Antioxidant Activity of Diatomic Phenols

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Abstract—Nine compounds are studied for antioxidant activity, including those from the class of catechol-amines containing 3,4-hydroxyphenyl (catechol) as a common structural fragment, which imparts antioxidant properties to the compounds in the reactions of hydrocarbon substrate oxidation. The antiradical activity is determined by the chemiluminescent method by the interception of peroxyl radicals in the model reaction of the initiated oxidation of ethylbenzene (RH). The mechanism of the inhibition of chain oxidation processes by diatomic phenol compounds is provided by the presence of two active hydroxy groups with a possible intramolecular hydrogen bond, leading to a weakening of the O−H bond and a high rate constant of hydrogen abstraction in the reaction with peroxyl radicals (k_{inh}). This reaction is dominant and determines the inhibitory activity of antioxidants in oxidation processes. The maximum inhibitory activity is shown by 3,5- and 3,6-di-*tert*-butylpyrocatechins, dopamine, and epicatechin.

INTRODUCTION

Diatomic phenols containing OH groups in the *ortho*-position are widespread in wildlife. Fragments of pyrocatechol are present in many flavonoids. Such compounds play an important role in inhibiting unwanted oxidative processes in living systems, protecting them from the effects of oxidative stress. There is substantial interest in studying polyphenols due to the ability of these compounds to reduce the risk of atherosclerosis, cancer, and cardiovascular diseases. Until the 1990s, there was practically no information in the literature on the antioxidant and biological properties of sterically complicated diatomic phenols of the catecholamine group. Recently, a great deal of attention has been paid to determine the antioxidant activity (AOA) of these compounds. A variety of approaches are used in the research to assess the AOA. However, the results of many works seem to be ambiguous. The AOA of natural compounds and their analogs is most often studied in heterogeneous systems that mimic the structure of a cell of a living organism: micelles and liposomes. However, the AOA determined under these conditions depends on various factors. In order to exclude the influence of these factors, it is necessary to carry out studies on the oxidation of model systems in a homogeneous system. The study of the AOA of polyphenols upon inhibition of oxidation in solution seems to be relevant.

The search for drugs among compounds of diatomic phenols and their derivatives seems to be one of the most promising ways to create new drugs for the treatment of diseases such as polyneuropathy of various etiologies, Parkinson's disease, Alzheimer's, and complications after a disease caused by ther coronavirus infection COVID-19.

Catecholamines are a group of biogenic amines containing 3,4-dihydroxyphenol (catechol) as a common structural fragment, which gives these compounds the status of antioxidants in the oxidation of organic compounds. Catecholamines are water-soluble compounds that function as natural neurotransmitters in living organisms (adrenaline, norepinephrine, dopamine) [1−6]. This circumstance raised the problem of determining the key kinetic characteristics of the antioxidant action and developing convenient and reliable instrumental methods for their determination.

