Synthesis and Characterization of Tris(tetraethylammonium) Pentacyanoperoxynitritocobaltate(III)

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We report the first synthesis of a stable complex of peroxynitrite coordinated to a transition-metal ion. Solid tris(tetraethylammonium) pentacyanosuperoxocobaltate(III) reacts with 1 equiv. of gaseous nitrogen monoxide to yield tris(tetraethylammonium) pentacyanoperoxynitritocobaltate(III) (**1**). This novel complex is characterized by a UV absorption band at 280 nm ($\varepsilon \approx 2000\ \text{M}^{-1}\,\text{cm}^{-1}$) in H_2O. The IR spectrum of the sodium salt of the complex, **2**, shows vibration bands due to peroxynitrite. Nitrated and hydroxylated products are observed when the complex is dissolved in H_2O in the presence of phenol. The rate constant of hydrolysis is $k = 4.9 \times 10^{-6}\ \text{s}^{-1}$. The complex is less stable in MeCN and in MeOH and perhaps reacts with these solvents.

Introduction. – Nitrogen monoxide ($NO^{\bullet}$) reacts very rapidly with superoxide ($O_2^{\bullet-}$) to yield peroxynitrite (oxoperoxonitrate(1–), $ONOO^-$) [1–3]. The anion is relatively stable in alkaline solution (pH $\geq$ 12) and shows an absorption band in the UV ($\lambda_{max} = 302$ nm, $\varepsilon = 1705\ \text{M}^{-1}\,\text{cm}^{-1}$) [4]. Peroxynitrous acid (hydrogen oxoperoxonitrate, ONOOH) isomerizes to nitrate at a rate of 1.2 s^{-1}; its pK_a is 6.5 at low phosphate concentration [3]. The acid is a powerful oxidant [5] that damages biological compounds [6–9]. Aside from one-electron and two-electron oxidations, it reacts with phenolic compounds to form nitrated, hydroxylated, and dimerized products [10][11]. Indeed, the nitration of free tyrosine, or tyrosine in proteins, has served as a marker of peroxynitrite formation *in vivo* [12]. In this work, we argue that nitration of phenol is evidence that peroxynitrite was formed.

The mechanism of isomerization of peroxynitrous acid to nitrate is not known. Two mechanisms have been proposed: *1*) homolysis of the O–O bond within a solvent cage, followed by rearrangement of the radicals [13] and *2*) an intramolecular rearrangement of the terminal O-atom to the N-atom [14]. Like protons, metals can also catalyze the isomerization. Nitrogen monoxide reacts with coordinated dioxygen in oxyhemoglobin to form an intermediate peroxynitrito complex [15]. In this particular case, the positive charge on the metal center is so high that isomerization of the coordinated peroxynitrite is induced. This suggests that the ligands around a metal center may play an important role in the stabilization of peroxynitrite coordinated to metal. We assumed that reduction of the *Lewis* acid character of a metal center could stabilize coordinated peroxynitrite.

Several methods exist for the synthesis of peroxynitrite. It has been shown that peroxynitrous acid is formed from the reaction of H_2O_2 with nitrous acid [16][17].

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Peroxynitrite can be stabilized in alkaline solution. Irradiation of solid nitrates with UV light also results in the formation of peroxynitrite. In this way, it was possible to record the IR spectrum of peroxynitrite [18] and peroxynitrous acid [19][20] in a solid Ar matrix. The heterogeneous reaction between superoxide salts and gaseous nitrogen monoxide can be used to generate relatively pure solutions of peroxynitrite [21][22]. Recently, the single-crystal structure of tetramethylammonium peroxynitrite was determined [23].

End-on superoxo complexes are rare. They often decompose or dimerize to form μ-bridged peroxo or superoxo complexes. For instance, pentacyanocobaltate(II) is oxidized in H_2O to pentacyanosuperoxocobaltate(III), which decomposes afterward [24]. However, its tetraethylammonium salt is stable in several organic solvents, such as MeCN [25]. An X-ray crystal-structure determination has confirmed that the complex is an η^1-dioxygen complex [26]. We show here that pentacyanosuperoxocobaltate(III) reacts with nitrogen monoxide to pentacyanoperoxynitritocobaltate(III) **1**.

