Regiochemical Variations in Reactions of Methylcubane with *tert*-Butoxyl Radical, Cytochrome P-450 Enzymes, and a Methane Monooxygenase System

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Abstract: Reactions of methylcubane (**1**) with the *tert*-butoxyl radical (*t*-BuO•), with cytochrome P-450 enzymes, and with a methane monooxygenase (MMO) system have been studied. For the purpose of product characterization, authentic samples of 2-methylcubyl and 4-methylcubyl derivatives were prepared. 2-Methylcubanecarboxylic acid (**9b**) is a new compound prepared from cubanecarboxylic acid. The key synthetic reactions were (1) metalation and subsequent iodination of the 2-position of (diisopropylcarbamoyl)cubane to effect the initial functionalization, (2) lithium-for-iodine exchange and methylation followed by reduction to give 2-methyl-1-[(diisopropylamino)methyl]-cubane, and (3) dimethyldioxirane oxidation of this amine to give **9b**. The known 4-methylcubanecarboxylic acid (**9d**) was prepared here by a route related to that employed for **9b**. Reactions of acids **9b** and **9d** with methyllithium gave the corresponding methyl ketones which were oxidized by *m*-chloroperoxybenzoic acid to provide authentic samples of 2- and 4-methylcubanol acetates (**3b** and **3d**). Reaction of **1** with *t*-BuO• in the presence of 2,2,5,5-tetramethylisoindole-*N*-oxyl radical (TMIO•) at 40−55 °C gave mainly cube-substituted products in confirmation of the report (Della, E. W.; Head, N. J.; Mallon, P.; Walton, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 10730) that hydrogen atom abstraction by the electrophilic alkoxyl radical at low temperature occurs at the cubyl C−H positions. In a competition experiment at 42 °C, methylcubane was at least 3.5 times more reactive toward *t*-BuO• than cyclohexane, indicating that the cubyl positions in **1** are ≥40 times more reactive than the methyl positions in **1** (per hydrogen) toward the alkoxyl radical. Oxidation of **1** by enzymes gave alcohol products that were converted to their acetate derivatives for identification and quantitation. Microsomal cytochrome P-450 enzymes from rat and the rat purified P-450 isozyme CYP2B1 hydroxylated **1** at all positions, whereas the reconstituted MMO system from *Methylococcus capsulatus* (Bath) hydroxylated **1** only at the methyl position. The differences in regioselectivity suggest that the transition states for hydrogen abstraction by the alkoxyl radical and for enzyme-catalyzed hydroxylation differ considerably. The results are consistent with a model for concerted enzyme catalyzed hydroxylation of **1** involving "side-on" approach to the C−H bond of substrate.

Enzyme-catalyzed oxidations of unactivated hydrocarbons to alcohols at ambient temperatures are among the most remarkable biochemical conversions known, especially since the C−H bond dissociation energies (BDE) of the hydrocarbons range from 93 kcal/mol for a tertiary position to 105 kcal/mol for methane.[2] Two families of iron-containing enzymes effect hydrocarbon hydroxylations. The ubiquitous cytochrome P-450 enzymes contain a single iron atom, in the iron protoporphyrin IX prosthetic group, at the active site.[3] The hydroxylase enzymes in the soluble methane monooxygenase (MMO) systems of methanotrophic bacteria are dimeric protein assemblies with carboxylate bridged diiron active sites,[4−6] and X-ray crystal structures of the reduced and oxidized hydroxylase enzyme from one species, *Methylococcus capsulatus* (Bath), are now available.[7−9] A consensus mechanism for hydrocarbon hydroxylation by P-450 enzymes that evolved over the past two decades involves hydrogen atom abstraction from substrate by an electrophilic high-valent iron−oxo species to give a radical intermediate that is subsequently trapped in a pseudo-first-order hydroxyl transfer process, the so-called oxygen rebound step (k_{ox}) (Scheme 1).[10−13] Recent results indicate, however, that cytochrome P-450 hydroxylation occurs in a non-synchronous

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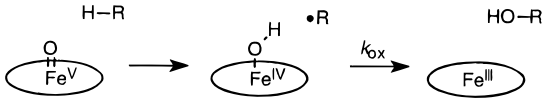
Scheme 1



concerted process with a very short lived radical-like moiety formed in the reactive ensemble.[14]

Despite the differences in the active sites of the P-450 and MMO hydroxylase enzymes, the mechanism of MMO hydroxylation might resemble that of the P-450 enzymes. Evidence has been reported for the intermediacy of radicals in MMO hydroxylations[15−20] and in hydroxylation by the alkane hydroxylase in *Pseudomonas oleovorans*,[21] but a hypersensitive probe gave little and no rearranged alcohol when oxidized by the soluble MMOs of *Methylosinus trichosporium* OB3b and *M. capsulatus* (Bath), respectively.[22] These same soluble MMOs give only small amounts (ca. 20−35%) of inverted ethanol products in oxidations of chiral ethane (CH_3CHDT),[23,24] and interestingly, the copper-containing particulate MMO from *M. capsulatus* (Bath) has been reported to oxidize chiral ethane with no inversion.[25]

The mechanisms of enzymatic hydroxylations have been investigated by a number of approaches. One experimental design involves comparisons of substrate reactivities toward enzymes and mechanistically well-characterized chemical reagents. Enzyme catalyzed hydroxylations can be influenced by steric effects in the enzyme-active site, but the hepatic P-450 enzymes are typically broad-spectrum oxidants that display the property of metabolic switching (or isotopically sensitive branching).[11,26−29] This property requires that multiple positions of the substrate can be selectively accessed by the enzyme's active site after the point of catalytic commitment. The MMO hydroxylases, for which the native substrate is methane, may be more restricted. For example, the MMO system from *Methylococcus capsulatus* (Bath) did not display metabolic switching in hydroxylation of *trans*-2-phenylmethylcyclopropane,[22] a substrate for which metabolic switching by P-450 enzymes has been demonstrated.[29]

Despite the steric *caveat*, hydroxylations of simple alkanes by hepatic P-450 enzymes generally follow the pattern of reactivity observed in hydrogen atom abstraction reactions of the alkanes with alkoxyl radicals. For example, microsomal rat liver P-450 and a purified P-450 isozyme were reported to hydroxylate octane at C(1) and C(2) in statistically adjusted ratios of 0.13:1 and 0.03:1, respectively.[26,30] These results are qualitatively consistent with the observed reactivity of the *tert*-butoxyl radical (*t*-BuO•) toward alkanes;[31] at 40 °C, this radical abstracts hydrogen atoms from primary and secondary centers with a statistically corrected ratio of 0.08:1. The C−H reactivity ratios are in step with the bond dissociation energy (BDE) values of primary and secondary C−H bonds,[2] and because one expects only slight variations in the transition states for almost any type of functionalization reaction occurring at primary and secondary C−H bonds, the results are not highly informative.

Recent studies of methylcubane (**1**) indicate that this compound is special with regard to its C−H bond energies versus C−H reactivities. Cubyl C−H BDE values have been computed to be about 104 kcal/mol,[32−34] significantly greater than the 98 kcal/mol BDE of a methyl C−H.[2] Nonetheless, reaction of **1** with *t*-BuO• at low temperatures, as observed by EPR spectroscopy, has been reported to give only cube-centered radicals.[35] *Ab initio* calculations of the transition states for hydrogen atom abstraction from the cubyl and methyl positions of **1** by an alkoxyl radical provide an explanation.[36] Polarization in the transition states for cubyl C−H bond abstractions results in considerable positive charge development at cubyl carbon and reduced activation energies in comparison to that for C−H abstraction from the methyl group, for which no special stabilizing mechanism is available.[36]

Thus, methylcubane is a potentially sensitive probe for studying the polarization of C−H functionalization reactions. Hydrogen atom abstraction by an electron-deficient center should result in predominant (or even exclusive) functionalization of the cubyl C−H positions as reported for the alkoxyl radical abstraction reaction.[35] In the present work, we have confirmed the high reactivity of the cubyl C−H bonds in **1** toward the *t*-BuO• radical in reactions conducted at 40−55 °C, and we have investigated oxidation of **1** by P-450 enzymes and an MMO system.

