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# Kinetic Model for the Antiradical Activity of the Isolated *p*-Catechol Group in Flavanone Type Structures Using the Free Stable Radical 2,2-Diphenyl-1-picrylhydrazyl as the Antiradical Probe

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The time evolution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) concentration in four solvents (methanol, ethanol, propanol, and acetonitrile) during its reduction by three flavanones containing an isolated *p*-catechol group (taxifolin, eriodyctiol, and fustin) as well as the time evolution of the mass spectra of the reaction mixture has been determined by spectrophotometry and liquid mass spectrometry, respectively. In alcoholic solvents the reduction curves consisted of an initial short but fast kinetics step followed by a longer slow kinetics step; in contrast, in acetonitrile the reduction curves completely lacked the slow kinetics step. From the results, a kinetic model for the reaction of reduction of the DPPH• by the isolated *p*-catechol group in flavanone type structures is proposed. According to this model, the *p*-catechol group rapidly transfers two hydrogen atoms to DPPH•, through a fast rate constant $k_1$, yielding the corresponding *o*-quinone. Then, the intermediate *o*-quinone forms an adduct with the alcoholic solvent, through a slow rate constant $k_2$, and regenerates the *p*-catechol group. The regenerated *p*-catechol group reduces additional DPPH• through a fast rate constant $k_3$, yielding the corresponding *o*-quinone, which can form a new adduct with the solvent to regenerate the *p*-catechol group, and so on. From the kinetics model, two explicit kinetics equations have been derived that fit very well the experimental data points acquired from all assayed compounds in all of the experiments carried out, thus allowing an accurate determination of the corresponding rate and stoichiometric constants.

**KEYWORDS: Antiradical activity; kinetics; *p*-catechol; flavanone; DPPH•**

## INTRODUCTION

Most edible plants as well as foodstuff derived from plants contain components (e.g., ascorbic acid, flavonoids, carotenoids, anthocyanidins, hydroxycinnamic acid derivatives, etc.) that exhibit antiradical activity (*1*−*6*). There is partial evidence that many of these compounds could be bioactive against different free radical mediated diseases, such as cancer, cardiovascular diseases, arthritis, and diabetes as well as premature body aging (*7*−*10*). Hence, the intake of antiradicals present in food is thought to be an important health-protecting factor (*11*).

It is known that antiradical activity mainly depends on a reduced number of "active antiradical groups", having at least a free hydroxyl group, which are contained within the chemical structure of active compounds, rather than on their full chemical structure. Among these active antiradical groups, the vinyl-alcohol and the *p*-catechol (3,4-dihydroxybenzene) groups are noticeable due to their intense antiradical activities. It is also recognized that the conjugation of isolated active antiradical groups results in a new extended active antiradical group with enhanced antiradical activity. Hence (see **Figure 1**), the antiradical activity of eriodyctiol (5,7,3′,4′-tetrahydroxyflavanone), which contains an isolated *p*-catechol group in the B-ring, is enhanced by the presence of a double bond between C2 and C3 in the C-ring (luteolin, 5,7,3′,4′-tetrahydroxyflavone) and even more enhanced by the additional presence of a hydroxyl on C3 (quercetin, 3,5,7,3′,4′-pentahydroxyflavanone), that is, a conjugated vinyl-alcohol (*12*, *13*).

Among the different published methodologies for determining the antiradical activity of both isolated compounds and complex mixtures of antiradicals, the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay, initially developed by Blois (*14*) and more recently adapted by Brand-Williams et al. (*15*), has been widely used due to its simplicity. This methodology is based on the reduction of the free stable radical DPPH•, which strongly absorbs at 515 nm, to the corresponding hydrazine, which is almost transparent at this wavelength, by the transfer of hydrogen atoms from the antiradical. Hence, the time evolution of the absorbance, subsequently converted to DPPH• concentration, is the parameter monitored.

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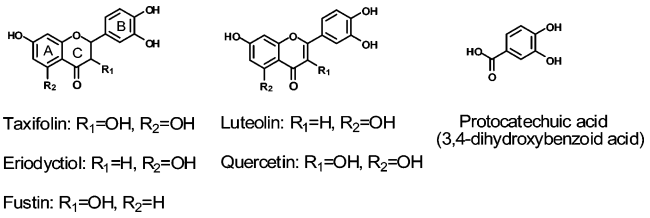


**Figure 1.** Chemical structures of the experimental and reference compounds.

There is abundant scientific literature dealing with the determination of the antiradical activity of plant extracts, juices, and isolated compounds by means of the DPPH• assay. However, the explicit kinetic equations for these reactions are not known, and so the fitting of the experimental data points to a given ad hoc equation to extract kinetic data lacks reliability and is hardly justifiable. As a consequence, the kinetic rate constants remain unknown, the antiradical activity is quantified by means of empirical parameters (i.e., the antiradical activity $EC_{50}$), the influence of the molecular structure and solvents on this activity has been only qualitatively determined, and even the determined total stoichiometric constants are largely dependent on the experimental reaction time (i.e., the value assigned to the true reaction asymptote) (*15*).

In an attempt to improve the determination of the antiradical activity of citrus juices, Sendra et al. (*6*) determined the time evolution curves of the DPPH• concentration during its reduction by different antiradicals present in these juices. According to the observed experimental kinetics of the reduction process in methanol, these authors roughly grouped the antiradicals into three main groups: fast kinetics, fast+slow kinetics, and slow kinetics. The components belonging to the fast kinetics group, which contain isolated vinyl-alcohols (e.g., ascorbic acid), exhibit a single short but very fast kinetics step; the components belonging to the fast+slow kinetics group, which contain at least a *p*-catechol group (e.g., chlorogenic acid), exhibit an initial short but fast kinetics step followed by a longer slow kinetics step; finally, the components belonging to the slow kinetics group, which contain isolated phenols (e.g., hesperitin), exhibit only a single slow kinetics step.

The isolated *p*-catechol group can be found in several families of naturally occurring compounds such as flavanones, isoflavanones, protocatechuic (3,4-dihydroxybenzoic) acid derivatives, and chalcones among others. The scientific literature is rather confusing about the antiradical activity of this group, which could be due to the fact that its antiradical activity also depends on the chemical structure of the family. For instance, the total stoichiometric constant of the isolated *p*-catechol group was determined to be about 4 in flavanone type structures (*13*) but >5 in protocatechuic acid alkyl esters (*16*) when methanol was used as solvent. In nonalcoholic solvents, such as ethyl acetate or acetonitrile, the determined total stoichiometric constant was about 2 in both cases. There seems to be consensus, however, that the *p*-catechol group reduces the DPPH• by the transfer of two hydrogen atoms and its subsequent conversion to the corresponding *o*-quinone (*16*−*18*).

In the present work the time evolution of the DPPH• concentration in four solvents (methanol, ethanol, propanol, and acetonitrile) during its reduction by three flavanones containing an isolated *p*-catechol group (taxifolin, eriodyctiol, and fustin) has been determined by spectrophotometry, and the time evolution of the mass spectra of the reaction mixture has been determined by liquid mass spectrometry. From the results, a kinetics model for the reaction of reduction of the DPPH• by the isolated *p*-catechol group in flavanone type structures is proposed. Moreover, two explicit kinetic equations have been derived from the kinetics model, which fit very well the experimental data points acquired from all of the assayed compounds, thus allowing the accurate determination of the corresponding rate and stoichiometric constants.

## MATERIALS AND METHODS

**Reagents and Standards.** Spectrophotometric grade methanol, ethanol, propanol, and acetonitrile were from Sigma (Sigma-Aldrich Co., St. Louis, MO). Taxifolin and DPPH• (94.6% purity) were from Fluka (Fluka AG Chemische, Buchs, Switzerland). Fustin was from Roth (Carl Roth GmbH, Karlsruhe, Germany), and eriodyctiol was from Extrasynthèse (Genay, France). Anhydrous sodium sulfate was from Panreac (Panreac Química S.A., Barcelona, Spain).

