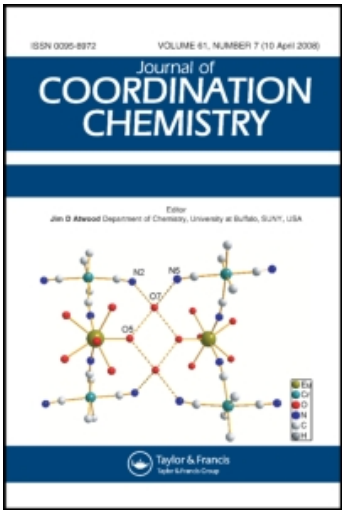

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

AN EQUILIBRIUM AND RATE STUDY OF THE INTERACTION OF AQUEOUS CHROMIUM(III) ION WITH ADENINE

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To cite this Article Krishnamoorthy, C. R. and Harris, G. M.(1980) 'AN EQUILIBRIUM AND RATE STUDY OF THE INTERACTION OF AQUEOUS CHROMIUM(III) ION WITH ADENINE', Journal of Coordination Chemistry, 10: 1, 55 — 65
To link to this Article: DOI: 10.1080/00958978008079851
URL: http://dx.doi.org/10.1080/00958978008079851

J. Coord. Chem., 1980, Vol. 10, pp. 55–65
0095-8972/80/1001-0055 $04.50/0

AN EQUILIBRIUM AND RATE STUDY OF THE INTERACTION OF AQUEOUS CHROMIUM(III) ION WITH ADENINE

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(Received July 9, 1979)

Potentiometric titration data obtained at 50° for aqueous solutions containing aquochromium(III) ions and adenine were analyzed to yield values for the acid dissociation constants of adenine and the formation constants of 1:1 and 1:2 mononuclear complexes. There is also evidence for a 2:2 dimer for which elemental analysis data suggest a structure of the form (HL = neutral adenine): $[(H_2O)_2(HL)Cr\text{-}\mu(OH)_3\text{-}Cr(HL)(H_2O)_2]^{3+}$. The kinetic studies indicate that the 1:1 complex is formed in excess adenine with a half-time of about two hours at pH = 4.4 and 60°. The proposed mechanism involves an outer-sphere associative intermediate of appreciable stability ($K_A \sim 200$) followed by rate-determining substitution of adenine for ligand water to produce the 1:1 complex. A much slower subsequent reaction yields the dimer as the final stable product. The formation of the 1:1 complex is an equilibration and both forward and reverse rate constants were determined. The pH dependence data indicate that the reactive species in the system are HL, $Cr(H_2O)_5OH^{2+}$, and $Cr(H_2O)_4(OH)_2^+$.

INTRODUCTION

A number of studies have been made of the complexation of various metal ions by purine and pyrimidine bases and some of their biologically significant derivatives. In the case of the simple bases, the earliest studies were carried out in mixed dioxane-water media,[1-3] so are not particularly relevent to any natural system. More recently, the interaction of a number of bivalent metals with these bases in aqueous systems has been investigated,[4] and the various equilibrium constants determined by potentiometric methods. The goal of the present study has been to determine the nature of the complexation process between a typical "inert" trivalent aquated metal ion, $Cr(H_2O)_6^{3+}$, and the heterocyclic bases cytosine and adenine, both from the equilibrium and kinetic point of view. Preliminary experiments soon demonstrated that no readily detectable interaction takes place between the metal center and cytosine, so attention was focussed on the interaction with adenine, which is accompanied by substantial spectral changes. No previous work along these lines has been attempted, although Cleland and co-workers[5] have made considerable use of Cr(III)-nucleotide complexes in exploring the kinetics of certain enzyme reactions. However, in their studies, the metal ion binding is through the phosphate grouping of the adenine derivative, a linkage which of course is not available with the simple base as employed in our study.

EXPERIMENTAL

Materials Anhydrous adenine and all other chemicals were of reagent grade. Fresh solid ligand was weighed out for each experiment in order to avoid possible photochemical decomposition. A stock solution of chromium(III) (~0.2 M) was prepared from crystalline $Cr(NO_3)_3 \cdot 9H_2O$ and estimated volumetrically by persulfate oxidation and iodimetric titration of the resultant chromate. Carbonate-free NaOH solutions and de-ionized water were utilized in all the experiments.

Equilibrium measurements The relevant data were obtained by potentiometric titration of the protonated ligand (achieved by addition of one equivalent of acid) in presence and absence of the metal ion. The measurements were made by use of a Fisher Model 420 digital pH meter, with the calomel/glass electrode system calibrated as previously described.[4] The ionic strength remained almost constant during titration by use of a 0.50 M KNO_3 medium and

relatively low concentrations of ligand and metal ion (10^{-3} to 10^{-4} M). The reactant mixtures were first heated at about 70° until complexation appeared to have reached equilibrium, as shown by constancy of the pH. The temperature was then maintained at 50.0 ± 0.1°, and the reactants were allowed to equilibrate at this temperature until the pH became constant (several hours) before commencing the titration, and also between each further addition of NaOH titrant (0.1 M).

Rate measurements The reactions were followed spectrophotometrically in the UV-visible range by means of a Cary 118 spectrophotometer. A typical fully-scanned spectral record of the complexation process at pH = 4.0 and temperature 45° is shown in Figure 1. In most runs, absorbance changes were recorded digitally at 380 nm, where the change during reaction is greatest (see Figure 1). A few runs, made at 360 nm, yielded rate constants identical with those of duplicate runs at 380 nm. The rate studies were carried out at ionic strength 0.5 M (KNO_3) by a batch sampling technique, with the thermostat controlled to ±0.1. The initial pH adjustment was achieved by additions of 0.01 M NaOH or HNO_3, employing the self-buffering capacity of the excess adenine in the experimental pH range. The complexation occurs in two steps. The first is much more rapid than the second, so proceeds with excellent retention of the three isosbestic points for several half-times as seen in Figure 1, and the derived rate data refer to this process. The best-fit observed rate constants were obtained by a computerized non-linear first-order kinetics curve-fitting procedure.[6] The slow second reaction, requiring as many as ten additional first-reaction half times for completion, yields a somewhat shifted final spectrum with a higher final absorbance (the curve labelled D_∞ in Figure 1). Some of a final solution containing excess adenine was evaporated to small volume and cooled to precipitate most of the excess ligand. After filtration and further evaporation, a deep violet solid was isolated, which was purified by several repetitions of the isolation procedure. So far, no crystalline material suitable for X-ray structure determination has been obtainable. However, CHN analyses (duplicate determinations) rule out the possibility of a mononuclear 1:1 metal:ligand complex, any kind of 1:2 complex, and singly or doubly bridged binuclear complexes. But triply-bridged $\mu(OH)_3$ binuclear structures[7] of the types (monodentate neutral adenine (HL) in the first, bidentate in the second) $[(H_2O)_2(HL)Cr\mu\text{-}(OH)_3Cr(HL)(H_2O)_2](NO_3)_3$ and $[(H_2O)(HL)Cr\mu\text{-}(OH)_3Cr(HL)(H_2O)](NO_3)_3 \cdot 2H_2O$ are reasonably compatible with the analytical data, viz.: Calcd: C, 17.6%; H, 3.1%; N, 26.7%. Found:[8] C, 18.5%; H, 2.8%; N, 26.6%.

