Photochemistry of 2-Azido-1-methylimidazole in Aqueous Solutions. Observation of the 1-Methyl-2-imidazolylnitrenium Ion

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Abstract: Irradiation of 2-azido-1-methylimidazole (**12**) in aqueous solution gives products from two reaction channels. One pathway involves a ring opening typical of azidoheterocycles. The observed products are glyoxal bis-hydrate, the methylammonium ion, and cyanamide; a glyoxal bis-oxime is presumed to be the intermediate initially formed in the ring opening. The other pathway leads to products that retain the five-membered ring, the 2-amino-4,5-dihydro-4,5-dihydroxy-1-methylimidazolium ion **3**, its monophosphate ester **6** when the irradiation is carried out in phosphate buffer, and glutathione adducts **7** and **8** when glutathione (GSH) is present. These products have been previously observed in the reactions of 2-hydroxylamino-1-methylimidazole in aqueous solution, and arise from reaction of the 1-methyl-2-imidazolylnitrenium ion ($\mathbf{2}^+$) with water, phosphate, and GSH. This pathway is therefore proposed to involve formation of the cation $\mathbf{2}^+$ via protonation of the singlet 1-methyl-2-imidazolylnitrene **13** formed upon irradiation of the azide. A single transient species undergoing exponential decay with λ_{max} at 230−235 nm is observed with flash photolysis. This transient is assigned to $\mathbf{2}^+$ on the basis of the pH dependence of the yields of products, and especially because of the correspondence of $k_2(GS^-)$:k_s ratios measured directly with flash photolysis and by competition kinetics starting from the hydroxylaminoimidazole. The cation $\mathbf{2}^+$ has a lifetime in water of 100 ms, and shows a high selectivity for GSH with $k_2(GS^-) = 3 \times 10^7\ M^{-1}\ s^{-1}$. There is evidence that this class of nitrenium ion is formed upon reductive metabolism of 2-nitroimidazoles. Thus this class of drugs is capable of producing a relatively long-lived electrophile in biological systems.

Nitroimidazoles are long established antibiotics effective against anaerobic bacterial and protozoal infections.[1] They have also undergone extensive clinical examination as radiation sensitizers of hypoxic (oxygen-deficient) tumor cells.[2] Experiments with 2-nitroimidazoles, the derivatives more commonly employed in the latter studies, have revealed several biological properties that appear to be associated with reductive metabolism.[3] These include a preferential toxicity toward hypoxic cells as compared to normal aerated ones,[4] mutagenicity,[5] potentiation of the effect of other chemotherapeutic agents,[6] depletion of glutathione (γ-L-glutamyl-L-cysteinylglycine, GSH) and other cellular thiols,[4b,7] and selective binding to cellular macromolecules,[8] an effect that can be applied in the imaging of tumor hypoxia.[9] Like the other classes of bioreductive drugs, such as the mitomycins[10] and the benzotriazene di-*N*-oxides,[11] these phenomena are usually interpreted in terms of a biologically active metabolite formed via reduction, obviously of the nitro group in the case of the nitroimidazoles.[12]

One candidate for such a species is a 2-hydroxylaminoimidazole **1**, the product of a four-electron reduction of the nitro group. Several examples of **1** have been prepared.[13] They are unstable in aqueous solution at pH 7, reacting with half-lives of the order of 1−10 min.[14] In the absence of added nucleophiles, the products are a cis:trans mixture of **3**.[13b−d,15] These 4,5-dihydroimidazolium ions equilibrate with glyoxal bis-hydrate **4** and the guanidinium ion **5**. This equilibrium favors **3** to the extent that the cyclic system can be quantitatively prepared by mixing aqueous solutions of **4** and **5**.[13b−d,15,16] The cyclic **3** can

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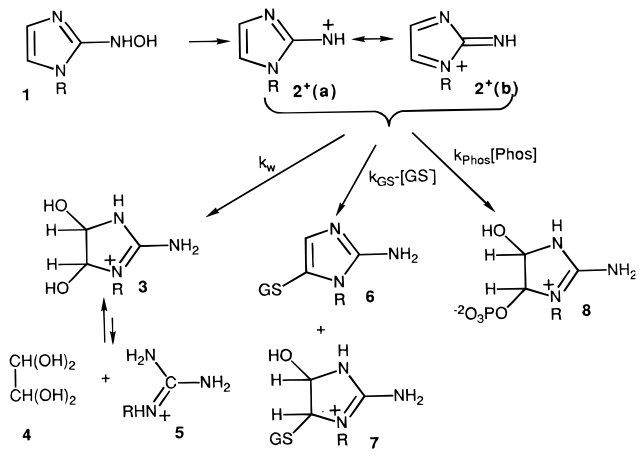
Scheme 1

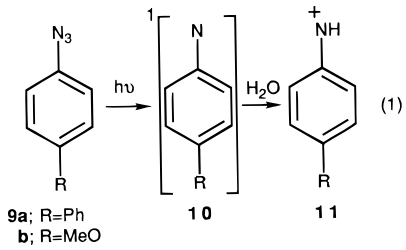


however serve as a source of glyoxal, forming appropriate derivatives with reagents capable of reacting with glyoxal, including guanosine.[13,17] A product capable of reacting like glyoxal is seen in cells[18] and *in vivo* in man[19] following treatment with the 2-nitroimidazole misonidazole.

