Heteroatom Chemistry
Volume 17, Number 6, 2006

Organotins-Promoted Peroxidation of Unsaturated Fatty Acids: A New Antioxidative Scavenger for Promoters

Elena R. Milaeva, Vladimir Yu. Tyurin, Dmitry B. Shpakovsky, Olga A. Gerasimova, Zhang Jingwei, and Yulia A. Gracheva

Department of Organic Chemistry, Moscow State Lomonosov University, 119992 Moscow, Russia

Received 17 June 2005; revised 7 September 2005

ABSTRACT: *The organotin compounds R_nSnX_{4-n} are promoters of lipids peroxidation. The influence of $(CH_3)_2SnCl_2$, $(C_2H_5)_2SnCl_2$, and $SnCl_2$ upon the radical chain oxidation of oleic acid as model substrate $R'H$ for lipid peroxidation in the simultaneous presence of porphyrins (free bases of meso-tetrakis(3,5-di-tert-butyl-4-hydroxyphenyl)porphyrin (R''_4PH_2) and of meso-tetraphenylporphyrin ($TPPH_2$)) has been studied. The monitoring of the unsaturated acid peroxidation level has been performed by the determination of the total concentration of isomeric hydroperoxides as well as of the thiobarbituric acid reactive substances, as markers of carbonyl compounds formation following the hydroperoxides decomposition. The organotin compounds demonstrate prooxidative activity. The promoting effect of these compounds decreases in the presence of $TPPH_2$. The free-base porphyrin R''_4PH_2, containing the antioxidative phenolic moieties (2,6-di-tert-butylphenol), demonstrates the acute inhibitory effect upon the acid's peroxidation. The analogous results have been achieved when compared with the influence of CH_3HgI and $HgCl_2$ upon the acids peroxidation of oleic acid in the presence of porphyrins. This fact points out that meso-tetrakis(3,5-di-tert-butyl-4-hydroxyphenyl)porphyrin shows the activities of both the antioxidant and of the scavenger for metals and might be used as a new antioxidative scavenger preventing lipids peroxidation.* Heteroatom Chem 17:475–480, 2006; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20269

INTRODUCTION

The organometallic compounds of main group elements (R_nMX_m) are broad-spectrum biocidal agents whose toxic effect is manifested at the membrane level due to the lipophilic properties of their molecules [1]. Among them, organotins (R_nSnX_m) are supposed to induce membrane associated oxidative stress in living organism through different mechanisms including the enhancement of the lipid peroxidation and intracellular generation of reactive oxygen species (ROS), H_2O_2, $O_2^{-\bullet}$, $HO^\bullet$ [2–5]. The perturbation of antioxidative defense system and the peroxidation of unsaturated fatty (oleic, linoleic, linolenic, and arachidonic) acids in a membrane lipid bilayer are consequences of this impact [6,7].

It has been observed that $[(n\text{-}C_4H_9)_3Sn]_2O$ caused a significant decrease in the total lipids content in body tissues of the estuarine edible clam [8] that might be explained as a consequence of the degradation of the unsaturated fatty acids promoted by organotins. The influence of $(C_6H_5)_3SnCl$

Correspondence to: Elena R. Milaeva; e-mail: milaeva@org.chem.msu.ru.

Contract grant sponsor: Russian Foundation for Basic Research.
Contract grant numbers: 06-03-32731, 06-03-32773, and 05-03-32864.

and $(C_6H_5)_2SnCl_2$ on fatty acids composition in a marine chlorophyte and a marine diatom was studied [9]. The results show a decrease in total polyunsaturated fatty acids that are more easily oxidized substrates. Enhanced lipid peroxidation in liver, kidney, and brain of mice was observed after exposure to CH_3HgCl as well [10].

The influence of organometallic compounds R_nSnX_{4-n} bearing various organic groups R as well as RHgX and R_2Hg upon the lipid peroxidation level was studied using model compounds—oleic acid and methyl oleate as substrates R′H [11–14].

