Combined inhibitory effect of sulfur-containing phenol SO-4 with natural and synthetic antioxidants in the oxidation of methyl oleate

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In a model system of initiated oxidation of methyl oleate, the antioxidant activities of 3-hydroxy-2-ethyl-6-methylpyridinesuccinate (mexidol) and bis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl] disulfide (SO-4) were studied and compared with those of α-tocopherol and 1-hydroxy-2,6-di-*tert*-butyl-4-methylbenzene (dibunol). A linear pattern of dependence of the inhibitory effect on the concentration of compounds was established. The ability of antioxidants to decompose hydroperoxides and inhibit their accumulation was revealed. The combined inhibitory effects of SO-4 with mexidol, α-tocopherol, and phospholipids were described for the first time. The rate constant for disproportionation of the SO-4 phenoxyl radicals, $k_9 = 0.90 \cdot 10^3$ L mol^{-1} s^{-1}, was determined by steady-state photolysis. The rate constant k_{10}^{eff} for the reactions of SO-4 phenoxyl radicals with lipids characterized by different unsaturation degrees were determined for methyl oleate, linolic and arachidonic acids containing one, two, and four multiple bonds, and phospholiopids containing polyunsaturated fatty acids.

Key words: α-tocopherol, sulfur-containing antioxidants, 3-hydroxypyridine derivatives, mexidol, phospholipids.

The oxidation of food and cosmetic oils or fatty bases of pharmaceuticals is often inhibited using synergistic compositions, either consisting of several oxidation inhibitors that enhance the action of one another or including an antioxidant (AOx) and a synergist. As a rule, the latter does not exert a self-sustained inhibitory action, but its presence markedly enhances the efficiency of the inhibitor (phospholipids). The range of nontoxic AOx has markedly extended in recent years. Despite the wide use of 3-hydroxypyridine derivatives, *viz.*, mexidol (3-hydroxy-2-ethyl-6-methylpyridinesuccinate) and emoxipin, in clinical practice where they showed good therapeutic effect, the mechanism of the antioxidant effect of these compounds is little studied.[1—3] Sulfur-containing phenols also arouse considerable interest because they efficiently retard oxidation by several mechanisms including the reaction with peroxyl radicals and destruction of hydroperoxides to give molecular products.[4—6] A sulfur-containing phenol, bis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl] disulfide (SO-4) has been synthesized at the Novosibirsk Institute of Organic Chemistry, Siberian Branch of the RAS. This compound does not have any local or general toxicity, does not affect the embryogeny or development of the descendants, which allows its use in clinical practice.[6]

Thus, the purpose of this work was to study the antiradical and antioxidant activities of mexidol and the sulfur-containing phenol SO-4 in comparison with known inhibitors, and to study the prospects of combined use of AOx of different classes as additional drugs for nonspecific therapy of many diseases and for stabilization of model and natural unsaturated lipids against oxidation.

Experimental

The antiradical activity of the AOx was tested in the system of initiated oxidation of ethylbenzene using chemiluminescence (CL).[7] The luminescence intensity was measured on a

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photometric setup designed at the Institute of Chemical Physics of the RAS. The oxidation of ethylbenzene was carried out in a glass cell located in a light-tight chamber of the photometric setup equipped with a FEU-29 photoelectron multiplier. The cell has a thermostatically controlled shell. Dust- and water vapor-free air was passed through the cell. The substance under study was injected into the oxidation cell in the course of the reaction by means of a syringe unit. The emitted light was focused on the photoelectron multiplier by a system of spherical dishes. The oxidation was initiated by azobisisobutyronitrile (AIBN) (concentration $3 \cdot 10^{-3}$ mol L^{-1}) at 60 °C. The rate of free radical generation measured experimentally using a reference inhibitor, chromane C, was $2.3 \cdot 10^{-8}$ mol L^{-1} s^{-1}. The luminescence was enhanced by the luminophore 9,10-dibromoanthracene (concentration $5 \cdot 10^{-4}$ mol L^{-1}), which does not affect the oxidation kinetics. The concentration of the inhibitor was $(1—5) \cdot 10^{-4}$ mol L^{-1}. The resulting kinetic curves have a typical S-shape. The key kinetic characteristic of the CL curve is the slope of the tangent line in the inflection point, which is proportional to the maximum rate of AOx consumption $[d(I_0/I)/dt]_{max}$. This value was used to calculate k_7 (see Ref. 7) taking into account the equation

$$[d(I_0/I)/dt]_{max} = (0.22 \pm 0.02) k_7 \left(\sqrt{W_i} \Big/ \sqrt{k_6} \right),$$

where k_6 is the rate constant for recombination of peroxide radicals (for ethylbenzene, $k_6 = 4.1 \cdot 10^8 \exp[-2100/(RT)]$).[8a]

The oxidation kinetics was measured by monitoring the absorption of oxygen in Warburg type gage setup during oxidation of a model substrate, methyl oleate, in the presence of an inert solvent (chlorobenzene).[9] The process was initiated by thermal decomposition of AIBN at 60 °C, the initiation rate under the experimental conditions was $4.2 \cdot 10^{-8}$ mol L^{-1} s^{-1}. The induction period (τ_i) was found as the intercept on the *x*-axis made by the perpendicular from the point of intersection of the tangents to the kinetic curve.[8b]

