Density Functional Theory Study of the Conformational, Electronic, and Antioxidant Properties of Natural Chalcones

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Chalcones are natural compounds that are largely distributed in plants, fruits, and vegetables. They belong to the flavonoid group of molecules, and some of them exhibit numerous biological activities. The results of quantum chemical calculations (based on density functional theory, using the B3P86 exchange-correlation potential) are reported for 11 chalcones, in the gas phase and in the presence of an implicit solvent (using the conductor-like polarizable continuum model, C-PCM). These results are discussed in regard to the capacity of these chalcones to scavenge the 2,2-diphenyl-1-pycril-hydrazyl (DPPH) free radical. The O−H bond dissociation enthalpy (BDE) parameter, which is calculated for each OH group, seems to be the best indicator of the anti-radical property of these compounds. This demonstrates the importance of the H atom transfer mechanism to explain their capacity to scavenge the free radicals. The active sites are identified as the 6′-OH group and the 3,4-dihydroxy-catechol. The α,β-double bond is influential in determining the activity.

Introduction

Chalcones (or 1,3-diaryl-2-propen-1-one) form a group of open-chain flavonoids, in which two aromatic rings are linked by a three-carbon α,β-unsaturated carbonyl system (see Figure 1). The most simple chalcone (i.e., without any OH and OCH_3 substitution on the aromatic rings) has not been encountered as a naturally occurring compound; however, numerous derivatives have been identified in fruits, vegetables, and various plants. They are classified according to the A- and B-ring substitution by OH and OCH_3 groups. Aglycone as well as glycoside forms naturally occur in plants. They are precursors in flavonoid biosynthesis: the enzymatic cyclization of the 6′-hydroxychalcones leads to the formation of flavanones, and subsequently to a large number of flavonoid groups, including flavones, flavonols, dihydroflavonols, aurones, and isoflavones.[1] In addition to their biosynthetic importance, they are responsible for the yellow color of many plant organs.

The presence of a α,β-unsaturated bond and the absence of the central C-ring are two specific characteristics of chalcones, making these compounds chemically different from the other flavonoids. Numerous biological activities have been observed for these compounds over the past 20 years, including antioxidant, chemopreventive, antiproliferative, antimicrobial, and antiviral activities.[2−4] For some of those activities, experimental data succeeded in establishing the structure−activity relationships (SARs) and the role of the OH groups has been noted.[5,6]

Chalcones, similar to other flavonoids, can also mimic estrogen and andrenal androgens (see, for example, estradiol in Figure 1). As a consequence, they demonstrate abilities to bind to the estrogen receptor[7−9] or to inhibit aromatase (i.e., estrogen synthase),[10] thus decreasing estrogen production and subsequently inhibiting the proliferation of the hormone-dependent breast cancer cells (the MCF-7 cancer cell line).[11] The anti-estrogenic activity of chalcones has also been correlated to their redox potential, measured by the capacity to inhibit the 2,2-diphenyl-1-pycril-hydrazyl (DPPH) radical.[12] Indeed, the SARs are very similar for the three tests (radical scavenging, anti-estrogenic, and antiproliferative on MCF-7). Of the different chalcones reported by Calliste et al.,[12] naringenin chalcone (compound **8** in the present paper) is the most active. The presence of both the α,β-double bond and the 6′-OH group has been proposed to explain that activity. Therefore, the elucidation of the redox capacity of chalcones to scavenge free radicals is of prime importance for understanding their antioxidant activity, but also for understanding other aspects of their biological activity, including their capacity to inhibit hormone-dependent cancer cell proliferation.

Quantum chemistry is a powerful approach to investigate the redox properties of phenolic compounds;[13,14] our objective here is to clarify the role of the double bond and the OH groups, especially the 6′-OH group, in the redox activity of chalcones. For this purpose, we quantum-chemically evaluate the O−H bond dissociation enthalpy (BDE) and the ionization potential (IP) in a series of 11 chalcones (4-hydroxychalcone, 2′-hydroxychalcone, 4′-hydroxychalcone, 2′,4-dihydroxychalcone, 2′,3′,4′-trihydroxy-chalcone, 2′,4′,4-trihydroxy-chalcone (or isoliquiritigenin), 2′,4′,3,4-tetrahydroxy-chalcone (or butein), 2′,4′,6′,4-trihydroxy-chalcone (or naringenin chalcone), 3,4,2′,4′,6′-pentahydroxychalcone, 3-methoxy-2′,4′,4-trihydroxychalcone (or homobutein), and phloretin; these are identified as compounds **1**−**11** in Figure 1. These properties are rationalized in terms of the radical scavenging activity evaluated from the capacity to inhibit the DPPH radical, to establish a hierarchy between the 11 chalcones, on the basis of coherent IC50 values (i.e., the chalcone concentration required to reduce the DPPH electron spin resonance (ESR) signal by 50%) (see Table 1). The DPPH

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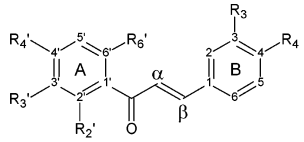
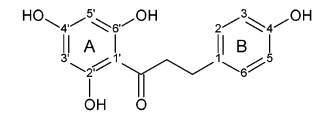
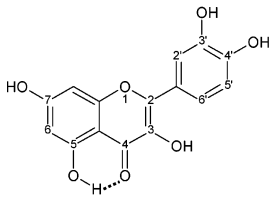


