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Antiradical Activity of Morpholine- and Piperazine-Functionalized Triphenylantimony(V) Catecholates

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Abstract—The antiradical activity of the functionalized triphenylantimony(V) catecholates Ph_3Sb[4-$O(CH_2CH_2)_2N$-3,6-DBCat] (**I**), Ph_3Sb[4,5-Piperaz-3,6-DBCat] (**II**), and Ph_3Sb[4-$PhN(CH_2CH_2)_2N$-3,6-DBCat] (**III**) (where $[4\text{-}O(CH_2CH_2)_2N\text{-}3,6\text{-}DBCat]^{2-}$, $[4,5\text{-Piperaz-}3,6\text{-DBCat}]^{2-}$, and $[4\text{-}PhN(CH_2CH_2)_2N\text{-}3,6\text{-DBCat}]^{2-}$ are the dianionic ligands 3,6-di-*tert*-butyl-4-(morpholin-1-yl)-, 3,6-di-*tert*-butyl-4,5-(piperazine-1,4-diyl)-, and 3,6-di-*tert*-butyl-4-(4-phenylpiperazin-1-yl)catecholates, respectively) was studied in reactions with the diphenylpicrylhydrazyl radical during autooxidation of unsaturated fatty (oleic and linoleic) acids with lipid peroxidation of Russian sturgeon (*Acipenser gueldenstaedti* B.) sperm and human blood erythrocytes in vitro as examples. The EC_{50} and n_{DPPH} values obtained indicate the high antiradical activity of complexes **II** and **III** in the reactions with the stable radical. On the whole, complexes **I–III** inhibit the lipid peroxidation in both model (oxidation of unsaturated fatty acids) and in vitro experiments. The inhibiting effects of the complexes are comparable with and even, in some cases, higher than those of the known antioxidant ionol.

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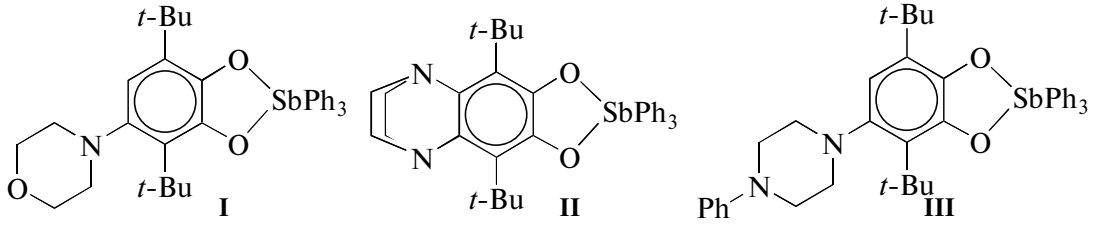
The coordination chemistry of antimony compounds is an extensively developed field in modern organometallic chemistry. Antimony(III/V) complexes are attractive by their multiple ways of coordination and pharmacological activity [1–4]. Organoantimony compounds find use in organic synthesis as well [5]. The well known antimony complexes include those employed as antihelminthics and in the treatment of leishmaniasis (potassium antimony(III) tartrate, antimony(III) gluconate, meglumine antimonate(V), and sodium stibogluconate) [6, 7]. Inorganic antimony(III/V) derivatives are known to be genotoxic in vivo and in vitro; their toxic effect is due to irreversible binding to thiol-containing fragments of proteins and enzymes [8]. At the same time, data on the toxicity of organoantimony compounds are scarce [9, 10]. Antimony(V) derivatives are less toxic than antimony(III) ones and are considered to be precursors of pharmacologically active forms [1].

Currently available data on the mechanism of the toxic action of antimony compounds are fragmentary. In relevant studies, the toxicity of antimony(III) compounds is attributed to the generation of reactive oxygen species (ROS) and the initiation of oxidative stress [11–14]. The toxicity and physiological effect of antimony complexes depend on both the oxidation state of antimony and the organic ligands attached to it. The properties of antimony complexes vary with the ligand nature, so the toxic effect can intentionally be canceled by making changes to the coordination environment of antimony. A combination of redox-active ligands with heavy metals gives rise to unusual physicochemical properties [15–19]. Main group metal complexes with redox-active ligands is characterized by more active participation of the ligands (rather than the metal) in chemical transformations [20–22].

Earlier, triphenylantimony(V) complexes with catecholate and *o*-amidophenolate ligands have been found to exhibit antiradical activity [23]. These complexes can reversibly bind molecular oxygen [24–26]. In a search for new antimony derivatives with unusual properties, we obtained triphenylantimony(V) complexes with catecholate ligands containing electron-donating fragments in positions 4 and 4,5 of the benzene ring (**I–III**) [27]:



In this work, we further studied the antiradical activity of triphenylantimony(V) complexes with substituted catecholate ligands in reactions with the radical DPPH, in the inhibition of the oxidation of oleic (*cis*-octadeca-9-enoic) and linoleic (*cis*,*cis*-octadeca-9,12-dienoic) acids, and in the in vitro oxidation of Russian sturgeon (*Acipenser gueldenstaedti* B.) sperm lipids and human blood erythrocytes in vitro. The possible existence of the ligands in several redox forms (catecholate/*o*-semiquinolate/*o*-benzoquinone) and introduction of a nitrogen-containing redox site as the piperazine (N-phenylpiperazine) and morpholine fragments into the ligand structure (these fragments are found in many drugs) enable complexes **I–III** to react with free radicals and to be regarded as potential inhibitors of free-radical processes.

EXPERIMENTAL

Commercial chemicals Ph_3Sb (99%, Aldrich), Ph_3SbCl_2 (99%, Aldrich), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich), 2,6-di-*tert*-butyl-4-methylphenol (ionol, 99%, Fluka), $[(C_5H_5)_2Fe]BF_4$ (Aldrich), oleic (*cis*-octadeca-9-enoic) acid (97%, Acros Organics), and linoleic (*cis*,*cis*-octadeca-9,12-dienoic) acid (99%, Acros Organics) were used as purchased. Complexes **I–III** and 5,8-di-*tert*-butyl-1,4-ethano-2,3-dihydroquinoxaline-6,7-dione (4,5-Piperaz-3,6-DBBQ) were prepared as described in [27, 28].

