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Photochemistry of *N*-Hydroxy-2(1*H*)-pyridone, a More Selective Source of Hydroxyl Radicals Than *N*-Hydroxypyridine-2(1*H*)-thione

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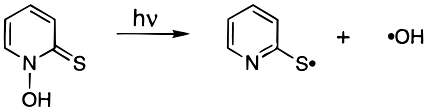
Abstract: The primary and subsequent photochemistry of *N*-hydroxy-2(1*H*)-pyridone (N-HP) has been investigated in aqueous and nonaqueous media by laser flash photolysis ($\lambda_{exc} = 308$ nm). In organic solvents, as well as in buffers at pH ≤ 7, the initial photochemistry of N-HP consists of homolytic N−O bond cleavage leading to the formation of the 2-pyridyloxyl (PyO$^\bullet$) and hydroxyl ($^\bullet$OH) radicals, the quantum yield ($\Phi_{N-O} = \Phi_{^\bullet OH}$) varying from 0.25 to 0.6, depending on the solvent. Quenching experiments have demonstrated that PyO$^\bullet$ is relatively unreactive and is removed mainly *via* a bimolecular radical reaction. In highly basic aqueous media, N-HP exists in the anionic form and is much less photolabile. At pH = 10, in addition to a low yield of N−O bond cleavage ($\Phi_{N-O} = 0.037$), N-HP undergoes photoionization, but solvated electron production was found to be very inefficient ($\Phi_{e^-} = 0.003$). Thus, under biologically relevant conditions, N-HP has a much simpler photochemical behavior than that of the closely related *N*-hydroxypyridine-2(1*H*)-thione (N-HPT) and may be more useful as a specific generator of hydroxyl radicals in chemical and biological systems.

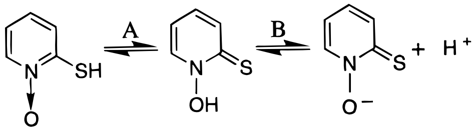
Introduction

Despite the number and variety of methods developed for generating the highly reactive hydroxyl radical ($^\bullet$OH),[1,2] a clean and facile source remains desirable for the understanding of $^\bullet$OH reaction mechanisms leading to oxidative damage *in vivo*.[1,2] In this context, several potential photoactive agents have recently been reported as $^\bullet$OH generators.[3−5] One of them, *N*-hydroxypyridine-2(1*H*)-thione (N-HPT), has been proposed as a mild photolytic source for investigation of $^\bullet$OH reactions[6−9] and their consequences in biological systems.[3] For N-HPT, $^\bullet$OH photoproduction is expected to occur *via* homolytic cleavage of the N−O bond, resulting also in the formation of the 2-pyridylthiyl radical (PyS$^\bullet$), as depicted in eq 1.



(1)

However, we have recently shown by laser flash photolysis that N-HPT is far from being a specific $^\bullet$OH precursor.[10,11] Indeed, the primary photochemistry of N-HPT proved to be highly solvent dependent, behavior which was rationalized in terms of the known thione/thiol tautomerism (A) and acid/base equilibrium (B) (pK_a = 4.67[12]) of this molecule (eq 2).



(2)

Detailed flash photolysis studies in aqueous solution as a function of pH allowed the differentiation of the photochemistry of the anionic and neutral forms of N-HPT.[10,11] The negatively-charged structure, which predominates at neutral pH,[12] undergoes monophotonic photoionization, giving rise to the hydrated electron (e_{aq}^-) with a relatively high efficiency ($\Phi_{e^-} = 0.1$ using $\lambda_{exc} = 308$ nm).[10] In addition to $^\bullet$OH and e_{aq}^-, N-HPT generates two distinct thiyl radicals, an excited triplet state and an *N*-oxide radical, under various conditions. In nonaqueous media, although the primary photoprocesses undergone by N-HPT (scission of the N−O bond and triplet-state formation) are essentially identical, regardless of the solvent, the nature of the secondary reactions was found to be affected by the polarity of the environment.[11]

In work analogous to a previous study concerning the photochemical mechanisms in ester derivatives of N-HPT (Barton salts),[13] we have recently investigated the closely related hydroxamic esters, or Taylor salts.[14,15] Similar to their sulfur analogues, homolytic N−O bond cleavage, as depicted in eq 3,

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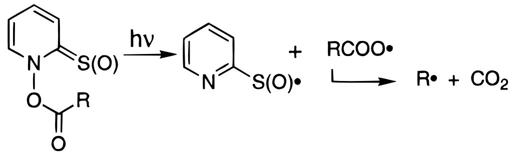
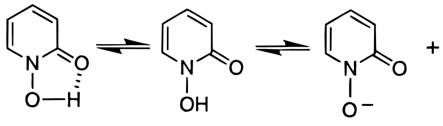
was observed to be the only primary process induced by flash photolysis (λ_{exc} = 308 nm) of these compounds.[13,16] In addition,



(3)

the 2-pyridyloxyl radical (PyO$^•$),[17] generated along with RCOO$^•$ by irradiation of Taylor salts, was found to be much less reactive than PyS$^•$. This lower reactivity appears as a potential advantage in the use of UV excitation of hydroxamic esters as a means to selectively study the reactivity of various radical types (RCOO$^•$ or R$^•$) and their deleterious effects in biological systems.[16] By analogy, we have investigated *N*-hydroxy-2(1*H*)-pyridone (N-HP), the parent molecule of the Taylor salts and the oxygen analogue of N-HPT, as a potentially cleaner and simpler photolytic source of $^•$OH.

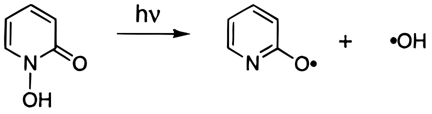
Similar to N-HPT, *N*-hydroxy-2(1*H*)-pyridone exhibits an acid/base equilibrium (between structures **2** and **3**, with a reported pK_a of 6.0[18,19]) and a keto/enol tautomerism. However, in solution, the keto form predominates over the enol tautomer.[18] In addition, in aprotic solvents, N-HP was observed to be strongly intramolecularly hydrogen-bonded (structure **1**).[18]



(4)

1 **2** **3**

We report here a laser flash photolysis study of the primary photochemistry of N-HP. Using different solvents, we have investigated the photochemical behavior of each of the three main forms of N-HP found in solution: the intramolecularly hydrogen-bonded (**1**), the neutral (**2**), and the negatively-charged (**3**) structures. Irrespective of the form, our results demonstrate the advantages of this compound compared to N-HPT as a $^•$OH generator. In organic solvents and in aqueous media with pH values in the physiological range, UV excitation of N-HP causes homolytic N−O bond cleavage (eq 5) without the additional competitive processes exhibited by N-HPT. In most solvents, N-HP generates $^•$OH with yields similar to those determined for its sulfur analogue. In addition, PyO$^•$, a poorly reactive radical species, which is also formed in the scission process, is unlikely to contribute to any damage in biological systems, allowing the observed effects to be correctly and exclusively assigned to $^•$OH.



(5)

Experimental Section

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

Chemicals. N-HP was prepared in a manner similar to its sulfur analogue,[20,21] by acidification of the commercially available aqueous solution (20% of N-HP, from Aldrich Chemical Co. (Milwaukee, WI)) and recrystallization of the precipitate from ethyl acetate. N-HP was characterized by ^{1}H NMR using deuteriochloroform (99.8 atom %, from Aldrich) with a tetramethylsilane (TMS) internal reference, as solvent. The ^{1}H NMR spectrum displays signals with chemical shifts at 6.32 (1H, dt), 6.70 (1H, dd), 7.36 (1H, dt), 7.75 (1H, dd), and 7.90 ppm (1H, broad, s). N-HP was stored in solid form at −20 °C. Purity was checked by absorption spectroscopy and HPLC. No degradation with time was observed under these storage conditions. Solution samples were prepared immediately before use and were protected from light at all times.

All other reagents were purchased from Aldrich or Fisher Chemical (Pittsburgh, PA) and were of highest purity grade. Buffers (10 mM; NaCl/HCl for pH = 2; potassium hydrogen phthalate/HCl for pH = 3; potassium hydrogen phthalate/NaOH for pH = 4 and 5; KH_2PO_4/NaOH for pH = 6, 7, and 8: and $NaHCO_3$/NaOH for pH = 10) were prepared using deionized water.

Laser Flash Photolysis. The experiments were carried out using the apparatus previously described.[22,23] A Lambda Physik EMG 103 MSC XeCl excimer laser was employed for excitation at 308 nm (8 ns pulse duration, up to 18 mJ cm^{-2} pulse^{-1}). The 320 nm output of a Quanta-Ray MOPO-710 optical parametric oscillator (Spectra Physics Lasers, Inc., Mountain View, CA) equipped with a MOPO-FDO frequency doubler was also used. For energy dependence experiments, the laser beam was attenuated by quartz slides. Transient absorption spectra and kinetic signals were recorded under continuous flow conditions, and a high flow rate was maintained to ensure irradiation of a completely fresh volume of sample with each laser pulse. Quenching rate constants were measured using static cells. Unless otherwise stated, samples were deaerated by bubbling nitrogen, prior to and during the experiment.

