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Mechanistic study of the structure–activity relationship for the free radical scavenging activity of baicalein

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Abstract Density functional theory calculations were performed to evaluate the antioxidant activity of baicalein. The conformational behaviors of both the isolated and the aqueous-solvated species (simulated with the conductor-like polarizable continuum solvation model) were analyzed at the M052X/6-311+G(d,p) level. The most stable tautomers of various forms of baicalein displayed three IHBs between O4 and OH5, O5 and OH6, and O6 and OH7. The most stable tautomer of the baicalein radical was obtained by dehydrogenating the hydroxyl at C6, while the most stable anion tautomer was obtained by deprotonating the C7 hydroxyl in gaseous and aqueous phases. The expected antioxidant activity of baicalein was explained by its ionization potentials (IPs) and homolytic O–H bond dissociation enthalpies (BDEs), which were obtained via the UM052X optimization level of the corresponding radical species. Heterolytic O–H bond cleavages (proton dissociation enthalpies, PDEs) were also computed. The calculated IP, BDE, and PDE values suggested that one-step H-atom transfer, rather than sequential proton loss–electron transfer or electron transfer–proton transfer, would be the most favorable mechanism for explaining the antioxidant activity of baicalein in the gas phase and in nonpolar solvents. In aqueous solution, the SPLET mechanism was more important.

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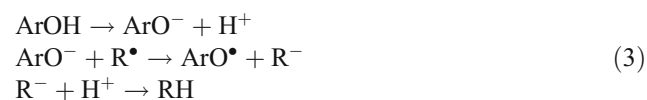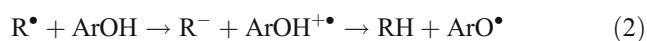Boston, MA 02115, USA

Introduction

The flavonoid family is a vast and important group among the phenolics, comprising more than several thousand known compounds [1–3]. They play essential roles in plant physiology, as they are involved in the light phase of photosynthesis, the attraction of pollinating insects, growth, reproduction, the regulation of iron channels associated with phosphorylation, resistance to pathogens and predators, and the protection of plants from UV [4]. This variety of beneficial actions is believed to be closely related to their pronounced antioxidant activity, which operates at different levels in the oxidative process, such as when scavenging for free radicals, chelating metal ions, scavenging for lipid peroxyl radicals, and removing oxidatively modified and damaged biomolecules [5–11]. Their in vivo activity is largely dependent on bioavailability, the heterogeneity of natural media, in vivo redox status, and factors related to their basic structure, including structural determinants such

as molecular size, degree of glycosylation, hydroxylation pattern, presence of the C4 carbonyl group, presence of the double bond between C2 and C3 that is conjugated to the 4-oxo group, the C3 hydroxyl group present in flavonols, the degree of conjugation with other polyphenols, and their interactions with surrounding molecules [12–15]. Among the major determinants in vivo, the pH of the medium (which varies from pH 1 in the stomach to pH 5.3 in the small intestine, pH 6.8 in mouth saliva, pH 7.4 in blood and tissue fluid, pH 8 in the large intestine, pH 7–8.7 in the pancreas, to pH 8.3–9.3 in the duodenum) [16] influences the basic flavonoid structure [17, 18] as well as the oxidation site and the mechanism through which the oxidation of flavonoids takes place.

Although the mechanism of flavonoid antioxidant activity has been extensively studied over several decades, there are still different perspectives on the molecular mechanisms of radical scavenging by flavonoids and the structural dependency of their antioxidant action. It is known that phenolic antioxidants generally act as very good hydrogen and electron donors. Also, their radical intermediates are relatively stable due to resonance delocalization and a lack of suitable sites for attack by molecular oxygen [19, 20].

There are at least three fundamental and widespread reaction pathways through which flavonoids (ArOH) and other phenolic compounds act as antioxidants [21]. Besides the possibility of direct free radical scavenging through the rapid donation of the hydrogen atom to a radical (the so-called HAT mechanism; see Eq. 1) and the chain-breaking mechanism that leads to indirect H abstraction [the electron transfer–proton transfer (ET-PT) mechanism; see Eq. 2], flavonoids can also undergo a sequential proton loss–electron transfer (SPLET) mechanism, which takes place once the anion (ArO$^-$) has formed. All three of these mechanisms may take place in parallel, but at different rates.

$$R^\bullet + ArOH \rightarrow ArO^\bullet + RH \tag{1}$$

$$R^\bullet + ArOH \rightarrow R^- + ArOH^{+\bullet} \rightarrow RH + ArO^\bullet \tag{2}$$

$$\begin{aligned} ArOH &\rightarrow ArO^- + H^+ \\ ArO^- + R^\bullet &\rightarrow ArO^\bullet + R^- \\ R^- + H^+ &\rightarrow RH \end{aligned} \tag{3}$$

The reaction implicated in the HAT mechanism is governed by the bond dissociation enthalpies (BDEs) of ArOH and RH, as the BDE is the molecular property that is used to assess the possible radical scavenging potential of the molecule. To a first approximation, if the BDE of the parent molecule is smaller than the BDE of the protonated

radical form, the reaction is allowed. The first step in the ET-PT mechanism (Eq. 2) is an electron-transfer reaction, where the corresponding controlling parameter is the ionization potentials (IPs) of the ArOH and R$^-$ species. A prerequisite for this reaction would be that the IP of the parent molecule is lower than the IP of the radical ion, R$^-$. Finally, mechanism 3 is mainly governed by the O–H heterolytic bond dissociation enthalpy (the proton dissociation enthalpy, PDE), although the IP of the ArO$^-$ species is another influential parameter.

