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CHEMISTRY

Synthesis and Antioxidant Activity of New Organotin Compounds Containing a 2,6-Di-*tert*-butylphenol Moiety

E. M. Mukhatova[a], V. P. Osipova[b], M. N. Kolyada[b], N. O. Movchan[b], D. B. Shpakovsky[c], Yu. A. Gracheva[c], S. I. Orlova[c], and E. R. Milaeva[c]

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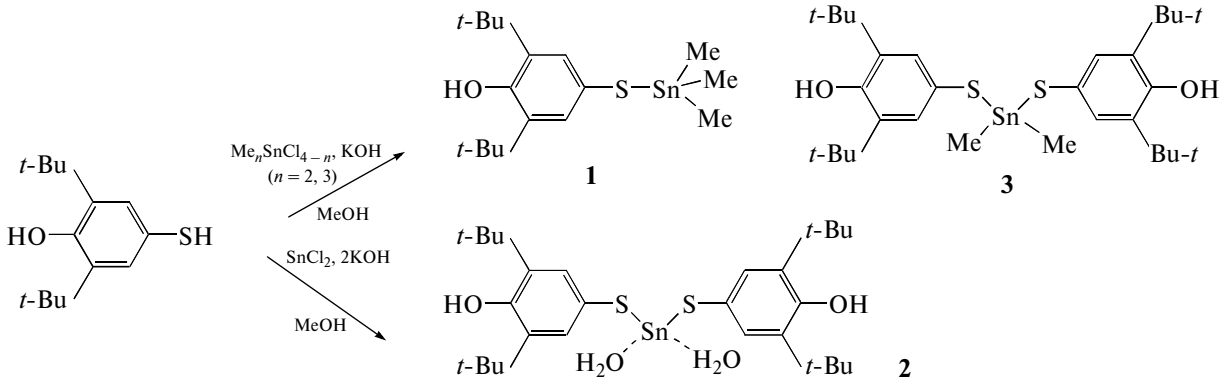
Organotin compounds show biological activity, high toxicity, and nonspecific action. The toxicity of these compounds is caused by a number of factors: binding of Sn atom to SH groups of proteins, biomembrane damage, induction of oxidative stress in organism, etc. [1]. At the same time, the ability of organotin compounds to accumulate in cell as well as their toxicity provide an opportunity to consider them as promising pharmaceuticals of specific activity, for example, as cytotoxic antitumor agents in cancer chemotherapy [2–4].

An organic fragment in tin compounds plays an important role in their physiological activity. The introduction of sterically hindered 2,6-dialkylphenol fragment σ-bonded to Sn atom, which is vitamin E mimetic showing antioxidant activity, may decrease the overall toxicity of metal compounds [5, 6]. The combined use of the cytotoxic potential of tin compounds and an antioxidant functional group in the ligand structure to design promising antitumor agents will allow one to avoid undesired side effects typical of currently used platinum derivatives.

The aim of this work is to study the effect of new tin(IV) and tin(II)compounds of general formula $Me_nSn(SR)_{4-n}$ (n = 2, 3) and $Sn(SR)_2$ containing a 2,6-di-*tert*-butylphenol fragment (R = 3,5-di-*tert*-butyl-4-hydroxyphenyl) on the processes of nonenzymatic and enzymatic peroxidation of unsaturated fatty acids and binding to SH groups of tubulin.

In this work, we synthesized novel tin compounds **1**–**3** by the reaction of appropriate tin chlorides and 2,6-di-*tert*-butyl-4-mercaptophenol [7] (RSH) in methanol in the presence of potassium hydroxide (Scheme 1).



Scheme 1.

The structures of the compounds were confirmed by IR and NMR spectroscopy and elemental analysis data.

