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Why B-Ring is the Active Center for Genistein to Scavenge Peroxyl Radical: A DFT Study

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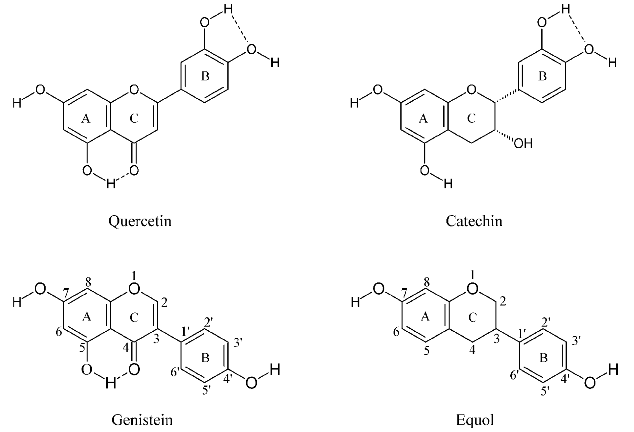
Abstract—The structure–activity relationship for genistein to scavenge peroxyl radical was clarified by density functional theory (DFT) calculations using the B3LYP/6-31G(d,p) method. It was revealed that the conjugation of an electron-withdrawing 1,4-pyrone group with A-ring of genistein was not beneficial to enhance the radical-scavenging activities. Thus, hydroxyl in B-ring became the active center of genistein to scavenge peroxyl radical.

Flavonoids and isoflavonoids (Scheme 1) are the most abundant natural antioxidants (ArOH) and have received considerable attention in recent years.[1,2] Many efforts have been made to summarize and elucidate the structure–activity relationships (SARs) for them. The most interesting finding may be that B-ring is the active center for flavonoids and isoflavonoids to scavenge free radicals, despite their distinct molecular structures. Namely, the first radical-scavenging step for the both kinds of antioxidants is, taking peroxyl radical as an example, a H-abstraction reaction (eq 1) or a proton concerted electron transfer (eq 2) reaction occurred on hydroxyl of B-ring.[1,2]

$$\mathrm{ROO^\bullet + ArOH \rightarrow ROOH + ArO^\bullet} \qquad (1)$$

$$\mathrm{ROO^\bullet + ArOH \rightarrow ROO^- + ArOH^{\bullet +}}$$
$$\mathrm{\rightarrow ROOH + ArO^\bullet} \qquad (2)$$

As most flavonoids contain a catecholic B-ring (Scheme 1), it is easy to understand why this moiety is the active center. That is, the radical generated after donating a hydrogen atom can be well stabilized by the *ortho* hydroxyl group and an intramolecular hydrogen bond.[3] However, it is difficult to understand why B-ring is also the active center for isoflavonoids, especially for isoflavone, for example genistein.[2] First, isoflavonoids have no catecholic group at all. Second, according to the theory proposed by Bors and co-workers,[1a] a 1,4-pyrone group extends the conjugation system of phenoxyl radical and is helpful to stabilize the radical, thus, is beneficial to enhance the radical-scavenging activity



Scheme 1. Molecular structures of several typical flavonoids and isoflavonoids.

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of phenol. Therefore, the hydroxyls in A-ring of genistein should be more active than the counterpart in B-ring to donate a hydrogen atom, however, which is inconsistent with the experimental finding.[2] Taking into account that theoretical methods have been successfully used to elucidate the SAR for antioxidants,[4] we attempt to clarify the SAR in this letter by means of quantum chemical calculations.

Considering the accuracy and conveniency of density functional theory (DFT),[5] B3LYP function on the basis set of 6-31G(d,p) was employed to do calculations. The molecular geometries were optimized, firstly, by the molecular mechanic method MMX,[6] and then, by the semiempirical quantum chemical method AM1.[7] Finally, the B3LYP/6-31G(d,p) was used for the full geometry optimization in gas phase. The zero point vibrational energy (ZPVE) and the vibrational contribution to the enthalpy were scaled by a factor of 0.9805.[8] The quantum chemical calculations were accomplished by Gaussian 94.[9]

To elucidate the SAR, we have to select appropriate theoretical indexes to characterize the radical-scavenging activity at first. Although both mechanisms give identical products (eqs 1 and 2), the theoretical indexes are different. For the first pathway (eq 1), the reactivity can be measured by O–H bond dissociation enthalpy (BDE) to a large extent.[4,10] The lower the O–H BDE is, the higher the activity. In fact, this parameter has been successfully used to explain the SAR for phenolic antioxidants and to design novel antioxidants.[4] For the second pathway (eq 2), adiabatic ionization potential (IP) and O–H proton dissociation enthalpies (PDEs) are proper parameters, and the former is more important than the latter.[11] The lower the parameters are, the faster the reaction. Thus, three kinds of indexes were calculated and listed in Table 1.

