

Regular article

Density functional computations of the energetic and spectroscopic parameters of quercetin and its radicals in the gas phase and in solvent

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Received: 15 January 2003 / Accepted: 30 April 2003 / Published online: 29 December 2003
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Abstract. Quercetin and its radicals were investigated at the B3LYP density functional level with the aim of determining the energetic and spectroscopic parameters and the factors that influence their antioxidant activity in the gas phase and in aqueous solution. The results indicated that the antioxidant ability of quercetin is mainly due to the orthodiphenolic moiety but also to the presence of the C=C double bond of the pyrone ring. Differences in the stability order of the isomers of quercetin's radicals were found in going from vacuum to solvent. Hyperfine coupling constants were computed to help the interpretation of the intricate ESR spectrum of quercetin.

Keywords: Antioxidants – Density functional theory – Bond dissociation energy – Ionization potential – Solvent effects

Introduction

Quercetin is a member of a group of naturally occurring compounds, the flavonoids, which have a common flavone nucleus composed of two benzene rings linked through a heterocyclic pyrone ring. Quercetin is widely distributed in the plant kingdom and is the most abundant of the flavonoid molecules. It is found in many often-consumed foods, including apple, onion, tea, red wine, berries, and brassica vegetables, as well as many seeds, nuts, flowers, barks, and leaves. It is also found in medicinal botanicals, including *Ginkgo biloba*, *Hypericum perforatum*, *Sambucus canadensis*, and many

others. It is often a major component of the medicinal activity of the plant, and has been shown in experimental studies to have numerous effects on human health, including cardiovascular protection, anticancer activity, antiulcer effects, anti-allergy activity, cataract prevention, antiviral activity, and anti-inflammatory effects. It also acts as an antioxidant [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12].

All flavonoids have the same basic chemical structure, a three-ringed molecule with hydroxyl (–OH) groups attached. A multitude of other substitutions can occur, giving rise to the many types of flavonoids. Flavonoids often occur in foods as a glycoside, meaning they have a sugar molecule (rhamnose, glucose, galactose, etc.) attached to the center (C) ring. Quercetin is the aglycone of a number of other flavonoids, including rutin, isoquercetrin, and hyperoside. These molecules have the same structure as quercetin except they have a specific sugar molecule in place of one of quercetin's hydroxyl groups on the C ring, which dramatically changes the activity of the molecule. Activity comparison studies have identified other flavonoids as often having similar effects as quercetin, but quercetin usually has the greatest activity.

Flavonoids, as a rule, are antioxidants, and a number of quercetin's effects appear to be due to its antioxidant activity. Quercetin scavenges oxygen radicals [1, 2], and inhibits xanthine oxidase [3] and lipid peroxidation in vitro [4]. As another indicator of its antioxidant effects, quercetin inhibits oxidation of LDL cholesterol in vitro, probably by inhibiting LDL oxidation itself, by protecting vitamin E in LDL from being oxidized or by regenerating oxidized vitamin E [5]. By itself, and paired with ascorbic acid, quercetin reduces the incidence of oxidative damage to neurovascular structures in skin, and inhibits damage to neurons caused by experimental glutathione depletion [6].

Quercetin's anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects on inflammation-producing enzymes (cyclooxygenase, lipoxygen-

Contribution to the Jacopo Tomasi Honorary Issue

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ase) and the subsequent inhibition of inflammatory mediators, including leukotrienes and prostaglandins [7, 8]. Inhibition of histamine release by mast cells and basophils [9, 10] also contributes to quercetin's anti-inflammatory activity.

Quercetin is a strong inhibitor of human lens aldose reductase [11]. Furthermore it exerts antiviral activity against reverse transcriptase of HIV and other retroviruses, and was shown to reduce the infectivity and cellular replication of herpes simplex virus type 1, poliovirus type 1, parainfluenza virus type 3, and respiratory syncytial virus (RSV) [12, 13, 14, 15, 16, 17, 18, 19, 20, 21].

