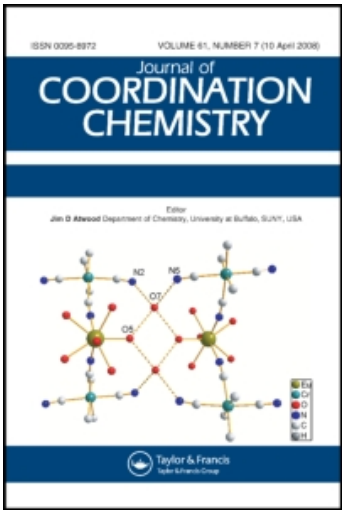

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REACTIONS OF THE RHODIUM TRIFLUOROACETATE DIMER WITH NUCLEOSIDES AND NUCLEOTIDES

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Complexes of rhodium trifluoroacetate with adenosine (ado), adenosine-5'-momophosphate (AMP), cytidine (cyd), guanosine (guo) and inosine (ino) have been prepared from aqueous or methanolic solutions. They were characterized by elemental analysis, magnetic susceptibility measurements, mass spectra, infrared, visible and nuclear magnetic resonance spectroscopies. In some cases their formation was followed by NMR prior to isolation. They all proved to be diamagnetic and in every compound contain two ligand molecules per rhodium dimer except for the ado complex where the ratio is 1:1. In the solid state it was found that adenosine interacts with the metal via the N(1) and N(7) atoms, its nucleotide through N(7), whereas in the case of cyd, guo and ino their exocyclic oxygen atoms take part in coordination. Solution study seem highly interesting since in some cases more than one species appear.

Keywords: rhodium; triflouroacetate; dimer nucleosides; nucleotides; complexes

INTRODUCTION

The discovery[1–5] that tetrakis (μ-carboxylato)dirhodium(II) complexes function as antitumour agents against many types of tumours has prompted investigations into the chemical properties and nature of complexes formed with ligands of biological importance.[6–10] Since they mainly act by inhibiting DNA and protein synthesis,[5, 7] their reaction with nucleic acid bases has gained considerable attention.[2, 6–7, 11–14] The dominant compound in these studies is the rhodium acetate dimer. It appears to react specifically only with adenosine and not with other nucleosides or polynucleosides.[2, 7, 14] Replacement of the methyl group by the highly electronegative function CF_3 enhances the Lewis acidity of the rhodium atoms so as to allow

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interactions with relatively weak donor molecules.[15] This kind of behaviour led us to investigate the complexing ability of the rhodium trifluoroacetate dimer, henceforth abbreviated as RTD, with nucleosides and nucleotides, in an attempt to form complexes other than those with adenine derivatives.

EXPERIMENTAL

Materials and Equipment

Rhodium trifluoroacetate dimer (RTD) was synthesized according to the literature.[16] Adenosine was purchased from Calbiochem, AMP from Sigma, guanosine and trifluoroacetic acid from Aldrich, cytidine from Carlo Erba and inosine from Theodor Schuchard GMBH. Deuterated DMSO and MeOH were from Aldrich, water from SIC and acetone from Jannsen. The non-deuterated water used was distilled.

^{1}H and ^{13}CNMR measurements were carried out on a 250 MHz Bruker spectrometer. Chemical shifts were referenced relative to TMS or DSS. The ambient temperature was 20° C. ^{19}FNMR spectra were obtained at 25° C on a 300 MHz Varian spectrometer with $CFCl_3$ as external reference. Infrared spectra were recorded on a Nicolet 205 spectrophotometer. Electronic absorption spectra were measured on a Shimatzu UV-160A instrument. Fast atom bombardment (FAB) mass spectrometry studies were performed at the Mass Spectrometry Laboratory of the Catholic University of Louvain, Louvain-la-Neuve, Belgium; the samples were dissolved in a *meta*-nitrobenzyl alcohol matrix. Elemental analyses were performed at the NCSR 'Demokritos', Analytical Chemistry Dept.; the fluorine determination was carried out at the National Centre of Scientific Research, Vernaison, France. Magnetic susceptibility measurements were performed on a Cahn/Vetron Faraday balance in combination with a Hewlett-Packard 3465A digital multimeter. Melting points were determined on a Gallencamp melting point apparatus.

Preparations

$Rh_2(CF_3CO_2)_4.Ado.2H_2O$

The complex RTD (0.5 mmol, 0.3290 g) was dissolved in 100 cm^3 of water and 0.5 mmol (0.1336 g) of adenosine was dissolved in 25 cm^3 of water. On mixing the solutions, with continuous stirring, a pink precipitate appeared. The mixture was stirred for *ca* 2 h to achieve complete precipitation and the product was collected by centrifugation at 10000 rpm. The solid was washed with water and dried at 110° C for 1 h and *in vacuo* at room temperature.

Yield: 0.2614 g (54%). Anal. calcd. for $C_{18}H_{17}N_5O_{14}F_{12}Rh_2$ (%): C, 22.49; H, 1.78; N, 7.28; F, 23.72. Found: C, 22.44; H, 1.50; N, 6.63; F, 23.92. Fast atom bombardment mass spectrometry (FAB-MS): highest peak 1581.9 for $Rh_2(CF_3CO_2)_4C_{10}H_{13}N_5O_4$–$Rh_2(CF_3CO_2)_4$ (minus H). The peak at 924 corresponding to $Rh_2(CF_3CO_2)_4$–$C_{10}H_{13}N_5O_4$ (minus H) also was observed. Decomposition point: 232° C.

$Rh_2(CF_3CO_2)_4.(AMP)_2.10H_2O$

The complex RTD (0.5 mmol, 0.3290 g) was dissolved in 100 cm^3 of water and 0.5 mmol (0.1942 g) of the monosodium salt of 5'-adenosinomonophosphoric acid (AMP) was dissolved in 2 cm^3 of water. Upon mixing the solutions the colour became pink. The solution was stirred for a few hours and the solvent was removed *in vacuo* at 45° C. The product was washed with water and acetone several times. It was then dried *in vacuo* at room temperature in a desiccator over P_2O_5. Yield: 0.1227 g (31%). Anal. calcd. for $C_{28}H_{46}N_{10}O_{32}F_{12}P_2Na_2Rh_2$ (%): C, 21.33; H, 2.94; N, 8.88; F, 14.46. Found: C, 21.57; H, 2.10; N, 7.99; F, 14.34. The product was insoluble so that no FAB-MS spectrum could be obtained. Decomposition point: 228° C.

$Rh_2(CF_3CO_2)_4.(Cyd)_2$

The complex RTD (0.25 mmol, 0.1645 g) was dissolved in 5 cm_3 of methanol and 0.5 mmol (0.1216 g) of the nucleoside was added. The solution was left to stir. Some 2 days later all of the cytidine was dissolved and the solution colour had changed from blue to mauve. It was then filtered, concentrated *in vacuo* and passed through a microcrystalline cellulose column. A blue band (unreacted RTD) was eluted by CH_2Cl_2, while the product was obtained as a mauve band with methanol. The mauve band was collected and the solvent removed under reduced pressure. The blue residue was lyophilised over P_2O_5. Yield: 0.2245 g (78%). Anal. calcd. for $C_{26}H_{26}N_6O_{18}F_{12}Rh_2$ (%): C, 27.29; H, 2.29; N, 7.34. Found: C, 26.77; H, 2.71; N, 7.28. FAB-MS: highest peak 1144.4 for $Rh_2(CF_3CO_2)_4$–$(C_9H_{13}N_3O_5)_2$. Decomposition point: 273° C.

$Rh_2(CF_3CO_2)_4.(Guo)_2.2H_2O$

In this case, 1 mmol (0.2832 g) of guanosine was added in a methanolic solution of RTD (0.5 mmol, 0.3289g of RTD in 12.5 cm^3 of methanol). The solution was left to stir, filtered after two days, concentrated to minimum amount and passed through a microcrystalline cellulose column. Unreacted RTD was eluted with

$CHCl_3$, whereas the reaction product eluted as a green band with methanol. The green band was collected and the solvent removed under reduced pressure. The green residue was lyophilised over P_2O_5. Yield: 0.4591 g (73%). Anal. calcd. for $C_{28}H_{30}N_{10}O_{20}F_{12}Rh_2$ (%): C, 26.68; H, 2.40; N, 11.11. Found: C, 25.91; H, 2.40; N, 10.89. FAB-MS: highest peak 1223.1 for $Rh_2(CF_3CO_2)_4$–$(C_{10}H_{13}N_5O_5)_2$ (minus H). Decomposition point: 280° C.

$Rh_2(CF_3CO_2)_4.(Ino)_2$

In this case, 0.25 mmol (0.1645 g) of RTD was dissolved in 5 cm^3 of methanol and 0.5 mmol (0.1340 g) of inosine was added. After 3 days stirring the nucleoside was completely dissolved and the colour of the solution became green. It was filtered, concentrated and passed through a microcrystalline cellulose column. Unreacted RTD was again removed with $CHCl_3$, whereas the product was eluted with $CHCl_3$:MeOH (2:1) as a green band. This was collected and the solvent removed under reduced pressure. The residue was lyophilised over P_2O_5. Yield: 0.1830 g (61%). Anal. calcd. for $C_{28}H_{24}N_8O_{18}F_{12}Rh_2$ (%): C, 28.16; H, 2.02; N, 9.38. Found: C, 27.59; H, 2.89; N, 9.89. FAB-MS: highest peak 1118.1 for $Rh_2(CF_3CO_2)_4$–$(C_{10}H_{12}N_4O_5)_2$ (minus $CFCO_2H$). Decomposition point: 290° C.

