Photochemistry of 5-Methylphenazinium Salts in Aqueous Solution. 2. Optical Flash Photolysis and Fluorescence Results and a Proposed Mechanism[†]

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The photochemistry of 5-methylphenazinium (MP^+) salts in aqueous solutions for pH 2–7 has been studied by using fluorescence, optical flash photolysis, and electron paramagnetic resonance (EPR) techniques. A complete mechanism has been proposed which accounts for all of the known observations on this reaction. The fluorescence yield is ~1% and independent of pH for pH > 4 but drops sharply for pH < 3 presumably because of the formation of the nonfluorescent protonated excited state $MPH^{2+}(S_1)$. This state also appears to be photochemically inert as the quantum yield of formation of $MPH^{+}\cdot$ also drops for pH < 3. The triplet state $MP^+(T_1)$ has likely been detected by its triplet–triplet absorption and is found to have a lifetime in solution of ~0.5 ms. The triplet state is quenched efficiently by oxygen; however, oxygen does not quench the photochemistry; hence, it is concluded that photochemistry occurs via the excited $n\pi^*$ singlet state $MP^+(S_1)$. The novel feature of the proposed mechanism is that the primary photochemical step is proposed to involve the addition of water to $MP^+(S_1)$ with the concomitant loss of a proton to form an adduct X. X is a strong oxidizing agent and appears to react as an OH radical addition reagent in its reactions with MP^+, formate, and acetate. In the absence of added reagents, X reacts with MP^+ to initiate a complex series of reactions. Most of the rate constants and some equilibrium constants have been determined for these reactions and are summarized in Table II. It is possible that this dye may be useful as a photochemical oxidizing agent in the sensitized photochemical decomposition of water.

Introduction

In part 1[1] it was demonstrated that irradiation of aqueous solutions of 5-methylphenazinium (MP^+) salts results in the reaction

$$3MP^+ + H_2O \xrightarrow[\lambda < 500\ \mathrm{nm}]{h\nu} 2MPH^{+}\cdot + PYH^+$$

where $MPH^{+}\cdot$ is the protonated semireduced form of MP^+ and PYH^+ is pyocyanine (1-hydroxy-5-methylphenazinium cation) (see Figure 2 of ref 1 for structures and symbols). The quantum yield for formation of $MPH^{+}\cdot$ was found to be 0.29 ± 0.03 at pH 7.0 and 1.1 ± 0.1 at pH 3.0. Irradiation in the presence of the spin trap 5,5-dimethyl-1-pyrroline 1-oxide (DMPO) produced a spin adduct indistinguishable from the ·OH radical adduct of DMPO. In this paper, we will describe the fluorescence properties of MP^+ and report an extensive study of the photochemistry of MP^+ by optical flash photolysis. We will also present a mechanism which we believe accounts for all of the known observations. It is important to understand the mechanism of this reaction since it appears that MP^+ is behaving as a strong photochemical oxidizing agent and thus could be a sensitizer in a photochemical scheme to decompose water to hydrogen and oxygen.[2]

To our knowledge, there have been no previous thorough studies of the mechanism of the photochemistry of MP^+ in aqueous solution; however, there have been some studies of the related phenazine photochemistry.

The photoreduction of phenazine (P) in strongly acidic methanol (0.1–1.0 M HCl in methanol) and weakly acidic methanol (0.1 M acetic acid and 0.1 M sodium acetate in methanol) was investigated by Bailey et al.[3] They proposed that the reactive state is the lowest $n\pi^*$ singlet excited state with the doubly protonated $PH_2^{2+}(S_1)$ species reacting in the strongly acidic case and the monoprotonated $PH^+(S_1)$ species reacting in the weakly acidic case. The semireduced species $PH_2^{+}\cdot$ is then produced by electron transfer from methanol. The protonated triplet state $PH^+(T_1)$ was found to be unreactive in their study. Using kinetic and flash-photolysis methods, Davis et al.[4] and Japar and Abrahamson[5] provided further evidence that the $n\pi^*$ singlet state is the reactive state and that the $\pi\pi^*$ triplet state is unreactive in the photoreduction of P by electron donors. When a nitrogen-purged solution of P in 2 M aqueous phosphoric acid was irradiated, Wake et al.[6] obtained a green solution and a green precipitate which was shown to be the semireduced $PH_2^{+}\cdot$ cation radical salt. They also obtained 1-hydroxyphenazine as a product. They proposed a hydrated addition product as an intermediate.

Experimental Section

1. Fluorescence Apparatus. A Perkin-Elmer fluorescence spectrophotometer Model MDF-4 was used to record fluorescence spectra and to determine fluorescence quantum yields. The latter were determined by the relative method of Heller et al.[7] using quinine sulfate in 0.5 M sulfuric acid as the standard, for which $\phi_f = 0.55 \pm 0.03$.[8]

2. Optical Flash-Photolysis Apparatus. The flash-photolysis apparatus employed was a standard setup for optical detection of intermediates by kinetic spectrophotometry.[9] An FX-1C-6 flash lamp from E, G & G, Inc., was used as the excitation source. The electrical-discharge energy at 9 kV was ~100 J, and the time required for the flash to drop to half the peak intensity was ~20 μs. A 100-W quartz-halogen projector lamp was used as the

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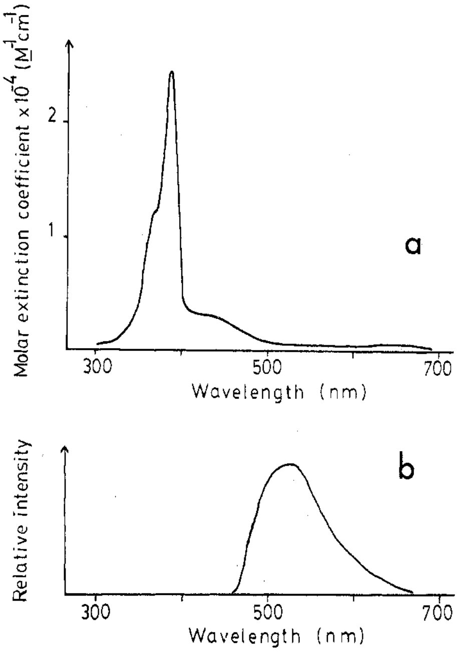


Figure 1. (a) Optical absorption spectrum of an aqueous solution of MPMS at pH 7. (b) Fluorescence emission spectrum of the same solution; excitation wavelength, 360 ± 4 nm.

monitoring source; and a Bausch and Lomb Model 33-87-07 high-intensity grating monochromator, an IP-28 photomultiplier, and a Tektronix Model 7613 storage oscilloscope were employed to detect and record the kinetic response at fixed wavelengths set by the monochromator. A Tektronix Model C12 oscilloscope camera was attached to the screen to record the results photographically. Some of the early results were conducted by using an Applied Photophysics optical flash-photolysis apparatus.

