.84	54.95	33.56
C(3′)	65.38	65.61	55.43	56.19	70.76	71.37	65.19	65.36	55.09	55.79	70.48	71.03	32.04
C(4′)	19.54	19.60	10.95	11.88	26.83	27.49	19.38	19.39	10.66	11.51	26.58	27.20	29.32
C(5′)	67.11	67.55	57.61	58.43	72.48	73.30	66.11	66.47	56.38	57.11	71.50	72.22	32.04
C(6′)	45.28	45.73	33.92	34.95	50.85	51.69	45.89	46.41	34.44	35.44	51.48	52.35	33.56
H(2)	23.69	23.90	23.44	23.67	23.48	23.69	23.48	23.68	23.18	23.40	23.25	23.45	29.10
H(5)	23.10	23.25	22.58	22.73	22.83	22.98	23.18	23.33	22.63	22.78	22.90	23.05	29.11
H(6)	25.00	25.12	24.60	24.72	24.79	24.91	24.58	24.71	24.15	24.28	24.35	24.48	29.22
H(7)	27.23	27.04	27.03	27.36	27.07	27.33	26.21	26.44	25.96	26.28	26.04	26.28	–
H(8)	24.66	24.84	24.26	24.42	24.46	24.63	24.51	24.70	24.10	24.26	24.30	24.47	29.23
H(2′)	24.34	24.48	23.80	23.96	24.06	24.21	24.16	24.29	23.59	23.75	23.87	24.01	29.23
H(3′)	24.59	24.74	24.14	24.28	24.37	24.52	24.47	24.63	24.00	24.16	24.24	24.40	29.17
H(4′)	27.59	27.85	27.39	27.73	27.43	27.69	26.74	26.98	26.49	26.81	26.56	26.81	–
H(5′)	24.92	25.07	24.48	24.63	24.71	24.86	24.60	24.76	24.14	24.29	24.38	24.40	29.17
H(6′)	23.41	23.37	22.89	22.96	23.04	23.02	23.78	23.70	23.18	23.20	23.41	23.37	29.23
RMS	7.58	7.51	9.10	8.76	7.63	7.68	7.54	7.40	8.77	8.45	7.41	7.41	
RMS(C)	9.56	9.50	9.94	9.82	9.34	9.26	9.41	9.28	10.28	9.88	9.20	9.08	
RMS(H)	0.69	0.74	0.50	0.75	0.74	0.79	0.46	0.52	0.52	0.55	0.51	0.53	

Table 3 ^{13}C and ^{1}H chemical shifts of daidzein determined theoretically and experimentally (in ppm)

