

Research Article**Theoretical Study on the Radical Scavenging Activity of Shikonin and its Ester Derivatives**¹Fuyu Sun and ²Ruifa Jin¹College of Physical and Electronic and Information Engineering,²College of Chemistry and Chemical Engineering, Chifeng University, Chifeng 024000, China

Abstract: Aim of study is to get a further insight on the contribution of the structural features to the radical scavenging potential of shikonin and its ester derivatives. The radical scavenging activity of shikonin and its ester derivatives have been studied by using density functional theory. The hydrogen bond property of the studied structures has been investigated by using the Atoms in Molecules (AIM) theory. The calculated results reveal that the hydrogen bond is important for good scavenging activity. The hydrogen atom transfer mechanism for shikonin and its ester derivatives are difficult to occur. However, shikonin and its ester derivatives appear to be good candidates for the one-electron-transfer mechanism. The shikonin ester derivatives investigated in this study are expected to be of high radical scavenging activity compared with parent compound shikonin. Taking this system as an example, we present an efficient method for the investigation of radical scavenging activity from theoretical point of view.

Keywords: Bond Dissociation Energy (BDE), Ionization Potential (IP), radical scavenging activity, shikonin derivatives, spin density

INTRODUCTION

Alkannin and shikonin are isohexenylnaphthazarin derivatives of hydroxynaphthoquinones. These two compounds are enantiomers (*S/R*) and mostly present as ester derivatives in the outer surface of the roots of at least 150 species belonging to the genera *Alkanna*, *Lithospermum*, *Echium*, *Onosma*, *Anchusa* and *Cynoglossum* of the Boraginaceae family (Akgun *et al.*, 2009). Recently interest on these two compounds increased because of their pharmacological properties (Deng *et al.*, 2010; Noula *et al.*, 2010; Ordoudi *et al.*, 2011). Phenolic compounds can act as antioxidants, by inhibiting biomolecules from undergoing oxidative damage through free radicals mediated reactions (Chang *et al.*, 2007; Visioli *et al.*, 1998). The antioxidant activity of the phenolic constituents is related to their structures. The intramolecular hydrogen bonds play important roles in stability of the radicals (Nenadis and Sigalas, 2008; Russo *et al.*, 2000). However, to the best of our knowledge, no calculation about the antioxidant activity of shikonin and its ester derivatives has been reported so far.

Recently, (Albrecht *et al.*, 2009) reported a series of shikonin ester derivatives (Fig. 1). Herein the structural and electronic properties of shikonin and its ester derivatives and their radicals were investigated at

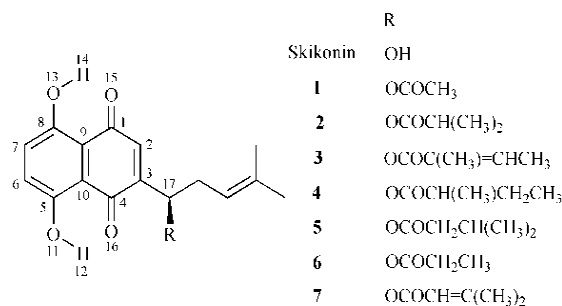


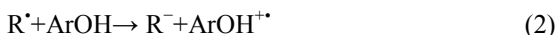
Fig. 1: Geometries of shikonin and its ester derivatives, along with atom numbering

density functional level. The aim is to get a further insight on the contribution of the structural features to the radical scavenging potential of the particular compounds and elucidate their scavenging capacity through calculation of proper molecular descriptors.

Computational details: Phenoxy radicals represent important intermediates in many biological applications (Halliwell and Gutteridge, 1989). Two main kinds of mechanism are reported for the radical scavenging processes of chain-breaking antioxidants ArOH (Leopoldini *et al.*, 2004): H-atom transfer, Eq. 1, and one-electron transfer, Eq. 2.

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In the H-atom transfer mechanism, the Bond Dissociation Energy (*BDE*) of the O-H bonds is an important parameter to evaluate the antioxidant action, because the weaker the O-H bond the easier the reaction of free radical inactivation will be. In the one-electron transfer mechanism, the radical cation arising from the electron transfer must be stable, so it does not react with substrate molecules. In this case, the Ionization Potential (*IP*) is the most significant energetic factor for the scavenging activity evaluation. Molecules with the low *IP* and *BDE* values are expected to have high activity.