3. *Chemicals and Sample Preparations.* 5-Methylphenazinium methyl sulfate (MPMS) was obtained from Sigma and used without further purification.[10] For the optical flash-photolysis experiments, 1 × 10^{-5} M aqueous MPMS was used so that about half the photolysis light was absorbed. The solutions were buffered by using 5 × 10^{-3} M phosphate for pH > 5, 5 × 10^{-3} M phthalate for 3.0 < pH < 5.5, and solutions of hydrochloric acid for pH < 3. The MPMS solution and the reaction vessel were purged with nitrogen for at least 15 min before irradiation.[12]

4. *Flash-Photolysis–Electron Paramagnetic Resonance (EPR) Measurements.* These measurements were carried out on a Varian E12 EPR spectrometer using a Model 610A Photochemical Research Associates pulsed-light source with a Xenon Corp. Model 185 Novatron xenon flash lamp and a Fabritek Model 1072 computer of averaged transients. The technique is described more fully by Bolton and Warden.[13]

5. *Potentiometric Titrations.* Potentiometric titrations of MP$^+$ were carried out by using a saturated calomel reference electrode and a platinum measuring electrode. A Model 8691-2 Leeds and Northrup millivolt potentiometer was employed. Reductive titrations were carried out by using nitrogen-purged solutions of chromous chloride. Further details on the techniques used can be found in ref 14.

Results and Discussion

We have obtained a wealth of data on this system; hence, in order to organize the best presentation of this data, we have chosen the unusual procedure of presenting our proposed mechanism first. In the presentation of data and discussion which follow, the reader will find it convenient to refer back repeatedly to this mechanism as each step in turn is justified.

1. *The proposed photochemical mechanism* is as follows:

$$\mathrm{MP^+(S_0)} \xrightarrow[h\nu]{k_1} \mathrm{MP^+(S_1)} \qquad (1)$$

$$\mathrm{MP^+(S_1)} \xrightarrow{k_2} \mathrm{MP^+(T_1)} \qquad (2)$$

$$\mathrm{MP^+(T_1)} \xrightarrow{k_3} \mathrm{MP^+(S_0)} \qquad (3)$$

$$\mathrm{MP^+(S_1) + H_2O} \xrightarrow{k_4} \mathrm{X + H^+} \qquad (4)$$

$$\mathrm{X + MP^+} \xrightarrow{k_5} \mathrm{MP\cdot + PYH_2{}^{+}\cdot} \qquad (5)$$

$$\mathrm{X + MP^+} \xrightarrow{k_6} \mathrm{2MP^+ + OH^-} \qquad (6)$$

$$\mathrm{MP\cdot + H^+} \xrightarrow{k_7} \mathrm{MPH^{+}\cdot} \qquad (7)$$

$$\mathrm{PYH_2{}^{+}\cdot + MP^+} \xrightarrow{k_8} \mathrm{PYH^+ + MPH^{+}\cdot} \qquad (8)$$

$$\mathrm{PYH\cdot + MP^+} \xrightarrow{k_9} \mathrm{PYH^+ + MP\cdot} \qquad (9)$$

$$\mathrm{PYH_2{}^{+}\cdot} \underset{k_{-10}}{\overset{k_{10}}{\rightleftharpoons}} \mathrm{PYH\cdot + H^+} \qquad (10)$$

$$\mathrm{2MPH^{+}\cdot} \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} \mathrm{MP^+ + MPH + H^+} \qquad (11)$$

$$\mathrm{2MP\cdot + H^+} \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} \mathrm{MPH + MP^+} \qquad (12)$$

The overall stoichiometric reaction is

$$\mathrm{3MP^+ + H_2O} \xrightarrow{h\nu} \mathrm{2MPH^{+}\cdot + PYH^+} \qquad (13)$$

MP$^+$ is assumed to be in the S$_0$ ground state unless otherwise indicated. MP$^+$(S$_1$) is the first excited singlet state (an nπ^* state) of MP$^+$, and MP$^+$(T$_1$) is the first excited triplet state (a $\pi\pi^*$ state) of MP$^+$. X is an intermediate adduct of MP$^+$ with H$_2$O of unknown structure. It will be shown that the reactive state is the singlet state, disproportionation is unimportant at pH < 7, and reaction 6 is a possible side reaction. Hence, reactions 2, 3, 6, 11, and 12 have been omitted in determining the overall reaction 13. Note that if reactions 2, 3, and 6 were negligible, that is, a quantum yield of 1.0 for the photochemistry, the quantum yield of formation of MPH$^{+}\cdot$ would be 2.0.

2. *Excited-State Behavior.* (*a*) *Fluorescence Measurements.* The absorption and fluorescence-emission spectra of MP$^+$ are shown in Figure 1. The latter is seen to have a broad peak of 525 nm. The fluorescence excitation spectrum is virtually identical with the absorption spectrum. Excitation was not carried out at the absorption peak of 385 nm, but rather at 360 nm because of too rapid a photolysis by the excitation beam at 385 nm.

The pH dependence of the relative fluorescence intensity was determined at 525 nm. The time dependence of the fluorescence intensity was measured and then extrapolated to zero time to determine each fluorescence intensity at various pH values. The results are shown in Figure 2. It is seen that the fluorescence intensity starts to drop around pH 4 and then drops sharply for pH < 3. Rubaszewska and Grabowski[15] report that the pK_a of the protonated form (MPH^{2+}(S$_1$)) of the excited state is 3.3. Hence, for pH < 3.3, MPH^{2+}(S$_1$) is expected to be the predominant excited state. The drop-off of the 525-nm fluorescence of MP$^+$ (see Figure 2) matches the drop-off of the quantum yield of formation of MPH$^{+}\cdot$ (see Figure 6 of part 1)[1] for

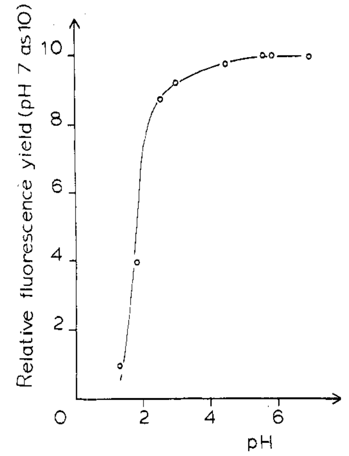


Figure 2. The effect of pH on the fluorescence intensity of MP$^+$ relative to that of MP$^+$ at pH 7 (arbitrarily taken to be 10). Excitation wavelength, 360 nm; emission wavelength, 525 nm; $[MP^+] = 10^{-5}$ M.

pH < 4.[16] It would thus appear that the $MPH^{2+}(S_1)$ state is nonfluorescent and photochemically inactive and conversely that $MP^+(S_1)$ state is the photochemically active state.

