

Kinetic Simulation of Methane and Ethane Hydroxylation by Methane Monooxygenase

E. I. Karasevich*, Yu. K. Karasevich**, A. F. Shestakov***, and A. E. Shilov*

* Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, 119991 Russia

** Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, 119991 Russia

*** Institute of Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Moscow oblast, 142432 Russia

Received July 22, 2002

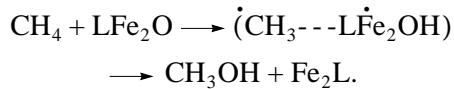
Abstract—According to the mechanism of alkane hydroxylation, whose main postulate is the formation of an intermediate complex containing pentacoordinated carbon, the hydroxylation of methane and ethane by methane monooxygenase was kinetically simulated by the numerical method. The published data on the kinetic isotope effects of oxidation of deuterium-substituted methane molecules (CHD_3 , CH_2D_2 , and CH_3D) and the distribution of products of chiral ethane (*R*- and *S*-MeCHDT) oxidation by methane monooxygenase were examined. The kinetic models proposed for the oxidation of isotopically substituted methane and ethane are in good agreement with experimental data.

INTRODUCTION

The enzyme of methanotrophic bacteria, methane monooxygenase (MMO) [1], catalyzes one of the most remarkable chemical reactions, namely, the oxidation of methane by molecular oxygen under mild conditions according to the equation



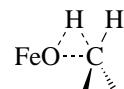
The active site of MMO contains a binuclear iron complex LFe_2O (Fig. 1). This enzyme can exist in both membrane-bound and cytoplasmic (soluble) forms. In addition to methane, the enzymatic system of MMO can oxidize many other hydrocarbons [2], for example, ethane. The hydroxylation of chiral (^1H , ^2H , ^3H) ethane is sometimes characterized by the complete retention of a configuration [3], and in some cases its partial inversion is observed [4]. The experimental observation of the racemization of the oxidation products has been seen by now as a convincing argument for the oxygen rebound mechanism [5]



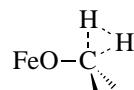
However, this mechanism disagrees with experimental data on the oxidation of deuterium-substituted methanes (CH_3D , CH_2D_2 , and CHD_3) by the enzymatic system of MMO from *Methylosinus trichosporium* [6]. The kinetic isotope effect (KIE) calculated from the ratio of the reaction products taking into account the numbers of C–D and C–H bonds in a methane molecule substantially increases with an increase in the number of deuterium atoms. This nontrivial result indicates that the mechanism of reaction of an MMO active site with

a hydrocarbon substrate is not reduced to the simple interaction with one H (or D) atom. A nonradical mechanism has been proposed [7] for the oxidation of hydrocarbons by natural monooxygenases and their chemical models. A simple kinetic model of this mechanism is in good agreement with all experimental data obtained for hydrocarbons with one C–H and one C–D bond at the oxidized carbon atom [8]. Racemization is assumed to occur due to the coexistence of two forms of intermediate complex containing pentacoordinated carbon, namely,

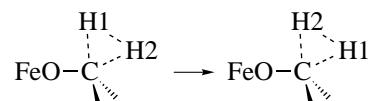
species F1



and species FII



Hydrogen atoms in FII can readily exchange their positions:



If one C–H or C–D bond reacts with the active intermediate of the MMO catalytic cycle, the first step is the formation of F1. If two C–H (C–D) bonds are involved simultaneously, the first step is the formation of FII. The insertion of an oxygen atom into the C–H bond upon complex formation results in the formation of a hydroxylation product. The kinetic schemes of this mechanism were considered in [9]. Analysis showed that simple schemes involving one or two hydrogen

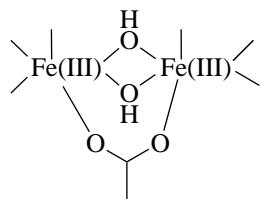


Fig. 1. Binuclear structure of the active site of MMO.

atoms in the first step explain an increase in the KIE values per one C–H bond with an increase in the number of deuterium atoms in a methane molecule. However, the values of the distribution of products of partially deuterated methane oxidation, which coincide with experimental values within the experimental error, cannot be obtained in the framework of these schemes. More complicated kinetic schemes should be considered to eliminate this contradiction. The purpose of this work is to perform the numerical kinetic simulation of the oxidation of isotopically labeled substrates, namely methane (CH_3D , CH_2D_2 , and CHD_3) and ethane (*R*-MeCHDT and *S*-MeCHDT), in the framework of a mechanism including the formation of an intermediate containing the formation of an intermediate pentacoordinated carbon.

CALCULATION PROCEDURE

To elucidate the dynamics of methane and ethane oxidation by MMO, calculations were carried out using specially developed algorithms. The problem requires determining the rate constants of the process steps and, hence, is kinetic parameter estimation. Since only the ratios of the oxidation products are known, only the ratios of model parameters rather than their absolute values are significant for mathematical description. The kinetic schemes of methane and ethane oxidation were calculated by the numerical method using a standard program for kinetic parameter estimation adapted to the case under consideration. The ratios of model parameters were varied. Of all the solutions obtained for different kinetic parameters, we chose those corresponding within the experimental error to the experimental data on methane monooxygenase oxidation of isotopically substituted methane or ethane molecules. A set of kinetic parameters corresponding to the steady-state regime of the oxidation process was selected for each model of the mechanism. In each case, the correspondence to the steady-state regime was monitored by the unchanged ratio of the reaction products with a change in the conversion of the oxidized substrate from 0.1 to 99.9%.

RESULTS AND DISCUSSION

The following distribution of the $\text{CH}_{3-n}\text{D}_n\text{OH}$ and $\text{CH}_{4-n}\text{D}_{n-1}\text{OH}$ products of the oxidation of the C–H and C–D bonds of the $\text{CH}_{4-n}\text{D}_n$ methane molecule was

obtained in the study of the oxidation of isotopically labeled methane (CH_3D , CH_2D_2 , and CHD_3) by soluble MMO from *Methylosinus trichosporum*:

$$\begin{aligned}
 & \text{for } \text{CH}_3\text{D} \\
 \rho_1 &= [\text{CH}_2\text{DOH}]/[\text{CH}_3\text{OH}] = 11.7 \pm 3.0, \\
 & \text{for } \text{CH}_2\text{D}_2 \\
 \rho_2 &= [\text{CHD}_2\text{OH}]/[\text{CH}_2\text{DOH}] = 9.3 \pm 0.54, \\
 & \text{for } \text{CHD}_3 \\
 \rho_3 &= [\text{CD}_3\text{OH}]/[\text{CHD}_2\text{OH}] = 4 \pm 0.33.
 \end{aligned} \tag{1}$$

The $\text{CH}_{4-n}\text{D}_{n-1}\text{OH}$ alcohols are formed with a high rate constant from $\text{CH}_{4-n}\text{D}_{n-1}\text{OD}$ in the reaction of isotope exchange with water molecules.

The KIE values calculated from the ratio of the reaction products taking into account the number of the C–H and C–D bonds in the methane molecule,

$$\begin{aligned}
 \sigma_n &= \{n/(4-n)\}\rho_n \\
 &= ([\text{CH}_{3-n}\text{D}_n\text{OH}]/[\text{CH}_{4-n}\text{D}_{n-1}\text{OH}]),
 \end{aligned}$$

increase with an increase in the number of deuterium atoms (n) and are

$$\begin{aligned}
 & \text{for } \text{CH}_3\text{D} \\
 \sigma_1 &= (1/3)\rho_1 = (1/3)[\text{CH}_2\text{DOH}]/[\text{CH}_3\text{OH}] = 3.9 \pm 1.0, \\
 & \text{for } \text{CH}_2\text{D}_2 \\
 \sigma_2 &= \rho_2 = [\text{CHD}_2\text{OH}]/[\text{CH}_2\text{DOH}] = 9.3 \pm 0.54, \\
 & \text{for } \text{CHD}_3 \\
 \sigma_3 &= 3\rho_3 = 3[\text{CD}_3\text{OH}]/[\text{CHD}_2\text{OH}] = 12.0 \pm 1.0.
 \end{aligned} \tag{2}$$

As we mentioned above, such a change in the KIE values calculated per one C–H bond contradicts the oxygen rebound mechanism. Indeed, in this case, $\sigma_n = [n/(4-n)]\rho_n = k_{\text{H}}/k_{\text{D}}$, that is, it is only determined by the ratio of the rate constants of the homolytic cleavage of the C–H and C–D bonds in the methane molecule. In contrast to this, a simple kinetic scheme of the nonradical mechanism for methane oxidation by MMO involving two hydrogen atoms in the first step [9] explains an increase in the KIE values calculated taking into account the number of C–D and C–H bonds in a methane molecule with an increase in the number of deuterium atoms. This scheme does not contain mutual transformations of intermediate compounds. The F_I and F_{II} species are assumed to be kinetically indistinguishable, and F_{II} is the main species determining the statistical coefficients at the rate constants of reaction steps. The kinetic parameters calculated by the method of quasistationary concentrations are in good agreement with the character of the plot of the KIE value of methane oxidation vs. the number of the ¹H isotopes in the substrate molecule. According to the scheme, for

each two ρ parameters the region of acceptable values of the parameter is determined as follows:

$$\begin{aligned} 5.9 < \rho_2 < 7.9 & \text{ for } \rho_1 = 11.7 \text{ and } \rho_3 = 4, \\ 5.8 < \rho_3 < 8 & \text{ for } \rho_1 = 11.7 \text{ and } \rho_2 = 9.3, \quad (3) \\ 16.7 < \rho_1 < 25.4 & \text{ for } \rho_2 = 9.3 \text{ and } \rho_3 = 4. \end{aligned}$$

Evidently, the experimental distribution of the products of methane oxidation (see expressions (1)) does not correspond to the region of acceptable calculated values in (3). Therefore, the distribution of the oxidation products of partially deuterated methane corresponding to the experimental value cannot be obtained in the framework of this scheme.

