Ab initio and Density Functional Theory Studies for the Explanation of the Antioxidant Activity of Certain Phenolic Acids[1]

Evangelos G. Bakalbassis[a,*], **Argero Chatzopoulou**[a], **Vasilios S. Melissas**[b,2],
Maria Tsimidou[c,*], **Matina Tsolaki**[a], **and Anastasios Vafiadis**[a]

[a]Laboratory of Applied Quantum Chemistry, School of Chemistry, Aristotle University of Thessaloniki, GR-540 06 Thessaloniki, Greece, [b]Molecular Modeling of Materials Laboratory, Institute of Physical Chemistry, National Research Center for Physical Sciences "Demokritos", GR-153 10 Aghia Paraskevi Attikis, Greece, and [c]Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, GR-540 06 Thessaloniki, Greece

ABSTRACT: *Ab initio* and density functional theory molecular orbital calculations were carried out at both the HF/6–31+G(d) and B3LYP/6–31+G(d) levels for the four antioxidants, *p*-hydroxycinnamic acid derivatives, namely, the *p*-coumaric, caffeic, ferulic, and sinapinic acid and the corresponding radicals, in an attempt to explain the structural dependency of the antioxidant activity of these compounds. Optimized resulting geometries, vibrational frequencies, absolute infrared intensities, and electron-donating ability are discussed. Both the high degree of conjugation and the extended spin delocalization in the phenoxyl radicals offer explanation for the scavenging activity of the four acids. In structurally related compounds, the calculated heat of formation value in radical formation appears as a meaningful molecular descriptor of antioxidant activity in accordance with experimental data. This becomes more clear at the B3LYP level.

Paper no. L8594 in *Lipids 36,* 181–190 (February 2001).

Structure-activity relationship studies using theoretical methods are gaining interest among scientists for prediction or elucidation of differences in the activity of series of molecules (1–6). Phenolic antioxidants such as tocopherols, flavonoids, and phenolic acids are the most intensively examined categories because of their broadly accepted biological function (7–10). Difficulties in theoretical approach are related to the various factors that determine the performance of a molecule as an antioxidant; scavenging of free radicals, metal chelation, and lipophilicity are the most important ones (11,12). Although a considerable number of molecular descriptors have been examined, only a few of them prioritize the molecules in accordance to conclusions derived by experimental data. Various factors have been considered so far for the characterization of free radical scavenging activity of antioxidants, for instance, (i) the difference in the heat of formation (ΔHOF) between the antioxidant and the free radical (1–4), (ii) the spin distribution in the radical (4), (iii) the dissociation energy of the phenolic O–H bond (5,6,13) and (iv) the energy-eigenvalue of the highest-occupied molecular orbital (HOMO) (2,13–16). Owing to the large size of the antioxidant molecules, semiempirical quantum-chemical methods have been employed in most cases and *ab initio* calculations are quite rare (4–6,13,17–19).

Korzekwa *et al.* (20) proposed that a linear relationship exists between stability of radicals and activation energies of hydrogen abstraction in the reaction of a flavonoid with a radical in a series of analogous substrates. Consequently, the calculated enthalpy difference (ΔHOF) between the parent antioxidant molecule and its potential radical indicates the relative order of hydrogen atom abstraction, and the difficult task of determining transition states could be avoided for the moment. Based on these, the above hydrogen abstraction scheme is generally accepted (1,21) for the phenolic acid antioxidants, the parent molecule phenoxyl radical couple exhibiting the lowest theoretical ΔHOF value will be considered the one that most easily allows hydrogen atom abstraction by any free radical. The derived radical could then scavenge another one. Hydrogen atom abstraction depends mostly on enthalpy differences (as reflected by calculated ΔHOF values), whereas radical scavenging depends on the spin distribution. The calculated ΔHOF values of the parent molecule phenoxyl radical couples correlate well with the experimental antioxidant activity trend of the same molecules. This was also found by both Zhang (1,2) for certain phenolic antioxidants and van Acker *et al.* (4) for certain flavonoids. Zhang derived ΔHOF values from semiempirical calculations, whereas van Acker *et al.*, from *ab initio* ones; still the antioxidants studied by both exhibited profound structural differences. Moreover, van Acker *et al.* effectively used the radical spin distribution to

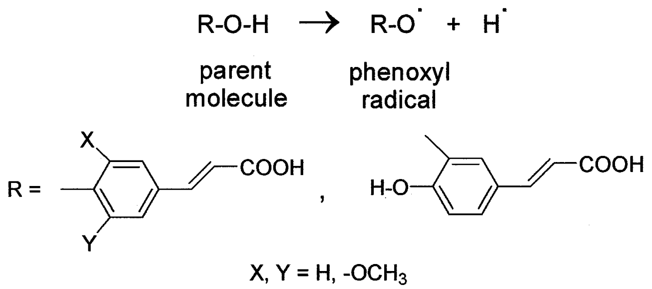
---

[1] Supplementary material, Tables S1–S9, are available from the author E.G.B. upon request.

*To whom correspondence should be addressed at Laboratory of Applied Quantum Chemistry, School of Chemistry, Aristotle University of Thessaloniki, P.O.B. 135, GR-540 06 Thessaloniki, Greece.
E-mail: bakalbas@chem.auth.gr or tsimidou@chem.auth.gr

[2] Formerly at Laboratory of Applied Quantum Chemistry, School of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Abbreviations: ARP, antiradical power; B3LYP, Becke's three parameter hybrid functional using the Lee-Yang-Parr correlation functional; DFT, density functional theory; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; EC_{50}, efficient concentration; HF, Hartree-Fock; HOMO, highest occupied molecular orbital; IP, induction period; PF, protection factor.



SCHEME 1

account for the ease of a second radical scavenging. Hence, the molecular descriptors considered to be worth examining are the ΔHOF values and the radical spin distribution.

In the present study, high level *ab initio* and density functional theory (DFT) molecular orbital calculations, at both the HF/6-31+G(d) and B3LYP/6-31+G(d) levels, are carried out to optimize the geometries of four structurally related antioxidants, *p*-hydroxycinnamic acid derivatives, namely caffeic, sinapinic, ferulic and *p*-coumaric acids (parent molecules) and the corresponding phenoxyl radicals (Scheme 1). The geometries of the parent molecules and their phenoxyl radicals, the vibrational frequencies and the electron-donating ability of the parent molecules are examined and discussed.

The ΔHOF values, giving information about the ease with which the radical is formed, are compared, too. The radical spin distributions, which are measures of the radical stability since they provide information about the degree of delocalization as well as the electron-donating ability, are also calculated and considered. Calculations for the molecular descriptors considered in this study are CPU-intensive and physical memory-demanding. To our knowledge, no *ab initio* and/or DFT results are available regarding energetics and structural parameters for the molecules under examination. The only relative paper is that of Hueso-Urea *et al.* (22), in which, some molecular descriptors have been calculated, using semiempirical approaches, to establish structure-liquid chromatographic retention time relationships for certain phenolic compounds. Neither structural nor detailed frequency data were given in that paper. The only experimental data available for the four acids under study are solid-state X-ray crystallographic studies for caffeic and ferulic acids (23,24) and solid-state infrared spectroscopic data for all of them (25).

The *p*-hydroxycinnamic acids under study are widely examined experimentally, and the order of their activity is well established in practice. Additional experiments are also carried out to support theoretical results.

