Articles

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Kinetics for pH-Dependent Complexation of Aquated *cis*-Diammineplatinum(II) with Inosine and 1-Methylinosine

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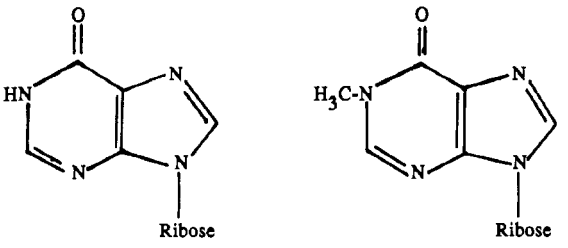
Kinetics of the complex formation between aquated *cis*-$Pt^{II}(NH_3)_2$ and inosine or 1-methylinosine has been studied by HPLC in aqueous solution over a pH range of 4.1–8.6 at 298.2 K. The complexation of aquated *cis*-$Pt^{II}(NH_3)_2$ can be completely explained by substitution of the aqua ligand(s) with incoming nucleosides. Deprotonation of one of the aqua ligands results in a 10-fold decrease in the displacement rate of the remaining one. In ligand excess, a subsequent formation of 1:1 and 1:2 complexes takes place. 1-Methylinosine forms only N7-bound complexes, which is also the predominant binding mode of neutral inosine. Deprotonation of inosine N1H makes this site available for Pt(II) and also facilitates N7 coordination. With anionic inosine, the intrinsic formation rates of the N1- and N7-bound 1:1 complexes appear to be equal. Nevertheless, the N7 binding mode predominates in the first complexation step throughout the pH range studied. With 1-methylinosine, the binding of the second nucleoside depends only on deprotonation of the remaining aqua ligand in the 1:1 complex. By contrast, introduction of the second ligand to the N7-bound inosine 1:1 complex is facilitated by deprotonation of N1H with concomitant attachment of the proton to the deprotonated OH group in the Pt(II) moiety. This gives a substitutionally labile aqua ligand, the reactivity of which is about one-third of that for the dication. In the N7-bound inosine 1:1 complexes, the N1H proton is acidified by 1.8 or 1.2 log units when the fourth ligand is a water molecule or a hydroxide ion. Intrinsically, anionic inosine exhibits almost an equal tendency to bind through the N1 and N7 sites to the differently charged N7-bound 1:1 complexes.

Introduction

Coordination of Pt(II) to 6-oxo-substituted purine nucleosides and related compounds has been the topic of numerous studies in the past two decades.[1] With neutral ligands, preferential binding to the N7 position of the base moiety is well established.[1-4] The prevailing keto tautomer requires proton at N1, which effectively prevents platination at this site, whereas coordination to N3 is blocked by the ribose group.[3] N1 binding mode may, however, become significant in neutral and slightly basic solution upon deprotonation of N1H. Excess of monofunctional Pt(II) usually leads to mixtures of N1-platinated, N7-platinated, and N1,N7-diplatinated complexes[5] or in some cases to N1,N3,N7-triplatinated complexes.[6] Bifunctional platinum(II) yields in equimolar mixtures of the ligand and Pt polymeric species involving Pt(II) N1,N7 bridging[7] or N1,N7-diplatinated compounds in excess Pt.[7b,8] An exclusive N1 coordination has been observed with ligands bearing an alkyl group at N7.[9]

Despite these observations, little is known about the quantitative distribution of Pt(II) between the N1 and N7 sites in 6-oxo-substituted purine nucleosides. The lack of thermodynamic data can be attributed to the general inertness of Pt(II) compounds toward substitution reactions,[10] whereas quantitative kinetic studies deal only with the complexation of Pt(II) with nucleosides[11] or nucleotides[12] in acidic medium. In this paper, we wish to report the kinetics for the formation of 1:1 and 1:2 complexes of aquated *cis*-$Pt^{II}(NH_3)_2$ with inosine and 1-methylinosine in the pH range 4.1–8.6 in aqueous solution at 298.2 K (Chart I). The main purpose was to study in detail the reaction pathway for different complexes giving special emphasis to (i) the effect of pH on the reactivity of the aqua ligands in *cis*-$Pt^{II}(NH_3)_2$ and in 1:1 complexes, (ii) the pH-dependent distribution of various Pt(II) species between the N1 and N7 sites in inosine, and (iii) the influence of N7-bound Pt(II) charge type on the acidity of inosine N1H.

Chart I



Inosine (InoH) 1-Methylinosine (MeIno)

Experimental Section

Materials. Inosine and 1-methylinosine were purchased from Sigma and they were used as received.[13] Aquated *cis*-$Pt^{II}(NH_3)_2$ and its N7-bound 1:1 complex with inosine were prepared as previously described.[14]

Kinetic Measurements. The complexation of aquated *cis*-$Pt^{II}(NH_3)_2$ with both nucleosides in buffered aqueous solution (pH 4.2–8.6) was