This contradiction can be eliminated by assuming a more complex kinetic model of this nonradical mechanism, and the latter results in a situation where an analytical solution cannot be obtained and calculations are needed. The results of kinetic simulation of methane oxidation of active iron-oxygen complexes (LFeO) through the intermediate formation of a complex with pentacoordinated carbon are presented below. In the models considered, the irreversible formation of the FI or FII species with the rate constants k_1 is accepted, for simplicity, as the first step. In all models, the products are formed in the second step due to the direct insertion of an oxygen atom into a C–H or C–D bond with the rate constants k_2 .

KINETIC MODEL I

The following assumptions are made for kinetic model I. The FI and FII conformations are sufficiently different in energy to be kinetically distinguishable in spite of their fast mutual transformations. The first step is the formation of FI, which reversibly transforms into FII. Each hydrogen isotope of the methylene unit in FII can form FI according to the reactions $\text{FI}_{\text{OH}} \rightleftharpoons \text{FII}_{\text{HD}} \rightleftharpoons \text{FI}_{\text{OD}}$. The FII conformation with the same methylene units formed from different FI are similar. The mutual transformations of FII with different methylene units occur without isomerization: hydrogen isotopes do not exchange their positions, but some hydrogen bonds are replaced by others. The rate constants of the second step are only determined by the isotopic composition of FI.

Figure 2 presents the kinetic scheme of CH_2D_2 oxidation corresponding to model I. The kinetic schemes of the oxidation of CHD_3 and CH_3D only differ from the scheme of CH_2D_2 oxidation by the values of coefficients at the rate constants of particular steps (see table). Of course, the species with two H atoms in the methylene unit (FII_{HH}) is excluded from the kinetic scheme for CHD_3 , and the species with two D atoms (FII_{DD}) is excluded for CH_3D .

The calculation of the kinetic schemes of CH_2D_2 , CHD_3 , and CH_3D oxidation corresponding to model I

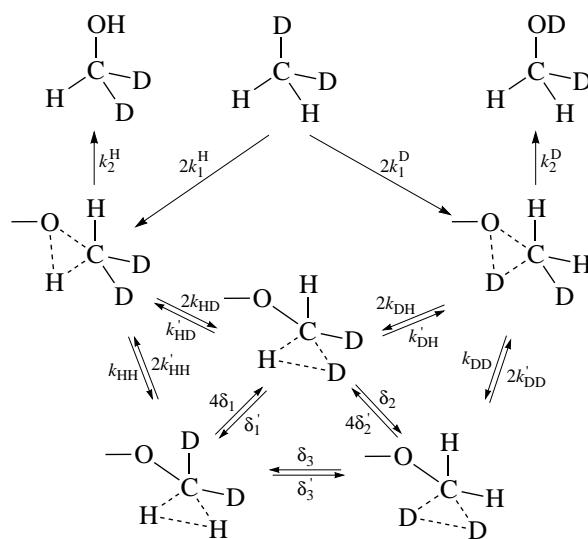


Fig. 2. Scheme of CH_2D_2 oxidation in the framework of kinetic model I.

gave the distribution of the products of oxidation of the C–H and C–D bonds in methane, which almost coincided with the experimental value. For example, the values $\rho_3 = 4.0$, $\rho_2 = 9.2$, and $\rho_1 = 11.8$ are obtained by calculating the kinetic scheme using the ratios of kinetic parameters presented below. The ratio $k_1^{\text{H}}/k_1^{\text{D}} = 1$ corresponds to the first step, which is the formation of FI_{OH} and FI_{OD} . The second step, alcohol formation from FI_{OH} and FI_{OD} , is characterized by $k_2^{\text{H}}/k_2^{\text{D}} = 3$. The ratio of the rate constants of the steps of formation of FII from FI is $k_{\text{HH}} : k_{\text{DD}} : k_{\text{HD}} : k_{\text{DH}} = 3 : 3 : 1 : 1$. The ratio of the rate constants of the mutual transformations of the FII forms is $\delta_1 : \delta_1' : \delta_2 : \delta_2' : \delta_3 : \delta_3' = 6 : 10 : 1 : 3 : 100 : 20$, where δ_1 and δ_1' are the rate constants of transformations of FII_{HH} into FII_{HD} and back, δ_2 and δ_2' are the rate constants of transformations of FII_{HD} into FII_{DD} and back, and δ_3 and δ_3' are the rate constants of transformations of FII_{DD} into FII_{HH} and back. The ratio of the rate constants of FI formation from FII is $k'_{\text{HH}} : k'_{\text{HD}} : k'_{\text{DD}} : k'_{\text{DD}} = 2 : 2 : 1 : 1$, and $k_{\text{HH}}/k_2^{\text{H}} = 10$, $k_{\text{HH}}/k'_{\text{HH}} = 3$, and $k_{\text{HH}}/\delta_1 = 10$. In the absence of the transformation of FII into FI, the statistical distribution of the FII species with different methylene units determined by the ratio of the δ values (ignoring the number of C–H and C–D bonds) is $\text{FII}_{\text{HH}} : \text{FII}_{\text{HD}} : \text{FII}_{\text{DD}} = 5.1 : 3.0 : 1 = 56\% : 33\% : 11\%$. The values of the reaction rate constants were specified taking into account that the conditions of the quasistationary hydroxylation of methane are fulfilled:

$$\begin{aligned} k_1^H, k_1^D &\ll k_2^H, k_2^D, k_{HH}, k_{HD}, k_{DH}, k_{DD}, \\ k'_{HH}, k'_{HD}, k'_{DH}, k'_{DD}, \delta_1, \delta'_1, \delta_2, \delta'_2, \delta_3, \delta'_3. \end{aligned} \quad (4)$$

Thus, the unusual distribution of alcohols during the hydroxylation of partially deuterated methane by MMO in the framework of the proposed kinetic model finds a reasonable explanation. Note that we specified the ratios corresponding to the equality of the rate constants of the steps of formation of FI_{OH} from FII_{HH} and FII_{HD} and the formation of FI_{OD} from FII_{HD} and FII_{DD} . In this case, we can designate

$$k'_{HH} = k'_{HD} = k_{OH} \quad \text{and} \quad k'_{DH} = k'_{DD} = k_{OD}. \quad (5)$$

Then we obtain the following ratios of the corresponding parameters:

$$k_{HD}/k_{OH} = 1; \quad k_{OH}/k_{OD} = 2.$$

Numerical values of coefficients at the rate constants of the elementary steps of the oxidation of isotopically labeled methane in the framework of kinetic model I

Rate constants of steps	CH_3D	CH_2D_2	CHD_3
k_1^H	3	2	1
k_1^D	1	2	3
k_{HH}	2	1	0
k_{HD}	1	2	3
k_{DH}	3	2	1
k_{DD}	0	1	2
k'_{HH}	2	2	0
k'_{HD}	1	1	1
k'_{DH}	1	1	1
k'_{DD}	0	2	2
δ_1	3	4	0
δ'_1	3	1	0
δ_2	0	1	3
δ'_2	0	4	3
δ_3	0	1	0
δ'_3	0	1	0
k_2^H	1	1	1
k_2^D	1	1	1

Analysis showed that the isotope effect of the step of the process insignificantly influences the distribution of products of partially deuterated methane oxidation. Therefore, for simplicity the calculation was performed at $k_1^H/k_1^D = 1$. The authors of [6], whose results are used for the numerical experiment, found the kinetic isotope effect $\sigma_4 = \rho_4 = [\text{CH}_3\text{OH}]/[\text{CD}_3\text{OH}] = 19.3 \pm 3.9$ for the $\text{CH}_4 + \text{CD}_4$ mixture. It is reasonable to assume that this value reflects the KIE value of the first step, and the problem can be solved under the condition of $k_1^H/k_1^D = 19.3$.

