Peroxyl-Radical-Scavenging Activity of Garlic: 2-Propenesulfenic Acid versus Allicin

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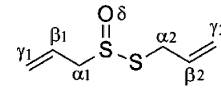
The OOH radical reactions with allicin and its Cope elimination products (2-propenesulfenic acid and thioacrolein) in aqueous solution have been studied. The CBS-QB3 quantum chemistry method has been used, with geometries and frequencies at BHandHLYP/6-311++G(d,p) level and conventional transition state theory. 2-Propenesulfenic acid is predicted to be over 1000 times more reactive toward OOH radical than allicin (2.60×10^7 vs 7.38×10^3 L mol^{-1} s^{-1}, at 298 K). Accordingly, our results strongly support the novel suggestion by Vaidya et al. (*Angew. Chem., Int. Ed.* **2009**, *48*, 157) that the active ingredient responsible for the free radical scavenging activity of garlic is actually 2-propenesulfenic acid and not allicin. In addition, direct reaction branching ratios and product distribution for the three studied reactions are proposed for the first time.

Introduction

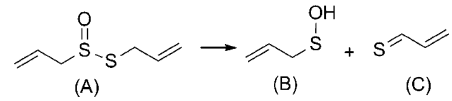
Garlic constitutes one of the first documented examples of plants used for its medicinal properties.[1] Among many other benefits, it has been proposed that garlic shows antioxidant activity.[2] This valuable effect is of particular importance since it means that garlic can be effective at fighting oxidative stress, which in the last decades has been related to the development of a large number of health disorders.[3]

Allicin (2-propenyl 2-propenethiosulfinate) is the most abundant thiosulfinate in fresh garlic,[4] and it has been proposed to be responsible for the free radical scavenging activity of garlic.[5−8] Okada et al.[7,8] suggested that the antioxidant activity of allicin occurs through a mechanism involving the abstraction of the allylic H atom adjacent to the divalent sulfur atom (site α2, Scheme 1). However, in a very recent work, Vaidya et al.[9] have argued that such a mechanism is unlikely for a peroxyl radical scavenger. They have based their statement on two facts: (i) rate constants for H atom transfer from hydrocarbons to peroxyl radicals are much lower than those reported for allicin-inhibited autoxidation reactions; and (ii) carbon-centered radicals generally undergo diffusion-controlled reactions with O_2 to yield peroxyl radicals, which continue to propagate the autoxidation chain reaction. They have proposed a very interesting alternative mechanism accounting for the antioxidant activity previously ascribed to allicin: that the allicin precursor and decomposition product, 2-propenesulfenic acid, is in fact the active ingredient involved in the free radical scavenging activity of garlic.[9] These authors computed O−H bond dissociation energies (BDE) of some sulfenic acids and their analogous hydroperoxides and demonstrated that the BDEs of the latter are systematically higher.[9] They also calculated some of the possible energy barriers in the gas phase and based on their values predicted the reactions to be diffusion controlled.[9]

SCHEME 1



SCHEME 2



Despite of the information so far, there are no previous studies addressing the kinetics of allicin versus that of 2-propenesulfenic acid. Such information would be very useful to quantify the difference on their reactivity toward a peroxyl radical and hopefully demonstrate which species is responsible for the antioxidant activity of garlic. There is no information either on the product distribution of the corresponding reactions, in spite of its importance for the understanding of the further fate of the studied systems. Therefore, it is the main aim of this work to perform a comparative study on the reactivity of allicin (A) versus its Cope elimination products: 2-propenesulfenic acid (B) and thioacrolein (C) (Scheme 2) toward the smallest peroxyl radical (•OOH). With that purpose in mind, different mechanisms of reaction have been tested, and thermodynamic and kinetic calculations have been performed to help identify the main channels of reaction in aqueous solution. In addition, the product distribution for the three studied reactions has been calculated and proposed for the first time.

