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INTERACTION OF MERCURIC CHLORIDE (MERCURY II) AND ACETATE WITH PURINE AND PYRIMIDINE NUCLEOSIDES

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The interaction of mercuric chloride with nucleosides guanosine, inosine, cytidine and uridine give complexes where the nitrogen of an excocyclic NH_2 group or ring nitrogen takes part in coordination to Hg(II). In the reaction of mercuric acetate with guanosine and inosine, chelates involving the coordination of Hg(II) with C_6O^- and N_7 are formed. In the case of cytidine and uridine, however, mercuric acetate forms an organometallic compound with a η^1 bond to the C_5 carbon atom of the nucleosides. All the complexes were characterised by elemental analysis IR, ^1H nmr and ^{13}C nmr.

Key Words : $HgCl_2$; $Hg(OAC)_2$; Guanosine; Inosine; Cytidine; Uridine

INTRODUCTION

MERCURY is a hazardous environmental pollutant. Most of the toxicity of mercury in river water and lakes is due to the presence of CH_3Hg which has a greater mutagenic effect and is more toxic than covalent mercury compounds.[1,2] The interaction of Hg(II) compounds with nucleic acid and their constituents have gained much importance after the report by Katz[3] that $HgCl_2$ changes the properties of DNA solution. Mercury is unique in its form complexes with natural DNA at room temperature whereas other metal ions like Cu(II) require heating for base binding.[4] The interaction of Hg(II) with nucleic acid bases and the nucleosides and nucleotides have been studied by a number of investigators[5–10] where the coordination of Hg(II) with the N atom of the heterocyclic bases were reported.

In this paper, the interaction of $HgCl_2$ and $Hg(OAC)_2$ with the purine nucleosides, guanosine and inosine and the pyrimidine nucleosides, cytidine and uridine are reported. The complexes were characterised on the basis of elemental analysis, IR, proton and ^{13}C nmr spectroscopy.

EXPERIMENTAL

The nucleosides were purchased from Sigma Chemicals, USA. Mercuric chloride and mercuric acetate were obtained from BDH Chemicals. Microanalyses were performed at CSIRO (Australia) and CDRI (Lucknow). The conductivity

data were obtained in DMSO using a Digisun Conductivity Bridge. The IR spectra were recorded on a Nicholet Model SX-200 spectrophotometer. The proton and ^{13}C spectra were recorded on a Jeol FX-100 spectrophotometer at CSMCRI, Bhavnagar, Gujarat and a Bruker 257MHz spectrophotometer at I.I.Sc., Bangalore. The elemental analyses of the complexes confirm the formulations *1–8.* complexes.

Preparation of the complexes

1. *Dichloroguanosinemercury(II)*—Guanosine 0.32g (1mM) was dissolved in hot dilute HCl and to this a solution of mercuric chloride (0.27g 1mM) dissolved in 5ml of H_2O was added. The *p*H of the solution was adjusted to 3. It was refluxed for 2 hours on a water bath and was allowed to cool at 0° overnight when a white precipitate was obtained. The precipitate was filtered, washed with ice cold water and dried. Yield : 70 per cent.

2. *Bis(inosine)dichloro-μ-dichlorodimercury(II)*—Inosine (0.28g 1mM) was dissolved in 15ml of H_2O and to this a solution of $HgCl_2$ (0.27g 1mM) dissolved in 20ml of H_2O was added. The solution was acidified with 1N HCl to *p*H 3 and was refluxed for five hours at 120°. On cooling the solution overnight to 0° a white precipitate was obtained which was filtered washed with cold H_2O and dried. Yield : 75 per cent.

3. *Bis(cytidine)dichloro-μ-dichlorodimercury(II)*—Cytidine (0.24g 1mM) was dissolved in 5ml of H_2O and to this $HgCl_2$ (0.27g 1mM) dissolved in 5ml of H_2O was added. The solution was acidified with 1N HCl to *p*H 4.5 and was refluxed for 4 hours. On cooling the solution overnight a white precipitate was obtained which was washed with acetone and dried. Yield : 65 per cent.

