A DFT Computational Study of the Antioxidant Activities Exhibited by 3-aryl-4-hydroxycoumarin Derivatives

Research Article

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Abstract

Density functional theory (DFT) was employed to obtain energy (E), ionization potential (IP), bond dissociation enthalpy (BDE) of O-H bond and stabilization energy (ΔE_{iso}) in order to infer the scavenging activity of 3-phenyl-4-hydroxy coumarin derivatives. Spin density calculations were also performed for the proposed antioxidant activity mechanism. The unpaired electron formed by the hydrogen abstraction from the phenolic hydroxyl group of 3-phenyl-4-hydroxy coumarin derivatives localized on the phenolic oxygen at positions 4 and 3', the C_3 carbon, the $C_{2'}$ and $C_{6'}$ carbon atoms at ortho positions and $C_{4'}$ carbon atom at para position. The lowest phenolic oxygen contribution corresponded to the highest scavenging activity value. It was found that antioxidant activity depends on the presence of a phenyl group at position 3 of coumarin skeleton and presence of a hydroxyl at the C_4 and $C_{3'}$. In addition, the presence of chlorine atom at position 6 on the coumarin skeleton leads to decrease O-H BDE and increase antioxidant activity significantly. There is a correlation between IP and O-H BDE and the capacity of scavenging peroxy radicals and the percentage of the hydroxyl radical scavenging activity.
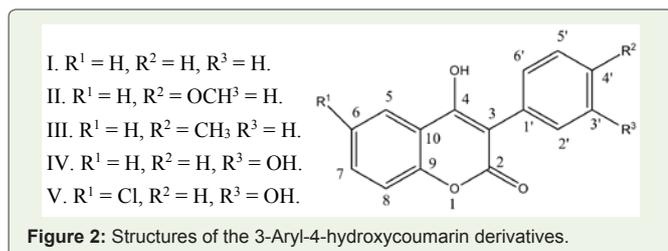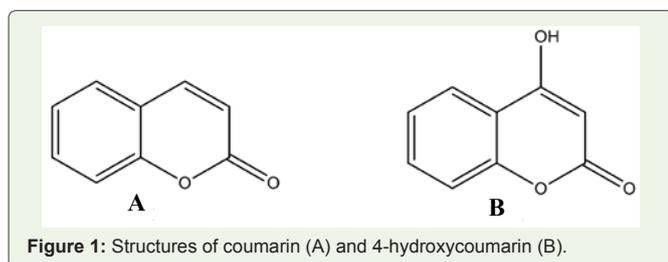
Key words: Hydroxycoumarin; Scavenging activity; Antioxidant activity

Introduction

Coumarins (known as 1,2-benzo pyrones) comprise an important group of low molecular weight phenolics that have been widely used for prevention and treatment of many diseases [1], Figure 1 shows the structure of the parent compound, coumarin." Coumarins possess anti-inflammatory, antioxidants, anticancer, and antiviral activities. Several recent reviews summarize advances in the application of coumarins, particularly concentrating their antioxidant properties [1]. The hydroxy coumarins, which are derivatives of the parent compound coumarin (Figure 1), are phenolic compounds known for acting as potent metal chelators and free radical scavengers. Hydroxy coumarins have attracted intense interest in recent years since they exhibit diverse pharmacological properties. Notable among these properties are their antioxidant effects that were extensively examined [1], and 3-aryl-4-hydroxycoumarin derivatives that proved to be good antioxidants [2,3].

DFT calculations were used in studying the antioxidant activities of many phenolic compounds such as flavonoid [4,5] as well as dihydrochalcone derivatives [6]. In contrast, very few studies have been reported on using DFT calculations to study the antioxidant activity of coumarins [7], and their derivatives such as 4-methyl coumarins [8] and some 4-hydroxy coumarin derivatives [9]. A brief computational study of the O-H bond dissociation energy of some 3-aryl-4-hydroxycoumarin derivatives was recently conducted [2].

Up to our knowledge, there is no computational study on antioxidant activity of 3-aryl-4-hydroxycoumarin derivatives.In this work, quantum mechanical calculations at the B3LYP/6-31G (d,p) [10-24] level of theory shall be employed to obtain the energies (E), ionization potentials (IP) in the gas phase and implicit water solvent using the polarized continuum model (PCM), the bond dissociation enthalpy (BDE) of O-H bond and the stabilization energies (ΔE_{iso}) of several 3-aryl-4-hydroxycoumarins as shown in Figure 2. The six

Figure 1: Structures of coumarin (A) and 4-hydroxycoumarin (B).

