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PEROXIDES-XI

The Role of Peroxides in the Mechanism of Low-Temperature Autooxidation of Methyl Oleate and Its Solutions with Lipids

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Abstract—The kinetics of low-temperature autooxidation of methyl oleate for a bimolecular mechanism of the degenerate branched reaction were analyzed taking into account changes in the methyl oleate concentration for different amounts of peroxides formed. This analysis made it possible to explain the experimental data. The role of the initial peroxide concentration in the mechanism and kinetics of the chain degenerate branched reaction of methyl oleate autooxidation was studied in the steady-state approximation and in the course of establishing a steady-state concentration of radicals. A systematic approach to estimating the antioxidant properties of lipids on the basis of the methyl oleate model was proposed.

The fundamental theoretical study of the kinetics of chain reactions of hydrocarbon oxidation in the liquid phase was performed in the 1950s–1970s by Emanuel and his scientific school [1–3]. The mechanism of liquid-phase oxidation of organic compounds proposed by Emanuel and coworkers is considered as the main mechanism for lipid peroxidation in biological membranes [4–8]. To evaluate the antioxidant properties of lipids, a methyl oleate model was proposed and the antioxidative activity of lipids (*AOA*) was introduced [6]. The latter was determined as $AOA = (\tau_{exp} - \tau_{RH})/C$, where τ_{exp} is the induction period for a solution of methyl oleate with lipids, τ_{RH} is that for methyl oleate, and C is the concentration of lipids. Later the *AOA* was normalized by standard methyl oleate: $AOA = (\tau_{exp} - \tau_{RH})w_0/(Cw_{0,\,ref})$, where w_0 is the chain initiation rate in a pilot sample of methyl oleate, and $w_{0,\,ref}$ is that for methyl oleate taken as a reference [9]. It is shown that the main primary products of methyl oleate oxidation are peroxides and oxides. However, the formation of more than 80% of the oxidation products is caused by further peroxide transformations [6].

It was found for the methyl oleate model that lipids of gram-negative bacteria and blood and brain erythrocytes of animals manifest only prooxidant properties regardless of the type and initiation rate of free radicals in the model system [10]. Attempts to explain these results using only habitual characteristics were unsuccessful. The development of a systematic approach to the investigation of the oxidation mechanism in complex systems requires studying the detailed kinetics of autooxidation of methyl oleate and its solutions with lipids.

The detailed analysis of the kinetics of peroxide accumulation upon the thermal autooxidation of methyl oleate solutions of lipids from microorganismic cells and animal tissues [10] showed that the ability of lipids to be involved in low-temperature autooxidation processes at radical initiation and chain propagation steps resulted in the following characteristics of lipids along with the *AOA*: the degree of oxidation inhibition ($DOI = \tau_{exp}/(\tau_{RH}C)$) by lipids of methyl oleate, the initial amount of peroxides ($[ROOH]_0$), the antiperoxide activity (*APA*), the composition of lipids, the degree of their unsaturation, and the ratio of sums of more readily oxidizable to less readily oxidizable fractions of phospholipids.

The purpose of this work is to study the role of peroxides in the autooxidation of methyl oleate and its solutions with lipids, taking into account the nonzero initial content of peroxides, a relation of the change in the methyl oleate concentration to the peroxide content, and the antiperoxide activity of lipids for a bimolecular mechanism of the degenerate branched reaction.

EXPERIMENTAL DATA

Chain initiation in methyl oleate autooxidation was experimentally found to occur via the trimolecular reaction [11]: $2RH + O_2 \longrightarrow 2R^{\cdot} + H_2O_2$. The rate of free radical formation w_0 in the autooxidation of methyl oleate at 37°C may change by an order of magnitude within $w_0 \times 10^{10} \simeq 0.30$–$3.47$ mol l^{-1} s^{-1} due to variations in the initial concentration of peroxide and content of a minor amount of methyl linoleate [10]. Experimental data [12] on peroxide accumulation upon methyl oleate autooxidation have a pronounced exponential character ($[ROOH] = [ROOH]_0 + a(\exp(kt) - 1)$), which indicates a bimolecular mechanism of the degenerate branched reaction. The exponent value k (h^{-1}) varies within a factor of 18.3, and the preexponential factor a (mmol/g) varies within three orders of magnitude. These values change for the same sample of methyl oleate: k changes within 5–6%, and a changes proportionally with w_0. In

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addition, analysis of the whole data body asserts that k and a increase with an increase in w_0 [10].

Experimental data on peroxide accumulation during the oxidation of methyl oleate solutions with lipids show that lipids can either inhibit (antioxidant effect) or accelerate methyl oleate oxidation (prooxidant effect) depending on the isolation source. This is illustrated in Fig. 1 for methyl oleate solutions with the lipids from spleens, blood erythrocytes, and livers of SHK mice of the line (female). Similar data have previously been obtained by studying the antioxidant properties of lipids isolated from other biological objects [12]. A substantial influence of the substrate oxidizability on the induction period of inhibited hydrocarbon oxidation and on the efficiency of the inhibitors [13–15] suggests that the prooxidant activity of lipids is caused by the high degree of their unsaturation, along with their depletion in antioxidants. In fact, the prooxidant effect was observed when lipids contained α-tocopherol, whose presence was experimentally confirmed and whose contribution to the total effect of natural antioxidants was most significant [16].

The lipids affect the induction period of oxidation, the overall oxidation rate, and the concentration of peroxides, because the initial level of peroxides can significantly change immediately after lipids are introduced into methyl oleate (at $t = 0$) (Fig. 1). The initial amount of peroxides in the lipids was estimated from an increase in the peroxide concentration referred to the weight unit of lipids added. The antiperoxide activity of lipids, (that is, their ability to decompose peroxides to molecular products) was estimated from a decrease in the peroxide concentration in methyl oleate immediately after the lipids were introduced [12, 17]. The ability of natural components of the cell to interact directly with peroxide was experimentally confirmed in the reactions of methyl oleate peroxide with α-tocopherol, 20-hydroxyecdysone, vitamin A palmitate, choline chloride, and β-carotene [10].

Thus, lipids are involved in the low-temperature autooxidation of methyl oleate as substrates even in the case when their concentration does not exceed 3%, which corresponds to a lipid concentration of ~25 mg/ml of methyl oleate.

Because of the above facts, there is a need for a quantitative theory of methyl oleate autooxidation under nonzero initial conditions imposed on peroxides.

