Antioxidant Properties of Phenolic Compounds: H-Atom versus Electron Transfer Mechanism

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Received: October 28, 2003; In Final Form: April 2, 2004

Density functional theory (DFT) calculations were performed to evaluate the antioxidant activity of molecules commonly present in many Mediterranean foods. A series of interesting systems, including tyrosol, hydroxytyrosol, gallic, and caffeic acids, belonging to the family of phenols, resveratrol of the stilbenes family, epicatechin, kaempferol, and cianidin as examples of flavonoids and, at last, a simplified model of α-tocopherol (6-hydroxy-2,2,5,7,8-pentamethylchroman (HPMC)) were studied. Conformational behavior was examined at the B3LYP/6-311++G(3df,2p) level of theory, in the gas phase and in two solvents with different polarity (water and benzene), with the aim to compute the bond dissociation enthalpy (BDE) for the O−H bonds and the adiabatic ionization potentials (IP). BDE and IP for these systems do not follow the same trends in gas and solution phases: the major differences with respect to vacuum are found as when water computations are performed. On the basis of the computed BDE and IP values, the most active systems able to transfer an H-atom seem to be α-tocopherol followed by hydroxytyrosol, gallic acid, caffeic acid, and epicatechin. Instead, kaempferol and resveratrol appear to be the best candidates for an electron-transfer mechanism.

1. Introduction

Interest in food phenolics has grown greatly because of their antioxidant and free radical scavenging ability and potential health benefits. Attention has increased mostly in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants that, in some cases, have been reported to be carcinogenic.[1]

Antioxidants are compounds that, in low concentration, can prevent biomolecules (proteins, nucleic acids, polyunsaturated lipids, sugars) from undergoing oxidative damage through free radical mediated reactions.[2] They can inhibit oxidizing chain reactions in several ways, including direct quenching of reactive oxygen species, inhibition of enzymes, and chelation of metal ions (Fe^{3+}, Cu^{+}). Their beneficial effects are related to diseases in which oxidative processes are remarkable, i.e., atherosclerosis, coronary heart disease, certain tumors, and aging itself.[3,4] In fact, a diet rich in fruits, vegetables, cereals, and olive oil can prevent cardiovascular diseases and certain forms of cancer.[5] The major antioxidant components of these common foods are the phenolic compounds. Their antioxidant activity seems to be related to their molecular structure, more precisely to the presence and number of hydroxyl groups, and to conjugation and resonance effects.[5] Recently, a quantum-mechanical investigation[6] has shown that the antioxidant action of flavonols is related to radicals showing a planar conformation that allows extended electronic delocalization between adjacent rings.

Two main mechanisms by which antioxidants can play their protective role have been proposed.[7] In the first one, the free radical removes a hydrogen atom from the antioxidant (ArOH) that itself becomes a radical:

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

This mechanism is referred to as H-atom transfer. A higher stability of the radical $ArO^{\bullet}$ corresponds to a better efficiency of the antioxidant ArOH, so that it is unlikely to react with the substrate. Generally, hydrogen bonds, conjugation, and resonance make it a nonreactive phenoxyl radical.

In this mechanism, the bond dissociation enthalpy (BDE) of the O−H bonds is an important parameter in evaluating the antioxidant action, because the weaker the OH bond the easier will be the reaction of free radical inactivation.

In the second mechanism (the one-electron transfer), the antioxidant can give an electron to the free radical becoming itself a radical cation:

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

Also in this case, the radical cation arising from the electron transfer must be stable, so it does not react with substrate molecules. According to these latter proposals, the ionization potential (IP) is the most significant energetic factor for scavenging activity evaluation. The lower the ionization potential, the easier is the electron abstraction. So, the calculation of BDEs and IPs includes interesting information about the efficiency and the activity of the phenolic antioxidants.

The aim of our investigation is to rationalize the correlation between molecular/electronic structure and scavenging activity, on the basis of BDE and IP values, and to establish a relative trend of reactivity among the compounds analyzed.

2. Theoretical Calculations

All calculations were performed by the Gaussian 98 code.[8] The B3LYP[9,10] exchange correlation potential was used for optimizing geometries in connection with the 6-31G basis set.[11,12] Harmonic vibrational frequencies were computed at the

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10.1021/jp037247d CCC: $27.50

same level of theory for both parent (ArOH) molecule and ($ArO^{\bullet}$ and $ArOH^{\bullet +}$) radicals to characterize all their conformations as minima or saddle points and to evaluate the zero-point energy (ZPE) corrections. Single point energy refinement on the 6-31G optimized geometries was performed with use of the 6-311++G-(3df,2p) basis set. Relative energy and ionization potential 6-311++G(3df,2p) values were then corrected by ZPE.