Quantum yields (Φ) of photoprocesses undergone by N-HP were determined by comparative actinometry as previously described.[13,24] Φ values were calculated for optically matched solutions of sample and standard (absorbance at 308 nm between 0.3 and 0.5) using the following equation:

$$\Phi_S = \Phi_{T(R)}(A_S/A_{T(R)})(\epsilon_{T(R)}/\epsilon_S) \qquad (6)$$

where the subscript T(R) refers to the triplet state of the reference used[25] and S refers to the species produced or consumed by the photoprocess considered. In this relationship, ϵ values correspond to 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. The appropriate ϵ values are given in the text.

High-Pressure Liquid Chromatography. HPLC analyses were performed using the setup described previously.[11] A mixture of deionized water and acetonitrile (3:1, vol:vol) was employed as eluent at a flow rate of 0.15−0.2 mL/min. Product identities were established by comparison of their absorption spectra obtained from the photodiode array detector and their retention times with those of commercially available authentic samples.

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(16) The results of the investigation of the photochemistry of hydroxamic esters by laser flash photolysis will be published elsewhere.

(17) Similar to N-HPT, the N-centered radical generated by N−O bond cleavage of N-HP undergoes a fast intramolecular rearrangement and the unpaired electron localizes mainly on the oxygen atom, to give PyO$^•$.

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(25) (a) 2'-Acetonaphthone (for which $\Phi_T = 0.84^{25b}$ and ϵ_T = 10 500 M^{-1} cm^{-1} ^{24} at 430 nm) and benzophenone (for which $\Phi_T = 1.0$ ^{25c} and ϵ_T = 7220 M^{-1} cm^{-1} ^{25d} at 530 nm) in deaerated benzene were used as standards. (b) Gorman, A. A.; Hamblett, I.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1984**, *106*, 4679-4682. (c) Inbar, S.; Linschitz, H.; Cohen, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 1048-1054. (d) Hurley, J. K.; Sinai, N.; Linschitz, H. *Photochem. Photobiol.* **1983**, *38*, 9-14.

Table 1. Absorption properties of N-HP in various solvent systems[a]

		λ_{max} (nm) [ϵ (M^{-1} cm^{-1})][b]		
1	cyclohexane		235 [4100]	314 [4000]
	benzene			314 [4800]
	acetonitrile	210	235 [7100]	310 [5000]
2	2-propanol	212	232 [10400]	308 [4000]
	methanol	208	230 [6500]	306 [5000]
	buffer (pH = 2)	202	228 [5600]	298 [5100]
3	buffer (pH = 10)	220	shoulder	316 [5000]

[a] The absorption maxima (λ_{max}) and corresponding molar absorption coefficients (ϵ) are reported for structures **1**, **2**, and **3** in buffers of pH = 2 and 10 and in different organic solvents. [b] The absorption properties were studied for concentrations of N-HP between 10 and 500 μM in aqueous media and between 10 and 300 μM in organic solvents. These concentrations correspond to those used in laser flash photolysis experiments. Errors on ϵ values are ≤5%.

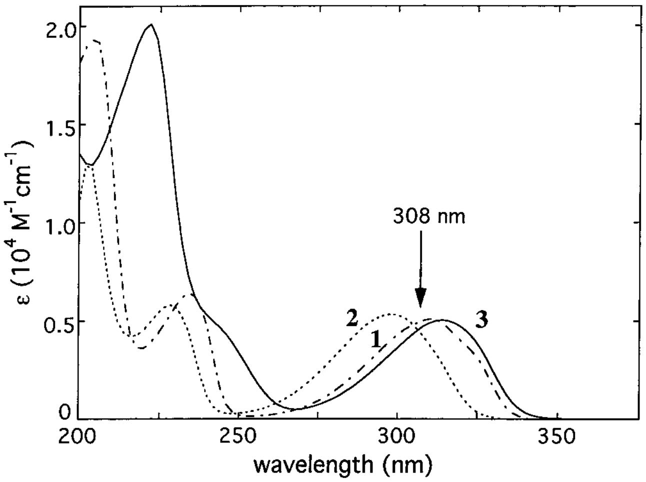

Figure 1. Ground-state absorption properties of N-HP. The ground-state absorption spectra of structures **1**, **2** and **3** were recorded in acetonitrile (— •—), in buffer at pH = 2 (- - -) and pH = 7 (—), respectively.

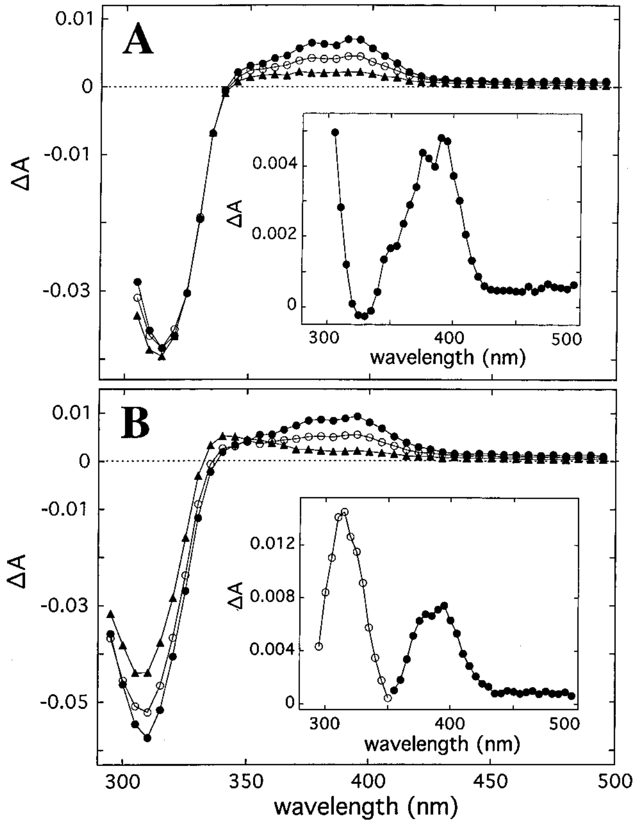

Figure 2. Spectra recorded for N-HP in deaerated acetonitrile (A) and methanol (B), 6 (●), 24 (○) and 72 μs (▲) after excitation (λ_{exc} = 308 nm). The absorbance of the solution at λ_{exc} was 0.33 in acetonitrile (~66 μM) and 0.37 in methanol (~88 μM), and the laser intensities were 8.5 and 10 mJ cm^{-2} $pulse^{-1}$, respectively. The inset in A displays the absorption spectrum of $PyO^{\bullet}$ in acetonitrile, obtained by difference of the spectra recorded at 6 and 72 μs. The inset in B presents the spectra of $PyO^{\bullet}$ (●) and of the photoproduct formed at λ = 310−315 nm (○) in methanol.

Results

Structure 1. In aprotic media (cyclohexane, benzene, and acetonitrile), N-HP is strongly intramolecularly hydrogen-bonded and exists as form **1** (see eq 4).

The ground-state absorption of N-HP in organic solvents and in aqueous buffers follows linear Beer−Lambert behavior in the measured range of concentrations (from 10 to 500 μM). The maxima (λ_{max}) and corresponding molar absorption coefficients (ϵ) determined for N-HP are reported in Table 1. In apolar media, the spectrum of **1** displays three distinct absorption bands with maxima around 210, 235, and 310−315 nm (Figure 1).

The time-dependent transient absorption spectrum formed on flash photolysis (λ_{exc} = 308 nm) of **1** in acetonitrile is presented in Figure 2A. The spectrum was found to be identical under aerated and deaerated conditions and was independent of laser energy. The same behavior was observed in benzene and cyclohexane. The transient spectrum displays an absorption band with a maximum at 390 nm. This band, which is also observed in protic organic solvents (*vide infra*) was assigned to $PyO^{\bullet}$ for the following reasons. (a) The presence of an absorption band with a maximum at 390 nm is a common feature of the spectra recorded after flash photolysis (λ_{exc} = 308 nm) of various hydroxamic esters in acetonitrile. Under UV excitation, these compounds undergo a homolytic scission of the N−O bond, leading to $PyO^{\bullet}$ and $RCOO^{\bullet}$, according to eq 3.[16] (b) A similar spectrum with a maximum at 402 nm has been recorded for the closely related phenoxyl radical ($PhO^{\bullet}$), formed either by chemical oxidation of the phenolate ion with the oxide radical ($O^{\bullet -}$, generated by pulse radiolysis)[26] or by laser flash photolysis of phenol in aqueous media.[27] In the latter case, depending on the pH of the solution, $PhO^{\bullet}$ is produced by O−H bond cleavage or by photoionization.[27] (c) We have observed the formation of the same absorption band at 390 nm by pulsed irradiation (λ_{exc} = 308 nm) of 2-hydroxypyridine in acetonitrile or benzene. Similar to phenol, $PyO^{\bullet}$ produced from this compound is expected to result from O−H bond cleavage.

