implemented on our laboratory computer using a general scientific/engineering analysis/data acquisition program called ASYST. Signal averaging 100 experiments, each with laser pump energies ≤5 mJ gave 8192 point decay curves, each of which produced pseudo-first-order rate constants with correlation coefficients (square root) better than 0.99.

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Supplementary Material Available: The data used to generate Figures 1 and 2 and time evolution data and plots for the photooxidation of **1b** in acetone-d_6 at −29 °C, in $CDCl_3$ at −60 °C, and in CD_3OD at −60 °C (12 pages). Ordering information is given on any current masthead page.

Photochemistry and Photophysics of Purine Free Base and 6-Methylpurine

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Abstract: A comprehensive study of the photochemistry and photophysics of purine free base and 6-methylpurine has been carried out. The presence of the purine triplet state was determined by using the energy-transfer technique for sensitizing the crocetin triplet. The purine triplet quenching rate coefficients have been determined to be 7.1×10^9, 3.4×10^9, and 2.0×10^8 M^{-1} s^{-1} for crocetin (C), O_2, and Mn^{2+}, respectively. The decay time of the triplet under deoxygenated conditions and the triplet–triplet (T–T) molar absorption coefficient at 390 nm are, respectively, 1.7 μs and $(2.0 \pm 0.5) \times 10^3$ M^{-1} cm^{-1}. The intersystem crossing rate constant obtained from picosecond laser experiments, 1.4×10^8 s^{-1}, allowed an estimate of the singlet excited state (S_1) decay time, 7 ns, in favorable agreement with the value of the fluorescence decay time, 5 ns, obtained from single photon counting experiments. Photoionization of the purines was observed at an excitation energy of 4.7 eV, which is lower than their gas-phase photoionization energy. The purine radical cation was produced by oxidation of the purine by radiolytically generated azide radical. Its absorption maximum appears at 290 nm. The purine anion radical, which was generated from the radiolytically produced carbon dioxide radical anion, had an absorption band maximum and decay time of 275 nm and 16 μs, respectively. An intermediate species resulting from a fast reaction of the radical anion is postulated to account for the formation of the band at 320 nm. The electron scavenging rate coefficient for purine is 2.9×10^{10} M^{-1} s^{-1}.

Excellent reports describing the physical properties of the electronic states of purine bases are readily available.[1-8] However, the reactivity and dynamics of the electronically excited purines, especially in aqueous solutions, have not been studied to the same extent. Perhaps the extremely low emission yields of purines at 300 K and the lack of structure in their ground-state absorption spectra observed in aqueous solutions have discouraged this type of study. Traditionally, purine bases have been regarded as photochemically inert.[9] Nonetheless, it has been established that they are photochemically reactive[10-14] in recent reports. In the case of purine free base, Connolly[15] and Arce et al.[16] have made qualitative assignments of the transient absorption bands. No kinetic or quantitative data on reaction rates or excited-state parameters were reported, nor were the identities of the transient species definitely established. Under low- and high-pH conditions, the photoionization of the purine was postulated[16] to proceed through a purine triplet excited state and the presence of the purine radical cation and radical anion was inferred from an increase in absorbance.

Low-temperature UV–visible absorption and electron paramagnetic resonance techniques have been used[17-19] to characterize and quantify the intermediates produced during the continuous UV irradiation of purine free base in acid, neutral, and basic aqueous glasses at 77 K. A biphotonic ionization of the base through a triplet intermediate was established as one of the principal photodestruction paths in basic and neutral glassy solutions.

From the photochemical standpoint, we found it interesting that the title purines have three singlet excited-state components that are almost isoenergetic and oriented perpendicularly to each other: namely, two (π, π^*) and the (n, π^*)[5] states. Furthermore, theoretical works predict the participation of the (π, π^*) and Rydberg states,[1] as well as vibronic–electronic coupling that assists intersystem crossing processes[20] in this family of compounds. Mixing of these electronic states could lead to different photophysical processes as manifested by this work.

In this paper we report a study on the identification of the purine free base and 6-methylpurine intermediate species produced in aqueous media by 266-nm laser flash photolysis. A goal of our research is to provide kinetic data leading to the description of photophysical processes such as intersystem crossing, triplet and singlet state decay times, energy-transfer processes, and photoionization. Some of the reactive intermediate species have been

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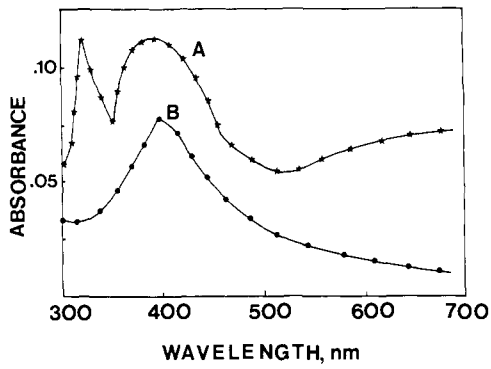

