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Structure−DPPH• Scavenging Activity Relationships: Parallel Study of Catechol and Guaiacol Acid Derivatives

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The scavenging behavior of a series of catechol and guaiacol acid derivatives toward DPPH• was examined having as a starting point the order of activity derived on the basis of theoretically calculated BDE values. The studied compounds were protocatechuic, homoprotocatechuic, dihydrocaffeic, and caffeic acids and also vanillic, homovanillic, dihydroferulic, and ferulic acids. Catechol and guaiacol were used as reference compounds. Observations from the parallel study were made with regard to structural features (number and position of hydroxyl groups and the side-carbon chain characteristics) that regulated the behavior of the compounds experimentally. The exceptional DPPH• scavenging behavior observed for homoprotocatechuic acid in ethanol and for caffeic acid in acetonitrile could not be supported by the respective BDE values. Ferulic was the most active among guaiacolic acids, whereas dihydroferulic exhibited the highest stoichiometry. Ionizable carboxylic groups seem to affect considerably the relative order of activity as was also evidenced using the ORAC assay. Questions raised about the validity of widely accepted views on criteria for SARs are discussed with regard to literature findings.

KEYWORDS: Hydroxybenzoic acids; hydroxyphenylacetic acids; hydroxypropanoic acids; hydroxycinnamic acids; structure−activity relationships; DPPH•; ORAC; BDE; DFT

INTRODUCTION

o-Dihydroxyl configuration (also referred to as a catecholic group) is commonly regarded as an essential feature ruling the antiradical activity of phenolic antioxidants (see, e.g., refs *1*−*3*). Concerning the reaction of catechol with free radicals, the active site, where O−H bond cleavage occurs, is indistinguishable due to the equivalence of both hydroxyl groups (*4*). In catechol acid derivatives, however, the two hydroxyl groups become different due to the presence of a carboxylic group attached to or linked with a side-carbon chain of the phenyl ring. Field inductive and resonance effects, depending on the structural features of the chain, stabilize/destabilize the respective radical formed after abstraction of one hydrogen atom (*5*−*7*). In particular, as frequently expressed, in the case of 3,4-dihydroxyl acid derivatives, H-atom abstraction is expected to occur at the 4-position, although documentation is rather limited.

One of the most frequently used tools for testing the radical scavenging activity of phenolic antioxidants is the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) assay, despite the considerations for the prevailing mechanism of hydrogen abstraction or the factors that may influence analytical results (*8*, *9*). On the other hand, theoretically derived bond dissociation enthalpy (BDE) values have been reported as supportive means to investigate the potential of a compound to act as a radical scavenger (*4*) and are in good correlation with relevant kinetic parameters (*10*, *11*).

Continuing our effort to examine differences in activity among structurally related compounds (*12*−*16*), the theoretically derived order of activity was used as the starting point to examine the scavenging behavior of a series of catechol and guaiacol acid derivatives (AHs) (**Figure 1**) toward DPPH•. The respective simple phenols were used as reference compounds. Observations from the parallel study are made with regard to structural features that regulated the behavior of the studied compounds experimentally. Questions raised about the validity of widely accepted views on criteria for structure−activity relationships (SARs) are discussed with regard to literature findings.

MATERIALS AND METHODS

Quantum Chemical Calculations. All geometries of molecules studied were fully optimized with the density functional theory (DFT), using the B3LYP functional (UB3LYP for the resulting radicals), and a 6-31+G(d) basis set as implemented in the Gaussian 98 computational programs suite (Gaussian Inc., Pittsburgh, PA) (*17*). The d polarization functions had five components. The C_1 point group symmetry for each species was assumed as the initial geometry of the optimization procedure, and all redundant internal coordinates were fully optimized.

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	-X	
(1)	-H	**(1′)**
(2)	-COOH	**(2′)**
(3)	-CH_2COOH	**(3′)**
(4)	-CH_2CH_2COOH	**(4′)**
(5)	-CH=CHCOOH	**(5′)**

Figure 1. Structural formulas and numbering of the AHs studied: catechol (**1**); protocatechuic (**2**); homoprotocatechuic (**3**); dihydrocaffeic (**4**); caffeic acid (**5**); guaiacol (**1′**); vanillic (**2′**); homovanillic (**3′**); dihydroferulic (**4′**); ferulic acid (**5′**).

