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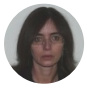

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Radical Scavenging Ability of Gallic Acid toward OH and OOH Radicals. Reaction Mechanism and Rate Constants from the Density Functional Theory

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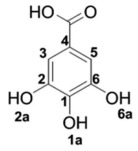
Supporting Information

ABSTRACT: Gallic acid is a ubiquitous compound, widely distributed in the vegetal kingdom and frequently found in the human diet. In the present work, its primary antioxidant activity has been investigated using the density functional theory (DFT), and the quantum mechanics-based test for overall free radical scavenging activity (QM-ORSA) protocol. It was found that gallic acid is a better antioxidant than the reference compound, Trolox, regardless of the polarity of the environment. In addition, gallic acid is predicted to be among the best peroxyl radical scavengers identified so far in nonpolar (lipid) media. This compound is capable of scavenging hydroxyl radicals at diffusion-limited rates, and hydroperoxyl radicals with rate constants in the order of 10^5 M^{-1} s^{-1}. The deprotonation of gallic acid, in aqueous solution, is predicted to increase the protective action of this compound against oxidative stress. Gallic acid was also identified as a versatile scavenger, capable of rapidly deactivating a wide variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) via electron transfer at physiological pH.

INTRODUCTION

Gallic acid (3,4,5-trihydroxybenzoic acid, Scheme 1) is a naturally occurring phenolic compound widespread in the vegetal kingdom. It is found in nuts,[1] grapes,[2,3] cherries, naseberry,[4] pomegranate,[5] honey,[6] green tea,[7] wine,[8] among many other natural sources. It has been reported that gallic acid has numerous beneficial effects on human health including antiallergic,[9] anti-inflammatory,[10] antiviral,[11,12] antifungal,[13] antimicrobial, antimutagenic,[14] anticarcinogenic,[15−18] cardioprotective,[19] and neuroprotective[20] activities. In addition, it has been extensively demonstrated that gallic acid is a potent antioxidant.[2,8,14,17−24] This is a particularly appealing property because it makes gallic acid a good protector against oxidative stress (OS), which constitutes a major health problem currently associated with the development of several diseases.[25−34] OS is a chemical stress triggered by an excess of reactive oxygen species (ROS) and often involves reactions between free radicals and molecules of high biological importance such as lipids, proteins, and DNA. Thus, dietary products that behave as good radical scavengers can help in counteracting the detrimental and cumulative effects of OS in humans.

Scheme 1. Structure of Gallic Acid (H_4GA), and Atoms Numbering



It has been shown that gallic acid has the ability of scavenging diverse free radicals such as hydroxyl,[35] singlet oxygen,[36] alkyl peroxyl,[37,38] peroxyl,[38,39] and long-lived mutagenic radicals.[21] It has also been demonstrated that gallic acid is capable of protecting cells from damage induced by UV or ionizing radiation,[40] which are known to produce free radical species. Moreover, it has been proposed that the antioxidant protection exerted by gallic acid is directly related to its direct action as free radical scavenger, which was described to be stronger than that of Trolox.[41]

Accordingly, it can be stated that there are no doubts regarding the antioxidant activity of gallic acid. However, there are many aspects of this activity that still remain to be elucidated. As it is the case for many other scavengers,[42−48] different reaction mechanisms may contribute to the overall free radical scavenging activity of gallic acid. Despite this, their relative importance has not been quantitatively assessed yet. In addition, the kinetic data directly related to the antioxidant activity of gallic acid are rather scarce. Even though the term "antioxidant" is used rather freely, the currently most accepted definition is "any substance that, when present at low concentrations compared with those of an oxidizable substrate,

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significantly delays or prevents oxidation of that substrate". Such a definition implies that kinetic data on the reactions with free radicals are crucial to establish the efficiency of a particular compound as antioxidant, that is, to assess if it will be capable of protecting biological targets by reacting faster than them.

Dwibedy et al.[30] reported that the rate constants for the gallic acid + $^{\bullet}$OH reaction is 1.1×10^{10} M^{-1} s^{-1}, at pH = 6.8 (the closest to the physiological pH investigated in that work). Under the same conditions, these authors found that the rate constants for the reactions with $^{\bullet}N_3$ and $^{\bullet}Br_2^-$ are 1.3×10^9 and 3.3×10^9 M^{-1} s^{-1}, respectively. Benitez et al.[49] determined the same value for the gallic acid + $^{\bullet}$OH reaction (1.1×10^{10} M^{-1} s^{-1}). Caregnato et al.[50] obtained rate constants equal to 6.3×10^8 and 2.9×10^9 M^{-1} s^{-1}, for the reactions of sulfate radical with gallic acid and its anion, respectively. The rate constants for the reactions of gallic acid with $^{\bullet}CCl_3O_2$ and ozone have been reported by Aruoma et al.[51] and Beltran et al.[52] to be 4.47×10^5 and 1.3×10^4 M^{-1} s^{-1}, respectively. To our best knowledge, they are the only kinetic data reported so far for the reactions of gallic acid with free radicals.

In addition, it has been established that the rate constants corresponding to the $^{\bullet}$OOH damage to polyunsaturated fatty acids are $(1.18-3.05) \times 10^3$ M^{-1} s^{-1}.[53] This value has been proposed as a threshold for identifying which compounds are expected to act as efficient antioxidants[54] because most of the potential biological targets are, fortunately, less reactive than bis-allylic hydrogens in polyunsaturated acids. However, the rate constant for the reaction of gallic acid with this radical has not been estimated yet.

