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Theoretical elucidation of activity differences of five phenolic antioxidants[1]

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KEY WORDS antioxidants; ferulic acid; salvianic acid A; rutin; *L*-EGCG; paeonol; structure-activity relationship; computer-aided design

ABSTRACT

AIM: To verify the effectiveness of structure-activity relationship (SAR) and theoretical calculation methods for antioxidants. **METHODS**: Preliminary elucidation on the differences of activities of 5 antioxidants was performed by SAR. Then semiempirical quantum chemistry method AMI was employed to calculate the ΔHOF value, the difference between the heat of formation of antioxidant and its free radical, which was used as a theoretical parameter to elucidate the differences of activities of the antioxidants thoroughly. **RESULTS**: ΔHOF values of antioxidants were obtained as follows: ferulic acid, 150.58 kJ·mol^{-1}; anion of ferulic acid, 122.64 kJ·mol^{-1}; modified ferulic acid, 137.70 kJ·mol^{-1}; anion of modified ferulic acid, 118.99 kJ·mol^{-1}; salvianic acid, 134.17 kJ·mol^{-1}; rutin, 137.83 kJ·mol^{-1}; *L*-EGCG, 124.39 kJ·mol^{-1}; paeonol, 176.79 kJ·mol^{-1}. The differences of the antioxidant activities were elucidated, and how to further enhance the antioxidant activity was investigated as well. **CONCLUSION**: The SAR and calculation methods are rather effective to elucidate the differences of antioxidant activities, and present some new clues for structural modification of antioxidants to increase their activities.

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INTRODUCTION

Since free radicals play important roles in food oxidation and can induce many diseases, much attention have been paid on selecting cheap and low toxic antioxidants[1,2]. Preliminary structure-activity relationship (SAR) for phenolic antioxidants have been achieved[3,4]. Meanwhile, theoretical methods, such as quantum chemistry calculations, have been proven to be very efficient to predict the activities of antioxidants[3,5]. In fact, SAR for tocopherolic and flavonoid antioxidants have been explained successfully by theoretical results[3,6], and theoretical investigations on the difference between activities of xanthonoid and flavonoid antioxidants are also significant[7]. To verify further the effectiveness of SAR and theoretical methods, the experimental differences of activities of 5 phenolic antioxidants, such as ferulic acid (FA), salvianic acid A (SAA), rutin, *L*-epigallocatechin gallate (*L*-EGCG, main ingredient of green tea polyphenols), and paeonol was investigated and elucidated in this study.

MATERIALS AND METHODS

Firstly, SAR was used to give a preliminary elucidation of the differences of antioxidant activities. Secondly, semiempirical Quantum chemistry method AMI was employed to give an accurate calculation.

Former studies indicated that ΔHOF value, the difference of the heat of formation (HOF) between the mother molecule and its free radical, was the best parameter to represent the activity of phenolic antioxidants to scavenge free radicals[3,5]. The lower the ΔHOF, the more stable the semiquinonoid free radical was, and the more active the antioxidant.

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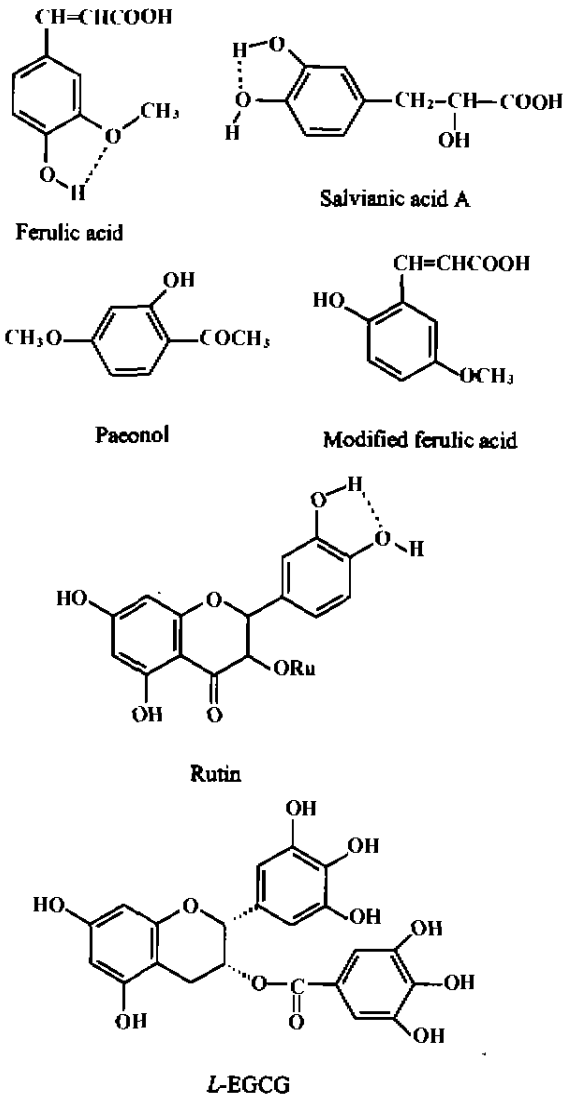

Structures of phenolic antioxidants.