Results

Syntheses and Product Identifications. Some of the cubane derivatives used in this work were reported previously, but most of the synthetic preparations are new. Methylcubane (**1**) has been prepared by several routes,[35,37−40] but we prepared it by $LiAlH_4$ reduction of cubanecarboxylic acid, mesylation of the alcohol thus formed, and reduction of the mesylate with

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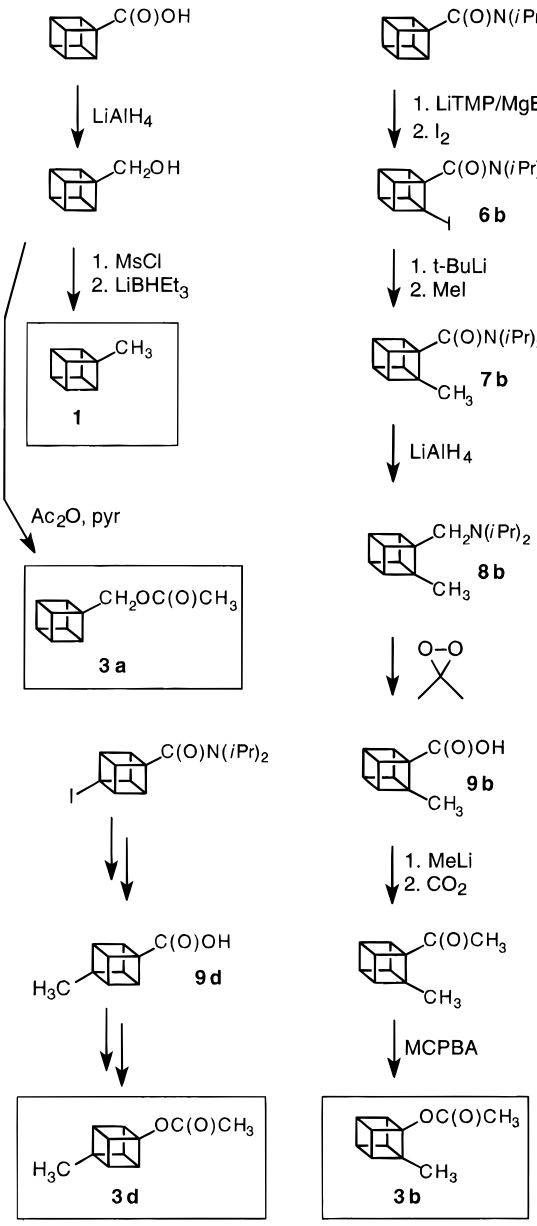
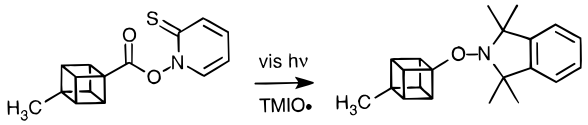
Scheme 2



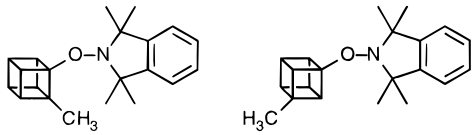
$LiBHEt_3$. Scheme 2 summarizes most of the synthetic reactions for production of the cubyl derivatives. For continuity, we have adopted a numbering scheme wherein all methyl-substituted derivatives of **1** are designated with the identifier **a** and all 2-, 3- and 4-substituted derivatives of **1** are designated with identifiers **b**, **c**, and **d**, respectively. Cubylmethanol acetate (**3a**) was readily prepared from cubanecarboxylic acid. The complete synthetic pathway for production of 2-methyl-substituted cubane derivatives is shown in the scheme. We note that 2-methylcubanecarboxylic acid is a new compound that was fully characterized. A similar sequence was employed for the production of 4-methyl-substituted cubane derivatives *via* the known 4-methylcubanecarboxylic acid.[35,41−43] Methods for production of 3-methyl-substituted cubane derivatives are currently under development, and authentic samples in this series are not yet available.

As discussed below, we studied reactions of **1** with *t*-BuO• generated *in situ* at 40−55 °C in the presence of the nitroxyl radical 2,2,5,5-tetramethylisoindole-*N*-oxyl (TMIO•).[44] Insofar as possible, we isolated and identified the TMIO adducts formed in coupling reactions of TMIO• with the alkyl radicals produced by hydrogen atom abstraction from **1**. A commonly employed alternative route to nitroxyl radical coupling products involves production of an alkyl radical by decarboxylation of an acyloxyl radical in the presence of TMIO•,[45] and Barton has demonstrated that *N*-acyloxypyridine-2-thione derivatives (or PTOC esters)[46] can be used as alkyl radical sources in the presence of a nitroxyl radical.[47,48] Thus, for comparison purposes, an authentic sample of the 4-methylcubyl adduct **2d** was prepared by photochemical decomposition of the PTOC ester from 4-methylcubanecarboxylic acid in the presence of TMIO•.



2 d

Attempts to produce an authentic sample of the 2-methylcubyl TMIO adduct **2b** from 2-methylcubanecarboxylic acid via its PTOC ester derivative were unsuccessful. We presume that production of the desired cubyl radical occurred as expected but that the TMIO adduct **2b** did not form or was unstable.[49,50] Because 3-methylcubanecarboxylic acid is not available as a starting material, we could not prepare the 3-methylcubyl TMIO adduct **2c** by an independent route. Compound **2c** was identified as a product from the reaction of **1** with *t*-BuO• in the presence of TMIO•, however (see below).



2 b **2 c**

Alkoxyl radical abstraction of a hydrogen atom from the methyl group on **1** will give the cubylcarbinyl radical (**4**). Radical **4** rearranges to **5** so fast that it cannot be trapped by TMIO• at the concentrations employed in our studies.[51,52] Radical **5** is relatively long-lived[51] and should be trapped by TMIO•. Because even the hydrocarbon products produced by hydrogen atom trapping of radical **5** are known to be unstable,[51] however, TMIO adducts from **5** cannot be expected to be stable. As discussed below, a mixture containing several unstable TMIO adducts in which the cubane nucleus was destroyed was isolated from reaction of **1** with *t*-BuO•.[53]

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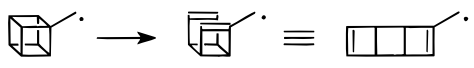
(53) In an attempt to characterize some of the components in this mixture, we allowed the PTOC derivative from cubylacetic acid (a source of **4**)[51,52] to react in the presence of TMIO•. A mixture of products was obtained, and ^{1}H-NMR spectral analysis indicated that no component contained an intact cube. These products slowly decomposed on standing. Further, they



4 **5**

In the enzyme-oxidation reactions of **1**, alcohol products (cubylmethanol and methylcubanols) were formed. Cubylmethanol can be analyzed by GC, and some enzyme product mixtures were analyzed by GC-MS immediately after the oxidation reactions. Cubyl alcohols are fragile,[54,55] and one should not expect these products to survive a GC analysis. Therefore, the alcohol products were quantitated by conversion to the acetate derivatives (products **3**) and subsequent GC and GC-mass spectral analysis. Authentic samples of three of the four possible products, acetates **3a**, **3b**, and **3d**, were prepared (see Scheme 2). No authentic sample of 3-methylcubanol acetate (**3c**) was available, but in the GC-mass spectral analyses of the P-450 oxidation products, a base-line resolved component with a retention time slightly shorter than those of **3b** and **3d** was observed. We concluded that this component was acetate **3c** on the basis of its GC retention time and its mass spectral fragmentation pattern, which was quite similar to those of **3b** and **3d** and included a highly characteristic $(M - 43)^+$ peak from loss of acetate (see Experimental Section).

