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KINETICS AND MECHANISM OF CHEMICAL REACTIONS. CATALYSIS

Kinetic Isotope H/D Effect in the Oxidation of Ethers of Linoleic Acid in Solutions

I. V. Tikhonov[a], I. V. Moskalenko[a], E. M. Pliss[a, *], M. A. Fomich[b], A. V. Bekish[c], and V. V. Shmanai[b]

[a] *Demidov Yaroslavl University, Yaroslavl, 150000 Russia*

[b] *Institute of Physical Organic Chemistry, National Academy of Sciences of Belarus, Minsk, 220072 Belarus*

[c] *Belarus State University, Minsk, 220030 Belarus*

**e-mail: e.pliss@mail.ru*

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Abstract—It is shown that the initiated oxidation of deuterated esters of linoleic acid proceeds as a radical chain process. The value of the kinetic isotope effect for hydrogen atom abstraction from the bisallyl group of linoleic acid esters is determined: 3.0 for *tert*-butyl peroxy and cumylperoxy radicals.

Keywords: kinetic isotope effect, oxidation, polyunsaturated fatty acids, hydrogen atom abstraction

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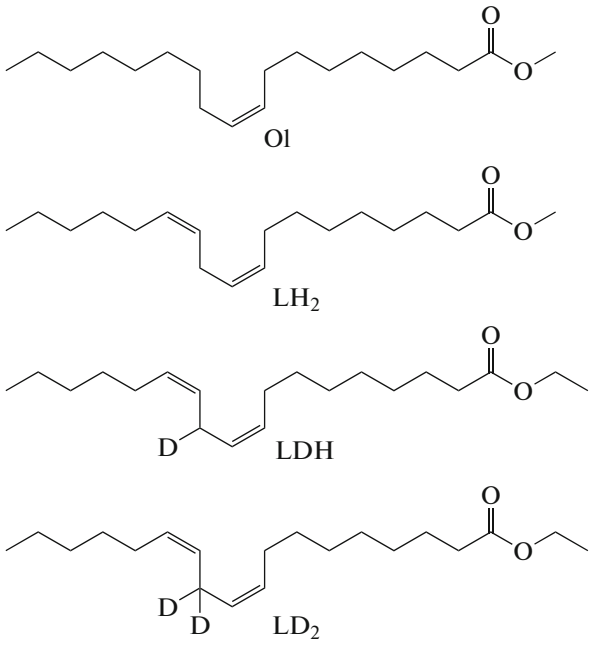
Polyunsaturated fatty acids (PUFAs) of the arachidonic family are well known as highly important components for the normal functioning of living organisms. However, these components are susceptible to oxidation accompanied by the generation of active forms of oxygen. The latter is directly related to oxidative stress, which disrupts the normal functioning of the cell and provokes numerous dangerous consequences (DNA damage, cancer, Alzheimer's and Parkinson's diseases, heart failure and atherosclerosis) [1–7]. The goal of many efforts to study the chemical mechanism of the oxidation of PUFAs is to find means to control or, at least, inhibit their oxidative metabolism and to prevent dangerous medical consequences.

The main reaction sites in the oxidation of PUFAs are bisallyl CH_2 groups. The C–H bond energy at the bisallylic position in the PUFA molecules is low, only 315 kJ/mol, as compared to 364 kJ/mol for allylic fragments [8]. Replacing hydrogen atoms by deuterium at the bisallylic position leads to a sharp decrease in the rate of many reactions [9], for example, the reaction of catalytic oxidation of PUFAs with soy lipoxygenase [10–12] and the abstraction of the hydrogen atom from PUFAs by the α-tocopherol radical [13]. Moreover, the introduction of PUFAs deuterated at the bisallylic position into cell membranes [14] and mitochondria [15, 16] protects the cell against oxidative stress. Note that, although many experimental [10, 12, 17] and theoretical works [18–20] have been devoted to studying the enzymatic oxidation of PUFAs, there are only some contradictory publications on the oxidation of deuterated PUFAs in organic solutions at 310 K [16, 21]. The current situation requires further research in this direction, which is the subject of the present work.

