JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Gas and Liquid Phase Acidity of Natural Antioxidants

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The gas phase and in solution absolute and relative acidities of nine natural systems contained in red and white wines were determined through theoretical B3LYP/6-311++G** calculations. The aim was to correlate these thermodynamic quantities to the ability that some of these compounds show in chelating metals ions to carry out an antioxidant action following a mechanism recently reported in the literature. Results indicated that both absolute and relative values are affected by molecular features such as electronic delocalization and conjugation and intramolecular hydrogen bonds. Polyphenols characterized by the *ortho*-dihydroxy functionality were found to be good candidates to act as metal cation chelating ligands. Some differences in absolute acidities values were encountered in going from vacuum to water solution.

KEYWORDS: Gas phase; liquid phase; natural antioxidants

INTRODUCTION

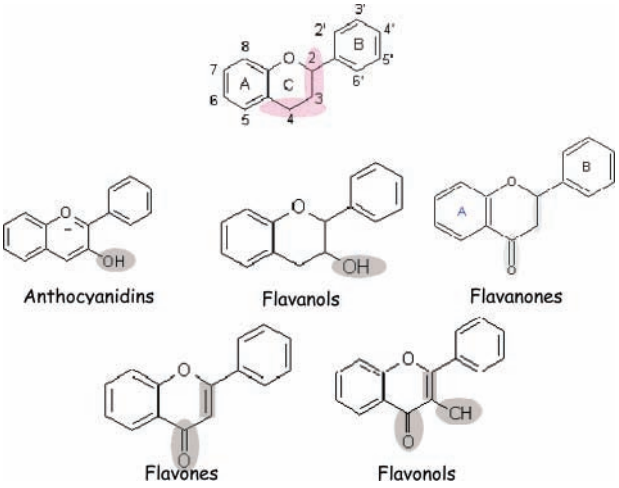
Flavonoids represent a class of naturally occurring compounds, mainly found in fruits, vegetables, and cereals (*1*). Most of them belong to the Mediterranean food culture (*1*). During the last few decades, they attracted great interest because of their beneficial effects on human health (*2*). Epidemiological studies evidenced that flavonoids exhibit vasoprotective, antinflammatory, antiviral, antifungal, antihepatotoxic, antiallergic, and anticancer activities (*3*−*13*). Many of these beneficial effects are related to their antioxidant properties, which may be due to their ability to scavenge free radicals, and to synergistic effects with physiological antioxidants (glutathione, α-tocopherol) and several enzymes. In fact, free radicals can damage biomolecules such as proteins, membrane lipids, and nucleic acids, thus making them involved in several diseases and aging itself (*14*, *15*).

All polyphenolic flavonoids (see **Scheme 1**) consist of a benzene ring (A) condensed with a six-membered ring (C), which carries a phenyl group (B) as a substituent in the 2-position. C is either a γ-pyrone ring (flavonols and flavones) or its dihydro derivative (flavanols and flavanones) (*16*). In the literature, two main mechanisms by which antioxidants can play their protective role were proposed and widely analyzed (*17*−*20*) including: the H-atom transfer, in which a free radical $R^{\bullet}$ removes an hydrogen atom from the antioxidant (ArOH):

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet}$$

and the one-electron transfer mechanism, according to which

Scheme 1. Flavonoids Classes



the antioxidant can give an electron to the free radical:

$$R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet+}$$

The radicals arising from both reactions ($ArO^{\bullet}$ and $ArOH^{\bullet+}$) must be stable in order to prevent chain radical reactions.

Another antioxidant mechanism, not exhaustively studied, results from their ability to chelate transition metals ions (especially iron and copper), which gives rise to stable complexes that avoid these metals, to participate in free radicals generation (*21*). In fact, during the Fenton reaction (*22*, *23*), hydroxyl radicals are produced from hydrogen peroxide in the

