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New Approaches to the Development of Biologically Active Water-Soluble Antioxidants

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Abstract

Results obtained by the authors in the investigations in the area of synthesis and examination of the antioxidant properties of polyfunctional water-soluble antioxidants based on alkylated phenols are presented in the review. The promising character of the use of synthesized compounds for corrections of pathological states connected with the development of oxidative stress is demonstrated.

Key words: phenols, polyfunctional phenolic antioxidants, water-soluble antioxidants, antioxidant activity, free radical pathologies

Contents

INTRODUCTION

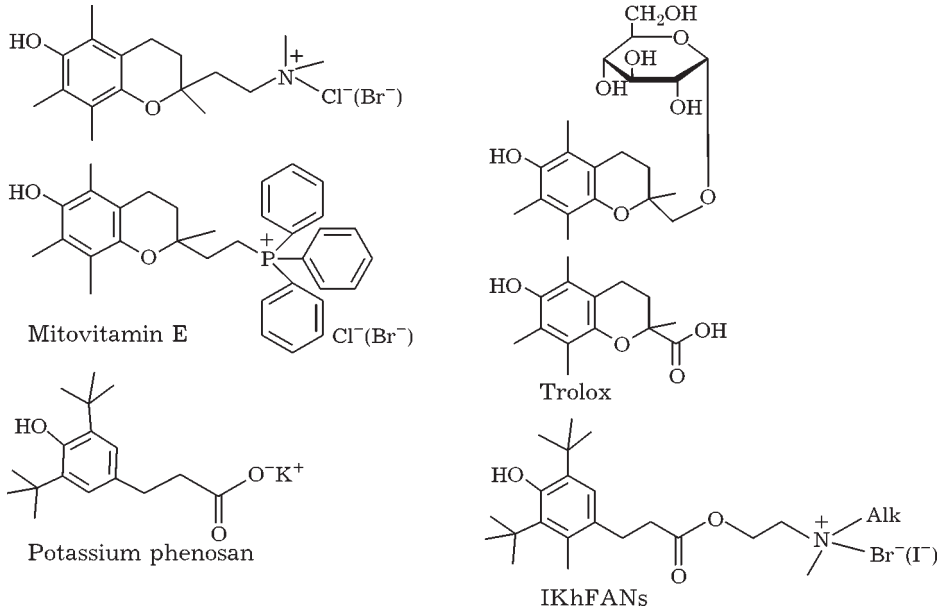
Modern science enrols more than 200 diseases and pathological states arising and developing in conjunction with the intensification of the processes of non-enzymatic oxidation, or oxidative stress. These pathologies include widespread cardiovascular, inflammatory, oncological, endocrine diseases, as well as disorders connected with the unfavourable action of the environment (ecological pathologies) and associated with ageing (age-related pathologies). This explains the urgency of the development of medicinal preparations based on the compounds that possess antioxidant activity [1, 2].

Important role is played in the system of the natural protection of organisms from the hazardous action of the oxidative stress by natural phenol compounds (tocopherols, flavonoids, ubiquinols *etc.*). Their synthetic analogues – alkylated phenols – are efficient bioantioxidizers, too [3]. In this connection, it is not surprising that the antioxidants of phenol type are mainly used as remedies [4].

The majority of phenol compounds that are in use in practice and/or have been studied in laboratories as bioantioxidizers possess lipophilic properties. At the same time, it is more efficient to use hydrophilic forms in biology, veterinary and medicine because these forms are characterized by higher biological availability and convenient introduction procedures.

The problem connected with the development of water-soluble bioantioxidants is being solved by introducing hydrophilic groups – ionogenic groups or carbohydrate residues – into the molecules of efficient natural and synthetic antioxidants (Scheme 1).

This modification allows one not only to render water solubility to phenol antioxidants but also to make antioxidants of directed action. For instance, the substitution of the aliphatic tail of α-tocopherol by the tetraalkyl ammonium group allowed the synthesis of hydrophilic



Scheme 1.

preparations with directed cardioprotective action [5, 6], while in the case of its substitution by triphenylphorphonium group, a mitochondrially addressed antioxidant mitovitamin E was obtained [7, 8]. It was demonstrated that antioxidants similar to mitovitamin E are accumulated in mitochondria in concentrations exceeding those in blood by a factor of 100–500. In this situation, they provide better protection of mitochondria from oxidative damage than usual α-tocopherol does. Thanks to works [9, 10], mitochondrially addressed antioxidants based on plastoquinone became especially widely known.

It is known [3, 11] that the mechanism of the action of phenol antioxidants (ArOH) is based on their ability to interact with radicals that drive oxidation chains, in particular lipoperoxide radicals ($LOO^{\bullet}$):

$$ArOH + LOO^{\bullet} \rightarrow ArO^{\bullet} + LOOH \quad (1)$$

The high rate of this reaction and low activity of phenoxyl radicals $ArO^{\bullet}$ in the reactions of oxidation chain propagation define antioxidant action of phenol compounds [11, 12].

