Contribution from the Department of Chemistry,
University of Maryland, College Park, Maryland 20742

Preparation and Characterization of Chromium(III) and Cobalt(III) Complexes of Adenosine 5′-*O*-(1-Thiodiphosphate)

IRENE LIN, AMY HSUEH, and DEBRA DUNAWAY-MARIANO*

Received August 19, 1983

Reaction of adenosine 5′-(1-thiodiphosphate) (ADPαS) with $[Cr(H_2O)_4Cl_2]Cl$ at pH 3, 80 °C, followed by ion-exchange chromatography provided α,β-bidentate $Cr(H_2O)_4$(ADPαS) in 20% yield. Reaction of ADPαS with $[Cr(NH_3)_4Cl_2]Cl$ under the same conditions provided α,β-bidentate $Cr(NH_3)_4$(ADPαS) in 30% yield. The α-P screw sense isomers of these complexes were separated by using reverse-phase HPLC techniques and were shown to possess Cr(III)–O coordination at the α-P rather than Cr(III)–S coordination by demonstrating that the (*S*)-ADPαS isomer gives rise exclusively to the Λ-$Cr(H_2O)_4$(ADPαS) and Λ-$Cr(NH_3)_4$(ADPαS) isomers and that (*R*)-ADPαS gives rise to the corresponding Δ isomers. In contrast, reaction of $Co(NH_3)_4(H_2O)_2^{3+}$ with ADPαS yielded two sets of α-P screw sense isomers. The Λ-$Co(NH_3)_4$ADPαS isomer derived from the (*R*)-ADPαS isomer and the Δ-$Co(NH_3)_4$(ADPαS) isomer derived from the (*S*)-ADPαS isomer gave as expected ^{31}P NMR and absorption spectral data consistent with Co(III)–S coordination. The second set of $Co(NH_3)_4$(ADPαS) isomers had a longer retention time on the reverse-phase HPLC column, and we judged both on the basis of the stereochemical correlation and spectral data to have Co(III)–O coordination at α-P. In interconversion experiments the sulfur-coordinated α,β-bidentate $Co(NH_3)_4$(ADPαS) complex was shown to be thermodynamically more stable than the oxygen-coordinated α,β-bidentate $Co(NH_3)_4$(ADPαS) complex.

Introduction

Nucleoside di- and triphosphates (e.g., adenosine 5′-triphosphate (ATP) and adenosine 5′-diphosphate (ADP)) play central roles in the bioenergetics of the cell. In the cell these nucleotides are chelated by Mg(II), and it is in this form that they are most often recognized by the enzymes that act upon them. The determination of the mechanism of action of these enzymes has received much attention in recent years and has been greatly aided by the availability of nucleotide analogues, phosphorothioates in particular, for use as mechanistic probes.[1]

In the phosphorothioate analogue, a nonbridging phosphoryl oxygen is replaced by a sulfur atom. When the substitution is made at the β position of the nucleoside triphosphate or α position of the nucleoside di- or triphosphate, a chiral center is formed. Since sulfur and oxygen differ dramatically in their H-bonding and metal ion coordinating properties, the selectivity of an enzyme for a given phosphorothioate diasteromer may be used to probe stereospecific interactions between the polyphosphate moiety and the enzyme active site important for substrate binding and catalysis.

If the thiophosphoryl center of adenosine 5′-(2-thiotriphosphate) (ATPβS) or adenosine 5′-(1-thiodiphosphate) (ADPαS) is, along with a neighboring phosphoryl center, coordinated to the obligatory metal ion, a six-membered chelate ring is formed, the screw sense of which is defined by the configuration of the thiophosphoryl phosphorus and the actual atom that is coordinated, viz. O vs. S. An enzyme will discriminate between a set of screw sense isomers on the basis of the orientation of the sulfur or oxygen atom vs. the adenosine 5′-monophosphate (in the case of ATPβS) or adenosine (in the case of ATPαS or ADPαS) moiety relative to the chelate ring.

Previous studies have shown that Mg(II) coordinates to β-thiophosphoryl of ATPβS (or α-thiophosphoryl of ADPαS) via the oxygen atom, while Cd(II) coordinates via the sulfur atom.[2] The β,γ-bidentate β-P screw sense isomers formed from a single ATPβS diastereomer by using Mg(II) and Cd(II) are therefore opposite in chelate-ring stereochemistry. Similarly, the α,β-bidentate α-P screw sense isomers formed from a single ADPαS diastereomer with Mg(II) and Cd(II) are opposite in configuration.

Many ATP/ADP-dependent enzymes have been shown to react with one ATPβS, ATPαS, or ADPαS isomer faster than the opposite stereoisomer, and in instances where the thiophosphoryl group of the nucleotide is metal coordinated, these enzymes show a reversal of stereospecificity when the metal ion cofactor is switched from Mg(II) to Cd(II). In general, however, the stereospecificity that is observed is not an absolute one,[1,3-5] and there are several possible sources of the stereochemical "slippage".[6] Because divalent cation chelates of polyphosphates undergo rapid ligand exchange, the structural and stereochemical isomers of these complexes are not kinetically stable and cannot be easily applied in studies designed to examine the source of and degree of stereospecificity. An alternate approach, which we have selected, is to examine enzyme specificity toward phosphorothioate complexes with fixed structure and stereochemistry that have been prepared from metal ions that have slow ligand exchange rates, viz. Co(III) and Cr(III). The present studies focus on the preparation and characterization of the α,β-bidentate M(III) complexes of adenosine 5′-(1-thiodiphosphate).[7]

Experimental Section

General. ^{31}P NMR spectra were recorded at 25 °C by using either a Varian XL-100 (operating at 40.51 MHz) or an IBM WP 20054 (operating at 81.02 MHz) NMR spectrometer. NMR samples contained 0.3 mM EDTA in 10% D_2O. Chemical shifts are reported in ppm downfield (+) or upfield (–) from a 0.1 M D_3PO_4 external standard. CD spectra were recorded on a Jasco 500-C spectropolarimeter and UV/visible absorption spectra on a Perkin-Elmer 552 spectrophotometer. Concentrations of the solutions of Co(III) and Cr(III) complexes of ADP and ADPαS were determined by measuring the absorbance at 260 nm and assuming a molar extinction coefficient

