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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4095–4098

Proton dissociation is important to understanding structure–activity relationships of gallic acid antioxidants

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Received 15 February 2006; revised 20 April 2006; accepted 28 April 2006
Available online 18 May 2006

Abstract—Gallic acid derivatives (GADs) can efficiently scavenge free radicals, which is partially responsible for their neuroprotective effects. As GADs tend to deprotonate to give birth to GAD anions, which has big influence on the radical-scavenging behaviors of GADs, to understand the structure–activity relationships (SARs) of GAD antioxidants, the anions should be taken into consideration. In this paper, a combined density functional theory method, labeled as (RO)B3LYP/6-311 + G(2d,2p)//AM1/AM1, was employed to calculate homolytic O–H bond dissociation enthalpies and adiabatic ionization potentials for GADs and derived anions in solvent (ethanol), by which the experimentally observed SARs of GADs were better elucidated.

As free radicals play a key role in pathogenesis of neurodegenerative diseases (NDs),[1] antioxidants are expected to serve as potent complementary agents to the therapy of NDs.[1,2] Hence, finding novel antioxidants, especially naturally originated, with neuroprotective effect is of great interest.

As widespread naturally occurring antioxidants, gallic acid derivatives (GADs) have attracted much attention.[3] Recently, the antioxidant potential and neuroprotective effects of eight GADs (Scheme 1) were evaluated in detail.[4] Most of them were identified as efficient scavengers of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in ethanol solution and in liposome as well, which in combination with molecular polarity defines the neuroprotective effects of these GADs.[4] In addition, much theoretical effort was devoted to elucidating the structure–activity relationships (SARs) of these GAD antioxidants.[4] The theoretical treatment successfully explained the activity difference of a part of gallic acids (**1**–**4**) in terms of H-atom-donating ability that is characterized by O–H bond dissociation enthalpy (BDE). However, the theoretical calculation failed to elucidate the weaker activity of gallic acid esters (**5**–**8**) than that of gallic acids (e.g., **1** and **2**), despite the lower O–H BDEs of the former than those of the latter.[5]

As the pK_a values of gallic acids are around 4.0, the anions derived from proton dissociation will dominate in neutral system (occurrence >99.5%). Therefore, gallic acid anions might be responsible for the inexplicable SARs. In fact, carboxyl itself is an electron-withdrawing group,[6] which does not benefit radical scavenging. However, the deprotonated carboxyl becomes an electron-donating group,[6] which favors H-atom-transfer- and electron-donation-based radical scavenging. Therefore, we attempted to re-elucidate the SARs of the GADs by taking GAD anions into consideration.

According to the current understanding of radical-scavenging processes of phenolic antioxidants (ArOH), H-atom donation is the dominant mechanism, which involves two pathways, that is, one-step H-atom transfer (HAT) (Eq. 1) and stepwise electron-transfer/proton-transfer (ET/PT) (Eq. 2).[7] Moreover, if the phenols tend

Abbreviations: ArOH, phenolic antioxidants; BDE, bond dissociation enthalpy; DPPH, 11-diphenyl-2-picrylhydrazyl; EA, electron affinity; DFT, density functional theory; ET/PT, electron-transfer/proton-transfer; GAD, gallic acid derivative; HAA, H-atom affinity; HAT, H-atom transfer; IHB, intramolecular hydrogen bond; IP, ionization potential; ND, neurodegenerative disease; PCM, polarizable continuum model; SAR, structure–activity relationship; SCRF, self-consistent reaction field; SPE, single-point electronic energy; TCE, thermal contribution to enthalpy; ZPVE, zero point vibrational energy.
Keywords: Gallic acid derivatives; Antioxidant; Structure–activity relationships; Density functional theory; Bond dissociation enthalpy; Ionization potential.
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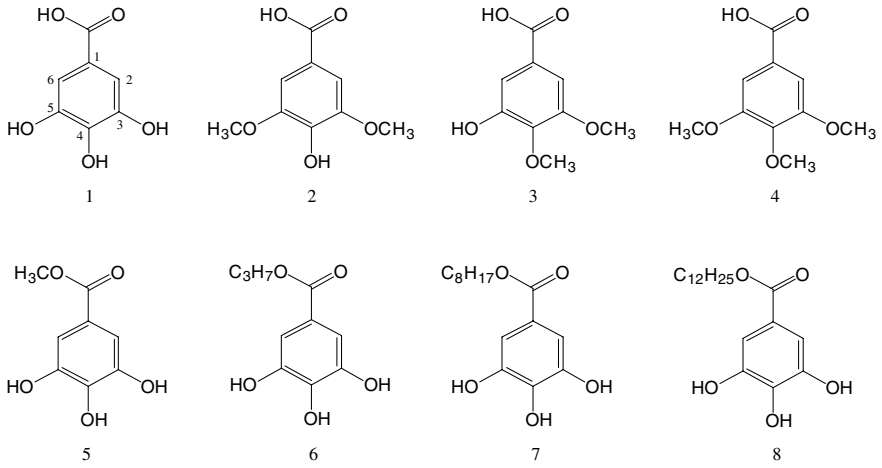
doi:10.1016/j.bmcl.2006.04.096