This paper considers a possible explanation for the potential antioxidant activity of each reactive site of baicalein, and investigates the correlation between the structure and the scavenging activity of baicalein in a molecular manner. Baicalein and its glycozidized form baicalin are two of the major bioactive flavone compounds found in a traditional Chinese medicinal herb, the Baikal skullcap (*Scutellaria baicalensis* Georgi). Baicalein has been the subject of a number of theoretical [22] and experimental studies that have produced promising results in different areas, such as the inhibition of iron-induced lipid peroxidation and anticancer, antiinflammatory and antioxidant activities [23–25]. Medical interest in it arises from its numerous beneficial effects; among the most striking of these are the treatment of disease-related symptoms such as insomnia, fever, and copious perspiration. It is considered a relatively potent antioxidant, even though the A ring is not considered a good electron donor.

Computational details

The conformations of different forms of baicalein (neutral, radical, radical cation, and anion) were fully optimized with a new local density functional method (M05-2X) that was recently developed by the Truhlar group [26, 27] using the 6-311+G(d,p) basis set, as implemented in the Gaussian 09 package [28]. This new hybrid meta exchange correlation functional is parameterized so that it includes both nonmetallic and metallic compounds. This functional also yields satisfactory overall performance for main-group thermochemistry and thermochemical kinetics, as well as for organic, organometallic, biological and noncovalent interactions [26, 27, 29, 30]. To calculate the thermodynamic properties in the solvent environment (water), a conductor-like polarizable continuum solvation model (CPCM) [31–35]—as implemented in Gaussian 09—was used in combination with DFT calculations at the M05-2X/6-311+G(d,p) level.

The vibrational frequencies were obtained by diagonalizing the corresponding M05-2X Hessian matrices. The nature of the stationary points was determined by analyzing the number of imaginary frequencies: 0 for a minimum and

1 for a transition state. Relative energies were calculated at 298 K.

Potential energy surfaces were obtained in relation to the torsion angle τ between the rings B and C, as defined by C3–C2–C1′–C2′ (Fig. 1). The torsion angle τ was scanned in steps of 10° without constraining any other geometric parameters. The effects of the following torsion angles rotations were also studied: C4–C5–OH, C5–C6–OH, and C6–C7–OH. Afterwards, the structures were further optimized without any constraint around each potential minimum. Particular attention was devoted to the DFT interpretation of the reactivities of the OH groups in baicalein and the radicals and cations formed after removing H from the molecule.

The bond dissociation enthalpy (BDE) for baicalein was calculated using the following equation:

$$BDE = H_{BOH} - H_{BO\bullet} - H_{H\bullet} \qquad (4)$$

where H_{BOH}, $H_{BO\bullet}$, and $H_{H\bullet}$ represent the enthalpies of baicalein, the baicalein radical, and the hydrogen atom, respectively. The ionization potential (IP) was obtained as the energy difference between the BOH and BOH$^{+\bullet}$ species.

The proton dissociation enthalpy (PDE) is defined as $PDE = H_{BO^-} + H_{H^+} - H_{BOH}$, where H_{BO^-} is the enthalpy of the molecule derived from proton dissociation and H_{H^+} is the enthalpy of the proton (−0.00236 au).

IPs and O–H BDEs were obtained by carrying out unrestricted complete UM05-2X/6-311+G(d,p) optimizations for the radical species formed after the ionization or O–H bond cleavage experienced by the most stable conformer of each of the baicalein forms. In the gas phase, IP, PDE and BDE values were obtained by including ZPVE and other thermal corrections to the enthalpies (TCEs) of the final and initial states. The M05-2X/6-311+G(d,p) values were corrected by using the recommended scaling factor 0.9631 [36].

As previously proposed [36], gas-phase corrections were employed to calculate these parameters in the aqueous phase, because the calculation of vibrational frequencies

with CPCM is not very accurate and thermal corrections are expected to be rather similar in the gas and solvated phases.

In the conformational analysis performed here, all of the conformations obtained by combining antiperiplanar and synperiplanar arrangements of hydrogen atoms for the dihedral angles $\omega 1$ (C4–C5–O–H), $\omega 2$ (C5–C6–O–H) and $\omega 3$ (C6–C7–O–H) were considered, as well as the important dihedral angle O1–C2–C1′–C2′ (hereafter denoted τ), which defines the coplanarity of the AC and B systems (Fig. 1).

## Results and discussion

Conformational analysis

Since the behavior of the different OH groups in polyphenolic compounds is largely influenced by electronic effects of the neighboring groups and the overall geometry of the molecule, the conformation can be regarded as the first parameter of importance when analyzing the antioxidant capacity of any polyphenolic molecule, and baicalein in this case.

A detailed conformational analysis of baicalein in gaseous and aqueous phases (applying the CPCM model) was performed, and the most important results are presented in the sections that follow.

Neutral form

According to the general rules indicated in the "Computational details" section, four different initial conformations (Fig. 1) were optimized in the gas phase. Analysis of the geometries of baicalein rotamers showed that all of them were nonplanar, implying that the dihedral angle between the bicycle AC and the ring B was nonzero. Rotamer 1 in Fig. 2 (see also Table 1) was found to be the most stable conformer of baicalein.

