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Influence of the Environment on the Protective Effects of Guaiacol Derivatives against Oxidative Stress: Mechanisms, Kinetics, and Relative Antioxidant Activity

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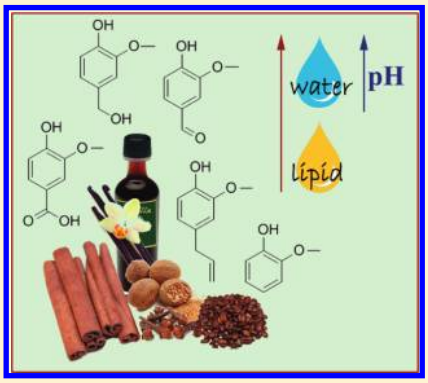
ABSTRACT: The peroxyl radical scavenging activity of five guaiacol derivatives (GD) has been studied in nonpolar and aqueous solutions, using the density functional theory. The studied GD are guaiacol, vanillin, vanillic alcohol, vanillic acid, and eugenol. It was found that the environment plays an important role in the peroxyl scavenging activity of these compounds. They were all found to react faster in aqueous solution than in nonpolar media. The order of reactivity in nonpolar environments was found to be vanillic alcohol > eugenol > guaiacol > vanillin > vanillic acid, while, in aqueous solution, at physiological pH, it becomes vanillic acid > vanillic alcohol > guaiacol ≈ eugenol > vanillin. It was also found that in aqueous solution as the pH increases so does the reactivity of GD toward peroxyl radicals. The environment also has important effects on the relative importance of the hydrogen transfer (HT) and the sequential proton electron transfer (SPET) mechanisms, which are the ones relevant to the peroxyl radical scavenging activity of GD. The HT from the phenolic OH was identified as the main scavenging process in nonpolar media, and in aqueous solution at pH ≤ 4. On the other hand, SPET is proposed to be the one contributing the most to the overall peroxyl scavenging activity of GD in aqueous solution at pH ≥ 6.



INTRODUCTION

Oxidative stress (OS) is a major health problem currently associated with the development of numerous diseases such as cancer,[1] cardiovascular disorders,[2] atherosclerosis,[3] fetal growth restriction and preeclampsia,[4] and several neurological disorders including Parkinson's and Alzheimer's diseases.[5] This chemical stress is triggered by an excess of free radicals, which reach the cells due to a wide variety of exogenous and endogenous processes. Endogenous free radicals are generated from immune cell activation, inflammation, mental or physical stress, excessive exercise, ischemia, infection, cancer, and aging.[6] Exogenous sources of free radicals are environmental pollution, cigarette smoke, alcohol, heavy or transition metals, certain drugs, and radiation.[7] Therefore, keeping free radicals at healthy low/moderate concentrations is a difficult task in the modern world. As a result, finding efficient strategies for detoxifying free radicals has become a subject of great interest.

One way of counteracting the detrimental and cumulative effects of OS is the intake of free radical scavengers, which are frequently referred to as antioxidants. Humans obtain a wide variety of these compounds from their diet. In fact, some of the beneficial effects of consuming fruits, vegetables, and grains have been attributed to the antioxidant activity of some of their components. In particular, phenolic compounds are widespread in the plant kingdom and among the most abundant and active antioxidants in the human diet.[8] Moreover, the antioxidant activity of natural products is frequently correlated with their total phenolic contents.[9] This has attracted a great deal of attention toward this kind of compounds. There are thousands of phenols in nature, with distinctive structural characteristics. Guaiacol derivatives are among the least studied.[10] Those investigated in the present work are guaiacol (2-methoxyphenol; HGua), vanillin (4-hydroxy-3-methoxybenzaldehyde; HVan), vanillic alcohol (4-hydroxymethyl-2-methoxyphenol; HVal), vanillic acid (4-hydroxy-3-methoxybenzoic acid; H_2Vac), and eugenol (2-allyl-4-methoxyphenol; HEug). Their structures and site numbering are shown in Figure 1. Guaiacol is found in roasted coffee; vanillin, vanillic acid, and vanillic alcohol are present in vanilla extracts; and eugenol is found in clove, cinnamon, and nutmeg.

All these guaiacol derivatives (GD) have been reported to be efficient antioxidants, able to scavenge a wide variety of free radicals.[10−29] However, there is still some lack of information regarding such activity. The relative activity of GD as free radical scavengers has been addressed in six previous works. Ordoudi et al.[22] proposed that the DPPH scavenging activity of guaiacol is higher than that of vanillic acid in ethanol or acetonitrile solutions. Ogata et al.[14] reported that eugenol is

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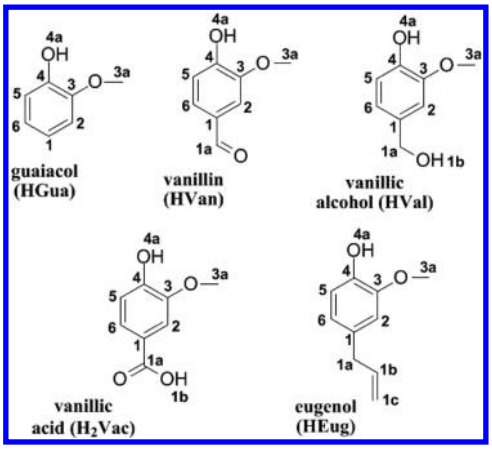

Figure 1. Structures, site numbering, and acronyms.

more efficient than guaicol for inhibiting lipid peroxidation. Tsujimoto et al.[13] found the same trend (eugenol > guaiacol) for the inhibitory effect on $O_2^{\bullet-}$. Chou et al.[26] reported that vanillic acid is a stronger antioxidant than vanillin in terms of free radical scavenging activity, reducing power, and inhibition of lipid peroxidation. The same order of reactivity was reported for the reactions of these two GD with DPPH by Mourtzinos et al.[24] These results are in line with the order vanillic alcohol > vanillic acid > vanillin reported by Shyamala et al.[23] However, there are no previous investigations on their relative order of reactivity when scavenging peroxyl radicals, nor on the role of the environment on the antioxidant efficiency of these compounds.

