J. Am. Chem. Soc. **1987**, *109*, 3748-3754

Complex Formation between Excited-State Saturated Amines and Water in *n*-Hexane Solution

Arthur M. Halpern,* Christopher J. Ruggles, and Xing-kang Zhang

Contribution from the Department of Chemistry, Northeastern University, Boston, Massachusetts 02115. *Received December 17, 1986*

Abstract: Fluorescence spectra and decay curves of dilute solutions ($<3 \times 10^{-4}$ M) of triethylamine (TEA), tri-*n*-propylamine (TPA), and 1,4-diazabicyclo[2.2.2]octane (DABCO) in H_2O- and D_2O-saturated *n*-hexane reveal the presence of a complex formed between the electronically excited amine and water. The decay curves, measured between 273 and 323 K (and at 280 and 360 nm; 300 and 400 nm for DABCO), conform to the standard monomer–excimer photokinetic scheme and are analyzed accordingly. These results indicate that the binding energy of the excited TEA–H_2O complex (B^*) is ca. 7.8 kcal/mol, which is larger than that of the ground-state TEA hydrate. B^* for the TPA and DABCO–H_2O complexes is estimated to be ca. 10 and 8.8 kcal/mol, respectively. Stationary-state measurements are consistent with these assignments. The activation energy for the diffusion of water in *n*-hexane (assumed to be monomeric) appears to be very small (<1 kcal/mol). The decay constants of the three complexes studied are ca. 3.4×10^7 s^{-1} for amine–H_2O and 2.9×10^7 s^{-1} for the amine–D_2O systems. Intrinsic fluorescence quantum efficiencies of the amine–H_2O complexes are 0.17, 0.23, and 0.28 for TEA, TPA, and DABCO, respectively, at 303 K. A Förster cycle analysis of the dry and H_2O-saturated fluorescence spectra of TEA, when taking the ground-state hydrate into account, indicates that the repulsion energy of the post-fluorescence (TEA–H_2O) complex is ca. 10 kcal/mol. A bonding mechanism between the excited-state amine and H_2O involving both the O and H atoms is proposed. Although differences could not be discerned between B^* values of the amine–H_2O and amine–D_2O complexes, the dissociation constants for the amine–D_2O complexes are all *larger* (by ca. 1.4 kcal) than those for the H_2O species.

The photophysical properties of saturated tertiary amines, both in the vapor phase and especially in solution, are of particular interest because they provide insight into the characteristics and behavior of molecular Rydberg excited states.[1] These compounds are highly fluorescent in the vapor phase; for example, trimethylamine (TMA) and triethylamine (TEA) have reported quantum efficiencies of unity when subject to long-wavelength excitation (e.g., 240–250 nm) under low-pressure conditions.[2] In dilute nonpolar solution (e.g., *n*-hexane) the quantum efficiency of TEA is 0.69 at 300 K. This important experimental advantage has allowed the observation and characterization, for example, of both intra- and intermolecular excimer formation, both in the vapor phase and in solution.[3] In addition, processes such as exciplex formation (and emission) and electronic energy transfer have been investigated in systems involving photoexcited saturated tertiary amines.[4]

In view of the well-known role of ground-state saturated amines as hydrogen bond acceptors (i.e., Brønsted bases), it is of fundamental importance to establish the hydrogen bonding behavior of saturated amines in their lowest electronically excited state. In general, the question arises regarding the nature of the association of electronically excited amines with protic species. The interaction between these excited states and protic molecules is particularly interesting because the relatively long singlet lifetimes of the amines in nonpolar solution (e.g., 25–60 ns) permit considerable encounter frequencies with potential protic quenchers. Moreover, the dramatic changes in electronic structure and geometry which occur in the amines upon photoexcitation point to possibly different modalities of interaction with protic species. The lowest excited state in the simplest trialkylamine, TMA, is assigned (in the vapor phase) as 3s Rydberg;[5] this is presumably the case in the other trialkylamines as well. Moreover, in the case of TMA (and related, conformationally unhindered amines) the $^1(n_N,3s)$ state is *planar*, as distinct from the pyramidal ground state.[6] Questions thus arise concerning the energetics of hydrogen bonding (or complexation) between water and the excited state of saturated tertiary amines and the structure of this complex.

We report here the results of a study in which the protic species is water and the saturated amines are triethylamine (TEA), tri-*n*-propylamine (TPA), and the bicyclic amine 1,4-diazabicyclo[2.2.2]octane (DABCO). The monoaza analogue, ABCO, was also investigated, but these studies were abandoned because of the competition, and thus complication, of excimer formation in this amine[3c] (even at 8×10^{-5} M to below this concentration the signal to noise quality was too low). The solvent medium used in this study is *n*-hexane; it was chosen to avoid complications which result from the strong interactions between the excited amine and polar solvent environments.[6] For example, dramatic photophysical changes are observed for saturated amines in even moderately polar solvents (e.g., ethers).[7]

Also, because of the relatively low solubility of water in *n*-hexane (e.g., 4×10^{-3} M at 300 K, see below), the role of dimers and higher oligomers can be largely ignored; hence, the amine photophysics studied in this system can be interpreted in terms of the involvement of the water monomer itself. A study dealing with intramolecular association in amino alcohols, as well as intermolecular interactions between tertiary amines and ethanol in *n*-hexane solution, has been carried out[8] and will be reported separately.

Experimental Section

The amines (Aldrich) were distilled over CaH_2, TPA at reduced pressure. DABCO and ABCO were dried over CaH_2 in concentrated 2-methylbutane solution. After the solvent was evaporated, the amine was sublimed in vacuo. Deionized, distilled H_2O was used; D_2O (99.7%) was obtained from Merck Sharp & Dohme. *n*-Hexane (Burdick and Jackson) was distilled from CaH_2 for the use in the "dry" experiments; this solvent was used as received for the water-saturated measurements. To prepare the water-saturated samples, ca. 20 μL of N_2-deaerated H_2O (or D_2O) was added to the N_2-deaerated solution of the amine in *n*-hexane by using a syringe equipped with a long tip. The water droplet was deposited on the bottom of the fluorescence cell (1 cm^2 × 5 cm) which was fitted with a Teflon stopcock. This procedure was carried out in a N_2-purged drybox. Before the cell stopcock was tightly sealed, the solution was briefly deaerated with dry N_2; a gentle stream was used to avoid dispersing the water droplet. After being heated and cooled, the samples revealed that the water phase coated the inner surface of the cell

---

[1] Robin, M. B. *Higher Excited States of Polyatomic Molecules*; Academic: New York, 1974; Vol. 1, pp 208–229.

[2] Halpern, A. M.; Gartman, T. *J. Am. Chem. Soc.* **1974**, *96*, 1393.

