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Reduction of Osmium(VIII) to Osmium(II) by Reaction with an Olefin and EDTA. Formation of an Os^{II}–EDTA Complex

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The complex $Na_6H_4[Os^{II}(EDTA)_3]$ is formed by reaction between osmium tetroxide, an olefin, and EDTA. The same complex is produced by reaction between potassium osmate and EDTA. The EDTA analogue, trans-*1,2-diaminocyclohexane-N,N,N′,N′-tetraacetate, forms a similar complex.*

When osmium tetroxide, an olefin, and ethylenediamine tetraacetate (EDTA) were allowed to react at 25 or 100 °C in aqueous solutions pH 4–6, we observed the formation of a compound which contained only osmium and EDTA. This compound was not formed, however, in the absence of the olefin. The electronic spectrum of the reaction mixture showed an initial appearance of a maximum at 460 nm which diminished with time and which was replaced by a maximum at 378 nm characteristic of the new complex. The complex was isolated following purification by gel chromatography. Elemental analysis of the complex agreed reasonably well with the formula: $Na_6H_4[Os(EDTA)_3]$ although the Na_7H_3 and Na_5H_5 species cannot be excluded.

The ir spectrum of this complex showed no evidence for osmium–oxygen linkages. Bands characteristic [1] of the *trans*-osmyl stretch (O=Os=O) near 830 cm^{-1}, of the Os=O stretch near 1000 cm^{-1}, and of the Os–O single bond stretch near 580 cm^{-1} were all absent. A set of bands appeared, however, in the 300–400 cm^{-1} region which were markedly different in shape and intensity from the Na_2H_2EDTA pattern [2] (Fig. 1). Osmium–ethylenediamine complexes show bands in this region [3]. This is also the region in which many metal–nitrogen stretches and deformations appear [4].

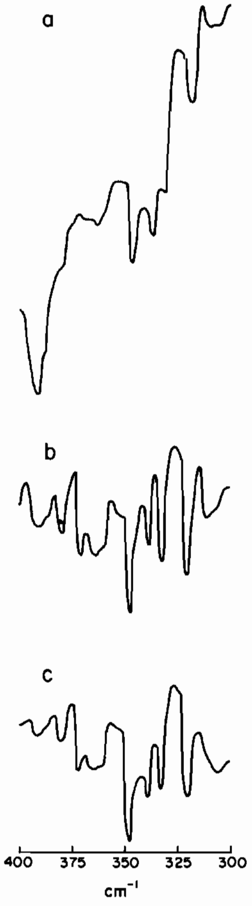

Figure 1. Low-frequency ir spectra: a) $Na_2H_2EDTA \cdot 2H_2O$. b) $Na_6H_4[Os(EDTA)_3]$. c) $Na_5H_5[Os(DCTA)_3]$. Absorption increases downwards.

The ^1H nmr spectrum in D_2O, in the pD range 3–12, showed only two sharp singlets corresponding closely to the chemical shifts characteristic of Na_2H_2EDTA. Only small shifts are observed for more typical di- and trivalent metal-ion–EDTA complexes [5]. The proton-decoupled ^{13}C nmr spectrum also suggests a single environment for the EDTA ligands in this complex since only three narrow resonances were observed at pD 6.5 in D_2O. We assume that the osmium is octahedrally bonded to all six nitrogens. The absence of line-broadening in both the ^1H and ^{13}C nmr spectra strongly suggests that the complex is diamagnetic as would be expected for an octahedral Os(II) species.

A few experiments were carried out to test the scope of the reaction. When N,N,N′,N′-tetramethylethylenediamine reacted with osmium tetroxide in the presence of an olefin, a complex formed which

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TABLE I. Vanadometric Titrations.

Compound	μ mol cmpd. taken	μ mol VO_3^- consumed	$\frac{\text{mol } VO_3^-}{\text{moles cmpd.}}$
$[Os(NH_3)_5N_2]Cl_2$[a]	8.98	36.5	4.06
	8.53	35.2	4.13
OsO_2[b]	9.36	18.3	1.96
$Na_6H_4[Os(EDTA)_3]$	5.80	24.4	4.21
	4.43	17.9	4.04
	4.51	18.0	3.99
$Na_5H_5[Os(DCTA)_3]$	3.86	14.6	3.78
	3.99	15.2	3.81
	4.43	16.0	3.61

[a] Ref. 14. [b] Ref. 10.

showed a strong band at about 835 cm^{-1} typical of the *trans*-osmyl stretch of an oxo-osmium(VI) ester [1] although we note that tetramethylethylenediamine has a band of medium intensity in this region. This complex gave a pink color with the thiourea–HCl reagent (see below). When, however, the EDTA analogue *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetate (DCTA) was used in a reaction with osmium tetroxide and cyclohexenecarboxylic acid, an osmium(II) complex was formed with properties similar to those of the EDTA complex, (Table I and Fig. 1), although in this case the Na_5H_5 species fits the analytical data better than the Na_6H_4 formulation. The Na^+/H^+ proportion is presumably merely a reflection of the pH of isolation. When nitrilotriacetic acid was used as the nitrogen component a light-blue complex was formed which did not titrate as an Os(II) species although its ir spectrum in the 300–400 cm^{-1} region was similar to the EDTA complex.

