Photoinduced Double Proton Transfer in a Model Hydrogen Bonded Base Pair.[1] Effects of Temperature and Deuterium Substitution

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Abstract: 7-Azaindole hydrogen bonded dimers and complexes of 7-azaindole with alcohols undergo a unique photoinduced double proton transfer reaction to form tautomers. In order to provide more understanding of this phenomenon, we have investigated the effects of solvent, concentration, pH, temperature, excitation wavelength, and deuterium substitution on the fluorescence of 7-azaindole solutions. By examining the temperature dependence of the relative intensities of the normal violet fluorescence (F_1) and the green tautomer fluorescence (F_2) in 3-methylpentane, a lower limit of 1.4 kcal/mol was estimated for the barrier to proton transfer in the dimer. It is shown that proton tunneling (through the barrier) is the dominant mechanism at 77°K. The effects of deuterium substitution of the pyrrolic hydrogen on the magnitude of F_2/F_1 ratio at 77°K are consistent with current theories of proton tunneling. The similarities between the 7-azaindole dimer and the DNA base pairs are discussed in terms of double proton transfer as a possible mechanism for photomutagenesis.

Solutions of 7-azaindole (7AI) in nonhydrogen bonding solvents exhibit two fluorescence bands.[3-5] In addition to the normal violet fluorescence (F_1) in the region 320–360 nm, there is a second green fluorescence (F_2) with a maximum near 480 nm. The relative intensity of F_1 and F_2 depends on concentration, excitation wavelength, and temperature. The second band F_2 was suggested[3,4] to originate from a tautomer formed by a fast intermolecular double proton transfer reaction in the excited state of the double hydrogen bonded dimer as shown below.
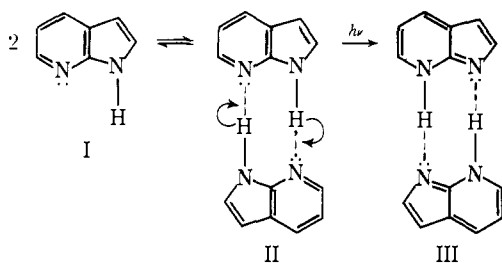
This interpretation was nicely confirmed[5] by a comparison of the fluorescence of 7AI (I) with that of its two N-methyl derivatives, N^1-methyl-7-azaindole (IV) and 7-methyl-7H-pyrrolo[2,3-b]pyridine (V). In particular, the latter compound, which has a formal electronic structure similar to species III, was shown to have a fluorescence spectrum very similar to F_2.
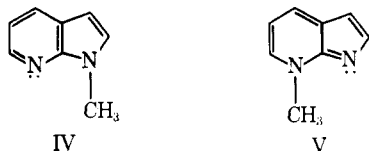
Solutions of 7AI in ethanol also exhibit two fluorescence bands similar to those observed in hydrocarbon solvents. However, in this case the ratio F_2/F_1 is not dependent on concentration or excitation wavelength but does depend on temperature. In ethanol there is no evidence for dimerization and in this case the green fluorescence is attributed[2] to a double proton transfer reaction occurring in the excited state of a 1:1 complex between ethanol and 7AI as shown below.
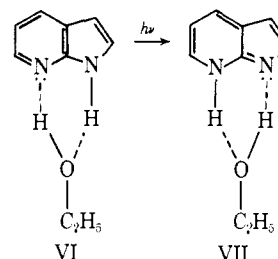
Since the tautomer fluorescence (F_2) is well separated (by about 7000 cm^{-1}) from the normal fluorescence (F_1), it is possible to monitor the extent of the reaction as a function of various experimental parameters and thus obtain information about the energetics and dynamics of double proton transfer. We previously reported[5] some initial results on the effect of temperature on the relative intensity of F_2 and F_1 in 3-methylpentane (3MP) which indicated the presence of an activation barrier to double proton transfer in the excited state of the dimer and suggested that proton tunneling was an important mechanism for the reaction at low temperature. Temperature studies as well as deuterium substitution effects on F_2/F_1, which are presented here, confirm these qualitative conclusions and allow a more detailed description of the barrier.

Experimental Section

Solvents were purified as follows. Phillips pure grade 3-methylpentane (3MP) was first washed extensively with a mixture of concentrated nitric and sulfuric acids followed by dilute base and then water. After drying over $CaCl_2$ and refluxing over Na–Pb alloy for at least 24 hr, the 3MP was distilled directly through a 4-ft vacuum jacketed column. The resulting solvent was free from
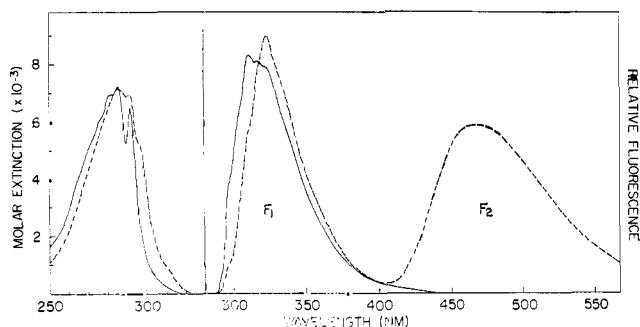
(1) Supported by U. S. Atomic Energy Commission Contract No. AT (11·1)2039.

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(3) C. A. Taylor, Masters Thesis, Florida State University, Tallahassee, Fla., 1969.

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Figure 1. Room temperature absorption (left) and fluorescence (right) of 7AI in 3MP at 1.0×10^{-5} (———) and 1.0×10^{-2} M (---). The excitation wavelength was 285 nm. Front surface excitation was used for the concentrated sample. The fluorescence spectra are uncorrected for instrument response, and the signals were arbitrarily adjusted to give comparable intensity in the F_1 region.

emitting impurities in the region of interest. For the low temperature work, extreme care was required to ensure the absence of protic impurities in the 3MP. This was accomplished by using only 3MP which was freshly distilled after refluxing over Na-Pb alloy and by drying all cells extensively in a desiccated oven.

Ethanol was distilled slowly through a 4-ft column, discarding the initial fractions until no benzene was evident in the absorption spectrum using a 10-cm cell. Carbon tetrachloride (Eastman spectroquality grade) was used without further purification. 7-Azaindole (Aldrich) was recrystallized several times from cyclohexane and dried extensively. Both N'-methyl-7-azaindole (IV) and 7-methyl-7H-pyrrolo[2,3-b]pyridine (V) were prepared according to the method of Robison and Robison;[6] IV was purified by vacuum distillation and V by paper chromatography.

N'-Deuterated 7-azaindole was prepared by refluxing 7-azaindole in alkaline D_2O for 1 hr. Mass spectral analysis gave an isotopic purity of 91%.

