Liquid-Phase Theoretical Antioxidant Activity Trend of Some Cinnamic Acid Antioxidants

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ABSTRACT: The trend in antioxidant activity of four cinnamic acid antioxidants, derived on the basis of their liquid-phase phenolic O–H bond dissociation enthalpies, is determined theoretically, with the aid of the polarizable continuum model at the density functional theory level. Three solvents, differing in their hydrogen-bonding ability and polarity—*n*-heptane, acetonitrile, and ethanol—were used to model different environmental situations. The trends in solution-phase antioxidant activity appeared different in each solvent; still, the theoretical antioxidant activity trend derived in *n*-heptane was in excellent agreement with the experimental one in the lipid systems and the gas-phase system. Different *ortho* substituents show notably different solvent effects. These results could lead to the determination of reliable antioxidant activity trends in real-solution environments of interest in food or biological science frameworks.

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KEY WORDS: Antioxidant activity trend, B3LYP, DFT, 3,4-dihydroxycinnamic acid, 4-hydroxycinnamic acid, 4-hydroxy-3,5-dimethoxycinnamic acid, 4-hydroxy-3-methoxycinnamic acid, liquid-phase BDE, PCM, solvent effect.

The main mechanism of action of phenolic antioxidants (ArOH) is considered to be the scavenging of free radicals by donation of their phenolic hydrogen atoms (1), namely,

$$ROO^\bullet + ArOH \rightarrow ROOH + ArO^\bullet \qquad [1]$$

where $ROO^\bullet$, ArOH, ROOH, and $ArO^\bullet$ are a lipid peroxide radical, the parent phenolic antioxidant, the lipid hydroperoxide, and the antioxidant aroxyl radical, respectively. A molecular descriptor that seems to correlate well with the experimental antioxidant activity results is the difference in the heat of formation (ΔHOF) value between the parent antioxidant molecule and its respective radical (2–4) and/or the phenolic O–H bond dissociation enthalpy (BDE) value (5). Whereas the vast majority of the BDE determinations reported for antioxidants refer to gas-phase reactions, most of the chemistry to which they are applied occurs in solution. However, solute–solvent interactions have a significant effect on the behavior of molecular systems. It was found (6) that the range of the solution values for the phenolic BDE (O–H) (abbreviated as BDE_{solv}) is well outside the claimed experimental errors of *ca.* ±2 kcal/mol for simple phenolic molecules. On the basis of such evidence, a theoretical investigation is presented in this paper on the bulk solvent effects on the BDE_{solv} determination of four phenolic antioxidants (*p*-hydroxycinnamic acids). We determined their gas-phase O–H BDE (hence, their gas-phase antioxidant activity trend) theoretically (2), and the main question is whether the solution-phase BDE and/or the antioxidant activity trend in solution follows the same trend that is found in the gas phase.

In an attempt to obtain reliable results on solvent effects, approaches employing a polarizable continuum description of the solvent appear to be accurate and efficient. The polarizable continuum model (PCM) (7) has become a standard (8) for the calculation of structural and energetic properties of molecules in solution. It allows for fast and reliable liquid-phase calculations, even if the solute is a large system, and for the calculation of solvation free energies (ΔG_{solv}) with chemical accuracy for a large number of chemical systems at the density functional theory (DFT) level. Moreover, we (9) used the PCM model at the DFT level for the accurate determination of absolute and relative liquid-phase O–H BDE of some simple phenols. Hence, we decided to apply it also to the calculation of the BDE_{solv} of the cinnamate antioxidants (hereafter denoted as cinnamates). However, in the present paper the 6-31+G(d) basis set was used, instead of that used before [6-31+G(,3pd)] (9). This choice was made because the aim of the present study was to make comparisons between the gas- and the liquid-phase antioxidant activity trends of the four cinnamates. Because there are no experimental BDE values for the antioxidants under study, an accurate determination of their BDE_{solv} seems meaningless.