The fluorescence quantum yield was determined to be ~0.5% at pH 7. However, because of the low yield, a higher concentration of MP$^+$ had to be used such that the optical density was ~0.3. Since the method used requires that the optical density not exceed 0.05, we feel that the fluorescence quantum yield has been somewhat underestimated but is probably not higher than ~1%.

(*b*) *Effect of Oxygen.* Using the flash-photolysis–EPR technique with the magnetic field set at the peak of the overmodulated MPH$^+\cdot$ signal (see part 1),[1] we found the concentration of MPH$^+\cdot$ formed within ~1 ms after the flash to be the same in nitrogen-purged, air-saturated, and oxygen-saturated solutions, even though the MPH$^+\cdot$ EPR signal does decay faster (in seconds) in the oxygenated solutions. Also, the fluorescence yields were found to be the same for these three solutions, indicating that oxygen is not quenching the singlet excited state.

Since oxygen is an efficient quencher of triplet states and since oxygen did not affect the initial amount of MPH$^+\cdot$ formed in a flash, we take this as strong evidence that photochemistry is occurring via the S_1 singlet state of MP$^+$. Thus, in this regard, MP$^+$ is behaving in an analogous manner to the behavior of phenazine as noted in the Introduction.

(*c*) *Effect of Light Intensity.* The rate of formation of MPH$^+\cdot$ was measured at different light intensities by placing neutral density filters in front of a krypton ion laser beam at 476.2 nm. The rate was found to be a linear function of the light intensity, and hence we conclude that the excitation process involves only one photon.

(*d*) *Mechanistic Conclusions.* The data presented in this section establish the validity of reactions 1 and 4. It is assumed that water is one of the reactants in reaction 4 as later it will be shown that an OH group is introduced at this stage. The nature of the products of reaction 4 will be discussed later.

3. *Redox Behavior.* The redox behavior of pyocyanine (PYH$^+$) has been thoroughly studied by Friedheim and Michaelis.[17] We have carried out parallel studies of the redox behavior of MP$^+$. Both PYH$^+$ and MP$^+$ undergo two separate one-electron reductions at pH < 7. As expected from the reactions

$$MP^+ + e^- + H^+ \rightleftharpoons MPH^+\cdot$$

$$PYH^+ + e^- + H^+ \rightleftharpoons PYH_2{}^+\cdot$$

the first reduction potential in each case is pH dependent and follows the relation

$$E_1 = E_1{}^0 - 0.059\text{pH}$$

whereas E_2 is pH independent. Table I summarizes the reduction potentials for MP$^+$ and PYH$^+$. From these results, it is clear that reaction 8 is thermodynamically allowed since

$$E_1(PYH^+) < E_1(MP^+)$$

TABLE I: Reduction Potentials of MP$^+$ and PYH$^+$ at Various pH Values

$Z^+ + e^- + H^+ \rightleftharpoons ZH^+\cdot$ $E_1{}^a$
$ZH^+\cdot + e^- \rightleftharpoons ZH$ $E_2{}^a$
where Z = MP or PYH

	MP$^+$		PYH$^+$	
pH	E_1,[a,b] V	E_2, V	E_1,[c,d] V	E_2,[d] V
2	0.38 ± 0.03	0.16 ± 0.01	0.262	0.097
3	0.31 ± 0.01	0.18 ± 0.01	0.207	0.097
7	0.099 ± 0.007[e,f]		-0.34[e]	

[a] All potentials are referred to the standard hydrogen electrode. [b] $E_1 = E_1{}^0 - 0.059$ pH where $E_1{}^0 = 0.49 \pm 0.04$ V. [c] $E_1 = E_1{}^0 - 0.059$ pH where $E_1{}^0 = 0.38 \pm 0.01$ V. [d] From ref 17. [e] Two-electron reduction potentials. [f] Rao and Hayon[19] have determined a value of 0.08 V at pH 7 for MP$^+$.

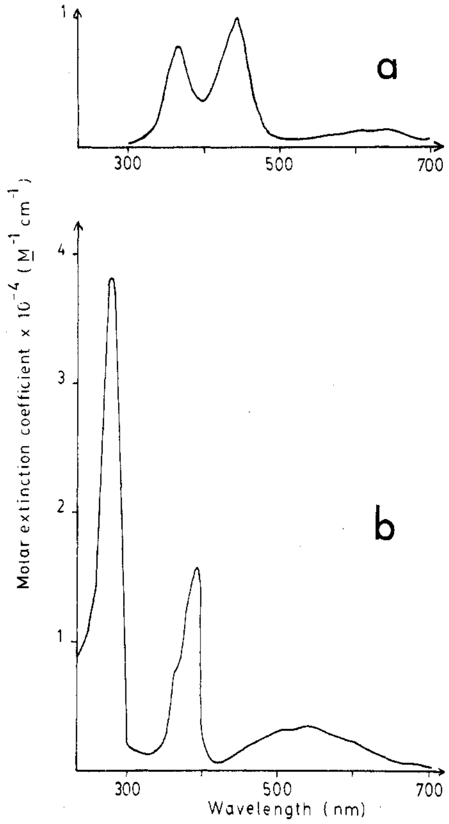


Figure 3. Optical absorption spectra at pH 3 for (a) MPH$^+\cdot$ and (b) PYH$^+$.