Regarding the reaction mechanisms involved in the antioxidant activity of GD, two of them have been proposed: the hydrogen transfer (HT) from the phenolic OH[10,20,22] and the single electron transfer (SET) from the GD to the radical.[10,17,18] That is probably why previous theoretical investigation on this subject has been mainly based on bond dissociation energies of the OH group[10,22] and ionization energies.[10] However, there are no reports on the relative importance of these two mechanisms of reaction depending on the reacting GD or on the environment. The possible importance of other mechanisms has not been investigated either.

Regarding the kinetic data for the reactions of GD with peroxyl radicals, it has been reported that guiacol[10] and eugenol[20] have rate constants in the order of 10^2–10^3 M^{-1} s^{-1} when reacting with ROO$^{\bullet}$. In addition, when GD reacts with a more electrophilic peroxy radical, $CCl_3OO^{\bullet}$, the rate constants become several orders of magnitude higher. For eugenol[17] and vanillin,[18] they have been reported to be 7.5×10^8 and 8×10^8 M^{-1} s^{-1}, respectively. There is not a systematic study, however, dealing with the kinetics of a series of GD to investigate the possible structure–activity relationship. With respect to the influence of the environment, the information gathered so far is more limited. There are only two reports indicating that the free radical scavenging activity of GD increases with the pH,[17,18] which suggests that the anionic forms of these compounds are more active than the neutral ones. However, there is no information at all on the influence of the polarity of the environment or on whether the influence of the pH changes for different GD.

In summary, according to the above discussion, while there are abundant data supporting the antioxidant activity of GD, there is still a lack of information on the kinetics and mechanisms involved in the reactions of these compounds with reactive oxygen species (ROS). There is also very little information on their relative activity, as well as on how much it is affected by the polarity of the environment and by the pH. Such information is particularly scarce for the reactions of GD with peroxyl radicals (ROO$^{\bullet}$). Therefore, it is the main goal of the present work to provide new data that help clarifying these aspects that are directly related to the free radical scavenging activity GD under physiological conditions.

To that purpose, we have chosen the hydroperoxyl radical (HOO$^{\bullet}$), which is the simplest of the ROO$^{\bullet}$. These free radicals are among those of biological relevance that can be effectively scavenged to retard OS.[30] This is because they have not too short half-lives, which is a requisite for efficient interception by phenolic compounds.[31] Therefore, ROO$^{\bullet}$ have been proposed as major reaction partners for this kind of compounds.[31] ROO$^{\bullet}$ have also been proposed to be involved in the oxidation of lipoproteins and biological membranes and had been held responsible for microvascular damage.[32] They are formed within living organisms, where they are involved in DNA cleavage and protein backbone modification.[33] It has even been proposed that the main antioxidant function of phenolic compounds is to trap ROO$^{\bullet}$.[34] In the particular case of HOO$^{\bullet}$, it has been suggested to be central to the toxic side effects of aerobic respiration.[35] It has also been pointed out that more information on the reactivity of this species is needed.[35] In addition, radicals of intermediate to low reactivity have been recommended for studying the relative scavenging activity of different compounds,[36,37] since using highly reactive radicals that usually react at diffusion-limited rates might lead one to misconclude that all the tested compounds are equally efficient as antioxidants. Moreover, it has been proposed that such highly reactive radicals cannot be intercepted in biological systems with reasonable efficiency.[35]

■ COMPUTATIONAL DETAILS

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional[38] and the 6-311+G(d,p) basis set, in conjunction with the SMD continuum model[39] using pentyl ethanoate and water as solvents to mimic lipid and aqueous environments, respectively. The M05-2X functional has been recommended for kinetic calculations by their developers,[38] and it has also been successfully used by independent authors to that purpose.[40−43] It is also among the best performing functionals for calculating reaction energies involving free radicals.[44] SMD is considered a universal solvation model, due to its applicability to any charged or uncharged solute in any solvent or liquid medium for which a few key descriptors are known.[39]

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). In the case of the transition states, it was verified that the imaginary frequency corresponds to the expected motion along the reaction coordinate, by intrinsic coordinate calculations (IRC). All the electronic calculations were performed with the Gaussian 09 package of programs.[45] Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. In addition, the solvent cage effects have been included according to the corrections proposed by Okuno,[46] taking into account the free volume theory.[47]