(1) Eckstein, F. *Acc. Chem. Res.* **1979**, *12*, 204.
(2) Jaffe, E. K.; Cohn, J. *J. Biol. Chem.* **1979**, *254*, 10839.
(3) Eckstein, F.; Goody, R. S. *Biochemistry* **1976**, *15*, 1685.
(4) Cohn, M. *Acc. Chem. Res.* **1982**, *15*, 326.
(5) Armstrong, V. W.; Lee, D.; Eckstein, F. *Biochemistry* **1979**, *18*, 4120. Connolly, B. A.; Eckstein, F. *J. Biol. Chem.* **1981**, *256*, 9450.
(6) First, the enzyme may be capable of binding and catalyzing the reaction of the "wrong" screw sense isomer in much the same way as it does with the opposite isomer, however, at a reduced efficiency. Alternatively, an enzyme active site residue may substitute for the metal ion in interacting with the thiophosphoryl center of the wrong isomer. Last, the enzyme may be recognizing the small amount of Mg(II)–S-coordinated or Cd(II)–O-coordinated complex that is present in the solution of the less active ATPβS or ATPαS diastereomer, and that has the correct screw sense.
(7) These complexes are particularly important in that they can be used to generate via elimination of the adenosine residue single enantiomeres of M(III) thiopyrophosphate. These enantiomers can in turn be used to assign the configuration of M(III) thiopyrophosphate enantiomers generated from diastereomers of bidentate M(III) complexes of ADPβS and ATPγS in which the configurations at the thiophosphoryl centers are unknown.

0020-1669/84/1323-1692$01.50/0

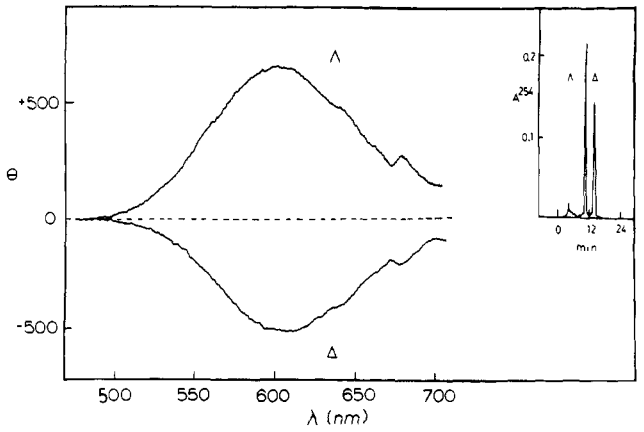


Figure 1. CD spectra of the Λ and Δ isomers of α,β-bidentate Cr-$(H_2O)_4$(ADPαS) measured at pH 5. The inset shows the reverse-phase HPLC elution profile of the Cr$(H_2O)_4$(ADPαS) reaction mixture after having passed it through Dowex cation- and anion-exchange columns (see Experimental Section for details).

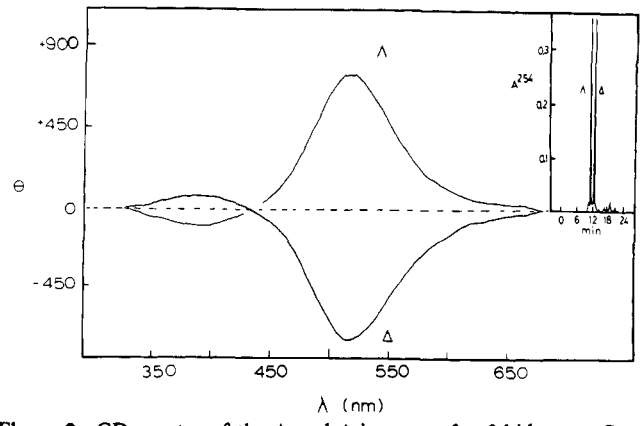


Figure 2. CD spectra of the Λ and Δ isomers of α,β-bidentate Cr-$(NH_3)_4$(ADPαS) measured at pH 5. The inset shows the reverse-phase HPLC elution profile of the reaction mixture after having passed it through Dowex cation- and anion-exchange columns (see Experimental Section for details).

of 15 400. High-pressure liquid chromatography was carried out with use of an IBM LC19533 or Beckman 332 HPLC equipped with an Hitachi 100-10 variable-wavlength detector, an Altex C-18 reverse-phase analytical column (25 cm) or Whatman C-18 reverse-phase preparative column (50 cm), and with 0.02–0.10 M potassium methanesulfonate (with or without 5% MeOH) at pH 2.2 as an isocratic eluant. The cycloheptaamylose column (1.5 × 245 cm) was prepared as previously described.[8] All enzymes, nucleotides, buffers, and Dowex resins were purchased from Sigma Chemical Co. Prior to use, the Dowex resins were bleached with Br_2 as previously described.[9] α,β-Bidentate Co$(NH_3)_4$(ADP) was prepared according to the method of Cornelius et al.[10] while α,β-bidenate Cr$(H_2O)_4$(ADP) was prepared according to the method of Dunaway-Mariano and Cleland.[9] The screw sense isomers of these complexes were separated on a cycloheptaamylose column as previously described[8] or on the reverse-phase HPLC column as described below.

Preparation of Cr$(H_2O)_4$(ADP) (1). Twenty-milliliter solutions, 10 mM in ADP and either 50, 30, 20, and 10 mM in [Cr$(H_2O)_4Cl_2$]Cl at pH 3, were heated at 80 °C for 10 min. After cooling to 25 °C, the reaction mixtures were adjusted to pH 4.5 with KOH solution and passed through a Millipore filter and then through a Dowex 50-X2 (NH_4^+) column (10 × 1 cm). The green eluant was then washed through a Dowex 1-X2 (Cl^-) column (10 × 1 cm) with water. The purity of the Cr$(H_2O)_4$(ADP), which was passed through the anion-exchange column, was determined by using reverse-phase HPLC techniques. The green material that remained absorbed on the anion-exchange column, and which is thought to be **3**, was eluted with 0.5 M $NaClO_4$ and treated with a 40-fold excess of ethylenediaminetetraacetate (EDTA) (pH 5) at 100 °C for 2 min. The resulting solution was loaded onto a cycloheptaamylose column (prepared from a disposable pipet) and eluted with 10 mM K^+MES (2-(*N*-morpholino)ethanesulfonate) (pH 5.8). The colorless fractions following the purple fractions containing the Cr–EDTA complex were combined and concentrated in vacuo. The material obtained was shown by silica gel thin-layer chromatographic analysis to be ADP. The amount of **2** (which binds to the Dowex 50 column) present in each reaction mixture was estimated from the difference in total o.d. units of the reaction mixture measured at 260 nm before and after it was passed through the Dowex 50 column.