(1) For a recent review, see: Lippert, B. In *Progress in Inorganic Chemistry*; Lippard, S. J., Ed.; Wiley: New York, 1989; Vol. 37, pp 1–97.
(2) (a) Reedijk, J. *Pure Appl. Chem.* **1987**, *59*, 181–192. (b) Lippard, S. J. *Pure Appl. Chem.* **1987**, *59*, 731–742. (c) Lippert, B. *Gazz. Chim. Ital.* **1988**, *118*, 153–165.
(3) Marzilli, L. G. In *Advances in Inorganic Biochemistry*; Eichhorn, G. L., Marzilli, L. G., Eds.; Elsevier/North-Holland: New York, 1981; Chapter 2.
(4) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.
(5) (a) den Hartog, J. H. J.; Salm, M. L.; Reedijk, J. *Inorg. Chem.* **1984**, *23*, 2001–2005. (b) van der Veer, J. L.; van den Elst, H.; Reedijk, J. *Inorg. Chem.* **1987**, *26*, 1536–1540. (c) Arpalahti, J.; Lehikoinen, P. *Inorg. Chem.* **1990**, *29*, 2564–2567.
(6) Raudaschl-Sieber, G.; Schöllhorn, H.; Thewalt, U.; Lippert, B. *J. Am. Chem. Soc.* **1985**, *107*, 3591–3595.
(7) (a) Chu, G. Y. H.; Tobias, R. S. *J. Am. Chem. Soc.* **1976**, *98*, 2641–2651. (b) Chu, G. Y. H.; Mansy, S.; Duncan, R. E.; Tobias, R. S. *J. Am. Chem. Soc.* **1978**, *100*, 593–606.
(8) Dijt, F. J.; Canters, G. W.; den Hartog, J. H. J.; Marcelis, A. T. M.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 3644–3647.
(9) Shinozuka, K.; Wilkowski, K.; Heyl, B. L.; Marzilli, L. G. *Inorg. Chim. Acta* **1985**, *100*, 141–150 and references cited therein.
(10) Basolo, F.; Pearson, R. G. *Mechanisms of Inorganic Reactions*; Wiley: New York, 1967; Chapter 5.
(11) (a) Eapen, S.; Green, M.; Ismail, I. M. *J. Inorg. Biochem.* **1985**, *24*, 233–237. (b) Arpalahti, J.; Lippert, B. *Inorg. Chem.* **1990**, *29*, 104–110.
(12) (a) Evans, D. J.; Ford, N. R.; Green, M. *Inorg. Chim. Acta* **1986**, *125*, L39–L40. (b) Evans, D. J.; Green, M.; van Eldik, R. *Inorg. Chim. Acta* **1987**, *128*, 27–29. (c) Inagaki, K.; Dijt, F. J.; Lempers, E. L. M.; Reedijk, J. *Inorg. Chem.* **1988**, *27*, 382–387. (d) Laoui, A.; Kozelka, J.; Chottard, J.-C. *Inorg. Chem.* **1988**, *27*, 2751–2753.
(13) Both nucleosides were found to be free from contaminants of crystallization liquids.[11b]
(14) Arpalahti, J.; Lehikoinen, P. *Inorg. Chim. Acta* **1989**, *159*, 115–120.

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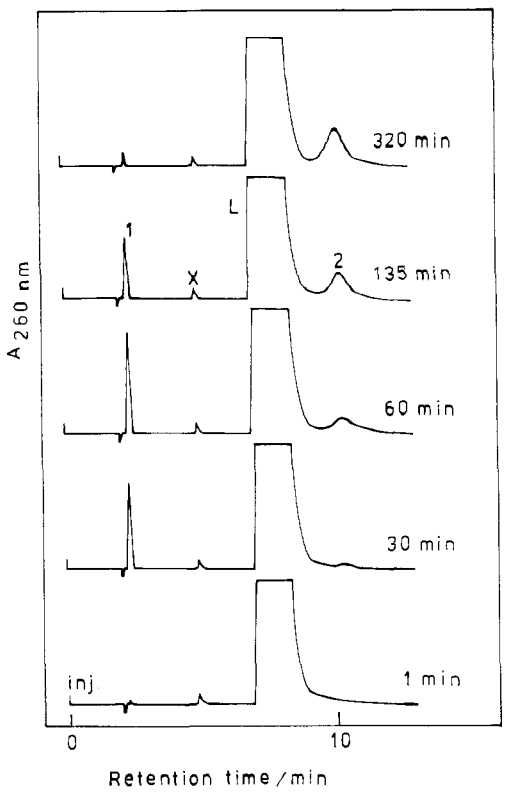


Figure 1. LC elution profiles of the mixture of aquated *cis*-$Pt^{II}(NH_3)_2$ (4×10^{-4} M) and 1-methylinosine (0.038 M) in triethanolamine buffer (pH 6.92) at selected time intervals using aqueous $NaNO_3$ (0.05 M) in 1 mM HNO_3 containing 9% MeOH as an eluant. Notation: (1) N7 bound 1:1 complex; (2) N7 bound 1:2 complex; (X) unknown impurity of 1-methylinosine (L).[24]

followed at 298.2 K by HPLC as previously described.[5c] High excess of the ligand ($[L]_T$:$[Pt]_T \geq 20:1$) provided pseudo-first-order conditions for the complexation, while the total concentration of Pt(II) in the reaction mixture was kept below 0.4 mM to avoid the influence of possible side reactions on the complex formation.[15] Buffers prepared from sterically hindered nitrogen bases and HNO_3 were employed to maintain the pH during kinetic runs.[5c] Samples withdrawn from the reaction mixture at suitable time intervals were made alkaline to stop the complex formation (pH >11), after which the samples were chromatographed on an RP-18 column. The eluents, 0.05 M $NaNO_3$ and 1 mM HNO_3 in water–methanol mixtures (100:0–82:18), were thoroughly degassed by sonication under reduced pressure. Signal height was used as the measure of concentration in all cases.

