ISSN 1070-3632, Russian Journal of General Chemistry, 2012, Vol. 82, No. 5, pp. 880–884. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © N.I. Belaya, A.V. Belyi, A.I. Paschenko, 2012, published in Zhurnal Obshchei Khimii, 2012, Vol. 82, No. 5, pp. 777–781.

Reactions of Unsubstituted Hydroxybenzenes with 2,2-Diphenyl-1-picrylhydrazyl in the System Water–Aprotic Solvent

N. I. Belaya, A. V. Belyi, and A. I. Paschenko

Donetsk National University, ul. Universiteteskaya 24, Donetsk, 83110 Ukraine
e-mail: sschemy@dongu.donetsk.ua

Received February 21, 2011

Abstract—The reactivity of natural unsubstituted di- and trihydroxybenzenes in the reaction with a stable free radical 2,2-diphenyl-1-picrylhydrazyl in a mixed solvent water–dimethyl sulfoxide (2:1) was investigated. Kinetic and stoichiometric parameters of the studied reaction were estimated. In the water environment the studied hydroxybenzenes were found to dissociate to form ions, which reacted rapidly with the radical by the mechanism of single electron transfer with subsequent adding a proton. The corresponding rate constants were calculated taking into account the concentration of the ionized form of phenol.

DOI: 10.1134/S1070363212050131

One of the most popular model systems for the investigation of the antiradical activity of phenolic compounds (PhOH) is based on their ability to react with the stable free radical diphenylpicrylhydrazyl (Dpph$^{\bullet}$) [1, 2]. Commonly, to study such reactions solvents where the radical Dpph$^{\bullet}$ is well soluble and stable for a long period (ethanol, benzene, hexane, dimethyl sulfoxide, etc.) are used [3, 4]. Such reactions are artificial and cannot reproduce the situation *in vivo*. In this regard, attempts are continued to improve this method of determining the antiradical activity of substances by changing the experimental conditions, of which the main one is the presence of the water environment. This would allow to approach the conditions of reactions in biological systems.

Currently, there is little information on the research of antioxidant properties of natural phenols in reaction with Dpph$^{\bullet}$ in the water environment, and the available data are debatable. Natural phenolic compounds are known [1, 5] to react with the radical Dpph$^{\bullet}$ in water by the mechanism of single electron transfer followed by proton transfer from the resulting phenol radical cation:

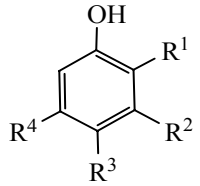
$$PhOH + Dpph^{\bullet} \rightarrow (PhOH)^{\bullet+} + Dpph^{-} \rightarrow PhO^{\bullet} + Dpph\text{–}H. \quad (1)$$

At the same time, a number of studies [2, 3] proposed another mechanism of this reaction in polar solvents based on the reaction of Dpph$^{\bullet}$ with the phenolate ion (PhO^{-}):

$$PhO^{-} + Dpph^{\bullet} \rightarrow PhO^{\bullet} + Dpph^{-} (\rightarrow Dpph\text{–}H). \quad (2)$$

The aim of this paper is to study the antiradical activity of the natural unsubstituted di- and trihydroxybenzenes in the reaction with Dpph$^{\bullet}$ and to establish the mechanism of their action in the water environment.

Since the Dpph$^{\bullet}$ radical is insoluble in water, the kinetics of its reaction with the given phenolic compounds was studied in the mixed solvent water – DMSO (2:1).



I, $R^2 = R^3 = R^4 = H$, $R^1 = OH$ (pyrocatechol); **II,** $R^1 = R^2 = R^4 = H$, $R^3 = OH$ (hydroquinone); **III**, $R^1 = R^3 = R^4 = H$, $R^2 = OH$ (resorcynol); **IV**, $R^3 = R^4 = H$, $R^1 = R^2 = OH$ (pyrogallol); **V**, $R^2 = R^4 = H$, $R^1 = R^3 = OH$ (oxyhydroquinone); **VI**, $R^1 = R^3 = H$, $R^2 = R^4 = OH$ (phloroglucinol).

The numbering of the investigated phenols corresponds to their numbering in Tables 1–3.