MATERIALS AND METHODS

Quantum-chemical calculations. The geometries of all minimum energy structures for all four hydroxycinnamic acid derivatives and the corresponding radicals were fully optimized, employing the Hartree-Fock (HF) (26) theory (namely, the restricted HF for the parent molecules and unrestricted HF for the radicals) and the Becke's Three Parameter Hybrid Functional using the Lee-Yang-Parr correlation functional theory (B3LYP) (27) of available DFT ones, both with the 6–31+G(d) basis set (28–33). To form the radical, an $H^{\bullet}$ was removed from the phenolic hydroxyl group in each acid. Diffuse functions (32) were added to heavy atoms, e.g., C and O, and inclusion of *d* polarization functions (33) on heavy atoms was considered necessary for the calculation of molecular properties. Pure *d* functions were used in the *d* shells, i.e., the *d* functions have five components. C_1 point group symmetry for each species was assumed as the initial geometry of the optimization procedure, and all redundant internal coordinates (34) were fully optimized (35). All calculations investigating the structural parameters of the molecules addressed here are based on final frequency calculations that provide energy minima with certainty. For each molecule full optimization calculations were performed in an attempt to determine the closest structure to the global minimum. The nonappearance of negative frequencies was assumed to be evidence for a global potential energy minimum of each calculated structure. The GAUSSIAN-94 software package (36), installed on a Hewlett-Packard 9000 series workstation (model J210 with 128 MB of memory; Hewlett-Packard, Palo Alto, CA), was used throughout this study.

The 298 K theoretical difference of heat of formation, ΔHOF, between the parent phenolic antioxidant, HOF*m*, and its free radical, HOF*f*, produced after the H-abstraction equals

$$\Delta\text{HOF} = \text{HOF}f - \text{HOF}m + \text{E}h + RT \qquad [1]$$

where HOF*f*, HOF*m*, and E*h* are the sum of electronic, zero-point and thermal energies of the radical, the parent molecule, and the H atom, respectively; *R* is the gas constant, and *T* is the absolute temperature.

Samples and standards. Commercial refined olive oil was from Elais S.A. (Piraeus, Greece). Sinapinic acid and *p*-coumaric acid were from Sigma Chemical Co. (St. Louis, MO), whereas ferulic and caffeic acids were from Fluka (Buchs, Switzerland). Silicic acid (100–200 mesh size, Sigma Chemical Co.), celite (Riedel de Häen, Seelze, Germany), commercial sucrose, activated carbon (100 mesh size, Aldrich, Dorset, England), and *n*-hexane (Riedel de Häen) were used for column chromatography. 2,2-Diphenyl-l-picrylhydrazyl radical (DPPH) (approximately 90%) was from Sigma Chemical Co., and 2-propanol for spectroscopy was from Merck (Darmstadt, Germany). All solvents used were of appropriate grade.

Estimation of antiradical activity of the four p-*hydroxycinnamic acids by DPPH and antioxidant activity on olive oil triacylglycerol fraction.* The antiradical activity of the molecules under study was determined using the free radical DPPH (6×10^{-5} M) in ethanol (37). The reduction of DPPH was followed by monitoring the decrease in its absorbance at 515 nm, automatically recorded every 60 s. The exact initial DPPH concentration (C_{DPPH}) in the reaction medium was calculated from a calibration curve using the equation

$$\text{Abs}_{515\,\text{nm}} = 11{,}589 \times (C_{\text{DPPH}}) + 0.0027$$

(correlation coefficient, $r = 0.999$) [2]

where Abs = absorbance.

Different concentrations (expressed as the number of antioxidant moles per mole of DPPH) were used, and for each antioxidant, the reaction kinetics were plotted. From these graphs, the percentage of DPPH remaining at the steady state was determined. These values were transferred onto another graph showing the percentage of residual stable radical at the steady state as a function of the molar ratio of antioxidant to DPPH. The latter was used to determine the efficient concentration (EC_{50}), which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. The lower the EC_{50}, the higher the antioxidant activity is. Then, the antiradical power (ARP) was calculated as ARP = $1/EC_{50}$.

Commercial refined olive oil was purified in our laboratory using column chromatography (38) and stored at −18°C till use. The quality characteristics of the substrate were evaluated as reported elsewhere (39). Samples (2.5 g) of purified olive oil containing phenolic antioxidants dissolved in ethanol at a concentration of 2.8×10^{-3} M and control samples were prepared, and induction periods (IP) at 120°C were estimated using a Rancimat apparatus (679 Rancimat; Metrohm Ltd., Herisau, Switzerland.). IP is considered to be the time period over which the oil is resistant to oxidation with or without the presence of antioxidants. Protection factors (PF) were calculated as the ratio of IP in the presence of the antioxidant/IP of control, i.e., PF = IP (time units)/$IP_{control}$ (time units).

RESULTS AND DISCUSSION

Equilibrium geometries. All tables with the structural data for all acids and phenoxyl radicals under examination are given as Supplementary Material (Tables S1–S5), available upon request from the author. Owing to the lack of structural experimental studies in the gas phase for the same acids, the X-ray solid-state ones for caffeic (23) and ferulic (24) acids are also given in Table S1. Although comparison between results in the gas and the solid state is not allowed, it is simply mentioned that the corresponding values do not deviate more than 1%. The resulting structures for the parent molecules (**I**, *p*-coumaric acid; **II**, caffeic acid; **III**, ferulic acid; **IV**, sinapinic acid), together with the adopted numbering scheme, are given in Figure 1; those of their radical species (**V**, *p*-coumaric acid radical; **VI**, caffeic-H acid radical; **VII**, caffeic-open radical; **VIII**, caffeic acid radical; **IX**, ferulic acid radical; **X**, sinapinic acid radical) in Figure 2. This was easily accomplished, since in all structures, with only a few exceptions, the dihedral angle values were either ±180° or 0°; planar structures were derived for the substituents OH and OMe, in excellent agreement with assumptions made by Wu and Lai (40). Moreover, owing to the very low deviations derived between the corresponding structural values of the parent molecules and the phenoxyl radicals, calculated at a particular level, mean structural values are given in both Figures 1 and 2. In the case of caffeic acid, possessing two neighboring hydroxyl groups in the phenyl ring, three different radical structures were considered. The first two correspond to the radical structures deriving from the OH group at C(3), in which the intramolecular hydrogen bond is retained (**VI**), or eliminated (**VII**), the third to the structure in which an $H^{\cdot}$ was eliminated from the OH group at C(4) (**VIII**), leading also to a nonhydrogen bonding structure. The reason for this separate consideration stems from the fact that an intramolecular hydrogen bond further stabilizes any molecule.

An unexpected discrepancy, found between the HF and B3LYP levels of theory geometrical parameters, lies in the dihedral angle between the phenyl ring and the carboxyl group of the caffeic acid, being 8.5° in the former and zero in the latter. This discrepancy leads to a symmetry point group of C_1 for the caffeic acid at the HF level and a corresponding one of C_s at the B3LYP level. The planar calculated structures for all acids and radicals at the latter imply that the molecules are completely conjugated, hence a C_s symmetry point group is also assumed for them.

As shown in both Figures 1 and 2, the phenyl ring and the carboxyl groups are *trans* to each other around the connecting carbon-carbon double bond. This is the case at both levels of theory. A *trans* configuration around the connecting carbon-carbon double bond accounts well for the correctness of our results concerning both series of compounds studied, since a *trans* configuration leads, in general, to a more energetically stable structure than a *cis* one.

An inspection of the numbers appearing in Figures 1 and 2 clearly shows that the computed bond distances at the B3LYP level are constantly slightly higher than those calculated at the HF level. With only a few exceptions, the computed bond lengths obtained from both levels of theory are in agreement within 1.5 %.