At the same time, lipoperoxides formed in reaction (1) are low-stable compounds and can decompose with the initiation of new oxidation chains:

$$LOOH \rightarrow LO^{\bullet} + {}^{\bullet}OH$$

The introduction of functional groups (in particular sulphide), able to reduce hydroperoxides, into the molecules of phenol antioxidants causes a substantial increase in the efficiency of antioxidants [2, 13–15].

It is assumed [13, 14] that the high efficiency of the antioxidant action of sulphur-containing phenol antioxidants is connected with cell effects. The occurrence of hydroxyphenyl and sulphhydryl fragments in one molecule results in the reduction of hydroperoxide formed at the phenol OH group according to reaction (1) without going out into the volume through reaction with sulphur atom:

$$RSR' + LOOH \rightarrow RS(O)R' + LOH \quad (2)$$

Due to this process, the possibility of LOOH decomposition into free radicals is prevented.

It was demonstrated that lipophilic sulphur-containing phenol antioxidants are efficient bioantioxidizers. For example, bis(3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propyl)sulphide (CO-3, thiophane) protects the cells of *S. typhimurium* from the damaging action of H_2O_2 in Ames paste more efficiently than trolox does [15] and exhibits pronounced protective action *in vivo* in various free radical pathologies [16–22]. Dodecyl-(3,5-dimethyl-4-hydroxybenzyl)sulphide also provides efficient protection of cell cultures from H_2O_2 [23], exhibits hemorheological, antiaggregatory and antiplatelet activity *in vivo* [24], decreases the accumulation of lipoperoxidation products during experimental brain ischemia [25].

In this connection, it appears promising to make water-soluble phenol antioxidants that, unlike previously proposed analogues, along with antiradical activity would exhibit also antiperoxide properties. This problem was solved by us through introducing sulphur (selenium, nitrogen, phosphorus)-containing ionogenic fragments, as well as additional sulphide and selenide groups.

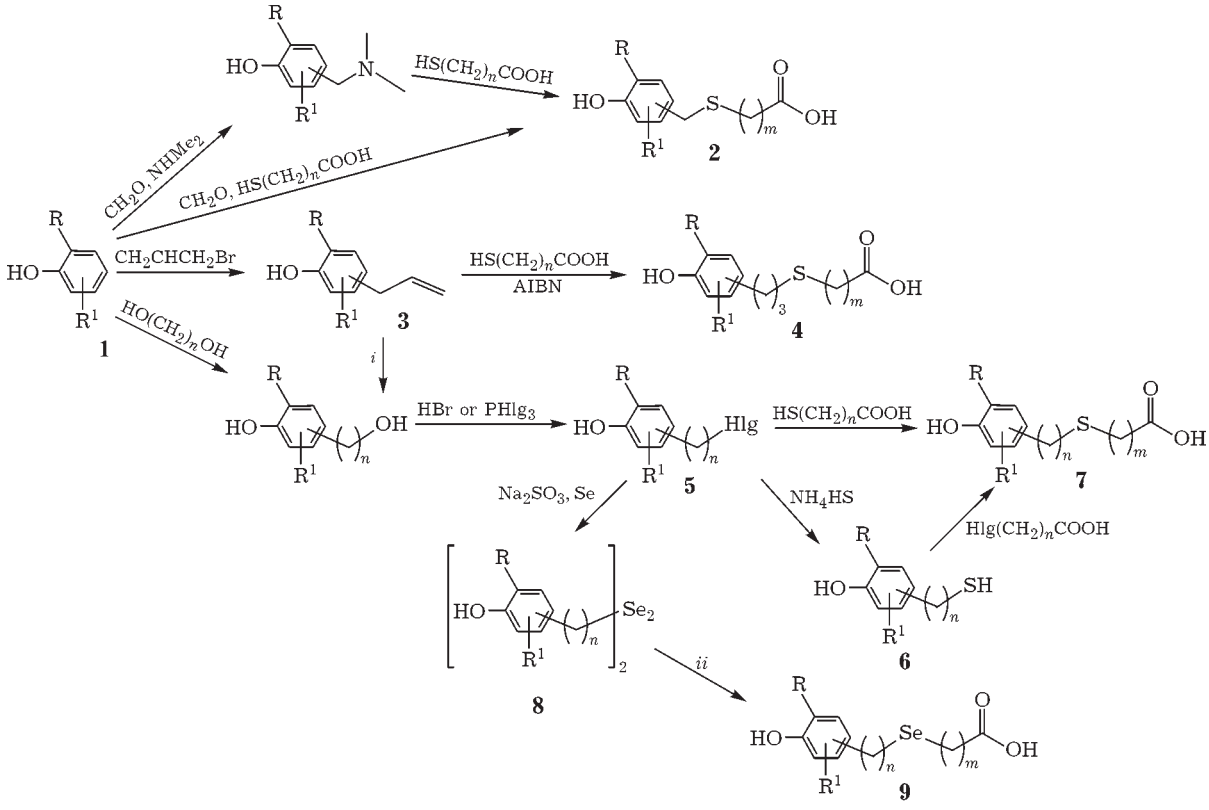
ROUTES OF THE SYNTHESIS OF POLYFUNCTIONAL HYDROPHILIC ANTIOXIDANTS

Different synthesis approaches were used for the synthesis of sulphur (selenium, nitrogen, phosphorus)-containing hydrophilic alkyl phenols; the choice of approaches was determined both by the structure of target compounds and by the availability of initial reagents. For example, the synthesis of hydroxyaryl thioalkane acids from 2,6(2,4)-dialkyl phenols **1** was carried out according to Scheme 2.