The inhibitory effect was estimated based on the antioxidant activity (A) determined quantitatively by the formula $A = (\tau_i - \tau_S)/\tau_S$ (τ_S and τ_i are the induction periods of substrate oxidation in the absence and in the presence of the AOx under study, respectively) and was compared with the action of the reference inhibitor by using the τ_i/τ_{ref} ratio where τ_{ref} is the induction period for the reference inhibitor.[9] The accumulation kinetics of hydroperoxide was studied by iodometric back titration with methyl oleate autoxidation at 60 °C in chlorobenzene.[8c] The reference inhibitors used included 6-hydroxy-2,5,7,8-tetramethyl-2-phytylchromane (α-tocopherol, α-TP) and 1-hydroxy-2,6-di-*tert*-butyl-4-methylbenzene (dibunol) present in comparable concentrations. The efficiency of combined inhibitory effect of the mixture was either characterized quantitatively by the absolute value ($\Delta\tau$) of the difference between the induction times of methyl oleate oxidation in the presence of AOx (τ_Σ) and the simple sum of the components ($\Sigma\tau_i$, additive action, $\Delta\tau = \tau_\Sigma - \Sigma\tau_i$) or expressed in relative units $(\Delta\tau/\Sigma\tau_i) \cdot 100\%$.

The phenoxyl radicals were obtained by steady-state photolysis and identified by UV spectroscopy using a Specord M-40 spectrophotometer with automated recording and computer processing of the spectra. The amount of phenols was estimated by IR spectroscopy (at 3620 cm^{-1}) on a Specord IR-75 spectrophotometer; the same method was used to study the reaction of phenoxyl radicals with oxidation substrates. The photolysis of phenols was carried out in specially designed chamber equipped by two 250-W mercury lamps.

Commercial α-TP and dibunol (Serva, Germany) were used. Methyl oleate synthesized at the Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the RAS and purified by vacuum distillation in an argon flow at 105 °C was used as the oxidation substrate. The purity of phospholipids (egg phosphatidylcholine) was determined by TLC. Mexidol was synthesized at the N. M. Emanuel´ Institute of Biochemical Physics (IBCP), SO-4 was prepared at the N. N. Vorozhtsov Institute of Organic Chemistry, Siberian Branch of the RAS. The purity of antioxidants was determined by UV and IR spectroscopy and HPLC on a Milichrome A-02 chromatograph with a spectrophotometric detector composed of a double-beam UV spectrophotometer and a Nucleosil 100-5 column. The chromatograms were recorded using gradient elution by water, methanol, and acetonitrile (supply flow rate 100 μL min^{-1}, cell volume 1.2 μL). The content of the major AOx was ≥99.9%.

Results and Discussion

The rate constant of the elementary reaction of AOx with peroxyl radicals k_7 was determined by CL (according to the generally accepted scheme,[7] this is reaction (7)):

$$InH + RO_2^{\bullet} \xrightarrow{k_7} ROOH + In^{\bullet}, \qquad (7)$$

where InH is the oxidation inhibitor, $In^{\bullet}$ is the inhibitor radical, and $RO_2^{\bullet}$ is the peroxyl radical. The results are summarized in Table 1.

The antiradical activity (k_7) was compared with that for α-TP and dibunol. The rate constants k_7 for SO-4 and dibunol were similar, being equal to $1.3 \cdot 10^4$ and $1.4 \cdot 10^4$ L mol^{-1} s^{-1}, respectively. According to published data,[10] the k_7 value for dibunol is $2.0 \cdot 10^4$ L mol^{-1} s^{-1}. In the case of mexidol, $k_7 = 2.8 \cdot 10^4$ L mol^{-1} s^{-1}, which is consistent with published data.[11] The k_7 value for α-TP is equal to $3.6 \cdot 10^6$ L mol^{-1} s^{-1}, which is 250 times higher than those for SO-4 or dibunol.

The kinetic curves for the oxidation of methyl oleate in the presence of equal concentrations of various AOx are shown in Fig. 1. The introduction of the AOx not only

Table 1. Kinetic characteristics of antioxidants of various chemical structures[a]

Antioxidant	$k_7 \cdot 10^{-4}$ /L mol^{-1} s^{-1}	τ_{ind}[b] /min	A[c]
Mexidol	2.80	110	3.2
SO-4	1.30	240	8.2
Dibunol	1.40	190	6.3
α-Tocopherol	360.00	160	5.2

[a] Here and in Tables 2—4, the significance of the differences was evaluated using the Student criterion; an error probability less than 0.05 was considered to be acceptable.

[b] The AOx concentration was $2 \cdot 10^{-4}$ mol L^{-1}.

[c] The oxidation conditions are presented below Fig. 1, $T = 60$ °C.

