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Photoinduced Release of Nitroxyl and Nitric Oxide from Diazeniumdiolates

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Abstract

Aqueous photochemistry of diazen-1-ium-1,2,2-triolate (Angeli's anion) and (Z)-1[N-(3-aminopropyl)-N-(3-aminopropyl)amino]diazen-1-ium-1,2-diolate (DPTA NONOate) has been investigated by laser kinetic spectroscopy. In neutral aqueous solutions, a 266 nm photolysis of these diazeniumdiolates generates a unique spectrum of primary products including the ground state triplet (³NO[•]) and singlet (¹HNO) nitroxyl species, and nitric oxide (NO[•]). Formation of these spectrophotometrically invisible products is revealed and quantitatively assayed by analyzing a complex set of their cross-reactions leading to the formation of colored intermediates, the N₂O₂^{•-} radical and N₃O₃⁻ anion. The experimental design employed takes advantage of the extremely slow spin-forbidden protic equilibration between ³NO[•] and ¹HNO and the vast difference in their reactivity toward NO[•]. To account for the kinetic data, a novel combination reaction, ³NO[•] + ¹HNO, is introduced and its rate constant of 6.6 × 10⁹ M⁻¹ s⁻¹ is measured by competition with the reduction of methylviologen by ³NO[•]. The latter reaction occurring with 2.1 × 10⁹ M⁻¹ s⁻¹ rate constant and leading to the stable, colored methylviologen radical cation is useful for detection of ³NO[•]. The distributions of the primary photolysis products (Angeli's anion: 22% ³NO[•], 58% ¹HNO, and 20% NO[•]; DPTA NONOate: 3% ³NO[•], 12% ¹HNO, and 85% NO[•]) show that neither diazeniumdiolate is a highly selective photochemical generator of nitroxyl species or nitric oxide, although the selectivity of DPTA NONOate for NO[•] generation is clearly greater.

Introduction

Diazeniumdiolates, also known as NONOates, are compounds of the general structure X[N(O)NO]⁻, where X is a strongly nucleophilic group, such as aminyl (R₁R₂N) or oxyl (O⁻);¹ in the latter case, the species is commonly referred to as Angeli's anion. These compounds are extensively used in biochemical, physiological, and pharmacological studies due to their ability to slowly release nitric oxide (NO[•]) and/or its congeneric nitroxyl (HNO) in neutral media.²⁻⁶ As shown in Scheme 1 for the two diazeniumdiolates that are investigated in this work, their thermal decompositions in neutral solutions both require a source of protons but are believed to occur through different pathways leading to different products.⁶⁻⁹ Apparently the nature of the nucleophile covalently linked to the nitrogen atom of the [N(O)NO]⁻ group is a key factor determining thermal reactivity of diazeniumdiolates.¹

The [N(O)NO]⁻ group is photosensitive, decomposing upon illumination in its characteristic mid-UV absorption band.¹⁰⁻¹⁵ Recently, we have used flash photolysis of Angeli's anion to generate HNO and its conjugate anion (NO[•]) and to investigate their reactivities.^{12,13,15} These

species comprise a unique acid-base pair in which the acid ground state is singlet (^1HNO) and the base has a triplet ground state ($^3\text{NO}^-$). We have shown that despite the high pK_a ($^1\text{HNO} = ^3\text{NO}^- + \text{H}^+$) ≈ 11.4 , the spin prohibition makes this acid-base equilibration so slow that ^1HNO and $^3\text{NO}^-$ coexist even in the strongly buffered neutral solutions. The rapid photochemical generation of ^1HNO and $^3\text{NO}^-$ has allowed us to investigate these unstable species. It would be impossible to obtain this type of insight with the far too slow thermal decompositions of Angeli's anion.

In addition to its utility for fundamental reactivity studies, the photochemical decomposition of diazeniumdiolates, especially if it can be photosensitized, holds the potential for highly-controlled photoactivated delivery of the biologically active $\text{NO}^\cdot$ and/or HNO to particular physiological targets. This approach is analogous to photodynamic therapy, and there is a significant ongoing effort toward the development of efficient and selective photochemical $\text{NO}^\cdot$ donors.^{11,14,16-21} In each case, it is important to know the distribution of primary products resulting from the donor excited states. This is particularly so for diazeniumdiolates, when both $\text{NO}^\cdot$ and HNO/NO^- can be generated and these species have been reported to produce distinctly different physiological effects.^{5,6,22,23} In our previous work, we found that both ^1HNO and $^3\text{NO}^-$ are primary products of the photolysis of Angeli's anion and that their relative yields depend on the Angeli's anion protonation state.¹² Toscano and co-workers have reported that the steady-state UV photolysis of $\text{Et}_2\text{N}[\text{N}(\text{O})\text{NO}]^-$ generates $\text{NO}^\cdot$ as the major final product and that N_2O is formed in minor quantities.¹⁴ However, the nature of the primary products of this photochemical process remains speculative.

