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Photochemistry of the Nonspecific Hydroxyl Radical Generator, *N*-Hydroxypyridine-2(1*H*)-thione

Béatrice M. Aveline, Irene E. Kochevar, and Robert W. Redmond*

Contribution from the Wellman Laboratories of Photomedicine, Department of Dermatology, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114

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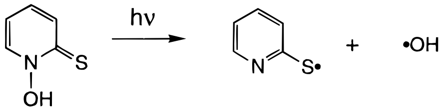
Abstract: The photochemistry of *N*-hydroxypyridine-2(1*H*)-thione (N-HPT) has been investigated in aqueous and organic solvents using laser flash photolysis ($\lambda_{exc} = 308$ or 355 nm). Independent of the environment, UV excitation of N-HPT causes homolytic N−O bond cleavage, which leads to formation of the 2-pyridylthiyl (PyS$^\bullet$) and hydroxyl ($^\bullet$OH) radicals. In aqueous media, this process occurs efficiently from both the anionic and neutral forms ($\Phi_{N-O} \approx 0.20-0.30$). In addition to N−O bond scission, N-HPT undergoes other primary photoprocesses which are pH-dependent. At pH = 7, photoionization ($\Phi_{e^-} = 0.09$ ($\lambda_{exc} = 308$ nm) and 0.05 ($\lambda_{exc} = 355$ nm)) of the anionic form generates the hydrated electron as well as the semioxidized radical of N-HPT. Fast rearrangement of the latter species produces the *N*-oxy-2-pyridylthiyl radical. At pH = 2, where the uncharged structure predominates, formation of an excited triplet state ($E_T \geq 59.5$ kcal mol^{-1}) is observed ($\Phi_T \geq 0.05$ using $\lambda_{exc} = 355$ nm) but photoionization does not take place. The neutral form of N-HPT displays similar photochemical behavior in organic solvents, generating $^\bullet$OH, PyS$^\bullet$, and the triplet state ($\Phi_{N-O} = 0.30-0.45$ and $\Phi_T = 0.03-0.05$ using $\lambda_{exc} = 355$ nm). In nonpolar, aprotic media, the subsequent reaction of PyS$^\bullet$ with the thiol tautomer of N-HPT leads to the formation of an unsymmetric disulfide radical. Reactivities of the different transient species toward potential scavengers have also been investigated. The results obtained show that the simultaneous generation of several highly reactive intermediates is a major drawback in the use of N-HPT as a clean and simple photolytic source of $^\bullet$OH.

Introduction

Recently, UV excitation of *N*-hydroxypyridine-2(1*H*)-thione (N-HPT) has been proposed as an attractive method for the production of hydroxyl radicals ($^\bullet$OH).[1−3] N-HPT, an analogue of aspergillic acid, is known for its antimicrobial, anticancer, and antifungal activities,[4−6] and the industrial use of its zinc and sodium salts in antidandruff shampoos.[7] This compound is also the starting material in the preparation of Barton salts, which are extensively used as radical generators for synthetic purposes (see review[8] and references therein), and, more recently, for kinetic investigations of radical reactivity.[9−11] Although the photosensitivity of N-HPT has long been recognized,[12,13] its ability to generate $^\bullet$OH under irradiation has only been discovered in the last few years.[1−3,14] ESR[14] and chemical trapping[3] experiments have shown that formation of $^\bullet$OH from this molecule occurs in aqueous media[3,14] as well as in organic solvents[1,2,15] *via* homolytic cleavage of the N−O bond (eq 1).



(1)

N-HPT was consequently concluded to be a versatile reagent which can generate $^\bullet$OH under a variety of mild conditions suitable for both chemical[15] and biological[3] investigations. This result appeared all the more important since none of the existing methods developed for producing $^\bullet$OH are fully satisfactory for the *selective* study of $^\bullet$OH reactions.[16]

However, we recently communicated a preliminary investigation of the photochemistry of N-HPT in neutral aqueous solution[17] where the molecule was indeed demonstrated to produce $^\bullet$OH, but not in a selective manner. Photoionization was an equally efficient process generating highly reactive solvated electrons (e_{aq}^-) in good yield. The 2-pyridylthiyl radical (PyS$^\bullet$), formed concomitantly with $^\bullet$OH, as a result of N−O bond cleavage (eq 1), was also found to possess a high reactivity toward various substrates. These results indicate that, at least under neutral pH conditions, N-HPT cannot be considered as an ideal photolytic $^\bullet$OH generator.

In addition to complications arising from production of multiple reactive species, other factors may exert a significant influence on the photochemistry of N-HPT. This compound is

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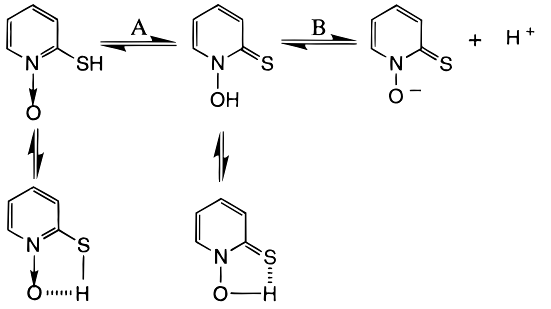
potentially tautomeric (eq 2) and the thione/thiol equilibrium (A) is solvent-dependent: the thione structure predominates in polar media while the less polar thiol tautomer is more likely to exist in nonpolar and aprotic solvents.[18] Furthermore, in the latter environments, N-HPT can undergo intramolecular hydrogen bonding (eq 2). In aqueous solutions, the predominance of the thione form over the thiol has been demonstrated, and the molar ratio between the two tautomeric structures was determined to be 54.[18] As shown by eq 2, N-HPT also exhibits an acid/base equilibrium (B) with a reported pK_a of 4.67.[18]



(2)

Since N-HPT is soluble in both water and organic media, its localization in biological systems may vary from hydrophilic to hydrophobic sites. Therefore, we have extended our preliminary investigation and studied the influence of the nature of an aqueous and a nonaqueous environment on its photochemistry. We report here a comprehensive study of the effects of acid/base equilibrium and thione/thiol tautomerism on the photoprocesses undergone by N-HPT. This compound, far from leading to clean $^{\bullet}$OH formation, exhibits a rich photochemistry. The transient intermediates produced by the different forms of N-HPT have been identified and spectroscopically characterized. We have also quantified their generation and studied their reactivity toward various substrates. The detailed description of the photochemistry of N-HPT given in this paper is aimed at providing new information which should be taken into account when UV excitation of N-HPT is used as a means to generate $^{\bullet}$OH.

Experimental Section

General. ^{1}H NMR spectra were measured with a Varian XL 500 instrument or a Brucker AC 200 spectrometer. Ground-state absorption properties were studied using a Cary 2300 UV−visible spectrophotometer or a Hewlett-Packard HP8451A UV−visible diode array spectrophotometer.

Materials. N-HPT was purchased from Aldrich and recrystallized twice from aqueous ethanol before use (^{1}H NMR (CDCl$_3$) δ 6.30 (1H, dt), 6.67 (1H, dd), 7.34 (1H, dt), 7.74 (1H, dd) and 8.91 ppm (1H, broad, s)). 2-Mercaptopyridine (**1**), 2,2′-dipyridyl disulfide (**2**), and 2,2′-dithiobis(pyridine *N*-oxide) (**4**), whose chemical structures are presented in Figure 1, were also supplied by Aldrich. Compound **2** was recrystallized twice from methanol/water before use. All pyridine derivatives were stored in solid form at −20 °C. No degradation with time was observed under these storage conditions, as verified by absorption spectroscopy and HPLC. Solution samples were prepared immediately before use and were protected from light at all times. 2,2′-Dithiobispyridine mono-*N*-oxide (**3**) was prepared using a solution of an equimolar mixture of compounds **2** and **4**, known to result in the formation of the unsymmetric disulfide **3**.[1,2]

All other reagents were supplied by Aldrich, Kodak, Sigma, or Fisher. Before use, *trans*-β-carotene was purified by recrystallization from benzene/methanol.[19] Solvents were of spectroscopic grade from Fisher, and buffers (10 mM; NaCl/HCl for pH = 2; potassium hydrogen phthalate/NaOH for pH = 4.5, 5 and 5.5; KH_2PO_4/NaOH for pH = 6, 7, and 8, and $NaHCO_3$/NaOH for pH = 10) were prepared using deionized water.

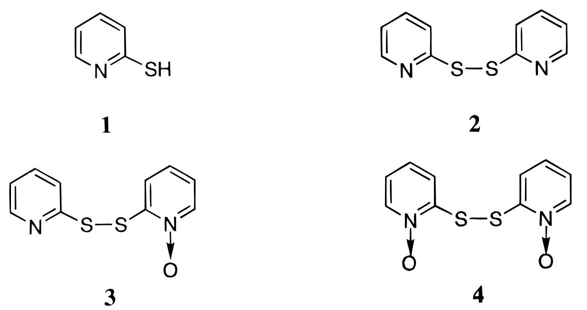


Figure 1. Chemical structures of 2-mercaptopyridine (**1**), 2,2′-dipyridyl disulfide (**2**), 2,2′-dithiobispyridine mono-*N*-oxide (**3**) and 2,2′-dithiobis(pyridine *N*-oxide) (**4**).

Laser Flash Photolysis. The equipment has been described in detail elsewhere.[20,21] Excitation was provided by either the frequency-tripled output of a Quantel YG660A Nd/YAG laser (355 nm, 8 ns duration pulse, up to 10 mJ cm^{-2} pulse^{-1}) or a Lambda Physik EMG 103 MSC XeCl excimer laser (308 nm, 8 ns pulse duration, up to 18 mJ cm^{-2} pulse^{-1}). Quenching rate constants were measured using static samples whereas transient absorption spectra and kinetic signals were recorded employing a flow system ensuring irradiation of a completely fresh volume of solution with each laser pulse. In all cases, the sample was contained in a 10 mm × 10 mm quartz cuvette.

