# Theoretical Methods Used in Elucidating Activity Differences of Phenolic Antioxidants

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**ABSTRACT:** The effectiveness of the existing theoretical methods used in prediction of antioxidant activity, including structure-activity relationships (SAR) and theoretical index, were evaluated. In most cases, it was found that the theoretical predictions were consistent with the experimental data. Although only one mechanism, free radical scavenging, was considered in the theories, the methods were valuable in predicting the activity of antioxidants to protect lipid oxidation.

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**KEY WORDS:** Antioxidant, flavonoids, ΔHOF value, phenols, structure-activity relationships.

Free radicals play an important role in food oxidation and can contribute to many diseases. Much attention has been paid to the selection of inexpensive antioxidants with low toxicity. Recently, preliminary structure-activity relationships (SAR) of phenolic antioxidants to scavenge free radicals have been achieved (1–3). It was found that many factors influenced the scavenging activity such as the location of OH, the property of substituting groups, and the hydrogen bond formation. A theoretical parameter, the difference of heat of formation between the phenolic antioxidant and its free radical produced after H-abstraction (ΔHOF), appeared to be a good index to measure the scavenging activity of antioxidants (1,4). Consequently, quantum chemistry calculations are helpful to investigate the activity differences of antioxidants. In fact, SAR for tocopherolic and flavonoid antioxidants have been explained successfully by theoretical calculations (1,3), and the activity differences of various phenolic antioxidants have been elucidated by using ΔHOF as an index (5–7). However, it must be pointed out that the aforementioned SAR and ΔHOF index characterize only the ability of antioxidants to scavenge free radicals. Other antioxidant mechanisms are also in use, such as chelating metals to reduce the Fenton reaction (8). Further studies are required to determine whether the theoretical methods including SAR and ΔHOF index are still effective to predict the activity of antioxidants in a complex system, e.g., in the experiments that show how antioxidants protect lipid oxidation. Therefore, activity differences of various antioxidants in the protection of lipid oxidation determined by Ogata *et al.* (9) were employed to evaluate the effectiveness of the existing theoretical methods, which might be helpful in order to further the study in this field and accelerate the selection of antioxidants.

## MATERIALS AND METHODS

SAR was used to give a preliminary elucidation of the differences of antioxidant activities and semiempirical method Austin Model 1(AM1) was employed to calculate the ΔHOF values. Former studies indicated that ΔHOF value was a good parameter to represent the activity of phenolic antioxidants in scavenging free radicals (1,4). The lower the ΔHOF, the more stable the semiquinonoid free radical was, and the more active the antioxidant.

The calculating procedure was as follows: Molecular mechanic method MMX in program PCMODEL (10) was used to optimize the molecular structures. The semiempirical method AM1 in MOPAC7 (11) was employed to perform a complete geometry optimization. AM1 was selected because it was found to be better than other semiempirical methods, such as Modified Neglect of Diatomic Overlap (MNDO) and Parametric Method 3(PM3), to calculate ΔHOF (4). Heat of formation of the parent molecules (HOF*m*) and the free radicals (HOF*f*) were obtained to calculate the index: ΔHOF = HOF*f* – HOF*m*. To make a comparison, parent molecules and free radicals were all calculated by Restricted Hartree Fock (RHF). ΔHOF values obtained by the RHF method were almost equal to those obtained from the Unrestricted Hartree Fock (UHF) after recalculation by one self-consistent field (SCF) Restricted Open Shell (ROHF) (1). Overall, the RHF method for calculating ΔHOF values was more self-consistent.

In the calculations, only the most stable conformations of antioxidants and their free radicals were taken into consideration. For instance, conformations possessing hydrogen bonds were selected in the calculations because hydrogen bonds stabilized the molecules in energy.

## RESULTS AND DISCUSSION

*Elucidation of the experimental results by means of SAR*. The existing SAR of antioxidants to scavenge free radicals indicate that: (i) the activities of OH in different positions are in the order

