Electrochemical method in determination of antioxidative activity using ferrocene derivatives as examples

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Electrochemical method for the evaluation of antioxidative activity of compounds based on their reaction with the stable radical, 2,2-diphenyl-1-picrylhydrazyl, was suggested with monitoring of the reaction by cyclic voltammetry (CVA). Antioxidative properties of new ferrocene derivatives Fc(L)R (where Fc is the ferrocenyl, R is the fragment of 2,6-di-*tert*-butylphenol or its aromatic analog, L is the spacer) were studied. Anodic oxidation of the compounds Fc(L)R, which contain azomethine and 2,6-di-*tert*-butylphenol moities, proceeds in three steps, that suggests a possibility of intramolecular proton-coupled electron transfer process. Conjugates of ferrocene and 2,6-di-*tert*-butylphenol are efficient antioxidants.

Key words: cyclic voltammetry, ferrocenes, 2,6-di-*tert*-butylphenol, antioxidative activity, 2,2-diphenyl-1-picrylhydrazyl.

Nowadays, there are several methods for the evaluation of antioxidative activity of compounds, however, a search for the new efficient methods is still of significant interest.[1—2] Lately, a reaction with the stable radical, *viz.*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), is used as a fast and convenient test, which is based on the hydrogen atom transfer from the antioxidant molecule to the DPPH radical.[3—5] This reaction is usually monitored by spectrophotometry from the decrease in intensity of the absorption band of DPPH at 512 nm. However, this method has significant disadvantages. First, it cannot be used for the chromophoric substances having an absorption band close to the absorption band of DPPH itself. Second, since DPPH possesses a high extinction coefficient[4] ($\varepsilon = 1.16 \cdot 10^4$ L mol^{-1} cm^{-1}), the method is limited to the range of low concentrations of DPPH and antioxidant.

We suggested to use CVA for monitoring of this reaction. The voltamgram of DPPH in the anodic region shows two one-electron reversible waves, corresponding to the oxidation and reduction of the radical[6,7] (Fig. 1). From the Randles—Shevchik equation[8] it follows that for the given surface of the electrode and speed of potential sweep, the proportions of currents in the peak of oxidation or reduction of DPPH is determined only by the ratio of concentrations

$$I/I_0 = C/C_0,$$

where I_0 is the current at the initial concentration of DPPH (C_0), I is the current at the concentration of DPPH in the given moment of time (C). Consequently, if the I value is known for some given concentration of DPPH and a decrease of this value can be tracked with time, a kinetic curve of concentrations of DPPH versus time can be plotted. The relative change of concentration of DPPH in the

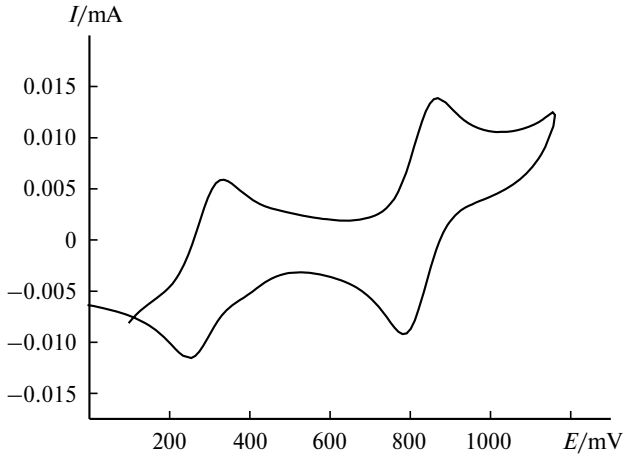

Fig. 1. Cyclic voltamgram of DPPH in MeCN at the speed of potential sweep 100 mV s^{-1} (Pt-electrode, the concentration was $0.5 \cdot 10^{-3}$ mol L^{-1}, $Bu^n_4NBF_4$, Ag/AgCl).

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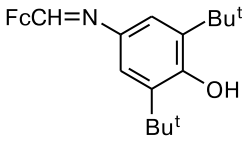
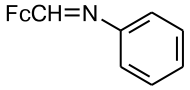
course of the reaction can be used for the evaluation of antioxidative activity of compounds under study.

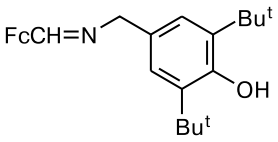
We set a task to develop a new method for the monitoring of this reaction by CVA and its testing on the redox-active compounds, for which spectrophotometric DPPH-test is of little use. Organometallic derivatives of 2,6-di-*tert*-butylphenol, in which a proton-coupled electron transfer (PCET) process is possible, have been chosen as such compounds. These processes play an important role in biochemistry and are of significant practical and theoretical interest from the point of view of the search for new model systems.[9—16] In the present work, we studied ferrocene conjugates Fc(L)R containing redox-active 2,6-di-*tert*-butylphenol substituents R and spacers L. Such processes as intramolecular electron transfer with subsequent proton abstraction, or proton abstraction and electron transfer, or proton-coupled electron transfer in one elementary step can take place in such polytopic systems.[9,10] Note that these processes are responsible for the mechanism of the antioxidant action,[17—19] which include 2,6-di-*tert*-