To study the kinetics of oxidative processes, including those involving antioxidants, it is most convenient to use chemiluminescent methods [7−15]. In this study, we consider the possibilities of using the chemiluminescent method based on measuring the intensity of chemiluminescence (CL) accompanying the initiated oxidation of hydrocarbons to obtain quantitative characteristics of the antiradical activity of diatomic phenols: the rate constants of the reaction of a peroxide radical with an inhibitor molecule (k_{inh}) and stoichiometric inhibition coefficient (f). The structural formulas of the compounds studied in this work are shown in Fig. 1.
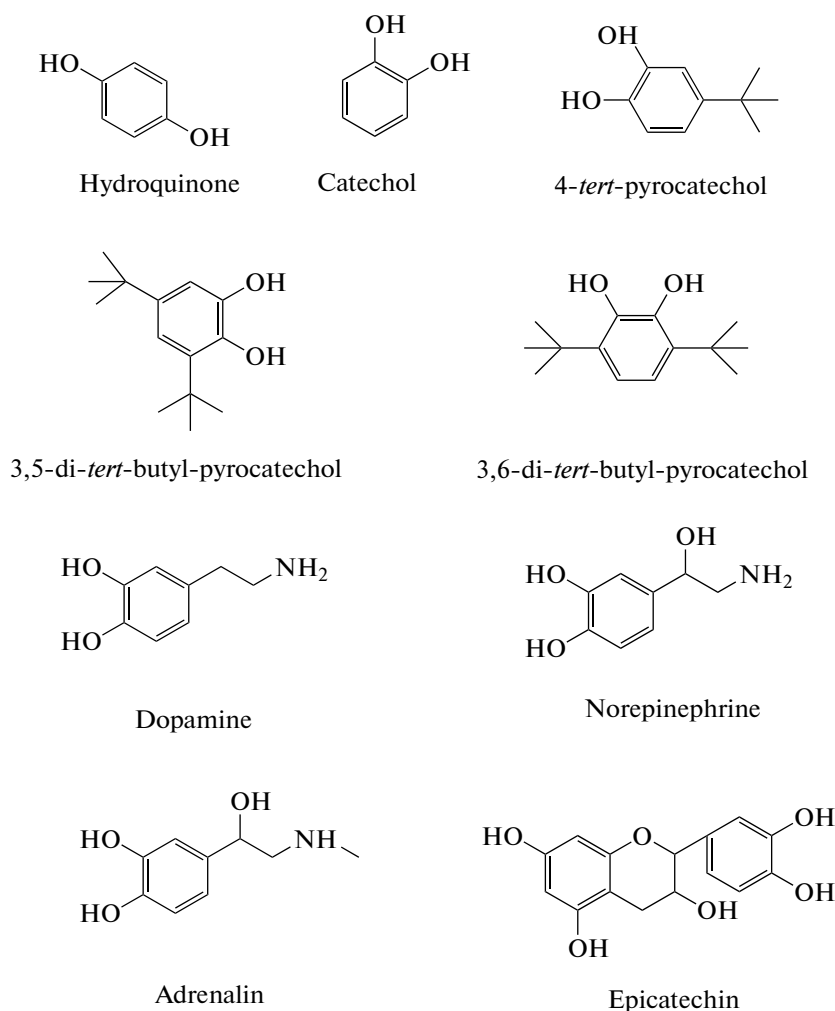
Fig. 1. Structural formulas of the studied diatomic phenols.

EXPERIMENTAL

In this study we investigated five compounds of diatomic phenols, homologs of pyrocatechol, synthesized and provided by the laboratory for the synthesis of complicated phenols (Institute of Biochemical Physics, Russian Academy of Sciences (IBCP RAS)), and four compounds of a number of natural catecholamines (Fluka) for antiradical activity in relation to peroxyl radicals leading the oxidation chain. The antiradical properties of diatomic phenols were investigated by the effect of inhibition of the liquid-phase oxidation of ethylbenzene (RH), initiated by the thermal decomposition of the initiator 2,2'-azobisisobutyronitrile (AIBN). The initiator was recrystallized twice from ethanol, followed by drying in a vacuum to a constant weight. The solvent chlorobenzene (Merck) and the model hydrocarbon ethylbenzene (Aldrich, 99.8%) were used without preliminary purification. The oxidizing reaction mixture (5 mL) was placed in a chemiluminometer cuvette thermostated at 50°C and saturated with oxygen by bubbling air with an injection compressor. Weak primary luminescence (triplet-singlet emission of light by an excited product, acetophenone) was enhanced by transferring energy to an effective phosphor, 9,10-dibromoanthracene (DBA), and recorded on a chemiluminometer with an H7467 photosensor module (Hamamatsu, Japan) with an RS-232C interface.

The initiated oxidation rate, W_i, was calculated using the ratio

$$W_i = 2 f_{cell} k_0 \left[AIBN \right]$$
$$= 1.2 \times 1.58 \times 10^{15} \exp(-30800/RT)\left[AIBN \right], \quad (1)$$

where f_{cl} is the exit of radicals from the cell ($f_{cl} = 0.6$), and k_0 is the rate constant of the decomposition of the initiator, which is practically independent of the nature of the solvent [16−21].

