一种新型细梗胡枝子黄酮类提取物的结构和抗氧化活性

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摘要: 利用密度泛函理论的 B3LYP 交换相关泛函对从细梗胡枝子中提取的一种新型黄酮类化合物的分子结构和抗氧化活性进行了研究, 获得了该化合物的中性分子、阴离子、自由基和自由基阳离子的稳定几何构型和能量. 通过分析前线分子轨道特征, 确定了与实验结果一致的现象: A 环是参加化学反应的活性部位, 并发现 A' 环也是重要的抗氧化活性部位. 为判断其抗氧化活性, 预测其水溶液中, 中性和阴离子的电离势分别为 509.0 和 432.2 kJ·mol⁻¹, 均裂 O—H 键解离能为 347.3 kJ·mol⁻¹, 羟基自由基电子亲和势和氢原子亲和势分别为 -620.6 和 -487.5 kJ·mol⁻¹. 通过理论分析比较, 该黄酮类化合物清除羟基自由基的三种机理即 H 原子转移、电子转移-质子转移和质子丢失-电子转移在热力学上并存, 其中质子丢失-电子转移是热力学最有利的机理. 本文为设计新型高效黄酮类抗氧化剂, 研究黄酮类化合物的构效关系和抗氧化机理提供了理论依据.

关键词: 密度泛函理论; 细梗胡枝子黄酮类提取物; 键解离能; 电离势; 抗氧化活性

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Structures and Antioxidant Activities of Lespedezaflavonone from *Lespedeza Virgata*

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Abstract: The molecular structures and antioxidant activities of a novel flavonoid-type compound (lespedezaflavonone) from *Lespedeza virgata* were studied using density functional theory (DFT) with the B3LYP exchange correlation functional. The optimized geometries of neutral, radical cation, radical, and anion forms of lespedezaflavonone were obtained. Ring A was found to be responsible for the high activity of the flavonoids by an analysis of the character of the frontier molecular orbital, which was consistent with what was observed experimentally. Furthermore, it was noteworthy that ring A' was firstly found to be the important part for the potent antioxidant activity of lespedezaflavonone. To quantify the antioxidant activities, we determined the adiabatic ionization potential (IP, 509.0, 432.2 kJ·mol⁻¹ for the neutral and anion forms, respectively), the homolytic O—H bond dissociation enthalpy (BDE, 347.3 kJ·mol⁻¹) for lespedezaflavonone, the adiabatic electron affinity (EA, -620.6 kJ·mol⁻¹) and the H-atom affinity (HA, -487.5 kJ·mol⁻¹) for hydroxyl radical in aqueous solution. Our theoretical analysis shows that H-atom transfer, stepwise electron-transfer-proton-transfer, and sequential proton-loss-electron-transfer mechanisms for lespedezaflavonone to scavenge hydroxyl radical may occur thermodynamically in parallel, while the last process is the most favorable. These findings are helpful for further study on the design of novel efficient flavonoid-type antioxidants, the structure-activity relationship and the antioxidant mechanism of flavonoid-type compounds.

Key Words: Density functional theory; Lespedezaflavonone; Bond dissociation enthalpy; Ionization potential; Antioxidant activity

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Lespedeza species (Leguminosae), which distributed in East Asia and North America, have been used as folk medicine for the treatment of nephritis, azothemia, and diuresis^[1]. Many low-toxic antioxidants have been isolated, including stilbenoids, flavonoids, and other phenolic compounds^[2-6]. *Lespedeza virgata* (Thunb.) DC., a Chinese herb, is used in treatment of various nephropathies in central China. Potent antioxidants with low toxicity are desired to be used as food additives and medical substances. Thus interests in search for potent antioxidants from *Lespedeza virgata* have start up since 2007^[6-10].