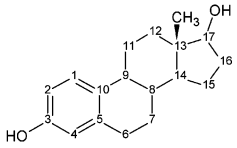
1 *(4-hydroxy-chalcone)*:
R2'=H, R3'=H, R4'=H, R6'=H, R3=H, R4=OH
2 *(2'-hydroxy-chalcone)*:
R2'=OH, R3'=H, R4'=H, R6'=H, R3=H, R4=H
3 *(4'-hydroxy-chalcone)*:
R2'=H, R3'=H, R4'=OH, R6'=H, R3=H, R4=H
4 *(2',4-dihydroxy-chalcone)*:
R2'=OH, R3'=H, R4'=H, R6'=H, R3=H, R4=OH
5 *(2',3',4'-trihydroxy-chalcone)*
R2'=OH, R3'=OH, R4'=OH, R6'=H, R3=H, R4=H
6 *(2',4',4-trihydroxy-chalcone or isoliquiritigenin)*:
R2'=OH, R3'=H, R4'=OH, R6'=H, R3=H, R4=OH
7 *(2',4',3,4-tetrahydroxy-chalcone or butein)*:
R2'=OH, R3'=H, R4'=OH, R6'=H, R3=OH, R4=OH
8 *(2',4',6',4-tetrahydroxy-chalcone or naringenin chalcone)*:
R2'=OH, R3'=H, R4'=OH, R6'=OH, R3=H, R4=OH
9 *(2',4',6',3,4-pentahydroxy-chalcone)*:
R2'=OH, R3'=H, R4'=OH, R6'=OH, R3=OH, R4=OH
10 *(3-methoxy-2',4',4-trihydroxy-chalcone or homobutein)*:
R2'=OH, R3'=H, R4'=OH, R6'=H, R3=OCH_3, R4=OH



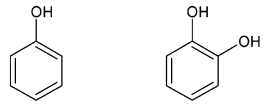
11 *(phloretin – 2',4',6'-trihydroxy-3-(4-hydroxyphenyl)propiophenone)*:
R2'=OH, R3'=H, R4'=OH, R6'=OH, R3=H, R4=OH



Quercetin (flavonol)

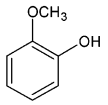


Estradiol



Phenol

Catechol



Guaiacol

Figure 1. Structures of the studied chalcones and related compounds. Note that, in chalcones, the numbering of the substituent positions is reversed, compared to the other flavonoids.

TABLE 1: IC50 Values of the Antioxidant Activity on DPPH[a]

compound	IC50 ($\times 10^{-5}$ M)	compound	IC50 ($\times 10^{-5}$ M)
1	$\gg$500.0 [9%][b]	quercetin	2.5
2	$\gg$500.0 [0%][b]	guaiacol	30.0
3	$\gg$500.0 [1%][b]	catechol	4.5
4	$\gg$500.0 [7%][b]	phenol	$\gg$500.0 [0%][b]
5	5.0		
6	$\gg$500.0 [11%][b]		
7	2.7		
8	100.0		
9	3.1		
10	13.0		
11	440.0		

[a] IC50 represents the chalcone concentration required to reduce the DPPH electron spin resonance (ESR) signal by 50%. Standard deviations are <10%. [b] Values given in brackets represent the inhibition percentage at 5×10^{-3} M for compounds with IC50 >500.0×10^{-5} M.

scavenging activity provides reliable information concerning the H atom abstraction capacity. It does not give any information on the inhibition of enzymes or chelation of metals involved in oxidative stress; in that sense, this is not an indicator of the total antioxidant activity in vitro and in vivo. Nonetheless, it gives a generally good indication of the scavenging capacity toward oxidative species, including hydroxyl and peroxyl radicals. As a consequence, the DPPH activity often correlates well with the inhibition of the lipid peroxidation process.[15,16] The IC50s of three model phenolic compounds (phenol, catechol, and guaiacol; see Figure 1) have also been measured and will be useful in the discussion, which is organized as follows: first, the conformational behavior is analyzed. The contribution of each OH group to the radical scavenging activity is then established, supported by the theoretical BDEs obtained in the gas phase or in the presence of an implicit solvent (using the conductor-like polarized continuum method (C-PCM) approach). The role of the α,β-double bond is also discussed, and, finally, we address the electron-transfer mechanism.

Experimental Methods

DPPH Radical Scavenging Activity. Because of its paramagnetic properties, DPPH exhibits a characteristic ESR signal. The ESR spectra were obtained with a Bruker model ESP300E spectrometer, using microsampling pipets at room temperature under the following conditions: modulation frequency of 100 kHz, microwave frequency of 9.78 GHz, microwave power of 2 mW, modulation amplitude of 1.97 G, and time constant of 10.24 ms. The ESR spectra were recorded immediately after sample preparation. Mixtures that contained 50 μL of chalcone, dissolved in methanol at different concentrations, and 50 μL of DPPH ethanolic solution (5×10^{-4} M) were tested.

The inhibition ratio (IR) was calculated as follows:

$$\mathrm{IR} = \frac{\mathrm{ref} - \mathrm{chalcone}}{\mathrm{ref} - \mathrm{bg}}$$

where ref and chalcone are the values of the double integrals for the ESR spectrum of the reference (DPPH + solvent) and the tested solution (DPPH + solvent + chalcone), respectively; bg represents the background signal. The data were the average of three measurements. The IC50 values (Table 1) were calculated from the IR = *f*(concentration) curves.

Preparation of Naringenin Chalcone and Structural Identification. Naringenin chalcone (compound **8**) was obtained from the corresponding flavanone (naringenin). Naringenin was dissolved in methanol and was added to a solution of potassium hydroxide (KOH). The reaction mixture was heated at 40 °C for 10 min, then diluted with water and acidified with HCl (pH 6). After an addition of ethyl acetate, the organic layer was separated, washed with water, dried over $MgSO_4$, and evaporated to dryness. Purification of the residue was performed via medium-pressure liquid chromatography (MPLC), using a Polygoprep (Ø 60−20 μm) column (200 × 20 mm) with CH_2Cl_2/CH_3OH as mobile phases.

The identification was performed via nuclear magnetic resonance (NMR), using a Bruker model DPX Avance spectrometer with tetramethylsilane (TMS) as the internal standard. The NMR characteristics of compound **8** are as follows: ^{1}H NMR (400 MHz, CD_3COCD_3): 10.56 (4H, br s, OH), 8.12 (1H, d, J = 15.5 Hz, H-α), 7.76 (1H, d, J = 15.6 Hz, H-β), 7.57 (2H, d, J = 8.6 Hz, H-2, H-6), 6.91 (2H, d, J = 8.7 Hz, H-3, H-5), 5.97 (2H, s, H-3′, H-5′). ^{13}C NMR (100 MHz, CD_3COCD_3): 193.3 (C=O), 165.8 (C-2′, C-6′), 165.5 (C-4′), 160.7 (C-4), 143.3 (C-β), 131.3 (C-2, C-6), 128.3 (C-1), 125.4 (C-α), 116.9 (C-3, C-5), 105.8 (C-1′), 96.1 (C-3′, C-5′).

