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Nickel(III) Complexes of Histidine-Containing Tripeptides and Bleomycin. Electron Spin Resonance Characteristics and Effect of Axial Nitrogen Donors

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The Ni(III) complexes of various histidine-containing tripeptides and bleomycin show electron spin resonance spectra which are characteristic of tetragonal geometry ($g_{xx}, g_{yy} > g_{zz}$). The Ni(III) complexes with sulfhydryl groups in equatorial positions exhibit larger g_{xx} values and more rhombic symmetry than those with the amino ligands. The trend in equatorial donor strength is in the order $S^- > NH_2$. The 1:1 bleomycin–nickel(III) complex gives an electron spin resonance spectrum with nearly axial symmetry ($g_{xx} \simeq g_{yy}$) and five-line hyperfine splittings in the g_{zz} region, indicating that two nitrogen atoms are coordinated in the axial positions. In the present Ni(III) complexes, as the number of axial nitrogen ligands increases, the g_{xx}, g_{yy} values decrease and the g_{zz} value increases.

Introduction

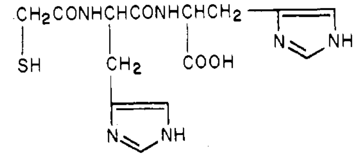
Bleomycin (BLM), which was originally isolated as a Cu(II) complex from a culture of *Streptomyces verticillus*,[1,2] is a histidine-containing glycopeptide antibiotic for the treatment of selected human neoplastic diseases.[3] The antibiotic has both metal-binding[4] and DNA-interacting sites,[5] and its biological activity is probably related to this bifunctionality. We have already reported a stable Cu(II) complex, the oxygen adduct of the Co(II) complex, and the iron(II)–nitrosyl complex of BLM.[6] In fact, the histidine portion of BLM was found to be an important metal-binding site.

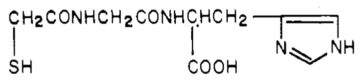
The Ni(III) has been considered to be a relatively rare oxidation state, and pulse radiolysis has been used to generate the Ni(III) complexes of ethylenediamine and glycine in an aqueous solution.[7] Recently, Margerum and his collaborators showed that Ni(III) complexes of oligopeptides containing glycine are easily oxidized by electrochemical or chemical methods to form the corresponding Ni(III) complexes and that the Ni(III) could be expected to form in biological systems.[8] They have characterized the Ni(III) state by cyclic voltammetry[9] and by electronic, circular dichroic,[10] and particularly electron spin resonance spectroscopy.[8] Tetragonally distorted octahedral geometry is the most common, although square-planar geometry[11] has been also observed.

Herein, the Ni(III) complexes of various histidine-containing peptides have been investigated by electron spin resonance (ESR) spectroscopy. The axial coordination of the BLM ligand, the characteristics of equatorial sulfur coordination, and the correlation between g_{av} and g_{zz} values are discussed on the basis of the present ESR results.

Experimental Section

N-Mercaptoacetyl-DL-histidyl-DL-histidine (MAHH) and *N*-mercaptoacetylglycyl-L-histidine (MAGH) were synthesized by using a previously reported method.[12] Their oxidized compounds (disulfide), ox-MAHH and ox-MAGH, were prepared by the treatment of MAHH and MAGH with H_2O_2 in an aqueous solution and checked by elemental analysis and proton nuclear magnetic resonance spectroscopy.[13] Purified bleomycin A_2 (BLM-A_2) and its biosynthetic intermediate (P-3A) were kindly supplied from Nippon Kayaku Co. Ltd. Glycylglycyl-L-histidine (GGH) and sodium hexachloroiridate(IV) ($Na_2IrCl_6 \cdot 6H_2O$) were purchased from Sigma and Alfa, respectively. A stock solution of Ni(II) prepared from nickel nitrate was standardized by titration with EDTA.