The antiradical activity of complexes **I–III** in reactions with DPPH in CH_2Cl_2 was determined as described in [23]. Electronic absorption spectra were recorded on an SF-103 spectrophotometer (220–1100 nm) at room temperature. EPR spectra were recorded on a Bruker EMX spectrometer (~9.5 GHz); HFC constants were determined by modeling theoretical EPR spectra with the Simfonia program (Bruker).

The reduction potential of 4,5-Piperaz-3,6-DBBQ was measured by cyclic voltammetry (CV) in a three-electrode cell on an IPC-pro potentiostat in acetonitrile under argon. The working electrode was a stationary glassy carbon electrode 2 mm in diameter, the auxiliary electrode was a platinum plate (S = 18 mm^2), and the reference electrode was Ag/AgCl/KCl with a waterproof bag. The concentration of 4,5-Piperaz-3,6-DBBQ was 0.003 mol/L. The number of electrons transferred in the electrode reaction was assessed with respect to ferrocene used as a standard. The potential scan rate was $0.2\ V \cdot s^{-1}$. A 0.1 M solution of Bu_4NClO_4 (99%, Acros) was used as a supporting electrolyte.

Oleic acid was oxidized in the presence of complexes **I–III**, 4,5-Piperaz-3,6-DBBQ, and ionol in a temperature-controlled cell (60°C) through which air was bubbled at a rate of 2–4 mL/min for 5 h. Because this process follows an autooxidation pattern, addition of the reagents was preceded by 2-h air bubbling. The concentration of the additives was 1 mmol/L. The activity of the compounds under study was estimated according to a standard procedure [29] by measuring the amounts of isomeric hydroperoxides L'OOH formed in the oxidation of oleic acid

Autooxidation of linoleic acid in the presence of complexes **I** and **II**, Ph_3Sb, Ph_3SbCl_2, and ionol was carried out in a thermostat (30°C) for 120 h. After 24 h, three samples were withdrawn to determine the concentration of the isomeric hydroperoxides by iodometric titration. Carbonyl compounds were quantified in a test with thiobarbituric acid; the concentration of the resulting colored pigment was determined by spectrophotometry for a wavelength of 532 nm [30].

We used packed red blood cells extracted from the venous blood of healthy donors to study the lipid peroxidation (LP) of human blood erythrocytes. The compounds under study were dissolved in ethanol (0.01 mL) and added to specimens. The concentration of the complexes in the system was 0.1 mmol/L. The level of the LP of erythrocytes was determined as described in [31].

The concentration of L'OOH in the LP was determined by the standard thiobarbiturate assay [32] for the sperm of Russian male sturgeons (*Acipenser gueldenstaedti* B.) provided by the fish hatcheries Bertyul'skii and Lebyazhii in 2011.

A 1-mL sample of Russian sturgeon sperm was diluted with a cooled (0–4°C) 1.2% solution of KCl (20.5 mL). The working solution was divided into six parts: one blank and five test specimens containing the compounds under study (**I**, **II**, Ph_3Sb, Ph_3SbCl_2, and ionol). The compounds were dissolved in $CHCl_3$ (0.01 mL) and added to specimens. The concentration of the compounds in the system was 0.1 mmol/L. Five 2.0-mL samples of each test specimen were withdrawn and transferred to test tubes. Then solutions of ascorbic acid (0.1 mL), Mohr's salt (0.1 mL), and trichloroacetic acid (1 mL) were added and the test tubes were heated on a water bath at 37°C for 10 min and centrifuged at 3000 rpm for 10 min. The supernatants (2 mL) were sampled and transferred to clean test tubes and a solution of thiobarbituric acid (1 mL) was added in each tube. The samples were heated on a boiling water bath for 10 min and cooled in ice water to room temperature. Then the samples were diluted with chloroform (1.0 mL) to make them transparent and the resulting solutions were centrifuged at 3000 rpm for 15 min. The supernatants were sampled and the molar absorption of each sample was measured on an SF-103 spectrophotometer at 532 nm and compared with that of the blank specimen. The concentration was determined at time intervals of 1, 3, 24,

Table 1. Antiradical activity of complexes **I**–**III** in the reactions with DPPH*

Complex	E^{ox}, V [23, 27]	$k\ 10^{-2}$, s^{-1}	EC_{50}, μmol	n_{DPPH}
$Ph_3Sb[4\text{-}O(CH_2CH_2)_2N\text{-}3,6\text{-}DBCat]$ (**I**)	0.76	0.91	25.5	1.0
$Ph_3Sb[4,5\text{-}Piperaz\text{-}3,6\text{-}DBCat]$ (**II**)	0.74	0.98	11.1	2.2
$Ph_3Sb[4\text{-}PhN(CH_2CH_2)_2N\text{-}3,6\text{-}DBCat]$ (**III**)	0.70	1.64	15.3	1.7
$Ph_3Sb[3,6\text{-}DBCat]$	0.89	0.66	17.3 [23]	1.5
Ph_3Sb [AP-Me]	0.55	1.89	14.0 [23]	1.9

* The electrochemical oxidation potentials determined by cyclic voltammetry (glassy carbon electrode, CH_2Cl_2, 0.1 M n-Bu_4NClO_4, $c_i = 3 \times 10^{-3}$ mol/L, Ar, vs. Ag/AgCl/KCl (sat.)); E^{ox} is the peak potential of the first anodic process; k is the pseudofirst-order rate constant; EC_{50} is the antiradical activity index of complexes **I**–**III** in the reactions with DPPH; n_{DPPH} is the average stoichiometric number of DPPH molecules per complex molecule.

and 48 h; the samples were incubated at 0–3°C between the concentration measurements.