4. *Bis(uridine) dichloro-μ-dichlorodimercury(II)*

Uridine (0.24g 1mM) was dissolved in 5ml of H_2O and acidified with 0.2 N HCl to *p*H 4. Mercuric chloride (0.27g 1mM) was dissolved in 5ml of H_2O and acidified to *p*H 3. The two solutions were mixed, the *p*H adjusted to 3 and the solution refluxed for 4 hours. The solution was concentrated to 1/3 of its volume and kept overnight at 0 °C. The white precipitate obtained was filtered, washed with acetone-ether and dried. Yield : 70 per cent

5. *Guanosinatoacetoxymereury(II)*

6. *Inosinatoacetoxymercury(II)*

Guanosine (0.32g 1mM) or inosine (0.27g 1mM) was dissolved in 10 ml of water and to this a solution of mercuric acetate (0.32g 1mM) in glacial acetic acid was added. The *p*H of the solution was brought to *p*H 2.5 for guanosine and *p*H 4.5 for inosine and the solution heated on a water bath for 4 hours. The solution was concentrated to half its volume, kept overnight at 0 °C and precipitated with acetone. The precipitate was filtered, washed with water and acetone and dried. Yield : 70 per cent.

7. *n^1-Cytidineacetoxymercury(II)*

Cytidine (0.244g 1mM) was dissolved in 5ml of H_2O and to this mercuric acetate (0.319g 1mM) in water glacial acetic was added. The *p*H of the solutian was made 4.5. The solution was refluxed for one hour at 130 °C when a white precipitate was obtained. It was kept overnight at 0 °C, filtered, washed with water and methanol and dried.

8. *n^1-Uridinatoacetoxymercury(II)*

Uridine (0.244g 1mM) was dissolved in 5ml of H_2O and to this mercuric acetate (0.319g 1mM) in water glacial acetic acid was added. The *p*H of the solution was made to 5.5–6.0. It was refluxed for 4 hours at 140 °C when a white precipitate was obtained. It was kept overnight at 0 °C, filtered washed with water and methanol and dried.

Results and Discussion

The two reagents $HgCl_2$ and $Hg(OAC)_2$ exhibit different electrophilicity on reaction with purine and pyrimidine nucleosides. $HgCl_2$ being more electrophilic than $Hg(OAC)_2$ prefers to coordinate with the ring nitrogens whereas the acetoxymercury group coordinates to nitrogen and exocyclic oxygen. In some cases such as the pyrimidine nucleosides, cytidine and uridine organometallic compounds are found with n^1-coordination of Hg to C_5.

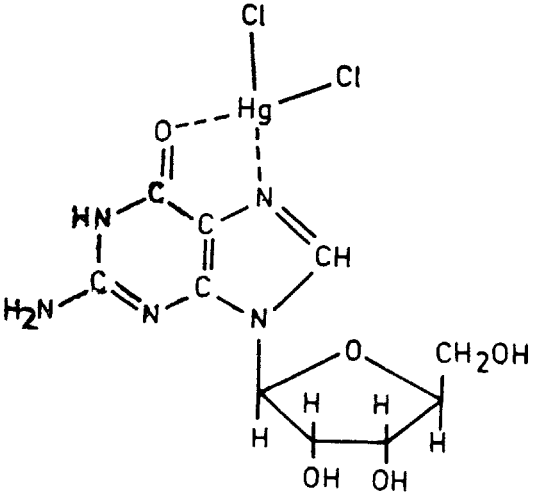
The infrared spectrum of the complex *1* shows a broad band in the region 3100cm^{-1} which is assigned N–H stretching frequency of the NH_2 group. The NH_2 deformation mode is observed at 1680cm. The $C_6 = O$ stretching frequency is lowered by about 20cm^{-1} in reference to the ligand and is observed at 1660cm^{-1} indicating the involvement of $C_6 = O$ in coordination to the metal ion. The ring C = N and C = C stretching frequencies are observed at 1420 and 1540cm^{-1}, respectively, with a shift of 30–50cm^{-1} to lower frequency indicating the coordination of ring nitrogen to the metal ion. The involvement of $C_6 = O$ favours the chelate structure of the complex. The νM–N and νM–Cl stretching frequencies were observed at 512cm^{-1} ν(M–N) and 362 and 400cm^{-1} for νM–Cl, respectively.

The peak observed at 262nm in the electronic spectrum of the complex is assigned to the π–π^* transition of guanosine.

The proton nmr spectrum of guanosine shows resonance peaks at 7.93ppm and 6.44ppm, assigned to C_8–H and NH_2, protons, respectively. In the ^{1}H nmr spectrum of the complex the C_8–H proton was observed at 8.16ppm with a down field shift of 0.23ppm indicating N_7 as the site of coordination to the metal ion. The NH_2 protons were observed at 6.54ppm with a down field shift of 0.10ppm.