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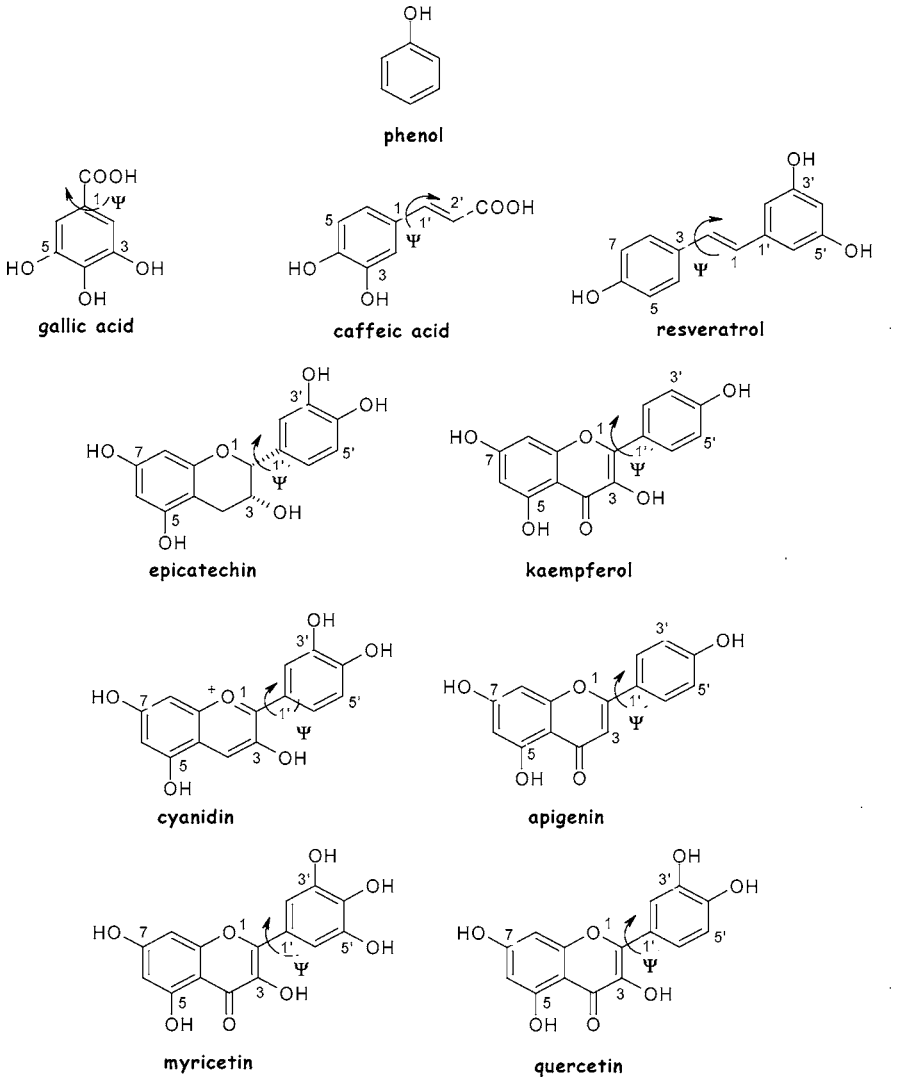
10.1021/jf053180a CCC: $33.50
Published on Web 03/24/2006

Scheme 2. Studied Polyphenols



presence of a metal in a low oxidation state:

$$H_2O_2 + M^{n+} \rightarrow HO^- + HO^\bullet + M^{(n+1)+}$$

Fenton chemistry may occur in dopaminergic neurons of nervous tissue, where normally dopamine catabolism produces some levels of hydrogen peroxide (*24*). The accumulation of free radicals in these neurons may be recognized as the main aetiological agent of Parkinson's disease (*24*).

In the "metals chelation" mechanism, the loss of a proton in the polyphenol is crucial for its antioxidant ability, because the cation's chelation often occurs through at least one deprotonated ligand (*25*). So, the determination of the acidity of these compounds is an important thermodynamic parameter to be taken into account, since the smaller the phenolic OH acidity value is, the easier the deprotonation and the metals chelation will be.

In this paper, we have undertaken a theoretical systemic study to determine the acidity of some polyphenols (**Scheme 2**): gallic and caffeic acids, resveratrol, epicatechin, kaempferol, cyanidin, apigenin, myricetin, quercetin, and phenol, used as a reference compound. The polyphenols chosen for this investigation are the main nonalcoholic constituents of wine, especially Italian red and white wines (*26*).

COMPUTATIONAL DETAILS

All of the calculations reported in this study were performed with the Gaussian03 code (*27*). The selected flavonoids' neutral molecules and their anions were optimized without constraints at the B3LYP level, employing the 6-311++G** basis set (*28*−*33*). The inclusion of diffuse functions in the basis set can properly describe the long-range behavior of molecular orbitals associated with anionic systems.