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The rate constants (k) were calculated using the conventional transition state theory (TST)[48] and 1 M standard state. Reaction path degeneracies and tunneling corrections have been included in the calculations. The tunneling corrections, defined as the Boltzmann average of the ratio of the quantum and the classical probabilities, were calculated using the zero curvature tunneling corrections (ZCT).[49] For the mechanisms involving electron transfers, the barriers of reaction were estimated using the Marcus theory.[50] Some of the calculated rate constants (k) are close to the diffusion limit. Accordingly, the apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. In the present work, the Collins–Kimball theory is used to that purpose:[51]

$$k_{app} = \frac{k_D k_{act}}{k_D + k_{act}} \quad (1)$$

where k_{act} is the thermal rate constant, obtained from TST calculations, and k_D is the steady-state Smoluchowski[52] rate constant for an irreversible bimolecular diffusion-controlled reaction:

$$k_D = 4\pi R D_{AB} N_A \quad (2)$$

where R denotes the reaction distance, N_A is the Avogadro number, and D_{AB} is the mutual diffusion coefficient of the reactants A (free radical) and B (GD). D_{AB} has been calculated from D_A and D_B according to ref 53, and D_A and D_B have been estimated from the Stokes–Einstein approach:[54]

$$D = \frac{k_B T}{6\pi\eta a} \quad (3)$$

where k_B is the Boltzmann constant, T is the temperature, η denotes the viscosity of the solvent, in our case water ($\eta = 8.91 \times 10^{-4}$ Pa s) and pentyl ethanoate ($\eta = 8.62 \times 10^{-4}$ Pa s), and a is the radius of the solute.

RESULTS AND DISCUSSION

The experimental values of the first pK_a's of the GD studied in this work are reported in Table 1, together with the molar fractions of the neutral (N), monoanionic (A), and dianionic (D) species, when possible, at physiological pH (7.4). They have been estimated from the acid constants calculated from the pK_a values, using the average values when there are more than one pK_a reported for the same acid–base equilibrium. According to the values in Table 1, under physiological conditions, the main form of guaiacol, vanillic alcohol, and eugenol is the neutral one. For vanillin, both the neutral and the monoanion are present to significant extents (~60 and ~40%, respectively), while the prevailing form of vanillic acid is the monoanion. Accordingly, these are the species included in the present study for the reactions in aqueous solution, unless specified otherwise, while in nonpolar (lipid) media only the neutral forms are used.

Table 1. pK_a Values and Molar Fractions at pH 7.4

	ref	pK_{a1}	pK_{a2}	mF(N)[a]	mF(A)[a]	mF(D)[a]
HGua	55[b]	10.09				
	56	9.47				
	average	10.03		0.998	0.002	
HVan	55	7.38				
	56	7.78				
	average	7.58		0.602	0.398	
HVal	55	9.84				
	56	9.75				
	average	9.80		0.996	0.004	
H_2Vac	55	4.44	9.44			
	56	4.45				
	57	4.16	8.96			
	58	4.31	8.81			
	average	4.34	9.07	0.001	0.978	0.021
HEug	55	10.24				
	56	10.29				
	average	10.27		0.999	0.001	

[a]N = neutral, A = monoanion, D = dianion. [b]Extrapolated to 100% water.

Regarding the reaction mechanisms, the free radical scavenging activity of GD can take place though a variety of them, as is the case for many other scavengers.[59−65] Those considered in this work are radical adduct formation (RAF), hydrogen transfer (HT), and single electron transfer (SET) in aqueous solution, while in nonpolar solution only RAF and HT have been considered. The SET mechanism has not been included in this case, since nonpolar environments do not promote the necessary solvation of the intermediate ionic species yielded by this mechanism. However, just to prove this point, the energies of reaction for the SET process were calculated and found to be larger than 65 kcal/mol in all cases. In addition, in aqueous solution, the SET from the dianion of vanillic acid has also been considered, as well as from the monoanions of guaiacol, vanillic alcohol, and eugenol. This process actually corresponds to a sequential proton electron transfer mechanism (SPET) involving the dominant species at physiological pH. It should be noticed that the products yielded via SPET are identical to those yielded by HT from the phenolic OH (radical 4a). The SPET has been included for vanillic acid because its dianion has a low, but not negligible, population (2.1%) at physiological pH and, based on charge considerations, it is expected to be a better electron donor than the monoanion. Therefore, albeit $HVac^-$ is the dominant species under physiological conditions, based on their relative populations, the contributions of Vac^{2-} to the overall activity of the studied compounds could be significant provided that it reacts fast enough. Moreover, even though the populations of the monoanions of HGua, HVal, and HEug are 300–700 times smaller than those of the corresponding neutral species, they might still significantly contribute to the overall reactivity of these GD, provided that their rate constants are at least 300–700 times larger than those of the fastest reactions involving the neutral species. This is particularly probable for the SET mechanism, due to the known electro-donating capacity of the phenoxide anions. Therefore, the SPET has been considered not only for Van^- but also for HGua, HVal, and HEug.

The Gibbs free energies of the studied reaction paths involving electron transfers are reported in Table 2. The SET processes from HGua, HVan, HVal, $HVac^-$, and HEug in aqueous solution were found to be endergonic with ΔG values ranging from 25 to 34 kcal/mol. On the other hand, the SPET processes, which actually involve Vac^{2-}, Gua^-, Val^-, and Eug^-, are slightly endergonic or almost isoergonic, with ΔG values ranging from 0.97 to −3.50 kcal/mol. For vanillin, on the other hand, the ΔG value for SPET is significantly higher but lower than the other SET process (~9 kcal/mol). Accordingly, SET is not expected to contribute to the $HOO^\bullet$ radical scavenging activity of the studied GD, while SPET might be important.

