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## References and Notes

(1) B. H. Howard and H. Ralstrick, *Biochem. J.*, **44**, 227 (1949).
(2) F. Arcamone, G. Cassinelli, G. Fantini, A. Grein, P. Orezzi, C. Pol, and C. Spalla, *Biotechnol. Bioeng.*, **11**, 1101 (1969).
(3) S. Gatenbeck, *Acta Chem. Scand.*, **14**, 296 (1960).
(4) (a) M. Tanabe, T. Hamasaki, and H. Seto, *Chem. Commun.*, 1539 (1970); (b) H. Seto, L. W. Cary, and M. Tanabe, *Ibid.*, 867 (1973); (c) *Tetrahedron Lett.*, 4491 (1974); (d) *J. Antibiot.*, **27**, 558 (1974); (e) H. Seto and M. Tanabe, *Tetrahedron Lett.*, 651 (1974).
(5) (a) O. A. Gansow, A. R. Burke, and W. D. Vernon, *J. Am. Chem. Soc.*, **94**, 2550 (1972); (b) S. Barcza and N. Engstrom, *Ibid.*, **94**, 1762 (1972); (c) O. A. Gansow, A. R. Burke, and G. N. LaMar, *J. Chem. Soc., Chem. Commun.*, 456 (1972); (d) G. C. Levy and J. D. Cargioli, *J. Magn. Reson.*, **10**, 231 (1973).
(6) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N.Y., 1972.
(7) A referee has advanced this explanation based on the qualitative observation that the satellite peaks appeared larger than they should have were only possibility (1) important.

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## Interaction of *cis*-Diammineplatinum(II) with Nucleosides. Evidence for Bifunctional Electrophilic Attack and Base Stacking in the Binding to Inosine

*Sir:*

On the basis of Raman and $^1$H NMR studies on the reactions of *cis*-$(H_3N)_2Pt^{II}$ and $enPt^{II}$ with nucleosides in aqueous solution, we have obtained evidence for a strong bifunctional interaction with inosine in neutral solution which stabilizes the normal stacking of the bases. Coordination of the bifunctional platinum(II) electrophiles in neutral solution is considerably more complex than has been assumed in earlier work. Because of the proton loss from inosine and, by analogy, guanosine accompanied by stacking, it is questionable how much relevance studies on nucleosides and mononucleotides have to the stereochemistry of the interaction of bifunctional electrophiles with native polynucleotides.

Since the discovery by Rosenberg and coworkers[1] in 1969 that *cis*-$[PtCl_2(NH_3)_2]$ was a potent antitumor agent against Sarcoma 180 in Swiss White mice, there has been considerable interest in the activity of compounds of this type.[2-6] Studies of tissue cultures,[7-8] viral inhibition,[9] and bacterial transforming DNA[10] in the presence of active platinum(II) complexes suggest binding of the complex to nuclear DNA is responsible for the cytotoxic effect.

Reactions of *cis*-$[PtCl_2(NH_3)_2]$ and $[PtCl_2en]$ with nucleosides and nucleotides have been studied with uv,[11] NMR,[12,13] and mass spectra[14] and the reactions with dinucleotides by CD spectra.[15] With purines, coordination generally has been suggested to involve N(7) of inosine or guanosine and N(7) together with 6-$NH_2$ of adenosine; while for the pyrimidines, cytidine binds via N(3), and uridine or thymidine have been reported not to react. The primary reaction with native DNA's appears to be with guanine base.[16] Goodgame et al.[17] have reported the preliminary crystal structure of a nonstoichiometric complex $Na_{2.88}[Pt(NH_3)_2]_{0.56}(IMP)_2 \cdot 16H_2O$ which has two neutral inosines bound to platinum via N(7).

We have systematically mapped the perturbations of the base vibrations caused by heavy metal coordination at different sites.[18-22] These results, obtained with $CH_3Hg^{II}$ as a probe ion, can be used as an aid in interpreting Raman spectra for platinum binding.

