Structural Evidence for a New Metal-Binding Mode for Guanine Bases: Implications for the Binding of Dinuclear Antitumor Agents to DNA

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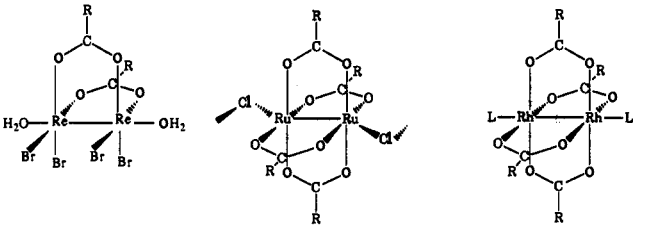
The recognition by Rosenberg *et al.* in 1969 that *cis*-$(NH_3)_2PtCl_2$ (cisplatin) is an antitumor agent[1] and its eventual approval as a chemotherapeutic drug triggered an enormous response in the chemical and medical research communities.[1-6] During the last two decades, inorganic chemists have sought to gain insight into the *mechanism* whereby Pt(II) and Pt(IV) complexes inhibit DNA replication by designing and structurally characterizing key model compounds.[7,8] In recent years, increasing emphasis has been placed on the screening and tailoring of non-platinum complexes in the hope of discovering drugs that are effective against cancers other than those treated by cisplatin.[9]

Among the inorganic compounds that have been documented to exhibit substantial carcinostatic activity are dinuclear complexes of Re,[10] Ru,[11] and Rh;[9,12-18] a common feature of these is the presence of at least two bridging carboxylate ligands as depicted in the molecular drawings in Scheme 1. A goal of our research in this area is to develop the substitution chemistry of these and other biologically active dinuclear compounds with purine bases and their corresponding nucleosides and nucleotides. At the outset of our investigation, we were aware of reports that the antitumor agent dirhodium tetraacetate reacts with adenine but not guanine bases. The latter conclusion was based partly on the observation that, upon addition of guanine and guanosine, the blue-green color of the tetraacetate complex persists. This is in contrast to the corresponding reactions of $Rh_2(O_2CCH_3)_4$ with adenine bases, which undergo a dramatic color change from blue-green to pink.[14-18] We have recently discovered, however, that $Rh_2(O_2CCH_3)_4(MeOH)_2$ does in fact react with guanine bases in H_2O or MeOH to yield substitution products that contain two purine bases per Rh_2 unit. Two equivalents of 9-ethylguanine (9-EtGH) or guanosine were added to an aqueous suspension of $Rh_2(O_2CCH_3)_4(MeOH)_2$ and heated at 60 °C for ~48 h. During this time, the solution color slowly turned from cloudy blue-green to clear green. Analogous results were obtained for reactions carried out at lower temperatures (35–40 °C) although the reaction rates under these conditions were much slower. The products were isolated by evaporation and lyophilized twice with D_2O before ^1H NMR measurements were performed. Two downfield resonances in an approximately 1:1 ratio were observed in the H8 region of the ^1H NMR spectra measured in D_2O (9-ethylguanine δ = 8.20, 8.30; guanosine δ = 8.48, 8.55). FAB-MS studies of the products dissolved in a nitrobenzyl alcohol matrix revealed parent ion peaks corresponding to the formula $Rh_2(O_2CCH_3)_2(purine)_2$ (purine = 9-ethylguanine, m/e = 681.0 $[M + H]^+$, 60% abundance; guanosine, m/e = 889.2 $[M]^+$, 20% abundance). Reactions of the trifluoroacetate derivative $Rh_2(O_2CCF_3)_4(Me_2CO)_2$ with 2 equiv of 9-EtGH in refluxing acetone for 24 h produced a green compound whose ^1H NMR spectrum displays a single H8 resonance at δ = 8.78.

Scheme 1. Molecular Drawings of the Three Structural Types Encountered for Dinuclear Carboxylate Compounds That Exhibit Carcinostatic Activity