RESULTS AND DISCUSSION

Reactions of DPPH with complexes **I**–**III** were studied in deaerated CH_2Cl_2 at 298 K. Addition of complexes **I**–**III** to a solution of DPPH lowers the intensity of the absorption peak at 527 nm, which suggests their interactions. A kinetic study of reactions of DPPH with complexes **I**–**III** used in excess showed that these are pseudofirst-order reactions with respect to DPPH (Table 1).

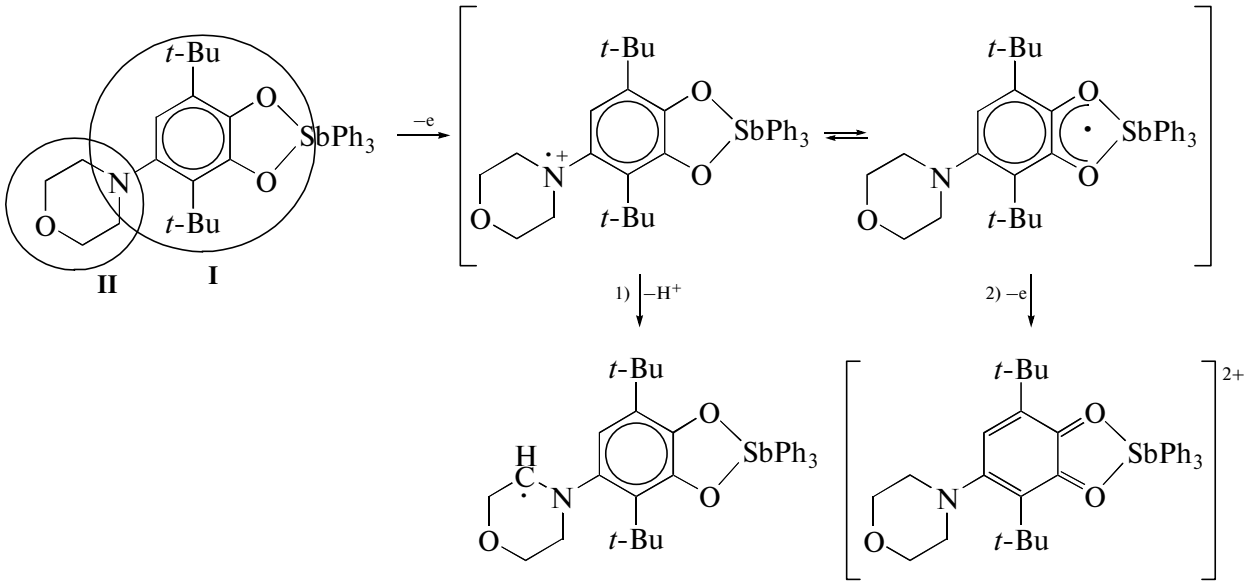
The oxidation potentials of the complexes are more electropositive (Table 1) than the reduction potential of DPPH (0.3 V). Therefore, the Gibbs energy increases during this reaction [33]. Reactions of DPPH with the complexes studied are thermodynamically unfavorable; however, the course of the reaction suggests that triphenylantimony(V) complexes are efficient radical scavengers. When moving from complex **I** to **III**, the pseudofirst-order rate constant (k) increases, with an accompanying decrease in the oxidation potential. The oxidation potentials of complexes **I**–**III** are shifted to more negative values compared to those of known antioxidants (e.g., 1.50 (ionol) and 0.97 V (α-tocopherol) [34]). Obviously, the easier the oxidation, the higher the probability of reactions with radicals. As expected, complex **III** is most reactive because its anodic oxidation potential is the lowest among the antimony complexes under consideration.

The possibility of reactions occurring between the complexes and DPPH allows complexes **I**–**III** to be regarded as efficient radical scavengers, which is confirmed by EC_{50} values (Table 1). The lower EC_{50} values of complexes **II** and **III** (Table 1) suggest their higher efficiency in reactions with DPPH compared to the morpholine derivative. The antiradical activity of complexes **II** and **III** is close to that of triphenylantimony(V) derivatives with sterically hindered *o*-amidophenolate (Ph_3Sb[AP-Me]) and catecholate ligands (Ph_3Sb[3,6-DBCat]) (Table 1).

There is a correlation between the average stoichiometric number of converted DPPH molecules (n_{DPPH}) and the number of electrons transferred in the electrochemical process [35]. For complexes **I**–**III**, the total number of electrons involved in the anodic reactions varies from 1.5 to 2 [27]. In the case of complexes **II** and **III**, the number of electrons transferred in the anodic reactions is nearly equal to n_{DPPH}. The EC_{50} and n_{DPPH} values of complex **I** indicate its moderate antiradical activity.

To determine the site under initial radical attack, we studied a reaction of complex **I** with silver(I) triflate (CF_3SO_2OAg), a one-electron oxidant, by EPR spectroscopy. The complicated EPR spectrum of the reaction mixture (Fig. 1a) is a superposition of two spectra with $g_i = 2.0034$ (the extreme components of this spectrum are indicated with arrows in Fig. 1b) and $g_i = 2.0032$; one of these spectra can obviously be assigned to the degradation product of the complex. The second spectrum has a complicated hyperfine structure (HFS), which is resolved in dilute solution (Fig. 1b) and is due to a hyperfine coupling (HFC) of an unpaired electron with the magnetic nuclei of antimony (^{121}Sb, ^{123}Sb), nitrogen, the proton of *o*-semiquinone ring, and a group of the protons at the α-C atoms of the morpholinyl substituent. The exact values of the HFS constants cannot be determined (their approximate values are 4, 2.1, 3.4–3.6, 0.4–0.6, and 0.6–0.8 Oe for A(^{121}Sb), A(^{123}Sb), A(^{1}H), A(^{14}N), A(4 ^{1}H), respectively).