It has been proposed that the formation of active free radicals $R^\bullet$ as a result of carbon to metal bond's homolytic cleavage in the oxidative/free radical medium is responsible for the promotion of the unsaturated fatty acids peroxidation. Therefore, the involvement of lipophilic organometallic compounds in cellular radical and redox processes means the promotion of membrane lipid bilayer oxidative destruction due to the generation of ROS and other active radical species. These events might be prevented or inhibited by the antioxidants. On the other hand, the metals themselves are also capable of participating in oxidative/free radical processes and promoting them and the addition of chelating agents as metals scavengers seems to be of importance.

This study is oriented toward a new route for the prevention of the prooxidative activity of organometallics.

RESULTS

The oxidation of unsaturated fatty acids serves as a model reaction for the lipid peroxidation in cellular membranes bilayer. The oxidation of oleic and linolenic acids as substrates R′H by molecular oxygen involves the generation of substituted allyl radicals that interact with O_2 to produce peroxyl radicals $R'OO^\bullet$. The corresponding *cis*- and *trans*-isomeric hydroperoxides (R′OOH) are the main products of the initial reaction period [15,16], and the rate of $R'OO^\bullet$ transformation to hydroperoxides R′OOH might be used as a criterion of the initial rate of the peroxidation [17].

Figure 1 presents the effect of organotin compounds $(CH_3)_2SnCl_2$ and $(C_2H_5)_2SnCl_2$ that increase the amount of oleic acid hydroperoxides formed at 65°C in the initial period (Fig. 1, curves 2 and 3). The data presented do not show the significant influence of the organic group nature upon the effectiveness of R_2SnCl_2. The total content of R′OOH after 2 h when compared with oleic acid without additives is 149.8 and 102.1% higher in the presence of $(CH_3)_2SnCl_2$ and $(C_2H_5)_2SnCl_2$, respectively. However, the influence of $SnCl_2$ is more significant (Fig. 1, curve 4), and the total concentration of R′OOH is 240.5% higher than in the oleic acid autooxidation. The relative rate constants of the hydroperoxides formation are given in Table 1.

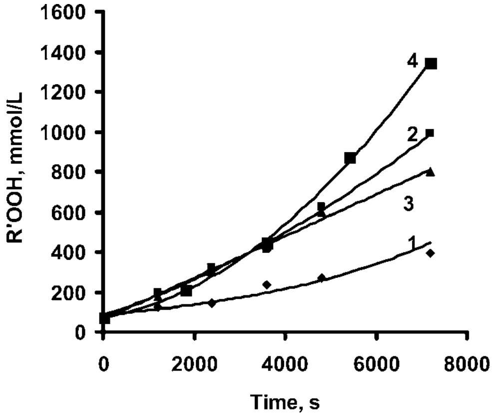


FIGURE 1 Kinetic curves of R′OOH formation in the presence of 1 mmol/L additives at 65°C: (1) oleic acid without additives; (2) $(CH_3)_2SnCl_2$; (3) $(C_2H_5)_2SnCl_2$; (4) $SnCl_2$.

The role of free base porphyrins as additives in the peroxidation of oleic acid is presented in Fig. 2.

meso-Tetraphenylporphyrin ($TPPH_2$) does not influence the level of oleic acid hydroperoxides, and the kinetic curves of R′OOH formation are almost identical in the case of acid's autooxidation and in

TABLE 1 The Kinetic Parameters of Oleic Acid Peroxidation in the Presence of 1 mmol/L Additives at 65°C

Additives	k_i/k_0
Without additives	1.00
$SnCl_2$	1.58
$(CH_3)_2SnCl_2$	1.4
$(C_2H_5)_2SnCl_2$	1.27
$TPPH_2$	1.05
R''_4PH_2	0.69
$(CH_3)_2SnCl_2$ and $TPPH_2$	1.05
$(CH_3)_2SnCl_2$ and R''_4PH_2	0.3
$(C_2H_5)_2SnCl_2$ and $TPPH_2$	1.17
$(C_2H_5)_2SnCl_2$ and R''_4PH_2	0.15
$HgCl_2$	1.37
CH_3HgI	1.29
CH_3HgI and $TPPH_2$	1.19
CH_3HgI and R''_4PH_2	0.24

k_i and k_0 are initial rate constants of R′OOH accumulation in the presence of additives and without additives, respectively.