The study of the inhibiting activity of compounds **1**–**3** in the nonenzymatic oxidation of *cis*-9-octade-

[a] *Astrakhan State Technical University, ul. Tatishcheva 16, Astrakhan, 414025 Russia*
[b] *South Research Center, Russian Academy of Sciences, ul. Chekhova 41, Rostov-on-Don, 344006 Russia*
[c] *Moscow State University, Moscow, 119991 Russia*

Effect of tin compounds on the accumulation of oleic acid hydroperoxides (1 μM, 65°C, 5 h)

Compound	[LOOH], % toward control
Me_3SnCl	174
A mixture of Me_3SnCl + RSH	18
Me_3SnSR (**1**)	16
$SnCl_2 \cdot 2H_2O$	210
A mixture of $SnCl_2 \cdot 2H_2O$ + RSH	20
$Sn(SR)_2 \cdot 2H_2O$ (**2**)	23
Me_2SnCl_2	188
A mixture of Me_2SnCl_2 + RSH	28
$Me_2Sn(SR)_2$ (**3**)	26
RSH	14

cenoic (oleic) acid was carried out in a thermostated apparatus at 65°C for 5 h. The effect of compounds was assessed from the accumulation of peroxides LOOH, primary oxidation products of oleic acid (LH), in comparison with the corresponding tin chlorides both separately and in the combination with RSH.

The kinetic curves of LOOH accumulation without additives and in the presence of tin compounds have exponential character, which corresponds to the initial period of *S*-shaped curve typical of chain radical reactions with degenerate branching. The addition of tin compounds containing no phenol fragment promotes the oxidative processes (table), which agrees well with the literature data [8]. Combined addition of tin chlorides and RSH (in 1 : 1 ratio for Me_3SnCl_2 and 1 : 2 for Me_2SnCl) leads to a fivefold decrease in the LOOH accumulation level as compared with the control and to a 6–11-fold decrease as compared with the accumulation level in the presence of tin compounds.

Sulfur-containing derivatives of sterically hindered phenols are known to be polyfunctional antioxidants that can display internal synergism of antioxidant activity [9]. The compounds under study behave as inhibitors of mixed type; the phenolic fragment participates in the termination of kinetic chain of oxidation in the reaction with peroxide radicals while the sulfide fragment of the molecule degrades hydroperoxides without radical formation.

Our data indicate that LOOH accumulation level in the presence of tin thiolates **1**–**3** is comparable with that in the presence of a mixture of tin chlorides with RSH. The addition of single mercaptophenol proved to be more efficient than in the mixture with organotin compound. Thus, the activity of compounds **1**–**3** in the nonenzymatic peroxide oxidation of oleic acid is determined by the presence of antioxidant fragment in molecule.

Next, we studied the effect of tin compounds on the initial accumulation rate of isomers: 9-hydroperoxy-*trans*-10,*cis*-12-octadiene and 13-hydroperoxy-*cis*-9,*trans*-11-octadienoic acids (HPOD), the products of *cis*-9,*cis*-12-octadecadienoic (linoleic) acid oxidation under the action of lipoxygenase-1 (LOX 1-B), which was used as a model enzyme in the studies of homologous family of lipoxygenases. The initial rate of HPOD accumulation in the presence of the studied compounds (v_0) was calculated by the formula:

$$v_0 = \Delta c/\Delta t = \Delta A/(\Delta t \varepsilon) = \tan\alpha/(\Delta t \varepsilon),$$

where c is the hydroperoxide concentration, t is the reaction time, A is the optical density, ε is the molar absorption coefficient, and α is the slope of the kinetic curve.

All studied compounds were found to decrease the rate of the enzymatic reaction of HPOD accumulation (Fig. 1). The largest descent (~50%) is observed in the presence of $Sn(SR)_2 \cdot 2H_2O$ (**2**).

The decrease in the HPOD accumulation rate in this enzymatic reaction may be owing to enzyme inhibition. The revealed inhibiting effect of the studied organotin thiolates in the reaction of HPOD formation in the presence of LOX 1-B is less expressed than the effect of complexes of organotin compounds with thioamides [10].

Thus, our study showed that organotin compounds with Sn–S bond containing a 2,6-di-*tert*-butylphenol fragment can inhibit the enzymatic reaction resulting in linoleic acid hydroperoxide and behave as antioxidant agents.

The mitotic spindle (MS) of dividing cells can be considered as the biochemical target for a number of antitumor drugs [11]. The formation of MS is contributed by microtubes that form in tubulin polymerization. Therefore, the inhibition of tubulin polymerization may be one of the mechanisms of anticancer activity. Compounds that bind to SH groups of proteins can damage the mitotic spindle and disorganize the microtube system, which causes apoptosis [12]. According to literature data [13], di- and trisubstituted methyl, phenyl and butyl derivatives of tin chlorides were shown to inhibit tubulin polymerization [13]. The modification of thiol groups of F actin was shown to lead to the depolymerization of this protein under the action of *n*-Bu_3SnCl [14].