Reactions of Methylcubane with *t*-BuO•. Della, Walton, and co-workers observed an EPR spectrum from reaction of methylcubane (**1**) with *t*-BuO• at −120 °C consistent with production of cube-centered radicals in a 3:3:1 ratio corresponding to the statistics of the cubyl hydrogen types.[35] The EPR spectrum lacked any evidence for production of the cubylcarbinyl radical (**4**) or its rearrangement products. We wanted to confirm the high reactivity of the cubyl hydrogens toward *t*-BuO• at temperatures similar to those employed in enzyme studies. Thus, we examined reactions of **1** with *t*-BuO• at 40−55 °C. The *t*-BuO• radical was generated by thermolysis of di-*tert*-butyl hyponitrite.[56] The nitroxyl radical TMIO• at 0.3 M concentration was used as a radical trap.[44,45]

The reactions gave complex mixtures of unreacted **1** and TMIO adducts. Preparative TLC (silica gel) gave two fractions containing TMIO adducts. The faster eluting TLC fraction contained two cubyl products in a 4:1 ratio by both HPLC and ^{1}H NMR spectral analysis. The minor product was **2d**, and the major product was identified as **2c**. The ^{1}H NMR spectrum for the major product clearly indicated a cubane nucleus by the presence of characteristic signals in the cubyl region (δ 3.4−4.3). The pattern consisted of a set of resonances in the 1:2:2:1 ratio characteristic of 3-methylcubyl derivatives; the corresponding signals in a 2-methylcubyl product appear in a 2:1:1:2 ratio.

The slower eluting TLC fraction consisted of several TMIO adducts that obviously arose from radicals in which the cubane nucleus had been destroyed. The ^{1}H NMR spectrum of this fraction contained no signals in the cubyl C−H region and no cubylmethyl singlet but, instead, complex sets of vinyl proton signals. One of the minor sets of vinyl resonances from this mixture corresponded to signals from a product obtained from the reaction of cubylcarbinyl radical in the presence of TMIO•,[53] but we estimate that the component giving rise to this set of vinyl proton signals constituted less than 5% of the total amount of isolated products. We ascribe the major portion of the "noncubyl" TMIO adducts to products resulting from the 2-methylcubyl radical, for which we were unable to obtain a stable TMIO adduct.

Several reactions of **1** with *t*-BuO• in the presence of TMIO• were conducted at both 40 and 55 °C. The relatively slow thermolysis of di-*tert*-butyl hyponitrite, the *t*-BuO• precursor, at these temperatures required that the reactions be conducted over several days. The TLC fraction containing rearranged TMIO products (slower eluting) was considerably reduced in yield in those reactions conducted at 55 °C rather than 40 °C. Apparently, over time, the rearranged TMIO adducts gave hydrocarbon or polymeric products. Therefore, the yields of rearranged TMIO adducts given below are less than the actual yields. In contradistinction, the cubyl TMIO adducts **2c** and **2d** were stable upon standing.

When the *t*-BuO•/TMIO• reaction of **1** was conducted at 40 °C, GC analysis of the crude product mixture showed that 42% of unreacted methylcubane remained. Preparative TLC gave the TMIO adducts in 65% isolated yield based on unrecovered methylcubane. HPLC analysis of the product mixture showed that it contained **2c**, **2d**, and the mixture of rearranged TMIO adducts in absolute yields of 38%, 9%, and 18%, respectively. It is clear that at 40 °C the alkoxyl radical abstracted cubyl hydrogens in large part.

Given the large amount of unidentified and unstable TMIO adducts obtained from the reactions of **1** with *t*-BuO• and the fact that TMIO• cannot trap the cubylcarbinyl radical (**4**) before rearrangement, we resorted to a competition experiment in order to establish the relative reactivity of the C−H positions in **1**. A limiting amount of *t*-BuO• was produced in a benzene solution containing **1** (0.14 mM), cyclohexane (0.19 mM), and TMIO• in a reaction conducted at 42 °C. Preparative TLC isolation of the TMIO adducts followed by HPLC analysis showed that the product mixture contained **2c** (7%), **2d** (2%), rearranged TMIO adducts from **1** (7%), and the cyclohexyl TMIO adduct (6%). Independent experiments showed that the cyclohexyl TMIO adduct was stable to the reaction and isolation conditions. Adjusting for initial concentrations, **1** is at least 3.5 times as reactive as cyclohexane toward *t*-BuO• at 42 °C.

The computational results of Hrovat and Borden indicate that the transition state for alkoxyl radical abstraction of a methyl C−H in **1** does not enjoy any unusual polar stabilization.[36] Therefore, the methyl group in **1** should react with *t*-BuO• with about the same rate constant as does a typical alkane methyl group. At 40 °C, secondary hydrogen atoms of alkanes are about 12 times as reactive toward *t*-BuO• as primary hydrogen atoms.[31] From this fact and the results of the competition study with **1** and cyclohexane, we conclude that the cubyl hydrogens in **1** are ≥40 times as reactive (per hydrogen) as the methyl hydrogens in **1** at 42 °C; *i.e.* $\Delta\Delta G^{\ddagger} \geq 2.3$ kcal/mol. This conclusion agrees with the low-temperature EPR results of Della, Walton, and co-workers.[35] The cubyl hydrogens in **1** are much more reactive toward an electrophilic agent than one would predict solely from BDE values.

Enzyme-Catalyzed Hydroxylations of Methylcubane. Methylcubane was subjected to enzymatic oxidations by liver microsomal P-450 from phenobarbital-treated rats, a purified rat P-450 isozyme (CYP2B1),[57] and the methane monooxygenase from *M. capsulatus* (Bath). The crude product mixtures were treated with acetic anhydride and pyridine to convert the alcohol products to acetates, and the resulting mixtures were analyzed by GC and GC-mass spectrometry for identification and quantitation. Table 1 contains the results. The total yields of oxidation products obtained in the P-450 studies were less

were demonstrated to be unstable when subjected to the conditions used for the alkoxyl radical reaction with **1**. Nevertheless, one set of vinyl ^{1}H-NMR signals appeared to match those of a minor component isolated from the reaction of **1** with *t*-BuO• in the presence of TMIO•.

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Table 1. Products from Enzymatic Oxidations of Methylcubane

	normalized yields[a]					
enzyme	**3a**	**3b**	**3c**	**3d**	C/M[b]	total % yield[c]
microsomes[d]	1.00	0.89	1.30	1.40	1.2	0.5
	1.00	0.79	1.36	1.29	1.1	0.5
	1.00	0.82	1.32	1.29	1.1	0.4
CYP2B1[e]	1.00	0.63	1.44	1.90	1.2	0.4
	1.00	0.43	1.40	1.50	1.0	0.5
	1.00	0.70	1.07	1.70	1.0	1.1
MMO[f]	1.00	0	0	0	0	0.5[g]

[a] Normalized relative yields (per hydrogen) of acetate products after derivatization of the enzyme product mixtures are given. [b] Normalized ratio (per hydrogen) of cubyl to methyl hydroxylation. [c] Total yield of acetate products. [d] Microsomes from livers of phenobarbital treated rats. [e] Reconstituted purified rat P-450 isozyme. [f] Reconstituted methane monooxygenase system from *M. capsulatus* (Bath). [g] Average yield for four individual experiments.

than those we typically observe (ca. 2%) with other substrates under the same conditions, indicating that methylcubane was a poor substrate. This result might have been a consequence of the poor solubility of methylcubane in the buffer solutions or the intrinsic reactivity of the substrate, but the origin of the effect is not important for the interpretation of the results. The good agreement in the results from the microsomal P-450 and the purified isozyme oxidations is satisfying because CYP2B1 is the predominant isozyme expressed in livers of phenobarbital-treated rats.[57]

The P-450 enzyme oxidations of **1** afforded nearly a statistical ratio of alcohols, and the total amount of cubyl oxidation to methyl oxidation was close to unity when computed on a per hydrogen basis. Assuming for the sake of argument that intermediate radicals were produced in the P-450 oxidations, then a portion of the cubylcarbinyl radicals (**4**) resulting from hydrogen abstraction at the methyl group might have rearranged in competition with rebound resulting in a reduced yield of **3a**.[58,59] Irrespective of any possible reduction in yield of the methyl functionalized product **3a**, the important result in the P-450 hydroxylations is the *large* amount of methyl oxidation that was observed.

