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Electron-Transfer Reaction of Cinnamic Acids and Their Methyl Esters with the DPPH• Radical in Alcoholic Solutions

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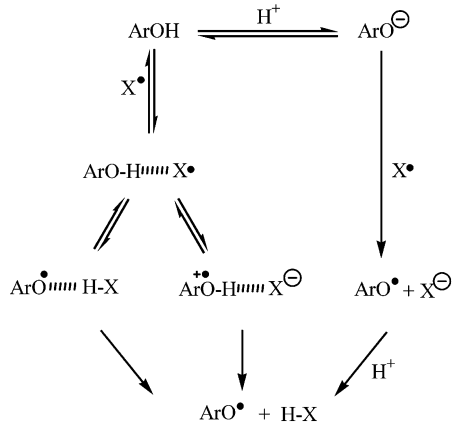
The kinetic behavior of cinnamic acids, their methyl esters, and two catechols **1**−**10** (ArOH) in the reaction with DPPH• in methanol and ethanol is not compatible with a reaction mechanism that involves hydrogen atom abstraction from the hydroxyl group of **1**−**10** by DPPH•. The rate of this reaction at 25°C is, in fact, comparatively fast despite that the phenolic OH group of ArOH is hydrogen bonded to solvent molecules. The observed rate constants (k_1) relative to DPPH• + ArOH are 3−5 times larger for the methyl esters than for the corresponding free acids and, for the latter, *decrease* as their concentration is *increased* according to the relation $k_1 = B/[\mathrm{ArOH}]_0^m$, where k_1 is given in units of $M^{-1}\ s^{-1}$, m is ca. 0.5, and B ranges from 0.02 (*p*-coumaric acid) to ca. 3.48 (caffeic acid) in methanol and from 0.04 (*p*-coumaric acid) to ca. 13 (sinapic acid) in ethanol. Apparently, the reaction mechanism of DPPH• + ArOH involves a *fast* electron-transfer process from the phenoxide anion of **1**−**10** to DPPH•. Kinetic analysis of the reaction sequence for the free acids leads to an expression for the observed rate constant, k_1, proportional to $[\mathrm{ArOH}]_0^{-1/2}$ in excellent agreement with the experimental behavior of these phenols. The experimental results are also interpreted in terms of the influence that adventitious acids or bases present in the solvent may have. These impurities *dramatically* influence the ionization equilibrium of phenols and cause a reduction or an enhancement, respectively, of the measured rate constants.

Introduction

Free oxygen-centered and nitrogen-centered radicals (ROO•, RO•, and 2,2-diphenyl-1-picrylhydrazyl (DPPH•)) (hereafter simply X•) react with phenols (ArOH) via two different mechanisms: (i) a direct abstraction of phenol H-atom by X• (HAT reactions) and (ii) an electron-transfer process from ArOH or its phenoxide anion (ArO^-) to X• (ET reactions), see Scheme 1.[1−14] The contribution of one or the other pathway depends on the nature of the solvent and/or the redox potentials of the species involved.[12−14] Generally, in apolar solvents the HAT mechanism is predominant, but with strongly oxidizing radicals such as Cl_3COO•, the mechanism of electron transfer can be the preferential route even in these media.[14]

SCHEME 1



The text above implies that polar solvents (*S*) may have a strong influence on the rates of these reactions. Ingold

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and co-workers,[1−8] but also other groups,[10] have recently shown that nonprotic polar solvents reduce the rate of many ArOH/X• reactions. This has been explained by considering that most of the molecules of ArOH are hydrogen bonded to the solvent (ArOH···*S*), and these species are unable to react by HAT with X•. Only the *free*, i.e., non-hydrogen-bonded, fraction of ArOH is capable of transferring an H-atom to X•. Therefore, the observed value of the rate constant for this reaction ($k^{S}_{ArOH/X}$) is dependent on the strength of the hydrogen bond in the complex, ArOH···*S*. The stability of this 1:1 complex depends on both the hydrogen-bond basicity, β^{H}_{2}, of the solvent[15] and the hydrogen-bond acidity, α^{H}_{2}, of ArOH.[16] Experimentally, it was found that log $k^{S}_{ArOH/X}$ correlates linearly with these two parameters (for many phenols and solvents).[7,8] The fact that the value of $k^{S}_{ArOH/X}$ for a particular ArOH/X• couple in a given solvent is either subject or not subject to the restriction of this free-energy relationship[7,8] may be taken as evidence for a HAT or ET mechanism. More recently, in fact, Litwinienko and Ingold[17] observed that the reactions ArOH + DPPH• in methanol and ethanol were faster than predicted. This observation demanded a non-HAT mechanism, which was shown to be an oxidation of the phenoxide anion by the DPPH• radical.[17]

