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H-Atom Acceptor Capacity of Free Radicals Used in Antioxidant Measurements

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ABSTRACT: Antioxidants are well-known for their beneficial effects on human health. Their capacity to scavenge free radicals mainly depends on their capacity to transfer H atoms to free radicals ($R^{\bullet}$), and indirectly to labile H-atoms of the $R^{\bullet}$-parent molecule (R—**H**). The aim of this study was to select an accurate method to estimate bond dissociation enthalpies (BDEs) of ROO—**H**, LOO—**H** and DPPH—**H**. $ROO^{\bullet}$ and $LOO^{\bullet}$ are radicals involved in lipid peroxidation and DPPH is a free radical used to measure antioxidant activities. BDE(ROO—**H**) was systematically calculated with more than 20 different methods (HF, post-HF and DFT). This methodology was performed on three ROO—**H** prototypes (HOO—**H**, CH_3OO—**H**, and CH_3CH_2OO—**H**) for which theoretical BDEs were compared to experimental BDEs. B3P86 and BHandH/6-31+G(d,p) appeared as the best compromises. The influence of R chain length and conjugation degree on BDEs was studied with DFT. Both parameters did not significantly change BDE(ROO—**H**), except when intra H bonding was formed. The DPPH—**H** BDE was accurately calculated with B3P86. This functional could definitely be considered as a good compromise since it also gave a reliable estimation of O—H BDE for polyphenols. Int J Quantum Chem 111: 1131–1142, 2011

Key words: free radical; antioxidants; BDE; DFT; lipid peroxidation; DPPH; peroxyradical

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Additional Supporting Information may be found in the online version of this article.

1. Introduction

Antioxidants are well-known for their beneficial effects on human health. An antioxidant is defined as a compound able to decrease oxidative stress, e.g., metal chelator, inhibitor of enzymes involved in oxidative stress, lipid peroxidation inhibitor, free-radical scavenger. The free-radical scavenging capacity is the primary antioxidant feature, and the structure activity relationship obtained is often correlated to the other antioxidant activities. Many of antioxidants are natural, e.g., vitamins and natural polyphenols. Over the past 30 years numerous experimental studies have described the antioxidant activities of polyphenols that are largely distributed in fruit, vegetables, spices, fruit juice, tea, chocolate, red wine, medicinal plants [1].

Free-radical scavenging capacity is evaluated *in vitro* and *in vivo*. The literature is rich in studies that report on (i) DPPH scavenging capacity and (ii) lipid peroxidation inhibition. The former measures the capacity of the compound to scavenge the DPPH [2] (2,2-diphenyl-1-picryl-hydrazyl) radical. This radical is relatively stable in solution [3] and free-radical scavenging measurements can easily be achieved to give a quick but reliable evaluation of the radical scavenging capacity. From an experimental/technical point of view the latter evaluation (lipid peroxidation inhibition) is slightly more sophisticated [4, 5]. It is directly achieved with lipid membranes (e.g., microsomes). The oxidative process is initiated by free radicals such as $^{\bullet}OH$ (e.g., produced by irradiation) or AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride). These species initiate the formation of carbon-centered radicals on lipids followed by peroxy radical ($LOO^{\bullet}$) formation after O_2 addition. Afterward radical propagation may occur between lipid chains (propagation phase). The antioxidant may scavenge either the initiator or the peroxy radicals. Both tests (DPPH scavenging and lipid peroxidation inhibition) are often correlated together except that results obtained from the latter are modulated by compound lipophilicity. Nonetheless for a compound able to approach or to incorporate in the membrane, the sheer free-radical scavenging property is the major parameter that drives the antioxidant capacity.

Natural polyphenols (ArOH) have been widely evaluated by these two tests. They may scavenge free radicals following four different mechanisms.

1. HAT (H Atom Transfer) from the molecule to the radical (direct O—H bond breaking) [6, 7]:

$$ArOH + R^{\bullet} \rightarrow ArO^{\bullet} + RH$$

2. ET-PT (Electron Transfer—Proton Transfer), in which electron transfer is followed by a proton release [8, 9]:

$$ArOH + R^{\bullet} \rightarrow ArOH^{+\bullet} + R^{-} \rightarrow ArO^{\bullet} + RH$$

3. SPLET (Sequential Proton Loss Electron Transfer), in which a proton is first lost [10–12]:

$$ArOH \rightarrow ArO^{-} + H^{+};$$
$$ArO^{-} + R^{\bullet} \rightarrow ArO^{\bullet} + R^{-};$$
$$R^{-} + H^{+} \rightarrow RH$$

4. Adduct formation, which is also a free radical scavenging mechanism. This may lead to the formation of relatively stable compounds after re-organization and H-transfer from solution [13].

$$ArOH + R^{\bullet} \rightarrow [ArOH - R]^{\bullet} \rightarrow \text{stable metabolites}$$

The last mechanism is relatively specific and is mainly observed in solutions rich in reactive species, e.g., radiolytic solutions. The first mechanism is directly driven by the O—H bond dissociation enthalpy (BDE = H[ArOH,298K] − H[$ArO^{\bullet}$,298K] − H[$H^{\bullet}$,298K]), while the second one is governed by the ionization potential (i.e., the $ArOH^{+\bullet}$ stability), and the third one depends on the antioxidant compound pKa and pH conditions of the solution. It must be stressed that the first three mechanisms are fundamentally different, but from the thermodynamic point of view they are similar i.e., same enthalpy of reaction (i.e., $\Delta H^{HAT} = \Delta H^{ET\text{-}PT} = \Delta H^{SPLET}$). The reaction between antioxidants (e.g., ArOH) and free radicals is thermodynamically favorable (ΔH = H[products] − H[reactants] = H[$ArO^{\bullet}$ + RH] − H[ArOH + $R^{\bullet}$] < 0) if BDE(R—**H**) is higher than BDE(ArO—**H**).

