

for carbonylmetal (0) complexes of the parent heterocycle of lumazines and pterines, viz., pteridine;^{27b} however, attempts to isolate tetracarbonylmolybdenum and -tungsten chelate complexes of **1** were unsuccessful because of the high lability of M⁰-O bonds.

In summary, organometallic complexes from alkylated derivatives of biochemically relevant molecules can be studied in aprotic media and may serve as starting systems for subsequent studies that involve real coenzymes under physiological conditions.

Acknowledgment. This work was generously supported by grants from the Deutsche Forschungsgemeinschaft and Stiftung Volkswagenwerk. We also thank Degussa AG for donation of chemicals.

Registry No. 1, 13401-18-8; [(1)Cu(PPh₃)₂](BF₄), 124021-09-6; [(1)Ru(bpy)₂](PF₆)₂, 124021-11-0; [(1)Re(CO)₃Cl], 124021-12-1; tetrakis(triphenylphosphine)copper(I) tetrafluoroborate, 34010-82-7; bis-(2,2'-bipyridine)ruthenium(II) chloride, 15746-57-3.

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Preparation and Properties of the Nitrido-Bridged Osmium(IV) Binuclear Complexes [Os^{IV}₂N(NH₃)_{10-n}Cl_n]Cl_{5-n} (n = 2, 3)

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Received February 8, 1989

The nitrido-bridged osmium(IV) binuclear complexes [Os₂N(NH₃)₈Cl₂]Cl₃·2H₂O (I) and [Os₂N(NH₃)₇Cl₃]Cl₂·H₂O (II) have been shown to be products of the ammonolysis of sodium hexachloroosmate(IV) (Na₂[OsCl₆]) in aqueous ammonia at elevated temperatures. Spectral (infrared and UV-vis) and electrochemical properties of the complexes have been explored. In aqueous solution, I and II undergo aquation reactions involving the chloro ligands, and the resulting aqua complexes of I and II exhibit respectively two and three ionizable protons with pK_a values in the range 3.6-7.3. In purified form, the complexes I and II plus their aquated derivatives were found to be ineffective as DNA-selective, electron-dense stains for electron microscopy, whereas effective staining was observed with unpurified mixtures.

Although polynuclear nitrido-bridged complexes of osmium were first encountered over a century ago, the study of their syntheses and properties has primarily been limited to a small number of bi- and trinuclear compounds.²⁻¹¹ Most of the binuclear examples belong to the series [Os^{IV}₂N(NH₃)_{10-n}X_n]Y_{5-n} when n = 0 and n = 2; a variety of ligands X and counterions Y are possible. Our interest in the nitrido-bridged polynuclear

complexes of osmium has arisen from the need for an electron-microscopy stain having DNA-staining properties improved over those of the ill-defined "osmium ammine" complex^{9,11-15} currently in use. Because of similarities in the preparations of the binuclear complexes⁶ and "osmium ammine",¹³ we have been led to a closer examination of [Os₂N(NH₃)₈Cl₂]Cl₃·2H₂O (I).¹⁶

Experimental Section

Chemicals and General Procedures. All chemicals were of reagent quality and were used without further purification. Water was doubly distilled.

IR and UV-visible spectra were recorded on Digilab FTS-60 FTIR and Cary 219 spectrophotometers, respectively. Cyclic voltammograms were obtained at a carbon-paste working electrode¹⁷ vs a saturated sodium chloride calomel (NaSCE) reference electrode with use of a PAR Model 173 potentiostat and a PAR Model 175 universal programmer; solutions were purged with argon and scanned at 50-500 mV s⁻¹ at ambient temperature (23 ± 2 °C). Solution conductivities (ambient temperature) were determined by using a Type CDM 2d conductivity bridge (The London Co.). Molar conductivities at infinite dilution (Λ_{M,0}) were determined by extrapolation of plots of Λ_M vs C^{1/2} to zero. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