Harmonical vibrational frequencies were computed at the same level of theory for both parent and anionic molecules, with the aim to characterize them as minima and saddle points and to estimate zero point energy and thermodynamic corrections. Solvent effects were computed in the framework of a self-consistent reaction field polarized continuum model (PCM) (*34*−*36*), using the UAHF (*37*) set of solvation radii to build the cavity for the solute, in the gas phase equilibrium geometries. The dielectric constant of 80 was chosen to reproduce the water medium. In the PCM method, the variation of the free energy when going from vacuum to solution is composed of the work required to build a cavity in the solvent (cavitation energy, G_{cav}) together with the electrostatic (G_{el}) and nonelectrostatic work ($G_{disp} + G_{rep}$).

Natural bond orbital analysis (*38*−*45*) implemented in the Gaussian03 package was used to better characterize the electronic structures of the systems under investigation. The gas

phase acidity was computed at 298 K as the enthalpy difference between the anion (A^-) and its neutral species (HA):

$$\Delta H_{acidity} = H_{A^-} - H_{HA}$$

For the calculations in the condensed phase, the acidities were computed in terms of total free solvation energies (ΔG).

pK_a values in solution were obtained by dividing the relative total free energies by 1.37 as a consequence of the following relation:

$$pK_a = \Delta G/\ln(10)RT$$

where R is the gas constant and T is the absolute temperature. The relative pK_a between the various antioxidants and phenol is calculating using

$$\Delta pK_a = \Delta G/1.37$$

RESULTS

Polyphenols. Gallic acid, caffeic acid, and resveratrol do not share the basic nucleus of flavonoids, and they are generally indicated as polyphenols. All of them, as gas phase neutral species, are completely planar systems, characterized by a torsional angle Ψ (see **Scheme 2**) equal to 0 (*19*). They show the features of conjugation and electronic delocalization, especially in the case of the resveratrol system (*19*).

In resveratrol, the mutual position of hydroxyls does not allow the formation of any weak interactions. In gallic and caffeic acids, the hydroxyl groups are instead involved in hydrogen bonds (the H···O lengths are 2.195 and 2.196 Å for gallic acid and 2.152 Å for caffeic acid) (*19*). By abstracting an H^+, two and three anions were obtained from gallic and caffeic acids and from resveratrol, respectively. All anionic forms share with the parent molecule the planar conformation.

For gallic acid, the 4-OH anion (throughout the text, the anions will be indicated as the original deprotonated hydroxyl group) is more stable than the 3-OH one by about 9.5 kcal/mol because the negative charge is stabilized by two hydrogen bonds (≈2.054 Å) and several resonance structures (see **Table 1**). When deprotonation occurs at the 3-OH position, the resulting anion rearranges itself (see **Scheme 3**) so as to maximize the number of hydrogen bonds (1.864 and 2.247 Å), through a rotation around the C_5-OH single bond, which requires ≈3−4 kcal/mol (*19*).

The caffeic acid minimum energy anion is the 4-OH species, which is favored with respect to the 3-OH one (ΔE = 5.8 kcal/mol) by a better electronic delocalization involving the −CH═CH− bridge and by the presence of a further H-bond (1.926 Å).

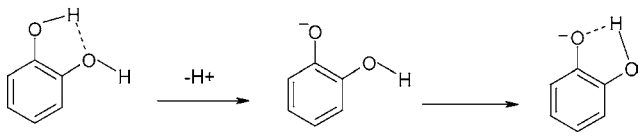
The energetic gaps of resveratrol 3′-OH and 5′-OH anions with respect to the global minimum 6-OH are 8.7 and 8.1 kcal/mol, respectively. The minor stability of the former must be researched in the lack of the resonance effects due to the position of the −CH═CH− group. This bridge in the 6-OH anion lies in the para-position, thus allowing a complete delocalization over the entire molecule.

The energetic trends among anions are retained in going from the gas phase to the water solution, even if some decrease of the energetic gaps occurs. In particular, the energy separation between the two anionic forms of gallic and caffeic acids decreases, respectively, by 3.8 and 1.6 kcal/mol with respect to the vacuum. For resveratrol, the reduction is sensibly higher, being the 6-OH and 3′-OH, and the 6-OH and 5′-OH gaps are smaller by 6.9 and 6.2 kcal/mol, in that order. Because all anionic systems have the same negative charge (−1), the stabilizing effect due to the ion−dipole interaction can be considered quite equal for all systems. The reason for the more pronounced reduction of gaps among resveratrol anions must be searched elsewhere. A look at the dipole moments (**Table 1**) of polyphenols suggests that a further stabilization of resveratrol anions can be derived from the higher values of these quantities, which makes the dipole solute−dipole solvent interaction significant.

