Solvation of 7-Azaindole in Alcohols and Water: Evidence for Concerted, Excited-State, Double-Proton Transfer in Alcohols

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Received February 17, 1993®

Abstract: The proton inventory technique is used for the first time to investigate excited-state proton-transfer processes. The nonradiative pathways of the biological probe, 7-azaindole, in methanol, ethanol, and water are examined. Results in methanol and ethanol demonstrate the involvement of two protons in the transition state for the excited-state double-proton transfer process. These data provide the first experimental evidence suggesting a concerted tautomerization reaction of 7-azaindole in alcohols. The data for 7-azaindole in water are interpreted in terms of a nonradiative pathway that is qualitatively different from that in alcohols. We propose abstraction of the N_1 hydrogen by water as a possible nonradiative decay process.

Introduction

We have recently presented the nonnatural amino acid, 7-azatryptophan, as a noninvasive optical probe of protein structure and dynamics that is distinguishable in both absorption and emission from tryptophan.[1,2] The chromophoric moiety of 7-azatryptophan, 7-azaindole, has undergone considerable study in nonpolar solvents.[3-5] Kasha and co-workers discovered that 7-azaindole can form dimers that undergo excited-state tautomerization[3] (Figure 1). It has also been demonstrated that excited-state tautomerization occurs for 7-azaindole in alcohols.[5-10]

In alcohols, the fluorescence spectrum of 7-azaindole is bimodal. In methanol, for example, the maximum of the higher energy band is at 374 nm and that of the lower energy band is at 505 nm. The former band arises from the so-called "normal" species that decays into the latter band by double-proton transfer. In alcohols, the tautomerization or double-proton transfer reaction has been traditionally depicted (Figure 1) as being mediated by one solvent molecule, which forms a cyclic complex with the solute. There has been, however, no experimental evidence to verify the concerted nature of this reaction or the involvement of the cyclic complex; and it seems possible that more than one solvent molecule could be involved in the shuttling of the proton from N_1 to N_7 in the excited state. The model of excited-state tautomerization of 7-azaindole in alcohols being mediated by a cyclic solute–solvent complex suggests that *only two protons are involved in the transition state for this nonradiative decay process.* In water, on the other hand, we have suggested that the majority of 7-azaindole molecules *do not* execute concerted excited-state double-proton transfer.[11-15] The significantly different behavior observed in water is illustrated by the fluorescence emission with a single maximum at 386 nm and the single-exponential fluorescence decay when emission is collected with a wide bandpass, 910 ps.[11,12] We and Chou et al.[14] have attributed the different behavior in alcohols and water to fundamentally different types of solvation. In this work, we present experimental evidence that supports the model of the double-proton transfer reaction in

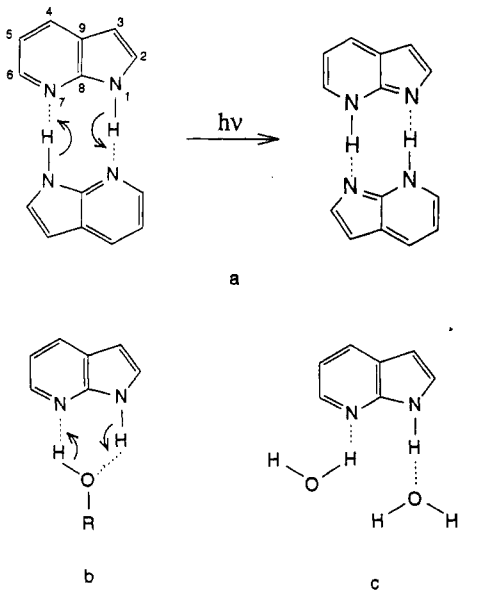

Figure 1. Idealized structures for excited-state tautomerization in (a) dimers of 7-azaindole and (b) complexes of 7-azaindole with linear alcohols. We have argued[12] that water solvates 7-azaindole in such a fashion (c) that excited-state tautomerization is frustrated. We suggest, however, that abstraction of the N_1 proton by the coordinated water molecule is an important nonradiative pathway (see Figure 6 and the text).

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® Abstract published in *Advance ACS Abstracts,* September 15, 1993.

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(15) This viewpoint is in agreement with that of Chou et al.[14] Chapman and Maroncelli (*J. Phys. Chem.* **1992**, *96*, 8430), however, propose that the entire 7-azaindole population in water undergoes excited-state tautomerization.

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alcohols arising from a two-proton, concerted process that is consistent with a cyclic solute–solvent complex.

Our experimental procedure consists of measuring the fluorescence lifetimes of 7-azaindole in solvent mixtures that vary in the ratio of the amount of the protiated to deuterated component. There are several examples of using ROH/ROD solvent mixtures to elucidate reaction mechanisms in double-proton transfer reactions.[16-27] As we discuss below, the interpretation of our results depends on whether the time scale for proton/deuteron exchange between solute and solvent is shorter than the excited-state lifetime of the solute. If it is, the measured fluorescence lifetimes are rigorously single-exponential; and the data may be interpreted in terms of the Gross–Butler equation.[16,18,19] If on the other hand proton exchange is slow, interpreting the excited-state kinetics is more complicated, even if the data are fortuitously well-described by a single, exponentially decaying component. In the Discussion, we consider the results in the limit of both fast and slow exchange with solvent. We conclude that the limit of slow exchange is probably more appropriate. Regardless, however, of whether exchange is rapid or not, the data for alcohols are qualitatively different from those for water and are suggestive of a two-proton, concerted process.

Experimental Section

Fluorescence lifetimes of 7-azaindole are obtained by time-correlated single-photon counting.[11-13] 7-Azaindole was obtained from Sigma. Solvents were purchased from Aldrich (MeOD and EtOD > 99.5% D; D_2O, 99.9% D). The reported results are the average of three to four runs for the MeOH/MeOD and the EtOH/EtOD experiments and six to eight runs for the H_2O/D_2O experiments. The error in the measured lifetime was never more than 2% of the lifetime. The quality of fit was measured by the χ^2 criterion.[11-13] 7-Azaindole was either purified as described elsewhere[12] or used without purification. The lifetime results were identical. Regardless of the state of purification of the sample, a small percentage of a long lifetime component was always present in the alcohol data (but not in the water data). An extra lifetime component was required in all the fits to take into account its presence (Figure 2). Thus, in the Discussion, when we refer to single- and double-exponential fits, we implicitly ignore the long-lived component. In previous work,[10] we had been led to believe that the long-lived component arose from an impurity in the sample. Fluorescence lifetime measurements of the isolated impurity,[11,12] the absence of the long-lived component in water,[10-12] the recognition of states of solvation that block tautomerization,[12,14] and the temperature dependence of the long-lived component (unpublished results and ref 10) contribute to the notion that it arises from a real solute–solvent interaction in alcohols.

