

Structural and electronic features of baicalein and its radicals

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Abstract Baicalein (5,6,7-trihydroxy-2-phenyl-4*H*-chromen-4-one) was investigated by using the B3LYP/6-311+G** level of theory in the gas phase. The results for bond order, bond dissociation enthalpy, highest occupied molecular orbital (HOMO), and Mulliken spin density for baicalein are presented. The structure–activity relationship was examined in the light of these results. Particular attention was devoted to the density functional theory (DFT) interpretation of the reactivity of the OH groups in baicalein, and the radicals formed after hydrogen removal from this molecule. A detailed analysis of the obtained results revealed that the 6-OH group is responsible for the significant antioxidant activity of this molecule.

Keywords Baicalein · Antioxidant activity · BDE · HOMO · DFT

Introduction

Reactive oxygen and nitrogen radical species can potentially damage almost all types of biologically important molecules like lipids (causing lipid peroxidation), amino acids, carbohydrates, and nucleic acids (causing mutations). An imbalance between antioxidants and reactive oxygen species results in oxidative stress, which is implicated in many diseases, suggesting that free radicals participate as fundamental components in a large majority of, if not all, human diseases [1–3]. A variety of naturally occurring and synthetic antioxidant molecules have been shown to quench free radicals, reduce lipid peroxidation, and detoxify hydrogen peroxide through non-enzymatic defense mechanisms. The flavonoid family is a vast and major group among the phenolics with more than several thousand known compounds. Numerous investigations provided some circumstantial evidence that flavonoids exhibit a variety of beneficial actions generally related to their pronounced antioxidant activity, which operates at different levels in the oxidative process including scavenging free radicals, scavenging lipid peroxy radicals, or removing oxidatively changed and damaged biomolecules [4–9]. The chemical behavior of flavonoids as antioxidants has therefore become the subject of intense experimental and theoretical research.

There are generally two fundamental and widespread mechanisms through which flavonoids (ArOH) and other phenolic compounds can play a preventive role: (1) HAT (hydrogen atom transfer) mechanism leading to the direct O–H bond breaking by rapid donation of a hydrogen atom to a radical to form a new radical, more stable than the initial one ($\text{ArOH} + \text{HO}\cdot \rightarrow \text{ArO}\cdot + \text{HOH}$) and (2) the chain-breaking mechanism leading to indirect hydrogen abstraction, by which the primary antioxidant transforms

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into a radical cation by donating an electron to the free radical present in the system ($\text{ArOH} + \text{HO}\cdot \rightarrow \text{ArOH}^+ + \text{OH}^- \rightarrow \text{ArO}\cdot + \text{HOH}$). The net result of reactions (1) and (2) is the same and both hydrogen atom transfer and electron transfer occur in parallel at different rates. Besides the hydrogen atom transfer (HAT) mechanism, where the proton and the electron are transferred together, many important biochemical processes proceed via proton-coupled electron transfer (PCET) mechanism, which occurs when a proton and electron are transferred between different sets of molecular orbitals [10–13]. Both HAT and PCET mechanisms depend upon the medium investigated. In general they are governed by the strength of the phenolic O–H bond, i.e., by the O–H bond dissociation enthalpy (BDE), the molecular property used in the assessment of possible radical scavenging potential of the molecule. BDE is calculated as the difference in enthalpy between the molecule (baicalein in this case) and its radicals, implicating its correspondence to the OH bond breaking energy (the weaker the OH bond is, the smaller BDE is and the faster HAT mechanism will be leading to the faster reaction with free radicals). The electron-transfer mechanism (2) is governed by a one-electron transfer process with both the ionization potential and reactivity of the radical cation ArOH^+ playing important roles. Whatever mechanism is involved, the formed radical species $\text{ArO}\cdot$ needs to be relatively stable, so that reactions (1) and (2) could be thermodynamically favorable in the sense that

it is easier to remove a hydrogen atom from ArOH than from HOH . In this way an antioxidant molecule will react slowly with the substrate and faster with the radical form preventing or postponing toxic effects (such is the oxidative stress) of its reaction with the substrate molecules, thereby justifying the term “chain-breaking antioxidant”. The antioxidant molecule and the final product obtained from it should be nontoxic and should not exert a pro-oxidant effect [10, 14].