A kinetic study has suggested that **1** reacts by initial formation of the cationic intermediate $\mathbf{2}^+$.[14] At pH 7, this occurs by simple N−O bond heterolysis, although under appropriate conditions there are pathways involving catalysis by H^+ and added buffer acids. Low concentrations of thiols such as GSH divert $\mathbf{2}^+$ to **6** and the cis:trans forms of **7**.[13d,20] This occurs with no change in the rate constant for the disappearance of **1**,[14] classic evidence for a reaction where the nucleophile reacts after the rate-limiting step. Even phosphate buffer is capable of trapping the cation, forming the cis:trans adducts **8**.[13d]

Although theoretical calculations suggest that the structure more closely resembles $\mathbf{2}^+\mathbf{(b)}$,[21] the cation can also be viewed as a nitrenium ion when written as $\mathbf{2}^+\mathbf{(a)}$. Such electrophiles have been implicated in the DNA binding observed with carcinogenic amines such as 4-aminobiphenyl and 2-aminofluorene.[22] Reduction products of nitroimidazoles have also been found to bind to DNA.[8] Although the species responsible for this has not been identified, the cation $\mathbf{2}^+$ is an obvious candidate.

The technique of flash photolysis has recently been used to generate and directly study arylnitrenium ions,[23] including **11a** derived from 4-aminobiphenyl.[24] This cation was observed in aqueous solution upon irradiation of the azide **9a**, being formed by protonation by the solvent of the initially formed singlet nitrene **10a**. A similar approach has been described with 2-azidofluorene[24] and with *p*-methoxyphenyl azide **9b**.[25] Singlet nitrenes are highly reactive species,[26,27] but they are also very basic[24] and thus can be trapped by protonation.[24,25,27,28]



In this paper we report a study of the aqueous solution photochemistry of 2-azido-1-methylimidazole **12**. Our objective was to see if this system produces the cation $\mathbf{2}^+$, in the same manner as the aryl azides **9**. There is indeed evidence from both product analysis and flash photolysis experiments that this does happen, although cation formation competes with a ring opening typical of heterocyclic azides.[29]

Results

Acidity Constant. A spectroscopic titration curve was constructed working at 290 nm. At this wavelength, there is a factor of 2 difference in the extinction coefficients of the imidazole and imidazolium forms of **12**. Fitting with the appropriate equation gave $pK_a = 4.05 \pm 0.05$. A value in satisfactory agreement of 3.95 was obtained from the pH of an 0.1 M solution of the HCl salt of **12** that had been half-neutralized with NaOH.[30]

Products. (a) Acid Solution. These experiments were carried out directly in an NMR tube, with solutions of the HCl salt of **12** in D_2O containing small amounts of DCl. Upon irradiation, the characteristic imidazole ring protons give way to upfield signals at 4.5−5.5 ppm, accompanied by three new methyl singlets also upfield from the original. As summarized below, two of these match the methyl peaks of cis−trans **3**; the doublets for the ring protons are also present.[13d] The third methyl peak is at 2.54 ppm and corresponds to the methylammonium ion, as was verified in the ^{13}C NMR spectra. There is no change in the position or ratio of the three methyl peaks with time, as is also true for the ring protons. Recording immediately after irradiation, however, results in a pair of doublets at 4.55 and 4.91 ppm that on standing convert to a singlet at 4.75 ppm. The latter corresponds to glyoxal bis-hydrate **4**, whose presence was also confirmed in the ^{13}C NMR. The total area of the signals at 4.55, 4.75, and 4.91 ppm remains constant, equal to two-thirds the methylammonium peak. A final piece of the puzzle is the observation in the ^{13}C NMR of a weak peak at 159 ppm, matching the carbon of cyanamide.

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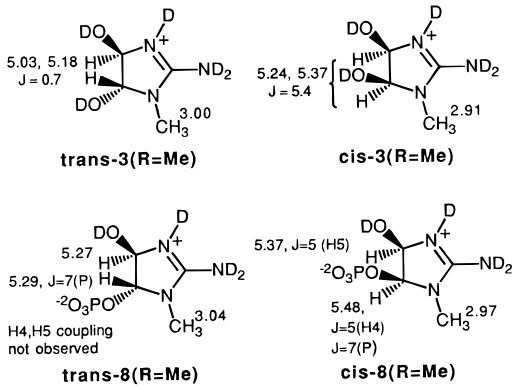
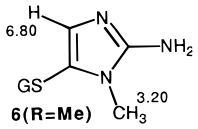


(b) Phosphate Buffer. A pattern similar to that in acids is observed. The methylammonium ion and glyoxal bis-hydrate are present, although the precursor to glyoxal is not observed even immediately after irradiation. Also present are the two isomers of **3**, and a second cis:trans mixture that corresponds to **8**. The characteristic NMR peaks that identify the latter pair[13d] are summarized on the structures above. Of note is the observation that the ratio **3**:**8** with the azide is very similar to that obtained with the hydroxylamine **1**.

