Structure, Conformation, and Electronic Properties of Apigenin, Luteolin, and Taxifolin Antioxidants. A First Principle Theoretical Study

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The structural and electronic properties of apigenin, luteolin, and taxifolin and their radicals were investigated at density functional level of theory employing the B3LYP exchange-correlation potential coupled with the 6-311++G** basis set. Results indicated that the presence of a dihydroxy functionality increases the radical stability through H-bonds formation and favors hydrogen atom abstraction. Bond dissociation energy and ionization potential were also determined in order to know if the antioxidant activity of these compounds proceeds via an H-atom or an electron-transfer mechanism.

Introduction

Flavonoids are a group of naturally occurring compounds, mainly found in a wide variety of fruit, vegetables, leaves, and flowers.[1] They attracted growing global interest during the last years, and the number of known flavonoids has increased considerably. In fact, starting from the work of Kühnau[2] published in 1976, about 800 different compounds were discovered.

Interest was also due to the so-called "French paradox", a phenomenon for which in France, the mortality rate from coronary heart diseases (CHD) is lower than in other European countries, despite the smoking tendency and a diet rich in saturated lipids and fats, which is, however, quite similar to that of the other countries.[3]

The paradox was considered a direct consequence of the French daily consumption of red wine, rich in antioxidative phenolics.[4,5] The work of Hertog and co-workers[5] showed the inverse correlation between flavonoids intake and CHD mortality. Furthermore, it was demonstrated that plant-derived foodstuffs, already considered healthy, contained relatively high amounts of flavonoids. Flavonoids intake and their effects on CHD and cancer mortality were investigated in a number of epidemiological studies and summarized in two important reviews.[6,7]

Flavonoids can act as antioxidants, by inhibiting biomolecules from undergoing oxidative damage through free radicals mediated reactions.[8] They can act in several ways, including direct quenching of reactive oxygen species, inhibition of enzymes, chelation of metal ions (Fe^{3+}, Cu^{+}), promotion of radical production, and regeneration of membrane-bound antioxidants such as α-tocopherol. Their beneficial effects are related to diseases in which oxidative processes are remarkable, i.e., atherosclerosis, coronary heart disease, certain tumors, and aging itself.[9] Flavonoids represent the most common and active edible antioxidants.[10] While fat-soluble tocopherols can exhibit their antioxidant power especially in hydrophobic systems, flavonoids can act both in hydrophilic and hydrophobic environments.

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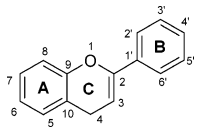
SCHEME 1: Flavonoids Basic Structure



Several studies carried out on flavonoids showed that they have medicinal properties, including antiinfiammatory, antiallergic, antiviral, antibacterial, and antitumor activities.[11,12]

Flavonoids occur as aglycones, glycosides, and metilated derivatives.[1] All the aglycone flavonoids consist of a benzene ring (A) condensed with a six-membered ring (C) which carries a phenyl group (B) as a substituent in the 2-position.[13] C is either a γ-pyrone ring (flavonols and flavones) or its dihydro derivative (flavanols and flavanones) (see Scheme I).

Antioxidant properties were mainly ascribed to flavonols, flavones, and catechins.[14]

The antioxidant ability was related to the number and mutual position of hydroxyl groups and to conjugation and resonance effects.[15] Recently, a quantum-chemical investigation[16] has shown that in quercetin flavonol the planar conformation of radicals allows an extended electronic delocalization.

In literature are reported two main mechanisms by which antioxidants can play their role.[17]

In the H-atom transfer, a free radical $R^{\bullet}$ removes a hydrogen atom from the antioxidant (ArOH):

$$R^{\bullet} + ArOH \rightleftharpoons RH + ArO^{\bullet}$$

The efficiency of the antioxidant ArOH depends on the stability of the radical $ArO^{\bullet}$, which in turn is determined by the number of hydrogen bonds, conjugation, and resonance effects.

The bond dissociation enthalpy (BDE) of the O−H bonds is an important parameter to evaluate the antioxidant action, because the weaker the OH bond the easier the reaction of free radical inactivation will be.

