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A Theoretical Investigation on DPPH Radical-Scavenging Mechanism of Edaravone

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Abstract—The mechanism of edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) to scavenge DPPH radical is clarified by density functional theory (DFT) calculations. It is revealed that H-atom-abstraction rather than electron-transfer reaction is involved in the radical-scavenging process of edaravone, and H-atom at position 4 is readily to be abstracted. The C–H bond dissociation enthalpy (BDE) of edaravone is higher than the O–H BDE of α-tocopherol, accounting for the activity difference between the two antioxidants. As substituents have little influence on the C-H BDE, 2-pyrazolin-5-one is recognized as the active center for edaravone.

Edaravone (3 - methyl - 1 - phenyl - 2 - pyrazolin - 5 - one, Scheme 1) is a novel neuroprotective agent that was approved for the acute therapy of embolic stroke, and has great potential to protect against various radical-induced toxicity.[1] The pharmacological effect of edaravone arises from its radical-scavenging activity. In fact, it is efficient to scavenge hydroxyl radical (HO·) and DPPH radical (DPPH·).[1a] As HO· is one of the most reactive chemical species in nature, it is not surprising to see edaravone possesses high HO·-scavenging activity. However, it is unexpected to find that edaravone is effective to scavenge DPPH· ($IC_{50} = 29.3$ μmol L^{-1}), because edaravone has no phenol, amino or thiophenol group that are commonly known to be the active group for scavenging DPPH·.[2] Therefore, it is very interesting to clarify the DPPH·-scavenging mechanism for edaravone, which will be helpful to elucidate the structure–activity relationship (SAR) for the antioxidant and beneficial to design novel edaravone-derived antioxidants with better pharmacological effect as well. In view of the successful use of theoretical methods in elucidating radical-scavenging mechanisms and SAR for various antioxidants,[3] we attempt to achieve the goal by density functional theory (DFT) calculations.

At first, we have to select proper theoretical parameters to characterize the radical-scavenging pathways. Generally speaking, there exist two mechanisms for antioxidants to scavenge DPPH·. The first is a direct H-atom-abstraction process (eq 1), and the second is a proton concerted electron-transfer process (eq 2).[4]

$$\mathrm{DPPH}\cdot + \mathrm{RXH} \rightarrow \mathrm{DPPHH} + \mathrm{RX}\cdot \quad (1)$$

$$\mathrm{DPPH}\cdot + \mathrm{RXH} \rightarrow \mathrm{DPPH}^- + \mathrm{RXH}^{\cdot +} \rightarrow \mathrm{DPPHH} + \mathrm{RX}\cdot \quad (2)$$

In which, X represents O, N, S or C. The first pathway is governed to large extent by X–H bond dissociation enthalpies (BDEs) of RXH and DPPHH. Only if the BDE of the former is lower than that of the latter, the reaction is permitted. While, the second pathway is determined by ionization potentials (IPs) of RXH and $DPPH^-$. The prerequisite for the reaction is that the IP of RXH is lower than that of $DPPH^-$.

In this paper, BDEs were calculated according to the following procedures. The molecular geometries were optimized firstly, by molecular mechanic method MMX,[5] and then, by semiempirical quantum chemical

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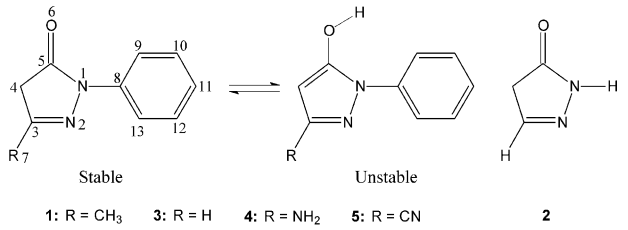


Scheme 1. Molecular structures of edaravone and model compounds.