The unrestricted open-shell approach was used for radical species. No spin contamination was found for radicals, being the $\langle S^2 \rangle$ value about 0.750 in all cases.

Natural bond orbital (NBO) analysis implemented in the Gaussian 98 package was used to evaluate bond order in all systems. Tables containing bond order values are available as Supporting Information.

Solvent (water and benzene) effects were computed in the framework of the self-consistent reaction field polarized continuum model (SCRF-PCM)[13-15] implemented on the Gaussian 98 package. The default UAHF[16] set of solvation radii was used in the calculations to build the cavity according to the molecular topology, hybridization, and formal charge.

Gas-phase bond dissociation enthalpy (BDE) and adiabatic ionization potential (IP) were calculated at 298 K as the enthalpy difference for the reaction:

$$ArOH \Rightarrow ArO^{\bullet} + H^{\bullet}$$

and as the energy difference between the ArOH and $ArOH^{+\bullet}$ species, respectively.

For the computation in the solution phase, the BDE values are reported in terms of total free solvation energy, ΔG.

3. Results and Discussion

The studied compounds are depicted in Chart 1.

For clarity we will discuss separately the conformational properties and the relative stabilities of radicals for each system and the BDE and IP trends.

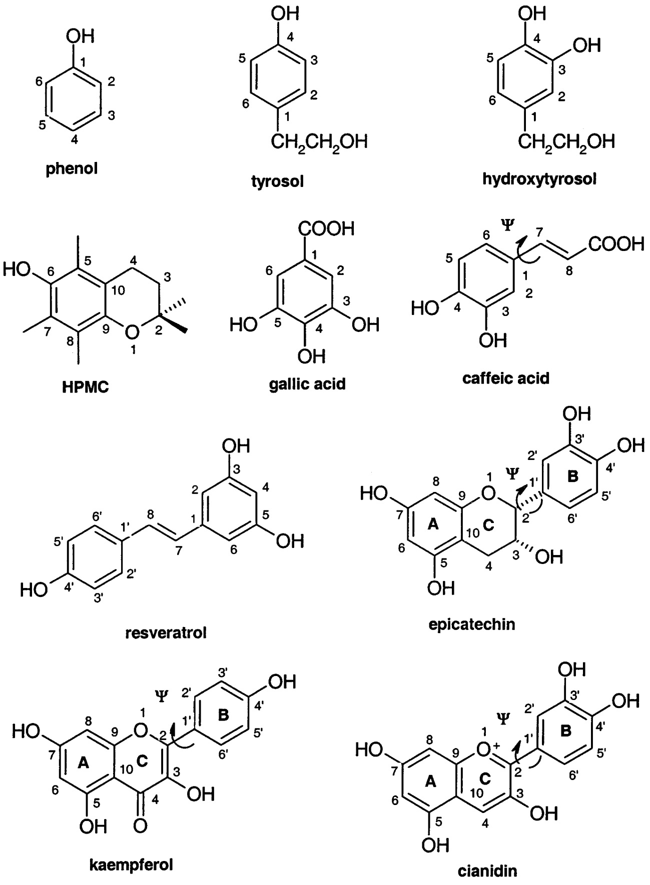
3.1. Conformations and Radical Stabilities. *Tyrosol and Hydroxytyrosol.* Virgin olive oil hydrophilic extracts contain a large number of phenolic compounds, mainly 3,4-dihydroxyphenylethanol, or hydroxytyrosol, and *p*-hydroxyphenylethanol, or tyrosol, as well as phenyl acids.[17] HPLC/UV and GC-MS[18] studies had led to the conclusion that hydroxytyrosol and tyrosol predominate among the simple phenols. Tyrosol also inhibits cytokines release in atherosclerosis.[19]

As Chart 1 shows, tyrosol has only a phenolic group that represents the antioxidant active site according to the H-atom transfer mechanism. We have performed a conformational analysis at the B3LYP/6-311++G(3df,2p) level of theory for all configurations arising from the possible orientations of the flexible side chain $-CH_2CH_2OH$.

The most stable structure in a vacuum is characterized by the folded gauche conformation of the flexible side chain stabilized by a hydrogen bond-like interaction between the alcoholic OH group and the aromatic ring. The relative energies of the other conformers in which such a type of hydrogen bond is absent are slightly higher (0.76 and 1.20 kcal/mol above the absolute minimum), underlining the stabilizing role of this interaction. Previous HF/6-31G* and MP2/6-31G* computations on tyrosol indicated this conformation was the preferred one.[20]

Starting from the gas-phase absolute minimum of tyrosol, an H atom has been removed giving rise to the phenoxyl radical. A CO double bond is formed, with a quinoid-like structure, while the unpaired electron appears delocalized over all the ring. The radical is stabilized by several resonance structures whose number is enhanced by the presence of the electron-donor alcoholic group in the para position with respect to the phenoxyl.

