Theoretical Study for Exploring the Diglycoside Substituent Effect on the Antioxidative Capability of Isorhamnetin Extracted from *Anoectochilus roxburghii*

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ABSTRACT: Radical-scavenging activity of isorhamnetin (1) and its diglycosides, named isorhamnetin-3,5′-O-β-D-diglucoside (2) and isorhamnetin-3,7-O-β-D-diglucoside (3) extracted from *Anoectochilus roxburghii*, has been studied through three main antioxidant pathways: hydrogen atom transfer (HAT), single electron transfer followed by proton transfer, and sequential proton loss electron transfer (SPL). All thermodynamic parameters related to these radical-scavenging mechanisms were computed at the B3LYP/6-311G(d,p) level of theory both in the gas phase and in solution. The results suggest that HAT is the predominant mechanism in the gas phase, while SPL is supported in an aqueous environment. In addition, the stability of radicals has also been explored by electron spin density and intramolecular hydrogen bonding. The potential energy profiles and kinetic calculations for the reactions between the selected compounds and the CH₃OO• radical were calculated at 298.15 K. Among all investigated, compound 2 has the highest antioxidant activity with the lowest Gibbs free energy (−4.05 kcal/mol) and the highest hydrogen atom transfer rate constant (3.61 × 10⁷ M⁻¹ s⁻¹). Substitution of the OH and OMe groups by two glycoses at the 3 and 5′ sites of isorhamnetin has a significant impact on its antioxidant activity.

1. INTRODUCTION

Human body has natural immune systems to protect our health to avoid the aging process and diseases. When the immune system gets weaker, the development of numerous diseases increases through oxidative stress. Oxidative stress is known as a "chemical silent killer". This process arises from oxidative damage of biomolecules (such as proteins, lipids, enzymes, and DNA) by the presence of reactive free radicals. Oxidative stress is caused by an imbalance between antioxidants and oxidants (free radicals or reactive species) in favor of oxidants.

Antioxidants play a significant role in oxidative stress resistance, also labeled as "free radical scavengers". Recently, antioxidants such as natural flavonoids have grown greatly due to their high antioxidant activity, nontoxic effects on human beings, and safety to the environment. They are mainly found in vegetables, fruits, cereals, and medicinal plants.

In natural compounds, *Anoectochilus roxburghii* is known as a good source of organic compounds, which are used as traditional medicines for the treatment of diabetes, cancer, liver diseases, cardiovascular diseases, and so on. Recent research has revealed that flavonoids and their glycosides have been isolated from *A. roxburghii* that have remarkable bioactivities, allowing the scavenging of free radicals. Water solubility of flavonoid glycosides is better than that of flavonoids because sugar moietyes have a hydrophilic nature. Moreover, many studies show that the presence of the glucosidic group at different positions in flavonoids may affect the antioxidant activity and the mechanism of their biological activities. For example, Zheng et al. demonstrated that the presence of glucosidic group at the 4′ and 5′ positions of quercetin would enhance the antioxidant activity, while the substitution on other sites would reduce the antioxidant activity. However, no data are available in the literature to...
predict the radical-scavenging activity for diglycoside flavonoids at the molecular level. Therefore, an understanding of the diglycoside substitution effect on the antioxidative capability of flavonoids is important.

According to the literature, the radical-scavenging activity of flavonoid antioxidants was analyzed via three of the most useful mechanisms as follows:15–17 hydrogen atom transfer (HAT), single electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET). All thermodynamic parameters descriptor for each step of these mechanisms including bond dissociation enthalpy (BDE), ionization energy (IE), proton dissociation enthalpy (PDE), proton affinity (PA), and electron-transfer enthalpy (ETE) were used to elucidate the antioxidative potential of the investigated compounds.

In this work, the antioxidative activity of isorhamnetin (1) and its diglycosides, named isorhamnetin-3,5'-O-β-D-diglucoside (2) and isorhamnetin-3,7-O-β-D-diglucose (3) (Figure 1) that had been extracted from A. roxburghii, were studied in detail using the density functional theory (DFT). Relying on the thermodynamic descriptors concerned to the mechanisms of antioxidative action, the purpose of this study is to evaluate their radical-scavenging capacity through three mechanisms as mentioned above in the gas phase and in solution. Finally, the potential energy surfaces and rate constants (k) of the reaction between diglycosyl flavonoids and hydroperoxy radicals (CH3OO•) were evaluated for an insight into their mechanism of action.