Results and Discussion. – The radical character of the pentacyanosuperoxocobaltate(III) complex **1** was determined by *White et al.* [25] and confirmed by us. The broad ESR signal shows no hyperfine structure and is centered at $g = 2.026$. In analogy to free superoxide, we expected that a superoxo complex would react with nitrogen monoxide to form a peroxynitrito complex. Indeed, we found that pentacyanosuperoxocobaltate(III) reacts with 1 equiv. of nitrogen monoxide in MeCN. The reaction, which is faster than ligand exchange, ended within minutes. No ESR signal was detected after the reaction with nitrogen monoxide. In addition, the red color of the superoxo complex ($\lambda_{max} = 400$ nm, $\varepsilon = 1000$ м$^{-1}$ cm^{-1}; $\lambda_{max} = 320$ nm, $\varepsilon = 2800$ м$^{-1}$ cm^{-1} [24]) changed to yellow, and a new absorption band was observed, a shoulder at 279 nm, which, in MeCN, disappeared within 1 h (*Fig. 1*). We assign this new absorption to coordinated peroxynitrite.

IR Spectroscopy of the sodium salt **2** of the complex **1** provided evidence for a coordinated peroxynitrite. Because the IR spectrum of tetraethylammonium cation would be expected to show several interfering lines below 2000 cm^{-1}, the cation was exchanged for sodium. The O–O stretching frequency at 915 cm^{-1} is evidence of formation of a peroxynitrito complex (*Fig. 2*). Furthermore, the locations of the two other major bands at 1621 cm^{-1} and 1399 cm^{-1} are in the range expected for the N=O and N–O stretching freqencies of peroxynitrite bound to a transition metal ion, respectively [18][20][22][27]. Isomerization of peroxynitrite to nitrate within the coordination sphere is excluded, because IR absorptions of the nitrato ligand would be expected near 1475 cm^{-1}, 1280 cm^{-1}, 1017 cm^{-1}, and 800 cm^{-1}, as determined for $[Co(NH_3)_5NO_3]^{2+}$ [28][29].

The isolation and characterization of compound **1** from the reaction of nitrogen monoxide with pentacyanosuperoxocobaltate(II) in MeCN were not reproducible. We, therefore, attempted synthesis by conversion of solid tris(tetraethylammonium) pentacyanosuperoxocobaltate(III) with gaseous nitrogen monoxide. As in solution, 1 equiv. of nitrogen monoxide was absorbed per cobalt, and the powder became yellow. Surprisingly, this powder was insoluble in MeCN or EtOH. Instead, decomposition of the complex took place, which may explain why the results obtained in MeCN were variable.

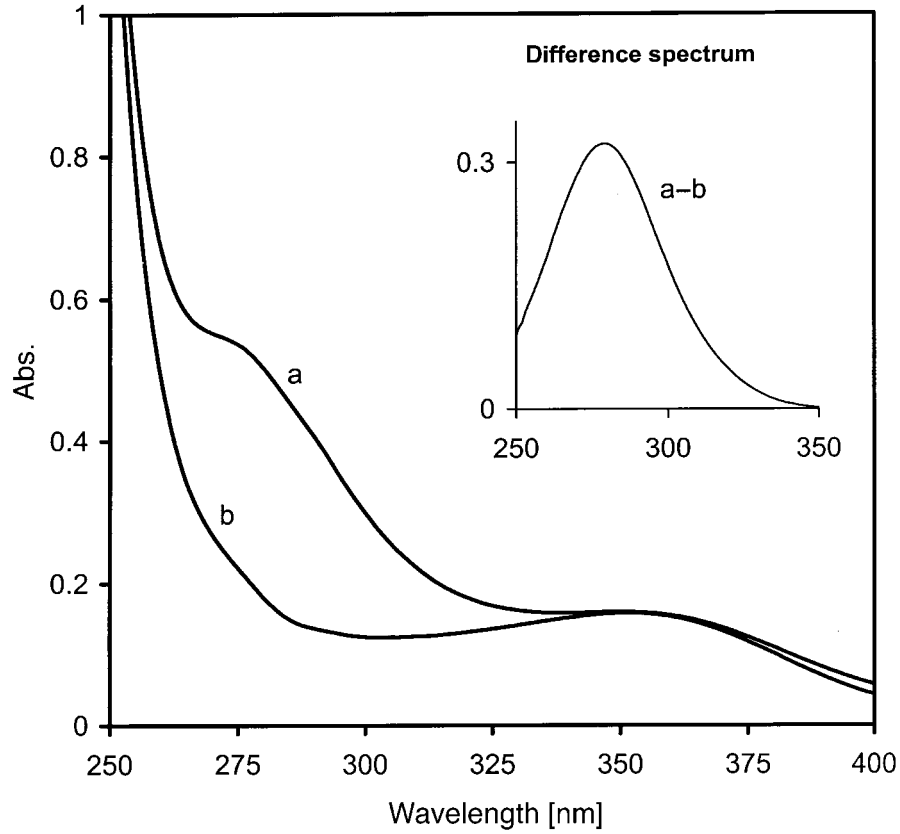

Fig. 1. *The UV spectra showing the shoulder at 279 nm at 0 min* (a) *and after 45.5 min* (b). The difference spectrum (a – b) shows the absorption of coordinated peroxynitrite. With respect to free peroxynitrite in MeCN, λ_{max} shifts from *ca.* 315 nm to 279 nm ($\varepsilon \approx 2000$ M^{-1} cm^{-1}).