CHART 1



phenol tyrosol hydroxytyrosol

HPMC gallic acid caffeic acid

resveratrol epicatechin

kaempferol cianidin

Hydroxytyrosol differs from tyrosol by a further OH group in position 3 on the aromatic ring (ortho diphenolic functionality). The different conformers of this compound arise from the relative disposition of OH groups that depend on their H-bond donor or acceptor role, as well as from the flexibility of the side chain.

The B3LYP/6-311++G(3df,2p) gas-phase optimization yields as the preferred structure the conformation in which the orientation of the side chain is the same as that in tyrosol and the hydroxyl groups are oriented in such a way as to realize a hydrogen bond in which the O_4-H hydroxyl plays the H-bond donor function. Recently, a conformational study[21,22] on the noradrenaline (a catechol-type neurotransmitter) proposed the same orientation for the catechol hydroxyl groups. However, for noradrenaline, the stability of the conformers depends on the intramolecular H-bonds in a side chain that is obviously different from that in hydroxytyrosol. As far as the ortho diphenolic functionality is concerned, after the O−H bond is broken, the radical is able to rearrange itself to assume the most stable conformation. The free rotation, around the C_3-OH bond, involved in this rearrangement was reported to require an energy of 3 kcal/mol,[7] which, at 298 K, represents a very small amount of energy. So, when the OH group in position 4 is radicalized, the radical again establishes a hydrogen bond. The corresponding radical 4-OH missing this hydrogen bond is less stable by about 8.83 kcal/mol. This value can be considered as an indirect measure of the stability arising from the hydrogen bond.

The radical 3-OH, formed after the breaking of the O−H bond in the 3 position, is less stable with respect to the 4-OH radical by about 1.40 kcal/mol (see Table 1); this fact can be

TABLE 1: Relative Energies for Some Phenolics Radicals in Gas-phase, Water and Benzene. All Values Are in Kcal/Mol

	ΔE		
compd	gas phase	water	benzene
hydroxytyrosol radicals			
radical 4OH	0.00	0.76	0.00
radical 3OH	1.40	0.00	0.75
gallic acid radicals			
radical 4OH	0.00	0.00	0.00
radical 3OH	6.40	5.14	6.37
caffeic acid radicals			
radical 3OH	6.62	3.03	2.80
radical 4OH	0.00	0.00	0.00
resveratrol radicals			
radical 3-OH	6.33	3.96	6.40
radical 5-OH	5.82	5.33	5.67
radical 4′-OH	0.00	0.00	0.00
epicatechin radicals			
radical 3′OH	0.00	0.00	0.00
radical 4′OH	0.09	1.60	0.04
radical 5OH	8.29	3.86	9.00
radical 7OH	10.61	5.20	12.68
kaempferol radicals			
radical 4′OH	0.00	6.71	1.35
radical 3OH	0.24	0.00	0.00
radical 5OH	13.48	13.49	14.20
radical 7OH	5.69	13.80	7.34
cianidin radicals			
radical 3′OH	4.32	0.63	3.01
radical 4′OH	3.06	0.00	1.69
radical 3OH	0.00	1.68	0.00
radical 5OH	2.53	5.23	1.88
radical 7OH	5.74	3.83	4.00

explained because only in the case of radical 4-OH does one of the resonance structures have the unpaired electron on the tertiary carbon C_1. This electron deficiency can be immediately supplied by the presence of the electron-donor $-CH_2CH_2OH$ group with a consequent stabilization of the conformer.

In water solution, the radical 3-OH becomes the most stable one and the energetic gap is reduced by about half. In benzene, the gas-phase stability trend is kept and the gap again reduced as in the case of water.

α-Tocopherol. A new population-based study of antioxidants[23] suggests that a diet rich in foods containing vitamin E may help to protect some people against Alzheimer’s disease (AD). Researchers suggested testing the effectiveness of vitamin E and other antioxidants in preventing or postponing cognitive decline and AD.[23] Vitamin E is one of the nonenzymatic antioxidant systems in living organisms. It is characterized by a “phytyl tail” (a $C_{16}H_{33}$ group) that enhances the solubility in lipid membranes but does not affect the antioxidant activity. For our computational purposes we have considered a simplified model of α-tocopherol (6-hydroxy-2,2,5,7,8-pentamethylchroman, HPMC), in which the phytyl tail is replaced by a CH_3 group (see Chart 1). As confirmed by bond order computations, this compound has the features of an aromatic system, in which there is a complete electronic delocalization over one of the two rings; the alkyl substituents increase the charge density on this same ring.

The HPMC radical is obtained when an H atom is removed from the OH group present in the molecule; the bond order calculation underlines a delocalization of the unpaired electron over the whole aromatic system, coupled with a CH_3 groups electron-releasing effect.