In the one-electron transfer mechanism, the antioxidant can give an electron to the free radical:

$$R^{\bullet} + ArOH \rightleftharpoons R^{-} + ArOH^{\bullet+}$$

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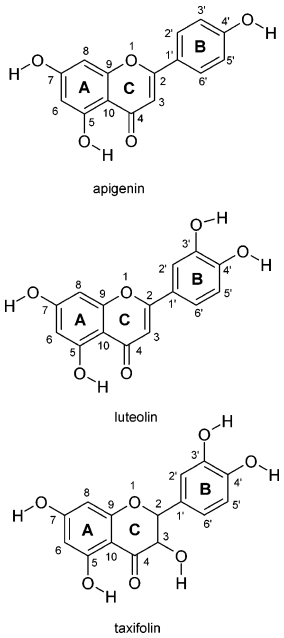

Figure 1. Apigenin, luteolin, and taxifolin structures.

Again, the radical cation arising from the electron transfer must be stable, so it does not react with substrate molecules. In this case, the ionization potential (IP) is the most significant energetic factor for the scavenging activity evaluation.

In this work, we have investigated at density functional level the conformational and electronic features of three flavonoids: apigenin, a member of flavones, luteolin, also belonging to the same class but with an additional hydroxyl group in B ring, and taxifolin, as a flavanone. They were chosen for their peculiar chemical structure, to evaluate the effect of the functional groups on the antioxidant ability. BDE and IP values computed for these systems were used as indicators of the ease by which flavonoids can deactivate free radicals. The spin densities were reported to give better insight into delocalization of the unpaired electron and conjugation effects.

Computational Methods

All calculations reported here were performed by Gaussian 98 code.[18] B3LYP[19,20] exchange correlation potential, in connection with the 6-311++G**basis set,[21,22] was used for optimizing geometries. Harmonic vibrational frequencies were computed for both parent (ArOH) molecule and ($ArO^{\bullet}$ and $ArOH^{+\bullet}$) radicals, to characterize all their conformations as minima or saddle points and to evaluate the zero-point energy (ZPE) corrections, which we have included in all the relative energies, bond dissociation energies, and ionization potentials.

The gas-phase bond dissociation enthalpy (BDE) was calculated at 298 K as the enthalpy difference for the reaction

$$ArOH \Rightarrow ArO^{\bullet} + H^{\bullet}$$

The adiabatic ionization potential (IP) was obtained as the energy difference between the ArOH and $ArOH^{+\bullet}$ species.

Results and Discussion

Flavonoids. The flavonoids studied are depicted in Figure 1. Tables containing optimized geometries of the molecules and their relative radicals are available as supporting information.

Apigenin and luteolin differ for a hydroxyl group in the 3′-position of the B ring. Upon comparative analysis of these two structures, it is possible to evaluate the effect of the catechol moiety (ortho-dihydroxy functionality) on the antioxidant power. Similarly, through a comparison between luteolin and taxifolin, which differ by a C_2–C_3 double bond in C ring, it is possible to understand the influence of the unsaturation in the C ring.

B3LYP/6-311++G** computations suggest that apigenin and luteolin in their equilibrium structure are planar molecules with $C_3-C_2-C_{1'}-C_{2'}$ torsional angles of 0.0° in both cases.

The corresponding conformers with dihedral angles of 180.0° represent two relative minima whose energy values are 0.10 (apigenin) and 0.23 kcal/mol (luteolin) higher than those of the most stable forms.

Transition states on going from 0.0° to 180.0° were found at 4.04 and 3.69 kcal/mol and characterized by torsion angles of 90.9° and 90.8°, for apigenin and luteolin, respectively. As can be noted from energy differences and from interconversion barriers, both the molecules can exists in two conformations that are practically isoenergetic.

Density functional structural features are in disagreement with those suggested by a previous theoretical[23] study. The authors of this last investigation report for flavones a nonplanar conformation, with torsional angles that assume values of 16.5° and 16.3° for apigenin and luteolin, respectively. They explain these results on the basis of those obtained for quercetin in which an OH group in the 3-position forces the system to assume a planar structure because of the presence of a hydrogen-like interaction between the 3-OH moiety and the $C_{6'}$ carbon atom. So, flavones lacking of the 3-OH group should be slightly twisted (≈20°). Our treatment is generally more accurate than the RHF/STO-3G, as confirmed by the previous B3LYP/6-311++G** analysis of quercetin[24] for which we found a torsional angle very similar to the experimental indication.