Figure 1a shows the kinetic curves of the Dpph$^{\bullet}$ radical concentration in the reaction with di- and trihydroxybenzenes in the water environment. As seen,

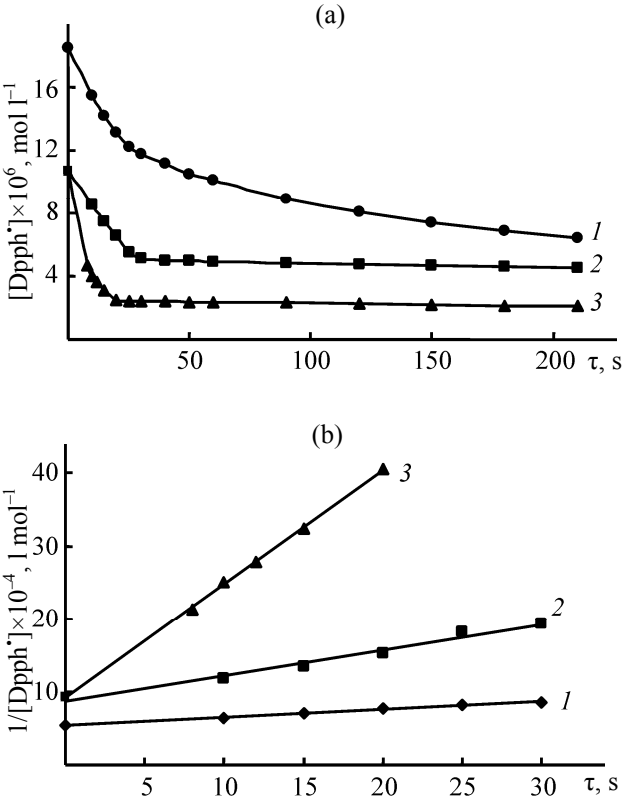


Fig. 1. Kinetic curves of consumption of diphenylpicrylhydrazyl in the reaction with di- and trihydroxybenzenes (a) and anamorphoses of the kinetic curves in coordinates of second-order reaction (b). (*1*) catechol (c 1.85×10^{-5} M), (*2*) hydroxyhydroquinone (c 7.1×10^{-5} M), (*3*) pyrogallol (c 7.1×10^{-5} M) . Solvent water–DMSO (2:1), $T = 293 \pm 2$ K.

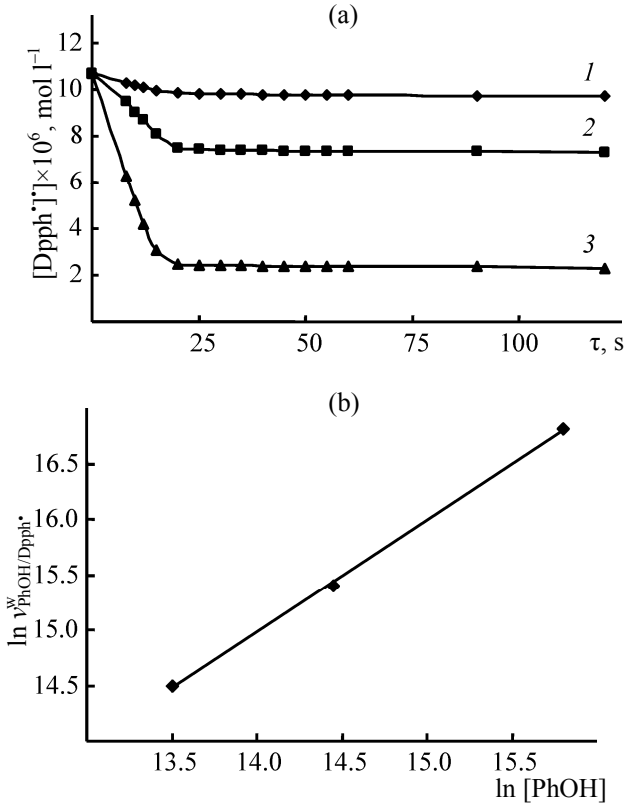


Fig. 2. Kinetic curves of diphenylpicrylhydrazyl consumption in the reaction with pyrogallol (a), and the logarithm of the reaction rate $v^{w}_{PhOH/Dpph\bullet}$ vs the concentration of pyrogallol (b). [DPPH] = 1.07×10^{-5} M, solvent water–DMSO (2:1), $T = 293 \pm 2$ K. Phenol concentration, M: (*1*) 0.26×10^{-6}, (*2*) 0.53×10^{-6}, (*3*) 1.07×10^{-6}.

all the studied phenols react actively with the radical. While determining the reaction stoichiometric parameters, we found that the kinetic curves can be linearized in the coordinates of the second-order reaction (*n*) (Fig. 1b). By the excess method [6] we found the pseudo-first order of the reaction towards both the radical ($n_{Dpph\bullet}$) and phenol (n_{PhOH}) (Fig. 2). Regardless of the phenol structure, the reaction order with respect to the substance remains unchanged. (Table 1).

