Semiempirical Molecular Modeling into Quercetin Reactive Site: Structural, Conformational, and Electronic Features

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The conformational behavior, molecular geometry and electronic structure of quercetin were investigated using the semiempirical AM1 and PM3 methods. Results reveal that quercetin has a nonplanar molecular structure, with cross-conjugation occurring at the C ring. Calculations were also performed for quercetin radical species at the OH groups, showing the presence of three radicals in a narrow range of energy. An interpretation of the antioxidative process mechanism, exerted by quercetin as a free radical scavenger, relies on two isoenergetic radicals with extended electronic delocalization between adjacent rings, also having cross-conjugated systems and being affected by the experimental environment influencing their relative order.

Keywords: *Quercetin; structure; conformation; semiempirical calculations; reactive sites*

INTRODUCTION

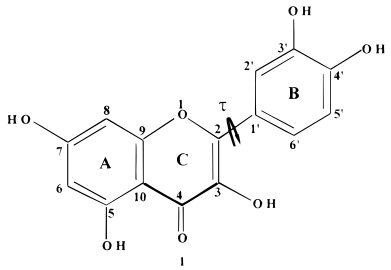
Biophenols (BP) are natural hydroxyaromatic derivatives widely provided in significant amounts by plant material in traditional Mediterranean food culture (Romeo and Uccella, 1996), i.e., olive oil and table olives (Bianco et al., 1998; Esti et al., 1998; Aruoma et al., 1998), grape and wine (Kanner et al., 1994), and fruits and vegetables (Gordon and An, 1995). Many BP-phytochemicals are known to be found in the plant kingdom, with simple and complex biomolecular structures, such as hydroxy benzoic, cinnamic, stilbenic, secoiridoid-monoterpenic and flavonoid moieties.

BPs and their oxidation products can be involved in the defense mechanism of plants against attaching pathogens such as viruses, bacteria (Garcia et al., 1997) and insects (Friedman, 1997).

The functionality of some BP derivatives is well-known to be associated with sensorial perception, giving rise to bitterness and astringency (Bianco et al., 1997; Vekej et al., 1997) and to the food organoleptic quality (Montedoro et al., 1993). Furthermore, they play a fundamental role in the aging of wine (Sato et al., 1996), as they are effective antioxidants through free radical scavenging and metal chelation. In fact, the hydroxyl functional groups, attached to aromatic rings, are responsible for BP antioxidant activity (Saija et al., 1998). The agrifood BP content exhibits many biological effects (Ho et al., 1992; Kinsella et al., 1993) including vasodilatatory, anticarcinogenic and antiinflammatory benefits (Duarte et al., 1993).

Red wine BPs inhibit in vitro the oxidation of low-density lipoprotein considered a primary event in the pathogenesis of coronary heart disease (CHD), providing a molecular interpretation to the origin of the "French Paradox" (Frankel et al., 1993; Kinsella et al., 1993) i.e., the phenomenon of very high fat consumption and low CHD mortality, as in the epidemiological observation in Toulose, France. BPs are minor molecular components of functional foods (Goldberg, 1994), and the flavonoids, mainly quercetin (**1**) (see Chart 1), are the most active compounds from a biological point of view (Okuda et al., 1989; Nakane et al., 1990; Middleton and Kandaswami, 1992; Bourne and Rice-Evans; 1998). In particular, they behave as antioxidant in the red wine and table olives (Esti et al., 1998; Okuda et al., 1989) and exhibit antiviral activity against HIV (Nakane et al., 1990) and Herpes Simplex (Middleton and Kandaswami, 1992).

Chart 1. Schematic Drawing of Quercetin (1)



The physiological effect of **1** has been related to its functional (Rice-Evans et al., 1996) and geometric (van Acker et al., 1996) molecular structure. The antioxidant properties results from the formation of stable radicals, due to the hydroxyaromatic groups at each of the A, B and C rings (van Acker et al., 1996; Jovanovic et al., 1996) and to an extensive electronic delocalization through the whole system. The delocalization has been proposed to spread over the entire BP molecule, involving the A and B rings via C. The absence of C_2-C_3 double bond in C ring is believed to be the main cause of a minor delocalization and thus of a lower biological activity of the other flavonoids (Rice-Evans et al., 1996).