Gallic Acid. Gallic acid (3,4,5-trihydroxybenzoic acid) is a strong natural antioxidant. Gallate esters antioxidant ability against hydroxyl, azide, and superoxide radicals has been reported.[23] It is widespread in plant foods and beverages such as tea and wine and has been proven to be one of the anticarcinogenic polyphenols present in green tea.[24]

The gas-phase minimum geometry shows two hydrogen bonds with 2.196 Å between the hydroxyl groups. From gallic acid it is possible to obtain two radicals by abstraction of a hydrogen atom from the 3-OH (5-OH) and 4-OH groups. The central O−H bond is the weakest one as confirmed by the binding energy value. The radical 4-OH is a symmetric species stabilized by two hydrogen bond interactions (2.152 Å). It has been postulated that the stability of the free radicals is enhanced by the presence at the radical center of both an electron-donor and an electron-withdrawing group.[25] This is called the *push−pull* or *captodative* effect. In our case the effect arises from the presence of the electron-donating OH group in the ortho position and the electron-withdrawing −COOH group in the para position that stabilize the radical with a large number of resonance forms.

When an outer O−H group is radicalized, the arising radical arranges itself so as to maximize the number of hydrogen bonds. As in the case of hydroxytyrosol, the rearrangement occurs through a rotation around the C_5-OH single bond that requires about 3 kcal/mol. The radical 3-OH is less stable than 4-OH by 6.40 kcal/mol (see Table 1). In both radicals the unpaired electron appears delocalized over all the ring. Computations in solution do not introduce significant news. The stability trend and the energetic gap are practically the same as those obtained in the gas phase although in water the relative minimum 3-OH gains 1.36 kcal/mol with respect to 4-OH.

Caffeic Acid. Hydroxycinnamic acids are widely distributed among the plant kingdom, especially coumaric, caffeic, and ferulic acids, produced from the shikimate pathway from L-phenylalanine or L-tyrosine.[5] In caffeic acid (3,4-dihydroxy-*trans*-cinnamic acid) (see Chart 1), the presence of the −CH═CH− bridge between phenyl and carboxyl groups favors resonance and conjugation effects. The geometry optimization was carried out on all conformers arising from the rotation around the C_1-C_7 bond and from the mutual orientation of the hydroxyls. As expected, all stable configurations contain the H-bond in the carboxyl group and the absolute minimum shows the hydroxyls involved in a further H bond of 2.152 Å and pointing toward the substituent in C_1. The gas-phase absolute minimum is a completely planar system in which the torsion angle Ψ is 0.

With an expense of only 6.14 kcal/mol, which leads the ethylene group perpendicular to the plane of ring, a relative minimum lying at 0.37 kcal/mol and characterized by a torsion angle Ψ of 180° is reached. The existence of such a low barrier suggests the probable coexistence of the two conformers.

Our findings are in good agreement with those of VanBesien and co-workers,[26] who have performed a conformational analysis at the B3LYP/6-31G(3df,2p) level of theory, by also taking into account the conformers arising from the *cis* and *trans* conformations around the −CH═CH bond. We did not explore this situation since our investigation was focused on the most common naturally occurring conformers, i.e., the trans forms.

Caffeic acid radicalization yields the 3-OH and 4-OH radicals. The latter is the most stable one (about 6.62 kcal/mol) because of the electronic delocalization effect due to the position of the −CH═CH−COOH group. This contribution is missing in radical 3-OH since the unpaired electron cannot travel across the ethylene bridge.

The energetic gaps are reduced in water and benzene solutions although the trends of stability remain the same (see Table 1).

Resveratrol. Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a natural product present in grapes and other food products. It is characterized by phenolic rings and a double bond in the middle of the "dumbell" that makes it a very efficient antioxidant. Its special action can be explained by considering two factors. When a free radical is generated at the phenolic oxygen, it is stabilized by delocalization over the aromatic ring, the double bond, and the second ring; furthermore, the unsaturated portion has good affinity to (aliphatic) parts of the cell walls, which are made essentially of unsaturated lipids. Some harmful effects of unstabilized free radicals are attributed to reactions at these parts of the cell walls, causing a breakdown. Over 100 papers[27] have proven the anticarcinogenic, antiinfiammatory, and antioxidant action of resveratrol.

The B3LYP/6-311++G(3df,2p) optimization of resveratrol in vacuo yields an absolute minimum characterized by planarity, conjugation, and electronic delocalization as underlined by the bond order analysis. The mutual position of the hydroxyls does not allow any hydrogen bond interactions.