Absorption measurements were made on a Cary Model 15 spectrophotometer. Fluorescence spectra were obtained on an Aminco-Keirs spectrofluorimeter or on a component system equipped with phase-sensitive detection.[7] The latter was useful for eliminating interferring phosphorescence at low temperature. Corrected excitation spectra were obtained on an instrument built by Dr. J. Holland at Michigan State University.[8] When necessary, samples were degassed by the freeze-pump-thaw technique.

In the study of the effect of temperature on F_2/F_1, the temperature was controlled by varying the rate at which cold N_2 was blown into the optical dewar. There was a constant outflow of N_2 from the dewar which prevented condensation on the cell. The temperature was monitored with a thermocouple immersed directly in the sample. Corning excitation and emission filters served to reduce the amount of scattered light. Front face excitation was used to minimize inner filter effects. F_2/F_1 ratios were determined by comparing intensities at the maxima of the corresponding bands and were uncorrected for differences in instrument response at the two wavelengths. Quantum yields were estimated by comparison to quinine sulfate in 0.1 N H_2SO_4 on the Aminco-Keirs instrument which was calibrated according to Melhuish.[9] A value of 0.54 was used for the quantum yield of quinine sulfate.

Hydrogen Bonding Equilibria

A. 7-Azaindole Dimers. It was known from previous work[10] that 7AI forms hydrogen bonded dimers in nonpolar solvents like 3MP and CCl_4. The equilibrium constant for this self-association was estimated to be 118 M^{-1} based on an infrared study in CCl_4 at 35°. Since most of the luminescence work utilized 3MP as a solvent, it was felt worthwhile to study the equilibrium
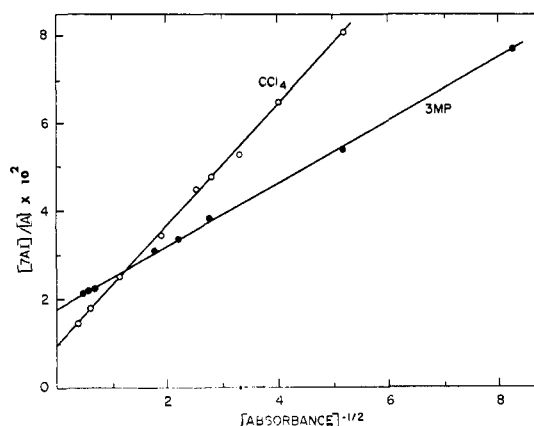
(6) M. M. Robison and B. L. Robison, J. Amer. Chem. Soc., **77**, 6554 (1955).

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(8) J. F. Holland, R. E. Teets, and A. Timnick, Anal. Chem., **45**, 145 (1973).

(9) H. Melhuish, J. Opt. Soc. Amer., **52**, 1256 (1962).

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Figure 2. Application of eq 3 to determine the dimer association constant for 7AI in 3MP (●) and CCl_4 (○) at 25°. Absorbance values were determined at 315 nm and correspond to a path length of 1 cm. The straight lines, obtained by linear regression, have correlation coefficients of 0.9996 (3MP) and 0.9997 (CCl_4).

in more detail. The effect of concentration on the room temperature absorption spectrum of 7AI in 3MP is shown in Figure 1. At 10^{-2} M there is an increased intensity on the long-wavelength side of the absorption band. In dilute solution there is very little absorption at 315 nm while in the more concentrated solution a distinct shoulder appears at this wavelength. This absorption is due to the formation of hydrogen bonded dimers in the more concentrated solutions and is similar to the absorption in ethanol.[10]

The equilibrium expression for the monomer–dimer association can be written as

$$K = \frac{[D]}{[M]^2} = \frac{[D]}{(C - 2[D])^2} \qquad (1)$$

where K is the association constant, [M] and [D] are the molar concentrations of monomer and dimer, respectively, and C is the actual concentration of 7AI in the solution. Since at sufficiently long wavelength (315 nm) the absorption is due almost exclusively to dimers, one can write

$$A = \epsilon[D]l \qquad (2)$$

where A is the absorbance at 315 nm, ϵ is the molar extinction coefficient of the dimer at 315 nm, and l is the path length. Combining eq 1 and 2 leads to (for $l = 1$ cm)

$$\frac{C}{A} = \frac{2}{\epsilon} + \left(\frac{1}{\epsilon K}\right)^{1/2}\left(\frac{1}{A}\right)^{1/2} \qquad (3)$$

Figure 2 illustrates the application of eq 3 to data obtained at 315 nm in CCl_4 and 3MP solutions at room temperature. The straight line behavior is sufficiently good to confirm the assumptions of a 1:1 complex and negligible monomer absorption at 315 nm. The absorbance of 10^{-5} M solution of 7-azaindole in 3MP in a 10-cm cell is 0.0065 at 315 nm. From the intercept in Figure 2 we obtain an ϵ value of 1110 l. mol^{-1} cm^{-1} for the dimer at 315 nm. Using our estimate of $K = 1.8 \times 10^3$, we calculate that about 33% of the above absorption is due to the small concentration of dimers present at 10^{-5} M. This leads to an estimate of 44 l. mol^{-1} cm^{-1} for the extinction of the monomer at 315 nm. Thus the neglect of monomer absorption in eq 2 is justified.
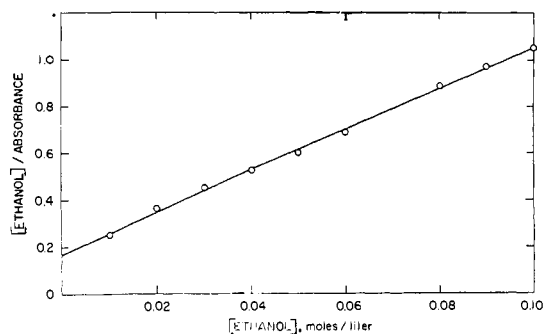
Figure 3. Application of eq 4 to determine the association constant of the 7AI–ethanol complex at 25°. Absorbance values were determined at 310 nm and correspond to a path length of 1.0 cm. The 7AI concentration was 1.0×10^{-4} M. The straight line, obtained by linear regression, has a correlation coefficient of 0.9992.

The association constants determined from the data in Figure 2 are 1.8×10^3 and 2.5×10^2 M^{-1} in 3MP and CCl$_4$, respectively. These correspond to ΔG values of -4.5 and -3.3 kcal/mol at room temperature. The association is much stronger than would be anticipated simply from the number and type of hydrogen bonds involved. For instance, Fritzche[11] reported a value of $\Delta G = +0.81$ kcal/mol for the indole–pyridine complex in CCl$_4$. The pyrrole–pyridine complex is reported to have ΔG values of -0.54 kcal/mol in CCl$_4$[12] and -1.9 kcal/mol in cyclohexane.[13] The larger values for 7AI dimerization suggest a cooperative effect perhaps resulting from a resonance interaction between the rings mediated by the hydrogen bonds as suggested by Pullman[14] for the DNA base pairs.

