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Carboxyl Group as a Radical Scavenging Moiety: Thermodynamics of $2H^+/2e^-$ Processes of Phloretic Acid

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Abstract: Phloretic acid is one of the most abundant colon catabolites of various classes of polyphenols (*e.g.*, polymeric proanthocyanidins, tea catechins, and ellagitannins). In this paper thermodynamics of $2H^+/2e^-$ radical scavenging mechanisms of phloretic acid was studied. For the first time the involvement of carboxyl group in double hydrogen atom transfer (dHAT), double electron transfer-proton transfer (dET-PT) and sequential double proton loss double electron transfer (SdPLdET) processes was investigated. Obtained results indicate that phloretic acid possesses potential for inactivating radicals of different characteristics ($HO^\bullet$, $HOO^\bullet$, $CH_3O^\bullet$, $CH_3OO^\bullet$, $CH_2{=}CH{-}O{-}O^\bullet$, $PhO^\bullet$, $Cl_3COO^\bullet$ etc.) *via* dHAT and SdPLdET mechanisms. Because phloretic acid is usually better absorbed than its precursor molecules, it may contribute to health benefits associated with regular intake of polyphenol-rich diet by direct scavenging of radicals.

Keywords: phloretic acid, radical scavenging, DFT, dHAT, SdPLdET.

INTRODUCTION

PHLORETIC acid (3-(4-hydroxyphenyl)propanoic acid; dihydro-*p*-coumaric acid) can be found in very small quantities in plant kingdom[1] where it is involved as a precursor in the biosynthesis of cyclic diarylheptanoids, a class of natural products.[2] Some processed fruits, such as black brined olives and raisins, contain phloretic acid as a product of bacterial activity on phenolic acids.[3,4] In man and animal, among numerous products formed during colonic microbial catabolism of various (poly)phenols (*e.g.*, polymeric proanthocyanidins, acylated flavonoid glycosides, tea catechins, gallotannins, ellagitannins and hydroxycinnamates),[5–7] phloretic acid is one of the most abundantly produced.[8] Increasing evidence suggests that health benefits of (poly)phenol-rich diet (*e.g.*, reduced risk of cardiovascular diseases and cancer) are related to highly bioavailable colonic catabolites (such as phloretic acid) rather than to parent molecules present in vegetables, fruits, grains, olive oil, red wine and tea which usually possess very low bioavailability.[9–11] Concentrations of intact (poly)phenols in systemic circulation are mainly in nM to very low μM range,[10] which is insufficient to exert *in vivo* direct radical scavenging (antioxidant) activity.[12] However, colonic catabolites produced in high μM concentrations[8] have potential to promote human health *via* several possible *in vivo* mechanisms.[13]

Phloretic acid is a weak acid (pK_{a1} (–COOH) = 4.76;[14] pK_{a2} (–OH) = 10.1).[15] It is slightly soluble in water and at the physiological pH of 7.4 (pH of colon is in the range of 6.6 to 7.5)[16] the majority of phloretic acid is in the ionized form, *i.e.* as a carboxylate anion. Its radical scavenging activity has been investigated by inhibition of chemiluminescence in human granulocytes.[17] Phloretic acid concentration in colon may reach values of 200 μM,[8] which could be sufficient to exert biological activity and effective direct radical inactivation.

Radicals are continuously produced in all cells as a part of normal cellular function. They are essential for human health because of involvement in specific metabolic processes (such as energy production), intercellular signalling and destroying of pathogenic microbes.[18] Body defence mechanisms ensure delicate balance between the production and the removal of radicals, maintaining their optimal concentrations.[19,20] If this homeostasis is interrupted, excess of radicals initiate a chain of oxidative reactions, giving rise to protein, lipid and DNA damage. (Poly)phenolic colon catabolites produced in high μM concentrations could be important endogenous antioxidants capable to scavenge excess of radicals and suppress their deleterious effects.

Various reaction mechanisms involved in radical scavenging by (poly)phenols have been proposed, mostly depending on the environment polarity and radical characteristics.[21–23] They can be grouped into two types of processes: H-atom abstraction and radical adduct formation (RAF). H-atom abstraction processes may occur *via* at least three different mechanisms: hydrogen atom transfer (HAT), electron transfer followed by proton transfer (ET-PT) and sequential proton loss electron transfer (SPLET). These and some other possible mechanisms (such as proton-coupled electron transfer (PCET) and sequential proton-loss hydrogen-atom transfer (SPLHAT)) have been recently reviewed.[24] Already published studies dealing with radical scavenging mechanisms of natural (poly)phenols have mostly been based on single, $1H^+/1e^-$ processes.[25] However, depending on a number and position of radical scavenging moieties such as phenolic O–H and imino N–H groups, they may also proceed as double (multiple) sequential $1H^+/1e^-$ mechanisms.[26–29] Recently, we have performed a DFT investigation of radical scavenging potency of phloretic acid by considering single $1H^+/1e^-$ mechanisms related to homolytic and heterolytic O–H bond cleavage in phenolic OH group.[30] In this study, we have computationally investigated energetics of double, *i.e.*, $2H^+/2e^-$ mechanisms (two sequential $1H^+/1e^-$ processes) by including carboxyl group of phloretic acid in consideration. To the best of our knowledge, involvement of carboxyl group as a radical scavenging moiety in such processes has not been studied before.