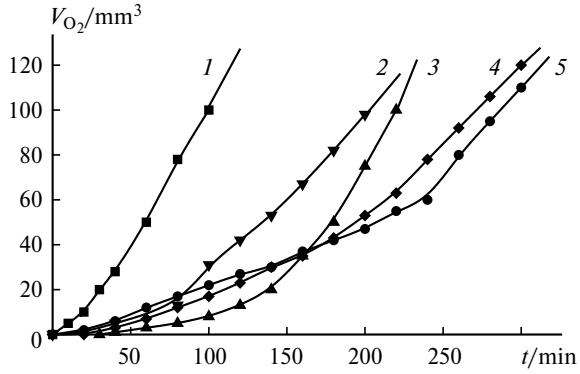

Fig. 1. Kinetic curves for the absorption of oxygen by methyl oleate in chlorobenzene in the presence of various AOx: (*1*) reference, (*2*) mexidol, (*3*) α-TP, (*4*) dibunol, (*5*) SO-4; C_{AOx} = const = $1 \cdot 10^{-4}$ mol L^{-1}, $W_i = 4.2 \cdot 10^{-8}$ mol L^{-1} s^{-1}, $T = 60$ °C.

increases the induction period with respect to the control experiment but also decreases the initial and the highest oxidation rates. When the compound concentration was $1 \cdot 10^{-3}$ mol L^{-1}, the highest oxidation rate decreased 1.3- and 8.3-fold in the presence of SO-4 and mexidol, respectively. For α-TP and dibunol, this phenomenon was not observed. Presumably, SO-4 and mexidol can destroy hydroperoxides.

This hypothesis was verified by measuring the accumulation kinetics of hydroperoxides in the autoxidation of methyl oleate. In a specified instant, AOx was added, in a concentration of $2 \cdot 10^{-4}$ mol L^{-1}, to relatively oxidized substrate. It can be seen from Fig. 2 that the hydroperoxide concentration decreases during the first hour and remains low for the subsequent 8 h, whereas in the blank experiment (in the absence of AOx), hydroperoxides continue to accumulate. The most pronounced hydroperoxide destruction was found for SO-4.

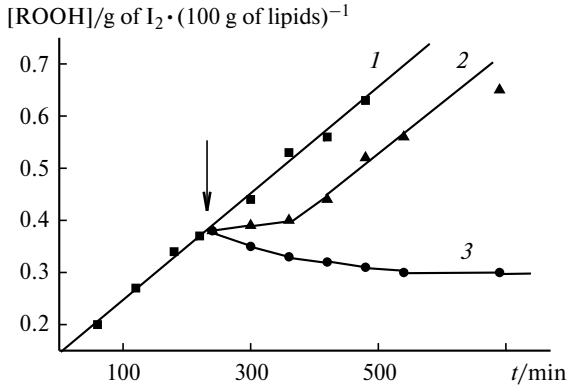

Fig. 2. Destruction kinetics of hydroperoxides during autoxidation of methyl oleate in the presence of equal concentrations of AOx: (*1*) reference, (*2*) mexidol, (*3*) SO-4. The arrow shows the injection point of AOx; C_{AOx} = const = $2 \cdot 10^{-4}$ mol L^{-1}, $T = 60$ °C.

The pattern of hydroperoxide transformations has a substantial effect on the kinetics and mechanism of oxidation as a whole; therefore, studies of the effect of inhibitors on the behavior of peroxides receives much attention.[12—15] It was shown[16,17] that sulfur-containing compounds that combine different functional groups in one molecule promote heterolytic cleavage of the peroxide bond and, under certain conditions, they can exhibit substantial synergistic effects in the antioxidant action. Thus, these AOx can efficiently terminate the oxidation chains upon the reaction with the peroxyl radicals and also prevent the secondary initiation of the process through hydroperoxide destruction by a molecular route.

In recent years, it has been shown[18—20] that for some natural AOx, the direct proportion between the induction times and the concentration of compounds holds only for low doses, while with an increase in the AOx concentration, the efficiency may decrease. Therefore, for these AOx it is important to study the variation of the induction time *vs* their content in the substrate.

The inhibitory effect of AOx was studied over a broad concentration range. It was shown that for dibunol, SO-4, and mexidol, the induction time increases in direct proportion to their concentration in the model system, whereas for α-TP this dependence passes through a maximum as described previously[19,20] and reproduced in this study (Fig. 3). Under our experimental conditions, the maximum was observed at a concentration of $2.5 \cdot 10^{-3}$ mol L^{-1}. The antioxidant activity of mexidol is commensurable with the efficiency of α-TP. It can be seen in Fig. 3 that SO-4 ensures longer retardation times than dibunol or α-TP.

The efficiency of the AOx is also related to the high reactivity of the phenoxyl radicals formed on their oxidation by reactions (8), (8′), (9), (9′) and on the reaction with the oxidation substrate (10).