Hydroxybenzyl thioalkane acids **2** were obtained directly from dialkyl phenols **1** by condensation with formaldehyde and thioalkane acids, as well as through the intermediate formation of Mannich bases [26]. Propyl thioalkane acids **4** were obtained through allyl phenols **3**, while acids **7** with different number of methylene links separating the aromatic centre and sulphur atom were synthesized through halogenoalkyl phenols **5**. On the basis of the latter, thiols **6** and diselenides **8** were synthesized; from these compounds using reactions with halogenoalkane acids, corresponding thio- and selenoalkane acids **7** and **9** were synthesized [27].

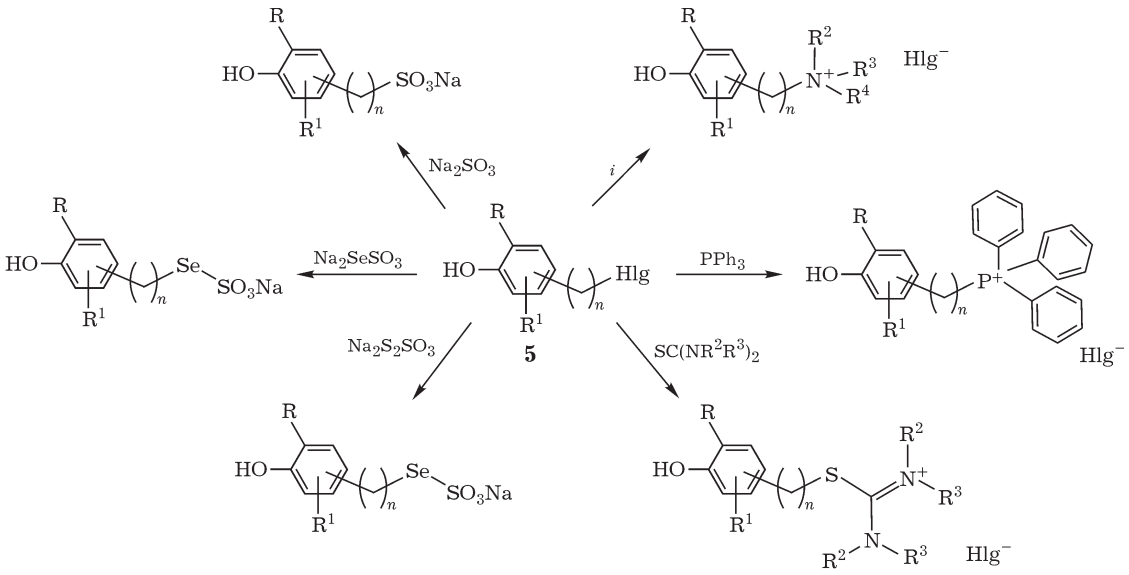
On the basis of halogenoalkyl phenols **5**, also other classes of water-soluble antioxidants were synthesized (Scheme 3). They contain alkylammonium [28, 29], thio- and selenosulphate, as well as sulphonate [31–33] groups as ionogenic fragments.

It should be noted that previously researchers from our institute together with the colleagues from the Novosibirsk Institute of Organic Chemistry, SB RAS, developed efficient



