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CHEMISTRY

Electrochemical and Antioxidant Activity of 2,6-di-*tert*-Butylphenols with Phosphonate Groups

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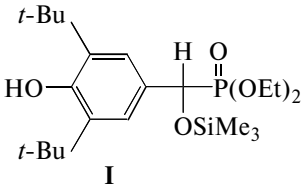
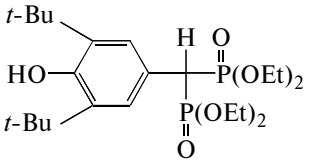
One of the methods of design of new efficient antioxidants—biomimetics of natural phenol compounds—is combination of several biologically active centers in one molecule [1]. As similar compounds, *para*-substituted phosphorus-containing derivatives of the widely used antioxidant ionol can act, which contain antioxidant groups of substituted 2,6-di-*tert*-butylphenol and fragments of phosphomimetics based on phosphonic acid and its esters [2].

The antioxidant properties of phenol compounds are determined by the O–H bond dissociation energy when the hydrogen atom is taken away by active lipid radicals [3], or by how easy the one-electron oxidation of phenols and phenolate anions occurs upon deprotonation [4–6]. A correlation between the antioxidative activity and the oxidation potential has been found for some 2,6-di-*tert*-butylphenols and thio(amino)alkylphenols [7, 8].

This study has addressed the electrochemical and antioxidant activities of phenols containing one or two phosphonate groups (**I**–**IV**). To elucidate the effect of the phosphonate group, these phenols were compared to ionol (2,6-di-*tert*-butyl-4-methylphenol). To block removal of the hydrogen atom from the hydroxyl group, phenols **I**–**IV** were compared to their analogues, phosphonic acids containing anisole fragments (**V**, **VI**).



I



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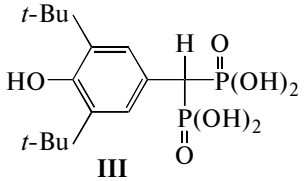

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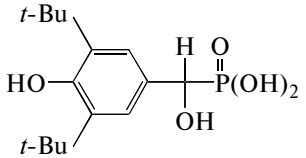
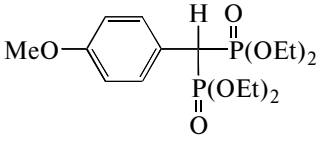
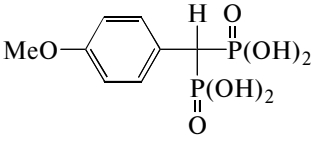


IV



V



VI

The oxidation potentials were measured by cyclic voltammetry (CVA) in a three-electrode cell in CH_3CN using an IPC-pro potentiostat (Volta, Russia). The working electrode was a stationary Pt electrode 3 mm in diameter. The counter electrode was a platinum plate ($S = 18$ mm^2). The reference electrode was (Ag/AgCl/KCl) with a waterproof diaphragm. The potential sweep rate was 0.2 V s^{-1}. The supporting electrolyte was 0.1 M Bu_4NClO_4 (99%, Acros) recrystallized twice from aqueous EtOH and dried in vacuum for 48 h at 50°C. Acetonitrile was dried and purified by a known procedure [9]. The concentration of compounds was 5 mmol/L.

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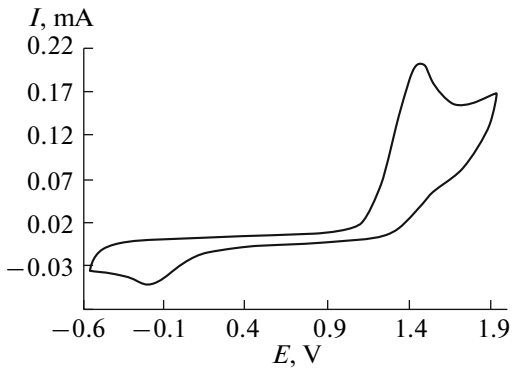

Fig. 1. Cyclic voltammogram of the oxidation of **I**.

The antioxidant activity of the compounds was determined from the decrease in the lipid peroxidation (LPO) rate in the liver homogenate of female Russian sturgeon (*Acipenser gueldenstaedti Brandt*) in vitro. Solutions of the compounds (with a concentration of 0.1 mmol/L) in EtOH were added to the liver homogenate, and the mixture was incubated for 48 h at 5°C. Then, a portion of the mixture was sampled and the level of malondialdehyde (MDA), a TBA-reactive product, in liver was evaluated by a routine procedure with the use of thiobarbituric acid (TBA) [10]. From these data, the antioxidant efficiency (AE) of compounds **I**–**VI** was calculated [11].

