Interactions of Tetrakis-μ-acetato-dirhodium(II) with Adenine Nucleosides and Nucleotides

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Reactions of the antitumour complex $[Rh_2(O_2CMe)_4]$ with adenine nucleosides and nucleotides have been studied in aqueous solutions. Both 1 : 1 and 1 : 2 solid adducts have been isolated and characterized by elemental analyses and i.r., visible, and ^1H n.m.r. spectra. The results indicate that the 1 : 1 adenine derivatives form polymeric bridged adducts with both N(1) and N(7) sites bonded to Rh^{II}. The only exception is tetra-acetyladenosine which forms 1 : 2 adducts in which only the N(7) site is involved in bonding to Rh^{II}.

THE acetate derivatives of certain bivalent metals, such as Cu, Cr, Mo, *etc.*, of formula $[M_2(O_2CMe)_4]$ are dimeric and contain a metal–metal bond.[1,2] Chernyaev *et al.*[3] reported the analogous rhodium(II) dimer, which corresponds to the same formula.[4] Adducts of formula $[Rh_2(O_2CMe)_4]\cdot 2L$ (L = *N*-, *S*-, or *O*-donor ligand at the axial positions) are also known.[5-7]

Recently, Bear and his co-workers [8-10] discovered that $[Rh_2(O_2CMe)_4]$ exhibited anticancer activity against many types of tumours. The same workers have also studied the interaction of this complex with several molecules of biological importance.[10,11] They found that the complex inhibited DNA synthesis and that it reacted mainly with poly-A but not with poly-C and poly-G.[10] This group also reported [12-14] the formation constants of the complex with ado-5′-*P* (adenosine 5′-monophosphate), ado-5′-*PP* (adenosine 5′-diphosphate), ado-5′-*PPP* (adenosine 5′-triphosphate), imidazole, *etc.*

In this paper, we report the reactions of $[Rh_2(O_2CMe)_4]$ with adenine nucleotides and nucleosides in aqueous solutions, the nature of the isolated adducts, and the bonding sites of the metal with the bases. Such studies may also help in obtaining a better understanding of the antitumour properties of this and other similar complexes.[10]

RESULTS AND DISCUSSION

Rhodium(II) acetate may form 1 : 1 or 1 : 2 adducts with ligands occupying the two axial positions. The complexes 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.[6,7,10,12] On mixing $[Rh_2(O_2CMe)_4]$ with the ligands in water the colour of the solutions became pink with subsequent precipitation of the adducts. This indicates that nitrogen is involved in the bonding of Rh^{II} with the ligands. The adenine derivatives used in this study were 9-methyladenine (9Me-ade), adenosine (ado), triacetyladenosine (trado), tetra-acetyladenosine (tetado), disodium salt of adenosine 5′-monophosphate [Na_2(ado-5′-*P*)], disodium salt of adenosine 5′-diphosphate [Na_2-(ado-5′-*PP*)], disodium salt of adenosine 5′-triphosphate [Na_2(ado-5′-*PPP*)], and adenosine 5′-dihydrogenphosphate (H_2ado-5′-*P*). The chemical analyses correspond to 1 : 1 adducts with all the ligands used, except the 1 : 2 adduct obtained with tetado, irrespective of the metal-to-ligand ratio (see Experimental section).

The nitrogen involvement in the bonding of the metals with the bases is also confirmed by the visible spectra of the complexes. Anhydrous $[Rh_2(O_2CMe)_4]$ gives two bands at 617 and 442 nm in the visible region.[7] The first of these bands is shifted to lower wavelengths on adduct formation, while the second remains essentially constant.[7] The spectral shifts are related to the donor atoms in the general order: $O < S < sp^3$ $N < sp^2$ $N \approx As < >S{=}O$. Thus the ado-5′-*PPP* adduct of $[Rh_2(O_2CMe)_4]$ has a band at 559 nm, that of ado-5′-*PP* has a band at 555 nm, and that of ado-5′-*P* has a band at 560 nm in aqueous solutions compared with 584 nm for $[Rh_2(O_2CMe)_4(OH_2)_2]$.[7] The ado adduct shows this band at 557 nm in dimethylformamide (dmf), while the trado and tetado adducts have bands at 540 and 530 nm in chloroform solutions.