The transient absorption spectrum (Figure 2A) also exhibits a marked negative band with a maximum around 315 nm, due to photoinduced ground-state depletion. The corresponding kinetic signal is a step function, suggesting that there are no short-lived transient species strongly absorbing in that region. The only process consuming the starting material is N−O bond cleavage occurring within the laser pulse. At lower wavelengths ($\lambda \le$ 310 nm), the kinetic signal is composed of an instantaneous step, attributed to the photodecomposition of N-HP, followed by a second, slower process. Since no consumption of the starting N-HP due to chemical reaction was observed at $\lambda >$ 310 nm, this slow process corresponds to the decay of a transient species absorbing in the UV ($\lambda <$ 310 nm). Increasing the laser energy or ground-state concentration (from 30 to 250 μM) had identical quenching effects on the decay rate constants of $PyO^{\bullet}$

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at 390 nm and on that of the slow process at $\lambda < 310$ nm. This indicates that PyO$^\bullet$ has a second absorption band with a maximum at $\lambda < 300$ nm (as shown on the inset of Figure 2A). The presence of this UV band in the spectrum of PyO$^\bullet$ is not surprising: in addition to the 402 nm band, the closely related phenoxyl radical (PhO$^\bullet$) was reported to exhibit another absorption maximum at 299 nm.[27]

In aprotic media, the generation of PyO$^\bullet$ from N-HP was complete within the pulse duration and under the experimental conditions used to record the spectra, PyO$^\bullet$ decays by second-order kinetics.

In addition to PyO$^\bullet$, photoinduced N−O bond cleavage of N-HP also gives rise to $^\bullet$OH (eq 5). Since $^\bullet$OH exhibits only a weak absorption in the far UV,[28] it cannot be observed directly. In aqueous media, different acceptors, such as various ionic species (*e.g.* SCN^-, Br^-, I^-) can be used to probe the presence of $^\bullet$OH.[2] They react with $^\bullet$OH to give species which are strongly absorbing in the visible region. *N*,*N*-Dimethyl-*p*-nitrosoaniline (RNO) is known to be similarly useful in organic solvents.[29−33] The consumption of RNO, by chemical reaction with $^\bullet$OH,[34] can be followed by the bleaching of its characteristic 420 nm absorption band.[33]

In the presence of RNO, the transient absorption spectrum recorded after pulsed excitation ($\lambda_{exc} = 308$ nm) of N-HP in deaerated acetonitrile displays a negative band with a maximum at 420 nm. This bleaching was not observed when DMSO or phenol (known to be $^\bullet$OH scavengers) were added to the solution. Since RNO absorbs some light at 308 nm, the concentrations of acceptor which were used in these experiments were chosen so that the difference of absorbance between the solution samples in the presence and the absence of RNO was ≤ 0.05 at 308 nm. In the corresponding range of concentrations ([RNO] $\leq 45\ \mu$M), the excitation of the aniline derivative alone did not lead to significant ground-state depletion. We have also verified that RNO has no quenching effect on PyO$^\bullet$. Furthermore, as no singlet oxygen formation takes place from N-HP, RNO cannot be consumed by reaction with O_2 ($^1\Delta_g$).[35] It can therefore be concluded that the bleaching observed at 420 nm is only due to the reaction of RNO with $^\bullet$OH, produced by homolytic N−O bond cleavage of N-HP.

The rate constant (k_{RNO}) of reaction between $^\bullet$OH (generated by N-HP) and RNO was determined by monitoring the bleaching at 420 nm in the presence of several acceptor concentrations ($\leq 45\ \mu$M). Since the intermediates formed by interaction of $^\bullet$OH and RNO were reported to undergo further reaction resulting in the recovery of the aniline derivative,[30,33] only the initial part of the kinetic signals recorded at 420 nm was analyzed.[36] The experimental pseudo-first-order rate constants were plotted against [RNO] to give k_{RNO} from the slope. A value of $(1.0 \pm 0.15) \times 10^{10}$ M^{-1} s^{-1} was obtained in acetonitrile, which is in reasonable agreement with the data reported in the literature (1.25×10^{10} M^{-1} s^{-1} in water[34] and 3.8×10^9 M^{-1} s^{-1} in acetonitrile[33]).

Table 2. Quantum yields of homolytic N−O bond cleavage[a] (Φ_{N-O}) Determined for N-HP and N-HPT in Different Solvent Systems Using $\lambda_{exc} = 308$ nm

			Φ_{N-O}	
			N_2	air
N-HP	**1**	benzene	0.57	0.58
		acetonitrile	0.36	0.32
	2	methanol	0.56	0.54
		buffer pH = 2	≥0.28	≥0.26
	3	buffer pH = 10	∼0.037	
		buffer pH = 7	0.24	0.25
		(KSCN)	0.11	0.11
N-HPT		acetonitrile		0.61[b]
		methanol		0.49[b]
		buffer pH = 2		0.27[b]
		buffer pH = 7	0.28	0.26
		(KSCN)	0.11	0.14

[a] Except where (KSCN) is indicated, the Φ_{N-O} values were determined using the comparative technique, by following the variations of the amplitude of initial photoinduced ground-state consumption with laser energy in the case of N-HP and by following the variations of the maximum amplitude of PyS$^\bullet$ with laser energy in the case of N-HPT. [b] This value was determined under O_2-saturated conditions in order to avoid interference from the triplet state of N-HPT which was found to absorb at the detection wavelength (see ref 11). Errors on Φ values are <10%.

As $^\bullet$OH and PyO$^\bullet$ are the only species detected in aprotic solvents, homolytic scission of the N−O bond appears to be the only primary photoprocess undergone by N-HP in these media. The hypothesis that negligible transient absorption occurs at the maximum of ground-state depletion, *i.e.* 315 nm, has been verified for all the aprotic solvents used. Φ_{N-O}, the quantum yield of initial consumption of N-HP by N−O bond cleavage (which is equivalent to $\Phi_{^\bullet OH}$, the quantum yield of $^\bullet$OH production) was determined by comparative actinometry. The Φ_{N-O} values determined for **1** in acetonitrile and in benzene, and calculated using eq 6, are reported in Table 2.

The experimental data show that, as expected (by analogy with N-HPT,[11] hydroxamic,[16] and thiohydroxamic[13] esters), O_2 does not affect the efficiency of homolytic cleavage of the N−O bond. Similar behavior was observed in all the solvents studied.

The molar absorption coefficient of PyO$^\bullet$ at 390 nm ($\epsilon_{PyO^\bullet}$) in aprotic media, was determined by a comparative method. Assuming that the scission of one N−O bond of starting material leads to the formation of one PyO$^\bullet$ and no additional absorbing species at 390 nm (*i.e.* the detection wavelength), $\epsilon_{PyO^\bullet}$ can be calculated using the following relationship:

$$\epsilon_{PyO^\bullet} = \epsilon_{GS}(A_{PyO^\bullet}/A_{GS}) \qquad (7)$$

where $A_{PyO^\bullet}$ and A_{GS} are the initial slopes of the laser energy dependence plots of the maximum absorbance of PyO$^\bullet$ at 390 nm and the amplitude of ground-state depletion due to photoinduced N−O bond cleavage. $\epsilon_{PyO^\bullet}$ values of 1100 and 1350 M^{-1} cm^{-1} were obtained for PyO$^\bullet$ in acetonitrile and benzene, respectively. These values are in the same range than those reported for the phenoxyl radical (2400 M^{-1} cm^{-1} at 400 nm[26]) and for the $^\bullet$OH adducts of 4-pyridone and 2-pyridone (1500 M^{-1} cm^{-1} at 370 nm and 1460 M^{-1} cm^{-1} at 350 nm, respectively[37]).

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(35) Irrespective of the environment (i.e. irrespective of the form considered), N-HP does not undergo intersystem crossing leading to triplet state formation. This was verified by a lack of triplet sensitization of merocyanine 540 in the case of aqueous and organic protic media and of β-carotene in the case of aprotic solvents.

(36) To avoid interference with secondary kinetic processes.

(37) Steenken, S.; O'Neill, P. *J. Phys. Chem.* **1979**, *83*, 2407−2411.

As demonstrated above, PyO$^{\bullet}$ and $^{\bullet}$OH are the only species generated by UV irradiation (λ_{exc} = 308 nm) of N-HP in aprotic media. No spectral evidence for the subsequent chemical reaction of $^{\bullet}$OH with the starting material was observed. Quenching experiments were undertaken in order to elucidate possible reaction pathways of PyO$^{\bullet}$, and determine the fate of this radical in the system. More importantly, investigation of the reactivity of PyO$^{\bullet}$ is necessary to draw conclusions concerning the potential of UV excitation of N-HP as a clean and specific photolytic source of $^{\bullet}$OH to study $^{\bullet}$OH-induced damage in biological systems.