	Theoretical												Experimental	
	Isolated molecule						In methanol-d_4							
	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	This work	Ref. [13]
C(2)	157.60	156.53	170.88	169.32	156.20	154.77	161.59	159.74	174.97	172.76	160.21	158.08	154.81	152.78
C(3)	133.35	131.97	144.91	143.70	130.74	128.96	134.02	131.93	144.89	143.24	131.26	128.89	124.46	122.52
C(4)	179.76	178.28	195.41	192.70	177.61	175.76	183.71	181.13	179.07	181.16	181.23	178.45	178.34	174.66
C(4a)	125.91	124.29	138.10	136.50	123.54	121.48	125.71	123.59	137.33	135.46	123.21	120.72	118.29	116.61
C(5)	135.88	134.97	151.65	150.06	135.42	134.06	135.49	134.29	150.98	149.34	135.00	133.39	128.65	127.26
C(6)	115.92	114.83	128.95	127.67	115.00	113.55	119.54	118.01	132.57	131.01	118.62	116.78	116.66	114.90
C(7)	168.14	167.12	181,80	180.02	165.57	163.94	170.53	169.02	184.13	182.08	168.06	165.98	164.95	162.46
C(8)	106.61	105.69	119.23	118.01	105.73	104.49	107.79	106.38	119.98	118.49	106.94	105.27	103.39	102.06
C(8a)	165.84	164.13	178.37	176.19	162.89	160.70	167.05	164.84	179.20	176.72	164.08	161.44	159.99	157.40
C(1′)	131.54	130.76	144.49	143.51	129.10	127.82	132.86	131.33	145.45	143.98	130.23	128.30	126.10	123.46
C(2′)	134.41	13,312	149.57	148.09	133.56	131.87	137.13	135.21	152.07	150.16	136.22	133.99	116.37	130.04
C(3′)	119.12	118.19	133.38	132.14	118.36	117.05	120.26	118.93	134.31	132.87	119.59	117.91	131.58	115.01
C(4′)	164.96	164.20	177.86	176.45	162.29	160.93	166.07	164.90	178.74	177.15	163.48	161.74	158.85	157.12
C(5′)	117.39	116.25	131.20	129.90	116.64	115.12	119.34	117.82	133.02	131.55	118.56	116.72	131.58	115.01
C(6′)	139.22	138.07	154.89	153.38	138.27	136.73	139.56	137.88	154.96	153.22	138.58	136.59	116.37	130.04
H(2)	8.05	7.92	8.39	8.25	8.09	7.97	8.25	8.13	8.64	8.50	8.30	8.19	8.13	8.29
H(5)	8.64	8.57	9.25	9.19	8.74	8.68	8.55	8.48	9.19	9.12	8.65	8.59	8.05	7.97
H(6)	6.74	6.70	7.23	7.20	6.78	6.75	7.15	7.10	7.67	7.62	7.20	7.16	6.94	6.94
H(7)	4.51	4.78	4.80	4.56	4.50	4.33	5.52	5.37	5.86	5.62	5.51	5.36	–	10.76
H(8)	7.08	6.98	7.57	7.50	7.11	7.03	7.22	7.11	7.72	7.64	7.25	7.17	6.85	6.86
H(2′)	7.40	7.34	8.03	7.96	7.51	7.45	7.57	7.52	8.23	8.15	7.68	7.63	6.85	7.39
H(3′)	7.15	7.08	7.69	7.64	7.20	7.14	7.26	7.18	7.82	7.74	7.31	7.24	7.37	6.81
H(4′)	4.15	3.97	4.44	4.19	4.14	3.97	4.99	4.83	5.33	5.09	4.99	4.83	–	9.52
H(5′)	6.82	6.75	7.35	7.29	6.86	6.80	7.13	7.05	7.68	7.61	7.18	7.24	7.37	6.81
H(6′)	8.33	8.45	8.94	8.96	8.53	8.64	7.95	8.11	8.64	8.70	8.14	8.27	6.85	7.39
RMS	7.82	7.74	8.68	8.62	7.69	7.59	7.63	7.50	9.72	9.19	7.51	7.38		
RMS(C)	9.57	8.85	9.96	9.84	9.35	9.27	9.42	9.29	10.30	9.90	9.21	9.09		
RMS(H)	0.67	0.72	0.72	0.74	0.72	0.77	0.44	0.50	0.50	0.54	0.49	0.52		



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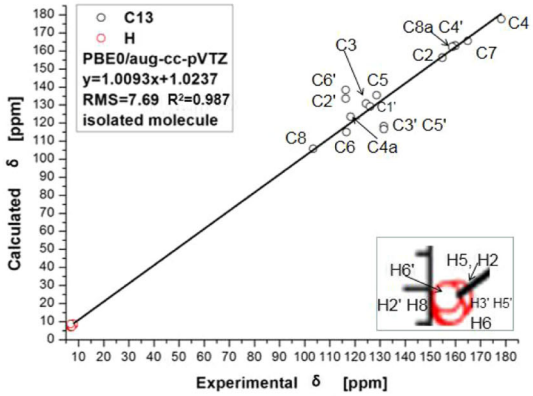

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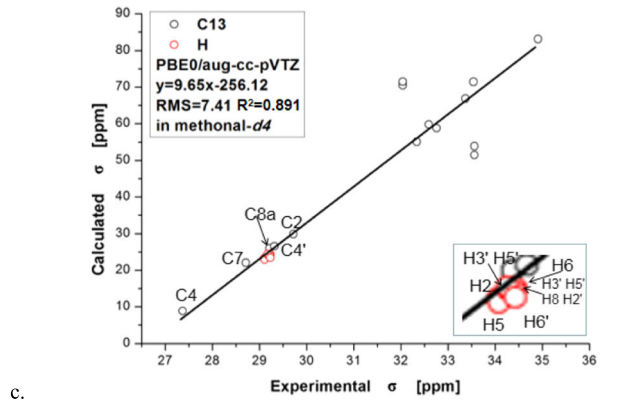

