Theoretical Model for an Alternate Mechanism for the Cytochrome P-450 Hydroxylation of Quadricyclane

Robert D. Bach,* Iliana Mintcheva, Carlos M. Estévez, and H. Bernhard Schlegel

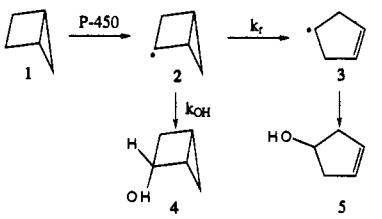
Department of Chemistry, Wayne State University Detroit, Michigan 48202-3489

Received May 23, 1995

During the past decade the mechanism of enzymatic hydroxylation of saturated hydrocarbons has come under increased scrutiny.[1] The widely accepted mechanism for the catalytic cycle for cytochrome P-450 monooxygenase hydroxylation of alkanes is thought to involve the recombination of an iron-bound hydroxyl radical with a carbon-centered free radical in a process termed "oxygen rebound".[2] Another related area of chemistry that has developed simultaneously is the use of "radical clock" substrates to estimate the magnitude of the rate constant for the oxygen rebound step (k_{OH}). If alkane hydroxylation is initiated by hydrogen abstraction, then the free-radical intermediate may be hydroxylated directly (k_{OH}) or it may undergo rearrangement (k_r) and subsequent recombination to afford a rearranged alcohol as exemplified for the P-450 hydroxylation of bicyclo[2.1.0]pentane (Scheme 1). Ortiz de Montellano and Stearns[3a] reported a 7:1 ratio of unrearranged (**4**) to rearranged (**5**) alcohols, implying that the oxygen rebound step is about seven times faster than the ring-opening of bicyclo[2.1.0]pent-2-yl radical (**2**) in Scheme 1.

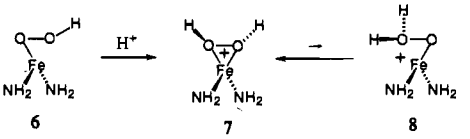
We predict a classical activation barrier for rearrangement of **2** to cyclopenten-4-yl radical **3** of 6.8 kcal/mol ($\Delta G^{\ddagger}_{298}$ = 6.23 kcal/mol) at the PMP4 level and a rate constant at 25 °C of $k_r = 1.7 \times 10^8\ s^{-1}$.[4] A recent estimate of the rate constant for radical recombination to form unrearranged alcohol **4** is $k_{OH} = 2.2 \times 10^{10}\ s^{-1}$.[3c] During the past several years the kinetic scale for these very rapid radical reactions has approached the theoretical limit for the frequency factor $kT/h \cong 10^{13}$. Measured unimolecular rate constants for rearrangement of aryl-substituted cyclopropylcarbinyl radical of $5 \times 10^{11}\ s^{-1}$ have been reported[3b,7] requiring an estimated rebound rate constant for enzymatic hydroxylation[8] with methane monooxygenase of $k_{OH} > 10^{13}\ s^{-1}$.

Scheme 1



A mechanism was proposed that does not involve radicals or carbocations. It became obvious to us several years ago that a single ferryl oxygen precursor could not account for all P-450 alkane hydroxylation reactions. Consequently, we started to examine mechanistic possibilities for alkane hydroxylation other than the "oxygen rebound".[9]