(c) With GSH. This experiment involved an argon-saturated solution containing 0.0005 M **12**·HCl and 0.004 M GSH with the pH adjusted to 9.0. After irradiation, the water was removed by lyophilization, and the ^{1}H NMR spectrum was recorded in D_2O. The spectrum is complex because of the multiple signals associated with the GS portion. However, the major product is **6**, as evidenced by its methyl peak and in particular a characteristic singlet in the aromatic region.[20c] Traces of the methylammonium ion and **7** may be present, but there is no **3** (<5%). The absence of this product is also characteristic of the hydroxylamine **1** when it reacts in a solution of this GSH concentration.[14,20c]



(d) Ring-Retained versus Ring-Opened Products. As is apparent from the products, there are two photoreactions, one that leaves the imidazole ring intact and a second involving ring fragmentation. Since the methylammonium ion is the indicator of the latter, the ratio of the two can be obtained from the relative integration of the methylammonium peak versus the methyl peaks for **3** and, in phosphate buffer, **8**. The results are that in acid (pH 1−3.5), ring retention accounts for 35 ± 5% of the products, and in phosphate buffers (pH 6.5−7.5), 70 ± 5%.[31]

Spectroscopy. (a) UV Spectra. The azide **12** has λ_{max} at 254 nm (acid form) and 258 nm (base form) with extinction coefficients of 6700 and 6100, respectively. When **12** (0.1−0.5 mM) in phosphate buffer or HCl is irradiated at 254 nm in a UV cuvette and the spectra recorded, the band for **12** simply decreases until there is no residual absorbance above 220 nm.

(30) NMR spectra were also consistent with a pK_a in this range. In 0.01 M DCl the two ring hydrogens are at 7.22 and 7.19 ppm, while in both NaOD and a 1:1 NaD_2PO_4:Na_2DPO_4 buffer they appear at 6.90 and 6.81 ppm. Thus, **12** exists in the imidazole form in the neutral solution. Solutions in CD_3COOD:CD_3COONa buffers do show NMR signals at intermediate positions, but these experiments were not performed with sufficient accuracy to furnish a quantitative value for the acidity constant.

(31) This calculation was not performed for the experiment with GSH, since it appeared as if the lyophilization removed most of the methylamine.

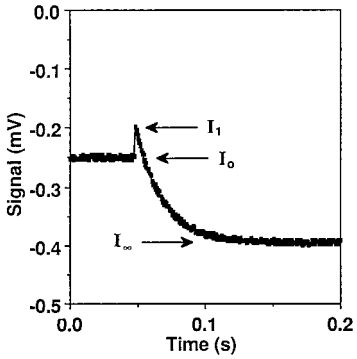


Figure 1. Lamp flash photolysis of 2-azido-1-methylimidazolium chloride (2.5×10^{-5} M) in 0.002 M HCl. The monitoring wavelength is 230 nm.

This is consistent with the products in these solutions, since none absorb above 220 nm.

(b) Flash Photolysis. Initial experiments involved laser flash photolysis with 248 nm excitation. These revealed changes from 220 (lowest λ employed) to 300 nm that were complete within the 20 ns laser pulse, an absorbance increase from 220 to 245 nm, and a decrease from 245 to 300 nm. Above 300 nm there was no change. The increase at lower wavelengths indicates the presence of some intermediate, since, as just discussed, the final products do not absorb. However, working to the maximum time resolution of the equipment (100 μs), there was no decay.

This could be seen at longer times with a lamp flash photolysis apparatus. A representative trace is shown in Figure 1. The initial reading I_o is the voltage output from the monitoring system before irradiation; this signal is based on transmittance through a solution that contains absorbing substrate. The reading I_1 is the voltage immediately after the flash. The solution becomes less transmitting, since at this wavelength irradiation produces a species that absorbs more strongly than the precursor. An exponential decay then results in a final reading I_∞ that is considerably smaller than I_o. Thus the solution at the end of the decay is more transmitting than it was before irradiation. The final products do not absorb above 220 nm, so that the net result of irradiation is an increase in transmittance due to the removal of the azide.

Equation 2 is the absorbance change that occurs in the decay, i.e., from I_1 to I_∞. This provides the absorbance of the transient, since it is decaying to transparent products. Equation 3 calculates a negative number, the absorbance decrease that occurs from I_o to I_∞ as a result of the depletion of the azide.

$$\text{Abs(transient)} = \log(I_\infty/I_1) \quad (2)$$

$$\text{Abs(bleach)} = \log(I_o/I_\infty) \quad (3)$$

Figure 2 shows the wavelength dependence of these two quantities in an acidic and a neutral solution, with Abs(bleach) plotted as a positive number. As expected, the latter results in spectra that are the same as those of the azide. Decay is observed over the range 220−300 nm,[32] and the rate constant is independent of wavelength. The latter is consistent with a single transient species being observed.

The shape of the absorption band of the transient is the same in both acidic and neutral solutions, with λ_{max} at 230−235 nm.

(32) At 245−300 nm, the azide precursor absorbs more strongly than the transient species. Thus, irradiation causes a prompt decrease in optical density, as observed in the laser flash photolysis experiments. This is followed by the slow exponential decay as the transient converts to the nonabsorbing products.

