

Studies on Antioxidative Properties of Nitrones from 3-Imidazoline-3-oxide, Dihydropyrazine-1,4-dioxide and 2H-imidazole-1-oxide Series in the Reactions with Peroxyde Radicals

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Abstract

Comparative studies have been carried out for the antiradical activity of nitrones from 3-imidazoline-3-oxide, dihydropyrazine-1,4-dioxide and 2H-imidazole-1-oxide series in model reactions of the initiated oxidation of cumene and methyloleate in chlorobenzene media at 60 °C. For the compounds under investigation, reaction rate constants of the interaction with peroxyde radicals resulted from cumene and methyloleate have been determined.

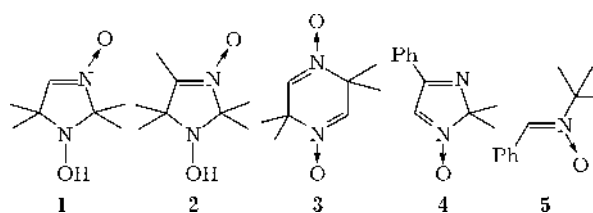
INTRODUCTION

The characteristic feature of nitrones (azomethine-N-oxides) consists in a high reactivity with respect to active radicals [1]. Free radicals play a significant role in the development of a wide range of pathological states, such as atherosclerosis, Alzheimer's disease, cancer, ischemia, diabetes, ageing, etc. The ability of nitrones to be scavengers of active oxygen containing radicals allows one to use them as antioxidant species those, in turn, could be considered as a possible therapeutic agent in pathological states under the conditions of oxidative stress [2–14]. N-hydroxylamine derivatives can exhibit a high antioxidant activity, as well [15–17].

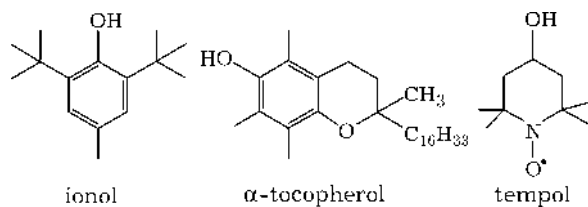
It has been shown earlier that the series of the derivatives 3-imidazoline-3-oxide [18, 19], dihydropyrazine-1,4-dioxide [19] and 2H-imidazole-1-oxide [20, 21] could serve as efficient spin

traps for a wide range of free radicals including oxygen-centered radicals, too.

In the present work, a comparative study of the antiradical (antioxidant) activity of nitrones, the derivatives of 3-imidazoline-3-oxide **1** and **2**, dihydropyrazine-1,4-dioxide **3**, 2H-imidazole-1-oxide **4** and α -phenyl-*N*-*tert*-butylnitron **5** is reported. It should be noted as well, that both the derivatives **1** and **2** contain the N-hydroxylamine group that also exhibits antioxidant properties, which allows one to expect a high antioxidant activity of these compounds.



As reference antioxidants, widely known inhibitors such as 2,6-di-*tert*-butyl-4-methylphenol (ionol), α -tocopherol and tempol were used:



EXPERIMENTAL

The investigation was carried out for two model systems such as cumene and methyloleate in chlorobenzene media at 60 °C exposed to oxidation initiated with azobis-*iso*-butyronitrile (AIBN, Acros Organics, the USA). The following reagents were used in the experiments: cumene (99.6 %, Acros Organics, the USA) and methyloleate (Acros Organics, the USA), containing, according the results of chromatography–mass spectrometry analysis, 75.7 % main substance, 9.4 % methylinoleate and 14.9 % esters of saturated fatty acids. The intensity of oxidation processes was determined basing on the rate of oxygen absorption with the use of a high-sensitivity microcapillary volumometer. The plotting and processing of kinetic curves was carried out using Origin 6.0 software. The induction period was determined as an intersection point of two tangents to a kinetic curve; the slope ratio values for those were equal to 0.5-fold and 0.75-fold slope ratio value for a forward non-inhibited reaction [22].

