Kinetics and Catalysis, Vol. 45, No. 4, 2004, pp. 488–496. Translated from Kinetika i Kataliz, Vol. 45, No. 4, 2004, pp. 519–527.

Interrelation between the Inhibiting Properties and the Activity of Phenoxy Radicals of Antioxidants Different in Structure

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Received January 15, 2003

Abstract—Efficiency of the inhibiting action of phenol-based antioxidants (AO) was studied in initiated oxidation of methyl oleate (MO). The following AO were examined: hydroxybenzene (I), tyrosol (4-2'-hydroxyethylphenol) (II), 2-*tert*-butyltyrosol (III), 2,6-di-*tert*-butyltyrosol (IV), 4-3'-hydroxypropylphenol (V), 2,6-di-*tert*-butylphenol (VI), dibunol (2,6-di-*tert*-butyl-4-methylphenol) (VII), and α-tocopherol (VIII). The rate constants k_7 determining the activity of AO in the reaction with peroxide radicals were equal to (0.94, 0.97, 0.93, 0.75, 0.92, 1.30, and 360.00) × 10^4 M^{-1} s^{-1} for compounds II, III, IV, V, VI, VII, VIII, respectively. For inhibitors II–VI, VIII, the dependence of the induction period on the AO concentration was of an extreme character, and the most efficient concentrations of AO (C_{max}) were equal to (0.7, 1.0, 1.6, 1.0, 1.1, and 2.5) × 10^{-3} M, respectively. The efficiency of inhibitors (τ_{max} and C_{max}) was demonstrated to rise with increasing extent of shielding of the OH group and shortening of the chain of a substituent in the para position. Using the stationary photolysis method, the rate constants of disproportionation (k_9) of phenoxy radicals of inhibitors I, III, VII, and VIII were evaluated and equal to (0.52, 2.38, 3.40, and 0.94) × 10^3 M^{-1} s^{-1}, respectively, at 20°C. It was shown that the k_9 value depended on the extent of shielding of phenoxy radicals. The rate constants ($k_{10, eff}$) were determined for the reaction of phenoxy radicals with lipids with various extents of unsaturation: MO, linoleic and arachidonic acids. The values of $k_{10, eff}$ for the reaction of phenoxy radicals of I, III, VII, and VIII with linoleic acid (20°C) were equal to (0.69, 0.48, 0.09, and 0.49) × 10^2 M^{-1} s^{-1}, respectively. It was found that in MO oxidation, the $k_{10, eff}$ values decreased in proportion to the number of *ortho-tert*-butyl substituents in an AO molecule. For the reactions of phenoxy radicals of inhibitor VII with MO, linoleic and arachidonic acids, the values of $k_{10, eff}$ increased with the number of double bonds in a substrate and were equal to (0.09 ± 0.01, 0.09 ± 0.01, and 0.64 ± 0.04) × 10^2 M^{-1} s^{-1}, respectively. The $k_{10, eff}$ rate constants for the reaction of phenoxy radicals of compound VIII with the same lipids were equal to (0.20 ± 0.04, 0.49 ± 0.01, and 0.74 ± 0.12) × 10^2 M^{-1} s^{-1}, respectively.

INTRODUCTION

It is well known that natural and synthetic AO are used to stabilize foodstuffs, medicinal and cosmetic preparations, polymers, oils, and fuels to avoid oxidative conversions. Therapy with the use of AO finds increasing application in medicine, in the method of nonspecific complex correction of a wide variety of diseases that are accompanied by enhanced free-radical oxidation of biological membranes of lipids and their supramolecular complexes with proteins and nucleic acids.

Peroxide radicals, as highly reactive species, are known to carry out oxidation of hydrocarbons and other organic compounds both *in vitro* and *in vivo*, involving a great number of substrate molecules in the process (reaction II) (hereafter, the numeration of elementary reactions corresponds to the generally accepted scheme [1]). Radicals $RO_2^{\bullet}$ disappear as a result of disproportionation (VI) and interaction with inhibitors of radical processes (VII).

$$RO_2^{\bullet} + RH \xrightarrow{k_2} ROOH + R^{\bullet}, \qquad \text{(II)}$$

$$RO_2^{\bullet} + RO_2^{\bullet} \xrightarrow{k_6} \text{molecular products}, \qquad \text{(VI)}$$

$$InH + RO_2^{\bullet} \xrightarrow{k_7} In^{\bullet} + ROOH. \qquad \text{(VII)}$$

The efficiency of AO is quantitatively estimated by the product $k_7 f$[InH] where k_7 is the rate constant of elementary reaction (VII) and f is the inhibition factor. The k_7 value for the key natural AO—α-tocopherol (α-TP)—is 150 times greater than the corresponding

rate constants for the known inhibitors [2]. Based on this fact, it is believed that exogenous phenols have a competitive effect on free-radical oxidation of lipids of biological membranes, only entering an organism together with food, water, and drugs in relatively high amounts [3].