Table 1. Dipole (in Debye), and Gas Phase and in Water Relative Energies (ΔE in kcal/mol) of Selected Polyphenol Anions

		ΔE	
compound	dipole moment	gas phase	water
	gallic acid		
4-OH	2.659	*0.0*	*0.0*
5-OH	4.772	9.5	5.7
	caffeic acid		
3-OH	3.626	5.0	3.4
4-OH	4.773	*0.0*	*0.0*
	resveratrol		
3′-OH	17.235	8.7	1.8
5′-OH	13.169	8.1	1.9
6-OH	13.752	*0.0*	*0.0*
	epicatechin		
3′-OH	15.196	*0.0*	*0.0*
4′-OH	15.905	0.4	0.3
5-OH	14.269	10.3	4.3
7-OH	15.422	12.6	4.7
	kaempferol		
4′-OH	12.177	0.3	2.1
3-OH	8.587	10.3	3.3
5-OH	11.497	14.8	5.8
7-OH	12.691	*0.0*	*0.0*
	cyanidin		
3′-OH	11.967	14.1	9.4
4′-OH	10.209	0.6	0.7
3-OH	5.867	2.9	3.5
5-OH	3.884	*0.0*	0.1
7-OH	5.054	1.2	*0.0*
	apigenin		
4′-OH	11.253	*0.0*	0.7
5-OH	13.904	25.0	8.3
7-OH	15.525	6.3	*0.0*
	myricetin		
3′-OH	9.906	10.7	5.8
4′-OH	9.495	*0.0*	*0.0*
5′-OH	12.157	11.7	6.0
3-OH	10.071	21.5	7.2
5-OH	13.578	25.8	9.7
7-OH	14.042	10.8	4.0
	quercetin		
3′-OH	11.301	6.8	*0.0*
4′-OH	10.413	0.0	1.6
3-OH	9.360	16.6	1.5
5-OH	11.558	20.9	4.0
7-OH	12.113	6.0	1.7

Scheme 3



Flavonoids. On the basis of unsaturation degree and oxidation of the 2, 3, 4 three-carbon segment (see **Scheme 1**), flavonoids are divided into several classes: flavanols, flavanones, anthocyanidins, flavones, and flavonols (*16*). Epicatechin belongs to

the flavanols class, characterized by a saturated C-ring and an OH group in position 3; kaempferol, quercetin, and myricetin belong to the flavonols class, in which the 4-keto and 3-OH groups are simultaneously present in ring C; cyanidin is a member of the anthocyanidins class, with a charged oxygen in the C-ring; and finally, apigenin is a flavone showing the 4-keto functional group (*16*).

The results of the optimization show that, except for the case of epicatechin, all molecules adopt a planar conformation, in which the torsional angle between the C-ring and the B-ring assumes a value of 0.0°. In all of them, the π-electron delocalization effects exceed the steric hindrance destabilization. This also occurs for the apigenin molecule, which is the only system lacking the 3-OH functionality (*18*−*20*).

Our results are completely in disagreement with ab initio HF/6-31G(d) ones (*46*) that predict for both flavonols and flavones a nonplanar conformation. Minor disagreement exists with the B3LYP/6-31G(d) computations (*46*), according to which apigenin has a torsional angle of 16.4° between C- and B-rings. However, these discrepancies are not so relevant since one has to remember that HF, as well as semiempirical AM1 and PM3 approaches, underestimate the stabilizing π-delocalization contributions with respect to the density functional methods (*47*−*49*). Concerning the comparison between B3LYP results, it is worth noting that the employment of an extended basis set should provide a better description of the electronic structure.

Because of the absence of the C_2-C_3 double bond in the C-ring that prevents any electronic conjugation, epicatechin is characterized by a great conformational flexibility, which is translated in an equilibrium geometry with a torsional angle of 92.5°.