Table 1. O–H BDE (kcal/mol), adiabatic IP (kcal/mol) and O–H PDE (kcal/mol) calculated by means of B3LYP/6-31G(d,p)

	Genistein	**1**	**2**	**3**	Equol
O–H BDE (4′)[a]	81.81				82.30
O–H BDE (5)	99.70	82.51	79.06	106.01	
O–H BDE (7)	88.34		83.14	88.64	81.89
IP[b]	162.54	178.21	168.12	179.86	160.68
O–H PDE (4′)[c]	233.26	218.28			235.58
O–H PDE (5)	251.14		225.07	240.05	
O–H PDE (7)	239.78		229.15	222.69	235.17

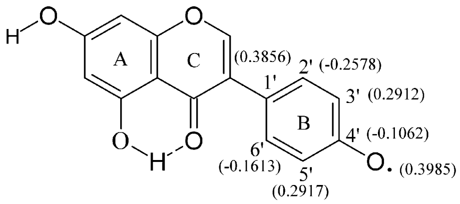
[a] O–H bond dissociation enthalpy. BDE $= H_r + H_h - H_p$, in which, H_r is the enthalpy for radical generated after H-abstraction, H_h is the enthalpy for hydrogen atom, −0.49792 hartree, and H_p is the enthalpy for parent molecule. The positions for hydroxyl groups are in parentheses.

[b] Adiabatic ionization potential. IP $= (TE_c + ZPVE_c \times 0.9805) - (TE_p + ZPVE_p \times 0.9805)$, in which, TE_c is the total energy for cation radical, $ZPVE_c$ is the zero point vibrational energy for cation radical, TE_p is the total energy for parent molecule, and $ZPVE_p$ is the zero point vibrational energy for parent molecule.

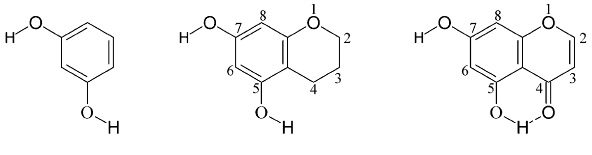
[c] O–H proton dissociation enthalpy. PDE $= H_r + H_p - H_c$, in which, H_r is the enthalpy for radical generated after proton dissociation, H_p is the enthalpy for proton, 0.00236 hartree, and H_c is the enthalpy for cation radical. The positions for hydroxyl groups are in parentheses.

As shown in Table 1, the O–H BDE and O–H PDE in B-ring of genistein are much lower than those in A-ring, in good agreement with the experimental finding that B-ring is the active center, which results from the fact that the genistein radical generated after the H-abstraction in B-ring is more stable than the counterpart radicals in A-ring. Thus, it seems the conjugation of 1,4-pyrone to A-ring is not beneficial to stabilize the phenoxyl radical and to enhance the radical-scavenging activity. Besides, the spin density distributions indicate that positions 1′, 3′ and 5′ have the highest unpaired electron density in carbons of genistein radical (Scheme 2), suggesting these positions are most probable to be attacked by other radicals, which is also consistent with the succeeded radical-adducting mechanisms of genistein radical revealed previously.[2b]

To gain a deeper insight into the SAR for genistein and other isoflavonoids, we attempt to evaluate the 1,4-pyrone effect further. 1,4-Pyrone contains an electron-donating part, O1, due to the *p*-type lone pair, and an electron-withdrawing part, 2,3-double bond with a 4-oxo-function. Thus, three model molecules were designed (Scheme 3), and the corresponding theoretical parameters were calculated (Table 1) to evaluate the 1,4-pyrone effect on the theoretical parameters. It has been revealed that the electron-donating group reduces the O–H BDE and adiabatic IP, and increases the O–H PDE, while the electron-withdrawing group has opposite effects.[5,10,11] As **2** contains an electron-donating moiety, chroman, it has lower O–H BDE and adiabatic IP than **1**, and its O–H PDE is higher than that of **1**. However, when a 2,3-double bond with a 4-oxo function is incorporated into **2**, giving **3**, the O–H BDE and adiabatic IP get much higher,[12] consistent with the fact that a 2,3-double bond with a 4-oxo function is a strong electron-withdrawing group. And accordingly, O–H PDE (7) gets lower compared with that of **2**.[13] From the differences of parameters between **1** and **3**, it is clear that the overall effect of 1,4-pyrone is electron-withdrawing, and this group is not beneficial to enhance the radical-scavenging activity of A-ring.[14] Thus, it is understandable why A-ring is not the active center of genistein.



Scheme 2. Spin density distributions in genistein radical.



1 **2** **3**

Scheme 3. Designed model molecules.

It is also interesting to find that the O–H BDE for B-ring of genistein is ~1 kcal/mol lower than that of phenol,[15] which arises from the electron-donating property of O1. Considering the O–H BDEs for flavonoids containing a catecholic group, such as quercetin or catechin, are 6–12 kcal/mol lower than that of phenol,[3,16] the radical-scavenging activity of genistein should be much lower than the activity of flavonoids, which really has been observed by experiments.[17]

Last but not least, according to the above result, if an isoflavonoid contains a chroman moiety, the A-ring may be the active center too. For instance, the O–H BDE and O–H PDE in A-ring of equol are comparable to those in B-ring (Table 1). We hope this will arouse the interest of experimentalist who can verify the prediction by experiments.

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12. The intramolecular hydrogen bond between 5-OH and 4-oxo also results in the high O–H BDE (5) in **3**.
13. The higher O–H PDE (5) in **3** than that in **2** stems from the intramolecular hydrogen bond between 5-OH and 4-oxo.
14. A similar effect of 1,4-pyrone has been observed on catechols contained in flavonoids.[3d]
15. O–H BDE of phenol calculated by B3LYP/6-31G(d,p) is 82.83 kcal/mol.[3d]
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