Quantum yields (Φ) of photoprocesses undergone by N-HPT were determined by comparative actinometry as previously described.[22] Φ values were calculated for optically matched solutions of sample and standard (absorbance at λ_{exc} between 0.3 and 0.5) using the following equation:

$$\Phi_S = \Phi_{T(R)}(A_S/A_{T(R)})(\epsilon_{T(R)})/\epsilon_S) \tag{3}$$

where the subscript T(R) refers to the triplet state of the reference used and S refers to the species produced or consumed by the photoprocess considered. ϵ values are the molar absorption coefficients of the appropriate species at their monitoring wavelengths, and A values are the slopes of the linear energy dependence plots of their maximum absorbance following excitation. In these experiments, 2′-acetonaphthone (for which $\Phi_T = 0.84$[23] and $\epsilon_T = 10\,500$ M^{-1} cm^{-1} at 430 nm[22]) and benzophenone (for which $\Phi_T = 1.0$[24] and $\epsilon_T = 7220$ M^{-1} cm^{-1} at 530 nm[25]) in deaerated benzene were used as standards.

Pulse Radiolysis. The setup at the Paterson Institute for Cancer Research (Manchester, U.K.) has been described previously.[26] Experiments were carried out under flow conditions. In aqueous solution, radiolysis results in the formation of several reactive species including $^{\bullet}$OH and e_{aq}^-. The reaction rate constants (k_Q) of e_{aq}^- were determined using deaerated solutions containing *tert*-butyl alcohol (*t*-BuOH, 10 mM) to remove $^{\bullet}$OH. The k_Q values for $^{\bullet}$OH were measured under N_2O-saturated conditions to scavenge e_{aq}^-.

High-Pressure Liquid Chromatography. HPLC analyses were performed on a Hewlett-Packard HP1090 liquid chromatograph equipped with a HP1040 UV−visible diode array detector and controlled using a HP300 computer. Separation was achieved on a 250 mm × 4.5 mm

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Table 1. Absorption Properties (λ_{max} and ϵ) of N-HPT in Different Environments

	λ_{max} (nm) [ϵ (M^{-1} cm^{-1})]^a		
hexane	220	292 [16800]	361 [3800]
cyclohexane	220	292 [20900]	361 [3550]
benzene		290 [17800]	360 [3900]
2-propanol	223	283 [16350]	350 [4550]
methanol	224	284 [11850]	348 [5150]
acetonitrile	224	282 [14150]	348 [5000]
buffer (pH = 2)	216	274 [11450]	334 [5900]
buffer (pH = 7)	246	283 [11600]	334 [3600]

[a] The absorption properties were studied for concentrations of N-HPT between 10 and 500 μM in aqueous media and between 10 and 300 μM in organic solvents. Experimental errors on ϵ values are $\leq$5%.

LC18 (5 μm) column preceded by a 50 mm × 4.5 mm guard column (Waters). A mixture of deionized water and acetonitrile (3:1, vol:vol) was used as eluent at a flow rate of 0.15−0.2 mL/min. The identity of the products was established by comparison of their absorption spectra and retention times with those of authentic samples.

Solutions of N-HPT (~1 mM) in buffer were irradiated in a static cell or a flow-through system immediately before injection (100 μL aliquots) on to the HPLC column. The irradiated sample was treated with 2-3 drops of an aqueous solution of $FeCl_3$ (0.15 M) and the purple precipitate formed, which corresponds to the chelate, tris-(N-HPT)-iron(III),[27] was removed by filtration prior to injection. This treatment simplifies the analysis of the chromatogram by eliminating the starting material, which is otherwise not eluted as a sharp peak but rather over several minutes. The photoproducts of N-HPT were verified to be unaffected by addition of $FeCl_3$ by use of authentic standards. When the irradiations were carried out in organic solvents, the samples, which turned purple after addition of $FeCl_3$, were passed through a 10 mm × 8 mm silica gel column in order to remove the chelate (under these conditions, the photoproducts of N-HPT are quickly eluted).

Results

The primary photochemistry of N-HPT was found to be strongly affected by the acid/base equilibrium undergone by the molecule in aqueous media, whereas the variation in the thione/thiol tautomerism with organic solvents had greater influence on the secondary reaction processes.

(1) N-HPT in Aqueous Media. Ground-State Absorption. The absorption properties of N-HPT were studied in aqueous buffers of pH = 2−8. In all cases, the Beer−Lambert plots were found to be linear in the measured range of ground-state concentrations (from 10 to 500 μM). The maxima (λ_{max}) and corresponding molar absorption coefficients (ϵ) determined for N-HPT are reported in Table 1. Figure 2A displays the absorption spectra of the anionic and neutral forms of N-HPT (eq 2) recorded in buffer at pH = 7 and pH = 2, respectively (*i.e.* under conditions where they predominate over their conjugate).

Laser Flash Photolysis. The results obtained using 308 and 355 nm excitation were virtually identical except for quantum yield values of the primary photoprocesses of N-HPT, as given in the text.

(a) Photochemistry of the Anionic Form of N-HPT. Figure 3 shows the time-dependent transient absorption spectrum recorded after flash photolysis (λ_{exc} = 308 nm) of N-HPT in deaerated buffer at pH = 7. As reported in our preliminary study,[17] the broad band with a maximum around 720 nm, which is quenched in the presence of O_2 or N_2O and decays with a time constant of 450 ns in deaerated solution, is assigned to e_{aq}^-.[28] The transient spectrum (Figure 3A) also displays a strong absorption at 375 nm which decays very rapidly with a lifetime of 130 ns. Increasing the N-HPT concentration (up to 500 μM) or laser intensity (from 1 to 18 mJ cm^{-2} $pulse^{-1}$) does not modify this time constant, suggesting that the species at 375 nm decays through an intramolecular process. Since *trans,-trans*-2,4-hexadien-1-ol (2,4-HD), an efficient thiyl radical quencher,[29,30] does not affect the absorption band at 375 nm, the corresponding species is assigned to the one-electron semioxidized radical of N-HPT, generated on photoionization (eq 4).

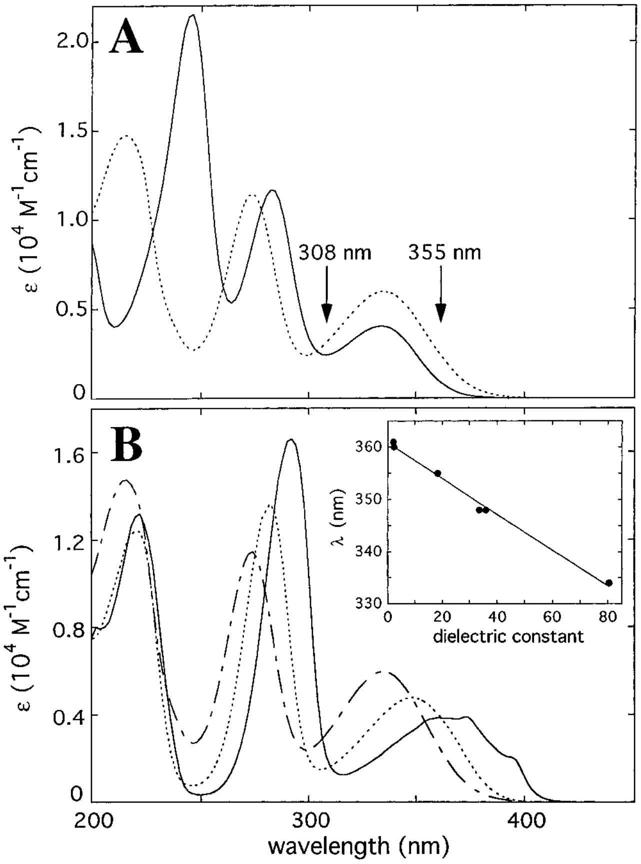
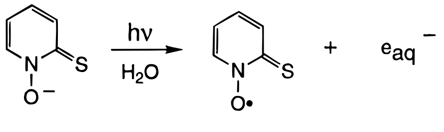


Figure 2. (A) Absorption spectra of the anionic and neutral forms of N-HPT, recorded in aqueous buffer at pH = 7 (—) and pH = 2 (- - -), respectively. (B) Absorption spectra recorded for N-HPT in hexane (—), in acetonitrile (- - -), and in buffer at pH = 2 (— - —). The inset presents the relationship between the longer wavelength absorption maxima and the dielectric constants of the solvents used.



