

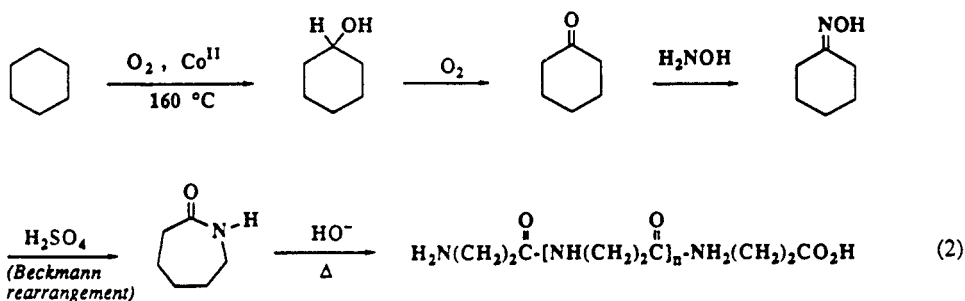
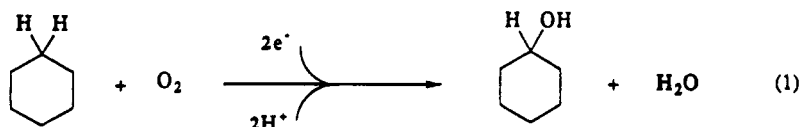
Calibration of very fast alkyl radical "clock" rearrangements using nitroxides

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Abstract - The nitroxide radical, Tempo, has been shown to combine with alkyl radicals to form hydroxylamines with a rate constant of ca. $10^9 \text{ M}^{-1} \text{ s}^{-1}$ at room temperature and this reaction has been used to measure the rate of some very fast alkyl radical rearrangements. These calibrated "clock" reactions have then been used to investigate the "hydroxyl rebound" reaction in the cytochrome P-450 induced hydroxylation of alkanes.

The oxidation of cyclohexane to cyclohexanol using molecular oxygen, two protons and two electrons is one of the simplest imaginable reactions (eq. 1). It is also an extremely important commercial reaction since ca. 10^6 tons of cyclohexanol are made per year worldwide for conversion to caprolactam and hence to nylon 6 (eq. 2). The commercial oxidation of cyclohexane must be the least efficient of all major industrial chemical processes. Typically cyclohexane is air-oxidized at 160°C (some 80°C above the b.p. of cyclohexane) in enormous pressurized tanks using Co^{II} as a catalyst. Because the desired oxidation products, cyclohexanol and cyclohexanone, are both much more susceptible to oxidation than cyclohexane under these conditions the reaction is run only to 4% conversion, meaning that 96% of the cyclohexane must be separated from the products and recycled. However, even at this low conversion the desired compounds constitute only 85% of the products.



In contrast to the commercial oxidation of cyclohexane all of us can oxidize this compound to cyclohexanol with 100% efficiency. This oxidation occurs mainly in our livers and we use an iron, rather than a cobalt catalyst, cytochrome P-450. This is an iron protoporphyrin IX (Figure 1) embedded in a protein with the "back" of the iron atom (i.e., the 5th coordination site) being protected by a thiolate ligand and the "front" being accessible via a hydrophobic pocket in the protein.¹ The catalytic cycle which converts an alkane, RH, to the alcohol, ROH, is shown in Figure 2. Reading this cycle as a clock, our current concern lies between 9:00 and 10:30. The intermediates from 10:30, $[\text{Fe}^{\text{II}}]$ the resting enzyme, to 6:00 can be observed but the 9:00 species,

$[\text{Fe}^{\text{IV}}=\text{O}][\text{RH}]$, "inserts" an oxygen atom into RH too rapidly for this species to be observed. The mechanism by which the oxygen is "inserted" into a C-H bond was originally inferred to be just that, i.e., an insertion. However, as Groves and coworkers² have demonstrated, with an appropriate choice of substrate the hydroxylation can occur with a loss of stereo- and regio-selectivity. It has therefore been inferred that the hydroxylation of RH involves an initial hydrogen abstraction to form a carbon-centered radical, R^\cdot (eq. 3), followed by oxygen (hydroxyl) "rebound" from iron to carbon (eq. 4).

