(14) We thank Professor R. L. Augustine for supplying us with a sample of dihydrocinchonamine.
(15) Additional general conditions are described in the first paper of this series.
(16) According to *Chemical Abstracts* systematic nomenclature, dihydrocinchonamine is named 1*H*-indole-3-ethanol, 2-(5-ethyl-1-azabicyclo[2.2.2]oct-2-yl)-, [1*S*-(1α,2α,4α,5β)], and the numbering refers to this name.

Heavy Metal Nucleotide Interactions. 11. Stereochemical and Electronic Effects in the Electrophilic Attack of *cis*- and *trans*-Diammineplatinum(II) on 5'-Guanosine Monophosphate and Polyguanylate in Aqueous Solution[1,2]

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Abstract: *cis*- and *trans*-$(H_3N)_2Pt^{II}$ react in acidic solution with 5'-GMP to produce mono and bis complexes with GMP bound via N(7). As the pH is increased for solutions with r (Pt(II):phosphate) = 0.5, proton transfer from the phosphate occurs from pH 5 to 7 (D_2O) and from the N(1)-H above pH 9. The decrease in pK, N(1)-H, which is a measure of the inductive effect of the platinum(II) complex, is 0.3, 0.6 log unit for *cis*- and *trans*-$(H_3N)_2Pt^{II}$, respectively. This is much smaller than ΔpK for N(7) methylation, 2.5 log units. N(7) platination has a much smaller effect on the electron distribution and chemical reactivity of 5'-GMP than either protonation or methylation. The use of Raman spectra to assign binding sites is outlined. As the r value is increased above 0.5, pH $\geq$7, quite different reactions are observed for the cis and trans isomers. *trans*-$(H_3N)_2Pt^{II}$ appears at r = 1 to form primarily a mononuclear complex with N(7) binding. At pH >9, the principal species is *trans*-$[(H_3N)_2Pt(OH)(GMPH_{-1})]^{2-}$. When r = 2, $[\{trans\text{-}(H_3N)_2Pt(OH)\}_2GMPH_{-1}^{-}]$ with N(1)-N(7) binding is produced. *cis*-$(H_3N)_2Pt^{II}$ or enPt^{II} at r = 1 causes a large decrease in the N(1)-H pK, >2.8 log units, as has been observed to occur also with inosine. Solubility is much lower than in the trans system. Possible structures for the product of this unique *cis*-$(H_3N)_2Pt^{II}$ interaction are discussed. A cooperative process involving deprotonation, the formation of N(1), N(7) or O(6) interactions, and a rather high degree of polymerization is suggested for the product $[cis\text{-}(H_3N)_2Pt(GMPH_{-1})^{-}]_n$. The data indicate that N(7) is a strong nucleophile for platinum(II), but because $(H_3N)_2Pt(OH)_2$, a poor electrophile, is produced at high pH, attack on deprotonated GMP at N(7) is not favorable and at N(1) it is even less so. Reactions of *cis*-$(H_3N)_2Pt^{II}$ with poly(rG) at 37 °C are very slow because of the multistranded, strongly hydrogen-bonded structure of poly(rG). The *cis*-$(H_3N)_2Pt^{II}$ appears to be strongly hydrogen bonded to the polymer via the ammine ligands.

A number of experiments have indicated that the guanine base is probably the preferred site of electrophilic attack by *cis*-$[PtCl_2(NH_3)_2]$ on native polynucleotides. Mansy[4] reported that the rate of reaction with DNAs increased with increasing G + C content, Stone et al.[5] found that the increase in buoyant density upon reaction was proportional to G + C content, and Munchausen and Rahn[6] found, using ^{195m}Pt, an increase in platinum binding with increasing G + C content.

Because different experiments have pointed to the guanine base as the preferred binding site for platinum(II) complexes, considerable attention has been focused on the mode of binding. The two isomers, *cis*- and *trans*-$[PtCl_2(NH_3)_2]$, exhibit quite different physiological effects,[7] although the only chemical experiment of which we are aware that shows a difference is the DNA binding study of Grant et al.[8] where cis but not trans binding was observed. Although there are many possible explanations for the different physiological behavior of these two isomers, almost all attention has been directed to potential stereochemical differences in their binding to the guanine base. Lately, there have been several publications that suggest the important difference is the ability of *cis*-$(H_3N)_2Pt^{II}$ to form an intramolecular N(7)-O(6) chelate in its binding to the guanine base,[9-11] thereby altering interbase hydrogen bonding. It would be very interesting if the physiological difference between these isomers has its origin in such a simple stereochemical effect.

Although metal ion chelation involving the guanosine or inosine N(7)-O(6) atoms has been proposed many times, it generally has been rejected on steric grounds.[12] Recently, Szalda, Kistenmacher, and Marzilli[13] synthesized a theophyllinato-Cu^{II} complex in which such an interaction was forced. The crystal structure showed a Cu-N(7) distance of 1.956 (3) Å, while the Cu-O(6) distance was much longer, 2.919 (3) Å. Chelation has been suggested both with the neutral guanosine and in some cases for the N(1) deprotonated conjugate base. In a detailed spectroscopic study of the *cis*-$(H_3N)_2Pt^{II}$-inosine reaction in dilute aqueous solution, we found no evidence for such an intramolecular interaction at any pH.[14] In the case of the binding of *cis*-$(H_3N)_2Pt^{II}$, the evidence presented for N(7)-O(6) chelation appears to be summarizable as follows.

(1) The N(7)-O(6) chelation was first proposed for $(H_3N)_2Pt^{II}$ and the neutral guanine by Goodgame et al.[9] in a paper dealing with the structure of $[Pt(NH_3)_2(5'\text{-}IMP)_2]^{2-}$. It should be noted that the crystal structure showed *only* interaction at N(7) of inosine.

(2) The oxygen 1s binding energy is reported to be 532.7 eV for DNA, 532.3 eV for DNA + *trans*-$[PtCl_2(NH_3)_2]$ (r = 0.82), and 531.7 eV for DNA + *cis*-$[PtCl_2(NH_3)_2]$ (r = 0.82).[10a] The decrease in binding energy of ca. 1.0 eV was attributed to a "chelation of Pt with the N(7) Gua and O(6) Gua sites". It should be noted that the guanine O(6) would be expected to contribute approximately 4% of the intensity of the oxygen 1s envelope, since this is due to the phosphate diester oxygens plus those on cytosine and thymine as well as guanine O(6). Consequently, a shift in the oxygen binding energy does not seem to point to a specific interaction with the guanosine O(6).

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(3) Reaction of salmon sperm DNA with 2.6×10^{-4} M *cis*-$[PtCl_2(NH_3)_2]$, $r = 0.2$, was reported to release two chlorides per bound platinum at pH ca. 6 on the basis of emf measurements using a concentration cell with $Ag|AgCl|Cl^-$ electrodes.[10]

(4) Recrystallization of $[Pt(dat)Guo]Cl_2$, dat = 3,4-diaminotoluene, from ethanol–diethyl ether caused an increase of ca. 100 cm^{-1} in the main IR absorption band of the solid compounds in the 1600–1700-cm^{-1} region.[11] This was attributed to displacement of the O(6) in a chelated guanosine complex by penetration of chloride ion into the first coordination sphere of platinum, i.e. transformation from $[Pt(DAT(Guo]Cl_2$ to $[Pt(dat)(Guo)Cl]Cl$.

Since much importance has been attached to the formation of an N(7)–O(6) chelate in the attack of *cis*-$(H_3N)_2Pt^{II}$ but not *trans*-$(H_3N)_2Pt^{II}$ on the guanine base of polynucleotides in aqueous solution, we have attempted to design some simple experiments to test this hypothesis. The principal assumption involved is that a platinum(II)–O(6) interaction will shift significantly the frequency of the 5′-guanosine monophosphate vibration at ca. 1670 cm^{-1}. This mode is almost purely C(6)=O bond stretching. For example, the band that is observed at 1665 cm^{-1} in the infrared spectrum of ^2H_2O solutions of [6-^{16}O]guanosine decreases 14 cm^{-1} with [6-^{18}O]guanosine, while other frequencies remain essentially unchanged.[15] In the Raman spectrum of [6-^{16}O]GMP we observe this band at 1665 cm^{-1} with medium intensity. Since any chelate interaction depends only upon the guanine moiety, we have examined the Raman spectrum of moderately dilute aqueous solutions of 5′-GMP, 25 mM, with different concentrations of *cis*-$(H_3N)_2Pt^{II}$ and different pH. Solution studies avoid the problems of lattice effects on the vibrational spectrum that occur with solid samples.

In addition, since the reactions with guanosine of *cis*- and *trans*-$(H_3N)_2Pt^{II}$ have been suggested to differ in that the former can form the N(7)–O(6) chelate while the latter cannot, we have used Raman and ^1H NMR spectroscopy to search for differences in the binding reactions of these two complexes. Because coordination of strong electrophiles to nucleotides is known to have a marked effect on their electronic configurations and, consequently, on their chemistry,[16] we have sought to measure properties, namely the N(1)–H dissociation constant and vibrational spectrum, that will reflect changes in electron distribution. This allows a comparison of the *cis*- and *trans*-$(H_3N)_2Pt^{II}$ electrophiles with one another and with other electrophiles such as alkylating agents. Finally, to examine the effect of constraining the base along a phosphate diester backbone, we have examined some reactions with polyguanylate.

Experimental Section

Platinum Complexes. Solutions of *cis*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ as the perchlorate and nitrate were prepared from *cis*-$[PtCl_2(NH_3)_2]$ as described previously[14,17] in H_2O or D_2O (99.8%, Columbia Organic Chemicals, Columbia, S.C.). Solutions of $[Pten(OH_2)_2](F_3CSO_3)_2$ also were prepared as described previously.[17] *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ nitrate and perchlorate were prepared from *trans*-$[PtCl_2(NH_3)_2]$ synthesized according to Kauffman and Cowan[18] and $AgNO_3$ or $AgClO_4$ solution. Crystalline *trans*-$[(H_3N)_2Pt(OH_2)_2](ClO_4)_2$ was isolated as described previously for the cis isomer.[14] Anal. Calcd for $PtN_2O_{10}Cl_2H_{10}$: Pt, 42.0; N, 6.04; Cl, 15.3; H, 2.17. Found: Pt, 42.0; N, 5.94; Cl, 15.5; H, 2.43. The same procedure employed for preparation of solutions of the cis isomer was followed with the trans complex. The sterochemical purity of the *cis*- and *trans*-$PtCl_2(NH_3)_2$ starting materials was checked by comparing the Raman spectra of the crystalline compounds, which differ substantially, with literature spectra [19,20] and spectra of authentic samples.

5′-Guanosine Monophosphate and Polyguanylate Solutions. The disodium salt of 5′-GMP obtained from Aldrich Chemical Co. (Milwaukee, Wis.) was dissolved in H_2O or D_2O. In the latter case, solutions were prepared shortly before a given experiment to minimize exchange of deuterium onto the C(8) position. Poly(rG) was obtained as the sodium salt from Miles Laboratories (Elkhart, Ind.) and weighed amounts were dissolved in distilled water.

Binding Reaction Studies. Solutions were prepared by mixing stock solutions of the platinum(II) complex with a GMP solution in H_2O or D_2O to give the desired r = Pt(II):nucleotide ratio. The pHs (pDs) of the solutions were adjusted with $HClO_4$ ($DClO_4$) or NaOH (NaOD). Since the sequence of mixing and the initial pH of the solutions can be important, this is described below with the results. Solution pH values were measured with a Radiometer PHM-4 meter, and for D_2O solutions the meter reading was corrected as described by Glascoe and Long.[21] In many instances, the pH-stat technique was employed becuase of the slow reactions involved. The total perchlorate concentration was adjusted to 100 mM using $NaClO_4$. Poly(rG) (organic phosphorus 2.77 μmol/mg) was dissolved in 0.05 M $NaNO_3$ to give 25 mM total phosphate. A concentrated solution of *cis*-$[(H_3N)_2Pt(OH_2)_2](NO_3)_2$ was prepared, and an aliquot was added to the poly(rG) solution using an ultramicro buret. The Raman spectrum was recorded. A second aliquot was added to the solution to increase the concentration of *cis*-$(H_3N)_2Pt^{II}$, and a new Raman spectrum measured. This procedure was repeated to give the series of spectra for the Raman titration. This technique is used to minimize the quantity of valuable samples, but it has the disadvantage that the polynucleotide concentration decreases during the series of measurements.