All structures were true minima on the calculated potential energy surface (PES), verified by final frequency calculations that provide energy minima with certainty. All possible conformers of the phenols and the respective phenoxyl radicals under study (amounting to a total of 104 structures) were optimized, using tight convergence criteria. All of the final conformers and their energies in the gas phase are available upon request from E.G.B. The bond dissociation enthalpies (BDE) for the homolytic O−H bond cleavage in the parent phenolic molecules were calculated as the sum of the enthalpy of the radical resulting from the hydrogen atom abstraction and that of the hydrogen atom minus the enthalpy of the parent molecule. The H• enthalpy value was calculated to be −0.497912 hartree.

Experimental Procedures. *Materials.* DPPH•, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3,4-dihydroxybenzoic acid (protocatechuic acid, **2**), 3,4-dihydroxyphenylacetic acid (homoprotocatechuic acid, **3**), 3-(3,4-dihydroxyphenyl)propanoic acid (dihydrocaffeic acid, **4**), 4-hydroxy-3-methoxybenzoic acid (vanillic acid, **2′**), 4-hydroxy-3-methoxycinnamic acid (ferulic acid, **5′**), 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, **3′**), and 2-methoxyphenol (guaiacol, **1′**) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). 3,4-Dihydroxycinnamic acid (caffeic acid, **5**) (98%) was obtained from Riedel de Haën (Seelze, Germany), and 3-(4-hydroxy-3-methoxy)propanoic acid (dihydroferulic acid, **4′**) (98%) was obtained from Alfa Aesar (Heysam, Lancaster). Pyrocatechol (**1**, >98%) and 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH, >98%) were from Fluka Chemie (Buchs, Switzerland). Fluorescein was from Panreac Quimica SA (Barcelona, Spain). Ethanol (absolute >95%) was purchased from Riedel de Haën and acetonitrile (HPLC grade) from Panreac Quimica SA.

DPPH Assay. Quantification of the radical scavenging activity was based on the procedure used by Nenadis et al. (*18*) with some modifications. The solutions of the test compounds (AH) in ethanol or acetonitrile (0.1 mL) were added to the respective solution of the stable free radical DPPH• (2.9 mL, 100 μM) at different molar ratios. [DPPH•] reduction (ΔC) was monitored by absorbance measurement at 516 nm, automatically recorded every 6 s on a UV-1601 Shimadzu spectrophotometer (Kyoto, Japan). The reaction solution was thermostated at 25 ± 0.5 °C by an outer water-circulating bath. Calculation of the kinetic parameters EC_{50} (efficient concentration of the antioxidant necessary to decrease the initial [DPPH•] by 50%) and T_{EC50} (reaction time needed to reach the steady state at EC_{50}) were based on the estimation of the [DPPH•] at steady state as follows: [DPPH•] reduction (ΔC_b) of five blank ethanolic solutions (containing only DPPH•) was monitored over 5 h. The average ΔC_b was calculated for various time intervals along with the corresponding SD_b value. Thus, the steady state was defined as the time interval (at least 20% of the overall monitoring time) during which $\Delta C \leq \Delta C_b + 10SD_b$. EC_{50} and T_{EC50} values were then estimated graphically as described previously (*19*). Using the graphically estimated EC_{50} value, triplicate kinetic tests were carried out for each AH. The [DPPH•] remaining value at the steady state was then included in the plots $\%[DPPH^•]_{rem}$ versus $[AH]/[DPPH^•]_0$ to correct EC_{50} and T_{EC50}. Finally, the corrected EC_{50} and T_{EC50} values were used to calculate the antiradical index AE [antiradical efficiency, $AE = 1/(EC_{50} \times T_{EC50})$]. Stoichiometric values (moles of DPPH• scavenged per mole of AH) were also calculated.

