The *Briggs-Rauscher* Reaction as a Test to Measure the Activity of Antioxidants

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A new method for monitoring the relative activity of antioxidants is presented, and its advantages and limits are discussed. The method is based on the previously reported inhibitory effects of free-radical scavengers on the oscillations of the *Briggs-Rauscher* reaction. The effect consists of an immediate cessation of oscillations, an inhibition time that linearly depends on the concentration of the antioxidant added, and subsequent regeneration of oscillations. Here the effects of ten antioxidants (pyrocatechol (= benzene-1,2-diol), ferulic acid (= 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid), caffeic acid (= 3-(3,4-dihydroxyphenyl)prop-2-enoic acid), 2,6-, 3,4-, 2,4-, 3,5-, and 2,5-dihydroxybenzoic acids, homovanillic acid (= 4-hydroxy-3-methoxybenzene-acetic acid), and resorcinol (= benzene-1,3-diol)) were studied in detail. Relative antioxidant activities of these substances with respect to resorcinol were determined in different ways on the basis of inhibition times. The limits of the calculated values of relative activity based on the *Briggs-Rauscher* reaction are the same as those obtained with other analytical procedures and are discussed here. The new method is inexpensive: reagents and apparatus are commonly used in all chemical laboratories. The thermochemical behavior of the *Briggs-Rauscher* reaction and the dependence of inhibition time on the temperature were also carefully investigated and taken into account. A semiquantitative mechanistic interpretation of the inhibitory effects based on a suitable kinetic model is given.

1. Introduction. – The involvement of O-free radicals in the development of several pathological states such as cancer, rheumatoid arthritis, arteriosclerosis, and post-ischaemic reoxygenation injury of liver and other organs is well-established [1]. A number of analytical techniques to measure the antioxidant capacity of free-radical scavengers have been proposed, some of which are widely used in chemical-clinical laboratories [2] [3].

Recently, we reported [4] the inhibitory effects of soy antioxidants on an oscillating reaction, the *Briggs-Rauscher* (*BR*) reaction [5]. The *BR* oscillating system consists of the iodination and oxidation of an organic substrate (in general, malonic acid (MA) or its derivatives) by acidic iodate in the presence of H_2O_2 and with the Mn^{2+} ion as catalyst. The *Briggs-Rauscher* reaction can be considered a 'hybrid' between the well-known *Belousov-Zhabotinsky* [6] [7] reaction and the *Bray-Liebhafsky* [8] [9] reaction.

The main intermediates for which concentrations oscillate in the *BR* reaction are: iodine, iodide ion, the oxyiodine species HOI, HOIO, and $IO_2^•$, and the hydroperoxyl radical $HOO^•$. Decisive indirect evidence for the involvement and important role played by $HOO^•$ radicals in the onset of oscillations is given by the inhibitory effects observed when glycosides contained in the soy wholeflour (as malonyldaidzin or malonylgenistin) are added to an active *BR* mixture [4]. The effect consists of an immediate cessation of oscillations, but, after some time, the oscillatory behavior is

regenerated with amplitude, frequency, and duration different from those observed in a reference mixture. Since it has been established that a number of glycosides contained in the soy show strong O-free-radical-scavenging activity [10] [11], inhibitory effects on the oscillations of the *BR* reaction were ascribed to scavenging of $HOO^{\bullet}$ radicals by the glycosides [4].

The dependence of the inhibition time (*i.e.* the time elapsed between the cessation and the regeneration of the oscillatory regime) on the concentration of glycosides added was found to be linear over a wide range of concentration [4]. This is an indication of the possibility of using the oscillating *BR* reaction as a test for the activity of antioxidants. The main goal of the present work is to present and to discuss a new analytical method to determine the relative activity of H_2O-soluble antioxidant scavengers of free radicals. Another goal is to give a mechanistic interpretation of the inhibitory effects on the basis of a suitable model for the oscillating behavior of the *BR* system.

2. Current Analytical Procedures for the Determination of Antioxidant Status. – Many analytical methods have been developed for the determination of the antioxidant activity of substances. All the methods are based on the generation of free radicals in the reaction mixture and their detection. In the presence of antioxidants, the amount of the free radicals detected is much less in comparison with that of a reference mixture. The main differences are in the methods of radical production and detection. Some procedures are more suitable for H_2O-soluble substances and others more for lipophilic substances; also, the pH varies from method to method.

The *Frankel* method [12] uses the copper(II) ion to initiate oxidation of human LDL (low-density lipoproteins) and the measurements of hexanal formation (a secondary lipid-oxidation product) at pH 7.4 in presence and absence of antioxidants (head-space gas-chromatography measurements). *Marco* [13] has used the β-carotene bleaching in a mixture of β-carotene/linoleic acid for the determination of antioxidant capacity. The *Pryor* rapid screening test [14] consists of the reaction between linoleic acid and 2,2′-azobis[2-amidinopropane] dihydrochloride (ABAP = 2,2′-azobis[2-methylpropanimidamide] dihydrochloride) which forms free radicals at pH 7.4. Two other chemiluminescent assays are reported by *Roda et al.* [3]: an enhanced chemiluminescence system based on horseradish peroxidase and a luminol/oxidant/enhancer reagent, which works at pH 8.6, and the hypoxanthine/xanthine oxidase/Fe^{2+}-EDTA/luminol system, which works at pH 10.3. The TEAC (trolox equivalent antioxidant capacity) method of *Miller et al.* [15] is used to calculate the total antioxidant status of samples by the inhibited formation of the colored 2,2′-azinobis[3-ethyl-2,3-diyhdrobenzothiazole-6-sulfonic acid] radical cation ($ABTS^{\bullet+}$) in the reaction mixture at pH 7.4. This method has become the most common procedure quoted in the literature because there is a test kit available from *Randox Laboratories* (Ireland) to determine the total-antioxidant status. Recently, some of these testing methods have been compared on the basis of their response to different antioxidants and their suitability for screening [16].

3. Experimental. – 3.1. *Materials and Apparatus.* Malonic acid (*Merck*; reagent grade, > 99%), manganese(II) sulfate monohydrate (*Merck*; reagent grade, > 99%), and $NaIO_3$ (*Merck*; reagent grade, > 99.5%) were

used without further purification. $HClO_4$, H_2O_2, and other chemicals were of anal. grade. All stock solns. were prepared from doubly distilled, deionized H_2O. Perchloric acid was analysed by titration *vs.* standard 0.1M NaOH (*Merck*). H_2O_2 was standardized daily by manganometric analysis.