Over the past 5 years DFT (Density Functional Theory) gave very accurate BDE evaluations, especially for polyphenols [14–24]. The BDE

parameter (capacity to lose an H atom) strongly correlated with experimental free-radical scavenging capacity, whatever the three modes of action. The BDE parameter appeared as a major descriptor in structure-activity-relationships for antioxidant activities. Nonetheless, only a few studies systematically dealt with the thermodynamics of the radical reactions involved in the antioxidant action. The theoretical BDEs of antioxidants were considered as intrinsic parameters. To the best of our knowledge no study has reported on the systematic theoretical evaluation of R—**H** BDE as compared to antioxidant BDEs. In this publication BDE(ROO—**H**) was systematically evaluated by more than twenty different methods (HF, post-HF, and DFT). This methodology was performed on three ROO—**H** prototypes (HOO—**H**, CH_3OO—**H**, and CH_3CH_2OO—**H**) for which experimental BDEs were available. The chain-size effects were evaluated, i.e., BDE(ROO—**H**) was estimated for different R lengths. The chains were chosen in the presence or absence of a carboxylic acid group. In the presence of this group and when R is large enough ROO—**H** simulates a hydroperoxy issued from saturated and mono-unsaturated lipids and could be quoted LOO—**H**. To simulate poly-unsaturated fatty acids of cellular membranes, the BDE of the LOO—**H** hydroperoxy issued from arachidonic acid (AA) was calculated. It is well-known from the literature that four different AA conformers exist, i.e., angle-iron shape (linear), J-shape, U-shape, and helical shape. Molecular dynamic simulations demonstrated that the linear shape is the major conformer in lipid-bilayer membranes while the U-shape is the main conformation that exists in solution [25]. This study proposed the BDE(LOO—**H**) evaluation for these two conformers. Influence of the solvent on BDE was also calculated. The most accurate method and the best compromise were chosen to compare O—H BDEs of polyphenol-antioxidants. The BDE of DPPH—**H** was evaluated with different DFT methods and was compared to the experimental value. Stabilities and spin densities of the different radicals (DPPH and ROO$^\bullet$) were also determined.

Finding an accurate method of calculation but not time- and memory-consuming appeared crucial to evaluate thermodynamics of reactions involving antioxidants and free radicals. This is of great importance to predict the free-radical scavenging capacity of large series of potent-antioxidants.

2. Methodology

BDEs were calculated at 298K. If the free radical is quoted R$^\bullet$ (e.g., ROO$^\bullet$ and DPPH), the corresponding BDE of R—**H** is calculated as follows:

$$\mathrm{BDE}(\mathrm{R}-\mathbf{H}) = \mathrm{H}[\mathrm{R}-\mathbf{H}, 298\mathrm{K}] - (\mathrm{H}[\mathrm{R}^\bullet, 298\mathrm{K}] + \mathrm{H}[\mathrm{H}^\bullet, 298\mathrm{K}]),$$

where each H value corresponds to the sum of electronic and thermal enthalpies (i.e., electronic energy + ZPE + $E_{vibration}$ + $E_{rotation}$ + $E_{translation}$ + RT). Unrestricted DFT was used to describe radicals. No spin contamination was observed for the different radicals (i.e., $< S^2 >$ around 0.75 for a spin multiplicity of 2). Radical spin densities were evaluated by Mulliken population analysis. (The Mulliken spin density is defined as the difference of Mulliken charges of α and β electrons, on each atom).

Different approaches were used including (i) the sheer Hartree–Fock (HF) formalism, (ii) post-HF methods (MP2, CCD, CCSD), (iii) G1, G2MP2, and G3 which are optimized procedures mixing optimization and single point calculations at different levels, (iv) pure DFT functionals (BP86, BLYP, HCTH, HCTH147, HCTH407, HCTH93, VSXC, SVWN) and (v) hybrid functionals (B3LYP, B3P86, B1B95, B1LYP, O3LYP, B3PW91, B971, B972, B98, BMK, MPW1K, MPW1PW91, PBE1PBE, BHandH and BHandHLYP) including more or less HF exchange.

Basis set is known to be crucial to calculate energy, especially for radicals and for delocalized and polarized systems. The increase in basis-set size was systematically evaluated (from 6-31G to 6-311++G(2d,3pd)).

Calculations were carried out in the gas phase and in the presence of solvent. All structures were optimized in solvent phase from gas geometries. We used the integral-equation-formalism polarizable continuum model (IEF-PCM) [26, 27], as implemented in the Gaussian03 program [28]. From a fundamental point of view, in PCM models, the studied structure(s) is (are) embedded in a cavity surrounded by a dielectric continuum characterized by its dielectric constant. Different dielectric constants were used with permittivity ε equal to 2.27, 4.71, 24.85, 32.61, 78.35 for benzene, chloroform, ethanol, methanol, and water, respectively.

