Theoretical Studies on Cytochrome P-450 Mediated Hydroxylation: A Predictive Model for Hydrogen Atom Abstractions

Kenneth R. Korzekwa,*,† Jeffery P. Jones,‡ and James R. Gillette†

Contribution from the Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892, and the Institute for Enzyme Research, University of Wisconson, Madison, Wisconson 53705. *Received January 12, 1990*

Abstract: The semiempirical quantum chemical method, AM1, was used to develop a predictive model for cytochrome P-450 hydrogen abstraction reactions. Initially, a general rank-order correlation was observed between the calculated stability of a radical and the tendency of a carbon–hydrogen bond to undergo hydrogen atom abstraction by cytochrome P-450. Of several oxygen radicals studied, the *p*-nitrosophenoxy radical has the most appropriate transition-state symmetry for use as a model for P-450-mediated hydrogen atom abstractions. With this model, a linear correlation was observed between $\Delta H^{\ddagger}$ and a combination of ΔH_R and either the modified Swain–Lupton resonance parameter or the ionization potential of the radical formed. The latter relationship gave an estimated standard deviation of the predicted $\Delta H^{\ddagger}$ of 0.8 kcal/mol. This suggests that it may be possible to obtain an estimate at the relative ability for any carbon–hydrogen bond to undergo P-450-mediated hydrogen atom abstraction by calculating the relative stability and ionization potential of the resulting radical.

Introduction

The ubiquitous superfamily of enzymes, the cytochrome P-450s, continues to be the focus of many diverse research efforts. Interest in these heme-containing monooxygenases stems from their ability to catalyze the oxidation of a wide variety of lipophilic endogenous (i.e., steroids, prostaglandins, and fatty acids) and exogenous (drugs and environmental contaminants) compounds.[1,2] The task of metabolizing a vast number of compounds with a limited number of isoenzymes appears to be accomplished by relatively broad substrate specificities and regiospecificities coupled to a versatile oxygenating species. Although the active oxygenating form of the enzyme is too short-lived to be fully characterized,[3] it appears to be an iron–oxo species that acts in a "triplet-like" manner. For example, hydroxylation reactions occur by an initial hydrogen abstraction followed by the recombination of the resulting hydroxy radical equivalent.[4] Other mechanisms suggesting that the active oxygen has radical characteristics include stepwise addition to olefins[5,6] and to aromatic compounds.[7,8]

Both the short lifetime and the unusual versatility of the active oxygen species invite the use of theoretical methods, and several quantum chemical mechanistic studies have been previously reported. These include studies on the charge densities and energies of substrates or products,[9-11] as well as studies on the potential rearrangement mechanisms of intermediated in the oxidative process.[12,13] Other calculations have modeled the initial oxidative step in an attempt to probe the nature of the oxygenating species. In these studies, either triplet or singlet atomic oxygen was used as a model for the oxygenating species.[14-19]

Certain characteristics of these enzymes add to the potential validity of using theoretical calculations to model their reactivity. First, the principle *catalytic* steps in the enzymatic cycle generate the active oxygenating species. The substrate is not directly involved in these steps, other than by possible initiation of the cycle. After generation of the active species, the reaction with substrate appears to be "chemical-like", with the apoprotein primarily influencing only the often broad regioselectivity of the reaction. Second, since the oxygen is thought to have radical characteristics, many of the reactions probably do not involve substantial charge development during the initial step of substrate oxidation. Furthermore, the active site is thought to be hydrophobic in nature, which is more closely approximated by gas-phase calculations than a more polar environment.

Since almost any new drug or xenobiotic will serve as a substrate for the cytochrome P-450 system, a predictive model for reactions catalyzed by these enzymes would be an extremely useful tool. It can be expected that the ability of a drug or foreign compound to be metabolized by a particular isozyme will be a function of both its binding characteristics (steric factors) and the tendency of various sites within a molecule to undergo oxidation (electronic factors). The research presented here describes a theoretical approach to defining the electronic factors that govern the general susceptibility of any given C–H bond toward cytochrome P-450 mediated hydrogen atom abstraction.

Methods

The semiempirical quantum chemical method AM1,[20] as provided in MOPAC 4.0 (QCPE No. 455), was used for all calculations. All calculations were performed on a Microvax II. The UHF Hamiltonian was used for all open-shell calculations. Stable geometries were optimized from approximate starting geometries; reaction coordinates were generated by constraining and varying the appropriate oxygen–hydrogen and/or carbon–hydrogen bond lengths. Approximate transition states were optimized with the gradient minimization routines provided by MOPAC. SCF calculations were optimized to $<10^{-8}$ kcal/mol and

† National Institutes of Health.
‡ University of Wisconson.