The decay of PyO$^{\bullet}$ formed by pulsed irradiation (λ_{exc} = 308 nm) of **1** in aprotic solvents was found to be unaffected by the presence of O_2 in the solution. The same behavior was observed when PyO$^{\bullet}$ was generated by flash photolysis of N-HP in protic organic solvents or in aqueous media and when hydroxamic esters were used as photolytic PyO$^{\bullet}$ precursors.[16] The closely related phenoxyl radical (PhO$^{\bullet}$) was similarly reported to be unreactive toward O_2.[38]

All the potential quenchers selected, namely ascorbic acid 6-palmitate, methyl methacrylate, and benzhydrol (chosen to test the ability of the 2-pyridyloxyl radical for electron transfer, addition on double bonds, and hydrogen abstraction, respectively) failed to quench PyO$^{\bullet}$ to any detectable extent.[39] Similarly, PyO$^{\bullet}$ displayed very low reactivity toward biomolecules such as cholesterol and fatty acids (rate constants with upper limits of 10^4 M^{-1} s^{-1} were estimated in all cases). Since the phenoxyl radical (PhO$^{\bullet}$) was recently reported to possess a surprisingly high reactivity toward biologically important phenols and related compounds, α-tocopherol (vitamin E), found to be the most efficient scavenger in the case of PhO$^{\bullet}$, was also tested as a potential quencher of PyO$^{\bullet}$.[40] Formation of the α-tocopheryl radical by hydrogen abstraction of PyO$^{\bullet}$ from vitamin E[40] was evidenced by the presence of its characteristic absorption band with a maximum around 425 nm.[41] Quenching rate constants (k_Q) were determined by monitoring the growth at 425 nm for different concentrations of α-tocopherol over the range from 30 μM to 1 mM (Figure 3). These experiments were carried out using an irradiation wavelength of 320 nm (see Experimental Section) in order to prevent direct excitation of vitamin E. Values for k_Q of (1.15 ± 0.07) × 10^9 and (3.7 ± 0.1) × 10^9 M^{-1} s^{-1} were obtained in acetonitrile and in methanol, respectively.[42] These values are higher than those reported for the phenoxyl radical (3.1 × 10^8 M^{-1} s^{-1} in acetonitrile and 1.1 × 10^9 M^{-1} s^{-1} in benzene).[40]

In the absence of quencher, irrespective of the aprotic solvent used, the decay of PyO$^{\bullet}$ follows second-order kinetics. The transient signal at 390 nm was recorded for several N-HP concentrations (from 30 to 300 μM). In acetonitrile, $2k/\epsilon$ was determined to be (5.45 ± 0.3) × 10^6 cm s^{-1}. Using $\epsilon_{PyO^{\bullet}}$ previously determined, one derives a $2k$ of (6 ± 0.3) × 10^9 M^{-1} s^{-1}. This value is close to the radical self-quenching rate constant of PhO$^{\bullet}$, for which $2k$ was reported to be 1.3 × 10^9 M^{-1} s^{-1} in benzene.[43]

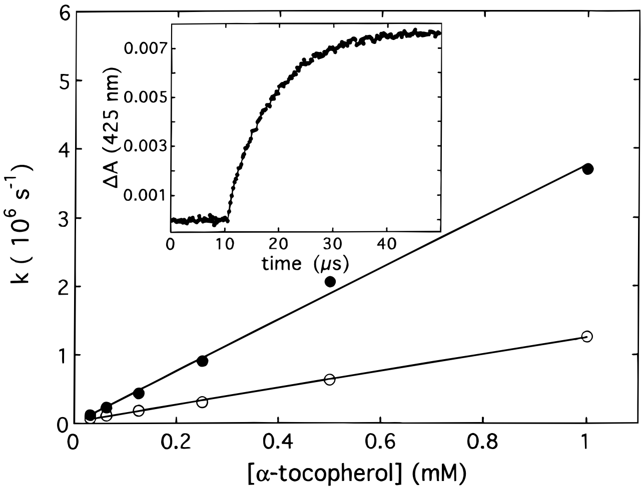

Figure 3. Variation in the experimental first-order growth rate constant as a function of the α-tocopherol concentration in acetonitrile (O) and in methanol (●). The inset presents the growth of the α-tocopheryl radical recorded at 425 nm following 320 nm excitation of a deaerated solution of N-HP in acetonitrile containing 62 μM of vitamin E.

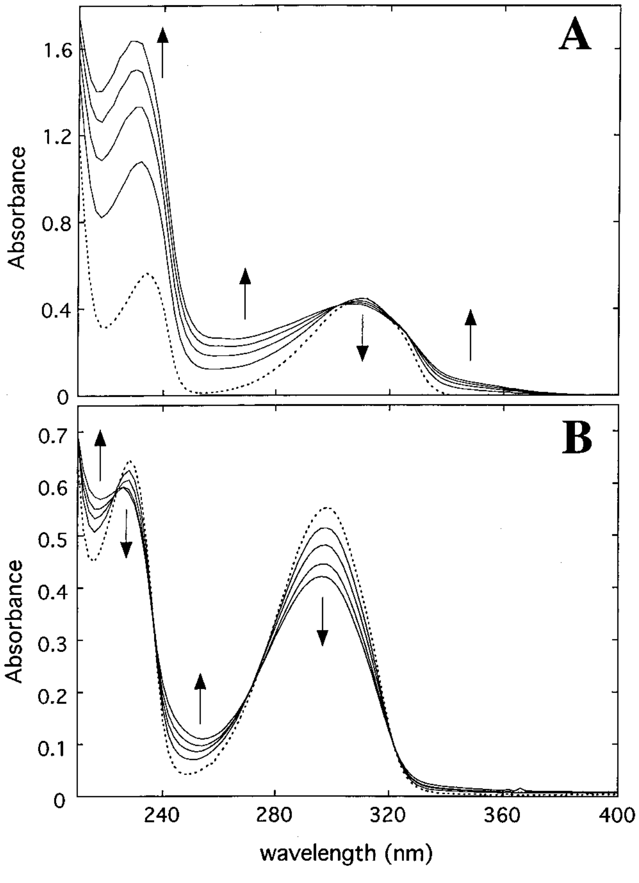

Figure 4. Photodegradation of N-HP followed spectrophotometrically. The starting solutions in acetonitrile (A) and in buffer at pH = 2 (B) had an absorbance of 0.44 at 308 nm. The spectra were recorded before irradiation (- - -) and after (A) 25, 50, 75, and 100 shots and (B) 50, 100, 150, and 200 shots. In both cases, the laser energy was 4 mJ cm^{-2} $pulse^{-1}$.

The photodegradation of N-HP under pulsed excitation (λ_{exc} = 308 nm) was also followed spectrophotometrically. Figure 4A displays the spectral changes observed in acetonitrile. In organic solvents, the spectra of irradiated solutions show a strong absorption around 230 nm and a weaker one at longer wavelengths.

Structure 2 in Protic Organic Solvents. The neutral form of N-HP (**2**) was studied in methanol and 2-propanol. Similar ground-state spectra, with three distinct absorption bands, were

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[38] Jonsson, M.; Lind, J.; Reitberger, T.; Eriksen, T. E.; Merenyi, G. *J. Phys. Chem.* **1993**, *97*, 8229–8233.

[39] Ascorbic acid 6-palmitate, methyl methacrylate, and benzhydrol were used at concentrations up to 0.2, 1, and 0.5 M, respectively.

[40] Foti, M.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1994**, *116*, 9440–9447.

[41] See ref. 2, p 138.

[42] These experiments were carried out in the presence of *t*-BuOH or DMSO to quench $^{\bullet}$OH and prevent its reaction with α-tocopherol.

[43] Mahoney, L. R.; Weiner, S. A. *J. Am. Chem. Soc.* **1972**, *94*, 1412–1413.

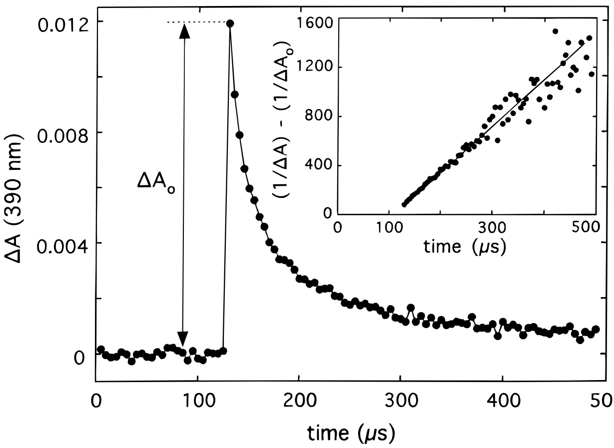


Figure 5. Second-order decay of PyO$^•$ recorded at 390 nm, after photolysis (λ_{exc} = 308 nm) of N-HP in deaerated methanol. The ground-state concentration of the solution sample was 150 μM and the laser intensity, 8 mJ cm^{-2} pulse^{-1}. The inset shows the analysis of the kinetic signal.

