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Metal Ion-Biomolecule Interactions. Synthesis, Spectroscopic, and Magnetic Resonance Investigations of Methylmercury(II) Complexes of the Nucleosides Guanosine and Inosine

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Solid complexes of the type $[MeHg(NucH_2)]NO_3$, [MeHg(NucH)], and $[(MeHg)_2(NucH)]NO_3$ ($NucH_2$ = guanosine or inosine) have been prepared by the reaction of the nucleosides and $MeHgNO_3$ in aqueous solution at the appropriate pH and mole ratios of constituents. In addition, a 3:1 MeHg-inosine complex, $[(MeHg)_3(Ino)]NO_3$, involving C_8 bonding has been prepared under relatively mild conditions in aqueous solution. The complexes have been characterized by their ^1H and ^{13}C NMR spectra in $(CD_3)_2SO$ and also by IR spectroscopy. Possible implications for the mutagenic action of organomercurials, particularly $MeHg^{II}$, are noted.

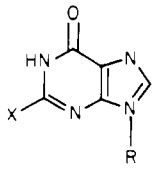
Introduction

In vivo investigations have proven the toxic nature of many heavy-metal ions, which has been thought to result from strong binding to S-donor atoms of proteins, with concomitant inhibitory enzymatic action.[2] However, consequences of "secondary" interactions involving nucleic acids are also manifest, in view of the known antineoplastic activity of certain cis-Pt^{II} complexes[3] and the chromosomal damage caused by some organomercurials,[4] both of which are most probably the results of direct interaction of the metal ions with the DNA constituents.

Nucleic acid constituents can be considered as naturally occurring ligands possessing a variety of metal ion binding sites. Not only do the ribose and ribophosphate groups of the nucleosides and nucleotides contain potential coordination sites but also the purine and pyrimidine bases possess several potential N- and O-donor atoms.[5]

In terms of the hard and soft acid and base (HSAB) principle, natural distinctions exist between the type of coordination encountered with hard donor atoms such as oxygen and that experienced with the softer nitrogen atoms. Soft heavy-metal ions such as Hg^{II}, Pt^{II}, and Ag^{I} are therefore expected to preferentially bind to the N atoms of the base moiety. As Klopman and co-workers point out,[6a] further diversity in binding charactristics arise because of the varying degrees of "hardness" or "softness" of individual base donor atoms. However, because of the possibility of delocalization of electrons in these systems, the difference between the N and O centers in these molecules may not be all that large, while steric factors may also be important.[6b] Such considerations make difficult the prediction of metal ion binding sites in all but a limited number of systems.

Except in the case of certain modified nucleosides that contain the very soft sulfur atom which provides the dominant site of interaction,[7,8] the mode of interaction of heavy-metal ions with the base portions of nucleic acids is generally not clearly predictable. In the present paper, the methylmercury(II) cation ($MeHg^{II}$) is used to probe the nature of the binding of heavy-metal ions to the base portions of guanosine (**1**: R = ribose, X = NH_2) and inosine (**1**: R = ribose, X = H). The soft characteristics of $MeHg^{II}$, its unifunc-



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tionality,[9] and its highly suitable NMR characteristics make it an ideal choice for such a study. The present study emphasizes the isolation of complexed species from aqueous solution under varying conditions of pH and metal ion:nucleoside mole ratio and their spectroscopic characterization.

During the early stages of this study, some aspects relating to the preparation of several complexes from the $GuoH_2$-RHg^{II} (R = Me and Ph) systems were reported.[10,11] While some of the results of the present study for the $GuoH_2$-$MeHg^{II}$ system confirm those results, additional novel C_8-bonded purine organomercurials have been obtained in this work, brief details of which have already been communicated.[12]

Experimental Section

^1H NMR spectra were measured in $(CD_3)_2SO$ on a Bruker HX-60 instrument operating at 60 MHz in the Fourier transform mode, using $(CH_3)_4Si$ (Me_4Si) as internal standard. ^{13}C NMR spectra were recorded at 15.09 MHz and were also referenced to Me_4Si. All spectra were recorded at room temperature (25 ± 2 °C). Infrared spectra were recorded as 1% KBr disks on the Perkin-Elmer 180 spectro-

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(9) Evidence has been presented for the existence of three-coordinate complexes of CH_3Hg^{II} in the solid state: A. J. Canty and B. M. Gatehouse, *J. Chem. Soc., Dalton Trans.*, 2018 (1976). However, there appears to be no definitive evidence for the existence of three-coordinate CH_3-Hg^{II} complexes in solution: A. J. Canty and A. Marker, *Inorg. Chem.*, **15**, 425 (1976).
(10) Abbreviations used for the nucleosides have been made essentially according to IUPAC rules as outlined in *Pure Appl. Chem.*, **40**, 279 (1974). For assistance in distinguishing between the various degrees of deprotonation of the nucleosides, neutral guanosine and inosine are designated as $GuoH_2$ and $InoH_2$, respectively, and deprotonation of N_1 leads to $GuoH^-$ and $InoH^-$, while further deprotonation of C_8, applicable to inosine, results in Ino^{2-}.
(11) A. J. Canty and R. S. Tobias, *Inorg. Chem.*, **18**, 413 (1979).
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photometer. Micranalyses were performed by Galbraith Laboratories Inc., the Guelph Chemical Laboratory, and the microanalytical section of the Chemistry Department of the University of Surrey.

