BIOPOLYMERS VOL. 16, 1863–1878 (1977)

Spectroscopic Studies on the Interaction of Au(III) with Nucleosides, Nucleotides, and Dimethyl Phosphate

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Synopsis

A series of complexes of Au(III) with nucleosides and nucleotides and their methyl derivatives in different stoichiometry have been prepared. Ultraviolet, visible, ir, and nmr studies have been performed to determine the site of binding of these ligands with the metal ion. In (1:4) Au(III): guanosine complex, N_7 is the binding site, whereas at 1:1 complex, a bidentate type of chelation through C_6O and N_7 is observed. C_6-NH_2 is favored over N_1 as coordinating site at all stoichiometry in the adenosine complex. Inosine binds through N_1 at $r = 1$. In cytidine, N_1 is the binding site, whereas thymidine reacts only at high pH. In the case of nucleotides a bidentate type of chelation through the phosphate and the ring nitrogen occurs. The phosphate binding ability of Au(III) was further confirmed by studying the interaction of Au(III) with dimethyl phosphate—a conformational analog of the phosphate backbone in DNA chain.

INTRODUCTION

Metal ions are known to bind with nucleic acids and thereby alter their conformation and biological function.[1] Various studies in the past[2,3] have shown that alkali and alkaline earth metal ions interact only with the phosphate moiety while the transitional metal ions interact with both the phosphate and bases of nucleic acids. The metal ion–base interaction depends on the nature of both metal and bases; a certain site of coordination is preferred. This base-specific interaction has, therefore, been exploited recently for the sequencing of DNA by electron microscopy, when DNA is labelled with heavy metal ions like Au and Pt.[4,5] This, together with the recent finding that certain transitional metal complexes have been found to be potentially useful in cancer chemotherapy,[6,7] created a renewed interest in the study of the interaction of heavy metal ions with DNA or its components, with respect to (a) the site of binding and (b) the structure and stability of the complex.

Au(III) isoelectronic with Pt(II)-ad[8] system usually forms square planar complexes in solution. Since the square planar geometry of Pt(II) is important for its action as an anticancer drug,[8] Au(III) salts also can be used for the same purpose with the added advantage of decreased toxicity.

Furthermore, it has recently been shown that Au(III) interacts quite strongly with adenine nucleotides[9] and DNA[10] and binds to both bases and phosphates of DNA. So far nothing has been reported as to how the bases are involved in the binding. This work was motivated by the thought that information on the nature of interaction of Au(III) with DNA can be obtained by studying the properties of Au(III) complexed with various ligands like nucleosides, nucleotides, and dimethyl phosphate with the help of uv, ir, and ^1H nmr spectroscopy. It may be emphasized that the phosphate binding ability of Au(III) can be better examined with dimethyl phosphate anion $(CH_3O)_2PO_2^-$.[11,12]

EXPERIMENTAL SECTION

Materials

Commercially available nucleosides and nucleotides (P-L Biochemicals, Wisconsin) were used without further purification. $HAuCl_4$ crystals (Johnson Matthey Chemicals Ltd., England) were crystallized from doubly distilled water before use, and purity was checked by the molar extinction coefficient value at 313 nm. Solvents Me_2SO and methanol were dried and distilled immediately before use. Both nmr spectroscopy and the Fischer reaction suggest that Me_2SO contains not more than 1% H_2O. Water used in the study was always doubly distilled. Methyl derivatives of nucleosides were prepared according to standard methods.[13-15] Dimethyl phosphate has been prepared in the usual way described by Tsuboi et al.[11] Purity of all ligands was checked by their extinction value in the uv region.

Equipment

The spectral measurements were made with a Cary 14/Unicam SP-700A spectrophotometer. Infrared spectra were taken in solid phase in dry KBr pellets using a Karl Zeiss-Jena spectrometer. ^1H nmr measurements were performed with Varian HA-100 and T-60 spectrometers in both D_2O and Me_2SO-d_6 solvents using sodium 2,2,dimethyl-2-silapentane-5-sulfonate and Me_4Si as internal standards, respectively.

Preparation of the Complexes

Complexes were prepared by following the procedure described by Cattalini and Tobe.[16] An aqueous solution of $HAuCl_4$ of known concentration (0.1*M*) was neutralized carefully to pH 6.5–7 by the gradual addition of solid Na_2CO_3. To this a stoichiometric amount of nucleoside or nucleotide was added in the solid form. In the case of purine nucleosides, a red color developed within 5–10 min and the complex was insoluble in H_2O. Purine nucleotide-Au(III) complex also developed a red color which was soluble in H_2O and subsequently precipitated by adding cold ethanol.

Complexes of Au(III) with cytidine and its derivatives formed soluble, orange complexes in H_2O which were isolated by evaporating the solvent under reduced pressure. An orange solution of Au(III)-5′dCMP was also precipitated by adding cold ethanol. Au(III)-thymidine complex was only detected at high pH. It could not be isolated due to the formation of metallic gold during solvent evaporation. The precipitated complexes were collected by centrifugation, dried *in vacuo*, and stored over P_2O_5. They were dissolved in fresh solvents prior to measurements. The complexes were reprecipitated slowly by ethanol or reevaporated for elemental analysis, performed by Bhabha Atomic Research Centre, Bombay, India.