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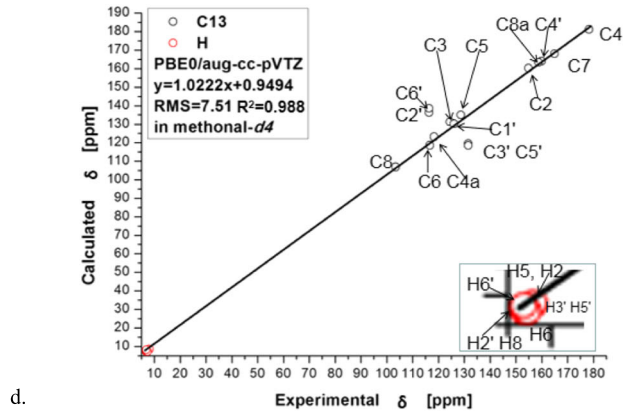


d.

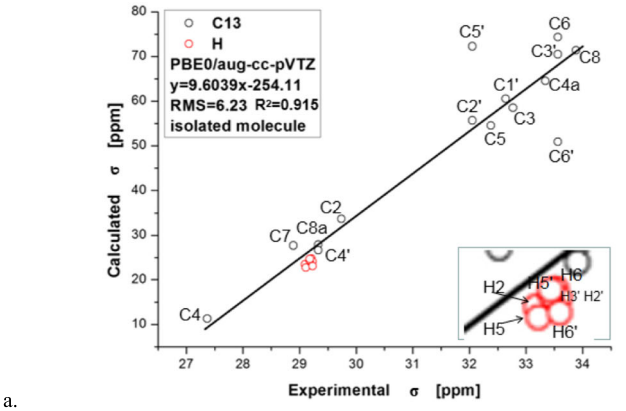
Fig. 2 **a**–**d** Correlations between experimental and theoretical shielding constant (σ) and chemical shift (δ) for daidzein. *Atomic numbers* refer to the corresponding data in Fig. 1a, and Tables 2 and 3

Table 4 ^{13}C and ^{1}H shielding values of puerarin determined theoretically and experimentally (in ppm)