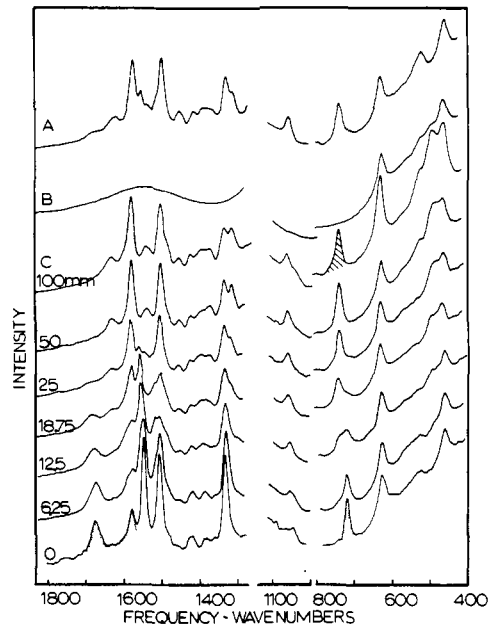


**Figure 1.** Raman spectra of 25 m*M* inosine, 25 m*M* $(H_3N)_2Pt^{II}$, and 25 m*M* inosine with $(H_3N)_2Pt^{II}$ or $enPt^{II}$ in $D_2O$ at 25°, pD 7.6: A, 25 m*M* Ino + 25 m*M* $enPt^{II}$; B, 25 m*M* $(H_3N)_2Pt^{II}$; C, Raman spectrophotometric titration of 25 m*M* inosine with $(H_3N)_2Pt^{II}$. Shaded bands indicate modes which can be used to determine the stoichiometry of the reactions.

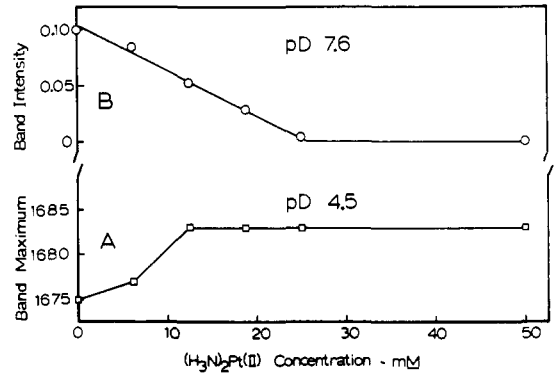


**Figure 2.** Changes in spectral parameters of 25 m*M* inosine upon the addition of $(H_3N)_2Pt^{II}$ in $D_2O$ at 25°. A (pD 4.5), values of the frequency at the maximum intensity showing the frequency shift in the 1650-1700-$cm^{-1}$ region. Overlapping bands are not resolved at 6.25 m*M* $(H_3N)_2Pt^{II}$. B (pD 7.6), values of the integrated intensity showing the disappearance of the 1675-$cm^{-1}$ band.

Raman spectra of solutions 50 m*M* in cytidine and in $(H_3N)_2Pt^{II}$, pH 7, 25°, 24 hr after mixing, are almost the same as those for cytidine quantitatively mercuriated at N(3).[18] Similar measurements with uridine show that there is only a slight decrease in the scattering at 1684 $cm^{-1}$ but none of the changes characteristic of quantitative mercuriation at N(3) with displacement of the proton. These results are consistent with previous reports on platinum coordination. We attribute the difference in platinum and methylmercury binding in these two cases to the reactions being kinetically controlled in the former, thermodynamically controlled in the latter case. In this respect platinum(II) binding resembles the reactions of alkylating agents.[23] Cytidine should be a good nucleophile using N(3); uridine is not, and the conjugate base of uridine ($pK_a$ = 9.5), a very good nucleophile, is not kinetically important in neutral solution.