(1) Nelson, S. D. *J. Med. Chem.* **1982**, *25*, 753.
(2) Ortiz de Montellano, P. R. *Cytochrome P-450, Structure, Mechanism, and Biochemistry*; Plenum Press: New York, 1987; p 217.
(3) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315.
(4) Grooves, J. T.; McClusky, G. A.; White, R. E.; Coon, M. J., *Biochem. Biophys. Res. Commun.* **1978**, *81*, 154.
(5) Hanzlik, R. P.; Shearer, G. O. *Biochem. Pharmacol.* **1978**, *27*, 1441.
(6) Ortiz de Montellano, P. R.; Correia, A. C. *Annu. Rev. Pharmacol. Toxicol.* **1983**, *23*, 481.
(7) Bush, E. D.; Trager, W. F. *J. Med. Chem.* **1985**, *28*, 992.
(8) Korzekwa, K. R.; Swinney, D. C.; Trager, W. F. *Biochemistry* **1989**, *28*, 9019.
(9) Kamiya, M.; Akahori, Y. *Chem. Pharm. Bull.* **1972**, *20*, 677.
(10) Loew, G. H.; Goldblum, A. *Mol. Pharmacol.* **1985**, *27*, 375.
(11) Koymans, L.; van Lenthe, J. H.; van de Straat, R.; Donne-Op den Kelder, G. M.; Vermeulen, N. P. E. *Chem. Res. Toxicol.* **1989**, *2*, 60.
(12) Korzekwa, K.; Trager, W. F.; Gouterman, M.; Spangler, D.; Loew, G. H. *J. Am. Chem. Soc.* **1985**, *107*, 4273.
(13) Tsuda, M.; Oikawa, S.; Okamura, Y.; Kimura, K.; Urabe, T.; Nakajima, M. *Chem. Pharm. Bull.* **1986**, *34*, 4457.
(14) Pack, G. R.; Loew, G. H. *Int. J. Quantum Chem., Quantum Biol. Symp.* **1979**, *6*, 381.
(15) Pudzianowski, A. T.; Loew, G. H. *J. Mol. Catal.* **1982**, *17*, 1.
(16) Pudzianowski, A. T.; Loew, G. H. *Int. J. Quant. Chem.* **1983**, *23*, 1257.
(17) Pudzianowski, A. T.; Loew, G. H. *J. Phys. Chem.* **1983**, *87*, 1081.
(18) Pudzianowski, A. T.; Loew, G. H.; Mico, B. A.; Branchflower, R. V.; Pohl, L. R. *J. Am. Chem. Soc.* **1983**, *105*, 3434.
(19) Shea, J. P.; Nelson, S. D.; Ford, G. P. *J. Am. Chem. Soc.* **1983**, *105*, 5451.
(20) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.

0002-7863/90/1512-7042$02.50/0

Table I. Calculated (AM1) Thermodynamic Parameters for Parent Compounds and their Radicals

	substrate[f]	$\Delta H_{f(sub)}$[a]	$\Delta H_{f(rad)}$[b]	radical stability[c]	exptl BDE[d]	$IP_{(rad)}$[e]
1.	ethylamine (1)	−13.52	3.76	17.27		7.65
2.	ethylmethylamine (1)	−11.81	6.47	18.28		7.57
3.	cyclohexadiene (3)	17.48	28.81	11.33	70	8.30
4.	methylamine	−7.38	14.27	21.65		7.77
5.	dimethylamine	−5.63	16.95	22.58		7.68
6.	*N*-methylcarbazole (α)	72.79	95.11	22.31		7.72
7.	ethanol (1)	−62.66	−37.24	25.43	90	8.36
8.	*p*-hydroxyethylbenzene (α)	−35.57	−16.66	18.90		8.26
9	ethylbenzene (α)	8.66	28.32	19.66	85[g]	8.49
10.	*p*-cyanoethylbenzene (α)	39.77	59.26	19.49		8.90
11.	methyl ethyl ether (1)	−58.79	−32.19	26.60		8.26
12.	propene (3)	7.12	30.19	23.07	89	9.04
13.	*tert*-butane (2)	−29.37	−6.24	23.13	92	8.48
14.	chloroform	−29.00	−7.03	21.96	96	9.51
15.	propane (2)	−24.26	3.42	27.69	95	8.77
16.	ethane	−17.41	15.49	32.90	98	9.21
17.	cyclohexanone (2)	−63.33	−34.88	28.46	98[h]	9.78
18.	cyanomethane	19.28	52.33	33.06		10.39
19	methane	−8.78	29.95	38.73	104	9.89
20.	nitromethane	−9.93	25.48	35.41		11.72

[a] Heat of formation of the parent compound. All energy values in kilocalories per mole. [b] Heat of formation of the radical. [c] Relative radical stability ($\Delta H_{f(sub)} - \Delta H_{f(rad)}$). [d] Experimental bond dissociation energies of representative compounds from ref 23. [e] Ionization potential of the radical. [f] Value in parentheses designates position of radical. [g] Value given is for toluene. [h] Value given is for acetone.