All the benefits of quercetin can be deduced by the above mentioned experimental studies and by many others that concern its electronic and spectroscopic features [22, 23, 24, 25, 26, 27, 28], its content in red wines [29], its mono- and two-electron oxidation in protic and nonprotic media [30, 31, 32], and its redox properties [33, 34, 35, 36, 37, 38]. From a theoretical point of view, quercetin was investigated at the semiempirical [25, 39, 40] and ab initio HF [24] levels. To our knowledge, no density functional work exists on this molecule.

The aim of this first DFT study is to give better insight into the gas-phase electronic and spectroscopic properties of quercetin and its radical species using a high level of theory and to individuate the eventual changes of these properties in solvent.

Method and computational details

Becke3 (B3) exchange [41] and Lee-Yang and Parr (LYP) correlation [42] potentials, in connection with the internal 6-311++G** [43] orbital basis set as implemented in the Gaussian 98 code [44], were used for the full geometry optimization of quercetin and its 3-OH, 3'-OH, 4'-OH, 5-OH, and 7-OH radical species. Natural bond orbital (NBO) analysis was employed to obtain bond order values. Vibrational frequencies were computed using the 6-31G* basis set and then used to correct all energetic terms by the zero point energy.

The polarizable continuum model (PCM) [45] was used for the computation of quercetin and its radicals in aqueous solution at the B3LYP/6-311++G** level of theory. The bond dissociation enthalpy (BDE) for quercetin was computed using the following expression:

$$\text{BDE} = H_{\text{que}} - H_{\text{querad}} - H_{\text{H}}$$

where H_{que} , H_{querad} , and H_{H} are the quercetin, quercetin radical, and hydrogen enthalpy, respectively. The ionization potential (IP) of quercetin was determined optimizing both its neutral and cationic species. Hyperfine coupling constants for quercetin radicals were computed at the equilibrium geometries obtained using the EPR-II [46] basis set in the gas phase and in solvent following the usual procedure [46].

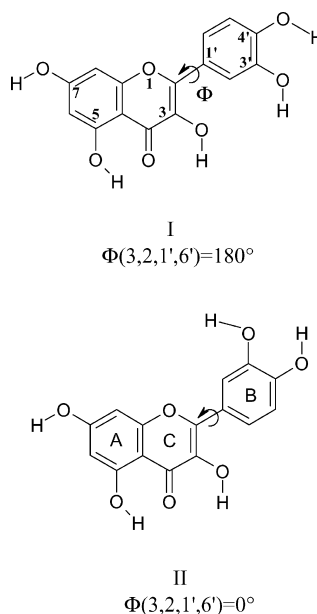
Results and discussion

Quercetin

The different conformations of the quercetin molecule are dictated by the possibility of rotation around the $C_2-C_{1'}$ (see Scheme 1 for atoms and labels) single bond connecting the B and C rings and by the disposition that each -OH group can assume in the system. The optimization at the B3LYP level with the 6-311++G** basis set yields as preferred structure a planar conformation ($\Phi=180^\circ$) in which the hydroxyl groups are oriented in such a way as to form the maximum number (three) of hydrogen bonds. Equilibrium B3LYP/6-311++G** geometrical parameters of the absolute minimum (I) are reported in Table 1 together with bond order values and data coming from crystallographic measurements [23]. If we take into account the known differences between the vacuum and condensed phase environments, the geometry appears to be quite well reproduced, and the small discrepancies easily ascribable to the packing in the crystal.

Bond order analysis, as well as the planarity of the molecule, precludes a possible extended delocalization with a consequent good stabilization of the radical species eventually originating from the hydrogen abstraction from the -OH groups of all the rings. As indicated by Rice-Evans et al. [47] and by van Acker et al. [25], the antioxidant properties of flavonoids can be derived just from their good delocalization possibilities.