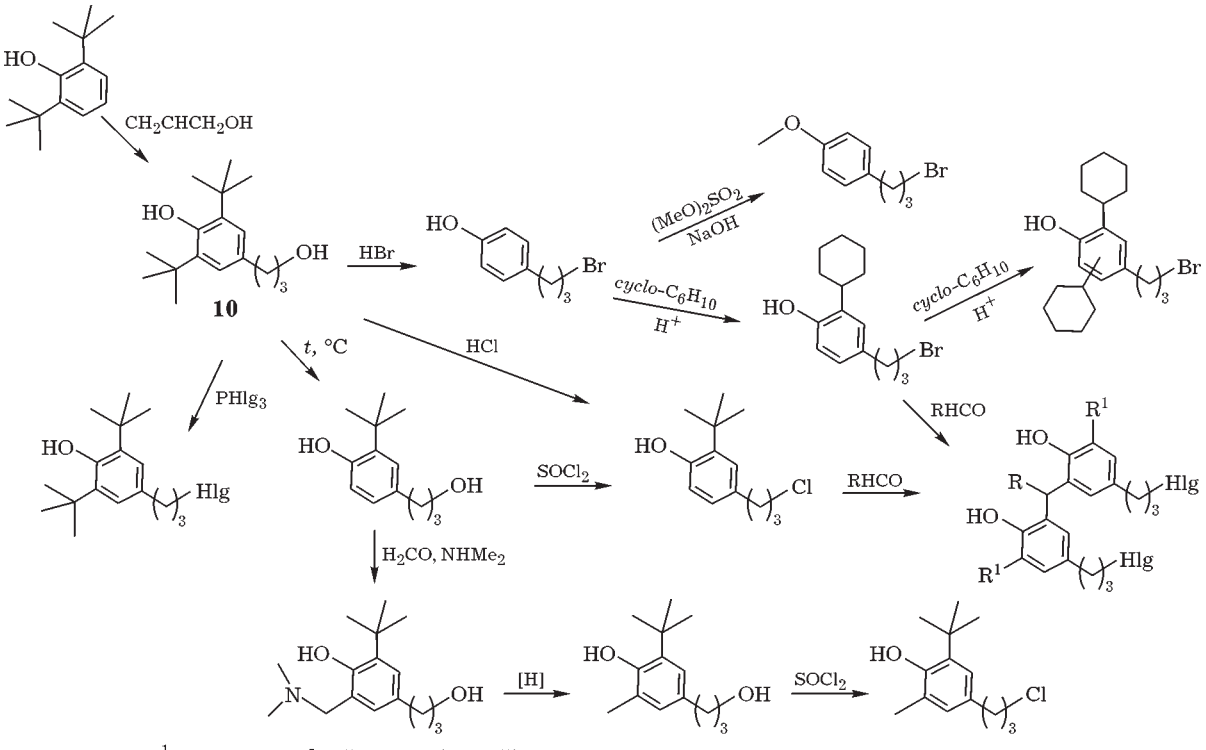
R, R^1 = Me, *t*-Bu, *cyclo*-C_6H_{11}; n = 2–4; m = 1–4; Hlg = Cl, Br; *i*: 1) $NaBH_4$, $(MeO)_2SO_2$; 2) H_2O_2, NaOH; *ii*: 1) $NaBH_4$, 2) $Br(CH_2)_nCOOH$

Scheme 2.



R, R^1 = H, Me, *t*-Bu, *cyclo*-C_6H_{11}; R^2, R^3, R^4 = H, Me; n = 2–4; Hlg = Cl, Br, J; *i*: 1) $C_6H_4(CO)_2NK$, 2) N_2H_4 H_2O, 3) Hlg or 1) $NHMe_2$ (or NH_2Me), K_2CO_3; 2) Hlg (or EtJ)

Scheme 3.



R = H, Me; R^1 = *t*-Bu, *cyclo*-C_6H_{11}; Hlg = Cl, Br

Scheme 4.

methods of functionalization of dialkyl phenols **1** on the basis of the introduction of hydroxyalkyl substituent into the aromatic nucleus and subsequent transformation of hydroxyalkyl phenols into halides **5**. Scheme 4 shows the routes of the synthesis of *para*-halogenopropylphenols with different *ortho*-substituents from 2,6-di-*tert*-butylphenol through alkanol **10** (γ-propanol). Separate stages of these transformations were described in detail in [33–38].

The use of halogenoalkyl phenols **5** as semiproducts for water-soluble antioxidants allowed obtaining several tens target compounds that, on the one hand, are characterized, by substantial structural diversity, and on the other hand, form series with variations in the structure of separate structural fragments. This serves as the basis to study the structure – properties dependences in the series of synthesized compounds and then to use the revealed regularities for molecular design and directed synthesis of new compounds with required properties.

ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS

Molecular design of synthesized hydrophilic antioxidants implies the presence of two types of antioxidant activity: antiradical activity of phenol OH group and antiperoxide activity of sulphur (selenium, nitrogen, phosphorus)-containing groups. In this connection, investigation of the antioxidant properties of synthesized compounds in comparison with structural analogues proposed previously was carried out in different model systems allowing investigation of both antiradical or antiperoxide activity and total inhibiting activity (Total Antioxidant Activity).

The antiradical activity of synthesized compounds was assessed on the basis of the rate constants of their interaction with lipoperoxide radicals (k_1) that were measured under the conditions of methyloleate oxidation initiated by azo compounds in homogeneous (in chlorobenzene) and microheterogeneous solutions (aqueous solutions of surfactants).

In the number of hydrophilic derivatives of 3-(4-hydroxyaryl)propyl series with different number and structure of *ortho*-substituents, the lowest k_1 values characterize *ortho*-unsubstituted compounds, while the highest k_1 values were characteristic of the derivatives with methyl and cyclohexyl groups [33].