The UV/VIS spectrum of compound **1** in MeOH did not show an isolated absorption band near 280 nm. However, the absorption at 280 nm decreased with a rate constant of $k = 1.7 \times 10^{-4}$ s^{-1}. This decay may be due to isomerization of peroxynitrite, a reaction with the solvent, or a ligand exchange reaction.

In H_2O, the existence of a peroxynitrite complex was demonstrated clearly. The complex is quite soluble and stable for hours. The UV/VIS spectrum shows an absorption band at $\lambda_{max} = 280$ nm (*Fig. 3*), similar to that in MeCN and to the spectrum of $[Co(CN)_5OOH]^{3-}$ with $\lambda_{max} = 272$ nm [30]. The kinetics of the disappearance of the 280 nm absorption (*Fig. 4*) is compatible with hydrolysis, because the rate constant of 4.9×10^{-6} s^{-1} is typical for ligand-exchange reactions for pentacyanocobaltate(III) complexes in H_2O [31]. We concluded that homolysis of coordinated peroxynitrite to nitrogen monoxide and superoxide did not occur, because neither the UV nor the ESR spectrum of tris(tetraethylammonium) pentacyanosuperoxocobaltate(III) was observed.

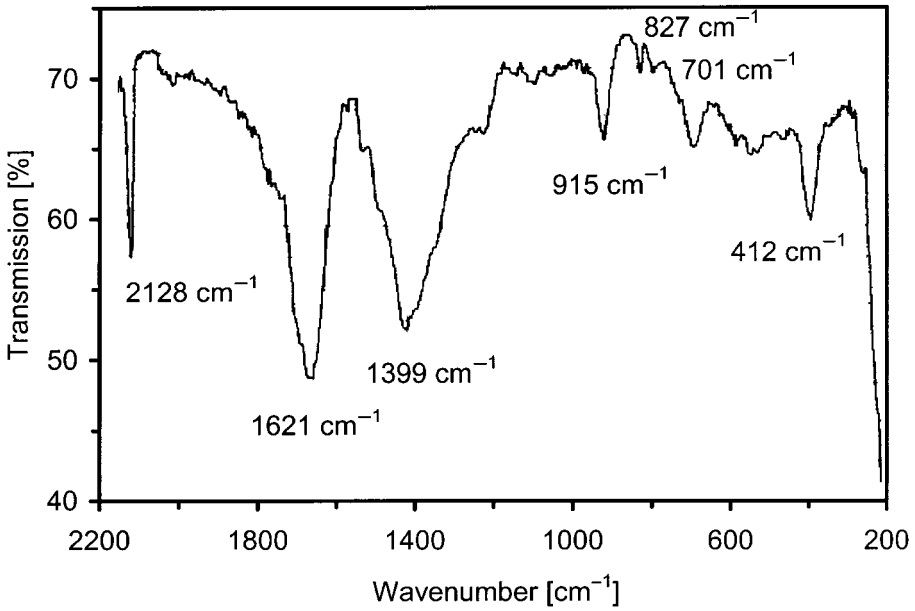

Fig. 2. *IR Spectrum of compound* **2**. The peaks at 1621 cm^{-1}, 1399 cm^{-1}, 915 cm^{-1}, 827 cm^{-1}, 701 cm^{-1}, and 412 cm^{-1} are assigned to coordinated peroxynitrite. The sharp peak at 2128 cm^{-1} is assigned to cyanide.

Compound **2**, dissolved in H_2O in the presense of phenol, leads to nitrated and hydroxylated products; benzene-1,4-diol (hydroquinone), benzene-1,2-diol (catechol), 4-nitrophenol, and 2-nitrophenol were identified by HPLC [10]. Three additional, unidentified peaks were assigned to phenol dimers [11].