EXPERIMENTAL

Methyl oleate (Ol, 99%, Sigma-Aldrich) and methyl linoleate (LH_2, 99%, Sigma-Aldrich) were used without further purification. The compounds 11-D-ethyl linoleate (LDH) and 11,11-D_2-ethyl linoleate (LD_2) were synthesized according as described in [13, 22]:



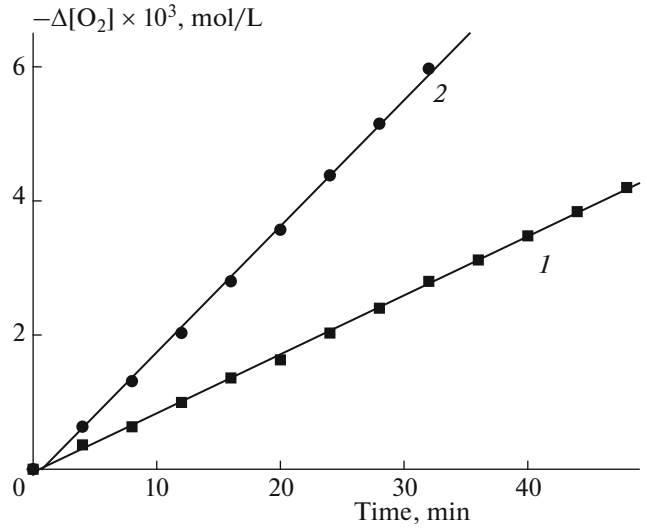


Fig. 1. Kinetic curves for oxidation in air; $[LD_2] = 1$ mol/L, $W_i = (1)\ 9.5 \times 10^{-8}$ and $(2)\ 3.8 \times 10^{-7}$ mol/(L s).

The initiator was 2,2-azobis(2,4-dimethyl-valeronitrile) (AMVN, Polyscience Inc.), and chlorobenzene (Reactive) was the solvent. *Tert*-butyl hydroperoxide (TBHP) and cumyl hydroperoxide (CHP) were purchased from Sigma-Aldrich. The purity of the oxidation substrates was monitored chromatographically with a Flexar high-performance liquid chromatograph (PerkinElmer, USA) and a Clarus 680T MS gas chromatography-mass spectrometer (PerkinElmer, USA). The content of basic substance in the oxidation substrates was not less than 99%. The kinetics of oxygen uptake was monitored using a capillary microvolumeter, as in [23]. The rate constants for the abstraction of hydrogen and deuterium atoms by peroxy radicals (k_2') were determined by the Howard–Ingold method [24]. All the experiments were carried out under conditions of saturation of the samples with air at a temperature of 310 K.

RESULTS AND DISCUSSION

The mechanism of the oxidation of the PUFAs in organic solutions is widely known [25–27], being described by Scheme 1.

Table 1. Dependence of W_{O_2} on W_i and $[LD_2]$

$[LD_2]$, mol/L	$W_i \times 10^7$, mol/(L s)	$W_{O_2} \times 10^6$, mol/(L s) (air)	$W_{O_2} \times 10^6$, mol/(L s) (oxygen)
1.0	1.0	1.5	1.5
1.0	4.0	3.0	3.0
0.5	4.0	1.5	–

$$\text{(i)}\quad \text{AMVN} \xrightarrow{+O_2,\ +LH_2} LH^{\bullet} \qquad (W_i),$$
$$\text{(1)}\quad LH^{\bullet} + O_2 \rightarrow LHO_2^{\bullet} \qquad (k_1),$$
$$\text{(2)}\quad LHO_2^{\bullet} + LH_2 \rightarrow LH^{\bullet} + LHOOH \qquad (k_2),$$
$$\text{(3)}\quad LHO_2^{\bullet} + LHO_2^{\bullet} \rightarrow \text{Products} \qquad (2k_3).$$

Scheme 1

The rate of the chain oxidation of the PUFAs is described by the equation [28]

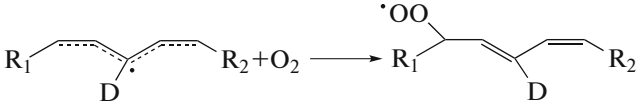
$$W_{O_2} = k_2(2k_3)^{-0.5}[LH_2]W_i^{0.5}. \qquad (1)$$

The oxidation of LD_2 in the presence of the initiator proceeds in the chain-reaction mode. This is evidenced by the following results: the kinetic curves of oxygen consumption are linear (Fig. 1); the oxidation rate is directly proportional to the substrate concentration and the square root of W_i, but is independent of the oxygen concentration (Table 1), which fully agrees with Eq. (1). Thus, the mechanism of LD_2 oxidation is described by Scheme 1 (with the replacement of LH_2 by LD_2). The ratio $k_2/(2k_3)^{0.5}$ for LD_2 calculated from the above results is 4.7×10^{-3} $(L/(mol\ s))^{0.5}$, which is 4.6 times lower than the analogous value for LH_2 $(2.2 \times 10^{-2}$ $(L/(mol\ s))^{0.5})$. The value of $k_2/(2k_3)^{0.5}$ for LDH is 9.5×10^{-3} $(L/(mol\ s))^{0.5}$, i.e., noticeably lower than the half sum of the values for LD_2 and LH_2. Thus, the oxidation rate is not an additive function of the number of deuterium atoms in the molecule. The oxidation of LDH proceeds through a more complex mechanism and, given nonequivalence of the hydrogen atoms, should be a co-oxidation process (Scheme 2).