In this work, we employ UV laser flash photolysis to quantify the primary release of $\text{NO}^\cdot$, ^1HNO and $^3\text{NO}^-$ from the photochemical decomposition of the two diazeniumdiolates shown in Scheme 1. We find that all these primary photolysis products are generated, but their distribution is quite different and depends on the structure of the parent compound.

Experimental Section

Sample Solutions

Analytical grade chemicals and Milli-Q purified (ASTM type I) water were used throughout. Disodium diazen-1-ium-1,2,2-triolate (Angeli's salt, $\text{Na}_2\text{N}_2\text{O}_3$) and (Z)-1[*N*-(3-aminopropyl)-*N*-(3-aminopropyl)amino]diazen-1-ium-1,2-diolate (DPTA NONOate) from Cayman Chemical were used as received. Relatively stable stock solutions of Angeli's salt and DPTA NONOate in 0.01 M NaOH were prepared daily; concentrations of these solutions were determined spectrophotometrically using molecular absorptivities $\epsilon_{248} = 8300 \text{ M}^{-1} \text{ cm}^{-1}$ (for $\text{N}_2\text{O}_3^{2-}$) and $\epsilon_{252} = 7860 \text{ M}^{-1} \text{ cm}^{-1}$ (for DPTA NONOate).^{24,25} Unstable neutral solutions of Angeli's salt and DPTA NONOate were prepared by flow-mixing equal volumes of an Angeli's salt or DPTA NONOate stock solution and a buffer solution; prior to mixing, both solutions were saturated with argon, nitrous oxide, or nitric oxide. Nitric oxide (Matheson) was purified by passing through a scrubbing column with 5 M NaOH and then through water. All solutions and the scrubber were thoroughly purged with argon before introducing nitric oxide. The nitric oxide solubility was taken as 1.9 mM/atm. All reported kinetic data pertain to 0.1 M phosphate buffers at pH 7. In a typical experiment, concentrations of Angeli's anion and DPTA NONOate were 0.3 and 0.1 mM, respectively, to obtain matching optical densities of the sample solutions at a 266 nm photolysis wavelength.

Laser Flash Photolysis with Flow Premixing

Transient absorption spectra were recorded by using a computerized kinetic spectrometer system described in detail elsewhere.^{12,13} Briefly, a 266 nm Nd:YAG laser operating at 20 Hz was used to photolyse the samples in a 0.4×1 -cm quartz flow cell. A polarization

beam splitter cube was used to adjust the laser energy incident on the cell in the 40 - 4 mJ/(cm² pulse) range, as measured by a thermoelectric bolometer. To allow for solution replacement between the laser shots the sample excitation frequency was reduced to 1 Hz by using a computer-controlled electromechanical shutter. Alkaline diazeniumdiolate and buffer, each saturated with the desired gas mixture, were forced by a small positive gas pressure into a 12-jet tangential mixer and then through the flow cell at a 12 mL/min flow rate; the laser excitation occurred within 1 s after mixing. The transient absorption was probed along a 1-cm optical path by a light beam from a 75-W xenon arc lamp, which was pulsed for short time scales. The kinetic traces were recorded by a Tektronix TDS 5052 oscilloscope operating in its oversampling mode, which typically allowed a suitable signal/noise ratio after a single laser shot. All flash photolysis experiments were performed at ambient temperature of 22 ± 2 °C. Kinetic modeling was carried out with the INTKIN software²⁶ developed at the Brookhaven National Laboratory by H. A. Schwarz.

Results and Discussion

Quantification of NO[•], ¹HNO, and ³NO[•] photolysis products

None of the three anticipated products of photodecomposition of diazeniumdiolates (NO[•], ¹HNO and ³NO[•]) exhibits molecular absorptivities in the near-UV-Vis spectral range that are characteristic and strong enough to allow their direct spectrophotometric detection on a short time scale. The same is true for the corresponding co-products (OH[•], NO₂[•], R₂NH, and R₂N-N=O) of the NO[•] and ¹HNO or ³NO[•] release from diazeniumdiolates. However, in our previous work we have found that the appearance of nitroxyl species can be detected through their reactions with NO[•], wherein concatenation of two NO[•] radicals sequentially produces the strongly-absorbing N₂O₂^{•-} radical and N₃O₃⁻ anion with absorption maxima at 280 and 380 nm, respectively (Figure 1).^{12,15,27} The major reactions involved are shown on the right side of Scheme 2 and their previously determined rate constants are summarized in Table 1 along with other minor reactions, which have little, if any, effect on data analysis under the prevailing conditions of this work, but are included for completeness.