The stable radical DPPH• is widely used to "evaluate" the antiradical/antioxidant properties of synthetic and natural phenols using methanol or ethanol as the most convenient solvents.[17,18] The validity of the conclusions that are drawn from these studies is, therefore, open to question because the antioxidant properties of ArOH are related to their ability to transfer their phenolic H-atom to a peroxyl radical (ROO•).[19]

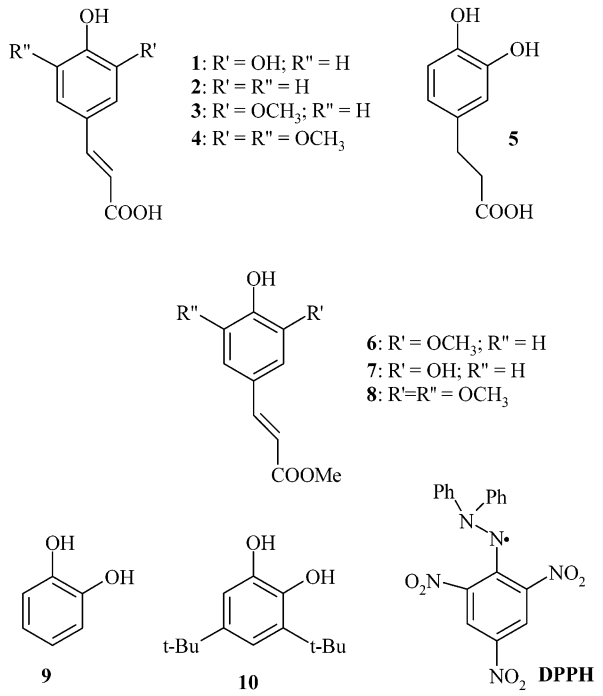
We intend to show in this paper that the cinnamic acids and cinnamic acid derivatives (**1**−**8**) and catechols **9** and **10** (see Chart 1) react with DPPH•, in methanol and in ethanol, reaction 1, by an electron-transfer mechanism.

$$ArOH + DPPH^{\bullet} \rightarrow\rightarrow ArO^{\bullet} + H\text{-}DPPH \qquad (1)$$

$$ArO^{\bullet} + DPPH^{\bullet} \rightarrow \text{products} \qquad (2)$$

The reactions of dietary phenols and polyphenols with DPPH• have been the subject of many contradictory investigations[20] and will continue to be until there is general recognition of the possible multiple paths for reaction 1 (see Scheme 1) and the fact that *the rates of this reaction can be dramatically increased and decreased by basic and acidic, respectively, impurities in the solvent* (vide infra). In the present work, we show that despite the presence of the carboxylic acid group in **1**−**5**, small but kinetically significant quantities of phenoxide anions (ArO^{-}) are responsible for the observed *fast* reactions with DPPH•, reactions for which the measured rate constants, k_1, *decrease as the phenol concentrations increase!*

CHART 1



Results

The rate constants for reaction 1 were determined in methanol and ethanol at 25 °C by following the decrease of DPPH• absorbance over time after the addition of phenols **1**−**10** in a cuvette of 1 cm optical path. The concentration of DPPH• was in the range 50−100 μM, while the phenols were added in the range 0.1−1 mM (pseudo-first-order kinetics) or in the range 5−100 μM (second-order kinetics).