2. RESULTS AND DISCUSSION

2.1. Thermodynamic Calculation. The calculated results of the reaction enthalpies for the investigated compounds in gas and solvent phases related to three radical-scavenging mechanisms (HAT, SET-PT, and SPLET) are presented in Tables 1 and S1 (Supporting Information). Based on thermodynamic parameters (BDE, IE, PDE, PA, and ETE), we can predict the most-active site for the radical-scavenging reaction of the investigated compounds and the thermodynamically preferred reaction pathway.

The different positions of OH and glucosidic groups on A, B, and C rings of the flavonoid skeleton will impact the radical-scavenging potency of 1, 2, and 3 compounds. The most-active OH group of the title compounds was determined by the minimal sum of the enthalpies of the specific reaction pathways including BDEmin, (IE + PDE)min, and (PA + ETE)min.18 Based on the BDEmin, (IE + PDE)min, and (PA + ETE)min values shown in Tables 1 and S1, the preferred OH site of each of the title compounds was the same in HAT, SET-PT, and SPLET mechanisms.

For specific compounds, the calculated BDEs of OH groups in the gas phase and in solution are shown in Table 1 in the following order

No sugar group at different rings of isorhamnetin (compound 1): 3-OH ≈ 4'-OH < 7-OH < 5-OH.
For sugar groups at B and C rings of isorhamnetin (compound 2): 4'-OH < 7-OH < 5-OH.
Sugar groups present at A and C rings of isorhamnetin (compound 3): 4'-OH < 5-OH.

In compound 1, BDE(O–H)s at O4' and O3 sites are lower than one at other sites in the gas phase (Table 1). It means that H-atom-transfer process from O4' and O3 sites is more preferred than that from other positions. From data in Table 1, it is seen that the difference between the BDEs for 4'-OH and 3-OH groups is quite low, about 1.1 kcal/mol, corresponding to the gas phase. Many previous research works have also demonstrated that the role of 3-OH site and C2–C3 double bond in the flavonoid core has a significant impact on their antioxidant activity.19–21 The preferred site for compound 1 is 3-OH because the 3-O• phenoxyl radical is formed more stably by expanding delocalization of electrons.

![Figure 1. Structures and atom numbering for isorhamnetin and its glucosides.](image-url)

Table 1. Thermodynamic Descriptors (kcal/mol) Related to the Three Major Antioxidative Mechanisms for the Studied Compounds Computed at the B3LYP/6-311G(d,p) Level in the Gas Phase and in Solution

| Compounds | BDE(O–H) | IE | PA | ETE | PDE |
|-----------|----------|----|----|-----|-----|
|           | gas      | water | ethanol | gas | water | ethanol | gas | water | ethanol | gas | water | ethanol |
| 1         |          |       |       |     |       |       |     |       |       |     |       |       |
| 4'-OH     | 80.1     | 83    | 82.3 | 160.8 | 102.6 | 110.4 | 334.5 | 35.4 | 47.3 | 61.5 | 74.8 | 80.6 | 235.2 | 7.6 | 17.5 |
| 3-OH      | 79.0     | 79.0  | 78.5 | 338.9 | 35.3 | 47.5 | 56.0 | 70.9 | 76.6 | 234.1 | 13.7 |
| 5-OH      | 94.8     | 92.9  | 92.5 | 345.4 | 39.1 | 51.4 | 65.2 | 81.7 | 86.7 | 249.8 | 17.6 |
| 7-OH      | 86.4     | 88.5  | 87.9 | 331.6 | 30.9 | 42.9 | 70.6 | 84.8 | 90.6 | 241.4 | 13.1 |
| 2         |          |       |       |     |       |       |     |       |       |     |       |       |
| 4'-OH     | 75.3     | 77.8  | 77.2 | 311.3 | 21.9 | 33.6 | 79.9 | 83.1 | 89.1 | 231.6 | 0.4 | 9.9 |
| 5-OH      | 98.1     | 95.7  | 95.4 | 346.1 | 41.6 | 53.9 | 67.9 | 81.3 | 87.1 | 254.4 | 17.9 | 28.1 |
| 7-OH      | 87.9     | 90.7  | 90.1 | 329.9 | 31.3 | 43.2 | 73.8 | 86.7 | 92.4 | 244.1 | 12.9 | 22.8 |
| 3         |          |       |       |     |       |       |     |       |       |     |       |       |
| 4'-OH     | 81.0     | 83.9  | 83.3 | 322.4 | 33.0 | 44.6 | 74.4 | 78.2 | 84.3 | 230.5 | 2.0 | 11.8 |
| 5-OH      | 96.9     | 95.9  | 95.5 | 335.4 | 39.6 | 51.6 | 77.4 | 83.6 | 89.5 | 246.5 | 14.0 | 24.0 |