Baicalein (5,6,7-trihydroxy-2-phenyl-4*H*-chromen-4-one) and its glucuronide baicalin are two of the major bioactive compounds found in the traditional Chinese medicinal herb Baikal skullcap (*Scutellaria baicalensis* Georgi). They are widely used in the treatment of copious and disease-related symptoms such as insomnia, fever, and perspiration. Baicalein was also the subject of numerous studies which gave promising results in different areas, such as inhibition of iron-induced lipid peroxidation, anticancer, anti-inflammatory, and antioxidant activities [15–17]. This paper addresses the potential reactivity of each of the baicalein reactive sites and stability of the corresponding radicals formed after hydrogen abstraction from its various OH groups. The structure–activity relationship is examined in the light of the results obtained by applying B3LYP/6-311+G** level of theory in the gas phase.

Results and discussion

The majority of theoretical investigations of flavonoids concerning BDE are focused only on the B ring, particularly the catechol moiety [18–30]. The present study is focused on ring A because baicalein has OH groups only in this ring (Fig. 1). In order to find the most stable conformation of baicalein and to determine the preferred relative positions between rings B and C the conformational space of structure **1** (Fig. 2) was analyzed as a function of torsional angle τ (C3–C2–C1'–C2') between these rings. By removing constraint for the torsional angle the conformational absolute minima are found at $\tau = 21.00^\circ$ and at

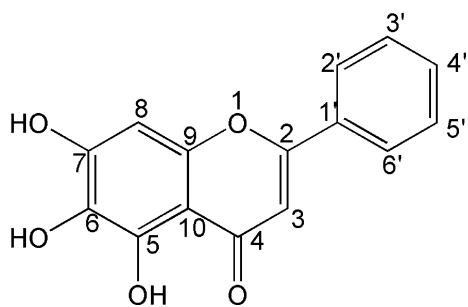
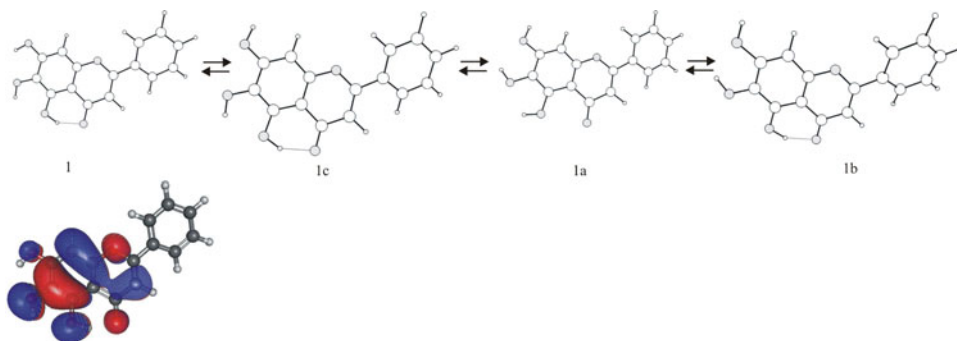


Fig. 1 Atomic numbering of baicalein

Fig. 2 Most stable conformation of the baicalein molecule (**1**). The structures of baicalein in the absence of the C7–OH–C6–O (**1c**), C5O–H–C4O (**1a**), and C6O–H–C5O (**1b**) hydrogen bonds. Calculated HOMO orbital of structure **1**



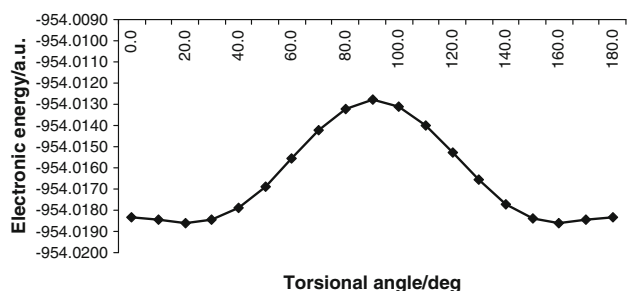


Fig. 3 Energy profile for rotation barrier around the inner bond C2–C1' for structure **1**

$\tau = 158.95^\circ$. The maximum of the potential energy lies at $\tau = 90^\circ$ and the interconversion barrier between the two minima is about 15.1 kJ mol^{-1} (Fig. 3).