X-ray crystallographic studies were performed on crystals from the two 9-ethylguanine reactions. Single crystals of $Rh_2(O_2CCH_3)_2(9\text{-EtG})_2(MeOH)_2\cdot 2MeOH$ (**1**) were obtained from a MeOH solution of the compound by slow evaporation in air.[19] The neutral compound **1** was found to contain an unprecedented *bridging* form of *deprotonated* 9-ethylguanine (9-EtG) involving the N7 and O6 positions. Two such groups span the dirhodium unit in a *cis* disposition and in a "head-to-tail" orientation as can be clearly seen from the labeled ORTEP diagram in Figure 1. The axial positions are occupied by MeOH, and the dinuclear unit is flanked by two additional MeOH molecules involved in hydrogen bonding to the bridging acetate ligands (O1···O9 = 2.81(3) Å and O4···O10 = 2.77(3) Å). The Rh–O6 distances to the 9-EtG ligands are 2.023(7) Å (Rh1–O8) and 2.025(7) Å (Rh2–O7). The assignment of the oxopurine as the anion form is based on the neutrality of the Rh_2 core as evidenced by the lack of counterions in the crystal. Support for the preservation of this structure in solution was obtained from conductivity measurements performed in MeOH (34 S cm^2 mol^{-1}) which indicates that **1** is essentially nonconducting. Crystals of **1** exhibit a single resonance in the H8 region at δ = 8.28 ppm corresponding to one of the two resonances observed for the crude product (vide supra). We conclude that both head-to-head and head-to-tail isomers are being formed at essentially the same rate in these reactions; attempts to isolate and characterize the head-to-head isomer are in progress.

X-ray quality crystals of $[Rh_2(O_2CCF_3)_2(9\text{-EtGH})_2(Me_2CO)_2][CF_3CO_2]_2\cdot(Me)_2CO$ (**2**) were obtained by slow evaporation of a solution of the compound in acetone/dichloromethane (1:1

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(19) Crystal data for **1**: T = −110 ± 1 °C, Mo Kα (λ_α = 0.710 69 Å) radiation, a = 13.430(5) Å, b = 14.680(4) Å, c = 10.121(2) Å, α = 106.02(2)°, β = 108.92(3)°, γ = 62.98(2)°, V = 1662(2) Å^3, $P\bar{1}$, 5834 unique data in the range 6° ≤ 2θ ≤ 50°, 3852 observations with $F_o^2 \geq 3\sigma(F_o^2)$. Calculations were performed with the TEXSAN package by standard procedures.[23,24] An empirical absorption correction based on three azimuthal scans was applied. Final residuals, R = 0.053, R_w = 0.072.

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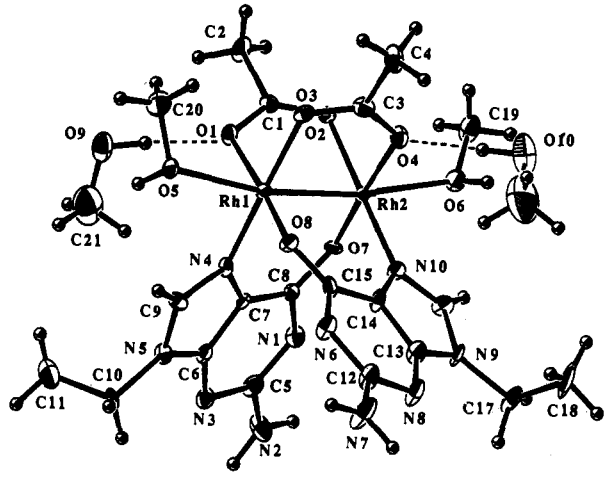

Figure 1. ORTEP drawing of a molecule of $Rh_2(O_2CCH_3)_2(9\text{-EtG})_2(MeOH)_2\cdot 2MeOH$ with thermal ellipsoids drawn at the 30% probability level. Selected bond lengths (Å) and angles (deg): Rh1–Rh2, 2.483(2); Rh1–O1, 2.031(6); Rh1–O3, 2.071(7); Rh1–O5, 2.315(7); Rh1–O8, 2.025(7); Rh1–N4, 1.973(8); Rh2–O2, 2.063(7); Rh2–O4, 2.041(7); Rh2–O6, 2.317(7); Rh2–O7, 2.023(7); Rh2–N10, 1.991(8); O4- - -O10, 2.77(3); O1- - -O9, 2.81(3); Rh2–Rh1–O5, 169.0(2); Rh1–Rh2–O6, 171.7(2); O5–Rh1–N4, 91.6(3); O6–Rh2–N10, 90.5(3).

v/v).[20] The cation $[Rh_2(O_2CCF_3)_2(9\text{-EtGH})_2(Me_2CO)_2]^{2+}$, displayed in Figure 2, is ligated by two *neutral* 9-ethylguanine groups bridging the dinuclear unit in a head-to-tail arrangement, two *cis*-CF_3COO^- groups, and axial acetone molecules. The conclusion that the ligand is neutral and not deprotonated as in the acetate compound **1** is supported by the presence of two outer sphere trifluoroacetate anions and by conductivity measurements in MeOH (110 S cm^2 mol^{-1}). The Rh1–O6 distance is 2.00(2) Å, which is a remarkably short distance for a metal–ketone interaction.