The ^{13}C nmr spectrum of guanosine showed resonance peaks for C_2, C_4, C_5, C_6, C_8 carbon atoms at ppm values of 153.5, 151.2, 116.4, 156.7 and 135.7, respectively. The ribose carbon atom C'_1, C'_2, C'_3, C'_4 and C'_5 were observed at ppm values of 86.2, 70.3, 73.5, 85.1 and 61.2ppm, respectively. The ring carbons

C_2, C_3, C_4, C_6, C_8 resonance in the complex were observed at 154.0, 151.4, 116.4, 157.2 and 136.4ppm. The C_6 and C_8 carbon atoms thus show a down field shift of 0.5 and 0.6ppm as compared to other carbon atoms supporting the formation of a chelate ring with the N–7 and $C_6=O$ group. The ribose carbon atoms C'_1, C'_2, C'_3, C'_4 and C'_5 in the complex were observed at 86.8, 70.75, 74.2, 85.26 and 61.31ppm indicating the absence of binding of the ribose groups to the metal ion. The structure of the complex is depicted in I.



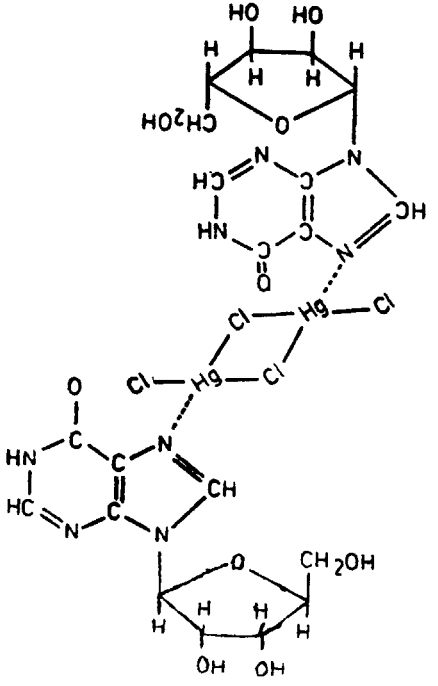
STRUCTURE I

The $C_6=O$ stretching frequency in complexes *2* was observed at 1670cm^{-1} without any shift on coordination to the metal ion. The ring C=N and C=C stretching frequencies were observed at 1410, 1450 and 1580cm^{-1} showing a lowering of these frequencies by about 20cm^{-1} on coordination to the metal ion. The νM–N stretching frequency was observed at 560cm^{-1}. The peaks at 350cm^{-1} and 245cm^{-1} in the I.R. spectrum of the complex are assigned to terminal and bridging chlorides.

The proton nmr spectrum of inosine exhibits the C_8H and C_2H protons at 8.22 and 8.0 ppm, respectively. In complex *2* the C_8H proton is shifted downfield by about 0.26ppm and was observed at 8.45ppm indicating the participation of N_7 as the site of coordination to the metal ion.

The ^{13}C nmr of inosine shows resonance peaks of C_2, C_4, C_5, C_6, C_8 carbon atoms at 142.2, 150.8, 126.0, 159.2 and 140.0ppm, respectively. The peaks for ribose carbon atoms C'_1, C'_2, C'_3, C'_4 and C'_5 were observed at 87.6, 74.1, 70.3, 85.6 and 61.3ppm, respectively. In complex *2* the peaks for C_2, C_4, C_5, C_6 and C_8 carbon atoms were observed at 142.2, 150.8, 126.02, 159.2 and 141.2ppm, respectively. The C_8 carbon resonance is thus shifted downfield by 1.2ppm and supports N_7 as the site of coordination to the metal ion.

There is no change in the resonance of ribose carbon atoms indicating the non-involvement of the hydroxyl groups of the sugar moiety in metal bonding. Based on these observations structure II is proposed for complex *2*.



STRUCTURE II

In the IR spectrum of complex *3* a medium intensity band at 3300cm^{-1} was assigned to the N–H ligational stretching frequency which is lowered by about 100cm^{-1} on coordination to the metal ion. The NH_2 deformation mode observed at 1620cm^{-1} showed a 20cm^{-1} lowering of frequency on coordination to the metal ion. The ring C = N and C = C stretching frequencies were observed at 1480 and 1580cm^{-1} and do not show a considerable shift in coordination supporting the non-involvement of ring nitrogens in coordination to the metal ion. The C_2O stretching frequency was observed at 1710cm^{-1}. The νM–N stretch was observed at 540cm^{-1} and the νM–Cl peaks corresponding to end and bridged chlorides at 320 and 285cm^{-1}, respectively.

