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

Samir Mansy, Thomas E. Wood, Jolyon C. Sprowles, and R. Stuart Tobias*

Contribution from the Department of Chemistry, Purdue University, West Lafayette, Indiana 47907. Received October 12, 1973

Abstract: Using data on the equilibrium constants for hydrolysis of CH_3Hg^+ and for its binding to uridine and cytidine, solutions in the 10–100 mM range have been prepared for which a particular complex should predominate. Raman spectra and particularly Raman difference spectra have been used to determine the perturbations of the cation and of the nucleoside ligand vibrations upon metal binding. The difference technique, applied here for the first time, is particularly effective for observing small spectral changes. Spectra have been obtained for the complex with polyuridilic acid to show that the perturbations are very similar with polynucleotides. A procedure for determining heavy metal binding sites on polynucleotides with two or more base moieties in aqueous solution is outlined. The methylmercury(II) ion binds to uridine (Urd) with displacement of a proton and coordination to $N_{(3)}$. Binding to cytidine also occurs at $N_{(3)}$, although, at pH 7, coordination to Urd is favored. The behavior of CH_3Hg^+ and Hg^{2+} are compared.

The binding of metals by nucleosides and nucleotides has been investigated for a number of years. Because of recent observations that CH_3Hg^+ causes chromosome damage and consequently is mutagenic[2,3] and that certain platinum(II) compounds inhibit mitosis by selective inhibition of DNA synthesis,[4,5] there is renewed interest in the binding of heavy metals to polynucleotides.

In this work we have examined the interaction between CH_3Hg^{II} and pyrimidine nucleosides and nucleotides. Equilibria of CH_3Hg^+ are relatively simple, since it is primarily a unifunctional electrophile. Consequently, the methylmercury cation should serve as a model for the binding of heavy metals to nucleosides, nucleotides, and polynucleotides. The mutagenic effect of the unifunctional CH_3Hg^+ electrophile compared to the antimitotic effect in tumor tissue of the (presumed) bifunctional $(NH_3)_2Pt^{II}$ parallels the activity of uni- and bifunctional organic alkylating agents.[6,7]

In 1961, Ferreira, *et al.*,[8] noted that CH_3Hg^+ and $C_2H_5Hg^+$ formed complexes with dThd,[9] and the competition reaction between H^+ and CH_3Hg^+ was studied.

Because of the proton dependence, binding was assumed to occur at $N_{(3)}$. In 1966, Gruenwedel and Davidson[10] obtained a value for the equilibrium constant for dThd reacting with CH_3HgOH by measuring the effect of dThd on the distribution between an aqueous and a toluene phase. Simpson[11] in 1964 used uv spectrophotometric measurements to obtain equilibrium constants for binding to Urd and to Cyd. Binding was assumed to occur at $N_{(3)}$ in both cases. With Cyd at high pH, a second reaction was observed, and this was assumed to be CH_3Hg^+ binding to the $C_{(4)}NH_2$ group.

Carrabine and Sundaralingham[12] recently determined the structure of the crystalline adduct $HgCl_2 \cdot 2Ura$ (Ura = uracil). This consists of a linear $HgCl_2$ molecule coordinated to one oxygen ($C_{(4)}$=O) from each of two Ura molecules with rather short Hg–O interactions of 2.71 (2) Å. In addition there are two chlorides from adjacent $HgCl_2$ molecules coordinated about mercury giving distorted octahedral coordination.

On the basis of the $HgCl_2 \cdot 2Ura$ structure, Carrabine and Sundaralingham suggested that reaction of mercury(II) with Ura, Urd, and dThd in aqueous solution occurs by coordination only with the oxygen of the $C_{(4)}$=O group. The pH dependence of the binding was suggested to be caused by proton transfer from a water molecule in the first coordination sphere of the bound Hg^{2+}. This, however, does not account for the similar proton dependence observed for CH_3Hg^+ and $C_2H_5Hg^+$[8] where the second principal coordination site of mercury is blocked by the inert carbanion ligands.

Recently the usefulness of Raman spectroscopy in

(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-23208.

(2) G. Löfroth, "Methylmercury," Bulletin No. 4, Swedish National Science Research Council, Stockholm, 1969.

(3) J. J. Mulvihill, *Science*, 176, 132 (1972).

(4) H. C. Harder and B. Rosenberg, *Int. J. Cancer*, 6, 207 (1970).

(5) G. R. Gale J. A. Howle, and E. M. Walker, Jr., *Cancer Res.*, 31, 950 (1971).

(6) W. C. J. Ross, "Biological Alkylating Agents," Butterworth, London, 1962.

(7) A. Haddow, *Advan. Cancer Res.*, 16, 181 (1972).

(8) R. Ferreira, E. Ben-Zvi, T. Yamane, J. Vasilevskis, and N. Davidson, "Advances in the Chemistry of the Coordination Compounds," S. Kirschner, Ed., Macmillan, New York, 1961, p 457.

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

(10) D. W. Gruenwedel and N. Davidson, *J. Mol. Biol.*, 21, 129 (1966).

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

(12) J. A. Carrabine and M. Sundaralingham, *Biochemistry*, 10, 292 (1971).

polynucleotide chemistry has been demonstrated amply.[13] The binding of H^+, Na^+, Mg^{2+}, and Ca^{2+} to the phosphate of ATP has been studied,[14,15] and considerable progress has been made in using Raman spectroscopy to follow conformational changes in polynucleotides.[16-18]

The present work was begun with several aims in mind. Firstly, we wished to examine the reactions of CH_3Hg^+ because of its known physiological effects. Secondly, it appears that this cation can serve as a model for heavy metal binding to nucleosides. Consequently, if information on the perturbation of the nucleoside or nucleotide vibrations caused by metal coordination at specific sites can be cataloged, Raman spectroscopy can be used in a straightforward fashion to ascertain the sites at which other heavy metals bind. In this context, we were interested in resolving the question concerning the site of the binding of Hg(II) to Urd and dThd. Finally, it has been shown that the principal spectral features of polynucleotides can be obtained by summing the spectra of the constituent nucleotides in the proper ratio.[19] Similarly, it should be possible to reproduce the spectra of polynucleotides which have a heavy metal bound to *some* of the base moieties by summation of the spectra of metallated and unmetallated nucleotides in the proper ratios. On this basis, laser Raman spectroscopy would appear to be a very useful tool for determining the bases to which binding occurs preferentially and in establishing conformational changes brought about by metal binding.

Mansy, et al.,[20] recorded Raman spectra of several synthetic polynucleotides as well as calf thymus DNA in the presence of cis- and trans-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and suggested binding sites and conformational changes on the basis of changes in the spectra from those of the pure polynucleotides. Lord and Thomas[21] investigated the interaction of $HgCl_2$ with the nucleosides Cyd and Urd using Raman spectroscopy. Changes in the spectrum of Cyd were observed upon addition of $HgCl_2$ which were similar to those occurring upon protonation, and $N_{(3)}$ was assigned as the binding site. No interaction could be detected with Ura, Urd, 1-MeUra, or 1,3-Me$_2$Ura.

In this work, we also give the first demonstration of the usefulness of Raman difference spectroscopy (RDS) in the study of changes in biological molecules.

