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Capsaicin, a Tasty Free Radical Scavenger: Mechanism of Action and Kinetics

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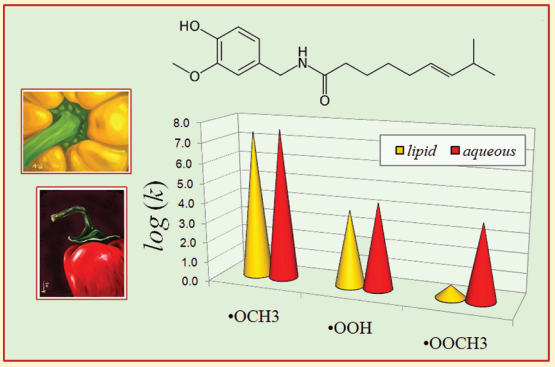
ABSTRACT: The free radical scavenging activity of capsaicin (CAP), which is the pungent component of hot chili peppers, has been studied in aqueous and lipid solutions, using the density functional theory. Different mechanisms of reaction have been considered: single electron transfer (SET), hydrogen transfer (HT), and radical adduct formation (RAF). Rate constants and branching ratios of the different channels of reaction are provided, as well as an interpretation of the UV−vis spectra. CAP is predicted to react faster in aqueous solution than in nonpolar media with oxygenated free radicals, and it was found to be a more efficient scavenger than melatonin and caffeine. It was also found that while SET does not contribute to the overall reactivity of CAP toward $^{\bullet}OOH$, $^{\bullet}OOCH_3$, and $^{\bullet}OCH_3$ radicals, it might be important for the reactions with more electrophilic radicals such as $^{\bullet}OH$, $^{\bullet}OCCl_3$, and $^{\bullet}OOCCl_3$. The main process, responsible for the peroxyl scavenging activity of CAP, was found to be the HT from the OH phenolic group. For the reaction with $^{\bullet}OCH_3$, on the other hand, the HT from allylic sites are predicted to be the main channels of reaction. In this particular case a wider product distribution is predicted. This supports the role of the reacting free radical on the preponderant mechanism of action of free radical scavengers.



INTRODUCTION

Capsaicin (trans 8-methyl-*N*-vannilyl-6-nonemide, CAP, Figure 1), also known as NCI-C56564, axsain, mioton, and zostrix; is the pungent component of hot chili peppers of the plants genus Capsicum.[1] Even though it is mainly known for its flavor, which makes it a frequent ingredient in human diet, its health-related beneficial effects are much more important. CAP is currently used in topical creams and gels to mitigate neurogenic pain because of its analgesic[2] and anti-inflamatory properties.[3] It also has some therapeutic effects on the treatment of arthritis,[4] diabetic neuropathy,[5] gastric lesions,[6] and cardiac excitability.[7] CAP has been proven to inhibit bacterial growth,[8] platelet aggregation,[9] and the expansion of different cancer cells.[10] It has been described to effectively inhibit tumor growth and to induce apoptosis in vivo with no toxic effects.[11] On the basis of these findings it was proposed as a novel therapeutic agent for the treatment of leukemia.[11] CAP also shows significant chemopreventive and therapeutic properties against certain mutagens and carcinogens.[12] In addition, CAP has been proposed to reduce adipose tissue and triglycerides,[13] to stimulate carbohydrate oxidation,[14] and to decrease appetite.[15]

Another interesting property of CAP is the antioxidant activity,[16−20] which is particularly important since oxidative stress (OS) is recognized as a major health problem involved in the development of several diseases such as cancer,[21]

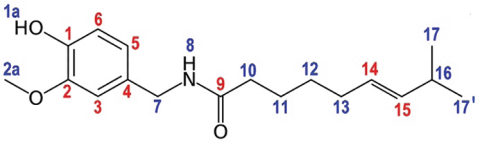


Figure 1. Capsaicin (CAP). Structure and site numbers.

cardiovascular disorders,[22] atherosclerosis,[23] and several neurological disorders including Parkinson's and Alzheimer's diseases (AD).[24] It has been hypothesized that the antioxidant activity of CAP might be the reason for the high consumption of capsicum in certain regions of the world.[19] Moreover, due to the antioxidant activity of CAP, Dairam et al.[20] proposed that this compound might have important implications in the prevention, or treatment, of neurodegenerative diseases such as AD. De and Ghosh[25] showed that pretreatments with CAP have protective effects against free radical induced lung damage, while Shimeda et al.[26] found that it protects against cisplatin-induced nephrotoxicity. CAP has been reported to inhibit the oxidation of various lipids,[27−37] which has been explained based on the

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potential of this compound to mitigate OS as a result of its free radical scavenging activity.[33] In particular, Lee et al.[38] proposed that capsaicin can be a potent antioxidant, lowering low-density lipoprotein (LDL) levels even when it is consumed for a short period. They also found that CAP improves the antioxidant status of blood and brain.[39] Anandakumar et al.[40] highlighted the role of CAP in mitigating the oxidative stress mediated damage produced during induced lung cancer. Regarding its relative efficiency as antioxidant, Henderson and Slickman[41] found that this substance is more effective than melatonin in suppressing the formation of lipid hydroperoxides, and Kogure et al.[33] reported that CAP inhibits lipid-peroxidation in a more efficient way than α-tocopherol.