KINETIC MODEL OF METHYL OLEATE AUTOOXIDATION

1. System of Equations

Based on experimental data, we can write the following system of reactions for the low-temperature autooxidation of methyl oleate:

(I.0)	$2RH + O_2 \longrightarrow 2R^{\bullet} + H_2O_2$	radical initiation
(I.1)	$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet}$	radical initiation
(I.2)	$RH + RO_2^{\bullet} \longrightarrow ROOH + R^{\bullet}$	chain propagation
(I.3)	$ROOH + ROOH \longrightarrow RO_2^{\bullet} + RO^{\bullet} + H_2O$	degenerate branching
(I.6)	$RO_2^{\bullet} + RO_2^{\bullet} \longrightarrow ROOR + O_2$	chain termination

When the concentration of dissolved oxygen is high, chain termination proceeds via bimolecular reaction (I.6). Free radicals $R^{\bullet}$ and $RO_2^{\bullet}$ are very active and rapidly disappear. Therefore, their kinetic-equilibrium concentration is rapidly established in autooxidation. According to the Bodenstein–Semenov method of steady-state concentrations, the derivatives $d[R^{\bullet}]/dt$ and $d[RO_2^{\bullet}]/dt$ can be made equal to zero, and then

$$d[R^{\bullet}]/dt = w_0 - k_1[O_2][R^{\bullet}] + k_2[RH][RO_2^{\bullet}] = 0,$$

$$d[RO_2^{\bullet}]/dt = k_1[O_2][R^{\bullet}] - k_2[RH][RO_2^{\bullet}] - k_6[RO_2^{\bullet}]^2 + \alpha_3 k_{33}[ROOH]^2 = 0,$$

where w_0 is the rate of $R^{\bullet}$ radical formation in reaction (I.0), k_i are the rate constants of the corresponding reactions, and α_3 is a constant within 0.5–2.

Summing these two equations, we have

$$w_0 = k_6[RO_2^{\bullet}]^2 - \alpha_3 k_{33}[ROOH]^2.$$

For low peroxide concentrations (when degenerate branching is neglected), one can consider that $w_0 \cong k_6[RO_2^{\bullet}]^2$, and the steady-state concentration of the $RO_2^{\bullet}$ radicals is determined from the expression

$$[RO_2^{\bullet}]_{st} = (w_0/k_6)^{1/2}.$$

The rate of methyl oleate oxidation (w_m) is determined by the slow step, which is chain propagation (Eq. (I.2)), and for low concentrations of peroxides

$$w_m = k_2[RH][RO_2^{\bullet}] = k_2[RH](w_0/k_6)^{1/2}.$$

2. The Steady-State Approximation

Let us consider the kinetics of methyl oleate autooxidation when the steady-state concentration of radicals has already been established and the steady-state approximation can be applied. As already mentioned, slow step (I.2) exists among the reactions in system (I) and determines the reaction rate. For the slow step, we can write

$$dx/dt = k_2 sy,$$

where s, y, and x are the concentrations of methyl oleate RH, $RO_2^{\cdot}$, and peroxide ROOH, respectively, at the moment t.

To determine the concentration of an intermediate product, the steady-state approximation can be used

$$dy/dt = w_0 - k_6 y^2 + \alpha_3 k_{33} x^2 = 0,$$

$$d[R^{\cdot}]/dt = w_0 - k_1[O_2][R^{\cdot}] + k_2[RH][RO_2^{\cdot}] = 0,$$

where w_0 is the rate of $R^{\cdot}$ radical formation via reaction (I.0), which is also constant when the oxygen and methyl oleate concentrations are constant ($[O_2] = b_0 = \text{const}$ and $s_0 = [RH]_0 = \text{const}$, respectively): $w_0 = k_0 s_0^2 b_0 = \text{const}$.

To change the methyl oleate concentration at low concentrations of peroxides, a linear approximation can be used: $s = s_0 - x/\alpha_0$, where $\alpha_0 \cong 0.5 - 2$. Then, the expression for the rate of $R^{\cdot}$ radical formation w_0 takes the following form:

$$w_0 = k_0(s_0 - x/\alpha_0)^2 b_0.$$

Based on these assumptions, we obtain the set of equations

$$\begin{gathered}(1.0)\ dx/dt = k_2 sy,\\ (1.1)\ dy/dt = w_0 - k_6 y^2 + \alpha_3 k_{33} x^2 = 0,\\ (1.2)\ x|_{t=0} = x_0,\quad y|_{t=0} = 0,\quad s|_{t=0} = s_0,\\ (1.3)\ s = s_0 - x/\alpha_0.\end{gathered} \tag{1}$$

Set (1) results in an equation for the dimensionless value $\pi = x/s$, the effective constant k_{eff}, and a dimensionless parameter ω_0:

$$\begin{gathered}\exp\left(k_{eff} s_0 t (1 + \omega_0/\alpha_0^2)^{1/2}\right)\\ = (\omega_0 + \alpha_0\pi)(\alpha_0 - \pi_0)(\omega_0 + \alpha_0\pi_0)^{-1}(\alpha_0 - \pi)^{-1},\end{gathered} \tag{2}$$

where $\omega_0 = w_0/\alpha_3 k_{33} s_0^2$, $k_{eff} = k_2(\alpha_3 k_{33}/k_6)^{1/2}$.

Exact analytical solution is possible in five cases:

(1) under the condition that $\pi^2 \ll \omega_0$, $\pi_0^2 \ll \omega_0$, $\pi \ll \alpha_0$:

$$\pi = \pi_0 + \{\pi_0 + \omega_0/\alpha_0 + \omega_0^{1/2}(1 + \omega_0/\alpha_0^2)^{1/2}\} \times \{\exp(k_{eff} s_0 t(1 + \omega_0/\alpha_0^2)^{1/2}) - 1\};$$

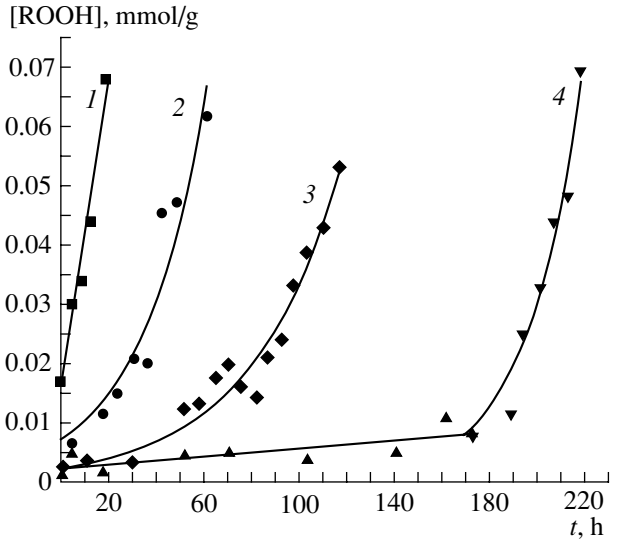

Fig. 1. Kinetics of peroxide accumulation upon the thermal autooxidation of (*3*) methyl oleate and its solutions with lipids from (*1*) spleens, (*2*) erythrocytes, and (*4*) livers of SHK mice (female).