Oxidations of **1** by the MMO system from *M. capsulatus* (Bath) gave cubylmethanol exclusively. When the reaction mixture was treated with acetic anhydride and pyridine, the only detectable acetate product was **3a**.

Cubane was a poor substrate for P-450, requiring extended reaction times or addition of a small amount of organic co-solvent to the buffer to effect hydroxylation in low yields (ca. 0.05%) even in comparison to the already low yields of oxidation products from **1**. On the basis of the successful oxidation of cubane when the organic co-solvent was used and the fact that **1** was oxidized at the cube positions successfully, we conclude that the small amount of cubane oxidation reflects poor solubility of the substrate in buffer.

Cubane was *not* a substrate for the MMO system. This result might again reflect poor solubility of cubane in the buffer. It is also possible, however, (1) that the substrate accessed the enzyme but was precluded from the active site or, more likely, (2) that the substrate reached the active site and simply was not oxidized.

(58) Recent attempts to calibrate the rate constant for oxygen rebound gave apparent k_{ox} values that varied by nearly three orders of magnitude.[59] The "best value" for k_{ox} selected by Atkinson and Ingold was 2.4×10^{11} s^{-1}, about 7 times as fast as radical **4** rearranges.[52] The slowest apparent k_{ox} value was 1.4×10^{10} s^{-1}, which is about 0.4 times as fast as rearrangement of **4**.

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Discussion

We begin our discussion by considering the present results for methylcubane reactions. The observed changes in regioselectivity for hydroxylation by *t*-BuO•, P-450, and MMO suggest significant differences in the mechanisms. This conclusion follows from the computational results of Hrovat and Borden on the reactions of methylcubane with radicals[36] and, for the P-450 reactions, from the well-established fact that the isozyme we employed is a broad-spectrum oxidant that has demonstrated metabolic switching with a wide range of substrates.

In the computed transition states for hydrogen abstraction at the cubyl positions in **1** by the methoxyl radical, high positive charge develops at the cubyl carbons (on the order of +0.3), but little charge development occurs at the methyl carbon in the corresponding transition state for methyl C−H abstraction.[36] The net result is that, despite the fact that the cubyl bonds are stronger,[32−34] the relative transition state energies for cubyl C−H abstractions are 2.0−2.4 kcal/mol *less* than that for abstraction of a methyl C−H. This computational difference in relative transition state energies, which is remarkably similar to the limit of $\Delta\Delta G^{\ddagger} \geq 2.3$ kcal/mol established by our competition study with **1** and cyclohexane, reflects an inherent property of methylcubane, specifically the high stability of (incipient) cubyl cations. In fact, even the nucleophilic methyl radical is predicted to abstract the cubyl C−H at C(4) in **1** in preference to the methyl C−H in **1** ($\Delta\Delta G^{\ddagger} = 0.4$ kcal/mol).[36]

In the cytochrome P-450 hydroxylations of **1**, the ratio of reactions at the cubyl positions to reaction at the methyl position, statistically corrected for the number of hydrogens, is approximately unity. Despite the lack of a crystal structure, the particular isozyme studied here, CYP2B1, almost certainly has a large and open active site. It oxidizes many substrates and, more importantly, demonstrates metabolic switching with isotopically labeled substrates. As noted earlier, this phenomenon requires that the position of oxidation is determined after the commitment to catalysis; that is, a selection between multiple oxidation sites is made *after* substrate binding. This feature rules out the possibility that the enzyme can bind substrate in a variety of orientations that predetermine the positions of oxidation. If the enzyme does not enforce regioselectivity by binding, and if the inherent reactivity of methylcubane with an electrophilic agent (as the iron−oxo at the active site of P-450 is presumed to be) strongly favors abstraction reactions at cubyl C−H positions, then the observed regioselectivity in reaction of **1** with the enzyme presents a conundrum. A possible explanation for the observed regiochemistry is that *conventional* hydrogen atom abstraction to give substrate radicals from **1** does not occur in the P-450 hydroxylation reaction.

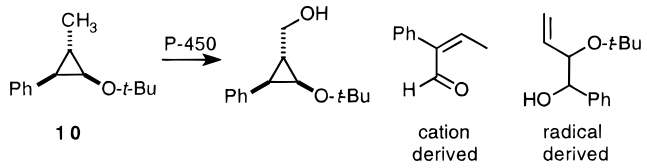
A similar case can be made for the MMO hydroxylation results although it is less secure in that metabolic switching has not been observed with this enzyme. We detected products corresponding only to oxidation at the methyl position of **1**, and we conservatively estimate the methyl-to-cubyl reactivity in **1** to be ≥20:1. This behavior represents a *change* in the relative activation free energies for reactions at the cubyl and methyl positions of >4 kcal/mol for the MMO hydroxylase enzyme in comparison to *t*-BuO• if the various sites in methylcubane had equivalent access to the active site diiron center. The MMO hydroxylase can oxidize a variety of substrates,[22] and modeling studies of the enzyme crystal structure suggest that cubane will fit easily into the active site.[60] Of course, substrate **1** does access the active site of the MMO because it is oxidized, and the hydrogens on the C(2) positions must have been brought into close proximity to the diiron core

(60) Rosenzweig, A. C.; Lippard, S. J. Unpublished results.

when the methyl C−H position was oxidized. Thus, we conclude that the failure of the MMO to hydroxylate the cubyl positions in **1** (and also in cubane) most likely indicates a true lack of reactivity of the C−H bonds at the cubyl positions. Again, the lack of reactivity at the cubyl C−H positions indicates that a conventional hydrogen abstraction to form a substrate radical intermediate is not involved in the hydroxylation mechanism.

If conventional hydrogen atom abstractions are not involved in the enzymatic hydroxylations of **1**, is there an inherent feature of this substrate that might explain the regioselectivity in the context of an acceptable alternative mechanism? We believe that there is.

A recent study of P-450 hydroxylation of a probe substrate (**10**) designed to distinguish between radical and cationic intermediates indicated that the "radical" involved in the process was actually a component of the transition state of a non-synchronous insertion reaction with a lifetime of only 70 fs.[14]

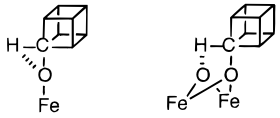


10 cation derived radical derived

Similarly, an extremely short lifetime (<100 fs) of any "radical" species in hydroxylation of probe substrates was indicated in studies of the MMO from *M. capsulatus* (Bath).[22] In the case of hydroxylations by a closely related MMO, from *Methylosinus trichosporium* OB3b, a "radical" lifetime of only 170 fs was implicated *both* from probe studies[22] and from studies of the oxidation of chiral CH_3CHDT.[23] These very short lifetimes preclude conventional hydrogen abstraction reactions which involve linear arrays of C−H−O in the transition state geometries. The distance between carbon and oxygen in minimized computed linear transition structures for hydrogen abstractions by alkoxyl radicals is about 2.5 Å,[36,61] and the translation of 1 Å necessary to form a C−O bond cannot occur on the subpicosecond time scale.[14] Subpicosecond phenomena include vibrations, and it is most reasonable to conclude that the short radical lifetimes in the hydroxylation reactions indicate that carbon and oxygen are at bonding distances to one another (ca. 1.5 Å) in the transition states and that "capture" of the radical involves a single Fe−O stretch.

The model for hydroxylation deduced from the above considerations is that attack of the C−H bond involves a triangular, as opposed to linear, C−H−O assembly in the transition state. Abstraction of hydrogen and collapse to form the O−C bond are concerted but non-synchronous due to the fact that a C−H vibration is "faster" than an Fe−O stretch. This description is in essence the "oxenoid" model for hydrocarbon oxidation presented by Hamilton over two decades ago.[62] Our more detailed description of atomic organization is similar to that recently proposed by Shteinman[63] for MMO hydroxylations and by Shestakov and Shilov[64] for both P-450 and MMO hydroxylations.