COMPUTATIONAL DETAILS

Phloretic acid (HO–Phl–COOH) could be able to scavenge two radicals *via* double HAT (dHAT) mechanism. The first step in this mechanism is the homolytic cleavage of phenolic O–H bond which results in formation of phenoxyl radical (•O–Phl–COOH) (Eq. 1). In the second step the homolytic cleavage of O–H bond of carboxyl group occurs producing dienone lactone (•O–Phl–COO•), Eq. (2).

$$\mathrm{HO-Phl-COOH} \rightarrow {}^{\bullet}\mathrm{O-Phl-COOH} + \mathrm{H}^{\bullet} \quad (1)$$

$${}^{\bullet}\mathrm{O-Phl-COOH} \rightarrow {}^{\bullet}\mathrm{O-Phl-COO}^{\bullet} + \mathrm{H}^{\bullet} \quad (2)$$

The HAT mechanism can be characterized by the bond dissociation enthalpy (BDE). The first and the second BDE are calculated according to Eqs. (3) and (4), respectively.

$$\mathrm{BDE1} = H\left({}^{\bullet}\mathrm{O-Phl-COOH}\right) + H\left(\mathrm{H}^{\bullet}\right) - H\left(\mathrm{HO-Phl-COOH}\right) \quad (3)$$

$$\mathrm{BDE2} = H\left({}^{\bullet}\mathrm{O-Phl-COO}^{\bullet}\right) + H\left(\mathrm{H}^{\bullet}\right) - H\left({}^{\bullet}\mathrm{O-Phl-COOH}\right) \quad (4)$$

where H(HO–Phl–COOH), H(•O–Phl–COOH), H(•O–Phl–COO•) and H(H•) are enthalpies of phloretic acid, phenoxyl radical, dienone lactone and H-atom, respectively.

The double ET-PT mechanism (dET-PT) proceeds in four steps. The first step is transfer of an electron from HO–Phl–COOH by which the radical cation (HO–Phl–$\mathrm{COOH}^{\bullet+}$) is formed, Eq. (5). The second step is deprotonation of HO–Phl–$\mathrm{COOH}^{\bullet+}$, which results in formation of phenoxyl radical, Eq. (6). Electron transfer from phenoxyl radical (the third step, Eq. (7)) gives cationic intermediate ([O–Phl–COOH]$^+$) which produces dienone lactone by the proton transfer in the final step, Eq. (8).

$$\mathrm{HO-Phl-COOH} \rightarrow \mathrm{HO-Phl-COOH}^{\bullet+} + \mathrm{e}^- \quad (5)$$

$$\mathrm{HO-Phl-COOH}^{\bullet+} \rightarrow {}^{\bullet}\mathrm{O-Phl-COOH} + \mathrm{H}^+ \quad (6)$$

$${}^{\bullet}\mathrm{O-Phl-COOH} \rightarrow \left[\mathrm{O-Phl-COOH}\right]^+ + \mathrm{e}^- \quad (7)$$

$$\left[\mathrm{O-Phl-COOH}\right]^+ \rightarrow {}^{\bullet}\mathrm{O-Phl-COO}^{\bullet} + \mathrm{H}^+ \quad (8)$$

The reaction enthalpies related to these processes can be calculated by the following equations:

$$\mathrm{IP1} = H\left(\mathrm{HO-Phl-COOH}^{\bullet+}\right) + H\left(\mathrm{e}^-\right) - H\left(\mathrm{HO-Phl-COOH}\right) \quad (9)$$

$$\mathrm{PDE1} = H\left({}^{\bullet}\mathrm{O-Phl-COOH}\right) + H\left(\mathrm{H}^+\right) - H\left(\mathrm{HO-Phl-COOH}^{\bullet+}\right) \quad (10)$$

$$\mathrm{IP2} = H\left(\left[\mathrm{O-Phl-COOH}\right]^+\right) + H\left(\mathrm{e}^-\right) - H\left({}^{\bullet}\mathrm{O-Phl-COOH}\right) \quad (11)$$

$$\mathrm{PDE2} = H\left({}^{\bullet}\mathrm{O-Phl-COO}^{\bullet}\right) + H\left(\mathrm{H}^+\right) - H\left(\left[\mathrm{O-Phl-COOH}\right]^+\right) \quad (12)$$

IP1 (IP2) and PDE1 (PDE2) are ionization potential and proton dissociation enthalpy related to the first (second) step. H(HO–Phl–COOH$^{\bullet+}$), H([O–Phl–COOH]$^{+}$), H(e^{-}) and H(H^{+}) are enthalpies of radical cation, cationic intermediate, electron and proton, respectively.

