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ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Antiradical and Antioxidant Activity of Phenylhydrazones of Aromatic Aldehydes

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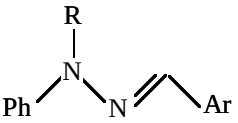
Abstract—With the aim of searching for new antioxidants, including those of the biogenic type, the antioxidant properties of phenylhydrazones of some aromatic aldehydes were studied under conditions of ascorbate-dependent peroxide oxidation of oleic acid residues in Tween-80. Also the reactivity of these hydrazones toward a stable radical, diphenylpicrylhydrazyl, was studied. The correlation between the antioxidant and antiradical activities of phenylhydrazones was examined.

The reactions of free-radical peroxide oxidation of lipids (POL) in biomembranes not only play an important role in the normal cell physiology but also often act as key units of the mechanisms of various pathological states, including the radiation sickness, infection and oncological diseases, etc. [1]. Therefore, search for physiologically active substances exhibiting antioxidant and antiradical properties and favoring normalization of the level of peroxide oxidation of lipids in biomembranes is an urgent problem. It is interesting to study in this respect phenylhydrazones, which exhibit a wide spectrum of physiological activity. It is known that phenylhydrazones (PHs) of some ketones exhibit antitubercular and antiviral activity [2, 3]. Salicylaldehyde benzoylhydrazone inhibits DNA synthesis in cells of radiation-sensitive tissues and is an effective low-toxic radioprotector [4]. It is believed that the pharmacological effect of phenylhydrazone compounds is associated, directly or indirectly, with their effect on the free-radical processes occurring in a living body. Data on the antioxidant activity of phenylhydrazones were obtained only recently [5]. The quantitative evaluation of the antioxidant and antiradical activity of phenylhydrazones will give insight into the mechanisms of their biological effect, on pathways of their transformations in radical processes, and on the structure–antioxidant activity relationships.

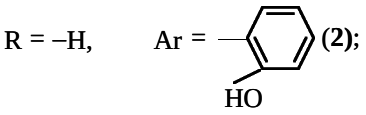
In this work we studied the antiradical and antioxidant activity of phenylhydrazones and the correlation of their reactivity in radical reactions with their composition and molecular structure.

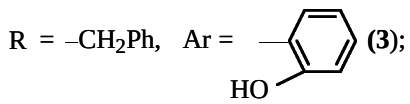
EXPERIMENTAL

The antiradical activity of phenylhydrazones was studied relative to a stable radical, diphenylpicrylhydrazyl (DPPH), as substrate. The reaction was monitored photocolorimetrically with a KFK-3 device. Phenylhydrazones [6] of the formulas



R = –H, Ar = –Ph (**1**);

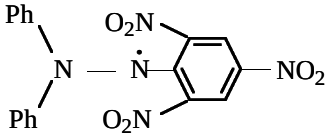




R = –CH_2Ph, Ar = (**3**);

R = –CH_2Ph, Ar = –Ph (**4**)

and DPPH [7]



were prepared and purified by standard procedures. The purity of phenylhydrazones was evaluated by elemental analysis and IR spectroscopy, and that of DPPH, by IR, UV, and ESR spectroscopy. Solutions

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of DPPH in hexane ($\varepsilon = 1.4 \times 10^3$ l mol^{-1} mm^{-1}), benzene ($\varepsilon = 1.24 \times 10^3$ l mol^{-1} mm^{-1}), and methanol ($\varepsilon = 1.18 \times 10^3$ l mol^{-1} mm^{-1}) exhibit an absorption maximum in the visible range (520 nm) and are stable in storage. Solutions of phenylhydrazones and DPPH were mixed in an equimolar ratio at 293 K in the range of reactant concentrations 10^{-4}–10^{-5} M, after which variation of the optical density of the DPPH solution was monitored in time and the kinetic curves of DPPH consumption were constructed (DPPH solutions in this concentration range obey the Lambert–Beer law).

The antioxidant activity of phenylhydrazones was studied in oxidation of Tween-80 (polyoxyethylene sorbitan monooleate), which is a model of lipid oxidation. Tween-80 purchased from Laba Chemie Fischamend, Austranal Präparate was used without additional purification. Oxidation of Tween-80 in dilute aqueous solutions was performed for 48–50 h at 313 K in the presence of optimal concentrations of Fe(II) and ascorbic acid. The concentration of malondialdehyde (MDA), which is the peroxidation product, was determined photocolorimetrically from the absorption of a pink complex of MDA with thiobarbituric acid ($\lambda = 540$ nm, $\varepsilon = 1.56 \times 10^4$ l mol^{-1} mm^{-1}) [8]. The performance of phenylhydrazones as antioxidants was evaluated from the decrease in the concentration of malondialdehyde as compared to the control experiment.