Table 1. B3LYP/6-31+G(d) Calculated BDEs and Literature Hammett/Brown Parameters

	BDE[a]			BDE	Hammet/Brown parameters[b]		
AH	4-r	3-r	AH	iHB	σ_p^+	σ_p	σ_m
1	*70.9*[c]	*70.9*	**1′**	78.4			
2	72.9 (2.0)[d]	*73.7* (*2.8*)	**2′**	81.1 (2.7)	0.42 0.34 + 0.08[e]	0.45 0.34 + 0.11	0.37
3	70.7 (−0.2)	*71.4* (*0.5*)	**3′**	78.6 (0.2)	−0.01	–[f]	–
4	*69.4* (*−1.5*) [70.2][g] [(−0.7)]	70.0 (−0.9) – –	**4′**	76.9 (−1.5)	–	−0.07 0.02–0.09	−0.03
5	69.6 (−1.3)	*72.4* (*1.5*)	**5′**	77.6 (−0.8)	–	0.03[h] 0.27–0.24	0.19

[a] All values in kcal/mol. [b] From ref *19*. [c] ΔBDE values [estimated as $\Delta BDE = BDE_X - BDE_1$ (X = **2**, **3**, **4**, **5**), for the catecholic compounds, and $\Delta BDE = BDE_X - BDE_{1'}$ (X = **2′**, **3′**, **4′**, **5′**) for the 3-methoxy-4-hydroxy ones] are given in parentheses. [d] BDEs of the most stable parent–radical pairs are given in italics. [e] Sum of the inductive (F) and resonance (R) effects. [f] No literature values available. [g] BDEs of the gauche conformers are given in brackets. [h] Values are referred to the –CH=CH–COOH substituent.

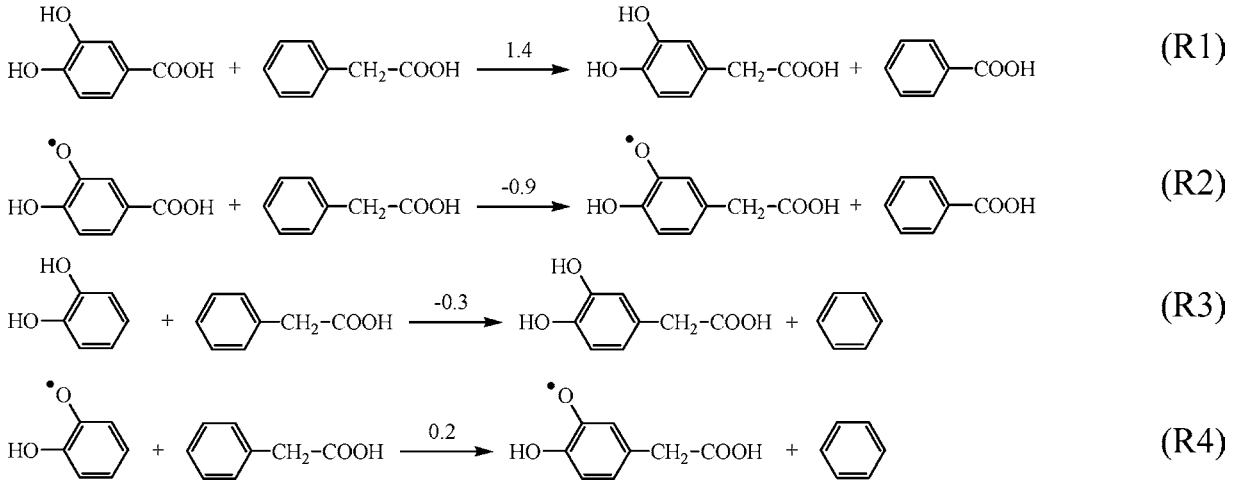
Statistical Analysis. Statistical comparisons of the mean values for each experiment were performed by one-way analysis of variance (ANOVA), followed by the multiple Duncan test ($p \leq 0.05$ confidence level). Precision was tested for two molar ratios (0.2 and 0.25 mol of AH/mol of DPPH•) on three consecutive working days (CV % < 7%).