Our experimental design takes advantage of the two unusual kinetic features in Scheme 2. The first is the absence of acid-base equilibration between ¹HNO and ³NO[•] on the time scale of interest due to spin prohibition involved in this reaction.¹³ This allows ¹HNO and ³NO[•] to be treated as the two completely independent reagents. The second feature is the vastly different rates for addition of the first NO[•] to ³NO[•] (reaction 1) and to ¹HNO (reaction 2). As a result, absorption of the long-lived N₃O₃⁻ anion grows in two well-resolved time windows, first from ³NO[•] and then from ¹HNO. With NO[•] added in large excess, the corresponding signal amplitudes allow straightforward quantification of ³NO[•] and ¹HNO. Finally, if NO[•] appears in solution along with either ³NO[•], or ¹HNO, or both, the concentration of NO[•] can be quantified from the rates of formation of the N₂O₂^{•-} and N₃O₃⁻ species. As described below, consistent application of this plan has revealed the existence of a previously unrecognized reaction 10 shown on the left side in Scheme 2.

Photolysis of Angeli's anion

Because of the large separation between successive pK_a (2.5 and 9.7) for H₂N₂O₃,²⁸ Angeli's anion is completely monoprotonated in neutral media, that is, it is present as HN₂O₃⁻. In Ar-saturated solutions, a 266 nm laser excitation of HN₂O₃⁻ induces a strong, prompt bleaching of its mid-UV absorption band (Figure 2) with near unit quantum yield.¹²

The transient absorption spectra in Figure 2 clearly show that N₂O₂^{•-} and N₃O₃⁻ with their characteristic absorptions at 290 and 380 nm, respectively, are formed following the bleaching. A 10 nm red shift of the maximum corresponding to N₂O₂^{•-} compared to its authentic 280 nm

position in Figure 1 is due to the overlap of this band with the bleaching of HN_2O_3^- . Absorption of N_2O_2^- rapidly grows to its maximum value at 8 μs , after which it decays symbatically with the rise of the N_3O_3^- signal (Figure 3). We consider the appearance of N_2O_2^- and N_3O_3^- as evidence that both $\text{NO}^\cdot$ and $^3\text{NO}^-$ are generated during the laser pulse, and attribute the observed kinetics to the sequential addition of two $\text{NO}^\cdot$ to $^3\text{NO}^-$ (Scheme 2, reactions 1 and 3). The minimal initial $\text{NO}^\cdot$ concentration required to generate the N_3O_3^- absorbance in Figure 3 is about $[\text{NO}^\cdot]_0 = 2 \times [\text{N}_3\text{O}_3^-]_{\text{max}} = 3 \mu\text{M}$. We have previously shown,^{12,15} that ^1HNO is also generated under these conditions and in an amount exceeding that of $^3\text{NO}^-$. However, the slower kinetic component of the N_3O_3^- growth associated with $\text{NO}^\cdot$ addition to ^1HNO (Scheme 2, reactions 2 and 3) is not observed, apparently because the $\text{NO}^\cdot$ level is too low for the N_3O_3^- growth to compete with its decay (reaction 4) occurring with the characteristic time of 4 ms.

To quantitatively intercept the ^1HNO and $^3\text{NO}^-$ species and thereby determine their initial concentrations, an NO -saturated solution was employed; a kinetic trace observed at 380 nm under these conditions is shown in Figure 4. Now, the rise of the N_3O_3^- transient absorption occurs in two well-resolved time windows; the fast step originates with $^3\text{NO}^-$ and corresponds to reactions 1 and 3 and the slow step reveals the presence of ^1HNO via reactions 2 and 3 in Scheme 2. The even more slow decay of N_3O_3^- through reaction 4 then follows, completing the process. The concentrations at the end of the pulse obtained from the N_3O_3^- signal amplitudes at 1.5 μs and 0.45 ms are $[^3\text{NO}^-]_0 = 12$ and $[^1\text{HNO}]_0 = 33 \mu\text{M}$, respectively.

We observe the same bleaching of Angeli's anion absorption in NO -saturated and in Ar -saturated solution and it corresponds to approximately 50 μM HN_2O_3^- decomposition. Because the photodecomposition stoichiometries are