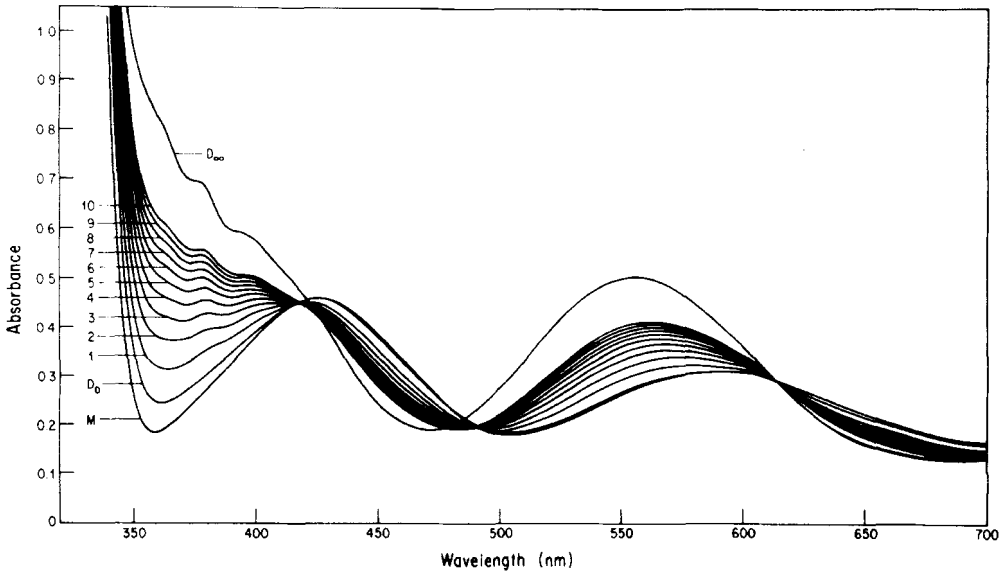


FIGURE 1 Successive scans of the spectrum of typical reactant mixture at pH = 4.0, and t = 45°. $[Cr(H_2O)_6]_0$ = 7.0×10^{-4} M; $[\text{Adenine}]_0 = 2.8 \times 10^{-2}$ M.

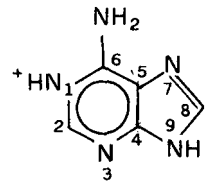
RESULTS AND DISCUSSION

Equilibrium Studies

The titration of protonated adenine alone is illustrated in Figure 2, and involves two buffer regions and a steep inflection at unit hydroxide-to-adenine stoichiometry (a = 1 in the Figure). This is as expected for a reaction of the type:

$$H_2L^+ \overset{K_{1a}}{\rightleftarrows} HL \overset{K_{2a}}{\rightleftarrows} L^- \qquad (1)$$

where HL represents neutral adenine. The two pK's were evaluated both by the direct algebraic method[4] and by the Bjerrum method.[9] The results are seen to agree well with each other (see Table IA) and are consistent with earlier published data.[10] These previous studies have established that the deprotonations involve successively the N(1) and N(9) protons of the purine base, as identified in the following formula:



The titration curves obtained with equilibrated mixtures of Cr(III) and adenine of various stoichiometries are also presented in Figure 2, the dotted portions beyond a = 2 indicating the appearance of a solid phase before an inflection point is reached. However, stability constants could be computed from the titration data available prior to precipitation, again utilizing both the algebraic[4] and the Bjerrum[9] techniques. It was found possible to evaluate constants for 1:1 and 1:2 metal-to-ligand complexes, and these are given in Table IB for one set of concentration conditions. The 1:3 data are inconclusive, however, suggesting that the 1:2 ratio is the maximum possible for this system.

Additional measurements, presented in Figure 3, indicate that the stability constants for 1:1 stoichiometry are dependent on reactant concentration when calculated in the conventional manner, as shown in Table IB. Such behavior suggests the possibility of dimerization of the 1:1 complex,[11] other evidence for which has already been outlined. The system involved is assumed[12] to consist of the following reactions and equilibrium constants, together with

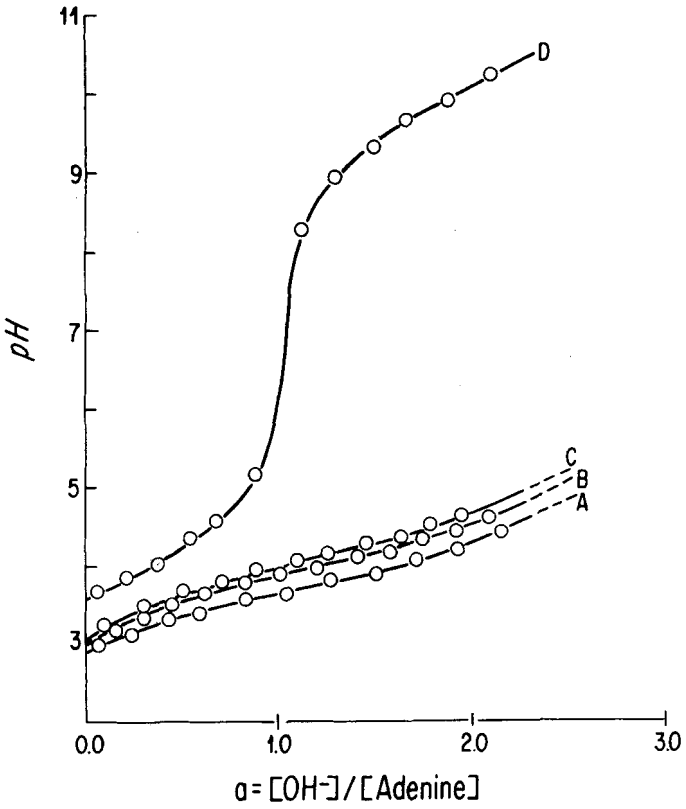


FIGURE 2 Titration data at 50° for adenine alone (Curve D) and for adenine/chromium(III) mixtures (1:1, Curve A; 2:1, Curve B; 3:1, Curve C; $[Cr(H_2O)_6^{3+}]_0 = 1.70 \times 10^{-3}$ M).