MAHH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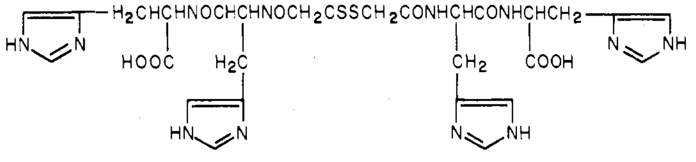
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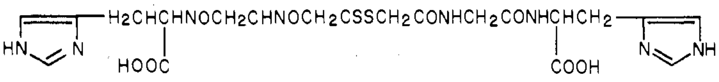
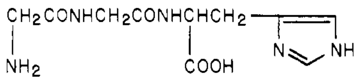

ox-MAGH


GGH

The 1:1 Ni(III) complexes of these histidine-containing peptides were prepared in aqueous solution by oxidation of the corresponding Ni(II) complexes[12] with $Ir^{IV}Cl_6^{2-}$,[8] and the solutions were immediately frozen in liquid nitrogen. Although the Ni(III) complexes of MAHH and MAGH containing sulfhydryl group were remarkably unstable, the rate of decomposition of other nickel(III)–oligopeptide complexes was relatively slow. X-Band electron spin resonance (ESR) spectra of magnetically dilute aqueous glasses containing the Ni(III) complexes ($<10^{-3}$ M) were measured at 77 K by using a JES-FE-3X spectrometer operating at 100 kHz magnetic field modulation. The g values were determined by taking Li–TCNQ ($g = 2.0026$) as a standard, and the magnetic fields were calculated by the splitting of Mn(II) in MgO ($\Delta H_{3-4} = 86.9$ G).

Results and Discussion

Ni(III) Complexes of Histidine-Containing Tripeptides. Figure 1 shows the ESR spectra for the Ni(III) complexes of MAHH, MAGH, and their disulfide compounds at 77 K. All ESR spectra are consistent with Ni(III) in a tetragonal geometry ($g_{xx}, g_{yy} > g_{zz}$) rather than square-planar geometry ($g_{zz} > g_{xx}, g_{yy}$).[8] It is known that a tetragonally distorted octahedral geometry is the most common for the Ni(III) state. In the Ni(III) complexes of MAHH and ox-MAHH, in fact, the three-line hyperfine splittings in the g_{zz} region strongly suggest a species which has a single nitrogen nucleus (^{14}N, $I = 1$) bound in an axial position. Presumably, water occupies the other axial coordination site. For the 1:1 Cu(II) complexes

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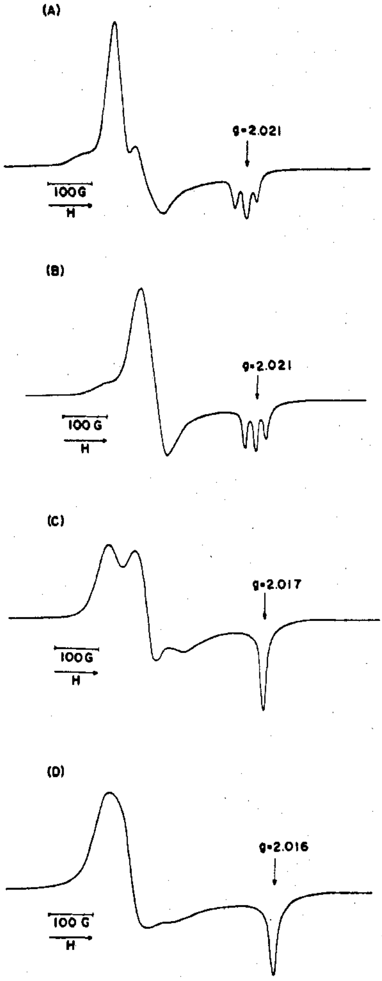


Figure 1. ESR spectra for Ni(III) complexes at 77 K of (A) MAHH, (B) ox-MAHH, (C) MAGH, and (D) ox-MAGH.

of MAHH and MAGH, the square-pyramidal and square-planar configurations, respectively, have been already proposed on the basis of the ESR results.[12] Bossu et al.[8] observed that the g_{xx} value increases with increasing strength of the equatorial binding and that the general trend in the g values reflects the trend in donor strength of the functional groups: $N^- > NH_2 > Im \sim COO^-$. The Ni(III) complexes with sulfhydryl sulfur as equatorial ligands showed larger g_{xx} values and more rhombic symmetry than those with the corresponding amino nitrogen, suggesting the order of $S^- > NH_2$ in the equatorial donor strength. The present result corresponds well to the previous observation that the exchange of a termal amino group by a sulfhydryl group gives a blue shift of about 25 nm in the d–d transition of Cu(II) and that the trend in the strength of the ligand field around the central Cu(II) is in the order $S^- > NH_2$.[12]

