diluted with 50 ml of water. The aqueous solution was washed with 10% sodium bicarbonate and water and dried over magnesium sulfate. Evaporation of the solvent gave 3-phenyl-Δ^3-cyclopentenylacetaldehyde as a colorless liquid. A 2,4-dinitrophenylhydrazone was prepared and recrystallized from ethanol to give an analytical sample: mp 132–133°.

Anal. Calcd for $C_{19}H_{18}N_4O_4$: C, 62.28; H, 4.95. Found: C, 62.54; H, 4.89.

The infrared spectrum has absorption bands at 3.45, 3.70, 5.78, 6.10, 13.31, and 14.40 μ. The mass spectrum shows the parent ion at *m/e* 186 and has the base peak at *m/e* 142. The 100-MHz nmr spectrum shows multiplets centered at τ 7.80 (4 H) and 7.30 (3 H), a triplet at τ 4.12 (1 H, J = 2.0 Hz), a multiplet at τ 2.90 (5 H), and a triplet at τ 0.48 (1 H, J = 1.5 Hz).

Quantum Yield Determinations. All quantitative measurements were made on a rotating assembly with a central light source (internal water-cooled mercury arc lamp, Hanovia Type L-450-W). Samples in 13-mm Pyrex ampoules were placed in holders on the assembly approximately 6 cm from the immersion well. The light was filtered by circulation of solution containing 46 g of nickel sulfate hexahydrate and 14 g of cobaltous sulfate heptahydrate in 100 ml of water through the inner jacket.[20] This solution permitted the following wavelength distribution to pass through: 6% 2967 Å, 20% 3025 Å, 62% 3130 Å, 10% 3340 Å. All studies were made at room temperature. Samples in 13-mm Pyrex test tubes were degassed to 5×10^{-3} mm in three freeze–thaw cycles and then sealed. Benzophenone–benzhydrol actinometry was used for quantum yield determinations.[22] Reliably reproducible output rates of 4.86×10^{16} quanta sec^{-1} were recorded. After the irradiation the degree of reaction was determined by quantitative vapor-phase chromatography. The conversions in the irradiations were run to 15% or less. The mass balance in these runs were generally better than 95%.

Quenching Studies. Benzoylbicyclo[2.1.1]hexane (90 mg) was dissolved in 5 ml of benzene. To each of five Pyrex tubes was added 1 ml of the above solution. To four of the tubes was added respectively 2 ml of a 0.05, 0.10, 0.15, and 0.20 M solution of piperylene in benzene. One tube without any piperylene was set aside as a control, and the four degassed, sealed tubes were placed in a turntable surrounding a 450-W Hanovia medium-pressure Hg lamp in a quartz immersion well. The turntable was rotated slowly and the tubes were irradiated for 10% conversion of starting material. At the end of this time, an internal standard (biphenyl) was added to each of the tubes, and the solvent was removed at reduced pressure at room temperature until the residual volume was about 0.1 ml. The mixture was analyzed by glpc on a 5 ft × 0.25 in. 5% Degs on 60–80 mesh Chromosorb W column at an oven temperature of 150° and a helium flow rate of 60 cc/min. The area under the peaks due to the standard (biphenyl) and products were measured using a planimeter after triangulation. Each set of chromatograms was measured twice in this way, and the results were converted to per cent reaction based on starting ketone.

Phosphorescence Emission Studies. The emission spectra were made on an Aminco-Bowman spectrophotofluorometer with a phosphoroscope and transmission attachments. The spectrophotofluorometer was equipped with a 1P21 photomultiplier and a high-pressure xenon lamp, as supplied by the manufacturer. All emission spectra were recorded using EPA (ethyl ether–isopentane–ethanol, 5:5:2 volume ratio) as solvent. The solvent was checked for emission each time a spectrum was recorded. No interference due to solvent was found at any time. All compounds having relatively long radiative lifetimes were recorded on a x–y plotter. Samples having short radiative lifetimes (<100 sec) were measured by photographing the decay curve on an oscillograph. The chopper was rotated manually to obtain the decay curve. The logarithmic intensities of the decay curve were plotted *vs.* time and the slope of the line at a logarithmic value of 2.303 gave the mean lifetime (τ_0).

Acknowledgment. We wish to thank the National Science Foundation (Grant GP-24449) for financial support.

Nature of the Reactive Species in the Photohydration of Uracil and Cytosine Derivatives

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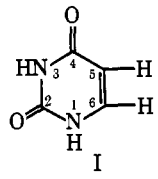
Abstract: The photohydration rates as functions of pH of 3-methyluracil, 5-fluorouracil, cytidine, deoxycytidine, uridine 2′-phosphate, and uridine 5′-phosphate are presented and discussed; the functions are sigmoidal and the inflection points of the curves are interpreted to reflect the pK values of the singlet excited molecules. From these curves, it is suggested that in general for uracil and cytosine derivatives, the neutral excited molecule is the species which reacts fastest with water; cationic and anionic species react more slowly. The curves for uracils substituted in the 3 position or unsubstituted in the 1 and 3 position reflect the pK for loss of the 1 proton (pK = 4–5); those for 1-substituted uracils reflect the pK for loss of the 3 proton (pK = 6–7). These values agree fairly well with the pK* values calculated for the singlet excited states of uracil and thymine, and suggest strongly that the reactive state for photohydration does not resemble the ground state electronically, but does resemble a singlet excited state. This evidence is supported by the observation of negative temperature coefficients for photohydration, analogous to the negative temperature coefficients observed for fluorescence.