On the basis of the above discussion, the main goal of the present work is to perform a detailed study on the free radical scavenging activity of gallic acid. To that purpose, we have modeled its reactions with $^{\bullet}$OOH and $^{\bullet}$OH radicals, in polar and nonpolar environments. Five different reaction mechanisms have been considered, as well as the influence of the pH in aqueous solution.

Thermodynamic and kinetic data are provided, as well as the contributions of the different mechanisms to the $^{\bullet}$OOH and $^{\bullet}$OH free radical scavenging activity of gallic acid. We have chosen $^{\bullet}$OH for being the most reactive and damaging free radical in biological systems, and $^{\bullet}$OOH because there is threshold value to identify efficient protectors. In addition, this radical has been suggested to be central to the toxic side effects of aerobic respiration.[53] It has also been pointed out that more information on the reactivity of this species is needed.[48]

COMPUTATIONAL DETAILS

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional[55] and the 6-31+G(d,p) basis set, in conjunction with the SMD continuum model[56] using benzene and water as solvents to mimic lipid and aqueous environments, respectively. The M05-2X functional has been recommended for kinetic calculations by their developers,[51] and it has been also successfully used by independent authors to that purpose.[57−60] It is also among the best performing functionals for calculating reaction energies involving free radicals.[61] 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.[52]

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 of the electronic calculations were performed with the Gaussian 09 package of programs.[62] 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,[63] taking into account the free volume theory.[64] The rate constants (k) were calculated using the Conventional Transition State Theory (TST)[65−67] and 1 M standard state, following the quantum mechanics-based test for overall free radical scavenging activity (QM-ORSA) protocol.[49] This computational protocol has been validated by comparison with experimental results, and its uncertainties have been proven to be no larger than those arising from experiments.[49]

The Gibbs free energy of activation, for the single electron transfer (SET) reactions, were calculated using the Marcus theory[68] as:

$$\Delta G^{\ddagger} = \frac{\lambda}{4}\left(1 + \frac{\Delta G}{\lambda}\right)^2$$

where ΔG is the free energy of reaction and λ is a reorganization term.

Gibbs free energies of reaction (ΔG, kcal/mol), reorganization energy (λ, kcal/mol), and Gibbs free energy of activation ($\Delta G^{\ddagger}$) related to the examined forms of gallic acid with different free radicals modeled for the electron transfer reactions are reported in Tables S1−S3 of the Supporting Information.

RESULTS AND DISCUSSION

The experimental values of the pK_a's of gallic acid are reported in Table 1, together with the molar fractions of the neutral

Table 1. pK_a Values of Gallic Acid, and Molar Fractions (*mf*) at pH = 7.4

		ref
pK_{a1}	4.24	2
	4.4	
average	4.32	
pK_{a2}	8.27	
	8.2	
average	8.24	
pK_{a3}	9.23	
	10.7	
average	9.97	
*p*Ka4	13.1	
mf (H_4GA)	0.001	
mf (H_3GA^-)	0.871	
mf (H_2GA^{2-})	0.127	
mf (HGA^{3-})	<0.001	
mf (GA^{4-})	<0.001	

(H_4GA), and the anionic (H_3GA^-, H_2GA^{2-}, HGA^{3-}, and GA^{4-}) species, at physiological pH (7.4). The later have been calculated using the average value for each pK_a when there is more than one available. The first deprotonation involves the carboxylic group; thus the monoanion corresponds to the carboxylate.

To assess which phenolic OH is involved in the second deprotonation, the three possible processes were investigated. It

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was found that the Gibbs free energy of the deprotonation from site 1a is 3.23 kcal/mol lower than those corresponding to sites 2a and 6a, which are identical by symmetry. Therefore, the dianion used in this work, when necessary, corresponds to gallic acid deprotonated from the carboxylic group and from site 1a.

According to the molar fractions, in aqueous solution (at physiological pH) the dominant form of gallic acid is the carboxylate monoanion (H_3GA^-), with a population equal to 87.1%. For the dianion (H_2GA^{2-}) and the neutral species (H_4GA), the populations are 12.7% and 0.1%, respectively, while the populations of HGA^{3-} and GA^{4-} are negligible (Figure 1). Accordingly, H_4GA, H_3GA^-, and H_2GA^{2-} are

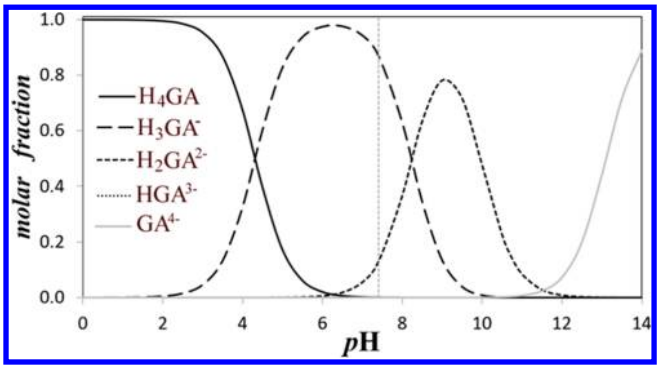

Figure 1. Distribution diagram and molar fractions of neutral and anionic species of gallic acid. The vertical line landmarks the physiological pH.

included in the present study for the reactions in aqueous solution. H_4GA and H_2GA^{2-} are taken into account despite their lower population under physiological conditions to investigate the influence of the pH on the radical scavenging activity of gallic acid. On the other hand, in nonpolar (lipid) media, only the neutral form is used, because such media do not promote the necessary solvation to stabilize the ionic species.