I. $R^1 = H$, $R^2 = H$, $R^3 = H$.

II. $R^1 = H$, $R^2 = OCH^3 = H$.

III. $R^1 = H$, $R^2 = CH_3$ $R^3 = H$.

IV. $R^1 = H$, $R^2 = H$, $R^3 = OH$.

V. $R^1 = Cl$, $R^2 = H$, $R^3 = OH$.

Figure 2: Structures of the 3-Aryl-4-hydroxycoumarin derivatives.

3-aryl-4-hydroxycoumarin derivatives (compounds I through V as shown in Figure 2) were studied, in addition to 4-hydroxycoumarin (Figure 1) as a reference. The corresponding values of these were utilized for the purpose of evaluating their relative scavenging activities. Calculations of the spin densities of these derivatives were also performed with the intent of exploring the stability of formed free radicals.

Computational Methods

Geometry optimization of the coumarin derivatives was carried out using density functional theory (DFT). It offers an excellent compromise between computational time and description of electronic correlation. The calculations were performed utilizing the Gaussian09 (G09) Quantum package [25]. Prior to any DFT calculations, all proposed structures were submitted to PM3 geometry conformational search. After the PM3 initial optimization, the conformer with the lowest electronic energy was selected. And then the structure was re-optimized with the B3LYP hybrid density functional utilizing the 6-31G(d,p) basis set. This level of theory was used previously to investigate antioxidant activity of various compounds [6,7]. The B3LYP optimized structure was ascertained to conform to a real minimum utilizing frequency calculation (no imaginary frequency). The radicals were treated as open shell systems. The OH bond dissociation enthalpies (**BDE**), for hemolytic O-H bond cleavage in the gas phase at 298.15 K was calculated using the sum of electronic and thermal enthalpies according to

$$BDE\ (OH) = Hr + H - Hp$$

Where **Hr** is the enthalpy of the radical resulting from hydrogen atom abstraction, **H** is the enthalpy of hydrogen atom (-0.49765 Hartrees same as reference [6]), while **Hp** is the enthalpy of the parent molecule.

The ionization potential (**IP**) in the gas phase and in implicit water was estimated using the polarized continuum model (CPCM). It was calculated as the enthalpy difference between a radical cation (**Hc**) and the respective parent molecule (**Hp**)

$$IP = Hc - Hp$$

The radical stability was determined through the calculations of the stabilization energy (ΔE_{iso}) (as shown below), in which it represents the hydrogen transfer in the coumarin. The coumarin derivatives are represented by **ArOH** while the phenol molecules are represented by **PhOH**, according to

$$\Delta E_{iso} = [ArO^\bullet] + [PhOH] - [ArOH] + [PhO^\bullet]$$

Results and Discussions

Figure 3 shows the optimized structures of 3-Aryl-4-hydroxycoumarin derivatives at B3LYP/6-31G(d,p) level of theory. The stabilization energy (ΔE_{iso}) is used as a simple yet very useful method for predicting the ability of antioxidants to trap free radicals of phenolic derivatives, which was previously utilized to study the ability of several phenolic compounds [6]. The stabilization energies of the phenoxy radicals were calculated and the values of ΔE_{iso} are shown in Table 1. From this table, we conclude that the presence of hydroxyl group on the 3'-phenyl ring increases the ΔE_{iso} due to the fact that more oxygen atoms of the phenolic hydroxyl groups can donate electrons to stabilize the semiquinone form. In addition, the presence of chlorine atom at position 6 on the coumarin skeleton of

Table 1: Stabilization energies of phenoxy radicals relative to phenol. The position of the most stable hydroxyl radical is given in parenthesis. The energies were calculated at B3LYP/6-31G(d,p) level of theory.

Compound	Energy of Parent Compound (Hartrees)	Energy of Radical (Hartrees)	ΔE_{iso} kcal/mol
phenol	-307.473071	-306.8353848	0.00
4-hydroxy coumarin	-572.2264418	-571.56559	14.54
I	-803.3117541	-802.6492861	15.55
II	-917.8326438	-917.17148	14.73
III	-842.6320917	-841.9701245	15.24
IV-OH(4)	-878.5315122	-877.8685027	15.89
IV-OH(3')	-878.5315122	-877.8761878	11.07
V-OH(4)	-1338.052429	-1337.421799	-4.43
V-OH(3')	-1338.052429	-1337.391787	14.41

Table 2: Ionization potentials (IP) and bond dissociation energies (BDE) for 3-aryl-4-hydroxycoumarin derivatives.

