Heavy Metal–Nucleotide Interactions. II. Binding of Methylmercury(II) to Purine Nucleosides and Nucleotides Studied by Raman Difference Spectroscopy[1]

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Abstract: Literature values of equilibrium constants for binding CH_3Hg^{II} to guanosine and adenosine have been used to construct models describing the species distribution as a function of pH in these systems. Raman difference spectra have been determined for H_2O and D_2O solutions of CH_3Hg^{II} and Guo-5′-P as well as CH_3Hg^{II} + Ado-5′-P and Ado to determine the perturbations of both cation and nucleotide vibrations upon metalation at different sites. At approximately neutral pH, Guo-5′-P mercuriates at $N_{(1)}$ with displacement of a proton giving a spectrum rather like that of $[GuoH_{-1}\text{-}5'\text{-}P]^-$. This behavior is very similar to that observed for mercuriation of Urd at $N_{(3)}$. At low pH, $N_{(1)}$ is blocked and mercuriation occurs at a second site. The perturbations of the spectrum are similar to those caused by protonation at $N_{(7)}$, and the same site is suggested for mercuriation. Mercuriation of Ado-5′-P at pH 3.5 gives a complex with a spectrum similar to that of $[AdoD\text{-}5'\text{-}P]^+$. Consequently, mercuriation is assigned at $N_{(1)}$. This is not in agreement with recent calculations which suggest hard acids should bind preferentially to $N_{(1)}$ and soft acids to $N_{(7)}$. This coordination also causes a very large and unusual hyperchromic effect on the (Hg–C) stretching band. There is no involvement of the phosphate group in coordination with either Guo-5′-P or Ado-5′-P. When 1-MeAdoH$^+$ reacts with CH_3Hg^{II} at pH 3.5, only small spectral perturbations result. The vibrations associated primarily with CH_3Hg^{II} suggest that coordination is to a weakly basic site, *i.e.*, coordination appears to occur at the $N_{(7)}$ position. Applications of Raman difference spectroscopy in the determination of structures for metal–nucleotide and –nucleoside complexes in dilute aqueous solutions are discussed.

In a previous paper,[2] we reported on the Raman spectral changes which occur upon coordination of the methylmercury(II) cation to Cyd, Urd, and poly(U)[3] in moderately dilute aqueous solution. This involved the first use of Raman difference spectroscopy (RDS) to study the behavior of biological molecules in aqueous solution. It was shown that CH_3Hg^+ binds strongly to uridine and by inference to thymidine with transfer of a proton as has generally been assumed in discussions of the reversible reaction of mercury(II) compounds with DNA.[4–6] This interpretation had been brought into question, because earlier Raman studies had shown no interaction between $HgCl_2$ and Ura, Urd, 1-MeUra, and 1,3-Me_2Urd in aqueous solution,[7] and the crystal structure of $HgCl_2 \cdot 2Ura$[8] showed that mercury(II) coordinated to the $C_{(4)}{=}O$ of the nonionized Ura. Lord and Thomas[7] did find evidence for reaction of $HgCl_2$ with Cyd but were unable to obtain spectra for solutions containing Ado or Guo derivatives.

The present work has two principal objectives. The first is to obtain information on the binding of a heavy metal ion to purine nucleotides because of the current interest in mutagenic and cytotoxic effects of heavy metals, particularly platinum, arising by interaction with nuclear DNA.[9,10] The methylmercury(II) cation which binds much as other heavy metals, *e.g.*, platinum and gold, serves as a good probe ion because it is unifunctional. Additionally, the reaction can be monitored not only by the perturbations of the nucleic acid base vibrations but also by shifts in the CH_3Hg^{II} modes. The binding sites on nucleosides are generally taken to be those suggested by Simpson[11] in his uv spectrophotometric study. Simpson[11] assigned the sites by assuming that the isomers of the mercuriated forms were analogous to those of the protonated forms. For example, CH_3Hg^+ was assumed to bind to $N_{(1)}$ of Ado. Although extensive quantum mechanical calculations of the stable tautomers of nucleic acid bases have been made,[12] there now is a considerable body of evidence which indicates that many metals interact at sites different from the proton. For example, while protonation of Ado occurs at $N_{(1)}$ ($pK = ca.\ 3.5$[13]), Cu^{2+} appears to bind at both $N_{(1)}$ and $N_{(7)}$.[14] With Ado-3′-P, Ado-5′-P, dAdo-5′-P and poly(A), Cu^{2+} binding is preferentially at $N_{(7)}$.[14] Glassman, *et al.*,[15] have used Klopman's perturbation theory to predict the site(s) of protonation and of metalation on the bases of purine nucleotides. It was predicted that hard acids will bind to $N_{(1)}$ of Ado, while softer acids will tend to bind to $N_{(7)}$. Since CH_3Hg^+ is the prototype soft acid,[16] there is a

(1) Work supported by Public Health Service Grant AM-16101 from the National Institute for Arthritis, Metabolism, and Digestive Diseases and by the National Science Foundation Grant GP-4002X.

(2) S. Mansy, T. E. Wood, J. C. Sprowles, and R. S. Tobias, *J. Amer. Chem. Soc.*, **96**, 1762 (1974).

(3) The IUPAC–IUB abbreviations for nucleosides, etc., are employed throughout; see *Biochemistry*, **9**, 4022 (1970).

(4) D. W. Gruenwedel, *Eur. J. Biochem.*, **25**, 544 (1972).

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(6) S. Katz, *Biochim. Biophys. Acta*, **68**, 240 (1963).

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(9) K. V. Shooter, R. Howse, R. K. Merrifield, and A. B. Robins, *Chem.-Biol. Interactions*, **5**, 284 (1972).

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(11) R. B. Simpson, *J. Amer. Chem. Soc.*, **86**, 2059 (1964).

(12) See, for example, A. Pullman and B. Pullman, *Advan. Heterocycl. Chem.*, **13**, 77 (1971).

(13) R. M. Izatt, J. J. Christensen, and J. H. Rytting, *Chem. Rev.*, 71, 439 (1971).

(14) N. A. Berger and G. L. Eichhorn, *Biochemistry*, **10**, 1847 (1971).

(15) T. A. Glassman, G. Klopman, and C. Cooper, *Biochemistry*, **12**, 5013 (1973).

(16) For a general discussion of this, see R. G. Pearson, *J. Chem. Educ.*, **45**, 585, 645 (1968).

Table I. Equilibrium Constants[a] (25°) Used in the Description of the $CH_3Hg(II)$–Nucleoside Systems. Previously Assigned Sites of Binding are in Parentheses

Reaction	μ	Log K_{eq}	Ref
CH_3Hg^{II}			
$CH_3Hg^+ + H_2O \rightleftharpoons CH_3HgOH + H^+$	0.1	−4.59	22
$CH_3HgOH + CH_3Hg^+ \rightleftharpoons (CH_3Hg)_2OH^+$	0.1	2.37	22
Guo			
$GuoH_{-1}^- + H^+ \rightleftharpoons Guo\ (N_1)$	0.1	9.24	13
$Guo + H^+ \rightleftharpoons GuoH^+\ (N_7)$	0.1	2.23	13
$GuoH_{-1}^- + CH_3Hg^+ \rightleftharpoons GuoH_{-1}HgCH_3\ (N_1)$	Var	8.1	11
$Guo + CH_3Hg^+ \rightleftharpoons GuoHgCH_3\ (N_7)$	Var	4.5	11
$GuoH_{-1}^- + 2CH_3Hg^+ \rightleftharpoons GuoH_{-1}(HgCH_3)_2^+ + (N_1, N_7)$	Var	12.6	11
Ado			
$Ado + H^+ \rightleftharpoons AdoH^+\ (N_1)$	0.1	3.55	13
$Ado + CH_3Hg^+ \rightleftharpoons AdoHgCH_3^+\ (N_1)$	Var	3.0	11
$Ado + CH_3Hg^+ \rightleftharpoons AdoH_{-1}HgCH_3 + H^+\ (C_6NH_2)$	Var	−3.49[b]	11
$Ado + 2CH_3Hg^+ \rightleftharpoons AdoH_{-1}(HgCH_3)_2^+ + H^+\ (N_1, C_6NH_2)$	Var	0.31[b]	11
1-MeAdo			
$1\text{-MeAdo} + H^+ \rightleftharpoons 1\text{-MeAdoH}^+\ (C_4\text{-imino})$	Var	*ca.* 7.6	23

[a] Dissociation of the protons from the 2′ and/or 3′ OH groups for which pK is *ca.* 12.3 (ref 13) is ignored, since it was not considered by Simpson.[11] [b] Computed from Simpson's equilibrium constants[11] which involved CH_3HgOH as the reactant together with the hydrolysis constant of Schwarzenbach and Schellenberg.[22]

question as to the correctness of Simpson's structures for the methylmercury(II)–nucleoside complexes.