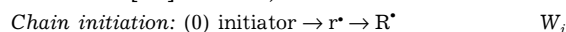
In the studies of cumene oxidation the following experimental concentrations of components in the sample were used: for cumene 6.9 mol/L, for AIBN 2.42–4.82 mmol/L, for the compounds under investigation $5 \cdot 10^{-5}$ – $1 \cdot 10^{-4}$ mol/L. The pressure of O₂ in the system amounted to 1 atm, the volume of the sample was 2 mL. The initiation reaction rate calculated according [22] was about $5 \cdot 10^{-8}$ mol/(L · s).

Methyloleate oxidation in chlorobenzene medium was performed at the following concentration of components in the sample: for methyloleate 1.47 mol/L, for AIBN 3.05–3.24 mmol/L, and for the compounds under investigation the concentrations ranged from $1.02 \cdot 10^{-5}$ to $2.14 \cdot 10^{-4}$ mol/L. The pressure of O₂ in the system amounted to 1 atm, the volume

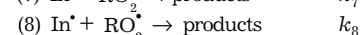
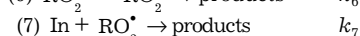
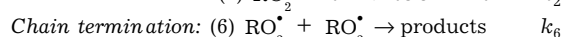
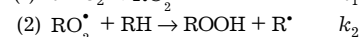
of the sample was 2 mL. The initiation reaction rate W_i determined with the use of the inhibitor method basing on the termination time of the induction period of methyloleate oxidation reaction inhibited with α -tocopherol, amounted to $2.8 \cdot 10^{-8}$ mol/(L · s).

RESULTS AND DISCUSSION

The AIBN initiated oxidation of hydrocarbon substrates occurs according to a free radical being in general agreement with the following kinetic scheme (the standard numeration of reactions [23] is used):



Chain propagation:



where r^{\bullet} is a radical formed from the initiator; RH is a hydrocarbon; R^{\bullet} and RO_2^{\bullet} are free alkyl and radicals, respectively; In is an inhibitor; In^{\bullet} is a radical resulted from the inhibitor; W_i is the initiation reaction rate; k_1 – k_8 are the rate constants of corresponding reactions.

The kinetic analysis of the present scheme results in the following equation for rate of oxygen absorption of oxygen in the process of inhibited oxidation:

$$\frac{\Delta[\text{O}_2]}{[\text{RH}]} = -\frac{k_2}{k_7} \ln\left(1 - \frac{t}{\tau}\right) \quad (\text{I})$$

where $\Delta[\text{O}_2]$ is the amount of oxygen absorbed normalized to the sample volume; k_7 and k_2 are the rate constants for the reactions of peroxide radicals with the molecules of an inhibitor and of a substrate under oxidation, respectively; τ is the induction period; t is time; [RH] is the concentration of a substrate in the sample.

The initial portions of the kinetic curves of oxygen absorption for all the inhibitors under investigation, for the model oxidation reactions of cumene and methyloleate in chlorobenzene were quite linear related to the coordinates corresponding to the eq. (I) (Fig. 1). This fact indicates that the inhibition of the reaction proceeds according to the reaction scheme considered above. Basing on slope ratio for the