RESULTS AND DISCUSSION

Magnetic susceptibility measurements showed that all the complexes are diamagnetic. IR assigmments for RTD are: 1681vs, 1665vs, ν_{as}—Co_2; 1459w, ν_{sym}—CO_2; 1191vs, 1169vs, ν_{as}—CF_3; 860s, ν—CC; 786s, ν_{sym}—CF_3; 739s, δ—OCO; 540m, 525m, δ—CF_3; 383m, ν_{as}—RhO; 325m, ν_{sym}—RhO. The ^{19}F-NMR spectrum of RTD in methanol referenced to $CFCl_3$ as external standard gives a sharp singlet at –75.50 ppm, corresponding to the four equivalent bridging fluoroacetate groups.

$Rh_2(CF_3CO_2)_4.Ado.2H_2O$

IR data for the complex as well as the free ligand are given in Table 1. Band assignments were based on previous studies for ado[17, 19] and for RTD.[20] It is observed that the exocyclic amino group does not interact with the metal. Asymmetric and symmetric stretching vibrations of the C(6) – NH_2 group in the region of 3320 and 3160 cm^{-1} shift toward higher wavenumbers. If a bond through this group existed then the shift should be in the opposite direction.[21] The spectrum also shows that the C=N and C=C bonds have a more pronounced

double bond character than in the free ligand, since their bands are shifted to higher frequencies in relation to their positions in the IR spectrum of the free ado.[17] Such shifts suggest that coordination of the nucleoside to the rhodium dimer takes place through a ring nitrogen atom.[17] The bands corresponding to CF_3 vibrations of free RTD remain unaltered after complex formation.

TABLE I Infrared data for the RTD complexes (cm^{-1}). Bands corresponding to the complexed RTD molecule are depicted in italics*

Adenosine	*RTD-adenosine*	*Band Assignments*
3335s, 3168s	3478s, 3382s	ν_{as}—NH_2, ν—OH, ν_{sym}—NH_2
2920m, 2843m	2925w, br	ν—CH
1667s	1664vs	δ—NH_2, ν_{as}—CO_2
1650s, sh, 1605s, 1573s	1650s, sh, 1605s, 1588sh,	ν—C=C, ν—C=N, ring vibration
1537w, 1475m, 1455m, sh,	1495w, 1462m, 1432w,	
1415m, 1388w, 1333m, 1303s	1334m, 1324m, sh	
1210s	1210s, sh	ν—C_6—NH_2
	1192vs, 1165sh	ν_{as}—CF_3
1109s, 1071s, 1056s, 1038s	1114m, sh, 1089m, 1054m	γ—OH ribose ring
1011m, 978m, 905m	1013m, sh, 980w, 900w	ribose ring
	*861*s	ν—*CC*
	*785*m	ν_{sym}—CF_3
	*740*s	δ—*OCO*
	*540*m, *525*m	δ—CF_3
AMP	*RTD-AMP*	
3416s	3473s	ν_{as}—NH_2
3377s	3419s	ν_{sym}—NH_2
3276s, 3219s	3260sh, 3160sh	ν—NH_2, 2xδ—NH_2
3205s, 3145s	3160sh	ν—C_8 — H
2970m	2950m	ν_{as}—CH_2
2900m	2925w, sh	ν_{sym}—CH_2
2784m, sh, 2720m, sh	2725w, sh	ν—CH
1685sh, 1656s	1693s, 1658s	δ—NH_2
	1683s, 1667s	ν_{as}— CO_2
1607s	1606m	ν—C_5—C_6, δ—NH_2
1577m	1578m, sh	ν— C_4—C_5, ν—N_3—C_4—C_5
1510w		ν—C_8—N_7, δ—C_8—H
1480m	1493m	δ—C_8—N_7, ν—C_8—N_9, δ—C_8—H, δ—C_2—H
1425m, 1398w	1428m, 1405w, sh	ν—N_1—C_6—N_6
1379w	1389w, sh	ν—pyrimidine ring
1337m	1357m, sh	ν—C_8—N_9, ν—C_2—N_3, ν—C_5—N_7
1305m	1334m	δ—C_8—H , ν—N_7—C_8
1240m, sh		ν—N_7—C_8—N_9, ν—C_2—H, δ—N_1—C_8
1214s	1215sh	ν—N_7— C_8, δ—C_8—H
	*1193vs, 1170*s	ν_{as}—CF_3
1175s		ν—C_8—N_7, δ—C_6—NH
1105s	1110w, sh	ν —C —O
1084s, 1067s, 1055s	1063m, br	γ_{as}—PO_3
989m	994w	γ_{sym}—PO_3
925w, sh, 865w, 810m	915w, sh, 870w, sh, 825w	ν—ribose phosphate
	860m	ν—*CC*

TABLE I (*Continued*)

AMP	*RTD-AMP*	
790m	788w, sh	ν—P—O
	*784*m	ν_{sym}—CF_3
760m		ν—ribose phosphate
	*739*s	*δ—OCO*
715m	720w, sh	breathing mode
640m, 610w	630w, sh	δ—NH, δ—C_6—NH_2
575w	570w, sh	PO_3 deg. def
	538m	*δ—CF_3*
525	525m	δ—NH, ribose def.
500m, sh	490sh	γ_{as}—PO_3
Cytidine	*RTD-Cytidine*	
3446s		ν—OH ribose
3349s	3385–3355s, br	ν_{as}—NH_2
3259s		ν_s—NH_2
3233s		ν—N—H
3088s	3114s	ν—CH_2 ribose
2963s		ν—C—H
2952s	2950s	ν—C—H
2920s		ν—C—H
2896s		ν—C—H
1693m, sh		δ—NH_2
	1678s	ν_{as}—CO_2
1660s		ν—C_2=O
1647s	1650s	
1605s	1570s	ν—C_5=C_6
1532s		δ—N—H in plane
1500s	1508s	ν—C_4=N_3 + ν—C_4—N_4
1464m, sh	1460w	ν—C_4—N_3 + ν—C_2—N_3
1432m		ribose
1404m	1423m	ribose
1397m, sh		
1380m	1404m, sh	δ—C=CH + ribose
1343w		ribose
1311m	1331w, br	ribose
1291s	1286m	ν—C_2—N_1 + ν—C_6—N_1 + ribose
1250m	1228m, sh	ν—C_4—N_4 + ribose
1213m		ribose
1193m	1201s	ν—C_1, —N_1 + ν_{as}—CF_3
1155m		ν—C—O + ribose
1136s	1137s	ribose
1102s	1106s	r—NH_2
1055s	1059s	δ—N_1—C_1,—H
1035s	1039m sh	ribose
985m	995w	δ—C_4—C_5—H + ribose
945m	950w	ribose
878m	900w	ribose
855m	869m	δ—C—N_1—C_1,
845w		δ—C_2, —C_1, —O_4,
817s	841m	δ—N—H out of plane
791s	803m	breathing mode
757w	786m	ν-ring + ribose
736m, sh	775m	δ—C_2, —C_1, —N_1

TABLE I (*Continued*)

Cytidine	*RTD-Cytidine*	
	*743*s	*δ—OCO*
716s	724s	ν—C_5—C_4—N_4
664w	681w	
630		ribose
618m	628w	δ—N_1—C_1, —O_4
600m	604w	ν— C=O in phase
	*533*w	*δ—CF_3*
550m	516w	δ—C_2—N_3=C_4 + δ—N_1—C_2—N_3
Guanosine	*RTD-Guanosine*	
3575m		ν_{as}—NH_2
3466s	3500–3300s, br	ν—OH
3327s		ν_s—NH_2
3217s	3228s, sh	ν—CH
2970m, 2930m, 2900m, 2860m	2937m	ν—NH
	2880m, sh	
2740m	2760sh	ν—CH
1733s, 1693s	1674s	ν—C=O1
	*1677*s	*ν_{as}—CO_2*
1641s	1651s	δ—NH_2
1625s	1636s	ν—C_2—N_2
1610s, 1575m	1610m, sh, 1585m, sh	ν— C_2=N_3
1538s	1538m, 1533m	ν—C=C
1488s	1487m	ν—N_7=C_8 + ν—C_8—N_9
	*1456*m	*ν_s—CO_2*
1425m	1428m	δ—N_7=C_8—H + ribose
1397s, 1375m, 1353m	1398m, 1386m, 1361m, 1338w	ribose
1338m, 1322m, 1279w		
1261m		ν—C_5—N_7 + ν—C_4—N_9
1248m, 1228m		ribose
1210w		ν—C_2—NH_2
1180m	1202s	ν—C_1, —N_9 + ν_{as}—*CF_3*
1131s, 1083s, 1064m	1137s, 1086m, sh	ribose
1050s	1056m	δ—N_9—C_1, —H
1033m	1033m	r—NH_2
1019m	1021m, sh	ribose
1005m	990w	δ—O_4, —C_1, —H
919m, 900m	910w, 888w	ribose
881m	871m	ribose + δ—N_9—H out of plane
857w		ribose
827m	841m	δ—C—N_9—C
807m	801m	ribose plus; νs—*CF_3*
776s	778m	δ—N_1—H
748m	743m	ribose
736m	722m	δ—O_4, —C_1, —N_9 + *δ—OCO*
713s		ribose
695s	680w	*δ*—C_2, —C_1, —N_9
688s	671w	*δ*—ring
653m, sh		breathing + ribose
Inosine	*RTD-Inosine*	
3543m, 3307s	3405s, br	ν—OH, H_2O
3147s, 3118s, 3085s, 3062s	3132m, sh	ν—N_1—H