It is also observed that, when a substituent hydroxyl or methoxy group is added to the *p*-coumaric acid at position *ortho* to the aroxyl group (leading to any of the three other acids under study), particular distortions at bond lengths and angles appear. It is noteworthy that the existing C(4)–O bond distance becomes longer when a hydroxyl group is added, e.g., caffeic acid by *ca.* 0.01 Å at both levels of theory, and shorter when a methoxy is added, e.g., ferulic and sinapinic acids. Furthermore, the existing phenolic O–H bond distance is slightly shorter in caffeic acid and slightly longer in ferulic and sinapinic acids, as compared to the *p*-coumaric one, at both levels of theory. This molecular descriptor may be useful in the explanation of the antioxidant activity in a series of homologs, considering that for larger bond length the hydrogen can be removed more easily, thus forming the phenolic hydroxyl. Phenolic OH bond lengths along with the inhibition of lipid oxidation values of all parent molecules studied are given in Table 1.

An inspection of the figures presented in the table clearly shows that the B3LYP O-H bond-length values are consistently higher than the HF ones. It is clear that caffeic acid, the experimentally most active molecule, is the one having the shortest OH bond length, whereas *p*-coumaric acid, the weakest antioxidant experimentally among the four studied, ex-

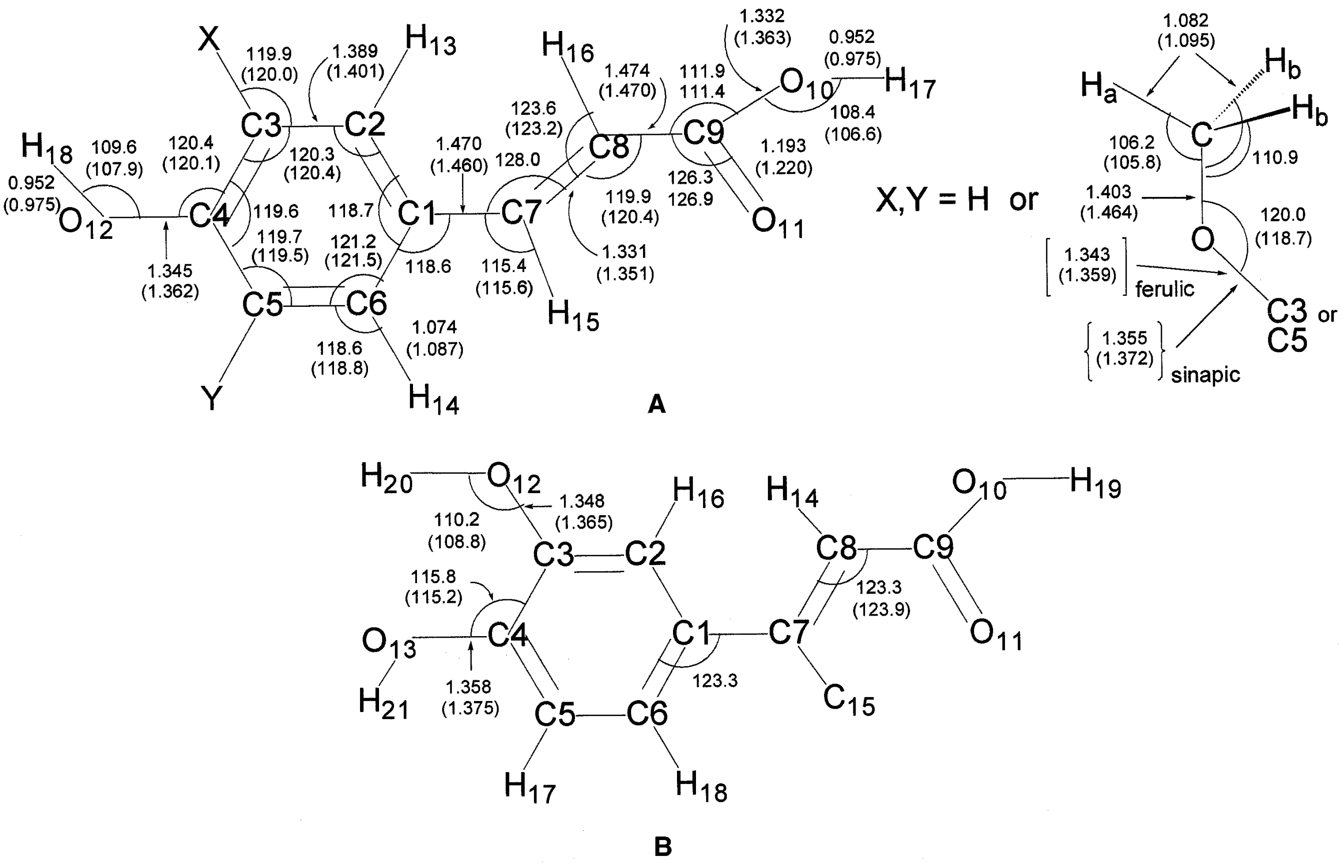


FIG. 1. Equilibrium structural parameters for the **I**, **III** and **IV** (A), and **II** acids (B). Density functional theory (DFT) values are given in parentheses. Where a particular structural parameter had identical values at both levels of theory, this is given once. Absence of a particular structural parameter value denotes identity to the corresponding one of the other acids. The C2-C3-X angle shown is the one for X = H; the one for X = O is 125.8° (126.1°).

hibits the second-shortest OH bond length. This could mean that phenolic OH bond length is not a fruitful molecular descriptor by itself, at this level of theory, to compare antioxidant activity of phenolic antioxidants. Zhang also reached an analogous conclusion by performing Austin Model 1 (AM1) semiempirical calculations on flavonoid antioxidants (1).

TABLE 1
O—H Bond Length and HOMO Values, and Inhibition of Lipid Oxidation of the Four *p*-Hydroxycinnamic Acid Antioxidants

	II	IV	III	I
O-H[a,b]	0.9694	0.9744	0.9742	0.9704
O-H[c]	0.9475	0.9502	0.9503	0.9478
HOMO[d]	−0.3090	−0.3029	−0.3021	−0.3100
HOMO[e]	−0.2294	−0.2236	−0.2245	−0.2333
Inhibition (PF)[f]	6.2	2.4	1.1	1.05
Inhibition (ARP)[g]	4.5	2.5	1.8	0.008

[a] All O-H bond lengths in Å.
[b] Parent molecule phenolic O-H bond lengths (DFT level).
[c] Parent molecule phenolic O-H bond lengths (HF level).
[d] Parent molecule HOMO energy (eV) values (HF level).
[e] Parent molecule HOMO energy (eV) values (DFT level).
[f,g] This work. HOMO, highest occupied molecular orbital; PF, protection factor; ARP, antiradical factor; DFT, density functional theory; HF, Hartree-Fock.

Formation of a hydrogen bond in both caffeic and ferulic acids is substantiated by the increase of the C(2)-C(3)-O angle by approximately 6.0° when a methoxy group is added to *p*-coumaric acid, compared to a 6.6° decrease of the C(3)-C(4)-O angle when a hydroxyl group is added instead. Sinapinic acid exhibits the same hydrogen bond structural effects with ferulic acid, as expected.