Although ΔHOF was only a thermodynamic parameter, there was a good correlationship between ΔHOF and lgk_3/k_I: $lgk_3/k_I = 14.6491 - 0.0955\ \Delta HOF$, $r = 0.9491$[5], in which, k_3/k_I was relative rate constant of antioxidants to scavenge free radical of methyl linoleate. Therefore, we calculated ΔHOF values of aforementioned antioxidants and used the values to determine the antioxidant activities, since scavenging free radical directly through hydroxyl was the most important means in antioxidant mechanisms.

The procedure was as follows. Molecular mechanic method MMX[8] was used to optimize the molecular structures preliminarily, and then semi-empirical method AM1[9] was employed to perform a complete geometry optimization and calculate the parameter, because AM1 was better than other semiempirical methods such as MNDO and PM3 in calculating ΔHOF[5]. Heats of formation of the mother molecules and the states of removing phenolic H were obtained to calculate ΔHOF. Mother molecules and free radicals were both calculated by RHF (restricted Hartree Fock). ΔHOF values obtained by this method were almost equal to those calculated firstly by UHF (unrestricted Hartree Fock) and then calculated by a 1SCF restricted Open Shell (ROHF)[3]. But this method was easier to be self-consistent.

All calculations were carried out by an IBM PC with a pentium MMX 166MHz processor.

RESULTS AND DISCUSSION

Elucidation by SAR Experimental results indicated that FA was the most active one in 5 antioxidants, SAA, rutin, and *L*-EGCG were similar to one another, and the activity of paeonol was the lowest[10]. From the observation of the molecular structure, SAA, rutin, and *L*-EGCG all possess catecholic or pyrogallolic hydroxyls. Former experiments and theoretical calculations have all indicated that the two kinds of hydroxyls were very active to scavenge free radicals[4,6], besides, pyrogallolic –OH was more active than catecholic –OH[6]. The reason for the phenomenon is, firstly, that the semiquinonoid free radicals of the molecule produced after H-abstraction can be stabilized by forming an intramolecular hydrogen bond with –OH at *ortho* position (Fig 1-I). Secondly, it is because the semiquinonoid free radicals can form *o*-benzoquinonoid structure through resonance (Fig 1-II), which also can stabilize the radical in a certain degree. Obviously, paeonol has only one –OH, and its semiquinonoid free radical can not be stabilized by the two mechanisms above-mentioned. In this sense, its activity is the lowest. However, it's rather complicated to elucidate the high activity of FA. According to the existing theory[3], $-OCH_3$ at *ortho* position will stabilize the semiquinonoid free radical of FA, but the intramolecular hydrogen bond between –OH and $-OCH_3$, and the slight electron-attracting ability of –CH=CHCOOH will decrease FA scavenging activity on free radicals. So, if we only take the normal form of FA into consideration, the high activity of FA

becomes somewhat a puzzle. However, since FA displays acidity in solution, a part of FA will exist as anion (FA^-). According to the theory[3], the electron-donating property of $-CH=CHCOO^-$ probably increases the hydroxyl activity, but which still needs accurate calculation to verify.

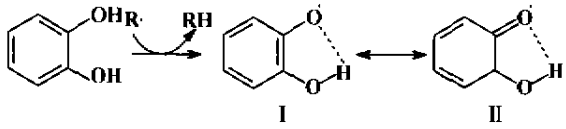

Fig 1. Stabilizing mechanisms of phenolic free radical.

Elucidation by AM1 calculation ΔHOF values of 5 antioxidants calculated by AM1 indicate that SAA, rutin, and *L*-EGCG are indeed much more active than paeonol, while FA is not active, which is consistent with the above conclusion drawn by SAR. However, ΔHOF value of FA^- decreases markedly, lower than that of any other antioxidants. Therefore, the scavenging activity of FA^- will be the highest, which is a verification of the above suggestion (Tab 1).

Tab 1. ΔHOF values and k_3/k_1 of antioxidants.

	Mother molecules HOF* /kJ·mol^{-1}	Free radicals HOF/kJ·mol^{-1}	ΔHOF /kJ·mol^{-1}	k_3/k_1†
FA	−569.86	−419.28	150.58	1.86
FA^-	−652.12	−529.48	122.64	864.93
SAA‡	−889.00	−754.83	134.17	68.53
Rutin‡,§	−840.98	−703.15	137.83	30.64
L-EGCG‡	−1586.03	−1461.64	124.39	588.65
Paeonol	−418.76	−241.97	176.79	0.01
MFA	−563.08	−425.38	137.70	31.53
MFA^-	−635.24	−516.25	118.99	1929.99

*HOF is abbreviation of heat of formation. † k_3/k_1 is relative rate constant for scavenging free radical of methyl linoleate, and is calculated by the equation: $\lg k_3/k_1 = 14.6491 - 0.0955$ ΔHOF. ‡ For SAA, rutin, *L*-EGCG, only the lowest ΔHOF values are listed. § Data of rutin is from ref[20].