The torsional angle values (C3–C2–C1'–C2') of 20° and 159° were found for structurally very similar molecules: quercetin (using AM1) and fisetin (using PM6 methods) [18, 19]. Similarly, the minimization procedure for baicalein finds a nonplanar minimum. It is worth mentioning that in going from $\tau = 0^\circ$ to $\pm 40^\circ$, the potential energy curve is very flat with an energy variation of about 0.59 kJ mol^{-1} ; this indicates that the planar conformation is easily obtained, requiring a negligible amount of energy.

Density functional theory (DFT) calculations imply that baicalein is as polar as quercetin [18] and fisetin [19] with a dipole moment of 3.26 D. Results of the bond order calculations (Table 1) indicate that the double bonds are strongly localized in the C2–C3 and C4–O positions of ring C. The bond order values also suggest a highly independent electronic delocalization only in rings B and A. The small deviation from planarity, as well as the low energy value necessary for interconversion from structure **1** to the planar structure (Fig. 2), do not prevent the electronic delocalization in the molecule of baicalein. The C2–C1' bond is in the chromone plane because the C4–C3–C2–C1' torsional angle is close to 180° . This bond has a length of 1.473 \AA (Table 2) and the bond order is close to 1 (Table 1). The single character of the C2–C1' bond shows that the conjugation of the π system across all the rings is very small. The double bonds around the carbonyl group in ring C indicate a cross-conjugated system [31] in which the delocalization is allowed only between C and A or C and B rings, but not for rings A and B. This finding is indirectly confirmed by the investigation in which biphenyl molecules were found to be not completely planar [31].

There are three hydrogen bonds present in structure **1** (Fig. 2). The formation of the hydrogen bond between the C5–OH and C4–O carbonyl group has a stabilizing effect. This OH group is additionally stabilized by the formation of another hydrogen bond between the oxygen of the C6–OH group and the C5 oxygen. The conformations lacking these

Table 1 Bond order values of baicalein (**1**) and its radical species (C5, C6, and C7)

Wiberg bond order	Baicalein	C5–OH	C6–OH	C7–OH
O1–C2	1.014	0.972	0.991	1.013
C2–C3	1.609	1.658	1.620	1.594
C3–C4	1.139	1.089	1.135	1.147
C4–C10	1.107	1.053	1.103	1.093
C5–C10	1.250	1.111	1.266	1.327
C5–C6	1.358	1.083	1.117	1.281
C6–C7	1.293	1.268	1.082	1.069
C7–C8	1.363	1.421	1.453	1.144
C8–C9	1.394	1.219	1.308	1.554
C9–O1	0.971	1.004	1.003	0.960
C9–C10	1.292	1.442	1.283	1.173
C2–C1'	1.067	1.065	1.073	1.074
C1'–C2'	1.366	1.368	1.363	1.361
C2'–C3'	1.447	1.445	1.448	1.448
C3'–C4'	1.430	1.431	1.430	1.429
C4'–C5'	1.422	1.422	1.421	1.420
C5'–C6'	1.455	1.456	1.457	1.458
C6'–C1'	1.362	1.362	1.358	1.357
O4–C4	1.532	1.690	1.539	1.536
O5–C5	1.071	1.568	1.180	1.077
O6–C6	1.000	1.105	1.516	1.123
O7–C7	1.052	1.042	1.112	1.582
H5–C5	0.637		0.632	0.630
H6–C6	0.729	0.681		0.728
H7–C7	0.737	0.741	0.701	
DH(O4–H5)	0.084		0.003	0.088
DH(O5–H6)	0.006	0.040		0.008
DH(O6–H7)	0.006	0.004	0.033	