After the addition of the platinum complex, solutions were stored in the dark.

Raman Spectra. The general procedures for the Raman and Raman difference spectral determinations have been given previously.[14,17,22] Most of the spectra were recorded with a computerized Raman difference spectrophotometer using a Spex 1400 monochromator.[23] Some spectra also were recorded with a Jobin-Yvon Ramanor HG-2 instrument interfaced to the same computer system. The samples containing the mononucleotide were passed through 250-nm pore size microfilters. Spectra were processed off-line using PROGRAM RAMAN. They were subjected to a 25 point quartic smooth.

Nuclear Magnetic Resonance Spectra. The ^1H spectra were determined with a Varian XL-100 (100 MHz) spectrometer using a CAT, and the general procedure has been described previously.[14,17,22] From 20 scans (25 mM 5′-GMP) to over 200 scans with the broad signals in the *cis*-$(H_3N)_2Pt^{II}$ system, $r > 0.5$, pH > 7, were accumulated.

Data and Results

The pH Profile and Stoichiometry of the Reactions of *cis*- and *trans*-$(H_3N)_2Pt^{II}$ with 5′-Guanosine Monophosphate at 25 °C. Raman Difference Spectra. In order to obtain a "fingerprint" for platination of GMP in H_2O at 25 °C, pH 7, Raman difference spectra (RADS) were recorded. Figure 1 illustrates both parent and difference spectra for *cis*-$(H_3N)_2Pt^{II}$ + GMP vs. GMP, $r = 0.5$. Frequency data from the parent spectra are tabulated in Table I in the microfilm edition (see paragraph at end of paper). The data indicate a quantitative reaction, i.e. formation of a bis complex. While significant shifts for most of the 5′-GMP bands are observed in the 1177 to 1576 cm^{-1} region upon reaction with *cis*-$(H_3N)_2Pt^{II}$, no significant change occurs in the carbonyl mode at 1680 cm^{-1}. A spectrum that is almost identical was obtained with $r = 0.5$, pH 8.7. The only difference in frequency larger than 2 cm^{-1} was in the ν(Pt–N) band which shifted from 535 (pH 7.0) to 527 cm^{-1} (pH 8.7). Intensities were all the same within experimental error. When r was increased to 1, pH 7, precipitation occurred. The spectrum of the filtrate was generally similar to the r = 0.5, pH 7 spectrum, although there appears to be some decrease in the intensity of the ν(CO) scattering at 1680 cm^{-1}, slight broadening and shifts in the bands in the double bond region, and a decrease of ν(Pt–N) to 527 cm^{-1}. On this basis, it appears that the material remaining in solution has GMP bound in much the same way that it is in the bis complex. Data are collected in Table I available in the microfilm edition.

RADS also were determined for *trans*-$(H_3N)_2Pt^{II}$ in H_2O at 25 °C, $r = 0.5$, pH 7. The only significant difference from

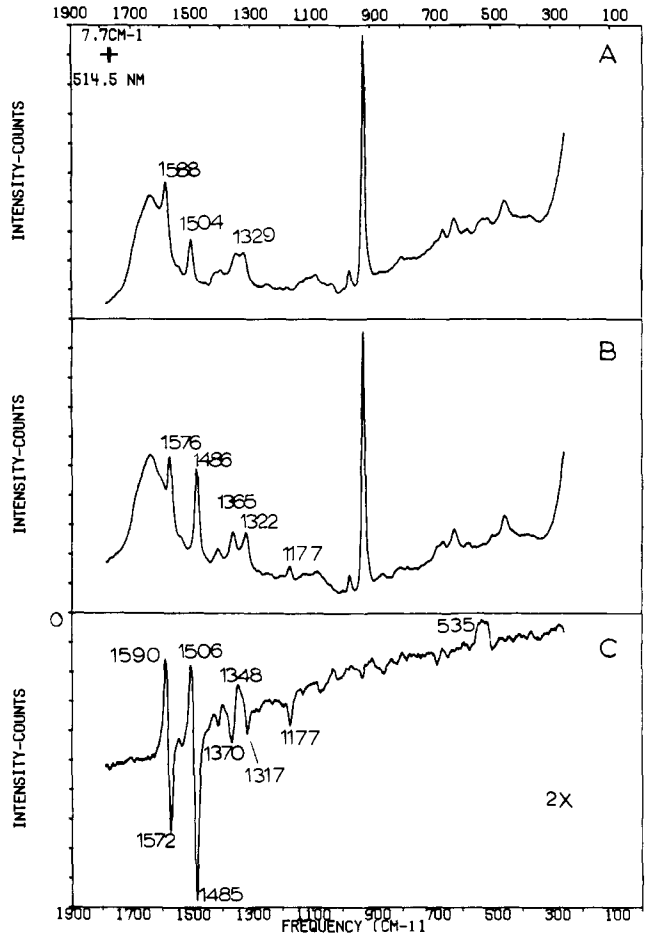


Figure 1. Raman difference spectrum (RADS) for the *cis*-$(H_3N)_2Pt^{II}$-GMP system, $r = 0.5$, H_2O, pH 7, $[ClO_4^-] = 100$ mM, 25 °C: (A) 12.5 mM $(H_3N)_2Pt^{II}$ + 25 mM GMP; (B) 25 mM GMP; (C) A − B. The ordinate of the difference spectrum is expanded by a factor of 2 compared to the parent spectra.

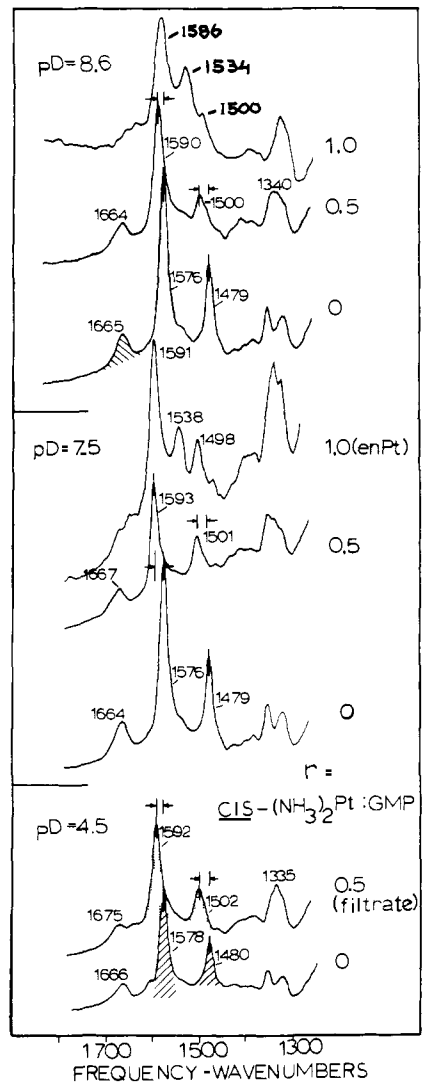


Figure 2. Raman titrations: spectra of 25 mM 5'-guanosine monophosphate with *cis*-$(H_3N)_2Pt^{II}$ in D_2O at different pDs, $[ClO_4^-] = 100$ mM. The solution at pD 7.5, $r = 1$, contains $enPt^{II}$ to avoid precipitation. Bands that can be used to monitor the reactions are shaded. With the exception of the $enPt^{II}$, all spectra are scaled so the intensities are comparable.

the spectrum of the corresponding cis complex is in the ν(CO) region. Whereas cancellation of the 1680-cm^{-1} band was observed in the difference spectrum of the cis complex, a weak derivative feature with a maximum at 1700 cm^{-1} occurs with the trans complex. Thus, while no significant shift in the carbonyl stretching mode occurs upon formation of the bis complex with *cis*-$(H_3N)_2Pt^{II}$, there is a slight shift to higher frequency with the trans isomer.

Raman Titrations: Cis Isomer. Spectra were obtained with D_2O, since the carbonyl region was of particular interest. The solutions contained 25 mM 5'-GMP, with varying concentrations of *cis*-$(H_3N)_2Pt^{II}$ at pD 4.5, 7.5, 8.5, and 11. The solubilities are quite limited in this system, especially where the phosphate group is partially protonated. Spectra are illustrated in Figure 2. Data are tabulated in Table II, available in the microfilm edition.

pD 4.5. The solution at pD 4.5, $r = 0.5$, gave a trace of a precipitate upon standing. The mixture was filtered, and ths solution spectrum indicated essentially quantitative formation of the bis complex, $[(H_3N)_2Pt(5'\text{-GMPH})_2]^0$. At this hydrogen ion concentration, the phosphate should be almost entirely in a monoprotonated form. The most intense phosphate mode occurs at ca. 1082 cm^{-1}, consistent with the presence of $ROPO_3H^-$.

The complex formation can be monitored easily using the GMP bands at 1480 and 1578 cm^{-1} which shift to ca. 1502 and 1592 cm^{-1}, respectively, upon metallation. The frequency of the 1666-cm^{-1} carbonyl mode increases by ca. 9 cm^{-1}. Clearly the principal reaction at pD 4.5, $r \leq 0.5$, is eq 1.

$$(r \leq 0.5)\ cis\text{-}[(H_3N)_2Pt(OH_2)_2]^{2+} + 2GMPH^- \rightleftharpoons 2H_2O + cis\text{-}[(H_3N)_2Pt(GMPH)_2]^0 \quad (1)$$

pD 7.5. At pD 7.5, the reaction is much slower. The solution with $r = 0.5$ is clear, and the frequencies are all the same as at pD 4.5 except the carbonyl stretch which is ca. 10 cm^{-1} lower. With $r = 1.0$ extensive precipitation occurs. The analogous system prepared from the ethylenediamine complex, enPt(II), exhibits higher solubility, and the spectrum, $r = 1$, pD 7, in Figure 2 was obtained in this way. At this pH, the phosphate is unprotonated as indicated by the vibration at 975 cm^{-1} characteristic of $ROPO_3^{2-}$. While the shifts in the GMP modes upon formation of the bis complex by platinum(II), $r = 0.5$, are essentially the same as those observed at pD 4.5, the spectrum for $r = 1$ is quite different. The 1664-cm^{-1} band had disappeared, and the bands at 1576 and 1479 cm^{-1} are replaced by intense, broad bands at 1591 and 1538 cm^{-1} plus a band at 1498 cm^{-1}. Quite similar changes were observed in the Raman spectrum of inosine[14] upon reaction with $(H_3N)_2Pt^{II}$, $r > 0.5$, pH ≥7. Disappearance of the ~1665-cm^{-1} band is generally diagnostic of deprotonation at N(1) and also has been observed in the substitution of the N(1)-H of GMP by CH_3Hg^{II}.[24]

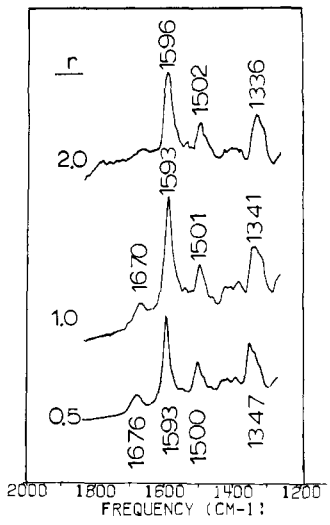


Figure 3. Raman titrations: spectra of 25 mM 5′-guanosine monophosphate with *trans*-$(H_3N)_2Pt^{II}$ in D_2O at pD 8.3; $[ClO_4^-]$ = 100 mM.

