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# The Redox Properties and Antiradical Activity of Terpenophenols

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**Abstract**—The redox properties and antiradical activity of terpenophenols (2,6-diisobornyl-4-methylphenol and 3-isocamphyl-2-naphthol) in comparison with BHT (butylhydroxytoluene, 2,6-di-*tert*-butyl-4-methylphenol) have been studied. The terpenophenols have been shown to react more readily in electron transfer processes as compared with BHT, and they have been found to react with electrochemically generated superoxide radical anion ($O_2^{\bullet-}$). The effect of the compounds on the rate of $O_2^{\bullet-}$ generation upon adrenaline oxidation in an alkaline medium and the ability of biopreparations based on Russian sturgeon liver and gonad homogenates to deactivate $O_2^{\bullet-}$ have been studied. Adrenaline oxidation inhibition and the increase in superoxide dismutation activity of the biopreparations in the presence of terpenophenols has been shown, and this fact can indicate the ability of these compounds to decrease the probability of oxidative stress enlargement. A correlation has been established between the redox properties and antioxidant activity of terpenophenols in the model system of adrenaline oxidation in the presence of the biopreparations.

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Recently, there has been a tendency to substitute synthetic antioxidants by less toxic products of natural origin [1], which can be produced from renewable feedstock [2].

Promising semisynthetic antioxidants are terpenophenols and terpenonaphthols obtainable from terpenoids, in particular camphene or pinenes, the products of wood processing industry [3]. The antiradical and antioxidative activity of certain terpenophenols have been demonstrated in chemical model systems [4] and experiments in animals [5]. 2,6-Diisobornyl-4-methylphenol **1** (Dibornol®) exhibiting antioxidative, antitoxic, antithrombotic, hemorheologic, and neuroprotective activity [6, 7] is a well-studied compound. The biological activity of phenol **1** is combined with low toxicity, which causes the necessity of its further studies.

The antioxidative action of the compounds is mainly determined by their antiradical activity toward reactive oxygen species (ROS). The initial ROS in living organism is the superoxide anion radical (superoxide anion $O_2^{\bullet-}$), which plays an important role in the initiation of different vital or pathologic processes.

The aim of this work is to compare the reactions of 2,6-diisobornyl-4-methylphenol **1**, 3-isocamphyl-2-naphthol **2**, and 2,6-di-*tert*-butyl-4-methylphenol **3** (BHT) (Fig. 1) with electrochemically generated superoxide anion radical, their effect on the rate of $O_2^{\bullet-}$ generation upon adrenaline oxidation in an alkaline medium and on the ability of Russian sturgeon liver and gonad homogenates to deactivate this ROS.

It is known that the redox properties of compounds often correlate with their antioxidant activity; therefore, the use of electrochemical methods provides a possibility to obtain valuable information on the reducing ability of antioxidants [8]. To predict the antioxidative ability of compounds **1**–**3**, we studied their electrochemical parameters by cyclic voltammetry ($CH_3CN$, Pt, 0.1 M $^nBu_4NClO_4$, $C$ = 5 mM, Ag/AgCl, $V = 0.2\ V\ s^{-1}$).

It was found that phenol **1** and naphthol **2,** when oxidized at potentials of 1.23 and 1.13 V, respectively, more easily participate in electron transfer processes as compared with ionol **3**, which undergoes oxidation at a potential of 1.52 V. Compound **1** undergoes irreversible oxidation in one two-electron stage to give aroxyl cation localized on the oxygen atom, which agrees with previously obtained data [9]. Compound **2** also undergoes irreversible oxidation in one one-electron stage to give a cation radical, which eliminates proton to form a radical (Scheme 1).