Since the high scavenging activity of the molecule was (due to the increased conjugation) correlated with the planarity (as measured by the torsion angle) of ring B compared with the rest of the molecule (AC bicycle), the conformational space of all the structures in Fig. 2 was analyzed as a function of the torsional angle τ. By removing any constraint on the torsional angle, the conformational absolute minimum for structure 1 was found to occur at τ=24.48°. The maximum of the potential energy lies at τ=90°, and the interconversion barrier between the two minima is about 3.88 kcal mol^{-1} (Fig. 3). In order to verify the values obtained for the energy barrier, a single point energy calculation using a larger basis set, 6-311++G(3df,2pd), was performed. The value found in the gas phase was slightly higher:
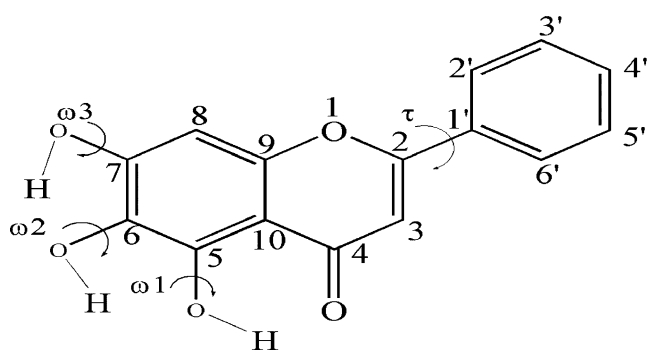
Fig. 1 Atomic numbering for baicalein, and its main dihedral angles ($\omega 1$–$\omega 3$ and τ)
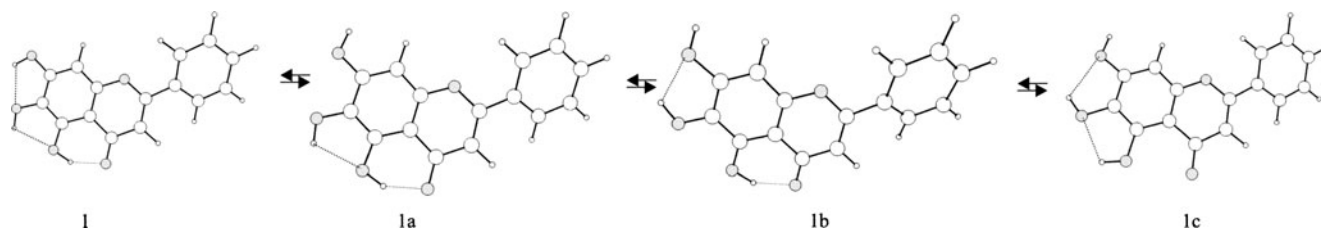
Fig. 2 The most stable conformation of baicalein molecule is conformer *1*. *1a*, *1b*, and *1c* are less stable rotamers

4.17 kcal mol^{-1} (Fig. 1). It is worth mentioning that in going from $\tau=0°$ to $\pm40°$, the potential energy curve is very flat, with an energy variation of about 0.34 kcal mol^{-1}, which indicates that the planar conformation is easily obtained and requires a negligible amount of energy. Similar degrees of nonplanarity between the rings B and C (from 24.28° to 25.08°) were found for all of the other baicalein rotamers, which indicates that they cannot use their full delocalization potentials and are less active as scavengers. Nonplanarity can also be caused by the lack of a 3-OH group, which causes slight twisting of the ring [37].

In the most stable structure, labeled 1 in Fig. 2, there are three internal hydrogen bonds (IHB) that all produce a stabilizing effect, especially the bond between the 5-OH and the C4–O carbonyl group. Conformations that lack these bonds (the other rotamers: 1a, 1b and 1c in Fig. 2) are less stable with respect to the absolute minimum by 5.34, 7.02 and 15.61 kcal mol^{-1}, respectively. On the basis of these values, it is clear that the hydrogen bond between 5-OH and C4–O has the strongest stabilizing effect, which agrees with the fact that this hydrogen bond is shorter than other two (see Table 1 of the "Electronic supplementary material"). The NBO analysis of baicalein revealed that lone pair–antibonding orbital interactions between the carbonyl oxygen and the adjacent OH bond are responsible for hydrogen bond formation. Since the O4(2p) and O4(1p) orbitals have abundant electrons while the O5–H(σ*) orbital is electron deficient, it was found that charge transfer from O4(2p) to the O5–H(σ*) orbital is a favorable donor–acceptor interaction. This is one of the most important influences on the strength of the hydrogen bond delocalization energy. Also, there is significantly weaker charge transfer from O4(1p) to O5–H(σ*). Both charge transfers contribute to the strength of this IHB. Almost

negligible charge transfer is observed for O6(1p) and O7–H (σ*), and for O5(1p) and O6–H(σ*). This finding is in accord with the IHB lengths (see Table 1 in the "Electronic supplementary material").

The four stationary points found in the gas phase were also optimized by simulating aqueous solvation with the CPCM method. The lowest energy in CPCM was found for rotamer 1. The energies of the three rotamers 1, 1a, and 1b are very close and vary within an interval of about 2 kcal mol^{-1} (Table 1). The relative energies are shrunk by CPCM with regard to the gas phase, which is the usual consequence of the interactions of every OH group with the solvent. Since structure 1 is the most stable conformation of baicalein, all further discussion will focus on this rotamer.