Preparation of Cr$(H_2O)_4$(ADPαS) (4). ADPαS (triethylamine salt) was prepared according to the method of Eckstein and Goody.[3] A 20-mL solution, 10 mM in ADPαS (as a mixture of *R* and *S* isomers) and in [Cr$(H_2O)_4Cl_2$]Cl, was heated at pH 3, 80 °C for 10–25 min and then chromatographed on a Dowex 50 and a Dowex 1 column in the same manner as that described for Cr$(H_2O)_4$(ADP). The green material that remained on the Dowex 1 column was eluted with 0.5 M $NaClO_4$ and treated with EDTA to remove Cr(III) (vide supra).

The resulting material was shown by silica gel thin-layer chromatographic analysis to be ADPαS, and therefore in analogy to the Cr-$(ADP)_2$ complex we assume that the green material remaining on the Dowex 1 column is Cr$(ADP\alpha S)_2$. The green material that washed through the Dowex 1 column when treated with EDTA also yielded exclusively ADPαS. The purity of Cr$(H_2O)_4$(ADPαS) (**4**) obtained from the Dowex 1 column chromatography was determined by using reverse-phase HPLC techniques (see Figure 1). The screw sense isomers of **4** were separated on a Whatman C-18 reverse-phase column (50 cm, 4 mL/min) using 5% MeOH in 0.1 M potassium methanesulfonate (pH 2.2) as eluant. The CD spectra of the pure isomers are shown in Figure 1. The UV absorption spectra of both isomers at pH 5 closely resemble that of ADPαS. The visible spectra show a λ_{max} at 596 nm (ϵ = 22) and at 430 nm (ϵ = 31) and a λ_{min} at 514 (ϵ = 9).

Preparation of Cr$(NH_3)_4$(ADPαS) (5). A 20-mL solution, 10 mM in [Cr$(NH_3)_4Cl_2$]Cl and ADPαS at pH 3, was heated at 80 °C for 10 min and then cooled to room temperature. The solution was adjusted to pH 5 with KOH and passed through a Millipore filter and then through a 1 × 10 cm Dowex 50-X2 (NH_4^+) column. Fifty percent of the adenosine-containing material [presumably (Cr-$(NH_3)_4)_2$(ADPαS)] remained bound to the Dowex 50 column. The eluant was then passed through a 1 × 10 cm Dowex 1-X2 (Cl^-) column. Forty percent of the adenosine-containing material (ADPαS and Cr$(NH_3)_4(ADP\alpha S)_2$) eluted from the Dowex 50 column remained bound to the Dowex 1 column. The eluate was shown by using reverse-phase HPLC techniques (see Figure 2) to be essentially pure Cr$(NH_3)_4$(ADPαS) (**5**) (30% yield). The two screw sense isomers of **5** were separated on a Whatman C-18 reverse-phase column (50 cm, 4 mL/min) using 5% MeOH in 0.1 M potassium methanesulfonate (pH 2.2) as eluant. The CD spectra of the purified isomers are shown in Figure 2. The UV absorption spectra of the isomers at pH 5 closely resemble that of ADPαS. The visible absorption spectra are characterized by a λ_{max} at 535 nm (ϵ = 32) and at 395 nm (ϵ = 33) and by a λ_{min} at 460 nm (ϵ = 16).

Preparation of Co$(NH_3)_4$(ADPαS) (6). [Co$(NH_3)_4CO_3$]NO_3 was dissolved in a 1 M excess of HCl, and the resultant mixture was diluted to a final concentration of 20 mM, adjusted to pH 3 with KOH, and added to an equal volume (20 mL) of 20 mM ADPαS (pH 3). The resulting solution was heated at 80 °C for 5 min, allowed to cool to room temperature, adjusted to pH 5 with KOH, and passed through a Dowex 50-X2 (NH_4^+) column (1 × 10 cm) and then through a Dowex 1-X2 (Cl^-) column (1 × 10 cm). The reverse-phase HPLC elution profile of the Dowex 1 eluate is shown in Figure 3. The overall yield of the Co$(NH_3)_4$(ADPαS) was determined to be 30%, and the ratio of isomers A plus B to C plus D, 7:3. In order to determine the relative rates of isomer formation during the reaction, the reaction was repeated at 70 °C and analyzed at varying conversion by using HPLC techniques. The results are presented in Figure 4. All four isomers could be resolved on a cycloheptaamylose column (1.5 × 245 cm) using 10 mM K^+MES (pH 6, 4 °C) as eluant. The approximate elution volumes were as follows: A, 400 mL; B, 480 mL; D, 680 mL; C, 960 mL. Alternatively, the four isomers could be separated by using a Whatman C-18 reverse-phase HPLC column (50 cm, 4

(8) Cornelius, R. D.; Cleland, W. W. *Biochemistry* **1978**, *17*, 3279.
(9) Dunaway-Mariano, D.; Cleland, W. W. *Biochemistry* **1980**, *19*, 1496.
(10) Cornelius, R. D.; Hart, P. A.; Cleland, W. W. *Inorg. Chem.* **1977**, *16*, 2799.

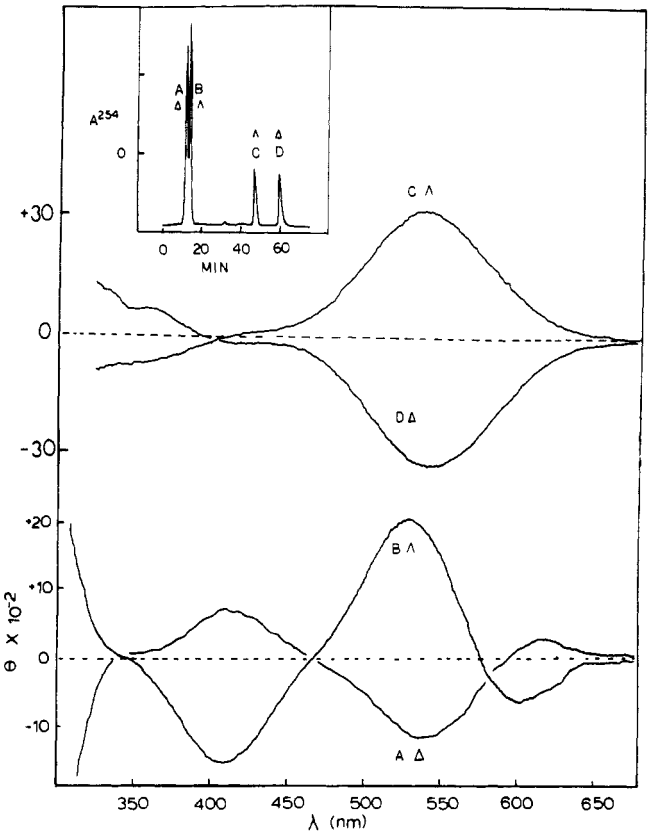


Figure 3. CD spectra of the four α,β-bidentate $Co(NH_3)_4(ADP\alpha S)$ isomers measured at pH 6. The inset shows the reverse-phase HPLC elution profile of a reaction mixture heated at pH 3, 80 °C for 5 min and then passed through Dowex cation- and anion-exchange columns (see Experimental Section for details).

