Oxygen Atom Transfer into C−H Bond in Biological and Model Chemical Systems. Mechanistic Aspects

ALEXANDER E. SHILOV* AND
ALBERT A. SHTEINMAN*

Institute of the Chemical Physics Problems, 142432 Chernogolovka, Russia

Received June 16, 1998

Introduction

Selective hydroxylation of organic compounds, particularly of C−H bonds in alkanes, remains a very interesting theoretical problem and also has important practical implications.[1,2]

In traditional radical chain oxidation of alkanes (RH), dioxygen causes an initiation and propagation of chains:

$$RH + O_2 \rightarrow R^{\bullet} + HO_2^{\bullet}$$

$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}; \quad RO_2^{\bullet} + RH \rightarrow RO_2H + R^{\bullet}$$

Transition metal compounds (M) can participate in the chain initiation and branching via $1e^-$ stages, e.g.,

$$RO_2H + M^n \rightarrow M^{n+1} + RO^- + {}^{\bullet}OH$$

The whole process is usually nonselective, since radicals, particularly such active ones as $^{\bullet}OH$, do not discriminate different C−H bonds; for less active radicals, the secondary (2°) and tertiary (3°) C−H bonds are more reactive than the primary (1°) one; i.e., bond selectivity is 1° < 2° < 3°.

Another mechanism of alkane reactions with transition metal compounds involves direct interaction of an alkane with a metal core in a $2e^-$ process (Figure 1).[1,3] The first example of such a mechanism was found by us for the reaction of Pt(II) complexes[3a] with alkanes via oxidative addition, presumably initially forming a complex with an alkane molecule (Figure 1a). The selectivity turned out to be essentially the opposite of that for radical reactions:

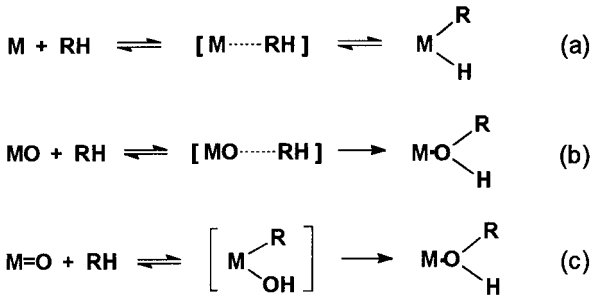

FIGURE 1. $2e^-$ mechanisms for alkane reactions with transition metal complexes.

1° > 2° ≫ 3°. Apparently, steric factors play an important role in determing this unusual selectivity.

In *aerobic* oxidation by monooxygenase enzymes, the living nature has found yet another but probably a somewhat similar mechanism for the process. While initially it is dioxygen that is activated,[4] the selectivity in these oxidations is very different from that observed in free radical reactions. Thus, ω-hydroxylases selectively hydroxylate terminal methyl groups in alkyl chains, and methane monooxygenase (MMO[5]) is most active toward methane, having the strongest C−H bonds as compared with those of other alkanes.[6]

To get insight into the reasons for such selectivity and to find ways to realize it in chemical alkane oxidation, the mechanism of oxygen atom insertion into a C−H bond has to be understood.

Monooxygenases hydroxylate the C−H bond of a hydrocarbon (RH) in a coupled reaction with the oxidation of $2e^-$ donors NADH[5] or NADPH[5] according to the following scheme:

$$RH + 2e^- + 2H^+ + O_2 \rightarrow ROH + H_2O$$

Recently, various monooxygenases underwent vigorous investigations by several scientific groups.[1,2a,7−14] At present, the structures of active centers of these enzymes are known, and attempts are made to visualize the mechanism of their reactions with different substrates. Many effective purely chemical systems have been proposed as structural and functional models of corresponding enzymes.[1,2a,c,9d,11,12]

At the same time, despite great efforts to elucidate the mechanism of monooxygenases action, it remains somewhat obscure, and controversial ideas concerning the mechanism have been put forward.

In our studies in the last 25 years, we carried out research on chemical model systems possessing monooxygenase activity. In this Account, we consider several of those and discuss the mechanism of their action. The systems investigated more recently will be primarily discussed, although some earlier works important for understanding the mechanism will be also mentioned to present a more general picture.

Alexander E. Shilov graduated from Kiev University (Ukraine) and since 1952 has worked at the Institute of Chemical Physics (Moscow). Now he is director of the Institute of Biochemical Physics (Moscow) and Professor at Moscow University. He is a member of the Russian Academy of Sciences and Academia Europaea. His research interests span the fields of kinetics and mechanism of chemical reactions, catalysis, and enzyme mimics.

Albert A. Shteinman graduated from Moscow University in 1961 and since 1963 has been working at the Institute of Chemical Physics (Chernogolovka). He received his Ph.D. in 1968 for the reaction of carbenes with N_2 and Sc.D. in 1978 for alkane activation on Pt and Pd complexes in solution. His research has been focused on biomimetic catalysis, with the present accent on monooxygenase mechanism.

10.1021/ar980009u CCC: $18.00
Published on Web 06/17/1999

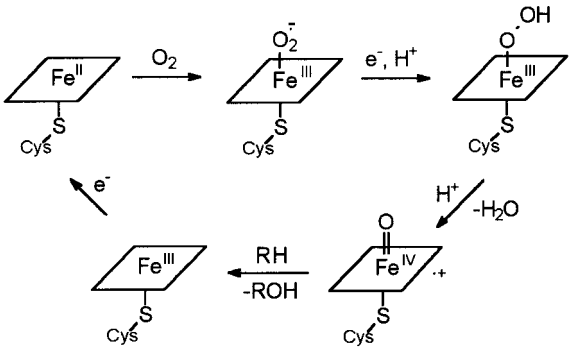

FIGURE 2. Catalytic cycle of CP450.

Heme-Containing Systems: Evidence for Proton Tunneling

Among monooxygenases, heme-containing CP450[5] has been studied in particular detail (see reviews[7]). The reaction cycle for CP450 is shown in Figure 2: the active species is thought[3] to involve the ferryl core $P^{+\bullet}Fe^{IV}O$. The latter reacts with the C−H bond, transferring an O atom, and this results in C−H hydroxylation to form an alcohol. Iron porphyrin complexes have been widely used as catalysts for hydrocarbon oxidation.[1,2c,11] To simplify oxidizing systems, donors of single oxygen atom are used, such as iodosylbenzene, hydrogen peroxide and alkyl hydroperoxides, sodium hypochlorite, potassium persulfate, amino *N*-oxides, and nitrous oxide. In such cases, no reducing agent to induce the coupled hydrocarbon oxidation is necessary.

