

## Electrochemical Antioxidative Activity Assay of Metalloporphyrins Bearing 2,6-Di-*tert*-butylphenol Groups Based on Electrochemical DPPH-Test

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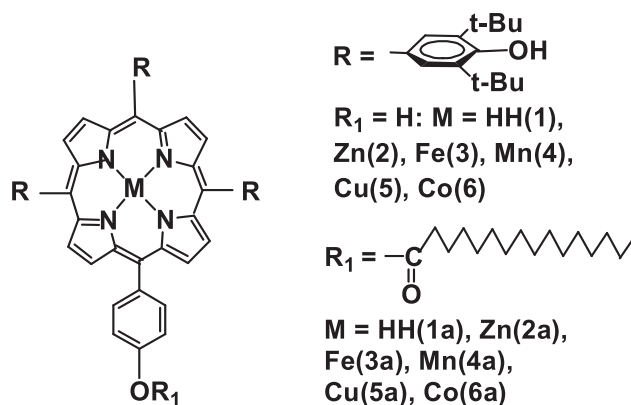
The electrochemical behaviour of metalloporphyrins with 2,6-di-*tert*-butylphenol pendants **1-6** and **1a-6a** was studied in CH<sub>3</sub>CN and their antioxidative activity was evaluated using a reaction with a stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) monitored by CVA. Depending on the nature of metalloporphyrins various numbers of reversible or irreversible waves in anodic range of potentials appears which can be attributed to ligand or metal-centered oxidation. It was shown that antioxidative activity of compounds **1-6** and **1a-6a** depends strongly on the nature of metal and the presence of palmitoyl moiety in the molecule.

**Keywords:** Metalloporphyrins, cyclic voltammetry, antioxidative activity, DPPH-test.

The oxidative stress causes various pathologies and fastens organism ageing in general.<sup>[1]</sup> The search for new antioxidants and methods of antioxidative activity evaluation is an important task.<sup>[2]</sup> In previous studies we offered a novel method of antioxidative activity assay based on electrochemically monitored reduction of a stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH).<sup>[3]</sup> This method has some advantages before traditionally used spectrophotometrically monitored DPPH-test:<sup>[4]</sup> it is applicable for chromophores and, in addition, it can be used with relatively high concentrations of components. Porphyrins have intensive adsorption band close to the band of DPPH (512 nm) and consequently their antioxidative activity cannot be studied by spectrophotometric DPPH-test. In this work the electrochemical behaviour of transition metal porphyrins with 2,6-di-*tert*-butylphenol pendants **1-6** and **1a-6a**<sup>[5]</sup> (Scheme 1) was studied in CH<sub>3</sub>CN and their antioxidative activity assay was completed using electrochemical DPPH-test.<sup>8</sup>

The redox-potentials values of compounds **1-6** and **1a-6a** are listed in Table 1. The shape of CVA curves depends strongly on the nature of metalloporphyrins and their oxidation has a complicative character: various number of irreversible or reversible oxidation waves in anodic range of potentials was observed (Table 1). In cathodic range the waves of ligand reduction appeared.

<sup>8</sup>Voltammograms were measured in a three electrode cell using an IPC pro potentiostat (Volta, Russia) under argon. A stationary platinum electrode 2 mm in diameter was used as the working electrode and a platinum wire served as the auxiliary electrode. An (Ag/AgCl/KCl) electrode was used as the reference electrode. The potential sweep rate was 0.1 V·s<sup>-1</sup>. The supporting electrolyte was a 0.5 M solution of Bu<sub>4</sub>NBF<sub>4</sub> (99%, Acros, Russia) twice recrystallized from aqueous EtOH and dried in vacuum for 48 h at 50°C. CH<sub>3</sub>CN was purified and dried by a known procedure.<sup>[6]</sup> The concentration of the compounds was 1 mM. For the reaction of these porphyrins with DPPH the concentration ratio was 1:1 (C<sub>0</sub> = 0.1 mM) by measuring CVA curves in certain intervals of time (10 s, 30 s, 1, 3, 5 and 10 min) during 1 h. The compounds under investigation were obtained by procedures described in our previous work.<sup>[5]</sup>