4. *Optical Flash-Photolysis Results.* (*a*) *Optical Spectra and Monitoring Wavelengths.* The two major products of this reaction have been established as MPH$^+\cdot$ and PYH$^+$ (see part 1).[1] The optical absorption spectra for these two species at pH 3 are shown in Figure 3. The kinetics of formation of MPH$^+\cdot$ and PYH$^+$ were monitored at 443 and 525 nm, respectively. These wavelengths were chosen so that the desired component is the predominent

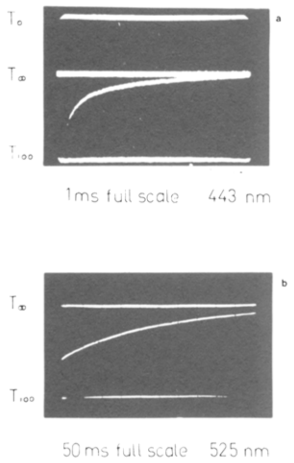


Figure 4. Oscilloscope traces of the kinetics at (a) 443 nm and (b) 525 nm for a 10^{-5} M aqueous solution of MP^+ at pH 3.0.

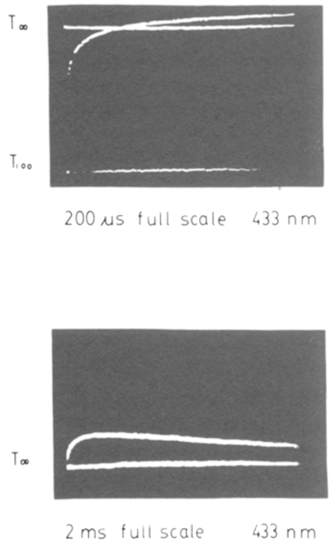


Figure 5. Oscilloscope trace of the kinetics at 433 nm for a 10^{-5} M aqueous solution of MP^+ at pH 3.0.

absorber with minimal interference from other species.

(*b*) *Rate Constants at pH 3.* Nitrogen-purged 10^{-5} M solutions of MP^+ at pH 3 were flashed (once per solution). Typical traces at 443 and 525 nm are shown in Figure 4 (note the different time scales). It is clear from Figure 4a that $MPH^{+}\cdot$ *cannot be a primary product of the photochemical reaction*, otherwise all of the $MPH^{+}\cdot$ would have been formed during the time of the flash (~20 µs). (We have already ruled out any role of triplet states in the photochemistry.) It was this observation which forced us to reinterpret the results of the spin-trapping experiment (see part 1).[1]

When the data in Figure 4 were converted to absorbance, they were found to exhibit linear semilog plots. Thus, the formation processes are either first order or pseudo first order. When experiments were carried out at various concentrations of MP^+, it was found that the first-order constants for *both* the formation of $MPH^{+}\cdot$ *and* the formation of PYH^+ are a linear function of $[MP^+]$. Thus, the formation reactions for $MPH^{+}\cdot$ and PYH^+ must each involve MP^+ as a reactant. From these measurements, the second-order rate constants for the formation of $MPH^{+}\cdot$ and PYH^+ were found to be $(7.7 \pm 1.1) \times 10^8\ M^{-1}\ s^{-1}$ and $(5.8 \pm 0.9) \times 10^6\ M^{-1}\ s^{-1}$, respectively. The fact that PYH^+ forms much more slowly than does $MPH^{+}\cdot$ suggests that the formation of $MPH^{+}\cdot$ preceeds that of PYH^+ in the mechanism.

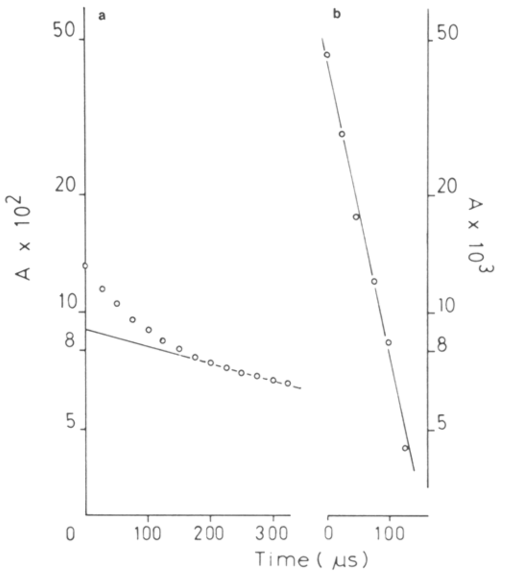


Figure 6. Semilog plot of the decay of the transient observed at 525 nm with pH 4.75: (a) the full decay and (b) the decay of the fast component. The rate constant of the slow-decay was determined from the data for longer times in (a). The contribution from this slow decay portion was subtracted from the data at shorter time in (a) to obtain the points in (b).

It was discovered that the kinetics monitored at 443 nm are complex (see Figure 5). First there is a fast rise followed by a slow decay. The fast rise matches very well the fast rise of $MPH^{+}\cdot$ seen at 443 nm and the slow decay matches well the slow rise of PYH^+ seen at 525 nm. Zaugg[18] has reported that semireduced $PYH_2^{+}\cdot$ absorbs at 433 nm. Thus, we propose that $PYH_2^{+}\cdot$ is formed in reaction 5 and reacts with MP^+ in reaction 6 since the rise time of $PYH_2^{+}\cdot$ is the same as that of $MPH^{+}\cdot$ and the decay of $PYH_2^{+}\cdot$ matches the rise of PYH^+. These results also suggest that protonation (reaction 7) at pH 3 is rapid so that the rise kinetics of $MPH^{+}\cdot$ matches that of $PYH_2^{+}\cdot$. This point will be expanded on later.

The flash kinetics were monitored from 320 to 680 nm at pH 3 in 10-nm intervals to search for a species with a decay time similar to the rise time of $MPH^{+}\cdot$ and $PYH_2^{+}\cdot$ but without success. However, any species absorbing in the 350–400-nm region would be masked by the bleaching of the ground state of MP^+.

(*c*) *Transient Species at 525 nm.* In the course of pH-dependence studies (to be described later), a transient species was detected accidentally around 525 nm. This species decays in the microsecond region and thus decays much faster than the rate of formation of PYH^+ which is in the millisecond region. Figure 6 shows that the decay kinetics at pH 4.75 at 525 nm for the log A vs. time plot are nonlinear and can be decomposed into two first-order decay components. When the same experiment is carried out without nitrogen purging, the fast-decay component disappears, leaving a simple single first-order decay. This suggests that the biphasic character of the decay kinetics at 525 nm is due to the presence of two species, only one of which is sensitive to oxygen.

At first we thought that one of these components might be the species X; however, variation of $[MP^+]$ showed that both the fast-decay and the slow-decay components are independent of $[MP^+]$; whereas, from reaction 5 it is clear that the pseudo-first-order rate constant for the decay of X should be a linear function of $[MP^+]$.