Effect of Additional Nitrogen Donors. The addition of *N*-methylimidazole (*N*-MeIm) or ammonia to the Ni(III) complexes of MAHH and GGH resulted in pronounced ESR spectral changes (see Figure 2). In the case of the MAHH–Ni(III) complex, the spectral feature in the g_{xx}, g_{yy} region was transformed from an anisotropic to isotropic pattern and the hyperfine splitting in the g_{zz} region changed from three lines into five lines. The ammonia adduct of the GGH–Ni(III) complex also displayed an ESR spectrum which has five-line hyperfine splittings in the g_{zz} region and nearly axial symmetry ($g_{xx} \simeq g_{yy}$). In both complexes, the ratio of relative amplitudes of the five-line pattern was approximately 1:2:3:2:1, indicating axial coordination of two magnetically equivalent nitrogen nuclei toward Ni(III).

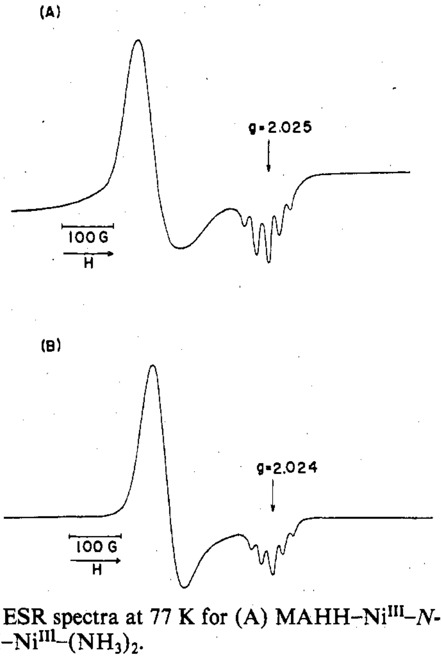


Figure 2. ESR spectra at 77 K for (A) MAHH–Ni^{III}–*N*-MeIm and (B) GGH–Ni^{III}–(NH$_3$)$_2$.

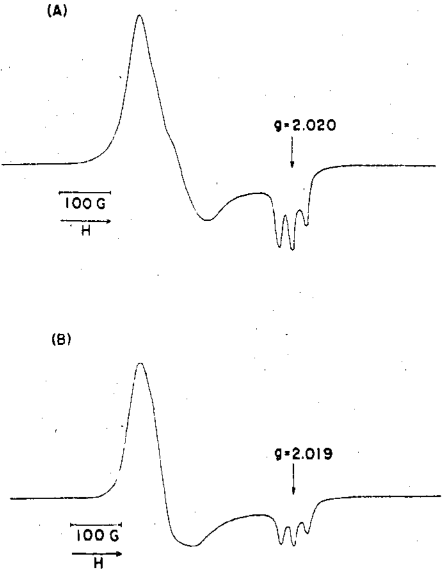


Figure 3. ESR spectra at 77 K for the proposed (A) (ox-MAGH)$_2$–Ni^{III} and (B) (GGH)$_2$–Ni^{III} complexes.

The ESR spectra of the 2:1 Ni(III) complexes of ox-MAGH and GGH clearly show three-line hyperfine splittings in the g_{zz} region, in contrast with those of their 1:1 Ni(III) complexes (see Figure 3). This result suggests a single nitrogen bound in the axial position and chelation of a second peptide molecule in these bis complexes. It has been observed that Ni(III) complexes with five or more nitrogen donors are slower to undergo self-decomposition reactions and that the initial Ni(III) complexes of oligopeptides such as glycylglycylglycinamide and glycylglycylglycine also are stabilized by chelation of a second oligopeptide to form bis complexes with five nitrogen donors.[8] Ni(III) complexes with axial nitrogen coordination are known to be both thermodynamically and kinetically more stable than those with axial water molecules.[8,14]

Characteristics of Ni(III) Complexes of Bleomycin and Its Biosynthetic Intermediate. The oxidation of the 1:1 Ni(II) complexes of BLM and its biosynthetic intermediate (P-3A) in aqueous solution also yielded paramagnetic products characterized as Ni(III) complexes by their ESR spectra (see Figure 4). The Ni(III) complex of BLM gave an ESR

Table I. ESR Parameters for Ni(III) Complexes of Histidine-Containing Peptides and Bleomycin

