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A DFT Study on the Radical Scavenging Activity of Maritimetin and Related Aurones

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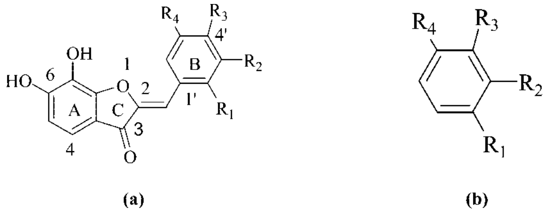
The radical scavenging activity of maritimetin and a series of synthetic aurones has been studied by using density functional theory with the B3LYP exchange correlation functional. The computation of various molecular descriptors that could assist the elucidation of hydrogen atom and electron donating ability of the selected compounds was carried out in the gas phase and in the liquid phase (benzene, methanol, water) with the aid of IEF-PCM. For reasons of comparison a series of simple phenols of known activity were also included in the study. The results are discussed with regards to the structure−activity relationship principles of flavonoids and in particular to the capacity of the selected aurones to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and superoxide anion ($O_2^{-\bullet}$) radicals. The O−H bond dissociation enthalpy (BDE) seems to be the most proper parameter to characterize the antiradical properties of the studied compounds. The hydroxylation pattern in ring B defines the order of activity, while the extended conjugation and especially the presence of a catechol moiety in ring A are responsible for the high activity observed experimentally for the selected aurones.

Introduction

Aurones [2-benzylidenebenzofuran-3(2*H*)-ones] are naturally occurring flavonoids found mainly in fruits and flowers contributing to their coloration.[1,2] They are biosynthesized from chalcones via auresidin synthase[2] and in addition to their role as pigments they seem to be a group of compounds having promising biological potential. Anticancer, antihormonal, antidiabetic, antimicrobial, and antiparasitic are some of the properties ascribed so far to members of this group of compounds.[3−7] The observed biological potential is postulated to be due to inhibition of the activity of ATP-dependent enzymes and proteins by mimicking the adenine of ATP, which is essential for the function of enzymes and receptors.[8] In particular, the benzofuranone structure of aurones is assumed to mimic better the adenine than the benzopyranone part of the corresponding flavones, indicating that structural conformation may be a key for the efficiency of aurones. However, as stated recently,[8] the understanding of the biological potential of aurones is yet to come. This may account for the few existing data on the antioxidant activity of aurones,[9,10] a property that has been related to various health promoting effects attributed to other flavonoid classes when consumed through diet and that could further broaden the therapeutic potential of aurones.



(a) (b)

A) R$_1$=R$_2$=R$_4$=H, R$_3$=OH
B) R$_1$=R$_4$=H, R$_2$=OCH$_3$, R$_3$=OH
C) R$_1$=R$_4$=H, R$_2$=R$_3$=OH (Maritmetin)
D) R$_1$=R$_3$=OH, R$_2$=R$_4$=H
E) R$_2$=R$_3$=R$_4$=OH, R$_1$=H

A) R$_1$=R$_2$=R$_4$=H, R$_3$=OH (Phenol)
B) R$_1$=R$_4$=H, R$_2$=OCH$_3$, R$_3$=OH (Guaiacol)
C) R$_1$=R$_4$=H, R$_2$=R$_3$=OH (Catechol)
D) R$_1$=R$_3$=OH, R$_2$=R$_4$=H (Resorcinol)
E) R$_2$=R$_3$=R$_4$=OH, R$_1$=H (Pyrogallol)

Figure 1. Structures of (a) aurones and (b) related simple phenols under study.

Considering the above, and the importance of the antioxidant activity of phenols in the protection from the in vivo deleterious effect of free radicals,[11] an attempt was made to elucidate the structure-radical scavenging activity of selected aurones by means of density functional theory. The study regarded maritimetin (3′,4′,6,7-tetrahydroxyaurone), a compound that has been isolated in the past from *Bidens frondosa*,[12] and a series of related analogues (Figure 1a) recently synthesized and screened for their antioxidant potential toward 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) and superoxide anion ($O_2^{-\bullet}$) radicals.[10] Although the substitution pattern in ring B varies in these compounds, all presented a catechol moiety in ring A, a structural feature that so far has been reported in few flavonoids (i.e., baicalin, scutellarin).[13] Our aim was to get a further insight on the contribution of the structural features to the radical scavenging potential of the particular compounds and elucidate their DPPH$^{\bullet}$ and $O_2^{-\bullet}$ scavenging capacity through calculation of proper molecular descriptors. Focus on radical scavenging was given as this is considered to be the main mechanism of antioxidant activity of phenols, although other modes of action have also been reported (oxidative enzyme inhibition, metal chelation).[14] For reasons of comparison a group of simple phenols structurally related to the particular aurones (Figure 1b) were also studied. In an effort to approximate the activity in solution or in a lipid medium, solute−solvent interactions were considered, using appropriate theoretical models. With the current work we hope to highlight the radical scavenging potential of this series of aurones and stimulate the interest for further studies and exploitation in food and pharmaceutical industries.

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Computational Methods

At least two mechanisms are reported for the radical-scavenging processes of chain-breaking antioxidants (AH):[14] a one-step H-atom transfer (eq 1) and a proton-coupled electron transfer (eq 2):

$$\mathrm{AH} + \mathrm{ROO}^{\bullet} \rightarrow \mathrm{A}^{\bullet} + \mathrm{ROOH} \qquad (1)$$

$$\mathrm{AH} + \mathrm{ROO}^{\bullet} \rightarrow \mathrm{ROO}^{-} \cdots \mathrm{AH}^{\bullet +} \rightarrow \mathrm{ROOH} + \mathrm{A}^{\bullet} \qquad (2)$$

Both mechanisms are important for the scavenging activity of reactive species by an AH in a certain chemical or biological system and may occur in parallel.[15] In the case where the first mechanism is followed, the reactivity of an AH can be estimated by calculating the O−H bond dissociation enthalpy (BDE),[16] where the lower the BDE value the higher the expected activity. When the second mechanism is preferred, adiabatic ionization potential (IP)[17] and O−H proton dissociation enthalpy (PDE) values are suitable to characterize the potency, with the former being more important than the latter since electron transfer is expected to be the rate-determining step.[18] Molecules with the low IP and PDE values are expected to have high activity. Thus, in the present study BDE, IP, and PDE values were used as molecular descriptors in an effort to elucidate the radical scavenging activity of compounds under investigation.