Since the fast-decay component at 525 nm is quenched by oxygen and we have already shown that the fluorescence yield is unaffected by oxygen, we conclude that the fast-

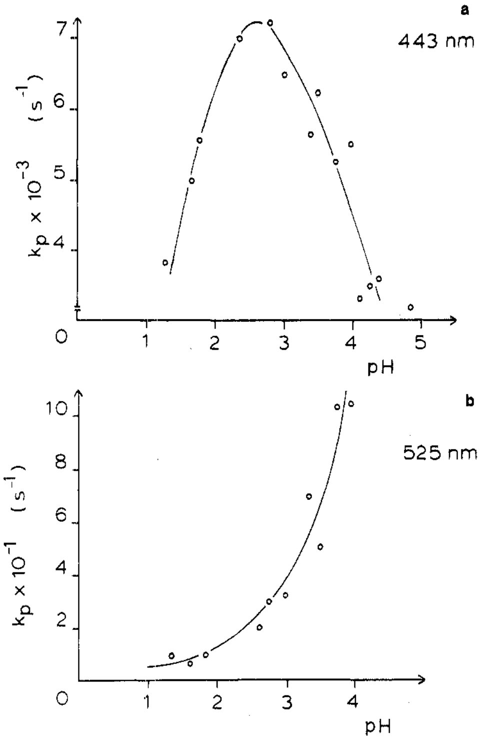


Figure 7. The effects of pH on the pseudo-first-order rate constants (a) for formation of $MPH^{+}\cdot$ and (b) for formation of PYH^+. $[MP^+] = 10^{-5}$ M.

decay component probably arises from triplet–triplet absorption in the triplet state of MP^+. From the measurements, we derive $k_3 = (2. \pm 0.2) \times 10^4\ s^{-1}$. As further confirmation, k_3 is found to be independent of pH.

The pH dependence of the slow-decay component is quite different from that of the fast-decay component. However, the determination of the slow-decay rate constant is complicated by the rise of the $PYH^{+}\cdot$ absorption. This problem was minimized by carrying out the measurements in air-saturated solutions so that the fast-decay component would be eliminated. Hence, many more points in the early-time region could be used to determine the rate constant.

A plot of log k_p vs. pH (where k_p is the pseudo-first-order rate constant) has a slope of -0.9 ± 0.1, which can be taken to be -1. This suggests that the reaction is one in which H^+ is a reactant; probably the reaction is an approach to an acid–base equilibrium. An estimate of the second-order rate constant was evaluated from the average value of $k_p/[H^+]$ and found to be $(6.6 \pm 1.4) \times 10^7\ M^{-1}\ s^{-1}$. This value will be refined later in a computer simulation analysis.

Among all of the known reactants, products, and intermediates, only PYH^+ and $MP\cdot$ absorb near 525 nm. Since the decay occurs at a much earlier time than the formation of PYH^+, we assume that $MP\cdot$ is probably the species involved in the reaction. This would fit with the observed independence of the rate on $[MP^+]$ and the linear dependence on $[H^+]$. We thus propose that $MP\cdot$ and $PYH_2^{+}\cdot$ are the products of reaction 5 and that $MP\cdot$ is then protonated in the subsequent reaction 7 to give $MPH^{+}\cdot$.

(*d*) *pH Dependence of the Rate Constants and the Yield of* $MPH^{+}\cdot$. Reactions 5 and 8 do not involve H^+ as a reactant; hence, we would not expect a strong dependence of the rate constants on pH. The pH dependences of the pseudo-first-order rate constants for formation of $MPH^{+}\cdot$ and PYH^+ are shown in Figure 7, a and b, respectively. Although there is some pH dependence, one should note that the rate constants change by less than an order of magnitude over a pH range of 1–4. Thus, we can rule out H^+ as a direct reactant in reactions 5 and 8.

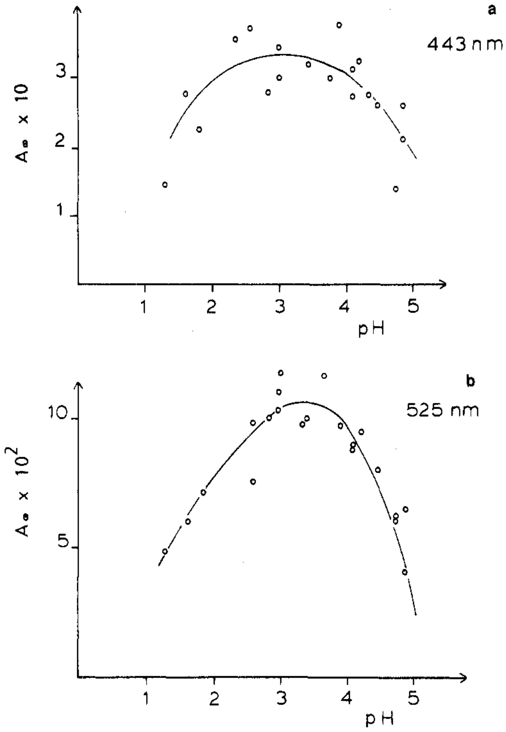


Figure 8. The effect of pH on the ultimate yield of (a) $MPH^{+}\cdot$ measured at 443 nm and (b) PYH^+ measured at 525 nm. $[MP^+] = 10^{-5}$ M.

Figure 8, a and b, display the effects of pH on the ultimate yield of $MPH^{+}\cdot$ and PYH^+ as measured at 443 and 525 nm, respectively. This behavior is very similar to the pH dependence of the quantum yield of formation of $MPH^{+}\cdot$ as determined by EPR (see Figure 6 of part 1)[1] as is expected providing that the stoichiometry remains 2:1 for $[MPH^{+}\cdot]:[PYH^+]$; k_5 also appears to follow this pH dependence (Figure 7a), but k_8 behaves quite differently (Figure 7b). The pH dependence of k_5 will be returned to in the discussion of possible competitive reactions of X.

We have already ascribed the drop-off in yield for pH < 3 to the acid–base equilibrium between $MP^+(S_1)$ and $MPH^{2+}(S_1)$, with the latter being nonfluorescent and photochemically inert. However, the drop-off in yield for pH 3–7 is somewhat of a puzzle. In the following discussion, we will explore some possible explanations.

(*1*) *Disproportionation of* $MPH^{+}\cdot$ *and* $MP\cdot$. Zaugg[18] reported that semireduced $MPH^{+}\cdot$ undergoes disproportionation at basic pH to form MP^+ and MPH and that $MPH^{+}\cdot$ is readily regenerated when such a solution is again made acidic.