Experimental Section

Methylmercury(II) Perchlorate. Methylmercuric iodide obtained from Alfa Inorganics was recrystallized from ethanol, mp 144° (lit. 143°). This was treated with a standard aqueous solution (H$_2$O or D$_2$O) of AgClO$_4$ (G. F. Smith) while stirring continuously to give a stock solution of CH$_3$HgClO$_4$. A test was run for unreacted Ag$^+$ by titrating the solution with base, log K_{soAgOH} = −7.4.[22]

(13) Raman spectra of biological molecules have been reviewed recently: J. L. Koenig, *J. Polym. Sci., Part D*, **6**, 59 (1972).
(14) L. Rimai and M. E. Heyde, *Biochem. Biophys. Res. Commun.*, **41**, 313 (1970).
(15) M. E. Heyde and L. Rimai, *Biochemistry*, **10**, 1121 (1971).
(16) E. W. Small and W. Peticolas, *Biopolymers*, **10**, 69 (1971).
(17) E. W. Small and W. Peticolas, *Biopolymers*, **10**, 1377 (1971).
(18) K. G. Brown, E. J. Kiser, and W. L. Peticolas, *Biopolymers*, **11**, 1855 (1972).
(19) M. Tsuboi, S. Takahashi, S. Muraishi, T. Kajiura, and S. Nishimura, *Science*, **174**, 1142 (1971).
(20) S. Mansy and B. Rosenberg, unpublished data.
(21) R. C. Lord and G. J. Thomas, *Biochim. Biophys. Acta*, **142**, 1 (1967).

Ligand Solutions. Cyd was obtained from Clyco Chemical, Los Angeles, Calif., and International Chemical and Nuclear, Irvine, Calif., Ura from Sigma Chemical, St. Louis, Mo., Urd from Aldrich Chemical, Milwaukee, Wis., and Poly U from Miles Laboratories, Kankakee, Ill. Weighed amounts were dissolved in deionized, doubly distilled H$_2$O or 99.8% D$_2$O to provide stock solutions. The pH's (pD's) of solutions containing nucleosides or nucleotides and CH$_3$Hg$^+$ 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.[23]

Raman Spectra. Spectra were excited with the 5145-Å line of a Coherent Radiation Model 52 Ar$^+$ laser at ca. 800 mW. The Raman scattering was dispersed with a Spex 1400 double monochromator and detected with an RCA 31034A photomultiplier tube. For the difference spectra, a programmed sample carrier for alternate positioning of reference and sample cells in the laser beam was employed. Single photon counting equipment was used to count for a preset period of time, usually 10 sec, at each wavelength value. This number and a digital signal from a photo diode laser power monitor used to normalize the spectra were punched on paper tape. Spectra were plotted and band maxima located routinely off-line with a Hewlett Packard 2116A computer (8K × 16 bit memory) system using PROGRAM RSPC written by R. W. Chrisman.[24] More complex spectral analyses were performed off-line with a CDC 6500 computer using PROGRAM RAMAN written by J. W. Lundeen.[24] A detailed description of the difference spectrophotometer has been given elsewhere.[25]

In order to compare intensities in a series of spectra, c.g., in a continuous variation experiment, the intensities were scaled so ν_1 of the internal ClO$_4^-$ reference was the same. This is an option in RAMAN, and the procedure involves the construction of a baseline and a numerical integration over the ν_1 envelope.

In general, solutions were clarified by filtration through 100-nm pore size ultrafilters or, with small samples, through fine frits as used for Rayleigh scattering studies on biological molecules.[26] The samples were contained in 1-ml cells with optically flat windows. The cell was maintained at 25 ± 1° by mounting it in a brass block through which water was circulated from a constant temperature bath. In the difference measurements, the sample temperature was ca. 22°.

Data and Results

Species Distribution. Since approximate equilibrium constants[11] for binding of CH$_3$Hg$^+$ to Urd and Cyd are known, we have attempted to obtain Raman spectra under conditions such that one methylmercury(II)-nucleoside complex predominates in the solution. The methylmercury(II) cation is rather acidic, and, unfortunately, upon hydrolysis it forms polynuclear complexes. In the determination of the hydrolysis constants,[27] CH$_3$Hg$^+$ concentrations of only up to 22 mM were employed; even at these concentrations the binuclear complex (CH$_3$Hg)$_2$OH$^+$ is an important species. In more concentrated solutions, it is likely that a trinuclear species also is produced, since [(CH$_3$Hg)$_3$O$^+$]ClO$_4^-$ precipitates when (CH$_3$Hg)$_2$O is treated with perchloric acid in aqueous solution.[28] Because of the uncertainty about the methylmercury(II) species present in solution at higher concentrations, the total stoichiometric concentration was no larger than 50 mM in any of the solutions studied.

(22) L. G. Sillén and A. E. Martell, *Chem. Soc., Spec. Publ.*, No. 17, 61 (1964).
(23) P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).
(24) Deck listings of all computer programs are available upon writing to R. S. T.
(25) J. W. Amy, R. W. Chrisman, J. W. Lundeen, T. Y. Ridley, J. C. Sprowles, and R. S. Tobias, *Appl. Spectrosc.*, in press.
(26) See S. N. Timasheff and R. Townend, "Physical Principles and Techniques of Protein Chemistry," Part B, S. J. Leach, Ed., Academic Press, New York, N. Y., 1970, Chapter 12.
(27) G. Schwarzenbach and M. Schellenberg, *Helv. Chim. Acta*, **48**, 28 (1965).
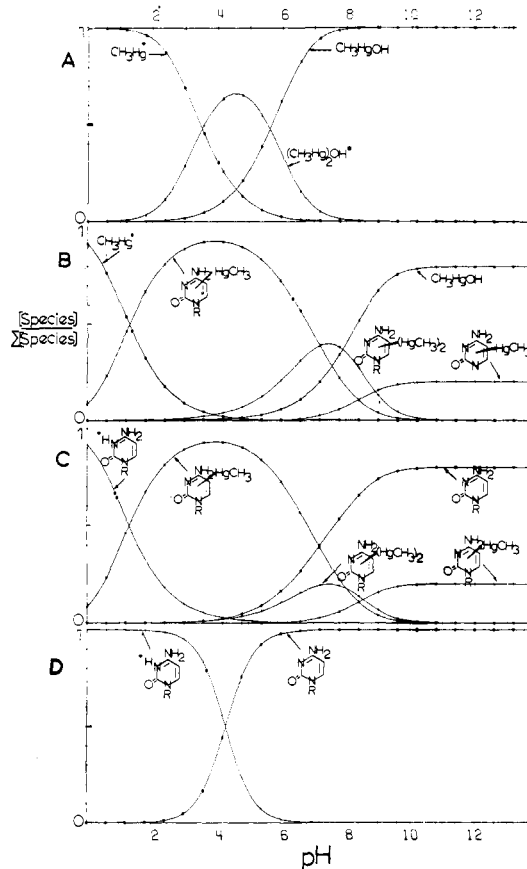(28) D. Grdenić and F. Zado, *J. Chem. Soc.*, 521 (1962).
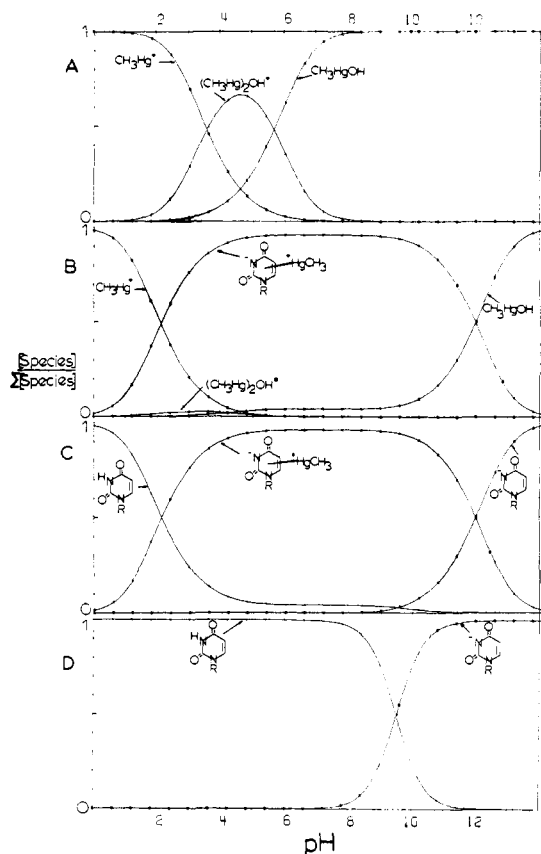
Figure 1. Species distribution in the CH_3Hg^+–Urd system computed from a model based on Simpson's equilibrium constants, proton transfer from ribose ignored: A, 50 mM CH_3Hg^+; 50 mM CH_3Hg^+ + 50 mM Urd, B, metal distribution, C, Urd distribution; D, 50 mM Urd.