Guanosine and inosine were obtained from the United States Biochemical Corp. Methylmercuric nitrate was prepared from methylmercuric chloride (Alfa) and silver nitrate, as described previously.[13] [8-^{2}H]Inosine was prepared by incubating a solution of inosine in D_2O for 24 h at 65 °C, removing the solvent by freeze-drying, and repeating the procedure once more. ^{1}H NMR was used to check the isotopic purity of the product (>98% deuteration at C_8).

Guanosine Complexes. 1. [MeHg(GuoH$_2$)]NO$_3$. A solution of methylmercuric nitrate (0.254 g, 0.92 mmol) in distilled water (0.5 mL) was added with stirring to a suspension of guanosine (0.259 g, 0.92 mmol) in water (1.0 mL). The guanosine dissolved to give a colorless solution, the pH of which was ca. 2–3. This was set aside to evaporate slowly. Within 1 day, small crystals started to form. Over a period of 1 month the crystals were collected by filtration, washed with water, and dried to constant weight in vacuo over silica gel. The yield was 0.445 g (86%). The IR spectrum showed 3340 (s), 3100 (s), 2930 (m), 2740 (m), 1685 (s), 1650 (m, sh), 1600 (m), 1575 (w), 1531 (m), 1480 (m), 1365 (s), 1226 (w), 1173 (m), 1120 (m), 1076 (m), 1043 (m), 1008 (w), 900 (m), and 775 (w–m) cm^{-1}. Anal. Calcd for $C_{11}H_{16}N_6O_8Hg$: C, 23.55; H, 2.85; N, 14.98. Found: C, 23.28; H, 3.11; N, 14.97.

2. [MeHg(GuoH)]·2H$_2$O. A solution containing methylmercuric nitrate (0.561 g, 2.02 mmol) in distilled water (3.0 mL) was added to a stirred solution of guanosine (0.572 g, 2.02 mmol) and tetramethylammonium hydroxide pentahydrate (0.366 g, 2.02 mmol) in distilled water (20 mL). Slow evaporation of the resultant slightly alkaline solution led to the formation of a syrupy residue, which was reduced to a solid in vacuo. The dry residue was stirred with ethanol (20 mL) for 10 min, during which time a thick white precipitate was produced. The precipitate was filtered off yielding a white solid (0.724 g, 72%), which was washed once with ethanol and once with water and dried in vacuo. Infrared absorptions: 3340 (s), 3290 (s), 2990 (m), 2690 (w–m), 1615 (s), 1575 (m), 1520 (w–m), 1487 (m), 1400 (m), 1380 (m), 1340 (m), 1307 (w, sh), 1227 (w), 1200 (w), 1175 (w), 1120 (s), 1076 (m), 1050 (m), 1020 (m), 984 (w), 948 (w), 900 (w), 860 (w), 779 (w) cm^{-1}. Anal. Calcd for $C_{11}H_{19}N_5O_7Hg$: C, 24.73; H, 3.56; N, 13.12. Found: C, 24.67; H, 3.35; N, 13.04.

3. [(MeHg)$_2$(GuoH)]NO$_3$. A solution of methylmercuric nitrate (0.486 g, 1.75 mmol) in water (1.0 mL) was added to a stirred suspension of guanosine (0.248 g, 0.875 mmol) in water (1.0 mL), and the pH was adjusted to ca. 3 with 1 M NaOH solution. A white precipitate was observed to form after a few hours, and this was collected by filtration after 2 weeks, washed quickly with cold water, and dried in vacuo (yield 0.377 g (57%)). Infrared absorptions: 3315 (s), 3190 (m), 3106 (m), 2910 (w), 1640 (s), 1593 (m), 1524 (w–m), 1498 (s), 1340 (s, br), 1170 (m), 1105 (m), 1073 (m), 1030 (m), 980 (w), 771 (w) cm^{-1}. Anal. Calcd for $C_{12}H_{18}N_6O_8Hg_2$: C, 18.58; H, 2.32; N, 10.84. Found: C, 18.46; H, 2.28; N, 10.53.

Inosine Complexes. 4. [MeHg(InoH$_2$)]NO$_3$. A solution of methylmercuric nitrate (0.155 g, 0.558 mmol) in water (1.0 mL) was added with stirring to an aqueous solution (2.0 mL) of inosine (0.150 g, 0.558 mmol). The resultant solution was set aside for 2 days, whereupon a syrupy residue was obtained. Complete solvent removal was effected in vacuo. The residue was then stirred with absolute ethanol (20 mL) for 2 h. The insoluble product was filtered off, washed with ethanol, and dried in vacuo to yield a white solid (0.186 g (61%)). Infrared absorptions: 3300 (s, br), 3110 (m), 3052 (m), 2910 (m), 2800 (m, br), 1765 (w), 1709 (s), 1597 (w), 1560 (m), 1515 (m), 1484 (w), 1440 (m), 1378 (s, br), 1343 (m), 1325 (m), 1282 (w–m), 1229 (m), 1188 (w–m), 1140 (m, br), 1114 (w), 1073 (m), 1045 (w), 980 (m), 949 (m), 896 (m), 865 (w), 843 (w), 819 (w), 783 (m), 740 (w), 690 (w–m, br), 623 (m), 569 (w), 550 (w) cm^{-1}. Anal. Calcd for $C_{11}H_{15}N_5O_8Hg$: C, 24.19; H, 2.75; N, 12.83. Found: C, 24.26; H, 2.48; N, 12.81.