[Os₂N(NH₃)₈Cl₂]Cl₃·2H₂O (I). A sealed, thick-walled glass tube containing Na₂[OsCl₆] (0.5 g, 1.114 mmol) in 5 mL of concentrated

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Table I. Electronic and Vibrational Spectral Data for Nitrido-Bridged Os(IV) Binuclear Complexes

aqueous ion	λ_{\max} , nm	$10^{-4}\epsilon$, M ⁻¹ cm ⁻¹
[Os ₂ N(NH ₃) ₈ Cl ₂] ³⁺	242 (242 ^a)	4.9 (4.9 ^a)
Os ₂ N(NH ₃) ₈ (OH ₂) ₂ ⁵⁺	233	5.3
[Os ₂ N(NH ₃) ₈ (OH) ₂] ³⁺	239	4.4
[Os ₂ N(NH ₃) ₇ Cl ₃] ²⁺	251	3.8
[Os ₂ N(NH ₃) ₇ (OH ₂) ₃] ⁵⁺	233	3.1
[Os ₂ N(NH ₃) ₇ (OH) ₃] ²⁺	244	3.1
[Os ₂ NCl ₈ (OH ₂) ₂] ³⁻	273 ^a	3.46 ^a

^a Previously published value.⁶

aqueous ammonium hydroxide was heated at 150 °C for 3 days in an oil bath. (*Caution!* The tube becomes pressurized. Perform heating in a ventilated hood behind a shield and cool before opening.) After a small amount of dark green solid was removed by filtration, the yellow solution was treated with ca. 4 mL of concentrated HCl to precipitate yellow microcrystals, which were collected by filtration, washed with a small quantity of HCl, and dried in vacuo; yield 0.40 g (68%). Anal. Calcd for [Os₂N(NH₃)₈Cl₂]₂·2H₂O: N, 16.95; H, 3.79; Cl, 23.83. Found: N, 17.43; H, 3.96; Cl, 23.94.

[Os₂N(NH₃)₇Cl₃]₂·H₂O (II). (NH₄)₂[OsCl₆] (1.5 g, 3.34 mmol) was heated in 40 mL of concentrated aqueous ammonia solution at 100 °C in a sealed, thick-walled glass tube under argon for 3 h (see caution for preparation of I). A small quantity of insoluble black solid was removed by filtration, and the remaining orange filtrate was evaporated to a small volume (10 mL), treated with ca. 6 mL of concentrated HCl, and evaporated to dryness; the resulting brownish yellow solid was thoroughly washed with ethanol to remove NH₄Cl. This product was dissolved in a minimum volume of 0.001 M HCl solution, which was then placed on a pretreated CM-cellulose cation-exchange column. The desired product was rapidly eluted with a 0.002 M HCl solution. The initial fractions showing a UV absorption band at 248–251 nm were combined, treated with concentrated HCl solution, and evaporated to a small volume to precipitate a yellow solid product, which was washed with ethanol and ether; yield 0.44 g (38%). Anal. Calcd for [Os₂N(NH₃)₇Cl₃]₂·H₂O: N, 15.81; H, 3.27; Cl, 25.01; O, 2.26. Found: N, 15.63; H, 3.34; Cl, 24.85; O, 2.79.

¹⁵N-Labeled Complexes. The procedures described above were followed on a reduced scale with use of ¹⁵NH₄OH.

Aqua Complexes of I and II. Aqueous solutions of I (0.05 g, 0.067 mmol) and AgClO₄ (0.069 g, 0.33 mmol) were mixed and stirred in the dark at 40 °C for 4 days. Upon removal of the white AgCl precipitate by filtration, the solution was used directly for spectral study. The chloro ligands of II were removed in a manner analogous to that for I.