TABLE I (*Continued*)

Guanosine	*RTD-Guanosine*	
2904s	2940m, sh	
2743s		ν—CH
1705s, 1694s	1669s, 1641s, sh	ν—C=O+ ν—C_6—C_5
1594s	1592s	ν—C=N + ν—C_4=C_5 + ν—N_1—H + ν—C_6—N_1
1553s	1567m, sh	ν—C—N + ν—(C_6=O)(C_4=C_5)
1522m	1519w	
1475m, 1468m	1470m	δ—C_8—H + ν—N_7—C_8
1427s	1431m	ν—N_7—C_5
1381m, 1353m, 1321m	1384w, 1345w, 1295w	ν—ring
1248s		ν—C_6—N_1 + ν—N_7—C_5
	*1202*s, *br*	ν_{as}—CF_3
1226s, 1200m, 1171m, 1135s	1161s, 1137s,	ν—ring
1120s, 1085s, 1056s, 982m	1102m, 1087m, 1059m, 986w	ribose
	*869*m	ν—*CC*
	785w	ν_{sym}—CF_3
	*742*m	δ—*OCO*
	*531*w	δ—CF_3

*Abbreviations: s, strong; vs, very strong; m, medium; w, weak; sh, shoulder; br, broad; ν, stretching; δ, bending; as, assymmetric; sym, symmetric; γ, deformation.

Rhodium(II) carboxylates form 1:1 or 1:2 adducts with molecules containing electron donor atoms and ligands occupy the two axial positions. Visible spectra of the complexes depend on the nature of the donor atom. [6, 11, 16, 22] The compounds formed are green or blue-green with oxygen donor ligands, rose-red, violet or pink with nitrogen donors and violet or orange with sulphur donors. The pink colour of the isolated compound RTD.Ado.$2H_2O$ is in agreement with an axial nitrogen donor ligand. Electronic spectra of RTD in some solvents have been discussed before.[23] Its visible spectrum in methanol consists of two bands at 576 nm (ε = 177 M^{-1} cm^{-1}) and at 459 nm corresponding to $\pi^*(Rh_2) \rightarrow \sigma^*(Rh_2)$ and $\pi^*(Rh_2) \rightarrow \sigma^*(RhO)$ transitions, respectively. The lower energy band exhibits a blue shift upon complexation with the nucleoside (λ_{max} = 571 nm, ε = 169 M^{-1} cm^{-1}) whereas the other band appears as a shoulder at 454 nm.

NMR spectra of the isolated compound show that complex behaviour varies in different deuterated solvents. DMSO replaces adenosine, so the ^{1}HNMR spectrum of the complex in DMSO shows no proton shift for adenosine hydrogen atoms (Table II).

TABLE II ^{1}H and ^{13}CNMR chemical shifts (ppm downfield TMS) of RTD-ado complex

Compound	*H(8)*	*H(2)*	*H(1')*	*Solvent*	*C(6)*	*C(2)*	*C(4)*	*C(8)*	*C(5)*	*C(1')*
Ado	8.35	8.14	5.88d	$(CD_3)_2SO$	156.2	152.4	149.1	140.0	119.4	88.0
RTD-ado	8.36	8.14	5.88d	$(CD_3)_2SO$						
RTD-ado	9.55	8.99	6.54	$(CD_3)_2CO$	158.3	155.9	150.8	146.2		92.0
Ado	8.30	8.17	5.96d	CD_3OD		153.5	142.0			91.2

TABLE II (*Continued*)

Compound	*H(8)*	*H(2)*	*H(1')*	*Solvent*	*C(6)*	*C(2)*	*C(4)*	*C(8)*	*C(5)*	*C(1')*
RTD-ado										
fresh	9.00sh	8.77br	6.33	CD_3OD		155.9				92.0
After 3 days	9.00sh	8.77br	6.33							
	9.61	8.05	6.20sh			147.7		144.1		91.7
		8.17sh	5.90sh							
After 14 days	9.61	8.03	6.19d			147.7		144.1	123.8	91.7
	8.75	8.18	6.23d			156.1		146.3		91.2
	8.73	8.82	5.99d			148.1		143.4		93.2
	8.70	8.14	5.86d							92.1

In deuterated acetone H(8) and H(2) shift by 1.20 and 0.85 ppm downfield (Table II) compared to free adenosine in DMSO; adenosine is insoluble in acetone and all efforts to obtain an ^{1}HNMR spectrum in this solvent were fruitless. Since DMSO and acetone have almost the same magnetic susceptibility it is possible to compare results between these two. Adenosine can act as a monodentate or a bidentate ligand, with N(1) and N(7) being the preferred binding sites.[24] Interaction through N(7) is characterized by a downfield shift of the H(8) and small or no shifts of H(2) and H(1').[25–28] When the metal binds to N(1), H(2) exhibits a greater downfield shift than H(8),[29–30] while simultaneous coordination of the metal to N(7) and N(1) atoms result in similar shifts for the two purine protons.[31–33]

In the case of the adenosine adduct of RTD in acetone-d_6 the purine shifts indicate that both N(1) and N(7) sites coordinate to Rh(II). Simultaneous binding through the two purine nitrogens is verified also by the ^{13}CNMR spectrum of the complex in deuterated acetone (Table II). The greatest shift is exhibited by C(8) which moves 6.2 ppm downfield, followed by C(1') and C(2) (4.0 and 3.5 ppm, respectively) and C(6) (2.1 ppm downfield). The C(5) atom is not seen; its signal is probably hidden in the background. Similar shifts have been found after the reaction of Pt(II) with ado[34] where binding through N(1) and N(7) was suggested. The CF_3 carbon atom appears as a quadruple peak centred at 111.0 ppm (J_{C-F} = 284 Hz) and the CO_2 carbon atom of the rhodium dimer is found at 177.1 ppm.

Reaction of the rhodium acetate dimer with adenine nucleoside and nucleotides[11] resulted in the formation of 1:1 adducts, having a polymeric bridging structure, involving both axial positions of the rhodium dimer and both N(1) and N(7) sites of the adenines. Analytical results, (1:1 complexes) the IR (non-involvement of the NH_2 group) and NMR spectra (simultaneous coordination through N(1) and N(7) atoms) are similar to those found for our RTD adduct. We thus propose this structure as the most probable for the ado complex with RTD. The polymeric structure is also evidenced by the FAB-MS spectrum of the

isolated compound. Here, except for the main peak of the complex (m/z = 924, M–H) we observe a peak at m/z = 1581.9 corresponding to one molecule of the nucleoside bound to two molecules of RTD and of course different fragments of the latter.

Methanol-d_4 seems to be a most interesting solvent, where more than one complexes appear with their concentrations being time dependent (Table II). When the solution is fresh, H(8) and H(2) shift by 0.70 and 0.60 ppm downfield compared to the free ligand in methanol, indicating that ado interacts via N(1) and N(7) with Rh(II). After 3 days in solution we observe new peaks at 9.61, 8.05 and 6.20 ppm, corresponding to H(8), H(2) and H(1′), of a new complex with the ligand interacting with Rh(II) only through N(7).[25–28] The H(8) downfield shift is 1.31 ppm whereas H(2) shifts upfield by only 0.12 ppm. A small quantity of a third new complex seems to be formed, whose peaks appear as shoulders at 8.17 and 5.90 ppm for H(2) and H(1′). As H(8) is not seen, no conclusion can be drawn about the site of interaction. The four ribose carbons C4′, C3′, C2′ and C5′ (not depicted in Table II), show the existence of two different complexes, as two peaks are observed for each of them. They appear at 88.2, 87.9 (C4′), 76.1, 77.2 (C3′), 72.6, 71.8 (C2′) and 63.3, 62.3 ppm (C5′). These carbon atoms in the non-complexed nucleoside dissolved in deuterated methanol appear at 88.2, 75.5, 72.7 and 63.5 ppm, respectively. The solution reaches equilibrium some days later and the NMR spectrum after 14 days remains unchanged even when recorded 3 months later. At equilibrium the H(1′) and the ribose carbons show that 4 different complexes exist in solution as 4 peaks correspond to each of them (Table II). The other ribose carbons of the four complexes appear at the following field values: 87.9, 87.7 86.7, 88.7 (C4′), 77.2, 76.9, 76.4, 75.7 (C3′), 71.8, 72.2, 72.7, 71.5 (C2′) and 62.2, 63.3, 63.5, 62.9 ppm (C5′). They exhibit downfield or upfield shifts which range between 0.0 and 2.0 ppm. Unfortunately no clear conclusions about the interaction site can be obtained from the ^{13}CNMR spectra since most of the purine carbon atoms are not seen in methanol.

The ^{19}FNMR spectrum of the complex in methanol shows the existence of bidentate CF_3CO_2 groups as only one peak appears at –75.50 ppm. This peak retains the same position as in the RTD ^{19}F-NMR spectrum, but is broader.