The other 10 chalcones were purchased from Indofine, and phenol, catechol, and guaiacol were purchased from Sigma.

Computational Methods. The redox reactivity of phenolic compounds (ArOH) can follow two different chemical pathways:

$$\mathrm{ArOH} + \mathrm{R}^{\bullet} \rightarrow \mathrm{ArO}^{\bullet} + \mathrm{RH} \quad (1)$$

$$\mathrm{ArOH} + \mathrm{R}^{\bullet} \rightarrow \mathrm{ArOH}^{+\bullet} + \mathrm{R}^{-} \rightarrow \mathrm{ArO}^{\bullet} + \mathrm{RH} \quad (2)$$

$R^{\bullet}$ can be any radical involved in the oxidative stress, including $^{\bullet}OH$, $O_2^{\bullet -}$, $ROO^{\bullet}$, $CH_3{}^{\bullet}CHOH$, ...

Reaction 1 is the homolytic dissociation of the O−H bond. This reaction can occur on each OH group of the phenolic compound (the chalcone, for example) and it is governed by the BDE of the OH groups and by the enthalpy ΔE_1 of reaction 1. The BDE is an intrinsic thermodynamical parameter, whereas ΔE_1 is dependent on the radical that is reacting with the chalcone. The lower the BDE, the easier the O−H bond breaking, and most important is its role in the antioxidant reactivity.

Redox properties of phenolic compounds have theoretically been established, using semiempirical Hartree−Fock methods[17−19] and more recently by density functional theory (DFT).[15,20−23] DFT seems to be the most reliable approach to obtain reliable BDE values that are close to experimental data, whereas semiempirical methods underestimate the π electron delocalization and, for example, yield nonplanar geometries for flavonols and flavones (i.e., flavonoids with a 2,3-double bond; see Figure 1). DFT recently produced a valid estimation of the BDE for phenolic compounds[14] and highlights the role of the OH groups of the B-ring[20,21] and the 3-OH group[22] in flavonoids. We have recently shown that a DFT approach, using the B3P86 functional,[24,25] is the most relevant for the BDE evaluation for phenol and catechol in the gas phase,[22] giving results that are very similar to the experimental values (with an accuracy of better than 1 kcal/mol). Compared to the widely used B3LYP functional, B3P86 calculations show a shift of BDEs by ∼4 kcal/mol, which is closer to the experiment data for phenol and catechol. On that basis, here, we extend the use of the DFT/B3P86 methodology to chalcones. We previously have reported B3P86 calculations on other flavonoids, and it must be noted that both theoretical approaches (B3LYP and B3P86) show the same trend.[20,22] To our knowledge, only one paper has reported on the use of DFT for the study of chalcones, showing that the electron affinities of different substituted chalcones are successfully reproduced.[26] That work did not address the antioxidant properties that we are investigating here.

Because of the importance of π electron delocalization in chalcones, theoretical investigations require an accurate description of the electron density over the entire molecule to take into account all the possible electronic effects; therefore, we have used the double-ζ basis set 6-31+g(d,p). This B3P86/6-31+G(d,p) scheme gives a BDE of 87.2 kcal/mol for phenol, which is very similar to the experimental value (87.0 ± 1.0 kcal/mol).[27] Such accuracy is attained by taking into account temperature corrections (zero point energy (ZPE), as well as translational, rotational, and vibrational energies). Because of the computational effort required for such calculations on the series of chalcones studied in this paper, those corrections have only been computed for compound **9**, which is a good model for the other 10 chalcones, because it possesses the maximum number of substituents. For each OH group of this compound, we obtained a correction of ∼8 kcal/mol (lower BDEs at 298 K, compared to those at 0 K) and we have then applied this value to the other 10 chalcones.

The geometries and energies of the phenoxyl radicals were obtained after the H atom was removed from each OH group of the optimized structure of the parent molecule and using an unrestricted approach, which is more relevant to DFT calculations.[28]

The geometry optimizations were performed with (U)B3P86/6-31+G(d,p), without and with taking the solvent effects into account. One most relevant study on solvent effects is that of Guerra et al.[29] They concluded that solvent molecules are arranged in cages (of at least 6 water molecules) in the vicinity of the OH group of phenol. Such a calculation for each OH group of the 11 chalcones would have been untractable. Thus, we decided to use a polarizable continuum model (PCM) for water and methanol. Continuum solvation models are usually based on polarizable dielectrics that are described by the dielectric constant of the solvent ($\epsilon = 78.39$ for water, $\epsilon = 32.63$ for methanol). A conductor-like scheme has been recently proposed,[30−32] which coincides with the dielectric scheme when $\epsilon \rightarrow +\infty$, that is, for very polar environments (as in our case). More recently, a conductor-like approach such as this has been implemented in the classical PCM formalism.[33] The resulting C-PCM (conductor-like PCM) procedure is then particularly relevant to estimate the solvent influence on the electronic properties.

All calculations were performed using the Gaussian 03 software.[34]

Results and Discussion

We calculated the BDE (i.e., $E(\mathrm{ArOH}) - E(\mathrm{ArO}^{\bullet}) - E(\mathrm{H}^{\bullet})$) for each OH group of the 11 chalcones. To evaluate the capacity of each group to react with the $R^{\bullet}$ free radicals, those BDEs were compared to the BDE of RH, relative to the following reaction:

$$\mathrm{RH} \rightarrow \mathrm{R}^{\bullet} + \mathrm{H}^{\bullet} \quad (3)$$

The reaction between the OH group and the $R^{\bullet}$ radical is thermodynamically favorable when the BDE of RH is greater than that of the OH group; in other words, for ΔE_1 (i.e., $E(\mathrm{ArO}^{\bullet} + \mathrm{RH}) - E(\mathrm{ArOH} + \mathrm{R}^{\bullet})$) negative and reaction (1) exothermic.