DH hydrogen bond

bonds (Fig. 2, **1a–1c**) are less stable with respect to the absolute minimum by 65.57, 26.61, and $19.99 \text{ kJ mol}^{-1}$, respectively. The lack of hydrogen bond between C5–OH and C4–O carbonyl group has the strongest destabilizing effect, which is in agreement with the fact that this hydrogen bond is shorter than other two (Table 2). The natural bond orbital (NBO) analysis of baicalein revealed that lone pair–antibond and bond–antibond interactions are responsible for hydrogen bond formation. Because O4(2p) and O5–H(σ^*) orbitals are the electron-abundant and electron-deficient components, respectively, the charge transfer from O4(2p) to O5–H(σ^*) orbital is a favorable donor–acceptor interaction, and is a relatively dominant term in determining the strength of hydrogen bond delocalization energy. Almost negligible charge transfer is observed for O6(1p) and O7–H(σ^*), whereas for O5(1p) and O6–H(σ^*) and for O5(2p) and O6–H(σ^*) charge transfer is not observed at all. This finding is in accordance with these hydrogen bonds lengths (Table 2).

Table 2 Bond lengths (nm) calculated using B3LYP/6-311+G** for baicalein and the corresponding radicals

	Baicalein	C5–OH	C6–OH	C7–OH
D(O1–C2)	0.1358	0.1370	0.1366	0.1358
D(C2–C3)	0.1359	0.1349	0.1356	0.1360
D(C3–C4)	0.1445	0.1466	0.1445	0.1441
D(C4–C10)	0.1452	0.1478	0.1455	0.1458
D(C5–C10)	0.1412	0.1456	0.1414	0.1396
D(C5–C6)	0.1386	0.1466	0.1450	0.1404
D(C6–C7)	0.1402	0.1400	0.1468	0.1473
D(C7–C8)	0.1394	0.1379	0.1375	0.1450
D(C8–C9)	0.1387	0.1424	0.1402	0.1361
D(C9–O1)	0.1373	0.1360	0.1360	0.1377
D(C9–C10)	0.1403	0.1385	0.1405	0.1430
D(C2–C1')	0.1474	0.1473	0.1472	0.1472
D(C1'–C2')	0.1403	0.1403	0.1403	0.1404
D(C2'–C3')	0.1391	0.1392	0.1391	0.1391
D(C3'–C4')	0.1394	0.1393	0.1393	0.1394
D(C4'–C5')	0.1395	0.1395	0.1395	0.1395
D(C5'–C6')	0.1390	0.1390	0.1390	0.1389
D(C6'–C1')	0.1403	0.1403	0.1404	0.1404
D(C2–C1')	0.1474	0.1473	0.1472	0.1472
D(O4–C4)	0.1249	0.1222	0.1247	0.1248
D(O5–C5)	0.1349	0.1466	0.1318	0.1346
D(O6–C6)	0.1370	0.1333	0.1249	0.1330
D(O7–C7)	0.1354	0.1355	0.1333	0.1240
D(H5–C5)	0.0996		0.1001	0.0997
D(H6–C6)	0.0967	0.0984		0.0971
D(H7–C7)	0.0967	0.0966	0.0980	

The torsional angle values (τ) between rings C and B in structures **1a–1c** are similar to the corresponding value for structure **1** (Fig. 2) implicating the importance of the conjugation stabilization between rings B and C in the structures lacking the hydrogen bonds.

Baicalein radicals

Geometry optimizations of the radicals are performed by starting from the optimized structure of the parent molecule, after hydrogen atom abstraction from the C5, C6, or C7 position. The radical formed by hydrogen removal from the C5–OH group of baicalein is called the C5–OH baicalein radical, and the same notation is used for the other two radicals (Fig. 4). No geometrical parameter constraint is imposed during the optimization. The OH group neighboring the primary radical site in the A ring is oriented in such way that hydrogen bonding is preserved.