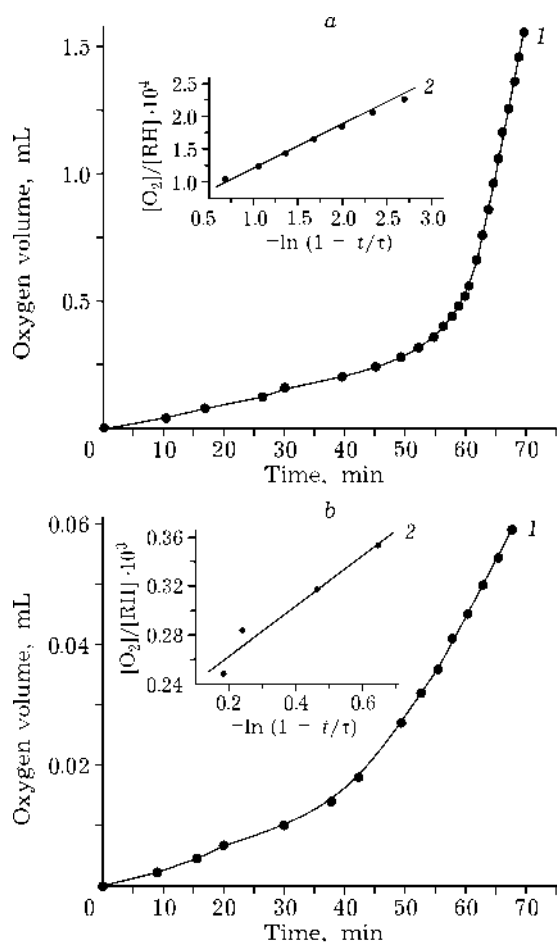


Fig. 1. Reactions of initiated oxidation of cumene in the presence of 1-hydroxy-2,2,4,4,5,5-pentamethyl-3-imidazoline-3-oxide **2** (a) and of methyloleate in chlorobenzene medium in the presence of 1-hydroxy-2,2,5,5-tetramethyl-3-imidazoline-3-oxide **1** (b): 1 - kinetic curves for oxygen absorption, 2 - anamorphoses of the kinetic curves.

linear anamorphosis, k_2/k_7 ratio value was found. Absolute values for k_7 were calculated on the assumption of the fact that for the model oxidation reaction of cumene, according to the data of [22] the reaction rate constant k_2 for oxidation chain propagation is 1.75 mol/(L · s).

TABLE 1

Reaction rate constant values k_7 for the interaction of the compounds under investigation with peroxide radicals at 60 °C, 10^4 L/(mol · s)

Initiator	Compounds					Ionol	α -Tocopherol
	1	2	3	4	5		
Cumene	25	22	3	1.6	3.2	2.2	20
	1.7	n/d					
Methyloleate in chlorobenzene medium	57	70	3.2*	1.5*	1.7*	2.6 [24]	140
						3.1*	120*

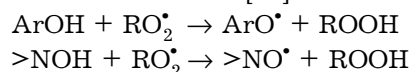
*Being determined from the eq. (V), the others were determined from the eq. (I).

According to [24], for the reaction of methyloleate oxidation in chlorobenzene media, in the case of ionol $k_7 = 2.6 \cdot 10^4$ mol/(L · s). This fact allowed us to determine the value of k_2 to be equal to 70 mol/(L · s).

As the result of the investigations carried out it has been shown that all the compounds under study, with the exception of tempol, exhibit a pronounced reactivity with respect to cumene peroxide radicals. The absence of inhibition effect in the case of tempol is in a good accordance with the data from the literature concerning the fact that stable nitroxides inhibit the process of oxidation due to the reactions with alkyl radicals, but do not interact with peroxide radicals [25].

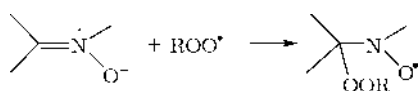
It has been shown that the compounds under investigation reacting with cumene peroxide radicals exhibit some difference in the efficiency. The hydroxylamine groups of compounds **1** and **2** exhibit higher reactivity that is comparable to the reactivity of α -tocopherol. The value of k_7 for these compounds amounts to $(2.2-2.5) \cdot 10^5$ mol/(L · s). The nitron groups in the molecules of compounds **1**, **3-5** are characterized by lower reactivity, the corresponding rate constant values k_7 are an order of magnitude lower, amounting to about $(1.6-3.2) \cdot 10^4$ mol/(L · s) (Table 1).

The mechanisms of oxidation chain termination for the inhibitors under our investigation may be different. The ability of phenolic compounds and hydroxylamines to decelerate free radical oxidation of cumene is connected with the presence of hydroxyl groups in these molecules [26]:



For the present reactions, the effect of inhibition is caused by a higher stability of phenoxyl (nitroxyl) radicals in comparison with alkylperoxide radicals responsible for the oxidation chain propagation.

The termination of the nitron oxidation chain occurs *via* addition of active nitron radicals to the double bond position resulting in the formation of stable nitroxyl radicals [25]:



It should be noted that there is a break observed on the kinetic curve anamorphosis for cumene oxidation inhibited with compound **1** which the break is dividing the curve into two linear portions (Fig. 2). This fact indicative of the presence of two types of functional groups in the structure of the compound those exhibit different values of reaction rate constant k_7 [27].