Oxygen Radical Absorbance Capacity (ORAC) Assay. Evaluation of peroxyl radical scavenging activity was based on the protocol described by Naguib (*20*) with slight modifications. Peroxyl radicals were generated at a controlled rate by thermal decomposition of AAPH. The test solutions (5 mL) contained fluorescein (4 mL, 15 nM) in phosphate buffer (75 mM, pH 7) along with 0.25 mL of AH solution (0.25−2 μM). The reaction started by the addition of AAPH (120 μL, 0.125 M) to the test solution. Decay of the fluorescein signal (exc, 490; em, 515 nm) was monitored until zero fluorescence occurred on a Shimadzu RF 1501 spectrofluorometer (Kyoto, Japan) equipped with a stirrer and temperature-controlled cell holder at 37 °C. Trolox was used as the reference compound. Experiments were carried out in triplicate. The net area under curve (AUC) calculated in the presence (AUC_{AH}) and in the absence of the test compounds (AUC_{blank}) was used to express the antiradical activity of AHs relative to Trolox: $[(AUC_{AH} - AUC_{blank})/(AUC_{Trolox} - AUC_{blank}) \times$ (mol of Trolox/mol of AH). Calculations were carried out by means of the RF 1501-PC software.

RESULTS AND DISCUSSION

DFT Calculations. Table 1 presents the calculated BDEs of both catechol and guaiacol acid derivatives; ΔBDEs are also given in parentheses. The calculated BDEs reflect the ease/difficulty of hydrogen radical elimination and, hence, the ease/difficulty in the free radical scavenging activity of the AH. In the same table, literature Brown (σ_p^+) and Hammett (σ_m) parameter values for some of the acids under study are also given. These electronic parameters provide information on the effect of para and meta substituents, respectively, on the activity of the phenyl ring (*5*, *21*).

Scheme 1



Formation of an intramolecular hydrogen bond (iHB) from either the 3- or 4-OH group is feasible in **2**−**5**, due to the 3,4-dihydroxyl configuration in their molecular structure (**Figure 1**). The H atom that is not involved in this bond will then be abstracted by free radicals, resulting in a stable phenoxyl radical. In our study, conformational stability of both parent compounds (**2**−**5**) and corresponding radicals indicated which of the two hydroxyl groups is more likely to participate in an iHB. Noticeably, the 4-OH of **2**, **3**, and **5** was found to be more susceptible to the formation of a hydrogen bond than the respective hydroxyl group of **4**. The most stable conformer of **4** corresponded to a gauche structure.

As far as the radicals formed from the compounds **2**−**4** are concerned, formation of an iHB at the 3-OH group is slightly favored toward that at the 4-position ($\Delta\Delta BDE = \Delta BDE_{3-r} - \Delta BDE_{4-r} = 0.6-0.8$ kcal/mol). The higher $\Delta\Delta BDE$ value (≈2.8 kcal/mol) observed in the case of **5** can be ascribed to additional resonance structures that favor stabilization of the phenoxyl radical at the 4-position. An inspection of BDE values for both series of different conformers revealed some differences in the order of potential radical scavenging activity.

In the case of H-radical elimination from the 4-OH-position, the theoretical free radical scavenging activity trend of the examined acids in descending order is **4** ≅ **5** > **3** ≅ 1 > **2**. As expected, the insertion of the carboxylic group to the catechol ring results in a less favorable H-radical elimination by ≈2 kcal/mol, as a result of the $-I$ effect of the substituent (*4*, *22*). Insertion of methylene and ethylene groups ($+I$) between the catechol ring and the carboxylic group favors the H-radical elimination, as it is also substantiated by the corresponding σ_p^+ Brown parameter and σ_p values (**Table 1**). Moreover, the insertion of a vinylidene group accounts for the lower BDE value of **5**, compared to those of **1** and **3**.

A quite different order in the activity of **2**−**5** is expected if H-radical elimination occurs at the 3-position: **4** > **1** > **3** > **5** > **2**. In this particular case, the corresponding phenoxyl radical is affected only by the inductive phenomenon of the carboxylic side-chain group. Such an effect can also explain the higher ranking in the activity of catechol (**1**), compared to those of **3** and **5**. The available Hammett parameter values (σ_m) of the carbon side chain are also indicative of these differences in activity (**Table 1**).