In the present paper, BDE_{solv} of **I**, 4-hydroxycinnamic acid (*p*-coumaric acid); **II**, 3,4-dihydroxycinnamic acid (caffeic acid); **III**, 4-hydroxy-3-methoxy-cinnamic acid (ferulic acid); and **IV**, 4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid) (Scheme 1) cinnamates are determined theoretically. Three solvents, differing in their hydrogen-bonding ability and polarity—*n*-heptane, acetonitrile, and ethanol—are used to model different environmental situations. This theoretical work could lead to the determination of reliable antioxidant activity trends in real solution environments.

THEORETICAL METHODS

Bulk solvent effects were calculated by using dielectric-PCM (D-PCM) approach, as implemented in the Gaussian-98 program suite (10). PCM allows solution-phase geometry optimization within the PCM-UAHF (united atom model for Hartree–Fock) framework, which visualizes the solute molecule

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*To whom correspondence should be addressed at Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Applied Quantum Chemistry, P.O.B. 135, GR-541 24 Thessaloniki, Greece. E-mail: bakalbas@chem.auth.gr in solution by enclosing it in a proper cavity. For all molecules, full geometrical optimizations were carried out in solution with tight convergence criteria by employing DFT calculations (11) and using the B3LYP functional (Becke's three-parameter hybrid functional combined with the Lee–Yang–Parr correlation functional) (unrestricted B3LYP, or UB3LYP, for the resulting radicals). For comparison with the calculated gas-phase BDE (2), the 6-31+G(d) basis set was used throughout. The method applied is our variation of the high-level model (HLM) (12), which constitutes a fully consistent calculation because both the parent molecule and its respective radical are calculated at the same level of theory.

As a consequence of the rather weak phenolic O–H BDE, the phenolic antioxidant character could be related to the readily abstractable phenolic H atom,

$$\mathrm{ArOH} \rightarrow \mathrm{ArO}^{\bullet} + \mathrm{H}^{\bullet} \quad [2]$$

Hence, $\mathrm{BDE}_{\mathrm{solv}}$ is defined by

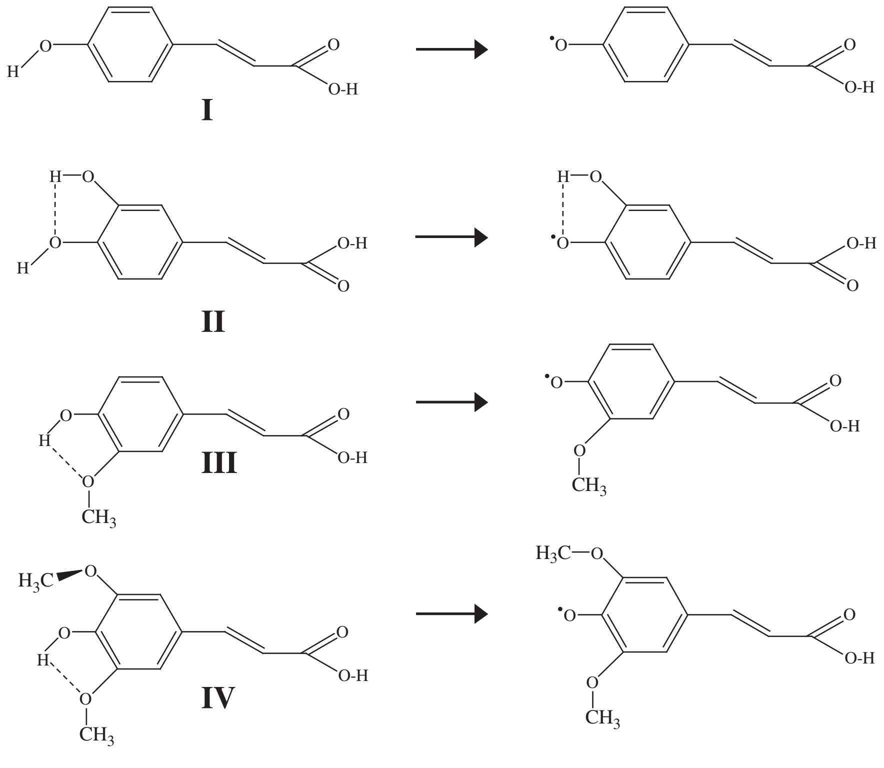
$$\mathrm{BDE}_{\mathrm{solv}}\,(\mathrm{ArO\text{-}H}) = H_f^S(\mathrm{ArO}^{\bullet}) + H_f^S(\mathrm{H}^{\bullet}) - H_f^S(\mathrm{ArOH}) \quad [3]$$