According to all the data gathered so far, it seems to be no doubts on the antioxidant capacity of CAP. However, in spite of these investigations the information on its reaction mechanism when acting as free radical scavenger, as well as on the kinetics of such process, is very scarce. In fact the necessity of obtaining reliable kinetic data, and elucidating the mechanisms involved in the CAP reactions with free radicals, has been recently pointed out.[42] Kogure et al.[33] demonstrated that CAP is able to scavenge DPPH radicals both in solution and in membranes. On the basis of UV−vis spectra, they proposed that, after reacting with DPPH, the OH phenolic group of CAP remains intact. From quantum chemical calculations, using the model compound *N*-vanillylacetamide (NVA) and based on the relative stability of the radical products, these authors identified site 7 as the most reactive one. On the contrary, Okada et al.[42] proposed that the OH phenolic group of CAP is mainly associated with the peroxyl radical scavenging activity of this compound. It should be noted that NVA is basically a fragment of CAP. They are identical from sites 1 to 9, but NVA ends at site 10, with a methyl group. Therefore, based on the CAP structure (Figure 1), it can be expected that the reactivity of sites 13 and 16 will be similar to that of site 7. Moreover, it has been previously proven that the nature of the reacting free radicals can have a role on the preponderant mechanisms of reaction, as well as on the site reactivity.[43−46] Therefore, more studies on CAP, using its whole structure and including different free radicals, are still needed to completely assess the relative site reactivity of this compound and to identify the main products of reaction. Regarding the kinetic data on the reactions of CAP with free radicals, the only rate constants available so far have been reported by Okada et al.[42,47] The first one[47] corresponds to the CAP reactions with hydroxyl radicals ($^{\bullet}$OH), and was estimated to be 3.5×10^{10} M^{-1} s^{-1}, which corresponds to the diffusion-limit. The second one[42] was obtained for the reaction of CAP with peroxyl radicals derived from cumene, and was found to be equal to 5.6×10^{3} M^{-1} s^{-1}.

On the basis of the scarce, and to some extent contradictory, information on the mechanism and kinetics of the reactions of CAP with free radicals, it is certain that more investigations are needed. For this reason, the main goal of the present work is to provide detailed and quantitative data on the antioxidant capacity of CAP. To that purpose the reactions of CAP with $^{\bullet}$OOH, $^{\bullet}OOCH_3$, and $^{\bullet}OCH_3$ radicals, in polar and nonpolar environments have been studied. Different mechanisms of reaction have been considered. They are as follows: hydrogen transfer (HT), radical adduct formation (RAF), and single electron transfer (SET). We have used the whole structure of CAP and performed quantum chemical calculations related to the thermochemistry and kinetics of the studied reactions. The relative importance of the different mechanisms has been assessed, and branching ratios for the different channels of reaction are provided for the first time. In addition, an analysis of the UV−vis spectra of the main products of reaction is provided.

COMPUTATIONAL DETAILS

Geometry optimizations and frequency calculations have been carried out using the M05−2X functional[48] and the 6-31G(d) basis set, in conjunction with the SMD continuum model,[49] using pentyl ethanoate (PE) and water (W) as solvents to mimic lipidic and aqueous environments, respectively. The electronic energies were improved by single point calculations using the 6-311+G(d,p) basis set. The M05−2X functional has been recommended for kinetic calculations by their developers,[48] and it has been also successfully used by independent authors to that purpose.[50−54] Local minima and transition states were identified by the number of imaginary frequencies (NIMAG = 0 or 1, respectively). Intrinsic reaction coordinate (IRC) calculations have been performed to confirm that the transition states properly connect reactants and products. All the electronic calculations were performed with Gaussian 09 package of programs.[55] Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. We have not corrected interaction energies for basis set superposition errors (BSSE) because it has been demonstrated that counterpoise corrections overcorrect the BSSE[56] and worsen the results.[57,58] The solvent cage effects have been included according to the corrections proposed by Okuno,[59] taking into account the free volume theory.[60]

The rate constants (k) were calculated using conventional transition state theory (TST)[61] and 1 M standard state as follows:

$$k = \sigma\kappa\frac{k_B T}{h}e^{-(\Delta G^{\neq})/RT} \quad (1)$$

Here k_B and h are the Boltzmann and Planck constants, $\Delta G^{\ddagger}$ 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 which were calculated using the zero curvature tunneling approach (ZCT).[62]

The electronic spectra have been computed using the time dependent density functional theory (TD-DFT), based on vertical excitations involving the three lowest lying excited states. TD-DFT efficiently and rapidly provides transition energies for monodeterminental systems in both gas and condensed phases.[63] Moreover, it has been demonstrated that he UV−vis spectra obtained from this approach frequently agree with experiments.[64]

On the basis of the pK_a value of CAP (10.10 ± 0.05),[65] under physiological conditions (pH = 7.4) the fraction of the phenolate anion is almost negligible (0.2%). Accordingly, the neutral form of capsaicin has been the only one considered in this work, regardless of the polarity of the environment. The antioxidant activity of CAP can take place through different mechanisms, as for many other compounds.[66−71] Those considered in this work are

$$\text{Single Electron Transfer (SET)}: \text{CAP} + {}^{\bullet}\text{R} \rightarrow \text{CAP}^{\bullet+} + \text{R}^{-}$$

Hydrogen Transfer (HT), from sites 1a, 2a, 7, 8, 10 to 13, 16, and 17 (Figure 1):

$$\text{CAP} + {}^{\bullet}\text{R} \rightarrow \text{CAP}(-\text{H})^{\bullet} + \text{HR}$$