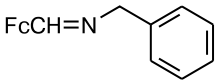
1a



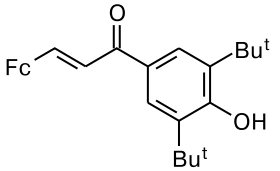
1b



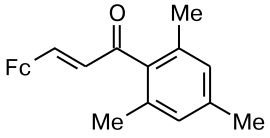
1c



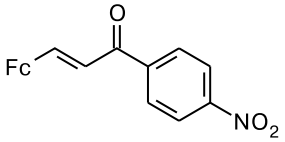
1d



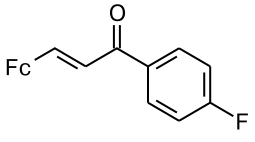
2a



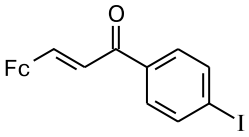
2b



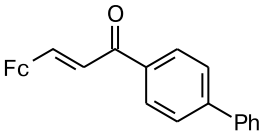
2c



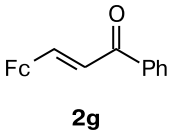
2d



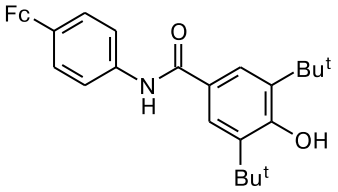
2e



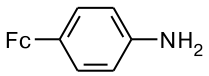
2f



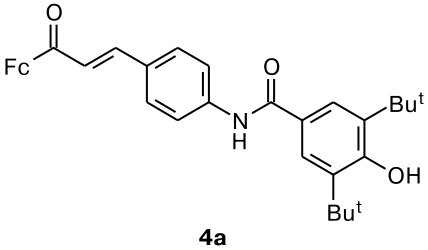
2g



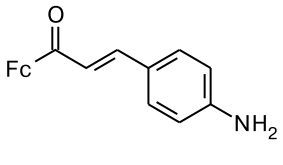
3a



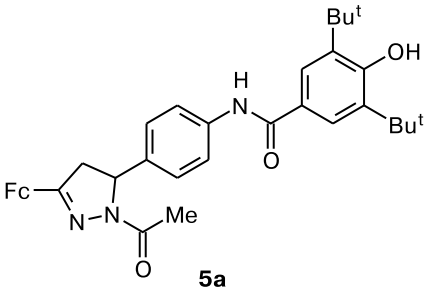
3b



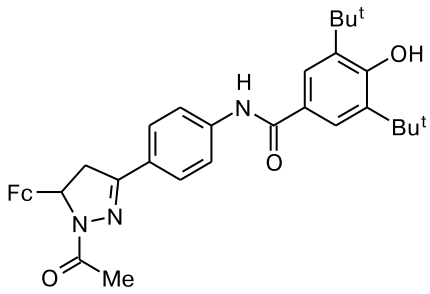
4a



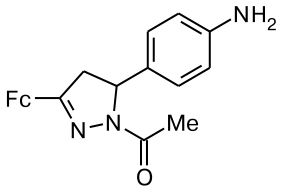
4b



5a



5b



5c

Fc stands for ferrocenyl

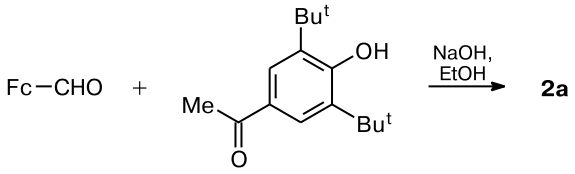
butylphenols. There is certain correlation between the redox-properties and antioxidative activity of these compounds.[20] Thus, it seems reasonable to study antioxidative properties of compounds together with their electrochemical behavior.

The present work is aimed at the study of electrochemical properties of ferrocenes Fc(L)R **1a—d**, **2a—g**, **3a**,**b**, **4a**,**b**, and **5a—c** (R is the fragment of 2,6-di-*tert*-butylphenol or its aromatic analog) in different solvents (MeCN, CH_2Cl_2, DMF) and evaluation of their antioxidative activity using the DPPH-test, whose monitoring was performed by CVA.

Results and Discussion

Chalcones **2b—g** were synthesized by the condensation of ferrocenecarboxaldehyde with substituted acetophenones in alkaline medium according to the method described earlier.[21] New α,β-unsaturated ferrocene carbonyl compound **2a** containing the 2,6-di-*tert*-butylphenol fragment was obtained under similar conditions (Scheme 1).

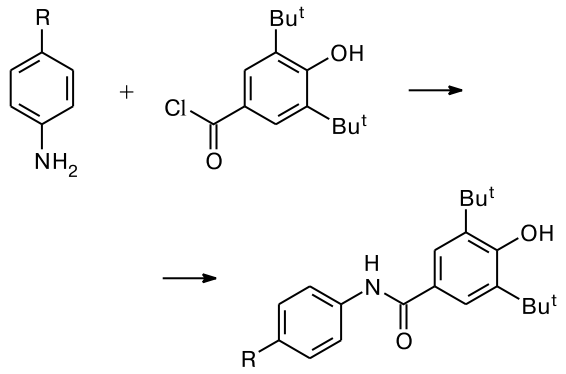
Scheme 1



Amino derivatives **4a**,**b** and **5c** were obtained by the reduction of the corresponding nitro-substituted phenylferrocene, ferrocenylchalcone, and ferrocenyl-4,5-dihydropyrazole with $SnCl_2$ in hydrochloric acid.[22] The synthesis of compounds **3a**, **4a**, and **5a**,**b** was accomplished by acylation of ferrocene amino derivatives with 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride (Scheme 2).

Electrochemical redox-potentials for compounds **1a—d**, **2a—g**, **3a**,**b**, **4a**,**b**, and **5a—c** were measured in different solvents. Similarly to the picture earlier observed[23] in MeCN, the voltamgram of compound **1a** in DMF in the anodic region has three waves of oxidation (Fig. 2). The irreversible character of the first one-electron wave indicates that the process of electron transfer is followed by the fast chemical or electrochemical step. The second wave is reversible and the third is irreversible. Such a shape of CVA can probably be explained by the following sequence of steps: oxidation of the ferrocene fragment to ferricinium cation with subsequent intramolecular reduction of the Fe^{3+} ion due to the electron transfer from the phenol fragment and elimination of proton (the first wave), repeated oxidation of Fe^{2+} to Fe^{3+} at more positive anode potential (the second wave), and oxidation of the phenoxy radical formed.[23] When MeCN is replaced by DMF and

Scheme 2



3a, **4a**, **5a**,**b**

R = Fc (**3a**), FcC(O)CH=CH (**4a**), 1-acetyl-3-ferrocenyl-4,5-dihydropyrazol-5-yl (**5a**), 1-acetyl-5-ferrocenyl-4,5-dihydropyrazol-3-yl (**5b**)