Figure 1 shows the kinetic curves of consumption of the DPPH radical in its reaction with phenylhydrazones of salicylaldehyde and benzaldehyde in hexane. Study of the reaction rate as a function of the DPPH and phenylhydrazone concentrations (Fig. 2) showed that the reaction is first-order with respect to both DPPH and phenylhydrazone. Such reaction orders are preserved in hexane for all the compounds studied. Thus, the kinetic equation for the reaction rate can be given as follows:

$$W = k_{app}[\text{DPPH}][\text{PH}].$$

The constants k_{app} calculated by this equation from the initial rates of DPPH consumption are listed in Table 1. It is seen that the rate constants of this reaction depend on the composition and structure of phenylhydrazones. Benzaldehyde benzylphenylhydrazone containing no labile hydrogen atom at nitrogen lacks antioxidant activity. This fact suggests that in the reaction with DPPH the reactive center in the phenylhydrazone molecule is the NH group of the hydrazone moiety. Thus, the reaction pattern can be represented

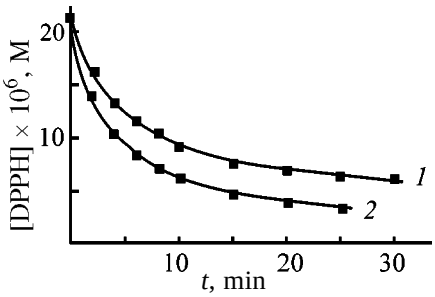


Fig. 1. Kinetic curves of the reactions of DPPH with phenylhydrazones of (*1*) benzaldehyde and (*2*) salicylaldehyde in hexane solution at 293 K. (*t*) Time.

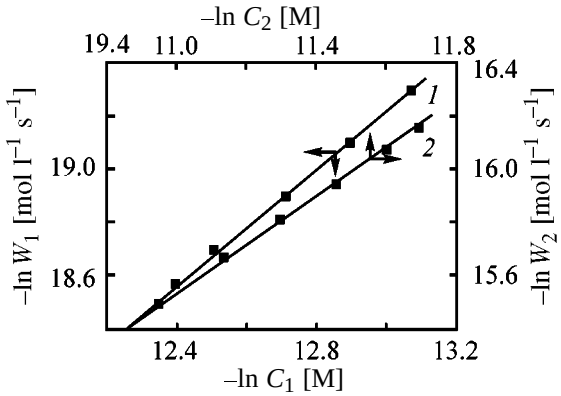
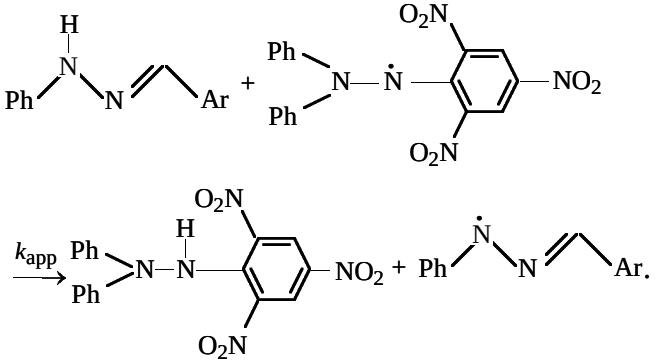


Fig. 2. Rate of reaction of DPPH with benzaldehyde phenylhydrazone as a function of the concentrations of (*1*) phenylhydrazone C_1 and (*2*) DPPH C_2.

as follows:



The final products of this reaction, along with the reduced form of diphenylpicrylhydrazyl (DPPH–H), are probably recombination products of the phenylhydrazone radicals with DPPH and of two phenylhydrazone radicals.

Comparison of the rate constants for different phenylhydrazones in hexane (Table 1) shows that introduction of the hydroxy group into the aldehyde moiety of the phenylhydrazone (compound **2**) significantly increases k_{app} as compared to **1**. This fact is

Table 1. Kinetic parameters of the reactions of aromatic aldehyde phenylhydrazones with DPPH in various solvents (T = 293 K)

Compd. no.*	k_{app}, l mol^{-1} s^{-1}		
	hexane	benzene	methanol
1	115 ± 5	11 ± 1	6.2 ± 0.6
2	201 ± 7	16 ± 20	155 ± 2
3	0.10 ± 0.01	Weak reaction	3.6 ± 0.5
4	No reaction with DPPH		
5	1.5 ± 0.2	0.27 ± 0.01	4.5 ± 0.6

* Compounds: (**1**) benzaldehyde phenylhydrazone, (**2**) salicylaldehyde phenylhydrazone, (**3**) salicylaldehyde benzylphenylhydrazone, (**4**) benzaldehyde benzylphenylhydrazone, and (**5**) Ionol.

due to additional contribution of the OH group to the reaction with DPPH, which occurs with the homolytic cleavage of the O–H bond. This contribution can be estimated by determining the antiradical activity of the hydrazone containing the hydroxy group in the aldehyde moiety but lacking the labile hydrogen atom at N (compound **3**). Table 1 shows that this activity is low, i.e., the main contribution to the reaction with DPPH (and hence to the antiradical activity) is made by the NH group.

Study of the solvent effect on the reaction kinetics showed that the reaction order is the same in all the tested solvents but the rate constant k_{app} strongly depends on the solvent.