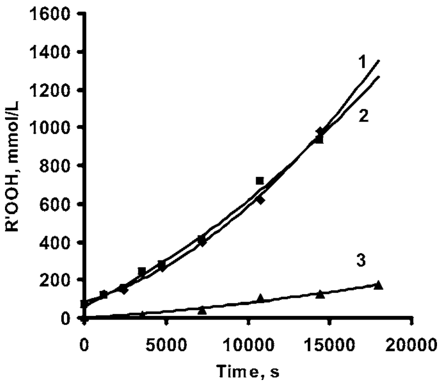


FIGURE 2 Kinetic curves of R′OOH formation in the presence of 1 mmol/L additives at 65°C: (1) oleic acid without additives; (2) *meso*-tetraphenylporphyrin; (3) *meso*-tetrakis-(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin (R''_4PH_2).

the presence of $TPPH_2$ (Fig. 1, curves 1 and 2). However, the addition of *meso*-tetrakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin (R''_4PH_2) decreases the hydroperoxides level (Fig. 1, curve 3): the content of R′OOH is eight times lower than that in oleic acid without additives. Therefore the data show an activity of R''_4PH_2 as an effective inhibitor.

The simultaneous presence of equimolar mixture of tin compound ($SnCl_2$ or $(CH_3)_2SnCl_2$ or $(C_2H_5)_2SnCl_2$) and *meso*-tetrakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin (R''_4PH_2) in oleic acid results in the significant decrease of hydroperoxides formation (Fig. 3a, curves 2 and 3). The total content of R′OOH after 4 h is more than 10 times lower than the experiment without any additives. However, the mixture of $(CH_3)_2SnCl_2$ or $(C_2H_5)_2SnCl_2$ with *meso*-tetraphenylporphyrin does not produce the inhibitory effect (Fig. 3b, curves 2 and 3), but the level of hydroperoxides is slightly lower than in the presence of organotins.

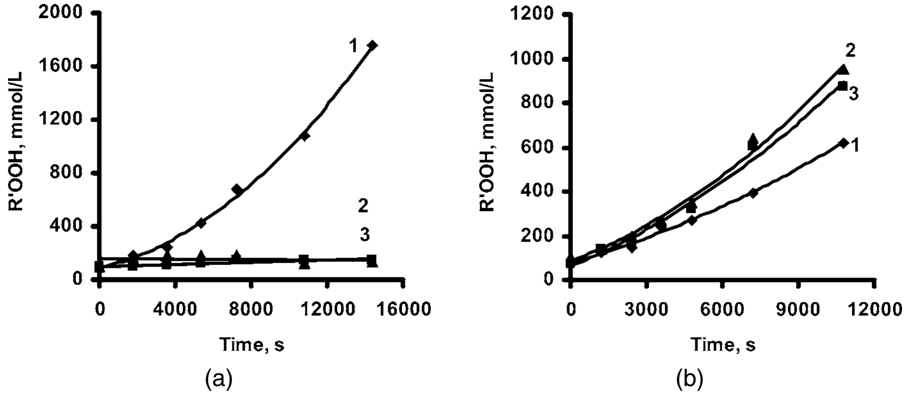
The comparison of tin and mercury compounds effects shows that $HgCl_2$ and CH_3Hgl are promoters of hydroperoxides accumulation. The relative values k_i/k_0 for the mixtures of mercury and tin compounds with porphyrins are close (Table 1).

The thiobarbituric acid reactive substances (TBARS) are markers of carbonyl compounds formation following the hydroperoxides decomposition. The accumulation of TBRAS in the presence of diorganotin compounds and their mixtures with porphyrins have been studied by UV–Vis spectrophotometry at λ_{max} 532 nm [17]. The data presented in Fig. 4 confirm the assumption that the influence of tin compounds and their mixtures with porphyrins are similar to that shown in Figs. 1–3. *meso*-Tetrakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin (R''_4PH_2) produces an inhibitory effect and prevents the promoting activity of tin additives, whereas in the presence of *meso*-tetraphenylporphyrin the content of TBRAS slightly increases.