The principal reactions at pH 7 are eq 2 for $r \leq 0.5$ and eq 3 for $r > 0.5$:

$$(r \leq 0.5)\ cis\text{-}[(H_3N)_2Pt(OH)(OH_2)]^+ + 2GMP^{2-} \xrightarrow{\text{slow}} cis\text{-}[(H_3N)_2Pt(GMP)_2]^{2-} + OH^- + H_2O \quad (2)$$

$$(r > 0.5)\ [enPt(OH)(OH_2)]^+ + GMP^{2-} \xrightarrow{\text{slow}} [enPt(GMPH_{-1})]^- + 2H_2O \quad (3)$$

Since this is in the GMP/GMPH (phosphate) buffer region, little change in pH occurs. It is to be presumed that the *cis*-$(H_3N)_2Pt^{II}$ behaves similarly to eq 3. The slowness of these reactions is ascribed to blocking by the hydroxo group of *cis*-$[(H_3N)_2Pt(OH)(OH_2)]^+$ and/or its polycondensed species which should predominate at pH 7.[25]

pD 8.6. The reactions also were studied at pD 8.6 where no precipitation occurred either at r = 0.5 or 1.0. The spectra also are illustrated in Figure 2. With $r \leq 0.5$, the spectral changes that occur are essentially the same as those at pD 4.5 and 7.5. The shifts relative to GMP over this wide pH range are very similar to those observed in the GMP-CH_3Hg^{II} reaction at pH 2[24] with both H_2O and D_2O solutions. A figure illustrating this is provided in the microfilm edition. In highly acidic solutions proton loss from N(1) is blocked, and CH_3Hg^{II} is presumed to bind at N(7), substituting for the N(7) proton (pK_a, N(7) deprotonation (H_2O) = 2.3[26]). Consequently the Raman spectra indicate simple platination of GMP at N(7) for all pH values when $r \leq 0.5$. It has been noted previously that mercuriation and platination at the same site of pyrimidines also cause very similar perturbations of the base Raman spectrum.[14,17] Platination in acidic solution at N(7) was demonstrated previously by Kong and Theophanides on the basis of ^{195}Pt-H(8) spin-spin coupling.[27]

The spectrum for the solution with r = 1.0 is similar to the one for enPt(II) + GMP, r = 1.0, pD 7.0. Complete deprotonation at N(1) is indicated.

At pD 8.5, the system can be described by reactions 4 and 5.

$$(r \leq 0.5)\ cis\text{-}[(H_3N)_2Pt(OH)_2]^0 + 2GMP^{2-} \rightarrow cis\text{-}[(H_3N)_2Pt(GMP)_2]^{2-} + 2OH^- \quad (4)$$

$$(r > 0.5)\ cis\text{-}[(H_3N)_2Pt(OH)_2]^0 + GMP^{2-} \rightarrow cis\text{-}[(H_3N)_2Pt(GMPH_{-1})]^- + OH^- + H_2O \quad (5)$$

When r was increased to 2.0 at pD 8.3, extensive precipitation occurred. The spectrum of the filtrate exhibited a band at 1595 cm^{-1} with a shoulder at ca. 1570 cm^{-1}, a very broad band at ca. 1490 cm^{-1}, and a band at 1329 cm^{-1} with a shoulder at ca. 1310 cm^{-1}, i.e. quite different from the r = 1 spectrum.

pD 11. Reaction with 5′-$GMPH_{-1}^{3-}$ deprotonated at N(1) (pK_a, N(1) deprotonation (H_2O) = 9.5[26]) might be expected to give a different isomer than produced by the reactions with 5′-GMP^{2-} described above. To examine this, solutions were prepared by mixing $(H_3N)_2Pt^{II}$, pD 11, where *cis*-$[(H_3N)_2Pt(OH)_2]$ should predominate,[28] with 5′-$GMPH_{-1}^{3-}$, pD 11, to give total GMP = 25 mM, r = 0.5, 1.0.

After 1 day, both spectra were characteristic of unreacted 5′-$GMPH_{-1}^{3-}$. After an additional 3 days the r = 1 solution showed broadening of the bands from 1300 to 1600 cm^{-1} with slight changes in frequency. After 3 more days, the $GMPH_{-1}^{3-}$ band at 1520 cm^{-1} had disappeared and the bands at 1591, 1468, and 1338 cm^{-1} had shifted by +9, +5, and −4 cm^{-1}, respectively. The r = 0.5 solution after 7 days was very similar to the r = 1 solution after 4 days. In all cases the bands were broad indicating that the solutions contained a mixture of species.

These solutions were acidified to give pD 7. The r = 1 solution gave a spectrum similar to that illustrated in Figure 2, pD 7.5, but with overlapping bands at ca. 1500, 1480, 1335, and 1315 cm^{-1}. This is to be expected because the ^1H NMR indicated significant deuteration at C(8), and this affects precisely these GMP bands.[29] The acidified r = 0.5 solution gave a spectrum similar to the corresponding one in Figure 2, again with effects due to deuteration at C(8).

Raman Titrations: Trans Isomer. A series of measurements similar to those described above for the cis isomer was carried out with *trans*-$(H_3N)_2Pt^{II}$. Spectra with r = 0.5 and 1.0, pD <8.5, are the same within the experimental error and essentially the same as the spectra of the complex formed by the cis isomer when $r \leq 0.5$. This indicates binding at N(7) of GMP with *no* deprotonation at N(1). Spectra for pD 8.3 are illustrated in Figure 3.

When r was increased to 2.0, pD 8.3, precipitation occurred, although to a much smaller extent than with the cis isomer. The spectrum of the filtrate also is illustrated in Figure 3. Noteworthy is the almost complete loss of the 1666-cm^{-1} (C=O) stretching mode which indicates deprotonation at N(1). Concurrent with this is a slight but significant increase in the frequency of the band in the 1590–1600-cm^{-1} region. This suggests that there is coordination of *trans*-$(H_3N)_2Pt^{II}$ to both the five- and six-membered rings at this high r value.

There is a slight difference in the shift in the ca. 1665-cm^{-1} carbonyl mode upon binding of GMP in the bis complexes with the cis and trans isomers. The values for the bis-trans complex are 1679 (pD 4.5) and 1676 cm^{-1} (pD 8.4), similar to the cis complex at pD 4.5 but ca. 10 cm^{-1} higher than the cis complex at pD 7 and higher.

These data indicate the following reactions (eq 6–10) are important with *trans*-$(H_3N)_2Pt^{II}$.

pD 4.5

$$(r \leq 0.5)\ trans\text{-}[(H_3N)_2Pt(OH_2)_2]^{2+} + 2GMPH^- \rightarrow 2H_2O + trans\text{-}[(H_3N)_2Pt(GMPH)_2]^0 \quad (6)$$

$$(r = 1.0)\ trans\text{-}[(H_3N)_2Pt(OH_2)_2]^{2+} + GMPH^- \rightarrow H_2O + trans\text{-}[(H_3N)_2Pt(GMPH)(OH_2)]^+ \quad (7)$$

pD 8.3

$$(r \leq 0.5)\ trans\text{-}[(H_3N)_2Pt(OH)_2]^0 + 2GMP^{2-} \rightarrow 2OH^- + trans\text{-}[(H_3N)_2Pt(GMP)_2]^{2-} \quad (8)$$

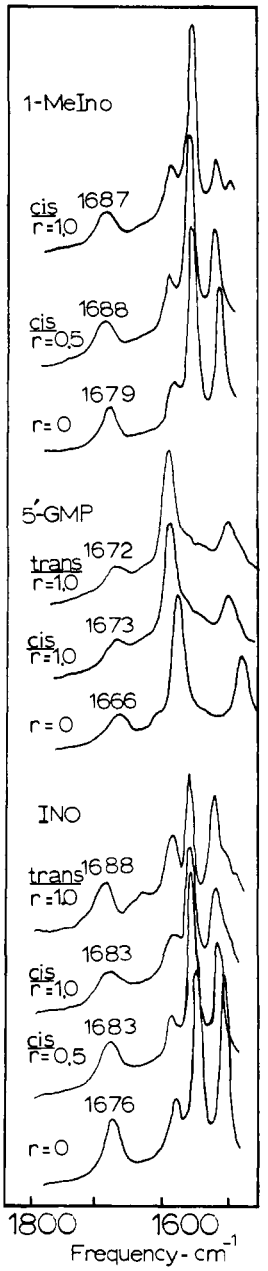


Figure 4. The ν(C(6)=O) band for acidic solutions of *cis*-$(H_3N)_2Pt^{II}$ with 5'-GMP, Ino, and 1-MeIno, r = 0.5 and 1.0. All solutions 100 mM in ClO_4^-, 25 mM in total ligand except for the GMP complexes which are 25 and 12.5 mM, respectively.

$$(r = 1.0)\ \textit{trans}\text{-}[(H_3N)_2Pt(OH)_2] + GMP^{2-} \rightarrow OH^- + \textit{trans}\text{-}[(H_3N)_2Pt(OH)(GMP)]^- \quad (9)$$

$$(r = 2.0)\ 2\ \textit{trans}\text{-}[(H_3N)_2Pt(OH)_2] + GMP^{2-} \rightarrow H_2O + OH^- + [(\textit{trans}\text{-}(H_3N)_2Pt(OH))_2GMPH_{-1}]^- \quad (10)$$

Search for Evidence of an N(7)–O(6) Intramolecular Interaction in Acidic Solution. Spectra were obtained for a number of systems to attempt to detect an interaction between platinum and the carbonyl oxygen of the neutral guanine base. Solutions with r = 1, where such an interaction would be favored, were studied for 5'-GMP and inosine, pD 4.5, with both *cis*- and *trans*-$(H_3N)_2Pt^{II}$. In addition, data are available for *cis*-$(H_3N)_2Pt^{II}$ + 1-MeIno, pD 7.7.[14]

The spectral features in the 1500–1700-cm^{-1} range are illustrated in Figure 4. There are no significant differences between systems with r = 0.5 where interaction with the carbonyl oxygen is blocked by bis complex formation and the corresponding system with r = 1. In addition no significant differences between *cis*- and *trans*-$(H_3N)_2Pt^{II}$ were observed.

The carbonyl stretching frequencies are all typical of a normal aquated carbonyl oxygen.[30]

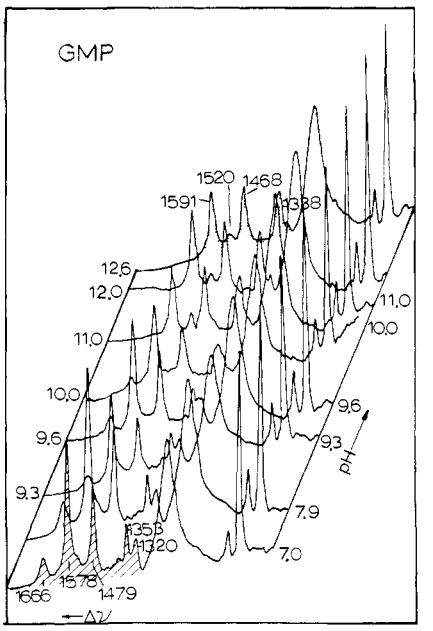


Figure 5. Raman spectra of 25 mM 5'-guanosine monophosphate in D_2O as a function of pD, 25 °C, $[ClO_4^-]$ = 50 mM. Shaded bands are shifted by ring deprotonation.

Comparison of the Effect of *cis*- and *trans*-$(H_3N)_2Pt^{II}$ on the N(1) Acidity of 5'-Guanosine Monophosphate in the Bis Complexes. Since the N(1)–H appeared to be dissociated in solutions containing the cis complex, r = 1, in neutral or alkaline solution but not with the trans isomer until pH 9, vide supra, the acid dissociation constants of the N(1)–H were determined for *cis*- and *trans*-$[(H_3N)_2Pt(GMP)_2]^{2-}$. By studying the bis complexes, one can measure the inductive effects of the two platinum electrophiles when they are bound *only* to N(7). The complex was assembled at pD 4.5 to ensure that coordination occurred via N(7), and then the pD value was adjusted to deprotonate the ligand. The pD values were checked after determination of the spectra and found to be constant.

These measurements were made using Raman spectroscopy to establish that it was N(1) that was being deprotonated and to follow electronic changes as reflected in the vibrational spectra. Spectra illustrating the deprotonation of 5'-GMP, *cis*-$[(H_3N)_2Pt(GMP)_2]^{2-}$, and *trans*-$[(H_3N)_2Pt(GMP)_2]^{2-}$ are illustrated in Figures 5–7. Data are for D_2O solutions 0.05 M in ClO_4^- at 25 °C. From these, the pK of 5'-GMP was calculated to be 9.8 ± 0.2. This is reasonable considering the literature values for dissociation of the N(1)–H in H_2O solutions which are ca. 9.5.[26] The platinum complexes exert only a very weak inductive effect. For *cis*-$[(H_3N)_2Pt(GMP)_2]^{2-}$, pK_a = 9.5 ± 0.2, while a value of pK_a = 9.2 ± 0.2 was obtained for the trans isomer. These correspond to decreases in ΔpK_a of 0.3 (cis) and 0.6 (trans).