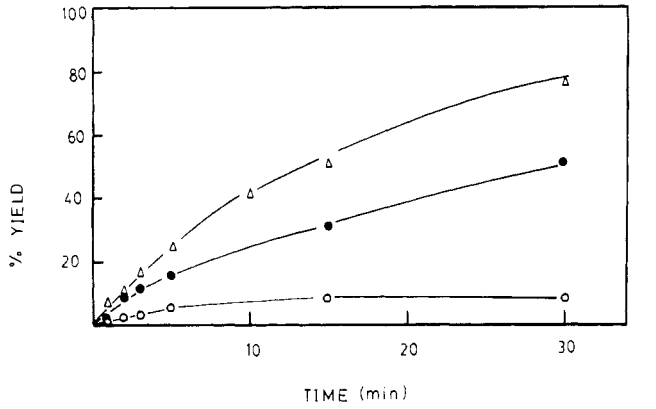
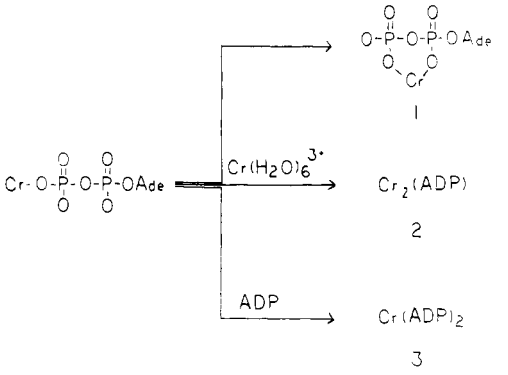


Figure 4. (Δ) Conversion of **6C** (1 mM initial concentration) to **6A** at pH 3, 70 °C; (●) formation of isomers **6A** and **6B** and (○) formation of isomers **6C** and **6D** in the $Co(NH_3)_4^{3+}/ADP\alpha S$ (10 mM initial concentration of each) reaction mixture at pH 3, 70 °C.

mL/min) and 0.1 M potassium methanesulfonate (pH 2.2) as eluant. The CD spectra of the purified isomers at pH 6 are shown in Figure 3. The UV absorption spectra of all four isomers at pH 6 closely resemble that of ADPαS. The visible absorption spectra for isomers A and B at pH 6 show a λ_{max} at 542 nm (ϵ = 71) and a λ_{min} at 56 nm (ϵ = 13). The visible absorption spectra for isomers C and D show a λ_{max} at 522 nm (ϵ = 63) and a λ_{min} at 438 nm (ϵ = 12). The ^{31}P NMR spectra of isomers A and B at pH 5.5 show a doublet at +35.5 ppm (α-P) ($J_{\alpha,\beta}$ = 17 Hz) and one at +6.0 ppm (β-P) while those of isomers C and D show a doublet at +62.6 ppm (α-P) ($J_{\alpha,\beta}$ = 25 Hz) and a doublet at +2.6 ppm (β-P).

Coordination Assignments. The *R* and *S* isomers of ADPαS were separated by using a Whatman C-18 reverse-phase HPLC column (50 cm, 4 mL/min) and 0.1 M potassium methanesulfonate (pH 2.2) as eluant. The retention time of the first isomer to elute was 24 min, while the retention time for the second isomer was 42 min. The

Scheme I



solutions of the two isomers were adjusted immediately to pH 7. The first isomer was shown to have the *S* configuration at α-P by demonstrating that it and not the second isomer served as substrate for rabbit muscle pyruvate kinase, which is specific for the *S* isomer.[3] The pyruvate kinase reaction was monitored at 340 nm by using a spectrophotometer and the standard lactate dehydrogenase/NADH coupled assay. A 0.22 mM solution of a mixture of the two isomers when treated with pyruvate kinase (5 units/mL), $MgCl_2$ (10 mM), NADH (0.3 mM), K^+(PIPES) (50 mM, pH 7), lactate dehydrogenase (5 units/mL), and pyruvate (10 mM) underwent a 0.5 absorbance unit decrease (for 50% reaction, a 0.7 absorbance unit decrease is predicted). Under these same conditions a 0.13 mM solution of the first ADPαS isomer underwent a decrease of 0.7 absorbance unit (expected for 100% conversion, 0.8 absorbance unit) while a 0.09 mM solution of the second isomer underwent no absorbance change (expected for 100% conversion, 0.56 absorbance unit). The stereochemical assignment of the ADPαS isomers was later confirmed by showing that these two isomers possessed the same relative retention times on a HPLC anion-exchange column (50 mM potassium phosphate, pH 6, as eluant) as were reported by Connolly and Eckstein[11] for the ADPαS isomers having known configurations. Solutions of the pure (*R*)- and (*S*)-ADPαS isomers were heated with solutions of $[Cr(H_2O)_4Cl_2]Cl$, $[Cr(NH_3)_4Cl_2]Cl$, and $[Co(NH_3)_4(H_2O)_2]Cl_2$ (generated from the carbonate complex), under the same conditions used in the original reactions. The configuration of the single screw sense isomer formed in each reaction was identified by using reverse-phase HPLC techniques.

Isomer Interconversions. The relative rates of isomer interconversions were examined by heating HPLC purified isomers of $Cr^{III}(ADP\alpha S)$ and $Co^{III}(ADP\alpha S)$ and cycloheptaamylose column purified isomers of $Cr^{III}(ADP)$ and $Co^{III}(ADP)$ at pH 3 and 80 °C for specified periods of time and then injecting the sample onto the Altex C-18 reverse-phase analytical column for analysis. The eluate was monitored at 260 nm and the relative amount of each isomer eluted calculated from its total of o.d. units, which in turn were determined from the relative areas of the peaks on the HPLC elution profile. In each study 0.1 M potassium methanesulfonate (pH 2.2) was used as eluant at a flow rate of 1 mL/min. Under these conditions the retention times of the isomers were as follows: $Co(NH_3)_4(ADP\alpha S)$ (**6**) *A* = 12.5, *B* = 14.5, *C* = 47.0, *D* = 59.5 min; $Cr(H_2O)_4(ADP\alpha S)$ (**4**) Λ = 35.4, Δ = 39.8 min; $Co(NH_3)_4(ADP)$ Λ = 6.4, Δ = 7.4 min; $Cr(H_2O)_4(ADP)$ Λ = 6.0, Δ = 7.0 min.