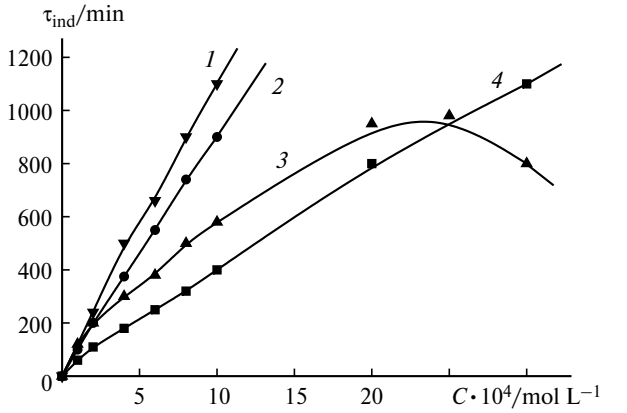

Fig. 3. Plot for the induction times *vs*. the concentration of AOx: (*1*) SO-4, (*2*) dibunol, (*3*) α-TP, (*4*) mexidol; $W_i = 4.2 \cdot 10^{-8}$ mol L^{-1} s^{-1}, $T = 60$ °C.

$$In^{\bullet} + R^{\bullet} \xrightarrow{k_8} P \qquad (8)$$

$$In^{\bullet} + RO_2^{\bullet} \xrightarrow{k_8'} P \qquad (8')$$

$$In^{\bullet} + In^{\bullet} \underset{k_9'}{\overset{k_9}{\rightleftarrows}} In{-}In + InH \qquad (9), (9')$$

$$In^{\bullet} + RH \xrightarrow{k_{10}} InH + R^{\bullet} \qquad (10)$$

RH is hydrocarbon (lipid), $RO_2^{\bullet}$ is peroxyl radical, InH is inhibitor, In—In is dimer, $In^{\bullet}$ is the inhibitor radical, P are molecular products

The activation energy of the reaction of phenoxyl radicals with peroxyl radicals (reaction (8)) is most often close to zero. The rate constants k_8 for sterically hindered phenols vary in a narrow range, depending only slightly on the nature of $In^{\bullet}$ or reactive radical $RO_2^{\bullet}$, and are usually $\sim 3 \cdot 10^8$ mol L^{-1} s^{-1}.[21] Thus, the crucial factor contributing to the antioxidant activity of phenols is played by the reactivity of the radicals they produce by reaction (9), which is recognized as the major route of the decay of the phenoxyl radicals under free-radical oxidation conditions.

The reactivity of SO-4 phenoxyl radicals with respect to model substrates with increasing degree of unsaturation was studied. The research was performed for the following series of phenolic AOx: hydroxybenzene, α-TP, SO-4, dibunol. The reactivity of the radicals of the most important natural AOx (α-TP) containing two Me groups in the *ortho*-position to the OH group was tested. The curve for AOx consumption was measured by IR spectroscopy in the phenolic hydroxyl region (3600 cm^{-1}) after exposure of samples to hard UV light. The kinetics of phenol transformations in two systems were compared, one system containing no substrate and the other containing lipids with different degrees of unsaturation. In the former system, the inhibitor was consumed due to photolysis to give radicals, involved mainly in reactions (9), while in the latter system, this route was supplemented by reaction (10), namely, phenoxyl reduction to phenols.

Figure 4, *a* shows the IR absorption spectra of dibunol solutions before and after irradiation for a specified period of time. The intensity of the OH absorption band decreased appreciably upon an increase in the time of photolysis due to the formation of phenoxyl radicals. The IR spectra of solutions of AOx with different concentrations allow one to determine the concentration of the inhibitor that did not reacted over a particular time of photolysis using the Bouguer—Lambert—Beer law. The resulting data were employed to plot the kinetic curves for AOx consumption.

The curves for SO-4 consumption during photoirradiation of solutions with equal initial concentrations of AOx are shown in Fig. 4, *b*. The rate of AOx consumption was determined from the initial segments of the kinetic curves (Table 2). Based on the AOx consumption rate (W_d), the effective constants k_9^{eff} were calculated taking into account the equation