Comparison of the ν(CO) bands in Figures 6 and 7 shows that there is a significant, reproducible difference in this vibration for the *cis*- and *trans*-$[(H_3N)_2Pt(GMP)_2]^{2-}$ complexes. Since platinum is coordinatively saturated, it is likely that the effect is due to differing solvation of the carbonyl oxygen.

Since studies on inosine have indicated that coordination of a heavy metal electrophile at N(7) increases the rate of exchange of the C(8)–H with solvent,[22] The solution of *trans*-

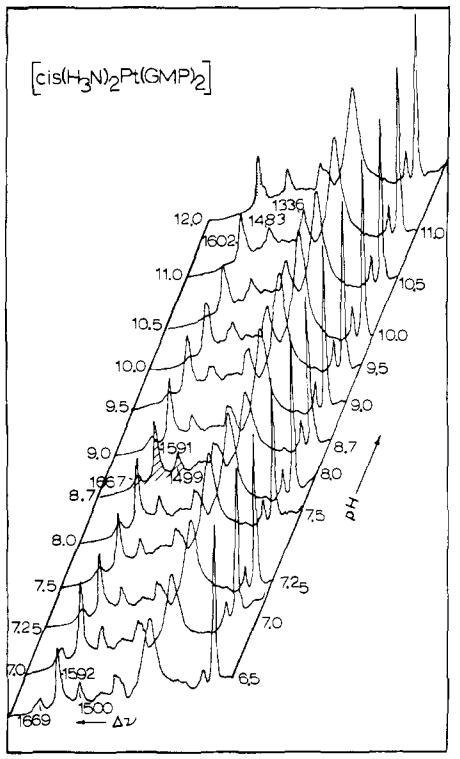


Figure 6. Raman spectra of 12.5 mM *cis*-$[(H_3N)_2Pt(GMP)_2]$ in D_2O as a function of pD, 25 °C, $[ClO_4^-]$ = 50 mM.

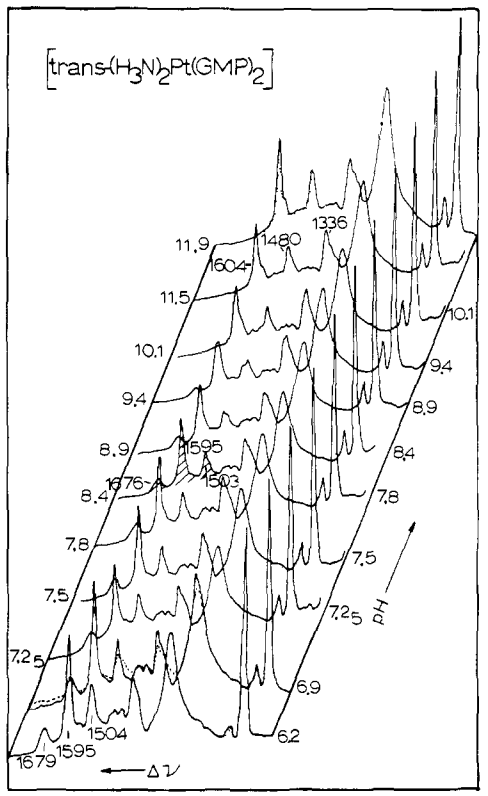


Figure 7. Raman spectra of 12.5 mM *trans*-$[(H_3N)_2Pt(GMP)_2]$ in D_2O as a function of pD, 25 °C, $[ClO_4^-]$ = 50 mM.

$[(H_3N)_2Pt(GMP)_2]^-$ which had the pD raised to 12 was reacidified to reduce the pD to 7, and the spectrum was recorded. This also is illustrated in Figure 7. The spectrum is essentially the same as that of the original acidified solution of the complex which had been raised to pD 7. Deuterium exchange onto C(8) is slight, and this reaction is considerably slower than with inosine. This was verified by measurement of the ^1H NMR spectrum.

^1H Nuclear Magnetic Resonance Spectra of Solutions of *cis*- and *trans*-$(H_3N)Pt^{II}$ and $enPt^{II}$ with 5'-GMP in D_2O. Survey spectra were run for solutions 25 mM in GMP, 25 mM in *cis*-$(H_3N)_2Pt^{II}$, D_2O, 25 °C at pD 2.3, 4.5, 8.8, and 9.8 where no precipitation occurred. At pD 2.3 and 4.5, the solutions exhibit sharp signals at 5.46 and 5.31 ppm in the H(8) region and sharp H(1') doublets at 2.86 and 2.72 ppm (J = 4 Hz) (downfield from $N(CH_3)_4^+$). The spectra are qualitatively very like the corresponding inosine spectra at pD 4.5 where the higher field signals are assigned to $[(H_3N)_2Pt(Ino)_2]^{2+}$ and the lower field resonances to $[(H_3N)_2PtIno(OH_2)]^{2+}$. At pD 8.8 and above very broad resonances were observed both in the H(8) and H(1') regions at approximately 5.5 and 2.7 ppm, respectively.

Since the ethylenediamine complexes were observed to have higher solubility in the pH 5–7 range, solutions 25 mM in GMP with enPt(II) to give r from 0 to 1.0 were prepared in D_2O, pD 7.0. This also gives a signal characteristic of the platinum electrophile, i.e. from the methylene protons of the ethylenediamine ligand. These spectra are illustrated in Figure 8. The solution with r = 0.25 exhibits H(8) signals of equal intensity at 4.94 due to unreacted GMP and at 5.46 ppm due to complexed GMP. When r = 0.50, only signals due to the bis complex are observed: H(8) 5.48; H(1') 2.74 ppm. The H(1') signal is at the same chemical shift as for unreacted GMP, although the spin-spin coupling constant has decreased from 6 to 4 Hz. The ethylendiamine resonance occurs at −0.34 ppm (upfield) from the $N(CH_3)_4^+$ reference for the solutions with r = 0.25 and 0.50, and the sidebands due to coupling with ^{195}Pt were not resolved. All line widths remain essentially constant as r increased from 0 to 0.5 indicating that simple mononuclear complexes are formed. When sufficient enPt(II) is added to give $r > 0.50$ marked changes occur in the spectrum. The signals due to H(8), H(1'), and the ethylenediamine methylene protons all broaden markedly. At r = 1, only broad signals remain. The resonance due to ethylenediamine increases from a full width at half-maximum intensity of 4 Hz as observed with r = 0.25 and 0.50 to a width of 16 Hz with r = 1.0. This is the same solution for which the Raman spectrum is illustrated in Figure 2. Since the $N(CH_3)_4^+$ line width remains constant, broadening due to effects such as introduction of a paramagnetic ion can be ruled out. Chemical shift data are summarized in Table I.

The broad resonances observed at higher pH are attributed to polymerization of the product of reactions 3 or 5 according to eq 11:

$$cis\text{-}[(H_3N)_2Pt(GMPH_{-1})]^- \rightleftharpoons (1/n)cis\text{-}[(H_3N)_2Pt(GMPH_{-1})^-]_n \quad (11)$$

Similar behavior was proposed for the inosine *cis*-$(H_3N)_2Pt^{II}$ system where broad signals also were observed at pD 7.6, $r > 0.5$.[14]

A similar study was carried out with 25 mM GMP and *trans*-$(H_3N)_2Pt^{II}$. Spectra for $0 \leq r \leq 1.0$, D_2O, pD 7, are illustrated in Figure 9, and the chemical shifts are collected in Table I. The basis for the assignments is similar to that for the ethylenediamineplatinum(II) species.

Comparison of the Binding of *cis*-and *trans*-$(H_3N)_2Pt^{II}$ to 5'-GMP in D_2O, $r > 0.5$, pD >7, 25 °C. The studies described above indicate that the cis and trans isomers under most conditions bind in a very similar fashion to N(7) of GMP and must exert a rather minor perturbation on the ligand electron distribution. With an excess of GMP, $r \leq 0.5$, the binding reactions as indicated by the Raman and ^1H NMR spectra remain similar to the highest pH examined, 12. On the other hand,

Table I. ^1H NMR Chemical Shifts[a] for Mononuclear 5′-GMP Complexes of Ethylenediamineplatinum(II) and *cis*- or *trans*-Diammineplatinum(II)

Species	pD	H(8), ppm	H(1′), ppm	en, ppm[e]
$5'\text{-GMP}^{2-}$	7.0	4.97	2.74	
$5'\text{-GMPH}^{-}$	4.5[b,d]	5.02	2.78	
$5'\text{-GMPH}_2$	2.0	5.82	2.90_5	
$enPt^{II}$	7.0			−0.65[c]
$enPt^{II}$	4.1			−0.66
cis-$[(H_3N)_2Pt(GMP)_2]$	8.0	5.49	2.70	
cis-$[(H_3N)_2Pt(GMPH)OH_2]^+$	4.5	5.46	2.86	
cis-$[(H_3N)_2Pt(GMPH)_2]^0$	4.5	5.31	2.72	
$[enPt(GMP)_2]^{2-}$	7.0	5.48	2.74	−0.34
$[enPt(GMP)_2]^0$	4.0	5.32	2.76	−0.35
$[enPt(GMPH)OH_2]^+$	2.0	5.43	2.86	−0.50
$[enPt(GMPH)_2]$	2.0	5.28	2.75	−0.35
trans-$[(H_3N)_2Pt(GMP)_2]^{2-}$	8.0	5.75	2.83_5	
trans-$(H_3N)_2Pt(GMPH)(OH_2)$	4.5	5.46	~2.85	
trans-$[(H_3N)_2Pt(GMPH)_2]^0$	4.5	5.59	2.85	

[a] Relative to internal $N(CH_3)_4^+$. [b] Change in shift from pD 7 to 4.5 is due to protonation of the phosphate. [c] This resonance has $^3J(^{195}Pt\text{-}H)$ = 52.5 Hz, and it is similar to the resonance for $[Pten(OH_2)_2]^{2+}$, pD 3.1, δ −0.66 ppm, $^3J(^{195}Pt\text{-}H)$ = 52.8 Hz. An additional resonance presumed due to a polycondensed species is observed at δ −0.80, $^3J(^{195}Pt\text{-}H)$ = 49.0 Hz. [d] Estimated from measurements of δ as a function of pH from 2 to 7, [GMP] = 25 mM. [e] No $^3J(^{195}Pt\text{-}H)$ observed.

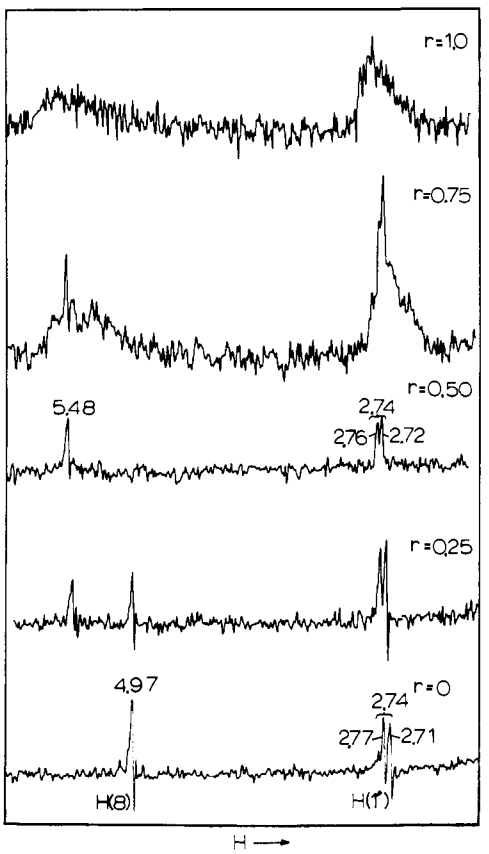


Figure 8. ^1H NMR titration: spectra (100 MHz) of 25 mM 5′-GMP + $enPt^{II}$, D_2O, pD 7, as a function of *r*.

