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Comparison of Flavonoids and Isoflavonoids as Antioxidants

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The isoflavonoid genistein was found to be a better antioxidant than the isomeric flavonoid apigenin in phosphatidyl liposomes at pH 7.4. The higher antioxidation activity of genistein compared with apigenin is in agreement with its lower oxidation potential (0.73 vs 0.86 V as determined by cyclic voltammetry in aqueous solution of pH= 7.4), lower dissociation enthalpy (87.03 vs 87.88 kcal mol^{-1} as calculated for the more reducing 4′-hydroxyl group), and higher radical scavenging capacity in the TEAC assay. On the basis of quantum mechanical calculations for genistein and apigenin in comparison with the flavonoid naringenin and the isoflavonoids puerarin, daidzein, and equol, a lower dipole moment and a larger deviation for the A-to-B dihedral angle from coplanarity (39.3° for genistein, 18.5° for apigenin) are suggested to be important for the increased antioxidant efficiency at water/lipid interfaces among (iso) flavonoids with an equal number of phenolic groups.

KEYWORDS: (Iso)flavonoid; liposome; radical scavenging; antioxidation

INTRODUCTION

Flavonoids and other plant phenols are important constituents in vegetables used for human consumption (*1*, *2*). Flavonoids and the isomeric isoflavonoids have been found to influence intercellular redox status, to interact with specific proteins in intracellular signaling and with nucleic acids, and to have antioxidant properties (*3*, *4*). According to recent epidemiological investigations, isoflavonoids abundant in certain plant foods such as soybeans are more beneficial for human health than flavonoids. Such positive effects, including protection against cardiovascular diseases, are partly due to their antioxidant activities (*5*). The bioavailability of flavonoids and isoflavonoids depends, however, on their presence as glycosides, and detailed investigations of various factors influencing their antioxidant activities are still required.

Isoflavonoids consist of a phenyl ring (the A-ring, **Figure 1**) fused with the six-membered heterocyclic C-ring and another phenyl ring (the B-ring) at the C3 position, whereas for flavonoids, the B-ring is substituted to the C2 position. Despite the subtle structural differences, some isoflavonoids have been experimentally found to be more active as antioxidants than the corresponding flavonoids (*6*, *7*). From the molecular structure point of view, the detailed difference between flavonoids and isoflavonoids needs to be further explored.

We have selected the isoflavonoid genistein and the isomeric flavonoid apigenin for a study of the importance of C3 versus C2 substitution in the C-ring for antioxidative efficiency using some of the experimental and computational methods recently developed for the study of the isoflavonoid C-glucoside puerarin (*8*−*10*). To highlight the effect of specific structural features on the antioxidation properties of the pair of (iso)flavonoids, the present study also included a few additional (iso)flavonoids as shown in **Figure 1**. We expect to gain a deeper insight into the structure−activity relationship through a detailed investigation of the physicochemical properties of the group of structurally correlated (iso)flavonoids and their radical scavenging and antioxidation activities.

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MATERIALS AND METHODS

Chemicals. The natural (iso)flavonoids apigenin, genistein, naringenin, puerarin, daidzein, and equol were purchased from Huike Plant Exploitation Inc. (Shanxi, China) and used as received. Phosphoric acid, acetic acid, hydrochloric acid, potassium persulfate, and NaCl from Beijing Chemical Plant (>99%, Beijing, China) were used directly. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Water was supplied by a Milli-Q apparatus (Millipore Corp., Billerica, MA). Methanol of high-performance liquid chromatography (HPLC) grade (Caledon Laboratories, Georgetown, ON, Canada) for spectroscopy was used as received. Chloroform (>99.0%, Beijing Chemical Plant) was purified before use by passing it through an alumina column (Wusi Chemical Reagent Ltd., Shanghai, China).

