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A theoretical study on the structure–activity relationships of metabolites of folates as antioxidants and its implications for rational design of antioxidants

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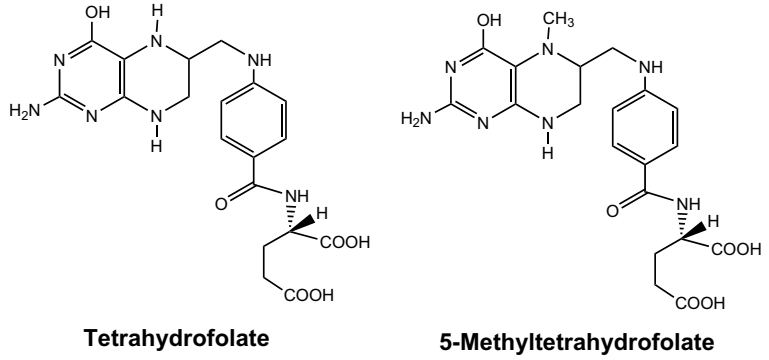
Abstract—To elucidate the structure–activity relationships of metabolites of folates as antioxidants, the O–H bond dissociation enthalpies (BDEs) and ionization potentials (IPs) for these compounds were calculated by density functional theory (DFT) on B3LYP/6-31+G(,3pd) level. Accordingly, the antioxidant activity difference for metabolites of folates can be elucidated by O–H BDE and IP values and can be further explained in terms of electronic effect and intramolecular hydrogen bond effect of substituents. Furthermore, the potential of the active center of metabolites of folates, 4-hydroxypyrimidine (4-HP), as lead antioxidant, was evaluated by comparing the BDEs and IPs of 4-HP with those of 5-hydroxypyrimidine (5-HP). It was revealed that 4-HP and 5-HP held identical IPs, but the O–H BDE of the former was 22.84 kcal/mol higher than that of the latter, which meant 4-HP was inert in H-atom donation. Nevertheless, the O–H BDE of 4-HP was very sensitive to the substituents, which made NH_2-derivatives of 4-HP very active as antioxidants. Therefore, 4-HP is also a potential lead antioxidant and deserves attention in rational design of antioxidants.

1. Introduction

In recent years, there has been growing interest in finding novel antioxidants, especially natural compounds, to meet the requirements of pharmaceutical, chemical and food industries.[1–3] Thus, it is very interesting to notice that Rezk et al., indicated that some physiological metabolites of folates, such as tetrahydrofolate and 5-methyl-tetrahydrofolate, had high antioxidant activity (Scheme 1),[4] and the pharmacophore was recognized



Scheme 1. Molecular structures of tetrahydrofolate and 5-methyltetrahydrofolate.

Keywords: Antioxidant; Folate; 4-Hydroxypyrimidine; 5-Hydroxypyrimidine; Phenol; Quantum chemical calculation.
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to be 4-hydroxy-2,5,6-triaminopyrimidine (**4**, Scheme 2).[4] The unique structure and the great potential of these compounds as antioxidants aroused our interest in elucidating their structure–activity relationships (SARs) by means of quantum chemical calculations, which have been successfully used for a variety of antioxidants.[5–8]

On the other hand, the active center of the pharmacophore, 4-hydroxypyrimidine (4-HP, **1**, Scheme 2), likely provides a novel lead antioxidant. Recently, to accelerate the discovery of antioxidants, rational design strategy has been proposed and applied in the practice.[9–11] A major challenge in this area is to find promising novel lead structures. Although much effort has been devoted to this subject, only a few novel lead structures were proposed, such as 5- hydroxypyrimidine (5-HP),[12,13] 1,8-naphthalenediol[14] and 1,4-dihydropyridine (Scheme 3).[15] Therefore, it is interesting and important to evaluate the potential of 4-HP as a lead antioxidant, which is also a motive of this study.