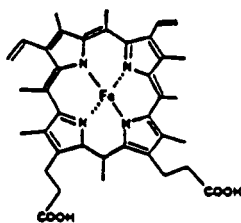


figure 1

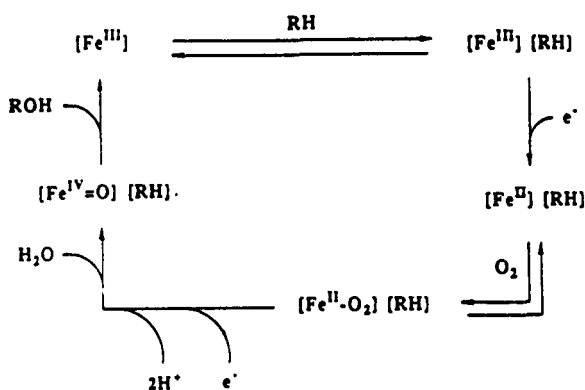
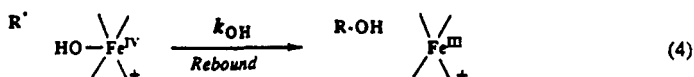
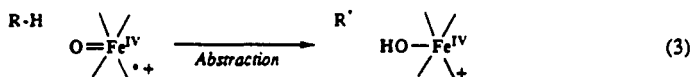
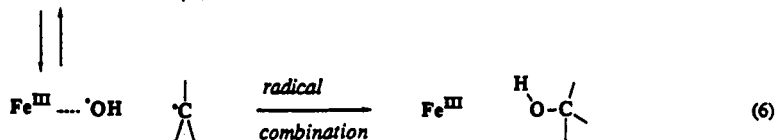
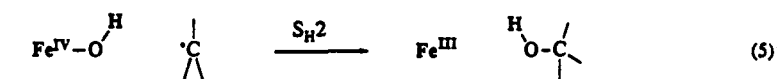


figure 2



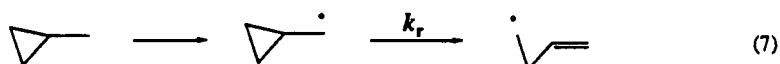
The interesting question relates to the nature of the "rebound" process. The two most obvious mechanisms are:

1. A bimolecular homolytic substitution ($\text{S}_{\text{H}}2$) by the carbon-centered radical at oxygen for iron (eq. 5);
2. Prior dissociation of the Fe-OH bond to form a "free" hydroxyl radical which combines with the adjacent carbon-centered radical (eq. 6).

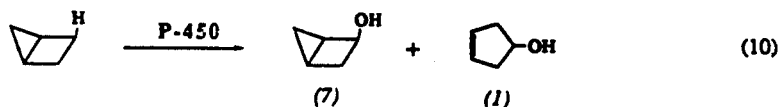
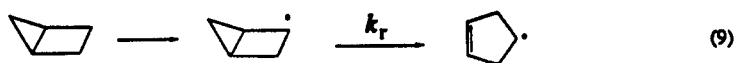


Mechanisms are deduced from product and kinetic studies. In a general sense the products are known, viz., $[\text{Fe}^{\text{III}}]$ and ROH. Kinetic studies must be concerned with *rate* rather than with *order*. That is, the hydroxylation of R^\bullet is clearly a bimolecular reaction but it will obey first-order rather than second-order kinetics because there is strong evidence that the carbon-centered radical will be hydroxylated much more rapidly than it can escape from the hydrophobic pocket in the enzyme. The only reasonable way to measure the rate of hydroxylation of R^\bullet is to use an alkane which will yield a *calibrated free-radical clock*³, i.e., R^\bullet must be an alkyl radical which undergoes an irreversible unimolecular rearrangement⁴ at a rate which has been measured³ and which will compete with the hydroxylation process.

In 1987, Ortiz de Montellano and Stearns⁵ applied the radical-clock method to alkane hydroxylations at 37 °C using rat liver microsomes enriched in cytochrome P-450 by pretreatment of the rats with phenobarbital. The fastest calibrated clock was the ring-opening of the cyclopropylmethyl radical to yield the 3-butenyl radical³ (eq.7).



This reaction has a rate constant of $1.2 \times 10^8 \text{ s}^{-1}$ at 37 °C but, despite its speed, methylcyclopropane yielded only cyclopropylmethanol⁵ (eq. 8). Fortunately, a faster alkyl radical rearrangement was known⁶ (eq. 9) but it had proved to be too fast to calibrate by the usual³ electron spin resonance (ESR) spectroscopic method. That is, the cyclopropylmethyl clock was originally calibrated⁷ by generating this radical photochemically in an ESR spectrometer in an inert solvent and measuring its absolute concentration at low temperatures where the 3-butenyl radical could also be observed and quantified. These two radicals were present at approximately equal concentrations at ca. 140 K⁷ with the unrearranged cyclopropylmethyl radical becoming the only species detectable at temperatures below ca. 120 K. By way of contrast,⁶ the bicyclo[2.1.0]pent-2-yl radical could not be detected by ESR spectroscopy even at temperatures as low as 110 K; only the rearranged cyclopentene-4-yl radical was observed from which it was concluded that $k \geq 10^9 \text{ s}^{-1}$ at ambient temperatures. The action of microsomal P-450 on bicyclopentane gave a 7:1 ratio of the unrearranged and rearranged alcohols⁵ (eq. 10) which left us with the problem of calibrating this rearrangement to turn it into a useful clock.