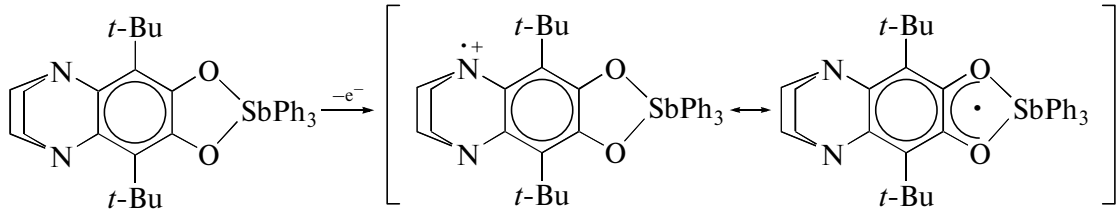
The reaction of DPPH with complex **I** proceeds through the formation of an intermediate radical cation in which the electron density is delocalized over the morpholine (II) and catecholate fragments (I):



The existence of two resonance forms affords two possible pathways of subsequent transformations. Quasireversible oxidation of *o*-semiquinone takes place in an electrochemical cell (pathway 2) [27]. A reaction with DPPH, as suggested by the n_{DPPH} value, follows pathway 1 with subsequent transformation of the morpholine ring.

Oxidation of complex **II** with ferricenium tetrafluoroborate in toluene gives a paramagnetic product (Fig. 2). The HFC of an unpaired electron with the magnetic nuclei of antimony, two nitrogen atoms, and four protons of the piperazinyl substituent in combination with the relatively wide own EPR line ($\Delta H \approx 2$ Oe) precludes determination of the HFC constants. However, the spectral pattern suggests delocalization of the unpaired electron over both the *o*-semiquinone fragment and the N atoms and the protons of the piperazine group, which are out of the plane of *o*-semiquinone ring and can immediately overlap the *p*-MO of the unpaired electron.

Electrochemical oxidation of complex **II** is irreversible in the first step, with possible intramolecular electron transfer between the N atom and the five-membered chelate ring through the aromatic linker, which gives rise to coordinated *o*-semiquinolate [27]:



In this case, secondary interactions with DPPH can involve the *o*-semiquinone fragment, which is an additional electron donor; this accounts for the n_{DPPH} value of complex **II**. According to the n_{DPPH} value of complex **III**, two spatially distant redox sites should participate in the reaction with DPPH. The antiradical activity of complexes **II** and **III** is close to that of known antioxidants: $n_{DPPH} = 2.0$ for ascorbic acid and $n_{DPPH} = 2.3$ for pyrocatechol [35].

A study of the oxidation of unsaturated fatty acids is a model reaction for lipid peroxidation in cell membranes. Oxidation of unsaturated acids L'H with oxygen generates substituted allylic radicals, which react with O_2 to produce peroxyl radicals L'OO$^{\cdot}$. Hydroperoxides L'OOH are main products in the initial step of the process, so the rate of the transformation L'OO$^{\cdot}$ L'OOH can serve as a criterion for estimation of the initial rate of peroxide oxidation [36, 37]. We studied the effect of complexes **I**–**III** and 4,5-Piperaz-3,6-DBBQ on the peroxide oxidation of lipids in such model reactions as oxidation of oleic acid at 60°C and autooxidation of linoleic acid at 30°C.

Complexes **I**–**III** inhibit the oxidation of oleic acid (Fig. 3). In the presence of complexes **I** and **III**, the concentration of hydroperoxides remains nearly the same as in the blank entry during the first hour. Addi-

tion of complex **I** lowers the concentration of L'OOH (Fig. 3, curve *1*), producing an inhibiting effect on the radical chain process compared to the blank experiment (Fig. 3, curve *5*). In the presence of complex **III**, the concentration of L'OOH remains virtually unchanged, which corresponds to the induction period (Fig. 3, curve *3*). Addition of complex **II** monotonically lowers the concentration of L'OOH in the system; moreover, the hydroperoxides contained in oleic acid at the instant the complex was added decompose slightly. However, complex **II** is inferior in hydroperoxide decomposition to previously studied triphenylantimony(V) catecholates [23].

In the presence of the *o*-quinone 4,5-Piperaz-3,6-DBBQ, the concentration of L'OOH increases in the early reaction (0–2 h); i.e., this compound acts as a promoter (Fig. 3, curve *4*). Depending on the conditions, quinones can show anti- and/or prooxidant activity. During the reaction, the solution decolorizes because of diminution of the absorption peak (λ_{max} = 420 nm) characteristic of the *o*-quinone system [28]. Reduction of 4,5-Piperaz-3,6-DBBQ leads to a catechol with inhibiting activity, which subsequently lowers the level of hydroperoxides in the system.

The effects of the compounds under study on the oxidation of oleic acid are compared in Fig. 4. We found that complexes **II** and **III** and the *o*-quinone 4,5-Piperaz-3,6-DBBQ are comparable to ionol in antioxidant activity.

We studied the effects of complexes **I** and **II**, ionol, Ph_3Sb, and Ph_3SbCl_2 on the hydroperoxide concentration in the autooxidation of linoleic acid at 30°C for 120 h (Fig. 5). Unstable hydroperoxides of unsaturated fatty acids readily decompose into various products (alcohols, aldehydes, and ketones), malonic dialdehyde and 4-hydroxynonenal being most toxic. For this reason, we used a thiobarbiturate assay also to assess the formation of carbonyl compounds from the amount of thiobarbituric acid reactive substances (TBARS).

In a blank entry, the concentrations of hydroperoxides and TBARS increase monotonically (Figs. 5 and 6). In [23], the promoting effect of triphenylstibine has been noted in the oxidation of oleic acid. In the autooxidation of linoleic acid, Ph_3Sb increases the concentrations of L'OOH and TBARS, thus acting as a promoter. Addition of Ph_3SbCl_2 considerably increases the concentration of L'OOH (840 mmol/L) and somewhat increases the concentration of TBARS compared to that in the blank entry (c_{TBARS} = 810 nmol/mL). The compounds Ph_3Sb and Ph_3SbCl_2 behave like organometallic derivatives of heavy metals and promote the peroxide oxidation of lipids [38].

