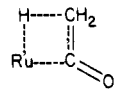
Monitoring by FTIR spectroscopy the reaction of equimolar amounts of $Ru(CO)_2$(triphos) (ν_{CO} = 1941, 1858 cm^{-1}) and acetyl chloride (ν_{CO} = 1803 cm^{-1}) in methylene chloride at room temperature resulted in observation of the smooth disappearance of the carbonyl stretching bands of the reactants and the smooth appearance to the two ν_{CO} of **3** (2057, 2010 cm^{-1}). Interestingly, a weak shoulder on the high-frequency side of the 2057-cm^{-1} band was observed to appear and remain throughout the reaction. Subtraction of the two ν_{CO} of **3** subsequently made possible the observation of two new, weak bands at 2085 and 2056 cm^{-1}. As the acetyl group is more electronegative than hydrogen, it seems reasonable to assign the weak bands to **2**, formed in a very low steady-state equilibrium concentration.

If these assignments are correct, the rate-determining step in the formation of ketene would be the conversion of **2** to products, a β-elimination reaction involving migration of hydrogen from carbon to ruthenium. A primary kinetic isotope effect might be expected, and accordingly a competition reaction was carried out between $Ru(CO)_2$(triphos) and a 2:1 mixture of CH_3COCl and CD_3COCl. The resulting ketenes were trapped as described above and treated with water, and the ratio of CH_3CO_2H to CD_2HCO_2H was determined mass spectrometrically. The ruthenium-containing products were also isolated, and the ratio of $[RuH(CO)_2(triphos)]^+$ to $[RuD(CO)_2(triphos)]^+$ was determined by ^1H NMR spectroscopy. The kinetic isotope effect obtained by these methods was 3.0 ± 1.0, certainly a primary isotope effect and consistent with a nonlinear C--H--Ru transition state[19] of the type expected for a β-elimination reaction, i.e.,



4

Indeed, although not very precise, the measured kinetic isotope effect is similar to kinetic isotope effects reported for reactions involving β-elimination of olefins from alkyl compounds of cobalt (2.30 ± 0.05),[20] iridium (2.28 ± 0.20),[21] and palladium (1.4 ± 0.1).[22] Again, a nonlinear C--H--M transition state is favored.[23]

Although elimination of ketenes from acetyl complexes appears to be unprecedented, there have been two reports of ketene insertions into metal–hydrogen bonds,[24,25] i.e.,

$$HMn(CO)_5 + CH_2{=}C{=}O \rightarrow MeCOMn(CO)_5$$

$$HCo(CO)_4 + R_1R_2C{=}C{=}O \rightarrow R_1R_2CHCOCO(CO)_4 \quad (4)$$

$$R_1, R_2 = H, Me, Et$$

In addition, we note a crystal structure of an acetyl compound, $Mo(COMe)(S_2CNMe_2)(CO)(PMe_3)_2$, in which there is a strong, attractive interaction between the metal atom and one of the hydrogen atoms of the acetyl group.[26] Although it has been suggested[26] that the structure provides a possible model for the transition state (or intermediate) of the migratory insertion of carbon monoxide into metal–carbon bonds, the structure possibly provides a model for the elimination reaction described above. Similar metal–β-CH interactions of alkylmetal compounds, reported in the literature, have been cited as models of the transition state for olefin β-elimination reactions.[27–29]

Acknowledgment. We thank the Natural Sciences and Engineering Research Council for financial support, Queen's University and the NATO Science Fellowship Program for scholarships awarded to S.I.H., and Johnson Matthey and the International Nickel Co. of Canada for loans of ruthenium trichloride. We are also much indebted to J. A. Stone for running the mass spectra.

Registry No. 3, 95123-22-1; $Ru(CO)_2$(triphos), 37843-33-7; $CH_3C(O)Cl$, 75-36-5; $CD_3C(O)Cl$, 19259-90-6; $[RuD(CO)_2(triphos)]Cl$, 95123-23-2; $CH_2{=}CO$, 463-51-4; D_2, 7782-39-0.

