Hydrolysis and Photolysis of Tris(tetraethylammonium) Pentacyanoperoxynitritocobaltate(III): Evidence for a Novel Complex, Pentacyanonitratocobaltate(III)

by **Patrick K. Wick**, **Reinhard Kissner**, and **Willem H. Koppenol***

Laboratorium für Anorganische Chemie, ETH Hönggerberg, CH-8093 Zürich
(Phone: +41-1-632-2875; fax: +41-1-632-1090; e-mail: koppenol@inorg.chem.ethz.ch)

Dedicated to the memory of Prof. *Luigi M. Venanzi*, a friend and colleague

At pH 2, the rate constant of hydrolysis of tris(tetraethylammonium) pentacyanoperoxynitritocobaltate(III) in H_2O is 9.6×10^{-6} s^{-1}. In the absence of any light, $ONOO^-$ is not replaced by H_2O and isomerizes within the coordination sphere to NO_3^-. The novel complex $[Co(CN)_5NO_3]^{3-}$ released NO_3^- slowly, as detected by ion chromatography. At pH 6, no hydrolysis is observed. Direct photolysis, both at pH 2 and pH 6, of tris(tetraethylammonium) pentacyanoperoxynitritocobaltate(III) by irradiation (YAG laser) at 355 nm destroys the coordinated $ONOO^-$ and releases $NO_2^{\bullet}$, which hydrolyzes to NO_3^- and NO_2^-. We also measured the ^{59}Co-NMR spectra of $[Co(CN)_5OONO]^{3-}$ and $[Co(CN)_5H_2O]^{2-}$; the chemical shifts correspond very well to those predicted and are in agreement with the expected contribution to the ligand field by H_2O and $ONOO^-$.

Introduction. – Nitrogen monoxide ($NO^{\bullet}$) reacts very rapidly with superoxide ($O_2^{\bullet -}$) to yield $ONOO^-$ (oxoperoxonitrate(1–)) [1][2]. The anion is relatively stable in alkaline solution ($pH \geq 12$) and shows an absorption band in the UV ($\lambda_{max} = 302$ nm, $\varepsilon = 1705$ M^{-1}cm^{-1}) [3]. The conjugate acid HOONO (hydrogen oxoperoxonitrate) isomerizes to NO_3^- at a rate of 1.2 s^{-1} (25°); its pK_a is 6.5 at low phosphate concentrations [2]. The acid is a powerful oxidant [4] that damages biological compounds [5–8]. Aside from carrying out one-electron and two-electron oxidations, it reacts with phenolic compounds to form nitrated, hydroxylated, and dimerized products [9][10]. Indeed, the nitration of tyrosine residues in proteins has served as a marker of $ONOO^-$ formation *in vivo* [11].

We are interested in stabilizing $ONOO^-$ in the coordination sphere of a suitable transition metal such as Co or Ti. Recently, we reported the first synthesis and characterization of a stable complex between a metal and $ONOO^-$, namely tris(tetraethylammonium) pentacyanoperoxynitritocobaltate(III) (**1**) [12]. The UV/VIS spectrum of **1** shows a band at 280 nm and a shoulder at 350 nm. These absorptions are assigned to a transition within coordinated $ONOO^-$, and to a d-d transition ($^1A_{1g} \rightarrow {}^1T_{1g}$). The complex is soluble in H_2O and MeOH. In unbuffered H_2O, it decomposes with a rate constant of 4.9×10^{-6} s^{-1} and, in MeOH, with $k = 1.7 \times 10^{-4}$ s^{-1}. When PhOH is present, nitrated and hydroxylated products are found. We assume that coordinated $ONOO^-$ first exchanges with H_2O before it reacts with PhOH. Given the higher rate constant, $ONOO^-$ probably reacts with MeOH while it is coordinated to the metal. In this paper, we discuss hydrolysis and photolysis of **1** in buffered solutions at pH 2 and 6.

We also used ^{59}Co-NMR to characterize **1** and the product of the photolysis in more detail. *Au-Yeung* and *Eaton* [13] have described a method to estimate chemical shifts and line widths of Co^{III} complexes. One chemical shift (S_L) and one line width (γ_L) parameter are required for each different type of ligand. These parameters are related to each other, and to the crystal-field-splitting parameters (f_L) of the ligand. The calculation can be readily applied to complexes of any symmetry, and, generally, there is good agreement between calculated and experimental values. Relative chemical shifts are calculated with considerably higher accuracy for complexes with similar ligands.