Table 2. Gibbs Free Energies of Reaction (kcal/mol), at 298.15 K, for SET and SPET in Aqueous Solution

		SET	SPET
HGua	*a*	71.82	
HGua	*b*	27.90	−0.06
HVan	*a*	79.24	
HVan	*b*	34.24	9.21
HVal	*a*	67.12	
HVal	*b*	26.15	−1.08
H_2Vac	*a*	79.63	
$HVac^-$	*b*	27.97	0.97
HEug	*a*	68.65	
HEug	*b*	25.29	−3.50

[a]Pentyl ethanoate solution. [b]Aqueous solution.

The same behavior is also expected for other ROO•, where R is an alkyl or alkenyl group. However, SET can be viable for the reactions of these compounds with other free radicals with higher electrophilic character. In addition, it is interesting to notice that a molecular charge equal to −1 is not enough to guarantee a viable electron transfer. The SET from $HVac^-$, which corresponds to the carboxylate anion, is significantly endergonic (ΔG = 27.97 kcal/mol), while the electron transfer from the Gua^-, Val^-, and Eug^- were found to be viable processes. Therefore, what seems to be required for the electron transfer to be viable is the formation of the phenoxide anion. It is also interesting to notice that in the particular case of Van^-, the feasibility of the electron transfer is significantly lower than that of the other studied phenoxide anions. This can be explained by the presence of the electro-withdrawing carbonyl group located in *para* position with respect to the phenoxide. The relevance of the groups in *para* sites to the feasibility of the electron transfer from phenoxide anions is also evident in the finding that the largest exergonicity corresponds to eugenol (−3.50 kcal/mol).

Regarding RAF, all the reaction paths were found to be endergonic, regardless of the polarity of the environment (Table 3). Consequently, this mechanism is not expected to contribute to the HOO• radical scavenging activity of the studied GD. For the HT mechanisms, on the other hand, there are some thermochemically viable paths. For guaiacol, the HT from the phenolic OH (path 4a) was found to be exergonic by ∼4 kcal/mol in aqueous solution, and almost isoergonic in nonpolar media. For vanillin, path 4a was also found to be exergonic in aqueous solution, although less exergonic than for guaiacol. In nonpolar media, this path is predicted to be slightly endergonic (ΔG = 2.3 kcal/mol). The difference of ΔG values for vanillin, with respect to guaicol, can be explained by the presence of the carbonyl group in *para* position to the phenolic OH. The electro-withdrawing character of this group causes a destabilization of the radical product arising from HT. On the contrary, the ΔG values for vanillic alcohol are lower than those of guaiacol, also because of the substituent group. In this case, the effect is the opposite. The $-CH_2-OH$ group has electro-donor character, which stabilizes the radical product yield by path 4a.

For HVal, path 1a was also found to be exergonic (Table 3). In nonpolar media, its exergonicity is slightly higher than that of path 4a, which can be explained by the formation of a benzylic radical causing an extended conjugation of the π system. In aqueous solution, on the other hand, the polarity of the solvent inverts the relative stability of the radicals yielded by paths 1a and 4a, because the solvation of the O-centered radical (4a) is higher than that of the C-centered radical (1a). For vanillic acid, path 4a in nonpolar media is endergonic with ΔG 2.5 kcal/mol higher that of guaiacol, because of the electro-withdrawing character of the carboxyl group. In aqueous solution, where the reacting species is the monoanion ($HVac^-$), the ΔG value for vanillic acid is very similar to that of guaiacol, which indicates that the deprotonation turns off the deactivating effect of the carboxyl group. For eugenol, paths 1a and 4a were found to be exergonic, with the first one being the most exergonic one, regardless of the polarity of the environment (Table 3). This can be explained on the basis of the formation of a bis-allylic-like radical in site 1a. The exergonicity of this particular path is the largest among all the studied reactions, which indicates the high importance of this structural feature. Compared to that of vanillic alcohol, the ΔG values of HT from site 1a in eugenol are lower by at least 5 kcal/mol, which is justified by the extra-extension of the π conjugated system.

For the kinetic study, we have not included 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

Table 3. Gibbs Free Energies of Reaction (kcal/mol), at 298.15 K, for HT and RAF Mechanisms

	$HGua^a$	$HGua^b$	$HVan^a$	$HVan^b$	$Van^{-\,b}$	$HVal^a$	$HVal^b$	H_2Vac^a	$HVac^{-\,b}$	$HEug^a$	$HEug^b$
						HT					
site 1a			5.91	5.74	4.86	−3.24	−4.81			−8.28	−11.30
site 1b						16.73	17.52	26.03		4.39	1.76
site 1c										2.71	1.78
site 3a	13.78	11.05	14.26	11.30	10.09	13.66	12.07	14.38	11.28	14.36	10.95
site 4a	0.49	−4.01	2.26	−1.09		−1.43	−5.31	3.04	−4.16	−0.56	−6.45
						RAF					
site 1	13.99	11.94	20.10	19.02	17.06	12.64	11.40	21.36	14.63	12.47	10.26
site 2	17.26	14.96	16.10	13.03	12.11	15.52	14.23	15.66	14.06	16.79	14.65
site 3	13.10	11.51	14.43	13.52	7.98	12.86	10.57	15.05	12.37	12.72	11.18
site 4	12.27	10.07	10.58	8.39	12.80	10.84	9.85	11.88	8.59	11.93	9.80
site 5	16.50	14.46	18.24	16.09	12.18	15.88	14.53	18.21	14.92	16.60	14.53
site 6	15.70	12.78	13.15	9.40	11.95	12.61	11.54	14.67	11.92	14.33	11.86
site 1a			19.15	21.05	1.24			33.67	35.72		

[a]Pentyl ethanoate solution. [b]Aqueous solution.

