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SCIENCE DIRECT®
Food Chemistry 97 (2006) 679–688
Food Chemistry
www.elsevier.com/locate/foodchem

Rapid communication

A DFT study of the reactivity of OH groups in quercetin and taxifolin antioxidants: The specificity of the 3-OH site

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Received 21 April 2005; accepted 20 May 2005

Abstract

Over the past decade, the chemical behaviour of flavonoids as antioxidants has become the subject of intense experimental research. In this paper, we use a quantum-chemical approach to shed light on the reactivity of two flavonoids, quercetin and taxifolin. We particularly focus on the 3-OH site and the role played by the 2,3-double bond in the reactivity of that site. In order to establish the most efficient theoretical methodology, different methods, either Hartree–Fock-based or derived from density functional theory, and different basis sets (from 6-311G(d) to 6-311++G(2d,p)) were tested on phenol and catechol, for which experimental bond dissociation enthalpy (BDE) values are available. It appears that (U)B3P86/6-311+G(d,p) is the most relevant method for BDE prediction of these phenolic compounds and it has, therefore, been used for an extensive study of the two flavonoids.

The analysis of the theoretical BDE values, for all OH sites of quercetin and taxifolin, clearly shows the importance of the B-ring and the 3-OH group only when the 2,3-double bond is present (i.e. in quercetin). We have also considered the importance of keto–enol tautomerism (present in quercetin but not in taxifolin) to rationalize the difference in reactivity between the two compounds. Our analysis also includes the Mulliken spin density distribution for the radicals formed after H-removal on each OH site of both flavonoids. The results clearly show that the 3-OH quercetin radical possesses a large spin density on the C-2 atom, which explains the C-ring opening process observed in different redox systems, including metabolization.

Keywords: Flavonoid; Antioxidant; DFT; BDE; Spin density

1. Introduction

Over the past decade, a number of epidemiological studies (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Trichopoulou & Vasilopoulou, 2000) have contributed to build the consensus that diets rich in fruits and vegetables have beneficial effects on human health. The subsequent decrease in the risk of certain pathologies, including cardiovascular diseases and cancer, is attributed in part to phenolic compounds contained in such food. Those molecules have demonstrated multiple in vitro and in vivo biological properties including antioxidant activities (Cadenas & Packer, 2002).

Among the natural phenolic antioxidants, the flavonoid family is the most important class. A number of in vitro studies have established the hierarchy of flavonoids in terms of their antioxidant activities (Cos et al., 1998; Rice-Evans, Miller, & Paganga, 1996) and

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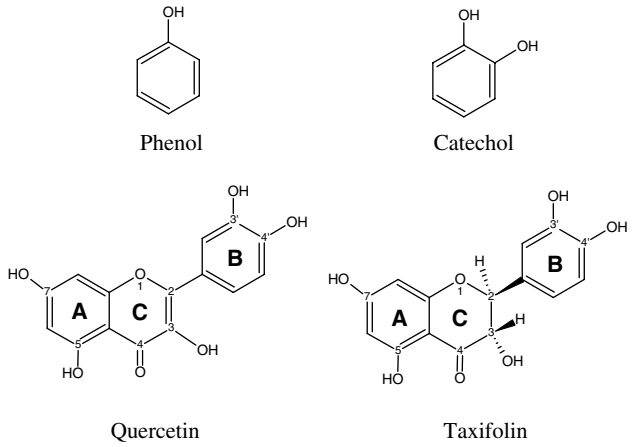
doi:10.1016/j.foodchem.2005.05.042



Scheme 1.

the corresponding structure–activity relationships. It has been clearly proved that the B-ring is the most important site for H-transfer and consequently for the antioxidant capacity. This is particularly true when the B-ring is a catechol moiety, as in the flavonol quercetin (Scheme 1). In contrast, the A-ring seems to be less important. The 2,3-double bond also contributes to the antioxidant activity, as it ensures π-electron delocalization between the B- and C-rings, which contributes to the stabilization of RO$^{\bullet}$, after H-abstraction (Van Acker et al., 1996).

An important issue that is still under debate is the role of the 3-OH group. In vitro studies demonstrated that 3-OH contributes to the antioxidant potential. Indeed, blocking the 3-OH group with a sugar moiety, as in rutin, or removing this group, as in luteolin, significantly decrease the activity (Rice-Evans et al., 1996). However, the effectiveness of this group seems to be dependent on the presence of the 2,3-double bond and the 4-carbonyl group. 3-OH is also thought to be involved in the metabolization by copper-containing quercetin 2,3-dioxygenase issued from human intestinal bacteria. Mechanisms have been proposed for the quercetin metabolism pathway, in which the copper-containing quercetin 2,3-dioxygenase binds to the molecule (Steiner, Kalk, & Dijkstra, 2002). Quercetin is believed to coordinate to the copper atom of the dioxygenase enzyme, via the 3-OH and 4-carbonyl groups. The consequence of this complex-formation is H-removal from the 3-OH group and some authors proposed that C-ring opening takes place after a second redox attack by O_2 on the C-2 atom (Balogh-Hergovich, Kaiser, & Speier, 1997). This leads to the formation of the corresponding depside (phenolic carboxylic acid ester) and releasing of carbon monoxide. Afterwards, small phenol compounds such as phenol acids can be formed and are more easily absorbed. Thus, this group is particularly important; however, to our knowledge, no fundamental study is available to explain its redox capacity.