Results

The normal band of 7-azaindole in MeOH/MeOD and EtOH/EtOD mixtures can be fit relatively well to a single-exponential fluorescence decay, although a double-exponential always provides a slightly better fit and yields physically reasonable parameters. Results from both fitting procedures are cited (Tables I–IV). The double-proton transfer rate in the alcohols was estimated to be the inverse of the fluorescence lifetime. For water, the nonradiative decay was also taken to be the inverse of the fluorescence lifetime. Subtraction of the radiative rate[12] has a negligible effect on k_n and k_0. For water, a double-exponential fit was never significantly better than a single-exponential fit and always yielded two lifetimes of the same duration. Figure 3 presents the solvent isotope effect k_n/k_0, where k_0 is the proton-transfer rate in the pure undeuterated solvent and k_n is the proton-transfer rate in the solvent mixture whose mole fraction is n in

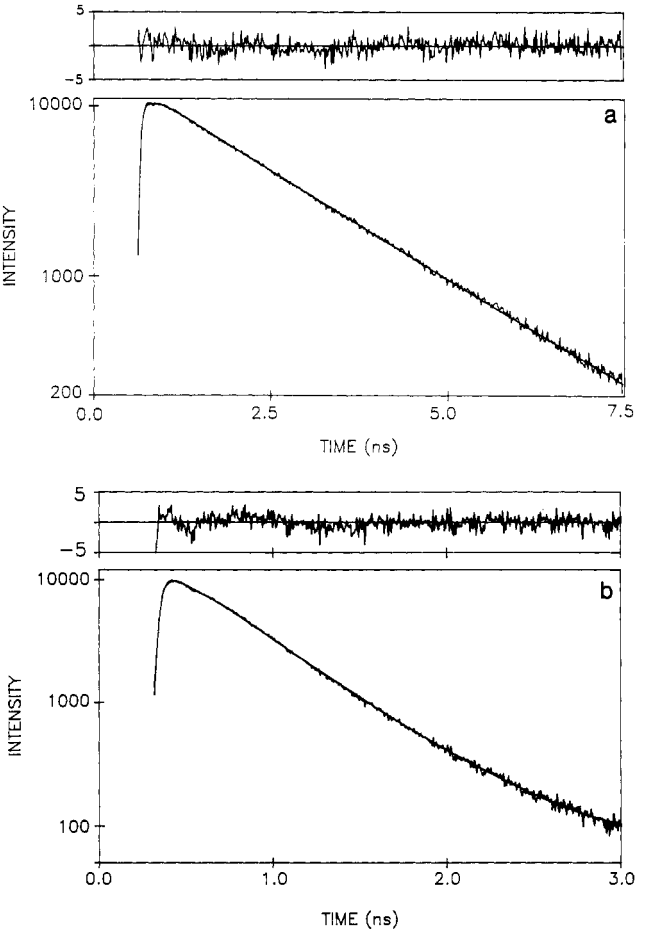

Figure 2. (a) Fluorescence decay of 7-azaindole in H_2O/D_2O (n = 0.5). A single-exponential fit yields $F(t) = \exp(-t/1665\text{ ps})$, $\chi^2 = 1.20$. A double-exponential fit yields $F(t) = 0.5\exp(-t/1665\text{ ps}) + 0.5 \times \exp(-t/1650\text{ ps})$, $\chi^2 = 1.20$. The figure displays the single-exponential fit. (b) Fluorescence decay of 7-azaindole in EtOH/EtOD (n = 0.8). The figure displays the result for a fit to a sum of three exponentials: $F(t) = 0.199\exp(-t/271\text{ ps}) + 0.798\exp(-t/455\text{ ps}) + 0.003\exp(-t/3600\text{ ps})$, $\chi^2 = 1.10$. The double-exponential fit yields $F(t) = 0.95\exp(-t/401\text{ ps}) + 0.05\exp(-t/852\text{ ps})$, $\chi^2 = 1.23$. See the Experimental Section for a discussion of the significance of the third and second lifetime components in b.

Table I. Single-Exponential Fluorescence Lifetimes of 7-Azaindole in H/D Solvent Mixtures[a]

n[b]	MeOH/MeOD	EtOH/EtOD	H_2O/D_2O
0.0	139 ± 2	184 ± 2	885 ± 5
0.1	152 ± 2	201 ± 2	988 ± 3
0.2	166 ± 3	219 ± 3	1122 ± 23
0.3	185 ± 2	240 ± 2	1262 ± 6
0.4	196 ± 4	260 ± 2	1453 ± 27
0.5	217 ± 3	292 ± 4	1675 ± 15
0.6	239 ± 2	314 ± 3	1873 ± 17
0.7	267 ± 2	346 ± 4	2178 ± 48
0.8	289 ± 3	395 ± 4	2466 ± 30
0.9	327 ± 3	462 ± 3	2829 ± 23
1.0	365 ± 4	519 ± 4	3247 ± 13

[a] Lifetimes are given in picoseconds (ps). Data are reported for 20 °C. [b] Mole fraction of deuterated solvent (atom fraction of deuterium in solvent).

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Table II. Double-Exponential Fit of 7-Azaindole Fluorescence Lifetimes in MeOH/MeOD Mixtures[a]

n^b	τ_1 (ps)	τ_2 (ps)	n^b	τ_1 (ps)	τ_2 (ps)
0.1	139 ± 2	232 ± 3	0.6	172 ± 7	270 ± 4
0.2	144 ± 2	232 ± 10	0.7	184 ± 4	288 ± 5
0.3	155 ± 2	245 ± 2	0.8	199 ± 2	309 ± 4
0.4	166 ± 3	243 ± 6	0.9	207 ± 11	330 ± 3
0.5	167 ± 15	262 ± 5			

[a] Data are obtained at 20 °C. Fluorescence lifetimes are fit to the functional form $F(t) = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t)$. The interpretation of the rate constants (the inverse fluorescence lifetimes) is given in the text. Fluorescence lifetimes for the pure solvents ($n = 0$ and $n = 1.0$) are well described by a single-exponential and are reported in Table I. [b] Mole fraction of deuterated solvent.

Table III. Double-Exponential Fit of 7-Azaindole Fluorescence Lifetimes in EtOH/EtOD Mixtures[a]

n^b	τ_1 (ps)	τ_2 (ps)	n^b	τ_1 (ps)	τ_2 (ps)
0.1	187 ± 3	315 ± 3	0.6	234 ± 4	398 ± 10
0.2	193 ± 4	339 ± 8	0.7	261 ± 3	420 ± 3
0.3	203 ± 4	350 ± 1	0.8	275 ± 8	453 ± 3
0.4	217 ± 4	356 ± 2	0.9	287 ± 1	484 ± 11
0.5	225 ± 17	385 ± 25			

[a] Data are obtained at 20 °C. Fluorescence lifetimes are fit to the functional form $F(t) = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t)$. The interpretation of the rate constants (the inverse fluorescence lifetimes) is given in the text. Fluorescence lifetimes for the pure solvents ($n = 0$ and $n = 1.0$) are well described by a single-exponential and are reported in Table I. [b] Mole fraction of deuterated solvent.

Table IV. Rate Constants for Proton-Transfer Steps[a]

rate constant ($s^{-1} \times 10^{-9}$)	MeOH/MeOD	EtOH/EtOD	H_2O/D_2O
k^{HH}	7.19 ± 0.10	5.43 ± 0.08	1.13 ± 0.02
k^{DD}	2.74 ± 0.04	1.93 ± 0.02	0.31 ± 0.01
$(k^{HH}k^{DD})^{1/2}$	4.43 ± 0.05	3.24 ± 0.03	0.59 ± 0.01
k^{HD} [b]	4.42 ± 0.06	3.25 ± 0.02	
k^{DH} [b]	4.59 ± 0.04	3.27 ± 0.02	
k^{HD} [c]	4.29 ± 0.11	3.24 ± 0.09	0.48 ± 0.02

[a] Fluorescence lifetime measurements from which the rate constants were obtained were performed at 20 °C. [b] Obtained from eqs 18 and 19 (see text). Because this method requires fitting the data to a double-exponential fluorescence decay, the corresponding rate constants could not be determined for water, where a single-exponential is sufficient to describe the decay curves. The rate constants were determined by assuming that $\phi^R = 1$ in both the ground and the excited states. If, on the other hand, $\phi^R = 1.6$, then, for example, an ethanol mixture where $n = 0.5$ yields $k^{HD} = (3.22 \pm 0.03) \times 10^9\ s^{-1}$ and $k^{DH} = (4.78 \pm 0.04) \times 10^9\ s^{-1}$. Eliason and Kreevoy[39] have discussed mechanisms by which values for ϕ^R other than 1 may result. [c] Obtained from eq 22. This method of analysis assumes that $k^{HD} = k^{DH}$. The reported results were determined by assuming that $\phi^R = 1$ in both the ground and the excited states. If $\phi^R = 1.6$, then, for $n = 0.5$, eq 22 becomes $k_{n=0.5} = (0.5)(0.385)k^{HH} + (0.5)(0.615)k^{HD} + (0.385)(0.5)k^{DH} + (0.5)(0.615)k^{DD}$. For methanol, ethanol, and water, this value of the fractionation factor yields $k^{HD} = (4.88 \pm 0.16) \times 10^9\ s^{-1}$, $(3.71 \pm 0.13) \times 10^9\ s^{-1}$, and $(5.78 \pm 0.11) \times 10^9\ s^{-1}$, respectively.