The proton nmr spectrum of cytidine shows two doublets centered at 5.69 and 7.89ppm assigned to C_5–H and C_6–H protons, respectively. The peak observed at 7.15ppm is assigned to NH_2 protons. In complex *3* the NH_2 protons resonances was observed at 7.50ppm and were shifted down field by about 0.35ppm supporting NH_2 as the site of coordination to the metal ion. There is a small downfield shift of C_5–H and C_6–H protons by about 0.11 and 0.06ppm, respectively. The large shift in the NH_2 resonance as compared to C_5–H or C_6–H resonances further supports NH_2 as the site of coordination.

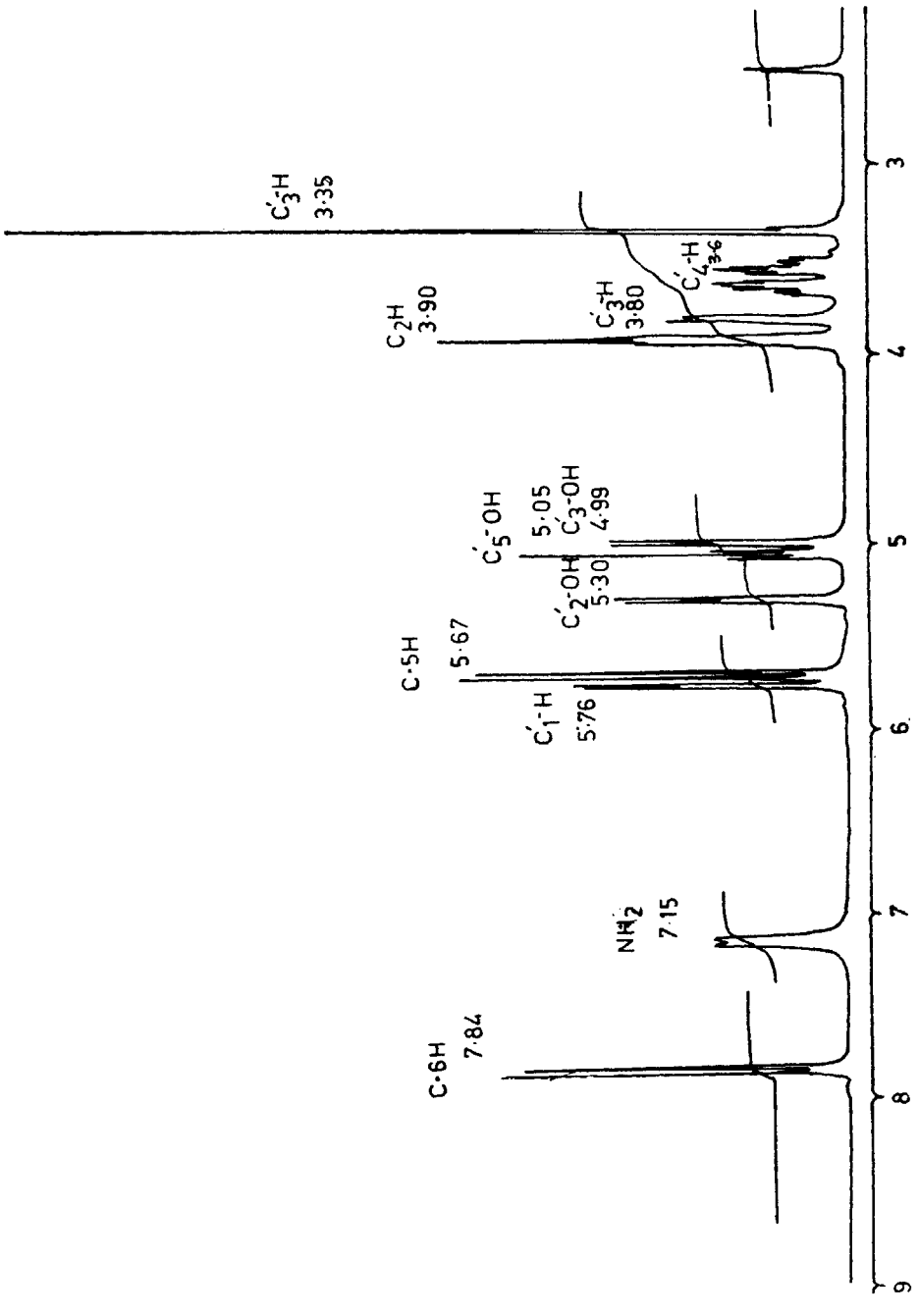
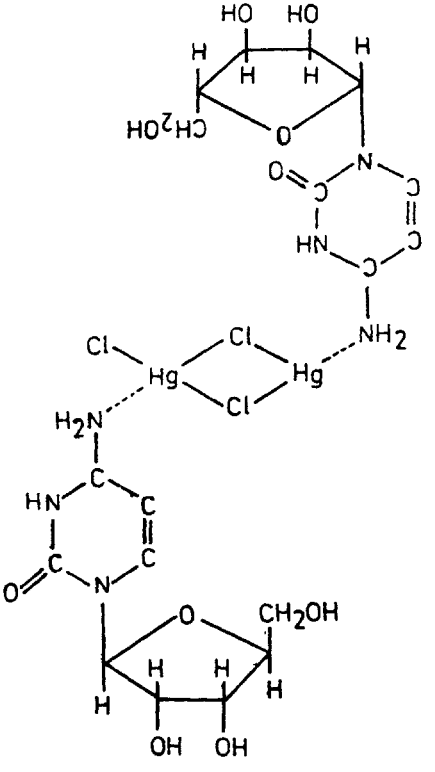