Taking into account (5), under the conditions of quasi-stationary methane hydroxylation (4), the ratios of the kinetic parameters can be written as

$$\begin{aligned} k_1^H/k_1^D &= 19.3; \quad k_2^H/k_2^D = 4; \quad k_{HH}/k_2^H = 7.5; \\ k_{HH}/k_{OH} &= 3; \quad k_{HH}/\delta_1 = 3; \\ k_{HD}/k_{OH} &= 1; \quad k_{OH}/k_{OD} = 2; \\ k_{HD}/k_2^H &= 2.5; \quad k_{HD}/\delta_1 = 1; \\ k_{HH} : k_{DD} : k_{HD} : k_{DH} &= 3 : 1.8 : 1 : 1; \\ \delta_1 : \delta'_1 : \delta_2 : \delta'_2 : \delta_3 : \delta'_3 &= 1 : 1 : 1 : 1 : 8 : 1. \end{aligned}$$

They give the following distribution of the oxidation products of the C–H and C–D bonds in methane: $\rho_3 = 4.0$, $\rho_2 = 9.4$, and $\rho_1 = 11.8$.

In the absence of the transformation of FII into FI, the statistical distribution of FII (ignoring the number of C–H and C–D bonds) is

$$\text{FII}_{\text{HH}} : \text{FII}_{\text{HD}} : \text{FII}_{\text{DD}} = 5.1 : 3.0 : 1 = 56\% : 33\% : 11\%.$$

The ratio of the kinetic parameters selected for the case of $k_1^H/k_1^D = 19.3$ are close to the above-presented ratios for $k_1^H/k_1^D = 1$. In these two cases, the agreement of the calculated and experimental values of the distribution of the oxidation products of partially deuterated methane C_{4-n}D_n is achieved if the conditions $\delta_3 > \delta'_1$ and $k_{DD} > k_{HD}$ are fulfilled. This ratio of the kinetic parameters increases the contribution of the channel $\text{CH}_{4-n}\text{D}_n \rightarrow \text{FI}_{\text{OD}} \rightarrow \text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{HH}} \rightarrow \text{FI}_{\text{OH}} \rightarrow \text{CH}_{(3-n)}\text{D}_n\text{OH}$ to the formation of products. Since this channel is absent for CHD_3 and CH_3D , its occurrence increases the ρ value only for CH_2D_2 , extending the region of acceptable ρ_2 values at the unchanged ρ_1 and ρ_3 values (see condition (4)). Note that the condition $k_{DD} > k_{HD}$ is formal with no obvious physical sense.

KINETIC MODEL II

As in the case of model I, we assumed for model II that the FI and FII species are kinetically distinguishable, the first step is FI formation, and the rate constants of the second step are only determined by the isotopic composition of FI. Unlike model I, we accepted for

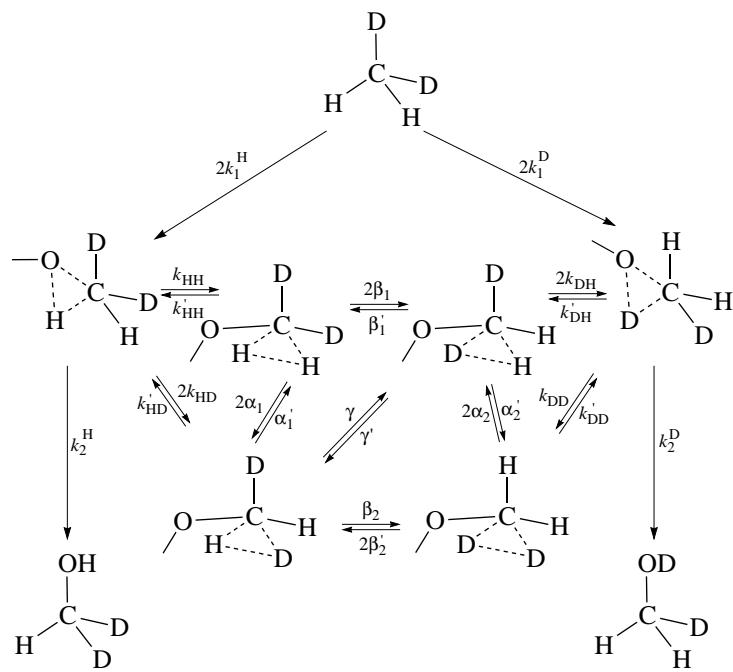


Fig. 3. Scheme of CH_2D_2 oxidation in the framework of kinetic model II.

model II that the oxygen atom can be inserted only into the bond subjected to the attack from the active species in the first step; that is, each FII species yields only one FI species. One of the positions of the FII species is distinguished, so to speak, “bound” to the oxygen atom. In this case, the FII species with the H and D atoms formed from different FII species differ: $\text{FI}_{\text{OH}} \rightleftharpoons \text{FII}_{\text{HH}}$, $\text{FI}_{\text{OD}} \rightleftharpoons \text{FII}_{\text{DH}}$. All mutual transformations of the FII species with different methylene units occur with isomerization: the hydrogen isotopes exchange their positions. Thus, kinetic model II is sterically more “rigid” than model I.

The kinetic scheme of CH_2D_2 oxidation corresponding to model II is presented in Fig. 3. According to this scheme, the first step is the formation of FI_{OH} and FI_{OD} with the k_1^{H} and k_1^{D} rate constants, respectively. The FI_{OH} species with the rate constants of the forward reactions k_{HH} and k_{HD} and backward reactions k'_{HH} and k'_{HD} transforms into FII_{HH} and FII_{HD} . The FI_{OD} species with the k_{DH} , k_{DD} , k'_{DH} , and k'_{DD} rate constants is reversibly transformed into FII_{DH} and FII_{DD} . The FII species with different methylene units reversibly transform into each other due to the exchange of positions of the hydrogen isotopes. The exchange of isotopes in the methylene unit with free (out of the methylene unit) isotopes occurs with the rate constants β_1 , β'_1 , β_2 , and β'_2 for the isotopes “bound” to oxygen and with the rate constants α_1 , α'_1 , α_2 , α'_2 for the isotopes “unbound” to oxygen. The hydrogen isotopes of the methylene unit in the FII

species with the rate constants γ and γ' exchange their positions. As a result of this exchange, the “bound” isotope becomes “unbound” and vice versa. Thus, this kinetic scheme is characterized by three types of exchange of positions of hydrogen isotopes, α , β , and γ . The second step is the formation of products from FI_{OH} and FI_{OD} with the rate constants k_2^{H} and k_2^{D} .

Similarly to model I, the kinetic schemes of CHD_3 and CH_3D oxidation only differ from the scheme of CH_2D_2 oxidation in terms of the values of coefficients at the reaction rate constants (which can easily be obtained by analogy with the data in the table).

The calculation of the kinetic schemes of CH_2D_2 , CHD_3 , and CH_3D oxidation corresponding to model II under the quasistationary conditions

$$(k_1^{\text{H}}, k_1^{\text{D}} \ll k_2^{\text{H}}, k_2^{\text{D}}, k_{\text{HH}}, k_{\text{HD}}, k_{\text{DH}}, k_{\text{DD}}, k'_{\text{HH}}, k'_{\text{HD}}, k'_{\text{DH}}, k'_{\text{DD}}, \alpha_1, \alpha'_1, \alpha_2, \alpha'_2, \beta_1, \beta'_1, \beta_2, \beta'_2, \gamma, \gamma') \quad (6)$$

gave the distribution of products of the oxidation of the C–H and C–D bonds in methane, which almost coincided with the experimental distribution.