Determination of the Stoichiometry: Amount of Nucleoside/Tide per Mole of Au(III)

A known amount of the Me_2SO or H_2O solution of complexes (purine nucleosides are soluble in Me_2SO) was transferred to 3–5 ml of 0.1*N* HCl and kept overnight for the complete breakdown of the complexes. The absorption of the solution was then measured in a cell of 1-cm pathlength at two different wavelengths, namely 270 and 313 nm. Since the absorption of nucleic acid components at 313 nm can be neglected, the amount of Au(III) was determined from its known extinction value.[17] The stoichiometric amount of nucleoside or nucleotide was then calculated by using the following two equations:

$$A_{313} = \epsilon_{313}^{Au} c_{Au} \tag{1}$$

$$A_{270} = \epsilon_{270}^{Au} c_{Au} + \epsilon_{270}^{N} c_N \tag{2}$$

where A, ϵ, and c are absorbance, molar extinction, and concentration, respectively. N denotes base unit. The extinction values of nucleosides and nucleotides at pH 1 were taken from the literature.[13–15,18]

RESULTS AND DISCUSSION

Interaction of Au(III) with Nucleosides and Nucleotides

Analytical data of the complexes and their stoichiometry are reported in Table I. Spectral studies can be broadly classified under three groupings: visible and uv, ir, and ^1H nmr.

Visible and Ultraviolet Spectra

In Figs. 1 and 2 visible spectra of various Au(III)-nucleoside/(tide) complexes in 1:1 CH_3OH/Me_2SO (v/v) mixture and H_2O are shown. It is seen that a broad band appears far outside the range of charge-transfer bands (300–330 nm) from ligand to metal ion. In the case of $AuCl_4^-$ a monotonic increase of absorbance was observed and appeared as a tail of highly intense charge-transfer band. The broad band in the visible region

TABLE I
Analytical Data of the Complexes[a]

Compounds	%C		%H		%N		Stoichiometry Au^{+3}:n
	Calcd.	Obsd.	Calcd.	Obsd.	Calcd.	Obsd.	
$[Au(G)_4]Cl_2OH$	33.85	33.78	3.74	3.7	19.74	19.64	1:4
$[Au(G)Cl_2]$	22.81	21.85	2.2	2.44	12.72	12.74	1:1
$[Au(A)_3Cl](OH)_2$	33.7	33.62	3.84	3.79	19.66	19.51	1:3
[Au5′dGMP Cl_2]Na	18.86	18.65	1.88	2.02	10.99	10.89	1:1
[Au5′dAMP Cl_2]Na	19.23	19.2	1.92	1.88	11.69	11.75	1:1
[Au ICl_2]	22.42	22.39	1.87	1.82	10.46	10.38	1:1
$[AuC_2Cl_2]OH$	28.07	28.1	3.51	3.42	10.91	10.95	1:2
[Au5′dCMP Cl_2]Na	18.05	18.01	2.0	1.98	7.02	7.00	1:1

[a] Nucleoside/nucleotide = n, G = guanosine, A = adenosine, 5′dGMP = 2′-deoxy guanosine 5′monophosphoric acid, C = cytidine, 5′dAMP = 2′-deoxy adenosine 5′monophosphoric acid, I = inosine, and 5′dCMP = 2′-deoxy cytidine 5′monophosphoric acid.

could be detected because the charge-transfer band shifted to lower wavelength in nucleoside/(-tide) complexes. This is what one would expect if "N" atom of ligand coordinated to central metal atom as lone pair of "N" has greater ionization potential than the Cl^- ion.[17]

Because of the overlapping of the highly intense charge-transfer band, the assignment of the d–d transition in Au(III) complexes was rather difficult. Recently Mason and Gray[19] have shown that at 77°K, the d–d transition can be resolved in the cases of $(C_4H_9)_4NAuX_4$ (where X = Cl^-, Br^-, SCN^-) complexes. They found d–d transitions along with the charge-transfer band and characterized them in the range of 330–600 nm. The least intense band at the longest wavelength (21,000–26,000 cm^{-1}) was assigned to $^1A_{1g} \rightarrow {}^1A_{2g}$, and $^1A_{1g} \rightarrow {}^1E_g$. By analogy one could ascribe the broad visible band above 400 nm (see Figs. 1 and 2) to $^1A_{1g} \rightarrow {}^1A_{2g}$ and $^1A_{1g} \rightarrow {}^1E_g$. An attempt to resolve them further at room temperature by varying solvent composition with different dielectric constants was not successful; however, the d–d band of Au(III)-5′dGMP complex at $r = 1$ (where r = (Au^{+3})/(nucleoside/(-tide)) in Me_2SO is much more intense and resolvable than that in H_2O (see Fig. 1). The derivative plot ($\Delta\epsilon/\Delta\lambda$ vs λ) (Fig. 2) clearly shows two bands whose respective positions and intensity of absorption are indicated in Table II. It is interesting to note that the intensities of the band depend not only on the ratio of Au(III) to nucleic acid bases, but also on the nature of the nucleoside/(-tide). The intensities of d–d transitions of the complexes decrease in the following order: (1:1) Au-guanosine < (1:3)Au-adenosine < (1:4)Au-guanosine < (1:1)Au-3′dGMP < (1:1)Au-5′dAMP. This is presumably due to the fact that a nucleotide can act as a stronger bidentate ligand.

