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Mechanistic Variations in the Oxidation of Piloty's Acid by Metal Complexes†

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The reactions of *N*-hydroxybenzenesulfonamide (Piloty's acid, PA) with a variety of metal oxidants are reported. Either nitric oxide or nitrite is the final reaction product, along with benzenesulfinate and the reduced metal compound. The nitrogen product depends on the oxidation potential of the metal oxidant and its ability to further oxidize NO to nitrite. The observation and preliminary interpretation of unusual kinetic behavior of Piloty's acid as a reductant is also described. Analogues of PA were also prepared and found to show similar reactivity.

Introduction

Although Piloty's acid (PA), *N*-hydroxybenzenesulfonamide, was first prepared in 1896,[1] it was not until the 1970s that *N*-hydroxysulfonamides were observed to be inhibitors of Zn metalloenzymes such as carbonic anhydrase and matrix metalloproteinases.[2] Piloty's acid and some of its acylated derivatives have also been shown to be good inhibitors of aldehyde dehydrogenase and potent vasodilators.[3] Recently, PA and its derivatives have been shown to decompose to produce HNO in aqueous solution or release NO upon oxidation.[4a,5] In view of the biomedical relevance of these reactions, PA and its derivatives have been explored as potential delivery agents for NO and HNO.[3] It is surprising, however, that the oxidation kinetics of PA with various oxidants has received only limited attention.[5]

The decomposition of PA in aqueous solution has been shown to produce benzenesulfinate, HNO/ NO^-, and finally nitrous oxide according to the reactions[4a]

$$C_6H_5SO_2NHOH + OH^- \rightleftharpoons C_6H_5SO_2NHO^- + H_2O$$

$$C_6H_5SO_2NHO^- \rightarrow HNO + C_6H_5SO_2^-$$

$$2HNO \rightarrow N_2O + H_2O$$

These decomposition studies were carried out at high pH (9−13), and from the inverse proton dependence of the reaction along with ^{15}N NMR data, it was concluded that the deprotonated form, $C_6H_6SO_2NHO^-$, was the reactive species.[4] However, PA is relatively stable to decomposition to HNO at physiological pH under anaerobic conditions (at pH 7, $t_{1/2} = 92$ h).[4b]

Recently, it was shown that, under both anaerobic and aerobic conditions at pH 7−9, oxidative decomposition of PA by O_2, H_2O_2, or $[Fe(CN)_6]^{3-}$ takes place with the production of nitric oxide.[5] The production of NO in the oxidation of PA was also reported in studies to determine whether NO or HNO (NO^-) was responsible for the vasorelaxant properties of the sulfonamide.[5]

As part of an ongoing study into selective chelation of actinides and transition metals, the interaction of aqueous iron(III) with *N*-hydroxysulfonamides, which are structurally similar to hydroxamic acids, was investigated. No complex formation between PA and iron(III) was evidenced by

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Table 1. Rate Constants for PA Oxidations at 25 °C

oxidant[a]	pH[b]	k (M^{-1} s^{-1})	k_H (M^{-1} s^{-1})	k_O (M^{-1} s^{-1})	$E°$ (V vs NHE)	k_{exc} (M^{-1} s^{-1})
$[Fe(bipy)_2(CN)_2]^{+c}$	7.0	610	11	1.2×10^5	0.78[d]	$\sim 10^8$ [e]
	6.5	195				
	6.0	75				
$[IrCl_6]^{2-}$	7.1	1.2×10^3			0.957[d]	2.3×10^5 [d]
$[IrBr_6]^{2-}$	7.1	1.6×10^3			0.882[d]	
$[Fe(CN)_6]^{3-}$					0.361[f]	5×10^3 [e]
$[Fe(CN)_5N_3]^{3-}$					0.33[e]	10^5 [e]
$[Fe(H_2O)_6]^{3+}$	3.0[i]	145			0.771[f]	4.0 [g]
Mb^+	7.5	$\sim 1.4 \times 10^{-4}$			0.06[h]	