Due to the excellent property displayed by FA, it is interesting and significant to investigate further the factors that influence FA activity. Firstly, effect of forming intramolecular hydrogen bond on the activity is to be discussed. Calculations on the two states, forming the hydrogen bond or not (Fig 2-I, II), show

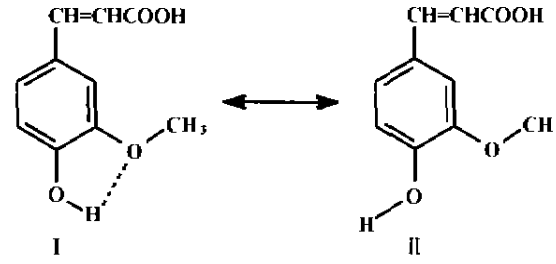

Fig 2. Two conformations of ferulic acid.

that HOF of conformation II is −555.69 kJ·mol^{-1}, which is 14.17 kJ·mol^{-1} higher than that of conformation I. As the difference of HOF between the two conformations mainly arises from forming hydrogen bond, the bond energy can be estimated to be 14.17 kJ·mol^{-1}. So, forming hydrogen bond will raise the ΔHOF value approximately 14.17 kJ·mol^{-1}. Secondly, an evaluation of the effect of $-OCH_3$ is to be carried out. According to the theory[3], $-OCH_3$ is helpful to stabilize semiquinonoid free radical at *ortho* or *para* position. For instance, the oxygen of six-member ring of vitamin E (V_E) can stabilize the semiquinonoid free radical at *para* position, because p-type lone pair of the oxygen overlaps with the SOMO (singly occupied molecular orbital) of the radical, and the radical can be stabilized through resonance[3]. Calculations indicate that methoxyl oxygens of radicals of FA and FA^- possess fairly high spin density distribution, 0.067 and 0.040 respectively, suggesting that $-OCH_3$ indeed stabilizes the free radical. Comparing the structure, we find that FA is similar to paeonol, but the latter has no $-OCH_3$ at *para* or *ortho* position, so the later ΔHOF value is 26 kJ·mol^{-1} higher than the former. Therefore, if −OH of FA is changed to *para* position of $-OCH_3$, the stabilizing effect of $-OCH_3$ is retained and the formation of hydrogen bond is avoided, so it is possible for FA to increase its activity further. Results of calculation on the modified FA (MFA, Fig 1) show that its ΔHOF value is 12.88 kJ·mol^{-1} lower than that of FA, and ΔHOF of MFA anion (MFA^-) is also 3.65 kJ·mol^{-1} lower than that of FA^-, indicating MFA is more active than FA to scavenge free radicals.

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The distributions of spin density on methoxyl oxygens of MFA and MFA^- radicals are 0.069 and 0.040 respectively, similar to the values of FA and FA^-, which suggests the stabilizing effect of $-OCH_3$ on the semiquinonoid free radical is slightly related to whether $-OH$ is at *para* or *ortho* position. Therefore, ΔHOF of MFA 12.88 $kJ \cdot mol^{-1}$ lower than that of FA mainly arises from the fact that there is no hydrogen bond formed in MFA. In fact, the difference between ΔHOF values of MFA and FA is close to the hydrogen bond energy.

The SAR and calculation methods for antioxidants are rather effective to elucidate the differences of antioxidant activities, and present some new clues for structural modification of antioxidants to increase their activities.

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363~366

五种酚类抗氧化剂活性差异的理论解释[1]

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关键词 抗氧化剂; 阿魏酸; 丹参素; 芦丁; *L*-EGCG; 芍药酮; 结构-活性关系; 计算机辅助设计

目的: 验证目前关于抗氧化剂的结构-活性关系及理论计算方法的有效性. **方法**: 首先用结构-活性关系初步解释五种抗氧化剂的活性差异, 其次用半经验量子化学方法 AM1 计算抗氧化剂与其自由基生成热的差值(ΔHOF 值), 并以此为理论指标深入阐释五种抗氧化剂的活性差异. **结果**: 计算得出几种抗氧化剂的 ΔHOF 值为: 阿魏酸, 150.58 $kJ \cdot mol^{-1}$; 阿魏酸负离子, 122.64 $kJ \cdot mol^{-1}$; 阿魏酸修饰物, 137.70 $kJ \cdot mol^{-1}$; 阿魏酸修饰物负离子, 118.99 $kJ \cdot mol^{-1}$; 丹参素, 134.17 $kJ \cdot mol^{-1}$; 芦丁, 137.83 $kJ \cdot mol^{-1}$; *L*-EGCG, 124.39 $kJ \cdot mol^{-1}$; 芍药酮, 176.79 $kJ \cdot mol^{-1}$. 据此解释了它们抗氧化活性的差别, 并探讨了如何进一步提高其抗氧化活性. **结论**: 现有的关于抗氧化剂的结构-活性关系和理论研究方法可以较好地解释实验现象, 并可为其结构修饰, 进一步提高抗氧化活性提供指导.

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