The initiation rate, W_i, was additionally monitored and was measured directly in the reaction mixture before and after the experiment on the CL kinetics after the introduction of a known amount of the stan-

Table 1. The parameters of inhibition of the chemiluminescent process of ethylbenzene oxidation by antioxidants obtained by processing the data in Fig. 3

Curve no. in Fig. 3	Antioxidant	[AO], M	f	k_{inh}, M^{-1} s^{-1}	W_i (M s^{-1}) on $\tau_{0.5}$
1	α-naphthol	1.96×10^{-6}	2.0	3.6×10^5	6.54×10^{-9}
2	Hydroquinone	9.77×10^{-6}	2.0	5.5×10^5	6.78×10^{-9}
3	Dopamine	1.44×10^{-5}	1.9	1.5×10^5	5.88×10^{-9}
4	Ionol	1.48×10^{-5}	2.0	2.4×10^4	5.88×10^{-9}

dard chroman inhibitor (CrC$_1$), 6-hydroxy-2,2,5,7,8-pentamethylchroman, which also made it possible to record the effect of oxidation products on the oxidation rate (W_{O_2}).
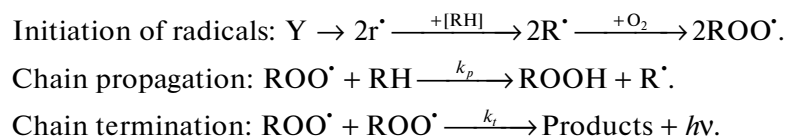
The main problem arising in the study of the AOA of polyphenols in a nonpolar medium is related to their extremely low solubility under these conditions. To prepare working solutions of polyphenols, it is necessary to use additives of polar solvents. However, the polarity of the medium can have a significant effect on the activity of inhibitors. The reason for this effect is the formation of a hydrogen bond between the OH group of phenol and the solvent. This leads to a complication of the mechanism of the action of antioxidants and an additional error in the measurement of elementary inhibition constants. Therefore, an attempt was made to select a solvent that would combine good solubility of the compounds under study and the minimal effect on the measurement parameters. A suitable solvent was found to be dimethyl sulfoxide (DMSO) in combination with acetonitrile

(ACN). First, the compound was dissolved in DMSO to a concentration of 1×10^{-3} M, then a diluted base solution in acetonitrile (1×10^{-4} M) was prepared by dilution by a factor of 10. DMSO and ACN in the control measurements did not affect the initiation rate in the oxidation of hydrocarbons; therefore, subsequently, solutions of the inhibitors under study were prepared using DMSO and ACN.

The effective antiradical activity was measured by the chemiluminescent method, which records the CL intensity accompanying the initiated oxidation of hydrocarbon (RH) at the known initiation rate (W_i). With the introduction of an inhibitor, the oxidation rate decreases, and the CL intensity changes proportionally, which is then analyzed [17].

The oxidation of hydrocarbons and lipids (RH) develops according to the free-radical chain mechanism, the key stages of which are initiation, continuation, and termination of the chain [18−21]:

Scheme 1

Initiation of radicals: $Y \rightarrow 2r^{\bullet} \xrightarrow{+[RH]} 2R^{\bullet} \xrightarrow{+O_2} 2ROO^{\bullet}$.

Chain propagation: $ROO^{\bullet} + RH \xrightarrow{k_p} ROOH + R^{\bullet}$.

Chain termination: $ROO^{\bullet} + ROO^{\bullet} \xrightarrow{k_t} Products + h\nu$.

Here $R^{\bullet}$ and $RO_2^{\bullet}$ are free radicals, Y is the initiator, and ROOH is hydroperoxide, the primary oxidation product. At moderate temperatures (40–60°C), low conversion levels, and a constant oxidation rate, the formed hydroperoxides do not affect chain initiation and are neutral oxidation products, and the reaction itself proceeds in the oxidation mode with degenerate branching [21−23].

The stationary concentration $RO_2^{\bullet}$ in a chain process is

$$\left[RO_2^{\bullet} \right] = \left(W_i / 2k_t \right)^{1/2}, \quad (2)$$

and the oxidation rate is described by the equation

$$W_{O_2} = \frac{k_p}{2k_t} [RH] (W_i)^{1/2}. \quad (3)$$

It was found that in the processes of the oxidation of hydrocarbons, the source of CL is the exothermic reaction of the recombination of the peroxyl radicals $\left(RO_2^{\bullet} \right)$, passing through the formation of an intermediate tetroxide followed by decomposition into products [19, 22]:

$$ROO^{\bullet} + ROO^{\bullet} \leftrightarrows ROOOOR$$

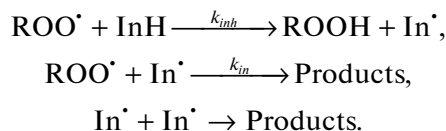$$\rightarrow (1 - \eta) P + \eta P^* + M + O_2 \uparrow.$$
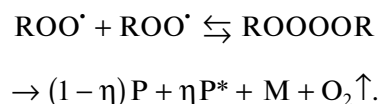
In this reaction, an energy of 100 to 120 kcal /mol is usually released, and in each such elementary act, one molecule of the product, a carbonyl compound P* (in the case of ethylbenzene oxidation, it is acetophenone) is excited, which emits a CL light quantum. The

speed of this reaction $W_i = 2k_t[RO_2^\bullet]^2$. Consequently, the measurement of the CL intensity can be used as a convenient kinetic method, since it is proportional to $k_t[RO_2^\bullet]^2$.