$Rh_2(CF_3CO_2)_4.(AMP)_2.10H_2O$

Infrared data for the nucleotide and its complex are presented in Table I. The most characteristic bands are those due to the purine ring, the phosphate group and the phosphoric ester in the regions 1700–1500, 1100–900 and 820–800 cm^{-1}, respectively. Their assignment was based on previously published studies.[35–37]

The monosodium salt of adenosine-5′-monophosphate shows a strong absorption at 1656 cm^{-1} with a shoulder at 1685 cm^{-1} due to NH_2 bending and two other

bands at 1607 and 1577 cm^{-1} due to stretching of C_5—C_6 and C_4—C_5 respectively, and are strongly coupled. Upon complexation the first band remains practically unaltered, while the shoulder becomes sharper and is displaced towards higher frequencies by some 8 cm^{-1}. In the same region we observe two new bands at 1683 and 1667 cm^{-1}, which correspond to the asymmetric stretching vibration of the RTD carboxyl group. The band intensity at 1607 cm^{-1} is considerably less, while the fourth band becomes a shoulder at 1578 cm^{-1} losing intensity as well. In the IR spectrum of $[NiAMP.(H_2O)_5].H_2O$ whose structure determination proved coordination through N(7),[38] the band at *ca* 1650 cm^{-1} was shifted a few wavenumbers to higher frequencies, while the absorption bands at *ca* 1610 and 1580 cm^{-1} did not show any considerable change. According to several investigators[35, 39–42] complex formation through N(7) of the purine ring of adenine nucleosides or nucleotides decreases the intensity of the band found in the region of 1610 cm^{-1}. Thus it seems reasonable to suggest that coordination takes place through the N(7) atom. Moreover, the possibility that the metal ion interacts with N(1) is excluded. It has been suggested that N(1) coordination causes a split of the absorption band at 1577 cm^{-1} to yield a doublet at higher frequencies.[43] No split or shift was observed for this band upon interaction with the metal. Changes upon complexation of the absorption bands at 1510 and 1480 cm^{-1} indicate that the metal coordinates with the N(7) atom.[35, 37] The absorption bands at 1305, 1240, 1214 and 1175 cm^{-1} show that the electron distribution of the imidazole ring changes upon complexation, such distortion being expected by metallation to the N(7) site.[37]

The phosphate group of the monosodium salt of 5′-AMP exhibits three absorption bands between 1090 and 1050 cm^{-1} (strong bands related to PO_3^{2-} degenerate asymmetric vibration), at 989 cm^{-1} (medium band assigned to PO_3^{2-} degenerate symmetric vibration) and at 790 cm^{-1} (medium band due to P-O stretching). It was found that these bands decrease in intensity as the pH of the solution is lowered,[44] that is, when the phosphate group is protonated. For the AMP complex the aforesaid bands appear weaker, suggesting that water molecules are probably hydrogen bonded to the phosphate group. Ni(II) and Ba(II) are indirectly bonded to the phosphate group through hydrogen-bonded water molecules in $NiAMP.6H_2O$ and $BaAMP.7H_2O$, respectively.[45, 38] Small shifts with no or some splitting were observed in the last two cases.

The weak absorption bands at 930–800 cm^{-1} assigned to sugar phosphate vibrations shift by 5–15 cm^{-1}. This is due to conformational changes around the phosphate-ribose bond upon direct or indirect phosphate coordination.[46]

Few modifications appeared in the out-of-plane deformation modes of NH_2 and NH at 650–520 cm^{-1}.

The AMP complex was insoluble, so no NMR spectrum could be recorded. Its formation was followed by NMR at different ratios, r, of nucleotide/metal.

The nucleotide was dissolved in D_2O and RTD in CD_3COCD_3. The two solutions were mixed a few minutes before recording the spectrum. The final solvent was a mixture of deuterated water and acetone in a ratio 9:2. We did not use water alone because the aqueous solubility of RTD is poor. Starting from the solution colour which became pink upon mixing the nucleotide with the metal, we have a first indication that the atom taking part in complexation is a nitrogen atom.[6, 11, 16, 22] Since the spectra were recorded at temperatures up to 45° C, the free AMP NMR spectrum was also recorded at 20 and 45° C. At room temperature (Figure 1) the peak values are 8.53(s), 8.20(s), 6.11(d), 4.75(t), 4.52(q), 4.39(m), 4.15(q) ppm for H(8), H(2), H(1′), H(2′), H(3′), H(4′) and H(5′), respectively (where s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet). Peak assignments were based on previous studies.[47–49] If the temperature is raised no great changes are observed for the intensity or the position of the peaks and the multiplicity remains. At the highest temperature used, the protons are found at 8.52, 8.24, 6.12, 4.75, 4.52, 4.38, and 4.14 ppm.

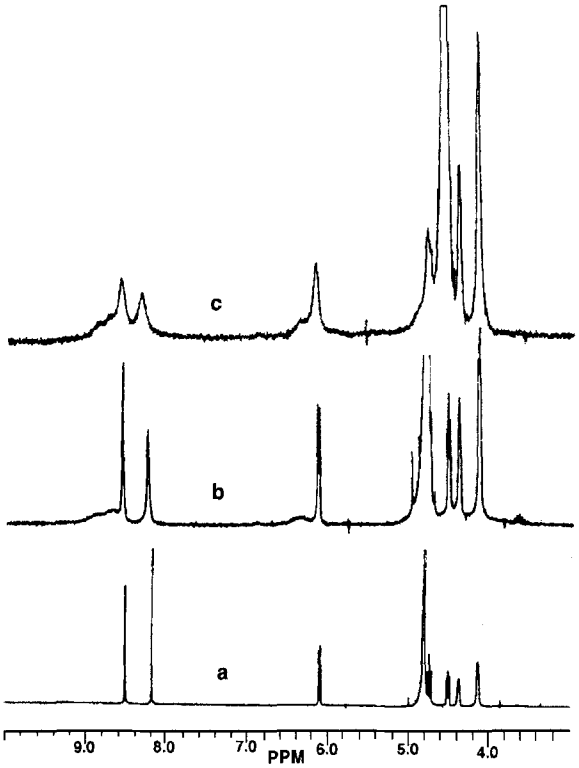

FIGURE 1 ^{1}HNMR spectra of (a) AMP at 20°C, (b) AMP:RTD = 3:1 at 20°C, (c) AMP:RTD = 3:1 at 45° C in D_2O/CD_3COCD_3 = 9/2 using DSS as standard.

When metal is added to the nucleotide solution at a ratio AMP:RTD = 3:1 (Figure 1), at 20° C, the two purine of the free nucleotide retain their positions (at 8.52 and 8.27 ppm) but appear broadened, with H(2) being most affected. At the same time the protons of the complex formed appear as broad peaks at 8.88, 8.75 and 6.40 ppm corresponding to H(8), H(2) and H(1′). The downfield shift of the purine protons (0.35 and 0.55 ppm), indicates coordination of the metal to both N(7) and N(1).[31–33] Integration of the peaks show that free nucleotide is three times more concentrated than the complexed. The peak broadening is explained by exchange between uncomplexed and complexed ligand, behaviour that is further verified by the temperature increase, where the peaks of uncomplexed AMP broaden more and shift a little downfield to 8.61, 8.35 and 6.20 ppm. Ribose peaks lose their multiplicity at 45° C. The ribose proton signals of the complex appear together with these of the free AMP furanose hydrogen atoms. In the case when AMP:RTD = 2:1 (Figure 2), we observe the same phenomena. One third of the nucleotide exists in the complexed form with its proton signals at 8.90, 8.74 and 6.40 ppm. Increasing temperature results in further broadening of the free AMP signals and increase in the quantity of complex. The ratio of complexed and uncomplexed AMP becomes 1:1.

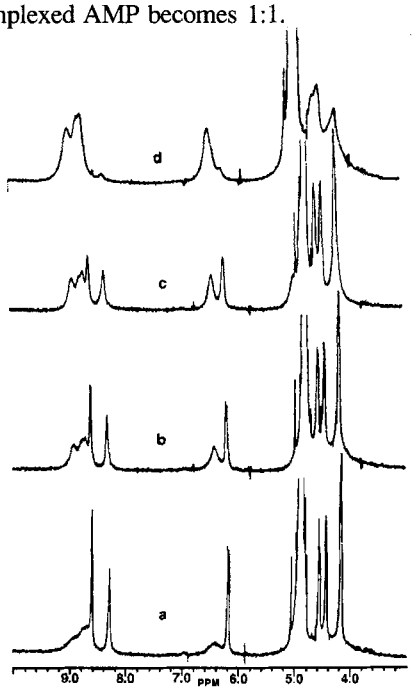

FIGURE 2 ^{1}HNMR spectra of solutions formed when RTD and AMP are mixed in D_2O/CD_3COCD_3 = 9/2 in different ratios r = [AMP]/[RTD] (a) r = 2:1 at 20°C, (b) the same at 30°C, (c) the same at 35°C and (d) r = 1:1 at 20°C.

Complexation of the nucleotide with the rhodium dimer results in downfield shifts, 0.42 and 0.52 ppm for the H(8) and H(2), respectively, at 20° C and for r = 1:1, indicating again interaction of the metal with both N(1) and N(7). This type of interaction is also supported by the ^{13}CNMR spectrum of the solution, which shows greatest downfield shifts for C(2), C(8) and C(6) (4.0, 3.6 and 1.5 ppm, respectively). Conclusions are based on previous studies which show that the signals of the carbon atoms adjacent to the coordination site exhibit greater downfield shifts compared with the uncomplexed ligand.[30–31] The peak values for all the carbon atoms are (in ppm downfield from DSS) 159.1 C(6); 158.9 C(2); 151.9 C(4); 146.0 C(8); 121.4 C(5); 91.5 C(1′); 86.4 C(4′); 77.3 C(2′); 73.2 C(3′); 67.6 C(5′). AMP gives rise to peaks at 157.6, 154.9, 151.2, 142.4, 120.8, 89.8, 86.7, 77.2, 73.1 and 66.9 ppm.