Another peculiar property of biological AOs involves inversion of their action from antioxidative to oxidative. Such a phenomenon was noted in a series of studies of α-TP and carotinoids *in vitro* [2–5] and *in vivo* [6]. These properties of AOs appear to be connected with the formation (during their oxidation) of rather active phenoxy radicals involved in reactions (VIII), (VIIIa), (IX), and (X) [3, 4]:

$$In^{\cdot} + R^{\cdot} \xrightarrow{k_8} \text{molecular products}, \quad \text{(I)}$$

$$In^{\cdot} + RO_2^{\cdot} \xrightarrow{k_8'} \text{molecular products}, \quad \text{(VIIIa)}$$

$$In^{\cdot} + In^{\cdot} \underset{k_9'}{\overset{k_9}{\rightleftarrows}} \text{molecular products (dimers D)}, \quad \text{(IX)}$$

$$In^{\cdot} + RH \xrightarrow{k_{10}} InH + R^{\cdot}. \quad \text{(X)}$$

In recent years, the rate constants of reaction (X) for tocopheroxy radicals were estimated using the kinetic methods, EPR, and flash photolysis [7–10]. Data obtained by these methods differ from one another by three orders of magnitude. Adequate explanation of this fact is lacking in the literature. The interrelation between the inhibiting action of AOs (that are structurally different) and the activity of their phenoxy radicals was not systematically studied either. The above problems are widely discussed in the literature.

In this work, the efficiency of the inhibiting action of a homologous series of phenol-based AOs with different extents of shielding is studied. An important role of elementary reaction (X) resulting in partial regeneration of the AO during oxidation was shown. The relation between the $k_{10,\text{eff}}$ value, the structure of inhibitors, and the extent of unsaturation of oxidized substrates is revealed.

EXPERIMENTAL

The inhibiting action of AOs was studied in initiated oxidation of methyl oleate (MO). A homogeneous solution of MO in chlorobenzene (at a molar ratio of 1 : 1) was used; the AO was introduced into the solution in a concentration ranging from 5×10^{-5} to 5×10^{-3} M. The kinetics of oxidation were traced by oxygen consumption in a Warburg-type gauge setup. Azobisisobutyronitrile (AIBN) was used as an initiator. The rate of initiation at 60°C was equal to 5.3×10^{-8} M s^{-1}. The efficiency of AO action was quantitatively estimated by the values of the induction period (τ_{ind}), which were found from the kinetic curves of MO oxidation in the presence of an inhibitor [11]. The activity of the AO in reactions with peroxide radicals was determined by the chemiluminescence method [12] in cumene oxidation initiated by AIBN ($T = 60$°C, $w_i = 2.3 \times 10^{-8}$ M s^{-1}) on a SNK-7 setup constructed at the Semenov Institute of Chemical Physics, Russian Academy of Sciences. The setup was equipped with a FEU-29 photomultiplier. We used 9,10-dibromoanthracene (5×10^{-4} M) as a luminophor.

Phenoxy radicals were generated by the stationary photolysis method [13] and identified using UV spectroscopy. To determine the amount of phenol (at 3620 cm^{-1}) and to study the interactions between phenoxy radicals and oxidized substrates, IR spectroscopy was used [14]. Photolysis of phenols was performed in a special chamber equipped with two mercury lamps (250 W). These lamps each were offset by 5 cm from the sample under study.

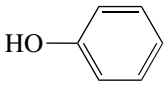
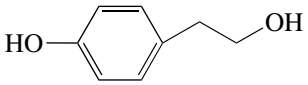
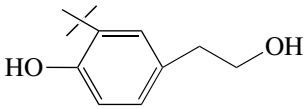
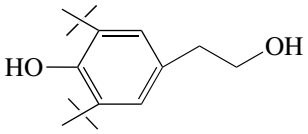
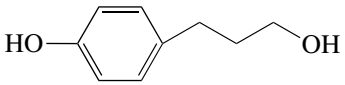
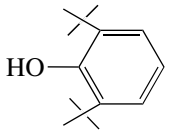
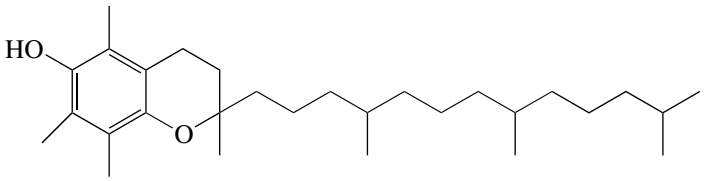
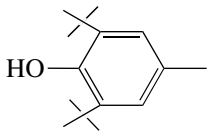
We used α-TP produced by Serva (USA). Tyrosol (4-(2'-hydroxyethyl)phenol), 2,6-di-*tert*-butylphenol, 2-*tert*-butyltyrosol, and 2,6-di-*tert*-butyltyrosol were synthesized at the Institute of Organic Chemistry (Siberian Division, Russian Academy of Sciences). Chroman C_1 and dibunol were prepared at the Moscow State Academy of Fine Chemical Technology and at the Emanuel' Institute of Biochemical Physics (Russian Academy of Sciences), respectively. The solvents (chlorobenzene, tetrachloromethane, heptane, tetrahydrofurane) were distilled before use.

