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Methane Monooxygenase Catalyzed Oxygenation of 1,1-Dimethylcyclopropane. Evidence for Radical and Carbocationic Intermediates†

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ABSTRACT: Methane monooxygenase catalyzes the oxygenation of 1,1-dimethylcyclopropane in the presence of O_2 and NADH to (1-methylcyclopropyl)methanol (81%), 3-methyl-3-buten-1-ol (6%), and 1-methylcyclobutanol (13%). Oxygenation by $^{18}O_2$ using the purified enzyme proceeds with incorporation of ^{18}O into the products. Inasmuch as methane monooxygenase catalyzes the insertion of O from O_2 into a carbon–hydrogen bond of alkanes, (1-methylcyclopropyl)methanol appears to be a conventional oxygenation product. 3-Methyl-3-buten-1-ol is a rearrangement product that can be rationalized on the basis that enzymatic oxygenation of 1,1-dimethylcyclopropane proceeds via the (1-methylcyclopropyl)carbinyl radical, which is expected to undergo rearrangement with ring opening to the homoallylic 3-methyl-3-buten-1-yl radical in competition with conventional oxygenation. Oxygenation of the latter radical gives 3-methyl-3-buten-1-ol. 1-Methylcyclobutanol is a ring-expansion product, whose formation is best explained on the basis that the 1-methylcyclobutyl tertiary carbocation is an oxygenation intermediate. This cation would result from rearrangements of carbocations derived by one-electron oxidation of either radical intermediate. The fact that both 3-methyl-3-buten-1-ol and 1-methylcyclobutanol are produced suggests that the oxygenation mechanism involves both radical and carbocationic intermediates. Radicals and carbocations can both be intermediates if they are connected by an electron-transfer step. A reasonable reaction sequence is one in which the cofactor (μ-oxo)diiron reacts with O_2 and two electrons to generate a hydrogen atom abstracting species and an oxidizing agent. The hydrogen-abstracting species might be the enzymic radical or another species generated by the iron complex and O_2. Oxygenation then could proceed by abstraction of a hydrogen atom from the substrate to form a radical, followed by electron transfer from the radical to the oxidizing species to form a carbocation. The carbocation would be quenched by oxygen associated with the oxygenation cofactor to generate the product.

Methane monooxygenase catalyzes the oxygenation of methane in methanotrophic bacteria according to eq 1.[1] The

$$H^+ + CH_4 + NADH + O_2 \rightarrow CH_3\text{–}OH + H_2O + NAD^+ \quad (1)$$

enzymes from *Methylococcus capsulatus* (Bath) and *Methylosinus trichosporium* (OB3b) are complexes of the following three proteins: (a) An oxygenase designated as component A that contains a (μ-oxo)diiron complex and an organic radical. The oxygenase has an overall M_r of 220 000 and comprises three subunits of M_r 54 000, 42 000, and 17 000, respectively, with two copies of each subunit and two (μ-oxo)diiron complexes in each enzyme particle. (b) A flavo-

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[1] Abbreviations: NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NMR, nuclear magnetic resonance; GC, gas chromatography; GC-MS, gas chromatographic mass spectroscopy.

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protein of M_r of 44000 designated component C that contains an iron–sulfur cluster (Fe_2S_2). (c) A coupling factor designated component B of M_r of 16000 (Dalton, 1980; Woodland & Dalton, 1984; Green & Dalton, 1985; Woodland et al., 1986; Fox et al., 1988, 1989; Prince et al., 1988). Little is known about the oxygenation mechanism, about the nature of reaction intermediates, or about the roles of the cofactors in this reaction. The overall reaction is analogous to cytochrome P-450 catalyzed oxygenations. However, methane monooxygenase contains no heme cofactor. The (μ-oxo)diiron complex presumably reacts with O_2 to form an oxygenating species. The structure of this species is unknown.

We here report the results of initial experiments designed to generate information about the nature of substrate-derived reaction intermediates. Methane monooxygenase catalyzed oxygenation of 1,1-dimethylcyclopropane produces (1-methylcyclopropyl)methanol, 3-methyl-3-buten-1-ol, and 1-methylcyclobutanol. The latter two products stem from carbon skeletal rearrangements of reaction intermediates, which are likely to be substrate-derived radicals and carbocations on the mechanistic pathway for oxygenation.