The lower association constant in CCl$_4$ can be rationalized on the basis of the high polarizability of this solvent relative to 3MP. The dipole-induced dipole solvation forces for the monomer are increased on changing from 3MP to CCl$_4$. By contrast the effect will be much smaller for the dimer which, because of its symmetry, has no net dipole moment. The net result is a preferential stabilization of the monomer and thus a lowering of the dimerization equilibrium constant in CCl$_4$.

In order to be able to estimate the extent of dimerization at lower temperatures, the association constant in 3MP was determined as a function of temperature between 20 and 50°. This resulted in an estimate of -9.6 kcal/mol for ΔH and -18 eu for ΔS. Using the value of 1.8×10^3 M^{-1} for the association constant in 3MP at 25°, one can calculate that at 10^{-5} M only about 4% of the 7AI molecules are dimerized, compared to 85% at 10^{-2} M. Thus the solid curve in Figure 1 is due mainly to monomer while the dashed curve represents the dimer. Because of the large negative ΔH, the extent of dimerization will increase dramatically on cooling. This was confirmed by measuring the absorption spectrum in 3MP at 77°K, where the spectra were identical at 10^{-4} and 10^{-2} M.

B. 7-Azaindole–Ethanol Complex. Addition of small amounts of ethanol to a dilute (1.0×10^{-4} M) solution of 7AI in 3MP at room temperature perturbs the absorption spectrum in a fashion similar to that shown in

(11) Von H. Fritzche, *Ber. Bunsenges. Phys. Chem.*, 68, 459 (1964).
(12) H. J. Wimmette and R. H. Linnell, *J. Phys. Chem.*, 66, 546 (1962).
(13) J. A. Happe, *J. Phys. Chem.*, 65, 72 (1961).
(14) B. Pullman, *J. Chem. Phys.*, 43, 5233 (1965).

Figure 1, for dimerization. These effects are attributed to the formation of a cyclic hydrogen bonded 1:1 complex between 7AI and ethanol (species VI). Similar results are observed with other alcohols. The absorption at 310 nm is due primarily to the complex and can be used to determine the association constant by the method of Benesi and Hildebrand[15] as modified by Scott,[16] according to eq 4 (assuming a 1:1 complex)

$$\frac{C_A}{A} = \frac{C_A}{C_I l \epsilon} + \frac{1}{\epsilon l K C_I} \tag{4}$$

where C_A is the ethanol concentration, C_I is the 7AI concentration, l is the path length, ϵ is the molar extinction coefficient of the complex at 310 nm, and K is the association constant. A plot of C_A/A vs. C_A is shown in Figure 3 for data obtained at room temperature and 310 nm. The good linearity of the plot confirms the assumption of a 1:1 complex since higher order complexes would produce curvature. From the ratio of intercept to slope we obtain $K = 49$ M^{-1}. This value must be considered a lower limit since at the 7-azaindole concentration used, about 20% of the 7-azaindole molecules are initially present as dimers which have appreciable absorption at 310 nm. It should be noted that K for the alcohol complex is much lower than that for the dimer. Apparently no cooperative effects are involved here.

Fluorescence Spectra

A. Effect of Concentration and Solvent. The fluorescence of 7AI at room temperature in 3MP is shown in Figure 1 at two different concentrations. The solid curve corresponds to a dilute (10^{-5} M) solution consisting mainly of monomers. Here, only the normal fluorescence (F_1) is observed. The dashed curve corresponds to a 10^{-2} M solution in which about 85% of the 7AI molecules are dimerized. The F_1 component which is due almost exclusively to the remaining (15%) monomers appears red shifted. However, this is due to self-absorption of the short-wavelength fluorescence by the high concentration of dimers whose absorption spectrum extends out to around 325 nm. The dimers make very little contribution to F_1 under these conditions because of the high efficiency of the double proton transfer process at room temperature (*vida infra*). At intermediate concentrations one obtains fluorescence spectra in which F_2/F_1 varies continuously between the extremes shown in Figure 1.

The room temperature fluorescence of 7AI in three additional solvents is shown in Figure 4. In diethyl ether it is possible to observe an F_2 component although much higher concentrations (0.1 M) are required than in 3MP. This is attributed to a much lower dimer association constant in ether compared to 3MP, a result which is expected on the basis of the hydrogen bond accepting properties of this solvent. No cyclic complex with ether is possible since ether serves only as hydrogen bonding acceptor. The relative contributions of F_2 and F_1 in ether depend on excitation wavelength and temperature in addition to concentration.

Two fluorescence bands are also observed in ethanol. However, in this solvent the spectrum is independent of

(15) H. A. Benesi and J. H. Hildebrand, *J. Amer. Chem. Soc.*, 71, 2703 (1949).
(16) R. L. Scott, *Recl. Trav. Chim. Pays-Bas*, 75, 787 (1956).
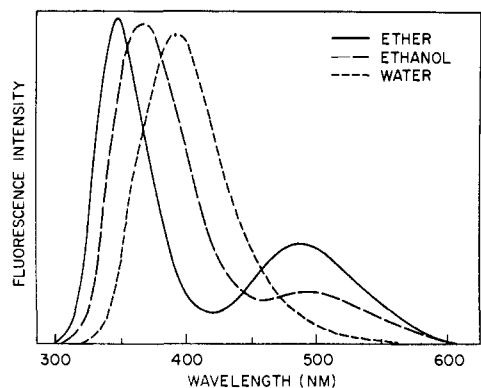
Figure 4. Effect of solvent on the room temperature fluorescence of 7AI. The concentrations and excitation wavelengths were as follows: ether 0.1 M, 315 nm; ethanol, 1.0 $\times$ 10^{-4} M, 290 nm; water, 1.0 $\times$ 10^{-4} M, 290 nm. The curves are not corrected for instrumental response and have been arbitrarily adjusted to give comparable F_1 intensities. Front surface excitation was used for the ether solution.