A measure of the antioxidant activity of the inhibitors can be the degree of quenching of CL by them. In the presence of inhibitors (InH), the chain termination rate increases due to the participation of the inhibitor (InH) in reactions with peroxyl radicals:

Scheme 2

$$ROO^\bullet + InH \xrightarrow{k_{inh}} ROOH + In^\bullet,$$

$$ROO^\bullet + In^\bullet \xrightarrow{k_{in}} Products,$$

$$In^\bullet + In^\bullet \rightarrow Products.$$

The straight chain termination reactions on the inhibitor lead to a decrease in the concentration $RO_2^\bullet$ and the rate of inhibited oxidation, which in the simplest case, not complicated by the side reactions involving an inhibitor, is determined by the relation

$$W_{O_2} = \frac{k_p[RH]W_i}{k_{inh}f[InH]}, \tag{4}$$

where f is the stoichiometric inhibition coefficient showing the number of chain breaks per [InH] molecule (usually $1 < f < 2$).

For pyrocatechol derivatives, the mechanism Scheme 3 of interaction with peroxyl radicals is presented as follows [23, 24]:

Scheme 3
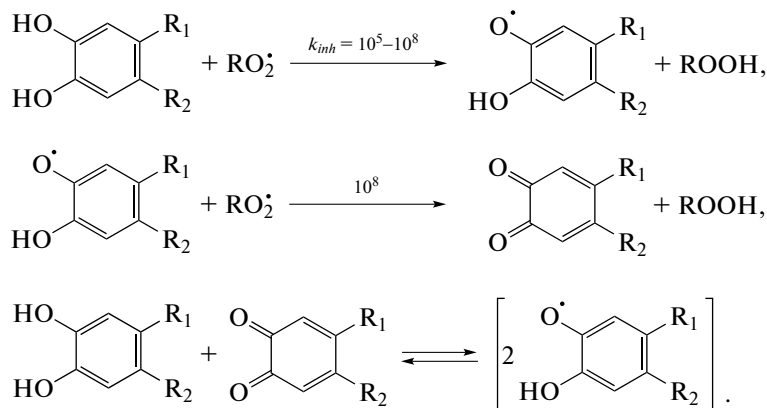
The end product of the reaction is a quinone, which does not interact with peroxyl radicals (it reacts only with alkyl radicals), but can be reduced to an inhibitory radical when interacting with the starting catechol compound. The stoichiometric coefficient of inhibition is $f = 2$.

When studying the kinetics of oxidation, it is convenient to use not absolute (I) but relative (i) values of the CL intensity determined by the relation

$$i = I/I_0 = \left[RO_2^\bullet\right]^2 \Big/ \left[RO_2^\bullet\right]_0^2 = k_t\left[RO_2^\bullet\right]^2 \Big/ W_i, \tag{5}$$

where I is the current value of the absolute intensity and I_0 is the intensity value in the absence of the inhibitor.

The area of the CL light sum above the kinetic curve of CL quenching by the inhibitor shows how many peroxyl radicals died in the inhibitor from the moment of administration until its complete consumption, and is a quantitative characteristic of the stoichiometric coefficient of inhibition f independent of k_{inh}.