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should be noted 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 their barriers of reactions are low. That is expected to be the case for the SET mechanism, since the formed radicals can spontaneously deprotonate; that is why SET from Van^- and from Vac^{2-} (SPET) have also been considered. In addition, for the reactions of HGua, HVan, and H_2Vac in pentyl ethanoate solution, the paths with the lowest ΔG value have also been included in the kinetic calculations, since no exergonic channels were found.

The fully optimized geometries of the transition states (TS) are shown in Figures 2 and 3 for pentyl ethanoate and aqueous

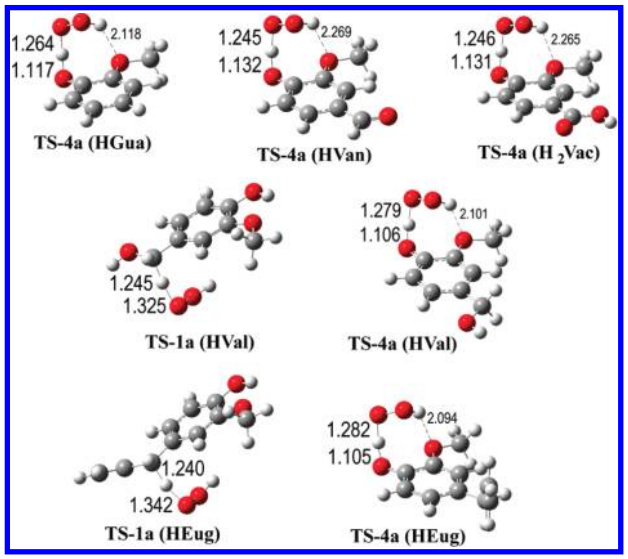

Figure 2. Optimized geometries of the HT transition states in pentyl ethanoate solution.

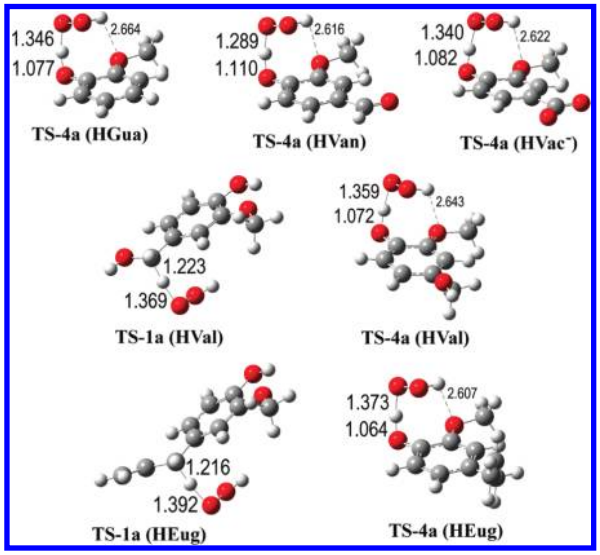

Figure 3. Optimized geometries of the HT transition states in aqueous solution.

solution, respectively. All the transition states corresponding to HT from the phenolic OH (site 4a) present hydrogen-bond-like interactions between the H in the OOH radical and the O in the methoxy group of the GD. On the basis of the H···O distances, these interactions are predicted to be particularly strong in nonpolar media but much weaker in aqueous solution. In the first case, d(H···O) ranges from 2.09 to 2.27 Å, with the shortest distance for eugenol and the longest one for vainillin. For the TSs optimized in aqueous solution, the H···O distances becomes larger and about 2.6 Å, for all the studied GD. On the other hand, for the TS corresponding to HT from site 1a, such stabilizing interaction is not present. Since this kind of interaction is expected to lower the barrier height, it should increase the importance of HT from sites 4a over HT from sites 1a.

The importance of this structural feature of the TSs (4a) is reflected in the values of the Gibbs free energies of activation ($\Delta G^{\ddagger}$) that are reported in Table 4. While the Gibbs free

Table 4. Gibbs Free Energies of Activation (kcal/mol), at 298.15 K

				HT	
		SET	SPET	site 1a	site 4a
HGua	*a*				16.13
HGua	*b*	29.40	3.87		14.45
HVan	*a*				18.00
HVan	*b*	38.10	9.84		17.54
HVal	*a*			17.00	15.33
HVal	*b*	26.63	3.81	16.30	14.08
H_2Vac	*a*				19.17
$HVac^-$	*b*	29.31	4.60		15.66
HEug	*a*			18.20	15.71
HEug	*b*	26.27	2.63	14.52	14.16

[a]Pentyl ethanoate solution. [b]Aqueous solution.

energies of path 1a were found to be lower than those of path 4a for vanillic alcohol in nonpolar media, and for eugenol in both pentyl ethanoate and aqueous solutions, the Gibbs energies of activation of path 4a are systematically lower than those of the corresponding 1a paths. Therefore, it is expected that for HT the kinetics would favor path 4a over 1a, for all the studied GD. The barriers of the SET processes were found to be significantly higher than those of the other studied reactions, indicating that SET would be too slow to significantly contribute to the overall $HOO^{\bullet}$ scavenging activity of GD. On the contrary, the lowest values of $\Delta G^{\ddagger}$, in aqueous solution, systematically correspond to the SPET process, i.e., the electron transfer from the phenoxide anions.