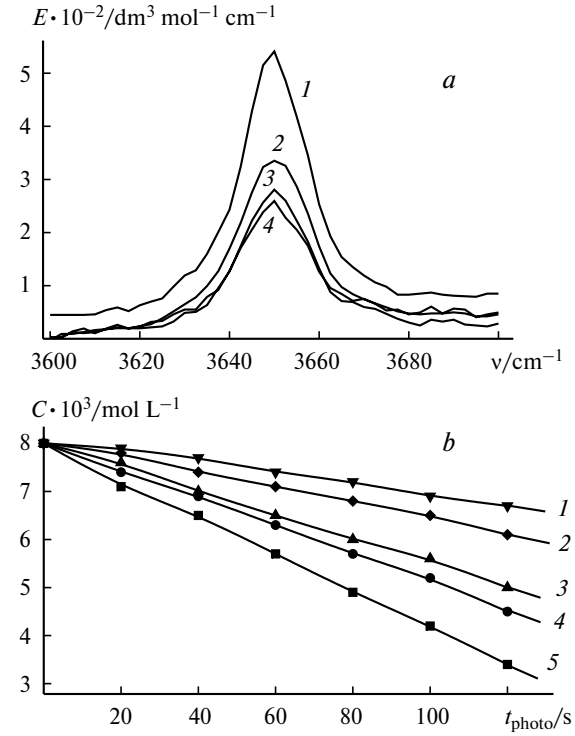

Fig. 4. *a*. IR absorprion spectra of solutions of dibunol in hexane depending on the photoirradiation time: (*1*) 0, (*2*) 20, (*3*) 40, (*4*) 60 s; $C_{AOx} = 8 \cdot 10^{-3}$ mol L^{-1}, $T = 20$ °C; *b*. Variation of the concentration of SO-4 during photoirradiation of its solutions in hexane: (*1*) SO-4 + arachidonic acid; (*2*) SO-4 + phospholipids; (*3*) SO-4 + linolic acid; (*4*) SO-4 + methyl oleate; (*5*) SO-4. Concentrations of SO-4 and oxidation substrates $8 \cdot 10^{-3}$ mol L^{-1}, $T = 20$ °C.

$$W_d = k_9^{eff}[In^{\bullet}]^2.$$

The steady-state concentration $[In^{\bullet}]$ was determined by UV spectroscopy at $\lambda = 410$—425 nm from the relation $[In^{\bullet}] = D/(l\varepsilon)$, which takes into account the cell length l, the observed absorbance D, and the molar extinction coefficient ε, which was taken to be 4000 L mol^{-1} s^{-1} by analogy with the extinction coefficient of the model radicals derived from α-TP.[22] The k_9^{eff} values that we found for all inhibitors were of the same order of magnitude (see Table 2). The published value for α-TP is also of the same order of magnitude, namely, $3.3 \cdot 10^3$ L mol^{-1} s^{-1} (25 °C) and $2.2 \cdot 10^3$ L mol^{-1} s^{-1} (50 °C). For dibunol phenoxyl radicals, $k_9^{eff} = 4.7 \cdot 10^3$ L mol^{-1} s^{-1}.[23] It can be seen that the disproportionation constants for tocopheroxyl radicals are comparable with published data. Hence, the approach we used appears to be well-posed enough to estimate the phenoxyl dimerization constant. The lower rate

Table 2. Kinetic characteristics of phenoxyl radicals derived from phenols of various structures*

Anti-oxidant	$k_9^{eff} \cdot 10^3$ /L mol^{-1} s^{-1}	$W_d \cdot 10^{-5}$ /mol L^{-1} s^{-1}					$W_r \cdot 10^{-5}$ /mol L^{-1} s^{-1}				$k_{10}^{eff} \cdot 10^2$ /L mol^{-1} s^{-1}			
		I	II	III	IV	V	II	III	IV	V	II	III	IV	V
Hydroxy-benzene	0.75	3.0	2.7	2.0	1.4	1.6	3.6	8.0	10.5	9.5	0.21	0.48	0.63	0.57
α-TP	2.08	8.3	5.0	2.5	0.6	1.0	2.5	5.5	7.0	6.5	0.16	0.34	0.44	0.31
SO-4	0.90	3.6	2.9	2.2	1.1	1.5	1.8	2.3	6.2	4.5	0.11	0.14	0.39	0.28
Dibunol	2.78	16.0	12.5	10.0	5.6	6.7	1.5	1.7	6.0	4.3	0.08	0.09	0.31	0.22

* $C_{AOx} = C_{PL} = 8 \cdot 10^{-3}$ mol L^{-1}, $T = 20$ °C; I — no substrate; oxidation substrate: II — methyl oleate (one π-bond), III — linolic acid (two π-bonds), IV — arachidonic acid (four π-bonds), V — phospholipids containing polyunsaturated fatty acids; W_d and W_r are consumption and recording rates, respectively.

constant k_9^{eff} for SO-4 is due, most likely, to the great bulk of the *para*-substituent, which creates steric hindrance to the formation of dimers upon disproportionation (see reaction (9)), as was also demonstrated for other groups of compounds.[6]

It was found that the degree of hindrance has a substantial influence on the rate of AOx consumption. Indeed, unhindered phenol present in the same concentration is consumed 5 times more slowly than sterically hindered dibunol (see Table 2).

The phenoxyl radicals formed in the steady-state photolysis of solutions of phenols can not only undergo disproportionation in the presence of unsaturated compounds but can also be consumed in the reactions with the substrate. Reaction (10) results in the recovery of the phenolic form of AOx. It was shown that photolysis of solutions of AOx containing additionally an oxidation substrate shows a different consumption kinetics of the inhibitor. The kinetic curves for the consumption of SO-4 with addition of methyl oleate, linolic acid, arachidonic acid, or phospholipids (PL) are shown in Fig. 4, *b*. It can be seen that the slope of the kinetic curves and the consumption rate of AOx in the presence of an unsaturated compound decrease. The decrease in the consumption rate of the inhibitor is attributable only to the reduction of phenoxyl radicals to phenol. The amount of phenol (Δ[InH]) reduced in reaction (10) over a certain period of time is determined by the difference between the phenol concentrations in the presence ($[InH]_S$) and in the absence ([InH]) of the substrate related to the same irradiation time as $\Delta[InH] = [InH]_S - [InH]$. The AOx consumption rates in the presence and in the absence of the substrate and the rate of phenoxyl reduction, $\Delta[InH]/\Delta t$, were found from the kinetic curves (see Fig. 4, *b*).