geometries were optimized to $<10^{-4}$ Å. Most stable geometries and all transition states have been verified by the presence of 0 and 1 negative eigenvalues of the force constant matrix, respectively. Vibrational frequencies from the force constant calculations were used to calculate kinetic isotope effects as described previously.[21]

Results and Discussion

The thermodynamic and electronic properties of 20 radicals and their parent compounds are given in Table I. With the exception of chloroform,[22] the calculated radical stabilities are in good agreement with experimental bond dissociation energies.[23] Although to our knowledge a quantitative scale that precisely described the susceptibility of any specific C–H bond to be oxidized by cytochrome P-450 does not exist, it is generally accepted that α-carbon oxidation leading to N-dealkylation > O-dealkylation, and that benzylic oxidation > ω − 1 hydroxylation > ω hydroxylation. The data in Table I suggest that the calculated stabilities of the radicals relative to their parent compounds correspond to this general tendency (see "radical stability", Table I). The origin of this type of relationship, $\Delta H^{\ddagger} \propto \Delta H_R$, is usually discussed in terms of the Hammond postulate.[24] This postulate states that for certain similar reactions, exothermic reactions tend to have earlier transition states that are of lower energy than endothermic reactions. Linear relationships between activation energies (rates of reaction) and energies of reaction (relative stabilities of reactants and products) have been described previously for certain closely related reactions and are generally referred to as Brønsted relationships. The conceptual basis for these rate–equilibrium relationships are based on the Hammond postulate, Leffler principle,[25] and works by Evans,[26] Bell,[27] and others.[28,29] Most reported Brønsted relationships are associated with closed-shell reactions, i.e., acid/base catalysis or substitution reactions, rather than radical reactions. This, however, is presumably due to the analytical difficulties associated with measuring the stabilities of radicals rather than to the absence of such relationships. Studies have been reported that describe linear free energy relationships for certain radical reactions.[30] These include correlations of log of rates of hydroxy radical mediated hydrogen atom abstraction with bond dissociation energies.[31-33] For a reaction series where the abstracting species is constant, dissociation energies are a relative measure of radical stability.[34] Also, Hammett plots have been generated for some gas-phase radical abstraction reactions.[35] Although it is possible to have linear Hammett plots with no Brønsted relationship, this usually occurs when there are interactions of functional groups at the transition state that are not present in reactants or products.[36]

The requirements for linear Brøsted plots are stringent and many deviations have been observed and described.[36,37] Marcus has applied the relationships derived for electron transfer to describe the nonlinearity of proton-transfer reactions,[38] and Murdoch and others have extended these relationships to group-transfer and other reactions.[39-42] Beyond the possibility of nonlinear relationships, *any* relationship requires that the series of reactions have similar potential energy surfaces, with changes in energy due to stabilizing and destabilizing factors being related to changes in the geometry along the reaction coordinate. The lack of a precise linear relationship may be expected for P-450-mediated hydrogen abstraction reactions because of the variety of stabilizing and destabilizing effects of the various possible substrates. In addition, experimental correlations may be expected to deviate from theoretical calculations because of apoprotein-mediated substrate specificity (steric effects). However, the general correlation between radical stability and tendency for oxidation as well as the previously reported studies on gas-phase hydrogen atom ab-