[a] Anaerobic conditions. [b] Buffer = 0.1M sodium phosphate. [c] bipy = 2,2′-bipyridine. [d] Reference 11. [e] Reference 12. [f] Reference 13. [g] Reference 14. [h] Reference 15. [i] 10^{-3} M HCl, $I \approx 0.01$ M.

changes in the UV−vis spectrum of a mixture of these two reactants around pH 3. However, a redox reaction was observed to form iron(II) and nitric oxide. Because of the novel nature of this reaction and the potential therapeutic uses of synthetic NO and HNO/NO^- donors,[6] a systematic study of the oxidation of PA and some of its derivatives was undertaken.

Experimental Section

Oxidants were either purchased from Aldrich (K_2IrCl_6, K_2IrBr_6) or Fluka ($K_3Fe(CN)_6$) or prepared ($[Fe(bipy)_2(CN)_2]^+$ and $[Fe(CN)_5N_3]^{3-}$) by literature methods.[7] Myoglobin (equine) was purchased from Sigma. Piloty's acid was purchased from Acros and recrystallized from EtOH/water before use. The other *N*-hydroxysulfonamides were synthesized by the method of Blackburn.[2a] All other chemicals were of the highest purity commercially available. Solutions of $[Fe(CN)_5N_3]^{3-}$ and $[IrCl_6]^{2-}$ were used immediately after preparation to limit possible hydrolysis. Solutions were prepared fresh daily and were maintained under an Ar atmosphere.

The kinetics data were collected on a Dionex stopped-flow instrument interfaced with an OLIS data acquisition/data reduction system or on a Hewlett-Packard model 8452A diode array spectrophotometer followed by data reduction using OLIS fitting routines. These routines fit the absorbance change data to the exponential function $\Delta Abs = \exp(-k_1 t)$ for pseudo-first-order data sets and $\Delta Abs = \exp(-k_1 t) + k_o t$ for mixed zeroth- and first-order data sets. The fitting routine documentation along with the kinetic data are available in the Supporting Information. All reactions were studied at the absorption maximum of the metal complex and were carried out at 25.0 °C and in 0.1 M sodium phosphate to control pH. For aqueous iron(III), the reaction was studied using a 20-fold excess of phenanthroline. This rapidly produces a red species at 510 nm. The phosphate buffer also set the ionic strength to ∼0.2 M over the limited pH range for this study. For the aquairon(III) study, the pH was set using HCl, and the ionic strength was approximately 0.01 M in the absence of added salts or 0.2 M in the presence of added KCl or $NaClO_4$. The kinetic data were fitted using Origin linear fitting routines. The first-order rate constants are typically ±10%. The errors on the zeroth-order rate constants vary widely and are typically ±20−30%. Concentration ranges of the oxidants are given in the Supporting Information and are typically a 10- to 100-fold excess over the Piloty's acid to comply with pseudo-first-order conditions.

Ionic reaction products were identified chromatographically on a Dionex model 2020i ion chromatograph using a CO_3^{2-}/HCO_3^- eluent solution. Details of this separation are available in the Supporting Information.

Results and Discussion

The oxidation of PA by aqueous iron(III) in acid produced iron(II) and NO; the latter was identified by scavenging with metmyoglobin, Mb^+. Addition of Mb^+ initially gave the metmyoglobin−NO (Mb^+NO) adduct that underwent a slow base-catalyzed reductive nitrosylation to form the deoxymyoglobin−NO adduct (Mb°NO). Both adducts were identified by their visible absorption spectra.[8] The scavenging of NO occurred much faster than the reduction of iron(III) by PA.