2. Methods

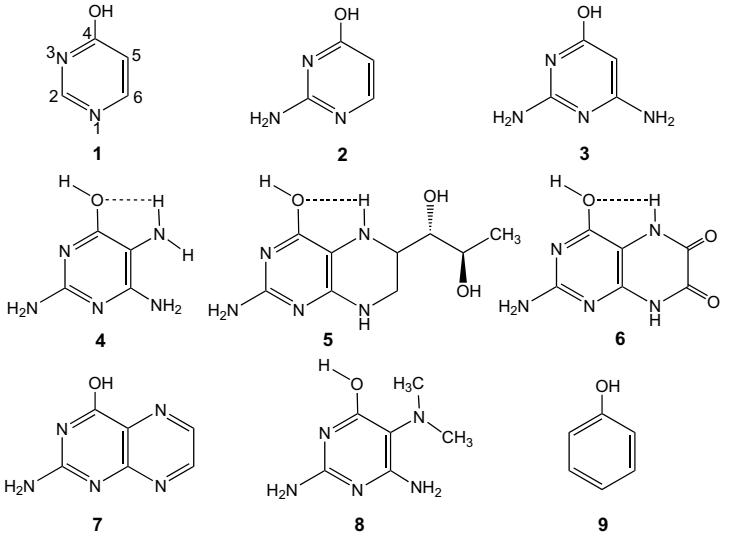
2.1. Theoretical models

At first, we have to select proper theoretical parameters to characterize the radical-scavenging activity of folates and their metabolites. Generally speaking, there exist two mechanisms for antioxidants to scavenge radicals. Taking peroxy radical ($ROO^{\cdot}$) as an example, the first mechanism is a direct H-atom-abstracting process (Eq. 1), and the second is a proton-coupled electron-transfer process (Eq. 2).

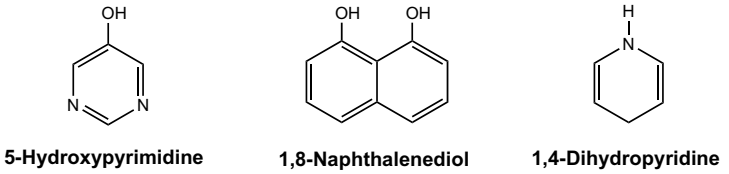
$$ROO^{\cdot} + RXH \rightarrow ROOH + RX^{\cdot} \quad (1)$$

$$ROO^{\cdot} + RXH \rightarrow ROO^{-} + RXH^{\cdot+} \rightarrow ROOH + RX^{\cdot} \quad (2)$$

In which, X represents an O–, N–, S– or C-atom. The first pathway (Eq. 1) dominates in nonpolar solvents[16–19] and can be characterized by the homolytic X–H bond dissociation enthalpy (BDE) of the antioxidant,[20–25] whereas the second (Eq. 2) dominates in polar solvents[26–28] and can be measured by the ionization potential (IP) of the antioxidant or anions derived thereof.[24,29] Obviously, low X–H BDE values are beneficial to enhance the H-atom-donating power of the antioxidants. However, although low IP values are also favorable to raise the electron-transfer reactivity, they enhance the danger of generating a superoxide anion radical through transfer of the electron directly to surrounding O_2.[24] Therefore, the elucidation on the SARs of antioxidants and the evaluation on a lead structure mainly focus on the BDE and IP values. Nevertheless, in practice, the rational design of antioxidants also takes the solubility, bioavailability and toxicity of the compound into consideration.



Scheme 2. Molecular structures of metabolites of folates and some model molecules.



Scheme 3. Molecular structures of rationally designed lead antioxidants.