$$\text{(i.1)}\quad \text{AMVN} \xrightarrow{+O_2,\ +LDH} LD^{\bullet} \qquad (W_i),$$
$$\text{(i.2)}\quad \text{AMVN} \xrightarrow{+O_2,\ +LDH} LH^{\bullet} \qquad (W_i),$$
$$\text{(1.1)}\quad LH^{\bullet} + O_2 \rightarrow LHO_2^{\bullet} \qquad (k_{1.1}),$$
$$\text{(1.2)}\quad LD^{\bullet} + O_2 \rightarrow LDO_2^{\bullet} \qquad (k_{1.2}),$$
$$\text{(2.1)}\quad LDO_2^{\bullet} + LDH \rightarrow LD^{\bullet} + LDOOH \qquad (k_{2.1}),$$
$$\text{(2.2)}\quad LDO_2^{\bullet} + LDH \rightarrow LH^{\bullet} + LDOOD \qquad (k_{2.2}),$$
$$\text{(2.3)}\quad LHO_2^{\bullet} + LDH \rightarrow LD^{\bullet} + LHOOH \qquad (k_{2.3}),$$
$$\text{(2.4)}\quad LHO_2^{\bullet} + LDH \rightarrow LH^{\bullet} + LHOOD \qquad (k_{2.4}),$$
$$\text{(3.1)}\quad LHO_2^{\bullet} + LHO_2^{\bullet} \rightarrow \text{Products} \qquad (2k_{3.1}),$$
$$\text{(3.2)}\quad LHO_2^{\bullet} + LDO_2^{\bullet} \rightarrow \text{Products} \qquad (2k_{3.2}),$$
$$\text{(3.3)}\quad LDO_2^{\bullet} + LDO_2^{\bullet} \rightarrow \text{Products} \qquad (2k_{3.3}).$$

Scheme 2

This scheme includes a number of reactions for which the contribution of the secondary isotopic effect, although insignificant, is possible [29]. For example, the reactions of oxygen addition:



On the other hand, the rates of reactions (3.1)–(3.3) should not differ significantly due to the secondary isotopic effect, since there are five other atoms between the deuterium atom and the reaction site. A similar conclusion can be drawn for pairs of reactions (2.1), (2.3) and (2.2), (2.4).

It was interesting to investigate the kinetics of the oxidation of LD_2 (LDH)–LH_2 mixtures, especially in the light of information on a significant inhibition of oxidation upon addition of small amounts of deuterated PUFAs under oxidation conditions in cell membranes [14]. Figure 2 displays the dependence of the rate of the oxidation of PUFA mixtures on their composition. As can be seen, the oxidation rate is not an additive function of the mixture composition. At the same time, it is hardly possible to claim a significant inhibition of the oxidation of LH_2 with small amounts of deuterated PUFAs. In the first approximation, the co-oxidation of LD_2 and LH_2 is described by Scheme 3.

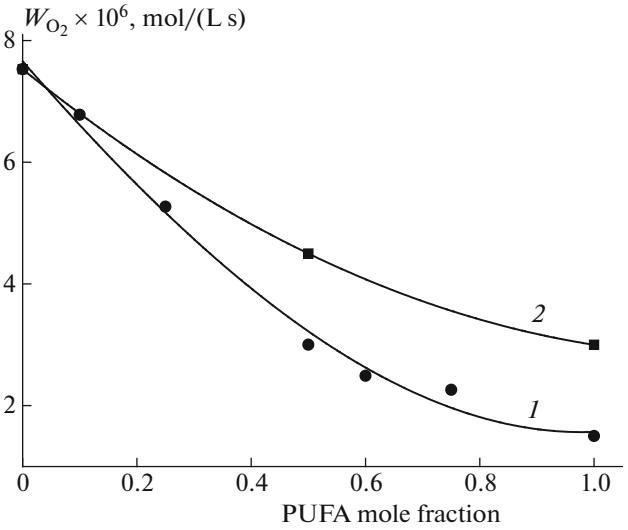


Fig. 2. Dependence of the rate of the oxidation of (*1*) LH_2 + LD_2 and (*2*) LH_2 + LDH mixtures on their composition. The total concentration of PUFA esters is 1.0 mol/L, $W_i = 1 \times 10^{-7}$ mol/(L s).