There is considerable C–C bond length alteration in the benzene ring on going from the parent molecules to the phenoxyl radicals. As a matter of fact, contrary to the equality of all C–C bonds in the acids [*ca.* 1.390 (1.400) Å], the two pairs of C–C bonds, C(1)-C(2), C(1)-C(6) and C(4)-C(3), C(4)-C(5) in the benzene ring in the radicals are longer than the C(2)-C(3) and C(5)-C(6) ones by *ca.* 0.04–0.06 Å, indicating quinoid structure. These deviations however, were larger for the former pair of C–C bonds corresponding to the C(4)-O$^{\bullet}$ edge, than the latter corresponding to the C(1)-chain edge. Wu and Lai (40) also found very similar geometries for the B3LYP/6-31G(d) level radical structures of both phenol and anisole. Moreover, the C–O bond of the phenoxyl radical has considerable double-bond character, as indicated by the short bond length of *ca.* 1.238 (1.250) Å. The increase of the C(3)-C(4)-O(13) angle, on going from **VI** to **VII**, is probably

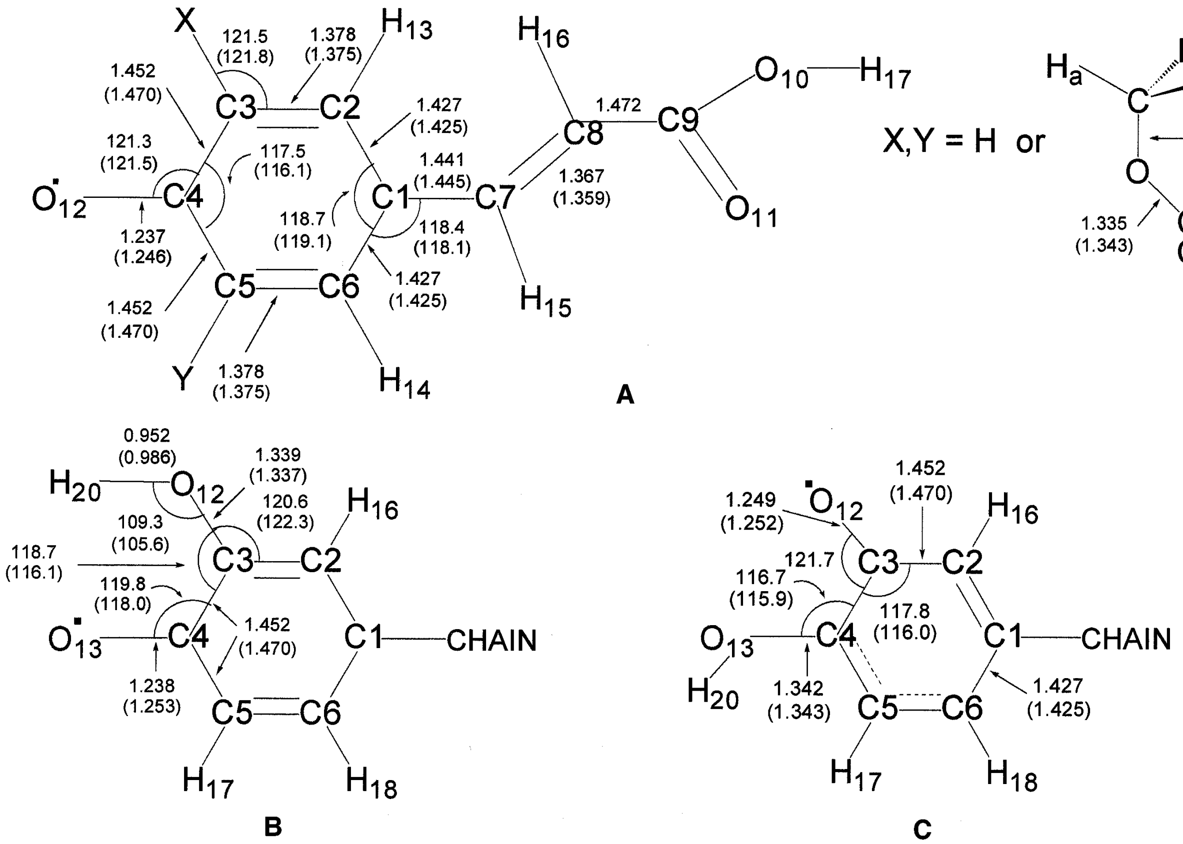


FIG. 2. Equilibrium structural parameters for the **V**, **IX** and **X** (A), **VI** (B) and **VII** (C) radicals. DFT values are given in parentheses. Where a particular structural parameter had identical values at both levels of theory, this is given once. Absence of a particular structural parameter value denotes identity to the corresponding one of the other acids. The C2-C3-X angle shown is the one for X = H; the one for X = O is 125.3° (125.5°). The C2-C3-O12, O13-C4-C3, C3-O12-H20, and C4-C3-O12 angle values for the **VIII** radical are 123.6° (123.9°), 121.7°, 111.3° (110.4°), and 116.2° (115.2°), respectively. For abbreviation see Figure 1.

due to the elimination of the O(13)...(H(20) hydrogen bonding. Moreover, the reduction of the C(4)-C(3)-O(12) angle and the concomitant increase of the C(2)-C(3)-O(12) one could be attributed to the H(20)...(H(16) repulsion.

Agreement between the computed values for the bond angles, obtained at both levels of theory, is within 1% or better. Dihedral angles calculated at both levels of theory are identical. However, this is mainly observed between *p*-coumaric and ferulic molecules and less for the sinapinic and caffeic ones (within 1%). The C–C and C–C–C phenyl-ring bonds and angles, and the phenolic C–O and O–H bonds are in excellent agreement with others derived theoretically (41) for the 2,6-dimethylphenol at the UHF/6–31G(d) level of theory; still, this is also the case with the carboxylic C–O, C=O and O–H bonds (42).

Vibrational frequencies. Frequency values calculated at the HF level contain known systematic errors (electron correlation is not taken into account, whereas B3LYP calculations include it) that produce an overestimate of about 10% compared to the experimental values. Therefore, it is usual to scale frequencies predicted at the HF level by an empirical factor of 0.8929 (43–45) along with one of 0.9613 (43–45) for the B3LYP model of DFT theory. The harmonic oscillator approach, which is used for calculated frequencies, usually produces higher values than the fundamental ones. Owing to the large size of the frequency tables for all acids under study, their unscaled calculated harmonic frequencies and infrared intensities are given as Supplementary Material (Tables S6–S9), available upon request from the author. An inspection of the figures presented in those tables clearly shows that the B3LYP frequency values are, contrary to those of the bond lengths, consistently lower than the HF ones with a minor exception for those between 200 and 450 cm^{-1}.

From all frequency values in Tables S6–S9 (available from the author upon request), only a few have been properly scaled and their values are given in Table 2. Those were chosen based upon the importance of the bond and the intensity of the corresponding absorption. Carboxylic acids, for instance, are mostly characterized by the O–H and C=O stretching bands (46). Table 2 includes the scaled calculated frequency values for these latter bands, as well as those for the phenolic O–H ones, along with their intensities, vibrating atoms, corresponding experimental frequencies in the vapor phase (47,48), and percentage differences between scaled calculated and experimental ones. The above frequencies are well characterized as stretch bands, since both their atom's

TABLE 2
The Most Important Scaled (sc.) Calculated Harmonic Frequencies of the Four Acids, at Both Levels of Calculations, Along with Their Corresponding Experimental (exp.) Ones and the Unscaled Infrared Intensities[a]

		HF				DFT	
Acid	Bond	$\nu_{exp.}$[b] (cm^{-1})	A[c]	$\nu_{sc.}$ (cm^{-1})	DP	$\nu_{sc.}$ (cm^{-1})	DP
I	O–H(20)	3651	119	3656	0.14	3596	1.53
	O–H(19)	3582	166	3606	0.66	3545	1.04
	C=O	1762	478	1762	0.00	1715	2.74
II	O–H(21)	3651	143	3665	0.38	3616	0.97
	O–H(20)	3651	151	3638	0.36	3574	2.15
	O–H(19)	3582	169	3614	0.88	3556	0.73
	C=O	1762	923	1772	0.56	1710	3.04
III	O–H(24)	3582	172	3630	1.32	3552	0.84
	O–H(23)	3582	169	3606	0.67	3544	1.07
	C=O	1762	482	1763	0.06	1714	2.80
IV	O–H(28)	3582	178	3633	1.40	3547	0.99
	O–H(27)	3582	171	3606	0.67	3546	1.01
	C=O	1762	477	1764	0.11	1715	2.74

[a] The % $\nu_{sc.}/\nu_{exp.}$ discrepancy percentage (DP) is also shown.
[b] The corresponding experimental values are from References 46–48.
[c] Intensity of absorption at the HF level (arbitrary units).

orientation and eigenvector orientation lie on a single plane.