2.2. Calculation methods

In this study, homolytic BDEs and IPs were calculated by density functional theory (DFT) on B3LYP/6-31+G(,3pd) level, which is more accurate than other DFT methods in calculating BDEs for intramolecular-hydrogen-bonded groups.[30] The detailed calculation procedures are as follows. Each molecule was optimized, firstly by semi empirical quantum chemical method AM1,[31] to obtain the most stable structure. Then, using these structures as starting points, the molecules were fully optimized by B3LYP/6-31+G(,3pd). For each optimized structure, a frequency analysis at the same level of theory was performed to verify that it corresponded to a stationary point in the potential energy surface. Employing the sum of electronic and thermal enthalpies (H) in the gas phase at 298.15 K, the homolytic BDE is equal to $H_r + H_h - H_p$ where H_r is the enthalpy of the radical generated after H-atom abstraction, H_h is the enthalpy of the H-atom (−0.497912 hartree), and H_p is the enthalpy of the parent molecule. The IP is equal to $H_c - H_p$ where the subscripts 'p' and 'c' indicate the parent molecule and the corresponding cation radical generated after electron transfer, respectively. All of the calculations were performed with the GAUSSIAN-98 program package.[32]

3. Results and discussion

3.1. Elucidation on the structure–activity relationships of folates as antioxidants

Table 1 lists the calculated BDEs and IPs for 4-HP and derivatives and the corresponding antioxidant activity as well. It can be seen that both parameters correlate well with the antioxidant activity. Namely, the lower the BDE or the IP is, the higher the activity, which justifies the effectiveness of BDE and IP in characterizing the antioxidant activity.

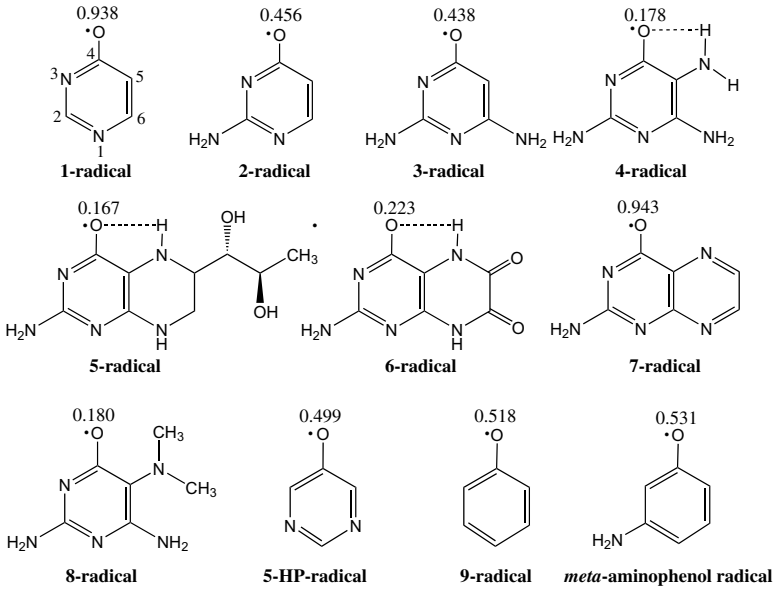
Furthermore, it is evident that electron-donating substituents, such as amino group, tend to lower the BDE and IP (1 vs 2 or 3 vs 4), whereas electron-withdrawing substituents, such as keto group, incline to enhance both parameters (4 vs 6), which has also been recognized by previous studies on phenolic antioxidants.[24,33,34] A deeper insight into the substituent effect on BDE can be gained by the substituent effect on spin density of O-atom of the radicals. That is, electron-donating groups tend to stabilize the radical and thus decrease the BDE, whereas electron-withdrawing groups have an opposite effect. This is clearly illustrated in Scheme 4 that the higher the spin density on the O-atom remains, the higher the BDE.

A further analysis indicates that the substituent effect on O–H BDE of 4-HP is much different than that on O–H BDE of phenol. For instance, from the O–H BDEs of **1** and **2**, one can deduce that the 2-NH_2 reduces the BDE by 12.17 kcal/mol, which is much stronger than the *meta*-NH_2 effect on O–H BDE of phenol, −0.2 kcal/mol.[24] A straightforward explanation of the phenomenon is that due to the electron-demanding state of both nitrogens in 4-HP, the 2-NH_2 gets the opportunity to exhibit its strong electron-donating effect, while in phenol the *meta*-NH_2 has no this kind of chance. This is supported by the spin density distribution in 4-HP, phenol and amino substituted counterparts. As shown in Scheme 4, the spin density on O-atom of 4-HP-derived radical can be efficiently attenuated by the substitution of 2-NH_2, but in phenol *meta*-NH_2 has little influence