Experimental Procedures

Methylosinus trichosporium (OB3b) were grown with methane as the sole carbon source in a medium containing mineral salts. Methane monooxygenase was assayed with propene as the oxygenation substrate and by measurement of propene oxide formation by gas chromatography (Woodland et al., 1986). The components of methane monooxygenase were purified essentially as described by Green and Dalton (1985) and modified by Fox et al. (1989). 1,1-Dimethylcyclopropane was purchased from Pfaltz and Bauer, Inc.; (1-methylcyclopropyl)methanol, 3-methyl-3-buten-1-ol, 3-methyl-2-buten-1-ol, 2-methyl-3-buten-1-ol, and 2-methyl-3-buten-2-ol were purchased from Aldrich. All were redistilled before use. 1-Methylcyclobutanol was synthesized by heating (1-methylcyclopropyl)methanol with 1 M HCl at 100 °C for 60 min. The product was isolated by distillation and characterized by comparison of its mass spectrum and NMR spectrum with published spectra for this compound (Diekman et al., 1968).

The enzymatic reactions were conducted in solutions in which the enzyme complex was reconstituted by mixing the three components together with 1,1-dimethylcyclopropane, O_2, and NADH in MOPS buffer. The oxygenation reaction mixtures contained air as the O_2 source, 1.5 mM 1,1-dimethylcyclopropane, 0.33 mg/mL component A, 0.028 mg/mL component B, 0.07 mg/mL component C, 5 mM NADH, 20 mM MOPS buffer, and 0.08 M NaCl at pH 7.5 in a total volume of 0.4 mL. In the usual incubation the reaction was terminated by extraction with chloroform, and the products in the extracts were analyzed by GC and GC-MS and compared with standards. In a few experiments methylene chloride, which emerged earlier than chloroform from the GC column, was used in the extraction to determine whether any enzymatic products had been masked by the chloroform peak. No such product appeared. In the experiment of Figure 1 the reactions were terminated by extraction with chloroform at times ranging from 30 s to 10 min. GC analysis was carried out on a Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector, a recorder, and a $^1/_8$ in. × 30 ft stainless steel column packed with Carbopack c 80/100 (Supelco) coated with 0.2% Carbowax-1500. The chromatograph was operated at 130 °C (160 °C injection) and a flow rate of 10 mL/min, with N_2 as the mobile phase. GC-MS analysis was carried out on a Kratos MS25 system in the Department of Chemistry at the University of Wisconsin—Madison.

Characterization of Oxygenation Products. Oxygenation products derived from 1,1-dimethylcyclopropane were identified by comparison of their GC retention times and mass spectra with those of authentic samples. Compound **1** in Figure 1 exhibited a GC retention time of 52 min, and the mass spectrum by GC-MS analysis consisted of the following fragments (M^+, intensity relative to that of the base ion): 71, 1.0 (base); 59, 1.0; 58, 19.7; 57, 1.5; 43, 9.7; 41, 1.7; 39, 1.0; 31, 0.7; 27, 1.0. The GC retention time and mass spectrum were found to be identical with those of 1-methylcyclobutanol. Compound **2** in Figure 1 exhibited a GC retention time of 65 min, and the mass spectrum consisted of the following fragments (M^+, intensity relative to that of the base ion): 71, 1.0 (base); 68, 0.6; 67 <0.5; 59, <0.5; 58, 8.8; 57, 4.5; 56, 4.1; 55, 2.0; 54, <0.5; 53, 0.9; 45, <0.5; 44, 0.6; 43, 1.2; 42, <0.5; 41, 2.5; 40, 0.7; 39, 1.7; 31, 1.4; 30, 0.7; 29, 2.1; 28, 0.5; 7.0, 1.4. The GC retention time and mass spectrum were found to be identical with those of (1-methylcyclopropyl)methanol. Compound **3** in Figure 1 exhibited a GC retention time of 89 min, and the mass spectrum consisted of the following fragments (M^+, intensity relative to that of the base ion): 71, 1.0 (base); 69, 0.5; 68, 3.8; 67, 4.5; 57, 1.0; 56, 3.9; 55, 2.1; 53, 2.2; 51, 0.9; 50, 0.8; 44, 0.4; 43, 2.7; 42, 0.9; 41, 10; 40, 2.5; 39, 9.7; 38, 1.3. The GC retention time and mass spectrum were identical with those of authentic 3-methyl-3-buten-1-ol. No other products were observed, and none of the three products exhibited the GC retention times of 2-methyl-3-buten-2-ol, 2-methyl-3-buten-1-ol, or 3-methyl-2-buten-1-ol.