Molar Absorptivities and Partition Coefficients of (Iso)flavonoids. The molar absorptivities of (iso)flavonoids in aqueous solution were determined according to a standard method, and the results are summarized in **Table 1**. Partition coefficients

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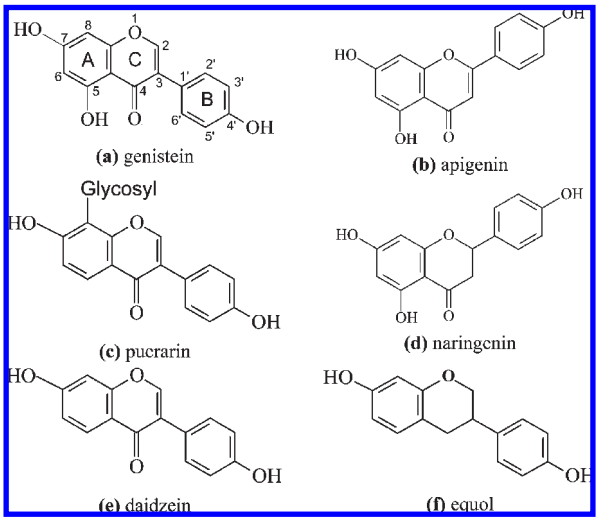

Figure 1. Structures of genistein (**a**), apigenin (**b**), puerarin (**c**), naringenin (**d**), daidzein (**e**), and equol (**f**).

in **Table 1** were determined for the (iso)flavonoids in *n*-octanol/water binary solvent by using the relationship $\log P = \log[A_0/(A_0 - A)]$, in which A_0 and A, respectively, stand for the absorbance of (iso)flavonoids in water-saturated *n*-octanol before and after being extracted by the same amount *n*-octanol-saturated water.

Determination of pK_a. The pK_a values of (iso)flavonoids were determined at 25 °C following the method of ref *8* by fitting the dependence of the absorbance (A) on pH at a specific wavelength using the following equations:

$$A = A_{\mathrm{FOH}} \times \frac{10^{-\mathrm{pH}}}{10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_a}} + A_{\mathrm{FO}^-} \times \frac{10^{-\mathrm{p}K_a}}{10^{-\mathrm{pH}} + 10^{-\mathrm{p}K_a}} \quad (1)$$

or

$$A = A_{\mathrm{FOH}} \times \frac{10^{(-2\times\mathrm{pH})}}{10^{(-2\times\mathrm{pH})} + 10^{(-\mathrm{pH}-\mathrm{p}K_{a1})} + 10^{(-\mathrm{p}K_{a1}-\mathrm{p}K_{a2})}} + A_{\mathrm{FO}^-} \times \frac{10^{(-\mathrm{pH}-\mathrm{p}K_{a1})}}{10^{(-2\times\mathrm{pH})} + 10^{(-\mathrm{pH}-\mathrm{p}K_{a1})} + 10^{(-\mathrm{p}K_{a1}-\mathrm{p}K_{a2})}} + A_{\mathrm{FO}^{2-}} \times \frac{10^{(-\mathrm{p}K_{a1}-\mathrm{p}K_{a2})}}{10^{(-2\times\mathrm{pH})} + 10^{(-\mathrm{pH}-\mathrm{p}K_{a1})} + 10^{(-\mathrm{p}K_{a1}-\mathrm{p}K_{a2})}} \quad (2)$$

A_{FOH}, A_{FO^-}, and $A_{\mathrm{FO}^{2-}}$ represent the absorbance of neutral, monoanionic, and dianionic forms of (iso)flavonoids, respectively.

Determination of Oxidation Potentials. Cyclic voltammetry was performed on a CHI 660B electrochemical analyzer (CH Instruments Inc., Austin, TX) with a three-electrode configuration (*11*), for which the solutions of (iso)flavonoids at a concentration of 2.0×10^{-5} M in Britton−Robinson (B-R) buffer were used (pH 3.4 and pH 7.4). The working electrode was a glassy carbon piece (diameter, 4 mm), the reference electrode was of the Ag/AgCl type (KCl-saturated), and the counter electrode was a platinum wire.