The simplest calibration procedure appeared to us to be to generate the bicyclopentyl radical in the presence of Tempo and measure the relative yields of the Tempo-trapped unrearranged and rearranged radicals at known concentrations (Figure 3). We chose Tempo as the trap because we had previously determined the absolute rate constants for its reaction with a variety of alkyl radicals by laser flash photolysis (LFP) and found that these rate constants were all about $1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at room temperature, showing little dependence on the nature of the carbon-centered radical.⁸ Three Tempo adduct radical products were detected which were identified as the *exo* and *endo* (2.4:1 ratio) adducts of the unrearranged radical and the adduct of the rearranged radical.⁹ From the ratio of the two unrearranged adducts to the rearranged adducts we determined that $k_r/k_T = 1.6 \text{ M}$ at 37 °C⁹. Taking $k_T = 1.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (the value found by LFP for trapping the cyclobutyl radical¹⁰) yielded $k_r = 2.4 \times 10^9 \text{ s}^{-1}$. Combination with the P-450 derived alcohol product ratio found by de Montellano and Stearns⁵ gave the rate constant for oxygen rebound, $k_{\text{OH}} = 1.7 \times 10^{10} \text{ s}^{-1}$.

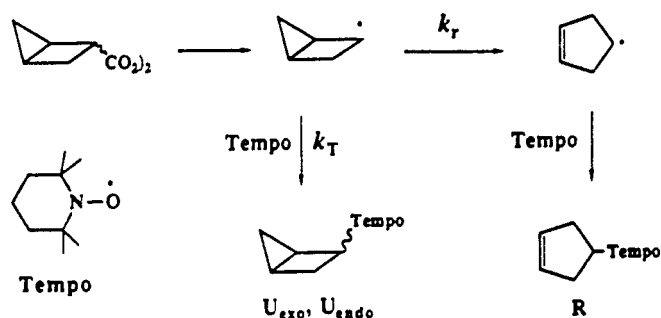


figure 3

Of course, a single rate constant demonstrates nothing except that oxygen rebound is a very rapid process. We have therefore extended our studies to half a dozen polymethyl-substituted cyclopropanes. Rate constants have been measured by the Tempo method for the ring opening of methyl-substituted cyclopropylmethyl radicals and when two ring-opened radicals can be produced their ratio has been determined.¹⁰ The same compounds have been hydroxylated with P-450 using phenobarbital-induced rat liver microsomes.¹⁰ Most interestingly, *cis*- and *trans*-1,2-dimethylcyclopropane give the same secondary alcohol/primary alcohol ratio as found for the Tempo alkyl radical adducts, viz., 3:1 and 1:1, respectively. That is, *cis*- and *trans*-2-methylcyclopropylmethyl radicals generated by P-450 oxidation of the parent hydrocarbons partition between the two ring-opening reactions to form the secondary and the primary alkyl radical in just the same ratio as when these radicals are formed in homogeneous solution. This demonstrates that the enzyme's hydrophobic pocket does not influence the ring-opening partitioning of either of these two 2-methylcyclopropylmethyl radicals but, nevertheless, the calculated rate constants for oxygen rebound are only about half as large as the value calculated from the bicyclopentane data. It seems improbable that k_{OH} would depend on the nature of the substrate if hydroxylation involved combination of R^\cdot with a free HO^\cdot radical since the rate-controlling step would be fission of the iron-oxygen bond — a process which would be expected to be independent of the substrate. We therefore conclude that the most probable mechanism for the P-450 hydroxylation of alkanes involves the $\text{S}_\text{H}2$ reaction (eq. 5). Work is continuing to calibrate other rapid alkyl radical rearrangements by the Tempo method and to submit the parent hydrocarbons as substrates for hydroxylation to cytochrome P-450. The results obtained to date¹⁰ remain consistent with hydroxylation via an $\text{S}_\text{H}2$ process but this "consistency" could be destroyed tomorrow.

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