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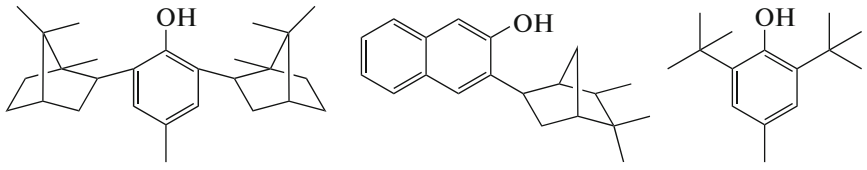



**1** **2** **3**

**Fig. 1.** Chemical structures of 2,6-diisobornyl-4-methylphenol (**1**), 3-isocamphyl-2-naphthol (**2**), and 2,6-di-*tert*-butyl-4-methylphenol (**3**).

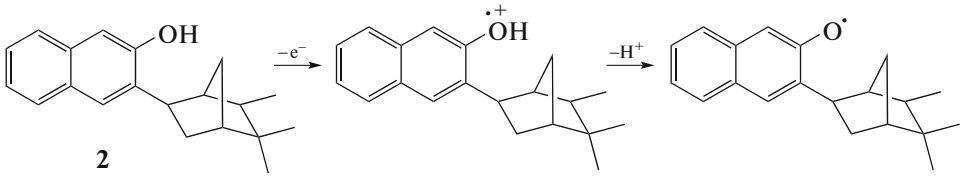
Starting from the obtained electrochemical data and taking into account that the antioxidative action of the majority of biologically active compounds is related to their ability to undergo easy oxidation [10], one can expect the expressed antioxidative activity of compound **2**. However, it should be taken into account that the presence of two bulky substituents in compound **1** increases steric hindrances in reactions involving the hydroxyl H atom and enhances the stability of resulting aroxyl radical.

The reaction of superoxide anion radical with antioxidants is one of the ways of its neutralization [11]. Since $O_2^{\bullet-}$ forms due to one-electron reduction of oxygen, electrochemical methods can be used to assess the antioxidant efficiency; the methods are highly sensitive, precise, and fast and produce no byproducts [12].

Electrochemical reduction of oxygen on a Pt electrode in acetonitrile produces $O_2^{\bullet-}$. It has been shown that the reaction of compounds **1**–**3** with $O_2^{\bullet-}$ is accompanied by the disappearance of reversibility of the oxygen reduction stage and the emergence of the anodic peak at a potential of +0.2 V in the cyclic voltammetric curve, which probably results from the formation of the phenolate anion in the following reaction (Scheme 2).

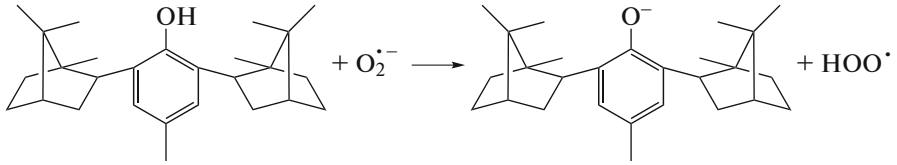
The antiradical activity of compounds **1**–**3** toward $O_2^{\bullet-}$ was studied using the superoxide producing reaction of adrenaline autooxidation in an alkaline medium (pH 10.65), and the formation of adrenochrome was detected at a wavelength of 347 nm [13]. This model system provides a possibility to assess the effect of compounds on the superoxide dismutase activity of biopreparations, i.e., the ability to inhibit the $O_2^{\bullet-}$ production. It is known that the cytoplasm of eukaryotic cells contains only one of the enzymes that deactivate $O_2^{\bullet-}$, superoxide dismutase SOD (Cu/ZnSOD), whose activity is independent of pH. Another enzyme that promotes the reaction of one-electron reduction of $O_2^{\bullet-}$ was revealed only in prokaryotes and unicellular eukaryotes [14].