Radical Scavenging Assay (Determination of TEAC Value). Trolox equivalent antioxidant capacity (TEAC) assay was based on the stoichiometry of the radical scavenging of isoflavones toward $ABTS^{\bullet+}$ (*12*). To generate $ABTS^{\bullet+}$, potassium persulfate was added to a solution of 7 mM ABTS (final concentration, 2.45 mM), which was kept at room temperature for 12 h in the dark (*13*). The kinetics of (iso)flavonoids scavenging $ABTS^{\bullet+}$ at various concentrations were monitored at the characteristic absorption wavelength of $ABTS^{\bullet+}$ (734 nm) for 10 min. The concentrations of antioxidants were 4.2×10^{-6} M for genistein, 5.2×10^{-6} M for apigenin, 4.6×10^{-6} M for naringenin, 11.7×10^{-6} M for puerarin, 7.2×10^{-6} M for daidzein, and 27.9×10^{-6} M for equol. The TEAC values of six (iso)flavonoids were determined according to standard methods.

Evaluation of Antioxidation in Liposome. Liposome was prepared as described by Roberts and Gordon (*14*): 13.5 mg of soybean L-R-phosphatidylcholine (PC, 99%, from Sigma-Aldrich Chemic GmbH; the molecular mass of soybean PC was taken as 900) and 7.9 mg of lipophilic radical initiator 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN, from Huichang Petrochemical Auxiliary Co. Ltd., Zibo, Shandong, China), were dissolved in 30 mL of chloroform. Each antioxidant in methanol was added to 3 mL of the combined soybean PC/AMVN solution. Solvent was then removed under reduced pressure with a rotary evaporator at a water bath temperature of 30 °C. Nitrogen gas was introduced to re-establish atmospheric pressure, and the flask was covered with aluminum foil. Then an oil-free vacuum pump was used to maintain the flask vacuum at < 0.5 mmHg for ~2 h. The lipid residue was rehydrated with phosphate buffer (10 mL, 10 mM, pH 7.4). The flask was then shaken while being sonicated for 1 h, producing a homogeneous white suspension of multilamellar liposomes. Unilamellar liposomes were obtained by pushing the multilamellar liposome solutions through the polycarbonate membrane with 200 nm sieve pores (Whatman, Maidstone, U.K.) 20 times. The final concentration of the antioxidants in the liposome suspension in mole percent of the lipid fraction was 1.0.

Lipid peroxidation was followed by monitoring the formation of conjugated dienes using the absorbance change at 234 nm (A_{234}). The unilamellar liposome suspension (3.5 mL) of six antioxidants was pipetted into a quartz cuvette and incubated at 43 °C, and A_{234} was recorded every 10 min in sequence. The lag phase (LP) was determined as the evolution time to the point where a tangent to the propagation phase intercepted that of the initial phase with little or no oxidation (*14*).

Quantum Chemical Calculations. Structural optimization of the six compounds was performed using the Gaussian 03 package with the UB3LYP density functional method in conjunction with the 6-31++G (d, p) basis set (*15*−*19*). The bond dissociation enthalpy (BDE) were calculated as the gas phase enthalpy difference for the reaction $ArOH \rightarrow ArO^{\bullet} + H^{\bullet}$.

RESULTS AND DISCUSSION

Genistein as an isoflavonoid differs from the flavonoid apigenin only by the position for the attachment of the B-ring to the C-ring (cf. **Figure 1**); still, significant differences in the properties of the two isomers have been noted (*6*, *7*, *20*). To provide a direct comparison of the physicochemical properties of the two compounds in aqueous solution, *n*-octanol/water partition coefficients, the pH-dependent distribution coefficients, pK_a values, oxidation potentials, and antioxidative activities were determined under similar conditions together with those of naringenin, puerarin, daidzein, and equol. Naringenin was included in the comparison because it has the same structure as apigenin, except that the conjugation between the A- and B-rings through the C-ring is absent. Daidzein and the *C*-glucoside of daidzein, puerarin, were