Results and Discussion

Preparation of α,β-Bidentate $Cr(H_2O)_4(ADP\alpha S)$ and $Cr(NH_3)_4(ADP\alpha S)$. For the purpose of conserving ADPαS, reaction conditions and purification procedures that would be used in the preparation of Cr(ADPαS) were first tested with ADP. Two procedures had been reported for the preparation of α,β-bidentate $Cr(H_2O)_4(ADP)$.[9,12] These procedures involve reaction of a solution of ADP and $[Cr(H_2O)_4Cl_2]Cl$ (10 mM in each) at pH 3 and 80 °C or at pH 5.5 and 4 °C. The product formed initially in the Cr(III)/ADP reaction mixture is the β-monodentate complex.[9,13] As indicated in Scheme

(11) Connolly, B. A.; Eckstein, F. *Biochemistry* **1982**, *21*, 6158.
(12) DePamphilis, M. L.; Cleland, W. W. *Biochemistry* **1973**, *12*, 3714.

I the complex may undergo reaction intramolecularly to give the desired α,β-bidentate complex **1** or may undergo reaction intermolecularly with Cr(III) or ADP to give $Cr_2(ADP)$ (**2**) and $Cr(ADP)_2$ (**3**), respectively.[12]

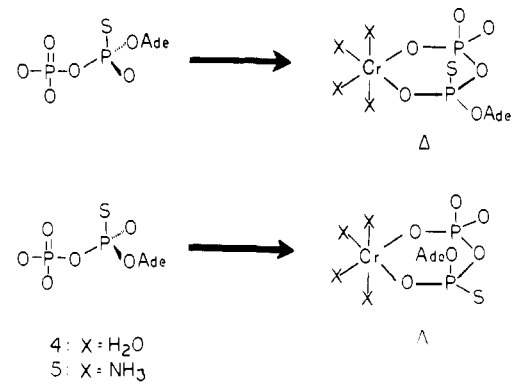
We found that the desired compound **1** could be obtained in relatively pure state, as judged by HPLC analysis, simply by passing the crude reaction mixture (adjusted to pH 4.5) through a Dowex cation-exchange column ($Cr(H_2O)_6^{3+}$ and $Cr_2(ADP)$ are absorbed) and then through a Dowex anion-exchange column ($Cr(ADP)_2$ and ADP are absorbed). The yields of $Cr(H_2O)_4(ADP)$ obtained from the reactions carried out at pH 3 (80 °C) or at pH 5.5 (4 °C) were, however, somewhat lower than expected, viz. 26% and 20%, respectively. Increasing the reaction time had no effect on the product yield. In search of a method to optimize the conversion of ADP to **1** we examined the product yield from reactions carried out by using Cr(III):ADP stoichiometries greater than unity. The reaction mixture (pH 3) in which this ratio was 1:1 contained 37% adenine-containing material that remained bound to the Dowex cation-exchange column and is assumed to be at least in part **2** and 37% material that was retained by the Dowex anion-exchange column and is assumed to be $Cr(ADP)_2$ and ADP. At a Cr(III):ADP ratio of 2:1 the approximate percentages of **1**–**3** (plus ADP) in the reaction mixture were determined to be 13%, 55%, and 32%, for a ratio of 3:1, 16%, 66%, and 18%, and for a ratio of 5:1, 5%, 90%, and 5%. It was thus concluded that the maximum yield of $Cr(H_2O)_4$-(ADP) from ADP could not be increased by using a Cr-(III):ADP stoichiometry greater than 1:1.

Reaction of $[Cr(H_2O)_4Cl_2]Cl$ with ADPαS (1:1 stoichiometry) at pH 3, 80 °C for 10 min resulted in 20% yield of α,β-bidentate $Cr(H_2O)_4(ADP\alpha S)$ (**4**). In this case 53% of the adenine-containing material ($Cr_2(ADP\alpha S)$) was retained by the cation-exchange column and 27% ($Cr(ADP\alpha S)_2$ and ADPαS) by the anion-exchange column. Neither the product yield nor composition was altered by increasing the reaction time to 25 min.

The reverse-phase HPLC elution profile of $Cr(H_2O)_4$-(ADPαS) (**4**) shown in Figure 1 indicates that this material is relatively pure and that it can be resolved into two α-P screw sense isomers. In an earlier study it had been shown that the two α-P screw sense isomers of $Cr(H_2O)_4(ADP)$ (**1**) could be separated on cycloheptaamylose columns.[9] While we were able to cleanly separate the isomers of **1** on our own column, we could not resolve the isomers of **4** and therefore instead relied on reverse-phase HPLC (preparative scale) techniques to effect the desired separation. The CD spectra of the pure diastereomers of **4** are shown in Figure 1. The screw sense, based on the definition provided by Cornelius and Cleland,[8] was assigned to these isomers by making a comparison of their CD spectral properties to those of the $Cr(H_2O)_4(ADP)$ isomers whose configurations are known.[9] In correlating the screw sense to observed Cotton effects it is assumed that substitution of an oxygen atom at α-P with a sulfur atom will not perturb the CD spectral properties of the isomer within the visible region to the extent of reversing the sign of the Cotton effect (i.e., the electronic environment about the metal is dominated by the relative orientation of the adenine moiety rather than that of the nonbridging α-P oxygen atom). Indeed, the CD spectra of the isomers of **4** do closely resemble those of the isomers of **1**.[9] The Λ isomer of **1** like that of **4** was found to elute from the reverse-phase HPLC column before the corresponding Δ isomer.