(4)

The negative band around 335 nm, also visible in Figure 3A, is due to photochemical consumption of starting material. Kinetic signals recorded on a short time scale (time window of 1 μs) in this spectral region (~300−350 nm) are composed of an instantaneous depletion followed by a rapid growth in absorbance with a rise time of 100−150 ns. Due to the

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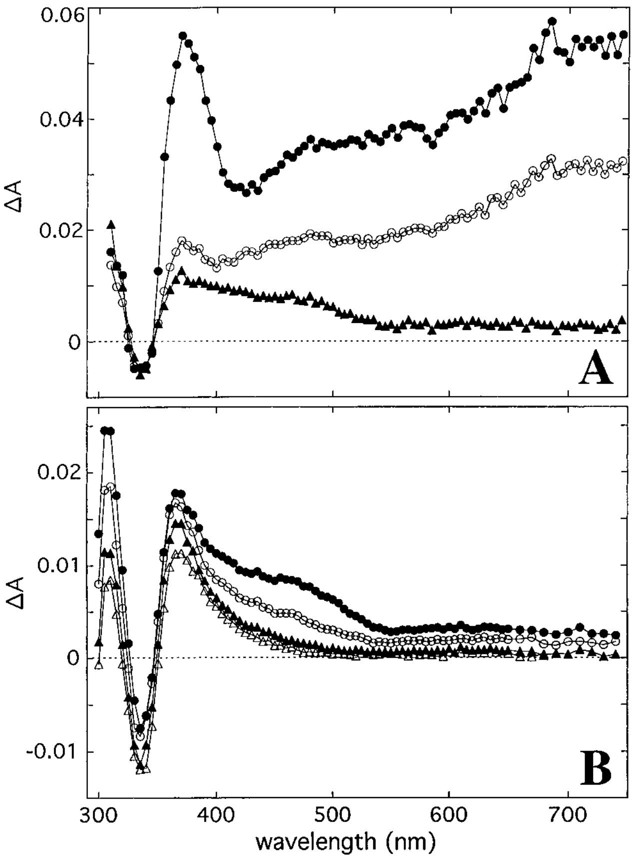

Figure 3. Spectra recorded for 308 nm excitation (8 mJ cm^{-2} $pulse^{-1}$) of 160 μM N-HPT in deaerated aqueous buffer at neutral pH (absorbance = 0.35 at 308 nm): (A) 100 ns (●), 200 ns (○), and 1 μs (▲); (B) 6 μs (●), 20 μs (○), 70 μs (▲) and 350 μs (△) after the laser pulse (λ_{exc} = 308 nm).

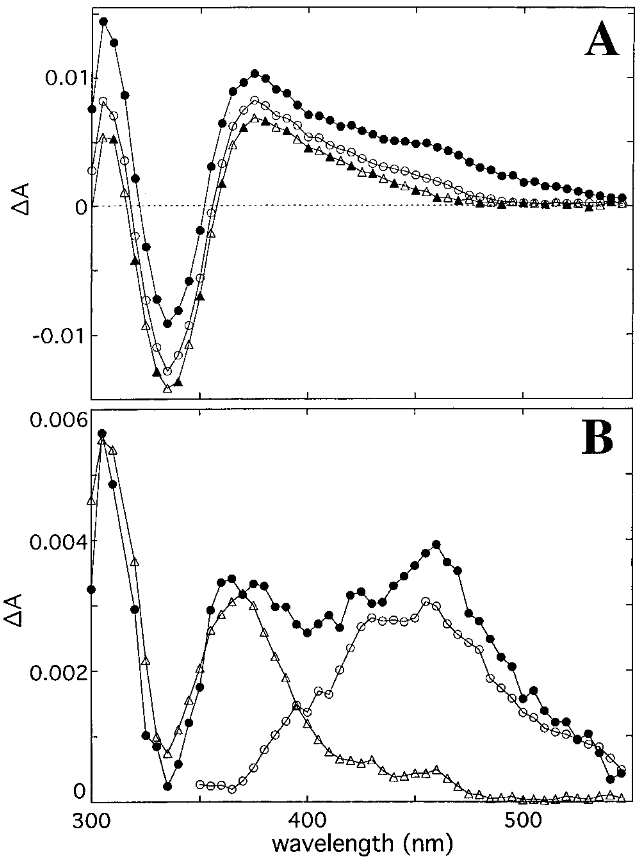

Figure 4. Identification of the transient and permanent species produced by pulsed excitation (λ_{exc} = 308 nm, 8 mJ cm^{-2} $pulse^{-1}$) of 140 μM N-HPT in N_2O-saturated buffer (pH = 7), containing 1 M *t*-BuOH. (A) Spectra recorded for N-HPT in the presence of 2,4-HD (400 μM), 500 ns (△), and in the absence of 2,4-HD, 12 μs (●), 40 μs (○) and 80 μs (▲) after the excitation pulse. (B) The composite spectrum of the transient species (●) corresponds to the sum of the spectrum of $PyS^{\bullet}$ (○), generated by irradiation of a thiohydroxamic ester (λ_{exc} = 308 nm) in neutral buffer, and that of $PyNOS^{\bullet}$ (△), generated by excitation of 2,2′-dithiobis(pyridine *N*-oxide) (λ_{exc} = 308 nm) in aqueous solution (pH = 7).

similarity in time constant for this growth and the decay at 375 nm, we concluded that these two processes are correlated, *i.e.* the intramolecular decay process undergone by the semioxidized radical of N-HPT generates a species which absorbs around 300−350 nm. The species formed was observed to be unaffected by the presence of O_2 but very efficiently scavenged by 2,4-HD, *i.e.* thiyl radical type behavior.[29−31]

Following decay of e_{aq}^- and semioxidized radical of N-HPT, longer-lived absorption bands with maxima at 370 and 460 nm become visible (Figure 3B). The transient spectrum also displays another maximum at 305 nm. Under the conditions used to record the spectrum, the analysis and identification of these absorption bands are complicated by secondary reactions involving the highly reactive e_{aq}^- and $^{\bullet}$OH, the starting material, and/or its photoproduct(s). In order to simplify the system, different experimental conditions were employed.

Figure 4A presents the transient spectrum obtained after excitation (λ_{exc} = 308 nm) of N-HPT in a N_2O-saturated neutral solution containing *t*-BuOH and 2,4-HD.[32] In addition to the negative band at 335 nm, the transient spectrum recorded 500 ns after the pulse displays maxima at 305 and 375 nm. These bands exhibit no absorption changes over several seconds, indicating formation of a stable photoproduct. This spectrum is identical to that recorded for a similar solution in the absence of 2,4-HD, after decay of all the transient absorption bands (*i.e.* $t \geq 80$ μs) (Figure 4A). Investigation of the kinetics of formation of the photoproduct is complicated by the semioxidized radical of N-HPT which absorbs strongly at 375 nm (see Figure 3A) and could not be efficiently quenched out. However, as shown on Figure 4A, the generation of the stable product is already complete 500 ns after the excitation pulse. This product formation was also found to be independent of the excitation wavelength and of the laser energy. Involvement of e_{aq}^-, $^{\bullet}$OH, and thiyl radicals in the formation reaction was excluded by specific quenching experiments. These results suggest that the stable species is a primary photoproduct of the starting material. Although we have insufficient information to identify the reaction, an intramolecular process (rearrangement) of N-HPT is the most likely hypothesis, due to the short time needed for its completion ($\leq$500 ns).

Figure 4A also presents the time-dependent transient absorption spectrum recorded after flash photolysis (λ_{exc} = 308 nm) of N-HPT in a N_2O-saturated solution containing *t*-BuOH (1 M). Under these conditions, the absorption band at 370 nm and the shoulder at 460 nm decay with similar monoexponential kinetics (lifetime of ~25 μs) although a large residual absorption (due to the permanent photoproduct) is present at 370 nm. These absorption bands were also observed to be efficiently quenched by 2,4-HD but unaffected by the presence of O_2, behavior which is typical of similar thiyl radicals.[30,31] The composite spectrum of these species is presented in Figure 4B. The band at 460 nm is attributed to $PyS^{\bullet}$ since an identical absorption was formed

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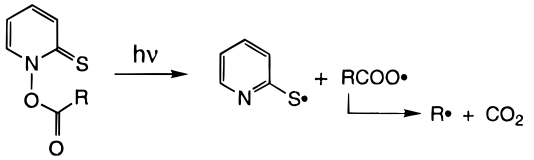
(32) Under these conditions, e_{aq}^- reacts with N_2O and $^{\bullet}$OH is scavenged by reaction with *t*-BuOH. In the presence of 2,4-HD (~300 μM), the species absorbing at 370 and 460 nm are quenched.

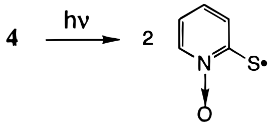
by excitation of various thiohydroxamic esters (Barton salts) in aqueous solution (pH = 7).[33] As shown by eq 5, under



(5)

irradiation, these compounds undergo homolytic N−O bond cleavage leading to the formation of PyS$^\bullet$.[11,30,34] A blue shift of the absorption spectrum of PyS$^\bullet$ in aqueous media (compared to that in organic solvents where the maximum is situated around 490 nm[30]) is not surprising since a similar shift is also observed in the case of the closely related benzenethiyl radical.[35]

The spectrum of the other transient species (Figure 4B) displays absorption bands at 305 and 370 nm and was assigned to the *N*-oxy-2-pyridylthiyl radical (PyNOS$^\bullet$), since it is in full agreement with the spectrum recorded following photolysis (λ_{exc} = 308 nm) of compound **4** in buffer at pH = 7. Similar to the closely related disulfide **2**,[29] under UV irradiation, compound **4** undergoes a S−S bond cleavage which generates PyNOS$^\bullet$ (eq 6), as recently reported.[36]



(6)

To verify that homolytic scission of the N−O bond is the photoprocess responsible for PyS$^\bullet$ formation, we looked for evidence of concomitant production of $^\bullet$OH. Due to its absorption properties,[28] $^\bullet$OH cannot be observed directly. Thiocyanate ions (SCN$^-$) were used to probe the presence of $^\bullet$OH: the reaction with $^\bullet$OH, according to eqs 7 and 8, gives (SCN)$_2$$^{\bullet-}$, which is easily detectable by its absorption around 480 nm.[37]

$$\mathrm{SCN^- + {}^\bullet OH \rightarrow SCN^\bullet + OH^-} \tag{7}$$

$$\mathrm{SCN^- + SCN^\bullet \rightleftharpoons (SCN)_2^{\bullet -}} \tag{8}$$

The transient spectrum recorded following flash photolysis of a neutral solution of N-HPT in the presence of KSCN displays a broad band with a maximum around 480 nm,[38] which is characteristic of (SCN)$_2$$^{\bullet-}$.[37] When DMSO, *t*-BuOH, phenol, or ascorbic acid (known to be efficient $^\bullet$OH scavengers) are added to the solution, the band is not observed, clearly indicating that $^\bullet$OH is generated upon UV excitation of N-HPT. The formation of (SCN)$_2$$^{\bullet-}$ was found to be a rapid process following pseudo-first-order kinetics. No additional detectable production of (SCN)$_2$$^{\bullet-}$ was observed, suggesting that the generation of $^\bullet$OH is solely due to photoinduced N−O bond cleavage. In the presence of N$_2$O (which scavenges e_{aq}^- and gives additional $^\bullet$OH[28]), the intensity of the absorption band at 480 nm was found to be significantly enhanced.