In the double SPLET mechanism deprotonation of carboxyl group occurs first followed by the heterolytic cleavage of phenolic O–H bond in the second step. Obtained dianion ($^{-}$O–Phl–COO^{-}) by two-step process of electron donation produces dienone lactone:

$$\mathrm{HO-Phl-COOH} \rightarrow \mathrm{HO-Phl-COO^- + H^+} \quad (13)$$

$$\mathrm{HO-Phl-COO^- \rightarrow {}^-O-Phl-COO^- + H^+} \quad (14)$$

$$\mathrm{{}^-O-Phl-COO^- \rightarrow {}^{\bullet}O-Phl-COO^- + e^-} \quad (15)$$

$$\mathrm{{}^{\bullet}O-Phl-COO^- \rightarrow {}^{\bullet}O-Phl-COO^{\bullet} + e^-} \quad (16)$$

Accordingly, this mechanism can be abbreviated as SdPLdET because sequential double proton loss followed by double electron transfer occurs. Related reaction enthalpies are as follows:

$$\mathrm{PA1} = H(\mathrm{HO-Phl-COO^-}) + H(\mathrm{H^+}) - H(\mathrm{HO-Phl-COOH}) \quad (17)$$

$$\mathrm{PA2} = H(\mathrm{{}^-O-Phl-COO^-}) + H(\mathrm{H^+}) - H(\mathrm{HO-Phl-COO^-}) \quad (18)$$

$$\mathrm{ETE1} = H(\mathrm{{}^{\bullet}O-Phl-COO^-}) + H(\mathrm{e^-}) - H(\mathrm{{}^-O-Phl-COO^-}) \quad (19)$$

$$\mathrm{ETE2} = H(\mathrm{{}^{\bullet}O-Phl-COO^{\bullet}}) + H(\mathrm{e^-}) - H(\mathrm{{}^{\bullet}O-Phl-COO^-}) \quad (20)$$

PA1 (PA2) and ETE1 (ETE2) are proton affinity and electron transfer enthalpy related to the corresponding steps. H(HO–Phl–COO^{-}), H($^{-}$O–Phl–COO^{-}) and H($^{\bullet}$O–Phl–COO^{-}) are enthalpies of carboxylate anion, dianion and radical anion, respectively. The net result of all three mechanisms is the same, that is, dienone lactone production.

Inactivation of radicals is a very complex process influenced by many factors.[31] Among them, chemical nature of scavenged radicals particularly influences scavenging processes.[24,32] To investigate radical scavenging potential of phloretic acid we used the same set of radicals as in our recent papers.[29,30] It embraces oxygen-derived radicals with very different characteristics: $^{\bullet}$OH (hydroxyl), $^{\bullet}$OOH (hydroperoxyl), $^{\bullet}OCH_3$ (methoxyl), $^{\bullet}OC(CH_3)_3$ (*t*-butoxyl), PhO$^{\bullet}$ (phenoxyl), CH_3–O–O$^{\bullet}$ (methyl peroxyl), CH_2=CH–O–O$^{\bullet}$ (vinyl peroxyl), CH_2=CH–CH_2–O–O$^{\bullet}$ (allyl peroxyl), Cl_3C–O–O$^{\bullet}$ (trichloromethyl peroxyl) and $O_2^{\bullet-}$ (superoxide anion). The $^{\bullet}$OH radical is the main source of biological damage in living organisms because it is the most reactive and electrophilic of the oxygen-centered radicals. It may withdraw an electron or H-atom from almost any compound in its vicinity.[33] Radicals $^{\bullet}OCH_3$ and $^{\bullet}OC(CH_3)_3$ are examples of alkoxyl radicals. The simplest alkoxyl radical $^{\bullet}OCH_3$ is abundant damaging radical in the human body. In comparison, peroxyl radicals $^{\bullet}$OOH, CH_2=CH–O–O$^{\bullet}$ and CH_2=CH–CH_2–O–O$^{\bullet}$ are less reactive. They may mimic lipid peroxyl radicals LOO$^{\bullet}$ which are abundantly formed in biological systems. Cl_3C–O–O$^{\bullet}$ is very electronegative, highly reactive peroxyl radical. $O_2^{\bullet-}$ is an important radical with rather low reactivity that can occur during *in vivo* metabolism. Phenoxyl type radicals (PhO$^{\bullet}$) are involved in biological redox processes and in the biosynthesis of natural products.[34]

To take into account electronic properties of selected radicals on reaction with phloretic acid, calculations of Gibbs free energy of reactants and products involved in studied mechanisms have been performed. In general, Gibbs free energy of a reaction represents the criterion for definition of thermodynamically preferred process. The reaction of phloretic acid with particular radical (RO$^{\bullet}$) is thermodynamically favourable if it is exergonic:

$$\Delta_r G^{\circ} = \left[G^{\circ}(\text{products}) - G^{\circ}(\text{reactants}) \right] < 0 \quad (21)$$

$\Delta_r G^{\circ}_{\mathrm{BDE1}}$ and $\Delta_r G^{\circ}_{\mathrm{BDE2}}$ represent the free energies of double HAT mechanism (Eqs. 1 and 2) and are given by Eqs. 22 and 23:

$$\Delta_r G^{\circ}_{\mathrm{BDE1}} = \left[G^{\circ}(\mathrm{{}^{\bullet}O-Phl-COOH}) + G^{\circ}(\mathrm{ROH}) \right] - \left[G^{\circ}(\mathrm{HO-Phl-COOH}) + G^{\circ}(\mathrm{RO^{\bullet}}) \right] \quad (22)$$

$$\Delta_r G^{\circ}_{\mathrm{BDE2}} = \left[G^{\circ}(\mathrm{{}^{\bullet}O-Phl-COO^{\bullet}}) + G^{\circ}(\mathrm{ROH}) \right] - \left[G^{\circ}(\mathrm{{}^{\bullet}O-Phl-COOH}) + G^{\circ}(\mathrm{RO^{\bullet}}) \right] \quad (23)$$

Lower $\Delta_r G^{\circ}_{\mathrm{BDE1}}$ and $\Delta_r G^{\circ}_{\mathrm{BDE2}}$ values are assumed to correspond to a higher reactivity of phloretic acid with a radical considered.