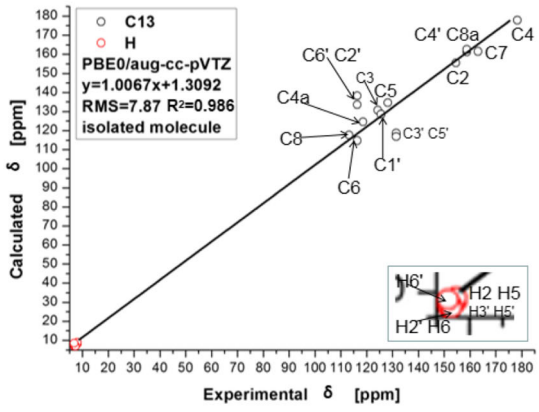
	Theoretical												Experimental
	Isolated molecule						In methanol-d_4						
	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	This work
C(2)	27.65	27.00	18.64	19.38	33.62	34.04	24.65	25.05	15.24	16.39	30.58	31.31	29.74
C(3)	51.33	52.09	44.14	44.92	58.52	59.56	51.91	52.54	44.64	45.61	59.10	60.13	32.77
C(4)	4.49	5.29	−6.75	−4.57	11.30	12.45	1.54	2.93	−10.46	−7.69	8.63	10.28	27.37
C(4a)	57.53	58.21	49.55	50.29	64.55	65.58	58.81	59.40	51.19	51.89	65.97	66.98	33.34
C(5)	49.56	49.72	38.07	39.14	54.55	55.14	50.73	50.75	39.03	39.94	55.71	56.14	32.38
C(6)	68.83	69.05	60.34	61.00	74.40	75.08	66.28	66.59	57.28	57.99	71.80	72.46	33.56
C(7)	20.24	19.97	12.15	12.81	27.67	28.06	18.61	18.44	9.89	10.68	25.86	26.29	28.89
C(8)	64.18	63.80	56.63	56.79	71.43	71.74	65.44	65.24	58.34	58.46	72.70	72.97	33.88
C(8a)	20.19	20.19	12.37	13.53	27.91	28.88	20.06	20.64	12.35	13.63	27.79	28.85	29.33
C(1′)	53.52	53.53	44.93	45.45	60.60	61.24	52.82	53.24	44.18	44.95	60.06	60.93	32.64
C(2′)	50.19	50.66	39.58	40.62	55.70	56.66	48.49	49.11	37.47	38.73	53.97	54.95	32.05
C(3′)	65.17	65.42	55.12	55.89	70.55	71.14	65.11	65.30	54.96	55.66	70.41	70.98	33.56
C(4′)	19.34	19.45	10.64	11.56	26.59	27.23	19.31	19.30	10.58	11.39	26.51	27.10	29.33
C(5′)	66.98	67.47	57.50	58.29	72.33	73.12	66.06	66.36	56.36	57.08	71.42	72.11	32.05
C(6′)	45.37	45.76	34.10	35.13	50.93	51.72	45.99	46.40	34.43	35.51	51.54	52.34	33.56
H(2)	22.65	23.77	23.38	23.61	23.44	23.65	23.44	23.65	23.15	23.36	23.22	23.42	29.10
H(5)	23.03	23.20	22.50	22.95	22.74	22.88	23.12	23.26	22.56	22.71	22.83	22.97	29.11
H(6)	24.94	25.07	24.54	24.66	24.73	24.85	24.54	24.66	24.10	24.22	24.31	24.43	29.21
H(7)	27.04	27.39	26.82	27.23	26.85	27.18	26.01	26.32	25.73	26.12	25.81	26.12	–
H(2′)	24.33	24.47	23.78	23.94	24.05	24.21	24.15	24.30	23.59	23.74	23.86	24.02	29.23
H(3′)	24.57	24.74	23.78	24.27	24.35	24.51	24.46	24.63	23.99	24.15	24.23	24.40	29.18
H(4′)	27.55	27.82	27.35	27.69	27.38	27.64	26.73	26.97	26.73	26.80	26.55	26.80	–
H(5′)	24.90	25.06	24.45	24.60	24.68	24.84	24.59	24.75	24.12	24.28	24.36	24.52	29.18
H(6′)	23.40	23.35	22.85	22.92	23.03	23.02	23.73	23.70	23.18	23.19	23.39	23.38	29.23
RMS	6.15	6.17	7.77	7,48	6,23	6.30	5.07	5.81	7.96	7.52	5.73	5.78	
RMS(C)	7.53	7,55	7.86	7.75	7.32	7.30	5.95	7.00	7.46	7.29	6.89	6,84	
RMS(H)	0.79	0.79	0.77	0.76	0.80	0.84	0.50	0.55	0.55	0.58	0.54	0.68	

Table 5 ^{13}C and ^{1}H chemical shifts of puerarin determined theoretically and experimentally (in ppm)