With pyrimidine nucleosides and nucleotides, the shoulder in the visible region could not be isolated, but the extinction values of Au(III) complexes are higher than that of $AuCl_4^-$, and also the extinction value for one complex

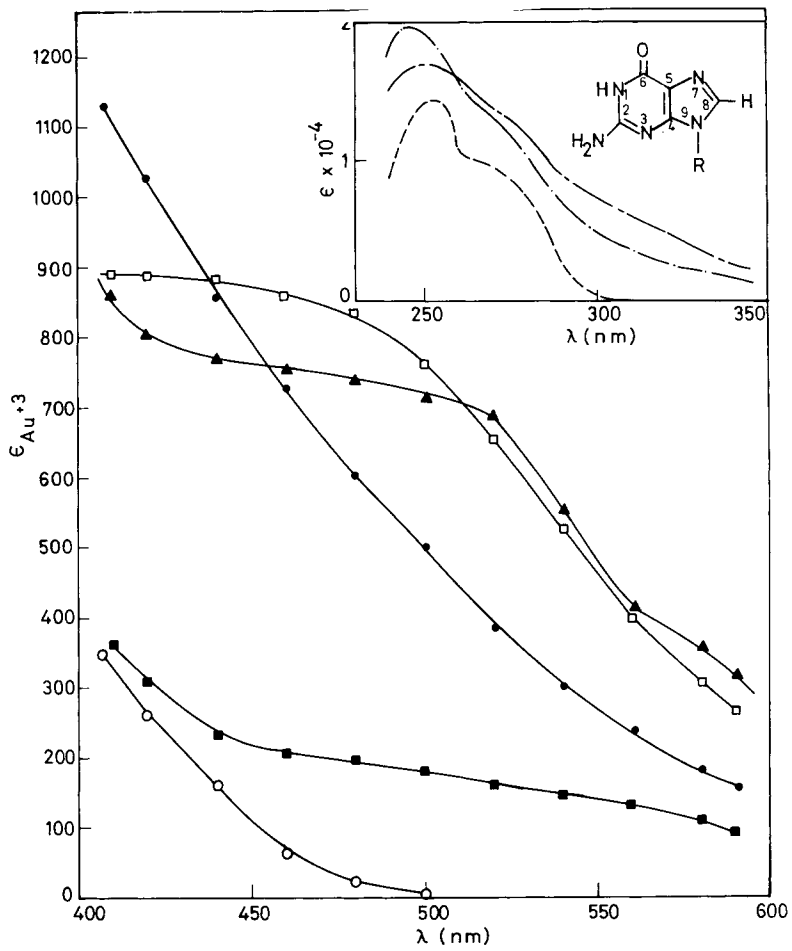


Fig. 1. Visible spectra of Au(III) complexes in (1:1) Me_2SO/methanol (v/v). -O- neutralized Au(III) solution; -■- (1:1) Au(III)-guanosine; -●- (1:1) Au(III)-5′dGMP in H_2O; -□- (1:1) Au(III)-5′dGMP; -▲- (1:4) Au(III)-guanosine. Inset shows the uv spectra of the complexes along with the structure of guanosine, R = β-D-ribofuranoside, and for 5′dGMP, R = 2′-deoxy-β-D-ribofuranoside 5′-phosphoric acid. - - - 5′dGMP in H_2O; – – – Au(III)-5′dGMP in H_2O; —·— Au(III)-guanosine in Me_2SO/methanol (1:9 v/v).

differs from others, indicating that different types of complexes were formed. In the case of Au(III)-thymidine complex this change was observed when the pH of the solution was changed from 6.5 to 8 (not shown).

A prior knowledge of the sites of coordination, however, is necessary to correlate visible band position and structure. The following sections describe ir and ^1H nmr studies carried out to obtain this information.

IR Spectra

We were mainly concerned with the qualitative change in the ir band in the region 900–1300 cm^{-1} (phosphate region) and 1500–1800 cm^{-1} (cor-

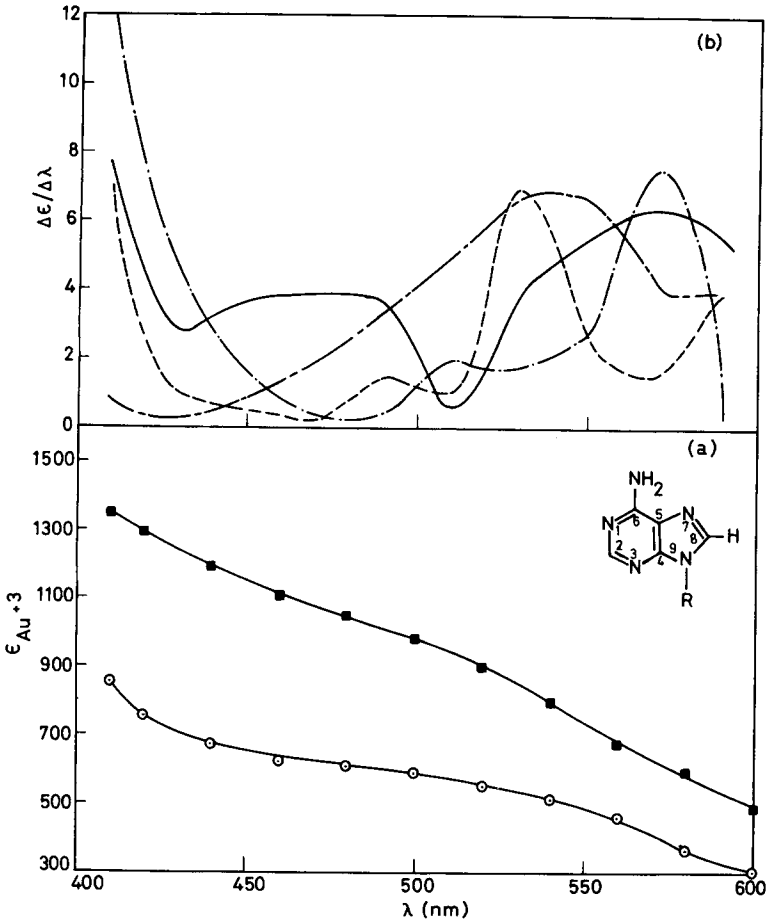


Fig. 2. (a) Visible spectra of Au(III) complexes in Me_2SO/methanol (1:1 v/v). —⊙— (1:3) Au(III)-adenosine; —■— Au(III)-5′dAMP and the structure of adenosine, R = β-D-ribofuranoside, and for 5′dAMP, R = 2′deoxy-β-D-ribofuranoside 5′-phosphoric acid. (b) Derivative plot of absorption spectra shown in Figs. 1 and 2(a). – (1:1) Au(III)-5′dAMP; -•- (1:3) Au(III)-adenosine; – – – (1:1) Au(III)-5′dGMP; - - - (1:4) Au(III)-guanosine.

responding to the in-plane vibrational modes of C=O, C=C, C=N, and NH_2)[20] due to the formation of the complex. Hartman[21] has studied the ir spectra of $AuCl_3$ with nucleosides and nucleotides but no detailed analysis has been given. Tsuboi et al.[22] elaborately described the characteristic ir spectra of nucleic acid–base residues and assigned the spectra by normal coordination analysis. Change in the spectra of complexes of Au salts is compared here with the assignments of Tsuboi et al.[20] to find the site of complexation as given in Table III.