The only other structurally characterized compound, to our knowledge, in which the O6 atom of an oxopurine is bonded to a metal is the molecule Cp_2Ti(theophylline), for which the Ti(IV)–O6 bond distance is 2.278(2) Å.[21] The participation of the O6 atom in the chemistry of Pt antitumor compounds has been debated for years, with recent data arguing against O6 platination.[6] The chelate formed in the present study differs from that dictated by a mononuclear complex, in that it involves two metals and a six-membered ring, *viz.*, Rh–Rh–N–C–C–O with the bridging N–C–C–O unit spanning Rh–Rh distances of 2.483(2) (**1**) and 2.520(5) Å (**2**).

The results of the present investigation lend themselves to comparison, in a general sense, with the mechanism of cisplatin binding to DNA. As in the mononuclear platinum chemistry, we have demonstrated that dinuclear antitumor agents are also capable of opening up *cis* positions to bind purines; furthermore,

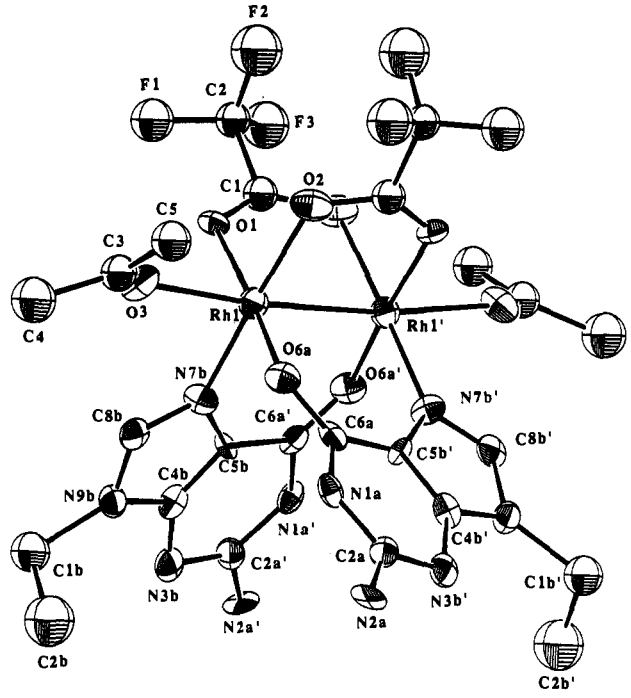

Figure 2. ORTEP diagram depicting the molecular cation $[Rh_2(O_2CCF_3)_2(9\text{-EtGH})_2]^{2+}$ with thermal ellipsoids drawn at the 40% probability level. Selected bond lengths (Å) and angles (deg): Rh1–Rh1′, 2.520(5); Rh1–O1, 2.03(2); Rh1–O2, 2.05(2); Rh1–O3, 2.18(1); Rh1–O6a, 2.00(2); Rh1–N7b, 1.94(2); O6a–C6a, 1.22(4); N7b–C5b, 1.43(4); O3–Rh1–Rh1′, 174.1(5); O3–Rh1–N7b, 88.4(8); O3–Rh1–O6a, 84.1(7).

in sharp contrast to the established dirhodium tetraacetate chemistry with adenine, we see no evidence for guanine reactions proceeding by axial ligand displacement. Extensions of these reactions to $[Re_2]^{6+}$ and $[Ru_2]^{5+}$ carboxylate antitumor agents have produced similar structures.[22] Details of these studies will be published in due course.

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Supplementary Material Available: Tables of fractional coordinates, isotropic and anisotropic thermal parameters, bond distances, and bond angles for **1** and **2** (29 pages); tables of structure factors for **1** and **2** (36 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(20) Crystal data for **2**: $T = -90 \pm 2$ °C, Mo Kα ($\lambda_{\bar{a}} = 0.710\,73$ Å) radiation, $a = 21.15(2)$ Å, $b = 13.436(6)$ Å, $c = 16.099(7)$ Å, $\beta = 99.41(6)°$, $V = 4514(9)$ Å^3, $C_{2/c}$, 3236 unique data in the range $4° \le 2\theta \le 45°$, 1286 observations with $F_o^2 \ge 3\sigma(F_o^2)$. Calculations were performed with the TEXSAN package by standard procedures.[23,24] DIFABS was used to correct for absorption after isotropic convergence.[25] The CF_3 groups were disordered and therefore refined as rigid groups. Final residuals, $R = 0.094$ and $R_w = 0.102$; these values reflect the fact that 20 atoms were refined isotropically due to a low data-to-parameter ratio (6:1).

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