It is well-established that phenolic compounds (ROH) scavenge free radicals (OH$^{\bullet}$ in the following examples) according to two possible reducing pathways:

(i) H-transfer from the molecule to the radical (direct O–H bond breaking)

$$ROH + OH^{\bullet} \rightarrow RO^{\bullet} + HOH \quad (1)$$

(ii) Electron-transfer from the molecule to the radical, leading to indirect H-abstraction

$$ROH + OH^{\bullet} \rightarrow ROH^{+\bullet} + OH^{-} \rightarrow RO^{\bullet} + HOH \quad (2)$$

The first mechanism is governed by the O–H bond dissociation enthalpy (BDE), while the second one is governed by the ionization potential and by the reactivity of the $ROH^{+\bullet}$ cation. In any case, the formed radical (RO$^{\bullet}$) must be relatively stable, so that: (i) reactions (1) and (2) are thermodynamically favourable (in the sense that it is easier to remove a hydrogen atom from ROH than from HOH) and (ii) it reacts slowly with neighbouring molecules, without toxic effects, such as oxidative stress.

Theoretical calculations of the BDE, using either density functional theory (DFT) or semi-empirical Hartree–Fock methods, have been useful for elucidating the high capacity of the OH groups of phenolic antioxidants to react by H-transfer (Leopoldini, Pitarch, Russo, & Toscano, 2004; Leopoldini, Marino, Russo, & Toscano, 2004; Lemaska et al., 2001; Lucarini, Pedulli, & Guerra, 2004; Priyadarsini et al., 2003; Russo, Toscano, & Uccella, 2000; Trouillas et al., 2004; Wright, Jonhson, & DiLabio, 2001; Zhang, Sun, & Chen, 2001; Zhang, Sun, & Wang, 2003; Zhang, 2004). Nevertheless quantum-chemical studies on flavonoids are far from complete: most theoretical investigations have focussed only on the B-ring (particularly the catechol moiety) and the most reliable methodology still has to be established. In the present study, we decided to focus on the 3-OH group and its surroundings, in order to elucidate the reactivity of this group and the corresponding radical formed after H-abstraction. We compare the properties of quercetin with those of taxifolin, the corresponding dihydroflavonol (Scheme 1), in order to gain insight on the role of the 3-OH group and to understand the interplay between the 2,3-double bond and the 3-OH group. Quercetin is a flavonol predominantly derived from onions, apples, and red wine. It possesses all requirements (*ortho*-OH groups on the B-ring, 2,3-double bond, 3-OH group) to be a reference phenolic antioxidant. Taxifolin is mainly found in *Citrus* fruits, especially grape fruits and oranges (Bohm, 1975). Its antioxidant activity is twice lower than that of quercetin, even though it remains quite interesting (Rice-Evans et al., 1996).

Due to the importance of π-electron delocalization in flavonoids, theoretical investigations require high-level

calculations over the entire molecules, in order to take into account all the possible electronic effects and their influence on the redox reactivity of the 3-OH group. To select the most appropriate theoretical approach, we performed a comprehensive methodological study on two model compounds: phenol and catechol (Scheme 1).

In this paper, we first present the results of BDE calculations for phenol and catechol, obtained by using different methods (ab initio Hartree–Fock (HF) and DFT) and different basis sets. Afterwards, we develop the selected methodology for quercetin and taxifolin, according to the results obtained on phenol and catechol. The results are compared and analyzed in terms of BDE, electron distribution in the singly occupied molecular orbital (SOMO) or the α-highest occupied molecular orbital (α-HOMO), and Mulliken spin density distribution in the radical species. The structure–activity relationship is examined in the light of those results and we pay particular attention to quantum-chemical interpretation of the reactivity of the 3-OH group in quercetin and the radical formed after H-removal from this molecule. Keto–enol tautomerism before H-abstraction is also discussed for explaining the role of this group. Finally, the calculated electronic structures allow as to elucidate the chemical reaction that leads to the C-ring opening of quercetin in various oxidative systems, including metabolization.

2. Theoretical methodology

2.1. General procedure

BDE was calculated as the difference in total enthalpy between the corresponding radical (formed after H-abstraction) and the flavonoid, according to the following reaction:

$$ROH \rightarrow RO^{\bullet} + H^{\bullet}$$

As a starting point, we calculated the energies by using the ab initio (U)HF method. HF theory has long been used for the investigation of open-shell systems with unpaired electrons and the performance and limits of this approach are well known (Szabo & Ostlund, 1982, Chap. 3). In order to obtain a more accurate description of the BDE, one needs to go beyond the HF level, to take into account electron correlation. MP2 allows taking such correction into account.