The degree of the influence of ionogenic fragment on k_1 value depended on its remoteness from the aromatic nucleus and on oxidation conditions. Thus, for the oxidation of methyl oleate in chlorobenzene, in the series of *N*,*N*-dimethyl-ω-(3,5-di-*tert*-butyl-4-hydroxyphenyl)alkyl ammonium chlorides, an increase in the number of methyl links in the *para*-substituent caused an increase in k_1 values. In this situation, benzyl ammonium chloride was characterized by lower (by a factor of 1.7) k_1 value in comparison with the corresponding amine, while an increase in the distance of nitrogen atom from the aromatic nucleus caused levelling of differences in k_1 values for alkyl amines and their salts [39]. This effect is likely to be connected with the electron acceptor influence of the ammonium nitrogen atom.

On the other hand, for the oxidation of methyl oleate in chlorobenzene, 2,6-di-*tert*-butyl-4-methyl phenol (ionol, or dibunol) and its hydrophilic derivatives, in particular sodium S-[3-(3,5-di-*tert*-butyl-hydroxyphenyl)propyl]-thiosulphate (11), were characterized by almost the same k_1 values [40]. For methyl oleate oxidation in the aqueous solution of surfactant, k_1 value for ionol is about 5 times higher than that for thiosulphate **11** [33]. It was demonstrated with the help of UV spectroscopy that thiosulphate **11** in the two-phase system methyl oleate–water is present mainly in water, while ionol passes completely into methyl oleate. So, k_1 values measured experimentally in water-lipid systems are likely to be essentially dependent on the distribution of antioxidant molecules between the lipid and aqueous phases.

Antiperoxide activity of the synthesized compounds was studied in the model reaction of cumene hydroperoxide (CHP) decomposition. As expected, addition of phenosan (3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propane acid) had no effect on the stability of CHP, while in the presence of its sulphur- and selenium-containing analogues **12–15** a decrease in CHP concentration was observed (Fig. 1).

The kinetic curves of CHP decomposition in the presence of benzylthioethane acid **13** had pronounced S-like character, which is the evi-

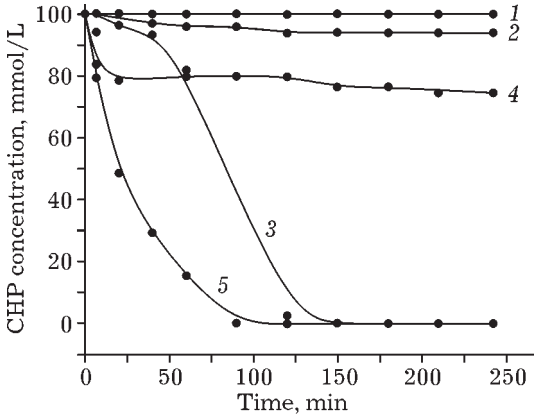


Fig. 1. Kinetic curves of CHP decomposition under the action of 10 mM phenosan (*1*) and ω-(4-hydroxyaryl)alkylthio(seleno)alkane acids **12–15** (**2–5**, respectively) at 60 °C.

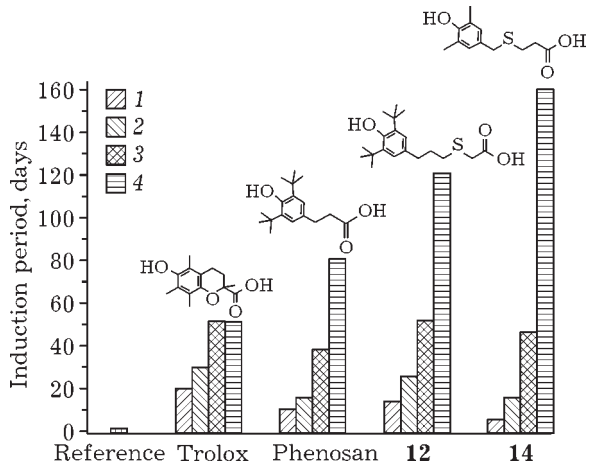
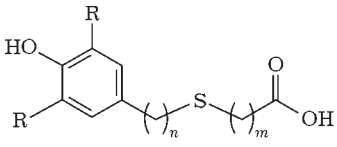
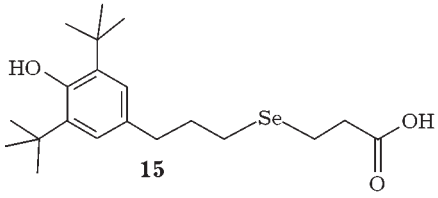


Fig. 2. Diagram of induction periods of the oxidation of methyl oleate (60 °C) inhibited by hydroxyarylalkane acids. Acid concentration, μmol/g: 0.25 (*1*), 0.5 (*2*), 1.0 (*3*), 2.5 (*4*).

dence of autocatalytic reaction [26]. Possible catalysts of CHP decomposition are sulphonic acids formed in the oxidation of sulphide group of acid **13**. The possibility of the formation of sulphonic acids during the oxidation of the structural analogue of acid **13** – bis(3,5-di-*tert*-butyl-4-hydroxybenzyl)sulphide and their ability to catalyse the decomposition of hydroperoxides were described previously by the authors of [41, 42].