Table 1. B3LYP/6-31+G(,3pd)-calculated homolytic O–H bond dissociation enthalpies (BDEs) and ionization potentials (IPs) of 4-hydroxypyrimidine derivatives and phenol, $T = 298.15$ K

	H_{ArOH}[a] (hartree)	$H_{ArO\cdot}$[b] (hartree)	$H_{ArOH\cdot^+}$[c] (hartree)	O–H BDE (kcal/mol)	IP (kcal/mol)	IC_{50} PON[d] (μM)	IC_{50} LPO[e] (μM)
1	−339.394731	−338.714530 (0.7536)[f]	−339.052660 (0.7586)[f]	114.39	214.65	>500	>500
2	−394.746800	−394.085996 (0.7891)[f]	−394.434461 (0.7747)[f]	102.22	196.00	>500	>500
3	−450.094952	−449.435445 (0.7538)[f]	−449.802271 (0.7811)[f]	101.40	183.66	143 ± 18	>500
4	−505.422457	−504.790529 (0.7568)[f]	−505.168549 (0.7553)[f]	84.10	159.33	0.8 ± 0.3	55 ± 2
5	−851.113671	−850.485412 (0.7790)[f]	−850.885041 (0.7654)[f]	81.79	143.47	0.5 ± 0.2	86 ± 1
6	−730.814653	−730.170505 (0.7648)[f]	−730.518176 (0.7595)[f]	91.76	186.04	27 ± 8	>500
7	−580.370267	−579.689268 (0.7569)[f]	−580.055871 (0.7543)[f]	114.89	197.29	>500	>500
8	−583.958953	−583.319607 (0.7571)[f]	−583.709108 (0.7551)[f]	88.75	156.78		
9	−307.322821	−306.683825 (0.7886)[f]	−307.013622 (0.7633)[f]	88.53	194.03		

[a] The sum of electronic and thermal enthalpies for parent molecules.
[b] The sum of electronic and thermal enthalpies for radicals generated after H-atom abstraction.
[c] The sum of electronic and thermal enthalpies for cation radicals generated after electron transfer.
[d] Antioxidant activity evaluated by synthesis of peroxynitrite.[4]
[e] Antioxidant activity evaluated by lipid perioxidation assay.[4]
[f] The total spin, $\langle S^2 \rangle$, for each radical is indicated in parentheses, from which one can conclude that the spin contamination is negligible, because the value of $\langle S^2 \rangle$ differs from $s(s + 1)$ (0.75) by less than 10%.



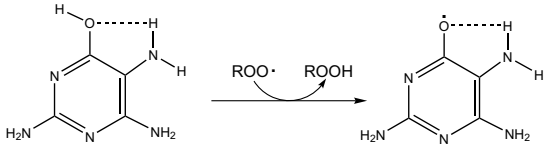
Scheme 4. Spin density on O-atom of folate-metabolite-derived radicals and model molecule-derived radicals.

on the spin density of O-atom. More interestingly, if a second *meta*-NH_2 group is inserted in 4-HP, giving **3**, the O–H BDE drops to 101.40 kcal/mol, indicating that in this case the *meta*-NH_2 has slight effect on the BDE, which results from the fact that **2** is not as electron-demanding as **1**.

From the BDE difference between **3** and **4**, it can be found that the 5-NH_2 reduces the BDE by 17.30 kcal/mol, which should result not only from the strong electron-donating property of NH_2 but also from the intramolecular hydrogen bond (IHB) in **4** (Scheme 5). Through a (RO)B3LYP/6-311+G(2d,2p)//AM1/AM1 calculation, Wright et al., suggested that an *ortho*-NH_2 reduced the O–H BDE of phenol by 11.5 kcal/mol,[24] viz., ~6 kcal/mol lower than the *ortho*-NH_2 effect in 4-HP. The difference could come from the distinct electronic states and thus distinct substituent sensitivity of 4-HP and phenol. If the 5-amino group in **4** is replaced by a $N(CH_3)_2$ group, giving **8**, the IHB effect does not exist. Thus, this substitution is not beneficial to enhance the activity of **4** (Table 1), despite the electron-donating effect of methyl. This provides an alternative explanation for the experimental observation that the lipid-peroxidation-inhibiting activity of 5-methyltetrahydrofolate is lower than that of tetrahydrofolate (Scheme 1).[4]