With an expenditure of 5.59 kcal/mol, which leads the B ring perpendicular to the plane of the A and C rings, the form II ($\Phi=0^\circ$), lying at only 0.55 kcal/mol above the absolute minimum, is quickly reached (see Table 2). Previous semiempirical [39, 40] and ab initio



Scheme 1

Table 1. B3LYP/6-311++G** geometrical parameters of the absolute minimum ($\Phi = 180^\circ$) of quercetin in the gas-phase and in water. Distances are in Å and valence angles in degrees. Experimental data are crystallographic measurements(ref. 23)

Bond lengths	Gas phase	EXP ^a	Solvent	Bond order	gas phase	Angles	Gas phase	EXP ^a	Solvent
C ₂ -O ₁	1.376	1.371	1.374	0.983		C ₂ -O ₁ -C ₉	122.8	121.7	122.7
C ₂ -O ₁ '	1.462	1.469	1.463	1.106		O ₁ -C ₂ -C ₁ '	112.4	111.5	112.7
C ₂ -C ₃	1.371	1.362	1.372	1.485		C ₃ -C ₂ -C ₁ '	128.6	128.4	128.0
C ₃ -C ₄	1.450	1.431	1.446	1.119		C ₂ -C ₃ -O ₃	124.0	121.1	123.0
C ₄ -C ₁₀	1.433	1.451	1.431	1.151		C ₂ -C ₃ -C ₄	121.6	121.2	121.4
C ₁₀ -C ₅	1.424	1.418	1.423	1.220		C ₃ -C ₄ -O ₄	118.4	120.6	119.0
C ₅ -C ₆	1.390	1.365	1.384	1.397		C ₃ -C ₄ -C ₁₀	116.9	116.7	116.9
C ₆ -C ₇	1.405	1.396	1.407	1.345		O ₅ -C ₅ -C ₆	119.9	120.2	119.8
C ₇ -C ₈	1.399	1.385	1.401	1.352		C ₆ -C ₅ -C ₁₀	119.7	120.8	120.0
C ₈ -C ₉	1.390	1.381	1.391	1.391		C ₅ -C ₆ -C ₇	119.6	119.5	119.6
C ₉ -C ₁₀	1.408	1.390	1.408	1.267		C ₆ -C ₇ -O ₇	121.4	120.3	120.5
C ₉ -O ₁	1.360	1.368	1.362	1.001		C ₆ -C ₇ -C ₈	122.0	121.8	121.8
C ₁ -C ₂ '	1.413	1.397	1.413	1.318		C ₇ -C ₈ -C ₉	117.7	117.3	117.7
C ₂ '-C ₃ '	1.384	1.383	1.386	1.436		O ₁ -C ₉ -C ₈	117.4	117.1	117.6
C ₃ '-C ₄ '	1.407	1.392	1.409	1.275		O ₁ -C ₉ -C ₁₀	120.4	119.7	120.1
C ₄ '-C ₅ '	1.393	1.378	1.394	1.366		C ₈ -C ₉ -C ₁₀	122.2	123.2	122.2
C ₅ '-C ₆ '	1.391	1.380	1.392	1.453		C ₄ -C ₁₀ -C ₅	122.0	122.2	121.9
C ₆ '-C ₁ '	1.408	1.398	1.408	1.348		C ₄ -C ₁₀ -C ₉	119.2	120.5	119.6
C ₃ -O	1.377	1.373	1.367	0.986		C ₅ -C ₁₀ -C ₉	118.7	117.4	118.5
C ₄ -O	1.358	1.374	1.358	1.047		C ₂ -C ₁ -C ₂ '	121.3	119.7	121.3
C ₃ -O	1.359	1.358	1.357	1.031		C ₂ -C ₁ -C ₆ '	120.3	121.6	120.3
C ₄ -O	1.263	1.244	1.265	1.466		C ₁ '-C ₂ -C ₃ '	120.2	120.7	120.6
C ₅ -O	1.341	1.352	1.347	1.108		C ₂ '-C ₃ '-O ₃ '	124.3	122.2	124.1
C ₇ -O	1.359	1.359	1.352	1.043		C ₂ '-C ₃ '-C ₄ '	121.1	120.0	120.5
						C ₃ '-C ₄ '-O ₄ '	120.5	117.9	120.9
						C ₃ '-C ₄ '-C ₅ '	119.0	119.5	119.2
						C ₄ '-C ₅ '-C ₆ '	120.3	120.9	120.5
						C ₁ '-C ₆ '-C ₅	121.0	120.2	120.8