The rate of reaction 1 was defined as

$$-d[DPPH^{\bullet}]/dt = n \times k_1[DPPH^{\bullet}] \times [ArOH] \qquad (3)$$

where n is the stoichiometric factor of ArOH (vide infra). The values of k_1 were calculated either from the initial rates or using the integrated eqs 4 and 5 valid for pseudo-first and second-order kinetics, respectively, in the first seconds of reaction.

$$\ln A = \ln A_0 - k_1[ArOH]_0 \times t \qquad (4)$$

$$\ln\left\{\frac{A}{A - \Delta_0\epsilon}\right\} = \ln\left\{\frac{[DPPH^{\bullet}]_0}{n \times [ArOH]_0}\right\} + \Delta_0 k_1 \times t \qquad (5)$$

In these equations, A represents the DPPH• absorbance, t the time, ϵ the extinction coefficient of DPPH•,[21] and $\Delta_0 = [DPPH^{\bullet}]_0 - n \times [ArOH]_0$.

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TABLE 1. Observed Rate Constants[a,b], k_1 (M^{-1} s^{-1}), and Stoichiometric Factors,[c] *n*, for the Reaction of DPPH• with 1−10 at 25 °C in Methanol and Ethanol

	methanol		ethanol	
phenol	k_1	*n*	k_1	*n*
1 caffeic acid	900−105	2.2	2000−200	2.1
	8000−660[d]	2.3[d]		
2 *p*-coumaric acid	0.9−0.22	1.0	1−0.30	0.9
3 ferulic acid	120−10	1.2	240−25	1.1
4 sinapic acid	4100−310	1.3	4000−550	1.2
5 dihydrocaffeic acid	180−30	2.1	540−35	2.0
6 ferulic ester	200	1.2	278	1.0
7 caffeic ester	9100	2.2	1.1×10^4	1.8
	1.9×10^4 [d]	2.2[d]		
8 sinapic ester	2.0×10^4	1.0	2.0×10^4	0.9
9 catechol	300	3.2	960	2.5
10 3,5-di-*tert*-butylcatechol	750	2.2	2100	2.0

[a] Experimental error, ca. ±20%. [b] Reported rate constants for **1**−**5** are the observed values at the phenol concentrations of 20 μM (the highest rate constant) and 1 mM (the lowest rate constant); in the case of **6**−**10**, the reported rate constants were determined in the range of phenol concentration 1−50 μM (see Results). [c] Values are the average of 8−10 determinations, and the standard deviation was ca. ±15%. [d] In CH_3OD.

The transient aryolxyl radicals (ArO•) generated in reaction 1 are generally able to quench another DPPH• radical, reaction 2. The total number of DPPH• radicals quenched per molecule of phenol, i.e., the stoichiometric factor (*n*), was determined by allowing a small quantity of phenol to react with an excess of DPPH•. Typically, the concentration of DPPH• was 2−10 times larger than that of **1**−**10**. The curves *A* vs time were usually characterized by a *fast* initial decrease of the DPPH• absorbance followed by a *slow* subsequent disappearance of DPPH•. The initial tract was attributed to reactions 1 and 2, while the subsequent decay was attributed to secondary slow reactions from the products of dimerization (or disproportionation) of ArO• or from the products of reaction 2. The values of *n* were evaluated after the end of the initial fast reaction and are reported in Table 1.

The rate constants k_1 for the methyl esters **6**−**8** and catechols **9** and **10** were determined in the concentration range of 1−50 μM in which the reaction orders with respect to **6**−**10** were close to unity. At higher concentrations (100−1000 μM), the rate of reaction 1 tended to reach a constant value and the orders of reaction decreased, therefore, considerably. Table 1 gives the values of k_1, which range from ca. 200 (methyl ester **6** in methanol) to ca. 20 000 M^{-1} s^{-1} (methyl ester **8**).

The kinetic behavior of the phenols with free carboxylic acid group, i.e., **1**−**5**, was very surprising because *the values of k_1 were strongly dependent upon their initial concentration*. In fact, it turned out that k_1 decreased monotonically as the concentration of **1**−**5** was increased. Figure 1 reports the two most impressive cases relative to sinapic acid **4** and caffeic acid **1** (the graphs relative to **2**, **3**, and **5** are given in Supporting Information) for which k_1 decreased by more than 1 order of magnitude as the concentration of the phenols was increased from ca. 1−10 μM to ca. 1000 μM. For instance, k_1 was 1100 M^{-1} s^{-1} for 14.5 μM caffeic acid **1** but dropped to 98 M^{-1} s^{-1} when the initial concentration of **1** was 1.19 mM. These changes were independent of $[DPPH•]_0$ and followed the relation