The double ET-PT mechanism (Eqs. 5–8) is characterized by $\Delta_r G^{\circ}_{\mathrm{IP1}}$, $\Delta_r G^{\circ}_{\mathrm{PDE1}}$, $\Delta_r G^{\circ}_{\mathrm{IP2}}$ and $\Delta_r G^{\circ}_{\mathrm{PDE2}}$ which are calculated by Eqs. 24–27, respectively:

$$\Delta_r G^{\circ}_{\mathrm{IP1}} = \left[G^{\circ}(\mathrm{HO-Phl-COOH^{\bullet+}}) + G^{\circ}(\mathrm{RO^-}) \right] - \left[G^{\circ}(\mathrm{HO-Phl-COOH}) + G^{\circ}(\mathrm{RO^{\bullet}}) \right] \quad (24)$$

$$\Delta_r G^{\circ}_{\mathrm{PDE1}} = \left[G^{\circ}(\mathrm{{}^{\bullet}O-Phl-COOH}) + G^{\circ}(\mathrm{ROH}) \right] - \left[G^{\circ}(\mathrm{HO-Phl-COOH^{\bullet+}}) + G^{\circ}(\mathrm{RO^-}) \right] \quad (25)$$

$$\Delta_r G^\circ_{IP2} = \left[G^\circ\left(\left[O-Phl-COOH \right]^+ \right) + G^\circ\left(RO^- \right) \right] - \left[G^\circ\left({}^\bullet O-Phl-COOH \right) + G^\circ\left(RO^\bullet \right) \right] \quad (26)$$

$$\Delta_r G^\circ_{PDE1} = \left[G^\circ\left({}^\bullet O-Phl-COO^\bullet \right) + G^\circ\left(ROH \right) \right] - \left[G^\circ\left(\left[O-Phl-COOH \right]^+ \right) + G^\circ\left(RO^- \right) \right] \quad (27)$$

In the case of the SdPLdET mechanism (Eqs. 13–16), $\Delta_r G^\circ_{PA1}$, $\Delta_r G^\circ_{PA2}$, $\Delta_r G^\circ_{ETE1}$ and $\Delta_r G^\circ_{ETE2}$ are related to involved processes, and are calculated by Eqs. 28–31, respectively:

$$\Delta_r G^\circ_{PA1} = \left[G^\circ\left(HO-Phl-COO^- \right) + G^\circ\left(ROH \right) \right] - \left[G^\circ\left(HO-Phl-COOH \right) + G^\circ\left(RO^- \right) \right] \quad (28)$$

$$\Delta_r G^\circ_{PA2} = \left[G^\circ\left({}^- O-Phl-COO^- \right) + G^\circ\left(ROH \right) \right] - \left[G^\circ\left(HO-Phl-COO^- \right) + G^\circ\left(RO^- \right) \right] \quad (29)$$

$$\Delta_r G^\circ_{ETE1} = \left[G^\circ\left({}^\bullet O-Phl-COO^- \right) + G^\circ\left(RO^- \right) \right] - \left[G^\circ\left({}^- O-Phl-COO^- \right) + G^\circ\left(RO^\bullet \right) \right] \quad (30)$$

$$\Delta_r G^\circ_{ETE2} = \left[G^\circ\left({}^\bullet O-Phl-COO^\bullet \right) + G^\circ\left(RO^- \right) \right] - \left[G^\circ\left({}^\bullet O-Phl-COO^- \right) + G^\circ\left(RO^\bullet \right) \right] \quad (31)$$

All three radical scavenging mechanisms have the same reactants and the same products, and hence they have the same net thermodynamic balance, Eq. (32):

$$\Delta_r G^\circ_{dHAT} = \Delta_r G^\circ_{dET\text{-}PT} = \Delta_r G^\circ_{SdPLdET} \quad (32)$$

Accordingly, they may proceed in parallel and could be competitive.

All electronic calculations were performed using the Gaussian 09 program package.[35] The density functional theory (DFT)-based methods are able to accurately predict antioxidant potencies and reaction mechanisms involved in radical scavenging reactions of (poly)phenolic compounds.[24] Geometry optimizations and frequency calculations for phloretic acid and its radical cation, radicals, anions, radical anions, as well as for ten selected radicals and their species involved in radical scavenging pathways were carried out using the M06-2X functional[36] and the 6-311++G(d,p) basis set. Among the suite of density functionals, M05-2X, M06-2X and M-11L Minnesota functionals are particularly suitable for systems where main-group thermochemistry, kinetics, and noncovalent interactions are important.[37] The M06-2X has been chosen because it is among the best performing functionals for modelling reaction energies involving radicals.[38] The influence of water and pentyl ethanoate as solvents was calculated with an implicit continuum solvation model – SMD,[39] which takes into account the full solute electron density in estimation of energy of solvation. SMD is a universal solvation model and in conjunction with the M06-2X density functional has been successfully used for study of thermodynamics and kinetics of radical scavenging mechanisms.[40] Spin unrestricted calculations were used for radical species. Since spin contamination can affect the accuracy of enthalpies of open-shell systems, the spin operator $\langle S^2 \rangle$ values for all the open-shell species have been checked. If there is no spin contamination, this should equal $s(s+1)$, where s equals ½ times the number of unpaired electrons. In all cases, the deviations from the ideal value ($\langle S^2 \rangle = 0.75$) were lower than 4.87 % and 0.11 % before and after annihilation of the first spin contaminant. Because spin contamination can be considered negligible if the value of $\langle S^2 \rangle$ differs from $s(s+1)$ by less than 10 %,[41] the obtained enthalpy values of radical species studied in this work are reliable. Enthalpies and energies were calculated at 298.15 K. Published values of the gas-phase enthalpy of proton and electron as well as of the solvation enthalpies of hydrogen atom, proton and electron were used.[42–45]