Table 1. Molar Absorptivities (ε), *n*-Octanol/Water Partition Coefficients (log *P*), and Distribution Coefficients at pH 7.4 (log $D_{7.4}$) pK_a, Oxidation Potentials (E° vs NHE), the Energy (Gap) of the Frontier Orbitals E_{HOMO}, E_{LUMO}, and $E_{LUMO} - E_{HOMO}$, Radical Scavenging Capacity (TEAC) in Aqueous Solution at 25 °C, and Lag Phase (LP) As Initiated by Radical Initiator (AMVN) at 43 °C in Liposome for Isoflavonoids and Flavonoids

	genistein	apigenin	naringenin	puerarin	daidzein	equol
ε (L mol^{-1} cm^{-1})	36500_{260nm}	14000_{290nm}	15600_{290nm}	28000_{250nm}	23600_{250nm}	4200_{280nm}
log *P*	2.82	2.20	1.68	−0.35	1.73	>3.00
log $D_{7.4}$[a]	2.69	1.71	1.16	−0.76	1.46	>3.00
pK_{a1}	7.84	7.08	7.03	7.20[b]	7.47	9.84
pK_{a2}	9.34	8.51	11.71	9.84[b]	9.65	9.84
pK_{a3}	12.9[c]	12.0[c]	12.5[c]			
E_{HOMO}/eV	−5.9088	−6.2435	−6.3273	−5.9477	−6.0343	−5.8487
E_{LUMO}/eV	−1.5397	−1.8126	−1.4820	−1.7563	−1.7987	−0.7040
$H_{LUMO} - E_{HOMO}$	4.3691	4.4309	4.8453	4.1914	4.2356	5.1447
E° (pH 3.4)	1.02	1.09	1.08	1.00	1.00	0.95
E° (pH 7.4)	0.73	0.86	0.85	0.83	0.84	0.73
TEAC (pH 7.4)	12.7	4.1	2.9	2.1	0.3	0.5
LP/min[d]	336	304	325	251	53	311

[a] $\log D = \log P - \log(1 + 10^{pH-pKa1})$ obtained from ref *21*. [b] From ref *8*. [c] Slightly out of the pH range of B-R buffer. [d] $LP_{control}$ = 240 min. A similar hierarchy of LP was obtained in three independent experiments.

studied as isoflavonoids with only two hydroxyl groups, together with equol, a metabolite of daidzein, which like naringenin also lacks the conjugation between the A- and B-rings.

The *n*-octanol/water partition coefficient, log *P*, is considered to be one of the principal parameters for the evaluation of the lipophilicity of a chemical compound, and it significantly influences the activity of an antioxidant in heterogeneous systems. However, the partition coefficient describes only the partitioning of a neutral form compound. For partially ionized compounds, the effect of ionization must be considered. For the (iso)flavonoids except equol investigated in the present study, about ~50% neutral molecules deprotonate and transform into monoanions at a physiological pH of 7.4 as estimated on the basis of the corresponding pK_a value (vide infra).

Accordingly, the pH-dependent distribution coefficient, log *D*, was calculated following the relationship (*21*), $\log D = \log P - \log(1 + 10^{pH-pKa1})$. It is seen from **Table 1** that the distribution coefficient is lower than the partition coefficient, indicating that deprotonation of (iso)flavonoids reduces their lipophilicity. However, the log $D_{7.4}$ values for all of the antioxidants follow the same sequence as the log *P* values do. The more symmetric genistein was found to be more hydrophobic than apigenin as evidenced by its larger log *P* and log $D_{7.4}$ (**Table 1**), which is in agreement with a more specific hydration as expected for the less symmetric flavonoid. Equol with only two hydroxyl groups is an even more hydrophobic isoflavonoid, whereas the *C*-glycoside puerarin is relatively strongly hydrophilic.