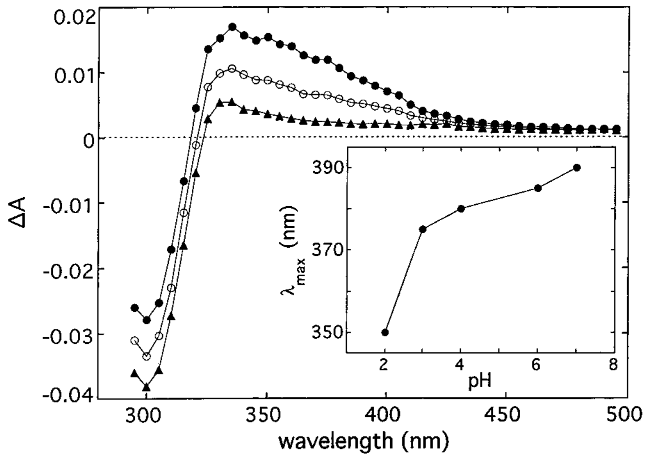


Figure 6. Spectra recorded for 90 μM N-HP in deaerated buffer of pH = 2, 10 (●), 40 (○), and 140 μs (▲) after excitation (λ_{exc} = 308 nm, 12 mJ cm^{-2} pulse^{-1}). The inset presents the variations of the absorption maximum (λ_{max}) of the transient species as a function of pH.

recorded for N-HP in these protic organic media. As shown by the data reported in Table 1, the two longer wavelength absorption maxima of **2** undergo a blue shift as the polarity of the solvent is increased.

Figure 2B presents the time-dependent transient absorption spectrum recorded after flash photolysis (λ_{exc} = 308 nm) of **2** in methanol. This spectrum is similar to that obtained in 2-propanol. In addition to the spectral features described for aprotic media (Figure 2A) (*i.e.* absorption band at 390 nm assigned to PyO$^•$ and negative band around 310 nm due to photochemical ground-state consumption), the transient spectrum of N-HP in protic organic solvents also displays an isosbestic point at 350 nm.

PyO$^•$, generated by **2**, is formed within the laser pulse and decays with second-order kinetics (see Figure 5) under the experimental conditions used to record the spectrum. At 310 nm, the kinetic signal is composed of an instantaneous step due to photoinduced ground-state consumption of starting material and a subsequent slow growth in absorbance. This slow process follows second-order kinetics and then does not undergo any changes over several hundreds of microseconds, suggesting the formation of a stable product. As shown in the inset of Figure 2B, the spectrum of the corresponding photoproduct displays an absorption band with a maximum at 310−315 nm.

In protic organic solvents, Φ_{N-O} (which is equivalent to $\Phi_{•OH}$) was determined by comparative actinometry, and the experiment was carried out using a time scale short enough to avoid interference from the secondary growth observed at 310 nm. The Φ_{N-O} values measured for **2** in methanol and calculated using eq 6 are reported in Table 2. Values of 0.54 and 0.56 (identical, within experimental error) were obtained under aerated and deaerated conditions, respectively.

Using the method previously described and eq 7 to calculate the molar absorption coefficient of PyO$^•$ at 390 nm in methanol, $\epsilon_{PyO^•}$ of 1000 M^{-1} cm^{-1} was obtained, which is very similar to the values measured in aprotic media.

The second-order decay of PyO$^•$ at 390 nm was recorded for several N-HP concentrations (from 30 to 300 μM). Analysis of the kinetic signals obtained led to a $2k/\epsilon$ value of $(4 \pm 0.5) \times 10^6$ cm s^{-1} in methanol (see inset of Figure 5). Using ϵ determined previously for PyO$^•$, $2k$ was found to be $(4 \pm 0.5) \times 10^9$ M^{-1} s^{-1}. Thus, in protic as well as in aprotic organic solvents, PyO$^•$ disappears mainly *via* a bimolecular radical reaction. However, on the time scales used in laser flash photolysis experiments, the generation of a photoproduct (with an absorption maximum around 310−315 nm) was detected in methanol and 2-propanol but not in aprotic organic solvents, suggesting either that the photoproduct formation is solvent-dependent, or that the absorption properties of the photoproduct are highly solvent-dependent.

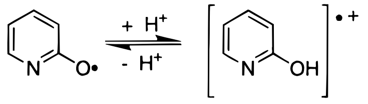
HPLC analysis of N-HP samples irradiated in protic organic solvents (methanol and 2-propanol) indicates the presence of a product which is not observed when the irradiation is carried out in aprotic media (cyclohexane and benzene). By comparison of its retention time and absorption spectrum with those of a pure standard, this photoproduct was unambiguously identified as 2-hydroxypyridine. Thus, the absorption band with a maximum around 310−315 nm, which was detected solely in protic organic solvents by laser flash photolysis and was attributed to the formation of a stable photoproduct, can be assigned to the 2-hydroxypyridine.

N-HP in Aqueous Media. The neutral form of N-HP (**2**) predominates in buffer of pH values below the pK_a of 6. As the pH is increased above the pK_a, the negatively-charged structure (**3**) becomes the major species present in solution.

Figure 1 displays the ground-state absorption spectrum of N-HP in acidic aqueous buffer (pH = 2). With its three distinct absorption bands, it is similar to that recorded for structures **1** and **2** in organic solvents. An increase of pH leads to a red-shift and a modification of its profile. As shown by Figure 1, in neutral buffer, the spectrum is composed of two absorption bands with maxima around 220 and 315 nm and a shoulder on the red side of the shorter wavelength peak. Under these conditions, the anionic form **3** predominates, although the amount of conjugate acid **2** (∼10%) contributing to the absorption observed is not negligible. The unambiguous spectrum of **3** was recorded in aqueous buffer at pH = 10, and the maxima (λ_{max}) and corresponding molar absorption coefficients (ϵ) are reported in Table 1.

Figure 6 presents the time-dependent transient absorption spectrum obtained by flash photolysis (λ_{exc} = 308 nm) of the neutral form (**2**) of N-HP in buffer at pH = 2. The spectrum recorded under these conditions (Figure 6) differs from those obtained for N-HP in organic solvents (Figure 2). At pH = 2, the transient spectrum displays a negative band at 300 nm which is due to photochemical consumption of starting material and a broad absorption band with a maximum around 335 nm. All signals between 295 and 430 nm were observed to follow identical first-order kinetics with a time constant of 36 μs, which indicates the presence of only one transient species absorbing in that region. The kinetic signals recorded around 335 nm also possess a residual absorption which does not decay over several seconds, suggesting the formation of a stable product. After correction for the absorption due to the photoproduct, the actual spectrum of the transient species displays an absorption band with a maximum at 350 nm. This band was not affected by the presence of O_2 or other potential quenchers (ascorbic acid, phenol, and *trans,trans*-2,4-hexadien-1-ol (2,4-HD)) in the solution, a behavior identical to that observed for PyO$^{\bullet}$ in organic solvents. A similar broad absorption band at 350 nm was obtained by pulsed irradiation (λ_{exc} = 308 nm) of several hydroxamic esters in buffer of pH = 2.[44] In all cases (*i.e.* for N-HP as well as for its ester derivatives), addition of one drop of concentrated NaOH in the solution sample (increasing the pH from 2 to 6−7) causes a narrowing of the transient absorption band and a shift of its maximum from 350 to 390 nm. Furthermore, the spectrum recorded after pulsed excitation of N-HP in acetonitrile containing trifluoroacetic acid ([TFA] ~100 mM), displays a transient absorption with a maximum which is also blue-shifted to 340-350 nm compared to that obtained in the absence of TFA (see Figure 2A).

In order to test the hypothesis of a pH effect on the absorption properties of PyO$^{\bullet}$, transient spectra were recorded after laser flash photolysis (λ_{exc} = 308 nm) of N-HP in buffers of pH between 2 and 7. As the pH of the buffer is increased, the maximum of the transient absorption band undergoes a marked red-shift. The inset in Figure 6 presents the observed maxima (λ_{max}) of this absorption peak as a function of pH. The graph obtained appears as a titration curve with an inflection point between 2 and 3. Thus, at pH = 2, the 2-pyridyloxyl radical generated by N−O bond cleavage of N-HP (or its ester derivatives) exists mainly as PyOH$^{\bullet+}$, the protonated form (eq 8), with the pK_a of the radical cation situated around pH = 2−3.



(8)

UV excitation of **2** in acidic buffer (pH = 2) is expected to produce $^{\bullet}$OH concomitant to PyOH$^{\bullet+}$. In aqueous media, several acceptors can be used to probe the formation of $^{\bullet}$OH. Thiocyanate ions are known to react with $^{\bullet}$OH, according to eqs 9 and 10, to give $(SCN)_2^{\bullet-}$, which is easily detectable by

$$\mathrm{SCN^- + {}^{\bullet}OH \rightarrow SCN^{\bullet} + OH^-} \tag{9}$$

$$\mathrm{SCN^- + SCN^{\bullet} \rightleftharpoons (SCN)_2^{\bullet-}} \tag{10}$$

its absorption around 480 nm.[45]

The spectrum recorded after pulsed excitation (λ_{exc} = 308 nm) of an acidic aqueous solution of N-HP in the presence of KSCN (0.2 M) was found to display a strong absorption band with a maximum at 480 nm.[45] This band[46] is indicative of the trapping of $^{\bullet}$OH and was not formed when $^{\bullet}$OH scavengers (*t*-BuOH, DMSO, ascorbic acid, or phenol) were added to the solution.