Figure 1. Transient absorption spectra resulting from the laser photolysis of a 2.1×10^{-4} M purine free base aqueous solution: A (★), at neutral pH, 0.40 μs after the pulse; B (●), at neutral pH, N_2O-saturated solution containing 0.5 M *tert*-butyl alcohol, 0.40 μs after the pulse.

identified by monitoring their selective reactions with added solutes and by selectively producing them with use of pulse radiolysis techniques. Another purpose of this work is to use faster techniques in order to obtain a better time resolution for the detection of intermediates than that obtained in our previous microsecond flash photolysis experiments. We also compare the room-temperature laser flash results with the low-temperature EPR and UV–visible results reported from our laboratory.[17-19]

Experimental Section

Chemicals: Purine and 6-methylpurine, Sigma; NaOH, NaN_3, $NaHCO_2$, $Mn(ClO_4)_2$, Na_2HPO_4, and NH_2PO_4, Fisher Scientific ACS. Crocetin (Sigma) was purified according to the procedure established by Craw and Lambert.[21]

Methods and Instruments. Samples were prepared by dissolving purine in purified water. Water was purified through a Millipore filtration system. Solutions were prepared in acetonitrile (Fisher) and 2-propanol (Fisher) without further purification of these solvents. Oxygen was removed by bubbling nitrogen gas through the sample. The concentrations were determined from their absorption spectra recorded with a HP-8450A UV–visible spectrophotometer.

Samples were contained in a quartz cuvette of 1-cm excitation optical path and 0.5-cm optical path for analyses. The excitation was provided by a Quantel ND:Yag Q switch laser delivering 60 mJ in an 11-ns pulse at 266 nm. The beam profile was Gaussian, unfocused, and circular with a 0.8-cm diameter at the sample position. The beam energy was attenuated by the use of metal screens. Time-resolved absorption measurements were made perpendicular to the excitation beam by using a conventional xenon lamp, a monochromator, and a R928 Hamamatsu photomultiplier tube.[22] The output of the multiplier was digitized and stored in a PDP11/70 minicomputer, which controlled the data acqueisition and analysis.[23] A second Nd:Yag (Quantel) laser operating at the same wavelength and producing 200-ps pulses was used for the experiments with 6-methylpurine. The pulse radiolysis experiments were performed with a Van de Graff type electron generator producing 4-MeV electrons with a pulse width of 30–300 ns and delivering a dose of 50 rad ns^{-1} A^{-1} into a target of unit density. The experiment and the analysis of the data produced were performed at the Center for Fast Kinetics Research of the University of Texas, Austin. Low-temperature EPR experiments were performed in a Varian Model E-9 spectrometer irradiating inside the cavity with a 1000-W Xe–Hg lamp (Oriel Co.). The emission data were collected at 77 K in a Perkin-Elmer fluorometer using 0.3-cm Suprasil cells inside a Suprasil liquid N_2 Dewar. The description of the single photon counting experiments can be obtained elsewhere.[24]

Results

Nanosecond Laser Flash Photolysis of Purine Free Base at Neutral pH Conditions. The transient absorption spectrum of a N_2-saturated unbuffered (pH 5.9) 2.1×10^{-4} M purine free base solution observed after excitation at 266 nm with 11-ns pulses is shown in Figure 1A. At 0.40 μs the spectrum (1A) depicts three

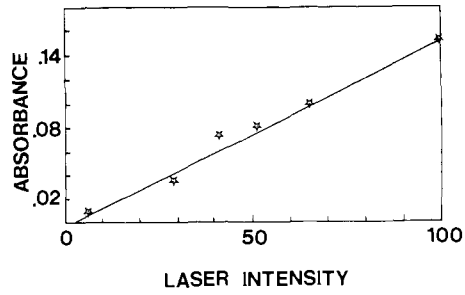

Figure 2. Hydrated electron absorbance at 700 nm as a function of the relative laser intensity. Attenuation of the laser beam was achieved with use of neutral density filters in a random fashion. Each point represents the average absorbance of six experiments performed at the same laser intensity.

major absorption regions: (1) a broad band with maximum at 700 nm characteristic of the hydrated electron (e^-_{aq}) with a decay time of 0.70 μs; (2) a band from 300 to 450 nm with maximum absorption around 390 nm, which also follows a first-order decay process; and (3) a long-lived sharp band with maximum at 320 nm. This latter absorption is still present 13 μs after the excitation, whereas the others have already disappeared. The entire absorption band of the hydrated electron was not included in Figure 1A for convenience in the presentation of the spectrum. Similar transient absorption spectra were observed in a 50 μM aqueous purine solution at pH 5.9, 20 μs after irradiation with a conventional flash lamp.[16] Maxima were observed at 300, 360, 390, and 700 nm.[16]

Yamashita et al.[25] recently reported the emission of the 9-methyladenine excimer working at concentrations of the order of 10^{-3} M. No excimer emission was observed by these researchers at lower concentrations (10^{-4} M). In view of these results, the concentrations used in our experiments make such bimolecular interactions negligible as compared to the photophysical processes observed.