All calculations were performed by the Gausssian 03W ver. 6.0 set of programs.[19] The B3LYP exchange correlation potential[20,21] was used for geometry optimization and computation of harmonic vibrational frequencies using the 6-31G basis set[22,23] (UB3LYP was used for the resulting radicals). Then, single-point electronic energies (SPEs) were obtained by using the 6-311++G(2d,2p) basis set. The molecular enthalpy in the gas phase at 298 K consisted of B3LYP/6-311++G(2d,2p) calculated SPE values and B3LYP/6-31G thermal contributions to enthalpy (TCE, in which the vibrational contributions include zero-point vibrational energy) were used to calculate the BDE values according to the formula BDE = $H_\mathrm{r} + H_\mathrm{h} - H_\mathrm{p}$, where H_r is the enthalpy of the radical generated by H-abstraction, H_h is the enthalpy of the H-atom (−0.499897 hartree at this level of theory), and H_p is the enthalpy of the parent molecule. In the case of O−H BDE values in the gas phase the basis set superposition error (BSSE) was also considered using the counterpoise method.[24−26] The IP values were determined according to the equation IP = $E_\mathrm{cr} - E_\mathrm{p}$, where p and cr indicate the parent molecule and the corresponding cation radical generated after electron transfer. The O−H PDE values were calculated by using the formula PDE = $H_\mathrm{r} + H_\mathrm{p} - H_\mathrm{cr}$, in which, H_r is the enthalpy for radical generated after proton dissociation, H_p is the enthalpy for proton (0.00236 hartree), and H_cr is the enthalpy for the cation radical. BDE and PDE values have been calculated for all O−H groups in each molecule and the values given and discussed hereafter are those having the lowest absolute value regarding the most active O−H group located in the molecule. No spin contamination was found for radicals, with the $\langle S^2 \rangle$ values being about 0.750 in all cases. Solvent (benzene, methanol, and water) effects were computed by using the integral equation formulation−polarized continuum model IEF-PCM[27] and the UAHF solvation radii.[28]

Results and Discussion

To compute all the appropriate values of the selected parameters that may characterize the radical scavenging activity, all the possible conformers were optimized to locate the one with the lowest energy. The presence in all the compounds of various hydroxyl groups capable of participating in hydrogen bonds plus a double bond at C-2 gave rise to numerous conformers (102 possible structures totally).

The computational model selected for this potential energy surface (PES) was evaluated by comparing calculated optimized values for bond lengths and angles with experimental ones reported in the literature for *Z*-2-*p*-methoxyaurone.[29] Some selected calculated and experimental structural parameters are reported in Table S1 in the Supporting Information. In general, and taking into account that the experimental data were obtained by crystal X-ray analysis and not in the gas phase, one can observe quite a satisfactory agreement. Bond distances agree within 0.016 Å, while the largest deviation of bond angles appears to be about 0.5°. Thus, the selected method was considered accurate and it was applied in the PES search.

The most stable conformers found (optimized structures are shown in Figure 2) were those possessing intramolecular hydrogen bonds (IHBs). The energy spectrum of the conformers located is shown in Figure S1 in the Supporting Information. All but aurone A adopted the E configuration, with the energy differences from the most stable Z conformers being less than 1 kcal/mol. The global minimum structures of all aurones were planar, except for aurone D. In the latter, the B ring deviated from the plane of the rest of the molecule by 18.1°. This conformation is stabilized by a hydrogen bond formation between a hydroxyl group of the B ring and the keto group of the C ring.

Hydrogen Atom Transfer. The BDE values that characterize the hydrogen atom donating ability of simple phenols and aurones in the gas phase as well as in solvent are presented in Table 1. In the same table experimental findings[10] on the radical scavenging activity of selected aurones are also given in terms of inhibition concentration (IC_{50}), which is the necessary concentration of a phenol needed for the scavenging or inhibition of radical formation by 50%. Simple phenols are discussed first due to the simplicity of their structure and the known experimental trend on activity.[30−34] The particular trend could serve as a means to evaluate whether the selected methodology could provide accurate energetic results, being therefore suitable for the investigation of the structure−activity relationship of the selected aurones. On the basis of gas phase BDE values, it is evident that pyrogallol and secondly catechol are predicted to be the most efficient radical scavengers among the simple phenols, in line with experimental findings.[30−34] Such efficiency is related to the further stabilization of the derived phenoxy radical via formation of IHB.[35,36] Resorcinol, guaiacol, and phenol are expected to be of low activity since the corresponding BDE values were ∼8−14 kcal/mol higher that those obtained for pyrogallol and catehol. However, although resorcinol is a poor antioxidant as is phenol[31,32] due to the electron-withdrawing effect of the second hydroxyl group and the inability to form IHB, this is not true for guaiacol. The latter is known to be active and could scavenge, i.e., 2 mol of DPPH per mol AH.[30] Such activity is related of course to the presence of the methoxy group. As recently reported for a series of chalcones, the one bearing a guaiacol moiety could donate a second hydrogen atom to free radicals but from the methoxy group, forming, thus, a cyclic compound with a low enthalpy value (BDE = 48.9 kcal/mol).[34] Such a mechanism, which was considered thermodynamically favorable in the presence of oxidizing species, could account for the higher activity estimated experimentally in comparison to that of the monohydroxy chalcone. Indeed this could be verified even in the case of the simple guaiacol (BDE = 44.0 kcal/mol) as illustrated in Scheme 1. When the basis set superposition error was taken into account in the calculation