To start with, we would like to mention our results for iron porphyrin complexes in a biphasic system, water−benzene, which readily hydroxylate alkanes by hypochlorite.[15,16a] The system is very stereoselective: up to 92% retention of configuration is observed for hydroxylation of the tertiary C−H bond in *cis*- and *trans*-1,2-dimethylcyclohexane. The kinetic H−D isotope effect (KIE, k_H/k_D) is often used as an indicator for the reaction mechanism. A high KIE (k_H/k_D = 21.9 at 20 °C) was observed for cyclohexane (C_6H_{12} vs C_6D_{12}) in the system mentioned. The Arrhenius temperature dependence of the KIE shows the ratio of preexponential factors $A_H/A_D = 0.01$, and $E_D - E_H = 4.5 \pm 0.5$ kcal/mol. These data strongly indicate the involvement of *proton tunneling*. The criterion for tunneling besides the high value of KIE is $A_H/A_D \leq 0.7$. The intramolecular KIE obtained later[16b] for a specially prepared substrate (adamantane) confirmed the implication of the tunneling. As will be shown below, this fact is very significant for understanding the mechanism of O transfer to a C−H bond.

Full Model of CP450: 5/6 Parameter in the System Iron Porphyrin−O_2−Carboxylic Acid−Zn Powder

The kinetics of alkane oxidation was investigated in detail in this system in CH_3CN with methyl viologen, MV^{2+}, as electron-transfer agent.[17,18] Noticeable concentrations of H_2O_2 (ca. 10^{-2} M) are detected in the reaction solution. Presumably, the role of metalloporphyrin is to form an active particle with H_2O_2, produced in the dioxygen reaction with the reduced methyl viologen in the presence of an acid. The formation of HO_2 radicals and H_2O_2, which could be obvious intermediates, may lead to the suggestion that free radicals ($^\bullet$OH or RO$^\bullet$) interact with a hydrocarbon molecule.

To make a definite conclusion about the mechanism(s), stereoselectivity, KIE, and substrate selectivity were tested. Racemization was observed in the oxidation of *cis*- and *trans*-1,2-dimethylcyclohexane, and relatively low KIE values were detected for the oxidation of cyclohexane and cyclohexane-d_6 (about 2). This may be explained on the basis of both free radicals and iron porphyrin oxocomplexes as active species. Substrate selectivity measurements witness against a free radical mechanism, however, at least as the sole reaction mechanism. These were made for competitive oxidation of C_5H_{10} and C_6H_{12}: the so-called 5/6 parameter was determined, i.e., the ratio of respective rate constants corrected for the number of C−H bonds.

The ratio of hydroxylation products for the C_5H_{10}/C_6H_{12} mixture provides indirect evidence for the reaction mechanism. Bond dissociation energy is somewhat higher in cyclohexane than in cyclopentane; therefore, free radical abstraction from C_5H_{10} must be easier than that from C_6H_{12}, and the 5/6 parameter should be more than 1. However, the values of the 5/6 parameter turned out to be less than unity and strongly dependent on the nature of the metalloporphyrin. For different metalloporphyrins, they vary in the range 0.38−0.50, that is, characteristic of electrophilic attack by positively charged reagent. Thus, evidently a nonradical mechanism functions at least partially in this system.

Non-Heme Systems: Hydroxylation by Dinuclear Iron Complexes

It is not difficult to induce oxidation of alkanes in a coupled reaction with the oxidation of simple metal salts, e.g., chlorides, by molecular oxygen. Thus, coupled oxidation of C_1-C_4 alkanes and $SnCl_2$ or $FeCl_2$ was found to occur in acetonitrile at room temperature via a free radical mechanism.[1a] In our early investigations of monooxygenase models based on Sn(II) compounds, which are $2e^-$ reductants, we concluded that a shift from a $1e^-$ (radical) process to a $2e^-$ (oxenoid) one is stimulated by *soft ligands on metal and aprotic nonpolar solvents*.[19]

In efforts to model MMO, which is clearly different with regard to the mechanism of C−H attack, we turned to dinuclear iron complexes, since MMO is known to involve a dinuclear iron active center.[8,9] A number of such complexes were reported as structural models of enzymes with carboxylate-bridged non-heme diiron sites.[20] Although no effective biomimetic methane oxidation has been found yet with the use of such complexes, there is progress in the understanding of factors determining the catalytic activity.

Taking into account that only H_2O_2 among other oxygen atom donors can replace O_2/NADH in the case of MMO,[10] we concentrated our attention on this oxidant.

Table 1. Use of Substrate Probes To Determine the Mechanism of Alkane Oxidation by Diiron Complexes with Substituents X in Ligands[23,25]

test	X = Me	X = H	X = NO_2
KIE(C_6H_{12})	3.1	2.4	2.0
2°/3°	0.20	0.30	0.62
I/II	1.7	1.9	2.7
RC (%)	48		72

Because labile coordination sites should be necessary to bind and activate H_2O_2, the complex $[Fe_2O(dpy)_2(OCOR)_2(H_2O)_2]X_2$ was prepared and characterized.[21] In the presence of H_2O_2, this complex catalyzes the oxidation of C_1−C_6 alkanes in MeCN at room temperature. Cyclohexane produces alcohol and ketone (the ratio about 2 and 25% yield to $[H_2O_2]_0$) with the rate ca. 60 turnovers/h. The following reactions were proposed:

$$Fe{-}O{-}Fe(H_2O) + H_2O_2 \rightarrow$$

$$Fe{-}O{-}Fe(OOH) \rightarrow Fe{-}O{-}Fe^{VI}{=}O + HO^{\bullet} \quad (1)$$

$$\rightarrow Fe{-}O{-}Fe^{V}{=}O + HO^{-} \quad (2)$$

Some H_2O_2 is probably consumed in reaction 1; however, an increase in the alcohol yield upon acidification by $HClO_4$ suggested also an involvement of reaction 2, producing a metal-based active intermediate which attacks the C−H bond. This mechanism supported by KIE = 3.0 observed in cyclohexane oxidation and the low value (0.3) of the 5/6 parameter for cyclopentane/cyclohexane oxidation seemingly witness against the involvement of OH radical as *the only* intermediate attacking alkane. The above complex was also able to catalyze the methane oxidation, albeit with a small turnover number (2−3).