[3] (a) Halpern, A. M.; Chan, P. P. *J. Am. Chem. Soc.* **1975**, *97*, 2971. (b) Halpern, A. M. *J. Am. Chem. Soc.* **1974**, *96*, 7655. (c) Halpern, A. M.; Ravinet, P.; Sternfels, R. J. *J. Am. Chem. Soc.* **1977**, *99*, 169. (d) Frye, S. L.; Ko, J.; Halpern, A. M. *Photochem. Photobiol.* **1984**, *40*, 555.

[4] (a) Halpern, A. M.; Wryzykowska, K. *Chem. Phys. Lett.* **1981**, *22*, 82. (b) Frye, S. L. Ph.D. Dissertation, Northeastern University, 1985.

[5] Halpern, A. M.; Ondrechen, M. J.; Ziegler, L. D. *J. Am. Chem. Soc.* **1986**, *108*, 3907.

[6] Halpern, A. M.; Wryzykowska, K. *J. Photochem.* **1981**, *15*, 147.

[7] Halpern, A. M. *J. Phys. Chem.* **1981**, *85*, 1682.

[8] Legenza, M. W. Ph.D. Dissertation, Northeastern University, 1984.

0002-7863/87/1509-3748$01.50/0

in small "puddles"; these islets did not interfere with the fluorescence measurements (i.e., scattered light was not a serious problem). Samples cooled from higher temperatures became turbid, but, interestingly, decay curves of these turbid samples were consistent with those of clear solutions (at the same temperature). Fluorescence spectra were obtained by using a homemade dc fluorimeter. Excitation (60-W D_2 lamp) was at 230 nm (300 nm for DABCO). Quantum efficiencies were measured in an instrument in which the transmitted light of the sample was measured directly in the cavity. Since the presence of water did not interfere with optical excitation, the very small decrease in free amine concentration was taken directly into account. TEA was used as a standard (0.69, 1.5×10^{-4} M in *n*-hexane). Fluorescence decay curves were measured by using a time-correlated photon counting apparatus employing a 30-kHz gated D_2 (0.5 atm) flashlamp. Excitation light was isolated via a monochromator (3.2-nm band-pass); fluorescence was detected through interference filters (Corion Corp.). When the long-wavelength decay curves were analyzed separately, and the amplitude ratio allowed to "float", values very close to -1 were obtained. All decay curves were analyzed as a pair, monomer and excited complex, by using a dual analysis reconvolution program installed on a dedicated LSI 11/23 microcomputer.[3d] The short- and long-lived components of both decay curves were constrained to be equal to each other. For consistency, the amplitude ratio in the long-wavelength decay curves was constrained to -1. Typical χ^2 values for the *pair* of decay curve reconvolutions were 3–5; the fitting range started well before the rising edges of the decay curves.

Results and Discussion

Amine concentrations were kept low ($<3 \times 10^{-4}$ M) to minimize the extent of ground-state hydrogen bonding with water. At this concentration, fluorescence decay curves of all the amines studied in rigorously dried *n*-hexane follow single-exponential decay for at least 3 decades; moreover, the amine self quenching under these conditions is unimportant. For example, the self-quenching efficiency ranges from 4% for TEA to 1% for DABCO at 300 K.[9]

In wet solvent, even when the water level is far below saturation, nonexponential decay curves are observed when fluorescence is monitored at λ_{max} (e.g., 282 nm for TEA). This effect can be rather subtle; i.e., reasonably acceptable single-exponential decay curves ($\chi^2 < 3$) can be obtained. Double-exponential reconvolution analysis, however, clearly reveals two components having similar components.

Because our objective is to characterize the photokinetics and thermodynamics of the amine–water system in *n*-hexane solution, it is important to identify the conditions under which the fluorescence spectra and decay curves could be meaningfully and reproducibly measured as a function of water composition and temperature. We found that fluorescence decay curves (which are readily characterized quantitatively) could not be reproduced when the water composition was below the saturation level. In addition, the fluorescence spectrum and decay curve of a given sample changed after standing for a few hours. These problems presumably arise from the tendency of water to adhere to the inner (hydrophilic) surface of the fluorescence cell. To avoid these undesirable complications, we opted to study systems in which the water was *saturated*, and thus its composition was determined thermodynamically. This approach assumes that the presence of the amine ($1–3 \times 10^{-4}$ M) does not alter the water–solvent equilibrium and hence the water composition and its temperature dependence. Thus *two-phase* systems were prepared by admitting small amounts of water (ca. 20 μL) to deaerated *n*-hexane solutions of the amine. Over the maximum temperature range studied (273–353 K), there was always a finite, but small, amount of liquid water in contact with the *n*-hexane phase. The water droplet was deposited at the bottom of the cell and thus out away from the optical excitation and analysis paths. The dissolution and equilibration of water into the organic phase could be monitored in real time by observing the quenching (and broadening) of the amine fluorescence spectrum. Equilibration was reached within 30 min. Shorter equilibration times could be achieved by mixing the solution gently, but this technique was not employed in these studies.

The partitioning of the amine into the water phase, and thus the lowering of the concentration in *n*-hexane, was found to depend on the size of the water droplet present in the cell. In no case did the decrease in amine concentration exceed ca. 5% relative to the initial amine composition in dry *n*-hexane. In any case this effect is of no consequence in our studies because (1) the photokinetic results are independent of amine concentration and (2) the quantum efficiency measurements are based on the actual amount of light absorbed in a given sample.

The effect of water on the absorption spectra of the amines was examined by quantitatively comparing the absorption spectra of an amine in dry, dearated solvent relative to water-saturated solvent. With the exception of DABCO and TPA, no measurable changes in the position or absorbance could be detected (after accounting for the small volume increase, and hence dilution of the amine, in the water-saturated systems). This is attributed to the small hydration constant and low water composition of the ground-state amine–water system. For example, for TEA, the equilibrium constant for the 1:1 hydrate is reported to be 7.01 in cyclohexane at 298 K.[10] With this value, along with the solubility of water in *n*-hexane at this temperature (assuming that it is unaffected by the presence of the small amount of amine), ca. 3% of the amine exists as the 1:1 hydrate. The presence of higher (2:1) hydrates can be neglected. In any case, the expected (and observed) blue shift in the absorption of the amine hydrate further lessens the role of this species from the photophysics.

The question also arises as to the nature of the water species which exist in *n*-hexane under saturated conditions, that is, whether dimers or higher adducts, or even water–solvent complexes, might play a role in the quenching of the photoexcited amine. From an infrared study of the dimerization of water in CCl_4 at 298 K, the dimerization constant is reported to be 2.2.[11] Assuming a similar value in *n*-hexane, the fraction of water dimer relative to the bulk water composition is ca. 0.01. We are thus justified in ignoring the role of water dimers and higher oligomers in these experiments.