Antioxidants used: 2,4-dihydroxybenzoic acid (2,4-DHBA) (*Fluka*; reagent grade, ≥98%), 2,5-DHBA (*Aldrich*; reagent grade, 98%), 2,6-DHBA (*Aldrich*; reagent grade, 98%), 3,4-DHBA (*Acros Organics*; reagent grade, 97%), 3,5-DHBA (*Merck*; reagent grade, >98%), caffeic acid (= 3-(3,4-dihydroxyphenyl)prop-2-enoic acid; *Merck*; reagent grade, >98%), ferulic acid (= 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid; *Fluka*; reagent grade, ≥98%), homovanillic acid (=4-hydroxy-3-methoxybenzeneacetic acid; *Fluka*; reagent grade, ≥99%), pyrocatechol (=benzene-1,2-diol; *Fluka*; reagent grade, ≥98%), resorcinol (=benzene-1,3-diol; *Fluka*; reagent grade, ≥98%).

Oscillations in the *BR* mixtures were followed potentiometrically by recording the potential of a iodide-ion-selective electrode (*Orion*, model *9453*) or the potential of a bright-platinum electrode. As reference electrode, we used a double-junction Ag/AgCl electrode (*Ingold*, model *373-90-WTE-ISE-S7*). Electrodes were connected to a pH multimeter (*WTW*, model *pH 540 GLP*) controlled by an *IBM*-compatible PC. The accuracy of the multimeter was ±1 mV. The suitable data-acquisition program Multi Achat II (*WTW*) was used. The multimeter was equipped with a temp. sensor with an accuracy of ±0.1°.

All soln. and reaction mixtures were maintained at constant temp. by means of a suitable thermostating system (accuracy ±0.1°).

Fig. 1 shows a simultaneous recording of the potentials of the iodide-selective electrode and the bright-platinum electrode for a typical *BR* mixture under batch conditions.

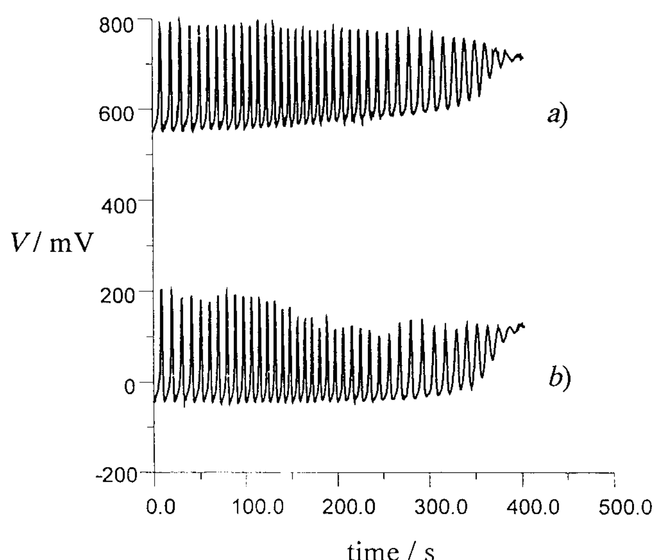


Fig. 1. *Simultaneous recordings of the potentials* a) *of the bright-platinum electrode and* b) *of the iodide-selective electrode* vs. *time for a typical batch* BR *mixture.* Initial conditions: $[H_2O_2] = 1.20$M, $[HClO_4] = 0.0266$M, $[IO_3^-] = 0.0667$M, [MA] = 0.050M, $[Mn^{2+}] = 0.00667$M.

BR Mixtures oscillate in a narrow pH range (typically 0.5 – 2.5 at 25°), *i.e.* at the concentrations used in the present work. We found good amplitude, frequency, and duration of oscillations at pH 1.55 ± 0.05.

3.2. *Thermochemistry of the* BR *Reaction.* 3.2.1. *Non-thermostated, Non-inhibited System.* Doubly distilled H_2O (1.0 ml) was added to an active, well-stirred *BR* mixture (30 ml; initial composition: $[H_2O_2] = 1.5$M, $[HClO_4] = 0.0334$M, $[IO_3^-] = 0.0667$M, [malonic acid] = 0.05M, and $[Mn^{2+}] = 0.0063$M), after the third oscillation. Simultaneous recordings of the behavior of the bright-platinum electrode potential and temp. of the mixture show that the oscillating *BR* reaction is exothermic (*Fig. 2*). The rise of the temp. is similar to that observed and described by *Cooke* [17]. He ascribed this relative great exothermicity to oxidation of the organic substrate by

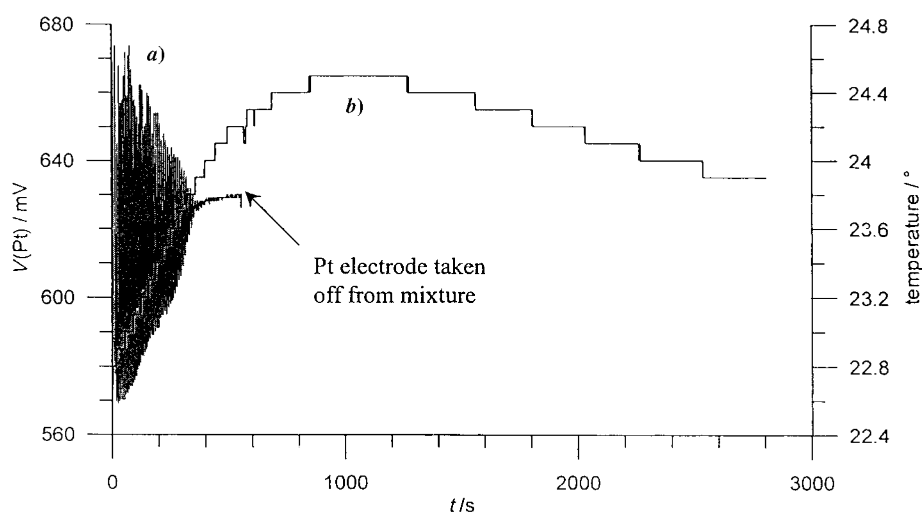


Fig. 2. *Simultaneous recordings of* a) *the potential of the bright-platinum electrode and* b) *the temperature* vs. *time when 1.0 ml of doubly distilled water was added to 30 ml of a non-thermostated* BR *mixture.* Initial conditions: $[H_2O_2] = 1.50$M, $[HClO_4] = 0.0334$M, $[IO_3^-] = 0.0667$M, $[MA] = 0.050$M, $[Mn^{2+}] = 0.0063$M.

free radicals. Indeed, the heat output does not stop immediately at the end of the oscillations but continues during the decomposition of the iodinated product. This indicates that another, different exothermic reaction not associated with oscillating behavior occurs. At the end of the decomposition and oxidation of the organic material to CO_2, the temp. decreases to r.t.