FIG 1 Proton NMR spectra of cytidine

Cytidine gives a well resolved spectrum for the sugar protons (Fig. 1). A doublet of doublets centered at 3.90 and 3.81ppm were assigned to C_2'-H and C_3'-H protons of ribose ring. A triplet of doublet centred at 3.60ppm was assigned to C_4'-H protons. A doublet of doublet centered at 3.35ppm was assigned to C_5-H protons. In the complex these protons were observed at the same position as in the ligand. Therefore, coordination through ribose ring is over-ruled.

In the ^{13}C nmr spectrum of cytidine the C_2, C_4, C_5, C_6 ring carbon atoms exhibit the resonance peaks at 155.3, 165.3, 93.5 and 141.3 ppm, respectively. The ribose ring carbon atoms C_1', C_2', C_3', C_4', C_5' were observed at 89.0, 69.3, 73.9, 84.0 and 60.5ppm, respectively. In the complex the ring carbon atoms C_2, C_4, C_5, C_6 exhibit resonance peaks at 154.8, 166.80, 94.8 and 142.30ppm. The large downfield shift by about 1.5ppm of C_4 carbon atoms supports the site of coordination is C_4-NH_2. In the C_2 and C_5 carbon atoms the shifts are about 0.5 to 1.0ppm, respectively. The ribose carbon atoms C_1', C_2', C_3', C_4', C_5' exhibit the resonance peaks at 89.50, 69.3, 73.79, 84.29 and 60.5ppm, respectively. The



STRUCTURE III

negligible shifts in these carbon atoms exclude the ribose ring participation in coordination to the metal ion.

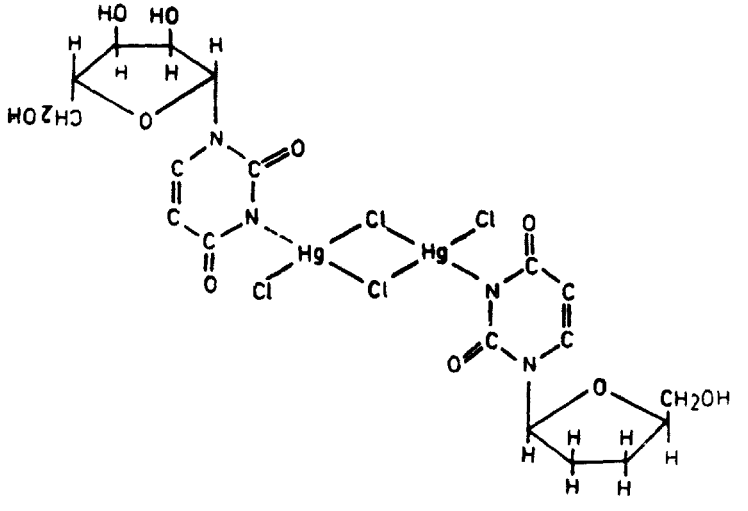
Based on the analytical and spectral data structure III is proposed for the complex.

In complex 4 the C = O stretching frequency in the region 1650–1700cm^{-1} of the $C_4 = O$ and $C_2 = O$ groups of uridine is not affected by complexation of the ligand to the metal ion. The C = N and C = C ring stretching frequencies, observed at 1390 and 1460cm^{-1}, respectively, indicate a lowering of 60–80cm^{-1} on complexation to the metal ion. The νM–N stretching frequency was observed at 580cm^{-1}. The terminal and bridging chlorides were observed at 360 and 280cm^{-1}, respectively.