Hydrated Electron Band. The decay of the absorption signal at 700 nm fits a first-order decay process with a rate constant of 5.8×10^6 s^{-1}. This rate constant can be regarded as a pseudo-first-order constant by assuming that the electron decays mainly by the reaction with the neutral purine base according to eq 1.

$$HP + e^-_{aq} \rightarrow HP^{\bullet -} \qquad (1)$$

Through the use of the analytical concentration of purine, a rate constant of 2.9×10^{10} M^{-1} s^{-1} was obtained for the reaction (1). This value for the purine scavenging constant is in favorable agreement with the value of 2.1×10^{10} M^{-1} s^{-1} measured by Hayon et al.[26] at pH 6.0 using the pulse radiolysis technique. In our experiments a 6.6% ionization of the purine base was calculated at time zero after the laser pulse. This calculation was derived from the value of the absorbance at 700 nm, the analytical concentration of the base, and the reported molar absorption coefficient of the hydrated electron, 18 500 M^{-1} cm^{-1},[27] at the same wavelength. Reaction 1 was selectively studied by generating the hydrated electron radiolytically in a purine aqueous solution containing *tert*-butyl alcohol (0.5 M), which acts as a scavenger of $OH^{\bullet}$ radicals. The resulting transient absorption spectrum observed immediately after the pulse radiolysis of this solution (not shown) presents only the hydrated electron band, implying that reaction 1 is not responsible for the band at 320 nm.

A plot of the variation of the maximum intensity of the signal at 700 nm (Figure 2) against the laser power shows a linear functionality. Within experimental errors, this straight line passes through the origin when it is extrapolated to zero laser intensity. This is characteristic of a monophotonic electron-ejection mechanism[28] resulting from an S_1 or T_1 state. At the intensities used,

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deviations from linearity due to saturation effects were not observed. This should have been the case for saturation of a monophotonic process at high laser intensities. Laser excitation (60 mJ) at 266 nm of a cuvette containing purified water did not produce a transient absorption at any wavelength, corroborating the notion that the transient absorptions result from excitation of the purine. Similar transient spectra were also observed at the lower intensities used in the conventional microsecond flash apparatus.[16] Thus, we can disregard the two-photon ionization of the water or of the purine as contributors to the observed hydrated electron signal. A ionization potential of 9.5 eV has been determined by HeI photoelectron spectroscopy for gaseous purine.[29] Since the laser excitation energy is 4.7 eV, the photoionization of purine in aqueous solution is occurring at a much lower energy. On the other hand, Arce et al.[17,19] found a biphotonic ionization mechanism when purine was irradiated at rigid media such as 12 M LiCl, 8 M $NaClO_4$, and 8 M NaOH at 77 K. The biphotonic ionization favored at low temperature could be explained in terms of the triplet lifetime (1.0 s) at 77 K, which happens to be 10^6 times longer than at 300 K. This long radiative lifetime ($T_1 \rightarrow S_0$) allows the excited triplet state to be populated with an excitation source of moderate intensity at 254 nm. Absorption of a photon by T_1 produces an excited triplet state from which ionization occurs, thus yielding the trapped electrons observed in glassy solutions at 77 K. A few low-temperature EPR experiments exciting purine in 8 M NaOH at 254 nm were performed, and the rise time of T_1 was observed. These experiments allowed us to estimate the intersystem crossing rate constant k ($S_1 \rightarrow T_1$) at 77 K, 1 s^{-1}. The decrease in the intersystem crossing rate constant, as well as the corresponding decrease in the triplet decay rate observed when the temperature is lowered, suggest that triplet–singlet and singlet–triplet crossings are affected by changes in the viscosity of the solvent.

The presence of the solvated electron was further confirmed by adding N_2O as an electron scavenger. In its presence, bands resulting from the absorption of the electron or from intermediate species resulting from the reaction of the electron with a neutral purine base or with a geminate radical cation should be affected also. This was the case for the 320- and 700-nm bands, which are eliminated from the transient absorption spectrum in a N_2O-saturated solution (Figure 1B). In fact, at 0.40 μs after the pulse, the absorptions at 320 and 700 nm were reduced to 67% and 85%, respectively. The reduction of the signal at 700 nm is due to the following process:[30]

$$N_2O + e^-_{aq} + H_2O \rightarrow N_2 + OH^- + OH^\bullet \qquad (2)$$

The hydroxyl radical produced in reaction 2 was removed in our experiments by the addition of *tert*-butyl alcohol (0.5 M), which reacts with $OH^\bullet$ producing an unreactive radical, and the rate constant describing that reaction is 2.8×10^8 M^{-1} s^{-1}. Recently, Vieira and Steenken[31] reported the oxidation reactions between $OH^\bullet$ and some purine bases, firmly establishing the importance of the added *tert*-butyl alcohol.