(21) Dewar, M. J. S.; Ford, G. P. *J. Am. Chem. Soc.* **1977**, *99*, 822.
(22) Whether the discrepancy with chloroform is associated with the experimental or the theoretical value is unknown. The error is not due to spin contamination associated with the UHF calculation ($\langle S^2 \rangle = 0.76$).
(23) Kerr, J. A.; Trotman-Dickenson, A. F. In *Handbook of Chemistry and Physics*, 53rd ed. Weast, R. C., Ed.; Chemical Rubber Co.: Cleveland, OH, 1972; p F189.
(24) Hammond, G. S. *J. Am. Chem. Soc.* **1955**, *77*, 334.
(25) Leffler, J. E. *Science* **1953**, *117*, 340.
(26) Evans, M. G.; Polanyi, M. *Trans. Faraday Soc.* **1936**, *32*.
(27) Bell, R. P. *Proc. R. Soc. A* **1936**, *154*, 414.
(28) (a) Hammett, L. P. *Chem. Rev.* **1935**, *17*, 125. (b) Hammett, L. P. *Physical Organic Chemistry*; McGraw Hill: New York, 1970; p 215.
(29) For reviews, see: Bell, R. P. In *Correlation Analysis in Chemistry*; Chapman, N. D., Shorter, J., Eds.; Plenum: New York, 1978; p 55. In *Correlation Analysis in Chemistry*; Chapman, N. D., Shorter, J., Eds.; Plenum: New York, 1978; p 55. Kresge, A. J. *Chem. Soc. Rev.* **1973**, *2*, 475.
(30) For a review, see: Gaffney, J. S.; Bull, K. In *Chemical Kinetics of Small Organic Radicals*; Alfassi, Z. B., Ed.; CRC Press: Boca Raton, FL, 1988; Vol. II.
(31) Gaffney, J. S.; Levine, S. Z. *Int. J. Chem. Kinet.* **1979**, *11*, 1197.
(32) Martin, J.; Paraskevopoulos, G. *Can. J. Chem.* **1983**, *61*, 861.
(33) Gurvinder, S. J.; Paraskevopoulos, G.; Singleton, D. L. *Int. J. Chem. Kinet.* **1985**, *17*, 1.
(34) Nicholas, A. M.; Arnold, D. R. *Can. J. Chem.* **1984**, *62*, 1850.
(35) Zavitsas, A. A.; Pinto, J. A. *J. Am. Chem. Soc.* **1972**, *94*, 7390.
(36) Pross, A. *J. Org. Chem.* **1984**, *49*, 1811.
(37) Jencks, W. P. In *Nucleophilicity*; Harris, J. M., McManus, S. P., Eds.; Advances in Chemistry 215; American Chemical Society: Washington, DC, 1987; p 155.
(38) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891.
(39) Murdoch, J. R.; Magnoli, D. E. *J. Am. Chem. Soc.* **1982**, *104*, 3792.
(40) Murdoch, J. R. *J. Am. Chem. Soc.* **1983**, *105*, 2660.
(41) Pellerite, M. J.; Brauman, J. I. *J. Am. Chem. Soc.* **1983**, *105*, 2672.
(42) Donnella, J.; Murdoch, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4724.

Table II. Calculated AM1 Thermodynamic Parameters for the Reaction of the *p*-Nitrosophenoxy Radical with Various Substrates

	substrate[h]	$\Delta H_{f(ts)}$[a]	$\Delta H^{\ddagger}$[b]	ΔH_R[c]	R[d]	r_{tot}[e]	r_{CH}/r_{tot}[f]	intr bar.[g]
1.	ethylamine (1)	21.78	16.26	−16.42	−2.93	2.64	0.48	24.5
2.	ethylmethylamine (1)	24.14	16.91	−15.41		2.63	0.49	24.6
3.	cyclohexadiene (3)	53.58	17.06	−22.36		2.62	0.49	28.2
4.	methylamine	29.44	17.78	−12.04	−2.52	2.60	0.49	23.8
5.	dimethylamine	31.56	18.14	−11.11		2.60	0.49	23.7
6.	*N*-methylcarbazole (α)	110.81	18.98	−11.38		2.58	0.50	24.7
7.	ethanol (1)	−24.50	19.12	−8.26	−2.30	2.58	0.50	23.3
8.	*p*-hydroxyethylbenzene (α)	3.56	20.09	−14.79		2.60	0.50	27.5
9.	ethylbenzene (α)	48.23	20.53	−14.03	−0.78	2.59	0.50	27.3
10.	*p*-cyanoethylbenzene (α)	79.88	21.06	−14.20		2.59	0.50	28.2
11.	methyl ethyl ether (1)	−18.48	21.27	−7.09	−2.09	2.57	0.50	24.8
12.	propene (3)	47.70	21.53	−10.62		2.57	0.50	26.8
13.	*tert*-butane (2)	11.56	21.88	−10.56	−1.23	2.59	0.50	27.2
14.	chloroform	12.29	22.25	−11.73	−0.72	2.56	0.52	28.1
15.	propane (2)	17.74	22.96	−6.00	−0.82	2.57	0.51	26.0
16.	ethane	26.20	24.57	−0.79	−0.41	2.54	0.51	25.0
17.	cyclohexanone (2)	−19.16	25.13	−5.23		2.55	0.52	25.4
18.	cyanomethane	64.65	26.33	−0.63	0.71	2.54	0.52	26.6
19	methane	37.26	26.99	5.04	0.00	2.52	0.52	24.5
20.	nitromethane	36.34	27.23	1.72	1.00	2.53	0.54	26.4

[a] All energy values in kilocalories per mole. [b] ΔH_f for the *p*-nitrosophenoxy radical is 19.04 kcal/mol. [c] ΔH_f for *p*-nitrosophenol is −14.65 kcal/mol. [d] The modified Swain–Lupton resonance parameter (see text). [e] Total C–H–O transition-state distance in angstroms. [f] Fraction of transition-state distance associated with the C–H bond. [g] Estimated intrinsic barrier as calculated in ref 43d. [h] Value in parentheses designates hydrogen atom being removed.

stractions[31-33,35] suggests that a broad relationship may exist and that the application of quantum chemical methods to model the transition states may provide useful insights into these reactions.