Apigenin presents an hydrogen bond between 5-OH and the 4-keto groups. In the luteolin molecule all the most stable conformations are characterized by a hydrogen bond in the ortho-dihydroxy functionality and by another similar interaction as in apigenin.

For the saturated taxifolin, the absence of the double bond in the C ring does not allow any electronic delocalization, so a strong deviation from ring coplanarity happens as for other saturated flavonoids (e.g., catechin). Our B3LYP value for the dihedral angle is 101.0°. Van Acker and co-workers[23] suggest in this case a dihedral of 152.4° that again is very different from our value.

The conformation of taxifolin in which the torsional angle $C_3-C_2-C_{1'}-C_{2'}$ is −93.0°, was found at 0.29 kcal/mol with respect to the global minimum. Transition state between absolute and relative minimum structure is located at 0.79 kcal/mol above the most stable conformer. Also in this case, like for apigenin and luteolin, the energetic parameters strongly suggest the coexistence of the two examined forms. The expense for the interconversion between the lowest lying isomers of apigenin and luteolin is, however, slightly higher than for taxifolin because in the first two cases the rotation around the $C_2-C_{1'}$ single bond implies a loss of conjugation and electron delocalization.

In the conformation of minimum energy of taxifolin, the hydroxyl groups are oriented in a such a way to maximize H-bond-like interactions. Taxifolin has three hydrogen bonds established between the hydroxyls and the 4-keto group and between the ortho-dihydroxyls in the B ring, respectively.

In taxifolin, due to the saturation in the C ring, the twisting of the B ring relative to the rest of the molecule does not affect

TABLE 1: Relative Energies for Apigenin, Luteolin, and Taxifolin Radical Species

apigenin	ΔE, kcal/mol	luteolin	ΔE, kcal/mol	taxifolin	ΔE, kcal/mol
4′-OH	0.00	3′-OH(I)	2.28	3′-OH	0.59
5-OH	23.84	4′-OH(I)	0.00	4′-OH	0.00
7-OH	5.19	5-OH(I)	31.45	5-OH	22.40
		7-OH(I)	12.86	7-OH	29.72

the electronic π-delocalization, which takes place individually in the B ring and the A ring.

Flavonoid Radicals. Starting from the absolute minima of each system, three radicals from apigenin and four from luteolin and taxifolin were obtained upon H-atom abstraction from every hydroxyl phenolic group, while a single radical cation for each parent molecule was obtained by removing one electron.

The most stable radical arising from apigenin is the 4′-OH, obtained by abstraction of a hydrogen atom from the hydroxyl attached to the carbon $C_{4'}$. The others species lie at 5.19 (7-OH) and 23.84 kcal/mol (5-OH) (see Table 1). The radicalization of the 5-OH group involves the breaking of the hydrogen bond established with the 4-keto group, so this radical is the less stable one.

In Figure 2 are reported the spin densities for the most stable radicals. For apigenin the 4′-OH radical, the unpaired electron is delocalized over the B and C rings because of the planar conformation assumed by this species. In particular, the spin distribution indicates the carbon $C_{1'}$ as the most probable radical center (Figure 2), followed by the oxygen atom from which the H$^{\bullet}$ is removed.

Also the luteolin 4′-OH radical is completely planar and conjugated; the hydrogen bond in the B ring between the 3′-OH group and the oxygen from which the H atom is removed contributes to the radical stability. In contrast to the apigenin 4′-OH species, in which the odd electron leaves the radicalized oxygen and localizes mainly to the carbon $C_{1'}$, in the luteolin 4′-OH radical the unpaired electron remains on the oxygen atom from which we remove the H$^{\bullet}$. We think that this occurs because the H-bond between the remaining OH group and the neighboring oxygen atom confines the spin density on this last site. Van Acker and co-workers[23] classifies this finding as unexpected, because flavonoids are usually considered good radical scavengers thanks to their excellent delocalization possibility. They observed that when oxidation takes place in the B ring (which is normally the case), almost all spin (84%) remains on the B ring, even for quercetin that is completely conjugated.

The energy gaps among the 4′-OH species and the other isomers are listed in Table 1. The species 3′-OH is very near in energy to the absolute minimum (ΔE = 2.28 kcal/mol), underlining that the hydrogen bond in ring B influences decidedly the stability of the radical. In this species ring B is the most important site for the H-atom transfer. 5-OH and 7-OH species are found at 31.45 and 12.86 kcal/mol. These radicals are characterized by a scarce delocalization. Abstraction of the H atom from the hydroxyl in position 5 implies the breaking of the hydrogen bond formed with the 4-keto group.