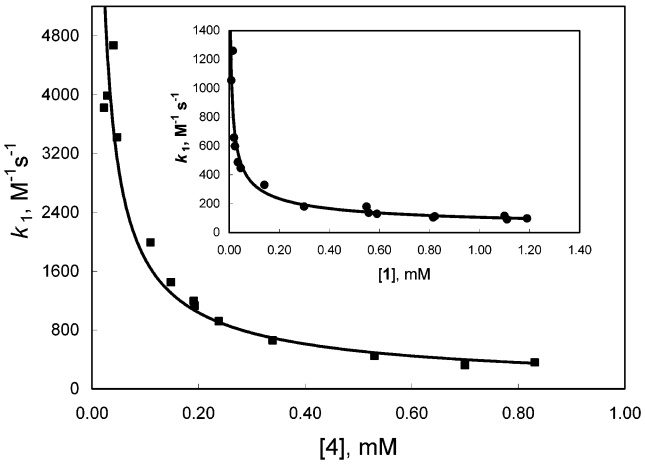

FIGURE 1. Observed rate constants k_1 in methanol at 25 °C versus concentration of sinapic acid **4**. Inset: Observed k_1 in methanol at 25 °C versus concentration of caffeic acid **1**. In both cases, the solid line represents the (best-fit) equation, $B[ArOH]^{-m}$ (see Results and Table 2).

$$k_1 = B \times [ArOH]_0^{-m} \quad (6)$$

where k_1 is given in units of M^{-1} s^{-1}, the exponent *m* changed in the limited range 0.34−0.71, i.e., *m* was on average ca. 0.5, while *B* ranged from 0.02 (*p*-coumaric acid **2**) to ca. 3.5 (caffeic acid **1**) in methanol and from ca. 0.04 (*p*-coumaric acid **2**) to ca. 13 (sinapic acid **4**) in ethanol. These parameters, reported in Table 2, show that sinapic acid **4** is the most active DPPH• scavenger among **1**−**5**. More explicitly, Table 1 reports the values of k_1 determined at low concentration (2×10^{-5} M), which demonstrate that **4** is 4.6−4560 times more reactive in methanol and 2−4000 times more reactive in ethanol than the other acids.

In conclusion of this section, we report that the overall order of reactivity found for cinnamic acids and relative derivatives **1**−**8** is **8** > **7** > **4** > **1** > **6** ≈ **5** > **3** ≫ **2** (see Tables 1 and 2). Although the mechanism of reaction of these compounds with DPPH• has been elucidated (see Discussion), we will not try to rationalize this order because the complexity of these reactions and their sensitivity to adventitious acids (or bases) (see Discussion) make any attempt very likely to fail.

Discussion

The antioxidant activity of ArOH is evaluated by measuring the rate constant of ArOH + ROO•($k_{ArOH/ROO}$)[19] which, for an H-atom transfer mechanism, has its maximum value[7] ($k^0_{ArOH/ROO}$) in apolar media (see Introduction). However, it is a common practice lately to *estimate* the antioxidant activity of phenols from the rate by which they react with the stable and colored radical DPPH•, reaction 1, using methanol or ethanol as a solvent.[17,18] In hydrocarbon solvents, $k^0_{ArOH/ROO} \propto k_1{}^0$,[22] but when k_1 is determined in *polar* solvents, its value may result "abnormally"[17] high. Especially in alcohols,[17] k_1

(21) Extinction coefficients of DPPH• are: 10870 ± 200 (515 nm, methanol) and 11500 ± 150 M^{-1} cm^{-1} (516 nm, ethanol).

TABLE 2. Values of B,[a] B',[a,b] and m[a] Relative to Eq 6 Determined in a Range c of Concentrations (μM) for 1−5 at 25 °C

	methanol				ethanol			
phenol	B	m	B' [b]	c	B	m	B' [b]	c
1	3.475	0.495	2.93	10−1190	3.780	0.588	11.50	20−2000
	7.470[c]	0.645[c]	39.57[c]	18−1370[c]				
2	0.022	0.341	0.005	500−4900	0.035	0.325	0.005	400−4900
3	0.100	0.661	0.70	100−2300	0.149	0.687	1.10	90−2500
4	1.996	0.710	18.50	5−1700	13.03	0.549	26.94	5−1650
5	0.903	0.493	0.85	29−3300	0.392	0.668	2.94	4−560

[a] Error has been estimated to be ca. ±20%. In all cases, R^2 was ≥0.95. [b] Values B' have been obtained by setting $m = 0.5$, $k_1 = B'[\mathbf{1}-\mathbf{5}]^{-0.5}$, where k_1 is given in units of M^{-1} s^{-1}. [c] In CH_3OD.

may not be representative of the rate of H-atom abstraction from ArOH by DPPH• (or ROO•[24]).