In the i.r. spectra of $[Rh_2(O_2CMe)_4]$–amine adducts the $\nu_{asym}(CO_2^-)$ band found at 1 580 cm^{-1} in the dimer is displaced to higher wavenumbers by 5 cm^{-1}, while $\nu_{sym}(CO_2^-)$ is displaced to lower wavenumbers by 10—20 cm^{-1}.[5] In the complexes with adenine derivatives, $\nu_{asym}(CO_2^-)$ is found in the range 1 584—1 592 cm^{-1} (see Table 1), shifted by 4—12 cm^{-1} to higher wavenumbers. On the other hand, for the different adducts, $\nu_{sym}(CO_2^-)$ occurs in the range 1 420—1 436 cm^{-1} displaced by 9—25 cm^{-1} towards lower wavenumbers. Also, both $\delta_{asym}(CH_3)$ and $\delta_{sym}(CH_3)$, which occur at 1 415 and 1 356 cm^{-1} for $[Rh_2(O_2CMe)_4]$, are shifted to lower frequencies by *ca.* 5 and 4—18 cm^{-1} respectively [5] upon amine-adduct formation. The $\delta_{asym}(CH_3)$ band cannot be assigned in the adenine nucleoside adducts, possibly coinciding with $\nu_{sym}(CO_2^-)$, but $\delta_{sym}(CH_3)$ is displaced by 19—22 cm^{-1} towards lower frequencies (see Table 1).

The i.r. spectra also indicate the non-involvement in bonding of the amine-group at position 6 of the adenine derivatives of Rh^{II}. The NH_2 vibration may be assigned to a strong band at *ca.* 1 660 cm^{-1} for all the complexes, which disappears on deuteriation. The complexes with ado-5′-*P*, ado-5′-*PP*, and ado-5′-*PPP* show two bands in this region (Table 1) which correspond to an un-co-ordinated NH_2 group.[15,16] Free adenosine, for example, shows the $\delta(NH_2)$ vibration coupled with a ring-stretching vibration at 1 665 and 1 605 cm^{-1}.[17] The NH_2 group

TABLE 1

Characteristic i.r. bands of the adducts

Complex	$\nu_{asym}(CO_2)$	$\nu_{sym}(CO_2^-)$	$\delta_{asym}(CH_3)$	$\delta_{sym}(CH_3)$	$\nu_{sym}(C-C)$	$\gamma(CH_3)$	$\nu(NH_2)$, $\nu(NH)$, $\nu(OH)$, $\nu(CH)$	$\nu(C=O)$	$\delta(NH_2)$ or $\delta(NH)$	$\nu(P-O-P)$
$[Rh_2(O_2CMe)_4]$ (anhydrous) *	1 579	1 445	1 417	1 354	720, 707	632 603				
$[Rh_2(O_2CMe)_4(OH_2)_2]$ *	1 580	1 445	1 415	1 356	717, 700	630 603				
$[Rh_2(O_2CMe)_4]$·9Me-ade	1 592	1 420		1 348	717	610	3 456, 3 360 3 285, 3 220 3 136, 3 017 2 935, 2 852		1 655	
$[Rh_2(O_2CMe)_4]$·ado	1 586	1 428		1 334	699		3 364, 3 215 2 936		1 660	
$[Rh_2(O_2CMe)_4]$·trado	1 592	1 431		1 353	699	629	3 346, 3 280 3 210, 3 000 2 937	1 745	1 668	
$[Rh_2(O_2CMe)_4]$·2 tetado	1 585	1 422		1 354			3 328, 3 280 3 000, 2 937	1 749 1 701	1 604	
$[Rh_2(O_2CMe)_4]$·ado-5′-*P*	1 584	1 428		1 333	700	628	3 370, 3 214 2 936		1 672	1 100
$[Rh_2(O_2CMe)_4]$·H_2ado-5′-P	1 588	1 424		1 336	696	628	3 372, 3 209 2 932		1 692 1 669	1 209, 1 120 1 047, 953
$[Rh_2(O_2CMe)_4]$·ado-5′-*PP*	1 592	1 432		1 337	700	629	3 384, 3 215 2 936		1 694 1 676	1 216, 1 067
$[Rh_2(O_2CMe)_4]$·ado-5′-*PPP*	1 592	1 432		1 336	700	630	3 372, 3 209 2 936		1 695 1 652	1 214, 1 067 936

* Taken from ref. 3.

is also not involved in bonding in platinum(II) complexes with adenine derivatives,[15,16] as well as in complexes with thiamine and its phosphates.[18] The NH_2 stretchings are also unchanged on complex formation, and other characteristic bands are given in Table 1.