The ratios of the kinetic parameters

$$k_1^{\text{H}}/k_1^{\text{D}} = 1; \quad k_2^{\text{H}}/k_2^{\text{D}} = 5; \quad k_{\text{HH}}/k_2^{\text{H}} = 2;$$

$$\alpha_1 = \gamma = \gamma' = 0.2k_{\text{HH}};$$

$$k_{\text{HH}} : k_{\text{HD}} : k_{\text{DH}} : k_{\text{DD}} : k'_{\text{HH}} : k'_{\text{HD}} : k'_{\text{DH}} : k'_{\text{DD}} \\ = 1 : 1 : 1 : 1 : 1 : 1 : 1 : 1;$$

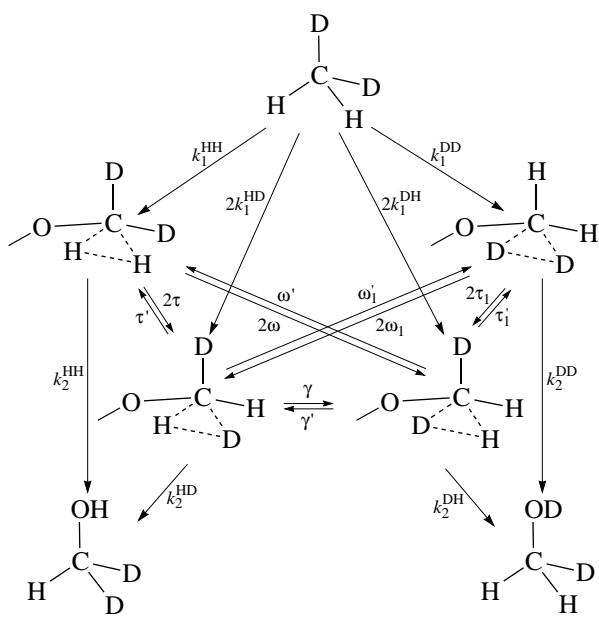


Fig. 4. Scheme of CH_2D_2 oxidation in the framework of kinetic model III.

$$\begin{aligned} \alpha_1 : \alpha'_1 : \alpha_2 : \alpha'_2 : \beta_1 : \beta'_1 : \beta_2 : \beta'_2 \\ = 1 : 1 : 1 : 15 : 1 : 1 : 1 : 3.75 \end{aligned}$$

give $\rho_3 = 4.0$, $\rho_2 = 9.3$, and $\rho_1 = 11.7$. In the absence of transformations of FII into FI, the statistical distribution of FII species with different methylene units, determined by the ratio of the kinetic parameters α , β , and γ (ignoring the number of C–H and C–D bonds), is $\text{FII}_{\text{HH}} : \text{FII}_{\text{HD}} : \text{FII}_{\text{DH}} : \text{FII}_{\text{DD}} = 4.4 : 7.8 : 1 : 4.8 = 25\% : 44\% : 6\% : 27\%$.

The distribution of the products of methane oxidation corresponding to the experimental one can also be obtained in the framework of kinetic model II using other sets of kinetic parameters. Note that in kinetic model II, unlike model I, the direct route $\text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{HH}}$ is absent, and the FII_{HD} and FII_{DH} species differ. To provide agreement of the calculated and experimental values of distributions of the oxidation products of partially deuterated methane $\text{CH}_{4-n}\text{D}_n$, one has to increase the fraction of products formed in the channel $\text{CH}_{4-n}\text{D}_n \rightarrow \text{FI}_{\text{OD}} \rightarrow \text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{HD}} \rightarrow \text{FII}_{\text{HH}} \rightarrow \text{FI}_{\text{OH}} \rightarrow \text{CH}_{3-n}\text{D}_n\text{OH}$, which is absent for CHD_3 and CH_3D . This channel increases the ρ_2 value at the unchanged ρ_1 and ρ_3 values if FII_{DD} predominantly produces the FII_{HD} species rather than FII_{DH} . To this end, in the case of exchange of the α type, an α'_2 value (the rate constants of replacement of the H atom by the D atom) higher than α_2 is chosen, while in the case of

β -type exchange, on the contrary, a β'_2 value (the rate constants of replacement of the D atom by the H atom) higher than β_2 is chosen. In the particular case when

exchange reactions between free hydrogen isotopes and isotopes bound to the oxygen atom in the methylene unit are absent ($\beta_1 = \beta'_1 = \beta_2 = \beta'_2 = 0$), this channel is not effective. In this case, to provide agreement of the calculated and experimental values of the distribution of the oxidation products of partially deuterated methane $\text{CH}_{4-n}\text{D}_n$, one has to increase the fraction of products formed in the channel $\text{CH}_{4-n}\text{D}_n \rightarrow \text{FI}_{\text{OD}} \rightarrow \text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{DH}} \rightarrow \text{FII}_{\text{HH}} \rightarrow \text{FI}_{\text{OH}} \rightarrow \text{CH}_{3-n}\text{D}_n\text{OH}$, which is absent for CHD_3 and CH_3D . The occurrence of this channel increases the ρ value for CH_2D_2 if $k'_1 < k'_2$.

KINETIC MODEL III

The basic difference between kinetic model III and the above-considered models I and II is that FII and FI are kinetically indistinguishable, as in the earlier proposed simple kinetic model [9]. The difference from the simple model is in the assumption that two hydrogen atoms are simultaneously involved in the reactions of an active species with hydrocarbon, that is, the first step is the formation of FII. The main state of the intermediate complex is FII, and, therefore, the formation of products from species of this type was formally accepted. As in kinetic model II, one of the positions of the methylene unit is distinguished as bound to oxygen. According to the difference in positions of the isotopes in the methylene unit, two types of exchange with free isotopes in FII are assumed: τ is the exchange of free and oxygen-unbound isotopes of the methylene unit, and ω is the exchange of free and oxygen-bound isotopes in the methylene unit. In addition, as for other models, there is an exchange of the γ type between isotopes bonded by the hydrogen bond in the methylene unit of FII. The rate constants of the second step are determined by the isotopic composition of FII.

The kinetic scheme of CH_2D_2 oxidation corresponding to model III is presented in Fig. 4. According to this scheme, the first step is the formation of FII_{HH} , FII_{HD} , FII_{DH} , and FII_{DD} with the rate constants k_1^{HH} , k_1^{HD} , k_1^{DH} , and k_1^{DD} , respectively. The FII_{HH} species transforms into FII_{DH} and FII_{HD} with the rate constants of forward reactions τ and ω and backward reactions τ' and ω' . The FII_{DD} species reversibly transforms into FII_{DH} and FII_{HD} with the rate constants τ_1 , ω_1 , τ'_1 , and ω'_1 . The FII_{HD} and FII_{DH} species transform into each other with the rate constants γ and γ' . The oxidation products are formed in the second step from the FII_{HH} , FII_{HD} , FII_{DH} , and FII_{DD} species with the rate constants k_2^{HH} , k_2^{HD} , k_2^{DH} , and k_2^{DD} , respectively.

The kinetic schemes of CHD_3 and CH_3D oxidation differ from the scheme of CH_2D_2 oxidation in terms of the coefficients at the reaction rate constants according

to the number of H and D atoms in the oxidized substrate (which can easily be obtained by analogy with the data in the table).

The calculation of the kinetic schemes of CH_2D_2 , CHD_3 , and CH_3D oxidation corresponding to model III made it possible to select the ratios of the kinetic parameters, which provide the distribution of the products of oxidation of the C–H and C–D bonds of methane coinciding with the experimental distribution.

For example, under quasistationary conditions for methane hydroxylation

$$k_1^{\text{HH}}, k_1^{\text{HD}}, k_1^{\text{DH}}, k_1^{\text{DD}} \ll k_2^{\text{HH}}, k_2^{\text{HD}}, k_2^{\text{DH}}, k_2^{\text{DD}}, \quad (7)$$

$$\omega, \omega_1, \tau, \tau_1, \omega', \omega'_1, \tau', \tau'_1, \gamma, \gamma',$$

the ratios of the kinetic parameters

$$k_1^{\text{HH}} = k_1^{\text{HD}} = k_1^{\text{DH}} = k_1^{\text{DD}},$$

$$k_2^{\text{HH}} : k_2^{\text{HD}} : k_2^{\text{DH}} : k_2^{\text{DD}} = 33.3 : 16.7 : 1.7 : 1,$$

$$k_2^{\text{HH}} / \tau = 2.0;$$

$$\tau = \tau' = \tau_1 = \tau'_1; \quad \omega = \omega' = \gamma = \gamma'; \quad \omega_1 = \omega'_1; \\ \tau / \omega = 10; \quad \tau_1 / \omega_1 = 0.2$$

give $\rho_3 = 4.1$, $\rho_2 = 9.3$, and $\rho_1 = 12.0$.

