CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF CALIFORNIA, BERKELEY, CALIFORNIA

Air Oxidation of Hydroxylamine-N-sulfonate

BY MARTIN N. ACKERMANN AND RICHARD E. POWELL

Received May 17, 1967

The reaction of gaseous O_2 with alkaline hydroxylamine-N-sulfonate leads to N-nitrosohydroxylamine-N-sulfonate, $ON(SO_3^-)NO^-$, by the stoichiometry

$$O_2 + 3HONHSO_3^- + 4OH^- \longrightarrow NO_2^- + ON(SO_3^-)NO^- + 2SO_3^{2-} + 3H_2O$$

The rate-determining step of the reaction is the formation of peroxynitrite from O_2 and the conjugate base of hydroxylamine-N-sulfonate, with a half-life of 23 sec.

In the course of our investigation of the alkaline hydrolysis of hydroxylamine-N-sulfonate,[1] we found that air had to be excluded in order to avoid a rapid side reaction with atmospheric oxygen. We report here the stoichiometry and rate of that side reaction.

Experimental Section

Materials.—The preparation of potassium hydroxylamine-N-sulfonate has been described earlier.[1] Solutions of peroxynitrite were prepared by slow addition of cold acidic hydrogen peroxide to cold alkaline nitrite, then freeing the resultant alkaline solution from other species by the ion-exchange method of Anbar and Yagil,[2] using BioRad AG-1-X4 anion-exchange resin with an eluent 1 *M* in hydroxide and 0.1 *M* in formate. Peroxynitrite solutions were usable for several days if stored at −10°. Dipotassium N-nitrosohydroxylamine-N-sulfonate, $K_2N_2O_2SO_3$, was prepared by bubbling nitric oxide into alkaline sulfite solution;[3] the solid was stable when stored over potassium hydroxide pellets in a vacuum desiccator at room temperature. *Anal.* Calcd for $K_2N_2SO_5$: S, 14.70; N, 12.84. Found: S, 14.55; N, 12.55. Sodium nitrohydroxamate, $Na_2N_2O_3$, was prepared as described by Smith and Hein,[4] using commercial ethyl nitrate. *Anal.* Calcd for $Na_2N_2O_2$: N, 23.0. Found: N, 22.2. Sodium hyponitrite, NaN_2O_2, was a gift from Dr. James D. Ray. It assayed spectrophotometrically at 90% purity.

Analytical Procedure.—Aliquots were analyzed for unconsumed hydroxylamine-N-sulfonate by acidification and titration, as described earlier.[1] The apparent rate of its disappearance, as measured by such titration, is higher than the true rate by a factor very near 4:3, because for each 3 moles of it consumed there is 1 mole of nitrite produced, and, upon acidification, most of this nitrous acid reacts to remove 1 additional mole of hydroxylamine-N-sulfonate.[5]

All other species were determined with a Cary Model 14 spectrophotometer: sulfite, after acidification to 0.8 *N* with H_2SO_4, at 2760 A (ϵ 403 cm^{-1} M^{-1}); nitrite in alkaline solution, at 3650 A (ϵ 23); peroxynitrite at 3030 A (ϵ 1300); hyponitrite at pH greater than 11, at 2480 A (ϵ 6500); nitrohydroxamate at 2480 A (ϵ 8300); and N-nitrosohydroxylamine-N-sulfonate (see text) at 2580 A (ϵ 7140).

Kinetic Procedure.—The apparatus previously described[1] was used for the kinetic study of oxygen with hydroxylamine-N-sulfonate. The initial concentration of hydroxylamine-N-sulfonate was approximately 0.01 *M*, the pH was adjusted with NaOH, carbonate, or phosphate, and the ionic strength was adjusted to 1.6 *M* with Na_2SO_4. The reaction was followed for at least 2 half-lives.

The kinetics of decomposition of nitrohydroxamate and of N-nitrosohydroxylamine-N-sulfonate were followed spectrophotometrically, for at least 3 half-lives.

Results and Discussion

Isolation of the Desired Reaction.—Preliminary experiments showed that the reaction of oxygen with hydroxylamine-N-sulfonate has a rather small activation energy—the reaction is about twice as fast at 65° as at 25°. By contrast, the alkaline hydrolysis has an activation energy of 25–28 kcal.[1] Consequently, the oxygen reaction can be studied near room temperature without significant interference from the alkaline hydrolysis. The data reported here were taken at 25.2°.

Transient Product.—When a diluted (10^{-3} *M* or less) alkaline solution of hydroxylamine-N-sulfonate was shaken with oxygen, a spectroscopic absorption near 3030 A (the absorption peak of peroxynitrite) appeared. In a matter of minutes or less, this peak decayed, with an accompanying growth of an absorption peak at 2580 A ascribable to the ultimate product of reaction. In more concentrated solutions, peroxynitrite disappeared too rapidly to be detected. An authentic sample of peroxynitrite, freed of nitrite, peroxide, and oxygen, was treated with solid $HONHSO_3K$; the yellow peroxynitrite color immediately disappeared, and the resulting solution showed an intense absorption at 2580 A.