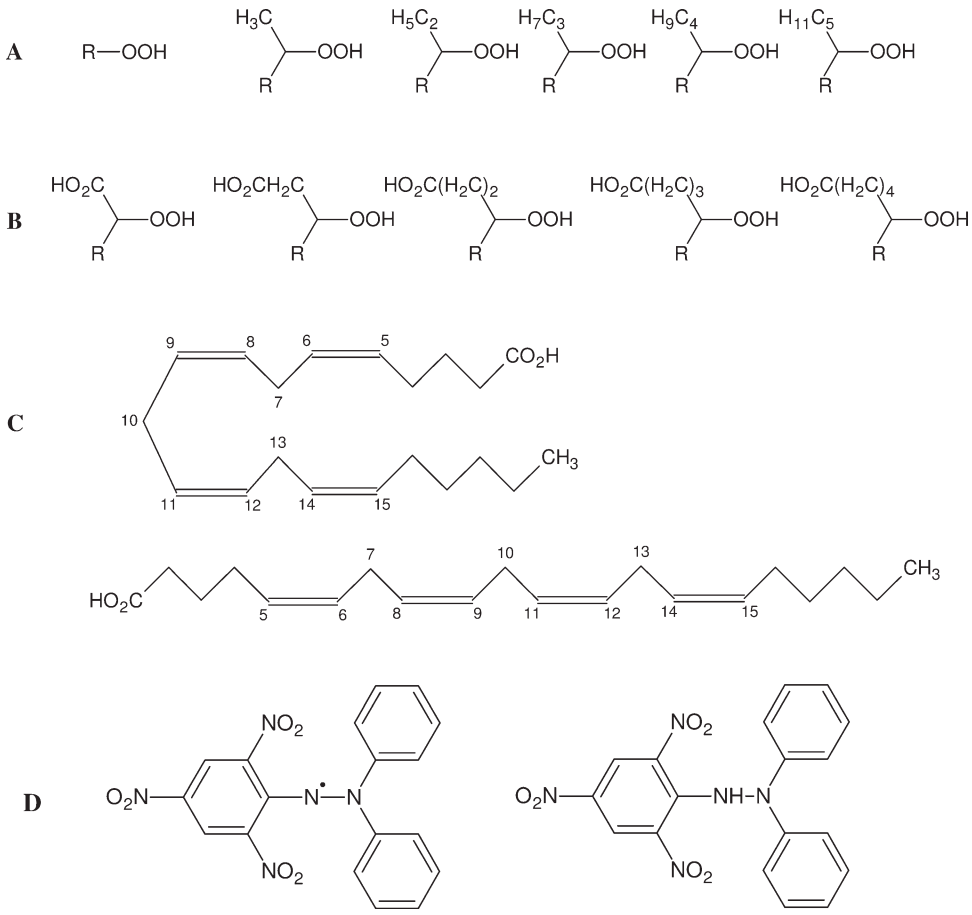

FIGURE 1. Structures of (A) alkyl compounds, (B) carboxylic derivatives, (C) arachidonic acid (U- and linear shapes) and (D) DPPH and DPPH—**H**. For series **A** and **B**, chain size is R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$ (chain size contains less than 12 C-atoms).

Concerning $ROO^{\bullet}$ and $LOO^{\bullet}$, the different structures that were studied are shown in Figure 1. HOO—**H**, CH_3OO—**H,** and CH_3CH_2OO—**H** are the three prototypes for which all the methods cited above were used. For the other compounds, only B3P86/6-31+G(d,p) and BHandH/6-31 +G(d,p) were used to determine the role of size and position of the aliphatic chain. Series **A** corresponded to a series of alkyl chains with a hydroperoxyl group (—OOH) located at different positions (Figure 1). Series **B** was built according to series **A** except that a carboxylic acid group was included at position 1 (Figure 1). The number of C-atoms was less than 12.

Conformational analysis demonstrated that all LOO—**H** structures were linear in shape, except for AA. Stabilizing H bonding occurred in some structures (discussion below). The AA conformers were optimized at the B3P86/6-31+G(d,p) level from starting geometries calculated by Barnett-Norris [25]. During lipid peroxidation, C—H bond rupture may occur at C4, C7, C10, C13, and C16. Dioxygen may thus add at different positions of AA (i.e., C5, C6, C8, C9, C11, C12, C14, and C15) to form the corresponding $LOO^{\bullet}$. BDE(LOO—**H**) was estimated for the eight isomers and for both conformers (U and linear shapes).

All calculations were performed by Gaussian03 [28].

3. Results and Discussion

3.1. BDE OF ROO—H SMALL PROTOTYPES

The intrinsic BDE(ROO—**H**) was experimentally estimated, mainly for HOO—**H,** but also for

TABLE I

Experimental BDEs for the different ROO-H prototypes.

ROO—H	BDE (kcal/mol)	Method	References
HOO—**H**	88.2 ± 1.0	Review	[29]
	87.2 ± 1.0	Laser magnetic resonance	[30]
	87.9 ± 0.8	Negative-ion photoelectron spectroscopy	[31]
	87.8 ± 0.5	Negative-ion photoelectron spectroscopy	[32]
CH_3OO—**H**	88.5 ± 0.5	Very low pressure reactor technic	[33]
	87.8 ± 1.0	Negative-ion photoelectron spectroscopy	[34]
CH_3CH_2OO—**H**	84.8 ± 2.2	Negative-ion photoelectron spectroscopy	[34]

other peroxy radicals including CH_3OO—**H** and CH_3CH_2OO—**H** [29–34]. The values obtained in the gas phase by different methods (photoelectron and photoionization mass spectroscopies, and VLPR (very low pressure reactor)) were relatively homogeneous. BDE(HOO—**H**) and BDE(CH_3OO—**H**) were around 88 ± 1 kcal/mol and BDE(CH_3CH_2OO—**H**) was around 85 ± 2 kcal/mol (Table I).

To correlate these values, theoretical gas-phase BDEs were calculated for these three compounds and are given in Tables II and III. The general conclusion that could be drawn from Table II was that the theoretical estimation of BDE(ROO—**H**) was relatively easy since classical methods failed. The pure HF method failed dramatically, giving a BDE value 30 kcal/mol lower than the expected value (i.e., experimental value). This essentially came from the lack of correlation effect that did not lead to a reliable description of $ROO^{\bullet}$ radicals. Stabilization of radicals was actually underestimated. The introduction of the correlation contribution according to the second-order Moller-Plesset formalism (MP2) definitely improved the quality of the calculation. The corresponding estimation appeared to be closer to the experimental value (e.g., BDE(HOO—**H** = 87.4 kcal/mol). As a consequence the G1, G2MP2, and G3 optimized procedures gave very accurate values, since they include Möller-Plesset correlation effects. These methods dramatically increased the computational time and were memory-consuming. Since the primary aim of this study was to extrapolate the method of calculation to bigger radicals and to natural antioxidants, methods including MP-corrections appeared to be irrelevant. The results were, however, an accurate reference for theoretical comparisons, e.g., comparison with DFT calculations.

The DFT results ranged from 80 to 94 kcal/mol and for most of the functionals (pure or hybrid) BDEs ranged from 80 to 82 kcal/mol. This was globally closer to the experimental value as compared to HF, thus improving the radical description. Nonetheless, DFT functionals (especially pure functionals) still underestimated radical stabilization and consequently BDEs. Only four functionals gave BDEs higher than 85 kcal/mol and closer to experimental values. The B3P86 and BHandH functionals resulted in the best agreement with the experimental value.

Table III shows BDE versus basis-set with B3P86 and BHandH. It must be stressed that by using the larger basis set we attainted a threshold (around 85.5 kcal/mol) that corresponded to the theoretical limit of accuracy for the method used (B3P86 and BHandH in this case). Convergence (to this threshold) was almost reached with 6-31+G(d,p). The use of polarization functions on the hydrogen atom was crucial, since without the p function on the H atoms, the BDE was lowered by about 3 kcal/mol. Adding more polarization functions on H atoms only slightly increased BDEs. Adding a diffuse function also significantly contributed to convergence, i.e., BDE increased by about 2 kcal/mol. The use of triple-ζ basis set did not significantly increase BDE, (less than 0.5 kcal/mol).