(19) Melander, L.; Saunders, W. H. In "Reaction Rates of Isotopic Molecules"; Wiley: New York, 1980; p 157.
(20) Ikariya, T.; Yamamoto, A. *J. Organomet. Chem.* **1976**, *120*, 257.
(21) Evans, J.; Schwartz, J.; Urquhart, P. W. *J. Organomet. Chem.* **1974**, *81*, C37.
(22) Ozawa, F.; Ito, T.; Yamamoto, A. *J. Am. Chem. Soc.* **1980**, *102*, 6457.
(23) Collman, J. P.; Hegedus, L. S. In "Principles and Applications of Organotransition Metal Chemistry"; University Science Books: Mill Valley, CA, 1980; p 294.
(24) Lindner, E.; Berke, H. *Z. Naturforsch. A*, **1974**, *29A*, 275.
(25) Ungvâry, F. *J. Chem. Soc., Chem. Commun.* **1984**, 824.
(26) Carmona, E.; Sânchez, L.; Mârin, J. M.; Poveda, M. L.; Atwood, J. L.; Priester, R. D.; Rogers, R. D. *J. Am. Chem. Soc.* **1984**, *106*, 3214.
(27) Dawoodi, Z.; Green, M. L. H.; Mtetwa, V. S. B.; Prout, K. *J. Chem. Soc., Chem. Commun.* **1982**, 802.
(28) Cracknell, R. B.; Orpen, A. G.; Spencer, J. L. *J. Chem. Soc., Chem. Commun.* **1984**, 326.
(29) As suggested by a referee, a transition state as in **4** requires a vacant site on the ruthenium. We doubt that phosphorus atom dissociation occurs during elimination and suggest that a CO dissociates but does not escape from the solvent cage before the ketene dissociates. Alternatively, reactions 3 and 4 may occur via radical processes as, apparently, can "insertion" reactions of dienes with some metal hydrides.[30] The significance of a primary kinetic isotope effect in the reverse reactions would be unclear.
(30) Fergusson, S. B.; Sanderson, L. J.; Shackleton, T. A.; Baird, M. C. *Inorg. Chim. Acta* **1984**, *83*, L45. Connolly, J. W. *Organometallics* **1984**, *3*, 1333. Thomas, M. J.; Shackleton, T. A.; Baird, M. C., unpublished results.

Mechanism of Cytochrome P-450 Catalysis. Mechanism of N-Dealkylation and Amine Oxide Deoxygenation

Leo T. Burka*

Toxicology Research and Testing Program
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina 27709

F. Peter Guengerich* and Randolf J. Willard

Department of Biochemistry and Center in Environmental Toxicology, Vanderbilt University
Nashville, Tennessee 37232

Timothy L. Macdonald*

Department of Chemistry, University of Virginia
Charlottesville, Virginia 22901

Received July 13, 1984

The cytochrome P-450 enzymes, which catalyze a variety of oxidative and reductive transformations, have received considerable attention with regard to their catalytic mechanisms.[1] These studies suggest that carbinolamine formation is the penultimate step in the mechanism for N-dealkylation, a representative oxidative heteroatom dealkylation process. The oxygen atom in the carbinolamine is derived from O_2;[2] however, the sequence of events leading to the carbinolamine intermediate has not been completely resolved. Relatively small *intermolecular* deuterium isotope effects have been observed for N-dealkylation indicating that breaking the α-carbon–hydrogen bond is not rate determining in the catalytic mechanism.[3–6] Moderate *intramolecular* isotope effects

(1) For leading references, see: (a) Guengerich, F. P.; Macdonald, T. L. *Acc. Chem. Res.* **1984**, *17*, 9–16. (b) Ullrich, V. *Top. Curr. Chem.* **1979**, *83*, 67–104. (c) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 3154–3156.
(2) (a) Shea, J. P.; Valentine, G. L.; Nelson, S. D. *Biochem. Biophys. Res. Commun.* **1982**, *109*, 231–235. (b) Kedderis, G. L.; Dwyer, L. A.; Rickert, D. E.; Hollenberg, P. F. *Mol. Pharmacol.* **1983**, *23*, 758–760.
(3) (a) Thompson, J. A.; Holtzman, J. L. *Drug Metab. Dispos.* **1974**, *2*, 577–582. (b) Nelson, S. D.; Pohl, L. R.; Trager, W. F. *J. Med. Chem.* **1975**, *18*, 1062–1065.
(4) (a) Miwa, G. T.; Walsh, J. S.; Kedderis, G. L.; Hollenberg, P. F. *J. Biol. Chem.* **1983**, *258*, 14445–14449. (b) Miwa, G. T.; Garland, W. A.; Hodshon, B. J.; Lu, A. Y. H.; Northrop, D. B. *J. Biol. Chem.* **1980**, *255*, 6049–6054. (c) Abdel-Monem, M. M. *J. Med. Chem.* **1975**, *18*, 427–430.
(5) Heimbrook, D. C.; Murray, R. I.; Egeberg, K.,D.; Sligar, S. G.; Nee, M. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1984**, *106*, 1514–1515.