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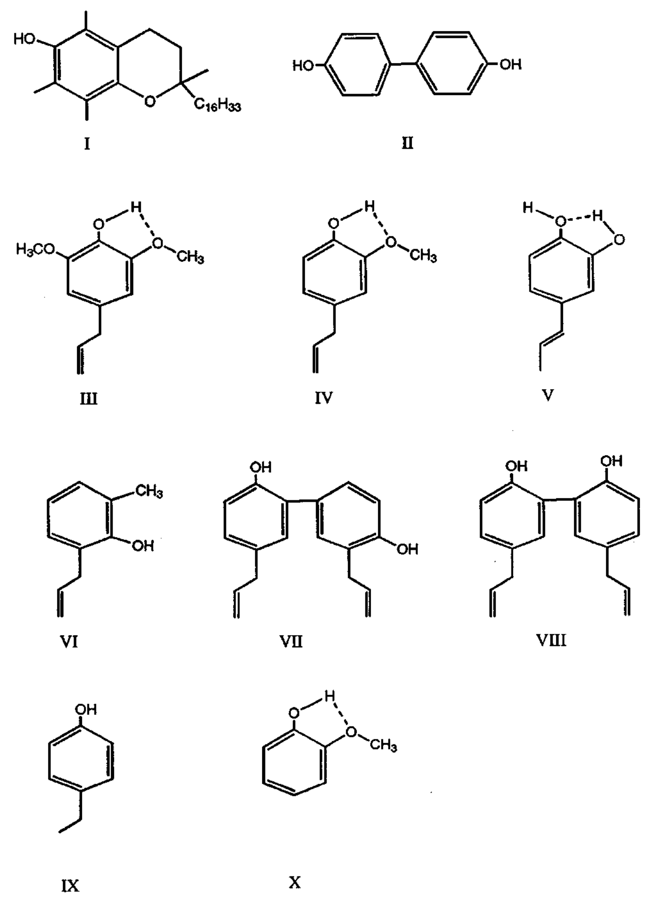

**FIG. 1.** The structures of phenolic antioxidants. In the calculations, $C_{16}H_{33}$ of molecule I was substituted with $CH_3$, as $C_{16}H_{33}$ had little influence on the scavenging activity.

pyrogallol > catechol > phenol > resorcinol (2,3,12); (ii) heteroatom, O or N, at the para or ortho position of OH will enhance the activity, owing to the resonance between the p-type lone pair of the heteroatom and the radical (1,13,14); (iii) electron-donating groups will increase the antioxidant activity while electron-attracting groups decrease the activity (1,3); (iv) hydrogen bonds formed in the antioxidants do not enhance the activity (1,6), but hydrogen bonds formed in the radical produced after H-abstraction will stabilize the radical and are favorable to increase the activity (3,5,6,12); (v) OH with two neighboring bulky groups, i.e., $-C(CH_3)_3$, will be overprotected, so it will be rather passive. But appropriate groups, i.e., $-CH_3$, in the neighboring positions can enhance the activity of OH (1).

From observation of the structures of 10 phenolic antioxidants (Fig. 1), one sees that molecules III, IV, and X all possess the heteroatom, oxygen, at the ortho position of OH. Consequently, they will be rather active in scavenging free radicals, and may be effective in protecting lipid oxidation. Molecule I has $-OCH_2-$ at the para position and two $-CH_3$ at the neighboring positions of OH. According to the SAR, molecule I will be an active radical scavenger. In addition, molecule V possesses catecholic OH, which will certainly result in high activity. Other molecules have no structural factors that enhance their activity markedly, so they will be rather passive in protecting lipid oxidation. The experimental results obtained by Ogata *et al.* (9) showed that molecules I, II, III, IV, and V were rather effective in protecting lipid oxidation, as their inhibitory effects all exceeded 50% (Table 1). However, molecules VI, VII, VIII, IX, and X were rather passive, since their inhibitory effects were all less than 40%. It is evident that the predictions on eight compounds are consistent with the experiments, except molecule II and X.

A similar analysis can be carried out on the flavonoids (Fig. 2). According to the SAR, epigallocatechin-gallate (EGCG) and epigallocatechin (EGC) will be the most active of the seven flavonoids because both of them possess pyrogallic OH. Baicalein has a pyrogallic OH, but it will be less active than EGCG and EGC owing to the electron-attracting property of the chromonoid ring that is conjugated with the pyrogallic ring. The third most active one will be catechin because of the catecholic OH. Similar to baicalein, baicalin will be less active than catechin owing to the electron-attracting property of chromonoid ring. Puerarin and wogonin will be the least active flavonoids, with wogonin being less active than puerarin because wogonin possesses many unfavorable factors: (i) possessing resorcinolic OH, (ii) forming hydrogen bonds before the radical is produced, and (iii) having chromonoid ring conjugated with resorcinol. In summary, the order of activities of the flavonoids may be EGCG ~ EGC > catechin > baicalein > baicalin > puerarin > wogonin. The predictions on the flavonoids are in good agreement with the experiments with the exception of baicalein and baicalin (Table 2).