Recently, a novel flavonoid-type compound 5-hydroxy-6-methyl-2'-methoxy-[6,6''-dimethylpyrano(2'',3'':7,8)]-[6''',6'''-dimethylpyrano(2''',3''':4',5')](2S) flavonone (lespedezaflavonone, Fig.1) from *Lespedeza virgata* was isolated and identified by Liu's group^[6]; and lespedezaflavonone is found experimentally to have the strongest antioxidant activity among the flavonoids extracted from *Lespedeza virgata*.

The previous experimental studies have contributed positively to the search for potent antioxidants from *Lespedeza virgata*^[6-10]. Nevertheless, it is worth mentioning that no study has provided structure information, the antioxidant activity mechanism, and structure-activity relationship of the new flavonoid-type compound.

Flavonoid is a group of polyphenolic compounds which can be found in seeds, fruits, tea, vegetables, and green leaves^[11-14]. They execute a wide range of biological effects, such as antibacterial, antiviral, anti-inflammatory, antiallergenic, and vasodilatory actions^[15-17]. Based on the predicted physico-chemical properties, some theoretical studies on the antioxidant activities of flavonoids were done^[11,18-23].

This paper focuses on the molecular structures and antioxidant activities of lespedezaflavonone. The character of the frontier molecular orbital was analyzed; and BDE and IP values for lespedezaflavonone and EA and HA values for the scavenged hydroxyl radical in aqueous solution were predicted. On the basis of the values, discussion was then made about the hydroxyl radical scavenging mechanisms of lespedezaflavonone. With the current work we hope to highlight the potent antioxidative activities of compounds from *Lespedeza virgata* and stimulated the interest for further studies and exploitation in food and pharmaceutical industries of extracts in *Lespedeza virgata*.

1 Computational details

All calculations were performed by the Gaussian 03 suite of

Fig.1 Structure of lespedezaflavonone from *Lespedeza virgata*

programs^[24]. Conformation analyses and geometry optimizations were carried out using the B3LYP hybrid density functional^[25-26] with the 6-31g(d) basis set. The optimized structures were confirmed as true minima by vibrational analysis at the same level. Frequency calculations were carried out and performed on all of the species to confirm convergence to appropriate local minima of the energy surface and to extract zero-point, enthalpy and entropy corrections. Single-point electronic energies (SPEs) were then calculated at the B3LYP/6-311++g(2df,2p) levels to the most stable conformer. Next SPEs were converted to the enthalpy values at 298 K, 101329 Pa, by adding the thermal contributions to enthalpy (TCE, in which the vibrational contributions include zero-point vibrational energy). The B3LYP/6-311++g(2df,2p)/B3LYP/6-31g(d) method was successfully used for the study on the radical scavenging activity of maritimetin and related aurones by Nenadis's group^[27].

Finally, the O—H BDE^[28-29], IP^[30-31], EA, and HA can be computed using Eqs.(1-4), respectively.

$$\text{BDE} = H_{A\cdot} + H_{H\cdot} - H_{AH} \quad (1)$$

$$\text{IP} = E_{AH^{+}} - E_{AH} \text{ or } \text{IP} = E_{A\cdot} - E_{A^{-}} \quad (2)$$

$$\text{EA} = E_{RO^{-}} - E_{RO\cdot} \quad (3)$$

$$\text{HA} = H_{ROH} - H_{H\cdot} - H_{RO\cdot} \quad (4)$$

where, BDE is homolytic bond dissociation enthalpy. $H_{A\cdot}$, H_{AH} and $H_{H\cdot}$ are the enthalpies of radical, neutral of an antioxidant, and hydrogen radical in Eq.(1), respectively. In Eq.(2), IP is the adiabatic ionization potential; E_{AH} , $E_{AH^{+}}$, $E_{A^{-}}$, and $E_{A\cdot}$ are the energies of neutral, cation free radical, anion, and radical of an antioxidant, respectively. In Eq.(3), EA is adiabatic electron affinity; $E_{RO^{-}}$ and $E_{RO\cdot}$ are the energies of anion and radical of a scavenged radical, respectively. In Eq.(4), HA means H-atom affinity; H_{ROH} and $H_{RO\cdot}$ are the enthalpies of neutral and radical of a scavenged radical. Solvent effects were computed on the single-point level by using the integral equation formulation-polarized continuum model IEF-PCM^[32] and the united atom for Hartree-Fock (UAHF) cavity model in aqueous solution ($\epsilon=78.39$).