RESULTS AND DISCUSSION

The most stable structures of phloretic acid in water and pentyl ethanoate obtained by the conformational analysis are presented in Figure 1. Conformational analysis indicates that the difference in energy between them is small which indicates that structures interconvert easily at room temperature. All obtained results and related discussion presented in this paper are based on *syn* conformation in both solvents. It should be noted that more than a half century ago Iwasaki et al. indicated that *syn* conformation of phloretic acid converts into its dienone lactone *via* $2H^+/2e^-$ oxidation.[46] As mentioned in *Introductory section*, recently we investigated single, *i.e.*, $1H^+/1e^-$ radical

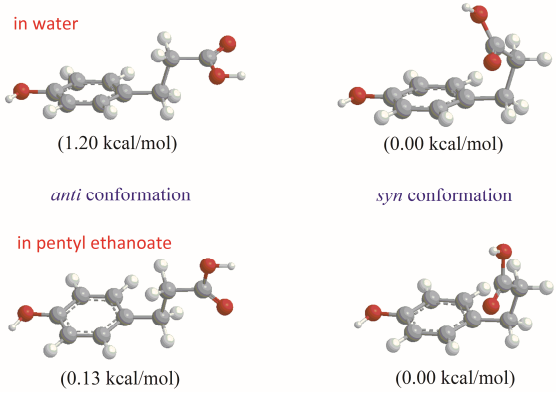

Figure 1. Optimized structures of phloretic acid calculated by SMD/M06-2X/6-311++G(d,p) level of theory in water and pentyl ethanoate as solvents.

Table 1. The SMD/M06-2X/6-311++G(d,p) reaction enthalpies (in kcal/mol) for dHAT, dET-PT and SdPLdET mechanisms of radical scavenging by phloretic acid in water and pentyl ethanoate

			dHAT	dET-PT		SdPLdET	
solvent	site		BDE	IP	PDE	PA	ETE
water		1st		107.29			
	4'–OH	1st	86.48		–3.23	26.58	77.48
	–COOH	1st	104.53		14.84	15.79	106.35
		2nd		107.21			
	–COOH	2nd	61.89		–27.72	15.73	63.77
pentyl ethanoate		1st		136.77			
	4'–OH	1st	87.15		10.65	69.34	78.09
	–COOH	1st	112.73		36.22	63.31	109.69
		2nd		134.38			
	–COOH	2nd	61.56		–12.55	60.81	61.01

scavenging mechanisms of *anti* conformation of phloretic acid[30] by considering solely phenolic O–H group as antiradical moiety. Here presented results additionally embrace contribution of carboxyl moiety in radical inactivation by *syn* conformation of phloretic acid *via* $2H^+/2e^-$ mechanisms.

The major problem in evaluation of correctness of theoretical results related to reaction enthalpies of radical scavenging mechanisms of (poly)phenolic compounds is lack of corresponding experimental results. Only limited number of published data for simple phenolic compounds can be found in literature. To ascertain reliability of applied approach experimentally determined BDE for phenol and 3,5-di-*tert*-butylcatechol may be used. For these compounds BDE values of 88.30 kcal/mol and 79.30 kcal/mol have been obtained in benzene as a solvent, respectively.[47,48] We recently showed that chosen level of theory (SMD/M06-2X/6-311++G(d,p)) may be considered as appropriate because obtained calculated values of 89.40 kcal/mol and 77.29 kcal/mol in the same solvent are in good agreement with experimental results.[30]

Reaction enthalpies related to dHAT, dET-PT and SdPLdET mechanisms of phloretic acid calculated at the SMD/M06-2X/6-311++G(d,p) level of theory are listed in Table 1. Calculations were performed in water and pentyl ethanoate, representing biological liquids and membrane lipids, respectively, *i.e.*, the two kinds of natural environments of radical inactivation.

To conclude which radical scavenging mechanism represents the most probable reaction pathway from the thermodynamic point of view, BDE, IP, PA and ETE should be considered. Their values may indicate thermodynamically preferred mechanism and point out the active site for initial radical inactivation.[23,43] Data presented in Table 1 indicate that in both solvents dET-PT mechanism (Figure 2) is thermodynamically unfavourable pathway because IP values (for the first and second $1H^+/1e^-$ process) are much higher than the corresponding BDE and PA (ETE) values.