Figure 5. Corrected fluorescence excitation spectra of 7AI (1.0 $\times$ 10^{-4} M) in 3MP at 25°. The fluorescence wavelengths were 325 nm (solid curve, F_1) and 475 nm (dashed curve, F_2).

concentration and excitation wavelength. Similar results are observed in other alcohols. A comparative study in several alcohols revealed a slight tendency for F_2/F_1 to decrease on changing from primary to secondary to tertiary alcohols (Table I). One might expect

Table I. F_2/F_1 Ratios in Alcohols

Solvent	F_2/F_1	Solvent	F_2/F_1
Primary alcohols		Secondary alcohols	
Methanol	0.17	2-Butanol	0.14
Ethanol	0.18	2-Propanol	0.14
1-Propanol	0.19	Tertiary alcohols	
1-Butanol	0.16	tert-Butyl alcohol	0.11

that F_2/F_1 would increase in going from primary to tertiary alcohols because of the increased acidity. The opposite trend suggests that steric factors are involved. The F_2/F_1 ratio in all the alcohols is small suggesting that double proton transfer in the 7AI–alcohol complexes is much less efficient than in the 7AI dimer.

In water, 7AI exhibits only one fluorescence band (F_1) with a maximum near 390 nm; no F_2 was detected under any conditions. One possible explanation is that no double proton transfer occurs in this solvent. This would be expected if each 7AI molecule were hydrogen bonded to two water molecules as opposed to forming a 1:1 cyclic complex similar to that in ethanol. The absence of a cyclic complex in water could be rationalized in terms of the greater tendency of water to self-associate. Another possibility is that proton transfer occurs but the tautomer thus formed has a negligible fluorescence yield in this solvent. This would be manifested by a quenching of F_1 and is supported by the observation that the fluorescence yield of 7AI actually decreases approximately threefold on going from 3MP to water while that of the N^1-methyl derivative increases. However, the N^7-methyl tautomer has a higher yield in water than in 3MP arguing against this interpretation. Further studies are required to explain the absence of F_2 in water.

There is a dramatic red shift in the maximum of F_1 as the polarity of the solvent increases. Similar results
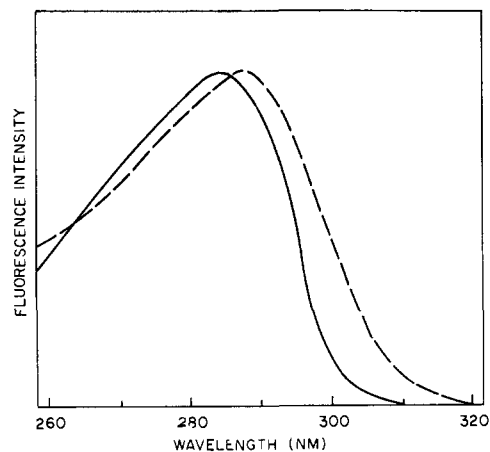
have been reported for other indole derivatives[17–19] and probably reflect a large increase in the permanent dipole moment in the emitting singlet state. N^1-Methyl-7-azaindole (IV) exhibits similar behavior. By contrast, the fluorescence of the N^7-methyl tautomer is insensitive to solvent polarity as is the F_2 component of the various 7AI solutions. This suggests that the tautomeric structure is associated with a small permanent dipole moment in its lowest excited singlet state.

B. Effect of Excitation Wavelength. The room temperature corrected excitation spectra corresponding to F_1 and F_2 in 3MP are shown in Figure 5. Although the resolution is poor, the F_2 excitation spectrum is distinctly red shifted relative to that of F_1. Recall that the absorption spectrum of the dimer is also red shifted relative to that of the monomer (Figure 1). Thus the excitation spectra are compatible with the interpretation that F_1 is predominantly monomer fluorescence and F_2 arises from dimers which have undergone double proton transfer. By choosing a sufficiently long excitation wavelength (315 nm) and sufficiently high concentration (10^{-2} M), one obtains a fluorescence spectrum which is almost exclusively F_2. This argues for a highly efficient double proton transfer and further supports our conclusion that dimers do not contribute significantly to F_1 at room temperature.

The fluorescence spectrum of 7AI in ethanol is independent of excitation wavelength indicating that a single species is responsible for the fluorescence. This species is presumably the 1:1 cyclic hydrogen bond complex between 7AI and ethanol (species VI). In ether, no dependence of the fluorescence spectrum on excitation wavelength is observed except in very concentrated (0.1 M) solutions where one begins to see effects similar to those observed in 3MP. In H_2O at neutral pH the fluorescence is independent of excitation wavelength.

C. Effect of pH. The effect of pH on the fluorescence of aqueous 7AI is illustrated in Figure 6. In the alkaline range the fluorescence is quenched with a pK of about 12.3. Similar results are observed with other

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(18) M. S. Walker, T. W. Dednar, R. Lumry, and F. Humphries, *Photochem. Photobiol.*, **14**, 147 (1971).

(19) B. L. Van Duuren, *J. Org. Chem.*, **26**, 2954 (1961).
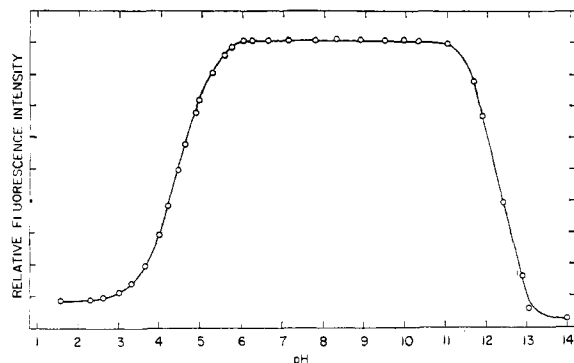
Figure 6. Effect of pH on the fluorescence of an aqueous solution of 7AI at 26°. The concentration of 7AI was 5×10^{-5} M. The buffers were 0.01 M phosphate, 0.01 M acetate, and 0.01 M bicarbonate. The excitation wavelength was 280 nm and the emission wavelength 390 nm.
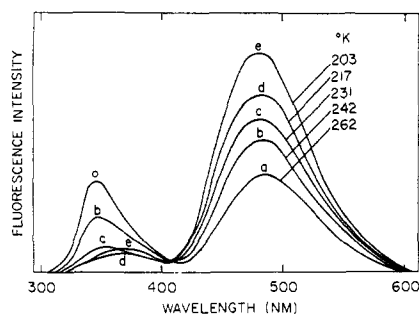
Figure 7. Effect of temperature on the fluorescence spectrum (uncorrected) of 7AI (10^{-2} M) in 3MP. The degassed sample was excited at 285 nm using front surface excitation. A Corning filter (CS-054) was placed in front of the emission monochromator.

indole derivatives.[20-22] Since no change in the absorption spectrum occurs even at much higher pH values, quenching is attributed to excited-state dissociation of the indolic proton at the N^1 position to form the corresponding anion which is nonfluorescent. The pK of 12.3 is identical with that reported by Vander Donckt[21] for the excited state of indole suggesting that aza substitution in the 7 position has very little effect on the excited-state charge density at the N^1 position.