$Rh_2(CF_3CO_2)_4.(Cyd)_2$

The most common metal coordination site for cytosine, cytidine and its nucleotide is the N(3) atom.[50] Monodentate binding through O(2) or the exocyclic aminogroup, bidentate or bridging *via* N(3) and O(2) or N(3) and N(4) are also possible modes found in the literature.[50, 51] The infrared spectral data for cytidine and it's complex are reported in Table I. Band assignments were based on previous studies.[52, 53] The ν-C=O stretch shifts to lower frequency by 10 cm^{-1} and is observed at 1650 cm^{-1} indicating that the exocyclic oxygen atom is involved in coordination. The exclusive interaction of a metal ion with the O(2) atom of the cytosine ring has a very small effect on the ν-C=O vibration. No shift[54] or a small one to lower frequency[51, 55] as a result of the slight decrease of double bond character is observed for this type of coordination.

N(3) does not participate in bonding with the metal; the stretching vibrations at 1500 and 1464 cm^{-1} exhibit no significant shift or change in intensity. The exocyclic aminogroup does not bond to the metal as its rocking vibration remains unshifted, with no change in intensity, while the shifts observed for N-H in plane and out of plane bending are explained by the fact that the lone pair of electrons present on the nitrogen atom interacts with the ring.[56]

A methanolic solution of the complex exhibits one band at 570 nm (ε = 159 M^{-1} cm^{-1}) due to $\pi^*(Rh_2) \rightarrow \sigma^*(Rh_2)$. The colour is bluish, indicating interaction of the metal with an oxygen atom.[11, 16]

The ^{1}HNMR spectrum of the complex in deuterated methanol (Figure 3) shows the existence of four different complexes (characterized as **a, b, c** and **d** further on). The pyrimidine hydrogen atoms of cytidine in the same solvent appear as doublets at 8.10 and 5.95 ppm (H(6) and H(5)). In the complex solution the shifts exhibited for H(6) are 0.33, 0.29, 0.28 and 0.23 ppm downfield, respectively, for **a, b, c, d.**

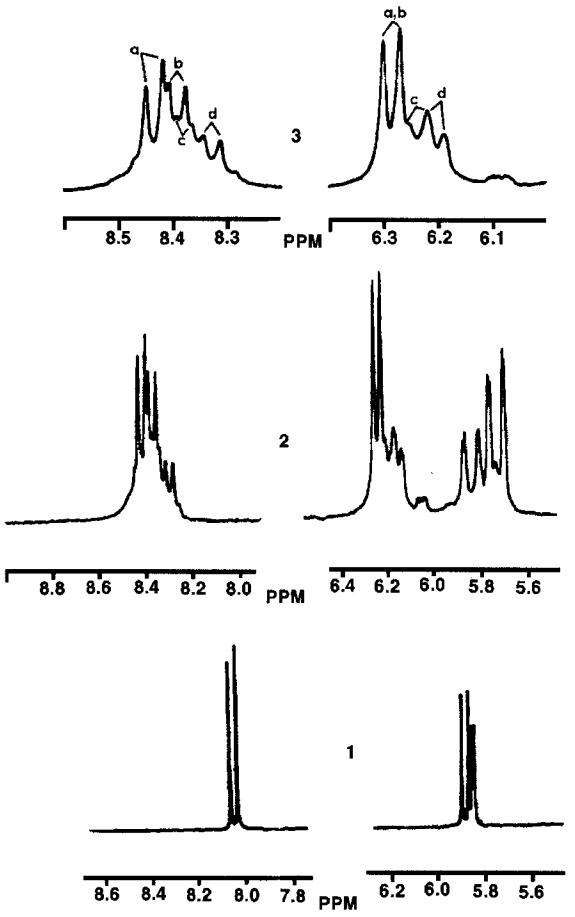

FIGURE 3 ^{1}HNMR spectra of RTD-cytidine complexes in MeOD-d_4 solutions; (1) free cytidine, (2) $Rh_2(CF_3CO_2)_4(cyd)_2$, (3) the H(5) and H(6) regions where the peak assignment for the four different species (**a, b, c, d**) is seen.

The ratio is **a:(b+c):d** = 4:2:1. Efforts to separate the four species using column chromatography were fruitless. Similar shifts to the H(6) atom were observed for H(5) atom as well, 0.36, 0.36, 0.28 and 0.25 for each complex, respectively. The shift assignments have been made on the basis of decoupling procedures.

Coordination of the metal to N(3) or O(2) or to both atoms of the cytidine molecule results in electron density withdrawal from about the same region of the ring. In all three binding cases downfield shifts for the pyrimidine hydrogens are expected. The downfield shifts of H(5), 0.33 ppm and of H(6), 0.11 ppm, upon complexation of cytosine with Ru(III)edta were attributed to simultaneous binding through N(3) and O(2).[57] In the same article the downfield shifts of the H(6) and

H(5) atoms of 2-thiocytosine (0.11 and 0.13 ppm, respectively) were also explained by interaction with N(3) and C(2)SH. In the complex K[Ru(edta)(cyd)] the shifts for H(5) and H(6) were 0.77 and 0.56 ppm. Coordination through N(3) and O(2) was suggested.[27] The shifts exhibited by the ribose atoms were less than 0.2 ppm. Generally similar downfield shifts of the two cytosine residue hydrogen atoms were explained by N(3) and O(2) binding. When the H(5) shift was greater than that of H(6) N(3) only coordination was suggested.[25, 40, 58] Cases where exclusive binding at O(2) takes place are rare.[51] The aforesaid remarks do not constitute a strict rule. There are cases where H(5) and H(6) were shifted to the same extent and no bidentate binding occurred.[33, 58–60] Solution studies of the cytosine derivatives appear to be complicated, because of the small distance between the two coordination sites N(3) and O(2). The two positions are electronically conjugated and the expected metal-O(2)-C(2) angle brings the metal ion close to the position expected when the metal binds to N(3). Hence ^{1}HNMR spectra alone are inadequate to ascertain metal binding sites in solution. A much better approach is by the aid of ^{13}CNMR spectra.

It is known that the shifts of the heteroaromatic ring carbon atoms are related to their electronic structures. The first spectroscopic criterion for distinguishing N(3) and O(2) as based on the direction of shift of the C(2) resonance.[61, 62] An upfield shift signified strong N(3) binding and a downfield shift was indicative of primary binding at O(2). Of course the resonances of the other pyrimidine carbon atoms should be taken into consideration, as there are reports of N(3) binding with the C(2) resonance exhibiting a downfield shift.[58]

The ^{13}CNMR spectrum of the complex in deuterated methanol (Figures 4, 5), verifies the existence of four species in solution. The resonance assignment for the species **a, b, c** and **d** has been made according to their relative intensity, with **a** being found in greatest proportion and **d** in smallest proportion. NMR values (in ppm downfield from TMS) for the four species are as follows; **a:** 170.4, C(6); 165.7, C(2); 142.0 C(4); 98.8 C(5); 93.1, C(1′); 86.0, C(4′); 76.9, C(3′); 69.6, C(2′); 60.7, C(5′); **b:** 170.3, C(6); 165.6, C(2); 142.0, C(4); 92.8, C(1′>; 86.3), C(4′); 76.8, C(3′); 69.9, C(2′), 60.8, C(5′); **c:** 170.1, C(6); 163.5, C(2); 143.3, C(4); 98.6, C(5); 93.0, C(1′): 85.9, C(4′); 76.6, C(3′); 61.0, C(5′); **d:** 162.9, C(2); 140.6 and 93.3, C(1′). In the same solvent, free cytidine exhibits peaks at 167.6, 156.3, 143.1, 95.8, 92.4, 85.8, 76.2, 70.7 and 61.9 for the carbon atoms 6, 2, 4, 5, 1′, 4′, 3′, 2′ and 5′, respectively. In all the cases the C(2) atom shifts to the greater extent (9.4, 9.3, 7.2 and 6.6 ppm downfield for **a, b, c** and **d**, respectively). It is thus concluded that a strong interaction exists between this carbon atom and RTD. The C(6) atom shifts upfield by about 2.5 ppm and C(5) by 3 ppm. The ribose carbon atoms exhibit non-uniform shifts of less than 1.3 ppm, indicating that the ribose ring does not participate in metal binding.

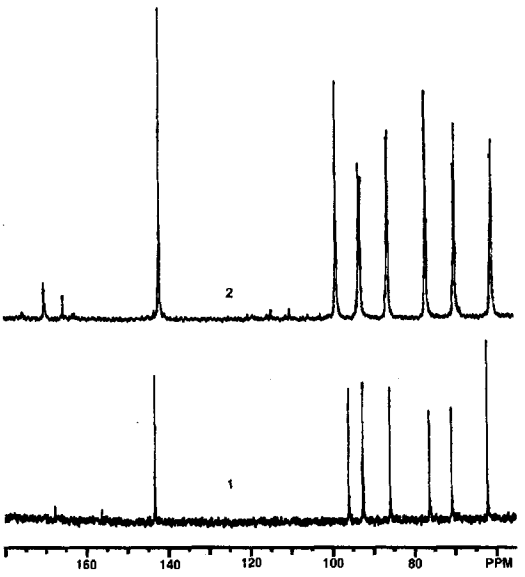

FIGURE 4 ^{13}CNMR spectra of RTD-cytidine complexes in MeOD-d_4 solutions; (1) free cytidine, (2) $Rh_2(CF_3CO_2)_4(cyd)_2$.