Thus, the results obtained suggested some contribution of the oxenoid mechanism with participation of high-valent iron−oxo species.[1c,21] To check this suggestion more directly, the simplest diiron complexes with labile coordination sites of the general formula $[Fe_2O(L)_4(H_2O)_2](ClO_4)_4$, where L = dpy, 4,4′-$Me_2$dpy, 4,4′-$(ClCH_2)_2$dpy, phen, and 5-$NO_2$phen, were synthesized, and the effect of substituents on ligands on such parameters as catalytic activity, KIE, and others (Table 1) was studied.[22] The opposite dependences of the catalytic activity observed for methane (X = NO_2 > H > CH_3) and cyclohexane (X = CH_3 > H > NO_2) were interpreted on the basis of the superposition of the O transfer and usual radical chain oxidation.[22] The maximum contribution of metal-based O transfer was expected for the strongest C−H bond (methane) and the most electrophilic species (X = NO_2).

We have found further that the values of testing parameters 2/3 (rate constant ratio for attack onto 2° and 3° C−H bonds of adamantane) and I/II (the same for two different double bonds of limonene) depend strongly on the dioxygen concentration in the catalytic solution, likely due to the contribution of the radical chain process. Evacuation of O_2 during the reaction allowed us to get 2/3 and I/II parameters which are independent of dioxygen concentration and characteristics of the MO active intermediate (Table 1). A good correlation of these characteristics with Hammett constants of the substituents in the ligands of iron complexes (Figure 3) proves the O transfer for these complexes.[23] The retention of configuration during oxidation of *trans*-1,4-dimethylcyclohexane[24] and partial retention of the cyclopropane ring during oxidation of a very fast radical-clock substrate, *trans*-1-methyl-2-phenyl-cyclopropane[24] (Figure 4), are evidence for a contribution of 2e⁻ mechanism of the oxygen atom transfer from hydrogen peroxide to the C−H bond of an alkane via a metal complex.[23] This mechanism takes place in both alkane hydroxylation and olefin epoxidation in chemical model systems against a background of radical chain mechanism.[23,25]

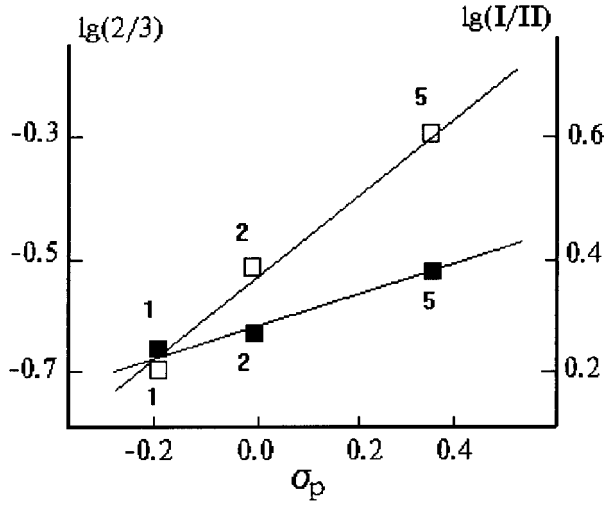

FIGURE 3. Correlation of lg(2/3) (□) and lg(I/II) (■) with Hammett constant (σ_p) of substituent X in the ligand.

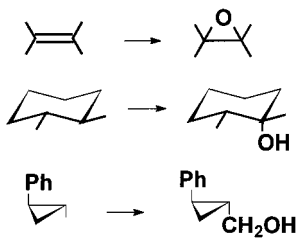

FIGURE 4. Tests for 2e⁻ mechanism of O transfer.

While iron porphyrin complexes are good models for hemoproteins, there are no simple diiron complexes which could be considered as adequate model for the carboxylate-bridged non-heme diiron class of metalloproteins. The above-mentioned diiron complexes synthesized by self-assembly method suffer from kinetic instability of their coordination sphere.[20] The stability of metal surroundings in non-heme diiron proteins is provided by immobilization of donor groups via their attachment to the polypeptide frame. To stabilize and control the coordination sphere of diiron models, we synthesized new polydentate framework ligands which contain bridging carboxylates linked with terminal donor groups.[26] However, the first generation of these ligands turned out to be too flexible, and the diiron models prepared showed rather poor catalytic activity in methane oxidation. Next we started synthesis of the second generation of diiron models, more preorganized for catalysis.

Functional Model of MMO: Hydroxylation of Methane by Iron Complexes in Zeolites

Nitrous oxide, N_2O, can be used as an O-donor for hydroxylation of a C−H bond in the presence of metal complexes for heterogeneous catalysts at high temperatures. Panov et al.[27] have shown that, at temperatures higher than 573 K, N_2O decomposes at Fe-containing ZSM5 zeolite (FeZSM5) by loading active centers (called α-centers) with oxygen. At temperatures lower than 573 K, α-oxygen-containing centers are thermally stable, but at 573−623 K they catalyze direct oxidation of benzene to phenol with N_2O, ensuring almost 100% selectivity.

We collaborated with Panov and his group when the same system was used for hydroxylation of methane.[28,29] Methane was found to react with α-oxygen at room temperature and lower temperatures. The product of the reaction is bound to the surface and cannot be identified by its desorption. At 520−570 K, the product decomposes, and formation of carbon monoxide is observed. But at low temperatures, the product can be extracted by a mixture of water and acetonitrile, and turned out to be methanol. *The amount of CH_3OH produced corresponds (92−100%) to methane consumed.* Methanol is observed in reaction with methane only when α-oxygen is loaded onto the surface.

To exclude any accidental source of methanol, experiments were carried out with methane-^{13}C and $(^{18}O)_\alpha$, given quantitative evidence that methanol is formed from methane and $(O)_\alpha$.

Intramolecular KIE was measured using the competition of C−H and C−D bonds in CH_2D_2 in the reaction with α-oxygen.[29] CHD_2OH and CH_2DOD were the only reaction products, their ratio being measured by ^1H NMR spectroscopy. In the temperature range 223−373 K, KIE changed from 5.5 to 1.9. These values mean that the rate-determining step of methane oxidation involves C−H bond cleavage.