Radicalization of the 4′-OH group generates the most stable radical (see Table 1); its major stability of about 6.33 and 5.82 kcal/mol with respect to radicals 3-OH and 5-OH can be explained by taking into account the resonance effects due to the position of the $-CH{=}CH-$ group that allows complete delocalization over the whole molecule. The other radicals exhibit a spin distribution that leaves the odd electron on the same oxygen atom from which hydrogen has been removed.

In water and benzene solutions, the 4′-OH is also the most stable radical. The stability of the 3-OH and 5-OH species is reversed in water but retained in benzene. So, the site responsible for the H-atom transfer for resveratrol should be the hydroxyl group in position 4′.

Epicatechin. The flavonoid structure consists of a benzene condensed with a γ-pyrone ring (flavonols and flavones or its dihydro derivates flavanols and flavanones) to which a phenyl group in position 2 is attached. Individual differences within each group arise from the number and position of the hydroxyls[5] that can be present on every ring. Flavonoids can occur as glycosides in nature.[28] Antioxidant properties have been mainly ascribed to flavonols, flavones, and catechins.[29] Several functions of flavonoids in plants have been proposed, such as protection from UV light, insects, viruses, and bacteria, plant hormones controllers, and enzymes inhibitors.[30]

Flavonoids represent the most common and active edible antioxidants.[31] Several studies carried out on flavonoids have shown that they have medicinal properties, including antiinfiammatory, antiallergic, antiviral, antibacterial and antitumor activities.[32,33] Epicatechin, kaempferol, and cyanidin belong to this class of compounds.

Green tea polyphenols, i.e., (−)-epicatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate, are strong antioxidants against linoleic acid peroxidation in homogeneous solutions.[34]

(−)-Epicatechin contains four phenolic groups, on rings B and A, respectively (Chart 1). The OH group on ring C is an alcoholic group to which no antioxidant ability can be ascribed. Among all the conformers arising from the arrangement of the hydroxyl groups and by the rotation around the $C_2-C_{1'}$ bond, the gas-phase absolute minimum is that in which the 3′-OH and 4′-OH groups are oriented in such a way as to realize a hydrogen bond interaction. Equilibrium geometry is characterized by a dihedral angle Ψ of about 92.49°. This value indicates that for epicatechin there is no possibility of conjugation between the rings, due to the saturation of the C ring. The other conformer, with a torsion angle Ψ of 270.14°, lies at only 0.35 kcal/mol; the energetic expense of only 1.08 kcal/mol for the interconversion of conformers suggests that they may coexist.

Upon radicalization, epicatechin can give four radicals whose relative energies are listed in Table 1. The most stable is the radical 3′-OH, practically isoenergetic with the 4′-OH one (0.00 and 0.09 kcal/mol). The stabilization factor is the hydrogen bond (2.041 Å for 3′-OH and 2.000 Å for 4′-OH) built after an H atom is removed. For both radicals, the unpaired electron is delocalized over the B ring to which the radicalized hydroxyl group is attached; the torsion angles Ψ for these radicals are 80.25° and 86.48°, respectively.

Removing an H atom from the 5-OH group we obtain a radical whose stability is mainly due to the electronic delocalization on the aromatic ring. Contrary to the former radicals, the mutual position of the hydroxyl groups in this ring does not allow the hydrogen bond interaction.

Radical 7-OH shows the same features as the 5-OH radical.

In Table 1 are also reported the radical stability trends found in water and in benzene. In aqueous solution, the energetic gaps among radicals fall in a very small range of only 5 kcal/mol. All the computations indicate the 3′-OH radical as the most stable species.

Through a comparison between all species deriving from epicatechin radicalization, it is clear that hydrogen bond interactions are essential for enhancing the stability of the radical, so the catechol (ortho diphenolic) functionality is the main feature that affects the antioxidant activity.

Kaempferol. The absence of the ortho diphenolic structure in ring B determines a scarce efficiency of kaempferol as a hydrogen donor.[5] This flavonol occurs mainly in endive, leek, broccoli, radish, grapefruit, and black tea.

The structure of the global minimum is characterized by two hydrogen bonds between 3-OH and 5-OH hydroxyls and the C_4-O carbonyl of 1.997 and 1.761 Å, respectively. The dihedral angle Ψ of 180° allows a good electronic delocalization and conjugation of the π electrons that involve rings A and B independently, as indicated by bond order computations.

Radicals 4′-OH and 3-OH have practically the same stability being separated in energy by only 0.24 kcal/mol (Table 1). Even if the abstraction of a H atom from the hydroxyl group in position 3 implies the breaking of a hydrogen interaction, the radical formed is quite stabilized by electronic delocalization. For both radicals, the bond order underlines that the unpaired electron travels over rings C and B thanks to the planarity of the species (torsion angle of 180°). For kaempferol radicals, missing the possibility of hydrogen bonds, the main factor affecting the radical stability is the electronic delocalization over two rings.