Five different mechanisms of reaction have been considered: hydrogen transfer, radical adduct formation (RAF), SET, sequential proton loss electron transfer (SPLET), and sequential double proton loss electron transfer (SdPLET). The two latter correspond to SET processes from the mono- and dianions, respectively. The SPLET mechanism was first proposed by Litwinienko and Ingold for the reactions of substituted phenols with the DPPH radical.[70−73] The SdPLET mechanism is a particular case of SPLET that becomes important for phenolic acids because they present more than one acid/base site: the carboxyl group, which deprotonate first, and the phenolic OH, which is involved in the second pK_a. SdPLET has been included because, based on charge considerations, and on previous studies, the most active species is expected to be the dianion, because phenolic compounds increase their activity after the formation of the phenolate moiety. On the other hand, in nonpolar solution, only RAF and HT mechanisms have been considered. The electron transfer mechanisms have not been included in this case because nonpolar environments do not promote the necessary solvation of the ionic species involved in these processes. However, just to prove this point, the SET reaction energy in benzene solution was calculated, and found to be larger than 90 and 100 kcal/mol for $^\bullet$OOH and $^\bullet$OH radicals, respectively.

The electron transfers involving the OH radical yield the OH anion. Because it has been previously reported that the largest errors derived from using solvent continuum models to properly describe the solvation energies of anions arise for those that concentrate charge on a single exposed heteroatom,[74] these species have been modeled including four solvation water molecules. This is attributed to strong short-range H bonding interactions between the anion and the solvent. A recommended strategy to overcome this issue is to combine continuum models with explicit water molecules.[75] It was previously demonstrated[76] that including one water molecule has a huge effect in this regard, while the effect of including subsequent water molecules is almost negligible. However, because it is generally accepted that the first solvation shell of OH^- contains four water molecules, we have used this particular model in the present work.

In aqueous solution, the endoergonicity of the SET process is significantly lower than that in nonpolar media, but it still remains endergonic for the reaction with $^\bullet$OOH (36.8 kcal/mol). As successive deprotonation takes place, the electron transfer becomes more thermochemically feasible, with Gibbs free energies of reaction (ΔG) for the SPLET and SdPLET reactions being 29.9 and 7.3 kcal/mol. The same trend was found for the reactions with $^\bullet$OH. In this case, the ΔG values for the electron transfers form H_4GA (SET), H_3GA^- (SPLET), and H_2GA^{2-} (SdPLET) are 0.6, −6.4, and −28.9 kcal/mol, respectively. The lower values of these energies as compared to those of the reactions involving $^\bullet$OOH can be explained on the basis of the fact that $^\bullet$OH is the most electrophilic,[77] and reactive, of the oxygen-centered radicals. In fact, these features cause $^\bullet$OH to have a very short half-life ($\sim 10^{-9}$ s).[78]

The higher reactivity of $^\bullet$OH, as compared to $^\bullet$OOH, is also evidenced in the thermochemical data of the hydrogen transfer (HT) and radical adduct formation (RAF) mechanisms. All of the reaction channels involving $^\bullet$OH were found to be exergonic (Table 2), while for the reactions of $^\bullet$OOH only the HT from the phenolic sites are thermochemically viable. Moreover, even for these cases the ΔG values of the $^\bullet$OH reactions are significantly more negative than those corresponding to $^\bullet$OOH. Another interesting finding is that the ΔG values for the reactions of H_4GA are systematically lower in aqueous

Table 2. Gibbs Free Energies of Reaction (kcal/mol), at 298.15 K, for HT and RAF Reactions

	benzene H_4GA + $^\bullet$OOH	water H_4GA + $^\bullet$OOH	water H_3GA^- + $^\bullet$OOH	benzene H_4GA + $^\bullet$OH	water H_4GA + $^\bullet$OH	water H_3GA^- + $^\bullet$OH
HT-1a	−1.76	−3.40	−6.46	−35.02	−36.86	−39.92
HT-2a	−0.38	−0.72	−2.87	−33.64	−34.17	−36.33
RAF-1	8.43	7.08	7.45	−21.12	−21.49	−20.59
RAF-2[a]	14.32	13.28	12.58	−15.45	−15.15	−15.63
RAF-3[b]	10.29	9.65	11.17	−19.41	−17.36	−15.75
RAF-4	19.75	18.68	14.30	−8.27	−8.45	−12.79

[a]Identical to RAF-6 by symmetry. [b]Identical to RAF-5 by symmetry.

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than in benzene solution, suggesting that the reactivity of gallic acid toward oxygenated free radicals increases with the polarity of the environment. The effect of deprotonation, in aqueous solution, is not so straightforward. For the HT reaction channels, deprotonation lowers the ΔG values, while for the RAF mechanism, its effect varies with the reaction site (lower for sites 2 and 4, higher for sites 1 and 3).

For the kinetic study, we have included only the reaction channels above-described as exergonic. This is because, even if endergonic channels take place at a significant rate, they would be reversible, and therefore the formed products will not be observed. However, it 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 electron transfer mechanisms (SPLET and SdPLET), because the formed intermediates are usually very reactive radicals. That is why they have also been included in the kinetic study.

The fully optimized structures of the transition states (TS) are shown in Figures 2−4.