Computational Details

Electronic structure calculations have been performed with the Gaussian 03 program.[10] The high-level composite method CBS-QB3[11] has been used for all of the calculations. The complete basis set (CBS) methods are compound methods that extrapolate to the CBS limit by using a N-1 asymptotic convergence of MP2 pair energies calculated from pair natural orbital expansions.[11,12] CBS methods were developed to over-

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come the truncation of the basis sets, which is a major source of error in quantum mechanical calculations. They also correct for spin contamination. CBS-QB3 is a five-step method that starts with geometry and frequency calculations using the B3LYP functional, followed by CCSD(T), MP4SDQ, and MP2 single-point calculations and a CBS extrapolation.[11] However, the B3LYP functional is known for underestimating barrier heights by an average of 4.4 kcal/mol for a database of 76 barrier heights.[13] This makes B3LYP a nonadequate method of choice for studies involving accurate kinetic calculation. To overcome this issue, all of the computed species were first optimized at BHandHLYP/6-311++G(d,p) level of theory followed by a frequency calculation at the same level. CBS-QB3 calculations were then carried out using these geometries and frequencies with the STARTMP2 option. The BHandHLYP functional has been chosen because it has been proven that the differences in geometries between several DFT methods compared to CCSD and QCISD are minimal for BHandHLYP.[14]

Unrestricted calculations were used for open shell systems, and local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). Thermodynamic corrections at 298 K were included in the calculation of relative energies. The stationary points were first modeled in the gas phase (vacuum), and solvent effects were included a posteriori by single-point calculations using a polarizable continuum model, specifically the integral equation formalism (IEF-PCM)[15] and RADII = UAHF, with water as solvent.

Relative Gibbs free energies in solution have been computed using the Hess law and thermodynamic cycles, explicitly including solvation free energies. For example, for H abstractions from A From this cycle, the Gibbs free energy of reaction

$$\begin{array}{ccccccc} A_{gas} & + & {}^{\bullet}OOH_{gas} & \longrightarrow & A(-H)^{\bullet}_{gas} & + & H_2O_{2\,gas} \\ \uparrow -\Delta G_s(A) & & \uparrow -\Delta G_s({}^{\bullet}OOH) & & \downarrow \Delta G_s(A_{(-H)}{}^{\bullet}) & & \downarrow \Delta G_s(H_2O_2) \\ A_{sol} & + & {}^{\bullet}OOH_{sol} & \longrightarrow & A(-H)^{\bullet}_{sol} & + & H_2O_{2\,sol} \end{array}$$

in solution (ΔG_{sol}) can be obtained as the sum of the Gibbs free energy of reaction in vacuum (ΔG_{gas}) and the difference in solvation free energies ($\Delta\Delta G_s$):

$$\Delta G_{sol} = \Delta G_{gas} + \Delta\Delta G_s \quad (1)$$

where $\Delta\Delta G_s$ is calculated as

$$\Delta\Delta G_s = \Delta G_s(A(-H)^{\bullet}) + \Delta G_s(H_2O_2) - \Delta G_s(A) - \Delta G_s({}^{\bullet}OOH) \quad (2)$$

with ΔG_s representing the free energies of solvation for each species. In all of the cases, the reference state is 1M. In a similar way, the free energies of activation ($\Delta G sol^{\ddagger}$) were calculated as

$$\Delta G^{\ddagger}_{sol} = \Delta G^{\ddagger}_{gas} + \Delta\Delta G^{\ddagger}_s \quad (3)$$

with

$$\Delta\Delta G^{\ddagger}_s = \Delta G_s(TS) - \Delta G_s(A) - \Delta G_s({}^{\bullet}OOH) \quad (4)$$

The solvent cage effects have been included according to the corrections proposed by Okuno,[16] taking into account the free volume theory.[17] These corrections are in good agreement with those independently obtained by Ardura et al.[18] and have been successfully used by other authors.[19]