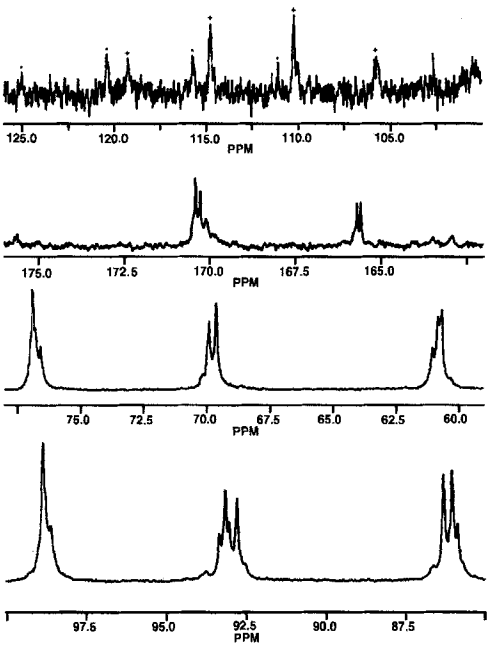

FIGURE 5 Extended regions of the spectrum (2) shown in Figure 4. The peaks marked + have J_{C-F} = 286 Hz, while those marked * have J_{C-F} = 292 Hz.

The small peak at 175.6 ppm corresponds to the rhodium dimer CO_2 carbon atom. Resonances observed in the region 105–125 ppm correspond to CF_3 group. The carbon atom appear as a quadruplet due to coupling with the flourine atom. In Figure 5 two such quadruplets are shown, indicating that two kinds of CF exist. The more intense is centered at 112.5 ppm (J_{C-F} = 286 Hz) and the other at 113.4 ppm (J_{C-F} = 292 Hz).

The ^{19}FNMR spectrum of the complex in methanol exhibits three peaks at –75.75, –75.17 and –75.08 ppm in the ratio 7:1.5:3. Assignment of these peaks is difficult; presumably, they correspond to mono- and bidentate $CF_3CO_2^-$.[16]

$Rh_2(CF_3CO_2)_4.(Guo)_2.2H_2O$

Potential coordination sites for the guanosine molecule are N(1), N(3), N(7) as well as the exocyclic O(6) atom and the aminogroup. In order to determine the actual donor group, i.r. spectra of the free and coordinated ligand (Table I) were compared. Band assignments are in agreement with previous publications.[19, 63]

The two strong bands at 1733 and 1693 cm^{-1} in the spectrum of free guanosine have been assigned to C(6)=O stretching. Upon metallation, the first band disappears while the second shifts to lower frequency by 19 cm^{-1}. The new strong band observed at 1677 cm^{-1} in the complex is due to the presence of asymmetric RTD CO_2 stretching. Bands in the region 1630–1540 cm^{-1} corresponding to C=N vibrations of the six-membered guanosine ring appear shifted to higher frequencies after complex formation, showing electronic enforcement of the purine ring system. The strong band at 1538 cm^{-1} loses intensity and splits.

The position of the ν-C=O vibration of the guanine moiety has been considered as being indicative of the coordination state of the nucleobase.[40, 64, 65] Specifically, any shift of this band to lower energy has been interpreted in terms of N(7), O(6) chelate formation when also accompanied by shifts of the C=C, C=N vibration bands. Hydrogen bonding of O(6) is sufficient to shift ν-C=O to lower frequencies and over-rides the expected shift (in opposite direction) on coordination of Pt(IV) at N(7) of guanine.[66] The i.r. spectrum of the RTD-Guo complex therefore suggests interaction of the metal with the exocyclic oxygen atom, either by a direct bond or through a hydrogen bond.

Only one band in the visible region (λ_{max} = 587 nm, ε = 81 M^{-1} cm^{-1}) was observed for a methanolic solution of the complex. The green colour of the solution indicates metal binding through an oxygen atom.[11, 16]

The ^{1}HNMR spectrum of the complex in methanol-d_4 is shown in Figure 6. The most significant atoms are H(8) and H(1'). The former, a singlet at 7.99 ppm, exhibits two peaks in the complex, while the second being a doublet centred

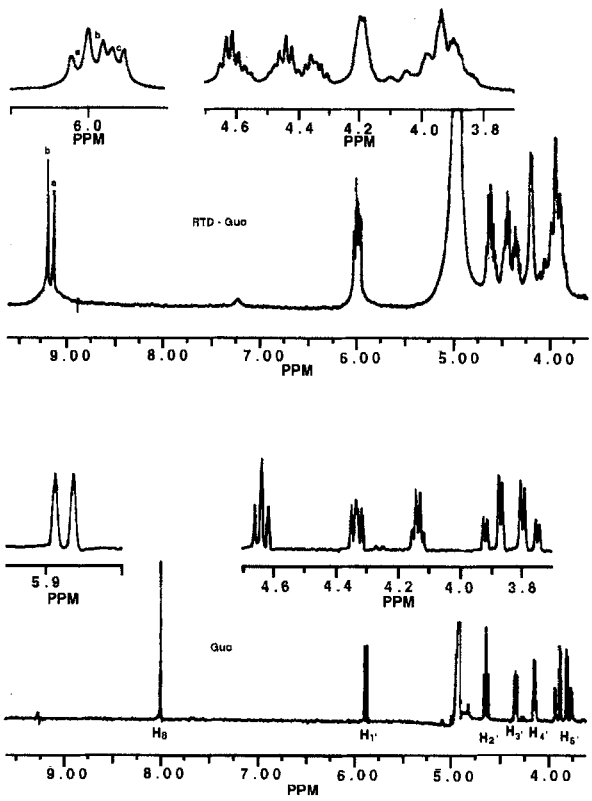

FIGURE 6 ^{1}HNMR spectra of free guanosine and its RTD complex in MeOD-d_4. By the letter a, b and c indicate the three species found in solution.

at 5.88 ppm when free, appears as three doublets after metallation. A possible explanation is the existence of three species, **a, b** and **c**, in solution; integration shows that they are found in about the same ratio, **a:b:c** = 4.9:5.3:5.7. These could not be rotameric forms since the difference in proton chemical shifts is rather large.[67] Proton-proton coupling constants of the metallated species compared with the free nucleoside are smaller. $J_{H1'-H2'}$ decreases from 5.9 Hz (free guanosine) to 5.3, 4.7 and 3.8 Hz (in the complexes **a, b** and **c** respectively).

The H(8) atom is found downfield (1.15 ppm for complex **a** and 1.21 ppm for **b**, relative to the free ligand. The same atom of the third complex is not seen probably because it merges with one of the purine protons of the mentioned two complexes. H(1') is also shifted downfield, but to a smaller extent, 0.13, 0.11 and 0.08 ppm, respectively, for **a, b** and **c**. The other ribose hydrogens do not exhibit shifts greater than 0.12 ppm. Since the largest chemical shift change was observed H(8), the ribose ring is excluded from metal binding.

In DMSO (Figure 7a) guanosine shows peaks at 10.61 (NH), 7.93 (H_8), 6.44 (NH_2), 5.68 ($H_{1'}$ with $J_{H1'-H2'}$ = 5.9), 5.38 ($OH_{3'}$), 5.11, 5.03 ($OH_{2'}$,. $OH_{5'}$,), 4.37 ($H_{2'}$) 4.07 ($H_{3'}$), 3.86 ($H_{4'}$) and 3.56 ppm ($H_{5'}$). The ^{1}HNMR spectrum of the complex in the same solvent (Figure 7b) is rather complex, showing the existence of four species in solution. The H(1') atoms of these species appear as four doublets centered at 5.88, 5.85, 5.83 and 5.78 ppm. $J_{H1'-H2'}$ values are 5.7, 5.2, 4.1 and 4.7 Hz respectively. H(8) of the four species gives two peaks at 9.06 and 9.01 ppm. The NH band disappears due either to deprotonation of N(1)H atom or to fast exchange with small amounts of D_2O present in DMSO-d_6. Three sharp peaks at 7.34, 7.14 and 6.94 ppm correspond to the NH_2 groups of the three species, while a broad peak is also observed at 7.55 ppm being probably the NH_2 group of the fourth isomer. The integrated intensity of the signal (two protons) rules out the possibility that a deprotonated amino group is involved in bonding to the rhodium moiety. Other signals are found at 5.66 ($OH_{3'}$), 5.33($OH_{2'}$., $OH_{5'}$), 4.45 ($H_{2'}$), 4.01 ($H_{3'}$), 3.75 ($H_{4'}$) and 3.42 ppm ($H_{5'}$) for all species. The addition of two drops of deuterated water to the solution of the complex Figure 7c results in the disappearance of NH_2 and OH peaks. The form of the H(1') peak remains the same in position and intensity. If we call the four species a, b, c, d, with a being the most abundant and a4 the least abundant, their ratio will a b c d are 2.6:1.8:1.6:1.5. The H(8) peaks appear at 9.04, 9.03, 9.00 and 8.99 ppm, respectively.