In order to distinguish between sulfur atom and oxygen atom coordination at the α-P of $Cr(H_2O)_4(ADP\alpha S)$, the configuration of an individual ADPαS isomer was correlated with the

Scheme II



screw sense of the chelate ring of the α,β-bidentate Cr-$(H_2O)_4(ADP\alpha S)$ isomer derived from it. The ADPαS isomers were separated by using reverse-phase HPLC techniques and assigned stereochemistry at α-P on the basis of their observed reactivity with pyruvate kinase and relative retention times on a reverse-phase C-18 HPLC column (see Experimental Section). *S*-ADPαS was shown by using HPLC techniques to give rise specifically to the Λ-$Cr(H_2O)_4(ADP\alpha S)$ isomer while the *R*-ADPαS isomer was shown to produce the Δ-$Cr(H_2O)_4(ADP\alpha S)$ isomer (see Scheme II). On the basis of these observations it is concluded that the thiophosphoryl group of **4** is coordinated at the oxygen atom and not at the sulfur atom. The visible absorption spectrum of **4**, which closely resembles that of **1**, is consistent with oxygen atom coordination.

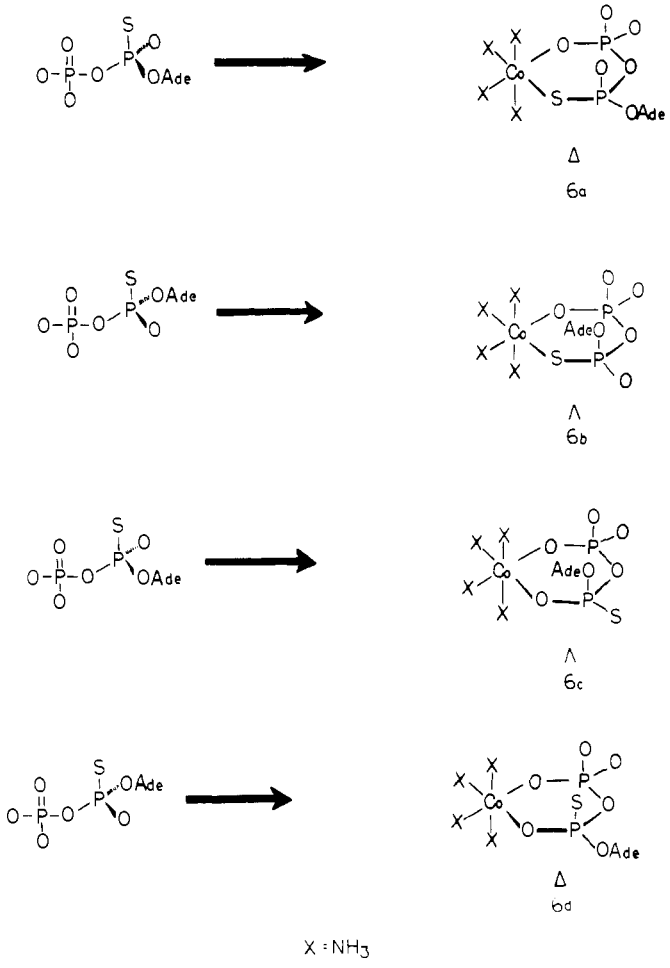
$Cr(NH_3)_4(ADP\alpha S)$ (**5**) was prepared by reaction of [Cr-$(NH_3)_4Cl_2]Cl$ with ADPαS at pH 3, 80 °C for 10 min. The reverse-phase HPLC elution profile of **5**, obtained in 30% yield following the Dowex cation- and anion-exchange steps described previously, is shown in Figure 2. Increased reaction periods resulted in loss of NH_3 from the coordination sphere and in formation of what appeared to be the corresponding triammine complexes.

The CD spectra of the purified isomers (preparative HPLC) of $Cr(NH_3)_4(ATP\alpha S)$ are included in Figure 2. The screw sense of these isomers was assigned by comparing their CD spectral properties with the CD spectral properties of the corresponding α,β-bidentate $Cr(NH_3)_4(ADP)$ isomers, which have known configurations.[9] As in the case of the Cr-$(H_2O)_4(ADP\alpha S)$ isomers the $Cr(NH_3)_4(ADP\alpha S)$ isomers are oxygen coordinated at α-P. This was demonstrated by showing that (*S*)-ADPαS specifically gives rise to Λ-$Cr(NH_3)_4$-(ADPαS) and (*R*)-ADPαS gives rise to Δ-$Cr(NH_3)_4$-(ADPαS). Likewise, the visible absorption spectrum of **5** closely resembles that of $Cr(NH_3)_4(ADP)$.[12]

Preparation of α,β-Bidentate $Co(NH_3)_4(ADP\alpha S)$. Reaction of $[Co(NH_3)_4(H_2O)_2]^{3+}$ with ADPαS for 5 min at pH 3 and 80 °C gave, upon Dowex cation- and anion-exchange chromatography, $Co(NH_3)_4(ADP\alpha S)$ (**6**) in 30% yield. The reverse-phase HPLC elution profile of $Co(NH_3)_4(ADP\alpha S)$ is provided in Figure 3 along with the CD spectra of the four purified isomers (A–D). The UV/visible absorption spectra of isomers **6A** and **6B** are characterized by a λ_{max} at 542 nm compared to 517 nm for α,β-bidentate $Co(NH_3)_4(ADP)$. Examination of the ^{31}P NMR spectra of isomers **6A** and **6B** reveals that the resonance of the α-P is shifted 6 ppm upfield relative to the α-P resonance from ADPαS (measured at pH 8) while the β-P resonance from these isomers is shifted 12 ppm downfield from that of ADPαS. On the basis of studies of Cornelius et al.,[10] with $Co(NH_3)_4(ADP)$ we expect that coordination of the α-P and β-P oxygens by Co(III) would lead to a 10–12 ppm downfield shift of both phosphorus resonances. On the other hand, Jaffe and Cohn[2] have shown that coor-

(13) Bossard, M. J.; Samuelson, G. S.; Schuster, S. M. *J. Inorg. Biochem.* **1982**, *17*, 61.

Scheme III



dination of divalent cations to the sulfur atom of ADPαS leads to an upfield shift of the α-P resonance. The ^{31}P NMR and UV/visible absorption spectral data for isomers **6A** and **6B** suggest that these are α,β-bidentate complexes in which the sulfur atom at the α-P is coordinated rather than the oxygen atom. Sulfur coordination was confirmed by demonstrating (see Scheme III) that isomer **6A**, which on the basis of its negative Cotton effect at 540 nm (compared to the negative Cotton effect of the $Co(NH_3)_4(ADP)$ isomer at 540 nm)[8,9,14] is assigned the Δ configuration, is derived exclusively from (*S*)-ADPαS. Similarly, isomer **6B**, which shows a positive Cotton effect at 540 nm (compared to the positive Cotton effect of the $Co(NH_3)_4ADP$ isomer at 540 nm)[8,9,14] and is assigned the Λ configuration, is derived exclusively from (*R*)-ADPαS. In contrast, isomers **6C** and **6D** show positive and negative Cotton effects at 540 nm, respectively, and therefore they are assigned the Λ and Δ configurations, respectively. Since isomer **6C** was shown to derive exclusively from (*S*)-ADPαS and isomer **6D** from (*R*)-ADPαS (see Scheme III), these two α,β-bidentate isomers are coordinated to α-P via the oxygen atom. The UV/visible absorption spectra of isomers **6C** and **6D** that show a λ_{max} at 522 nm and the ^{31}P NMR spectra that show a 20 ppm downfield shift of the α-P resonance relative to that of ADPαS (at pH 8) and a 9 ppm downfield shift of the β-P resonance relative to that of ADPαS support this assignment.