As an alternative to post-HF methods, DFT has recently been developed and has succeeded in BDE estimation and in the description of radical species; nevertheless, the choice of the functional has been shown to significantly influence the accuracy of the results (DiLabio, Pratt, LoFaro, & Wright, 1999; Feng, Liu, Wang, Huang, & Guo, 2003; Fox & Kollman, 1996; Marsal, Roche, Tordo, & De Sainte Claire, 1999). Following Pople, Gill, and Handy (1995), we have decided to use an unrestricted scheme for open-shell DFT calculations, instead of a restricted open-shell scheme, and we have tested a number of exchange-correlation potential schemes. Previous calculations have shown the interest of combining Hartree–Fock and Becke exchange to provide accurate BDE values (Feng et al., 2003; Marsal et al., 1999). BDE is also very sensitive to the correlation potential. In order to evaluate this effect, we decided to use the Lee–Yang–Parr correlation potential, either with the three-parameter scheme for exchange (B3LYP), or with the one-parameter (B1LYP) developed by Adamo and Barone (Adamo & Barone, 1997). We also tested the Perdew–Wang and the Perdew correlation potentials, in combination with the three-parameter scheme (B3PW91 and B3P86, respectively).

BDE values were corrected to take into account the zero point energy (ZPE) and the contributions from translational, rotational, and vibrational degrees of freedom in the heat of reaction at 298 K. The use of scaling factors for vibrational frequencies and ZPE was not considered because the basis sets we used with DFT (from 6-311G(d) to 6-311++G(2d,p)) would lead to negligible rescaling.

Concerning the general methodology, another important structural issue must be taken into account. Indeed, three of the molecules studied here (catechol, quercetin and taxifolin) possess a catechol-like moiety in which a stabilizing effect exists due to H-bonding between the two adjacent OH groups. This structural feature must be taken into account in the molecule but also in the radicals originating from the catechol moiety (Russo et al., 2000; Wright et al., 2001). Therefore, in those radicals, we took care that H-bonding is maintained between the O-atom where H-abstraction takes place and the *ortho*-OH group.

2.2. Test calculations on phenol and catechol

We compare our theoretical results on flavonoids with the structure–activity relationship obtained from many experimental studies. However, since no experimental values of BDE and IP are available for flavonoids, it is difficult to directly give quantitative support to the calculations. As we have seen above, the antioxidant activity of flavonoids is attributed, in part, to H-transfer from the B-ring. Depending on its degree of oxidation, the B-ring is equivalent to phenol or catechol, for which experimental data indeed exist, and can, therefore, be used for testing the theoretical methodology. The OH groups of A-ring and the 3-OH group are significantly different from the OH groups of phenol and catechol. Nonetheless we assume that, if the method gives a reliable evaluation of the BDE in phenol and catechol, it will give valuable results for the A-ring and 3-OH as well.

In the recent literature, there are several experimental reports on the O–H BDE determination of phenols, based on a photoacoustic calorimetry technique (Borges dos Santos & Martinho Simoes, 1998). Among the recent values, Wayner et al. (1995) obtained 87.0 ± 1.0 kcal/mol (Wayner et al., 1995). More recently, De Heer, Korth, and Mulder (1999) proposed correcting that value by taking into account the enthalpy of intermolecular hydrogen bonding with benzene (i.e., the solvent used for the measurements); they obtained 86.2 kcal/mol. Concerning the BDE in catechol, there are very few quantitative studies. Brigati, Lucarini, Mugnaini, and Pedulli (2002) reported experimental values measured by an EPR equilibration technique for various phenolic antioxidants containing two or more hydroxyl groups. From that work, the BDE of catechol is 79.3 ± 0.3 kcal/mol. The study was performed in benzene solution; because of the small dielectric constant of that solvent, the authors suggested that the values are probably close to those expected in the gas phase.

In order to obtain an accurate description of the BDE, we tested several quantum-chemical methods and basis sets. All the details concerning the results that we have obtained on phenol and catechol with (U)HF, (U)MP2, (U)B1LYP, (U)B3LYP, (U)B3P86, and (U)B3PW91 and the different basis sets (from 6-311G(d) to 6-311++G(2d,p)) are available as ****supplementary material. Globally, it appears that, for phenol and catechol, the DFT/B3P86/6-311G(d,p) scheme is sufficient to give an accurate description of the BDE, since it performs within ~1 kcal/mol of the most extended scheme compared to the experimental gas phase value. Nevertheless, we decided to include diffuse functions for the flavonoids, in order to obtain a better description of the delocalization effects that are crucial for the geometry as well as for the electronic structure; we consequently used 6-311+G(d,p) in the following of the study.