	Theoretical												Experimental	
	Isolated molecule						In methanol-d_4							
	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	B3LYP/aug-cc-pVTZ	B3LYP/6-311G (2d,2p)	M06-2X/aug-cc-pVTZ	M06-2X/6-311G (2d,2p)	PBE0/aug-cc-pVTZ	PBE0/6-311G (2d,2p)	This work	Ref. [14, 15]
C(2)	156.85	156.80	170,17	168.95	155.50	154.38	160.80	159.24	174.16	172.27	159.48	157.63	154.61	153.09
C(3)	133.17	131.71	144,67	143.41	130.60	128.86	133.54	131.75	144.76	143.05	130.96	128.81	124.30	123.61
C(4)	180.01	178.51	195,56	192.90	177.82	175.97	183.91	181.36	199.86	196.35	181.43	178.66	178.39	175.47
C(4a)	126.97	125.59	139,26	138.04	124.57	122.84	126.64	124.89	138.21	136.77	124.09	121.96	118.59	117.35
C(5)	134.94	134.08	150,74	149.19	134.57	133.28	134.72	133.54	150.37	148.72	134.35	132.80	128.22	126.78
C(6)	115.67	114.75	128,47	127.33	114.72	113.34	119.17	117.70	132.12	130.67	118.26	116.48	116.37	115.52
C(7)	164.26	163.83	176,66	175.52	161.45	160.36	166.84	165.85	179.51	177.98	164.20	162.65	163.11	161.58
C(8)	120.32	120.00	132,18	131.54	117.69	116.68	120.01	119.05	131.06	130.20	117.36	115.97	113.24	113.10
C(8a)	164.31	163.61	176,44	174.80	161.21	159.54	165.39	163.65	177.05	175.03	162.27	160.09	158.78	156.63
C(1′)	130.98	130.27	143,88	142.88	128.52	127.18	132.63	131.05	145.22	143.71	130.00	128.01	125.62	123.03
C(2′)	134.31	133.14	149,23	147.71	133.42	131.76	136.96	135.18	151.93	149.93	136.09	133.99	116.37	130.51
C(3′)	119.33	118.38	133,69	132.44	118.57	117.28	120.34	118.99	134.44	133.00	119.65	117.96	131.49	115.52
C(4′)	165.16	164.35	178,17	176.77	162.53	161.19	166.14	164.99	178.82	177.27	163.55	161.84	158.78	157.61
C(5′)	117.52	116.33	131,31	130.04	116.79	115.30	119.39	117.93	133.04	131.58	118.64	116.83	131.49	115.52
C(6′)	139.13	138.04	154,71	153.20	138.19	136.70	139.46	137.89	154.89	153.15	138.52	136.60	116.37	130.51
H(2)	9.09	8.05	8,45	8.31	8.13	8.01	8.29	8.16	8.67	8.54	8.33	8.22	8.15	8.21
H(5)	8.71	8.62	9,33	8.97	8.83	8.78	8.61	8.55	9.26	9.19	8.72	8.67	8.03	8.06
H(6)	6.80	6.75	7,29	7.26	6.84	6.81	7.19	7.15	7.72	7.68	7.24	7.21	6.97	7.09
H(7)	4.70	4.43	5,01	4.69	4.72	4.48	5.72	5.49	6.09	5.78	5.74	5.52	–	–
H(2′)	7.41	7.35	8,05	7.98	7.52	7.45	7.58	7.51	8.23	8.16	7.69	7.62	6.84	4.26
H(3′)	7.17	7.08	8,05	7.65	7.22	7.15	7.27	7.18	7.83	7.75	7.32	7.24	7.35	3.62
H(4′)	4.19	4.00	4,48	4.23	4.19	4.02	5.00	4.84	5.09	5.10	5.00	4.84	–	–
H(5′)	6.84	6.76	7,38	7.32	6.89	6.82	7.14	7.06	7.70	7.62	7.19	7.12	7.35	3.62
H(6′)	8.34	8.47	8,98	9.00	8.54	8.64	8.00	8.11	8.64	8.71	8.16	8.26	6.84	3.89
RMS	8.01	7.95	8.92	8.85	7,87	7,76	7.79	7,69	8.67	8.53	7.67	7.55		
RMS(C)	9.38	9.37	9.71	9.57	9.16	9.10	9.26	9.16	9.70	9.47	9.08	8.98		
RMS(H)	0.78	0.79	0.77	0.76	0.79	0.84	0.50	0.55	0.55	0.58	0.54	0.59		



a.