(2) under the condition that $\omega_0 \ll \pi^2$, $\omega_0 \ll \pi_0^2$, $\pi \ll \alpha_0$:

$$\pi = \pi_0 + \pi_0\{\exp(k_{eff} s_0 t(1 + \omega_0/\alpha_0^2)^{1/2}) - 1\};$$

(3) for the zero initial conditions with respect to the concentration of peroxides $\pi_0 = 0$:

$$\pi = \{\omega_0/\alpha_0 + \omega_0^{1/2}(1 + \omega_0/\alpha_0^2)^{1/2}\} \times \{\exp(k_{eff} s_0 t(1 + \omega_0/\alpha_0^2)^{1/2}) - 1\},$$

(a) if $\alpha_0^2 \ll \omega_0$ $\pi = (2\omega_0/\alpha_0)(\exp(k_{eff} s_0 \omega_0^{1/2} t/\alpha_0) - 1)$,

(b) if $\omega_0 \ll \alpha_0^2$ $\pi = (\omega_0/\alpha_0 + \omega_0^{1/2})(\exp(k_{eff} s_0 t) - 1)$;

(4) in the particular case of $[RH] = [RH]_0 = s_0 = \text{const}$, the integration of Eq. (2) is considerably simplified, and π is determined from the equation

$$\pi + (\pi^2 + \omega_0)^{1/2} = (\pi_0 + (\pi_0^2 + \omega_0)^{1/2})\exp(k_{eff} s_0 t). \tag{3}$$

Two exact solutions can be obtained under the conditions

(a) $\pi^2 \ll \omega_0$, $\pi_0^2 \ll \omega_0$: $\pi = \pi_0 + (\pi_0 + \omega_0^{1/2})(\exp(k_{eff} s_0 t) - 1)$.

(b) $\omega_0 \ll \pi^2$, $\omega_0 \ll \pi_0^2$: $\pi = \pi_0 + \pi_0(\exp(k_{eff} s_0 t) - 1)$;

(5) if $[RH] = s_0 = \text{const}$ and $\pi_0 = 0$, Eq. (3) is simplified as $\pi + (\pi^2 + \omega_0)^{1/2} = \omega_0^{1/2}\exp(k_{eff} s_0 t)$ and is divided into two cases:

(a) if $\pi^2 \ll \omega_0$, then $\pi = \omega_0^{1/2}\exp(k_{eff} s_0 t) - 1)$,

(b) if $\omega_0 \ll \pi^2$, then $\pi = 0.5\,\omega_0^{1/2}\exp(k_{eff} s_0 t)$.

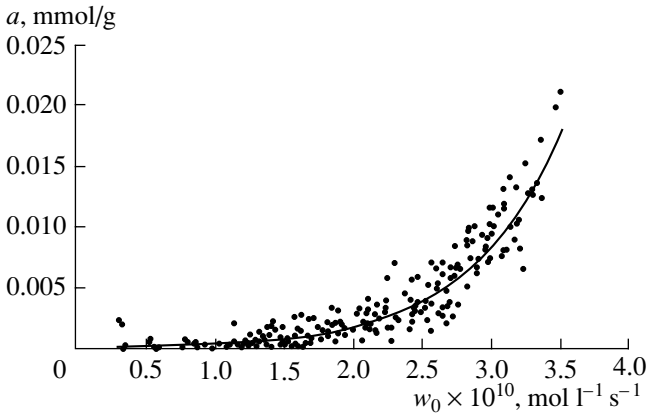

Fig. 2. Influence of the initiation rate of radicals w_0 on the preexponential factor a of the kinetic functions of peroxide accumulation upon methyl oleate thermal autooxidation (T = 37°C, n = 208).

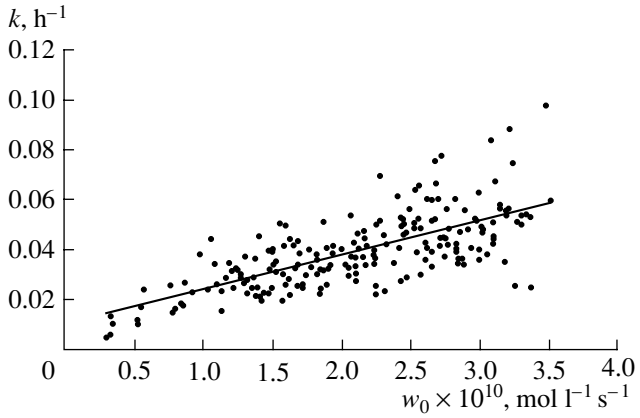

Fig. 3. Influence of the initiation rate of radicals w_0 on the exponent k of the kinetic functions of peroxide accumulation upon methyl oleate thermal autooxidation (T = 37°C, n = 208).

Note that case (5b) coincides with a known result [1, p. 121].

Thus, the analytical problem describing the regularities of the chain degenerate branched autooxidation of methyl oleate is solved, taking into account the nonzero initial conditions with respect to the concentration of peroxides and changes in the methyl oleate concentration for different amounts of peroxides. This solution makes it possible to explain the experimental plots of peroxide accumulation [ROOH] = $x = \pi s = x_0 + a(\exp(kt) - 1)$ [12] and the plots of the exponent k and preexponential factor a vs. w_0 (Figs. 3 and 2, respectively).

This analytical solution leads to the following conclusions:

1. The accumulation of peroxides with time is exponential, and the rate of radical initiation w_0 generally contributes to both the exponent k and preexponential factor $a = f(w_0)$ (Figs. 2 and 3). The function $f(w_0)$ that expresses the a value contains w_0 to the powers 1 and 1/2.

2. The exponent of the exponential function of peroxide accumulation vs. time is proportional to the effective reaction rate constant $k_{\text{eff}} = k_2(\alpha_3 k_{33}/k_6)^{1/2}$. The ratio $k_2/k_6^{1/2}$ enters only into the exponent, while the rate constant of degenerate branching $\alpha_3 k_{33}$ also contributes to the preexponential factor.

3. The exponent is proportional to the methyl oleate concentration s_0 only when it can be considered constant under the experimental conditions. In the simplest case when the linear relation s = [RH] = $s_0 - x/\alpha_0$ is taken into account, the exponent already contains the effective value $s_0(1 + \omega_0/\alpha_0^2)^{1/2}$, which is responsible for an increase in the exponent k with an increase in w_0 (Fig. 3).