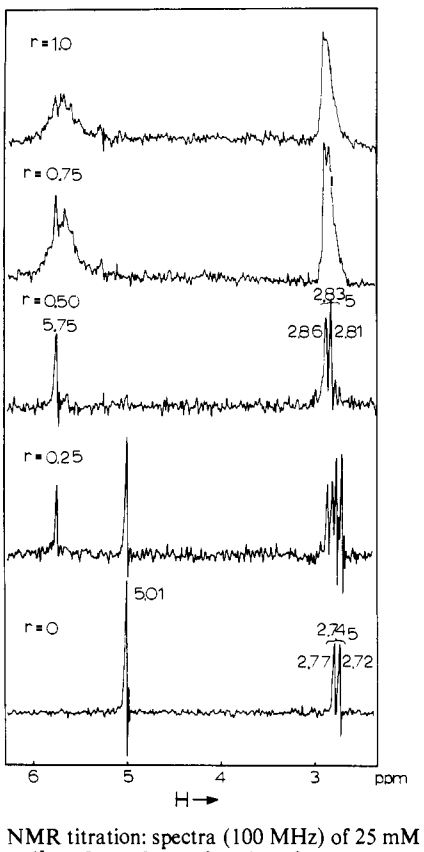


Figure 9. ^1H NMR titration: spectra (100 MHz) of 25 mM 5′-GMP + *trans*-$(H_3N)_2Pt^{II}$, D_2O, pD 8, as a function of *r*.

there are significant differences in the reactions of the two isomers at pH 7 and above when $r = 1$.

In order to study these differences, Raman and ^1H NMR spectra were obtained using solutions prepared from the same GMP stock solution with the same sequence of reagent addition and with pH values measured with the same instrument and in rapid succession. The Raman spectra for solutions with $r = 1$, pD 8.3 and ~9.6, are illustrated in Figure 10. These spectra also were followed as a function of time to establish when they were essentially time independent.

At pD 8.3, the solution containing the trans isomer gives a Raman spectrum similar to that exhibited in acidic solution and by the bis complexes of both the cis and trans isomers up to pD 9. See, for example, the spectra in Figures 2, 6, and 7. The somewhat low intensity at 1668 cm^{-1} suggests partial deprotonation of the N(1) position has occurred. Clearly, the *trans*-$(H_3N)_2Pt^{II}$ is coordinated to N(7) of the GMP, and the N(1) position still is at least partially protonated. Spectra determined 2 and 5 days after mixing were identical. At this

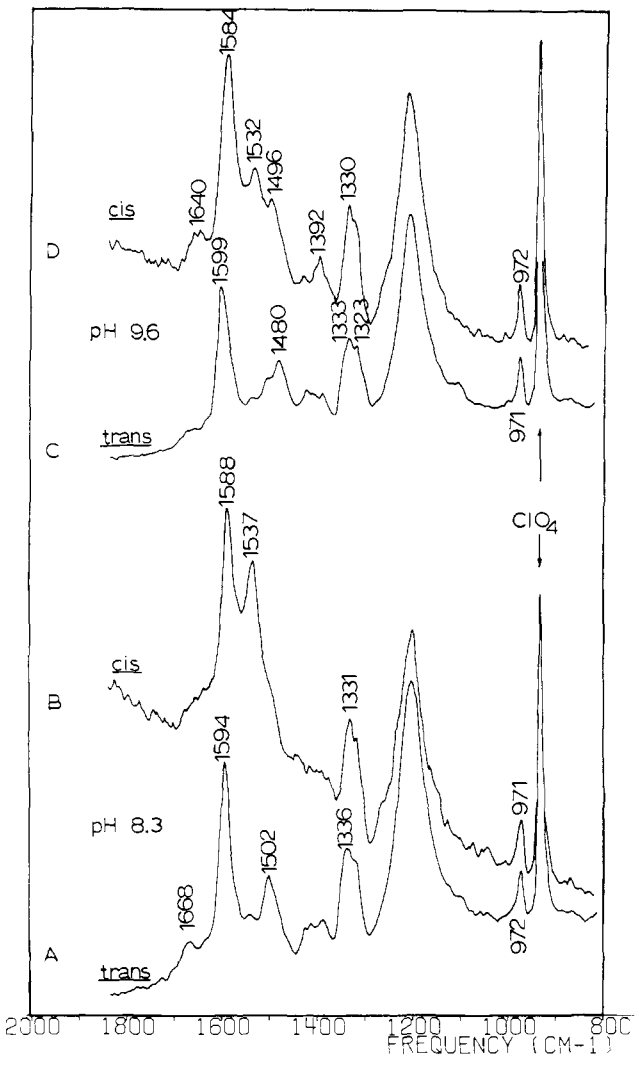


Figure 10. Raman spectra of 25 mM 5′-GMP + 25 mM *cis*- or *trans*-$(H_3N)_2Pt^{II}$, D_2O, 25 °C: (A) trans, pD 8.3; (B) cis, pD 8.3; (C) trans, pD 9.4; (D) cis, pD 9.8.

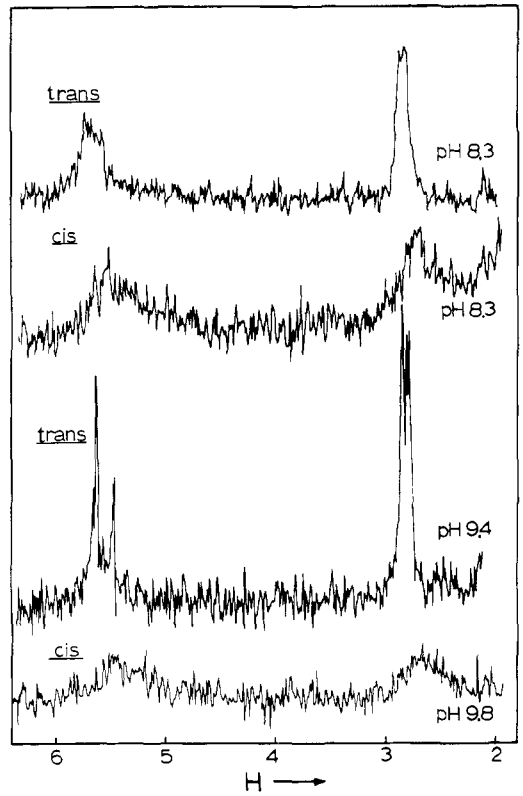


Figure 11. ^1H NMR spectra (100 MHz) of 25 mM 5′-GMP + 25 mM *cis*- or *trans*-$(H_3N)_2Pt^{II}$, D_2O, 25 °C. The solutions are the same as those of Figure 10.

same pD 8.3, the cis complex gives a spectrum similar to the trans complex shortly after mixing, but after 2 days at 25 °C, the 1665-cm^{-1} (C=O) stretching band has disappeared, probably shifting to give the broad scattering at ca. 1630 cm^{-1}. The change was complete in 5 days. Other pronounced differences from the spectrum of free GMP or of GMP platinated at N(7) occur. The disappearance of the 1665-cm^{-1} band suggests deprotonation at N(1) has occurred. Since the en-Pt(II)-5′-GMP complex at r = 1, pD 7, gives an essentially identical spectrum (see Figure 2) either *cis*-$(H_3N)_2Pt^{II}$ or $enPt^{II}$ causes a much larger decrease in the pK_a value of the N(1)-H than the trans isomer does. The decrease in pK_a of N(1) in the presence of the cis or ethylenediamine complexes at r = 1 at least 2.8, somewhat greater than observed for *cis*-$(H_3N)_2Pt^{II}$ and inosine.[14]

At pD ca. 9.6, the Raman spectral changes indicate extensive deprotonation of N(1) with both *cis*- and *trans*-$(H_3N)_2Pt^{II}$. With the trans isomer, the most significant change that occurs upon increasing the pH is the disappearance of the 1675-cm^{-1} (CO) stretching mode. The frequencies are essentially the same as those for *trans*-$[(H_3N)_2Pt(GMPH_{-1})_2]^{4-}$ (see Figure 7), suggesting platinum is interacting primarily at N(7). The decrease in pK_a of N(1) in the presence of the trans isomer at r = 1.0 is ca. 1 unit. The spectrum of the solution containing the cis isomer at pD 9.8 is similar to the spectrum at pD 8.3. The weak band at ca. 1496 cm^{-1} was observed to decrease with time. A comparable band was observed for the solution containing the cis isomer at pD 8.3 when the spectrum was scanned initially, but this decreased with time and eventually disappeared. It probably is due to a small amount of GMP that is normally N(7) bonded to *cis*-$(H_3N)_2Pt^{II}$. From these data, it is clear that the *cis*- and *trans*-$(H_3N)_2Pt^{II}$ have markedly different effects in stabilizing the deprotonated GMP in the complex with 1:1 $GMPH_{-1}^{3-}$:$(H_3N)_2Pt^{II}$ stoichiometry.

The ^1H NMR spectra also were obtained for these same pairs of solutions, and these are illustrated in Figure 11. The solutions containing the cis complex at pD 8.3 and 9.8 both exhibit the same very broad signals at ca. 5.5 (H(8)) and 2.7 ppm (H(1′)) described above for solutions of enPt(II) with r = 1.0, pD 7. The resonances of the other ribose protons were broadened comparably. With the trans isomer, complex multiplets were obtained at pD 8.3, but the bandwidths of the individual components are essentially the same as the $N(CH_3)_4^+$ internal reference. These data suggest that there is extensive polymerization of the *cis*-$[(H_3N)_2PtGMPH_{-1}]^-$ product. With *trans*-$[(H_3N)_2PtGMPH_{-1}]^-$, there appears to be some aggregation at pD 8.3 but much less than in the case of the cis isomer, and almost complete depolymerization at pD 9.4. The presence of an H(8) resonance in all cases rules out direct coordination of platinum(II) to C(8).

Reactions of *cis*-$(H_3N)_2Pt^{II}$ with Polyguanylate in H_2O, pH 7. In order to assess the effect of constraining the guanine bases along a phosphate diester backbone on the reaction with *cis*-$(H_3N)_2Pt^{II}$, solutions of poly(rG) were prepared with r values up to 0.60. At appreciably higher r values, precipitation of the polynucleotide occurs. Since the poly(rG) concentration was no greater than 25 mM, difference spectra were obtained to subtract the H_2O + 50 mM $NaNO_3$ reference background. The r = 0 spectrum is the same as that obtained by Rice et al.[31] for the high salt form of poly(rG), pH 7. In marked contrast to the roughly comparable GMP, pD 7.6, titration (Figure 2),

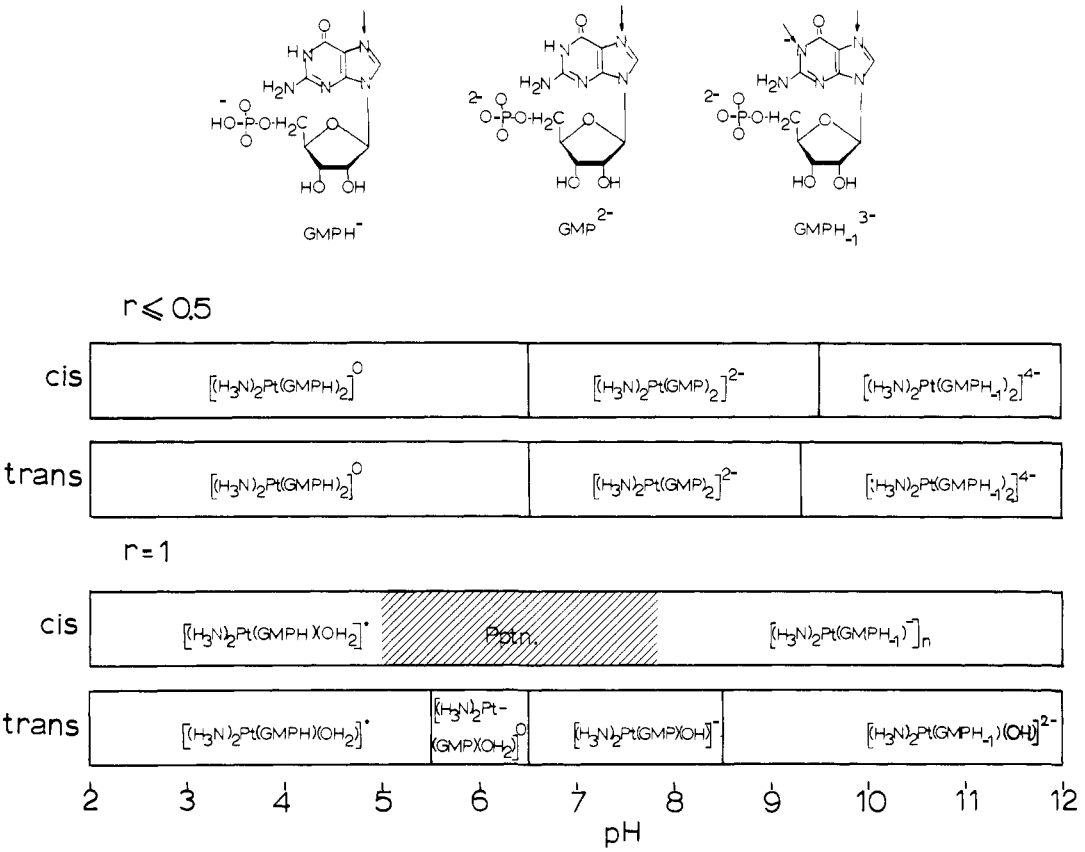


Figure 12. Distribution of species in the *cis*- and *trans*-$(H_3N)_2Pt^{II}$-5′-GMP systems in D_2O, 25 °C, as a function of r and pH.

only very minor changes in the poly(rG) frequencies and intensities occur as r increases. An asymmetric band assigned to (Pt-N) stretching is observed at ca. 570 cm^{-1}. The spectra are illustrated in the microfilm edition, Figure 2.