The time-dependent concentration of the N7-bound 1:1 complex was employed to calculate the pseudo-first-order rate constants for the formation and disappearance of this species. The rate constants were obtained by eq 1 using least-squares fitting.[16] Here $[M]_0$ is the initial

$$[ML]_t = [M]_0 \frac{k'_{(a,N7)obs}}{k'_{b,obs} - k'_{a,obs}} (e^{-k'_{a,obs}t} - e^{-k'_{b,obs}t}) \quad (1)$$

concentration of Pt(II), and $[ML]_t$ stands for the concentration of the N7-bound 1:1 complex at the moment t. $k'_{(a,N7)obs}$ denotes the rate constant for the formation of N7-bound 1:1 complex, while $k'_{a,obs}$ is the total rate constant for the formation of all 1:1 complexes. The term $k'_{b,obs}$ represents the total rate constant for the conversion of the N7-bound 1:1 complex to 1:2 complexes. A calibration sample of ML, prepared at pH 3 from a known amount of ligand in Pt(II) excess, was used to transform peak heights into concentrations. It is known that Pt(II) predominantly forms N7-bound 1:1 complex under these conditions.[5c]

The employment of the isolated N7-bound 1:1 complex as a starting material provided an alternative way to study the formation of different 1:2 complexes. Both inosine and its methyl derivative were employed as an incoming ligand. Pseudo-first-order rate constants, $k'_{i,obs}$, for the disappearance of the complex were obtained from eq 2, in which $[ML]_0$

$$\ln [ML]_t = -k'_{i,obs}t + \ln [ML]_0 \quad (2)$$

denotes the initial concentration of the 1:1 complex. A calibration sample, prepared at pH 3 from a known amount of ML in excess of inosine, was used to calculate the extent of N7 coordination in inosine 1:2 complexes.

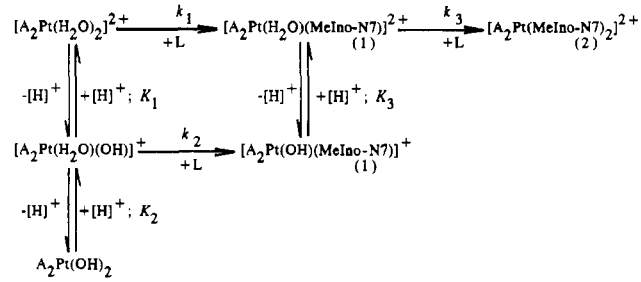
(15) The ionic strength was adjusted to 0.1 M with $NaClO_4$.
(16) Ruckdeschel, F. R. *BASIC Scientific Subroutines*; BYTE/McGraw-Hill: Peterborough, NH, 1981; Vol. II, p 75.

Table I. Observed Second-Order Rate Constants, $k_j/10^{-3}$ M^{-1} s^{-1}, for the Formation of 1:1 and 1:2 Complexes between Aquated *cis*-$Pt^{II}(NH_3)_2$ Ion and 1-Methylinosine in Buffered Aqueous Solution (pH 4.5–8.3) at 298.2 K[a]

pH	$k_{a,N7}$	k_b	pH	$k_{a,N7}$	k_b
4.58	104	76	6.21	30	23
4.82	103	78	6.54	17	13
5.21	85	63	6.92	12	5
5.52	71	53	7.34	5	1.5
5.66	57	50	8.30	1	0.3
6.18	30	25			

[a] In 0.1 M $NaClO_4$. Data obtained by eq 1.

Scheme I



Results and Discussion

1-Methylinosine. Figure 1 depicts the chromatographic analysis of the reaction mixture of aquated *cis*-$Pt^{II}(NH_3)_2$ and 1-methylinosine in a triethanolamine buffer (pH 6.92). In excess of the ligand consecutive formation of 1:1 and 1:2 complexes was observed by HPLC. On the basis of the elution order, compound **1** is assigned to a 1:1 complex and **2** to a 1:2 complex. These were the only products detected throughout the pH range studied.[17] In both complexes, Pt(II) is assumed to exhibit N7 coordination, analogous to that observed previously for the monofunctional Pt^{II}(dien).[5c] The observed second-order rate constants, $k_{(a,N7)obs}$ and $k_{b,obs}$, for the formation of compounds **1** and **2** are recorded in Table I. The data were obtained by eq 1, where the rate constants $k_{a,obs}$ and $k_{(a,N7)obs}$ are in this case equal, because of a single available coordination site. Similarly, $k_{b,obs}$ represents only the 1:2 complex, in which both ligands are coordinated to Pt through N7. Attempts to study the complex formation in excess Pt(II) were unsuccessful. The disappearance of the free ligand, L, did not obeyed the pseudo-first-order rate law under these conditions. Rather than being linear, the plots of ln [L] vs t showed upfield curvature in the progress of the reaction, especially at higher pH. This suggests a decrease in the effective Pt(II) concentration during the complex formation. It is known that aquated *cis*-$Pt^{II}(NH_3)_2$ forms dimers and higher aggregates through OH bridges in neutral solution.[18] Although these OH-bridged species are quite stable and inert toward substitution reactions, the suggested intermediates containing aqua ligands may react with nucleosides.[19] Hence, the complex formation in excess Pt(II) becomes difficult to analyze on the basis of the