Reaction of $(H_3N)_2Pt^{II}$ with inosine or guanosine at pH 7, 25°, by analogy, should involve electrophilic attack at N(7), although $CH_3Hg^{II}$ binds almost quantitatively both

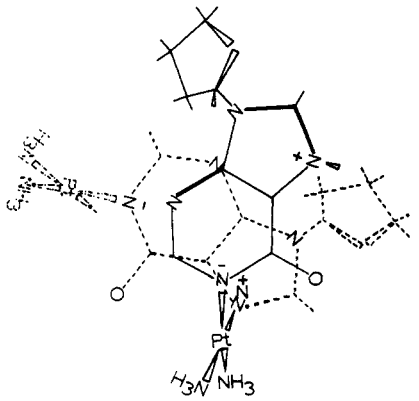

**Figure 3.** Proposed model for bridging of adjacent stacked bases in solutions of 1:1 *cis*-$(H_3N)_2Pt^{II}$:inosine. Only two units in the stack are illustrated.

to N(1) and N(7). Raman spectra are illustrated in Figure 1 for 25 m*M* inosine binding *cis*-$(H_3N)_2Pt^{II}$ and $enPt^{II}$. In $D_2O$ at pD <5, the addition of *cis*-$(H_3N)_2Pt^{II}$ has little effect on the *intensity* of the band in the 1650–1700-$cm^{-1}$ region, although the *frequency* increases slightly. This is illustrated in Figure 2, and the complex formed at pD 4.5 clearly has 2:1 inosine–platinum stoichiometry. The frequency shifts throughout the spectrum are very similar to those caused by $CH_3Hg^{II}$ coordination at N(7).[22] When proton transfer from the N(1) position of inosine occurs, the band in the 1650–1700-$cm^{-1}$ region disappears. With solutions at pH 7.6, the variation in integrated intensity of the 1675- or the 720-$cm^{-1}$ bands indicates proton loss is complete in solutions with *equimolar* concentrations of inosine and *cis*-$(H_3N)_2Pt^{II}$. This also is illustrated in Figure 2. The scattering at other frequencies, e.g., 1580 $cm^{-1}$, indicates, however, that this is not a simple 1:1 reaction. The intensity passes through a minimum in a solution with 2:1 inosine: Pt(II) as can be seen in Figure 1. As more *cis*-$(H_3N)_2Pt^{II}$ is added, the 1580 $cm^{-1}$ intensity increases up to 1:1 stoichiometry when the spectrum is very like inosine mercuriated at both N(1) and N(7).[22] The addition of more $(H_3N)_2Pt^{II}$ has little effect on the spectrum indicating that inosine interacts only weakly, if at all, at sites other than N(1) and N(7).

$^1H$ NMR studies on the same solutions show that up to 12.5 m*M* $(H_3N)_2Pt^{II}$, sharp resonances due to H(2) and H(8) of free and bound inosine are present at −5.01, −5.13 and −5.04, −5.50 ppm, respectively. (An internal $N(CH_3)_4^+$ reference was used.) At higher concentrations, new signals appear at −5.28, −5.36 ppm, and at 25 m*M* $(H_3N)_2Pt^{II}$ and above a broad resonance at −5.34 ppm is observed. In addition, all of the sugar protons become complex multiplets and then broad signals. This behavior is in marked contrast to that observed for $CH_3Hg^{II}$ binding[22] where sharp signals are present throughout. It suggests a number of slightly different environments exist for inosine in the 1:1 complex and that these are not time averaged in the $^1H$ NMR experiment as occurs in the normal nucleoside stacking.

The Raman and $^1H$ NMR data can be explained by a vertical stacking of the inosine with adjacent bases bridged by the bifunctional $(H_3N)_2Pt^{II}$. At these concentrations, stacking occurs with inosine itself.[24] Coordination of the positive platinum center at N(7) with proton loss from N(1) should result in a highly polar complex which should have a tendency to stack with the same orientation as 7-methylinosine.[25] The stacking distance, ca. 3.4 Å corresponds to the "bite" of the platinum complex, and we suggest that adjacent nucleosides are bridged as illustrated in Figure 3. The major binding site of *trans*-$PtCl_2(NH_3)_2$ to yeast $tRNA^{Phe}$ appears to involve N(7) of each of the adjacent bases Gm34 and A35 in both the crystal and solution,[26] so the trans isomer forms bridges too.