The rate constants (k) were calculated using conventional transition state theory (TST)[20−22] and 1M standard state as

$$k = \sigma\kappa\frac{k_B T}{h}e^{-(\Delta G^{\ddagger})/RT} \quad (5)$$

where k_B and h are the Boltzmann and Planck constants, respectively, $\Delta G^{\ddagger}$ is the Gibbs free energy of activation, σ represents the reaction path degeneracy, accounting for the number of equivalent reaction paths, and κ accounts for tunneling corrections. The tunneling corrections defined as the Boltzmann average of the ratio of the quantum and the classical probabilities were calculated using the Eckart method.[23]

Results and Discussion

On the basis of the structure of the reactants shown in Scheme 2, three different mechanisms have been considered for their reaction with the OOH radical:

radical adduct formation (RAF):

$$X + {}^{\bullet}OOH \rightarrow XOOH^{\bullet} \quad (I)$$

hydrogen atom transfer (HAT):

$$X + {}^{\bullet}OOH \rightarrow X(-H)^{\bullet} + H_2O_2 \quad (II)$$

single electron transfer (SET):

$$X + {}^{\bullet}OOH \rightarrow X^{\bullet+} + OOH^{-} \quad (III)$$

with X = A, B, or C. It should be noticed that proton loss can occur combined with SET in a coupled or sequential way. This deprotonation step would correspond to

$$X^{\bullet+} \rightarrow X(-H)^{\bullet} + H^{+} \quad (IIIa)$$

and would lead to the same radical product as HAT.

The SET processes were found to be endergonic by 47.8, 38.2, and 47.4 kcal/mol for A, B, and C, respectively, indicating that electron transfers from the studied reactants to the OOH radical are not feasible. However, attention must be called on the fact that this is valid only for this radical because the nature of free radicals may play an important role on the relative importance of the competing mechanisms.[24] Electron transfers to other radicals with higher $R^{\bullet}/R^{-}$ reduction potential might be feasible. Hydroxyl radical for example has an $OH^{\bullet}/OH^{-}$ potential of 1.89 V vs NHE,[25] significantly higher than the $OOH^{\bullet}/OOH^{-}$ potential (0.79 V vs NHE[26]). Another important point is that the above-mentioned Gibbs free energy values rule out the SET, from A, B, and C to $^{\bullet}OOH$ process when it occurs as an isolated process, but it can still be feasible if this process takes place coupled with deprotonation (PCET). Since PCET energetic data (heats of reaction, energy barriers, etc.) are identical to those of HAT mechanism, from this point, we will

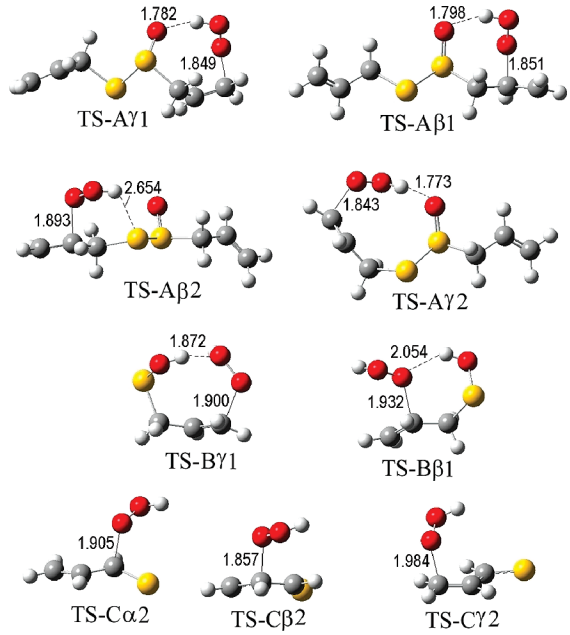

Figure 1. Optimized geometries of the transition states corresponding to ˙OOH addition reactions (RAF mechanism). A, B, and C are for allicin, 2-propenesulfenic acid, and thioacrolein, respectively.