^aFrom ref. 23

Table 2. Relative energies (ΔE) of quercetin from B3LYP computations in the gas-phase and in solvent. Free hydration energies (ΔG) are reported together with cavitation, dispersion, and repulsion contributions. All data are in kcal/mol

Property	6-311++G**		
	I	II	Barrier
ΔE_{gas}	0.0	0.55	5.59
ΔE_{solv}	0.0	0.82	2.41
ΔG	-20.80	-20.69	-23.18
Cav	33.71	33.56	34.27
Disp	-36.09	-37.07	-36.65
Rep	6.43	7.36	4.80

[24] studies suggested for the absolute minimum geometrical features very similar to those obtained in the present investigation. The only exception is the value of the Φ torsion angle which, at the AM1 and RHF/6-31G* levels, was found to be 153.3° [39] and 162.3° , respectively. The density functional B3LYP value (180°) was much nearer to the experimental torsion of 175.1° [23]. The AM1 [39] and RHF/6-31G* [40] energy difference between the I and II conformations was estimated to be 0.23 and 0.20 kcal/mol, respectively, and the rotation barrier to pass from conformation I to II, 2.5 and 4 kcal/mol, respectively.

Although the sets of theoretical data are slightly different, they underline univocally an easy rotation around the Φ angle and a very small energy gap between the two stable conformations, suggesting their probable coexistence. Optimization in water solution does not change the stability order of the I and II conformers and

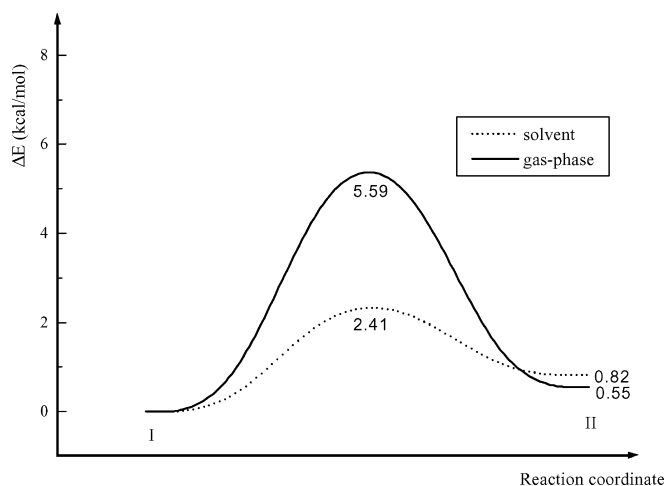


Fig. 1. Energy profile for I-II interconversion process of quercetin in the gas phase and in solvent

leaves the ring structures practically unaltered, as can be noted by the values reported in Tables 2 and 1, respectively. Nevertheless, the solvent seems to favor the I-II transformation because of the decrease of the barrier from 5.59 to 2.41 kcal/mol (see Fig. 1). In fact, the results reported in Table 2 indicate that there are differences of about 2.5 kcal/mol between the free hydration energy values of minima with respect to the transition state. Furthermore, for this latter species the repulsion energy term, related to the noncovalent interactions, has the smallest value. The dihedral Φ assumes a value of 171.9° in the absolute minimum and of 8° in the relative

one, suggesting a slight deviation from planarity in agreement with the experimental findings [23]. The loss of planarity of forms I and II and the major structural similarity between transition states, in which we have a torsion of about 90°, and both the reactant and product could explain why the barrier becomes energetically lower in solvent than in vacuo. The three O₅–H–O₄ (1.762 Å), O₄–H–O₃ (1.970 Å), and O₃–H–O_{4'} (2.120 Å) hydrogen bonds present in the gas-phase minimum are still retained in solution. The first one becomes shorter by 0.034 Å and the others longer by 0.076 and 0.051 Å, respectively.