The rate constants of each reaction path, as well as the overall rate coefficients ($k_{overall}$), at physiological pH, are reported in Table 5. The values of $k_{overall}$ have been calculated as the sum of the rate constants of each path, and all the reported values include the molar fractions of the reacting species. In non polar environments, where the neutral species are the reacting ones, the $k_{overall}$ values range from 10^1 to 10^3 M^{-1} s^{-1}, depending on the GD. These values are in agreement with those previously reported for the reactions of guaiacol and eugenol with other peroxy radicals.[20,25] The $k_{overall}$ values from the present work are slightly higher, which is a logical finding, since $HOO^{\bullet}$ is the most reactive of the $ROO^{\bullet}$ radicals, provided that R = alkyl or alkenyl groups.

The efficiency of these compounds as $HOO^{\bullet}$ scavengers decreases in the following order: HVal > HEug > HGua > HVan > HVac, in nonpolar environments. This order is in line with the nature of the group in *para* position to the phenolic OH. Those GD with electro-donating groups (HVal and

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Table 5. Rate Constants and Overall Rate Coefficients (M^{-1} s^{-1}), at 298.15 K

			HT		
		SPET	site 1a	site 4a	overall
HGua	*a*			1.55×10^3	1.55×10^3
HGua	*b*	2.35×10^6		3.59×10^4	2.38×10^6
HVan	*a*			9.75×10^1	9.75×10^1
HVan	*b*	1.53×10^5		8.70×10^2	1.54×10^5
HVal	*a*		1.36×10^2	5.54×10^3	5.67×10^3
HVal	*b*	4.06×10^6	3.74×10^2	6.34×10^4	4.12×10^6
H_2Vac	*a*			1.29×10^1	1.29×10^1
$HVac^-$	*b*	1.65×10^7		9.07×10^3	1.65×10^7
HEug	*a*		2.05×10^1	2.47×10^3	2.49×10^3
HEug	*b*	1.52×10^6	5.48×10^3	2.52×10^4	1.55×10^6

[a]Pentyl ethanoate solution. [b]Aqueous solution.

HEug) are more reactive than guaiacol, and those with electron-withdrawing groups (HVan and HVac) are less reactive than guaiacol. This order is also in agreement with that found by Ogata et al.[14] and Tsujimoto et al.[13] for HEug and HGua. Taking into account that the rate constants corresponding to the HOO• damage to polyunsaturated fatty acids are in the range $(1.18–3.05) \times 10^3$ M^{-1} s^{-1},[35] HVal, HEug, and HGua are predicted to be moderate protectors against peroxyl oxidation of lipids. On the contrary, HVan and HVac are not expected to be efficient for that purpose. This is in line with the results reported by Shyamala et al.,[23] who found that vanillin and vanillic acid are not potent enough to cause 50% inhibition in the β-carotene linoleate method.

Compared to other antioxidants, in nonpolar environments, the peroxyl radical scavenging activity of HVal, HEug, and HGua ($\sim 10^3$ M^{-1} s^{-1}) was found to be lower than that of carotenes ($\sim 10^5–10^6$ M^{-1} s^{-1}),[66] canolol (6.8×10^5 M^{-1} s^{-1}),[67] hydroxytyrosol (6.4×10^5 M^{-1} s^{-1}),[68] sesamol (3.3×10^4 M^{-1} s^{-1}),[69] and sinapinic acid (1.7×10^4 M^{-1} s^{-1});[70] similar to that of α-mangostin (7.8×10^3 M^{-1} s^{-1});[71] and higher than that of tyrosol (7.1×10^2 M^{-1} s^{-1}),[68] melatonin (3.1×10^2 M^{-1} s^{-1}),[72] and caffeine (3.2×10^1 M^{-1} s^{-1}).[64]

In aqueous solution, the overall rate constants of the reactions of the studied GD with HOO• are significantly higher ($10^5–10^7$ M^{-1} s^{-1}) than in nonpolar media. This is mainly caused by the SPET process, but the HT reactions also become faster as the polarity of the environment increases. Therefore, it can be stated that the phenoxide anions are much better HOO• scavengers than the neutral species of GD. The polarity of the environment was also found to change the reactivity order. In aqueous solution, it becomes vanillic acid > vanillic alcohol > guaiacol ≈ eugenol > vanillin, which is in agreement with the findings of Chou et al.,[26] Mourtzinos et al.,[24] and Shyamala et al.[23] for vanillic acid and vanillin.

In addition, we have also estimated the rate constants for the SPET reactions with CCl_3OO•. At pH 7.4, they were found to be 3.83×10^8, 3.31×10^6, 2.09×10^7, 2.39×10^7, and 6.86×10^6 M^{-1} s^{-1} for vanillin, guaiacol, vanillic acid, eugenol, and vanillic alcohol, respectively. They are higher than those corresponding to the equivalent reactions with HOO•, in line with the higher electrophilicity of CCl_3OO•. In addition, the calculated rate constant for vanillin agrees well with that experimentally obtained by Mahal et al.[18] at pH 7.3 (8×10^8 M^{-1} s^{-1}). The value previously reported by Guha et al.[17] for the reaction between CCl_3OO• and eugenol (7.5×10^8 M^{-1} s^{-1}) was measured at pH 10.5; thus, for fair comparison, we have calculated this rate constant at the same pH. It was found to be equal to 6.16×10^8 M^{-1} s^{-1}, i.e., about 26 times faster than the one calculated at pH 7.4, and in excellent agreement with the experimental value.