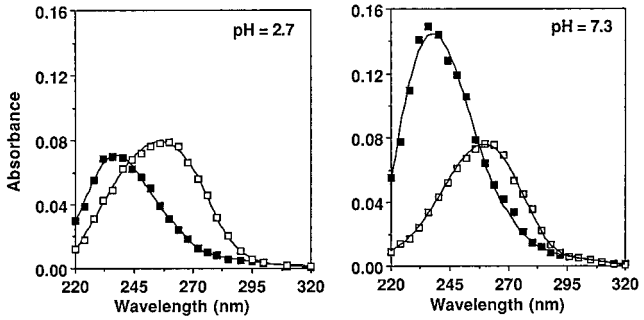


Figure 2. Spectra obtained with 2-azido-1-methylimidazolium chloride (2.5×10^{-5} M) in 0.002 M HCl (pH 2.7) and 0.005 M NaH_2PO_4: 0.005 M Na_2HPO_4 buffer (pH 7.3). The points ■ are Abs(transient) calculated by eq 2 and the points □ are the absolute values of Abs(bleach) calculated by eq 3.

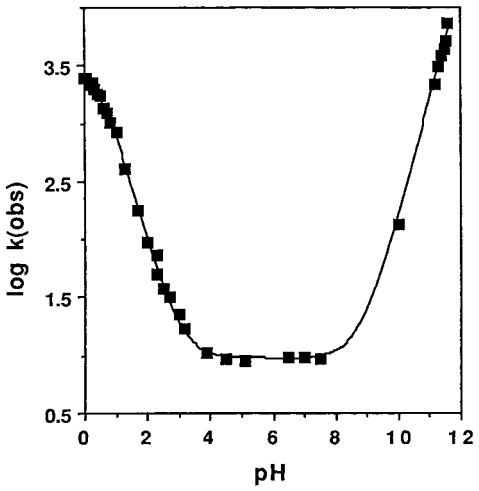


Figure 3. Log *k*(obs)−pH profile for the decay at 230 nm of the transient observed on photolysis of 2-azido-1-methylimidazole in aqueous solution (20 °C, ionic strength = 1.0 M maintained with $NaClO_4$). The data in solutions with pH <3 were obtained in $HClO_4$ solutions, pH 4−5 in acetate buffers, pH 6−8 in phosphate buffer, pH 10 in carbonate buffer, and pH >11 in NaOH solutions. The rate constants in the buffers have been extrapolated to zero buffer concentration. The line is based on Scheme 3 and the parameters given in Table 1.

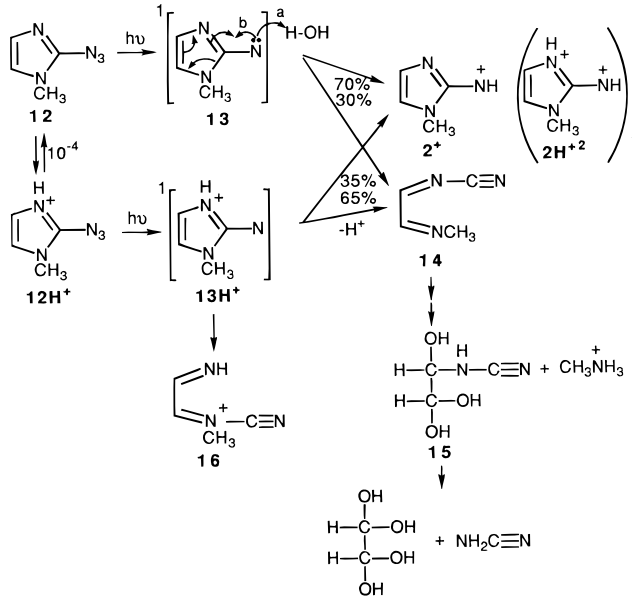
However, the magnitude is about double in the neutral solution. This must be due to a doubling in the quantum yield for the formation of this species. This statement can be made since the depletion of the precursor is essentially the same in the two solutions, especially when it is recognized that the azide is slightly less absorbing in the neutral solution.

(c) Kinetics of Decay. First-order rate constants for the decay of the transient were obtained as a function of pH and added buffers/nucleophiles at 20 °C, and an ionic strength of 1.0 ($NaClO_4$). The rate−pH profile (Figure 3) shows a pH-independent region from pH 4−8, with increases in acid and base; there is a suggestion of leveling below pH 1. The rate is increased by buffers. The nature of this acceleration was investigated in detail in neutral phosphate buffers. The form responsible is HPO_4^{2-}, with $k_2(HPO_4^{2-}) = 3.1 \times 10^2$ M^{-1} s^{-1}. The other reagent investigated in detail was GSH. It is the anionic form of this species that quenches, and it does so very efficiently, with $k_2(GS^-) = 3 \times 10^7$ M^{-1} s^{-1}.

Discussion

Mechanism. There can be no doubt that irradiation of **12** follows the pattern of behavior shown by the azides **9**,[24,25] with

Scheme 2



the singlet nitrene being in part trapped by protonation to form $\mathbf{2}^+$. This is the intermediate formed in the ground-state reactions of the hydroxylamine **1**, and the same products **3** and **6**−**8** are observed with the azide precursor, with similar patterns of behavior. The competing pathway is a ring opening, a reaction that also has precedence (with heterocyclic azides).[29] Combining these pathways, the mechanism of Scheme 2 can be proposed.