Figure 2. Species distribution in the CH_3Hg^+–Cyd system computed from a model based on Simpson's equilibrium constants, proton transfer from ribose ignored: A, 50 mM CH_3Hg^+; 50 mM CH_3Hg^+ + 50 mM Cyd, B, metal distribution, C, Cyd distribution; D, 50 mM Cyd.

Table I. Equilibrium Constants (25°) Used in the Description of the CH_3Hg^{II} Nucleoside Systems. Assigned Sites of Binding Are in Parentheses

Reaction	μ	Log K_{eq}	Ref
CH_6Hg^{II}			
$CH_3Hg^+ + H_2O \rightleftharpoons$ $CH_3HgOH + H^+$	0.1	-4.59	27
$CH_3HgOH + CH_3Hg^+ \rightleftharpoons$ $(CH_3Hg)_2OH^+$	0.1	2.37	27
Urd			
$UrdH_{-1}^- + H^+ \rightleftharpoons$ $Urd(N_3C_4O)$	0.1	9.51	29
$UrdH_{-1}^- + CH_3Hg^+ \rightleftharpoons$ $UrdH_{-1}HgCH_3(N_3C_4O)$	Var	9.0	11
dThd			
$dThdH_{-1}^- + H^+ \rightleftharpoons$ $dThd(N_3C_4O)$	0	9.79	29
$dThd_{-1}^- + CH_3Hg^+ \rightleftharpoons$ $dThdH_{-1}HgCH_3(N_3C_4O)$	Var	9.49	8
	Var	9.2	10
Cyd			
$Cyd + H^+ \rightleftharpoons CydH^+(N_3)$	0.1	4.22	29
$Cyd + CH_3Hg^+ \rightleftharpoons$ $CydH_{-1}HgCH_3(N_3) + H^+$	Var	4.6	11
$Cyd + CH_3Hg^+ \rightleftharpoons$ $CydH_{-2}HgCH_3(C_4NH_2) + H^+$	Var	-3.8	11
$CydHgCH_3^+(N_3) + CH_3Hg^+ \rightleftharpoons$ $CydH_{-1}(HgCH_3)_2^+(N_3,C_4NH_2) + H^+$	Var	-2.9	11

The equilibrium constants used in constructing models of the Urd– and Cyd–CH_3Hg^+ systems are sum-

marized in Table I. Data also are included for dThd, since its reactions are similar to Urd. Values for the ligand–proton equilibrium constants were taken from the tables of Izatt, Christensen, and Rytting.[29] Species distributions as a function of pH were computed and plotted with PROGRAM QUARK[24] using Purdue University's CDC 6500 computer. These are illustrated in Figures 1 and 2 for the CH_3Hg^+–Urd and –Cyd systems, respectively.

Raman Spectra. The Raman studies, in general, 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, and, in continuous variation experiments, the maximum total concentration was 50 mM.

Methylmercury Perchlorate and Methylmercury Hydroxide. On the basis of the equilibrium constant data of Schwarzenbach and Schellenberg,[27] the aquo cation predominates in solutions with pH ≤ 2 and the hydroxide when pH ≥ 7. In the intermediate range, polynuclear complexes exist so the species distribution also is a function of the total CH_3Hg^{II} concentration.

Woodward and coworkers have reported the Raman spectrum of CH_3HgClO_4[30] in 4 M solution and of CH_3-

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

(30) P. L. Goggin and L. A. Woodward, *Trans. Faraday Soc.*, **58**, 1495 (1962).

HgNO₃³¹ from 6.45 to 2.15 M, and the frequencies reported by them for the vibrations of interest here are very similar to those obtained in this work in the 10–100 mM range: ν(Hg–O) 451 bd, ν(Hg–C) 566, δ_s(CH₃) 1207 cm⁻¹. Goggin and Woodward[32] measured values for CH₃HgOH using a 4 M aqueous solution. Because of reports that the reaction of Ag₂O with RHgI compounds gives mixtures of [(RHg)₃O)]OH and (R-Hg)₂O,[28,33] we were concerned that the frequencies reported for the concentrated solution would not be those, strictly, of CH₃HgOH. Nevertheless, the spectrum of a 50 mM solution is qualitatively very similar, although the frequencies differ somewhat from those reported for the 4 M solution: (ref 32) ν(Hg–O) 511, ν(Hg–C) 577, δ_s(CH₃) 1211 cm⁻¹; this work 505, 569, 1211 cm⁻¹, respectively. All of our solutions contained 0.100 M ClO₄⁻ which serves as a check on our absolute frequencies. In all of these measurements ν_1 of ClO₄⁻ was observed at 931.5 ± 0.5 cm⁻¹.

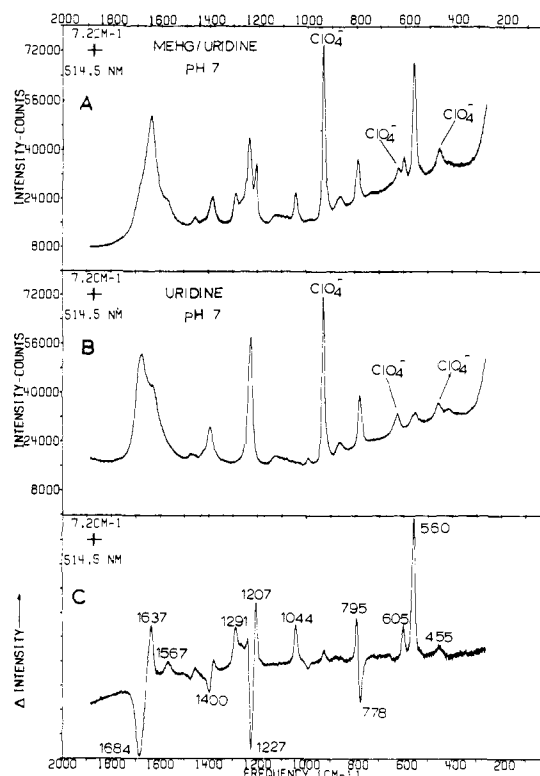Clarke and Woodward[31] also examined partially hydrolyzed 2 M CH₃Hg^{II} solutions and assigned a band at 415 cm⁻¹ as characteristic of the binuclear complex (CH₃Hg)₂OH⁺. No such band was observed in any of our spectra indicating that significant amounts of the binuclear complex are absent. The distribution of CH₃Hg^{II} illustrated in Figures 1 and 2 was calculated for a 50 mM solution. The maximum fraction of (CH₃Hg)₂OH⁺ decreases from 0.66 for 50 mM to 0.40 for 10 mM CH₃Hg^{II}.