Results and Discussion. – We reported previously [12] that **1** is unstable in H_2O and in MeOH. However, in both solvents, its lifetime is much longer than that of uncoordinated ONOOH. In MeOH, we measured a rate constant of 1.7×10^{-4} s^{-1} and, in H_2O, 4.9×10^{-6} s^{-1}; the decrease in absorption at 280 nm (*Fig. 1,b*) fits a first-order dependence very well. In H_2O, the reaction appears to be faster in the beginning and slower afterwards, but these observations may well reflect the instability of the lamp over 120 h and that these experiments were carried out in unbuffered solutions. For this reason, further experiments were performed in 20 mM phosphate-buffered solutions. The UV/VIS spectrum of **1** does not change with pH, and, therefore, all kinetic experiments were carried out at 280 nm. We repeated the measurement of the hydrolysis of **1** at pH 2 and at pH 6. At the lower pH, the rate constant was 9.6×10^{-6} s^{-1} (*Fig. 1,a*). The first-order fit matches the kinetic trace better than in unbuffered solution [12], and the rate constant is higher. Protonation of the O-atom coordinated to Co, as has been described for the μ-bridged peroxo Co-complex [14], as well as protonation of the axial CN^-, would weaken the ligand bond to $ONOO^-$ and initiate the exchange of this ligand. We therefore propose that the ligand exchange is catalyzed by acid.

At pH 6, no decrease in the size of the band at 280 nm nor any change in the overall spectrum from 250 to 500 nm was observed: **1** appears to be stable in weakly acidic or

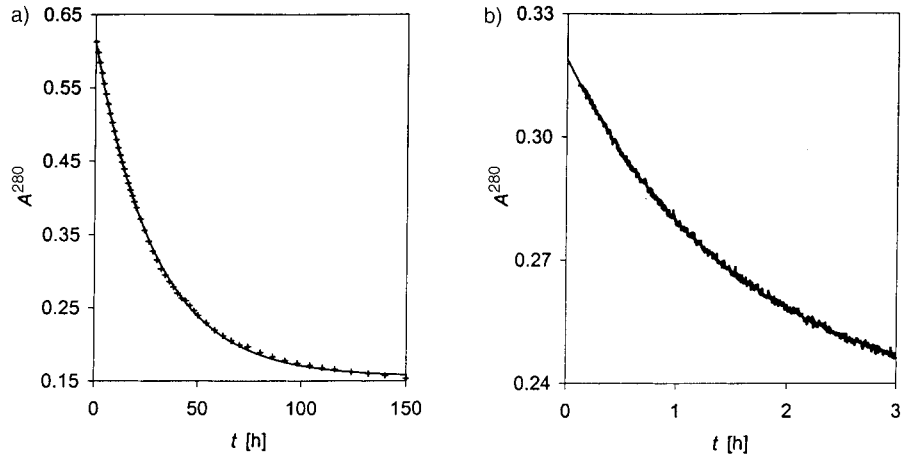


Fig. 1. *Kinetic traces of the decomposition of* **1** *at 280 nm* a) *in* H_2O (with first-order fit) *and* b) *in MeOH*

basic solutions. This conclusion is supported by a lack of nitration and hydroxylation of PhOH when **1** and PhOH are kept in a buffered solution at pH 6 for 6 days: $ONOO^-$ is not released, and coordinated $ONOO^-$ does not react with PhOH. However, in an unbuffered solution, nitration and hydroxylation were observed [12].

During previous experiments [12], the shutter between the lamp and sample was closed between spectral measurements to prevent photolysis of **1**. To determine whether **1** is indeed sensitive to photolysis, we flashed a 0.4 mM solution of **1** with 355-nm light from a YAG laser and measured the UV/VIS spectrum after every fifth flash (*Fig. 2,a*). As shown, the spectrum changes considerably. The band at 280 nm, which was assigned to coordinated $ONOO^-$ [12], disappeared, and the shoulder at 350 nm shifted to 380 nm. The spectrum after photolysis is identical to the spectrum after hydrolysis and is ascribed to $[Co(CN)_5H_2O]^{2-}$ [15]. The red-shift illustrates the change in the ligand field, which is caused by $ONOO^-$ making a stronger contribution than H_2O to the ligand field.