The role of oxidation products was minimized by using a low initiation rate $W_i \sim 10^{-9}$ M s^{-1}, which was additionally controlled by measuring it directly in the reaction mixture before and after the experiment on the kinetics of CL quenching with the standard inhibitor, a synthetic analog of α-tocopherol–chroman (CrC$_1$) in accordance with the relation

$$W_i = f[InH]_0 \big/ \tau_{0.5}, \tag{6}$$

in which for chroman $f = 2$, $[InH]_0$ is the concentration of the inhibitor, $\tau_{0.5}$ is the deceleration period in seconds from the moment that chroman is introduced until the glow intensity reaches the level of $0.5I_0$ as the antioxidant is consumed.
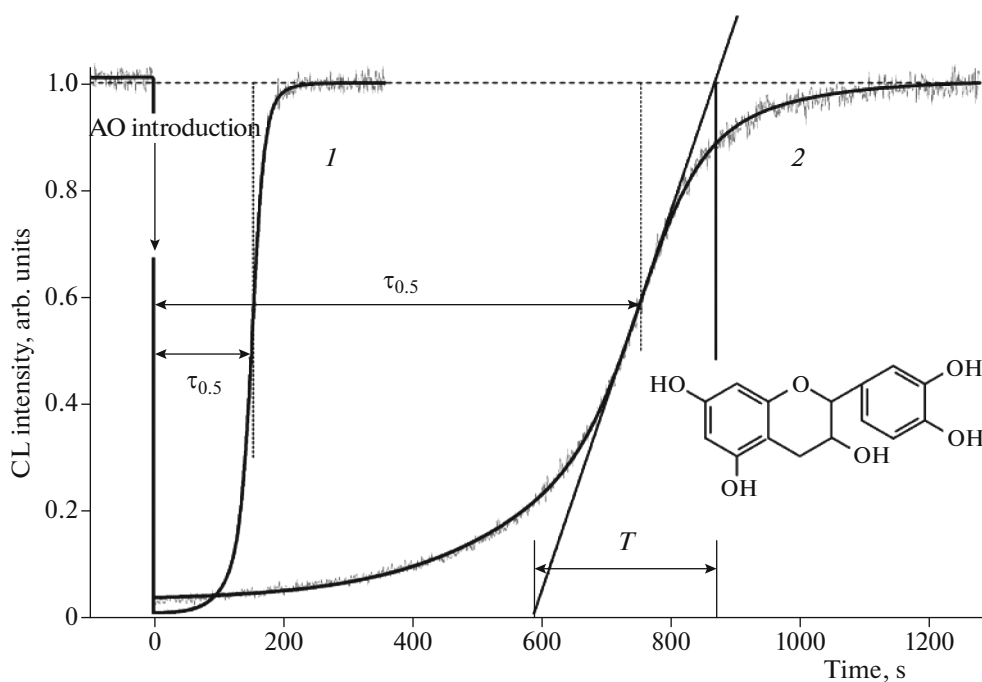
Fig. 2. Kinetic curves of CL quenching during the oxidation of ethylbenzene (20% solution in chlorobenzene, $W_i = 4.8 \times 10^{-9}$ M s^{-1}, [DBA] $= 2.0 \times 10^{-4}$ M, 50°C) after the introduction of antioxidants: *1*, chroman ([CrC$_1$] $= 3.8 \times 10^{-7}$ M); *2*, epicatechin ([EPC] $= 4 \times 10^{-6}$ M). The arrows on the kinetic curves indicate the braking periods $\tau_{0.5}$ and the inverse slope of the kinetic curve of the CL intensity recovery ($T = 1/\tan\varphi$, where φ is the slope of the curve at the inflection point).

RESULTS AND DISCUSSION

Figure 2 shows the kinetic curves of CL quenching in the process of ethylbenzene oxidation by additions of chroman (CrC$_1$) and a sample of catecholamine (epicatechin). As the antioxidant is consumed, the CL intensity is restored according to the S-shaped symmetric curve determined by the rate constant (k_{inh}) of the reaction of an antioxidant with a peroxide radical, i.e., the individual kinetic characteristics of the antioxidant. The maximum slope of the luminescence recovery curve CL $i(t)$ at the inflection point is defined as

$$(di/dt)_{max} = 1/T = 0.273 W_i^{1/2} k_{inh}/(2k_t)^{1/2}. \quad (7)$$

From expression (7), the calculated relation for k_{inh}

$$(k_{inh})\big|_{\tan\varphi} = \frac{(2k_t)^{1/2}\tan\varphi}{0.273 W_i^{1/2}}. \quad (8)$$

