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Bis(2,2′-(alkylimino)diethanolato)cobalt(III) Chelates. Characterization of Hydrolytic Stability and Interactions with Acetylcholinesterase

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Evreev and co-workers report[1-4] the preparation and structural characterization of several cobalt(III) chelates, including the bis(2,2′-(alkylimino)diethanolato)cobalt(III) sodium salts

$$Na\{[RN(CH_2CH_2O)_2]_2Co^{III}\}$$

where R = methyl, propyl, allyl, and the like. The title compounds are inherently interesting and novel. In a practical context, however, interest in the (alkylimino)diethanolato chelates derives from reports by Evreev et al.[5-8] that bis(2,2′-(methylimino)diethanolato)cobalt(III), $Co(MIDE)_2$, bis(2,2′-(allylimino)diethanolato)cobalt(III), $Co(AIDE)_2$, and related compounds possess antidotal properties in the therapy of intoxication by the organophosphorus pesticide dimethyl dichlorovinyl phosphate (DDVP).

DDVP and related organophosphorus esters owe their toxicity to phosphorylation of a serine hydroxyl at the active site of acetylcholinesterase, AChE (reaction 1), where EOH is

$$(RO)_2P(O)OR' + E\text{–}OH \rightarrow E\text{–}OP(O)(OR)_2 + R'OH \quad (1)$$

active enzyme and $EOP(O)OR)_2$ is inactive, phosphorylated enzyme.[9-11] Although phosphorylated AChE is inert to aqueous hydrolysis, incubation of inhibited enzyme with powerful nucleophiles (such as oximes) restores enzymatic activity via S_N2 attack on phosphorus (reaction 2).

$$EOP(O)(OR)_2 + RCHNOH \rightarrow EOH + RCHNOP(O)(OR)_2 \quad (2)$$

Although 2-((hydroxyimino)methyl)-1-methylpyridinium iodide (2-PAM) and other AChE "reactivators" are used clinically to treat anticholinesterase agent poisoning,[11-14] previous attempts to employ metal complexes for reactivating inhibited AChE have failed.[15,16] Thus the claims of Evreev et al., if verified, could establish the foundation for an entirely new approach to therapy of organophosphorus ester intoxication.

Observations[17,18] that various cobalt(III) chelates catalyze the hydrolysis of organophosphorus esters lend credence to the claims of Evreev et al. regarding antidotal properties for $Co(MIDE)_2$ and $Co(AIDE)_2$. However, we were concerned that the reported[1,2] hydrolytic instability of $Co(MIDE)_2$ and $Co(AIDE)_2$ in alkaline and acidic solutions might extend to physiological pH and thereby limit the activity of these complexes as AChE reactivators. Accordingly we have undertaken a brief examination of the hydrolysis of $Co(MIDE)_2$ and $Co(AIDE)_2$ at near-neutral pH and of the interactions of these two chelates with AChE and phosphorylated AChE.

Experimental Details

Materials. The following were used as supplied by the manufacturer: 3-(*N*-morpholino)propanesulfonic acid, MOPS (Sigma); diisopropyl phosphorofluoridate, DFP, 2-((hydroxyimino)methyl)-1-methylpyridinium iodide, 2-PAM, bis(2-hydroxyethyl)methylamine, and bis(2-hydroxyethyl)amine (Aldrich); lyophilized electric eel acetylcholinesterase (Worthington). We prepared $[Co(NH_3)_5Cl]Cl_2$ by the method of Schlessinger[19] and bis(2-hydroxyethyl)allylamine by the method of Ford-Moore, Lidstone, and Waters.[20]

$Na\{Co[CH_3N(C_2H_4O)_2]\}\cdot 6H_2O$. A mixture of 2 equiv of bis(2-hydroxyethyl)methylamine with 1 equiv of $[Co(NH_3)_5Cl]Cl_2$ and 10 equiv of NaOH were dissolved in a minimum of water and heated 20 h at 60 °C, by the method of Evreev and Golub.[1] The resulting crystalline material was dissolved in 1 M NaOH, precipitated by addition of concentrated NaOH solution, and dried in a vacuum desiccator. Infrared spectrum (Nujol mull): 3300 (s, br), 1650 (m), 1385 (vw), 1158 (s), 1093 (m), 1074 (w, sh), 1010 (sh), 998 (m), 932 (m), 925 (m), 884 (w), 775 (w, sh), 723 (m) cm^{-1}. Anal. Calcd for $C_{10}H_{34}N_2O_{10}CoNa$: C, 28.31; H, 8.08; N, 6.66; Co, 13.89; Na, 5.42. Found: C, 28.22; H, 7.93; N, 6.47; Co, 13.69; Na, 5.74.