The pK_a values of genistein, apigenin, and equol were determined spectrophotometrically as shown in **Figure 2**. As for equol it was not possible to assign the individual value to the two hydroxyl groups on the basis of the spectrophotometric measurement and the method of calculation used for the other (iso)flavonoids. An estimation of pK_a ~ 9.8 is considered to be common for the two hydroxyls, taking equol as a monoprotic acid; this value is close to that of an unsubstituted phenol and is inconsistent with the almost equivalent position of the two hydroxyl groups for equol without the conjugation between the rings. For the other (iso)flavonoids the assignment of the macroscopic pK_a values to the individual hydroxyl groups was based on a comparison with the pK_a values determined for the isomeric monoalkylated forms of the isoflavonoids puerarin and daidzein, which

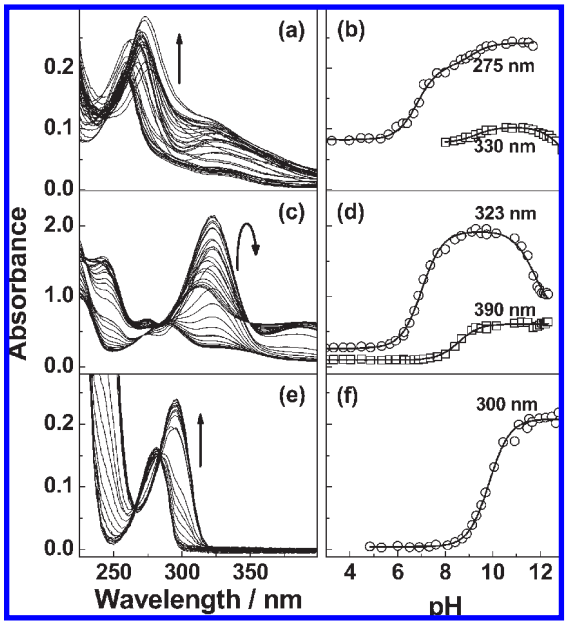

Figure 2. Absorption spectra of genistein (**a**), apigenin (**c**), and equol (**e**) in aqueous solution of ionic strength 0.1 at 25 °C at various pH values (arrows indicating tendency of absorbance change on pH increase). A_{275nm} and A_{330nm} for genistein and A_{323nm} and A_{390nm} for apigenin were fitted by using eqs **1** and **2** as shown in (**b**) and (**d**), respectively, to give the values reported in **Table 1**. A_{300nm} for equol was fitted by using eq **1** as shown in (**f**). For the two pK_a values of equol, the value pK_a = 9.84 ± 0.03 was calculated as a common estimation.

is also in agreement with other evidence from the literature (*8*, *22*, *23*). As a result, pK_{a1} is attributed to the more acidic 7-hydroxyl, whereas pK_{a2} and pK_{a3} are attributed to 4′-OH and 5-OH, respectively.

The flavonoid apigenin is more acidic than the isoflavonoid genistein for all three ionization steps, whereas the flavonoid naringenin is more acidic only for the first ionization step corresponding to pK_{a1} (**Table 1**). It had been found for six flavonols and dihydroflavonols that the relative values of pK_{a1}, pK_{a2}, and pK_{a3} could be accounted for by use of the Hammett σ-parameters and the Hammett equation (*23*). A similar relationship is not clearly seen when the pK_a values of the flavonoids and the isoflavonoids in **Table 1** are compared. For example, the pK_{a1} of genistein and apigenin should

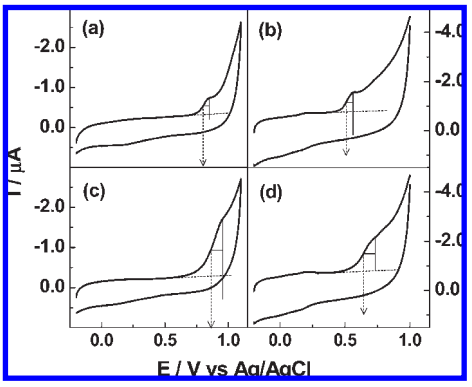

Figure 3. Cyclic voltammograms of 5×10^{-5} M genistein (**a**, **b**) and apigenin (**c**, **d**) at pH 3.4 (**a**, **c**) and pH 7.4 (**b**, **d**) obtained at room temperature in aqueous solution with an ionic strength of 0.1. Half-potentials versus Ag/AgCl at pH 3.4 (7.4) were determined to be $E_{1/2}$ = 0.80 V (0.51 V) for genistein and $E_{1/2}$ = 0.87 V (0.64 V) for apigenin.