Guanosine and its derivatives. Infrared spectra of the complexes of Au(III) and guanosine (r = 0.25,1), 7-methyl guanosine (r = 1), inosine (r = 1), and 5′dGMP (r = 1) have been studied. We were mainly interested in N_1H stretching and bending frequencies (around 3150 and 1450 cm^{-1}), C_6=O, stretching frequency (1690 cm^{-1}), C_2-NH_2 scissoring vibration (1640

TABLE II
Energy of Absorption of the Complexes in Me_2SO/Methanol

Complex	Energy of Absorption (cm^{-1})
Au(III)-guanosine(1:4)	2,088 (725), 1,870 (610)[a]
Au(III)-5′ dGMP(1:1)	1,850 (640)
Au(III)-guanosine(1:1)	2,000 (190)
Au(III)-adenosine(1:3)	1,960 (590), 1,754 (450)
Au(III)-5′ dAMP(1:1)	2,130 (1100), 1,754 (630)

[a] Numbers in the parenthesis indicate the extinction values.

cm^{-1}), and ring vibration of C=C and C=N (around 1600 cm^{-1}). In the Au(III) complex of guanosine at $r = 0.25$ these above-mentioned bands were not changed except for the decrease in intensity of the 1600 cm^{-1} band which appeared as a shoulder. Since coordination through nitrogen is expected, the only possible sites of binding are N_3 and N_7, which would disturb the ring vibration. Due to the basicity, N_7 was earlier assumed to be the most potent metal binding site,[2] which is even true for the gold complex at small r values, later proved by nmr studies. At $r = 1$, the 1600 cm^{-1} band was greatly reduced, as found in the Au(III)-guanosine ($r = 0.25$) complex. The additional change was observed for C_6=O frequency (1690 cm^{-1}), which completely disappeared in the complex. In contrast, the 1640 cm^{-1} band of C_2-NH_2 was shifted by 20 cm^{-1} in the Au(III)-7 methyl guanosine complex ($r = 1$), along with a change of N_1H group frequency. In both the cases it can be assumed that a bidentate type of bonding has taken place, either through C_6=O, N_7 or through N_1H and C_2-NH_2.

In spite of the basicity at the N_7 site, inosine binds through N_1 in (1:1) complex. The changes in spectra are similar to the (1:1) Au(III)-7-methyl inosine complex as given in Table III. In 5′dGMP complexes of Au(III) 1230 cm^{-1} band of P=O was absent, with the appearance of a small band around 1200 cm^{-1}, which can be attributed to phosphate binding. The changes in ring vibrations were also observed.

Adenosine and its derivatives. Infrared spectra of Au(III)-adenosine at $r = 0.25$, 0.33, and 1 were found to be identical to each other. The intense band around 1680 cm^{-1} of adenosine was found to be greatly reduced in all these complexes with no other appreciable change. We have attributed this to the C_6-NH_2 coordination in the metal complex. The change in 1680 cm^{-1} NH_2 scissoring vibration was identical to that of Au(III)-1-methyl adenosine complex at $r = 1$. On the other hand, ir data show that binding through N_1 or N_7 and phosphate took place in the (1:1) Au(III)-5′dAMP complex.

Cytidine and its derivatives. The strong bands of cytidine around 1650 cm^{-1} (C_2=O stretching), and 1670 cm^{-1}, 1600 cm^{-1} (C_6-NH_2 coupled to ring vibration) did not change appreciably at $r = 0.25$, 0.5, or 1, except the reduced intensity of the 1600 cm^{-1} band showing Au^{+3} is not coordinated

TABLE III
Infrared Absorption Bands of the Nucleosides and Nucleotides and their Complexes in Solid Phase in Dry KBr Pellets

