Brief Communications

Synthesis of 5-(4-hydroxyphenyl)-10,15,20-tris[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphine and 5-(4-palmitoyloxyphenyl)-10,15,20-tris[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphine and generation of phenoxyl radicals from them

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A porphyrin with a lipophilic hydrocarbon substituent, 5-(4-palmitoyloxyphenyl)-10,15,20-tris[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphine, was synthesized for the first time by acylation of 5-(4-hydroxyphenyl)-10,15,20-tris[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphine. Oxidation of these compounds with PbO_2 in toluene leads to the corresponding phenoxyl radicals.

Key words: porphyrins, 2,6-di(*tert*-butyl)phenol, phenoxyl radicals, EPR, lipophilic groups.

Porphyrins are parts of a numerous biochemical systems, while their synthetic analogs find application in pharmacology and medicine.[1—3] Lipophilic properties of porphyrins and their complexes with various metals ensure the accumulation of these compounds in the lipid bilayer of cell membranes and transport into the living cells. At the same time, the structural and functional similarity of synthetic metalloporphyrins with the active centers of oxidoreductases is the reason for their catalytic activity in oxidation reactions of organic substrates.[4] Interaction of exogenous porphyrins with membrane-bound proteins and transport of porphyrins into the cells can be accompanied by chemical processes resulting in the destruction of the protein structure and in the formation of by-products.[5] In this connection, the use of porphyrins, for example, as sensitizers in the diagnostics of tumor diseases is complicated by side processes of oxidative destruction of biological substrates. In this case, the presence of antioxidants is required.

Earlier, it was shown that polyfunctional systems are formed upon introduction of antioxidant fragments, 2,6-dialkylphenols, into complexes of metals with macrocyclic ligands, porphyrins and phthalocyanines.[6,7] Such compounds show catalytic or antioxidant properties de-

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pending on the metal nature. A model reaction of lipid peroxidation with the use of (*Z*)-octadec-9-enoic acid demonstrated[8] that tetrakis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphyrin shows high antioxidant activity.

The present work is aimed at the synthesis of polyfunctional porphyrins combining, in one molecule, 2,6-di(*tert*-butyl)phenol moieties as antioxidant substituents and a lipophilic fragment, which enables incorporation of such a porphyrin into the lipid bilayer of cell membranes.

Results and Discussion

The synthesis of non-symmetrical porphyrin **1a** containing three residues of sterically hindered 2,6-di(*tert*-butyl)phenol and one phenol fragment in *meso*-positions of the macrocycle[9] was modified enabling us to increase the product yield. Acylation of **1a** with palmitoyl chloride leads to porphyrin **2a** containing a higher saturated fatty acid residue (Scheme 1).

Compounds **1a** and **2a** are crystalline substances stable in air and in solutions. The structures of the compounds obtained are confirmed by the IR, UV, ^{1}H and ^{13}C NMR spectroscopic data, as well as by analytical data. In the IR spectra, sharp absorption bands are present in the region 3620—3640 cm^{-1} corresponding to the stretching vibrations of the O—H bond of the spatially hindered nonassociated phenolic group and in the region 3403—3426 cm^{-1} (associated OH group of phenol). In the UV spectra, the absorption bands characteristic of the free porphyrin bases[10,11] are observed: the Soret bands at 424 and 427 nm and four bands of lower intensity in the regions 652—522 and 650—521 nm, respectively, for compounds **1a** and **2a**, which confirms the formation of the porphyrin ring. Introduction of a long-chain hydrocarbon substituent into the porphyrin molecule results in a slight shift of the absorption bands toward the shortwave