Figure 3 shows four characteristic kinetic curves of CL quenching after the introduction of the studied antioxidants into the model reaction of ethylbenzene oxidation. The results of measuring the parameters of inhibition are summarized in Table 1 together with the experimental conditions.
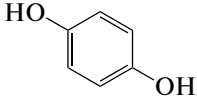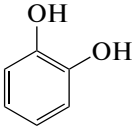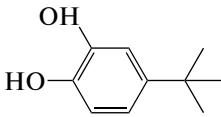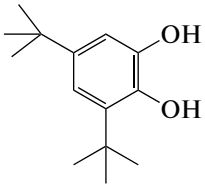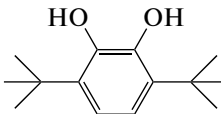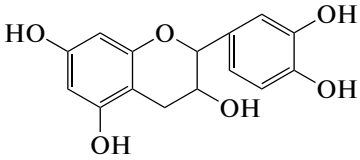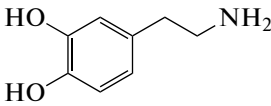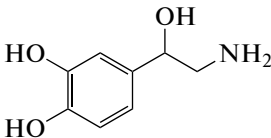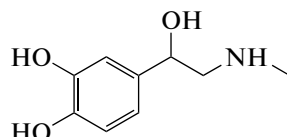
As we can see from Table 1, the initiation rates for all AO are similar, which made it possible to combine them on one graph (Fig. 3).

Table 2 shows the results of determining the parameters of the antioxidant activity of diatomic phenols: the rate constants of interaction with peroxyl radicals k_{inh} and the stoichiometric inhibition coefficients f when using relations (8) and (6), respectively. For the data presented in Tables 1 and 2, the measurement error was ≤15%.

The presence of two phenolic OH groups in adjacent positions in the structure of the pyrocatechol fragment leads to the formation of an intramolecular hydrogen bond, due to which one of the bonds of the hydroxy group is weakened. This leads to a sharp increase in the rate constant of the interaction with peroxyl radicals in comparison with phenol and other diatomic phenols. It was shown that the mentioned property is preserved for di-*tert*-butyl-substituted pyrocatechols. Thus, the 3,5- and 3,6-di-*tert*-butylpyrocatechols in the rate constant of the interaction with peroxyl radicals significantly exceed the di-*tert*-substituted phenols and are similar in efficiency to αα-tocopherol [23–27]. In this case, the stoichiometric coefficient of inhibition $f = 2$.

A feature of pyrocatechins is the low stability of the products formed in the process of inhibition of the *ortho*-quinones, which determines their high level of activity with respect to alkyl radicals when measuring the AOA of catecholamines in aqueous micellar substrates [27–31, 33].

Table 2. Parameters of inhibition of the chemiluminescent process of ethylbenzene oxidation by diatomic phenols

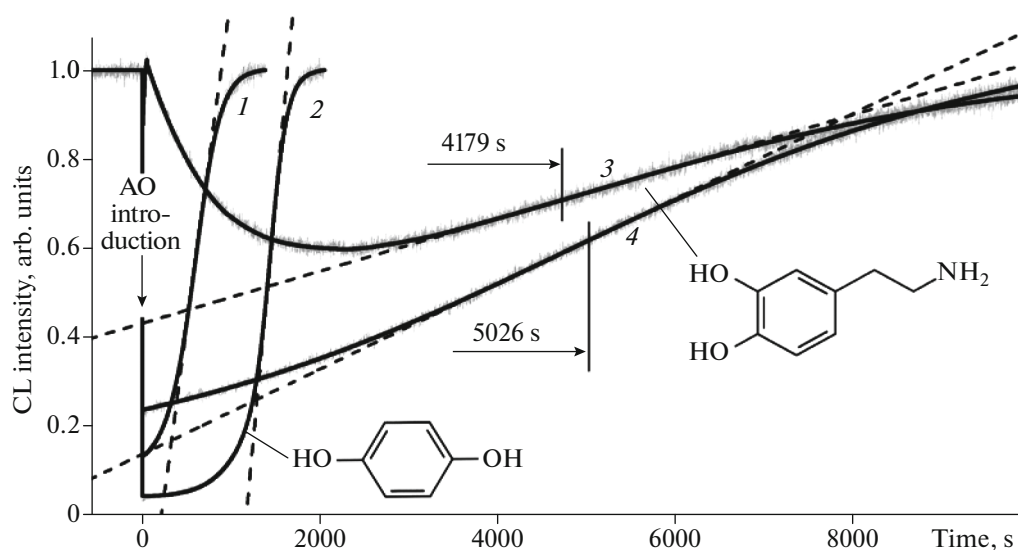| Structural formula	Name	k_{inh}, M^{-1} s^{-1}	f
	n-dihydroxybenzene (hydroquinone)	5.5×10^5	2.0
	o-dioxybenzene (catechol)	2.8×10^6	2.0
	4-$tert$-butyl pyrocatechol	4.0×10^6	2.1
	3,5-di-$tert$-butyl pyrocatechol	7.7×10^6	2.1
	3,6-di-$tert$-butyl pyrocatechol	6.8×10^6	1.8
	Epicatechin	7.0×10^5	2.0
	Dopamine	1.5×10^5	1.9
	Norepinephrine	2.4×10^3	0.5
	Adrenalin	4.9×10^3	1.6