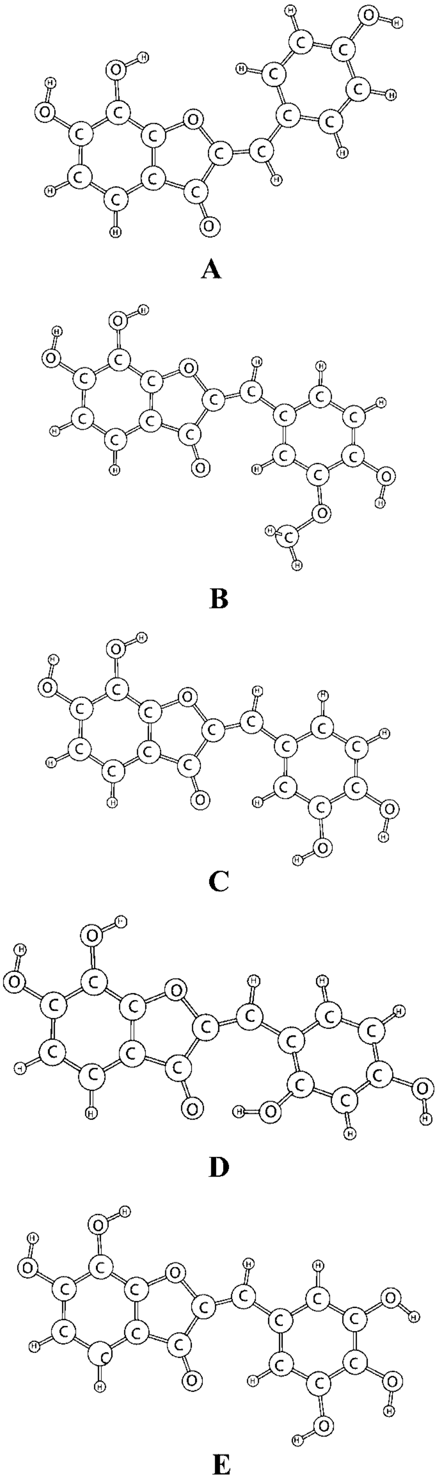


Figure 2. Optimized structures of the aurones studied at the B3LYP/6-31G level in the gas phase.

of BDE values an insignificant correction to enthalpy was achieved (0.2−0.3 kcal/mol) and therefore no BSSE was calculated in the PCM calculations. All these findings for the simple phenols suggested that the selected method could give reliable results and could be applied to the study of the selected aurones.

The Role of the B Ring. Ring B is considered as the primary target for radical attack in flavonoids.[14] Its hydroxylation pattern is of major importance for the scavenging of free radicals by flavonoids and is expected to define the relative order of activity of the compounds under study. According to BDE values obtained for the hydroxyl group, which was found as the most favorable hydrogen atom donor in each aurone, it is obvious that the order of activity is similar to the one obtained for the simple phenols. This is in accord with the order of activity reported for other flavonoids like myricetin, quercetin, isorhamnetin, and kaempferol having a similar substitution pattern in the B ring as the investigated aurones.[14] Therefore, aurone E is expected to be the most active one, with aurone C to follow in the activity. Formation of a second IHB in the case of the former aurone resulted in a decrease of BDE value by 7 kcal/mol. In the same way formation of a IHB is responsible for the relatively low BDE value calculated for aurone C, as it may confer higher stability to the phenoxy radical (BDE = 81.7 kcal/mol without IHB). With regard to aurones A and B found to be equally potent, an investigation similar to that made for phenol and guaiacol was attempted. Indeed a low BDE value was obtained for the formation of a cyclic analogue (BDE = 48.1 kcal/mol). The value was close to the one reported in the literature for a structurally related chalcone.[34] When the BSSE was also considered in BDE values, the corrected enthalpy values were 0.2−0.3 kcal/mol lower.

The Role of Extended Conjugation. A close inspection of aurone structures reveals that conjugation is further extended from ring B throughout ring C. Such a structural feature is necessary for stabilization of the derived phenoxy radicals minimizing, thus, the probability to act as pro-oxidants after their formation. The stability of the radicals due to the extended conjugation is better illustrated in Figure 3, where the spin distribution of phenoxy radicals is given for each aurone in comparison to the corresponding one for the structurally related simple phenols.

The lower stability of the phenoxy radical of aurone D contrary to that of aurone A and B radicals is not only related to the electronic phenomena of the substituents, but also to the planarity of the radical. The latter is crucial for the stability as it may affect spin delocalization.[37] In aurone D there is a deviation of ring B from the plane as mentioned earlier that is retained in the derived radical, whereas the radicals of aurones A and B are planar. This may account for the higher BDE value computed for the particular O−H group of aurone D.

