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STRUCTURE OF CHEMICAL COMPOUNDS, METHODS OF ANALYSIS AND PROCESS CONTROL

QUANTITATIVE ANALYSIS OF THE ANTIOXIDANT ACTIVITY OF MEXIDOL

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The antioxidant activity of the drug Mexidol was quantitatively measured using an objective method. The rate constant for the reaction of Mexidol with peroxyl radicals of 1,4-dioxane was measured in 1,4-dioxane solution. Mexidol was found to inhibit oxidation of 1,4-dioxane and showed good antioxidant activity comparable to that of ionol. The obtained data were interesting for studying the mechanism of action of the antioxidant in biological systems because the physicochemical properties of 1,4-dioxane are close to those of water, in which processes associated with oxidative stress occur. Measurement of the rate constant for the reaction of the inhibitor with peroxyl radical also had fundamental importance for establishing the structure—reactivity relationship in order to predict the antioxidant activity of compounds.

Keywords: radical-chain oxidation, 1,4-dioxane, Mexidol, peroxyl radical, antioxidant activity.

Mexidol is a derivative of 2-ethyl-6-methyl-3-hydroxypyridine and succinic acid. 2-Ethyl-6-methyl-3-hydroxypyridine has a chemical structure that can be considered a nitrogenous heterocyclic analog of aromatic phenols and exhibits the properties of both pyridine and phenols. It reacts with the peroxyl radical of ethylbenzene with rate constant $k_7 = 4.7 \times 10^4$ $M^{-1} \cdot s^{-1}$ [1]. Few quantitative data exist for the reaction of Mexidol, the active ingredient of which is 2-ethyl-6-methyl-3-hydroxypyridine succinate. In particular, the rate constant for the reaction of Mexidol with peroxyl radical in a model system of methyl oleate radical-chain oxidation was measured as $k_7 = 2.8 \times 10^4$ $M^{-1} \cdot s^{-1}$ [2]. The studies were hindered because the drug was poorly soluble in the commonly used substrates for quantitative determination of antioxidants, i.e., cumene, ethylbenzene, and styrene. Many antioxidants breaking the chain are water-soluble but little is known about their reactions with peroxyl radicals because of the limited applicability of practical research methods. In the present work, the antioxidant activity of Mexidol was studied in a model system of controlled radical-chain oxidation of 1,4-dioxane, which was simultaneously the solvent and the oxidation substrate. The drug was soluble in it over a rather broad range of concentrations. 1,4-Dioxane is the optimal substrate for studying reactions of peroxyl radical with antioxidants because several physicochemical properties of dioxane solutions are close to those of water [3], in which processes associated with oxidative stress occur. Therefore, the found trends would also be valid for biological systems. This was important because inhibitors that are active in organic solvents (cumene, ethylbenzene, styrene) are often inactive in aqueous solutions [4]. For example, the rate constant for reaction of the most active inhibitor α-tocopherol with methyl linoleate peroxyl radical in its solution was 1.3×10^6 L/(mol·s). The rate constant for the reaction of it with this same peroxyl radical in aqueous solution decreased to 2.3×10^4 L/(mol·s) [5]. A study of the properties of water-soluble antioxidants in a medium simulating physiological conditions would have practical interest. A reliable and

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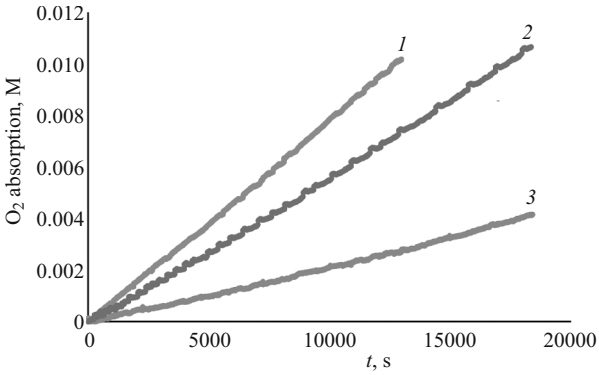

Fig. 1. Typical kinetic curves for oxygen absorption during oxidation of 1,4-dioxane without inhibitor (*1*) and with Mexidol: 7.3×10^{-4} (*2*) and 27.7×10^{-4} M (*3*). Reaction conditions: [1,4-dioxane] = 9.7 M, $w_i = 10^{-7}$ mol/(L·s), 333 K.

objective measurement of antioxidant activity would have fundamental significance for evaluating antioxidants. Therefore, the present work used a method based on the model reaction of radical-chain oxidation of 1,4-dioxane and the rate of oxygen absorption during inhibited oxidation. This method was most suitable for evaluating the antioxidant activity of water-soluble compounds used for medical purposes [6].