The apparent rate constants of the reaction of DPPH with benzaldehyde phenylhydrazone in various solvents (k_{app}, l mol^{-1} s^{-1}) were measured at various temperatures in the range 293–323 K, and the following relations were obtained:

$$k_{app} = 2.29 \times 10^3 \exp[(-6800 \pm 380)/RT] \quad \text{(hexane)},$$

$$k_{app} = 2.27 \times 10^7 \exp[(-36\,000 \pm 1200)/RT] \quad \text{(benzene)},$$

$$k_{app} = 2.33 \times 10^4 \exp[(-20\,000 \pm 1000)/RT] \quad \text{(methanol)}.$$

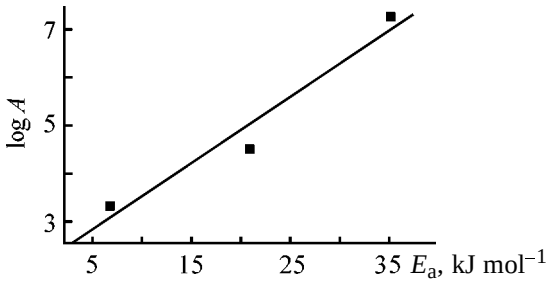


Fig. 3. Correlation between the preexponential term A and activation energy E_a of the reaction of DPPH with benzaldehyde phenylhydrazone in various solvents.

The decrease in the apparent rate constants, increase in the activation energy, and variation of the preexponential term in going from hexane to benzene and methanol show that the reactivity of the hydrazones decreases in going from hexane to benzene and methanol. The logarithm of the preexponential term linearly correlates with the activation energy (Fig. 3), which suggests the presence of a compensating effect. This effect is due to the localized interaction of the reagent with the solvent. For example, in benzene the decrease in the reaction rates is due to the well-known capability of DPPH to form π complexes with aromatic hydrocarbons, in which the radical is considerably less reactive than in the nonsolvated state [9]. In methanol, the reaction center of phenylhydrazone is blocked as a result of hydrogen bonding with the solvent [10]. The decrease in k_{app} and increase in E_a are in this case due to a decrease in the concentration of free, more active phenylhydrazone molecules. A considerably higher rate constant of the reaction with **2** in methanol, as compared to **1**, is due to active participation in the reaction of the hydroxy group in the aldehyde fragment of **2**. Owing to the high dielectric permittivity of methanol, the hydroxy group dissociates in this solvent to a greater extent.

The activity of the examined phenylhydrazones in the reaction with DPPH considerably exceeds that of the commercial inhibitor Ionol.

Our data on the antiradical activity of phenylhydrazones correlate with data on their antioxidant activity in oxidation of ethylbenzene [5]. This fact shows that the antioxidant properties of these compounds are due to their capability for reacting with radicals and confirms the suggested mechanism of the antioxidant action of these compounds in oxidation of hydrocarbons. Study of aromatic aldehyde phenylhydrazones as antioxidants of the ascorbate-dependent peroxide oxidation of oleic acid residues in Tween-80 showed, however, that their activity parameters do not always correlate with the characteristic of the antiradical activity (Table 2). This may be due to specific features of oxidation in this system, and also to possible operation of alternative mechanisms of the antioxidant effect of phenylhydrazones. At present, the mechanism of lipid oxidation in the presence of peroxide oxidation cofactors, Fe(II) and ascorbic acid, is not fully understood. Along with the radical-chain mechanism [1], oxidation by the ionic mechanism [11] is also probable. As seen from Table 2, the antioxidant activity of phenylhydrazones increases in the order **1** < **2** < **4** < **3**. The high antioxidant activity of **3** and **4**, with their low antiradical effect, is due to the possi-

Table 2. Effect of phenylhydrazones of aromatic aldehydes on the rate of accumulation of malondialdehyde (MDA) in oxidation of Tween-80

Compd. no.	MDA concentration $C \times 10^6$, M		Decrease in MDA accumulation rate, %
	control	experiment	
1	1.94	1.46	25
2	2.50	1.47	41
3	2.35	0.67	71
4	1.93	0.65	66

bility of their reaction with hydroperoxides initiating POL and of complexation with iron ions affecting the reaction rate. Also, interaction of oleic acid residues with phenylhydrazones, accompanied by structural changes, can enhance the oxidation resistance of the system.

CONCLUSIONS

(1) The kinetic study of the reactions of aromatic aldehyde phenylhydrazones with a stable radical, diphenylpicrylhydrazyl, in various solvents showed that the parameters of the antiradical activity of phenylhydrazones depend both on the compound structure and on the solvent.

(2) Reactions of diphenylpicrylhydrazyl with phenylhydrazones involve abstraction of hydrogen atom from the NH group of the hydrazone moiety. The contribution of the hydroxy group in the aldehyde moiety to the antiradical activity of phenylhydrazones is significant only in methanol.

(3) The antiradical activity of phenylhydrazones correlates with their antioxidant activity in oxidation of hydrocarbons and does not fully correlate with their antioxidant activity in ascorbate-dependent peroxide oxidation of oleic acid residues in Tween-80 in aqueous solutions. This is due to specific features of oxidation of this system and to possible operation of alternative mechanisms of antioxidant action.

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