2.3. Calculations on quercetin and taxifolin

A semi-empirical study of the conformation of quercetin has previously been published (Russo et al., 2000) and we recently applied the same method for taxifolin (Trouillas et al., 2004). In those papers, the potential energy surfaces were investigated along the torsion angle τ, defined by the C3–C2–C1′–C2′ atoms (Scheme 1). For quercetin, the energy minimum is found for a torsion angle of 153° (Russo et al., 2000). Taxifolin is a dihydroflavonol and it must be stressed that the dihydroflavonol structure possesses two chiral centres, C-2 and C-3, leading to the existence of the four diastereomers 2S3S, 2R3R, 2R3S and 2S3R. The 2S3S isomer shows a torsion angle of 100° at the AM1 level and the conformational behaviour of the 2S3S compound is a mirror image of that of 2R3R. Moreover, since the antioxidant activity has only been reported for a racemic mixture of both *trans*-taxifolin isomers (2S3S and 2R3R), we did not consider the *cis*-isomers (2S3R and 2R3S) in this study. Therefore, the present study is restricted to the 2S3S isomer.

B3LYP/6-311+G(d,p) and B3P86/6-311+G(d,p) geometry optimizations were carried out and torsion angles, τ, of 180° and −78° were found for quercetin and taxifolin, respectively. Thus, as expected, quercetin is a planar molecule, in which electron delocalization between the B-ring and the C-ring is favoured (the fact that AM1 gives a non-planar geometry is probably due to the underestimation of the π-electron delocalization at the semi-empirical level, along with the flat shape of the potential energy surface in the surroundings of 180°).

The BDE values are calculated with B3P86/6-311+G(d,p), according to what was shown above for phenol and catechol. We also used B3LYP/6-311+G(d,p), since it is the most common functional used in the literature; this allows us to compare our results with those that are likely to appear in the future at the B3LYP level. Nevertheless, the discussion is essentially based on the B3P86 values. All calculations correspond to systems in vacuum, i.e., no solvent effects were taken into account. Influence of solvent on phenolic compounds has been discussed by Wright et al. (2001) and, more recently, by Guerra, Amorati, and Pedulli (2004) for para-substituted phenols and they concluded that this correction is below 2 kcal/mol.

Geometry optimizations on the radicals were performed, starting from the optimized structure of the parent molecule, after the H-atom was removed from the 3, 5, 7, 3′, or 4′ position. In the discussion, the radical formed by H-removal from the 3-OH group of quercetin is called 3-OH quercetin radical. The same notation is used for the other four radicals. No geometrical parameter constraint was imposed during the optimization. We took care only that the OH group neighboring the primary radical site in the B-ring is oriented in such a way that H-bonding is preserved. Indeed, after H-abstraction from 4′-OH, the *ortho*-OH group was tilted to form a H-bond with the remaining O-atom.

3. Discussion

3.1. BDE values for quercetin and taxifolin

The middle part of Table 1 shows the (U)B3P86/6-311+G(d,p) and (U)B3LYP/6-311 + G(d,p) calculated BDE values for the five radicals formed by H-abstraction on quercetin and taxifolin. A systematic increase in energy of about 4 kcal/mol is observed from B3LYP to B3P86, as in phenol and catechol. Even if no experimental data are available for comparison for

Table 1
Calculated values of the BDE for the different OH positions in quercetin and taxifolin

		BDE (kcal/mol)					
		3-OH	3′-OH	4′-OH	5-OH	7-OH	H-2 keto form
B3P86/6-311+G(d,p)	Quercetin	83.7	77.0	74.6	99.3	88.6	64.3
	Taxifolin	106.7	76.0	76.0	99.6	92.0	–
B3LYP/6-311+G(d,p)	Quercetin	79.7	73.6	71.1	94.7	84.4	61.6
	Taxifolin	103.0	72.9	72.7	95.0	87.8	–

The middle part (from column 3 to column 7) is related to the enol form of quercetin. The last column reports the BDE of the H-atom bound to the C-2 atom in the keto form of quercetin.

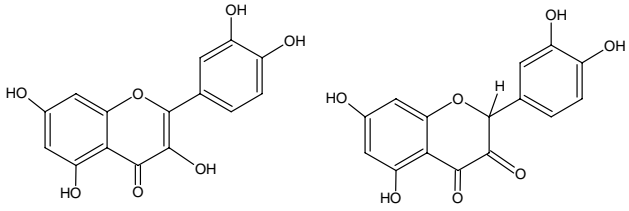
flavonoids, these DFT results appear quite realistic for phenol compounds.

Both methods give the following BDE sequence for the OH groups: 4′-OH < 3′-OH < 3-OH (quercetin) < 7-OH < 5-OH < 3-OH (taxifolin). This clearly confirms that H-transfer from the B-ring (4′-OH and 3′-OH groups) is easier than from the A-ring (7-OH and 5-OH groups), consistent with what is known from the literature concerning structure–activity relationships of antioxidant flavonoids (Rice-Evans et al., 1996). According to the B3P86 calculations, the BDE values for the OH sites on the A-ring are higher than those on the B-ring, by: (i) 22 kcal/mol between 3′-OH and 5-OH; (ii) l2 kcal/mol between 3′-OH and 7-OH; (iii) 25 kcal/mol between 4′-OH and 5-OH; (iv) 14 kcal/mol between 4′-OH and 7-OH for quercetin, and by: (i) 16 kcal/mol between the OH groups on the B-ring and 7-OH; (ii) 24 kcal/mol between the OH groups on the B-ring and 5-OH for taxifolin. From such differences, there is no doubt that the reactivity of the B-ring is higher than that of the A-ring, whatever the kind of oxidative system involved. 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.