otherwise be identical due to the presence of the same substituent in the A-ring; however, the value of genistein is in fact larger by 0.76. Likewise, both pK_{a2} and pK_{a3} should be identical for genistein and apigenin according to the Hammett formalism, which is clearly not observed. In contrast, pK_{a1} of naringenin is very similar to the value of apigenin, whereas pK_{a2} is larger for naringenin than for apigenin, in agreement with what is expected from the Hammett equation. The pK_{a2} values of daidzein, puerarin, and equol are, contrary to pK_{a1} for the same three compounds, expected to have similar values according to the Hammett formalism, which is in agreement with the experimental results (**Table 1**). The substitution effect of the hydroxyl groups in the ring under consideration may be accounted for within series of flavonoids or within series of isoflavonoids but not for the case of flavonoids and isoflavonoids together. The lower acidity of the isoflavonoid genistein compared to the flavonoid apigenin may be understood on the basis of a smaller difference in hydration in aqueous solution between the anionic and the neutral form of the more symmetric isoflavonoid, which is in agreement with its higher hydrophobicity compared to the flavonoid apigenin.

Cyclic voltammograms of genistein and apigenin at pH 3.4 and 7.4 are shown in **Figure 3**. The isoflavonoid genistein is found to be more reducing than the flavonoid apigenin, and generally the $E°$ values of the isoflavonoids are smaller than those of the flavonoids at pH 3.4 (**Table 1**). Under such moderate acidic conditions >99.9% of each (iso)flavonoid is fully protonated, allowing a direct comparison between the compounds. For $E°$ determined at pH 7.4, more relevant to physiological conditions, the differences in distribution between the acid/base forms of the individual compounds makes a comparison more difficult. The calculated BDE for the two or three hydroxyl groups of the compounds shown in **Table 2** corresponding to the reaction in gas phase, ROH → $RO^{\bullet}$ + $H^{\bullet}$, allows the assignment of the 4′-OH as the more reducing hydroxyl for all compounds except for equol, of which the 7-OH is marginally more reducing. Daidzein is the more reducing of the compounds, in agreement with the lowest BDE followed by equol and puerarin. Genistein has a comparable $E°$ value with daidzein, which is also in agreement with their almost identical BDE values. The flavonoids apigenin and naringenin are less reducing and have larger BDE values. The 7-OH and 5-OH of genistein, naringenin, and apigenin, the compounds with three hydroxyl groups, have very similar BDE values; however, it should be noted that this observation is more of theoretical interest, because the potential for further oxidation of each molecule will depend on the actual properties of the form with the first phenol oxidized and not on those of the parent molecule (*22*).

The radical scavenging capacity of the six (iso)flavonoids were measured in the TEAC assay, which is based on scavenging of the $ABTS^{\bullet+}$ radical cation, and the scavenging capacity is related to a aqueous vitamin E analogue Trolox (*13*). The pH was carefully controlled at 7.4, allowing for a direct comparison with the values determined for $E°$. On the one hand, the number of hydroxyl groups available is seen to be of importance to the radical scavenging capacity of (iso)flavonoids. On the other hand, for (iso)flavonoids with the same three hydroxyl groups, the isoflavonoid genistein has a significantly larger radical scavenging capacity than the flavonoids apigenin and naringenin, which is in agreement with the lower oxidation potential of genistein (**Table 1**). A radical scavenging capacity higher than the number of hydroxyl groups present in any polyphenol indicates further oxidative degradation of the antioxidant during the radical scavenging, and such reactions clearly must occur, especially for genistein, which was found to have the highest radical scavenging capacity among the compounds investigated (**Table 1**).

