ISSN 0012-5008, Doklady Chemistry, 2013, Vol. 450, Part 2, pp. 152–155. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © V.Yu. Tyurin, Zhang Jingwei, A.A. Moiseeva, E.R. Milaeva, D.V. Belykh, E.V. Buravlev, T.K. Rocheva, I.Yu. Chukicheva, A.V. Kuchin, 2013, published in Doklady Akademii Nauk, 2013, Vol. 450, No. 5, pp. 543–546.

CHEMISTRY

Comparative Study of Redox Characteristics and Antioxidant Activity of Porphyrins with 2,6-Dialkylphenol Groups

V. Yu. Tyurin[a], Zhang Jingwei[a], A. A. Moiseeva[a], E. R. Milaeva[a, b], D. V. Belykh[c], E. V. Buravlev[c], T. K. Rocheva[c], I. Yu. Chukicheva[c], and Corresponding Member of the RAS A. V. Kuchin[c]

Received December 13, 2012

DOI: 10.1134/S0012500813060025

The oxidative stress of an organism is a result of the acceleration of lipid peroxidation (LPO) in cells and the formation of reactive oxygen species and free radicals. This process leads to destruction of membranes, the emergence of numerous pathologies, and premature aging of the organism. The search for new antioxidants, as well as for methods to assess the antioxidant activity, is an important and interesting task [1]. Of particular interest are synthesis and study of properties of polyfunctional compounds with several pharmacophoric centers in the molecule, because such a combination is able not only to enhance the known physiological activity but also to cause the appearance of new types of physiological activity [2–4]. Ionol **I** (2,6-di-*tert*-butyl-4-methylphenol) is a known antioxidant used in the manufacture of foodstuffs (food additive E321). 2,6-Diisobornyl-4-methylphenol (dibornol) **IV** is currently undergoing preclinical trials as a promising drug [5].

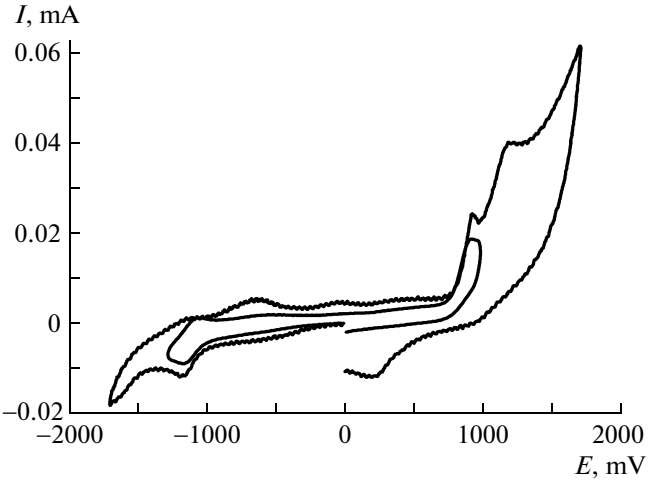


Fig. 1. Cyclic voltammogram of free base of porphyrin *V* in DMF at a potential sweep rate of 100 mV/s (Pt, porphyrin concentration 10^{-3} M, *n*-Bu_4NBF_4, Ag/AgCl).