$Na\{Co[C_3H_5N(C_2H_4O)_2]_2\}\cdot 4H_2O$. Following the method described above for $Na\{Co[CH_3N(C_2H_4O)_2]_2\}\cdot 6H_2O$, we were unable to isolate the bis(allylamino)diethanolato chelate in pure form. Repeated attempts to crystallize the desired product by addition of concentrated NaOH solution yielded solid material contaminated with NaOH. The UV–visible (see Results section) and infrared spectra (below) and C/H, C/N ratios from elemental analysis (below) indicated the presence of the desired chelate. However, the C, H, N analyses were low by a constant amount. Because of the difficulties associated with purifying this chelate, kinetic studies focused on the bis(methylimino)diethanolato chelate described above. Infrared spectrum (Nujol mull): 3250 (vs, br), 1640 (m, br), 1370 (sh), 1340 (w), 1287 (m), 1158 (w), 1092 (m, sh), 1075 (m), 1031 (w), 1000 (ms), 973 (w), 931 (s), 915 (ms), 723 (m) cm^{-1}. Anal. Calcd for $C_{12}H_{30}N_2O_8CoNa$: C, 34.97; H, 7.34; N, 6.79. Found: C, 31.8; H, 6.51; N, 5.01.

Methods. We obtained NMR spectra (in D_2O solvent, $Me_3SiCH_2CO_2Na$ internal reference, δ 0.0) with a Varian Model XL-100-15 spectrometer and visible spectra with a Perkin-Elmer Model 575 spectrophotometer. We followed enzyme kinetics in 1-cm path length cuvettes using a Gilford Model 2000/Beckman DU spectrophotometer equipped with a four-position sample changer. Unless otherwise noted, all experiments were conducted at 25 ± 0.5 °C.

Details of the experimental procedures for enzyme inhibition and assay were as previously reported.[21] Briefly, the enzyme was incubated with excess DFP for 3 min to completely inhibit AChE activity. Following this, the inhibited enzyme was separated from unreacted DFP by gel permeation chromatography and incubated with test compounds (2-PAM, $Co(MIDE)_2$, or $Co(AIDE)_2$) for timed intervals prior to dilution and colorimetric assay for activity. For the examination of the interaction of $Co(MIDE)_2$ and $Co(AIDE)_2$ with non-

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Table I. Time-Dependent Spectral Changes at 25 °C and pH 7.6 for $Co(MIDE)_2$ in Aqueous Solutions Containing Various Buffers and Salts

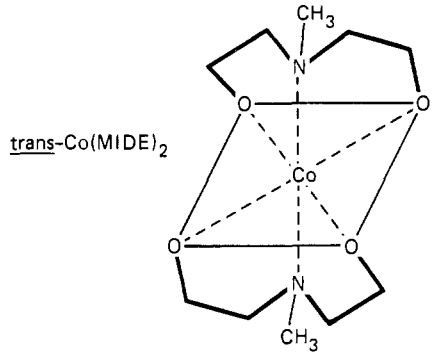
$10^3[Co(MIDE)_2]$, M	buffer (concn, M)	salt (concn, M)	product[a] designation	λ_{max}, nm	10^4k_5,[b] s^{-1}	10^5k_6,[c] s^{-1}	remarks
0.70	MOPS (0.1)	$NaClO_4$ (0.5)	I	564	0.93	3.1	red to green color change
			II				green color fades
5.2	MOPS (0.1)	MOPS (0.5)	I	563	1.00	3.5	red to green color change
			II				green color fades
15.0	MOPS (0.1)	$NaClO_4$ (0.5)	I	563	1.1		red to green color change
			III				λ_{max} shifts to 580 nm
0.71	NaH_2PO_4 (0.1)	$NaClO_4$ (0.5)	I	568	0.52		red to green color change
			IV				green precipitate formed
0.79	MOPS (0.1)	$NaClO_4$ (0.5), NaCl (0.1)	I	572	0.59	5.9	red to green color change
			II				green to colorless solution

[a] See eq 5 and 6 for definitions. [b] Calculated according to eq 7. [c] Calculated according to eq 8.

inhibited AChE, the test compounds were incubated for timed intervals with active enzyme. Following this the solutions were assayed for activity either with or without 40-fold dilution.