12–14



15

R = *t*-Bu, n = 3, m = 1 (**12**);
R = Me, n = 1, m = 1 (**13**), m = 3 (**14**)

The diagram of induction periods for autooxidation of methyl oleate inhibited by the addition of antioxidants containing the carboxyl group as the hydrophilic fragment is presented in Fig. 2. One can see that phenosan within the whole range of concentrations studied – from 0.25 to 2.5 μmol/g – exhibits lower antioxidant activity than its sulphur-containing analogue **12**. In the region of low concentrations, water-soluble analogue of α-tocopherol trolox exhibits higher efficiency than the *o*-dimethyl-substituted acid **14**. However, within the concentration range 1–2.5 μmol/g the antioxidant activity of acid **14** increases sharply, while no enhancement of inhibiting action is observed for trolox.

It is known that α-tocopherol, which is considered to be one o the most efficient natural antioxidants, exhibits high antioxidant activity exactly in low concentrations. In the region of high concentrations (according to estimates reported in [43], at the ratio of α-tocopherol to fatty acid > 1 : 100), it exhibits pro-oxidant properties due to participation of tocopheryl radicals in the propagation of oxidation chains:

$$\alpha\text{-Tp-O}^{\bullet} + \text{LH} \rightarrow \alpha\text{-Tp-OH} + \text{L}^{\bullet} \qquad (3)$$

This concentration-related inversion of the antioxidant action into pro-oxidant is characteristic of many natural antioxidants, and this is believed to be the reason of failures in the attempts to treat free radical pathologies using antioxidant vitamins [1]. Different types of the dependencies of antioxidant action on concentration for trolox and thioalkane acids **12** and **14** point to the fact that, unlike natural antioxidants (in particular, α-tocopherol and its hydrophilic derivatives), dose-dependent inversion of antioxidant action is not characteristic of the compounds synthesized by us.

TABLE 1

Antioxidant activity of sodium S-[3-hydroxyaryl)propyl]thiosulphates and [3-(hydroxyaryl)propane]-1-sulphonates *in vitro* (concentrations providing 50 % inhibition of oxidation intensity, μM)

Compounds	System 1		System 2	System 3
	Cu^{2+}	Fe^{2+}		
11	10	1.5	123	10.7
16	30	18	525	9.7
17	400	380	27	2.9
18	11 800	$>1 \cdot 10^6$	74	16.5
19	15	3.6	631	24.7
20	43	32	1170	10.1
21	2770	2650	214	5.9
22	Dose-dependent stimulation			
Potassium phenosan	13	1.8	800	21.3

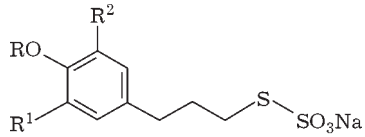
BIOLOGICAL ACTIVITY OF NITROGEN- AND SULPHUR-CONTAINING HYDROPHILIC ANTIOXIDANTS

The authors of [44] studied the ability of *N*,*N*-dimethyl-(4-hydroxyaryl)alkylammonium chlorides of different structures to protect the cells of *Escherichia coli* of two strains, AB1157 of the wild type and its isogenic mutant BH910, which is defective at the system of oxidative damage reparation, from the negative effect of H_2O_2. It was established that the salts of 3-methyl-5-*tert*-butyl-4-hydroxybenzyl- and 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propylamines protect the mutant cells of BH910 from H_2O_2 with higher efficiency than trolox does, while *N*,*N*-dimethyl-(3,5-dimethyl-4-hydroxy-benzyl)ammonium chloride exhibits higher protective action than trolox with respect to both strains.

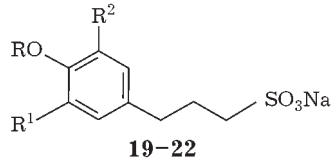
Bio-antioxidant properties of thiosulphates **11**, **16–18** and sulphonates **19–22** were studied *in vitro* as their effect on the oxidation of low-density lipoproteins (LDLP) during their incubation with the ions of metals with variable valence (system 1), on the generation of active oxygen metabolites (AOM) by stimulated neutrophils of blood (system 2) and on the formation of peroxynitrite anion ($ONOO^-$) through decomposition of morpholinosydnonimine (system 3) [32, 45, 46]. The results are presented in Table 1.

In all the cases, a 50 % inhibition of the intensity of oxidation processes was achieved using lower concentrations of thiosulphates **11**, **16–18** in comparison with sulphonates **19–22** of similar structure (see Table 1). Potassium phenosan, which was used in that study as the reference antioxidant, is close in activity to sulphonate **19** but lags behind the corresponding thiosulphate **11**. These data provide evidence that the presence of divalent sulphur atom in the structure of thiosulphates **11**, **16–18** enhances their antioxidant properties.