In the taxifolin 4′-OH most stable radical species, the electronic flow cannot leave the ring in which the radicalization takes place. In the absence of delocalization, the H-bonding in the B ring affects very substantially the radical stability. For this reason, 3′-OH and 4′-OH are found to be practically isoenergetic (0.59 and 0.00 kcal/mol, respectively). The isomers 5-OH and 7-OH are found at 22.40 and 29.72 kcal/mol, respectively, above the absolute minimum (Table 1). The relative

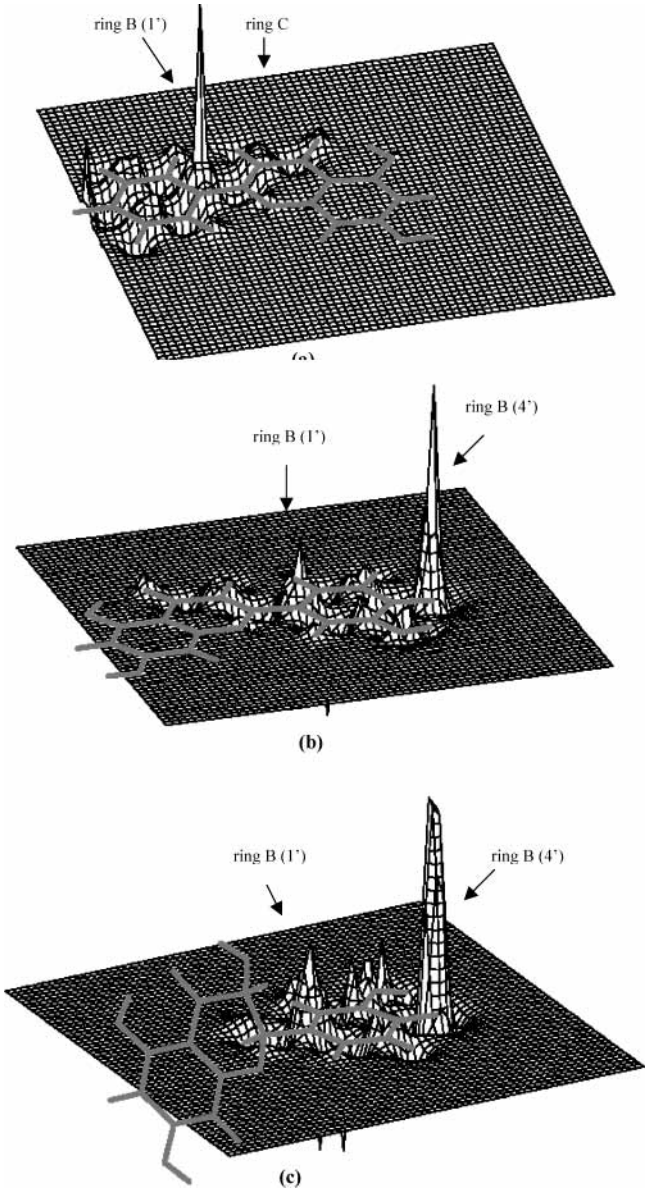

Figure 2. Spin densities for the most stable radical of (a) apigenin, (b) luteolin, and (c) taxifolin.

energy of these latter isomers is quite unexpected because the 5-OH radical is now more stable than the 7-OH, even if its generation involves the breaking of the H-bond. The taxifolin 7-OH radical retains the H-bond between the 5-OH and C_4═O groups, but the presence of the more flexible unsaturated C ring causes a slight distortion of ring A, allowing a better mutual disposition of the 5-OH and the C_4═O carbonyl. The distortion requires an amount of energy that is not compensated by the hydrogen bond formation. The computed H-bonds for the three 7-OH radicals from apigenin, luteolin, and taxifolin of 1.695, 1.697, and 1.739 Å, respectively, confirm that in taxifolin this interaction is weaker than in the other two compounds. In the 4′-OH species, as shown in Figure 2, the spin density remains on the B ring, mainly on the radicalization site. Although, in principle, extended resonance and conjugated effects are not possible in taxifolin due to the saturation of the C ring, it can be considered a good antioxidant among the H-atom transfer mechanism, as its BDE value shows (Table 2).