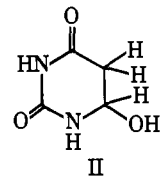
Photolysis of uracil (I) and uracils substituted in the 1 and 3 positions converts them into mixtures of cyclobutane type photodimers and 5,6-dihydro-6-hydroxyuracil derivatives (II) "photohydrates."[2] The ratio of photohydrate to dimer obtained depends upon the nature of the uracil derivative and the photolysis conditions.[2] Cytosine and thymine and their derivatives form analogous photoproducts. These types of photoproducts are known to have important roles in the inactivation and mutation of living organisms and viruses by ultraviolet light.[2] Information about the charge on the reactive species and about the pK of the excited species may be gained by examining the

(1) University of Oklahoma.

(2) (a) J. G. Burr, *Advan. Photochem.* 6, 193 (1968), and general references cited therein; (b) K. C. Smith and P. C. Hanawalt, "Molecular Photobiology," Academic Press, New York, N. Y., 1969.



I



II

rate of these photolyses as functions of the pH of the solution. Earlier papers from this laboratory[3,4a] and from others[4b,5] have shown that the rate of photohydration of uracil is a sigmoid function of the pH of the solution. We have also shown[2,3] that the rate of this reaction is independent of ground state UH^+ concentration, exhibits no neutral salt effects, and is first order in water concentration (in acetonitrile–water mixtures). The photohydration rates of 1-ethyluracil, 1-cyclohexyluracil, and uridine were similar functions of the pH of the solutions, although the dependencies were less well marked than that of uracil.

As a result of these earlier preliminary studies,[3,4a] we concluded that the most reactive uracil species in the photohydration reaction was the protonated singlet excited state. We report in this paper additional studies of the variation of photohydration rates with pH for 3-methyluracil, 5-fluorouracil, cytidine, deoxycytidine, uridine 2′-monophosphate (UMP-2), and uridine 5′-monophosphate (UMP-5′), the results of which considerably modify the earlier conclusion.

Experimental Section

Materials. Cytidine, uridine, and the uridine phosphates were from CalBiochem. Corp. 5-Fluorouracil was from Columbia Organic Chemicals, Inc. 3-Methyluracil was from Cyclo Chemical Co. Water was double distilled.

Photolysis Procedures. The procedures for photolysis of 5-fluorouracil, cytidine, deoxycytidine, and the uridine phosphates in aqueous solution were similar to those reported previously for the photolysis of uracil derivatives[3] using a 1000-W GE BH6 mercury arc, a Bausch and Lomb high intensity monochrometer, 1-cm quartz cuvettes, and a photolyzing wavelength of 270 nm. The photolysis source for 3-methyluracil was 1–4 15-W GE germicidal lamps, held in a circular array of sockets, and the photolyzing wavelength was that of the resonance line of mercury, 253.7 nm. The photolysis cells used in the 1000-W source were standard 1-cm path length quartz cuvettes contained in a thermostated holder whose temperature was controlled by a refrigerated circulating system. The temperature dependence of photohydrate formation in uracil and cytidine was studied in this system since temperature was controlled and easily varied. The solutions in these cells were stirred magnetically and saturated with O_2 through a rubber septum prior to beginning the photolysis. The cells used for photolyses in the germicidal lamp sources were jacketed Vycor cylinders, closed at one end, containing 30 ml of solution. These solutions were stirred by a stream of gas introduced at the bottom of the cell. The cell was held parallel to and 20-mm distant from each of the lamps in the semicircular array of lamps. Except where otherwise noted, all the photolyses reported here were run in oxygen-saturated solution at 5 or 25°.

A standard solution of 1,3-dimethyluracil (DMU), 10^{-4} M in double distilled water, was used to measure the amount of light absorbed by these solutions. The volume of DMU solution, the cell, and the position of the cell, were always the same for the photolysis of the actinometer as for the photolysis of the target pyrimidine. The λ_{max} and the extinction coefficient for DMU are very similar to those of the other pyrimidines investigated. We have previously measured[3a] the quantum yield of this DMU actinometer as $3.86 \pm 0.07 \times 10^{-3}$ over the concentration range 1×10^{-4}–1×10^{-3} M.[6] The intensity of the light beam was monitored at the beginning and end of each run, using a standard 15-min exposure of the solution; these values are referred to as ΔA_{15}^{DMU} values. The data obtained from this actinometry were used to calculate the amount of light absorbed by the actinometer solution, I_a, assuming a quantum yield of 3.86×10^{-3} for DMU disappearance. This value of I_a was used to calculate the quantum yield of photohydrate formation for the other pyrimidines, relative to that of DMU photohydration.

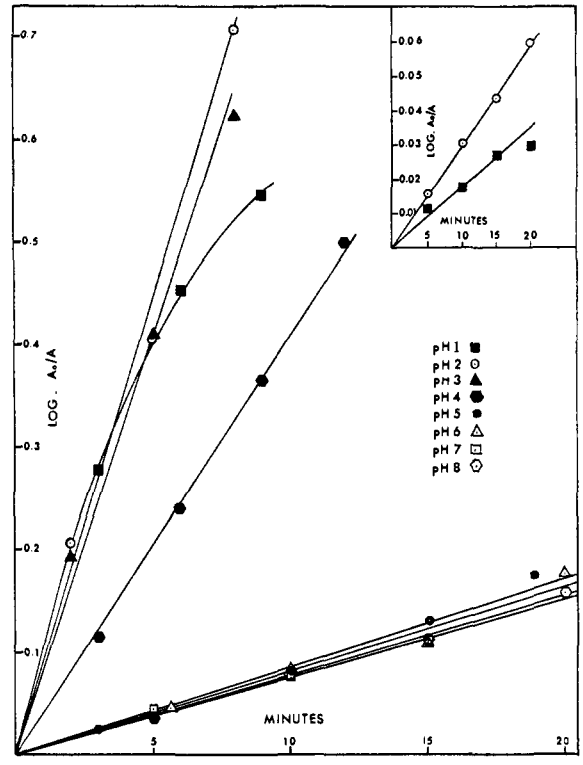


Figure 1. Photohydration rates for 3-methyluracil, 10^{-4} M in oxygen-saturated water solution at 5°.