Besides practical value, studies of the dependence of antioxidant effectiveness on its structure and the solvent effect were timely.

EXPERIMENTAL PART

1,4-Dioxane was purified as before [7]. 2,2′-Azo-*bis*-isobutyronitrile (AIBN, $C_8H_{12}N_4$; REAKHIM) was recrystallized twice from freshly distilled EtOH and then dried under vacuum. An aqueous solution of Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate, $C_8H_{11}NO$, 50 mg/mL) in 2-mL ampuls (Farmasoft) was used. The water was evaporated. Then, the contents were dissolved in 1,4-dioxane.

Oxidation of 1,4-dioxane initiated by AIBN was performed at 333 K. The oxidant was atmospheric oxygen, absorption of which was monitored using a universal differential manometer system [8]. Experiments used a glass reactor that was loaded with 1,4-dioxane (4.4 – 4.95 mL), thermostatted, and treated with a solution of the initiator in chlorobenzene (1 mL). Mexidol was added to the 1,4-dioxane solution (0.05 – 0.6 mL) 15 min after the start of the oxidation. The inhibitor concentration was varied in the range $(0.1 – 2.8)\cdot10^{-3}$ M. The O_2 absorption rate in the liquid phase was calculated by the literature method [8, 9]. The initial rate of inhibited oxidation was determined from the slope of the time dependence of the amount of absorbed O_2.

The rate of initiation was calculated from the equation $w_i = k_i[\text{AIBN}] = 2ek_p[\text{AIBN}]$. The rate constant for decay of AIBN in 1,4-dioxane, $\log k_p = 15.8 – 132.9/\theta$ [s^{-1}], $\theta = 2.303RT \times 10^{-3}$ kJ/mol, was used in the calculations [10]. The radical yield in bulk 1,4-dioxane was set to $2e = 1$ [11, 12].

RESULTS AND DISCUSSION

Liquid-phase inhibited oxidation of 1,4-dioxane under the conditions of our experiment [333 K, $w_i = 1.0 \times 10^{-7}$ mol/(L·s), substrate concentration 9.7 M] occurred according to Scheme 1 [13].

Scheme 1

$$\text{AIBN} \longrightarrow \text{r}^{\bullet} \xrightarrow{\text{RH}} \text{R}^{\bullet}, \quad \text{(i)}$$

$$\text{R}^{\bullet} + \text{O}_2 \longrightarrow \text{RO}_2^{\bullet}, \quad \text{(I)}$$

$$\text{RO}_2^{\bullet} + \text{RH} \longrightarrow \text{ROOH} + \text{R}^{\bullet}, \quad \text{(II)}$$

$$\text{RO}_2^{\bullet} + \text{RO}_2^{\bullet} \longrightarrow \text{P}_6, \quad \text{(VI)}$$

$$\text{RO}_2^{\bullet} + \text{InH} \longrightarrow \text{P}_7. \quad \text{(VII)}$$

Here AIBN was the initiator; RH, the 1,4-dioxane substrate being oxidized; RO_2^{*}, peroxyl radical formed from 1,4-dioxane; P_6 and P_7, products inactive in the chain propagation reaction; and InH, inhibitor (in this instance, Mexidol).

Figure 1 shows typical kinetic curves for O_2 absorption.

The induction period (τ) was calculated by processing the kinetic curves using an integral method and the formula [14]:

$$\tau = \int_0^{\infty} \left(1 - \left(\frac{w}{w_0}\right)^2\right) dt, \quad (1)$$

where w is the inhibited oxidation rate and w_0, the uninhibited oxidation rate.

Table 1 presents the experimental τ values.