The lag phase (LP) of lipid peroxidation in phosphatidylcholine liposomes was used for evaluation of the antioxidation activity. For each (iso)flavonoid investigated, oxidation was initiated in the lipid phase by the lipophilic azo initiator AMVN. The lag phase until oxidation starts progressing, as evidenced by the formation of conjugated dienes, was monitored spectrophotometrically by the absorbance at 234 nm. The effect of the antioxidants except daidzein was clear for oxidation initiated in the lipid phase as seen in **Figure 4**, and the individual compounds had rather different effects. From the lag phase of (iso)flavonoids collected in **Table 1**, it is seen that isoflavonoid genistein is the best antioxidant among all of the (iso)flavonoids examined, which is consistent with the highest radical scavenging capacity in aqueous solution (**Table 1**). The dihydroflavone naringenin is better than apigenin, and the isoflavandiol equol is remarkably better than isoflavonones puerarin and daidzein; however, daidzein is found even to be pro-oxidative. Equol, with the absence of a carbonyl group, C2=C3 double bond, and hydroxyl groups in the pyran ring, was recently found to have an anomalous and higher antioxidant inhibitory effect toward LDL oxidation (*24*).

The radical scavenging capacity of the (iso)flavonoids was determined in homogeneous solution, whereas the antioxidant activity was determined in a heterogeneous system with water/lipid interfaces. Quantum mechanical calculations were performed to assist a comparison of the oxidation potentials and the radical scavenging capacity in homogeneous solution with antioxidative activity in the structured medium. Among the compounds with three hydroxyl groups, genistein is the most lipophilic as evidenced by the partition coefficient and the highest antioxidation activity (**Table 1**). High lipophilicity has recently been demonstrated to be crucial for activity of antioxidant bioconjugates at lipid/water interfaces (*25*). The high lipophilicity is, however, not solely in control of an optimal function in the water/lipid interface for these compounds.

The partition coefficient is a macroscopic property of the compounds, whereas the dipole moment is a microscopic property of the compounds. It had been reported that the dipole moment defines molecular orientation and is important in driving molecular interactions and that the magnitude of dipole moment for flavonoids may inversely correlate to the

Table 2. Calculated A-to-B Dihedral Angles (α, Degrees) and Dipole Moments (μ, Debye) for (Iso)flavonoid Parent Molecules (F) and Their Phenoxyl Radicals and Bond Dissociation Enthalpies (BDE, Kilocalories per Mole) for Hydroxyls of (Iso)flavonoids

	genistein			apigenin			naringenin			puerarin			daidzein			equol		
	α	μ	BDE	α	μ	BDE	α	μ	BDE	α	μ	BDE	α	μ	BDE	α	μ	BDE
F	39.3	3.70		18.5	6.70		79.5	3.44		37.4	5.40		38.4	5.25		81.4	2.57	
7-OH/-O•	41.0	2.57	92.42	14.7	4.54	91.81	67.5	2.83	92.35	37.2	5.47	95.05	37.9	4.65	91.74	78.7	6.98	87.80
4′-OH/-O•	32.3	9.38	87.03	8.5	5.67	87.88	84.1	6.90	88.63	31.4	10.38	87.04	31.2	8.14	86.86	81.7	4.05	88.24
5-OH/-O•	40.4	6.46	92.22	18.2	8.85	92.87	91.5	8.21	91.90									

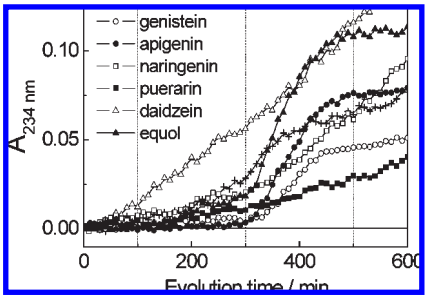

Figure 4. Oxidation of phosphatidylcholine in liposomes at 43 °C as monitored spectrophotometrically at 234 nm through formation of conjugated dienes following initiation by lipophilic free radical initiator AMVN. The lag phase for oxidation in the absence or presence of (iso)flavonoids was determined as the intercept between the slope of absorption during progressing oxidation and the curve for absorption during lag phase. A sample without antioxidants was used as a control.