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over the C ring via 4-keto, C2–C3 double bond. In both compounds 2 and 3, the lowest BDEs are found for the 4′-OH group at B ring. Conversely, BDE values for 5-OH of 1, 2, and 3 are remarkably larger than the BDE values of other OH groups. This can be explained by the formation of hydrogen bonds between 5-OH of A ring and C=O of C ring. Especially, BDE for 4′-OH of compound 2 (75.3 kcal/mol) is the lowest compared to the BDE(O−H) values of compounds 1 and 3. This means that the H-atom donation ability to scavenge the radical at the O4′ site of compound 2 is more preferred than for other compounds. The high reactivity of this site could be explained by the glycosyl substitution at O-5, and O-3 site decreases the negative charge on the oxygen atom at O-4′ and increases polarization of the O–H bonds. Additionally, the 4′-O• phenoxyl radical of compound 2 is more stable by the intramolecular hydrogen bond because of the presence of the glycosyl group. This will be further demonstrated in Section 2.2.

The BDEmin is an important parameter to evaluate the H-atom-transfer ability to scavenge the free radical.22,23 As shown in Table 1, the lowest O–H BDE values for the investigated compounds in the gas phase can be ranged in the following sequence: 2 < 1 < 3. On comparing the BDEmin value of compound 2 with those of 1 and 3, the value decreases by 3.7 and 5.7 kcal/mol, respectively. Thus, the obtained result shows that compound 2 has a higher radical-scavenging potency than that of the compounds 1 and 3. In other words, the glycosyl groups present at the B and C rings of isorhamnetin are better antioxidants than the one at A and C rings of isorhamnetin. In this work, the computed BDE in solution (water and ethanol) is due to the reason that these solvents are commonly used to extract flavonoid glucosides from A. roxburghii.7 In the solvents, these BDE values tended to slightly increase by 2–3 kcal/mol, except the 5-OH BDE. Unlike other OH groups, the 5-OH BDE in the gas phase is higher by 1–3 kcal/mol than that in solution. This shift may be related to the distortion of the intramolecular hydrogen bond between 5-OH and C=O group in solvents. As can be concluded, substituting the hydroxyl group by the glycosyl group in flavonoids has a strongly influence on their antioxidant activity through the HAT mechanism.

For SET-PT and SPLET mechanisms including multiple steps, the first step plays the most important role from the thermodynamic viewpoint. Corresponding to the first steps of these mechanisms, the lowest IE and PA values indicate the predominant mechanism and reaction pathway from the thermodynamic point of view.24 From Table 1, it can be seen that the thermodynamic descriptors related to SET-PT and SPLET are greatly impacted by the solvation enthalpies due to these mechanisms involving charged species.25

For the SET-PT mechanism, IE is a key factor showing the range of electron donation. It is clearly from Table 1, the IEs of investigated compounds arranged in the order of gas > ethanol > water. When there is a change from the gas phase to water, the IE decreased dramatically with an average deviation of 56.3 kcal/mol. Thus, the electron-transfer process is favorable in the solvent. Based on the IE values of the studied compounds, the antioxidant activity can be ranged in the following order: 2 > 1 > 3. From the calculated data in Table 1, it is obtaining that the trend of the IEs and BDEs is the same. However, the calculated IE values are significantly larger than the corresponding BDE values, thus indicating that HAT is a more thermodynamically favorable mechanism than SET-PT in all of the studied environments.