When the e^-_{aq} scavenging rate constant of N_2O,[29] 5.9×10^9 M^{-1} s^{-1}, and its concentration at 1 atm, 3.4×10^{-2} M, are taken into consideration, it can be noted that the initial rate of reaction of (2) is 300 times larger than that of (1). Under these conditions, the reaction of e^-_{aq} with the neutral base or with a geminate radical cation are inhibited. The formation of intermediate species absorbing at 320 nm may be due to these reactions.

Nitrogen-Saturated Solution at pH 2. At pH 2 the transient absorption spectrum of purine free base is similar to the one observed under N_2O-saturated conditions (Figure 1B) in the sense that neither the absorption at 320 nm nor the band with maximum at 700 nm is observed. Nonetheless, the absorption band at 390 nm is still present under these acidic conditions (data not shown). The absence of the e^-_{aq} band can be explained in terms of a rapid reaction with the hydronium ion. It should be pointed out that

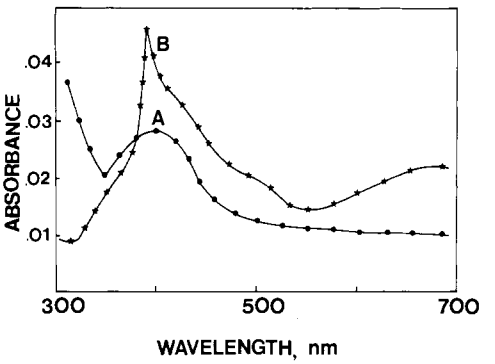

Figure 3. Absorption spectra of transient species produced in the 266-nm laser photolysis of a purine aqueous solution in the presence of additives: A (●), neutral pH, O_2-saturated solution, 0.12 μs after the pulse; B (★), neutral pH, 10^{-2} M $Mn(ClO_4)_2$, 0.10 μs after the pulse.

at pH 2 the predominant species of purine is H_2P^+ ($pK_a = 2.39$),[32] and perhaps the photochemical behavior of this protonated species is not similar to that of the neutral base, HP. However, the absence of the band at 320 nm in the acidic medium is consistent with its assignment to possible reaction products between e^-_{aq} and (a) the neutral purine or (b) a radical cation or assignment to secondary intermediate products resulting from the reactions of the species resulting from (a) or (b). A discussion on the kinetics of formation of this band is deferred for another section.

In glassy acid solutions (6 M H_3PO_4)[18,19] the intersystem crossing and photodestruction yields were found to be 0.1 and 0.001, respectively, being smaller than the corresponding yields observed in neutral and basic glasses. Only solvent radicals and the purine triplet state were observed in the continuous UV irradiation at 77 K.

Short-Lived Species at 390 nm. As expected for a first-order decay process, the lifetime of the species absorbing at 390 nm (1.7 ± 0.2 μs) remains unchanged upon varying its initial concentration by changing the laser power. The decay of this signal is not affected by the presence of an electron scavenger while its decay rate is increased in the presence of O_2 or Mn^{2+} as discussed below. These results indicate that the band at 390 nm can be assigned to a T–T absorption band.

Oxygen-Saturated Solutions. The transient absorption spectrum of a purine oxygenated solution consists of a long-lived band that extends below 300 nm, a short-lived species absorbing in the region of 380 to 480 nm, and a weak absorption at 700 nm (Figure 3A). The lifetimes of the signals at 390 and 700 nm are 0.19 and 0.05 μs, respectively. In the presence of O_2, these latter signals are produced within the duration of the laser pulse (11 ns), as in the case of the deoxygenated solutions.

The disappearance of the purine triplet in the O_2-saturated solutions can be represented according to

$$HP(T_1) \rightarrow HP(S_0) \qquad (3)$$

$$HP(T_1) + O_2 \rightarrow HP + O_2 \qquad (4a)$$

$$\rightarrow HP + O_2(^1\Delta_g) \qquad (4b)$$

Furthermore, the oxygen quenching constant of the purine triplet can be calculated since $k(3)$ was determined in deaerated solutions. The transient absorption at 390 nm in the presence of O_2 decays following pseudo-first-order kinetics, 5.1×10^6 s^{-1}. With eq 3 and 4 the oxygen quenching constant of the purine triplet was calculated as 3.4×10^9 M^{-1} s^{-1}. This rate constant is of the same order of magnitude as that reported for the quenching by O_2 of the pyrazine triplet, 3.2×10^9 M^{-1} s^{-1},[33] and other organic triplets.[34,35]

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Low-temperature emission measurements in aqueous glasses (12 M LiCl, 8 M NaOH, and 5 M H_2SO_4) showed phosphorescence maxima around 400 nm; i.e., the vibrationally relaxed T_1 state has an approximate energy of 3.1 eV. Therefore, the generation of O_2 ($^1\Delta_g$, 0.98 eV) based on the reaction energy defect cannot be ruled out. If the $O_2(^1\Delta_g)$ formation channel is a major reaction pathway, the reaction of $O_2(^1\Delta_g)$ with the neutral base could be responsible for the formation of stable adducts:

$$HP + O_2(^1\Delta_g) \rightarrow \text{photoadduct} \quad (5)$$

The formation of an absorption band with the onset at 300 nm observed only in O_2-containing solutions could indicate the generation of a transient species resulting from reaction 5.