A comparison with other antioxidants in aqueous solution has also been performed. It was found that the peroxyl radical scavenging activity of GD is much higher than that of melatonin (2.0×10^1 M^{-1} s^{-1}),[72] caffeine (3.3×10^{-1} M^{-1} s^{-1}),[64] allicin (7.4×10^3 M^{-1} s^{-1}),[73] and thioacrolein (2.9×10^4 M^{-1} s^{-1});[73] similar to that of α-mangostin (1.4×10^6 M^{-1} s^{-1}),[71] 2-propenesulfenic acid (2.6×10^7 M^{-1} s^{-1}),[73] and glutathione (2.7×10^7 M^{-1} s^{-1});[74] and lower than that of sesamol (2.4×10^8 M^{-1} s^{-1}).[69] On the basis of these results, it can be stated that GD are among the best peroxyl radical scavengers in aqueous solution, at physiological pH.

To analyze the relative importance of the different mechanisms and reaction paths on the overall HOO• scavenging activity of the studied GD, the branching ratios have been estimated according to

$$\Gamma_i = \frac{k_i}{k_{\text{overall}}} \times 100 \quad (4)$$

where *i* represents each particular path.

In nonpolar media, HGua, HVan, and H_2Vac have only one thermochemically viable channel of reaction (4a). Therefore, under such conditions, their HOO• scavenging activity takes place exclusively via HT from the phenolic OH. For HVal and HEug, on the other hand, there are two possible reaction paths (1a and 4a). Their branching ratios (Table 6) show that the contributions of path 1a to the overall reactivity of these two GD toward HOO• are almost negligible. The largest contribution of this path was found for HVal, and it represents only 2.4% of the total scavenging activity. Therefore, it can be stated that in nonpolar media, such as the lipid solution, the HOO• scavenging activity of GD occurs almost exclusively via HT from the phenolic OH. Consequently, this is proposed to be the key structural feature for the ROO• scavenging activity of GD in nonpolar environments, as long as R = alkyl or alkenyl groups.

Table 6. Branching Ratios (%), at 298.15 K

			HT	
		SPET	site 1a	site 4a
HGua	*a*			100
HGua	*b*	98.5		1.5
HVan	*a*			100
HVan	*b*	99.4		0.6
HVal	*a*		2.4	97.6
HVal	*b*	98.5	~0	1.5
H_2Vac	*a*			100
$HVac^-$	*b*	99.9		0.1
HEug	*a*		0.8	99.2
HEug	*b*	98.0	0.4	1.6

[a]Pentyl ethanoate solution. [b]Aqueous solution.

On the contrary, when the reactions take place in aqueous solution, at physiological pH, the HT mechanism is no longer the most important one. Under such conditions, the SPET processes were found to be responsible for most of the HOO• scavenging activity of the studied GD (Table 6). The contributions of this mechanism to the overall reactivity of

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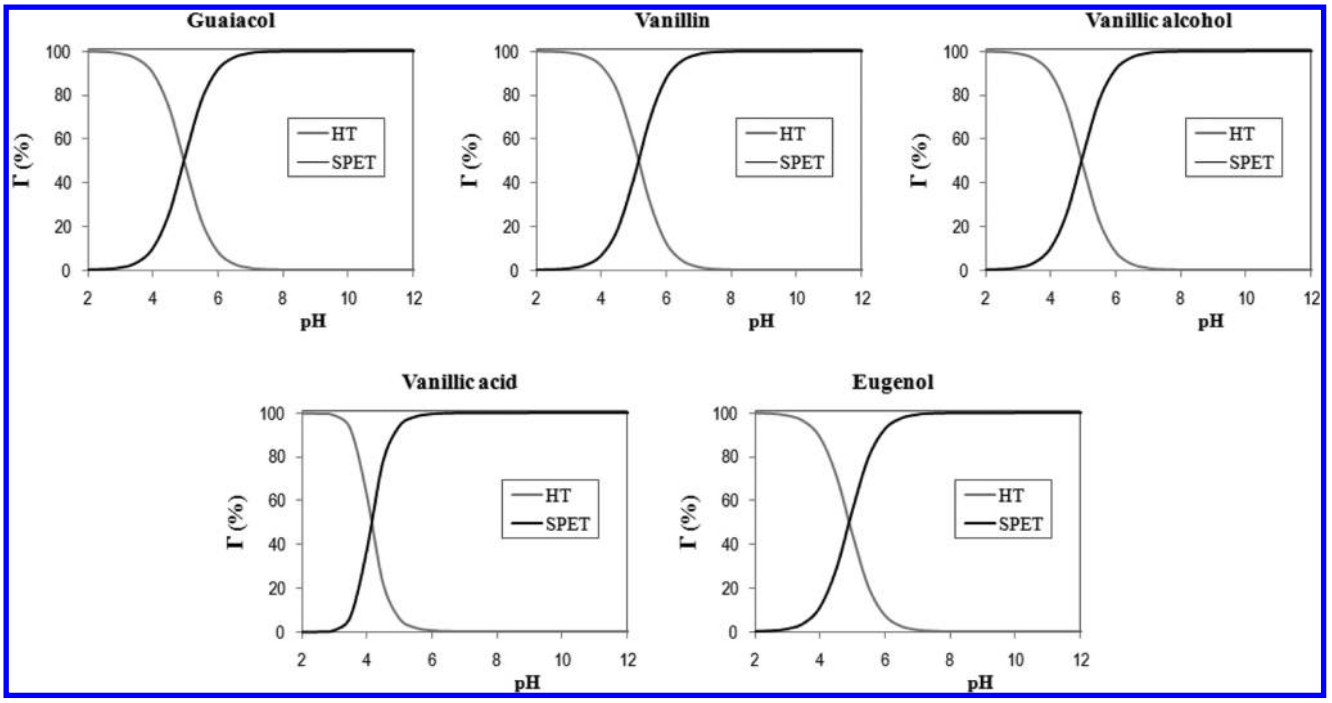

Figure 4. Influence of the pH on the relative importance of HT and SPET mechanisms for the reactions of GD with HOO• in aqueous solution.

the studied compounds were found to be >98% in all the cases. This means that in aqueous solution, at physiological pH, the key species relevant to their HOO• scavenging activity are the phenoxide anions. This is also expected to be the case for other free radicals with electrophilicity similar to, or higher than, that of HOO•.