In the case of SPLET mechanism, the order of PA values in the studies environments is gas > ethanol > water for the same molecules. From the gas phase to solution, the PA values decrease drastically because of the relatively high enthalpies of proton and anion solvation. The average deviation between the gas phase and water is 298.3 kcal/mol. This means that the protonation process is more preferred to occur in polar solvents. In the gas phase, the calculated PAs are extraordinarily high compared to the BDE and IE values. On the other hand, in water, PAs are significantly lower than BDEs and IE values, meaning that SPLET is a more favorable mechanism than HAT and SET-PT. The antioxidant activity of the studied compounds can be ranked according to the lowest PA values in the following sequence: 2 > 3 > 1. Therefore, the substitution of the OH and OMe groups by two glucoses at the 3 and 5′ positions would increase the antioxidant ability of isorhamnetin via the SPLET mechanism in solution.

Based on the analysis above, we can draw conclusions that HAT is the most predominant reaction pathway in the gas phase and SPLET is the most preferred mechanism in water from the thermodynamic viewpoint.

2.2. Electron Spin Density and Atoms in Molecules (AIM) Analysis. To further gain an understanding of the stability of the radicals, the electron spin density and intramolecular hydrogen bonds were investigated by AIM analysis. Since these are other important factors that influence the radical-scavenging ability of studied compounds. The more stable the free radical, the stronger the antioxidative capability. Electron spin density can be used to explain the stability of formed radical species. The more extended the electronic delocalization, the more stable the formed radical.27,28

The data in Figure 2 shows that spin density distributions in the O3 favorable mechanism than SET-PT in all of the studied environments.

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The data in Figure 2 shows that spin density distributions in the O3 and O4′ sites of all of the investigated compounds are 0.310, 0.320, 0.322, and 0.340, corresponding to 2-O4′, 1-O3, 1-O4′, and 3-O4′ radicals, respectively. This shows that the stabilization of formed radicals decreases in the following sequence: 2-O4′ > 1-O3 > 1-O4′ > 3-O4′. Therefore, the formation of 2-O4′ radical is more favorable than other radicals.

In addition, the AIM topological analysis was selected to investigate the characteristics of intramolecular bond for the
formed radicals (Figure 3). These intramolecular hydrogen bond plays a more important role in stabilizing the radical. To evaluate the stabilization effect of the radical, the topological parameters of the bond critical points (BCPs) including electron density ($\rho$), Laplacian of the charge density ($\nabla^2\rho(r)$), local potential energy density ($V(r)$), local gradient kinetic energy density ($G(r)$), and total energy density ($H(r)$) are summarized in Table 2. According to the study of Rozas et al., the covalent nature of bonding in these systems is classified as a weak hydrogen bond because of the positive values of $\nabla^2\rho(r)$ and $G(r)$ and the negative values of $V(r)$ and $H(r)$. However, the weak interactions of the radical of the studied compounds include not only hydrogen bonding but also another interaction type called "tetrel bonding". The tetrel bonding is defined as a subtype of the simple electrostatic interaction. For instance, in the 3-O4' radical, no hydrogen bond is formed in which the O4'...BCP-C7' path is dictated by the topology of the electron density. Moreover, the sum of the $E$ values of the contacts are more negative, the radicals are more stable. Therefore, based on the total $E$, we can predict that the order of stability for radicals is 2-O4' > 1-O3 > 1-O4' > 3-O4', respectively. Substitution of hydroxyl and methoxyl groups at 3 and 5' sites by glucose has a significant impact on the stabilization of 2-O4' and thus the lower BDE.