The k_{10}^{eff} values were determined from the equation $\Delta[InH]/\Delta t = k_{10}^{eff}[RH][In^{\bullet}]$. The results are given in Table 2. The tocopheroxyl radicals are more reactive than the phenoxyl radicals derived from sterically hindered AOx, namely, dibunol and SO-4.

The variation of the consumption rate and the AOx regeneration rate in the presence of substrates with different unsaturation degrees has been studied. The substrates used included methyl oleate and linolic and arachidonic acids containing one, two, and four double bonds, respectively, and PL containing polyunsaturated fatty acids.

The data given in Table 2 show that during oxidation AOx are regenerated through the reaction of phenoxyl radicals with unsaturated compounds. The radicals derived from slightly hindered α-TP are reduced much more easily than phenoxyl radicals of sterically hindered AOx, dibunol and SO-4. It can be seen that the rate of regeneration of AOx and the reactivity of phenoxyl radicals in reaction (10) decrease as the OH group becomes more shielded and increase with an increase in the number of double bonds in the oxidation substrate (see Table 2). The possibility of regeneration of the active form of AOx during oxidation may also be favorable for efficient inhibition.

However, this occurs only in the case of low activity of alkyl and peroxyl radicals ($R^{\bullet}$ and $RO_2^{\bullet}$) in chain propagation reactions

$$In^{\bullet} + RH \xrightarrow{k_{10}} InH + R^{\bullet} \xrightarrow{O_2} RO_2^{\bullet} \xrightarrow{RH,\ k_2} ROOH + R^{\bullet}.$$

If the resulting radicals ($R^{\bullet}$ and $RO_2^{\bullet}$) are more reactive in reaction (2) than phenoxyl radicals, and the inhibitor reactivity in reaction (7) is relatively low, then the oxidation will be accelerated due to reaction (10). Thus, the inhibitory action of AOx may be related not only to their reactivity toward peroxyl radicals but also to the reactivity of phenoxyl radicals toward the oxidation substrate. Synergism will be observed if the radicals formed in the presence of the synergist are less reactive than $RO_2^{\bullet}$.

Synthetic AOx are widely used to stabilize systems containing natural inhibitors, for example, α-TP. Therefore, we studied the combined action of SO-4 and α-TP and determined their optimal concentrations, ensuring the highest inhibitory effect.

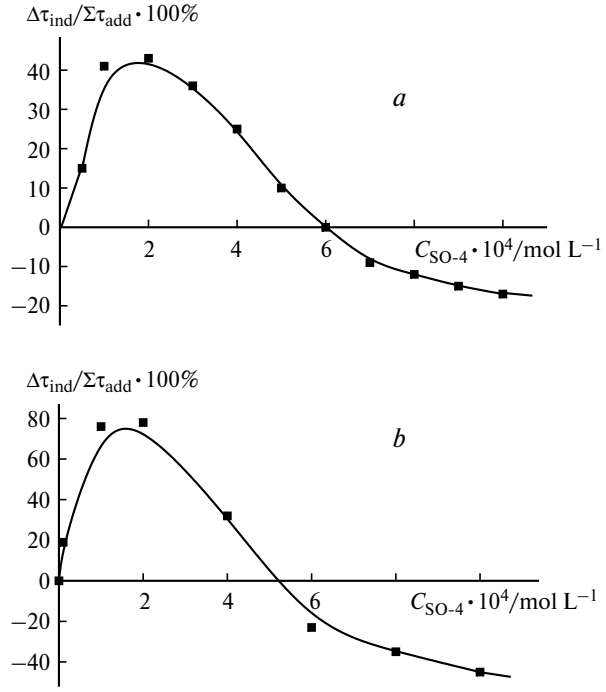

Fig. 5. Synergistic effect in α-TP + SO-4 (*a*) and mexidol + + SO-4 (*b*) blends *vs.* concentration of SO-4; $W_i = 4.2 \cdot 10^{-8}$ L mol^{-1} s^{-1}, T = 60 °C. Concentrations of α-TP (*a*) and mexidol (*b*) are $2.5 \cdot 10^{-4}$ and $1 \cdot 10^{-4}$ mol L^{-1}, respectively.

The efficiency of the binary mixture was studied in comparison with the expected additive effect based on individual AOx. The dependence of the synergistic effect on the concentration of each component was studied. The optimal concentration range of SO-4 is $(1.5—2.0) \cdot 10^{-4}$ mol L^{-1}. These concentrations of SO-4 ensure the highest synergistic effect (65—68%). The synergism increases with an increase in the α-TP concentration in the mixture (Fig. 5, *a*, Table 3).