(17) This observation suggests that the buffer–Pt interactions are negligible. Although triethanolamine is not absorbing at 260 nm, the replacement of the coordinated aqua ligand in 1:1 complex with a buffer molecule gives a UV-absorbing product, the retention time of which should be different from the aquated 1:1 complex.
(18) (a) Gray, H. B.; Olcott, R. J. *Inorg. Chem.* **1962**, *1*, 481–485. (b) Lim, M. C.; Martin, R. B. *J. Inorg. Nucl. Chem.* **1976**, *38*, 1911–1914. (c) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Rosenberg, B. *J. Am. Chem. Soc.* **1977**, *99*, 777–781; *Inorg. Chem.* **1977**, *16*, 1192–1196. (d) Boreham, C. J.; Broomhead, J. A.; Fairlie, D. P. *Aust. J. Chem.* **1981**, *34*, 659–664. (e) Appleton, T. G.; Hall, J. R.; Ralph, S. F.; Thompson, C. S. M. *Inorg. Chem.* **1989**, *28*, 1989–1993.
(19) Appleton, T. G.; Berry, R. D.; Davis, C. A.; Hall, J. R.; Kimlin, H. A. *Inorg. Chem.* **1984**, *23*, 3514–3521.

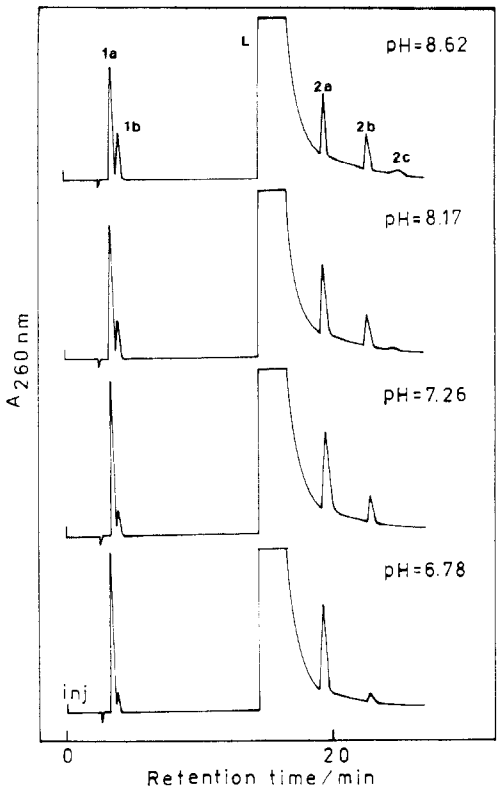


Figure 2. LC gradient elution profiles of the mixtures of aquated *cis*-$Pt^{II}(NH_3)_2$ (4×10^{-4}) and inosine (L, 0.04–0.1 M) in triethanolamine buffers of different pHs using 0.05 M $NaNO_3$ and 1 mM HNO_3 in water–methanol mixtures as an eluent (linear gradient from 100:0 to 82:18 in 10 min, 7-min initial delay, flow rate 0.8 mL/min). The chromatograms were recorded at the moment peak **1a** reached its maximum, i.e. 30, 45, 60, and 90 min after mixing (from bottom to top). Notation of the complexes as Pt(II) binding sites: (**1a**) N7, (**1b**) N1, (**2a**) N7,N7, (**2b**) N1,N7, (**2c**) N1,N1.

time-dependent concentration of the ligand.

As can be seen from Table I, the rate constants for both complexes decrease with increasing pH. Since 1-methylinosine acts as a neutral ligand throughout the pH range studied, the observed decrease in the complexation rate must be attributed to deprotonation of the Pt(II) diaqua cation, which yields substitution-inert hydroxo species.[18a,b] Accordingly, the stepwise complex formation of aquated *cis*-$Pt^{II}(NH_3)_2$ with 1-methylinosine (L) may be depicted by Scheme I. The observed rate constant, $k_{(a,N7)obs}$, for the formation of the N7-bound 1:1 complex, can be expressed by eq 3. Here K_1 and K_2 denote the acidity constants of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and *cis*-$[Pt(NH_3)_2(H_2O)(OH)]^+$ ions, respectively. Least-squares fitting to the kinetic data gave the value 0.114 M^{-1} s^{-1} for k_1 and 8.3×10^{-3} M^{-1} s^{-1} for k_2. The former agrees well with the value of 0.126 M^{-1} s^{-1} reported earlier for k_1 at pH 4.[11b] The acidity constants, $K_1 = 10^{-5.64}$ M and $K_2 = 10^{-7.40}$ M, are in a good agreement with those reported in the literature.[20] The values obtained for k_1 and k_2 show that the aqua ligand is displaced from the *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ ion 10 times more readily than from the monocation *cis*-$[Pt(NH_3)_2(H_2O)(OH)]^+$.

$$k_{(a,N7)obs} = \frac{k_1[H^+]^2 + k_2K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (3)$$