Following the results achieved for organotin compounds as promoters of peroxidation, a special experiment for $SnCl_2$ was carried out to compare the activity of R''_4PH_2 in the prevention of the prooxidative effect of inorganic tin compound. Figure 5 illustrates the activity of R''_4PH_2 as a scavenger for metal and as an inhibitor of oxidation.

DISCUSSION

The metal compounds might act as prooxidants when involved in the oxidation. Moreover, the donors of active free radicals are the promoters



(a)

(b)

FIGURE 3 Kinetic curves of R′OOH formation in the presence of equimolar mixture of 1 mmol/L additives at 65°C: (1) oleic acid without additives (a) and (b); (2) $(CH_3)_2SnCl_2$ and R''_4PH_2 (a); (2) $(CH_3)_2SnCl_2$ and $TPPH_2$ (b); (3) $(C_2H_5)_2SnCl_2$ and R''_4PH_2 (a); $(C_2H_5)_2SnCl_2$ and $TPPH_2$ (b).

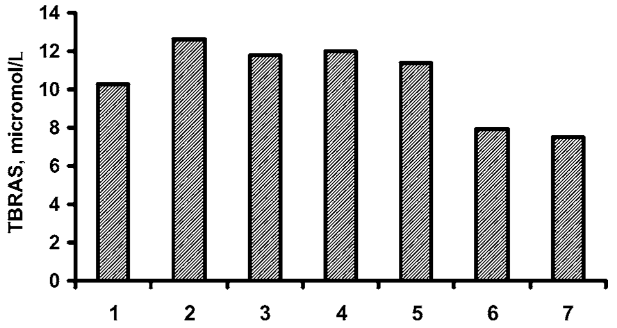


FIGURE 4 The diagram showing TBRAS content in the presence of equimolar mixture of 1 mmol/L additives after 3 h of heating of the oleic acid at 65°C: (1) oleic acid without additives; (2) $(CH_3)_2SnCl_2$; (3) $(C_2H_5)_2SnCl_2$; (4) $(CH_3)_2SnCl_2$ and $TPPH_2$; (5) $(C_2H_5)_2SnCl_2$ and $TPPH_2$; (6) $(CH_3)_2SnCl_2$ and R''_4PH_2; (7) $(C_2H_5)_2SnCl_2$ and R''_4PH_2.

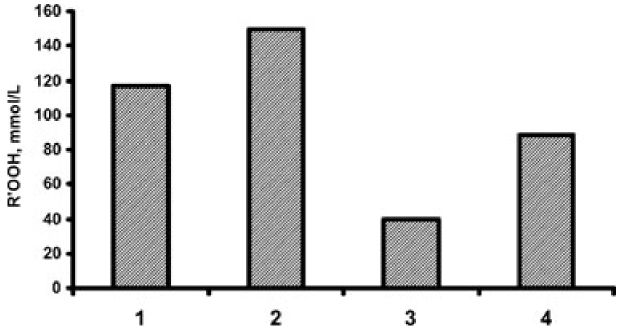


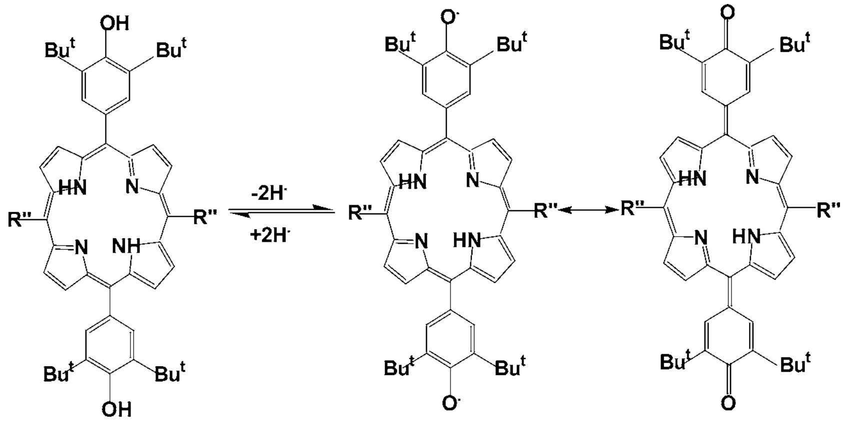
FIGURE 5 The diagram showing R′OOH content in the presence of equimolar mixture of 1 mmol/L additives after 3 h of heating of the oleic acid at 65′C: (1) oleic acid without additives; (2) $SnCl_2$; (3) R''_4PH_2; (4) $SnCl_2$ and R''_4PH_2.