Since, except for $^{\bullet}$OH and PyOH$^{\bullet+}$, no evidence for the presence of other transient species was observed in buffer at pH = 2, homolytic N−O bond cleavage is the only primary photoprocess undergone by structure **2**.[35] Φ_{N-O} was determined using the comparative method, by following variations of initial photoinduced consumption of N-HP (at 300 nm) with laser energy. The values obtained in buffer at pH = 2 and calculated using eq 6 are reported in Table 2.[47] Since PyOH$^{\bullet+}$ absorbs at the maximum depletion of starting material (see Figure 6), these Φ_{N-O} values must be considered as lower limits.

Due to the uncertainty of the pK_a value of the radical cation (between pH = 2 and 3) and to the error in the determination of the quantum yield of N−O bond cleavage, the molar absorption coefficient of PyOH$^{\bullet+}$ at 350 nm ($\epsilon_{PyOH^{\bullet+}}$) cannot be obtained by the comparative technique outlined previously. However, it is possible to get an estimation of this ϵ value by using hydroxamic esters in buffer at pH = 2.[44] Assuming that the cleavage of one N−O bond of a molecule of ester derivative of N-HP leads to the formation of one PyO$^{\bullet}$, which then undergoes a rapid acid/base equilibrium with PyOH$^{\bullet+}$, $\epsilon_{PyOH^{\bullet+}}$ can be calculated using eq 7. An upper limit of 3000 M^{-1} cm^{-1} was obtained using pK_a = 2.5.

UV irradiation of N-HP in buffer of pH = 2 does not lead to any permanent photoproduct formation detectable by flash photolysis. Figure 4B presents the spectral changes undergone by N-HP in acidic aqueous solution (pH = 2) under pulsed excitation (λ_{exc} = 308 nm). These modifications, which are similar to those recorded in aqueous media of pH $\leq$ 7, are different from those in organic solvents (see Figure 4A for comparison).

As the pH of the solvent is increased above 2, the transient absorption spectrum recorded after excitation (λ_{exc} = 308 nm) of N-HP presents new features. In addition to the aforementioned changes (in intensity, width and λ_{max}) undergone by the absorption band of the 2-pyridyloxyl radical (PyO$^{\bullet}$ or PyOH$^{\bullet+}$), the transient spectrum also shows a red shift of the negative peak, which parallels the shift observed for the longer wavelength absorption band of the ground state of starting material. At neutral pH, *i.e.* under biologically relevant conditions, where N-HP exists as a mixture of the anionic form (~90%) and conjugate acid (~10%), the spectrum was found to be identical for aerated and deaerated solutions, and its profile was also unaffected by an increase of laser energy. The transient spectrum obtained is also very similar to that recorded for **2** in protic organic solvents (Figure 2B), although at neutral pH, only 10% of N-HP should exist as the uncharged form. The same spectral features are present: an absorption band at 390 nm assigned to PyO$^{\bullet}$, a negative band with a maximum at 305 nm due to photoinduced ground-state consumption, and an isosbestic point at 345 nm.[48] No detectable absorption was observed at $\lambda \geq$ 450 nm,[49] indicating that N-HP does not undergo photoionization at pH = 7 or, at least, that the process is too inefficient for detection (*i.e.* $\Phi_{e^-} \leq 0.001$).

Contrary to PyO$^{\bullet}$ generated from **1** or **2** in organic solvents, PyO$^{\bullet}$ produced at neutral pH decays monoexponentially with 44 μs lifetime under the experimental conditions used to record the spectrum. Under the same conditions, the stable species formed at 310−315 nm presents a risetime of 38 μs. The

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[44] (a) Since the ester derivatives of N-HP are hydrolyzed in aqueous media, cold buffer was used as solvent to retard the degradation. (b) Hydroxamic esters with R = $(CH_3)_3C$ and R = $(C_6H_5)CH_2$ (see eq 3) were studied in buffer at pH = 2 or pH = 10.

[45] Ellison, D. H.; Salmon, G. A.; Wilkinson, F. *Proc. R. Soc. London, Ser. A.* **1972**, *328*, 23−36.

[46] The band with a maximum at 480 nm observed in the presence of KSCN at pH = 2 is similar to that recorded under the same conditions but at neutral pH and displayed in Figure 7.

[47] Since the molar absorption coefficient of $(SCN)_2^{\bullet-}$ has only been determined in neutral aqueous solution, the KSCN method cannot be used to determine $\Phi_{^{\bullet}OH}$ values under acidic conditions.

[48] The similarity between the transient absorption spectra recorded for N-HP at pH = 7 and in protic organic solvents suggests that the photochemistry of the negatively-charged structure is either identical to that of the neutral form or different, but very inefficient.

[49] e_{aq}^- is known to present a broad absorption band with a maximum around 720 nm in aqueous solution.[34]

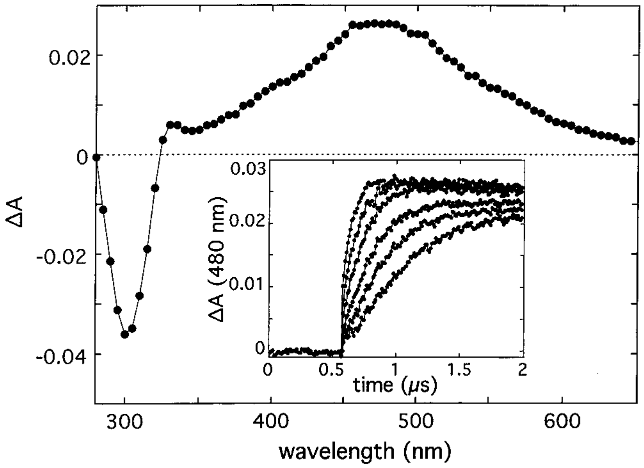

Figure 7. Spectrum recorded 8 μs after flash photolysis ($\lambda_{exc} = 308$ nm) of N-HP in deaerated buffer at neutral pH in the presence of 0.2 M of KSCN. The absorption of the solution at 308 nm was 0.4 and the laser intensity was 10 mJ cm^{-2} $pulse^{-1}$. The inset displays the kinetic signals recorded at 480 nm, the absorption maximum of $(SCN)_2^{\bullet-}$, as [KSCN] is increased from 0.25 to 1.5 M, using a constant laser intensity of 10 mJ cm^{-2} $pulse^{-1}$.

kinetics of the decay at 390 nm and of the growth at 310−315 nm were observed to be identical within experimental error over a wide range of laser energies and of ground-state concentrations, both gradually becoming more second-order in character with increasing laser energies or N-HP concentrations.[50] This suggests that these two processes are correlated: PyO$^\bullet$ is involved in the formation of the photoproduct absorbing around 315 nm (identical to the behavior observed in protic organic solvents). Similar to methanol and 2-propanol, HPLC analysis of N-HP irradiated in buffers of pH $\leq$ 7 shows that this photoproduct is 2-hydroxypyridine.

Figure 7 presents the spectrum recorded after pulsed excitation ($\lambda_{exc} = 308$ nm) of a neutral aqueous solution of N-HP containing KSCN (0.2 M). The presence of a strong absorption band with a maximum around 480 nm,[45] which is characteristic of $(SCN)_2^{\bullet-}$ (produced by eqs 9 and 10), demonstrates the generation of $^\bullet$OH by UV irradiation of N-HP at pH = 7. This band was not observed when $^\bullet$OH scavengers (*t*-BuOH, DMSO, ascorbic acid, or phenol) were added to the solution.

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 due only to photoinduced N−O bond cleavage.

Under experimental conditions where all the hydroxyl radicals generated by N-HP are quenched by KSCN, the quantum yield of $(SCN)_2^{\bullet-}$ formation is equivalent to $\Phi_{^\bullet OH}$. As shown in the inset of Figure 7, as the concentration of KSCN is increased, the kinetic signal due to the production of $(SCN)_2^{\bullet-}$ reaches a plateau corresponding to a total trapping of $^\bullet$OH. The $\Phi_{^\bullet OH}$ values determined for N-HP in buffer at neutral pH and calculated using eq 6[51] are reported in Table 2. Since the generation of $^\bullet$OH from N-HP is solely due to photoinduced N−O bond cleavage, $\Phi_{^\bullet OH}$ (which is equivalent to Φ_{N-O}) was also determined by monitoring the amplitude of ground-state depletion with laser energy. The experiment was carried out using a time scale short enough to avoid interference from the subsequent recovery. The results obtained with this method are also reported in Table 2. The absence of noticeable nitrous oxide effects on Φ_{N-O} (which was determined to be 0.12 under N_2O-saturated conditions) confirms that photoionization of N-HP at pH = 7 is not significant to any detectable extent (N_2O is known to react efficiently with e_{aq}^- to generate $^\bullet$OH[52]).