4. The initial concentration of peroxides ($x_0 = \pi_0 s_0$) also contributes to the preexponential factor ($a = f(w_0, x_0)$), and, in some cases, it is precisely the high initial amount of peroxides that explains the prooxidant effect of solutions of lipids in methyl oleate (see below).

3. Characteristic Parameters and Critical Phenomena

Let us analyze the results obtained for the accumulation of peroxides in the steady-state approximation from the viewpoint of characteristic parameters and critical phenomena.

The analytical solutions to variants (1), (2), and (3) (see above) makes it possible to monitor the role of the dimensionless parameter $\omega_0 = w_0 s_0^{-2}(\alpha_3 k_{33})^{-1}$ and to determine its critical value $\omega_{0,\text{cr}} = 1$. If $\omega_0 > \omega_{0,\text{cr}}$, the exponent ($k_{\text{eff}} s_0 t$) stops to depend linearly on the initial methyl oleate concentration, and its value ($k_{\text{eff}} s_0 t(1 + \omega_0/\alpha_0^2)^{1/2}$) increases stepwise. That is, a decrease in the methyl oleate concentration cannot be neglected even in the initial period of autooxidation.

For variants (4) and (5), when a change in the methyl oleate concentration with a change in the peroxide concentration can be neglected ([RH] = s_0 = const, [ROOH] $\ll$ [RH]) for the dimensionless value $\pi = x/s_0$ and parameter $\gamma = s_0(\alpha_3 k_{33})^{1/2}$ under the condition that π_0, $\pi \ll \omega_0^{1/2}$, we obtain the following expression:

$$\pi = \pi_0 + (\pi_0 + w_0^{1/2}/\gamma)(\exp(\gamma k_2 k_6^{-1/2} t) - 1).$$

An increase in the parameter γ leads to an increase in the exponent and a decrease in the preexponential factor. A high initial concentration of peroxides ($\pi_0 \gg w_0^{1/2}$) results in the situation when an increase in the parameter γ ($\gamma \gg w_0^{1/2}$, which corresponds to $\omega_0 \ll 1$) only increases the exponent, and the w_0 value stops directly influencing an increase in the peroxide concen-

tration: a high concentration of oxides becomes responsible for an increase in the amount of free radicals due to the degenerate branching reaction

$$\pi = \pi_0 + \pi_0(\exp(\gamma k_2 k_6^{-1/2} t) - 1).$$

This transition is characterized by the critical value of the initial peroxide concentration: $\pi_{0,\,cr} = w_0^{1/2}$. Thus, a sufficiently high initial concentration of peroxides results in the transition of methyl oleate autooxidation to the regime of initiated oxidation.

4. Establishment of the Steady-State Concentration of Radicals

For the process of establishing a steady-state concentration of radicals in the simplest case when (1) there is no degenerate branching, (2) the rate of radical initiation (w_0) is constant, (3) the initial conditions with respect to the peroxide concentration are zero, and (4) the methyl oleate concentration is constant, [RH] = const = $[RH]_0$, the following system of equations was obtained and solved [1]:

$$\begin{gathered} d\xi/d\tau = 1 - \xi^2, \\ d\eta/d\tau = \xi, \\ \xi|_{\tau=0} = \eta|_{\tau=0} = 0, \end{gathered} \quad (4)$$

where $\tau = t/t_s$, $t_s = (k_6 w_0)^{-1/2}$, $\xi = [RO_2^{\cdot}]/[RO_2^{\cdot}]_{st}$, $[RO_2^{\cdot}]_{st} = (w_0/k_6)^{1/2}$, and $\eta = k_6[ROOH]/k_2[RH]$.

The solution to this system is as follows:

$$\xi = (e^{2\tau} - 1)/(e^{2\tau} + 1),$$

$$\eta = \ln(e^{2\tau} + 1) - \tau - \ln 2, \text{ and for } \tau \longrightarrow \infty$$
$$\eta \longrightarrow \tau - \ln 2;$$

for $\tau = 1$, $\xi = 0.76$. This shows the significance of the correlation of w_0 and k_6 for understanding whether or not the time of establishing the steady-state concentration of radicals can be ignored. For instance, if for $w_0 = 10^{-6}$ and $k_6 = 10^6$ the time of establishing the radical concentration $\xi = 0.76$ (t_s) is 1 s, then for other values $w_0 = 10^{-10}$ and $k_6 = 10^6$ this time is 100 s and, hence, cannot be neglected when considering the reaction kinetics.

Let us consider a more complex example of establishing the steady-state concentration of radicals for the complete system of reactions (I). In the general case, system of equations (4) transforms into (5)

$$\begin{gathered} d\xi/d\tau = (1 - \xi^2)(1 + x^2/\beta), \\ dx/d\tau = \xi(\alpha_0 s_0 - x)k_{eff} t_s (x^2 + \beta/\alpha_0)^{1/2} - \alpha_3 k_{33} t_s x^2, \\ \xi|_{\tau=0} = 0, \quad x|_{\tau=0} = x_0, \quad s|_{\tau=0} = s_0 - x/\alpha_0, \end{gathered} \quad (5)$$

where the designations $x = [ROOH]$, $s = [RH]$, and ξ, τ, and t_s are identical to those in Eq. (4), but the expression for the stationary radical concentration changed, $[RO_2^{\cdot}]_{st} = ((w_0 + \alpha_3 k_{33} x^2)/k_6)^{1/2}$, and the following parameters were introduced: $\beta = w_0/\alpha_3 k_{33}$ and $k_{eff} = k_2(\alpha_3 k_{33}/k_6)^{1/2}$.

System (5) cannot be solved analytically, but some approximations can be obtained.

The very first approximation to an exact solution at the zero initial conditions ($\xi_0 = x_0 = 0$), a constant rate of free radical formation (w_0), and a constant concentration of methyl oleate (s_0) can be obtained if one determines conditions under which the fraction of chain branching in the overall rate of free radical formation in the period of establishing their stationary concentration is low. The reaction can be considered as nonbranched chain when

$$\sqrt{w_0/k_6} > \alpha_3 k_{33} \int_0^{t_s} [ROOH]^2 dt.$$

After integration under the condition of a linear increase in the free radical concentration, we have

$$w_0 > \alpha_3 k_{33} k_2^2 [RH]^2/20 k_6.$$

The fulfillment of this correlation makes it possible to derive a simple formula for the oxidation kinetics, taking into account the establishment of the stationary radical concentration

$$dx/dt = d[ROOH]/dt = k_2[RH]_0[RO_2^{\cdot}],$$

$$y_{st} = [RO_2^{\cdot}]_{st} = ((w_0 + \alpha_3 k_{33} x^2)/k_6)^{1/2},$$

$$\xi = y/y_{st} = (e^{2\tau} - 1)/(e^{2\tau} + 1)$$
$$= (\exp(2t(w_0 k_6)^{1/2}) - 1)/(\exp(2t(w_0 k_6)^{-1/2}) + 1),$$

$$dx/dt = k_2[RH]_0((w_0 + \alpha_3 k_{33} x^2)/k_6)^{1/2}$$
$$\times (\exp(2t(w_0 k_6)^{1/2}) - 1)/(\exp(2t(w_0 k_6)^{1/2}).$$