TABLE I
Equilibrium constants for complexation of aquochromium(III) by adenine[a]

A. *Acid dissociation constants of adenine*

pK_{1a}	pK_{2a}
4.10 ± 0.05[b]	9.30 ± 0.05[b]
4.05 ± 0.05[c]	9.35 ± 0.05[c]

B. *Formation constants for aquochromium(III)/adenine complex ions*[b]

$T_L = T_M$(M)	log K_{MHL}	log $K_{M(HL)_2}$
1.70×10^{-3}	3.7 ± 0.2	
3.40×10^{-3}	4.0 ± 0.2	3.1 ± 0.2
3.40×10^{-3}	3.8 ± 0.1[c]	2.9 ± 0.2[c]
6.81×10^{-3}	4.2 ± 0.2	
10.21×10^{-3}	4.5 ± 0.2	

[a] Temp., 50°; I = 0.5 M (KNO_3).
[b] Algebraic method[4].
[c] Bjerrum's method[9].

the equilibria and constants defined by Eq. (1) above:

$$M + HL \rightleftarrows MHL \qquad K_{MHL} \qquad (2)$$

$$2M + 2HL \rightleftarrows M_2(HL)_2 \qquad K_D \qquad (3)$$

In these, M represents the Cr(III) species and HL neutral adenine. Following the procedure of Rajan and Martell,[11] one can derive an expression which takes the form:

$$(T_M - XY)/X^2YZ = K_D X^2 YZ + K_{MHL} \qquad (4)$$

In this, T_M is the total metal ion concentration, $X = (1 - a)T_L - [H^+]$, a is the stoichiometric ratio for a given point as already defined, T_L is the total ligand concentration (= T_M in these experiments), $Y = 1 + K_{1a}/[H^+]$ and $Z = K_{1a}/[H^+]$. (The constant K_{2a} is not involved since in the pH range of this study the second part of Eq. (1) can be ignored.) A plot of the l.h.s. of Eq. (4) *vs* X^2YZ should yield a straight line of slope K_D and intercept K_{MHL}. Such a plot is given in Figure 4, and the values of the constants so derived by least squares analysis are as follows:

$$\log K_{MHL} = 3.5 \pm 0.1; \log K_D = 10.8 \pm 0.1$$

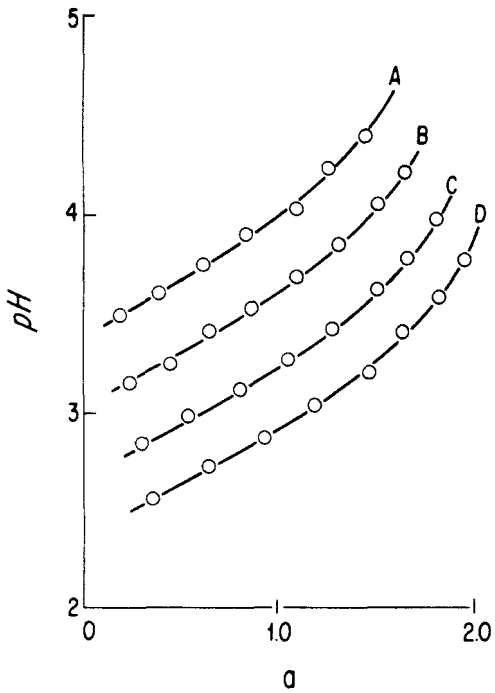


FIGURE 3 Variation of titration behavior of equimolar adenine/chromium(III) mixtures at 50° with concentration. (Curve A, 1.70×10^{-3} M; Curve B, 3.40×10^{-3} M; Curve C, 6.81×10^{-3} M; Curve D, 10.21×10^{-3} M).

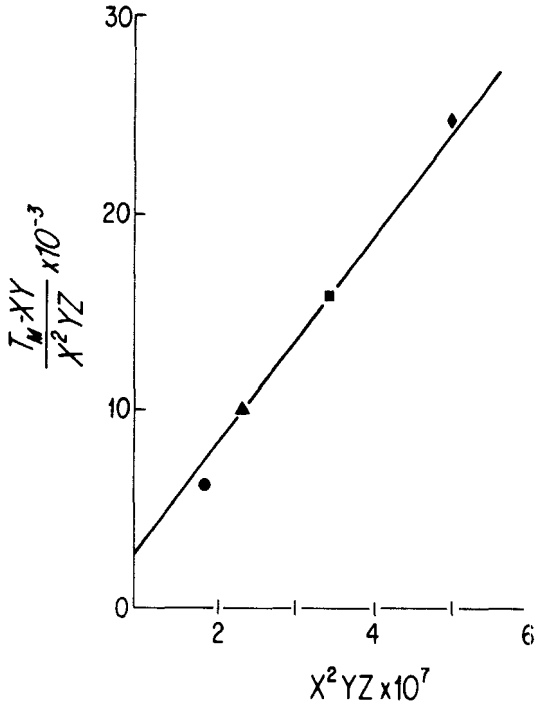


FIGURE 4 Graphical demonstration of binuclear 1:1 adenine/chromium(III) complex utilizing Eq. (4). •, 1.70×10^{-3} M; ▲, 3.40×10^{-3} M; ■, 6.81×10^{-3} M; ♦, 10.21×10^{-3} M.

The equilibrium constant data enable reasonable justification of the elemental analysis findings mentioned above. These were seen to support the concept of a $\mu(OH)_3$ 1:1 metal-to-ligand final product when aquochromium(III) is evaporated to small volume in excess of adenine. Knowing the low solubility of adenine (< 0.03 M), one can determine on the basis of the known approximate K_{MHL} and $K_{M(HL)_2}$ values (see Table IB) that at equilibrium the concentrations of both uncomplexed aquochromium(III) and 1:2 complex are negligible as compared to that of 1:1 complex. Furthermore, since the equilibrium constant for the reaction

$$2[MHL] \rightleftarrows [M_2(HL)_2] \qquad K'_D \qquad (5)$$

has a value of $\sim 10^4$ ($K'_D = K_D/K^2_{MHL}$), even at low [MHL] the final product should be largely in the binuclear form on completion of equilibration.