The fraction of photohydrate formed in the photolysis products was estimated as before[3,4a] by the comparison of absorbances before photolysis; after photolysis; and after photolysis, acidification, and heating.[2] The gross rates of pyrimidine disappearance, from ΔA measurements, were multiplied by the fraction of photohydrate formed to obtain the rates of photohydrate formation. Thin layer chromatography showed that in most cases a number of minor photolysis products were also formed, but these were all formed in only trace amounts. The fraction of photohydrate formed was usually well over 90%, and never less than 85%. None of these minor photoproducts absorbed in the region 250–300 nm since the result

(3) (a) J. G. Burr, B. R. Gordon, and E. H. Park, *Advan. Chem. Ser.*, No. 81, 418 (1968); (b) J. G. Burr and E. H. Park, *ibid.*, 81, 435 (1968).

(4) (a) J. G. Burr and E. H. Park, *Photochem. Photobiol.*, 8, 73 (1968); (b) I. H. Brown and H. E. Johns, *ibid.*, 8, 273 (1968).

(5) S. Y. Wang and J. C. Nnadi, *Chem. Commun.*, 1160 (1968).

(6) We have since made multiple determinations of this value using both uranyl oxalate and ferrioxalate actinometers, both at 265–270 nm with the high intensity lamp and monochromator combination, and at 254 nm with the mercury resonance lamp apparatus (the value obtained here was corrected for the 10% smaller absorbance of DMU at 254 nm relative to that at the absorption maximum). The range of values thus observed was 3.75×10^{-3}–4.4×10^{-3}. These seemed in reasonable agreement with the value reported by Wang[7] of 4.63×10^{-3} for 10^{-4} M DMU, although somewhat lower than the early value of Moore and Thompson[8] of 10.4×10^{-3}. However, Johns[9] has recently reported a value of 14.0×10^{-3} for 10^{-3} M DMU, and we note that the values reported by Wang[7] for DMU solutions which were 0.5–5.0×10^{-3} M were about 12×10^{-3}. Although the DMU actinometer is quite suitable for the measurement of relative quantum yields for different pyrimidines, as used here, the quantum yield of DMU photohydration does not seem well enough known to make this process a suitable secondary actinometer for the measurement of absolute quantum yields. The data in this paper are therefore reported simply in terms of quantum yields relative to that of DMU photohydration, *i.e.*, ϕ/ϕ_{DMU}.

(7) S. Y. Wang, *Photochem. Photobiol.*, 1, 135 (1962).

(8) A. M. Moore and C. H. Thompson, *Progr. Radiobiol.*, 4, 75 (1956).

(9) H. E. Johns in "Creation and Detection of the Excited State," A. A. Lamola, Ed., Marcel-Dekker, New York, N. Y., 1971.

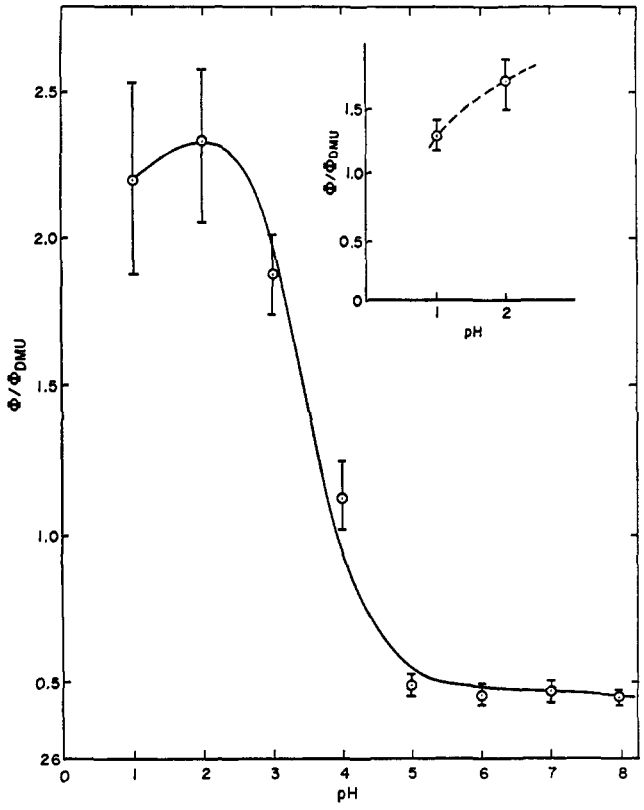


Figure 2. Quantum yields for photohydration of 3-methyluracil as functions of the pH, 10^{-4} M in oxygen-saturated water solution at 5°.

of exhaustive photolysis was complete disappearance of the pyrimidine peaks at 255–275 nm with no new peaks appearing in this region.