The iron(III)/PA reaction at $I \approx 0.01$ M is relatively rapid (Table 1), and a plot of k_{obs} vs the PA concentration is linear with a very small intercept ($\sim 6 \times 10^{-3}$ s^{-1}) compared to observed rate constants between 0.1 and 1.3 s^{-1}; however, it is unlikely that this is an equilibrium given that the reverse reaction is thermodynamically unfavorable. Because the reaction was carried out at pH 3 and the pK_a of PA is 9.29, the electron-transfer reaction must occur between $[Fe(H_2O)_6]^{3+}$ or $[Fe(H_2O)_5(OH)]^{2+}$ and $C_6H_5SO_2NHOH$, as very little deprotonated PA is present at pH 3.

At an ionic strength of 0.2 M the k_{obs} vs PA concentration plot for the iron(III) oxidation of PA exhibits saturation behavior. In contrast to the observation at low ionic strength, this indicates formation of a weak Fe^{3+}−PA "intermediate" at high ionic strength. The data conform to the expression

$$k_{obs} = \frac{k_{et} K_{eq}[PA]}{1 + K_{eq}[PA]}$$

where k_{et} is the first-order rate constant for electron transfer and K_{eq} is the formation constant for the Fe^{3+}−PA intermediate. At pH 3, the values for k_{et} and K_{eq} are 0.85 and 1.0 s^{-1} and 290 and 250 M^{-1} for 0.2 M Cl^- and ClO_4^- solutions, respectively. This indicates that observation of the Fe^{3+}−PA intermediate is a consequence of the high ionic strength and not a specific ion effect.

Because of the ambiguity with respect to the nature of the oxidant, i.e., the extent of deprotonation, and the

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possibility of both inner- and outer-sphere electron transfer with iron(III), the investigation of known outer-sphere oxidants was undertaken. The results of the oxidation of PA by a variety of oxidants under anaerobic conditions and over a range of pHs were examined. The evolution of nitric oxide was directly observed with some oxidants, whereas other oxidants led to the formation of nitrite, presumably by rapid further oxidation of the initially produced NO.

Nitric Oxide Producing Oxidants. Oxidation of PA by $[Fe(CN)_6]^{3-}$ or $[Fe(CN)_5N_3]^{3-}$ produced the reduced metal complex, benzenesulfinate, and NO via a single electron transfer

$$[Fe(CN)_6]^{3-} + C_6H_5SO_2NHO^- \rightarrow [Fe(CN)_6]^{4-} + C_6H_5SO_2{}^- + NO + H^+$$

The gas bubbles observed in the product solutions were again shown to be nitric oxide by scavenging with metmyoglobin (Mb^+)

$$Mb^+ + NO \rightarrow Mb^+NO \xrightarrow{OH^-} Mb^0NO$$

Although Mb^+ can oxidize PA directly to form Mb^0NO, this reaction is very slow (Table 1) and does not interfere with the scavenging studies. The rapid formation of the Mb^+NO adduct therefore shows that NO production is from the primary reaction and not from the added Mb^+ ($t_{1/2}$ for Mb^+NO formation <10 s, pH 7 and 7.5).[8]

The kinetic experiments were performed under pseudo-first-order conditions with PA in excess. Reactions were followed by absorbance decreases for the formation of the iron(II) products. The rate data are reported in Table 1 along with relevant thermodynamic and kinetic information for each oxidant.

Although the oxidation of NO to NO_2^- is extremely facile ($E° = -0.46$ V vs NHE in base[9]), neither $[Fe(CN)_6]^{3-}$ nor $[Fe(CN)_5N_3]^{3-}$ rapidly oxidizes NO further under the conditions of this study. However, when $[Fe(CN)_6]^{3-}$ was used in excess, a second slower conversion of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ was observed, presumably because of further oxidation of the NO produced in the first electron-transfer step. No further studies of this reaction were undertaken. With excess PA, only benzenesulfinate and NO are produced.

Surprisingly, when $[Fe(CN)_6]^{3-}$ or $[Fe(CN)_5N_3]^{3-}$ was the oxidant, the reaction is mixed zeroth- and first-order in [Fe(III)] complex, and the order is somewhat dependent on pH and ionic strength; see Figure 1a. Because it is difficult to ascribe zeroth-order behavior to either oxidant in this reaction, the effect likely arises from a rate-determining production of an "active form" of PA.