The function of antioxidants is to intercept and react with free radicals at a rate faster than a substrate. There are two possible pathways for oxidation in which antioxidants can play their preventive role. The first consists of an H-atom transfer from the antioxidant molecule to the free radical and the second is an electron transfer between the same couple of species. It is clear that as far as specific molecular properties are concerned, the bond dissociation enthalpy (BDE) and ionization potential (IP) are of particular importance to decide which mechanism is the favored one. For this reason, we have computed both these quantities and compared them with the data available in the literature for the system generally used as reference (i.e., phenol) in such a type of study [48]. The gas-phase B3LYP/6-311++G** BDE value of quercetin, computed with reference to its most stable radical species, is 72.35 kcal/mol. This value is smaller by 10.54 kcal/mol with respect to the phenol BDE (82.89 kcal/mol) computed by us at the same theoretical level, indicating a better activity of quercetin as radical scavenger.

The phenol experimental [49] BDE of 87.30 ± 1.50 kcal/mol seems to be quite different from our theoretical value, but it is a mean of experimental measurements by photoacoustic calorimetry in five solvents having very different hydrogen bond accepting properties. The B3LYP/6-311++G** BDE of 87.10 kcal/mol obtained in previous theoretical work [48], although nearer the experimental value, was obtained by a computational procedure in which the radical is treated using a restricted open-shell approach instead of an unrestricted one. Furthermore an H-atom correction of 1.4 kcal/mol was also introduced.

However, appropriate comparisons should be done between ΔBDE values but this is not possible in the present case because of the lack of literature data concerning the quercetin BDE evaluation. In water, the B3LYP/6-311++G** BDE of quercetin and phenol were found to be 86.72 and 96.59 kcal/mol, respectively. ΔBDE in solution (9.86 kcal/mol) is substantially not too different from the gas-phase value. IP values of quercetin and phenol computed at B3LYP/6-311++G** were found to be 166.08 and 192.08 kcal/mol, respectively. As in the case of the H-atom transfer also in the electron-transfer mechanism, quercetin seems to be more effective than phenol in its antioxidant action.

Quercetin radicals

Starting from the two minima (I and II) of quercetin, which are practically coexistent, ten radical species obtained breaking the 3-, 3', 4', 5', and 7-OH bonds, can be derived. Gas-phase B3LYP computations performed by 6-311++G** and EPR-II basis sets indicate that all these species are planar and characterized by a variable delocalization of the unpaired electron.

In Table 3 the stability order of the radicals is reported at the two levels of theory. The results show that, although the energy gaps between minima are slightly different in the two methods, the trend is the same and the most stable species arise mainly from radicalization on the B and C rings. This finding is substantially in agreement with previous experimental [34] and theoretical [24, 25, 27] indications. In particular, the absolute minimum is the 4'-OH (II) radical which is obtained by the relative minimum II of the parent molecule, followed by the 4'-OH (I) species at only 0.23 kcal/mol at both 6-311++G** and EPR-II levels.

Radicalization occurring on ring A produces four species, 5-OH (I), 5-OH (II), 7-OH (I), and 7-OH (II), which are always very high in energy. These latter radicals exhibit a spin distribution that leaves the odd electron on the same oxygen atom (i.e., O₇ or O₅) from which hydrogen was extracted. This fact is not surprising because of the presence in the adjacent C ring of the –C=O and –O– moieties that work like electron-withdrawing groups, preventing the complete delocalization and blocking the conjugation. On the contrary, conjugation and delocalization effects are very significant on the orthodiphenolic B ring and the portion of C containing the C₂=C₃ double bond. For this reason, 3', 4', and 3-OH radicals present a spin distribution which collocates the unpaired electron on all oxygen and carbon atoms on the right of –C=O and –O– groups, irrespective of its original position. The 4'-OH (II) species is the most favorable by the conjugation, delocalization, and spin-distribution factors, hence it should be the most active radical scavenger. Nevertheless, the other 4'-OH (I), 3'-OH (II), and 3'-OH (I) radicals seem to be good candidates to participate in the antioxidant

Table 3. Relative energies (ΔE in kcal/mol) of quercetin radicals from B3LYP computations with different basis sets