In the acid range the fluorescence is also quenched although not entirely. At pH 4 a new band is observed with a maximum near 450 nm corresponding to the cation with the proton at the N^7 position. The ground state pK for this reaction was reported by Adler and Albert[23] to be 4.59, essentially the same as that observed here by fluorescence. Thus, there is either no increase in the basicity of the aza nitrogen on excitation or excited-state protonation at this position is simply too slow to compete with fluorescence.

D. Fluorescence of Solid 7AI. Several attempts were made to observe evidence for double proton transfer in solid samples of 7AI. Single crystals grown in 3MP as well as powder samples both show a weak F_1 component with a maximum near 370 nm. Similar results were observed in a sample which was melted and pressed between two Suprasil plates. No evidence for an F_2 component was observed in any of the solid sam-

(20) E. Vander Donckt, *Progr. React. Kinet.*, **5**, 294 (1970).
(21) E. Vander Donckt, *Bull. Soc. Chim. Belg.*, **78**, 69 (1969).
(22) J. W. Bridges and R. T. Williams, *Biochem. J.*, **107**, 225 (1968).
(23) T. K. Adler and A. Albert, *J. Chem. Soc.*, 1794 (1960).
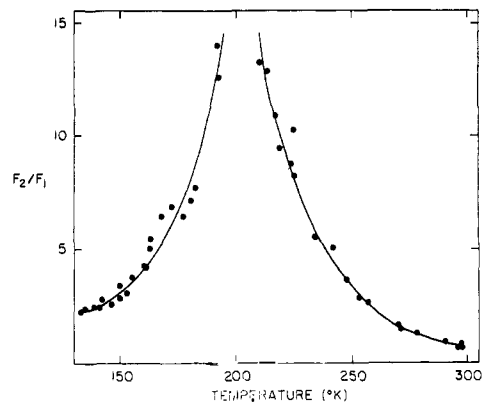
Figure 8. Effect of temperature on the F_2/F_1 ratio of 7AI (10^{-2} M) in 3MP. Conditions are identical with those in Figure 7. The F_2/F_1 ratios were estimated by comparing the maximum intensities (uncorrected) of F_2 and F_1.

ples, even at 77°K. Apparently dimers are either not present in these solid preparations or, if present, are associated with a much lower efficiency of double proton transfer. In this regard it may be pertinent that we have observed strong F_2 in solid solutions of 7AI in rigid plastic media such as poly(methyl methacrylate) and polystyrene. The alternative explanation that double proton transfer occurs in the solid preparations but that the tautomer thus formed has an extremely low fluorescence quantum yield is unlikely since powder samples of the N^7-methyl tautomer (V) yield a bright green fluorescence.
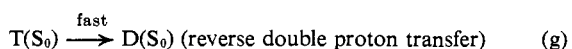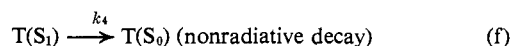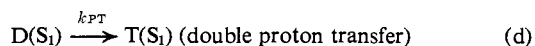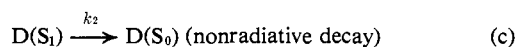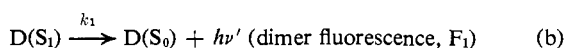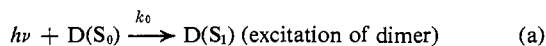
Temperature Dependence of Proton Transfer in the the 7AI Dimer

The effect of temperature on the fluorescence of 7AI in 3MP (10^{-2} M) is illustrated in Figure 7. As the solution is cooled the F_1 component is diminished and the F_2 component enhanced. The effects of temperature on the F_1 component are interpreted as follows. At room temperature, F_1 is due primarily to monomer fluorescence even though 85% of the 7AI molecules are in the dimer form at this concentration. As stated before the dimers undergo efficient double proton transfer in the excited state and hence the dimer fluorescence is low. However, at room temperature the F_1 component is still strong because of the higher yield of the monomer relative to the tautomer. As the temperature is lowered the equilibrium shifts toward the dimer side causing a decrease in monomer fluorescence and unmasking the weak dimer fluorescence which occurs at longer wavelength. Near 200°K this dimer fluorescence begins to increase on further cooling due to the diminished efficiency of the double proton transfer reaction.

The ratio F_2/F_1 is plotted in Figure 8 over the range from room temperature to 130°K. These results confirm our initial report[5] and we now have more data in the important range below 200°K. The initial rise in F_2/F_1 on cooling is due to the previously mentioned shift in the dimer equilibrium. Near 200°K the curve goes through a maximum rapidly on further lowering the temperature. Further lowering of the temperature to 77°K or even 4°K has little additional effect[5] on F_2/F_1. One should mention, however, that there is some concentration dependence of F_2/F_1 at 77°K. This could be

due to residual amounts of protic solvent impurities which may interfere with dimer equilibration leading to the trapping of some monomers.

Using our value of $\Delta H = -9.6$ kcal/mol for the dimerization, we estimate an association constant of 10^6 at 200°K. At 10^{-2} M essentially all of the 7-azaindole molecules exist in the dimer form at this temperature. Thus, the interpretation of the drop in F_2/F_1 below 200°K should not be complicated by shifts in the monomer–dimer equilibrium. The results in this range can be analyzed in terms of the following kinetic scheme for the dimer D (species II) and the tautomer T (species III)

$$h\nu + D(S_0) \xrightarrow{k_0} D(S_1) \text{ (excitation of dimer)} \quad \text{(a)}$$

$$D(S_1) \xrightarrow{k_1} D(S_0) + h\nu' \text{ (dimer fluorescence, } F_1) \quad \text{(b)}$$

$$D(S_1) \xrightarrow{k_2} D(S_0) \text{ (nonradiative decay)} \quad \text{(c)}$$

$$D(S_1) \xrightarrow{k_{PT}} T(S_1) \text{ (double proton transfer)} \quad \text{(d)}$$

$$T(S_1) \xrightarrow{k_3} T(S_0) + h\nu'' \text{ (tautomer fluorescence, } F_2) \quad \text{(e)}$$

$$T(S_1) \xrightarrow{k_4} T(S_0) \text{ (nonradiative decay)} \quad \text{(f)}$$

$$T(S_0) \xrightarrow{\text{fast}} D(S_0) \text{ (reverse double proton transfer)} \quad \text{(g)}$$