There are three possibilities for the observed zeroth-order behavior. The first is the hydrolysis of PA into hydroxylamine and benzenesulfinate followed by rapid reduction of Fe(III) by the free hydroxylamine. This pathway can be excluded from consideration, however, given that the hydrolysis reaction is too slow around pH 7 ($t_{1/2} \approx 90$ h) and hydroxylamine was not found as a decomposition product by other investigators.[5a] Because it is also well-known that aqueous metal ions can catalyze hydrolysis, this pathway must be considered. Hence, we have briefly studied the oxidation of hydroxylamine by ferricyanide and showed with the Mb^+ scavenging technique that NO was not produced at pH 7.5. Therefore, any mechanism involving the initial formation of hydroxylamine can be discounted.

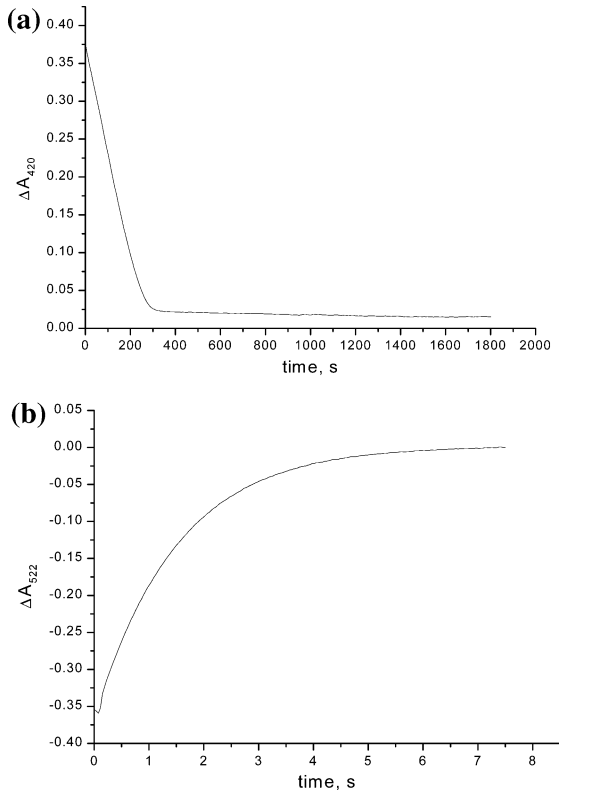

Figure 1. (a) Zeroth-order trace for reaction of $[Fe(CN)_6]^{3-}$ (3.5×10^{-4} M) with PA (1.0×10^{-3} M) at pH 7.5, $T = 25$ °C, [phosphate] = 0.1 M, and 420 nm. (b) First-order trace for reaction of $[Fe(bipy)_2(CN)_2]^+$ (5.7×10^{-5} M) with PA (1.2×10^{-3} M) at pH 7, $T = 25$ °C, [phosphate] = 0.1 M, and 522 nm.

The second possibility is a rate-determining breakdown of an "aggregate" form of PA (dimer, trimer, etc.) into a monomeric form that reacts with the metal complex. If true, then at high pH (>9.5), where the PA is deprotonated, electrostatic repulsion should augment aggregate breakdown, and the reaction profile should "reduce" to simple first-order behavior. This is what is observed. The amount of first- and zeroth-order behavior should also depend on the PA concentration under investigation. At the lowest PA concentrations studied, where more aggregate dissociation occurs, the amount of zeroth-order character for the disappearance of ferricyanide is significantly less than at higher concentrations. This observation is consistent with PA existing in an equilibrium involving an associated form in solution. It is tempting to ascribe this behavior to hydrogen bonding, but such an interaction seems unlikely to be strong in aqueous media. However, the crystal structure of PA has been solved, and the authors point out that there is "extensive hydrogen bonding" both within and between the layers of PA

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molecules in the solid state.[10] This hydrogen-bonding network might not apply to the solution state. As an initial probe toward better defining this aggregation, the reaction was carried out in 0.25 M urea (pH 7.5) with ferricyanide. Neither the zeroth- nor the first-order portion of the reaction was affected.