To achieve the best BDE(ROO—**H**) estimation, a post-HF approach (e.g., G1 or G2MP2) combined with a 6-311+G(2d,3pd) basis set would have been the most accurate. Nonetheless, this method would be difficult to use on bigger systems (i.e., lipid peroxy radicals and antioxidants such as vitamins and polyphenols). DFT is an alternative method but the choice of functional appeared crucial to estimate BDE(ROO—**H**), since it was not systematically accurately described. B3P86 and BHandH were the two best functionals to approximate the experimental BDE of the prototypes (i.e., HOO—**H**, CH_3OO—**H**, CH_3

TABLE II

BDEs of HOO—H, CH_3OO—H and CH_3CH_2OO—H obtained with different methods of calculation in gas phase using the 6-31+G(d,p) basis set.

	Methods	BDE (kcal/mol)		
		HOO—**H**	CH_3OO—**H**	CH_3CH_2OO—**H**
Pure DFT functionals	HCTH	80.3	78.2	77.5
	HCTH147	80.9	78.7	78.1
	HCTH407	80.3	78.2	77.5
	HCTH93	80.3	78.2	77.5
	VSXC	82.4	80.7	80.2
	SVWN	93.8	91.6	91.1
	BLYP	81.7	79.4	78.8
	BP86	84.0	81.9	81.3
Hybrid DFT functional	B1B95	82.2	80.6	80.1
	B1LYP	81.7	80.0	79.6
	B3LYP	83.0	81.2	80.7
	O3LYP	82.8	80.9	80.3
	B3P86	85.5	83.8	83.3
	B3PW91	81.8	80.1	79.6
	B971	81.6	79.9	79.4
	B972	81.4	79.7	79.1
	B98	82.6	80.9	80.5
	BHandH	87.0	86.1	85.8
	BHandHLYP	81.5	80.4	80.0
	BMK	83.5	81.8	81.5
	MPW1K	80.9	79.8	79.4
	MPW1PW91	81.1	79.5	79.1
	PBE1PBE	81.1	79.6	79.1
HF	HF	57.8	57.8	57.5
	MP2	87.4	86.7	86.4
	CCD	85.4	–	84.4
	CCSD	82.0	–	80.3
G – methods	G1	87.3	85.7	–
	G2MP2	88.2	86.6	–
	G3	86.7	85.1	–

CH_2OO—**H**). The 6-31+G(d,p) basis set was the best compromise (accurate and less computational consuming) for the basis set.

Solvent influence was studied by increasing dielectric permittivity from benzene to water (Table IV). As expected for HOO—**H**, CH_3OO—**H** and CH_3CH_2OO—**H**, the higher the permittivity, the higher the BDE and the higher the difference between experimental gas phase values. BDE increased by 3-4 kcal/mol from benzene to water.

3.2. INFLUENCE OF R-CHAIN POSITION AND LENGTH ON BDE(ROO—H)

The effect of system size on BDE(ROO—**H**) was estimated by increasing the length of the R-alkyl chains without (series **A**) and with a carboxylic acid group at C1 (series **B**). The presence of the carboxylic acid group was introduced so that series **B** simulated the oxidized saturated and monounsaturated fatty acids as they exist in the organism, mainly in membranes and lipoproteins. Calculations were performed with BHandH/6-31+G(d,p) and are given in Figure 2.

It must be stressed for the ROO—**H** compound without the carboxylic acid group (series **A**), that BDE was influenced neither by peroxide position nor by alkyl chain size. All BDEs were around 85 kcal/mol [Figure 2(a)]. Only a very slight decrease was observed when the number of C-atoms was higher than 3, which could be due to a very weak electronic effect along the alkyl chain.

TABLE III

Influence of basis set on BDEs of HOO—H, CH_3OO—H and CH_3CH_2OO—H at the DFT level in gas with (3a) B3P86 and (3b) BHandH.

Basis set	BDE (kcal/mol) HOO—**H**	CH_3OO—**H**	CH_3CH_2OO—**H**
3a			
6-31G	81.8	80.3	79.5
6-31G(d)	80.2	79.4	78.9
6-31G(d,p)	83.4	82.5	82.1
6-31+G(d,p)	85.5	83.8	83.3
6-31++G(d,p)	85.6	83.9	83.4
6-311G	81.1	79.7	79.1
6-311G(d)	79.4	78.4	78.0
6-311G(d,p)	83.7	82.5	82.1
6-311+G(d,p)	85.1	83.3	82.8
6-311++G(d,p)	85.1	83.3	82.8
6-311+G(d,2p)	85.9	84.1	83.6
6-311++G(d,2p)	85.9	84.1	83.6
6-311+G(2d,3p)	85.6	84.0	83.5
6-311++G(2d,3p)	85.6	83.8	83.5
6-311+G(2d,3pd)	86.0	84.3	83.9
6-311++G(2d,3pd)	86.0	84.3	83.9
3b			
6-31G	83.4	82.8	82.2
6-31G(d)	81.8	81.6	81.3
6-31G(d,p)	85.1	84.9	84.6
6-31+G(d,p)	87.0	86.1	85.8
6-31++G(d,p)	86.3	85.3	85.0
6-311G	82.4	81.9	81.4
6-311G(d)	80.6	80.3	80.1
6-311G(d,p)	85.2	84.6	84.4
6-311+G(d,p)	86.4	85.3	85.0
6-311++G(d,p)	86.3	85.3	85.0
6-311+G(d,2p)	87.2	86.1	85.8
6-311++G(d,2p)	87.2	86.1	85.8
6-311+G(2d,3p)	87.0	85.9	85.7
6-311++G(2d,3p)	86.9	85.9	85.6
6-311+G(2d,3pd)	87.4	86.4	86.1
6-311++G(2d,3pd)	87.4	86.3	86.1

TABLE IV

Influence of PCM solvent at the BHandH/6-31+G(d,p) level.