There is some evidence favoring a dinuclear structure of the active iron complexes in the ZSM5 matrix. Results obtained by Mössbauer spectroscopy (Figure 5)[28,30] can be interpreted from the point of view of the FeZSM5 similarity with MMO. The Mössbauer spectral characteristics of iron complexes in both reduced and oxidized states in MMO and FeZSM5 are very close to each other.[28] Acceptance of an O atom from N_2O occurs only at the coordinatively unsaturated Fe(II) centers, and the intermediates formed have Mössbauer parameters[31] resembling the corresponding values for MMO intermediates **P** and **Q**.[8,9] The differences observed could be explained on the basis of a distinction in the coordination surroundings of iron in FeZSM5 and MMO.

We conclude that the FeZSM5−N_2O system may be considered as a good functional model of MMO for the step of methane oxidation.

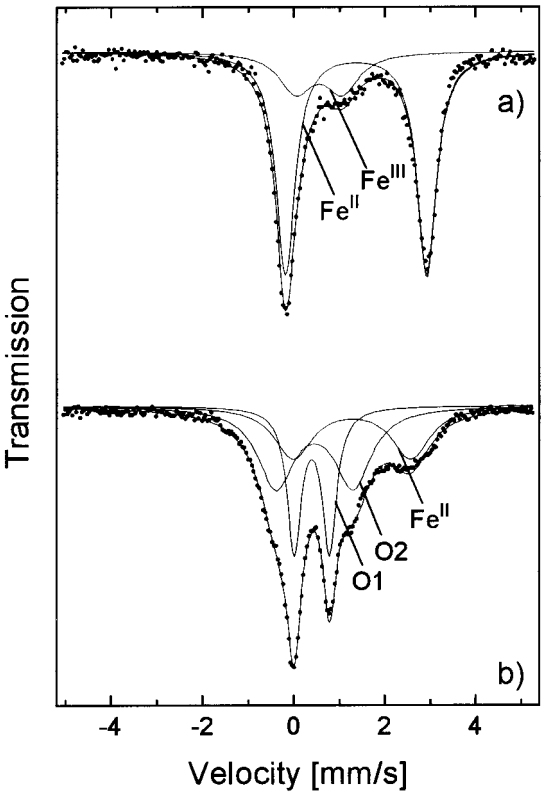

FIGURE 5. Mössbauer spectra of FeZSM5 before (a) and after (b) reaction with N_2O; **O1** and **O2** are CH_4 oxidizing intermediates.

Nature of Active Species and Mechanism of Its Formation from Dioxygen

Studies of iron porphyrin models of CP450 have led to the spectroscopic characterization of the active intermediate as an oxo-ferryl porphyrin π-cation radical, $P^{+\bullet}Fe^{IV}{=}O$.[11] Although this species has not been directly detected in the native CP450 system, recent spectroscopic data for the *m*-chloroperbenzoic acid-shunted system strongly support the possibility of its formation.[32] It has also been shown in iron porphyrin model systems that O^{2-} acceptors such as protons[33] or acyl cations[34] are essential for heterolytic cleavage of the O−O bond in the peroxide intermediate with formation of ferryl species and H_2O or acyl-OH.

While no transient intermediates of the CP450 catalytic cycle beyond oxy-CP450[5] have been detected yet,[7b] there is already good evidence for such intermediates in the case of MMO.[8,9] The most characterized of them are peroxide intermediate **P** and high-valent diiron intermediate **Q**, which were trapped by the freeze−quench kinetic technique and have been examined by a number of spectroscopic approaches.[8a,9a,12a] On the basis of the observation a single narrow quadrupole doublet in the Mössbauer spectrum of compound **Q**, this intermediate was concluded[8c] to have a symmetric structure, either diferryl or $Fe^{IV}{-}O{-}Fe^{IV}$. In 1995, a new mechanism for MMO, different from that for CP450, was proposed by A. A. Shteinman.[35] This so-called "bridged" mechanism involves the formation of a bis-μ-oxo-μ-carboxylatodiiron(IV) species **Q** directly from peroxide intermediate **P**

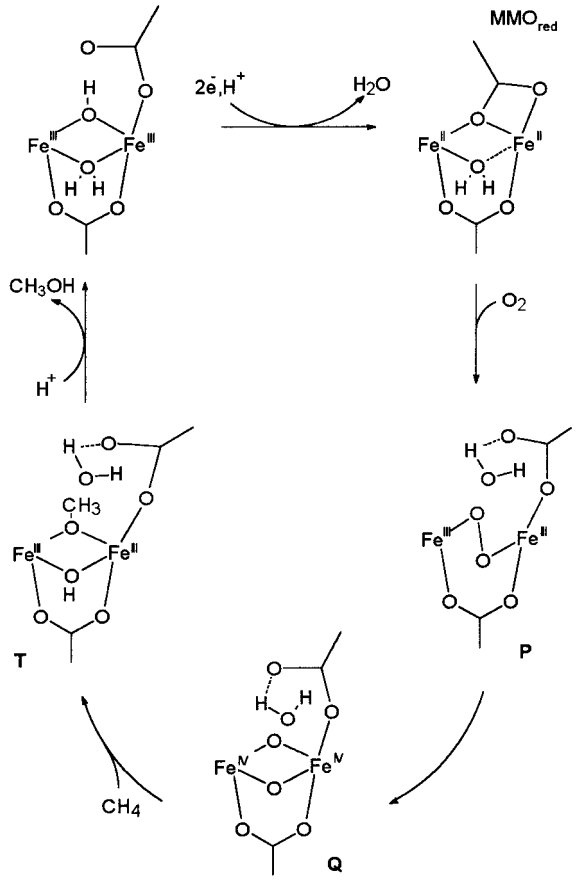

FIGURE 6. Bridge mechanism[35] of MMO.