An experiment was set up to test whether the decrease in the $MPH^{+}\cdot$ yield at high pH might be due to this disproportionation reaction (reaction 11 in the mechanism). The equilibrium constant for reaction 11 is

$$K_{11} = \frac{[MP^+][MPH][H^+]}{[MPH^{+}\cdot]^2}$$

or

$$\log \frac{2A}{A_0 - A} = -0.5\text{pH} - 0.5 \log K_{11}$$

where A_0 is the absorbance at 443 nm if all of the species were in the form $MPH^{+}\cdot$ and A is the actual absorbance at various pH values.

We must consider the possibility that the deprotonation reaction (reaction 7) may also be important

$$K_7 = [\mathrm{MPH}^+\cdot]/[\mathrm{MP}\cdot][\mathrm{H}^+]$$

or

$$\log \frac{2A}{A_0 - A} = +\mathrm{pH} + \mathrm{p}K_\mathrm{a} + \log 2$$

(Note that $K_\mathrm{a} = K_7^{-1}$.) pK_a has been reported to be 5.7[15] or 6.8.[19] The latter value is probably more reliable as it was determined by using a pulse-radiolysis technique.

Hence, the slope of a plot of log $[2A/(A_0 - A)]$ vs. pH should permit a test of which reaction is important. Indeed, the experimental plot is linear with a slope of -0.6 ± 0.1 and an intercept of 3.8 ± 0.2 from which we obtain $K_{11} = 10^{-7.6}$. These results suggest that disproportionation (reaction 11) is the more important reaction. But we still cannot rule out the possibility that deprotonation (reaction 7) is followed by disproportionation of MP· (reaction 12) which gives the same overall reaction as reaction 11.

The value of $K_{11} = 10^{-7.6}$ agrees with Zaugg's[18] observation that most of the MPH$^+$· undergoes disproportionation between pH 7 and 8 in aqueous solution. However, the EPR spectrum in this pH range, although decreasing in intensity, does not show any change which would indicate a conversion of MPH$^+$· to MP·.[20] If pK_a is indeed ~6.8, then we must favor the model in which any MP· which is formed immediately disproportionates to MP$^+$ and MPH. Indeed, if we combine eq 7 and 11, we obtain $K_{12} = 10^{+6.0}$. This would indicate that MP· should be strongly disproportionated for pH > 6.

In order to determine whether disproportionation is important in our present study, we calculated a conversion factor α defined as

$$\alpha = [\mathrm{MPH}]/[\mathrm{MPH}^+\cdot]_0$$

where $[\mathrm{MPH}^+\cdot]_0$ is the initial concentration of MPH$^+$· assuming no disproportionation. The results (see ref 14 for details) indicate that under the conditions of our experiments where $[\mathrm{MP}^+] \gg [\mathrm{MPH}^+\cdot]$, disproportionation cannot explain the drop in the yield of MPH$^+$· in the pH range 3–7.

(2) *Competitive Reactions Involving the Intermediate X.* Figure 7a indicates that for pH > 3, k_5, the rate constant for formation of MP·, decreases with increasing pH but that k_5 is not directly proportional to [H$^+$]. A possible alternative is that k_5 is linearly dependent on pH, that is, $k_5 = k_5' + k_5''[\mathrm{H}^+]$. Indeed, a plot of k_5 vs. [H$^+$] indicates a possible linear relation with $k_5' = 2.6 \times 10^3$ s^{-1} and $k_5'' = 1.7 \times 10^7$ M^{-1} s^{-1}. However, a decrease in the rate constant k_5 would not account for a drop in the yield of MPH$^+$· unless there is a competitive reaction with X to return the species to the starting reactants. This competitive reaction cannot be a unimolecular reaction since, if it were, the quantum yield of formation of MPH$^+$· should depend on [MP$^+$], which it does not. A possible competitive reaction is

$$\mathrm{X} + \mathrm{MP}^+ \xrightarrow{k_6} 2\mathrm{MP}^+ + \mathrm{OH}^- \qquad (6)$$

This reaction involves the same reactants as reaction 5, that is, in the transition state, the activated complex can follow either reaction 5 or 6. If k_6 were independent of pH, or only slightly dependent, then as pH increases, k_5/k_6 would decrease and the quantum yield of MPH$^+$· would decrease for pH > 3 in the same way as k_5 decreases.

For pH < 3, we can understand the drop in the quantum yield of MPH$^+$· as being due to the formation of the inactive MPH^{2+}(S$_1$) state. However, we should not expect a decrease in k_5 for pH < 3 as is observed (see Figure 7a). A possible explanation is

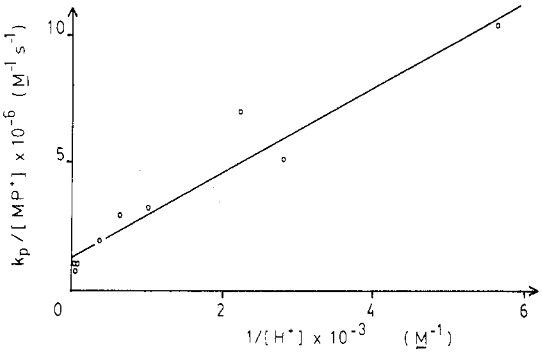


Figure 9. A plot of $k_\mathrm{p}/[\mathrm{MP}^+]$ vs. $1/[\mathrm{H}^+]$. λ = 525 nm, $[\mathrm{MP}^+] = 10^{-5}$ M.

$$\mathrm{X} + \mathrm{MP}^+ \xrightarrow{k_5} \mathrm{MP}\cdot + \mathrm{PYH}_2^+\cdot \qquad (5)$$

$$\mathrm{H}^+ \downarrow\uparrow -\mathrm{H}^+$$

$$\mathrm{XH}^+ + \mathrm{MP}^+ \xrightarrow{k_{14}} \mathrm{MPH}^+\cdot + \mathrm{PYH}_2^+\cdot \qquad (14)$$

At low pH, reaction 14 dominates, and we propose that

$$k_{14} = k_{14}' + \frac{k_{14}''}{[\mathrm{H}^+]}$$

so that as [H$^+$] increases, k_{14} decreases. This would explain the apparent drop in k_5 as pH decreases below 3.