Attack of a C−H bond in a "side-on" manner in the hydroxylation reactions provides two possible explanations for the observed regioselectivity in enzyme-catalyzed hydroxylations of methylcubane. On the one hand, one might envision transition state structures such as **11** for P-450 and **12** for MMO



11 **12**

hydroxylation in which the bonding about carbon is proceeding toward trigonal bipyramidal. Such a geometry would impart considerably increased strain on the already strained cubyl structures. Alternatively, even without bond reorganization about carbon, it is possible that charge development on the cubyl carbons depends on the C−H−O angle. Without significant positive charge development for cubyl positions, abstraction of these hydrogens in **1** will not be favored over abstraction of the methyl hydrogens. The effects of an acute C−H−O angle on the transition state energies for cubyl hydrogen abstractions would be worth computing.

We now consider more generally the mechanisms of enzyme-catalyzed hydroxylation. Does the model discussed above disregard the large body of evidence that led to the deduction of the hydrogen abstraction−oxygen rebound mechanism for P-450 hydroxylations shown in Scheme 1? We believe it does not.

The present consensus mechanism for P-450 hydroxylation[65] involving formation of a radical intermediate is deduced from the following line of reasoning. (1) The observation of scrambled stereochemistry and rearrangements of probe substrates in a number of P-450 oxidations requires some type of intermediate. (2) A *requisite* cationic intermediate is excluded by the observation that probes could be functionalized at a cyclopropylcarbinyl position without ring opening, which must occur if a cation is involved. (3) Primary deuterium kinetic isotope effects (KIEs) are usually large, and secondary KIEs, when determined, are normal. (4) C−H bond reactivity in P-450 functionalizations correlates with the bond energies; one notes that this point applies for reactions of simple alkanes and is not followed for substrate **1**. Interestingly, retention of stereochemistry observed in a number of P-450 hydroxylations, which is inconsistent with the observations involved with point 1 above, is dismissed in mechanistic considerations, if addressed at all, by attributing it to structural constraints of the enzyme. MMO hydroxylations are less thoroughly studied, but the results generally follow those of P-450. For example, the observed intramolecular deuterium KIEs for a common substrate were similar in P-450 and MMO hydroxylations,[22,29] although the primary and secondary KIEs could not be factored out for the MMO hydroxylation because it did not display metabolic switching.[22]

The details of the mechanism for enzyme hydroxylation we presented above are consistent with this accumulated evidence. Until recently, all probe studies of P-450 hydroxylations in which rearrangements (allylic shifts[65] and cyclopropylcarbinyl ring openings[29,59,66,67]) were observed involved substrates which would give radicals and cations that undergo the same skeletal reorganizations. The results with such substrates do not exclude the possibility that the observed rearrangements were due in part to cationic species formed in a *competing* process, and this is an important distinction from the exclusion of *requisite* cationic intermediates. In the only case yet reported of P-450

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(65) An excellent discussion of the early evidence and current reasoning for the consensus mechanism for P-450 hydroxylation is given in ref 11.
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hydroxylation of a probe that permitted a distinction between radicals and cationic intermediates *and* in which rearrangement was observed, the major amount of rearrangement involved cationic species formed during the hydroxylation reaction, and the "radical" lifetime was very short;[14] such a short lifetime is an essential component of our model.

In regard to isotope effects, one is hard-pressed to imagine any C−H bond functionalization process that cannot, in principle, display a primary KIE. In theory, the magnitude of a primary KIE might be different for two processes, but since the evidence for our model implicates hydrogen abstraction preceding C−O bond formation in a non-synchronous process,[14] it seems unlikely that the primary KIE should differ considerably from that in a conventional abstraction. Normal secondary KIEs imply a change in the hybridization about carbon such that p-character in the C−H(D) bond decreases in the transition state. Again, this feature is consistent with bonding changes in structures such as **11** and **12**. Rehybridization of carbon to $sp^2 + p_z$ (or dsp^3) in order to obtain (hypervalent) trigonal bipyramidal bonding results in a decrease in p-character for bonds to the equatorial groups. C−H bond rupture is necessary in a side-on approach for hydroxylation, as it must be in any other C−H functionalization, and reactivity is expected to follow BDE values in alkane hydroxylations.

Our model for hydroxylation is not a vast departure from the consensus mechanism for P-450 hydroxylation shown in Scheme 1. The primary difference is that, in our model, the lifetime of the radical has been shortened from that of an intermediate to that of a vibrational component in a transition state, thus returning to the essence of the "oxenoid" model.[62] The other major point, that the atomic organization in the transition state is described in some detail, is one often not addressed in the abstraction−recombination picture except by default in that abstractions of hydrogen atoms are usually thought to occur with linear C−H−O arrays.

Conclusions

The unusual combination of BDE values for the cubyl and methyl positions in methylcubane (**1**), coupled with the highly polarized and relatively stabilized transition states for hydrogen atom abstractions at the cubyl positions by an electrophilic agent, renders it a useful substrate for studies of enzyme-catalyzed hydroxylations. At 40 °C, a strong preference for cubyl hydrogen abstraction in **1** by *t*-BuO$^\bullet$ was observed, consistent with previously reported results from reactions at low temperature and recent computational studies of the reaction. The regioselectivity obtained upon enzymatic hydroxylation of **1** differs considerably from that found in alkoxyl radical abstractions. The results with the enzymes are explained by the recently implicated non-synchronous concerted mechanism for P-450 oxidation.[14] A "side-on" approach to the C−H bonds in hydroxylations could result in significantly increased strain energy at cubyl positions as carbon approached trigonal bipyramidal bonding in the transition state, or to altered charge development at cubyl carbons in comparison to that obtained in linear approaches. Other potentially useful applications of methylcubane include oxidations by P-450 and MMO mimics that are more tractable than the enzymes. Methylcubane may not be an ideal mechanistic probe because rearranged products have not been isolated or identified, but if a mimic were to produce the unique product signature found for the P-450 enzyme, then one would have strong evidence that the mechanisms of oxidation were quite similar.

Experimental Section

Caution. Most cubanes are quite stable kinetically. Nonetheless, as they are high-energy materials, it is prudent to run reactions thereof behind safety shields. Crude reaction mixtures should not be concentrated at elevated temperature, particularly in the presence of acidic or metallic contaminants.

General. Reactions were conducted in oven-dried (145 °C) or flame-dried glassware under a positive nitrogen atmosphere. Transfers of anhydrous solvents or mixtures were accomplished with oven-dried syringes or cannulae. THF was distilled from potassium benzophenone ketyl, benzene was distilled over $CaCl_2$, and methylene chloride was distilled from P_2O_5 under nitrogen. Triethylamine was distilled from KOH pellets, and methanesulfonyl chloride was freshly distilled prior to use. Unless noted, reagents were supplied by Aldrich Chemical Co. and used as obtained. Silica gel 60A (Whatman) was used for preparative TLC, and silica gel 60 (Merck) was used for column chromatography. Melting points were obtained in open capillary tubes on a Thomas Hoover capillary melting point apparatus and are uncorrected. "Standard workup" refers to extraction with an organic solvent, washing the extract with saturated aqueous NaCl solution, drying over Na_2SO_4 or $MgSO_4$, and removal of solvents on a rotary evaporator with the bath at ambient temperature.

Unless noted, NMR samples were prepared in $CDCl_3$ with *ca.* 1% Me_4Si as an internal reference. Chemical shifts for ^{1}H NMR (300, 400, or 500 MHz) and ^{13}C NMR (75 or 100 MHz) spectra are reported in δ units relative to Me_4Si (δ 0.0) and the center line of $CDCl_3$ (δ 77.0), respectively.