Table 1. Gibbs Free Energies of Reaction (ΔG), at 298.15 K, in kcal/mol, with Respect to the Isolated Reactants

	$^{\bullet}OOCH_3$		$^{\bullet}OOH$		$^{\bullet}OCH_3$	
	PE	W	PE	W	PE	W
SET	73.81	30.18	72.22	28.33	63.75	21.63
HT						
site 1a	1.37	−2.36	−0.95	−4.18	−18.72	−21.73
site 2a	16.59	13.15	14.27	11.33	−3.50	−6.22
site 7	−0.76	−2.53	−3.07	−4.35	−20.84	−21.89
site 8	24.74	31.04	22.42	29.22	4.65	11.67
site 10	8.59	6.42	6.27	4.60	−11.50	−12.95
site 11	14.15	11.25	11.83	9.43	−5.94	−8.12
site 12	14.34	12.34	12.02	10.52	−5.75	−7.03
site 13	0.54	−0.80	−1.77	−2.62	−19.55	−20.17
site 16	−2.50	−4.89	−4.81	−6.71	−22.59	−24.25
site 17	17.69	15.80	15.37	13.98	−2.40	−3.56
RAF						
site 1	15.04	12.46	12.37	9.67	−3.70	−5.97
site 2	15.09	14.07	12.32	11.66	−3.62	−5.65
site 3	19.90	18.67	15.79	15.39	0.60	0.62
site 4	15.30	16.60	11.76	10.81	−5.80	−3.78
site 5	17.24	17.19	13.28	13.14	−0.35	−1.49
site 6	19.21	18.30	15.57	14.31	0.71	−0.02
site 9	40.44	40.01	36.75	36.59	19.70	20.61
site 14	7.32	4.19	3.71	3.34	−10.78	−10.22
site 15	6.28	5.31	5.37	3.24	−10.44	−10.97

Radical Adduct formation (RAF), on sites 1 to 6, 9, 14, and 15 (Figure 1):

$$CAP + {}^{\bullet}R \rightarrow [CAP{-}R]^{\bullet}$$

RESULTS AND DISCUSSION

The Gibbs free energies of the different channels of reaction (ΔG) for CAP with different free radicals are reported in Table 1. The electron transfers from CAP to the studied free radicals were found to be thermochemically unfeasible, regardless of the polarity of the environment. Accordingly, this mechanism is not expected to significantly contribute to the scavenging processes of $^{\bullet}OOH$, $^{\bullet}OOCH_3$ and $^{\bullet}OCH_3$ free radicals by CAP. It should be noted, however, that this does not mean that the SET mechanism could be neglected for other free radicals. In particular it might be viable when CAP reacts with free radicals that are more electrophilic than those studied in the present work, such as $^{\bullet}OH$ and halogenated alkoxyl and peroxyl radicals. To prove this point SET from CAP to $^{\bullet}OH$, $^{\bullet}OCCl_3$, and $^{\bullet}OOCCl_3$ have also been modeled. They have been studied only in aqueous solution, since nonpolar environments do not promote the necessary solvation of the intermediate ionic species yielded by the SET mechanism, as demonstrated by the values in Table 1. The calculated ΔG values for the SET reactions of CAP with $^{\bullet}OH$, $^{\bullet}OCCl_3$, and $^{\bullet}OOCCl_3$ were found to be 5.24, −32.78, and 7.97 kcal/mol, respectively. The corresponding rate constants, calculated using the Marcus theory,[72] are: 4.85×10^8, 1.08×10^9, and 7.50×10^5 M^{-1} s^{-1}. Accordingly, the SET mechanism is expected to significantly contribute to the overall reactivity of CAP toward $^{\bullet}OH$, $^{\bullet}OCCl_3$, and $^{\bullet}OOCCl_3$ radicals.

Regarding the HT mechanism, it was found that the polarity of the environment increases the thermochemical viability of the studied reactions (Table 1). For the HT products that are carbon-centered free radicals, the reaction feasibility is directly related to their chemical nature, i.e. tertiary C-centered free radicals are more stable than secondary, which in turn are more stable than primary ones. In addition an extra stabilization is expected for the allylic C sites. Accordingly HT from site 16 is predicted as the most reactive position since it yields a tertiary allylic C-centered radical. The results reported in Table 1 concur with this hypothesis. In all cases this channel was found to have the largest exergonicity. The fact that site 16 is a tertiary C atom explains why its exergonicity is larger than those of the allylic sites 7 and 13 which are secondary. In turn the stabilization of the radicals formed in these two sites is larger than those of the radicals formed by HT from secondary C atoms, which are not allylic (10, 11, and 12). This explains the results in Table 1, showing these three channels as exergonic only for the most reactive of the studied free radicals ($^{\bullet}OCH_3$).

Comparing the HT processes from the secondary carbon atoms (sites 7, 10, 11, 12, and 13), it was found that the reaction involving the C directly connected to the N atom (site 7) is thermochemically more favored. The presence of the N atom, that is more electronegative than the C atom, weakens the C−H bond facilitating its rupture. On the other hand, in site 1a, the H to be transferred is directly bonded to an O phenolic atom. All the reactions between the free radicals and the H atom at this position are exergonic, except with $^{\bullet}OOCH_3$ in PE. The HT reactions from sites 2a and 17, which correspond to primary C atoms, were found to be endergonic with the studied peroxyl radicals but not with $^{\bullet}OCH_3$. HT processes from site 8, which is the only amino site in CAP, were found to be endergonic for all the studied radicals, regardless of the polarity of the environment.