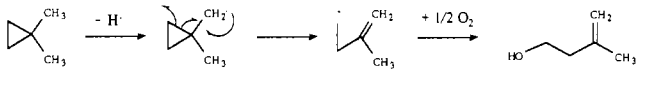
Preparation of 1-Methylcyclobutanol in $H_2{}^{18}O$ and in $^{18}O_2$. 1-Methylcyclobutanol was prepared from (1-methylcyclopropyl)methanol in $H_2{}^{18}O$ by acid-catalyzed isomerization and by methane monooxygenase catalyzed oxygenation of 1,1-dimethylcyclopropane. In the nonenzymatic isomerization the reaction mixture contained 20 μL of (1-methylcyclopropyl)-methanol, 50 μL of H_2O, 100 μL of $H_2{}^{18}O$ (97% ^{18}O), and 18 μL of concentrated HCl. After heating at 100 °C for 100 min, the solution was extracted with 0.2 mL of $CDCl_3$. In the enzymatic oxygenation the reaction mixture contained 45 μL of MOPS buffer (75 mM at pH 7.0 with 0.3 M NaCl), 155 μL of methane monooxygenase (495 μg of component A, 28 μg of component B, and 53 μg of component C), 20 μL of NADH (0.1 M), 200 μL of $H_2{}^{18}O$ (97% ^{18}O), and 4 mL of 1,1-dimethylcyclopropane gas in a sealed 8-mL reaction vessel. After 15 min at 30 °C, the solution was extracted with 0.2 mL of $CDCl_3$. The $CDCl_3$ extracts from the two reactions were subjected to analysis by GC-MS to determine the ^{18}O content of 1-methylcyclobutanol and (1-methylcyclopropyl)-methanol.

The enzymatic reaction was repeated inside a glass vial containing Ar and $^{18}O_2$ (98% enrichment) under conditions otherwise as nearly identical as possible with those of the experiment in $H_2{}^{18}O$. The chloroform extract of products was analyzed by GC-MS to determine the ^{18}O contents of 1-methylcyclobutanol and (1-methylcyclopropyl)methanol.

Results

Oxygenation of 1,1-Dimethylcyclopropane. 1,1-Dimethylcyclopropane is an interesting substrate for monooxygenases because of the propensity for cyclopropylcarbinyl radicals to undergo ring opening to homoallylic radicals on the nanosecond time scale (Griller & Ingold, 1980). Should a cyclopropylcarbinyl radical lie on the catalytic pathway, there is a reasonable chance for it to rearrange to a homoallylic radical and give a noncyclic product. In the case of 1,1-di-

Scheme I



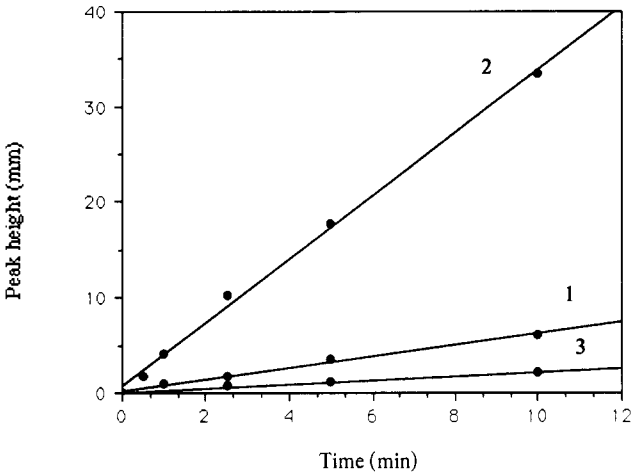
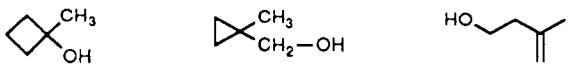