<table>
<tr><th>no.</th><th>complex</th><th>g_{xx}</th><th>g_{yy}</th><th>g_{zz}</th><th>g_{av}</th><th>A^N, G</th><th>no. of lines at g_{zz}</th></tr>
<tr><td>1</td><td>MAHH-Ni^{III}</td><td>2.264</td><td>2.196</td><td>2.021</td><td>2.160</td><td>23.8</td><td>3</td></tr>
<tr><td>2</td><td>MAHH-Ni^{III}-*N*-MeIm</td><td colspan="2">2.175</td><td>2.025</td><td>2.125</td><td>22.4</td><td>5</td></tr>
<tr><td>3</td><td>MAHH-Ni^{III}-NH_3</td><td colspan="2">2.168</td><td>2.025</td><td>2.120</td><td>22.4</td><td>5</td></tr>
<tr><td>4</td><td>ox-MAHH-Ni^{III}</td><td>2.237</td><td>2.176</td><td>2.021</td><td>2.145</td><td>23.8</td><td>3</td></tr>
<tr><td>5</td><td>MAGH-Ni^{III}</td><td>2.274</td><td>2.206</td><td>2.017</td><td>2.167</td><td></td><td>1</td></tr>
<tr><td>6</td><td>ox-MAGH-Ni^{III}</td><td>2.240</td><td>2.284</td><td>2.016</td><td>2.179</td><td></td><td>1</td></tr>
<tr><td>7</td><td>(ox-MAGH)$_2$-Ni^{III}</td><td>2.170</td><td>2.220</td><td>2.020</td><td>2.140</td><td>23.8</td><td>3</td></tr>
<tr><td>8</td><td>GGH-Ni^{III}</td><td>2.254</td><td>2.277</td><td>2.016</td><td>2.182</td><td></td><td>1</td></tr>
<tr><td>9</td><td>GGH-Ni^{III}-$(NH_3)_2$</td><td colspan="2">2.162</td><td>2.024</td><td>2.116</td><td>22.0</td><td>5</td></tr>
<tr><td>10</td><td>(GGH)$_2$-Ni^{III}</td><td>2.177</td><td>2.218</td><td>2.019</td><td>2.138</td><td>23.8</td><td>3</td></tr>
<tr><td>11</td><td>BLM-A_2-Ni^{III}</td><td colspan="2">2.169</td><td>2.027</td><td>2.122</td><td>22.4</td><td>5</td></tr>
<tr><td>12</td><td>P-3A-Ni^{III}</td><td>2.235</td><td>2.163</td><td>2.022</td><td>2.140</td><td>23.5</td><td>3</td></tr>
</table>

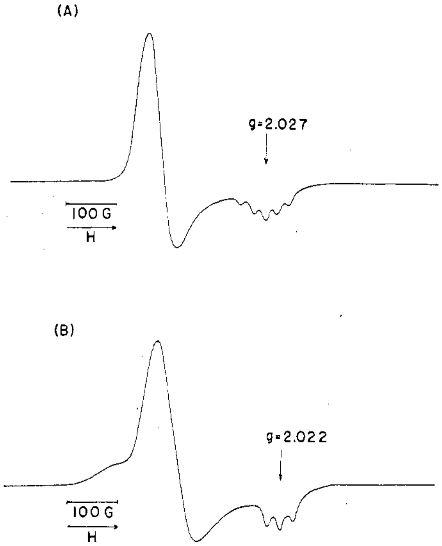
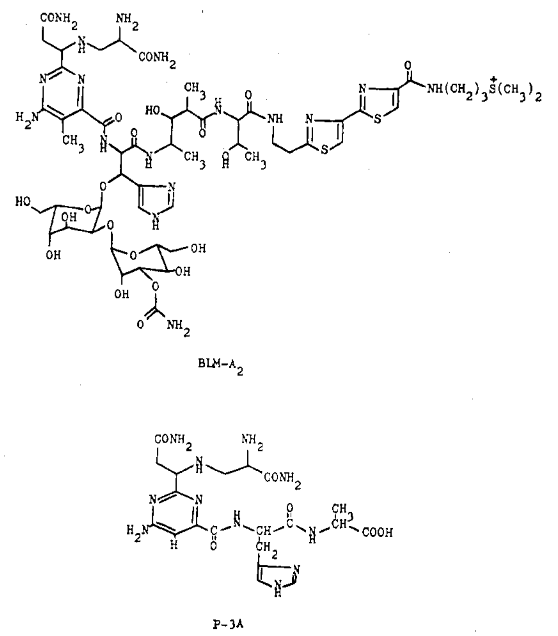


Figure 4. ESR spectra at 77 K for Ni^{III} complexes of (A) BLM-A_2 and (B) P-3A.