of radical chain oxidation as well [17]. An intriguing aspect of the organotin compounds R_nSnX_{4-n} behavior is their capability to perform both the activity of metal-containing prooxidants and radical promoters due to the homolytic cleavage of the carbon metal bond that leads to the formation of active organic radicals $R^\bullet$ [2]. On the other hand, the exogenous compounds might play the role of inhibitors if their molecules possess the antioxidative moieties. Among the classes of well-known antioxidants, the substituted 2,6-dialkylphenols, analogues of vitamins E group, are widely used as inhibitors of free radicals formation in the oxidative destruction of natural and synthetic substrates [18].

Recently, we have reported the results of the kinetic study of free base porphyrins $R''PH_2$ and their metal complexes behavior in oleic acid peroxidation and described the acute inhibitory effect that is associated with the key role of 2,6-di-*tert*-butylphenol moieties in their molecules [19]. In this paper, we consider another possibility, which is connected with the capability of free base porphyrins to incorporate metal ions in their core [20] when these macrocyclic compounds are involved in oxidative/radical processes.

The experimental results of oleic acid peroxidation prove that whereas *meso*-tetraphenylporphyrin does not influence the hydroperoxides accumulation, *meso*-terakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin acts as an effective inhibitor both in pure oleic acid in the presence of R_2SnCl_2 (Figs. 2 and 3a).

The hydrogen atom abstraction from the phenol fragments in R''_4PH_2 gives relatively stable biradicals



R'' = 3,5-di-*tert*-butyl-4-hydroxyphenyl

SCHEME 1

that produce porphodimethene diquinomethide derivative (Scheme 1) [21]. The further oxidation leads to the formation of end product—*meso*-tetrakis(3,5-di-*tert*-butyl-4-quinomethide)porphyrinogen [22]. The oxidation steps are reversible, and the quinomethide porphodimethene and porphyrinogen derivatives might be converted easily back to the starting phenolic compound.

The action of R''_4PH_2 might be independent of the presence of R_2SnX_2 in the reaction mixture. However, the relative values of k_i/k_0 for the mixtures of R''_4PH_2 and R_2SnX_2 are 0.3 ($R = CH_3$) and 0.15 ($R = C_2H_5$), which are in each case lower than for pure oleic acid with R''_4PH_2 (0.69) (Table 1). Since there is no direct evidence of the formation of stable porphyrin R_4PSnR_2 at least when R_2SnX_2 reacts with tetra-arylporphyrins free bases [23], this effect might correspond to the incorporation of tin into the porphyrin core and to the formation of R_4PSn [21] as a result of the oxidative dealkylation of R_2SnX_2. Moreover, this fact is in agreement with the increase in the free radical stability of phenoxyl porphyrin, which is responsible for the inhibitory effect of the substituted phenol (Scheme 1) when the metal complex is formed [24].

The comparison of the k_i/k_0 values for the hydroperoxides accumulation in the presence of the mixture of R_2SnX_2 and $TPPH_2$ with that of R_2SnX_2 only shows no inhibitory effect (Table 1, Fig. 3b). However, the k_i/k_0 values are lower than in the case of pure R_2SnX_2 as additives. This might be explained by the generation of tin tetraphenylporphyrin as well. It is clear from these values that the efficiency of $TPPH_2$ is linked to its action as scavenger for tin. The same effect is observed for the prevention of the mercury compounds activity as promoters of the peroxidation.

The identical activity of free base porphyrins $TPPH_2$ and R''_4PH_2 as scavengers for tin was displayed when the monitoring of the TBRAS was performed during 3 h of the oleic acid oxidation (Fig. 4).