Structural aspects of **1**, investigated at a theoretical level (van Acker et al., 1996; van Acker et al., 1998), have shown a completely planar structure, characterized by an extended conjugation over the whole molecule.

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A quantum-mechanical investigation has thus been undertaken in order to correlate previous experimental findings and to rationalize the mechanism affecting the biological activity of **1**. The following aspects have been analyzed: 1) the stability order of all possible conformations; 2) the role of ring C in allowing extended conjugation over the whole biomolecule; and 3) the energetic differences between possible radical forms obtained by H• removal from OH groups.

MATERIALS AND METHODS

Calculations have been performed employing the semiempirical Austin Method (AM1) (Dewar et al., 1985) and PM3 (Stewart, 1989) implemented in the GAUSSIAN 94 package (Frisch et al., 1995) and successfully applied to biological systems (Russo et al., 1996). The species with even and odd numbers of electrons have been described with RHF and UHF treatments, respectively.

Optimizations have been made without symmetry constraints.

Although it is well-known that ab initio methods are better than semiempirical ones, they require, to obtain reliable information, the use of large basis sets and the introduction of the configuration interaction (CI). For systems of medium-large dimensions, such as quercetin and its radical species, these choices imply a considerable amount of computation time. The advanced semiempirical methods bypass the correlation problem, because it is introduced via parametrization and often gives reliable results with relatively low computational cost.

A wide variety of literature on the performances of AM1 and PM3 methods exists relative to conformational (Topiol et al., 1990; Voets et al., 1990) and spectroscopic (Karaman et al., 1991; Seeger et al., 1991; Coolidge et al., 1991) features. Recently it is been shown that the conformational behavior of DNA base pairs obtained at AM1 level is comparable to the corresponding MP2 one (Hobza et al., 1997).

RESULTS AND DISCUSSION

Quercetin. Two previous theoretical works (van Acker et al., 1996; van Acker et al., 1998) have already related the planarity of **1** to the presence of 3-OH group on ring C: the oxygen atom of this group interacting with the hydrogen attached at $C_{6'}$ or $C_{2'}$, forces ring B to assume a planar position with respect to rings A and C. This fact was indirectly confirmed by the investigation in which BP molecules, lacking the latter group and belonging to flavones, were found to be neither completely planar nor conjugated (Hutter and Bodenseh et al., 1993).

The planar geometry of **1** was correlated to the scavenging activity of the molecule due to the increased conjugation which the planarity offers (Rice Evans et al., 1996; van Acker et al., 1998).

As the first step, the conformational space of **1** was explored as a function of the torsional angle τ between rings C and B, to determinate their preferred relative positions.

The potential energy surface was obtained by a scan of the angle τ in steps of 30° whithout constraints on all other geometrical parameters. The AM1 results are depicted in Figure 1. Removing the constraint also for the torsional angle, the conformational absolute minimum was found at $\tau = 153.3°$ (see Figure 2) followed by a relative minimum at $\tau = 27°$ with an energy difference of only 0.23 kcal/mol. The maximum lies at $\tau = 90°$, and the interconversion barrier between the two minima is about 2.5 kcal/mol.

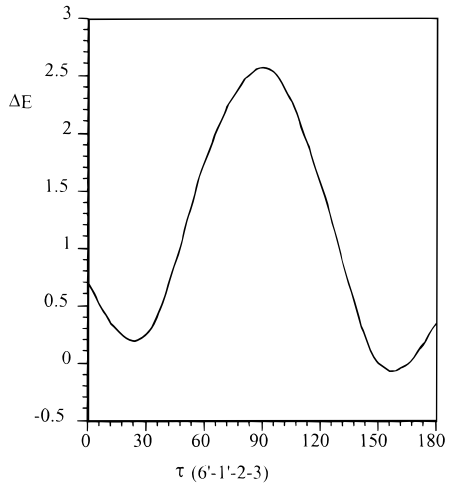

Figure 1. AM1 potential energy surface of quercetin obtained in the framework of flexible rotor model. ΔE is in kcal/mol.