Epicatechin, kaempferol, cyanidin, apigenin, myricetin, and quercetin show a variable number of hydrogen bonds (*18*−*20*). For epicatechin and cyanidin, these bonds involve the *ortho*-dihydroxy functionality in the B-ring (2.158 and 2.117 Å, respectively). For kaempferol, the 3-OH and the 4-keto (1.997 Å) and the 4-keto and the 5-OH (1.761 Å) groups are implicated. As far as apigenin is concerned, the H-bond occurs between the 4-keto and the 5-OH groups (1.687 Å). The trihydroxy functionality (2.204 and 2.200 Å), the 3-OH and the 4-keto (2.021 Å), and the 4-keto and the 5-OH (1.767 Å) groups are involved in myricetin. Finally, for quercetin, we found H-bonds between the 3′-OH and the 4′-OH (2.151 Å), the 3-OH and the 4-keto (2.019 Å), and the 4-keto and the 5-OH (1.765 Å) groups.

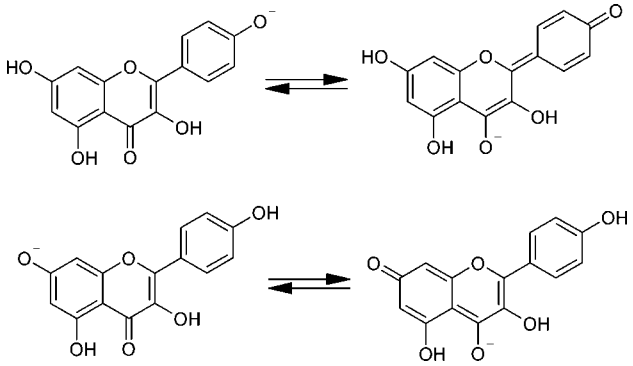
The optimized equilibrium geometries of all anionic species arising from the parent molecule by removing an H^+ adopt planar conformations that in principle can allow the delocalization of the negative charge over the entire molecule. The exceptions are again the epicatechin anions, showing very different values of the torsional angle Ψ: 90.1, 90.5, 83.8, and 79.6°, for 3′-OH, 4′-OH, 5-OH, and 7-OH anions, respectively.

The patterns of hydrogen bonding are retained in going from the parent molecule to the deprotonation products, even if deprotonation on the B-ring entails the rearrangement to the most stable conformer.

The epicatechin 4′-OH anion is almost isoenergetic with the 3′-OH one (0.4 kcal/mol). In both, the negative charge is stabilized by a hydrogen bond occurring on the B-ring. The 5-OH and 7-OH anions lie at 10.3 and 12.6 kcal/mol above the 3′-OH. In the absence of the 4-keto group in the C-ring, the main factor governing the anion stability is represented by the H-bonding. In a water solution, 3′-OH is retained as the global minimum, while the gap among anions is sensibly reduced.

The most stable 7-OH anion and the first relative minimum

Scheme 4



4′-OH of kaempferol lying at 0.3 kcal/mol are stabilized by the delocalization of the negative charge that spreads from the deprotonation site to the 4-keto group in the C-ring, as can be seen from **Scheme 4**. The other anions 3-OH and 5-OH are found 10.3 and 14.8 kcal/mol above the global minimum, because of the electronic repulsion between two adjacent oxygen atoms. This difference in the stabilization of the anions is to some extent reduced by the presence of the water medium (the gaps are 2.1, 3.3, and 5.8 kcal/mol for 4′-OH, 3-OH, and 5-OH, as compared to the 7-OH minimum, respectively).

For cyanidin, the most stable anion in the gas phase is the 5-OH one. At 14.1, 0.6, 2.9, and 1.2 kcal/mol above the global minimum, we found the 3′-OH, 4′-OH, 3-OH, and 7-OH species, respectively. The very low stability of the 3′-OH species can be explained by the fact that it is the only anion in which the negative charge is confined in ring B as can be argued by analyzing its resonance structures. For the other species, the negative charge can in principle be delocalized over the entire molecule, with a resulting stabilization of the anion. The in water solution relative energy values indicate the 7-OH anion as the most stable conformer even if it is practically isoenergetic with the 5-OH one ($\Delta E = 0.1$ kcal/mol). The 3′-OH, 4′-OH, and 3-OH groups are found at 9.4, 0.7, and 3.5 kcal/mol above the minimum 7-OH.

The flavone apigenin anions are obtained upon H^+ removal from 4′-OH (0.0 kcal/mol), 5-OH (25.0 kcal/mol), and 7-OH (6.3 kcal/mol) groups, respectively. The 5-OH species is energetically less-favored because of the electronic repulsion between the negatively charged oxygen attached to the C_5 carbon and the carbonyl oxygen in ring C. The stability order between 4′-OH and 7-OH is reversed in the presence of water. The energetic gap between all anions appears to be decidedly smaller.