The C6–OH radical, like parent molecule **1**, retains nonplanarity with a torsional angle of approximately 20.3° (Table 3). Energy minima for two other isomers generated

by the loss of the hydrogen from the C7–OH and C5–OH groups are higher than that for C6–OH form by 44.37 and 58.79 kJ mol⁻¹, respectively. These two forms are also nonplanar, because torsional angle values between rings B and C are approximately 19.3° (C7–OH) and 20.4° (C5–OH) (Table 3).

Careful inspection of Table 1 allows further comments on the electronic structure of three possible radical isomers of baicalein. The C5–OH, C6–OH, and C7–OH radicals are characterized by two hydrogen bonds (Fig. 4) that contribute to their stabilities. In the C6–OH radical the complete delocalization involves only ring B, whereas ring C is characterized by two double bonds strongly localized on the carbonyl group and C2–C3 bond. The C6–OH radical also has strong localized double bonds in ring A, on the carbonyl group at C6, and between C7 and C8 atoms, which is the significant difference in comparison to the parent molecule **1** (Fig. 2). The same bond order value for the C2–C1' bond in the parent molecule and in the C6–OH radical indicates a weak electron delocalization over ring B.

In the C5–OH radical the complete delocalization involves only ring B, whereas rings A and C are characterized by five double bonds strongly localized on the carbonyl groups C3–O and C5–O, as well as on the C2–C3, C6–C7, and C9–C10 bonds. Moreover, as a result of the nonplanarity of this radical the electronic flow between rings A and C is prevented, a conclusion also supported by the C2–C1' bond order value (Table 1).

Significant geometrical changes in ring A are also present in the structure of the C7–OH radical. In the C7–OH radical, like in the C6–OH radical, the C6–C7 bond becomes significantly longer (Table 2), whereas the bond order values (Table 1) show that the C7–O and C8–C9 double bonds in ring A are almost localized. The bond order between C2 and C1' is close to 1, implying that conjugation between rings C and B is hindered.

BDE values

The DFT calculated BDE values (Table 4) for the three radical forms give the following BDE sequence for the present OH groups: C5–OH > C7–OH > C6–OH. This sequence clearly confirms that hydrogen transfer from C6–OH is easier than that from the other two OH groups. The obtained results are consistent with the literature data concerning structure–activity relationships of antioxidant flavonoids [32].

According to the performed calculations, the BDE values for the C5–OH and C7–OH sites on the A ring are higher than those for the C6–OH form by 44.4 and 58.8 kJ mol⁻¹. Whatever the kind of oxidative system is involved, these results clearly implicate the higher reactivity of the C6–OH group compared with the other two

Fig. 4 Optimized structures of baicalein radicals. Values of corresponding spin densities are presented as *numbers*. Hydrogen bonds are presented as *dashed lines*