Figure 1a shows that the dependence of Dpph$^{\bullet}$ consumption on time in the initial stage corresponds to rapid decrease in the radical concentration, followed by a slow progressive disappearance of the radical. The initial part of the kinetic curve on the basis of the

Table 1. Stoichiometric and kinetic parameters of reaction of di- and trihydroxybenzenes with the radical Dpph$^{\bullet}$ in DMSO and DMSO–water mixture (2:1), $T = 293\pm2$ K

Comp. no.	Water–DMSO (2:1)			DMSO		
	$n_{Dpph\bullet}$	n_{PhOH}	$k^{w}_{PhOH/Dpph\bullet}$, l mol^{-1} s^{-1}	$n_{Dpph\bullet}$	n_{PhOH}	$k^{DMSO}_{PhOH/Dpph\bullet}$, l mol^{-1} s^{-1}
I	1.12	0.93	$(6.72\pm0.19)\times10^{2}$	0.99	0.98	$(1.27\pm0.03)\times10^{2}$
II	1.14	0.91	$(3.80\pm0.11)\times10^{2}$	1.00	0.99	$(4.76\pm0.14)\times10^{1}$
III	1.11	0.92	$(3.19\pm0.09)\times10^{1}$	0.98	1.00	2.34±0.07
IV	1.01	0.98	$(4.41\pm0.08)\times10^{3}$	0.99	1.10	$(9.87\pm0.21)\times10^{2}$
V	1.00	1.00	$(2.34\pm0.05)\times10^{3}$	0.97	1.06	$(7.34\pm0.14)\times10^{2}$
VI	1.02	0.99	2.31±0.05	0.99	1.10	1.17±0.03

Table 2. Calculated energy of homolytic rupture of O–H bonds (D_{PhO-H}) and ionization potentials of molecules of phenols (IP_{PhOH}) and phenolate ions (IP_{PhO^-})

Comp. no.	D_{PhO-H}, kJ mol^{-1}	IP_{PhOH}, eV	IP_{PhO^-}, eV
I	313.5	8.88	2.74
II	320.9	8.73	2.63
III	331.7	8.98	2.92
IV	312.0	8.96	2.67
V	304.4	8.68	2.69
VI	333.2	9.23	3.16

stoichiometric parameters obtained can be described by reactions either (1) or (2), while the slow consumption of the radical is due to the secondary reactions of phenoxyl radical with Dpph$^\bullet$ and further reaction products.

On the ground of the determined reaction order with respect to the substance (Table 1), the rate of the reaction ($v^{w}_{PhOH/Dpph\bullet}$) can be described by the equation:

$$v^{w}_{PhOH/Dpph\bullet} = k^{w}_{PhOH/Dpph\bullet}[PhOH]\cdot[Dpph^\bullet]. \quad (3)$$

As the Dpph$^\bullet$ is unstable in the water environment, in the expression (3) the rate of reaction of the radical with water ($v^{w}_{Dpph\bullet}$) must be taken into account:

$$v^{w}_{PhOH/Dpph\bullet} - v^{w}_{Dpph\bullet} = k^{w}_{PhOH/Dpph\bullet}[PhOH]\cdot[Dpph^\bullet]. \quad (4)$$

The rate constants ($k^{w}_{PhOH/Dpph\bullet}$), calculated with Eq. (4), are listed in Table 1. We can see that all the investigated di- and trihydroxybenzenes show pronounced activity in the reaction with the radical. The pyrogallol with its OH groups in *o*-positions possesses the highest antiradical activity, the lowest activity belongs to phloroglucinol with its hydroxy groups located in *meta*-positions. A similar pattern is observed in the case of dihydroxybenzenes. In general, trihydroxybenzenes are more active than the phenols with two OH groups.