All these results are logical and in line with chemical intuition. What seems to be a peculiarity of CAP, that tells it apart from other phenolic scavengers, is that the HT from the phenolic OH moiety (site 1a) is not the channel with the largest exergonicity. This can be explained by the presence of a tertiary allylic carbon (site 16), which is an interesting peculiarity not frequently found in this kind of compounds. It also supports the importance of modeling the whole structure of CAP since this site is absent in the model compound VMA previously used.[33]

Concerning the radical adduct formation (RAF) mechanism, it was found that the feasibility of the RAF processes increases with the polarity of the environment for most of the studied channels of reactions. For the reaction of CAP with $^{\bullet}OOH$ and $^{\bullet}OOCH_3$, all the reaction channels were found to be endergonic (Table 1). For the reactions with $^{\bullet}OCH_3$, on the other hand, radical additions to sites 1, 2, 4, 14, and 15 were found to be exergonic, regardless of the polarity of the environment; with the largest exergonicities corresponding to sites 14 and 15. RAF on sites 3 and 6 were found to be almost isoergonic in both kinds of environment, polar and nonpolar, being on site 6 slightly exergonic in aqueous solution. RAF on site 9 was found to be significantly endergonic in both media. Accordingly the RAF mechanism is described as viable for the reactions of CAP with $^{\bullet}OCH_3$ but not for its reactions with $^{\bullet}OOH$ and $^{\bullet}OOCH_3$. This is a reasonable result based on the relative reactivity of these radicals.

For the kinetic study, we have not include the channels of reaction described above as endergonic because even if they take place at a significant rate, they would be reversible and therefore

the formed products will not be observed. However, it should be noticed that they might still represent significant channels if their products rapidly react further. This would be particularly important if these later stages are sufficiently exergonic to provide a driving force, and if they have low barriers of reactions. In addition, it should be noted that even when endergonic processes might play important roles in biological systems, this particular behavior is expected only when there are not parallel reactions more energetically favored. This is not the case of the systems studied in this work since for all of them there are some thermochemically feasible ($\Delta G < 0$) channels.

The fully optimized geometries of the transition states (TS) are shown in Figures 2−5. In general, they are earlier in aqueous solution than in nonpolar environments, suggesting that the reactivity of CAP toward the studied free radicals increases with the polarity of the solvent. For the CAP + $^{\bullet}OOCH_3$ reaction TS-1a presents a H bond like interaction involving one of the H atoms of the radical and the O atom in the methoxy moiety of CAP (Figure 2). A similar interaction was found in TS-1a for the reaction with $^{\bullet}OOH$, but in this case the interaction distance is shorter (Figure 3). For the CAP + $^{\bullet}OOH$ reaction, TS-7 also presents a H bond like interaction. It involves the H atom of the radical and the O atom of the amide functional group of CAP, and has a H···O distance shorter than that in TS-1a. None of the transition states involving $^{\bullet}OCH_3$ (Figures 4 and 5) present this kind of interactions.

The barriers, or Gibbs free energies of activation ($\Delta G‡$), of the different reaction channels are reported in Table 2. They are systematically lower for the CAP reactions with $^{\bullet}OCH_3$, as expected based on its relative higher reactivity. For the reaction with this radical the lowest barrier corresponds to channel 13, both in aqueous and in pentyl ethanoate solutions but the barrier of channel 16 is very similar. For the CAP reaction with $^{\bullet}OOH$, on the other hand, the lowest barrier is for channel 1a, regardless of the polarity of the environment. For the CAP + $^{\bullet}OOCH_3$ reaction channel 1a has the lowest barrier in aqueous solution, but very similar to that of channel 16, while in a nonpolar

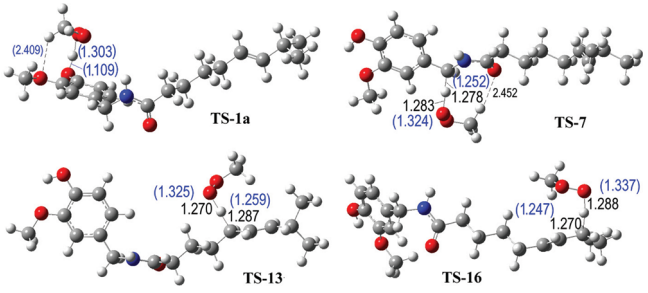

Figure 2. Optimized geometries of transition states involved in the HT reactions of CAP with $^{\bullet}OOCH_3$, in pentyl ethanoate (water) solution. Only thermochemically feasible ($\Delta G < 0$) channels are considered. The distances are reported in Å.

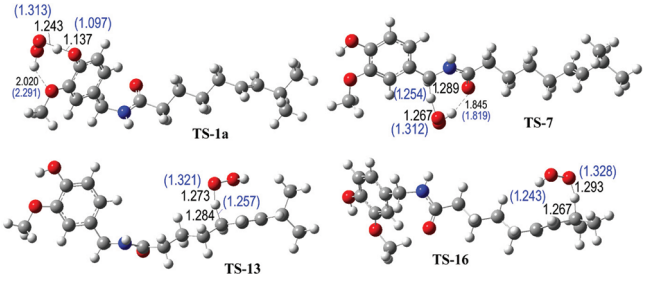

Figure 3. Optimized geometries of transition states involved in the HT reactions of CAP with $^{\bullet}OOH$, in pentyl ethanoate (water) solution. Only thermochemically feasible ($\Delta G < 0$) channels are considered. The distances are reported in Å.