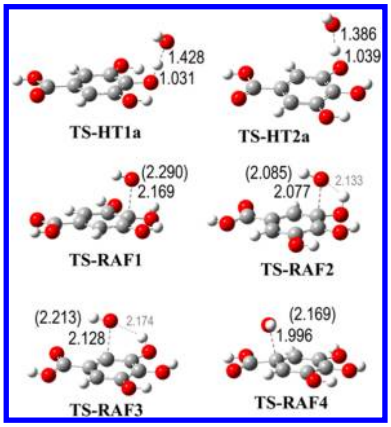

Figure 2. Optimized geometries of the transition states in benzene solution for the HT and in benzene (water) solution for the RAF reactions involving H_4GA and $^{\bullet}OH$. Distances are reported in angstroms.

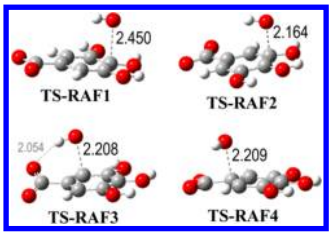

Figure 3. Optimized geometries of the transition states in aqueous solution for the HT and RAF reactions involving H_3GA^- and $^{\bullet}OH$. Distances are reported in angstroms.

It was not possible to locate the TSs corresponding to the HT channels for the reactions of OH with H_4GA and H_3GA^- in aqueous solution. Using partial optimizations with frozen O---H and H---OH bond distances, we obtain structures that present a single imaginary frequency corresponding to the desired transition vector. Unfreezing these two distances, during a saddle point optimization, invariably led to an increase of the H----OH distance, and the corresponding decrease of the

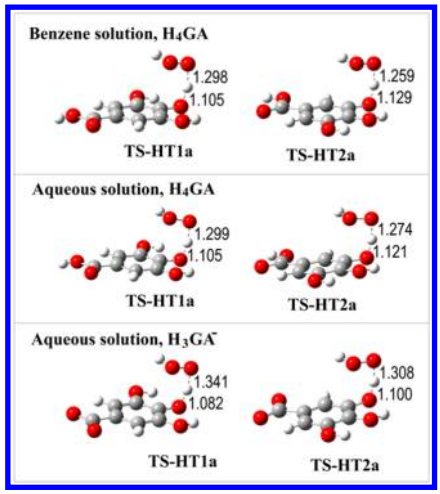

Figure 4. Optimized geometries of the transition states for the HT reactions involving H_4GA and $^{\bullet}OOH$. Distances are reported in angstroms.

imaginary frequency and gradient, yielding the separated reactants. A relaxed scan, obtained by decreasing the H---OH distance, produces a similar result; that is, the energy decreases until the H atom is completely transferred. This means that these reactions are barrierless and strictly diffusion-controlled. In other words, every encounter is effective in producing the conversion of reactants into products.

The geometrical features of the TSs indicate that for the HT mechanism the transition states corresponding to site 1a are systematically earlier than those for site 2a. This trend is the same regardless of the polarity of the environment, the reacting free radical, and the acid/base form of gallic acid reacting in aqueous solution. This suggests that site 1a should be the most reactive site via HT. In addition, for H_4GA, increasing the polarity of the environment seems to have a negligible effect on the distances corresponding to the breaking and forming bonds in TS1a for the $^{\bullet}OOH$ reactions (Figure 4). On the other hand, deprotonation leads to a significantly earlier transition state, suggesting that it should increase the reactivity of site 1a via HT. TS2a becomes earlier when increasing the environment's polarity and also after deprotonation. Thus, both factors seem to promote the reactivity of this site. In addition, all of the TS for the $^{\bullet}OH$ reactions are earlier than the corresponding ones involving $^{\bullet}OOH$, which is in agreement with the relative reactivity of these radicals.

The trends in Gibbs free energies of activation ($\Delta G^{\ddagger}$, Table 3) are in line with the above-described geometrical features of the transition states. As expected, on the basis of the known higher reactivity of $^{\bullet}OH$, for all of the studied reaction channels, the barriers are lower for the reactions with $^{\bullet}OH$, as compared to those involving $^{\bullet}OOH$. In general, it was found that the activation energies become lower as the polarity of the environment increases. In aqueous solution, the $\Delta G^{\ddagger}$ values decrease after deprotonation. The lowest $\Delta G^{\ddagger}$ values for the reactions of H_4GA with $^{\bullet}OOH$ correspond to channel HT1a in both studied environments. For the reactions with H_3GA^- in aqueous solution, the same behavior was found. However, after the second deprotonation, that is, when H_2GA^{2-} is formed, the SdPLET mechanisms have the lowest barrier. For the reactions involving $^{\bullet}OH$, on the other hand, the lowest $\Delta G^{\ddagger}$ values correspond to the RAF mechanism in nonpolar environment, and to the HT in aqueous solution. The effects of the polarity

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

	benzene H_4GA + •OOH	water H_4GA + •OOH	water H_3GA^- + •OOH	benzene H_4GA + •OH	water H_4GA + •OH	water H_3GA^- + •OH
SET		43.44			5.33	
SPLET			32.29			2.55
SdPLET[a]			8.25			1.42
HT-1a	11.14	13.50	11.96	4.65	barrierless	barrierless
HT-2a	13.49	16.22	14.78	3.32	barrierless	barrierless
RAF-1				3.34	2.37	0.02
RAF-2[b]				3.79	4.34	3.27
RAF-3[c]				3.26	2.42	1.59
RAF-4				8.25	6.05	4.54

[a]SET from H_2GA^{2-}. [b]Identical to RAF-6 by symmetry. [c]Identical to RAF-5 by symmetry.