FIGURE 1: Time course for methane monooxygenase catalyzed formation of oxygenation products from 1,1-dimethylcyclopropane. The reaction mixtures contained methane monooxygenase, 1,1-dimethylcyclopropane, and oxygenation cosubstrates as described under Experimental Procedures, except that the enzyme level was increased. At the indicated times the reactions were arrested and the oxygenation products isolated by extraction of the solutions with chloroform. The chloroform extracts were analyzed for compounds **1**, **2**, and **3** by gas chromatography as described under Experimental Procedures. The relative amounts of oxygenation products were determined by measurements of peak height on the chart record and plotted versus time in the figure.

methylcyclopropane, radical formation and isomerization should follow the course of Scheme I. If the homoallylic radical should be oxygenated, the product would be 3-methyl-3-buten-1-ol. Monooxygenation of alkanes by cytochrome P-450 and other monooxygenases is thought to involve radicals as intermediates; however, evidence for radicals is limited and difficult to obtain, owing in part to their extreme instability and the attendant difficulties in directly detecting them. Substrates such as 1,1-dimethylcyclopropane offer a means to probe the reaction mechanism when rearrangements such as that in Scheme I can be observed.

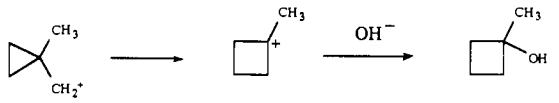
Methane monooxygenase acts on hydrocarbons containing up to eight carbons (Dalton, 1980). As an oxygenation substrate 1,1-dimethylcyclopropane reacts at a rate of about one-third that of propene under comparable conditions. Propene is an epoxidation substrate that reacts slightly faster than methane and is often used as the substrate for assaying the enzyme. Monooxygenation of 1,1-dimethylcyclopropane gives three products that are characterized by GC retention times of 52 (**1**), 65 (**2**, major product), and 89 min (**3**). As



1 1-methylcyclobutanol; **2** (1-methylcyclopropyl)methanol; **3** 3-methyl-3-buten-1-ol

shown by Figure 1, products **1**, **2**, and **3** are generated in constant ratios over a period of 10 min at relative rates of 1:7.5:0.5, respectively. In 12 trials the product compositions averaged 13% **1**, 81% **2**, and 6% **3**. Compounds **1**, **2**, and **3** are 1-methylcyclobutanol, (1-methylcyclopropyl)methanol, and 3-methyl-3-buten-1-ol, respectively, as determined by comparisons of their GC retention times and mass spectra with those of authentic samples of these compounds (see Experimental Procedures).

Scheme II



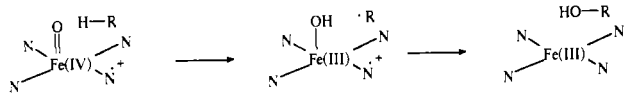
The major product, (1-methylcyclopropyl)methanol (**2**), is the conventional oxygenation product resulting from insertion of an oxygen into a carbon–hydrogen bond of a methyl group, as in the oxygenation of methane in eq 1. It is reasonable to assume that this product is formed by a mechanism very similar to that for the oxygenation of methane. The other two compounds are unlike conventional products, in that their carbon skeletons have undergone rearrangements. These rearrangements presumably occurred at points on the catalytic pathway that lie between the cleavage of a carbon–hydrogen bond and the insertion of oxygen. That is, the rearrangement products may be derived from one or another reaction intermediate on the pathway to the formation of (1-methylcyclopropyl)methanol. Isomerization of a reactive intermediate to another reactive species, followed by oxygenation of the isomerized form, can account for isomeric products.