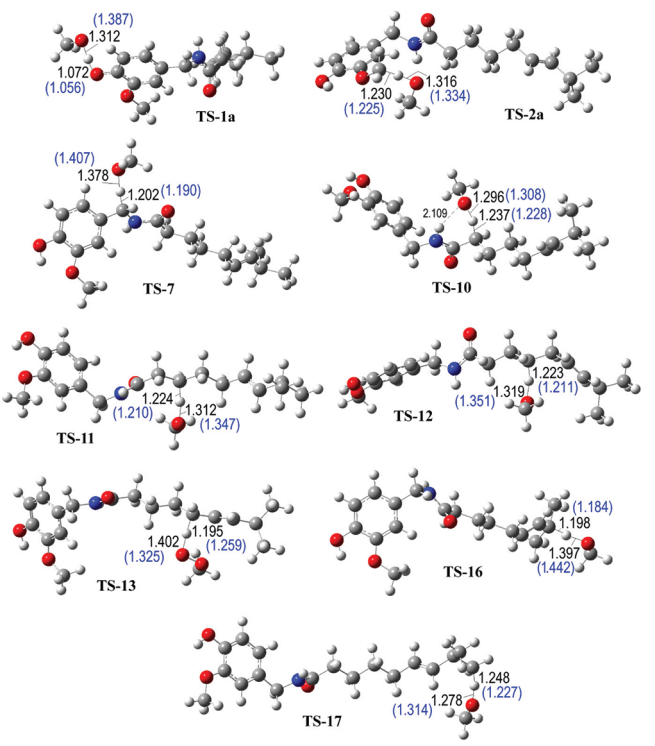

Figure 4. Optimized geometries of transition states involved in the HT reactions of CAP with $^{\bullet}OCH_3$, in pentyl ethanoate (water) solution. Only thermochemically feasible ($\Delta G < 0$) channels are considered. The distances are reported in Å.

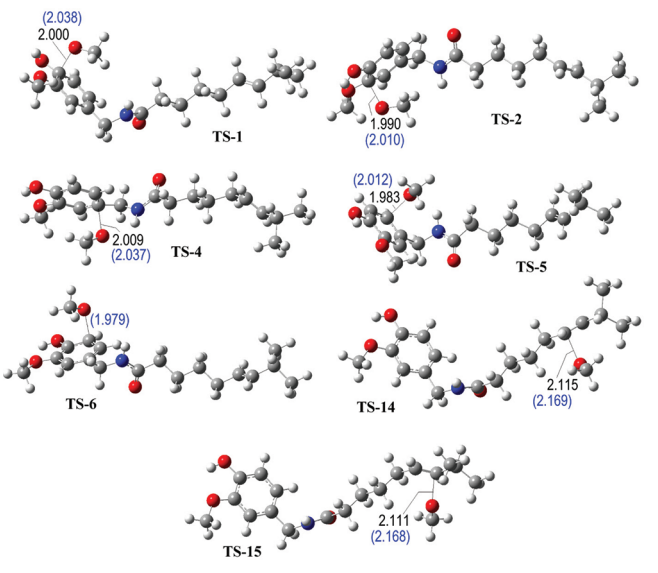

Figure 5. Optimized geometries of transition states involved in the RAF reactions of CAP with $^{\bullet}OCH_3$, in pentyl ethanoate (water) solution. Only thermochemically feasible ($\Delta G < 0$) channels are considered. The distances are reported in Å.

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environment the latter has the lowest barrier. Site 1a in PE is not analyzed since the Gibbs free energy of reaction at this position is positive (Table 1). In general the barrier heights of the reaction channels involving $^{\bullet}OOCH_3$ are higher than those of the equivalent channels with $^{\bullet}OOH$.

The rate constants for the different channels of reaction, in aqueous and lipid solutions, are reported in Table 3, together with the overall rate coefficients, which have been calculated as the sum of the rate constants of each path, for example for the CAP + $^{\bullet}OCH_3$ reaction

$$k_{overall} = k_{app}^{HT} + k_{app}^{RAF} \quad (2)$$

where

$$k_{app}^{HT} = k_{app}^{p1a} + k_{app}^{p2a} + k_{app}^{p7} + k_{app}^{p10} + k_{app}^{p11} + k_{app}^{p12} + k_{app}^{p13} + k_{app}^{p16} + k_{app}^{p17} \quad (3)$$

$$k_{app}^{RAF} = k_{app}^{p1} + k_{app}^{p2} + k_{app}^{p4} + k_{app}^{p5} + k_{app}^{p6} + k_{app}^{p14} + k_{app}^{p15} \quad (4)$$

According to the overall rate coefficients, CAP is predicted to react faster in aqueous solution than in nonpolar media with oxygenated free radicals. The overall rate coefficients in aqueous solution were found to be about 1566, 13.2, and 1.5 times higher than in nonpolar media for the reactions of CAP with $^{\bullet}OOCH_3$, $^{\bullet}OOH$, and $^{\bullet}OCH_3$, respectively (Table 3). The order of the free radical scavenging activity of CAP toward these radicals was found to be $^{\bullet}OCH_3$ > $^{\bullet}OOH$ > $^{\bullet}OOCH_3$, in agreement with the relative reactivity of the involved free radicals. CAP was found to rapidly react with $^{\bullet}OCH_3$ with rate coefficients in the order of 10^7 M^{-1} s^{-1} in both environments. For the reaction with $^{\bullet}OOH$ the overall rate coefficients were found to be 2.1×10^4 M^{-1} s^{-1} and 6.5×10^3 M^{-1} s^{-1} in aqueous and pentyl ethanoate solutions, respectively, while with $^{\bullet}OOCH_3$ the overall rate coefficient in aqueous solution was found to be 6.6×10^3 M^{-1} s^{-1} and much lower in nonpolar media (Table 3). This can be justified by the fact that the HT from the phenolic OH is endergonic in the latter case, which means that it would be reversible and the formed product is not expected to be detected. The values of the overall rate coefficients for the peroxyl radicals are in general agreement with the value reported by Okada et al.[42] These values are not