### Table 2. Selected Parameters at the BCPs Presenting at Intramolecular Contacts for the Radicals of the Studied Compounds at the B3LYP/6-311G(d,p) Level

| contacts | $R$ (Å) | $\rho(r)$ (au) | $\nabla^2\rho(r)$ (au) | $G(r)$ | $V(r)$ | $G(r)/V(r)$ | $H(r)$ | $E$ (kcal/mol) |
|----------|---------|----------------|------------------------|--------|--------|-------------|--------|----------------|
| C6'−H−O3 | 2.0891  | 0.0225         | 0.0848                 | 0.0185 | -0.0158| 1.1709      | 0.0027 | -5.0          |
| C7'−O4'  | 2.7345  | 0.0156         | 0.0637                 | 0.0141 | -0.0122| 1.1557      | 0.0019 | -3.8          |
| C1″−H−O4' | 2.2012 | 0.0205         | 0.0792                 | 0.0173 | -0.0148| 1.1689      | 0.0025 | -4.6          |
| C2″−O4'  | 2.9175  | 0.0125         | 0.0484                 | 0.0106 | -0.0091| 1.1648      | 0.0015 | -2.9          |
| O2″−H−O4' | 2.4308 | 0.0098         | 0.0363                 | 0.008  | -0.0070| 1.1429      | 0.0010 | -2.2          |
| C7′−O4'  | 2.7316  | 0.0157         | 0.0641                 | 0.0141 | -0.0123| 1.1463      | 0.0018 | -3.9          |

Local gradient kinetic energy density. Local potential energy density. Total energy density. Individual energy of each intramolecular contact.

2.3. CH$_3$OO$^*$ Radical-Scavenging Capability. The previous studies showed that the contribution of the rate constants following the radical adduct formation (RAF) mechanism between phenolic compounds and ROO$^*$ radicals (i.e., HOO$^*$) into the overall rate constants was minor. Thus, in this study, our interest is focused on the investigation of the interaction of the CH$_3$OO$^*$ radical with the antioxidant molecules via the HAT mechanism. Based on the calculated BDE values, the weakest O=H bonds are found at O4' position of compounds 1, 2, and 3 and O3 position of compound 1. Thus, to better understand the H donation process, the potential energy surfaces were calculated for the reaction between CH$_3$OO$^*$ and the studied compounds (Figure 4). All structural optimizations were performed in the gas phase at the B3LYP/6-311G(d,p) level. Cartesian coordinates and molec-
the next step, the relative energies of the second intermediates named Int2-1-O3, Int2-1-O4’, Int2-2-O4’, and Int2-3-O4’ are predicted to be about 9.5, 6.0, 4.1, and 4.9 kcal/mol in comparison to the reactants. Finally, the products are formed with relative energies equal to 0.7, 0.5, 4.3, and 1.4 kcal/mol, respectively.

Moreover, activation ($\Delta G^\ddagger$) and reaction ($\Delta G$) Gibbs free energies and rate constants ($k$) calculated for the reaction between the studied compound and the CH$_3$OO$^\cdot$ radical are summarized in Table 3. Analyzing the data from Table 3 shows that the reactions of CH$_3$OO$^\cdot$ with the O4$^-$–H bonds of compounds 1 and 3 are not favorable due to the high activation Gibbs free energies at 13.8 and 14.7 kcal/mol, respectively, and the almost positive values of Gibbs energies ($\sim 0.13$ and $0.85$ kcal/mol). However, the reaction of 1-O3$^-$$H$ and 2-O4$^-$$H$ with CH$_3$OO$^\cdot$ following the HAT mechanism is supported by the lower $\Delta G^\ddagger$ values ($11.5$ kcal/mol) and negative $\Delta G$ values from $\sim 1.08$ to $\sim 4.05$ kcal/mol. It is noticing that the rate constants of the 1-O3$^-$$H$ + CH$_3$OO$^\cdot$ reaction ($k = 9.64 \times 10^4$ M$^{-1}$ s$^{-1}$) are lower than that of the 2-O4$^-$$H$ + CH$_3$OO$^\cdot$ reaction ($k = 3.61 \times 10^5$ M$^{-1}$ s$^{-1}$) despite of the same $\Delta G^\ddagger$ values of for both the reactions. That is because of the different tunneling corrections (the $\kappa$ for 1-O3$^-$$H$ site is 4.3, compared with 15.6 for the 2-O4$^-$$H$) (Table S3). In Table 3, it is clearly observed that the reaction between 2-O4$^-$$H$ and CH$_3$OO$^\cdot$ has the highest rate constant ($k = 3.61 \times 10^5$ M$^{-1}$ s$^{-1}$). Comparing with the typical antioxidant