5. [MeHg(InoH)]·H$_2$O. A solution of methylmercuric nitrate (0.486 g, 1.75 mmol) in distilled water (5.0 mL) was added to a stirred solution of inosine (0.469 g, 1.75 mmol) and tetramethylammonium hydroxide pentahydrate (0.317 g, 1.75 mmol) in water (10 mL). Slow evaporation of the neutral solution resulted in the formation of a syrupy residue which was subsequently reduced to a solid in vacuo. The dry residue was stirred with absolute ethanol (20 mL) for 10 min, during which time a thick white precipitate was obtained. The precipitate was filtered off, washed with further ethanol and a small quantity of water, and finally dried in vacuo to yield a white solid (0.688 g (82%)). Infrared absorptions: 3350 (s, br), 3100 (s, sh), 2905 (w m), 1635 (s), 1556 (vw), 1526 (m), 1482 (m), 1422 (w–m), 1370 (m), 1330 (m), 1312 (m), 1284 (m), 1218 (m), 1125 (m, br), 1077 (m), 1050 (m, br), 980 (w), 944 (w), 895 (w), 860 (w), 790 (w–m), 666 (w), 642 (w–m) cm^{-1}. Anal. Calcd for $C_{11}H_{16}N_4O_6Hg$: C, 26.36; H, 3.20; N, 11.19. Found: C, 26.69; H, 3.31; N, 11.58.

6. [(MeHg)$_2$(InoH)]NO$_3$·H$_2$O. Methylmercuric nitrate (0.305 g, 1.10 mmol) in distilled water (1.0 mL) was added to a stirred solution of inosine (0.147 g, 0.55 mmol) in water (2.0 mL) and the pH of the resultant solution adjusted to ca. 3 with 1 M NaOH solution. The white solid remaining after the bulk of the solvent had evaporated (after 2 days) was filtered off, quickly washed with cold water, and dried in vacuo. The yield was 0.109 g (26%). Infrared absorptions: 3350 (s, br), 3100 (m, sh), 2995 (w), 2910 (w–m), 1646 (s), 1572 (w), 1572 (m), 1484 (m), 1431 (m, sh), 1335 (s, br), 1210 (w–m), 1140 (m), 1113 (m), 1075 (m), 1040 (m), 974 (w), 890 (w), 858 (w), 815 (w, sh), 780 (m), 621 (w–m) cm^{-1}. Anal. Calcd for $C_{12}H_{19}N_5O_9Hg_2$: C, 18.50; H, 2.44; N, 9.00. Found: C, 18.36; H, 2.10; N, 8.61.

7. [(MeHg)$_3$(Ino)]NO$_3$. (a) A solution of methylmercuric nitrate (1.07 g, 3.85 mmol) in distilled water (1.5 mL) was added to a stirred solution of inosine (0.344 g, 1.2, mmol) in water (1.0 mL). The pH of the resultant solution was increased to 7 with 1 M NaOH, and the solution was heated at 50 °C for 30 min. After the solution was allowed to stand, a white solid precipitated, and this was collected by filtration after 2 days, washed with water, and dried in vacuo. The yield was 0.885 g (71%). Infrared absorptions: 3360 (s, br), 3190 (s, sh), 2915 (w–m), 1644 (s), 1574 (w, sh), 1512 (s), 1445 (w, sh), 1370 (s), 1297 (s), 1162 (w), 1116 (m), 1071 (s), 1043 (m–s), 979 (w), 895 (w), 865 (w), 783 (m), 545 (w, br) cm^{-1}. Anal. Calcd for $C_{13}H_{19}N_5O_8Hg_3$: C, 16.00; H, 1.95; N, 7.18; Hg, 61.74. Found: C, 16.16; H, 1.96; N, 7.02; Hg, 61.67.

(b) A reaction essentially identical with that described in section 7a was performed by using [8-^{2}H]inosine as the substrate (0.149 g, 0.546 mmol) along with methylmercuric nitrate (0.452 g, 1.63 mmol) in D_2O/OD$^-$ solution. The product (0.366 g (67%)) exhibited a ^{1}H NMR spectrum essentially identical with that given by the product in section 7a[12] (differences only resulting from deuteration of the ribose hydroxyl groups were evident).

Results and Discussion

Complex Formation. The early work of Simpson[14] using UV difference spectroscopy assigned the pH dependence of the spectra to the formation of several complexed species in the Guo– and Ino–MeHg^{II} and other systems. More recently, Tobias and his co-workers used Simpson's formation constants to construct species distribution curves for the same Guo[15] and Ino[16] systems. For equimolar (50 mM) mixtures of $MeHgClO_4$ and nucleoside (i.e., $r = 1$)[17] the concentration of the species [MeHg(NucH$_2$)]$^+$ reached a maxium at pH ca. 2, and N_7 binding was suggested since N_1 is protonated at this pH ($pK_a \approx 9$[18]). The corresponding species formed by displacement of the N_1 proton, [MeHg(NucH)], predominates at pH 8–9, since the pK_a values for N_1 deprotonation lie in this region.[18] In the high-pH region, complex formation involving the Guo and Ino conjugate bases would be expected to be very favorable, and this is indeed reflected in the magnitude of the relevant formation constants.[14]

It seemed to us that the information derived from the above studies could be utilized toward isolation of specific complexes which should be present in solution under the different conditions. It has been found in fact that, by adjustment of the conditions (i.e, initial pH and r values) under which the for-

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(17) r is defined as the ratio [total MeHg^{II}]/[total nucleoside].
(18) J. J. Christensen, J. H. Rytting, and R. M. Izatt, *Biochemistry*, **9**, 4907 (1970).