Table 2. Gibbs Free Energies of Activation ($\Delta G^{\neq}$), at 298.15 K, in kcal/mol

	$^{\bullet}OOCH_3$		$^{\bullet}OOH$		$^{\bullet}OCH_3$	
	PE	W	PE	W	PE	W
HT						
site 1a		15.06	15.23	14.76	12.74	9.08
site 2a					12.85	15.31
site 7	20.91	20.62	17.83	16.46	11.73	12.37
site 10					14.01	13.71
site 11					13.43	11.15
site 12					11.70	11.60
site 13	21.44	17.96	19.15	16.71	8.76	8.66
site 16	19.02	15.27	16.53	15.81	8.80	9.10
site 17					15.14	13.28
RAF						
site 1					13.70	10.00
site 2					12.00	10.75
site 4					10.44	9.15
site 5					12.92	11.06
site 6						12.15
site 14					13.24	11.53
site 15					12.79	10.85

Table 3. Rate Constants (k) of the Different Channels of Reaction and Overall Rate Coefficient (M^{-1} s^{-1}), at 298.15 K

	$^{\bullet}OOCH_3$		$^{\bullet}OOH$		$^{\bullet}OCH_3$	
	PE	W	PE	W	PE	W
HT						
site 1a		5.28×10^3	6.28×10^3	1.89×10^4	1.17×10^6	4.65×10^6
site 2a					1.31×10^5	3.06×10^3
site 7	6.02×10^{-1}	1.49E+00	7.08×10^1	9.74×10^2	3.02×10^5	1.03×10^5
site 10					9.54×10^3	1.80×10^4
site 11					2.18×10^4	4.01×10^5
site 12					3.67×10^5	2.77×10^5
site 13		3.97×10^1	5.85E+00	2.45×10^2	1.34×10^7	2.40×10^7
site 16	3.63E+00	1.30×10^3	1.87×10^2	6.24×10^2	8.81×10^6	3.48×10^6
site 17					6.26×10^3	1.09×10^5
RAF						
site 1					1.12×10^3	5.79×10^5
site 2					1.98×10^4	1.64×10^5
site 4					2.74×10^5	2.41×10^6
site 5					4.18×10^3	9.75×10^4
site 6						1.53×10^4
site 14					2.46×10^3	4.39×10^4
site 15					5.25×10^3	1.38×10^5
overall	4.23E+00	6.62×10^3	6.54×10^3	2.07×10^4	2.45×10^7	3.65×10^7

identical since the reactions involve different peroxyl radicals, and are studied in different media.

A very important aspect of studying the antioxidant activity of chemical compounds is to identify those with higher activity. To that purpose it is recommended to compare different scavengers using their reactions with free radicals that are not particularly reactive.[73] This recommendation is based on the fact that reactions with $^{\bullet}OH$, for example, are often diffusion-controlled, and as a result comparisons based on reactions with this free radical might be misleading. Therefore, we prefer to use a less reactive species ($^{\bullet}OOH$).

In nonpolar environments the peroxyl radical scavenging activity of CAP was found to be lower than that of carotenes ($\sim 10^5-10^6$ M^{-1} s^{-1})[74] and canolol (6.8×10^5 M^{-1} s^{-1});[75] similar to that of sinapinic acid (1.7×10^4 M^{-1} s^{-1}),[76] sesamol (3.3×10^4 M^{-1} s^{-1}),[46] and α-mangostin (7.8×10^3 M^{-1} s^{-1});[77] and higher than that of melatonin (3.1×10^2 M^{-1} s^{-1})[44] and caffeine (3.2×10^1 M^{-1} s^{-1}).[45] In aqueous solution the peroxyl radical scavenging activity of CAP was found to be lower than that of α-mangostin (1.4×10^6 M^{-1} s^{-1}),[70] and sinapinic acid (5.4×10^5 M^{-1} s^{-1});[69] similar to that of allicin (7.4×10^3 M^{-1} s^{-1}) and thioacrolein (2.9×10^4 M^{-1} s^{-1});[78] much higher than that of melatonin (2.0×10^1 M^{-1} s^{-1})[44] and caffeine (3.3×10^{-1} M^{-1} s^{-1});[45] and significantly lower than that of 2-propenesulfenic acid (2.6×10^7 M^{-1} s^{-1}),[71] glutathione (2.7×10^7 M^{-1} s^{-1}),[79] and sesamol (2.4×10^8 M^{-1} s^{-1}),[46] which are excellent for scavenging $^{\bullet}OOH$. According to these comparisons CAP is a good free radical scavenger, with intermediate activity, in both polar and nonpolar environments. In addition CAP can be present in both lipid and aqueous media and is nontoxic, which are desirable characteristic of good antioxidants for human consumption.[66] In addition, the relative scavenging activity of CAP, with respect to melatonin, is in agreement with what was previously reported by Henderson and Slickman.[41]