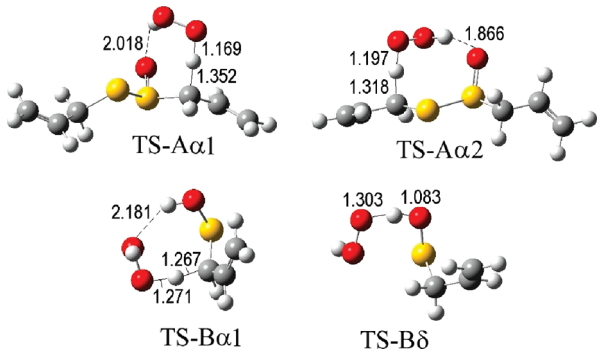

Figure 2. Optimized geometries of the transition states corresponding to H abstraction reactions (HAT mechanism). A, B, and C are for allicin, 2-propenesulfenic acid, and thioacrolein, respectively.

analyze only RAF and HAT processes, and any information on the latter will also be valid for PCET.

The optimized structures of the transition states (TS) corresponding to the RAF mechanism are shown in Figure 1. All those corresponding to ˙OOH additions to allicin (A) and 2-propenesulfenic acid (B) show H bond intramolecular interactions leading to cyclic TS structures. For the thioacrolein (C) transition states, on the other hand, no intramolecular interactions were found. The longest forming C−O bond distance was found for TS-Cγ2, indicating that this is the earliest TS and suggesting that the addition of the OOH radical to the γ site in thioacrolein should be the most feasible among all the studied RAF processes. The TS structures, corresponding to the HAT mechanism (Figure 2), also show H bond intramolecular interactions, with the exception of TS-Bδ. The longest forming O−H bond distance was found for TS-Bδ, which suggests that this is the most reactive site among all of the computed HAT reactions. It should be mentioned that H abstractions from vinylic sites were also computed, but they are not going to be discussed here because they were all found to be endergonic (Table S1, Supporting Information).

Gibbs free energies of reaction (ΔG) and barriers ($\Delta G^{\ddagger}$) at 298.15 K are reported in Table 1. According to these values,

TABLE 1: Gibbs Free Energies of Reaction (ΔG) and Barriers ($\Delta G^{\ddagger}$) at 298.15 K (all in kcal/mol) Corresponding to All Studied Channels of Reaction in Aqueous Solution

	RAF			HAT	
	ΔG	$\Delta G^{\ddagger}$		ΔG	$\Delta G^{\ddagger}$
		allicin			
Aβ1	1.00	17.26	Aα1	−6.03	18.19
Aγ1	−0.97	14.81	Aα2	−7.62	17.97
Aβ2	1.97	21.71			
Aγ2	−0.39	15.05			
		2-propenesulfenic acid			
Bβ1	0.66	19.92	Bα1	−6.51	21.10
Bγ1	−0.26	18.94	Bδ	−17.59	10.17
		thioacrolein			
Cα2	−12.88	18.25			
Cβ2	−2.40	22.29			
Cγ2	−15.27	13.67			

all of the HAT processes are significantly exergonic. For RAF reactions, on the other hand, three of the modeled channels were found to be endergonic: Aβ1, Aβ2, and Bβ1. Four other channels were found to be exergonic but with the energy of the products only slightly lower than those of the starting reactants: Aγ1, Aγ2, Bγ1, and Cβ2. Therefore, these channels of reaction are expected to correspond to reversible processes. Only ˙OOH additions to sites α and γ in thioacrolein were found to be significantly exergonic.