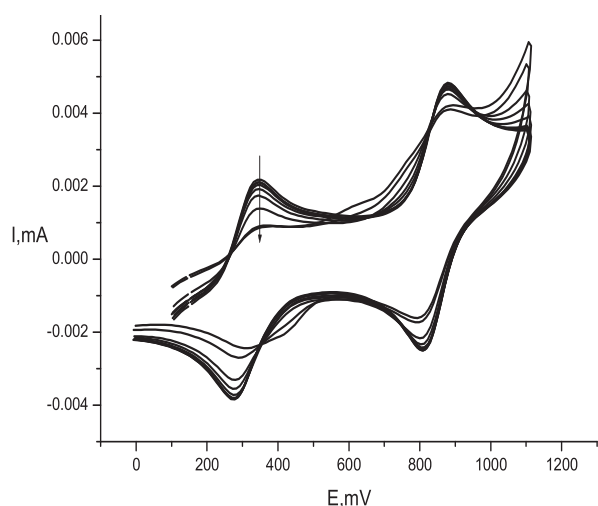
Scheme 1.

**Table 1.** Redox potentials and values of scavenging activities of compounds **1-6** and **1a-6a** in CH<sub>3</sub>CN (Pt electrode, C = 10<sup>-3</sup> M, 0.5 mM Bu<sub>4</sub>NBF<sub>4</sub>, 100 mV/s, vs. Ag/AgCl).

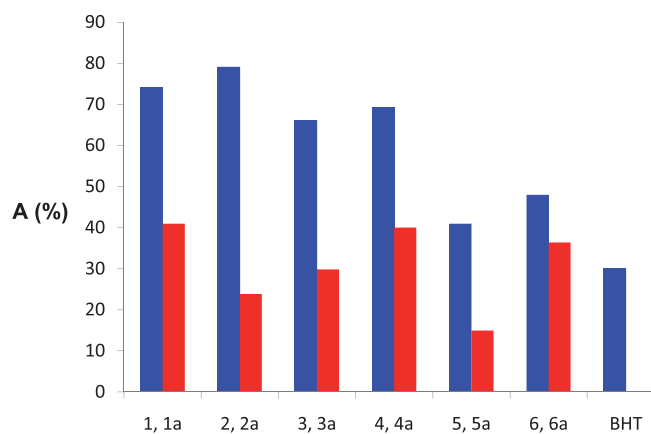
Compound	E <sup>ox</sup>	E <sup>red</sup>
<b>1</b>	0.84; 1.03/0.88; 1.44; 0.14*	-1,18 (irr.)
<b>1a</b>	0.79/0.40; 0.97/0.95; 1.42/1.23	-1,14 (irr.)
<b>2</b>	0.7/0.45; 1.46 (irr.)	-0.89/-0.5
<b>2a</b>	0.74/0.65; 0.42*; 1.47 (irr.)	-0.87/-0.63
<b>3</b>	1.03/0.8	-0.3/-0.25
<b>3a</b>	1.01/0.78**; 1.18 (irr.)	-0.26/-0.18
<b>4</b>	1.05; 1.30/1.13; 1.55/1.4	-0.76/-0.61
<b>4a</b>	1.07; 1.33/1.13; 1.56/1.43	-0.72/-0.58
<b>5</b>	0.88/0.65; 1.54 (irr.)	-1.2 (irr.)
<b>5a</b>	0.92/0.7; 1.5 (irr.)	-0.107 (irr.)
<b>6</b>	0.42/0.31; 1.02; 0.58**	-0.84/-0.64
<b>6a</b>	0.45/0.37; 1.10/0.97	-0.8/-0.62
BHT	1.52	

\*at reverse scan after triggering potential at 0.9 V;

\*\*at reverse scan after triggering potential at 1.1 V.