The second objective of this study is to catalog the perturbations of the Raman spectrum for each of the pertinent bases so that Raman spectroscopy can be used to determine the preferred sites for binding on a native polynucleotide chain. As noted above, Hg^{II} and CH_3Hg^{II} frequently have been used in the characterization of DNA.[4–6] Recently, CH_3HgOH has been used as a chemical probe for unpaired bases in superhelical DNA.[17]

In recent years, ^1H and ^{13}C nmr spectroscopy has proved to be one of the most powerful techniques for determining the binding sites of metal ions to nucleosides and nucleotides. The rapid, on the nmr time scale, exchange of acidic protons is a problem, and many of these investigations have been made on solutions with solvents such as DMSO. In general, these solutions are very different systems from the aqueous ones. Much more success has been achieved with paramagnetic ions such as Mn^{2+}, Co^{2+}, Ni^{2+}, and especially Cu^{2+} where the chemical shifts are much larger than with a diamagnetic ion. The soft metal ions which interact strongly with DNA, *e.g.*, Ag^+ and Hg^{2+}, are diamagnetic. RDS is a sensitive technique for detecting metal ion coordination to nucleosides and nucleotides and has proved to be quite useful in establishing binding sites for diamagnetic ions.[2]

Experimental Section

Methylmercury(II) Perchlorate. This was prepared as a standard aqueous solution from methylmercuric iodide purchased from Alfa Inorganics using the procedure described earlier.[2]

Nucleoside and Nucleotide Solutions. Ado-5′-P, Guo-5′-P, and 1-MeAdo were obtained from Sigma Chemical Co., St. Louis, Mo., and Ado was purchased from International Chemical and Nuclear Corp., Irving, Calif. Weighed amounts were dissolved in deionized, doubly distilled H_2O or 99.8% D_2O to provide stock solutions. The pH's (pD's) of solutions containing nucleosides or nucleotides and CH_3Hg^+ were adjusted with $HClO_4$ ($DClO_4$) or NaOH (NaOD) solutions using a Radiometer PHM-4 pH meter. For the deuterium oxide solutions, a standard glass electrode was used, and the meter reading was corrected by the procedure of Glascoe and Long.[18]

Raman Spectra.[19] **Solutions.** Spectra were excited with the 514.5-nm line of a Coherent Radiation Model 52 or a Control Laser Model 400 Ar^+ laser at *ca.* 700 mW. The Raman scattering was dispersed with a Spex 1400 double monochromator and detected with an RCA 31034A photomultiplier using photon counting equipment. Difference spectra were obtained by photon counting for a precise time interval, usually 10 sec, first with the sample in position, then with the reference in the laser beam. Each of these signals was ratioed to a digital signal from a photodiode laser power monitor obtained while the sample or reference was being counted. This corrects for any long term fluctuations in the laser intensity. The sample is returned to its position in the laser beam, the monochromator is advanced one step (usually *ca.* 1 cm^{-1}), and the process is repeated. The difference spectrum is obtained by subtracting the normalized reference photon count from the normalized sample count. The difference spectrophotometer and PROGRAM RAMAN used for data processing have been described elsewhere.[20] Solution temperature in difference spectra was maintained at 25 ± 1° by carrying out the measurements in a constant temperature room. For continuous variation measurements, the cell was contained in a brass block through which water thermostated at 25 ± 0.01° was circulated. The general procedures for solution preparation have been described previously.[2]

Crystal Powder Spectra. Samples of a few milligrams were sealed in capillaries and excited with a Spectra Physics Model 112 He–Ne laser (632.8 nm). The power at the sample was *ca.* 10 mW. The apparatus was essentially that described by Ramos and Tobias[21] except that a small two-prism monochromator was used to remove background plasma lines rather than a spike filter.

Data and Results

Species Distribution. As in our previous work,[2] we have used literature values of the equilibrium constants for protonation and reaction with methylmercury(II) to construct models for the behavior with pH of the Guo– and Ado–CH_3Hg^+ systems (Table I). The equilibrium constants of Schwarzenbach and Schellenberg[22] were used to describe the hydrolysis of CH_3Hg^+. Values for the ligand–proton equilibrium constants were taken from the tables of Izatt, Christensen, and Rytting[13] or the "Handbook of Biochemistry."[23] Species dis-

(17) T. A. Beerman and J. Lebowitz, *J. Mol. Biol.*, **79**, 451 (1973).
(18) P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).
(19) Since the spectra discussed herein are stored on magnetic tape, large copies of any of them may be obtained, at cost, plotted on a Gould or Calcomp plotter by writing to R. S. T.
(20) J. W. Amy, R. W. Chrisman, J. W. Lundeen, T. Y. Ridley, J. C. Sprowles, and R. S. Tobias, *Appl. Spectrosc.*, **28**, 262 (1974).
(21) V. B. Ramos and R. S. Tobias, *Inorg. Chem.*, **11**, 245 (1972).
(22) G. Schwarzenbach and M. Schellenberg, *Helv. Chim. Acta*, **48**, 28 (1965).
(23) H. A. Sober, Ed., "Handbook of Biochemistry," Chemical Rubber Publishing Co., Cleveland, Ohio, 1970, p G. 23.

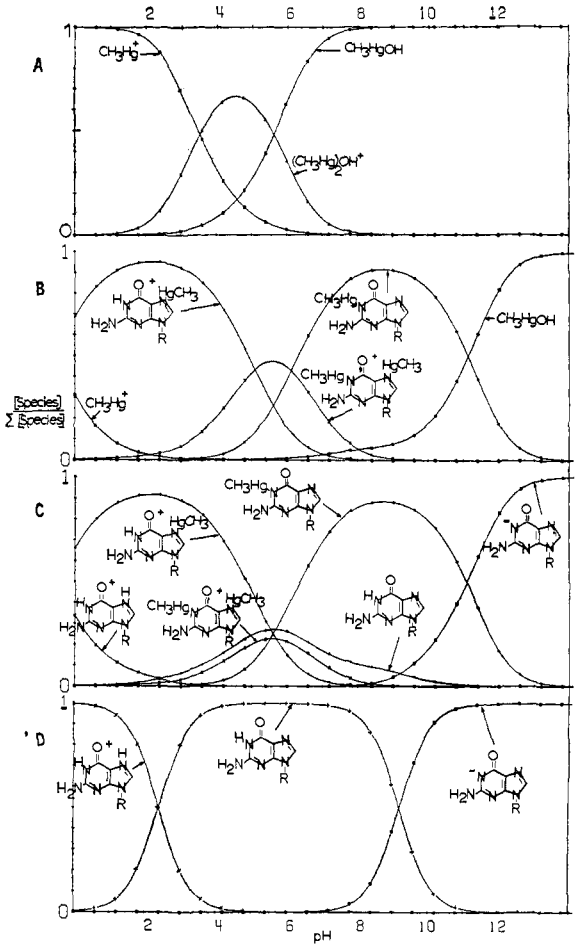


Figure 1. Species distribution in the CH_3Hg^+–Guo system computed from a model based on Simpson's[11] equilibrium constants. Proton transfer from ribose is ignored: A, 50 m*M* CH_3Hg^+; B, 50 m*M* CH_3Hg^+ + 50 m*M* Guo, metal distribution; C, 50 m*M* CH_3Hg^+ + 50 m*M* Guo, Guo distribution; D, 50 m*M* Guo.

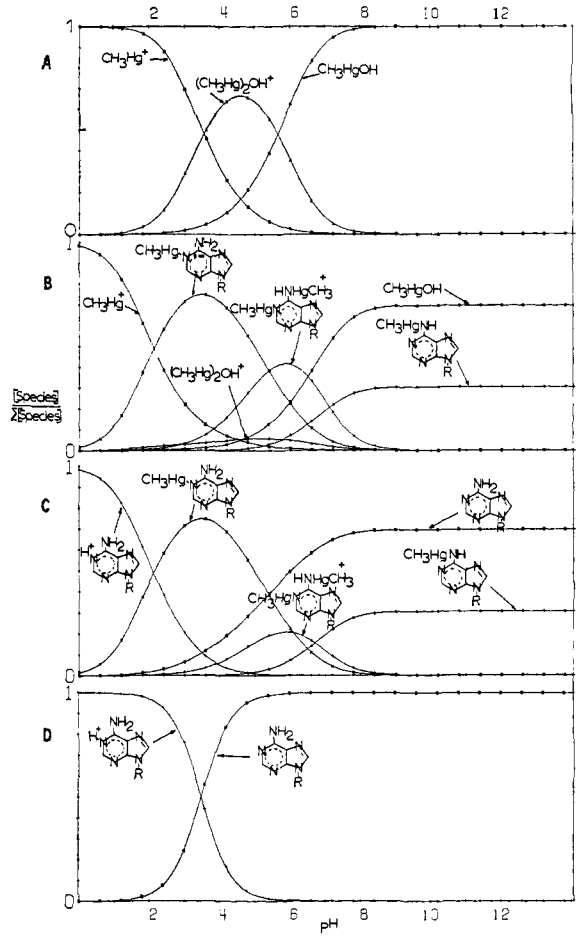


Figure 2. Species distribution in the CH_3Hg^+–Ado system computed from a model based on Simpson's[11] equilibrium constants. Proton transfer from ribose is ignored: A, 50 m*M* CH_3Hg^+; B, 50 m*M* CH_3Hg^+ + 50 m*M* Ado, metal distribution; C, 50 m*M* CH_3Hg^+ + 50 m*M* Ado, Ado distribution; D, 50 m*M* Ado.

tributions were computed and plotted with PROGRAM QUARK[24] using the university's CDC-6500 computer. These are illustrated in Figure 1 for Guo and Figure 2 for Ado.

Raman Spectra. The Raman studies were designed to use solutions which were similar to those normally employed in studies of complex formation by potentiometric and spectrophotometric techniques. A 0.1 *M* $(Na)ClO_4$ constant ionic medium was used.[25] The perchlorate bands, particularly ν_1, also serve as internal frequency and intensity standards. In all of these measurements, ν_1 of ClO_4^- was observed at 932 ± 1 cm^{-1}. Spectra of CH_3Hg^{II} under these experimental conditions have been discussed previously.[2]

Guanosine 5′-Phosphate + CH_3Hg^{II}, pH 8.5. According to Simpson,[11] Guo mercuriates at both $N_{(1)}$ and $N_{(7)}$. Mercuriation at $N_{(1)}$ is blocked by protonation at low pH, while at high pH, hydroxide coordination to CH_3Hg^{II} supposedly blocks mercuriation at $N_{(7)}$. These effects are illustrated in Figure 1. At pH 8.5, *ca.* 92% of the Guo of a 50 m*M* Guo–50 m*M* CH_3Hg^{II} mixture should be mercuriated at $N_{(1)}$ and the remaining Guo present as the free base.

(24) Deck listings of all computer programs are available upon writing to R. S. T.

(25) See the discussion in F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants," McGraw-Hill, New York, N. Y., 1961, Chapter 2.

The Raman spectra of Guo and Guo-5′-P in H_2O and D_2O solutions have been examined carefully by Lord and Thomas.[26] Although both the proton on $N_{(1)}$ and the $C_{(2)}$–NH_2 protons are exchanged in D_2O, only rather small frequency shifts were observed in the 1200–1700-cm^{-1} region. More obvious are the changes in the relative intensities of the bands. This stands in marked contrast to the behavior of Urd[2,26] and Urd-5′-P[26] which undergo large frequency and intensity changes when the proton on $N_{(3)}$ is replaced by a deuteron. Because of the low solubility of Guo in neutral solutions Guo-5′-P was employed in this work. Aside from the reactions involving the phosphate, the protonation constants and presumably the mercuriation constants are sufficiently similar so Figure 1 should apply.