According to the one-electron transfer, an electron is removed from the HOMO of the parent molecules, giving rise to radical cation species.

TABLE 2: BDE and IP Values in kcal/mol for Flavonoids[a]

species	BDE, kcal/mol	IP, kcal/mol	ΔBDE, kcal/mol	ΔIP, kcal/mol
apigenin	82.20	176.05	−0.69	−16.00
luteolin	74.54	174.44	−8.53	−17.61
taxifolin	74.73	182.84	−8.16	−9.21
tocopherol[b]	71.67	154.90	−11.22	−37.15
quercetin[c]	72.35	166.08	−10.54	−25.97
epicatechin[b]	73.72	170.85	−9.17	−21.20
kaempferol[b]	80.94	167.99	−1.95	−24.06
cianidin[b]	79.37	246.17	−3.52	54.12

[a] ΔBDE and ΔIP are referred to phenol. Gas-phase BDE for phenol is 82.89 kcal/mol; gas-phase IP for phenol is 192.05 kcal/mol. [b] Reference 25. [c] Reference 24.

Even though in the literature some IP calculations are available,[17] no detailed description of these radical species has been carried out.

Except for taxifolin, whose radical cation torsion angle is 101.0°, all cationic radical species are planar and thus completely conjugated. Hydrogen bonds are normally retained as in the parent molecule, contributing to a further stabilization.

The spin densities for the radical cation species arising from apigenin, luteolin, and taxifolin are shown in Figure 3.

Luteolin and apigenin radical cations show a higher degree of conjugation and delocalization with respect to taxifolin. The spin distribution for these two species shows the unpaired electron delocalized over the entire molecules. This is more evident for luteolin, whose radical cation shows a spin distribution that involves A, B, and C rings, in proximity to carbons $C_{4'}$, C_3, and C_5. For apigenin, the oxygen of the 5-OH hydroxyl group can be considered the more accredited radical center, even if there is a possibility of finding the spin density on the C_3 atom of the C ring, the C_8 atom of A ring, and the $C_{1'}$ atom of B ring.

For taxifolin, where the π-flow is more limited, the spin distribution indicates oxygen O_1 and carbon C_6 alone as radical centers.

Our results are in good agreement with that obtained in a recent experimental work,[15] which has elucidated the structure−activity relationships for different classes of flavonoids, through the TEAC (Trolox equivalent antioxidant activity) value, that reflects the ability of hydrogen-donating in scavenging the $ABTS^{\bullet+}$ (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation.

Looking at the structure of quercetin (see Figure 4), which literature indicates as the most active flavonoid, the maximum effectiveness as antioxidant requires the simultaneous presence of the catechol-type structure in the B ring and the 3-OH group attached to the 2,3 double bond and adjacent to the 4-carbonyl in the C ring.

The lack of one of these functionalities reduces the antioxidative ability, as happens in luteolin for the absence of the 3-OH group, in taxifolin for the absence of the 2,3 double bond, and in apigenin for the absence of the ortho-diphenolic structure.

Table 2 lists the BDE (relative to the most stable radical species) and IP values for apigenin, luteolin, and taxifolin. ΔBDE values are referred to phenol BDE, generally chosen as the zero compound.[17] In the same table the values computed for members of other classes of flavonoids[24,25] (see Figure 4 for schemes and labels) are also reported.

BDEs for taxifolin and luteolin are very low, 74.73 and 74.54 kcal/mol, respectively. For these systems, the particular H-atom donating ability can be attributed to the catechol moiety, as confirmed also by the values computed for quercetin, epicatechin, and cianidin (Figure 4), characterized by the same functional group, and tocopherol,[24,25] which is the biological reference compound for the antioxidant activity.