In summary, we conclude that compound **1** is tris(tetraethylammonium) pentacyanoperoxynitritocobaltate(III), the first stable metal complex with peroxynitrite as a ligand. The UV spectrum shows an absorption in a region expected for coordinated peroxynitrite. The IR spectrum unambiguously indicates the presence of a peroxynitrito ligand and the absence of nitrate, and only peroxynitrite would be expected to lead to nitro derivatives of phenol. The rate of decay indicates that peroxynitrite first undergoes exchange with H_2O before reacting with phenol.

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Experimental Part

Materials. All reagents except nitrogen monoxide (99.5% purity, *Linde AG*, D-Höllriegelskreuth) were of anal. grade (*Fluka Chemie AG*, CH-Buchs).

Tris(tetraethylammonium) Pentacyanosuperoxocobaltate(III) ($[N(CH_2CH_3)_4]_3[Co(CN)_5(O_2)]\cdot 1.5\,H_2O$) was prepared as described in [25]: Tetraethylammonium cyanide (1.4829 g, 9.205 mmol) and anh. $CoCl_2$ (0.2390 g, 1.841 mmol) were dissolved in 50 and 20 ml of deoxygenated MeCN, resp., and the blue $CoCl_2$ soln. was added to the cyanide solution with stirring. After the addition, the yellow solution of $[Co(CN)_5]^{3-}$ was oxygenated. The red-brown pentacyanosuperoxocobaltate(III) complex was precipitated with Et_2O and dried *in vacuo*. Yield: 0.8034 g (74%).

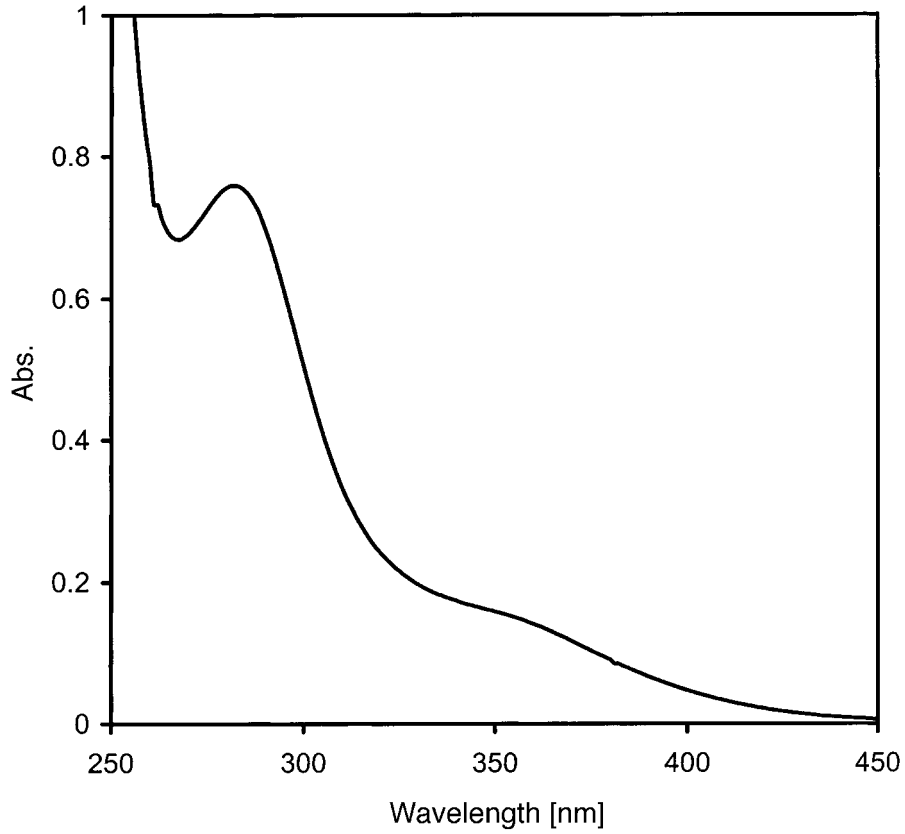

Fig. 3. *UV/VIS Spectrum of compound* **1** *in* H_2O. The two bands at 350 and 280 nm are assigned to a d-d transition ($^1A_{1g} \rightarrow {}^1T_{1g}$) and an inner ligand transition.

Tris(tetraethylammonium) Pentacyanoperoxynitritocobaltate(III) ($[N(CH_2CH_3)_4]_3[Co(CN)_5(OONO)]$; **1**). Nitrogen monoxide (1 equiv.) was passed over solid NaOH, before addition to dried tris(tetraethylammonium) pentacyanosuperoxocobaltate(III) in a flask under Ar. After two days, all nitrogen monoxide was absorbed, and the color of the solid had changed from red-brown to yellow.