Estimating the outlooks for the practical use of synthetic compounds as biologically active substances, along with their specific activity it is important to take into account also the safety of their application. As a result of the investigation of acute toxicity of the hydrochloric salts of *N*,*N*-dimethyl-3-(4-hydroxyaryl)-propyl)isothiuronium, it was shown that the toxicity decreases with the removal of *tert*-butyl *ortho*-substituents or with their replacement by methyl groups [29, 31]. In the sequence of



11, 16–18



19–22

R = H, $R^1 = R^2$ = *t*-Bu (**11, 19**)
R = R^1 = H, R^2 = *t*-Bu (**16, 20**)
R = $R^1 = R^2$ = H (**17, 21**)
R = Me, $R^1 = R^2$ = H (**18, 22**)

TABLE 2

Semilethal doses (LD_{50}) for hydrophilic derivatives of 3-(4-hydroxyaryl)propyl series (mice, intraperitoneal introduction), mg/kg

Ortho-substituents	Hydrophilic fragment in the *para*-substituent				
	$NMe_2 \cdot HCl$	$SC(NH_2)_2^+Cl^-$	SSO_3Na	SO_3Na	SCH_2COONa
t-Bu	80	30	175	275	200
Me	70	110	1000	>3000	950
H	45	80	800	1800	900

the derivatives of 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl series, toxicity decreased with the replacement of alkyl ammonium and isothiuronium groups by thiosulphate and sulphonate [31]. This allowed assuming that the safest compounds for application will be the derivatives of 2,6-dimethylphenol containing hydrophilic fragments of anion type in the *para*-alkyl substituent. The correctness of this assumption was confirmed after the synthesis of corresponding S-[3-(4-hydroxyaryl)propyl]-thiosulphates, -sulphonates and thioethanoates (Table 2). According to the accepted classification [47], alkyl-substituted phenols containing SSO_3Na, SO_3Na and $SCH_2COOK(Na)$ groups in their structure belong to IV–V classes of toxicity, respectively.

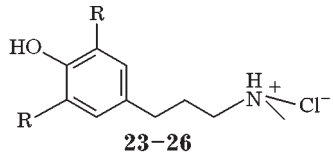
Hydrophilic derivatives of ω-(4-hydroxyaryl)alkyl series with different ionogenic fragments exhibited clearly pronounced hepatoprotective activity *in vivo* [40]. For example, ω-(3,5-dialkyl-4-hydroxyaryl)alkylammonium chlorides in low doses (1/10 of LD_{50}) decreased the hepatotoxic action of CCl_4, which was exhibited as a reliable decrease in the activity of hepatocellular enzyme (alanine aminotransferase, ALAT) in blood serum and a decrease in the concentration of malonic dialdehyde (MDA) in the liver of experimental animals [29]. The most efficient chloride in the given study was *N*,*N*-dimethyl-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl]ammonium chloride **23** which decreased the activity of ALAT by 58 % and the concentration of MDA almost by a factor of 2. This chloride exceeded in hepatoprotective properties the water-soluble antioxidant applied in medicine – emoxipin (3-hydroxy-6-methyl-2-ethylpyridine hydrochloride).

Differences in hepatoprotective activity of hydrochlorides **23–27** [29] corresponded to the differences in the ability of corresponding amines and N-oxide to inhibit auto-oxidation of lard [48] (Fig. 3).

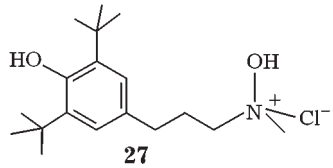
The existence of correlation between antioxidant activity of compounds in simple oxidation systems and hepatoprotective action *in vivo* is in good agreement with generally accepted notions of the mechanism of damaging action of CCl_4. Thus, according to these notions, CCl_4 metabolises in liver microsomes with the formation of trichloromethyl radicals, and the latter induce the processes of peroxide oxidation of lipids in membranes and cause the death of hepatocytes [3].

The fact that the oxidation of nitrogen atom when passing from **23** to **27** causes a decrease in the antioxidant activity provides evidence that the salts of ω-(hydroxyaryl)alkyl amines are polyfunctional antioxidants, and the efficiency of their action is determined by the activity of both phenol and alkyl ammonium groups.

With the model of isolated heart (rats of Wistar and OXYS lines), the existence of cardioprotective activity was revealed for thiosulphate **11** [49]. For example, perfusion of isolated hearts with the solution of thiosulphate **11** caused a stable increase in their work (150–160 % with respect to the initial level) with si-



23–26



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R = *t*-Bu (**23**), *cyclo*-C_6H_{11} (**24**), Me (**25**), H (**26**)

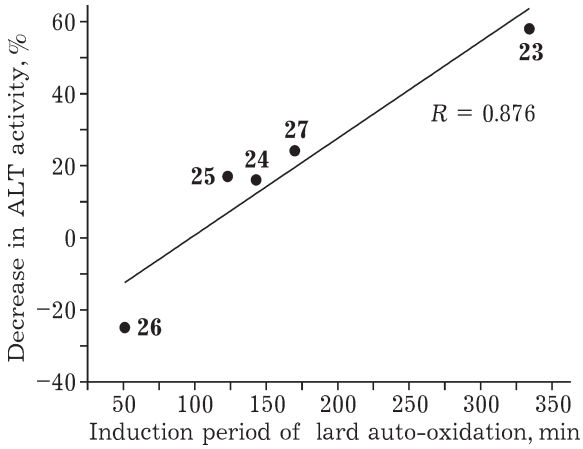

Fig. 3. Dependence between the hepatoprotective activity of *N*,*N*-dimethyl-3-(4-hydroxyaryl)propylammonium chlorides and the antioxidative activity of the corresponding amines.

multaneous enhancement of oxygen consumption; after 30-min long ischemia of myocardium thiosulphate **11** recovered heart work to the initial parameters.