Another consideration in these studies is the extent to which water is bound to, or formally associates with, the solvent. This is relevant in connection with the mobility (and activation thereto) of free water in *n*-hexane. In discussing the thermodynamics of water solubility in nonpolar solvents, Kirchnerova and Cave have assumed that water–solvent complexes are not formed in saturated hydrocarbons (cyclohexane and hexadecane) because of the small polarization interaction.[12] This is unlike the case of water in CCl_4 and benzene in which, because of the larger polarization interaction, discrete 1:1 complexes are presumed to exist (hence the much larger bulk solubilities of water in these solvents vis-a-vis saturated hydrocarbons).[13]

In summary thus far, we assume, based on the above discussion, that the saturated amine–water/*n*-hexane systems studied here are characterized as follows: (1) the composition of 1:1 amine hydrates is negligible, (2) nearly all of the water present in the hydrocarbon phase is monomeric, and (3) the water monomer is unassociated with the solvent.

Fluorescence Spectra. Figure 1 shows the fluorescence spectra of TEA, TPA, and DABCO in water-saturated *n*-hexane as well as in dry solvent at 303 K. It should be noted that the "wet" spectra, as presented, indicate the extent of fluorescence quenching relative to the emission in the dry solvent. These observations are consistent with the earlier report of Muto et al.[14] Pairs of wet and dry spectra were normalized at the high energy end (e.g., between 267 and 270 nm for TEA) and then subtracted in order to illustrate the residual emission due to the presence of water. Thus, these subtracted spectra provide an indication of the emission

---

(9) The quenching efficiency is $k_Q[A]/k_A$; $k_Q = 6 \times 10^9$ and 2×10^7 M^{-1} s^{-1} for TEA and DABCO, respectively. k_A values are given in the text.

(10) Gregory, M. D.; Christian, S. D.; Affsprung, H. E. *J. Phys. Chem.* **1967**, *71*, 2283.

(11) Magnusson, L. B. *J. Phys. Chem.* **1970**, *74*, 4221. (b) Yamazaki, S.; Ikawa, S.; Kimura, M. *J. Quant. Spectrosc. Radiat. Transfer* **1980**, *24*, 421.

(12) Kirchnerovà, J.; Cage, G. C. B. *Can. J. Chem.* **1976**, *54*, 3909.

(13) Bonner, O. D.; Choi, Y. S. *J. Phys. Chem.* **1974**, *78*, 1723.

(14) Muto, Y.; Nagato, Y.; Tsubomura, H. *Chem. Phys. Lett.* **1971**, *9*, 597.

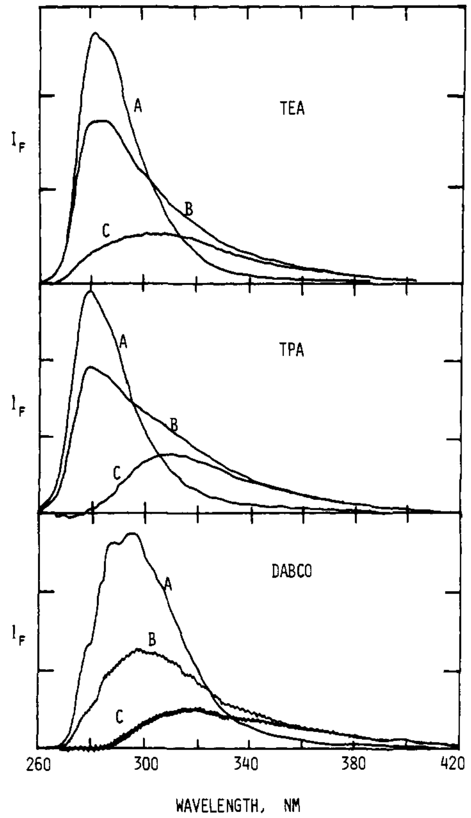


Figure 1. Fluorescence spectra of TEA, TPA, and DABCO in dry (A) and H_2O-saturated (B) *n*-hexane solution at 303 K. The latter spectra indicate the extent of quenching. Difference spectra (C) are also presented.

component associated with the excited hydrated amine (this assumes that emission of the hydrated amine is negligible at the normalization wavelength(s)). The difference spectra of TEA, TPA, and DABCO reveal broad structureless emission having λ_{max} values of 309, 312, and 322 nm, respectively. Details concerning the quantitative quenching of the amine fluorescence in the presence of water, as well as the emission yields and efficiencies of the red-shifted amine hydrates, will be presented below.

Fluorescence Decay Curves. As mentioned above, fluorescence decay curves of the amines in the water-saturated solvent were observed to be nonexponential. For example, at 303 K the fluorescence decay of TEA (1.0×10^{-4} M) in water-saturated *n*-hexane, excited at 230 nm, is

$$I_f(t) = \exp(-t/3.32) + 1.71 \exp(-t/26.9) \quad (\chi^2 = 1.7)$$

when analyzed at 290 nm (near the emission maximum) and

$$I_f(t) = \exp(-t/26.9) - \exp(-t/3.32) \quad (\chi^2 = 1.8)$$

when analyzed at 340 nm (at the low-energy tail, see figure 1); *t* is in ns. These decay curves are illustrated in Figure 2. In all of the amine–water systems reported here, we observed decay curves at 280–290 and 340–360 nm for which consistent values of both the short- and long-lived components were obtained from the simultaneous reconvolution analysis of the data. Moreover, and significantly, the decay curves measured at the long-wavelength position gave acceptably good statistical fits in which the amplitude ratio of the two components was −1 (i.e., $\chi^2 < 2$); see the Experimental Section for details.

These observations indicate that the species responsible for the long-wavelength emission in these systems is formed between the amine and water *after* photoexcitation of the amine. Thus a kinetic analysis of the decay data can be performed by using the photoassociation mechanism, i.e., exciplex formation and emission. We will refrain, however, from referring to the species emitting at long wavelength as an exciplex per se because a bound state exists between water and ground-state amine (the hydrate) even though its equilibrium composition in these systems is negligible; in addition, its structure may not be the same as that of the excited state complex. An exciplex is generally intended to denote an electronically excited complex which is *dissociative* in the ground state. As will be discussed below, however, the ground- and excited-state amine hydrates may have different structures, and thus the term exciplex may formally apply.

