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A theoretical study of the conformational behavior and electronic structure of taxifolin correlated with the free radical-scavenging activity

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Abstract

Based on the most stable conformations of taxifolin, its electronic structure was calculated using AM1 and RHF/6-31G* treatments. BDE of the OH groups was calculated and this confirms the predominant H-transfer capacity of the 3′-OH and 4′-OH groups, compared to the 3-OH group and the OH groups of the A-ring. HOMO, characteristic of the electron-transfer capacity, is delocalized in the B-ring but, compared to quercetin, π-electron delocalization does not occur to the 2,3-bond and the 4-keto group, which confirms the minor role of the 3-OH group in taxifolin. Also the singly-occupied molecular orbital (SOMO) of the 3-OH radical are localized on the O-atom, whereas SOMO of the 3′-OH and the 4′-OH radicals are delocalized. This is in agreement with high BDE for the first one and low BDE for the latter two. All calculations are in agreement with the structure–activity relationship established for taxifolin in the literature concerning its antioxidant activity.

Keywords: Taxifolin; Antioxidant; Molecular modeling; Ab initio calculations; AM1; HOMO; BDE; SOMO

1. Introduction

Many epidemiological studies have demonstrated correlation between the dietary content of antioxidant nutrients and various pathologies, including cardiovascular diseases, cancer, Alzheimer disease (for a review see Halliwell, Asechbach, lLoliger, & Aruoma, 1995). The flavonoid family, including flavanones, dihydroflavonols, flavones, flavonols, isoflavones and catechins, has been identified as a major source of antioxidants in fruits, vegetables and beverages. For the past decade, beneficial aspects of diets rich in fruits and vegetables have been attributed to the presence of these antioxidants. Estimates of daily intake range from 20 mg to 1 g (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). Quercetin (predominantly found in onions, apples, red wine) and catechin (in green and black teas) are the most studied molecules, due to their strong antioxidant capacities (Rice-Evans, 2001). Flavanones and dihydroflavonols, such as taxifolin, are mainly found in citrus fruits, especially grapefruit and orange (Bohm, 1975); they also possess antioxidant properties.

Structure–activity relationship (SAR) has been established from many in vitro assays (for reviews see Rice-Evans, Miller, & Paganga, 1996; Van Acker et al., 1996a). Flavonoids can act by radical-scavenging or by inhibition of radical formation (metal chelation, enzyme inhibition). Three criteria for effective radical-scavenging are: (i) the ortho-dihydroxyl structure in the B-ring, (ii) the 2,3-double bond, which allows π electron delocalization from the B-ring and (iii) the 3- and 5-OH with 4-oxo function in the A- and C-rings (Bors, Heller, &

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Saran, 1990). Theoretical studies have partially confirmed these criteria, especially for quercetin (Russo, Toscano, & Uccella, 2000; Van Acker et al., 1996b). Quantitative SAR (QSAR) calculations have recently correlated TEAC (trolox equivalent antioxidant capacity) with the three following parameters: number of OH groups, the presence of the 2,3-double bond, and the presence of the 3,5,7-OH groups and the 3′,4′,5′-OH groups (Lien, Ren, Bui, & Wang, 1999).

Quantum mechanical models can provide further interesting information concerning the relationship between the geometric and electronic structures and the antioxidant properties. Van Acker, Koymans, and Bast (1993) have proposed MNDO semi-empirical calculations correlating the ionization potential (IP) and ΔH_f (difference in heat of formation between the radical and the parent molecule) with the activity of vitamin E. Electronic parameters, such as lipophilicity, enthlapy of oxidation, ionization potential, enthalpy of hydrogen abstraction, are included in QSAR studies concerning furanones (Weber et al., 2002) and trimetazidine derivative antioxidants (Ancerewicz et al., 1998).

The aim of the present study is to elucidate the geometric and electronic structures of taxifolin, by an appropriate molecular model, in order to explain its reactivity with free radicals. The stable conformations of taxifolin are calculated for the different diastereoisomers. The electronic density distribution in the HOMO (highest occupied molecular orbital) level and the hydrogen dissociation energy for OH groups are used to determine the most probable redox sites, which are then compared with SAR results. The electronic density distribution in the singly-occupied molecular orbital (SOMO) level is analyzed for the radicals obtained after H-transfer and stabilization of the molecule and the radicals by H-bonding is discussed.

2. Methodology

The lowest energy conformation of taxifolin was determined by employing a combination of esff force field (extensible and systematic force field implemented in the Insight II package v.2000) and quantum-chemical semi-empirical (Dewar, Zoebisch, Healy, & Stewart, 1985) and ab initio Hartree–Fock calculations. Potential energy surfaces were obtained versus the torsion angle τ between ring B and C, defined by C3–C2–C1′–C2′ atoms (Fig. 1). τ was scanned in steps of 20° without constraint on all other geometrical parameters. The effect of the following torsion angle rotations was also studied: C4–C3–OH, C10–C5–OH, C8–C7–OH, C3′–C4′–OH and C2′–C3′–OH. Afterwards, the structures were further optimized without any constraint around each potential minimum. AM1 calculations were performed, using the restricted Hartree–Fock (RHF) method. Optimizations were made without symmetry constraints and with a 0.01 kcal/mol · Å gradient and a 10^{-5} kcal/mol energy convergence criterion. Geometrical analysis was performed independently, with esff and AM1 leading to a series of potential curves (Fig. 2). To confirm the position of the minima, ab initio RHF/6-31G* geometry optimizations were performed, starting from the AM1 most stable conformations. The convergence criteria