3.2.2. *Nonthermostated, Inhibited System.* Caffeic acid sodium salt soln. (1.0 ml; 0.03 mg/ml) was added to an active, well-stirred *BR* mixture (30 ml; initial composition as reported in *3.2.1.*), after the third oscillation. Simultaneous recordings of the behavior of the potential and temp. show that the temp. of the mixture rises immediately at the beginning of the oscillating reaction (*Fig. 3*). After the addition of the antioxidant, the temp. remains constant during the inhibition time. When the oscillations restart, the temp. reincreases and continues to increase also after the end of the oscillations. This behavior is similar to that of the nonthermostated, non-inhibited system. Finally the temperature decreases when the reaction is complete.

3.2.3. *Thermostated, Non-inhibited System.* The procedure described in *3.2.1.* was repeated, thermostating the reaction vessel at $25 \pm 0.1°$. *Fig. 4* shows that the temp. rises in spite of the thermostasis in a noninhibited *BR* mixture, but the increase is much less than in the nonthermostated system. However, it is obvious that even under thermostated conditions, the temp. does not remain constant; therefore, the temp. variation has to be considered.

3.2.4. *Thermostated, Inhibited System.* The procedure described in *3.2.2.* was repeated, thermostating the reaction vessel at $25 \pm 0.1°$. As can be noted in *Fig. 5*, the temp. begins to rise immediately with the start of the oscillations. After the addition of the antioxidant (ferulic acid) the oscillations stop, and, after a short period, the temp. remains constant during the whole inhibition time. At the end of the inhibition period, the temp. increases at once with the restart of oscillations in spite of the thermostasis. Moreover, in the first phase of the inhibition period, there is a difference between the thermostat temp. and that of the reaction mixture. Therefore, the dependence of the inhibition time on the temp. was studied.

The trend of the inverse of the inhibitory time *vs.* T, starting from *ca.* 20.0° and going up to about 40.0° shows an *Arrhenius*-type temp. dependence. The inhibitory time becomes increasingly shorter as the temp. increases. However, the portion of the curve in a narrow temp. range (2–3°) around 25.0° is well-approximated by a straight line for all the antioxidants studied (an example is reported in *Fig. 7,a*). We used these linear relationships to obtain 'corrected' inhibition times at exactly 25.0°.

As an example, the following relationship for $t_{inhib.}$ *vs.* temp. for caffeic acid was found:

$$t_{inhib}\ [s] = -200.1\ [s/°] \cdot temp.\ [°] + 7960\ [s] \qquad R^2 = 0.994$$

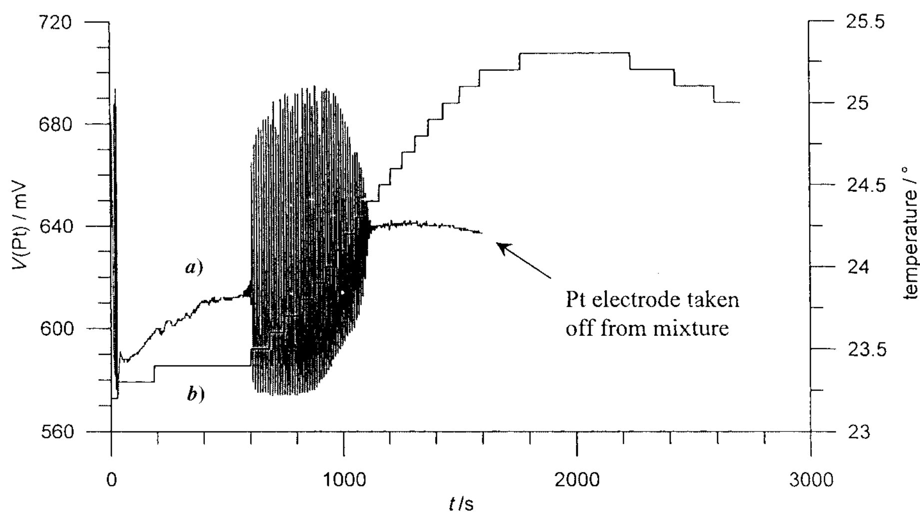


Fig. 3. *Simultaneous recordings of* a) *the potential of the bright-platinum electrode and* b) *the temperature* vs. *time when 1.0 ml of a solution of caffeic acid* ($c = 0.03$ mg/ml) *was added to 30 ml of a nonthermostated* BR *mixture.* Initial conditions: the same as in *Fig. 2.*

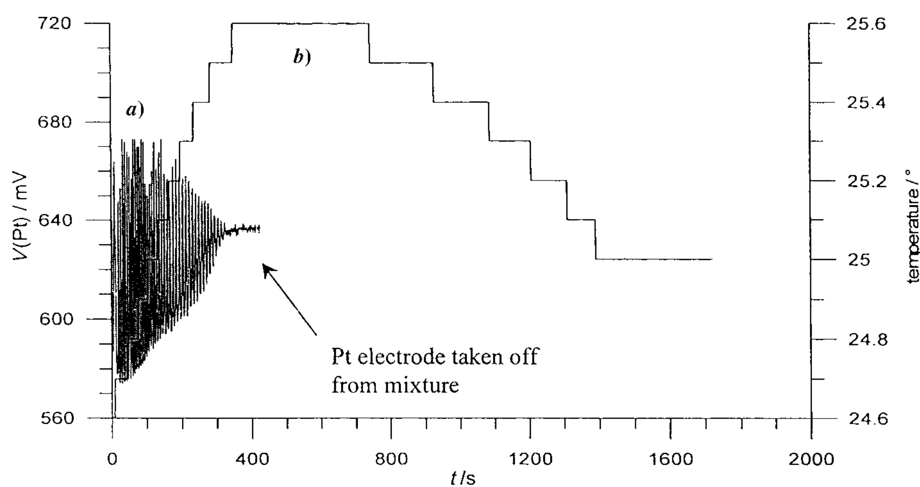


Fig. 4. *Simultaneous recordings of* a) *the potential of the bright platinum electrode and* b) *the temperature* vs. *time when 1.0 ml of doubly distilled water was added to 30 ml of a thermostated* BR *mixture.* Temperature of the thermostatic bath, 25.0 ± 0.1°. Initial conditions: the same as in *Fig. 2.*