Electrochemical phenol oxidation depends on the electronic and steric properties of substituents in the benzene ring and experimental conditions (solvent, electrode material, pH) and can occur as one-electron oxidation, through the formation of a radical cation, or as two-electron oxidation resulting in the phenoxonium cation [12]. The electrochemical oxidation of compounds **I**–**IV** is an irreversible two-electron process (Fig. 1). On the reverse scan of the cyclic voltammograms, the proton reduction peak is observed, which was identified by introducing acids ($HClO_4$, CF_3COOH) into the system. The oxidation potentials of the compounds are summarized in the table.

Compounds **V** and **VI** are more easily oxidized than anizole, which has an oxidation potential of 1.9 V under analogous conditions [13]. Ethyl esters **II** and **V** are oxidized at more positive potentials than corresponding acids **III** and **VI**, and phenols **I**–**IV** are more easily oxidized than ionol. The electrochemical data obtained allowed us to assume that phosphorus-containing phenols **I**–**IV** should have higher antioxidant activity than ionol and analogues **V** and **VI**.

The results of the in vitro study of the antioxidant activity of the compounds with the use of liver homogenate confirmed this assumption. As compared to ionol, phenols **I**–**IV** exhibited higher antioxidant activity in all steps of LPO (Fig. 2), which is likely due to the presence of two reaction centers, phenol and phosphonate group [14]. Among the phenols studied, the highest AE value was obtained for compound **III**, which has the lowest oxidation potential (table). Methyl esters of phosphorus-containing phenols **V** and **VI** exhibit low antioxidant activity: their AE values are lower than that of ionol. Our results are evidence of the combined action of the phenol moiety and phosphonic acid residue in the in vitro inhibition of the lipid oxidative destruction in liver homogenate.

Thus, the correlation found between the oxidation potential of the phosphorus-containing phenols under consideration and their antioxidant efficiency in the model system of LPO in the Russian sturgeon liver makes it possible to use CVA for primary bioassay of the antioxidant activity of 2,6-di-*tert*-butylphenols.

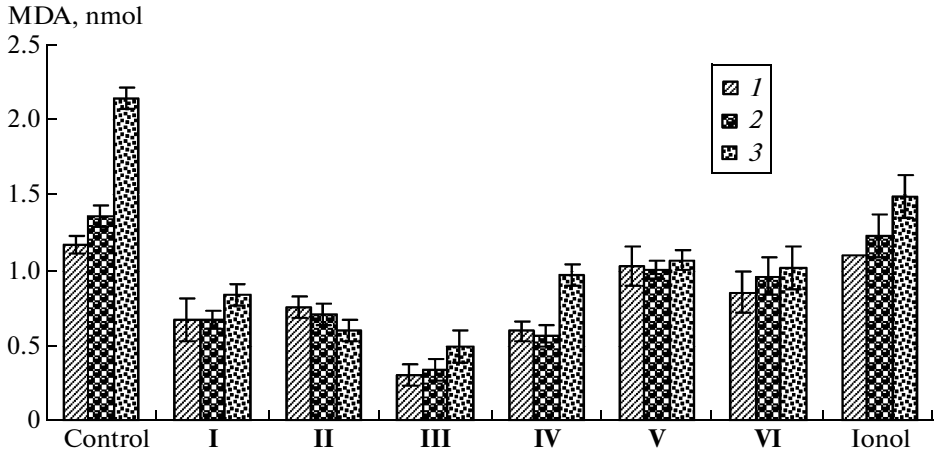

Fig. 2. Change in the MDA accumulation rate in the presence of antioxidants in the Russian sturgeon liver in vitro over a period of (*1*) 3, (*2*) 24, and (*3*) 48 h.

Table 1. E_a and AE for compounds **I**–**VI** and ionol

E_a, V	AE, %		
	3 h	24 h	48 h
1.44 (**I**)	40.6	50	61
1.48 (**II**)	35.5	48.1	72
1.40 (**III**)	73.3	75	76.8
1.38 (**IV**)	48.8	57.6	54.8
1.79 (**V**)	9.2	11.05	7.86
1.65 (**VI**)	13.15	14.94	12.36
1.52 (ionol)	35.4	42.4	38.9

Note: AE was calculated relative to the control from the change in the MDA level.

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