Therefore, the existing SAR data are valuable in investigating and predicting activities of antioxidants to protect lipid

**TABLE 1**
**ΔHOF Values and Inhibition of Lipid Oxidation of the Phenolic Antioxidants**

| | I[e] | II | III | IV | V | VI | VII | VIII | IX | X |
|---|---|---|---|---|---|---|---|---|---|---|
| HOF*m* (kJ/mol)[a] | –380.83 | –167.92 | –344.81 | –203.05 | –249.20 | –72.09 | –72.11 | –62.21 | –148.84 | –250.71 |
| HOF*f* (kJ/mol)[b] | –253.09 | –13.98 | –211.09 | –56.38 | –120.46 | 79.11 | 87.12 | 97.65 | 3.48 | 101.61 |
| ΔHOF (kJ/mol)[c] | 127.74 | 153.94 | 133.72 | 146.67 | 128.74 | 151.20 | 159.23 | 159.86 | 152.32 | 149.10 |
| Inhibition (%)[d] | 97.2 | 81.7 | 78.8 | 63.2 | 53.7 | 38.7 | 32.7 | 21.6 | 14.8 | 6.6 |

[a] Heat of formation of parent molecule.
[b] Heat of formation of free radical produced after H-abstraction.
[c] HOF*f* – HOF*m*.
[d] Data are from Reference 9.
[e] Data are from Reference 4.

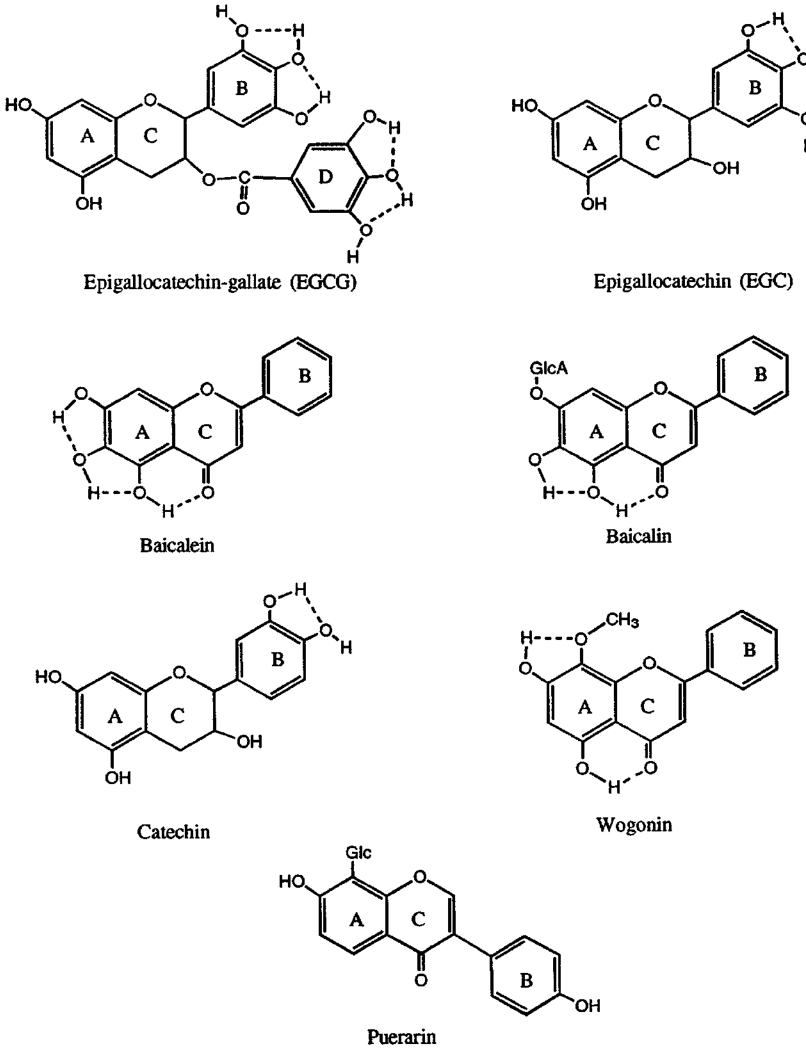

**FIG. 2.** The structures of flavonoid antioxidants. In the calculations, the glucuronate (GlcA) of baicalin and the glucose (Glc) of puerarin were substituted with $CH_3$, as the two groups had little influence on the scavenging activity.

oxidation.

*Elucidation of the experimental results by means of ΔHOF values.* ΔHOF values of 10 phenolic antioxidants (Fig. 1) were calculated (Table 1). For the molecules possessing two OH groups, such as molecules II, V, VII, and VIII, only the lower ΔHOF was considered. Table 1 shows that ΔHOF values of molecules I, III, IV, V, and X are less than 150 kJ/mol, and the values of other molecules are higher than 150 kJ/mol.