3-Methyl-3-buten-1-ol (**3**) is the expected product derived from the intermediate formation and rearrangement of a (4-methylcyclopropyl)carbinyl radical to a homoallylic radical as in Scheme I. If this radical were generated as an intermediate, it could be expected to rearrange very rapidly, perhaps at a rate that would compete with the oxygenation rate. If the rearrangement and oxygenation rates were comparable, both 3-methyl-3-buten-1-ol and (1-methylcyclopropyl)-methanol would be produced by oxygenation of the homoallylic and cyclopropylcarbinyl radicals. Both compounds are produced, indicating that the (1-methylcyclopropyl)carbinyl radical is an intermediate.

1-Methylcyclobutanol as an Oxygenation Product. 1-Methylcyclobutanol (**1**) is not expected as a product of the rearrangement of a radical intermediate. Its formation is most simply rationalized on the basis of the involvement of a carbocationic intermediate. For example, the (1-methylcyclopropyl)methyl carbocation would undergo a ring expansion typical of species of this type to form a methylcyclobutyl carbocation, as shown in Scheme II (Caserio et al., 1960). The latter would then be oxygenated to 1-methylcyclobutanol. Other reaction routes are included in Scheme IV. The basic characteristic of all pathways leading from 1,1-dimethylcyclopropane to 1-methylcyclobutanol is that all reasonable pathways involve carbocations as intermediates.

The appearance of 1-methylcyclobutanol as a major product suggests that the 1-methylcyclobutyl carbocation in Scheme II may have been generated in the oxygenation reaction. Is this cation an oxygenation intermediate? This cation can be produced from (1-methylcyclopropyl)methanol by acid catalysis, and it is the intermediate in the isomerization of (1-methylcyclopropyl)methanol to 1-methylcyclobutanol. Since (1-methylcyclopropyl)methanol is the major oxygenation product, it is conceivable that 1-methylcyclobutanol might have been produced by a secondary isomerization of (1-methylcyclopropyl)methanol under the conditions of the enzymatic reaction. We know, from experiments in which (1-methylcyclopropyl)methanol was incubated under the enzymatic oxygenation conditions, that 1-methylcyclobutanol is produced from (1-methylcyclopropyl)methanol, albeit at a rate that is slower than the overall oxygenation rate. Since only 13% of the total oxygenation products from 1,1-dimethylcyclopropane is 1-methylcyclobutanol, it is possible that a significant fraction of this product arises from isomerization of (1-methylcyclo-

Scheme III



propyl)methanol, the primary oxygenation product.

Nonenzymatic isomerization of cyclopropylcarbinols to cyclobutanols has been explained on the basis of initial acid-catalyzed dehydration to a primary carbocation, such as that shown in Scheme II, followed by ring expansion to the tertiary methylcyclobutyl cation and quenching with water (Caserio et al., 1960). This would lead to the incorporation of oxygen from water into 1-methylcyclobutanol. We have verified this for (1-methylcyclopropyl)methanol by carrying out the isomerization in $H_2{}^{18}O$ (62% ^{18}O) and analyzing the 1-methylcyclobutanol for ^{18}O. The analysis confirmed the incorporation of ^{18}O into 1-methylcyclobutanol. The ratios M/M + 2 for the fragments *m/e* 58 and 43 were 0.62 and 0.66, respectively, corresponding to 61% ^{18}O enrichment.