Compounds	IR Bands (cm^{-1})[a]	Special Remarks
Guanosine	1540(Sh,ms), 1600(b,s), 1640(Sh,s), 1690(b,s), 3150(b,s)	1540 corresponds to N_1H lending, 3150 . . . N_1H stretching, 1600 . . . ring vibration due to C=C, C=N, 1640 . . . NH_2 vibration, 1690 . . . C=O stretching
Au(III)-guanosine (r = 0.25)	1540(Sh,ms), 1600(b,w), 1640(Sh,s), 1690(b,s) 3150(b,s)	Coordination through N_7
Au(III)-guanosine (r = 1)	1540(Sh,ms), 1600(b,w), 1640(Sh,s), 3150(b,s)	Coordination through C_6-O and N_7
7 methyl guanosine	1540(Sh,ms), 1590(b,ms), 1640(Sh,w), 1690(b,s), 3150(b,s)	Assignments are similar to guanosine, 1590 corresponds to C=C, C=N
Au(III)-7 methyl guanosine (r = 1)	1540(shoulder), 1590(b,ms), 1660(b,w), 1690(b,s)	Coordination through N_1H and C_2-NH_2
Inosine	1540(Sh,s), 1610(b,s), 1690(b,s), 3150(b,s)	1610 corresponds to ring C=C, C=N, others similar to guanosine
Au(III)-inosine (r = 1)	1540(Shoulder), 1610(b,s), 1690(b,s)	Coordination through N_1H
7 methyl inosine	1540(Sh,s), 1600(Sh,ms), 1700(b,s), 3150(b,s)	1600 corresponds to ring C=C, C=N, 1700 . . . C=O, others similar to guanosine
Au(III)-7 methyl inosine (r = 1)	1540(Shoulder), 1600(Sh,ms), 1700(b,s)	Coordination through N_1
5′dGMP	980(Sh,s), 1100(b,s), 1190(Sh,w), 1230(b,s), 1400(Sh,ms), 1540(Sh,ms), 1610(b,s), 1660(Sh,s), 1700(b,s), 3150(b,s)	900–1300 cm^{-1} phosphate region, 1230 corresponds to P=O stretching, other bands are same as guanosine
Au(III)-5′dGMP	980(w), 1100(b,s), 1200(w,Sh), 1400(Sh,s), 1550(Sh,ms), 1610(Shoulder), 1660(Sh,s), 1700(b,s), 3150(b,s)	Coordination through phosphate and N_7
Adenosine	1575(Sh,s), 1610(b,s), 1680(b,s)	1610 corresponds to ring vibration, 1680 band is due to NH_2 scissoring
Au(III)-adenosine (r = 0.33)	1575(Sh,s), 1610(b,s), 1680(Shoulder)	Coordination through C_6-NH_2
1-methyl adenosine	1600(b,ms), 1690(b,s)	1600 corresponds to ring vibration, 1690 band is due to NH_2 vibration
Au(III)-1 methyl adenosine (r = 1)	1600(b,ms), 1700(shoulder)	Coordination through C_6-NH_2
5′dAMP	930(Sh,s), 980(Sh,ms), 1100(Sh,s), 1220(Sh,s), 1610(Sh,s), 1700(b,s)	900–1300 cm^{-1} is phosphate region with 1220 cm^{-1} band due to P=O stretching, 1610 . . . ring vibration, 1700 . . . NH_2

TABLE III (*continued*)

Compounds	IR Bands (cm^{-1})[a]	Special Remarks
Au(III)-5′dAMP (r = 1)	930(Sh,s), 980(Sh,ms), 1100(w,s), 1230(w,b), 1600(w,b), 1700(b,s)	Coordination through phosphate and N_1 or N_7
Cytidine	1530(Sh,ms), 1600(b,ms), 1650(Sh,s), 1670(b,s)	1530 corresponds to ring vibration, 1670 and 1600 . . . coupled vibration of C_6-NH_2 and ring C=C and C=N
Au(III)-cytidine (r = 0.5)	1600(shoulder), 1650(Sh,s), 1670(b,s)	Coordination through N_1
1-methyl cytidine	1540(b,w), 1600(b,w), 1670(b,s), 1640(Sh,s)	1540 corresponds to ring vibration, 1670 and 1600 . . . coupled vibration of C_6-NH_2 and C=C, C=N, 1640 . . . C=O stretching
Au(III)-1-methyl cytidine (r = 1)	1540(b,w), 1600(b,w), 1640(Sh,s), 1700(b,w)	Coordination through C_6-NH_2
5′dCMP	960(Sh,s), 1100(Sh,s), 1230(Sh,s), 1540(Sh,ms), 1600(b,ms), 1650(Sh,s), 1670(b,s)	Assignments are same as other nucleotides and cytidine
Au(III)-5′dCMP (r = 1)	950(Sh,s), 1100(b,w), 1220(shoulder), 1600(w,b), 1650(Sh,s), 1670(b,s)	Coordination through N_1 and phosphate

[a] S = strong, Sh = sharp, ms = medium strong, w = weak, b = broad.

to any of these groups. The band of the ligand around 1530 cm^{-1} disappeared in the complex. This can be attributed to coordination through N_1. In (1:1) 1-methyl cytidine-Au(III) complex, the band around 1670 cm^{-1} disappeared and the 1600 cm^{-1} band was further reduced, indicating in this case that C_6-NH_2 takes part in binding to metal.

^1H NMR Studies

Due to the limited solubilities of several complexes in Me_2SO, the nmr spectra were only analyzed for Au(III)-guanosine (r = 0.25, 1), Au(III)-5′dGMP (r = 1), Au(III)-adenosine (r = 0.33), Au(III)-5′dAMP (r = 1), Au(III)-cytidine (r = 0.5), and Au(III)-5′dCMP (r = 1) compounds. Changes in resonance positions were also noted in the original solution of Au(III) with thymidine (stoichiometry maintained as 1:1) at pH 8. The assignments of the nmr lines given in the figures were established previously.[23-25]

Guanosine and 5′dGMP complexes. Figure 3 shows the change in nmr spectra of guanosine in the Au-complex at different stoichiometries. The peaks due to the sugar portion of nucleoside did not change appreciably and are not reported here. This is what one would expect, since the sugar ring is not participating in coordination. In r = 0.25 Au(III)-guanosine,