RESULTS AND DISCUSSION

With the aim of determining the interrelation between the efficiency of inhibiting action of AOs and the activity of their phenoxy radicals, we studied several phenols, differing in their nature and the number of substituents in the ortho position relative to the characteristic OH group. Hydroxybenzene (I), tyrosol (2'-hydroxyethylphenol) (II), 2-*tert*-butyltyrosol (III), 2,6-di-*tert*-butyltyrosol (IV), 4-3'-hydroxypropylphenol (γ-propanol) (V), 2,6-di-*tert*-butylphenol (VI), dibunol (2,6-di-*tert*-butyl-4-methylphenol) (VII), and α-tocopherol (VIII) were used as model compounds. Structures of the inhibitors studied are given in Table 1. As is seen from Table 1, the above series involves hydroxybenzene, tyrosol (an active component of *Rhodiola rosea L* [15]), homologs of tyrosol containing one or several bulky *tert*-butyl substituents in the ortho position relative to the OH group, and dibunol. α-Tocopherol belonging to the homologous series of chroman contains two *ortho*-methyl groups as shielding substituents.

The data in Table 1 show that the constants k_7 for the tyrosol homologs agree very well and do not exceed 1×10^4 M^{-1} s^{-1}. In other words, the activity of the above inhibitors is lower than that of α-TP by a factor of 360. The k_7 value for α-TP obtained in this work virtually coincides with the value determined previously [16, 17]. The k_7 value for dibunol is 1.5 times higher than those for tyrosol homologs but it is more than

Table 1. Kinetic characteristics of antioxidants with various structures

No.	Phenol	Formula of the compound	$k_7 \times 10^4$, $M^{-1}\ s^{-1}$	τ_{max}, min	$C_{max} \times 10^{-4}$, M	$\frac{\tau_{AO} - \tau_{MO}}{\tau_{MO}}$
I	hydroxybenzene		0.24	70	5.0	1.5
II	tyrosol (4-2'-hydroxyethylphenol)		0.94	220	7.2	8.2
III	2-*tert*-butyltyrosol		0.97	355	10.0	13.8
IV	2,6-di-*tert*-butyltyrosol		0.93	480	16.0	19.0
V	4-3'-hydroxypropylphenol (γ-propanol)		0.75	124	10.0	3.6
VI	2,6-di-*tert*-butylphenol		0.92	300	11.0	10.5
VII	2,6-di-*tert*-butyl-4-methylphenol (dibunol)		360	980	25.0	39.8
VIII	α-tocopherol		1.3	1650	30.0	67.8

two orders of magnitude lower than the k_7 constant for α-TP (Table 1). Thus, all compounds studied readily react with peroxide radicals (reaction (VII)), which results in chain termination.

To elucidate the mechanism of the action of phenols, the kinetics of initiated oxidation of MO was studied under various conditions.

It is well known that with high concentrations of oxygen, long chains of oxidation, and a constant rate of initiation ($w_i = k_i[AIBN]$), provided that $w_i \gg w_3$, the rate of oxidation ($w_{O_2} = -dO_2/dt$) is determined by the equation [1]:

$$-dO_2/dt = k_2/k_6^{-1/2} w_i^{-1/2}[RH]. \quad (1)$$

The rate of chain initiation w_i and the value of the induction period (τ_{ind}) in the presence of AO can be represented as

$$w_i = f[InH]/\tau_{ind}, \quad (2)$$

$$\tau_{ind} = f[InH]/w_i. \quad (3)$$

Provided that $fk_7[InH] \gg k_6[RO_2^{\cdot}]$, the equation for the oxidation rate takes the form

$$-dO_2/dt = k_2/k_7[RH]w_i/f[InH], \quad (4)$$

where k_2, k_6, and k_7 are the rate constants of the corresponding elementary reactions of oxidation; f is the factor of inhibition determining the number of radicals, termination of which involves one AO molecule. For the compounds under study, $f = 2$.