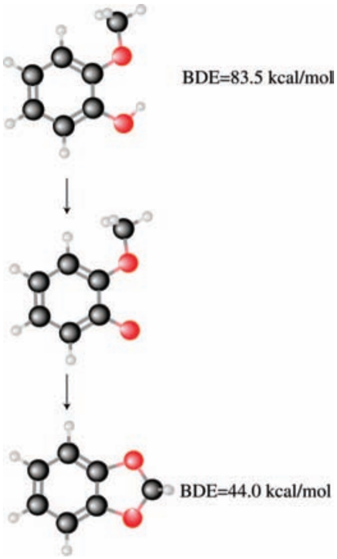
The Role of Catechol Moiety in the A Ring. Structure−activity relationship studies on flavonoids suggest that the substitution pattern in the A ring correlates poorly with antioxidant activity.[14] This has been ascribed to the presence of a resorcinol moiety or various methoxy groups and/or sugars in the corresponding ring in most of the known flavonoids.[14] Contrary to that, maritimetin and related aurones bear a catechol moiety in the ring A, a feature that has been reported so far in few flavonoids (i.e., baicalin, scutellarin).[13] Calculation of BDE values revealed that the ease of hydrogen atom donation is favored from the hydroxyl group at the 7 position with the BDE value to range between 74.8 and 75.2 kcal/mol for all aurones studied. Such values were close to those computed for the simple catechol, as well as the catechol moiety in the B ring of aurone C, respectively. These data suggest that the 6,7-dihydroxy group in ring A may greatly affect the radical scavenging activity of the compounds, and thus their total activity. In this way the high efficiency observed experimentally for the five aurones with regard to that of the known antioxidants such as vitamins C and E, BHT, and resveratrol may further be supported.[10] Besides, the 6,7-dihydroxy group of aurones is expected to be a more efficient hydrogen atom donor than the corresponding catechol moiety in ring A of scutellarin and baicalin. The corresponding BDE values reported for these flavonoids are ∼16 kcal/mol

TABLE 1: B3LYP/6-311++G(2d,2p)//B3LYP/6-31G Bond Dissociation Enthalpy (BDE) Values at 298 K of Simple Phenols and Selected Aurones in the Gas Phase, Benzene, Methanol, and Water

	BDE (kcal/mol)[a]					IC_{50}[c]	
AH	gas	gas_{BSSE}[b]	benzene	methanol	water	DPPH$^{\bullet}$	$O_2^{\bullet -}$
phenol	84.3	84.1	85.2	85.4	85.7		
guaiacol	83.5	83.2	83.5	80.7	81.0		
catechol	75.2	75.0	76.8	77.9	78.8		
recorcinol	83.8	83.6	84.3	84.5	84.9		
pyrogallol	69.4	69.2	71.6	76.4	74.5		
A	80.9	80.7	82.2	82.6	85.7	10.4	10.0
B	81.3	81.1	81.4	78.6	81.0	8.7	9.0
C	75.0	74.7	76.0	76.8	78.8	8.3	6.5
D	84.6	84.4	85.3	84.6	84.9	10.1	12.3
E	69.7	69.4	71.4	72.5	74.5	7.9	4.3

[a] Bond Dissociation Enthalpy for the most active hydroxyl group of simple phenols and the most active one in ring B for aurones. [b] BDE corrected values considering BSSE. [c] Inhibition concentration values in μM obtained from ref .

SCHEME 1



higher than that of simple catechol due to the formation of a strong IHB between the O−H at position 5 and the oxygen of the keto group in ring C.[38] At this point it should be stressed that according to BDE values, ring A seems to be the active site at least in the case of aurones A, B, and D when the hydrogen atom transfer mechanism is preferred. Lack of this structural feature would result in poor activity for these compounds.

Influence of the Solvent. When the solvent was taken into consideration an increase of 0.7−7.0 kcal/mol was obtained for BDE values when computation was carried out in polar solvents. No or smaller differences were found in the nonpolar benzene. This is rather expected since both methanol and water employed in the calculations tend to form intermolecular hydrogen bonds with solutes,[39] thus affecting hydrogen donation. An exception was the case of guaiacol and aurone B, where a decrease of ∼3 kcal in BDE values was observed in the polar media. A close inspection on the BDE values in structurally related chalcones revealed a similar decrease.[34] The latter may be related to the better stabilization of the parent compound in the presence of methanol or water but mainly to that of the corresponding radical as shown from the spin density distribution of the phenoxy radical of aurone B in the gas phase and, i.e., in methanol (Figure 4). The stability of the phenoxy radical is known to be more important for a low BDE value.[40−42] The decrease in BDE values for guaiacol and aurone B resulted in a clear differentiation in activity of the particular compounds from the other analogues bearing one hydrohyl group (phenol, aurone A) and a resorcinol moiety (resorcinol, aurone D) in ring B. Despite this fact and for reasons of comparison with gas phase, calculation of BDE values for donation of a second hydrogen atom but from the methoxy group were also carried out. Results

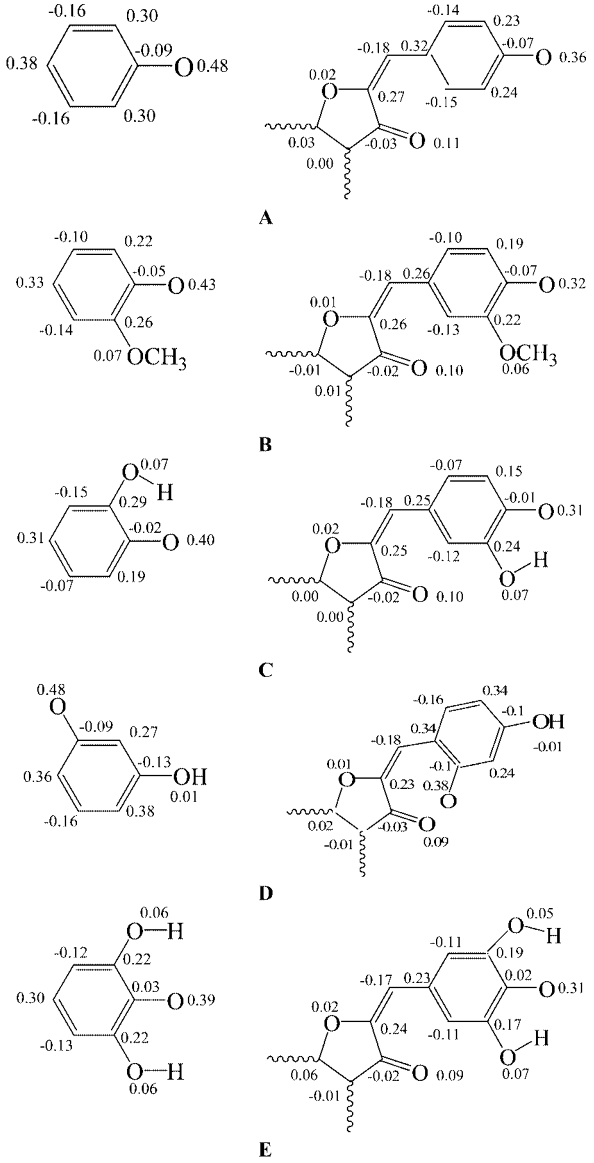


Figure 3. Spin density distribution in phenoxy radicals of simple phenols and selected aurones.