$$
\begin{aligned}
&(\text{i.1})\quad \mathrm{AMVN} \xrightarrow{+O_2,\,+LD_2} LD^{\bullet} && (W_i),\\
&(\text{i.2})\quad \mathrm{AMVN} \xrightarrow{+O_2,\,+LH_2} LH^{\bullet} && (W_i),\\
&(1.1)\quad LH^{\bullet} + O_2 \rightarrow LHO_2^{\bullet} && (k_{1.1}),\\
&(1.2)\quad LD^{\bullet} + O_2 \rightarrow LDO_2^{\bullet} && (k_{1.2}),\\
&(2.1)\quad LDO_2^{\bullet} + LD_2 \rightarrow LD^{\bullet} + LDOOD && (k_{2.1}),\\
&(2.2)\quad LDO_2^{\bullet} + LH_2 \rightarrow LH^{\bullet} + LDOOH && (k_{2.2}),\\
&(2.3)\quad LHO_2^{\bullet} + LD_2 \rightarrow LH^{\bullet} + LHOOD && (k_{2.3}),\\
&(2.4)\quad LHO_2^{\bullet} + LH_2 \rightarrow LD^{\bullet} + LHOOH && (k_{2.4}),\\
&(3.1)\quad LHO_2^{\bullet} + LHO_2^{\bullet} \rightarrow \text{Products} && (2k_{3.1}),\\
&(3.2)\quad LHO_2^{\bullet} + LDO_2^{\bullet} \rightarrow \text{Products} && (2k_{3.2}),\\
&(3.3)\quad LDO_2^{\bullet} + LDO_2^{\bullet} \rightarrow \text{Products} && (2k_{3.3}).
\end{aligned}
$$

Scheme 3

It is noteworthy that the rate of the oxidation of individual LDH is almost equal to the oxidation rate of an equimolar mixture of LD_2 and LH_2. Consequently, in both cases, co-oxidation takes place, proceeding through identical mechanisms with similar rate constants. In this case, Schemes 2 and 3 are actually the same. Given the expected similar reactivity of the $LHO_2^{\bullet}$ and $LDO_2^{\bullet}$ radicals the nonadditivity of the oxidation rate of the mixture is eigenvalues of a more complex mechanism of the oxidation of these substrates. In particular, the participation of allylic fragments in the oxidation process is possible. The contribution of this reaction can be quite significant in the case of deuterated substrates. Taking into account these reactions makes the kinetic scheme much more complicated, so that it becomes impossible to determine the rate constants of elementary stages from the results of kinetic experiments. At the same time, the reactivity of C–H and C–D bonds with respect to the same radical, for example $(CH_3)_3COO^{\bullet}$ or $Ph(CH_3)_2COO^{\bullet}$ can turned out to be a more suitable parameter for estimating the kinetic isotopic effect (CIE) under radical oxidation conditions. It becomes possible to take into account the abstraction of hydrogen atoms from the allyl groups of the linoleates (the corresponding rate constants can be set equal to the rate constants for methyl oleate).

The rate constants for hydrogen atom abstraction from PUFA by these peroxy radicals (k_2') were determined using the Howard–Ingold method [24]. The mechanism of the initiated oxidation of a mixture of LH_2 and ROOH is given below.

$$
\begin{aligned}
&(\text{i})\quad \mathrm{AMVN} \xrightarrow{+O_2,\,+LH_2} LH^{\bullet} && (W_i),\\
&(1)\quad LH^{\bullet} + O_2 \rightarrow LHO_2^{\bullet} && (k_1),\\
&(2)\quad LHO_2^{\bullet} + LH_2 \rightarrow LH^{\bullet} + LHOOH && (k_2),\\
&(\text{exchange})\quad LHO_2^{\bullet} + ROOH \rightarrow RO_2^{\bullet} + LHOOH && (k_e),\\
&(2')\quad RO_2^{\bullet} + LH_2 \rightarrow LH^{\bullet} + ROOH && (k_2'),\\
&(3')\quad RO_2^{\bullet} + RO_2^{\bullet} \rightarrow \text{Products} && (2k_3').
\end{aligned}
$$