0002-7863/85/1507-2549$01.50/0

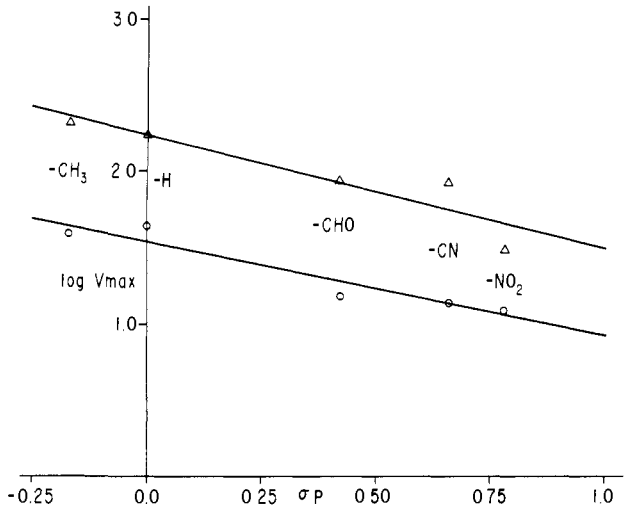


Figure 1. Correlation of log V_{max} for N-demethylation of para-substituted dimethylanilines by cytochrome P-450$_{PB-B}$ to ρ_{para}: (○) O_2/NADPH; (△) iodosylbenzene.

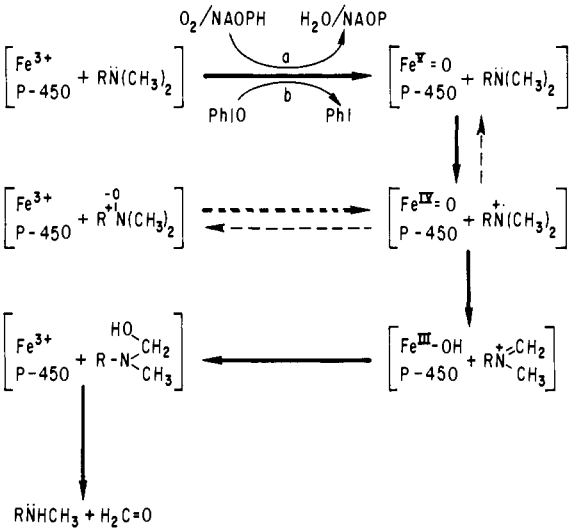
(k_H/k_D = 1.3–3.9)[4,6] imply that while hydrogen abstraction is not rate determining, there is discrimination in the α-CH bond cleavage. We,[1a,7] as well as others,[6,8] have suggested that these observations reflect the rate-determining formation of a nitrogen-centered radical cation intermediate.

To investigate the demethylation mechanism further, the rates of demethylation by highly purified cytochrome P-450$_{PB-B}$[9] of five para-substituted *N,N*-dimethylanilines were determined by monitoring the rate of formaldehyde production.[10] Two series of studies were done, one in which the oxidation was supported by O_2/NADPH and one in which the oxygen source was iodosylbenzene. The Michaelis–Menten parameters, V_{max} and K_m, for demethylation were determined.

Correlation of log V_{max} to the Hammett substituent parameter σ_{para} was found for both studies (Figure 1). In the O_2/NADPH-supported dealkylation, a correlation coefficient (r) of 0.953 was obtained, while r for the iodosylbenzene-supported process was 0.925. The slope (ρ) of the regression line in both cases was negative, −0.61 for the O_2/NADPH series and −0.74 for the PhIO series, indicating that the rate-determining step is facilitated by electron-donating substitutents. Another observation is that the magnitude of ρ is nearly identical in both studies, suggesting that the electron demand in the intermediate for both oxygenation processes in virtually the same. This observation is consistent with a common oxygenating intermediate (a formal Fe^V=O species) in both the O_2/NADPH- and PhIO-supported processes which then effects catalysis via identical pathways.[11]