where H_f^S are the theoretically calculated enthalpies of solvation (in kcal/mol at 298 K). The total enthalpy of solvation for the parent molecules and the radicals is the sum of the thermal correction to the enthalpy and the B3LYP or the UB3LYP energy, respectively (13). For the PCM calculation of the hydrogen radical, a van der Waals radius [Bondi's (14) hydrogen radius] was assigned to the hydrogen in order to built its cavity. All $\mathrm{BDE}_{\mathrm{solv}}$ refer to the most stable pairs of parent and respective radical conformer, in which all but the parent and the radical conformers of **I** possess intramolecular hydrogen bonding (see Scheme 1). All parent and radical molecules studied herein have a rather flexible side chain, which, along with the toward and away orientations of the OH and OMe substituents of the benzene ring, give rise to several possible conformations. The most probable conformations of each parent and radical molecule were identified by a preliminary molecular dynamics search in the gas phase. These structures were further refined by a single-point calculation search in solution and in each of the three solvents selected, using the B3LYP method with a 6-31+G(d) basis set. Finally, the global minima on the calculated potential surface were determined by final frequency calculations in solution that provide energy minima with certainty. Owing to program limitations, the frequencies were calculated numerically, resulting in more time-consuming calculations. Calculated structures and energetic data of all parent and radical compounds are available from author E.G.B. upon request

The selection of the three different solvents was based on both their polarity and hydrogen-bonding ability, leading to a nonpolar, aprotic solution environment (*n*-heptane), a dipolar aprotic one (acetonitrile), and a polar, protic one (ethanol). Moreover, since in the PCM continuum model the dielectric constant of each solvent is an essential parameter of the calculation, a wide spectrum of dielectric constant (ε) values was used, ranging from 1.92 (*n*-heptane) to 24.55 (ethanol) and to 36.64 (acetonitrile).

RESULTS AND DISCUSSION

Fully planar calculated structures were derived for all of the parent molecule–radical couples studied except the parent molecule of **IV**. Planar structures strongly support complete conjugation



SCHEME 1

TABLE 1
The B3LYP/6-31+G(d) BDE, Δ(BDE), and Δ*G* Values for Compounds I–IV

ArOH	Solvent	E_r^a	E_h^a	E_p^a	BDE^b	Δ(BDE)^b	ΔG^b
I	Gas phase	–572.673768	–0.497912	–573.296217	78.15	0	69.88
	n-Heptane	–572.680203	–0.496407	–573.303454	79.60	1.45	71.31
	Acetonitrile	–572.677329	–0.495442	–573.301521	80.79	2.64	72.48
	Ethanol	–572.695773	–0.495970	–573.322718	82.19	4.04	75.01
II	Gas phase	–647.902003	–0.497912	–648.510964	69.68	0	61.82
	n-Heptane	–647.908664	–0.496407	–648.519668	71.91	2.23	63.45
	Acetonitrile	–647.905475	–0.495442	–648.519777	74.59	4.90	66.64
	Ethanol	–647.926874	–0.495970	–648.549098	79.23	9.54	70.68
III	Gas phase	–687.165329	–0.497912	–687.788461	78.58	0	70.21
	n-Heptane	–687.172556	–0.496407	–687.795216	79.22	0.65	70.66
	Acetonitrile	–687.169289	–0.495442	–687.790566	78.96	0.39	70.92
	Ethanol	–687.189739	–0.495970	–687.815282	81.31	2.73	72.97
IV	Gas phase	–801.655353	–0.497912	–802.270827	73.77	0	65.45
	n-Heptane	–801.662569	–0.496407	–802.278105	74.75	0.98	66.95
	Acetonitrile	–801.656938	–0.495442	–802.271892	74.99	1.22	67.25
	Ethanol	–801.679051	–0.495970	–802.294888	75.22	1.45	68.25