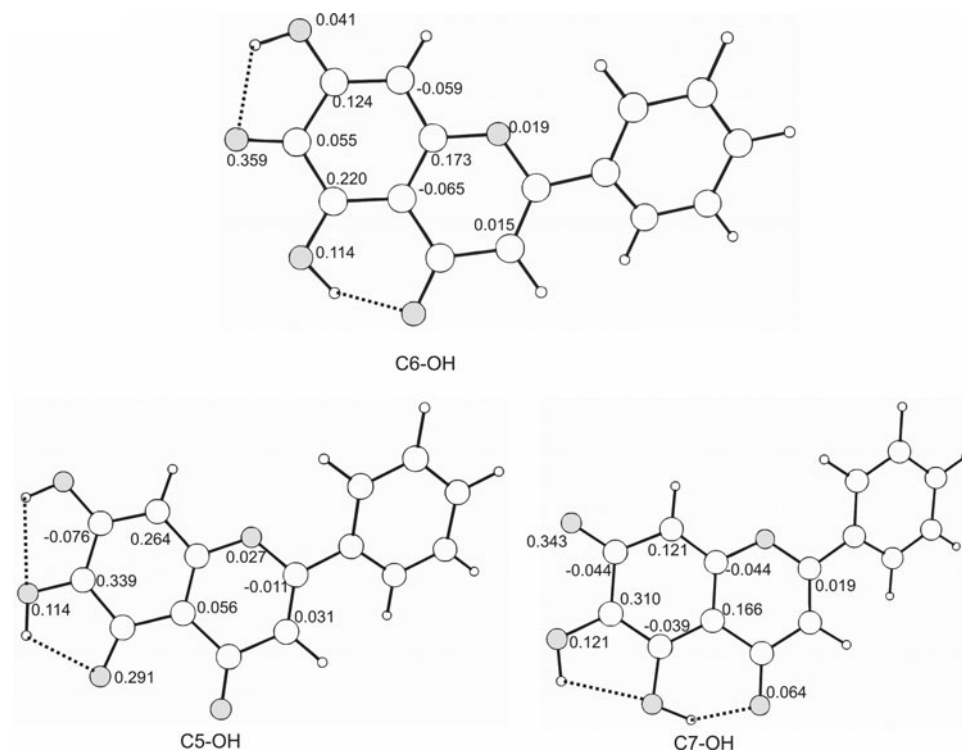


Table 3 Bond angle and dihedral angle values (°) for baicalein and the corresponding radicals

Angle	Baicalein	C5-OH	C6-OH	C7-OH
A(O1-C2-C3)	121.9	120.8	121.4	122.2
A(C2-C3-C4)	121.6	123.1	121.5	121.5
A(C3-C4-C10)	115.0	113.4	115.4	115.4
A(C4-C10-C5)	121.0	123.5	120.8	120.6
A(C10-C5-C6)	119.9	117.1	120.0	120.2
A(C5-C6-C7)	120.2	123.2	116.6	121.1
A(C6-C7-C8)	121.0	118.1	123.0	116.6
A(C7-C8-C9)	118.1	120.0	117.7	120.2
A(C8-C9-C10)	122.4	124.6	123.5	122.9
A(C9-O1-C2)	120.7	120.5	121.3	121.4
A(C1'-C2'-C3')	120.4	120.4	120.4	120.4
A(C2'-C3'-C4')	120.3	120.3	120.3	120.3
A(C3'-C4'-C5')	119.7	119.6	119.7	119.7
A(C4'-C5'-C6')	120.3	120.3	120.2	120.2
A(C5'-C6'-C1')	120.5	120.5	120.4	120.5
A(C6'-C1'-C2')	118.9	118.8	118.9	118.9
A(C6'-C1'-C2)	120.8	120.6	120.6	120.8
A(C3-C4-O4)	123.3	121.8	123.0	123.5
A(C10-C5-O5)	122.1	127.4	121.4	122.9
A(C5-C6-O6)	122.4	116.7	125.0	120.3
A(C6-C7-O7)	119.7	121.5	115.0	120.6
τ (O1-C2-C1'-C2')	20.96	21.36	20.34	19.29

Table 4 Some of the calculated energies of the baicalein molecule (**1**) and its radical isomers (C5-OH, C6-OH, and C7-OH) in their ground state