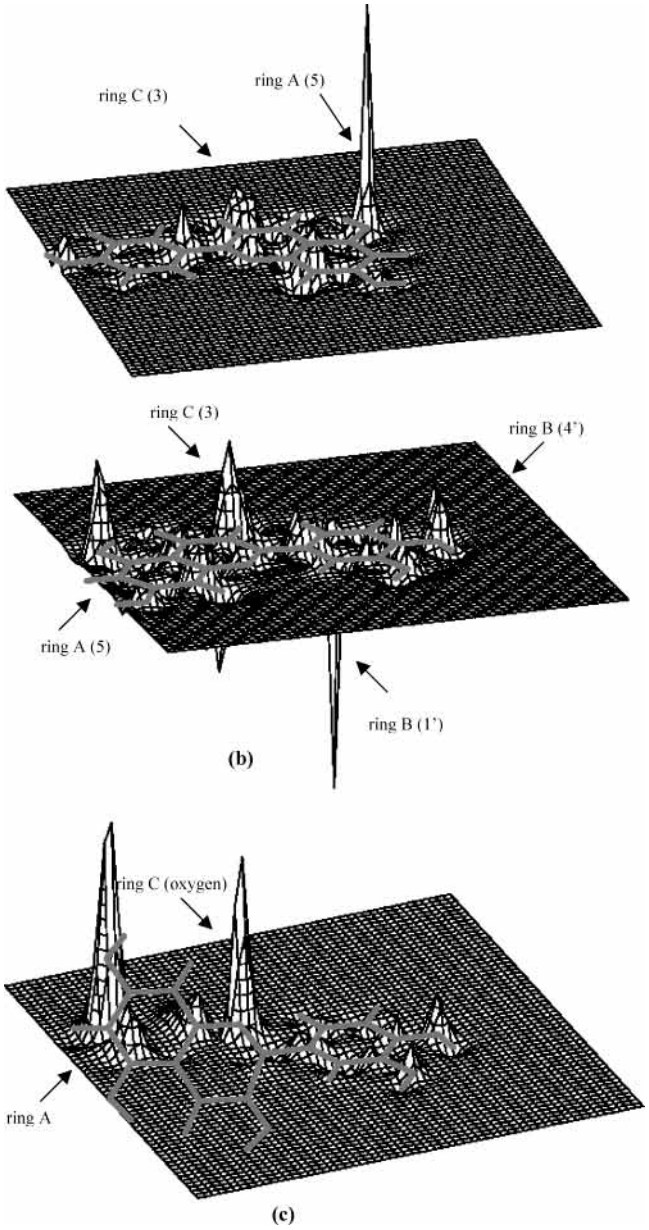

Figure 3. Spin densities for the radical cation of (a) apigenin, (b) luteolin, and (c) taxifolin.

Burton, Ingold, and co-workers[26] have shown that α-tocopherol (vitamin E) is the major lipid-soluble chain-breaking antioxidant normally in human blood plasma.

Apigenin misses this functionality, consequently its BDE is higher (82.20 kcal/mol) and comparable to the kaempferol value.[25] The trend of BDE values for the examined compounds suggests that an essential factor for a good activity as antioxidant is the catechol-type structure, and it can be further improved by the presence of the 2,3 double bond.

The trend for computed IPs is slightly different from that of BDEs. The lowest value is found for luteolin (174.44 kcal/mol), followed by apigenin (176.05 kcal/mol), and taxifolin (182.84 kcal/mol). The electron donating ability of flavonoids seems to be related to an extended electronic delocalization over all the molecule. Systems having a high degree of π-delocalization, such as quercetin, epicatechin, and kaempferol, are the most active among the one-electron-transfer mechanism. The IP, as

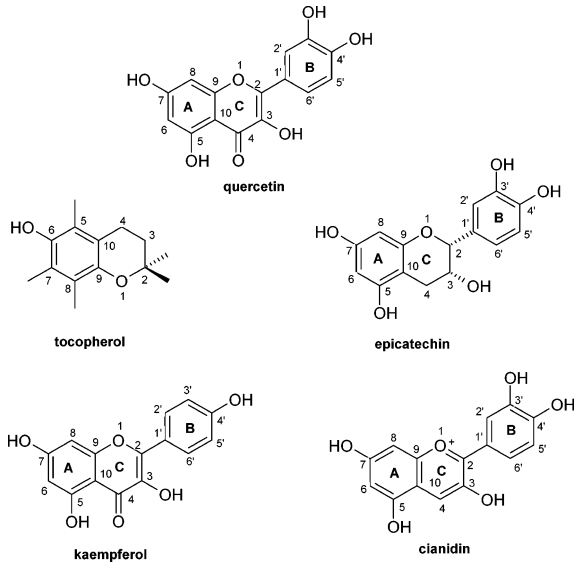

Figure 4. Structures of quercetin, tocopherol, epicatechin, kaempferol and cianidin.

a rule, is dependent on the HOMO energy of the parent molecule, that for conjugated system, is raised in energy, so the abstraction of an electron becomes very easy.