The first band examined corresponds to the phenyl O–H stretching frequencies. Those appear in the region of 3665–3630 cm^{-1}, where caffeic and ferulic acids define its two far ends, respectively. The corresponding absorption of *p*-coumaric acid is found at 3656 cm^{-1}, since there are no neighboring groups that would lead to a variation of the expected value. The formation of an intramolecular hydrogen bond between the two properly oriented neighboring hydroxy groups in caffeic acid leads to both a strengthening of the free O–H bond and a concomitant weakening of the one participating in the hydrogen bond, as compared to that of *p*-coumaric acid. In effect, this leads to both a shift toward higher frequency values (3665 cm^{-1}) for the former bond and a shift toward lower frequency values (3638 cm^{-1}) for the latter, followed by a simultaneous narrowing of both bands. This narrowing is further substantiated by its frequency intensity, since a high frequency intensity results in a narrow absorption bandwidth. This band narrowing will be discussed in the following section with respect to the corresponding absolute infrared intensities. Owing to the intramolecular hydrogen bond formation, the existing O–H covalent bond in the newly formed five-membered ring becomes weaker in all acids except *p*-coumaric. The corresponding absorption appears at a lower value (3633 cm^{-1}) in sinapinic acid rather than in *p*-coumaric one (3656 cm^{-1}). The O–H phenyl group frequency value for ferulic acid (3630 cm^{-1}) is very close to that of sinapinic acid because of the formation of an analogous five-membered intramolecular hydrogen-bonded ring. The lower frequency values derived for these latter two acids, as compared to that of caffeic (3665 cm^{-1}), is in excellent agreement with the known fact that the hydroxy group is a better electron donor than the methoxy one (49).

In a similar manner, the scaled calculated carboxylic O–H stretching frequency values range between 3606–3614 cm^{-1} and are always lower than the scaled phenolic ones (see also Table 2). Moreover, it is easily seen that there are no large discrepancies between the carboxylic carbonyl C=O group stretching bands for all acids.

So far, each particular theoretical frequency value shown in Table 2 has been both assigned and explained regarding its relative shift in the infrared spectrum region. Frequency information from infrared and Raman spectra in the gas phase was not available for any of the molecules under study. Vapor phase frequency values (46–48) of the most characteristic groups closely match the calculated ones of the four acids under study. In particular, the OH stretching frequency of *m*- and *p*-substituted alkylated phenols, in vapor phase, appears as a single band in the region of 3360–3642 cm^{-1} (48), and its mean value, 3651 cm^{-1}, is representative for comparison with *p*-coumaric acid. A frequency value lower than 3651 cm^{-1} should be considered for the vapor phase of caffeic acid owing to formation of an intramolecular hydrogen bond (46). The OH stretching frequency is found at 3595–3569 cm^{-1}, when phenol exhibits an –OR group as an *o*-substituent (48). Hence, the mean value, 3582 cm^{-1}, of this latter frequency range was considered as the corresponding one for the vapor phase phenolic OH frequency values for both ferulic and sinapinic acids. The OH stretching frequency is found at 3585–3580 cm^{-1}, in the α,β-unsaturated monomeric carboxylic acids (47); its mean value, 3582 cm^{-1}, was considered as the corresponding one for the vapor-phase carboxylic OH frequency values for all acids. Finally, the vapor phase C=O band is near 1764–1760 cm^{-1} in α,β-unsaturated carboxylic and its mean value, 1762 cm^{-1}, was considered as the vapor phase carboxylic C=O frequency for all acids.

An inspection of the values given in Table 2 clearly indicates that: (i) the calculated results coming from either level of theory are in excellent agreement with those in the vapor phase to within 1.5 %; (ii) most of the calculated frequency values are larger than the available vapor phase ones; (iii) the scaled calculated frequencies at the HF level better approximate vapor phase ones; (iv) scaled calculated HF frequencies are always larger than the DFT ones.

Absolute infrared intensities. The *ab initio* unscaled absolute infrared intensities for the four acids under study are also given in Table 2. An inspection of those values clearly shows that the intense infrared modes occur within the 1500–1800 and 3500–3700 cm^{-1} regions. Unfortunately, there are no available experimental intensities in the gas phase for any of the four acids under study to compare with. Usually, individual band intensities cannot be accurately measured experimentally due to overlap with neighboring frequencies. However, calculated intensities of nonoverlapping bands occasionally deviate more than 50% from experimental ones (50).

As it was discussed before, formation of a five-membered intramolecular hydrogen-bond ring results in both a shift and a narrowing of the band corresponding to the covalent participating OH bond. The narrowing of the band is also substantiated by its frequency intensity, since a high frequency intensity leads to a narrow absorption bandwidth. Based upon the calculated frequency intensities, it was shown that sinapinic acid exhibits the largest intensity value for the O–H stretching frequency, whereas *p*-coumaric acid the smallest. As a matter of fact, the effect of hydrogen bond formation is to increase the IR intensity and decrease the O–H stretching frequency (46). Sinapinic and ferulic acids, and to a minor extend caffeic acid, do exhibit stronger intensities of absorption as compared to those of *p*-coumaric acid due to intramolecular hydrogen-bond ring formation. It is well known that the stronger the hydrogen bond, the larger the OH stretching frequency shift toward a lower value (46), which predicts that hydrogen bonds formed in ferulic and sinapinic acids will be stronger than those in caffeic acid.

Heat of formation, ΔHOF values. Both Figures 1 and 2 clearly show that planar structures were derived. Structure planarity strongly supports complete conjugation within parent and radical molecular species. Intramolecular H-bond interactions found between the two neighboring phenolic hydroxyl groups in caffeic acid and between the phenolic hydroxyl group and the methoxy one in ferulic and sinapinic acids, as well as in caffeic-H acid radical, further strengthen stabilization of these molecules. The calculated ΔHOF values of the parent molecule-radical couples are shown in Table 3, which clearly states that the latter exhibits the lowest ΔHOF value among all radicals studied at both levels of theory. Caffeic acid has been reported to be more active than its counterparts (51–57). Sinapinic and ferulic acids present some antioxidant activity, whereas *p*-coumaric is almost inactive. These findings also are in agreement with experimental data given in Table 3.

ΔHOF numbers in Table 3 also indicate that there are significant energy differences between most phenoxyl radicals studied; the corresponding energy differences are in the range of 2–19 kJ/mol (DFT) and 3–23 kJ/mol (HF). The significant energy differences between the ΔHOF values at both levels of theory provide a secure and ready way to put the structurally related compounds under study in an order according to their antioxidant activity. Such differences have not been found by using semiempirical levels of theory (1). The inconsistency observed at the HF level, between the calculated ΔHOF value-trend and the experimental scavenging activity trend for some of the radicals studied, could be due to the fact that at this theoretical level electron correlation is not taken into account, whereas DFT calculations include it. The energy differences between ferulic and *p*-coumaric (DFT) and sinapinic and ferulic (HF) acids are small (2–3.3 kJ/mol). This is also the case with the experimental PF and ARP values of the former pair of compounds. The hydrogen-bonding interaction has a large stabilizing effect of *ca.* 23 kJ/mol (HF) or 34 kJ/mol (DFT) on the **VI** as compared to that of **VII**. Analogous stabilization effects have been observed in the case of the catechol radical where the corresponding energy gain was in the range of 15–25 kJ/mol (4). The higher value derived for the stabilization effect of the H-bond at the DFT level could be attributed to the functional model used. Actually, the precise choice of nonlocal functionals remains a matter of some uncertainty for the H-bonding interaction calculation. According to Gresh *et al.* (58) the Local Density Approximation seriously overestimates at 37 kJ/mol, whereas various nonlocal functionals provide lower energy results.