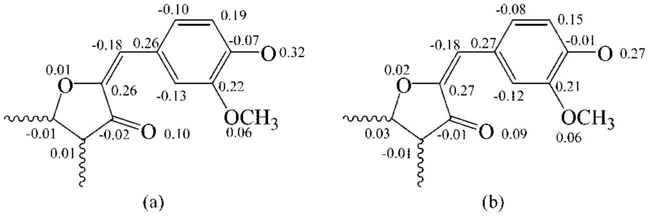


Figure 4. Spin density distribution in phenoxy radical of aurone B in (a) the gas phase and (b) in methanol.

revealed that the process is feasible in the presence of either nonpolar or polar solvents (BDE_{benz} = 46.6 kcal/mol, BDE_{alc} = 47.9 kcal/mol, and BDE_{aq} = 44.3 kcal/mol for guaiacol; BDE_{benz} = 50.0 kcal/mol, BDE_{alc} = 49.3, and BDE_{aq} = 49.5 kcal/mol for aurone B).

Single Electron Transfer. Scavenging of free radicals by phenols may also be achieved via donation of a single electron. The values for the adiabatic ionization potential IP of simple phenols and aurones in the gas phase as well as in solvent are given in Table 2. In the same table PDE values are also included. Considering the IP values in the gas phase it is obvious that phenol is expected to be the least active from the group of simple phenols. On the other hand, guaiacol seems to donate an electron easily, followed in activity by pyrogallol and catechol. The order of activity is in close agreement with that derived for pyrogallol, catechol, and resorcinol by using cyclic voltametry.[31] A close look at the PDE values reveals that the effect of substituents is rather different than that in the IP values. Thus, guaiacol cation radical is not easily deprotonated even in comparison to simple phenol. From the values of both IP and PDE it can be generally concluded that pyrogallol and catechol are expected to be potent compounds since both IP and PDE values are rather low. In the case of aurones any differences observed in activity were rather small in comparison to those observed for the simple phenols. Since differences were not great it was assumed that maybe the deprotonation step could determine the activity of compounds. On the basis of PDE values, however, it could be concluded that aurone E should be the most efficient one as, except for electron donation, cation radical deprotonation was easier than that of the rest of the aurones. From the results it is evident that the derived trend of activity for the compounds under investigation is different from that obtained according to calculated BDE values. This can be attributed to the fact that BDE is affected by the local phenomena induced by the substituents, whereas IP values are affected by the structure of the whole molecule.[17] The same seems to apply in the case of PDE values, since for compounds A–D deprotonation is expected to occur at the same hydroxyl group of ring A.

Considering the above it is difficult to conclude on any characteristic differences concerning the electron donating activity of the compounds, except maybe that aurone E should be the most potent. However, a close look at the IP values of aurones reveals that they are ~22–28 kcal/mol lower than that of phenol. Such a difference is rather small in comparison to those reported for other flavonoids which usually have 30–40 kcal/mol lower IP value than that of phenol.[43] The latter is an indication that when the electron transfer mechanism predominates for the scavenging of free radicals, aurones are not expected to be as efficient as other flavonoids. On the other hand, the rather high IP values could suggest that the possibility to donate an electron directly to oxygen thus promoting oxidation[17] will be low.

Influence of the Solvent. When the solvent effect was taken into account in both simple phenols and aurones a drastic influence on IP values was observed. Contrary to BDE values, where the solvent effect was not great, IP values were severely affected because the charge separation process is quite sensitive to the polarity of solvents.[14] As expected in the polar solvents lower IP values were obtained. The opposite trend was obtained for PDE values. The findings suggest that action of aurones via electron donation is facilitated in an aqueous environment (physiological liquids) rather than in a lipophilic one (lipid membranes, bulk oils). However, even the consideration of the solvent effect did not result in a clear differentiation in the activity of the investigated compounds.

Comparison with Experimental Data. As mentioned in the introduction, maritimetin and related analogues were synthesized and then screened for their ability to scavenge selected free radicals. It would be therefore interesting to investigate whether the data obtained through calculation of the values for the various physicochemical parameters could shed light on experimental findings.