Presentation of Data. At the concentrations of pyrimidine, the temperatures, and the light intensities used in these experiments, the reactions were usually observed to be first order, *i.e.*, log (A_0/A) was a linear function of the time of exposure (= dose). This means that the value of the exponential in eq 1 was small enough that the

$$dC/dt = \phi I_a = \phi I_0[1 - \exp(-2.303\epsilon Cl)] \quad (1)$$

lower limiting form, eq 2, was actually applicable; I_a = rate of light

$$dC/dt = 2.303\phi I_0 \epsilon Cl = K'C \quad (2)$$

absorption in units compatible with dC/dt, and ϵ = molar decadic extinction coefficient. The absorption curves of the several pyrimidine derivatives discussed in this paper are slightly different from that of DMU. Differences in light absorption by the pyrimidine relative to that of the DMU actinometer were estimated from the relative extinction coefficients of the pyrimidine and DMU at the photolysis wavelength. Since the absorption curves of these pyrimidines are all broad peaks with half-widths typically about 30 nm, the corrections involved were very small.

The data which are important for the discussion in this paper are the variations in relative quantum yields of photohydration with variations in the pH of the solution, *i.e.*, the sigmoidal shapes of such curves and the pH value at the midpoint of these curves.

An example of the raw data for calculation of photohydrate yields in 3-methyl uracil is shown in Figure 1. Five such runs were made, and the average quantum yields calculated from these data are shown in Figure 2. It is evident from the data in Figure 1, that the plot of log (A_0/A) shows a curvature at pH values of 1 and 2. This curvature is caused by the hydrogen ion catalyzed thermal conversion of photohydrate back to 3-methyluracil, according to eq 3, and the reaction scheme

$$Py \xrightarrow{h\nu} Py^*$$

$$Py^* \xrightarrow{K_1} Py$$

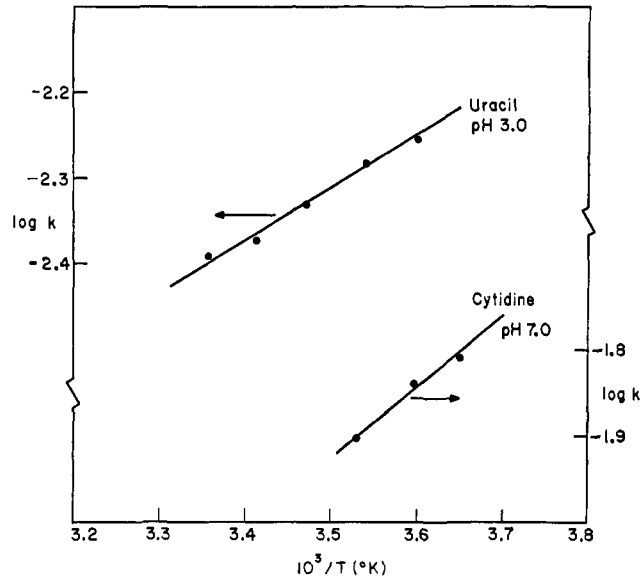


Figure 3. Rate constants (I_a = 5.36 × 10^{20} quanta $l.^{-1}$ min^{-1}) for photohydration of uracil and cytidine in oxygen-saturated water solution as functions of the temperature.

$$Py^* + H_2O \xrightarrow{K_2} H$$

$$H \xrightarrow{K_3} Py + H_2O$$

$$dH/dt = \frac{2.3K_2W\epsilon I_0\phi_e}{K_2W + K_1}(Py) - K_3(H) \quad (3)$$

where H = photohydrate, Py = pyrimidine, W = water, and ϕ_e = quantum yield for excitation. At low concentrations of photohydrate, the formation of photohydrate is first order, and $dH/dt = -dPy/dt$. If the concentration of photohydrate is higher, and if $K_3(H)$ approaches $K_2(Py^*)(H_2O)$ in magnitude, then $dH/dt \neq -dPy/dt$ and log (A_0/A) will deviate from a linear relationship with dose (the initially straight lines will begin to bend over). The absence of such curvature in the other lines in Figure 1 is evidence that thermal photohydrate reversal is not taking place, and that the yield of photohydrate can be measured by the slopes of such lines. However, the presence of such curvature at pH 1 and 2 demonstrates that the rate of thermal reversal was competing with the rate of photohydrate formation, and that the yield of photohydrate is only given by the initial slope of the line. Such slopes are hard to estimate; quantum yields calculated from the intial slopes of five runs similar to that illustrated in Figure 3 are shown in Figure 2. Although the error limits were very large, there was an indication that the yield at pH 1 was less than that at pH 2. Since the rate of the thermal reversal reaction is simply a function of the photohydrate concentration (at constant temperature and pH), the error in the measurements at pH 1 and pH 2 was reduced by carrying out several runs at a much lower light intensity.[10] This lower intensity, obtained by using only one resonance lamp instead of four, corresponded to ΔA_{15}^{DMU} ~0.07 compared to the ΔA_{15}^{DMU} ~0.35 for the four-lamp system. For photolyses in the low intensity source, plots of log (A_0/A) were linear with time; two such runs are shown in the inset in Figure 3. Quantum yields calculated from two such sets of runs are shown in Figure 2, and it can be seen that the photohydrate yield at pH 1 is indeed lower than the yield at pH 2. The quantum yield appears to be somewhat lower at the lower light intensity, but this may simply reflect the difficulty of making quantum yield measurements at such low pyrimidine conversions. This apparent intensity effect is being investigated separately.

(10) It was suggested by a referee that a higher light intensity should have been used so that the forward reaction would become much larger than the backward reaction; the objection to this procedure is that the reaction is then so fast that at any practical stopping point, the conversion of uracil into photohydrate is already large, the thermal reversal reaction is quite fast and the time between cessation of irradiation and measurement becomes important. Furthermore the forward reaction is then so fast that the linearity of log (A_0/A) *vs.* time plots cannot be tested at low yield of photohydrate.