Similarly, the observed decrease of $k_{b,obs}$ with increasing pH refers to deprotonation of the remaining aqua ligand in the 1:1 complex. Hence, $k_{b,obs}$ can be expressed by eq 4, where K_3 stands

$$k_{b,obs} = k_3\frac{[H^+]}{[H^+] + K_3} \quad (4)$$

Scheme II

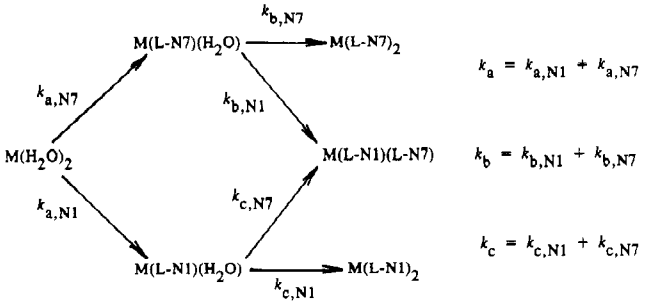


Table II. Observed Rate Constants, $k_{j,obs}/10^{-3}$ M^{-1} s^{-1}, for the Formation of Different 1:1 and 1:2 Complexes between Aquated *cis*-$Pt^{II}(NH_3)_2$ Ion and Inosine in Buffered Aqueous Solution (pH 4.1–8.6) at 298.2 K[a]

pH	k_a	$k_{a,N1}$[b]	$k_{a,N7}$	k_b	$k_{b,N1}$	$k_{b,N7}$[c]	k_d[d]
4.12[e]							74.1
4.20[e]	122		*f*	73.6			
4.48	120			74.5			72.4
4.51	116			70.0			
4.78							68.6
4.82	112			67.3			
5.13							61.7
5.30	86.1			62.3			
5.46	78.0			55.0			
5.49							50.4
5.68	64.4			45.2			
5.80	52.6			36.6			
5.95							30.6
6.02	40.3			29.1			
6.16	34.1			24.8			
6.27							18.6
6.53	23.2			13.9			11.6
6.78	21.2	1.8[g]	19.4	9.9			
6.87				7.1[d]	1.1	6.0	
6.99							4.8
7.25				4.1[d]	0.9	3.2	
7.26	11.7	1.6[g]	10.1	4.3			
7.49				3.2[d]	0.7	2.5	
7.52							1.7
7.54	8.7	1.4	7.2	3.5			
7.75				2.5[d]	0.6	1.9	
7.82	6.6	1.4	5.2	2.8			
7.98				2.1[d]	0.6	1.5	0.6
8.17	4.4	1.3	3.1	1.9			
8.35				1.6[d]	0.5	1.1	
8.60							0.2
8.62	2.9	1.0	1.9	1.1			

[a] See footnote *a* in Table I. [b] $k_a = k_{a,N1} + k_{a,N7}$. [c] $k_b = k_{b,N1} + k_{b,N7}$. [d] Obtained by eq 2. [e] Unbuffered solution. [f] $k_a = k_{a,N7}$ below pH 6.5. [g] Inaccurate, not included in computations.[20]

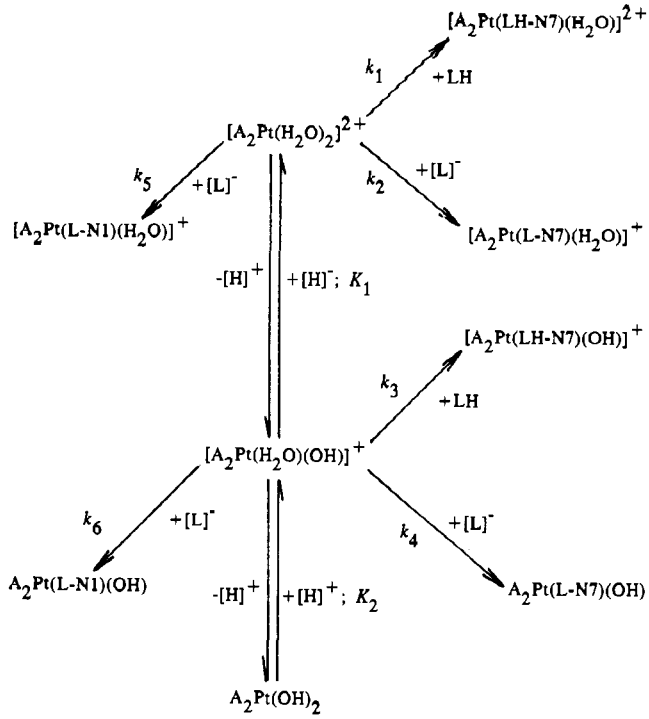
for the acidity constants of the aqua ligand in compound **1**. The values obtained by least-squares fitting were $k_3 = 0.081$ M^{-1} s^{-1} and $K_3 = 10^{-5.79}$ M. The former agrees well with the value 0.079 M^{-1} s^{-1} found earlier for the same system at pH 4.[11b] The acidity constant of the coordinated aqua ligand in compound **1** is similar to the pK_a value of 5.9 reported for the aqua ligand in the corresponding complex of 1-methylcytosine.[21]

Inosine. Chromatographic analysis of the mixtures of aquated *cis*-$Pt^{II}(NH_3)_2$ and inosine at different pH values are shown in Figure 2. Below pH 6.5 only products **1a** and **2a** are detected. On the basis of their time-dependent concentration, the former is assigned to 1:1 and the latter to 1:2 complex. Most probably both **1a** and **2a** have Pt(II) coordinated to the N7 site of inosine. At pH >6.5 three additional products appear. Compound **1b** is assigned to the N1-platinated 1:1 complex. Compound **2b** denotes a 1:2 complex, in which one ligand binds to Pt(II) through N7

(20) $pK_1 = 5.56$; $pK_2 = 7.32$. Jensen, K. A. *Z. Anorg. Allg. Chem.* **1939**, *242*, 87–91. See also ref 18e.