The putative ferryl (Fe = 0) complex involved in the P-450 enzymatic cycle is thought to be derived from an iron(III) hydroperoxide. In a recent theoretical study using diamidoiron(III) hydroperoxide (**6**) as a model oxygen atom donor we made the surprising observation that the accepted catalytic process involving protonation of the hydroperoxide with loss of water to yield a monooxygenase iron(V) donor is energetically highly unfavorable.[9] The proton affinity of **6** is predicted



to be 214.3 kcal/mol at the PMP4//MP2/WH level.[6] However, bridged hydrogen peroxide Fe(III) complex **7** is 21.5 kcal/mol lower in energy than water oxide complex **8**. Since a 1,2-hydrogen shift equilibrating **7** and **8** has a prohibitively high barrier (~50 kcal/mol),[9a,c] formation of **8** must involve protonation−deprotonation or a proton relay. At equilibrium the concentration of **8** would be extremely low, and in addition the energy required for subsequent heterolytic O−O bond cleavage in **8** to yield water and a ferryl oxygen intermediate must be considered. Consequently loss of water from **8** would be associated with a high barrier height in the absence of a major stabilizing influence at the active site. This observation prompted us to suggest that oxygen atom transfer could occur from a symmetrically bonded hydrogen peroxide porphyrin iron(III) adduct in concert with O−O bond cleavage.[9a,10] The net nuclear event in such an oxygen transfer from a protonated iron(III) hydroperoxide is the insertion of a hydroxyl cation (HO^+) into a σ bond. In earlier studies we have used H_2O_2 complexed or bonded to H^+, Li^+, and $(NH_2)_2Fe^+$ as the incipient HO^+ donor.[9]

To lend credibility to this postulate we chose as substrate a hydrocarbon that is believed to undergo P-450 hydroxylation by an adaptation of the "oxygen rebound" mechanism. Sterns and Ortiz de Montellano[11] suggested that highly strained quadricyclane is oxidized initially to a radical cation that is captured in a distinct step by the hydroxyl form of the activated iron(V) porphyrin complex to form the nortricyclyl cation **11**, which is known to afford a rearranged protonated aldehyde **12**. Alternate mechanisms such as concerted exo addition to give the epoxide of norbornadiene or insertion of oxygen into the

(1) (a) Cytochrome P-450: *Structure Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum, New York, 1986. (b) Ortiz de Montellano, P. R. *Acc. Chem. Res.* **1987**, *20*, 289. (c) Ortiz de Montellano, P. R. *Trends Pharmacol. Sci.* **1989**, *10*, 354. (d) Poulos, T. L.; Raag, R. *FASEB J.* **1992**, *6*, 674. (e) Guengerich, F. P.; MacDonald, T. S. *FASEB J.* **1990**, *4*, 2453. (f) Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5699. (g) Hayano, M. In *Oxygenases*; Hayaishi, O., Ed.; Academic Press: New York, 1986; pp 181−240.

(2) Groves, J. T.; McClusky, G. A.; White, R. E.; Coon, M. *Biochem. Biophys. Res. Commun.* **1978**, *81*, 154.

(3) (a) Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415. (b) Atkinson, J. K.; Ingold, K. U. *Biochemistry* **1993**, *32*, 9209. (c) Bowry, V. W.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5687.

(4) Calculations were executed with the Gaussian 92 program,[5] and all energies cited in the text are at the MP4SDTQ//MP2/6-31G* level unless noted otherwise. The iron basis set is a contraction of the [14s, 11p, 5d] primitive set of Wachters[6a] with the Hay diffuse function[6b] and a one Gaussian 4f polarization function (exponent = 1.339) added to the valence shell. This 48 basis function set is referred to as WH.[6] For a more complete description of this basis set, see ref 9a. PMP4 refers to spin-projected MP4 (for details, see: Schlegel, H. B. *J. Phys. Chem.* **1988**, *92*, 3075). Vibrational frequency calculations on all stationary points were carried out at MP2/6-31G*.

(5) (a) Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, B. G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andrés, J. L.; Raghavachari, K.; Binkley, J. S.; Gonzalez, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A. *GAUSSIAN 92*; Gaussian, Inc.: Pittsburgh, PA, 1992. (b) Schlegel, H. B. *J. Comput. Chem.* **1982**, *3*, 214.