In the case of compound **2**, only one k_7 value could be determined that is corresponding, obviously, to the reactivity of the hydroxylamine group (see Table 1). It seems likely that the methyl group at the double bond position provides a steric hindrance for the interaction of the nitron group with cumyl peroxide radicals.

The kinetic curves for the oxidation of methyloleate in chlorobenzene medium under inhibition by compounds **3–5** exhibited no prominent induction period, on the grounds of this the absolute k_7 values were determined basing on the dependence of the initial rate of inhibited oxidation process on the inhibitor concentration according to [28].

For the reaction of methyloleate oxidation in chlorobenzene media the reaction rate value for non-inhibited process is proportional to W_i , which gives an indication of square-law chain termination according to the reaction (6). Basing on the analysis of the general kinetic scheme for the process of non-inhibited oxidation of methyloleate in chlorobenzene medium consisting of the reactions such as (0), (1), (2), (6), the following expression was obtained for the rate of oxygen consumption in the chain process [26]:

$$W_0 = -\frac{d[O_2]}{dt} = k_2 [RH][RO_2^*] = k_2 [RH] \sqrt{W_i / (2k_6)} \quad (\text{II})$$

where W_0 is the reaction rate for non-inhibited oxidation process; $d[O_2]$ is the amount of oxygen absorbed during the time dt , normalized to the sample volume; $[RO_2^*]$ is the concentration of peroxide radicals.

With an inhibitor introduced into the system, the kinetic scheme should be supplemented with the reactions (7) and (8).

The solution of the corresponding set of differential equations basing on quasi-steady-state approximation taking into account all free radical species (in view of the fact that the inhibitor is spent only due to the reaction (7)) results in the equation

$$W = -\frac{d[O_2]}{dt} = \frac{k_2 [RH] W_i}{2k_7 [In]} \quad (\text{III})$$

where W is the rate of the inhibited oxidation process.

From the expressions (II) and (III) we obtain

$$\frac{W_0}{W} = k_7 [In] \sqrt{\frac{2}{k_6 W_i}} \quad (\text{IV})$$

Substituting the expression for k_6 taken from (II) in equation (IV) shows that

$$W = \frac{W_i k_2 [RH]}{2k_7 [In]} \quad (\text{V})$$

The eq. (V) allows one to determine k_7 value basing on experimentally measured reaction rate for inhibited oxidation process (W) at low levels of inhibitor consumption.

By the example of α -tocopherol, the rate constant values for k_7 , determined basing on both the eq. (I) and the eq. (V), are demonstrated to be close to each other.

It is shown that for the process of methyloleate oxidation in chlorobenzene medium as compared to the oxidation of cumene, the hydroxylamine groups of compounds **1** and **2** exhibit higher reactivity being characterized by the reaction rate constant values k_7 ranging from $5.7 \cdot 10^5$ to 7.0 quasi-steady-state approximation 10^5 L/(mol · s). For α -tocopherol k_7 value is almost an order of magnitude higher, too. The nitron groups $RCH=N(R') \rightarrow O$ in the molecules of compounds **3–5** react with peroxide radicals of methyloleate exhibiting the reaction rate constant value $k_7 = (1.5-3.2) \cdot 10^4$ L/(mol · s),

which is corresponding to the antiradical activity of these compounds in the model reaction of cumene oxidation.

CONCLUSION

As a whole, the results obtained point to the fact that nitrones **1–5** exhibit a pronounced inhibition activity with respect to peroxide radicals of cumene and methyloleate. There are two types of antiradical centres present in the structure of the compounds **1** and **2** under investigation. They are the nitrone groups for those k_7 value amounts to $(1.6–3.2) \cdot 10^4$ L/(mol · s), and the hydroxylamine groups characterized by higher k_7 values of about $(2.2–7.0) \cdot 10^5$ L/(mol · s). According to the values of the reaction rate constants k_7 , the compounds under investigation are quite comparable with widely used phenolic antioxidants. It may allow one to consider the nitrones **1–5** to be promising as potential practical antioxidant species for the further research.

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