**Determination of the Antiradical Activity.** *Sample Preparation.* For spectrophotometric determinations, the solvent to be used (methanol, ethanol, propanol, or acetonitrile) was dried overnight over anhydrous sodium sulfate, and the working solutions of the antiradical and DPPH• were freshly prepared before analysis. A volume of the antiradical solution (between 5 and 40 $\mu$L) was added in situ, using a chromatographic syringe, into a thermostated (22 °C) and stirred (600 rpm) quartz spectrophotometric cuvette (3.5 mL of capacity and 1 cm path length) containing an appropriate volume of DPPH• to yield a final volume of 2 mL (the final concentration of DPPH• was around 100 $\mu$mol/L), and the spectrophotometric cuvette was immediately end-capped again. The analysis time commenced with the addition of the antiradical. As a general rule, those samples yielding an asymptotic value of the DPPH• concentration of <10% or >90% of its initial concentration were discarded.

*UV*−*Vis Analysis.* Absorbance was measured using a model 8453 UV−Vis spectrophotometer (Agilent Technologies GmbH, Karlsruhe, Germany) equipped with a diode array detector and a thermostated cell holder with magnetic stirring. Operating conditions were as follows: vis lamp, on; UV lamp, off; wavelength, 515 nm; slit width, 1 nm; and data acquisition rate, 2.1 s/data point. Automatic acquisition of data was stopped after a reaction time of 60−90 min, depending on the speediness of the kinetics. Thus, each set of data contained over 1500 data points. All samples were analyzed in duplicate.

Prior to the experiments on antiradical activity, a calibration curve of absorbance versus concentration of DPPH• in all of the assayed solvents was obtained to determine the molar extinction coefficient ($\epsilon$) of DPPH•. From the linear fitting of data, the values determined for $\epsilon$ were as follows: methanol, $1.09 \times 10^4$; ethanol and propanol, $1.08 \times 10^4$; and acetonitrile, $1.06 \times 10^4$ L/(mol cm).

**Determination of the Mass Spectra.** *Sample Preparation.* For mass spectrometry determinations, the working solutions of the antiradical and DPPH• were freshly prepared using anhydrous methanol from a recipient opened immediately before analysis. A volume of the antiradical solution (between 2 and 5 $\mu$L) was added, using a chromatographic syringe, into a cuvette containing an appropriate volume of DPPH• to yield a final volume of 2 mL (the final concentration of DPPH• was around 25 $\mu$mol/L). The cuvette was end-capped immediately and shaken by hand, and then an aliquot of the reaction mixture was transferred into the infusion syringe (500 $\mu$L capacity) for analysis. Reaction time commenced with the mixing of the antiradical and DPPH•. Mass spectra were manually acquired from 5 min of reaction time onward, at a sampling rate of 5 min/mass spectrum. Data acquisition was stopped after a reaction time of about 80 min.

*Mass Spectrometry Analysis.* Mass spectra were obtained using an LCQ Advantage (Thermo Finnigan, San Jose, CA) mass spectrometer, equipped with an electrospray ionization source and ion trap detector. Instrument control and analysis of data were carried out using a PC loaded with the LCQ Tune/Excalibur software. The sample was introduced into the mass spectrometer by direct infusion (syringe) at a flow rate of 5 $\mu$L/min. Operating conditions were as follows: mode,

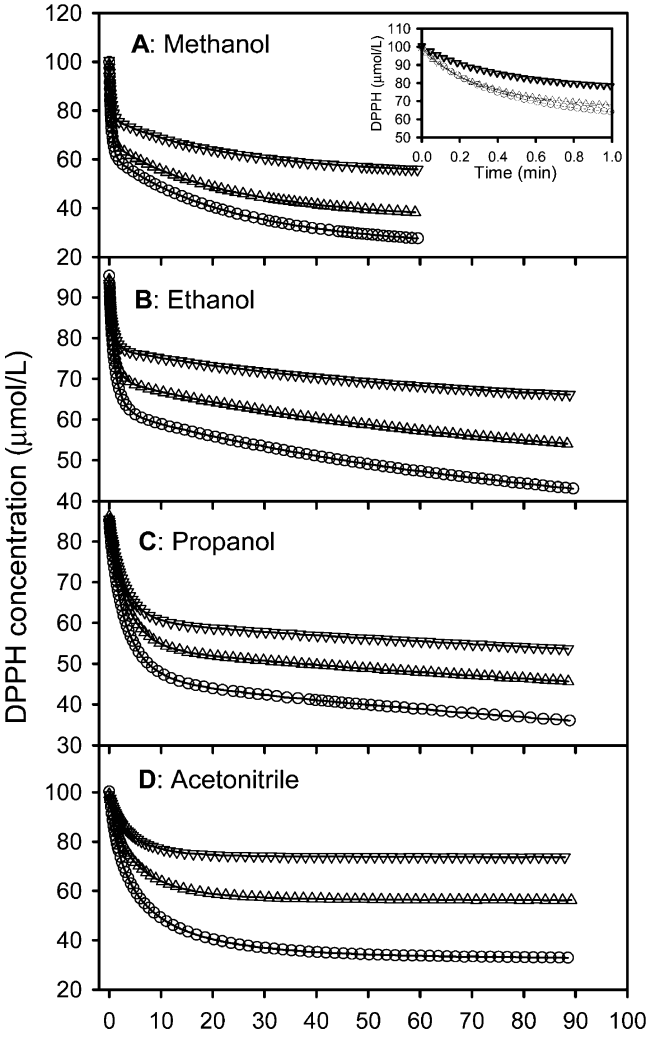


**Figure 2.** Time evolution of the DPPH• concentration in (**A**) methanol, (**B**) ethanol, (**C**) propanol, and (**D**) acetonitrile during its reduction by three different initial concentrations of taxifolin. (Inset) Zooming of the first minute of the reaction. See **Table 1** for the initial concentrations of DPPH• and taxifolin in the assayed solvents.

negative; scan mode, full scan MS ($m/z$ 200−800); number of microscans, 5; maximum inject time, 150 ms; sheath gas ($N_2$) flow rate, 20 units; auxiliary/sweep flow rate, 0 units; ionization spray voltage, 3.9 kV; capillary temperature, 160 °C; capillary voltage, −20 V; and tube lens offset, −50 V.

## RESULTS AND DISCUSSION

**Figure 1** shows the chemical structures of the assayed flavanones (taxifolin, eriodyctiol, and fustin) as well as the structures of some referenced compounds (luteolin, quercetin, and protocatechuic acid).

**Antiradical Activity of Taxifolin in Methanol. Figure 2A** shows the time evolution of the concentration of DPPH• during its reduction by three different initial amounts of taxifolin, as well as a zooming of the first minute of the reaction. The sets of experimental data points were fitted using the kinetic equation proposed by Sendra et al. (*6*) for the determination of the antiradical activity of citrus juices (see later for the derivation of this equation from the kinetic model)

$$y - y_s = \frac{y_1(y_0 - y_1)}{y_1 - y_o(1 - e^{(k_1/\sigma_1)y_1 t})} + \frac{y_2(y_o - y_2)}{y_2 - y_o(1 - e^{(\rho_2/\sigma_2)y_2 t})} \quad (1)$$

with the constraints

$$y_2 = y_0 + y_s - y_1 = y_o - \sigma_2 a_0$$
$$y_0 - y_1 = \sigma_1 a_0 \quad (2)$$
$$y_0 - y_s = (\sigma_1 + \sigma_2) a_0$$

where $y$ is the time-dependent concentration of DPPH•, $y_0$ is the initial concentration of DPPH•, $a_0$ is the initial concentration of the antiradical, $t$ is the reaction time, $k_1$ is the fast kinetics rate constant, $y_1$ is the asymptote that would be reached due solely to the fast kinetics antiradical activity, $\rho_2$ is the slow kinetics pseudo-rate constant, $y_2$ is the asymptote that would be reached due solely to the slow kinetics antiradical activity, $y_s$ is the experimental asymptote of the reaction, and $\sigma_1$ and $\sigma_2$ are the stoichiometric constants of the fast and slow kinetics, respectively. The results are given in **Table 1**.