The most easily explained situation is the one where the neutral form **12**[33] is the precursor, so that an uncharged nitrene **13** is the intermediate. This partitions between pathways (a) and (b) where an electron pair takes a proton from the solvent forming $\mathbf{2}^+$ or moves internally to participate in ring opening. The protonation by solvent is indicated by the observation that "nitrenium" products are observed at neutral pH. This same observation was also made with the azides **9**, where with ps LFP the protonation of **10** (R = Ph) was found to have a rate constant of 5×10^9 s^{-1} (in 20% acetonitrile).[24a] While this rate constant for **13** is not known, it should also be very large because of the electron-rich imidazole ring. It can also be noted that an experiment in 100% acetonitrile results in no products derived from $\mathbf{2}^+$, as expected because of the requirement for water.

Under acidic conditions where the protonated $\mathbf{12H}^+$ is being irradiated, the competition switches to favor ring opening, but there is still clearly some protonation. The initial intermediate that is formed here is $\mathbf{13H}^+$, and the protonation mechanism is uncertain. The one shown in Scheme 2 involves a proton switch, which could involve several water molecules. There is a possibility of direct protonation to form a dication $\mathbf{2H}^{2+}$, a species for which there is evidence (see later). The 65:35 ring opening:protonation ratio would also be explained by a 50:50 competition between ring opening of $\mathbf{13H}^+$ and deprotonation to form **13**, which would then partition 30:70 between ring opening and protonation as in neutral solutions. It can be noted that the partitioning between ring opening and protonation remains the same from pH 1 to pH 3, ruling out a role for H^+ in the latter. This is not unexpected if water protonation is as fast as it is with the biphenylylnitrene, since even 0.1 M H^+ is not going to compete effectively.

As shown in Scheme 2, the initial product of the ring opening is a glyoxal bis-imine **14**, whose hydrolysis would give the

(33) (a) 1-Azido-2-methylimidazole exists in the azide form, and not as its tetrazole isomer.[33b] (b) Granados, R.; Rull, M.; Vilarrasa, J. *J. Heterocycl. Chem.* **1976**, *13*, 281.

observed glyoxal bis-hydrate, methylammonium ion, and cyanamide. The intermediate in acid is proposed to be **15**. This species has lost the methylammonium ion, but retains nonequivalent CH protons whose chemical shifts are suggestive of hydrated carbons, i.e., not aldehyde or imine. Under neutral conditions this intermediate is not observed, presumably because its rate of breakdown to glyoxal and cyanamide is more rapid.

Under acidic conditions where **13H**$^+$ is the intermediate, the possibility exists of ring opening at the C_2-N_3H bond to give **16**, and ultimately methylcyanamide. This is not observed, or at least occurs to less than 10%. This can be explained by a mechanism for cleavage of the C_2-N_1Me bond where the proton at N3 is lost simultaneously. This nitrogen in **14** is very weakly basic because of the adjacent nitrile group, and there will be a powerful driving force for it to be removed before the ring-opened bis-imine is formed. If, on the other hand, C_2-N_3H is cleaved, the species formed must retain the positive charge.

There has been speculation as to whether the ring opening of azidoheterocycles occurs at the stage of the nitrene, or whether it is concerted with loss of nitrogen from the azide.[29,34] Our observation of ring-retained products establishes that the azide **12** does form nitrene on irradiation, although it does not unambiguously establish that this intermediate is also the precursor of the ring-cleavage products. The alternative however is to say that the outcome is determined during irradiation. In other words, as N_2 is lost there is one channel forming nitrene and from it only **2**$^+$, and a second channel involving ring opening. Although this scenario cannot be unequivocally dismissed, it seems less likely than one where the competition occurs at the nitrene stage.

Identity of Transient. The quantum yield for the transient is about twice as much in neutral solution as it is in acid. This correlates with the chemical yield of the ring-retained products, ~70% in neutral solution, and ~35% in acid. Thus, the transient must be an intermediate of the protonation pathway of Scheme 2, and not of the ring opening.

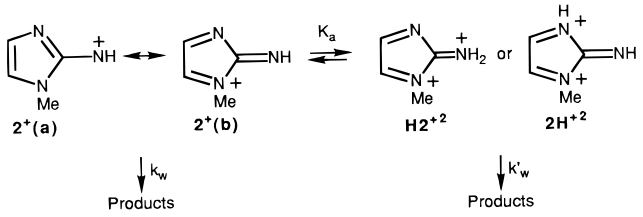
The question then arises as to whether the transient is the cation **2**$^+$ or some later intermediate. In favor of **2**$^+$ is the observation that the species is formed very quickly, within the 20 ns laser pulse. This implies that it represents an intermediate early in the reaction mechanism. More compelling evidence however takes the form of the GSH quenching. In a previous study with the hydroxylamine **1** (R = Me) as a precursor, a selectivity k_2 (GS$^-$):$k_s = 5 \times 10^5$ M^{-1} was obtained for the reactions of **2**$^+$.[14] With the transient, k_2(GS$^-$) is directly measured as 3×10^7 M^{-1} s^{-1}. The value of k_s in a buffer of the type employed in that previous study (0.1 M Na_2HPO_4, 0.05 M NaH_2PO_4) is 41 s^{-1}. Thus k_2(GS$^-$):k_s from the absolute rate constants is 7.5×10^5 M^{-1}. Considering the uncertainty in the previous ratio,[35] the agreement is excellent. The conclusion is that the transient is the same as the intermediate derived from the hydroxylamine, i.e., the cation **2**$^+$.