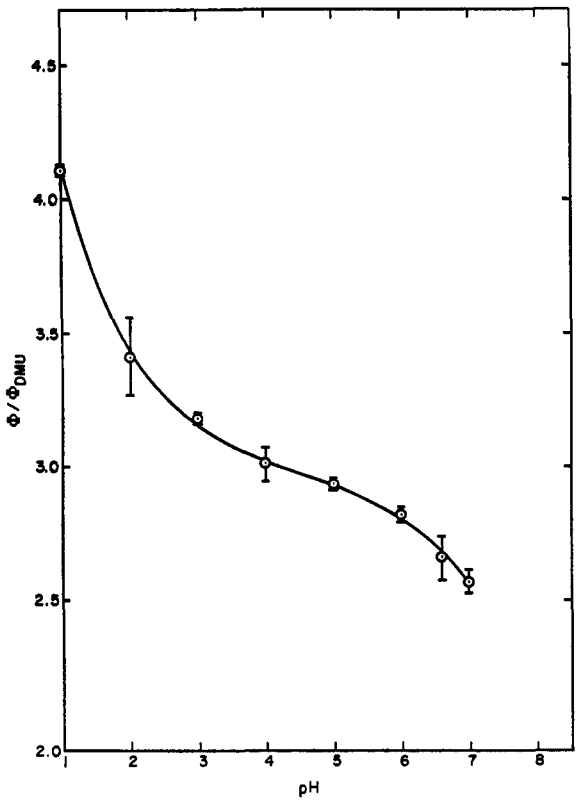


Figure 4. Quantum yields for formation of 5-fluorouracil photohydrate as a function of pH, 10^{-4} M in oxygen-saturated water at 5°.

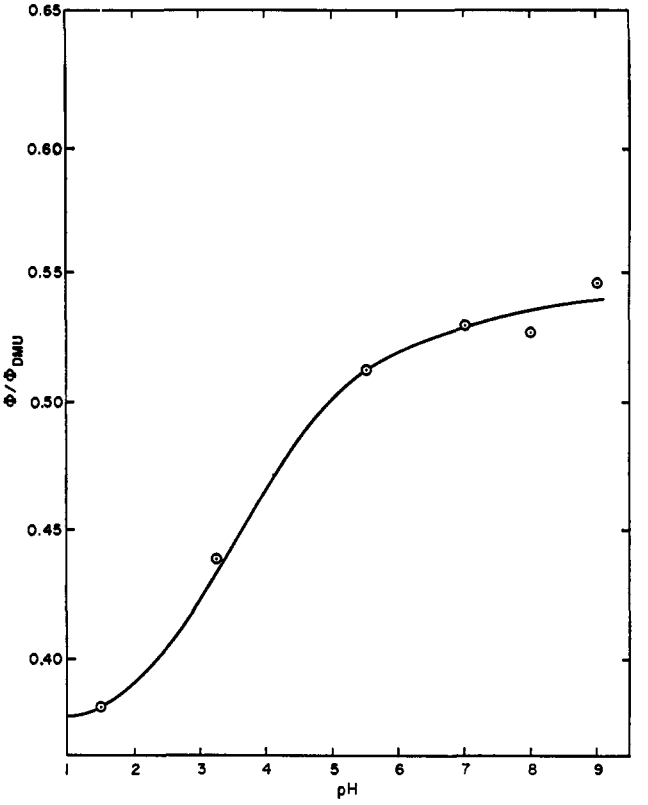


Figure 5. Quantum yields for photohydration of cytidine as functions of the pH, 10^{-4} M in oxygen-saturated water solution at 5°.

Results

Photohydration quantum yields for 5-fluorouracil are shown in Figure 4, relative to the DMU photohydration yield. We did not try to measure the yields of photohydrate at pH values above 7 since the large shift in the absorption maximum of the uracil at high pH values made the rate of photolysis in our resonance lamp apparatus very slow. The values shown are the averages of two runs and the error shown is the average deviation. Relative photohydration quantum yields for photolysis of 3-methyluracil are shown in Figure 2; each data point is the average of three runs, and the deviations shown are average deviations. Relative photohydration quantum yields for cytidine are shown in Figure 5; the curve for deoxycytidine was very similar except that the quantum yields were uniformly somewhat lower. Relative photohydration yields for the uridylic acids are shown in Figure 6, and previously obtained data for uracil photohydration,[3a] converted from rate constants to relative quantum yields, are included for purposes of comparison. The shape of our curve for uracil photohydration as a function of pH has been confirmed by others.[4b,5] Wang and Nnadi[5] have also studied the pH dependence of 3-methyluracil photohydration. Our values differ somewhat from theirs, but the trends are roughly the same; our studies cover a larger pH range and at the temperature (5°) which we used, the photohydrate was stable enough at the lowest pH values to enable accurate measurement of the photohydrate quantum yield. Temperature dependencies of the rate constants (I_a = 5.23 × 10^{20} quanta $l.^{-1}$ min^{-1}) are shown in Figure 3.