Methylcubane (**1**) was prepared by reduction[68] of the mesylate[69] from cubylmethanol.[51] Alternative methods of synthesis exist.[35,37−40] To a solution of cubylmethanol (350 mg, 2.6 mmol) and Et_3N (359 mg, 3.9 mmol) in 10 mL of CH_2Cl_2 at −10 °C was added via syringe methanesulfonyl chloride (327 mg, 2.86 mmol). After being stirred for 30 min, the reaction mixture was diluted with 10 mL of cold CH_2Cl_2 and transferred to a chilled separatory funnel. The solution was extracted with ice water and 0 °C aqueous solutions of 10% HCl, saturated $NaHCO_3$, and saturated NaCl. The mixture was dried ($MgSO_4$) and concentrated at reduced pressure (0 °C bath). The crude mesylate was dissolved in 5 mL of cold THF, and the solution was transferred into a dry, N_2-flushed flask in a low-temperature bath (*ca.* −5 °C). $LiBHEt_3$ (6 mL of a 1 M solution in THF) was added in one portion via a syringe. The mixture was stirred for 4 h at −5 °C and then overnight at room temperature. The reaction was quenched by dropwise addition of water, and the mixture was treated with 2 mL of 3 N aqueous NaOH solution and 2 mL of 30% H_2O_2. The mixture was heated at reflux for 1 h. The reaction mixture was poured into 20 mL of water, and the mixture was extracted with pentane. The organic phase was dried ($MgSO_4$) and *carefully* concentrated on a rotary evaporator (bath at −2 °C). The crude product was vacuum transferred (0.05 Torr) to a liquid nitrogen trap to give 200 mg (65% yield) of **1**. ^{1}H NMR: δ 4.05 (m, 1 H), 3.96 (m, 3 H), 3.65 (m, 3 H), 1.25 (s, 3 H). ^{13}C NMR: δ 55.5, 49.9, 48.7, 43.8 (cubyl), 19.8 (methyl).

2-Iodo-1-(diisopropylcarbamoyl)cubane (**6b**). A solution of *n*-BuLi (40 mL, 2.5 M in hexanes, 0.10 mol) was added slowly to a solution of anhydrous 2,2,6,6-tetramethylpiperidine (17 mL, 0.10 mol) in THF (100 mL) in a vessel maintained at −78 °C. The mixture was allowed to warm to 0 °C, left there for 20 min, re-cooled to −78 °C, and transferred by cannula to a suspension of anhydrous $MgBr_2{\cdot}OEt_2$ (18.1 g, 0.0709 mol) and diisopropylcarbamoylcubane[70] (2.31 g, 0.010 mol) stirred together in THF (50 mL) at −78 °C. The heterogeneous mixture was warmed to 0 °C, stirred until homogeneous, and then allowed to warm to room temperature. After being stirred for 1 h, the solution was transferred slowly by cannula into a stirred solution of iodine (75 g) in THF (200 mL) at −78 °C. The mixture was stirred overnight at room temperature and then diluted with water (200 mL). Saturated aqueous Na_2SO_3 solution was added until the iodine color disappeared. The solution was extracted twice with $CHCl_3$. The combined extract was washed with aqueous 1 N hydrochloric acid (200 mL) and dried over $MgSO_4$. Evaporation under reduced pressure left a brown oil. Column chromatography on silica gel with 10−15 vol % EtOAc in hexanes provided 2.76 g (78%) of a white solid. Mp 153−154 °C. ^{1}H-NMR: δ 1.31 (d, 6 H), 1.45 (d, 6 H), 3.38 (m, 1 H), 3.57

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(70) Eaton, P. E.; Castaldi, G. *J. Am. Chem. Soc.* **1985**, *107*, 724−726.

(m, 1 H), 4.07 (m, 1 H), 4.13 (m, 1 H), 4.30 (m, 2 H), 4.43 (m, 2 H). ^{13}C-NMR: δ 20.6, 21.9, 39.1, 44.3, 46.2, 47.6, 48.8, 50.7, 54.8, 65.7, 167.8. Compound **6b** was used in the next reaction without further purification.

2-Methyl-1-(diisopropylcarbamoyl)cubane (7b). A solution of *t*-BuLi in THF (1.7 M in pentanes, 10.5 mL, 14.6 mmol) was added to a stirred solution of **6b** (2.37 g, 6.64 mmol) in THF (100 mL) at −78 °C. CH_3I (1.2 mL, 19 mmol) was added after 5 min. The mixture was allowed to warm to room temperature, and water (0.5 mL) was added. Most of the solvent was removed in vacuo. Cold aqueous 0.5 N hydrochloric acid was added with stirring, and the mixture was extracted twice with CH_2Cl_2. Standard workup gave 1.67 g of a crude solid product which was flash chromatographed on silica gel with 5:1 hexanes/ethyl acetate elution to give 1.49 g (92%) of white, crystalline **7b**. Mp 68−70 °C. ^{1}H-NMR: δ 1.22 (d, 6 H), 1.42 (d, 6 H), 1.27 (s, 3 H), 3.32 (m, 1 H), 3.54 (m, 1 H), 3.65 (m, 2 H), 3.80 (m, 1 H), 3.94 (m, 1 H), 4.11 (m, 2 H). ^{13}C-NMR: δ 18.0, 20.8, 21.5, 43.0, 44.7, 45.7, 46.6, 47.6, 48.0, 58.4, 60.8, 170.5.

2-Methyl-1-[(diisopropylamino)methyl]cubane (8b). A solution of **7b** (1.35 g, 5.51 mmol) in dry THF (20 mL) was added to a suspension of $LiAlH_4$ (0.37 g, excess) in THF (30 mL). The mixture was heated at reflux for 16 h and then cooled to room temperature. Saturated aqueous Na_2SO_4 (1 mL) was added carefully, followed by solid Na_2SO_4. The mixture was filtered, and the collected salts were washed thoroughly with THF (70 mL). The combined filtrate was dried over Na_2SO_4. Evaporation left **8b** (1.26 g, 99%) as pale yellow oil. ^{1}H-NMR: δ 0.97 (d, 12 H), 1.21 (s, 3 H), 2.65 (s, 2 H), 3.52 (m, 2 H), 3.71 (m, 2 H), 3.82 (m, 1 H), 3.88 (m, 1 H). ^{13}C-NMR: δ 16.2, 20.6, 43.6, 44.2, 45.3, 46.6, 47.5, 47.6, 55.1, 58.4.

2-Methylcubanecarboxylic Acid (9b). Compound **8b** (1.26 g) was stirred for 4 h with freshly prepared dimethyldioxirane[71] in wet acetone (350 mL, 0.05 M).[72] The solvent was removed. More dimethyldioxirane/acetone solution (150 mL) was added. The solution was kept overnight in a freezer. The solvent was removed in vacuo. CH_2Cl_2 (10 mL) was added, and the mixture was dried with Na_2SO_4. After filtration, evaporation left pale yellow, crystalline **9b** (840 mg, 95%). A sample for analysis was prepared by crystallization from hexanes. Mp 134−135 °C. ^{1}H-NMR: δ 1.36 (s, 3 H), 3.70 (m, 2 H), 3.82 (m, 1 H), 4.03 (m, 1 H), 4.15 (m, 2 H). ^{13}C-NMR: δ 16.97, 44.13, 46.07, 46.11, 48.15, 56.80, 58.31, 177.59. Anal. Calcd for $C_{10}H_{10}O_2$: C, 74.06; H, 6.21. Found: C, 73.97; H, 6.38.

4-Methylcubanecarboxylic Acid (9d).[35,41−43] This compound was synthesized in 88% overall yield from 4-iodo-1-(diisopropylcarbamoyl)-cubane[73,74] following the procedure given above for the synthesis of the **9b**. Intermediates **7d** and **8d** made on route were partially characterized.

4-Methyl-1-(diisopropylcarbamoyl)cubane (7d). ^{1}H-NMR: δ 1.20 (d, 6 H), 1.30 (s, 3 H), 1.43 (d, 6 H), 3.30 (m, 1 H), 3.56 (m, 1 H), 3.60 (m, 3 H), 4.03 (m, 3 H).

4-Methyl-1-[(diisopropylamino)methyl]cubane (8d). ^{1}H-NMR: δ 0.98 (d, 12 H), 1.26 (s, 3 H), 2.64 (s, 2 H), 2.94 (m, 2 H), 3.47 (m, 3 H), 3.59 (m, 3 H). ^{13}C-NMR: δ 20.0, 20.9, 44.5, 46.5, 46.9, 47.8, 56.0, 59.9.