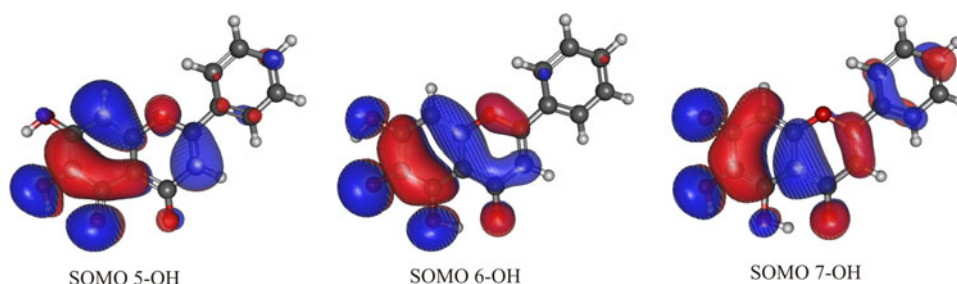
	C5-OH	C6-OH	C7-OH	1
$E + ZPE$ (a.u.)	-953.1631	-953.1849	-953.1683	-953.8061
BDE (kJ mol ⁻¹)	89.89	75.85	86.44	
E_{HOMO} (eV)	-0.2460	-0.2570	-0.2605	-0.228
E_{LUMO} (eV)	-0.080	-0.092	-0.094	-0.085
E_{SOMO} (eV)	-0.197	-0.182	-0.200	
IP (kJ mol ⁻¹)				166.30

ZPE zero point energy, *BDE* bond dissociation enthalpy, E_{HOMO} energy of the highest occupied molecular orbital, E_{LUMO} energy of the lowest unoccupied molecular orbital, E_{SOMO} energy of the singly occupied molecular orbital, *IP* ionization potential

hydroxyl groups of ring A. This hierarchy may only be overcome if the oxidation of the molecules takes place via an enzymatic action for which the binding configuration with the protein receptor governs the location of the redox reactions.

The BDE values for C5-OH and C7-OH groups of baicalein are very similar to the corresponding values obtained for quercetin at the same level of theory [21]. Also, a similar value for BDE is obtained for the C7-OH group of fisetin [33]. On the other hand, the BDE value for the C6-OH group is significantly lower, and can be

Fig. 5 SOMO of radicals formed by hydrogen removal from baicalein



compared with the values of catechol OH groups of quercetin and fisetin [21, 33]. The calculated BDE value for the C6–OH form is in agreement with the experimental TEAC (Trolox equivalent antioxidant activity) value of baicalein [34].

On the basis of the ionization potential value for structure **1** ($696.82 \text{ kJ mol}^{-1}$), which is slightly higher than the corresponding value for quercetin [20], one can expect a lower antioxidant activity for baicalein in comparison to quercetin. This means that baicalein, undergoing whatever mechanism of action, hydrogen atom or electron transfer, would be less effective in terms of antioxidant activity compared with quercetin. The experimental value of TEAC confirms this assumption [32, 34].

The difference in antioxidant activity, which is reflected in the BDE values, is often attributed to the π -electron delocalization leading to the stabilization of the radical forms obtained after hydrogen abstraction. This conclusion is made on the basis of an assumption that if π -electron delocalization exists in the parent molecule it also exists in the corresponding radical forms. The somewhat higher BDE value for the C6–OH group of baicalein, compared with the BDE values of quercetin and fisetin catechol hydroxyl groups [21, 33], could be explained by the lower delocalization of this radical form in comparison to the corresponding radicals of quercetin and fisetin.

The HOMO of baicalein is presented in the lower part of Fig. 2. As expected, the greatest contribution to the HOMO comes from ring A and oxygen atoms O6, O5, and O7. The shape of the HOMO undoubtedly reveals that O6, O5, and O7 atoms are suitable for the formation of stable radical forms (corresponding to hydrogen removal from one of the three OH groups). This assumption is in agreement with the calculated BDE values, especially with the lowest one, obtained for the formation of the C6–OH radical, because the greatest contribution to the HOMO comes from this oxygen atom. The shapes of the SOMO of the radical forms are presented in Fig. 5.

Within an unrestricted scheme, the spin density is often considered to be a more realistic parameter which provides a better representation of the reactivity [35]. The importance of the spin density for the description of flavonoids has been pointed out by recent literature data [20, 21].

Therefore, the spin density on the various baicalein radical forms is also analyzed to help understand the differences in reactivity of the various OH groups, and consequently the differences in BDE values. It should be pointed out that the more delocalized the spin density in the radical form is, the easier the radical is formed, and thus the lower the BDE value is [20, 21].