the deuterated solvent. Similar data are presented for 7-azaindole in H_2O/D_2O in Figure 4. The methanol, ethanol, and water data all exhibit downward-bulging curves, suggesting the involvement of a multiproton transition state in the nonradiative process of 7-azaindole in these solvents.[16]

Discussion

I. Application and Appropriateness of the Gross–Butler Equation. A. 7-Azaindole in Methanol and Ethanol. The isotope effect on proton-transfer reactions is rarely a linear function of solvent deuterium content. Gross and Butler explained this phenomenon by noting that either the H/D composition in the proton site can be different with respect to the solvent or more than one proton is in flight during the rate-limiting step. References 16, 18, and 19 provide discussions and derivations of

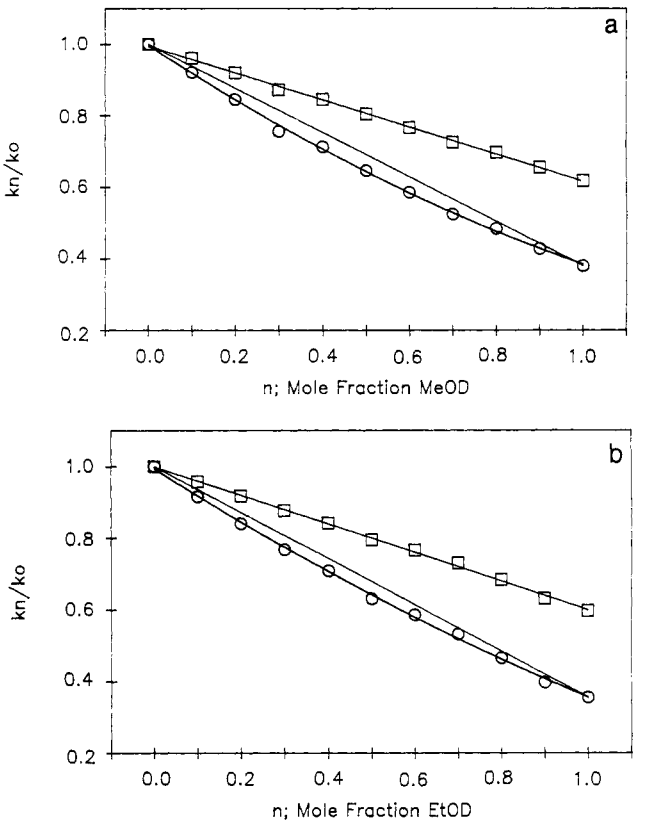

Figure 3. (a) Ratio of tautomerization rate of 7-azaindole in MeOH, k_0, to that in a mixture of protiated and deuterated methanol that is mole fraction n in MeOD, k_n. The open circles represent k_n/k_0 vs n. The solid line through the data represents the fit assuming a two-proton process with $\phi^T = 0.62$. Directly above is plotted the straight line that would result from a one-proton process, i.e., the average of k_0 and k_1 weighted by the respective mole fractions of protiated and deuterated solvents.[16] The open squares represent $(k_n/k_0)^{1/2}$ vs n. The linearity of this plot verifies the two-proton process in methanol, assuming the validity of the Gross–Butler equation. (b) Similar to part a only here the rates are measured in EtOH and EtOD. These data are also consistent with a two-proton process with $\phi^T = 0.60$.

what has come to be called the Gross–Butler equation. This equation relates the rate of the process in the protiated solvent, k_0, to the rate in a solution of mole fraction n of the deuterated solvent and to all the protons in the reactant and transition states involved:

$$k_n = k_0 \frac{\prod_i^{\nu}(1 - n + n\phi_i^T)}{\prod_i^{\nu}(1 - n + n\phi_i^R)} \simeq k_0 \prod_i^{\nu}(1 - n + n\phi_i^T) \quad (1)$$

where ν is the total number of protons involved. The $\phi^{T,R}$ are the fractionation factors in the transition and the reactant states, respectively. ϕ is the ratio of the preference in a site in a molecule for deuterium over protium relative to the preference for deuterium over protium in a solvent molecule.[16,18,19] In other words, ϕ is the equilibrium constant for the generalized reaction: XH + ROD $\rightleftarrows$ XD + ROH. It is customary in most analyses to take $\phi^R = 1$ for an NH or an OH site, as indicated above. For 7-azaindole, we suggest that $\phi^R = 1$ is a reasonable approximation for N_1L (L = H, D) since $\phi^R = 0.92$ for R_2NL and $\phi^R = 0.97$ for R_3N^+L for water.[16] (These ϕ^R are for the *ground state*. In order to apply them directly to our problem, we must assume that the ϕ^R are identical in the excited state. We have considered this possibility elsewhere[10,12] and also discuss it below.)

We must also take into account that fractionation factors are often estimated for weak acids from the empirical equation ΔpK_a

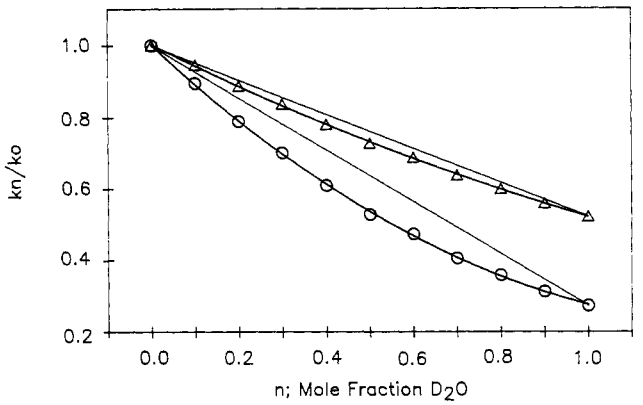

Figure 4. Proton inventory data for 7-azaindole in H_2O and D_2O at 20 °C. The open circles represent k_n/k_0 vs n. The pH at n=0 is 6.8. The solid line through these data represents the fit assuming a three-proton process: $k_n/k_0 = (1 - n + 0.48n)(1 - n + 0.69n)^2$. The straight line plotted directly above is the result expected for a one-proton process. The open triangles represent $(k_n/k_0)^{1/2}$ vs n. The solid line through the open triangles is only meant to guide the eye. This plot deviates significantly from the straight line just above it. Hence, the proton inventory data in water are different from those in the alcohols. Assuming the validity of the Gross–Butler equation, the water data are inconsistent with a two-proton process.

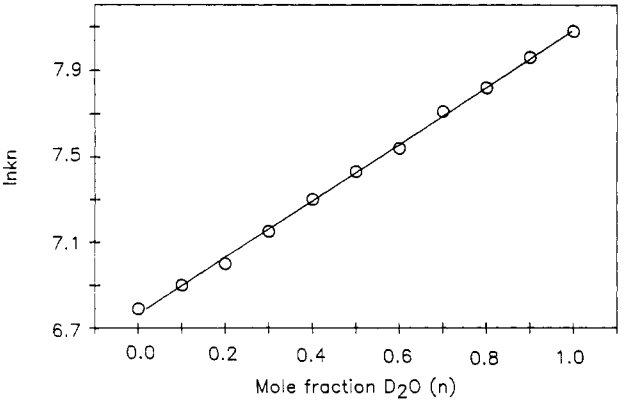

Figure 5. Plot of ln k_n vs n for the proton inventory data of 7-azaindole in water (Figure 3). The linear plot is consistent with an "infinite-proton" process.[16] Because the proton inventory data can be fit to a form that is identical with that observed in ribonuclease and because the details of these two systems are so similar (compare Figure 5 with those in refs 16 and 17), we have chosen to interpret the data in terms of the more intuitive three-proton process discussed in the text.