In quercetin, it is interesting to note that the BDE is lower for 4′-OH by 2.4 kcal/mol compared to 3′-OH, whereas the values are identical for both groups in taxifolin. An interpretation for this small but significant difference will be proposed later in the paper, in the light of the electronic structure.

As expected, the behaviour of the 3-OH group is dramatically different in quercetin and taxifolin. The 3-OH-BDE of quercetin (83.7 kcal/mol with B3P86) is intermediate between those on the B-ring and those on the A-ring. It is only 7 and 9 kcal/mol higher than the 4′-OH and 3′-OH values, 5 and 16 kcal/mol lower than 7-OH and 5-OH. In strong contrast, O–H bond dissociation from the 3-OH group is highly thermodynamically unfavourable in taxifolin (BDE = 106.7 kcal/mol with B3P86), which could explain, in part, the marked difference in antioxidant activity between quercetin and taxifolin.

The present DFT BDE values clearly demonstrate that H-transfer is more energetically favourable from the B-ring and that H-transfer from the 3-OH group is also possible in quercetin, depending on the oxidative system. This confirms several in vitro studies that have shown the participation of that group in redox reactions (Balogh-Hergovich et al., 1997; Marfak, Trouillas, Allais, Calliste, & Duroux, 2004). At this stage, we also considered another possible mechanism for explaining the reactivity of the 3-OH group and the 2,3-double bond in quercetin. Indeed keto–enol tautomerism can take place in this molecule (Scheme 2), which is not the case for taxifolin. We have, therefore, calculated the BDE of H-transfer from the C-2 atom in the keto form for both enantiomers and we have obtained a value close to 64 and 62 kcal/mol with B3P86 and B3LYP, respectively (right part of Table 1). This very low BDE indicates the high capacity of H-transfer from the C-2 site in the keto form. However, it must be noted that the keto form of quercetin is markedly less stable than the enol form, the latter being stabilized by π-conjugation from the B-ring to the C-4 carbonyl group through the 2,3-double bond. The difference in stability is such (about 20 kcal/mol) that the contribution of the keto form can be considered negligible for quercetin as a free molecule. Nevertheless, one may speculate that, in some enzymatic environments, the stability difference between the two forms is reduced (for instance, upon the loss of the molecular planarity), so that the keto form becomes relevant, and then H-abstraction from the C-2 atom can



Enol form of quercetin

Keto form of quercetin

Scheme 2.

take place easily, thereby contributing to the antioxidant properties.

3.2. Importance of spin densities for the description of flavonoid radicals

3.2.1. General

The difference in antioxidant activity between quercetin and taxifolin, which is reflected in the BDE values calculated above, is often attributed to π-electron delocalization, which leads to the stabilization of the radicals obtained after H-abstraction. This conclusion is drawn assuming that, if π electron delocalization exists in the parent molecule, it also exists in the corresponding radical. In order to understand the relationship between the electron delocalization and the reactivity of the radicals, one can examine the electron distribution in the singly occupied molecular orbital (SOMO), also called, in this case, the α-highest occupied molecular orbital (α-HOMO). For quercetin, the α-HOMO is delocalized over the entire molecule (we have checked that the α-HOMO indeed corresponds to the orbital containing the unpaired electron, by comparing its shape to that of the first unoccupied beta orbital (Ohta, 2002)). Its shape is quite similar for the five radicals (corresponding to H-removal from one of the five OH groups) and it does not exhibit sufficient variations for explaining the differences in activity between those OH groups (Fig. 1). Therefore, the shape of the α-HOMO is not a reliable indicator for the reactivity of flavonoids.

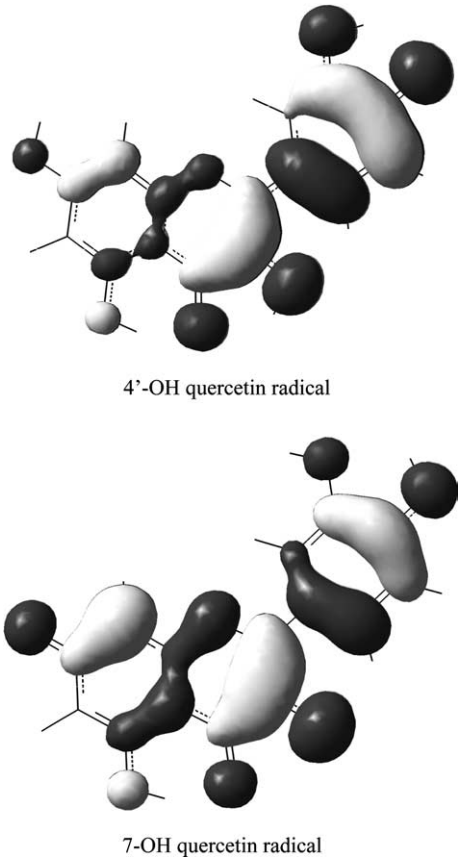


Fig. 1. α-HOMO of the 4'-OH and the 7-OH radicals formed by H-removal from quercetin. The α-HOMO for the other radicals issued from quercetin (not shown here) are quite similar (i.e. delocalized over the entire molecule).