Difference Spectra. In order to determine the shifts which occur upon mercuriation at $N_{(1)}$, Raman difference spectra were obtained for solutions 50 m*M* in Guo-5′-P and 50 m*M* in CH_3Hg^{II} *vs.* 50 m*M* Guo-5′-P, both at pH 8.5. The difference spectrum is illustrated in Figure 3B, and the spectrum of Guo-5′-P run *vs.* solvent is shown for comparison in Figure 3A. The extent of cancellation of the very intense ν_1 of ClO_4 at 932 cm^{-1} is a good measure of the match of sample and reference.

In the Guo-5′-P + CH_3Hg^{II} *vs.* Guo-5′-P difference

(26) R. C. Lord and G. J. Thomas, Jr., *Spectrochim. Acta, Part A*, **23**, 2551 (1967).

spectrum, any vibrations localized in the CH_3Hg^{II} ion will appear as positive features. Intense bands are observed at 1207 ($\delta_s(CH_3)$) and 560 cm^{-1} (ν(Hg–C)). New bands characteristic neither of Guo-5′-P nor of CH_3Hg^{II} also will appear as positive bands, and one example occurs at 616 cm^{-1}. Bands characteristic of Guo-5′-P which are not present in the complex will give negative bands in the difference spectrum. One such band occurs at 1690 cm^{-1}. Finally small shifts in Guo-5′-P bands will give derivative features such as are observed at 1586 (maximum) and 1569 (minimum) cm^{-1}. This arises from an increase in frequency of a Guo-5′-P band from 1576 to 1581 cm^{-1}.

A difference spectrum also was measured for 50 m*M* Guo-5′-P + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* CH_3Hg^{II}. This is illustrated in Figure 3C. The large negative band at 500 cm^{-1} indicates displacement of the hydroxo group from CH_3Hg^{II}. Both $\delta_s(CH_3)$ and ν(Hg–C) appear as unsymmetric derivatives indicating a decrease in the frequencies of both of these vibrations upon coordination to Guo-5′-P and hyperchromism in the complex.

Both of the above described data sets were combined, and a total difference spectrum was computed. From the sum of the Guo-5′-P spectrum plus the $NaClO_4$ solvent spectrum were subtracted the CH_3Hg^{II} and the Guo-5′-P spectra. This is illustrated in Figure 3D. This should give complete solvent and internal reference compensation, but it will be somewhat less precise than the difference spectra described above because it involves two independent scans with the instrument. The perturbations of the Guo-5′-P and CH_3Hg^{II} modes discussed are clearly visible.

With the information provided by the difference spectrum it is possible to examine the important regions of the parent spectra where perturbations occur upon reaction. All of these parent spectra are available in the microfilm edition.[27]

For comparison, the Guo-5′-P + CH_3Hg^{II} *vs.* Guo-5′-P difference spectrum also was recorded with D_2O as the solvent at pD 8.5. This is illustrated in Figure 4A. Although the ligand gives rather similar spectra in H_2O and D_2O, the complex spectra are somewhat different; however, the complex frequencies rather closely resemble those of [$GuoH_{-1}$-5′-P]$^-$ in the same solvent.[26]

Phosphate Binding. A question which clearly must be answered is whether CH_3Hg^{II} binds significantly to the phosphate in these solutions. Nucleotides containing the $ROPO_3^{2-}$ group show $\nu_s(PO_3)$ at *ca.* 980 cm^{-1} plus a very weak band above 1100 cm^{-1}.[26] Rimai, *et al.*,[28] have investigated these modes carefully for Raman spectra of Ado-5′-P, and they assigned bands at 850, 882, and 980 (ν_s) and 1123 and 1170 (ν_{as}) to the $ROPO_3^{2-}$ moiety. Protonation to give $ROPO_2OH^-$ causes a large perturbation, and bands are observed at 817, 858, and 1082 cm^{-1} (ν_s).

The spectrum of Guo-5′-P exhibits bands at 812, 868, and 978 cm^{-1} which do not correspond to Guo modes. Of these, 978 cm^{-1} clearly is ν_s $ROPO_3^{2-}$. An examination of the 980-cm^{-1} region in the Guo-5′-P + CH_3Hg^{II} *vs.* Guo-5′-P difference spectra, Figures 3B, 3D, 4A, shows that there is no perturbation of ν_s $ROPO_3^{2-}$, and

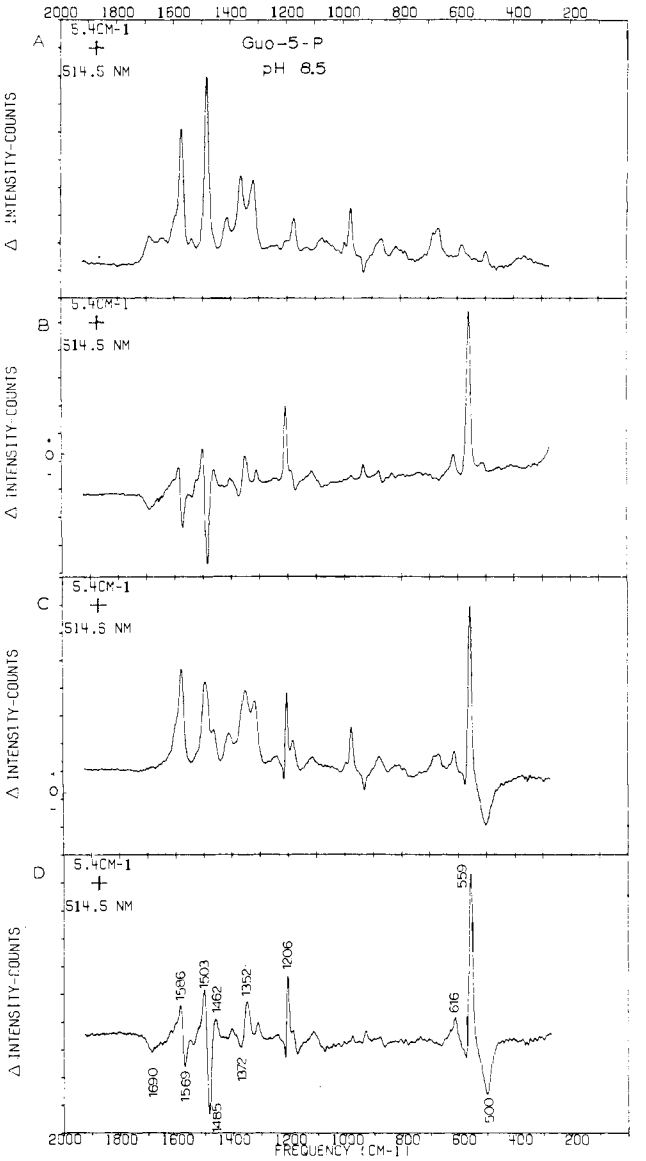


Figure 3. Raman difference spectra, Guo-5′-P + CH_3Hg^{II} at pH 8.5: A, 50 m*M* Guo-5′-P *vs.* solvent; B, 50 m*M* Guo-5′-P + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* Guo-5′-P; C, 50 m*M* Guo-5′-P + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* CH_3Hg^{II}; D, [(50 m*M* Guo-5′-P + 50 m*M* CH_3Hg^{II}) + (solvent)] − [(50 m*M* Guo-5′-P) + (50 m*M* CH_3Hg^{II})]. All solutions are 0.1 *M* in $NaClO_4$. Scan conditions: 0.25 Å steps, 10 sec count time.

on this basis it may be concluded that no interaction with the phosphate occurs in the solution.

Double-Bond Stretching Region, 1800–1500 cm^{-1}. Since the bands in this region are similar in both H_2O and D_2O solution, it was concluded that they arise from ring modes not coupled to NH_2 deformations.[23] The highest frequency (1644 (D_2O), 1690 cm^{-1} (H_2O)) assigned to $C_{(6)}$=O stretching disappears completely in the complex, probably shifting to *ca.* 1600 cm^{-1}. The band at 1576 cm^{-1}, H_2O (1577, D_2O), shifts to 1581 cm^{-1}, H_2O (1580, D_2O), in the complex.

1500–700 cm^{-1}. The most significant change in this region is the replacement of the 1487-cm^{-1} band of Guo-5′-P by two weaker bands at 1466 and 1497 cm^{-1}. This causes a large negative feature in the difference spectrum, Figures 3B and 3D, at 1484 cm^{-1}. There are shifts in all of the other Guo-5′-P bands in the 1200–1500-cm^{-1} region. In addition, the band at 1177 cm^{-1}

(27) See paragraph at end of paper regarding supplementary material.
(28) L. Rimai, T. Cole, J. L. Parsons, J. T. Hechmott, and E. B. Carew, *Biophys. J.*, **9**, 320 (1969).

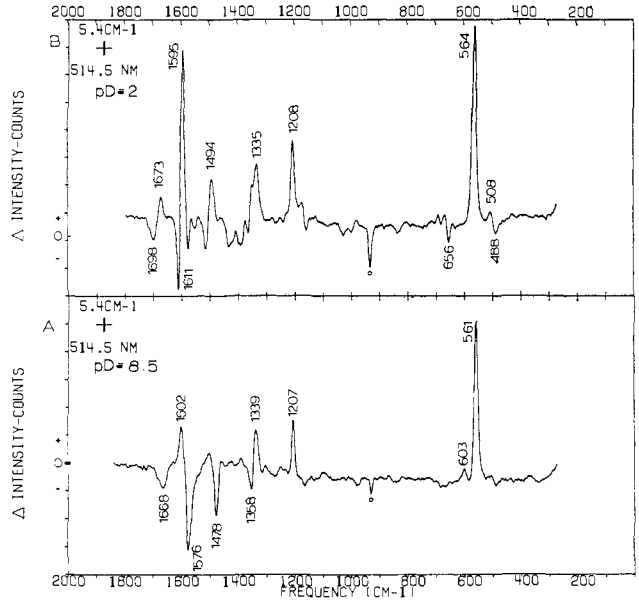


Figure 4. Raman difference spectra, 50 m*M* Guo-5′-P + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* Guo-5′-P in D_2O: A, pD 8.5; B, pD 2. Solutions are 0.1 *M* in $NaClO_4$.