The relative ratio of the sulfur-coordinated isomers (**6A** and **6B**) to the oxygen-coordinated isomers (**6C** and **6D**) formed in the reaction was dependent on how long the reaction mixture was heated. The HPLC elution profile shown in Figure 3 is that of a reaction mixture that was heated for 5 min at 80 °C.

(14) Coderre, J. A.; Gerhlt, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 6594.

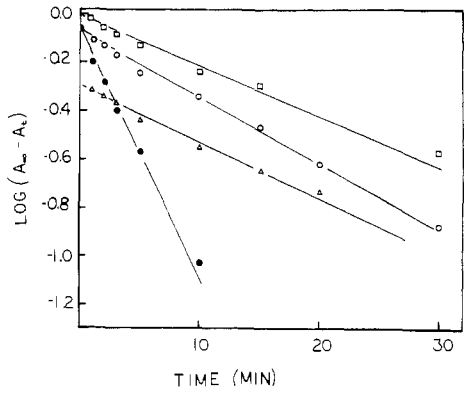


Figure 5. (●) Epimerization of the Λ isomer of α,β-bidentate $Co(NH_3)_4(ADP)$ at pH 3, 80 °C (k = 0.10 min^{-1}) and (○) at 70 °C (k = 0.03 min^{-1}); (△) epimerization of the Λ isomer of α,β-bidentate $Cr(H_2O)_4(ADP)$ at pH 3, 80 °C (k = 0.04 min^{-1}); (□) conversion of **6C** to **6A** at pH 3, 70 °C (k = 0.03 min^{-1}). At equilibrium the ratio of the Λ and Δ isomers of $Co(NH_3)_4(ADP)$ is 1:1, and the ratio of the Λ and Δ isomers of $Cr(H_2O)_4(ADP)$ is 1:1. A_∞ refers to the millimolar concentration of the isomer at equilibrium while A_t refers to the millimolar concentration of the isomer at time t.

By comparison, reaction mixtures heated for 30 min contain virtually none of **6C** and **6D**. Purified isomers of **6** were heated at pH 3 and 80 °C and analyzed by using HPLC techniques. We found that isomers **6A** and **6B** were stable for up to 20 min of heating while isomer **6C** was quantitatively converted to **6A**, and **6D** to **6B** (at 10 min the conversion was ca. 50% complete). These results indicate that isomers **6A** and **6B** are the thermodynamic products of the reaction.

In search of reaction times that would lead to a maximum yield of **6C** and **6D**, the relative amounts of the isomers formed in the $Co(NH_3)_4^{3+}$/ADPαS reaction at 70 °C (pH 3) were monitored at varying conversion. As indicated by the data presented in Figure 4 the sulfur-coordinated isomers **6A** and **6B** appear to predominate at all percent conversions. The oxygen-coordinated isomers, **6C** and **6D**, appear to reach a steady-state level of 10% yield, while the percent yield of **6A** and **6B** continually increases with reaction time. Under identical conditions the rate of conversion of **6C** to **6A** was measured. The data shown in Figure 4 indicate that this conversion is faster than the rate of formation of **6A** and **6B** in the ADPαS reaction mixture. Thus, the yield of the **6C** and **6D** isomers is indeed limited to their steady-state level, which in turn is determined by the relative rates of their formation and conversion to **6A** and **6B**.

In contrast to $Co(NH_3)_4(ADP\alpha S)$ (**6**), $Cr(H_2O)_4(ADP\alpha S)$ (**4**) is stable as the fully oxygen-coordinated species. When single isomers of $Cr(H_2O)_4(ADP\alpha S)$ were heated at pH 3, 80 °C for up to 40 min and analyzed by using HPLC techniques, we found that no change had taken place. The contrasting behavior between the Co(III) and Cr(III) complexes could potentially be rationalized in two different ways. First, conversion of the oxygen-coordinated isomer of $Cr(H_2O)_4$-(ADPαS) to the sulfur-coordinated species requires Cr–O bond cleavage, which in turn could be much less facile than Co–O bond cleavage. In order to test this possibility we examined the relative rates of α,β-bidentate $Co(NH_3)_4(ADP)$ α-P epimerization and α,β-bidentate $Cr(H_2O)_4(ADP)$ α-P epimerization at pH 3, 80 °C (and 70 °C). The results shown in Figure 5 indicate (1) that epimerization of the Λ isomer of $Co(NH_3)_4(ADP)$ occurs at ca. the same rate as the conversion of **6C** to **6A** and (2) that the rate of epimerization of the Λ isomer of $Cr(H_2O)_4(ADP)$ at 80 °C although somewhat slower than that of the corresponding $Co(NH_3)_4(ADP)$ isomer is not slow enough to account for the lack of conversion of the oxygen-coordinated $Cr(H_2O)_4(ADP\alpha S)$ isomer to the sulfur-coordinated isomer.

An alternative explanation of the lack of formation of the Cr–S-coordinated species is that the oxygen-coordinated complex, **4**, is both the kinetic and thermodynamic product of the Cr(III)/ADPαS reaction. Because the thermodynamically most stable form of $Cr(NH_3)_4$(ADPαS) also requires oxygen coordination at the α-P (**5**) the difference in the behavior of **4** and **6** cannot be directly attributed to differences in ligands, viz. H_2O vs. NH_3. In addition, since the X-ray structures of the analogous P^1,P^2-bidentate $Co(NH_3)_4PP$[15] and $Cr(NH_3)_4PP$ complexes are essentially identical,[16] it is unlikely that steric factors contribute to a large extent to the difference in behavior of the Cr(III) and Co(III) complexes. The preference for Co–S vs. Co–O coordination might instead derive from the nephelauxetic effect.