Conclusions

The antioxidant properties of some flavones and flavanones were investigated at the B3LYP/6-311++G** level of theory, to establish what and how the functional groups can affect the radical scavenging activity. On the basis of the obtained results, it can be summarized that two requisites are important for good antioxidant activity:

the ortho-dihydroxy structure in the B ring which confers high stability to the radical species through H-bond formation;

the C_2-C_3 double bond in conjugation with the 4-oxo function in the C ring, which is responsible for the electronic delocalization starting from the B ring.

Moreover, flavonoids with the dihydroxy functionality are the most active in donating an H atom, as confirmed by their low BDE values. So, taxifolin and luteolin are expected to act as hydrogen donors. Luteolin and apigenin appear to be good candidates for the one-electron-transfer mechanism. Their planar conformation and the extended electronic delocalization between adjacent rings determines low IP values.

Comparison between the three considered molecules indicates luteolin as the flavonoid that requires the lowest energy for both H-atom and electron-transfer mechanisms.

Although our results are obtained in vacuum, while the flavonoids act normally is solution, we think that gas-phase BDE and IP are, however, excellent primary indicators of free radical scavenging activity.

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Supporting Information Available: Tables containing Cartesian coordinates for optimized luteolin, apigenin, and taxifolin and their radicals. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hsieh, R. J.; Kinsella, J. E. *Adv. Food Nutr. Res.* **1989**, *33*, 233.
(2) Kühnau, J, *World Rev. Nutr. Diet.* **1976**, *24*, 117.
(3) Renaud, S.; de Lorgeril, M. *Lancet* **1992**, *339*, 1523.
(4) Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. *Lancet* **1993**, *341*, 454.
(5) Hertog, M. G. L.; Feskens, E. J. M.; Hollmann, P. C. H.; Katan, M. B.; Kromhout, D. *Lancet* **1993**, *342*, 1007.
(6) Hollman, P. C. H.; Katan, M. B. *Food Chem. Toxicol.* **1999**, *37*, 937.
(7) Böhm, H.; Boeing, H.; Hempel, J.; Raab, B.; Kroke, A. *Z. Ernährungswiss.* **1998**, *37*, 147.
(8) Visioli, F.; Bellomo, G.; Galli, C. *Biochem. Biophys. Res. Commun.* **1998**, *247*, 60.
(9) Visulli, F.; Galli, C. *J. Agric. Food Chem.* **1998**, *46*, 4292.
(10) Kuehnau, J. *World Rev. Nutr. Diet* **1976**, *24*, 117.
(11) Middleton, E. *Flavonoids TIPS* **1984**, *5*, 335.
(12) Harborne, J. B. *The Flavonoids: Advances in research since 1980*; Chapman and Hall: London, 1988.
(13) Geissmann, T. A. *The Chemistry of Flavonoids Compounds*; Geissmann, T. A., Ed.; Pergamon Press: Oxford, 1962; p 1.
(14) Herrmann, K. *Z. Lebensm. Unters. Forsch.* **1970**, *144*, 191.
(15) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radicals Biol. Med.* **1996**, *7*, 933 and references therein.
(16) Russo, N.; Toscano, M.; Uccella, N. *J. Agric. Food Chem.* **2000**, *48*, 3232.
(17) Wright, J. S.; Johnson, E. R.; Di Labio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173.
(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millan, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malich, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzales, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andreas, J. L.; Head-Gordon, M.; Reploge, E. S.; Pople, J. A. *Gaussian 98*; Gaussian Inc.; Pittsburgh, PA, 1998.
(19) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(20) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
(21) McLean, A. D.; Chandler, G. S. *J. Chem. Phys.* **1980**, *72*, 5639.
(22) Krisknan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. *J. Chem. Phys.* **1980**, *72*, 650.
(23) van Acker, S.; de Groot, M. J.; Van den Berg, D. J.; Tromp, M. N. J. L.; Donné-OP den Kelder, G.; Wim, J. F.; van Der Vijgh, W. J. F.; Bast, A. *Chem. Res. Toxicol.* **1996**, *9*, 1305.
(24) Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. *Theo. Chem. Acc.*, in press.
(25) Leopoldini, M.; Marino, T.; Russo, N.; Toscano, M. *Phys. Chem. Chem. Phys.*, submitted for publication.
(26) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194.