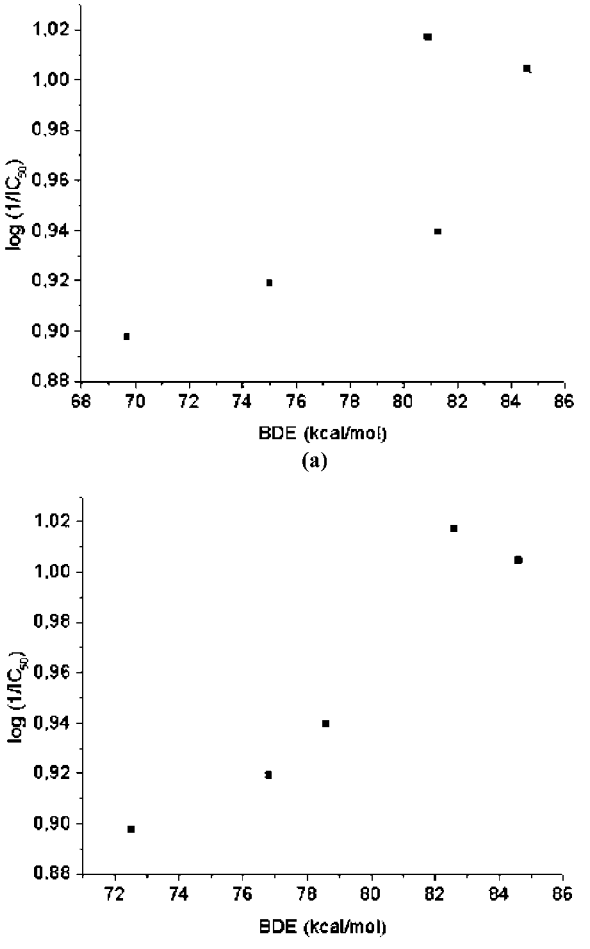
The Case of DPPH. It is the most common assay employed by the researchers in the field of natural antioxidants. The mechanism of the scavenging has been suggested to be carried out through either hydrogen or electron donation.[30] However, experimental and theoretical findings regarding the enthalpy of the bond DPPH–H, as well as theoretical data concerning the calculation of energy for the formation of $DPPH^-$, suggest that the most favorable mechanism should be through hydrogen atom donation from an AH.[34,43,44] Although, the size of the data sample does not allow the establishment of a valuable QSAR equation, comparison of the order of activity obtained experimentally with that derived on the basis of calculated BDE values in both gas phase (where calculated values may serve as good primary indices for radical scavenging)[45] and methanol (the common solvent used in the assay) revealed a good agreement, as shown in Figure 5, panels a and b. An improved agreement was exhibited when the solvent effect was taken into account, due to the fact that the higher efficiency of aurone B toward that of A (on the basis of IC_{50} values) was found only when computation was carried out in methanol. However, it should be stressed that differences in activity between aurones are rather small on the basis of IC_{50} values. The latter is better evidenced when the corresponding IC_{50} values obtained for the reference compounds BHT, vitamins C and E, and resveratrol were considered.[10] The reference compounds were found 24–54 times less potent from the least active of the aurones. This potency is expected to be due to contribution of the catechol moiety in ring A. The particular moiety, except for an enhancement in activity, may mask the contribution of other structural features as evidenced for other phenolic compounds.[46] Furthermore, the mechanism of reaction is complex, as usually the stoichiometry of the reaction is higher than the number of available hydroxyl groups for many phenols.[30,46] Taking this into account, as well as the fact that solvent may affect the ultimate performance of compounds with $DPPH^•$,[4748] it can be argued that finding meaningful results in SAR studies is a difficult task.

The case of ($O_2^{-•}$). Contrary to $DPPH^•$, the scavenging of $O_2^{-•}$ could be an index for a possible in vivo potency. The particular radical though of low activity may give rise to highly reactive species leading thus to deleterious effects in the human body. The mechanism of scavenging of the particular radical by phenolics is rather complex. Electron donation has been proposed as well as inhibition of radical formation in the case where enzymes and not chemicals are used for $O_2^{-•}$ production in various experimental protocols. Electron donation is further supported by the fact that the variety of pH values that are

TABLE 2: B3LYP/6-311++G(2d,2p)//B3LYP/6-31G Ionization Potential (IP) and Proton Dissociation (PDE) at 298 K of Simple Phenols and Selected Aurones in the Gas Phase, Benzene, Methanol and Water

	IP (kcal/mol)				PDE (kcal/mol)[a]			
AH	gas	benzene	methanol	water	gas	benzene	methanol	water
phenol	191.8	166.5	141.3	140.3	207.7	232.6	259.3	260.6
guaiacol	177.8	155.0	132.6	131.5	220.8	242.4	263.3	264.6
catechol	183.4	158.5	133.2	131.7	206.9	232.2	259.9	262.2
recorcinol	185.7	160.7	135.4	134.8	213.3	239.1	264.2	265.3
pyrogallol	182.0	157.4	134.9	131.2	202.5	228.1	256.7	258.4
A	170.0	151.0	126.7	130.7	220.0*	237.8*	262.0*	261.5*
B	162.7	145.1	124.3	126.9	227.3*	243.7*	267.7*	265.2*
C	165.5	147.0	129.7	130.2	224.6* 224.7**	241.6* 241.9**	263.6* 263.3**	264.8* 264.3**
D	165.3	147.3	128.7	127.5	225.0*	241.8*	262.6*	262.2*
E	165.2	146.7	128.3	126.8	219.6**	237.0**	259.8**	261.1**

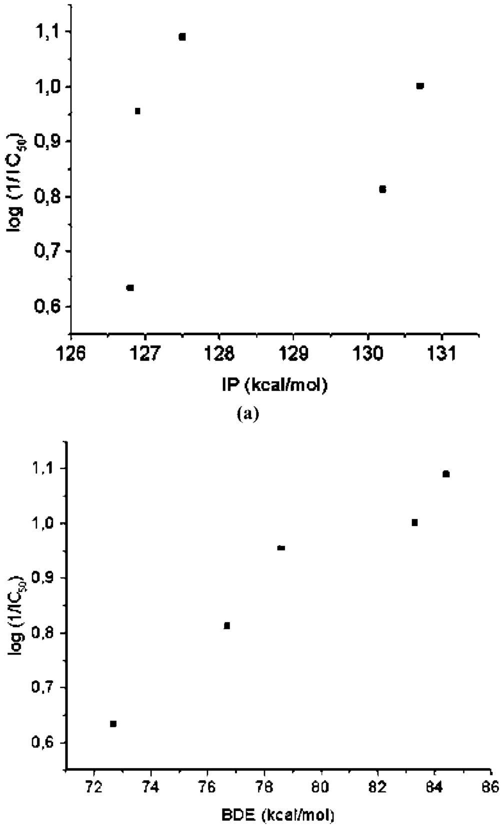
[a] PDE values are referred to the O−H group that can be most easily ionized, which is located in either in ring A (*) or/and ring B (**).