To assess the effect of acid–base properties of water on the activity of phenols we studied stoichiometric and kinetic parameters of the Dpph$^\bullet$ reaction with hydroxybenzenes in the aprotic solvent DMSO (Table 1). We found that the reactivity of phenols in DMSO ($k^{DMSO}_{PhOH/Dpph\bullet}$) falls sharply compared to that in water environment, whereas the stoichiometric parameters remain the same. This may indicate a change in the reaction mechanism conserving the same stoichiometry. Since DMSO is an aprotic solvent and unsubstituted hydroxybenzenes have high values of pK_a, whose value in the aprotic solvent is even higher [7], the reaction in DMSO follows most likely the mechanism of direct abstraction of H atom from a phenol molecule by the Dpph$^\bullet$ radical:

$$PhOH + Dpph^\bullet \rightarrow PhO^\bullet + Dpph\text{–}H. \quad (5)$$

Reaction (5) is much slower than reaction (2) because the phenol molecule is less reactive than ion, as confirm the corresponding values of the reaction rate constants in water and DMSO (Table 1).

To prove the suggested mechanism of single electron transfer from the phenoxide ion to the radical in an aqueous medium we performed quantum-chemical calculations of the energy of homolytic rupture (D_{PhO-H}) of the O–H bond in the phenol molecule, as well as the ionization potentials of phenol (IP_{PhOH}) and phenoxide ions (IP_{PhO^-}). The lowest energy of the O–H bond cleavage in the molecule of phenol should characterize the reactivity of hydroxylbenzenes ($k^{DMSO}_{PhOH/Dpph\bullet}$) in the reaction (5) at the direct abstraction of the H atom in an aprotic solvent. The lowest ionization potential of the phenolate ions indicates activity of phenols ($k^{DMSO}_{PhOH/Dpph\bullet}$) in the reaction (2) of the one-electron transfer in the water environment.

To estimate the energy gap of the O–H bond we calculated formation enthalpies of phenols and the corresponding radicals in the unrestricted Hartree–Fock approximation using the semiempirical method AM1 within the software package MOPAC 2009 [8]. Ionization potentials of molecules and ions of phenols were calculated according to Koopmans' theorem [9] as the energy of the highest occupied molecular orbital taken with the opposite sign. The applicability of the AM1 method to the calculation of the enthalpies of formation and ionization potentials of molecules of phenols and phenoxide ions was investigated separately. For this purpose we compared the experimental values of heats of formation and ionization potentials of substituted phenols of different structures, taken from [10, 11], with calculated values. The data obtained by the AM1 approximation are in satisfactory agreement with experimental values.

The results of calculations of structure of the phenol molecules and ions are listed in Table 2. As seen, the lowest values of IP_{PhOH} and IP_{PhO^-} depend on the structure of the phenol molecules and ions, and the decrease in these quantities corresponds to increased reactivity of the substances in the reaction with Dpph$^\bullet$ (Table 1). The findings correlate with the constants determined in pure DMSO and DMSO–water 1:2. The

values $k^{DMSO}_{PhOH/Dpph\bullet}$ correlate better with the energy of homolytic rupture of O–H bond in the molecule hydroxybenzene (D_{PhO-H}), suggesting a mechanism of direct H atom abstraction from PhOH by the Dpph$^\bullet$ radical in aprotic solvents:

$$k^{DMSO}_{PhOH/Dpph\bullet} = (80.22\pm10.41) - (0.24\pm0.03)\, D_{PhO-H},\ r\ 0.965.$$

For the constants $k^{w}_{PhOH/Dpph\bullet}$ a better correlation was observed with the ionization potential of the phenolate ion (IP_{PhO^-}):

$$k^{w}_{PhOH/Dpph\bullet} = (42.10 \pm 7.29) - (13.06 \pm 2.60)\, IP_{PhO^-},\quad r = 0.929. \qquad (6)$$

Clearly, the dependence (6) is consistent with the hypothesis about the mechanism of single electron transfer in the reaction between PhO^- and the radical Dpph$^\bullet$ in the water environment. The role of water is its ability to ionize the molecule of phenol thus providing a very fast reaction course in the initial period (Fig. 1a). This is confirmed by the dependence of the $k^{w}_{PhOH/Dpph\bullet}$ value on the fraction of water in the mixed solvent. It can be seen (Fig. 3) that the higher is the water content in the mixture with DMSO, the higher is the reactivity of the phenol.