(21) Britten, J. F.; Lippert, B.; Lock, C. J. L.; Pilon, P. *Inorg. Chem.* **1982**, *21*, 1936–1941.

Scheme III



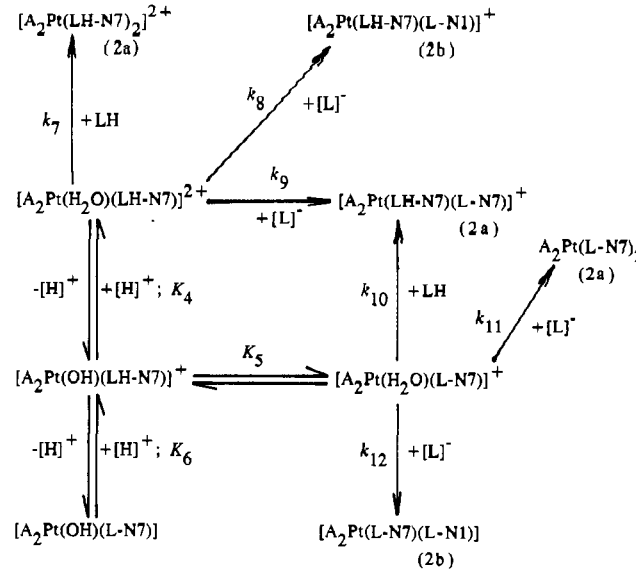
and the other through the N1 site. The fact that isolated **1a** gave in an excess of inosine product **2b** at pH >6.5 strongly supports this assignment. Product **2c** is formed in detectable amounts only at pH >8. Evidently this compound is a 1:2 complex, in which the *cis*-$Pt^{II}(NH_3)_2$ unit links two inosine molecules through their N1 sites. The simplified reaction pathway of aquated *cis*-$Pt^{II}(NH_3)_2$ (M) in excess of inosine (L) may thus be expressed by Scheme II, in which charges and protolysis of the aqua ligands are omitted for clarity.

Table II records the observed second-order rate constants for the formation of different complexes. Since the N7 binding mode predominates at pH <6.5, $k_{(a,N7)obs}$ and $k_{a,obs}$ are approximately equal under these conditions. Similarly, $k_{b,obs}$ represents only the rate constant for compound **2a** under these conditions, though at pH 6.5 a small amount of compound **2b** already can be detected. At pH >6.5, instead, the N1 binding mode must be taken into account. Thus $k_{a,obs}$ stands for the total rate constant for the formation of N7- and N1-bound 1:1 complexes, while $k_{b,obs}$ is the total rate constant for the binding of the second nucleoside, either through N7 or N1, to compound **1a**. Table II also lists the rate constants $k_{(b,N7)obs}$ and $k_{(b,N1)obs}$ for the formation of **2a** and **2b**, respectively, which were obtained from the sum constant $k_{b,obs}$, as shown in Scheme II. For this purpose $k_{b,obs}$ was measured in high ligand excess by using a known amount of isolated **1a** as a starting material. The amount of N7,N7-bound 1:2 complex formed was obtained by employing a standard sample of **2a** for calibration. As can be seen in Table II, the values obtained for $k_{b,obs}$ by different methods agree rather well, which lends support to the validity of the data.

Although the complex formation via the N7-bound 1:1 complex 1a could be independently studied in detail, the situation involving the N1-bound 1:1 complex **1b** is more complicated. Attempts to isolate chromatographically compound **1b** in preparative scale gave a mixture of both 1:1 complexes even at pH >8, because of insufficient resolution. Hence, we were not able to transform the signal height of **1b** to the concentration with reasonable accuracy.[14] For the same reason the overall rate constant k_c could not be bisected to its components, $k_{c,N1}$ and $k_{c,N7}$. Inspection of the complex formation via the N1-bound species is thus limited to the formation of compound **1b**. The observed rate constant, $k_{(a,N1)obs}$, for the formation of this species is readily obtained from the rate constants $k_{a,obs}$ and $k_{(a,N7)obs}$ as shown in Scheme II.

The pH-dependent rate data in Tables I and II reveal that the complexation rate of both nucleosides decreases with increasing pH. However, the involvement of N1 as an additional binding site makes the complex formation of inosine far more complicated than that of 1-methylinosine. The formation of 1:1 and 1:2 complexes are thus treated separately in the following. The assumed pathway for the formation of different 1:1 complexes is depicted in Scheme III. The observed second-order rate constants, $k_{(a,N1)obs}$ and $k_{(a,N7)obs}$, for the formation of N1- and N7-bound species may be expressed by eqs 5 and 6, respectively.