The mechanism of combined action of components of the binary mixture is apparently as follows. The rate constant k_7 of α-TP-phenol (R^1OH) is high, and the antiradical activity of sterically hindered SO-4 (R^2OH) is much lower. The oxidation of α-TP gives reactive tocopheroxyl radicals,[22,24,25] while oxidation of hindered phenols yields unreactive phenoxyl radicals.[21,26,27] The first oxidation stages consume, first of all, the more reactive inhibitor, and the resulting tocopheroxyl radicals rapidly exchange hydrogen atoms with the hindered phenol by the reaction

$$R^1O^{\bullet} + R^2OH \rightleftharpoons R^1OH + R^2O^{\bullet}.$$

The reduced species of more reactive AOx can again terminate the oxidation chains:

$$R^1OH + R^2O_2 \longrightarrow R^2OOH + R^1O^{\bullet}.$$

Table 3. Synergistic effect *vs.* concentration of the antioxidants*

$C_{AOx} \cdot 10^{-4}$ /mol L^{-1}	τ_{ind}	τ_{add}	$\Sigma\tau_i$	$\Delta\tau$	$(\Delta\tau/\Sigma\tau_i) \cdot 100\%$
	min				
The α-TP + SO-4 mixture, C_{SO-4} = const = $1 \cdot 10^{-4}$ mol L^{-1}, τ_{ind} = 130 min					
0.25	60	230	190	40	17.4
2.50	160	510	290	220	76
5.00	350	970	480	490	102
7.50	450	1200	580	620	107
10.00	600	1210	730	480	66
15.00	800	980	930	50	5.4
The α-TP + SO-4 mixture, $C_{\alpha\text{-TP}}$ = const = $2.5 \cdot 10^{-4}$ mol L^{-1}, τ_{ind} = 160 min					
0.10	75	280	235	45	19
1.00	130	510	290	220	76
2.00	240	710	400	310	78
4.00	500	870	660	210	32
6.00	760	710	920	210	−23
8.00	900	690	1060	370	−35
The mexidol + SO-4 mixture, C_{SO-4} = const = $1 \cdot 10^{-4}$ mol L^{-1}, τ_{ind} = 130 min					
1.0	60	190	270	80	42.1
2.0	110	240	360	120	50.0
5.0	220	350	540	190	54.3
7.0	280	410	680	270	65.9
8.0	320	450	710	260	57.8
10.0	400	530	840	310	58.5
16.0	650	780	1230	450	57.7
20.0	800	930	1460	530	57.0
25.0	950	1080	1680	600	55.6
30.0	1100	1230	1910	680	55.3
35.0	1300	1430	2210	780	54.6
The mexidol (**A**) + SO-4 mixture, C_A = const = $1 \cdot 10^{-4}$ mol L^{-1}, τ_{ind} = 60 min					
0.5	80	140	160	20	14.2
1.0	130	190	270	80	42.1
2.0	240	300	430	130	43.3
4.0	500	560	740	180	24.3
5.0	580	640	720	80	11.1
7.0	800	860	790	−70	−8.9
10.0	1100	1160	980	−180	−18.4

* Conditions: methyl oleate as the oxidation substrate, $W_i = 4.2 \cdot 10^{-8}$ mol L^{-1} s^{-1}, T = 60 °C.

The phenoxyl radicals $R^2O^{\bullet}$ have low reactivity and they hardly participate in the subsequent chain process.

A study of the efficiency of combined action of binary mixtures comprising SO-4 and mexidol has shown that the concentration range of choice for SO-4 is $(1—5) \cdot 10^{-4}$ mol L^{-1} (see Fig. 5, *b*), and that for mexidol is $(2—20) \cdot 10^{-4}$ mol L^{-1} (see Table 3). These concentrations of SO-4 and mexidol ensure the highest synergistic effect (50%). These data show that the hindered phenols form highly efficient synergistic mixtures with hydroxypyridine derivatives.

Apparently, the synergistic effect for mexidol and SO-4 mixtures is enhanced owing to the ability of these AOx to destroy hydroperoxides without giving rise to free radicals, excluding the additional consumption route of AOx in the reaction with alkoxyl radicals.

The maximum efficiency of inhibition provided by the mixtures can be attained with neat mexidol in a concentration 2.5 as high as that in the synergistic blend.

Here we studied the action of PL blended with the oxidation inhibitor SO-4. The action of the mixture was compared with the effect of the reference AOx (dibunol and α-TP), which were shown previously to exhibit the synergistic effect with PL.[19,20] The combined action of AOx with PL was compared with the antioxidant effect from the individual components that form the mixture. It is known that PL do not inhibit the oxidation but can act as synergistic agents for AOx.

The kinetic parameters of oxygen absorption by methyl oleate in the presence of AOx and their mixtures with PL are given in Table 4. A comparison of the induction periods shows that a mixture of PL and AOx is more efficient than the single AOx. In addition, an increase in the induction times ($\Delta\tau$) and a decrease in the highest oxidation rate ($\Delta W_{O_2}^{max}$) were observed.

The following relations indicative of a synergistic action hold for AOx and PL mixtures: $\tau_i < \tau_\Sigma$ (τ_i and τ_Σ are the induction periods for methyl oleate oxidation in the presence of AOx and an AOx + PL mixture, respectively); $(\Delta W_{O_2}^{max})^1 > (\Delta W_{O_2}^{max})^2$ ($(\Delta W_{O_2}^{max})^1$ and $(\Delta W_{O_2}^{max})^2$ are the oxidation rates of methyl oleate in the presence of AOx and an AOx + PL mixture, respectively).