Table I. ^{1}H NMR Chemical Shifts and Coupling Constants for the $MeHg^{II}$–Guanosine Complexes[a]

compd	δ[b,c] N_1H	NH_2	H_8	$H_{1'}$	N–HgMe	$^2J(^{199}Hg-^1H)$, Hz
$GuoH_2$	10.67	6.48	7.95	5.71 (d)		
$[MeHg(GuoH_2)]NO_3$	11.43 (11.45)	6.92 (7.02)	8.63 (8.69)	5.86 (d) (5.90 (d))	0.86 (0.89)	228.0 (229)
[MeHg(GuoH)]	...	6.49 (6.38)	7.92 (7.85)	5.70 (d) (5.72 (d))	0.76 (0.76)	206.2 (206.5)
$[(MeHg)_2(GuoH)]NO_3$	...	7.02 (6.97)	8.60 (8.60)	5.87 (d) (5.88 (d))	0.83 (0.85)	218.7 (221)

[a] Data in parentheses are taken from ref 11. [b] In $(CD_3)_2SO$; chemical shifts are measured from $(CH_3)_4Si$ internal standard at 60 MHz. The absence of a signal due to complex formation is indicated (. . .). [c] All resonances are singlets unless otherwise indicated: d = doublet.

mation of a particular species would be anticipated, isolation of various solid $MeHg^{II}$–nucleoside complexes could be realized.

It has thus been possible to isolate the two 1:1 $MeHg^{II}$ complexes of Guo and Ino derived from coordination at N_1 and N_7. Additionally, for Guo at pH 7 and $r = 2$ the product $[(MeHg)_2(GuoH)]NO_3$ was isolated as found also by Canty and Tobias.[11] Moreover, the pH 3, $r = 2$ system has been observed to yield the same product. The reaction at low pH probably occurs by initial formation of the N_7-bound MeHg complex (eq 1); the presence of the electrophile coordinated

$$MeHg^+ + NucH_2 \rightarrow [MeHg(NucH_2)]^+ \quad (1)$$

to N_7 increases the acidity of the N_1 proton (vide infra), facilitating displacement of the proton by a second $MeHg^{II}$ cation (eq 2). This is different from the process occurring

$$[MeHg(NucH_2)]^+ + MeHg^+ \rightarrow [(MeHg)_2(NucH)]^+ + H^+ \quad (2)$$

at high pH, in which initial reaction involving N_1 occurs with MeHgOH (eq 3). Subsequent reaction with further

$$MeHgOH + NucH_2 \rightarrow [MeHg(NucH)] + H_2O \quad (3)$$

MeHgOH leads to formation of the 2:1 complex.

In a ^{1}H NMR study of the reaction between Ino and $MeHg^{II}$ in D_2O (pD 8), Mansy and Tobias observed the rapid disappearance of the H_8 resonance for reaction solutions with $r \geq 2$, due to exchange with solvent deuterium.[16,19] The increased exchange rate with respect to inosine was attributed to $MeHg^{II}$ coordination at N_7, after the initial substitution of the N_1 proton by $MeHg^{II}$, hence the need for an $r \approx 2$ system. The increased positive charge thereby created at the N_7 site facilitates hydroxide ion abstraction of the C_8 proton, leading to exchange via the well-established pathway.[20] Further examples of the activation of purine nucleoside C_8–H bonds resulting from metal ion coordination to N_7 have been identified and quantified.[21]

Thus in our initial investigation of the Ino pH 7, $r = 2$ system, we observed the formation of a 3:1 adduct, in low yield, which was identified as the complex **6**. This could be explained as a result of the enhanced acidity of the C_8 proton on metal ion coordination at N_7, as shown in Scheme I. The observation that **6** was formed in the $r = 2$ system was, however, a fortuitous result of the relatively low solubility of this particular complex, and this has warranted an extended study under other conditions, including when $r = 3$.

Thus, from Ino, the complexes (inosine-N_7)methylmercury(II) nitrate and (inosine-N_1)methylmercury(II) have been isolated from $r = 1$ mixtures of Ino and $MeHgNO_3$ at low and high pH, respectively. (Inosine-N_1,N_7)bis(methylmercury(II)) nitrate resulted from $r = 2$ mixtures of Ino and $MeHgNO_3$ at either pH 3 or 7. Heating an $r = 3$ aqueous

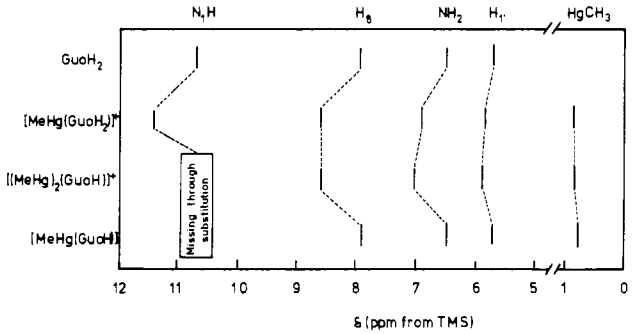

Figure 1. ^{1}H NMR spectral chart for the methylmercury(II)–guanosine complexes.

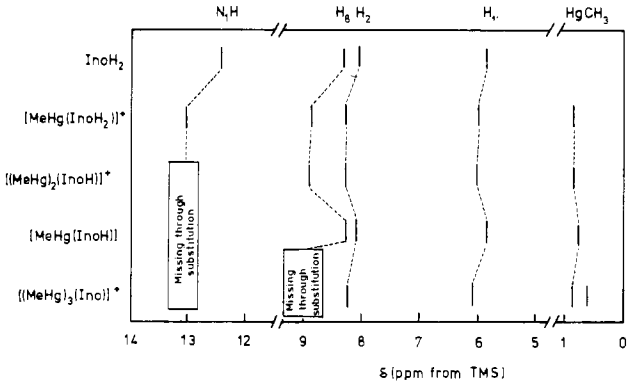

Figure 2. ^{1}H NMR spectral chart for the methylmercury(II)–inosine complexes.

mixture of Ino and $MeHgNO_3$ at pH 7 resulted in the formation of (inosine-N_1,N_7,C_8)tris(methylmercury(II)) nitrate. The corresponding Guo complexes, excluding the latter 3:1 adduct, have also been isolated under the same conditions as described for the Ino complexes.