Table 4. Rate Constants, Total, and Overall Rate Coefficients (M^{-1} s^{-1}), at 298.15 K

	benzene H_4GA + •OOH	water H_4GA + •OOH	water H_3GA^- + •OOH	benzene H_4GA + •OH	water H_4GA + •OH	water H_3GA^- + •OH
SET		8.88×10^{-20}			$7.02 \times 10^{+08}$	
SPLET			1.33×10^{-11}			$6.83 \times 10^{+09}$
SdPLET[a]			$5.52 \times 10^{+06}$			$7.32 \times 10^{+09}$
HT-1a	$4.54 \times 10^{+05}$	$4.54 \times 10^{+04}$	$1.77 \times 10^{+05}$	$1.57 \times 10^{+09}$	$4.40 \times 10^{+09}$	$4.50 \times 10^{+09}$
HT-2a	$5.07 \times 10^{+04}$	$2.96 \times 10^{+03}$	$1.57 \times 10^{+04}$	$4.02 \times 10^{+09}$	$4.40 \times 10^{+09}$	$4.50 \times 10^{+09}$
RAF-1				$3.14 \times 10^{+09}$	$3.34 \times 10^{+09}$	$3.46 \times 10^{+09}$
RAF-2				$1.57 \times 10^{+09}$	$1.40 \times 10^{+09}$	$1.67 \times 10^{+09}$
RAF-3				$1.64 \times 10^{+09}$	$1.68 \times 10^{+09}$	$1.73 \times 10^{+09}$
RAF-4				$1.11 \times 10^{+07}$	$4.04 \times 10^{+08}$	$2.17 \times 10^{+09}$
RAF-5				$1.64 \times 10^{+09}$	$1.68 \times 10^{+09}$	$1.73 \times 10^{+09}$
RAF-4				$1.57 \times 10^{+09}$	$1.40 \times 10^{+09}$	$1.67 \times 10^{+09}$
[t]otal	$5.04 \times 10^{+05}$	$4.83 \times 10^{+04}$	$1.92 \times 10^{+05}$	$1.52 \times 10^{+10}$	$1.94 \times 10^{+10}$	$2.83 \times 10^{+10}$
overall[b]		$8.71 \times 10^{+05}$			$2.56 \times 10^{+10}$	

[a]SET from H_2GA^{2-}. [b]At pH = 7.4.

of the environment and deprotonation on the $\Delta G^{\ddagger}$ for the reactions with this radical are the same as found for the reactions with •OH.

The rate constants for each reaction path, as well as the total coefficients (k_{tot}) in benzene solution, are reported in Table 4. For the reactions in aqueous solution, the overall rate coefficients ($k_{overall}$), at physiological pH, are also reported. The values of k_{tot} are calculated as the sum of the rate constants of each path, for the dominant species, under the different conditions. $k_{overall}$ is obtained considering the molar fractions of the mono- and dianions at the investigated pH. Accordingly, the values of $k_{overall}$ are the ones that can be directly related to the observable behavior of the studied reactions (in aqueous solution, at buffered pH = 7.4). They were calculated according to the following expressions:

$$k^{Bz,H_4GA}_{tot(OOH)} = \sum k^{HT}_{i(H_4GA)} \quad (1)$$

$$k^{Bz,H_4GA}_{tot(OH)} = \sum k^{HT}_{i(H_4GA)} + \sum k^{RAF}_{i(H_4GA)} \quad (2)$$

$$k^{W,H_4GA}_{tot(OOH)} = k^{SET}_{(H_4GA)} + \sum k^{HT}_{i(H_4GA)} \quad (3)$$

$$k^{W,H_4GA}_{tot(OH)} = k^{SET}_{(H_4GA)} + \sum k^{HT}_{i(H_4GA)} + \sum k^{RAF}_{i(H_4GA)} \quad (4)$$

$$k^{W,H_3GA^-}_{tot(OOH)} = k^{SPLET}_{(H_3GA^-)} + \sum k^{HT}_{i(H_3GA^-)} \quad (5)$$

$$k^{W,H_3GA^-}_{tot(OOH)} = k^{SPLET}_{(H_3GA^-)} + \sum k^{HT}_{i(H_3GA^-)} + \sum k^{RAF}_{i(H_3GA^-)} \quad (6)$$

$$k^{W,pH=7.4}_{overall(OOH)} = mf^{pH=7.4}_{(H_4GA)}\, k^{W,H_4GA}_{tot(OOH)} + mf^{pH=7.4}_{(H_3GA^-)}\, k^{W,H_3GA^-}_{tot(OOH)} + mf^{pH=7.4}_{(H_2GA^{2-})}\, k^{SdPLET} \quad (7)$$

$$k^{W,pH=7.4}_{overall(OH)} = mf^{pH=7.4}_{(H_4GA)}\, k^{W,H_4GA}_{tot(OH)} + mf^{pH=7.4}_{(H_3GA^-)}\, k^{W,H_3GA^-}_{tot(OH)} + mf^{pH=7.4}_{(H_2GA^{2-})}\, k^{SdPLET} \quad (8)$$