the amount of $\text{NO}^\cdot$ produced cannot exceed about 10 μM . However, application of a numerical integration that includes only the previously identified reactions 1-6 in Table 1 does not correctly describe the observed kinetics for Ar -saturated solution when $[^3\text{NO}^-]_0 = 12$ and $[\text{NO}^\cdot]_0 = 10 \mu\text{M}$ (dash lines in Figure 3). First, the expected yield of N_3O_3^- is much greater than the observed yield at 380 nm and, second, the calculated kinetics for N_2O_2^- does not have the observed decay component. Also, comparison of the N_3O_3^- signal amplitudes in Figures 3 and 4 shows that only 13% of the initially formed $^3\text{NO}^-$ is converted to N_3O_3^- by $\text{NO}^\cdot$ photogenerated from Angeli's anion in the Ar -saturated solution. It would appear that reaction 1 is not the only pathway consuming $^3\text{NO}^-$, and that an additional, previously unrecognized $^3\text{NO}^-$ -consuming reaction occurs. The existence of such a reaction becomes evident from the dependence of the N_3O_3^- yields on the concentration of $\text{NO}^\cdot$ added to the solution. As shown in Figure 5, the N_3O_3^- yield falls steeply at $\text{NO}^\cdot$ concentrations below 0.5 mM. Contrary to this data, simulations with reactions 1-6 do not predict more than 10% drop in the N_3O_3^- yield down to 0.03 mM $\text{NO}^\cdot$ concentration. Thus an $\text{NO}^\cdot$ level that is 40 times greater than $[^3\text{NO}^-]_0$ is required to suppress some competing $^3\text{NO}^-$ -consuming reaction.

To gain insight into this reaction we have explored its competition with the reduction of methyl viologen (MV^{2+}) by $^3\text{NO}^-$. As a strong one-electron reductant ($E^\circ(\text{NO}^\cdot/^3\text{NO}^-) = -0.81 \text{ V}$, $\text{NHE}^{12,29,30}$), $^3\text{NO}^-$ should readily reduce methyl viologen ($E^\circ(\text{MV}^{2+}/\text{MV}^\cdot) = -0.448 \text{ V}$, NHE^{31}) to produce a stable radical cation, that is,

This reaction is indeed observed by monitoring the strong characteristic $\text{MV}^{\cdot+}$ absorption at 396 nm ($\epsilon = 42.1 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$)³², as shown in Figure 6A, inset. In contrast, ^1HNO , a weaker reductant ($E^\circ(\text{NO}^\cdot, \text{H}^+/^1\text{HNO}) = -0.14 \text{ V}$, NHE^{12}) does not reduce MV^{2+} with observable

rate. In spite of both products of reaction 9 being radicals, they do not recombine and MV^{+} persists in the $NO^{\cdot}$ presence.³³

Following flash photolysis of Angeli's anion, the MV^{+} radical signal grows exponentially and the observed rate constant for this process (k_{obs}) increases with concentration of added MV^{2+} , becoming practically linear at $[MV^{2+}] > 30 \mu M$ (Figure 6A). The value for $k_9 = (2.1 \pm 0.4) \times 10^9 M^{-1} s^{-1}$ has been estimated from the slope of the k_{obs} vs. $[MV^{2+}]$ plot. At low MV^{2+} concentrations ($< 30 \mu M$), reaction 9 does not efficiently compete with other pathways of $^3NO^{\cdot}$ decay, and MV^{+} forms with almost constant $k_{obs} \sim 2.4 \times 10^5 s^{-1}$. Under these conditions, the yields of MV^{+} are very small ($\sim 1 \mu M$), that is, less than 10% of initially formed $^3NO^{\cdot}$ is consumed in reaction 9. Taking into account that $k_1[NO^{\cdot}]_0$ is $3 \times 10^4 s^{-1}$, at most, the limiting value of k_{obs} measured at 10 - 20 μM of added MV^{2+} gives a crude estimate of $2 \times 10^5 s^{-1}$ for the pseudo-first order rate constant of $^3NO^{\cdot}$ decay through a pathway other than reaction 1.

In considering this pathway, our choices are limited to assume that $^3NO^{\cdot}$ reacts either with the parent Angeli's anion or with one of its photolysis products. To discriminate between these possibilities, we have investigated the effects of laser energy and concentration of $HN_2O_3^-$ on the MV^{+} radical formation. At constant laser energy, the value of k_{obs} monotonically increases with the concentration of $HN_2O_3^-$ (Figure 6B), which is in a qualitative accord with both possibilities. However, the monotonic increase of k_{obs} with laser energy at a constant $HN_2O_3^-$ concentration shown in Figure 6C is a clear indication that $^3NO^{\cdot}$ decays by reaction (s) with a photolysis product.

We have therefore examined several eventualities by numerical integration of the mechanism comprising reactions 1-6 in Table 1 plus *one* of the following: recombination of two $^3NO^{\cdot}$, reaction of $^3NO^{\cdot}$ with $N_2O_2^{\cdot-}$, or with $NO_2^{\cdot}$, or with 1HNO . The first two reactions would explain the observed kinetics only if their rate constants are at the level of $(2 - 5) \times 10^{10} M^{-1} s^{-1}$, which certainly exceeds a diffusion-controlled limit for the like-charged species. Similarly, addition of excess nitrite up to 1 mM does not alter the observed kinetics. In contrast, if we introduce the reaction

and include it in the mechanism with $k_{10} = 6.6 \times 10^9 M^{-1} s^{-1}$, the kinetics of $N_3O_3^-$ accumulation and decay as observed at 380 nm (where $N_2O_2^{\cdot-}$ and $N_3O_3^-$ are the only absorbing species, with their molar absorptivities accurately known^{15,27}) in Ar-saturated solution can be satisfactorily reproduced by simulations (Figure 7). Moreover, the characteristic times of the signal rise, decay, and maximum observed at 290 nm (mainly due to $N_2O_2^{\cdot-}$, but other species might also contribute, most notably, the bleaching of parent $HN_2O_3^-$) are also accounted for by including reaction 10.