It was shown that, under the chosen conditions of initiated oxidation of MO, Eqs. (1)–(3) are fulfilled. This fact suggests that the process proceeds via the free-radical mechanism. The oxidation mechanism containing elementary reactions (VII)–(IX) should show a positive correlation between the AO concentration and the value of the induction period. However, in the study of AO effects over a wide range of inhibitor concentrations (7×10^{-5}–1×10^{-3} M), we only observed the linear dependence of the induction period on C_{AO} in the region of relatively low AO concentrations (Fig. 1). In most cases (except for dibunol), the dependence of τ_{ind} on C_{AO} passes through a maximum (Fig. 1). Thus, an increase in the AO concentration on some interval causes a decrease in the induction period instead of the growth of this value. According to the known classification, such AOs are assigned as "inefficient" inhibitors [18]. Although the curves in Fig. 1 have identical character, the coordinates of the extreme points (the values of τ_{max} and C_{max}) are different for the compounds under study. The efficiency of inhibitors (τ_{max}) increases as the extent of shielding of the OH group of phenols grows, and the chain length of the *para*-substituent becomes shorter (Table 1). Analysis of the inhibiting effect of the tyrosol analogs, containing variable numbers of substituents (with identical structures) showed that the induction period became 60% longer when each *ortho-tert*-butyl substituent was introduced into a molecule. Such a relationship is not inherent to dibunol and α-TP. At comparable concentrations, the efficiency of the above inhibitors is substantially higher (by a factor of 4.5–7.5) than the maximum efficiency of sterically nonhindered homologs of tyrosol.

The deviation of the τ_{ind}–C_{AO} relations from the linear ones (Fig. 1) suggests that for most AOs, the scheme of oxidation involving reactions (VII)–(IX) fails to describe inhibited oxidation of the substrate. An additional reaction (X) (chain propagation) occurs in the presence of the AOs studied. It is reasonable to suggest that in excess oxygen, peroxide radicals are formed by reaction (X) if the activity of radicals of an inhibitor is rather high. This reaction is additionally chain initiation. Thus, the appearance of a maximum on the concentration curve indicates an acceleration of oxidation and a decrease in the efficiency of the AO in the region of its high concentrations (Fig. 1).

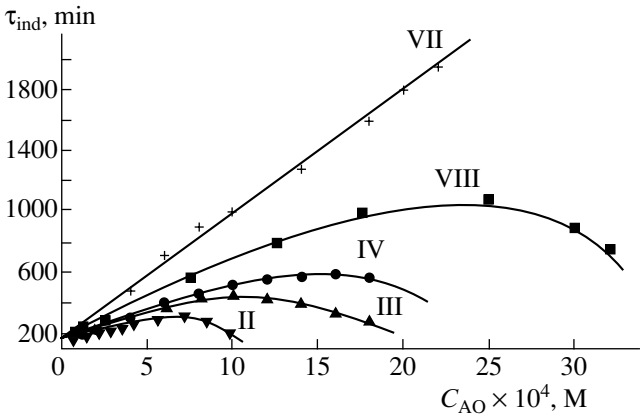


Fig. 1. Dependence of the induction period τ_{ind} on the AO concentration: dibunol (VII); α-TP (VIII); 2,6-di-*tert*-butyltyrosol (IV); 2-*tert*-butyltyrosol (III); tyrosol (II); $C_{AIBN} = 3 \times 10^{-3}$ M; $T = 60°C$.

Reaction (X) can also result in partial regeneration of AOs, which are capable of repeatedly terminating oxidation chains and inhibiting the process. The overall result of reaction (X) (acceleration or retardation of the process) is determined by the ratio between the gains in the rate of chain propagation ($k_2\Delta[RO_2^{\cdot}][RH]$) and in chain termination ($k_7f\Delta[InH]$) with the participation of the AO, which is reduced during the process. Acceleration of inhibited oxidation (the occurrence of a maximum in the concentration curve in Fig. 1) appears to be a manifestation of the phenoxy radical reaction with a substrate.

In this work, the above hypothesis was tested experimentally. We assumed that if reaction (X) occurred under the experimental conditions to give the reduced active form of AO, we could determine experimentally an increase in the AO amount by comparison of the IR spectra of phenols exposed to photoirradiation with and without lipids.

Photoirradiation of AO solutions resulted in the formation of phenoxy radicals that absorbed at 407–430 nm. A solvent (heptane) had no effect on the kinetics of phototransformation of inhibitors. Figure 2 shows the IR spectra of absorption by AO solutions in the course of photoirradiation. Based on spectral data, we obtained the kinetic curves of inhibitor consumption. The amount of phenols was evaluated from the Beer-Lambert law with the consideration of the intensity of absorption of the OH group. We compared the kinetic curves of phenol consumption in the substrate-free system with those in the system containing lipids with various degrees of unsaturation. The above method allowed us to experimentally show the possibility of reduction of the phenolic form of AO. In the first system, AO consumption was due to the formation of radicals during photolysis, which undergo disproportionation by reaction (IX). Supplementary reaction (X)