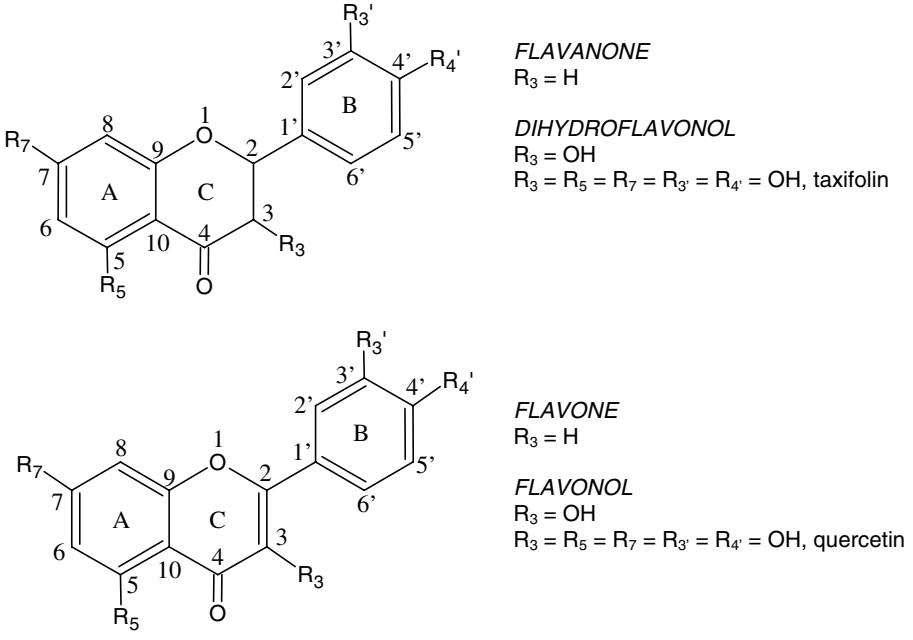

Fig. 1. Flavonoid chemical structures.

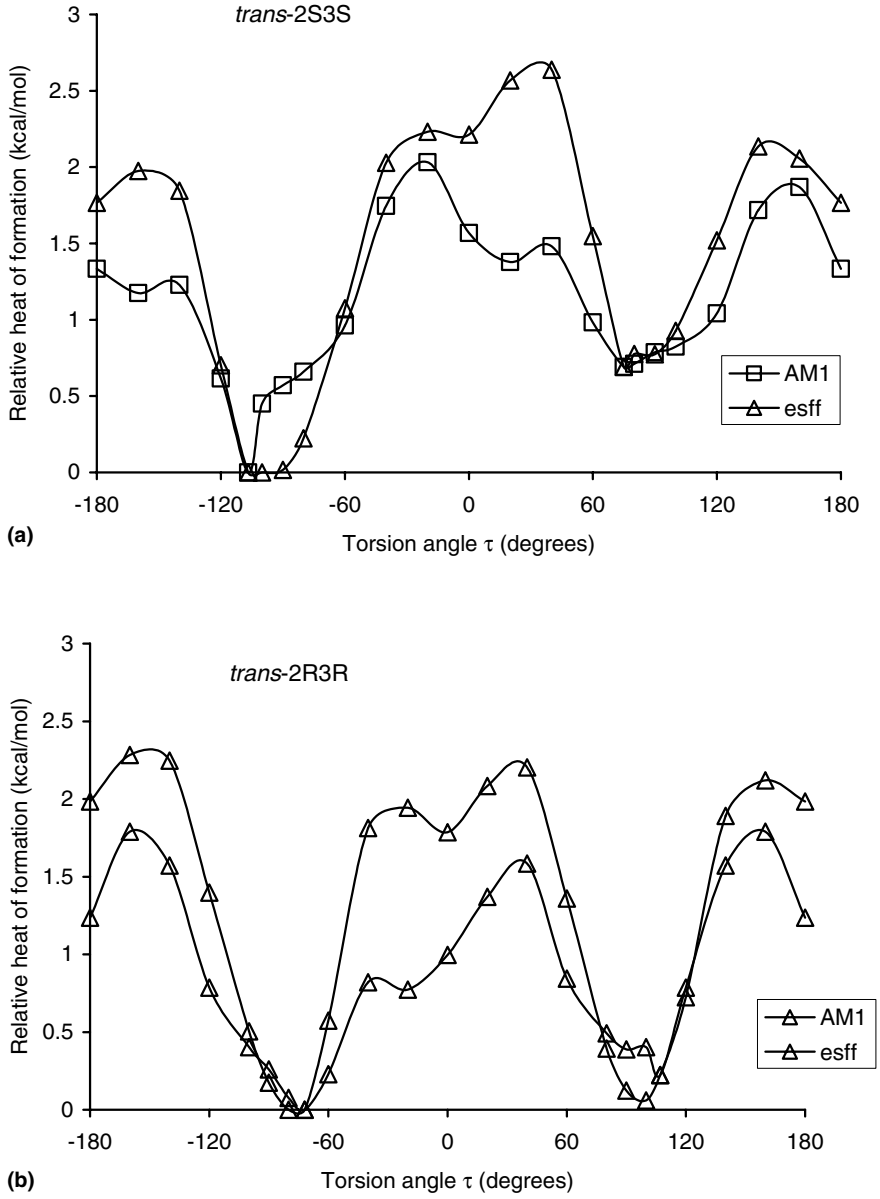

Fig. 2. Potential energy surface versus the dihedral angle τ (see text), calculated with esff force field and AM1 for 2S3S (a) and 2R3R (b).

were 6×10^{-4} kcal/mol for the energy and 0.3 kcal/mol $\cdot$ Å for the gradient. We tested more drastic convergence criteria, but energy values were not changed.

The IP value is equal to the opposite of the HOMO energy, according to the Koopman's theorem (Leach, 1996). The spatial distribution of the HOMO level was analyzed via the linear combination of atomic orbitals (LCAO) scheme and was represented graphically using the ZOA software (Calbert). We have found excellent agreement between the AM1 and RHF/6-31G* results, in terms of molecular conformation and LCAO description of the molecular orbitals. Moreover, the trends found for the IP values are very similar for both techniques. For the sake of consistency with the BDE data (see below), we will mostly present the AM1 data here.