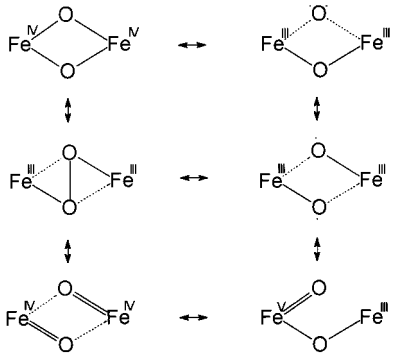

FIGURE 7. Resonance structures of putative core of intermediate **Q.**

without water molecule release (Figure 6). The variety of resonance structures for the proposed bis-μ-oxo-diiron-(IV) core of intermediate **Q** (Figure 7) indicates that it should be capable of displaying high reactivity together with good relative stability. There is some experimental support for the above-mentioned structure of this intermediate.[9d,12b]

The O_2 activation may involve initial end-on peroxide formation, followed by its transformation into a side-on peroxide. The bridge mechanism of O_2 activation in the active site of MMO permits both iron atoms to bind O^{2-} during the O−O bond cleavage in the **P** → **Q** transformation. Thus, there is no need for the participation of protons or the release of a water molecule in this stage, which is essential for the corresponding stage of the catalytic cycle of CP450. In contrast to the CP450 mechanism (Figure 2), a water molecule, another product of monooxygenase reaction, would be released during the reduction of the diferric core into the corresponding diferrous form in the bridge mechanism for MMO (Figure 6). This conclusion is supported by X-ray crystal structures of the resting diferric and reduced diferrous forms of MMO hydroxylase.[9b]

Mechanism of Oxygen Atom Transfer

Two mechanisms are usually considered for alkanes hydroxylation in biological systems: direct insertion of an oxygen atom into a C−H bond (*oxenoid mechanism*) and abstraction of a hydrogen atom with subsequent recombination of the free radical formed with an OH group on the metal (*oxygen rebound mechanism*).

High KIE is considered to be evidence for a linear transition state, which seems incompatible with a direct insertion mechanism, whereas isomerization in the process of hydroxylation is explained on the basis of the formation of an intermediate capable of forming an isomerized product, which is proposed to be a free radical. Thus, the oxygen rebound mechanism has been widely accepted recently for various hydroxylation systems.[11]

In model chemical systems, a traditional free radical mechanism often functions with OH radicals as the active species produced (vide supra). This mechanism can be preferable in polar media in the presence of water.

Apart from this radical mechanism, there exists clearly another mechanism, particularly in less polar media and hydrophobic surroundings, which is more selective and electrophilic and can easily involve such strong C−H bonds as that in methane.

In both biological and model chemical systems, the oxygen rebound mechanism, which is proposed as an alternative to the traditional free radical mechanism, at present gives rise to serious doubts. First, the evidence usually given for this mechanism no longer looks very convincing. As we have seen, a very high KIE was explained as due to involvement of proton tunneling, which does not necessarily require a linear transition state.[1a,15] When the KIE values become smaller (e.g., in more polar media) and can be attributed to the classic picture with a linear transition state, actually the tunneling can still be involved, since it is more likely that its contribution is decreased gradually than that it suddenly completely disappears.

In the full model system O_2 + HOAc + Zn + MV^{2+} in acetonitrile, KIE is low (as is often the case in polar solutions), but the 5/6 factor does not correspond to an H atom abstraction mechanism; it rather suggests electrophilic addition as the first step.[18] A high rate, even at very low temperatures (up to −50 °C) and 100% selectivity of methane hydroxylation in an iron zeolite system with α-oxygen,[29] is difficult to reconcile with any radical mechanism, taking into account the high C−H bond energy of methane.

It should be noted that the isomerization observed in biological and model chemical systems, while demon-

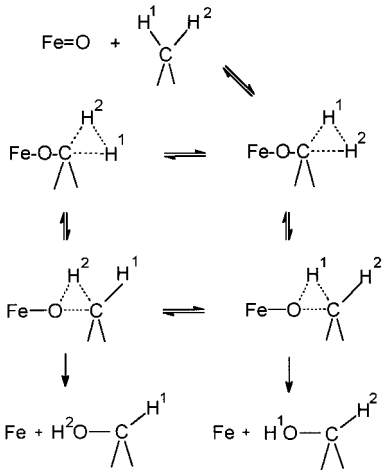

FIGURE 8. Mechanism of C−H bond hydroxylation via a five-coordinate carbon intermediate.[36]

strating the *stepwise* character of the process, is not necessarily due to free radicals as intermediates. Epoxidation of olefins as well as hydroxylation of aromatics is often accompanied by isomerization (formation of aldehydes alongside epoxides in the case of epoxidation and so-called NIH shift in the case of aromatics hydroxylation), but the origin of the isomerization is not formation of free alkyl radicals. Rather, the oxygen atom *bound to metal* adds to a double bond or to an aromatic molecule, giving intermediates capable of isomerizing. If a similar addition of the oxygen atom takes place also in the case of a C−H single bond, then the intermediate formed might be able to isomerize without free radical formation. Different from a free radical (which is a three-coordinate carbon compound), the product of oxygen atom addition to the C−H bond is a five-coordinate carbon intermediate, and A. F. Shestakov and A. E. Shilov proposed to call this mechanism a "five-coordinate carbon mechanism" (Figure 8).[36a]

The formation of a five-coordinate carbon intermediate is also possible for a bridging oxygen in $Fe^{IV}_2(\mu\text{-}O)_2$ active species.[35a] For methane, the smallest of the alkanes, one may suggest a nucleophilic co-action of the second O-bridge atom, maybe via H-bonding. This should stabilize the five-coordinate carbon intermediate. It may explain the possible hydrogen tunneling effect that has been found only in the case of methane.[8a] For larger alkanes, steric hindrances diminish the probability for the formation of such a doubly bonded intermediate.

The analysis of the isomerization results published in the literature from the point of view of the oxygen rebound mechanism and from the mechanism of five-coordinate carbon often witnesses in favor of the latter, for both biological and model systems.[1a] Let us consider, for example, a remarkable case of ethylbenzene hydroxylation by iodosylbenzene with chiral binaphthyl iron porphyrin (Figure 9), investigated by Groves and Viski.[11b] Ethylbenzene afforded 1-phenylethanol. Both *(R)*- and *(S)*-(1-deuterioethyl)benzenes give all possible isomers of 1-phenylethanol, *(R)*- and *(S)*-enantiomers, each containing either deuterium or protium next to the OH group. The major product in both cases (with retention of configuration) is