In addition to k_{10} , the only other adjustable parameter in these simulations is the initial $NO^{\cdot}$ concentration, which comes out as $[NO^{\cdot}]_0 = 11 \mu M$. The yield of $N_3O_3^-$ at low $NO^{\cdot}$ concentration is almost entirely controlled by the competition between reactions 1 and 10 but as the concentration of $NO^{\cdot}$ rises above 0.5 mM, reaction 10 becomes practically inconsequential. As shown by the solid line in Figure 5, the yield dependence on the $NO^{\cdot}$ concentration is well described by using all the reactions in Table 1.

Reaction 10 is formally spin-forbidden, and yet, it is suggested to be quite rapid. We feel that the high rate is likely due to the very large driving force for this reaction, which is estimated as $\Delta_{10}G = -80$ kcal/mol based on our prior considerations of the $^3NO^{\cdot}/^1HNO$ energetics¹² and the tabulated data.³⁴ Our previous examination of the mechanism for acid-base equilibration involving $^3NO^{\cdot}$ and 1HNO has led us to a conclusion that their reactions, even if spin-forbidden,

can be close to diffusion-controlled when these reactions are sufficiently exergonic;¹³ reaction 10 may be an example of this behavior. Given the complexity of this reaction and its high rate, the intermediacy of an adduct between $^3\text{NO}^-$ and ^1HNO appears likely.

Photolysis of DPTA NONOate

Undoubtedly, both amino end groups in DPTA NONOate are fully protonated at pH 7. As for the $[\text{N}(\text{O})\text{NO}]^-$ functional group, the available data suggest that this group remains essentially deprotonated in neutral media.^{9,35} A cursory examination has not revealed noticeable differences in the flash photolysis behavior of DPTA NONOate at pH 9 and pH 7, indicating no consequential variation in the protonation state in this range. Therefore, below we only report results for pH 7.

As with Angeli's anion, laser excitation induces bleaching of the UV absorption band of DPTA NONOate (Figure 8) with near unity quantum yield. On the submicrosecond time scale, a broad and rapidly decaying absorption band in the 500 - 700 nm range is also observed. Based on its spectral properties³⁶ and on the fact that introduction of N_2O (an efficient hydrated electron scavenger)³⁶ completely eliminates this band (Figure 8, inset), we assign this absorption to the hydrated electrons produced via photoionization of DPTA NONOate. The photoionization occurs with very low efficiency; about 40 μM of DPTA NONOate is destroyed by the laser pulse but less than 0.8 μM of the hydrated electron ($\epsilon_{650} = 1.8 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$)³⁷ is produced.

Beyond the first microsecond, the transient absorption spectra clearly show formation of N_3O_3^- with its characteristic absorption band at 380 nm (Figure 8). The $\text{N}_2\text{O}_2^{\cdot -}$ radical precursor to N_3O_3^- cannot be detected at 290 nm because the small $\text{N}_2\text{O}_2^{\cdot -}$ absorption is overwhelmed by bleaching of DPTA NONOate, which is much stronger than for Angeli's anion. As with Angeli's anion however, the N_3O_3^- formation occurs in the two well-resolved time domains (Figure 9), and we interpret this pattern as an indication that photolysis of the DPTA NONOate also results in formation of $^3\text{NO}^-$, ^1HNO , and $\text{NO}^\cdot$. To determine the end-of-flash concentrations, $[^3\text{NO}^-]_0$ and $[^1\text{HNO}]_0$, the DPTA NONOate photolysis was performed in NO -saturated solution (Figure 4), from which $[^3\text{NO}^-]_0 = 2.5 \mu\text{M}$ and $[^1\text{HNO}]_0 = 9 \mu\text{M}$ have been obtained. That is, the yields of $^3\text{NO}^-$ and ^1HNO from DPTA NONOate photolysis are by about a factor of 4 less than for Angeli's anion, which suggests that $\text{NO}^\cdot$ can be a major remaining product.