S_0 and S_1 are ground and first excited singlet states, respectively. Here, we have neglected any direct absorption by the tautomer, as well as the reverse of step d. Both of these assumptions will be discussed later. Under steady-state illumination the intensity of F_1 will be given by

$$F_1 = G_1 k_0 \left(\frac{k_1}{k_1 + k_2 + k_{PT}} \right) = G_1 k_0 k_1 \tau_D \quad (5)$$

where the quantity in parentheses is the dimer fluorescence quantum yield, τ_D is the dimer fluorescence lifetime, and G_1 is a constant which includes the geometrical arrangement of the cell and detection system, phototube response, and other *temperature independent* factors. Similarly, for F_2 we have

$$F_2 = G_2 k_0 \left(\frac{k_{PT}}{k_1 + k_2 + k_{PT}} \right) \left(\frac{k_3}{k_3 + k_4} \right) =$$

$$G_2 k_0 \tau_D k_{PT} \phi \quad (6)$$

where ϕ is the fraction of excited tautomers which fluorescence. The ratio F_2/F_1 then becomes

$$\frac{F_2}{F_1} = \left(\frac{G_2}{G_1} \right) \left(\frac{\phi}{k_1} \right) k_{PT} \quad (7)$$

The radiative rate constant k_1 is not expected to depend on temperature. If we assume for the moment that ϕ is also independent of temperature, then the only temperature dependence of F_2/F_1 will be due to k_{PT} which may be expressed as

$$k_{PT} = A \exp(-E_a/RT) \quad (8)$$

where E_a is the activation barrier for double proton transfer. Combining eq 7 and 8 one obtains

$$\log (F_2/F_1) = \text{constant} - \frac{E_a}{2.3RT} \quad (9)$$

A plot of eq 9 using the data below 200°K is shown in Figure 9. The straight line drawn through the some-
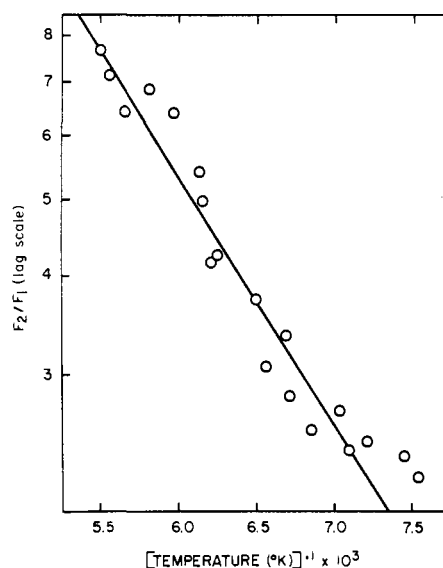

Figure 9. Arrhenius plot for estimation of the barrier to double proton transfer in 7AI dimers (data from Figure 8). The straight line was obtained by linear regression analysis and has a correlation coefficient of 0.93.

what scattered data points has a slope corresponding to an activation barrier of 1.4 kcal/mol.

Let us return now to some of the assumptions made in this analysis. Since the tautomer is chemically unstable it is impossible to test the assumption that its quantum yield is independent of temperature. In the experiments by which the data in Figure 9 were obtained, the overall quantum yield $(F_1 + F_2)$ did not vary substantially over the temperature range studied, although attempts to measure it accurately were frustrated by changes in lamp intensity and the impossibility of removing the sample periodically for comparison with a standard. We have investigated[24] the temperature dependence of the fluorescence of the N^7-methyl tautomer (V), in which case there is about a threefold increase between 200 and 130°K. If the 7AI tautomer behaves similarly then the above value of E_a must be taken as a lower limit.

It was also assumed that reaction g in the kinetic scheme was sufficiently rapid to prevent a significant accumulation of ground-state tautomer and to prevent its direct excitation. Several observations support this assumption. Attempts to trap the tautomer in the ground state by prolonged intense illumination at 77°K were unsuccessful. No absorption of the transient tautomer in the 400-nm region could be detected in a sample which was simultaneously exposed at right angles to intense excitation below 300 nm. Thus, the possibility of a contribution to F_2 arising from direct excitation of transient ground-state tautomers seems unlikely.

Finally, the neglect of excited-state proton transfer in the reverse direction is justified on the following basis. At room temperature, it is possible to choose a sufficiently long excitation wavelength such that only dimers are excited. Under those conditions, the amount of F_1 is negligible compared to F_2. Thus, the equilibrium at room temperature is overwhelmingly in favor of tautomer so that the forward rate constant for double pro-

(24) K. C. Ingham, J. Kordas, and M. A. El-Bayoumi, unpublished results.

Figure 11. Double minimum potential well describing the energetics of double proton transfer in the 7AI dimer (see text).
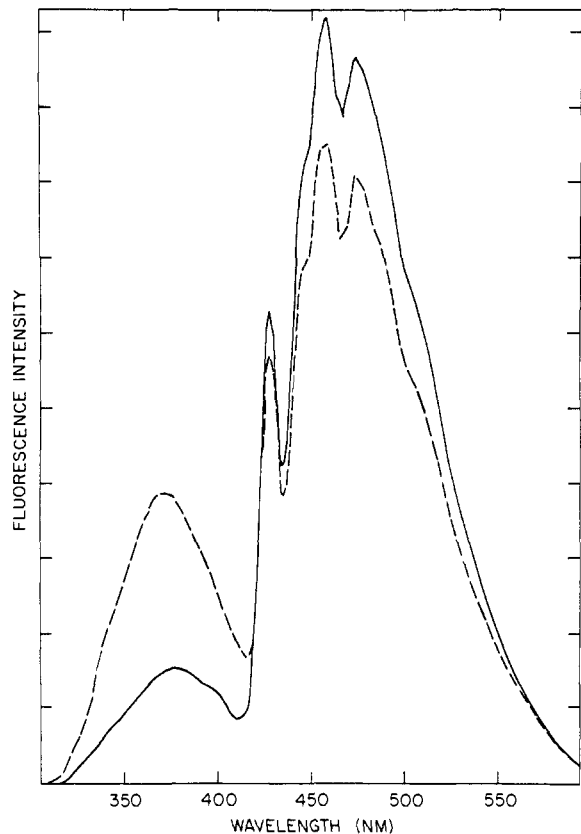
Figure 10. Effect of deuterium substitution on the fluorescence of 7AI (10^{-2} M) in 3MP at 77°K. The degassed solutions were excited at 285 nm using front surface excitation: solid curve 7AI; dashed curve 7AI-d_1.

ton transfer is large compared to the rate constant for reverse double proton transfer.

Double Proton Tunneling and the Deuterium Isotope Effect

In the previous section, the effects of temperature on the F_2/F_1 ratio in 7AI solutions were interpreted in terms of an activation barrier for double proton transfer. A lower limit of 1.4 kcal/mol (500 cm^{-1}) was estimated for the barrier in the 7AI dimer in 3MP. Using this barrier height one would predict a value of 0.03 for F_2/F_1 at 77°K and even lower at 4°K. Thus, at these temperatures thermal energy is not sufficient to allow appreciable transfer over the barrier. However, substantial F_2 is observed, even at 4°K. This points to proton tunneling as a dominant mechanism for tautomerization at low temperatures.