Although this is the first reported isolation of a purine nucleoside–organomercurial complex involving C_8 binding, it should be pointed out that is is not the first observation of direct Hg^{II}–C_8 bonding in purines. Beck and Kottmair,[22] following an earlier related study by Schönherr and Wanzlick,[23] succeeded in isolating a bis(1,3,7,9-tetramethylxanthine)–mercury(II) adduct, in which the metal ion bridged two xanthine molecules via the C_8 positions, from the reaction between the xanthine derivative and mercuric acetate in $(CH_3)_2SO$. The reaction plausibly proceeds by a mechanism analogous to that proposed in Scheme I to account for formation of the $[(MeHg)_3(Ino)]NO_3$ complex.

Nature of the Complexes. ^{1}H NMR, ^{13}C NMR, and infrared spectroscopy have been used to characterize the complexes. Not only do the ^{1}H NMR spectra yield information relating to the ligand but also effects of complexation on the metal ion can be monitored via (i) the chemical shift of the methyl protons and (ii) the two-bond coupling constant be-

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(21) J. R. Jones and S. E. Taylor, *J. Chem. Soc., Perkin Trans. 2*, in press.
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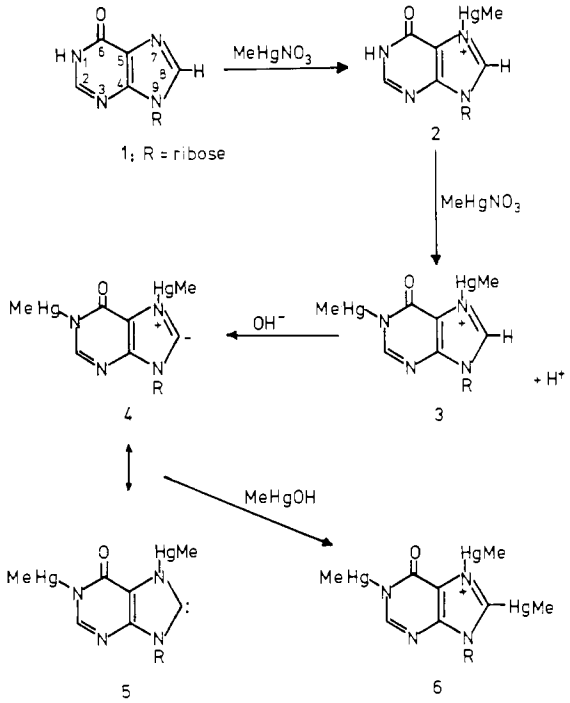
Table II. ^{1}H NMR Chemical Shifts and Coupling Constants for the $MeHg^{II}$–Inosine Complexes

compd	δ [a, b] N_1H	H_8	H_2	$H_{1'}$	N-HgMe	C-HgMe	$^2J(^{199}Hg-^1H)$, Hz N bound	C bound
$InoH_2$	12.47[c]	8.33	8.10	5.86 (d)				
$[MeHg(InoH_2)]NO_3$	13.08[c]	8.92	8.34	6.05 (d)	0.88		233.3	
[MeHg(InoH)]	...	8.24	8.09	5.84 (d)	0.78 (0.78)[d]		207.5 (211)[d]	
$[(MeHg)_2(InoH)]NO_3$	...	8.93	8.32	6.05 (d)	0.85		221.2	
$[(MeHg)_3(Ino)]NO_3$	...	...	8.24	6.09 (d)	0.85	0.62	215.8	159.5

[a] In $(CD_3)_2SO$; chemical shifts are measured from $(CH_3)_4Si$ internal standard at 60 MHz. The absence of a signal due to complex formation is indicated (. . .). [b] All resonances are singlets unless otherwise indicated: d = doublet. [c] Very broad. [d] See ref 16 for D_2O solution.

Scheme I



tween the ^{199}Hg isotope ($I = {}^1/_2$, isotopic abundance 16.9%) and the methyl protons, $^2J(^{199}Hg-^1H)$. The latter parameter is inherently more sensitive to the strength of the metal–ligand bond, as is reflected in correlations between the coupling constant and the logarithm of the stability constant of the metal–ligand complex.[2c] The results of Simpson's study[14] reflect the different stability constants of N_1- and N_7-bound $MeHg^{II}$ in Guo and Ino, and on this basis one would expect this to manifest itself in the magnitude of the observed $^2J(^{199}Hg-^1H)$ values. Tables I and II summarize the ^{1}H NMR data for these complexes in $(CD_3)_2SO$, and spectral comparisons are shown in Figures 1 and 2. The absence of a ligand proton resonance (N_1–H and H_8 for Ino, N_1–H for Guo) is attributable to $MeHg^{II}$ binding at that site, except for N_7 binding for which the large downfield shifts (ca. 0.7 ppm) of the H_8 resonances implicate the N_7 position. In addition, large downfield shifts of the N_1–H resonances of Guo and Ino and the NH_2 resonance of Guo in the N_7-bonded complexes reflect the decrease in electron density in the pyrimidine ring accompanying the introduction of a positive charge into the imidazole ring. Such effects due to metallation or alkylation have been observed previously.[24,25] Apart from the removal of the H_1 resonance, $MeHg^{II}$ complexation to N_1 has no significant effect on the ^{1}H NMR spectra of Guo and Ino.