Scheme 1

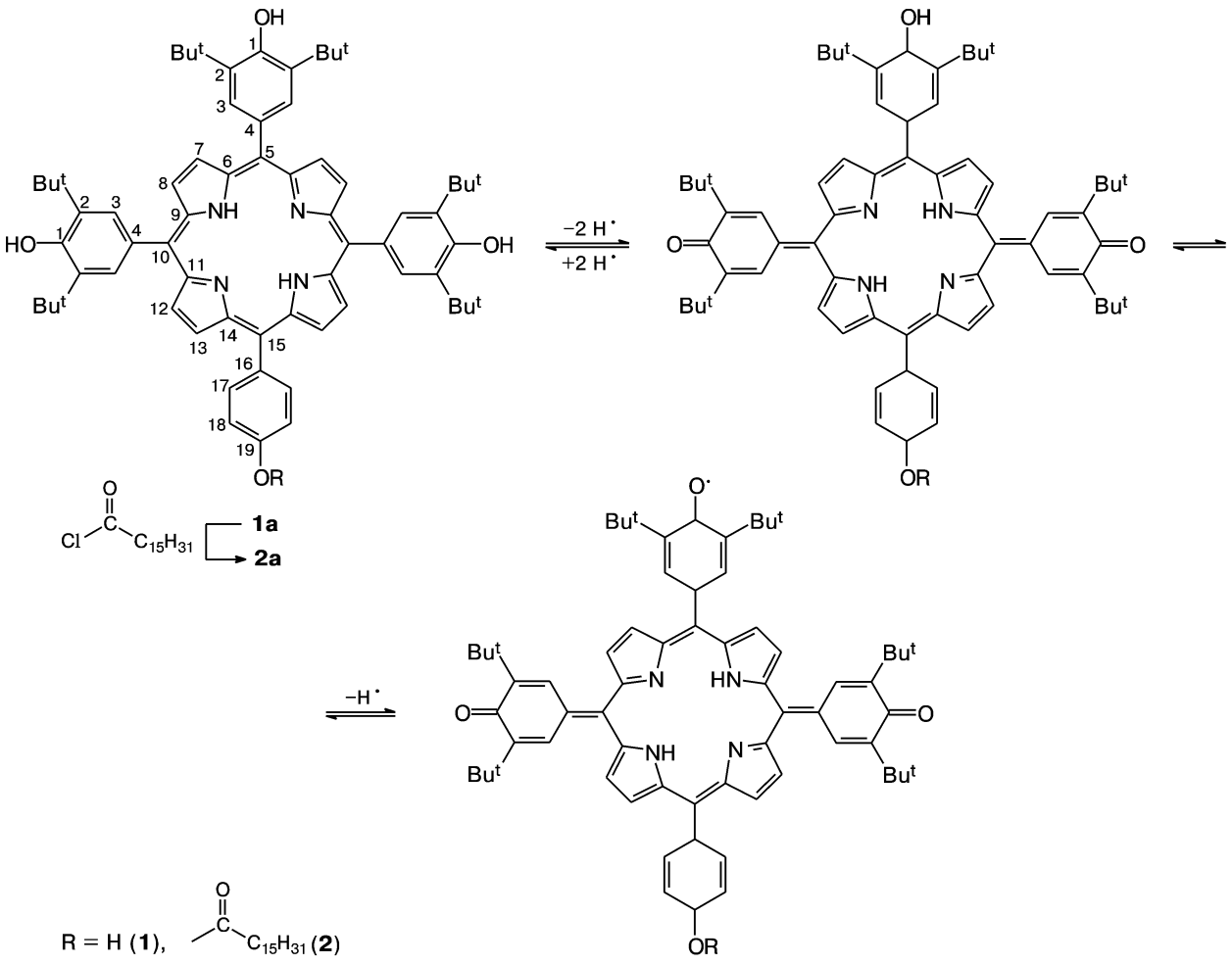

Fig. 1. EPR spectrum of radical **1b**• (toluene, 295K).

region. ^{1}H and ^{13}C NMR spectroscopic data also confirm the structures of the compounds obtained.

It is known that antioxidant activity of 2,6-di(*tert*-butyl)phenols depends on the stability of the corresponding phenoxyl radicals. In this connection, we carried out the oxidation of compounds **1a** and **2a** and studied the thus formed radical species by EPR method. It is known[12] that the oxidation of the free base of tetrakis[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphyrin affords a biradical, which is converted into a diamagnetic derivative of porphodimethenediquinomethide in the fast step. The oxidation steps are reversible and porphodimethenediquinomethide and porphyrinogene derivatives can be reduced back to the starting porphyrin. The presence of three sterically hindered phenolic groups in molecules of compounds **1a** and **2a** suggests the possibility of registering the monoradical species **1b**• and **2b**• (see Scheme 1).

Indeed, the EPR spectra of radicals **1b**• and **2b**• were registered during oxidation of **1a** and **2a** by PbO_2. They show triplets (Figure 1) characterizing interaction of a spin of the unpaired electron with two equivalent *meta*-protons in the phenoxyl residue. Radicals **1b**• and **2b**• are stable in solution in the absence of oxygen at room temperature for a few days. The values of isotropic *g*-factor are 2.0047 and 2.0048, the superfine coupling constant values on the a_H (^{1}H) nuclei are equal to 0.19 and 0.18 mTl for **1b**• and **2b**•, respectively.