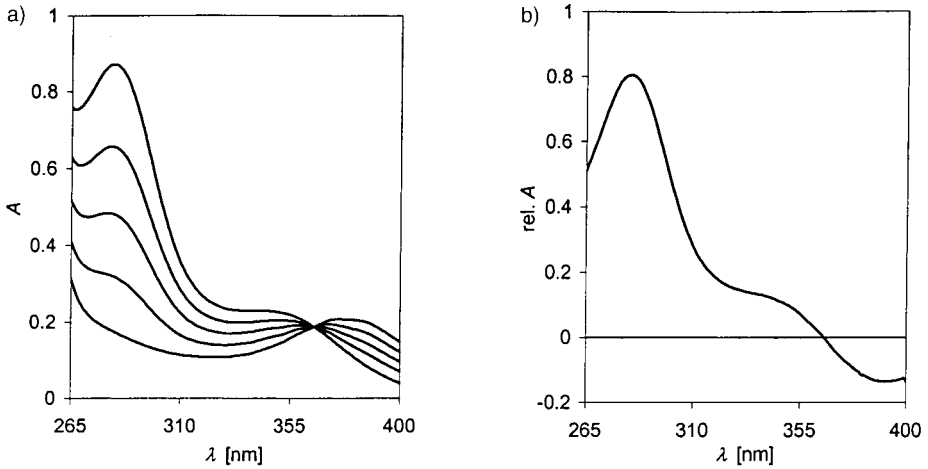


Fig. 2. a) *Initial spectrum of* **1** *at pH 2 with the two bands at 350 nm and 280 nm assigned to a d-d transition* ($^1A_{1g} \rightarrow {}^1T_{1g}$) *and to a transition within ONOO⁻, respectively.* The $ONOO^-$ transition at 280 nm disappears when the solution is flashed with the YAG laser (spectra after 5, 10, 20, and 50 flashes). b) *Difference spectrum of the initial and the final spectrum of* **1** *after complete photolysis*

We measured ^{59}Co-NMR to obtain more information about **1** and the final product of the photolysis. Before photolysis, we found a signal at 1386 ppm, and, afterwards, at 1780 ppm, that we assigned to $[Co(CN)_5H_2O]^{2-}$. *Au-Yeung* and *Eaton* [13] described a method to estimate chemical-shifts for Co^{III} complexes. For $ONOO^-$ as a ligand, no chemical-shift parameters (S_L) are known. From the signal at 1386 ppm, we calculated a chemical-shift parameter S_L of 2.373×10^{-5} ppm^{-1}. This value lies somewhat above the value for O_2^{2-}, $S_L = 2.169 \times 10^{-5}$ ppm^{-1}, as expected for a ligand with a stronger contribution to the ligand field. After photolysis the chemical shift was 1780 ppm, in good agreement with the calculated value of 1734 ppm [13], and the experimental value of 1840 ppm ([16] for $[Co(CN)_5H_2O]^{2-}$). Close agreement between the different experiments is not expected because the peaks in the ^{59}Co-NMR spectrum are broad.

We repeated the hydrolysis at pH 2 without continuous collection of UV/VIS spectra, because of the sensitivity of **1** to light, and found that the spectrum after hydrolysis in the dark was not the same as that after photolysis. Even though the band of coordinated $ONOO^-$ had disappeared, the shoulder assigned to the d-d transition shifted only to 366 nm (*Fig. 3*). The spectrum is not compatible with that of $[Co(CN)_5H_2O]^{2-}$ [15]. We assume that $ONOO^-$ isomerized within the coordination sphere and is then replaced by H_2O. This substitution takes place more slowly in the dark than in light. The spectrum was assigned to the novel complex $[Co(CN)_5NO_3]^{3-}$ (**2**) according to the product analysis described below. The contribution to the ligand field by coordinated NO_3^- lies between that of H_2O and that of $ONOO^-$. If a solution of **2** is left standing for one week in daylight, then the spectrum of $[Co(CN)_5H_2O]^{2-}$ is observed.

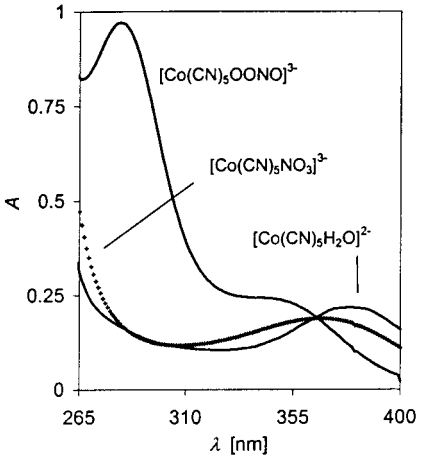
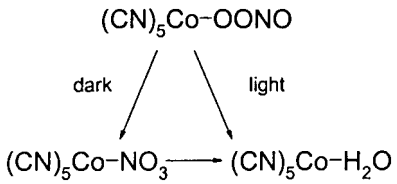