Solvent	ε	BDE (kcal/mol) HOO—**H**	CH_3OO—**H**	CH_3CH_2OO—**H**
Benzene	2.27	91.0	90.5	90.1
Chloroform	4.71	92.0	92.0	91.5
Ethanol	24.85	93.0	93.7	93.2
Methanol	32.61	93.1	93.8	93.3
Water	78.35	93.8	94.9	94.3

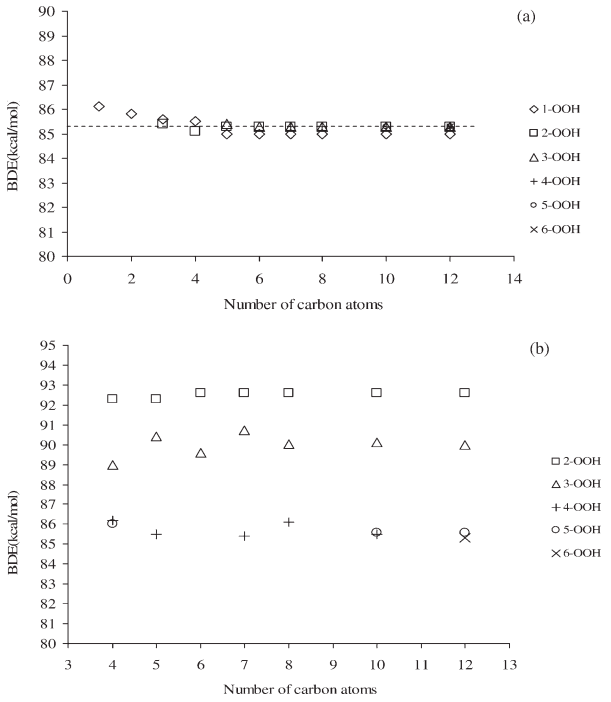

FIGURE 2. BDEs (BHandH/6-31+G(d,p) calculations) versus number of carbon atoms for (a) series **A** and (b) series **B**. Each curve corresponds to the different positions of the hydroperoxyl group, e.g., 2-OOH corresponds to compounds with OOH group on C2.

However, the difference was very close to the limit of chemical accuracy.

The very weak influence of R-alkyl-chain length was very consistent with the spin density distribution since no delocalization effect was observed. As expected spin density remained strongly localized on both O-atoms (0.98) for all structures (Figure 3).

The OOH position of carboxylic derivatives (series **B**) significantly impacted on ROO—**H** and LOO—**H** BDEs. The differences were due to the possibility of H bonding between hydroperoxy and carboxylic groups. As shown in Figure 4, the most stable H-bond was formed with the C=O group. Such an H bond was only possible if both groups were separated by less than one C-atom i.e., when the hydroperoxy group was located at position 2 or 3. This H bond stabilized the ROO—**H** molecule compared to the radical $ROO^{\bullet}$ thus increasing the BDE. The stabilizing effect was greater for the 2-hydroperoxy than for the 3-hydroperoxy radical since the H bond was stronger. The stabilizing enthalpy was around 7 and 4

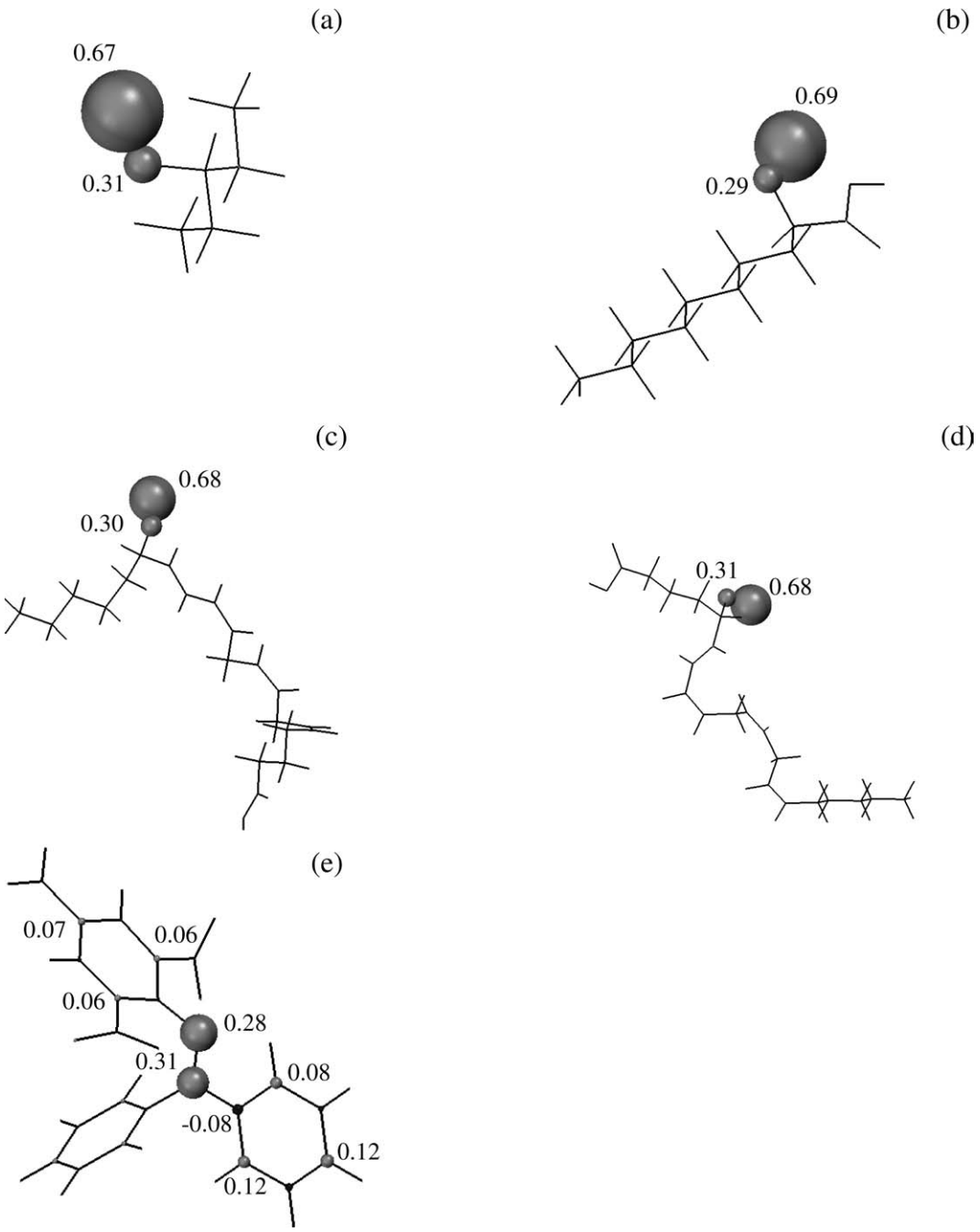

FIGURE 3. Spin density distribution of peroxyradicals (ROO•) for (a) a small prototype from series **A**, (b) an oxidized form of a saturated or monounsaturated fatty acid (series **B**), (c) an oxidized form of the polyunsaturated fatty acid AA in linear shape, (d) an oxidized form of the polyunsaturated fatty acid AA in U-shape. Figure 3(e) shows the spin density distribution of DPPH.

kcal/mol, and the H bond distance was 2.01 and 2.33 Å, respectively. In the absence of this H-bond BDE(ROO—**H**) was around 85 kcal/mol without any influence of the hydroperoxy position. As for series **A**, R chain length did not impact on BDEs. For all ROO• radicals from series **B**, the spin density on both O atoms was 0.98 (Figure 3).