The final possibility involves a rate-determining proton migration from nitrogen to oxygen of the anion of PA prior to the oxidation. To explore this possibility, we examined the ferricyanide oxidation of PA in D_2O at a pD equivalent to pH 7.6. The ratio k_H/k_D from these studies was 6.9, which is surprisingly high. This suggests that proton migration might be the cause of the zeroth-order behavior. To confirm that deuterium exchange was rapid, a sample of PA was dissolved in deuterated chloroform, and a drop of D_2O was added. Deuterium exchange was complete within 5 min, the time required to acquire a new spectrum. Similar zeroth order behavior has been observed for the oxidation of phosphorous acid.[16] This was ascribed to an active and inactive form of the P(III) center. The zeroth-order component was ascribed to migration of a hydrogen from the phosphorus to oxygen. It is possible that analogous behavior might be observed in the ferricyanide oxidation of PA.

Nitrite/Nitrous Acid Producing Oxidants. Oxidation of PA by $[Fe(bipy)_2(CN)_2]^+$, $[IrBr_6]^{2-}$, or $[IrCl_6]^{2-}$ was rapid and formed 2 mol equiv of reduced metal complex, one of benzenesulfinate, and one of nitrous acid as shown below. The benzenesulfinate and nitrite products were identified and quantified by capillary ion chromatography.

$$2[Fe(bipy)_2(CN)_2]^+ + C_6H_5SO_2NHOH + H_2O \rightarrow 2[Fe(bipy)_2(CN)_2] + C_6H_5SO_2^- + HNO_2 + 3H^+$$

$$2[IrCl_6]^{2-} + C_6H_5SO_2NHOH + H_2O \rightarrow 2[IrCl_6]^{3-} + C_6H_5SO_2^- + HNO_2 + 3H^+$$

The 2:1 oxidant-to-PA stoichiometry in these reactions was established by the spectral changes observed for the $[Fe(bipy)_2(CN)_2]^+$ to $[Fe(bipy)_2(CN)_2]$, $[IrBr_6]^{2-}$ to $[IrBr_6]^{3-}$, and $[IrCl_6]^{2-}$ to $[IrCl_6]^{3-}$ conversions.

As in the earlier systems, all kinetic experiments were performed under pseudo-first-order conditions with PA in excess over oxidant, and the electron-transfer rate constants were determined by observing the absorbance change associated with formation of the Fe(II) complex or loss of the Ir(IV) complex. The data for loss of oxidant at each PA concentration were fit by a single exponential, and a typical trace is shown in Figure 1b. No zeroth-order behavior was observed. This indicates that the "inactive PA" described above could react directly with the oxidant rather than just the "active" PA. This could be due to the higher reduction potentials of these oxidants increasing the driving force for the oxidation. Similar behavior has been observed for the ferricyanide oxidation of dithionite ion.[17]

Plots of the rate constants for all of the oxidants vs PA concentration were linear over a 10-fold concentration range of the sulfonamide and exhibited a zero *y* intercept. The data conform to the rate expression

$$-d[\text{oxidant}]/dt = k[\text{oxidant}][\text{PA}]$$

The second-order rate constant is inversely proportional to the H^+ concentration for $[Fe(bipy)_2(CN)_2]^+$ and conforms to the following rate expression

$$k = k_H + k_O K_{AH}[H^+]^{-1}$$

From the k vs inverse H^+ concentration data, the following values were obtained: $k_H = 11\ M^{-1}\ s^{-1}$ and $k_O K_{AH} = 6.0 \times 10^{-5}\ s^{-1}$. As in the previous section, deprotonation of PA was assumed, and k_O is calculated to be $1.2 \times 10^5\ M^{-1}\ s^{-1}$.