Sodium Pentacyanoperoxynitritocobaltate(III) ($Na_3[Co(CN)_5(OONO)]$; **2**). The tetraethylammonium cation was exchanged for sodium in a strongly acidic cation-exchange column (*Amberlite IR-120*) in H_2O. Compound **2** was detected at 283 nm, collected, and dried.

Spectroscopy and Kinetics. Spectra for identification of the products and for kinetic measurements in MeOH were recorded on a double-beam *Kontron Uvikon 820* instrument (CH-Basel). Spectra for kinetic measurements in H_2O were obtained from a single-beam diode array *J & M TIDAS* instrument (D-Aalen). Spectra (200–450 nm) were recorded every 10 min. Between measurements, the shutters of the light sources were closed to prevent photolysis. The IR spectrum of compound **2** (KBr disc) was recorded on a *Perkin-Elmer 883* IR spectrophotometer (Norwalk, USA). ESR Experiments were carried out at r.t. in the TM110 cavity of a *Bruker EMX 080* instrument (Billerica, MA, USA).

HPLC. A soln. containing 21 μmol of compound **2** and 210 μmol of phenol in 10 ml of H_2O was stirred for three days, before the phenolic products were extracted with Et_2O. These products were analyzed by HPLC (*Vydac Protein & Peptide C18* column, *Hewlett-Packard Series 1050* HPLC, CH-Urdorf). The mobile phase consisted of 10% MeOH, 10% MeCN, and 0.1% CF_3COOH in H_2O. The flow rate was 1 ml/min. Peaks

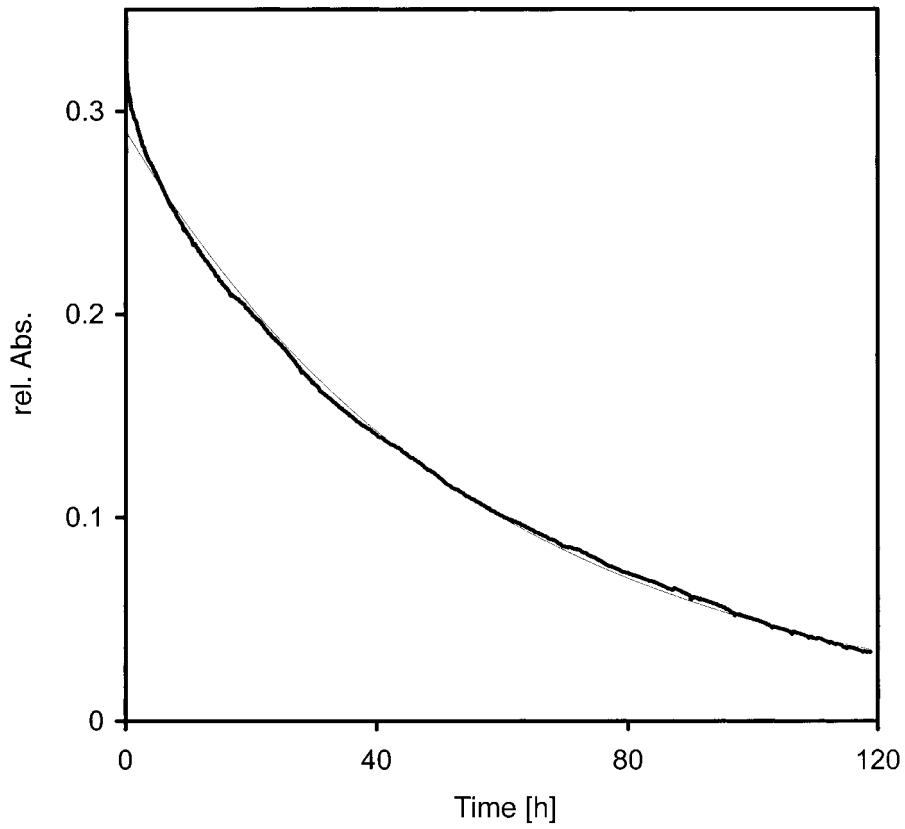

Fig. 4. *Kinetic measurement of the hydrolysis of compound* **1** *at 280 nm*. The kinetics fit a first-order reaction with $k = 4.9 \times 10^{-6}\ s^{-1}$.

measured at 250, 270 and 320 nm were identified with external standards. The t_R values of benzene-1,4-diol (hydroquinone), benzene-1,2-diol (catechol), 4-nitrophenol, and 2-nitrophenol were 0.42, 0.60, 1.49, and 2.14, relative to the t_R value of phenol.

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