Acid 9d. Mp 139.5−140.5 °C (lit.[35] mp 143−144 °C; lit.[42] mp 139.5−141.0 °C). ^{1}H-NMR: δ 1.28 (s, 3 H), 3.66 (m, 3 H), 4.13 (m, 3 H). ^{13}C-NMR: δ 19.3, 45.8, 47.4, 56.1, 56.2, 178.7.

Cubylmethanol Acetate (3a). A mixture of cubylmethanol (8.5 mg, 0.063 mmol), pyridine (25 mg, 0.32 mmol), and acetic anhydride (32 mg, 0.32 mmol) in 1 mL of CH_2Cl_2 was stirred at room temperature for 30 min. Water was added, and the organic layer was separated, dried ($MgSO_4$), and concentrated at reduced pressure to give 8.2 mg (75%) of **3a**. ^{1}H-NMR: δ 4.22 (s, 2 H), 4.04−4.08 (m, 1 H), 3.88−3.94 (m, 6 H), 2.07 (s, 3 H).

2-Methylcubanol Acetate (3b).[75] CH_3Li (1.4 M in pentanes, 0.22 mL, 0.30 mmol) was added to a solution of 2-methylcubanecarboxylic acid (**9b**) (16 mg, 0.10 mmol) in ether (1 mL) in a vessel cooled in a −78 °C bath. The white suspension was stirred at room temperature for 1.5 h, re-cooled to −78 °C, and quenched with crushed dry ice (ca. 1 g). The mixture was poured into an aqueous saturated $(NH_4)_2SO_4$ solution. Standard workup with ether gave a yellow oil identified as methyl (2-methyl)cubyl ketone (15 mg, 94% yield). ^{1}H-NMR: δ 1.33 (s, 3 H), 2.09 (s, 3 H), 3.67 (m, 2 H), 3.84 (m, 1 H), 3.97 (m, 1 H), 4.16 (m, 2 H). ^{13}C-NMR: δ 17.4, 26.9, 44.3, 45.5, 46.3, 47.6, 206.6. The compound was used below without further purification.

The crude ketone was dissolved in CH_2Cl_2 (2 mL), and *m*-chloroperoxybenzoic acid (35 mg, 0.20 mmol) was added to the mixture. The clear solution was stirred overnight at room temperature, diluted with $CHCl_3$ (5 mL) and extracted twice with vigorous shaking with saturated aqueous K_2CO_3 solution. The organic phase was dried over $MgSO_4$, and the solvent was removed to leave acetate **3b** as a clear oil (15 mg, 91%) of reasonable purity. ^{1}H-NMR: δ 1.28 (s, 3 H), 2.08 (s, 3 H), 3.59 (m, 2 H), 3.74 (m, 1 H), 3.94 (m, 1 H), 4.19 (m, 2 H). ^{13}C-NMR: δ 15.1, 21.0, 42.9, 44.1, 45.2, 61.9, 88.4, 169.7.

4-Methylcubanol Acetate (3d). This compound was obtained as a colorless oil in 80% overall yield from 4-methylcubanecarboxylic acid (**9d**) following the procedure used for the 2-methyl analog **3b**. ^{1}H-NMR: δ 1.28 (s, 3 H), 2.08 (s, 3 H), 3.53 (m, 3 H), 4.10 (m, 3 H). ^{13}C-NMR: δ 19.4, 21.0, 44.1, 50.9, 55.7, 89.0, 169.3.

The intermediate methyl 4-methylcubyl ketone was identified by its NMR spectra. ^{1}H-NMR: δ 1.30 (s, 3 H), 2.12 (s, 3 H), 3.62 (m, 3 H), 4.11 (m, 3 H). ^{13}C-NMR: δ 19.4, 24.6, 54.9, 46.9, 56.4, 64.5, 207.0.

***N*-(4-Methylcubyloxy)-2,2,5,5-tetramethylisoindole (2d).** In a 5-mL flask equipped with a $CaCl_2$ drying tube, a mixture of 4-methylcubanecarboxylic acid (20 mg, 0.123 mmol), oxalyl chloride (16 μL, 0.185 mmol), DMF (0.05 mL), and dry CH_2Cl_2 (0.25 mL) was stirred at 0 °C for 10 min. The reaction was allowed to warm to room temperature over a period of 30 min. Excess oxalyl chloride and solvent were distilled at reduced pressure. The residual acid chloride was dissolved in dry benzene (5 mL) under nitrogen. The solution was added via cannula to a cooled (5 °C) mixture of the sodium salt of *N*-hydroxypyridine-2-thione (22 mg, 0.15 mmol, 1.2 equiv) (Olin Chemical), 2,2,5,5-tetramethylisoindole-*N*-oxyl[44] (TMIO$^\bullet$, 70 mg, 0.37 mmol), and a few milligrams of DMAP in 3 mL of dry benzene. The mixture rapidly turned a yellow color. The reaction mixture was warmed in a 60−70 °C bath and irradiated with a 150-W tungsten filament lamp at a distance of *ca.* 0.5 m. After 30 min of gentle reflux, the solution was cooled and concentrated under reduced pressure to give the crude TMIO adduct **2d**. Preparative TLC (hexanes−ethyl acetate, 10:1) gave 15.7 mg (41%) of **2d**. ^{1}H NMR: δ 7.13 (m, 2 H), 7.26 (m, 2 H), 4.07−4.09 (broad m, 3 H), 3.38−3.39 (m, 3 H), 1.47−1.48 (broad m, 12 H), 1.28 (s, 3 H).

Attempted preparations of ***N*-(2-Methylcubyloxy)-2,2,5,5-tetramethylisoindole (2b)** from 2-methylcubanecarboxylic acid by the method employed above for **2d** were unsuccessful. Several slight variations of this method also gave none of the desired product. In addition to the preparation of **2d** described above, these methods successfully gave the 1-adamantyl and cyclohexyl TMIO adducts from the corresponding carboxylic acids.

Reaction of Radical 4 in the Presence of TMIO$^\bullet$. The PTOC ester precursor to radical **4**, 1-[[(cubylmethyl)carbonyl]oxy]-2(1*H*)-pyridinethione, was prepared from cubylacetic acid as previously described.[51,52] A mixture of this precursor (20 mg, 0.08 mmol) and TMIO$^\bullet$ (57 mg, 0.3 mmol) in 1 mL of benzene was sealed in a tube and then irradiated with a 150-W tungsten filament lamp. Concentration of the reaction mixture and preparative TLC (hexanes−ethyl acetate, 10:1) gave 16 mg (65%) of a mixture of TMIO adducts. The vinyl proton region in the ^{1}H NMR spectrum of this mixture contained signals at δ 6.33 (t), 6.23 (t), 5.93 (t), 5.23 (t), 4.90 (t), and 4.71 (t). The signals at δ 6.33, 6.23, 4.90, and 4.71 coincided with a minor set of signals in the mixture of ring-opened TMIO adducts from the reaction of methylcubane with *t*-BuO$^\bullet$ in the presence of TMIO$^\bullet$.

Mass spectra of various acetates, obtained with an HP 5890 Series II gas chromatograph interfaced to an HP 5791A mass selective detector

(71) Eaton, P. E.; Wicks, G. E. *J. Org. Chem.* **1988**, *53*, 5353−5355.

(72) This procedure for conversion of a diisopropylamide (nearly impossible to hydrolyze) into a carboxylic acid by first reducing the amide to the amine and subsequent oxidation with dimethyldioxirane was developed by Dr. A. Bashir-Hashemi when at The University of Chicago.

(73) The amide was derived[70] from the iodoacid.[74]

(74) Tsanaktsidis, J.; Eaton, P. E. *Tetrahedron Lett.* **1989**, *30*, 6967−6968.

(75) Eaton, P. E.; Yang, C.-X.; Xiong, Y. *J. Am. Chem. Soc.* **1990**, *112*, 3225−3226.

(EI, 70 eV), are listed below. The spectra for **3a**, **3b**, and **3d** are from authentic samples; the spectrum for **3c** is that for the product from P-450 oxidations assigned as **3c**. The order of elution for compounds **3** on an SE-54 column was **3c**, **3d**, **3b**, and **3a**.