After integration we have

$$x + (x^2 + \beta)^{1/2}$$
$$= \beta^{1/2}\exp((\ln(e^{2\tau} + 1) - \tau - \ln 2)k_{eff} s_0/(w_0 k_6)^{1/2}).$$

This equation resembles (5) for long oxidation times

$$x + (x^2 + \beta)^{1/2} = \beta^{1/2}\exp(k_{eff} s_0 t - k_{eff} s_0 \ln 2/(w_0 k_6)^{1/2}).$$

If $x^2 \ll \beta$, $x_0 = 0$: $x = \beta^{1/2}(\exp(k_{eff} s_0 t - k_{eff} s_0 \ln 2/(w_0 k_6)^{1/2}) - 1)$.

The lower the w_0 and k_6 values, the slower the establishment of the stationary radical concentration and the slower the accumulation of peroxides.

Let us try to obtain another analytical approximation for the case of slow peroxide accumulation taking into account the nonzero initial conditions with respect to the peroxide concentration and a change in the methyl oleate concentration. If $x^2 \ll \beta$ and $x_0^2 \ll \beta$, the variable in the set of equations (5) are separated to yield an exactly solvable equation in x:

$$dx/d\tau = (\alpha_0 s_0 - x)k_{eff} t_s (x^2 + \beta)^{1/2}(e^{2\tau} - 1)/(e^{2\tau} + 1)\alpha_0,$$

$$x|_{\tau=0} = x_0.$$

The solution of this equation is

$$x = x_0 + \{x_0 + \beta\alpha_0^{-1} s_0^{-1} + \beta^{1/2}(1 + \beta\alpha_0^{-2} s_0^{-2})^{1/2}\} \times \{\exp\{(\ln(e^{2\tau} + 1) - \tau - \ln 2)k_{\text{eff}} \times (s_0^2 + \beta\alpha_0^{-2})^{1/2}/(w_0 k_6)^{1/2}\} - 1\}.$$

In the general case, the set of equations (5) is numerically solvable if the rate constants are experimentally determined.

PARAMETERS OF THE KINETIC MODEL AND METHODS FOR THEIR DETERMINATION

Autooxidation of Methyl Oleate in the Presence of Ionol. Determination of w_0

In the experiment w_0 is determined by the inhibitor method using methyl oleate oxidation in the presence of ionol. Ionol (InH) is an efficient inhibitor, and it only reacts with free radicals and does not react with either methyl oleate or peroxides, which significantly simplifies analysis of this system of reactions

(II.0)	$2RH + O_2 \longrightarrow 2R^{\cdot} + H_2O_2$	radical initiation	
(II.1)	$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot}$	radical initiation	
(II.2)	$RH + RO_2^{\cdot} \longrightarrow ROOH + R^{\cdot}$	chain propagation	
(II.3)	$ROOH + ROOH \longrightarrow RO_2^{\cdot} + RO^{\cdot} + H_2O$	degenerate branching	(II)
(II.6)	$RO_2^{\cdot} + RO_2^{\cdot} \longrightarrow ROOR + O_2$	chain termination	
(II.7)	$InH + RO_2^{\cdot} \longrightarrow ROOH + In^{\cdot}$		
(II.8)	$In^{\cdot} + RO_2^{\cdot} \longrightarrow ROOH$ + molecular products.		

Using the steady-state approximation with respect to the concentrations of free radicals $In^{\cdot}$ ($[In^{\cdot}] = (k_7/k_8)[InH]$), we obtain the complete system of equations for methyl oleate autooxidation in the presence of ionol

$$\begin{aligned}
&(6.1)\ dx/dt = k_2 sy + 2k_7 iy - \alpha_3 k_{33} x^2,\\
&(6.2)\ dy/dt = w_0 - k_6 y^2 + \alpha_3 k_{33} x^2 - 2k_7 iy = 0,\\
&(6.3)\ x|_{t=0} = x_0,\quad y|_{t=0} = 0,\quad i|_{t=0} = i_0,\\
&(6.4)\ s = s_0 - x/\alpha_0,\\
&(6.5)\ di/dt = -k_7 iy,
\end{aligned} \tag{6}$$

where [InH] is designated through i, and the rate constants of reactions (II.7) and (II.8) are given as k_7 and k_8, respectively.

System (6) is considerably simplified in several limiting cases.

(A) When the inhibitor is very efficient and terminates all chains, the following system of equations is valid in the induction period:

$$\begin{aligned}
&(7.1)\ dx/dt = 2k_7 iy - \alpha_3 k_{33} x^2,\\
&(7.2)\ dy/dt = w_0 + \alpha_3 k_{33} x^2 - 2k_7 iy = 0,\\
&(7.3)\ -di/dt = k_7 iy,\\
&(7.4)\ x|_{t=0} = x_0,\ y|_{t=0} = 0,\ i|_{t=0} = i_0,
\end{aligned} \tag{7}$$

from which we have

$$y = (w_0 + \alpha_3 k_{33} x^2)/2k_7 i,$$

$$x = x_0 + w_0 t,$$

$$di/dt = (w_0 + \alpha_3 k_{33}(x_0 + w_0 t)^2)/2,$$

and the induction period τ_i corresponding to the time when ionol is entirely consumed in the reaction is expressed by the cubic equation

$$\alpha_3 k_{33}(w_0)^2 \tau_i^3/6 + \alpha_3 k_{33} w_0 \tau_i^2 + w_0 \tau_i/2 + \alpha_3 k_{33} x_0^2 - i_0 = 0, \tag{8}$$

which is simplified at $x_0 = 0$:

$$\alpha_3 k_{33}(w_0)^2 \tau_i^3/6 + w_0 \tau_i/2 - i_0 = 0. \tag{9}$$

If the value τ_i is experimentally known, then for $x_0 = 0$ w_0 can be determined as $w_0 = 3(1 + 8\alpha_3 k_{33}[InH]_0 \tau_i/3)^{1/2}/2\alpha_3 k_{33}\tau_i^2$.