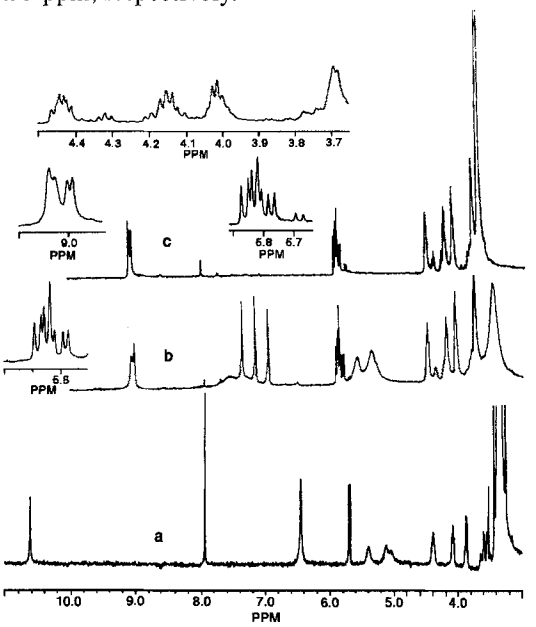

FIGURE 7 ^{1}HNMR spectra of (a) free guanosine in DMSO-d_6 (b) guanosine-RTD complex in DMSO-d_6 and (c) the same as in (b) plus two drops of D_2O.

The most frequently found metal binding position for guanine compounds is N(7). NMR studies show downfield shifts of adjacent H(8) signals.[68–72] Sometimes, shift in the opposite direction are also observed.[72] In some cases, where metallation occurs at the sixth position of the purine ring, the H(8) proton exhibits an upfield shift, especially when it is accompanied by deprotonation of N(1)H.[70, 72] Judging only from the H(8) proton shift we cannot be certain which site the purine ring offers for binding. More information can be derived from the ^{13}CNMR spectrum (Figure 8) of the solution.

The carbon atoms of guanosine in deuterated methanol appear at 159.2, 155.3, 152.5, 136.8, 118.4, 90.2, 87.3, 75.5, 72.3 and 63.2 being respectively C(6), C(2), C(4), C(8), C(5), C(1'), C(4'), C(2'), C(3') and C(5'). The spectrum of RTD-Guo shows two new peaks at 121.0 and 115.2 ppm which are attributed to CF_3 carbon atoms of the rhodium trifluoroacetate dimer. This peak is normally a quadruplet but in this case only the two stronger peaks are seen, with $J_{C—F} = 292$ Hz. The fact

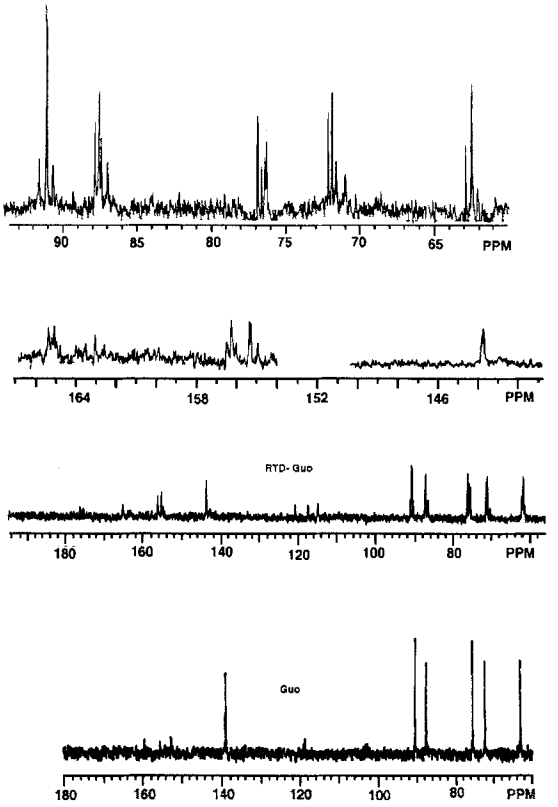

FIGURE 8 ^{13}CNMR spectra of free guanosine and its RTD complex in MeOD-d_4 with extended NMR regions of the latter.

carbon atom of the complexed RTD carbonyl group is observed at 176 ppm. The that four peaks appear for most of the ribose carbon atom shows the existence of four species **a, b, c** and **d**. Taking into account the ^{1}HNMR spectrum (Figure 6) where the H(8) and H(1') of the fourth isomer are not seen, we conclude that these bands are most probably hidden by peaks from the other three species. Shifts of ribose carbons lie between 0.1 and 1.4 ppm. More pronounced are these of the purine carbons with the C(6) and C(8) atoms exhibiting the largest. In the case of the most abundant complex, C(6) is shifted downfield by 15.8 ppm while the position of C(8) changes only by 7.0 ppm. It is concluded that metal is binding through the O(6) atom of the nucleoside. C(8) of the next complex was most affected (6.9 ppm downfield shift) by complexation, while C(6) was less shifted (5.8 ppm). The most probable binding site seems to be N(7) in this case.[71] The formation of a chelate through N(7) and O(6) is not excluded since the ^{19}F-NMR spectrum of the complex shows the existence of mono- and bidentate CF_3CO_2- (three sharp peaks at –75.76, –74.87 and –74.84 ppm in methanol at 25° C and with, relative intensities 4.2:2:1.5). The C(2) atom of both species shifts less than 1.0 ppm, so N(1) does not interact with the metal. For the two complexes found in the smallest quantity no conclusions can be derived since their purine carbon atom signals are not seen in the spectrum.

$Rh_2(CF_3CO_2)_4(Ino)_2$

Table I records i.r. data for the compound obtained with inosine. The assignment has been carried out according to the literature.[19, 36, 39, 73]

C=O bands show significant changes. The first appearing at 1705 cm^{-1} shifts to lower frequencies by 36 cm^{-1} and retains intensity probably because in the same region appears, the ν_{as}–CO_2 of the metal complex. The second band is found as a shoulder at 1641 cm^{-1} with reduced intensity and displaced downwards by 53 cm^{-1}. These variations, together with the fact that the band due to C=C and C=N ring vibration at 1592 cm^{-1} remains unaltered, point to C(6)=O coordination.[73]

Several studies have dealt with the carbonyl group as a possible binding site in complexes with inosine. Small shifts of the ν–C=O band shows that this group is not involved in metal coordination.[33, 36, 64, 74–76] On the contrary, considerable shifts to lower frequencies are explained by metallation at this position;[73] if at the same time significant changes in the ring bands take place, then bidentate coordination through the O(6) and a ring nitrogen atom is suggested.[31, 62, 64]

The bands observed at 1475 and 1427 cm^{-1} attributed to vibrations related with N(7) do not change appreciably on formation of the complex, corroborating the non-involvement of this atom in metal binding.

Methanolic solution of the RTD-inosine complex presents one visible absorption peak at 583 nm (ε = 91 M^{-1} cm^{-1}). Their green colours indicates interaction of the rhodium dimer with an oxygen atom of the nucleoside.[11, 16]

The ^{19}F–NMR spectrum of the inosine complex shows three singlets at –75.77, –74.90 and –74.89 ppm with relative intensities 1.9:1.4:1. These three signals result from the existence in solution of three chemically different types of coordinated CF_3CO_2 –.[16] The ^{1}HNMR spectrum of the complex in methanol–d_4 was difficult to interpret and since in the ^{13}CNMR spectrum the most critical peaks were not seen, we recorded the ^{1}H and ^{13}CNMR spectra of an aqueous solution of the nucleoside while adding the metal dissolved in acetone, with various ratios r = [ino]/[RTD].

Chemical shifts of the inosine protons upon addition of the rhodium dimer are seen in Figure 9. When r = 3:1 the H(8) peak loses half its intensity and shifts downfield by 0.03 ppm Figure 9b. Very small shifts (0.02 ppm) are also observed for H(2) and H(1') with no significant change in intensity. Recording of the spectrum 5 h later shows further shifts of the protons; 0.07 ppm for H(8) and 0.04 ppm for H(2) and H(1'). The reaction reaches equilibrium at this point when r = 2:1, Figure 9c broadening and decrease in intensity is observed for the H(8) peak. The displacement reaches 0.16 ppm, while at the same time the H(2) peak loses intensity and exhibits a downfield shift of 0.07 ppm. A similar shift (0.08 ppm) and broadening is observed for the H(1') ribose proton doublet. When r = 1:1 H(8) peak appears as doublet and is shifted by 0.23 ppm. H(2) atom loses intensity and is displaced by 0.11 ppm. H(1') shows similar changes to H(2). In all cases the other ribose hydrogen atoms do not exhibit significant changes. Their shifts do not exceed 0.03 ppm indicating that binding involves the purine part of the nucleoside.

Previous NMR studies have revealed the binding sites of inosine derivatives with metal ions. Large downfield shifts of the H(8) resonance with no or very little shift of the N(2) peak implicate metal binding to the N(7) atom.[25, 27–28, 75, 77–80] When the greatest shift is exhibited by H(2) coordination through N(1) is suggested.[77–80] In some cases N(1) coordination had no significant effect on the H(2) resonance, only the N(1)-H proton.[80] Simultaneous shifting of the two purine protons is usually attributed to the N(7), O(6) chelate.[81] It is difficult using only ^{1}HNMR results to clearly distinguish a N(7), O(6) chelate from an N(7) unidentate.[82] They both induce shifts of H(8) and H(2) signals, as charge redistribution can be transfered to the latter from N(7) through the purine ring or in the chelate case from O(6) to the six-membered ring.