The oxidation state of the metal in the EDTA complex appears to be +2 on the basis of the following data. The compound does not oxidize iodide ions in acid solution. Oxidation is expected for a species containing osmium in an oxidation state of +5 or greater [6]. Incubation of the complex with thiourea in the presence of HCl does not give the distinctive pink color produced by osmium species with oxidation state +3 or greater [7]. Treatment of the Os–EDTA complex with chlorine in water produced a color change of brown to yellow–green. The oxidized complex had uv shoulders at 330 and 260 nm. These absorbances decreased and the characteristic maximum absorbance at 378 nm of the Os(II) complex reappeared when the oxidized complex was heated in water. The oxidized complex reacted with thiourea and HCl in water upon heating to give a pink color. These observations are consistent with the idea that chlorine oxidizes the Os(II) complex to an Os(III) species [8].

The complex titrates as an Os(II) species with Syrokomskii's metavanadate reagent [9]. Syrokomskii reported the use of this reagent to oxidize Os(IV) to Os(VI). We confirm this finding and report in addition its application to Os(II) species (Table I).

A stoichiometric study on the formation of the complex was carried out by following the reaction at 378 nm with varying amounts of EDTA and fixed quantities of OsO_4 and olefin. The results showed that four mol of EDTA were required per mol of OsO_4. Since only three mol of EDTA are found in the complex, we assume that the other mol serves as a reducing agent. In this regard, we have observed the evolution of CO_2 from the reaction mixture as shown by trapping it in the form of barium carbonate. The organic products of the reaction have not been investigated.

The molecular weight of the complex was estimated by vapor phase osmometry. An apparent molecular weight of 157 was found. The calculated value for the complex (six Na ions plus one complex anion) is 1197/7 = 171.

Fifty mg of the complex dissolved in 50 ml water to give a pH of 6.7. When this solution was acidified to pH3 and back-titrated with base, a pK_a was observed at pH 6.3 which corresponded to 1.09×10^{-4} mol of base. On the assumption of three equivalent carboxyl groups, this corresponds to MW = 1377 ± 140.

We view the role of the olefin in this reaction as a requirement to carry out an initial reduction to Os(VI). Ligand-free Os(VI) species undergo disproportionation reactions easily under mildly acidic conditions [10]. In agreement with this idea, we have observed the formation of the same Os(II)–EDTA complex at 100 °C in the absence of olefin from Os(VI), in the form of potassium osmate, and EDTA in acetate buffer, pH5. The same complex is also formed when maleic acid is employed as the olefin in the reaction between OsO_4 and EDTA.

Although it is convenient to carry out the reaction at 100 °C, the same complex forms readily at 25 °C. The maximum at 378 nm is observed in the electronic spectrum and tlc shows a major component with an identical R_f value. The reaction time at 25 °C, however, is much longer as would be expected. Isolated, purified yields were 70% at 100° and 30% at 25° after 24 hours.

The fact that these Os(II) complexes form easily at 25 °C may be important in understanding the complexities of the reactions which occur when osmium tetroxide is used to fix tissues for electron microscopic examination. White *et al.* [11] have recently used photoelectron spectroscopy to examine the oxidation states of osmium in such specimens. They find that in addition to the expected Os(VI) species, Os(IV) and Os(III) oxidation states are present as well. We note, however, that their reported difference in binding energy between the Os(II) reference compound and one of the Os(III) compounds is only 0.1 eV greater than the difference between the two Os(III) standards. It does not seem to us, therefore, that the data of White *et al.* [11] (see especially Fig. 4c and 6a) necessarily exclude the presence of substantial quantities of Os(II) species. The very recent report by Battistoni *et al.* supports this view [12].*

Experimental

Chemicals were reagent grade and were used without further purification. Carbon, hydrogen, and nitrogen analyses were carried out by Galbraith Laboratories as were molecular weight determinations (vapor phase osmometry). Vanadometric titrations were carried out following a slight modification of the procedure of Syrokomskii [9]. The bismuth reduction step was omitted. The osmium-containing compound was dissolved in 30 ml of 6 *N* sulfuric acid. A 3–4-fold excess of ammonium metavanadate (NH_4VO_3) solution in 10 ml of 6 *N* sulfuric acid was added. The remaining metavanadate was back-titrated after 24 h at room temperature using ferrous ammonium sulfate in 6 *N* sulfuric acid. The color change with N-phenylanthranilic acid was from red to green and sharp. Low-frequency infrared spectra were obtained (KBr) with a Beckman 4350 instrument. Electronic spectra were recorded on a Cary 118 spectrophotometer. Proton nmr spectra were taken on a Varian Associates T-60 instrument, and the ^{13}C spectrum on a Brucker 90. Osmium analyses were done by Daniel's modification [13] of the Criegee and Goldstein techniques. We found, however, that in contrast to the 30–60 min oxidation time which was satisfactory for the oxo-osmium(VI) standards, the Os–EDTA complex required about 8 h for complete oxidaton to OsO_4. These long reaction times result in some uncertainty in the osmium analyses and imply marked inertness of these complexes [14]. Sodium analyses were carried out by atomic absorption spectroscopy.