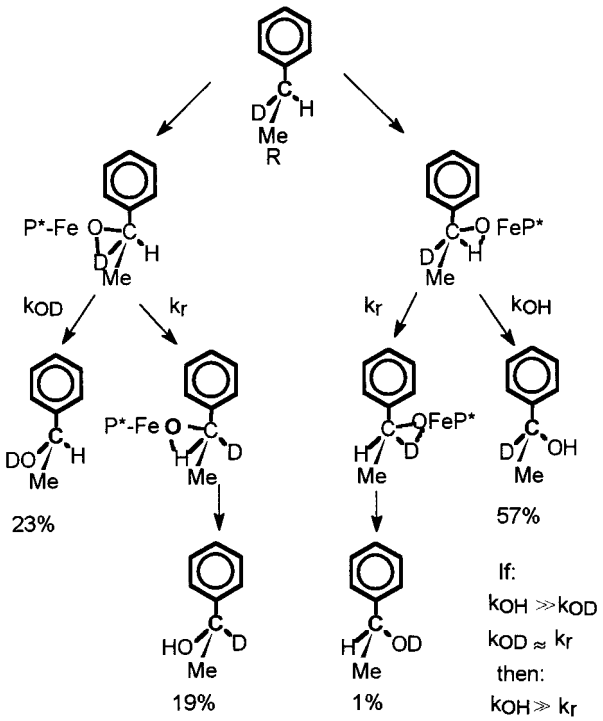

FIGURE 9. Scheme for hydroxylation of chiral deuterioethylbenzenes by iron(III) binaphthylporphyrin/PhIO.

Table 2. Observed and Calculated Distribution of the Products in Chiral Deuterioethylbenzene Hydroxylation

		1-phenylethanols			
$C_6H_5CHDCH_3$		R_H	R_D	S_H	S_D
R	exptl	23	19	1	57
	calcd	22.9	19.1	1.1	56.9
S	exptl	2	87	6	5
	calcd	1.7	87.3	6.0	5.0

virtually pure monodeuteriophenylethanol, whereas the second (minor) product, with the inverse configuration, contains nearly equal amounts of hydrogen and deuterium. This result leads to a natural explanation in the framework of the five-coordinate carbon mechanism. If the insertion of the O atom is accompanied by a considerable H−D isotope effect, then in the case of the C−H bond the insertion can be much faster than the isomerization, while in the case of the C−D bond insertion and isomerization can occur in parallel with comparable rates. Therefore, in the case of C−H hydroxylation, there will be retention of configuration at the C atom, while for C−D hydroxylation there will be partial inversion, leading to racemization.

The ratios of the products obtained for both (*R*)- and (*S*)-isomers make it possible to calculate the rate constant ratios for elementary steps in the hydroxylation.[11b] The obtained values of the parameters from the results of, for example, the (*R*)-enantiomer *allow us to predict the distribution of the products for the (S)-enantiomer without any additional postulate.* The results obtained in this way are in very good agreement with those from experiment (Table 2). The authors[11b] who analyze their results in the framework of the oxygen rebound mechanism regard the inversion leading to racemization as the process proceed-

ing *after* the abstraction of H or D from the substrate molecule. To explain the drastic difference in the behaviors of the radicals formed in these two reactions, they have to postulate that, for hydrogen atom abstraction from the *pro-R* position of ethylbenzene, the recombination of the radical formed with an OH group at iron proceeds without the radical rotation, while H abstraction from the *pro-S* position is accompanied by a high probability of rotation.

The KIE found for O atom insertion ($k_H/k_D = 23.3$) is close to that for cyclohexane hydroxylation with sodium hypochlorite in the biphasic system mentioned above.[16] In the case of a chiral porphyrin complex, voluminous naphthyl substituents connected with the porphyrin ring evidently create nonpolar surroundings in the vicinity of the active center, which leads to a high H−D kinetic isotope effect.

This example helps us to understand how, even for similar systems, one can observe both full retention of configuration and a considerable extent of isomerization. Thus, whereas for soluble MMO (sMMO), considerable isomerization is observed for chiral ethane substituted by D and T atoms in one of the methyl groups, for particular MMO (pMMO) there is 100% retention of configuration. It is more likely that the insertion-to-isomerization rate constant ratio changes with some structural and polar effects changes than that the mechanisms are completely different for pMMO as compared with sMMO.

Recently, the results obtained by several authors[9c,13] with the use of substituted cyclopropanes (radical clock method) also caused them to reconsider the mechanism of biological as well as model chemical hydroxylations. The use of more and more sensitive probes which had to isomerize within reasonable times of rebound steps did not lead to the expected isomerization of the substrates chosen. The combined experimental evidence compels authors[9c] to conclude that radical intermediates are not formed.

Conclusion

Analysis of the results described in this Account as well as those published in the literature for monooxygenases and their chemical analogues shows that, besides the traditional radical mechanism of C−H hydroxylation in the presence of metal complexes, another, five-coordinate, carbon mechanism exists which involves addition of an O atom to a C−H bond, followed by insertion into the C−H bond to form an alcohol (Figure 1b).

However, the exact nature of the latter mechanism remains to be elucidated and may be somewhat different for different systems. For example, the metal atom bound with oxygen might take part in the reaction, forming a M−C bond (Figure 1c). According to recent quantum chemical calculations, this is the case for MMO.[14]

It should be mentioned that the five-coordinate carbon mechanism for C−H bond activation apparently takes place also for metal complexes other than iron. Rhodium complexes are particularly interesting because they can activate methane similarly to iron complexes considered above. For example, such a mechanism can be suggested for oxidation and oxidative carbonylation of methane in the presence of rhodium complexes discovered recently by Sen et al.,[37] where oxorhodium active species may be proposed.

This work was supported by grants from the Russian Foundation for Basic Research and INTAS.

References

(1) (a) Shilov, A. E. *Metal Complexes in Biomimetic Chemical Reactions*; CRC Press: New York, 1997. (b) Karasevich, E. I.; Kulikova V. S.; Shilov, A. E.; Shteinman, A. A. Biomimetic alkane oxidation involving metal complexes. *Russ. Chem. Rev.* **1998**, *67*, 335−355. (c) Shteinman, A. A. Oxygen transfer catalysis by chemical analogs of monooxygenases. *Russ. Chem. Bull.* **1993**, *42*, 227−234.

(2) (a) Valentine, J. S., Foote, C. S., Greenberg, A., Liebman, J. F., Eds. *Active Oxygen in Biochemistry*; Chapmen and Hall: London, 1995. (b) Sheldon, R. A.; Kochi, J. K. *Metal-Catalyzed Oxidations of Organic Compounds*; Academic Press: New York, 1981. (c) Meunier, B. Metalloporphyrins as Versatile Catalysts for Oxidation Reactions and Oxidative DNA Cleavage, *Chem. Rev.* **1992**, *92*, 1411−1456.