In water solution the SdPLdET mechanism is more probable than the dHAT mechanism because PA values are much lower than the corresponding BDE values. This mechanism starts with deprotonation of carboxyl group, which is more acidic than the phenolic OH group (PA1 values 15.79 kcal/mol *vs* 26.58 kcal/mol, respectively). Obtained carboxylate anion easily deprotonates (abstracts phenolic proton) rather than undergoes electron transfer due to the much lower PA2 value (15.73 kcal/mol) than

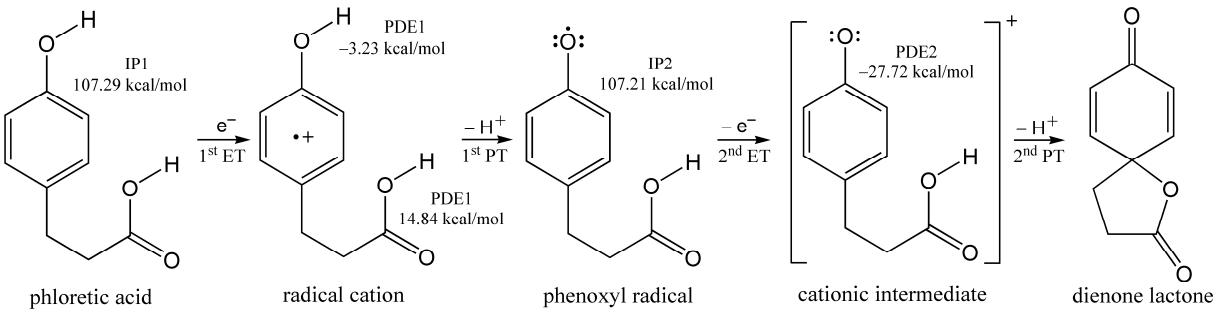

Figure 2. The dET-PT mechanism in water.

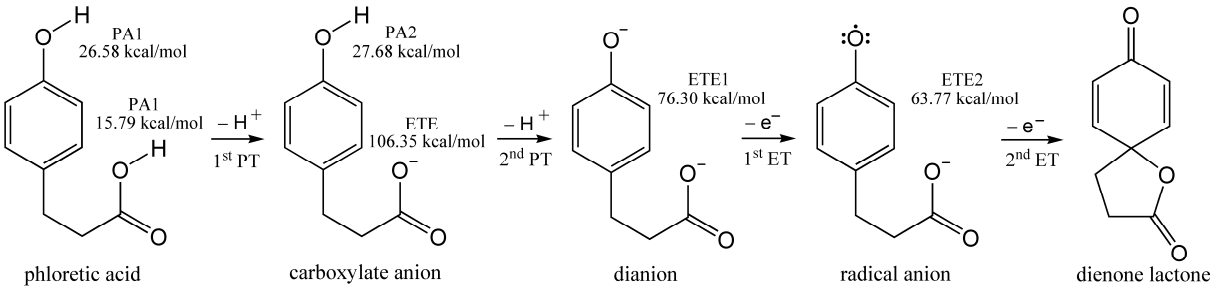

Figure 3. The SdPLdET mechanism in water.

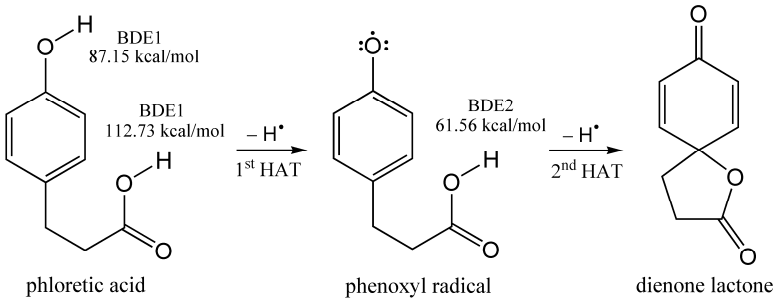

Figure 4. The dHAT mechanism in pentyl ethanoate.

ETE1 value (77.48 kcal/mol). Then, resulting dianion yields dienone lactone by the double electron transfer (Figure 3).

Regarding the dHAT mechanism, data presented in Table 1 indicate that it is a favourable mechanism in non-polar medium (pentyl ethanoate). The first H-atom abstraction occurs from phenolic O–H group due to the lower BDE1 value (87.15 kcal/mol *vs* 112.73 kcal/mol for –OH and –COOH group, respectively). The second H-atom abstraction from –COOH group, which is less energy demanding than the first one by 25.59 kcal/mol (61.56 kcal/mol *vs* 87.15 kcal/mol), gives dienone lactone (Figure 4). Lower energetic costs of second processes, which indicate $2H^+/2e^-$ processes as plausible, have been recognized earlier in the case of guaiacyl moiety,[49] catechol moiety[27,29] and uric acid.[28]