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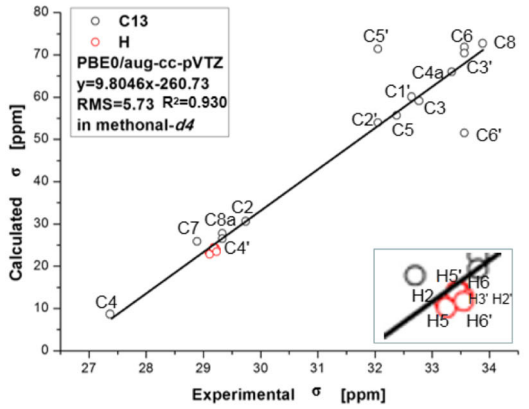

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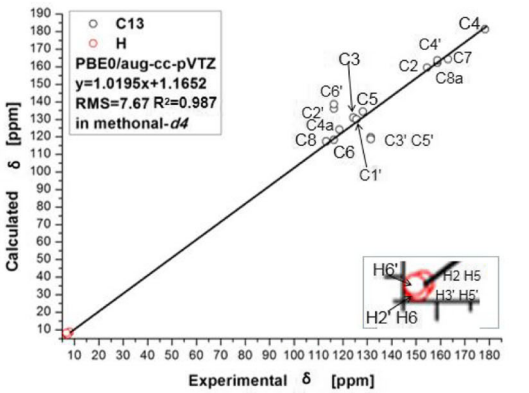

d.

Fig. 3 **a**–**d** Correlations between experimental and theoretical shielding constant (σ) and chemical shift (δ) for puerarin. *Atomic numbers* refer to the corresponding data in Fig. 1b, and Tables 4 and 5

Table 6 Selected structural parameters in daidzein and puerarin (Fig. 1) and corresponding radicals (from B3LYP/aug-cc-pVTZ)Angles in degree, and distances in Ångstrom

		Dihedral angle (2,3,1′,2′)	A (C7−O)	B (C4'−O)
Daidzein	Molecule	40.54	1.361	1.367
	Radical (C7)-O•	39.15	1.249	1.365
	Radical (C4')-O•	33.07	1.358	1.248
Puerarin	Molecule	40.28	1.364	1.366
	Radical (C7)-O•	38.53	1.250	1.364
	Radical (C4')-O•	32.83	1.363	1.248

−6.37 ppm [H(5)]. Results in Table 4 in methanol-d_4 are much better than in vacuum for the same method PBE0/aug-cc-pVTZ, with values ranging from −18.74 ppm to 38.82 ppm. In Table 5, the PBE0 method also presents better RMS results compared to the other method, RMS values in methanol-d_4 are 7.67 for PBE0/aug-cc-pVTZ and 7.55 for PBE0/6-311G(2d,2p). It seems that our experimental data for chemical shifts compared to results of Xu et al. [14] match our calculated values well. For example, differences between experimental data and calculated values with PBE0/aug-cc-pVTZ method methanol-d_4 are smaller than differences between data of of Xu et al. [14] and our calculated values (only values C(2′), C(3′), C(5′), and C(6′) are higher).

Comparing Tables 3 and 5, experimental data of shielding changes between daidzein and puerarin for each atom are quite small, ranging from −1.52 ppm [C(3′)] to 1.51 ppm [C(2′)] based on their similar chemical structure. However, RMS values of puerarin present better results than daidzein with every method used. For instance, the RMS value of puerarin is 5.73 ppm for PBE0/aug-cc-pVTZ in methanol-*d4* with 1.68 less than daidzein, and RMS differences of shielding between daidzein and puerarin ranging from 0.93 ppm [M06-2X/6-311G(2d,2p) in methanol-d4)] to 2.47 ppm (B3LYP/aug-cc-pVTZ in methanol-*d4*). Differences of chemical shifts in Tables 4 and 6 from experimental results are a little higher than shielding comparing the same atom between daidzein and puerarin.

Antioxdative properties

The optimization of daidzein and puerarin structures leads to a nonplanar flovone backbone with a dihedral angle between benzopyrene and phenyl moieties of about 40° (Table 6). The corresponding radicals, resulting from hydrogen abstraction from phenolic groups, are only slightly more planar. The transformation of −OH into =O• substituent results in a shorter C–O bond with a significant double bond character. The effect, however, is not strong enough to flatten the flavone skeleton. Puerarin, which is daidzien modified by a glucoside group, possesses similar structural characteristics. The charge extraction from the flovone ring is too small to cause significant geometrical effects (Table 6).