Discussion

Species Stoichiometry. Figure 12 summarizes the species distributions as a function of pH for both *cis*- and *trans*-$(H_3N)_2Pt^{II}$, $r = 0.5$ and 1.0. It should be noted that the order of addition of reagents and of pH adjustment can affect the nature of the species produced, since the products in these systems are largely kinetically controlled. The species in Figure 13 are those produced by mixing solutions of *cis*- or *trans*-$[(H_3N)_2Pt(OH_2)_2]^{2+}$ and of 5′-GMP at the equilibrium pH (pD) values, slightly acidic and slightly alkaline, respectively, followed by adjustment of pH (pD) via the pH-stat technique. In addition, because of the formation of polycondensed hydrolysis products,[25] the reaction rates will differ with freshly prepared and aged solutions of the *cis*-$(H_3N)_2Pt^{II}$ or $enPt^{II}$ complexes.

A number of proton transfer reactions occur as the pH is raised above 4 with solutions of *cis*- or *trans*-$(H_3N)_2Pt^{II}$ plus 5′-GMP. The acid dissociation constants for the water ligand of the mono-GMP complexes were assumed to be similar to $^*K_{11}$ ($pK = 5.6$)[28] for the cis diaquo complexes where the water molecules are trans to a nitrogen donor. Proton transfer from the phosphate groups of the bis complexes in D_2O is observed in the range pD 5-7, the normal range for free 5′-GMP ($pK \simeq 6.7$,[26] H_2O). Similarly, deprotonation of N(1) of the GMP ligand occurs above pD 9 with the bis complexes. When $r \leq 0.5$, cis and trans isomers behave similarly, and both form complexes over a wide pH range. When $r = 1$, both systems are characterized by decreased solubility. The most significant difference in the behavior of the two isomers is the marked decrease in the pK for deprotonation of N(1) in the presence of the cis but not the trans complex at $r = 1$. Similar behavior was observed for *cis*-$(H_3N)_2Pt^{II}$ and inosine or 5′-IMP.[14] Since solutions at $r = 0.5$ containing *cis*-$[(H_3N)_2Pt(GMP)_2]$ are produced without precipitation while the system at $r = 1$ gave precipitation in the range pD 4.5-7.5, it appears that coordination of the second GMP ligand is kinetically favored over the precipitation process.

Assignment of Structures of Complexes in Aqueous Solution from Raman and ^1H NMR Data. It is now apparent that heavy metals cause relatively minor perturbations of the vibrations of nucleotides upon coordination to the rings. The spectrum of a complexed neutral nucleotide will resemble the free nucleotide, while the complex of a nucleotide deprotonated on the ring will give a spectrum not unlike the sodium or potassium salt. For example, the highest frequency band of Urd in H_2O which involves mainly ν(C(2)=O) occurs at 1680 cm^{-1}, the highest frequency band of $UrdH_{-1}$ is at 1635 cm^{-1},[32] and the corresponding band of $CH_3HgUrdH_{-1}$ is at 1637 cm^{-1}.[33]

Only two forms of 5′-GMP appear to be important to discussions of platinum(II) binding, I and the N(1) deprotonated form II. Relatively little is known about the charge distribution in II. No crystallographic data seem to be available for any salts or complexes containing II. Canonical form IIA often is drawn, but because no IR band or strong Raman line appears above 1600 cm^{-1} a high degree of delocalization of charge from N(1) is assumed.[34] Miles, who originally established the stable tautomer using IR spectra, chose to emphasize IIB because the anion spectrum was very like the 6-methoxyenol model and the ^{16}O and ^{18}O anions had similar spectra above 1500 cm^{-1}.[15,35]

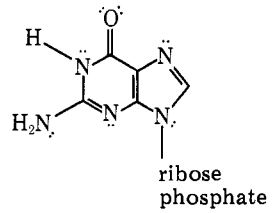
As a result of the uncertainty about charge distribution, heavy metal complexes of this type have been assigned structures with the metal bound at C(6)O (Hg(II)-$IMPH_{-1}^{3-}$),[36] C(6)O-N(7) chelating or binding two metals in a dimer

Table II. Fingerprint Frequencies for Coordination of Electrophiles to Different Sites on 5′-GMP in D_2O

Electrophile coordinated to		Frequency, cm^{-1}		
N(7)	N(1)	I	II	III
CH_3^+	$^2H^+$ [a]	1693	1608	1535
$^2H^+$	$^2H^+$ [b]	1695	1607	1515
CH_3Hg^+	$^2H^+$ [c]	1677	1598	1493
cis-$(H_3N)_2Pt(GMPH)^+$	$^2H^+$ [d]	1675	1592	1502
trans-$(H_3N)_2Pt(GMPH)^+$	$^2H^+$ [d]	1679	1593	1503
cis-$(H_3N)_2PtGMP^0$	$^2H^+$ [e]	1666	1591	1499
trans-$(H_3N)_2PtGMP^0$	$^2H^+$ [e]	1675	1593	1500
trans-$(H_3N)_2Pt(OH)^+$	*trans*-$(H_3N)_2Pt(OH)^+$ [f]		1596	1502
cis-$(H_3N)_2Pt(GMPH_{-1})^-$			1602	1483
trans-$(H_3N)_2Pt(GMPH_{-1})^-$			1604	1483
	$^2H^+$	1666	1578	1478
	CH_3Hg^+ [g]		1600	1500, 1466
			1591	1468

[a] 7-MeGuo; ref 30, pD 4. [b] Reference 31, pD 1. [c] Reference 24, pD 2. [d] pD 4.5. [e] pD 7.5. [f] pD 8.3, $r = 2$. [g] Reference 24, pD 8.5. The complexity of the CH_3Hg^{II}-5′-GMP spectra compared to the $(H_3N)_2Pt^{II}$ spectra and the CH_3Hg^{II} or $(H_3N)_2Pt^{II}$-Ino spectra suggests that mercury is not binding to a unique site on 5′-GMP. See also Simpson, ref 41.

(Ag^+-$GuoH_{-1}^-$, $InoH_{-1}^-$),[37] C(6)O-N(7) chelating (Pt(II) + $2InoH_{-1}^-$, $GuoH_{-1}^-$),[38] and N(7)-N(1) with polynuclear complex formation.[14] In all cases, infrared or Raman spectra indicated no ν(C(6)=O) mode above 1635 cm^{-1}.



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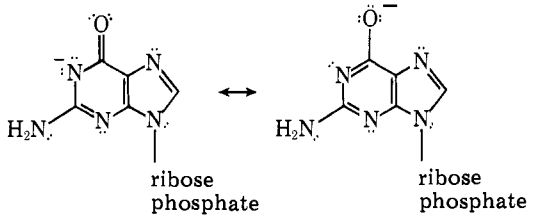


IIA IIB

Chemical evidence based on the reactions of alkylating agents suggests that N(7) is the major nucleophilic site in acidic or neutral solution, while the major site in alkaline solutions is N(1).[16] Nevertheless, there is a definite dependence on the nature of the electrophile. Table II contains a list of Raman frequencies for 5′-GMP with electrophiles bound at different sites. The three modes listed may be described as follows: I, ν(C(6)=O)[39]; II, ν(C(4)=C(5)) + ν(C(5)—C(6)) in phase[39]; III, a purine mode that is affected by coordination to N(7) or isotopic substitution to C(8) and consequently one that involves N(7)-C(8) motion.[40] A number of patterns can be discerned by examination of these data. Considering the values for coordination of $^2H^+$, CH_3^+, and H_3CHg^+ it is seen that the first two have a much greater electron localizing effect than the heavy metal. Species protonated at N(1) have a medium intensity band in the range 1666–1695 cm^{-1}, mode I, regardless of whether N(7) is coordinated to an electrophile or not. Mode II is highest with strong electrophiles at N(1). Mode III generally appears at ca. 1500 cm^{-1} when electrophiles are bound at N(1) and N(7) and near 1480 cm^{-1} when an electrophile is bound at either N(1) *or* N(7).

In a comparison of the Raman and ^1H NMR data in this work, it should be noted that the perturbations in the Raman spectra reflect primarily electronic changes in the purine base and are relatively little affected by the environment of the ligand.[14,17,22] Consequently, the vibrational spectrum of a coordinated GMP ligand is essentially the same in both a mono and a bis complex so long as both involve coordination through the same donor atom. On the other hand, the ^1H NMR spectrum is influenced by diamagnetic anisotropy effects, and the chemical shifts depend markedly upon the other ligands in the first coordination sphere of the metal and the overall complex structure.[14,17,22]

The Bis Complexes

N(7) Coordination and Its Effect on the Electronic Structure of 5′-GMP. Solutions prepared by mixing 5′-GMP and *cis*- or *trans*-$(H_3N)_2Pt^{II}$ in acidic solution, $r \leq 0.5$, contain *cis*- or *trans*-$[(H_3N)_2Pt(GMPH)_2]^0$. Gellert and Bau[42] determined the crystal and molecular structure of $[Pten(Guo)_2]Cl_{1.5}I_{0.5}$. The complex cation was found to possess a C_2 axis which passes through the platinum atom and the midpoint of the ethylenediamine C-C bond. The Pt-N(en) and Pt-N(7) (Guo) distances are 2.036 and (17) 1.967 (15) Å, and the C(6) carbonyl oxygen is hydrogen bonded to ethylenediamine. Slightly shorter distances have been observed for *cis*-$[(H_3N)_2Pt(Guo)_2]Cl_{3/2}(ClO_4)_{1/2}\cdot 7H_2O$:Pt-N ammine (2.077 and 2.068 Å) and Pt-N(7) (1.987 and 2.027 Å).[43] A similar coordination geometry was observed for $[(H_3N)_2Pt(IMP)_2]^{2-}$ in the nonstoichiometric compound $Na_{2.88}\{Pt(NH_3)_2\}_{0.56}(5'\text{-}IMP)_2\cdot 16H_2O$.[44] The Pt-$NH_3$ and Pt-N(7) distances are 2.05 and 2.02 Å, respectively. The Raman spectra of *cis*- and *trans*-$[(H_3N)_2Pt(GMPH)_2]^0$ as well as of *cis*- and *trans*-$[(H_3N)_2Pt(GMP)_2]^{2-}$ are all very similar in this region and very similar to the species with CH_3Hg^+ bound at N(7) of GMP. In addition, as described above, Kong and Theophanides[27] observed ^{195}Pt-H(8) satellites with rather concentrated solutions of *cis*-$[(H_3N)_2Pt(Guo)_2]Cl_2$ in D_2O, and this provided the original evidence for attack at N(7) by the platinum(II) electrophiles. Consequently, it can be assumed safely that these bis complexes have the GMP ligands platinated at N(7) and protonated at N(1).