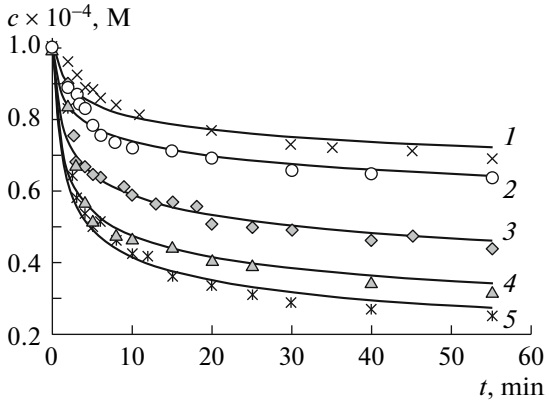
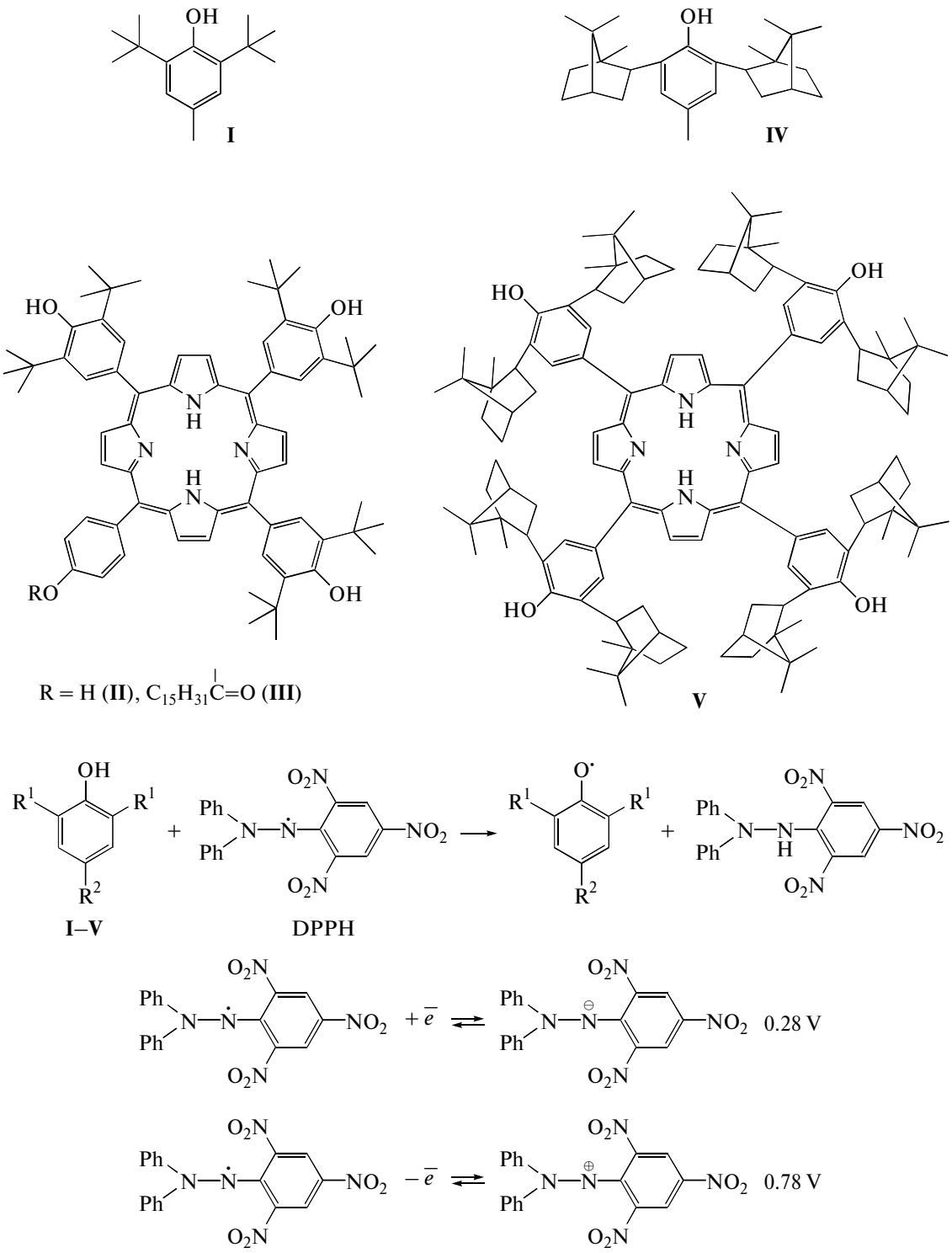


Fig. 2. Kinetic curves of DPPH concentration vs. time in the presence of additives: (*1*) compound **I** ([DPPH]/[additive] = 1 : 4), (*2*) **IV** ([DPPH]/[additive] = 1 : 4), (*3*) **III** ([DPPH]/[additive] = 1 : 1), (*4*) **V** ([DPPH]/[additive] = 1 : 1), (*5*) **II** ([DPPH]/[additive] = 1 : 1); DMF, Pt, 0.05 M *n*-Bu_4NBF_4, Ag/AgCl/KCl, c_0 = 0.1 mM. Reaction time, 60 min.

[a] *Moscow State University, Moscow, 119992 Russia*
[b] *Institute of Physiologically Active Substances, Russian Academy of Sciences, Severnyi proezd 1, Chernogolovka, Moscow oblast, 142432 Russia*
[c] *Institute of Chemistry, Komi Scientific Center, Ural Branch, Russian Academy of Sciences, ul. Pervomaiskaya 48, Syktyvkar, 167982 Russia*



Scheme 1.

Previously, it was shown that tetra(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin (**II**), containing ionol moieties, and its analogue with the palmitic acid residue in the molecule (**III**) exhibit high antioxidant activity in the model oxidation reaction of (*Z*)-9-octadecenoic (oleic) acid, as well as in the LPO processes in Wistar rat liver homogenates [6–8]. Tetra(3,5-diisobornyl-4-hydroxyphenyl)porphine (**V**), containing bulky bicycloalkyl substituents on the phenyl ring, has been recently synthesized [9].