Optimization in water solution leaves the ring structures practically unaltered, as seen from the values reported in Table 1 of the "Electronic supplementary material." The water does not favor internal rotation about the C2–C1′ bond, because the energy barriers obtained in the gas and aqueous phases are nearly identical (3.88 kcal mol^{-1}). The main reason for this is that there are no OH groups in the ring B, so there is no significant interaction of ring B with water as a solvent, as in the case of quercetin [38]. This means that there are no differences between the hydration energy values of minima with respect to the transition state. A single point energy calculation with a larger basis set 6-311++G(3df,2pd) gives a somewhat higher value for the energy barrier of 4.38 kcal mol^{-1} in water (Fig. 1 of the Electronic supplementary material). The dihedral angle τ

Table 1 M05-2x/6-311+G(d,p) relative energies (kcal mol^{-1}) for isolated and solvated baicalein rotamers

Rotamer	ΔE	ΔH	ΔE^{CPCM}	$E^{CPCM} - E$	ΔG_{solv}
1	0.0	0.0	0.0	8.22	−8.43
1a	5.29	5.34	1.98	14.81	−11.01
1b	6.91	7.02	2.25	13.08	−10.55
1c	15.26	15.61	8.81	11.65	−13.97
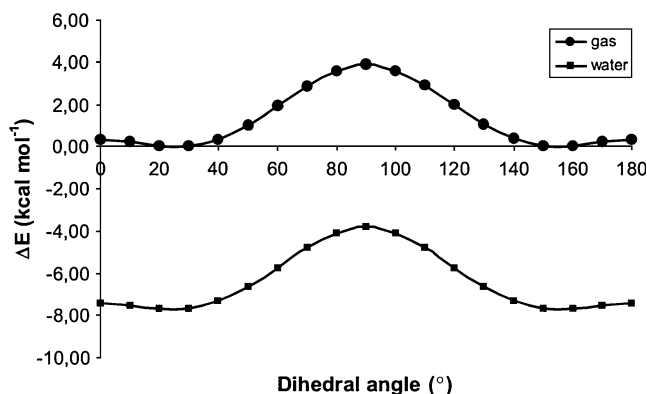
Fig. 3 Energy profile for rotation around the C2–C1′ bond of baicalein in the gaseous and aqueous phases

assumes a value of 23.6° for the absolute minimum, suggesting a slight deviation from planarity, which is in agreement with the findings for similar compounds [37–39]. Three hydrogen bonds present at the gas-phase minimum, O5–H–O4 (1.725 Å), O6–H–O5 (2.238 Å) and O7–H–O6 (2.151 Å), are also retained in solution. The second of these bonds lengthens by 0.031 Å, while the other two are almost the same length as in the gas phase (see Table 1 in the "Electronic supplementary material").

Homolytic and heterolytic O–H breaking

The energies of homolytic and heterolytic O–H bond cleavage may point to the predominant antioxidative mechanism of baicalein. Those O–H bond-breaking energies are given as BDE and PDE values in Tables 2 and 3.

Homolytic O–H bond breaking in baicalein can give rise to three different radical forms: O5R, O6R and O7R (named after the O–H bond that is broken). Each radical form can adopt several conformations (3, 4, and 3, respectively). In the case of O5R, these rotamers are named O5R1, O5R2 and O5R3, respectively. A similar notation is used for the other two radicals. All possible radical forms and their corresponding rotamers are shown in Fig. 4. The order of stability for the radical species obtained from the most stable rotamer of baicalein in the gas phase is O6R> O7R>O5R, implying that homolytic cleavage of the 6-OH bond is favored. The most stable radical, O6R1, is stabilized by two IHBs (O5–H–O4 and O7–H–O6). For the O7R and O5R radical forms, the most stable rotamers are O7R2 and O5R1, which are 7.3 and 13.6 kcal mol^{-1} higher than the O6R1 form (Table 2). The sequence obtained is consistent with the literature data on structure–activity relationships of antioxidant flavonoids [40–42].

The order of stability for the radical forms computed in the aqueous phase is basically the same as that in the gas phase. Again, the O6R1 tautomer is the most stable. Moreover, all of the O6R rotamers are more stable than the other radical rotamers (Table 2). It should be pointed out that this radical retains a nonplanar structure in the gas phase and in water. The nonplanarity could imply decreased conjugation and consequently decreased antioxidant activity (TEAC value 2.56±0.03 mM) in comparison with quercetin (TEAC value 4.42±0.08 mM) [43]. The values of the corresponding dihedral angles are 24.07° and 22.82° in gas and water. Based on PES analysis, it is clear that water does not favor internal rotation about the C2–C1′ bond, because the energy barriers obtained in the gas phase and in water are almost identical: 3.93 and 4.00 kcal mol^{-1}, respectively Fig. 2 of the Electronic supplementary material.