The presence of O_2 also increases the decay rate of the e^-_{aq} absorption at 700 nm by 1 order of magnitude, 2.6×10^7 s^{-1}. This effect could be caused by two factors: (a) the large oxygen e^-_{aq} scavenging rate constant, 1.8×10^{10} M^{-1} s^{-1},[30] or (b) the purine triplet being the e^-_{aq} precursor. The fact that the initial absorbances of the time-resolved absorption measurements taken at 700 nm in the presence and absence of O_2 are the same within experimental error suggests that the triplet state is not the precursor of the e^-_{aq}. In other words, the initial e^-_{aq} should not be affected by the presence of O_2 provided there is no interaction between O_2 and the electronic state precursor of e^-_{aq}.

Manganese Perchlorate N_2-Saturated Solution. Manganese(II), like O_2, has been extensively used as a triplet quencher. Experiments with added Mn^{2+} were also performed since its concentration and, therefore, its quenching reaction rate can be controlled much more easily than in the case of O_2. Working with 10^{-2} M Mn^{2+} solutions, we were able to record triplet waveforms with satisfactory signal to noise ratios. Since neither Mn^{2+} nor ClO_4^- is expected to undergo chemical reactions with purine, a comparison of the transient absorption spectra of irradiated solutions containing Mn^{2+} with those saturated with O_2, which is likely to be reactive, could give information on the reactive intermediates produced by the reactions of O_2 with purine excited states and/or intermediates. However, the shapes of both transient absorption spectra were similar (Figure 3B) except for the presence of the band extending below 300 nm. Another consideration for using Mn^{2+} was its small electron scavenging rate constant, 7.7×10^7 M^{-1} s^{-1},[30] as compared to that of N_2O or O_2. The Mn^{2+} quenching constant of the purine triplet was calculated by the same procedure followed for O_2. This value was found to be 2.0×10^8 M^{-1} s^{-1}. The Mn^{2+} quenching constant of the naphthalene triplet reported by Norrish and Porter,[36] 2.8×10^7 M^{-1} s^{-1}, is smaller than the corresponding value for purine, as a consequence of a larger spin–orbit coupling interaction.

Sensitization of the Crocetin Triplet State. Triplet crocetin was sensitized by the purine triplet state, and the resulting T–T absorption spectrum was identical with that reported by Craw and Lambert.[21] Purine free base was excited at 266 nm in the same manner as the previously discussed experiments. The pH of the purine and crocetin solution was adjusted to 8.5 with a phosphate buffer (50 mM) in order to reproduce the sensitized crocetin T–T absorption spectrum obtained by exciting psoralen at 337 nm.[21] It should be pointed out that the band at 390 nm assigned to the purine triplet appeared at all the pH's tested in the range of 2–12; i.e., the one neutral and two ionic forms of purine produced the triplet when excited at 266 nm. At room temperature, the state of protonation affected the relative triplet quantum yields but no effect on the T–T absorption properties was observed. Differences in triplet parameters were observed in glassy matrices having different OH^- or H_3O^+ concentrations.[18] In the low-temperature glasses, the intersystem crossing yield decreased 0.62, 0.37, and 0.10 for 8 M NaOH, 8 M $NaClO_4$, and 6 M H_3PO_4 glasses, respectively. A similar trend was observed in the room-temperature laser photolysis. Variations in decay lifetimes and root mean square zero field splitting parameters were also observed for the different states of protonation of the purine in the low-temperature glasses. In addition, the cationic and anionic forms presented similar triplet state characteristics such as decay lifetimes and zero field splitting parameters.[18]

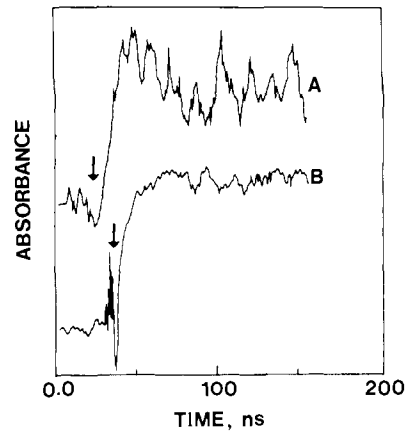

Figure 4. Oscilloscope traces resulting from the 200-ps laser irradiation (15 mJ) of a 6-methylpurine aqueous solution. Arrows indicate the time at which the excitation pulse was applied. Key: A, transient signal at 700 nm (hydrated electron); B, transient signal at 390 nm (triplet state).