Because BDE(ROO—**H**) was accurately calculated with B3P86 for the three prototypes and because this functional also demonstrated a good

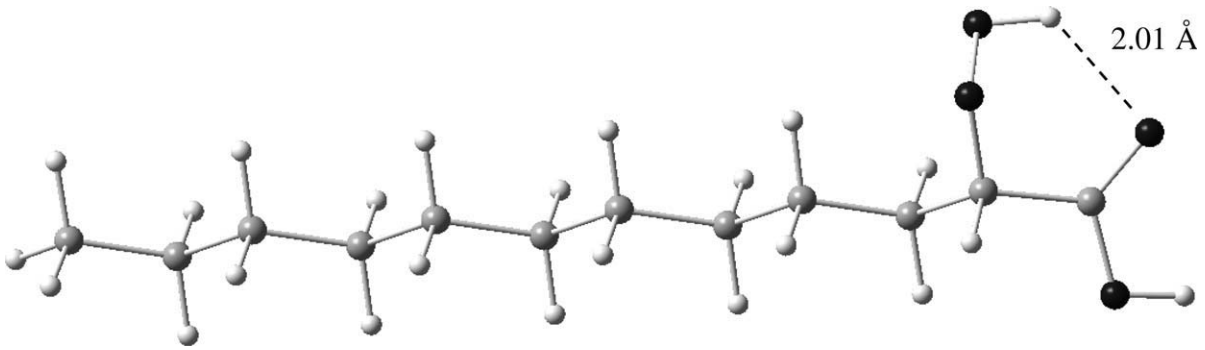

FIGURE 4. 3D conformation of a hydroperoxide from series **B** (hydroperoxyl group at C2) exhibiting an H bond with carboxylic acid.

accuracy to estimate polyphenol BDEs, R chain length was also calculated with B3P86 (data not shown). An identical trend was observed as for BHandH, except that the average value was decreased by about 3 kcal/mol.

3.3. ARACHIDONIC ACID BDE

As described above, series **B** gave a reliable description of BDE(LOO—**H**) in oxidized saturated and monounsaturated fatty acids. To estimate electronic effects that may influence BDE(LOO—**H**) in polyunsaturated fatty acids, this BDE was calculated for hydroperoxy compounds issued from AA oxidation. Eight plausible positions of the hydroperoxy group were evaluated (i.e., C5, C6, C8, C9, C11, C12, C14, and C15).

As for series **A** and **B**, position only slightly influenced the BDE that was around 85 kcal/mol (Figure 5). The only significant change was observed when the hydroperoxy group was at C14 in the U-shape. In this case a stabilizing H bond was formed between the hydroperoxy and the carboxylic-acid groups [Figure 6(a)]. BDE increased by about 6 kcal/mol. In all LOO$^{\bullet}$ radicals the spin density was strongly localized on both O-atoms and was around 0.98.

It must be emphasized that the U-shape conformation was partially lost when the hydroperoxy group was added to C11 and C12 [Figure 6(b)]. This addition resulted in π electron conjugation [Figure 6(c)] and would be crucial in solution in which AA is U in shape. Even in the presence of neighboring lipids, C11 and C12 oxidation would induce new steric interactions and a global membrane disorganization, which should partly explain membrane denaturation during lipoperoxydation.

3.4. DPPH RADICAL AND DPPH—H BDE

DPPH is a stable free radical widely used to measure antioxidant capacity. Its stability was partly explained by its capacity for spin density delocalization. As shown in Figure 3(e) spin density was delocalized over the entire molecule and was relatively low (0.28) on the N-atom. This could partly explain DPPH stability in solution as well as in solid phase. It was also demonstrated that, due to steric hindrance, DPPH dimerization was not favored [35].

Three dimension geometries of DPPH and its parent molecule (DPPH—**H**) were very similar, especially around the two N-atoms. This observation demonstrated that the presence or absence of the H atom (in DPPH—**H** or DPPH, respectively)

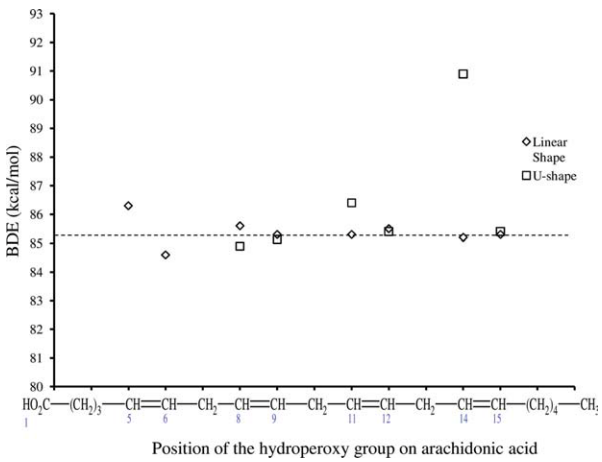

FIGURE 5. BDEs (BHandH/6-31+G(d,p) calculations) versus hydroperoxy group position for series C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