Cubylmethanol Acetate (3a). MS: *m/z* (rel intensity) 133 (9), 115 (100), 103 (16), 91 (18), 77 (18), 65 (4), 51 (8).

2-Methylcubyl Acetate (3b). MS: *m/z* (rel intensity) 133 (65), 119 (74), 105 (80), 94 (36), 91 (100), 77 (80), 51 (30).

3-Methylcubyl Acetate (3c). MS: *m/z* (rel intensity) 133 (60), 119 (83), 105 (73), 94 (43), 91 (100), 77(65), 51 (23).

4-Methylcubyl Acetate (3d). MS: *m/z* (rel intensity) 133 (56), 119 (76), 105 (92), 94 (83), 91 (100), 77 (87), 65 (28), 51 (31).

Cubanol Acetate. MS: *m/z* (rel intensity) 119 (22), 103 (16), 94 (32), 91 (100), 65 (49).

Reactions of Methylcubane with *tert*-Butoxyl Radical. The following is representative. A mixture of methylcubane (27.5 mg, 0.233 mmol), di-*tert*-butyl hyponitrite[56] (18.3 mg, 0.11 mmol), TMIO$^{\bullet}$ (58 mg, 0.30 mmol), and 1 mL of benzene was placed in a dry 20-cm Pyrex tube (10 mm ID) containing a small Teflon-coated stir bar. The mixture was stirred with a vortex stirrer and degassed (freeze-thaw), and the tube was sealed under vacuum (liquid nitrogen bath). The reaction ampule was equilibrated at room temperature for 5 min and then placed in a temperature-regulated bath at 40 ± 3 °C. The reaction mixture gradually darkened over a 7-day reaction period. The tube was cooled in a liquid nitrogen bath and opened. A weighed amount of octane was added as a standard, and the amount of unreacted methylcubane (15.1 mg, 0.128 mmol) was determined by GC using predetermined response factors. The reaction mixture was concentrated at reduced pressure, and the residual TMIO$^{\bullet}$ was separated from the reaction products by chromatography on a short silica gel column. Preparative TLC (hexanes−ethyl acetate, 10:1) gave two major bands with R_f of 0.75 and 0.50 which were isolated and analyzed by 500-MHz ^{1}H-NMR spectroscopy. Naphthalene was added as a standard to each fraction, and the resulting mixtures were analyzed by HPLC (Waters Millennium system, photodiode array detection, 250 mm × 4.6 mm reverse phase C18 column, CH_3CN-H_2O, 85:15, v:v).

The R_f 0.75 mixture contained two components by HPLC in a 4:1 ratio and in a total yield of 47% based on unrecovered methylcubane. (The 4:1 product ratio was observed in a total of four experiments conducted at 40 and 55 °C.) The minor component coeluted with authentic **2d**. The ^{1}H-NMR spectrum of this mixture contained signals from **2d** and another component assigned as **2c** in a 1:4 ratio. The ^{1}H-NMR spectrum assigned to **2c** was as follows: δ 7.26 (m, 2 H), 7.13 (m, 2 H), 4.23 (m, 1 H), 3.90 (m, 2 H), 3.62 (broad m, 2 H), 3.55 (broad m, 1 H), 1.43 (broad s, 12 H), 1.28 (s, 3 H).

The R_f 0.50 mixture contained several components by ^{1}H-NMR analysis. A minor set of signals in the vinyl proton region (*ca.* 5% of the total) coincided with the vinyl proton signals observed from the products from reaction of radical **4** with TMIO$^{\bullet}$. HPLC analysis showed at least five components in a total yield of 18% assuming that the relative response factors versus naphthalene for the products were the same as that for **2d**. The products in this mixture decomposed on standing. The vinyl proton region in the ^{1}H-NMR spectrum contained the following signals: δ 6.72 (m), 6.56 (m), 6.42 (m), 6.36 (m), 6.32 (d), 6.19 (m), 6.16 (m), 6.01 (m), 5.36 (d), 5.31 (m), 5.22 (m), 5.10 (d), 5.05 (s), 4.94 (m), 4.82 (d), 4.70 (m).

Competition Reaction of Methylcubane and Cyclohexane. Following the method described above, a mixture of methylcubane (17.7 mg, 0.14 mmol), cyclohexane (12.6 mg, 0.19 mmol), di-*tert*-butyl hyponitrite (10.9 mg, 0.06 mmol), and TMIO$^{\bullet}$ (38 mg, 0.20 mmol) in 1 mL of benzene was allowed to react at 42 °C for 4 days. TMIO adducts were isolated as above and analyzed by HPLC employing naphthalene as a standard. Cyclohexyl-TMIO (^{1}H-NMR: δ 7.24 (m, 2 H), 7.10 (m, 2 H), 3.73 (broad s, 1 H), 2.1 (broad s, 2 H), 1.77 (broad s, 2 H), 1.56−1.54 (broad s, 6 H), 1.34−1.49 (broad s, 12 H)), **2c**, **2d**, and rearranged TMIO adducts were present.

Microsomal Cytochrome P-450 Hydroxylations. Microsomes were prepared[76] from livers of inbred Fischer 344 male rats (8−10 weeks old) which were fed rodent lab chow and water *ad libitum*. The rats were pre-treated with phenobarbital (0.1% in drinking water for 10 days). The microsomes were suspended in 100 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA and 20% glycerol and stored at −70 °C.

Reaction mixtures (total volume of 2 mL in an 8-mL Pyrex tube with a Teflon stopper) contained a total of 10 nmols (5.0 nmol/mL) of liver microsomal cytochrome P-450 and approximately 2 mg of substrate (neat methylcubane, neat cubane, or cubane dissolved in a 2% solution of MeOH or acetone) in 50 mM potassium phosphate buffer, pH 7.4, containing 1 mM desferroxamine and 0.075 M KCl. The reaction mixtures were pre-incubated at 37 °C in a shaking water bath, and the reaction was initiated by the addition of 110 μL of a 22 mM NADPH solution in buffer (final concentration, 1.2 mM NADPH). Reaction mixtures were incubated at 37 °C for 30 min and terminated by placing the reaction tubes on ice and adding 50 μL of 0.5 M sodium dodecyl sulfate solution in water. After addition of 2 mL of distilled methylene chloride, the mixture was transferred to a test tube, mixed with a vortex stirrer for 1 min, and centrifuged at 2000 rpm for 10 min using a clinical centrifuge. The methylene chloride layer was removed, and the extraction was repeated twice more. The methylene chloride pool (6 mL) was then dried ($MgSO_4$), and 2 mg of octane was added as an internal standard. The mixture was concentrated under a nitrogen stream to a volume of 250 μL and analyzed by GC for unreacted substrate. Pyridine (2 drops) and acetic anhydride (2−3 drops) were added, and the mixture was stirred for 30 min. Water (1 mL) was added, and the mixture was extracted thrice with 2 mL of CH_2Cl_2. The combined organic phase was dried ($MgSO_4$), concentrated to *ca.* 250 μL under a nitrogen stream, and analyzed by GC and GC-MS. GC analyses were performed on an FID equipped instrument with a bonded phase, wide bore capillary column (Alltech, SE-30, 15 m × 0.53 mm); signal integration was achieved with the ChromePerfect (Justice Innovation, Inc.) software package. Analytical GC-MS was accomplished with a 30 m × 0.25 mm low-polarity column (Alltech, SE-54, 30 m × 0.25 mm).

CYP2B1 Hydroxylations. Purified CYP2B1[77] (0.6 nmol) was reconstituted with reductase[78] (1.2 nmol) as previously described.[14] Reactions with **1** were conducted as described above. For the third CYP2B1 entry in Table 1, the amounts of enzyme and reductase were doubled.

Reconstituted MMO Hydroxylations. Oxidations were performed with the reconstituted methane monooxygenase system from *M. capsulatus* (Bath) by the method previously reported.[22] GC-MS analyses were performed on the product mixtures both before and after treatment with acetic anhydride by the above method.

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