As can be seen in **Figure 2**, the fittings were excellent in all cases ($r^2 > 0.999$, nonlinear parametric fitting using SigmaPlot 8.02). The resulting curves consisted in a short initial fast step, which is due to a fast kinetics with a rate constant $k_1$, followed by a longer slow step up to reach the asymptote of the reaction, which is due to a slow kinetics with a pseudo-rate constant $\rho_2$. The determined value of the fast kinetics rate constant [$k_1 = 60.9 \times 10^3$ L/(mol min)] was greater by far than that of the slow kinetics pseudo-rate constant [$\rho_2 = 937$ L/(mol min)], but the determined value of both fast and slow kinetics stoichiometric constants ($\sigma_1 = 1.97$ and $\sigma_2 = 2.04$, respectively) was very close to 2, indicating that the value of the total stoichiometric constant ($\sigma_t = \sigma_1 + \sigma_2 = 4.01$) of the reaction was very close to 4.

**Influence of the Solvent on the Antiradical Activity of Taxifolin.** The influence of the solvent on the reaction kinetics was determined by reducing the DPPH• with taxifolin in four different solvents, namely, methanol, ethanol, propanol, and acetonitrile. Panels **B**, **C**, and **D** of **Figure 2** show the reduction curves in ethanol, propanol, and acetonitrile, respectively, and data on the determined rate and stoichiometric constants are given in **Table 1**. For comparison purposes, **Figure 3** shows one reduction curve per solvent when using approximately the same initial concentrations of DPPH• and taxifolin. As can be seen, there was a remarkable difference between the alcoholic solvents and acetonitrile. For all of the alcoholic solvents assayed, the short initial fast kinetics step was always followed by a longer slow kinetics step, but this later step was completely lacking for acetonitrile. Data in **Table 1** indicate that within the alcoholic solvents, the values of both fast rate ($k_1$) and slow pseudo-rate ($\rho_2$) kinetics constants were dependent on the protic power of the alcohol, in the order $k_1$(methanol) = $60.9 \times 10^3 > k_1$(ethanol) = $28.1 \times 10^3 > k_1$(propanol) = $8.97 \times 10^3$ L/(mol min) and $\rho_2$(methanol) = 937 > $\rho_2$(ethanol) = 286 > $\rho_2$(propanol) = 88 L/(mol min). On the other hand, the value of the stoichiometric constants of both fast kinetics ($\sigma_1 = 1.97$, 2.01 and 2.03 for methanol, ethanol, and propanol, respectively) and slow kinetics ($\sigma_2 = 2.04$, 1.95, and 1.92 for methanol, ethanol, and propanol, respectively) was close to 2 for all of the assayed alcohols. In the case of acetonitrile, in contrast, the fast kinetics step, which was slower than those from methanol and ethanol, was composed of the sum of two components, each of them having a different fast rate constant [$k_1(1) = 9.22 \times 10^3$ and $k_1(2) = 1.25 \times 10^3$ L/(mol min)], but the same stoichiometric constant with a value very close to 1 [$\sigma_1(1) = \sigma_2(1) = 1.03$)], whereas the value of its slow kinetics pseudo-rate and stoichiometric constants was zero ($\rho_2 = \sigma_2 = 0$). It

**Table 1.** Rate (Mean ± SD) and Stoichiometric (Mean ± SD) Constant from the Reduction of DPPH• in Methanol, Ethanol, Propanol, and Acetonitrile by Taxifolin

| | | methanol[a] | ethanol[a] | propanol[a] | acetonitrile[a] |
|---|---|---|---|---|---|
| initial concentration ($\mu$mol/L) | DPPH• | 99.872 (○)<br>98.755 (△)<br>100.464 (▽) | 95.305 (○)<br>94.291 (△)<br>93.346 (▽) | 85.168 (○)<br>86.165 (△)<br>85.430 (▽) | 100.242 (○)<br>99.186 (△)<br>98.976 (▽) |
| | taxifolin | 19.31 (○)<br>16.10 (△)<br>11.59 (▽) | 16.104 (○)<br>12.078 (△)<br>8.052 (▽) | 19.46 (○)<br>15.81 (△)<br>12.16 (▽) | 32.867 (○)<br>20.542 (△)<br>12.325 (▽) |
| mean rate constant [L/(mol min) from eq 27] | $k_1$ | $(60.9 \pm 7.2) \times 10^3$ | $(28.1 \pm 4.1) \times 10^3$ | $(8.97 \pm 0.3) \times 10^3$ | |
| | $\rho_2$ | $937 \pm 29$ | $286 \pm 37$ | $88 \pm 4$ | 0 |
| | $k_1(1)$ | | | | $(9.22 \pm 0.6) \times 10^3$ |
| | $k_1(2)$ | | | | $(1.25 \pm 0.3) \times 10^3$ |
| mean stoichiometric constants | $\sigma_1$ | $1.975 \pm 0.031$ | $2.010 \pm 0.029$ | $2.030 \pm 0.035$ | |
| | $\sigma_2$ | $2.038 \pm 0.028$ | $1.955 \pm 0.019$ | $1.922 \pm 0.072$ | 0 |
| | $\sigma_1(1)$ | | | | $1.029 \pm 0.013$ |
| | $\sigma_1(2)$ | | | | $1.028 \pm 0.013$ |
| | $\sigma_t$ | $4.013 \pm 0.034$ | $3.965 \pm 0.044$ | $3.952 \pm 0.090$ | $2.058 \pm 0.025$ |
| mean values of $k_2$[ROH] ($min^{-1}$) and $k_2$ [L/(mol min)] from eq 19 | $k_2$[ROH] | $0.0434 \pm 0.0013$ | $0.0127 \pm 0.0019$ | $(3.65 \pm 0.07) \cdot 10^{-3}$ | 0 |
| | $k_2$ | $(1.76 \pm 0.05) \times 10^{-3}$ | $(7.39 \pm 1.12) \times 10^{-4}$ | $(2.73 \pm 0.06) \times 10^{-4}$ | 0 |

[a] Symbols refer to the corresponding curves in **Figure 2**.

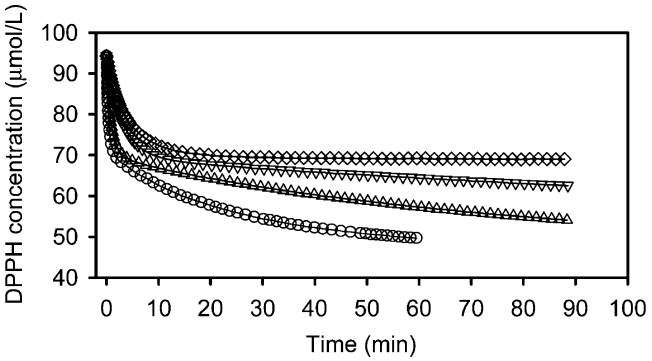


**Figure 3.** Time evolution of the DPPH• concentration in methanol (○), ethanol (△), propanol (▽), and acetonitrile (◇) during its reduction by taxifolin, at approximately the same initial concentrations of DPPH• (≈ 94.3 $\mu$mol/L) and taxifolin (≈ 12 $\mu$mol/L).

must be pointed out that the reduction curves for acetonitrile were initially fitted using the single-term kinetics equation proposed by Sendra et al. (*6*) for components belonging to the fast-kinetics antiradical group (one rate/one stoichiometric constant), but the fittings were not as good as they should be ($r^2 < 0.99$), indicating the presence of two different but close rate constants. In contrast, the fittings using eq 1 were excellent for all curves ($r^2 > 0.999$). It seems that the theoretically expected antiradical nonequivalence of both hydroxyls of the *p*-catechol group, which could not be detected in alcoholic solvents, becomes detectable in acetonitrile and, in general, in nonalcoholic solvents. This could probably be due to the fact that in acetonitrile all of the experimental data points strictly belong to the fast kinetics, because there is no contaminating slow kinetics, and that the fast kinetics step was significantly slower than those from methanol and ethanol. The conjunction of both factors probably allows the fitting to be much more discriminating.