In order to obtain further support for the occurrence of proton tunneling at low temperature, we prepared a sample of 7AI in which the hydrogen atom at the N^1 position was replaced by deuterium. The room temperature fluorescence of solutions of the deuterated and nondeuterated compounds in 3MP are indistinguishable within experimental error. However, at 77°K where proton transfer over the barrier is negligible, there is a large effect on the F_2/F_1 ratio as shown in Figure 10. Note that deuterium substitution enhances F_1 at the expense of F_2 indicating that the proton transfer reaction is less efficient in the deuterated sample. One possible explanation for this observation is that deuterium substitution causes an increase in the F_1 quantum yield and a simultaneous decrease in the F_2 quantum yield. How-
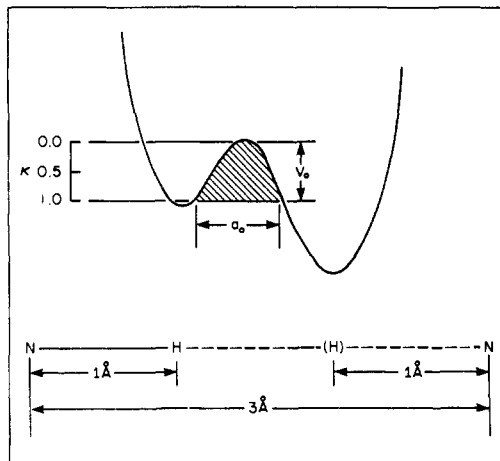
ever, intramolecular deuterium isotope effects on fluorescence quantum yields have been shown to be rare,[25,26] at least for aromatic compounds. In order to test this possibility in 7AI, we measured the quantum yields of F_1 in dilute 3MP solutions at room temperature as well as the phosphorescence/fluorescence ratios in ethanol at 77°K and found no measurable difference between the deuterated and nondeuterated compounds. Both of these results suggest the absence of an intramolecular deuterium isotope effect on the fluorescence yield of F_1. If a similar result applies to the tautomer, we can assume that the rate constants k_1, k_2, k_3, and k_4 in the kinetic scheme of the previous section are unaffected by deuterium substitution. This enables us to relate our observations directly to the ratio of proton tunneling rates for the deuterated and nondeuterated compounds.

From eq 7 we obtain

$$\frac{k^{\mathrm{h}}_{\mathrm{PT}}}{k^{\mathrm{d}}_{\mathrm{PT}}} = \left(\frac{F_2}{F_1}\right)^{\mathrm{h}}\left(\frac{F_1}{F_2}\right)^{\mathrm{d}}$$

where the superscripts refer to the deuterated (d) and nondeuterated (h) compounds. The quantities in brackets can be obtained directly from the spectra in Figure 10 to give the value of 2.9 for the ratio $k^{\mathrm{h}}_{\mathrm{PT}}/k^{\mathrm{d}}_{\mathrm{PT}}$ which must be a lower limit since the deuterated material contained about 9% of the normal form.

It is pertinent to ask whether a threefold reduction in the tunneling rate constant is compatible with current theories of proton tunneling. According to Löwdin,[27] the rate constant for tunneling of a particle of mass m through a double minimum potential barrier can be expressed as

$$k_{\mathrm{PT}} = \nu \exp\left[\frac{-\pi^2 a_0\kappa}{h}(2mV_0)^{1/2}\right] \qquad (10)$$

where ν is the "hit frequency," i.e., the number of times per second that the particle falls incident on the barrier. This equation assumes that the barrier can be described by an inverted parabola of height V_0 and width a_0 as shown in Figure 11. κ is the fraction of energy mea-

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(26) J. D. Laposa, E. C. Lim, and R. E. Kellogg, J. Chem. Phys., 42, 3025 (1965).
(27) P. O. Löwdin, Advan. Quantum Chem., 2, 213 (1965).

sured from the top of the barrier, also shown in Figure 11. Since at $77\,°K$ ($kT = 50\ cm^{-1}$) most of the protons will be near the bottom of the well, we will assume $\kappa = 1$.

Using eq 10 the following expression for the effect of deuterium substitution on proton tunneling can be obtained

$$\frac{k^h{}_{PT}}{k^d{}_{PT}} = \frac{\nu_h}{\nu_d}\exp[0.38a_0V_0{}^{1/2}(\mu_d{}^{1/2} - \mu_h{}^{1/2})] \qquad (11)$$

where a_0 is in Å, V_0 in cm^{-1}, and $\mu = m_1m_2/(m_1 + m_2)$ is the reduced mass in daltons. The latter has been included to reflect the fact that eq 10 applies to a single proton whereas our system involves a simultaneous transfer of two protons.

Since the protons and deuterons are attached to a large massive framework, the frequencies ν_h and ν_d should be inversely proportional to the square root of the reduced mass of the proton and deuteron pairs, respectively.[28] This gives $\nu_h/\nu_d = \sqrt{2}$. We can estimate a_0 to be about 0.5 Å from Figure 11 where the N-H and N-H$\cdots$N distances are assigned values typical of other hydrogen bonded base pairs.[29] Note that the right-hand potential minimum is much lower than that on the left reflecting the fact that the forward rate constant for double proton transfer far exceeds that for the reverse reaction as discussed in the previous section.

If we now use our estimate of $E_a = 1.4\ kcal/mol$ (500 cm^{-1}) for V_0, we calculate a value of 4.9 for the ratio $k^h{}_{PT}/k^d{}_{PT}$ which is larger than the experimental value of 2.9. However, considering the extreme sensitivity of the calculation to the values of the exponential parameters, the agreement is not bad. We do not wish to attach large significance to the values obtained. The main point we wish to make is that the magnitude of the observed deuterium isotope effect is reasonable based on current theories of proton tunneling.