Since the anionic species are the ones involved in the SPET processes, the relative importance of such a mechanism on the scavenging activity of GD is expected to be ruled by the pH of the environment. Therefore, we have also investigated this point, by including the corresponding molar fractions at different pHs ranging from 2 to 12 (Figure 4). As expected at the lowest pH's, where the populations of the anions are negligible, the dominant mechanism is the HT, while at the highest pHs, where the dominant form of the GDs is anionic, the main mechanism is the SPET. It was found that the behavior for guaiacol, vanillic alcohol, vanillin, and eugenol is almost identical, with the turning point around pH 5. At pH $\leq$ 3, their HOO• scavenging activity occurs almost exclusively via HT, while, at pH $\geq$ 7, SPET becomes the key mechanism. Within the pH range 3−7, both mechanisms are predicted to have significant contributions to their overall activity. For vanillic acid, on the other hand, the turning point was found at pH ~4, the HT zone is at pH $\leq$ 3, the SPET zone at pH $\geq$ 5.5, and the mixed zone at pH between 3 and 5.5. The preponderance of SPET at physiological pH (7.4) can be explained by the finding that the SPET rate constants, under such conditions, are enough times larger than the HT ones to overcome the population differences between protonated and deprotonated species. Moreover, it seems that predictions of the relative importance of these two mechanisms, in aqueous solution, should be based on the multiplication of the corresponding rate constant by the molar fractions of the different species at each particular pH ($k_i \times mF$).

The influence of the pH has also been analyzed for the overall reactivity of the GD toward HOO• in aqueous solution (Figure 5). It was found that as the pH increases so does the

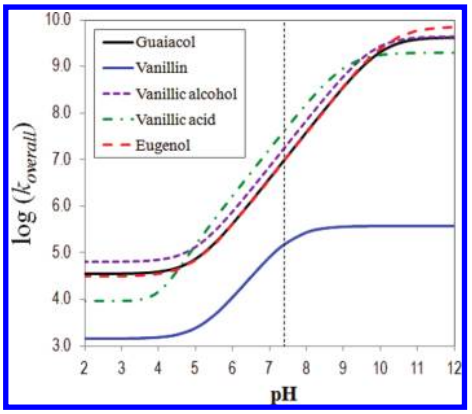

Figure 5. Influence of the pH on the overall rate coefficients of GD + HOO• reactions in aqueous solution.

HOO• scavenging efficiency of GD, in agreement with previously reported experimental evidence for other free radicals.[17,18] This strongly supports that the anionic species are more active than the neutral ones, regarding the peroxyl scavenging activity of these compounds. However, the pH not only alters the overall reactivity of GD and the relative importance of HT and SPET but also the order of reactivity of the studied compounds toward HOO•. At the most acidic pHs, where HT prevails, it was found to be HVal > HGua ≈ HEug > H_2Vac > Van, while, at the most basic pHs, it becomes Eug^- > Val^- ≈ Gua^- > Vac^{2-} > Van^-. The feature that was found to remain in the whole pH range is that vanillin is the least efficient HOO• scavenger among the studied GD. The presence of the electro-withdrawing carbonyl group lowers the electro-donating capability which is directly related to the reactivity via SPET (higher pHs), and also increases the strength of the O−H bond in the phenolic OH, lowering the HT reactivity of GD toward the electrophilic radicals (lower pHs).

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CONCLUSIONS

The peroxyl radical scavenging activity of guaiacol, vanillin, vanillic alcohol, vanillic acid, and eugenol has been studied in lipid and aqueous solutions. Different mechanisms and reaction sites have been considered as well as the pH in aqueous solution. It was found that the environment plays an important role in the free radical scavenging activity of these compounds, which becomes orders of magnitude higher in aqueous solution, compared to pentyl ethanoate solution.

The HT from the phenolic OH was identified as the main mechanism of reaction in nonpolar media and in aqueous solution at pH $\leq$ 4. On the other hand, the SPET is proposed to be the mechanism contributing the most to their peroxyl scavenging activity in aqueous solution at pH $\geq$ 6. In between, both mechanisms are predicted to contribute to a significant extent to the overall reactivity of GD toward peroxyl radicals.

The environment also influences the relative efficiency of the studied compounds as peroxyl radical scavengers. In nonpolar media, their activity follows the order vanillic alcohol > eugenol > guaiacol > vanillin > vanillic acid, while, in aqueous solution, at physiological pH, it becomes vanillic acid > vanillic alcohol > guaiacol $\approx$ eugenol > vanillin. This order can also be slightly different at extreme pH ($\leq$3 and $\geq$10).

In addition, on the basis of kinetic considerations, the studied GD are proposed to be among the best peroxyl radical scavengers in aqueous solution, at physiological pH.

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Notes

The authors declare no competing financial interest.

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