The α-HOMO is the highest-occupied molecular orbital of spin α. In that, it does not describe the global electronic behaviour of the radical. Within an unrestricted scheme, the spin density is often considered to be a more realistic parameter and provides a better representation of the reactivity (Szabo & Ostlund, 1982, Chap. 3). The importance of the spin density for the description of flavonoids has been pointed out by the recent paper of Leopoldini et al. (2004). We have, therefore, decided to analyze the spin density on the various quercetin and taxifolin radicals, in order to rationalize the differences in reactivity of the OH sites in flavonoids and consequently the differences in BDE.

3.2.2. Comparison between the spin densities of the radicals formed from the B- and A-rings

It must be stressed that the more delocalized the spin density in the radical, the easier is the radical formed, and thus the lower is the BDE (Parkinson, 1999). The spin population (on the remaining O-atom after H removal and on the neighbouring C-atoms) appears to be slightly more delocalized for radicals issued from the B-ring (3′-OH and 4′-OH) than for those located on the A-ring (5-OH and 7-OH) (Fig. 2). For example, the spin density is 0.28 on the O-atom in the 4′-OH quercetin radical whereas it is 0.37 for the 7-OH quercetin radical. Consistently, one can observe some delocalization beyond the ring bearing the O-atom (smaller spin densities on the surrounding atoms). As a consequence, the BDE is lower in the B-ring than in the A-ring.

Nevertheless, differences in the BDE cannot be explained only on the basis of the spin density value on the O-atom where H-abstraction occurred: the BDE of 5-OH is about 10 kcal/mol higher than that of 7-OH, whereas the spin density on the O-atom of the 5-OH radical is lower than that on the O-atom of the 7-OH radical. That difference is related to the fact that a H-bond exists between the 5-OH group and the carbonyl group on C-4. As a consequence, the BDE on that site is higher because H-removal also implies the breaking of the H-bond.

On the basis of the spin density description, let us now compare quercetin and taxifolin. In quercetin, the BDE of 4′-OH (74.6 kcal/mol) is lower than that of 3′-OH (77.0 kcal/mol) by 2.4 kcal/mol, whereas the two values are the same in taxifolin (76.0 kcal/mol). The spin density is actually more delocalized in the 4′-