For the Guo system, retention of the exocyclic amino protons is observed in all cases except for mixtures with $r > 2$, in which

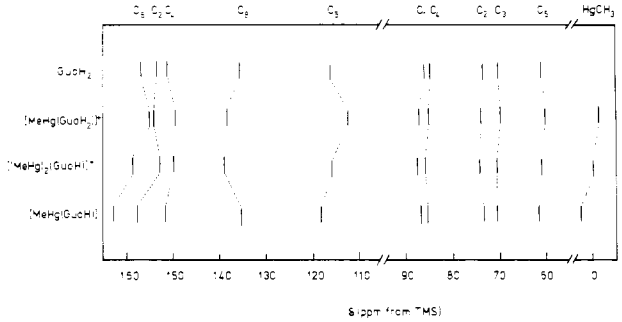


Figure 3. ^{13}C NMR spectral chart for the methylmercury(II)–guanosine complexes.

simultaneous C_8 and amino substitution occurs.[26] In the corresponding Ino ($r = 3$) system no such complication arises, and complete C_8 substitution results, as indicated by the ^{1}H NMR spectrum of the product.[12] That C_8 is the third binding site is based on two observations. First, only one resonance (δ 8.24) is observed at low field, very close to the value assigned to H_2 (δ 8.32) in the 2:1 complex (Table II). Moreover, it has been found that this is the only low-field signal appearing in a complex prepared under the same experimental conditions when [8-^{2}H]Ino was used as the substrate, indicating that the resonance is not due to H_8.[27] Second, at high field, two separate $MeHg^{II}$ methyl resonances are observed (Table II). This is suggestive of two very different types of coordination behavior, one of which involves rapidly exchanging (presumably) N-bound $MeHg^{II}$ (assignable to δ 0.85; 6 H) and the other involving more strongly bound C-bonded $MeHg^{II}$ (δ 0.62; 3 H). The strength of binding is reflected in the magnitude of the coupling constants associated with each signal. $^2J(^{199}Hg-^1H)$ for the proposed N- and C-bound $MeHg^{II}$ are 215.8 and 159.5 (±0.5) Hz, respectively. In contrast, the positions of the respective methyl resonances are only marginally affected by the site of complexation (Table II). The lower values of the coupling constants associated with N_1-bound $MeHg^{II}$ systems are in accord with the known greater stability of these complexes[14] and fit very well with Rabenstein's least-squares regression equation[2c] (eq 4) found for

$$J = -5.09 \log K + 249 \quad (4)$$

ligands of varying donor atom, where K is the formation constant for binding at a given site. Moreover, the coupling constants for the 2:1 complexes are intermediate between the values observed for the individual 1:1 complexes. This reiterates the idea that the observed $MeHg^{II}$ signal is an average, due to rapid exchange (on the NMR time scale at this temperature) of N_1- and N_7-coordinated $MeHg^{II}$ and that in $(CD_3)_2SO$ solution no $MeHg^{II}$ exchange with the solvent occurs, since $^2J(^{199}Hg-^1H)$ for $MeHg[(CD_3)_2SO]^+$ is 260.6 Hz.[28]

(24) M. J. Clark and H. Taube, *J. Am. Chem. Soc.*, **96**, 5413 (1974).
(25) M. Nakanishi, M. Tsuboi, and I. Nakigawa, *Bull. Chem. Soc. Jpn.*, **49**, 2011 (1976).
(26) S. E. Taylor, unpublished observations.
(27) This experiment was performed following a suggestion by a reviewer of our preliminary communication.[12]
(28) A. J. Brown, O. W. Howarth, and P. Moore, *J. Chem. Soc., Dalton Trans.*, 1589 (1976).

Table III. ^{13}C NMR Chemical Shifts for the $MeHg^{II}$–Nucleoside Complexes

	δ^a											
	base carbon atoms					ribose carbon atoms					methylmercury(II) carbon atoms	
compd	C_6	C_2	C_4	C_8	C_5	$C_{1'}$	$C_{4'}$	$C_{2'}$	$C_{3'}$	$C_{5'}$	$N_{1,7}$–$HgCH_3$	C_8–$HgCH_3$
$GuoH_2$	156.7	153.4	151.2	135.7	116.4	86.2	85.1	73.5	70.3	61.2		
$[MeHg(GuoH_2)]NO_3$	155.3	154.7	149.9	138.8	(113.2)[b]	87.8	85.9	74.5	70.3	60.9	−0.65	
[MeHg(GuoH)]	162.3	157.3	151.2	135.2	118.0	86.4	85.1	73.2	70.4	61.4	2.33	
$[(MeHg)_2(GuoH)]NO_3$	158.5	152.7	149.8	138.8	114.6	87.6	85.8	74.1	70.3	60.8	−0.13	
$InoH_2$	158.0	149.3	147.0	140.2	125.2	88.8	86.8	75.3	71.5	62.4		
$[MeHg(InoH_2)]NO_3$	(155.2)[b]	(147.5)[b]	146.8	141.4	121.8	88.8	86.0	74.5	69.9	60.7	−0.71	
[MeHg(InoH)]	161.4	151.9	147.9	138.2	125.4	87.5	85.5	73.7	70.3	61.4	0.97	
$[(MeHg)_2(InoH)]NO_3$	159.4	154.3	146.6	141.2	(122.4)[b]	88.0	86.0	74.3	70.0	60.9	−0.13	
$[(MeHg)_3(Ino)]NO_3$	159.2	152.6	147.4	201.0	124.3	90.7	86.4	73.2	70.1	61.4	−0.19	5.95

[a] In $(CD_3)_2SO$; chemical shifts measured from $(CH_3)_4Si$ internal standard at 15.09 MHz. [b] Tentative assignments.