We found that complexes **I** and **II** differently affect the autooxidation of unsaturated acid. Throughout the experiment, the concentration of L'OOH in the presence of complex **II** virtually does not grow, so the complex shows an inhibiting effect similar to that observed for oleic acid (Fig. 5, curve *3*). Ionol inhibits the formation of L'OOH (Fig. 5, curve *4*) more efficiently than does complex **II**. In the initial step (0–48 h), morpholine-containing complex **I** has a short-time inhibiting effect fading with time (Fig. 5, curve *2*); then it acts as a promoter and the concentration of L'OOH in the system exceeds that in the control experiment.

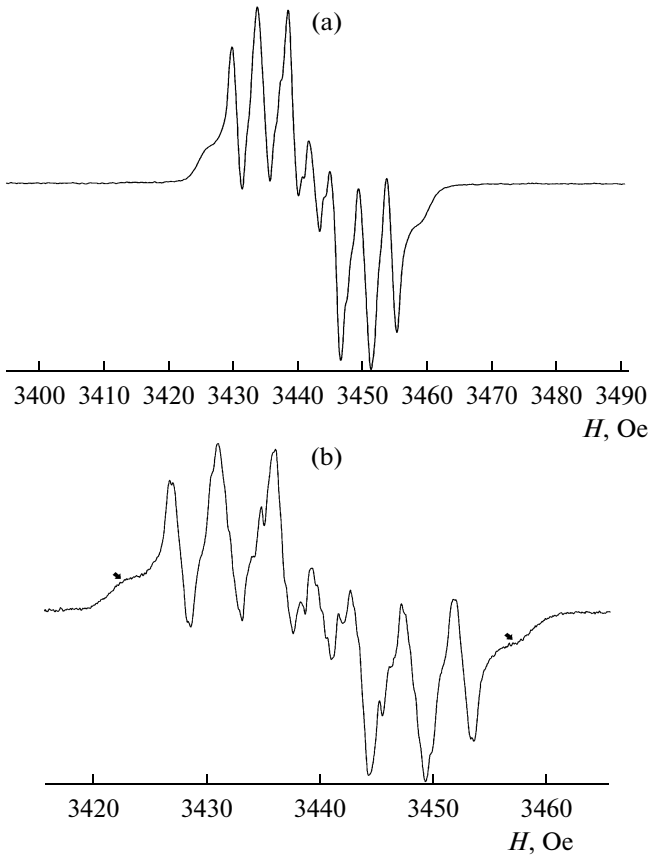


Fig. 1. EPR spectra of (a) the system $Ph_3Sb[4\text{-}O(CH_2CH_2)_2N\text{-}3,6\text{-}DBCat]{-}CF_3SO_2OAg$ and (b) the same system diluted with toluene (293 K).

When measuring the TBARS level after 72 and 120 h, we found that this level in the presence of complexes **I** and **II** and ionol is lower than that in the blank entry; i.e., these compounds have inhibiting effects (Fig. 6). After 72 h, the concentration of TBARS differs only slightly from the initial TBARS level (1 h) in the blank entry (72 nmol/mL); the increase in the concentration of carbonyl compounds in the final step (120 h) indicates the weakened inhibiting effects of these complexes and ionol. Compared to the blank entry, the TBARS concentration in the presence of morpholine-containing complex **I** does not increase in the final step; in contrast, the hydroperoxide concentration does go up. The discrepancy between the levels of TBARS and L'OOH can be attributed to the higher rate of hydroperoxide accumulation compared to the rate of hydroperoxide decomposition into molecular products.

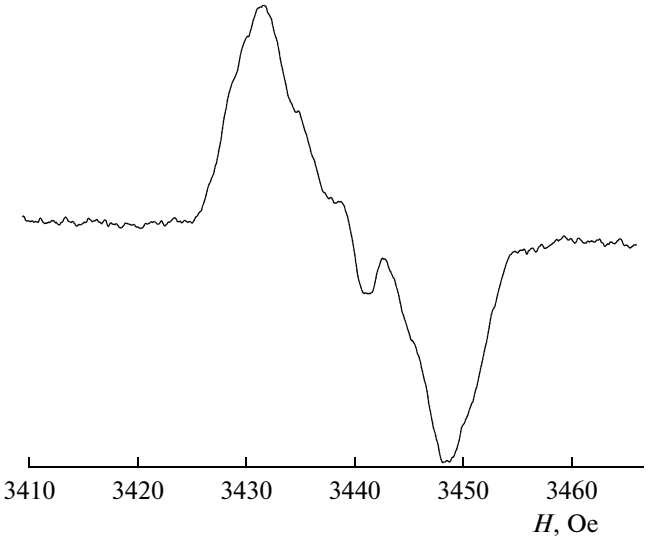
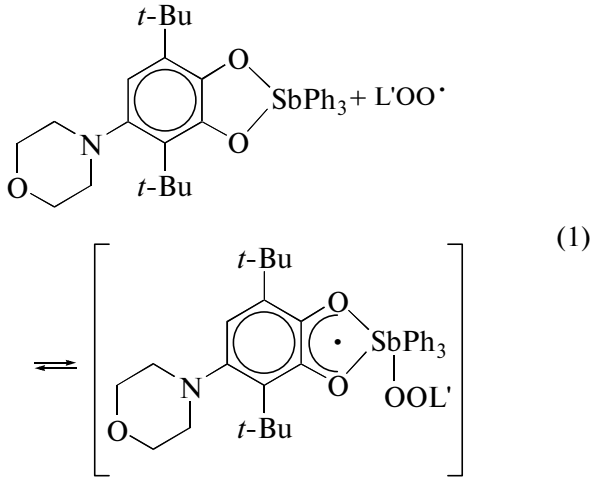


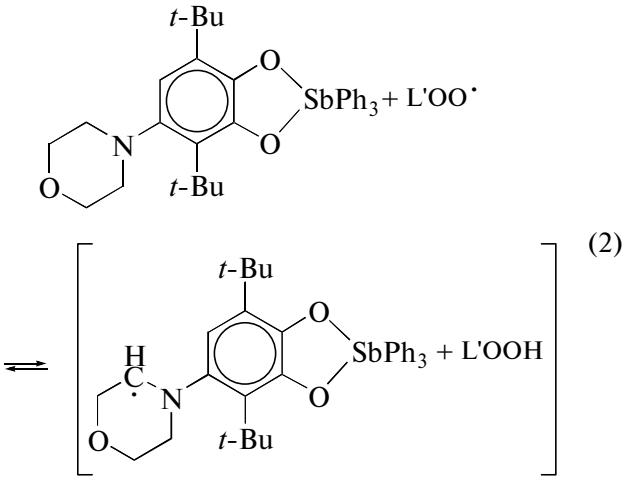
Fig. 2. EPR spectrum of the system Ph_3Sb[4,5-Piperaz-3,6-DBCat]–$FcBF_4$ (toluene, 293 K).