Complete delocalization in the most stable O6R1 radical rotamer in both the gaseous and aqueous phases involves only ring A, while ring C is characterized by two double bonds that are strongly localized on the carbonyl group and the C2–C3 bond. In this form, partly localized double bonds are located on ring A, on the C–O group at C6, and between the C7 and C8 atoms. The bond lengths of these bonds are significantly different from those of the parent molecule, 1 (see Fig. 2 and Table 1 of the "Electronic supplementary material"). The same bond length of 1.470 Å for the C2–C1′ bond in the O6R1 rotamer was found in both the gas and the water phases. The length of this bond lies between the bond lengths characteristic of aromatic and single C–C bonds, indicating weak electron delocalization between rings B and C. This assumption is supported by the NBO analysis. A slightly greater p-orbital contribution on C1′ in the C2–C1′ bond ($sp^{1.79}$–$sp^{2.28}$) could be the main reason for this bond being slightly longer than a double bond.

Table 2 M05-2x/6-311+G(d,p) relative energies (kcal mol^{-1}) for rotamers of baicalein radicals in the gaseous and aqueous phases

Rotamer	ΔE	ΔH	ΔE^{CPCM}	$E^{CPCM} - E$	ΔG_{solv}	BDE	BDE^{CPCM}
O5R1	13.56	13.90	10.14	14.05	9.60	93.53	87.70
O5R3	23.78	24.31	14.22	20.19	13.54	103.94	91.82
O5R2	17.48	17.26	11.19	16.92	10.75	96.89	88.67
O6R1	0.00	0.00	0.00	10.62	0.00	79.63	77.23
O6R2	13.71	13.93	8.49	15.85	8.06	93.56	85.91
O6R3	6.20	6.35	5.36	11.47	4.91	85.98	82.79
O6R4	9.65	9.77	4.06	16.21	4.03	89.40	81.34
O7R1	11.22	11.35	10.80	11.04	10.66	90.98	88.17
O7R2	7.29	7.31	8.16	9.75	8.10	86.94	85.44
O7R3	17.59	17.91	16.19	12.02	15.64	97.54	93.72

ΔE denotes the difference in energy with respect to the most stable conformer, corrected with ZPVE (including the correction factor) [36]. The electronic energies for the most stable radicals are E(O6R1)=−953.28261552 a.u. in the gas phase and E(O6R1) = −953.298919744 a.u. in water. ΔH is the difference in enthalpy with respect to the most stable conformer, with thermal correction for enthalpy accounted for. ΔG_{solv} denotes the difference in free energy relative to the most stable conformer, with thermal correction to the Gibbs free energy (including the appropriate correction factor) accounted for [36]

Table 3 M05-2x/6-311+G(d,p) relative energies (kcal mol^{-1}) for isolated and solvated baicalein anion rotamers

Rotamer	ΔE	ΔH	ΔE^{CPCM}	$E^{CPCM} - E$	ΔG_{solv}	PDE	PDE^{CPCM}
O5A1	11.93	12.19	8.36	57.25	-57.03	339.33	282.07
O5A3	17.95	18.39	10.51	61.12	-60.66	345.54	284.18
O5A2	27.48	28.03	14.98	66.18	-65.90	355.18	288.88
O6A1	8.06	8.05	5.01	56.73	-56.56	335.20	278.39
O6A2	21.66	21.96	13.37	61.97	-61.84	349.11	287.01
O6A3	9.41	9.48	8.15	54.94	-54.90	336.62	281.72
O6A4	24.33	24.57	12.50	65.51	-65.21	351.72	285.99
O7A1	5.58	5.74	3.44	55.81	-55.73	332.88	277.03
O7A2	0.00	0.00	0.00	53.68	-53.55	327.15	273.43
O7A3	8.21	8.42	7.05	54.83	-54.81	335.56	280.78

ΔE denotes the difference in energy with respect to the most stable conformer, corrected with ZPVE (including the correction factor) [36]. The electronic energy for structure 1 is $E(1)=-953.921756$ a.u. in the gas phase and $E(1) = -953.934016$ a.u. in the aqueous phase. ΔH is the difference in enthalpy with respect to the most stable conformer, with thermal correction for enthalpy accounted for. ΔG_{solv} denotes the difference in free energy relative to the most stable conformer, with thermal correction to the Gibbs free energy (including the appropriate correction factor) accounted for [36]