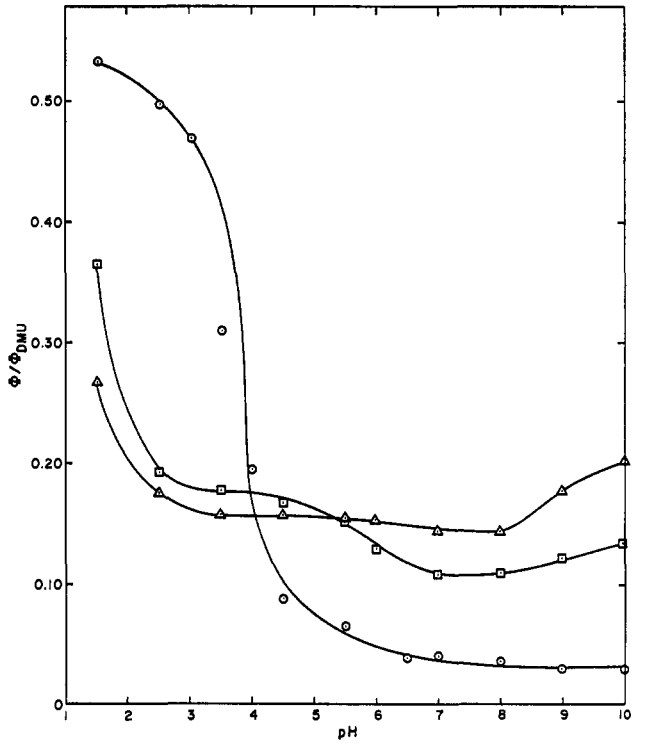


Figure 6. Quantum yields for photohydration of several uracil derivatives as functions of the pH, 10^{-4} M in oxygen-saturated water at 5°; uracil, ⊙; uridine 5′-phosphate, ⊡; uridine 2′-phosphate, △.

Discussion

Photohydrate formation from uracil and related pyrimidine derivatives has been tacitly assumed to be

a reaction of the singlet state,[2] which, from absorption and emission[11] spectra, is assumed to be the π,π^* singlet rather than any of the several n,π^* singlets. The evidence for this is principally negative, *i.e.*, it is evidence that the triplet state is not the precursor. For example, the photohydration reactions are not quenched by oxygen[2,3a,4a,12] or other triplet quenchers, and cannot be sensitized by known triplet donors.[2,4b,12] However, although transients identified as triplet excited uracil molecules have been observed in flash photolysis[13b] and pulse radiolysis[13a] of uracil, no other transients of an excited molecule, radical, or radical-ion nature have been observed.[14]

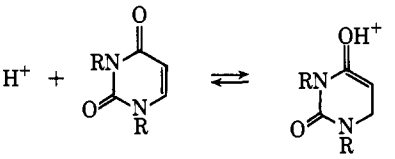
Whitten, *et al.*,[15] report that the quenching of 1,3-dimethyluracil fluorescence by nucleophiles, including water, does not appear to have a good correlation with the rate of the photochemical addition of such nucleophiles to the pyrimidine. A vibrationally excited ground state has been suggested as the reactive intermediate.[5]

Positive evidence for the nature of the state of these pyrimidines which is the precursor of photohydrate formation would clearly be helpful. We report here two observations of aspects of photohydration which bear clear analogy to similar aspects of fluorescence, a known reaction of excited singlet state molecules. The first of these aspects is that the photohydration reactions of uracil (at pH 3) and cytidine (at pH 7) exhibit negative temperature coefficients, Figure 3. These experiments were carried out because photohydration, intersystem crossing to the lowest triplet state, and radiationless decay to the ground state appear to be the only observable reactions of the excited singlet state of these molecules, since they fluoresce very weakly[11] or not at all in either liquid or condensed phase.[16] The negative temperature coefficient of photohydration then resembles the well-known negative temperature coefficient sometimes observed for fluorescence,[17-19] and probably has a similar cause, *i.e.*, the positive temperature coefficients of the radiationless processes which depopulate the excited singlet and possibly of the singlet–triplet intersystem crossing efficiency.[19]

The second observation is one which has been noted by many observers,[2] but to which we have paid particular attention,[3,4a] namely, that the photohydration quantum yield for many pyrimidines is sensitive to pH. This must mean that the charge or lack of charge on an excited pyrimidine molecule affects the rate of the photohydration process; neutral excited pyrimidines and charged excited pyrimidines appear to photohydrate at different rates. Immediate identification of the nature of the charged species from observation of the photohydration yield *vs.* pH curves is difficult because the pK values for proton loss or gain can be different in the excited states than for the ground states,[17-20] but a comparison of these curves with the known ground state pK values can help in characterizing the reactive state as like or unlike the ground state.

On the basis of rate *vs.* pH curves for uracil, 1-ethyluracil, 1-cyclohexyluracil, and uridine, it was suggested earlier[3,4a] that the protonated excited uracil molecule reacted faster with water than did the neutral excited uracil. The measurements reported in this paper, together with some additional data, and some reconsideration of earlier data have persuaded us to come to a different point of view about the nature of the reactive species in the two pH ranges, 1.5–4.5 and 4.5–8.

In the first place, the corresponding inflection points in the rate *vs.* pH curves for photohydration of 1-ethyluracil[2a] and 1-cyclohexyluracil[3a] came respectively at pH values of 6.5 and 7.5. The differences in these inflection points presumably reflected differences in the pK values for protonation of the substituted uracils, eq 4. However, the pK value for protonation of 1-ethyluracil has been recently reported[21] as 0.45, and that for 1,3-dimethyluracil[21] as 0.81. These are little different from the reported[22] pK value for protonation of uracil, 0.5. The presence of electron-donor alkyl groups on one or both of the two nitrogen atoms has very little effect on the pK value for protonation of the carbonyl oxygen atoms, and, even though these values are for ground state pyrimidines and not excited state pyrimidines, it still becomes hard to attribute the large change in the inflection points of the photohydration



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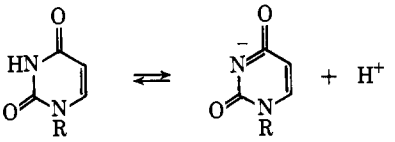

(5)

rate *vs.* pH curves to a change in the pK values for protonation of the excited states, since the absorption spectra of the three substances are so similar.