The ^1H n.m.r. spectra reveal that the 1 : 1 adducts of the adenine derivatives are bonded through both their N(1) and N(7) sites to Rh^{II}. The assignments are given in Table 2. In free adenosine, in $S(CD_3)_2O$ solutions, the H(2) proton appears at 8.15 p.p.m. and H(8) at 8.36 p.p.m. The ^1H n.m.r. spectrum of the ado adduct of $[Rh_2(O_2CMe)_4]$ also consists of two resonances in the aromatic proton region, at 8.90 and 8.66 p.p.m. assigned to H(8) and H(2) respectively. These assignments were based on the ^1H n.m.r. spectrum of the complex with 8-deuterioadenosine,[19] where the resonance due to H(8) disappears. When metals co-ordinate to the ring nitrogen of purines the adjacent protons become more acidic and shift downfield in the n.m.r. spectra.[19-22] In the case of the ado adduct of $[Rh_2(O_2CMe)_4]$, both H(8) and H(2) shift by 0.54 and 0.51 p.p.m. downfield compared to free adenosine and this indicates that both the N(1) and N(7) sites co-ordinate to Rh^{II}. This shift of *ca.* 0.50 p.p.m. is less than that on co-ordination to Pt^{II} (*ca.* 1 p.p.m.),[19,21,23] but comparable with that caused by co-ordination to Pd^{II} (*ca.* 0.5—0.6 p.p.m.).[24,25] This also indicates that Pt^{II} forms more stable complexes with the purine bases than the other two metals.

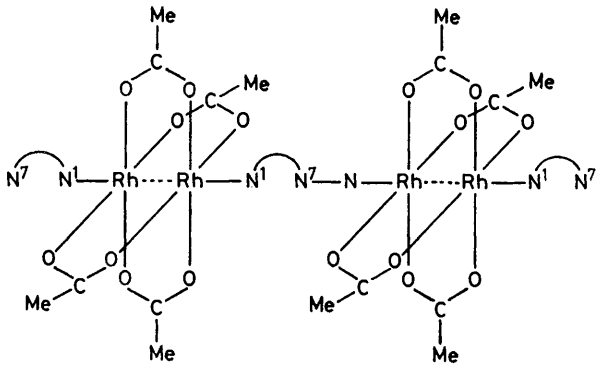
The H(2) resonance of trado occurs at 8.35 p.p.m. and H(8) at 8.02 p.p.m. in $CDCl_3$ solutions, as is revealed by deuteriation of H(8). The trado adduct of $[Rh_2(O_2CMe)_4]$, in $CDCl_3$ solution, shows H(2) at 8.85 p.p.m., shifted by 0.60 p.p.m., and H(8) at 8.60 p.p.m., also shifted by 0.58 p.p.m. compared with the free ligand. Here, again, both N(1) and N(7) co-ordination is implied. The same is true for all the adenine adducts which were sufficiently soluble for their n.m.r. spectra to be recorded (Table 2). The only exception is tetado which shows H(2) at 8.76 p.p.m. and H(8) at 8.42 p.p.m., while its 1 : 2 adduct with $[Rh_2(O_2CMe)_4]$ exhibits only a single resonance at 8.93 p.p.m. assigned to both these protons.[23] Since H(8) shifts downfield by 0.51 p.p.m., while H(2) is shifted by only 0.17 p.p.m., we conclude that the N(7) atom is the only binding site in the 1 : 2 complex.

TABLE 2

Hydrogen-1 n.m.r. chemical shifts (p.p.m.)

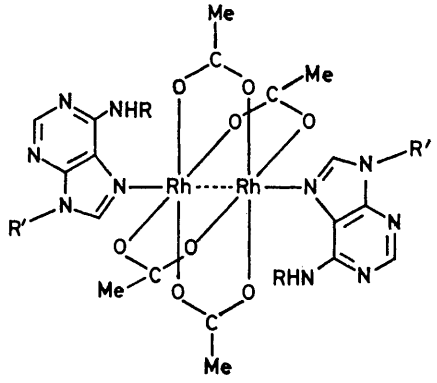
Compound	H(2)	H(8)	NH or NH_2	H(1′)	Solvent
ado	8.15	8.36	7.33	5.90 (d) 5.80	$S(CD_3)_2O$
$[Rh_2(O_2CMe)_4]$·ado	8.66	8.90	7.81	6.31 (d) 6.23	$S(CD_3)_2O$
trado	8.35	8.02	6.47	6.30 (d) 6.22	$CDCl_3$
$[^2H_8]$trado	8.41	8.04	6.50	6.30 (d) 6.23	$CDCl_3$
$[Rh_2(O_2CMe)_4]$·trado	8.85	8.60	7.17	6.50 (d) 6.40	$CDCl_3$
$[Rh_2(O_2CMe)_4]$·$[^2H_8]$trado (80%)	9.08	8.80	7.20	6.56 (d) 6.49	$CDCl_3$
tetado	8.76	8.42	9.83	6.37 (d) 6.30	$CDCl_3$
$[Rh_2(O_2CMe)_4]$·tetado	8.93	8.93	9.64	6.67 (d) 6.59	$CDCl_3$
ado-5′-*PPP*	8.08	8.41			D_2O
$[Rh_2(O_2CMe)_4]$·ado-5′-*PPP*	8.50	8.73		6.17 (d) 6.08	D_2O