Upon deprotonation of myricetin hydroxyls, six anions are obtained, whose relative energies fall in a range of 25.8 kcal/mol. 4′-OH is a planar species stabilized by electronic delocalization effects and hydrogen bonds involving hydroxyls on $C_{3'}$ and $C_{5'}$ carbon atoms. The H-bonding interactions are missing in the other species that are found at 10.7 (3′-OH), 11.7 (5′-OH), 21.5 (3-OH), 25.8 (5-OH), and 10.8 (7-OH) kcal/mol above the 4′-OH. Computations in condensed phases indicate again the 4′-OH species as the global minimum, and the other species closer in energy, with respect to the vacuum.

Quercetin anions are all planar systems in which the negative charge can travel across two rings: from ring B to ring C in the case of 4′-OH and 3-OH or from ring A to ring C for 5-OH and 7-OH anions. Again, deprotonation at the 4′-site yields to the most stable anion in which the H-bonding pattern is the main stabilizing effect. The H-bonding interaction becomes less important in water medium where ion−dipole interactions predominate. The 3′-OH species becomes the most stable one.

Table 2. Gas Phase (Enthalpies) and Water (in Parentheses) (Free Energies) Acidities of Selected Polyphenols[a]

	acidities			relative acidities	relative pK_a in solution
compound	B3LYP/6-311++G**	B3LYP/6-311+G(d,p) (*38*)	MP2/6-311+G(d,p) (*42*)	B3LYP/6-311++G**	B3LYP/6-311++G**
			phenol		
OH	345.1 (310.0)			0.0 (0.0)	0.0
			gallic acid		
4-OH	*317.9 (292.2)*			–27.2 (–17.8)	–13.0
5-OH	327.4 (297.9)			–17.7 (–12.1)	–8.8
			caffeic acid		
3-OH	323.8 (297.2)			–21.3 (–12.8)	–9.3
4-OH	*318.0 (293.8)*			–27.1 (–16.2)	–11.8
			resveratrol		
3′-OH	336.2 (303.7)			–8.9 (–6.3)	–4.6
5′-OH	335.6 (303.8)			–9.5 (–6.2)	–4.5
6-OH	*327.5 (301.9)*			–17.6 (–8.1)	–5.9
			epicatechin		
3′-OH	*327.2 (299.7)*			–17.9 (–10.3)	–7.5
4′-OH	327.6 (300.0)			–17.5 (–10.0)	–7.3
5-OH	337.5 (304.0)			–7.6 (–6.0)	–4.4
7-OH	339.8 (304.4)			–5.3 (–5.6)	–4.1
			kaempferol		
4′-OH	323.0 (298.6)	327.1	328.9	–22.1 (–11.4)	–8.3
3-OH	333.0 (299.8)	338.0	336.3	–12.1 (–10.2)	–7.4
5-OH	337.5 (302.3)	342.2	340.3	–7.6 (–7.7)	–5.6
7-OH	*322.7 (296.5)*	328.1	327.3	–22.4 (–13.5)	–9.8
			cyanidin		
3′-OH	251.8 (294.6)			–93.3 (–15.4)	–11.2
4′-OH	238.3 (285.9)			–106.8 (–24.1)	–17.6
3-OH	240.6 (288.7)			–104.5 (–21.3)	–15.5
5-OH	*237.7* (285.3)			–107.4 (–24.7)	–18.0
7-OH	238.9 *(285.2)*			–106.2 (–24.8)	–18.1
			apigenin		
4′-OH	*321.3* (297.1)	324.4	327.0	–23.8 (–12.9)	–9.4
5-OH	346.3 (304.7)	349.6	346.2	1.2 (–5.3)	–3.9
7-OH	327.6 *(296.4)*	328.8	330.3	–17.5 (–13.6)	–9.9
			myricetin		
3′-OH	323.2 (298.4)	326.5		–21.9 (–11.6)	–8.5
4′-OH	*312.5 (292.6)*	314.8		–32.6 (–17.4)	–12.7
5′-OH	324.2 (298.6)	326.9		–20.9 (–11.4)	–8.3
3-OH	334.0 (299.8)	334.8		–11.1 (–10.2)	–7.4
5-OH	338.3 (302.3)	340.9		–6.8 (–7.7)	–5.6
7-OH	323.3 (296.6)	327.3		–21.8 (–13.4)	–9.8
			quercetin		
3′-OH	323.3 (298.3)	326.6	339.8	–21.8 (–11.7)	–8.5
4′-OH	*316.5* (299.9)	319.4	321.8	–28.6 (–10.1)	–7.4
3-OH	333.1 (299.8)	337.0	336.3	–12.0 (–10.2)	–7.4
5-OH	337.4 (302.3)	343.5	340.8	–7.7 (–7.7)	–5.6
7-OH	322.5 *(296.6)*	328.2	327.8	–22.6 (–13.4)	–9.8

[a] Values are in kcal/mol.