Independently of the position of H-radical elimination, dihydrocaffeic acid (**4**) possessing a propanoic side chain is expected to exhibit the highest activity among these compounds, whereas the opposite is expected for protocatechuic acid (**2**). The relative order of activity of **1**, **3**, and **5** in the case of hypothetical abstraction from the 3-position is the reverse of that in the case of radical formation at the 4-position. In such a case, the characteristics of the side chain of the parent molecule are not a prerequisite for the behavior of phenoxyl radicals and, if abstraction occurs at the 3-position, then classical criteria for antiradical activity become irrelevant.

In the case of guaiacol acid derivatives (**2′**−**5′**), for which only the 4-OH group is available for H-atom donation, the theoretical free radical scavenging activity trend in descending order is **4′** > **5′** > **1′** ≅ **3′** > **2′**, in line with the σ_p^+ Brown parameter values trend (**Table 1**). Compared to the respective order within the series of catecholic acids, the above trend showed more clearly the contribution of the side-chain length to the activity of the tested compounds. This is due to the presence of only one −OH, forming an iHB, in the series of guaiacolic acids, which, in turn, means that an additional energy, identical to the iHBstrength, is necessary for the H-radical elimination. This is not the case with the catecholic acid series, from which the non-iHB is usually eliminated. Because both the −OH and $-OCH_3$ groups introduce similar electronic phenomena to the ortho and para positions [$\sigma_p^+(OH) = \sigma_p^+(OCH_3) = 0.78$] (*23*), no significant differences should be expected for the BDE values of both of the two AH series. Nevertheless, the ΔBDEs observed are significant, amounting to ≈3−3.6 kcal/mol, due to the iHBs formed by the second −OH group present only in the series of catecholic radicals.

Overall, it seems that the electron-withdrawing properties of the −COOH group present in both series of phenylacetic and phenylpropanoic acids affect only moderately the activity of the phenyl ring. Thus, phenylpropanoic acids could be more efficient radical scavengers than the respective phenols. The activity of cinnamic acid derivatives, possessing a double bond in the side chain, is not expected to highly differentiate from that of phenylpropanoic ones. On the other hand, H-atom abstraction is favored in cinnamic acids rather than in phenylacetic ones.

A quantitative confirmation of the.BDE values, already presented in **Table 1**, is attempted next, based upon the stabilization/destabilization (enthalpic contributions, ΔHs), of the parent compound/respective radical pairs, with the aid of hypothetical isodesmic reactions (*24*). Abstraction of the ΔH value of the former compound from that of the latter affords the ΔBDE value.

In particular, the hypothetical isodesmic reactions R1 and R2 (shown in **Scheme 1**) afford ΔH values of 1.4 and −0.9 kcal/