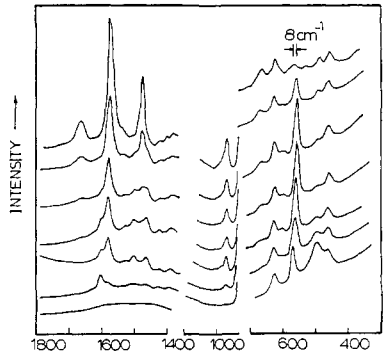


Figure 5. Continuous variation data, Guo-5′-P in D_2O at 25.0°, pH 8.5. Total concentration of Guo-5′-P + CH_3Hg^{II} = 50 m*M*. [Guo-5′-P] from top to bottom: 50, 40, 30, 25, 20, 10, 0 m*M*. Solutions are 0.1 *M* in $NaClO_4$. Scan conditions are the same as for Figure 3.

increases in frequency to 1183 cm^{-1}, and the very broad band at 1084 cm^{-1} shifts to 1115 cm^{-1}, both giving rise to derivative features in the difference spectra. Shifts in the frequencies observed in this region with D_2O solutions also occur upon coordination.

Features Associated with CH_3Hg^{II}. There is a small decrease in $\delta_s(CH_3)$ upon coordination, from 1212 to 1207 cm^{-1} (H_2O and D_2O), and ν(Hg–C) also decreases from 570 to 559 (H_2O), 561 cm^{-1} (D_2O). There is a marked hyperchromic effect on the (Hg–C) stretching band as hydroxide is replaced by nucleotide. This results in a very large, positive band at 559 cm^{-1} in the Guo-5′-P + CH_3Hg^{II} *vs.* CH_3Hg^{II} difference spectrum, Figures 3C and D and Table II. This difference spectrum also shows a large negative feature at 500 cm^{-1} caused by the disappearance of the (Hg–O) stretching band observed at 504 cm^{-1} in the solution of CH_3HgOH.[2]

Table II. Raman Frequencies (cm^{-1}) for 1:1 Guo-5′-P Complex with CH_3Hg^{II}

Guo-5′-P + CH_3Hg^{II} H_2O, pH 2	Guo-5′-P + CH_3Hg^{II} D_2O, pD 2	Guo-5′-P + CH_3Hg^{II} H_2O, pH 8.5	Guo-5′-P + CH_3Hg^{II} D_2O, pD 8.5
1677 *3*	1677 *2*		
1599 *7*	1598 *10*	1581 *3*	1580 *4*
	1578 *0*		
	1545 *0*		
1496 *4*	1493 *2*	1497 *4*	1500 *1*
			1475 *1*
		1466 *0*	1466 *1*
1418 *2*	1425 *1*	1410 *1*	1426 *0*
			1390 *0*
1360 *3*	1392 *2*	1352 *3*	1339 *4*
1339 *4*	1340 *4*	1321 *1*	1323 *1* sh
1208[b] *4*	1207[b] *4*	1207[b] *5*	1207[b] *4*
1183 *1*	1180 *0* sh	1183 *1*	
1084[c] *2*	1082[c] *0*		
		976[a] *2*	975[a] *2*
810 *1*	800 *1*	878 *0*	871 *0*
			801 *0*
668 *1*	666 *1*	671 *0*	666 *0*
		616 *1*	604 *0*
561[b] *10*	564[b] *9*	559[b] *10*	561[b] *10*
508 *0*	508 *0*	501 *0*	

[a] $ROPO_3^{2-}$ mode. [b] CH_3Hg^{II} mode. [c] $ROPO_3H^-$ mode.

Continuous Variation Spectra. In order to examine the stoichiometry of the reaction at pH 8.5, the continuous variation method[2,29] was employed. Since the most prominent changes observed in the difference spectra, Figures 3B and 4A, occurred in the 1800–1300-cm^{-1} range, D_2O was chosen to minimize solvent interference. The results are illustrated in Figure 5. The integrated intensity of the Guo-5′-P band at 1680 cm^{-1} decreases linearly with increasing CH_3Hg^{II}–Guo-5′-P ratio, vanishing entirely at a ratio of 1. Reaction is quantitative, and the stoichiometry of the reaction causing disappearance of the 1680-cm^{-1} band is 1:1. The behavior of the band at 1577 cm^{-1} indicates a second kind of reaction occurs causing binding of more CH_3Hg^{II} at higher CH_3Hg^{II}:Guo-5′-P ratios, since this band is replaced by one at higher frequency, 1603 cm^{-1}.

Examination of the ν(Hg–OH) band at 505 cm^{-1} also indicates that more than one CH_3Hg^{II} binds at high mole ratios. The band intensity is greatly reduced even with the 40 m*M* CH_3Hg^{II}–10 m*M* Guo-5′-P solution, and the variation suggests a 2:1 complex.

The 975-cm^{-1} $\nu_s(ROPO_3^{2-})$ appears unshifted remaining at 974 ± 2 cm^{-1} in all of the six spectra of solutions containing Guo-5′-P indicating that no reaction is occurring with the phosphate.

Guanosine 5′-Phosphate + CH_3Hg^{II}, pH 2. The model based upon Simpson's data indicates that CH_3Hg^{II} should bind to Guo-5′-P almost quantitatively but without displacement of the $N_{(1)}$ proton over the range of pH 1–4. Although Figure 1 was computed for Guo rather than Guo-5′-P, the difference in the reactions in these two systems should be small. The p*K* for Guo-5′-P protonation as $N_{(7)}$ is 2.3, very similar to the value taken for Guo, 2.23. Although there has been some question about the site of this protonation, *i.e.*, $N_{(7)}$ or phosphate, the Raman measurements of Lord and Thomas[26] at pH 0.5 show *complete* protonation on the ring. The Raman difference spectra are illustrated in Figures 4B and 6. The Guo-5′-P reference spectrum at this pH is that of a mixture of Guo-5′-P and [GuoH-

(29) See the discussion in ref 25, p 47.

5′-P]$^+$. The spectrum of the complex in D_2O is rather similar to that in H_2O.

Phosphate Binding. Since the experimental evidence indicates that the protonation equilibrium with pK = 2.3 involves $N_{(7)}$, the phosphate should be in the form $ROPO_2(OH)^-$. A value of 0.7 for the pK for protonation to produce $RORO(OH)_2$ also has been reported.[13] The Raman spectra of Guo-5′-P in solution at pH 2 are in accord with this. A sharp band not characteristic of the solutions at high pH is observed at 1084 cm^{-1}. This corresponds closely to the value of 1082 cm^{-1} reported by Rimai, *et al.*,[28] for $\nu_s(PO_2)$ of the $ROPO_2(OH)^-$ group. This indicates that the phosphate is not involved in the binding.

Double-Bond Stretching Region, 1800–1500 cm^{-1}. The two intense bands in this region, 1677 (H_2O, D_2O) and 1599 (H_2O), 1598 cm^{-1} (D_2O), have values intermediate between those of Guo-5′-P and [GuoH-5′-P]$^+$. Protonation causes an increase in frequency of the free ligand modes.

1500–700 cm^{-1}. The bands in this region generally are shifted from the values of both Guo-5′-P and [GuoH-5′-P]$^+$, although they have some features in common with both of these species. There is a considerable increase in scattering at 1496 (H_2O), 1493 cm^{-1} (D_2O), a region where the neutral ligand has an intense band. The scattering also increases at 1339 (H_2O), 1340 cm^{-1} (D_2O), where only [GuoH-5′-P]$^+$ has a band.

Examination of regions where the ligand mixture scatters but the complex does not, negative bands in the difference spectra, *e.g.*, 1565 (H_2O), 1515 cm^{-1} (D_2O), indicates that the complexation reaction is nearly complete at pH 2.

Features Associated with CH_3Hg^{II}. The methylmercuric cation, $CH_3HgOH_2^+$, is present almost entirely at pH 2; see Figure 1. It is not as good a probe as CH_3HgOH, because $\delta_s(CH_3)$ and ν(Hg–C) are closer to the values observed for the nucleoside and nucleotide complexes, and the scattering associated with ν(Hg–O) is considerably lower in intensity for a coordinated water molecule compared to a hydroxo group.

A slight decrease of $\leq$6 cm^{-1} in ν(Hg–C) was observed coupled with some hyperchromism upon coordination. A broad negative feature was observed in the Guo-5′-P + CH_3Hg^{II} *vs.* CH_3Hg^{II} difference spectrum at 455 cm^{-1}, Figure 6C. The (Hg–O) stretch gives broad scattering at *ca.* 451 cm^{-1}, and this indicates that the coordinated water molecule has been displaced from the methylmercury cation.

Adenosine 5′-Phosphate + CH_3Hg^{II}, pH 3.5. According to the model based on Simpson's equilibrium constants, Ado in a 50 mM Ado–50 mM CH_3Hg^{II} mixture should mercuriate at $N_{(1)}$ in acidic solutions (pH <4), on the $C_{(6)}NH_2$ group at high pH (pH >8), and at both sites in the intermediate pH region. Since the equilibrium constants for protonation of the nucleic acid base in Ado-5′-P are similar to those for Ado, the model should apply approximately to the nucleotide as well. At pH 3.5, Ado should be present approximately as a 50:50 mixture of Ado and $AdoH^+$, pK = 3.55. With Ado-5′-P, the corresponding pK is slightly larger, *ca.* 4.0. Protonation is more nearly complete, and the phosphate group is present as $ROPO_2(OH)^-$.

Raman spectra of Ado and Ado-5′-P have been recorded by Lord and Thomas[26] for both H_2O and D_2O solutions. In general, there appears to be little mixing of NH_2 deformations and the ring modes, but coupling does appear to occur between the N–H in-plane deformation of the protonated Ado or Ado-5′-P and ring modes. Rimai, *et al.*,[28] also have made careful measurements of the phosphate modes of Ado-5′-P as a function of pH.