Table 3. Activation ($\Delta G^\ddagger$) and Reaction ($\Delta G$) Gibbs Free Energies and Rate Constants ($k$) Calculated at the B3LYP/6-311G(d,p) Level of Theory at 298.15 K in the Gas Phase

| reactions                  | $\Delta G$ (kcal/mol) | $\Delta G^\ddagger$ (kcal/mol) | $k$ (M$^{-1}$ s$^{-1}$) |
|----------------------------|------------------------|---------------------------------|--------------------------|
| 1-O3$^-$$H$ + CH$_3$OO$^\cdot$ | $\sim 1.08$            | 11.5                            | $9.64 \times 10^4$      |
| 1-O4$^-$$H$ + CH$_3$OO$^\cdot$ | $\sim 0.13$            | 13.8                            | $5.24 \times 10^3$      |
| 2-O4$^-$$H$ + CH$_3$OO$^\cdot$ | $\sim 4.05$            | 11.5                            | $3.61 \times 10^3$      |
| 3-O4$^-$$H$ + CH$_3$OO$^\cdot$ | 0.85                   | 14.7                            | $1.45 \times 10^3$      |
| BHT + CH$_3$OO$^\cdot$         | $\sim 7.4$             | 10.2                            | $13.85 \times 10^3$     |

Figure 4. Potential energy surface of the reaction between the selected compounds and CH$_3$OO$^\cdot$ radical.

As can be seen in Figure 4, the tendency for all reaction paths of the studied compounds with CH$_3$OO$^\cdot$ is quite similar. This theoretical observation is quite coherent with several previous studies. The first intermediates named Int1-1-O3, Int1-1-O4’, Int1-2-O4’, and Int1-3-O4’ are formed and lying lower than the reactants by an amount of $\sim 2.9$, $\sim 1.6$, $\sim 5.8$, and $\sim 1.9$ kcal/mol, respectively. Then, the channels of reactions through transition states (TS) are characterized by relative energies of 1.9, 4.5, 2.1, and 5.0 kcal/mol, corresponding to TS-1-O3, TS-1-O4’, TS-2-O4’, and TS-3-O4’, respectively. In these transition states, the H atom of the O3/O4’–H bond is located approximately midway between the O3/O4’ and the O atom of the CH$_3$OO$^\cdot$ radical. The H···OCH$_3$ and O3/O4’···H distances are in the ranges of 1.21–1.31 and 1.09–1.17 Å, respectively (as shown in Figure 5). In the next step, the relative energies of the second intermediates...
butylated hydroxytoluene (BHT) \((k = 13.85 \times 10^5 \text{ M}^{-1} \text{ s}^{-1})\), the radical-scavenging efficiency of compound 2 is almost similar to that of the BHT. From the above analysis, the results propose that compound 2 has the highest free-radical-scavenging activity in the investigated compounds because of the lower Gibbs free energies and higher rate constants.

3. CONCLUSIONS

In this paper, thermodynamic and kinetic calculations were applied to explore the diglycoside substituent effect on the antioxidative capability of isorhamnetin extracted from *A. roxburghii*. Several conclusions are drawn as follows:

Thermodynamic descriptors including BDE, IE, PDE, PA, and ETE have been used to evaluate the radical-scavenging action of the studied compounds through three main antioxidant pathways (HAT, SET-PT, and SPLET) both in the gas phase and in solution. The achieved results prove that the HAT action is thermodynamically preferred in the gas phase and SPLET is more preferred in water.

The influence of the diglycoside substituent on the stabilization of radicals has been analyzed through electron spin density and intramolecular hydrogen bond. The glucose groups in 3 and 5 sites of isorhamnetin have greatly impacted the stabilization of 2-O4' radical and thus the lower BDE (75.3 kcal/mol in the gas phase).