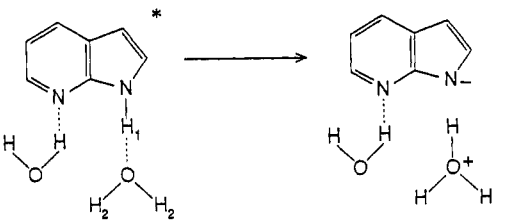

Figure 6. Nonradiative decay of the blocked species of 7-azaindole in water by means of deprotonation of N_1 by a water molecule. The blocked species of 7-azaindole is a complex that is solvated by water in such a fashion that concerted, two-proton transfer as depicted in Figure 1 is frustrated.[11-15]

$= 0.41 + 0.020\ pK_H$, where $\Delta pK_a = pK_D - pK_H$, and from the relationship $\phi^R = l^3(K_D/K_H)$, where l is the fractionation factor for the OH hydrogens of H_3O^+ and is equal to 0.69.[16,39] If the excited-state species is long lived enough to establish a proton-transfer equilibrium, then the excited-state pK_H of N_1 is somewhere between 10 and 13[12] (see Discussion) and ϕ^R lies between 1.3 and 1.6. Given, however, that the expression for ΔpK_a is approximately accurate[16] for only 70% of the compounds tabulated by Laughton and Robertson[28] and then only for pK values in the range 3–10, we tentatively discuss our results using $\phi^R = 1$ for both the ground and the excited state. A more serious problem in the interpretation of the data using eq 1 is the rate of ligand exchange with the solvent (see below).

The downward-bulging curves for 7-azaindole in methanol and ethanol (Figure 3) are both such that a plot of $(k_n/k_0)^{1/2}$ vs n yields a straight line. This result suggests that only two protons are involved in the excited-state tautomerization of 7-azaindole in alcohols. This result is also consistent with the "cyclic complex" of 7-azaindole and alcohol (Figure 1) that has been traditionally assumed to be required for the tautomerization to proceed.

B. 7-Azaindole in Water. The downward bulging of the curve obtained for 7-azaindole in H_2O/D_2O mixtures suggests that more than one proton is involved in the transition state of the nonradiative deactivation process. Fitting k_n/k_0 vs n to a quadratic model (i.e., a two-proton process) gives imaginary ϕ^T for the data in water ($\phi^T = 0.43 \pm 0.30i$). Imaginary ϕ^T can be obtained when there are two or more competing parallel pathways and if at least one of the transition states involves *at least* two protons.[16] We have, however, argued elsewhere[11-13] that not more than 20% of the 7-azaindole population in water is capable of executing double-proton transfer and that this process can be observed only under conditions of sufficient wavelength and time resolution.[11,12] In fact, double-proton transfer of 7-azaindole in water is a minor nonradiative pathway compared to monophotonic ionization.[11,29,30] The failure of the quadratic model to fit the proton inventory data coupled with the previous evidence against the importance of excited-state tautomerization in water argues against a concerted two-proton process in this solvent. (If two protons are being transferred by 7-azaindole in water, they are not transferred concertedly between N_1 and N_7.)

The data are consistent with an infinite number of protons involved in the transition state, whose ϕ^T are all very close to 1. For such a model ln k_n vs n is linear (Figure 5). The data are, however, also in agreement with any number of multiproton mechanisms ($\nu > 2$). For the sake of simplicity and because of experimental precedent with another system, we discuss the proton inventory data of 7-azaindole in water as a three-proton process. This three-proton process involves the abstraction of hydrogen from N_1 by a coordinated water molecule.

For the three-proton process shown in Figure 6, the data in Figure 4 yield an excellent fit to the equation $k_n/k_0 = (1 - n + 0.48n)(1 - n + 0.69n)^2$. Furthermore, a plot of $(k_n/k_0)^{1/2}$ vs n does not yield a straight line, which is inconsistent with a concerted two-proton process as observed in the alcohols.

Wang et al. observed essentially identical behavior in ribonuclease.[17] In this enzyme, there is an isomerization between two of its conformations that are characterized by $pK_a > 8$ and $pK_a = 6.1$. These workers measured a solvent isotope effect of 4.7 ± 0.4. Their proton inventory measurements were best described by the relation $k_n/k_0 = (1 - n + 0.46n)(1 - n + 0.69n)^2$. They assigned the rate-limiting step in this isomerization to proton transfer to a water molecule from the protonated imidazole group of a histidine.[16,17] Within experimental error, the proton inventory rate parameters for 7-azaindole in water are identical to those for the isomerization of ribonuclease. In both cases, the shuttling of a proton from nitrogen to a water molecule is proposed to be the rate-determining step.

A fundamental assumption made in deriving the Gross–Butler equation is that the rate of H/D exchange between the solute and the solvent is significantly greater than the rate of proton transfer being investigated. In other words, *the decay of the entire reactant*

(28) Laughton, P. M.; Robertson, R. E. In *Solute-Solvent Interactions*; Coetzee, J. F., Ritchie, C. D., Eds.; Marcel Dekker: New York; 1969, p 399.
(29) Négrerie, M.; Gai, F.; Lambry, J.-C.; Martin, J.-L.; Petrich, J. W. *J. Phys. Chem.* **1993**, *97*, 5046.
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population must be characterized by a rate constant that does not change with time; that is, first-order decay kinetics must be obtained. If solvent exchange is not rapid, then the observed decay is a superposition of the decays of the individual isotopically substituted species. For the case of 7-azaindole, at least four individual rate constants may be involved (see below). In practice, it is often very difficult to distinguish experimentally between genuine first-order kinetics, which are characterized by a single-exponential decay time, and the superposition of several single-exponential decays characterized by different time constants. For this reason, we present several different methods of analyzing the 7-azaindole data (Figures 2–4, Table I–IV).

In order for application of the Gross–Butler equation to the excited-state process of 7-azaindole to be valid, we require that 7-azaindole exchange its N_1 ligand with solvent protium or deuterium much faster than the actual tautomerization reaction discussed in the Introduction and depicted in Figure 1. Since the fluorescence lifetime of 7-azaindole in the solvents used here ranges from 140 to 900 ps, an appropriate time constant for ligand exchange with the solvent would be a few picoseconds. Such a rapid exchange seems unlikely. NMR measurements of ground-state indoles indicate that N_1 exchanges its proton on a time scale of seconds with the solvent.[31] The strong likelihood of slow exchange in the excited state requires us to consider the kinetics in more detail.

II. The Criteria for a Concerted Reaction. Figure 7 presents the four cases that may arise if two protons are involved in the deactivation of excited-state 7-azaindole. In Figure 7, the reactants and products are denoted A and D, respectively. B and C denote *intermediates* that would exist if the excited-state tautomerization of 7-azaindole proceeded by either the stepwise pathway ABD or ACD involving first the breaking of the N_1–H bond and then the formation of the N_7–H bond, and vice versa. Given such a reaction scheme, in order to demonstrate that the tautomerization is a concerted process, it is necessary, but not sufficient, to show that $k^{HD} = k^{DH}$ and that $k^{HD} = (k^{HH}k^{DD})^{1/2}$. This latter criterion is referred to as "the rule of the geometric mean" and was originally stated by Bigeleisen.[32] Use of the Gross–Butler equation (eq 1) assumes the applicability of the rule of the geometric mean (see below). As we shall see, this relationship is very restrictive and demands that many requirements be satisfied. For the examples illustrated in Figure 7, one of the most important of these requirements is that, for the concerted double-proton transfer, the secondary isotope effect at the N_7 (or N_1) site is *equal* to the primary isotope effect at the N_1 (or N_7) site. We shall also see that in order for this relationship to be satisfied, the reaction must be "symmetric"; that is, the rate constants for the decay of the intermediate B (or C) to A and D must be equal.