For the reaction of ˙OOH with allicin, the lowest barriers of reaction were found to correspond to addition processes (channels Aγ1 and Aγ2) and not to an abstraction reaction. Therefore, these channels are expected to be the fastest ones. However, since they are expected to be reversible, as discussed above, for long enough times of reaction, the most abundant products should be those formed through Aα1 and Aα2 channels (HAT). Since thioacrolein does not have any H susceptible to be abstracted in its structure, it can only react with ˙OOH through the RAF mechanism. The ˙OOH addition with lowest barrier for this reactant was found to be that corresponding to the γ site. This is also the C channel with largest exergonicity. Therefore, for the ˙OOH reaction with thioacrolein, the adduct formed by addition to the terminal carbon should be the major product. For the reaction of ˙OOH with 2-propenesulfenic acid, the H abstraction from the hydroxyl moiety was found to be, by far, the lowest energy barrier. As for thioacrolein, the B channel with the lowest barrier is also the most exergonic one. Consequently, the main product for the 2-propenesulfenic acid + ˙OOH reaction is proposed to be the radical formed through channel Bδ. According to our results, the OH group in 2-propenesulfenic acid is not only the most reactive site toward OOH radical in 2-propenesulfenic acid but also the most reactive of all the studied sites of reaction, including those in A and C. This supports the hypothesis by Vaidya et al.[9] that the active ingredient accounting for the peroxyl scavenging activity of garlic is actually 2-propenesulfenic acid, and not allicin, as previously thought.

Rate constant calculations have also been performed for each of the modeled RAF and HAT channels of reaction (Table 2). The overall rate constant for each reactant (A, B, and C) that measures the rate of ˙OOH disappearance has then been estimated by summing the rate coefficients calculated for all the corresponding channels. This approach implies that once a specific channel started it proceeds to completion, independently of the other pathways; that is, there is no mixing or crossover between different pathways. According to our results, the overall

TABLE 2: Rate Constants (k) at 298 K (L mol^{-1} s^{-1}) Corresponding to All Studied Channels of Reaction in Aqueous Solution

k^{RAF}		k^{HAT}	
	allicin		
Aβ1	6.79×10^{1}	Aα1	9.54×10^{1}
Aγ1	4.24×10^{3}	Aα2	1.51×10^{2}
Aβ2	3.67×10^{-2}		
Aγ2	2.82×10^{3}		
	2-propenesulfenic acid		
Bβ1	7.58×10^{-1}	Bα1	6.15×10^{-1}
Bγ1	$3.96 \times 10^{\circ}$	Bδ	2.60×10^{7}
	thioacrolein		
Cα2	1.27×10^{1}		
Cβ2	1.38×10^{-2}		
Cγ2	2.87×10^{4}		

TABLE 3: Branching Ratios (Γ) and Relative Product Population at Equilibrium Conditions (P^{MB}) for Reactions in Aqueous Solution at 298.15 K

k^{RAF}			k^{HAT}		
	Γ^a	P^{MB}[b]		Γ^b	P^{MB}[b]
			allicin		
Aβ1	0.92	~0	Aα1	1.29	6.38
Aγ1	57.50	~0	Aα2	2.05	93.61
Aβ2	~0	~0			
Aγ2	38.24	~0			
			2-propenesulfenic acid		
Bβ1	~0	~0	Bα1	~0	~0
Bγ1	~0	~0	Bδ	~100	~100
			thioacrolein		
Cα2	0.04	1.73			
Cβ2	~0	~0			
Cγ2	99.96	98.27			

[a] $\Gamma_i = k_i/k_{overall} \times 100$. [b] Maxwell−Boltzmann distribution.

rate constants at 298.15 K for the •OOH reactions with allicin, 2-propenesulfenic acid, and thioacrolein are 7.38×10^3, 2.60×10^7, and 2.87×10^4 L mol^{-1} s^{-1}, respectively.