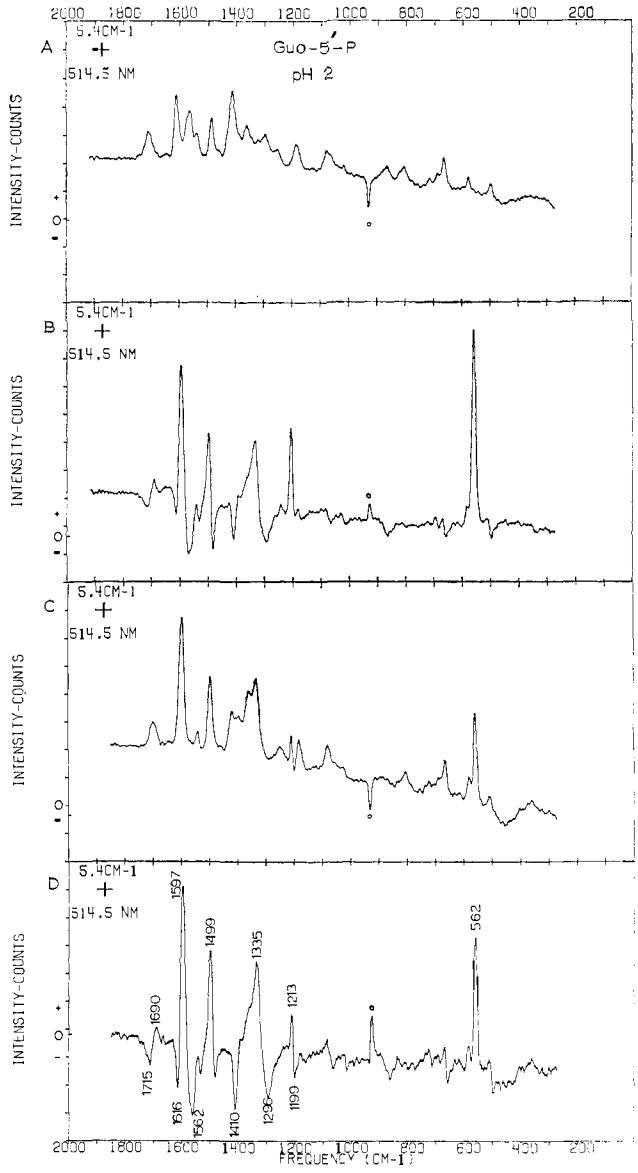


Figure 6. Raman difference spectra of Guo-5′-P + CH_3Hg^{II} at pH 2. The compositions of the systems and scan conditions are the same as those in Figure 3.

At pH 3.5, CH_3Hg^{II} is approximately equally distributed between CH_3Hg^+ and $(CH_3Hg)_2OH^+$.

Difference Spectra. Raman difference spectra were recorded for solutions 50 mM in Ado-5′-P and 50 mM CH_3Hg^{II} *vs.* 50 mM Ado-5′-P, both at pH 3.5, in order to search for perturbations of the Ado-5′-P vibrations. A difference spectrum also was run for 50 mM Ado-5′-P + 50 mM CH_3Hg^{II} *vs.* 50 mM CH_3Hg^{II} to determine perturbations of the methylmercury vibrations. As described above, these were combined to yield a ([Ado-5′-P + CH_3Hg^{II}]–Ado-5′-P–CH_3Hg^{II}) difference spectrum. These spectra plus that of Ado-5′-P *vs.* solvent are illustrated in Figure 7. Frequency data are tabulated in Table III. The pH adjustment was rather

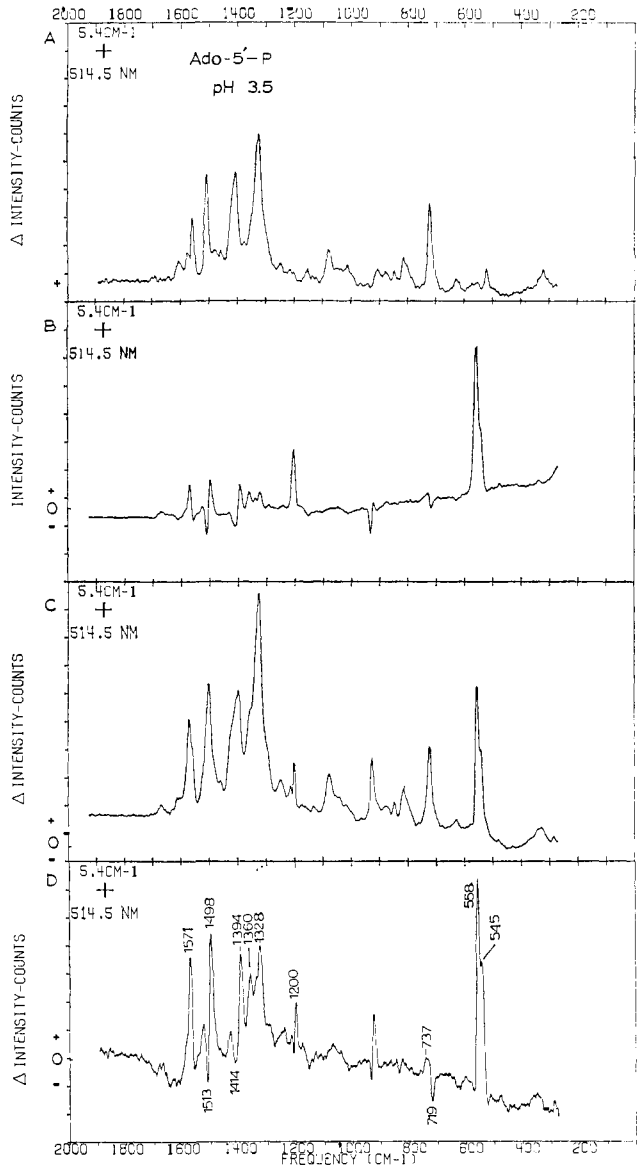


Figure 7. Raman difference spectra, Ado-5′-P + CH_3Hg^{II} at pH 3.5: A, 50 m*M* Ado-5′-P *vs.* solvent; B, 50 m*M* Ado-5′-P + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* Ado-5′-P; C, 50 m*M* Ado-5′-P + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* $CH_3Hg(II)$; D, [(50 m*M* Ado-5′-P + 50 m*M* CH_3Hg^{II}) + (solvent)] − [(50 m*M* Ado-5′-P) + (50 m*M* CH_3Hg^{II})]. All solutions are 0.1 *M* in $NaClO_4$. Scan conditions are the same as those in Figure 3.

critical in this study, since the value, 3.5, is near the p*K* for ring protonation, 4.0. As expected, the spectrum of the 50 m*M* Ado-5′-P reference shows mainly bands of the protonated base with weak contributions from the base itself.

Phosphate Binding. Again the first question to be considered is whether there is any interaction of CH_3Hg^{II} with the phosphate of the nucleotide. As discussed above, Rimai, *et al.*,[28] reported a polarized band at 1082 cm^{-1} characteristic of the $ROPO_2OH^-$ moiety. The spectra of the solutions containing CH_3Hg^{II} show a symmetric band at 1079 cm^{-1} which is canceled completely in the difference spectra. This shows that no interaction occurs at the phosphate group.

Double-Bond Stretching Region, 1800–1480 cm^{-1}. There is a decrease in the weak scattering at *ca.* 1620 cm^{-1} due to $AdoH^+$-5′-P, with a new weak band appearing at *ca.* 1673 cm^{-1}. This is best seen in the Ado-5′-P + CH_3Hg^{II} *vs.* Ado-5′-P difference spectrum, Figure Scattering at *ca.* 1560 cm^{-1}, where the protonated form of Ado-5′-P has a medium band, decreases, and scattering increases at 1571 cm^{-1}. Similarly a band at 1510 cm^{-1} shifts to 1503 cm^{-1} giving a derivative feature with extrema at 1513 and 1498 cm^{-1}. The pattern of Raman bands in this region is very similar to that for Ado-5′-P in DCl solution, pD 0.5,[26] *i.e.*, to that for $[AdoD-5'-P]^+$.

Vibrations below 1480 cm^{-1}. The changes in these ring modes are very similar to those which would be obtained simply by decreasing the pD of a solution of Ado-5′-P in D_2O.

Features Associated with CH_3Hg^{II}. At a pH of 3.5, the solutions should contain roughly equal amounts of CH_3Hg^{II} as the aquo cation and the binuclear species $(CH_3Hg)_2OH^+$, Figure 2. In spite of this, the CH_3Hg^{II} reference spectrum shows no evidence of a band at 415 cm^{-1} observed by Clarke and Woodward[30] with 2 *M* CH_3Hg^{II} solutions which had been titrated partially with base and which was assigned to $(CH_3Hg)_2OH^+$. Consequently, the absence of this band cannot be used as evidence for the absence of $(CH_3Hg)_2OH^+$ as had been assumed in our previous study.[2] The $\delta_s(CH_3)$ decreased from 1208 to 1205 cm^{-1}, although the difference spectra suggest that binding is not quantitative. The value of ν(Hg–C) decreases by *ca.* 6 cm^{-1}, but it is accompanied by a very marked hyperchromism which almost doubles the band intensity relative either to the $\nu_1(ClO_4^-)$ internal standard or the $\delta_s(CH_3)$ deformation band. This gives a very large positive feature in the Ado-5′-P + CH_3Hg^{II} *vs.* CH_3Hg^{II} difference spectra, Figures 7C and D. There is a low frequency shoulder, *ca.* 545 cm^{-1}, on this intense band which may arise from an increase in frequency of the 524-cm^{-1} Ado-5′-P band.

Adenosine + CH_3Hg^{II}. In order to test the assumption that Ado and Ado-5′-P would react similarly with CH_3Hg^{II}, *i.e.*, that phosphate coordination is not involved, difference spectra were determined for this system. The spectra for 50 m*M* Ado + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* Ado, both at pH 3.5, are illustrated in Figure 8. Spectra were obtained with both H_2O and D_2O solutions. The reference solutions showed spectra of mainly $AdoH^+$ ($AdoD^+$) rather than those of an equimolar mixture of Ado and $AdoH^+$. The complex spectrum in H_2O is virtually identical with the corresponding spectrum of Ado-5′-P, Figure 7B. The H_2O and D_2O spectra are surprisingly similar. The very large hyperchromic effect on the (Hg–C) stretching vibration is observed with both H_2O and D_2O solutions. Again, an examination of bands present for the ligand at pH 3.5 but absent from the complex spectrum, *e.g.*, 516 or 1554 cm^{-1}, indicates reaction is extensive. The frequencies of the ring modes above 1500 cm^{-1} are all much lower than for $AdoH^+$. This is best seen with the D_2O solution, Figure 8B, where derivative features occur in the difference spectra arising from the frequency decreases.