Secondly, the pK value for protonation of dimethyluracil is little different than that of uracil, yet the photohydration of uracil is very pH sensitive and that of dimethyluracil is independent of pH. This difference in the pH dependence of photohydration between these two compounds is more consistent with a proton loss process,[22b] eq 5, as the important one in the pH range 1.5–8.0, even though the pK for proton loss from

(11) M. Daniels and W. Hauswirth, *Science*, 171, 675 (1971).
(12) C. L. Greenstock, I. H. Brown, J. W. Hunt, and H. E. Johns, *Biochem. Biophys. Res. Commun.*, 27, 431 (1967).
(13) (a) E. Hayon, *J. Amer. Chem. Soc.*, 91, 5397 (1969); (b) D. W. Whillans, M. A. Herbert, J. W. Hunt, and H. E. Johns, *Biochem. Biophys. Res. Commun.*, 36, 912 (1969). The transients reported by each of these two groups appear to have different spectra; that reported by Whillans, *et al.*, having λ_{max} at 380 nm, and that reported by Hayon having λ_{max} at 289 nm.
(14) E. Hayon, personal communication; *cf.* also R. M. Danziger, E. Hayon, and M. E. Langmuir, *J. Phys. Chem.*, 72, 3842 (1968).
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Table I. Absorption and Emission Spectra of Uracil and Thymine Derivatives[a,b]

Compound	T	T^-	1-MT	1-MT^-	3-MT	3-MT^-	U	U^-	1-MU	1-MU^-	3-MU	3-MU^-
Absorption	37.5	34.5	36.2 (77)	37.2		34.4	38.5	35.1	37.3	37.6	40.0	35.2
Emission	29.8	26.0		29.7 (77)		26.0	32.1					

[a] Spectra measured in $cm^{-1} \times 10^{-3}$; temperatures are 300°K unless indicated otherwise in parentheses. [b] These data are taken from references 11, 20, 23, 24.

uracil is known to be 9.5 in the ground state. It seems likely that the predominant excited species in the pH range 1.5–4.5 is the neutral excited uracil molecule, and the predominant species in the pH range 4.5–8.0 is the excited uracil anion, and that the pK value for proton loss in uracil is shifted down from 9.5 in the ground state to 4.5 in the excited state. The insensitivity of dimethyluracil photohydration to pH is caused by the impossibility of proton loss process in this pH range.

From this point of view, the photohydration yield *vs.* pH curves for the 1-substituted uracils (1-ethyluracil, 1-cyclohexyluracil, and uridine) which exhibit inflection points at pH values of 6–7 suggest that the pK value for loss of the 3 proton from the excited molecule is about 6–7. The analogous curve for 3-methyluracil, Figure 4, has an inflection point at about pH 3.5; this curve suggests that the pK value for loss of the 1 proton is about 3.5. The analogous curves for uracil[3,4a] and 5-fluorouracil, Figure 3, have inflection points at low pH values; this suggests that the proton which is lost from the excited state of these pyrimidines, unsubstituted at either nitrogen, is mostly the 1 proton. Ionization of ground state uracil occurs about equally at the 1 position and the 3 position;[23c] thymine ionizes similarly in the ground state.[24] The pK values thus estimated for the excited singlet states of the substituted uracils can be compared with those obtained according to the expression proposed by Weller[20]

$$pK^* = pK - 0.625/T(\Delta\nu)$$

where pK^* = pK of the excited singlet state (for loss of the N-1 or N-3 proton), pK = pK for loss of the N-1 or N-3 protons in the ground state, T = absolute temperature, and $\Delta\nu$ = the difference in the frequency of the absorption or emission maximum between the un-ionized base (ground state or excited singlet state) and the ionized base and is usually taken as the arithmetic average of the shift in the fluorescence maxima and the shift in the absorption maxima. From the fluorescence spectrum of thymine at 300°K (emission max = 28,800 cm^{-1}) and of thymine monoanion[23a] (emission max = 26,800), and the corresponding shift in absorption maxima (3000 cm^{-1}), pK^* = 10.0 − 6.8 = 3.2. The absorption maximum of uracil (38,400 cm^{-1}), uracil anion (35,000), uracil fluorescence maximum (32,500 cm^{-1}),[11] and pK = 9.51 are known but the emission spectrum of the anion is not. However, it is probable that the pK^* of excited singlet uracil is about the same as that of excited singlet thymine, or about 3. Similar incomplete data for the N-1 and N-3 methylated thymines and uracils are shown in Table I. These data suggest that the values of pK^*, about 3–4, for the excited singlets of 3-methylthymine and 3-methyluracil, similar to those of uracil and thymine, represent the pK value for ionization of the N-1 proton from these molecules. The pK^* values for 1-methylthymine and 1-methyluracil are probably very close to the pK values for the ground state and suggest that the pK^* for ionization of the N-3 proton is only slightly less than the value of pK in the ground state. This is supported by the observation[25] that the pK^* for ionization of N-3 in uridine monophosphate is about 9.0, compared to 9.5 in the ground state.

The small discrepancies between pK^* values for loss of N-1 and N-3 protons calculated from the photohydration data and those calculated from absorbance or fluorescence data may reflect an incomplete equilibrium between the excited molecules and the protons in the environment.