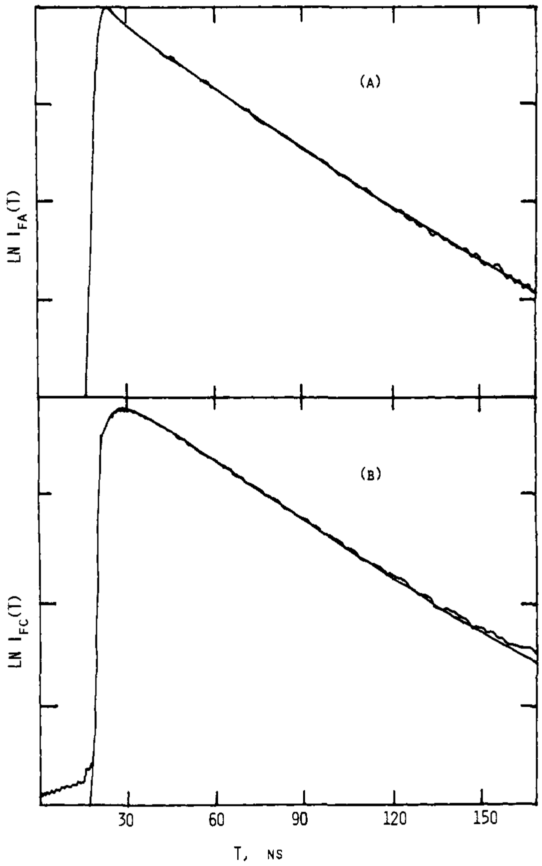


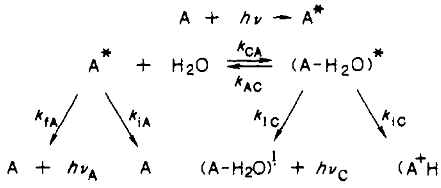
Figure 2. Fluorescence decay curves and reconvolutions of TEA (3×10^{-4} M) in H_2O-saturated *n*-hexane at 303 K excited at 230 nm: (a) decay curve monitored at 290 nm; (b) decay curve monitored at 340 nm. χ^2 values are 1.7 and 1.8, respectively. Each vertical block represents 3 decades.

Anticipating the discussion of the nature of the excited amine–water complex, we note now that the fluorescence spectra and decay curves of *n*-hexane solutions of amines in the presence of oxygen-containing, nonprotic species such as diethyl ether and tetrahydrofuran (at 300 K and 3×10^{-3} M) do not show the red shift and nonexponential decay described above.[7] At much higher ether concentrations (ca. 0.01 M), however, the spectra continuously broaden and shift to lower energies. These observations can be attributed to the changing bulk property (increasing dielectric constant) of the ether–hydrocarbon solvent. The addition of ethanol, however, does produce similar changes in the fluorescence spectra and decay curves as in water.[8,15] It thus appears that the excited complex between the amine and water (and ethanol) involves bonding in some way between the *hydrogen atom* and the excited state of the amine. With ethanol, however, complications arise due to self-association at higher concentrations. A study of this system has recently been reported by Köhler, who attributed the excited complex to association between the cationic-like core of the N atom and the O atom of the alcohol.[15b] Although it might be thought that the failure to observe the spectral broadening and decay curves described above for the water system in the ether-containing solutions could be the result of steric repulsion between the amine and the O substituents, it would be surprising if this repulsion were so strong in tetrahydrofuran.

---

[15] (a) Köhler, G. *Chem. Phys. Lett.* **1986**, *126*, 260. (b) Köhler, G. *J. Photochem.* **1986**, *35*, 189.

Scheme I



Presumably this molecule could coordinate with the (planar, or nearly planar) excited amine.

Kinetic and Thermodynamic Analysis. In part, the objective of this study is to determine the binding energy of the excited amine–water complex. We considered two approaches to obtain this information: steady-state and photokinetic measurements. In the latter method, which proved to be somewhat more definitive, fluorescence decay curves were obtained between 273 and ca. 323 K. The specific rate constants for photoassociation and dissociation were determined in this way. On the basis of the discussion presented above, we adopt the monomer–exciplex kinetic scheme for the free and water-complexed amine species, respectively (Scheme I), where A denotes the amine and $(A\text{-}H_2O)^{!}$ represents the ground-state hydrated amine formed directly after photon emission. This species does not necessarily have the same structure as the "normal" hydrogen-bonded amine. This point will be discussed in further detail below.

A steady-state analysis yields expression 1 for the ratio of fluorescence intensities of water-complexed amine to free amine,

$$I_{fC}/I_{fA} = (k_{fC}/k_{fA})\{k_{CA}[W]/(k_C + k_{AC})\} \qquad (1)$$

where [W] denotes the molar concentration of water. We will assume that the formation and dissociation rate constants, as well as the water concentration in *n*-hexane, can be expressed in Arrhenius form, i.e.

$$k_{CA} = A_{CA}\exp(-E_{CA}/RT) \qquad k_{AC} = A_{AC}\exp(-E_{AC}/RT)$$
$$[W] = A_W\exp(-E_W/RT)$$

It is necessary to take into account the temperature dependence of [W] because in the two-phase, alkane-aqueous system the water composition is a dependent variable. In these experiments, one degree of freedom is lost (the isothermal water composition). Because the information about the formation kinetics of the excited amine hydrate is obtained through the pseudo-first-order rate constant, $k_{CA}[W]$, $[W](T)$ must be known in order to obtain $k_{CA}(T)$ and hence E_{CA}.

An Arrhenius plot of (1) is expected to yield different activation parameters in the limit of low and high temperatures. For example, assuming that the radiative rate constants and k_C are temperature independent, the low-temperature-*limiting* slope is equal to $-E_{CA} - E_W$, while at high temperatures (where $k_{AC} \gg k_C$), the slope approaches $-E_{CA} - E_W + E_{CA}$. While in principle the excited complex binding energy, B^*, can be obtained from such an approach[16] ($B^* = E_{AC} - E_{CA}$), we found that Arrhenius plots of (1) were not reliable and accurate enough to furnish this information unambiguously. One complication is that one is not a priori justified in assuming that one measures the limiting slopes within the experimentally accessible temperature range. This plot can, nevertheless, be used as a confirmation of the data obtained from photokinetic experiments (see below). An Arrhenius plot for the TEA–H_2O system is shown in Figure 3.

Quantum Efficiencies. The fluorescence quantum yields of the quenched free amine, Q_A, and excited amine–water complexes, Q_C, were determined by comparing the fluorescence spectra of an amine in dry vs. H_2O-saturated *n*-hexane solution. The wet spectrum was decomposed into monomer and residual (excited complex) portions as described above. The monomer and excited complex quantum yields were obtained by comparing the areas of the two components with that of the dry monomer spectrum. These values were made quantitative by using free amine quantum efficiencies, q_A, of 0.69, 0.73, and 0.5 for TEA, TPA, and DABCO,[17] respectively. For these three amines at 303 K, quantum *yields*, Q_A, are 0.21, 0.18, and 0.23; valves for Q_C are 0.11, 0.17, and 0.15, respectively. The extraction of the excited complex fluorescence efficiencies from these data and their separation into radiative and nonradiative components will be presented below.