Our current results indicate that in these solvents, the main mechanism by which reaction 1 occurs does not involve a HAT mechanism. Generally, reaction 1 with ArOH = **1**−**10** was, in fact, fast both in methanol and in ethanol (see Table 1), and this was rather surprising because a reduction of the rate by kinetic solvent effect (KSE) was expected.[7,8] This situation is particularly evident for catechol **10**. The H-atom transfer from **10** to DPPH• is, in *n*-hexane, very fast, $k_1 = 2.1 \times 10^4$ M^{-1} s^{-1}.[8] However, this rate constant decreases as the hydrogen bond acceptor ability of the solvent (i.e., the β_2^H parameter)[15] increases, e.g., k_1 (in M^{-1} s^{-1}) is[8] 1400 (1-chlorobutane), 64 (acetonitrile), 52 (ethyl acetate), 70 (*n*-propanol), 44 (*tert*-butyl alcohol), and 12 (acetone). Our results show instead that this rate constant is comparatively large in ethanol since $k_1 = 2100$ M^{-1} s^{-1} (see Table 1). Thus, the rate decreases with respect to that in the hydrocarbon solvent by a factor of 21 000/2100 = 10 (28 in methanol), while in the other solvents with comparable β_2^H values, the KSEs are responsible for a ca. 400-fold reduction of the rate! This very large difference suggests that in ethanol and methanol the HAT mechanism has been replaced by a more efficient path that makes reaction 1 occur at a higher rate.

As reported in Table 1, caffeic acid **1**, its methyl ester **7**, and surprisingly[25] sinapic acids **4** and its methyl ester **8** are also *very* efficient DPPH• scavengers since the values of k_1, at lower concentrations of scavenger, ranged from 1300 to 20 000 M^{-1} s^{-1} (the esters being the most active compounds). We also observed that the rate constants k_1 for **1**−**5** decline as expressed by eq 6 and shown in Figure 1. Equation 6 shows that the order of reaction with respect to **1**−**5** is ca. 0.5 since $m \approx 0.5$

$$-\mathrm{d[DPPH]}/\mathrm{d}t = nB[\mathbf{1}-\mathbf{5}]_0^{1-m}[\mathrm{DPPH}]_0 \approx nB[\mathbf{1}-\mathbf{5}]_0^{0.5}[\mathrm{DPPH}]_0 \quad (7)$$

and this indicates that the reactive species is *not* the neutral form, ArOH, because in this case the reaction order would be unity with respect to both reactants. However, an order of reaction equal to 1 is certainly not sufficient for demonstrating a HAT mechanism. We noticed, in fact, that phenols **6**−**10**, at low concentration, had an order of reaction of ca. 1; however, even in this case, the HAT mechanism seems to be rather unlikely because the methyl esters were 3−5 times more reactive than the corresponding free acids (see Table 1), and this different reactivity is *not* explainable for a reaction of H-atom transfer to DPPH•.

The foregoing kinetic observations imply that the actual mechanism by which reaction 1 occurs is influenced by the carboxylic group of **1**−**5**, as has also been confirmed by two more experiments. First, when 30 μM caffeic acid **1** was allowed to react with DPPH• (90 μM) in methanol both in the absence and presence of 30 μM KOH,[27] the observed rate constant k_1 increased, respectively, from ca. 600 to 3310 M^{-1} s^{-1}. Second, substituting the base with 30 μM acetic acid[27] caused the rate of reaction 1 to decrease dramatically, and k_1 became ca. 32 M^{-1} s^{-1}.