For apigenin anions, the disagreement between the present B3LYP/6-311++G** calculations and the literature HF/6-31G-(d) data (*46*) arises again as in the case of neutral parent molecule. An explanation for the different planarity degree attributed to these molecules by the two methods can be found again in the different treatment of π-delocalization contributions.

Upon examination of energetic gaps among all anions (**Table 1**), one can argue that the 4′-position in ring B is the most favored deprotonation site, followed in energy by the 7-position in ring A. Both deprotonation sites allow an extended delocalization of the negative charge. Deprotonation of the 4′-OH is energetically preferred as hydrogen bond formation is possible, as indicated by ΔE values for myricetin and quercetin anions. The less acidic group is the 5-OH one because the generation of this anion leads to the disappearance of an H-bond and introduces an electronic repulsion between the negative charge of the deprotonated oxygen and the lone pairs on the carbonyl oxygen of the C-ring.

The presence of a water medium reduces the anion stability differences, especially for epicatechin, cyanidin, and apigenin systems. In some cases, an inversion occurs. A larger stabilization in aqueous solvent usually is associated with high dipole moment values coupled, however, to the presence of strong resonance and delocalization effects.

Gas Phase and in Water Acidities. The absolute and relative acidities in gas phase and in water solutions of the antioxidants depicted in **Scheme 2** are collected in **Table 2**. Literature B3LYP/6-311+G(d,p) (*46*) and MP2/6-311+G(d,p) (*50*) data are enclosed in the same table, for purpose of comparison. The pK_a values in solution are given with respect to phenol and reported as a further information for polyphenols acidities (*51*).

The B3LYP/6-311++G** gas phase acidity for phenol used as a reference system was found to be 345.1 kcal/mol, while the corresponding value in water solution is 310.0 kcal/mol. The experimental acidity, obtained by gas phase proton transfer equilibria, of 346.9 kcal/mol (*52*) appears to be well-reproduced.

In phenol, deprotonation of the acidic group leads to an anionic species in which the electron pair is delocalized over the aromatic ring. All of the relative values of acidity and pK_a were determined considering the most stable anion for every compound.

On the basis of increasing gas phase acidity values, polyphenols can be ordered as follows: cyanidin (237.7 kcal/mol) > myricetin (312.5 kcal/mol) > quercetin (316.5 kcal/mol) > gallic acid (317.9 kcal/mol) > caffeic acid (318.0 kcal/mol) > apigenin (321.3 kcal/mo) > kaempferol (322.7 kcal/mol) > epicatechin (327.2 kcal/mol) > resveratrol (327.5 kcal/mol). As can be noted, acidities fall in a range of 312.5−327.5 kcal/mol, with the exception of cyanidin (237.7 kcal/mol). However, this result is not surprising because cyanidin is a positively charged species (charge lies on O_1 in ring C), so the deprotonation gives rise to very stable neutral species.

By looking at their molecular structures, the most acidic systems are those characterized by an high π-delocalization. This occurs for cyanidin, myricetin, quercetin, and gallic and caffeic acids. For the first three flavonoid molecules, the delocalization involves rings B (on which we have the deprotonation site) and C, while for the latter, the electron pair may be delocalized over the aromatic ring and the substituents.

Further contributions to the anion stability arise from H-bond formation between the negative oxygen and the adjacent hydroxyl. The acidity values for apigenin, kaempferol, epicatechin, and resveratrol, which lack the *ortho*-dihydroxy functionality, confirm the stabilizing effect of hydrogen bonds.

B3LYP/6-311+G(d,p) literature indications (*46*) for kaempferol, apigenin, myricetin, and quercetin gave the same acidity order obtained in our investigation, even though some differences in absolute values were encountered. In fact, our gas phase acidities are lower. The gas phase MP2/6-311+G(d,p) study (*50*) suggested the same trend for kaempferol, apigenin, and quercetin, again with some differences in the absolute acidity values. We can explain these small discrepancies by considering the fact that both B3LYP (*46*) and MP2 (*50*) enthalpies were obtained as single-point energy evaluations on HF/6-31G(d)-optimized geometries. Furthermore, as mentioned before, these methods find nonplanar conformations; thus, the stabilizing contributions of π-delocalization are underestimated.