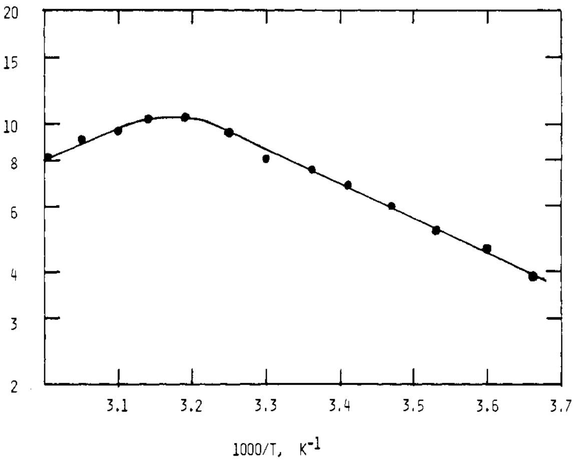


Figure 3. Plot of ln (I_{fC}/I_{fA}) vs. $1/T$ for TEA (3×10^{-4} M) in H_2O-saturated *n*-hexane. Monitoring wavelengths are 360 and 290 nm; excitation wavelength is 230 nm.

Water Composition. It is evident that reliable quantitative information regarding the solubility of water, *and* its temperature dependence, is needed in order to decouple the temperature dependence of $k_{CA}[W]$ and hence obtain B^*. Unfortunately, definitive data for the *n*-hexane–water system appear to be lacking.[18] The reported solubility of water in *n*-hexane at 298 K ranges from 3.27×10^{-3}[18f] to 5.48×10^{-3} M.[18d] For the purpose of further discussion here, we take the solubility of water in *n*-hexane at 298 K to be 4.5×10^{-3} M. This is based on water solubilities in cyclohexane (between 283 and 313 K) reported by Goldman;[19] the adjustment to *n*-hexane was obtained from the difference in surface tension of these liquids (at 298 K) according to the approach described by Uhlig.[20] Furthermore, the water solubility in *n*-hexane is assigned an activation energy of 7.8 kcal/mol; this is also based on Goldman's data to which an adjustment (the ratio of heats of vaporization) is applied.[21]

Photokinetics. As mentioned above, the nature of the decay curves acquired for the free and complexed amines justifies the application of simple exciplex photokinetics (see Scheme I). With the excited amine hydrate denoted as C*, the time dependences of the excited free amine, A*, and C* are as follows:

$$A^*(t) = \exp(-\lambda_1 t) + F\exp(-\lambda_2 t) \qquad (2)$$

$$C^*(t) = \exp(-\lambda_1 t) - \exp(-\lambda_2 t) \qquad (3)$$

where F is the relative amplitude of the short-lived component in the free amine decay curve. The time coefficients, λ_1 and λ_2, are expressed in terms of the rate constants and [W] through the quantities X and Y:

$$2\lambda_{1,2} = X + Y \mp \{(Y - X)^2 + 4k_{AC}k_{CA}[W]\}^{1/2}$$

---

(16) Stevens, B.; Ban, M. I. *Trans. Faraday Soc.* **1964**, *60*, 1515.

(17) Halpern, A. M.; Danziger, R. M. *Chem. Phys. Lett.* **1972**, *16*, 72.

(18) (a) Roddy, J. W.; Coleman, C. F. *Talanta* **1968**, *15*, 1281. (b) Schatzberg, P. *J. Phys. Chem.* **1963**, *67*, 776. (c) Gester, G. C., Jr. *Chem. Eng. Prog.* **1947**, *43*, 117. (d) Hoot, W. F.; Azarnoosh, A.; McKetta, J. J. *Pet. Refin.* **1957**, *36*, 255. (e) Black, C.; Jorris, G. G.; Taylor, H. A. *J. Chem. Phys.* **1948**, *16*, 537. (f) Polak, J.; Ju, B. C.-Y. *Can. J. Chem.* **1973**, *51*, 4018. (g) Englin, B. A.; Plate, A. F.; Tugolukov, V. M.; Pryanishnikova, M. A. *Khim. Tekhnol. Topl. Masel* **1965**, *10*, 42. Data were interpolated from: *Chem. Abstr.* **1965**, *63*, 14608f. The 40 °C datum in this citation is in error.

(19) (a) Goldman, S. *Can. J. Chem.* **1974**, *52*, 1668. (b) Goldman, S.; Backx, P. *J. Phys. Chem.* **1981**, *81*, 2975.

(20) Uhlig, H. H. *J. Phys. Chem.* **1937**, *41*, 1215.

(21) See ref 18b and: Hildebrand, J. H.; Scott, R. L. *Solubilities of Non-Electrolytes*, Reinhold: New York, 1950.

(22) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley-Interscience: London, 1970; pp 304–307.

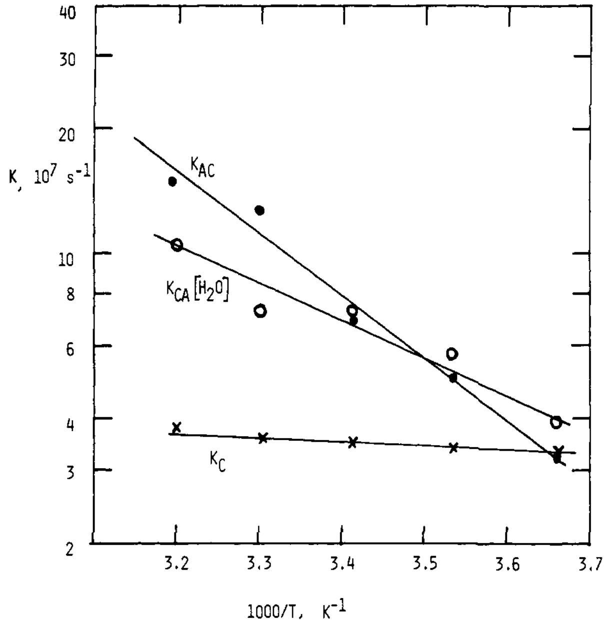


Figure 4. Arrhenius plots of the pseudo-first-order formation rate, k_{CA}[H_2O], the dissociation rate constant, k_{AC}, and the excited complex decay rate constant, k_C, for TEA (1×10^{-4} M) in H_2O-saturated *n*-hexane solution.

where $X = (F\lambda_2 + \lambda_1)/(F + 1)$ and $Y = (F\lambda_1 + \lambda_2)/(F + 1)$.