The branching ratios of the different reaction paths are reported in Table 4. They represent the percent contribution of the different channels to the overall reaction, and have been calculated as:

$$\Gamma_i = \frac{k_i}{k_{overall}} \times 100 \qquad (5)$$

According to the calculated branching ratios (Table 4) the hydrogen transfer is the main mechanism involved in the $^{\bullet}OCH_3$ scavenging activity of CAP, accounting for 98.7% and 90.5% of its overall reactivity toward this radical, in nonpolar and polar environments, respectively. Regarding the reaction sites, channel 13 was found to be the main one both in aqueous and pentyl ethanoate solutions. However, in the latter HT from site 16 was found to be the second major channel, while in aqueous solution the second most important channel is 1a. For the reaction with $^{\bullet}OOH$, HT from the phenolic OH (site 1a) is predicted to be the major channel of reaction, contributing by more than 90% to the overall reactivity of CAP toward this radical. Thus, it can be stated that the $^{\bullet}OOH$ scavenging activity of CAP takes place almost exclusively via channel 1a, in agreement with the proposal of Okada et al.[42] For the CAP reaction with $^{\bullet}OOCH_3$ the main HT channel is influenced by the polarity of the environment. In aqueous solution channel 1a plays the most important role, while in nonpolar environments channel 16 is the main one. Accordingly, it can be stated that the main site of reaction is not always the same for CAP. It depends on the nature of the reacting free radical, and is also moderately influenced by the polarity of the environment.

Table 4. Branching ratios (Γ) of the Different Channels of Reaction, at 298.15 K

	$^{\bullet}OOCH_3$		$^{\bullet}OOH$		$^{\bullet}OCH_3$	
	pe	w	pe	w	pe	w
ht						
site 1a		79.71	95.97	91.09	4.76	12.75
site 2a					0.53	0.01
site 7	14.24	0.02	1.08	4.71	1.23	0.28
site 10					0.04	0.05
site 11					0.09	1.10
site 12					1.50	0.76
site 13	~0.00	0.60	0.09	1.18	54.62	65.77
site 16	85.76	19.67	2.86	3.02	35.95	9.54
site 17					0.03	0.30
raf						
site 1					~0.00	1.59
site 2					0.08	0.45
site 4					1.12	6.61
site 5					0.02	0.27
site 6					~0.00	0.04
site 14					0.01	0.12
site 15					0.02	0.38

UV−Vis Spectra. Kogure et al.[33] and Okada et al.[42] based their proposal of the preponderant mechanism involved in the free radical scavenging of CAP on UV−vis spectra. Kogure et al.[33] recorded the UV−vis spectra of CAP at two different pHs, and observed that the band that appears at 280 nm, at pH = 7.4, is shifted to 295 nm when the pH increases to 12.8. Therefore, they assumed that this band is associated with the OH phenolic group. Since the band still appeared at 280 nm, after incubation with DPPH for 1 h, at pH = 7.4, and CAP should have scavenge this radical by then, they concluded that the OH phenolic group is not involved in the reaction. On the other hand, by comparing the UV−vis spectra of CAP and MeO-CAP, Okada et al.[42] concluded that the band at 280 nm is not due to the OH phenolic group. In addition they also observed that when the spectrum is recorded in the presence of AIBN, the band shape around 280 nm changes with the consumption of CAP. They also observed that when monitoring the 200−350 nm region for MeO-CAP in the presence of AIBN the shape of the spectrum remains almost unaltered even though the amount of MeO-CAP was significantly reduced. Accordingly they concluded that no valuable information, regarding the reaction mechanism, can be directly obtained from this region of the UV−vis spectra of CAP.

In an attempt to interpret the above-described features, the UV−vis spectra of CAP, and their main products of reaction with $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$ have been computed. In addition the UV−vis spectrum of the CAP's monoanion has also been calculated for comparison purposes with respect to the experiments. The band at 280 nm in the spectrum of CAP has been used to calibrate the calculated data. This band was found at 232 nm, in aqueous solution, i.e., 48 nm lower than the experimental values. Therefore, all the computed spectra have been scaled accordingly to facilitate comparisons with the