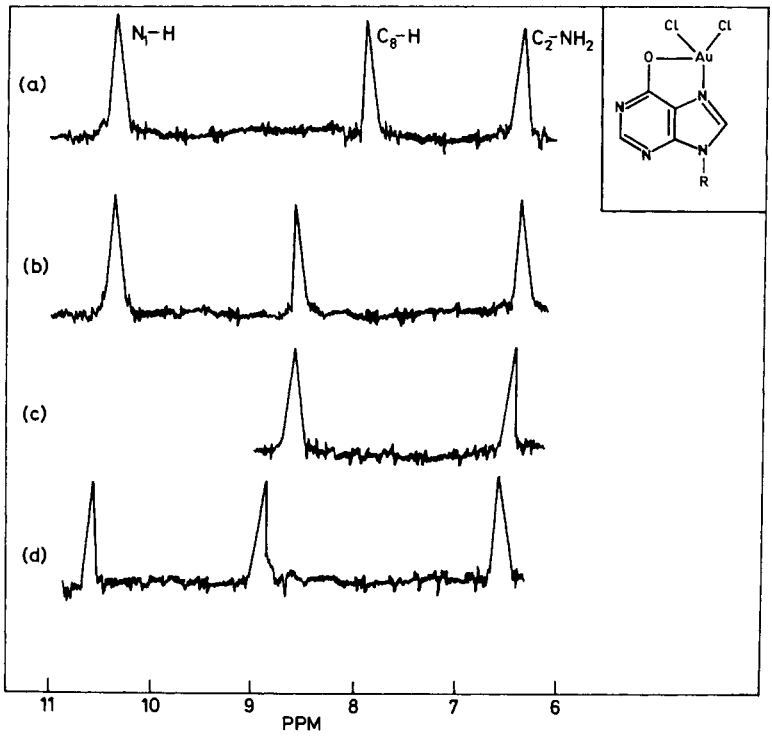


Fig. 3. ^{1}H nmr spectra of nucleosides, Au(III)-nucleoside, and Au(III)-nucleotide complexes in Me_2SO-d_6 using Me_4Si as an internal standard. (a) Guanosine, (b) Au(III)-guanosine (r = 0.25), (c) Au(III)-guanosine (r = 1), (d) Au(III)-5′dGMP (r = 1). Inset shows the structure of Au(III)-guanosine (r = 1).

C_8-H is shifted downfield without much change in other peak positions. This clearly indicates N_7 is the site for coordination and consistent with ir data. The donation of a lone pair of electrons from N_7 has its effect only on neighboring C_8, and as the N_1H and C_2-NH_2 are far away from N_7, no effect was produced. In a similar way, the C_8-H peak was also shifted downfield in the 1:1 Au(III)-guanosine complex and the C_2-NH_2 peak did not change at all. However, the absorption due to N_1H could not be detected downfield or at higher amplitude. These results are consistent with ir data. If we assume that a covalent and coordination type of bonding were present in the 1:1 complex through C_6-O and N_7, respectively, the absence of N_1H peak in nmr could be explained. At pH 6.5 $AuCl_4^-$ exists as $AuCl_2(OH)_2^-$ and forms square planar complexes. So a bidentate type of chelation through N_7 and C_6-O can take place by the liberation of $2OH^-$ groups from $AuCl_2(OH)_2^-$, similar to Pt(II) complexes.[8] The absence of the N_1H peak confirms the proton removal due to complexation, but for simultaneous OH^- liberation no quantitative pH-titration studies could be performed. The inset in Fig. 3 shows the possible structure for the 1:1 Au(III)-guanosine complex. N_7 coordination in metal binding is well

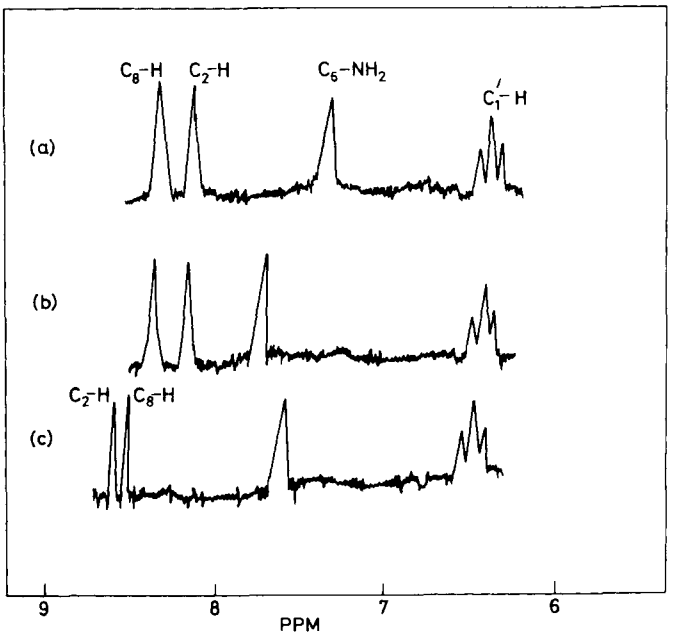


Fig. 4. ^{1}H nmr spectra of nucleosides, Au(III)-nucleoside, and Au(III)-nucleotide complexes in Me_2SO-d_6 using Me_4Si as an internal standard. (a) Adenosine, (b) Au(III)-adenosine (r = 0.33), (c) Au(III)-5′dAMP (r = 1).

documented in the literature, including a recent study[26] on Pt(II)-guanosine and -ionisine complexes where the guanosine was assumed to be a poor bidentate ligand. But our spectral results and analytical data show the above assumption is not true, and this kind of stoichiometry dependent different type of chelation is unique for guanosine. In the case of 5′-dGMP complex (cf. Fig. 3) C_8-H was again shifted downfield without any other change, indicating that N_7 is the preferable site for binding.

Adenosine and 5′dAMP complexes. In the complex of Au(III)-adenosine, C_6-NH_2 was shifted by 50 Hz (Fig. 4) downfield with no other change which can be attributed to the participation of a lone pair of electrons of the amino group in Au(III) chelation. This is in agreement with our ir data. The nmr spectra of nucleotides are not given in the figures. The only change in the nmr spectra one can observe between purine nucleoside and nucleotide is the downfield shift of C_8-H by 0.2δ. In the 1:1 complex of Au(III)-5′dAMP, this C_8-H peak around 8.5δ did not change its position significantly, whereas C_2H and C_6-NH_2 shifted downfield, showing that N_1 is the site for coordination (cf. Fig. 4). If the coordination had taken place through N_7, the peaks due to C_2-H and C_6-NH_2 need not be shifted. This indicates our assignments are correct. Adenosine has been reported to bind to different metal ions through both C_6-NH_2 and N_7.[27-29] Our results show that for Au(III)-adenosine complex at all r values, C_6-NH_2 is the only binding site.