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## References and Notes

(1) B. Rosenberg, L. Van Camp, J. E. Trosko, and V. H. Mansour, *Nature (London)*, **222**, 385 (1969).
(2) B. Rosenberg, *Naturwissenschaften*, **60**, 399 (1973).
(3) M. J. Cleare and J. D. Hoeschele, *Platinum Met. Rev.*, **17**, 2 (1973).
(4) M. J. Cleare, *Coord. Chem. Rev.*, **12**, 349 (1974).
(5) T. A. Connors and J. J. Roberts, Ed., "Platinum Coordination Compounds in Cancer Chemotherapy", Springer, Berlin, 1974.
(6) S. K. Carter and M. Slavik, *Annu. Rev. Pharmacol.*, **14**, 157 (1974).
(7) H. C. Harder and B. Rosenberg, *Int. J. Cancer*, **6**, 207 (1970).
(8) J. A. Howle and G. R. Gale, *Biochem. Pharmacol.*, **19**, 2757 (1970).
(9) K. V. Shooter, R. Howse, R. U. Merrifield, and A. B. Robins, *Chem. Biol. Interact.*, **5**, 289 (1972).
(10) L. L. Munchausen, *Proc. Nat. Acad. Sci. U.S.A.*, **71**, 4519 (1974).
(11) S. Mansy, B. Rosenberg, and A. J. Thomson, *J. Am. Chem. Soc.*, **95**, 1663 (1973).
(12) P. C. Kong and T. Theophanides, *Inorg. Chem.*, **13**, 1167 (1974).
(13) P. C. Kong and T. Theophanides, *Inorg. Chem.*, **13**, 1981 (1974).
(14) I. A. G. Roos, A. J. Thomson, and J. Eagles, *Chem. Biol. Interact.*, **8**, 421 (1974).
(15) I. A. G. Roos, A. J. Thomson, and S. Mansy, *J. Am. Chem. Soc.*, **96**, 6484 (1974).
(16) P. J. Stone, A. D. Kelman, and F. M. Sinex, *Nature (London)*, **251**, 736 (1974).
(17) D. M. L. Goodgame, I. Jeeves, F. L. Phillips, and A. C. Skapski, *Biochim. Biophys. Acta*, **378**, 153 (1975).
(18) S. Mansy, T. E. Wood, J. C. Sprowles, and R. S. Tobias, *J. Am. Chem. Soc.*, **96**, 1762 (1974).
(19) S. Mansy and R. S. Tobias, *J. Am. Chem. Soc.*, **96**, 6874 (1974).
(20) S. Mansy, J. P. Frick, and R. S. Tobias, *Biochim. Biophys. Acta*, **378**, 319 (1975).
(21) S. Mansy and R. S. Tobias, *Inorg. Chem.*, **14**, 287 (1975).
(22) S. Mansy and R. S. Tobias, *Biochemistry*, **14**, 2952 (1975).
(23) See the discussion by D. M. Brown, "Basic Principles in Nuclic Acid Chemistry", Vol. 2, P. O. P. Ts'o, Ed., Academic Press, New York, N.Y., 1974, p 20.
(24) P. O. P. Ts'o in "Basic Principles of Nucleic Acid Chemistry", Vol. 1, P. O. P. Ts'o, Ed., Academic Press, New York, N.Y., 1974, p 553.
(25) P. O. P. Ts'o, N. S. Kondo, R. K. Robins, and A. D. Broom, *J. Am. Chem. Soc.*, **91**, 5625 (1969).
(26) D. Rhodes, P. W. Piper, and B. F. C. Clark, *J. Mol. Biol.*, **89**, 469 (1974), and references therein.

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## Some Deficiencies of MINDO/3 Semiempirical Theory

*Sir:*

In a recent series of papers,[1] Bingham, Dewar, and Lo (BDL) have documented a new semiempirical molecular orbital method (MINDO/3) which attempts to provide an inexpensive and reliable theoretical technique for investigating energetic and structural features inaccessible to experimental study. The method is applied to a wide range of molecules where experimental data are available and the results are also compared with ab initio molecular orbital treatments in some cases. The purpose of this communication is to point out that MINDO/3, although low in cost and useful in reproducing broad features of electronic structure, is nevertheless unreliable for energy prediction. In spite of direct parameterization to experimental data, it fails to reproduce certain key energetic relationships which are handled quite satisfactorily without parameterization by ab initio techniques. For brevity we shall limit our remarks to the