In the first few minutes of reaction, SO_3^{2-} is readily identifiable as a product, *via* the absorption spectrum of aqueous SO_2 at 2760 A in an acidified aliquot. As time goes on, sulfite becomes undetectable, partly because it is fairly rapidly oxidized to sulfate by gaseous oxygen and partly because nitrite (another product of reaction) oxidizes it to sulfate during the analytical process.

On the basis of these observations, we propose that the initial net reaction is

$$O_2 + HONHSO_3^- + 2OH^- \longrightarrow OONO^- + SO_3^{2-} + 2H_2O$$

Ultimate Product.—At first the identity of the reaction product with the intense absorption at 2580 A was puzzling to us. It differed in spectrum and in kinetics of its acid decomposition from the well-known species hyponitrite, $^-ONNO^-$, or nitrohydroxamate, $^-ONNO_2^-$. It was not the substances reported by Addison[6] to be formed upon reaction of liquid N_2O_4

---

[1] M. N. Ackermann and R. E. Powell, *Inorg. Chem.*, **5**, 1334 (1966).

[2] G. Yagil and H. Anbar, *J. Inorg. Nucl. Chem.*, **26**, 453 (1964).

[3] S. Nyholm and L. Rannitt, *Inorg. Syn.*, **5**, 117 (1957).

[4] P. A. S. Smith and G. E. Hein, *J. Am. Chem. Soc.*, **82**, 5831 (1960).

[5] F. Seel and H. Pauschmann, *Z. Naturforsch.*, **17b**, 347 (1962).

[6] C. C. Addison, G. A. Gamlen, and R. Thompson, *J. Chem. Soc.*, 346 (1952).

with solid sodium hyponitrite or sodium nitrohydroxamate, which in our hands led to no spectroscopically distinguishable species. Eventually we identified it as N-nitrosohydroxylamine-N-sulfonate, $ON(SO_3^-)NO^-$, on the basis of the following evidence.

(1) **Spectroscopic Data.**—The absorption spectrum of authentic N-nitrosohydroxylamine-N-sulfonate (Figure 1) coincides with that of the reaction product of oxygen with hydroxylamine-N-sulfonate and also that of the reaction product of peroxynitrite with hydroxylamine-N-sulfonate. Oddly enough, the spectrum of this long-known substance does not seem to have been recorded previously. It was not included in Kortüm and Finckh's[7] classic study of the oxyacids of nitrogen; and Seel and Winkler,[8] in their study of its acid decomposition, used the rather tedious method of gas analysis instead of the quite expeditious method of spectrophotometry to follow the reaction.

(2) **Decomposition Kinetics.**—Upon acidification, the reaction product is irreversibly destroyed. We have determined the rate, from pH 5.5 to 14 (Figure 2). Both the rate and, what is more significant, the pattern of pH dependence are the same for our reaction product as for authentic N-nitrosohydroxylamine-N-sulfonate.[8] Both Seel and we observe a moderate decrease in the rate of decomposition if EDTA has been added, suggesting that heavy-metal ions play a catalytic role. For comparison, the patterns of pH dependence are also shown in Figure 2 for the decomposition of hyponitrite[9] and of nitrohydroxamate.[10]

(3) **Specific Chemical Properties.**—Our reaction product, like authentic N-nitrosohydroxylamine-N-sulfonate, is precipitated by Ba^{2+}. Like the authentic substance but unlike hyponitrite or nitrohydroxamate, it decomposes rapidly in the presence of borate,[8] so that borate buffers cannot be used to control its pH. Like the authentic substance, it is unaffected by Ag^+, whereas hyponitrite yields yellow $Ag_2N_2O_2$ and nitrohydroxamate yields metallic silver.

These observations, taken together, convince us that the course of reaction after the transient formation of peroxynitrite is

$$OONO^- + 2HONHSO_3^- + 2OH^- \longrightarrow NO_2^- + ON(SO_3^-)NO^- + SO_3^{2-} + 3H_2O$$

giving an over-all stoichiometry of

$$O_2 + 3HONHSO_3^- + 4OH^- \longrightarrow NO_2^- + ON(SO_3^-)NO^- + 2SO_3^{2-} + 3H_2O$$

This stoichiometry predicts that a maximum of 66.7% of nitrogen atoms can be converted to N-nitrosohydroxylamine-N-sulfonate. We observe yields of 65.8, 65.0, and 63.2%.