Fig. 3. *Spectrum of $[Co(CN)_5OONO]^{3-}$ and $[Co(CN)_5NO_3]^{3-}$ after hydrolysis of* **1** *in the dark, and that of $[Co(CN)_5H_2O]^{2-}$ after hydrolysis with light or photolysis of* **1**

Ion chromatography was carried out to investigate whether N-containing species were released from **1** by photolysis and hydrolysis. The initial solution of *ca.* 0.4 mM was flashed with the YAG laser at pH 2 until no further changes in the UV/VIS spectrum could be detected (*Fig. 2, a*). The difference spectrum (*Fig. 2, b*) represents the isolated transition band for coordinated $ONOO^-$ ($A = 0.807$; $\lambda_{max} = 284$ nm). The product analysis yields a total of 362 μM of NO_2^- and NO_3^-. Thus, the extinction coefficient of the differential maximum can be calculated: $\varepsilon = 2.2 \times 10^3\ M^{-1}\ cm^{-1}$ at 284 nm. Relative to the total amount of N-containing species, the amounts of NO_3^- and NO_2^- are 55 and 45%, respectively, from which we conclude that $NO_2^{\bullet}$ was liberated by light and $O^{\bullet-}$ remains bound to Co(III). In the end, $[Co(CN)_5H_2O]^{2-}$ is found. This reaction may proceed *via* peroxo species known to be unstable in H_2O [17–19].

Product analysis of the hydrolysis experiments at pH 2 yielded different results depending on whether or not the soluton was exposed to light or not (*Scheme*). Without light, exclusively NO_3^-, but only 6% relative to **1**, was found. When this solution is flashed, more NO_3^-, but no NO_2^-, is found (NO_3^- is reportedly stable to irradiation at wavelengths above 300 nm [20][21]). With light, the otherwise slow

Scheme



hydrolysis of coordinated $ONOO^-$ and of NO_3^- is accelerated, and up to 1 equiv. of NO_3^- per **1** is detected.

In summary, we conclude that, in aqueous solution, **1** is stable at pH $\geq$ 6. At pH 2, the rate constant for hydrolysis is $k = 9.6 \times 10^{-6}\ s^{-1}$ in daylight. Without irradiation, coordinated $ONOO^-$ isomerizes to NO_3^- within the ligand sphere and forms the novel complex $[Co(CN)_5NO_3]^{3-}$ (**2**), as follows from the results of ion chromatography and UV/VIS spectroscopy. When irradiated, NO_3^- is exchanged for H_2O, as detected by ion chromatography. Decomposition of **1** with 355-nm light from a YAG laser leads to the formation of NO_2^- and NO_3^-. We assume, therefore, that $NO_2^{\bullet}$ is released. ^{59}Co-NMR spectroscopy provided evidence for the formation of $[Co(CN)_5H_2O]^{2-}$.

We thank Drs. *T. Nauser* and *H. Rüegger* for introducing us to the YAG laser and the ^{59}Co-NMR measurements, respectively. These studies were supported by the ETH-Zürich and the *Swiss National Science Foundation*.

Experimental Part

General. All reagents except NO$^{\bullet}$ (99.5% purity, *Linde AG*) were of anal. grade (*Fluka Chemie AG*). Tris(tetraethylammonium) pentacyanoperoxynitritocobaltate(III) ($[N(CH_2Me)_4]_3[Co(CN)_5OONO]$; **1**) was synthesized according to [12] from solid tris(tetraethylammonium) pentacyanosuperoxocobaltate(III) and NO$^{\bullet}$.

Tris(tetraethylammonium) Pentacyanonitratocobaltate(III) ($[N(CH_2Me)_4]_3[Co(CN)_5NO_3]$; **2**) is formed when complex **1** is kept at pH 2 for 6 d in the dark.

Spectroscopy and Kinetics. UV/VIS Spectra: double-beam *Kontron Uvikon-820* instrument. Kinetic measurements: single-beam diode-array *J&M TIDAS* instrument. Spectra (200–450 nm) were taken every 20 min; between measurements, the shutters of the light sources were closed to prevent photolysis. Before and after the photolysis with the *Quantel Brilliant-BW* (F-Les Ulis) YAG-laser (355 nm, 5 ns, 200 mJ), spectra were collected. ^{59}Co-NMR Spectra: *Bruker Avance-250* (59,348 MHz).