1 2 3 4

5 6 7 8

Scheme 1. Structures of eight gallic acid derivatives.

to deprotonate, the HAT or ET from phenolic anions should be considered.[8,9] As the first pathway does not involve charge separation, it is preferred in non-polar solvents[10,11] and can be characterized by homolytic BDE.[12,13] Whereas, the second is favored in polar media,[14,15] due to the charge separation processes, and can be measured by ionization potential (IP).[9,12,13] The lower the parameters are, the stronger the radical-scavenging ability.

$$RO^{\cdot} + ArOH \rightarrow ROH + ArO^{\cdot} \quad (1)$$

$$RO^{\cdot} + ArOH \rightarrow RO^{-} + ArOH^{\cdot+} \rightarrow ROH + ArO^{\cdot} \quad (2)$$

In this paper, homolytic BDEs and adiabatic IPs of five GADs (**1**–**5**) and derived anions (if they have) were calculated by a combined density functional theory (DFT)[16,17] method, labeled as (RO)B3LYP/6-311 + G(2d,2p)//AM1/AM1.[12] The detailed calculation procedures are as follows. First, semiempirical method AM1[18] was used to optimize the molecular geometries and determine the vibrational frequencies. Then, single-point electronic energies (SPEs) were calculated by (RO)B3LYP functional[19] at 6-311 + G(2d,2p) level. Since solvent effect is critical to understanding antioxidant behaviors in polar environments,[20] the ethanol effect ($\varepsilon = 24.55$) was taken into consideration on the single-point level by employing the self-consistent reaction field (SCRF) method with a polarizable continuum model (PCM).[21,22] The effectiveness of the method has been verified by previous studies[9,23] and present higher-level calculations.[24]

According to the parameters' definitions, O–H BDE = $H_r + H_h - H_p$, in which, H_r is the enthalpy of radical generated through H-abstraction reaction, H_h is the enthalpy of H-atom, −0.49765 hartree, and H_p is the enthalpy of parent molecule. Molecular enthalpy (H) consists of (RO)B3LYP/6-311 + G(2d,2p)-calculated SPE, AM1-derived thermal contributions to enthalpy (TCE, in which the vibrational contributions including zero point vibrational energy were scaled by a factor of 0.973).[12,25] IP = $E_t - E_p$, where E_t is the energy of molecule derived from electron transfer and E_p is the energy of parent molecule. Molecular energy (E) consists of (RO)B3LYP/6-311 + G(2d,2p)-calculated SPE and AM1-derived zero point vibrational energy (ZPVE, scaled by a factor of 0.973).[12,25]

All of the quantum chemical calculations were accomplished by the Gaussian 98 program.[26]

As BDEs and IPs depend on electronic effects rather than steric effects of substituents, GADs **5**–**8** hold similar BDEs and IPs, which have been observed in the previous research.[4] As a result, only theoretical parameters for GADs **1**–**5** were calculated, which are listed in Table 1. Through comparing the BDEs of antioxidants (and derived anions) and H-atom affinity (HAA) of DPPH radical (which was calculated to be −82.6 kcal/mol in ethanol),[27] one can determine whether HAT is permitted; through comparing the IPs of antioxidants (and derived anions) and electron affinity (EA) of DPPH radical (which was estimated to be −93.9 kcal/mol in ethanol),[28] one can determine whether ET is possible. In addition, the differences in BDEs or IPs of GADs and anions thereof reflect the relative radical-scavenging potentials of GADs. Only if the theoretical calculation is in agreement with the experimental observation, the theoretical model can be justified and the SARs can be understood accordingly.