Because the scavenging processes are highly influenced by the properties of the scavenged radical species, we also calculated the free energy of reactions ($\Delta_r G^o$) of phloretic acid with each of ten selected radicals ($^\bullet$OH, $^\bullet$OOH, $^\bullet OCH_3$, $^\bullet OC(CH_3)_3$, PhO$^\bullet$, $O_2^{\bullet-}$, CH_3–OO$^\bullet$, CH_2=CH–OO$^\bullet$, CH_2=CH–CH_2–OO$^\bullet$ and Cl_3C–OO$^\bullet$) for dHAT, dET-PT and SdPLdET mechanisms. Obtained thermodynamic results for these mechanisms in water and pentyl ethanoate are summarized in Table 2 and Table 3, respectively. The second and seventh columns in Table 2 and Table 3 are related to free energy change of reactions of inactivation of radicals by phloretic acid *via* the first HAT ($\Delta_r G^o_{BDE1}$) and second HAT ($\Delta_r G^o_{BDE2}$) mechanisms, respectively. The sum of these numerical values is the total energy requirement for dHAT ($\Delta_r G^o_{HAT1} + \Delta_r G^o_{HAT2}$). It is equal to the total energy

Table 2. Standard Gibbs energies of reaction ($\Delta_r G^o$ in kcal/mol) for dHAT, dET-PT and SdPLdET mechanisms of radical scavenging by phloretic acid in aqueous medium

radical	$\Delta_r G^o_{HAT1}$	$\Delta_r G^o_{ET\text{-}PT1}$		$\Delta_r G^o_{SdPLdET1}$		$\Delta_r G^o_{HAT2}$	$\Delta_r G^o_{ET\text{-}PT2}$		$\Delta_r G^o_{SdPLdET2}$	
	$\Delta_r G^o_{BDE1}$	$\Delta_r G^o_{IP1}$	$\Delta_r G^o_{PDE1}$	$\Delta_r G^o_{PA1}$	$\Delta_r G^o_{PA2}$	$\Delta_r G^o_{BDE2}$	$\Delta_r G^o_{IP2}$	$\Delta_r G^o_{PDE2}$	$\Delta_r G^o_{ETE1}$	$\Delta_r G^o_{ETE2}$
HO$^\bullet$	–33.79	9.72	–43.51	–24.94	–12.48	–55.45	10.95	–66.40	–21.50	–30.31
$(CH_3)_3$C–O$^\bullet$	–20.87	25.03	–45.90	–27.33	–14.88	–42.53	26.26	–68.79	–6.19	–15.00
CH_3O$^\bullet$	–18.74	25.82	–44.55	–25.98	–13.53	–40.40	27.05	–67.44	–5.41	–14.21
CCl_3–O–O$^\bullet$	–9.50	10.63	–20.12	–1.56	10.90	–31.16	11.86	–43.02	–20.60	–29.40
PhO$^\bullet$	–2.53	26.65	–29.18	–10.61	1.84	–24.19	27.89	–52.07	–4.57	–13.37
HOO$^\bullet$	–2.08	31.72	–33.80	–15.23	–2.78	–23.74	32.95	–56.69	0.50	–8.31
CH_2=CH–O–O$^\bullet$	–1.81	27.11	–28.92	–10.35	2.11	–23.47	28.34	–51.81	–4.12	–12.92
CH_3–O–O$^\bullet$	–0.38	33.48	–33.86	–15.29	–2.83	–22.04	34.71	–56.75	2.25	–6.55
H_2C=CH–CH_2–O–O$^\bullet$	–0.32	32.90	–33.21	–14.65	–2.19	–21.98	34.13	–56.11	1.67	–7.13
O–O$^{\bullet-}$	14.59	70.02	–55.44	–36.87	–24.41	–7.08	71.25	–78.33	38.80	29.99

Table 3. Standard Gibbs energies of reaction (Δ_rG^o in kcal/mol) for dHAT, dET-PT and SdPLdET mechanisms of radical scavenging by phloretic acid in pentyl ethanoate

radical	$\Delta_rG^o_{HAT1}$	$\Delta_rG^o_{ET\text{-}PT1}$		$\Delta_rG^o_{SdPLdET1}$		$\Delta_rG^o_{HAT2}$	$\Delta_rG^o_{ET\text{-}PT2}$		$\Delta_rG^o_{SdPLdET2}$	
	$\Delta_rG^o_{BDE1}$	$\Delta_rG^o_{IP1}$	$\Delta_rG^o_{PDE1}$	$\Delta_rG^o_{PA1}$	$\Delta_rG^o_{PA2}$	$\Delta_rG^o_{BDE2}$	$\Delta_rG^o_{IP2}$	$\Delta_rG^o_{PDE2}$	$\Delta_rG^o_{ETE1}$	$\Delta_rG^o_{ETE2}$
HO$^\bullet$	−32.06	55.96	−88.02	−35.49	−16.85	−54.92	54.99	−109.9	−18.00	−16.65
$(CH_3)_3C–O^\bullet$	−19.19	64.44	−83.63	−31.10	−12.46	−42.05	63.47	−105.5	−9.52	−8.17
$CH_3O^\bullet$	−16.81	66.50	−83.31	−30.78	−12.15	−39.67	65.54	−105.2	−7.45	−6.10
$CCl_3–O–O^\bullet$	−6.20	42.89	−49.09	3.44	22.08	−29.06	41.92	−70.98	−31.07	−29.72
PhO$^\bullet$	−1.75	55.33	−57.07	−4.54	14.10	−24.61	54.36	−78.97	−18.63	−17.28
HOO$^\bullet$	−0.02	75.30	−75.32	−22.79	−4.15	−22.88	74.33	−97.22	1.34	2.69
$CH_2=CH–O–O^\bullet$	1.07	64.70	−63.63	−11.10	7.54	−21.79	63.74	−85.52	−9.25	−7.90
$CH_3–O–O^\bullet$	2.27	76.43	−74.17	−21.63	−3.00	−20.59	75.47	−96.06	2.48	3.83
$H_2C=CH–CH_2–O–O^\bullet$	2.50	75.04	−72.54	−20.01	−1.37	−20.36	74.08	−94.43	1.09	2.44
$O–O^{\bullet-}$	22.66	167.3	−144.7	−92.14	−73.51	−0.20	166.4	−166.6	93.38	94.74