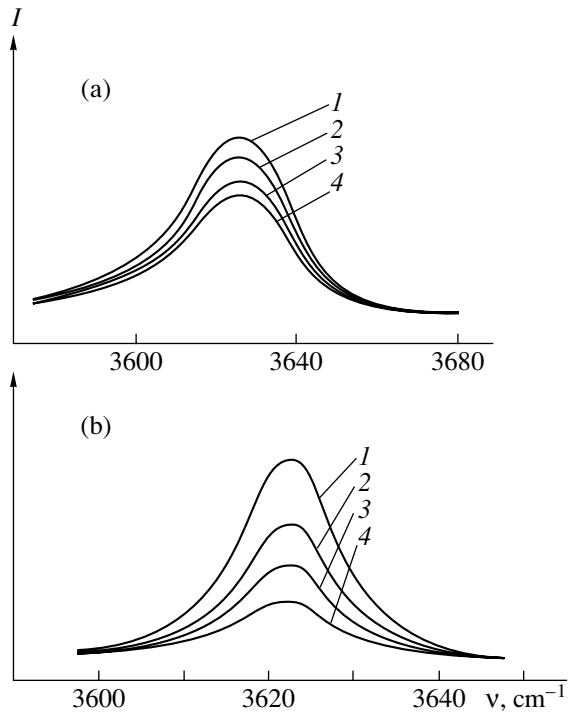


Fig. 2. IR spectra of absorption of solutions of (a) α-TP and (b) dibunol, depending on the time of their photoirradiation (s): (*1*) 0, (*2*) 20, (*3*) 40, (*4*) 60; $C_{\alpha\text{-TP}} = 8 \times 10^{-3}$ M; $T = 20 \pm 2$°C.

should be taken into account for the second system. When the reaction of phenoxy radicals with the oxidized substrate occurs, an increase in the AO amount may result from the regeneration of phenoxy radicals. The above approach was used to elucidate the role of reaction (X) in the mechanism of the action of AOs with different structures.

The value Δ[InH], which characterized the reduction of radicals of inhibitors, was estimated as the difference between the AO concentrations in the presence and in the absence of a substrate (S) for the same photolysis time: $\Delta[\text{InH}] = [\text{InH}]_S - [\text{InH}]$. The rate of phenoxy radical regeneration was determined as Δ[InH] (Table 2) taking into account the $w_{reg} = \Delta[\text{InH}]/\Delta t$ value.

α-Tocopheroxy radicals ($\text{In}^{\cdot}$) were identified on the basis of the differential spectrum described in the literature [10, 19]. Maxima of absorption of $\text{In}^{\cdot}$ of other phenols are found at 407–412 nm. The concentration of phenoxy radicals was determined from UV spectroscopic data with regard to the values of their molar extinction coefficients [19] and to the length of a cell.

An approach that we applied allowed us to determine the rate constants of reactions (IX) and (X). In the absence of a substrate, the rate of AO consumption should be proportional to the rate constant of self-disproportionation k_9:

$$w_{cons} = k_{9,\,eff}[\text{In}^{\cdot}]^2. \qquad (5)$$

Figure 3 shows the kinetic curves of the α-TP consumption, from which the w_{cons} values are estimated. Table 2 lists the values of the effective rate constants $k_{9,\,eff}$ calculated from Eq. (5). The $k_{9,\,eff}$ value for radicals of α-TP obtained in this work agrees well with the corresponding value (0.88×10^3 M^{-1} s^{-1}) reported in [20].

Table 2. Kinetic characteristics of phenoxy radicals of structurally different phenols ($T = 20 \pm 2$°C, $p < 0.05$)

Kinetic parameter	Number of π bonds in a substrate*	Antioxidant			
		hydroxybenzene (I)	2-*tert*-butyltyrosol (III)	α-TP (VIII)	dibunol (VII)
$w_{cons} \times 10^{-5}$, M s^{-1}	0	2.3	10.5	8.0	15.0
	1	–	–	7.0	14.0
	2	1.7	6.7	5.0	13.5
	4	–	–	0.5	7.5
$k_{9,\,eff} \times 10^3$, M^{-1} s^{-1}	0	0.52 ± 0.02	2.38 ± 0.01	0.94 ± 0.02	3.40 ± 0.03
$w_{reg} \times 10^{-5}$, M s^{-1}	1	–	–	2.0	1.0
	2	7.0	4.9	5.0	1.0
	4	–	–	7.5	6.4
$k_{10,\,eff} \times 10^2$, M^{-1} s^{-1}	1	–	–	0.20 ± 0.04	0.09 ± 0.01
	2	0.69 ± 0.02	0.48 ± 0.03	0.49 ± 0.01	0.09 ± 0.01
	4	–	–	0.74 ± 0.12	0.64 ± 0.04

Note: $C(S) = C(AO) = 8 \times 10^{-3}$ M. The criterion of confidence p was determined by the Student criterion at $f = n_1 + n_2 - 2$, where f is the first function of the normalized deviation, n_1 is the number of check tests, n_2 is the number of test samples. The constant values were taken reliable at $p < 0.05$.