Since in aqueous medium in the initial period the radical Dpph$^\bullet$, according to the data obtained, reacts with the phenoxide ions, the values of $k^{w}_{PhOH/Dpph\bullet}$ correspond to effective rate constants. For their refinement it is necessary to calculate concentration of the ionized form of phenol. To find it, the reaction was performed in a mixture of phosphate buffer–DMSO (2:1) at pH 7.4 (corresponding to the pH of the basic physiological fluid of the human body), which allowed an accurate calculation of the H^+ concentration, and using reference pK_a [12], the calculation of the concentration of phenoxide ions in the studied dilute solutions of di- and trihydroxybenzenes:

$$K_a = [PhO^-][H^+]/[PhOH],$$

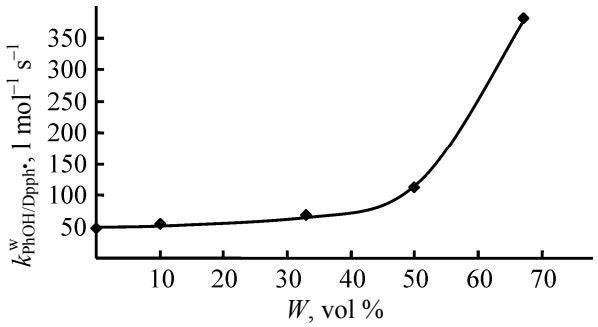


Fig. 3. The dependence of the reactivity of hydroxylbenzenes ($k^{w}_{PhOH/Dpph\bullet}$) on the water content (W) in its mixture with DMSO. $T = 293 \pm 2$ K.

$$[PhO^-] = K_a[PhOH]/10^{-pH} \qquad (7)$$

Rate of the reaction (2) with the ion concentration from Eq. (7) is described by the equation:

$$v^{buf}_{PhO^-/Dpph\bullet} = k^{buf}_{PhO^-/Dpph\bullet}[Dpph^\bullet]([PhOH]K_a/10^{-pH}),$$

$$k^{buf}_{PhO^-/Dpph\bullet} = (v^{buf}_{PhO^-/Dpph\bullet}\times10^{-pH})/[Dpph^\bullet][PhOH]K_a. \qquad (8)$$

Values of $k^{buf}_{PhO^-/Dpph\bullet}$ calculated from Eq. (8) (Table 3) are significantly higher than the $k^{w}_{PhOH/Dpph\bullet}$ (Table 1), but vary similarly. The exception is hydroquinone, which, according to the calculated data, is more active than hydroxyhydroquinone and pyrocatechol. This may be due to low ionization potential of its phenolate ion (Table 2). In general, the dependence $k^{buf}_{PhO^-/Dpph\bullet}$ vs. IP_{PhO^-} has an even higher correlation coefficient compared with Eq. (6), evidencing additionally in favor of the mechanism of single electron transfer from the phenolate ion to the radical in the water environment.

$$k^{buf}_{PhO^-/Dpph\bullet} = (56.60\pm2.38) - (17.01\pm0.85)\, IP_{PhO^-},\ r\ 0.995.$$

A similar structure–reactivity correlation of an antioxidant was found previously for other groups of natural phenols [13, 14], and may well be used to predict the antiradical activity of the substances belonging to one structural series, as well as for plan-

Table 3. The results of calculations of the reaction rate constants $k^{buf}_{PhO^-}$/Dpph$^\bullet$ [Eq. (2)] in a mixture of phosphate buffer–DMSO (2:1) at pH 7.4, $T = 293\pm2$ K[a]

Comp. no.	[Dpph], mol l^{-1}	[PhOH], mol l^{-1}	pK_a	[PhO^-], mol l^{-1}	$v^{buf}_{PhO^-/Dpph\bullet}$, mol l^{-1} s^{-1}	$k^{buf}_{PhO^-/Dpph\bullet}$, l mol^{-1} s^{-1}
I	2.58×10^{-5}	2.58×10^{-5}	9.36	2.83×10^{-7}	$(2.29\pm0.05)\times10^{-7}$	$(3.14\pm0.07)\times10^{4}$
II	2.58×10^{-5}	2.58×10^{-5}	9.91	7.97×10^{-8}	$(1.74\pm0.04)\times10^{-7}$	$(8.46\pm0.17)\times10^{4}$
III	8.80×10^{-5}	8.80×10^{-5}	9.44	8.03×10^{-7}	$(5.65\pm0.11)\times10^{-8}$	$(7.99\pm0.17)\times10^{2}$
IV	1.07×10^{-5}	1.07×10^{-5}	9.03	2.51×10^{-7}	$(2.76\pm0.06)\times10^{-7}$	$(1.03\pm0.03)\times10^{5}$
V	1.07×10^{-5}	1.07×10^{-5}	9.08	2.24×10^{-7}	$(1.27\pm0.03)\times10^{-7}$	$(5.29\pm0.11)\times10^{4}$
VI	8.80×10^{-5}	8.80×10^{-5}	8.45	7.85×10^{-6}	$(1.24\pm0.03)\times10^{-8}$	17.95±0.42

[a] pK_a are given for the first stage of the hydroxybenzene dissociation [12].

ning the synthesis of compounds with high antioxidant properties.

