Theoretical Elucidation of the Radical-Scavenging-Activity Difference of Hydroxycinnamic Acid Derivatives

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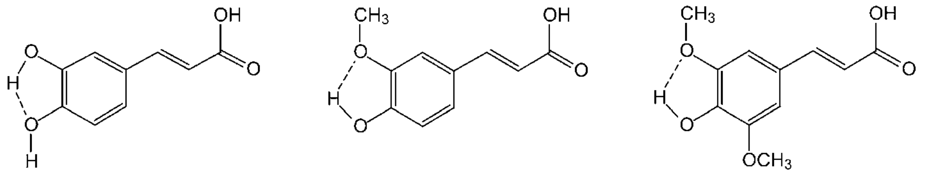
To elucidate the nitrogen dioxide radical-scavenging-activity difference of three hydroxycinnamic acid derivatives (HCAs), *i.e.*, caffeic acid (= 3-(3,4-dihydroxyphenyl)prop-2-enoic acid; **1**), ferulic acid (= 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid; **2**), and sinapic acid (= 3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoic acid; **3**), a combined density-functional-theory (DFT) method, labeled as B3LYP/6-31G(d)//AM1, was employed to calculate adiabatic ionization potentials (*IP*s) for parent molecules and anions derived by proton dissociation. It was found that not the *IP*s of the parent HCAs but those of the anions account well for the experimentally observed higher activity of caffeic acid as compared to those of the other two HCAs, suggesting that the antioxidant anion should be taken into consideration in the selection or rational design of novel antioxidants; this, however, was neglected in previous studies.

Introduction. – Hydroxycinnamic acid derivatives (HCAs), *e.g.*, caffeic acid (= 3-(3,4-dihydroxyphenyl)prop-2-enoic acid; **1**), ferulic acid (= 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid; **2**), and sinapic acid (= 3-(4-hydroxy-3,5-dimethoxyphenyl)-prop-2-enoic acid; **3**) (*Fig. 1*), are excellent antioxidants with great potential to be used as medicines or food additives [1–4]. Recently, it was found that they were rather active in scavenging nitrogen dioxide radicals with the activity order: **1** ($k_s \approx 8.6 \cdot 10^8$ M^{-1} s^{-1}) > **2** ($k_s \approx 7.4 \times 10^8$ M^{-1} s^{-1}) ≈ **3** ($k_s \approx 7.2 \cdot 10^8$ M^{-1} s^{-1}) [5]. Since the three HCAs have distinct structures, it is very interesting to elucidate why **1** is more active than **2** and **3** and why **2** is similar to **3**. This will give a deeper insight into the antioxidant mechanisms and provide new clues for selecting or designing better antioxidants. Considering the successful use of theoretical methods in elucidating the structure–activity relationships (SAR) for various antioxidants [6–14], we attempt to achieve the goal by means of quantum-chemical calculations.



1 **2** **3**

Fig. 1. *Structures of three hydroxycinnamic acid derivatives* (HCAs)

Results and Discussion. – It is known that there exist at least two mechanisms for phenolic antioxidants (ArOH) to scavenge free radicals. The first is, taking peroxyl radical (ROO$^\bullet$) as an example, a direct hydrogen-transfer process (*Eqn. 1*), and the second is a proton-coupled electron-transfer process (*Eqn. 2*).

$$\mathrm{ROO^\bullet + ArOH \rightarrow ROOH + ArO^\bullet} \tag{1}$$

$$\mathrm{ROO^\bullet + ArOH \rightarrow ROO^- + ArOH^{\bullet +} \rightarrow ROOH + ArO^\bullet} \tag{2}$$

The first pathway is preferred in nonpolar solvents [15] [16], which is well characterized by the O–H bond-dissociation enthalpy (BDE) of ArOH [17][18], while the second one predominates in polar solvents, which is governed by the ionization potential (*IP*) of ArOH [6]. Since the nitrogen dioxide radical scavenging experiments were carried out in H_2O, only the second mechanism was involved [5], and thus the *IP* will be an appropriate parameter to characterize the radical scavenging ability of the HCAs¹). Obviously, the lower the *IP* is, the more active the HCA.

As shown in the *Table*, the *IP* order for the three parent HCA is **1** > **2** > **3**, just opposite to their radical-scavenging-activity sequence, suggesting that the real nitrogen dioxide radical scavenging process for HCAs is more complex than a simple one-step electron-transfer reaction arising from parent molecules. In fact, HCAs are weak acids, so the HCA anions generated after proton dissociation from the carboxy group should be taken into consideration. Since the pK_a values of **1**, **2**, and **3** are 4.4, 4.2, and 4.2, respectively, most of the HCA (> 99.5%) existed as the anion under the experimental conditions (pH 7). Therefore, the *IP*s for the HCA anions were calculated (*Table*). It is interesting to note that the *IP*s of the anions are several kcal/mol higher than those of the parent molecules, contrary to the common idea that anions are much stronger electron-donors than their neutral counterparts. This is thought essentially arising from the extremely strong polarity of H_2O. As polar solvents are favorable to stabilize the charged molecule, according to the definition of *IP*, the *IP*s of parent molecules will

Table. *Ionization Potentials (IPs) of HCAs Calculated by the B3LYP/6-31G(d)//AM1 Method with Solvent Effect*