The BDEs of each OH group of the 11 chalcones are compared to the BDEs of (i) DPPH−**H**, (ii) **H**−OH, (iii) ROO−**H**, and (iv) $CH_3C\mathbf{H}_2OH$, which gives an indication of the reactivity with (i) the stable DPPH radical; (ii) $^{\bullet}OH$ (i.e., one of the most toxic radical species produced by oxidative stress); (iii) the peroxyl radical, which plays a major role in the lipid peroxidation process; and (iv) the carbon-centered $CH_3{}^{\bullet}CHOH$ radical, involved in various oxidative stress processes, including those initiating liver injuries.

Those BDEs have been experimentally estimated to be (i) 80 kcal/mol,[35] (ii) 118.8 ± 0.1 kcal/mol,[36] (iii) 88 kcal/mol,[37] and (iv) 96 ± 1 kcal/mol[38] for DPPH−**H**, **H**−OH, ROO−**H** ($R = CH_3$), and $CH_3C\mathbf{H}_2OH$, respectively. We calculated those four BDEs using the (U)B3P86/6-31+G(d,p) scheme and, taking the temperature correction into account, we obtained values of 79.3, 119.1, 84.1, and 94.8 kcal/mol for DPPH−**H**, **H**−OH, ROO−**H**, and $CH_3C\mathbf{H}_2OH$, respectively. These values are in very good agreement with the experimental data and confirm the relevance of the method for such comparisons.

Reaction 2 consists in an oxidation step to form an $ArOH^{+\bullet}$ cation, followed by the heterolytic dissociation of an O−H bond in the cation. The first step is governed by the ionization potential (IP) of ArOH and by the stabilization of the $ArOH^{+\bullet}$ cation. IP is a global property of the molecule; it is calculated as the difference in energy between the molecule (ArOH) and the cation ($ArOH^{+\bullet}$). The ΔE_2 (i.e., $E(\mathrm{ArOH}^{+\bullet} + \mathrm{R}^{-}) -$

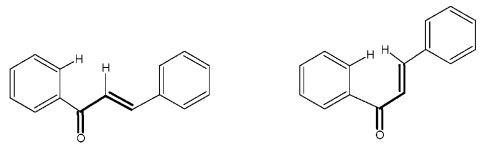


Figure 2. Structures of the *s*-cis and *s*-trans conformers of chalcones; *s*-cis and *s*-trans represent the relative position of the C=O and C=C double bonds, with respect to the single bond.

E(ArOH + R•)) values have also been calculated for DPPH to investigate, in that case, the importance of the second mechanism (i.e., electron transfer) compared to the first one (i.e., reaction 1). Previous theoretical studies demonstrated the predominant role of the latter[14,20,21] for other phenolic compounds, which is especially well-accepted for the reaction between phenols and DPPH radicals. Nonetheless, both mechanisms must be studied in detail for each group of polyphenols on each activity, because it is clear that the balance between reactions 1 and 2 could be influenced by the redox properties of the compound itself and by the chemical and biological environment (the type of R• radical, the temperature, the solvent, the presence in a receptor for an enzyme activity,...).

Conformational Study. In polyphenolic compounds, the behavior of the different OH groups is largely influenced by the neighboring groups and by the geometry. Thus, the first parameter of importance for explaining the redox capacity of chalcones is the conformational one. To our knowledge, no conformational study has been reported in the literature for chalcones. Thus, in the present study, we first focus on the geometrical characteristics obtained from the theoretical calculations. A complete conformational analysis has been achieved with compound **2** (the 2′-OH chalcone), which is used as a good model for the other chalcones. Indeed, the 2′-OH group is present in all the chalcones studied here, except for compounds **1** and **3** (the presence of an OH group on that position is actually common to numerous flavonoids). As a result, hydrogen bonding can occur between that OH group and the O atom of the neighboring carbonyl group, as illustrated for quercetin in Figure 1. That hydrogen bond is expected to favor coplanarity between the A-ring and the central part of the chalcone molecules.

The α,β-double bond is always considered to be in the trans configuration, because the cis configuration is very unstable, because of strong steric effects between the B-ring and the carbonyl group. Then two conformers must be taken into account: the *s*-cis and the *s*-trans compounds (see Figure 2). Those conformers correspond to two different conformations of the α,β-double bond and the keto group. Thus, we performed the conformational analysis of both conformers for compound **2**. Usually *s*-*trans*-buta-1,3-diene is more stable than its *s*-cis counterpart, because of lower steric hindrance and stronger conjugation. The case of chalcones is different: the *s*-cis conformer seems to be fully planar, whereas steric hindrance between H atoms (see Figure 2) leads the *s*-trans conformer to be nonplanar, with a torsion angle O−C−Cα−Cβ of approximately −142°. The difference in energy between the two conformers is 5.6 kcal/mol in favor of the *s*-cis compound, with a barrier of 8.3 kcal/mol (from *s*-cis to *s*-trans).

For all the other unsaturated chalcones, we extrapolated the results of this conformational analysis and we addressed the different hydrogen bonding that could exist: (i) between the ortho-OH groups in the B-ring for compounds **7** and **9**, (ii) between the adjacent OH groups in the A-ring for compound **5**, and (iii) between the 4-OH group and the O-atom of the neighboring OCH_3 group for compound **10**.

We have found that compounds **1** and **3** are also planar, despite the absence of an OH group on the 2′ position. This suggests that the planarity is essentially due to the π electron delocalization and that the participation to the planarity of the hydrogen bonding of the 2′-OH group is weak. Nonetheless, for compounds that possess the 2′-OH group, the hydrogen bond must exist; otherwise, the coplanarity is lost due to steric interactions between O-2′ and the O atom of the keto group, leading to a loss in stability of ∼15 kcal/mol.