2 Results and discussion

2.1 Conformational analysis and molecular geometries

The behavior of the hydroxyl group is largely influenced by the neighboring groups and the geometry. Thus, the conformation can be regarded as the first important parameter to analyze the antioxidant capacity of lespedezaflavonone. The optimized structures of the most stable conformers for neutral, radical cation, radical, and anion forms generated from lespedezaflavonone are shown in Fig.2.

The most stable conformers of neutral and radical cation are those possessing intramolecular hydrogen bonds (IHBs). In global minimum structures of neutral and radical cation molecules, the hydroxyl group in ring A can form a hydrogen bond with the keto group in ring C *via* a six-membered ring. The rings A, A', and C are planar in the global minimum structures of four molecules. The rings B and B' are planar in the most stable con-

Fig.2 The most stable conformers of neutral, radical cation, radical, and anion forms of lespedezaflavonone

formers of four molecules. The two planes are almost perpendicular. The coplanarity of the rings A and C could increase the antioxidant activity of lespedezaflavonone which is consistent with the previous studies^[11,18].

2.2 Character of the frontier molecular orbital

According to Fukui's frontier molecular orbital theory^[33-34], the

highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) play fundamental roles in the interpretation of chemical reactivity. The contours of HOMO and LUMO for neutral and anion forms of lespedezaflavonone, are displayed in Fig.3.

From Fig.3, it is observed that the HOMO electron density

Fig.3 HOMO and LUMO for neutral and anion forms of lespedezaflavonone

concentrated in rings A and A' and the LUMO electron density focused on rings A, A', and C for neutral molecule of lespedezaflavonone. These results show that ring A is responsible for the high activity for flavonoid-type compound, which is consistent with what is observed in previous studies^[18,35–36]. Furthermore, it is noteworthy that ring A' is firstly found to be the important part for the potent antioxidant activity of flavonoid-type compound. The atomic orbital distributions of ring A' in HOMO and LUMO are 51.7% and 20.5%, respectively, which also show that ring A' is the significant part for the potent radical scavenging activity.

The HOMO electron density concentrated in rings A and A' and the LUMO electron density focused on rings B, and B' for anion form of lespedezaflavonone (see Fig.3). It can be inferred that rings A, A', B and B' are responsible for the antioxidant activity of lespedezaflavonone in polar solvent, which is in agreement with the previous results^[18,35–36]. In view of the obtained results it is of interest to design new flavonoid-type compound with enhanced activity.

2.3 IP and O—H BDE for lespedezaflavonone, EA and HA for hydroxyl radical

As we know, there are at least three mechanisms reported for the radical scavenging processes of antioxidants (AH) by donating H-atom in physiological systems^[37–41]: (1) one-step H-atom transfer (HAT) (Eq.(5)); (2) stepwise electron-transfer-proton-transfer (ET-PT) (Eq.(6)); (3) sequential-proton-loss-electron-transfer (SPLET) (Eq.(7)).

All of them may occur in parallel, but with different rates in a certain chemical or biological system^[38,41]. In mechanism (1), the reactivity of an AH can be estimated by calculating the O—H BDE^[38,42], where the lower the BDE value, the higher the expected activity. IP of AH is the controlling parameter in mechanisms (2) and (3)^[38]. Molecules with the low IP values are expected to have high activity. Furthermore, the antioxidant activity is also governed by solvents, structure characteristics of the scavenged radicals, and the capacity of electron affinity and H-atom affinity^[38,43]. For radicals with high HA, the former mechanism is preferred, while for radicals with high EA, the latter two mechanisms are favored. In addition, the radicals are scavenged by antioxidant in biological system. The solvation effect of aqueous solution can not be ignored. Thus, in the present study BDE and IP values for lespedezaflavonone, EA and HA values for the scavenged radicals (hydroxyl radical) in aqueous solution are used to elucidate the radical scavenging activity of lespedezaflavonone.