(a)

(b)

Figure 5. Log (1/IC$_{50}$) obtained with DPPH$^•$ assay versus O−H BDE values for the most active hydroxyl group in ring B: (a) gas phase and (b) methanol.



(a)

(b)

Figure 6. Log (1/IC$_{50}$) obtained with $O_2^{-•}$ assay versus calculated in the presence of water (a) IP values and (b) O−H BDE values for the most active hydroxyl group in ring B.

employed by the researchers are high (pH 7.0−10.2)[49−54] affecting thus the ionization of hydroxyl groups. However, except for the physical state of a compound, pH affects also the physical state of the radical. In lower than physiological pH values (which are expected in close proximity to cellular membranes)[55] a portion of the radical is transformed to $HO_2^•$. The latter can pass through membranes and oxidize lipids.[55] Despite all these, it is generally accepted that the presence of a catechol moiety is necessary for an efficient scavenging of superoxide or even better the presence of a pyrogallol moiety.[50,52]

Considering that flavonoids generally present pK_a values higher than 8.0 with minor exceptions, as evidenced both experimentally and theoretically,[56−59] and that the existing data were obtained at a pH value of 7.0, the order of activity obtained experimentally was compared with the one derived according to the calculated BDE and IP values in the presence of water for the nonionized compounds. The data shown in Figure 6a,b revealed that a good agreement was achieved only with regard to the BDE values, suggesting that radical scavenging via hydrogen atom donation may be more likely.

Conclusions

The aurones under investigation are flavonoids, which are generally expected to be efficient hydrogen atom donors. The order of activity for the corresponding compounds is defined by the substitution pattern in the B ring according to O−H BDE values as the corresponding values in the A ring were similar. The presence of the catechol moiety in ring A is beneficial for the high scavenging efficiency of the compounds toward reactive free radicals. As a matter of fact due to the catechol moiety in ring A, aurones A and D are able to scavenge free radicals. The extension of conjugation to ring C contributes further to the activity due to a higher spin delocalization in the phenoxy radical. In this way stability of the derived phenoxy radicals is enhanced. The ability of compounds to form IHB is important. The contribution of the solvent should be taken into account as evidenced in the present study when an effort is attempted to address real problems. The radical scavenging ability of aurones should not be overlooked as it may contribute to the spectrum of their bioactivity. With the current work we hope to contribute to the ongoing interest on the understanding of the biological activity of aurones for better exploitation in the field of food chemistry and pharmacy.

Supporting Information Available: Experimental and calculated values of bond lengths and angles for *Z*-2-*p*-methoxyaurone (Table S1), energy spectrum of the conformers located for all aurones studied (Figure S1), and Cartesian coordinates for the most stable conformers (Tables S2−S6). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Ono, E.; Fukuchi-Mizutani, M.; Nakamura, N.; Fukui, Y.; Yonekura-Sakakibara, K.; Yamaguchi, M.; Nakayama, T.; Tanaka, T.; Kusumi, T.; Tanaka, Y. *Proc. Natl. Acad. Sci. U S A.* **2006**, *103*, 11075–11080.

(2) Nakayama, T. J. *Biosci. Bioeng.* **2002**, *94*, 487–491.

(3) Boumendjel, A.; Di Pietro, A.; Dumontet, C.; Barron, D. *Med. Res. Rev.* **2002**, *22*, 512–529.

(4) Chu, W. L. A.; Jensen, F. R.; Jensen, T. B.; McAlpine, J. B.; Sokilde, B.; Santana-Sorensen, A. M.; Ratnayake, S.; Jiang, J. B.; Noble, C.; Stafford, A. M. U.S. Patent US 6307070, 2001.

(5) Igaki, N.; Sakai, M.; Hata, H.; Oimomi, M.; Baba, S.; Kato, H. *Clin. Chem.* **1990**, *36*, 631–634.

(6) Kayser, O.; Kiderlen, A. F.; Brun, R. *Planta Med.* **2001**, *67*, 718–721.

(7) Ballinari, D.; Bonomini, L.; Ermoli, A.; Gude, M.; Menichincheri, M.; Jurgen, S.; Vanotti, E. PCT Int. Appl. WO2002083123.

(8) Boumendjel, A. *Curr. Med. Chem.* **2003**, *10*, 1241–1253.

(9) Westenburg, H. E.; Lee, K. J.; Lee, S. K.; Fong, H. H. S.; Van Breemen, R. B.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2000**, *63*, 1696–1698.

(10) Venkateswarlu, S.; Panchagnula, G. K.; Subbaraju, G. V. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2183–2185.

(11) Halliwell, B.; Aeschbacht, P.; Loligert, J.; Aruoma, O. I. *Food Chem. Toxicol.* **1995**, *33*, 601617.

(12) Romussi, G.; Pagani, F. *Boll. Chim. Pharm.* **1970**, *109*, 467–475.

(13) Horvath, C. R.; Martos, P. A.; Saxena, P. K. *J. Chromatogr. A* **2005**, *1062*, 199–207.

(14) Tsimidou, M.; Nenadis, N.; Zhang, H. Y. *Natural antioxidant phenols. Sources, structure-activity relationship, current trends in analysis and characterization*. Boskou, D., Gerothanassis, I., Kefalas, P., Eds.; Research Signpost: Kerala, India, 2006.

(15) Zhang, H. Y.; Wang, L. F. *J. Phys. Chem. A* **2003**, *107*, 11258–11259.

(16) Wright, J. S.; Carpenter, D. J.; McKay, D. J.; Ingold, K. U. *J. Am. Chem. Soc.* **1997**, *119*, 4245–4252.

(17) Wright, J. S.; Johnson, E. R.; DiLabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183.

(18) Zhang, H. Y.; Sun, Y. M.; Wang, X. L. *J. Org. Chem.* **2002**, *67*, 2709–2712.