Double Proton Transfer in DNA? There are a number of similarities between the 7-azaindole dimer and adenine-thymine (A-T) as well as guanine-cytosine (G-C) base pairs in DNA. All three complexes are joined by hydrogen bonds, the two N$\cdots$H-N hydrogen bonds in the 7AI dimer being formally identical with one of those in both the A-T and G-C pairs although the specific nitrogen atoms involved are not the same (Figure 12). The free energy of association of the 7AI dimer is greater than would be predicted simply on the basis of the number and type of hydrogen bonds involved, suggesting the presence of additional stabilizing forces. Similar conclusions[29] have been drawn for the complementary base pairs. Kyogoku, et al.,[30] observed that the free enthalpy of association of the adenine-uracil (A-U) pair in chloroform was about 50% greater than that for either base with itself, even though the number and type of hydrogen bonds were the same for all three complexes (A-U, A-A, and U-U). Similar results were reported[31] for the G-C pair. In view of the similarities between the DNA base pairs and the 7AI dimer,
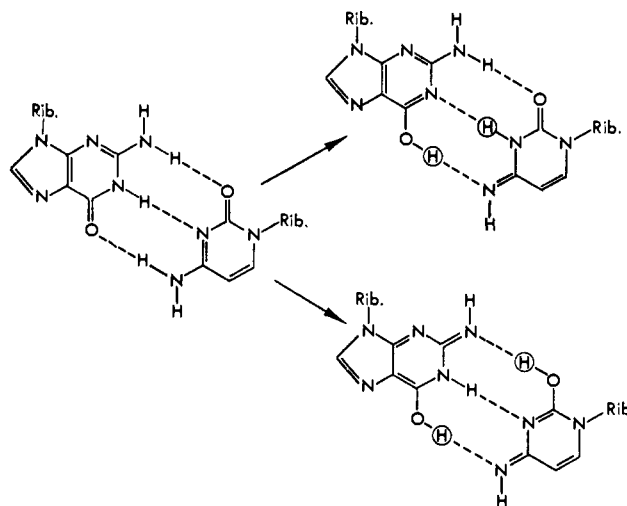

Figure 12. Photoinduced double proton transfer in guanine-cytosine DNA base pair tautomers resulting from the transfer of two different proton pairs is shown.

it is pertinent to consider the possibility of photoinduced double proton transfer in DNA.

Watson and Crick[32] in their original proposal of the double helix structure for DNA suggested the possible role of the rare tautomeric forms of the nucleotides in causing genetic mutations. Since the various tautomers have a different hydrogen bonding code than the corresponding normal forms, their presence could lead to errors in DNA replication. If even a minute fraction of the free cellular nucleotides existed in their rare tautomeric forms, the stability of the genetic code would be threatened. The fidelity of the genetic code suggests that either (a) the rare forms are extremely unstable under physiological conditions or (b) the enzymatic machinery responsible for DNA replication is endowed with the ability to select only the correct tautomeric forms.

It is well-known that when DNA is exposed to ultraviolet radiation, either in vivo or in vitro, significant photochemical damage is inflicted. The production of covalent thymine dimers is one example which is well documented.[33] Many cells are capable of repairing this type of damage through an excision process by which a series of enzymes remove the lesion and replace the damaged bases with normal ones. An alternative, more subtle type of damage would be the production of transient rare tautomers via an excited-state double proton transfer similar to that in the 7AI dimer. If such a reaction occurred and if the resulting tautomers survived sufficiently long, an error in replication could result. Since this type of transient photoproduct would not be expected to produce a lesion in the DNA molecule, it would probably not be subject to the repair mechanism.

The concept of double proton transfer in DNA as a mechanism of mutagenesis was put forth by Löwdin[34] who suggested that quantum mechanical tunneling of protons through a double minimum potential barrier could lead to tautomerization. He included the possibility that the shapes of the double-minimum potentials

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(29) D. Voet and A. Rich, Progr. Nucl. Acid Res. Mol. Biol., 10, 183 (1970).

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(32) J. D. Watson and F. H. C. Crick, Nature (London), 171, 964 (1953).

(33) R. B. Setlow, Progr. Nucl. Acid Res. Mol. Biol., 8, 257 (1968).

(34) P.-O. Löwdin, Advan. Quantum Chem., 2, 213 (1965).
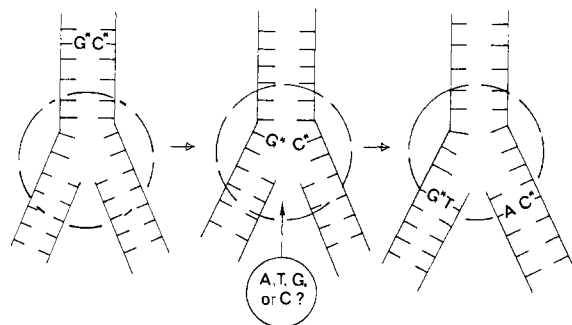
Figure 13. A schematic representation of a replicating double stranded DNA molecule and the possible insertion of incorrect bases as a result of photoinduced double proton transfer.

might be different in the excited state leading to increased tunneling probability. One may speculate about the way in which excited-state double proton transfer produces a genetic error. In Figure 13 we schematically depict a double stranded DNA molecule undergoing replication. Imagine that a particular base pair not too far from the replication fork suddenly absorbs a photon and undergoes excited-state double proton transfer to form the tautomers (indicated by asterisks). If these tautomers survive long enough to reach the point where the strands separate, the bases might be trapped in this form. Making the reasonable assumption that water is excluded at the interior of the enzyme complex, there would be no obvious mechanism for the protons to return to their original positions. Since the tautomers have a different hydrogen bonding specificity from the normal forms, an incorrect base pair could be inserted into the growing strand; mutation may thus be produced.

How long must a tautomer survive to produce an error in replication? This will depend on the replication rate and on the separation along the DNA strand between the point at which the tautomer is produced and the point where replication is taking place. Studies[35] with *E. coli* indicate that DNA replication

(35) J. Cairns, *J. Mol. Biol.*, **6**, 208 (1963).

occurs at a rate on the order of 10^3 nucleotides per second, suggesting that a tautomer lifetime of a few milliseconds might be sufficient for mutation production.

An obvious way to detect photoinduced double proton transfer in DNA would be to observe tautomer fluorescence as we have done with the 7AI system. However, the nucleic acids have an extremely weak fluorescence at room temperature[36] and most of the available luminescence data have been obtained at low temperatures (77°K). Unless the barrier were quite small as in the 7AI dimer, one would not expect to observe tautomer fluorescence at a low temperature. Furthermore, spectral studies of methyl-stabilized tautomers of adenosine,[37] uracil,[38] and cytidine[39] indicate that the nucleic acid tautomers have S_0-S_1 transition energies similar to those of the normal forms. Thus it would be difficult to detect a small amount of tautomer fluorescence superimposed on the background fluorescence of the normal forms, even though the difficulties of working with low fluorescence yields at room temperature might be overcome.

Daniels[40] recently reported the room temperature fluorescence of aqueous solutions of uracil and some of its methyl derivatives. He concluded that at least part of the weak room temperature fluorescence of uracil was due to the presence of uracil tautomers in solution. The possibility that the tautomers might have been formed in the excited state by a double proton transfer reaction with water was not considered.

Although to date there is no experimental evidence for photoinduced double proton transfer in DNA, the possibility is sufficiently interesting and important to warrant further investigation. The 7AI system is the only documented experimental observation of this reaction. The similarity of this system to the DNA base pairs should revive interest in the role of proton transfer in mutagenesis and aging.

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