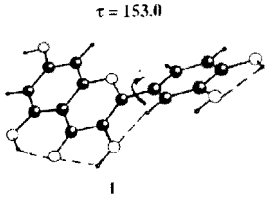

Figure 2. AM1 optimized structure of quercetin absolute minimum. The torsional angle τ is defined as the angle between the two planes 6′-1′-2 and 1′-2−3 atoms (see Chart 1). In the definition, τ (6′-1′-2−3) = 0° and τ (2′-1′-2−3) = 180° represent the planar conformations.

Although the minimization procedure finds a nonplanar minimum, it is worth noting that in going from $\tau = 150°$ to $\tau = 180°$, the potential energy curve is very flat with an energy variation of about 0.5 kcal/mol. This means that the planar conformation is easily obtained requiring a negligible amount of energy.

PM3 calculations indicate also a nonplanar conformation ($\tau = 121.1°$) with a maximum at $\tau = 180°$. These results appears to be unreliable because a heavy underestimation of the conjugation effects seems occur.

Results of the Mayer bond order analysis are collected in Table 1.

AM1 calculations indicate that quercetin is a polar molecule with a dipole moment of 3.409 D. Double bonds are strongly localized in the C_2-C_3 and C_4-O positions of ring C, while bond order values suggest a high independent electronic delocalization at rings B and A only.

The difference between our τ value and that from a previous ab initio STO-3G study (van Acker et al., 1996) is due to the different methods used for the calculations. Because the conformation of phenyl-like systems depends on a delicate balance between nonbonded and conjugative effects, the definitive answer to this problem can only be arrived at the use of high level theoretical methods such as Hartree−Fock plus CI or Density Fuctional.

The small deviation from planarity, due to the nonbonded effects, does not prevent, in principle, the electronic delocalization on the molecule. The structural moiety of the double bonds at ring C around the carbonyl unit indicates a cross-conjugated system (Hutter and Bodenseh, 1993) in which the delocalization is allowed only between C and A or C and B but not for rings A and B.

Table 1. AM1 Bond Order Values of Quercetin (1) and Its Radical Species (2-6)

bond order	1	2	3	4	5	6
$C_{1'}-C_{2'}$	1.365	1.241	1.159	1.231	1.307	1.305
$C_{2'}-C_{3'}$	1.433	1.428	1.599	1.382	1.426	1.425
$C_{3'}-C_{4'}$	1.357	1.287	1.025	1.331	1.321	1.319
$C_{4'}-C_{5'}$	1.290	1.214	0.985	1.010	1.247	1.247
$C_{5'}-C_{6'}$	1.395	1.377	1.369	1.049	1.377	1.373
$C_{6'}-C_{1'}$	1.364	1.254	1.263	1.405	1.319	1.329
$C_{1'}-C_2$	1.006	1.163	1.119	0.982	1.065	1.063
C_2-O_1	1.023	1.042	1.022	1.071	1.044	1.028
C_2-C_3	1.650	0.998	1.379	0.941	1.453	1.466
C_3-C_4	0.966	0.866	0.972	1.039	0.991	0.959
C_4-C_{10}	1.018	1.007	1.029	1.024	0.997	0.988
$C_{10}-C_9$	1.277	1.278	1.284	1.281	1.224	1.428
C_9-C_8	1.376	1.415	1.402	1.426	1.375	1.186
C_8-C_7	1.337	1.321	1.340	1.319	1.034	1.248
C_7-C_6	1.351	1.365	1.352	1.383	1.046	1.385
C_6-C_5	1.369	1.356	1.376	1.345	1.376	1.024
C_5-C_{10}	1.250	1.280	1.268	1.311	1.201	0.995
$C_{3'}-O$	1.037	1.057	1.108	1.739	1.059	1.059
$C_{4'}-O$	1.073	1.086	1.764	1.124	1.091	1.088
C_3-O	1.051	1.866	1.097	1.080	1.083	1.089
C_5-O	1.088	1.133	1.135	1.137	1.112	1.806
C_7-O	1.089	1.102	1.104	1.090	1.738	1.085
C_4-O	1.758	1.879	1.779	1.782	1.814	1.876
C_9-O	1.057	1.046	1.063	1.042	1.055	1.019