Amine oxide deoxygenation by purified cytochrome P-450$_{PB-B}$ was also investigated as a result of our interest in the mechanism of heteroatom deoxygenation and its relation to the catalytic cycle of cytochrome P-450.[11,12] We found that cytochrome P-450$_{PB-B}$ formed formaldehyde from *N,N*-dimethylaniline *N*-oxide and its *p*-CH_3- and *p*-CN-substituted derivatives,[13] but the turnover number was no greater than 0.5 min^{-1} in any case. Moreover,

Scheme I



none of these three *N*-oxides supported the demethylation of aminopyrine, a readily demethylated cytochrome P-450 substrate, at rates ≥2 min^{-1}. These values may be compared to the V_{max} values of 83–209 min^{-1} observed for the demethylation of the *N,N*-dimethylaniline derivatives in the PhIO-supported reactions (Figure 1). These *N,N*-dimethylaniline *N*-oxides do not appear to have sufficiently high potentials to oxidize cytochrome P-450 to the formal Fe^V=O form in the manner that PhIO does.[14] None of the three *N,N*-dimethylaniline *N*-oxides were able to oxidize either Cr^{III} or Mn^{III}–*meso*-tetraphenylporphinato chloride[15] to detectable levels of the Cr^V=O or Mn^V=O complexes under the conditions described by Groves,[16] although PhIO did so stoichiometrically. Further, neither of these metalloporphyrins supported detectable conversion of any of the three *N*-oxides to formaldehyde. The results suggest that *N,N*-dimethylaniline *N*-oxides are not really able to oxidize ferric cytochrome P-450 (or Mn^{III} or Cr^{III}) to +V hypervalent states.

A mechanistic scheme for N-dealkylation and N-deoxygenation is presented in Scheme I in which the solid arrows represent major pathways and the dashed arrows minor pathways. In the O_2/NADPH- and iodosylbenzene-supported processes (path a and b, respectively), the perferryl species undergoes rate-determining single-electron transfer (SET) oxidation of the amine to generate a formal Fe^{IV}=O species and a nitrogen radical cation. Evidence that SET oxidation of the nitrogen atom is the initial step in *N*-dealkylation has been obtained from studies of the mechanism-based inhibition of cytochrome P-450 by cyclopropylamines,[7,8] from correlation of deuterium isotope effects for electrochemical and microsomal N-dealkylation of a series of amines[6,17] and from spin trapping experiments using dihydropyridines as substrates.[18] The next step in the mechanism, is hydrogen atom transfer from the substrate to the +IV cytochrome to generate a Fe^{III}–OH iminium ion pair. To reconcile the incorporation of the iron-bound oxygen into the derived carbinolamine,[2] the ion pair must collapse to form the carbinolamine without exchange.

We propose that amine oxide deoxygenation proceeds via a rate determining homolytic cleavage of a [Fe–O–N] complex to form the Fe^{IV}=O amine cation radical pair, which then undergoes

(6) Shono, T.; Toda, T.; Oshino, N. *J. Am. Chem. Soc.* **1982**, *104*, 2639–2641.
(7) Macdonald, T. L.; Zirvi, K.; Burka, L. T.; Peyman, P.; Guengerich, F. P. *J. Am. Chem. Soc.* **1982**, *104*, 2050–2052.
(8) Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048–2050.
(9) Enzyme (cytochrome P-450$_{PB-B}$) purification and reconstitution; Guengerich, F. P.; Dannan, G. A.; Wright, S. T.: Martin, M. V.; Kaminsky, L. S. *Biochemistry* **1982**, *21*, 6019–1030.
(10) Formaldehyde Assay: Nash, T. *Biochem. J.* **1953**, *55*, 416–421. Cochin, J.; Axelrod, J. *J. Pharmacol. Exp. Ther.* **1959**, *125*, 105–115.
(11) Macdonald, T. L.; Burka, L. T.; Wright, S. T.: Guengerich, F. P. *Biochem. Biophys. Res. Commun.* **1982**, *194*, 620–625.
(12) Burka, L. T.; Thorsen, A.; Guengerich, F. P. *J. Am. Chem. Soc.* **1980**, *102*, 7615–7616.
(13) Prepared by hydrogen peroxide oxidation of the corresponding dimethylanilines and characterized by NMR and mass spectra.
(14) An alternate explanation—that the amine oxide cannot enter the active site of the enzyme—appears unlikely: Guengerich, F. P. *J. Med. Chem.* **1984**, *27*, 1103–1108.
(15) Prepared as described: Summerville, D. A.; Jones, R. D.; Hoffman, B. M.; Basolo, F. *J. Am. Chem. Soc.* **1977**, *99*, 8195–8202.
(16) Groves, J. T.; Kruper, W. J., Jr.; Haushalter, R. C. *J. Am. Chem. Soc.* **1980**, *102*, 6377–6380.
(17) Shono, T.; Hamaguchi, H.; Matsumura, J. *J. Am. Chem. Soc.* **1975**, *97*, 4264–4268.
(18) Augusto, O.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 11288–11295.