It is known that the solvent influences the value of the fast kinetics rate constant ($k_1$) in the order $k_1$(methanol) > $k_1$(ethanol) > $k_1$(propanol), although two different mechanisms have been proposed to explain this influence. According to Valgimigli et al. (*19*), the influence of the solvent is due to a differential stabilization of the charge in the DPPH• radical by hydrogen bonding from the solvent. In contrast, Litwinienko and Ingold (*20*) propose that this influence is due to the dielectric constant of the solvent which modifies the p$K_a$ of phenols. The mechanism of the influence of the solvent on the value of the slow kinetics pseudo-rate constant ($\rho_2$) is not so explicitly documented, but the work by Saito et al. (*16*) on the antiradical activity of the *p*-catechol group in protocatechuic (3,4-dihydroxybenzoic) acid alkyl ester gives conclusive information. The intermediate *o*-quinone forms an adduct with the alcoholic solvent, regenerates the *p*-catechol group, and allows the reaction to go on.

**Antiradical Activity of Eriodyctiol and Fustin in Methanol.** To quantify the individual influence of the removal of the hydroxyl on C3, in the C-ring, and the hydroxyl on C5, in the A-ring, the reduction curves of DPPH• by different amounts of eriodyctiol and fustin, respectively, were determined. The resulting curves are shown in **Figure 4**, and the results are given in **Table 2**. As can be seen, the removal of the hydroxyl on C3 (eriodyctiol) slightly increased the value of the fast kinetics rate constant [$k_1 = 70.2 \times 10^3$ L/(mol min)] and appreciably decreased the value of the slow kinetics pseudo-rate constant [$\rho_2 = 330$ L/(mol min)], but left unchanged the values of both fast and slow kinetics stoichiometric constants ($\sigma_1 = 2.04$ and $\sigma_2 = 2.00$, respectively). The removal of the hydroxyl on C5 (fustin) left unchanged the values of the fast kinetics rate [$k_1 = 59.5 \times 10^3$ L/(mol min)] and stoichiometric ($\sigma_1 = 2.02$) constants, but appreciably increased both the values of the slow kinetics pseudo-rate [$\rho_2 = 1335$ L/(mol min)] and stoichiometric ($\sigma_2 = 2.21$) constants. It is unclear how a hydroxyl on the A-ring can significantly affect the antiradical behavior of the *p*-catechol in the B-ring, but it seems that in fustin there exists a limited formation of a second adduct between a further intermediate *o*-quinone and the solvent, with a partial regeneration of the *p*-catechol group and the subsequent reduction of additional DPPH•. This would explain the greater values of both the slow kinetics pseudo-rate and stoichiometric constants of fustin when compared with those of taxifolin. In fact, this second adduct formation was already observed by Saito et al. (*16*) for the *p*-catechol group in protocatechuic acid alkyl esters during the reduction of the DPPH• in methanol. In the latter case, however, the second adduct formation seems to be much more favored

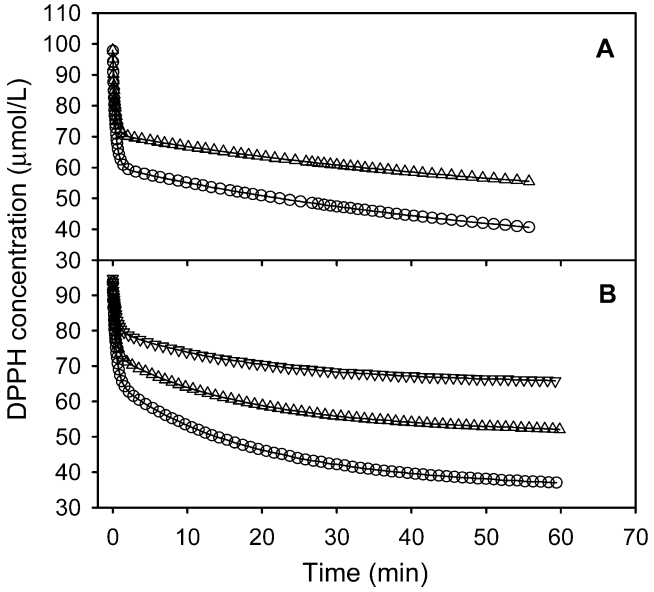


**Figure 4.** Time evolution of the DPPH• concentration in methanol during its reduction by (**A**) eriodyctiol and (**B**) fustin. See **Table 2** for the initial concentrations of DPPH•, eriodyctiol, and fustin, and the meaning of the symbols.

than in flavanone type structures, because the total stoichiometric constant of the *p*-catechol group was about 5, indicating that the value of the slow kinetics stoichiometric constant was about 3.

**Time Evolution of the Mass Spectra of the Reaction Mixture in Methanol. Figure 5** shows the mass spectra (electrospray, negative mode, sample introduction by infusion) of the reaction mixture acquired at 5, 20, and 60 min during the reduction of DPPH• in methanol by taxifolin and fustin (T5, T20, and T60 and F5, F20, and F60, respectively). Because the reaction must be carried out into the infusion syringe, reliable data could only be acquired from about 5 min of reaction time onward. At this initial time, the concentration of the parent molecule has decayed to zero and, consequently, its corresponding ion (*m*/*z* 303 and 287 for taxifolin and fustin, respectively) could not be detected. It must be observed that the ion corresponding to the reduced DPPH• (DPPH − H, *m*/*z* 394) as well as minor irrelevant ions corresponding to the formation of dimmers at the ionization source (*m*/*z* 603, 633, 635, 665, and 727 for taxifolin; *m*/*z* 571, 601, 633, 663, and 695 for fustin) has been removed from the mass spectra. If the ion corresponding to the parent molecule (*m*/*z* 303 and 287 for taxifolin and fustin, respectively) is denoted as **a**, it is known that the first step of the reduction process is a fast transfer of two hydrogen atoms from **a** to DPPH•. Hence, **a** is transformed into **a*** (**a*** = **a** − 2), which is clearly present in both mass spectra (**a*** = 301 and 285 for taxifolin and fustin, respectively). The ion **a*** decreases with time to give its adduct **b** with methanol (**b** = **a*** + 32), which is also very well observed in both mass spectra (**b** = 333 and 317 for taxifolin and fustin, respectively). The ion **b** decreases with time more slowly than **a*** because it is continuously being formed from **a*** but also rapidly destroyed by the fast transfer of two hydrogen atoms to the DPPH• and its subsequent transformation into **b*** (**b*** = **b** − 2), which is clearly seen in both mass spectra (**b*** = 331 and 315 for taxifolin and fustin, respectively). The ion **b*** increases exponentially with time, reaching an asymptote. Apparently, at this point the reaction is completed because the ion **c**, corresponding to the adduct between **b*** and methanol (**c** = **b*** + 32, **c** = 363 and 347 for taxifolin and fustin, respectively) behaves more as an adduct formed at the ionization source than a true reaction product. If **c** was mainly a true reaction product, it would transfer two hydrogen atoms to the DPPH•, thus decreasing with time and yielding a significant amount of **c*** (**c*** = **c** − 2, *m*/*z* 361 and 345 for taxifolin and fustin, respectively). However, because a small amount of **c*** was present in both mass spectra, it could not be disregarded that a small amount of **c** was the true reaction product. To clarify this question, the ionic abundance of **c*** was plotted against reaction time as shown in **Figure 6**. From the result, it seems clear that the ionic abundance of **c*** remains practically constant with time for taxifolin but slightly increases for fustin, indicating that in this latter case at least a small amount of **c** was the true reaction product. This result is in accordance with the previous finding that both the slow kinetics pseudo-rate and stoichiometric constants of fustin are greater than those of taxifolin.

**Kinetic Model.** From all results mentioned above and considering the available bibliographic information, the kinetic model depicted in **Figure 7**, for the reaction of reduction of DPPH• by the isolated *p*-catechol group in flavanone type structures, is proposed.

For a clearer and more concise discussion on the proposed model, it can be considered that the reaction of reduction is composed of successive steps as follows.