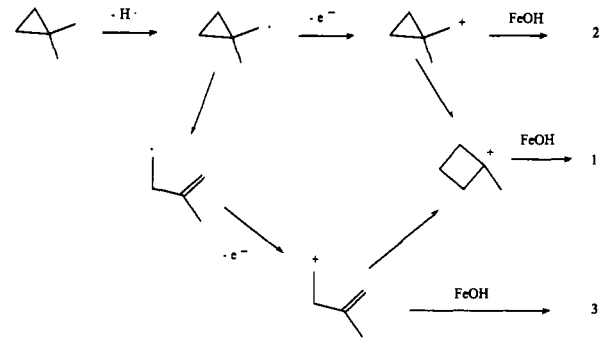
To examine the origin of oxygen in 1-methylcyclobutanol produced in the oxygenation of 1,1-dimethylcyclopropane, we conducted the reaction in $H_2{}^{18}O$ (48% ^{18}O) and analyzed 1-methylcyclobutanol for ^{18}O. In the mass spectroscopic analysis, observing the major ion fragments of masses 58 and 43 for unenriched samples, the ratios M/M + 2 (4/1) revealed a 20% enrichment of ^{18}O in 1-methylcyclobutanol. [(1-Methylcyclopropyl)methanol produced in the same reaction contained no ^{18}O above natural abundance.] Therefore, less than half of the 1-methylcyclobutanol (42%) was generated by nonenzymatic isomerization of (1-methylcyclopropyl)-methanol with incorporation of ^{18}O. The remainder (58%) must have been produced by another reaction that did not allow solvent oxygen to be incorporated. This other reaction was presumably enzymatic oxygenation of 1,1-dimethylcyclopropane by O_2.

To verify the origin of the balance of the oxygen in 1-methylcyclobutane, we carried out the oxygenation of 1,1-dimethylcyclopropane in $^{18}O_2$ (98%) and analyzed both 1-methylcyclobutanol and (1-methylcyclopropyl)methanol for ^{18}O. Oxygenation of hydrocarbons in extracts containing methane monooxygenase had previously been shown to proceed with incorporation of oxygen from O_2 into the product (Higgins & Quayle, 1970). The (1-methylcyclopropyl)methanol contained 98% ^{18}O, verifying that purified methane monooxygenase incorporates oxygen from O_2 into a conventional oxygenation product. The 1-methylcyclobutanol, analyzed as described above, contained 53% ^{18}O. Therefore, 54% of the oxygen in this compound was derived from O_2 and must have been incorporated by enzymatic oxygenation.

Discussion

Substrate-Derived Intermediates in Methane Monooxygenase Catalyzed Oxygenation. The methane monooxygenase catalyzed oxygenation of 1,1-dimethylcyclopropane proceeds with partial ring opening and partial ring expansion. These findings indicate that the reaction proceeds by a more complex mechanism than that proposed for oxygenation of hydrocarbons by cytochrome P-450 (McMurry & Groves, 1986; Ortiz de Montellano, 1986). The rebound mechanism for cytochrome P-450 is briefly outlined in Scheme III. In this mechanism an oxygenating P-450 heme species is formulated as an oxoiron(IV) cation radical, with an unpaired electron and a positive charge delocalized in the porphyrin ring. This species is often alternatively formulated as $Fe^{V}{=}O$, with the porphyrin ring in its usual oxidation state. Oxygenation

Scheme IV



proceeds by the mechanism in Scheme III, in which the oxygenating species first abstracts a hydrogen from the substrate, generating a substrate radical and a hydroxoporphyrin. In the rebound step the substrate radical abstracts a hydroxyl group from the hydroxoporphyrin to form the product. This mechanism is consistent with stereochemical evidence (Groves, 1978). Recent experiments show that bicyclo[2.1.0]pentane reacts as a substrate for cytochrome P-450 to form both 2-hydroxybicyclo[2.1.0]pentane and the radical rearrangement product 3-cyclopenten-1-ol (Ortiz de Montellano & Stearns, 1987). On the basis of the results of product partitioning experiments and measurement of the rate constant for the radical rearrangement and on the assumption that the rebound mechanism is applicable, the calculated rate constant for the rebound step in this reaction is 2×10^{10} s^{-1} (Bowry et al., 1989).