Note that the relation of w_0 to τ_i would be different in the case of monomolecular degenerate branching with the rate constant k_3

$$w_0 = k_3[InH]_0/((1 + k_3\tau_i)^2 - 1).$$

(B) When an inhibitor is very efficient and, in addition, $\alpha_3 k_{33} \times [ROOH]^2 \ll w_0$ is valid in the inhibition period, that is, peroxides are accumulated very slowly, then

$$-di/dt = k_7 iy \cong w_0/2 \quad \text{and} \quad \tau_i = 2[InH]_0/w_0,$$

and the τ_i and w_0 values are related by the simple correlation $w_0 = 2[InH]_0/\tau_i$.

Note that the fulfillment of a linear relationship between τ_i and $[InH]_0$ does not always prove that $-di/dt = k_7 iy \cong w_0/2$ and $\tau_i = 2[InH]_0/w_0$. If the inhibitor terminates all chains, then the induction period must be calculated by formula (8) from variant (A). If $[InH]_0$ is low, we obtain a linear relationship $\tau_i \sim [InH]_0$, and when $[InH]_0$ is high, no linear relationship is observed: $\tau_i \sim [InH]_0^{1/3}$ (for monomolecular branching, $\tau_i \sim [InH]_0^{1/2}$).

(C) In the opposite case when w_0 is low and peroxides formed in the presence of an inhibitor become the main source of free radicals very rapidly, that is, when $w_0 \ll \alpha_3 k_{33}[ROOH]^2$ for most of the induction period, we obtain the system

$$\text{(10.1)}\ dx/dt = k_2 sy + 2k_7 iy - \alpha_3 k_{33} x^2,$$

$$\text{(10.2)}\ dy/dt = w_0 - k_6 y^2 + \alpha_3 k_{33} x^2 - 2k_7 iy = 0,$$

$$\text{(10.3)}\ -di/dt = k_7 iy, \qquad (10)$$

$$\text{(10.4)}\ s = s_0 - x/\alpha_0,$$

$$\text{(10.5)}\ x|_{t=0} = x_0,\ y|_{t=0} = 0, \quad i|_{t=0} = i_0.$$

Substituting the y value expressed by quadratic equation (10.2),

$$y = k_7 i k_6^{-1} (\{1 + (w_0 + \alpha_3 k_{33} x^2) k_6 / k_7^2 i^2\}^{1/2} - 1),$$

we obtain the system

$$\text{(11.1)}\ dx/dt = (k_2 s + 2k_7 i) k_7 i k_6^{-1}$$

$$\times (\{1 + (w_0 + \alpha_3 k_{33} x^2) k_6 / k_7^2 i^2\}^{1/2} - 1) - \alpha_3 k_{33} x^2,$$

$$\text{(11.2)}\ -di/dt = k_7^2 i^2 k_6^{-1} \qquad (11)$$

$$\times (\{1 + (w_0 + \alpha_3 k_{33} x^2) k_6 / k_7^2 i^2\}^{1/2} - 1),$$

$$\text{(11.3)}\ s = s_0 - x/\alpha_0, \quad x|_{t=0} = x_0, \quad y|_{t=0} = 0, \quad i|_{t=0} = i_0.$$

According to particular experimental conditions, one has to solve the system of equations (6) in the general case or use approximations (A), (B), and (C) and go to the simplified system of equations.

Determination of Parameters and Reaction-Rate Constants

Let us analyze data obtained in [10] for the autooxidation of methyl oleate and its autooxidation in the presence of ionol at 37°C. The experimental data were processed in [9] as follows: w_0 was calculated from the formula

$$w_0 = 2[InH]_0/(\tau_i - \tau_0),$$

where τ_i is the induction period of methyl oleate autooxidation in the presence of ionol (the time during which the peroxide concentration reached 0.02 mmol/g was taken as the induction period), and τ_0 is the induction period of methyl oleate autooxidation. A particular sample of methyl oleate contains a minor fraction of methyl linoleate, and the τ_0 values differ for different samples of methyl oleate. Based on the relation of $1/w_0^{1/2}$ to τ_0, we calculated w_0 from the formula $w_0^{-1/2} = (2.8 + 0.015\tau_0) \times 10^4$ [9].

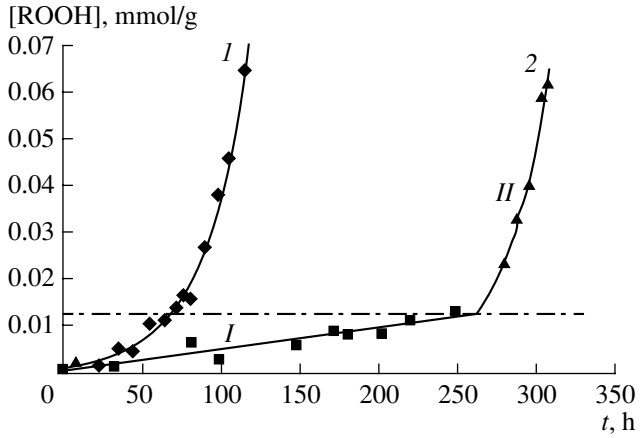

Fig. 4. Kinetic curves of peroxide accumulation upon the thermal autooxidation of (*1*) methyl oleate and (*2*) its solution in the presence of ionol (1.77×10^{-4} mol/l).

In this study, we propose to change the method for w_0 determination, according to the kinetic analysis performed, and to obtain the reaction parameters from the experimental data using the formulas obtained above.

For methyl oleate with a minor (3–5%) admixture of methyl linoleate, the autooxidation process can be described in the framework of "model methyl oleate" with elementary rate constants estimated from experimental data for chain propagation, degenerate branching, etc. The kinetics of peroxide accumulation during methyl oleate oxidation in the presence of minor amounts of methyl linoleate and the kinetics of single-substrate oxidation do not qualitatively differ. This makes it possible to choose the rate constants of elementary reactions in such a way that the peroxide accumulation curves during the oxidation of "model methyl oleate" would coincide with similar curves for the oxidation of two substrates.

To determine τ_i, w_0, and $\alpha_3 k_{33}$, let us use the experimental plots (Fig. 4) of the accumulation of peroxides vs. time $x(t) = [RH]\pi(t)$ for the autooxidation of model methyl oleate and its solutions in the presence of ionol ($x = [ROOH]$).

The induction period τ_i for methyl oleate autooxidation in the presence of ionol can be determined as described previously. However, τ_i can also be determined by the method of superposition of curves, because the relation $x(t)$ in the induction period is described by one function and, after the inhibitor was consumed, $x(t)$ should not differ (except for the shift along the time axis) from the autooxidation of methyl oleate itself, which is seen from Fig. 4 (curve *1*).