Compound **11** (phloretin) is the only saturated chalcone (it is a dihydrochalcone), and the conformational analysis has been conducted in an independent way, compared to the other chalcones. As a consequence of the loss of the double bond, the coplanarity of the A- and B-rings is lost: the most stable conformation is obtained with a torsion angle Cα−Cβ−C1−C2 of ∼94°. Coplanarity still remains between the A-ring and the keto group.

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, 4, 2′, 3′, 4′, and 6′ positions. No geometrical parameter constraint was imposed during the optimization, except those favoring the stabilizing effects due to hydrogen bonding between two adjacent OH groups. When existing, this structural feature must be taken into account in the molecule and in the corresponding radicals, especially those originated from the catechol moiety (compounds **7** and **9**) and from the tri-OH substitution in the A-ring (compound **5**). In those radicals, we were careful to maintain the hydrogen bonding between the O atom, where hydrogen abstraction occurred, and the adjacent OH group.

No significant geometrical change has been observed when going from the molecule to the phenoxy (ArO•) and the cation ($ArOH^{+\bullet}$) radicals obtained after hydrogen and electron abstraction, respectively, except for the 2′-OH radicals of the unsaturated chalcones. In that case, because of steric interaction between O-2′ and the O atom of the carbonyl group (and the loss of hydrogen bonding), the O−C−C1′−C2′ torsion angle drifts (in a barrierless process) from 0° to a value that is dependent on the presence of the 6′-OH group. The potential curves (E = f(O−C−C1′−C2′)) are plotted in Figures 3a and 3b for compounds **6** (without the 6′-OH group) and **8** (with the 6′-OH group), respectively. For chalcones that have not been substituted at C-6′, the structure again becomes planar, with a torsion angle of 180°, whereas for 6′-OH substituted chalcones, the most stable conformer is obtained for a torsion angle of ∼140°. In the second case (i.e., in the presence of both 2′-OH and 6′-OH groups in the parent molecule: compounds **8**, **9**, and **11**), another rearrangement can occur: a twist of the H−O−C6′−C1′ torsion angle occurs (Figure 3c) to form a new hydrogen bond between the 6′-OH and the keto groups. This second rearrangement has a very low energy barrier of ∼2 kcal/mol in the gas phase (Figure 3c) and ∼3 kcal/mol in the presence of a C−PCM solvent (water or methanol). This eventually gives a planar geometry for the 2′-OH radical, which is thus stabilized in the presence of the 6′-OH group.

Except for the specific case of the 2′OH radicals, the fact that no significant changes in geometry have been observed when going from the molecule to the ArO• and $ArOH^{+\bullet}$ radicals brings us to the conclusion that the entropic variation can be neglected. As a consequence, the energy barriers for the hydrogen transfer (reaction 1) and the electron transfer (reaction 2) are assumed to be negligible.

Redox Properties for the *s*-cis and *s*-trans Conformers. Although the *s*-trans conformer is less stable, the difference in

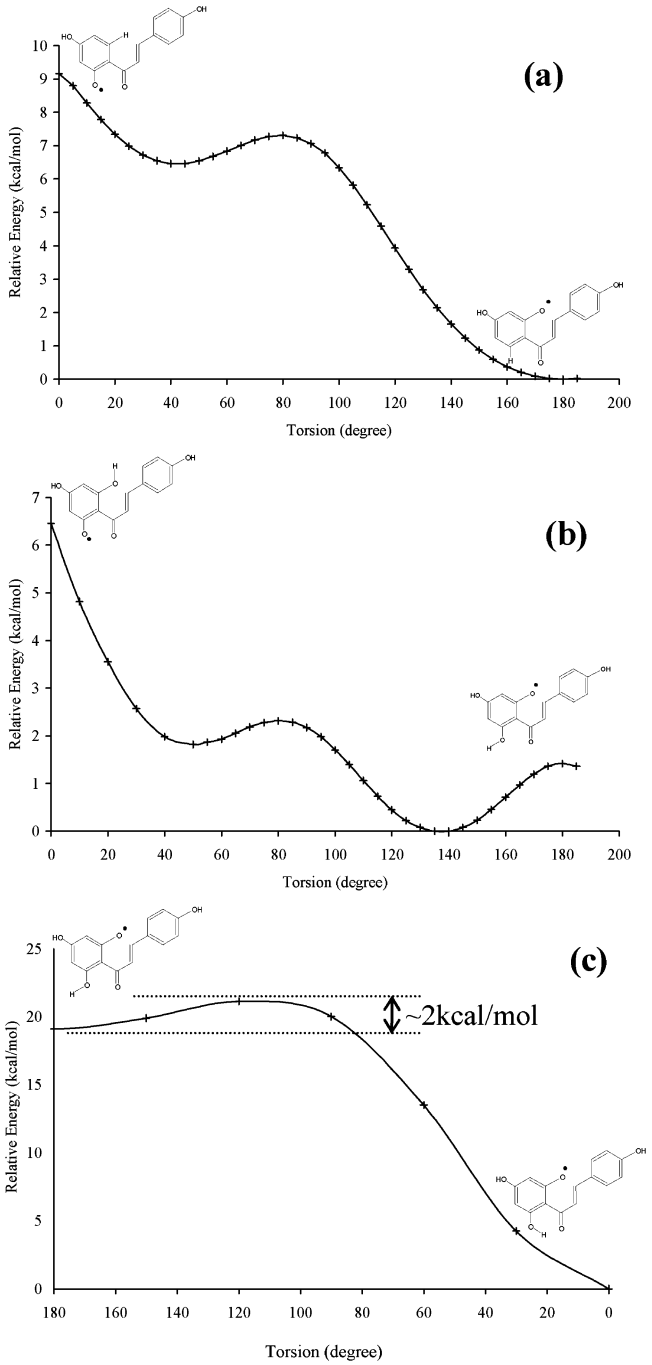


Figure 3. Potential curve of the O−C−C1′−C2′ torsion angle in the 2′-OH radical formed after hydrogen abstraction on (a) compound **6** and (b) compound **8**. Panel c shows the potential curve of the HO−C6′−C2′ torsion angle rearrangement in the 2′-OH radical formed after hydrogen abstraction on compound **8**.

stability is too small (∼3 kcal/mol) to exclude the presence of such species in the actual system. Therefore, we decided to examine the redox properties of both conformers for the most representative chalcone (in terms of the redox properties), i.e., compound **9**, which has the largest number of OH groups. Table 2 compares the BDE values calculated for the *s*-trans and the *s*-cis conformers of this compound. They are identical, which shows that the conformation has no influence on the redox properties. Consistently, no difference is observed in the spin density of the corresponding radical for the two conformations. Thus, we decided to focus on the *s*-cis conformer for compounds **1**−**10**. Compound **11** does not exhibit such a geometrical feature, because of the absence of the C−C double bond.