The homolytic O—H BDE, IP for lespedezaflavonone in aqueous solution were calculated. The calculated BDE is 347.3 kJ·mol⁻¹. The BDE is 23.7 kJ·mol⁻¹ lower than the experimental

data of phenol^[44]. The lower the BDE values, the easier the H atom abstraction. It is obviously that the H atom in the hydroxyl group of lespedezaflavonone is easier to be abstracted than that of phenol.

The adiabatic IP for neutral and anion form are 509.0 and 432.2 kJ·mol⁻¹, respectively. HA and adiabatic EA for hydroxyl radical in aqueous solution were also computed. The data are -487.5 and -620.6 kJ·mol⁻¹. The IP of lespedezaflavonone is lower than those widely used food synthetic additives such as butylated hydroxyanisole, nordihydroguaiaretic acid, and propyl gallate (638.3, 668.8, 701.4 kJ·mol⁻¹, respectively)^[37], and the natural polyphenolic flavonoid epigallocatechin-3-gallate (617.4 kJ·mol⁻¹)^[45], one of the most active antioxidant obtained from green tea. Apparently lespedezaflavonone seems to donate an electron easily, comparable to the activity of some food additives.

2.4 Antioxidant mechanisms

According to the results of Zhang *et al.*^[38], the mechanism dominating the antioxidant activity of an antioxidant can be inferred by the sum value of its BDE and HA of scavenged radicals and the sum value of its IP and EA of with scavenged radicals. If the sum value of the former is positive, HAT is thermodynamically forbidden. Otherwise, HAT is thermodynamically permitted. Likewise, if the sum value of the latter is positive, SPLET or ET-PT is permitted. Otherwise, SPLET or ET-PT is not permitted.

From the above results, it is observed that the O—H BDE of lespedezaflavonone, is 140.2 kJ·mol⁻¹ lower than the absolute HA of hydroxyl radical, which suggests that HAT is thermodynamically permitted in the scavenging of hydroxyl radical by lespedezaflavonone. The adiabatic IP of lespedezaflavonone and its anion are 111.6, 188.4 kJ·mol⁻¹ lower than absolute EA of hydroxyl radical. The sum values are both negative. It can be inferred that the electron transfer between neutral, anion, and hydroxyl radical are both thermodynamically permitted. The thermodynamically favored hydroxyl radical scavenging mechanisms for lespedezaflavonone are in the order of: ET-PT < HAT < SPLET since the sum data are 111.6, 140.2, 188.4 kJ·mol⁻¹, respectively.

Overall, three antioxidant mechanisms for lespedezaflavonone to scavenge hydroxyl radical may occur thermodynamically in parallel. Therein, SPLET is the most preferred one for lespedezaflavonone to scavenge hydroxyl radical in thermodynamics.

3 Conclusions

Using the B3LYP/6-311++g(2df,2p)/B3LYP/6-31g(d) method, the molecular structures and antioxidant activities of lespedezaflavonone were studied. The optimized geometries and energies of neutral, radical cation, radical, and anion forms from lespedezaflavonone were obtained. The ring A and A' are responsible for the high activity for flavonoid-type compound through the analysis of the character of the frontier molecular or-

bital. Then, IP (509.0, 432.2 kJ·mol⁻¹ for neutral and anion forms) and homolytic O—H BDE (347.3 kJ·mol⁻¹) for lespedezaflavonone and EA (−620.6 kJ·mol⁻¹) and HA (−487.5 kJ·mol⁻¹) for hydroxyl radical in aqueous solution were determined. Our theoretical analysis indicates that three antioxidant mechanisms for lespedezaflavonone to scavenge hydroxyl radical may occur thermodynamically in parallel and SPLET is the most favorable one. These findings are helpful in the further study on the design of novel efficient flavonoid-type antioxidants, the structure-activity relationship and the antioxidant mechanism of flavonoidtype compounds.

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