In order to avoid saturation problems of the purine T–T transition, the sensitization experiments were performed at 1% of the total laser pulse energy (60 mJ). The laser power was kept low since the magnitude of the crocetin and purine T–T annihilation constant is not known. A blank solution of crocetin was excited at the same laser power, and no evidence of direct excitation of crocetin was found. This test was important since crocetin has a weak absorption band at 250 nm. The reaction between triplet purine and crocetin can be written as follows:

$$^3HP + C \rightarrow HP + {}^3C \quad (6)$$

It is evident that reaction 6 should follow processes 7 and 8.

$$HP + h\nu\ (4.7\ eV) \rightarrow HP(S_1) \quad (7)$$

$$HP(S_1) \rightarrow HP(T_1) \quad (8)$$

Moreover, in order for reaction 6 to be observed, its reaction rate should be at least of the same order of magnitude, or greater, than that of reaction 3. When (6) and (3) are compared, it becomes evident that our selection of crocetin concentration was more appropriate in order to characterize longer lived species, i.e., with decay times in the range of 3.5×10^{-5} s. In order to avoid direct excitation of crocetin, its concentration was kept as low as possible. The rate constant for the formation of triplet crocetin, 7.1×10^9 M^{-1} s^{-1}, was calculated from the initial slope of a pseudo-first-order plot of the absorbance growth at 470 nm against time, the analytical concentration of crocetin, 4.3×10^{-6} M, and the available molar absorption coefficient, 73 000 M^{-1} cm^{-1}.[21] The decay rate of crocetin triplet calculated in the present work, $(1.4 \pm 0.8) \times 10^5$ s^{-1}, is similar to the crocetin triplet decay reported by Craw and Lambert,[21] 1.25×10^5 s^{-1}, indicating no interaction between triplet crocetin and ground-state purine. The purine T–T molar extinction coefficient at 390 nm, $(2.0 \pm 0.5) \times 10^3$ M^{-1} cm^{-1}, was estimated from the known sensitized crocetin triplet concentration and the absorbance of the purine T–T absorption for the same laser power.

Picosecond Laser Flash Photolysis. The excitation of purine with 200-ps pulses delivering only 15 mJ produced signals too weak to be analyzed. On the other hand, excitation of 6-methylpurine resulted in the oscillograms shown in Figure 4. The electron signal grows to a maximum within the first 10–15 ns after the pulse (Figure 4A). The instrument's response time is 1 ns. An intersystem crossing rate constant of 1.4×10^8 s^{-1} was determined from the initial growth of the triplet signal (Figure 4B). Although the signal to noise ratio of the electron signal is not as high as the triplet signal, it can be noted from these curves that it takes around 25 ns for the triplet signal to reach its maximum intensity,

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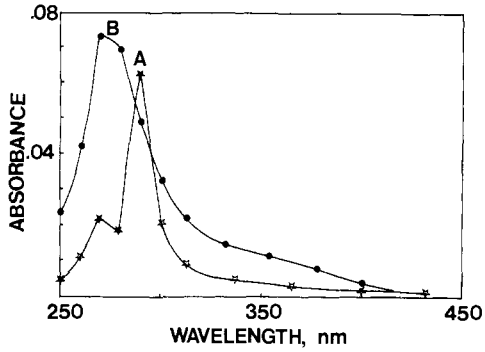

Figure 5. Transient absorption spectra resulting from the pulse radiolysis of a 10^{-4} M purine free base solution saturated with N_2O in the presence of the following: A (☆), 10^{-2} M NaN_3, 0.40 μs after the pulse; B (●), 10^{-2} M $NaHCO_2$, 1.0 μs after the pulse.

whereas for the electron this occurs within the first 15 ns. The monophototonic ionization mechanism found for purine at room temperature, together with the observation that the triplet signal reaches its maximum intensity at a later time than the e^-_{aq} (Figure 4B) signal, indicates that S_1 is the precursor of the e^-_{aq}.