The experimental value of 2276 s obtained at a mean temp. of 24.3° during the inhibitory phase was reported at 25° by means of the following equation:

$$t_{\mathrm{inhib}(25.0^\circ)}\ [\mathrm{s}] = 2276\ [\mathrm{s}] - 200.1\ [\mathrm{s}/^\circ] \cdot (25.0 - 24.3)\ [^\circ] = 2136\ [\mathrm{s}]$$

3.3. *Inhibitory Effects of a Series of Antioxidants.* Structures of antioxidants considered are reported in *Fig. 6*. For each antioxidant, we studied the dependence of the inhibition time on the concentration and the

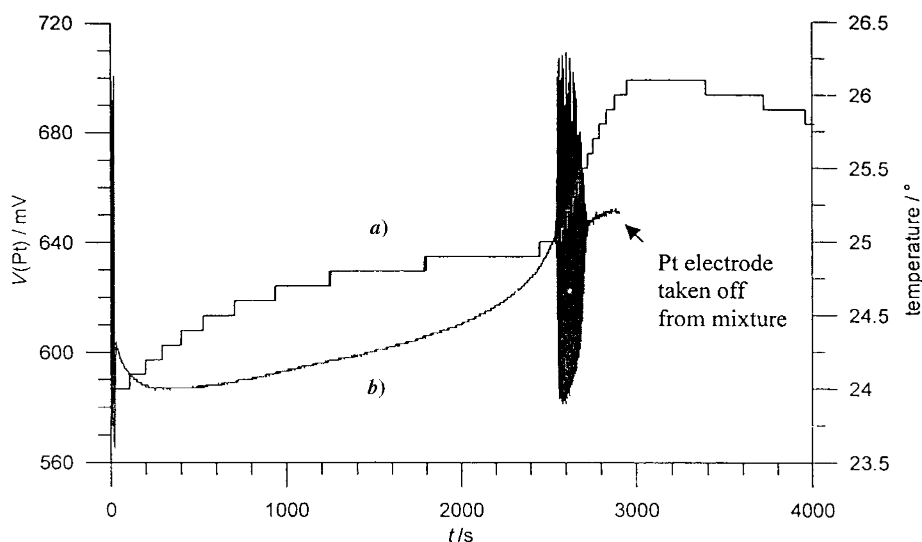


Fig. 5. *Simultaneous recordings of* a) *the potential of the bright platinum electrode and* b) *the temperature* vs. *time when 1.0 ml of a solution of ferulic acid* ($c = 0.04$ mg/ml) *was added to 30 ml of a thermostated* BR *mixture.* Temperature of the thermostatic bath, $25.0 \pm 0.1°$. Initial conditions: the same as in *Fig. 2*.

dependence of the inhibition time on the mean temp. of the inhibition phase. As an example, the graph t_{inhib} *vs.* concentration for caffeic acid is reported in *Fig. 7,b*[1]).

4. Relative-Activity Calculations. – The linear dependence of the t_{inhib} *vs.* concentration for all the substances studied are shown in *Fig. 8*. Below a certain concentration of antioxidant added (different for each antioxidant), the behaviour deviates from linearity. In fact, at low concentrations of antioxidant added, the inhibition times become too low to be measured, as shown in *Fig. 3* of [4]. There is a threshold under which inhibition time cannot be detected. We believe that, under these lower limits, the straight lines curve towards 0. As can be seen from *Fig. 8*, the slopes of the straight lines are different, so the calculation of the relative antioxidant activity will depend on the substance chosen as a standard and the concentration of the sample. The parameters of the straight lines together with the *R*-squared values are reported in *Table 1.*

It is possible that the antioxidants undergo also other reactions like oxidation or iodination, but we believe that the scavenging of the $HOO^{\bullet}$ radicals is the main reason for the inhibition of oscillations under the described experimental conditions.

We calculated the relative antioxidant activity in three ways:

i) Relative activity with respect to concentrations (*r.a.c.*): From the straight-line equation of a substance chosen as standard, the concentration of the standard that should give the same inhibition time of the sample was calculated. The ratio between this value and the concentration of the sample gives the relative activity; *i.e.*

$$r.a.c. = [\text{std}]/[\text{smp}]$$

[1]) For all the antioxidants studied, these graphs are available from the authors upon request.