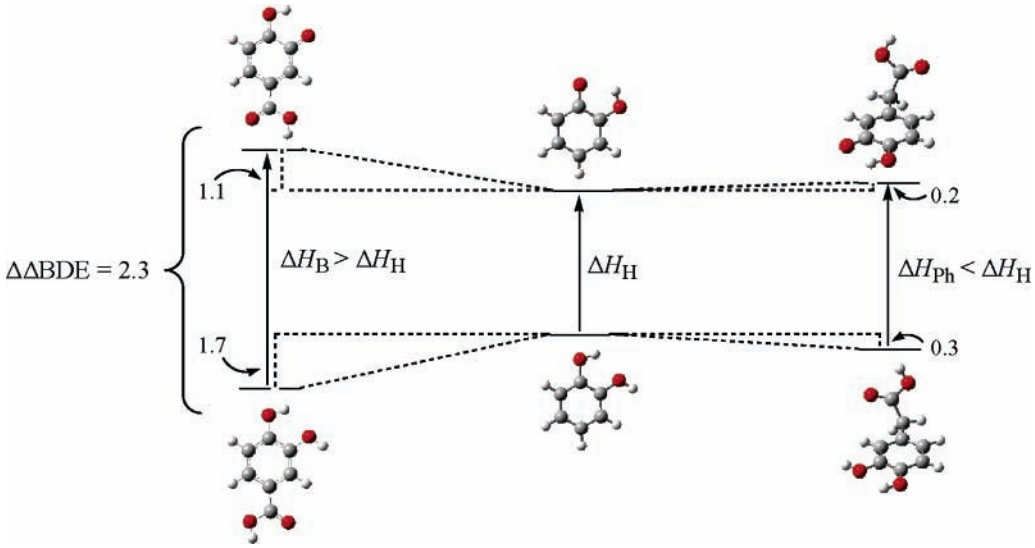


Figure 2. Schematic representation of the enthalpic effect of the –COOH and –CH_2COOH substituents on the parent and radical species of protocatechuic and homoprotocatechuic acids with regard to catechol (values in kcal/mol).

Table 2. DPPH• Scavenging Activity of Catechol and Guaiacol Acid Derivatives in Ethanol

AH	EC_{50} [a]	T_{EC50} [b] (min)	AE[c]	n[d]	AH	EC_{50}	T_{EC50} (min)	AE	n
1	0.20	12.3–17.0[b] (14.9 ± 0.1)[c]	0.36 ± 0.01	2.5	**1′**	0.90	120–150 (135.0 ± 0.0)	0.008 ± 0.000	0.6
2	0.19	75.0–91.0 (83.0 ± 0.0)	0.06 ± 0.01	2.6	**2′**	–[e]	–	0.00[e]	–
3	0.12	12.0–15.0 (13.5 ± 0.0)	0.63 ± 0.00	4.4	**3′**	0.46	280–340 (310.0 ± 0.0)	0.007 ± 0.000	1.1
4	0.22	30.0–42.0 (35.8 ± 2.6)	0.15 ± 0.01	2.3	**4′**	0.34	115–141 (128.0 ± 0.0)	0.025 ± 0.000	1.5
5	0.21	11.0–14.0 (12.5 ± 0.0)	0.38 ± 0.00	2.4	**5′**	0.48	46.2–56.8 (51.5 ± 3.0)	0.041 ± 0.003	1.0

[a] Molar ratios [AH]/[DPPH•] were in the range of 0.08–6 mol/mol. [b] Steady-state calculated from three experiments as described under Materials and Methods; mean ± SD values are given in parentheses. [c] Mean ± SD values. [d] Mol of DPPH•/mol of AH. [e] Vanillic acid was found to be nonreactive (%$[DPPH^•]_{rem}$ > 90% after 6 h).

mol for the parent compound and the formed radical of the protocatechuic acid, respectively, which, by abstraction, give a ΔBDE value of −2.3 kcal/mol, identical to that appearing in **Table 1** between the compounds **2** and **3**, differing by one methylene group. Moreover, the isodesmic reactions R3 and R4 afford Δ*H* values of −0.3 (R3) and 0.2 kcal/mol (R4), hence, a ΔBDE value of −0.5 kcal/mol, being also identical to that (70.9 − 71.4 = −0.5 kcal/mol) appearing in **Table 1** for compounds **1** and **3**, differing by one −CH_2COOH group.

Finally, **Figure 2** is a schematic representation of the enthalpic effect of the −COOH and −CH_2COOH substituents on the parent and the radical species of the protocatechuic (**2**) and homoprotocatechuic acids (**3**) with regard to catechol (**1**).