HCA	SPE_b [hartree]ᵃ)	$ZPVE_b$ [hartree]ᵇ)	SPE_a [hartree]ᶜ)	$ZPVE_a$ [hartree]ᵈ)	*IP* [kcal/mol]ᵉ)
1	– 648.677856	0.161337	– 648.478477	0.159117	123.76
1$^-$	– 648.205901	0.148707	– 648.003108	0.147118	126.28
2	– 687.974604	0.190252	– 687.776518	0.187849	122.83
2$^-$	– 687.504696	0.177817	– 687.299413	0.176045	127.74
3	– 802.484741	0.223346	– 802.291452	0.220962	119.84
3$^-$	– 802.016661	0.210876	– 801.810917	0.209066	128.00

[a]) Single-point energies for molecules before electron transfer. [b]) Zero-point vibrational energies for molecules before electron transfer. [c]) Single-point energies for molecules after electron transfer. [d]) Zero-point vibrational energies for molecules after electron transfer. [e]) $IP = [(SPE_a + ZPVE_a \cdot 0.973) - (SPE_b + ZPVE_b \cdot 0.973)] \cdot 627.5095$.

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[1]) In fact, the O–H BDEs for the parent HCAs and derived anions were also calculated (unpublished results). However, the parameters failed completely to explain the radical-scavenging-activity difference of the HCAs.

greatly decrease, while the *IP*s of anions will markedly increase in H_2O compared with those in the gas phase, which results in the reversed *IP* order for parent HCA and HCA anions. In contrast, in the gas phase, the *IP*s for HCA anions are around 100 kcal/mol lower than those of parent HCA[2]).

As indicated in the *Table*, the *IP* of caffeic acid anion ($\mathbf{1}^-$) is *ca.* 1.5 kcal/mol lower than those of the other two HCA anions, while the difference in the *IP*s of the **2**- and **3**-derived anions is slight, which agrees well with the radical-scavenging-activity order of the three HCAs. Furthermore, a quantitative correlation between *IP* and the logarithm of k_s (*Fig. 2*) reveals an extremely high linear coefficient indicating that HCA anions do play a key role in the radical-scavenging process. This finding also highlights the catechol moiety's effect in enhancing the radical-scavenging activity. Indeed, catechol (benzene-1,2-diol) has been recognized as a good lead structure in the rational design of excellent antioxidants [19][20]. In combination with the previous experimental finding that **1** is more active than **2** and **3** to scavenge the 2,2-diphenyl-1-picrylhydrazyl (=2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl) radical (DPPH$^•$) by donating a H-atom [21], caffeic acid holds more potential than the other two HCAs to be used as a medicine or food additive in practice.

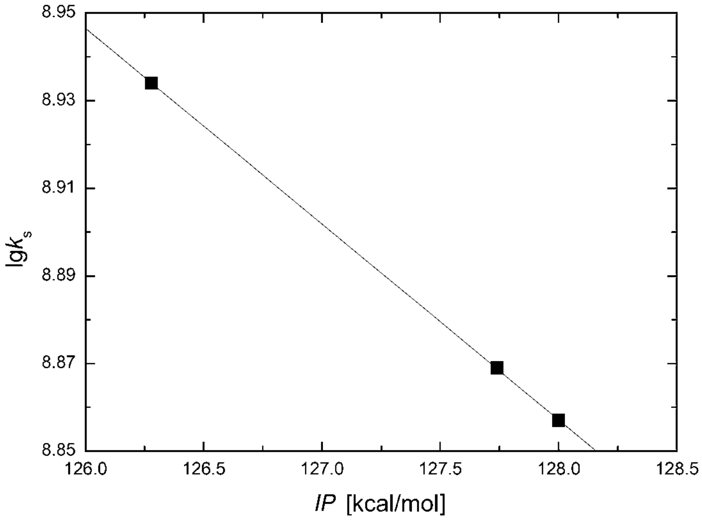

Fig. 2. *Correlation between the logarithm of the nitrogen dioxide radical scavenging rate constant* (lgk_s) *and* IP ($r = 0.99999$)

Recently, it was also found for simple monophenols that the anions played an important role in radical-scavenging processes in polar solvents [22]. Hence, to select or rationally design novel antioxidants, one should take the proton dissociation process into consideration, especially for the use in polar systems, which apparently was neglected in previous studies.

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2) The *IP*s of the parent **1**, **2**, **3**, and the corresponding anions in the gas phase calculated by B3LYP/6-31G(d)//AM1 are 171.32, 167.27, 162.94, 60.53, 70.77, and 70.32 kcal/mol, respectively.

Conclusion. – The quantum-chemical calculation suggests that HCA-derived anions play an important role in scavenging the nitrogen dioxide radical. This not only accounts for the higher activity of caffeic acid (**1**) than those of ferulic acid (**2**) and sinapic acid (**3**), but also provides further evidence to support the idea that phenols containing a catechol moiety have a great potential as excellent antioxidants.

Methods. – A kind of combined density-functional-theory (DFT) method was used to calculate the adiabatic *IP*s, which takes advantages of accuracy and economy [6][23][24]. The calculation procedures are as follows. The molecular geometries were optimized, first, by the molecular-mechanic method, and then, by the semiempirical quantum-chemical method AM1 [25]. Finally, B3LYP functional on basis set of 6-31G(d) was used to calculate the single-point energy (*SPE*) with solvent effect. The molecular energy in H_2O soln. consists of B3LYP/6-31G(d)-calculated *SPE* and AM1-calculated zero-point vibrational energy (*ZPVE*, scaled by a factor of 0.973) [6]. The solvent effect was taken into consideration by employing the self-consistent reaction-field (SCRF) method with the polarized continuum model (PCM). The quantum-chemical calculations were accomplished by the Gaussian 98 program [26].

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