The significance of the rule of the geometric mean is that if there is a concerted reaction, both protons must be "in flight" in the transition state. Under these circumstances and in the absence of other effects such as tunneling,[24] one thus expects the multiple sites in a single transition state to behave independently with respect to isotopic substitution.[16,18–22,32] It is useful in the course of this discussion to bear in mind Dewar's distinction between a concerted reaction and a synchronous reaction.[33] A concerted reaction takes place in a single kinetic step, with no reaction intermediate, where some of the changes in bonding take place to different extents in different parts of the reaction. A synchronous reaction is one where all the bond-making and bond-breaking processes take place at the same time and have all proceeded to the same extent in the transition state. Synchronicity is a much more restrictive condition than concertedness.[20,21,33] We shall now show what conditions are required so that the above

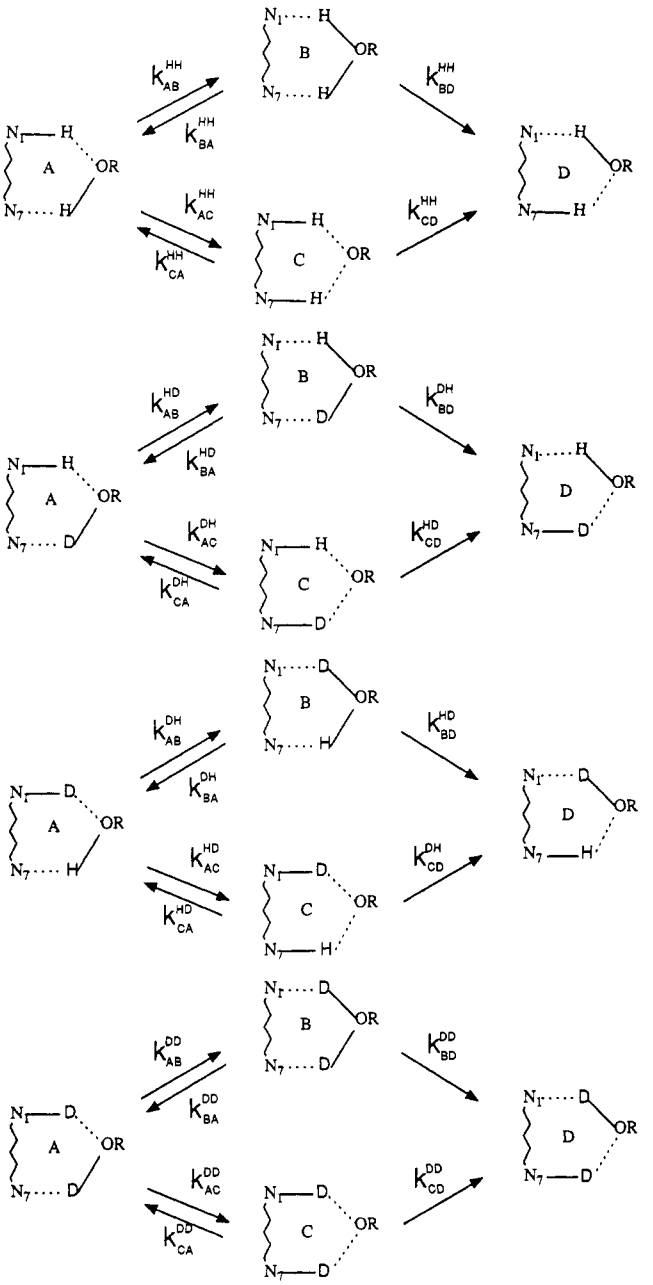

Figure 7. Excited-state tautomerization reactions for each of the four cases of isotopic substitution considered in the text: N_1HN_7···H, N_1HN_7···D, N_1DN_7···H, and N_1DN_7···D. For each case, the paths ABD and ACD represent *stepwise* processes where B and C are distinct intermediates. The criteria for a concerted reaction are discussed in the text.

relations are satisfied so that the tautomerization may be regarded as a concerted reaction. Our discussion is similar to that of Limbach and co-workers.[24,26,27]

If it is assumed that there is a fast equilibrium between the reactant and the first intermediate,[24,26,27] then the rate of tautomerization (Figure 7) is given by[34]

$$k_{AD} = \frac{k_{AB}k_{BD}}{k_{BA} + k_{BD}} + \frac{k_{AC}k_{CD}}{k_{CA} + k_{CD}} \qquad (2)$$

where $k_{AD} = k_{ABD} + k_{ACD} = k_{ABD}(1 + k_{ACD}/k_{ABD})$. We define the ratio of the overall tautomerization rates to be $k_{ACD}/k_{ABD} = \beta$, for any degree of isotopic substitution. *In order to obtain the desired kinetic relationships, it is necessary that*

(31) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986; Chapter 2. Wüthrich, K.; Wagner, G. *J. Mol. Biol.* **1979**, *130*, 1.
(32) Bigeleisen, J. *J. Chem. Phys.* **1955**, *23*, 2264.
(33) Dewar, M. J. S. *J. Am. Chem. Soc.* **1984**, *106*, 209.
(34) Fersht, A. *Enzyme Structure and Function*, 2nd ed.; W. H. Freeman: New York, 1985.

$$\frac{k_{ACD}^{HH}}{k_{ABD}^{HH}} = \frac{k_{ACD}^{HD}}{k_{ABD}^{HD}} = \frac{k_{ACD}^{DH}}{k_{ABD}^{DH}} = \frac{k_{ACD}^{DD}}{k_{ABD}^{DD}} = \beta \tag{3}$$

For a given single step represented by the generalized rate constant, k_{ij}, the first superscript H or D refers to the first ligand being transferred; the second superscript refers to the ligand that is not being transferred in the given single step. *For the overall rate constants, k_{ABD} and k_{ACD}, the first superscript refers to solute isotopic substitution; and the second, to solvent isotopic substitution.* This latter definition will be important when considering eqs 14–17.

The implications of the requirement that all the β are equal (eq 3) are *either* of the following: (1) The intermediates B and C in the upper and the lower branches of a scheme for a particular case of isotopic substitution decay with the same rates to the reactants or the products. The remaining conditions will require that these rates are equal in each of the four schemes displayed in Figure 7. We define a parameter α, which is the ratio of the rates of formation of D and A from the intermediate B (or C). For a "symmetric reaction", where the potential surface of the reactants and the products is identical in the reaction coordinate, $\alpha = 1$. In addition, all the secondary isotope effects are equal to one another, and all the primary isotope effects are equal to one another. (2) All the secondary isotope effects are equal to the primary isotope effects, which are in turn all equal to one another.