1-Methyladenosine + CH_3Hg^{II}, Solid State and Solution Spectra of 1-MeAdo. Very little information on 1-methyladenosine is available in the literature. A Raman spectrum of the microcrystalline compound was

(30) J. H. R. Clarke and L. A. Woodward, *Trans. Faraday Soc.*, 62, 3022 (1966).

Table III. Raman Frequencies (cm^{-1}) of Ado-5′-P, Ado, and 1-MeAdo Complexes with CH_3Hg^{II} and of 1-MeAdoH$^+$

Ado-5′-P + CH_3Hg^{II} H_2O, pH 3.5	Ado + CH_3Hg^{II} H_2O, pH 3.5	1-MeAdoH$^+$ H_2O, pH 3.5	1-MeAdo + CH_3Hg^{II} H_2O, pH 3.5	1-Me-6-iminoAdo, crystal
1673 *0*	1672 *0*		1656 *2*	1641 *1*
1571 *1*	1572 *2*	1579 *2*	1579 *0* sh	1583 *1*
1558 *0* sh	1560 *0* sh	1556 *2*	1565 *7*	1567 *2* bd
(1512) *0* sh	1528 *0* sh			
1503 *3*	1501 *3*	1512 *6*	1511 *6*	1522 *1*
1460 *0*		1464 *1*	1472 *0*	1467 *4*
1422 *0* sh	1410 *0* sh	1430 *1*	1430 *0* sh	1420 *1*
		1414 *3*	1417 *4*	1405 *0*, 1399 *0*
1397 *2*	1395 *2*			
1360 *0* sh	1360 *1* sh			1357 *2* bd
1327 *6*	1326 *6*	1336 *10*	1336 *10*	1326 *5*
1291 *0* sh	1291 *0* sh			1301 *0* bd
1250 *0*	1244 *0*			1247 *2*, 1241 *2*
1205 *4*[b]	1207 *4*[b]	1195 *1* bd	1207 *5*[b]	1203 *2* m, 1197 *2* m
		1042 *2*	1043 *1*	
1079 *1*[a]		961 *0*	1005 *1*	
878 *0*	864 *1* bd	868 *1*	866 *1*	1072 *2*
848 *0*				944 *1* bd
813 *1*			729 *2* bd	797 *2*
724 *3*	726 *3*	721 *4*	719	716 *10*, 708 *3*
		622 *1*		
560 *10*[b]	561 *10*[b]	597 *0* sh	563 *6*[b]	
				634 *2*, 625 *2*
545 *0* sh	546 *0* sh	548 *0* bd	508 *1*	559 *6*
		476 *0* bd		471 *2*
		332 *0* bd		329 *2*, 322 *2*

[a] $ROPO_2OH^-$ mode. [b] CH_3Hg^{II} mode.

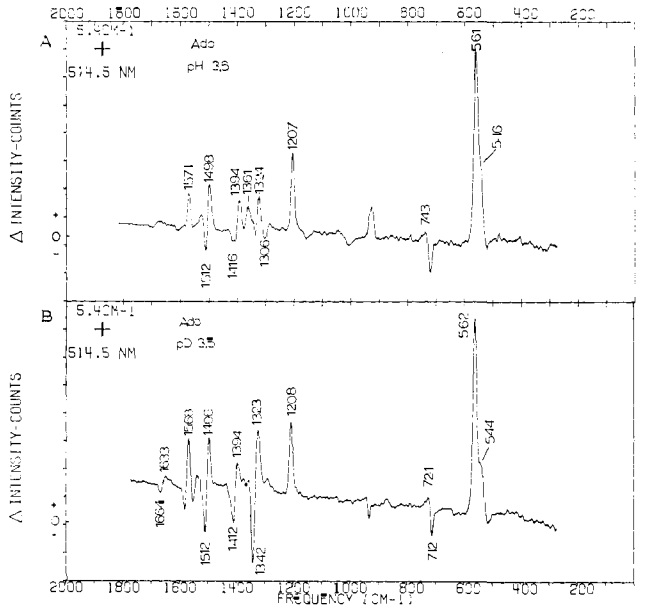


Figure 8. Raman difference spectra, 50 m*M* Ado + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* Ado at pH (pD) 3.5: A, H_2O; B, D_2O. Solutions are 0.1 *M* in $NaClO_4$. Scan conditions are the same as those in Figure 3.

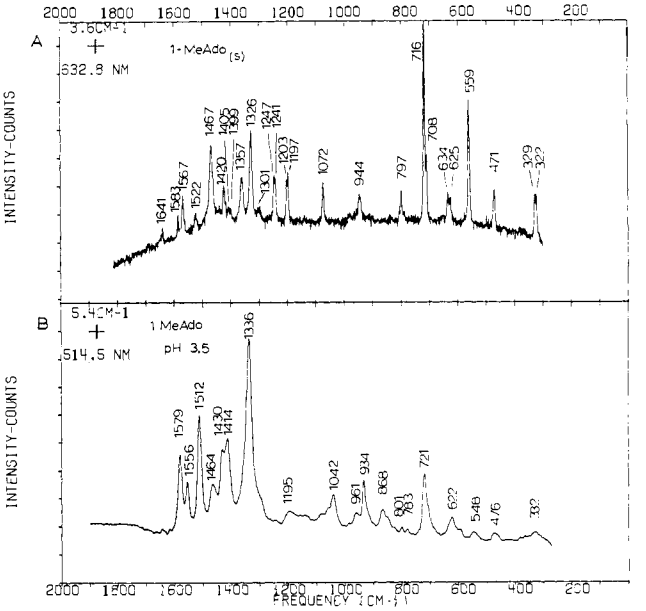


Figure 9. Raman spectra of 1-MeAdo: A, crystalline powder; B, difference spectrum, 50 m*M* 1-MeAdo *vs.* solvent, pH 3.5. Both solutions are 0.1 *M* in $NaClO_4$. Scan conditions are the same as those in Figure 3.

obtained for comparison with the solution spectra. The data are collected in Table III and illustrated in Figure 9.

The crystalline compound exists in the neutral, 1-methyl-6-iminoadenosine form. The powder spectrum only shows two of the four bands cited by Lord and Thomas[26] as characteristic of the adenine ring, and these are at considerably lower frequency than normal. An intense band occurs at 1326 cm^{-1} (typical value 1336 ± 12 cm^{-1})[26] and the second is at 712 cm^{-1} (average of doublet) (typical range 726 ± 7 cm^{-1}).[26] A number of the bands are doubled in the crystal spectra with separations of less than 9 cm^{-1}. These are likely to be correlation multiplets arising from more than one molecule in the Bravais cell. The extent of coupling suggests rather strong intermolecular interaction, probably arising from hydrogen bonding. In contrast to Ado, there is a band at 1641 cm^{-1}.

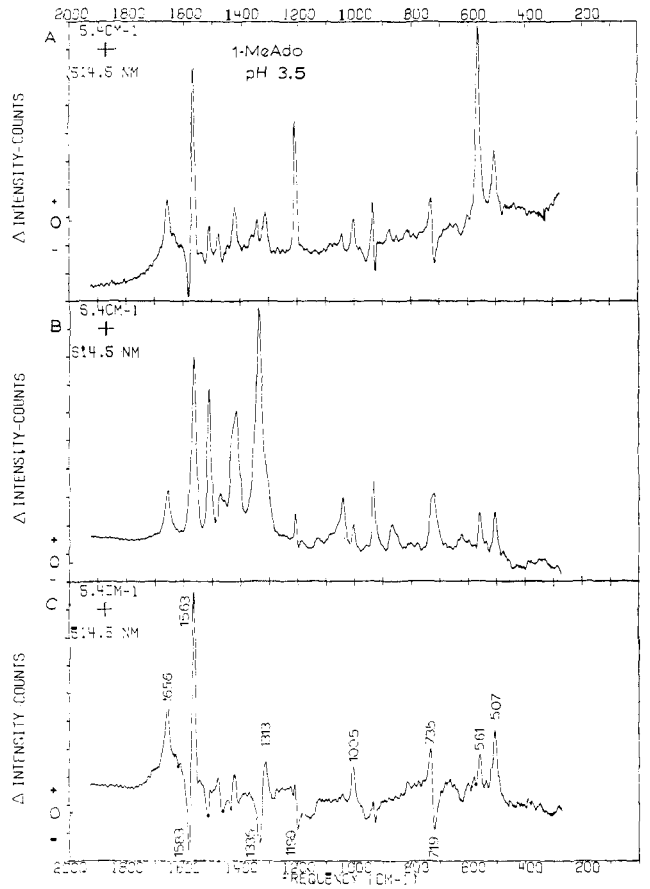


Figure 10. Raman difference spectra of 1-MeAdo + CH_3Hg^{II} at pH 3.5: A, 50 m*M* 1-MeAdo + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* 1-MeAdo; B, 50 m*M* 1-MeAdo + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* CH_3Hg^{II}; C, [(50 m*M* 1-MeAdo + 50 m*M* CH_3Hg^{II}) + (solvent)] − [(50 m*M* 1-MeAdo) + (50 m*M* CH_3Hg^{II})]. All solutions are 0.1 *M* in $NaClO_4$. Scan conditions are the same as those in Figure 3.

The spectrum of 1-MeAdo in solution at pH 3.5, Figure 9B, where it should be present as 1-MeAdoH$^+$, is rather similar to the spectrum of AdoH$^+$.[26]

Solution Spectra of 1-MeAdo + CH_3Hg^{II}. A difference spectrum was obtained for 50 m*M* 1-MeAdo + 50 m*M* CH_3Hg^{II}, pH 3.5, *vs.* 50 m*M* 1-MeAdo, and it is illustrated in Figure 10A. There are only very slight changes in the bands below 1550 cm^{-1} from the values of 1-MeAdoH$^+$. The minor changes cause several small derivative features in the difference spectra, Figures 10A and C. The principal change is replacement of the two medium intensity double bond stretches at 1556 and 1579 cm^{-1} with an intense band at 1565 cm^{-1} giving a very large positive feature in the difference spectrum, minimum 1583, maximum 1563 cm^{-1}. In addition, a new band appears at *ca.* 1656 cm^{-1}, while 1-MeAdoH$^+$ in solution shows no band in this region above 1600 cm^{-1}. Other new bands characteristic of the complex are observed at 1005 and 507 cm^{-1}. The medium intensity band at 721 cm^{-1} which is characteristic of the adenine ring is replaced by two overlapping bands of similar intensity at 719 and 729 cm^{-1}. This causes a distinct derivative feature with extrema at 735 and 719 cm^{-1}. This feature plus a shoulder at *ca.* 1580 cm^{-1} on the complex band at 1565 cm^{-1} suggests that reaction is incomplete.