Whereas previous studies have used a triplet atomic oxygen model, the high reactivity of this species, and therefore the exothermicity of its associated reactions, suggests that it would not be an ideal model for P-450. Very early transition states would be associated with the large negative heats of reaction, and the reactions would be insensitive to changes in the nature of the hydrogen atom being abstracted. For example, it has been shown that the Hammett ρ value decreases for abstraction of the benzylic hydrogen atoms from substituted toluenes as the exothermicity of the reaction increases.[35] Also, the correlation of rates of hydroxy radical abstractions with bond dissociation energies[33] has a slope of 0.04 (after converting the rate differences to ΔE). Since a more stable oxygen is required and any heme-containing model would be too large for transition-state calculations, smaller stabilized oxygen radicals, i.e., the substituted ethyleneoxy and phenoxy radicals, were used. Figure 1 shows a representative hydrogen atom abstraction reaction, and Figure 2 shows the theoretical Brønsted plots for the abstraction of the C_2 hydrogen from propane and the C_1 hydrogen from ethane with these radicals. Again, the data in Figure 2 suggest that linear or nonlinear relationships may exist between stability of radicals and activation energies of hydrogen abstractions.

Experimental data on the isotope effects associated with P-450 oxidations provide some information about the required thermodynamic characteristics for a valid model compound. It has been suggested that the transition state for the ω hydroxylation of aliphatic compounds is symmetrical since octane hydroxylation is accompanied by a large primary deuterium isotope effect and an intermediate secondary isotope effect.[43] Of the models in Figure 2, the most thermodynamically symmetrical ω-hydrogen abstraction reaction ($\Delta H_R \approx 0$) is seen with the *p*-nitrosophenoxy radical. For this radical, the calculated theoretical isotope effects for abstraction of hydrogen atoms from ethylamine, methylamine, ethane, and cyanomethane are 5.4, 6.0, 6.4, and 6.2, respectively. Although these values are somewhat lower than the experimental primary isotope effects,[43] the fact that the ethane reaction had the highest isotope effect suggests that the symmetry of the ethane/*p*-nitrosophenoxy radical reaction is manifest both thermodynamically and in its transition-state geometry.[44] Thus, the *p*-nitrosophenoxy radical may serve as a useful model for the first step in cytochrome P-450 mediated hydroxylation reactions.

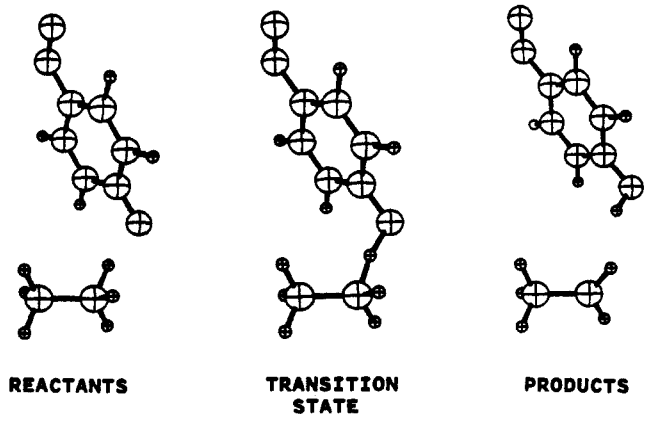

Figure 1. A representative hydrogen abstraction reaction: *p*-nitrosophenoxy radical abstraction of a hydrogen atom from ethane. The transition state has the following characteristics: r_{O-H} = 1.246, r_{H-C} = 1.297, θO–H–C = 168.9°.

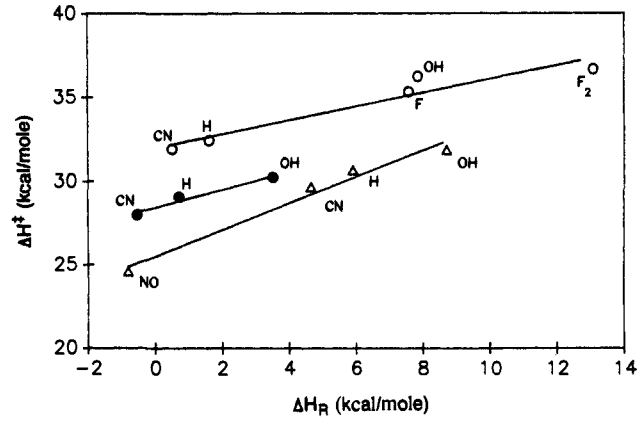

Figure 2. AM1 Brønsted relationships for hydrogen abstraction reactions. (○) abstraction of a C_2 hydrogen from propane with substituted ethyleneoxy radicals (XHC=CHO•). (●) abstraction of a C_2 hydrogen from propane with para-substituted phenoxy radicals. (△) abstraction of a hydrogen from ethane with para-substituted phenoxy radicals.