The spin density values of the atoms constituting the radicals studied are shown in Figure 3. An inspection of the numbers presenting in Figure 3 shows that the computed spin density values at the HF level are consistently higher than those calculated at the B3LYP level. If oxidation takes place in the phenolic OH, the spin delocalization spreads over all atoms participating in the extended conjugation system of the molecule. At each particular theoretical level, the spin density values on similar atoms are almost identical, so that the delocalization is large in all radicals studied. The planarity of the rad-

TABLE 3
ΔHOF Values of the Phenoxyl Radicals, and Inhibition of Lipid Oxidation of the Four *p*-Hydroxycinnamic Acid Antioxidants

	VI	X	IX	V	VII	VIII
HOF*m*[a,b]	–1,702,667.85	–2,106,364.34	–1,805,790.91	–1,505,191.55	–1,702,667.85	–1,702,667.85
HOF*f*[c]	–1,701,069.03	–2,104,748.41	–1,804,154.88	–1,503,557.31	–1,701,032.67	–1,701,021.89
HOF*m*[d]	–1,692,808.52	–2,094,074.43	–1,795,190.96	–1,496,289.63	–1,692,808.52	–1,692,808.52
HOF*f*[e]	–1,691,350.62	–2,092,592.33	–1,793,705.10	–1,494,819.00	–1,691,325.58	–1,691,318.76
ΔHOF[f,g]	291.56	308.66	328.77	326.97	327.91	338.70
ΔHOF[h]	156.00	180.19	183.94	168.72	181.03	187.86
Inhibition (PF)[i]	6.2	2.4	1.1	1.05	—	—
Inhibition (ARP)[j]	4.5	2.5	1.8	0.008	—	—

[a] All energies in kJ/mol.

[b] Sum of electronic and thermal energies of parent molecule (DFT level).

[c] Sum of electronic and thermal energies of free radical produced after H-abstraction (DFT level).

[d] Sum of electronic and thermal energies of parent molecule (HF level).

[e] Sum of electronic and thermal energies of free radical produced after H-abstraction (HF level).

[f] Calculated at the DFT level.

[g] The sum of electronic and thermal energies of the H atom are –1309.75 and –1304.39 kJ/mol in the DFT and the HF levels of theory, respectively.

[h] Calculated at the HF level.

[i,j] This work. For abbreviations see Table 1.

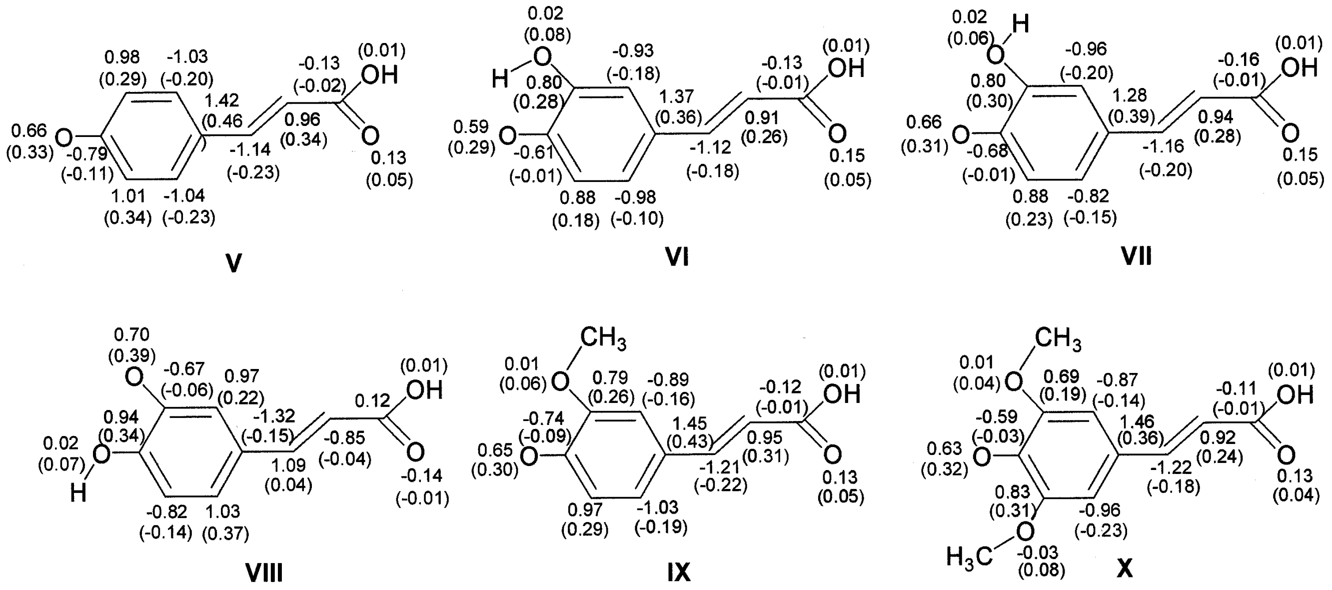


FIG. 3. Calculated atomic spin densities (DFT values are given in parentheses). Values of ≥0.01 are only shown; the benzene ring and the olefinic hydrogens, possessing 0.01 spin values, are omitted for clarity. For abbreviation see Figure 1.

icals leads to their full conjugation, and this, in turn, to an extended spin delocalization. Van Acker *et al.* (4) reached an analogous conclusion. The excellent delocalization possibilities of the radicals under study could account for their potential radical scavenging activity. Absence of a high amount of localized spin in these compounds may diminish the possibility to initiate a radical chain reaction (4). It is worth mentioning that, contrary to the HF spin density values for the **VIII**, the DFT ones clearly show that almost all spin remains in the benzene ring and its substituents, and the delocalization possibilities are limited. This fact could account for a lower antioxidant activity. The inferior antioxidant activity of **VIII**, as compared to that of **VII**, could be due to its higher calculated ΔHOF value, hence to its relatively harder tendency for hydrogen atom abstraction.

Molecular electron-donating ability. In an attempt to investigate whether the antioxidant activity correlates with the redox potentials, the HOMO energy-eigenvalues were calculated at both levels of theory. HOMO is a parameter representing molecular electron-donating ability. Calculated HOMO values are also listed in Table 1. Numbers in the table clearly show that *p*-coumaric acid, exhibiting the lowest experimental antioxidant activity, is the one with the lowest HOMO energy-eigenvalue at both levels of theory. This could account for its low experimental antiradical activity. However, caffeic acid, despite having the highest experimental antioxidant activity, exhibits the second-lowest HOMO energy-eigenvalue. Considering that an electron-donating group, for instance, the second hydroxyl of caffeic acid, should increase the HOMO energy-eigenvalue and reduce the O–H bond strength at the same time (16), the relative HOMO-energy order of the two acids appears correct. Nevertheless, the ferulic acid HOMO energy-eigenvalue, which involves a less electron donating group (methoxy) than the hydroxyl one of caffeic acid, should lie in between those of *p*-coumaric and caffeic acids. Consequently, HOMO energy-eigenvalue differences among those three acids are not suitable to predict antioxidant activity, although redox potential is expected to be directly related to their antioxidant activity. Zhang reached an analogous conclusion (16) by performing semiempirical calculations on phenolic antioxidants.