N-dealkylation. A small percentage of this pair may produce the perferryl species and free amine and thereby enable oxidation of alternate substrates. However, direct two-electron oxidative cleavage of the [Fe–O–N] complex to generate a formal Fe^{V}=O species and an amine appears incompatible with the low oxidation potential of amine oxides and with the mechanism proposed for the reverse reaction—amine oxygenation.

Acknowledgment. This work was supported by Grants ES01590, ES00267, and ES03181.

Supplementary Material Available: Table of Michaelis–Menten parameters (1 page). Ordering information is given on any current masthead page.

Decaammine(μ-dinitrogen-*N*,*N*')diosmium(III): Synthesis, Characterization, Reactivity, and Spectral Properties

Peter A. Lay, Roy H. Magnuson, and Henry Taube*

Department of Chemistry, Stanford University Stanford, California 94305

James Ferguson* and Elmars R. Krausz

Research School of Chemistry The Australian National University Canberra 4, ACT 2601, Australia

Received May 21, 1984

The electrochemical oxidation of the decaammine(μ-dinitrogen-*N*,*N*')diosmium(5+) ion (I) shows that the 6+ ion (II) has a short half-life in aqueous solution.[1] Disproportionation of II with cleavage of the N≡N bond would result in two nitridoosmium(VI) species, which makes this ion potentially interesting in relation to dinitrogen fixation. Species II is also interesting because it features rather strong metal–metal interactions, either directly or through the dinitrogen bridging ligand.

We have found that $[(NH_3)_5OsN_2Os(NH_3)_5]Cl_6$ is readily produced by solid-state oxidation of I with chlorine. The oxidation is rapid (~15 min for completion) and is readily observed as the solid changes color from an emerald green to light blue. The reaction has been shown to go virtually to completion (>95%) by microanalytical data[2] and by IR spectral measurements. Although the complex slowly decomposes in the solid state with the reemergence of the color of the mixed-valence ion, it is moderately stable and may be stored for weeks at 0 °C in the absence of water and light.

The mixed-valence ion I was prepared by the action of a reducing agent on an aqueous mixture of $[Os(NH_3)_5OH_2]^{3+}$ and $[Os(NH_3)_5N_2]Cl_2$,[1] by partial oxidation of $[Os(NH_3)_5N_2]Cl_2$ in deaerated CF_3SO_3H,[3,4] or by the reaction of $[Os(NH_3)_5N_2]^{2+}$ with $[Os(NH_3)_5(OSO_2CF_3)]^{2+}$ in nonaqueous solvents.[4] Cyclic voltammetry in 2 M HCl at a carbon paste electrode shows reversible 5+/4+ and 6+/5+ couples respectively at −0.16 and +1.05 V vs. the NHE, leading to a comproportionation constant (K_{com}) of 10^{21}. These values agree well with those reported previously[1] in 0.1 M HCl (−0.16, ~+1.04 V, K_{com} ~10^{20}). At scan rates >10 V/s, we find that the 6+/5+ couple is almost reversible, a result that is at variance with that of previous measurements[1] (but, it should be noted that these were made at a different acid concentration). At slower scan rates (<1 V/s) a new wave grows at 0.58 V, which corresponds to the $[Os(NH_3)_5N_2]^{3+/2+}$ couple, with concomitant diminution of the reduction wave of II. The electrochemical experiments provide no evidence for the reduction of dinitrogen. In searching further for dinitrogen reduction, I was oxidized to II in 2 M HCl with a stoichiometric amount of Ce^{4+} at 5 °C, using a hand-operating stopped-flow mixer.[5] The solution immediately changed color from green to the light blue characteristic of II.[6] The concentration of II decayed, following first-order kinetics with a half-life of 40 min at 5 °C, to yield a mixture containing I, $[Os(NH_3)_5N_2]^{2+}$, and unidentified osmium species. Chromatographic and UV–vis measurements indicated that I was the major product (>70%). The qualitative features of the stoichiometry can be accounted for by assuming that the unidentified products are comparised of osmium species in oxidation states above 3+. The possibility that a small amount of N≡N cleavage occurs is not excluded by our observations.