Results and Discussion

Physical Properties. $Co(MIDE)_2$ and $Co(AIDE)_2$ were prepared and isolated as the hydrated sodium salts as described by Evreev and Golub.[1] Visible spectra for $Co(MIDE)_2$ and $Co(AIDE)_2$ dissolved in distilled water exhibit the absorption maxima and extinction coefficients (nm (M^{-1} cm^{-1})) 526 (45), 690 (9.2) and 535 (42), 690 (ϵ not determined), respectively. These compare with values (nm (M^{-1} cm^{-1})) of 533 (52) for $Co(MIDE)_2$ and 541 (54), 694 (14) for $Co(AIDE)_2$ as reported by Evreev and Golub.[1] The spectra for both chelates are consistent[2,22] with octahedral geometry and transcoordinated nitrogen as shown for $Co(MIDE)_2$.



The sodium salts of $Co(MIDE)_2$ and $Co(AIDE)_2$ are basic; 10^{-2} M solutions of the chelates in water exhibit pH 10.6. This is due to equilibria for protonation and deprotonation of coordinated ligand oxygens, as shown in eq 3 and 4.

$$Co[RN(C_2H_4O)_2]_2 \overset{K_1}{\rightleftharpoons} [RN(C_2H_4O)_2]Co[RN(C_2H_4O)(C_2H_4OH)] \quad (3)$$

$$[RN(C_2H_4O)_2]Co[RN(C_2H_4O)RN(C_2H_4OH)] \overset{K_2}{\rightleftharpoons} [RN(C_2H_4O)_2]Co[RN(C_2H_4OH)_2] \quad (4)$$

Potentiometric titration of $Co(MIDE)_2$ in water gives $pK_1 = 7.7 \pm 0.06$ and $pK_2 = 4.7 \pm 0.19$. Thus in near-neutral solution $Co(MIDE)_2$ exists as a mixture of the fully deprotonated complex (shown on the left-hand side of eq 3) and the monoprotonated species shown on the right-hand side of the equation. $Co(AIDE)_2$ is expected to behave similarly. For conciseness, the abbreviations $Co(MIDE)_2$ or $Co(AIDE)_2$ are used below without reference to the degree of protonation of the chelates.

Reactions in Aqueous Solution. Evreev and Golub[1,2] report that $Co(MIDE)_2$ and $Co(AIDE)_2$ are unstable in acid (12 M HCl) or alkaline (pH 10–11) aqueous solution.

We examined the hydrolysis of $Co(MIDE)_2$ at pH 7.6 as a function of chelate concentration, buffer, and added salts. Table I gives the results of these experiments.

These results can be generally described by eq 5 and 6,

$$Co(MIDE)_2 \xrightarrow{k_5} I \quad (5)$$

$$I \xrightarrow{k_6} \text{II, III, or IV} \quad (6)$$

where I is the single green ($\lambda_{max} \approx 565$ nm) reaction product formed initially under all conditions, and II, III, and IV are different products formed in the slower, second stage of the reactions. Reactions 5 and 6 follow first-order kinetics, with rate constants determined according to eq 7 and 8. For eq

$$k_5t = \ln [(A_\infty - A_0)/(A_\infty - A_t)] \quad (7)$$

$$k_6t = \ln [(A_0 - A_\infty')/(A_t - A_\infty')] \quad (8)$$

7, A_0 is the absorbance of I at 620 nm immediately after solution preparation (the analytical wavelength being selected to avoid shifts in λ_{max} in 520–600-nm region), A_t is the absorbance at time t, and A_∞ is the absorbance after many half-lives. Values of A_∞ were adjusted to account for the slow disappearance of I. For eq 8, A_0 is the absorbance at λ_{max} (≈565 nm) for I, measured after complete disappearance of starting material. Absorbance of I at various times is given at A_t, and at long reaction times by A_∞'.