Synthesis of $Na_6H_4[Os(EDTA)_3]$

A 0.4 g (1.57×10^{-3} mol) sample of OsO_4 was dissolved in 20 ml of water. This solution was added to a solution of 2.34 g (6.28×10^{-3} mol) of Na_2H_2-EDTA and 0.2 g (1.59×10^{-3} mol) of 3-cyclohexene carboxylic acid (Aldrich) in 280 ml 0.1 *M* acetate buffer, pH 5.05. The solution was heated at 100 °C (oil bath) in a flask fitted with a reflux condenser. The solution, which was initially brown, changed to blue within fifteen minutes and then back to brown after approximately 90 min. The formation of blue intermediates in osmium reductions has previously been noted. It was also observed that at this point CO_2 evolution began. After 24 hr, the solvent was removed *in vacuo*, the dried residue powdered, and then extracted twice with boiling ethyl acetate. The suspension was filtered, dried, and 150 mg portions were dissolved in 3 ml of water and purified by chromatography on a Sephadex G-15 column (2.5 × 16 cm) using water as an eluant. The chromatography step was repeated several times in order to obtain pure product. The combined middle cuts of the single brown band upon drying (48 h, 56 °C, 25 mm, P_2O_5) yielded 1 g (70%) of the product. Tlc on silica gel using 60% ethanol–water as solvent showed a single spot, R_f 0.78. *Anal.* Calc. for $C_{30}H_{40}N_6O_{24}OsNa_6$: C, 30.1; H, 3.37; N, 7.02; Os, 15.9; Na, 11.5. Found: C, 30.33, 29.56; H, 3.88, 3.62; N, 7.43, 6.90; Os, 16 ± 1; Na, 10.5 ± 1%.

Ir (KBr): 3700–3100, 1625, 1400, 910 cm^{-1} (see Fig. 1)

Uv (H_2O): λ_{max} 378 nm ($\epsilon = 585 \pm 5$), 510 (sh) ($\epsilon = 120$)

pmr (D_2O): δ: 3.59 (s), 3.24 (s) ratio: 2:1 [internal acetone std.]

Synthesis of $Na_5H_5[Os(DCTA)_3]$

A 7.2×10^{-4} mol (0.262 g) sample of *trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid·H_2O (Aldrich) was dissolved in 15 ml of 0.1 *M* acetate buffer pH 5.05. To this solution a 1.8×10^{-4} mol (0.023 g) sample of 3-cyclohexene carboxylic acid was added with swirling. The pH was adjusted to 5.05 with 1 *N* NaOH. A 1.8×10^{-4} mol portion (0.046 g) of OsO_4 was dissolved in water (5 ml) and slowly added to the above mixture. The flask was fitted with

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*However, a related reaction has been reported by Saito *et al.* [12a] in which an osmium(III) complex, $H[OsCl_2H_2$-EDTA] is formed by heating H_4EDTA and $H_2[OsCl_6]$ in aqueous solution at 140 °C.

a reflux condenser and heated at 100 °C in an oil bath. The color of the solution went from brown, directly after mixing, to black. The reaction was allowed to proceed overnight, the solvent was removed *in vacuo*, and the residue was washed with hot ethyl acetate. After filtration and drying, the residue was dissolved in two ml of water and chromatographed on the Sephadex G-15 column using water as the eluant. The mixture separated into three bands: a black, a brick-red, and a light green band. Only the brick-red middle band, containing 200 mg (80%) was kept since the other two bands contained only minor amounts of material. Tlc on silica gel using 60% ethanol–water as solvent showed a single spot, R_f 0.67. This material was dried in the same way as the EDTA complex. *Anal.* Calc. for $C_{42}H_{59}N_6O_{24}OsNa_5$: C, 37.7; H, 4.45; N, 6.29. Found: C, 37.86; H, 5.03; N, 6.29%.

Ir (KBr): 3700–3100, 2930, 1625, 1400, 1110 (w), 980 (w), 915 (w) cm^{-1} (see Fig. 1).

Uv (H_2O) λ_{max} 383 nm, $\epsilon = 114$; 500 nm, $\epsilon = 72$.

Pmr (D_2O) δ: 3.57(A_2B_2), 3.13 (br.s.), 2.20–1.05 (m) ratio: 4:1:4.

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