In the case of the C6–OH radical, the highest spin density is located on the O6 atom while the rest of it is disposed over *ortho* and *para* carbon atoms (Fig. 4). This effect can be explained by using the classical resonance effects occurring in the phenoxy radical. Such a scheme explains the presence of the radical (high spin density) on the C5, C7, and C9 atoms.

In the case of the C5–OH radical the spin density is very high on the C6 atom (0.339) and concomitantly the delocalization is weak (Fig. 4). A somewhat better spin delocalization is found in the case of the C7–OH radical where the spin density is delocalized among the C6, C8, and C10 atoms. Because spin density delocalization is related to the ease of radical formation, it is clear that hydrogen removal from the C5–OH and C7–OH sites is not favored. Figure 4 shows that O7 and O6 atoms have higher spin density values than O5, implying that higher capacity for hydrogen removal from O7 and O6 can be expected.

Conclusions

On the basis of the results presented baicalein appears to be a nonplanar molecule exerting a cross-conjugation effect. The antioxidant action is generated by three radicals showing nonplanar conformations. The very flat potential energy curve, with an energy variation of about 0.59 kJ mol^{-1} , indicates that the planar conformation is easily obtained, requiring a negligible amount of energy. This fact means that radical structures allow extended electronic delocalization between adjacent rings.

The applied theoretical approach confirms the importance of the A ring and sheds light on the role of the C6–OH group, whose reactivity, influencing antioxidant properties of the molecule, is dependent upon the good electron delocalization.

Our results also point to the C5–OH radical, which lies at higher energetic values with the respect to the absolute minimum, indicating its presence in the antioxidant mechanism is rather improbable. The main reason justifying this assumption lies in the fact that the hydrogen atom of the C5–OH group forms a hydrogen bond with the O4 atom.

Methods

All calculations were performed by using the Becke's three-parameter (B3) exchange functional [36] associated with the Lee–Yang–Parr (LYP) gradient corrected correlation functional [37] denoted B3LYP with the standard split valence double zeta Gaussian basis set 6-31G of Peterson et al. [38, 39]. Furthermore, in order to obtain a better description of the delocalization effects that are crucial for the geometry and the electronic structure of baicalein, diffuse functions were added to the heavy atoms. The *p*, *d* polarization functions (***) were also added. Full geometry optimizations were undertaken without any symmetry constraints. A vibrational frequency calculation at the optimized geometry was performed to confirm that the structure obtained was a stationary point (characterized by no negative frequencies) on the potential energy surface. The natural bond orbital (NBO) [40–43] analysis of charges and spin densities of baicalein and corresponding radicals was performed. The Wiberg bond orders [44] were calculated by using the GenNBO program [45]. It should be noted that the Wiberg bond order (sometimes referred to as the Wiberg bond index) is equal to 1 and 2 for pure single and double bonds, respectively. In the case when a bond participates in some π -electron conjugation, its Wiberg bond order will assume a value between 1 and 2; the closer to 2, the stronger the character of a double bond. The bond dissociation enthalpy (BDE) for baicalein was calculated by using the following equation:

$$\text{BDE} = H_{\text{BOH}} - H_{\text{BO}\cdot} - H_{\text{H}\cdot},$$

where H_{BOH} , $H_{\text{BO}\cdot}$, and $H_{\text{H}\cdot}$ are the enthalpies of baicalein, baicalein radical, and hydrogen atom respectively. The ionization potential (IP) was obtained as the energy difference between the BOH and BOH^+ species. The NBO orbital occupancies associated with the distribution of the singly occupied molecular orbitals (SOMOs) were also used to identify the scavenging mechanism in both cases. All calculations were carried out using the Gaussian 09 computational package [46].

Potential energy surfaces were obtained in relation with the torsion angle τ between rings B and C, defined by the C3–C2–C1'–C2' atoms (Fig. 1). The torsion angle τ was scanned in steps of 15° without constraints on all other

geometrical 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 paid to the DFT interpretation of the reactivity of OH groups in baicalein and the radicals formed after hydrogen removal from the molecule.

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