The *p*-nitrosophenoxy radical was used to model hydrogen abstraction reactions in molecules containing various functional groups. The thermodynamic and electronic properties for 20

(43) Jones, J. P.; Trager, W. F. *J. Am. Chem. Soc.* **1987**, *109*, 2171.
(44) Bell, R. P. *Chem. Soc. Rev.* **1974**, *3*, 513.

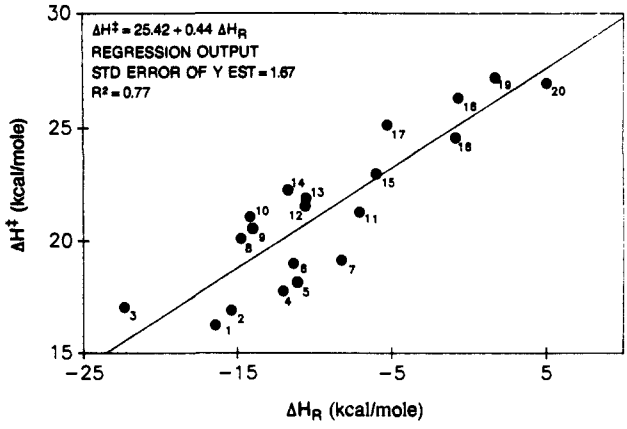

Figure 3. AM1 Brønsted relationships for hydrogen abstraction reactions with the *p*-nitrosophenoxy radical. The numbers correspond to the reactions in Table II.

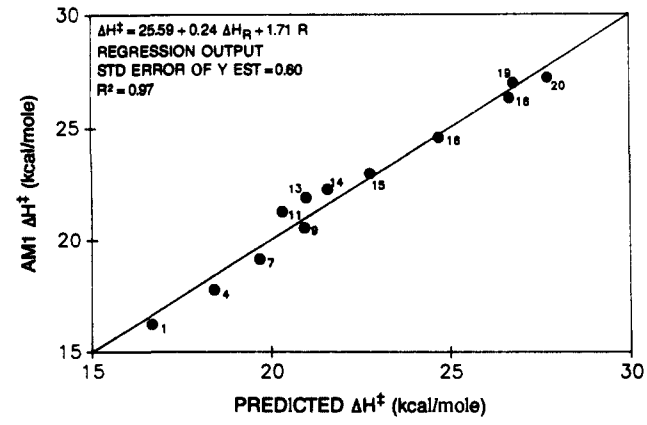

Figure 4. Predicted vs calculated enthalpies of activation for hydrogen abstraction reactions with the *p*-nitrosophenoxy radical. Calculated by regression of $\Delta H^{\ddagger}$ on both ΔH_R and the modified Swain–Lupton resonance parameter **R**. The numbers correspond to the reactions in Table II.

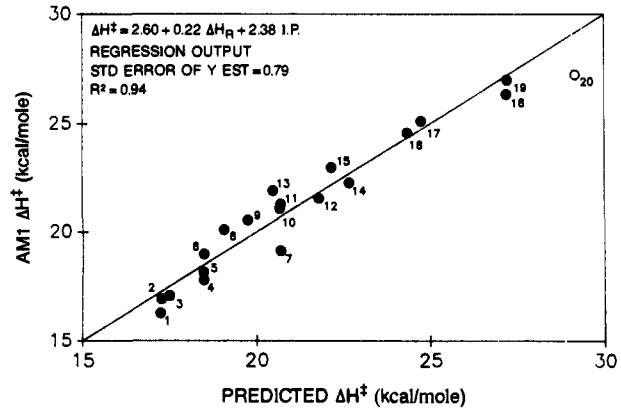

Figure 5. Predicted vs calculated enthalpies of activation for hydrogen abstraction reactions with the *p*-nitrosophenoxy radical. Calculated by regression of $\Delta H^{\ddagger}$ on both ΔH_R and the ionization potential of the product radicals. The numbers correspond to the reactions in Table II.

hydrogen abstraction reactions are given in Table II. The theoretical Brønsted plot for these reactions is shown in Figure 3, and regression analysis gives a modest linear correlation (R^2 = 0.77). The lack of a better correlation is not surprising, since the different functional groups will interact with the reaction center in different ways, resulting in differences in potential energy surfaces. One property that can be used in the characterization of potential energy surfaces for proton and group-transfer reactions is the intrinsic barrier.[40-42,45] The intrinsic barrier for a reaction is the hypothetical activation energy of a reaction when ΔH_R = 0. Linear Brønsted plots or Marcus-type relationships for group-transfer reactions are seen when the intrinsic barrier does not change within a reaction series. The approximate intrinsic barriers for the modeled hydrogen abstraction reactions (Table II) vary by as much as 20%.