Table I shows that k_5 is independent of chelate concentration over the range (0.7–5) × 10^{-3} M, as required for a first-order reaction. The value of $k_5 = (1.01 \pm 0.09) \times 10^{-3}$ s^{-1} for $Co(MIDE)_2$ in MOPS buffer falls slightly to (0.52–0.59) × 10^{-3} s^{-1} for the chelate in phosphate buffer or for the chelate in MOPS buffer plus added NaCl. Also, the absorption maximum for I shifts to slightly longer wavelength in phosphate or MOPS plus NaCl solution. The small changes in rate constant and absorption spectrum are consistent with medium or ion-pairing effects on the reaction. Reaction 8, which is an order of magnitude slower than reaction 7, gives products that are quite dependent on the initial chelate concentration and on added salts, suggesting the occurrence of various anation reactions.

A mechanism consistent with these observations includes complete loss of one 2,2′-(methylimino)diethanol ligand in reaction 5 to give the hydrolysis product I = $[CH_3N(C_2H_4O)_2]Co(OH_2)_3$ (where, as before, no distinction is made between the various protonated and deprotonated species), followed by substitution reactions, reaction 6, to give II–IV = $[CH_3N(C_2H_4O)_2]Co(OH_2)_2X$, where X = PO_4^-, Cl, or the like.

In accordance with the S_N1 mechanism generally accepted for cobalt(III) complex substitution reactions,[23] reaction 5

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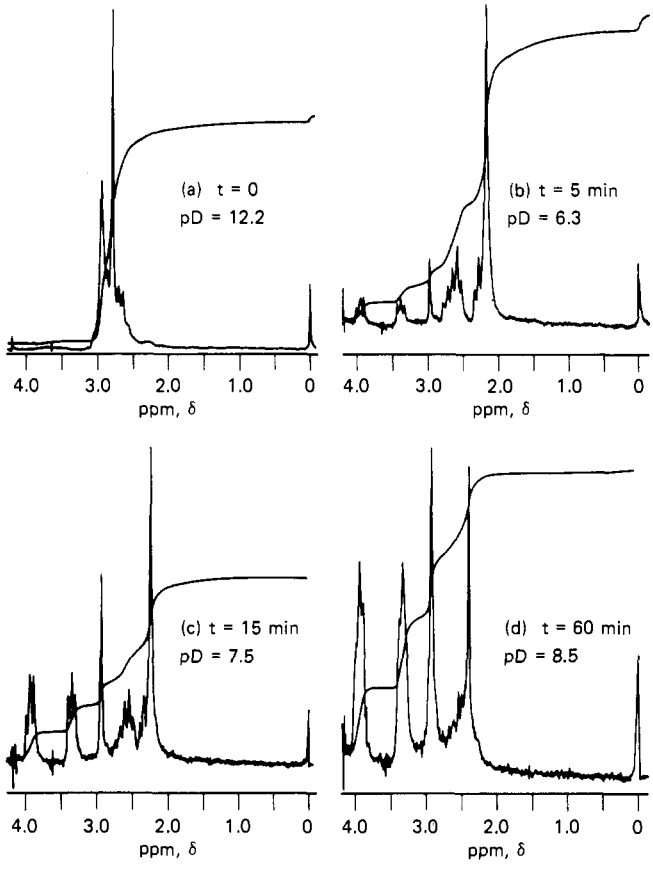

Figure 1. NMR spectrum (100 MHz) of $Co(MIDE)_2$ in D_2O at 25 °C as a function of time.

should exhibit first-order kinetics and be independent of added salt because of the high concentration of solvent water.

Although we did not identify the products of reaction 6, we did attempt to establish the mechanism of reaction 5 by confirming the production of free ligand using NMR spectroscopy. To do this, we first prepared a 1.0×10^{-1} M solution of $Co(MIDE)_2$ in D_2O (pD 12.1, where pD = pH + 0.4) and recorded the 100-MHz spectrum. We then added $DClO_4$ to pD 6.3 and recorded spectra at 5, 15, and 60 min. We also noted the pD of the solution at each time point. Figure 1 gives these data with the NMR spectra.