All calculations were performed by Gaussian 09 code (Frisch, 2009). The geometries of shikonin and 1-7 were optimized at the B3LYP/6-31+G (d) level without symmetry constraints. Single-point electronic energies (SPEs) were obtained at the B3LYP/6-311++G (d,p) level. The *BDE* and *IP* values were evaluated by the B3LYP/6-311++G (d,p) single-point energy calculations with the thermal contributions to enthalpy and the zero-point energy corrections at the B3LYP/6-31+G(d) level, respectively. The *BDE* values were calculated according to the formula $BDE = H_r + H_h - H_p$, where H_r is the enthalpy of the radical generated by H-abstraction, H_h is the enthalpy of the H-atom (-0.499897 hartree at this level of theory), and H_p is the enthalpy of the parent molecule. The *IP* values were determined according to the equation $IP = E_{cr} - E_p$, where p and cr indicate the parent molecule and the corresponding cation radical generated after electron transfer.

RESULTS AND DISCUSSION

Hydrogen atom transfer mechanism: Starting from the absolute minima of each species, two radicals from shikonin and 1-7 were obtained upon H-atom abstraction from every hydroxyl phenolic group, whereas a single radical cation for each parent molecule was obtained by removing one electron. Figure 2 presents the spin densities of 5-OH and 8-OH radicals for shikonin and 1-3 as representatives. Hydroxyanthraquinone derivatives are usually considered radical scavengers due to their excellent delocalization possibility. For both 5-OH and 8-OH radicals, the spin distribution indicates the oxygen atoms O₁₁ and O₁₃ from which the H atoms are removed as the most probable radical centers.

The calculated *BDE* and *IP* values of shikonin and 1-7 are presented in Table 1. The *BDE* values characterize the hydrogen atom donating ability of shikonin and 1-7. On the basis of *BDE* values, it is evident that the radical scavenging activity of 5-OH and

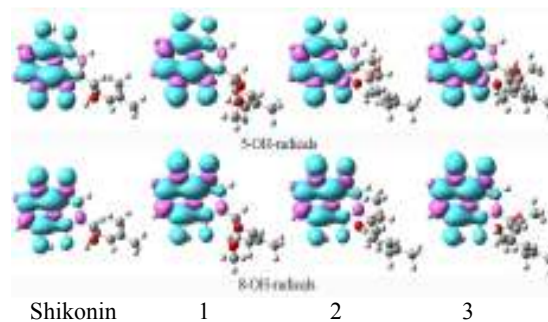


Fig. 2: The spin densities of 5-OH and 8-OH radicals for shikonin and 1-3

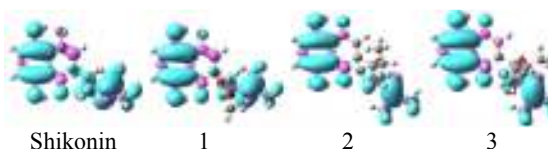


Fig. 3: The spin densities of radical cations for shikonin and 1-7

Table 1: The *BDE* and *IP* (both in kcal/mol) values for radicalas of shikonin and 1-7

Species	<i>BDE</i>		<i>IP</i>
	5-OH-radical	8-OH-radical	Radical cation
Shikonin	83.9 (0.0) ^a	85.4 (0.0) ^a	181.0 (0.00) ^a
1	84.2 (0.3)	85.2 (-0.2)	165.6 (-15.4)
2	84.2 (0.3)	84.0 (-1.4)	177.2 (-3.8)
3	83.9 (0.0)	84.0 (-1.4)	176.2 (-4.8)
4	84.2 (0.3)	84.1 (-1.3)	177.0 (-4.0)
5	84.1 (0.2)	84.7 (-0.7)	179.3 (-1.7)
6	84.2 (0.3)	84.7 (-0.7)	179.6 (-1.4)
7	83.9 (0.0)	84.0 (-1.4)	175.3 (-5.7)

^a: The relative *BDE* and *IP* (in the parentheses) are referred to shikonin

8-OH radicals for 1-7 is predicted to be almost equal to that of shikonin radical. Such efficiency is related to the further stabilization of the derived formation of an intramolecular hydrogen in both 5-OH and 8-OH radicals. The trend of *BDE* values for the examined compounds suggests that an essential factor for a good radical scavenging activity is the intramolecular hydrogen bond type structure, and the introduction of ester groups for shikonin decreases (increases) slightly the radical scavenging activity of 5-OH (8-OH) radicals for 1-7. However, abstraction of the H atom from the hydroxyl in positions 5 and 8 implies the breaking of the hydrogen bonds formed with the 4 and 1-keto groups, respectively. Hence, the hydrogen atom transfer for shikonin and 1-7 are difficult to occur.