Kinetic model III, like model II, does not contain the direct route $\text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{HH}}$, and the FII_{HD} and FII_{DH} species differ. To provide agreement of the calculated and experimental values of the distribution of the oxidation products of partially deuterated methane $\text{CH}_{4-n}\text{D}_n$, one should increase the fraction of product formation in the channel $\text{CH}_{4-n}\text{D}_n \rightarrow \text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{HD}} \rightarrow \text{FII}_{\text{HH}} \rightarrow \text{CH}_{3-n}\text{D}_n\text{OH}$, which is absent for CHD_3 and CH_3D . This channel increases the ρ value for CH_2D_2 if FII_{HD} rather than FII_{DH} is formed predominantly from FII_{DD} . To this end, the values of the constants corresponding to the conditions $\tau > \omega$ but $\tau_1 < \omega_1$ are specified in the kinetic model. In this case, the FII_{HH} species prefer the exchange of free and oxygen-unbound isotopes of the methylene unit of the τ type, and the ω -type exchange of free and oxygen-bound isotopes in the methylene unit is preferential for the FII_{DD} species. If reactions of this type are absent ($\omega = \omega' = \omega_1 = \omega'_1 = 0$), this channel cannot be effective. An increase in the fraction of product formation in the channel $\text{CH}_{4-n}\text{D}_n \rightarrow \text{FII}_{\text{DD}} \rightarrow \text{FII}_{\text{DH}} \rightarrow \text{FII}_{\text{HH}} \rightarrow \text{CH}_{3-n}\text{D}_n\text{OH}$, which is absent for CHD_3 and CH_3D , is required to provide agreement of the calculated and experimental values of oxidation product distribution of partially deuterated methane. In this case, the values of the constants corresponding to the conditions $\tau > \tau'$ and $\tau_1 > \tau'_1$ are specified. The rate constant of FII_{HD} formation from FII_{HH} is higher than the rate constant of FII_{HH} formation from FII_{HD} , while the rate constant of the formation of the

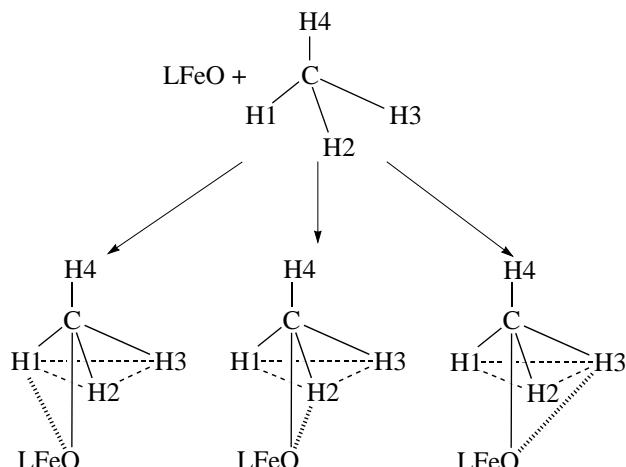


Fig. 5. Addition of the oxygen atom to the H1–H2–H3 face in the framework of kinetic model IV.

FII_{DD} species from FII_{HD} is lower than that of FII_{HD} formation from FII_{DD} .

Expressions (1) and (3) show that ρ for CH_2D_2 should be increased or ρ for CH_3D and CHD_3 should be decreased to obtain agreement of the calculated and experimental values. In the kinetic models involving simultaneously two C–H bonds in an elementary act, this can be carried out by an increase in ρ_2 due to the efficient transformation of the intermediate FII species with the DD isotopic composition of the methylene unit into the HH species. Let us consider a more complicated model assuming the participation of three hydrogen atoms in the reaction steps.

KINETIC MODEL IV

Kinetic model IV assumes the involvement of three C–H(D) bonds simultaneously in the elementary steps of the process. The first step is the formation of intermediate complexes due to the addition of an oxygen atom from the side of one of the structural faces of the hydrocarbon molecule accompanied by a change in the electron density of all hydrogen atoms that lie on this face (see the scheme for the H1–H2–H3 face in Fig. 5). One of the faces and one of the vertices of this (active) face, that is, the position bound to the oxygen atom, are thus distinguished. This is the position of the H1 isotope in Fig. 5. For the active faces with different isotopic compositions (HHH, HHD, HDD, and DDD), the parameters k_1^{HHH} , k_1^{HHD} , and k_1^{HDD} are the rate constants of the first step of the reaction between the O atom and H atom. The latter lies on one face with two H atoms, or H and D atoms, or two D atoms. The parameters k_1^{DDD} , k_1^{DHH} , and k_1^{DHD} are the rate constants of the first step of the reaction of the O atom with the D atom on the active faces DDD, DHD, and DHH, respectively. Three types

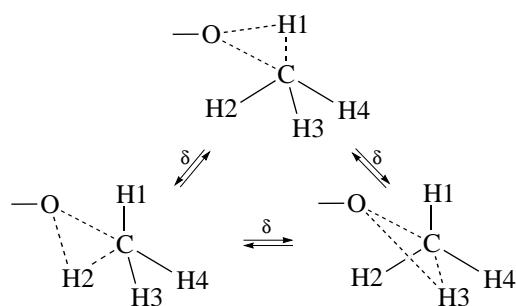


Fig. 6. Rebonding reactions of the oxygen atom with the hydrogen isotopes lying on the same H1–H2–H3 face in the framework of kinetic model IV.

of mutual transformations of these species are considered. First of all, the “rebonding” reaction of the oxygen atom with the hydrogen isotopes, which lie on the same face with the oxygen atom (without isomerization). The scheme of these reactions for the face with the vertices H1–H2–H3 is presented in Fig. 6. Then the isotopes laying on the same face with the oxygen atom exchange their positions (see the scheme for the H1–H2–H3 face in Fig. 7). In addition, the exchange reactions of the isotopes lying on the same face with oxygen with the isotope on the opposite vertex of the molecule are considered (see the scheme for the H1–H2–H3 face in Fig. 8). It is assumed for simplicity that the intermediate complexes transform into each other with rate constants whose values are only determined by their type and are independent of the isotopic composition of the active face: δ for the first type, γ for the second type, and α for the third type (Figs. 6–8). The oxidation products are formed in the second step due to the insertion of an oxygen atom into the C–H (C–D) bond corresponding to the “distinguished” position of the active face. According to the accepted model, the

electron density of all hydrogen isotopes lying on the same face with the oxygen atom changes in this process. The rate constants of the second step are determined by the isotopic composition of the active face of the alkane molecule with which oxygen reacts. The parameters k_2^{HHH} , k_2^{HHD} , and k_2^{HDD} are the rate constants of the second step, which is the insertion of the O atom into the C–H bond of the methane molecule $\text{CH}_{4-n}\text{D}_n$ to form the $\text{CH}_{3-n}\text{D}_n\text{OH}$ product. The parameters k_2^{DDD} , k_2^{DHH} , and k_2^{DHD} are the rate constants of the insertion of the O atom into the C–D bond to form the $\text{CH}_{4-n}\text{D}_{n-1}\text{OH}$ product.

Owing to the simultaneous participation of three C–H(D) bonds in the elementary steps of the process, the isotopic composition of the intermediate complexes for this kinetic model is more diverse than that of such complexes for the models considered above. According to model IV, these complexes have the composition of the active faces: HHH and HHD for CH_3D_2 , HHD and HDD for CH_2D_2 , and HDD and DDD for CHD_3 . According to models I–III, the isotopic composition of the methylene units of the FII forms of the intermediate complexes for CH_3D is HH and HD; HH, HD, and DD for CH_2D_2 ; and HD and DD for CHD_3 . Therefore, kinetic model IV can provide more approaches to obtain agreement with experimental data on the distribution of the oxidation products of partially deuterated methane molecules $\text{CH}_{4-n}\text{D}_n$. First, as in the case of models I–III, the ρ value for CH_2D_2 can be increased by the efficient transformation of a species with an isotopic composition of the active DDH face into a species with an HHD face composition. Then, the ρ values for CH_3D can be decreased, accepting the lowest rate of product formation of intermediate complexes with an HHH active face composition compared to species with

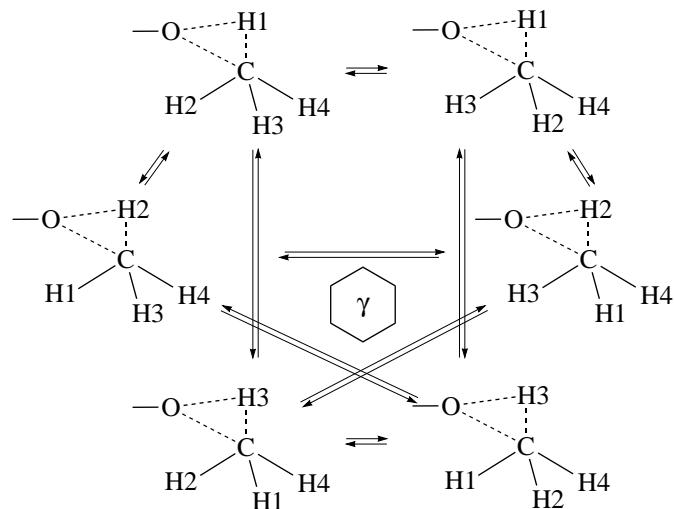


Fig. 7. Exchange of positions of the hydrogen isotopes lying on the same H1–H2–H3 face with oxygen in the framework of kinetic model IV.

another composition. In addition, the experimental distribution of the oxidation products of the C–H and C–D bonds of methane can be obtained by a simultaneous decrease in the ρ values for CH_3D and CH_2D_2 . In the framework of kinetic model IV, this is achieved under the condition that the $\text{CH}_{3-n}\text{D}_n\text{OH}/\text{CH}_{4-n}\text{D}_{n-1}\text{OH}$ ratio for alcohols formed from complexes with an HDD active face composition is higher than this ratio for complexes with an HHD face composition.