At pD 12.2 the spectrum is characterized by a broad multiplet at δ 2.5–3.1 with a CH_3 singlet at δ 2.8. On acidification to pD 6.3 (Figure 1b), the $Co(MIDE)_2$ spectrum shows the CH_3 resonance at δ 2.2 superimposed on a multiplet at δ 2.0–2.4. This multiplet is assigned to methylene protons α to coordinated N. A similar multiplet, perhaps a pair of superimposed triplets, appears at δ 2.5–2.8 and is assigned to methylene protons α to coordinated oxygen. The nonequivalence of the individual protons in either methylene group is rationalized in terms of anisotropy due to a rigid conformation of the coordinated ligands.

Figure 1b also reveals a small singlet at δ 2.9 and triplets at δ 3.4 and 3.9. These resonances are identical with proton signals in the spectrum of uncoordinated 2,2′-(methylimino)diethanol in D_2O at pD 7.

In Figure 1c,d the increase in the free 2,2′-(methylimino)diethanol protons with time is clearly seen and is consistent with hydrolytic cleavage of ligand from the complex. The increase in pD of the medium as the reaction progresses is explained simply by the liberation of base (i.e., free amine).

To further prove the mechanism, we repeated the NMR experiment, except that $DClO_4$ was continuously added to the reaction mixture to maintain the acidity at pD 7.6 ± 0.1. At each time point we integrated the proton signals (δ 2.9, 3.4, and 3.9) for noncoordinated 2,2′-(methylimino)diethanol as well as the total proton signal. Table II gives these data and the fraction, F_t, of total 2,2′-(methylimino)diethanol present as the noncoordinated ligand.

Table II. Time-Dependent Changes in the NMR Spectrum of $Co(MIDE)_2$ in D_2O at 25 °C, pD 7.6

time, s	integrated proton signal, arbitrary units				F_t[a]
	δ 3.9	δ 3.4	δ 2.9	total protons	
0	0.71	1.10	0.51	19.9	0.12
600	1.21	1.71	0.81	20.6	0.18
1200	1.00	1.39	1.09	13.0	0.26
1800	1.35	1.80	1.81	13.8	0.36
2400	2.41	2.91	1.85	21.2	0.34
3000	2.10	2.50	1.85	14.9	0.43
3600	2.50	2.80	2.32	16.7	0.46
4200	4.00	4.10	3.85	21.2	0.56

[a] F_t is the fraction of total 2,2′-(methylimino)diethanol present as noncoordinated ligand.

Table III. Inhibition of Acetylcholinesterase by $Co(MIDE)_2$ at 25 °C, pH 7.6

10^5(incubation concn of chelate), M	10^5(assay concn of chelate), M	incubation time, h	enzyme activity,[a] % of control
25.0	0.60	2	68
25.0	0.60	24	37
50.0	1.2	2	75 ± 2
50.0	1.2	24	45
125.0	3.1	2	64 ± 5
125.0	3.1	24	56
0.10	0.10	2	100 ± 4
1.0	1.0	2	105 ± 3
10.0	10.0	2	92 ± 2
100.0	100.0	2	58 ± 3

[a] Error limits indicate ±SD from the mean value of two determinations.

From the table it is clear that F_t increases with time and that it approaches a limiting value of $F_t = 0.51 \pm 0.07$ (averaged for the points at t = 3600 and 4200 s). This indicates that precisely half the coordinated ligand is hydrolyzed in 1 h, as required by the proposed mechanism.

A plot (not shown) of ln $[(0.51 - F_t)/(0.51)]$ vs. time is linear with slope $(0.53 \pm 0.08) \times 10^{-3}$ s^{-1}, in good agreement with the value of $k_5 = (0.52\text{–}1.0) \times 10^{-3}$ s^{-1} determined from visible spectra (Table I). Thus we conclude that the $Co(MIDE)_2$ chelate is labile at 25 °C, pH 7.6, and that the inherent instability is due to total hydrolytic cleavage of one ligand from the complex.

Acetylcholinesterase Inhibition. Before attempting to employ $Co(MIDE)_2$ and $Co(AIDE)_2$ as reactivators of phosphorylated acetylcholinesterase, we examined the possibility of direct inhibition of enzyme activity by the chelates themselves.