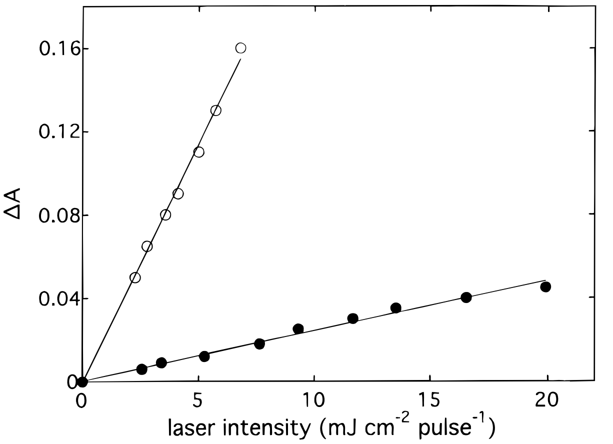


Figure 5. Laser energy dependence of the maximum absorbance of e_{aq}^- at 720 nm (●) under N$_2$-saturated conditions compared to the triplet state formation of 2′-acetonaphthone in deaerated benzene monitored at 430 nm (○). The samples were optically matched at 355 nm (absorbance = 0.3).

Table 2. Quantum Yields of Photoionization (Φ_{e^-}), N−O Bond Cleavage (Φ_{N-O}), and Triplet-State Formation (Φ_T) Determined for N-HPT in Aqueous Buffer (at pH = 7 and pH = 2) Using 308 or 355 nm as the Excitation Wavelength

		λ_{exc} = 308 nm	λ_{exc} = 355 nm
		pH = 7	
Φ_{e^-}	N$_2$	0.094	0.048
Φ_{N-O} (KSCN)	N$_2$	0.12	0.15
	air	0.14	0.13
	N$_2$O	0.24	0.21
Φ_{N-O} (cleavage)[a]	N$_2$	≤0.28	≤0.21
	air	≤0.26	≤0.19
		pH = 2	
Φ_T	N$_2$		≥0.05
Φ_{N-O} (KSCN)	N$_2$	0.19	
Φ_{N-O} (cleavage)[a]	O$_2$	0.27	0.31

[a] The second method used to measure Φ_{N-O} is based on the fact that, under the experimental conditions employed, $^\bullet$OH production is only due to photoinduced N−O bond cleavage, which also generates PyS$^\bullet$. The molar absorption coefficient of PyS$^\bullet$ necessary for the calculation of Φ_{N-O} was beforehand determined to be 600 and 450 M^{-1} cm^{-1} using various thiohydroxamic esters in buffer at pH = 7 and pH = 2, respectively (see ref 5). Errors on the Φ values are <10%.

On UV irradiation in buffer at neutral pH, N-HPT undergoes instantaneous electron ejection and homolytic N−O bond cleavage.[39] The quantum yield of photoionization (Φ_{e^-}) was determined using the comparative technique by monitoring the variation of the maximum absorbance of e_{aq}^- at 720 nm (where ϵ = 19 000 M^{-1} cm^{-1} [28]) with laser energy. Irrespective of the excitation wavelength used, the intensity dependence of the solvated electron absorbance was found to be linear (Figure 5), indicating that photoionization of N-HPT occurs *via* a monophotonic process. As shown by the data reported in Table 2, the Φ_{e^-} values obtained for N-HPT are wavelength-dependent, increasing with decreasing λ_{exc}.

For determination of the quantum yield of $^\bullet$OH generation ($\Phi_{^\bullet OH}$), enough KSCN was used to quench all the $^\bullet$OH produced. Under these conditions, the quantum yield of (SCN)$_2$$^{\bullet-}$ formation is equivalent to $\Phi_{^\bullet OH}$, which was calculated from eq 3 using ϵ = 6550 M^{-1} cm^{-1} at 500 nm[40] for (SCN)$_2$$^{\bullet-}$.[41]

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(33) Since the ester derivatives of N-HPT are hydrolyzed in aqueous media, cold buffer was used as solvent to retard the degradation. Thiohydroxamic esters with R = (CH$_3$)$_3$C and R = C$_6$H$_5$ (eq 5) were studied, using λ_{exc} = 308 or 355 nm.

(34) Bohne, C.; Boch, R.; Scaiano, J. C. *J. Org. Chem.* **1990**, *55*, 5414−5418.

(35) Scaiano, J. C. *Handbook of Organic Photochemistry*; CRC Press, Inc.: Boca Raton, FL 1988; Vol. II.

(36) Alam, M. M.; Watanabe, A.; Ito, O. *Photochem. Photobiol.* **1996**, *63*, 53−59.

(37) Ellison, D. H.; Salmon, G. A.; Wilkinson, F. *Proc. R. Soc. London, Ser. A* **1972**, *328*, 23−36.

(38) See Figure 3 in ref 17.

(39) The lack of energy transfer to merocyanine 540 was used to verify that N-HPT in buffer at pH = 7 does not undergo intersystem crossing, leading to triplet state formation.

Table 3. Reactivities of the Transient Species Formed by Pulsed Excitation of N-HPT in Aqueous Media

	k_Q (M^{-1} s^{-1})[a]				
	e_{aq}^-	$^•OH$	$PyNOS^•$	$PyS^•$	^{3}(N-HPT)*
O_2			nr[b]	nr[b]	2.5×10^9
2,4-HD			1×10^9	2.8×10^9	3.5×10^9
N-HPT (pH = 7)	8.1×10^9 9×10^9 [c]	 9×10^9 [c]	4×10^7	7×10^7	
N-HPT (pH = 2)				3.5×10^7	5.5×10^9

[a] Unless otherwise stated, the rate constants were determined by monitoring the decay of the appropriate transient absorption for different substrate concentrations (from 20 to 300 μM in N-HPT and from 40 to 450 μM in 2,4-HD). In the case of the thiyl radicals, a N_2O-saturated solution of N-HPT containing *t*-BuOH (1 M) was used. The k_Q values for reaction with oxygen were calculated using the approximation of a complete removal of O_2 under N_2-saturated conditions and O_2 concentration of 9×10^{-4} and 1.8×10^{-4} M in O_2- and air-saturated buffer, respectively. [b] The thiyl radicals were found to be nonreactive (nr) toward oxygen. [c] These k_Q values were determined by pulse radiolysis using N-HPT concentrations between 36 and 100 μM.

This method requires the presence of high salt concentrations which could influence the photoprocesses and affect the experimental results. Since generation of $^•OH$ from N-HPT was demonstrated to result solely from N−O bond cleavage, $\Phi_{^•OH}$ (therefore equivalent to $\Phi_{PyS^•}$, the quantum yield of $PyS^•$ formation) was also determined by monitoring the maximum absorbance of $PyS^•$ at 460 nm with laser energy. The data obtained by the latter method should be considered as an upper limit since $PyNOS^•$, the other thiyl radical produced, has a weak absorption at 460 nm (see Figure 4B). Table 2 presents the values of $\Phi_{^•OH}$ ($=\Phi_{N-O}$) determined for N-HPT in buffer at neutral pH. Taking into account the intrinsic limitations of the methods employed, $\Phi_{^•OH}$ is ≤ 0.27 using $\lambda_{exc} = 308$ nm and ≤ 0.22 using $\lambda_{exc} = 355$ nm. In contrast to a recent report,[14] O_2 was not found to affect the production of $^•OH$. It is also worth noting that $\Phi_{^•OH}$ measured in the presence of N_2O corresponds (within experimental error) to the sum of Φ_{e^-} and $\Phi_{^•OH}$ determined in the absence of N_2O.

As mentioned above, identification of the primary intermediates generated by UV excitation of N-HPT was complicated by the high reactivity of e_{aq}^- and $^•OH$ toward their precursor. The rate constants of reaction between e_{aq}^-, $^•OH$, and N-HPT were determined by flash photolysis and pulse radiolysis. The k_Q values obtained are reported in Table 3.[42]

We have previously noted for thiohydroxamic esters that secondary reactions with $PyS^•$ lead to further consumption of starting material.[30] As similar thiyl radicals are produced from N-HPT, the reactivity of $PyS^•$ and $PyNOS^•$ toward their precursor was investigated using N-HPT concentrations from 20 to 300 μM.[43] Very similar bimolecular k_Q values were obtained for $PyS^•$ and $PyNOS^•$ (Table 3). The reactions of these radicals with their precursor result in further loss of N-HPT which appears as a slow bleaching process subsequent to the instantaneous decrease in absorbance due to photochemical depletion. By monitoring the slow bleaching for several concentrations of N-HPT, a bimolecular rate constant of 5×10^7 M^{-1} s^{-1} is obtained, corresponding to the combination of the reactions of $PyS^•$ and of $PyNOS^•$ with their precursor.