The total rate constants for the reaction with •OH (Table 4) indicate that the reactions of gallic acid with this radical are diffusion-limited, regardless of the polarity of the environment, and of the dominant acid/base form of gallic acid, in aqueous solution, implicated in the reaction. This indicates that, as many other phenolic compounds, gallic acid is very efficient for scavenging this radical. The calculated overall rate coefficients (1.52×10^{10} and 2.56×10^{10} M^{-1} s^{-1}, in benzene solution and in aqueous solution at pH = 7.4, respectively) are in excellent agreement with the experimental values (1.1×10^{10} M^{-1} s^{-1}),[30,44] which supports the reliability of the calculations presented in this work. The overall reaction with •OOH has much lower rate coefficients, in both environments, in agreement with the relative low reactivity of this radical, as compared to •OH. However, this reaction is still fast enough, and gallic acid is proposed as an efficient •OOH scavenger in biological systems. This statement is based taking into account that the rate constants corresponding to the •OOH damage to polyunsaturated fatty acids are in the range $(1.18-3.05) \times 10^3$ M^{-1} s^{-1},[48] and the rate coefficients for gallic acid are 5.04×10^5 and 8.71×10^5 M^{-1} s^{-1}, in benzene solution and in aqueous solution at pH = 7.4, respectively. These values indicate that gallic acid reacts more than 100 times faster with •OOH than

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Table 5. Branching Ratios (%), at 298.15 K, in Benzene Solution and in Aqueous Solution at pH = 7.4

	benzene H_4GA + $^{\bullet}OOH$	water H_4GA + $^{\bullet}OOH$	water H_3GA^- + $^{\bullet}OOH$	benzene H_4GA + $^{\bullet}OH$	water H_4GA + $^{\bullet}OH$	water H_3GA^- + $^{\bullet}OH$
SET	~0.00	~0.00			~0.00	
SPLET			~0.00			23.25
SdPLET[a]			80.74			3.65
HT-1a	89.95	~0.00	17.69	10.36	0.01	15.33
HT-2a	10.05	~0.00	1.57	26.52	0.01	15.34
RAF-1				20.75	0.01	11.80
RAF-2				10.33	~0.00	5.70
RAF-3				10.81	~0.00	5.89
RAF-4				0.07	~0.00	7.39
RAF-5				10.81	~0.00	5.89
RAF-6				10.33	~0.00	5.70

[a]SET from H_2GA^{2-}.

the biological targets. Thus, it is capable of protecting them, even at lower concentrations.

The efficiency of gallic acid to that purpose can also be validated by comparisons with Trolox, which is frequently used as a reference antioxidant. The rate coefficients for the reactions between Trolox and $^{\bullet}OOH$ in nonpolar media (mimicking the lipid environment) have been reported to be 3.40×10^3 and 8.96×10^4 M^{-1} s^{-1}, in aqueous solution at pH = 7.4, respectively.[79] Therefore, gallic acid is predicted to react with this radical about 148 and 2 times faster than Trolox in nonpolar and aqueous solution, respectively. This indicates that gallic acid is a better antioxidant than this reference compound, in agreement with the previous findings by Kiranjit et al. [34] This agreement also validates the reliability of our calculations.

As compared to other antioxidants, in nonpolar media, the peroxyl radical scavenging activity of gallic acid was found to be similar to those of carotenes (~10^5 to 10^6 M^{-1} s^{-1}),[80,81] dopamine (8.2×10^5 M^{-1} s^{-1}),[82] canolol (6.8×10^5 M^{-1} s^{-1}),[83] and hydroxytyrosol (6.4×10^5 M^{-1} s^{-1});[84] and higher than those of sesamol (3.3×10^4 M^{-1} s^{-1}),[85] sinapinic acid (1.7×10^4 M^{-1} s^{-1}),[86] protocatechuic acid (5.1×10^3),[87] capsaicine (6.5×10^3),[88] α-mangostin (7.8×10^3 M^{-1} s^{-1}),[89] tyrosol (7.1×10^2 M^{-1} s^{-1}),[82] melatonin (3.1×10^2 M^{-1} s^{-1}),[90] and caffeine (3.2×10^1 M^{-1} s^{-1}).[48] Therefore, it can be stated that for nonpolar media, gallic acid is among the best peroxyl radical scavengers, identified so far. On the other hand, in aqueous solution, gallic acid is predicted to have higher peroxyl radical scavenging activity than those of melatonin (2.0×10^1 M^{-1} s^{-1}),[88] caffeine (3.3×10^{-1} M^{-1} s^{-1}),[48] allicin (7.4×10^3 M^{-1} s^{-1}),[91] and thioacrolein (2.9×10^4 M^{-1} s^{-1});[90] similar to those of dopamine (2.2×10^5 M^{-1} s^{-1});[80] and lower than those of canolol (2.50×10^6 M^{-1} s^{-1}),[81] α-mangostin (1.4×10^6 M^{-1} s^{-1}),[87] protocatechuic acid (1.3×10^7 M^{-1} s^{-1}),[85] 2-propenesulfenic acid (2.6×10^7 M^{-1} s^{-1}),[90] glutathione (2.7×10^7 M^{-1} s^{-1}),[92] and sesamol (2.4×10^8 M^{-1} s^{-1}).[83]

This comparisons have been performed for the reactions involving $^{\bullet}OOH$, and not for those with $^{\bullet}OH$, because free radicals of intermediate to low reactivity have been recommended for studying the relative scavenging activity of different compounds.[93,94]

To analyze the relative importance of the different mechanisms, and reaction paths, on the overall free radical scavenging activity of gallic acid, the branching ratios have been estimated (Table 5) according to:

$$\Gamma_i^{PE} = \frac{k_i}{k_{tot}} \times 100 \quad (9)$$

$$\Gamma_i^{W,pH=7.4} = \frac{k_i}{k_{overall}^{W,pH=7.4}} \times 100 \quad (10)$$

where i represents each particular reaction path.