The rate constants, finally, are obtained from λ_1, λ_2, X, and Y as given in (4)-(6). The decay constant, k_A, of the free amine

$$k_{CA}[W] = X - k_A \quad (4)$$

$$k_{AC} = (X - \lambda_1)(\lambda_2 - X)/k_{CA}[W] \quad (5)$$

$$k_C = Y - k_{AC} \quad (6)$$

is obtained from the fluorescence decay curve of dry, dilute solution of the amine in *n*-hexane. This is not the intrinsic amine decay rate but reflects the very slight degree of amine self-quenching at these concentrations (ca. 1×10^{-4} M). Between 273 and 333 K, k_A for TEA decreases very slightly, from 3.81×10^7 to 3.91×10^7 s^{-1}, this trend probably being the result of the decrease in the refractive index of *n*-hexane. From (4)-(6), the three rate constants (considering $k_{CA}[W]$ as a pseudo rate constant) are obtained at different temperatures as long as the free and hydrated amine decay curves are found to follow the respective decay laws (2) and (3) (see the Experimental Section).

Arrhenius plots of $k_{CA}[W]$, k_{AC}, and k_C for TEA-H_2O are shown in Figure 4. These data represent the results of several independent experiments. From these plots, $E_{CA} + E_W$, the latter representing activation energy for water solubility, is found to be ca. 6.5 kcal/mol, which is somewhat less than the (presumed) water solubility temperature coefficient presented above (7.8 kcal/mol). The reason for this discrepancy is unclear although several observations may be made. (1) The photokinetic activation energy for water solubility, which is smaller than the thermodynamic value, might imply the presence of a preequilibrium involving a ground-state amine–water complex. This is inconsistent, however, with the very low population of amine–water hydrate (ca. 3%) expected over the temperature range covered for TEA, based on the reported equilibrium constant,[10] enthalpy of formation,[23] and bulk water composition (see the discussion above). (2) The relatively low value of $E_{CA} + E_W$ implies a very small (or even zero) activation energy to water diffusion in *n*-hexane. This may be reasonable for a small molecule such as water which possesses little affinity for the *n*-hexane solvent medium[12] (e.g., consider the very low solubility—see the discussion above).

The values of k_{AC} displayed in Figure 4 show less scatter than the $k_{CA}[W]$ data despite the fact that the former rate constant is derived from the latter (see (4)-(6)). The activation energy for excited complex dissociation, E_{AC}, is found to be ca. 7.8 kcal/mol. Assuming a value of E_{CA} or (nominally) zero, this establishes an estimate (upper limit) of the binding energy of the excited TEA-H_2O complex of 7.8 kcal/mol. This can be compared with the value reported for the ground-state hydrate of 5.7-5.9 kcal/mol (in CCl_4).[23] Hence we see that the excited complex is somewhat more strongly bound relative to the ground-state species.[24] The excited complex decay parameter, k_C, is nearly temperature independent ($E_C < 1$ kcal/mol), as can be observed in Figure 3. The value for the H_2O complex is 3.4×10^7 s^{-1}. k_C for the D_2O complex, also temperature independent, is slightly smaller, 3.0×10^7 s^{-1}.

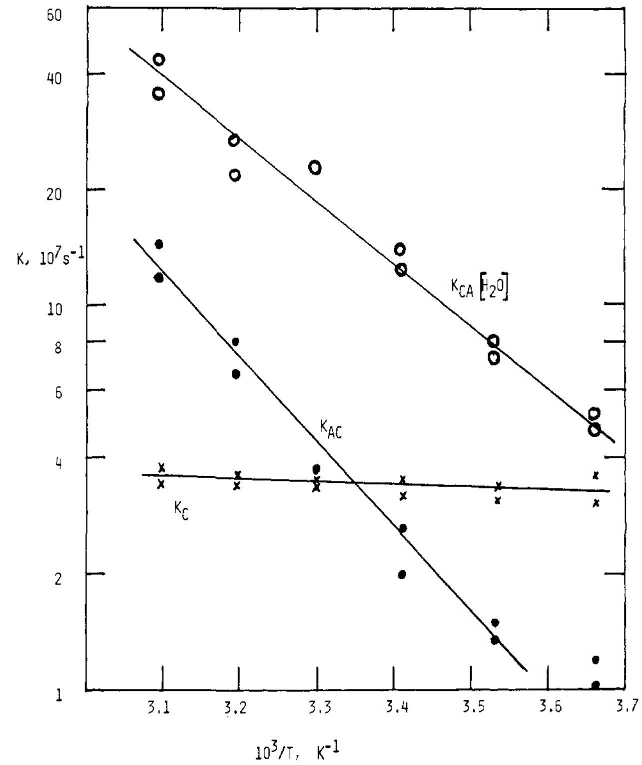


Figure 5. Arrhenius plots of $k_{CA}[H_2O]$, k_{AC}, and k_C for TPA (1×10^{-4} M) in H_2O-saturated *n*-hexane solution.

Similar photokinetic studies of TEA in D_2O-saturated *n*-hexane showed nearly identical behavior. Analysis of these results in terms of the monomer–exciplex scheme produced results which are indistinguishable (except for k_C) from the TEA-H_2O system. Moreover, the fluorescence spectrum of TEA in D_2O-saturated *n*-hexane revealed the same magnitude of red shift seen in the H_2O system. Thus the thermodynamics of complexation between excited TEA and D_2O vis-a-vis ground-state TEA appear to be very similar to H_2O. Although the ground-state TEA-D_2O hydrogen bonding enthalpy is not known, the difference in activation energies of solubility of D_2O and H_2O in *n*-hexane is presumably very small (in cyclohexane this difference is ca. 0.77 kcal/mol).[19]

The photokinetics of the TPA-H_2O system were also investigated, and, as for TEA-H_2O, decay curves adhering to the monomer/excimer-type scheme were observed at 280 and 360 nm, respectively. For TPA (1.0×10^{-4} M), $k_A = 3.1_3 \times 10^7$ s^{-1} and is nearly independent of temperature between 273 and 323 K. The rate constants $k_{CA}[W]$, k_{AC}, and k_C were determined as a function of temperature by the same method described above; the results are shown in Figure 5. For this system, $E_{CA} + E_W$ was found to be somewhat higher than that TEA-H_2O, ca. 7.4 kcal/mol; this is closer to the value of E_W (see above). The Arrhenius plot of k_{AC} appears to be nonlinear despite the scatter in the data. The

---

(23) Karzakin, A. V.; Murakova, G. A. *Zh. Fiz. Khim.* **1971**, *45*, 1054. See also ref 11.