The inorganic tin compound $SnCl_2$ is the most effective promoter of the R′OOH accumulation as it can be seen in Fig. 1 and the data of k_i/k_0 values in Table 1. This fact might be associated with the ability of inorganic tin to participate in both radical and electron transfer processes. Moreover, the involvement of tin ions in various oxidation states in redox reactions might influence the increase of ROS content in the reaction mixture.

Addition of the equimolar amount of R''_4PH_2 to the reaction mixture containing $SnCl_2$ leads to the significant decrease of R′OOH content (Fig. 5). Note that in this case R''_4PH_2 serves as a chelating agent (scavenger) for the metal itself.

The data obtained in this study showed that *meso*-tetrakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin exhibits the inhibitory activity and serves as an effective scavenger for metal. The most important fact is that the incorporation of metal into the porphyrin core might lead to the increase of the antioxidative activity.

EXPERIMENTAL

The free base porphyrins *meso*-tetraphenylporphyrin and *meso*-tetrakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)porphyrin were synthesized as described previously by the known procedures [25–28], purified by silica gel column chromatography using $CHCl_3$, 80% $CHCl_3$, and 20% hexane as the eluting solvents and identified by UV–Vis and IR spectroscopy. UV–Vis spectra were recorded on a Varian 100S spectrophotometer in CH_2Cl_2, benzene, $CHCl_3$, and oleic acid. Infrared (IR) spectra were recorded on a Perkin Elmer "Spectrum One" spectrophotometer in KBr, Nujol, and oleic acid.

Oleic acid, 18:1(Δ9), (Sigma, 99%), α-linolenic acid, 18:3(Δ9,12,15), (Sigma, 99%), $(CH_3)_2SnCl_2$, $(C_2H_5)_2SnCl_2$, $SnCl_2$, CH_3HgI, $HgCl_2$(Strem) were used as supplied.

The monitoring of oleic and linolenic acids peroxidation level was performed by the determination of the total concentration of the corresponding isomeric hydroperoxides using iodometric titration. The oxidation of constant volume of the acid (5 mL) was carried out in a thermostatic cell using an air flow with the constant rate 2–4 mL/min at 65°C. The oxidation proceeds in the "kinetic range" under these conditions; the oxidation rate is independent of air volume passing through the cell [29].

Since the oxidation proceeds as an autooxidation, the airflow has been passed through oleic acid during 2 h before addition of the compounds under investigation. The concentrations of the additives were 1 mmol/L compared with the initial concentration of hydroperoxides in the reaction mixture. The rate of hydroperoxides accumulation was determined by kinetic measurements. The approximation coefficients of kinetic curves were in a range of 0.9650–0.984. The kinetic curves of the acids oxidation in the presence of free base porphyrins and in the presence of both porphyrin and metal compound follow the exponential law. Kinetic investigation has shown the initial rate of the hydroperoxides accumulation to be pseudo-first-order in the air.

The monitoring of the total concentration of the thiobarbituric acid reactive substances, as markers of carbonyl compounds, has been performed by the known procedure in tris-buffer using UV–Vis spectrophotometry in the region of $\lambda = 532$ nm [15].

CONCLUSION

In conclusion, we have developed a new efficient route to prevent the prooxidative activity of organotin compounds and demonstrated the effectiveness of free base porphyrin containing the antioxidative phenolic groups as antioxidative scavenger for organometallics.

REFERENCES

[1] Crompton, T. R. Occurrence and Analysis of Organometallic Compounds in the Environment; Wiley: New York, 1998.

[2] Milaeva, E. R.; Petrosyan, V. S.; Berberova, N. T.; Pimenov, Yu. T.; Pellerito, L. Bioinorg Chem Appl 2004, 2, 69–91.

[3] Gennari, A.; Viviani, B.; Galli, C. L.; Marinovich, M.; Pieters, R.; Corsini, E. Toxicol Appl Pharmacol 2000, 169, 185–190.

[4] Regolia, F.; Gorbia, S.; Frenzillib, G.; Nigrob, M.; Corsic, I.; Focardic, S.; Winstond, G. W. Mar Environ Res 2002, 54, 419–423.