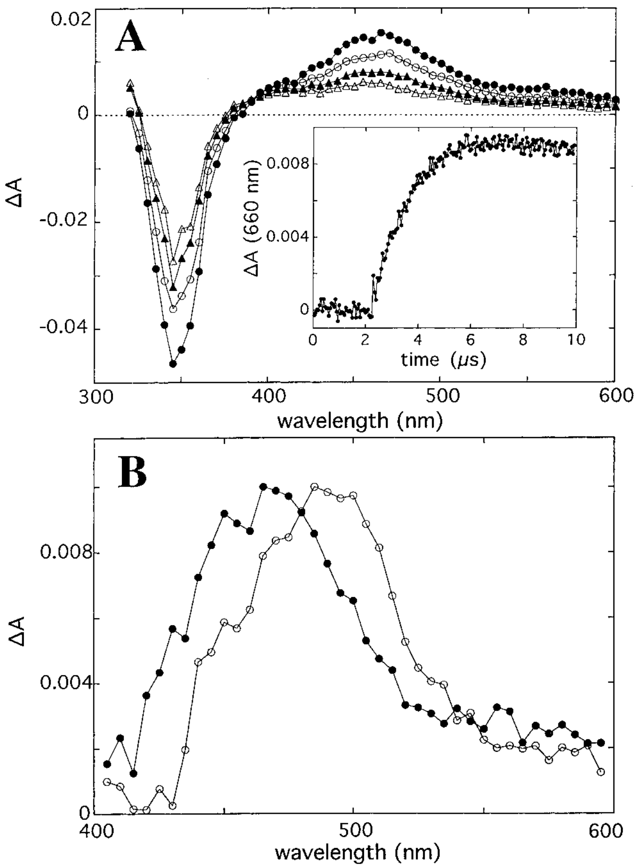

Figure 6. (A) Spectra recorded for 128 μM N-HPT (absorbance = 0.39 at 308 nm) in deaerated buffer at pH = 2, 350 ns (●), 1 μs (○), 2 μs (▲), and 3 μs (△) after the excitation pulse (λ_{exc} = 308 nm, 8.5 mJ cm^{-2} $pulse^{-1}$). The inset presents the kinetic signal corresponding to the sensitized formation of ^{3}(M540)* recorded at 660 nm for a solution of N-HPT (40 μM) in deaerated buffer at pH = 2 containing merocyanine 540. (B) Absorption spectra of ^{3}(N-HPT)* (●) and of $PyS^•$ (○) generated by flash photolysis of N-HPT at pH = 2.

The k_Q values measured for the reaction of the thiyl radicals with N-HPT are much lower than those determined for the addition of $PyS^•$ to thiohydroxamic esters in organic solvents [(3−4) × 10^9 M^{-1} s^{-1}].[30] Two different hypotheses can be considered to explain this discrepancy: the rate of addition reaction of thiyl radicals to carbon−sulfur double bond is solvent-dependent or the interactions of $PyS^•$ and $PyNOS^•$ with N-HPT and with its ester derivatives are of different nature. In order to distinguish between these hypotheses, the reactivity of $PyS^•$ toward thiohydroxamic esters was studied in aqueous media.[33] k_Q values of (2−5) × 10^7 M^{-1} s^{-1} (*i.e.* very similar to those determined in the case of N-HPT) were obtained, supporting the former hypothesis. This conclusion is also confirmed by the high rate constants measured for the reaction of $PyS^•$ with N-HPT in organic solvents (*vide infra*).

(b) Photochemistry of the Neutral Form of N-HPT. The transient absorption spectrum formed on 308 nm photolysis of a deaerated solution of N-HPT buffered at pH = 2 and recorded using a short time scale is shown on Figure 6A. This spectrum differs markedly from that obtained on excitation of the anionic form of N-HPT (see Figure 3). It displays an absorption band with a maximum at 470 nm. The corresponding kinetic signal

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(40) Lambert, C.; Sarna, T.; Truscott, T. G. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 3879−3882.

(41) In order to correct for the absorption of the thiyl radicals at that wavelength, two sets of experiments were carried out: one in the absence of KSCN (where only $PyS^•$ and $PyNOS^•$ absorb), the other in the presence of 0.2 M KSCN. $A_{(SCN)_2^{•-}}$ was calculated as the differential between the two initial slopes of energy dependence plots obtained.

(42) Pulse radiolysis experiments revealed that the interactions between the e_{aq}^-, $^•OH$, and N-HPT yield intermediates which absorb around 345 and 420 nm, respectively, and subsequently lead to the formation of other transient absorptions complicating the identification of the reaction products.

(43) For [N-HPT] > 300 μM (*i.e.* for high concentrations of radicals produced), the decays at 370 nm (for $PyNOS^•$) and at 460 nm (for $PyS^•$), which are originally first-order, become increasingly second-order in character as the radical-radical reaction processes become more probable.

is composed of two exponential decays (with time constants of 1 and 30 μs), indicating the presence of two different species absorbing around 470 nm. Both components were found to be efficiently quenched by 2,4-HD, but O_2 was observed to affect only the shorter-lived intermediate. Using O_2-saturated conditions, one can obtain the spectrum of the longer-lived species (Figure 6B). It was assigned to $PyS^\bullet$ as a similar absorption was formed by photolysis of different thiohydroxamic esters at $pH = 2$.[33]

A negative band with a maximum at 345 nm which corresponds to loss of starting material is also visible. From 320 nm to the isosbestic point at 390 nm, the instantaneous depletion of N-HPT is followed by a fast partial recovery of absorbance. The rise time of the secondary process, which is not observed in the presence of 2,4-HD (300 μM) or O_2 in the solution, was found to be 850 ns, *i.e.* close to that of the shorter-lived species absorbing at 470 nm. In addition, the time constants of the fast decay at 470 nm and of the growth at $\lambda <$ 390 nm were observed to be similarly strongly reduced with increasing N-HPT concentration (*vide infra*), which is typical of thione triplets.[44,45] Energy transfer was used to unambiguously identify the short-lived species as a triplet state. The transient spectrum recorded after photolysis of N-HPT in deaerated buffer at $pH = 2$ in the presence of merocyanine 540 (50 μM) exhibits an absorption band with a maximum around 660 nm, which is characteristic of ^{3}(M540)*, the triplet state of the cyanine dye.[46,47] The kinetic signal corresponding to the sensitized formation of ^{3}(M540)* is presented in the inset of Figure 6A. Therefore, the shorter-lived species (the absorption spectrum of which is displayed in Figure 6B) was identified as ^{3}(N-HPT)*, the triplet state of N-HPT and the growth observed between 320 and 390 nm (Figure 6A) was attributed to recovery of the ground state of N-HPT due to the intersystem crossing ($T_1 \rightarrow S_0$) of the triplet state.

Similar to N-HPT at neutral pH, the transient spectrum recorded in deaerated buffer at $pH = 2$ in the presence of KSCN displays the characteristic absorption band of $(SCN)_2^{\bullet -}$ at 480 nm. This band was significantly reduced by addition of $^\bullet OH$ quenchers. O_2 was found to have no effect on its intensity indicating that the triplet state of N-HPT is not involved in the generation of $^\bullet OH$.[48]

Thus, under UV excitation at $pH = 2$, N-HPT undergoes at least two different primary photoprocesses: homolytic cleavage of the N$-$O bond, which leads to the formation of $PyS^\bullet$ and $^\bullet OH$, and intersystem crossing producing ^{3}(N-HPT)*. The Φ_{N-O} values obtained are reported in Table 2. The quantum yield of intersystem crossing (Φ_T) cannot be determined accurately, as the molar absorption coefficient of the triplet state is unknown. However, Φ_T can be estimated by comparative actinometry, using the recovery process observed at 345 nm, assigned to $T_1 \rightarrow S_0$ intersystem crossing. The amplitude of this recovery was monitored as a function of laser energy.[49] The lower limit of Φ_T was calculated from eq 3 and found to be 0.05 using $\lambda_{exc} =$ 355 nm.

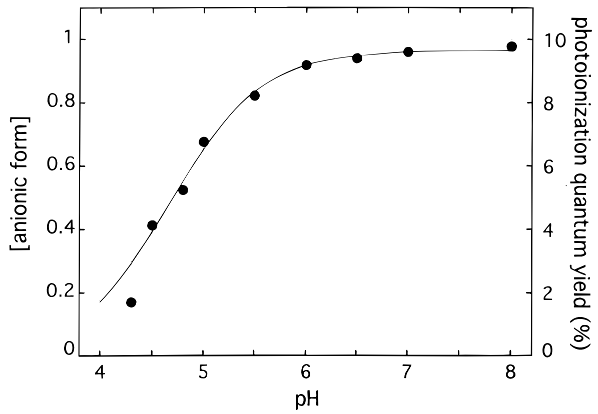

Figure 7. Variations of the photoionization quantum yield of N-HPT (●) as a function of pH compared to the corresponding variations of relative concentration of the anionic form present in the solution (—).

With the knowledge of Φ_T, an estimation of ϵ_T, the molar absorption coefficient of the triplet state of N-HPT in buffer at $pH = 2$ can be obtained using the comparative technique and benzophenone as actinometer. The maximum amplitude of the signal at 470 nm (*i.e.* the λ_{max} of ^{3}(N-HPT)*) was monitored as a function of laser energy. In order to correct for the absorbance of $PyS^\bullet$ at that wavelength, two sets of experiments were carried out: under N_2- and O_2-saturated conditions. Using eq 3, ϵ_T was determined to be $\leq 2100\ M^{-1}\ cm^{-1}$.