Two quantum properties are known to correlate with the free radical-scavenging activity:

1. The IP, because the HOMO level is involved in the electron transfer, according to the following reaction in the case of OH·:

$$ROH + OH^{\bullet} \rightarrow ROH^{+} + OH^{-}$$
$$(ROH = \text{phenol antioxidant})$$

2. The bond dissociation enthalpy (BDE), corresponding to the O–H bond breaking (H· abstraction) according to the following reaction:

$$ROH + OH^{\bullet} \rightarrow RO^{\bullet} + HOH$$

BDE is calculated as the difference in heat of formation between the flavonoid and the corresponding

radical. Because the semi-empirical AM1 method has given good results (Russo et al., 2000) and because ab initio methods require very large basis sets to provide reliable results, we decided to perform AM1 calculations on the radicals, in UHF (unrestricted Hartree–Fock) and ROHF (restricted open-shell Hartree–Fock) formalisms. The calculations on the radicals were performed from the optimized structure of the parent molecule, after an H atom was removed from the 3, 5, 7, 3′ and 4′ positions, successively. No geometrical parameter constraint was imposed during the calculation, except for the OH group neighboring the primary radical site. The SOMO distribution in radicals was visualized with the ZOA programme.

Because the UHF energy cannot be compared with the RHF energy from the mother molecule and due to spin contamination, we did not calculate BDE values but a qualitative parameter, ΔH_F, corresponding to the energy difference between the radical and the molecule, as obtained in our calculations. ROHF is a more reliable method in radical energy calculation and ΔH_F values are more satisfactory and probably close to exact BDE.

All calculations correspond to systems under vacuum at 0 K. Although the free radical-scavenging action always occurs in solution, no solvent effects were taken into account. Wright, Jonhson, and DiLabio (2001) have suggested that BDE values are only weakly solvent-dependent, whereas electron transfer is dependent, due to solvent stabilization of the charged species. However, the IP values in solution are assumed to correlate tightly with gas phase IP values. Therefore, the authors have concluded that the gas phase BDE and IP are excellent primary indicators of the free radical-scavenging activity.

3. Results and discussion

3.1. Geometrical analysis

Recent studies have reported conformational calculations of flavonoids. Van Acker et al. (1996b) have obtained a planar geometry for quercetin by using the ab initio method, with the STO-3G basis set. More recently, Russo et al. (2000) have reported that AM1 calculations give a non-planar geometry with a torsion angle, τ, of 153.3°. This result is important because planarity influences π electron delocalization, one of the important parameters for an antioxidant. However, the potential energy curve obtained by AM1 is very flat, from 150° to 180° (the energy variation is only 0.5 kcal/mol). The difference between the two results is attributed to over- or under-estimation of non-bond and conjugation effects. The AM1 method gives reliable thermodynamic and electronic results for phenol and other basic organic compounds (Dewar et al., 1985). Kondo, Kurihara, Miyata, Suzuki, and Toyoda (1999a) have used AM1 to calculate the conformations of epigallocatechin gallate and epicatechin gallate; they calculated the BDE of OH groups and proposed oxidative mechanisms supported by LC/MS results. It must also be noted that AM1 calculated torsion angles for morin and myricetin are within 10° from the molecular structures determined by X-ray analysis (Cody & Luft, 1994). Furthermore, in all AM1 studies, the H-bond stabilizing effects in flavonols are in agreement with the experimental results.

Flavanone and dihydroflavonols have been much less studied theoretically so far; only a few studies have been reported. Conformational calculations of a taxifolin structure were performed by ab initio STO-3G and led to a −27.6° torsion angle of the B-ring (Van Acker et al., 1996b). Due to the absence of the 2,3-double bond, the antioxidant activity is lower than that of flavonols: for instance, the quercetin TEAC is 4.7 ± 0.1 mM while that of taxifolin is 1.9 ± 0.1 mM (Rice-Evans et al., 1996). Nevertheless, the antioxidant capacity of those molecules remains interesting, even more so since flavanones and dihydroflavonols are widely distributed in fruits, especially *Citrus*. It must be stressed that the flavanone and dihydroflavonol structures are more complex than those of flavone and flavonol, due to the presence of two chiral centers. From NMR data, it was established (Clark-Lewis, Jackman, & Spotswood, 1964) that (i) for the *trans*-compound, the 2,3 substituents must be equatorial, (ii) the dihedral angle of 1,2-diaxial substituents is close to 180°, and (iii) the carbonyl group is coplanar with the aromatic A-ring. The authors suggested the presence of H-bonding between the 5-OH and the carbonyl groups. Little is known concerning the *cis*-compound because, for many years, it seemed difficult to obtain this stereoisomer. Note that recent studies report the presence of *cis*-dihydroflavonols in plants (Chosson, Chabond, Chulia, & Raynaud, 1998; Tofazzal & Satoshi, 2000).

As the 2 and 3 positions are stereo-centers, the following four compounds 2S3S, 2R3R, 2R3S and 2S3R were considered. The first two are *trans*-isomers, whereas the latter two are *cis*-isomers. Fig. 2 shows the evolution of the energy, with respect to the most stable conformation, vs. the C3–C2–C1′–C2′ torsion angle τ. The curves obtained for 2S3S with esff and AM1 are very similar (Fig. 2(a)) and the positions of the three main minima are confirmed by the ab initio calculations. The consistency between these three methods, in terms of the position of the minima, is a very interesting result, which confirms the validity of our approach.

The torsion angle of the absolute minimum is close to −100° for the three methods, which is very different from that obtained by RHF-STO-3G (Van Acker et al., 1996b) ($\tau = -27.6°$). This is probably due to the treatment of non-bonded interactions in the different meth-

ods. The force-field method has been parameterized to properly reproduce non-bonded interactions and AM1 has been designed to better take into account such interactions, compared to other semi-empirical HF techniques (Dewar et al., 1985). In contrast, ab initio methods do not explicitly account for non-bonded interactions; it is the extension and flexibility of the basis set that determines the quality of the calculation. In that regard, the 6-31G* basis set, used here, is expected to perform much better than the STO-3G basis set. Therefore, we believe that the value found here for τ (around $-100°$) is more reliable than the value found previously. The strong deviation from ring coplanarity we observed here is probably related to the fact that the steric hindrance between the B-ring and the C-ring (which tends to drive the rings out of planarity) is not compensated by electron conjugation effects (which would tend to keep the rings coplanar) in taxifolin, in contrast to quercetin ($\tau = 159.9°$, RHF/6-31G* calculation). Conjugation effects probably influence the energy calculation less than for quercetin, because π electron delocalization is confined to the B-ring here, due to the absence of the 2,3-double bond. The non-planar conformation of taxifolin is a clear geometrical indication that no extended electron delocalization takes place from the B-ring to the 4-keto group.