It is known that main group metal (Ge, Sn, and Pb) catecholates can scavenge various radicals to form mono- or bis(*o*-semiquinone) derivatives [22, 39–42]. Recent studies are devoted to the antiradical activity of triphenylantimony(V) complexes with redox-active ligands [23]. The mechanism of inhibition of free-radical processes by many phenolic compounds consists in transfer of a hydrogen atom or in sequential deprotonation and electron stripping leading to the formation of stable aroxyl radicals. Unlike phenol derivatives, the ligands of the compounds under study contain redox sites capable of interacting with radicals L'OO in two ways: by electron transfer to the catecholate chelate ring forming a complex with the coordinated *o*-semiquinolate upon the oxidation:



(1)

and by abstraction of a hydrogen atom from the morpholine (piperazine) fragment:



(2)

The presence of an additional redox site (morpholine or piperazine fragment) enables the second reaction to occur, through the activity of peroxyl and hydroperoxyl radicals toward the weakest α-C–H bond next to the amino group in substituted amines [43].

Intermediates in the reactions with radicals L'OO differ in stability. Unlike free *o*-semiquinone radicals, those coordinated to a metal center are relatively stable. The stabilization of the radical anion of a ligand via its coordination to a heavy metal atom greatly shifts the *o*-semiquinone/*o*-benzoquinone transition potential to more positive values, which affects the reactivity of the resulting species toward active radicals.

The secondary carbon-centered radical formed in the reaction is less stable and can, on the one hand, undergo further transformations involving the opening of the saturated ring or, on the other hand, react with oxygen to give α-aminoalkylperoxyl radical, which

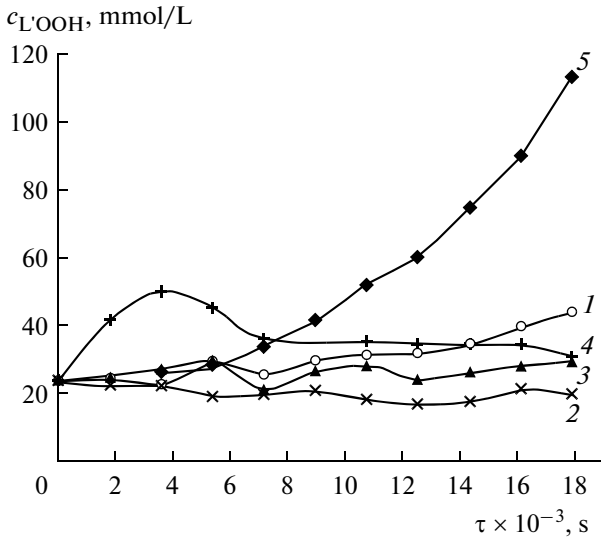


Fig. 3. Kinetic curves of L"OOH accumulation in the oxidation of oleic acid at 60°C in the presence of (*1*) **I**, (*2*) **II**, (*3*) **III**, and (*4*) 4,5-Piperaz-3,6-DBBQ (c = 1 mmol/L); curve (*5*) refers to the control.

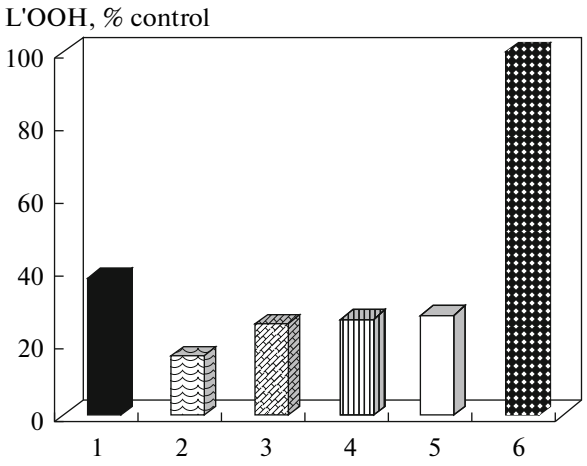


Fig. 4. Relative content of L'OOH in oleic acid oxidized at 60°C for 5 h in the presence of (1) **I**, (2) **II**, (3) **III**, (4) 4,5-Piperaz-3,6-DBBQ, and (5) ionol (c = 1 mmol/L); bar (6) refers to the control experiment without additives and is taken to be 100%.

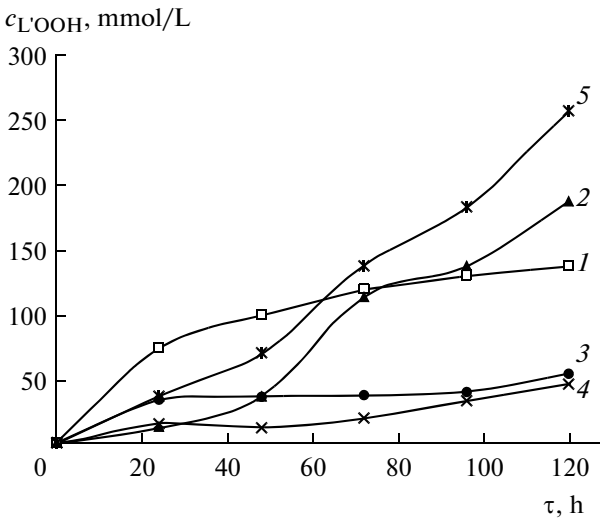


Fig. 5. Kinetic curves of L'OOH accumulation in the autooxidation of linoleic acid at 30°C in the presence of (*2*) **I**, (*3*) **II**, (*4*) ionol, and (*5*) Ph_3Sb (c = 1 mmol/L); curve (*1*) refers to the blank experiment.

initiates a chain oxidation process [43]. The inhibiting effect changed to a promoting one only for complex **I** in the autooxidation of linoleic acid. Such a decrease in the antiradical activity of complex **I** is probably due to secondary chemical transformations of the morpholine fragment.