Scheme 4

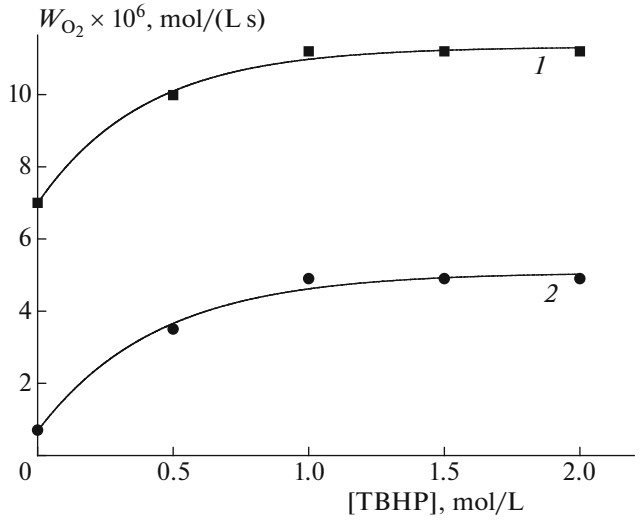


Fig. 3. Dependence of the rate of the oxidation of (*1*) LH_2 and (*2*) Ol on the TBHP; [Substrate] = 1 mol/L, $W_i = 1 \times 10^{-7}$ mol/(L s).

At a sufficiently high concentration of ROOH, practically all radicals of the substrate ($LHO_2^{\bullet}$) are exchanged for $RO_2^{\bullet}$, formed from the hydroperoxide, which are radicals that propagate the chain. In this case, the reaction rate is

$$W_{O_2} = k_2'(2k_3')^{-0.5}[LH_2]W_i^{0.5}, \qquad (2)$$

which makes it possible to determine $k_2'(2k_3')^{-0.5}$. At a known value of k_3', we calculated the rate constant k_2' for the abstraction of a hydrogen atom from PUFA. The ROOH concentration was selected so as to ensure that equation (2) holds, the criterion of which is the oxidation rate taking a constant value. Figure 3 shows typical dependencies of the rates of LH_2 and Ol oxidation on the ROOH concentration.

Table 2. Values of k_2' for the tested substrates prepared by the hydroperoxide method

Substrate	k_2', L/(mol s)	
	TBHP*	CHP**
Ol	2.19	2.03
LH_2	5.01	11.15
LD_2	3.13	5.07
LDH	3.94	8.10

* $k_6' = 2 \times 10^4$ L/(mol s) [31];
** $k_6' = 1.7 \times 10^5$ L/(mol s) [31].

Table 3. Rate constant for hydrogen (deuterium) abstraction from individual groups of the PUFAs

Hydroperoxide	k_{allyl}', L/(mol s)	K_H', L/(mol s)	k_D', L/(mol s)	KIE
TBHP	0.55	1.41	0.47	3.0
CHP	0.50	4.56	1.52	3.0

In view of additivity [30], the values of k_2' for individual substrates must be

$$\text{for Ol} \quad k_2' = 4k_{allyl}',$$
$$\text{for } LH_2 \quad k_2' = 4k_{allyl}' + 2k_H',$$
$$\text{for LDH} \quad k_2' = 4k_{allyl}' + k_H' + k_D',$$
$$\text{for } LD_2 \quad k_2' = 4k_{allyl}' + 2k_D',$$

where k_{allyl}' is the rate constant for hydrogen atom abstraction from the allyl group, k_H' is the rate constant for hydrogen atom abstraction from the bisallyl group, and k_D' is the rate constant for deuterium atom abstraction from the bisallyl group (all constants are calculated per hydrogen atom).

The measured values of k_2' are listed in Table 2, while the calculated rate constants for hydrogen atom abstraction from individual groups in the PUFA molecules calculated from them, in Table 3. It should be noted that the values calculated for LDH by using the additive scheme (4.07 L/(mol s) for TBHP and 8.27 L/(mol s) for CHP) are in good agreement with the experimental values.

In [30], for the process under consideration, the KIE was reported to be 6.0–6.8 (203–243 K). At the same time, extrapolation of this value to 310 K according to the Arrhenius equation leads to KIE = 4.9, which is somewhat higher than the value obtained in the present work, 3.0. However, these values are of the same order of magnitude and are much lower than the values KIE for enzymatic oxidation [9–12] and in those cases when the chain is propagated by some other radical (for example, the α-tocopherol radical [13]).

CONCLUSIONS

In general, the KIE for the non-enzymatic oxidation of PUFAs is much lower than that for enzymatic oxidation. In addition, in contrast to enzymatic oxidation, the value of KIE for non-enzymatic oxidation is temperature dependent, which indicates the absence of the tunneling effect in the chain propagation reactions. The oxidation of deuterated PUFAs itself is the co-oxidation of deuterated bisallylic sites and of non-deuterated allyl groups.

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