Table 3 presents the reaction rate constants, k_Q, measured for $PyS^\bullet$ and ^{3}(N-HPT)* generated by N-HPT at $pH = 2$. The bimolecular rate constant for the reaction of $PyS^\bullet$ with its precursor is very close to that obtained at $pH = 7$, suggesting that $PyS^\bullet$ reacts very similarly with the neutral and the anionic forms of starting material. The high k_Q value determined for the reaction of ^{3}(N-HPT)* with 2,4-HD, indicates that N-HPT has a triplet state energy, $E_T \geq 59.5$ kcal mol^{-1},[50] which is in agreement with the E_T upper limit value of 63 kcal mol^{-1} extracted from a published phosphorescence spectrum recorded for N-HPT in a methanol/ethanol mixture.[51]

(c) Influence of pH on Photoionization. The absence of the characteristic absorption band of e_{aq}^- at 720 nm in the transient spectrum recorded at $pH = 2$ is not itself indicative of a lack of photoionization since, in acidic media, any solvated electron produced would be rapidly quenched by protons. In order to determine whether or not N-HPT undergoes photoionization from the neutral form, Φ_{e^-} was measured, by comparative actinometry, in buffers of different pH values which bracket the pK_a. Figure 7 illustrates that the values of Φ_{e^-} obtained for N-HPT correlate with the relative concentration of anionic form present in solution as a function of pH. We conclude that (1) the anionic form of N-HPT is responsible for photoionization and (2) the efficiency of photoinduced electron ejection from this negatively-charged structure is identical, over the pH range studied.

Photoproducts of N-HPT. HPLC analysis of irradiated samples of N-HPT in aqueous media revealed that the main photoproducts are various disulfides. Irrespective of the irradiation conditions used,[52] compounds **3**, **4**, and **2** were found to be the major products. This observation is in agreement with results from previous published studies carried out under steady-

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(44) de Mayo, P. *Acc. Chem. Res.* **1976**, *9*, 52−59.

(45) Bhattacharyya, K.; Ramamurthy, V.; Das, P. K. *J. Phys. Chem.* **1987**, *91*, 5626−5631.

(46) Redmond, R. W.; Srichai, M. B.; Bilitz, J. M.; Schlomer, D. D.; Krieg, M. *Photochem. Photobiol.* **1994**, *60*, 348−355.

(47) When merocyanine 540 alone was excited under the same conditions, the intensity of the 660 nm band was about 5% of that recorded in the presence of N-HPT.

(48) The absorbances at 480 nm due to formation of $(SCN)_2^{\bullet -}$ under N_2- and O_2-saturated conditions were compared after correction for the absorption of the triplet state and $PyS^\bullet$ at this wavelength.

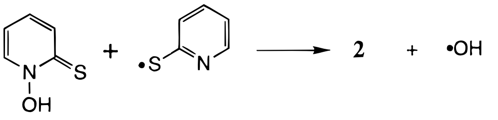
(49) The triplet state of N-HPT is assumed to have no absorption at 335 nm. In eq 3, an ϵ value of 5900 $M^{-1}\ cm^{-1}$ was used for the molar absorption coefficient of the ground-state.

(50) Murov, S. L. In *Handbook of Photochemistry*; Marcel Dekker: New York, 1973; p 29.

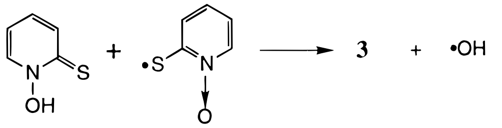
(51) Sakurai, T.; Inoue, H. *J. Chem. Soc., Perkin Trans. 2* **1984**, 2031−2036.

(52) N-HPT in N_2O-, N_2-, air, or O_2-saturated buffer at $pH = 2$, 7, or 10 was irradiated using 308 or 355 nm as excitation wavelength, the laser intensity was varied from 1 to 18 mJ cm^{-2} pulse^{-1}.

state irradiation conditions in aqueous media or in organic solvents.[1,2,12,13,15] As shown by eqs 9 and 10, addition of the



(9)



(10)

thiyl radicals, PyS• and PyNOS•, to the carbon−sulfur double bond of the parent molecule gives •OH and disulfides **2** and **3**, respectively. Formation of **4** can result from radical−radical recombination of PyNOS• and/or from the slow disproportionation undergone by the unsymmetric disulfide **3**.[1,2] As observed by HPLC, UV excitation of N-HPT in aqueous media also generates other products with very low yield. The presence of such minor compounds in the photoreaction mixture of N-HPT has previously been reported.[12] However, in biological systems, these product formations are likely to be less important as the thiyl radicals may be in the presence of better substrates.

(2) N-HPT in Organic Solvents. Ground-State Absorption. Figure 2B displays the spectra recorded for N-HPT in hexane and in acetonitrile and, for comparison, in aqueous buffer at pH = 2, where the same neutral form (eq 2) predominates. In all studied media (cyclohexane, benzene, hexane, methanol, acetonitrile, and 2-propanol), the Beer−Lambert plots were found to be linear in the measured range of concentrations (from 10 to 300 μM). The results obtained (Table 1) show that the absorption spectrum of N-HPT undergoes a blue shift with increasing solvent polarity and that this hypsochromic effect is more pronounced for the longest than the shortest wavelength bands. A linear relationship was observed between the longer wavelength absorption maxima of N-HPT and the dielectric constant of the solvent. As seen on the inset of Figure 2B, the absorption properties of N-HPT in aqueous solution fit perfectly into this trend. By analogy with other pyridine *N*-oxides, the thiol tautomer of N-HPT is expected to display no absorption above 340 nm, hence if present in solution, will not be excited at 355 nm.

Laser Flash Photolysis. (a) Photochemistry of N-HPT in Polar Solvents. The transient absorption spectra formed on photolysis of N-HPT in methanol, acetonitrile, and 2-propanol were very similar under aerated and deaerated conditions. Their profile was also unaffected by an increase of laser intensity (from 1 to 18 mJ cm^{-2} pulse^{-1}). Figure 8 shows an example of the time-dependent transient spectrum recorded for N-HPT in N$_2$-saturated methanol using 355 nm excitation. The spectrum displays an absorption band with a maximum at 495 nm which is assigned to PyS• since it is identical to that observed following pulsed irradiation of various thiohydroxamic esters[11,30,34] or by flash photolysis of 2,2′-dipyridyl disulfide[29] in organic solvents. The thiyl radical decays monoexponentially with a lifetime determined to be 2−3 μs under the experimental conditions used to record the spectrum. Monitoring the 495 nm decay as a function of N-HPT concentration (from 30 to 300 μM) gave a rate constant of 4×10^9 M^{-1} s^{-1} for the reaction of PyS• with its precursor. Similarly, k_Q values of 1×10^8 and 2.6×10^9 M^{-1} s^{-1} were obtained for the quenching of PyS• by methyl methacrylate (MMA) and 1,3-cyclohexadiene (1,3-CHD), respectively.[53] This reactivity is identical to that observed for PyS• when thiohydroxamic esters were used as photolytic precursors in organic solvents.[30] As previously noted,[30] the higher electrophilicity of PyS•, compared to that of substituted and unsubstituted benzenethiyl radicals, is responsible for its higher reactivity toward unsaturated systems.

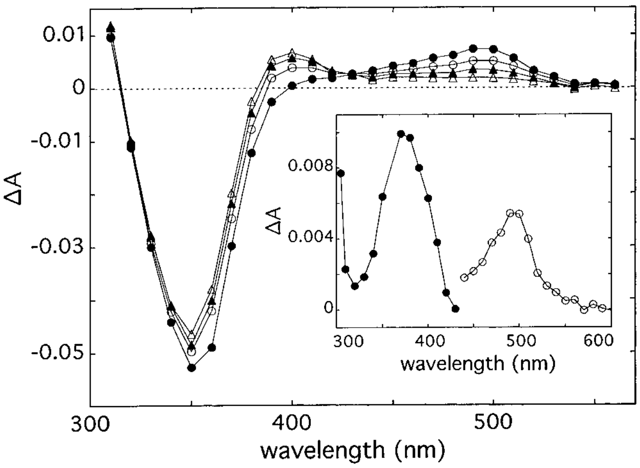


Figure 8. Transient absorption spectra recorded 300 ns (●), 1 μs (○), 2 μs (▲), and 3 μs (△) after excitation (λ_{exc} = 355 nm, 8 mJ cm^{-2} pulse^{-1}) of 90 μM N-HPT in deaerated methanol (absorbance = 0.37 at 355 nm). The inset presents the absorption spectrum of the photoproduct of N-HPT (●) and that of PyS• (○).

The transient absorption spectrum of N-HPT in methanol (Figure 8) also displays a negative band with a minimum at 350 nm (due to loss of starting material) and an isosbestic point at 430 nm. All the kinetic signals recorded between 300 and 430 nm have in common a fast growth in absorbance with rise time of 2−3 μs. Subsequent to this process (which is not observed in the presence of MMA but is unaffected by O$_2$), the signals do not change over several seconds. Increasing N-HPT ground-state concentration was found to have identical effect on the kinetics of decay of PyS• and the growth, suggesting that the thiyl radical mediates formation of a stable product (the absorption spectrum of which is presented in the inset of Figure 8). The ground-state absorption properties of this photoproduct and its retention time in HPLC correspond to those of the 2-mercaptopyridine (**1**).[54]

A transient spectrum was also recorded for N-HPT in the presence of 1,3-CHD (5 mM, in order to quench the transient species).[55] In addition to the precursor depletion, it exhibits an absorption with a maximum around 305 nm. This band is formed within the laser pulse and does not undergo any changes over several seconds. Similar to aqueous media, the corresponding product most probably results from an intramolecular rearrangement of N-HPT.

As the neutral form of N-HPT was observed to generate a triplet state in buffer at pH = 2, the same process was investigated in organic solvents. However, the absence of obvious spectral differences under aerated and deaerated conditions (*vide supra*) suggests an inefficient intersystem crossing. The inset A of Figure 9, which displays the kinetic signals recorded for static samples of N-HPT under N$_2$- and O$_2$-saturated conditions, shows evidence of the formation of a short-lived ($\tau \approx$ 500 ns), oxygen-dependent species. In addition, in the presence of merocyanine 540 (100 μM), the transient absorption

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[53] The concentration of MMA was varied from 10 to 100 mM, that of 1,3-CHD from 0.5 to 10 mM. Oxygen-saturated conditions were used to determined the k_Q values of PyS•.