3.2. Evaluation on the potential of 4-hydroxypyrimidine as a lead antioxidant

As a potential lead antioxidant, the molecule must hold rather low BDE to preserve the high H-atom-donating ability and relatively high IP to guarantee it is stable to air oxidation.[24] 5-HP has been proposed as a good lead antioxidant, because its O–H BDE is comparable with that of phenol[12,13,35] and its IP is much higher than that of phenol.[12,36] In addition, the substituent effect on the O–H BDE of 5-HP is similar to that on BDE of phenol,[12,13] which makes it possible to design novel 5-HP-derivatives with high radical-scavenging activity and good air stability.

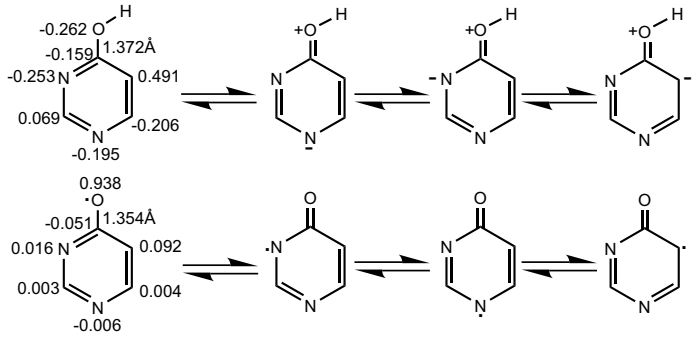


Scheme 5. Radical-scavenging process of **4**.

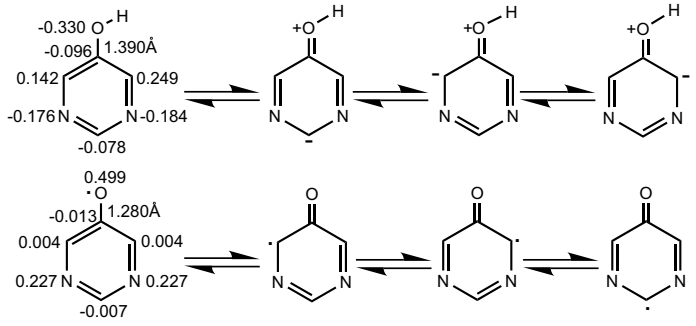
From Table 1, we can find that the O–H BDE of 4-HP is 25.76 kcal/mol higher than that of phenol, while the IP of 4-HP is 20.62 kcal/mol higher than that of phenol, which implies that 4-HP is as stable as 5-HP to air oxidation, however, its H-atom-donating ability is much weaker than that of 5-HP.

The difference must stem from the distinct structures of 4-HP and 5-HP. For the former molecule, both electron-deficient nitrogens are located at the *ortho* and *para* positions of the hydroxyl group, which allows nitrogens to exhibit the electron-withdrawing effect through resonance and thus to stabilize the parent 4-HP but destabilize the 4-HP-derived radical (Scheme 6).[37] While for 5-HP, both *meta*-nitrogens cannot participate in the resonance at all (Scheme 7),[38] implying that the *meta*-nitrogens have little effect on the O–H BDE of 5-HP. As a consequence, the O–H BDE of 5-HP is similar to that of phenol.[12,13,35]

In brief, the higher O–H BDE of 4-HP than that of 5-HP not only arises from the lower stability of 4-HP-derived radical but also from the higher stability of parent 4-HP. This is verified by the enthalpy difference between parent 4-HP and 5-HP, −339.394731 hartree versus −339.378193 hartree, and the enthalpy difference between 4-HP- and 5-HP-derived radicals, −338.714530 hartree versus −338.734542 hartree (Scheme 4). Nevertheless, although 4-HP is inert with respect to the H-atom-donating ability, its O–H BDE is,



Scheme 6. The resonance in 4-HP and derived radical, in which the charge density distribution in parent molecule, the spin density distribution in radical and the C–O bond length are indicated.