EXPERIMENTAL

We used the stable free radical Dpph$^{\bullet}$ and dimethylsulfoxide from Aldrich–Sigma. The Dpph$^{\bullet}$ solution in DMSO has an intense purple color with maximum absorption at 520 nm. We found that at the storage of Dpph$^{\bullet}$ in DMSO in the dark for 72 h, the intensity of the absorption maximum of its spectrum remains unchanged. The hydroxybenzenes of chemically pure grade were subjected to repeated recrystallizations from ethyl alcohol and dried at 40°C in a nitrogen atmosphere, and then sublimed in a vacuum. Hydroxyhydroquinone was synthesized as described in [15]. To prepare the phosphate buffer, we used double distilled water, the salts KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$ of reagent grade, twice recrystallized. The exact pH values were determined with a laboratory ionometer I-160MI. Investigations were carried out at 293±2 K in the range of the reactants concentrations 10^{-4}–10^{-5} M. Phenol in water (buffer) was mixed in equimolar concentrations with the Dpph$^{\bullet}$ solution in DMSO, and its optical density value was measured using a KFK-3 photocolorimeter. The concentration of Dpph$^{\bullet}$ was calculated from the optical density using the molar extinction coefficient equal to $\varepsilon = 1.2 \times 10^4$ l mol^{-1} cm^{-1}.

REFERENCES

1. Foti, M.C., Daquino, C., and Geraci, C., *J. Org. Chem*., 2004, vol. 69, no. 18, p. 2309.
2. Litwinienko, G. and Ingold, K.U., *J. Org. Chem*., 2004, vol. 69, no. 18, p. 5888.
3. Litwinienko, G. and Ingold, K.U., *J. Org. Chem*., 2005, vol.70, no. 68, p. 8982.
4. Sun, B., Spranger, I., Yang, J., Leandro, C., Guo, L., Canrio, S., Zhao, Y., and Wu, C., *J. Agric. Food Chem*., 2009, vol. 57, no. 18, p. 8623.
5. Villano, D., Fernández-Pachón, M.S., Moyá, M.L., Troncoso, A.M., and García-Parilla, M.C., *Talanta*, 2007, vol. 71, p. 230.
6. Emmanuel', N.M. and Knorre, D.G., *Kurs khimicheskoi kinetiki* (Course of Chemical Kinetics), Moscow: Vysshaya Shkola, 1974.
7. Bogolitsyn, K.G., Gorbova, N.S., and Kosyakov, D.S., *Zh. Fiz. Khim*., 2003, vol. 77, no. 4, p. 667.
8. Steward, J.J.P., *MOPAC 2009*, Colorado Springs, USA; *http://openmopac.net/*.
9. Citterio, A., Minisci, F., Parta, O., and Sesana, G., *J. Am. Chem. Soc*., 1977, vol. 99, no. 24, p. 7667.
10. Linstrom, P.J. and Mallard, W.G., *NIST Chemistry Web Book*, NIST Standard Reference Database, no. 69, July 2001, National Institute of Standards and Technology, Gaithersburg, USA.
11. Denisov, E.T., *Ingibitory v radikal'no-tsepnykh protsessakh* (Inhibitors in the Radical-Chain Processes), Moscow: Nauka, 1997.
12. Dean, J.A., *Lange's Handbook of Chemistry*, New York: McGRAW-HILL, INC., 5th ed., 1999.
13. Filippenko, T.A., Belaya, N.I., and Nikolaevskii, A.N., *Khim.-Farm. Zh*., 2004, vol. 38, no. 8, p. 34.
14. Belaya, N.I., Ovcharova, O.Yu., Filippenko, T.A., Nikolaevskii, A.N., and Palagushkina, L.A., *Zh. Prikl. Khim*., 2005, vol. 78, no. 11, p. 1912.
15. *Preparativnaya organicheskaya khimiya* (Preparative Organic Chemistry), Vul'fson, N.S., Ed., Moscow: Goskhimizdat, 1959.