Optical Pulse Radiolysis of Purine in Aqueous Solution. One-Electron Oxidation Reaction of Purine with $N_3^•$. In order to avoid the simultaneous generation of all the intermediates, the purine radical cation and radical anion were generated independently by the optical pulse radiolysis technique. In particular, this method eliminates the presence of the triplet and e_{aq}^- and possible species derived from them. The azide radical was prepared following Alfassi and Schuler's procedure.[37] This procedure is based on the radiolytical generation of the OH• radical produced by the irradiation of a N_2O-saturated solution in the presence of the azide anion, N_3^-. The bimolecular decay of the azide radical obtained from our experiments, $2k = 8 \times 10^9$ M^{-1} s^{-1}, is in favorable agreement with the value reported by Alfassi and Schuler,[37] $2k = (8.8 \pm 0.5) \times 10^9$ M^{-1} s^{-1}. The azide radical reacts with purine to produce the radical cation.

$$N_3^• + HP \rightarrow N_3^- + HP^{•+} \quad (9)$$

With the available azide radical molar absorption coefficient, 2029 M^{-1} s^{-1},[37] a 1.8×10^{-5} M concentration of the N_3^- species was estimated. In the presence of 2×10^{-5} M purine, the azide radical concentration is reduced to approximately 1×10^{-5} M due to reaction 9. The purine oxidation is evidenced by the appearance of a band at 290 nm (Figure 5A). The rate of formation of the purine radical cation (5×10^8 M^{-1} s^{-1}) was measured by monitoring the appearance of the absorption at 290 nm. This formation rate constant lies within the same range as those describing the formation rate of radical cations of substituted benzene compounds at pH 5.8, $k = (0.02–4.7) \times 10^9$ M^{-1} s^{-1}.[37] The azide radical was used in our experiments because of its low absorption above 275 nm;[37] nevertheless, the oxidation of adenine by $Br_2^{•-}$ has been reported.[38]

One-Electron Reduction Reaction of Purine with the Carbon Dioxide Radical Anion. The formation of $CO_2^{•-}$ was obtained by the oxidation of the formate anion[39] with radiolytically prepared OH•.

$$OH^• + HCO_2^- \rightarrow OH^- + CO_2^{•-} + H^+ \quad (10)$$

The generation of the purine radical anion was achieved through the following reaction:

$$CO_2^{•-} + HP \rightarrow CO_2 + HP^{•-} \quad (11)$$

The carbon dioxide radical anion absorption maximum occurs around 260 nm. In the presence of 10^{-4} M purine, the absorption at 260 nm is reduced, while the corresponding absorption due to the purine radical anion formation can be observed at 275 nm (Figure 5B). The decay time of the absorption at 275 nm was 16 μs. It is important to notice that neither the carbon dioxide radical anion nor the azide radical cation absorbs in the 320-nm region where strong absorptions by the purine intermediates are observed.

Discussion

When purine is excited by the fourth harmonic of the Nd:Yag laser (4.7 eV), the triplet state, the hydrated electron, and, therefore, the radical cation are produced. Unequivocal evidence of the presence of the triplet moiety was provided through sensitization experiments by use of crocetin. Additional evidence for the assignment of the 390-nm based to a purine T–T absorption (Figure 1) was obtained from O_2 and Mn^{2+} quenching experiments. The higher value of the quenching rate constant obtained for crocetin as compared to O_2 can be justified in terms of differences in their cross sections rather than in terms of an energy defect of reactions 6 and 4a. Matheson and Rodgers[40] showed that triplet crocetin can be sensitized by $O_2(^1\Delta_g)$ via a physical process analogous to reaction 4a involving triplet purine. Their results place the lowest energy triplet state of crocetin on the order of 1 eV; in which case, the energy defects for reactions 5 and 4a are almost identical. Furthermore, it must be noted that the decay of the 390-nm signal is not affected by the presence of an electron scavenger.

Arce et al.[16] assigned a band at 390 nm to a purine T–T transition in 8 M NaOH at 77 K. The long-lived emission with lifetimes on the order of seconds as well as the EPR spectra of the triplet state for some purine bases at 77 K have been reported.[3,19] The first-order decay of the high-field EPR signal ($t_{1/2} \sim 1$ s) corresponds to the visual estimate of the phosphorescence decay. These lifetimes and the zero field splitting parameters are characteristics of (π, π^*) triplet states.[41] No T–T emission was detected for either the low-temperature or the room-temperature studies. Moreover, deactivation of the triplet through eq 3 was found to proceed via nonradiative processes since the phosphorescence quantum yield was very small. According to our experiments on the effect of the variation of the laser intensity on the electron yield (Figure 2), the photoionization of purine occurs through a singlet state. Deactivation of the triplet by HP is expected to be fast, but its contribution is minor due to the low concentration of molecules in the triplet state and to the low analytical concentration of HP.

As demonstrated in the experiments done in the pH range of 2–12, the 266-nm laser excitation of the neutral and two ionic forms of purine produces the T–T absorption band. The triplet band was also observed in acetonitrile, which is an aprotic solvent. The fact that the decay of triplet absorption in acetonitrile is of the same order of magnitude as in the aqueous media tends to rule out the possibility of a protonation reaction to account for its decay in the aqueous environment. The possibility of an (n, π^*) triplet contributing to the absorption in the region of 390 nm was tested by exciting purine in 2-propanol/water mixtures of 10% and 20%. It is well-known that 2-propanol is an hydrogen atom donor and that (n, π^*) triplets react readily with hydrogen atom donors. A rate constant of 1.3×10^8 M^{-1} s^{-1} has been reported by Bent et al.[33] for the quenching of the pyrazine (n, π^*) triplet state by 2-propanol. The triplet band was observed in pure 2-propanol as well. Thus, no evidence for the presence of an (n, π^*) triplet was obtained.