The mechanism of the methane monooxygenase reaction appears to be more complex than that in Scheme III. The oxygenation products from 1,1-dimethylcyclopropane clearly indicate the involvement of both substrate radicals and substrate carbocations as intermediates. Both radicals and cations can be involved if they are connected on the catalytic pathway by a redox step. The most probable first step is abstraction of a hydrogen atom from a methyl group of 1,1-dimethylcyclopropane. The (1-methylcyclopropyl)carbinyl radical then can react by several pathways to give the observed products. The pathways shown in Scheme IV involve one-electron oxidations of radicals to carbocations. The cations react with oxygen, presumably with oxygen bound to the oxygenated (μ-oxo)diiron cofactor, to form the alcohol products. Scheme IV allows for the formation of 1-methylcyclobutanol by either of two routes. In one pathway the (1-methylcyclopropyl) carbinyl radical is oxidized to the (1-methylcyclopropyl)methyl carbocation, and this cation undergoes ring expansion to the more stable 1-methylcyclobutyl cation. In the other route the (1-methylcyclopropyl)carbinyl radical rearranges to the homoallylic radical, which undergoes oxidation to the homoallylic carbocation. The latter cation can undergo either oxygenation to 3-methyl-3-buten-1-ol or rearrangement to the more stable 1-methylcyclobutyl cation, which by oxygenation produces 1-methylcyclobutanol. The chemically favored route should be via the (1-methylcyclopropyl)methyl carbocation, since the (1-methylcyclopropyl)carbinyl radical should be more easily oxidized than the homoallylic radical.

Scheme IV accounts for the oxygenation of 1,1-dimethylcyclopropane in terms of the intermediate formation of radical and carbocationic intermediates. The generation of these intermediates at the enzymic active site is presumably the function of the (μ-oxo)diiron and the organic radical associated with component A of methane monooxygenase. A reduced form of the oxygenase, in which both iron ions are Fe(II) [Fe^{II}–O–Fe^{II}], reacts with O_2 and methane or propene to

produce methanol or propene oxide, respectively (Fox et al., 1989).

The transformations in Scheme IV could be brought about by the cofactors of methane monooxygenase if the oxygenated (μ-oxo)diiron and enzymic radical can generate a hydrogen atom abstracting species and an oxidizing agent. The hydrogen abstractor might be the enzymic radical itself. Alternatively, the hydrogen abstractor might be generated by an interaction of the enzymic radical with the oxygenated iron cofactor, or it might be derived from the oxygenated cofactor itself. The oxidizing species could be an oxygenated form of the cofactor (μ-oxo)diiron. The oxygenation mechanism utilizing these species can reasonably be formulated as follows: (a) An enzyme cofactor radical generates a substrate radical by abstraction of a hydrogen from the substrate. (b) The oxygenated iron cofactor oxidizes the substrate radical to a carbocation. (c) The carbocation is oxygenated by reaction with oxygen in the oxygenated iron complex. The last step essentially entails quenching of the substrate carbocation by the oxygenated iron complex, a process that can be expected to be facile. The oxidation of a substrate radical by the oxygenated iron complex is an electron transfer from a radical to a good oxidizing agent and is also expected to be facile. The difficult process is the first step, abstraction of a hydrogen atom from the substrate.

The results presented here suggest but do not directly prove that both substrate radicals and substrate carbocations are intermediates in methane monooxygenase catalyzed oxygenation. The rearrangement product 3-methyl-3-buten-1-ol observed in this work reflects the propensity of the (1-methylcyclopropyl)carbinyl radical to undergo rearrangement to the homoallylic radical in Scheme IV. The formation of 1-methylcyclobutanol as a major product indicates the involvement of one or more carbocationic intermediates in the reaction mechanism. The (methylcyclopropyl)carbinyl system is particularly suited to reveal the involvement of radicals and carbocations as intermediates, and for this reason 1,1-dimethylcyclopropane and related molecules may prove to be useful in studies of other enzymes.

While the reaction of 1,1-dimethylcyclopropane can reveal mechanistic details that are masked in the reactions of simple substrates such as methane, it is also conceivable that special properties of the (1-methylcyclopropyl)carbinyl radical, formed in the first chemical step, could perturb certain steps of the reaction mechanism. This radical is expected to be more easily oxidized than a simple alkyl radical, such as the methyl radical generated from methane. Given this fact, it is conceivable that the formation of carbocationic intermediates in the oxygenation of 1,1-dimethylcyclopropane may reflect the special redox properties of the (1-methylcyclopropyl)carbinyl radical rather than the reaction mechanism for the oxygenation of simple alkanes. It will be necessary to examine the reactions of other substrates for methane monooxygenase to determine whether carbocations are intermediates in the oxygenation of all substrates.

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