In the absolute minimum of **1** (see Figure 2) three hydrogen bonds are present. The formation of the hydrogen bond between the 3-OH and the C_4-O carbonyl groups has a stabilizing effect. This bond is responsible for a further interaction between the oxygen of 3-OH and the hydrogen atom of the $C_{2'}$ carbon, in agreement with previous findings (van Acker et al., 1998). The conformation lacking these bonds (see Figure 3a) is less stable with respect to the absolute minimum by 3.65 kcal/mol. In this case the torsional angle τ between rings C and B appears to be noticeably reduced (139.5°), making the conjugation stabilizing effect quite impossible. The absence of the 5-OH- -C_4-O or of the 4′OH- -3′OH hydrogen bonds (see Figure 3 (parts b and c, respectively)) determines an energy destabilization of 6.04 and 3.62 kcal/mol in the order with respect to the absolute minimum of **1**.

Radical Species of 1. Reduction potentials of quercetin radicals at different pH were determined (Jovanovic et al., 1996). Taking into account spectral and acid−base properties, the existence of two p*K* values of quercetin radicals assigned to the OH group at $C_{4'}$ and to the OH group at C_3 was determined. On the basis of the lowest reduction potential value, the ring B should be the most antioxidant active moiety in quercetin as well as in any flavonoid.

Conclusions about 4′-OH radical, as the most probable species deriving from **1**, were drawn without information on all the other ones by an ab initio quantum mechanical investigation (van Acker et al., 1996). In particular, the determination of the spin distribution, suggested that, upon oxidation of the ring B, the spin remains in ring B, namely on the oxygen atom which releases the H radical, also in the presence of a complete conjugation (van Acker et al., 1998).

In contrast, other experiments (Kano et al., 1994; Dangles et al., 1999a,b) indicate that the antioxidative mechanism of quercetin involves the C ring giving rise to its quinoid form. This situation cannot occur if the hydrogen radical is removed from the 4′-OH group but becomes possible in the case of radicalization of the 3-OH group.

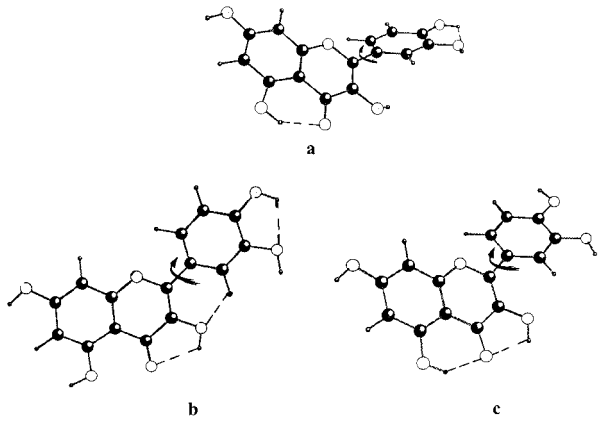

Figure 3. Quercetin structure in the absence of the 3-OH- -C_4O (a), 5-OH- -C_4O (b) and 4′OH- -3′OH (c) hydrogen bonds.