However, main group metal complexes with *o*-semiquinones can also be transformed through elimination of the trapped radical and undergo disproportionation or decoordination of the *o*-semiquinone [39–41, 44]. Triphenylantimony(V) complexes produced by reaction (1) are potential sources of free *o*-semiquinolate radical anions which can react with radicals L'OO, disproportionate into *o*-quinone and pyrocatechol, and exchange an electron with oxygen to generate a superoxide radical anion and *o*-quinone.

The equilibrium of the reaction "*o*-semiquinone radical anion–oxygen" also largely depends on the position of the reduction potential of the redox couple "*o*-quinone–*o*-semiquinone" relative to "oxygen–superoxide radical anion" [45]. If the reduction potential of *o*-quinone is more positive than that of oxygen, then the equilibrium is shifted toward the starting *o*-semiquinolate radical anion. Depending on the solvent used, the reduction potential of oxygen in nonaqueous media is –0.8 to –0.9 V. In the study of the electrochemical properties of 4,5-Piperaz-3,6-DBBQ, we found that the potential of the half-wave of the reversible reduction is –0.67 V (CH_3CN, vs. Ag/AgCl/KCl). The reduction potential of this *o*-quinone is more electropositive than that of molecular oxygen, so the equilibrium of its reaction with oxygen will shift toward the starting *o*-semiquinone radical anion. Because of this, the *o*-quinone liberated by the degradation of complex **II** will scavenge the superoxide radical anion, thus exhibiting antioxidant activity.

The toxic effect of unsubstituted quinones is often manifested in reactions with thiols (glutathione), which yield the corresponding catechols and disulfides; this can change the intracellular redox balance. The negative effect of quinones can also arise from their participation in the Michael reaction [46]. In complex **II**, the positions 4 and 5 of the aromatic ring are occupied, which precludes the addition of a nucleophile. *o*-Quinones produced by degradation of

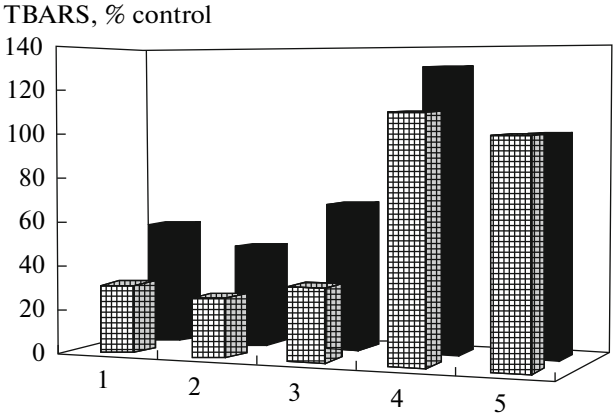


Fig. 6. Changes in the relative TBARS content in the autooxidation of linoleic acid at 30°C in the presence of (1) **I**, (2) **II**, (3) ionol, and (4) Ph_3Sb (c = 1 mmol/L); bar (5) refers to the control and is taken to be 100%. The absolute TBARS values are 91.52 ± 6.50 (1), 78.45 ± 1.12 (2), 98.10 ± 6.72 (3), 326 ± 7.54 (4), and 296 ± 13.1 nmol/mL (5) after 72 h (▦) and 222.20 ± 9.80 (1), 183.00 ± 13.20 (2), 267.90 ± 19.54 (3), 523.70 ± 10.30 (4), and 400.95 ± 33.6 nmol/mL (5) after 120 h (■).

Table 2. Concentration changes for the initial TBARS (malonaldehyde) in the Russian sturgeon (*Acipenser guldenstaedti* B.) sperm during the in vitro prolonged LP in the presence of the compounds under study (c_i = 0.1 mmol/L)

Compound	c_{TBARS}, nmol/mL			
	1 h	3 h	24 h	48 h
Ph_3Sb[4-O(CH_2CH_2)$_2$N-3,6-DBCat]	13.72 ± 0.62	6.23 ± 0.50	7.17 ± 0.56	12.39 ± 0.73
Ph_3Sb[4,5-Piperaz-3,6-DBCat]	8.26 ± 0.71	6.86 ± 0.56	6.60 ± 0.14	10.50 ± 0.89
Ph_3Sb	15.20 ± 1.15	18.80 ± 0.53	21.15 ± 0.92	20.40 ± 1.31
Ph_3SbCl_2	15.60 ± 0.92	18.69 ± 1.22	21.10 ± 1.32	22.03 ± 1.12
Ionol	17.70 ± 0.84	13.20 ± 0.67	17.20 ± 0.77	16.90 ± 0.69
Blank experiment	15.67 ± 1.18	18.95 ± 0.65	19.27 ± 0.56	20.30 ± 0.72

complexes **I** and **III** are very unlikely to react with nucleophiles since the nucleophilic substitution rate depends on the steric accessibility of position 5 and the vicinity of an electron-donating amino group in the heterocycle. The latter effect considerably weakens the electrophilicity of the carbon atom under attack and can decrease the probability of this reaction.