Experimental Procedures. Trends derived from computational data were regarded as a null hypothesis for the evaluation of SARs within each series of acids, using the DPPH• assay. The latter was employed in the present study because most published data on the in vitro radical scavenging potential of phenolic acids have been obtained using this assay. In our study, the kinetics of the DPPH• scavenging reaction was evaluated in terms of antiradical efficiency values. AE values together with EC_{50}, T_{EC50}, and stoichiometry values are presented in **Table 2**. Antiradical efficiency is an expression of results that takes into account both stoichiometry (in terms of EC_{50}) and time to reach steady state (T_{EC50}) (*19*). The AE value is the result of a combination of kinetic and static approaches to characterize the antioxidant efficiency of a molecule. Although questioned by some investigators (*9*, *25*), this parameter provides an indirect means to consider that low T_{EC50} and low amounts of a potent antioxidant are needed to prevent autoxidation of free radical mediated oxidation of a lipid substrate (*19*). Due to diverse expressions and analytical protocols in different DPPH• studies of phenolic acids, our findings were carefully interpreted by taking into account comparable literature data. It should be stressed, however, that the kinetics of the DPPH• scavenging reaction in terms of AE or rate constant values is scarcely discussed in the literature. Instead, stoichiometric factors (EC_{50} or IC_{50}) are most often used to describe differences in the scavenging potential of mono- and polyphenol acid derivatives, even though aspects other than stoichiometry, for example, rate of reaction, are also important in the evaluation of antiradical activity (*8*).

In view of the theoretically derived order of activity, small but clear differences should be expected in the radical scavenging activity among the acid derivatives of each series. Stoichiometric factors of **1**−**5** indicated that at least 2 mol of DPPH• was scavenged per mole of acid, in line with previous findings for **1**, **4**, and **5** (*26*, *27*). Side oxidative reactions (*28*) could be the reason for the abnormally high stoichiometry of **3** (≈4 mol of DPPH•/mol of AH). Such reactions could also play a role in the case of **2**, because its slightly higher stoichiometry compared to that of **1** could not be interpreted in terms of electronic

Table 3. DPPH• Scavenging Activity of Catechol Acid Derivatives in Acetonitrile

AH	EC_{50} [a]	T_{EC50} [b](min)	AE[c]	n[d]
1	0.24	37–52 (44.5 ± 2.1)	0.09 ± 0.00	2.1
2	0.21	12.5–18.5 (15.5 ± 0.0)	0.32 ± 0.00	2.4
3	0.20	31–46 (39.0 ± 0.9)	0.14 ± 0.00	2.5
4	0.18	46–63 (55.0 ± 0.0)	0.10 ± 0.00	2.8
5	0.23	1.2–1.6 (1.4 ± 0.1)	3.18 ± 0.13	2.2

[a] Molar ratios [AH]/[DPPH•] were in the range of 0.1–0.3 mol/mol. [b] Steady state was calculated from three experiments as described under Materials and Methods; T_{EC50} ± SD values are given in parentheses. [c] AE ± SD values. [d] Mol of DPPH•/mol of AH.

phenomena and no relevant literature data were available. Clear evidence on differences among the examined compounds was given when the overall kinetics of the reaction was considered. On the basis of AE values, the DPPH• scavenging activity of the catechol acid derivatives was ranked as follows: **3** > **5** ≅ **1** > **4** > **2**. Prioritization showed that the phenylacetic derivative (**3**) possessed the highest activity, almost twice as high as that of catechol (**1**) or even that of caffeic acid (**5**). Such a behavior was not inferred from the respective BDE values, so that it could not be solely attributed to electronic phenomena of the side-carbon chain but probably to reaction environment interferences. This was also suggested in the case of **4**, because it was ≈3 times less potent than **5**, as reported by Nenadis et al. (*14*). Dihydrocaffeic acid (**4**) was found to be even less active than catechol. A similar order was reported by Foti and co-workers (*27*) using rate constant values for the DPPH• reaction in ethanol. Catechol activity was 6-fold higher than that of **2**, indicating the prevailing role of the −COOH group attached to the catechol ring. This profound effect of the −COOH group is in line with the computational evidence, even though its size could not be predicted on the basis of the small ΔBDE value (2−3 kcal/mol) between **1** and **2**.