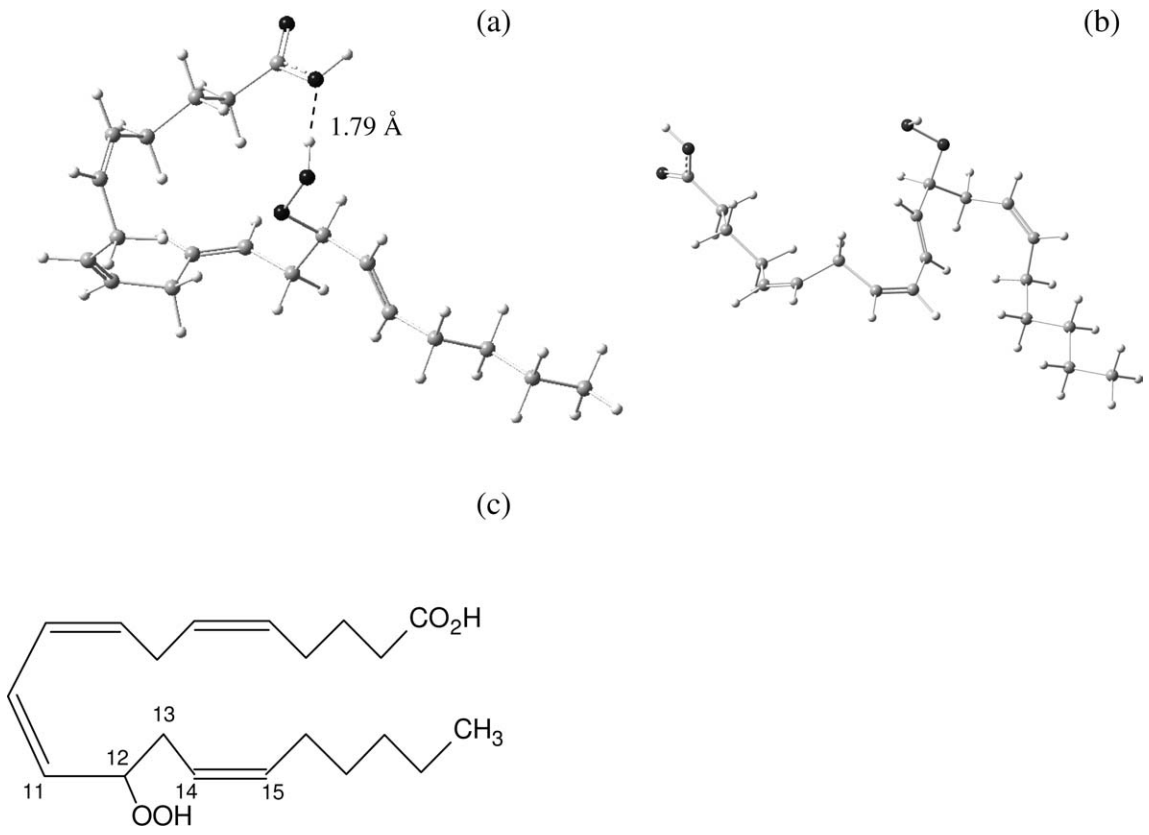

FIGURE 6. 3D conformations of oxidized AA form when the hydroperoxy group was at (a) C14 and (b) C12. Figure 6(a) shows the intra H bond between the hydroperoxy and carboxylic acid groups. Figure 6(c) shows the π electron conjugation possibility after O_2 addition on C12.

did not influence the electronic environment. This was confirmed by (i) very similar global charge distributions and (ii) N-atom hybridization characters for both DPPH and DPPH—**H**.

Antioxidants (e.g., polyphenols ArOH) are known to scavenge DPPH by HAT or SPLET [10, 36], following the global reaction:

$$\mathrm{ArO-\mathbf{H}+DPPH \rightarrow ArO^{\bullet}+DPPH-\mathbf{H}}$$

DPPH scavenging activity has been shown to be correlated with lipid peroxidation inhibition [21, 35, 37]. The former measurement (DPPH scavenging) is much easier to carry out than the latter (lipid peroxidation inhibition) and allows a quick but accurate estimation of global antioxidant capacity.

As explained in the introduction, the reaction between an antioxidant (e.g., ArOH) and DPPH would be thermodynamically favorable if BDE(DPPH—**H**) was higher than BDE(ArO—**H**). The experimental N—H BDE of DPPH—**H** obtained in benzene by calorimetry [38] was relatively low (around 80 kcal/mol), which explained that DPPH scavenging required very active compounds (i.e. compounds with low BDEs). By measuring DPPH scavenging hierarchies between different chemical compounds in terms of HAT capacity (i.e., structure activity relationship) can be established.

An accurate theoretical estimation of DPPH—**H** BDE was crucial to compare antioxidant and DPPH reactivity. It was calculated with the 6-31+G(d,p) basis set and different functionals. Gas phase BDEs were 79.3, 85.3, 76.0, 73.2, 72.0, 79.0, and 75.9 kcal/mol with B3P86, BHandH, B3LYP, PBE1PBE, HCTH407, BHandHLYP, and MPW1PW91, respectively.

To compare to the experimental BDE obtained in benzene, solvent influence was estimated and appeared to be weak, even by using a very polar dielectric constant. B3P86 BDEs were 79.3 and 80.4 kcal/mol in the gas phase and in the presence of PCM-like water, respectively. This very weak effect was confirmed by the small difference

(i) in dipole moment from gas to a polar solvent for both DPPH and DPPH—**H** (lower than 2 Debyes) and (ii) in geometry. For instance, the C—N—N—C dihedral angle in water was modified by only 1.5° for both DPPH and DPPH—**H**.

To conclude, the experimental value can be compared to the gas phase values. The best experimental agreement was obtained with B3P86, even better than for ROO—**H** and LOO—**H**. In contrast to ROO—**H** and LOO—**H** BDEs, BHandH failed in BDE(DPPH—**H**) estimation by about 5 kcal/mol. This definitely made the B3P86 functional a relevant compromise to evaluate H atom transfer capacity from/to free radicals, at least from the thermodynamic point of view.

4. Conclusion

The aim of this manuscript was to propose a reliable methodology to calculate the thermodynamic behavior of free radicals used to test antioxidant properties (ROO$^\bullet$ and DPPH). The main thermodynamic parameter that must be studied to understand the reaction between a free radical and a scavenger appeared to be the capacity to exchange H atoms (either by HAT, ET-PT or SPLET). The global capacity to exchange H atoms is measured by BDE, mainly O—H BDEs but in some cases N-H BDEs.

ROO—**H** BDE estimation failed with the Hartree-Fock formalism and correlation corrections were required to reach a better agreement with experimental data. Due to computational time and memory consumption, these calculations could not be used for larger free radicals (e.g., DPPH and LOO$^\bullet$) and for natural compounds (e.g., polyphenols ArO—**H**). When compared to experimental results, ROO—**H** and DPPH—**H** BDEs were accurately estimated by DFT methods. Globally speaking hybrid functionals appeared to be in better agreement with the available experimental data. The hybrid BHandH functional provided a very accurate evaluation of ROO—**H** BDEs but was less favorable for DPPH—**H**. The B3P86 hybrid functional was quite accurate for DPPH—**H** and ROO—**H** BDEs. This functional appeared to be a very good compromise since it also gave a reliable evaluation of O—H BDEs for polyphenols [19–21].