The proton nmr spectrum of uridine exhibits two doublets for C_5–H and C_6–H protons observed at 5.36 and 7.88ppm, respectively. In the complex the C_5–H proton is shifted downfield by about 0.28ppm which supports N_3 as the site of coordination.

The ^{13}C nmr spectrum of uridine exhibits resonance peaks for the C_2, C_4, C_6, carbon atoms at 150.60, 162.90, 101.60 and 140.60ppm. The ribose ring carbon atoms C_1', C_2', C_3', C_4' and C_5' are exhibited at 87.7, 69.8, 73.40, 84.70 and 60.80ppm, respectively. In complex *4* the ring corbon atom C_2, C_4, C_5, C_6 are exhibited at 151.2, 163.6, 102.05 and 149.90. The C_2, C_4 and C_5 carbon atoms are thus shifted downfield by about 0.6, 0.7 and 0.45pmm, respectively. Since there is a relatively larger shifts in the C_2 and C_4 carbon atom resonance as compared to C_5 the site of coordination is proposed as N_3.

Based on the analytical and spectral data structure IV is proposed for the complex.



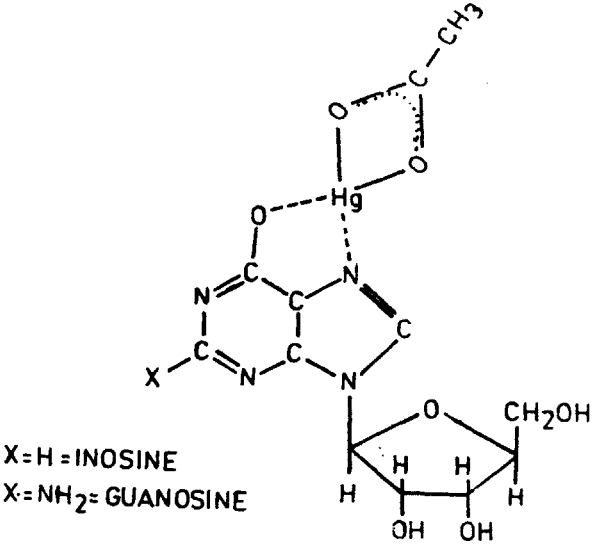
STRUCTURE IV

Acetoxymercury Complexes

The absence of an O-H stretch at 3300cm^{-1} and the ν $C_6 = O$ at 1680cm^{-1} is an indication of the coordination of guanosine to the metal ion through C_6-O^- group. NH_2 deformation mode was observed uncharged in complex *5* at 1620cm^{-1}. The ring C = N and C = C stretching frequencies were observed at 1400 and 1490cm^{-1} with a lowering of the frequencies by about 70–90cm^{-1} in the complex indicating the involvement of the ring nitrogen in coordination to the metal ion. The carboxyl stretching frequency of the CH_3COO^- group overlaps with that of CO group and appears at 1680cm^{-1}. The peaks observed at 512 and 432cm^{-1} were assigned to νM-N and νM-O stretching vibrations, respectively.

The ^{1}H nmr spectrum of guanosine shows a resonance peak at 7.34ppm which is assigned to the C_8-H proton. In the complex this peak is shifted downfield by about 1.01ppm and was observed at 8.35ppm, confirming the coordination of N_7 to the metal ion.

The ^{13}C nmr of guanosine complex could not be taken due to solubility problems in glacial acetic acid. Based on the I.R. and ^{1}H nmr the proposed structure of the complex is shown in V.



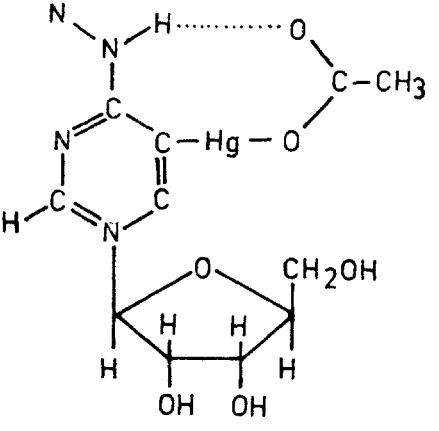
STRUCTURE V

In the I.R. spectrum of complex *6*, the band at 3300cm^{-1} by ν OH is completely absent. The broad band in the I.R. spectrum of complex at 2920cm^{-1} were assigned to N-H and C-H stretching frequencies of the ligand. The CO stretching frequency at 1680cm^{-1} was absent in the complex indicating the coordination of inosine through the C_6-O^- group. The ring C = N and C = C stretching frequencies were observed at 1400, 1450 and 1550cm^{-1} and were lowered by about 15–35cm^{-1} in the complex indicating the involvement of ring nitrogen in coordination

to the metal ion. The band observed at 1630cm^{-1} is assigned to the chelated corboxylato group in the complex. The ν M-N and ν M-O vibrations were observed at 530 and 430cm^{-1}, respectively.