Single electron transfer mechanism: Figure 3 presents the spin densities for the radical cations arising from shikonin and 1-3 as representatives. The unpaired electron delocalized over the naphthoquinones and the substituent groups on 3-position, as shown in Fig. 3. The results displayed in Table 1 reveals that the lowest

Table 2: Electronic density at BCP $\rho(r)_{bcp}$, the laplacian $\nabla^2\rho(r)_{bcp}$ (in au) and the hydrogen bond energy E_{HB} (in kcal/mol) of shikonin and 1-7

Species	$O_{16}\cdots H_{12}$			$O_{15}\cdots H_{14}$		
	$\rho(r)_{bcp}$	$\nabla^2\rho(r)_{bcp}$	E_{HB}	$\rho(r)_{bcp}$	$\nabla^2\rho(r)_{bcp}$	E_{HB}
Shikonin	0.0476	0.1400	-14.4	0.0472	0.1356	-14.0
1	0.0481	0.1402	-14.5	0.0467	0.1350	-13.8
2	0.0501	0.1432	-15.4	0.0470	0.1356	-13.9
3	0.0502	0.1432	15.4	0.0471	0.1358	-14.0
4	0.0501	0.1432	-15.4	0.0470	0.1357	-13.9
5	0.0493	0.1414	-15.0	0.0471	0.1356	-13.9
6	0.0493	0.1413	-15.0	0.0470	0.1356	-13.9
7	0.0502	0.1433	-15.4	0.0472	0.1359	-14.0

IP value is found for 1, followed by 7, 3, 4, 2, 5, and 6. The electron-donating ability of shikonin and 1-7 seems to be related to an extended electronic delocalization over all the molecules. Systems having a high degree of π -delocalization are the most active among the one-electron-transfer mechanism. Furthermore, the introduction of the ester groups for shikonin leads to a decrease the IP values compared with that of shikonin, particularly for 1. For conjugated system, the IP is raised in energy, so the abstraction of an electron becomes very easy. The trend of IP values for the examined compounds suggests that an essential factor for a good activity as antioxidant is the hydrogen bond type structure, and it can be further improved by the introduction of the ester groups for the molecules. The IP values of 1-7 are lower than that of shikonin, suggesting that 1-7 are expected to be of high radical scavenging activity compared with parent compound shikonin.

Atoms in molecules analysis: Atoms in molecules (AIM) (Bader, 1991) theory was applied at the B3LYP/6-311++G(d,p) level to investigate the topology of electron density and the OH \cdots O hydrogen bond properties of the studied structures. The electron densities, $\rho(r)_{bcp}$, their Laplacians, $\nabla^2\rho(r)_{bcp}$, and the potential electron energy densities $V(r)_{bcp}$ at bond critical points (BCPs) are frequently used as indicators of hydrogen bond. The hydrogen bond energy E_{HB} (defined as $-De$, where De is the hydrogen bond dissociation energy) in molecules can be estimated within the framework of the AIM analysis using the relationship: Espinosa *et al.* (1998).

$$E_{HB} = -De = 0.5V(r)_{bcp}$$

Table 2 presents the topological parameters and the E_{HB} values for shikonin and 1-7. As a general rule, hydrogen bonds are characterized by positive values of $\nabla^2\rho(r)_{bcp}$, low $\rho(r)_{bcp}$ values (<0.1). Inspection of Table 2 reveals clearly that the $\rho(r)_{bcp}$ values of the $O_{16}\cdots H_{12}$ hydrogen bonds in 1-7 are greater, while the corresponding values of $O_{15}\cdots H_{14}$ are lower than those in shikonin, respectively. It suggests that the strength of $O_{16}\cdots H_{12}$ hydrogen bonds in 1-7 are stronger, while the corresponding strength of $O_{15}\cdots H_{14}$ hydrogen bonds are weaker than those in shikonin, respectively. Furthermore, the E_{HB} values of $O_{16}\cdots H_{12}$ hydrogen bonds in 1-7 are greater, while the corresponding values of $O_{15}\cdots H_{14}$ are lower than those in 1, respectively.

Comparing the $\rho(r)_{bcp}$, their Laplacians $\nabla^2\rho(r)_{bcp}$, and E_{HB} in neutral forms with those in radical cations shown in Table 2 and SI, one can find that the strength of $H_{14}\cdots O_{15}$ hydrogen bonds in radical cations is stronger, while the corresponding strength of $H_{12}\cdots O_{16}$ hydrogen bonds is weaker than those in their neutral forms, respectively. An enhancement of the strength of hydrogen bond in radical cations confers high stability to radical cations of acylshikonin derivatives.

CONCLUSION

The introduction of ester groups for shikonin decreases/increases the radical scavenging activities of 5-OH/8-OH radicals slightly. Shikonin and its ester derivatives appear to be good candidates for the one-electron-transfer mechanism, particularly for acetylshikonin (1). The introduction of the ester groups for shikonin decreases the IP values compared with that of shikonin. All the shikonin ester derivatives are expected to be of high radical scavenging activity compared with parent compound shikonin.

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