To evaluate $[\text{NO}^\cdot]_0$, the kinetic traces for N_3O_3^- in the absence of the intentionally added $\text{NO}^\cdot$ have been simulated using the rate constants from Table 1 and slightly adjusting the initial concentrations of $^3\text{NO}^-$, ^1HNO , and $\text{NO}^\cdot$. The calculated absorption profiles at 380 nm conform to all experimental observations with the initial concentration values of 1.9 μM for $^3\text{NO}^-$, 8.6 μM for ^1HNO , and 60 μM for $\text{NO}^\cdot$ (Figure 9). This concentration of $^3\text{NO}^-$ is slightly less than the 2.5 μM value obtained from the data for NO -saturated solution; this difference can be accounted for by the contribution of hydrated electrons in the $^3\text{NO}^-$ formation at high $\text{NO}^\cdot$ concentration. Indeed, the hydrated electrons are extremely rapidly (at $2.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$)¹⁵ scavenged by $\text{NO}^\cdot$ to produce $^3\text{NO}^-$, so that at 1.9 mM $\text{NO}^\cdot$ (saturation) the scavenging is efficient. However, at low $\text{NO}^\cdot$ concentration, as in the DPTA NONOate photolysis in Ar -saturated solution, the hydrated electron contribution in the $^3\text{NO}^-$ formation is negligible. This is confirmed by an experiment with N_2O saturation; as shown in Figure 9, the "rapid" yields of N_3O_3^- in the N_2O - and Ar -saturated solutions are the same. We should mention that we have also detected 0.4 - 0.5 μM of hydrated electrons in the flash photolysis of Angeli's anion, but the signals are noisy and not fit to print. However, a negligible role of hydrated electrons was also confirmed by flash photolysis in N_2O -saturated solution.

Comparison and conclusions

It is clear from the data presented that the differences between the two diazeniumdiolates that have been investigated are more quantitative than qualitative; in both cases, the major photolysis products include $^3\text{NO}^-$, ^1HNO , and $\text{NO}^\cdot$, albeit in different proportions, as summarized in Figure 10. Thus, neither diazeniumdiolate is a highly selective photochemical generator of nitroxyl species or nitric oxide, although the selectivity of DPTA NONOate is clearly greater. In this respect, our results are similar to the steady-state photolysis data reported for diethylamine NONOate (DEA NONOate, $\text{R} = \text{CH}_2\text{CH}_3$ in Scheme 1) where $\text{NO}^\cdot$ and its attendant diethylamine have been analyzed as the major end products.¹⁴ Some formation of the nitroxyl species (without distinguishing $^3\text{NO}^-$ and ^1HNO) has also been inferred from diethylnitrosamine and N_2O found among the products. However, a quantum yield of only 0.18 has been reported for the DEA NONOate decomposition under a 266 nm irradiation (same as ours), whereas we observe a near unity quantum yield for the DPTA NONOate photobleaching. Moreover, a 2.5 fold decrease in the decomposition quantum yield is reported for DEA NONOate upon switching from argon-saturated to oxygen-saturated solution, while we do not observe any effect of oxygen on the DEA NONOate bleaching (data not shown). Considering the close similarity between the two NONOates, these variances are difficult to rationalize.

Spectral and kinetic evidence suggests that the same reactions occur following flash photolysis of Angeli's anion and DPTA NONOate both in the absence and in the presence of added $\text{NO}^\cdot$. A minimal reaction scheme quantitatively accounting for all observations is summarized in Table 1. The rate constants for reactions 1-6 have been independently obtained in our previous studies,^{12,13,15} and reaction 10 was introduced here to account for an additional pathway of $^3\text{NO}^-$ decay that clearly plays a role at very low $\text{NO}^\cdot$ concentrations. Detection and characterization of reaction 9 yielding a highly colored stable methylviologen radical gives a novel method for the spectroscopic monitoring of $^3\text{NO}^-$, which is a major challenge due to the lack of characteristic $^3\text{NO}^-$ absorption and its high reactivity. The high specific rate of reaction 9 together with the absence of detectable reverse reaction reported previously³³ are in line with the recent revision of $\text{NO}^\cdot$ reduction potential toward the more negative value $E^\circ(\text{NO}^\cdot/^3\text{NO}^-) \approx -0.81 \text{ V vs. NHE}$.^{12,29,30}

Implicit in our data analysis is the assumption that all $^3\text{NO}^-$, ^1HNO , and $\text{NO}^\cdot$ are photogenerated promptly, that is, before any of the reactions in Table 1 occur. In this sense, these species can be considered as the primary products and little can be said with certainty about their formation pathways. Some possibilities are depicted in Scheme 3.