The second minimum is located around $+85°$ and the energy difference, with respect to the global minimum, is small, i.e., lower than 0.8 kcal/mol, for all theoretical methods. This is consistent with the fact that these two minima actually correspond to the flipping of the B-ring clockwise or anticlockwise, relative to the rest of the molecule.

The local minimum at $\tau = 180°$ is destabilized by about 1.8 kcal/mol (esff), 1.3 kcal/mol (AM1) and 4.6 kcal/mol (RHF-6-31G*), relative to the global minimum. According to AM1, the potential barrier between the minima is around 2 kcal/mol, which is expected to allow interconversion at room temperature. In the analysis of the electronic properties detailed below, we worked with the most stable conformation. This conformation was obtained for H2 and H3 in axial positions. Calculations were also performed with AM1 for H2 and H3 in equatorial positions and gave a minimum at $\tau = -120°$, less stable than the previous structure but with a surprisingly small energy difference of about 0.5 kcal/mol.

Long-range interaction effects were also investigated by twisting the following torsion angles: C4–C3–OH, C10–C5–OH, C8–C7–OH, C3′–C4′–OH and C2′–C3′–OH. The most stable conformation exhibited three H-bonds, as shown in Fig. 3. By comparison, the taxifolin structure, without H-bonds between the 3′-OH and 4′-OH groups, the 5-OH and C4–O carbonyl groups, and the 3-OH and C4–O carbonyl groups, yielded energy differences of 3.5, 5.5 and 2.5 kcal/mol, respectively. These results thus confirm the role of H-bonding in the taxifolin stabilization, as for quercetin and catechin (Kondo, Kurihara, Miyata, Suzuki, & Toyoda, 1999b; Russo et al., 2000; Van Acker et al., 1996b). The conformational study of the 2R3R compound is presented in Fig. 2(b); H2 and H3 are in axial positions. The absolute minimum appears around $-80°$; another minimum, very close in energy (i.e. less than 0.3 kcal/mol) is located around $+100°$ and the third one, which is less stable, appears at 180°. This looks like a mirror image of the behavior of the 2S3S compound, as can clearly be seen when comparing the curves in Fig. 2(a) and (b). The three H-bonds, between the 3′-OH and 4′-OH groups, the 5-OH and C4–O carbonyl groups, and the 3-OH and

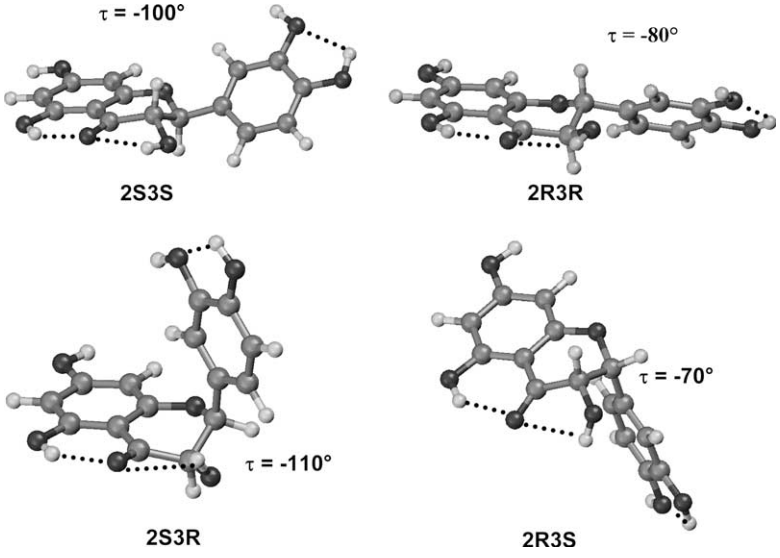

Fig. 3. Most stable conformations for the four taxifolin isomers, as calculated with AM1. Dotted lines represent H-bonds.

C4–O carbonyl groups, also stabilize the structure in this case.

The conformational behavior of the *cis*-compound was also investigated. The B-ring is axial and the 3-OH group is equatorial for both 2S3R and 2R3S. The potential curves of these compounds (not presented here) show two minima that are very close in energy (less than 0.5 kcal/mol). They are located around $\tau = -110°$ and $+70°$ in 2S3R while they appear around $\tau = -70°$ and $+115°$ in 2R3S. Again, the potential curves are thus mirror images of each other. According to both AM1 and ab initio calculations, the *trans*-dihydroflavonols are more stable than the *cis*-compounds. The difference in RHF-6-31G* total energy is 6.2 kcal/mol. This confirms that *trans*-compounds seem to be predominant in plants. Some authors (Clark-Lewis, Jemison, & Nair, 1968) suggested that H-bonds form less easily in *cis*-conformers than in *trans*-molecules. Interestingly, our quantum mechanical calculations suggest that the H-bonding possibility is the same in the four isomers. The following distances between hydrogen of the 3-OH group and oxygen of the 4-keto group are 2.26, 2.26, 2.46 and 2.21 Å for 2S3S, 2R3R, 2S3R and 2R3S, respectively. H bonds were discussed, for the *cis*-compounds, by Foo (1987). This author showed that a more planar configuration between the C3 hydroxyl and the 4-keto group may be obtained with the B-ring in the axial conformation and hence may promote more effective H-bonding between the two groups. Indeed, the *cis*-isomer conformations obtained present a steric hindrance pattern different from that of the *trans*-structure and characterized by the B-ring in the axial position; this could be very important for correlation with biological activity, especially for enzyme inhibition. Due to the lack of experimental data, it is difficult to establish the influence of chirality on the antioxidant properties. Nevertheless, it can be proposed on the basis of these results, that the antioxidant capacity, related to chemical reactions, is probably the same for *cis* and *trans*-compounds, because the quantum properties are the same. However, for antioxidant properties attributed to enzyme reactions, the capacity will be different due to conformational differences.