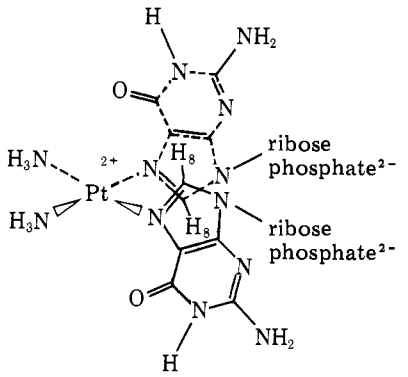
When the solution pH is raised to 7, the phosphate deprotonates with no significant effect on the platinum binding. At pH 9, the complexes begin to deprotonate at N(1). Platination at N(7) exerts a relatively minor effect on the electron distribution of the GMP ligand. The decreases in the pK of N(1) with the bis complexes are observed to be only 0.3 log unit for *cis*-$(H_3N)_2Pt^{II}$ and 0.6 unit for *trans*-$(H_3N)_2Pt^{II}$. Since platinum is coordinatively saturated in the bis complex, stabilization of the deprotonated species by N(1) coordination is avoided. Methylation at N(7) causes much larger shifts in the Raman bands than does platination. The pK of the 7-

methylguanosine cation is ca. 7.1 (H_2O),[45] i.e. $\Delta pK \simeq 2.5$. This is consistent with alkylation causing much larger changes in the electron distribution than platination.

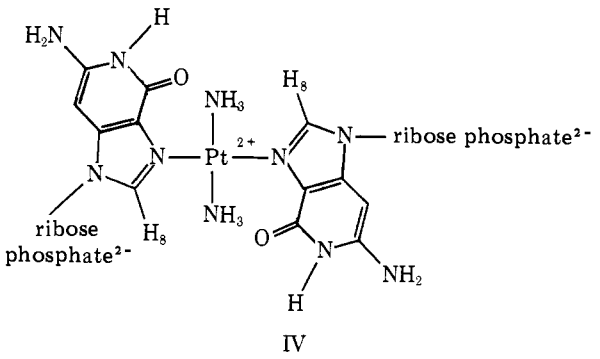
When stabilization of the conjugate base via coordination is possible, larger ΔpK values are observed. With H_3CHg^{II}, $r = 1$, ΔpK is *ca.* 6.5 log units, and the metal coordinates at N(1).[24] Even divalent, first-row transition metal ions exert a larger effect than the platinum complexes, presumably because they interact at N(1) as well as N(7); ΔpK values: Mn^{2+}, 0.43; Ni^{2+}, 1.15; Zn^{2+}, 1.40 log units with GTP.[46]

The ^1H NMR chemical shifts are very dependent upon the complex geometry because of the large diamagnetic anisotropy of the GMP ligand. Consequently the chemical shifts give little information about the inductive effects of the platinum electrophiles. The ca. 0.5-ppm downfield shift observed for H(8) at pH 7–8 for *cis*-$[(H_3N)_2Pt(GMP)_2]$ and $[enPt(GMP)_2]$ and 0.75-ppm shift for *trans*-$[(H_3N)_2Pt(GMP)_2]$ relative to GMP are consistent with the downfield shifts observed by Kong and Theophanides[27] for *cis*-$[(H_3N)_2Pt(Guo)_2]Cl_2{\cdot}2H_2O$ and $[enPt(Guo)_2]Cl_2{\cdot}2H_2O$ dissolved in water at unspecified pH. Protonation at N(7) gives a ca. 1-ppm downfield shift.

The chemical shift for H(8) is 0.26 ppm upfield for *cis*-$[(H_3N)_2Pt(GMP)_2]$ (III) relative to the trans isomer IV, at



III



IV

pD 8, consistent with a diamagnetic anisotropy due to the GMP ring current acting in the cis complex. Considering the virtually identical Raman spectra and N(1)H pK_as, it is unlikely that this shift is due to any electronic changes in the ligand from trans to cis complex.

Two structures for III can be drawn, and these would not be expected to interconvert rapidly on the NMR time scale. The two GMP ligands are related by a mirror plane in one and a twofold axis in the other. The fact that one sharp H(8) resonance is observed suggests that only one structure occurs in solution. The crystal structures of the compounds containing $[enPt(Guo)_2]^{2+}$ and $[(H_3N)_2Pt(IMP)_2]^{2-}$ both show only the isomer with an effective C_2 axis, so this is likely to be the predominant form in solution.

In previous studies on the interactions of H_3CHg^{II},[22] $(H_3N)_2Pt^{II}$,[14] and $enPt^{II}$ [14] with inosine, it was found that N(7) binding of the heavy metal catalyzed exchange of the C(8) proton with the solvent. At pD 8, exchange was quite rapid with CH_3Hg^{II}, $r > 1$. With *cis*-$(H_3N)_2Pt^{II}$, $r = 1$, exchange was not observed until pD $\geq$ 8.5, and it is much slower than with inosine. All ^1H NMR spectra of solutions with $r = 0.5$ exhibited H(8), H(1′) signals of approximately equal intensity.

In summary, it is clear that platination does not cause nearly as large an electronic perturbation as does alkylation, although N(7) is the principal target for both kinds of reagents in acidic and neutral solution.

Direct Reaction with Guanosinate at High pH. The Raman spectra of *cis*- and *trans*-$[(H_3N)_2Pt(GMP)_2]$ indicate that N(1) deprotonation occurs in alkaline solution with no isomerization. Since the uridine and thymidine conjugate bases have been found to bind *cis*-$(H_3N)_2Pt^{II}$ very strongly,[17] it seemed possible that the N(7) platinated, N(1) deprotonated GMP would be thermodynamically unstable to isomerization of platinum to N(1) or possibly O(6). Such a rearrangement would be expected to be very slow.

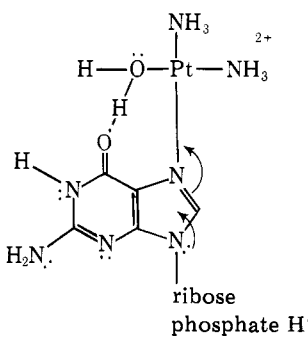
Solutions prepared by direct reaction of $[(H_3N)_2Pt(OH)_2]$ and $GMPH_{-1}^{3-}$ at pD 11 exhibited only a very slow reaction. The observed shifts in the band maxima indicated platination on the ring system, but the broad signals suggested a mixture of species was present. Clearly $[(H_3N)_2Pt(OH)_2]$ is not a good electrophile for $GMPH_{-1}^{3-}$ nor was it observed to be for $UrdH_{-1}^{-}$, at high pH.[17]

At this point, the general nuclophilic character of 5′-GMP toward platinum(II) may be summarized as follows: (i) the N(7) donor is a far better nucleophile than any other site in acid–neutral solutions; (ii) the deprotonated pyrimidine ring also would be expected to donate via N(1), but at pH 9 and above hydroxide competes quite effectively with 5′-GMP and the platinum complexes are poor electrophiles.

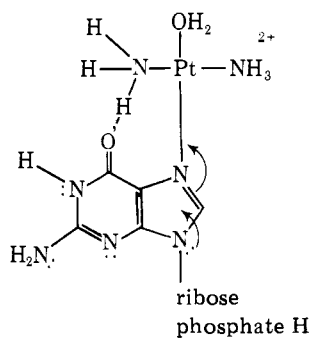
Coordination via O(6) is unlikely to be an important process in aqueous solution except in cases where favorable geometry forces an interaction at the oxygen atom. This oxygen can never be as good a donor as hydroxide, because of contributions of canonical forms such as IIA to the electronic configuration. Heavy metal binding to $GMPH_{-1}^{3-}$ when N(7) is blocked would be expected to occur at N(1). Nelson et al. suggested binding of Pd(II) to $GuoH_{-1}^{-}$ occurred at N(1) on the basis of the observaton that the only significant shifts in ^{13}C NMR signals were at C(2) and C(6).[47]

Mono Complexes

The Absence of N(7)–O(6) Chelation in Acidic Solution. Neither the interactions of *cis*- or *trans*-$(H_3N)_2Pt^{II}$ with 5′-GMP nor inosine in acidic solution at $r = 1$ show any evidence for a Pt(II)–O(6) interaction. The ν(C(6)=O) mode exhibits only a ca. 10-cm^{-1} increase due to the inductive effect of the platinum(II) electrophiles bound at N(7). This same shift is observed with the bis complexes, *cis*- and *trans*-$[(H_3N)_2Pt(GMPH)_2]$, where platinum is coordinatively saturated and cannot interact with O(6). It is possible that intramolecular hydrogen-bonded structures such as V and VI are formed;



V



VI

Table III. Factors Affecting the Formation of *cis*-$[(H_3N)_2PtGMPH_{-1}^-]_n$

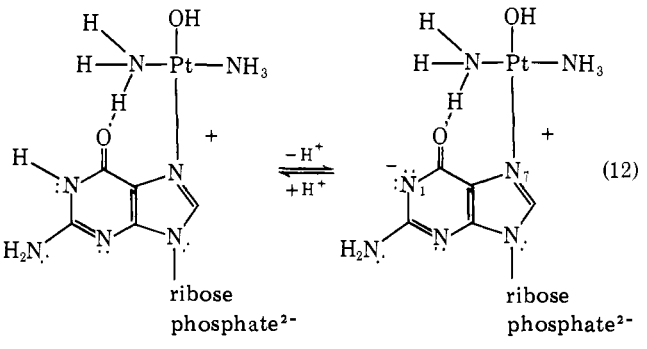
1. Ammine ligands are not essential; *cis*-$(H_3N)_2Pt^{II}$ and $enPt^{II}$ react similarly with Ino and GMP.
2. The phosphate group is not essential; similar reactions occur with Ino, IMP, GMP.
3. The C(2)NH_2 group of GMP is not essential; similar reactions occur with Ino and IMP where the amino group is absent.
4. Cis stereochemistry is essential; *trans*-$(H_3N)_2Pt^{II}$ does not form a comparable complex.
5. Attack on the deprotonated pyrimidine ring is involved; N(1) methylation blocks the reaction (observed with 1-MeIno) which also does not occur in acidic solution.
6. A cooperative process with polynuclear complex formation is indicated by very broad resonances for the base, sugar, and en protons.
7. Bridging via hydroxo groups of hydrolyzed platinum centers is not indicated, because no comparable reaction occurs with 1-methylinosine at pD 7.5 or with pyrimidine nucleosides.
8. The reaction appears to be kinetically and/or thermodynamically unfavorable relative to formation of the bis complex with N(7) donation.

however, the similarity of ν(C(6)=O) in solutions of the mono and bis complexes suggests the carbonyl is just solvated by water. If intramolecular hydrogen bonding were to occur, it would be expected to differ with the cis and trans isomers as illustrated in V and VI, because a coordinated water molecule will be a much better hydrogen-bond donor than an ammine group.

Since these complexes have positive charge more or less localized at the platinum atom and negative charge on the phosphate group, it is likely that there will be some intermolecular association. Raman[30] and infrared[48] studies of 5′-GMP indicate that it tends to associate because of a combination of hydrophobic interactions and hydrogen bonding.

Deprotonation at N(1) in Alkaline Solutions of the Trans Isomer. Since platinum is coordinatively unsaturated with respect to the nucleotide, reaction at N(1) should become possible as the pH approaches the pK of N(1)-H. At pD 8.3, solutions of *trans*-$(H_3N)_2Pt^{II}$ + GMP, r = 1, still show little evidence for deprotonation at N(1), and VII predominates.

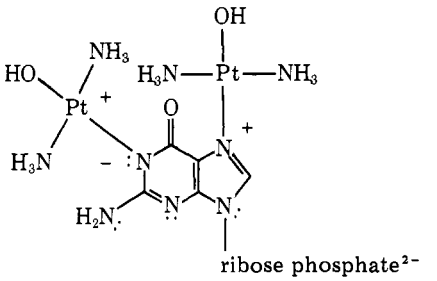
Deprotonation at N(1) can be brought about in either of two ways. If the pH is raised to above 9, proton loss occurs just as for the bis complex (reaction 12). Weak bands observed with this system at pD 9.4 are the same as those observed with pD 8.3 and are assigned to VII. The main signals are characteristic



(12)

VII VIII

of GMP platinated at N(7) and deprotonated at N(1), and these are assigned to VIII. Alternatively, increasing r from 1 to 2 at pD 8.3 leads to almost complete deprotonation at N(1). The Raman frequencies are very similar to those for GMP with H_3CHg^{II} bound at *both* N(1) and N(7). This decrease in the pK of GMP-N(1) with r = 2 indicates a stabilization of the ligand by coordination at N(1), structure IX.