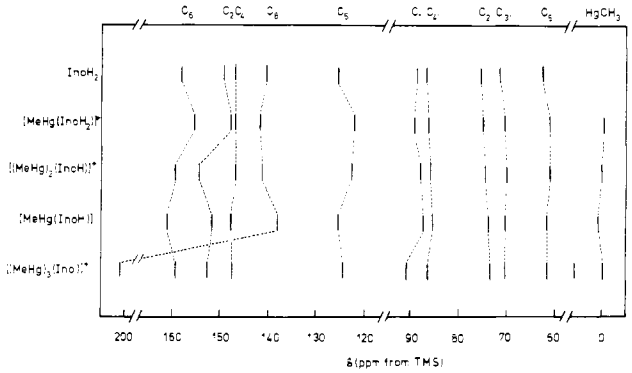


Figure 4. ^{13}C NMR spectral chart for the methylmercury(II)–inosine complexes.

Interestingly, in the 3:1 Ino complex, the observed coupling constant for the N-bound $MeHg^{II}$ is 5.4 Hz lower than for the 2:1 complex.

To complement the ^1H NMR data, ^{13}C NMR spectra have been obtained for the various complexes.[29] These will also serve to evaluate the usefulness of the latter technique for assigning the binding sites for methylmercury(II). The relevant data, together with the chemical shifts for the parent nucleosides are given in Table III. Spectral comparisons are made in Figures 3 and 4. The absence of binding to the ribose moiety follows from the observation that the chemical shifts of the sugar carbon atoms are only slightly influenced by complex formation. Complexation at N_7 is accompanied by reasonably small (ca. 3 ppm) downfield shifts of the C_8 resonances, the remaining signals being relatively unaffected. Complex formation at N_1, on the other hand, leads to more pronounced effects on the carbon atoms adjacent to the site of complexation. Thus, the C_2 and C_6 resonances experience downfield shifts of 3.9 and 5.6 ppm in Guo and 2.6 and 3.4 ppm in Ino. The remaining signals are relatively unaffected. As was found for the proton spectra, the ^{13}C spectra of the 2:1 complexes show intermediacy between the two monocomplexed situations. In the 3:1 Ino complex, in which C_8 binding was previously identified from ^1H NMR evidence, a 61-ppm downfield shift of the C_8 resonance further implicates this as the third binding site; all other resonances for this complex are essentially the same as in the 2:1 complex. The position of the $MeHg^{II}$ carbon resonance is seen not to vary greatly with the site of complexation, although the general trend is to parallel the $^2J(^{199}Hg-^1H)$ coupling constants, as demonstrated in Figure 5. Thus, the ^{13}C chemical shifts are inherently more sensitive to the environment of the metal ion than are the proton chemical shifts, for which much smaller effects of complexation are observed (Tables I and II).

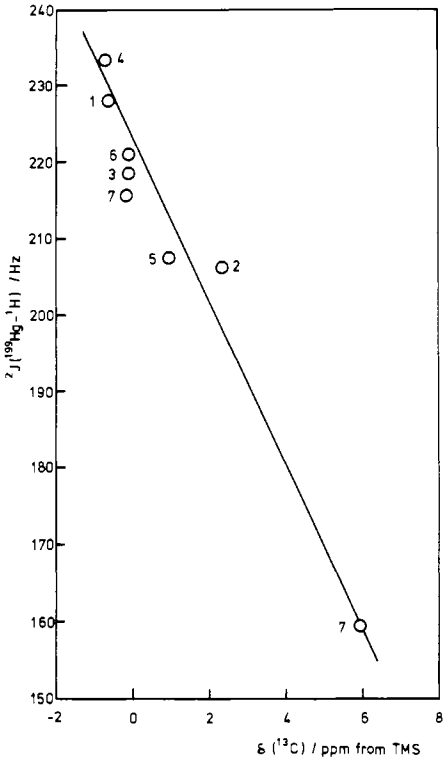


Figure 5. Correlation between $^2J(^{199}Hg-^1H)$ and the ^{13}C chemical shift of the methylmercury(II) carbon atom for the $MeHg^{II}$–nucleoside complexes. Numbers refer to the complexes as given in the Experimental Section.

Infrared spectra in the carbonyl stretching region of the nucleoside complexes are shown in Figures 6 and 7 for the Guo and Ino systems, respectively.[31] On complexation, the spectra of the nucleosides throughout the range 4000–400 cm^{-1} are greatly simplified, but specifically, changes in the region 1800–1600 cm^{-1} reflect effects of complexation on the C_6=O bond. In addition, the complexes which contain nitrate as the counterion exhibit intense absorptions in the region of 1350 cm^{-1}. In comparing the spectral changes where two possible binding sites exist, the greatest effects on $\nu(C_6{=}O)$ are observed for N_1-bonded $MeHg^{II}$, for which $\Delta\nu(C_6{=}O)$ values of −110 and −45 cm^{-1} are found for Guo and Ino, respectively. This is presumably due to the close proximity of the N_1 com-

(29) To our knowledge, only one other ^{13}C NMR study involving mercury(II)- or organomercury(II)–nucleoside complexation has been reported.[30]

(30) K. W. Jennette, S. J. Lippard, and D. A. Ucko, *Biochim. Biophys. Acta*, **402**, 403 (1975).