Adenosine is known to bind through only N(7) [19,23] or both N(1) and N(7) with Pt^{II} and Pd^{II},[21,24,25] while tetado co-ordinates only through N(7).[23] The non-involvement of the N(1) site in bonding to Pt^{II} has been attributed to steric effects caused by the adjacent bulky acetyl group.[23] In all the other cases, both the N(1) and N(7) sites of the adenine derivatives are equivalent and have equal chances of participating in metal complex formation.[19,21,24,25] On the other hand, both axial positions in $[Rh_2(O_2CMe)_4]$ are equivalent and may be simultaneously occupied by neutral ligands. Therefore, the most plausible structure for the isolated 1 : 1 adducts is a polymeric bridging one, involving both



(1)

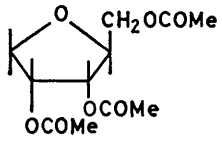
axial positions of the rhodium(II) dimer and both N(1) and N(7) sites of the adenines, as in (1). This structure could explain the analytical results (1 : 1 complexes), and



(2)

R = acetyl

R′ = 2,3,5,-*O*-triacetyl-β-D-*ribo*-furanosyl,



the i.r. (non-involvement of NH_2 group) and n.m.r. spectra [co-ordination of both N(1) and N(7) simultaneously]. The derivative tetado may form monomers, but again with both axial positions occupied by the ligand, and 1 : 2 derivatives bonded only through N(7), as in (2). The n.m.r. spectrum of the 1 : 2 tetado adduct of $[Rh_2(O_2CMe)_4]$ in $S(CD_3)_2O$ showed the presence of free tetado, indicating substitution of the ligand by the solvent. The polymeric 1 : 1 adducts, on the other hand, were stable in such solutions.

Bear and his co-workers [10,12,14] have reported the stepwise formation constants of 1 : 1 and 1 : 2 adducts of $[Rh_2(O_2CMe)_4]$ with adenine nucleotides, although they did not isolate the complexes. They also reported that rhodium(II) carboxylate dimers inhibit DNA and RNA synthesis *in vitro* and DNA synthesis *in vivo*,[9,10] but they are not reactive *in vitro* towards poly-C or poly-G.[10] Attempts to obtain complexes of $[Rh_2(O_2CMe)_4]$ with guanosine, inosine, or cytidine under the same conditions as used for the adenine adducts were unsuccessful. The availability of both N(1) and N(7) sites of the adenine derivatives for bonding with metals may be a plausible explanation for the easy formation of their stable 1 : 1 polymeric bridged adducts. We are now studying the reactions of the above three nucleosides with $[Rh_2(O_2CMe)_4]$.

EXPERIMENTAL

Materials.—The adenine nucleosides and nucleotides were purchased from Raylo Chemicals Ltd. and from Sigma Chemical Co. and used without further purification; $RhCl_3$(aq) was obtained from Fluka A.G. The complex $[Rh_2(O_2CMe)_4(HOMe)_2]$ was prepared according to the literature,[26] as were tri- and tetra-acetyladenosine.[27]

Methods.—Infrared spectra were recorded on a Beckman model 2050 spectrophotometer in KBr pellets and in Nujol mulls. The positions of the bands are given within ±2 cm^{-1}. Visible spectra were obtained with a Cary model 17D spectrophotometer. The ^1H n.m.r. spectra were recorded on a Varian T60 high-resolution spectrometer, $SiMe_4$ being used as external reference in D_2O and as internal reference in $CDCl_3$ or $S(CD_3)_2O$ solutions. Melting points were determined on a Fisher apparatus and are uncorrected. The microanalyses were performed in the Laboratories of the National Hellenic Research Foundation (N.H.R.F.), Athens.