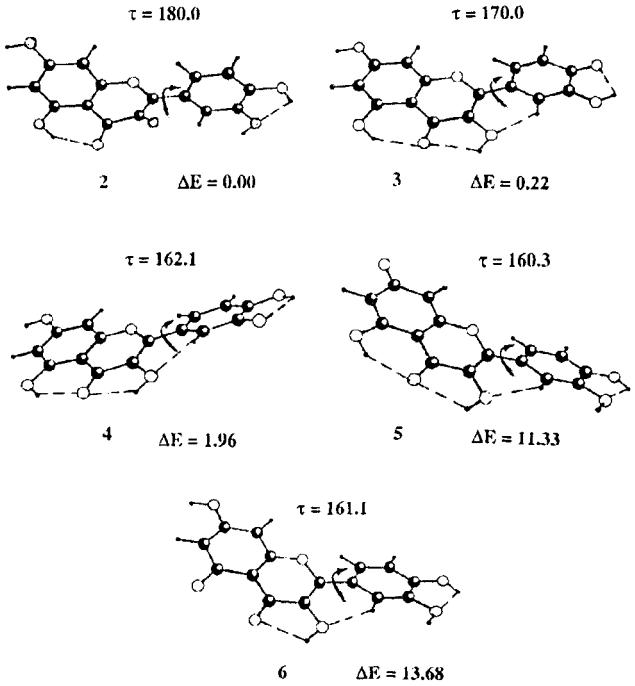

Figure 4. AM1 optimized structure of quercetin radical species. Relative energies (ΔE) with respect to two radicals are given in kcal/mol and torsional angle (τ) in degrees. For the numeration of atoms and the definition of τ see Chart 1 and Figure 2. Absolute energy of radical 2 is −0.311577 au.

Starting from the absolute minimum conformation of **1**, a hydrogen atom removal from the 3-OH and 4′-OH groups yields two radical forms **2** and **3** which are almost isoenergetic, the first one being more stable by 0.22 kcal/mol (see Figure 4).

This novel result could explain the existence of two p*K* values in the experimental work (Jovanovic et al. 1996) as well as the complexity of the ESR spectrum of the quercetin radical (van Acker et al., 1996). Furthemore, it is in agreement with the one-electron oxidation study of quercetin in protic and nonprotic media (Dangles et al., 1999) in which both the isomeric radical **2** and **3** are considered in order to rationalize the experimental measurements.

In any case, the present data refer to the vacuum, while, the experimental measurements were detected in the condensed phase, therefore, in specific chemical-physical conditions, solvent interactions could stabilize one radical more than the other.

Three other isomers, **4**−**6**, generated by the loss of the hydrogen radical from the 3′-OH, 7-OH, and 5-OH groups, are found at 1.96, 11.33, and 13.68 kcal/mol, respectively. The main structural feature of these three forms is a torsional angle differing by about 20° with respect to the absolute minimum.

All the radicals, **2**−**6,** are characterized by a variable number of hydrogen bonds (see Figure 4) that contribute to the stabilization of each species. Except in radical **2**, the hydrogen bond-like interaction, between the oxygen of the 3-OH group and the hydrogen atom attached to the $C_{2'}$ carbon, is always present. The lack of this interaction in the radical **2** is essentially due to the out of plane distortion of ring C.

Although forms **3**−**6** are not completely planar, the deviations from planarity could increase in absence of the latter interaction.

All the radical species of quercetin as well as quercetin have conformational isomers in which the torsional angle τ assumes values near to 0°. Their energy, with respect to the corresponding trans isomers, is lower of about 0.2−0.6 kcal/mol, but the stability order (**2** > **3** > **4** > **5** > **6**) is retained. The barrier for the cis−trans radical interconversion remains of the same height as in quercetin.

Inspection of Table 1 allows further comment on the electronic structure of the five possible radical isomers.

In species **2**, the complete delocalization involves only rings A and B, while ring C is characterized by two double bonds, strongly localized on the carbonyl groups. However, the electronic flow between the ortho-diphenolic moiety and the C ring is still possible, due to the planarity around the torsional angle τ (see Figure 4). This conclusion is supported by the spin density distribution that indicates the C_2 atom as the most probable radical center (78.4%), with the possibility of finding the spin density localized on the C_2', C_4' and C_6' carbon atoms (56.6% on each) of ring B and on the oxygen atom (21.2%) bearing the radical moiety. Radical **2** appears to be as well a cross-conjugated system as quercetin. The conjugation between rings A and C is still occurring (see Table 1), but the unpaired electron is travelling only between rings B and C.