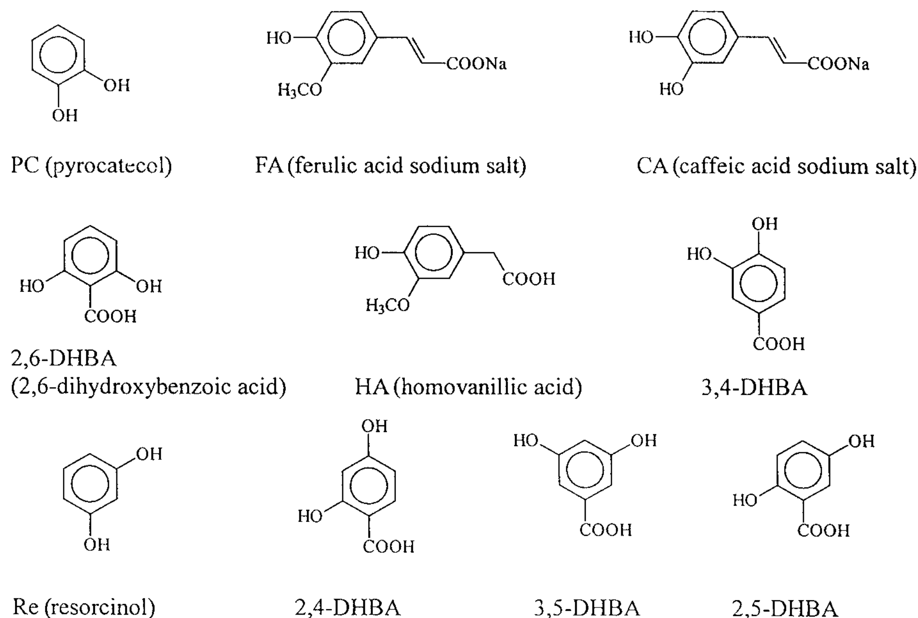


Fig. 6. *Structural formulae of the ten antioxidants studied*

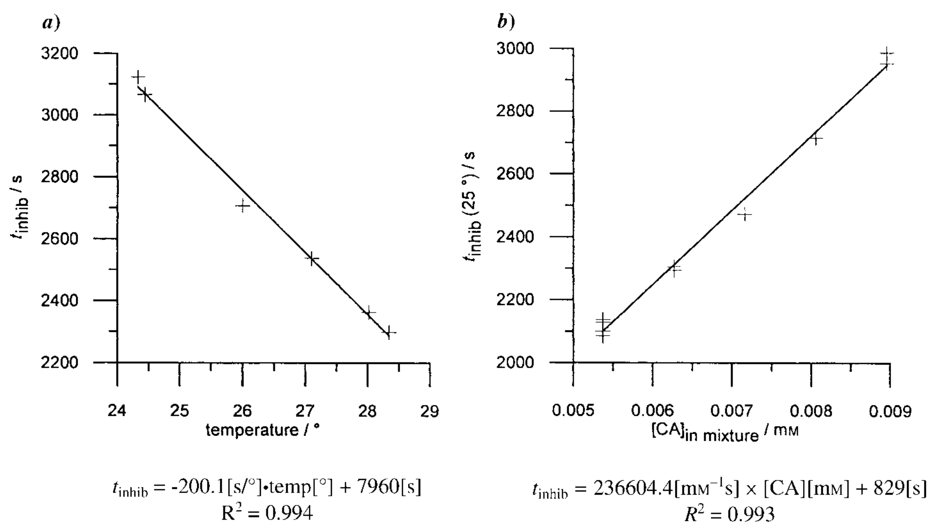


Fig. 7. a) *Straight line of* t_{inhib} vs. *mean temperature during the inhibitory phase for caffeic acid* ($[CA] = 8.95 \cdot 10^{-3}$ mM). b) *Straight line of* t_{inhib} vs. *concentration of caffeic acid* (in mM). The values of t_{inhib} are corrected at 25.0° with the equation for the temperature dependence (see *a*)).

where [smp] is the concentration of the sample added to the mixture and [std] is the concentration of the standard that should give the same inhibition time. Resorcinol was chosen as standard because the concentration intervals explored for almost all other

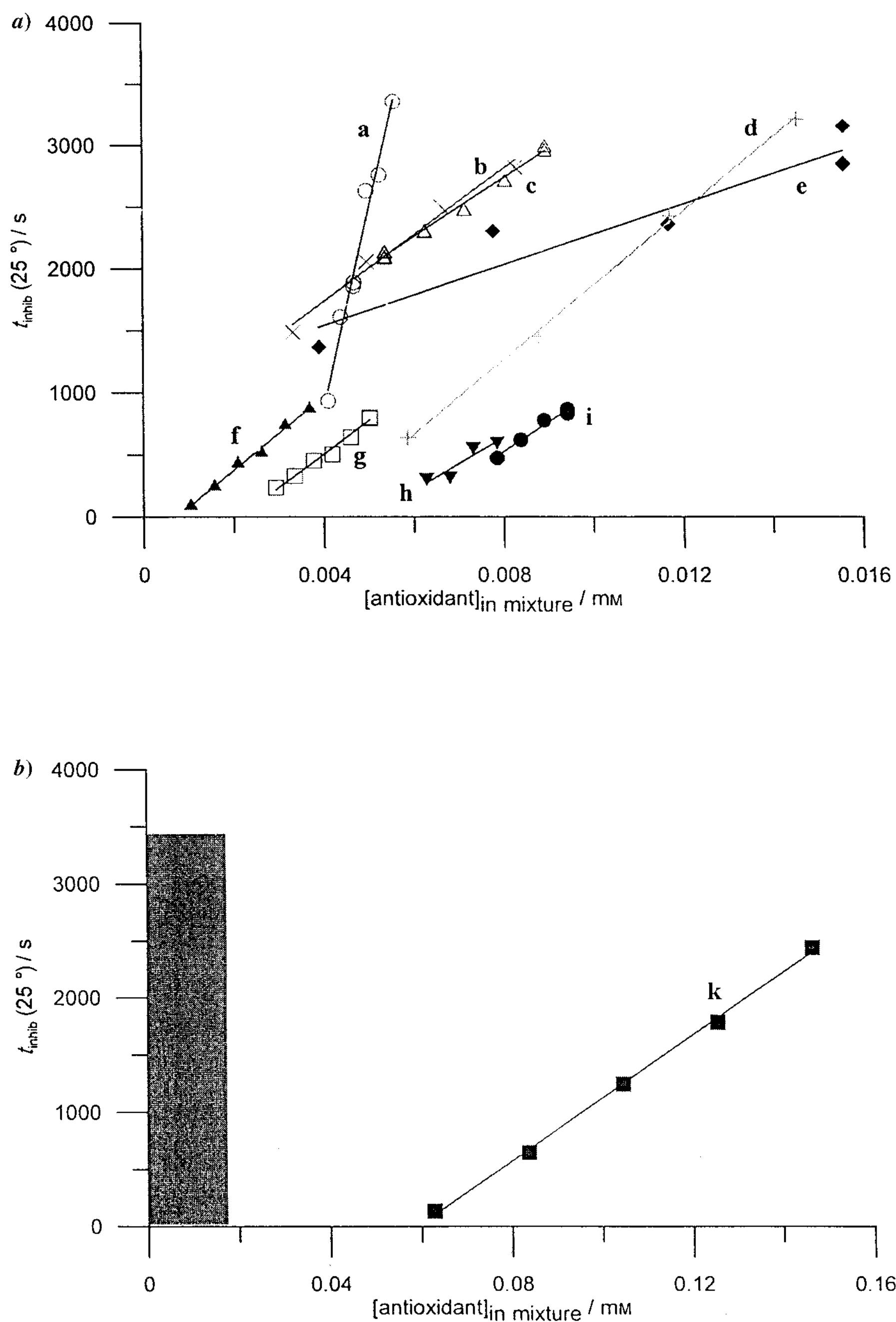


Fig. 8. a) *Straight line of* t_{inhib} vs. *concentration* (in mM) *for pyrocatecol (a), ferulic acid (b), caffeic acid (c), resorcinol (d), homovanillic acid (e), 2,6-DHBA (f), 3,4-DHBA (g), 2,4-DHBA (h), and 3,5-DHBA (i).* b) *Straight line of* t_{inhib} vs. *concentration* (in mM) *for 2,5-DHBA (k).* The rectangle shows the plane portion in which fall the straight lines reported in a).