That these conditions must be fulfilled can be seen from the following. Consider, for example, the leftmost equality of eq 3. The primary isotope effect on the step i → j is defined as[26] $P_{ij}^{H} = k_{ij}^{HH}/k_{ij}^{DH}$, $P_{ij}^{D} = k_{ij}^{HD}/k_{ij}^{DD}$. For the same step, i → j, the secondary isotope effect is defined as[26] $S_{ij}^{H} = k_{ij}^{HH}/k_{ij}^{HD}$, $S_{ij}^{D} = k_{ij}^{DH}/k_{ij}^{DD}$. Thus from the above requirements, $S^{H} = S^{D} = S$ and $P^{H} = P^{D} = P$. It follows that

$$\frac{k_{ACD}^{HD}}{k_{ABD}^{HD}} = \frac{k_{AC}^{HH}k_{CD}^{HH}}{\frac{1}{P}k_{CA}^{HH} + \frac{1}{S}k_{CD}^{HH}} \frac{\frac{1}{S}k_{BA}^{HH} + \frac{1}{P}k_{BD}^{HH}}{k_{AB}^{HH}k_{BD}^{HH}} \tag{4}$$

If the intermediates C and B decay into the products and reactants with the same rate, then $k_{CA}^{HH} = k_{CD}^{HH}$, $k_{BA}^{HH} = k_{BD}^{HH}$, and $k_{ACD}^{HH}/k_{ABD}^{HH} = k_{ACD}^{HD}/k_{ABD}^{HD}$. Alternatively, even if $k_{CA}^{HH} \neq k_{CD}^{HH}$ and $k_{BA}^{HH} \neq k_{BD}^{HH}$, if $S = P$, then we also obtain $k_{ACD}^{HH}/k_{ABD}^{HH} = k_{ACD}^{HD}/k_{ABD}^{HD}$. *In order to obtain the desired expression for the rule of the geometric mean, it will be shown that it is necessary that both conditions 1 and 2 above hold.*

Given these definitions,

$$k_{AD}^{HH} = (1+\beta)\frac{k_{AB}^{HH}k_{BD}^{HH}}{k_{BA}^{HH} + k_{BD}^{HH}} \tag{5a}$$

$$k_{AD}^{HD} = (1+\beta)\frac{k_{AB}^{HD}k_{BD}^{DH}}{k_{BA}^{HD} + k_{BD}^{DH}} \tag{5b}$$

$$k_{AD}^{DH} = (1+\beta)\frac{k_{AB}^{DH}k_{BD}^{HD}}{k_{BA}^{DH} + k_{BD}^{HD}} \tag{5c}$$

$$k_{AD}^{DD} = (1+\beta)\frac{k_{AB}^{DD}k_{BD}^{DD}}{k_{BA}^{DD} + k_{BD}^{DD}} \tag{5d}$$

It follows that

$$\frac{k_{AD}^{HD}}{k_{AD}^{HH}} = \frac{k_{AB}^{HD}k_{BD}^{DH}}{k_{AB}^{HH}k_{BD}^{HH}}\left(\frac{k_{BA}^{HH} + k_{BD}^{HH}}{k_{BA}^{HD} + k_{BD}^{DH}}\right) \tag{6}$$

For the rest of this discussion i and j = A, B, and D (that is, only the upper branches in Figure 7 are considered) and i ≠ j.

We may now write eq 6 (since $\alpha = k_{BA}^{HH}/k_{BD}^{HH}$) as

$$\frac{k_{AD}^{HD}}{k_{AD}^{HH}} = (S_{AB}^{H})^{-1}(P_{BD}^{H})^{-1}\frac{1 + k_{BD}^{HH}/k_{BA}^{HH}}{k_{BA}^{HD}/k_{BA}^{HH} + k_{BD}^{DH}/k_{BA}^{HH}} = (S_{AB}^{H})^{-1}(P_{BD}^{H})^{-1}\frac{1+\alpha^{-1}}{(S_{BA}^{H})^{-1} + (\alpha P_{BD}^{H})^{-1}} \tag{7a}$$

and similarly

$$\frac{k_{AD}^{DH}}{k_{AD}^{HH}} = (P_{AB}^{H})^{-1}(S_{BD}^{H})^{-1}\frac{1+\alpha^{-1}}{(P_{BA}^{H})^{-1} + (\alpha S_{BD}^{H})^{-1}} \tag{7b}$$

$$\frac{k_{AD}^{HH}}{k_{AD}^{DD}} = (S_{AB}^{H})(P_{AB}^{D})(S_{BD}^{H})(P_{BD}^{D})\frac{(P_{BA}^{D})^{-1} + (\alpha P_{BA}^{D})^{-1}}{S_{BA}^{H} + \alpha^{-1}S_{BA}^{H}} \tag{7c}$$

$$\frac{k_{AD}^{DH}}{k_{AD}^{DD}} = (S_{AB}^{H})(P_{BD}^{D})\frac{1+\alpha^{-1}}{S_{BA}^{D} + \alpha^{-1}P_{BD}^{D}} \tag{7d}$$

These equations present two extreme possibilities. First, if all the secondary isotope effects are equal to 1, then eqs 7a and 7b become

$$\frac{k_{AD}^{HD}}{k_{AD}^{HH}} = \frac{1+\alpha^{-1}}{P_{BD}^{H} + \alpha^{-1}} \tag{8a}$$

$$\frac{k_{AD}^{DH}}{k_{AD}^{HH}} = \frac{1+\alpha^{-1}}{1 + \alpha^{-1}P_{AB}^{H}} \tag{8b}$$

Under these conditions, when $\alpha = 1$ ($k_{AD}^{HD} = k_{AD}^{DH}$) and if $P_{AB}^{H} = P_{BA}^{H}$,

$$\frac{k_{AD}^{HD}}{k_{AD}^{HH}} = \frac{k_{AD}^{DH}}{k_{AD}^{HH}} = \frac{2}{1+P^{H}} \tag{9a}$$

Similarly,

$$\frac{k_{AD}^{DH}}{k_{AD}^{DD}} = \frac{k_{AD}^{HD}}{k_{AD}^{DD}} = \frac{2}{1+(P^{D})^{-1}} \tag{9b}$$

From eq 7c, $k_{AD}^{HH}/k_{AD}^{DD} = P_{BD}^{D} \doteq P^{D}$, which substituted into eq 9b yields

$$k_{AD}^{HD} = k_{AD}^{DH} = \frac{2k_{AD}^{DD}}{1 + k_{AD}^{DD}/k_{AD}^{HH}} \tag{10}$$

This is equivalent to the result obtained by Limbach and coworkers.[24,25] As they point out, the above equation clearly contravenes the rule of the geometric mean and is suggestive of a stepwise rather than a concerted process.

Second, if $\alpha = 1$, $S^{H} = S^{D} = S$, and $P^{H} = P^{D} = P$, then from eq 7a

$$\frac{k_{AD}^{HD}}{k_{AD}^{HH}} = \frac{2P^{-1}S^{-1}}{P^{-1} + S^{-1}} \tag{11}$$

and from eq 7c, $k_{AD}^{HH}/k_{AD}^{DD} = PS$, which upon substitution into eq 11 yields

$$\frac{k_{AD}^{HD}}{k_{AD}^{DD}} = \frac{2}{S^{-1} + P^{-1}} \tag{12}$$

For the concerted tautomerization, the secondary isotope effect is equal to the primary isotope effect ($S = P$), and we obtain from

eqs 11 and 12

$$\frac{k^{HD}}{k^{HH}} = \frac{k^{DD}}{k^{HD}} \quad (13)$$

Determination of the Individual Rate Constants, k^{HH}, k^{HD}, k^{DH}, k^{DD}. Formally, the excited-state tautomerization of 7-azaindole (N_1H) with alcohols or water (ROH) can be considered to be a bimolecular reaction. In pure protiated solvent,

$$d[N_1H]/dt = -k^{HH'}[N_1H][ROH] = -k^{HH}[N_1H] \quad (14)$$

where $k^{HH} = k^{HH'}[ROH]$ and the measured lifetime is $\tau_F^{HH} = 1/k^{HH}$. In pure deuterated solvent,

$$d[N_1D]/dt = -k^{DD'}[N_1D][ROD] = -k^{DD}[N_1D] \quad (15)$$

where $k^{DD} = k^{DD'}[ROD]$ and the measured lifetime is $\tau_F^{DD} = 1/k^{DD}$.