ROO—**H** BDEs were calculated on three prototypes, HOO—**H**, CH_3OO—**H**, and CH_3CH_2OO—**H**, for which experimental BDE values were available. Afterward, the influence of R chain size was studied. The key point of this study is that ROO—**H** and LOO—**H** BDEs were only slightly influenced by chain length. The BDE was around 85 kcal/mol for saturated/monounsaturated/polyunsaturated peroxy radicals containing from 3 to 20 C-atoms, except when intra H bonding was formed. This result may be of great importance for future studies on biological systems. Lipid peroxidation occurs on membranes and antioxidants such as polyphenols may act (inside or in the vicinity of membranes) during the propagation phase. In this case, antioxidants would scavenge LOO$^\bullet$ free radicals. To directly study these scavenging reactions from the quantum point of view, the whole system must be taken into account. This would not be feasible even with DFT methods. Nonetheless, our results suggest that only a small part of the peroxy radical needs to be examined with quantum chemistry. One can imagine QM/MM calculations, treating (i) the antioxidant and a small part of LOO—**H** (around the OO—H group) at the Quantum Mechanics level and (ii) the rest of the system (i.e., the rest of the lipid chain and neighboring chains) at the Molecular Mechanics level.

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References

1. Cos, P.; Ying, L.; Calomme, M.; Hu, J. P.; Cimanga, K.; Van Poel, B.; Pieters, L.; Vlietinck, A. J.; Vanden Berghe, D. J Nat Prod 1998, 61, 71.
2. Goldschmidt, S.; Renn, K. Ber Dtsch Chem Ges B 1922, 55B, 628.
3. Walter, R. I. J Am Chem Soc 1930, 1966, 88.
4. Halliwell, B.; Chirico, S. Am J Clin Nutr 1993, 57, 715S.
5. Gutteridge, J. M. C. Clin Chem (Washington, D. C.) 1995, 41, 1819.
6. Burton, G. W.; Doba, T.; Gabe, E.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. J Am Chem Soc 1985, 107, 7053.

7. De Heer, M. I.; Mulder, P.; Korth, H.-G.; Ingold, K. U.; Lusztyk, J. J Am Chem Soc 2000, 122, 2355.
8. Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. J Am Chem Soc 1994, 116, 4846.
9. Jovanovic, S. V.; Steenken, S.; Hara, Y.; Simic, M. G. J Chem Soc Perkin Trans 2 1996, 2497.
10. Zhang, H.-Y.; Ji, H.-F. New J Chem 2006, 30, 503.
11. Litwinienko, G.; Ingold, K. U. J Org Chem 2003, 68, 3433.
12. Foti, M. C.; Daquino, C.; Geraci, C. J Org Chem 2004, 69, 2309.
13. Anouar, E.; Kosinova, P.; Kozlowski, D.; Mokrini, R.; Duroux, J. L.; Trouillas, P. Phys Chem Chem Phys 2009, 11, 7659.
14. Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. Theor Chem Acc 2004, 111, 210.
15. Leopoldini, M.; Pitarch, I. P.; Russo, N.; Toscano, M. J Phys Chem A 2004, 108, 92.
16. Lemanska, K.; Szymusiak, H.; Tyrakowska, B.; Zielinski, R.; Soffers, A. E.; Rietjens, I. M. Free Radical Biol Med 2001, 31, 869.
17. Priyadarsini, K. I.; Maity, D. K.; Naik, G. H.; Kumar, M. S.; Unnikrishnan, M. K.; Satav, J. G.; Mohan, H. Free Radical Biol Med 2003, 35, 475.
18. Kozlowski, D.; Marsal, P.; Steel, M.; Mokrini, R.; Duroux, J.-L.; Lazzaroni, R.; Trouillas, P. Radiat Res 2007, 168, 243.
19. Kozlowski, D.; Trouillas, P.; Calliste, C.; Marsal, P.; Lazzaroni, R.; Duroux, J.-L. J Phys Chem A 2007, 111, 1138.
20. Trouillas, P.; Marsal, P.; Siri, D.; Lazzaroni, R.; Duroux, J.-L. Food Chem 2006, 97, 679.
21. Trouillas, P.; Marsal, P.; Svobodova, A.; Vostalova, J.; Gazak, R.; Hrbac, J.; Sedmera, P.; Kren, V.; Lazzaroni, R.; Duroux, J.-L.; Walterova, D. J Phys Chem A 2008, 112, 1054.
22. Belcastro, M.; Marino, T.; Russo, N.; Toscano, M. Theor Chem Acc 2006, 115, 361.
23. Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. J Phys Chem A 2004, 108, 4916.
24. Leopoldini, M.; Russo, N.; Toscano, M. J Agric Food Chem 2007, 55, 7944.
25. Barnett-Norris, J.; Guarnieri, F.; Hurst, D. P.; Reggio, P. H. J Med Chem 1998, 41, 4861.
26. Cossi, M.; Scalmani, G.; Rega, N.; Barone, V. J Chem Phys 2002, 117, 43.
27. Tomasi, J.; Mennucci, B.; Cammi, R. Chem Rev (Washington, DC, United States) 2005, 105, 2999.
28. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; 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.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision C. 02, ed. I. Gaussian: Wallingford, CT, 2004.
29. Shum, L. G. S.; Benson, S. W. Int J Chem Kinet 1983, 15, 433.
30. Howard, C. J. J Am Chem Soc 1980, 102, 6937.
31. Litorja, M.; Ruscis, B. J Electron Spectrosc Relat Phenom 1998, 97, 131.
32. Ramond, T. M.; Blanksby, S. J.; Kato, S.; Bierbaum, V. M.; Davico, G. E.; Schwartz, R. L.; Lineberger, W. C.; Ellison, G. B. J Phys Chem A 2002, 106, 9641.
33. Kondo, O.; Benson, S. W. J Phys Chem 1984, 88, 6675.
34. Blanksby, S. J.; Ramond, T. M.; Davico, G. E.; Nimlos, M. R.; Kato, S.; Bierbaum, V. M.; Lineberger, W. C.; Ellison, G. B.; Okumura, M. J Am Chem Soc 2001, 123, 9585.
35. Molyneux, P. Songklanakarin J Sci Technol 2004, 26, 211.
36. Musialik, M.; Litwinienko, G. Organic Lett 2005, 7, 4951.
37. Klein, E.; Lukes, V. J Phys Chem A 2006, 110, 12312.
38. Mahoney, L. R.; Mendenhall, G. D.; Ingold, K. U. J Am Chem Soc 1973, 95, 8610.