Table 1. *Parameters of the Straight-Line Equations* ($t_{inhib} = m \cdot [antioxidant] + q$) *and* R-*Squared Values*

Antioxidant	Straight line	m [mM^{-1} s]	q [s]	R^2
2,4-DHBA	h	213823	– 1071	0.8752
2,5-DHBA	k	27495	– 1628	0.9983
2,6-DHBA	f	297695	– 220	0.9929
3,4-DHBA	g	269469	– 573	0.9864
3,5-DHBA	i	236036	– 1362	0.9772
caffeic acid	c	236604	829	0.9934
ferulic acid	b	267055	663	0.9838
homovanillic acid	e	122092	1050	0.9039
pyrocatechol	a	1586532	– 5482	0.9754
resorcinol	d	299084	– 1131	0.9986

antioxidants fall into the concentration interval explored for resorcinol. The obtained *r.a.c.* values are reported in the third column of *Table 2*. Quoted errors were calculated by the procedure suggested by *Harris* [18]. As can be seen, in some cases (2,4-, 2,5-, and

Table 2. *Relative Activities with Respect to Concentrations, Slopes, and Inhibition Times*

Antioxidant	*r.a.c.* [a])		*r.a.s.* [b])	*r.a.t.* [c]) (conc. = 0.00785 mM)
	mM in mixture	activity		
2,4-DHBA	0.00682	0.71 ± 0.05	0.71 ± 0.19	0.50 ± 0.06
	0.00733	0.77 ± 0.05		
	0.00785	0.73 ± 0.05		
2,5-DHBA	0.06279	0.07 ± 0.01	0.092 ± 0.003	0.00 [d])
	0.10465	0.076 ± 0.004		
	0.14652	0.082 ± 0.003		
2,6-DHBA	0.00262	2.11 ± 0.14	1.00 ± 0.05	1.74 ± 0.08 [e])
	0.00314	1.99 ± 0.12		
	0.00366	1.83 ± 0.11		
3,4-DHBA	0.00335	1.46 ± 0.11	0.90 ± 0.05	1.27 ± 0.06 [e])
	0.00419	1.31 ± 0.09		
	0.00502	1.28 ± 0.08		
3,5-DHBA	0.00837	0.70 ± 0.05	0.79 ± 0.06	0.40 ± 0.03
	0.00890	0.72 ± 0.04		
	0.00942	0.70 ± 0.04		
Caffeic acid	0.00627	1.83 ± 0.08	0.79 ± 0.03	2.21 ± 0.10
	0.00716	1.68 ± 0.07		
	0.00895	1.53 ± 0.06		
Ferulic acid	0.00332	2.63 ± 0.13	0.89 ± 0.08	2.27 ± 0.12
	0.00498	2.14 ± 0.09		
	0.00831	1.59 ± 0.06		
Homovanillic acid	0.00390	2.14 ± 0.14	0.41 ± 0.07	1.65 ± 0.19
	0.0117	1.00 ± 0.05		
	0.0156	0.92 ± 0.05		
Pyrocatechol	0.00410	1.68 ± 0.10	5.30 ± 0.40	5.73 ± 0.27 [e])
	0.00469	2.15 ± 0.10		
	0.00527	2.46 ± 0.10		
Resorcinol		1	1	1

[a]) Relative activity with respect to concentrations. [b]) Relative activity with respect to slopes. [c]) Relative activity with respect to inhibition times. [d]) The considered concentration is below the linear range. [e]) The considered concentration is above the explored interval; then, this value is calculated from the equation of the straight line.

3,5-DHBA), *r.a.c.* values are the same (within the experimental errors) for different concentrations, but, in other cases, noticeable differences can be marked. Differences will occur when slope and intercept of the standard line are different from those of the sample line.

ii) Relative activity with respect to slopes (*r.a.s.*): This is simply the ratio between the slope of the straight line of the sample and that of the standard; *i.e.*

$$r.a.s. = \text{slope(smp)}/\text{slope(std)}.$$

The obtained *r.a.s.* values are reported in the fourth column of *Table 2*. The method is useful for comparison of the effect of *changes* in sample concentration with the effect of *changes* in the reference concentration, within the linear ranges.

iii) Relative activity with respect to inhibition times (*r.a.t.*): This is the ratio between the inhibition time of the sample and that of the standard at the same concentration; *i.e.*

$$r.a.t. = t_{\text{inhib}}(\text{smp})/t_{\text{inhib}}(\text{std}).$$

The chosen concentration must be specified together with the *r.a.t.* values. The concentration to choose for a *r.a.t.* calculation must be in the linear concentration range of the standard and of all the examined substances. The obtained *r.a.t.* values at concentration 0.00785 mM are reported in the last column of *Table 2*. Even if this method of relative-activity calculation has the same limitations as the *r.a.c.*, the advantage is that the activity is referred to a given specified concentration. On the basis of *r.a.t.* values, the order of antioxidant capacity of the studied substances is: pyrocatechol (5.73) $>$ ferulic acid (2.27) $\geq$ caffeic acid (2.21) $>$ 2,6-DHBA (1.74) $\geq$ homovanillic acid (1.65) $>$ 3,4-DHBA (1.27) $>$ resorcinol (1.00, std) $>$ 2,4-DHBA (0.50) $\geq$ 3,5-DHBA (0.40) $>$ 2,5-DHBA (*ca.* 0.0).

5. TEAC Measurements. – We studied also the dependence of the parameter used in the TEAC method on the concentration of our antioxidants, obtaining straight lines with different slopes. This means that also TEAC values depend on the concentration of the sample. As can be seen from the TEAC values in *Table 3*, there are differences both between the values reported in the literature and those measured in the present work. Our values come from a mean of different measurements over a wide range of concentration, while literature values are mean values of measurements at the same concentration.