(24) Köhler reports a binding energy of 2.9 kcal/mol for the TEA–ethanol excited complex (ref 16). This value, however, refers instead to the Gibbs free energy of formation of this complex. The binding energy is presumably larger (ca. 8 kcal).

Table I. Rate Parameters k_A, k_C, k_{fC}, k_{iC}, and k_{AC}, the Activation Energy to Excited Complex Dissociation, and Quantum Efficiencies q_A and q_C (See Scheme I) for TEA–, TPA–, and DABCO–H_2O (–D_2O) Systems[d]

amine	k_A	q_A[a]	k_C	q_C	k_{fC}	k_{iC}	k_{AC}[b]	E_{AC}, kcal/mol
TEA	3.83	0.67	3.4 (3.0)	0.17	0.57	2.8	9.7 (12.0)	7.8
TPA	3.13	0.71	3.4 (2.9)	0.23	0.78	2.6	2.9 (4.3)	10.0
DABCO	1.66	0.5[c]	3.3 (2.9)	0.28	0.92	2.4	10.0 (16.0)	9.0

[a] Corrected for self-quenching under the conditions studied. [b] Interpolated from k_{AC} vs. $1/T$ plots. [c] Reference 17. [d] All rate constants are at 298 K and are in units of 10^7 s^{-1}.

limited temperature range over which these studies can be carried out (273–323 K) precludes a more definitive analysis of the $k_{AC}(T)$ data. However, by excluding the 273 K values, an activation energy, E_{AC}, of ca. 10 kcal/mol is obtained. This result indicates a somewhat larger binding energy for the excited TPA–H_2O complex compared with TEA–H_2O (7.8 kcal/mol). The value of k_C is 3.4×10^7 s^{-1} (coincidentally identical with that of the TEA–H_2O complex) and is practically independent of temperature (between 273 and 323 K). For the TPA–D_2O complex, k_C is (again as in the case of TEA) smaller, 2.9×10^7 s^{-1}.

The higher binding energy of the TPA hydrate possibly reflects the slightly larger (gas-phase) proton affinity of TPA relative to TEA.[25] Stationary-state measurements of relative complex/monomer emission intensities were performed at 280 and 360 nm, respectively between 342 and 273 K. The low- and high-temperature-limiting slopes obtained are −6.3 and 3.0 kcal/mol, respectively. There is appreciable scatter (and some hysteresis) in the high-temperature data, possibly because of problems in achieving equilibrium. The difference in these slopes (3.0 − (−6.3)) compares reasonably well with E_{AC} obtained from the photokinetic data (10 kcal/mol).

The photokinetics of DABCO (5.6×10^{-5} M) in H_2O-saturated *n*-hexane solution, excited at 240 nm and analyzed at 300 (monomer) and 400 nm (excited complex), were examined between 273 and 323 K. The DABCO concentration was kept as low as possible to minimize complications from ground-state hydrate(s). The decay curves, well represented by the monomer–exciplex scheme presented above, were analyzed in the same manner as in the TEA and TPA systems. k_A (1.66×10^7 s^{-1}) was observed to be independent of temperature, while k_C, 3.3×10^7 s^{-1} at 293 K, was found to have an activation energy, E_C, of ca. 2 kcal/mol. As in the other amines, k_C for the D_2O complex is smaller, being 2.9×10^7 s^{-1} at 298 K. Thus we see that the k_C values for the three amines studies are very close to one another, both for the H_2O and D_2O complexes. The fact that k_C values are all about 10% smaller in the amine–D_2O excited complexes vis-a-vis H_2O suggests that a deuterium isotope effect acts to enhance radiationless decay from these complexes; this follows from the fact that $k_{iC} > k_{fC}$ (see below).

Arrhenius plots of $k_{CA}[H_2O]$ and k_{AC} were curved at low temperatures; the high-temperature-limiting slopes provide $E_{CA} + E_W$ and E_{AC} values of 8.0 and 9.0 kcal/mol, respectively. Photostationary measurements, analyzed at 300 and 400 nm and between 273 and 340 K, gave results consistent with $E_{AC} + E_C = 10$ kcal/mol. Rate parameters and the activation energy to complex dissociation for the amine–H_2O (D_2O) are summarized in Table I. It is interesting that the excited complex dissociation rate constants for the amine–D_2O complexes are all *larger* (by ca. 1.4) than those for the amine–H_2O species. That the activation energies are indistinguishable implies that the amine–D_2O complexes have larger activation entropies of dissociation.

The excited complex fluorescence quantum yields for the three amines studied were converted into the respective quantum efficiencies by using relationship 7 and the values of Q_A, q_A, and

$$Q_C/q_C = 1 - Q_A/q_A \qquad (7)$$

Q_C reported above. Radiative and nonradiative components of k_C were determined through $k_{fC} = q_C k_C$ and $k_{iC} = (1 - q_C)k_C$. These results are shown in Table I. As a test of the consistency between the stationary-state and photokinetic data, the free amine quantum yield/quantum efficiency ratio was calculated from eq 8, where $f = (k_C/k_A Y)(X - k_A)$.[22] Values of f determined from

$$Q_A/q_A = 1/(1 + f) \qquad (8)$$

the measured rate parameters for TEA, TPA, and DABCO at 303 K and used in eq 8 provided good agreement with Q_A/q_A quotients obtained from photostationary measurements.

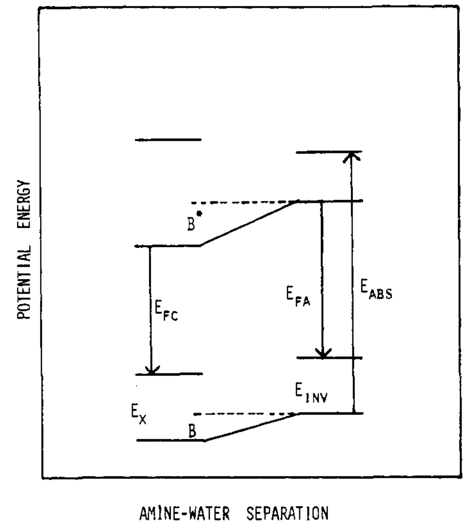


Figure 6. Schematic energy level diagram for the amine–water system. The right-hand states refer to the free amine and depict the absorption (E_{abs}), fluorescence (E_{fA}), and inversion (E_{inv}) energies. The left-hand states pertain to the amine–water complexes. B and B^* are the binding energies of the ground and excited complexes. E_{fC} is the energy of excited complex fluorescence, and E_X is the post-fluorescence repulsion energy of the amine–water complex.