The calculation taking into account the coefficients at the reaction rate constants (according to the numbers of H and D atoms in the oxidized substrate, analogously to the data in the table) for the kinetic schemes of oxidation of CH_2D_2 , CHD_3 , and CH_3D corresponding to model IV under the quasistationary conditions

$$k_1^{\text{HHH}}, k_1^{\text{HHD}}, k_1^{\text{HDD}}, k_1^{\text{DDD}}, k_1^{\text{DHH}}, k_1^{\text{DHD}} \ll k_2^{\text{HHH}}, k_2^{\text{HHD}},$$

$$k_2^{\text{HDD}}, k_2^{\text{DDD}}, k_2^{\text{DHH}}, k_2^{\text{DHD}}, \alpha, \gamma, \delta$$

made it possible to obtain the ρ values, which almost coincided with the experimental values.

For example, for the ratios of the kinetic parameters

$$k_1^{\text{HHH}} = k_1^{\text{HHD}} = k_1^{\text{HDD}} = k_1^{\text{DHH}} = k_1^{\text{DDD}} = k_1^{\text{DHD}};$$

$$k_2^{\text{HHH}} : k_2^{\text{HDD}} : k_2^{\text{HHD}}$$

$$= k_2^{\text{DHH}} : k_2^{\text{DDD}} : k_2^{\text{DHD}} = 3.74 : 2.5 : 1;$$

$$k_2^{\text{HHH}}/k_2^{\text{DHH}} = k_2^{\text{HDD}}/k_2^{\text{DDD}} = k_2^{\text{HHD}}/k_2^{\text{DHD}} = 15.0;$$

$$\alpha = \gamma; \quad \delta = 10\alpha;$$

$$k_2^{\text{HHH}}/\alpha = 20,$$

we obtained $\rho_3 = 4.0$, $\rho_2 = 9.2$, and $\rho_1 = 11.6$. In this case, an agreement of the calculated and experimental values of the distribution of the oxidation products of partially deuterated methane is achieved due to the fact that $k_2^{\text{HDD}} > k_2^{\text{HHD}}$ and $k_2^{\text{DDD}} > k_2^{\text{DHD}}$. The experimental distribution of the products of methane oxidation by MMO in the framework of kinetic model IV can also be obtained using other sets of kinetic parameters. Note that it is unnecessary to use all types of mutual transformations of intermediate compounds for that. The sets of the kinetic parameters providing the experimental distribution of the oxidation products of deuterated methane can be selected under the condition $\alpha = \delta = 0$ or $\gamma = \delta = 0$. In the first case, the correspondence of the calculated and experimental ρ values is possible, as in the general case, under the condition $k_2^{\text{HDD}} > k_2^{\text{HHD}}$, and in the second case it holds when $k_2^{\text{HHD}} > k_2^{\text{HHH}}$. Exchange reactions of the α or γ type are necessary to explain the racemization of the products of chiral ethane oxidation by MMO, while the case of $\alpha = \gamma = 0$ contradicts the experimental data.

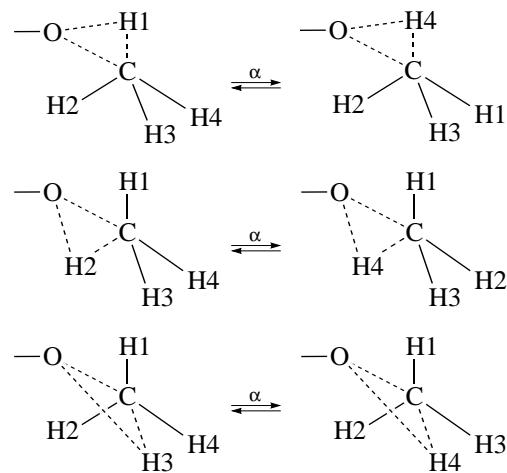


Fig. 8. Exchange of positions of the hydrogen isotopes on the H1–H2–H3 face with the isotope situated on the opposite vertex in the framework of kinetic model IV.

Thus, the kinetic schemes for methane, whose molecule contains four bonds with hydrogen isotopes at the same carbon atom, were considered in the framework of different kinetic models of the mechanism with the formation of an intermediate with pentacoordinated carbon. All the kinetic schemes proposed for the particular case of only one C–H bond and one C–D bond at the carbon atom are reduced to the previously proposed simple kinetic scheme involving one C–H or C–D bond in the first step. This simple scheme perfectly agrees with experimental data obtained for the oxidation of hydrocarbons with a similar structure by cytochrome P450 and its chemical models [8]. Let us consider the dynamics of the hydroxylation of ethane, whose molecule contains three bonds with hydrogen isotopes at the oxidized carbon atom.

The ethane molecule in which two hydrogen atoms in one of the methyl groups are replaced by deuterium and tritium exhibited a considerable isomerization during oxidation involving soluble MMO from *Methylosinus trichosporium* [4]. This experimental observation was explained by the intermediate formation of a free ethyl radical. In a similar experiment using membrane-bound, so-called particulate pMMO, from *Methylococcus capsulatus* (bath), the authors found the complete retention of the configuration [3], due to which the radical mechanism was rejected. These results were examined according to the nonradical mechanism through the formation of an intermediate complex with pentacoordinated carbon and showed [8] that both racemization and complete configuration retention during the oxidation of chiral ethane by MMO can be explained. The experimental data of ethane oxidation by soluble MMO [4] in the framework of the simple kinetic model seemed to be reasonable and needed no kinetic complication [8]. However, analysis [9] showed that the sim-

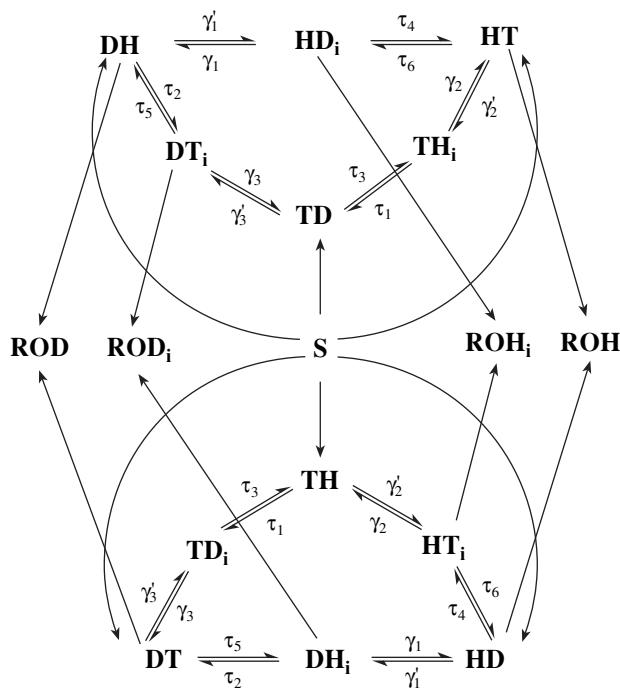


Fig. 9. Scheme of MeCHDT oxidation in the framework of kinetic model III (variant I).

ple model contradicted experimental data on methane oxidation by this enzymatic system. It seemed necessary to verify the kinetic models proposed for methane by kinetic calculations in the framework of these models of the oxidation of chiral ethane taking into account its isomerization. According to the models proposed, the isomerization of the *R* and *S* stereoisomers of ethane MeCHDT can occur in the transformations of the FII species containing different methylene units into each other and due to the exchange of positions of the hydrogen isotopes in their methylene units.

The kinetic parameters of oxidation were selected to provide agreement with the experimental distribution of the products of oxidation by MMO of the *R* and *S* stereoisomers of ethane MeCHDT [4]:

$$[\text{ROH}] : [\text{ROD}] : [\text{ROH}_i] : [\text{ROD}_i] = (52-53) : (12-14) : (26-27) : (7-9),$$

where ROH and ROH_i are the products of oxidation of the C–H bond of the methyl group, and CHDT, ROD, and ROD_i are the products of C–D bond oxidation. The subscript *i* refers to the isomerized products of stereoisomer oxidation. The products of C–T bond oxidation were not found. Since only the ratios of the products of chiral ethane oxidation are known, a set of kinetic parameters corresponding to the stationary regime of oxidation was selected for each model of the mechanism. The tritium atoms were assumed to participate in bond formation in the intermediate forms but, according to the experimental results, no product of C–T bond oxidation is formed.

KINETIC MODEL III FOR ETHANE

First, let us consider chiral ethane oxidation in the framework of kinetic model III, in which the FII and FI species are kinetically indistinguishable. For simplicity we consider the case of the absence of exchange of hydrogen isotopes in positions unbound to the oxygen atom. Let us take into account the exchange in positions of the hydrogen isotopes of the methylene unit in the FII species with the γ_i and γ'_i rate constants. This exchange results in the isomerization of the products of chiral ethane oxidation. Two variants of this kinetic scheme are possible for ethane because for this substrate the exchange of positions of the hydrogen isotopes in reactions similar to the steps of methane oxidation, which occur with the rate constants τ , τ' , τ_1 , and τ'_1 (see Fig. 4), may or may not result in the inversion of the chiral molecule.