The potential energy surfaces of reactions between the studied compounds and the CH3OO• radical have been investigated in detail. It is mentioned that reactions of CH3OO• into the O4'–H bonds of compound 2 are more favorable than other reactions with the lower Gibbs free energies \((-4.05 \text{ kcal/mol})\) and higher rate constants \((3.61 \times 10^5 \text{ M}^{-1} \text{ s}^{-1})\). Thus, this result suggests that compound 2 has the highest antioxidant activity in all of the studied compounds.

4. COMPUTATIONAL METHODS

In this study, all of the calculations were carried out using the Gaussian 09 software package.\(^{36}\) The geometry optimizations and the vibrational frequency calculations of the studied compounds and their radicals, cationic radicals, and anions were performed at the B3LYP/6-311G(d,p) level of the theory.\(^{37,38}\) The transition states, intermediates, and products of the reaction between the CH3OO• radical and the studied molecules were optimized and calculated at the same level of theory. The transition state for each reaction was confirmed by having single imaginary frequency. In addition, the intrinsic reaction coordinate (IRC) calculation was also performed to ensure each transition state connects to the expected reactant and product. To estimate the solvent effect on the enthalpies, the integral equation formalism of the polarizable continuum model was used for this system.\(^{39,40}\)

The thermodynamic parameters related to the three major radical-scavenging mechanisms were determined according to the following formula\(^{41,42}\)

\[
\begin{align*}
\text{BDE} &= H(\text{ArO}) + H(\text{H}) - H(\text{ArOH}) \\
\text{IE} &= H(\text{ArOH}^{*+}) + H(\text{e}^{-}) - H(\text{ArOH}) \\
\text{PDE} &= H(\text{ArO}^{*}) + H(\text{H}^{+}) - H(\text{ArOH}^{*+}) \\
\text{PA} &= H(\text{ArO}^{*}) + H(\text{H}^{+}) - H(\text{ArOH}) \\
\text{ETE} &= H(\text{ArOH}^{*}) + H(\text{e}^{-}) - H(\text{ArO}^{*})
\end{align*}
\]

where \(H(\text{ArOH}), H(\text{ArO}^{*}), H(\text{ArOH}^{*+})\), and \(H(\text{ArOH}^{*})\) denote the enthalpy of the neutral, radical, anion, and radical cation forms of flavonoids, respectively. The enthalpy of the hydrogen atom \((\text{H}^{+})\) was calculated at the same model chemistries. The enthalpy values for protons \((\text{H}^{+})\) and electrons \((\text{e}^{-})\) in the gas phase were taken from the literature.\(^{43-47}\) Proton and electron solvation enthalpies were calculated according to an approach recommended in previous studies.\(^{36}\) All reaction enthalpies defined in eqs 1–5 were calculated at 298.15 K and 1.0 atmosphere pressure.

All rate constants \((k)\) were estimated in the gas phase by the conventional transition state theory (TST) and 1 M standard state as

\[
k = \alpha_{k} \frac{k_{B}T}{h} e^{-\frac{(\Delta G^{\#})}{RT}}
\]

where \(k_{B}\) is the Boltzmann constant, \(T\) is the temperature, \(h\) is the Planck constant, \(R\) is the gas constant, \(\Delta G^{\#}\) is the Gibbs free energy of activation, \(\sigma\) is the reaction symmetry number that represents the number of different but equivalent reaction pathways that are possible, and \(\kappa\) accounts for tunneling corrections, which are calculated through the Wigner and Eckart approaches. All kinetic calculations were carried out with the Eyringpy code.\(^{51,52}\) The calculation of AIM analyses was carried out at the B3LYP/6-311G(d,p) level using the AIM2000 program.\(^{53}\)

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01780.

IRC plots for H-abstraction reactions; total energy requirements related to the SET-PT and SPLET mechanisms; Cartesian coordinates and molecular enthalpies of all parent molecules and resulted radicals and anions optimized at B3LYP/6-311G(d,p) level of theory; and data for rate constant calculations (PDF)

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### Notes

The authors declare no competing financial interest.

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