With the exception of the ΔHOF value, none of the remaining molecular descriptors by themselves could be safely used for the explanation of the antioxidant activity of the four molecules under examination. All parent-molecule phenoxyl-radical couples under study exhibit a high degree of conjugation, owing to their planarity. All radicals have almost equal, although large, spin delocalization. Both findings could account for their potential radical-scavenging activity. The higher antioxidant activity of caffeic acid, among the rest, should be attributed to its lower ΔHOF value, which arises from the less-energy-demanding hydrogen atom abstraction. It is also observed that relatively large ΔHOF value differences could lead to important changes in reactivity of a molecule *in vitro* and possibly *in vivo*. However, **VII** exhibits less antioxidant activity than **VI**, due to both its higher ΔHOF value and its limited spin delocalization. Considering that the structures of the four antioxidants studied exhibit slight differences and only one parameter is considered, the data obtained from experimental procedures and theoretical calculations are in good agreement. DFT calculations afford a good descriptor, ΔHOF, to correlate well with the antioxidant activity in molecules exhibiting similar structural parameters.

REFERENCES

1. Zhang, H.Y. (1998) Selection of Theoretical Parameter Characterizing Scavenging Activity of Antioxidants on Free Radicals, *J. Am. Oil Chem. Soc. 75*, 1705–1709.
2. Zhang, H.Y. (1999) Theoretical Methods Used in Elucidating Activity Differences of Phenolic Antioxidants, *J. Am. Oil Chem. Soc. 76*, 745–748.
3. van Acker, S.A.B.E., Koymans, L.M.H., and Bast, A. (1993) Molecular Pharmacology of Vitamin E: Structural Aspects of Antioxidant Activity, *Free Radical Biol. Med. 15*, 311–328.
4. 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., and Bast, A. (1996) A Quantum Chemical Explanation of the Antioxidant Activity of Flavonoids, *Chem. Res. Toxicol. 9*, 1305–1312.
5. Tanaka, K., Sakai, S., Tomiyama, S., Nishiyama, T., and Yamada, F. (1991) Molecular Orbital Approach to Antioxidant Mechanisms of Phenols by an *ab initio* Study, *Bull. Chem. Soc. Jpn. 64*, 2677–2680.
6. Tomiyama, S., Sakai, S., Nishiyama, T., and Yamada, F. (1993) Factors Influencing the Antioxidant Activity of Phenols by an *ab initio* Study, *Bull. Chem. Soc. Jpn. 66*, 299–304.
7. Rice-Evans, C.A., Miller, N.J., and Paganga, G. (1996) Structure-Antioxidant Activity Relationships of Flavonoid and Phenolic Acids, *Free Radical Biol. Med. 20*, 933–956.
8. Shahidi, F., Janitha, P.K., and Wanasundara, P.D. (1992) Phenolic Antioxidants, *Crit. Rev. Food Sci. Nutr. 32*, 67–103.
9. Brigelius-Flohe, R., and Traber, M.G. (1999) Vitamin E: Function and Metabolism, *FASEB J. 13*, 1145–1155.
10. Azzi, A., and Stocker, A. (2000) Vitamin E: Non Antioxidant Roles, *Prog. Lipid Res. 39*, 231–255.
11. van Acker, S.A.B.E., van den Berg, D.J., Tromp, M.N.J.L., Griffionen, D.H., van Bennekom, W.P., van der Vijgh, W.J.F., and Bast, A. (1996) Structural Aspects of Antioxidant Activity of Flavonoids, *Free Radical Biol. Med. 20*, 331–342.
12. Frankel, N.E. (1998) *Lipid Oxidation,* pp. 161–186, The Oily Press Ltd., Dundee.
13. Migliavacca, E., Carrupt, P.A., and Testa, B. (1997) Theoretical Parameters to Characterize Antioxidants. Part. 1. The Case of Vitamin E and Analogs, *Helv. Chim. Acta 80*, 1613–1626.
14. He, D., Wang, Z., and Mo, F. (1996) Studies on the Relationship Between Electronic Structure and Antioxidant Activity of Vitamin E, *Chin. J. Med. Chem. 6*, 157–161.
15. Liu, S.L., Pan, J.H., Shi, D.Y., Chen, K.X., Wang, Q.M., Chen, S.M., and Yan, X.M. (1998) Relationship Between Structure and Anti-Oxidation of Tocopherol with Molecular Orbital Theory, *Acta Pharmacol. Sin. 19*, 513–518.
16. Zhang, H.Y. (1999) Investigation of the Effectiveness of HOMO to Characterize Antioxidant Activity, *J. Am. Oil Chem. Soc. 76*, 1109–1110.
17. Nagaoka, S.I., Kuranaka, A., Tsuboi, H., Nagashima, U., and Mukai, K. (1992) Mechanism of Antioxidant Reaction of Vitamin E. Charge Transfer and Tunneling Effect in Proton-Transfer Reaction, *J. Phys. Chem. 96*, 2754–2761.
18. Nagaoka, S.I., Mukai, K., Itoh, T., and Katsumata, S. (1992) Mechanism of Antioxidant Reaction of Vitamin E. 2 Photoelectron Spectroscopy and *Ab Initio* Calculation, *J. Phys. Chem. 96*, 8184–8187.
19. Ono, S., Okazaki, K., Sakurai, M., and Inoue, Y. (1997) Density Functional Study of the Radical Reactions of 3-Methyl-1-phenyl–2-pyrazolin-5-one (MCI-186): Implication for the Biological Function of MCI-186 as a Highly Potent Antioxidant Radical Scavenger, *J. Phys. Chem. 101*, 3769–3775.
20. Korzekwa, K.R., Jones, J.P., and Gillette, J.R. (1990) Theoretical Studies on Cytochrome P-450 Mediated Hydroxylation: A Predictive Model for Hydrogen Atom Abstractions, *J. Am. Chem. Soc. 112*, 7042–7046.
21. Bors, W., Heller, W., Michel, C., and Saran, M. (1990) Flavonoids as Antioxidants: Determination of Radical-Scavenging Efficiencies, *Methods Enzymol. 186*, 343–355.
22. Hueso-Urena, F., Jimenez-Pulido, S.B., Moreno-Carretero, M.N., and Rodriguez-Avi, J. (1998) Quantitative Structure Liquid Chromatographic Retention Time Relationship on Natural Phenols Found in Olive Oil, *J. Am. Oil Chem. Soc. 75*, 793–799.
23. Garcia-Granda, S., Beurskens, G., Beurskens, P.T., Krishna, T.S.R., and Desiraju, G.R. (1987) Structure of 3,4-Dihydroxy-*trans*-cinnamic Acid (caffeic acid) and Its Lack of Solid-State Topochemical Reactivity, *Acta Cryst. C43*, 683–685.
24. Nethaji, M., Pattabhi, V., and Desiraju, G.R. (1988) Structure of 3-(4-Hydroxy-3-methoxyphenyl)-2 propenoic acid, *Acta Cryst. C44*, 275–277.
25. Pouchert, C.J. (1970) *The Aldrich Library of IR Spectra*, Aldrich Chemical Co., St. Louis.
26. Hehre, W.J., Random, L., Schleyer, P.v.R., and Pople, J.A. (1986) *Ab Initio Molecular Orbital Theory*, Wiley, New York.
27. Becke, A.D. (1993) Density-Functional Thermochemistry. III. The Role of Exact Exchange, *J. Chem. Phys. 98*, 5648–5652.
28. Ditchfield, R., Hehre, W.J., and Pople, J.A. (1971) Self-Consistent Molecular-Orbital Methods. IX. An Extended Gaussian-Type Basis for Molecular-Orbital Studies of Organic Molecules, *J. Chem. Phys. 54*, 724–728.
29. Hehre, W.J., Ditchfield, R., and Pople, J.A. (1972) Self-Consistent Molecular Orbital Methods. XII. Further Extensions of Gaussian-Type Basis Sets for Use in Molecular Orbital Studies of Organic Molecules, *J. Chem. Phys. 56*, 2257–2261.
30. Hariharan, P.C., and Pople, J.A. (1974) Accuracy of AHn Equilibrium Geometries by Single Determinant Molecular Orbital Theory, *Mol. Phys. 27*, 209–214.
31. Hariharan, P.C., and Pople, J.A. (1973) Influence of Polarization Functions on MO Hydrogenation Energies, *Theor. Chim. Acta 28*, 213–222.
32. Clark, T., Chandrasekhar, J., Spitznagel, G.W., and Schleyer, P.v.R. (1983) Efficient Diffuse Function Augmented Basis Sets for Anion Calculations. III The 3-21+G Basis Set for First-Row Elements, Lithium to Fluorine, *J. Comp. Chem. 4*, 294–301.
33. Frisch, M.J., Pople, J.A., and Binkley, J.S. (1984) Self-Consistent Molecular Orbital Methods 25. Supplementary Functions of Gaussian Basis Set, *J. Chem. Phys. 80*, 3265–3269.
34. Pulay, P., Fogarasi, G., Pang, F., and Boggs, J.E. (1979) Systematic *Ab Initio* Gradient Calculation of Molecular Geometries, Force Constants and Dipole Moment Derivatives, *J. Am. Chem. Soc. 101*, 2550–2560.
35. Peng, C., Ayala, P.Y., Schlegel, H.B., and Frisch, M.J. (1996) Using Redundant Internal Coordinates to Optimize Equilibrium Geometries and Transition States, *J. Comput. Chem. 17,* 49–56.
36. Frisch, M.J., Trucks, G.W., Schlegel, H.B., Gill, P.M.W., Johnson, B.G., Robb, M.A., Cheeseman, J.R., Keith, T., Petersson, G.A., Montgomery, J.A., *et al.* (1995) *Gaussian 94,* Revision D.4, Gaussian, Inc., Pittsburgh.
37. Brand-Williams, W., Cuvelier, M.E., and Berset, C. (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity, *Lebensm. Wiss. Technol. 28*, 25–30.
38. Lampi, A.M., Hopia, A., Ekholm, P., and Piironen, V. (1992) Method for the Preparation of Triacylglycerol. Fractions from Rape Seed and Other Oils for Autoxidation Studies. *Lebensm. Wiss. Technol. 25*, 386–388.
39. Psomiadou, E., and Tsimidou, M. (1998) Simultaneous HPLC Determination of Tocopherols, Carotenoids, and Chlorophylls, for Monitoring their Effect on Virgin Olive Oil Oxidation, *J. Agric. Food Chem. 46*, 5132–5138.
40. Wu, Y.-D., and Lai, D.K.W. (1996) Density Functional Study of Substituent Effect on the O-H and O-CH_3 Bond Dissociation Energies in Phenol and Anisole, *J. Org. Chem. 61*, 7904–7910.
41. Baesjou, P.J., Driessen, W.L., Challa, G., and Reedijk, J. (1997)