Radicals	ΔE	
	6-311++G**	EPRII
3-OH (I)	7.04	7.17
3'-OH (I)	2.91	3.24
4'-OH (I)	0.23	0.23
5-OH (I)	22.38	23.50
7-OH (I)	13.98	15.12
3-OH (II)	8.41	8.54
3'-OH (II)	2.55	2.64
4'-OH (II)	0.0	0.0
5-OH (II)	23.19	24.73
7-OH (II)	14.29	15.52

mechanism because of the small energy differences with respect to the absolute minimum. Since the basis set effect on the stability order of radical species is clearly marginal, we have computed the I–II interconversion barriers for the most stable radicals only at the B3LYP/6-311++G** level. Figure 2 shows that these barriers are quite high in the cases of the 4'-OH (II) → 4'-OH (I) (9.50 kcal/mol) and 3-OH (II) → 3-OH (I) (12.25 kcal/mol) interconversions but less important for the 3'-OH (II) → 3'-OH (I) (5.90 kcal/mol) transformation. Thus, taking into account both the energy differences and the facility to pass from the $\Psi=0$ to $\Psi=180$ conformation, the most active radicals should be essentially the 4'-OH (II), 3'-OH (II), and 3'-OH (I) species.

Computations in water were performed at the B3LYP/6-311++G** level without geometry optimization. The reason for this choice derives from the negligible influence of solvent effects on the geometrical parameters of quercetin, as it is evident from Table 1.

All the energetic contributions of solvation to the six most stable radicals of quercetin are reported in Table 4. As can be noted the stability order in solution is different from that obtained in the gas phase, although again the most stable species are those arising from the radicalization of the –OH groups present on the C and B rings. The energetic gap between the various species appears to be reduced with respect to that of the gas phase and they all fall in a range of about 4 kcal/mole. This fact further reinforces the hypothesis that the antioxidant effect of quercetin can be due to the presence of different radicals whose occurrence depends on the possible small variations of experimental conditions. From Table 4 it can be noted that cavitation, dispersion, and repulsion contributions are almost similar for all the considered radicals, so the relative stabilization mainly depends on the electrostatic terms.

Recently van Acker et al. [24] have evidenced the difficulty in interpreting the ESR spectrum of quercetin,

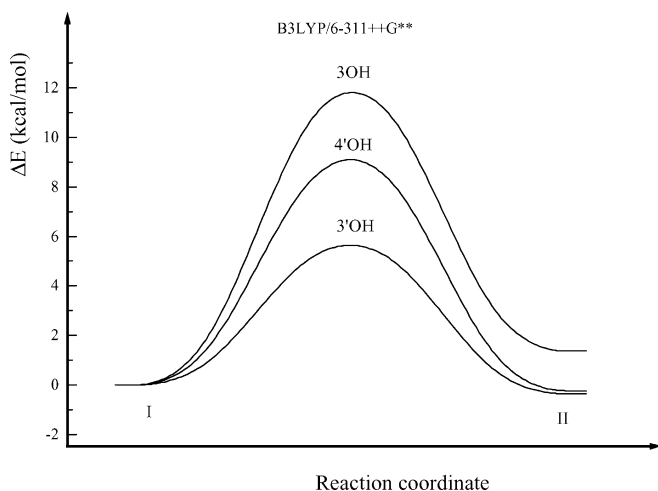


Fig. 2. Gas-phase energy profiles for I–II interconversion processes of 3-OH, 3'-OH, and 4'-OH quercetin radicals

Table 4. Relative (ΔE), hydration free (ΔG), cavitation (Cav), dispersion (Disp), and repulsion (Rep) energies of quercetin radicals in water from B3LYP/6-311++G** computations. All terms are given in kcal/mol