Formally, the formation of $^3\text{NO}^-$ or ^1HNO amounts to the heterolytic breakage of the N-N bond with nitrite (for Angeli's anion) or nitrosamine (for a NONOate) as the co-products. On the other hand, heterolysis of the X-N bond would lead to two $\text{NO}^\cdot$ (via an unstable $\text{NO}^\cdot$ dimer) and hydroxide ion (for Angeli's anion) or amine (for a NONOate). Alternatively, $\text{NO}^\cdot$ can be produced by a consecutive process that begins with the homolysis of the N-N bond followed by the rapid decomposition of the $\text{XNO}^\cdot$ radical, and this pathway has been postulated for DEA NONOate.¹⁴ These pathways, especially for the $\text{NO}^\cdot$ formation, are likely to be water-assisted, which would avoid formation of the high-energy oxide or amide bases. For the homolysis-initiated pathway, the $\text{NO}_2^{\cdot-}$ radical should be produced in the case of Angeli's anion; this radical indeed rapidly reacts with water releasing $\text{NO}^\cdot$ with about 0.4 μs half-life.³⁸ Although $\text{NO}_2^{\cdot-}$ reduces MV^{2+} , this reaction is too slow to betray the presence of $\text{NO}_2^{\cdot-}$, and only ~2% of $\text{NO}_2^{\cdot-}$ should yield $\text{MV}^{\cdot+}$, even at the highest concentrations of MV^{2+} in Figure 6.³⁸ Thus, there is no evidence to either support or rule out the photoinduced homolysis of Angeli's anion. The fate of the nitrosamine radical anion, $\text{R}_2\text{NNO}^\cdot$, expected for the NONOate homolysis-initiated pathway is less certain; while computations suggest a barrierless dissociation to $\text{NO}^\cdot$ and amine upon protonation of radical,¹⁴ pulse radiolysis data show that

the nitrosamine radical anion can be quantitatively scavenged at 10 mM scavenger,³⁹ which implies a significant radical lifetime.

As to the nature of the excited states that might be involved in Scheme 3, a considerable uncertainty also exists. Computations have predicted the lowest $^3(\pi, \pi^*)$ triplet excited state T_1 lying underneath the lowest $^3(n, \pi^*)$ singlet excited state S_1 for NONOates.¹⁴ If so, the $S_1 \rightarrow T_1$ transition would entail an $^3(n, \pi^*) \rightarrow ^3(\pi, \pi^*)$ orbital change, which is allowed by El-Sayed selection rules⁴⁰ and can be very facile. Then, the T_1 state can be involved and this has indeed been suggested for DEA NONOate and supported by the data on the benzophenone sensitization.¹⁴ The involvement of the T_1 state would certainly help to explain the appearance of $^3NO^-$ among the products. On the other hand, our discovery of the hydrated electrons among the primary photolysis products for both Angeli's anion and DPTA NONOate suggests that the photoactive absorption bands of diazeniumdiolates may be not intramolecular (as S_1 and T_1 above) but rather have a charge-transfer-to-solvent (CTTS) character. If that is the case and the molecular bonds break in this excited state, various contact, solvent-shared, and solvent-separated radical pairs may arise and play a key role. Clearly, discriminating between various possibilities for the excited states and pathways that may be involved in photodecomposition of diazeniumdiolates is neither possible nor wise with the available data.

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Figure 1. Absorption spectra of Angeli's anion, DPTA NONOate, and the $\text{N}_2\text{O}_2^{\cdot-}$ and N_3O_3^- transients^{15,27} derived from addition of NO^- to $^3\text{NO}^-/^1\text{HNO}$ species (Scheme 2) in neutral solutions (pH 7).

Figure 2. Transient absorption spectra recorded at the indicated delay times after laser flash photolysis of 0.3 mM HN_2O_3^- in Ar-saturated solution. Note the vertical axis break at -3.6 and the factor of 10 scale change.

Figure 3.

(Upper panel) Transient absorptions observed at 290 nm (mainly due to N_2O_2^- , blue) and 380 nm (from N_3O_3^- , red) following laser flash photolysis of 0.3 mM HN_2O_3^- in Ar-purged solution. (Lower panel) Simulated kinetics for the N_2O_2^- (blue) and N_3O_3^- (red) transients obtained by using reactions in Table 1 with their indicated rate constants and $[\text{}^3\text{NO}]_0 = 12$, $[\text{}^1\text{HNO}]_0 = 33$, and $[\text{NO}^-]_0 = 11 \mu\text{M}$. Dashed lines give the results for reactions 1-6 only and solid lines are obtained by also including reaction 10.

Figure 4.

Transient absorption profiles recorded at 290 nm (green) and at 380 nm (red) after laser flash photolysis of 0.3 mM HN_2O_3^- in a solution containing 1.9 mM $\text{NO}^\cdot$. The black trace shows the 380 nm absorption profile following photolysis of 0.1 mM DPTA NONOate under the same conditions. The three timescales are selected to show the fast and the slow portions of N_3O_3^- formation and subsequent decay; note the time axis breaks.

Figure 5.