For mixed solvents, ROH/ROD, we must consider the decay of at least four different species, $N_1HN_7\cdots H$, $N_1HN_7\cdots D$, $N_1DN_7\cdots H$, and $N_1DN_7\cdots D$, characterized by the *overall* bimolecular rate constants $k^{HH'}$, $k^{HD'}$, $k^{HD'}$, and $k^{DD'}$. *These rate constants refer to the sum of k_{ABD} and k_{ACD}, as mentioned earlier.* Similarly, the significance of the superscripts is different from that when *individual steps* are being considered (see above). If n is the mole fraction of the deuterated solvent, then [ROD] = n[ROL], [ROH] = $(1 - n)$[ROL], and [ROL] = [ROH] + [ROD], where L is H or D. The decay of both species where N_1 is bound to H is thus

$$d[N_1H]/dt = -k^{HH'}[N_1H][ROH] - k^{HD'}[N_1H][ROD] = -\{(1-n)k^{HH'}[ROL] + nk^{HD'}[ROL]\}[N_1H] = -\{(1-n)k^{HH} + nk^{HD}\}[N_1H] \quad (16)$$

where as above we have introduced the pseudo-first-order rate constants. For the two species where N_1 is bound to D,

$$d[N_1D]/dt = -\{(1-n)k^{DH} + nk^{DD}\}[N_1D] \quad (17)$$

Equations 16 and 17 do not depend on which ligand is at N_7 and therefore do not assume the presence of a cyclic complex (Figure 1), whose formation has been proposed to be necessary for the excited-state tautomerization (see below). We assume that the proton-exchange rate for N_1L is much smaller than $1/\tau_F$, the inverse of the fluorescence lifetime, and we then can apply the following treatment. If, in the ground state, the fractionation factor $\phi^R = 1$, then $[N_1H]/[N_1D] = [ROH]/[ROD]$. The rate of decay for protiated 7-azaindole (N_1H) may then be written as

$$d[N_1H]/dt = -k_1[N_1H], \quad \text{where} \quad k_1 = k^{HH} + (k^{HD} - k^{HH})n \quad (18)$$

Similarly,

$$d[N_1D]/dt = -k_2[N_1D], \quad \text{where} \quad k_2 = k^{DH} + (k^{DD} - k^{DH})n \quad (19)$$

The assumption that proton exchange is slow requires that the fluorescence decay curves, $F(t)$, be fit to a sum of two exponentials:

$$F(t) = A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t) \quad (20)$$

The ratio of the preexponential factors, A_1/A_2, is known from the solvent composition and the assumed value of ϕ^R. These parameters may thus be fixed during the fit, which yields k_1 and k_2. Since k^{HH} and k^{DD} are known from the experiments in the pure solvents, k^{HD} and k^{DH} may be obtained independently as demonstrated in Figures 8 and 9. We have assumed that for all cases of isotropic substitution the radiative rate is the same.[12]

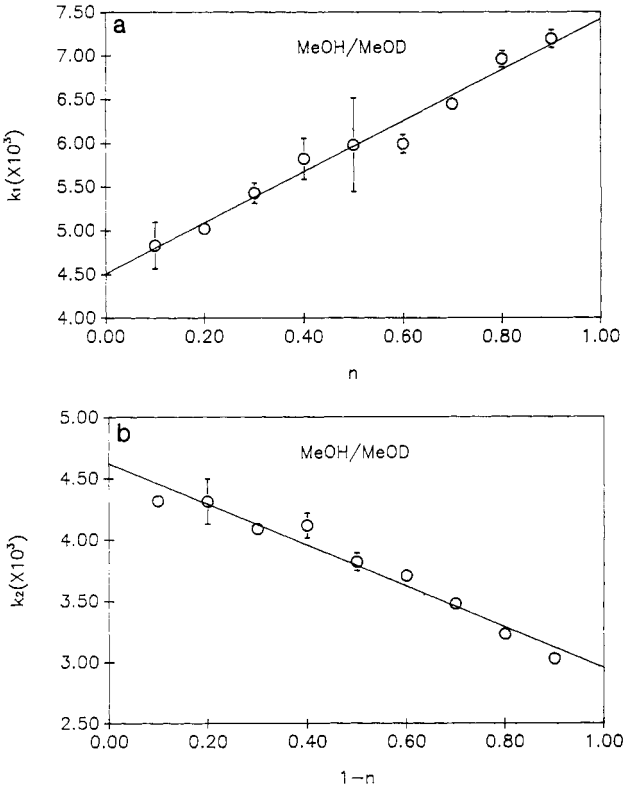

Figure 8. (a) k_1 vs $(1 - n)$ for MeOH/MeOD mixtures and (b) k_2 vs n for MeOH/MeOD mixtures, where n is the mole fraction of the deuterated solvent. The slopes and intercepts of these plots permit the determination of k^{HD} and k^{DH} (see text).

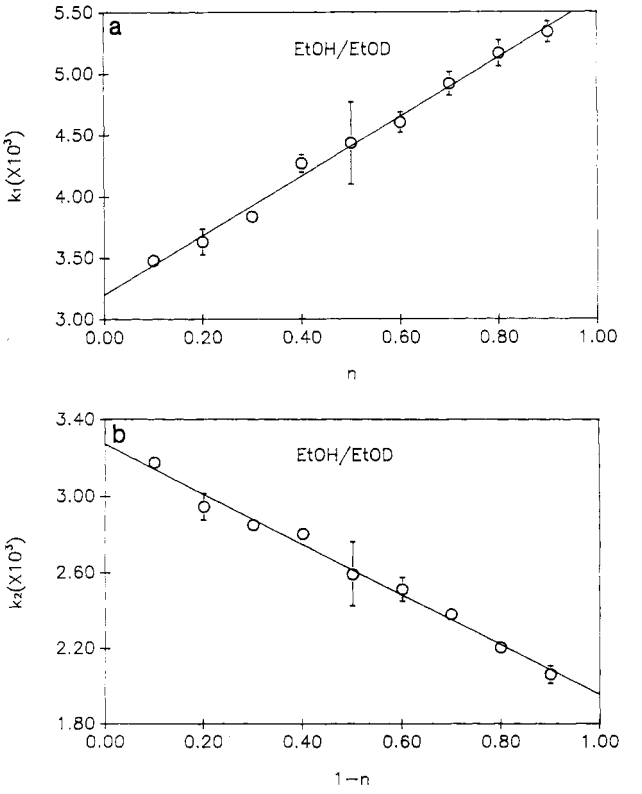

Figure 9. (a) k_1 vs $(1 - n)$ for EtOH/EtOD mixtures and (b) k_2 vs n for EtOH/EtOD mixtures, where n is the mole fraction of the deuterated solvent. The slopes and intercepts of these plots permit the determination of k^{HD} and k^{DH} (see text).

Another method of analyzing the data involves fitting (or force fitting) the data to a single-exponential decay. Even if proton

exchange is slow compared to the fluorescence lifetime, we can define an *apparent* first-order rate constant for the decay of the entire 7-azaindole population at time zero, $k_n(t=0)$, where $k_n(t=0)[N_1L] = k_1(1-n)[N_1L] + k_2n[N_1L]$. More specifically, if proton exchange is slow, the measured time constant for fluorescence decay is time dependent, $\tau_F = 1/k_n(t)$, and the decay of the excited-state population is, if $k^{HD} = k^{DH}$,

$$\exp[-k_n(t)t] = (1-n)^2 \exp(-k^{HH}t) + 2(1-n)n \exp(-k^{HD}t) + n^2 \exp(-k^{DD}t) \quad (21)$$

The prefactors in the above expression are a result of the assumption that $\phi^R = 1$. Differentiating this equation with respect to t and setting $t = 0$ yields

$$k_n(t=0) = (1-n)^2k^{HH} + 2(1-n)nk^{HD} + n^2k^{DD} \quad (22)$$

Using eq 22, k^{HD} may be extracted from a knowledge of the fluorescence lifetime, $1/k_n$, the lifetimes in the fully protonated and deuterated solvents, $1/k^{HH}$ and $1/k^{DD}$, and the mole fraction of deuterated solvent, n.