Scheme 7. The resonance in 5-HP and derived radical, in which the charge density distribution in parent molecule, the spin density distribution in radical and the C–O bond length are indicated.

as described above, very sensitive to the substituents, which makes some 4-HP derivatives, such as **4** and **5**, very active as antioxidants (Table 1). Considering the fact that **4** and **5** are physiological metabolites of folates, both of them are very promising to be applied in practice.

4. Conclusion

The antioxidant activity difference for 4-HP derivatives can be elucidated by O–H BDEs and IPs and can be further explained in terms of electronic effect and IHB effect of substituents, which provides a deeper insight into the SARs for folate-related antioxidants. Although 4-HP holds much higher O–H BDE than 5-HP and phenol, its BDE is more sensitive to substituents than that of phenol, which makes NH_2-derivatives of 4-HP very active as antioxidants. Therefore, 4-HP is also a potential lead antioxidant and deserves attention in the rational design of novel antioxidants.

Acknowledgements

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References and notes

1. Scott, G. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 165–170.
2. Halliwell, B.; Aeschbach, R.; Loliger, J.; Aruoma, O. I. *Food Chem. Toxicol.* **1995**, *33*, 601–617.
3. Aruoma, O. I. *Free Radical Biol. Med.* **1996**, *20*, 675–705.
4. Rezk, B. M.; Haenen, R. M. M.; van der Vijgh, W. J. F.; Bast, A. *FEBS Lett.* **2003**, *555*, 601–605.
5. Zhang, H. Y.; Wang, L. F. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 225–227.
6. Wang, L. F.; Zhang, H. Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3789–3792.
7. Wang, L. F.; Zhang, H. Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2609–2611.
8. Nenadis, N.; Zhang, H. Y.; Tsimidou, M. *J. Agric. Food Chem.* **2003**, *51*, 1874–1879.
9. Thomas, C. E. In *Handbook of Synthetic Antioxidants*; Packer, L., Cadenas, E., Eds.; Marcel Dekker, Inc.: New York, 1997, pp 1–52.
10. Noguchi, N.; Niki, E. *Free Radical Biol. Med.* **2000**, *28*, 1538–1546.
11. Zhang, H. Y.; Chen, D. Z. *Acta Biophys. Sinica* **2000**, *16*, 1–9.
12. Pratt, D. A.; DiLabio, G. A.; Brigati, G.; Pedulli, G. F.; Valgimigli, L. *J. Am. Chem. Soc.* **2001**, *123*, 4625–4626.
13. DiLabio, G. A.; Mastragostino, M.; Arbizzani, C.; Pratt, D. A. *Chem. Eur. J.* **2003**, *9*, 4997–5010.
14. Foti, M. C.; Johnson, E. R.; Vinqvist, M. R.; Wright, J. S.; Barclay, L. R. C.; Ingold, K. U. *J. Org. Chem.* **2002**, *67*, 5190–5196.