method AM1.[6] Finally, according to the proposal of Wright et al.,[3f] (RO)B3LYP functional[7] on basis set of 6-31G(d,p) was used for the full geometry optimization. The zero point vibrational energy (ZPVE) and the vibrational contribution to the energy were scaled by a factor of 0.9805.[8] According to the definition of BDE, $BDE = H_r + H_h - H_p$, in which, H_r is the enthalpy for radical generated after H-abstraction reaction, H_h is the enthalpy for hydrogen atom, −0.49792 hartree, and H_p is the enthalpy for parent molecule. Whereas, adiabatic IPs were calculated by means of a combined density functional theory (DFT) method, which takes advantages of accuracy and economy.[9] That is, (U)B3LYP functional on basis set of 6-31G(d) was used to calculate single point energy (SPE) on the basis of PM3 optimized structure.[10] Thus, the molecular energy (e) consists of (U)B3LYP/6-31G(d) calculated SPE and PM3 calculated ZPVE (scaled by a factor of 0.947). And $IP = e_c - e_p$, in which, e_c is the energy for cation radical, while e_p is the energy for parent molecule. All of the quantum chemical calculations were accomplished by Gaussian 98 program.[11] Considering the lipophilicity of edaravone, the calculations were achieved in gas phase. The results will be applicable in nonpolar solvents and lipid systems.

The IP of $DPPH^-$ is calculated to be 59.60 kcal/mol, while the IP for edaravone is 164.72 kcal/mol, indicating that the electron-transfer reaction between edaravone and DPPH· should be forbidden from the thermodynamic point of view.[12] On the other hand, the N–H BDE for DPPHH is 172.22[13] kcal/mol, much higher than the C–H BDEs of edaravone (Table 1).[14] Hence, H-atom abstraction rather than electron-transfer reaction is involved in the radical-scavenging process of edaravone.

As the C–H BDE corresponding to position 4 is much lower than that for position 7 (Table 1), the former position will be the H-atom-donating site, which arises from the fact that position 4-derived radical is more stable than the counterpart derived from position 7 (Table 1). As shown in Scheme 2, the former radical is indeed better stabilized through resonance effect than the latter one. According to the resonance theory, it is impossible to discriminate between resonance isomers by quantum chemical calculations, so position 4- or 7-derived radical is a mixture of different isomers. However, it is interesting to find from Table 2 that the spin density distribution in position 4-derived radical concentrates on N2, C4 and O6, corresponding to the three resonance modes, while the spin density in position 7-derived radical mainly populates on C2 and C7, in concordance with the two resonance isomers. More interestingly, the C-H BDE for position 4 relative to phenol, −7.33 kcal/mol (Table 1), is higher than the relative O–H BDE of α-tocopherol, ∼−10 kcal/mol,[3g] accounting for the higher activity of α-tocopherol ($IC_{50} = 23.8$ μmol L^{-1}) than edaravone to scavenge DPPH.[1a]

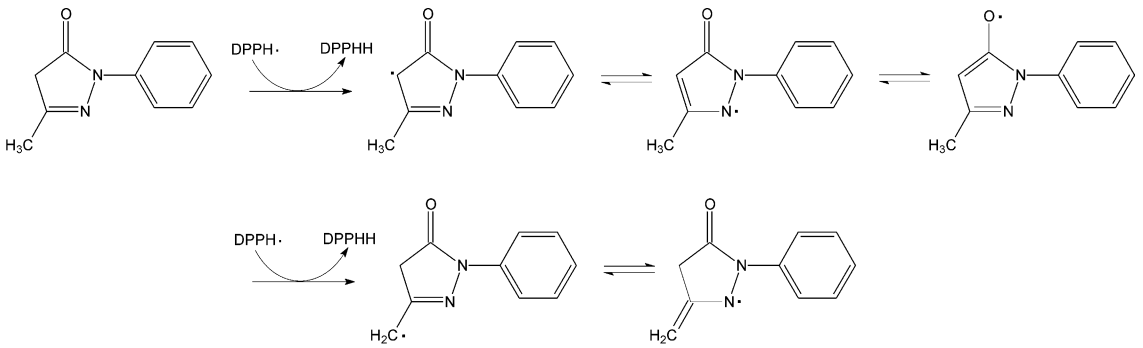
On the basis of the clarified radical-scavenging mechanism, we attempt to shed light on the SAR for edaravone. Thus, four model molecules were designed (Scheme 1) and the corresponding C–H BDEs were calculated (Table 1) to evaluate the contribution of each substituent to the C–H BDEs. It is unexpected to find that the C–H BDE of **2** is comparable with that of **1** and from the BDE difference between **2** and **3**, the benzene ring reduces the C–H BDE only by 0.5 kcal/mol, which is in accordance with the negligible spin density distribution on the benzene ring (Table 2). The slight difference between BDEs of **1**, **4**, **5** and **3** implies that the C–H BDE of edaravone is little influenced by the distinct electronic properties of substituents at position 3. Thus, it is clear that 2-pyrazolin-5-one (**2**) is the active center for edaravone as a radical-scavenger and further structural modification can be carried out on the benzene ring or position-3 to improve the absorbing and metabolic properties of edaravone.