The dependence of the synergistic effect on the PL concentration in the mixture was studied. It can be seen in Fig. 6, *a* that the curves for all of these AOx are similar. In the $(0—3)\cdot10^{-3}$ mol L^{-1} range, the action of the mixture increases in direct proportion to the concentration, the next range, $(3—9)\cdot10^{-3}$ mol L^{-1}, is matched by an efficiency plateau, and a further increase in the PL concentration induces a decrease in the synergistic effect. The dependences of the synergistic effect of the AOx and PL blends on the AOx concentration are shown in Fig. 6, *b*. The efficiency of synergism decreases in the following sequence: α-TP > SO-4 > dibunol. Thus, in the presence of the same PL, the magnitude of the synergistic effect is determined by the chemical structure of the inhibitor. Note that the effect is most pronounced for unhindered phenols and least pronounced for sterically substituted AOx.

Table 4. Kinetic characteristics of methyl oleate oxidation in the presence of AOx and PL* mixtures

Antioxidant	τ_{ind}	τ_Σ	$\Delta\tau$	$(\Delta\tau/\Sigma\tau_i)\cdot100\%$	$(\Delta W_{O_2}^{max})^1$	$(\Delta W_{O_2}^{max})^2$
	min				10^7 mol L^{-1} s^{-1}	
α-TP	160	200	40	25.0	6.50	6.00
SO-4	240	300	60	20.9	12.10	4.35
Dibunol	190	210	20	9.5	6.30	5.80

* The concentrations of AOx and PL were $2\cdot10^{-4}$ and $5\cdot10^{-4}$ mol L^{-1}, respectively, $W_i = 4.2\cdot10^{-8}$ mol L^{-1} s^{-1}, $T = 60$ °C; $(\Delta W_{O_2}^{max})^1$ and $(\Delta W_{O_2}^{max})^2$ are the oxidation rates in the presence of an AOx or its mixture with a PL, respectively.

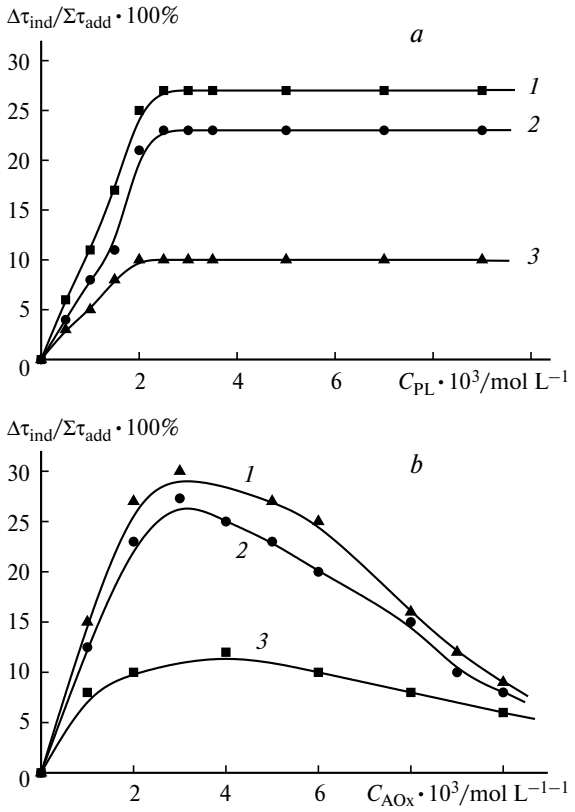

Fig. 6. *a*. Induction times in the synergistic compositions of various AOx with PL *vs.* concentration of PL: (*1*) α-TP, (*2*) SO-4, (*3*) dibunol; $W_i = 4.2\cdot10^{-8}$ mol L^{-1} s^{-1}, $C_{AOx} = 2\cdot10^{-4}$ mol L^{-1}, $T = 60$ °C. *b*. Synergistic effect in the combined action of PL with various AOx *vs.* their concentration: (*1*) α-TP, (*2*) SO-4, (*3*) dibunol; $W_i = 4.2\cdot10^{-8}$ mol L^{-1} s^{-1}, $C_{PL} = 5\cdot10^{-3}$ mol L^{-1}, $T = 60$ °C.

The mechanism of the synergistic effects in the combined action of α-TP and PL was studied previously.[9] It was shown by the direct method that the polyunsaturated fatty acids incorporated in PL promote the reduction of the reactive phenolic form of α-TP. This decreases participation of α-TP in side reaction (10) leading to additional initiation of the process. Amino alcohols (ethanolamine, choline) present in the PL structure can destroy hydroperoxides by a nonradical way. This excludes one more consumption route of α-TP in the reaction with hydroxyl and alkoxyl radicals formed upon homolytic decomposition of hydroperoxides.

Thus, the synergistic compositions show real prospects for wide use as agents for preservation of the proper-

ties and extension of shelf lives of biologically active lipids, foodstuffs, medicines, and cosmetics.

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