requirements for dET-PT ($\Delta_rG^o_{ET\text{-}PT1}$ + $\Delta_rG^o_{ET\text{-}PT2}$) and SdPLdET mechanism ($\Delta_rG^o_{SdPLdET1}$ + $\Delta_rG^o_{SdPLdET2}$), and may serve as a general parameter of antiradical potency regardless of underlying reaction mechanism.[50] It means that all studied mechanisms might take place and that the preferred one should be deduced from values of underlying processes, that is $\Delta_rG^o_{BDE}$, $\Delta_rG^o_{IP}$, $\Delta_rG^o_{PDE}$, $\Delta_rG^o_{PA}$ and $\Delta_rG^o_{ETE}$. More negative values indicate thermodynamically more preferred reactions. Significant endergonicity of the first step of dET-PT mechanism ($\Delta_rG^o_{IP1}$ > 10 kcal/mol) indicates this mechanism as thermodynamically unfavourable in both solvents. As can be seen from Table 2 and Table 3, reaction free energies for dHAT and SdPLdET processes ($\Delta_rG^o_{BDE1}$, $\Delta_rG^o_{BDE2}$, $\Delta_rG^o_{PA1}$, $\Delta_rG^o_{PA2}$, $\Delta_rG^o_{ETE1}$ and $\Delta_rG^o_{ETE2}$) are in the most cases exergonic. Consequently, considering electronic properties of scavenged radicals, dHAT and SdPLdET are competitive mechanisms and could be operative in both solvents. As can be seen from Figure 5, where overall energy requirements for scavenging of selected radicals *via* dHAT, dET-PT and SdPLdET are depictured, phloretic acid has potential to effectively scavenge hydroxyl, alkoxyl, phenoxyl, peroxyl and superoxide radicals (in descending order). As in the case of catecholic compounds,[29] $2H^+/2e^-$ mechanisms are less energy demanding in polar solvents.

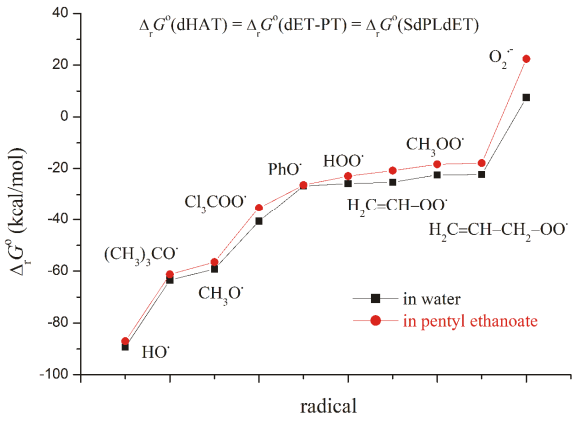

Figure 5. Overall energy requirements for scavenging of selected radicals *via* dHAT, dET-PT and SdPLdET by phloretic acid.

Obviously, here presented thermodynamic parameters may be important factors governing the radical scavenging reactions of phloretic acid, while a more complete understanding would require kinetic analysis. The knowledge of the kinetics of the processes involved in radical scavenging mechanisms is important because radicals are very short-lived species, what implies that the impact of an antioxidant depends on its high reactivity towards radicals. It should be noted that small positive values of Δ_rG^o (<10 kcal/mol) do not necessarily mean that the corresponding radical scavenging reactions should be neglected.[51] Such processes may represent notable reaction pathways if they take place at significant rates. On the other hand, it is expected that products yielded *via* significantly endergonic reaction pathways will not be experimentally detectable, even if they may occur at a significant rate.[52] Because it has been shown that BDEs of phenolic antioxidants vary linearly with logarithm of rate constants,[53–55] it could be predicted that kinetic results will follow trend displayed in Figure 5.

CONCLUSION

By investigating involvement of carboxyl moiety in radical scavenging mechanisms, we found on the basis of calculated reaction enthalpies that radical scavenging by phloretic acid *via* SdPLdET and dHAT mechanisms is thermodynamically feasible in polar and non-polar medium, respectively. The Gibbs free energy changes (Δ_rG^o) for reaction of inactivation of selected ten radicals of different nature indicate these antiradical mechanisms as competitive. Exergonicity of the calculated reaction free energies revealed that the reactivity of phloretic acid

toward radicals decreases as follows: hydroxyl >> alkoxyls > phenoxyl ≈ peroxyls >> superoxide. Because phloretic acid is produced in colon in high μM concentrations and is usually better absorbed than its precursor molecules, it has potential to contribute to health benefits associated with regular intake of polyphenol-rich diet *via* direct radical quenching.

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