The question of the point or points of attachment of adenine and its derivatives to a metal center has been discussed by a number of workers, notably in recent reviews by Hodgson[13(a)] and Marzilli.[13(b)] These authors discuss both solution and solid complex studies which have contributed evidence

toward the problem. Frequently, it appears that for unidentate complexes N(9) is the active site (see diagram above) for neutral adenine but when this site is blocked by a non-coordinating substituent (as in 9-methyl adenine or the nucleosides) N(7) is usually observed to become the coordination position. Chelation possibilities can occur involving either the N(6)/N(7) or the N(3)/N(9) combination. No examples have been reported for the first possibility suggested but N(3)/N(9) chelation is observed for several copper(II)/adenine species. Another type of complexing involves multinuclear bridging by adenine, which consequently requires that two nitrogens of the base be involved. Typically, the N(7) and N(1) sites are the ones utilized. Summarizing, it appears that while coordination at several adenine sites is possible, unidentate attachment at N(9) when not blocked is the most frequently observed possibility. Our proposed structures for the final product of the Cr(III)/adenine reaction therefore find support from the available data concerning metal ion/adenine interaction, with the chelated possibility less likely.

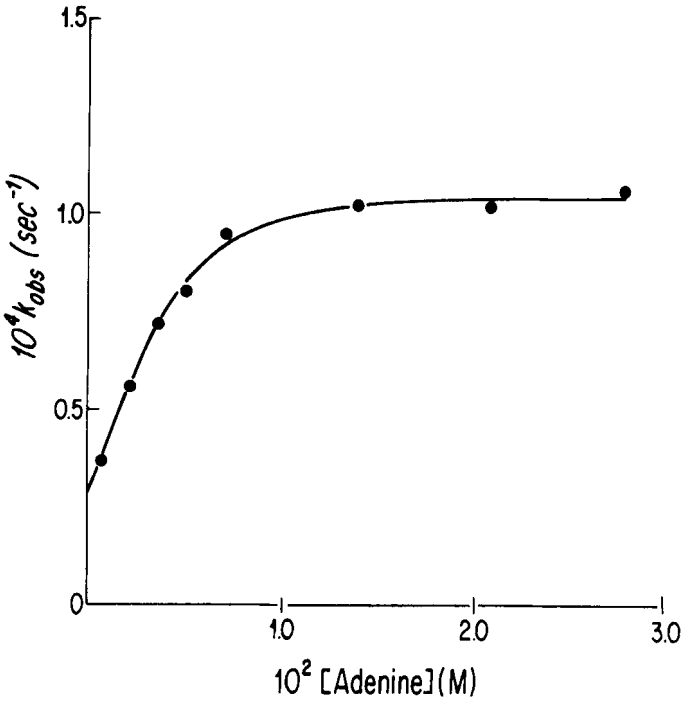


FIGURE 5 Variation of observed pseudo-first-order rate constant of reaction as a function of [adenine] at pH = 4.4, t = 60°, and $[Cr(H_2O)_6^{3+}]_0 = 7.0 \times 10^{-4}$ M.

Rate Studies

In view of the results already discussed, it may be logically assumed that the clean-cut and relatively rapid initial reaction consists of substitution of a adenine for water in the ionic aquochromium(III) species to form a 1:1 complex. The slow subsequent reaction probably involves structural rearrangements which appear from the data presented above to be formation of the stable μ-$(OH)_3$-bridged binuclear final product. Chelation of or bridging by adenine in the complex may also be involved in this process, though this seems to be unlikely as already pointed out.

Preliminary experiments showed that the complexation reaction is negligibly slow at room temperature whatever the pH, and that it occurs only to a slight extent (as shown by spectral measurements) below pH 3 even at 70°. Rate studies were therefore confined to the range $4 < pH < 5.5$,[14] and embraced the temperature range of 45° to 70°. In the first series of experiments, the dependence of the observed pseudo-first-order rate constant on total ligand concentration was studied at fixed pH (4.4) and temperature (60°). The rate data for these experiments[15] are presented in Figure 5, and are consistent with a mechanism involving a reverse path[16] and a limiting rate typical of the formation of an associative intermediate of the adenine ligand A (where A represents any of the three forms of adenine as defined by Eq. (1)) with the chromium-(III) species M prior to reaction. Such a mechanism can be symbolized in the form:

$$M + A \rightleftarrows M{\cdot}A \qquad K_A \qquad (6)$$

$$M{\cdot}A \rightleftarrows \text{Product} \qquad k_1, k_2 \qquad (7)$$

where M·A represents a relatively loosely-associated intermediate. The corresponding rate expression is:

$$k_{obs} = \frac{k_1 K_A [A]}{1 + K_A [A]} + k_2 \qquad (8)$$

which can be written in the inverse form

$$1/(k_{obs} - k_2) = \frac{1}{k_1 K_A [A]} + \frac{1}{k_1} \qquad (9)$$

One can obtain a value for k_2 by extrapolation of the curve in Figure 5 to zero ligand concentration ($k_2 \cong 2.9 \times 10^{-5}$ sec^{-1}). The data of Figure 5 (excluding the first point at lowest ligand concentration since it lies so far out on the inverse plot) can then be replotted according to Eq. (9). The values of the slope and intercept, as determined by least

TABLE II
Rate parameters for the interaction of aqueous chromium(III) with adenine

A. *Data obtained in presence of excess ligand concentration*

[Cr(III)] = 7.0×10^{-4} M; pH = 4.4; I = 0.5 M(KNO_3)

10^3 [Adenine]	$10^5 k_{obs}$ (sec^{-1})				
	45°	50°	60°	70°	80°
7.0	3.7	4.9	9.9	18.2	37.0
14.0	4.0	5.2	10.0	17.6	38.5
21.0	3.9	5.1	9.8	18.5	38.8
28.0	3.8	5.0	9.7	18.9	36.5
Averages	3.8 ± 0.1	5.0 ± 0.2	9.8 ± 0.2	18.3 ± 0.4	37.7 ± 0.8

B. *Data derived from Figure 6 at pH = 4.4 and various temperatures*

Temp. (°C)	$10^5 k_2$ (sec^{-1})	$10^3 k_1 K_A$ ($M^{-1} sec^{-1}$)	$10^5 k_1$[a] (sec^{-1})	K_A[b] (M^{-1})
45°	(0.8)[c]	(3.9)[c]	(3.0)	(130)
50°	1.2 ± 0.1	5.4 ± 0.4	3.8 ± 0.2	143 ± 13
60°	3.0 ± 0.3	10.9 ± 1.7	6.9 ± 0.4	158 ± 26
70°	6.7 ± 0.4	19.2 ± 1.2	11.6 ± 0.4	166 ± 12
80°	(14.6)[c]	(35.2)[c]	(23.1)	(152)
$\Delta H^{\neq}$ (Kcal mol^{-1})	18.2 ± 0.1	13.3 ± 0.9	11.6 ± 0.3	(1.7 ± 1.0)[d]
$\Delta S^{\neq}$ (cal deg^{-1} mol^{-1})	−24.8 ± 0.1	−27.9 ± 3.8	−43.0 ± 9.0	(15.1 ± 3.8)[d]