It is interesting to notice that not only 2-propenesulfenic acid reacts faster with •OOH than allicin but also thioacrolein does. This strongly supports the proposal that allicin decomposition products are better peroxyl radical scavengers than allicin itself.[9] It is also interesting that RAF processes (Aγ1 and Aγ2) for the allicin + •OOH reaction are the fastest and not the HAT ones. However, it seems worthwhile to emphasize again on the reversibility of the adduct formation (RAF), which is expected to make HAT products the most abundant ones under equilibrium conditions. In fact Maxwell−Boltzmann distribution, including all of the products of allicin + •OOH reaction (Table 3), shows that at equilibrium 93.6% of the product population would correspond to the species formed by abstraction of the allylic H atom adjacent to the divalent sulfur atom (α2). That is why this channel of reaction has been identified as the main channel for the allicin + •OOH reaction.[7,8]

Our calculated overall rate constant for 2-propenesulfenic acid + •OOH reaction is in line with the value estimation made by Koelewijn and Berger[27] for the reaction of 2-methyl-2-propanesulfenic acid with tetralyl peroxyl radicals (>10^7 L mol^{-1} s^{-1}). The calculated rate constant for the allicin + •OOH reaction is also in line with the value reported for the reaction of allicin with peroxyl radicals derived from cumene (2.6×10^3 L mol^{-1} s^{-1}).[7] The agreement with the experimental rate constants supports the results computed in the present work. The agreement between the calculated rate constant for the allicin + •OOH reaction and that estimated by Okada et al.[7] suggests that their work was performed under conditions that prevent the allicin Cope elimination reaction.

In addition to the continuum, another solvation model has been tested, as suggested by one of the reviewers. It includes an explicit water molecule in addition to the continuum. We have tested the influence of the different solvation approaches for the Gibbs free energy of reaction of two selected channels (Bα1 and Bδ) and for the barrier of the main one (Bδ). To calculate the ΔG of reaction, two different approaches have been tested: (i) with the water molecule in the vicinity of the OOH radical in the reactant valley and in the vicinity of H_2O_2 molecule in the product valley; and (ii) with the explicit water molecule in the vicinity of the 2-propenesulfenic acid and the corresponding product. For $\Delta G^{\ddagger}$, only one transition structure was located, with the water molecule in the vicinity of the OH moiety from where the H is being abstracted (Figure S1 in the Supporting Information). The comparison of the results computed within the different approaches is reported in Table S2 and shows that the values obtained within the approach (i) are systematically 1.48 kcal/mol higher than those obtained with the continuum approach. This is in agreement with what has been reported for guanosine + •OH reaction.[28] The average of the differences between the results computed with and without the explicit water molecules (including both aproaches (i) and (ii) together) is 1.41 kcal/mol. Since the differences in ΔG are about the same magnitude for the two tested systems, it is expected that the relative reactivity of the studied species (which is the main focus of the present investigation) would be the same, regardless of the solvation method. In addition, the best agreement with the estimation made by Koelewijn and Berger[27] is obtained with the continuum model. In general, and taking into account the above discussion, we found no evidence that the inclusion of an explicit water molecule would increase the accuracy of the performed calculation. We feel confident about the reliability of the kinetic and thermochemical data obtained within the continuum model alone (at least at a semiquantitative level), and certainly about the order of reactivity of the studied species toward peroxyl radicals.

The direct reaction branching ratios, which are a measurement of the kinetic site reactivity, together with products population, accounting for the relative distribution of products under equilibrium conditions, are reported in Table 3 for each channel of the studied reactions. Due to the reversibility of the RAF channels for allicin, discussed above, there is a significant difference between the kinetic site reactivity and the products that are expected to prevail under equilibrium conditions. Therefore, it can be established that the allicin + OOH radical reaction is thermodynamically controlled. For 2-propenesulfenic acid and thioacrolein, on the other hand, the most kinetically reactive site also corresponds to the product lowest in energy. Accordingly, under equilibrium conditions, the predominant products of reaction are predicted to be those formed through channels Aα2, Bδ, and Cγ2 for allicin, 2-propenesulfenic acid, and thioacrolein, respectively.

According to the overall rate constants reported above, 2-propenesulfenic acid is over 1000 times more reactive toward OOH radical than allicin. The difference in reactivity between these two molecules is so large that it can safely hypothesize that 2-propenesulfenic acid is significantly more reactive toward peroxyl radicals than allicin. Therefore, our results definitively support the recent proposal by Vaidya et al.[9] that 2-propene-

sulfenic acid is responsible for the antioxidant activity of garlic and not allicin.