Features Associated with CH_3Hg^{II}. As usual, these are best sought in the 50 m*M* 1-MeAdo + 50 m*M* CH_3Hg^{II} *vs.* 50 m*M* CH_3Hg^{II} difference spectrum. This is illustrated in Figure 10B. In this case neither the frequencies nor intensities of $\delta_s(CH_3)$ and ν(Hg–C) change significantly. Insofar as these vibrations are concerned, there is no evidence for any change in the CH_3Hg^{II} moiety in the presence of 1-MeAdo at pH 3.5, although there appears to be a slight decrease in scattering at *ca.* 450 cm^{-1} which would be expected if water were displaced from the first coordination sphere of CH_3Hg^{II}.

Discussion

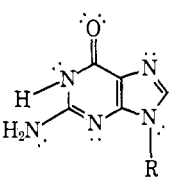
The results of the Raman studies on the Guo-5′-P reaction with CH_3Hg^{II} at pH 8.5 may be summarized as follows. As Guo-5′-P is titrated with CH_3Hg^{II}, a complex is formed. The variation of the integrated intensity of the 1664-cm^{-1} ligand band with stoichiometry indicates that the composition is 1:1 and that reaction is essentially quantitative. Examination of the intensity of the 978-cm^{-1} $\nu_s(ROPO_3^{2-})$ band shows that there is no involvement of the phosphate group. At CH_3Hg^{II}–Guo-5′-P ratios greater than 1, a second complexation reaction occurs. An examination of the variation in intensity with stoichiometry of the 504-cm^{-1} band associated with stretching of the Hg–O bond of CH_3HgOH suggests that this reaction is the formation of a $(CH_3Hg^{II})_2$Guo-5′-P$^+$ complex.

The disappearance of the 1664-cm^{-1} Guo-5′-P band upon formation of the 1:1 complex definitely indicates loss of the proton from N_1. The spectral changes accompanying this reaction are rather similar to those observed previously with Urd and CH_3Hg^{II} at pH 7.[2] In both cases, coordination is to the ring after displacement of a proton, reactions 1 and 2. The valence bond

$$CH_3HgOH + \text{Urd} \rightleftharpoons [H_3CHgUrdH_{-1}] + H_2O \quad (1)$$

$$CH_3HgOH + \text{Guo-5′-P} \rightleftharpoons [H_3CHgGuoH_{-1}\text{-5′-P}] + H_2O \quad (2)$$

structure of guanosine-5′-P, I, is illustrated showing all



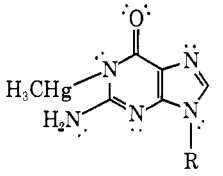
I

valence electrons for bookkeeping purposes. The ribose phosphate ester residue is merely represented schematically, since the spectra clearly show that the phosphate is not involved in the reaction. Formal charges are zero for all of the atoms in this canonical form, and this is a reasonable description of the neutral base.[26,31] The band at 1664 cm^{-1} (D_2O) which disappears is generally considered to derive its major contribution from $C_{(6)}$=O stretching. With Urd, it is the $\nu(C_{(2)}$=O) band at 1690 cm^{-1} which disappears. These changes are characteristic of increasing electron delocalization in the base. In fact, the general appearance of the $CH_3HgGuoD_{-1}$-5′-P complex spectrum resembles that of [$GuoD_{-1}$-5′-P]$^-$ observed by Lord and Thomas at pD 12.5.[26] The same similarity in the $CH_3HgUrdH_{-1}$ and [$UrdH_{-1}$]$^-$ spectra has been noted previously.[2]

(31) See, *e.g.*, M. Tsuboi, Y. Kyoguku, and T. Shimanouchi, *Biochim. Biophys. Acta*, **55**, 1 (1962).

The complexes with CH_3Hg^{II} both have a much more delocalized electronic structure than is true for the proton complexes, which results in a decrease in the C=O, C=N, and C=C bond order.

Binding is assigned to the $N_{(1)}$ position, II, by analogy



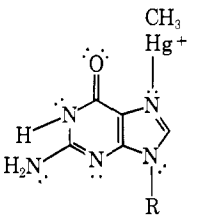
II

with the assignment for the $[CH_3HgUrdH_{-1}]$ complex. The effect of coordination on the ν(Hg–C) intensity is very similar in these two systems. The peak heights in the Guo-5′-P and Urd systems are 0.7$_0$ and 0.65, respectively, of the ClO_4^- ν_1 internal reference peak. Although the negative charge of the anionic base could be delocalized over the $N_{(1)}$—$C_{(6)}$=O portion of the molecule, it is much more likely that mercury will bind to a nitrogen in preference to an oxygen donor, thereby stabilizing the keto form, albeit with reduced bond order.

In contrast to the interpretation of the uv absorption spectra by Simpson,[11] the Raman spectra indicate that CH_3Hg^{II} binds to a second site at pH 8 and high CH_3Hg^{II}:Guo-5′-P ratios. This reaction appears to involve virtually quantitative coordination. The band at 1603 cm^{-1} increases in intensity at the expense of the *ca.* 1580-cm^{-1} band indicating coordination to the Guo base. The binding site is uncertain, although coordination may occur to the $C_{(2)}$–NH_2 group, since such a reaction is observed with Cyd at high pH, causing small shifts in the ring modes.[32]

At low pH, the Raman spectra indicate that there still is binding, in agreement with Simpson's[11] results using uv absorption measurements. The spectra also indicate that the nature of the interaction is quite different from that at high pH. Again, the phosphate group is not involved. In contrast to the effect of binding at $N_{(1)}$ which decreases the 1664-cm^{-1} carbonyl band to *ca.* 1600 cm^{-1}, the shift in this case is to higher frequency, 1677 cm^{-1}. A similar effect occurs upon protonation, and this band is observed for $[GuoH\text{-}5'\text{-}P]^+$ at 1710 cm^{-1} (H_2O), 1695 cm^{-1} (D_2O).[26] The other shifts observed for the $[CH_3HgGuo\text{-}5'\text{-}P]^+$ frequencies compared to Guo are smaller than those which occur upon protonation. For example, the intense band of Guo at 1578 cm^{-1} has counterparts in the complex at 1599 cm^{-1} (H_2O), 1598 cm^{-1} (D_2O) compared to 1607 cm^{-1} $[GuoD\text{-}5'\text{-}P]^+$ and 1612 cm^{-1} $[GuoH\text{-}5'\text{-}P]^+$. In acidic solution, $N_{(1)}$ is protonated and the metal is bound to one of the remaining sites.

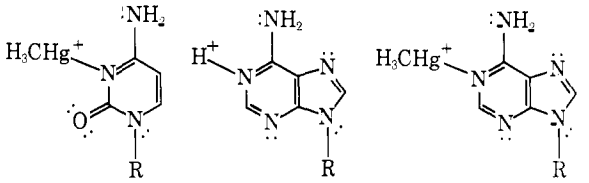
The spectra of the complex in H_2O and D_2O are much more like those of the free base than the $N_{(7)}$ protonated form. This strongly suggests that there is no protonation at $N_{(7)}$ and by inference that the metal is bound there, III. The crystal structure of $[GuaH^+]Cl^- \cdot 2H_2O$[33] shows protonation at $N_{(7)}$, and guanine at pH <0 also exhibits the high frequency band at 1710 cm^{-1} observed



III

for the complex and $GuoH\text{-}5'\text{-}P^+$.[26] Binding to the imidazole ring appears to reduce the delocalization of the nitrogen lone pair over the ring system, increasing particularly the $C_{(6)}$=O bond order.

The effect on the CH_3Hg^{II} vibrations is smaller for the acid solutions, because $CH_3HgOH_2^+$ vibrations are closer to those of the complex than is the case when CH_3HgOH is the reactant. The values of $\delta_s(CH_3)$ and ν(Hg–C) are nearly the same for the complex formed by mercuriation at $N_{(1)}$ and the complex formed in acid solution. The values of these frequencies for $CH_3Hg\text{-}Cyd^+$, IV, also are the same within the experimental



IV V VI

errors as for $[CH_3HgGuo\text{-}5'\text{-}P]^+$. The intensity of the (Hg–C) stretching band is significantly less than for the complex produced in alkaline solution; the peak height is 0.4$_0$ the value of the ClO_4^- ν_1. This may reflect decreased polarizability of Guo-5′-P compared to $[GuoH_{-1}\text{-}5'\text{-}P]^-$.

Ado and Ado-5′-P were studied at pH 3.5 where the base exists in the form V with $N_{(1)}$ protonated and the phosphate monoprotonated, *i.e.*, as $ROPO_2OH^-$. CH_3Hg^{II} clearly binds to the base, and the spectra show there is no interaction with the phosphate. The shifts from the free base frequencies upon mercuriation are somewhat like the shifts caused by protonation, although they are not as large and the intensity changes are not as great. The site of mercuriation cannot be assigned unequivocally, although the similarity of the spectrum of $[AdoD\text{-}5'\text{-}P]^+$ at pD 3.5, V, and that of the complex suggests that the complex has the analogous structure VI. The (Hg–C) peak height is 0.4$_8$ that of the perchlorate ν_1 reference. This is intermediate between the values for $[GuoH_{-1}\text{-}5'\text{-}P]^-$ and Guo-5′-P and somewhat less than the value for Cyd, 0.60.[2]

The indication that CH_3Hg^+ binds to the same site at which protonation occurs is in accord with Simpson's original assumption for the Ado reaction but at variance with the prediction of Glassman, *et al.*,[15] that soft acids will tend to bind to $N_{(7)}$ in preference to $N_{(1)}$. Kan and Li[34] also suggested that the sites for binding of $HgCl_2$ to Ado in DMSO solution were the $C_{(6)}$–NH_2 amino group and $N_{(7)}$ on the basis of observed shifts in the pmr signals of the amino and $C_{(8)}$–H protons. They did suggest that binding might be occurring at $N_{(1)}$, because there was some evidence for restricted rotation about the $C_{(6)}$–N bond as was observed for the Cyd·$HgCl_2$ com-

(32) S. Mansy, J. Frick, and R. S. Tobias, unpublished research.