Variant 1. The exchange of positions of the hydrogen atoms with the rate constants τ_i is assumed to result in the inversion of the chiral molecule. The kinetic scheme of ethane oxidation for this case is presented in Fig. 9. For simplicity, only bonds characteristic of the FII species are presented in this scheme instead of the structural formulas (see Fig. 10). In the scheme, subscript *i* is ascribed to the FII species corresponding to the isomerized stereoisomer. The first step yields the FII species with different methylene units in which one of the isotopes is bound to the oxygen atom. The rate constants of these steps are k_1^{HD} , k_1^{HT} , k_1^{DH} , k_1^{DT} , k_1^{TH} , and k_1^{TD} . The oxidation products are formed due to the insertion of the O atom into the bond corresponding to the “distinguished” position of the hydrogen isotope with the rate constants k_2^{HT} , k_2^{HD} , k_2^{DH} , and k_2^{DT} .

The calculation of the kinetic scheme of MeCHDT oxidation presented in Fig. 9 under the quasistationary conditions

$$k_1^{\text{HD}}, k_1^{\text{HT}}, k_1^{\text{DH}}, k_1^{\text{DT}}, k_1^{\text{TH}}, k_1^{\text{TD}} \ll k_2^{\text{HT}}, k_2^{\text{HD}}, k_2^{\text{DH}}, k_2^{\text{DT}}, \quad (9)$$

$$\tau_1, \tau_2, \tau_3, \tau_4, \tau_5, \tau_6, \gamma_1, \gamma'_1, \gamma_2, \gamma'_2, \gamma_3, \gamma'_3$$

for the ratios of the kinetic parameters

$$k_1^{\text{HD}} : k_1^{\text{DH}} : k_1^{\text{HT}} : k_1^{\text{TH}} : k_1^{\text{DT}} : k_1^{\text{TD}} = 30 : 30 : 25 : 25 : 1 : 1;$$

$$k_2^{\text{TH}} = k_2^{\text{TD}} = 0;$$

$$k_2^{\text{HD}} : k_2^{\text{HT}} : k_2^{\text{DH}} : k_2^{\text{DT}} = 30 : 30 : 1 : 1;$$

$$\gamma_1 : \gamma'_1 : \gamma_2 : \gamma'_2 : \gamma_3 : \gamma'_3 = 2.50 : 2.50 : 1.75 : 1.75 : 1 : 1;$$

$$\tau_1 : \tau_2 : \tau_3 : \tau_4 : \tau_5 : \tau_6 = 2000 : 700 : 700 : 50 : 20 : 1; \\ k_2^{\text{HT}} / \tau_1 = 1.5; \quad \tau_1 = 4\gamma_1$$

gave the distribution of the products of chiral ethane oxidation [ROH] : [ROD] : [ROH_i] : [ROD_i] = 52 : 12 : 26 : 10.

Variant 2. The exchange of hydrogen isotopes with the rate constants τ_i does not result in the inversion of the chiral molecule. In all other respects, the kinetic scheme for this variant (Fig. 11) is similar to the scheme of variant 1.

The calculation of the kinetic scheme of MeCHDT oxidation presented in Fig. 11 under the quasistationary condition (9) for the ratios of the kinetic parameters

$$\begin{aligned}
 k_1^{\text{HD}} : k_1^{\text{DH}} : k_1^{\text{HT}} : k_1^{\text{TH}} : k_1^{\text{DT}} : k_1^{\text{TD}} \\
 = 20 : 20 : 15 : 15 : 1 : 1; \\
 k_2^{\text{TH}} = k_2^{\text{TD}} = 0; \\
 k_2^{\text{HD}} : k_2^{\text{HT}} : k_2^{\text{DH}} : k_2^{\text{DT}} = 15 : 15 : 1 : 1; \\
 \gamma_1 : \gamma_1' : \gamma_2 : \gamma_2' : \gamma_3 : \gamma_3' \\
 = 2.5 : 2.5 : 1.5 : 1.5 : 1 : 1; \\
 \tau_1 : \tau_2 : \tau_3 : \tau_4 : \tau_5 : \tau_6 \\
 = 300 : 1000 : 300 : 100 : 10 : 1; \\
 k_2^{\text{HT}} / \tau_1 = 5; \quad \tau_1 = 0.6 \gamma_1
 \end{aligned}$$

provided the distribution of the oxidation products of chiral ethane $[ROH] : [ROD] : [ROH_i] : [ROD_i] = 52 : 14 : 27 : 7$.

Thus, using appropriate kinetic parameters, we succeeded in obtaining the product distribution of chiral ethane oxidation. It almost coincides with the experimental distribution. Similar results were obtained by the kinetic simulation of chiral ethane oxidation in the framework of other kinetic models proposed above. For simplicity, we present only the results obtained for the simplest kinetic model I.

KINETIC MODEL I FOR ETHANE

The scheme of chiral ethane oxidation corresponding to kinetic model I is presented in Fig. 12. Only the bonds characteristic of the FI and FII species are presented in the scheme instead of the structural formulas (see Fig. 10). The subscript i was ascribed in the scheme to the FI and FII species corresponding to the isomerized stereomer. The first step of oxidation is the formation of the FI_{OH} , FI_{OD} , and FI_{OT} species with the rate constants k_1^{H} , k_1^{D} , and k_1^{T} , respectively. The FI species transform into FII_{HD} , FII_{DH} , FII_{HT} , FII_{TH} , FII_{DT} , and FII_{TD} with the rate constants k_{HD} , k_{DH} , k_{HT} , k_{TH} , k_{DT} , and

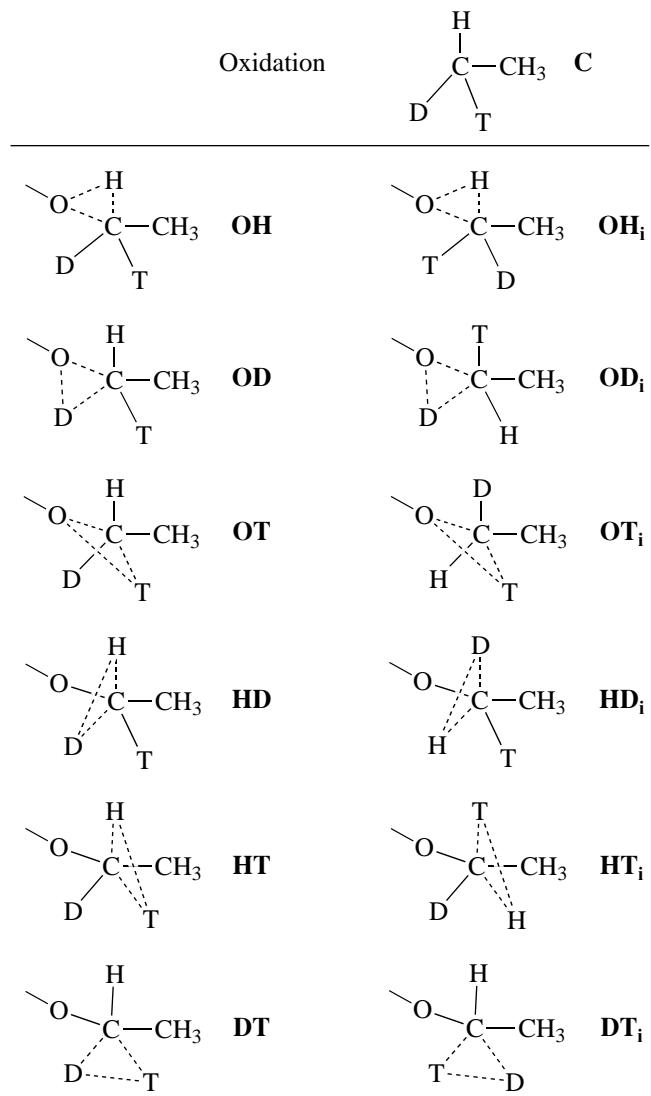


Fig. 10. Intermediate FI and FII complexes in MeCHDT oxidation.

k_{TD} , respectively. The FII species transform into the FI_{OH} , FI_{OD} , and FI_{OT} species with the rate constants k_{OH} , k_{OD} , and k_{OT} , respectively. We assume for simplicity that the values of the above-presented rate constants are only determined by the composition of the bond formed. According to the accepted scheme, isomerization occurs due to the exchange of positions of the hydrogen isotopes of the FII species with the γ rate constant, whose value is taken unchanged regardless of the isotopic composition of the methylene unit. The FII species transform into each other without changing stereochemistry with the rate constants, whose values are only determined by the isotopic composition of the methylene units in the initial and resulting forms: for $\text{FII}_{HD} \rightleftharpoons \text{FII}_{DT} - \delta_1$ and δ_1' ; for $\text{FII}_{DT} \rightleftharpoons \text{FII}_{HT} - \delta_2$

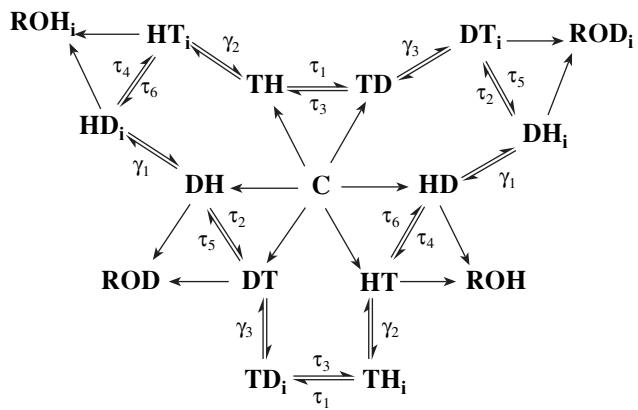


Fig. 11. Scheme of MeCHDT oxidation in the framework of kinetic model III (variant 2).