The entire picture described above led us to understand how the multifaceted aspects of reaction 1 are easily explained by taking into account the dissociation of the phenolic hydroxyl of **1**−**10**, equilibrium 8, and the subsequent cascade of reactions 9−11. The direction of the ET step from ArO^- to DPPH•, reaction 9, may be justified by the favorable $pK_a = 8.5$ of H-DPPH[17] with respect to that of phenols, $pK_a \approx 8.7-11$.

$$\mathrm{ArOH} \rightleftarrows \mathrm{ArO^-} + \mathrm{H^+} \qquad K_{OH} \quad (8)$$

$$\mathrm{ArO^-} + \mathrm{DPPH^\bullet} \rightarrow \mathrm{ArO^\bullet} + \mathrm{DPPH^-} \quad (9)$$

$$\mathrm{DPPH^-} + \mathrm{H^+} \rightarrow \mathrm{H\text{-}DPPH} \quad (10)$$

$$\mathrm{ArO^\bullet} + \mathrm{DPPH^\bullet} \rightarrow \mathrm{products} \quad (11)$$

In the case of **1**−**5** (which are relatively strong acids), the acidity of the medium is substantially determined by the dissociation of the carboxylic group, reaction 12,

(22) In apolar media, it is even possible to *calculate* the value of $k^0_{ArOH/ROO}$ at 30 °C (for ROO• = polyperoxylstyryl radicals), with good approximation, from the experimental value of k_1^0 at 25 °C. It turns out, in fact, that for nonhindered phenols, both the log $k^0_{ArOH/ROO}$ and the log k_1^0 are linearly dependent on the bond dissociation enthalpy of the phenolic O−H[23] involved in the reactions with ROO• and DPPH•, and this leads to the following empirical equation:[23] $\log k^0_{ArOH/ROO} = 3.61 + 0.66 \log k_1^0$.

(23) Foti, M. C.; Johnson, E. R.; Vinqvist, M. R.; Wright, J. S.; Barclay, L. R. C.; Ingold, K. U. *J. Org. Chem.* **2002**, *67*, 5190−5196.

(24) Peroxyl radicals ROO• are also able to abstract an H-atom from methanol or ethanol.

(25) Our surprise arose from the fact that our measurement of k_1(**8**) = 130 ± 20 M^{-1} s^{-1} in *n*-hexane at 20 °C indicates that the methyl ester **8** (and the corresponding acid **4**) is not an efficient scavenger of DPPH• by HAT. This is because the hydroxyl group is strongly hydrogen-bonded to one of the methoxyls in the ortho position. If the HAT mechanism were prevalent also in methanol or ethanol, this rate constant would be further decreased by a factor of ca. 6.[26] The observed value of k_1(**8**) in methanol and ethanol is instead 2.0×10^4 M^{-1} s^{-1} (Table 1), i.e., ca. 150 times *larger* than that in the hydrocarbon solvent.

(26) de Heer, M. I.; Mulder, P.; Korth, H.-G.; Ingold, K. U., Lusztyk, J. *J. Am. Chem. Soc.* **2000**, *122*, 2355−2360.

(27) Addition of small quantities of KOH or acetic acid did not cause any appreciable decay of DPPH• within the time of reaction 1.

(RCOOH = **1**−**5**),

$$\mathrm{RCOOH} \rightleftarrows \mathrm{RCOO^-} + \mathrm{H^+} \qquad K_{\mathrm{COOH}} \qquad (12)$$

which reduces the quantity of ArO^- in equilibrium with ArOH and causes, therefore, a reduction of k_1 (cf. free acids versus esters) and the peculiar behavior observed with these phenols (see Figure 1). The rate of reaction 1 is equal to

$$-\mathrm{d[DPPH^\bullet]}/\mathrm{d}t \approx nk_9[\mathrm{DPPH^\bullet}][\mathrm{ArO^-}] \qquad (13)$$

and since,[28,29] for **1**−**5**,

$$[\mathrm{ArO^-}] \approx K_{\mathrm{OH}}\,[\mathrm{ArOH}]/\sqrt{K_{\mathrm{COOH}} \times [\mathrm{RCOOH}]}$$

and

$$[\mathrm{RCOOH}] \equiv [\mathrm{ArOH}]_0$$

equation 13 becomes

$$-\mathrm{d[DPPH^\bullet]}/\mathrm{d}t \approx nk_9\frac{K_{\mathrm{OH}}}{\sqrt{K_{\mathrm{COOH}}}} \times \frac{[\mathrm{ArOH}]}{\sqrt{[\mathrm{ArOH}]_0}}[\mathrm{DPPH^\bullet}] \qquad (14)$$