CH_2Cl_2, the shift of potential peaks to the anodic region is observed (Table 1). Such a displacement was observed for the oxidation of other substituted ferrocenes, that is apparently due to the change of solvation energy when another solvent is taken.[24,25]

At the same time, two irreversible oxidation waves are observed on the CVA curve of compound **1c** in DMF in anodic region at low speeds of potential sweep (lower 200 mV s^{-1}) (Fig. 3). Apparently, at low speeds of potential sweep phenoxy radicals formed in the second wave of oxidation are unstable and readily involved into further chemical transformations. This agrees with the ESA spectroscopic data, according to which stability of phenoxy radical formed by oxidation of compound **1c** is low.[26] When the speed of potential scanning is higher, the third peak

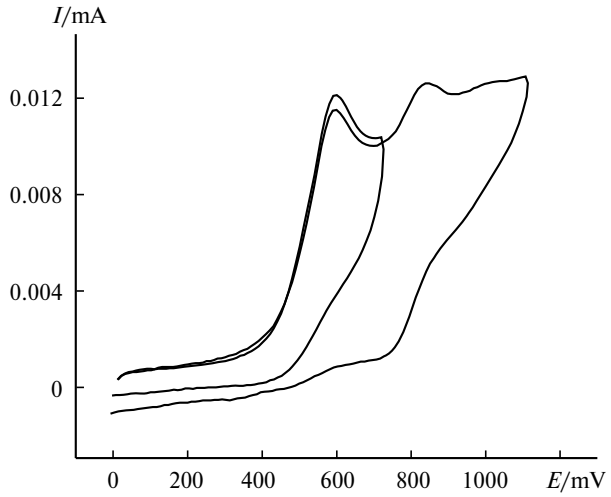


Fig. 2. Cyclic voltamgram of compound **1a** in DMF at the speed of potential sweep 100 mV s^{-1} (Pt-electrode, the concentration was $1 \cdot 10^{-3}$ mol L^{-1}, $Bu^n_4NBF_4$, Ag/AgCl).

Table 1. Electrochemical potentials of compounds **1a**,**c** (Pt-electrode, 10^{-3} mol L^{-1}, 0.5 *M* Bu_4NBF_4, Ag/AgCl/KCl, the scanning rate was 0.2 V s^{-1})

Compound	E^{ox_1}/E^{red_1}	E^{ox_2}/E^{red_2}	E^{ox_3}/E^{red_3}
		V	
		MeCN	
1a	0.48*	0.75/0.67	1.01/0.92
1c	0.64*	0.81/0.75	1.05/0.97
		DMF	
1a	0.60*	0.85/0.75	
1c	0.61*	0.78/0.61	1.03
		CH_2Cl_2	
1a	0.64	—	1.13
1c	0.77/0.68	0.96/0.88	1.13/1.03

* Irreversible wave of oxidation.

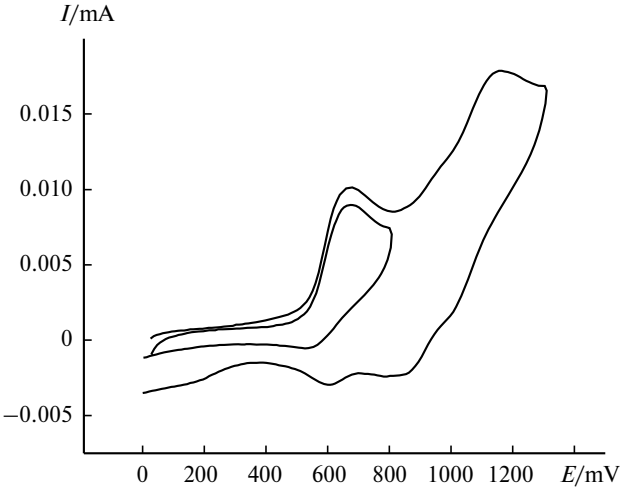


Fig. 4. Cyclic voltamgram of compound **1a** in CH_2Cl_2 at the speed of potential sweep 100 mV s^{-1} (Pt-electrode, the concentration was $1 \cdot 10^{-3}$ mol L^{-1}, $Bu^n{}_4NBF_4$, Ag/AgCl).

appears at the potential corresponding to the oxidation of phenoxy radicals. It is possible that when the speed of sweep increases, amount of unreacted phenoxy radicals increases, and potential of their oxidation is reached faster, that leads to the registration of the corresponding peak on the CVA curve.

When compound **1a** is oxidized in CH_2Cl_2, three peaks of oxidation on the direct scan of the cyclic voltamgram are observed (the second peak is of low intensity), while there are three waves on the reverse scan, apparently, corresponding to the conjugate reduction of the oxidation products of this substance (Fig. 4). Three reversible waves are observed on the CVA curve of derivative **1c** in CH_2Cl_2 (Fig. 5), with the height of the second wave of oxidation being considerably lower than that of the first and the third. Obviously, in this case the rate of chemical step, which follows the oxidation of ferrocenyl fragment of the molecule (*i.e.*, intramolecular electron transfer and proton abstraction or PCET), is low, which is indicated by the low values of current in the peak of the wave corresponding to the repeated oxidation $Fe^{2+} \rightarrow Fe^{3+}$. This agrees with the known data on higher deprotonating ability of acetonitrile as compared to CH_2Cl_2. Oxidation of thus formed phenoxy radical occurs at less positive potentials than in DMF, whereas the reversibility of the peak indicates formation of stable phenoxonium cation.