It has been shown that the addition of BHT **3** in an incubation medium has no effect on the kinetics of accumulation of adrenaline autooxidation products; studied terpene-containing antioxidants **1** and **2** inhibit adrenaline oxidation (Fig. 2). The addition of the supernatant of male sturgeon liver and gonad




**2**

**Scheme 1.**




**1**

**Scheme 2.**

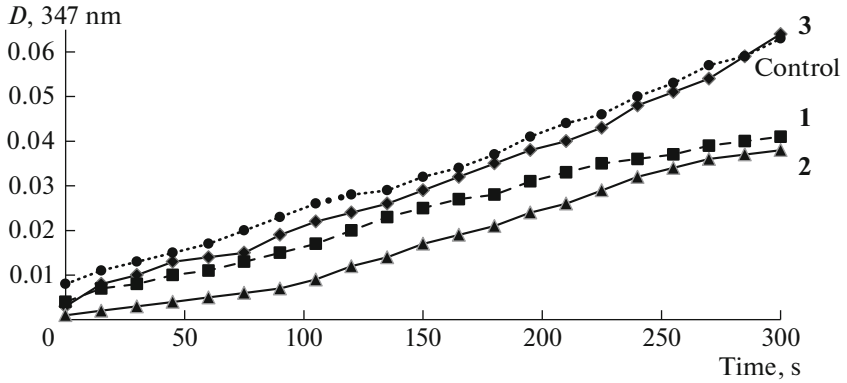



**Fig. 2.** Kinetic curves of adrenochrome accumulation upon adrenaline autooxidation in bicarbonate buffer (pH 10.65) at 25°C. Average values obtained in independent experiments for 3–5 parallel measurements in each experiment are given.

homogenates leads to inhibition of adrenaline oxidation, which indicates the utilization of $O_2^{\bullet-}$ by cytosol SOD. When calculated per 1 μL of biopreparation, the decrease of adrenaline oxidation rate in the presence of the supernatant of sturgeon liver homogenate is by one order of magnitude higher than that for gonads. Thus, a 50% decrease of the adrenaline oxidation rate is observed when 5 μL of liver homogenate biopreparation or 50 μL of gonad homogenate is added to a measurement medium. Thus, the superoxide dismutase activity of Russian sturgeon liver homogenate is considerably higher than that of gonad homogenate, which seems to be explained by the low content of cytoplasm in sturgeon gonad cells, which is the source of SOD [15].

According to the obtained results, 2,6-diisobornyl-4-methylphenol **1** increases the SOD activity of Russian sturgeon liver and gonad homogenates by 49 and 53%, while 3-isocamphyl-2-naphthol **2** increases the activity by 39 and 35% (Fig. 3). BHT **3** has no effect on the ability of the biopreparations to utilize $O_2^{\bullet-}$.

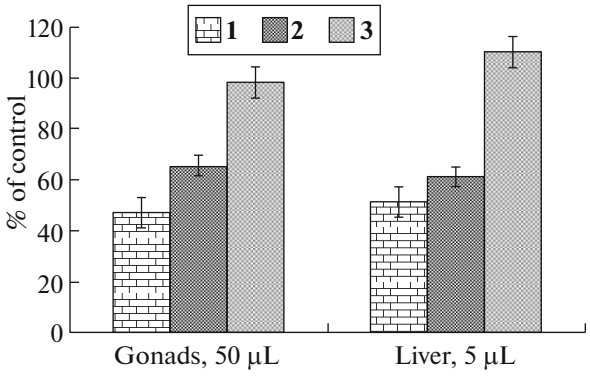



**Fig. 3.** Effect of compounds **1**–**3** on the level of accumulation of adrenaline autooxidation products in the presence of Russian sturgeon liver and gonad homogenates ($C$ = 25 μM, λ = 347 nm, $C_{adrenaline}$ = 5.46 mM). The level of accumulation of adrenaline autooxidation products in bicarbonate buffer in the presence of biopreparation is taken as 100% (control).

It has been found that the antioxidant properties of terpenophenols in the model system of adrenaline oxidation in the presence of biopreparations correlate with electrochemical data, which predict the larger antioxidant activity of the compounds as compared with BHT.

Thus, the antiradical activity of 2,6-diisobornyl-4-methylphenol and 3-isocamphyl-2-naphthol toward superoxide anion radical has been revealed. The ability of these compounds to increase the superoxide dismutase activity of the supernatant of Russian sturgeon liver and gonad homogenates has been demonstrated, which may indicate that these compounds can reduce the probability of oxidative stress development.

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