Earlier, it has been discovered that antimony(V) derivatives induce the generation of active oxygen forms (including superoxide radical anion, hydrogen peroxide, and nitrogen monoxide) in in vivo experiments and in human blood studied in vitro [47, 48]. The generation of ROS can indirectly be confirmed by measuring the concentration of their metabolites (specifically, LP products). We studied the effects of complexes **I**–**III**, 4,5-Piperaz-3,6-DBBQ, and ionol on the enzymatic and ascorbate-dependent peroxide oxidation of erythrocyte lipids of human blood taken from healthy donors (Fig. 7). The LP level was estimated from the accumulation of carbonyl compounds detected as colored complexes with thiobarbituric acid (TBARS).

We found that the TBARS level in an enzymatic reaction in the presence of the antimony complexes and substituted *o*-benzoquinone under study is lower than that in the blank entry. Complexes **I** and **III** inhibit LP more strongly than does ionol. In the metal-catalyzed reactions, addition of the antimony complexes virtually does not change the concentration of carbonyl compounds against that in the blank entry. Complex **III** has a slight inhibiting effect. Addition of Fe^{2+} ions to the system containing 4,5-Piperaz-3,6-DBBQ increases the TBARS level, which suggests its promoting effect.

For enzymatic reactions, the decrease in the TBARS concentration in the presence of the compounds under consideration is more pronounced; i.e., they can affect the activity of the enzymes lipoxygenases (cyclooxygenases). Free radical scavengers are known to inactivate lipoxygenases by reacting with radical intermediates or by reducing the Fe^{3+} ion in the active enzyme form into inactive Fe^{2+}. Apparently, the inhibiting effects of complexes **I**–**III**, as in the case of 4-aminophenol, catechol, and hydroquinone, are due to the reduction of Fe^{3+} in the active site of the enzyme and the formation of stable radical intermediates [49, 50].

A considerable concentration of polyunsaturated acids in sperm makes it very sensitive to harmful effects of free radicals; moreover, sperm contains few enzymes of the antioxidant protective system. Lipid oxidation products are toxic for sperm, thus diminishing its biological value [51]. For this reason, we studied the effects of complexes **I** and **II** and triphenylstibine on in vitro peroxide oxidation of Russian sturgeon sperm lipids for a long period of time when the LP becomes more intense. According to the data obtained (Table 2), the presence of complexes **I** and **II** in the system lowers the TBARS level throughout the experiment against that in the blank entry, which suggests their inhibiting effect on LP.

For complexes **I** and **II**, the most appreciable decrease in the sperm lipid peroxidation level (60–65%) compared to the blank entry was observed 3 and 24 h after the reaction started (Table 2); in the final step, their efficiency drops by 10–20%. Complex **II** proved to be the best inhibitor, which correlates with the results obtained in the oxidation of oleic and linoleic acids (Figs. 4–6).

Organoantimony compounds Ph_3Sb and Ph_3SbCl_2 do not change the concentration of TBARS in the initial step (1–3 h). However, the level of malonaldehyde increases with time, which reveals them as slight promoters. For ionol, its slight prooxidant activity in the early reaction (0–1 h) changes with time to an antioxidant effect (3–48 h). Complexes **I** and **II** are superior to ionol in the inhibition of the peroxide oxidation of Russian sturgeon sperm lipids.

On the whole, the results obtained show that Ph_3Sb and Ph_3SbCl_2 promote LP in the model systems and in vitro experiments. This agrees with the assumption of their toxicity due to generation of active oxygen forms and initiation of oxidative stress. The prooxidant activity of the organoantimony(V) derivative is higher than that of Ph_3SbCl_2. However, the promoting effects of these antimony compounds are less pronounced than those of organotin derivatives [52, 53].

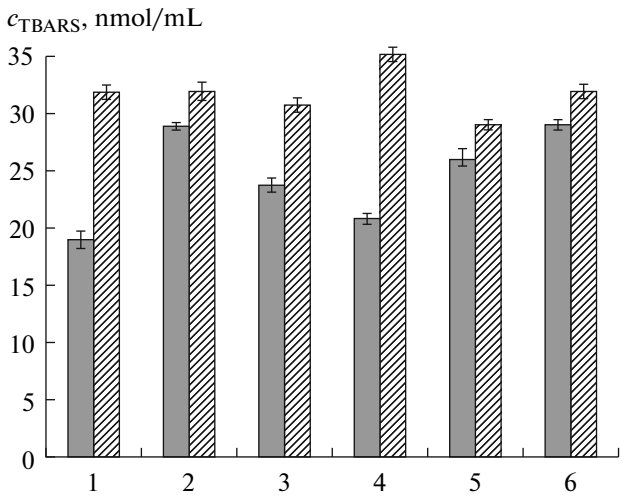


Fig. 7. Concentration of TBARS in the (▭) enzymatic and (▨) ascorbate-dependent lipid peroxidation of erythrocytes in the presence of (1) **I**, (2) **II**, (3) **III**, (4) 4,5-Piperaz-3,6-DBBQ, and (5) ionol ($c = 0.1$ mmol/L); bars (6) refer to the blank experiment.

The reactivity of metal complexes can substantially vary with ligands; therefore, their systematic toxic effect can intentionally be canceled by introduction of physiologically active fragments into the coordination sphere of the metal. The results obtained revealed that the complexes in question exhibit antiradical activity in reactions with DPPH, in the inhibition of the autooxidation of unsaturated fatty acids, and on lipid peroxidation in vitro. Thus, the decrease in the toxic effect of the organometallic fragment in these complexes is achieved by its combination with the catecholate fragment, which acts as an electron donor and contains the morpholine and piperazine (N-phenylpiperazine) pharmacophores.

The antiradical activity of complexes **I**–**III** in the reactions with DPPH is confirmed by model experiments (oxidation of oleic and linoleic acids) and by the LP of Russian sturgeon sperm and human blood erythrocytes, in which the oxidation level decreases by 60–65 and 25–35%, respectively. The inhibiting effects of complexes **I**–**III** are comparable with and, in some cases, are stronger than that of ionol. In all the model systems studied, complex **II** acts as an inhibitor. Its piperazine and catecholate fragments are fused together, which leads to greater delocalization of the electron density and, consequently, to a more stable oxidized form produced by reactions with radical intermediates via an intramolecular electron transfer between two redox sites.

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