To study the possible mechanisms of cytotoxic effect of new organotin thiolates, it is important to study the effect of these compounds on the content of SH groups in tubulin. In this part of work, we studied the effect of organotin thiolates **1**–**3** on the ability of tubulin SH groups to react with Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) [15].

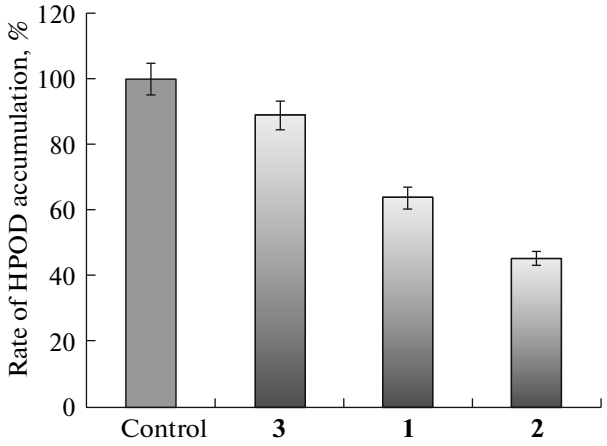


Fig. 1. Effect of organotin compounds **1**–**3** on the rate of accumulation of linoleic acid hydroperoxide. Acid concentration is 50 μM, borate buffer pH 9.

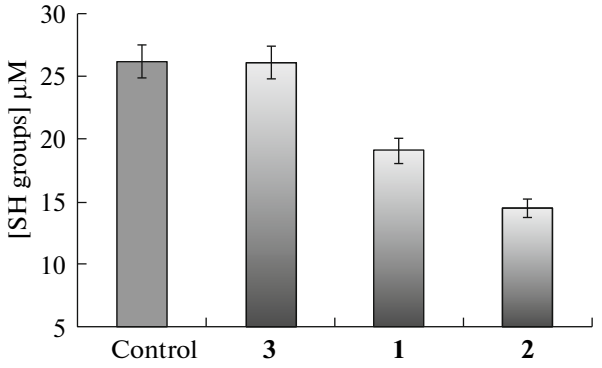


Fig. 2. Effect of organotin compounds **1**–**3** on the content of SH groups of tubulin. Concentration of compounds **1**–**3** is 50 μM, pH 7.10, tubulin concentration is 2 μM.

The method is based on the binding of free thiol groups of tubulin to DTNB in the reaction of thiol–disulfide exchange to form anion of 5-thio-2-nitrobenzoic acid (TNB) whose quinoid form has absorption maximum at 412 nm.

The study of the effect of tin compounds on the content of SH groups of tubulin showed that the concentration of SH groups in the presence of dimethyltin derivative **3** is constant, while trimethyltin derivative **1** decreases this parameter by 27% (Fig. 2). The largest drop in the content of SH groups (by 45%) is observed in the presence of compound **2**. This compound may be considered as a potential antimitotic agent.

Thus, we obtained the new derivatives of tin(IV) and tin(II) containing the 2,6-di-*tert*-butylphenol fragment. The antioxidant action of these compounds on the enzymatic and nonenzymatic formation of fatty acid hydroperoxides was shown. The ability of $Sn(SR)_2 \cdot 2H_2O$ to decrease the content of SH groups in tubulin allows us to consider this compound as a potential antimitotic agent with antioxidant activity.

EXPERIMENTAL

The following chemicals and solvents were used in the work as received: KOH, Me_2SnCl_2, Me_3SnCl, $SnCl_2 \cdot 2H_2O$ (Fluka), hexane, MeOH (reagent grade), petroleum ether 40/70, linoleic acid (Sigma, 99%), oleic acid (Sigma, 98%), lipoxygenase (Sigma Lipoxidase from Glycine max (soybean), Type I-B), DTNB (Aldrich, 99%), tubulin (Cytoskeleton, 99%).

Absorption spectra were recorded on an Evolution 300 Thermo spectrophotometer and a Zenyth 200rt Anthos spectrophotometer.