The molar absorption coefficient of PyO$^\bullet$ at 390 nm ($\epsilon_{PyO^\bullet}$) in neutral buffer was determined by the comparative method to be (680 ± 20) M^{-1} cm^{-1},[53] a value identical (within experimental error) to that measured for PyO$^\bullet$ in aqueous solution (pH = 7) when hydroxamic esters were used as precursors.[54]

Thus, PyO$^\bullet$ and $^\bullet$OH are the only species generated by UV irradiation ($\lambda_{exc} = 308$ nm) of N-HP in buffer at neutral pH. The lower reactivity of $^\bullet$OH toward water than organic solvents makes it more available to interact with the starting material. Pyridone-type molecules have been reported to present a high reactivity toward $^\bullet$OH[37,55] (*k* values of 4.4×10^9 and 3.6×10^9 M^{-1} s^{-1} were determined for the 2- and the 4-pyridone, respectively[37]). Therefore, one could expect similar behavior in the case of N-HP. Recording the kinetic signal at the maximum of ground-state depletion on a short time scale, one observes that the initial instantaneous negative step is followed by a slow bleaching process ($\leq$1 μs) rapidly masked by the growth attributed to formation of the photoproduct. This slow bleaching process, which consumes N-HP, does not take place in the presence of DMSO or 2,4-HD in the solution, suggesting that the corresponding chemical reaction involves $^\bullet$OH.[56] The reactivity of PyO$^\bullet$ toward its precursor was studied using a deaerated solution of N-HP buffered at pH = 7 and containing *tert*-butyl alcohol (*t*-BuOH, 1 M) in order to quench $^\bullet$OH and prevent secondary reactions. The decay of the 390 nm transient absorption was monitored for N-HP concentrations between 30 and 300 μM. However, as the ground-state concentration is increased (over 100 μM), the decay of PyO$^\bullet$ becomes second-order in character, suggesting that, under pulsed irradiation conditions, even if PyO$^\bullet$ can react with its precursor, it is a minor reaction compared to the bimolecular radical process.

Above neutral pH, where structure **3** is largely predominant, the transient absorption spectrum of N-HP presents significant modifications. By comparison with other solvents, in alkaline aqueous media, a large decrease of the intensity of all the bands is observed for similar laser energies. The time-dependent transient absorption spectrum recorded after flash photolysis (λ_{exc} = 308 nm) of **3** in deaerated buffer at pH = 10 is presented on Figure 8. The spectrum displays a negative band with a maximum at 315 nm due to photoinduced ground-state depletion. The corresponding kinetic signal is a step function over several seconds, suggesting no subsequent N-HP consumption or product formation. A broad band around 390 nm which extents out to 480 nm is also observed as well as a weak absorption with a maximum around 700 nm. The latter absorption band, which decays exponentially with a lifetime of 500 ns, was attributed to e_{aq}^- since it is efficiently quenched

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[50] In both cases, the effect is to enhance the concentrations of radicals with the consequence that the radical−radical reaction process becomes more probable.

[51] (a) A value of 6550 M^{-1} cm^{-1} [51b] was used for $\epsilon_{(SCN)_2^{\bullet-}}$, the molar absorption coefficient of $(SCN)_2^{\bullet-}$ at 500 nm. (b) Lambert, C.; Sarna, T.; Truscott, T. G. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 3879−3882.

[52] See ref 34, p 518.

[53] These experiments were carried out in the presence of *t*-BuOH in order to quench $^\bullet$OH and avoid secondary reactions with the starting material.

[54] The molar absorption coefficient of PyO$^\bullet$ at 390 nm was determined to be 700 M^{-1} cm^{-1} using different hydroxamic esters in buffer at neutral pH. Since the ester derivatives of N-HP are slowly hydrolyzed in aqueous media, cold buffer was used as solvent to retard the degradation. Hydroxamic esters with R = $(CH_3)_3C$ and R = $(C_6H_5)CH_2$ (see eq 3) were studied in buffer at pH = 7.

[55] Icli, S. *Tetrahedron* **1990**, *46*, 2891−2902.

[56] No detectable corresponding growth (with rise time $\leq$ 1 μs) in absorption was observed, preventing the identification of the product of reaction between $^\bullet$OH and the starting pyridone.

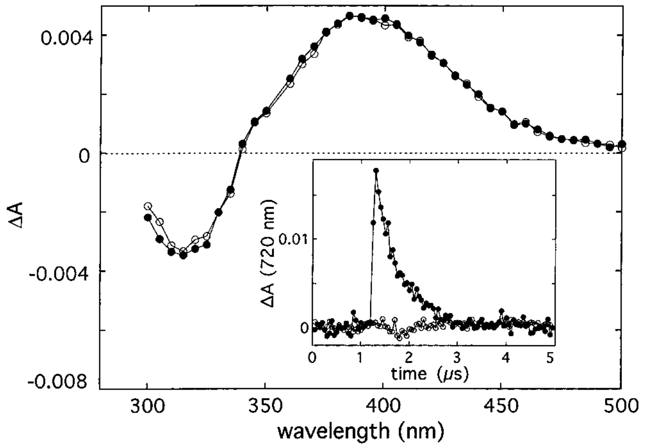
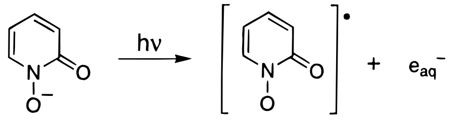


Figure 8. Spectra recorded for N-HP in deaerated aqueous buffer at pH = 10, 300 ns (●) and 70 μs (○) after excitation (λ_{exc} = 308 nm). The absorption of the solution sample was 0.45 at λ_{exc} and the laser intensity, 15 mJ cm^{-2} $pulse^{-1}$. The inset shows the monoexponential decay of e_{aq}^- (●), recorded at 720 nm after excitation of N-HP in deaerated buffer at pH = 10 and the signal obtained in the presence of N_2O (○).

by N_2O (see inset of Figure 8). The presence of e_{aq}^- indicates that structure **3** undergoes photoionization, as shown by eq 11.



(11)

The quantum yield of photoionization (Φ_{e^-}) was determined by monitoring the variation of the maximum absorbance of e_{aq}^- with laser energy. The intensity dependence was found to be linear in the whole range of energies studied, indicating that photoionization of N-HP occurs *via* a monophotonic process. Using eq 6,[57] Φ_{e^-} was calculated to be 0.003. This low value confirms the hypothesis of a different and inefficient photochemistry for structure **3** compared to that of the neutral form of N-HP (see ref 48). Due to the weak efficiency of photoionization and the limitation of the detection system, the species produced concomitant to e_{aq}^- by electron ejection from N-HP could not be observed.[58]

The transient absorption band at 390 nm decays very slowly (over several milliseconds). The corresponding species, generated within the laser pulse, is not affected by the presence of potential scavengers such as O_2, phenol, ascorbic acid or 2,4-HD in the solution. An identical long-lived, weak, and broad absorption band with a maximum at 390 nm was also recorded after flash photolysis (λ_{exc} = 308 nm) of various hydroxamic esters in buffer at pH = 10.[44] Since the only photoprocess undergone by these compounds is homolytic N−O bond cleavage (eq 3), this band can be attributed to PyO$^•$. The molar absorption coefficient at 390 nm for PyO$^•$ produced by hydroxamic esters at pH = 10 was found to be 3000 M^{-1} cm^{-1}. A similar value of 3100 M^{-1} cm^{-1} was measured for the species generated by N-HP, indicating that the absorption band at 390 nm (Figure 8) can be assigned to PyO$^•$.

The quantum yield of photoinduced consumption of starting material (Φ_{Bl}) was determined by monitoring the amplitude of initial ground-state depletion at 315 nm with laser energy. A Φ_{Bl} value of 0.04 (which was found to be identical in the presence of O_2 in the solution) was obtained. Therefore, under UV excitation in aqueous buffer at pH = 10, N-HP undergoes homolytic N−O bond cleavage (Φ_{N-O} = 0.037[59]) in addition to photoionization (Φ_{e^-} = 0.003).

Discussion

The results of our investigation clearly demonstrate the advantages of N-HP compared to N-HPT, as a simple and selective photolytic source of $^•$OH applicable in biologically relevant situations. With the exception of highly alkaline aqueous media, the *only* transient species produced by UV excitation (λ_{exc} = 308 nm) of N-HP are $^•$OH and PyO$^•$ (or PyOH$^{•+}$). In all cases, the homolytic N−O bond cleavage leading to generation of these radicals occurs in high yield. The absence of competitive photoprocesses which give rise to other reactive species in the case of N-HPT (*i.e.* hydrated electron, triplet state, and free radicals) simplifies the system. In addition, due to its poor reactivity, PyO$^•$ is unlikely to cause efficient biological damage, therefore allowing the observed effects to be unambiguously attributed to $^•$OH.