Kinetics of Reactions of 2$^+$. (a) pH Dependence. The rate−pH profile (Figure 3) shows the expected rate increase in base due to reaction of the cation with hydroxide. The increase in acid at first sight seems unexpected, but it was also seen with the 4-biphenylyl- and 2-fluorenylnitrenium ions.[24b] The explanation recognizes that these cations are not really nitrenium ions but are imines with a nearby positive charge.[24b,28b,36] In the case of the imidazole intermediate the major resonance contributor is **2**$^+$**(b)**.[21] This has two "imine" nitrogens, in each case two atoms removed from the positive charge. Either of these can be protonated forming a dication.

Table 1. Rate Constants and Dication Acidity Constant for the Transient Obtained at 230 nm on Flash Photolysis of 2-Azido-1-methylimidazole in Aqueous Solution (20 °C, ionic strength = 1.0 M with $NaClO_4$)

parameter	value
k_w	9.4 s^{-1}
k'_w	3.4×10^3 s^{-1}
pK_a	0.5
k_2($^-$OH)	1.5×10^6 M^{-1} s^{-1}

Scheme 3



The dication is more reactive toward water, and thus in acid solutions the rate is accelerated. In the more acidic solutions the acid−base equilibrium starts to shift in favor of the dication, and the rate begins to level. The kinetic expression is k(obs) = $(k_wK_a + k'_w[H^+])/(K_a + [H^+])$, and all three parameters can be determined by fitting the equation to k(obs). The constants so obtained, along with the hydroxide rate constant, are given in Table 1. It can be seen that the dication is 2 orders of magnitude more reactive than the monocation. A similar difference was seen between the di- and monocations of the 4-biphenylyl- and 2-fluorenylnitrenium ions.[24b] The pK_a of the dication is 0.5, consistent with the protonation of an imine where there is N$^+$ center two atoms distant.

(b) Reactivity. The selectivity ratio k_2(GS$^-$):k_w calculated with the absolute rate constants is 3×10^6 M^{-1}. This approaches the value of 10^8 M^{-1} predicted by N$^+$, a scale based on reactivities of highly stabilized carbocations.[37] It is of course not known whether cations such as **2**$^+$ actually follow N$^+$. Whatever the case, it certainly shows a very high selectivity toward GS$^-$, a selectivity that approaches that of a highly stable carbocation.

In our previous study the selectivity of 5×10^5 M^{-1} was employed to produce an estimate of 100 μs for the lifetime of **2**$^+$ in a neutral aqueous solution.[14] The actual lifetime is 100 ms, 1000-fold longer. That previous estimate was based on the assumption that GS$^-$ was reacting at the diffusion limit, with a rate constant of 5×10^9 M^{-1} s^{-1}. The direct measurements show that this is far from the case; the reaction is over 2 orders of magnitude slower than diffusion. This obviously accounts for the majority of the difference. The remainder is explained through our previous neglect of the phosphate component of the reaction.[35]

(34) (a) Spinelli, D.; Zanirato, P. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1129. (b) L'Abbe, G.; Dyall, L. K.; Meersman, K.; Dehaen, W. *J. Chem. Soc., Perkin Trans. 2* **1994**, *1994*, 2401. (c) Dyall, L. K.; L'Abbe, G.; Dehaen, W *J. Chem. Soc., Perkin Trans. 2* **1997**, 971.

(35) In our previous study, we treated "k_s" as the rate constant for the reaction of **2**$^+$ with water only. However, the selectivity was based on an assay that only looked at GSH depletion. Thus, reactions with water and with the phosphate employed as the buffer were not distinguished. The present study shows that both react, so that k_s was actually equal to $k_w + k_2(HPO_4^{2-})[HPO_4^{2-}]$. In fact the experimental partitioning ratios determined in the previous work show considerable scatter that can be attributed to changes in the concentration of HPO_4^{2-}.

(36) Srivasta, S.; Toscano, J. P.; Moran, R. J.; Falvey, D. E. *J. Am. Chem. Soc.* **1997**, *119*, 11552.

(37) Ritchie, C. D. *Can. J. Chem.* **1986**, *64*, 2239.