Product Analysis. A stock soln. of **1** in 0.02M phosphate buffer at pH 2 was adjusted such that an absorbance of *ca*. 0.8 was obtained. The concentration of **1** was *ca*. 0.4 mM. One aliquot of the stock soln. was kept in the dark for one week before the spectrum was measured. Another aliquot was flashed 50 times with a YAG-laser, and, after every fifth flash, a spectrum was collected. A third aliquot was kept in the spectrophotometer, and spectra were taken every 20 min (see above) to determine the rate of hydrolysis. Each aliquot was adequately diluted and the concentrations of NO_2^- and NO_3^- were determined by ion chromatography (*IC Anion Column SUPER-SEP* with precolumn, *IC Detector 732*, *Metrohm AG*). The mobile phase consisted of 2.5 mM phthalic acid, 5% MeCN and H_2O at pH 4.2 (*Tris*). The flow rate was 1.2 ml/min. The t_R values of NO_2^- and NO_3^- were 4.1 and 5.9 min, resp.

HPLC: Hewlett-Packard Series 1050; stationary phase: *Vydac Protein & Peptide C18* column; mobile phase: 10% MeOH, 10% MeCN and 0.1% TFA in H_2O; flow rate: 1 ml/min; t_R values were compared with those of authentic standards.

REFERENCES

[1] R. E. Huie, S. Padmaja, *Free Radical Res. Commun*. **1993**, *18*, 195.
[2] R. Kissner, T. Nauser, P. Bugnon, P. G. Lye, W. H. Koppenol, *Chem. Res. Toxicol*. **1997**, *10*, 1285.
[3] D. S. Bohle, P. A. Glassbrenner, B. Hansert, *Methods Enzymol*. **1996**, *269*, 302.
[4] W. H. Koppenol, R. Kissner, *Chem. Res. Toxicol*. **1998**, *11*, 87.
[5] W. H. Koppenol, J. J. Moreno, W. A. Pryor, H. Ischiropoulos, J. S. Beckman, *Chem. Res. Toxicol*. **1992**, *5*, 834.
[6] R. Radi, J. S. Beckman, K. M. Bush, B. A. Freeman, *J. Biol. Chem*. **1991**, *266*, 4244.
[7] R. Radi, J. S. Beckman, K. M. Bush, B. A. Freeman, *Arch. Biochem. Biophys*. **1991**, *288*, 481.
[8] S. V. Lymar, J. K. Hurst, *Chem. Res. Toxicol*. **1996**, *9*, 845.
[9] M. S. Ramezanian, S. Padmaja, W. H. Koppenol, *Chem. Res. Toxicol*. **1996**, *9*, 232.
[10] A. Daiber, M. Mehl, V. Ullrich, *Nitric Oxide: Biol. Chem*. **1998**, *2*, 259.
[11] J. S. Beckman, Y. Z. Ye, P. G. Anderson, J. Chen, M. A. Accavitti, M. M. Tarpey, C. R. White, *Biol. Chem. Hoppe Seyler* **1994**, *375*, 81.
[12] P. K. Wick, R. Kissner, W. H. Koppenol, *Helv. Chim. Acta* **2000**, *83*, 748.
[13] S. C. F. Au-Yeung, D. R. Eaton, *Can. J. Chem*. **1983**, *61*, 2431.
[14] H. Siebert, *Z. anorg. allg. Chem*. **1980**, *463*, 155.
[15] V. Miskowski, H. B. Gray, *Inorg. Chem*. **1975**, *14*, 401.
[16] T. Fujihara, S. Kaizaki, *J. Chem. Soc., Dalton Trans*. **1993**, 1275.
[17] M. Hoshino, M. Nakajima, M. Takakubo, M. Imamura, *J. Phys. Chem*. **1982**, *86*, 221.
[18] M. H. Gubelmann, S. Rüttimann, B. Bocquet, A. F. Williams, *Helv. Chim. Acta* **1990**, *73*, 1219.
[19] J. H. Bayston, M. F. Winfield, *J. Catal.* **1964**, *3*, 123.
[20] W.-J. Chen, W.-J. Lo, B.-M. Cheng, Y.-P. Lee, *J. Chem. Phys*. **1992**, *97*, 7167.
[21] C. E. Miller, H. S. Johnson, *J. Phys. Chem*. **1993**, *97*, 9924.

Received June 15, 2001