In this paper, we evaluated the antioxidant activity of compounds **I**–**V** from the hydrogen atom transfer

Oxidation and reduction potentials (E_{ox}, E_{red}) and antioxidant efficiency values (AE*) for compounds **I**–**V** determined from the rate of hydrogen elimination from DPPH by the rotating disc electrode method

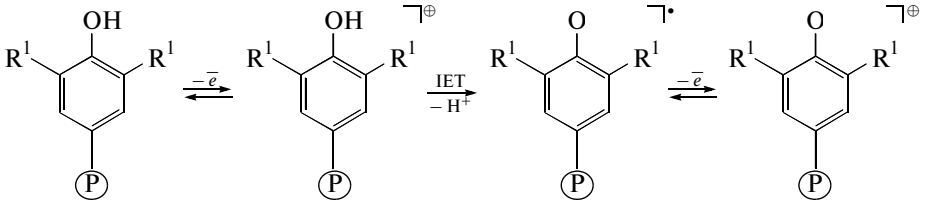
Compound	E_{ox}	E_{red}	AE, %
I	1.28	–	25.37
II	0.908 1.182	−1.167	77.36
III	0.961 1.246	−1.16	52.55
IV	1.23	−0.681 −1.782	30.98
V	0.912 1.247	−1.162	65.38

* The DPPH concentration is 0.1 mM. The additive-to-DPPH concentration ratio is 1 : 1 (compounds **II**, **III**, and **V**) and 4 : 1 (compounds **I** and **IV**). Reaction time 60 min, Pt, 0.05 M n-Bu_4NBF_4, DMF, Ag/AgCl.

rate in the reaction with the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH), Scheme 1. Since one of the absorption bands of porphyrins (λ_{max}, is 522.0 (**II**), 521.0 (**III**), and 522.5 (**V**) nm) is close to the absorption band of DPPH (517 nm), the reaction course was monitored using our proposed electrochemical DPPH test. The cyclic voltammogram (CV) of DPPH in the anode region shows two reversible waves corresponding to the reduction and oxidation of the DPPH radical. This allows us to study the kinetics of the reaction by monitoring the change in the CV peak current [10–13] or from the limiting diffusion current in the polarization curve obtained by the rotating disk electrode (RDE) method.

The use of the electrochemical DPPH test requires the absence of overlap of the oxidation–reduction peaks of DPPH and compounds under consideration. In this context, we studied the behavior of compounds **I**–**V** in DMF by cyclic voltammetry (the choice of a medium is due to the extremely low solubility of **V** in CH_3CN). The cyclic voltammograms of phenols **I** and **IV** in the anodic range show a wave of the irreversible two-electron oxidation localized on the hydroxyl group of the sterically hindered phenol moiety to give the phenoxylium cation (table).

The voltammograms of porphyrins **II**, **III**, and **V** in the anodic range are similar: there are two irreversible one-electron oxidation peaks (Fig. 1). The first peak is apparently due to the oxidation of the porphyrin ring to the radical cation, followed by its rapid reduction via intramolecular electron transfer (IET) from the phenol group (Scheme 2). The irreversible character of the peak is caused by the rapid chemical stage of proton elimination (the EC mechanism). The second peak corresponds to one-electron oxidation of the phenoxyl radical thus formed to the corresponding cation [13]. The oxidation peak of phenol groups are not observed because of the shift to the discharge region of the supporting electrolyte molecules.



II, **III**, **V**

Scheme 2.

Since the oxidation of porphyrins **II**, **III**, and **V** proceeds at more anodic potentials than the DPPH reduction wave potential (table) [14], it is possible to use the RDE method for estimating their antioxidant activity. The limiting diffusion current of the polarization curve on the rotating disk electrode is determined by the Levich equation [15]

$$I_d = 0.62nSFD^{2/3}\omega^{1/2}\nu^{-1/6}c_0,$$

where n is the number of transferred electrons, S is the electrode area (cm^2), F is Faraday's constant, D is the diffusion coefficient (cm^2/s), ω is the angular velocity of electrode rotation (rad/s), ν is the kinematic viscosity of a solution (cm^2/s), and c_0 is the depolarizer concentration (mol/cm^3).

For fixed values and the electrode area and rotation velocity, the ratio of the current at the beginning of the reaction (no additives) and in the end is determined by the DPPH concentration ratio:

$$I/I_0 = c/c_0,$$

where I_0 is the limiting current at the initial DPPH concentration c_0, I is the current at a DPPH concentration at a given moment of time.

As the reaction with DPPH proceeds, the limiting diffusion current decreases. Knowing the values I, I_0, and c_0 values, we construct kinetic curves of the DPPH concentration versus reaction time (Fig. 2). The antioxidant activity was quantified by determining the

antioxidant efficiency (AE) factor, which characterizes the content of the unreacted DPPH: AE = $(c_0 - c_{fin})/c_0 \times 100\%$, where c_0 is the initial concentration and c_{fin} is the final concentration of DPPH (reaction time, 60 min). The AE values for compounds **I**–**V** are presented in the table.