IX

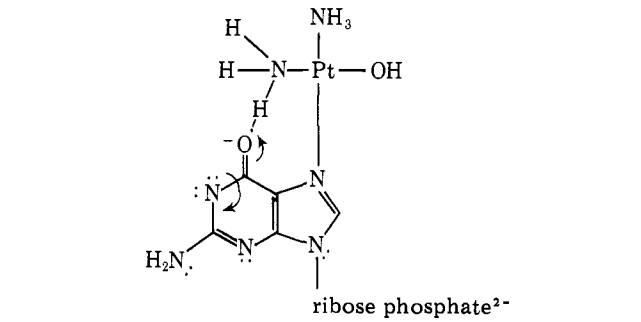
The 1:1 Reaction between *cis*-$(H_3N)_2Pt^{II}$ and GMP, pH >7, and the Structure of *cis*-$[(H_3N)_2Pt(GMP_{-1})]_n^-$. The one situation where significant differences in the complexes formed by the cis and trans isomers in solution are observed is with r = 1, pH ≥7. Firstly, the solubility around pH 7 is considerably lower with the cis than with the trans isomer. Secondly, while the trans isomer binds to GMP at N(7) very similarly at r = 1 compared to its behavior at r = 0.5, the cis isomer causes deprotonation at N(1). Thus, *cis*-$(H_3N)_2Pt^{II}$ causes a much larger increase in the N(1)-H dissociation constant, $\Delta pK >$ 2.8, compared to less than 1 log unit for the trans isomer. Similar behavior was observed with inosine where ΔpK was ca. 2 log units. These two species, *cis*-$[(H_3N)_2PtGMPH_{-1}]^-$ and *cis*-$[(H_3N)_2PtInoH_{-1}]^+$, and their ethylenediamine analogues all have a Raman band at 1630 cm^{-1} which probably involves C(6)=O stretching. The GMP system has intense bands at 1588 and 1537 cm^{-1}, and the intensity of the lower frequency band increases at the expense of the scattering at ca. 1500 cm^{-1}. This reaction is considerably slower and less complete at pD 9.8 than at pD 8.3. The ^1H NMR spectra are much broader than those of the corresponding systems with the trans isomer suggesting a considerably higher degree of polymerization occurs.

These species are obviously quite different from the bis inosine and GMP complexes of *cis*-$(H_3N)_2Pt^{II}$ and $enPt^{II}$ for which crystal-structure determinations have been carried out. A considerable amount of information on the conditions under which they are formed now has been accumulated. This is summarized in Table III.

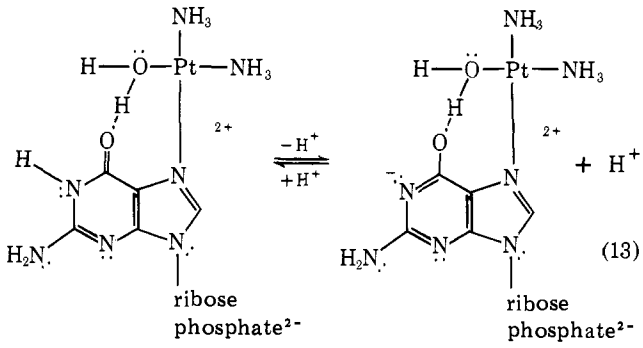
The difference between the cis and trans system at r = 1 could be attributed to (i) an unusually high inductive effect for *cis*-$(H_3N)_2Pt(OH)^+$ compared to the trans isomer, (ii) stabilization of either a deprotonated or tautomerized form of the ligand by an intramolecular interaction, or (iii) stabilization of same by an intermolecular interaction.

The simple inductive effect can be ruled out at once, because *cis*-$(H_3N)_2Pt^{II}$ has, if anything, a slightly smaller inductive effect than *trans*-$(H_3N)_2Pt^{II}$ vide supra. Intramolecular hydrogen bonding could stabilize the deprotonated form of the ligand slightly, but this should be similar with both *cis*- and *trans*-$(H_3N)_2Pt^{II}$; compare VIII and X.

Another possibility is the stabilization of the enol form of the ligand via a strong intramolecular hydrogen bond. The changes observed in the *cis*-$(H_3N)_2Pt^{II}$-GMP, -Ino, and -IMP systems take place at approximately the pH at which dissociation of a proton from an aquo group bound to platinum is expected to occur. This could occur by reaction 13 yielding XI. Interactions with this geometry are commonly found between water molecules in the first coordination sphere of metals bound at N(7) and O(6). The evidence against this is threefold. First, the reaction is a simple proton transfer and should be very fast. Experimentally, the changes in the GMP Raman spectrum are found to occur slowly. It also would be kinetically favored over bis complex formation. Secondly, the interaction at the carbonyl oxygen would have to be quite strong to bring



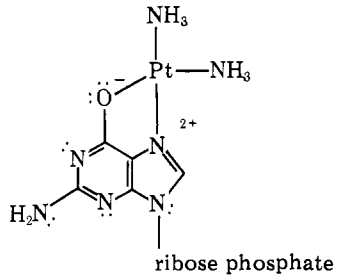
X



V XI (13)

about proton loss from N(1) rather than the coordinated water molecule when the acid dissociation constant of the latter normally would be three orders of magnitude larger. This is quite unlikely, because the *strong* binding at N(7) only approximately doubles the N(1)-H dissociation constant. The Raman spectra show no perturbation of the C=O in V. Thirdly, the NMR spectra indicate a wide range of environments for the protons exists in the complex. A structure such as XI should give a spectrum with sharp signals much as the bis complexes are observed to do.

Loss of the water molecule bound to platinum in XI would yield the internal chelate XII. Although Szalda et al.[13] were



XII

able to find only a weak interaction of the N(7)-C(6)=O chelating type when it essentially was forced in a crystalline complex, it might be expected to be more favorable in this case because of the delocalization of negative charge onto O(6) which would occur upon deprotonation. It should be noted that the formation of an internal chelate via reaction 12 would require a nucleophilic displacement of the coordinated aquo ligand by the C(6)=O and the formation of a complex with a considerably less favorable structure than XI. The geometry in XI has been shown to occur in many crystal structures; none of the type XII is yet known.

The internal chelate XII also could be formed by a direct nucleophilic displacement of OH^- from X by O(6). This should not be thermodynamically favorable, for OH^- should be a stronger base than the C(6)=O because of the electron delocalizing effect of the ring system. Because the hydroxo ligand is a poor leaving group and the C(6)=O a weak nucleophile, the substitution also should be a very slow process.

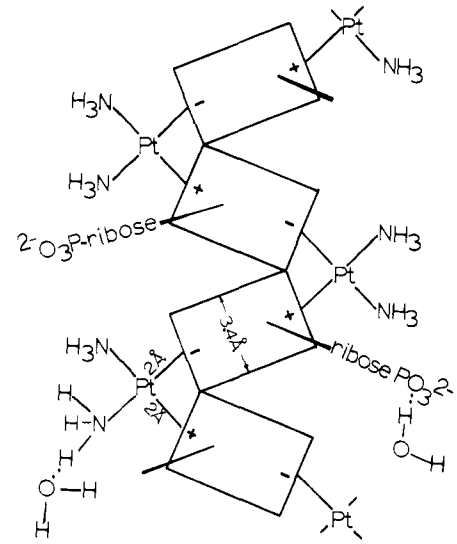


Figure 13. Possible structure for $[(H_3N)_2PtGMPH_{-1}]_n$.

Although anchimeric assistance[49] has been observed to increase rates in platinum substitution, the examples have involved flexible ligands rather than a rigid one such as GMP which also has unfavorable geometry for the chelation.

The experimental evidence is more consistent with stabilization of *cis*-$[(H_3N)_2Pt(GMPH_{-1})]^-$ by *intermolecular* interaction, i.e., by polynuclear complex formation. This was proposed for the analogous inosine complex, $[(H_3N)_2Pt\text{-}InoH_{-1}]^+$, because of the broad ^1H NMR signals. A similar NMR broadening effect has been observed with the 5-mercuric pyrimidine nucleotides which form polynuclear complexes,[50] presumably via C(5)-N(3) bridges. The broadening is considerably greater with *cis*- than with *trans*-$(H_3N)_2Pt^{II}$ suggesting that more extensive polymerization occurs in the cis case.

The occurrence of scattering at 1630 cm^{-1} with the cis isomer is suggestive of an interaction at N(1) and a rather high bond order for the carbonyl group. No comparable band is observed with $GMPH_{-1}^{3-}$ deprotonated at N(1),[32] with enol models (6-methoxy derivatives[35]), or with N(7)-bound *cis*- and *trans*-$[(H_3N)_2Pt(GMPH_{-1})_2]^{4-}$, but a band is found at 1633 cm^{-1} with $CH_3HgInoH_{-1}$ and $[(CH_3Hg)_2InoH_{-1}]^+$ where the unifunctional mercurial is presumed to bind at N(1) and N(1)-N(7), respectively.[22]

Previously we suggested a model for the analogous inosine complex which involved $(H_3N)_2Pt^{II}$ centers bridging N(1) and N(7) positions, Figure 13; see also Figure 15 of ref 14. This would tend to be stabilized by the relatively long-range Coulombic forces between the electron-rich pyrimidine rings and electron-deficient imidazole rings. Because of the complexity of the NMR spectra, simple dimer formation seems unlikely. In part, this was suggested originally by the report that the angle made by the two base planes in $[(H_3N)_2Pt(IMP)_2]^{2-}$ where platinum is coordinated to N(7)[9] was only 43°, much less than might be expected on the basis of the expected 90° bond angles at platinum (80° in this case). In this compound the N(7)-bound platinum atom is reported to be 0.59 Å out of the least-squares plane of the ring. No information is available for heavy metals bound to N(1) of the inosine or guanosine systems, but Hg^{2+} bound at N(3) of 1-methylthymine was found to be 0.30 Å out of the plane in $Hg(MeThyH_{-1})_2$.[51] These data suggest there is sufficient flexibility in these systems for hydrophobic interactions to help stabilize an aggregate of this type in solution. Since the purine ring system would be expected to have an effective thickness of ca. 3.4 Å,[52] solvent would be excluded rather effectively from this micelle-like

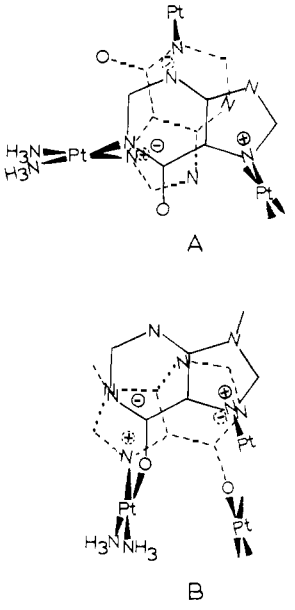


Figure 14. Polymerization via: (A) N(7)-N(1) bridges; (B) N(7)-O(6) bridges.

structure. Clearly, the trans isomer could form no comparable polynuclear complex. In addition, GMP itself is known to have a highly associated structure. A similar polymer can be drawn with platinum coordinated to N(7) and O(6) and with only a slightly different orientation of the rings; see Figure 14. This appears slightly more favorable and involves head-to-tail stacking as suggested for GMP itself. Recently Barton et al.[54] have demonstrated that bridging involving exocyclic oxygens, in this case of the conjugate base of 2-pyridone, is possible. Since these solutions exhibit only a weak absorption at the blue end of the spectrum, it is unlikely that direct Pt-Pt interactions of the type observed by Barton et al.[53] contribute significantly to the stability of the polynuclear complex.[54]

Comparison of the Reactions of *cis*-$(H_3N)_2Pt^{II}$ with GMP and Poly(rG). On the basis of Raman,[31] infrared,[15] and ultraviolet absorption studies,[55] poly(rG) has been reported to have a highly ordered structure. It is only partly melted at 90 °C. In the studies on the binding of *cis*-$(H_3N)_2Pt^{II}$ to 25 mM poly(rG), the solutions were maintained at 37 °C for 3 days, conditions under which extensive reaction with GMP occurs. With poly(rG), the Raman spectrum was hardly changed by addition of the platinum complex. It is concluded that the multistranded, strongly hydrogen-bonded structure of poly(rG) limits the availability of the N(7) sites for reaction. The high frequency of ν(Pt-N), 30-40 cm^{-1} higher than with GMP, suggests that the ammine groups are strongly hydrogen bonded to the polynucleotide.

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Supplementary Material Available: Raman spectra, 5 pages. Ordering information is given on any current masthead page.

References and Notes

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