Preparation of the Complexes.—(*a*) *General method for the tetra-μ-acetato-dirhodium(II) adducts with 9-methyladenine, adenosine, and tetra-acetyladenosine.* The complex $[Rh_2(O_2CMe)_4(HOMe)_2]$ (1 mmol, 0.605 8 g) was dissolved in water (150—200 cm^3) and 1 mmol of 9-methyladenine or adenosine was dissolved in the minimum amount of water. For tetra-acetyladenosine 2 mmol (0.870 4 g) were used. On mixing the solutions, the colour changed from green to pink and a precipitate appeared almost immediately. The mixture was stirred for *ca.* 2 h at room temperature to achieve complete precipitation, and the product was filtered off and washed with water and small quantities of acetone and diethyl ether. In the case of the tetado adduct, the precipitate was recrystallized from chloroform and diethyl ether, prior to washing it with acetone in which it is soluble. The products were then dried *in vacuo* at room temperature and then at 110 °C *in vacuo*. The yields were in the range 85—90% (Found: C, 24.65; H, 3.10; Rh, 29.25. Calc. for $C_{14}H_{19}N_5O_8Rh_2$: C, 24.3; H, 2.75; Rh,

29.8%. M.p. 245 °C with decomposition. Found: C, 26.6; H, 3.20; Rh, 25.25. Calc. for $C_{18}H_{25}N_5O_{12}Rh_2$: C, 26.7; H, 3.10; Rh, 25.45%. M.p. 215 °C with decomposition. Found: C, 37.7; H, 3.65; Rh, 14.35. Calc. for $C_{44}H_{54}N_{10}O_{24}Rh_2$: C, 37.4; H, 3.85; Rh, 14.6%. M.p. 130 °C).

(*b*) *Tetra-μ-acetato-dirhodium*(II)*–triacetyladenosine*. The complex $[Rh_2(O_2CMe)_4(HOMe)_2]$ (1 mmol 0.605 8 g) was dissolved in water (200 cm³), triacetyladenosine (1 mmol, 0.413 2 g), suspended in water (100 cm³) was added, and the mixture was stirred overnight at room temperature. The colour of the precipitate gradually changed to pink, as trado entered the solution by reacting with $[Rh_2(O_2CMe)_4(OH_2)_2]$ to finally produce the 1 : 1 adduct. The precipitate formed was filtered off, dissolved in chloroform, and reprecipitated with excess of diethyl ether. The complex was filtered off, washed with diethyl ether, and dried at 110 °C *in vacuo*, yield 90% (Found: C, 30.5; H, 3.55; Rh, 21.8. Calc. for $C_{24}H_{31}N_5O_5Rh_2$: C, 30.8; H, 3.35; Rh, 22.0%. M.p. 225 °C with decomposition).

(*c*) *General method for the adducts of adenosine 5′-mono-, 5′-di-, and 5′-tri-phosphate*. The complex $[Rh_2(O_2CMe)_4(HOMe)_2]$ (1 mmol, 0.605 8 g) was suspended in water (25 cm³) and 0.95 mmol of each nucleotide was added. The mixture was then stirred until dissolution was complete (*ca.* 5 h). The complexes were precipitated with excess of acetone. A slight excess of the starting complex was used in order to obtain complete consumption of the nucleotides, while the $[Rh_2(O_2CMe)_4(OH_2)_2]$ left at the end of the reaction was not precipitated since it is soluble in acetone. The chemical analyses and the ^1H n.m.r. spectra indicate the purity of the complexes isolated in this way (yield 80%) (Found: C, 23.05; H, 2.60. Calc. for $C_{13}H_{24}N_5Na_2O_{15}PRh_2$: C, 22.9; H, 2.55. M.p. 240 °C with decomposition. Found: C, 21.25; H, 2.55. Calc. for $C_{18}H_{25}N_5Na_2O_{18}P_2Rh_2$: C, 21.3; H, 2.50. M.p. 240 °C with decomposition. Found: C, 19.65; H, 2.30. Calc. for $C_{18}H_{26}N_5Na_2O_{21}P_3Rh_2$: C, 19.75; H, 2.40%. M.p. 245 °C with decomposition).

(*d*) *The* ND_2 *and* OD *derivatives*. The deuteriated derivatives were prepared by exposing the complexes to an atmosphere saturated with D_2O for 24 h, and the i.r. spectra were taken in Nujol mulls using KBr plates.

Attempts to prepare 1 : 2 adducts using a 1 : 2 mol ratio of $[Rh_2(O_2CMe)_4]$ to adenine derivative (other than tetado) in neutral or acid (0.3N HCl) solutions resulted in the 1 : 1 products.

We thank the National Hellenic Research Foundation for support, and Dr. Mantzos of the same Institute for the microanalyses.

[8/542 *Received*, 22*nd March*, 1978]

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