The methylmercury(II) cation is a good probe ion for Raman spectroscopy. If the coordinated water or hydroxo group is displaced by another ligand, a shift in ν(Hg–C) normally results. In addition, the bands assigned to stretching of the Hg–O bound to the water molecule or hydroxo group in the first coordination sphere are rather intense, and displacement of these oxygen ligands should lead to disappearance of these bands.

Uridine + CH₃Hg^{II}, pH 7. By examination of Figure 1, it is seen that ca. 95% of Urd and of CH₃Hg⁺ should be distributed in the complex when equimolar concentrations are present at pH 7. At this pH, the free ligand is present as the neutral molecule, and CH₃-HgOH is the only significant methylmercury species present. At pH values outside the 7–9 range, the complex either is a minor species or other ligand or methyl-mercury(II) species are present.

The Raman spectra of Urd in both H₂O and D₂O have been studied thoroughly by Lord and Thomas.[34] Because the proton on the N₍₃₎ position of the nucleoside is rapidly exchanged, the spectrum changes significantly from H₂O to D₂O solution.

Difference Spectra. In order to search for evidence of binding of CH₃Hg⁺ to Urd, we have used Raman difference spectroscopy (RDS). The difference spectrum for 50 mM Urd + 50 mM CH₃Hg⁺ vs. 50 mM Urd, both at pH 7, is illustrated in Figure 3. Any vibrations derived from CH₃Hg⁺ group vibrations should appear as positive features in the difference spectrum. Easily

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

(32) P. L. Goggin and L. A. Woodward, *Trans. Faraday Soc.*, **56**, 1591 (1960).

(33) G. E. Coates, M. L. H. Green, and K. Wade, "Organometallic Compounds," Vol. 1, Methuen, London, 1967, p 154.

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

Figure 3. Raman difference spectrum: A, 50 mM Urd + 50 mM CH₃HgClO₄; B, 50 mM Urd (both solutions 0.1 M in (Na)ClO₄); C, (A − B) difference spectrum. Solution pH 7. Scan conditions: 0.25 Å intervals, 10 sec counting time.

recognized are the ν(Hg–C) and δ_s(CH₃) modes at 560 and 1207 cm⁻¹. New bands arising from a metal-nucleoside complex and characteristic of neither CH₃-Hg^{II} nor nucleoside also will appear as positive features. Examples occur at 605, 1044, and 1291 cm⁻¹. Finally small shifts in Urd bands will give rise to derivative features the clearest example of which is centered at 786 cm⁻¹. These derivative curves make it possible to detect very small frequency shifts and/or intensity changes in broad bands. The feature at 786 cm⁻¹ arises from the shift in a Urd band from 780 to 791 cm⁻¹ upon coordination of CH₃Hg⁺.

A difference spectrum also was measured for 50 mM Urd + 50 mM CH₃Hg^{II} vs. 50 mM CH₃Hg^{II}, also at pH 7. This is illustrated in Figure 4. The large negative feature at 506 cm⁻¹ arises from the disappearance of a band characteristic of CH₃HgOH. Shifts in the ν(Hg–C) and δ_s(CH₃)Hg modes also are indicated.
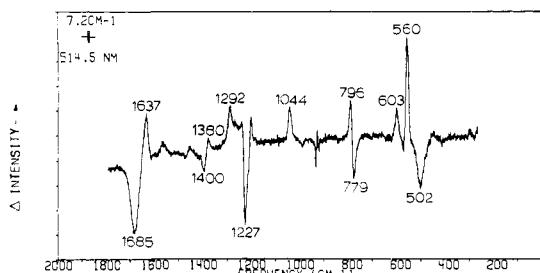
Finally a difference spectrum was computed using data from both of the RDS runs described above. This will be somewhat less accurate, since it involves data from three independent scans of the monochromator. It was computed in the following way. The Urd + CH₃Hg^{II} sample spectra from the two RDS runs were summed to improve signal/noise by $\sqrt{2}$, the sum was scaled on the basis of the ClO₄⁻ ν_1 internal reference and this was added to the spectrum for a 0.100 M NaClO₄ solution. From this was subtracted the sum of the Urd and the CH₃Hg^{II} reference spectra. This is illustrated in Figure 5. This procedure should give complete solvent and internal reference compensation. That this has been achieved can be seen from the absence of ClO₄⁻ vibrations in this difference
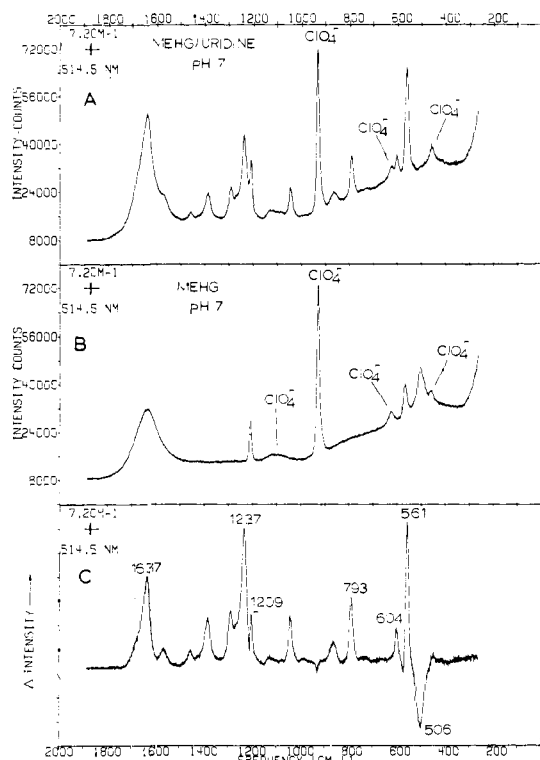
Figure 4. Raman difference spectrum: A, 50 mM Urd + 50 mM CH₃HgClO₄; B, 50 mM CH₃HgClO₄ (both solutions 0.1 M in (Na)ClO₄); C, (A − B) difference spectrum. Solution pH 7. Scan conditions as in Figure 3.

**Figure 5.** Raman difference spectrum: (50 mM CH₃HgClO₄ + 50 mM Urd) + (solvent) − (50 mM CH₃HgClO₄) − (50 mM Urd). All solutions 0.1 M in (Na)ClO₄. Solution pH 7. Scan conditions are the same as in Figure 3.

spectrum. The noise level is high at 931 cm⁻¹, since the very intense and consequently noisy ClO₄⁻ ν_1 signals occur there. The perturbations of the Urd and CH₃Hg^{II} vibrations described above are clearly visible in this spectrum.

Continuous Variation Spectra. In order to examine the stoichiometry of the reaction, the continuous variation method[35] was employed while recognizing that there are many limitations in its use. The results for H₂O and D₂O solutions are illustrated in Figures 6 and 7. Frequencies for the CH₃Hg^{II} complex with Urd are listed in Table II.