To sum up, the study of electrochemical behavior of ferrocene derivatives **1a** and **1c** in MeCN, DMF, and CH_2Cl_2 indicates that processes of intramolecular electron transfer and proton abstraction can proceed in these

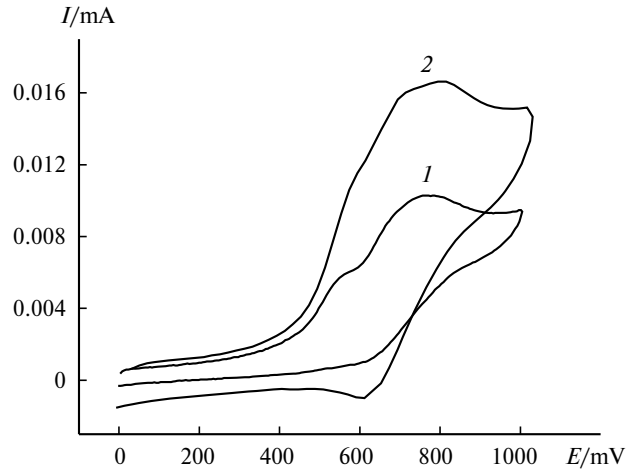


Fig. 3. Cyclic voltamgram of compound **1c** in DMF at the speeds of potential sweep 100 (*1*) and 300 mV s^{-1} (*2*) (Pt-electrode, the concentration was $1 \cdot 10^{-3}$ mol L^{-1}, $Bu^n{}_4NBF_4$, Ag/AgCl).

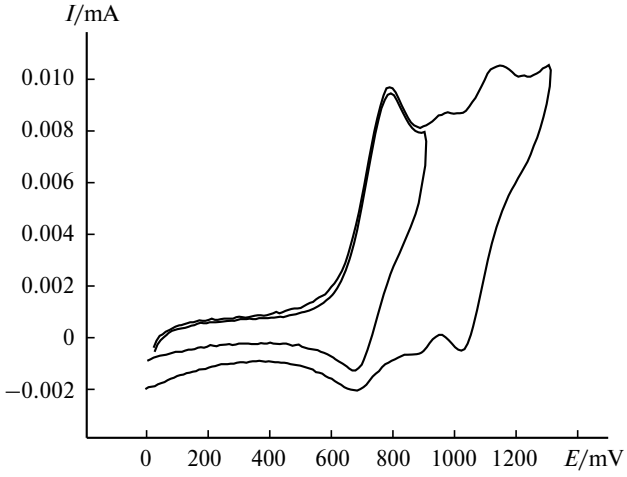


Fig. 5. Cyclic voltamgram of compound **1c** in CH_2Cl_2 at the speed of potential sweep 100 mV s^{-1} (Pt-electrode, the concentration was $1 \cdot 10^{-3}$ mol L^{-1}, $Bu^n{}_4NBF_4$, Ag/AgCl).

compounds with their rates being significantly dependent on the nature of the solvent. At the same time, compounds **1b** and **1d**, containing no phenol groups, undergo one-electron and reversible oxidation in MeCN, DMF, and CH_2Cl_2 at the potential close to the oxidation potential of unsubstituted ferrocene.

The CVA curve of compound **2a** in MeCN exhibits the oxidation peak of ferrocenyl fragment and two-electron irreversible wave of oxidation of phenol substituent. This indicates that the presence of the carbonyl group in the spacer between the phenol and ferrocenyl fragments of the molecule interferes with the intramolecular electron transfer.

The CVA curves of chalcones **2b**—**g** in MeCN, DMF, and CH_2Cl_2 exhibit a one-electron reversible wave in the anodic region, which corresponds to the oxidation $Fe^{2+} \rightarrow Fe^{3+}$ in ferrocene. Potentials of the oxidation peaks of chalcones **2b**—**g** are displaced toward the anodic region as compared to those for unsubstituted ferrocene (Table 2), that is due to the acceptor character of substituents in the cyclopentadienyl ring. In the case of compounds **2a** and **2c**—**e** in MeCN, there is linear correlation between the Brown constants[27] and potentials of oxidation peaks ($E_{ox} = 0.675 + 0.063\sigma^+$). This indicates the presence of conjugation of the substituent and ferrocenyl fragment of the molecule, which is stronger in the case of substituent with a lone electron pair on the heteroatom. It should be noted that in the case of compound **2a** in DMF and CH_2Cl_2, no oxidation wave of phenol group is observed in the operating region of potentials. Obviously, this is due to the significant electron-withdrawing effect of the oxidized ferrocene fragment, which shifts the peak potential to the region of discharge of the solvent molecules.

Compounds **4a**,**b** and **5a**—**c** containing an amide fragment also undergo one-electron and reversible oxidation in MeCN, DMF, and CH_2Cl_2 at potentials more positive than oxidation potential of ferrocene. The strongest shift of potential of the redox-transition $Fe^{2+} \rightarrow Fe^{3+}$ is observed in the case of compound **4a**, in which the ferrocenyl fragment is conjugated with the carbonyl group. The second one-electron wave is observed in oxidation of compounds **4a**,**b** and **5a**—**c**, which apparently is due to the oxidation process of the amino group.[28] Three waves of oxidation are observed on the CVA curve of compound **5c** in MeCN with the second wave having reversible character (Fig. 6), that indicates stability of the N-centered radical cation formed and slow rate of its deprotonation. The third peak, probably, corresponds to the oxidation of the radical formed (Scheme 3).

The CVA curves of compounds **3a**,**b** exhibit reversible one-electron wave corresponding to the redox-transition $Fe^{2+} \rightarrow Fe^{3}$. No peaks of oxidation corresponding to the N-centered oxidation were observed.

To sum up, research on electrochemical behavior of ferrocenes **1a**—**d**, **2a**—**g**, **3a**,**b**, **4a**,**b**, and **5a**—**c** by CVA showed that potential of the first oxidation peak of these compounds is more anodic than potential of the reduction peak of DPPH (Table 1, 2). Consequently, the compounds under study are convenient objects for the use in the suggested electrochemical method for evaluation of antioxidative activity. It should be noted that spectrophotometric method is little suitable in the case of ferrocenes **2a**, **3a**,

Table 2. Electrochemical oxidation potentials of compounds **1b**,**d**, **2a**—**g**, **3a**,**b**, **4a**,**b**, and **5a**—**c** (Pt-electrode, 10^{-3} mol L^{-1}, 0.5 *M* Bu_4NBF_4, Ag/AgCl/KCl, the scanning rate was 0.2 V s^{-1})