Excited Complex Structure. We now discuss the energetics of fluorescence in the free and water-complexed amines. The emission energies of the amine–water complexes, E_{fC}, can be estimated from the high-energy-normalized difference spectra shown in Figure 1. These values can be compared with the respective emission energies of the free amines, E_{fA}, obtained in dry *n*-hexane solution and analyzed in terms of binding and repulsion energies of the excited and ground-state complexes. There are two complications which must be considered here: the energetics (and structure) of the ground-state complex (hydrate) and the energy difference between the pyramidal and planar structures of the free amine. The latter consideration is necessary in this analysis because while the energy of the amine hydrate is referenced to the free, pyramidal amine, fluorescence presumably terminates in the planar structure. These different states and structures are portrayed schematically in Figure 6 in which the horizontal axis indicates the amine–water separation. On the right, the states representing the free amine are the pyramidal ground state and the pyramidal Franck–Condon (lowest) excited singlet state, as well as the relaxed, planar excited state and Franck–Condon (planar) ground state produced via photon emission. The fluorescence energy of the free amine (maximum) is denoted as E_{fA}. The elevation of the planar ground-state configuration vis-a-vis the pyramidal ground state (the inversion energy) is represented as E_{inv}.

The left-hand side of Figure 6 represents the H_2O- (or D_2O-) complexed amine in the ground and excited states. The fluorescence energy of the excited amine–water complex is E_{fC}. The binding energies of the ground- and excited-state hydrates

---

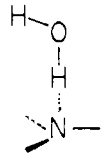
(25) Aue, D.; Webb, H. M.; Bowers, M. T. *J Am. Chem. Soc.* **1976**, *98*, 311.

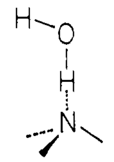
are symbolized as B and B^*, respectively. The energy difference between the amine–water complex which is produced subsequent to photon emission (and which presumably represents the equilibrium structure of the *excited* amine–water complex) and the "normal" ground-state amine–water complex is symbolized by E_X. A Förster cycle analysis of these state energies indicates that

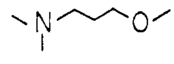
$$E_{fA} + E_{inv} = B^* + E_{fC} + E_X - B$$

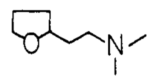
Since each of these terms can be measured (or estimated) except for E_X, a value of this quantity can be determined. It should be realized that E_X, ordinarily represented as a repulsion energy in exciplex and excimer systems (because the ground states of these species are dissociative), need not necessarily be very large. That is, the energy of the amine–water complex produced after photon emission may be nearly equal to that of the ground-state amine–water hydrate.

On the basis of the fluorescence energies of free and water-complexed TEA, the binding energies of the ground and electronically excited state TEA–water complexes, and the use of a value of 8 kcal/mol as the inversion barrier for TEA,[26] E_X is estimated to be ca. 13 kcal/mol. Data for the other amines suggest similar values for E_X. This value seems rather large if one assumes that the structure of the excited-state TEA–H_2O complex involves the water molecule with the O–H bond directed along the C_3 axis of the (planar) amine, I. The 13 kcal assigned to E_X represents the energy of this structure (in which, however, excitation energy is lost) *relative* to the (ground-state) TEA–H_2O hydrate II. In discussing the structure of the excited-state TEA–ethanol complex, Köhler proposed that the O atom is coordinated to the quasicationic N atom core of the Rydberg excited TEA molecule.[15] Were this to be true, it fails to explain why a donor such as tetrahydrofuran shows no evidence of complex formation with electronically excited TEA. Likewise, intramolecular complexation in amino ethers III and IV is not observed.[7] Thus only protic species (H_2O, D_2O, ROH) appear to form emissive stoichiometric complexes with electronically excited saturated tertiary amines.

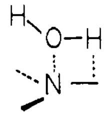

I


II


III


IV

In order to rationalize the relatively large repulsion energy assigned for the TEA–H_2O system, we propose that a very different bonding arrangement exists in the excited-state complex relative to the ground-state hydrate. One such possibility is the four-center structure, V, shown below. The electrostatic reorganization of the amine in the Rydberg excited state results in a quasicationic N atom core which coordinates with the O atom in H_2O (and presumably also in ethanol). Additional stability is achieved by the electrostatic attraction of the sterically unencumbered H atom with the quasianionic (nucleophilic) periphery of the amine, such as the C atoms. Model calculations using appropriately extended basis orbitals on structures such as V are needed to assess the reasonableness of this suggestion.


V

Acknowledgment. The donors of the Petroleum Research Fund, administered by the American Chemical Society, are acknowledged for partial support of this research.

---

[26] (a) Bushweller, C. H.; Fleischman, S. H.; Grady, G. L.; McGoff, P.; Rithner, C. D.; Whalon, M. R.; Brennan, J. G.; Marcantonio, R. P.; Domingue, R. P. *J. Am. Chem. Soc.* **1982**, *104*, 6224. (b) Fleischman, S. H.; Bushweller, C. H. *J. Comput. Chem.* **1985**, *6*, 249.

Metabolic Switching in Cytochrome P-450_{cam}: Deuterium Isotope Effects on Regiospecificity and the Monooxygenase/Oxidase Ratio

William M. Atkins and Stephen G. Sligar*

Contribution from the Departments of Biochemistry and Chemistry, University of Illinois, Urbana, Illinois 61801.

Received September 29, 1986

Abstract: Cytochrome P-450_{cam}, isolated and purified to homogeneity from the soil bacterium *Pseudomonas putida*, has been shown to catalyze the hydroxylation of the substrate analogue norcamphor to form three distinct products and yields: 5-*exo*-hydroxynorcamphor (45%), 6-*exo*-hydroxynorcamphor (47%), and 3-*exo*-hydroxynorcamphor (8%). Specific deuteriation of the norcamphor skeleton at the 5-, 6-, and 3-positions drastically alters this product distribution, indicating a substantial deuterium isotope effect. When the sum total of all oxygenated products formed in the presence of norcamphor is compared to the number of reducing equivalents consumed in the reaction (NADH), a striking unaccountability of electrons is observed. These are shown to reside in excess water produced by the four-electron reduction of atmospheric dioxygen by P-450_{cam}. Metabolism of specifically deuteriated norcamphor demonstrates a deuterium isotope effect on the branching ratio of substrate hydroxylation to excess water production and suggests that this oxidase activity of P-450_{cam} results from the two-electron reduction of a single oxygen–iron intermediate, $[FeO]^{3+}$.

Cytochrome P-450_{cam}, an extensively characterized monooxygenase derived from *Pseudomonas putida*, catalyzes the regio- and stereospecific hydroxylation of the monoterpene camphor to afford 5-*exo*-hydroxycamphor as the sole product.[1] In this tightly

0002-7863/87/1509-3754$01.50/0 © 1987 American Chemical Society