Experimental

The electron absorption spectra were recorded on a Varian Cari-219 spectrophotometer. The Fourier transform IR absorption spectra were recorded on a IR200 Thermo Nicolet spectrophotometer in KBr pellets. The NMR spectra were registered on a Bruker AMX-400 spectrometer in $CDCl_3$ (^{1}H, 400 MHz; ^{13}C, 100 MHz). Thin-layer chromatography was performed on Silufol UV-254 plates. Column chromatography was performed on alumina (neutral, Brockmann). The EPR spectra were registered on a Bruker ELEXSYS E-500-10/12 radiospectrometer in the X-range of 9.853 GHz (λ 3 cm). The measurements were carried out following evacuation of the ampoules with the sample solutions (concentrations of $1 \cdot 10^{-4}$ mol L^{-1}). The oxidizing reagent was used in tenfold excess.

5-(4-Hydroxyphenyl)-10,15,20-tris[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphine (1a).[9] A mixture of chloroacetic acid (2.6 g) and *o*-xylene (100 mL) was heated to reflux under argon. A solution of pyrrole (1 mL, 14.4 mmol), 4-hydroxybenzaldehyde (0.44 g, 3.6 mmol), and 3,5-di(*tert*-butyl)-4-hydroxybenzaldehyde (2.5 g, 10.8 mmol) in *o*-xylene (10 mL) was added to the resulting mixture. This was refluxed for 0.5 h under argon and for another 1.5 h with bubbling of air, cooled and neutralized with aq. ammonia. The solvent was evaporated by steam distillation, the precipitate was filtered off, washed with water, and dried in open air at 70 °C. The dried precipitate was dissolved in benzene (100 mL). The product was isolated by twofold column chromatography (benzene—methanol, 100 : 1). The eluate was concentrated to the minimum volume and porphyrin was precipitated by addition of methanol (30 mL). The precipitate was filtered off, washed with methanol, and dried in open air at 70 °C. The yield was 0.45 g (13.6%). R_f 0.60 (benzene—methanol, 10 : 1). Found (%): C, 80.44; H, 7.70; N, 5.34. $C_{68}H_{78}N_4O_4$. Calculated (%): C, 80.44; H, 7.74; N, 5.52. IR, ν/cm^{-1}: 3626 (nonassociated OH groups), 3403 (associated OH group). UV, λ_{max}/nm (logε): 652 (3.86); 595 (3.75); 560 (4.09); 522 (4.15); 425 (5.63). ^{1}H NMR ($CDCl_3$), δ: 1.70 (s, 54 H, 6 CMe_3); 5.59 (s, 3 H, 3 OH); 7.04 (d, 2 H, 2 C(17)H, J = 8.0 Hz); 8.09 (d, 2 H, 2 C(18)H, J = 8.0 Hz); 8.13 (s, 6 H, 6 C(3)H); 8.91 (d, 2 H, 2 C(12)H, J = 4.0 Hz); 9.0 (d, 2 H, 2 C(13)H, J = 4.0 Hz); 9.03 (s, 4 H, 2 C(7)H, 2 C(8)H). ^{13}C ($CDCl_3$), δ: 30.74 (6 C($\underline{C}H_3$)); 34.61 (6 $\underline{C}(CH_3)$); 113.55, 119.20, 121.27, 131.94, 132.04, 134.02, 135.60, (3 C_6H_2, C_6H_4, 4 C_4H_2N); 153.59 (C(1)); 155.27 (C(19)).