**TABLE 2**
**ΔHOF Values, HOMO Levels and Inhibition of Lipid Oxidation of the Flavonoid Antioxidants**

| | EGCG[f] | Baicalein | EGC[f] | Baicalin | Catechin[g] | Pueranin | Wogonin |
|---|---|---|---|---|---|---|---|
| HOF*m* (kJ/mol)[a] | –1586.03 | –528.66 | –1061.41 | –486.11 | –849.39 | –365.84 | –481.72 |
| HOF*f* (kJ/mol)[b] | –1461.64 | –391.59 | –934.10 | –348.07 | –719.30 | –212.36 | –322.41 |
| ΔHOF (kJ/mol)[c] | 124.39 | 137.07 | 127.31 | 138.04 | 130.09 | 153.48 | 159.31 |
| HOMO (ev)[d] | –9.1684 | –8.9670 | –9.0821 | –8.7130 | –8.9235 | –8.7425 | –8.7962 |
| Inhibition (%)[e] | 96.1 | 92.2 | 88.9 | 76.0 | 66.1 | 59.5 | 16.6 |

[a] Heat of formation of parent molecule.
[b] Heat of formation of free radical produced after H-abstraction.
[c] HOF*f* – HOF*m*.
[d] Highest occupied molecular orbital.
[e] Data are from Reference 9.
[f] Data are from Reference 7.
[g] Data are from Reference 3. Abbreviations: EGCG, epigallocatechin-gallate; EGC, epigallocatechin; for others see Table 1.

Hence, the former have higher scavenging activity and consequently have higher inhibitory effects on lipid oxidation. These results are consistent with the above results obtained by SAR, and are in agreement with the experimental data. In addition, the order of activities of molecules I, III, IV, VI, VII, and VIII is completely in line with the predictions by means of ΔHOF values (Table 1). The predictions on molecules II and X are inconsistent with the experiments, which indicates that consideration of only one mechanism cannot give a perfect prediction on the activity differences of antioxidants in complex systems. However, the prediction is helpful to obtain some qualitative conclusions on the antioxidant activities.

ΔHOF values of flavonoids were also calculated and only the lowest value of each molecules listed in Table 2. According to the SAR of flavonoid antioxidants (2,3), OH groups of ring B are their primary active site, thus only OH groups of ring B were considered in the calculation of EGC, catechin, and puerarin. OH groups in ring D of EGCG are less active than OH groups in ring B, owing to the electron-attracting property of -COO- that is conjugated with ring D. The results show that the lowest ΔHOF of each flavonoid is in the same order predicted by SAR. So, the calculations are also in concordance with the experiments. In addition, the order of ΔHOF of five flavonoids, EGCG > EGC > catechin > puerarin > wogonin, is in line with their activities, which indicates ΔHOF value is fairly helpful in predicting the protecting activities of flavonoid antioxidants. The higher than predicted antioxidant activities of baicalein and baicalin may result from their good lipid solubility, as the solubility of antioxidants can influence their activity to some extent. It has been found that in water systems the antioxidants possessing good water solubility were better than those possessing poor water solubility in scavenging free radicals (15). The experimental system of Ogata *et al.* (9) contained lipids, thus the good lipid solubility may enhance baicalein and baicalin antioxidant activity.

To investigate whether the antioxidant activity of flavonoids correlates with the redox potentials, highest occupied molecular orbital (HOMO) levels of the flavonoids were calculated by AM1 and listed in Table 2. We cannot find any relationship between the HOMO levels and the inhibitory effects. Thus for flavonoids, the protecting activity may not correlate with their electron donor activity, which is inconsistent with the suggestion of Ogata *et al.* (9).

Although only one mechanism, free radical scavenging, was considered, the existing theoretical methods including SAR and ΔHOF index were fairly effective to elucidate and predict the activity differences of antioxidants. However, for some antioxidants, such as molecule II, baicalein, and baicalin, the theoretical predictions are inconsistent with the experimental results, which indicates that the existing theoretical methods to predict the antioxidant activities in complex systems are still not perfect. The phenomenon arises from the fact that other mechanisms, such as metal chelating of the antioxidant or antioxidant solubility, may play a role in the process of protecting lipid oxidation. Also, a semiempirical method such as AM1 is not perfect for calculating ΔHOF. Hence, a comprehensive theoretical investigation and a high-level *ab initio* calculation are essential in this field.

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