Fig. 3. Kinetic curves of CL quenching during the oxidation of ethylbenzene (20% solution in chlorobenzene, $W_i = 4.8 \times 10^{-9}$ M s^{-1}, [DBA] $= 2.0 \times 10^{-4}$ M, 50°C) after the introduction of the studied antioxidants (1–4, see Table 1).

Thus, according to [32], the solubility of atmospheric oxygen in chlorobenzene at 50°C is 47 times higher than its concentration in the aqueous micellar substrate, which excludes the presence of a sufficient concentration of alkyl radicals to activate their reaction with *ortho*-quinone. Therefore, in our model reaction of the initiated oxidation of ethylbenzene, the interaction of catecholamines with alkyl radicals is not manifested. It is possible that the high level of activity of adrenaline in aqueous micellar media can manifest itself as a result of a more complex mechanism of the inhibition of the oxidation process and an increase in the stoichiometric coefficient due to the additional inhibition by oxidation products, in contrast to the model reaction of hydrocarbon oxidation [34–36].

CONCLUSIONS

A method is proposed for determining the true reactivity of diatomic phenols with respect to peroxyl radicals using a hydrocarbon–ethylbenzene model as a substrate for oxidation is proposed, based on which the parameters of the antioxidant activity (k_{inh} and f) are determined for nine compounds of catecholamines during the oxidation of ethylbenzene. The stoichiometric coefficient value $f = 2$, regardless of the number of hydroxyl groups.

It was found that diatomic phenols are strong antioxidants, comparable in the efficiency of inhibition of oxidation processes with α-tocopherol. The maximum inhibitory activity was shown by 3,5-di-*tert*-butyl pyrocatechol, 3,6-di-*tert*-butyl pyrocatechol, epicatechin, and dopamine.

REFERENCES

1. M. Laguerre, C. Bayrasy, A. Panya, et al., Crit. Rev. Food Sci. Nutr. **55**, 183 (2014).
2. O. S. Maldonado, R. Lucas, F. Comelles, et al., Tetrahedron **67**, 7268 (2011).
3. J. C. Ruiz-Ruiz, E. L. Ortiz-Vazquez, and M. R. Segura-Campos, Crit. Rev. Food Sci. Nutr. **57**, 1423 (2017).
4. C. Song, C. Shieh, Y. Wu, et al., Prog. Lipid Res. **62**, 41 (2016).
5. J. Koolman and K. Rohm, *Taschenatlas der Biochemie* (Thieme, Stuttgart, 2003).
6. T. A. Mikhas'ko, B. V. Dubovik, O. I. Shadyro, and G. I. Polozov, Med. Nov. (Minsk) **82** (4), 81 (2002).
7. G. F. Fedorova, V. A. Lapina, V. A. Menshov, et al., Photochem. Photobiol. **95**, 780 (2019).
8. R. F. Vasil'ev, A. V. Trofimov, and Yu. B. Tsaplev, Russ. Chem. Rev. **79**, 77 (2010).
9. Yu. B. Tsaplev and A. V. Trofimov, Russ. J. Phys. Chem. A **94**, 2369 (2020).
10. G. F. Fedorova, V. A. Menshov, and A. V. Trofimov, Photochem. Photobiol. **93**, 579 (2017).
11. Yu. B. Tsaplev, R. F. Vasil'ev, V. D. Kancheva, and A. V. Trofimov, Russ. J. Phys. Chem. B **14**, 431 (2020).
12. R. F. Vasil'ev, V. D. Kancheva, V. V. Naumov, A. K. Slavova-Kazakova, A. V. Trofimov, G. F. Fedorova, and O. I. Yablonskaya, Russ. J. Phys. Chem. B **14**, 479 (2020).
13. V. V. Naumov, G. F. Fedorova, R. F. Vasil'ev, V. D. Kancheva, T. L. Veprintsev, A. V. Trofimov, and