Double-Bond Stretching Region (1800–1500 cm⁻¹). Since the solutions were quite dilute, the bending fundamental interferes with the H₂O solutions in this region. This is removed in the difference spectrum, and Figures 3–5 show that there is a large decrease in the intense

(35) F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants," McGraw-Hill, New York, N. Y., 1961, p 47.
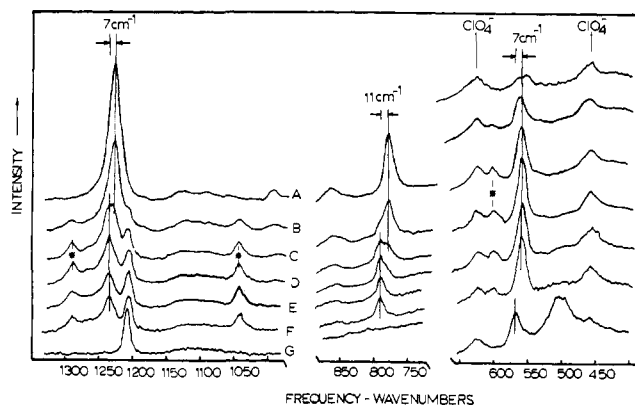
Figure 6. Continuous variation data, Urd–CH₃HgClO₄ in H₂O at 25°. Total concentration Urd + CH₃HgClO₄ = 50 mM. Urd: A, 50; B, 40; C, 30; D, 25; E, 20; F, 10; G, 0 mM. Asterisk indicates a new band characteristic of the complex. Scan conditions are the same as in Figure 3.
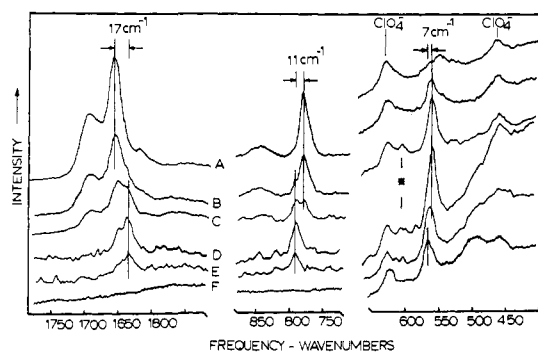
Figure 7. Continuous variation data, Urd–CH₃HgClO₄ in D₂O at 25° and pD 7. Total concentration Urd + CH₃HgClO₄ = 50 mM. Urd: A, 50; B, 40; C, 30; D, 20; E, 10; F, 0 mM. Asterisk indicates a new band characteristic of the complex. Scan conditions are the same as in Figure 3.

Table II. Raman Frequencies (cm⁻¹) for Ura, Urd, and Poly-U Complexes with CH₃Hg^{II} (Depolarization Ratios in Parentheses[a])

Ura–CH₃Hg⁺ D₂O, pD 6.9	Urd–CH₃Hg⁺ H₂O, pH 7.0	Urd–CH₃Hg⁺ D₂O, pD 7.0	Poly U–CH₃Hg⁺ H₂O, pH 6.9
1644 *5*[d]	~1630[b] [*6*]	1637 *8*	1637 *6*
	1574 *1*		
1454 *2*	1458 *1*	1459 *2*	1455 *1*, bd
1385 *3*	1386 *3*, pol	1388 *3*	1385 *3*
1258 [*7*][c]	1291 *3*, pol?	1290 *5*	1288 *3*
	1236 *5*, (0.6)	1236 [*6*][c]	1237 *7*
1207 [*7*][c]	1208 *4*, (0.7)	1206 [*6*]	1208 *6*
1057 *1*	1044 *2*, pol	1044 *3*	1044 *3*
	862 *1*, bd (0.0)		
			~800 sh
798 *3*	791 *3*, (0.3)	791 *5*	792 *4*
609 *2*	604 *2*, pol	606 *2*	604 *1*
560 *10*	561 *10* (0.5)	561 *10*	560 *10*

[a] For comparison, ν_1 of the ClO₄⁻ internal reference has ρ = 0.04. The values were obtained with solutions 25 mM in CH₃Hg^{II} and 25 mM in Urd. [b] Obtained from the difference spectrum, since there is extensive water scattering in this region. [c] The D₂O bending fundamental occurs in this region. [d] Relative intensities are given in italics. The most intense band was assigned a value 10.

ligand scattering at 1684 cm⁻¹ and an increase at 1637 cm⁻¹ where the free ligand exhibits a shoulder. A new, weak band characteristic of the complex appears at *ca.* 1574 cm⁻¹.

In D_2O solution, the band at 1690 cm^{-1} disappears in the complex and the 1654 cm^{-1} Urd band shifts to 1637 cm^{-1}. This is clearly seen in the continuous variation data, Figure 7.

(1500–700 cm^{-1}). The changes in this region are most clearly seen with the H_2O solutions, Figures 3, 4, and 6. The weak Urd band at 1475 cm^{-1} shifts to lower frequency, i.e., 1458 cm^{-1}. This band has been assigned to a vibration of the ribose residue. The most striking changes occur in the 1200–1300 cm^{-1} region where free Urd shows a very strong, polarized band at 1230 cm^{-1}. The complex has a weaker band at 1236 cm^{-1} together with a new one at 1291 cm^{-1}.

Since the proton on $N_{(3)}$ exchanges, the spectrum of Urd in D_2O is different from the H_2O spectrum. The deuterium derivative has intense bands at 1248 and 1303 cm^{-1}, rather similar to those of the CH_3Hg^{II} complex. From the data in Table II it is seen that the spectrum of the CH_3Hg^{II}–Urd complex is the same in both H_2O and D_2O solutions.

Examination of the intensities of the bands at 1044 and 1291 in the continuous variation experiments with the H_2O solutions, Figure 6, suggests that these bands arise from a 1:1 interaction, although this is quite imprecise with the signal:noise of these experiments. This is supported by the intensity of the band at 791 cm^{-1} which is a maximum for the solution with the equimolar concentrations of CH_3Hg^{II} and Urd; see Figure 6. The intense band at 778 cm^{-1} which is insensitive to deuteration increases in frequency upon metallation. It is observed at 791 cm^{-1} for both H_2O and D_2O solutions of the complex. The variation of the intensities of the bands characteristic of the complex with system stoichiometry is being studied in detail with much longer counting times.

Features Associated With CH_3Hg^{II}. There is a very small shift in $\delta_s(CH_3)$ from 1211 to 1208 Urd, Poly U in H_2O; 1206 Urd in D_2O. The $\nu(Hg-C)$ mode decreases 7 cm^{-1} to 560–561 cm^{-1} upon coordination, and there is a marked hyperchromic effect which can be seen in the difference spectra, Figures 4 and 5, and in the continuous variation plots, Figures 6 and 7. The most obvious change is the disappearance of the broad, intense band which has been assigned to $\nu(Hg-O)$ at ca. 511 (H_2O), 496 cm^{-1} (D_2O). The isotopic shift indicates that this vibration does, indeed, involve the coordinated OH$^-$ group. This band's absence leads to large negative features in the difference spectra in Figures 4 and 5.

An unusual feature is the disappearance of the Hg–O band even with the 40 mM CH_3Hg^{II}–10 mM Urd solution. The Hg–C frequency also has reached its limiting value even though there is an excess of CH_3Hg^{II}. This is most easily seen with Figure 6 for H_2O solutions. Apparently binding occurs initially at several sites and as the concentration of nucleoside increases, binding occurs preferentially at the most basic site.