Radical **3** shows electron delocalization between rings A and C and B and C individually, as indicated in Table 1. The radical center of **3** is not unequivocally established because C_3, C_1', C_3' and C_5' are equally involved (62.5%), along with the involved oxygen atom (31.7%). The previous HF study gives a spin population essentially concentrated on the oxygen center (84%), but this result is not surprising because the method employed generally overestimates the spin density on heteroatoms as recently demonstrated by a comparative density functional study on the phenoxyl radical (Adamo et al., 1998). On the other hand, the observation discussed above is in agreement with the flavonoids being good radical scavengers because of "their good delocalization possibilities" (Rice Evans et al., 1996; van Acker et al., 1998).

Therefore, an essentially planar radical ($\tau = 170.0°$), with all hydrogen bonds drawn in Figure 4 and also the hydrogen bond-like interaction between 3-OH and the C_2' proton, is in agreement with previous findings (van Acker et al., 1996; van Acker et al., 1998). The radical center is clearly spread over rings B and C without interfering with ring A.

The isomer **4** shows a partial charge distribution on rings A and B. The ring C is characterized by the C_4−O double bond only. Conjugation is not possible between ring C and the ortho-diphenolic system. Most of the spin density remains on the ring B, mainly on the C_2' and C_6' atoms (74.9%) and with a minor part on the C_4' (60%) and the involved oxygen atom (36.9%).

The radical **5** gives rise to good electron delocalization between rings C and B but, this time, C is not properly conjugated with A. The C_7−O carbonyl group, derived from the hydrogen removal, determines two separated branches of conjugation (see Mayer analysis reported in Table 1). The atomic spin density computations confirm that the radical center commutes from C_8 (or from C_6) (74.8%) to C_{10} (65.7%) to the oxygen of C_4−O group (20%). Furthermore, the spin density can concentrate on the oxygen of C_7−O (41.1%).

Finally, in form **6**, as in a typical cross-conjugated system, the electron delocalization is present between the A and C and C and B rings. Atomic spin density distribution involves only the A and C moieties. The radical center is delocalized between the C_8 (78.5%), C_6 (75.3%), C_{10} (56.3%), the oxygen atom of C_5−O group (34.6%) and the oxygen atom of carbonyl group (19.3%) of the C ring.

The high energetic values of **5** and **6** with respect to the most stable radical **2**, together with their spin distribution, should reduce their contribution to biological activity.

PM3 results are in qualitative agreement with the AM1 ones. In particular, the stability order of radicals is the same. ΔE values with respect to the most stable radical **2** are 1.71, 3.65, 13.6 and 16.6 kcal/mol for **3**, **4**, **5**, and **6** species, respectively. The difference with respect to the AM1 findings concerns the torsional angle τ. In fact, in all generated radicals we obtain the following values: 169.5° (**2**), 130.2° (**3**), 139.1° (**4**), 128.4° (**5**), and 130.7° (**6**). Also in this case it is evident that PM3 method underestimates the conjugation energy contribution, and consequently all the electronic properties will be influenced.

CONCLUSIONS

On the basis of these results, quercetin appears to be a nonplanar molecule, exerting a cross-conjugation effect. The antioxidant action is generated by two isoenergetic radicals showing a planar conformation that allows extended electronic delocalization between adjacent rings, also having cross-conjugated systems. All other radical species lie at higher energetic values with respect to the absolute minimum, so their presence, in the antioxidant mechanism, is rather improbable.

In the radical species **2**, the spin density calculation indicates C_2 as one of the most suitable centers for the unpaired electron.

Furthermore, radical **3** reveals that all C_1', C_3', and C_5' carbon atoms of ring B can equally support the unpaired electron.

Due to the small energy difference between radicals **2** and **3** the experimental conditions could reverse the stability order of the two species and so favor the formation of the compound that carries out its antioxidant activity preferentially involving the B ring.

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