(3) (a) Shilov, A. E.; Shteinman, A. A. Activation of Saturated Hydrocarbons by Metal Complexes in Solution. *Coord. Chem. Rev.* **1977**, *24*, 97−143. (b) Shilov, A. E.; Shul'pin, G. B. Activation of C−H Bonds by Metal Complexes. *Chem. Rev.* **1997**, *97*, 2879−2932.

(4) In this Account, we do not consider biological *anaerobic* oxidation, which possibly involves alkane reaction with a metal atom of a reaction center.[1a]

(5) Abbreviations used: CP450, cytochrome P-450; dpy, 2,2′-dipyridine; FeZSM5, Fe-containing ZSM5 zeolite; KIE, kinetic isotope effect; MMO, methane monooxygenase; oxy-CP450, O_2 complex of CP450; phen, 1,10-phenanthroline; RC, retention of configuration;

(6) This effect is not necessarily due to the protein matrix.[3a,35c]

(7) (a) Ortiz de Montellano, P. R., Ed. *Cytochrome P-450: Structure, Mechanism, and Biochemistry*, 2nd ed.; Plenum: New York, 1995. (b) Sono, M.; Roach, M. P.; Coulter, E. D.; Dowson, J. D. Heme-Containing Oxygenases. *Chem. Rev.* **1996**, *96*, 2841−2887.

(8) (a) Wallar, B. J.; Lipscomb, J. D. Dioxygen Activation by Enzymes Containing Dinuclear Non-Heme Iron Clusters. *Chem. Rev.* **1996**, *96*, 2625−2657. (b) Elango, N.; Radhakrishnan, R.; Froland, W. A.; Wallar, B. J.; Earhart, C. A.; Lipscomb, J. D.; Ohlendorf, D. H. Crystal structure of the hydroxylase component of methane monooxygenase from *Methylosinus trichosporium* OB3b. *Protein Sci.* **1997**, *6*, 556−568. (c) Lee, S. K.; Fox, B. G.; Froland, W. A.; Lipscomb, J. D.; Munck, E., A Transient Intermediate of the Methane Monooxygenase Catalytic Cycle Containing an $Fe^{IV}Fe^{IV}$ Cluster. *J. Am. Chem. Soc.* **1993**, *115*, 6450−6451.

(9) (a) Valentine, A. M.; Lippard, S. J. Principles of small molecule activation by metalloenzymes as exemplified by the soluble methane monooxygenase from *Methylococcus capsulatus* (Bath). *J. Chem. Soc., Dalton Trans.* **1997**, 3925−3932. (b) Rosenzweig, A. C.; Nordlund, P.; Takahara, P. M.; Frederick, C. A.; Lippard, S. J. Geometry of the soluble methane

monooxygenase catalytic diiron center in two oxidation states. *Chem. Biol.* **1995**, *2*, 409−418. (c) Valentine, A. M.; Wilkinson, B.; Liu, K. E.; Komar-Panicucci, S.; Priestley, N. D.; Williams, P. G.; Morimoto, H.; Floss, H. G.; Lippard, S. J. Tritirated Chiral Alkanes as Substrates for sMMO from *Methylococcus capsulatus* (Bath): Probes for the Mechanism of Hydroxylation. *J. Am. Chem. Soc.* **1997**, *119*, 1818−1827. (d) Lee, D.; Lippard, S. J. Structural and Functional Models of Dioxygen-Activating Centers of Non-Heme Enzymes RNR and sMMO. *J. Am. Chem. Soc.* **1998**, *120*, 12153−12154.

(10) Jiang, Y.; Wilkins, P. C.; Dalton, H. Activation of the hydroxylase of sMMO from *Methylococcus capsulatus* (Bath) by hydrogen peroxide. *Biochim. Biophys. Acta* **1993**, *1163*, 105−112.

(11) (a) Groves, J. T.; Han, Y. Z. Models and Mechanism of Cytochrome P-450 Action. In ref 7a, pp 3−48. (b) Groves, J. T.; Viski, P. Asymmetric Hydroxylation by a Chiral Iron Porphyrin. *J. Am. Chem. Soc.* **1989**, *111*, 8537−8538.

(12) (a) Que, L., Jr. Oxygen activation at non-heme diiron active sites in biology: lessons from model complexes. *J. Chem. Soc., Dalton Trans.* **1997**, 3933−3940. (b) Shu, L.; Nesheim, J. C.; Kauffmann, K.; Münck, E.; Lipscomb, J. D.; Que, L., Jr. An $Fe^{IV}_2O_2$ Diamond Core Structure for the Key Intermediate Q of Methane Monooxygenase. *Science* **1997**, *275*, 515−518. (c) Kim, C.; Chen, K.; Kim, J.; Que, L., Jr. Stereospecific Alkane Hydroxylation with H_2O_2 Catalyzed by an Iron(II) Tris(2-pyridylmethyl)amine Complex. *J. Am. Chem. Soc.* **1997**, *119*, 5964−5965.

(13) Newcomb, M.; Le-Tadic-Biadatti, M. H.; Chestney, D. L.; Roberts, E. S.; Hollenberg, P. F. A Nonsynchronous Concerted Mechanism for Cytochrome P-450 Catalyzed Hydroxylation. *J. Am. Chem. Soc.* **1995**, *117*, 12085−12091.

(14) (a) Yoshizawa, K.; Yamabe, T.; Hoffmann, R. Possible intermediates for the conversion of methane to methanol on dinuclear iron centers of MMO models. *New J. Chem.* **1997**, *21*, 151−161. (b) Siegbahn, P. E. M.; Crabtree, R. H. Mechanism of C−H Activation by MMO: Quantum Chemical Studies. *J. Am. Chem. Soc.* **1997**, *119*, 3103−3113.

(15) Khenkin, A. M.; Shilov, A. E. Biomimetic alkane oxidation in the presence of iron complexes. *New J. Chem.* **1989**, *13*, 659−667.

(16) (a) Sorokin, A. B.; Khenkin, A. M. Isotope Effect in Aliphatic Hydroxylation by a Cytochrome P-450 Models. *J. Chem. Soc., Chem. Commun.* **1990**, 45−46. (b) Sorokin, A.; Robert, A.; Meunier B. Intramolecular Kinetic Isotope Effects in Alkane Hydroxylations Catalyzed by Manganese and Iron Porphyrin Complexes. *J. Am. Chem. Soc.* **1993**, *115*, 7293−7299.