Although the direct detection of NO was not possible with these oxidants, from a mechanistic standpoint, one can infer that the initial one-electron oxidation of PA produces nitric oxide, which is then rapidly oxidized to NO^+ by a second mole of oxidant. The lifetime of the nitrosonium ion in water is very short ($\sim 3 \times 10^{-10}$ s), and NO^+ rapidly hydrolyzes to nitrite[18] as shown below for $[Fe(bipy)_2(CN)_2]^+$

$$[Fe(bipy)_2(CN)_2]^+ + C_6H_5SO_2NHO^- \rightarrow [Fe(bipy)_2(CN)_2] + C_6H_5SO_2^- + NO + H^+$$

$$[Fe(bipy)_2(CN)_2]^+ + NO \rightarrow [Fe(bipy)_2(CN)_2] + NO^+$$

$$NO^+ + H_2O \rightarrow HNO_2 + H^+$$

Although all of the metal complexes used in this study are outer-sphere oxidants, i.e., they exchange their ligands very slowly with respect to the electron-transfer rates, there is no consistent relationship among the oxidation rates of PA and the charge of the oxidant, the reduction potentials of the oxidants ($E°$), or the self-exchange rates (k_{exc}) of the redox couples (Table 1). This lack of correlation suggests that, rather than a "simple" outer-sphere electron-transfer process, the PA reactions are complicated by rapid intramolecular steps within the initial oxidation product, followed by an irreversible rapid breakdown to products. Because there are structural differences between the oxidized and reduced forms of PA, treatment with the Marcus cross relationship is inappropriate.

Other Substrates. To examine the effect of modifying the benzene ring in Piloty's acid, three related *N*-hydroxysulfonamides were synthesized, and their oxidation with ferricyanide was studied. Two compounds in which the para

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Table 2. Rate Constants for Other *N*-Hydroxysulfonamide Oxidations at 25 °C

oxidant[a]	pH[b]	k (M^{-1} s^{-1})
	p-bromoPA	
$[Fe(bipy)_2(CN)_2]^+$	7.0	1100
	p-methoxyPA	
$[Fe(bipy)_2(CN)_2]^+$	7.0	740
	N-hydroxy-*n*-butylsulfonamide	
$[Fe(bipy)_2(CN)_2]^+$	7.0	230
	6.0	53
$[IrCl_6]^{2-}$	7.0	290

[a] Anaerobic conditions. [b] Buffer = 0.1M sodium phosphate.

position in the benzene ring was substituted with either a Br or OCH_3 group showed little variation in rate and maintained a mixed first- and zeroth-order kinetic profile. No correlation with respect to electron-withdrawing or -donating character was observed. Replacement of the phenyl ring with an *n*-butyl group again showed little effect on the oxidation rate and an analogous mixed order behavior was observed. Judging from the similarities in rates of these widely different compounds, it is possible that the outer-sphere oxidation takes place directly at the nitrogen center and that conjugation between the N and the R group, through the sulfur, is not important in this system.

The oxidations of the same substrates with $[Fe(bipy)_2(CN)_2]^+$ and $[IrCl_6]^{2-}$ were also studied; see Table 2. Each reaction was nicely first-order with no evidence of a zeroth-order component. The rates varied by only 5-fold among the substituted analogues, which again suggests little conjugation between the nitrogen center and the R group attached to the sulfur.

Further attempts to examine the effects of structural changes on the oxidation processes were undertaken by preparation of *N*-methylated PA. Although the compound could be synthesized, it proved to be too unstable in aqueous media for study of its oxidation. Presumably, rapid hydrolysis of the S−N bond occurred to form *N*-methylhydroxylamine and benzenesulfinate.

Conclusions

As seen from this work, the nature of the oxidant is critical in the generation of NO from Piloty's acid. These preliminary kinetic studies show that these "simple" reactions actually occur via a series of steps that need to be systematically unraveled. Such investigations could drive the development of pharmaceutically useful NO precursors.

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Supporting Information Available: Listing of details for ion chromatography and tables of all kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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