*First Step*. According to this model, the first step of the reaction is a rather fast transfer of two successive hydrogen atoms from the *p*-catechol group **a** to the DPPH• [fast rate constants $k_1(1)$ and $k_1(2)$] and the subsequent transformation of **a** into its corresponding *o*-quinone **a***. It is postulated that the antiradical nonequivalence of both hydroxyls of the *p*-catechol group is not experimentally detectable in alcoholic solvents but becomes detectable in nonalcoholic solvents. Hence, in alcoholic solvents a cumulative fast kinetics rate constant [$k_1 = k_1(1) + k_1(2)$] and a fast kinetics stoichiometric constant with a value of 2 ($\sigma_1 = 2$) should experimentally be determined (i.e., reduction of DPPH• in alcoholic solvents by taxifolin, eriodyctiol, and fustin). In nonalcoholic solvents, such as acetonitrile, two fast kinetics rate constants [$k_1(1)$ and $k_1(2)$] and two stoichiometric constants with value 1 [$\sigma_1(1) = \sigma_2(1) = 1$] should experimentally be determined (i.e., reduction of DPPH• in acetonitrile by taxifolin). Moreover, and due to the influence of the solvent, it should experimentally be determined that $k_1$(methanol) > $k_1$(ethanol) > $k_1$(propanol) (i.e., reduction of DPPH• in methanol, ethanol, and propanol by taxifolin).

*Second Step*. In nonalcoholic solvents, such as acetonitrile, the reaction of reduction is already completed, and hence there is no further slow kinetics step (i.e., reduction of DPPH• in acetonitrile by taxifolin). In alcoholic solvents, in contrast, the intermediate *o*-quinone **a*** quantitatively reacts with the solvent (slow rate constant $k_2$) and fully regenerates the *p*-catechol group **b**. Hence, the total concentration of **b**, namely [**b**], coincides with the total concentration of **a** and **a***, that is, [**b**] = [**a***] = [**a**]. According to Saito et al. (*16*) the alkoxy group enters on the position C2′, in the B-ring.

*Third Step*. The regenerated *p*-catechol group **b** rapidly transfers two hydrogen atoms to the DPPH• [fast rate constants $k_3(1)$ and $k_3(2)$], yielding the corresponding *o*-quinone **b***. It is evident that this step cannot experimentally be detected as a fast kinetics step, with a rate constant $k_3$ [$k_3 = k_3(1) + k_3(2)$], but as a slow decreasing step with a rate constant of value close to $k_2$, because this slow rate constant is the limiting one. If the reaction of reduction is completed at this point, then a stoichiometric constant of value 2 ($\sigma_2 = 2$) should experimentally be detected (i.e., reduction of DPPH• in methanol by

**Table 2.** Rate (Mean ± SD) and Stoichiometric (Mean ± SD) Constants from the Reduction of DPPH• in Methanol by Eriodyctiol and Fustin

| | | DPPH• | eriodyctiol[a] | DPPH• | fustin[a] |
|---|---|---|---|---|---|
| initial concentration ($\mu$mol/L) | | 97.785 (○)<br>97.952 (△) | 18.04 (○)<br>13.53 (△) | 93.678 (○)<br>93.772 (△)<br>94.710 (▽) | 13.80 (○)<br>10.35 (△)<br>6.90 (▽) |
| average rate constant [L/(mol min)] | $k_1$ | | $(70.2 \pm 1.5) \times 10^3$ | | $(59.5 \pm 4.2) \times 10^3$ |
| | $\rho_2$ | | $330 \pm 5$ | | $1335 \pm 49$ |
| average stoichiometric constants | $\sigma_1$ | | $2.042 \pm 0.052$ | | $2.026 \pm 0.057$ |
| | $\sigma_2$ | | $1.996 \pm 0.001$ | | $2.206 \pm 0.054$ |
| | $\sigma_t$ | | $4.037 \pm 0.052$ | | $4.232 \pm 0.076$ |

[a] Symbols refer to the corresponding curves in **Figure 4**.

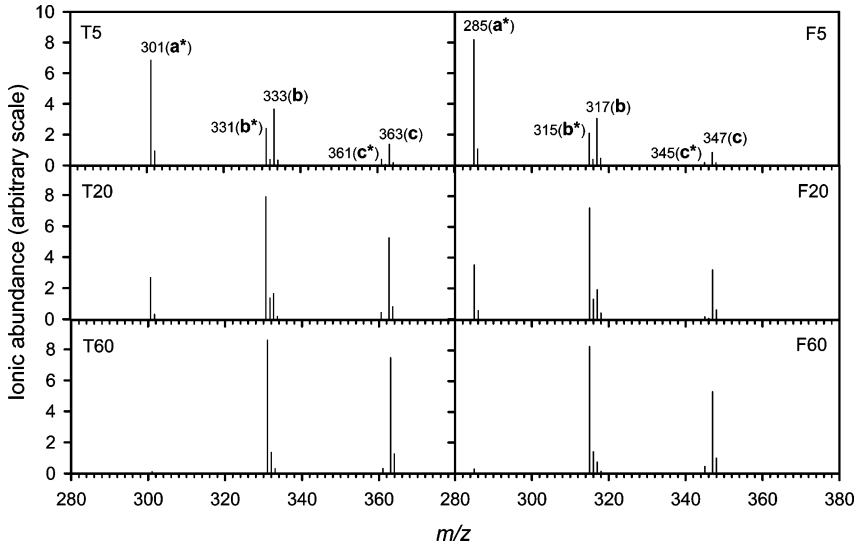


**Figure 5.** Mass spectra of the reaction mixture at 5, 20, and 60 min during the reduction of DPPH• (24.23 $\mu$mol/L) by taxifolin (3.04 $\mu$mol/L, T5, T20, and T60, respectively) and fustin (3.21 $\mu$mol/L, F5, F20, and F60, respectively).

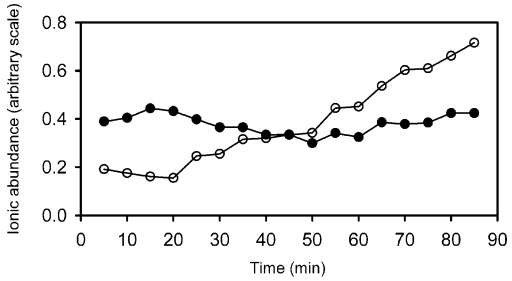


**Figure 6.** Time evolution of the abundance of the ion **c*** during the reduction of DPPH• by taxifolin (●, *m*/*z* 361) and fustin (○, *m*/*z* 345).

taxifolin and eriodyctiol; reduction of DPPH• in ethanol and propanol by taxifolin). On the other hand, because the value of the slow rate constant $k_2$ depends on the nucleophilic power of the alcohol in the order methanol > ethanol > propanol, and taking into account that $\rho_2$ and $k_2$ are directly correlated (see later), it should experimentally be found that $\rho_2$(methanol) > $\rho_2$(ethanol) > $\rho_2$(propanol) (i.e., reduction of DPPH• in methanol, ethanol, and propanol by taxifolin).

*Fourth Step*. A small amount of the intermediate *o*-quinone **b*** reacts with the solvent (slow rate constant $k_4$) and partially regenerates the *p*-catechol group **c**. Because the extent of this reaction is very limited in flavanone type structures, it is fulfilled that [**c**] < [**a**].

*Fifth Step*. The partially regenerated *p*-catechol group **c** transfers two hydrogen atoms to the DPPH• [fast rate constants $k_5(1)$ and $k_5(2)$], yielding the corresponding *o*-quinone **c***. Taking into account that [**c**] < [**a**] as well as the arguments given for the *third step*, a slow kinetics rate constant of value close to $k_4$ and a stoichiometric constant of value smaller than 2 ($\sigma_3 < 2$) should experimentally be detected. However, taking into account that it seems rather difficult to extract accurate values for $k_2$, $k_4$, $\sigma_2$, and $\sigma_3$ by means of a fitting, the experimentally determined value of the slow kinetics pseudo-rate constant, $\rho_2$(exp), should be, in these cases, close to the sum of the corresponding $\rho_2$ and $\rho_4$ [$\rho_2(\text{exp}) = \rho_2 + \rho_4$], and the experimentally determined value of slow kinetics stoichiometric constant, $\sigma_2$(exp), the sum of $\sigma_2$ and $\sigma_3$ [$\sigma_2(\text{exp}) = \sigma_2 + \sigma_3 = 2 + \sigma_3$] (i.e., reduction of DPPH• in methanol by fustin). It must be noted that the value of $\sigma_3$ can be any number between 0 and 2 ($0 \le \sigma_3 \le 2$) because this value depends on only [**c**], that is, on the extent of the adduct formation between **b*** and the solvent. In fact, the value of $\sigma_3$ [$\sigma_3 = \sigma_2(\text{exp}) - 2$] allows an approximate determination of [**c**], given that [**c**] = $(\sigma_3/2) \times$ [**a**] = $[(\sigma_2(\text{exp}) - 2)/2] \times$ [**a**].