(31) Spectra are available as supplementary material.

plexation site to the carbonyl group. The shift to lower wavenumber in the 2:1 Guo and Ino complexes is intermediate between the values for the individual 1:1 adducts. Little change in $\nu(C_6{=}O)$ between the 2:1 and 3:1 $MeHg^{II}$–Ino derivatives is observed, supporting the idea that the third binding site in the latter case is removed from the carbonyl group.

Concluding Remarks. It has frequently been the case in studies of metal ion complexation by nucleic acid constituents that single site specificity is not encountered and chelate formation results. Invariably, the effect of coordination on any given site is thus comprised of more than one contributing factor, and as a result the spectral properties, for example, reflect the *net* effect of chelation. Nevertheless, many examples of the successful identification of binding sites from spectroscopic considerations can be found in the literature (see for example the recent review by Marzilli[32]). However, in the present study, the use of the unidentate $MeHg^{II}$ cation has made it possible to probe *individual* binding sites in the purine nucleosides Guo and Ino.

It has been found possible to isolate the solid complexes which were previously predicted as existing in aqueous solution,[14-16] by selecting appropriate conditions under which complex formation was attempted. ^1H NMR, ^{13}C NMR, and IR spectroscopy have confirmed the identity of the complexes as being as suggested by Simpson.[14] However, in addition, activation of the C_8–H bond by prior $MeHg^{II}$ coordination to N_7 has led to the identification of a novel C-bonded $MeHg^{II}$–nucleoside derivative.

Each of the above-mentioned techniques has fulfilled a role in assigning and confirming the $MeHg^{II}$ binding site(s). Most valuable has been ^1H NMR through which effects of coordination on both the purine and the metal ion have been monitored, as well as confirming the stoichiometry of the complexes. Since it is not always possible to identify individual proton resonances (e.g., if overlapping or solvent-obscured regions exist in the spectrum), ^{13}C NMR can usefully supplement the proton data. In this way, binding to the ribose portion of the nucleoside has been discounted in the present work; thus N_1 and N_7 coordinations lead to specific shifts in the C_6/C_2 and C_8 resonances, respectively. From the effect on the $C_6{=}O$ stretching frequency, further confirmation of N_1 binding has been demonstrated, as has the presence in some of the complexes of the NO_3^- counterion.

In terms of the interaction of $MeHg^{II}$ with the guanine and hypoxanthine residues of nucleic acids at the molecular level, even though the strongest coordination site is believed to be N_1, since this site is already protonated at physiological pH, it is likely that N_7 will be the preferred position for binding. As is apparent from the ^1H NMR spectra of the complexes $[MeHg(GuoH_2)]NO_3$ and $[MeHg(InoH_2)]NO_3$, N_7 coordination has the effect of weakening the N_1–H bond, thereby either (i) facilitating deprotonation of N_1, so allowing reaction with further electrophiles at N_1, as in the case of the formation of the 2:1 complexes in the present work, or (ii) relaxing the hydrogen-bonding tendency of N_1–H, perhaps leading to a disruption of base-pairing capabilities of Guo and Ino. Furthermore, following N_7 coordination, C_8–H bond activation could lead to subsequent reactions at C_8, implying that the mutagenic nature of organomercurials (and heavy-metal ions in general) may be more far reaching than was at first envisaged.

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Registry No. $[MeHg(GuoH)]NO_3$, 68629-63-0; MeHg(GuoH), 68630-40-0; $[(MeHg)_2(GuoH)]NO_3$, 68629-65-2; $[MeHg(InoH_2)]NO_3$, 75311-39-6; MeHg(InoH), 75311-47-6; $[(MeHg)_2(InoH)]NO_3$, 75332-09-1; $[(MeHg)_3(Ino)]NO_3$, 72951-44-1; $GuoH_2$, 118-00-3; $InoH_2$, 58-63-9; $MeHgNO_3$, 2374-27-8.

Supplementary Material Available: Figures 6 and 7, infrared spectral data (1800–1400 cm^{-1}) for the methylmercury(II)–guanosine and methylmercury(II)–inosine complexes, respectively (2 pages). Ordering information is given on any current masthead page.

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Copper Complexes of the "Tripod" Ligand Tris(2-benzimidazolylmethyl)amine: Five- and Six-Coordinate Copper(II) Derivatives and Some Copper(I) Derivatives

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A series of five-coordinate trigonal-bipyramidal and six-coordinate pseudooctahedral copper(II) complexes of the title ligand are reported. These systems are characterized by analytical data, IR, far-IR, ESR, and electronic spectra and magnetic susceptibility measurements. Although six-coordinate structures may pertain in some cases, in the solid state, all the compounds appear to have trigonal-bipyramidal structures in solution in methanol, where characteristic ESR spectra are observed, with $g_{\|} < g_{\perp}$ and low values of $|A_{\|}|$. Cyclic voltammetry and rotating platinum electrode dc polarography indicate the accessibility of stable copper(I) species in solution, which may have five-coordinate structures. Carbonyl adducts of these copper(I) complexes are formed in both MeCN (K = 10 atm^{-1}) and DMF (K = 30 atm^{-1}). Solid copper(I) complexes can be obtained under reducing conditions from the corresponding copper(II) compounds and also by reacting the ligand with copper(I) salts.

Introduction

Histidine imidazole plays a key role in the coordination of metals at the active sites of numerous proteins. Its prevalence (but not ubiquity[2]) as a donor in copper proteins is evidenced by resonance Raman,[3] electron spin resonance,[4,5] and spin–echo[6] results, as well as by the crystallographic models for

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