Uracil (pH ~7). The reaction between CH_3Hg^{II} and Ura was examined with a D_2O solution 25 mM in each, pD 6.9. The extent of the reaction as indicated by the intensity of the bands at 798 (complex) and 783 (free Ura) was less than for Urd. Roughly equal concentrations of the complex and Ura appear to be present. The shifts in the Ura bands upon complexation are for the most part similar to those observed with
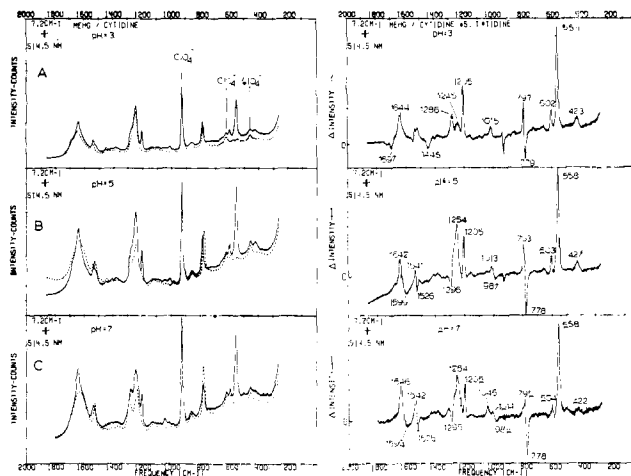
Figure 8. Raman spectra: Cyd, 50 mM, + CH_3HgClO_4, 50 mM, vs. Cyd, 50 mM, in H_2O: A, pH 3; B, pH 5; C, pH 7. Left side: solid line, solution spectrum; broken line, reference. Right side: difference spectra.

Urd. The complex vibrations are tabulated in Table II and the spectrum of the complex is available in the microfilm edition. See paragraph at end of paper regarding supplementary material. Lord and Thomas[34] have given frequencies for free Ura in D_2O.

Poly U (pH ~7). The Raman spectrum of poly U is almost identical with that of Urd itself, and the spectrum of the Poly U–MeHg^{II} complex at pH 6.9 is also almost the same as the Urd–MeHg^{II} complex spectrum. Frequencies are tabulated in Table II. The spectrum is illustrated in the microfilm edition.

Cytidine + CH_3Hg^{II}. The species distribution based on Simpson's[11] equilibrium constants as illustrated in Figure 2 is somewhat more complex than for the Urd–CH_3Hg^{II} system. Mercuriation appears to occur at two sites. Based on Simpson's constants, the concentration of the principal complex is maximized at pH 4, accounting for ca. 90% of metal or ligand. At this pH, Cyd is ca. 50% protonated, and appreciable amounts of $(CH_3Hg)_2OH^+$ exist in more concentrated solutions of methylmercury(II).

The Raman spectra of Cyd have been studied carefully in H_2O and D_2O by Lord and Thomas,[34] although it should be noted that the intensity values listed for the double bond region in D_2O solution do not correspond to their figure which is correct. The nucleoside has significantly different spectra in D_2O and H_2O because of exchange of the amino hydrogens and coupling of the $C_{(4)}$–NH_2 deformations and ring stretching vibrations.

Difference Spectra. Figure 8 illustrates CH_3Hg^{II}–Cyd vs. Cyd difference spectra for pH 3, 5, and 7. As judged from the relative intensities at 788 (complex) and 780 cm^{-1} (CydH$^+$), the extent of complex formation is greatest at pH 3, somewhat less at pH 5, and only partially complete at pH 7. This is roughly in accord with the predictions summarized in Figure 2. This effect can be seen clearly in the relative magnitudes of the two parts of the derivative signal centered at 785 cm^{-1} in the difference spectra.

The overall spectral changes in the 1200–1300 cm^{-1} region are much greater with the pH 5 and 7 solutions, because the CH_3Hg^+– and H$^+$–Cyd complexes have rather similar spectra in this range. From the data in
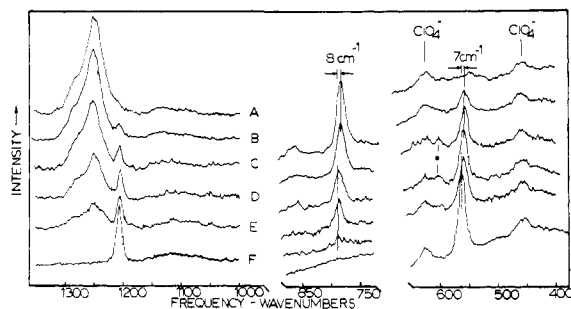
Figure 9. Continuous variation data, Cyd–CH₃HgClO₄ in H₂O at 25° and pH 3.8. Total concentration Cyd + CH₃HgClO₄ = 50 mM. Cyd: A, 50; B, 40; C, 30; D, 20; E, 10; E, 10; F, 0 mM. Asterisk indicates a new band characteristic of the complex. Scan conditions are the same as in Figure 3.

Figure 2, it can be seen that the pH 3 difference spectrum compares the complex with CydH⁺.

Continuous Variation Spectra. We chose to make our continuous variation measurements at pH 3.8, where complex formation is maximized, in order to avoid the possibility that the shifts in the Cyd vibrations would reflect some mercuration at a second site. These are illustrated in Figure 9. The spectral changes upon complexation in H₂O at this pH are not especially striking, because, as noted above, both Cyd and CydH⁺ are present. Table III gives the complex fre-

Table III. Vibrational Frequencies (cm⁻¹) of the Cyd–CH₃Hg^{II} Complexes in H₂O and D₂O[a]

CH₃Hg^{II}–Cyd H₂O, pH 4.0	CH₃Hg^{II}–Cyd D₂O, pD 3.9	CH₃Hg^{II}–Cyd H₂O, pH 4.0	CH₃Hg^{II}–Cyd D₂O, pD 3.9
1645 [4][b]	1653 6	1205 4	∼1204 5[c]
1542 2	1554 2	1015 0	1062 3
	1510 4	788 6	780 6
	1458 1	603 2	598 3
∼1286 4 sh	1292 8	559 10	559 10
1254 6	1258 7		

[a] The solvent and internal ClO₄⁻ frequencies have been deleted.
[b] Obtained from the difference spectra, since the H₂O bending fundamental occurs in this region. [c] Obscured by bending fundamental of D₂O.

quencies for both H₂O and D₂O solutions. Although there are minor differences in the spectra because of the exchange of the C₍₄₎–NH₂ protons, the complex spectrum is similar in the two solvents. The spectrum of a D₂O solution is illustrated in the microfilm edition. Continuous variation measurements also were made at pH 6.2 with Na[(CH₃)₂AsO₂] as a buffer. In spite of the fact that complex formation should be less complete, the spectral changes are more striking as expected from the pH 7 difference spectrum. Complexation leads to a decrease in scattering at 1295 cm⁻¹.

Double-Bond Stretching Region (1800–1500 cm⁻¹). A description of the vibrations for the D₂O solution is somewhat more straightforward. Here no coupling of the double-bond stretching with NH₂ scissoring occurs, and the vibrations in the 1500–1700 region are due to stretching vibrations of the ring. The band at ca. 1712 cm⁻¹, normally attributed to C₂=O stretching of CydH⁺, is absent in the complex, and one intense band occurs at 1653 cm⁻¹. This effect is similar to that observed with Urd. As with Urd, a new weak band

characteristic of the complex appears in the 1500–1600 region, here at 1554 cm⁻¹. In H₂O solution, there is an increase in the intensity of the scattering at 1644 cm⁻¹, and a new band appears at ca. 1542 cm⁻¹.