*Sixth Step*. For structures other than flavanones, the possible formation of a further and final adduct between **c*** and the solvent to regenerate the *p*-catechol group, with the subsequent reduction of additional DPPH•, cannot be disregarded.

Finally, it is important to emphasize that although this model envisages many rate and stoichiometric constants, the antiradical activity of the isolated *p*-catechol group in flavanone type structures behaves experimentally as a very simple "active antiradical group" exhibiting two rate and two stoichiometric constants [$k_1$, $\rho_2$(exp), $\sigma_1$, and $\sigma_2$(exp)]. This is of particular importance because it will allow the deduction of two different but equivalent explicit kinetic equations for the antiradical activity of this group against DPPH•.

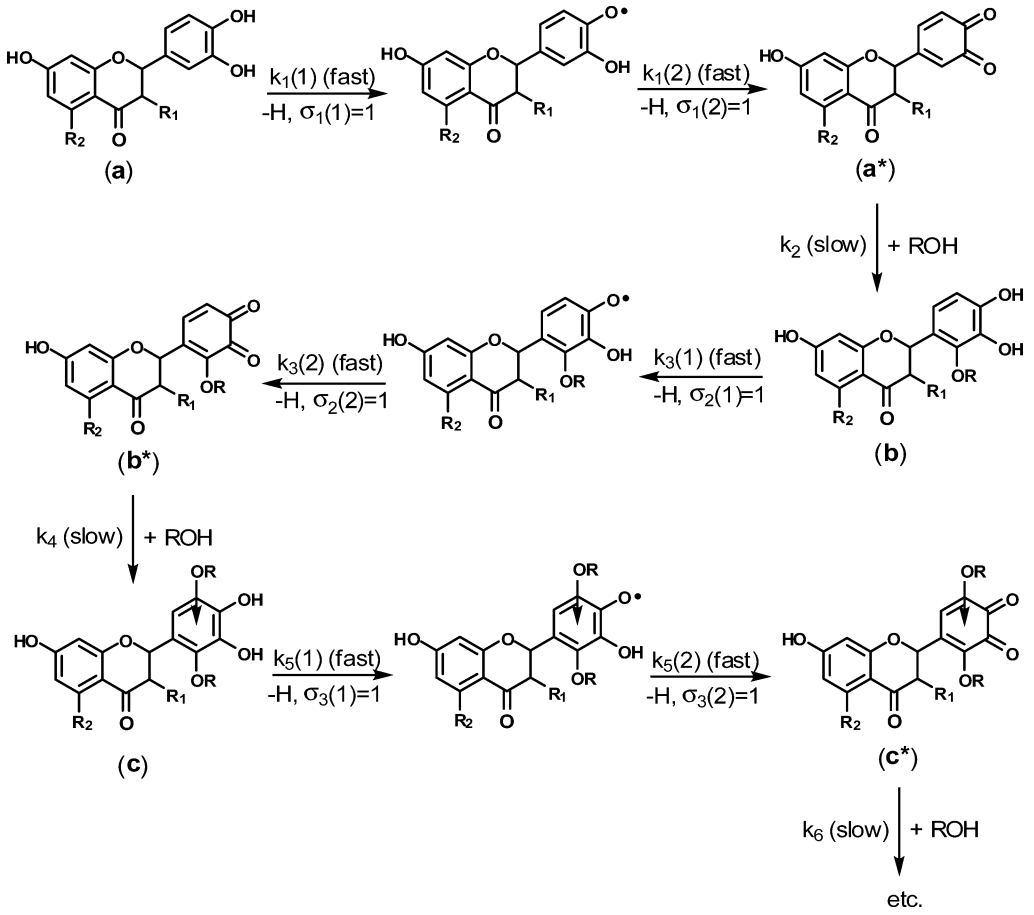


**Figure 7.** Kinetic model for the antiradical activity of the isolated *p*-catechol group in flavanone type structures using DPPH• as the antiradical probe.

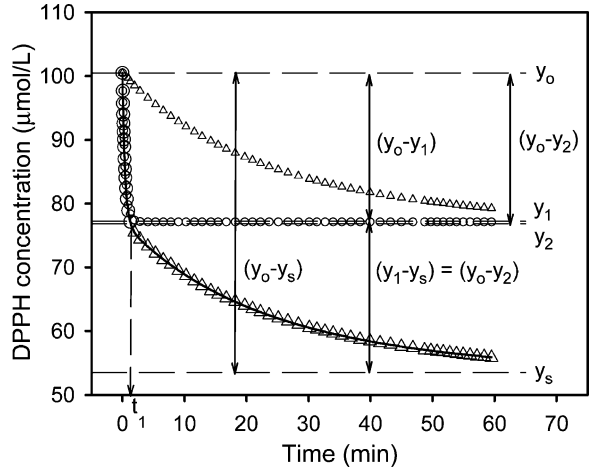


**Figure 8.** Time contribution and asymptote of the fast kinetics (small open circles) and slow kinetics (small open triangles) terms describing the fast kinetics (large open circles) and slow kinetics (large open triangles) steps, respectively, of the reduction curve of DPPH• (100.464 $\mu$mol/L) in methanol by taxifolin (11.59 $\mu$mol/L).

**Deduction of the Explicit Kinetic Equations from the Kinetic Model. Figure 8** shows the reduction curve of the DPPH• (100.46 $\mu$mol/L) in methanol by taxifolin (11.59 $\mu$mol/L), where the curve has been approximately divided into its fast (large blank circles) and slow (large blank triangles) kinetics steps, and the asymptotes corresponding to the fast ($y_1$), slow ($y_2$), and total ($y_s$) kinetics are shown as long dash lines.

Because the *p*-catechol group in flavanone type structures behaves experimentally as an "active antiradical group" exhibiting two rate and two stoichiometric constants, the kinetic model can reasonably be simplified to

$$y + a \xrightarrow{k_1} a^* + y - \mathrm{H} \quad (3)$$

$$a^* + \mathrm{ROH} \xrightarrow{k_2} b \quad (4)$$

$$y + b \xrightarrow{k_3} b^* + y - \mathrm{H} \quad (5)$$

The set of the corresponding differential equations has no analytical solution, and so it makes no sense to set it out. Instead, our aim is to deduce an approximate but accurate explicit analytical solution. To achieve this, it is important to observe that the rate constant $k_1$ is greater by far than the rate constant $k_2$ ($k_1 \gg k_2$). This allows uncoupling of reaction 3 (fast kinetics) from the reactions 4 and 5 (slow kinetics), solving them separately to obtain two terms (one for the fast kinetics and the other for the slow kinetics), and then coupling both terms. To uncouple reaction 3 from reactions 4 and 5, it is assumed that they are not simultaneous but successive or, in other words, that reaction 3 is so rapid when compared with reaction 4 that the latter does not start until the former is completed. Hence, reaction 4 starts after a very short time, $t_1$, at which $y$ in reaction 3 has already reached its asymptotic value $y_1$. To couple both solutions, $t_1$ in the second term (slow kinetics) must tend toward zero.