IR absorption spectra were recorded as KBr pellets on a Thermo Nicolet IR200 FT spectrophotometer. NMR spectra were recorded on a Bruker AMX-400 spectrometer (operating at 400 and 100 MHz for ^1H and ^{13}C, respectively) in $CDCl_3$ solutions. Elemental analysis was performed on a Carlo Erba EA MODEL 1108 microanalyzer.

Trimethyltin 3,5-di-*tert*-butyl-4-hydroxyphenylthiolate (1). A solution of KOH (1 M, 1.16 mL, 1.16 mmol) was added to a mixture of 200 mg (0.85 mmol) of trimethyltin chloride and 202 mg (0.85 mmol) of RSH in 4 mL of ethanol, the reaction mixture was stirred for 30 min. The resulting white precipitate was separated by filtration, washed with water and petroleum ether. The precipitate was dried in air for 12 h. Yield 233 mg (68.3%). T_m 120–123°C.

For $C_{14}H_{30}OSSn$ anal. calcd. (%): C, 50.90; H, 7.54.

Found (%): C, 51.07; H, 7.50.

^1H NMR (δ, ppm, *J*, Hz): 0.38 (s, 9H, $Sn(CH_3)_3$, $^2J_{H-Sn}$ 28 Hz), 1.43 (s, 18H, 4 $C(CH_3)_3$), 5.10 (s, 1H, 1OH), 7.21 (s, 2H, Ar).

^{13}C NMR (δ, ppm): 4.86 (2 CH_3Sn), 30.28 (6 CH_3, 2 Bu-*t*), 34.29 (2 $C(CH_3)_3$), 122.74, 131.15, 136.40, 152.54 (C_{arom}).

IR (ν, cm^{-1}): 3627.4 (OH), 2998.8–2869.6 (C–H), 1421.3, 1292.1, 1234.2, 1134, 885.2, 777.2, 536.1.

Tin(II) bis(3,5-di-*tert*-butyl-4-hydroxyphenylthiolate) dihydrate (2) was obtained similarly to compound **1** as a pale yellow precipitate. Yield 65%, T_m 202–205°C.

For $C_{28}H_{42}O_2S_2Sn$ anal. calcd. (%): C, 56.67; H, 7.13.

For $C_{28}H_{42}O_2S_2Sn \cdot 2H_2O$ anal. calcd. (%): C, 53.42; H, 7.37.

Found (%): C, 52.81; H, 6.77.

^{1}H NMR (δ, ppm): 1.42 (s, 36H, 4 $C(CH_3)_3$), 5.22 (s, 2H, 2 OH), 7.25 (s, 4H, 2 Ar).

^{13}C NMR (δ, ppm): 30.25 (12 CH_3, 4 Bu-*t*), 34.41 (4 $C(CH_3)_3$)), 118.51, 132.25, 136.65, 153.83 (C_{arom}).

IR (ν, cm^{-1}): 3635.2 (OH), 2998.8–2871.5 (C–H), 1423.2, 1234.2, 1155.15, 877.5, 715.5.

Dimethyltin bis(3,5-di-*tert*-butyl-4-hydroxyphenylthiolate) (3) was obtained similarly to compound **1** as a white precipitate. Yield 56%, T_m 115–118°C.

For $C_{30}H_{48}O_2S_2Sn$ anal. calcd. (%): C, 57.79; H, 7.76.

Found (%): C, 57.62; H, 7.61.

^{1}H NMR (δ, ppm): 0.43 (s, 6H, 2 CH_3), 1.42 (s, 36H, 8 $C(CH_3)_3$), 5.13 (s, 2H, 2 OH), 7.36 (s, 4H, 2 Ar).

^{13}C NMR ($CDCl_3$, δ, ppm): 1.94 (2 CH_3Sn), 30.27 (12 CH_3, 4 Bu-*t*), 34.34 ($C(CH_3)_3$), 120.88, 131.49, 136.58, 153.20 (2 C_{arom}).

IR (ν, cm^{-1}): 3633.3 (OH), 2998.0–2871.5 (C–H), 1423.2, 1309.4, 1230.4, 1155.1, 875.5, 765.6, 715.5.

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