(1500–700 cm⁻¹). The most intense bands are in the 1300–1200 cm⁻¹ region. Mercuration results in shifts for all of these bands compared with both CydH⁺ (CydD⁺) and Cyd. The H₂O spectra are simpler in this region. Compared to Cyd, there is an increase in the scattering at 1254 and a decrease at 1295 cm⁻¹.

A new band appears at 1062 (D₂O), 1015 (H₂O), characteristic of the complex analogous to the 1044 cm⁻¹ band with Urd. The medium band at 788 (H₂O), 780 cm⁻¹ (D₂O), is at higher frequency than for either HCyd⁺ or Cyd, again analogous to the shift in the Urd band in this region (791 cm⁻¹ in the complex). This is clearly indicated in the continuous variation spectra; cf. Figures 6 and 9.

Features Associated with CH₃Hg^{II}. Within the experimental error, these are the same as those observed for the Urd complex. The CH₃Hg^{II} is only partially hydrolyzed at pH 4. The hyperchromic effect on the (Hg–C) stretching band observed with Urd still can be seen in Figure 9, although to a reduced extent. Again the shift in the (Hg–C) stretching frequency has attained the limiting value in the 40 mM CH₃Hg^{II}–10 mM Cyd solution, indicating interaction at more than one site.

Discussion

The methylmercury(II) ion binds to Urd, contrary to the behavior observed by Lord and Thomas[21] for solutions of Urd and HgCl₂. In addition, CH₃Hg⁺ binds similarly to Ura and Poly U. The reaction at pH 7 of 25 mM CH₃HgClO₄ with the Ura moiety of 25 mM Urd is essentially quantitative, because the solution shows only bands characteristic of the complex. The continuous variation data suggest a 1:1 reaction with the base moiety.

The spectra of the 1:1 complex are identical for the H₂O and D₂O solutions. The spectra of Urd itself are rather different in these two solvents because of isotopic exchange of the proton on N₍₃₎. This indicates that the proton has been displaced from the N₍₃₎ position and that CH₃Hg^{II} binds to UrdH₋₁⁻ at pH 7 (reaction 1), as assumed originally by Ben-Zvi, et al.,[8] and by Simpson[11] but questioned by Carrabine and Sundaralingham.[12]

$$CH_3HgOH + Urd \rightleftharpoons CH_3HgUrdH_{-1} + H_2O \qquad (1)$$

The band at 1690 cm⁻¹ (D₂O), which is indicated by ¹⁸O isotopic substitution studies[36] to involve primarily C₍₂₎=O stretching, disappears in the complex, and the 1657-cm⁻¹ band generally described as in-phase stretching of C₍₅₎=C₍₆₎ and C₍₄₎=O shifts to 1637 cm⁻¹. The intensity of the ring stretching mode[37] at 1227 cm⁻¹ decreases, and the frequency of the mixed ring stretching–deformation mode at 782 cm⁻¹ increases. All of these changes are similar to those observed when Urd is deprotonated to produce UrdH₋₁⁻ or UrdD₋₁⁻. It appears that the methylmercury(II) cation stabilizes an electronic configuration for UrdH₋₁⁻ which involves increased electron delocalization similar to that of the

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(37) Approximate descriptions of the Ura modes based upon a normal coordinate analysis have been given by H. Susi and J. S. Ard, *Spectrochim. Acta, Part A*, 27, 1549 (1971).

conjugate base, I. It is unlikely that there would be significant coupling between the Hg–N coordinate and the ring coordinates, so the spectrum of the CH_3Hg^+ complex should resemble the conjugate bases rather than the protonated or deuterated form.

I   II   III
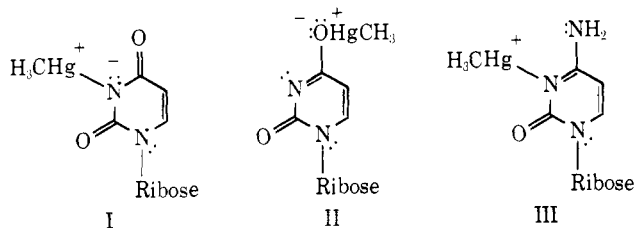
All electron pairs have been indicated in the structural formulas for bookkeeping purposes. There is, of course, extensive delocalization of the lone pairs drawn on nitrogen into the π system, and the Ura and Thy rings are almost planar.[38] The isomer, II, with coordination at the $C_{(4)}$–O is less likely for two reasons. Firstly, the $C_{(2)}$=O vibration would be expected to be at higher energy than with Urd itself. This is not observed to be the case. Secondly, it seems likely that OH⁻ would be a better base, and coordination to the nucleoside would not be favored. On the other hand, the ring nitrogen should be a much better base for CH_3Hg^{II}. Perturbations of the Ura and Poly U vibrations indicate that binding occurs at the same site as with Urd.

The observed value of ν(Hg–C), 560 cm⁻¹, suggests a rather weak mercury–ligand bond. The corresponding value for the pyridine complex is 550 cm⁻¹.[39] The (Hg–C) stretching frequency is moderately sensitive to the ligand trans to the methyl group, varying from 566 with H_2O to 526 with I⁻.

The observation that the hydroxo group is displaced from CH_3Hg^{II} even with a 4:1 CH_3Hg^{II}:Urd ratio indicates that binding occurs at other sites on the nucleoside besides the base, probably on the ribose residue. (This coordination is being investigated further.) As the relative concentration of Urd increases, the CH_3Hg^+ binds at the most basic site, $N_{(3)}$.

Since it has been demonstrated that CH_3Hg^+ binds to Urd with displacement of a proton, it is of interest to consider why Lord and Thomas[21] observed no Raman evidence for a reaction between Urd and a fivefold excess of $HgCl_2$. In addition, Carrabine and Sundaralingham[12] considered that binding to uracil and thymine bases occurred via the oxygens without displacement of the $N_{(3)}$ proton. At first, these results seem inconsistent with our data. The stability constants of the complexes of CH_3Hg^+ parallel those of Hg^{2+} and, in general, are slightly smaller.[40]

We recorded spectra in D_2O of 25 mM $HgCl_2$ + 50 mM Urd and 25 mM $HgCl_2$ + 25 mM Ura and observed, as did Lord and Thomas,[21] no evidence for a reaction. Upon consideration of the experimental conditions, this is not surprising. The Hg^{2+} cation is a rather strong aquo acid, and the solutions described above had pD 4.0. The distribution diagram in Figure 1 shows that CH_3Hg^+ competes effectively with the
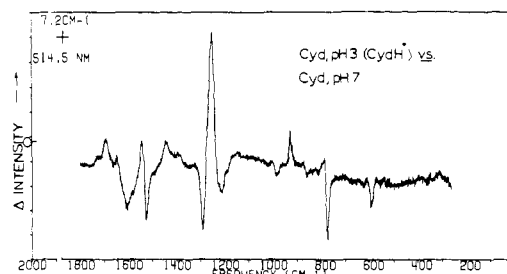
Figure 10. Raman difference spectrum: Cyd, pH 3 (CydH⁺), vs. Cyd, pH 7.

proton for $UrdH_{-1}^-$ only at pH values greater than ca. 3. The $HgCl_2$ case is complicated further because the stability constants for chloride complexing are rather large, log K_1 = 6.7 and log K_2 = 6.48.[22] Consequently, there is competition by H⁺ and Hg^{2+} for $UrdH_{-1}^-$ and by Cl⁻ and $UrdH_{-1}^-$ for Hg^{2+}, and little of the Hg–Urd complex is formed. Both of these $HgCl_2$ solutions showed a strong band at 318 cm⁻¹ characteristic of the undissociated species.