A simple comparison of this equation with eq 3 leads to the conclusion that the observed value of k_1 for **1**−**5** is given by

$$k_1 \approx \frac{k_9K_{\mathrm{OH}}}{\sqrt{K_{\mathrm{COOH}}}}[\mathrm{ArOH}]_0^{-1/2} \qquad (15)$$

This relationship matches eq 6, though the experimental value of m was found, in a few cases (see Table 2), to be slightly different from 0.5. Small differences in this exponent have, however, a strong effect on the values of B of eq 6 (see Table 2). Therefore, we have also reported the values, B', obtained by setting $m = 0.5$ and doing a linear regression of k_1 versus $[\mathrm{ArOH}]_0^{-0.5}$. The new B' values for **1**−**5** can be compared more effectively and represent the term

$$k_9K_{\mathrm{OH}}/\sqrt{K_{\mathrm{COOH}}}$$

of eq 15.

It is interesting to observe that in the case of **6**−**10** (which are all *very* weak acids), the concentration in solution of ArO^- is largely determined by the contents of acidic impurities in the alcohol, $[H^+]_{alc}$. Consequently, the observed value of k_1 at low [**6**−**10**] should essentially be concentration independent, eq 16, as in fact was the case, but dramatically dependent upon the nature and quantity of impurities.

$$k_1 \approx k_9K_{\mathrm{OH}}/[\mathrm{H^+}]_{\mathrm{alc}} \qquad (16)$$

(28) In the following equations, we use the molar concentration for the ionic species and not their activity. In other words, because the solutions are diluted, it is reasonable to assume that $\gamma_\pm \approx 1$.

(29) Concentration of ArO^- is given by $[\mathrm{ArO^-}] \approx K_{\mathrm{OH}}[\mathrm{ArOH}]/[\mathrm{H^+}]$. Because $[\mathrm{H^+}] \approx [\mathrm{RCOO^-}]$, it follows that $[\mathrm{H^+}] \approx (K_{\mathrm{COOH}}[\mathrm{RCOOH}])^{1/2}$ and $[\mathrm{ArO^-}] \approx K_{\mathrm{OH}}[\mathrm{ArOH}]/(K_{\mathrm{COOH}}[\mathrm{RCOOH}])^{1/2}$.

The ET mechanism for reaction 1 outlined above implies that the rate constants k_1 must be greater in solvents of higher dielectric constant (ϵ) since these solvents better support the ionization of Bronsted acids. In apparent contrast with this, we observed, however, that reaction 1 was for the same substrate faster in ethanol ($\epsilon = 24.30$) than in methanol ($\epsilon = 32.63$) roughly by a factor of 2 (see Tables 1 and 2). We think this discrepancy can be essentially attributed to different contents of water and acidic impurities in the two solvents used for the experiments. In fact, the titration of 50 mL of methanol and 50 mL of ethanol with 0.001 M NaOH in the presence of phenolphthalein required 15 ± 1 μmol and 9 ± 1 μmol of base, respectively. The comparison of the rate constants k_1 determined in *different* solvents or even in different lots of the *same* solvent must therefore be done with caution. In connection with this statement, we report that caffeic acid **1** and its methyl ester **7** reacted with DPPH$^\bullet$ *more* rapidly in deuterated methanol, CH_3OD, than in CH_3OH by a factor of ca. 8 and 2, respectively (inverse isotope effect), see Tables 1 and 2. It is for caution's sake that we do not attempt, therefore, to explain this inverse isotope effect since we believe it does not reflect anything but different levels of impurities in our CH_3OH and CH_3OD.

Conclusion

Kinetic analysis of the rates of DPPH$^\bullet$+ ArOH (**1**−**10**) in methanol and ethanol leads to the conclusion that the rate-determining step for this reaction consists of a *fast* electron-transfer process from the phenoxide anions of **1**−**10** to DPPH$^\bullet$. Therefore, the hydrogen-atom abstraction from neutral ArOH by DPPH$^\bullet$ becomes a marginal reaction path because in strong hydrogen-bond-accepting solvents, like methanol and ethanol,[15] it occurs *very* slowly. These two hydroxylic solvents have relatively high dielectric constants and thus support well the ionization of Bronsted acids and the ET mechanism.