In summary, at pH > 3, we propose that reaction 5 dominates but that k_5 is a linear function of [H$^+$]. Because of the competitive reaction 6, the quantum yield of formation of MPH$^+$· also drops. For pH < 3, reaction 14 becomes important, and we propose that the decrease in the apparent k_5 is due to a linear dependence of k_{14} on $[\mathrm{H}^+]^{-1}$. The drop in the yield of MPH$^+$· is due to the formation of the inactive MPH^{2+}(S$_1$) state. These proposals would explain why both the yield of MPH$^+$· (Figure 8a) and the rate constant k_5 follow bell-shaped curves.

(*e*) *Reactions of PYH$_2^+$·.* Reaction 8 suggests that PYH$_2^+$· is oxidized by MP$^+$ to give PYH$^+$ and MPH$^+$·. Figure 4a shows that the absorbance at 443 nm reaches a steady state after about 0.5 ms, whereas it takes PYH$^+$ (as measured at 525 nm) ~60 ms to reach its final concentration. This apparent discrepancy can be explained by the fact that both PYH$_2^+$· and MPH$^+$· absorb at 443 nm with extinction coefficients which are almost the same. Hence, decay of PYH$_2^+$· to form MPH$^+$· at this wavelength causes little change in absorbance.

Voriskova[21] has reported that the pK_a value of PYH$_2^+$· is 5.63 in aqueous solution. Since this pK_a is within the pH range of our measurements, the acid–base equilibrium (reaction 10) must be considered and may affect the rate of formation of PYH$^+$. The reactions of importance are

$$\mathrm{PYH}_2^+\cdot + \mathrm{MP}^+ \xrightarrow{k_8} \mathrm{PYH}^+ + \mathrm{MPH}^+\cdot \qquad (8)$$

$$\mathrm{PYH}\cdot + \mathrm{MP}^+ \xrightarrow{k_9} \mathrm{PYH}^+ + \mathrm{MP}\cdot \qquad (9)$$

$$\mathrm{PYH}_2^+\cdot \overset{k_{10}}{\rightleftharpoons} \mathrm{PYH}\cdot + \mathrm{H}^+ \qquad (10)$$

where PYH· is the semireduced pyocyanine neutral radical. From these equations, we can derive an expression for k_p, the pseudo-first-order rate constant for formation of PYH$^+$

$$k_\mathrm{p} = (k_8 + k_9 k_{10}/[\mathrm{H}^+])[\mathrm{MP}^+]$$

Thus, a plot of $k_\mathrm{p}/[\mathrm{MP}^+]$ vs. $1/[\mathrm{H}^+]$ should be a straight line with slope $k_9 K_{10}$ and intercept k_8. This plot is shown

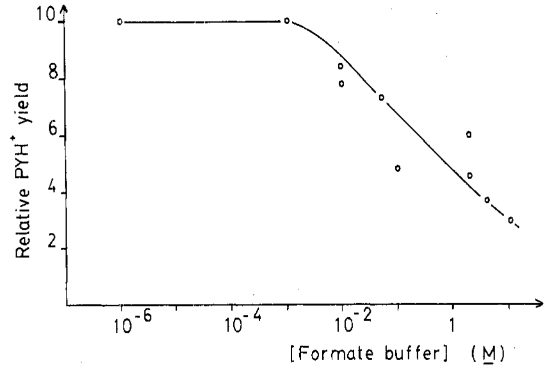


Figure 10. The effect of increasing the concentration of pH 3 formate buffer on the yield of pyocyanine, where 10 is the arbitrary yield of PYH^+ in a 10^{-6} M formate buffer at pH 3.

in Figure 9 from which we derive $k_8 = (1.3 \pm 0.4) \times 10^6$ $M^{-1}\ s^{-1}$ and $k_9 = (7.3 \pm 0.7) \times 10^8\ M^{-1}\ s^{-1}$ by using $K_{10} = 2.3 \times 10^{-6}$; k_8 might be expected to be less than k_9 because of the reaction of two cations in reaction 8. This analysis now completely explains Figure 7b as to why the pseudo rate constant for formation of PYH^+ increases with pH.

5. *Kinetic Simulations.* The following rate laws for reactions 5 and 7 were set up:

$$d[MP\cdot]/dt = k_5[X][MP^+] - k_7[MP\cdot][H^+]$$

$$d[MPH^{+}\cdot]/dt = k_7[MP\cdot][H^+]$$

The coupled differential equations were solved by numerical integration.[22] As the formation of $MPH^+\cdot$ due to reactions 8 and 9 is much slower than that in reaction 7, and since we wish to simulate only the first millisecond after the flash, we have neglected reactions 8 and 9. Our first estimate for k_7 was $6.6 \times 10^7\ M^{-1}\ s^{-1}$. However, using $k_5 = 7 \times 10^8\ M^{-1}\ s^{-1}$, we found that the simulated decay kinetics of MP· are slower than the observed decay. The difficulty seems to be that the rise and decay rates of MP· are comparable. The value of k_7 was then varied in the simulation until the simulated and observed decays matched in the pH 4–5 region; k_7 was then found to be $(1.7 \pm 0.2) \times 10^8\ M^{-1}\ s^{-1}$. The rates of formation and decay of MP· were then calculated. It was found that the decay rate of MP· is faster than its formation rate from pH 3 to 4.5. Hence, the rise kinetics of $MPH^+\cdot$ should match the rise kinetics of $PYH^+\cdot$ in this pH range as observed, and the rate constants obtained at 443 nm are thus reliably k_5 since reaction 5 is the rate-determining step.

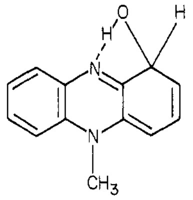
Next, the coupled differential equations involving reactions 5 and 7–11 were solved by numerical integration, and the concentrations of MP^+, $MPH^+\cdot$, PYH^+, and MPH were obtained at various times. The rate constants k_{-10} and k_{11} were assumed to be $10^9\ M^{-1}\ s^{-1}$, and rate constants k_{10} and k_{-11} were then estimated from the equilibrium constants. It was found that all of the simulated kinetic curves match the observed ones within ±10%. The [MPH] for pH 3–7 was less than 10^{-6} of that of $MPH^+\cdot$ which confirms our conclusion that disproportionation is not important in this pH range.