Because the rule of the geometric mean requires that[20-22]

$$\frac{k^{HD}}{k^{HH}} = \frac{k^{DD}}{k^{HD}} = \phi^T \quad \text{and that} \quad \frac{k^{DD}}{k^{HH}} = (\phi^T)^2 \quad (23)$$

substitution of eq 23 into eq 22 yields

$$k_n(t=0) = k^{HH}(1-n+\phi^Tn)^2 \quad (24)$$

which is formally equivalent to the Gross–Butler equation (eq 1) and is identical to it in the limit of fast exchange. Table IV summarizes the results of these analyses and indicates that the rule of the geometric mean is satisfied for methanol and ethanol, but not for water, assuming $\phi^R = 1$ (see above and notes to Table IV).

III. **The Nonradiative Process in 7-Azaindole.** Glasser and Lami[35] and Wallace and co-workers[36] have discussed the importance of fission of the NH bond as a nonradiative process in gas-phase indole. Barkley and co-workers[37] have performed detailed investigations of the deuterium isotope effect on the photophysics of tryptophan, indole, and some of their derivatives. They have proposed at least six different mechanisms to explain the isotope effect, such as photoionization, hydride transfer from the NH, proton transfer from the solvent to the ring, solvent-mediated NH exchange, tautomerization resulting in NH abstraction, and exciplex formation.

We propose that the isotope effect observed in indole derivatives can be rationalized by the same mechanism that we illustrate for 7-azaindole in Figure 6. We suggest that in indole this process is much less efficient because there is no N_7 nitrogen coordinated with a solvent proton. Such an interaction could establish a partial positive charge on N_7 that would help to stabilize the negative charge generated on N_1. Proton inventory experiments on indole could in principle help to sort out the numerous nonradiative mechanisms cataloged above for indole. Even with the excellent precision afforded by our experimental system, we have not, however, undertaken such experiments because the solvent isotope effect for indole is only 1.3.[12] Schowen has noted[16] that, for an isotope effect of 1.5, to distinguish between a one- and a two-proton process, precision in the data of 1% is required. To distinguish between a two- and a three-proton process, precision of 0.4% is required. It is important to comment on the "driving force" for the double-proton tautomerization of 7-azaindole in dimers and in alcohols (Figure 1) and on the proton abstraction by water (Figure 6). As we discuss in detail elsewhere,[12] there is a very small excited-state pK_a change in N_1 and N_7 of 7-azaindole. We have suggested that the nonequilibrium response of the solvent to the instantaneous charge redistribution in the excited state upon photon absorption may contribute to the proton-transfer reaction.[12] On the other hand, the most trivial way to reconcile the excited-state tautomerization reaction with the apparent similarity of ground- and excited-state pK_a values is to recognize that if the excited-state tautomer decays to the ground state on a time scale that is much more rapid[12] than the time scale for establishing a proton-transfer equilibrium, the pK_a can no longer be measured.

Finally, we must comment on the origin of the isotope effect. In large part because of the rapid (1.4 ps) tautomerization observed in dimers of 7-azaindole,[5] the tautomerization of dilute solutions of 7-azaindole in alcohols has been discussed in terms of a two-step process.[7-9,12] The first step involves obtaining the correct solvation of the solute by the alcohol; the second step, double-proton transfer. The interpretation of our isotopic substitution experiments depends on whether the two-step model is appropriate and, if it is, whether the solvation step is slow, fast, or comparable to tautomerization. If the rate-limiting step in the double-proton transfer reaction is the formation of the cyclic complex, then the isotope effects we discuss above require reinterpretation. Additional experimental and theoretical work is necessary in order to answer this question definitively. For the moment, we suggest that if solvation were the rate-limiting step in the excited-state tautomerization of 7-azaindole in alcohols, it would be extremely fortuitous that the rule of the geometric mean holds (Table IV). In addition, dimers of 7-azaindole may not be an appropriate paradigm for the tautomerization of the 7-azaindole–alcohol complex. For example, Fuke and Kaya[38] observe that in supersonic jets the rate of excited-state double-proton transfer of 7-azaindole dimers is 10^{12} s^{-1}, while in dimers of 1-azacarbazole and in complexes of 7-azaindole with 1-azacarbazole the rate is 10^9 s^{-1}. The reduction in rate by a factor of 10^3 is initially surprising given the very similar hydrogen bonding in the three types of complexes. It is therefore most likely premature to assume that tautomerization in a 7-azaindole complex occurs as rapidly as in a 7-azaindole dimer. Fuke and Kaya suggest that detailed considerations of the coupling of proton motion with intermolecular vibrational motion are required in order to predict the rate of such tautomerization reactions.[38]

Conclusions

1. We have performed the first application of the proton inventory technique to an excited-state process. The data suggest that the excited-state tautomerization of 7-azaindole in alcohols proceeds by a concerted, two-proton process that is consistent with the structure of the cyclic solute–solvent complex presented in Figure 1. (The data, however, do not prove the existence of a cyclic complex of one solvent molecule with the solvent. There is the possibility that the double-proton transfer involving N_1 and N_7 occurs via two different alcohol molecules that interact with each other sufficiently strongly to effect the concerted reaction.)

2. Interpretation of the data in terms of a concerted process is based on several methods of analysis. All of these methods require as a criterion of concertedness that the rule of the geometric mean be fulfilled. The first, employing the Gross–Butler equation, is the most easily interpreted but assumes rapid ligand exchange between solute and solvent, which may not be likely. Alternatively, explicit consideration of the double-proton transfer reaction in terms of nominally stepwise processes taking into account the four possible permutations of isotopic substitution (Figure 7) provides a similar result. The rule of the geometric mean is shown to hold within experimental error for methanol and ethanol, but

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not for water (Table IV). We note, however, that the rule of the geometric mean is satisfied only in very special cases: namely, that the reaction be symmetrical ($\alpha = 1$) and that $S = P$. There are also instances where the reaction is concerted but the rule of the geometric mean breaks down because of contributions from tunneling.[24,25] Finally, the rule of the geometric mean may apply even for a stepwise process, if it proceeds by what Limbach and co-workers refer to as a "compressed state",[27] an orientation where the reactants are so near that the barrier to proton transfer vanishes.[27] (In such a state there is no difference between a concerted and a stepwise transfer.)

3. Further evidence is provided to support the model (Figure 5) of 7-azaindole being solvated by water in such a way that double-proton transfer, as it occurs in alcohols, is negligible.[11-13]

4. Proton inventory experiments of 7-azaindole in H_2O/D_2O mixtures are interpreted in terms of a three-proton process involving the hydrogen of N_1 and the two protons of a water molecule coordinated to it. These data are consistent with measurements of proton abstraction in ribonuclease.[16,17]

5. The fundamentally different interactions of 7-azaindole with water and with alcohols, which are manifested in the photophysics,[11-13] demonstrate the sensitivity of 7-azaindole (and 7-azatryptophan) as an optical probe.[1,2]

Acknowledgment. J.W.P. is an Office of Naval Research Young Investigator. F.G. is a recipient of a fellowship from Phillips Petroleum. This work was partially supported by the ISU Biotechnology Council and University Research Grants. We thank R. L. Rich for a critical reading of the manuscript and for assistance with the figures. Alan Schwabacher provided many stimulating and helpful discussions.