For the H_4GA + $^{\bullet}OOH$ reaction, in benzene solution, the only important mechanism of reaction is the HT, with the major contribution corresponding to channel 1a. In aqueous solution, at physiological pH, H_2GA^{2-} and H_3GA^- are the key species to the overall reactivity of the gallic acid toward $^{\bullet}OOH$. The contributions from the neutral species, all combined, are still smaller than 0.01%. Under such conditions, the main reaction mechanism is SdPLET, supporting the idea that electron transfer processes from phenoxide anions are crucial to the peroxyl radical scavenging activity of phenolic compounds.

The reactivity of gallic acid toward $^{\bullet}OH$ is more diverse, regarding the different mechanisms and sites of reaction. In nonpolar media, where H_4GA is expected to be the dominant species, practically all of the reaction channels have significant contributions to the overall reactivity. The only exception is the RAF reaction at site 4. However, the HT channels contribute more than the RAF channels, when analyzed separately. On the contrary, combining all of the HT reaction channels together, and doing the same for the RAF channels, it becomes evident that both reaction mechanisms are similarly important to the $^{\bullet}OH$ scavenging activity of gallic acid. In aqueous solution, at physiological pH, SPLET becomes the major reaction channel, while the contributions of SdPLET, HT, and RAF are smaller but still important. This suggests that when gallic acid reacts with $^{\bullet}OH$, a wide variety of reaction products should be yielded.

Because it has been previously established that electron transfer is relevant to the free radical scavenging activity of phenolic compounds in general, we have extended the investigation on SET, SPLET, and SdPLET processes to a larger set of free radicals (Table 6). It has also been proposed that the nature of the reacting free radical may influence the relative importance of the different reaction mechanisms.[95] In the particular case of electron transfers from a specific compound toward free radicals, it is expected that its relative importance would increase with the electron-withdrawing character of the radical. To this part of the investigation, a wide variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been considered, because they are the most common oxidants in biological systems. The overall electron transfer (ET) process has been modeled by including the SET, SPLET, and SdPLET mechanisms. The corresponding rate coefficient is then calculated using the molar fractions

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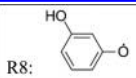
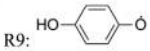
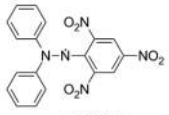
Table 6. Free Radicals Modeled for the Electron Transfer Reactions, Acronyms, and Formulas or Structures

R1: $^{\bullet}OH$ ($4H_2O$)	R8: 	R15: 
R2: $^{\bullet}OCH_3$	R9: 	R16: 
R3: $^{\bullet}OCH_2CH{=}CH_2$	R10: $^{\bullet}OOCH_3$	R17: 
R4: 	R11: $^{\bullet}OOCH_2Cl$	R18: 
R5: 	R12: $^{\bullet}OOCHCl_2$	R19: $^{\bullet}NO_2$
R6: 	R13: $^{\bullet}OOCCl_3$	R20: $^{\bullet}N_3$
R7: 	R14: $^{\bullet}OOCH_2CH{=}CH_2$	R21:  (DPPH)

of the involved acid base species (H_4GA, H_3GA^-, and H_2GA^{2-}, respectively) as:

$$k_{ET,overall}^{W,pH=7.4} = mf_{(H_4GA)}^{pH=7.4}\, k^{SET} + mf_{(H_3GA^-)}^{pH=7.4}\, k^{SPLET} + mf_{(H_2GA^{2-})}^{pH=7.4}\, k^{SdPLET} \quad (11)$$

The values of the overall rate coefficient, as well as the rate constants for the individual SET, SPLET, and SdPLET reactions, are reported in Table 7.

It was found that in general in aqueous solution, at pH = 7.4, as the deprotonation increases so does the free radical scavenging activity of gallic acid via electron transfer. This indicates that the pH of the environment plays a role in the antioxidant activity of this compound. Under physiological conditions, it was found that the order of reactivity of gallic acid toward the studied free radicals is: R1 > R17 > R19 > R18 > R13 > R5 > R3 > R12 > R2 > R20 > R8 > R11 > R4 > R15 > R21 > R7 > R6 > R16 > R14 > R10 > R9. All of the reactions are fast, with rate constants larger than 10^4 M^{-1} s^{-1}. In addition, under such conditions, gallic acid is very efficient for deactivating a wide variety of free radicals (R1−R5, R8, R11−R13, R15, R17−R20), with rate constants that are within, or close to, the diffusion limit regime ($k^{ET} \geq 10^8$ M^{-1} s^{-1}).

In addition, the Trolox equivalent antioxidant capacity (TEAC) assay is usually carried out using DPPH as the reacting free radical, which is expected to react mainly via electron transfer due to hindrance effects. Thus, we have compared the overall rate coefficient of gallic acid via electron transfer with that reported for Trolox (8.22×10^6 M^{-1} s^{-1}).[77] The comparison is expected to be fair because in both cases a similar methodology was used to obtain the kinetic data. Accordingly, it is estimated that in aqueous solution at physiological pH, gallic acid reacts 6.4 times faster than Trolox, which supports the higher antioxidant capacity of gallic acid.