From the data in Figure 3, it can be seen that all conjugated systems except nitromethane lie above the line, suggesting that resonance stabilization of the product radicals is greater than that for the transition states. A similar situation is discussed by Bernasconi[46] in which stabilizing or destabilizing properties of reactants or products are not proportionally manifested in the transition state. Bernasconi discusses these effects in terms of "the principle of non-perfect synchronization". This principle states that early destabilization of reactants or late stabilization of products will result in an increased intrinsic barrier. Analysis of the calculated intrinsic barriers in Table II shows that, indeed, the intrinsic barriers associated with conjugated systems are higher than those for unconjugated systems.

In order to correct for the disproportionate product stabilization, a total Swain–Lupton resonance parameter, **R**,[47] was calculated by adding the values for each group substituted on the carbon reaction center. Using the modified values provided by Swain et al.,[48] we obtained **R** values for 12 of the 20 reactions modeled. The calculated $\Delta H^{\ddagger}$ values were regressed on both ΔH_R and **R**, and the results are shown in Figure 4. The plot of predicted vs calculated $\Delta H^{\ddagger}$ gave an excellent linear relationship with an R^2 value of 0.97 and an estimated standard error for predicted activation energies of 0.60 kcal. When activation energies were regressed on field and resonance parameters (normal multiparameter Hammett), a poorer correlation (R^2 = 0.88) was obtained (data not shown). This suggests that the deviation from the strict Brønsted relationship is a result of nonlinear resonance effects along the reaction coordinate rather than effects on the transition state that are not present in reactants and products.

While the above correlation with ΔH_R and **R** is surprisingly good, it would be useful to find a more general measure of resonance stabilization, since the modified Swain parameters are not available for all possible radical species. The ionization potential of the radical may be a quantitative measure of this stabilization, since the energy of the singly occupied orbital is lowered through resonance. Since the ionization potentials of the product radicals are provided by the calculations (according to Koopmans' theorem[49]), these were available for regression analysis. The resulting linear relationship has an R^2 value of 0.92 and an estimated standard error for predicted activation energies of 0.99 kcal. If the point for nitromethane is excluded, a correlation coefficient of 0.94 is obtained with an estimated standard error for the predicted $\Delta H^{\ddagger}$ of 0.8, as shown in Figure 5. In order to show that the deviation for linearity is not due to the spin contamination associated with the UHF calculations, the $\langle S^2 \rangle$ values for radical products were used instead of ionization potential in the regression analysis. A regression coefficient of 0.80 was obtained, suggesting that spin contamination is not correlated with activation energy.

Although the exact nature of the apparent effect of resonance on the potential energy surfaces is not known, the authenticity of this relationship is verified by its ability to predict transition-state geometries. Since all of the transition states are approximately linear ($\theta \approx 170°$), the transition-state geometry can be defined by two parameters: r_{tot} (total O–H–C bond distance) and $r_{C\text{-}H}/r_{tot}$ (fraction associated with C–H bond length). These two parameters

(45) (a) Murdoch, J. R. *J. Am. Chem. Soc.* **1972**, *94*, 4410. (b) Murdoch, J. R.; *J. Am. Chem. Soc.* **1980**, *102*, 71. (c) Magnoli, D. E.; Murdoch, J. R. *J. Am. Chem. Soc.* **1981**, *103*, 7465. (d) Murdoch, J. R. *J. Phys. Chem.* **1983**, *87*, 1571.

(46) Bernasconi, C. F. In *Nucleophilicity*; Harris, J. M., McManus, S. P., Eds.; Advances in Chemistry 215; American Chemical Society: Washington, DC, 1987; p 115.

(47) Swain, G. C.; Lupton, E. C. *J. Am. Chem. Soc.* **1968**, *90*, 4328.

(48) Swain, C. G.; Unger, S. H.; Rosenquist, N. R.; Swain, M. S. *J. Am. Chem. Soc.* **1983**, *105*, 492.

(49) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. In *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986; p 24.

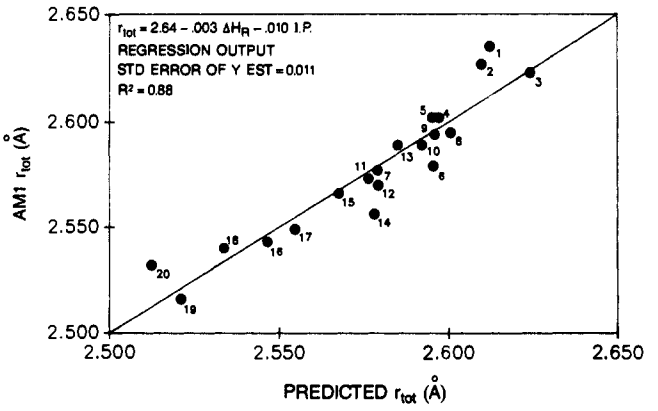

Figure 6. Predicted vs calculated r_{tot} (C-H-O bond transition-state distance) from regression on both ΔH_R and the ionization potential of the product radicals. The numbers correspond to the reactions in Table II.