**Figure 1.** Cyclic voltammograms of DPPH in presence of compound **2** (Pt electrode, 0.5 mM  $\text{Bu}_4\text{NBF}_4$ , 100 mV/s, vs. Ag/AgCl,  $C(\text{DPPH}) = 0.1$  mM, components ratio 1:1).



**Figure 2.** The values of scavenging activities  $A = [(I_0 - I)/I_0] \times 100\%$  of **1-6**, **1a-6a** and BHT determined by electrochemically monitored DPPH-test (Pt electrode, 0.5 mM  $\text{Bu}_4\text{NBF}_4$ , 100 mV/s, vs. Ag/AgCl,  $C(\text{DPPH}) = 10^{-4}$  M, components ratio 1:1, time of reaction 1 h).

From the character of CVA we can conclude that in the case of compounds **1-5** and **1a-5a** ligand-centered oxidation takes place. In case of compounds **6** and **6a** one-electron reversible wave appears at  $E = 0.46$  V which can be attributed to  $\text{Co}^{2+}/\text{Co}^{3+}$  process (Table 1). The two-electron irreversible wave at more positive potentials, obviously, corresponds to the oxidation of the phenolic moiety.

As can be seen from Table 1, all compounds under study are electrochemically oxidized at potentials more positive than the peak potential of the first wave at DPPH cyclic voltammograms ( $E = 0.35$  V). That makes them appropriate objects for antioxidative activity assay by electrochemically monitored DPPH-test. The cyclic voltammograms of DPPH in simultaneous presence of compound **2** are presented in Figure 2 ( $C(\text{DPPH}) = 0.1$  mM, components ratio 1:1).

The significant decrease of DPPH peak current  $I$  points out the proceeding of hydrogen atom transfer to

DPPH from **2**. Since the waves of DPPH reduction and oxidation<sup>[7,8]</sup> show reversible character at a specified electrode surface area and potential sweep rate, the ratio of the DPPH oxidation or reduction peak currents during the reaction is equal to the concentration ratio  $I/I_0 = C/C_0$ <sup>[3]</sup>, ( $I$  is the peak current for given DPPH concentrations,  $I_0$  is the peak current for the initial DPPH concentration). Hence, the change in the peak current is determined by the change of the DPPH concentration and can be used for evaluation of the antioxidative activity of the reacting substance. The scavenging activity of compounds under study was calculated as follows:  $A = [(I_0 - I)/I_0] \times 100\%$ . The data obtained are presented in Figure 3 and in Table 1. The results of DPPH test show that the activity of **1-6** and **1a-6a** depends strongly upon nature of metal and the presence of palmitoyl group in molecule. Accordingly to their scavenging activity  $A$  all compounds under study may be placed in the next order: **2 > 1 > 4 > 3 > 6 > 1a > 5 > 4a > 6a > 3a > BHT > 2a > 5a**.

It can be seen that in common the metalloporphyrins which contain a palmitoyl group in molecule demonstrate lower antioxidative activity than their analogs without this group. It may be connected with steric hindrances which arises at the DPPH reaction with these compounds. Nevertheless, it should be pointed out that porphyrins **1**, **1a**, **2**, **3**, **3a**, **4**, **4a**, **5**, **5a**, **6**, **6a** exhibit activity significantly higher than this of a known antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT) and therefore should be considered as promising antioxidants.

## Conclusions

Electrochemical properties of transition metal porphyrins with 2,6-di-*tert*-butylphenol pendants are studied by CVA and their antioxidative activity estimated using electrochemical DPPH-test. Antioxidative properties of these compounds depends strongly on metal nature and the presence of a palmitoyl group in molecular. Most compounds under study demonstrate activity higher than that of a known antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT).

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