Radicals	ΔE	ΔG	Cav	Disp	Rep
3-OH (I)	1.66	-21.90	33.61	-36.29	7.02
3'-OH (I)	3.91	-15.60	33.42	-35.19	5.95
4'-OH (I)	2.63	-14.24	33.45	-35.18	5.94
3-OH (II)	0.0	-24.92	33.60	-36.37	7.13
3'-OH (II)	4.02	-15.22	33.38	-35.23	6.02
4'-OH (II)	2.23	-14.40	33.42	-35.24	6.06

because at different scan speeds different spectra were recorded suggesting the presence of several consecutive reactions giving rise to several different radicals. From our computations the same conclusions can be drawn and thus, with the aim of contributing to the interpretation of this spectrum, we have computed the hyperfine coupling constants for the six radicals lying in a small energy range. The results concerning hydrogen atoms A_{iso} are listed in Table 5. From the observation of these values it is worth noting that there are no differences between A_{iso} when associated to the I or II conformer of a particular radical, but the hyperfine coupling constants of each 3-OH, 3'-OH, and 4'-OH species are quite different. The situation does not change in solvent although small variations from gas-phase values can be noted. We think that the present data can be useful for resolution of the ESR spectrum of quercetin.

Conclusions

The present study concerns the density functional determination of the spectroscopic and electronic features of quercetin and its radicals. On the basis of the obtained results we can conclude that:

- Quercetin is a planar molecule characterized by an extended delocalization and conjugation of the π electrons. It exists in two forms, practically isoenergetic and both containing three hydrogen bonds. The coexistence of the two conformers of quercetin is even more probable in water because of a smaller interconversion barrier between them.
- Radicalization of quercetin occurs favorably on the B and C rings, giving rise to six low-lying energetic forms that participate in the antioxidant activity of the molecule. The stabilization of the radical forms is due to the minor or major delocalization of the odd electron on the whole system. The most stable radical in the gas phase is the 4'-OH (II) species while, in solvent, the situation is less decisive because of the decrease of the energy gap between the various forms. However, in both phases, the spectroscopic features and the antioxidant activity can be influenced by the coexistence of many radicals originating from radicalization of –OH groups on the B and C rings.

Table 5. B3LYP/EPR2 hyperfine coupling constants of hydrogen atoms (A_{iso} in MHz) for I and (II) 3-OH, 3'-OH, and 4'-OH radicals of quercetin in the gas-phase and in water

	A_{iso} in gas phase			A_{iso} in solvent		
	3-OH-I (-II)	3'-OH-I (-II)	4'-OH-I (-II)	3-OH-I (-II)	3'-OH-I (-II)	4'-OH-I (-II)
H6'	-10.1 (-9.8)	-20.7 (-21.3)	4.8 (4.7)	-8.0 (-8.0)	-20.2 (-21.6)	0.6 (1.5)
H2'	-6.9 (-7.4)	-13.6 (-15.5)	7.5 (7.4)	-4.8 (-3.8)	-12.5 (-10.2)	6.2 (6.1)
H5'	3.8 (4.0)	6.1 (6.6)	-13.7 (13.4)	2.3 (1.8)	5.4 (5.7)	-9.6 (-10.4)
H8	-1.9 (-1.8)	0.2 (0.1)	-0.4 (-0.4)	-2.7 (-3.5)	0.0 (-0.1)	0.2 (0.4)
H6	-0.2 (-0.2)	0.4 (0.2)	-0.4 (-0.3)	-0.3 (0.2)	0.0 (0.1)	0.3 (0.3)
H3	-	-0.4 (0.2)	-3.4 (-3.2)	-	-0.6 (-0.7)	-4.0 (-3.0)
H3'	0.2 (0.2)	-	-5.3 (-5.1)	-0.1 (-0.4)	-	-5.4 (-5.9)
H4'	-2.6 (-2.5)	-6.3 (-6.0)	-	-4.5 (-1.9)	-6.9 (-7.0)	-
H5	-0.6 (-0.6)	-0.2 (-0.1)	-0.2 (-0.2)	-1.0 (-0.7)	0.0(-0.1)	-0.4 (-0.4)
H7	-0.6 (-0.6)	-0.2 (-0.1)	-0.1 (-0.2)	-0.8 (-0.7)	0.0 (-0.1)	-0.5 (-0.3)

– Hyperfine coupling constants computed for I and II radical conformers do not show particular differences, while different values are found in dependence on the radicalization position. This fact, together with the contemporary presence of several radicals, could be the possible cause of the difficult detection and interpretation of the ESR spectrum of quercetin.

Acknowledgements. We gratefully acknowledge the Università della Calabria and MIUR for financial support.

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