3.2. Electronic structure

To our knowledge, no detailed study of the electronic structure has been carried out for taxifolin. The only available theoretical data are those of Van Acker et al. (1996b) who have calculated the spin distribution for the radical of a single isomer at the RHF-STO-3G level. The authors did not give geometrical details, except for $\tau = -27.6°$, for the parent molecule. The calculated spin distribution of the radical suggested that, when oxidation takes place in the B-ring, 84% of all spin density remains on the O atom after H removal. The authors concluded that this result was "quite unexpected, as flavonoids are usually considered good radical-scavengers because of their excellent delocalization".

Here we present the electronic structure of *trans*-taxifolin, as calculated by AM1. For the electronic structure, AM1 has given reliable results for phenol compounds (Dewar et al., 1985) and, more recently, for quercetin (Russo et al., 2000) and for a broad class of molecules possessing a high degree of π conjugation (Cornil et al., 1999). Calculations have also been carried out for *cis*-taxifolin; however, since no experimental data exist for the antioxidant activity of *cis*-compounds, those theoretical results are not discussed here. The IP values of *trans*-conformations, deduced from the HOMO energy, are 8.91 and 8.96 eV for 2S3S and 2R3R, respectively. The quercetin IP is 8.79 eV; compared to that of taxifolin, the quercetin IP is lower by 0.2–0.3 eV, indicating a better ability for electron transfer. This is in accordance with the higher activity of quercetin. Electron transfer is one of the possible mechanisms by which an antioxidant can deactivate a free radical. It takes place from the molecule to the radical, giving two ions. The cation derived from the antioxidant is then rapidly deprotonated. For example, with the ROO· radical (involved in lipid peroxidation), the reaction with taxifolin (Tax-OH) is:

$$\mathrm{ROO^{\cdot} + Tax\text{-}OH \rightarrow ROO^{-} + Tax\text{-}OH^{+}} \quad \text{(electron transfer)}$$

$$\mathrm{Tax\text{-}OH^{+} + H_2O \leftrightarrows Tax\text{-}O^{\cdot} + H_3O^{+}} \quad \text{(deprotonation)}$$

The IP is generally considered as a global molecular property. However, because the electrons involved in the electron transfer are those of the highest energy level, the HOMO spatial distribution is an indication of electron transfer zones and probably of subsequent deprotonation sites. The more the HOMO level is delocalized, the more numerous are the electron transfer sites and more redox reactions will occur. π electron delocalization was calculated from the HOMO LCAO coefficients for the most stable conformation of 2S3S and 2R3R-taxifolin and quercetin. Fig. 4 represents a three-dimensional (3D) visualization of the HOMO. The size of the spheres is equal to $\sum c_i^2$, where c_i are the HOMO LCAO coefficients; it must be noted that both theoretical methods give the same spatial distribution for the HOMO. In order to give numerical support to this representation, we present, in Table 1 the AM1 p_z LCAO coefficients, which are the most representative coefficients for a π orbital. For the two-taxifolin isomers, 98% of the HOMO remains on the B-ring, while the HOMO of quercetin is delocalized from the B-ring to the C-ring and the 3-OH group. The presence of this molecular orbital on the 4-keto group represents only 0.2%. Before any oxidative process, the HOMO electrons likely to be involved in electron transfer are localized in the B-ring for taxifolin. This would influence

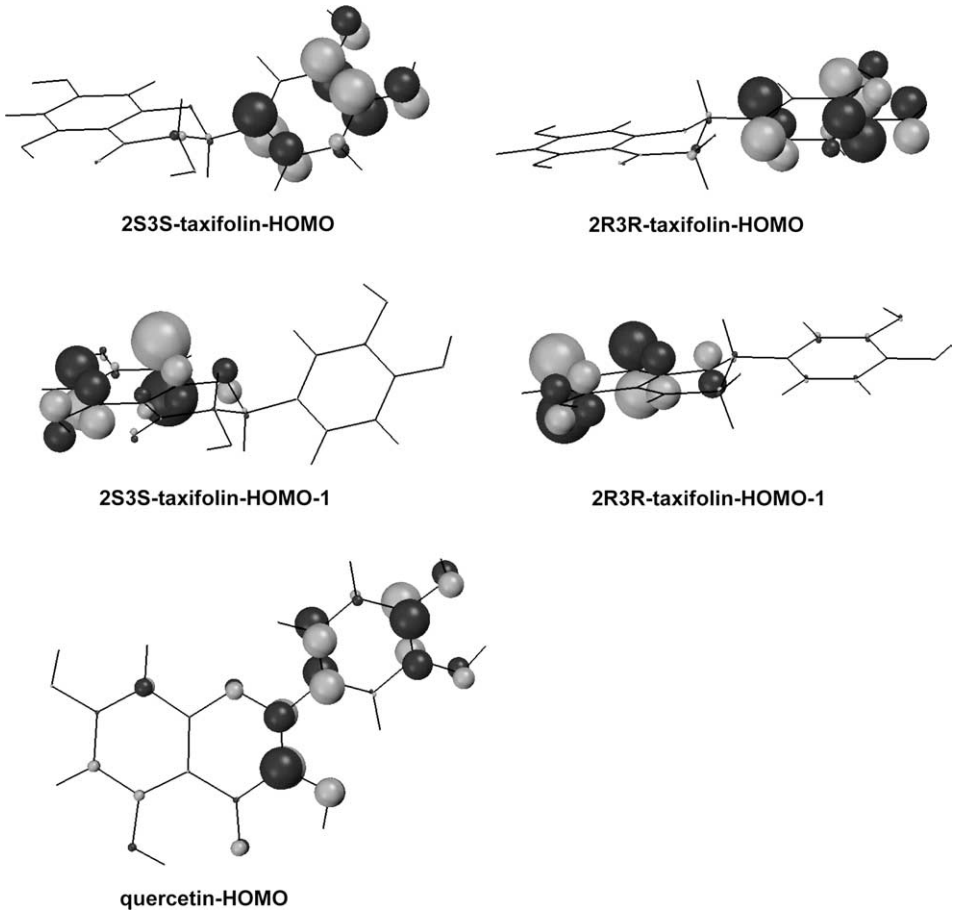

Fig. 4. HOMO spatial distribution for both *trans*-taxifolin isomers and quercetin, as obtained with AM1. The size of each ball is attributed according to the LCAO coefficients.