* 0 stands for the absence of a substrate; 1, 2, 4 denote that methyl oleate (one π bond), linoleic acid (two π bonds), and arachidonic acid (four π bonds), respectively, are used as substrates.

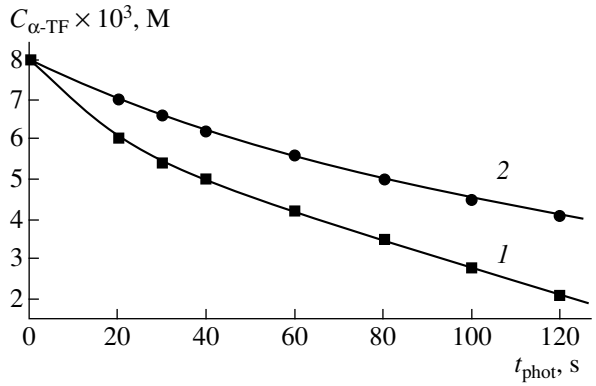


Fig. 3. Changes in the α-TP concentration in the course of photoirradiation of its solutions: (*1*) without a substrate, (*2*) in the presence of linoleic acid (LA); the concentrations of α-TP and LA are equal to 8×10^{-3} M, $T = 20 \pm 2$°C.

This fact suggested that the above approach is correct for evaluating the rate of disproportionation of phenoxy radicals with different structures and the rate of interaction between the radicals and substrate. We found that the $k_{9,\,eff}$ values depended on the extent of shielding of phenoxy radicals. For example, the rate constants of phenoxy radicals of hydroxybenzene, 2-*tert*-butyltyrosol, and dibunol are equal to (0.52, 2.38, and 3.40) × 10^3 M^{-1} s^{-1}, respectively (20°C). Note that the $k_{9,\,eff}$ value for dibunol determined in this work is close in magnitude to the corresponding rate constant of 3.4×10^3 M^{-1} s^{-1} reported in [20]. We found that the $k_{9,\,eff}$ value for radicals of the monoshielded tyrosol analog was equal to 1.25×10^3 M^{-1} s^{-1} at 20°C. This rate constant is available in the literature for radicals of 2,6-di-*tert*-butyltyrosol (1.1×10^3 M^{-1} s^{-1} at 22°C) [22]. In other words, the above values are of the same order. An increase in the temperature and a decrease in the extent of shielding are known to favor the growth of the $k_{9,\,eff}$ value [20]. The same relationships were observed in this work as well. Thus, the rate constants estimated above are comparable with the values reported in the literature for the previously studied AOs, suggesting that our estimate of $k_{9,\,eff}$ is correct for the inhibitors that are examined for the first time in this work.

Comparison between the kinetics of the consumption of various AOs during photolysis of substrate-free solutions and those containing unsaturated lipids (for example, linoleic acid) shows that in the latter case the kinetic curves are more flattened (Fig. 3). Consequently, AOs are consumed at a lower rate in the presence of the substrate (Table 2). The data obtained suggest that AOs are regenerated in the course of the reaction owing to the interaction of phenoxy radicals with the oxidized substrate.