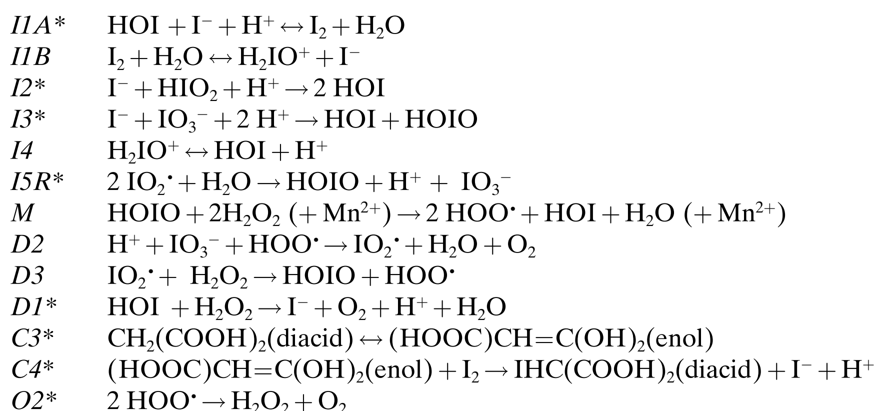
Table 3. *TEAC Values* [a])

Antioxidant	TEAC value
2,5-DHBA	1.01[b]), 1.04[c]), 0.78[d])
3,4-DHBA	0.99[b]), 1.19[c]), 0.87[d])
3,5-DHBA	2.15[c]), 0.66[d])
Caffeic acid	1.12[b]), 1.26[c]), 1.66[d])
Ferulic acid	1.70[b]), 1.90[c]), 2.07[d])
Homovanillic acid	1.72[c]), 1.34[d])

[a]) TEAC = Trolox equivalent antioxidant capacity. [b]) From [16]. [c]) From [2]. [d]) Present work.

6. Mechanistic Interpretation. – The mechanism of the *BR* reaction is quite complex; a first skeleton mechanism, proposed in 1982 by *Noyes* and *Furrow* (*NF* model) [19] was able to reproduce some of the basic features of the oscillations in the system. At the same time, *De Kepper* and *Epstein* developed a qualitatively similar mechanism (*DE* model) [20] that, taking into account flow terms, was able to model, besides oscillations, a variety of phenomena that appear in experiments performed in a continuous-flow stirred tank reactor. Subsequent attempts by *Furrow* to improve the *NF* skeleton mechanism have shown no major breakthrough in its prediction capabilities [21]. In 1996, *Sørensen* and co-workers [22] have presented a more detailed mechanism that well simulates a wide range of experimental results from flow and batch reactors. This mechanism, built starting from *NF* and *DE* models, contains their common fundamental steps. These fundamental steps have been briefly sketched in [4]. A principal-component analysis of the rate-sensitivity matrix of the *NF* model, made by *Turànyi* [23] showed that steps involving $HOO^{\bullet}$ radicals are mechanistically unimportant, so that $HOO^{\bullet}$ can be considered, in this model, as an end product not involved in propagation of radicals.

Very recently, *Furrow et al.* [24] have modified the original *NF* mechanism on the basis of experimental evidence of the important role played by $HOO^{\bullet}$ radicals in the oscillatory behavior of the *BR* reaction [4]. This new mechanism, now referred to as *FCA* model, has been qualitatively described in some detail in [4]. The reactions are the following:



*I1A** $HOI + I^- + H^+ \leftrightarrow I_2 + H_2O$
I1B $I_2 + H_2O \leftrightarrow H_2IO^+ + I^-$
*I2** $I^- + HIO_2 + H^+ \rightarrow 2\,HOI$
*I3** $I^- + IO_3^- + 2\,H^+ \rightarrow HOI + HOIO$
I4 $H_2IO^+ \leftrightarrow HOI + H^+$
*I5R** $2\,IO_2^{\bullet} + H_2O \rightarrow HOIO + H^+ + IO_3^-$
M $HOIO + 2H_2O_2\ (+Mn^{2+}) \rightarrow 2\,HOO^{\bullet} + HOI + H_2O\ (+Mn^{2+})$
D2 $H^+ + IO_3^- + HOO^{\bullet} \rightarrow IO_2^{\bullet} + H_2O + O_2$
D3 $IO_2^{\bullet} + H_2O_2 \rightarrow HOIO + HOO^{\bullet}$
*D1** $HOI + H_2O_2 \rightarrow I^- + O_2 + H^+ + H_2O$
*C3** $CH_2(COOH)_2$(diacid) $\leftrightarrow$ (HOOC)CH=C$(OH)_2$(enol)
*C4** (HOOC)CH=C$(OH)_2$(enol) $+ I_2 \rightarrow$ IHC$(COOH)_2$(diacid) $+ I^- + H^+$
*O2** $2\,HOO^{\bullet} \rightarrow H_2O_2 + O_2$

The notation follows that used previously [19][21]. Those steps that are retained from the original *NF* model are marked with an asterisk.

With the *FCA* mechanism, *Furrow et al.* [24] obtained not only a better agreement between experimental results and simulations for malonic acid and its derivatives, but the new model is able to simulate oscillations in *BR* systems with substrates that are iodinated in a different way than malonyl derivatives, as crotonic and acrylic acids, anisole, and 4-nitrophenol [25]. To try to simulate inhibitory effects by an antioxidant on the oscillations, the following step was added to the model:

IN $ArOH + HOO^{\bullet} \rightarrow ArO^{\bullet} + H_2O_2$ ($ArO^{\bullet}$ decays to products)

where ArOH indicates the antioxidant.

Caffeic acid was chosen for the simulations. Rate constants used in the simulations are those reported in [24], except for that of the inhibitory step that was allowed to vary for the best fit with the experimental behavior. With just that step, the inhibition time is very sensitive to the amount of inhibitor (roughly, increasing the inhibitor by 10% leads to an increase in inhibition time by nearly 50%). So we added a second step, 1st-order degradation of the inhibitor to unspecified products:

DEG ArOH → Products

The degradation may be due, for example, to iodination or oxidation of ArOH. The concentration of caffeic acid is very low, and the rate constant for step *IN* is getting very large in the simulations. Taking into account that caffeic acid is bifunctional and the effective concentration might be greater than the actual concentration, we increased the initial concentration in the simulations obtaining a reasonable value of k_{IN}. Experimental and simulated behaviors of log $[I^-]$ *vs.* time for a typical inhibited mixture are reported in *Fig. 9*. The very good agreement between the experimental and calculated inhibition time can be noted. The same agreement was obtained varying the initial concentration of caffeic acid. This is another strong indication of the capability of the *FCA* mechanism to account for the important role played by hydroperoxyl radicals in the reaction.

Diphenols are also subject to oxidation by other means. In particular, 2,5-DHBA, caffeic acid, ferulic acid, and 2,6-DHBA are all oxidized to quinones by acidic iodate at rates differing by more than two orders of magnitude, fastest to slowest in the order listed. We observed increased absorbance near 420 nm (quinone peak), decreased absorbance near 310–330 nm (diphenol peak). Since *m*-quinones are unknown, the slowness of oxidation of 2,6-DHBA is not unexpected, and the MeO group of ferulic acid would be much slower to oxidize than the comparable OH group of caffeic acid. The iodate is reduced at least to I(I), and that is known to undergo electrophilic substitution rather quickly in phenolic systems. Also diphenols decolorize I_2 solutions. Thus, some of the diphenols must be iodinated before becoming oxidized to quinones.