There is one observation that has not been fully explained: that is the kinetics of formation of the 320-nm absorption band. As mentioned earlier, that band is produced within the duration of the laser pulse (11 ns), its intensity is reduced to 67% when the solution is saturated with N_2O, and it is absent at pH 2 or in the presence of Mn^{2+} or O_2. These observations are consistent with its assignment to a radical anion or an intermediate product resulting from it. Nevertheless, the electron lifetime is 1.7 μs,

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and if the reaction of the neutral purine with e^-_{aq} occurs within its lifetime, one should be able to observe an increase in the intensity of the band at 320 nm as the e^-_{aq} signal decreases, i.e., approximately 2 μs after the laser pulse. This increase in absorption was not observed. A possible explanation is that, once formed, the radical anion reacts instantaneously to produce the species absorbing at 320 nm.

In the pulse radiolysis of 2′-deoxyadenosine Hissung et al.[42] observed that the radical anion presented a rather weak and featureless absorption above 300 nm which, in 0.2 μs, decayed into a neutral radical. At pH 5.2, Moorthy and Hayon[26] assigned the absorption maxima at 315 and 430 nm to the species H_3P^+, which resulted from the one-electron reduction of purine, HP, and subsequent protonation to form a dihydro radical cation.

Another possible species contributing to the absorption at 320 mn is the purine radical cation. During continuous UV irradiation of frozen purine solutions in 8 M $NaClO_4$, a permanent, faint blue coloration on the sample was observed as well as a decrease in absorbance of the purine absorption band at 264 nm. The appearance of a new band with a maximum near 320 nm was also observed.[17] The blue coloration was explained in terms of the presence of trapped electrons detected by EPR and their visible absorption band. Warming the irradiated solution to room temperature produced a partial regeneration of the purine absorption, while the absorption at 320 nm was no longer observed. This band was assigned to a purine radical cation. The radical cation was identified from its EPR spectra consisting of a singlet with line width of 22 G and g value of 2.004.[19] On the other hand, in the laser pulse experiments, irradiated purine solutions containing N_2O did not present an increase in the absorbance at 320 nm as should be expected if it were to correspond to the absorption of a radical cation.

In summary, simple models for one of the nucleic acid components, purine free base and 6-methylpurine, undergo monophotonic ionization as one of their primary photochemical processes. This is evidenced by the presence of a visible band assigned to the hydrated electron absorption band. This band is quenched by well-known electron scavengers, confirming the presence of the electron. Similarly, the effect of triplet quenchers such as O_2 and paramagnetic ions on the 390-nm band, as well as the sensitization of the crocetin triplet, provided evidence for the presence of a purine triplet state. The contribution of this state to the photoionization process is minor inasmuch as the electron initial yield is not affected by the presence of triplet quenchers. Furthermore, the triplet's appearance occurs 15 ns after that of the electron. The photoejected electron adds to purine at almost diffusion-controlled rates, implying the formation of radical anions and products resulting from their reaction with water.

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Characterization of the *Fusarium* Toxin Equisetin: The Use of Phenylboronates in Structure Assignment

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Abstract: *Fusarium equiseti* has repeatedly been identified in environments where several genetically unrelated individuals each developed leukemia. The screens on cultures of this fungus revealed interesting biological activities and turned up equisetin, a previously identified yet uncharacterized metabolite. The molecule has now been shown to contain two domains, a bicyclic hydrocarbon and an *N*-methyltetramic acid, connected by a bridging carbonyl. The steric requirements of the two bridging carbon–carbon bonds dictated much of the physical and chemical behavior of the toxin. A phenylboronic ester derivative proved to be essential in defining the enol form of the tetramic acid and in making possible the assignment of the scalar and dipolar spin exchange interactions in the complete characterization of the molecule.

Genetic and environmental factors are both known to contribute to the development of leukemia.[1] However, individual cases usually cannot be clearly attributed to either genetic predisposition or specific etiologic agents and probably result from several interacting factors.[2] In instances when "clusters" of genetically unrelated individuals living in the same environment all develop the same form of leukemia,[3,4] environmental leukemogens are more strongly implicated and can potentially be identified.

A striking medical report appearing in 1969 associated four cases of leukemia with a single home in Georgia.[4] Existing evidence suggesting that mycotoxins may be causative factors in environmentally induced leukemia[5] led Wray and O'Steen[6] to screen the house and surroundings for fungal species. Of 11 fungal isolates tested, an extract of a *Fusarium sp.* was shown to have the greatest toxicity to animals.[6] In a subsequent study, *Fusarium equiseti* was found in the home of a husband and wife who both

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