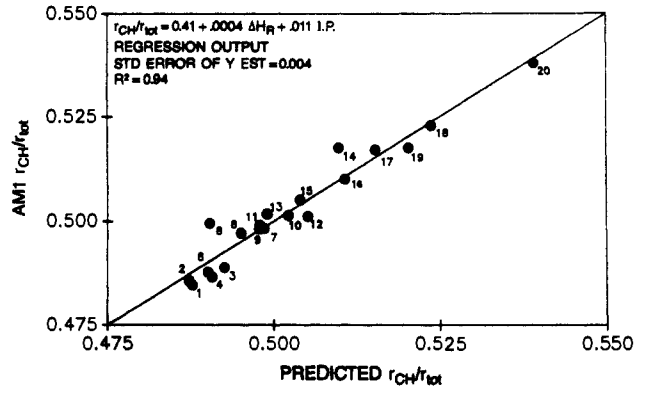

Figure 7. Predicted vs calculated r_{C-H}/r_{tot} (fraction of transition-state distance associated with the C-H bond) from regression on both ΔH_R and the ionization potential of the product radicals. The numbers correspond to the reactions in Table II.

were regressed independently on both ΔH_R and ionization potential of the resultant radicals. The predicted vs actual values are plotted in Figures 6 and 7. The coefficients for the two regression equations show that r_{tot} is a function of both ΔH_R and the radicals ionization potential while r_{C-H}/r_{tot} is almost completely predictable from ionization potential. As this study progressed, these relationships were used to predict transition-state geometries, eliminating the need for generating reaction coordinates, or in some more difficult cases, two-dimensional potential energy surfaces.

It should be noted that this model is only valid for the initial hydrogen atom abstraction. During hydroxylation by cytochrome P-450, initial hydrogen abstraction is followed by the recombination of a hydroxy radical equivalent. Two possible mechanisms for the generation of the hydroxy radical species are considered. First, an oxygen atom with one unpaired electron could abstract the hydrogen atom. A change in electronic configuration would then be necessary in order to provide the second unpaired electron required for recombination. A more likely mechanism is that the oxygen atom is initially involved in two molecular orbitals with unpaired electrons (triplet-like), with the necessary spin change occurring after hydrogen abstraction. The involvement of spin-orbit coupling with an iron, which is most likely paramagnetic, would greatly facilitate such a spin change.[50,51] Since the model oxidizing radical is not "triplet-like" but rather a doublet, the full process of abstraction and recombination cannot be modeled with this doublet radical.

Since the rates of recombination are fast, the initial hydrogen abstraction would be expected to be virtually irreversible, and therefore, the relative rates of hydroxylation would depend only on the first step. The question then is whether the doublet nitrosophenoxy radical model can place a reaction on a similar relative energy scale as exists for the triplet-like P-450 active oxygenating species. We are encouraged for the following reasons: First, Gaffney and Levine[31] have reported an excellent correlation between the rates for triplet oxygen, $O(^3P)$, gas-phase hydrogen atom abstractions vs the rates for the doublet hydroxy radical abstractions. Second, hydrogen atom abstraction reactions appear to be predictable within a wide range of electronic environments. Beyond the correlations presented herein, experimental relationships between activation energies for the hydroxy radical abstractions and bond dissociation energies can place various adjacent functional groups on the same line.[33] Finally, the relative rates for hydroxylation of octane appear to be consistent with the model. It has been shown through deuterium isotope effect experiments that rate of interchange between the substrate orientations that lead to ω and the $\omega - 1$ hydroxylation of octane is fast relative to the rate of hydrogen atom abstraction.[52] The relative amounts of products formed should depend primarily on the relative activation energies for hydrogen atom abstractions at different sites on the molecule. The ratio of ω to $\omega - 1$ hydroxylation products of octane varies between 5 and 23.[52] The ratio predicted from the difference in calculated activation energies is 14.

We are presently modeling aromatic addition reactions in order to develop a more complete model. Since there are additional data on the ratios of hydrogen abstractions relative to aromatic oxidation, the future model can be further tested with experimental results.

In summary, the relatively simple procedure of optimizing the geometry of any compound and its potential radicals may be useful in predicting the relative potential for hydrogen atom abstraction at the various positions, neglecting binding and steric constraints imposed by the enzymes. Since the optimization of stable geometries is much faster than the relatively arduous task of searching for and optimizing transition states, quick insight can be gained in addition to saving a substantial amount of computer time.

Acknowledgment. This research was supported in part by a PRAT fellowship (K.R.K.) from the National Institute of General Medical Sciences.

(50) Lowry, T. H.; Richardson, K. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper and Row: New York, 1987; p 983.
(51) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 4th ed.; Wiley: New York, 1980; p 651.
(52) Jones, J. P.; Korzekwa, K. R.; Rettie, A. E.; Trager, W. F. *J. Am. Chem. Soc.* **1986**, *108*, 7074.