The importance of spin density in determining the potential for delocalization and consequently the stability of flavonoids has already been pointed out by Leopoldini et al. [38, 44] and Trouillas et al. [45]. The spin distribution obtained by NBO analysis of the most stable O6R1 rotamer indicates that the oxygen atom bonded to C6 is the most probable radical center (spin density 0.37 in gas and 0.34 in water). Moreover, the rest of the spin density is delocalized over the C5, O5, C7, and C9 atoms of the A ring Fig. 3 of the Electronic supplementary material these have spin densities of 0.24/*0.25*, 0.10/*0.10*, 0.14/*0.13*, and 0.19/*0.19*, respectively (numbers in italics are the values in water). In
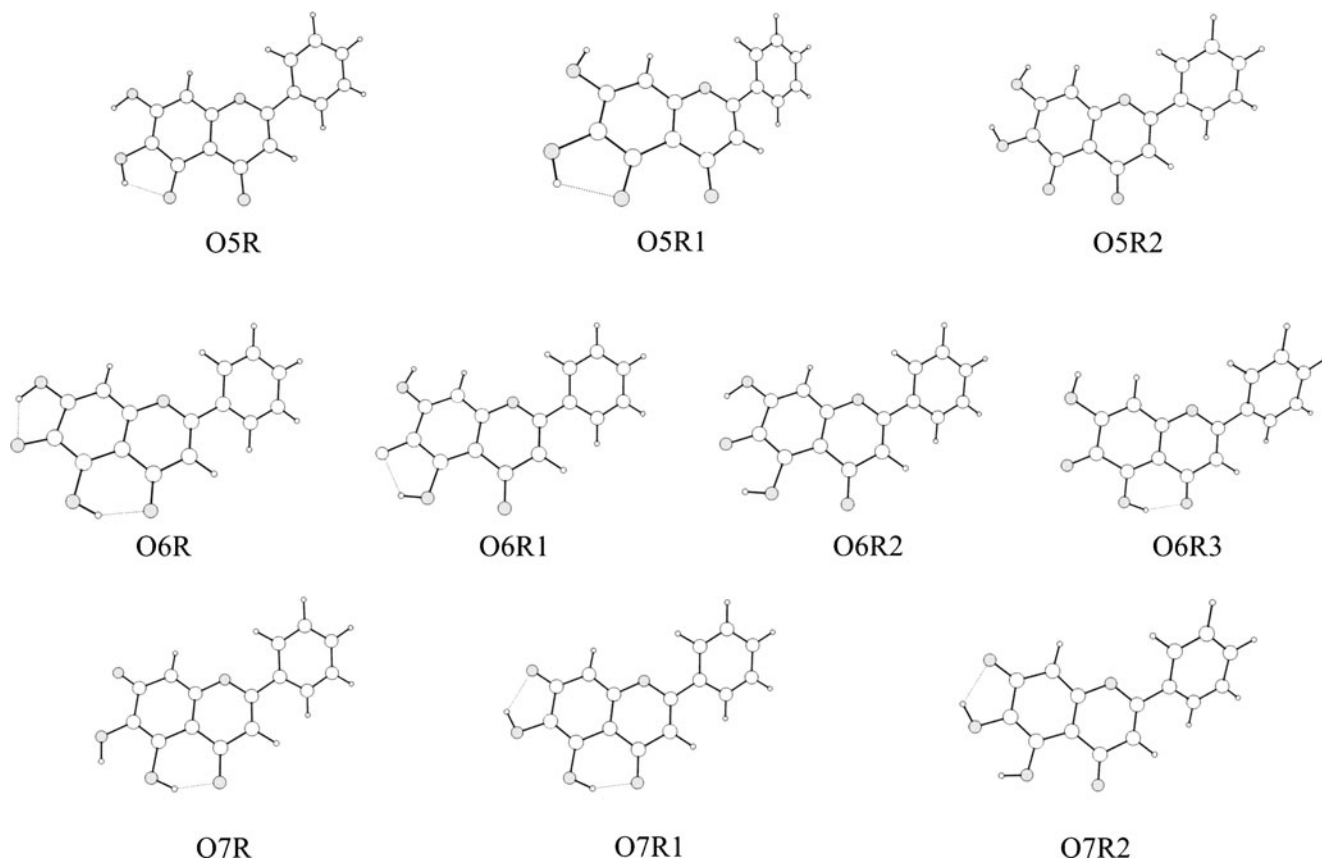
Fig. 4 The structures of the baicalein radicals O5R, O6R, and O7R. Each radical is presented with all of its rotamers

2581

gas and water, the most stable radical, O6R1, has the highest spin density on the radical oxygen, in contrast to the other two less stable rotamers Fig. 3 of the Electronic supplementary material. On the basis of the BDE values (Table 2), it is clear that the formation of O5 and O7 radicals is very unlikely in both the gaseous and the aqueous phases. For this reason, they are excluded from the discussion.

Heterolytic cleavage of the O–H bonds leads to the formation of three anions, O5A, O6A, and O7A, each of which is able to adopt several conformations. The ten anionic rotamers are denoted in the same way as the radical rotamers (Fig. 4), except that R (radical) is replaced by A (anion).

The stability sequence for the anion species differs slightly from that calculated for the species formed upon the homolytic breaking of an O–H bond. The order of stability for the anionic species in the gas phase is O7>O6 >O5, implying that the heterolytic cleavage of the 7-OH bond is favored. The most stable anion, O7A2, is stabilized by two IHBs (O5–H–O4 and O6–H–O7). The other two relatively stable anions, O6A1 and O5A1, are 5.0 and 8.4 kcal mol^{-1} higher than the O7A2 form (Table 3).

In spite of the fact that H-bonding interactions become less important in water, where ion–dipole interactions are predominant, the stability sequence for the anionic species obtained from structure 1 in water is the same as that seen in the gas phase. It is important to note that the values of electron energy are decreased by CPCM by about 55–66 kcal mol^{-1} compared to the gas phase (Table 3)—the usual consequence of the interactions of the anion and all OH groups of baicalein with water.

Complete delocalization in the most stable anion form, O7A2, involves only ring B; ring C is characterized by two strongly localized double bonds on the carbonyl group and the C2–C3 bond. The O7A2 anion form has strong localized double bonds in ring A between C7 and O, C5 and C6, and C8 and C9. These bonds are significantly shorter than those in the parent molecule, 1 (Fig. 2 and Table 1 of the "Electronic supplementary material"). It is clear that there is weak electron delocalization over ring B as a result of the nonplanarity of the anion structure, which is also the case with the radical species formed. As can be seen from Scheme 1, the O7A2 anion form is additionally stabilized by the delocalization of the negative charge that spreads from the site of deprotonation to the 4-keto group in ring C. The natural charge (NC) distribution obtained by the NBO analysis indicates that the oxygen atom bonded to C7 is the most probable center of negative charge (NC density is −0.78 in the gas phase and −0.87 in water). The increased negative charge on the O1, C8, C10, and O atoms bonded to C4 (Fig. 2 of the "Electronic supplementary material"), compared to the neutral form, could be a consequence of the possible presence of a resonance effect in the structures of these anion forms (Scheme 1).