[a] Calculated by subtracting k_2 values from average k_{obs} values of Table IIA.
[b] Calculated by dividing col. 3 by col. 4.
[c] Extrapolated by use of the $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values derived from the experimental data at 50°, 60°, and 70°.
[d] Calculated by subtracting col. 4 from col. 3.

squares analysis, are $k_1 = (1.0 \pm 0.1) \times 10^{-4}$ sec^{-1} and $K_A = 195 \pm 25$ M^{-1}. Since the limiting value of k_{obs} should be $(k_1 + k_2)$ (see Eq. (8)), with a value of $\sim 1 \times 10^{-4}$ sec^{-1} (see Figure 5) it is clear that the double reciprocal plot procedure is not yielding a consistent value for k_1. This is not unexpected, since such plots yield notoriously inaccurate values for the intercept ($1/k_1$ in this case), due to the inherently large errors in the k_{obs} values at low ligand concentration, and the large magnification of these errors in the reciprocal plotting process. It was therefore deemed advisable to develop an alternative procedure for the evaluation of k_1 and K_A. In the presence of large excess of ligand, k_{obs} has an accurately determinable constant value as illustrated in Figure 5. Sets of data of this type obtained at various temperatures are presented in Table IIA. These are seen to be internally consistent at each temperature, so provide accurate values of $(k_1 + k_2)$ as already explained.

Another series of runs were carried out at low ligand concentrations, where $k_{obs} \cong k_1 K_A [A] + k_2$, so straight-line plots could be made of k_{obs} *vs* [A], as shown in Figure 6 for several temperatures.[17] Least squares analysis of these curves yield the values of k_2 (intercept) and $k_1 K_A$ (slope) tabulated in Table IIB. In order to completely utilize the data of Table IIA, extrapolated values of k_2 at 45° and 80° have been included in Table IIB, utilizing the temperature variation parameters for k_2 derived from the observations at the other three temperatures, also recorded in the Table. Then, since $k_1 = k_{obs} - k_2$, this rate constant is readily evaluated, enabling determination of K_A, and both of these calculated variables are included in Table IIB, together with the appropriate temperature parameters.

The study was now expanded in an attempt to determine the acid dependence of the complexation reactions. To accomplish this, one must take cognizance of the acid/base equilibria for the ligand itself as given in Eq. (1). The other relevant equilibria are:

$$Cr(H_2O)_6^{3+} \overset{K_1}{\rightleftarrows} Cr(H_2O)_5OH^{2+} \overset{K_2}{\rightleftarrows} Cr(H_2O)_4(OH)_2^{+} \quad (10)$$

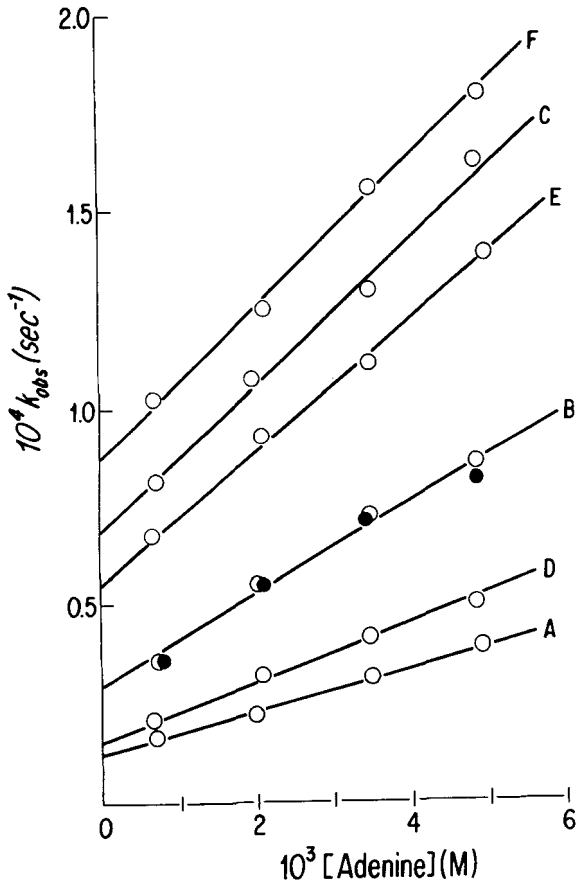
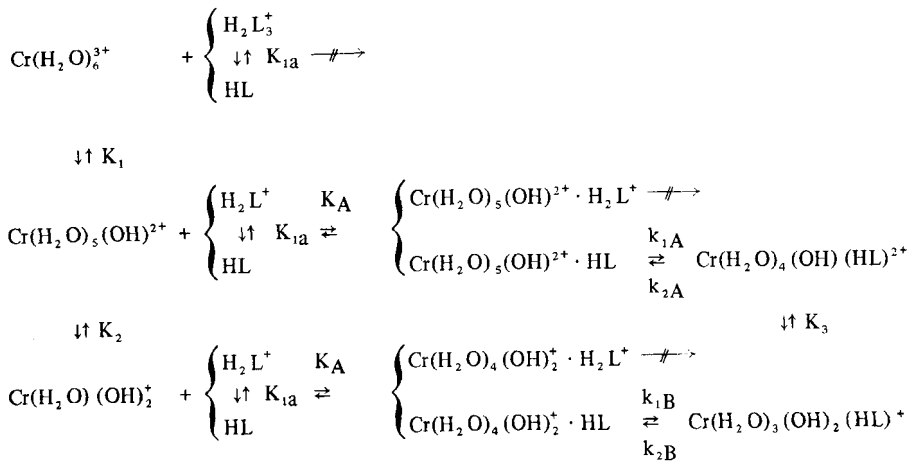


FIGURE 6 Variation of observed pseudo-first-order rate constant of reaction as a function of low values of [adenine] at various temperatures and pH values. Curve A, t = 50°, pH = 4.4; Curve B, t = 60°, pH = 4.4 (two independent sets of data); Curve C, t = 70°, pH = 4.4; Curve D, t = 60°, pH = 4.0; Curve E, t = 60°, pH = 4.7; Curve F, t = 60°, pH = 5.0. ($[Cr(H_2O)_6^{3+}]_0 = 7.0 \times 10^{-4}$ M in all runs).