Table 2. Rate Constants for the Reactions of Nitrenium Ions and Carbenium Ions with Water

cation	k_w, s^{-1}	k_w-RCHPh$^+$ / k_w-RNH$^+$
2-imidazolyl-NH$^+$ (**2**$^+$, R_1 = Me)	9.4×10^0 [a,b]	1.3×10^4
2-imidazolyl-CHPh$^+$ (**17**, R_1 = Me)	1.2×10^5 [a,c]	
2-imidazolyl-CH$_2^+$ (**18**, R_1 = Me)	6×10^7 [d,e]	
2-fluorenyl-NH$^+$ (**19**)	3.4×10^4 [a,f]	4.4×10^2
2-fluorenyl-CHPh$^+$ (**20**)	1.5×10^7 [a,g]	
4-methoxyphenyl-NH$^+$ (**11b**)	1.9×10^6 [a,h]	1.1×10^0
4-methoxyphenyl-CHPh$^+$ (**21**)	2.1×10^6 [a,i]	
4-methoxyphenyl-CH$_2^+$ (**22**)	2×10^8 [d,j]	

[a] Directly measured by flash photolysis. [b] This work. [c] R. A. McClelland and S. Steenken, unpublished results. [d] Estimate based upon the azide clock method. [e] Bolton, J. L.; McClelland, R. A. *Can. J. Chem.* **1989**, *67*, 1139. [f] Reference 24d. [g] Davidse, P. A.; Kahley, M. J.; McClelland, R. A.; Novak, M. *J. Am. Chem. Soc.* **1994**, *116*, 4513. [h] Reference 25. [i] McClelland, R. A.; Kanagasabapathy, V. M.; Banait, N.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 3966. [j] Amyes, T. L.; Richard, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 9507; solvent is 50:50 CF_3CH_2OH:H_2O.

The k_w for **2**$^+$ is compared in Table 2 with values for other nitrenium and carbenium ions. As noted previously,[14,23] arylnitrenium ions are usually longer-lived than their arylcarbenium analogues. Thus the 2-fluorenylnitrenium ion **19** is almost 3 orders of magnitude longer-lived than the carbenium analogue **20**, a cation that also has a stabilizing phenyl group. The same comparison in the 2-imidazoyl case, **2**$^+$ versus **17**, shows a difference of 4 orders of magnitude. In fact, with the imidazole, there is a comparison with a primary arylcarbenium ion **18**, and here the difference approaches a million-fold. Table 2 also shows that a 2-imidazoyl group is highly stabilizing toward an adjacent carbenium or nitrenium center. Thus the imidazoyl carbenium ion **17** is 2 orders of magnitude longer-lived than the 2-fluorenyl analogue **20**. In the same comparison involving nitrenium ions, **2**$^+$ versus **19**, the difference is over 3 orders of magnitude.

Another interesting comparison is that involving the 4-methoxyphenylnitrenium ion **11b**. Noyce and co-workers have previously assigned σ^+ values to heterocyclic groups by comparing solvolysis rate constants for S_N1 reactions. The value for the 1-methyl-2-imidazolyl group is -0.82.[38] This implies that this ring system is only slightly more stabilizing than 4-methoxyphenyl where σ^+ is -0.78. The cation **2**$^+$ however is over 5 orders of magnitude longer lived than **11b**. This is yet another example of the poor predictability of σ^+ for the water reactivities of nitrenium ions, a feature first noted by Novak and co-workers.[39]

(c) Biological Relevance. This study confirms the previous suggestion[14] that "nitrenium" ions derived from reduction of 2-nitroimidazoles are long-lived species in water. In fact they are 3 orders of magnitude longer-lived than the previous estimate. Also confirmed in this study is the very high reactivity with glutathione. One can consider, for example, a situation that mimics that in cells, pH 7 and 1 mM GSH, so that the concentration of GS$^-$ is 16 μM (using pK_a(GSH) = 8.8).[40] In such a solution the lifetime of **2**$^+$ is 2 ms, and 98% of its reaction occurs with GSH. Under hypoxic conditions where reduction can occur, 2-nitroimidazoles effectively deplete intracellular GSH.[7] There is also a correlation between the cytotoxicity of the drug and the GSH level, toxicity being observed only after GSH has been depleted to relatively low concentrations.[7] The addition of exogenous thiol is also known to have a protecting effect.[41] If reduction of the 2-nitroimidazole occurs to the hydroxylamine level, under physiological conditions this species will ionize to **2**$^+$ over 1−10 min.[14] In the presence of normal amounts of GSH there will be efficient scavenging of the nitrenium ion. However, as the GSH concentration is reduced because of this reaction, the cation becomes longer-lived, and it may find other biologically relevant sites. This study has established, for example, that phosphate is reactive, and thus it is possible that the nitrenium ion could find and react with the phosphate ester groups of DNA. This might account for the small amount of DNA binding that is observed with nitroimidazoles under reductive conditions.[8] The availability now of a flash photolysis method for studying nitrenium ions of this class means that it should be possible to address some of these questions by examining directly reactions with typical examples of biologically relevant nucleophiles. These experiments are currently in progress.

Experimental Section

2-Azido-1-methylimidazolium chloride was prepared by a modification of the literature procedure.[33b] In a typical reaction 2.2 g of 2-amino-1-methylimidazolium chloride[42] in 20 mL of 2 M H_2SO_4 was diazotized at −15 °C with 0.75 g of $NaNO_2$ dissolved in a minimum amount of water. A saturated solution of excess NaN_3 was then added dropwise, and after gas evolution ceased the solution was allowed to come to room temperature. Sodium bicarbonate was then added to neutralize the solution, and after filtration of solid material that had formed, the resulting solution was extracted repeatedly with chloroform. The combined chloroform layers were then dried with $MgSO_4$, and after filtration, the chloroform was removed. The resulting red oil was dissolved in dry ether, and after filtration to remove material that did not dissolve, ether·HCl was added to form a pale yellow precipitate. The NMR of this material in D_2O showed only peaks at δ 7.22 (1H, d, J = 1 Hz), 7.19 (1H, d, J = 1 Hz), and 3.58 (3H, s). The neutral imidazole form was reformed by neutralizing with aqueous NaOH, and extracting with chloroform. A yellow oil was obtained after drying ($MgSO_4$) and removal of the chloroform. This material had an NMR spectrum in $CDCl_3$ that was identical with that in the literature.[33b] We found that it was not stable and used it immediately after preparation.