[5] Mittler, R. Trends Plant Sci 2002, 7, 405–410.

[6] Sergent, O.; Morel, I.; Cillard, J. In Metal Ions in Biological Systems; Sigel, A.; Sigel, H. (Eds); Marcel Dekker: New York, 1999; Vol. 36, pp. 251–287.

[7] Hamilton, R. J.; Kalu, C.; Prisk, E.; Padley, F. B.; Pierce, H. Food Chem 1997, 60, 193–199.

[8] Shah, D. S. M.; Rojaramani, V.; Sathick, O.; Priya, R. S. J Environ Poll 2001, 8, 7–11.

[9] Mooney, H. M.; Cooney, J. J.; Baisden, C. M.; Patching, J. W. Bot Mar 1995, 38, 423–429.

[10] Huang, Y. L.; Cheng, S. L.; Lin, T. H. Biol Trace Element Res 1996, 52, 193–206.

[11] Milaeva, E. R.; Pimenov, Y. T.; Berberova, N. T.; Kirillova, L. B.; Gracheva, Y. A.; Tyurin, V. Y.; Kalyavin, V. A.; Petrosyan, V. S. Dokl Chem 2001, 379, 631–635 (Engl. Transl.).

[12] Milaeva, E. R.; Kirillova, L. B.; Berberova, N. T.; Pimenov, Y. T.; Tyurin, V. Y.; Grigor'ev, E. V.; Petrosyan, V. S. Russ J Gen Chem 2002, 72, 761–765 (Engl. Transl.) .

[13] Petrosyan, V. S.; Milaeva, E. R.; Gracheva, Y. A.; Grigor'ev, E. V.; Tyurin, V. Y.; Pimenov, Y. T.; Berberova, N. T. Appl Organomet Chem 2002, 16, 655–659.

[14] Petrosyan, V. S.; Milaeva, E. R.; Gracheva, Y. A.; Tyurin, V. Y.; Grigor'ev, E. V.; Pellerito, L. Russ J Org Chem 2003, 39, 384–388 (Engl. Transl.).

[15] Porter, N. A.; Mills, K. A.; Carter, R. L. J Am Chem Soc 1994, 116, 6690–6698.

[16] Adolf, R.; Lamm, T. J Chromatogr 1998, 799, 329–337.

[17] Frankel, E. N. In Autooxidation in Food and Biological Systems; Simic, M.; Karel, M. (Eds); Plenum Press: New York, 1980; p. 141.

[18] Denisov, E. Handbook of Antioxidants; CRC Press: Boca Raton, FL, 1995.

[19] Milaeva, E. R.; Gracheva, Yu. A.; Shpakovsky, D. B.; Gerasimova, O. A.; Tyurin, V. Yu.; Petrosyan, V. S. J Porphyrins Phthalocyan 2003, 8, 701–706.

[20] James, B. R. In The Porphyrins; Dolphin, D. (Ed.); Academic Press: New York, 1978.

[21] Milgrom, L. R. Tetrahedron 1983, 39, 3895–3912.

[22] Golder, A. J.; Milgrom, L. R.; Nolan, K. B.; Povey, D. C. Chem Commun 1989, 1751.

[23] Asadi, M.; Zabardasti, A.; Ghasemi, J. Polyhedron 2002, 21, 683–687.

[24] Milaeva, E. R. Russ Chem Bull 2001, 50, 573–589.

[25] Milgrom, L. R.; Jones, C. C.; Harriman, A. J Chem Soc, Perkin Trans II 1988, 71–83.

[26] Traylor, T. G.; Nolan, K. B.; Hildreth, R. J Am Chem Soc 1983, 105, 6149–6154.

[27] Rothemund, P. J Am Chem Soc 1935, 57, 2010–2017.

[28] Adler, A. D.; Longo, F.; Finarelli, J.; Goldmacher, J.; Assour, J.; Korsakoff, L. J Org Chem 1967, 32, 476–483.

[29] Emanuel, N. M.; Denisov, E. T.; Maizus, Z. K. Liquid Phase Oxidation of Hydrocarbons; Plenum Press: New York, 1967.