for which the values[18(a)] of pK_1 and pK_2 under the experimental conditions of our acid dependence study are 3.4 and 5.0. A comparable adjustment in pK_{1a} for the adenine from 50° to 60° suggests[18(b)] a value of 3.9. First, a series of runs at low ligand concentration were made at 60° and various acidities other than the fixed pH of 4.4 of the earlier experiments, leading to the second sets of curves in Figure 6. From these could be derived $k_1 K_A$ and k_2 values as was done in the case of the temperature variation experiments, and the relevant data appear in Table IIIA. In proposing a mechanism to account for the data, one recalls that the reaction becomes very slow at pH's appreciably below 4. This indicates that either or both of the species H_2L^+ and $Cr(H_2O)_6^{3+}$ are inactive. The protonation of adenine at N(1), however, to form H_2L^+ does not involve the probable active site N(9) (see above), so it is a possible reactant, both in forming the associative reaction intermediate of Eq. (6), and in the subsequent substitution for water. Increased reactivity of the deprotonated aquochromium(III) species is a possibility by analogy to other systems, as will be discussed later. A credible reaction mechanism, which assumes inactivity to inner-sphere complexation (but not outer-sphere association) for both H_2L^+ and $Cr(H_2O)_6^{3+}$, can be formulated as shown in Scheme I. This mechanism, unlike Eqs. (6) and (7), specifically defines the various Cr(III) and ligand species, thus enabling subdivision of k_1 and k_2 as shown. It is further assumed that the associative intermediate forms more or less equally well with both the deprotonated Cr(III) species, since the extra proton on H_2L^+ is rather remote from the incipient bonding site, as already explained.

We can now deal with the variation of the



SCHEME I

TABLE III
Acid dependence of the complexation of aqueous chromium(III) by adenine at 60°

A. *Data derived from Figure 6 at 60° and various pH values*

pH	$10^5 k_2$ (sec^{-1})	$10^3 k_1 K_A$ ($M^{-1} sec^{-1}$)	$10^5 k_1$ (calc.)[a] (sec^{-1})	K_A(M^{-1})
4.0	1.7 ± 0.1	6.9 ± 0.2	3.8	182
4.4	3.0 ± 0.3	10.9 ± 0.8	6.4	170
4.7	5.7 ± 0.2	16.6 ± 0.7	7.5	221
5.0	8.8 ± 0.2	18.9 ± 0.6	10.3	183

[a] Taken directly or interpolated from data of Table IIIB

B. *Data obtained in presence of excess ligand concentration*

pH	$10^5 k_{obs}$ (sec^{-1})	$10^5 k_2$ (calc.)[a] (sec^{-1})	$10^5 k_1$[b] (sec^{-1})
4.0	5.3	1.5	3.8
4.2	7.0	2.3	4.7
4.4	9.8	3.4	6.4
4.8	14.5	6.6	7.9
5.1	21.0	9.5	11.5
5.4	24.5	12.1	12.4

[a] Smoothed calculated values obtained by use of derived k_{2B} and K_3 values.
[b] $k_1 = k_{obs} - k_2$.

separately determined k_2 values of Table IIIA as a function of $[H^+]$. The proposed reaction scheme leads to the relation:

$$k_2 = (k_{2A}[H^+] + k_{2B}K_3)/([H^+] + K_3) \quad (11)$$

However, if $k_{2B}K_3 \gg k_{2A}[H^+]$, Eq. (11) becomes

$$k_2[H^+] = k_{2B}K_3 - k_2K_3 \quad (12)$$

An exploratory plot of $k_2[H^+]$ *vs* k_2 produced a reasonably good straight line, confirming the assumption that $k_{2A}[H^+]$ makes a relatively small contribution to the total rate except at the lowest pH. Least squares analysis of the data yield a slope and intercept from which are derived the values $K_3 = (1.0 \pm 0.3) \times 10^{-5}$ M and $k_{2B} = (17 \pm 4) \times 10^{-5}$ sec^{-1}.[19] It is of interest to note that $K_3 \cong K_2$, indicating that the presence of adenine in the chromium(III) coordination sphere has a negligible effect on the acidity of the remaining water molecules.

In another series of runs, excess ligand was present at the various acidities, leading to values of $k_{obs} = k_1 + k_2$, as previously explained. These are given in Table IIIB, along with the corresponding smoothed calculated k_2 values[20] and the k_1 values derived therefrom. These latter figures, interpolated as necessary, appear in Table IIIA, and enable calculation of K_A at the various pH values quoted. Bearing in mind the large errors inherent in the determinations of K_A ($\sim \pm 15\%$), they are seen to be essentially independent of pH, supporting in part our earlier assumption with respect to this constant. It follows therefore (see Scheme I) that the equilibrium constant for the process $Cr(H_2O)_5OH^{2+} + HL \rightleftarrows Cr(H_2O)_4(OH)_2^+ \cdot HL$ should be about the same as $K_2 \cong K_3 \cong 1.0 \times 10^{-5}$ M. Furthermore, as seen in Table IIB, K_A is only slightly temperature dependent in the range 45°–80°, with $\Delta H < 2$ kcal mol^{-1}, and all the values at 60° except one are nicely bracketed within the range 175 ± 10 M^{-1}.

The variation of k_1 with pH is quite substantial, as seen in Table IIIB. In terms of the proposed mechanism, this variation in presence of excess ligand[21] should be governed by the relation:

$$k_1 = \frac{k_{1A}K_1[H^+]\left(\frac{K_{1a}}{[H^+] + K_{1a}}\right) + k_{1B}K_1K_2\left(\frac{K_{1a}}{[H^+] + K_{1a}}\right)}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (13)$$

A plot of $k_1([H^+]^2 + K_1[H^+] + K_1K_2)([H^+] + K_{1a})/K_{1a}$ against $[H^+]$ should therefore yield a