Compared to its sulfur analogue, N-HP was found to possess much simpler photochemistry. Whether intramolecularly hydrogen-bonded (structure **1**) or not (structure **2**) (*i.e.* either in solution in aprotic or protic solvent), the only primary photoprocess undergone by the uncharged form of N-HP is homolytic N−O bond cleavage leading to concomitant formation of $^•$OH and PyO$^•$, according to eq 5. As shown by Table 2, similar Φ_{N-O} values were obtained for structures **1** and **2**, suggesting that the intramolecular hydrogen bond of N-HP in aprotic media and its intermolecular interaction with protic solvents have similar effects on the efficiency of the scission process. It is also worth noting that the Φ_{N-O} values determined for the neutral form of N-HP are very close to those measured for N-HPT under similar experimental conditions.[11]

The negatively-charged structure of N-HP was observed to be much less photolabile than its conjugate acid. The quantum yield of N−O bond cleavage caused by UV excitation of **3** at pH = 10 was determined to be 0.037. In addition to the homolytic scission, the anionic form of N-HP also undergoes monophotonic photoionization. However, even at pH = 10, where **3** is, by far, the major species present in solution, the photoinduced electron ejection was found to be very inefficient (Φ_{e^-} = 0.003). This quantum yield is low compared to that measured for the corresponding anionic form of N-HPT, for which Φ_{e^-} = 0.1 (using λ_{exc} = 308 nm). The difference between the oxygen and sulfur analogues can be tentatively rationalized in terms of the known increase of ground-state energy associated with the replacement of a C=O bond by a C=S bond.[60] Higher ground-state energies were recently shown to be, at least partially, responsible for the decrease in the homolytic bond dissociation energies in pyridones and related heterocycles.[60] A similar consequence on the ease of photoionization can reasonably be expected in the case of the compounds studied here.[61]

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[57] A molar absorption coefficient of 19 000 M^{-1} cm^{-1} was used for e_{aq}^- at 720 nm in water (see ref 34, p 515).

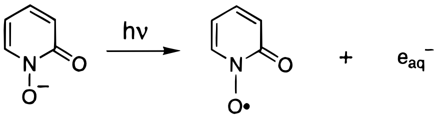
[58] (a) The species produced by photoionization could only be observed if its molar absorption coefficient at pH = 10 is in the same range than that of e_{aq}^- at 720 nm (*i.e.* ∼19 000 M^{-1} cm^{-1}). This ϵ is much higher than the usual values reported for radicals of pyridone-type molecules (*e.g.* for λ_{max} between 350 and 400 nm, $\epsilon \leq 8000$ M^{-1} cm^{-1},[55] $\epsilon \leq 2600$ M^{-1} cm^{-1},[58b] and $\epsilon \leq 3000$ M^{-1} cm^{-1} in our study), suggesting that, although present in solution, the species formed by photoionization of N-HP could not be detected by our system. (b) Danziger, R. M.; Hayon, E.; Langmuir, M. E. *J. Phys. Chem.* **1968**, *72*, 3842-3849.

[59] This value was calculated using eq 7 after correction for the photoionization process undergone by N-HP.

[60] Bordwell, F. G.; Singer, D. L.; Satish, A. V. *J. Am. Chem. Soc.* **1993**, *115*, 3543-3547.

Our results also demonstrate that only the negatively-charged structure of N-HP is capable of electron ejection under UV excitation. This is identical to our observations concerning N-HPT in aqueous media.[11] In the latter case, a parallel with the photoionization of phenols and related compounds (which undergo monophotonic electron ejection from the anionic form[62] and biphotonic photoionization[62−65] *via* the lowest triplet state[62,63] from the uncharged structure) led us to the conclusion that the absence of e_{aq}^- formation from the conjugate acid of N-HPT was not surprising since the absorption properties of its excited triplet state do not favor the absorption of a second photon (at 308 or 355 nm), to facilitate photoionization.[11] The absence of solvated electron production in the case of the neutral form of N-HP is even less surprising than for N-HPT as no triplet state was observed to be generated from structure **2**.[35]

Due to the low efficiency of electron ejection from N-HP, identification of the species generated by photoionization at pH = 10 (eq 11) cannot be achieved (*vide supra*). By analogy with N-HPT,[11] one can assume the formation of the semioxidized radical of starting material (eq 12).

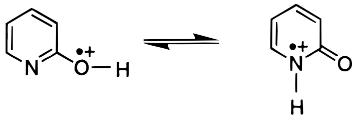


(12)

In all solvents (with the exception of basic aqueous solutions), PyO$^\bullet$ (or PyOH$^{\bullet+}$) is the only species produced concomitant to $^\bullet$OH, by UV irradiation of N-HP. Therefore, its properties are of importance to draw conclusions concerning the potential of UV excitation of N-HP as a clean and selective source of $^\bullet$OH.

In organic media, the presence of PyO$^\bullet$ was detected by its characteristic absorption band with a maximum at 390 nm. The shape and the position of this band were observed to be unaffected by the nature of the environment. This is not surprising since the ground-state absorption spectrum of N-HP does not show large solvent-dependent modifications (Table 1 and Figure 1). Similar behavior was also reported in the case of the closely related phenoxyl radical (PhO$^\bullet$).[26,27]

On the other hand, in aqueous media, differences in absorption properties were observed for the neutral radical (PyO$^\bullet$) and its conjugate acid (PyOH$^{\bullet+}$) (see eq 8). Decreasing the pH of the solvent causes a broadening of the absorption band, an increase of its intensity, and a blue shift of its maximum (λ_{max} = 390 nm for PyO$^\bullet$ and 350 nm for PyOH$^{\bullet+}$). The pK_a of the corresponding acid/base equilibrium was estimated to be between pH = 2 and 3 (see inset of Figure 6). By comparison to the phenoxyl radical cation, PhOH$^{\bullet+}$ (which was reported to have a pK_a of −2.1[66−68] in aqueous media), PyOH$^{\bullet+}$ appears as a weaker acid. If one assumes that PyOH$^{\bullet+}$ exists under form **a** (eq 13), an increase in acidity is expected, due to the electron-withdrawing inductive effect of the sp^2 ring nitrogen atom, known to be both cation[69] and radical[60] destabilizing (acid strengthening) relative to the phenyl analogue. The discrepancy between the expected and experimental results suggests that PyOH$^{\bullet+}$ rather exists as structure **b** (eq 13), where the unpaired electron and positive charge are localized on the nitrogen atom. This is supported by the fact that pK_a values of various hydroxylated pyridyl radical cations were reported to be ~2.5 for deprotonation of the nitrogen atom and <−1 for deprotonation of the oxygen atom.[37]



a **b**

(13)

Irrespective of its form (protonated or unprotonated), the 2-pyridyloxyl radical generated along with $^\bullet$OH by N−O bond cleavage of N-HP was found to be very poorly reactive. The difference in reactivity between PyO$^\bullet$ and PyS$^\bullet$ is best illustrated by the rate constants measured for their reaction with biological molecules such as cholesterol (k(PyO$^\bullet$) ≤ 10^4 M^{-1} s^{-1} and k(PyS$^\bullet$) = 5 × 10^6 M^{-1} s^{-1}) and fatty acids (*e.g.* for methyl linoleate, k(PyO$^\bullet$) ≤ 10^4 M^{-1} s^{-1} and k(PyS$^\bullet$) = 2 × 10^7 M^{-1} s^{-1}). However, similar to the closely related PhO$^\bullet$ recently reported to present a surprisingly high reactivity toward phenolic compounds,[40] PyO$^\bullet$ was found to be quenched very efficiently (k_Q = 1−3 × 10^9 M^{-1} s^{-1}) by α-tocopherol. In aqueous as well as in organic solvents, PyO$^\bullet$ was observed to decay mainly *via* a radical recombination reaction. This behavior is similar to that of PhO$^\bullet$, which was shown to produce carbon−carbon and carbon−oxygen dimers under irradiation.[43]

In conclusion, the present laser flash photolysis investigation has demonstrated that N-HP is a much simpler and more specific generator of $^\bullet$OH than its sulfur analogue, N-HPT. One of the only drawbacks of N-HP, compared to N-HPT, is its blue-shifted absorption spectrum. However, due to its intensity-independent photochemistry, N-HP is suitable for 313 nm excitation from a mercury lamp, irradiation conditions which limit the probability of direct absorption of UV light by biological chromophores associated with nucleic acids and proteins.[1,2]

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[61] (a) The acidifying effects brought about by the replacement of a C=O bond by a C=S bond (which were reported in the case of some pyridones[60] and which hold true for the molecules considered in this study) can also be explained by an increase in ground-state energies, together with the greater ability of sulfur than oxygen to accomodate a negative charge.[61b] (b) Bordwell, F. G.; Harrelson, J. A., Jr.; Lynch, T.-Y. *J. Org. Chem.* **1990**, *55*, 3337−3341.

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