Determination of pK_a Values. NaClO₄ (0.1 M) was used as an inert electrolyte for solutions of the aqua derivatives of I or II (3.4 × 10⁻⁵ and 4.5 × 10⁻⁵ M, respectively) with addition of either HClO₄ or NaOH to adjust the pH. UV spectra of these solutions were recorded over the pH range 1–9. Ionization constants were calculated by using nonlinear regression analyses (NLIN procedure of the Statistical Analysis System program, SAS Institute, Inc., Cary, NC) of absorbance at selected wavelengths as a function of pH. Regression analyses were conducted for two and three wavelengths simultaneously for the aqua derivatives of I and II, respectively; fitted parameters included a pK_a value for each of *n* ionizable protons assumed plus an extinction coefficient for each of the *n* + 1 species at each wavelength (see the supplementary material).

DNA Staining. Electron-microscopy tests of DNA-staining ability were conducted on epoxy-embedded rat liver exactly as described in an earlier publication.¹⁶

Results and Discussion

Synthesis, Separation, and Identification. Two compounds were isolated from the reaction of Na₂[OsCl₆] with aqueous ammonia. The yield, purity, and ease of preparation of the expected binuclear complex I could be maximized by holding the reactants at 150 °C for 3 days. By comparison with published data,^{3,5,6} I was identified by means of elemental analysis, FTIR spectrophotometry ($\nu_{\text{Os-N-Os}} = 1102 \text{ cm}^{-1}$, $\nu_{\text{Os-}^{15}\text{N-Os}} = 1069 \text{ cm}^{-1}$), UV-vis spectrophotometry (Table I), and aqueous conductivity ($\Delta_{\text{M},0} = 558$ and $495 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ in two trials; 1:3 electrolyte). With less severe reaction conditions (e.g., 90 °C, 12 h), we found it difficult to remove impurities ($\lambda_{\text{max}} = 320\text{--}340$ and $400\text{--}480 \text{ nm}$, $\nu = 1150, 1020\text{--}1070 \text{ cm}^{-1}$) by recrystallization, and yields declined.

Chromatographic separation of the reaction products by cation exchange (dilute HCl eluant) showed that the reaction mixtures contained significant products other than I, especially as the

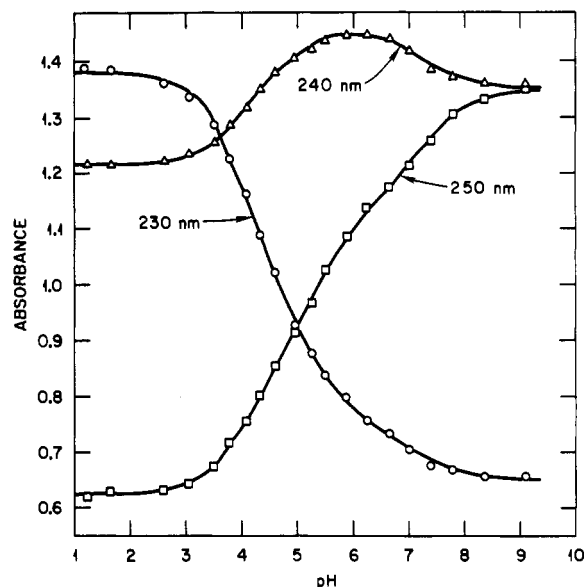


Figure 1. Absorbance vs pH profiles for the [Os₂N(NH₃)₇(OH₂)₃]⁵⁺ ion in aqueous solution (25 °C, 1-cm cells, [complex]_{total} = 4.5 × 10⁻⁵ M, [NaClO₄] = 0.1 M). The solid curves represent the least-squares optimized fit to the data.

reaction temperature and time were decreased. Eluting first was a new compound having an elemental analysis consistent with the formulation [Os₂N(NH₃)₇Cl₃]₂·H₂O (II). The compound was found to be a 1:2 electrolyte ($\Delta_{\text{M},0} = 280 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$) but otherwise had vibrational ($\nu_{\text{Os-N-Os}} = 1122 \text{ cm}^{-1}$, $\nu_{\text{Os-}^{15}\text{N-Os}} = 1084 \text{ cm}^{-1}$) and electronic (Table I) properties analogous to those of I. Eluting after II was the more highly charged complex I, followed by unidentified products in smaller quantities. Compound II was collected in greatest yield for the reaction at 100 °C for 3 h. Shorter reaction times and lower temperatures decreased the yields of both I and II due to the formation of copious unidentified precipitates.