The numerical calculation of the $x(t)$ plot for curve *1* in Fig. 4 gives

$$x(t) = 0.0013\exp(0.0334t),$$

and for time interval II in curve *2* (Fig. 4) after the shift along the time axis to the coincidence with curve *1* in the upper part a similar function has the form

$$x(t) = 0.0013\exp(0.0343t).$$

Thus, these plots are identical within experimental and numerical approximation errors and, hence, by using the method of superposition of curves we obtain both the method for determination of the induction period τ_i and the experimental evidence that the products of methyl oleate autooxidation in the presence of ionol exert no effect on methyl oleate autooxidation after the inhibitor was completely consumed.

Based on the system of equations (7) for an efficient inhibitor terminating all chains and using the first part of I in curve *2* (Fig. 4), we determine w_0 for a given methyl oleate sample from the slope of curve *2*: $x(t) = x_0 + w_0t$.

If we know experimental values of τ_i, w_0, and $i_0 = [InH]_0$, we can compare the results of different calculation methods [9] and obtain a new result, namely, the rate constant α_3k_{33}, from Eq. (8), and when $x_0 = 0$ the α_3k_{33} value can be obtained from Eq. (9): $\alpha_3k_{33} = 6(i_0 - w_0\tau_i/2)(w_0)^{-2}\tau_i^{-3}$.

Using the experimentally determined values of the preexponential factor and the exponent (in this case, $a = x_0 = 0.0013$ mmol/g, $k = k_{eff}s_0 = 0.0334$ h^{-1}) for the autooxidation of methyl oleate itself from curve *1* (Fig. 4), one can additionally estimate the constant $k_{eff} = k_2(\alpha_3k_{33}/k_6)^{1/2}$ and isolate the term $k_2(k_6)^{-1/2}$.

Analysis of kinetics obtained in [10] for the autooxidation of methyl oleate at 37°C and its autooxidation in the presence of ionol leads to the following relationship:

$$x = x_0 + a(\exp(kt) - 1),$$

where $a = 0–2 \times 10^{-2}$ mmol/g, and $k = (0.05–1) \times 10^{-1}$ h^{-1}.

Considering all these experimental results and taking into account data on the initial concentration of peroxides, we can quantitatively explain the plots $a(w_0)$ and $k(w_0)$ in Figs. 2 and 3 and determine the rate constants α_3k_{33} and $k_2(k_6)^{-1/2}$, the value of k_{eff}, and the parameters of autooxidation w_0 and τ_i.

Influence of Lipids on the Parameters of the Methyl Oleate Model

Lipids and especially phospholipids in the composition of lipids are oxidation substrates. On the other hand, some components dissolved in lipids and antioxidants inhibit oxidation due to a decrease in the rate of radical initiation and a decrease in the overall oxidizability of the system [10]. Since the antioxidant effect of components in complex systems is not additive, both synergism and antagonism appear, whose scales range strongly depending on the ratio of concentrations of phospholipids and natural or synthetic antioxidants, the degree of unsaturation of lipids or physicochemical characteristics of antioxidants, and the initiation rate of oxidative processes; they also depend substantially on the duration of oxidation [18].

To study the influence of lipids on the parameters of the methyl oleate model, we have to take into account data on the reactions involving different components of lipids in a solution of methyl oleate (in our experiments, the concentration of lipids is at most 3%); that is, we will consider a solution of methyl oleate with lipids as a complex multicomponent system. To systematize the influence of different characteristics of lipids (LH) on the antioxidant properties, let us write the system of reactions

(III.0)	$2RH + O_2 \longrightarrow 2R^{\bullet} + H_2O_2$	radical initiation	
(III.1)	$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet}$	radical initiation	
(III.2)	$RH + RO_2^{\bullet} \longrightarrow ROOH + R^{\bullet}$	chain termination	
(III.3)	$ROOH + ROOH \longrightarrow RO_2^{\bullet} + RO^{\bullet} + H_2O$	degenerate branching	
(III.6)	$RO_2^{\bullet} + RO_2^{\bullet} \longrightarrow ROOR + O_2$	chain termination	
(III.7)	$LH + RO_2^{\bullet} \longrightarrow ROOH + L^{\bullet}$	chain propagation	(III)
(III.8)	$L^{\bullet} + RO_2^{\bullet} \longrightarrow ROOL$		
(III.9)	$L^{\bullet} + O_2 \longrightarrow LO_2^{\bullet}$	involvement of lipids	
(III.10)	$LH + O_2 \longrightarrow LO_2^{\bullet} + H_2O_2$	in radical formation,	
(III.11)	$RH + LO_2^{\bullet} \longrightarrow LOOH + L^{\bullet}$	chain propagation, and *APA* of lipids	
(III.12)	$LH + ROOH \longrightarrow$ molecular products.		

Intermediate radicals $L^{\cdot}$, like $R^{\cdot}$, react rapidly and disappear, and radicals $RO_2^{\cdot}$ and $LO_2^{\cdot}$ are also very active. Therefore, we can state the fast establishment of a steady-state concentration of these radicals.

Let us take into account the contributions of different reactions of lipids (III.7)–(III.12) to the kinetics of methyl oleate autooxidation under the steady-state conditions.

The concentrations of radicals in the system will be designated as $y = [RO_2^{\cdot}]$, $n = [LO_2^{\cdot}]$, and $z = [L^{\cdot}]$. Using the steady-state approximation with respect to the concentrations of free radicals ($dn/dt = dy/dt = dz/dt = 0$), we obtain the complete set of equations for the autooxidation of solutions of methyl oleate with lipids

$$(12.1)\ dx/dt = k_2 sy + y\Sigma k_7^m l^m + s\Sigma k_{11}^p n^p - x\Sigma k_{12}^r l^r - \alpha_3 k_{33} x^2,$$

$$(12.2)\ dl/dt = -y\Sigma k_7^m l^m - [O_2]\Sigma k_{10}^f l^f - x\Sigma k_{12}^r l^r,$$

$$(12.3)\ dy/dt = w_0 - k_6 y^2 + \alpha_3 k_{33} x^2 - y\Sigma k_7^m l^m - y\Sigma k_8^i z^i = 0, \qquad (12)$$

$$(12.4)\ dn/dt = [O_2]\Sigma k_{10}^f l^f - s\Sigma k_{11}^p n^p + [O_2]\Sigma k_9^g z^g = 0,$$

$$(12.5)\ dz/dt = y\Sigma k_7^m l^m + s\Sigma k_{11}^p n^p - k_8 yz - [O_2]\Sigma k_9^g z^g = 0,$$

$$(12.6)\ x|_{t=0} = x_0, \quad n|_{t=0} = y|_{t=0} = z|_{t=0} = 0, \quad l|_{t=0} = l_0, \quad s|_{t=0} = s_0,$$

$$(12.7)\ s = s_0 - x/\alpha_0,$$

where the overall concentrations of all components of lipids and all peroxides are $l = [LH]$ and $x = [LOOH] + [ROOH]$, respectively; and the concentration of methyl oleate is $s = [RH]$. Particular components of lipids and their free radicals involved in each reaction are numbered by superscripts (the concentration of the mth component in lipids involved in reaction (III.7) is l^m and that involved in reaction (III.10) is l^f, etc.); the sign Σ implies summing over the components of lipids involved in reactions with the corresponding rate constants k_i^m, the initial overall concentrations of lipids and peroxides are l_0 and x_0, respectively; the initial concentration of methyl oleate is s_0; and the rate constants of reactions (III.7)–(III.12) are k_7 and k_{12}, respectively.