The other isomers, generated by the loss of the hydrogen atom from the 5-OH and 7-OH groups, are found at 13.48 and 5.69 kcal/mol, respectively. The electronic flow between ring A and ring B is broken up by the presence of ring C notwithstanding the planarity of these two species (torsion angle of 180°).

The radicalization of the hydroxyl group in position 5 requires the breaking of the hydrogen bond between this group and the C_4-O carbonyl. Thus, the quinoid-like structure that is formed (with the double bond strongly localized in C_5-O) on ring A and the C_4-O carbonyl of ring C give rise to a repulsion effect that turns into a destabilization of the radical itself.

For the radical 7-OH, the hydrogen bond interaction is retained and consequently it appears more stable than the 5-OH system. However, the high energetic values of these latter radicals should reduce their contribution to the biological activity.

The trend of stability of kaempferol radicals in water solution is different from that obtained in the gas phase because the radical 3-OH becomes the most stable one. The trend in water is found also in benzene.

Cyanidin. Cyanidin (Chart 1) belongs to the anthocyanin family present as pigments in berries and grape skin. Cyanidins are potent antioxidants as shown in recent studies performed in vivo by a group of Japanese scientists.[35−37] This aglycon has higher efficiency with respect to its glycosides, underlining that the antioxidant activity of anthocyanins is mainly ascribed to their aglycon moiety.[38]

The equilibrium gas-phase geometry of cyanidin presents a hydrogen bond between the 3′-OH and 4′-OH groups whose length is 2.117 Å. The torsion angle Ψ of 0° allows the electronic delocalization among all the rings. In fact, it can be noted from bond order analysis that, except for C_2-O_1 and C_9-O_1, all other couples of atoms form bonds with a mean order of 1.300. This confirms that cyanidin is a quite completely conjugated system with a very large stability. The absolute minimum is followed in energy by a species arising from the free rotation around the $C_2-C_{1'}$ bond, which lies at only 0.67 kcal/mol and shows a torsion angle Ψ of 180°. The barrier height for this rotation has been found to be 10.15 kcal/mol. This quite large value suggests that the second relative minimum cannot be formed easily from the absolute one through a rotation.

The order of stability for cyanidin radicals in the gas phase is 3OH > 5OH > 4′OH > 3′OH > 7OH (Table 1), which is different from those we have obtained previously for the other flavonoids. The radicalization of each hydroxyl group does not require the breaking of hydrogen bonds, so the energetic difference among all radicals is very small.

For the species 3-OH, the dihedral angle value and the bond order show that it is stabilized by electron delocalization over rings A and B, through ring C. Radical 4′-OH lies at 3.06 kcal/mol with respect to the global minimum 3-OH. Also in this case, there is a broad delocalization of the unpaired electron over all rings B, C, and A. The same finding was obtained for the 5-OH radical that we found at 2.53 kcal/mol. For radicals 3′-OH and 7-OH, the canonical forms do not allow electronic flow over the three rings. This is the reason for their minor stability (4.32 and 5.74 kcal/mol, respectively).

In water solution, the stability trend is quite different: the radical 4′-OH becomes the absolute minimum and is found very close in energy to the 3′-OH species (0.00 and 0.63 kcal/mol, respectively). The radical 3-OH follows at 1.68 kcal/mol. Although in the 3′-OH species there is no possibility of delocalization of the unpaired electron on rings C and A, the interaction with the solvent field stabilizes it probably because of its high dipole moment value. In benzene, the radical 3-OH remains the most stable one and all other isomers follow the same trend of the gas phase with smaller energetic gaps.

On the basis of the obtained results the antioxidant effect of cyanidin seems to be due to the presence of more than a radical, whose occurrence depends on the possible small variations of experimental conditions. As a consequence, every hydroxyl group can be responsible for the antioxidant activity of this flavonoid.

3.2. BDE and IP Evaluation. As mentioned before, phenolics and flavonoids can play their protective role by donating an H atom or acting as electron donors. It is clear that as far as specific molecular properties are concerned, the bond dissociation enthalpy (BDE) for the O−H bond and ionization potential (IP) are of particular importance in deciding which mechanism is the favored one for the radical scavenging activity.

TABLE 2: B3LYP/6-311++G(3df,2p) ΔBDE Values in the Gas Phase, Water, and Benzene for Some Phenolic Compounds (in kcal/mol)

compd	ΔBDE gas phase	ΔBDE water	ΔBDE benzene
phenol[a]	0.00	0.00	0.00
tyrosol	−0.93	−1.18	−1.22
hydroxytyrosol	−9.40	−8.60	−9.62
HPMC	−11.22	−12.07	−12.18
gallic acid	−10.64	−14.84	−11.51
caffeic acid	−9.28	−11.10	−9.64
resveratrol	−5.58	−8.97	−6.35
epicatechin	−9.17	−19.43	−10.36
kaempferol	−1.95	−10.11	−3.24
cianidin	−3.52	−7.12	−4.12

[a] BDE values for phenol are 82.89, 97.12, and 91.49 kcal/mol in the gas phase, in water, and in benzene, respectively.