Scheme IV



$$k_{(a,N1)obs} = \frac{(k_5[H^+]^2 + k_6K_1[H^+])K_a}{([H^+]^2 + K_1[H^+] + K_1K_2)([H^+] + K_a)} \quad (5)$$

$$k_{(a,N7)obs} = \frac{k_1[H^+]^3 + k_2K_a[H^+]^2 + k_3K_1[H^+]^2 + k_4K_1K_a[H^+]}{([H^+]^2 + K_1[H^+] + K_1K_2)([H^+] + K_a)} \quad (6)$$

In both equations, $K_1 = 10^{-5.64}$ M and $K_2 = 10^{-7.40}$ M denote the known acidity constants of the *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ ion (vide supra), and $K_a = 10^{-8.8}$ M is the known acidity constant of the ligand.[4] Least-squares fitting gave the value of 1.8 M^{-1} s^{-1} for k_5 and 0.04 M^{-1} s^{-1} for k_6. By contrast, a similar treatment of the rate data of the N7-bound species was less satisfactory. Although the fitted values, $k_1 = 0.12$ M^{-1} s^{-1}, $k_4 = 0.063$ M^{-1} s^{-1}, and $k_3 = 0.010$–0.013 M^{-1} s^{-1}, were practically independent of the guessed values given initially, no reliable value could be obtained for the term k_2. In fact, when the term $k_2K_a[H^+]^2$ was omitted from eq 6, the fitted values of k_1, k_3, and k_4 remained practically unchanged. Accordingly, the reaction between the Pt(II) diaqua ion and anionic inosine contributes only a little to the overall rate. This is expected, if the difference of about 3 log units in the pK_a values of these species is taken into account. The value found for k_1 is in reasonable agreement with that reported earlier[11b] for the same system, viz. 0.135 M^{-1} s^{-1}. Comparison of the rate constants k_4 and k_6 reveals that the *cis*-$[Pt(NH_3)_2(H_2O)(OH)]^+$ ion almost equally distributes between the N7 and N1 sites in anionic inosine, which parallels the behavior of aquated Pt^{II}(dien), though the latter more clearly favors N7 coordination.[5c] Unfortunately, the rate data do not permit any firm conclusions about the binding behavior of the Pt(II) diaqua cation with anionic inosine. Nevertheless, the substitution rates of the aqua ligands in different Pt(II) species seem to obey the same trend as observed for the complex formation with 1-methylinosine.

The complexation pathway assumed for the binding of the second inosine to the N7-bound 1:1 complex is depicted in Scheme IV. The observed second-order rate constants, $k_{(b,N1)obs}$ and $k_{(b,N7)obs}$ for compounds **2b** and **2a** may be expressed by eqs 7 and 8, respectively. Here K_a denotes the acidity constant of inosine

$$k_{(b,N1)obs} = \frac{(k_8[H^+]^2 + k_{12}K_4K_5[H^+])K_a}{\{[H^+]^2 + (K_4 + K_4K_5)[H^+] + K_4K_6\}([H^+] + K_a)} \quad (7)$$

$$k_{(b,N7)obs} = \frac{k_7[H^+]^3 + k_9K_a[H^+]^2 + (k_{10}[H^+] + k_{11}K_a)K_4K_5[H^+]}{\{[H^+]^2 + (K_4 + K_4K_5)[H^+] + K_4K_6\}([H^+] + K_a)} \quad (8)$$

N1H. The equilibrium constants K_4, K_5, and K_6 are those depicted in Scheme IV. Applying least-squares fitting directly to eqs 7 and 8 is, however, rather speculative. Although the number of adjustable parameters in eq 7 is reasonable, the kinetic data are obtained with sufficient accuracy only over a narrow pH range. On going to eq 8, the kinetic data are spread over a wider pH range, but the number of parameters is also increased. To improve the reliability of the numerical treatment, the constants K_4, K_5, and K_6 were calculated from the data observed for the binding of 1-methylinosine to the N7-bound 1:1 complex of inosine (Table II). The reaction pathway for the formation of this mixed-ligand 1:2 complex corresponds to that of compound **2b** in Scheme IV except that the incoming 1-methylinosine does not undergo side reactions under these conditions. Accordingly, the observed rate constant, $k_{d,obs}$, can be expressed by ignoring the term $K_a/([H^+] + K_a)$ in eq 7 and substituting the terms k_{d1} and k_{d2} for k_8 and k_{12}. The values obtained by least-squares fitting were $k_{d1} = 0.076$ M^{-1} s^{-1} and $k_{d2} = 0.032$ M^{-1} s^{-1} for the complexation of 1-methylinosine with $[A_2Pt(H_2O)(LH\text{-}N7)]^{2+}$ and $[A_2Pt(H_2O)(L\text{-}N7)]^+$ ions, respectively. The fitted values for the equilibrium constants were $K_4 = 10^{-5.78}$ M, $K_5 = 10^{-1.2}$, and $K_6 = 10^{-7.57}$ M. If the equilibrium described by K_5 in Scheme IV is neglected, the fitted values for k_{d1} and K_4 are not markedly changed, but K_6 becomes negative and the standard deviation of the fit considerably increases. It is therefore assumed that the reaction pathway depicted in Scheme IV is correct. The value obtained for K_4 is compatible with the acidity constant of the aqua ligand in the $[Pt(NH_3)_2(MeIno\text{-}N7)(H_2O)]^{2+}$ ion, viz. $10^{-5.79}$ M.