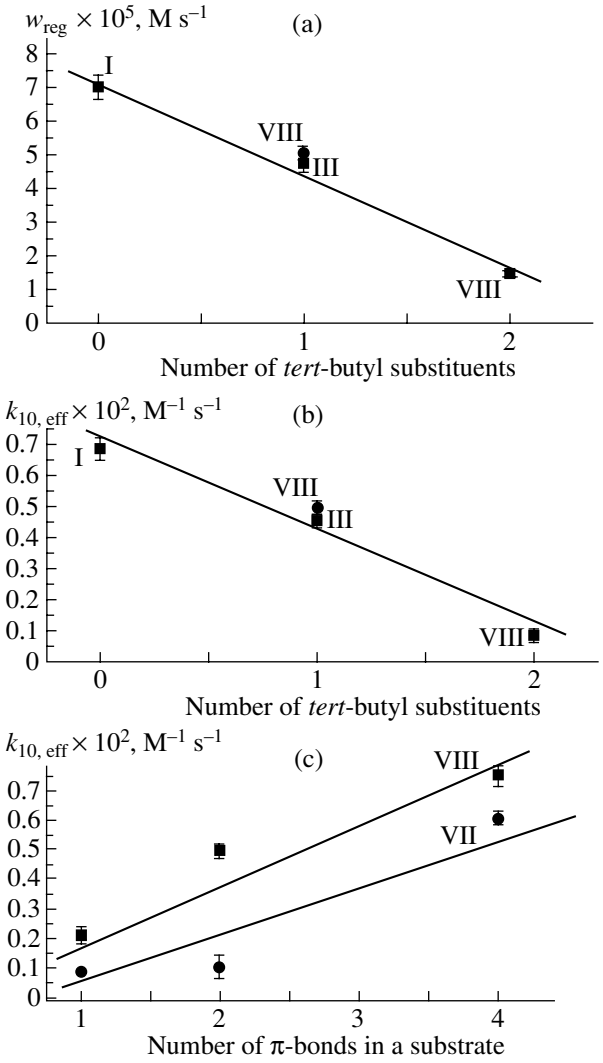


Fig. 4. Dependences of (a) the regeneration rate of AO and (b) the $k_{10,\,eff}$ value on the number of *ortho-tert*-butyl substituents in the AO structure; (c) interrelation between the $k_{10,\,eff}$ value and the extent of unsaturation of lipids. For designations of AOs see Fig. 1. Methyl oleate (one π bond), linoleic acid (two π bonds), and arachidonic acid (four π bonds) are used as substrates.

The values of $k_{10,\,eff}$, which characterize the activity of phenoxy radicals in reaction (X), can be obtained from kinetic data on AO reduction. The rate constants $k_{10,\,eff}$ are calculated from equation

$$\frac{\Delta[\mathrm{InH}]}{\Delta t} = k_{10,\,eff}[\mathrm{RH}][\mathrm{In}^{\cdot}] \qquad (6)$$

and given in Table 2.

Analysis of the results presented in Table 2 shows that the rate of phenoxy radical regeneration depends on the structure of inhibitors. The highest values of $k_{10,\,eff}$ were found for nonshielded phenols. In conjunction, it is important to relate the rate of phenoxy radical reduction to the extent of their shielding. The structures

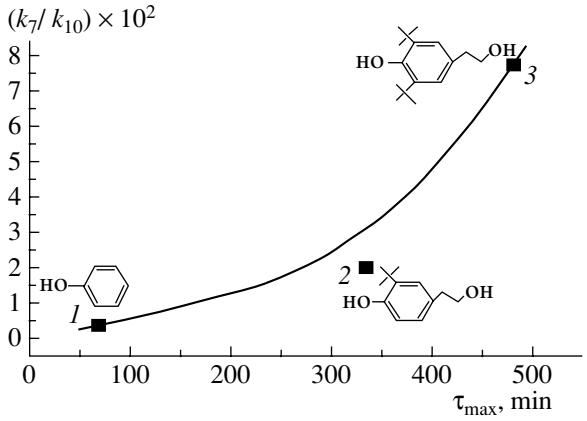


Fig. 5. Interrelation between the rate constant ratio k_7/k_{10} and the value τ_{max}: (*1*) hydroxybenzene, (*2*) 2-*tert*-butyltyrosol, (*3*) 2,6-di-*tert*-butyltyrosol.

of inhibitors under study allowed us to use these AOs as model compounds for solving this problem. The rate of regeneration of the active form of an AO and the $k_{10,\,eff}$ value are shown to be inversely proportional to the number of *ortho-tert*-butyl substituents (Figs. 4a and 4b). Nonshielded phenoxy radicals (or shielded only slightly) have the highest values of $k_{10,\,eff}$ and are readily reduced. The rate constant $k_{10,\,eff}$ for phenoxy radicals of dibunol is 7 times lower than that of nonshielded hydroxybenzene. Tocopherol radicals are regenerated more readily compared to phenoxy radicals of dibunol (Table 2). With methyl oleate as a substrate, the $k_{10,\,eff}$ values for the above AOs differ by a factor of 2, and with the use of linoleic acid the constants differ by a factor of 5. Thus, sterically hindered phenoxy radicals are the least active in the reaction with a substrate and virtually are not involved in reaction (X), as distinct from unsubstituted radicals.

One of the challenges was to study the dependence of phenoxy radical activity on the extent of unsaturation of lipids, as oxidized substrates. We showed that the $k_{10,\,eff}$ value is directly proportional to the number of double bonds in molecules of polyunsaturated lipids (Table 2, Fig. 4c). The results obtained are consistent with data reported in [21].

The values of the rate constants of the reaction between phenoxy radicals of hydroxybenzene and methyl linoleate obtained in this work and reported in [23] ($k_{10,\,eff}$ = 120 M^{-1} s^{-1} at 80°C) are of the same order of magnitude. However, the rate constant $k_{10,\,eff}$ determined under the same conditions for dibunol (0.41 M^{-1} s^{-1} at 80°C [23]) is 22 times less than that found from the relations presented in Fig. 4c for substrates containing several double bonds in a molecule ($k_{10,\,eff}$ = 9.00 M^{-1} s^{-1} at 80°C). Thus, a considerable decrease in the activity of phenoxy radicals in the reaction with the oxidized substrates with an increase in the degree of shielding is confirmed by data reported in [23]. The difference in the absolute values of the constants is due to the use of different methods in their determination.