From eq 3

$$\frac{dy}{dt} = -k_1 y a \tag{6}$$

with the boundary conditions at $t = 0$, $y = y_0$, $a = a_0$, and $a^* = 0$; and at $t = \infty$ (or $t > t_1$), $y = y_1$, $a = 0$, and $a^* = a_0$. Let $\sigma_1$ be the stoichiometric constant of the reaction, then (*6*)

$$\sigma_1 = \frac{y_0 - y_1}{a_0} = \frac{y - y_1}{a} \rightarrow a = \frac{y - y_1}{\sigma_1} \tag{7}$$

and eq 6 becomes

$$\frac{dy}{dt} = -\frac{k_1}{\sigma_1} y(y - y_1) \tag{8}$$

the solution of which, in its most explicit form, with the given boundary conditions, is well-known (*6*)

$$y - y_1 = \frac{y_1(y_0 - y_1)}{y_1 - y_0(1 - e^{(k_1/\sigma_1)y_1 t})} \tag{9}$$

and corresponds to the term (small blank circles in **Figure 8**) describing the fast kinetics step of the reaction.

From eqs 4 and 5

$$\frac{da^*}{dt} = -k_2 a^* [\mathrm{ROH}] \tag{10}$$

$$\frac{dy}{dt} = -k_3 y b \tag{11}$$

where [ROH] is the molar concentration of the alcohol and with the following boundary conditions: at $t = t_1$, $a^* = a_0$, $y = y_1$, and $b = b^* = 0$ and at $t = \infty$, $a^* = b = 0$, $y = y_s$, and $b^* = a_0$, $y_s$ being the experimental asymptote of the reaction. At any time $t \geq t_1$ it is fulfilled that $a_0 = a^* + b + b^*$, that is, $b = a_0 - b^* - a^*$. On the other hand, let $\sigma_2$ be the stoichiometric constant of the reaction. Then, at any time $t \geq t_1$ it is fulfilled that $(a_0 - b^*) = (y - y_s)/\sigma_2$ and consequently

$$b = (y - y_s)/\sigma_2 - a^* \tag{12}$$

The value of $a^*$ (a simple decaying exponential) can easily be deduced from eq 10

$$a^* = a_0 e^{-k_2[\mathrm{ROH}]t} \tag{13}$$

and substituted into eq 12 and the result substituted into eq 11, but the resulting differential equation, due to the exponential term, has no analytical solution. To overcome this difficulty there are two fine approximations.

*First Approximation.* Taking into account that the concentration of $b$ is very small at any time of the reaction process, because it is continuously and slowly formed from $a^*$ (slow rate constant $k_2$) but continuously and rapidly destroyed by the transfer of two hydrogen atoms to the DPPH$^\bullet$ (fast rate constant $k_3$), it would be reasonable to assume that its concentration remains practically constant with time, similarly to the concentration of the enzyme−substrate complex in simple Michaelis−Menten kinetics, and consequently $db/dt \approx 0$. Derivation of eq 12 leads to the immediate solution

$$y - y_s = \sigma_2 a_0 e^{-k_2[\mathrm{ROH}](t - t_1)} \tag{14}$$

Taking into account that it is fulfilled

$$\sigma_2 a_0 = y_1 - y_s$$

eq 14 can be rewritten as

$$y - y_s = (y_1 - y_s) e^{-k_2[\mathrm{ROH}](t - t_1)} \tag{15}$$

which would be the uncoupled term describing the slow kinetics step of the reaction.

To couple reactions 3 and 4 and make them simultaneous, the value of $t_1$ in eq 15 must tend toward zero. As shown in **Figure 8**, when $t_1$ tends toward zero in eq 15, the value of its $y_1$ tends toward $y_0$ and the value of its $y_s$ must tend toward a value $y_2$ (the asymptotic value of the small open triangles) such that it must be fulfilled

$$y_1 - y_s = y_0 - y_2 \rightarrow y_2 = y_o + y_s - y_1 \tag{16}$$

and so eq 15 becomes

$$y - y_2 = (y_0 - y_2) e^{-k_2[\mathrm{ROH}]t} = (y_1 - y_s) e^{-k_2[\mathrm{ROH}]t} \tag{17}$$

with the constraint for the value of $y_2$ given by eq 16. Hence, eq 17 is the coupled term (small open triangles in **Figure 8**) describing the slow kinetics step of the reaction.

Taking into account that for the full reaction

$$y_0 - y_s = y_0 - y_1 + y_1 - y_s = y_0 - y_1 + y_o - y_2 \tag{18}$$

the approximate kinetic equation describing this reaction is given by

$$y - y_s = \frac{y_1(y_0 - y_1)}{y_1 - y_0(1 - e^{(k_1/\sigma_1)y_1 t})} + (y_1 - y_s) e^{-k_2[\mathrm{ROH}]t} \tag{19}$$

with the constraints

$$y_0 - y_1 = \sigma_1 a_0$$

$$y_0 - y_2 = y_1 - y_s = \sigma_2 a_0$$

$$y_0 - y_s = (\sigma_1 + \sigma_2) a_0$$

*Second Approximation.* Taking into account that at any time of the reaction process the concentration of $b$ is smaller than that of $a^*$, depends directly on the concentration of $a^*$, is almost independent of the concentration of $y$, because $y$ is in excess and $k_3 \gg k_2$, and its time evolution is similar to that of $a^*$, it seems reasonable to assume that $b \approx Ka^*$, where $K \ll 1$. Because the concentration of $b$ is directly dependent on the rate constant $k_2$ but inversely dependent on the rate constant $k_3$, $K$ must also fulfill these same dependences and be also dependent on the solvent used, that is, $K = K(k_2, k_3, [\mathrm{ROH}])$. Hence

$$b \approx Ka^* \rightarrow a^* \approx \frac{1}{K} b \tag{20}$$

Substitution of eq 20 into eq 12 gives

$$b = \frac{K}{1 + K} \frac{(y - y_s)}{\sigma_2} \tag{21}$$

and substitution of eq 21 into eq 11 gives

$$\frac{dy}{dt} = -\frac{Kk_3}{1+K}\frac{1}{\sigma_2}y(y-y_s) \quad (22)$$

If a pseudo-rate constant $\rho_2$ is defined as

$$\rho_2 = \frac{Kk_3}{1+K} \quad (23)$$

eq 22 becomes

$$\frac{dy}{dt} = -\frac{\rho_2}{\sigma_2}y(y-y_s) \quad (24)$$

which is similar to eq 8 and its solution, taking into account the boundary conditions, is given by

$$y - y_s = \frac{y_s(y_1 - y_s)}{y_s - y_1[1 - e^{(\rho_2/\sigma_2)y_s(t-t_1)}]} \quad (25)$$

which is the uncoupled term describing the slow kinetics step of the reaction.

As in the case of the *first approximation*, to couple reactions 3 and 4 and make them simultaneous, the value of $t_1$ in eq 25 must tend toward zero. Hence, by following exactly the same considerations as above, it can be deduced that the coupled term is given by

$$y - y_2 = \frac{y_2(y_0 - y_2)}{y_2 - y_0(1 - e^{(\rho_2/\sigma_2)y_2 t})} \quad (26)$$

and the approximate kinetic equation describing this reaction is given by

$$y - y_s = \frac{y_1(y_0 - y_1)}{y_1 - y_0(1 - e^{(k_1/\sigma_1)y_1 t})} + \frac{y_2(y_0 - y_2)}{y_2 - y_0(1 - e^{(\rho_2/\sigma_2)y_2 t})} \quad (27)$$

with the constraints

$$y_2 = y_0 + y_s - y_1 = y_0 - \sigma_2 a_0$$

$$y_0 - y_1 = \sigma_1 a_0$$

$$y_0 - y_s = (\sigma_1 + \sigma_2)a_0$$

which coincides with eq 1.

In this work, eq 27 has been preferred to eq 19 for fitting the experimental data points, due to the reasons explained below.

First, both eqs 19 and 27 yield practically the same fitting in all cases, with excellent coefficients of correlation ($r^2 > 0.999$, although the values from eq 27 are slightly better than those from eq 19). The adjusted values of the parameters $k_1$, $\sigma_1$, and $\sigma_2$ are also practically identical from both fittings. However, the adjusted values of the slow kinetics rate ($k_2$, from eq 19) and the pseudo-rate ($\rho_2$, from eq 27) constants are extremely different, differing by >5 orders of magnitude.