Conclusions

2-Propenesulfenic acid has been found to be much more reactive toward peroxyl radicals than allicin, in agreement with what has been recently proposed by Vaidya et al.[9] It was found to react over 1000 times faster with OOH radical than allicin (2.60×10^7 vs 7.38×10^3 L mol^{-1} s^{-1}, at 298.15 K). In addition, the main channel for the $^{\bullet}$OOH + 2-propenesulfenic acid reaction was found to be, by far, the H abstraction from the hydroxyl moiety. When the $^{\bullet}$OOH reaction with allicin is carried out under conditions that prevent Cope elimination, 93.6% of the product population is expected to correspond to the species formed by abstraction of the allylic H atom adjacent to the divalent sulfur atom, under equilibrium conditions, as proposed by Okada et al.[7,8] For the reactions of 2-propenesulfenic acid and thioacrolein, almost 100% of the products is proposed to correspond to H abstraction from the OH moiety and $^{\bullet}$OOH addition to the terminal carbon, respectively. The agreement with the available experimental data supports the results from the present work.

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Supporting Information Available: Gibbs free energies of reaction for H abstractions from vinylic sites. Comparison of solvation models. Geometry of stationary points including one explicit water molecule. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Rivlin, R. S. *J. Nutr.* **2001**, *131*, 951Sand references therein.

(2) See for example: (a) Prasad, K.; Laxdal, V. A.; Yu, M.; Raney, B. L. *Mol. Cell. Biochem.* **1996**, *154*, 55. (b) Yin, M.-C.; Cheng, W.-S. *J. Agric. Food Chem.* **1998**, *46*, 4097.

(3) See for example: (a) Lin, M. T.; Beal, M. F. *Nature* **2006**, *443*, 787. (b) Reddy, P. H. *J. Neurochem.* **2006**, *96*, 1. (c) Schoeneich, C. *Biochim. Biophys. Acta* **2005**, *1703*, 111. (d) Giasson, B. I.; Ischiropoulos, H.; Lee, V. M.Y.; Trojanowski, J. Q. *Free Radical Biol. Med.* **2002**, *32*, 1264. (e) Aksenov, M. Y.; Aksenov, M. V.; Butterfield, D. A.; Geddes, J. W.; Markesbery, W. R. *Neuroscience* **2001**, *163*, 373. (f) Perry, G.; Raina, A. K.; Nunomura, A.; Wataya, T.; Sayre, L. M.; Smith, M. A. *Free Radical Biol. Med.* **2000**, *28*, 831. (g) Berlett, B. S.; Stadtman, E. R. *J. Biol. Chem.* **1997**, *272*, 20313.

(4) Rybak, M. E.; Calvey, E. M.; Harnly, J. M. *J. Agric. Food Chem.* **2004**, *52*, 682, and references therein.

(5) Prasad, K.; Laxdal, V. A.; Yu, M.; Raney, B. L. *Mol. Cell. Biochem.* **1995**, *148*, 183.

(6) Xiao, H.; Parkin, K. L. *J. Agric. Food. Chem.* **2002**, *50*, 2488.

(7) Okada, Y.; Tanaka, K.; Fujita, I.; Sato, E.; Okajima, H. *Org. Biomol. Chem.* **2006**, *4*, 4113.

(8) Okada, Y.; Tanaka, K.; Fujita, I.; Sato, E.; Okajima, H. *Org. Biomol. Chem.* **2008**, *6*, 1097.

(9) Vaidya, V.; Ingold, K. U.; Pratt, D. A. *Angew. Chem., Int. Ed.* **2009**, *48*, 157.

(10) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R. ; Montgomery J. A., Jr.; 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 E.01; Gaussian, Inc.: Wallingford, CT, 2004.