Ab Initio Calculations on 2,6-Dimethylphenol and 4-(2,6-Dimethylphenoxy)-2,6-dimethylphenol. Evidence of an Important Role for the Phenoxyonium Cation in the Copper-Catalyzed Oxidative Phenol Coupling Reaction, *J. Am. Chem. Soc. 119*, 12590–12594.

42. Huang, C.-L., Wu, C.-C., and Lien, M.-H. (1997) *Ab Initio* Studies of Decarboxylations of the β-Keto Carboxylic Acids $XCOCH_2COOH$ (X = H, OH and CH_3), *J. Phys. Chem. A 101*, 7867–7873.
43. Foresman, J.B., and Frisch, E. (1996) *Exploring Chemistry with Electronic Structure Methods*, Gaussian Inc., Pittsburgh.
44. Pople, J.A., Scott, A.P., Wong, M.W., and Random, L. (1993) Scaling Factors for Obtaining Fundamental Vibrational Frequencies and Zero-Point Energies from HF/6-31G-Asterisk and Mp2/6-31G-Asterisk Harmonic Frequencies, *Isr. J. Chem. 33*, 345–350.
45. Wong, M.W. (1996) Vibrational Frequency Prediction Using Density Functional Theory *Chem. Phys. Lett. 256*, 391–399.
46. Lin-Vien, D., Coltharp, N.B., Fateley, W.G., and Grasselli, J.G. (1991) *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*, Academic Press, San Diego.
47. Welti, D. (1970) *Infrared Vapor Spectra*, Heyden and Son, New York.
48. Nyquist, R.A. (1985) *The Interpretation of Vapor-Phase Infrared Spectra, Group Frequency Data*, The Sadtler Research Labs, Philadelphia.
49. Isaacs, N.S. (1995) *Physical Organic Chemistry*, 2nd edn., Longman Group Ltd., New York.
50. Papasavva, S., Illinger, K.H., and Kenny, J.E. (1996) *Ab Initio* Calculations on Fluoroethanes: Geometries, Dipole Moments, Vibrational Frequencies, and Infrared Intensities, *J. Phys. Chem. 100*, 10100–10115.
51. Papadopoulos, G., and Boskou, D. (1991) Antioxidant Effect of Natural Phenols on Olive Oil, *J. Am. Oil Chem. Soc. 68*, 669–671.
52. Marinova, E.M., and Yanishlieva, V.I.N. (1992) Effect of Temperature on the Antioxidative Action of Inhibitors in Lipid Autoxidation, *J. Sci. Food Agric. 60*, 313–318.
53. Marinova, E.M., and Yanishlieva, V. I. N., (1996) Antioxidative Activity of Phenolic Acids on Triacylglycerols and Fatty Acids Methylesters from Olive Oil, *Food Chem. 56*, 139–145.
54. Foti, M., Piattelli, M., Baratta, T.M., and Ruberto, G. (1996) Flavonoids, Coumarins, and Cinnamic Acids as Antioxidants in a Micellar System. Structure-Activity Relationship, *J. Agric. Food Chem. 44*, 497–501.
55. Terao, J., Karasawa, H., Arai, H., Nagao, A., Suzuki, T., and Takama, K. (1993) Peroxyl Radical Activity of Caffeic Acid and its Related Phenolic Compounds in Solution, *Biosci. Biotechnol. Biochem. 57*, 1204–1205.
56. Meyer, A.S., Donovan, J.L., Pearson, D.A., Waterhouse, A.L., and Frankel, E.N. (1998) Fruit Hydroxycinnamic Acids Inhibit Human Low-Density Lipoprotein Oxidation *in Vitro, J. Agric. Food Chem. 46*, 1783–1787.
57. Natella, F., Nardini, M., di Felice, M., and Scaccini, C. (1999) Benzoic and Cinnamic Acid Derivatives as Antioxidants: Structure–Activity Relation, *J. Agric. Food Chem. 47*, 1453–1459.
58. Gresch, N., Leboeuf, M., and Salahub, D. (1994) Energetics and Structure in Model Neutral, Anionic and Cationic Hydrogen-bonded Complexes: Combined ab initio SCF/MP2 Supramolecular, Density Functional and Molecular Mechanics Investiga-

tion, in *Modeling the Hydrogen Bond,* American Chemical Society Symposium Series (Smith, D.A. ed.), Vol. 569, pp. 82–112, American Chemical Society, Washington, DC.

[Received July 26, 2000, and in revised form January 8, 2001; revision accepted January 9, 2001]