Because the 5-OH group in baicalein is less acidic, the generation of the O5A form leads to the disappearance of very strong H-bonding, and introduces an electronic repulsion between the negative charge of the deprotonated oxygen and the lone pairs on the carbonyl oxygen of ring C.

Ionization potential, bond dissociation enthalpy and proton dissociation enthalpy

The IP values for the most stable structures of baicalein in the gaseous and aqueous phases were calculated as the difference between the electronic energy of the corresponding ionized form of the molecule, B$^{\cdot+}$, and that of the parent species, B, corrected with ZPVE (Table 4). The IP values of baicalein are somewhat higher (173.1 kcal mol^{-1} in gas and 137.1 kcal mol^{-1} in water) than those of widely used synthetic food additives such as butylated hydroxyanisole, propyl gallate and dihydroguaia-retic acid (152.7, 167.8 and 160.6 kcal mol^{-1}, respectively) [46], or the naturally occurring polyphenolic flavonoid epigallocatechin-3-gallate (147.7 kcal mol^{-1}), which is considered one of the most active antioxidants obtained from green tea [47, 48]. Because of the stabilization of charged systems in polar solvents, the IP value obtained from CPCM calculations is significantly different from that calculated in the gas phase.

The BDE values (calculated for the gas phase, with TCE corrections, as the change in enthalpy for reaction 1 at 298 K) of each of the OH groups present in all rotamers of the radical forms of baicalein are similar to, or smaller than, those of hydroxybenzoic acid (61.3–89.6 kcal mol^{-1}), cinnamic acid (83.7–105.6 kcal mol^{-1}) [48, 49] or phenol (values varying from 82.7 to 87.6 kcal mol^{-1}, depending upon the method employed) [50–52]. The lowest BDE value in both gas and water is obtained for the 6-OH group (Table 2). Except in the case of rotamer O7R2, the BDE values calculated in the gas phase are systematically larger, by a few kcal mol^{-1}, than those computed with CPCM.

The BDE values (Table 2) for all baicalein radical forms give the following BDE sequence for the present OH groups: O5>O7>O6. This sequence clearly shows that H transfer from the C6–OH group is the easiest. This sequence is also consistent with the literature data concerning structure–activity relationships of antioxidant flavonoids [40, 41]. The order of BDE values calculated for the aqueous phase with CPCM is the same as that for the gas phase. As a consequence of the interactions of the radical forms and all OH groups with the solvent molecules, those BDE values are decreased by about 2 kcal mol^{-1} compared to those for the gas phase. The somewhat changed order of stability of the radical rotamers under investigation can be related to the different influences of CPCM on the radical species involved in homolytic breaking.

Scheme 1 Resonance structures of O7R anion species
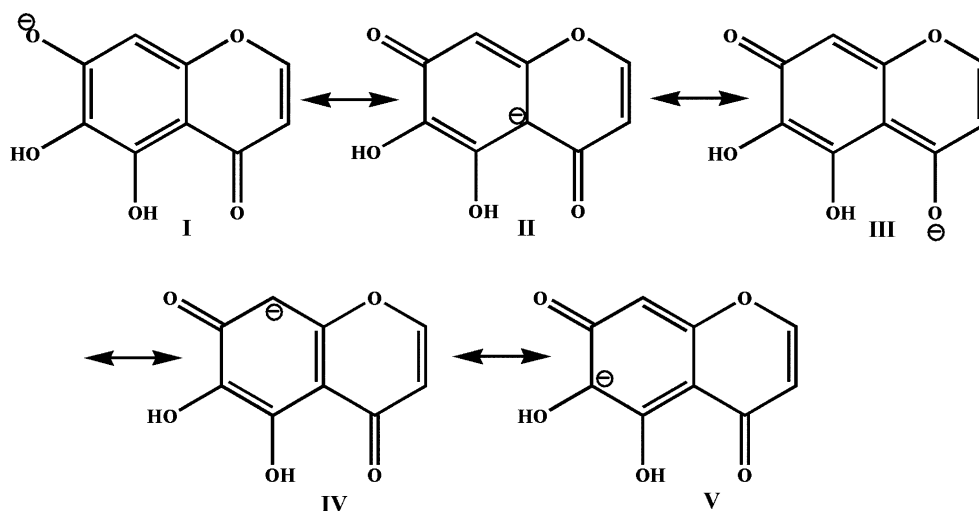
The PDE values were calculated for all of the OH groups on the rotamers of baicalein anions in both phases as the enthalpy difference at 298 K for reaction 3. TCE corrections were included in the calculations for both phases. Calculated PDE values in the gas phase are systematically larger, by several tens of kcal mol^{-1}, than those computed for water.

The calculated PDE values of all OH groups in all anion rotamers (Table 3) yield the sequence O5>O6>O7, indicating that H transfer from the C7–OH group is easier than that from the other two OH groups. Although they are several kcal mol^{-1} smaller than those in the gas phase, PDE values calculated for the aqueous phase follow the same sequence as those calculated for the gas phase. This is a consequence of the interactions of every OH group and O anion with the solvent molecules.