Reaction Mechanism.—The sequence which seems to us most plausible consists of a two-hydrogen-atom dehydrogenation by peroxynitrite to yield nitrosyl sulfonate, followed by addition of the conjugate base of hydroxylamine-N-sulfonate to the double bond of the intermediate nitrosyl sulfonate

$$OONO^- + HONHSO_3^- \longrightarrow NO_2^- + O{=}NSO_3^- + H_2O$$

$$O{=}NSO_3^- + {}^-N(OH)SO_3^- \longrightarrow ON(SO_3^-)NO^- + SO_3^{2-} + H_2O$$

Nitrosyl sulfonate, $O{=}NSO_3^-$, has never been isolated, but has been proposed by Seel[11] as an intermediate in the reaction of nitrous acid with sulfurous acid.

Stability of Product.—The alkaline solutions of N-nitrosohydroxylamine-N-sulfonate are stable for many

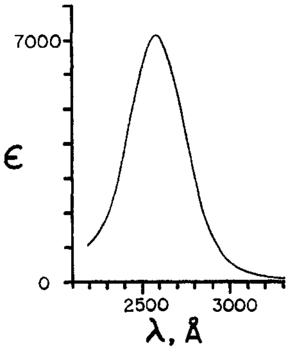

Figure 1.—Absorption spectrum of N-nitrosohydroxylamine-N-sulfonate ion, $ON(SO_3^-)NO^-$ (cm^{-1} M^{-1}).

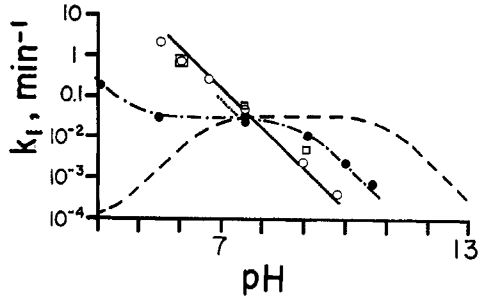

Figure 2.—Decomposition rate constant at 25° for the reaction product and some related compounds: open squares, reaction product; open circles, authentic N-nitrosohydroxylamine-N-sulfonate; short dotted line at pH 7, Seel's data on the same with added EDTA; dashed line, hyponitrite; dashed and dotted line with filled circles, nitrohydroxamate.

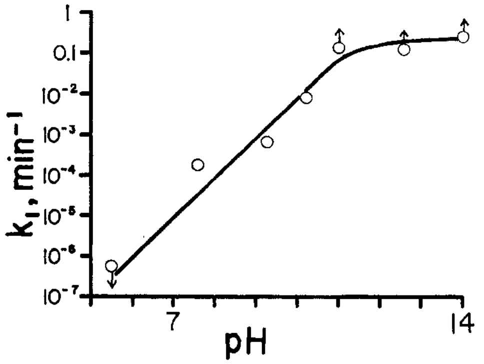

Figure 3.—Reaction rate of oxygen with alkaline hydroxylamine-N-sulfonate. At the lowest rate, we are unsure of our analytical accuracy, and at the highest rates, we are not sure that the stirring always sufficed to keep the solution saturated with oxygen, hence the arrows.

---

[7] G. Kortüm and B. Finckh, *Z. Physik. Chem.*, **B48**, 32 (1940).

[8] F. Seel and R. Winkler, *Z. Naturforsch.*, **18a**, 155 (1953).

[9] J. R. Buchholz and R. E. Powell, *J. Am. Chem. Soc.*, **85**, 509 (1963).

[10] Some scattered results are to be found in Smith and Hein,[4] in Seel and Winkler,[8] and in J. Veprek-Siska, V. Pliska, F. Smirous, and F. Vesely, *Collection Czech. Chem. Commun.*, **24**, 687 (1959). The data presented in Figure 2 were taken during the present investigation.

[11] F. Seel, *Fortschr. Chem. Forsch.*, **4**, 301 (1963).

weeks. If the solution is acidified or the original reaction carried out in other than strongly alkaline solution, it decomposes by the reaction whose kinetics is shown in Figure 2

$$ON(SO_3^-)NO^- \longrightarrow N_2O + SO_4^{2-}$$

Another decomposition takes place slowly if the alkaline solution is kept in contact with O_2, eventually converting all of the product to nitrite. We have observed this both with our reaction product and with the authentic material. In some prolonged runs we have found as many as 72.5% of the nitrogen atoms converted to nitrite.