To differentiate between reversible and irreversible inhibition of the enzyme, we incubated acetylcholinesterase with low concentrations ($(0.1\text{–}100) \times 10^{-5}$ M) of $Co(MIDE)_2$ or $Co(AIDE)_2$ for 2 h and assayed for activity directly and also incubated the enzyme for 2 or 24 h with high concentrations ($(25\text{–}125) \times 10^{-5}$ M) and diluted 40-fold for assay. Table III gives the results of the $Co(MIDE)_2$ experiments. $Co(AIDE)_2$ gave entirely analogous results, and we omitted these from Table III for conciseness.

The data show that $Co(MIDE)_2$ indeed inhibits acetylcholinesterase and that the degree of inhibition can be quite pronounced (e.g., 63% inhibition for 25×10^{-5} M $Co(MIDE)_2$ after 24 h). Moreover, the inhibition is progressive with respect to time and is not completely reversed by dilution. For example, 2-h incubation and assay with 1.0×10^{-5} M $Co(MIDE)_2$ gave 105 ± 3% of control activity, but incubation

for the same period of time with 50×10^{-5} M chelate followed by 40-fold dilution resulted in 75 ± 2% of control activity. In the same experiment, incubation with 50×10^{-5} chelate for 24 h yielded 45% of control activity.

These results are in marked contrast to observations made with various metal ions,[24,25] copper and nickel chelates,[15,26] and lanthanide ions,[16,27] all of which reversibly inhibit the enzyme. The irreversible inhibition exhibited by $Co(MIDE)_2$ and $Co(AIDE)_2$ suggests chemical modification of the enzyme. This, in turn, may be due to hydrolysis of the chelates to species that are highly reactive. $(H_2O)_6Co^{III}$, for example, is an extremely powerful oxidant, capable even of oxidizing water,[22,28] and if $(H_2O)_6Co^{III}$ or similar species are formed from hydrolysis of the (2,2′-(alkylimino)diethanolato)cobalt(III) chelates, it seems likely that sulfhydryl or other sensitive functional groups on the enzyme could be susceptible to oxidation with concomitant loss of enzyme activity.

Acetylcholinesterase Reactivation. We concluded our investigation by determining the degree to which $Co(MIDE)_2$ and $Co(AIDE)_2$ restore activity to AChE inhibited by DFP. In these experiments AChE was incubated with excess DFP, separated from unreacted inhibitor, and diluted immediately into solutions containing 20×10^{-5} or 100×10^{-5} M $Co(MIDE)_2$, $Co(AIDE)_2$, or 2-PAM. Aliquots were then withdrawn at 2-, 4-, and 24-h intervals and assayed for activity. At 20×10^{-5} and 100×10^{-5} M, 2-PAM restored AChE to greater than 96% of control activity for all incubation intervals; $Co(MIDE)_2$ and $Co(AIDE)_2$, however, were completely inactive as reactivators of AChE. In 12 experiments with the two chelates, AChE activity remained at 0.21 ± 0.2% of control activity for all concentrations and incubation periods investigated. Thus the chelates do not significantly restore activity to phosphorylated AChE under conditions where 2-PAM effectively reactivates inhibited enzyme.

Conclusions

$Co(MIDE)_2$ and $Co(AIDE)_2$ are unstable in near-neutral aqueous solution, hydrolytic cleavage of one of the two ligands proceeding at 25 °C with a half-life of 10–20 min depending on the reaction medium. The chelates or their solvolysis products irreversibly inhibit acetylcholinesterase, perhaps via oxidation of sulfhydryl or other sensitive functional groups on the enzyme.

Neither $Co(MIDE)_2$ nor $Co(AIDE)_2$ exhibit significant activity as reactivators of diisopropyl phosphorylacetylcholinesterase. These observations cast doubt on published claims[5-8] that $Co(MIDE)_2$ and $Co(AIDE)_2$ function as reactivators of dimethyl phosphorylacetylcholinesterase. We cannot attest to the claimed[5-8] antidotal efficacy of the chelates in dimethyl dichlorovinyl phosphate poisoning. However, our in vitro results with $Co(MIDE)_2$ and $Co(AIDE)_2$ largely exclude the possibility that any therapeutic properties derive from reactivation of inhibited acetylcholinesterase.