Acknowledgment. This investigation was supported by NIH Grant GM-28688, American Heart Grant-in-Aid 83918, and NIH Career Development Award (Grant ES-00111) to D. D.-M.

Registry No. **1**, 58642-43-6; Λ-**1**, 73037-58-8; Δ-**1**, 73000-97-2; **4**, 89791-44-6; Λ-**4**, 89885-94-9; Δ-**4**, 89885-95-0; **5**, 89791-45-7; Λ-**5**, 89885-96-1; Δ-**5**, 89885-97-2; **6**, 89791-46-8; Δ-**6a**, 89955-24-8; Λ-**6b**, 89908-10-1; Λ-**6c**, 89885-98-3; Δ-**6d**, 89885-99-4; $[Cr(H_2O)_4Cl_2]Cl$, 13820-85-4; $[Cr(NH_3)_4Cl_2]Cl$, 19706-96-8; $[Co(NH_3)_4CO_3]NO_3$, 15040-52-5.

(15) Merrit, E. A.; Sundaralingam, M. *Acta Crystallogr., Sect. B* **1980**, *B36*, 2576.
(16) Haromy, T.; Knight, W. B.; Dunaway-Mariano, D.; Sundaralingam, M. *Acta Crystallogr., Sect. B: Struct. Sci.*, in press.

Contribution from the Department of Chemistry, University of Rochester, Rochester, New York 14627

Flexibly Bridged Binuclear Rhodium and Iridium Complexes of *p*-Xylylenebis(3-(2,4-pentanedione))

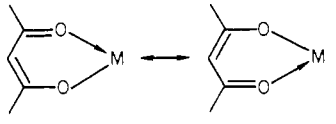
BRYAN C. WHITMORE and RICHARD EISENBERG*

Received September 30, 1983

Binuclear complexes of Rh and Ir containing a flexibly bridging bis(2,4-pentanedionato)ligand have been synthesized and characterized. The reaction of $[M(\mu\text{-Cl})(1,5\text{-COD})]_2$ (M = Rh, Ir) with *p*-xylylenebis(3-(2,4-pentanedione)), xyl(Hacac)$_2$, and 2 equiv of KOH results in the formation of the binuclear compounds $(M(COD))_2$(xyl(acac)$_2$). The cyclooctadiene ligand in these complexes is readily displaced from the metal centers by either CO or PPh_3, leading to the formation of $(M(CO)_2)_2$(xyl(acac)$_2$) and $(M(PPh_3)_2)_2$(xyl(acac)$_2$), respectively. The $(M(CO)_2)_2$(xyl(acac)$_2$) complexes react with excess triphenylphosphine, leading to the displacement of one CO from each metal center and the formation of $(M(CO)(PPh_3))_2$(xyl(acac)$_2$). The rhodium complex $(Rh(CO)_2)_2$(xyl(acac)$_2$) also reacts with triphenyl phosphite to produce the phosphite derivative, $(Rh(P(OPh)_3)_2)_2$(xyl(acac)$_2$), which is found to act as a catalyst precursor for propylene hydrogenation. At 24 °C and under 320 torr of $H_2 + C_3H_6$ (2.5:1), propane forms at the rate of 8 mol of product (mol of catalyst)$^{-1}$ h^{-1} in the presence of a 7.4×10^{-4} M solution of the phosphite derivative in toluene. The binuclear iridium complex $(Ir(CO)(PPh_3))_2$(xyl(acac)$_2$) undergoes oxidative-addition reactions with allyl bromide or benzyl bromide, producing the iridium(III) species $(IrR(CO)(PPh_3)Br)_2$(xyl(acac)$_2$) where R = σ-allyl and benzyl, respectively. The mononuclear iridium complex $Ir(PPh_3)_2$(acac) has also been synthesized and characterized. The reaction of this complex with H_2 results in the formation of $IrH_2(PPh_3)_2$(acac), whereas the reaction of Ir(COD)(acac) with H_2 in the presence of 2 equiv of PPh_3 leads to the formation of *mer*- and *fac*-$IrH_3(PPh_3)_3$ as determined by ^{1}H NMR spectroscopy. The significance of these reactions in terms of the stability of rhodium and iridium acac complexes in catalytic systems is discussed.

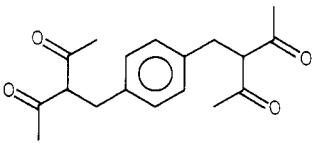
Introduction

Transition-metal complexes of β-diketonate ligands have been studied for many years because of the number and variety of stable complexes that they form and the spectroscopic and chemical properties that these complexes exhibit.[1,2] In these complexes, the six-membered chelate ring possesses a delocalized electronic structure, as suggested by resonance forms a and b, and exhibits partial aromatic character as evidenced by reaction chemistry of the acetylacetonate (acac) chelate ring.[3]



a b

We describe in the present study the preparation of binuclear complexes based on a ligand system that contains two acac moieties. The β-diketonate groups of the ligand system are connected by a xylylene bridge and are precluded from binding to a single metal center. The ligand, *p*-xylylenebis(3-(2,4-pentanedione))(xyl(HAcac)$_2$), shown as I, was first



I

prepared in 1959[4] and was studied briefly as a component in the formation of both organic[5] and coordination[6] polymers. We envisioned that this $(acac)_2$ compound would act as a flexible bridging ligand in the preparation of binuclear rhodium and iridium complexes, the mononuclear analogues of which have been studied extensively. Since 1964, when the rhodium complex $Rh(CO)_2$(acac) and closely related derivatives were first reported,[7] many rhodium and iridium acac complexes have been studied and described in thee literature. Some of the Rh

(1) Mehrotra, R. C.; Bohra, R.; Gaur, D. P. "Metal β-Diketonates and Allied Derivatives"; Academic Press: New York, **1978**.
(2) Graddon, D. P. *Coord. Chem. Rev.* **1969** *4*, 1.
(3) Collman, J. P. *Angew. Chem.* **1965**, *77*, 154.
(4) Martin, D. F.; Fernelius, W. C.; Shamma, M. *J. Am. Chem. Soc.* **1959**, *81*, 130.
(5) Korshak, V. V.; Krongauz, E. S.; Berlin, A. M. *J. Polymer Sci., Part A* **1965**, *3*, 2425.
(6) Korshak, V. V.; Krongauz, E. S.; Sheina, V. E. *Vysokomol. Soedin.* **1960**, *2*, 662.
(7) Bonati, F.; Wilkinson, G. *J. Chem. Soc.* **1964**, 3156.

0020-1669/84/1323-1697$01.50/0