(6) (a) Wachters, A. J. H. *J. Chem. Phys.* **1970**, *52*, 1033. (b) Hay, J. P. *Ibid.* **1977**, *66*, 4377. (c) Stewart, R. F. *Ibid.* **1970**, *52*, 431. (d) Huzinaga, S. *Ibid.* **1965**, *42*, 1293. (e) Dunning, T. H. *Ibid.* **1970**, *53*, 2823.

(7) (a) Newcomb, M. *Tetrahedron* **1993**, *49*, 1151. (b) Newcomb, M.; Johnson, C. C.; Manek, M. B.; Varick, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10915. (c) Newcomb, M.; Manek, M. B.; Glenn, A. G. *J. Am. Chem. Soc.* **1991**, *113*, 949.

(8) Liu, K. E.; Johnson, C. C.; Newcomb, M.; Lippard, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 939.

(9) (a) Bach, R. D.; Su, M.-D.; Andrés, J. L.; Schlegel, H. B. *J. Am. Chem. Soc.* **1993**, *115*, 8763. (b) Bach, R. D.; Schlegel, H. B.; Andrés, J. L. *J. Am. Chem. Soc.* **1994**, *116*, 3475. (c) Bach, R. D.; Su, M.-D. *J. Am. Chem. Soc.* **1994**, *116*, 10103.

(10) The PMP4//MP2/6-31G* bond dissociation energies for H_2O_2 and $Li^+{\cdot}H_2O_2$ are predicted to be 48.2 and 62.1 kcal/mol and the PMP4//MP2/WH O−O bond energy for **7** is predicted to be 22.3 kcal/mol, respectively.

(11) Stearns, R. A.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1985**, *107*, 4081.