A comparison of the $k_{10,\,eff}$ values found in this work (Table 2) and the rate constants determined previously by flash photolysis [9] shows substantial differences in the results obtained. According to flash photolysis data, the rate constants $k_{10,\,eff}$ for lipids involving from one to four π bonds differ by a factor of 100–1000 [8], whereas the constants found in this work are of the same order. The reasons for these differences can be due to different methods in which either the steady-state concentration or the nonequilibrium state (flash photolysis) are used. Note that considerable differences in the constant values (more than three orders of magnitude) were also observed for the reaction between the radicals of tocopherol and ascorbic acid using pulse radiolysis [24] and EPR [25, 26]. In the methods with equilibrium concentrations of phenoxy radicals, one should take into account (to obtain more accurate estimates) the kinetics of reversible dimerization of phenoxy radicals (IX) and the possibility for the shift of equilibrium under the action of unsaturated lipids according to the Le Chatelier principle.

$$D \overset{k_9,\,k_9'}{\rightleftharpoons} In^{\cdot} \xrightarrow{RH,\,k_{10}} InH\,.$$

This approach allows one to obtain comparable results independently of the method. To the best of our knowledge, no such estimates were reported in the literature.

Because with flash photolysis and pulse radiolysis, the formation of dimers in excess substrate is minimum, it is believed that the rate constants of reaction (X) can be estimated more exactly using these methods. Nevertheless, data on stationary photolysis are of interest as to the empirical characteristics of the reactivity of structurally different phenoxy radicals.

In light of the above results, the following problems are interesting: comparison between data obtained by various methods, the consideration of the activity of phenoxy radicals of AOs with different structures in side reaction (X), and the relation between radical activity and the efficiency of inhibition of the process. The above data show that sterically hindered phenols, giving low-activity radicals, are the most efficient inhibitors. In contrast, unshielded phenols, exhibiting rather high values of $k_{10,\,eff}$ are less appropriated as inhibitors of oxidation. These AOs are species that display a maximum on the concentration curves. The higher the $k_{10,\,eff}$ value (Table 2), the lower the concentration (C_{max}) at which a maximum is observed (Table 1), and the inversion in the inhibiting action becomes possible.

For AOs that were capable of showing inversion of their action, the important problems were to establish the interrelation between the τ_{max} values and the absolute values of k_7 and to relate the maximum efficiency (τ_{max}) to the rate constant ratio k_7/k_{10}. For inhibitors of one homologous series or for those of various series, no direct relation between the rate constants of reactions (VII)

and (X) and the value of τ_{max} was detected. Positive correlation was observed between the k_7/k_{10} ratio and τ_{max} (Fig. 5). Note that the same interrelation between parameters of inhibited oxidation and the k_7/k_{10} ratio was shown previously for a series of synthetic AOs (derivatives of nitriles) [27]. The above ratio was recommended by the authors of this report for more exact estimation of the AO action.

Thus, data obtained in this work show that for the evaluation of AO efficiency, not only should their activity in the reactions (k_7) with radicals be taken into account, but the activity of the products of AO conversion (k_{10}) must be considered as well. The presence of a maximum on the concentration curves reflects reaction (X) and points to the possibility for inversion of the AO action *in vivo*.

There is a possibility for improving the efficiency of the action of weak inhibitors by introducing substrates that produce inactive radicals into the system (synergism). A search for such substrates shows promise for α-TP (a natural AO), which is widely used for the stabilization of oxidation *in vitro* and *in vivo*. Data obtained in this work demonstrate that α-TP has high values of k_7 and k_{10}. In the course of oxidation, radicals of α-TP can be partially reduced to the active form that readily destroys peroxide radicals. Thus, when introducing a substrate (a source of low-active radicals), we can improve the efficiency of α-TP in oxidation. As such substrates, phospholipids (PL) are useful. In the course of simultaneous action of α-TP and Pl, synergism shows itself as an increase in the α-TP activity by a factor of 1.5–3.5 [28]. It was found experimentally that PL favored the regeneration of α-TP by multiple termination of the oxidation chains [29]. Molecules of α-TP and Pl are chemically bonded to form complex supramolecular structures with hydrogen bonds, which have at least two binding sites (the constant for one of them is 7.4×10^2 M^{-1} [27]). Radicals arising in the oxidation of such structures are stabilized by ionogenic fragments of polar molecules and, consequently, exhibit a low activity. Simultaneous localization of α-TP and PL in the biological membranes ensures the steady-state conditions of oxidation of the biological substrate.

Note that the relatively high activity of phenoxy radicals is mainly found for natural AOs (α-TP and tyrosol). The properties of these biological antioxidants make it possible to vary the capacity of the AO system depending on the AO amount. The peculiarities of the action of biological AOs is of great regulatory importance and may be considered as a mechanism that ensures the antioxidant homeostasis of an organism.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 02-04-49452) and by the Federal Program of Integration of Sciences and High Education (grant no. I0566/1653).

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