Considering previous studies and our experimental data, we suggest that the main coordination site for the RTD-ino complex is the N(7) atom when r = 3:1. There is rapid exchange between the complexed and uncomplexed ligands and

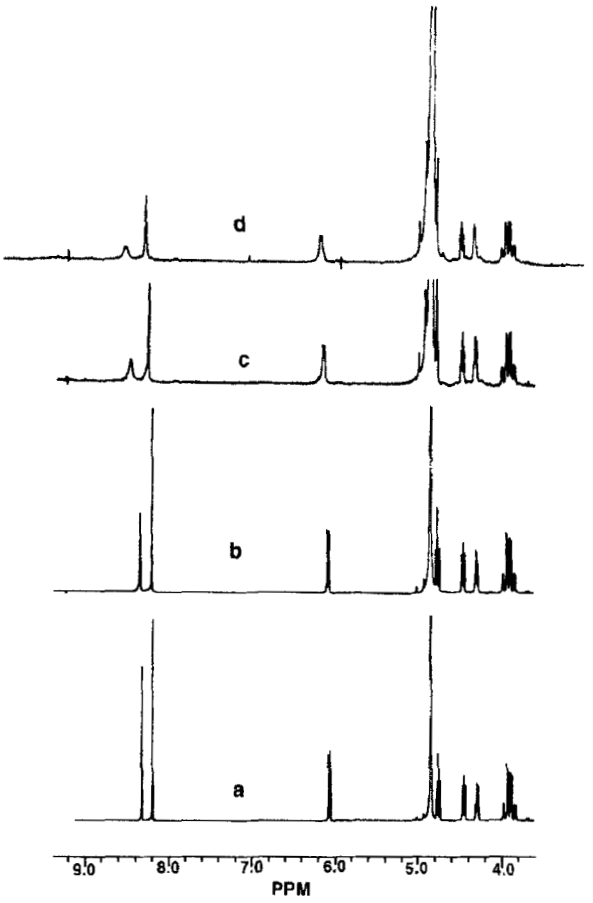

FIGURE 9 NMR spectra of solutions formed upon reaction of RTD (diluted in acetone-d_6) with inosine in D_2O with different ratios r = [ino]/[RTD]; (a) free inosine 0.05 M, (b) r = 3:1, (c) r = 2:1, (d) r = 1:1.

we record the result of the 'mean' situation. O(6) cannot be excluded from binding because the shift values for the aromatic ring protons are similar. These could be explained by the presence of non-complexed nucleoside ([free ligand]/[complexed ligand] = 3:1). When r is 2:1, then again the main coordination site seems to be the N(7) atom. The H(2) resonance decrease as well as its shift indicates that O(6) participates in binding. Chelate formation with N(7) and O(6) atoms seems unlikely since the H(8) and H(2) peaks are not equally influenced by the presence of the rhodium moiety.[81] As the i.r. spectrum of the isolated complex shows considerable displacement of the carbonyl stretching group and according to the elemental analysis the complex contains two ligand molecules

correspond per rhodium dimer, a probable explanation is that one inosine molecule is bound to the metal *via* N(7) and the other through O(6) with a rapid exchange between them as well as between the free ligand. This would also cause broadening of the proton signals. The same explanation holds for the third case where r is 1:1. Here the phenomena intensify and the H(8) resonance is split into two. In all cases the sugar hydrogens except that of H(1') remain almost the same, indicating that the ribose ring does not participate in metal bonding. H(1') exhibits shifts similar to H(2) thus implicating N(7) coordination.

^{13}CNMR spectra were also recorded Figure 10. The inosine spectrum shows signals at 161.13 (C6), 151.04 (C4), 148.79 (C2), 142.92 (C8), 126.85 (C5), 91.15 (C1'), 88.28 (C4'), 76.83 (C2'), 73.11 (C3') and 64.04 (C5'). For r = 3:1 we observe a decrease of the C(8) peak which also shifts downfield by 0.09 ppm. C(6) shifts upfield by 0.07 ppm. The shifts of the other carbon atoms are at the most 0.03 ppm. A few hours later, when reaction is complete the C(8) shift reached 0.22 ppm while that of C(6) was –0.14 ppm. The presence of a small peak at 92.49 ppm (corresponding to C(1') atom) indicating the presence of a second complex in the solution is noteworthy. When r = 2:1 the C(8) resonance diminishes significantly, the C(6) signal also decreases and the shifts exhibited are +0.50, –0.33 and +0.23 ppm for the C(8), C(6) and C(2) atoms, respectively. Quite important are the shifts of C(1') (+0.19 ppm) and C(4) (+0.16 ppm). The other carbon signals do not shift more than 0.11 ppm. New peaks emerge for the ribose carbons at 92.47 ppm (C1'), 77.74 ppm (C2'), 72.46 (C3') and 63.29 ppm (C5'), corroborating the existence of two species in solution. When r = 1:1, 5h after the metal addition, the shifts are +0.71, –0.57, +0.33, +0.18 and –0.09 ppm for C(8), C(6), C(2), C(4) and C(5), respectively. Carbon signals from ribose of the second complex appear at 152.97, 149.87, 149.78 and 148.20 ppm in addition to those already seen at r = 2:1. Bearing in mind the relative intensities of the purine atoms in inosine a possible assignment of these new signals could be for C(4), C(2), C(8) and C(6) or C(4), C(8), C(2) and C(6). The largest shift is experienced by the C(6) atom (–12.93 ppm), followed by the C(8) atom (6.86 or 6.95 ppm).

Reaction of Cu(II) with ino and 1-Me-ino in non-aqueous solutions, causes paramagnetic broadening of the carbon signals in order C(8) > C(4) > C(5) > C(6), while with IMP the broadening was in the order C(5) > C(4) > C(8) > C(6). C(2) and the ribose resonances were unaltered.[83] Binding *via* N(7) was suggested. Mn(II) ions influence to the same extent the IMP C(5), C(6) and C(8) signals indicating a O(6)—N(7) chelate.[84] O(6) binding is evidenced by pronounced C(6) shifts.[85] Complex formation at N(1) leads to significant effects on the carbon atoms adjacent to the site of complexation[80] while N(7) binding is accompanied by a reasonably small downfield shift of the C(8) resonance.

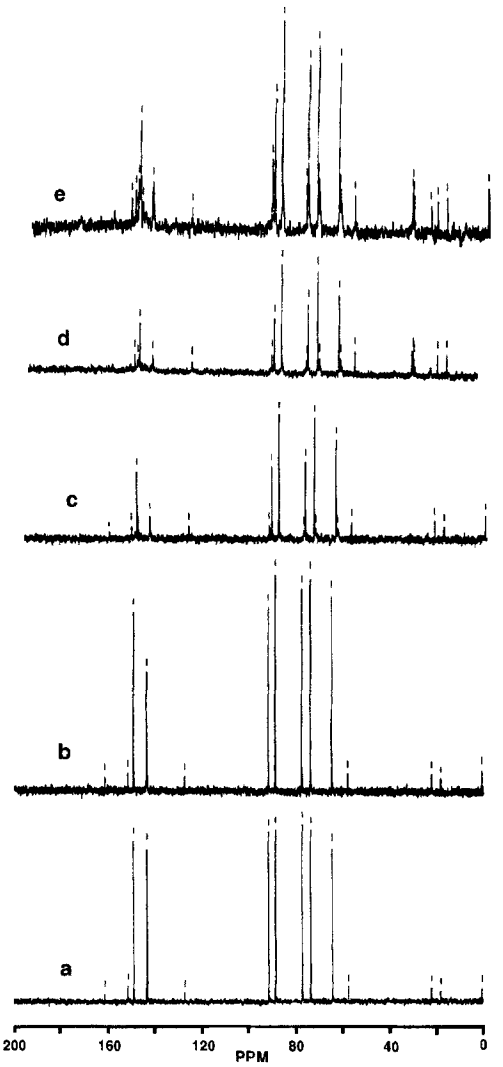

FIGURE 10 ^{13}CNMR spectra of solutions formed upon reaction of RTD (diluted in acetone-d_6) with inosine in D_2O with different ratios r = [ino]/[RTD]; (a) free inosine, 0.05 M, (b) r = 3:1, (c) r = 2:1, (d) r = 1:1, (e) the same as in d but recorded 4h later.

Our results indicate that at the beginning of the reaction the main site of metallation is at N(7). O(6) cannot be excluded as a possible binding site. On increasing the metal concentration (r = 2:1 or r = 1:1) the signals most affected by rhodium are those of C(6) and C(8). This implicates both N(7) and O(6) atoms in complexation. The absence of binding to the ribose moiety follows from the

observation that the chemical shifts of the sugar carbons are only slightly influenced by complex formation. The appearence of the second complex is remarkable. It produces more pronounced shifts for all its carbon atoms, except for that of C(4') which appears together with the C(4') signal of the first complex. With C(6) experiencing the greatest shift, the spectrum strongly indicates a metal —O(6) bond. N(1) atom is not considered to complex, as the C(2) signal is not greatly affected by the metal.

CONCLUSIONS

In view of the preceding discussion, the following points should be emphasized. First, rhodium trifluoroacetate dimer retains its 'cage' structure in the case of the adenosine complex where a polymeric structure is formed (RTD:Ado = 1:1) whereas cytidine, guanosine and inosine complexes give species mono- and bidentate CF_3CO_2 groups. Metallation takes place at the purine or pyrimidine ring and there is no involvement of the ribose moiety. Finally, solution studies usually reveal more than one species to be present under a variety of conditions.

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