As shown in Table 1, the IPs of GADs and derived anions are around 140 kcal/mol, ~50 kcal/mol higher than the absolute value of EA of DPPH radical (93.9 kcal/mol), suggesting that ET between GADs (and derived monovalent anions) and DPPH radical is not favored in terms of thermodynamics. Moreover, it is interesting to note that the IPs of GAD anions are several kcal/mol higher than those of parent GADs (Table 1), which is opposite to the electron-donating property of carboxylic anion. A similar phenomenon has been observed for hydroxycinnamic acid derivatives.[9a] This can be interpreted by the strong stabilizing effect of polar solvents on carboxylic anions, because in gas phase the IPs of GAD anions are still extremely (90 ~ 100 kcal/mol) lower than those of parent counterparts (see supporting information for the detail).[29]

Table 1. (RO)B3LYP/6-311 + G(2d,2p)//AM1/AM1-calculated BDEs and IPs of gallic acid derivatives and anions in ethanol (T = 298.15K)

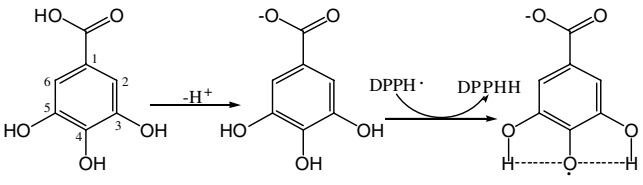
Compound	TE (hartree)[a]	ZPVE (hartree)[b]	TCE[c] (hartree)	O–H BDE[d] (kcal/mol)	IP[e] (kcal/mol)	IC_{50}[f] (μM)
1-parent	−646.744119	0.131503	0.143509	82.19	141.13	6.0 (7.3)
1$^{\cdot+}$[g]	−646.517635	0.129876				
1-radical (4)[h]	−646.102783		0.130449			
1^{-}[i]	−646.275695	0.118894	0.131000	77.67	144.74	
1^{-}-radical[j]	−646.044306	0.118145				
1^{-}-radical (4)[k]	−645.641671		0.118044			
2-parent	−725.339683	0.189013	0.203828	84.23	139.68	7.3 (7.4)
2$^{\cdot+}$[g]	−725.114764	0.186618				
2-radical (4)[h]	−724.694885		0.190550			
2^{-}[i]	−724.877673	0.176590	0.191281	82.96	141.93	
2^{-}-radical[j]	−724.650786	0.175870				
2^{-}-radical (4)[k]	−724.234875		0.177972			
3-parent	−725.342530	0.189181	0.203869	90.54	140.46	Inactive
3$^{\cdot+}$[g]	−725.116413	0.186843				
3-radical (5)[h]	−724.687504		0.190414			
3^{-}[i]	−724.875565	0.176849	0.191382	88.72	141.24	
3^{-}-radical[j]	−724.649429	0.175766				
3^{-}-radical (5)[k]	−724.223405		0.177896			
4-parent	−764.641937	0.217630		—	139.21	Inactive
4$^{\cdot+}$[g]	−764.417977	0.215449				
4^{-}[i]	−764.185479	0.205347			145.87	
4^{-}-radical[j]	−763.952009	0.204309				
5-parent	−686.047248	0.159673	0.173378	80.71	141.71	7.2 (8.4)
5$^{\cdot+}$[g]	−685.819753	0.157957				
5-radical (4)[h]	−685.408262		0.160315			

[a] Total electronic energy.
[b] Zero point vibrational energy.
[c] Thermal correction to enthalpy.
[d] Bond dissociation enthalpy.
[e] Ionization potential.
[f] DPPH radical-scavenging efficiency of GADs in ethanol solution and in liposome (in parentheses).[4]
[g] Cation radical generated by electron transfer from parent molecule.
[h] Radical generated by H-atom abstraction from parent molecule. The positions for the H-atom donation are indicated in parentheses.
[i] Anion generated by proton dissociation of carboxyl.
[j] Radical generated by electron transfer from anion.
[k] Radical generated by H-atom abstraction from anion. The positions for the H-atom donation are indicated in parentheses.