Table 7. Rate Constants of the Overall Electron Transfer Reactions between Gallic Acid and Different Free Radicals, in Aqueous Solution, at pH = 7.4 and 298.15 K

	k^{SET}	k^{SPLET}	k^{SdPLET}	$k_{ET,overall}^{W,pH=7.4}$
	H_4GA	H_3GA^-	H_2GA^{2-}	gallic acid
R1	$7.02 \times 10^{+08}$	$6.83 \times 10^{+09}$	$7.32 \times 10^{+09}$	$6.88 \times 10^{+09}$
R2	1.39×10^{-19}	2.55×10^{-08}	$7.29 \times 10^{+09}$	$9.29 \times 10^{+08}$
R3	3.69×10^{-17}	1.78×10^{-06}	$7.35 \times 10^{+09}$	$9.36 \times 10^{+08}$
R4	1.69×10^{-20}	7.86×10^{-10}	$4.82 \times 10^{+09}$	$6.14 \times 10^{+08}$
R5	7.62×10^{-25}	1.32×10^{-09}	$7.39 \times 10^{+09}$	$9.41 \times 10^{+08}$
R6	6.01×10^{-22}	2.90×10^{-12}	$5.83 \times 10^{+07}$	$7.43 \times 10^{+06}$
R7	4.73×10^{-21}	2.48×10^{-11}	$2.69 \times 10^{+08}$	$3.43 \times 10^{+07}$
R8	2.26×10^{-18}	2.06×10^{-08}	$5.91 \times 10^{+09}$	$7.53 \times 10^{+08}$
R9	5.02×10^{-27}	1.67×10^{-16}	$2.82 \times 10^{+05}$	$3.60 \times 10^{+04}$
R10	6.01×10^{-21}	5.93×10^{-13}	$3.16 \times 10^{+05}$	$4.02 \times 10^{+04}$
R11	5.07×10^{-09}	3.84×10^{-03}	$5.32 \times 10^{+09}$	$6.78 \times 10^{+08}$
R12	4.46×10^{-04}	$4.29 \times 10^{+01}$	$7.34 \times 10^{+09}$	$9.35 \times 10^{+08}$
R13	$6.22 \times 10^{+00}$	$8.35 \times 10^{+04}$	$7.40 \times 10^{+09}$	$9.43 \times 10^{+08}$
R14	9.96×10^{-20}	4.63×10^{-12}	$6.53 \times 10^{+05}$	$8.32 \times 10^{+04}$
R15	8.90×10^{-13}	6.04×10^{-06}	$1.81 \times 10^{+09}$	$2.31 \times 10^{+08}$
R16	8.28×10^{-20}	4.62×10^{-12}	$8.07 \times 10^{+05}$	$1.03 \times 10^{+05}$
R17	$7.64 \times 10^{+09}$	$7.51 \times 10^{+09}$	1.80×10^{-24}	$6.55 \times 10^{+09}$
R18	5.22×10^{-02}	$1.93 \times 10^{+03}$	$7.45 \times 10^{+09}$	$9.49 \times 10^{+08}$
R19	$6.14 \times 10^{+00}$	$1.15 \times 10^{+04}$	$7.57 \times 10^{+09}$	$9.65 \times 10^{+08}$
R20	$1.81 \times 10^{+02}$	$6.26 \times 10^{+07}$	$6.56 \times 10^{+09}$	$8.91 \times 10^{+08}$
R21	9.67×10^{-11}	4.13×10^{-05}	$4.10 \times 10^{+08}$	$5.23 \times 10^{+07}$

[a]From ref 77.

CONCLUSIONS

The free radical scavenging activity of gallic acid, which corresponds to its primary antioxidant activity, has been investigated. Five different reaction mechanisms have been considered, as well as the influence of the polarity of the environment, and the pH in aqueous solution for the reactions with $^{\bullet}OOH$ and $^{\bullet}OH$ radicals. The study of the electron transfer processes was extended to 21 other free radicals, including ROS, RNS, and DPPH.

For the gallic acid + $^{\bullet}OOH$ reaction, in nonpolar media, HT was identified as the only important mechanism of reaction. In aqueous solution, at physiological pH, the main reaction mechanism is SdPLET, supporting the idea that electron transfer processes from phenoxide anions are crucial to the peroxyl radical scavenging activity of phenolic compounds. For the $^{\bullet}OH$ scavenging activity of gallic acid, on the other hand, practically all of the reaction channels have significant contributions to the overall reactivity.

Gallic acid was found to react with $^{\bullet}OOH$ about 148 and 2 times faster than Trolox in nonpolar and aqueous solution, respectively, which indicates that gallic acid is a better antioxidant than this reference compound. In addition, in aqueous solution at physiological pH, gallic acid reacts 6.4 times faster than Trolox with DPPH. This result is directly comparable to the TEAC assay.

The electron transfer reactions from gallic acid to a wide variety of ROS and RNS were also investigated in aqueous solution at physiological pH. Under such conditions, all of the studied reactions are fast, with rate constants larger than 10^4 M^{-1} s^{-1}. Moreover, most of them are diffusion limited, which supports the versatility of gallic acid as free radical scavenger.

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The calculated data are in excellent agreement with the available experimental information, which support the reliability of the calculations presented in this work.

■ ASSOCIATED CONTENT

Supporting Information

Gibbs free energies of reaction (ΔG, kcal/mol), reorganization energy (λ, kcal/mol), and Gibbs free energy of activation ($\Delta G^{\ddagger}$) for the SET reactions involving H_4GA, H_3GA^-, and H_2GA^{2-} species collected in tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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