Compound	E^{ox_1}/E^{red_1}/V		
	MeCN	DMF	CH_2Cl_2
1b	0.72/0.64	0.72/0.64	0.85/0.72
1d	0.54/0.46	0.69/0.60	0.78/0.68
2a	0.62/0.55, 1.62*	0.70/0.64	0.76/0.69
2b	0.65/0.57	0.73/0.62	0.80/0.70
2c	0.72/0.64	0.70/0.63	0.61/0.54
2d	0.66/0.58	0.74/0.66	0.84/0.68
2e	0.68/0.59	0.67/0.68	0.85/0.72
2f	0.59/0.53	0.60/0.53	0.77/0.68
2g	0.74/0.63	0.76/0.65	0.79/0.70
3a	0.63/0.55	0.59/0.49	0.79/0.70
3b	0.45/0.35, 1.10/0.90	0.46/0.37, 1.02*	0.68/0.60, 1.31*
4a	0.74/0.65, 1.38*	0.82/0.71, 1.43*	0.85/0.76, 1.48*
4b	0.75/0.67, 1.15/1.07	0.78/0.68, 1.22*	0.82/0.75, 1.41*
5a,**b**	0.66/0.59, 1.30*	0.64/0.55, 1.36*	0.84/0.76, 1.44*
5c	0.56/0.48, 1.07/0.99, 1.33*	0.62/0.53, 1.12/1.11	0.71/0.62, 1.31*

* Irreversible wave of oxidation.

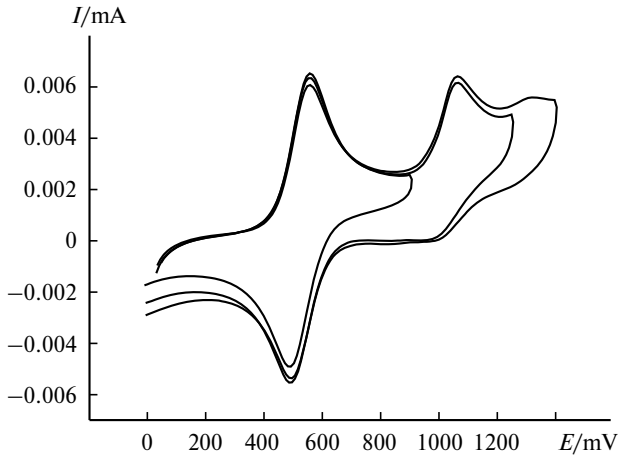
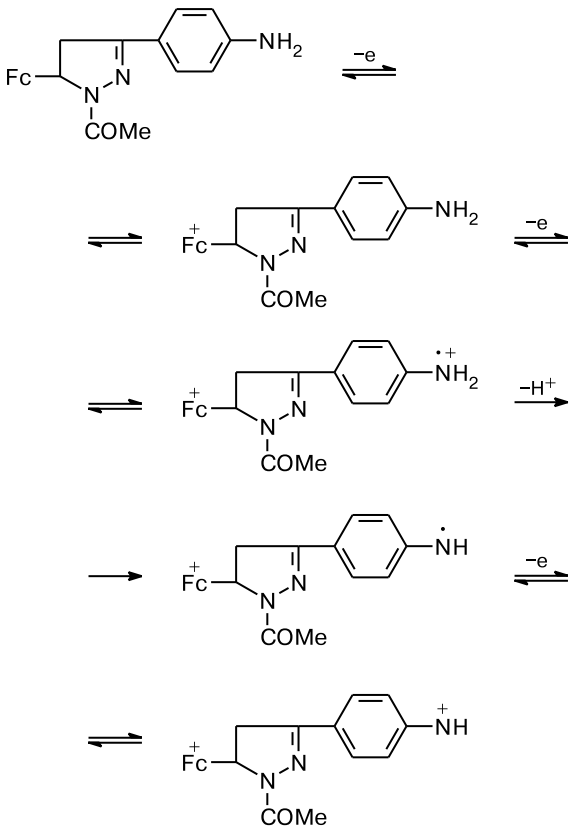


Fig. 6. Cyclic voltamgram of compound **5c** in MeCN at the speed of potential sweep 100 mV s^{-1} (Pt-electrode, the concentration was $1 \cdot 10^{-3}$ mol L^{-1}, $Bu^n_4NBF_4$, Ag/AgCl).

Scheme 3



4a, and **5a**,**b**, since the absorption band of these compounds virtually coincides with the absorption band of DPPH. Figure 7 shows the change of the reduction wave of DPPH in time when compound **4a** is added. It is seen that while the reaction progresses, a decrease in the value of current in the oxidation peak of the DPPH radical anion is observed. The data obtained were used for the plotting of kinetic dependencies and evaluation of antioxidative activity (A) of studied compounds. Figure 8 shows the A values determined in the test with DPPH obtained by CVA (the time from the beginning of the reaction is 10 min, $A = (1 - C/C_0) \cdot 100(\%)$).

Antioxidative activity of ferrocenes containing the 2,6-di-*tert*-butylphenol fragment changes in the following order:

4a > **3a** > **1a** > **1c** > **5a** **5b** > **2a**.

It is possible that such a dependence is due to the stability of phenoxy radicals formed in the reaction of these compounds with DPPH. Compounds **1c**, **1a**, **3a**, and **4a**, proceeding from the activity values $A > 50\%$ (56, 61, 64, 74%, respectively), can be related to the class of efficient antioxidants.

Ferrocene derivatives **1b**,**d** and **2b**—**e** containing no phenol fragments do not possess antioxidative activity (the A values are close to zero). At the same time, compounds bearing an amino group can be also involved into the reaction of hydrogen atom abstraction by the radical DPPH. However, the A values of compounds **3b**, **4b**, and **5c** are considerably lower (8, 6, and 6%, respectively) than those of the corresponding phenol derivatives (see Fig. 8). Consequently, a conclusion can be drawn that the fragment of spatially hindered phenol contributes the most to the antioxidative activity of these compounds.