In brief, edaravone scavenges DPPH radical through donating the H-atom at position 4. And its strong

Table 1. BDEs of edaravone and model compounds calculated by (RO)B3LYP/6-31G(d,p) ($T = 298.15$ K)

Compd[a]	TE (hartree)[b]	TCE (hartree)[c]	C(O)-H BDE (kcal/mol)
1	−571.817818	0.194202	
1-radical (4)	−571.183548	0.181800	77.93
1-radical (7)	−571.169412	0.181076	86.36
2	−301.430250	0.079443	
2-radical (4)	−300.797119	0.067222	77.33
3	−532.490508	0.164774	
3-radical (4)	−531.858090	0.152470	76.83
4	−587.859025	0.183450	
4-radical (4)	−587.225440	0.170838	77.37
5	−624.726778	0.165062	
5-radical (4)	−624.093521	0.152597	77.26
Phenol	−307.478467	0.110308	
Phenolic radical	−306.831578	0.096950	85.26

[a]Positions for H-atom abstraction are in parentheses.
[b]Total electronic energy.
[c]Thermal correction to energy.



Scheme 2. Resonance modes of edaravone-derived radicals.

Table 2. Spin density distributions in edaravone-derived radical calculated by (U)B3LYP/6-31G(d,p)

Atom number	Position 4-radical	Position 7-radical
1	0.111	0.092
2	0.402	0.507
3	−0.092	−0.221
4	0.249	0.010
5	−0.007	0.008
6	0.305	0.060
7	0.006	0.571
8	−0.028	−0.028
9	0.037	0.033
10	−0.021	−0.018
11	0.040	0.035
12	−0.018	−0.017
13	0.036	0.035

radical-scavenging activity mainly results from 2-pyrazolin-5-one and substituents have little influence on the activity, which provides new clues to modify edaravone to give better antioxidants.

Acknowledgements

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2. Although edaravone resonates between keto and enol forms (Scheme 1), the former is 8.17 kcal/mol more stable than the latter in enthalpy, calculated by (RO)B3LYP/6-31G(d,p) full geometry optimization (see the methodology part for the detail). Hence, the keto form predominates and the enol form can be neglected in solution.
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10. (a) Although AM1 is the preferred semiempirical method, it failed to give zero point vibrational energy of DPPH radical. So we had to use PM3 instead to perform the calculation.[10b] There is a little difference (1∼2 kcal/mol) between AM1- and PM3- based BDEs and IPs for edaravone, which suggests that PM3-based BDEs and IPs for DPPHH and $DPPH^-$ are applicable to elicit a qualitative conclusion when compared with those of edaravone. (b) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209.
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12. By employing the self-consistent reaction field (SCRF) method with polarized continuum model (PCM), the IPs for

$DPPH^-$ and edaravone in ethanol were calculated to be 89.76 kcal/mol and 125.08 kcal/mol, respectively, indicating that the electron-transfer reaction is forbidden even in polar solvent.

13. As (RO)B3LYP/6-31G(d,p)-full-geometry-optimization failed to give N–H BDE for DPPHH, a combined DFT method was used to calculate the value, in which, PM3 was employed to optimize the molecular structures and determine the zero point vibrational energy and vibrational contribution to the energy, then (RO)B3LYP/6-311 + G(2d,2p) was used to calculate single point electronic energy. This method was labeled as (RO)B3LYP/6-311 + G(2d,2p)/PM3/PM3.

14. The C-H BDE (position 4) for edaravone calculated by (RO)B3LYP/6-311 + G(2d,2p)/PM3/PM3 is 75.48 kcal/mol, similar to that calculated by (RO)B3LYP/6-31G(d,p).