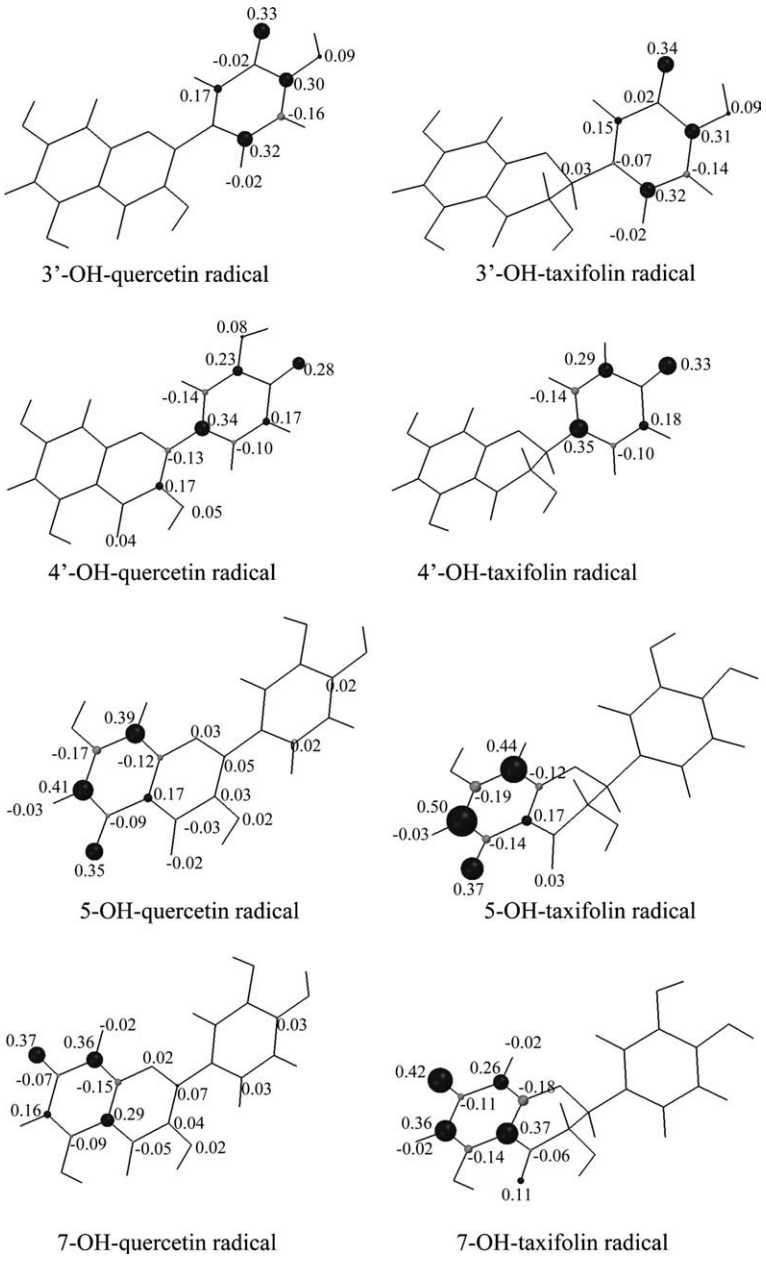


Fig. 2. Distribution of spin densities in the radicals formed by H-removal from the B-and the A-rings, for quercetin (left) and taxifolin (right).

OH quercetin radical than in its 3′-OH counterpart, in the 3′-OH taxifolin, and in the 4′-OH taxifolin radicals. Indeed, in the 4′-OH quercetin radical, the spin density on the remaining O-atom is 0.28, whereas it is 0.33 for the other three systems. This is a consequence of delocalization effects due to the presence of the 2,3-double bond, which allows for spin presence on the C-2 and C-3 atoms: 0.13 of β type and 0.17 of α type, respectively. This effect can be rationalized 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 C-1′ atom in *para*-position for the 4′-OH radicals, and the subsequent possible delocalization effect due to the presence of the 2,3-double bond in quercetin. It is to be noted that such delocalization cannot happen for the 3′-OH radical of quercetin and for the 3′-OH and 4′-OH taxifolin radicals.

Despite these clear differences, we believe that the delocalization effect acting for the 4′-OH quercetin radical is not sufficient to explain why the overall antioxidant activity is twice higher for quercetin than for taxifolin. Both in terms of BDE and spin density distribution, the redox action of the B-ring appears to be almost the same for both molecules. We have also checked that the ionization potentials (IP) of quercetin and taxifolin are very similar (the difference is only 0.3 eV at the

(U)B3P86 level), so that the difference in antioxidant activity cannot be ascribed to the predominance of the electron transfer mechanism, which is governed by the IP value. This leads us to the hypothesis that the higher antioxidant activity of quercetin is related to the concomitant presence of the 3-OH group and the 2,3-double bond, which confers high reactivity to that OH site.

3.2.3. The specificity of the 3-OH site

As mentioned above, the α-HOMO of the 3-OH radical is almost the same as that of the other radicals for quercetin. Thus, the α-HOMO localization cannot explain the reactivity of this site. In contrast, we find a very strong difference in the spin density distribution of the 3-OH radical: in taxifolin, the spin density is very high on the O-atom at C-3 (spin density = 0.81) and concomitantly the delocalization is weak (Fig. 3). Since spin density delocalization is related to the easiness of radical formation; this clearly confirms that, for taxifolin, H-removal from 3-OH is not favoured. For quercetin, the corresponding radical could be formed, either from the keto form or from the enol. It is nonetheless important to note that, whatever the mechanism of H-transfer, the radical formed is the same. Calculations show that the spin density on the O-3 atom is only 0.32, confirming the very high capacity for H-removal from the enol-quercetin 3-OH group or from the C-2 atom of keto-quercetin. This low value is a consequence of delocalization via the 2,3-double bond (after or before H-removal): the spin density is 0.44 on C-2, 0.13 on C-6′, 0.13 on C-4′ and 0.09 on C-1′ (Fig. 3). The presence of a high spin density (0.44) localized on the C-2 atom implies a high reactivity for that site, a feature that appears only upon formation of the radical. This is not the case for taxifolin (the spin density on C-2 is 0.07), due to the absence of the 2,3-double bond.

Along the same lines, we have recently shown that the 3-OH group is the primary site of reaction with radical species derived from alcohols (Marfak et al., 2004). The two following radicals: $CH_3{}^{\bullet}CHOH$ (α-hydroxyethyl radical or HER) and $^{\bullet}CH_3O$ (α-hydroxymethyl radical or HMR), were produced in radiolyzed alcohol solutions (ethanol and methanol, respectively) in the presence of oxygen. We demonstrated that those radicals stereospecifically attack the 3-OH group, yielding depside formation according to reaction Scheme 3. This mechanism is similar to that described for flavonoid degradation by dioxygenase, except that there is no CO releasing.