Quantitative analysis of the set of equations (12) will be discussed elsewhere. However, if the solution to this set is presented in the exponential form by analogy with the solutions to the set of equations considered above, it becomes evident that different components of lipids and free radicals formed in the reactions make both positive and negative contributions to both the preexponential factor and the exponent.

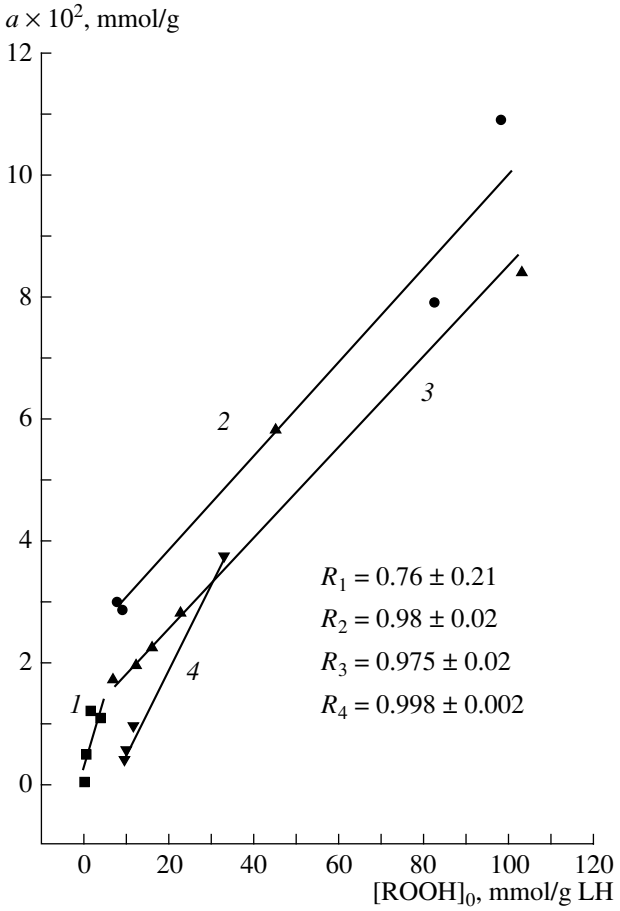

Fig. 5. Preexponential factor a of the kinetic curve of peroxide accumulation upon the oxidation of solutions of spleen lipids in methyl oleate as a function of the initial amount of peroxides in spleen lipids from (*1*) outbred rats, (*2*) mice of the CBA line, and (*3*) SHK mice at $w_0 = 1.57 \times 10^{-10}$ mol l^{-1} s^{-1} and (*4*) $w_0 = 1.18 \times 10^{-10}$ mol l^{-1} s^{-1}.

Prooxidant Effect Caused by a High Initial Concentration of Peroxides

Let us consider a particular case related to the critical value of the initial concentration of peroxides. This case was considered in detail using the methyl oleate model, and these calculations allowed us to assume that similar regularities are valid even for a more complex system. We examined the experimental curves of peroxide accumulation upon the oxidation of methyl oleate solutions with lipids from spleen (e.g., Fig. 1, curve *1*) with high initial concentrations of peroxides. These data are well approximated by the relation $[ROOH] = [ROOH]_0 + a(\exp(kt) - 1)$ with the correlation coefficient $R > 0.98$, and the plot of the preexponential factor a vs. initial concentration of peroxides $[ROOH]_0$ is linear (Fig. 5), like that for methyl oleate. This confirms the assumption that lipids are involved in radical initiation in reactions (III.9) and (III.10) and in chain propagation reaction (III.11).

In more complex cases, the involvement of lipids, which were isolated from other sources and possess different kinetic characteristics, in the low-temperature autooxidation of methyl oleate should be characterized by contributions from particular components on the basis of the system of equations (12).

CONCLUSIONS

Thus, for the low-temperature autooxidation of both methyl oleate and its solutions with lipids, the degenerate branching step is a bimolecular reaction. Analysis of the kinetics of methyl oleate autooxidation at 37°C taking into account the bimolecular mechanism of the degenerate branched reaction and the nonzero initial conditions substantially increases the applicability of the methyl oleate oxidation model for the estimation of both the antioxidant properties and kinetic characteristics of lipids as oxidation substrates.

In the case of a high degree of unsaturation of lipids resulting in the high concentrations of peroxides in them, the lipids manifest the prooxidant properties in the methyl oleate oxidation model due to their involvement in radical initiation and chain termination. When the initial concentration of peroxides exceeds a critical value, methyl oleate is oxidized in the regime of initiated oxidation, because radicals are initiated mostly due to the decomposition of peroxides. This effect is also observed for the autooxidation of complex systems, which was confirmed experimentally: direct correlation was observed between the preexponential factor of the curves of peroxide accumulation upon the oxidation of methyl oleate solutions with spleen lipids and the initial content of peroxides in the lipids.

The use of the method of superposition of curves for the comparative analysis of curves of peroxide accumulation upon the low-temperature autooxidation of methyl oleate and its solutions with ionol makes it possible to determine the induction period and radical initiation rates more exactly. This method is promising, from our point of view, for application in comparative analysis of the kinetics of the autooxidation of methyl oleate and its solutions with lipids or individual biologically active compounds, because it allows one to quantitatively estimate the involvement of lipids in radical initiation and determine both the effective rate constant of their reaction with peroxy radicals and the antiradical activity of lipids or other compounds in low-temperature autooxidation from experimental data.

Since lipids both are substrates of oxidation and contain some components inhibiting oxidation, including antioxidants extracted from biological objects, it is necessary to take into account the contributions from all these components to the reactions occurring in a solution of methyl oleate in the presence of even minor concentrations of lipids (at most 3%). The antioxidant effect of components in complex systems is not additive; hence, the antioxidant properties of complex systems should be efficiently estimated and predicted from their composition. The methyl oleate model provides a possibility of such a systematic approach (experiments with solutions with different compositions and kinetic analysis of contributions from components of a solution of methyl oleate with lipids) to estimate the antioxidant properties of components of complex systems by analysis of contributions from different components to the kinetics of methyl oleate autooxidation.

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