The results for Cyd + CH_3Hg^{II} are very similar to those found for Urd and CH_3Hg^{II}. This can be seen by comparing the difference spectra for $UrdHgCH_3$ vs. Urd and $CydHgCH_3^+$ vs. CydH⁺, Figures 3 and 8A. The principal difference is that replacement of the proton on Urd by CH_3Hg^+ causes a marked decrease in the Raman intensity in the 1200–1300-cm⁻¹ region, while replacement of the corresponding proton on CydH⁺ has little effect on the scattering in this region. As with Urd, complexation causes the disappearance of the scattering near 1700 cm⁻¹ associated with $C_{(2)}$=O stretching. Rather similar perturbations of the lower frequency ring modes[41] occur in both systems, and the shifts in the CH_3Hg^{II} frequencies are almost the same. This suggests strongly that coordination also occurs to the $N_{(3)}$ position, III.

While the Urd–CH_3Hg complex has a spectrum similar to the anion $UrdH_{-1}^-$, the Cyd–CH_3Hg^+ complex spectrum resembles that of CydH⁺ rather than Cyd. This can be seen by comparing the Cyd–CH_3Hg^+ vs. Cyd difference spectrum for pH 7, Figure 8C, with the CydH⁺ vs. Cyd difference spectrum, Figure 10.

The perturbation of the Cyd vibrations upon complexation with CH_3Hg^+ is similar to that observed by Lord and Thomas[21] upon reaction with $HgCl_2$. In this case Hg^{2+} competed favorably with the proton for $N_{(3)}$, because CydH⁺ is a much stronger acid than Urd.

The observation that the Hg–O stretching band disappears even with a 4:1 mole ratio CH_3Hg^{II}:Urd and that the (Hg–C) frequency has attained its limiting shift with 4:1 CH_3Hg^{II}:Urd or Cyd indicates that the cation interacts at more than one site on the nucleoside. Since the Urd band at 1475 cm⁻¹ which has been assigned to a vibration of the ribose shifts to 1451 cm⁻¹, it is possible that there is interaction with the sugar.

The cause of the hyperchromism of the ν(Hg–C) band upon replacement of H_2O or OH⁻ by the nitrogen bases has not been established. It seems likely that there is considerable mixing of the Hg–CH_3 and Hg–O coordinates in the two normal modes in the 500–600-

(38) D. Voet and A. Rich, Progr. Nucl. Acid Res. Mol. Biol., 10, 183 (1970).

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(40) R. S. Tobias, Organometal. Chem. Rev., 1, 93 (1966).

(41) Approximate descriptions of the Cyt normal modes have been given by H. Susi, J. S. Ard, and J. M. Purcell, Spectrochim. Acta, Part A, 29, 725 (1973).

cm^{-1} region. This would tend to account for the high intensity of the (Hg–O) stretch and low intensity of the (Hg–C) stretch of CH_3HgOH. With the heterocyclic base coordinated, one mode would be expected to involve almost pure (Hg–C) stretching leading to an increase in its intensity. Such mixing, however, is not supported by the observation that the stretching frequency at 568 cm^{-1} does not shift in going from CH_3HgOH to CH_3HgOD.

A second possible explanation would involve a preresonance effect. Coordination of CH_3Hg^+ to Urd or Cyd shifts the absorption bands toward the visible.[11] Such a preresonance effect has been suggested as the explanation for marked changes in the Raman intensities of polynucleotides with changes in conformation.[16] On the other hand, the absorption bands of the CH_3Hg^{II}–Urd and –Cyd complexes are at ca. 265 and 280 nm, far removed from the 514.5-nm exciting line. In addition, such an effect would require substantial interaction between the electronic transition originally occurring in the heterocyclic base and the (Hg–C) vibration. This hyperchromism is being studied further.

Mansy, et al.,[20] observed a decrease in the scattering of Poly U at 1681 cm^{-1} with solutions containing cis- and trans-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ which is similar to the effect observed here upon binding of CH_3Hg^+ to Urd and Poly U. The decrease in the scattering at 1291 cm^{-1} of Poly C upon reaction with the two platinum ammine isomers also is similar to that observed upon binding of CH_3Hg^+ to Cyd in H_2O at pH 7. It is probable that the platinum binds at the $N_{(3)}$ site of both of these bases too.

In summary, Raman spectroscopy and particularly Raman difference spectroscopy has been shown to be an effective technique for studying the interaction of heavy metals with nucleosides and establishing the binding site. The CH_3Hg^{II} cation binds both to Urd and Cyd at pH 7, although binding to Urd is more nearly complete at that pH. This is consistent with the results of Simpson's uv spectrophotometric investigation.[11] The suggestion of Nandi, et al.,[42] that Hg(II) binds to dThd at $N_{(3)}$ in AT rich DNA at pH 9 seems more likely than for coordination to occur at $C_4=O$.[12] The difference spectra of the complexes with the nucleosides exhibit bands characteristic of the metallated nucleoside. Consequently, this technique should be capable of establishing the binding sites in polynucleotides containing both the uracil and cytosine bases.

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Photochemistry of Perfluoroalkyl and Perfluoroacyl N-Chloramines. Reactions of N-Chloramines and N-Chlorimines in the Presence of Mercury

George H. Sprenger and Jean'ne M. Shreeve*

Contribution from the Department of Chemistry, University of Idaho, Moscow, Idaho 83843. Received October 15, 1973

Abstract: Photolysis of perfluoroalkyl(fluoroformyl) and bis(fluoroformyl) N-chloramines neat or with other similarly substituted chloramines resulted in the formation of a new family of hydrazines which includes CF_3-$(FCO)NN(FCO)CF_3$, $(FCO)_2NN(FCO)_2$, $(CF_3)_2NN(FCO)CF_3$, $(CF_3)_2NN(FCO)_2$, and $CF_3(FCO)NN(FCO)_2$. With $CF_3(FCO)NCl$, CO and SO_2 inserted into the nitrogen–chlorine bond to give $CF_3(FCO)NC(O)Cl$ and the unstable $CF_3(FCO)NSO_2Cl$. Bis(fluoroformyl), trifluoromethylfluoroformyl, and bis(trifluoromethyl) N-chloramines and hexafluoroisopropylidene(N-chloro)imine were treated with CF_3SCl and $CF_3C(O)Br$ in the presence of mercury to form $(FCO)_2NSCF_3$, $CF_3(FCO)NSCF_3$, $(CF_3)_2NSCF_3$, $(CF_3)_2C=NSCF_3$, and $(CF_3)_2C=NC(O)CF_3$ via the solid amido or imido mercuric chloride. Compounds which contain the fluoroformyl moiety tend to be less stable due to the ease of fluoride ion shift. Formation of chlorine pseudohalides when $(CF_3S)_2Hg$ and AgNCO were treated with the N-chloramines argues for the positive nature of the chlorine in these compounds.

luorinated N-chloramines have been obtained from proton abstraction reactions of fluorinated amines with ClF^1 or $Cl_2{}^{2-4}$ in the presence of alkali metal fluorides. More recently facile additions of ClF to fluoroalkylimines[1,5–7] and fluoroalkyl or acyl isocyanates[8] have resulted in a wide variety of N-chlor-

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