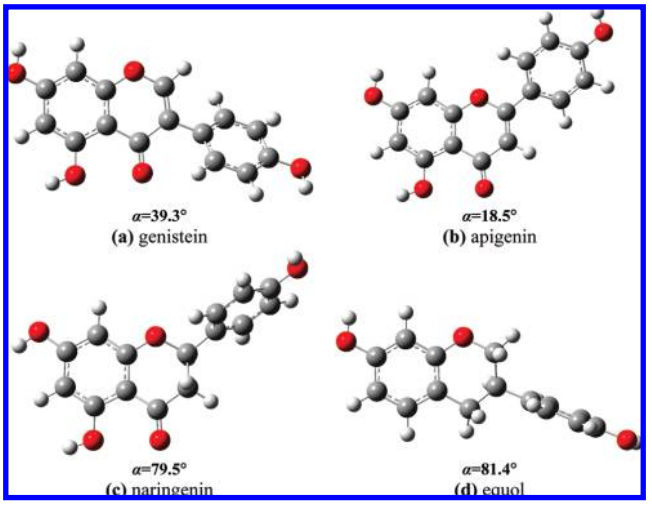

Figure 5. Molecular geometries for neutral genistein (**a**), apigenin (**b**), naringenin (**c**), and equol (**d**) as optimized by quantum chemical calculation. α is the A-to-B ring dihedral angle.

antioxidation activity (*26*). In the present study, a better agreement between the dipole moment acquired by the theoretically optimized structures and the antioxidative efficiency is noted (**Tables 1** and **2**). The more symmetric genistein, with a relatively lower dipole moment, is more efficient as an antioxidant than the less symmetric apigenin, which is further supported by a comparison with naringenin. Naringenin has a dipole moment more comparable with that of genistein (μ in **Table 2**) and clearly exhibits a higher antioxidation activity than apigenin. Equol has the lowest dipole moment and exhibits higher inhibition activity against lipid peroxidation among the examined antioxidants.

The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) provide the information on the electron-donating and electron-accepting characters of a molecule. It is known that the energy levels of HOMO and LUMO are crucial in governing the antioxidant activities (*27*). We have also attempted to examine the correlation between the frontier orbital energies (**Table 1**) and the activities of radical scavenging and inhibition of lipid peroxiation, and no general agreement was found for the (iso)flavonoids. From the values of E_{HOMO}, E_{LUMO}, and $E_{HOMO} - E_{LUMO}$ (H − L gap), genistein with higher E_{HOMO} has higher electron-donating ability than apigenin and naringenin in agreement with its observed higher radical scavenging and antioxidative activities. The rather small difference of the H − L gap between genistein and apigenin may imply that this particular parameter may be not suitable to characterize the relevant antioxidation activities.

The orientation and distribution of antioxidants in heterogeneous solution, which are known to play important roles in antioxidation, are highly dependent on their steric molecular structures (*28*, *29*). Comparing the optimized structures of (iso)flaovnoids (**Figure 5** and **Table 2**), the isoflavonones genistein, puerarin, and daidzein in both neutral and radical forms have larger A-to-B dihedral angles (30−40°) than the flavonone apigenin (α in **Table 2**). Apigenin and equol lacking C-ring conjugation have a more significant deviation from coplanarity (~80°) than the (iso)flavonones and show an outstanding antioxidant capacity. Notably, among the three isoflavonoids with two hydroxyl groups investigated, equol with the largest A-to-B dihedral angle is also the best antioxidant in support of the importance of this specific molecular feature for high antioxidative efficiency in interfaces. Thus, for equol the poorly conjugated 4′-hydroxyl in the B-ring with AC-rings may be an important structural factor responsible for its high antioxidant activity in liposome. Catechins, which are known as very efficient antioxidants, have a facile rotation of the B-ring relative to the AC-ring system and have accordingly similar structural flexibility for the ring with the most reducing hydroxyl group (*30*).

In conclusion, the isoflavonoid genistein is less acidic than the isomeric flavonoid apigenin due to the specific solvation effects but is more reducing than apigenin due to a lower bond dissociation enthalpy for the hydroxyl group in the B-ring. As an antioxidant in water/lipid interfaces, a low value of the dipole moment seems better correlated with efficiency as antioxidant than the partition coefficient as a macroscopic property. Deviation from coplanarity, moreover, seems to be of importance for an optimal orientation and function as antioxidants for flavonoids and isoflavonoids at interfaces.

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