The ^{1}H nmr spectrum of inosine in CH_3COOH-d_4 gives peaks at 8.11 and 8.22ppm assigned to C_2 and C_8 protons. These peaks appear in the complex at 8.32 and 8.46ppm shifted downfield by about 0.11 and 0.4ppm, respectively. As there is a larger downfield shift in the C_8-H proton as compared to the C_2-H proton the site of coordination to metal ion is suggested as N_7. This is further supported by ^{13}C nmr spectrum.

The ^{13}C nmr spectrum of inosine in CH_3COOH-d_4 shows the resonance peaks of C_2, C_4, C_5, C_6 and C_8 carbon atoms at 147.2, 150.8, 126.0, 157.0, 140.0ppm respectively. The C'_1, C'_2, C'_3, C'_4 and C'_5 carbon atoms of the ribose ring were observed at 91.21, 87.2, 76.71, 72.21 and 62.90ppm, respectively. In the ^{13}C nmr spectrum of the complex the ring carbon atoms C_2, C_4, C_5, C_6 and C_8 were observed at 147.1, 151.6, 128.1, 159.2 and 144.0ppm, respectively. The C_6 and C_8 carbon atoms are thus shifted downfield by about 2.1 and 4.0ppm, respectively indicating the involvement of C_6-O and N_7 groups in coordination to the metal ion to form a chelate ring. The structure of the complex shown as VI.



STRUCTURE VI

The IR spectrum of complex *7* shows a broad band in the region 3400–2920cm^{-1} for O-H, NH and C-H stretching frequencies. The 1630cm^{-1} peak for NH_2 deformation mode of the ligand is lowered in the complex by about 20cm^{-1} due to hydrogen bonding from the acetato group. The ring C = N and C = C stretching frequencies were lowered by about 70cm^{-1} in the complex and appear at 1400 and 1490cm^{-1}, indicating the involvement of the ring carbon in the η^1-coordination to the metal ion. The C_2 = O stretching frequency in the complex was observed at 1680cm^{-1} as in the ligand which shows that the group is not involved in coordination to the metal ion. The M-O stretching frequency in *I* was observed at 460cm^{-1} and ν M-C at 290cm^{-1}

The ^1H nmr spectrum of cytidine in DMSO-d_6 shows two doublets each for C_5-H and C_6-H protons centered at 5.69 and 7.89ppm, respectively. In the complex, the C_5-H proton resonance peak completely disappeared and the doublet for C_6-H proton collapsed to a broad and singlet observed at 7.64ppm. The NH_2 proton signal was shifted downfield by 0.13ppm and was observed at 7.28ppm. Since the C_5-H peak had completely disappeared in the complex, the site of coordination to the metal ion is suggested as C_5. As there is also a shift in the NH_2 proton it is proposed that there is hydrogen bonding with acetato oxygen of the acetoxymercury group.

The decoupled ^{13}C nmr spectrum of cytidine exhibits the resonance peaks for C_2, C_4, C_5, C_6 carbon atoms at 155.3, 165.3, 93.5, 141.3ppm, respectively. The ribose carbon atoms C_1', C_2', C_3', C_4', C_5' are observed at 89.0, 69.3, 73.9, 84.0, 60.5ppm, respectively. In the complex the ring carbons C_2, C_4, C_5, C_6 were observed at 155.06, 168.95, 142.26, 146.97ppm, respectively. The C_4, C_5 and C_6 carbon atoms shows a downfield shift of about 3.36, 48.46 and 5.6ppm respectively. The very large downfield shift of C_5 carbon of 48.46ppm thus supports the formation of Hg-C bond at C_5 carbon atom. The sugar ring exhibit the C_1', C_2', C_3', C_4', C_5' peaks at 89.3, 69.8, 73.8, 84.1 and 61.1ppm respectively. All these peaks were not shifted in the complex over-ruling the involvement of the sugar OH groups in bonding to the metal ion.

Based on the analytical and nmr spectral data structure VII is proposed for the complex.