15. Wang, L. F.; Zhang, H. Y.; Kong, L.; Chen, Z. W.; Shi, J. G. *Helv. Chim. Acta* **2004**, *87*, 1515–1521.
16. Burton, G. W.; Page, Y. Le.; Gabe, E. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1980**, *102*, 7791–7792.
17. Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053–7065.
18. de Heer, M. I.; Mulder, P.; Korth, H. G.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **2000**, *122*, 2355–2360.
19. Zhang, H. Y.; Wang, L. F. *J. Phys. Chem. A* **2003**, *107*, 11258–11259.
20. van Acker, S. A. B. E.; Koymans, L. M. H.; Bast, A. *Free Radical Biol. Med.* **1993**, *15*, 311–328.
21. Migliavacca, E.; Carrupt, P. A.; Testa, B. *Helv. Chim. Acta* **1997**, *80*, 1613–1626.
22. Zhang, H. Y. *J. Am. Oil Chem. Soc.* **1998**, *75*, 1705–1709.
23. Zhang, H. Y.; Sun, Y. M.; Zhang, G. Q.; Chen, D. Z. *Quant. Struct.-Act. Relat.* **2000**, *19*, 375–379.
24. Wright, J. S.; Johnson, E. R.; DiLabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183.
25. Bakalbassis, E. G.; Chatzopoulou, A.; Melissas, V. S.; Tsimidou, M.; Tsolaki, M.; Vafiadis, A. *Lipids* **2001**, *36*, 181–184.
26. Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846–4851.
27. Jovanovic, S. V.; Steenken, S.; Hara, Y.; Simic, M. G. *J. Chem. Soc., Perkin Trans. 2* **1996**, *11*, 2497–2504.
28. Nakanishi, I.; Miyazaki, K.; Shimada, T.; Ohkubo, K.; Urano, S.; Ikota, N.; Ozawa, T.; Fukuzumi, S.; Fukuhara, K. *J. Phys. Chem. A* **2002**, *106*, 11123–11126.
29. Kong, L.; Sun, Z. L.; Wang, L. F.; Zhang, H. Y.; Yao, S. D. *Helv. Chim. Acta* **2004**, *87*, 511–515.
30. Bakalbassis, E. G.; Lithoxoidou, A. T.; Vafiadis, A. P. *J. Phys. Chem. A* **2003**, *107*, 8594–8606.
31. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
32. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Cliord, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Salvador, P.; Dannenberg, J. J.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaroni, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian 98, Revision A 11. Gaussian Inc.: Pittsburgh, PA, 2001.
33. Wu, Y. D.; Wong, C. L.; Chan, K. W. K.; Ji, G. Z.; Jiang, X. K. *J. Org. Chem.* **1996**, *61*, 746–750.
34. Zhang, H. Y.; Sun, Y. M.; Wang, X. L. *Chem. Eur. J.* **2003**, *9*, 502–508.
35. The O–H BDE of 5-HP was estimated to be 89.6 kcal/mol by (RO)B3LYP/6-311+G(2d,2p)//AM1/AM1 calculation[12] and 90.3 kcal/mol by fitting of the experimental values and theoretical data for 5HP-analogs.[13] In this paper, through the B3LYP/6-31+G(,3pd) calculation, the O–H BDE of 5-HP was calculated to be 91.45 kcal/mol.
36. The IP of 5-HP was estimated to be 219.7 kcal/mol by B3LYP/6-31G(d)//AM1/AM1 calculation, which was 24.3 kcal/mol higher than that of phenol.[12] In the current study, through the B3LYP/6-31+G(,3pd) calculation, the IP of 5-HP was calculated to be 214.66 kcal/mol, 20.63 kcal/mol higher than that of phenol and identical to the IP of 4-HP (Table 1).
37. (a) 4-HP is stabilized by both electron-withdrawing nitrogen atoms,[37b] because they can accept negative charges, which is supported by the charge distribution in 4-HP that both nitrogens hold much negative charges (Scheme 6), especially comparing with the charge distribution in 5-HP (Scheme 7). However, 4-HP-radical is not stabilized by both nitrogen atoms, because the electron-withdrawing property of nitrogen makes it inert in donating an electron to stabilize the oxygen-centered radical, which is supported by the spin density distribution in 4-HP-radical that most of the unpaired electron concentrates on oxygen and both nitrogens possess slight spin density (Scheme 6), in contrast with the spin density distribution in 5-HP-radical (Scheme 7); (b) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165–195, from which, one can find that some chemicals with both *meta*-nitrogens have positive Hammett parameters, indicating their electron-withdrawing property.
38. The distinct resonance behaviors of 4-HP, 5-HP and related radical are manifested in their different C–O bond lengths that the C–O bond of 4-HP is shorter than that of 5-HP, opposite to the trend in C–O bonds of 4-HP- and 5-HP-derived radicals (Schemes 6 and 7).