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Registry No. $Na[Co(MIDE)_2]$, 59246-62-7; $Na[Co(AIDE)_2]$, 53770-42-6; $[Co(NH_3)_5Cl]Cl_2$, 13859-51-3; AChE, 9000-81-1.

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Crystal and Molecular Structure of $PPN[HRu_4(CO)_{13}]$

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The water-gas shift reaction ($CO + H_2O \rightleftharpoons CO_2 + H_2$) has been found to be catalyzed by many different metal carbonyl complexes, both mono- and polynuclear.[1] The ruthenium carbonyls are clearly some of the most studied systems,[1a,b,g] and several mechanisms have been proposed to account for the observations. These proposals emphasize the intermediacy of trinuclear or tetranuclear clusters.[1a,g] In one scheme[1a] the species $[HRu_4(CO)_{13}]^-$ is formed by CO substitution for H_2 in $[H_3Ru_4(CO)_{12}]^-$. The monohydrido cluster is believed to react with H_2O, giving $[H_2Ru_4(CO)_{12}(COOH)]^-$, which loses CO_2, regenerating $[H_3Ru_4(CO)_{12}]^-$.

The cluster $[HRu_4(CO)_{13}]^-$ has been found to be quite reactive,[2] and the determination of its molecular structure has not been reported. We have prepared $[HRu_4(CO)_{13}]^-$, by a serendipitous route starting with the isocyanato cluster $[Ru_4(NCO)(CO)_{13}]^-$, and report here its crystal and molecular structure.

Experimental Section

All reactions were carried out under an atmosphere of prepurified nitrogen, and the solvents were distilled under nitrogen from appropriate drying agents. The common reagents were obtained from the usual sources, and $Ru_3(CO)_{12}$ was synthesized by a literature procedure[3] with modifications suggested by Bradley.[4] $PPN[Ru_4(NCO)(CO)_{13}]$ was prepared from the reaction of $Ru_3(CO)_{12}$ and $PPN(N_3)$ as reported elsewhere.[5]

Synthesis of $PPN[HRu_4(CO)_{13}]$. A stock solution of KOH in methanol (0.099 M) was prepared, and 2.5 mL of this solution was deoxygenated and added to a 25-mL methanol solution of $PPN[Ru_4(NCO)(CO)_{13}]$ (80 mg, 0.059 mmol). After 20 min of stirring at room temperature, the methanol was removed under vacuum. The brown residue was extracted with ether (20 mL), giving a deep red solution. The volume of ether was reduced to 10 mL, and slow diffusion of hexane into the ether resulted in the formation of dark red crystals of $PPN[HRu_4(CO)_{13}]$ (9.1 mg, 12% yield).

The ether-insoluble material was extracted with tetrahydrofuran (20 mL); the resulting solution was concentrated to 15 mL and layered with 30 mL of hexane. Reddish brown crystals of $(PPN)_2[Ru_4(CO)_{13}]$ formed slowly (39 mg, 35% yield).

Collection and Reduction of the X-ray Data. A red crystal of $PPN[HRu_4(CO)_{13}]$ was mounted on a glass fiber and found to be triclinic by the Enraf-Nonius CAD4-SDP peak search, centering, and indexing programs and by a Delauney reduction calculation.[6] The centrosymmetric space group $P\bar{1}$ was chosen and data collection begun. Successful refinement of the structure verified the choice of this space group. A summary of the crystal data is presented in Table I. The intensity data were measured with ω–2θ scans. Background counts of duration equal to one-fourth of the scan time for each peak were measured at the two ends of the scan range. In this manner, the total

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(4) Bradley, J., personal communication.
(5) Fjare, D. E.; Jensen, J. A.; Gladfelter, W. L. *Inorg. Chem.*, in press.
(6) All calculations were carried out on PDP 8A and 11/34 computers using the Enraf-Nonius CAD 4-SDP programs. This crystallographic computing package is described in: Frenz, B. A. In "Computing in Crystallography"; Schenk, H., Olthof-Hazekamp, R., van Konigsvel, H., Bassi, G. C., Eds.; Delft University Press: Delft, Holland, 1978; pp 64–71. See also: "CAD 4 and SDP User's Manual"; Enraf-Nonius: Delft, Holland, 1978.

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