Table 1
HOMO p_z LCAO coefficients for 2S3S-taxifolin, 2R3R-taxifolin and quercetin, for all non-hydrogen atoms, as obtained with AM1

	C1′	C2′	C3′	O from C3′	C4′	O from C4′	C5′	C6′
B-ring								
2S3S-taxifolin	0.338	−0.017	−0.338	0.231	−0.348	0.273	−0.129	0.264
2R3R-taxifolin	0.348	−0.013	−0.338	0.239	−0.355	0.282	−0.145	0.263
Quercetin	−0.308	−0.032	0.245	−0.175	0.333	−0.217	0.099	−0.271
	O1	C2	C3	O from C3	C4	O from C4		
C-ring								
2S3S-taxifolin	0.026	−0.065	0.042	−0.019	0.004	−0.012		
2R3R-taxifolin	0.021	−0.060	0.041	−0.014	0.004	−0.013		
Quercetin	−0.173	0.290	0.408	−0.273	0.053	−0.153		
	C5	O from C5	C6	C7	O from C7	C8	C9	C10
A-ring								
2S3S-taxifolin	0.005	−0.004	0.014	0.001	−0.001	−0.013	−0.005	−0.008
2R3R-taxifolin	0.004	−0.003	0.013	0.003	−0.002	−0.010	−0.005	−0.008
Quercetin	−0.106	0.084	−0.115	0.047	−0.033	0.181	0.018	−0.033

the delocalization possibility in radicals formed after redox transfer. The calculations thus show that the absence of the 2,3-double bond makes electron conjugation to the C- and A-rings quasi-impossible. This is in agreement with all SAR studies, showing that dihydroflavonols are less active than flavonols.

Another possible mechanism by which an antioxidant molecule can react with radicals is by H-transfer. BDE for breaking the O–H bond is characteristic of this antioxidant pathway: the weaker the OH bond (low BDE), the faster is the reaction with free radicals. Due to the high BDE of the HO–H bond (119 kcal/mol in water)

(Berkowitz, Ellison, & Gutman, 1994), H-transfer from an antioxidant to the hydroxyl radical is a thermodynamically favorable event. For example, the phenol BDE is 88 ± 1 kcal/mol and H-transfer to OH$^{\cdot}$ is very exothermic and more favorable than electron transfer. In the case of taxifolin, the reaction will be

$$OH^{\cdot} + \text{Tax-OH} \rightarrow HOH + \text{Tax-O}^{\cdot}$$

By comparison, the BDE of ROO$^{\cdot}$ is about 88 kcal/mol and reaction with phenol is thermoneutral. In this case, it is difficult to decide which mechanism prevails. Many studies have reported the importance of an H-donor role for flavonoids, but according to the radical type and to experimental conditions, such as pH, the two mechanisms could occur in parallel and the balance between them is difficult to establish. To shed light on that aspect, in the case of taxifolin, it is interesting to consider bond dissociation of each hydroxyl group and formation of the corresponding radical.

3.3. Radical analysis

Because our calculations give qualitative rather than quantitative information, the ΔH_F term was used rather than BDE, generally compared with experimental data (Fox & Kollman, 1996). ΔH_F is the difference in the AM1 heat of formation between the radical and the parent molecule. ΔH_F values calculated in the UHF and the ROHF formalisms are shown in Table 2 for 2S3S and 2R3R-taxifolin and for quercetin. Our UHF-values for quercetin are consistent with those obtained by Russo et al. (2000), also based on the UHF formalism. It must be noted that the UHF and ROHF approaches yield a similar evolution as a function of the OH group position for the three molecules investigated here.

The radical with H-missing on the 3-OH group is called the 3-OH radical. The same notation is used for the other four radicals. The lowest values of ΔH_F are found for the 3′-OH and 4′-OH taxifolin radicals, which clearly demonstrates that the B-ring is the most important site for H-transfer. 4′-OH seems to be the most thermodynamically favorable site, as suggested by the small but systematic differences in ΔH_F between 3′-OH and 4′-OH radicals. Similar results are obtained for quercetin. This could be attributed to steric hindrance between the 3′-OH group and the rest of the molecule, leading to a small increase in interaction and thus a small increase of the corresponding ΔH_F. The role of the B-ring found here, especially that of the 4′-OH group, is in accordance with all SAR described in the literature (Rice-Evans et al., 1996). The role of ortho-substitution on the B-ring has also been largely discussed. In the 3′-OH and 4′-OH radicals, we observe H-bonding between the remaining OH group and the neighboring oxygen atom. After the O–H bond is broken in the parent compound, the radical is able to rearrange to the more stable conformation, corresponding to the formation of a new H-bond, as shown in Fig. 5. A decrease of about 3 kcal/mol in AM1 energy is observed between the molecule without (Fig. 5(a)) and with (Fig. 5(b)) this new H-bond. Wright et al. (2001) also observed this rearrangement between neutral catechol and the corresponding radical. They obtained a barrier height of about 4 kcal/mol. The consistency observed in barrier height between AM1 (our calculations) and DFT, (RO)B3LYP/6311+G(2d,2p)//AM1/AM1 (Wright et al.,

Table 2
UHF and ROHF AM1 ΔH_F values obtained from differences in heat of formation between radical and parent molecules

Radical	ΔH_F (kcal/mol)		
	2S3S-taxifolin	2R3R-taxifolin	Quercetin
3′-OH	22.6 (UHF); 32.1 (ROHF)	23.1 (UHF); 32.8 (ROHF)	24.9 (UHF); 32.8 (ROHF)
4′-OH	22.4 (UHF); 31.8 (ROHF)	22.8 (UHF); 32.4 (ROHF)	19.5 (UHF); 32.9 (ROHF)
3-OH	50.7 (UHF); 60.9 (ROHF)	50.8 (UHF); 60.9 (ROHF)	20.0 (UHF); 31.6 (ROHF)
5-OH	35.0 (UHF); 46.9 (ROHF)	35.2 (UHF); 47.2 (ROHF)	33.9 (UHF); 47.5 (ROHF)
7-OH	32.2 (UHF); 45.2 (ROHF)	32.1 (UHF); 45.1 (ROHF)	31.0 (UHF); 45.9 (ROHF)

The heat of formation values are −238.1, −238.1 and −214.3 kcal/mol for 2S3S-taxifolin, 2R3R-taxifolin and quercetin, respectively.