It can be seen that the activity of porphyrins with 2,6-dialkylphenol substituents changes along the series **III** < **V** < **II**. The decrease in antioxidant activity upon the introduction of the palmitic acid moiety is presumably associated with aggregation processes that occur due to hydrophobic interactions involving this substituent. It should also be noted that the antioxidant activity of **I**–**V** correlates with their oxidation potentials E_{ox} (table): compounds oxidized at more anodic potentials react with DPPH at a slower rate.

Noteworthy is the fact that the activity of porphyrins **II**, **III**, and **V** is clearly superior to the activity of compounds **I** and **IV**, even in the case where the concentration of **I** and **IV** exceeds the DPPH concentration (and the corresponding porphyrins) fourfold. It can be assumed that the antioxidant activity of **II**, **III**, and **V** is due not only to the combined effect of four phenol substituents but also to the presence of the porphyrin macrocycle in the molecule. The role of the macrocycle is that it is involved in unpaired electron delocalization, which enhances the stability of the resulting phenoxyl radical. Compounds for which AE exceeds 50% can be treated as promising antioxidants.

Voltammograms were measured in a three-electrode cell using an IPC-pro potentiostat (Volta, Russia) under argon. A stationary or rotating platinum electrode 3 mm in diameter was used as the working electrode and a platinum plate served as the auxiliary electrode. An (Ag/AgCl/KCl) electrode with a waterproof diaphragm was used as the reference electrode. The supporting electrolyte was a 0.5 M solution of Bu_4NBF_4 (99%, Acros) twice recrystallized from aqueous EtOH and dried in vacuum for 48 h at 50°C. The concentration of DPPH in all experiments was 1 mM. The EA was determined at various substrate-to-DPPH concentration ratios (table); the reaction time was 60 min.

ACKNOWLEDGMENTS

This work was supported by the Division of Chemistry and Materials Science of the RAS (program no. 9 "Medicinal and Biomolecular Chemistry," project no. 12-T-3–1020) and the Russian Foundation for Basic Research (project nos. 12–03–00937 and 12–03–00776).

REFERENCES

1. Zai-Qun Liu, *Chem. Rev.*, 2010, vol. 110, pp. 5675–5691.
2. Kuchin, A.V. and Chukicheva, I.Yu., *Vestn. Ural. Otd. Ross. Akad. Nauk,* 2011, no. 3 (37), pp. 43–57.
3. Buravlev, E., Chukicheva, I., Belykh, D., and Kuchin, A., *Chem. Natur. Comp.*, 2007, vol. 43, pp. 678–681.
4. Milaeva, E., *Curr. Top. Med. Chem.*, 2011, vol. 12, pp. 2703–2713.
5. Plotnikov, M.B., Chernysheva, G.A., Smol'yakova, V.I., et al., *Vestn. Ross. Akad. Med. Nauk*, 2009, no. 11, pp. 12–17.
6. Milaeva, E. Shpakovsky, D., Gracheva, Yu., et al., *J. Porph. Phthaloc.*, 2003, vol. 8, pp. 701–706.
7. Milaeva, E., Tyurin, V., Shpakovsky, D., et al., *Heteroatom. Chem.,* 2006, vol. 17, pp. 475–480.
8. Milaeva, E. and Gerasimova, O., Zhang Jingwei, et al., *J. Inorg. Biochem.,* 2008, vol. 102, pp. 1348–1358.
9. Belykh, D.V., Rocheva, T.K., Buravlev, E.V., et al., *Makrogeterotsikly*, 2012, vol. 5, pp. 121–124.
10. Tyurin, V.Yu., U Yaokhuan', Dolganov, A.V., Milaeva, E.R., *Dokl. Chem.*, 2011, vol. 436, part 2, pp. 31–33.
11. Tyurin, V.Yu., Meleshonkova, N.N., Dolganov, A.V., et al., *Izv. Akad. Nauk, Ser. Khim.*, 2011, vol. 60, pp. 633–641.
12. Tyurin, V.Yu., Jingwei, Z., Glukhova, A.P., and Milaeva, E.R., *Makrogeterotsikly*, 2011, vol. 4, pp. 211–212.
13. Meleshonkova, N.N., Shpakovsky, D.B., Fionov, A.V., et al., *J. Organomet. Chem.*, 2007, vol. 692, pp. 5339–5344.
14. Solon, E. and Bard, A.J., *J. Am. Chem. Soc.*, 1964, vol. 86, pp. 1926–1928.
15. Galus, Z., *Teoretyczne podstawy electroanalizy chemicznej*, Warszawa: Panstwowe Wydawnictwo Naukowe, 1971. Translated under the title *Teoreticheskie osnovy elektrokhimicheskogo analiza*, Moscow: Mir, 1974.

Translated by G. Kirakosyan