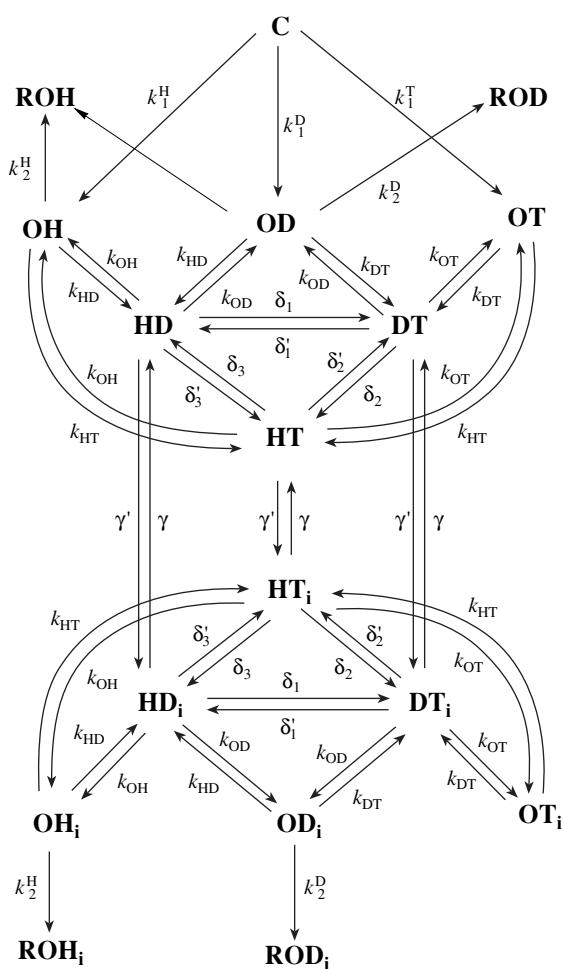


Fig. 12. Scheme of MeCHDT oxidation in the framework of kinetic model I.

and δ'_2 ; and for $\text{FII}_{\text{HT}} \rightleftharpoons \text{FII}_{\text{HD}} - \delta_3$ and δ'_3 for the forward and backward reactions, respectively.

Taking the ratios of kinetic parameters chosen in the framework of this kinetic model for methane

$$k_1^H/k_1^D = 19.3; \quad k_2^H/k_2^D = 4; \quad k_{HD}/k_2^H = 2.5; \\ k_{HD}/k_{OH} = 1; \quad k_{OH}/k_{OD} = 2; \quad k_{HD} = k_{DH},$$

as the initial ratios, one can select the ratios of other parameters, providing the specified product distribution of ethane oxidation. For example, the calculation of the kinetic scheme for MeCHDT oxidation presented in Fig. 12 under quasistationary conditions ($k_1^H, k_1^D, k_1^T \ll k_2^H, k_2^D, k_{HD}, k_{DH}, k_{HT}, k_{TH}, k_{DT}, k_{TD}, k_{OH}, k_{OD}, k_{OT}, \delta_1, \delta_1', \delta_2, \delta_2', \delta_3, \delta_3', \gamma$) for the ratios of kinetic parameters

$$k_1^{\text{H}} : k_1^{\text{D}} : k_1^{\text{T}} = 270 : 19.2 : 1;$$

$$k_2^T = 0; \quad k_2^H/k_2^D = 4;$$

$$k_{\text{HD}}/k_2^{\text{H}} = 2.5; \quad k_{\text{HD}}/k_{\text{OH}} = 1; \quad k_{\text{OH}}/k_{\text{OD}} = 2; \\ k_{\text{OH}}/k_{\text{OT}} = 3.3; \quad k_{\text{HD}}/\delta_1 = 0.5; \quad k_{\text{HD}}/\gamma = 1;$$

$$k_{\text{HD}} : k_{\text{DH}} : k_{\text{HT}} : k_{\text{TH}} : k_{\text{DT}} : k_{\text{TD}} = 10 : 10 : 3 : 3 : 1 : 1;$$

$$\delta_1 : \delta'_1 : \delta_2 : \delta'_2 : \delta_3 : \delta'_3 = 20 : 1 : 2 : 10 : 10 : 10$$

made it possible to obtain the product distribution of chiral ethane oxidation $[ROH] : [ROD] : [ROH_i] : [ROD_i] = 53 : 12 : 26 : 9$. Thus, the experimental data obtained for the oxidation of different substrates (methane and chiral ethane) by MMO can be explained in the framework of the general kinetic model for the mechanism.

CONCLUSIONS

Thus, we considered the variants of the nonradical mechanism of hydrocarbon oxidation simultaneously involving two or even three hydrogen atoms in the reaction with the active intermediate. Analysis of the results of the numerical experiment showed that the kinetic models proposed for the oxidation of isotopically substituted methane and ethane agreed with the experimental data, which were not explained in the framework of the oxygen rebound mechanism accepted by the majority of researchers. Note that the special ratios of the kinetic parameters were assumed to bring the calculated and experimental product distributions of partially deuterated methane into correspondence. For example, in the framework of model II for the exchange of the oxygen-bound isotopes of the methylene unit with the isotopes situated out of this unit, the rate constants of the $D \rightarrow H$ exchange were chosen higher than those for $H \rightarrow D$, whereas the situation is opposite for the oxygen-unbound isotopes. In the framework of model III, the exchange of the free and oxygen-unbound isotopes of the methylene unit is preferential for the $FII \rightarrow HH$ species, while the $FII \rightarrow DD$ species prefer the exchange of the free and oxygen-bound

isotopes of the methylene unit. The relation $k_{\text{DD}} > k_{\text{HD}}$ is chosen for kinetic model I, and the relation $k_2^{\text{HDD}} > k_2^{\text{HHD}}$ is chosen for model IV. These relations of the rate constants of particular steps of the kinetic models point to a more complicated mechanism of the process. Additional experimental and theoretical studies are needed to address such relevant questions.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 00-03-32316a.

REFERENCES

1. Karasevich, E.I., Kulikova, V.S., Shilov, A.E., and Shteynman, A.A., *Usp. Khim.*, 1998, vol. 67, no. 4, p. 376.
2. Valentine, A.M., Stahl, S.S., and Lippard, S.J., *J. Am. Chem. Soc.*, 1999, vol. 121, no. 16, p. 3876.
3. Wilkinson, B., Shu, M., Priestley, N.D., Nguen, H.-H.T., Morimoto, H., Williams, P.G., Chan, S.I., and Floss, H.G., *J. Am. Chem. Soc.*, 1996, vol. 118, no. 4, p. 921.
4. Priestley, N.D., Floss, H.G., Frohland, W.A., Lipscomb, J.D., Williams, P.G., and Morimoto, H., *J. Am. Chem. Soc.*, 1992, vol. 114, no. 19, p. 7561.
5. Groves, J.T., *J. Chem. Educ.*, 1985, vol. 62, no. 1, p. 928.
6. Nesheim, J.C. and Lipscomb, J.D., *Biochemistry*, 1996, vol. 35, no. 31, p. 10240.
7. Shestakov, A.F. and Shilov, A.E., *J. Mol. Catal.*, 1996, vol. 105, no. 1, p. 1.
8. Karasevich, E.I., Shestakov, A.F., and Shilov, A.E., *Kinet. Katal.*, 1997, vol. 38, no. 6, p. 852.
9. Karasevich, E.I., Karasevich, Yu.K., Shestakov, A.F., and Shilov, A.E., *Kinet. Katal.*, 2003, vol. 44, no. 1, p. 120.