(33) J. Iball and H. R. Wilson, *Proc. Roy. Soc., Ser. A*, **288**, 418 (1965).

(34) L. S. Kan and N. C. Li, *J. Amer. Chem. Soc.*, **92**, 4823 (1970).

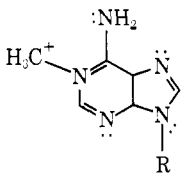
Table IV. Summary of Raman Spectra of Nucleosides and Nucleotides[a]

Base	pH	Site	Evidence	Marker[b] bands (cm^{-1})
Ado-5′-P	3.5	$N_{(1)}$	No phosphate binding. Complex spectrum much like that of [AdoD-5′-P]$^+$, similar to that of 1-MeAdoH$^+$. Hyperchromism of (Hg–C) stretch suggests binding to nitrogen heterocycle	1571+, 1513−, 1498+, 737+, 719−,
Ado	3.5	$N_{(1)}$	Complex spectrum almost identical with that formed by Ado-5′-P	Same as Ado-5′-P
1-MeAdo	3.5	Probably $N_{(7)}$	Binding perturbs nucleoside spectrum. No hyperchromism of (Hg–C) stretch. Weak interaction with protonated base	1656+, 1583−, 1563+, 735+, 719−
Cyd	7.0	$N_{(3)}$	Shifts somewhat like those for [CydH$^+$]. Very different shifts observed in basic solution where amino group was involved. Hyperchromism of (Hg–C) stretch indicates binding to ring nitrogen	1646+, 1254+, 795+, 778−, 603+
Guo-5′-P	8.5	$N_{(1)}$	No phosphate binding. Spectrum similar to that of [GuoH$_{-1}$-5′-P] with proton displaced from $N_{(1)}$. Hyperchromism in (Hg–C) stretch; absence of (Hg–O) stretch indicates binding to ring nitrogen	1690−, 1503+, 1485−, 616+
	8.5	N_1?	Shifts at high concentrations of CH_3Hg^{II}	1603+, 1577−
	2	$N_{(7)}$	No phosphate binding. Shifts in nucleotide modes similar to those which occur with protonation at $N_{(7)}$	1715−, 1597+, 1582−, 1499+, 1335+, 1296−
Urd	7.0	$N_{(3)}$	Spectrum very similar to that of [UrdH$_{-1}$]$^-$ with proton displaced from $N_{(3)}$. Complex spectra in H_2O and D_2O are identical. Marked hyperchromism in (Hg–C) stretch; absence of (Hg–O) stretch indicates binding to ring nitrogen	1685−, 1637+, 1227−, 796+, 779−, 603+
Poly(U)	7.0	$N_{(3)}$	Spectrum virtually identical with that of UrdHgCH$_3$	Same as Urd

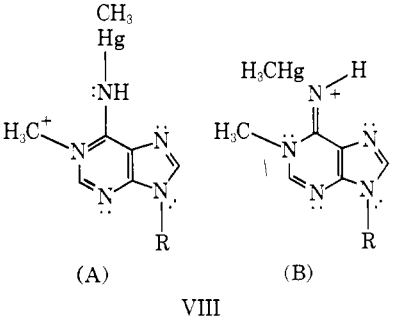
[a] Data obtained with solutions 0.1 *M* in $NaClO_4$. [b] The + indicates an increase in scattering, − a decrease in scattering at the listed frequency.

plex where coordination was assigned to $N_{(3)}$. Eichhorn and Clark[35] assigned the binding of $HgCl_2$ at pH 9 to the amino group of Ado, since no greater bathochromic shift in the uv spectrum was observed when $HgCl_2$ + formaldehyde was added to Ado compared to formaldehyde alone. Formaldehyde is assumed to block any interaction at the amino group and at the amino group only. Yamane and Davidson[36] examined the interaction of $Hg(ClO_4)_2$ and Ado, but concluded that the structure of the 1:1 complex was "unknown and puzzling." The large hyperchromic effect on the (Hg–C) stretch observed in this work upon coordination of CH_3Hg^+ to Ado indicates that there is a significant electronic effect on the methylmercuric moiety upon coordination.

The spectra of the solutions containing 1-MeAdo plus CH_3Hg^{II} at pH 3.5, VII, show that there is still an interaction with the Ado ring even when $N_{(1)}$ is blocked. Possible sites for the interaction are $N_{(3)}$, $C_{(6)}$–NH_2, and $N_{(7)}$. Of these, the amino group and $N_{(7)}$ are normally considered for metal ion binding. Simpson considered the second site for binding to Ado to involve the amino group with substitution of a proton by CH_3Hg^+, VIII,

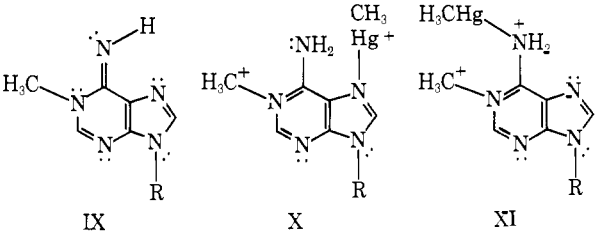


VII



(A) (B)

VIII



IX X XI

but with Ado, this interaction appeared to be significant only at pH *ca.* 4 and above. If VIII were a reasonable

(35) G. L. Eichhorn and P. Clark, *J. Amer. Chem. Soc.*, 85, 4020 (1963).
(36) T. Yamane and N. Davidson, *J. Amer. Chem. Soc.*, 83, 2599 (1961).

description, the complex might be expected to give a spectrum rather similar to the imino form of 1-MeAdo, IX, by analogy with the spectra of [$CH_3HgUrdH_{-1}$] and [$CH_3HgGuoH_{-1}$-5′-P]. Except for the appearance of a band at 1656 cm^{-1} in the complex in the region of a weak band of 1-MeAdo at 1642 cm^{-1}, the complex spectrum resembles 1-MeAdoH$^+$.

It is conceivable that mercuriation of 1-MeAdoH$^+$ could occur without displacement of a proton to give X or XI. The charge distribution of XI does not appear to be particularly favorable. Mercuriation at $N_{(7)}$ might be expected to have an effect similar to that assigned to mercuriation of Guo-5′-P at $N_{(7)}$, pH 2. In that case, shifts occurred in almost all of the bands compared either to [GuoH-5′-P]$^+$ or Guo-5′-P, unlike what is observed for coordination to [1-MeAdoH]$^+$. This also is the only system examined so far where coordination of CH_3Hg^{II} to a nucleic acid base does not give a significant hyperchromic effect in ν(Hg–C). This suggests that coordination occurs to a weakly polarizable site on the base and is consistent with a weak interaction without proton transfer, X or XI, of which the former seems more likely.

A summary of the Raman information on the CH_3Hg^{II} coordination to nucleosides and nucleotides is given in Table IV. Frequencies of difference spectra marker bands characteristic of metalation of particular bases also are tabulated. These are suitable for use in Raman spectrophotometric titrations of mixed polynucleotides.

Although a number of questions concerning the structure of these heavy metal nucleoside and nucleotide complexes can be settled on the basis of the Raman spectra alone, it is clear that a few model compounds of known structure would be of great help in further interpretations of the spectra. We are attempting to synthesize crystalline products which have spectra analogous to the solution species.

Supplementary Material Available. Reproductions of Raman spectra will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-6874.

Bis(cyclooctatetraenyl)neptunium(III) and -plutonium(III) Compounds[1,2]

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Abstract: Potassium bis(cyclooctatetraenyl)neptunium(III) ($KNp(COT)_2$, $COT = C_8H_8^{2-}$) and $KPu(COT)_2$ were prepared as bis(tetrahydrofuran) (THF) solvates by treating the trivalent actinide bromides or iodides with K_2COT in THF solution. X-Ray powder patterns indicate that these compounds are isostructural, and the similarity of the powder patterns of $KPu(COT)_2 \cdot (CH_3OCH_2)_2O$ with the Ce^{3+} analog suggests that the Pu^{3+} ion is in a D_{8d} ("sandwich") site in the molecule. The Mössbauer spectrum of the Np(III) compound has an isomer shift (δ) of +3.92 cm/sec, which confirms a +3 valence with only slight covalency, and a quadrupole splitting constant ($eqQ/4$) of 0.72 cm/sec with zero asymmetry. Magnetic susceptibility measurements on both compounds are reported and discussed in terms of crystal field models.

Mares, Hodgson, and Streitwieser have prepared and studied two series of compounds between the cyclooctatetraenyl dianion and trivalent lanthanide ions, $KLn(COT)_2$[3] and $[Ln(COT)Cl \cdot 2THF]$[4] (Ln = La^{3+}, Ce^{3+}, Pr^{3+}, Nd^{3+}, Sm^{3+}, Gd^{3+}, Tb^{3+}; $COT^{2-} = C_8H_8^{2-}$, the cyclooctatetraenyl dianion). The structure determination by single-crystal X-ray[5] of the mono-"diglyme" [$(CH_3OCH_2)_2O$] solvate of $KCe(COT)_2$ showed that the Ce^{3+} ion is at a site of D_{8d} symmetry between the two planar COT rings. In this structure, the carbon atoms of the COT rings are staggered, instead of eclipsed, like U^{4+} in $U(COT)_2$.[6,7] This paper reports synthesis and study of the analogous compounds $KNp(COT)_2$ and $KPu(COT)_2$.

Experimental Section

The methods used to purify solvents, analyze compounds, measure magnetic susceptibility, and obtain Mössbauer spectra have been previously reported.[8] All syntheses, transfer of compounds, and measurements of their properties were performed in a dry argon atmosphere. Qualitative tests for halides were performed by adding solid compounds to dilute HNO_3 under argon, filtering insoluble material, and adding $AgNO_3$ to the clear filtrate.

(1) Presented at the 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 26–31, 1973.

(2) This paper was prepared in connection with work under Contract No. AT(07-2)-1 with the U. S. Atomic Energy Commission.

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(4) F. Mares, K. O. Hodgson, and A. Streitwieser, *J. Organometal. Chem.*, **28**, C24 (1971).

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(8) D. G. Karraker, J. A. Stone, E. R. Jones, Jr., and N. Edelstein, *J. Amer. Chem. Soc.*, **92**, 4841 (1970).