(17) Karasevich, E. I.; Khenkin, A. M.; Shilov, A. E. A Chemical Model of Cytochrome P-450: Monooxygenase-like Activation of Dioxygen. *J. Chem. Soc., Chem. Commun.* **1987**, 731−733.

(18) Karasevich E. I.; Anisimova, B. L.; Rubailo, V. L.; Shilov, A. E. Kinetics of cyclohexane oxidation by molecular oxygen in the presence of iron porphyrins and reductant. *Kinet. Katal.* **1993**, *34*, 650−661.

(19) Muradov, N. Z.; Shilov, A. E.; Shteinman, A. A. New models of monooxygenase on the basis of Sn(II) compounds. *React. Kinet. Catal. Lett.* **1975**, *2*, 417−423.

(20) Kurtz, D. M., Jr. Oxo- and Hydroxo-Bridged Diiron Complexes: A Chemical Perspective on a Biological Unit. *Chem. Rev.* **1990**, *90*, 585−606.

(21) (a) Shteinman, A. A. Synthesis and Properties of Dinuclear μ-Oxo-μ-Carboxylato Iron(III) Complexes with Labile Coordination Site. *Mendeleev Commun.* **1992**, 155−157. (b) Shteinman, A. A. Alkane oxidation catalyzed by μ-oxo-μ-carboxylato-diiron(III) dipyridine complexes. *Dokl. Akad. Nauk SSSR* **1991**, *321*, 775−779 [*Dokl. Chem.*, **1991**, No. 4 (Engl. Transl.)]

(22) Gritsenko, O. N.; Nesterenko, G. N.; Shteinman A. A. Effect of substituents in the ligand on oxidation of alkanes catalyzed by dinuclear oxo-bridged iron complexes. *Russ. Chem. Bull.* **1995**, 2415−2417.

(23) Kulikova, V. S.; Gritsenko, O. N.; Shteinman, A. A. Molecular mechanism of alkane oxidation involving dinuclear iron complexes. *Mendeleev Commun.* **1996**, 119−120.

(24) No such effect was ever observed for chain radical oxidation.

(25) Gritsenko, O. N.; Nesterenko, G. N.; Kulikova, V. S.; Shteinman, A. A. Hydrocarbon Oxidation by Hydroperoxides Catalyzed by Binuclear Fe(III) Complexes. *Kinet. Catal.* **1997**, *38*, 699−706.

(26) Trukhan, V. M.; Polukhov, V. V.; Sulimenkov, I. V.; Ovanesyan, N. S.; Dodonov, A. F.; Shteinman, A. A. The first structural and functional model of MMO. *Kinet. Catal.* **1998**, *39*, 788−791.

(27) Panov, G. I.; Uriarte, A. K.; Rodkin, M. A.; Sobolev, V. I. Generation of active oxygen species on solid surfaces. *Catal. Today* **1998**, *41*, 365−385.

(28) Panov, G. I.; Sobolev, V. I.; Dubkov, K. A.; Parmon, V. N.; Ovanesyan, N. S.; Shilov, A. E.; Shteinman, A. A. Iron complexes in zeolites as a new model of methane monooxygenase. *React. Kinet. Catal. Lett.* **1997**, *61*, 251−258.

(29) Dubkov, K. A.; Sobolev, V. I.; Talsi, E. P.; Rodkin, M. A.; Watkins, N. H.; Shteinman, A. A.; Panov, G. I. Kinetic isotope effects and mechanism of biomimetic oxidation of methane and benzene on FeZSM-5 zeolite. *J. Mol. Catal.: A* **1997**, *123*, 155−161.

(30) Ovanesyan, N. S.; Sobolev, V. I.; Dubkov, K. A.; Panov, G. I.; Shteinman, A. A. The nature of the active oxidant in biomimetic oxidation of methane over FeZSM-5 zeolite. *Russ. Chem. Bull.* **1996**, *45*, 1509−1510.

(31) Ovanesyan, N. S.; Shteinman, A. A.; Dubkov, K. A.; Sobolev, V. I.; Panov, G. I. Mössbauer spectroscopic investigation of iron state in FeZSM-5 + N_2O system. *Kinet. Catal.* **1998**, *39*, 792−796.

(32) Egawa, T.; Shimada, H.; Ishimura, Y. Evidence for Compound I Formation in the Reaction of Cytochrome $P450_{cam}$ with m-Chloroperbenzoic Acid. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1464−1469.

(33) Weiss, R. Oxoiron Porphyrin Species with High-Valent Iron. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1709−1712.

(34) Khenkin, A. M.; Shteinman, A. A. Mechanism of alkane oxidation by peroxocomplexes of iron porhyrins in the presence of acylating agents: model for O_2 activation by Cytochrome P-450. *Chem. Commun.* **1984**, 1219−1220; *Oxid. Commun.* **1983**, *4*, 433−441.

(35) (a) Shteinman, A. A. The mechanism of methane and dioxygen activation in the catalytic cycle of methane monooxygenase. *FEBS Lett.* **1995** *362*, 5−9; *Russ. Chem. Bull.* **1995**, *44*, 975−984. (b) Shteinman, A. A. The $Fe^{IV}_2(\mu\text{-}O)_2$ cluster and bridge O_2 activation at the active center of methane monooxygenase. *Russ. Chem. Bull.* **1997**, *46*, 1599−1605. (c) Shtein-

man, A. A. Does the non-heme monooxygenase sMMO share a unified oxidation mechanism with the heme monooxygenase cytochrome P-450? *J. Biol. Inorg. Chem*. **1998**, *3*, 325−330.

(36) (a) Shestakov, A. F.; Shilov, A. E. Five-coordinate carbon hydroxylation mechanism. *J. Mol. Catal. A* **1996**, 105, 1−7; *Russ. J. Gen. Chem*. **1995**, *65*, 559−569. (b) Karasevich, E. I.; Shestakov, A. F.; Shilov, A. E. Catalytic alkane hydroxylation via five-coordinate carbon intermediate: dynamic aspects. *Kinet. Katal.* **1997**, *38*, 782−789.

(37) Lin, M.; Hogan, T. E.; Sen, A. Catalytic Carbon−Carbon and Carbon−Hydrogen Bond Cleavage in Lower Alkanes. Low-Temperature Hydroxylations and Hydroxycarbonylations with Dioxygen as the Oxidant. *J. Am. Chem. Soc.* **1996**, *118*, 4574−4580.

AR980009U