The scavenging of free OH• or OOH• radicals of antioxidants is controlled by their ability to transfer hydrogen of phenolic groups attached directly to aromatic rings according to the following reactions

$$\mathrm{ROH} + \mathrm{OH}^{\bullet} = \mathrm{RO}^{\bullet} + \mathrm{H_2O} \quad (3)$$

$$\mathrm{ROH} + \mathrm{OOH}^{\bullet} = \mathrm{RO}^{\bullet} + \mathrm{H_2O_2} \quad (4)$$

In the case of the studied compounds, the mechanism of the single-step hydrogen atom transfer dominates other mechanisms involving electron transfer. The thermodynamics of such processes is related directly to the delocalization of unpaired electron on aromatic moieties. The structures of the studied radicals possess significantly broken planarity, restricting the space for electron delocalization.

The mesomeric structures for daidzein rationalize the significant electron density delocalization (Fig. 4). This observation agrees well with the electron spin density distribution (Fig. 5, Fig. S11). Interestingly, some transfer of electron spin density is possible from the phenyl to the benzopyrene fragment. Of the many molecular properties, such as ionization energy, chemical hardness, or spin delocalization influencing the radical scaverging mechanism, the bond dissociation enthalpy (BDE), defined by the reaction

$$\mathrm{ROH} = \mathrm{RO}^{\bullet} + \mathrm{H}^{\bullet} \quad (5)$$

is probably the most important. The information provided by reaction (5) is equivalent to that carried out by (3) or (4). The enthalpy of cleaving the O–H bond by homolysis at a specific temperature is defined as

$$\mathrm{BDE} = \mathrm{H_{RO^{\cdot}}} + \mathrm{H_{H^{\cdot}}} - \mathrm{H_{ROH}} \quad (6)$$

The reference BDE for OH• or OOH• is adopted as

$$\mathrm{BDE_{ref}} = \mathrm{H_{OOH^{\cdot}}} + \mathrm{H_{H^{\cdot}}} - \mathrm{H_{HOOH}} \quad (7)$$

and the enthalpy of the resulting single-step hydrogen atom transfer (HAT) process is

$$\mathrm{H_{HAT}} = \mathrm{BDE} - \mathrm{BDE_{ref}}$$

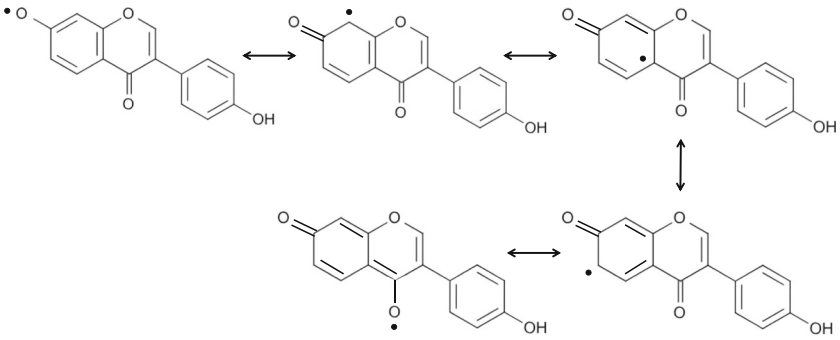

Fig. 4 Possible mesomeric structures of daidzein radical resulting from H dissociation at position C7-OH

The O–H BDEs are presented in Table 7. The hydrogen-donating capacities of daidzein and puerarin were calculated for positions (C)-OH and (4′)-OH. The results, when compared to reference OOH• and OH• radicals, indicate the increasing BDE upon hydrogen abstraction from the benzopyrene ring at position C7. Despite the fact that the actual mechanism of action of antioxidants is much more complicated, the HAT parameter constitutes the most important factor for the assessment of oxidative properties. The transition states (Fig. S12) for the hydrogen transfer in HAT favor the C4' position for the reaction (Table 7). Comparison of ^{13}C chemical shifts, in agreement with other suggestions [17], indicates a correlation with BDE (and HAT) (Fig. S13).