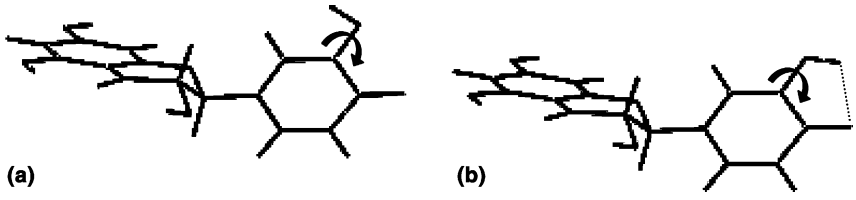

Fig. 5. Conformational rearrangement for H-bond stabilization by twisting the remaining OH group of the B-ring, upon radical formation on the 4′ site of taxifolin. The 4′OH radical is shown without (a) and with (b) the new H-bond.

2001) calculations, allows us to draw quantitative conclusions concerning the role of H-bonding. H-bonding has a stabilizing effect after radical formation on the B-ring and rearrangement, by twisting the remaining OH bond, is possible at room temperature. Nevertheless, the energetic contribution of this rearrangement is not sufficient to explain the predominant role of the 3′ and 4′-OH groups, compared to that of 5, 7 and 3-OH. Due to the large difference in ΔH_F (see Table 2), this just appears as a corrective effect to the low ΔH_F for 3′ and 4′-OH radicals.

For quercetin, the role of 3-OH is very important, as the corresponding 3-OH and 4′-OH radicals are almost isoenergetic (difference in ΔH_F of about 0.5 kcal/mol). In contrast, the 3-OH group of the two taxifolin-isomers is clearly the least favorable for H-donating. An increase in ΔH_F of approximately 30 kcal/mol is observed between 3-OH and 4′-OH radical formation. Most of the literature has suggested that H-donating comes from the B-ring. The 3-OH role has also been discussed and some studies indicate that the quercetin oxidative mechanism involves the C-ring, giving rise to the quinoid form (Dangles, Dufour, & Brett, 1999a, 1999b). 3-OH attack is also the primary mechanism for the in vivo quercetin metabolism pathway, leading to C-ring opening (Balogh-Hergovich, Kaiser, Speier, Fülöp, & Parkanyi, 1999). More recently, redox reaction with 3-OH, followed by C-ring opening and depside formation, has been observed in radiolyzed quercetin solutions (Marfak et al., 2002). The same study demonstrated that taxifolin was not degraded, confirming that H-transfer from the 3-OH group is a thermodynamically unfavorable event for dihydroflavonols. In the case of quercetin, the choice between 3-OH and B-ring sites for H-transfer probably depends on experimental conditions, such as the pH. After H removal of the 3-OH group, the stabilizing effect, due to an H-bond with the 4-keto group, disappears in taxifolin as well as in quercetin; clearly, the absence of the H-bond cannot be invoked to explain the difference in 3-OH-ΔH_F between taxifolin and quercetin. The role of the 2,3-double bond and the corresponding HOMO spatial distribution are essential, as discussed in the following.

5-OH and 7-OH radicals were also considered in the present study. ΔH_F values (Table 2) demonstrate that the role of the A-ring is clearly less important than that of the B-ring. A difference of more than 10 kcal/mol is observed between radicals issued from both rings. Again, this is in agreement with all SAR studies.

The predominant role of the B-ring in H-transfer is thus confirmed by the present calculations, but it is not only due to the presence of stabilizing effects of OH-bonds. Another contribution must be invoked to explain why ΔH_F are lower in the B-ring than in the A-ring. Interestingly, ΔH_F values and the HOMO distribution are well correlated. For quercetin, the HOMO is delocalized from the B-ring to the 3-OH group, corresponding to low ΔH_F of 3-OH, 3′-OH and 4′-OH. In contrast, for taxifolin, the HOMO is confined to the B-ring, corresponding to low ΔH_F only for 3′-OH and 4′-OH. As can be seen in Fig. 4, the electron density on the 3′-OH and 4′-OH groups strongly contributes to the HOMO in taxifolin. In turn, this means that the energy required for the cleavage of those bonds is related in some way to the energy of the HOMO. Instead, the OH groups in the A-ring participate to the HOMO-1 (see the distribution of the HOMO-1 level in Fig. 4). Because electrons in the HOMO-1 are more stable than those in the HOMO, the OH-ΔH_F of the A-ring are higher than those of the B-ring. This is why the spatial distribution of the highest-occupied levels correlates well with ΔH_F. The low ΔH_F for OH of the B-ring is not only due to the ortho-substitution, but also to the molecular orbital distribution. The taxifolin HOMO is localized on the B-ring and does not extend to the 3-OH group, therefore leading to a higher ΔH_F, than the same group in quercetin. Consistently, the 3-OH group strongly contributes to the HOMO in quercetin, which explains the low ΔH_F on that position, while that group appears neither in the HOMO nor in the HOMO-1 of taxifolin. As a consequence, the ΔH_F of the 3-OH site is the highest among the OH groups of taxifolin.