[a] Sum of electronic and thermal enthalpies of the radical (E_r), the hydrogen atom (E_h), and the parent molecule (E_p) of **I–IV** in hartrees.

[b] All BDE, Δ(BDE), and Δ*G* values are given in kcal/mol.

B3LYP, Becke's three-parameter hybrid functional combined with the Lee–Yang–Parr correlation functional; 6-31+G(d), split valence basis set with both d polarization and diffuse functions to heavy atoms; BDE, bond dissociation enthalpy; **I**, 4-hydrocinnamic acid (*p*-coumaric acid); **II**, 3,4-dihydroxycinnamic acid (caffeic acid); **III**, 4-hydroxy-3-methoxy-cinnamic acid (ferulic acid); **IV**, 4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid).

within these molecular species, in which the phenyl ring and the carboxyl groups are *trans* to each other around the connecting C=C bond (Scheme 1). This could account well for the correctness of our results, since a *trans* configuration is a more energetically stable structure than a *cis* one. Moreover, the intramolecular hydrogen-bonding found between the two neighboring phenolic hydroxy groups in the corresponding couple of **II** and between the phenolic hydroxy group and the methoxy group in **III** and **IV** further strengthens the stability of these molecules.

The calculated $<S^2>$ (spin operator) values of the phenoxyl radicals in the three different solvents tested ranged from 0.78 to 0.80, i.e., close to the expected value of a pure doublet wavefunction, 0.75. Hence, accurate $\mathrm{BDE}_{\mathrm{solv}}$ values should be expected, since the results of our DFT calculations are affected little by spin contamination (15).

Table 1 summarizes the calculated B3LYP/6-31+G(d) $\mathrm{BDE}_{\mathrm{solv}}$ for the three different solvents along with the corresponding gas phase. We know of no experimental $\mathrm{BDE}_{\mathrm{solv}}$ values for the antioxidants under study so that we could make comparisons. Nevertheless, their antioxidant activity trends have been well established experimentally in solution; the corresponding trend, in lipid systems (16) and in descending order, is **II** > **IV** > **III** ≅ **I**. We have simulated this trend through the calculated BDE in the gas phase (2). $\mathrm{BDE}_{\mathrm{solv}}$ values for the cinnamates in Table 1 appear larger than the corresponding gas-phase values, implying an inherent solvent effect. In particular, the more polar the solvent, the larger the $\mathrm{BDE}_{\mathrm{solv}}$ for **I–IV**; the calculated Δ(BDE) (= $\mathrm{BDE}_{\mathrm{solv}}$ – BDE) values range between 0.65 and 2.23, 0.39 and 4.90, and 1.45 and 9.54 kcal/mol for *n*-heptane, acetonitrile, and ethanol, respectively. For compounds **I** and **II**, this greater difference is the result of a stronger stabilization of the parent molecules in solution than of their radicals, evidenced by the E_p and E_r (liquid–gas phase) value differences of Table 1. In **III** and **IV**, with the exception of ethanol, a slightly stronger stabilization of the radicals is observed in solution than of their parent compounds.