Conclusions

Daidzein and puerarin represent two active compounds of Radix puerariae—an important member of the TCM collection. The medicinal activity of these compounds is believed to be related to their free radical scavenging activity. Puerarin and daidzein are isoflavones and are well known for their cardioprotective effects. These two chemicals were studied experimentally, providing a complete NMR analysis and determining the full set of ^{1}H and ^{13}C chemical shifts and corresponding shielding constants. The measurements were supported by theoretical considerations within DFT methodology. The correlation between calculated and experimental shielding parameters and chemical shifts is generally good,

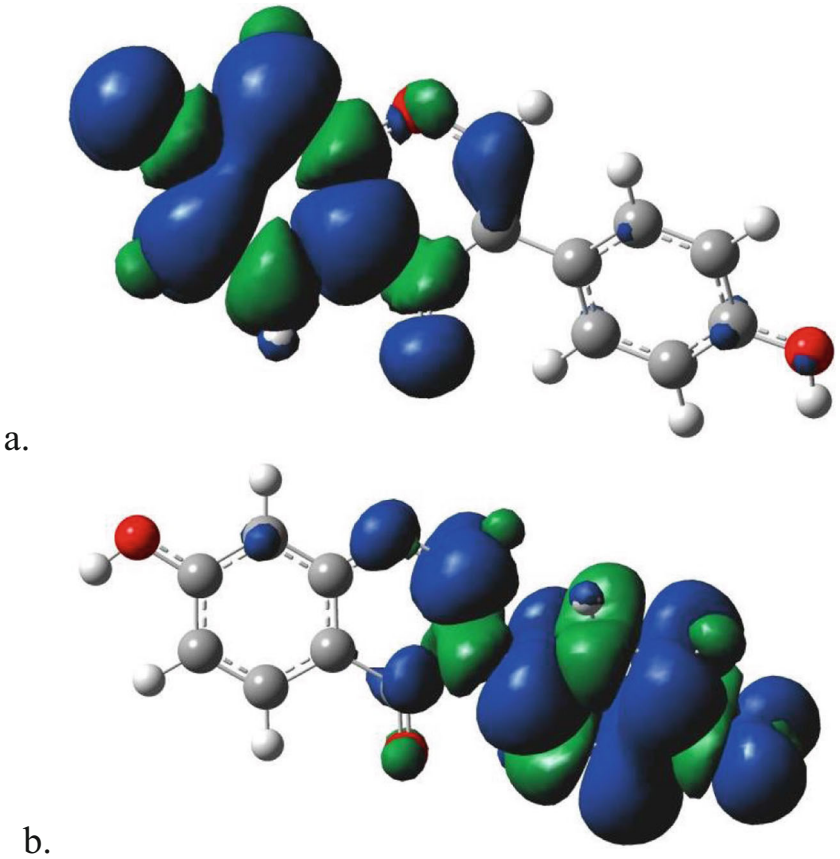

Fig. 5 Electron spin density distribution for daidzein radical phenolic hydrogen loss on oxygen at **a** C7 (benzopyrene ring) and **b** C4' (phenyl ring)

Table 7 Calculated ^{13}C and ^{1}H chemical shifts of puerarin and daidzein molecules (in C7 and C4' positions) in methanol-d_4, phenolic O–H bond dissociation enthalpies (BDE; kcal mol^{-1}), single-step hydrogen atom transfer (HAT), and transition state for the HAT reaction derived from B3LYP/aug-cc-pVTZ calculations

Site	C	H	BDE	HAT		TS
				H_2O_2[a]	H_2O[a]	
Daidzein (C7)	170.53	5.52	86.5	3.7	−13.0	9.3
Daidzein (C4')	166.07	4.99	82.2	−0.6	−17.3	5.8
Puerarin (C7)	166.84	5.72	84.3	1.5	−15.2	
Puerarin (C4')	166.14	5.00	82.5	−0.3	−17.0	
H_2O_2			82.8			
H_2O			99.5			

[a] Reference molecule (see text)

especially when the PBE0 approach is used. The antioxidative properties of daidzein and puerarin were studied with reference to hydroxyl and hydroperoxyl radicals. The calculated BDEs indicate the promising antiradical properties of these compounds. The single-electron hydrogen atom transfer molecular mechanism seems to be a suitable way to activate these molecules. The observed correlation between ^{13}C chemcial shifts and BDE indicates that NMR measurements may provide useful data for molecules of interest.

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