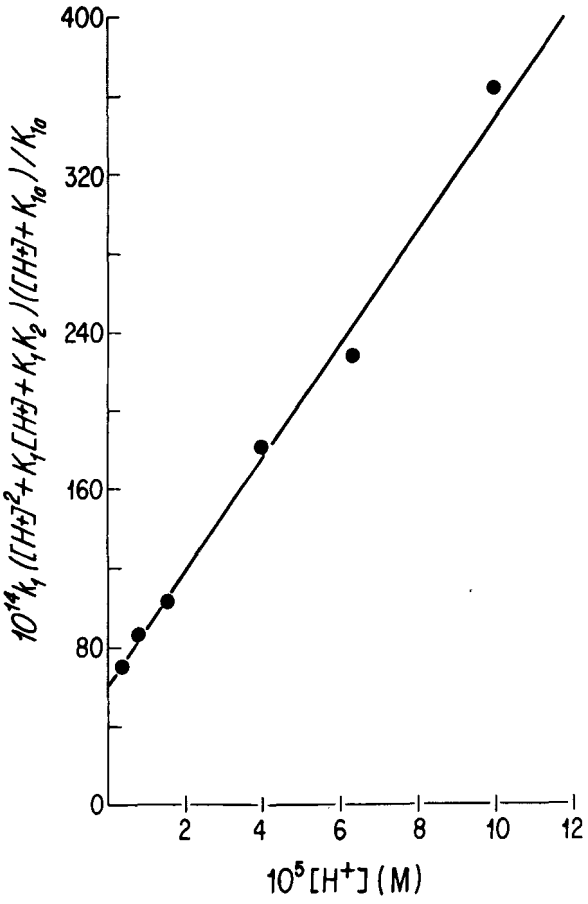


FIGURE 7 Graphical test of pH dependence of adenine/chromium(III) reaction at 60° according to Eq. (13) and data of Table IIIB.

straight line of intercept $k_{1B}K_1K_2$ and slope $k_{1A}K_1$. Such a plot is shown in Figure 7, and the values of k_{1A} and k_{1B} derived from least squares treatment of the data are $(7.5 \pm 0.4) \times 10^{-5}$ sec^{-1} and $(14.0 \pm 1.9) \times 10^{-5}$ sec^{-1}.

One final experimental test was made of the nature of the reaction mechanism by determining the variation of the value of k_{obs} in presence of excess ligand at 60° and pH = 4.4 as a function of the ionic strength. In all the rate experiments done so far, the latter was fixed at 0.5 M (KNO_3). Values of k_{obs} at this and other ionic strengths were as follows (the second k_{obs} value is from Table IIA and is more precise than the other):

$10^5 k_{obs}(sec^{-1})$				
	10.6 ± 0.5	9.8 ± 0.2	10.5 ± 0.5	9.2 ± 0.5
I(M)	0.25	0.50	0.75	1.00

These are seen to be practically invariant within experimental error. Since in our proposed mechanism $k_{obs} = k_1 + k_2$, and both k_1 and k_2 describe processes involving replacement of one uncharged species by another (adenine for H_2O and H_2O for adenine, respectively, within the coordination sphere of the complex ions involved) no appreciable ionic strength effects should indeed be observed.

In summary, our kinetics study shows that adenine forms a fairly stable outer-sphere complex with aquochromium(III) species, as indicated by the pH-independent association constant value of $K_A = 175 \pm 10\ M^{-1}$ at 60°. The lack of an appreciable pH dependence of this constant indicates that the adenine base shows little discrimination between the entities[22] $Cr(H_2O)_5OH^{2+}$ and $Cr(H_2O)_4(OH)_2^+$ so far as the associative reaction intermediate is concerned. However, the rates of substitution of adenine for water (k_{1A} and k_{1B}) do differ significantly ($k_{1B}/k_{1A} \sim 2$ at 60°), with the doubly deprotonated species having the advantage. The slowness of reaction with the hexaaquo parent ion is not unexpected, since such differences are frequently observed in the substitution reactions of octahedral aquo ions as compared to their deprotonated congeners. For example, in water exchange reactions of the type $Coen_2(H_2O)_2^{3+}/H_2^{18}O$ and $Rh(H_2O)_6^{3+}/H_2^{18}O$, the exchange rates increase by factors of about 100 for the $Co(en)_2(OH)(H_2O)^{2+}$ species[23] and of over one thousand for the $Rh(H_2O)_5OH^{2+}$ species.[24] Unfortunately, similar pH variation data are not available for the $Cr(H_2O)_6^{3+}$ ion, though its water exchange rate in acid solution is well established, as is the effect of added ions on this rate.[25] However, many kinetics studies have been made of the anation of both $Cr(H_2O)_6^{3+}$ and $Cr(H_2O)_5OH^{2+}$ ions. A fairly complete summation of such data[26] shows that the rate constants for such reactions are typically over 10^3 times greater for the deprotonated congener than for the parent aquo ion.

It is of value to compare in some detail the magnitudes of the rate constants obtained in our study with those derived from other relevant studies. Referring again to the ligand water exchange rate data[25] for $Cr(H_2O)_6^{3+}$, it is known that, with $[H^+] \sim 0.1$ M and $[NO_3^-] \sim 0.7$ M, $k_{ex} = 3.9 \times 10^{-6}$ sec^{-1} at 27°, with $E_a = 26.7$ kcal mol^{-1}. Extrapolation to 60° leads to a value of about 30×10^{-5} sec^{-1}, which somewhat exceeds both k_{1A} and k_{1B}, the constants which describe adenine-for-water interchange rates of $Cr(H_2O)_5OH^{2+}$ and $Cr(H_2O)_4(OH)_2^+$, respectively. The ligand water exchange rates of the latter two species would be expected to exceed

greatly the rate for the parent aquo ion, by analogy to the quoted water exchange data for cobalt(III) and rhodium(III) aquo complexes, and to the aquochromium(III) anation data. It is therefore clear that ligand water release is not the rate-determining step in the adenine interchange process. This concept is supported by the commonly held view[27] that chromium(III) complexes react by an associative interchange mechanism in which the nucleophilicity of the entering ligand is of major importance. The only other data involving replacement of water in an aquochromium(III) ion by an uncharged amine entity concern the $Cr(H_2O)^{3+}$/o-phenanthroline reaction,[28] which has a second order rate constant of 2.9×10^{-3} M^{-1} sec^{-1} at 60°. The appropriate comparable rate constants from the present study are $k_{1A}K_A$ and $k_{1B}K_A$, with values of 13×10^{-3} and 25×10^{-3} M^{-1} sec^{-1} at 60°, respectively. These, however, apply to replacement of water in $Cr(H_2O)_5OH^{2+}$ and $Cr(H_2O)_4(OH)_2^+$, respectively, so neither is directly comparable to the phenanthroline figure. It seems surprising, however, the o-phenanthroline should be so much more effective as a nucleophile than adenine, as evidenced by the negligible reactivity of the latter with $Cr(H_2O)_6^{3+}$.