While the reaction progresses, the height of the second oxidation wave of compound **1a** decreases in parallel with

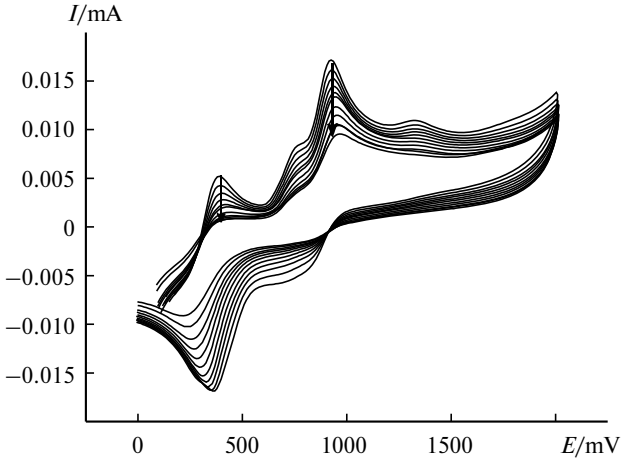


Fig. 7. The change of the CVA curves in the course of the reaction of DPPH with compound **4a** (Pt-electrode, the speed of potential sweep was 200 mV s^{-1}, concentration $0.5 \cdot 10^{-3}$ mol L^{-1}, the ratio of concentrations 1 : 1, $Bu^n_4NBF_4$, MeCN, Ag/AgCl, the reaction time was 20 min).

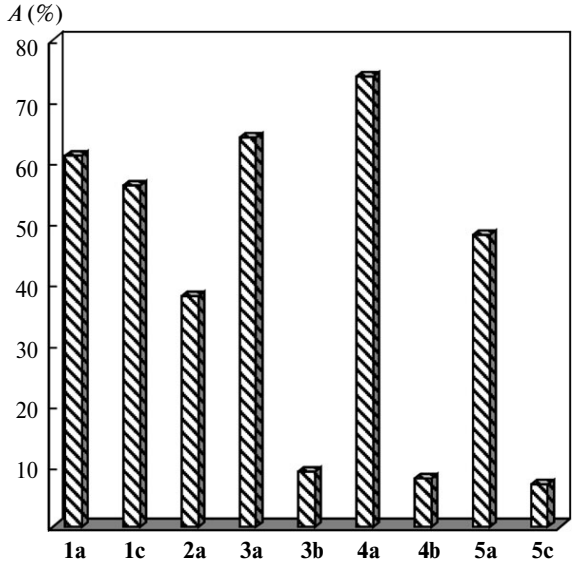


Fig. 8. The values of antioxidative activity of compounds **1a**,**c**, **2a**, **3a**,**b**, **4a**,**b**, and **5a**,**c** determined by CVA with the use of the DPPH-test (the time from the beginning of the reaction was 10 min, the ratio of concentrations was 1 : 1, $C_0 = 0.5 \cdot 10^{-3}$ mol L^{-1}).

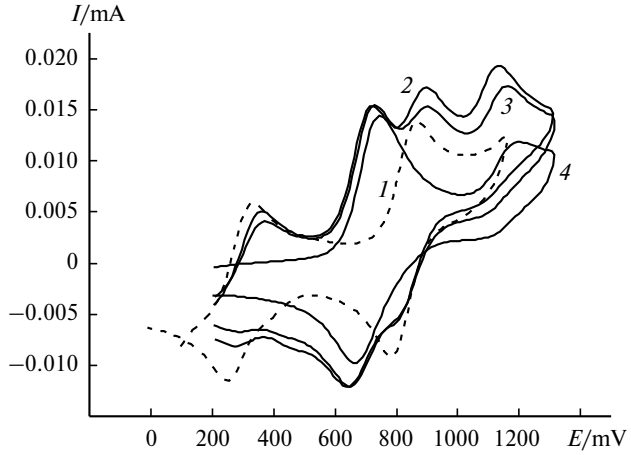
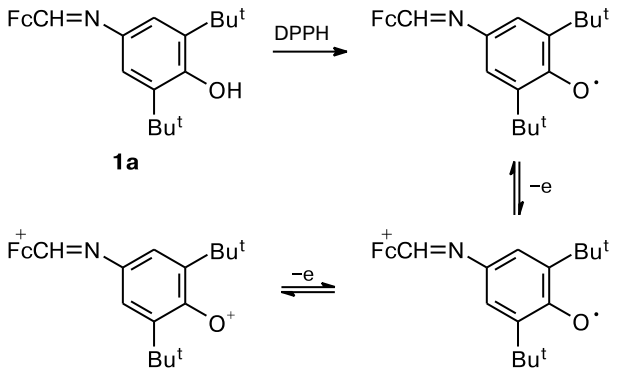


Fig. 9. The changes of the CVA curves in the course of the reaction of compound **1a** with DPPH: the CVA curve of DPPH in the absence of compound **1a** (*1*), the CVA curves in 0.5 (*2*), 1.5 (*3*), and 20 min (*4*) after the reaction begins (Pt-electrode, the speed of potential sweep was 200 mV s^{-1}, the concentration was $0.5 \cdot 10^{-3}$ mol L^{-1}, the ratio of concentrations was 1 : 1, $Bu^n_4NBF_4$, MeCN, Ag/AgCl).

the decrease of the DPPH reduction peak height (Fig. 9). It can be explained by the fact that the corresponding phenoxy radical is formed in the presence of DPPH, and oxidation of **1a** proceeds in two steps (Scheme 4): oxidation of ferrocenyl fragment and phenoxy radical to phenoxonium ion. Thus, there are no waves characteristic of the process of intramolecular electron transfer and proton abstraction on the CVA curves of **1a** and **1c** with DPPH, that confirms the mechanism of oxidation of these ferrocene derivatives suggested earlier.[23]

Scheme 4



Independent biological testing of compounds **1a** and **1c** on the degree of peroxide oxidation of lipids in the rat liver and brain homogenates showed that these compounds possess a significant antioxidative effect and inhibit a Ca^{2+}-dependent mitochondria swelling.[29] These data correlate with the results obtained by electrochemical method.

In conclusion, the present work showed that electrochemical method can be used for the quantitative evaluation of antioxidative activity of chromophoric substances by the reaction with DPPH, whereas ferrocenes **1a**,**c**, **3a**, **4a**, and **5a**,**b** having the 2,6-di-*tert*-butylphenol fragment as a substituent are efficient antioxidants.