We delayed addition of H_2O_2 to inhibited systems with the four antioxidants above, allowing some time for the acidic iodate diphenol reaction. The delayed addition of H_2O_2 did not alter the principle effect of inhibition followed by oscillations.

We have observed that a simple quinone, *p*-benzoquinone, will also incorporate an I-atom into its ring, and can inhibit I_2 production in the subsystem: acid, *p*-benzoquinone, iodate, manganous ion, hydrogen peroxide. One means of inhibition could be the reduction of quinone by $HOO^•$ radicals, which are thermodynamically good reducing agents. The reduction potential of $HOO^•$ radicals to H_2O_2 is 1.5 V. The potential for H_2O_2 to $HO^•$ radicals is 0.72 V. H_2O_2 is, therefore, a mild one-electron oxidizing agent, and a very weak one-electron reducing agent. $HOO^•$ Radicals have the potential to be rather strong oxidizing and reducing agents.

We have also observed that, in solutions containing caffeic acid or 2,5-DHBA and acid iodate, the characteristic yellow quinone color develops, then, on delayed addition of H_2O_2, the quinone colour above 400 nm gradually decreases as the absorbance at *ca.* 300 nm increases. There is some means of reduction of the quinone back to phenols, which are better inhibitors than quinones. Eventually, through coupling or other degradative processes in the phenol/quinone system, the concentration of inhibitors decreases to the point where $HOO^•$ radicals can multiply and oscillations resume. A

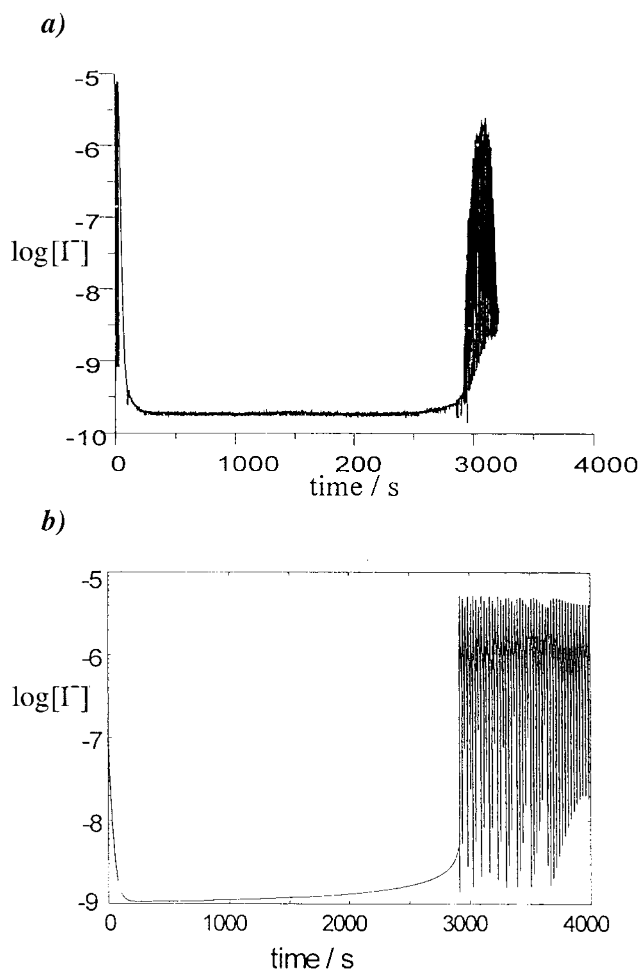


Fig. 9. a) *Experimental behavior of log [I⁻]* vs. *time for an inhibited* BR *mixture* (initial conditions: $[H_2O_2] = 1.20$M, $[HClO_4] = 0.0266$M, $[IO_3^-] = 0.0667$M, $[MA] = 0.050$M, $[Mn^{2+}] = 0.0067$M, 1.0 ml of caffeic acid ($c = 0.03$ mg/ml) added after the third oscillation). b) *Simulated behavior of log [I⁻]* vs. *time for a mixture of the same composition but with an 'effective' concentration of 0.056 mg of caffeic acid in 31 ml of mixture*; $k_{IN} = 1.0 \cdot 10^8$ M^{-1}s^{-1}, $k_{DEG} = 8.0 \cdot 10^4$ s^{-1}

detailed investigation of these complexities will be the subject of a further work. In any case, the scavenging action of HOO$^\bullet$ radicals by the diphenols is the main reason for the inhibition of oscillations.

7. Conclusion and Remarks. – From the experimental results and data treatment presented here, the oscillating *BR* reaction is suitable as an analytical method to measure relative activities of antioxidants. In fact, the reaction produces hydroperoxyl radicals that are quenched by antioxidants, so that the oscillations stop and restart after a period. These inhibition times at equal concentrations depend on the free-radical-

scavenging power of the antioxidants. Moreover, the inhibition time shows linear dependence on the antioxidant concentration over a wide range of concentrations.

The relative activity calculation methods based on the *BR* reaction are substantially the same as those used in other analytical methods, for example by the TEAC method [15]. Also the limits of the relative activity values are the same and have been discussed in detail here. Our method works for H_2O-soluble antioxidants in acidic medium at a pH value (*ca.* 2) that is similar to that of the fluids in the human stomach. Some other methods work at pH values near to the physiological value (*ca.* 7), and others at higher pH values. Since the antioxidant capacity of several substances depends on the pH, it is difficult to compare relative activities at different acidities.

Recently, a modified TEAC method that recognized the necessity to perform measurements at different antioxidant concentrations has been published [26]. This modified method is also suitable for lipophilic antioxidants. We are now studying the inhibitory effects by lipophilic antioxidants on the oscillations of the *BR* reaction in mixed organic-aqueous medium. Indeed the method described here is inexpensive: reagents and apparatus are commonly used in all chemical laboratories.

Finally the insertion of inhibitory steps in the *FCA* mechanism leads to simulated behaviours in very good agreement with the experimental ones. Further investigations in this direction could permit calculation of reliable values of the rate constants of the inhibitory reaction for different antioxidants. These kinetic constants would be, in fact, the true measure of the antioxidant power, the relative acitivities being only useful indicators of this capacity.

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