It is of further interest to note that the rate constants for anation of $Cr(H_2O)_5OH^{2+}$ by Cl^-, Br^-, and NCS^- are all close to 2.5×10^{-5} M^{-1} sec^{-1} at 25° and I = 1.00 M (perchlorate).[26] Since the $\Delta H^{\neq}$ values for such anations[29] are typically of the order of magnitude of 25 kcal mol^{-1}, the rate constants at 60° should be about 2×10^{-3} M^{-1} sec^{-1}, within an order of magnitude of the limiting second rate constants for adenine substitution, as just discussed. For $HC_2O_4^-$ substitution at 60°, a figure of $\sim 30 \times 10^{-3}$ M^{-1} sec^{-1} is indicated,[30] again within the same range of values. Clearly, the presence of negative charge on the entering species is not necessarily a significant factor in these processes, at least in terms of the overall second-order rate constant. If the mechanism is in general an associative interchange within an intermediate with a formation constant K, as we have shown to be true for the adenine reaction, the observed second-order rate constants are given by $k = k_iK$ where k_i is the rate constant for the interchange. These k_i's can vary considerably, depending on entering ligand's nucleophilicity, so that the constant K must vary more or less inversely for the various reactions mentioned in order to maintain the relative constancy of k_iK. Thus the fact that only second-order rate constants have been observed in anations by charged species such as Cl^-, Br^-, NCS^-, or $HC_2O_4^-$ suggests that these are all good nucleophiles but that the association constants K are quite small in spite of the opposite charges on the reactants. One must therefore conclude that the adenine molecule has much more effective outer-sphere bond-making capacity with the deprotonated aquochromium(III) entity than do simple inorganic anions of the type mentioned.

Finally, it is of interest to note that the kinetics data provide a means of estimating the equilibrium constant for formation of the first-formed complex which we symbolized above (see Eq. (2)) as K_{MHL}. From Scheme I one sees that an estimate of this is given by the ratio k_1K_A/k_2. From the data of Table IIB, this ratio is seen to be about 500 at 50°, or $pK_{MHL} = 2.7$. This differs by an appreciable amount from our titrimetric determination of this constant ($pK_{MHL} = 3.5$). However, such a discrepancy is not unexpected, considering the differences in the experimental approaches and the substantial inaccuracies inherent in both methods of determination.

ACKNOWLEDGEMENT

Financial support of this work from the John D. and Frances H. Larkin Foundation of the State University of New York at Buffalo is gratefully recognized, as is the grant of a sabbatical leave to C. R. Krishnamoorthy by the Indian Institute of Technology, Madras.

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12. This assumption is borne out by the titration curves of Figure 2, where an immediate decrease in pH is seen to occur when chromium(III) is added to the adenine solution, in keeping with the reaction $H_2L + M \rightarrow H^+ +$ MHL even at low pH.
13. (a) D. J. Hodgson, *Prog. Inorg. Chem.*, **23**, 211 (1977); (b) L. G. Marzilli, *Prog. Inorg. Chem.*, **23**, 255 (1977).
14. Above pH 5.5, precipitate formation begins to complicate the process during rather early stages of the reaction. In the equilibrium studies, precipitation became a problem even at pH $\geqslant$4.5 due to the higher chromium(III) concentration employed in those studies.
15. For the runs in which adenine was not in greater than five-fold excess ($\geqslant 3.5 \times 10^{-3}$ M), the values of k_{obs} were deduced from the initial portions of the rate data only, where reasonable first-order kinetics prevailed.
16. The existence of the reverse path was further supported by the spectral data, which indicated that the higher the ligand concentration, the more complete the reaction becomes, as shown by the final absorbance.
17. Two independent sets of data were obtained at pH 4.4 and 60°, and both are plotted in Figure 6. These confirm the good reproducibility of the experiments.
18. (a) Various authors agree on a value of 4.0 ± 0.1 for pK_1 at 25° in media up to I = 1 molar (see Chem. Soc. Special Publication, **17**, 48 (1964); see Chem. Soc. Special Publication, **25**, 20 (1971)), and temperature dependence studies suggest 3.4 as a reasonable estimate for pK_1 at 60°. Few determinations have been made of pK_2, but we have accepted the figure of 5.6 at 25° reported by C. Schenk, H. Stieger and H. Kelm, *Zeit. anorg. allg. Chemie.*, **391**, 1 (1972). Assuming the same temperature variation for K_2 as for K_1, we adopt the value of 5.0 at 60° for pK_2; (b) Izatt and co-workers (see Ref. 10) have measured $\Delta H^\circ = 4.8$ kcal mol^{-1} for the first deprotonation of adenine. We have used this figure to adjust our measured value for pK_{1a} at 50° (Table IA) to a value at 60° of 3.9. In the pH range of our experiments, pK_{2a} is not involved.
19. A further test that the k_{2A} path makes a small contribution to the process is that the quantity $k_2([H^+] + K_3)$ shows no trend in values and is constant within the range 1.7 ± 0.2 M sec^{-1} for each of the k_2 and $[H^+]$ values of Table IIIA.
20. These are readily obtained from Eq. (12), setting $k_{2A}[H^+] = 0$.
21. Since the ligand concentration is in excess in all these runs, the significant reactants are $[Cr(H_2O)_5OH \cdot HL]^{2+}$ and $[Cr(H_2O)_4(OH)_2 \cdot HL\}^+$ which will be present in equilibrium amounts dictated by K_{1a}, K_1 and K_2, but independent of the magnitude of K_A.
22. Nothing definitive can be said concerning the parent acidic form $Cr(H_2O)_6^{3+}$ in this respect since there appears to be no measurable substitution of adenine for water in this species, so no kinetic evidence is available from which to deduce K_A.
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29. It is noteworthy that our value for $\Delta H_1^{\neq}$ which relates to the adenine-for-ligand water substitution reaction is considerably smaller than 25 kcal mol^{-1}, even after adding in the ΔH for the association reaction. The slowness of the adenine reaction is a result of the very negative $\Delta S^{\neq}$ value, a possible indication of a highly structured transition state.
30. C. Schenk, H. Stieger and H. Kelm, *Zeit, anorg. allg. Chemie.*, **391**, 1 (1972). The authors quote a rate constant of 2.4×10^{-3} M^{-1} min^{-1} at 25°, pH = 2.7 and I = 1.0 M, with $\Delta H^{\neq} = 26.6$ kcal mol^{-1}. At the pH specified most of the oxalate exists as $HC_2O_4^-$ and the chromium(III) as $Cr(H_2O)_6^{3+}$.