Another aspect of this problem is seen in the curve for the photohydration yield *vs.* pH for cytidine, Figure 2. The most noteworthy aspect of this curve is that the photohydration of cytidine is slower at lower pH values than at higher pH values. Similarly shaped curves have been reported for cytidylic acid[26] and can be inferred for other cytosine derivatives.[2] In all of these cases the midpoints of the curves are at about pH 4, near the known pK for protonation of cytosine derivatives,[22] 4.1. A proton-loss process, similar to that shown in eq 5 for uracil, of course, is not probable in cytidine. The shape of this curve suggests that protonated cytidine photohydrates more slowly than does neutral cytidine, and that the pK value for protonation of the excited state is about the same as that of the ground state. Johns[26] has suggested that the reason for the slow photohydration of protonated cytidylic acid is that the protonated form exists in a tautomeric form in which the 5,6 double bond is absent. *Apparently the photohydration yields are low for both anionic and cationic forms of excited uracil and cytidine derivatives, and for both types of pyrimidines it is the neutral excited molecule which reacts fastest with water.*

Comparison of the pH dependences of photohydration for uridylic acids and cytidylic acids presents a problem. For uridylic acids, Figure 5, the steep drop in the photohydration yield between pH 1 and 2 apparently reflects loss of the first phosphoric acid proton (ground state pK = 1.6) and suggests that generation of a negative charge anywhere on the molecule retards the rate of photohydration. In contrast to this, the pH dependence of photohydration of cytidylic acid is very

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similar to that of cytidine over the pH range of 2–6, despite the negative charge on the cytidylic acid molecule over this pH range. The reason for this difference is not immediately apparent.

These observations can be summed up by stating the pH dependencies observed for photohydration of the uracil and cytosine derivatives agree in considerable detail with the behavior which might be expected for singlet excited molecules of these substances. Furthermore, the temperature dependence of photohydration bears an excellent analogy with that of fluorescence. We conclude that these observations constitute positive evidence that a singlet excited state is the precursor for photohydration.

It is not clear that this singlet state is the fluorescent state observed by Daniels and Hauswirth[11] since the lifetime of fluorescent state, for both uracil and thymine, was reported to be about 10^{-12} sec and not very many bimolecular collisions can take place during such a short time. It seems clear from the pH and temperature dependence studies reported here, from the dependence of photohydration yield upon water concentration in acetonitrile–water mixtures,[3b] and from the recent observation[27] that the quantum yield of 1,3-dimethyluracil photohydrate is independent of the viscosity of the medium (in glycerol–water mixtures) that the reactive state which leads to the photohydrate is long enough lived to be well aware of its bulk environment although it may not be in complete equilibrium with this environment.

We think that the data in this paper make a "hot" ground state[5] unlikely as the reactive state, but there are possibly other reactive states for photohydration which are not the fluorescent singlet states: (1) a hidden n,π^* singlet;[28] (2) a tautomeric form of the fluorescent singlet state; (3) singlet excited water–pyrimidine complex reached by vertical excitation of a ground state water–pyrimidine complex, *i.e.*, the photohydration reaction might then be simply a rearrangement of the excited water–pyrimidine complex. With this last in mind, we are presently investigating the nature of the water complexes of uracil and substituted uracils.

Acknowledgment. We would like to acknowledge helpful conversations with R. A. Holroyd, Brookhaven National Laboratory, and fruitful suggestions from several referees. This work was supported, in part, by Grant CA 11418 from the National Cancer Institute.

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Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs. Quaternized Bisnicotinamides[1-3]

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Abstract: Intramolecular interactions of quaternized nicotinamide groups have been studied in a series of compounds in which these two groups are held in close proximity by a polymethylene chain of length n linking the ring nitrogen atoms ($Nic^+-C_n-Nic^+\ 2Cl^-$, n = 2–6). *Hyperchromic* intramolecular perturbations of the 264-nm ($37.9 \times 10^3\ cm^{-1}$) ultraviolet bands of the Nic^+ groups were evaluated by comparing their oscillator strengths to that of 3-carbamoyl-1-propylpyridinium chloride ($Nic^+-C_3\ Cl^-$), a model for the isolated chromophore. A strong hyperchromic interaction (14%) was found for n = 2. The interaction decreased rapidly as the polymethylene chain was lengthened. Essentially the same hyperchromism values were obtained for a comparison series in which one nicotinamide group was replaced with a trimethylammonium group ($Nic^+-C_n-NMe_3^+\ 2Cl^-$, n = 2–4). In the $Nic^+-C_n-Nic^+\ 2Cl^-$ series, the hyperchromisms appeared to be due primarily to perturbations of the 264-nm transitions of the Nic^+ groups by the neighboring positively charged nitrogen atoms (either Nic^+ or NMe_3^+) rather than to intramolecular interactions of the electronic transition dipoles of the Nic^+ groups. The effects upon hyperchromism of changing the pH, the solvent, and the anions of the models are also discussed, and the study was extended to compounds in the $Nic^+-C_n-COO^-$ series (n = 2, 3).

Ultraviolet spectra of nucleic acids and other polynucleotides exhibit strikingly lower absorption intensities when compared with the constituent mononucleotides or to the same polymers in denatured form.[4-6] This loss of ultraviolet absorption intensity at a given wavelength is termed *hypochromicity*, while the total loss in intensity over the entire absorption band is called *hypochromism*. Significant hypochromic ef-

(1) The present paper is No. X in the series on Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs.

(2) For preceding papers (IX, VIII) in this series, see: (a) M. W. Logue and N. J. Leonard, *J. Amer. Chem. Soc.*, **94**, 2842 (1972); (b) J. A. Secrist III and N. J. Leonard, *ibid.*, **94**, 1702 (1972).

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