Second, the first approximation (eq 19) envisages the full reaction of reduction as "it really is". The fast step of the reduction curve is considered to be due to a true fast antiradical kinetics with a fast rate constant $k_1$ and thus fitted using the mathematical function derived from a second-order reaction. This allows the determination of the rate ($k_1$) and stoichiometric ($\sigma_1$) constants corresponding to the fast kinetics. The slow step of the reduction curve is considered to be due to a pseudo-slow antiradical kinetics, mainly modulated by the rate constant ($k_2$) of formation of the adduct, and thus fitted using a simple decaying exponential, which corresponds to a pseudo-first-order reaction. This allows the determination of the slow rate constant of the adduct formation ($k_2$) and the stoichiometric constant of the pseudo-slow kinetics ($\sigma_2$). However, this approximation, although very close to "reality", has the frustrating drawback that it does not allow a direct comparison of the speediness of both kinetics by direct comparison of the rate constants $k_1$ and $k_2$, because they differ by >7 orders of magnitude.

Third, the second approximation (eq 27) envisages the full reaction of reduction as "it behaves as", that is, as the conjunction of two true antiradical kinetics, the former being fast and the latter slow. Similarly to eq 19, the fast step of the reduction curve is considered to be due to a true fast antiradical kinetics with a fast rate constant $k_1$, and thus fitted using the mathematical function derived from a second-order reaction. This allows the determination of the rate ($k_1$) and stoichiometric ($\sigma_1$) constants corresponding to the fast kinetics. In contrast to eq 19, the slow step of the reduction curve is also considered to be due to a true slow antiradical kinetics with a slow pseudo-rate constant $\rho_2$, and thus fitted using the same mathematical function. This allows the determination of the slow kinetics stoichiometric ($\sigma_2$) and the pseudo-rate ($\rho_2$) constants. This approach clearly does not reflect "reality", because the *p*-catechol group is considered to have two different and independent "antiradical groups", one exhibiting a fast kinetics and the other a slow kinetics (due to the adduct formation, evidently), and so the value of the slow pseudo-rate constant $\rho_2$ does not correspond to any of the reactions of the reduction process. However, this approximation has an advantage of vital importance, because it allows the immediate comparison of the speediness of both kinetics by direct comparison of their corresponding rate ($k_1$) and pseudo-rate ($\rho_2$) constants. It must be emphasized, on the other hand, that this direct comparison, although quite important for isolated compounds, becomes essential to evaluate the antiradical activity of mixtures of antiradicals.

As indicated previously, the values of $\rho_2$ and $k_2$ are directly correlated. **Table 1** gives the adjusted values of $k_2$[ROH] ($min^{-1}$) and $k_2$ [L/(mol min)] for the reduction of DPPH$^\bullet$ by taxifolin in methanol, ethanol, and propanol (**Figure 2A**,**B**,**C**, respectively), using eq 19 for fitting. The resulting data demonstrate that there exists a linear relationship between $\rho_2$ and $k_2$[ROH] ($\rho_2 = mk_2$[ROH], $m = 21695$ and $r^2 = 0.9994$). Moreover, taking into account the definition of $\rho_2$ (eq 23)

$$\frac{Kk_3}{1+K} = mk_2[\text{ROH}] \rightarrow K = \frac{mk_2[\text{ROH}]}{k_3 - mk_2[\text{ROH}]}$$

that is, $K$ was directly dependent on $k_2$[ROH] and inversely dependent on $k_3$, and its value, determined by assuming that $k_3 \approx k_1$, was 0.0157, $9.87 \times 10^{-3}$, and $8.90 \times 10^{-3}$ for methanol, ethanol, and propanol, respectively, that is, $K \ll 1$ in all cases, as was expected. On the other hand, the determined values for the rate constant $k_2$ [$1.757 \times 10^{-3}$, $7.391 \times 10^{-4}$, and $2.726 \times 10^{-4}$ L/(mol min) for methanol, ethanol, and propanol, respectively] were very small and in the range of the published values of this rate constant for the adduct formation of quinomethanes (*21*).

Finally, it must be emphasized that neither eq 27 nor eq 19 is a general kinetics equation for determining the antiradical activity of the *p*-catechol group in any chemical structure. On the contrary, they are rather specific and should be used only

to determine the antiradical activity of "active antiradical groups" or "antiradical mixtures" that experimentally exhibit two rate and two stoichiometric constants. This explains why eq 27 works very well for determining the antiradical activity of citrus juices (*6*), because due to their high content in ascorbic acid, their cumulative antiradical activity experimentally behaves as an antiradical group exhibiting two rate and two stoichiometric constants (average constants in this case). This explains also why eq 27, or eq 19, should not be used for determining the antiradical activity of the *p*-catechol group in flavone type structures (e.g., luteolin). Preliminary but consistent data indicate that the presence of a double bond between C2 and C3, in the C-ring, influences the antiradical kinetics of the *p*-catechol group in such a way that it experimentally behaves as an antiradical group, exhibiting discernible three rate and three stoichiometric constants.

## ABBREVIATIONS USED

| | |
|---|---|
| $a$ | time-dependent concentration ($\mu$mol/L) of the antiradical |
| $a_0$ | initial concentration ($\mu$mol/L) of the antiradical |
| DPPH$^\bullet$ | 2,2-diphenyl-1-picrylhydrazyl |
| $k_1$ | cumulative fast kinetics rate constant [$= k_1(1) + k_1(2)$] [L/(mol min) in alcoholic solvents] |
| $k_1(1)$ | first component [L/(mol min)] of the fast kinetics rate constant in nonalcoholic solvents |
| $k_1(2)$ | second component [L/(mol min)] of the fast kinetics rate constant in nonalcoholic solvents |
| $k_2$ | rate constant [L/(mol min)] of the first adduct formation in alcoholic solvents [in eq 19, fraction of the cumulative pseudo-slow kinetics rate constant [L/(mol min)] due to the regenerated *p*-catechol group from the first adduct] |
| $k_4$ | rate constant [L/(mol min)] of the second adduct formation in alcoholic solvents [in eq 19, fraction of the cumulative pseudo-slow kinetics rate constant [L/(mol min)] due to the regenerated *p*-catechol group from the second adduct |
| $t$ | time (min) |
| $y$ | time-dependent concentration ($\mu$mol/L) of DPPH$^\bullet$ |
| $y_0$ | initial concentration ($\mu$mol/L) of DPPH$^\bullet$ |
| $y_1$ | DPPH$^\bullet$ concentration asymptote ($\mu$mol/L) that would be reached due solely to the antiradical activity of the fast kinetics |
| $y_2$ | DPPH$^\bullet$ concentration asymptote ($\mu$mol/L) that would be reached due solely to the antiradical activity of the slow kinetics |
| $y_s$ | experimental DPPH$^\bullet$ concentration asymptote ($\mu$mol/L) |
| $\rho_2$ | in eq 27, first component [L/(mol min)] of the slow kinetics pseudo-rate constant in nonalcoholic solvents |
| $\rho_4$ | in eq 27, second component [L/(mol min)] of the slow kinetics pseudo-rate constant in nonalcoholic solvents |
| $\rho_2$(exp) | in eq 27, cumulative slow kinetics pseudo-rate constant ($= \rho_2 + \rho_4$) [L/(mol min)] in alcoholic solvents |
| $\sigma_1$ | total stoichiometric constant of the fast kinetics [$= \sigma_1(1) + \sigma_1(2) = 2$] in alcoholic solvents |
| $\sigma_1(1)$ | stoichiometric constant ($= 1$) of the first component of the fast kinetics in nonalcoholic solvents |
| $\sigma_1(2)$ | stoichiometric constant ($= 1$) of the second component of the fast kinetics in nonalcoholic solvents |
| $\sigma_2$ | fraction of the slow kinetics stoichiometric constant ($= 2$) due to the regenerated *p*-catechol group from the first adduct |
| $\sigma_3$ | fraction of the slow kinetics stoichiometric constant ($0 \leq \sigma_3 \leq 2$) due to the partially regenerated *p*-catechol group from the second adduct |
| $\sigma_2$(exp) | total stoichiometric constant of the slow kinetics ($= \sigma_2 + \sigma_3 = 2 + \sigma_3$) |
| $\sigma_t$ | total stoichiometric constant [$= \sigma_1 + \sigma_2$(exp)] |

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