6. *Effect of Added Agents. Effect of Formate.* Formate is known to be a good scavenger of OH radicals, and, indeed, we found that the addition of formate/formic acid buffer at pH 3 did decrease the yield of PYH^+ as measured at 310 nm (see Figure 10). Presumably, formate or formic acid is competing for X[23] (eq 15). From the concentration

$$X + [HCOO^- \text{ or } HCOOH] \xrightarrow{k_{15}} MP\cdot + [COO^-\cdot \text{ or } COOH\cdot] + H_2O \quad (15)$$

of formate buffer which decreases the yield of PYH^+ to one half of the yield in the absence of formate, i.e., 0.8 M, we can derive $k_{15} = (9.6 \pm 0.5) \times 10^3\ M^{-1}\ s^{-1}$ by using $k_5 = 7.7 \times 10^8\ M^{-1}\ s^{-1}$. The rate constant for the reaction of formic acid with a free ·OH radical has been reported as $7.5 \times 10^7\ M^{-1}\ s^{-1}$ at pH 1.[24] This suggests that the intermediate behaves like an ·OH radical but is very much less reactive than free ·OH. Similar results are obtained by using pH 7 acetate buffers where the rate constant for the reaction of X with acetate was found to be $(2.5 \pm 0.3) \times 10^3\ M^{-1}\ s^{-1}$, again much slower than the rate constant for reaction of ·OH with acetate, viz. $1.4 \times 10^7\ M^{-1}\ s^{-1}$.[24] These results provide positive proof that X is *not* the free ·OH radical.

7. *Nature of the Intermediate X.* We propose that the structure of the intermediate X may be the following OH^- adduct to MP^+



Hydrogen bonding of the OH hydrogen with the lone pair on the nitrogen at the 10-position would stabilize the added OH. This would also explain why the protonated excited state $MPH^{2+}(S_1)$ is unreactive. Similar intermediates have been proposed by Wake et al.[6] for the photohydration of protonated phenazine, by Joussot-Dubien and Houdard-Pereyre[25] in the photohydration of aqueous solutions of pyridine, and by Wang[26] in the photohydration of pyrimidines. Ground-state hydrates of many nitrogen heterocyclic compounds have been well characterized.[27,28]

X behaves as an ·OH radical addition reagent as indicated by its reactions with formate and acetate. This behavior can be explained in terms of our proposed structure as a concerted reaction[29] in which the C–O bond is broken, transferring the OH radical to the acceptor molecule in the transition state. The presence of the nitrogen lone pair and the hydrogen-bonding possibilities may aid in this reaction. Our proposed structure is speculative, and there are undoubtedly other possible structures. All we can say for sure is that X is an adduct of water and MP^+ which loses a proton.

The photohydroxylation of the 9,10-anthraquinone-2-sulfonate anion (AQS) in basic aqueous solution seems to be a similar reaction and has been thoroughly studied by Clark and Stonehill.[30,31] They suggest two schemes: one involves a reaction of the triplet state to form a hydrate intermediate (similar to our species X) and the other involves the generation of free ·OH radicals. Recently, Clark and Stonehill[32] presented evidence to indicate that free ·OH radicals are not involved in the reaction. This view is supported by the work of Burchill et al.[33] and Charlton et al.[34] We have found that irradiation of sodium 9,10-anthraquinone-2-sulfonate aqueous solutions containing the spin trap DMPO produces the same OH spin adduct as is found in the MP^+ system. It may be that a similar intermediate is involved.

Another reaction in which a hydrated intermediate occurs may be the photohydroxylation of 7,8-dimethylalloxazine as studied by Dekker et al.[35] and Duren et al.[36] Thus, the formation of a hydrated intermediate may be a fairly common reaction in the aqueous photochemistry of heterocyclic dyes. The common feature appears to be a rapid reaction from the first excited singlet state, the exception being the 9,10-anthraquinone-2-sulfonate reaction.

TABLE II: Summary of the Rate Constants and Equilibrium Constants Determined in the Mechanism of the Photochemistry of 5-Methylphenazinium in Aqueous Solution

reaction	rate constant
$MP^+(T_1) \rightarrow MP^+(S_0)$	$k_3 = 2 \times 10^4\ s^{-1}$
$X + MP^+ \xrightarrow{k_5} MP\cdot + PYH_2^{+}\cdot$	$k_5 = 7.7 \times 10^8\ M^{-1}\ s^{-1\,a}$
$MP\cdot + H^+ \xrightarrow{k_7} MPH^{+}\cdot$	$k_7 = 2.0 \times 10^8\ M^{-1}\ s^{-1\,a}$
$PYH_2^{+}\cdot + MP^+ \xrightarrow{k_8} PYH^+ + MPH^{+}\cdot$	$k_8 = 1.0 \times 10^6\ M^{-1}\ s^{-1\,a}$
$PYH\cdot + MP^+ \xrightarrow{k_9} PYH^+ + MP\cdot$	$k_9 = 7.0 \times 10^8\ M^{-1}\ s^{-1\,a}$
reaction	equilibrium constants
$MPH^{+}\cdot \overset{K_7}{\rightleftharpoons} MP\cdot + H^+$	$K_7 = 1.6 \times 10^{-7\,b}$
$PYH_2^{+}\cdot \overset{K_{10}}{\rightleftharpoons} PYH\cdot + H^+$	$K_{10} = 2.3 \times 10^{-6\,c}$
$2MPH^{+}\cdot \rightleftharpoons MP^+ + MPH + H^+$	$K_{11} = 2.5 \times 10^{-8\,a}$
$2MP\cdot + H^+ \rightleftharpoons MP^+ + MPH$	$K_{12} = 6.3 \times 10^{5\,a}$

[a] This work. [b] Reference 19. [c] Reference 21.

Summary and Conclusions

We have presented a long and complex study of the photochemistry of 5-methylphenazinium; however, we believe that all of the observations we have reported are consistent with the mechanism we proposed at the beginning of the Results and Discussion section. Table II summarizes the rate constants and equilibrium constants that we have determined for the proposed mechanism.

$MPH^{+}\cdot$ is not stable under aerobic conditions and thus would not be suitable in a system designed to produce oxygen. However, X is a strong oxidizing agent and might be coupled to an oxygen generating charge-storage catalyst[2] if $MPH^{+}\cdot$ could be protected from attack by oxygen. Ishizu et al.[37] have reported that $MPH^{+}\cdot$ binds strongly to DNA where it appears to be protected from oxygen attack. Evans and Bolton[38] also found that $MPH^{+}\cdot$ binds strongly to anionic micelles. Thus, there is some hope that $MPH^{+}\cdot$ can be protected from oxygen in such a heterogeneous system. Further research must be conducted to see whether the MP^+ system can function as a photooxidation couple in a water decomposition reaction.

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