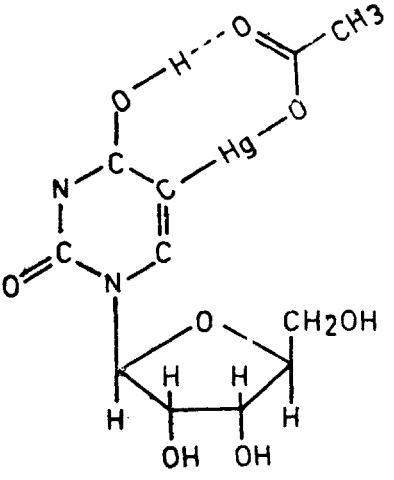
The IR spectrum of complex *8* shows two broad bands in the region 3450–3200cm^{-1} and 2900–2800cm^{-1} for O-H and N-H stretching frequency, respectively. The C = O stretching frequency of the $C_2 = O$ and $C_4 = O$ were observed at 1680cm^{-1}, at the same position as in the ligand indicating that the groups are not involved in coordination to the metal ion. The ring C = N and C = C stretching frequencies are shifted to lower frequency by about 40–80cm^{-1} and appear at 1430 and 1570cm^{-1}, respectively in the complex indicating the involvement of the ring carbon in the mercuration reaction. The νM-O and νM-C vibrations are observed at 430 and 270cm^{-1}, respectively.

The ^1H nmr spectrum of uridine in DMSO-d_6 shows two doublets each for C_5-H and C_6-H protons centered at 5.36 and 7.88ppm, respectively. In the complex the resonance peaks for C_5-H proton completely disappeared and the doublet for C_6-H proton collapsed to a sharp singlet peak at 7.07ppm. The complete disappearance of the C_5-H peak supports C_5 as the site of coordination to metal ion.

In ^{13}C nmr spectrum of uridine exhibits the C_2, C_4, C_5, C_6 carbon atoms at 150.60, 162.90, 101.60, 140.6. In the ribose ring the carbon atom C_1', C_2', C_3', C_4', C_5' were observed at 87.7, 69.8, 73.4, 84.7 and 60.8ppm, respectively. In complex *8* the ring carbons C_2, C_4, C_5, C_6, were observed at 154.28, 169.29, 123.05, 145.97ppm, respectively. The C_4, C_5, C_6 carbon atoms show large downfield shifts

by about 5.69, 21.0 and 5.00ppm, respectively. The large downfield shift of C_5 carbon atom of 21.5ppm as compared to C_4 and C_6 supports the formation of η^1-M-C bond at C_5 carbon atom. The ribose ring carbon atom C_1', C_2', C_3', C_4', C_5' exhibit the resonance peaks at 87.7, 69.80, 73.45, 84.7 and 60.63ppm, respectively. These peaks are not shifted in complex *8*.

Based on the analytical and spectral atom structure VIII is proposed for the complex.



STRUCTURE VII

The relative shifts of the C_2, C_4, C_5 and C_6 carbon resonance in cytidine and the corresponding $HgCl_2$ and Hg(OAC) complexes is shown in Fig. 2.

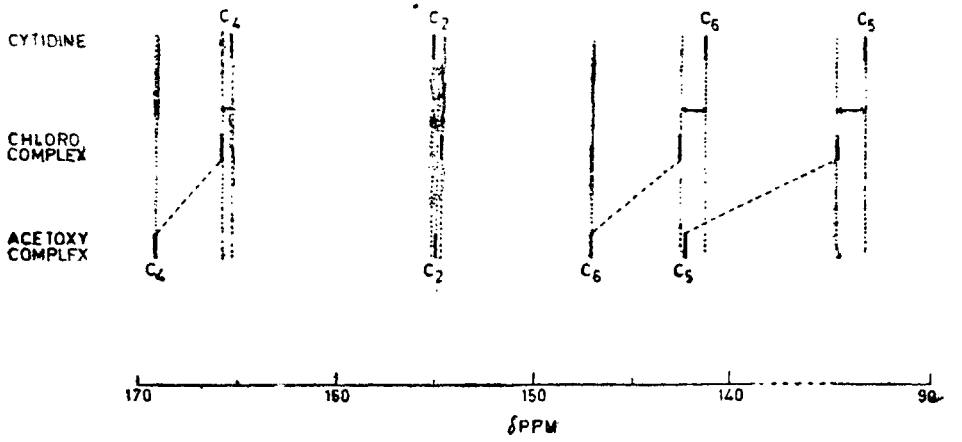


FIG 2 The shifts of ^{13}C in the NMR spectra of cytidine, mercury chloride and cytidine η^1 mercuric acetate complex

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