Rate constant k_7 was calculated using the equation [15, 16]:

$$\Delta[O_2] = -k_2(k_7)^{-1}[\text{RH}]\ln\left(1 - \frac{t}{\tau}\right), \quad (2)$$

where $\Delta[O_2]$ is the amount of absorbed O_2; k_2, chain-propagation rate constant [reaction (II) in Scheme 1]; k_7, chain-breaking rate constant of the oxidation by the inhibitor [reaction (VII) in Scheme 1].

The kinetic curves obtained in experiments in which the chain length in the inhibited oxidation was at least ~3 units (Table 1) [15] were processed using Eq. (2). The inhibition rate constant was calculated from the slope of the dependence of $\Delta[O_2]$ on $\ln(1 - t/\tau)$ in the part corresponding to ~80% of the length of the induction period (Fig. 2) [11].

Rate constant k_7 was calculated using $k_2 = 9.48$ L/(mol·s) [7]. Statistical processing of the results found the mean rate constant for the reaction of peroxyl radical with Mexidol $k_7^{\text{mean}} = 2.92 \times 10^4$ L/(mol·s) with standard deviation

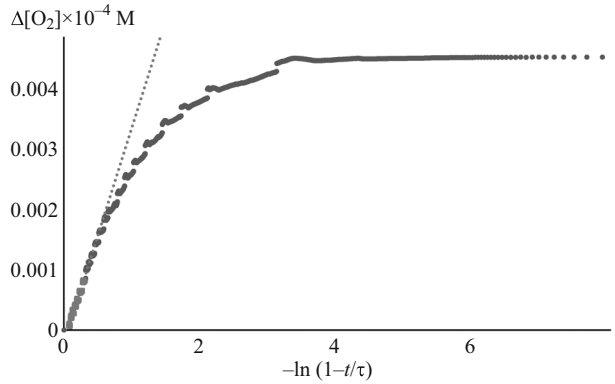

Fig. 2. Plot of kinetic curve 2 (Fig. 1) in coordinates of Eq. (1). Dashed line continues the segment of the dependence with a length less than ~80% of the induction period duration. The inhibition rate constant was calculated from the slope of this line.

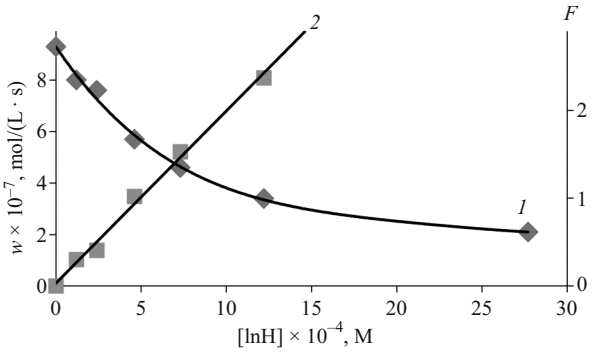

Fig. 3. Dependence (1) of initial oxidation rate of 1,4-dioxane on Mexidol concentration and anamorphosis (2, $r = 0.99$) of this dependence in coordinates of Eq. (3). Reaction conditions: [RH] = 9.7 M, $w_i = 10^{-7}$ mol/(L·s), 333 K.

$1.97 \cdot 10^3$ L/(mol·s). The standard error of the mean was $9.8 \cdot 10^2$ L/(mol·s). The resulting confidence interval was estimated as $k_7^{mean} = 2.92 \cdot 10^4 \pm (1.96 \times 9.8 \times 10^3)$ L/(mol·s) = $(2.9 \pm 0.2) \times 10^4$ L/(mol·s).

Effective rate constant fk_7 was determined by processing the dependence of the initial oxidation rate of 1,4-dioxane on Mexidol concentration in coordinates of the equation [16]:

$$F = w_0 w^{-1} - w (w_0)^{-1} = fk_7[\mathrm{InH}](2k_6 w_i)^{-0.5}, \qquad (3)$$

where w_0 and w are the initial rate of O_2 absorption without and with inhibitor, respectively; f, the inhibition stoichiometric coefficient; [InH], initial Mexidol concentration; $2k_6$, oxidation chain-breaking rate constant via recombination of 1,4-dioxane peroxyl radicals [reaction (VI) in Scheme 1], 10^9 L/(mol·s) in our instance [13].

Figure 3 shows that the dependence of parameter F on the initial concentration of Mexidol was satisfactorily linear.