Deprotonation of the aqua ligand in *cis*-$[Pt(NH_3)_2(H_2O)(LH\text{-}N7)]^{2+}$ ion followed by proton transfer formally from N1H to the OH group bound to Pt(II) represents, in fact, deprotonation of N1H in this compound. From the properties of a cyclic system the acidity constant of N1H can be obtained as $K_4K_5 = 10^{-6.98}$ M. Thus, in the *cis*-$[PtNH_3)_2(H_2O)(LH\text{-}N7)]^{2+}$ ion the inosine N1H proton is acidified by about 1.8 log units, which agrees with findings reported earlier for similar systems.[5-8,22] In contrast, the influence of Pt(II) on the N1H acidity in the *cis*-$[PtNH_3)_2(OH)(LH\text{-}N7)]^+$ ion is only about 1.2 log units, as evidenced by the value of the acidity constant K_6. This indicates that changes in the overall charge at the Pt(II) moiety markedly affect the acid–base properties of the N1 site.

Substitution of the values obtained for K_4, K_5, and K_6 in eq 7 gave $k_8 = 1$ M^{-1} s^{-1} and $k_{12} = 0.2$ M^{-1} s^{-1} for the formation of **2b**. Similarly, eq 8 gave the rate constants $k_7 = 0.08$ M^{-1} s^{-1}, $k_9 = 1$ M^{-1} s^{-1}, $k_{10} = 0.03$ M^{-1} s^{-1}, and $k_{11} = 0.3$ M^{-1} s^{-1} for the formation of **2a**. Comparison of the data allows the following conclusions. First, neutral inosine and 1-methylinosine behave kinetically in the same manner, as evidenced by the similarity of the rate constants k_{d1} and k_7, or k_{d2} and k_{10}. This is also evident on the basis of the data obtained for the 1:1 complexes. Second, anionic inosine exhibits almost equal tendency to bind to the N7-bound 1:1 complex through the N1 and N7 sites. And third, the substitution rate of the aqua ligand in *cis*-$[Pt(NH_3)_2(H_2O)(LH\text{-}N7)]^{2+}$ ion is higher than that in *cis*-$[Pt(NH_3)_2(H_2O)(L\text{-}N7)]^+$ ion. In other words, deprotonation of inosine N1H reduces the substitution rate of the aqua ligand, either by lowering the overall charge of the complex or by stabilizing the aqua ligand through enhanced H-bonding to C(6)O. H-bond formation has been reported to affect significantly the complexation of aquated *cis*-$Pt^{II}(NH_3)_2$ with 6-oxo-substituted purine derivatives.[11b,12d]

Concluding Remarks. The complexation of aquated *cis*-$Pt^{II}(NH_3)_2$ can be quantitatively explained by replacement of the aqua ligand with the incoming nucleoside, whereas the hydroxo group is inactive toward substitution reactions. The reactivity of the diaqua cation is about 10 times higher than that of the monohydroxo monoaqua cation. In excess ligand, the stepwise formation of 1:1 and 1:2 complexes is observed, whereas excess Pt(II) results in side reactions that affect the complexation rate of Pt(II) with nucleosides. 1-Methylinosine forms only N7-bound complexes, which is also the predominant binding mode of neutral inosine. With increasing pH, the complexation pathway with inosine becomes very complicated, because of the involvement of N1 as an additional binding site upon deprotonation of N1H. On the other hand, N7 coordination is facilitated by the loss of N1H proton, as well. With anionic inosine the intrinsic formation of the N1- and N7-bound 1:1 complexes appears to be equal, at least as far as complexing with the *cis*-$[Pt(NH_3)_2(H_2O)(OH)]^+$ ion is concerned. Nevertheless, in the first complexation step the N7 coordination predominates throughout the pH range studied. With 1-methylinosine binding of the second nucleoside depends only on deprotonation of the remaining aqua ligand in the 1:1 complex. By contrast, introduction of the second ligand to the N7-bound 1:1 complex of inosine is facilitated by the deprotonation of N1H with a concomitant proton attachment to the deprotonated OH group in the Pt(II) moiety. This gives substitution labile aqua ligand, the reactivity of which is about one-third of that in the dication. In the *cis*-$[Pt(NH_3)_2(H_2O)(LH\text{-}N7)]^{2+}$ ion the inosine N1H proton is acidified by 1.8 log units, whereas the effect of Pt(II) in the *cis*-$[Pt(NH_3)_2(OH)(LH\text{-}N7)]^+$ ion is only 1.2 units. Intrinsically, anionic inosine exhibits almost equal tendency to bind through the N1 and N7 sites to N7-bound 1:1 complexes of different charges.

Registry No. InoH, 58-63-9; MeIno, 2140-73-0; *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, 20115-64-4.

(22) (a) Lippert, B. *J. Am. Chem. Soc.* **1981**, *103*, 5691–5697. (b) Faggiani, R.; Lippert, B.; Lock, C. J. L.; Speranzini, R. A. *Inorg. Chem.* **1982**, *21*, 3216–3225. (c) Girault, J.-P.; Chottard, G.; Lallemand, J.-Y.; Chottard, J.-C. *Biochemistry* **1982**, *21*, 1352–1356. (d) Miller, S. K.; Marzilli, L. G. *Inorg. Chem.* **1985**, *24*, 2421–2425.

(23) The employment of entire rate data listed in Table II gave the value of 2.6 M^{-1} s^{-1} for k_5 with a concomitant loss of computational accuracy.

(24) It is assumed that compound X does not seriously affect the concentration of 1-methylinosine.