5-(4-Palmitoyloxyphenyl)-10,15,20-tris[3,5-di(*tert*-butyl)-4-hydroxyphenyl]porphine (2a). A solution of palmitoyl chloride (1.0 g, 3.64 mmol) in anhydrous pyridine (5 mL) was added to a solution of porphine **1a** (100 mg, 0.11 mmol) in anhydrous pyridine (15 mL) with stirring at ambient temperature. The mixture was stirred at this temperature for 20 h and then poured in water (100 mL). The precipitate was filtered off, washed with water, and dried in open air at ambient temperature. The product was dissolved in chloroform and isolated by column chromatography on Al_2O_3 (Brockmann) with chloroform as the eluent. The eluate was concentrated to the minimum volume and diluted with methanol (30 mL). The precipitate was filtered off, washed with methanol, and dried in open air at ambient temperature. The yield was 0.11 g (86.4%). Found (%): C, 80.51; H, 8.59; N, 4.28. $C_{84}H_{108}N_4O_5$. Calculated (%): C, 80.46; H, 8.68; N, 4.46. IR, ν/cm^{-1}: 3639 (nonassociated OH groups), 3426 (associated OH group). UV, λ_{max}/nm (logε): 650 (3.93); 593 (3.87); 558 (4.11); 521 (4.22); 424 (5.64). ^{1}H NMR ($CDCl_3$), δ: 0.93 (t, Me, J = 8.0 Hz); 1.26—1.99 (m, 26 H, $(CH_2)_{12}$); 1.69 (s, 54 H, 6 CMe_3); 2.80 (t, 2 H, CH_2CO, J = 8.0 Hz); 5.59 (s, 3 H, 3 OH); 7.55 (d, 2 H, 2 C(17)H, J = 8.0 Hz); 8.10 (s, 6 H, 6 C(3)H); 8.30 (d, 2 H, 2 C(18)H, J = 8.0 Hz); 8.91 (d, 4 H, 2 C(12)H, 2 C(13)H, J = 4.0 Hz); 8.99 (d, 4 H, 2 C(7)H, 2 C(8)H, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$), δ: 14.17 (Me); 29.75 (13 CH_2); 30.71 (6 C($\underline{C}H_3$)); 31.97 ($\underline{C}H_2CO$); 34.61 (6 $\underline{C}(CH_3)$); 119.81, 121.36, 131.90, 132.04, 133.32, 134.02, 135.29 (3 C_6H_2, C_6H_4, 4 C_4H_2N); 150.60 (C(19)); 153.59 (C(1)); 172.52 (CO).

Oxidation of porphyrins 1a and 2a to the corresponding radicals 1b$^{\bullet}$ and 2b$^{\bullet}$. Porphyrins **1a** or **2a** (0.1 mmol L^{-1}) were dissolved in anhydrous toluene (3 mL) and placed in tubes, then the tenfold excess of PbO_2 was added. The tubes with the sample solutions were evacuated three times at the temperature of liquid nitrogen, then the temperature was raised to 293 K and the EPR spectra of the corresponding radicals **1b$^{\bullet}$** and **2b$^{\bullet}$** were registered. Diphenylpicrylhydrazyl was used as a standard in determination of *g*-factor ($g = 2.0037$).

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References

1. B. Keppler, *Metal Complexes in Cancer Chemotherapy*, VCH, New York, 1993.
2. M. J. Perez and A. I. Cedelbaum, *Free Radical Biol. Med.*, 2002, **33**, 111.
3. E. Nyarko, T. Hara, D. J. Grab, M. Tabata, and T. Fukuma, *Chem.-Biol. Interactions*, 2002, 139, 177.
4. R. A. Sheldon and J. K. Koshi, *Metal-Catalysed Oxidation of Organic Compounds*, Academic Press, New York, 1981, 643.
5. Y. Kabe, M. Ohmori, K. Shinouchi, Y. Tsuboi, S. Hirao, M. Azuma, H. Watanabe, I. Okura, and H. Handa, *Biol. Chem.*, 2006, **281**, 31729.
6. E. R. Milaeva, *Russ. Khim. Zh.*, 2004, **4**, 20 [*Mendeleev. Chem. J.*, 2004, **4** (Engl. Transl.)].
7. E. R. Milaeva, *Izv. Akad. Nauk, Ser. Khim.,* 2001, 549 [*Russ. Chem. Bull., Int. Ed.*, 2001, **50**, 573].
8. E. Milaeva, D. Shpakovsky, Yu. Gracheva, O. Gerasimova, V. Tyurin, and V. Petrosyan, *J. Porphyrins Phthalocyanines*, 2003, **8**, 701.
9. L. R. Milgrom, N. Mofidi, C. C. Jones, and A. Harriman, *J. Chem. Soc., Perkin Trans. 2*, 1989, 301.
10. A. V. Melezhik and V. D. Pohodenko, *Zh. Org. Khim.*, 1982, **18**, 1054 [*J. Org. Chem. USSR*, 1982, **18** (Engl. Tramsl.)].
11. L. R. Milgrom, C. C. Jones, and A. Harriman, *J. Chem. Soc., Perkin Trans. 2*, 1988, 71.
12. L. R. Milgrom and C. J. Palmer, *Chem. Res. Synop.*, 1990, 66.

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