Chart I



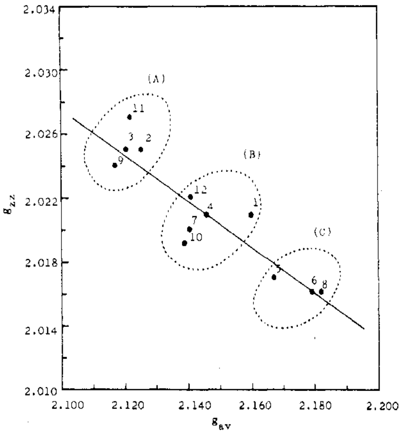


Figure 5. Correlation between g_{zz} and g_{av} values in the Ni(III) complexes of various histidine-containing peptides. The numbers represent the Ni(III) complexes shown in Table I.

spectrum with $g_{\perp} > g_{\parallel}(g_{zz})$ and five-line hyperfine splittings in the $g_{\parallel}$ region. The g_{xx} and g_{yy} values are approximately equal and are not resolved. On the other hand, the ESR spectrum of the 1:1 P-3A–Ni(III) complex showed g_{xx}, $g_{yy} > g_{zz}$ and three-line hyperfine patterns in the g_{zz} region. These results indicate species which have two and one nitrogen nuclei bound in the axial position, respectively, for the Ni(III) complexes of BLM and P-3A. P-3A is structurally related to BLM but lacks the sugar and bithiazole portions of BLM (see Chart I). The α-amino nitrogen atom of the β-aminoalanine portion is known to be the fifth axial coordination donor for the Cu(II) and Co(II) complexes of BLM.[6] The ESR spectrum of the 1:1 BLM–Co(II) complex clearly showed the three-line superhyperfine splittings from one axial ^{14}N atom, and then the 1:1 BLM–Co(II) complex also formed the oxygen adduct as demonstrated by the typical ESR spectrum of the monooxygenated low-spin Co(II) complex.[6] In addition, a recent X-ray crystallographic analysis for the 1:1 P-3A–Cu(II) complex clarified a distorted square-pyramidal structure which involves the secondary amine, the pyrimidine ring nitrogen, deprotonated peptide nitrogen of the histidine residue, and the histidine imidazole groups as planar donors and the α-amino group as the axial donor.[15] On the other hand, the sixth axial coordination of the carbamoyl group of the sugar has been presumed for the 1:1 BLM–Cu(II) complex.[6] Therefore, the fifth axial nitrogen donor for the Ni(III) complexes of BLM and P-3A is probably the α-amino nitrogen of β-aminoalanine portion, and the sixth nitrogen coordination donor for the BLM–Ni(III) complex may be the amide nitrogen of the carbonyl group in the sugar. The space-filling molecular model also supports a tetragonal configuration with axial nitrogen coordinations for the 1:1 BLM–Ni(III) complex.

Correlation between g_{av} and g_{zz} Values in Ni(III) Complexes of Histidine-Containing Tripeptides. Table I summarizes the ESR parameters for the Ni(III) complexes of various histidine-containing peptides and BLM. As the extent of the axial nitrogen coordination increases, the g_{xx}, g_{yy} values decrease and the g_{zz} value increases. Figure 5 shows the correlation between the g_{av} and g_{zz} values in the present Ni(III) complexes. In particular, the g_{zz} value is sensitive to the number of axial nitrogen donor atoms. The g_{zz} values of the Ni(III) complexes with two, one, and no axial nitrogen donors are approximately 2.025, 2.020, and 2.016, respectively. On the other hand, the g_{av} values are approximately 2.12, 2.14, and 2.17, respectively. As the binding in the axial direction increases, the energy of the d_{z^2} orbital increases relative to d_{xy}, d_{yz} and vibronic mixing of the d_{z^2} and $d_{x^2-y^2}$ ground states increases. Therefore, the g_{xx}, g_{yy} values decrease and the g_{zz} value increases as the complex approaches octahedral geometry.