Dependence of the “fast” N_3O_3^- yields derived from the 380 nm transient absorptions on the 0 - 100 μs time scale on the concentration of $\text{NO}^\cdot$ in a solution containing 0.3 mM HN_2O_3^- (circles) and yields calculated by using reactions in Table 1 with their indicated rate constants and $[\text{}^3\text{NO}^-]_0 = 12$, $[\text{}^1\text{HNO}]_0 = 33$, and $[\text{NO}^\cdot]_0 = 11 \mu\text{M}$. The dashed line gives the results for reactions 1-6 only and the solid line is obtained by also including reaction 10.

Figure 6.

Rate constants for MV^{2+} formation (k_{obs}) triggered by laser flash photolysis of HN_2O_3^- in Ar-saturated solution. Panel A: dependence of k_{obs} on the MV^{2+} concentration at constant $[\text{HN}_2\text{O}_3^-] = 0.3 \text{ mM}$ and the laser energies of 30 ($\circ$) and 15 ($\bullet$) mJ/pulse; the inset shows a kinetic trace at 394 nm due to MV^{2+} formation. Panel B: dependence of k_{obs} on the HN_2O_3^- concentration at constant $[\text{MV}^{2+}] = 10 \mu\text{M}$ and the laser energy of 30 mJ/pulse. Panel C: dependence of k_{obs} on the laser energy at $[\text{MV}^{2+}] = 10 \mu\text{M}$ and $[\text{HN}_2\text{O}_3^-] = 0.6 \text{ mM}$.

Figure 7.

Experimental (points) and simulated (lines) absorption kinetics at 380 nm associated with formation and decay of N_3O_3^- . The data are for Ar-purged solution containing 0.3 mM HN_2O_3^- . The simulations use all the reactions in Table 1 and assume that 12.5 μM of $^3\text{NO}^-$, 32.5 μM of ^1HNO , and 11 μM of $\text{NO}^\cdot$ are generated during the 6 ns laser pulse. The three timescales are selected to show the fast and slow portions of N_3O_3^- growth kinetics and subsequent decay; note the time axis breaks.

Figure 8.

Transient absorption spectra recorded at the indicated delay times after flash photolysis of 0.1 mM DPTA NONOate in anaerobic solution. Note the vertical axis break at -8 and the factor of 10 scale change. The inset shows kinetic traces at 650 nm recorded in Ar-purged (blue) and N_2O -saturated (black) solutions.

Figure 9.

Experimental (blue points) and simulated (red lines) transient absorption kinetics at 380 nm showing formation and decay of N_3O_3^- . The data are for Ar-purged solution containing 0.1 mM DPTA NONOate. The simulations use all the reactions in Table 1 and assume that $1.9 \mu\text{M } ^3\text{NO}^-$, $8.6 \mu\text{M } ^1\text{HNO}$, and $60 \mu\text{M NO}^\bullet$ are generated during the 6 ns laser pulse. Note the two time axis breaks. The black trace is recorded in N_2O -saturated solution and is vertically offset for clarity.

Figure 10.

Relative yields (the sum is 100%) of $^3\text{NO}^-$, ^1HNO and $\text{NO}^\cdot$ from flash photolysis of 0.3 mM Angeli's anion and 0.1 M DPTA NONOate in neutral solutions.

Angeli's anion

DPTA NONOate
(R = CH₂CH₂CH₂NH₂)

Scheme 1.

Scheme 2.

Scheme 3.

TABLE 1Reactions occurring in the $\text{NO}^-/{}^1\text{HNO}/{}^3\text{NO}^-$ mixtures

<i>N</i>	Reaction	<i>k</i> , $\text{M}^{-1} \text{s}^{-1}$	ref.
1	${}^3\text{NO}^- + \text{NO}^- \rightarrow \text{N}_2\text{O}_2^{2-}$	3×10^9	12,15
2	${}^1\text{HNO} + \text{NO}^- \rightarrow \text{N}_2\text{O}_2^{2-} + \text{H}^+$	5.6×10^6	12,15
3	$\text{N}_2\text{O}_2^{2-} + \text{NO}^- \rightarrow \text{N}_3\text{O}_3^-$	5.4×10^9	12,15
4	$\text{N}_3\text{O}_3^- \rightarrow \text{N}_2\text{O} + \text{NO}_2^-$	$260^{a)}$	12,15
5	${}^1\text{HNO} + {}^1\text{HNO} \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}$	8×10^6	12
6	$\text{N}_2\text{O}_2^{2-} + \text{N}_2\text{O}_2^{2-} \rightarrow 2\text{NO}^- + \text{N}_2\text{O}_2^{2-}$	8×10^7	27
10	${}^3\text{NO}^- + {}^1\text{HNO} \rightarrow \text{N}_2\text{O} + \text{HO}^-$	6.6×10^9	this work

^{a)} In units of s^{-1} ; this temperature-sensitive value is typically found in the 240 - 330 s^{-1} range at “room temperature”.