Another molecular parameter can be correlated with the free radical-scavenging activity: the SOMO of radicals. Lien et al. (1999) used this parameter in a QSAR study on phenolic compounds. The SOMO is important because it corresponds to the energy level of the unpaired electron. We have therefore analyzed the SOMO energy value and its spatial distribution. This parameter gives important information about possible stabilization, by delocalization, of the flavonoxy radical formed during oxidative processes. This is represented in Fig. 6 as a 3D-vizualization of the LCAO coefficients. First, we notice that the SOMO is delocalized over the B-ring for the 3′OH and 4′OH radicals of taxifolin and quercetin. This result is different from that obtained in a previous HF study (Van Acker et al., 1996b) in which radical spin distribution was localized on the O atom from which the H atom is removed. This difference is due to the method used (RHF-STO-3G), which probably underestimates delocalization effects. The AM1-calculated SOMO distribution seems to be in agreement with the excellent delocalization possibilities of flavonoxy radicals, suggested as a criterion of good flavonoid antioxidant activities. Second, the SOMO for the radical formed on the 3-OH group remains on the corresponding O atom for taxifolin, while it is delocalized in quercetin. Possibility of π electron delocalization in the radical, after redox transfer, largely influences the ΔH_F values. The AM1 SOMO distribution correlates well with low ΔH_F in quercetin and with large ΔH_F in taxifolin for the 3-OH group. This analysis supports the results obtained for

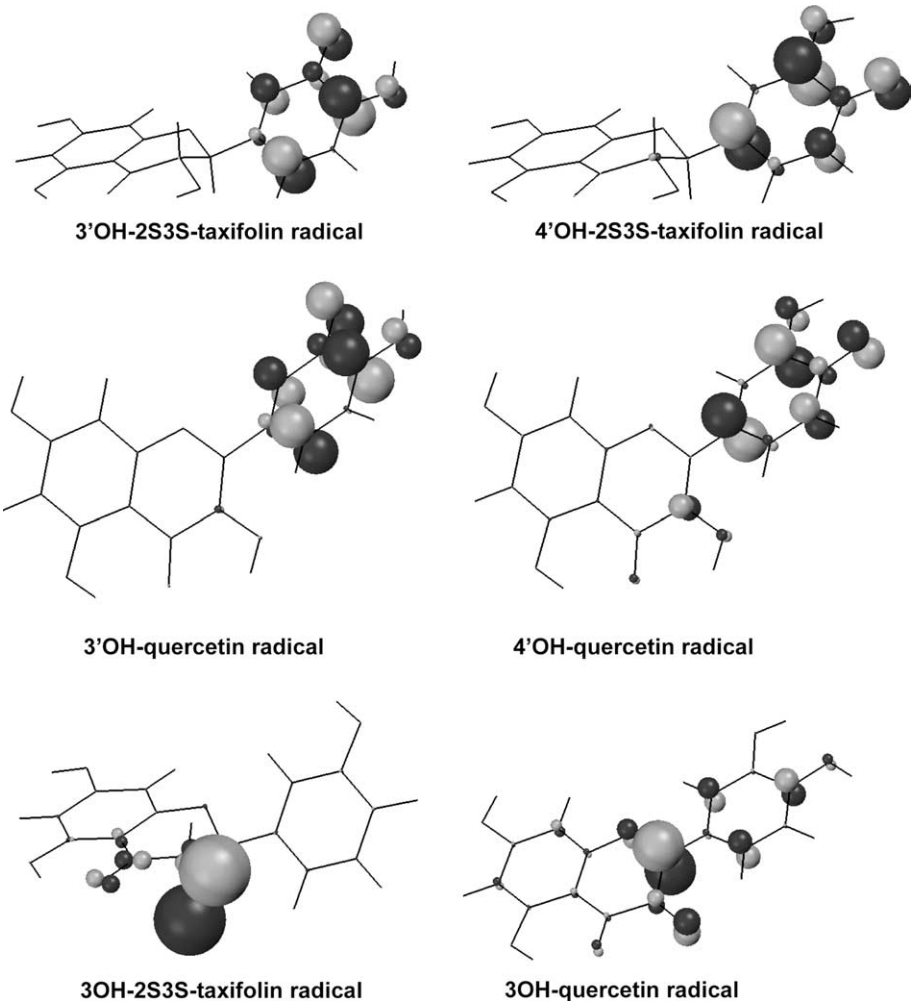

Fig. 6. SOMO spatial distribution for the 2S3S-taxifolin radical, as obtained with AM1. The size of each ball is attributed according to LCAO coefficients.

the HOMO distribution and ΔH_F values, which are in agreement with experimental SAR.

4. Conclusion

The antioxidant properties of dihydroflavonols have long been known and the structure–activity relationship has established the predominant role of the B-ring. In this paper, we have determined the geometric and electronic structure of taxifolin, one of the most common dihydroflavonols, in order to shed light on the molecular origin of such a relationship. The most stable conformations of the four taxifolin-isomers have been calculated. Two major minima are found, which correspond to a strong tilting (either clockwise or anticlockwise) of the B-ring out of the plane formed by the A- and C-rings. We also observe that the potential energy curve for the 2R3R (2S3R) isomer is a mirror image of that of its 2S3S (2R3S) counterpart. Stabilization, due to H-bonding, is also evidenced.

IP and ΔH_F were calculated and the importance of the B-ring has been theoretically confirmed by the low ΔH_F values found for the 3′ and 4′-OH radicals. The absence of the 2,3-double bond dramatically increases the ΔH_F value of the 3-OH radical compared to that of quercetin, making oxidation of this site impossible. The low ΔH_F values of 3′-OH and 4′-OH sites are attributed to stabilizing H-bond effects in the corresponding radicals, to the HOMO distribution of the parent molecule and to the SOMO delocalization.

On the basis of these results, it is still difficult to decide which mechanism (electron transfer or H-transfer) is the most important. The HOMO spatial distribution and BDE values are consistent with all SAR studies. Predominance of one mechanism probably depends on parameters including the radical type and experimental conditions. The differences found in the IP and ΔH_F values are in accordance with higher activity of quercetin, than of taxifolin. Even though quantitative analysis showed a very small difference in IP values, more reliable calculations must be done to confirm that H-

transfer is the predominant effect for taxifolin. Wright et al. (2001) have suggested that H-transfer is dominant for catechin, epigallocatechin and epicatechin gallate, whereas Jovanovic, Tosic, and Simic (1991) supposed that one-electron transfer is most likely the dominant reaction mechanism for gallocatechins. Understanding of the two mechanisms remains important due to possible parallel occurrence with regard to experimental conditions or biological context.

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