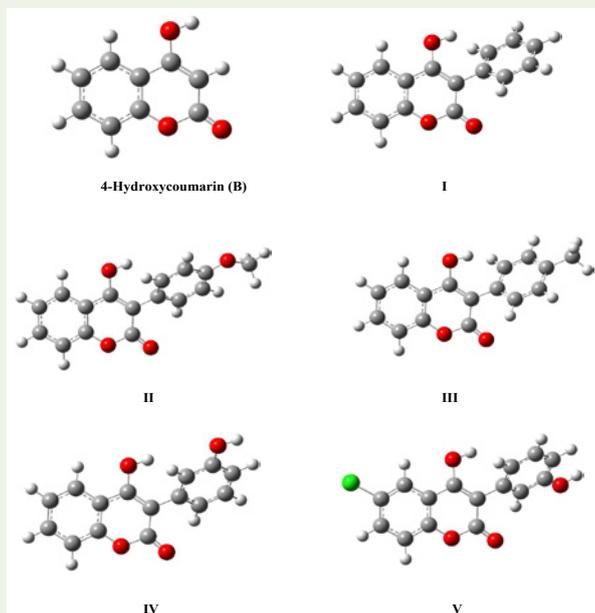| Compound	BDE (Kcal/mol)	IP in gas (kcal/mol)	IP in CPCM (Kcal/mol)	% Scavenging*	ORAC values*
4-hydroxy coumarin	112.36	214.90	144.99	61.9	4.2
I	79.29	172.04	136.10	45.1	4.4
II	76.17	161.94	128.09	46.3	5.7
III	78.29	168.16	133.33	34.6	6.5
IV-OH(4)	80.26	169.20	133.93	40.2	4.9
IV-OH(3')	82.96	169.20	133.93	40.2	4.9
V-OH(4)	80.15	173.44	136.81	100	7.7
V-OH(3')	46.43	173.44	136.81	100	7.7

Taken from reference [3]

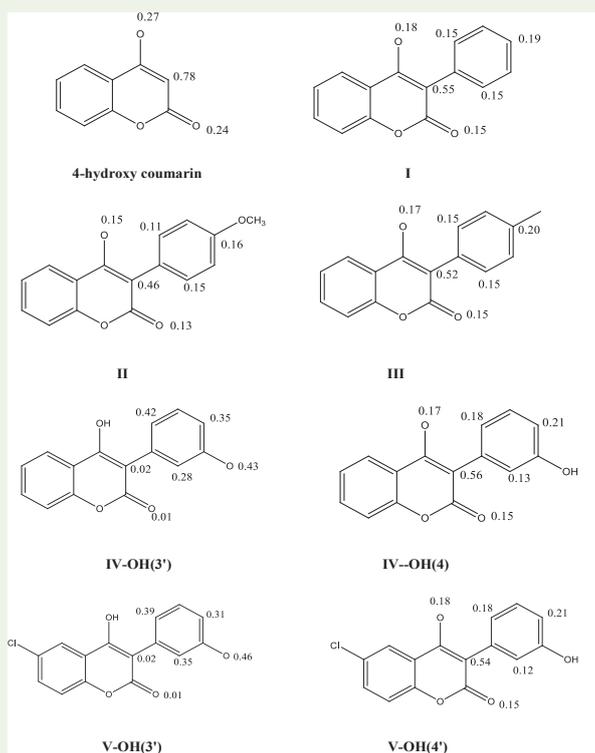**Figure 3:** Optimized structures of 3-Aryl-4-hydroxycoumarin derivatives.

Figure 4: The spin densities for the semiquinone derivatives.

compound **V** leads to increase the stability energy significantly. For **IV**-OH structure, the radical being on position 3' is more stable than on position 4. While for structure **V**-OH, the most stable radical is being at position 4 due to the effect of the chlorine atom on coumarin ring. For **V**-OH(4), the ΔE_{iso} reaches -4.43 kcal/mol. Adding phenyl group to 4-hydroxy coumarin (compound **B**) and adding electron donating group such as methoxy or methyl (compounds **II** and **III**) do not seem to be important on ΔE_{iso} values.