Cytidine and thymidine complexes. The nmr spectra of cytidine in D_2O have previously been assigned.[25] The H_4 is a downfield doublet and H_5 an upfield doublet close to the doublet of H_1'. On complexation the H_5 doublet shifted downfield, indicating it was close to the metal binding site (Fig. 5). Similar results were observed with 5′dCMP at $r = 1$. These

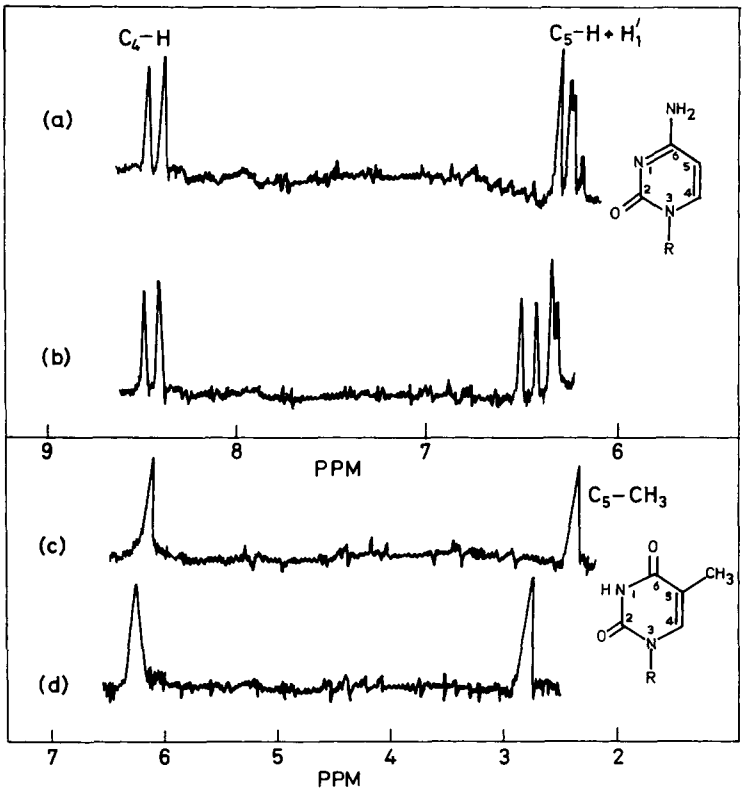


Fig. 5. ^1H nmr spectra of nucleosides, Au(III)-nucleoside, and Au(III)-nucleotide complexes in D_2O using sodium 2,2,dimethyl-2-silapentane-5-sulfonate as internal standard. (a) Cytidine; (b) Au(III)-cytidine ($r = 0.5$); (c) thymidine (at pH = 8); (d) Au(III)-thymidine ($r = 1$, pH = 8).

findings, along with the ir data, led us to conclude that N_1 in cytidine is the metal binding site like Cu(II).[30]

Some authors have found that uridine or thymidine do not bind to Zn(II), Hg(II), Cu(II), or Pt(II).[27,29,30] In the solution of the Au(III)-thymidine 1:1 stoichiometric mixture at pH 8, we observed a downfield shift of the thymidine CH_3 peak. This was not apparent at pH 6.5 of the same solution (cf. Fig. 5). This fact and visible spectral changes show that there is some interaction between Au(III) and thymidine, the exact nature of which cannot be concluded. But the 1-methyl thymidine and Au(III) mixture

did not show any change in the nmr spectra at any pH, indicating that N_1 of thymidine may be a possible site of chelation.

The Interaction of Au(III) with Dimethyl Phosphate

Figure 6 shows the change observed in the change-transfer band of $AuCl_4^-$ when mixed with dimethyl phosphate anion. Below pH 5, this change was

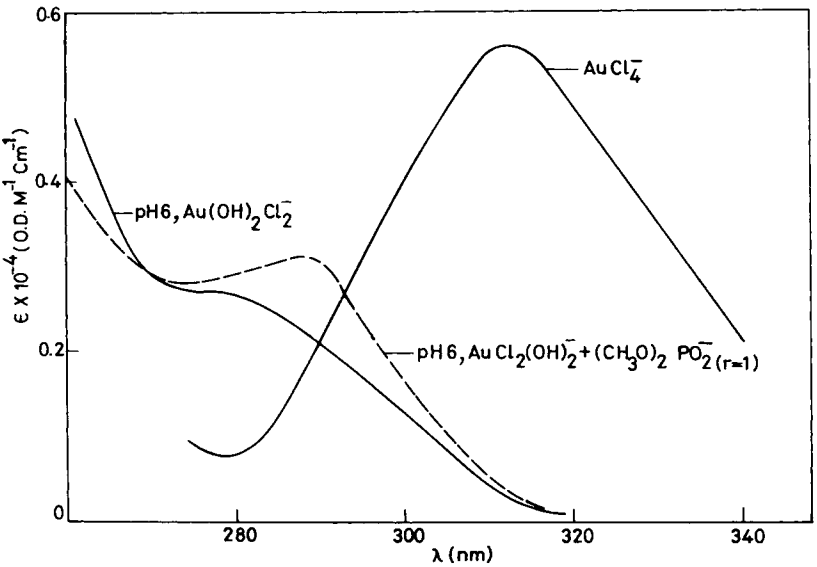


Fig. 6. Change in charge transfer band of $AuCl_4^-$ with pH of the medium and in presence of dimethyl phosphate.

not observable. This is presumably because of the fact that around pH 6 $AuCl_4^-$ exists as $AuCl_2(OH)_2^-$, which when ionized to $AuCl_2^+$ facilitates the reaction of two oppositely charged species, such as $AuCl_2^+$ with $(CH_3O)_2PO_2^-$. In fact, a drastic increase in pH in the solution was noticed when dimethyl phosphate was added to $Au(OH)_2Cl_2^-$.