To better appreciate the ranking of the catechol acid derivatives, a series of experiments were carried out in acetonitrile, a nonprotic solvent, in which the reaction kinetics is expected to be different from that in ethanol (*18*, *29*). Noticeable changes that were observed in the overall kinetics of the DPPH• scavenging resulted in the following order in terms of AE values: **5** ≫ **2** > **3** ≥ **4** ≅ **1** (**Table 3**). In contrast to the ranking in ethanol, phenylacetic acid did not show any exceptional activity in the nonprotic solvent. Caffeic acid presented the highest activity due to its rapid kinetic behavior, which has also been observed by Nenadis et al. (*14*). Moreover, the benzoic acid derivative (**2**) was unexpectedly potent, even more than **3**, **4**, or **1**. This finding, not in line with the null hypothesis, may be substantiated by recent NMR findings (*30*, *31*). The latter showed that a concomitant formation of quinoid structures that takes place after H-radical elimination might facilitate ionization of the −COOH group in a nonprotic solvent. This effect leads to a dramatic change in the inductive and resonance phenomena ($I_{COOH} = 0.44$ and $I_{COO^-} = -0.27$; $R_{COOH} = 0.66$ and $R_{COO^-} = 0.40$) (*32*). BDE values cannot account for such a contribution to the activity of **2**. It should be emphasized, though, that stoichiometry values were in line with the number of hydroxyl groups of all of the catechol acids, signifying a slightly higher activity of **4** in comparison to that of **3**, **5**, or **1**. This order did not contradict the one supported by BDE values.

Except for the effect of reaction environment, the effect of the type of radical on the activity of the compounds was also considered. The kinetics of **3**, **4**, and **5** toward alkylperoxyl radicals, expressed as ORAC values (4.5 ± 0.4, 3.3 ± 0.1, and 5.2 ± 0.2, respectively), indicated a trend similar to that found using the DPPH• in acetonitrile. The conditions of the ORAC assay favor the dissociation of the carboxylic group of acids, a phenomenon that is also reported to take place, although to a lesser extent, in the ethanolic environment of DPPH• assay (*27*). However, in such a case, electronic effects of the side-chain carbon that are changed dramatically may lead to either the hydrogen atom transfer (HAT) or single-electron transfer (SET) mechanism of H-atom abstraction (*27*, *34*). It should be noted here that the overall stoichiometry of the reaction is not dependent on the mechanistic model (*33*), so that any differences observed could be ascribed to parallel oxidation routes. On the other hand, differences in the kinetic behavior of these acids, if associated with the presence of $-COO^-$, could explain the high activity of **5** in both DPPH−acetonitrile and ORAC−aqueous buffered systems. In both of the latter cases, **3** was found to be equally or even more efficient as scavenger than **4**, a finding that could be related with the length of the side-chain carbon, taking into account the presence of the electron-donating $-COO^-$ group.

The order in DPPH• scavenging activity of guaiacol acid derivatives was **5′** > **4′** > **1′** ≅ **3′** > **2′** (**Table 2**). In the absence of the catechol moiety, AE values revealed a higher contribution of the side-chain characteristics, as suggested by BDE values. Ferulic acid (**5′**) showed the highest activity among the examined compounds owing to its faster kinetic behavior. However, the phenylpropanoic acid derivative (**4′**) was shown to scavenge ≈1.5 mol of DPPH•, exhibiting the highest stoichiometry, ≈3 times higher than that of guaiacol (**1′**) and 1.5 times higher than the respective value of ferulic acid. The latter finding is in accordance with that reported by Shimoji and co-workers (*35*) on the basis of IC_{50} values. Vanillic acid (**2′**) showed almost no activity. Homovanillic (**3′**) was as potent as guaiacol (**1′**) even though it was more efficient as a scavenger (1.1 and 0.6 mol of DPPH•/mol of AH, respectively). Taking into account that only one −OH group is present in both compounds, stoichiometry values reveal differences that are difficult to interpret. Still, the descending order is in accordance with the one based on BDE values. Due to limited information on the radical scavenging activity of phenylacetic acids, our findings can be used as the basis for further investigation.

Our findings imply that DPPH• scavenging activity of phenolic compounds, possessing structural features susceptible to analytical conditions, should be cautiously interpreted. The buildup SARs is becoming a terrain full of loopholes for those working in it.

ABBREVIATIONS USED

HAT, hydrogen atom transfer; ORAC, oxygen radical absorbance capacity; SET, single-electron transfer.

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