Since water is the main element of all the physiological liquids, we have computed BDE not only in the gas phase but also in water. In addition, since another possible site of action for antioxidants is the biological membranes, where unsaturated lipids are present, these quantities have been calculated in a nonpolar solvent like benzene. All the values of BDE are referred to the most stable radical species deriving from the absolute minimum of each antioxidant compound. Our results are then compared to a reference compound (phenol), and given in terms of ΔBDE and ΔIP values. The choice of a reference molecule, although arbitrary, is nor difficult to understand or original,[7] because it represents the basic nucleus of all phenolic compounds examined in this work and allows the evaluation of the effect of further OH groups and different substituents on the antioxidant activity.

Table 2 reports the ΔBDE in the gas phase for all the phenolics previously described, together with the values computed in water and in benzene. The gas-phase phenol BDE value of 82.89 kcal/mol computed by us seems to be quite different from the values previously reported in the literature (85.1 and 88.3 kca/mol[39−42] and 88.3 ± 0.8 kcal/mol[43]). However, we would underline that no direct determination in the gas phase was performed in these previous works. In fact, the values of 85.1 and 88.3 kcal/mol were estimated by subtracting the enthalpy of solvation of the hydrogen atom and the difference in the enthalpy of solvation of phenol and the phenoxyl radical from the BDE value in two different solvents. Instead, the value of 88.3 ± 0.8 kcal/mol[43] was taken in deoxygenated benzene solution supposing a negligible effect of the solvent because of its small dielectric constant. The B3LYP/6-311+G(3df,2p) BDE of 87.10 kcal/mol found by Wright and co-workers,[7] although nearer the experimental value, has been obtained by a computational procedure in which the radical is treated using the restricted open shell approach instead of the unrestricted one.

BDE values of phenol in water and in benzene were computed to be 97.12 and 91.49 kcal/mol, respectively.

Because our BDE values in solvent are given in terms of total free solvation energy, Δ*G*, a comparison with literature data, reported as solvation enthalpies,[43−46] is not consistent.

From Table 2, it can be seen that the most active systems able to work through the H atom transfer mechanism in the gas phase are those with the smallest value of BDE and the most negative value of ΔBDE; so HPMC, gallic acid, hydroxytyrosol, caffeic acid, and epicatechin are very efficient as hydrogen donors. The HPMC ΔBDE value of −11.3 kcal/mol, previously computed by Wright,[7] is in good agreement with our result.

TABLE 3: B3LYP/6-311++G(3df,2p) ΔIP Values in the Gas Phase, Water, and Benzene for Some Phenolic Compounds (in kcal/mol)

compd	ΔIP gas phase	ΔIP water	ΔIP benzene
phenol[a]	0.00	0.00	0.00
tyrosol	−10.33	−6.57	−7.97
hydroxytyrosol	−16.98	−11.00	−14.38
HPMC	−37.15	−15.01	−30.91
gallic acid	−2.96	−6.45	−2.59
caffeic acid	−10.90	−8.38	−8.24
resveratrol	−30.70	−20.96	−25.50
epicatechin	−21.20	−15.86	−15.31
kaempferol	−24.06	−13.23	−18.27
cianidin	54.12	−5.63	23.66

[a] IP values for phenol are 192.05, 145.14, and 166.99 kcal/mol in the gas phase, in water, and in benzene, respectively.

In water solution we found the same general indication but some stability inversion with respect to the gas phase occurs. In particular epicatechin, characterized by a bond dissociation free energy of 77.69 kcal/mol and a relative value with respect to the phenol of −19.43 kcal/mol, becomes the most reliable system acting as hydrogen donor.

The stability order in benzene proposes again HPMC, gallic acid, hydroxytyrosol, caffeic acid, and epicatechin as the most reactive systems.

The obtained results indicate that the catechol functionality (and the trihydroxy functionality for gallic acid) is the main cause of the stability of a radical and of the weakening of an O−H bond. The effect of electron-donating groups in ortho and para positions to the phenolic hydroxyl also contribute to lowering of the energetic expense for breaking the O−H bond and favors the H-atom transfer to free radicals. In fact, for HPMC, where there is no possibility of hydrogen bond interaction, the electronic releasing effects of the alkyl groups stabilize very well the electronic vacancy.