Further proof of the interaction came from ir and ^1H nmr studies. Infrared spectra of the Au(III)-dimethyl phosphate were taken in the solid phase, which was isolated by reacting the Au(III) ion with barium salt of dimethyl phosphate. Figure 7 shows the perturbation of the 1250 cm^{-1} band assigned to P=O of dimethyl phosphate.[31] The change in the P=O band indicates a coordination through phosphoryl oxygen.

The change in ^1H nmr spectra was found to be quite interesting (Fig. 7). Dimethyl phosphoric acid shows two doublets with an integration ratio 1:2 and P-C-H coupling constant for both cases was 10 Hz. In the Au(III) complex of dimethyl phosphate, only one doublet results with the same J value.

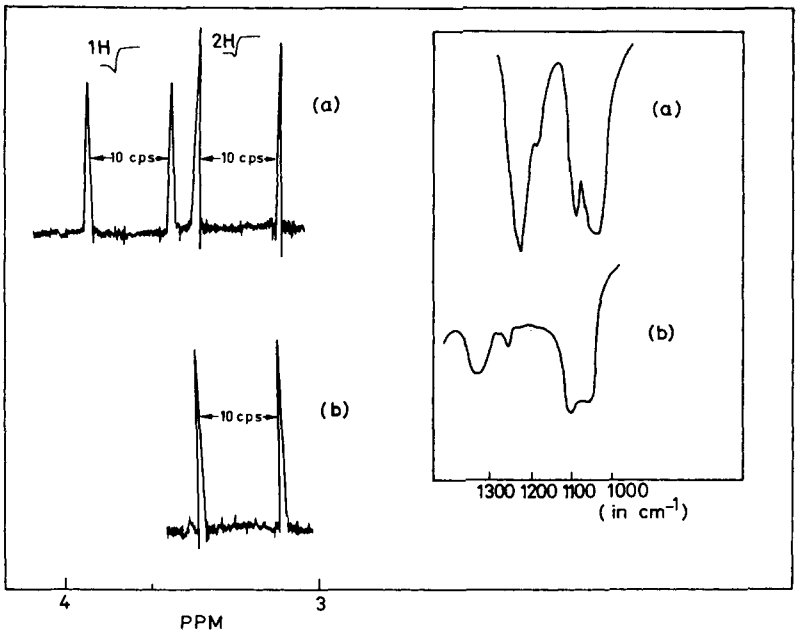
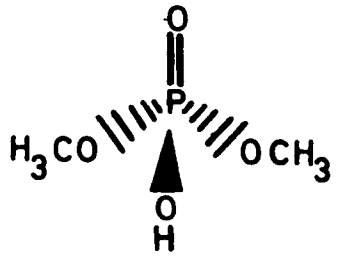
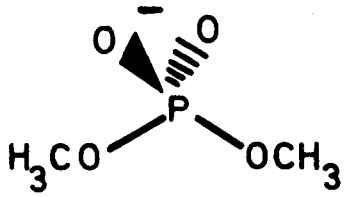


Fig. 7. ^1H nmr spectra of (a) dimethyl phosphoric acid, (b) (1:1) dimethyl phosphoric acid-$Au(Cl)_2(OH)_2^-$ at pH 6 in D_2O using sodium 2,2,dimethyl-2-silapentane-5-sulfonate as an internal standard. Inset shows the change ir spectra of (a) Ba-Salt of dimethyl phosphate and (b) Au complex of Ba-Salt of dimethyl phosphate in dry KBr pellet.

Dimethyl phosphoric acid has C_{3v} symmetry with the structure



In anionic form the symmetry changes to C_{2v}:



The geometry of dimethyl phosphoric acid induces the restricted rotation around the O—CH_3 bond due to P=O, as a result of which one hydrogen of each CH_3 group is more deshielded than the other two hydrogens, causing two doublets. In the Au(III) complex the symmetry changes (C_{2v}), due

to the square planar geometry of the Au(III) complex, two CH_3 groups were symmetrical, resulting in only one doublet.

CONCLUSION

From the above results the following general conclusions can be drawn:

1. The shoulder in the visible region could not be detected with the pyrimidine nucleoside/(nucleotide). This indicates that Au(III) forms stronger complexes with the purine nucleosides and nucleotides than with the pyrimidine nucleosides and nucleotides. Though the base free phosphates can interact with Au(III), the interaction is not as strong as the nitrogen-containing ligands.

2. A nucleotide always acts as a bidentate ligand through ring nitrogen and phosphate. Nucleosides act as a monodentate ligand except for Au(III)-guanosine in 1:1 ratio.

Spectroscopic studies with the Au(III) complexes of thymidine and methyl derivatives of nucleosides could not be performed in detail as these complexes are not stable in Me_2SO. This led us to study the kinetics of solvolysis and halide substitution to these complexes (results of which will be published elsewhere). The substitution was found to take place in a stepwise manner and follows the two-term rate law observed for d^8 square planar systems.[32] From the pK_a dependence of the rate constants, it was possible to identify the sites of coordination. These data are in good agreement with the results presented here.

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Received February 26, 1976
Accepted October 26, 1976