0002-7863/95/1517-10121$09.00/0

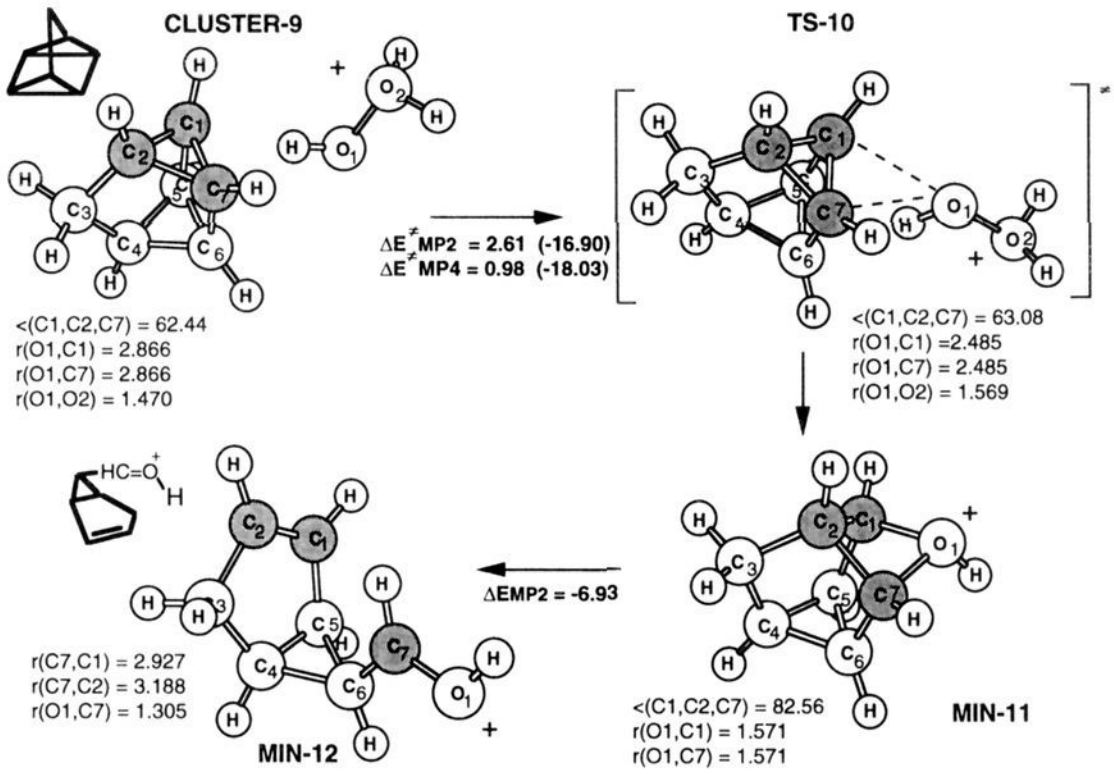


Figure 1. Insertion of hydroxyl cation (HO^+) into the C1−C7 bond of quadricyclane. Geometries are optimized at the MP2/6-31G* level of theory; energy differences (kcal/mol) are calculated at the MP2/6-31G* and MP4//MP2/6-31G* levels of theory. Bond distances are given in angstroms and angles in degrees. The energy differences in parentheses are calculated from isolated reactants.

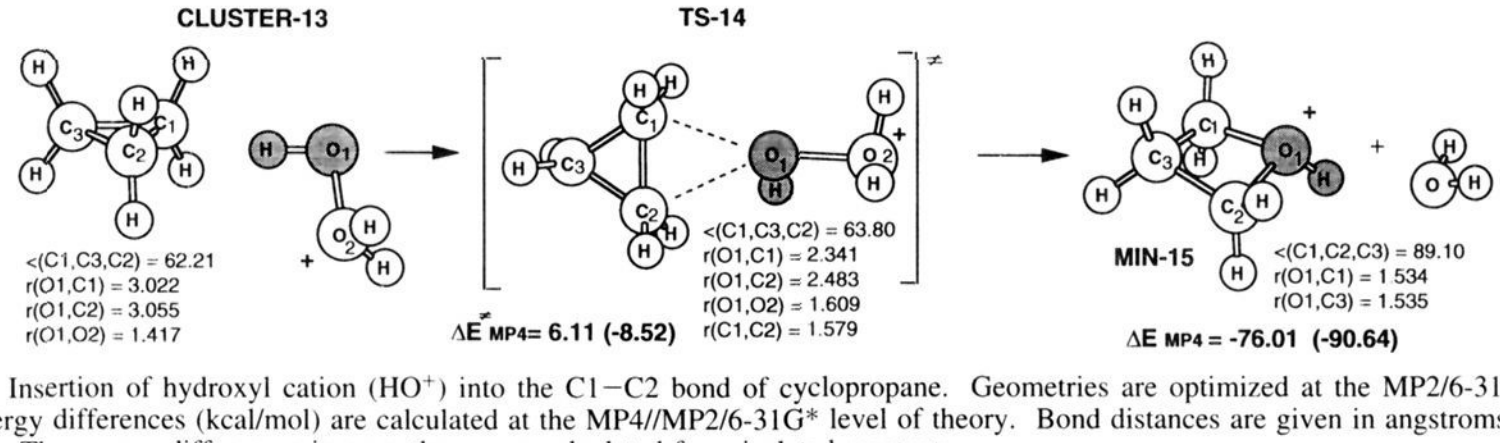


Figure 2. Insertion of hydroxyl cation (HO^+) into the C1−C2 bond of cyclopropane. Geometries are optimized at the MP2/6-31G* level of theory; energy differences (kcal/mol) are calculated at the MP4//MP2/6-31G* level of theory. Bond distances are given in angstroms and angles in degrees. The energy differences in parentheses are calculated from isolated reactants.

carbon−carbon bond to yield an oxetane were excluded because no chemical or biological precedent was known at that time. We provide theoretical evidence that concerted hydroxyl cation insertion into a C−C σ bond of cyclopropane is an extremely facile process providing an alternate pathway for oxidation of quadricyclane (Figure 1).