Based on the positive values derived for both ΔH and ΔG, the overall Reaction 2 is endothermic in both the gas and the liquid phases. Moreover, there is also a solvent effect in the case of ΔG, since, upon dilution, its value increases relative to that in the gas phase. One can also see that the more polar the solvent, the more endothermic Reaction 2 is for **I–IV**. The calculated ($\Delta G_{\mathrm{solv}} - \Delta G$) value differences range between 0.45 and 1.43, 0.71 and 4.80, and 2.80 and 8.86 kcal/mol for *n*-heptane, acetonitrile, and ethanol, respectively. The protic polar solvent (ethanol) shows the larger endothermic reactions, possibly due to a larger number of solute–solvent interactions (intermolecular hydrogen bonds, etc.). Hence, both the BDE and the ΔG values show analogous solvent effects. All these could also imply that the PCM model describes solvent-effect phenomena well. Moreover, all calculated ΔH_{solv} ($\mathrm{BDE}_{\mathrm{solv}}$) and ΔG_{solv} values for **I–IV** in all media are the first to be published.

Figure 1 illustrates the variation in B3LYP/6-31+G(d) $\mathrm{BDE}_{\mathrm{solv}}$ for **I–IV** as a function of the three solvents selected (solvent effect). Gas-phase BDE are also shown for comparison. The solvent effect is seen to be similar for **I** and **II** but different for **III** and **IV**. On going from *n*-heptane to acetonitrile and to ethanol, the $\mathrm{BDE}_{\mathrm{solv}}$ of **I** and **II** increase significantly [Δ(BDE) ≤ 4.04 and 9.54 kcal, respectively] relative to the gas-phase values, whereas those of **III** and **IV** increase moderately [Δ(BDE) ≤ 2.73 and 1.45 kcal/mol, respectively]; still, the increase in $\mathrm{BDE}_{\mathrm{solv}}$ of **II** is more pronounced than in **I**. Based on the calculated $\mathrm{BDE}_{\mathrm{solv}}$, the antioxidant activity trend derived in *n*-heptane (**II** > **IV** > **III** ≅ **I**) is identical to that in lipid systems and in the gas-phase system. The trends in acetonitrile

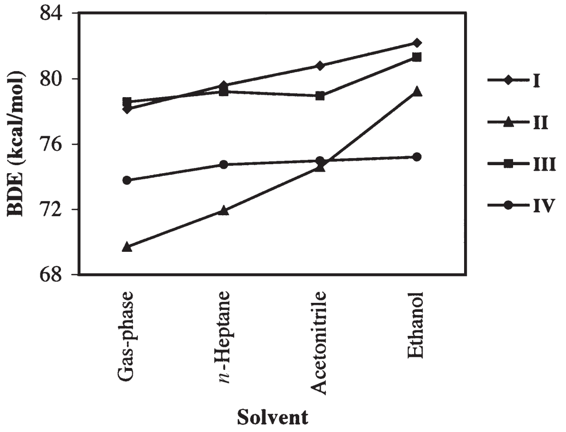


FIG. 1. Variation in bond dissociation enthalpy (BDE), of **I–IV**, denoting their solvent effects, in three different solvents: *n*-heptane, acetonitrile, and ethanol. Gas-phase BDEs are also included for comparison.

(**II** ≅ **IV** > **III** > **I**) and ethanol (**IV** > **II** > **III** ≥ **I**) appear different. In particular, $\mathrm{BDE}_{\mathrm{solv}}$ in *n*-heptane are nearly identical for **I** and **III** and different in acetonitrile, whereas the opposite is true for **II** and **IV** in the same solvents, respectively. In the case of ethanol, there is a significant inversion in the antioxidant activity trend: Caffeic acid shows a weaker activity than sinapic acid, in close agreement with the experimental results in an alcohol environment (17).

The presence of a substituent *ortho* to the phenolic OH group plays a key role in the solvent effect. For example, caffeic acid, **II**, and ferulic acid, **III**, possessing different *ortho* groups, show significantly different solvent effects, in close agreement with experimental results (18) with analogous phenolic molecules. Moreover, the *ortho* OH substituent leads to a better antioxidant activity than an *ortho* OMe one, being more pronounced in the case of the gas-phase and the nonpolar environments. However, their antioxidant activity differences become smaller on going from polar to protic media. This could account for the experimental finding that ferulic acid is 150% as efficient as caffeic acid in the aqueous phase (16).

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