The ionization potentials give a different trend of reactivity for our systems (see Table 3). HPMC, resveratrol, kaempferol, and epicatechin show small values of IP and very negative values of ΔIP referred to phenol, whose values are 192.05 (gas phase), 145.14 (water), and 166.99 kcal/mol (benzene). The model simulating vitamin E has the smallest value of IP, about 154.90 kcal/mol in vacuo. Cyanidin appears to be less active as an electron donor than phenol; we can explain this finding by considering that cyanidin is a charged system (see Chart 1), from which it is not so easy to abstract a further electron for generating a double positive charged system. Other (U)-B3LYP/6-31(d)//AM1/AM1 ΔIP values[7] are −36.1 kcal/mol for α-tocopherol, −33.1 kcal/mol for resveratrol, and −18.0 kcal/mol for epicatechin.

In water solution, resveratrol is the most active one, with a ΔIP of −20.96 kcal/mol, followed by epicatechin and α-tocopherol. It is noteworthy that cyanidin, with a positive value of ΔIP in the gas phase, now becomes more active (ΔIP value is −5.63 kcal/mol). This fact is due to the ability of a polar solvent to stabilize the charged systems such as cyanidin. As expected in nonpolar solvent, the gas-phase trend of ΔIP is retained in benzene.

Unfortunately, no experimental data are available in the literature for IP, so comparisons between computed and experimental values are not possible.

From Tables 2 and 3, the role of the solvent appears to be determinant for establishing a trend close to the biological environment in which these compounds normally act as antioxidants.

Our computations show that the abstraction of one electron is easier in phenols containing substituents which may conjugate with the aromatic systems and provide an increased electron density. So, for HPMC the stability of the radical cation arises from the hyperconjugation effect of several alkyl substituents, whereas for resveratrol, it arises from the motion of the unpaired electron over two rings and over the ethylene group.

The ΔBDE and ΔIP referring to phenol can give a relative trend of reactivity that in turn can be explained on the basis of molecular structure considerations.

An experimental study[5] was carried out on several flavonoids and phenolic acids employing the TEAC values to evaluate the H-donating ability with respect to the $ABTS^{\bullet +}$ radical cation of some compounds and to pick out some structural criteria for good radical scavenging.

The 2,3 double bond in connection with the 4-keto group in ring C is not so relevant without the ortho-diphenolic structure in ring B, as occurs when the kaempferol TEAC value is compared with that of its dihydro derivative, which were found very similar. This finding is also supported by our computational results. The monophenolic ring is not as effective; in fact, ΔBDE values of kaempferol (−1.95 kcal/mol), tyrosol (−0.93 kcal/mol), and resveratrol (−5.58 kcal/mol) are less negative with respect to the those of epicatechin and hydroxytyrosol (ΔBDE = −9.17 and −9.40 kcal/mol, respectively).

The presence of a third OH group was found not to enhance the hydrogen-donating ability, because myricetin and quercetin showed the same TEAC values, even if the former possess an additional hydroxyl group in ring B. We find similar results, because ΔBDE of gallic acid is −10.64 kcal/mol, very close to the ΔBDE values for the systems containing only the catechol moiety, such as hydroxytyrosol (ΔBDE = −9.40 kcal/mol), caffeic acid (ΔBDE = −9.28 kcal/mol) and epicatechin (ΔBDE = −9.17 kcal/mol).

Many studies have been undertaken in lipophilic systems with the aim to establish the features for a good radical scavenging ability.[47−55] The optimum antioxidant activity is associated with multiple phenolic groups, especially the catechol moiety, and with the 4-keto group in ring C. These findings are in good agreement with our results.

4. Conclusions

In this paper a density functional based method has been applied to study naturally occurring antioxidant compounds. These include virgin oil phenolics, vitamin E, tea polyphenols, substituted stilbenes, and some flavonoids. The study has concerned the determination of the bond dissociation enthalpy (BDE) and the adiabatic ionization potential (IP) according to the mechanism proposed in the literature for the radical scavenging activity.

On the basis of the obtained results in the gas phase, water, and benzene, we can draw the following conclusions:

Solution- and gas-phase BDE values do not always follow the same trends. In particular, some compounds that appear to be good candidates for H-atom or electron transfer in the gas phase, are less active in water.

The most efficient hydrogen-donor systems are characterized by the dihydroxy functionality, for which the BDE values are very small with respect to the phenol reference compound; radicalization of their hydroxyl groups gives rise to species in which the odd electron appears to be delocalized over the whole molecule and stabilized by hydrogen bond interactions.

An extended delocalization and conjugation of the π electrons, enhanced by resonance effects and planarity, favor the lowering of IP values and affect strongly the capacity of antioxidants to donate a single electron.

Acknowledgment. We gratefully acknowledge the Universita' della Calabria and MIUR (Progetto MEMOBIOMAR) for financial support.

Supporting Information Available: Tables containing bond order values for all the systems, parent molecules, and radicals. This material is available free of charge via the Internet at http://pubs.acs.org.

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