O. I. Yablonskaya, Russ. J. Phys. Chem. B **15**, 63 (2021).

14. V. A. Men'shov, V. D. Kancheva, O. I. Yablonskaya, and A. V. Trofimov, Russ. J. Phys. Chem. B **15**, 108 (2021).

15. V. D. Kancheva, M. A. Dettori, D. Fabbri, et al., Antioxidants **10**, 624 (2021).

16. N. M. Emanuel', E. T. Denisov, and Z. K. Maizus, *Chain Reactions of Liquid-Phase Oxidation of Hydrocarbons* (Nauka, Moscow, 1965) [in Russian].

17. V. Ya. Shlyapintokh, O. N. Karpukhin, L. M. Postnikov, I. V. Zakharov, A. A. Vichutinskii, and V. F. Tsepalov, *Chemiluminescence Methods of Investigation of Slow Chemical Processes* (Nauka, Moscow, 1966) [in Russian].

18. E. N. Frankel, *Lipid Oxidation,* 2nd ed. (Woodhead, Philadelphia, 2012), Chap. 1.

19. R. F. Vasil'ev and I. F. Rusina, Dokl. Akad. Nauk SSSR **156**, 1402 (1964).

20. I. F. Rusina, O. N. Karpukhin, and O. T. Kasaikina, Russ. J. Phys. Chem. B **7**, 463 (2013).

21. N. M. Emanuel' and D. Gal, *Oxidation of Ethylbenzene: A Model Reaction* (Nauka, Moscow, 1984) [in Russian].

22. G. F. Fedorova, A. V. Trofimov, R. F. Vasil'ev, and T. L. Veprintsev, ARKIVOC, No. **8**, 163 (2007).

23. N. A. Azatyan, G. V. Karpukhina, I. S. Belostotskaya, and N. L. Komissarova, Neftekhimiya, No. **3**, 435 (1973).

24. V. V. Ershov, G. A. Nikiforov, and A. A. Volod'kin, *Sterically Hindered Phenols* (Khimiya, Moscow, 1972) [in Russian].

25. I. V. Tikhonov, V. A. Roginskii, E. M. Pliss, and A. I. Rusakov, Bashkir. Khim. Zh. **13** (4), 112 (2006).

26. G.-C. Yen and C. L. Hsieh, Boisci. Biotechnol. Biochem. **61**, 1646 (1997).

27. S. E. Yagunov, S. V. Khol'shin, and N. V. Kandalintseva, Vestn. Novosib. Ped. Univ. **5** (15), 5 (2013).

28. I. P. Edimecheva, N. I. Ostrovskaya, G. I. Polozov, and O. I. Shadyro, Russ. J. Gen. Chem. **75**, 593 (2005).

29. M. B. Bel'kov, G. A. Ksendzova, P. V. Kuzovkov, G. I. Polozov, I. V. Skornyakov, V. L. Sorokin, G. B. Tolstorozhev, and O. I. Shadyro, J. Appl. Spectrosc. **74**, 635 (2007).

30. V. A. Roginskii, *Phenol Antioxidants: Reactivity and Efficiency* (Nauka, Moscow, 1988) [in Russian].

31. E. A. Mengele, I. G. Plashchina, and O. T. Kasaikina, Colloid. J. **73**, 815 (2011).

32. R. Battino, T. R. Rettich, and T. Tominaga, J. Phys. Chem. Ref. Data **12**, 163 (1983).

33. G. F. Fedorova, V. A. Menshov, A. V. Trofimov, et al., Analyst **134**, 2128 (2009).

34. R. F. Vasil'ev, V. D. Kancheva, G. F. Fedorova, D. I. Batovska, and A. V. Trofimov, Kinet. Catal. **51**, 510 (2010).

35. R. F. Vasil'ev, T. L. Veprintsev, L. S. Dolmatova, V. V. Naumov, A. V. Trofimov, and Yu. B. Tsaplev, Kinet. Catal. **55**, 148 (2014).

36. T. Shimizu, Y. Nakanishi, M. Nakahara, et al., J. Clin. Biochem. Nutr. **47**, 181 (2010).