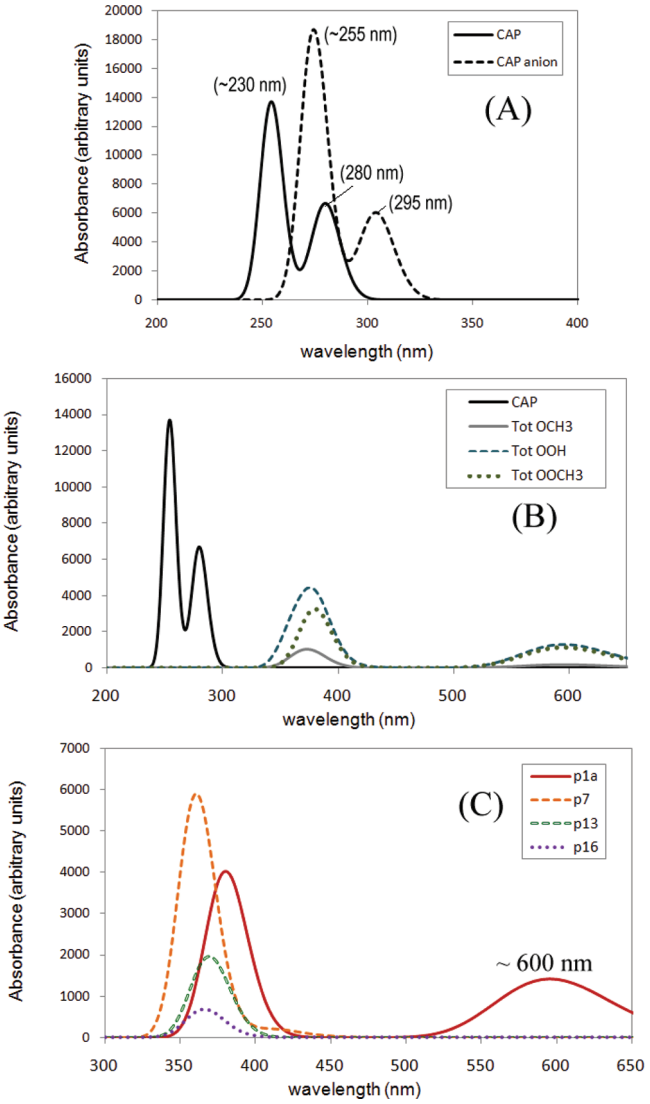

Figure 6. UV−vis spectra. CAP and its mono anion; total spectra from the reactions of CAP with $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$; and spectra of the main reaction products. They were all calculated in water solution. The values in parentheses are from experiments.

experiments ($\lambda_{reported} = \lambda_{calculated} + 48$ nm). The shape of the spectra of CAP and that of its monoanion (Figure 6A) are in excellent agreement with the experimental ones.[33] Both present two absorption bands which are red-shifted for the anion, by 15−20 nm, compared to the neutral species.

The spectra labeled as Tot (Figure 6B) represent the total spectra, which are those that would correspond to the observed ones. They were obtained using the additivity of absorbance and weighting the separated spectra according to the relative abundance of the formed products for the reactions with the three studied radicals. These spectra show that at 280 nm the only species that absorbs is CAP. Therefore, the observation of this band in the experiment is interpreted as corresponding to the CAP fraction that did not react. Accordingly, we agree with Okada et al.[42] This particular band is not useful to follow the evolution of the CAP reactions with free radicals, nor for elucidating the preponderant mechanism of action. There are two other bands that might be valuable for those purposes though. The first one is in the 360−380 nm region, which is predicted to be observed for incubation of CAP with any of the studied radicals ($^{\bullet}OCH_3$, $^{\bullet}OOH$, $^{\bullet}OOCH_3$), and probably with other alkoxyl or peroxyl radicals. Another band, less intense, was found around 600 nm that is predicted to be observed in the reactions with the least reactive radicals, but which would probably present almost negligible intensity for the reaction with $^{\bullet}OCH_3$, and possibly also with $^{\bullet}OH$.

To identify which products contribute to the above-mentioned bands, the separated spectra of the most abundant products are shown in Figure 6C. As this plot shows all of them absorb in the 350−400 nm region, which makes this band of little use to identify the main mechanism of reaction. On the contrary, the band around 600 nm is exclusive of product 1a, i.e., the one yielded by HT from the OH phenolic moiety. Accordingly this band is recommended for further confirmation, or refutation, on the role of this particular channel on the overall reactivity of CAP toward free radicals. According to our results it seems that for the less reactive, i.e., more selective free radicals, this channel is the main one, while for more reactive radicals a wider product distribution is expected and therefore the HT processes from the allylic C atoms in the side chain of CAP might become the most important path.

CONCLUSIONS

The reactions of CAP with $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$ free radicals, in lipid and aqueous media, have been studied. Three mechanisms of reaction have been considered: single electron transfer (SET), hydrogen transfer (HT), and radical adduct formation (RAF).

It was found that while SET does not contribute to the overall reactivity of CAP toward $^{\bullet}OOH$, $^{\bullet}OOCH_3$, and $^{\bullet}OCH_3$ radicals, it may be important for its reactions with $^{\bullet}OH$, $^{\bullet}OCCl_3$, and $^{\bullet}OOCCl_3$.

The order of the free radical scavenging activity of CAP toward the studied radicals was found to be $^{\bullet}OCH_3 > {}^{\bullet}OOH > {}^{\bullet}OOCH_3$, in agreement with the relative reactivity of the involved radicals. The overall rate coefficients in aqueous solution were found to be higher than those in nonpolar media for the reactions of CAP with $^{\bullet}OOCH_3$, $^{\bullet}OOH$, and $^{\bullet}OCH_3$. CAP is predicted to be a good scavenger for $^{\bullet}OCH_3$, and probably for other alkoxyl radicals, while its peroxyl radical scavenging activity was found to be only moderate.

According to the calculated branching ratios HT is the main mechanism involved in the free radical scavenging activity of CAP. However, regarding the reaction sites the nature of the reacting free radical was found to play an important role. For the CAP + $^{\bullet}OCH_3$, reaction channels 13 and 16, which correspond to HT from allylic C sites, were found to be the main ones. On the contrary for the reaction with peroxyl radicals the HT from the phenolic OH (site 1a) is predicted to be the major channel of reaction. The relative importance of the different channels of reaction was also found to be moderately influenced by the polarity of the environment.

The UV−vis spectra of CAP, and its main products of reaction have been computed, and interpreted. The band at 280 nm, has been attributed only to CAP, since none of the studied products absorb in this region. A band around 600 nm is recommended for further confirmation, or refutation, on the role of the HT from the phenolic OH on the overall reactivity of CAP toward free radicals.

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