Experimental

Electrochemical redox-potentials were measured in a three-electrode cell using an IPC Pro-M digital potentiometer-galvanometer (Vol´ta, Russia) connected to a personal computer. A stationary platinum electrode 2 mm in diameter was used as a reference electrode. Oxygen was removed from the cell by purging with dry argon. Potentials were measured relatively to the saturated Ag/AgCl electrode. Concentrations of solutions of compounds under study were $1 \cdot 10^{-3}$ mol L^{-1}. Platinum electrode was thoroughly purified after registration of each curve. Antioxidative activity of compounds **1a**—**d**, **2a**—**g**, **3a**,**b**, **4a**,**b**, and **5a**—**c** was studied by the reaction with stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in MeCN (the full reaction time was 20 min). The rate of the process was monitored by the change of the DPPH reduction current intensity (with the ratio of concentrations of compounds and DPPH being 1 : 1 ($C_0 = 0.5 \cdot 10^{-3}$ mol L^{-1})). The CVA curves were recorded after certain periods of time (30 s and 1 min). The values of antioxidative activity $A = (1 - C/C_0) \cdot 100(\%)$ of compounds under study were determined for the moment of time 10 min from the beginning of the reaction. The values of I_0, obtained by the equation of a calibrating curve at a given concentration of DPPH were used for the plotting of kinetic curves.

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz) in $CDCl_3$ relatively to Me_4Si. IR spectra were recorded on a IR 200 Fourier-spectrophotometer (Thermo Nicolet) in KBr pellets. The solvents used (MeCN, DMF, CH_2Cl_2) were purified according to the standard procedures.[27]

Compounds **1a**,**d** were obtained by the reaction of ferrocenecarboxaldehyde (Aldrich, 98%) with the corresponding amines according to the known procedures.[21,23] 4-Acetyl-2,6-di-*tert*-butylphenol was obtained according to the reported method.[26] 4-Aminophenylferrocene (**3b**), 3-(4-aminophenyl)-1-ferrocenylprop-2-en-1-one (**4b**), 1-acetyl-5-(4-aminophenyl)-3-ferrocenyl-4,5-dihydropyrazole (**5c**), 1-acetyl-3-(4-aminophenyl)-5-ferrocenyl-4,5-dihydropyrazole were obtained by the reduction of the corresponding nitro derivatives according to the reported method.[22,30]

1-(3,5-Di-*tert*-butyl-4-hydroxyphenyl)-3-ferrocenylprop-2-en-2-one (2a). Concentrated aqueous NaOH (1 mL) was added to a mixture of ferrocenecarboxaldehyde (0.11 g, 0.5 mmol) and 4-acetyl-2,6-di-*tert*-butylphenol (0.12 g, 0.5 mmol) in ethanol (7 mL). The mixture was refluxed for 8 h under argon. After cooling, the reaction mixture was weakly acidified with dilute aq. HCl, and the product was extracted with diethyl ether. After the solvent was evaporated, the product was isolated on a column with Al_2O_3 (Brockmann II activity, elution with diethyl ether—light petroleum, 1 : 1). First was eluted unreacted FcCHO, then product **2a** as a dark red oil. The yield was 0.13 g (59%). Found (%): C, 62.89; H, 9.86. $C_{17}H_{32}O_2Fe$. Calculated (%): C, 62.96; H, 9.95. ^{1}H NMR ($CDCl_3$), δ: 1.52 (s, 18 H, $C(CH_3)_3$); 4.20 (s, 5 H, C_5H_5); 4.48 (m, 2 H, C_5H_4); 4.61 (m, 2 H, C_5H_4);

5.73 (s, 1 H, OH); 7.14 (d, 1 H, =CH, J = 15.0 Hz); 7.73 (d, 1 H, =CH, J = 15.4 Hz); 7.89 (s, 2 H, C_6H_2). IR, ν/cm^{-1}: 3619 (OH); 2956, 2911 (CH, $C(CH_3)_3$); 1648 (CO); 1107 (C_5H_5).

4-(3,5-Di-*tert*-butyl-4-hydroxybenzoylamino)phenylferrocene (3a). Thionyl chloride (6 mL) was added to 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (Aldrich, 98%) (0.25 g, 1 mmol). The mixture was refluxed for 1 h, excess $SOCl_2$ was evaporated *in vacuo*. A solution of 4-aminophenylferrocene (0.14 g, 0.5 mmol) in CH_2Cl_2 containing Et_3N (2 mL) (to scavenge the liberated HCl) was added to the thus obtained white crystalline residue, the mixture was kept for 16 h. The solvent was evaporated *in vacuo*, the residue was separated on a column with Al_2O_3 (elution with diethyl ether—light petroleum, 2 : 1) to obtain product **3a** (0.24 g, 88%), m.p. 195—197 °C. Found (%): C, 73.20; H, 7.24; N, 2.45. $C_{31}H_{35}O_2NFe$. Calculated (%): C, 73.08; H, 6.97; N, 2.75. ^{1}H NMR ($CDCl_3$), δ: 1.51 (s, 18 H, $C(CH_3)_3$); 4.22 (s, 5 H, C_5H_5); 4.54 (m, 2 H, H(β), C_5H_4); 4.88 (m, 2 H, H(α), C_5H_4); 5.64 (s, 1 H, OH); 7.36, 7.54 (both d, 4 H, *n*-C_6H_4); 7.65 (m, NH); 7.70 (s, 2 H, C_6H_2). IR, ν/cm^{-1}: 3619 (OH); 3089 (NH); 2958, 2923 (CH, $C(CH_3)_3$); 1644 (CO); 1107 (C_5H_5).