Aqueous Solution Chemistry. As indicated by the absence of change in the UV spectra of fresh aqueous solutions of I or II vs pH in the range 1–9, the ammine ligands exhibit weak acidity compared with that of mononuclear Os(IV) halo ammine complexes,¹⁸ a fact easily explained by the strong π -donor properties of the nitrido ligand.¹⁹ On aging of the solutions under ambient conditions (23 ± 2 °C), the chloro ligands in both I and II undergo aquation as noted previously for I.^{3,6} In the case of I, a decrease in the intensity of the UV band at 242 nm occurred after several days, whereas the UV band of II shifted from 251 to 247 nm after 3.5 h. No change in the UV spectrum of either compound in 5 M HCl was observed after 5 days, whereas in 5 M HClO₄ spectral changes occurred as in water. The aquation reactions of I and II can be taken to completion by precipitating the Cl⁻ ion with Ag⁺ ion to form (under acidic conditions) the respective diaqua⁶ and triaqua complex ions [Os₂N(NH₃)₈(OH₂)₂]⁵⁺ (III) and [Os₂N(NH₃)₇(OH₂)₃]⁵⁺ (IV).

The UV spectra of IV in aqueous solution changed reversibly over the pH range 1–9. (Instability toward formation of a brown precipitate became noticeable as the pH was increased to 11–12; the precipitate was soluble in HCl solutions, giving back the spectrum of I.) As shown in Figure 1, the absorbance vs pH profile was consistent with three ionizable protons, giving on regression analysis the following consecutive pK_a values: 4.09 ± 0.02, 5.40 ± 0.04, and 7.22 ± 0.05. Fits assuming fewer ionizable protons clearly failed to reproduce the data. Reversible ionization of two protons in the case of III (stable up to pH 11 for at least 1 day) was similarly determined, yielding the pK_a values 3.62 ± 0.07 and

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5.17 ± 0.06. Selected spectral parameters are given in Table I.

The reversible processes occurring in the spectrophotometric titrations were formulated in terms of the stepwise deprotonation of the aqua ligand to give the corresponding hydroxo complexes. Accordingly, III gives $[\text{Os}_2\text{N}(\text{NH}_3)_8(\text{OH})_2]^{3+}$ at pH = 7, and IV gives $[\text{Os}_2\text{N}(\text{NH}_3)_7(\text{OH})_3]^{2+}$ at pH = 9. That the ammine protons probably remain intact is supported by the independence of the spectra of I and II over the pH range 1-9. Alternate formulations for the deprotonated complexes in terms of oxo ligands are unlikely since higher Os oxidation states are required to stabilize the coexistence of powerful electron-donating nitrido and terminal oxo ligands.^{2-10,18-22} In comparison with Os(III) aqua complexes, the existence of the high formal oxidation state of the metal in III and IV does not lead to particularly acidic aqua ligands.²⁰⁻²³ Thus, like the oxo ligand,²¹ the nitrido ligand moderates the otherwise expected intense polarization of other ligands in the coordination sphere.

Electronic Spectra. As shown in Table I, the nitrido-bridged Os(IV) compounds display an intense absorption band in the UV region, as do mononuclear halo ammine complexes of Os(III) and Os(IV). Since the assignment of halogen-to-metal LMCT attributed to the bands of the mononuclear complexes¹⁸ cannot be adopted for III and IV, an alternative assignment was sought. A molecular orbital scheme for the isoelectronic oxo-bridged $\text{Ru}_2\text{Cl}_{10}\text{O}^{4-}$ ion has been proposed²⁴ and applied to the spectra of a number of oxo-bridged complexes.^{25,26} Following these workers, we assign the intense bands in Table I as the metal-centered π^n to π^* transitions (e_g to e_u^*). The orbitals involved in the Os-N-Os π -bonding are the d_{xz} and d_{yz} orbitals on the Os atoms and the p_x and p_y orbitals on the N atom. Our assignment is consistent with the decrease in energy of the transition on changing from an aqua ligand to Cl^- or OH^- since π -donation from the latter ligands would destabilize the d_{xz} and d_{yz} orbitals.