TABLE 2: Bond Dissociation Enthalpy (BDE) in the Gas Phase for the Two Conformers of Compound 9

	BDE (kcal/mol)				
conformer	2′OH	4′OH	6′OH	3OH	4OH
s-cis conformer	90.3	97.9	90.3	85.6	82.4
s-trans conformer	90.3	98.0	90.3	85.6	82.4

TABLE 3: Bond Dissociation Enthalpy (BDE) at 298 K and Ionization Potential (IP) of Chalcones in the Gas Phase, in Water, and in Methanol

	BDE (kcal/mol)						
compound	2′OH	3′OH	4′OH	6′OH	3OH	4OH	IP (eV)
			(a) Chalcones in the Gas Phase				
1						83.9	6.82
2	96.5						6.95
3			87.5				7.12
4	96.5					84.5	6.82
5	89.7	79.7	83.9				6.78
6	98.3		90.0			84.2	6.79
7	98.7		90.1		78.0	75.0	6.75
8	81.9		89.9	81.9		83.5	6.69
9	82.3		90.0	82.3	77.6	74.4	6.64
10	97.5		89.8			84.4/84.1[a]	6.65
11	85.8		92.3	85.8		84.1	6.69
			(b) Chalcones in Water				
1						86.1	6.85
2	99.6						7.12
3			90.6				7.16
4	97.9					86.6	6.86
5	90.9	79.4	84.3				6.90
6	99.9		92.2			86.2	6.79
7	101.6		92.3			79.2	6.72
8	86.7		93.2	86.7		85.8	6.77
9	86.7		93.4	86.7	82.0	78.8	6.69
10	99.8		92.2			83.6	6.64
11	88.1		94.6	88.1	81.9	85.9	6.83
			(c) Chalcones in Methanol				
1	98.9					85.4	6.86
2							7.12
3	97.1		89.9				7.17
4	90.2					86.0	6.86
5	99.2	78.8	83.7				6.90
6	100.8		91.7			85.6	6.80
7	85.9		91.6		81.2	78.5	6.72
8	85.9		92.3	85.9		85.4	6.77
9	98.8		92.7	85.9	81.2	78.1	6.70
10	87.4		91.5			83.1	6.66
11	87.4		93.9	87.4		84.8	6.84

[a] Without/with a hydrogen bond between the OCH_3 and the 4′-OH groups in the radical.

Role of the B-ring. Table 3 shows the BDEs of the OH groups for the 11 chalcones in the gas phase, in water, and in methanol. The lowest BDEs are obtained for the B-ring, especially in the case of compounds **7** and **9**, in which a catechol moiety is present. This result is similar to those found for quercetin, taxifolin, luteolin, and eriodictyol (four other flavonoids that are characterized by the presence of the catechol moiety in the B-ring), for which the BDEs of the equivalent OH groups are ∼75−77 kcal/mol in the gas phase (data not shown). Therefore, the very important role of the catechol moiety in the antioxidant activity is confirmed to be as important for chalcones as for all the other flavonoids. Those BDE values are smaller than that of the DPPH−**H** species; as a result, reaction 1 is expected to be thermodynamically favorable. Consistently, compounds **7** and **9** are the most active chalcones toward DPPH and exhibit very small IC50 values of 2.7×10^{-5}

and 3.1×10^{-5} M, respectively, similar to that of quercetin (2.5×10^{-5} M) (see Table 1). Another indication of the importance of the catechol unit is the fact that the BDE of the 4-OH group in compounds **6** and **8**, which possesses only one OH group on the B-ring, is increased by ~9 kcal/mol. This result is consistent with the decrease in the antioxidant activity, compared to compounds **7** and **9**, respectively. Note that a similar BDE value (75 kcal/mol) is also obtained with the DFT/B3P86 methodology for catechol itself.[22] Thus, in flavonoids, it is the catechol moiety that governs the BDE, although electron delocalization effects occur over the entire molecule and stabilizes the phenoxyl radical obtained after the hydrogen abstraction.

The BDEs of the 4-OH groups having no neighboring substituted groups in the B-ring (compounds **1**, **4**, **6**, **8**, **10**, and **11**) are ~83−84 kcal/mol. Such values are still less than the BDE of ROO−**H** (88 kcal/mol), which means that those groups could also act in the antioxidant action toward the peroxyl radicals (ROO•). In regard to the reactivity with the DPPH radical, it is clear that a single OH group at C-4 only provides a low antioxidant activity. Compared to the BDE of DPPH−**H** (80 kcal/mol), such a BDE of 83−84 kcal/mol for 4-OH is not sufficient to provide a good activity; nonetheless, it is slightly more favorable, compared to the compounds with a single 4′-OH or 2′-OH groups (see, for example, the inhibition percentage of compound **1**, compared to compounds **2** and **3**, in Table 1).