TABLE 1. Dependence of 1,4-Dioxane Oxidation Rate (w) and Induction Period (τ) on Mexidol Concentration. Reaction Conditions: [RH] = 9.7 M, $w_i = 10^{-7}$ mol/(L·s), 333 K

[InH] · 10^{-4}, M	$w \cdot 10^7$, mol/(L·s)	τ, s	$k_7 \cdot 10^{-4}$,* L/(mol·s)	f**
0	9.3	–	–	–
1.2	8.0	2400	–	1.5
2.4	7.6	4550	3.7	1.3
4.6	5.7	6262	3.1	1.1
7.3	4.6	9527	2.5	1.1
12.2	3.4	13200	2.4	1.1
27.7	2.1	–	–	–

* Constant k_7 was calculated from Eq. (2).
** Inhibition coefficient f was determined using Eq. (4).

Its slope was used to determine the effective rate constant. The value $fk_7 = (2.1 \pm 0.3) \times 10^4$ L/(mol·s) was obtained considering a 95% confidence interval. Thus, the antioxidant activity of Mexidol was comparable to that of ionol, for which $fk_7 = 2.8 \times 10^4$ L/(mol·s) under these same conditions [13].

The stoichiometric coefficient of inhibition was measured based on the induction period using the equation:

$$\tau = f[\mathrm{InH}]/w_i. \qquad (4)$$

Parameter f was found to be 1.2 ± 0.1 (Table 1), according to which one peroxyl radical was consumed per Mexidol molecule.

As a rule, the inhibition stoichiometric coefficient was $f = 2$ if the following reaction besides reaction (VII) occurred:

$$RO_2^{\bullet} + In^{\bullet} \rightarrow P_8. \qquad (VIII)$$

If parameter $f \leq 1$, then either a) the radical formed from Mexidol was relatively stable and reaction (VIII) did not occur or b) the inhibitor was consumed in side reactions. For example, parameter $f \leq 1$ for radical-chain oxidation of 1,4-dioxane and ethylbenzene in the presence of 5-amino-6-methyluracil derivatives. InH was consumed through reactions with the peroxyl radical of the inhibitor itself [17]. Oxidation of 2-ethyl-6-methyl-3-hydroxypyridine was associated with the formation of rather active radicals [2]. Therefore, it could have reacted with peroxyl radical [reaction (VIII)]. However, hydroxypyrimidines form characteristic tautomers. The resulting inhibitor radical ($In^{\bullet}$) could be in equilibrium with its carbon-centered form ($In'^{\bullet}$). A new peroxyl radical ($In'O_2^{\bullet}$) formed in the presence of O_2 and could cleave an H atom from the inhibitor molecule. As a result, an additional channel for consumption of the inhibitor opened and led to a decrease of parameter f. The process could be expressed by Scheme 2, which was previously demonstrated for aminouracil [17].

Scheme 2

$$RO_2^{\bullet} + InH \longrightarrow In^{\bullet} + ROOH,$$

$$In^{\bullet} \rightleftharpoons In'^{\bullet},$$

$$In'^{\bullet} + O_2 \longrightarrow In'O_2^{\bullet},$$

$$In'O_2^{\bullet} + InH \longrightarrow In^{\bullet} + In'OOH.$$

Hence, Mexidol could exhibit pro-oxidant activity instead of antioxidant properties at sufficiently high concentrations. This should be taken into account if it is used. Also, this property is typical of α-tocopherol [18] because peroxyl radicals play a positive role in *in vivo* vitality [19]. If their concentration becomes less than the required level, tocopherol exhibits pro-oxidant properties. The concentration of peroxyl radicals exceeds the limiting allowed standard during diseases. The result of this is oxidative stress. Then, the antioxidant properties of tocopherol become prevalent.

It has been reported that neither Mexidol itself nor its generics have proven effective [20]. Currently, a method for measuring the antioxidant activity that is highly recommended in foreign practice is used [6, 18, 21]. This method is based on radical-chain oxidation of THF, a compound related to 1,4-dioxane, and is used to study the activity of water-soluble antioxidants [22]. The study performed by us showed convincingly that Mexidol was an effective antioxidant. It is used for diseases in which an excess of peroxyl radicals is formed and it just as potent as ionol.

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