(11) Montgomery, J. A.; Frisch, M. J.; Ochterski, J. W.; Petersson, G. A. *J. Chem. Phys.* **1999**, *110*, 2822.

(12) (a) Nyden, M. R.; Petersson, G. A. *J. Chem. Phys.* **1981**, *75*, 1843. (b) Al-Laham, M. A.; Petersson, G. A. *J. Chem. Phys.* **1991**, *94*, 6081. (c) Petersson, G. A.; Tensfeldt, T. G.; Montgomery, J. A. *J. Chem. Phys.* **1991**, *94*, 6091. (d) Petersson, G. A.; Malick, D. K.; Wilson, W. G.; Ochterski, J. W.; Montgomery, J. A.; Frisch, M. J. *J. Chem. Phys.* **1998**, *109*, 10570. (e) Montgomery, J. A.; Frisch, M. J.; Ochterski, J. W.; Petersson, G. A. *J. Chem. Phys.* **2000**, *112*, 6532.

(13) (a) Zhao, Y.; Gonzalez-García, N.; Truhlar, D. G. *J. Phys. Chem. A* **2005**, *109*, 2012. (b) Zhao, Y.; Truhlar, D. G. *Acc. Chem. Res.* **2008**, *41*, 157.

(14) Szori, M.; Fittschen, C.; Csizmadia, I. G.; Viskolcz, B. *J. Chem. Theory Comput.* **2006**, *2*, 1575.

(15) (a) Cances, M. T.; Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3032. (b) Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *106*, 5151. (c) Mennucci, B.; Cances, E.; Tomasi, J. *J. Phys. Chem. B* **1997**, *101*, 10506. (d) Tomasi, J.; Mennucci, B.; Cances, E. *J. Mol. Struct. (THEOCHEM)* **1999**, *464*, 211.

(16) Okuno, Y. *Chem.—Eur. J.* **1997**, *3*, 212.

(17) Benson, S. W. *The Foundations of Chemical Kinetics*; Krieger: Malabar, FL, 1982.

(18) Ardura, D.; Lopez, R.; Sordo, T. L. *J. Phys. Chem. B* **2005**, *109*, 23618.

(19) (a) Alvarez-Idaboy, J. R.; Reyes, L.; Cruz, J. *Org. Lett.* **2006**, *8*, 1763. (b) Galano, A. *J. Phys. Chem. A* **2007**, *111*, 1677; **2007**, *111*, 4726 (Addition/Correction). (c) Alvarez-Idaboy, J. R.; Reyes, L.; Mora-Diez, N. *Org. Biomol. Chem.* **2007**, *5*, 3682. (d) Galano, A.; Cruz-Torres, A. *Org. Biomol. Chem.* **2008**, *6*, 732. (e) Galano, A.; Francisco-Márquez, M. *Chem. Phys.* **2008**, *345*, 87. (f) Galano, A. *J. Phys. Chem. C* **2008**, *112*, 8922.

(20) Eyring, H. *J. Chem. Phys.* **1935**, *3*, 107.

(21) Evans, M. G.; Polanyi, M. *Trans. Faraday Soc.* **1935**, *31*, 875.

(22) Truhlar, D. G.; Hase, W. L.; Hynes, J. T. *J. Phys. Chem.* **1983**, *87*, 2664.

(23) Eckart, C. *Phys. Rev.* **1930**, *35*, 1303.

(24) Galano, A.; Alvarez-Diduk, R.; Ramirez-Silva, A.T.; Alarcon-Angeles, G.; Rojas-Hernández, A. *Chem. Phys.* **2009**, *363*, 13.

(25) Schwarz, H. A.; Dodson, R. W. *J. Phys. Chem.* **1984**, *88*, 3643.

(26) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637.

(27) Koelewijn, P.; Berger, H. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 1275.

(28) Galano, A.; Alvarez-Idaboy, J. R. *Org. Lett.* **2009**, DOI: 10.1021/ol901862h.

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