One intriguing observation is that compound **10**, for which the BDE of the 4-OH group is similar to that of compound **6** (84.4 and 84.2 kcal/mol, respectively) shows a much greater activity toward DPPH (see Table 1). Such a difference cannot be explained based on the BDEs of the 4-OH group. A very strong difference in IC50 is also observed between guaiacol and phenol, again with no difference in the BDE of the OH group (86.7 and 87.2 kcal/mol, respectively). The explanation definitely relates to the presence of the ortho-OCH_3 group in the B-ring (i.e., the only difference between compounds **8** and **10**). We first explored the possibility of a stabilizing effect in the phenoxyl radical due to the presence of this group. Such a stabilization could come from a rearrangement to form a hydrogen bond between the OCH_3 group and the remaining O-4 atom (see the structure at the center of Figure 4). The energy of the corresponding radical has been computed but an insignificant stabilization effect of 0.3 kcal/mol (BDE = 84.1 kcal/mol) is observed (Table 3). Thus, we explored the thermodynamical characteristics of a second hydrogen abstraction, from the OCH_3 group of the phenoxyl radical, to form a biradical. Actually, we find that such a hydrogen transfer from the twisted ortho-OCH_3 group is followed by cyclization (leading to the structure on the right in Figure 4). Interstingly, the global energetic cost of that process is only 48.9 kcal/mol, which makes it possible in the presence of reactive radical species. It is interesting to note that such cyclization naturally occurs in plants, confirming that this process is chemically feasible. This seems to be the explanation of the better activity obtained for compound **10**, compared to that of compound **6**.

The importance of OH and OCH_3 groups in the ortho configuration has previously been discussed for other natural phenolic compounds, based on experimental data[39] as well as theoretical calculations.[15,23] The authors attributed the good antioxidant activity of curcumin to the presence of such a 4-hydroxy,3-methoxy phenyl moiety. We believe that the rearrangement we have observed here could also contribute to explain those results.

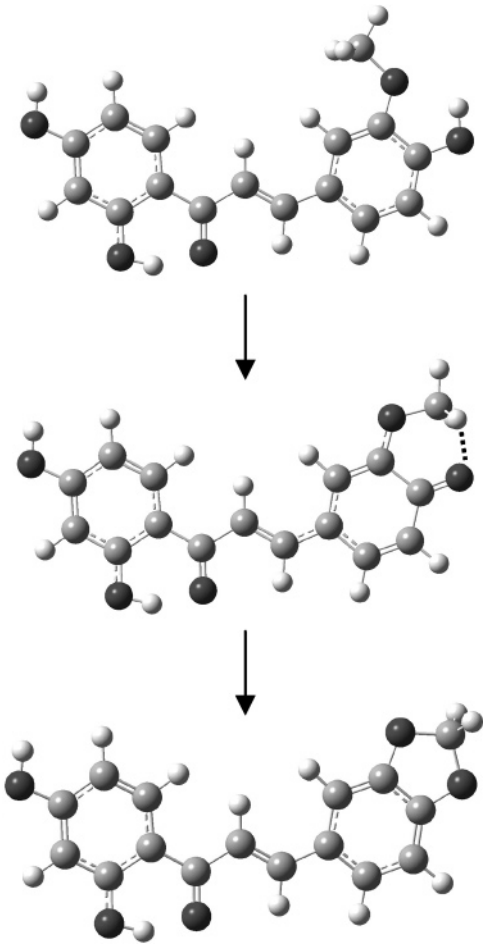


Figure 4. Reorientation of the OCH_3 group in the 4-OH radical of compound **10** favoring the formation of the cyclic compound.

Role of the A-ring. The role of the B-ring has been extensively studied in the literature, while, to our knowledge, no studies have theoretically explored the importance of the A-ring in flavonoids. The importance of the A-ring on the redox properties is essentially illustrated by compound **5**. As observed in Table 3, the BDEs are low, especially for the 3′-OH group. It is 79.7 kcal/mol in the gas phase, which is very similar to the BDEs obtained for the catechol moiety. This largely influences the antioxidant activity and leads to an IC50 of 4.9×10^{-5} M for compound **5**, which is similar to that of quercetin. That low BDE is related to the concomitant presence in the A-ring of the 4′-OH group, which makes it similar to catechol. Usually, the A-rings of flavonoids are only substituted at C-5 and C-7 (see the flavonoid numbering in Figure 1) or C2′ and C4′ (see the chalcone numbering in Figure 1). Substitution at C-6 (flavonoid numbering) or C3′ (chalcone numbering) is unusual; here, we show that this group could strongly participate in the antioxidant activity, in a manner similar to that of the catechol groups in the B-ring.

The highest BDEs are obtained for the 2′-OH groups of compounds nonsubstituted at C-6′ (ranging from 96.5 kcal/mol to 98.7 kcal/mol). Those BDEs are very high compared to the other OH groups of the A-ring, because the hydrogen bond between this group and the carbonyl group at C-4 has a strong stabilizing effect (~15 kcal/mol) in the parent molecule. Moreover, no significant differences are observed in the spin densities of the phenoxyl radicals obtained after hydrogen transfer from the 2′-OH, 4′-OH, and 6′-OH groups, indicating that there are no specific stabilizing effects in those different radicals, except the presence of hydrogen bonding. As previously

described, in the presence of the 6′-OH group, the 2′-OH radical could rearrange into a planar confomation with a new hydrogen bond between 6′-OH and the carbonyl group. This rearrangement provides a high stability to the 2′-OH radical, decreasing the BDE by ~15 kcal/mol, to reach 82 kcal/mol for compounds **8** and **9** and 86 kcal/mol for compound **11** (see Table 3).

Role of the α,β-Double Bond. The importance of the α,β-double bond in the redox capacity is illustrated by compounds **8** and **11**. The absence of the double bond in compound **11** decreases the antioxidant activity. Theoretical BDEs are consistent with this decrease in activity, because the three BDEs of the A-ring are increased from compound **8** to compound **11**. This is especially true for the 6′-OH group (from 81.9 kcal/mol to 85.8 kcal/mol), showing the concomitant role of this group and the α,β-double bond. This is due to better stabilizing effects in the phenoxyl radicals of **8** compared to **11,** because of the presence of the α,β-double bond.