(19) 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.; Millam, 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.; Salvador, P.; Dannenberg, J. J.; Malick, 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.; Komaroni, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian 03; Gaussian, Inc.: Pittsburgh, PA, 2003.

(20) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.

(21) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785–789.

(22) McLean, A. D.; Chandler, G. S. J. *J. Chem. Phys.* **1980**, *72*, 5639–5648.

(23) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A. *J. Chem. Phys.* **1980**, *72*, 650–654.

(24) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553–556.

(25) Davidson, E. R.; Chakravorty, S. *J. Chem. Phys. Lett.* **1994**, *217*, 48–56.

(26) Xantheas, S. S. *J. Chem. Phys.* **1996**, *104*, 8821–8824.

(27) Cances, E.; Mennucci, B.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3032–3041.

(28) Barone, V.; Cossi, M.; Tomasi, J. *J. Chem. Phys.* **1997**, *107*, 3210–3221.

(29) King, T. J.; Hastings, J. S.; Heller, G. H. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1455–1457.

(30) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. *Lebensm. Wiss. Technol.* **1995**, *28*, 25–30.

(31) Hotta, H.; Nagano, S.; Ueda, M.; Tsujino, Y.; Koyama, J.; Osakai, T. *Biochim. Biophys. Acta* **2002**, *1572*, 123–132.

(32) Nenadis, N.; Wang, L. F.; Tsimidou, M.; Zhang, H. Y. *J. Agric. Food Chem.* **2004**, *52*, 4669–4674.

(33) Ordoudi, S. A.; Tsimidou, M. Z. *J. Agric. Food Chem.* **2006**, *54*, 9347–9356.

(34) Kozlowski, D.; Trouillas, P.; Calliste, C.; Marsal, P.; Lazzaroni, R.; Duroux, J. L. *J. Phys. Chem. A* **2007**, *111*, 1138–1145.

(35) Ji, H. F.; Zhang, H. Y. *New J. Chem.* **2005**, *29*, 535–537.

(36) Zhang, H. Y. *New J. Chem.* **2003**, *27*, 453–454.

(37) Van Acker, S. A. B. E.; De Groot, M. J.; Van den Berg, D. J.; Tromp, M. N. J. L.; Den Kelder, G. D. O.; Van der Vijgh, W. J. F.; Bast, A. *Chem. Res. Toxicol.* **1996**, *9*, 13051312.

(38) Wang, L. F.; Sun, Z. L.; Zhang, H. Y. *J. Shandong Univ. Technol. (Sci. Technol. Ed.)* **2003**, *17*, 13–17.

(39) Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans.* **1990**, *2*, 521–529.

(40) Wu, Y. D.; Lai, D. K. W. *J. Org. Chem.* **1996**, *61*, 7904–7910.

(41) Brinck, T.; Haeberlein, M.; Johnson, M. *J. Am. Chem. Soc.* **1997**, *119*, 4239–4244.

(42) Borges Dos Santos, R. M.; Martinho-Simoes, J. A. *J. Phys. Chem. Ref. Data* **1998**, *27*, 707–739.

(43) Ji, H. F.; Tang, G. Y.; Zhang, H. Y. *QSAR Comb. Sci.* **2005**, *24*, 826–830.

(44) Mahoney, L. R.; Mendenhal, G. D.; Ingold, K. U. *J. Am. Chem. Soc.* **1973**, *95*, 8610–8614.

(45) Leopoldini, M.; Prieto-Pitarch, I.; Russo, N.; Toscano, M. *J. Phys. Chem. A* **2004**, *108*, 92–96.

(46) Nenadis, N.; Boyle, S.; Bakalbassis, E. G.; Tsimidou, M. *J. Am. Oil Chem. Soc.* **2003**, *80*, 451–458.

(47) Abe, N.; Nemoto, A.; Tsuchiya, Y.; Hojo, H.; Hirota, A. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 306–313.

(48) Nenadis, N.; Tsimidou, M. *J. Am. Oil Chem. Soc.* **2002**, *79*, 1191–1195.

(49) Cos, P.; Ying, L.; Calomme, M.; Hu, J. P.; Cimanga, K.; Van Poel, B.; Pieters, L.; Vlietinck, A. J.; Berghe, D. V. *J. Nat. Prod.* **1998**, *61*, 71–76.

(50) Furuno, K.; Akasako, T.; Sugihara, N. *Biol. Pharm. Bull.* **2002**, *25*, 19–23.

(51) Jovanovic, S. V.; Hara, Y.; Steenken, S.; Simic, M. G. *J. Am. Chem. Soc.* **1995**, *117*, 9881–9888.

(52) Yuting, C.; Rongliang, Z.; Zhongjian, T.; Yong, J. *Free Radical Biol. Med.* **1990**, *9*, 19–21.

(53) Cotelle, N.; Bernier, J. L.; Henichart, J. P.; Catteau, J. P.; Gaydou, E.; Wallet, J. C. *Free Radical Biol. Med.* **1992**, *13*, 211–219.

(54) Hanasaki, Y.; Ogawa, S.; Fukui, S. *Free Radical Biol. Med.* **1994**, *16*, 845–850.

(55) Halliwell, B.; Gutteridge, J. M. C. *Methods Enzymol.* **1990**, *186*, 1–85.

(56) Slabbert, N. P. *Tetrahedron* **1977**, *33*, 821–824.

(57) Jovanovic, S. V.; Steeden, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846–4851.

(58) Lemanska, K.; Szymusiak, H.; Tyrakowska, B.; Zielinski, R.; Soffers, A. E. M. F.; Rietjens, I. M. C. M. *Free Radical Biol. Med.* **2001**, *31*, 869–881.

(59) Leopoldini, M.; Russo, N.; Toscano, M. *J. Agric. Food Chem.* **2006**, *54*, 3078–3085.

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