In conclusion, the 1:1 Ni(II) complexes of various histidine-containing tripeptides are easily oxidized by Ir^{IV} ion to form the corresponding Ni(III) complexes. These Ni(III) complexes show ESR spectra which are typical of tetragonal geometry. The large g_{xx} value of the sulfur–nickel(III) complexes suggests the trend of $S^- > NH_2$ in the equatorial donor strength for Ni(III). The 1:1 Ni(III) complex of BLM, a histidine-containing glycopeptide antibiotic, has five-line hyperfine splittings in the g_{zz} region, indicating the presence of two axial nitrogen donor atoms. In the present Ni(III) complexes, the g_{zz} value is sensitive to axial nitrogen donors and a good correlation between the g_{av} and g_{zz} values is observed for axial nitrogen coordination.

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Registry No. Ni(III), 22541-64-6; MAHH, 66516-10-7; ox-MAHH, 69278-34-8; MAGH, 66516-06-1; ox-MAGH, 69278-35-9; GGH, 7451-76-5; BLM-A_2, 11116-31-7; P-3A, 68846-43-5.

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Organobis(dioximato)cobalt(IV) Complexes: Electron Paramagnetic Resonance Spectra and Electronic Structures

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Organobis(dioximato)cobalt(III) complexes can be oxidized either chemically (Br_2, PbO_2, or cerium(IV) nitrate) or electrochemically to generate radical cations, $[RCo(DH)_2L]^+$. One-electron oxidations have been confirmed by spectral titrations and cyclic voltammetry. While such radical cations are short-lived at 25 °C, they are quite stable at low temperatures (<−50 °C). $[RCo(DH)_2L]^+$ is paramagnetic exhibiting unusual EPR parameters ($g_{iso} = 2.0197$ to 2.0326, $g_{\parallel} = 2.016$ to 2.037, $g_{\perp} = 2.022$ to 2.031; $A_{iso}{}^{Co} = 11.5 \times 10^{-4}$ to 14.5×10^{-4} cm^{-1}, $A_{\parallel}{}^{Co} = 25.0 \times 10^{-4}$ to 35.0×10^{-4} cm^{-1}, $A_{\perp}{}^{Co} = 4.0 \times 10^{-4}$ to 5.5×10^{-4} cm^{-1}). Ligand superhyperfine parameters indicate that electron delocalization is small. Hyperfine equations are derived for a low-spin $3d^5$ electronic configuration with a $3d_{x^2-y^2}$ ground state. To account for the unusually small magnitude of $A_{\parallel}{}^{Co}$, $A_{\perp}{}^{Co}$ 3d/4p orbital mixing is incorporated into the hyperfine equation derivation. The experimentally observed $A_{\parallel}{}^{Co}$ and $A_{\perp}{}^{Co}$ values can be accommodated by this description by assuming that the orbital of the unpaired electron is approximately 70% 3d and 30% 4p in character. The EPR results support the formulation of these radical cations as organocobalt(IV) complexes.

Introduction

Considerable interest has recently been focused on the cleavage of transition-metal–carbon bonds. The formation and subsequent breaking of metal to carbon bonds is involved both in catalytic reactions[1] and in the biochemical reactions associated with vitamin B_{12} coenzymes.[2] Several modes for such cleavage reactions have already been described, including homolysis,[3] photolysis,[4] and reductive dealkylation.[5] However, the mechanism of oxidative dealkylation has only recently been investigated.[6] The chemistry of organomercurials[7] suggests that halogens attack the metal–carbon bond by electrophilic displacement with retention of configuration at the α carbon. The mechanistic aspects of cleavage of transition-metal alkyls are less clear and, at least in certain cases (e.g., $RCo(DMH)_2L$ where DMH_2 is dimethylglyoxime), inversion occurs at the α-carbon atom.[8-10] Anderson et al.[8] have suggested that in such cases the electrophile does not directly attack the α carbon but instead oxidizes the metal complex to a radical–cation, with metal–carbon bond cleavage resulting from subsequent nucleophilic attack at the α carbon thus explaining the observed inversion of configuration. Halpern et al.[11] have shown that $IrCl_6^{2-}$, a one-electron outer-sphere oxidant, oxidizes $RCo(DH)_2L$[12] according to the following scheme:

$$[RCo(DH)_2(H_2O)] \xrightarrow[1]{IrCl_6^{2-}} [RCo(DH)_2(H_2O)]^+ \xrightarrow[2]{X^-} Co^{II} + RX \quad (1)$$

A detailed examination of step **2** of the above sequence[6] has confirmed that Cl^- does induce the nucleophilic decomposition of $[RCo(DH)_2(H_2O)]^+$ and that cleavage of the cobalt–carbon

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