The bond dissociation energy (BDE) of a hydroxyl group involves

H-atom transfer whereas the ionization potential (IP) refers to an electron transfer process. Hence, there are two main theoretically accepted physical parameters for evaluating the possible antioxidant capacity of a molecule. In general, the weaker an O-H bond and the lower is the ionization potential of an antioxidant, the more active would the antioxidant be in reacting with radical molecules [8]. Hydroxycoumarins are believed to behave like classic phenol- or quinol-based antioxidants; in which the O-H group attached to an aromatic ring structure can take part in an H-atom transfer and/or an electron transfer process impacting the reduction of a free radical [8].

The capacity of scavenging peroxy radicals was studied experimentally through the oxygen radical absorbance capacity method using the fluorescence-based technology of detection measurements (ORAC-FL) [3]. The ORAC-FL assay depends on the free radical damage to a fluorescein. The presence of antioxidants results in an inhibition of the free radical damage to the fluorescent compound. This inhibition is observed as a preservation of the fluorescent signal. The area under the curve (AUC) from the experimental sample is calculated to quantitate the protection. AUC calculation indicates both the inhibition time as well inhibition percentage of free radical damage by the antioxidant [3]. Also, the percentage of the hydroxyl radical scavenging activity was studied previously by Perez-Cruz and coworkers [3]. The results of the bond dissociation energy (BDE), an ionization potential (IP), % scavenging and ORAC values are shown in Table 2. The 4-hydroxy coumarin is used as reference molecule. When a phenyl group is added at position 3 in the compound I, the delocalization of semiquinone radical increases; thus influencing ORAC values to be changed. As seen in Table 2, both the BDE and IP in the gas and solvent phases decrease significantly, which means an increase the antioxidant activity. In the compound II, in which the phenyl ring presents a p-methoxy group, and in the compound III with a p-methyl group, a decrease in BDE with respect to the compound I is observed and subsequently ORAC and antioxidant activity increase. This fact can be explained by the strong electron-donating effect that increases the electron density around the hydroxyl group, favoring the hydrogen atom transfer mechanism, which is detected by decreasing BDE. For compound IV, although the BDE for hydroxyl groups is higher than compound I, increasing the number of hydroxyl groups leads to increase antioxidant activity. Compound V has the lowest BDE of 46.4 kcal/mol. It is indicated that it has the highest antioxidant activity. These values agree with experimental results [3] since compound V has the highest % scavenging and the highest ORAC values. The mechanism which involved in radical scavenging process for these 3-aryl-4-hydroxycoumarin derivatives is mainly H-atom transfer [3]. The result in Table 2 shows that BDE has a better correlation with antioxidant activity than IP values.

Spin density is an important parameter for characterizing the stability of free radicals since the energy of a free radical can be substantially lowered wherever unpaired electrons become highly delocalized through a conjugated system, following hydrogen abstraction [6,26]. The spin densities for the semiquinone derivatives are shown in Figure 4. The calculated spin densities of hydrogen abstraction from phenolic hydroxyl group showed that the phenolic

oxygen contribution O_4 is between 15-27%. For phenolic oxygen O_3, the C_3 carbon, the C_2 and C_6 carbon atoms at ortho positions and C_4 carbon atom at the para position are between 43-46%, 2-78%, 11-42%, and 16-35%, respectively. The lowest contribution of phenolic oxygen (being between 15-18%) showed the highest scavenging activity values due to the electronegativity of oxygen and to the fact that their compounds have more resonance spin structure.

Conclusion

The antioxidant prediction of some 3-phenyl-4-hydroxycoumarin derivatives was investigated theoretically at B3LYP/6-31G(d,p) level of theory. Phenyl group at position 3 of coumarin skeleton has great importance in the resonance stabilization. The introduction of an electron-donating group such as methyl, methoxy, and hydroxyl group on the 3-phenyl ring decreases the BDE when compared to 4-hydroxy coumarin, resulting in better antioxidant activity. The presence of chlorine atom at position 6 on the coumarin skeleton is an important structural factor that decreases the BDE (O-H) significantly and increases the antioxidant activity. The phenolic oxygen with lowest spin density contribution has the highest scavenging activity values. Our results showed that hydrogen donation BDE (O-H) is more related to the scavenging activity of 3-phenyl-4-hydroxy coumarin derivatives.

Acknowledgment

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