Our calculations, which show a high spin density on the C-2 atom (0.44), thus indicate that C-2 is indeed the key site for redox reaction on the intermediate flavonoxy radical formed after H-removal on the 3-OH group of quercetin. Under certain experimental con-

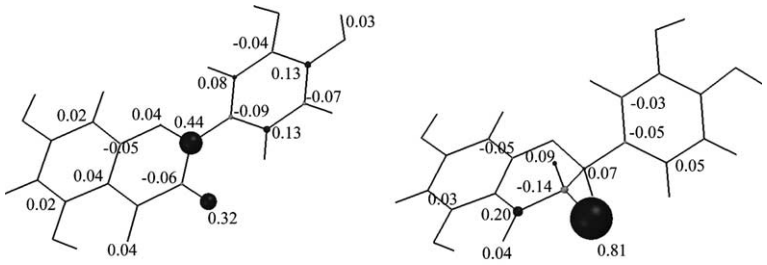
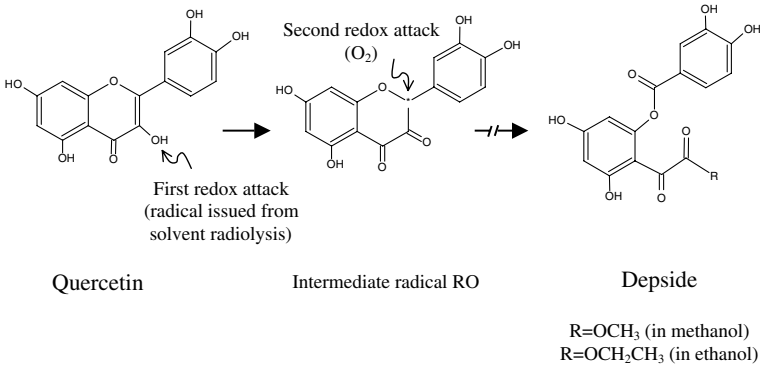


Fig. 3. Distribution of spin densities in the radicals formed by H-removal from the 3-OH group, for quercetin (left) and taxifolin (right).



Scheme 3.

ditions, O_2 is able to bind to C-2, thanks to the presence of the unpaired electron, to form a peroxide. Afterwards, the C-ring can open by the mechanism summarized on Scheme 3 (Marfak et al., 2004).

The absence of the 2,3-double bond in taxifolin precludes spin delocalization to the C-2 atom. Even though there is no available experimental data for that flavonoid, one can conclude that the metabolism pathway is most probably different from that of quercetin. The subsequent small phenol acids, formed after C-ring opening in quercetin, would hardly form, indicating that taxifolin would be less easily absorbed than quercetin. In addition, it must be stressed that such a mechanism (C-ring opening) has not been observed for taxifolin in radiolyzed alcohol solutions (Marfak et al., 2004).

4. Conclusion

Finding a reliable quantum-chemical method for describing the flavonoids is of particular interest because theoretical insight can contribute significantly to the understanding of the reactivity of those molecules in different oxidative systems. From the present results, DFT methods appear to be the most relevant for the description of phenolic compounds. Among the different functionals tested, B3P86 gave the best agreement with experimental data; for flavonoids, the 6-311+G(d,p) basis set appears as a good compromise between the quality of the results and the computational cost.

We used that theoretical approach to confirm the important role of the B-ring and to shed light on the role of the 3-OH group in the antioxidant properties. The reactivity of this site is turned on in the presence of the 2,3-double bond, as in quercetin. In this case, H-abstraction from the 3-OH group could be induced by two possible chemical pathways. The first one is H-abstraction from the 3-OH group of the enol form of quercetin, which induces spin delocalization to the C-2 atom. The second one is H-abstraction from the C-2 atom of the keto form of quercetin, which can form as a consequence of keto-enol tautomerism via the 2,3-double bond.

While the enol form is the most stable tautomer in the free molecule, the keto form, which possesses a much lower BDE for H-abstraction on the C-2 atom, might play a significant role in the real system, depending on the molecular environment of quercetin and on the oxidative system acting on the molecule. In any case, the high spin density found on the C-2 atom of the radical can open the way to C-ring opening. This reaction cascade is essential for flavonol degradation in radiolyzed solutions and for degradation during metabolization. Even though experimental data are missing for radicals issued from flavonoids, those two experimentally observed behaviours have led the authors (Balogh-Hergovich et al., 1997; Marfak et al., 2004) to propose a reaction pathway involving the C-2 atom, in accordance with our theoretical results.

We are now planning to extend our study to other natural phenolic compounds, in order to explain and predict their reactivity in different oxidative systems.

5. Supporting information available

Discussion and data corresponding to corrected and uncorrected BDE obtained on phenol and catechol with (U)HF, (U)MP2, (U)B1LYP, (U)B3LYP, (U)B3P86, and (U)B3PW91, for all the seven basis sets used (6-311G(d), 6-311G(d,p), 6-311+G(d), 6-311+G(d,p), 6-311++G(d,p), 6-311++G(2d,p), 6-311++G(2d,2p)).

Acknowledgements

The authors thank Victor Geskin (UMH) for stimulating discussions on quantum chemistry issues, the ''Conseil Régional du Limousin'' for financial support and IDRIS (Institut du Développement et des Ressources Informatiques Scientifiques, Orsay, Paris) for computing facilities. The work in Mons is partly supported by the Belgian Science Policy InterUniversity Attraction Pole Program (Project 5/3) and the Belgian National Science Foundation (FNRS). The research of P. Marsal has been made possible by a fellowship of the European Commission, under the 'LAMINATE' RTN network.

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