Importance of the Number of OH Groups. Initially, the number of OH groups seems to be important to increase the antioxidant activity of chalcones. This feature has been used for many years to establish the SARs of phenolic compounds.[40] Nonetheless, it is clear from the theoretical BDEs that the position of the OH groups is also crucial. What is important and must be taken into account to explain the antioxidant activity is the number of OH groups with a BDE lower than a given threshold, and this threshold is dependent on the radical that must be scavenged. For example, one additional OH group at C-6′ (i.e., compound **8** versus compound **6**), with a BDE of <82 kcal/mol, significantly increases the antioxidant activity (see Tables 1 and 3). In contrast, additional OH groups with BDEs higher than the threshold (from compound **2** to compound **4**, and from compound **4** to compound **6**) only weakly influence the antioxidant activity. Here, we clearly establish the importance of the 6′-OH group based on BDE calculations. An additional and general conclusion can be proposed: a BDE of ~82 kcal/mol is low enough to participate in the redox reaction with DPPH.

The presence of a catechol moiety (in compounds **7** and **9**) probably masks the role of the other groups, especially the 6′-OH group. Indeed, an additional OH group at that position in compound **9**, compared to compound **7**, does not increase the antioxidant activity. The very strong redox capacity of the catechol moiety probably "attracts" all the free radicals (high reaction rates) and hides reactions with all the other groups. Nonetheless, this conclusion is only true for a pure redox in vitro experiment and must be modulated for in vitro or in vivo experiments. Actually, what is important is to identify the groups that could be involved in a redox transfer, and our results indicate that the 6′-OH could.

Influence of the Solvent on the Calculated BDE Values. BDEs calculated in the presence of the C−PCM solvent are reported in Tables 3b and 3c for water and methanol, respectively. From the gas phase to water, BDEs are increased by ~3−4 kcal/mol for all groups, except for the 2′-OH group of chalcones nonsubstituted at the 6′ position, for which the increase is lower (in the range of 1−2 kcal/mol). In the case of methanol, the increase is less, by ~1 kcal/mol, compared to water. The increase in BDE is consistent with the increase observed for O−H BDE measurements of phenolic compounds from the gas phase to water.[41] The presence of the (implicit) solvent probably enables stronger stabilizing effects in the parent molecule, compared to the radical. Indeed, the interaction potential is taken into account between the continuum and the molecule, which is described as a dipole. Because of the presence of an additional polar OH group in the molecule compared to the radical, this interaction is increased. This effect is lower with a less-polar solvent (from water to methanol). In the case of the 2′-OH radical, this effect is also lower, probably due to a competition effect existing between intra- and inter-hydrogen bonding. In that case, the electrostatic C-PCM perturbation (i.e., the inter-counterpart) probably weakens the intra-hydrogen bonding with the carbonyl group in the molecule, thus decreasing the stabilizing effect in the parent molecule, compared to the radical.

TABLE 4: ΔE_2 Values for DPPH in the Gas Phase and in Methanol at 0 K

	ΔE_2 (kcal/mol)	
compound	DPPH in the gas phase	DPPH in methanol
1	99.7	29.4
2	99.3	39.7
3	104.8	38.0
4	94.4	30.2
5	90.9	23.5
6	95.8	28.9
7	94.5	26.8
8	93.5	28.0
9	92.3	26.1
10	92.2	25.8
11	97.0	29.2

Electron-Transfer Mechanism. Although the DPPH radicals usually react with phenols via hydrogen abstraction, we also performed calculations on the second possible redox mechanism (i.e., electron transfer from the molecule to the radical; see Table 4). Some studies reported on the role of this mechanism with DPPH, as well as with radicals involved in the lipid peroxidation.[42,43] Calculations in the gas phase clearly show that the second mechanism is endothermic, with ΔE_2 values in the range of 92−105 kcal/mol. Nonetheless, it must be noted that the influence of the solvent on ΔE_2 is very strong. For example, it is decreased from 92.3 kcal/mol to 26.1 kcal/mol for compound **9**. Such a difference has also been computed in water for (i) $^{\bullet}$OH, (ii) $CH_3{}^{\bullet}CHOH$, and (iii) ROO$^{\bullet}$, with the following results: (i) from 130.4 kcal/mol to 9.7 kcal/mol, (ii) from 180.7 kcal/mol to 81.1 kcal/mol, and (iii) from 147.8 kcal/mol to 38.5 kcal/mol. It is clear that the first mechanism (i.e., homolytic hydrogen transfer) strongly participates in the antioxidant activity, as shown by the strong correlation between the BDEs and the antioxidant activity on DPPH. However, the aforementioned results indicate that the importance of the second mechanism (i.e., electron transfer) could be dependent on the radical involved in the redox reaction. This could act as a secondary parameter under certain conditions. The chemical environment could influence the balance between the two mechanisms, and, for any biological system, the second mechanism must be considered rather than systematically excluded. To evaluate the relative importance of those two mechanisms, the influence of the solvent must clearly be taken into account.

Conclusions

Chalcones are flavonoids with a specific characteristic (open C-ring) that does not decrease electron delocalization, compared to flavonols such as quercetin, but allows a geometrical flexibility that could be of importance for increasing the ligand-binding affinity. Two conformers could exist, and we have demonstrated that, in term of the bond dissociation enthalpy (BDE) and all the energetic properties, the conclusions and the structure−activity relationship will not be modified from

the *s*-cis (presented here in detail) to the *s*-trans conformers. Nonetheless, before extrapolating to the redox behavior in biological systems such as enzymes, one must know which conformer is present in the active site. For such an activity which involves ligand-binding interactions, this geometrical feature is crucial. Therefore, we have demonstrated that, at least in solutions, there are no changes in the BDE (i.e., redox properties) from the *s*-cis to the *s*-trans chalcones. As for all the flavonoids, based on density functional theory (DFT) calculations, the role of the catechol moiety in the B-ring is confirmed for explaining the redox properties of chalcones; however, here, we also have noted the role of the other OH groups of chalcones, especially the 6′-OH group. The 6′-OH group has no counterpart in the other flavonoids (it has a carbon ring) and has been implicated in the anti-estrogenic action (correlated with DPPH scavenging activity) of chalcones. Its role in the redox capacity is weaker than that of the OH groups of the catechol moiety; however, its BDE (∼82 kcal/mol in the presence of the α,β-double bond) is sufficiently low to enable hydrogen transfer to a group with a relatively high H atom affinity (DPPH and ROO$^{\bullet}$ radicals and, perhaps, an estrogen receptor).

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