Product Analysis. Experiments in acid were performed by dissolving the imidazolium chloride (0.05 M) directly in a D_2O solution containing concentrations of DCl from 0.0005 to 0.1 M. For the phosphate buffer analysis the neutral form was dissolved in D_2O containing NaD_2PO_4:Na_2DPO_4, the latter prepared by dissolving NaH_2PO_4:Na_2HPO_4 in D_2O followed by lyophilization. The D_2O solutions were then placed in a NMR tube, which was suspended in a Rayonet reactor and irradiated for varying periods of time with 300 nm light. 200 MHz ^{1}H NMR spectra were recorded as soon as 5 min following completion of irradiation. Except for the change that was discussed in the Results section, and which occurs thermally, variation in the time of irradiation did not effect the relative amounts of products that formed. To conclusively establish the presence of **3** (prepared as in ref 13d), glyoxal, methylammonium ion, and cyanamide, authentic samples of these materials were added to the NMR tube containing the irradiated sample. The peaks assigned to the particular product were observed to increase in intensity, i.e, there was complete overlap with the signal or signals for the standard.

The experiments with GSH were performed by dissolving the substrate (0.0005 M) and GSH (0.004 M) in 30 mL of H_2O that had been degassed with argon. With the argon purging continuing, the pH of the solution was brought to 9.0 by the addition of a small quantity of 1 M NaOH. The solution was then irradiated at 300 nm under argon, until the UV spectrum showed essentially complete disappearance of

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the azide starting material. The solution was then transferred to a round-bottom flask and the solvent removed by lyophilization. D_2O (10 mL) was added, and the solution lyophilized again to convert exchangeable H to deuterium. D_2O (1 mL) was then added and the ^1H NMR spectrum recorded at 500 MHz.

Laser flash photolysis experiments involved ca. 20 ns pulses at 248 nm (60−120 mJ per pulse) from a Lumonics excimer laser (KrF) emission. The laser beam struck the 1×4 cm face of a $1 \times 1 \times 4$ cm cuvette. A pulsed Xenon lamp provided monitoring light, passing through the 1×1 cm face of the cuvette so that the path length was 4 cm. The monitoring beam was then passed through a monochromator onto a photomultiplier tube. The signal from this was amplified, digitized, and sent to a computer for analysis. The concentration of substrate used in this experiment was 50 μM. Experiments in both argon- and air-saturated solutions showed that there was no difference in the signal.

Conventional flash photolysis experiments were performed as previously described.[43] The sample was irradiated with broad-band irradiation from two flash lamps situated at either side of a cylindrical 10 cm long $\times$ 1.5 cm diameter cuvette, with the monitoring light passing through the 10 cm path length. The concentration of the azide was 5−10 μM. In constructing the spectra, one liter of a stock solution of 10 μM azide was prepared in the appropriate solvent and protected from light. A fresh solution of this stock solution was employed each time, with three flashes at each wavelength being averaged to provide the final OD readings. The rate−pH profile and the rate constant for HPO_4^{2-} were obtained with the ionic strength at 1.0 being maintained with $NaClO_4$. Five different buffer ratios of HPO_4^{2-}:$H_2PO_4^-$ were employed (from 4:1 to 1:4), with five different solutions at each ratio ranging up to 0.2 M total buffer concentration. For each buffer ratio a plot of k(obs) versus buffer concentration was linear. A plot of the slopes of these lines against the fraction of HPO_4^{2-} was linear with a zero intercept (within experimental error).

The rate constant for GS^- was obtained as follows. A stock solution of GSH of concentration 0.1 M was prepared in argon-saturated water. At the same time, solutions of 0.005 M 4:1 $NaHCO_3$:Na_2CO_3 (pH 9.3) and 0.004 M 1:1 $NaHCO_3$:Na_2CO_3 (pH 10) were prepared and sparged with argon. Just before irradiation a portion of the latter was transferred to a 100 cm volumetric flask under argon, to which was then added small volumes of the stock GSH solution and a stock solution of the substrate. The samples were transferred to the cuvette under argon, and immediately irradiated. The concentration of azide was 5 μM. Seven solutions varying in GSH concentration from 0 (none added) to 40 μM were employed. Two rate constants $k_2(GS^-)$ (3.4×10^7 at pH 9.3 and 2.6×10^7 at pH 10) were obtained from the slopes of the plots of the observed rate constants versus GSH concentration and averaged to give the value given in the results section. To establish that it was the anionic form of the GSH that was reacting, this experiment was repeated with a pH 6.8 phosphate buffer.

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Supporting Information Available: Representative ^1H NMR spectra following irradiation of 2-azido-1-methylimidazole in acid (Figure S1), in phosphate buffer (Figure S2), and in GSH solution (Figure S3) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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