3-[4-(3,5-Di-*tert*-butyl-4-hydroxybenzoylamino)phenyl]-1-ferrocenylprop-2-en-1-one (4a). Thionyl chloride (7 mL) was added to 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (0.375 g, 1.5 mmol). The mixture was refluxed for 1 h, then excess $SOCl_2$ was evaporated *in vacuo*. A solution of 3-(4-aminophenyl)-1-ferrocenylprop-2-en-1-one (0.33 g, 1 mmol) in CH_2Cl_2 (20 mL, containing a few drops of Et_3N to scavenge the liberated HCl) was added dropwise to the residue, 3,5-di-*tert*-butyl-4-hydroxybenzoyl chloride. A white smoke was observed together with a slight heating of the reaction mixture. The solution obtained was stirred for 30 min at room temperature, then refluxed for 1 h, and kept for 16 h. The reaction mixture was concentrated *in vacuo*, and the product was isolated by column chromatography on Al_2O_3 (Brockmann II activity, elution with diethyl ether). The starting compound (traces) was eluted first, then product **4a**. The yield was 0.3 g (53%), m.p. 190 °C (decomp.). Found (%): C, 72.43; H, 6.70; N, 2.36. $C_{34}H_{37}O_3NFe$. Calculated (%): C, 72.47; H, 6.61; N, 2.48. ^{1}H NMR ($CDCl_3$), δ: 1.51 (s, 18 H, $C(CH_3)_3$); 4.24 (s, 5 H, C_5H_5); 4.61 (m, 2 H, H(β), C_5H_4); 4.94 (m, 2 H, H(α), C_5H_4); 5.68 (s, 1 H, OH); 7.2 (d, 1 H, =CH); 7.67 (m, 2 H, C_6H_4); 7.73 (s, 2 H, C_6H_2); 7.77 (m, 2 H, C_6H_4); 7.80 (d, 1 H, =CH); 7.92 (br.m, 1 H, NH). IR, ν/cm^{-1}: 3625 (OH), 3299 (NH), 2954 (CH, $C(CH_3)_3$), 1640—1660 (C=O); 1100—1110 (C_5H_5).

1-Acetyl-5-[4-(3,5-di-*tert*-butyl-4-hydroxybenzoylamino)-phenyl]-3-ferrocenyl-4,5-dihydropyrazole (5a). Thionyl chloride (6 mL) was added to 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (0.375 g, 1.5 mmol). The mixture was refluxed for 1 h and excess $SOCl_2$ was removed *in vacuo*. A solution of 1-acetyl-5-(4-aminophenyl)-3-ferrocenyl-4,5-dihydropyrazole (0.16 g, 0.4 mmol) in CH_2Cl_2 (15 mL) and several drops of Et_3N was added to the acyl chloride obtained. A white smoke was observed. A dark red solution obtained was stirred for 1 h at room temperature and kept for 16 h. The solvent was evaporated *in vacuo*, the product was isolated on a column with Al_2O_3 (Brockmann II activity); traces of the starting amine were eluted first with diethyl ether, then product **5a** with the diethyl ether—ethanol mixture (20 : 1). The yield of compound **5a** was 0.2 g (80%), m.p. 175—177 °C. Found (%): C, 69.71; H, 6.91; N, 6.62. $C_{36}H_{42}O_3N_3Fe$. Calculated (%): C, 69.67; H, 6.82; N, 6.77. ^{1}H NMR ($CDCl_3$), δ: 1.48 (s, 18 H, $C(CH_3)_3$); 2.37 (s, 3 H, $COCH_3$); 2.95 (m, 1 H, CH_2, heterocycle); 3.52 (m, 1 H, CH_2, heterocycle); 4.15 (s, 5 H, C_5H_5); 4.42 (m, 2 H, H(β), C_5H_4); 4.53 (m, 1 H, H(α), C_5H_4); 4.69 (m, 1 H, H(α), C_5H_4); 5.49 (m, 1 H, CH, heterocycle); 5.62 (s, 1 H, OH); 7.20, 7.60 (both m, 4 H, C_6H_4); 7.71 (s, 2 H, C_6H_2); 8.07 (br.m, 1 H, NH). IR, ν/cm^{-1}: 3621 (OH), 3436 (OH and/or NH), 2956 (CH, $C(CH_3)_3$), 1646 (C=O), 1100—1110 (C_5H_5).

1-Acetyl-3-[4-(3,5-di-*tert*-butyl-4-hydroxybenzoylamino)-phenyl]-5-ferrocenyl-4,5-dihydropyrazole (5b). Thionyl chloride (4 mL) was added to 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (0.15 g, 0.6 mmol). The mixture was refluxed for 1 h under argon and excess thionyl chloride was evaporated *in vacuo*. A solution of 1-acetyl-3-(4-aminophenyl)-5-ferrocenyl-4,5-dihydropyrazole (0.19 g, 0.5 mmol) in CH_2Cl_2 (20 mL, containing several drops of Et_3N) was added to the residue with cooling. The mixture was refluxed for 1 h and kept for 16 h. The solvent was evaporated *in vacuo*. The residue was diluted with diethyl ether, the undissolved precipitate was filtered off, the solvent was evaporated. The residue was diluted with hexane, a precipitate formed was filtered off and washed with hexane to obtain compound **5b** (0.27 g, 87%), m.p. 163—165 °C. Found (%): C, 69.56; H, 6.75; N, 6.59. $C_{36}H_{42}O_3N_3Fe$. Calculated (%): C, 69.67; H, 6.82; N, 6.77. ^{1}H NMR ($CDCl_3$), δ: 1.51 (s, 18 H, $C(CH_3)_3$); 2.33 (s, 3 H, CH_3CO); 3.56 (ABX system, 1 H, H_a, heterocycle); 3.66 (ABX system, 1 H, $H_в$, heterocycle); 4.03 (m, 1 H, C_5H_4); 4.13 (m, 2 H, C_5H_4); 4.16 (s, 5 H, C_5H_5); 4.49 (m, 1 H, C_5H_4); 5.48 (ABX system, 1 H, H_x, heterocycle); 5.69 (s, 1 H, OH); 7.75 (s, 2 H, C_6H_2); 7.80 (m, C_6H_4). IR, ν/cm^{-1}: 3623 (OH), 3426 (OH and/or NH), 2960 (CH, $C(CH_3)_3$), 1648 (C=O), 1108 (C_5H_5).

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