Our paper shows that reaction 1 is therefore characterized by three important points that must be taken into account when the "antiradical/antioxidant activity" of phenols is evaluated in methanol or ethanol by means of the DPPH$^\bullet$ radical: (i) phenols *usually* react with peroxyl radicals ROO$^\bullet$ by a HAT mechanism, and therefore their *antioxidant* activity cannot be extrapolated from the rate of their reaction with DPPH$^\bullet$; (ii) the presence of free carboxylic acid groups in the phenol structure makes the observed rate "constants" k(ArOH + DPPH$^\bullet$) strongly dependent on the phenol concentration used in the experiment; and (iii) the presence in the solvent of adventitious acids or bases causes a reduction or an enhancement, respectively, of the observed value of the rate constants k(ArOH + DPPH$^\bullet$).

Experimental Section

General. Caffeic acid and the methyl ester of ferulic acid were purchased from Extrasynthese; *p*-coumaric and ferulic acids were from Aldrich, while dihydrocaffeic and sinapic acids, catechol, and 3,5-di-*tert*-butylcatechol were from Fluka. All phenols used had a purity of ca. 98−99%. Methanol was purchased from Romil (gradient quality), ethanol from Merck (spectroscopic grade), deuterated methanol, CH_3OD, from Aldrich (99% deuterium), and were all used as received.

Synthesis of the Methyl Esters 7 and 8. Sinapic or caffeic acid (500 mg, 2.23 and 2.78 mmol, respectively) were dissolved in methanol (30 mL) containing ca. 1 mL of sulfuric acid, and then the solution was heated at reflux for about 1 h. After cooling at room temperature, the solution was diluted with ethyl acetate (150 mL) and washed with an aqueous solution of $NaHCO_3$ (5% w/v) until neutral pH. The organic layer was then washed with distilled water and dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The residues were constituted by the pure methyl esters (HPLC-MS analysis). **7** (yield 90%; 99% pure): H^1 NMR (chloroform-*d*) δ 3.56 (s, 3H), 6.01 (d, J = 15.9 Hz, 1H), 6.64 (d, J = 7.5 Hz, 1H), 6.70 (d, J = 7.5 Hz, 1H), 6.87 (s, 1H), 7.34 (d, J = 15.9 Hz, 1H); ESI-MS m/z 193 = $[M - H]^-$. **8** (yield 94%; 99% pure): H^1 NMR (chloroform-*d*) δ 3.71 (s, 3H), 3.79 (s, 6H), 6.19 (bs, OH), 6.21 (d, J = 15.9 Hz, 1H), 6.66 (s, 2H), 7.50 (d, J = 15.9 Hz, 1H); ESI-MS m/z 237 = $[M - H]^-$.

Kinetics. Stock solutions of the phenols (range 0.05−0.1 M) were prepared and properly diluted in the solvent in use. Solutions of DPPH$^\bullet$ (ca. 100 μM) usually had an absorbance in its maximum of ca. 1 (methanol, max 515 nm and ϵ = 10870 ± 200 M^{-1} cm^{-1}; ethanol, max 516 nm and ϵ = 11500 ± 150 M^{-1} cm^{-1}). The procedure utilized to determine the value of k_1 was used for all phenols and solvents. Briefly, 2 mL of DPPH$^\bullet$ solution were put into a cuvette containing a small stirring bar, and a slow flow of nitrogen was then bubbled for a few minutes through the solution. While vigorously stirring the solution, various aliquots (10−80 μL) of the solutions of phenols were rapidly added and the absorbance of DPPH$^\bullet$ monitored with a spectrophotometer over time. The rate constants were thereby obtained from the decay traces using the initial rates or the integrated eqs 4 and 5. Because of the strong influence that water and acid impurities contained in the solvents have on the rates of reaction 1, the same lots of methanol and ethanol were used throughout the kinetic experiments (see Discussion).

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Supporting Information Available: Plots of k_1 versus [ArOH] for **2**, **3**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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