Since the O–H BDEs of **1**, **2**, **5**, and derived anions are comparable to the absolute value of HAA of DPPH radical (82.6 kcal/mol), we infer that HAT works for the three GADs and their monovalent anions as well. Thus, it is interesting to examine whether there exists a correlation between O–H BDEs and GAD antioxidant activities. For parent GADs, the order for BDEs is: **3** > **2** > **1** > **5**, which is not in agreement with GADs' activity difference (Table 1). However, if proton dissociates, the BDEs of GAD (**1**–**3**) anions get several kcal/mol lowered than those of parent molecules, indicating the electron-donating effect of carboxylic anion. As **5** cannot deprotonate, its BDE is not the lowest. The new order for BDEs is: **3** > **2** > **5** > **1**, which agrees well with the activity order that **1** is the strongest radical scavenger, **2** and **5** the middle, and **3** the weakest. This accordance justifies the theoretical model and indicates the importance of GAD anions in DPPH radical scavenging.

To provide some deeper insights into the SARs of GAD antioxidants, the difference in the O–H BDEs was further elucidated in terms of substituent effects. First, the quite low BDEs of **1** and **5** can be ascribed to the pyrogallol moiety, because the 4-O radical derived from H-atom abstraction from pyrogallol can be well stabilized by two intramolecular hydrogen bonds (IHBs) and by electron-donating effect of both *ortho*-hydroxyls as well (Scheme 2).[30–32] Second, as 3,5-OMe of **2** cannot offer IHB to stabilize the 4-O radical, its BDE is higher than those of **1** and **5**. Third, as 5-OH in **3** has only one radical-stabilizing factor, that is, 4-OMe,[33] its BDE is the highest.

In summary, the re-calculation of BDEs and IPs for GADs and anions thereof not only provides solid evidence to support HAT as DPPH radical-scavenging pathway in neutral environments, but also offers a deeper explanation to the SARs of these antioxidants, which clearly reveals the significance of considering proton dissociation in the elucidation of antioxidants' SARs. Since anion is also crucial to understanding the



Scheme 2. DPPH radical-scavenging mechanism of gallic acid.

photosensitive behaviors of some natural pigments,[34] we suggest that deprotonation should be kept in mind in interpreting pharmacological effects of natural products.

Acknowledgments

This work was supported by the National Basic Research Program of China (2003CB114400) and the National Natural Science Foundation of China (30100035 and 30570383).

Supplementary data

Optimized structures, in vacuo data and in solvent data calculated by higher-level method are available free of charge via the Internet at http://authors.elsevier.com/. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.04.096.

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6. (a) The Brown parameter σ_p^+ of COOH is 0.42, while the σ_p^+ of COO^- gets −0.02.[6b]; (b) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165.
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24. A high-level approach, labeled as (RO)B3LYP/6-311 + G(2d,2p)//(RO)B3LYP/6-31 + G(d)/(RO) B3LYP/6-31 + G(d) (which means that (RO)B3LYP/6-31 + G(d) was used to optimize molecular structures (in solvent) and obtain vibrational frequencies and then (RO)B3LYP/6-311 + G(2d,2p) was employed to give SPE (also in solvent)), was used to calculate O–H BDEs and IPs of GADs **1** (including anion) and **5**. Both methods give very similar O–H BDEs and IPs for the GADs, which partially arises from the fact that AM1 behaves as good as (RO)B3LYP/6-31 + G(d) in optimizing molecular structures (see Supporting information for detail).
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27. (a) HAA is defined as $H_n - H_r - H_h$, in which, H_n (−1418.589302 hartree) is the enthalpy of neutralized DPPH radical, H_r (−1417.960021 hartree) is the enthalpy of DPPH radical, and H_h (−0.49765 hartree) is the enthalpy of H-atom. The present value is comparable to the experimental value (−80 kcal/mol) determined in benzene.[27b]; (b) Mahoney, L. R.; Mendenhall, G. D.; Ingold, K. U. *J. Am. Chem. Soc.* **1973**, *95*, 8610.
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33. The 3-OMe in **3** is electron-withdrawing to the 5-OH, because they are in *meta*-position, which is well reflected by the Hammett parameters of OMe. Namely, the σ_p of OMe is −0.27, in contrast to the σ_m of OMe, 0.12.[6b]
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