Antioxidant mechanisms

According to Wright et al. [53], the main mechanism governing the antioxidant activity of a certain phenolic compound can be inferred from its ΔIP and ΔBDE values, which are calculated as the difference between the IP and BDE values of the phenolic compound (baicalein in this case) and phenol. For ΔIP≥-36 kcal mol^{-1} and for a ΔBDE value of around −10 kcal mol^{-1}, HAT is considered the dominant mechanism, whereas the predominant mechanism is SET for ΔIP≤-45 kcal mol^{-1}.

The IP and O–H BDE values computed for phenol at the M052X/6-311+G(d,p) level were 141.21 and 85.59 kcal mol^{-1}

(for the gas phase) and 146.11 and 84.71 kcal mol^{-1} (for the aqueous phase with the CPCM model), respectively.

The results for the gas phase only simulate the behavior in nonpolar solvents, where uncharged forms are preferred. The ΔIP value of about −32 kcal mol^{-1} and the ΔBDE value of about −6 kcal mol^{-1} point to HAT as the most favored antioxidant mechanism for baicalein. The SPLET mechanism (Eq. 3), which has been quoted by several authors [20, 52–56], could not be discarded as a possible mechanism for baicalein in water due to the IP values and relatively low O–H PDE values (lower than those of several polyphenols) found for all three species [38, 57].

According to Wright's conditions [52], the HAT mechanism seems to be the only practical one, or at least the preferred one, in the gas phase. However, it should be kept in mind that the relative importance of the HAT, ET-PT and SPLET mechanisms is determined not only by micro-environmental features (lipid phase, aqueous phase) but also the characteristics of the scavenged radical species [54]. It is important to note that, besides structural properties, it is necessary to consider how the electron affinity and H-atom affinity of the radical species that potentially reacts with baicalein affect these three mechanisms. It is also very important to analyze how the electron densities of both compounds change along the reaction coordinates [39, 57].

Besides the possibility of direct free radical scavenging through the mechanism mentioned above, flavonoids can also chelate potentially toxic transition metal ions [Fe(II),

Table 4 ΔBDE and ΔPDE values for all baicalein OH groups (with regard to phenol)[a]

	ΔBDE 5	ΔBDE 6	ΔBDE 7	ΔPDE 5	ΔPDE 6	ΔPDE 7	ΔIP
B^{gas}	7.94	-5.96	1.35	−6.27	−10.41	−18.46	−31.85
B^{CPCM}	4.82	-5.65	2.57	−9.11	−12.79	−17.74	8.98

[a] All theoretical values are given in relation to the values for phenol (calculated with the same method)

Fe(III), Cu(II)], thus preventing metal-catalyzed free radical generating reactions [58–62]. In the metal-assisted decomposition of hydroperoxides, which are the inevitable products of aerobic metabolism, poorly liganded iron species can lead to the catalytic production of the hydroxyl radical [Fe(II)+H$_2$O$_2$→Fe(III)+OH$^-$+OH$^\bullet$], which is very short-lived and consequently highly reactive [63–65]. In this "metal chelation" mechanism, the loss of a proton by the polyphenol flavonoid molecule is crucial to its antioxidant activity, because the cation's chelation often involves at least one deprotonated ligand [66]. Therefore, the acidity of these compounds is an important parameter to take into account, as lowering the acidity of the OH group makes deprotonation and metal chelation easier. Thus, the OH proton dissociation enthalpy (Table 3) was calculated. On the basis of the values obtained for PDA and ΔPDA, it is clear that the 7OH group should be the most reactive with metal ions in an acidic medium.

Conclusions

The results obtained using the M052X/6-311+G(d,p) level of theory imply that the baicalein rotamer 1 is the most stable. This form—which is significantly nonplanar in both gaseous and aqueous phases—is additionally stabilized by three IHBs, while the other rotamers are stabilized by two IHBs.

Position C6–OH is the most favored site for homolytic O–H breaking, while the C7–OH site is the most favored for heterolytic O–H breaking in the gaseous and aqueous phases. The spin density of the most stable radical C6 form indicates that the oxygen atom bonded to C6 is the most probable radical center in the gaseous and aqueous phases. The negative charge of the anionic form C7A1 is spread among five areas: the C7–O unit, from which the proton has been removed, and the C4–O, O1, C8, and C10 units. The electron distributions calculated with CPCM differ from those in the gas phase in their increased polarization, which gives rise to increased electron densities on the same units.

The results presented also indicate that the C5–OH site does not figure in the antioxidant mechanism due to its high BDE and PDE values. The main reason for this assumption is that the hydrogen atom of the C5–OH group forms a strong hydrogen bond with the O4 atom in the parent molecule.

It was found that IPs depend significantly on the solvent, because of the stabilization of charged species by polar solvents. Thus, the IPs of baicalein obtained using CPCM calculations were significantly lower (173.1 and 137.1 kcal mol^{-1}).

The gaseous and CPCM-computed values for IPs, BDEs and PDEs, which are compatible with a relatively high antioxidant activity, indicate that one-step HAT is the mechanism that best explains the radical-scavenging activity of baicalein in the gaseous phase, rather than ET-PT or SPLET. On the other hand, the significantly lower IP and PDE values calculated for aqueous solutions suggest that the SPLET mechanism is the most probable under these conditions.

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