[54] The identification of this compound is facile since it is the only photoproduct of N-HPT to present an absorption at long wavelength (≥350 nm).

[55] 1,3-CHD was used instead of MMA as only the former reagent quenches both PyS• and the triplet state (*vide infra*).

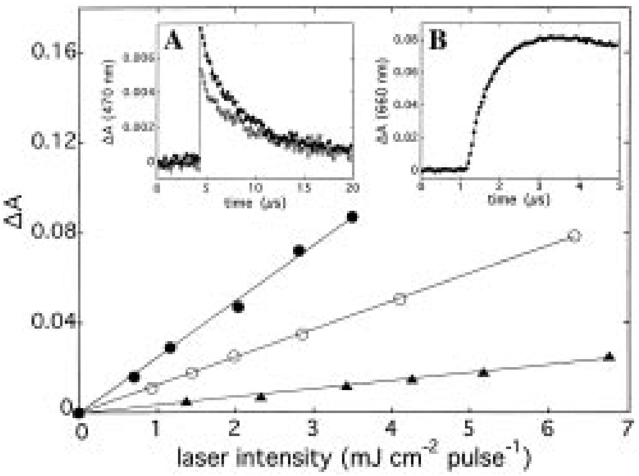

Figure 9. Determination of the quantum yield of intersystem crossing for N-HPT in methanol using 355 nm as excitation wavelength. Laser energy dependence of the maximum absorbance of the signal at 660 nm recorded for N-HPT in the presence of M540 (250 μM) (○) and for M540 alone (250 μM, see ref 58) (▲) compared to the triplet-state formation of benzophenone in deaerated benzene monitored at 530 nm (●). The samples were optically matched at 355 nm (absorbance = 0.35). Inset A presents the kinetic signals recorded for N-HPT at 470 nm in N_2- (●) and O_2- (○) saturated methanol. Inset B displays an example of signal corresponding to the sensitized formation of ^{3}(M540)*.

spectrum was found to display the characteristic absorption band of ^{3}(M540)* at 660 nm.[46] By monitoring the growth corresponding to the sensitized production of ^{3}(M540)* at 660 nm for several concentrations of cyanine (from 15 to 250 μM), the reaction rate constant of ^{3}(N-HPT)* with M540 was found to be 2×10^{10} M^{-1} s^{-1} in methanol and 9.2×10^9 M^{-1} s^{-1} in acetonitrile.

The results described above show that under UV excitation (λ_{exc} = 308 or 355 nm) in polar organic solvents, N-HPT undergoes intramolecular rearrangement which gives a photoproduct, homolytic N−O bond cleavage leading to the formation of $^\bullet$OH and PyS$^\bullet$ and intersystem crossing generating ^{3}(N-HPT)*. Φ_{N-O} was determined by following the variations of the maximum absorbance of PyS$^\bullet$ at 495 nm with laser energy, under O_2-saturated conditions. The Φ_{N-O} values calculated from eq 3 using the known molar absorption coefficients of PyS$^\bullet$ in organic media[29,30] were found to be 0.35 and 0.28 in acetonitrile and methanol, respectively (λ_{exc} = 355 nm).

Since ^{3}(N-HPT)* cannot be easily observed,[56] Φ_T was determined by an indirect method based on the sensitized generation of the triplet state of merocyanine 540. In the presence of enough cyanine dye to trap all ^{3}(N-HPT)* photoproduced (*i.e.* for [M540] = 250 μM),[57] the quantum yield of ^{3}(M540)* formation is equivalent to Φ_T. The variations of the amplitude of the signal recorded at 660 nm were monitored as a function of laser energy (Figure 9). Two sets of experiments were carried out: one in the absence of N-HPT[58] (where a small amount of ^{3}(M540)* is generated by direct excitation of the cyanine) and the other in the presence of N-HPT (where further ^{3}(M540)* is produced by sensitization). The *A* value used in eq 3 was calculated as the differential between the two slopes of the energy dependence plots obtained.[59] Φ_T determined by this method was found to be 0.030 in acetonitrile and 0.036 in methanol (λ_{exc} = 355 nm).

(b) Photochemistry of N-HPT in Nonpolar Solvents. The spectra recorded at the earliest detection times after pulsed excitation ($t \leq 300$ ns) of N-HPT in cyclohexane, benzene, or hexane resemble those obtained in polar media: they display the characteristic absorption of PyS$^\bullet$ at 495 nm, the ground-state depletion around 360 nm and another maximum at 305 nm. In these solvents, the presence of ^{3}(N-HPT)*, which is not easily observable, was evidenced by the sensitized formation of the triplet state of β-carotene at 540 nm.[16] By monitoring the kinetic signal at that wavelength for several concentrations of carotenoid (from 15 to 250 μM), a rate constant of 1.5×10^{10} M^{-1} s^{-1} was obtained for the reaction of ^{3}(N-HPT)* with β-carotene in benzene.[60] These results indicate that the primary photoprocesses undergone by N-HPT in nonpolar, aprotic media are identical to those occurring in polar solvents, *i.e.* intramolecular rearrangement, homolytic N−O bond cleavage and intersystem crossing. Using λ_{exc} = 355 nm and the method described above, Φ_{N-O} was determined to be 0.34 in benzene and 0.45 in hexane. Energy transfer to β-carotene was employed to measure Φ_T,[61] which was found to be 0.047 in benzene.

As seen in Figure 10A, on longer detection times, the transient absorption spectrum recorded for N-HPT in nonpolar media presents significant differences compared to that shown in Figure 8. Over the whole spectral domain considered (300 to 700 nm), the kinetic signals display a secondary growth in absorbance (with a rise time of 1−2 μs). This process generates a transient species ($\tau \sim 12$ μs), the absorption spectrum of which is presented in the inset of Figure 10A. The formation of this intermediate was found to be unaffected by the presence of O_2 or *t*-BuOH in the solution (excluding the involvement of ^{3}(N-HPT)* or $^\bullet$OH in the process). On the other hand, MMA, 1,3-CHD, and 2,5-dimethyl-2,4-hexadiene, known thiyl radical quenchers, inhibited this secondary reaction. In addition, by monitoring the growth (for example at 550 nm, as in the inset of Figure 10B) for several concentrations of N-HPT (from 30 to 300 μM), bimolecular rate constants of 1.3×10^{10} and 6.3×10^9 M^{-1} s^{-1} were obtained in hexane and in benzene, respectively (Figure 10B). It is concluded that the secondary species is formed by reaction of PyS$^\bullet$ with the ground state of N-HPT. However, addition of PyS$^\bullet$ on the carbon-sulfur double bond of the thione form of starting material cannot be responsible for the generation of the secondary intermediate as this reaction gives compound **2** and $^\bullet$OH, as depicted by eq 9. The amplitude of the secondary growth (which was not detected in polar media) was also observed to increase with decreasing solvent polarity (ethyl acetate ≪ benzene < cyclohexane < hexane), suggesting that the transient species rather may result from reaction of PyS$^\bullet$ with the thiol tautomer of N-HPT. Since the secondary intermediate is unaffected by O_2 and its absorption

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(56) In acidic buffer, the differences between the lifetimes of ^{3}(N-HPT)* (~1 μs) and PyS$^\bullet$ (~30 μs) make possible the observation of the triplet state even though the absorption spectra of the two species are very similar (see Figure 6B). In organic solvents, the decrease of the lifetime of the thiyl radical (~2−3 μs) complicates the observation of ^{3}(N-HPT)*.

(57) This method could not be used to determine the quantum yield of intersystem crossing of N-HPT in aqueous buffer at pH = 2 as the solubility of M540 in that solvent was found to be too low.

(58) In that case, 2-hydroxybenzophenone, a photoinert reagent, was added to the solution to mimic the filter effect due to N-HPT in the second experiment.

(59) An ϵ_T of 7.1×10^4 M^{-1} cm^{-1} was used for ^{3}(M540)* at 660 nm[46] to calculate Φ_T from eq 3.

(60) This experiment was carried out in the presence of methyl methacrylate in order to quench PyS$^\bullet$, which was also found to react efficiently ($k_Q = 7.3 \times 10^9$ M^{-1} s^{-1}) with β-carotene.

(61) This value was obtained for deaerated solutions of N-HPT containing 250 μM of β-carotene, by monitoring the amplitude of the signal at 540 nm with laser energy. The ϵ_T value for ^{3}(β-car)* at 540 nm was estimated from a published spectrum[16] to be 23 300 M^{-1} cm^{-1}. To simplify the analysis, MMA was added to the solution to quench PyS$^\bullet$ and prevent the formation of the secondary species which is otherwise visible at that wavelength.