In a recently reported frontier MO model for electrophilic ($\mathbf{E}^+$) insertion into C−H bonds we established a protocol where we first examined the TS for singlet methylene ($^1CH_2$) insertion and then used this geometry as a starting point for attack by $\mathbf{E}^+$.[12] The classical activation barrier for insertion of $^1CH_2$ into the C−C bond of ethane affording propane is predicted to be 32.9 kcal/mol.[13] However, the transition state for $^1CH_2$ insertion into the C−C bond of cyclopropane is 5.3 kcal/mol (QCISD-(T)//QCISD/6-31G*) above a reactant cluster. It should be noted here that $^1CH_2$ insertion into the C−H bond of an alkane at this level is barrierless.[12b] The classical activation barrier for formal transfer of hydroxyl cation (HO^+) from hydroperoxonium ion to cyclopropane (TS-**14**) is predicted to be 6.1 kcal/mol ($\Delta G^{\ddagger}_{298}$ = 7.8 kcal/mol). From these data we were encouraged to examine the feasibility of quadricyclane oxidation by a comparable concerted insertion mechanism.

The reactant cluster (**9**) between quadricyclane and hydroperoxonium ion (complexed to the least acidic hydrogen to HO−OH_2^+) exhibits a stabilization energy of 19.0 kcal/mol (Figure 1). The classical activation barrier for HO^+ insertion is predicted to be only 1.0 kcal/mol and $\Delta G^{\ddagger}_{298}$ = 1.5 kcal/mol. The classical barrier is reduced to 0.1 kcal/mol with ZPE. Formation of protonated oxetane **11**, the kinetic product, is exothermic by 109.2 kcal/mol.[14] A slight change in geometry of **11** from its equilibrium position resulted in optimization to protonated aldehyde **12**.

If we assume that a protonated porphyrin iron(III) hydroperoxide is a symmetrically bridged complex of H_2O_2 resembling **7** and that the activation barrier for heterolytic O−O bond cleavage with loss of water from **8** even approaches an estimated 40 kcal/mol, then a concerted oxidation pathway involving insertion of HO^+ into a C−C bond becomes a very attractive low-activation pathway for the oxidation of quadricyclane. An iron(III) hydroperoxide has also been implicated in cleavage of the C-17 side chain in 17-*O*-acetyltestosterone formation from progesterone.[15a] The role of available protons at the active site to regulate the branching between oxene (ferryl oxygen) and iron(III) hydroperoxide chemistry has also been described.[15b] We suggest that the protonated iron(III) hydroperoxide implicated in the present study could also serve as the proton source to catalyze Baeyer−Villiger rearrangement with C−C bond cleavage attending this C-17 side chain cleavage.

Acknowledgment. This work was supported in part by the National Science Foundation (CHE 90-20398) and a NATO collaborative research grant (900707). We are also thankful to the Pittsburgh Supercomputing Center, CRAY Research, and the Ford Motor Company for generous amounts of computer time.

JA9516726

(12) (a) Bach, R. D.; Owensby, A.; Andrés, J. L.; Su, M.-D.; McDouall, J. J. W. *J. Am. Chem. Soc.* **1993**, *115*, 5768. (b) Bach, R. D.; Su, M.-D.; Aldabbagh, E.; Andres, J. L.; Schlegel, H. B. *J. Am. Chem. Soc.* **1993**, *115*, 10237.

(13) For an earlier calculation at the HF level, see: Gordon, M. S.; Boatz, J. A.; Gano, D. R.; Friederichs, M. G. *J. Am. Chem. Soc.* **1987**, *109*, 1323.

(14) An unsymmetrical cation corresponding to **11** also exists as a minimum and coincidently has an identical total energy.

(15) (a) Mak, A. Y.; Swinney, D. C. *J. Am. Chem. Soc.* **1992**, *114*, 8309. (b) Swinney, D. C.; Mak, A. Y. *Biochemistry* **1994**, *33*, 2185.