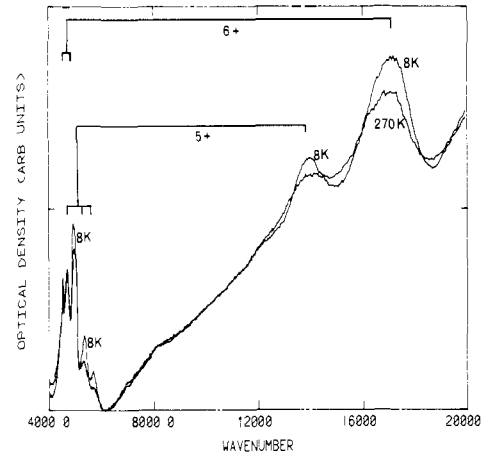


Figure 1. Electronic absorption spectra of the 6+ and 5+ ions in a KCl disk, measured at 270 and 8 K. The sharp line at 4500 cm^{-1} is ascribed to H_2O. The rising background is due to scattering.

The frequency of the dinitrogen stretch in the IR of II (2200 cm^{-1}) is much greater than that of I (2010 cm^{-1}), but still significantly lower than that of the free dinitrogen (2330 cm^{-1}), and is close to that observed for the mononuclear ions (2212 cm^{-1}).[7] The results indicate that both the mononuclear and binuclear Os(III) complexes are stabilized by π-back-bonding, but less than in I or the mononuclear 2+ ion. These results are in agreement with other chemical, structural, and spectral results on Os(III) complexes, all of which suggest[8-10] that Os(III), unlike Ru(III), is a rather good π-donor.

The coupling of osmium(VI) nitrido complexes is the reverse of the sought for disproportionation of a (μ-dinitrogen)diosmium(III) species to nitridoosmium(VI); it has been observed[11,12] (for compositions somewhat different from ours), and thus the reaction pathway, forward and reverse, has been demonstrated. Our failure to observe disproportionation of II to an ammine-nitridoosmium(VI) species cannot be taken to mean that the reaction is thermodynamically unfavorable. The principle of microscopic reversibility makes no statement about the rate of the disproportionation relative to others that are possible, and the failure to observe substantial N≡N bond cleavage may simply

(1) Richardson, D. E.; Sen, J. P.; Buhr, J. D.; Taube, H. *Inorg. Chem.* **1982**, *21*, 3136–3140.

(2) Anal. Calcd for $H_{30}N_{12}Cl_6Os_2$: C, 0.00; H, 3.82; N, 21.24; Cl, 26.88. Found: C, 0.00; H, 3.79; N, 19.72; Cl, 26.91. The low nitrogen figure found is a function of the automatic analysis (Lay, P. A.; Sargeson, A. M.; Skelton, R. H.; White, A. H. *J. Am. Chem. Soc.* **1982**, *104*, 6161–6164).

(3) Lay, P. A.; Magnuson, R. H.; Sen, J.; Taube, H. *J. Am. Chem. Soc.* **1982**, *104*, 7658–7659.

(4) Lay, P. A.; Magnuson, R. H.; Taube, H., manuscript in preparation.

(5) Similar in design to that reported by: Inoue, Y.; Perrin, D. D. *J. Phys. Chem.* **1962**, *66*, 1689–1693.

(6) Visible absorption spectrum, 2 M HCl, 5 °C (λ, nm (ε, M^{-1} cm^{-1})): 460 sh (340); 514 (1580); 600 (1760); 660 (1840); 794 sh (360).

(7) Buhr, J. D.; Taube, H. *Inorg. Chem.* **1980**, *19*, 2425–2434.

(8) The structure of $[(NH_3)_5OspzOs(NH_3)_5]Cl_6 \cdot 2H_2O$[9] indicates back-bonding in Os–N(p_z) bonds; $[Os(NH_3)_5(CH_3CN)]^{3+}$ is remarkably stable to hydrolysis[10] of nitrile in comparison to the Ru(III) analogue.

(9) Bino, A.; Lay, P. A.; Taube, H., manuscript in preparation.

(10) Lay, P. A.; Magnuson, R. H.; Taube, H., unpublished results.

(11) Buhr, J. D.; Taube, H. *Inorg. Chem.* **1979**, *18*, 2208–2212.

(12) Ware, D. C.; Taube, H., manuscripts in preparation.

0002-7863/85/1507-2551$01.50/0