Kinetics.—The rate-determining process in this sequence is the initial formation of peroxynitrite, the subsequent processes being observably much faster. We have measured the rate, in a well-stirred reactor at 25.2° and 1 atm of O_2, from pH 5.5 to 14. The results are shown in Figure 3. This pattern of pH dependence resembles closely that of the population of the conjugate base of hydroxylamine-N-sulfonate, given its pK_A of approximately 12.5.[1] We therefore propose that the species entering into the rate-determining process are molecular O_2 and the conjugate base, namely

$$O_2 + {}^-N(OH)SO_3^- \longrightarrow OONOH + SO_3^{2-}$$

Our numerical value for the rate constant of this reaction is 3×10^{-2} sec^{-1} atm^{-1} (half-life of some 23 sec at 1 atm of O_2), but, what with one one or another source of uncertainty in our experiments, we could be off by a factor of 2 either way.

Contribution from Westinghouse Electric Corporation, Research Laboratories, Pittsburgh, Pennsylvania 15235,
and the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

Mossbauer Spectra of Some Porphyrin Complexes with Pyridine, Piperidine, and Imidazole[1a]

By L. M. Epstein,[1b] Darel K. Straub,[1c] and C. Mariconi[1c]

Received February 6, 1967

The Mössbauer spectra of the imidazole, pyridine, and piperidine adducts of $\alpha,\beta,\gamma,\delta$-tetraphenylporphyriniron(II) and protoporphyriniron(II) and the imidazole and pyridine adducts of the corresponding porphyriniron(III) chlorides have been measured. Only the heme compound could be obtained with piperidine because piperidine causes spontaneous reduction of iron(III) to iron(II) in porphyrin complexes. For the iron(II) cases, the imidazole and pyridine adducts show similar isomer shifts, while the piperidine adducts have slightly larger isomer shifts. Imidazole gives the smallest quadrupole splitting; piperidine gives the largest. For the iron(III) cases, imidazole gives a somewhat larger isomer shift than pyridine and causes a much greater quadrupole splitting than pyridine, in contrast to the iron(II) cases. These results are discussed in terms of differences in π-bonding characteristics between the added ligand and the iron atom. Pyridine appears to have a greater affinity for heme than does piperidine.

Introduction

Because of their biological importance, iron porphyrin complexes have been studied extensively by a number of physical and chemical methods. Mössbauer spectroscopy is particularly suited to the study of these complexes, and the Mössbauer spectra of hemin,[2-7] hemin salts,[8] hematin,[5-6] hemoglobin and its derivatives,[6,8-10] cytochrome,[6,8] and catalase[8] have been reported in the literature.[11]

We have begun a systematic investigation of the Mössbauer spectra of some iron–porphyrin complexes and report in this paper a study of the spectra of the adducts of ferrous and ferric tetraphenylporphyrin, protoporphyrin, and protoporphyrin dimethyl ester complexes with pyridine, piperidine, and imidazole. These were selected as model compounds for the naturally occurring hemoglobins, cytochromes, and catalases which contain octahedrally coordinated iron with the fifth and sixth coordination positions being occupied by an amine (such as an imidazole nitrogen from histidine) and/or water. The only previous work on such compounds was done by Bearden, Moss, Caughey, and Beaudreau[12] on the bispyridine hemochromes of 2,4-diacetyldeuteroporphyrin dimethyl ester and mesoporphyrin dimethyl ester. Some related phthalocyanine complexes have been investigated by Hudson and Whitfield.[13]

---

(1) (a) Supported by AEC Contract AT(30-1)3514; (b) Westinghouse Electric Corp.; (c) University of Pittsburgh.

(2) L. M. Epstein, *J. Chem. Phys.*, **36**, 2731 (1962).

(3) R. G. Shulman and G. K. Wertheim, *Rev. Mod. Phys.*, **36**, 459 (1964).

(4) U. Gonser, *J. Phys. Chem.*, **66**, 564 (1962).

(5) P. G. Reizenstein and J. B. Swan, *Intern. Biophys. Congr.*, **1**, 147 (1961).

(6) J. E. Maling and M. Weissbluth in "Electronic Aspects of Biochemistry," B. Pullman, Ed., Academic Press Inc., New York, N. Y., 1964, p 93.

(7) P. P. Craig and N. Sutin, *Rev. Mod. Phys.*, **36**, 437 (1964).

(8) W. Karger, *Ber. Bunsenges. Physik. Chem.*, **68**, 793 (1964).

(9) U. Gonser, R. W. Grant, and J. Kregzde, *Science*, **143**, 680 (1964).

(10) G. Lang and W. Marshall, *Biochem. J.*, **95**, 3 (1965).

(11) For a brief review see U. Gonser and R. W. Grant in "Mössbauer Effect Methodology," Vol. 1, I. J. Gruverman, Ed., Plenum Press, New York, N. Y., 1965, p 21.

(12) A. J. Bearden, T. H. Moss, W. S. Caughey, and C. A. Beaudreau, *Proc. Natl. Acad. Sci. U. S.*, **53**, 1246 (1965).

(13) A. Hudson and H. J. Whitfield, *Chem. Commun.*, 606 (1966).