Electrochemistry. As shown by cyclic voltammetry in 5 N H_2SO_4 , the reversible IV,IV/IV,V 1e-oxidation couple ($\Delta E_p = 65$ mV) at 0.90 V vs NaSCE occurs for compound II, whereas irreversible oxidation at ca. 1.09 V vs NaSCE occurs for I. This contrasting behavior resembles results obtained with mononuclear osmium halo ammines, where the III/IV half-wave potentials increased as the ratio of ammine ligands to halogeno ligands was increased, with a transition to irreversible behavior occurring in the pentammine chloro case.¹⁸ A decrease in the cathodic current of the IV,IV/IV,V wave of II was observed relative to the anodic current as the acidity of the medium was decreased. Applying a method described earlier,¹⁸ we estimate that the pK_a of an ammine ligand on the 1e-oxidation product of II has the approximate value -1. This result represents a decrease in the pK_a of the ammine ligands of at least 10 units upon 1e oxidation of the metal, paralleling changes in ammine acidity on oxidation of the mononuclear Os(III) ammine complexes.¹⁸

DNA Staining. As described earlier,¹⁶ electron-microscopy tests showed that unpurified reaction products containing I and II plus unidentified components stained DNA with high contrast. Similar results were reported in the patent literature for a material obtained by an almost identical procedure except that anion metathesis was used to give the product as a nitrate salt.¹¹ Pure samples of I and II, however, showed little staining ability, as did the corresponding aqua complexes. Cation-exchange chromatography fractions containing primarily I also produced no significant staining. Thus, the active component of the DNA stain remains as yet unidentified. Although no useful DNA-staining behavior was found with complexes I-IV, the highly contrasting, specific DNA staining possible with unpurified mixtures represents strong motivation to continue to characterize this chemistry.

Acknowledgment. This research was sponsored by the U.S. Department of Energy under Contract No. DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., through the Exploratory Studies Program ("Seed Money") at Oak Ridge National Laboratory. This research was also supported in part by NSF Grant No. 8501261 (A.L.O.). We thank Dr. Donald E. Olins for suggestions made at the beginning of this project.

Supplementary Material Available: Figure 2, showing the UV absorbance changes of IV in the pH range 1-9, Figure 3, showing the absorbance data fitting for III, Table II, containing IR data for I and II, and a description of the SAS code containing experimental data and equations for pK_a calculations together with selected output (17 pages). Ordering information is given on any current masthead page.

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¹H NOE Studies on Dicopper(II) Dicobalt(II) Superoxide Dismutase

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Received December 28, 1988

Substitution of zinc(II) with cobalt(II) in copper zinc superoxide dismutase gives rise to a derivative suitable for ¹H NMR investigation owing to the magnetic coupling between the two metal ions. ¹H NOE measurements have been performed in order to relate them to interproton distances. Success has been obtained with pairs of protons less than 3.5 Å apart one from the other in the copper domain. The three signals for each histidine have been assigned on a firm basis and the implications for the interaction of anions with copper discussed.

Introduction

Bovine superoxide dismutase (SOD hereafter) is a dimeric enzyme, MW 32000, whose active site contains an heterodinuclear copper-zinc chromophore.^{1,2} The X-ray structure, solved at 2.0-Å

resolution, has shown that the copper ion is bound in a distorted five-coordinate geometry to a water molecule and to four histidines, one of them acting as a bridging ligand to the proximal zinc atom.³

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