The introduction of thiosulphate **11** (*per os* in the course dose of 25–45 mg/kg) promoted the recovery of the immune system of mice subjected to irradiation (200 Roentgen) or introduction of cyclophosphan (200 mg/kg). This is confirmed by an increase in the number of antibody-forming cells in the spleen of experimental animals at the background of immunization with a 2 % solution of sheep erythrocytes by a factor of 1.34 in comparison with the reference in the case of postradiation immunodeficiency and by a factor of 1.79 in comparison with the reference – in the case of postcyclophosphan recovery of immunity [50].

The addition of thiosulphate **11** (0.2 mg/mL) to the culture of mononuclear cells of the blood of patients suffering from chronic hepatitis C caused enhancement of their proliferative activity. This provides evidence that thiosulphate **11** can possess also antiviral activity along with immune-stimulating properties [51].

Thiosulphates **11**, **16–18** and sulphonates **19–21** exhibited reliable anti-inflammatory action in the model of carraginan-induced paw edema of legs in rats. The most efficient agent turned out to be mono-*tert*-butyl-substituted thiosulphate **16** which exhibited in efficiency either its analogues or potassium phenosan and aspirin [45]. No correspondence was observed between the anti-inflammatory and antioxidant properties of the preparations under study, which is the evidence that the effect of these preparations on an organism is not limited to their effect on the intensity of oxidation processes.

It was demonstrated in the recent years that the biological role of phenolic compounds in an organism is often determined by their regulatory action but not by antioxidant properties; this is so even for classical antioxidants such as α-tocopherol [52]. Important targets of exogenous phenolic antioxidants in cells are redox-sensitive transcriptional factors, first of all the antioxidant responsive element (ARE) [53].

In order to test the hypothesis concerning the ability of synthesized antioxidants to realize their action through activation of ARE, their ability to enhance transcription of GSTR1 gene coding glutathione-S-transferase P1 was studied with the culture of human hepatoma cells HepG2. It was established that all the studied compounds **11**, **16–21** in the concentration of 20 μM increased the expression of GSTP1 gene. The most active compound turned out to be partially screened thiosulphate **16**; its activity within the whole concentration range studied (10–100 μM) exceeded the activity of the classical ARE inductor *tert*-butyl-hydroquinone by a factor of 1.5 as an average. The efficiency of the anti-inflammatory action of thiosulphate **16** is likely to be based on its ability to induce expression of ARE-controlled genes coding the proteins that participate in the inflammatory process [45].

It is known [1] that the age pathologies (Alzheimer's disease, Parkinson's disease, myocardial infarction, type II diabetes, osteoarthritis, and rheumatoid arthritis) are connected with the development of oxidative stress. In this connection, it is interesting to study the possibility to use antioxidants as geroprotectors.

It was shown in [54] that the effect of thiosulphate **16** on the average life interval of different lines of *Drosophila melanogaster* is essentially dependent on the sex, genotype and environmental conditions. Under normal conditions, the average lifetime of long-living females and males of *Canton* S line varied under the action of antioxidant, while the flies of *Oregon* R line turned out to be insensitive to its action. At the same time, under the conditions of paraquat-induced oxidative stress

thiosulphate **16** increases the probability of survival for both lines of *D. melanogaster*. Paraquat gets oxidized and reduced in cells in the cyclic manner in the reactions participated by NAD(P)H forming superoxide anion radical. This causes damage of brain cells (*substantia nigra*), and processes characteristic of Parkinson's disease develop [55, 56]. The revealed protective effect of thiosulphate 16 under the conditions of paraquat action determines the promising character of the further studies of the preparation as the means to treat senile neurodegenerative diseases.

According to the data reported in [57], the derivatives of 2,6-dimethylphenol containing ionogenic groups SSO_3Na, SO_3Na, SCH_2COOK in the *p*-alkyl substituent exhibited fungistatic activity against microscopic fungi producing mycotoxins in compound feed used to grow chicken broilers. The introduction of the same antioxidants into the diet of chicken broilers subjected to intoxication by lead and cadmium compounds prevented the accumulation of heavy metals in organs and tissues [58] and had a positive action on the growth and development of chickens [59].

CONCLUSION

Results of the investigation of antioxidant activity of synthesized compounds in various model systems including *in vitro* and *in vivo* confirmed that the introduction of functional groups possessing antiperoxide activity into the molecules of water-soluble phenols promotes enhancement of total antioxidant activity of the compounds and allows obtaining antioxidants that exhibit higher efficiency than analogues proposed previously.

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