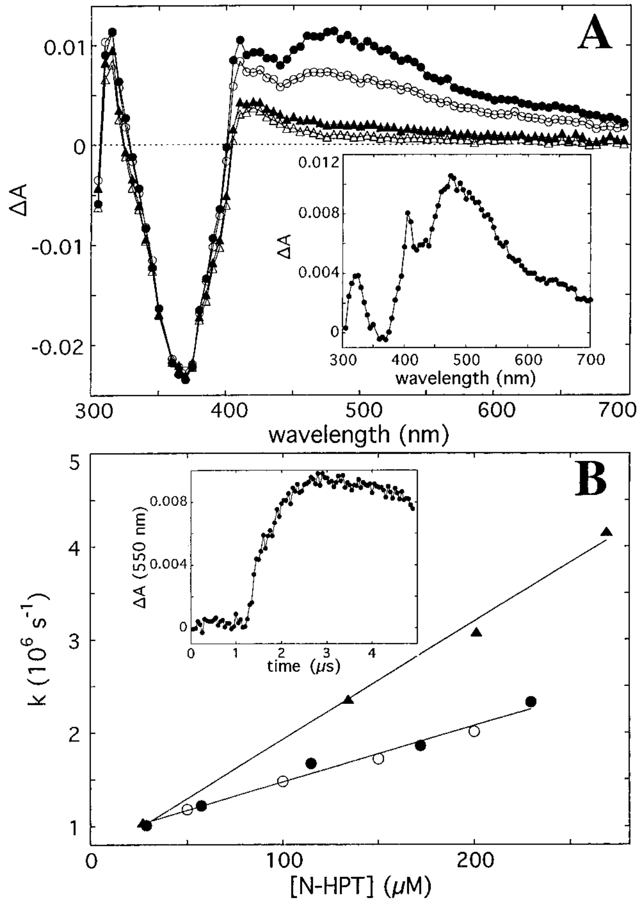
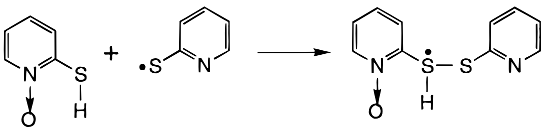


Figure 10. (A) Transient absorption spectra recorded 4 μs (●), 10 μs (○), 40 μs (▲), and 70 μs (△) after the photolysis (λ_{exc} = 355 nm, 8 mJ cm^{-2} pulse^{-1}) of 90 μM N-HPT in deaerated hexane (absorbance = 0.35 at 355 nm). The inset presents the absorption spectrum of the secondary transient species. (B) Variations of the experimental first-order growth constant recorded at 550 nm as a function of N-HPT concentration in N_2- (●) and O_2- (○) saturated benzene and in deaerated hexane (▲). The inset displays the growth corresponding to the formation of the secondary species at 550 nm following excitation of a deaerated solution of N-HPT in hexane.

spectrum is close to that reported for disulfide radical,[62] eq 11 can be tentatively proposed to describe the formation of this transient species.

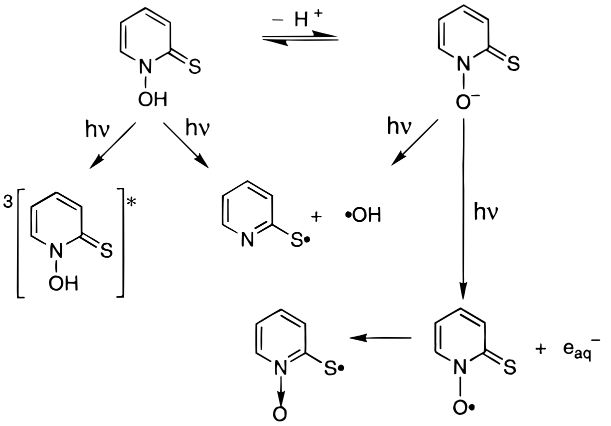


(11)

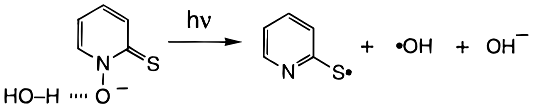
Discussion

A preliminary investigation of the photochemistry of N-HPT in neutral aqueous media[17] led us to the conclusion that the primary photoprocesses undergone by this compound on UV irradiation are much more complex than expected from published studies.[1−3] The detailed description given in this paper expands on this work by showing that the photochemical behavior of N-HPT is also pH-dependent and influenced by the polarity of the solvent. A consequence of the rich photochemistry of this molecule is that it cannot be considered as a clean photolytic source of $^\bullet$OH for the *selective* study of $^\bullet$OH reactions under any circumstances.

Scheme 1. Primary Photochemical Processes Undergone by the Anionic and Neutral Forms of N-HPT in Aqueous Media



Investigation of the photochemistry of N-HPT as a function of pH has demonstrated that the neutral and anionic forms involved in the acid/base equilibrium (eq 2) undergo different reactions upon UV excitation. Scheme 1 summarizes the results obtained. A common factor is that, irrespective of pH, irradiation of N-HPT in aqueous media causes homolytic scission of the N−O bond. This process takes place within the laser pulse, suggesting that PyS$^\bullet$ and $^\bullet$OH are primary intermediates and are not produced by long-lived excited state precursors. Although additional generation of $^\bullet$OH could possibly occur by reactions of PyS$^\bullet$ and PyNOS$^\bullet$ with N-HPT (eqs 9 and 10), their contribution to the formation of $^\bullet$OH was not observed by laser flash photolysis[63] and must be considered as negligible under these conditions. In the case of the neutral form of N-HPT, the photoinduced homolytic N−O bond cleavage can be depicted by eq 1. In order to rationalize the production of $^\bullet$OH from the anionic form, one may consider the involvement of a molecule of water (linked to N-HPT by hydrogen bonding[18]). As shown by eq 12, in addition to PyS$^\bullet$ and $^\bullet$OH, this process also generates the hydroxyl anion. Involvement of a molecule of water in the photoproduction of hydroxyl radicals has recently been proposed for another compound also bearing a nitrogen-oxygen bond.[64]



(12)

One of the main differences between the neutral and anionic forms of N-HPT is that, under UV excitation in aqueous media, only the latter photoionizes. This behavior can be compared to the photoionization of phenols and related compounds, which were reported to undergo monophotonic electron ejection from the anionic form[65] and biphotonic photoionization *via* the lowest triplet state from the uncharged structure.[65,66] Assuming a similar photochemical mechanism for N-HPT, the absence of electron ejection from the neutral form is not surprising since its excited triplet state displays no significant absorption at $\lambda_r \leq$ 400 nm (Figure 6B), therefore making unlikely the absorption of a second photon (λ_{exc} = 355 or 308 nm), to cause photoionization.

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[62] Elliot, A. J.; McEachern, R. J.; Armstrong, D. A. *J. Phys. Chem.* **1981**, *85*, 68−75.

[63] This is not surprising considering the low rate constants measured for the reactions of PyS$^\bullet$ and PyNOS$^\bullet$ with their precursor.

[64] Sako, M.; Nagai, K.; Maki, Y. *J. Chem. Soc., Chem. Commun.* **1993**, *9*, 750−751.

[65] Creed, D. *Photochem. Photobiol.* **1984**, *39*, 563−575.

[66] Feitelson, J.; Hayon, E.; Treinin, A. *J. Am. Chem. Soc.* **1973**, *95*, 1025−1029.

At neutral pH, in addition to e_{aq}^-, •OH, PyS•, and the semioxidized radical of starting material, UV excitation of N-HPT also produces the *N*-oxy-2-pyridylthiyl radical (PyNOS•), which is not observed at pH = 2. At least two possible formation pathways can be considered for the generation of PyNOS•. In neutral aqueous media, although the thione tautomer predominates,[18] the negative charge can also be localized on the sulfur atom. An electron transfer between this thiolate (which is a reducing agent[67]) and PyS• would produce PyNOS•. This hypothesis can be ruled out because the formation of PyNOS• would then be a slow process, even if it occurred at the maximum diffusion controlled rate, due to the low concentration of thiolate present in solution.[68] The actual mechanism for the formation of PyNOS• involves the short-lived species absorbing at 375 nm which was identified as the one-electron semioxidized radical of N-HPT (see eq 4). This oxygen-centered radical was found to undergo a fast intramolecular process yielding a species efficiently quenched by 2,4-HD but unreactive toward O_2, a thiyl radical behavior. A likely explanation for this process is given in Scheme 1, where the semioxidized radical of N-HPT converts to PyNOS•, a more stable chemical structure.

Another difference between the neutral and anionic forms of N-HPT is that triplet state formation only takes place from the former species. It is also worth noting that, irrespective of the nature of the solvent (aqueous or nonaqueous, polar or nonpolar), the uncharged structure displays identical primary photochemical behavior under UV excitation (λ_{exc} = 355 or 308 nm). In addition, the quantum yields of the photoprocesses undergone by the molecule (N−O bond cleavage and intersystem crossing) were found to be independent on the environment ($\Phi_{N-O} = 0.30-0.45$ and $\Phi_T = 0.03-0.05$ using λ_{exc} = 355 nm). The properties of the triplet state produced by UV irradiation of the neutral form of N-HPT in aqueous or organic solvents, in particular, its short lifetime (∼1 μs at pH = 2 and ∼500 ns in methanol) and its highly efficient quenching by the ground-state, are characteristic of those of a thione triplet.[44,45] The presence of ^{3}(N-HPT)* (with $E_T \geq 59.5$ kcal/mol) makes probable the formation of singlet oxygen (O_2, $^1\Delta_g$) by energy transfer to oxygen, further complicating the photochemistry of N-HPT and compromising its use as an unequivocal •OH generator.[69]

Discrepancies exist between our results and aspects of previous published reports. In particular, the production of an excited triplet state from the neutral form of N-HPT in aqueous and organic solvents has not been reported, and until very recent laser flash photolysis investigations,[17,36] photoionization of the negatively-charged structure of N-HPT was thought not to occur.[14] These discrepancies can be explained by the fact that, due to the high reactivity of the transient species generated, investigation of the primary photochemistry of N-HPT requires the use of conditions which prevent the occurrence of secondary photochemical reactions, precaution which was not taken in most published studies.

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(67) Surdhar, P. S.; Armstrong, D. A. *J. Phys. Chem.* **1987**, *91*, 6532−6537.

(68) This concentration can be determined to be ∼3 μM, based on the published molar ratio of 54 between the thione and the thiol forms.

(69) Due to the low quantum yield of intersystem crossing ($\Phi_T \approx 0.05$), the detection of the IR emission of singlet oxygen was not attempted.