The deprotonation energies (DE) for a series of hydroxyflavones, including apigenin, kaempferol, and quercetin molecules, were recently computed by Lemanska et al. (*53*) as B3LYP/6-311G(d,p)//B3LYP/6-31G(d) single-point values. Limited to these three antioxidants, they found the following acidity order: quercetin (331.6 kcal/mol) > kaempferol (338.7 kcal/mol) > apigenin (342.7 kcal/mol). As it can be noted, these energies are to some extent higher with respect to our gas phase values. The discrepancy can be in part due to the fact that the authors (*53*) did not include in the DEs the thermodynamic corrections (these are important contributions in decreasing the acidities by about 8−10 kcal/mol) (*46*) and in part to the use of a smaller basis set that usually leads to higher computed acidities values, as was recently demonstrated (*50*).

The in water solution trend is to some extent different from the gas phase one: cyanidin (285.2 kcal/mol) > gallic acid (292.2 kcal/mol) > myricetin (292.6 kcal/mol) > caffeic acid (293.8 kcal/mol) > apigenin (296.4 kcal/mol) > kaempferol (296.5 kcal/mol) > quercetin (298.3 kcal/mol) > epicatechin (299.7 kcal/mol) > resveratrol (301.9 kcal/mol). It is worth noting that the absolute acidity values for every compound are very much smaller than the corresponding ones in the gas phase. That is, solvent favors the deprotonation process by ≈30−40 kcal/mol.

Of course, the trends of ΔpK_a relative values reflect that of acidities in terms of ΔG and depend on the same effects, that is the delocalization of the negative charge (with the resulting stabilization of the anion) and the formation of the intramolecular hydrogen bonds. Similar conclusions were drawn by Himo et al. (*51*) for ortho-substituted phenols.

The absolute acidity for cyanidin in solution is now similar to the values found for the other polyphenols. The differences are reduced by the presence of water medium because the stabilization is mainly due to the electrostatic interactions with the neutral and charged systems. With respect to the values obtained in vacuo, the obtained order suggests that the most acidic polyphenols in the condensed phase have again a certain degree of π−delocalization, whereas the H-bonding pattern effect becomes less important with respect to the gas phase. The systems for which the acidity values are low can be easily deprotonated in vivo in physiological environments and so can chelate transition metal cations, inactivating them from giving radical reactions.

These B3LYP calculations on gas phase acidities match some previous results concerning the evaluation of OH bond dissociation enthalpies (BDEs) for these same systems (*18*−*20*). This is not surprising because the release of a hydrogen atom, as it occurs in the H-atom transfer mechanism, can be viewed as the simultaneous loss of a proton and an electron. So, the factors affecting the BDEs are strongly related to those relevant for acidities. We can indicate again the catechol functionality, and conjugation and delocalization of π-electrons, as determinant factors for a good activity performed through the chelation mechanism.

In conclusion, we have investigated by means of density functional theory the gas phase and in solution acidities of some polyphenolic systems widely recognized as very strong antioxidant molecules. The high level electronic calculations allowed us to compute the absolute acidities as well as the acidities of the various hydroxyl groups belonging to the same polyphenolic molecule. The hydroxyl groups showing the greater acidities are those para-positioned to the substituents, as it occurs for gallic acid, caffeic acid, and resveratrol. For flavonoids, the 4′-position on ring B and the 7-position on ring A are the most suitable deprotonation sites because of the better possibility to delocalize the electron pair. The former is favored when H-bonds between adjacent hydroxyls are present. The most acidic polyphenolic compounds are those characterized by a high degree of π-electron delocalization, for which deprotonation yields to anionic species stabilized by resonances phenomena. The stability of the anions is enhanced by the presence of a H-bonding pattern. This work may provide some useful information about the antioxidant mechanism that occurs through transition metals chelation.

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Received for review December 20, 2005. Revised manuscript received February 16, 2006. Accepted February 28, 2006. Financial support from the University of Calabria and Regione Calabria (POR Calabria 2000/2006 misura 3.16.d, progetto VACAVISS) is gratefully acknowledged.

JF053180A