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Reactivity Studies of Anticancer Active Dirhodium Complexes with 2-Aminothiophenol

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Introduction

The study of biologically active inorganic compounds has led to the development of several important chemotherapeutic drugs in the past few decades, the most notable example of which is *cis*-$[PtCl_2(NH_3)_2]$ (*cis*-DDP).[1] In addition to compounds of platinum, many other transition metal complexes have been found to exhibit considerable antineoplastic activities, including those of general formulae $Rh_2(\mu\text{-}O_2CR)_4$ and $[Rh_2(\mu\text{-}O_2CR)_2(N{-}N)_2L_2]X_2$ (N−N = 2,2′-bipyridine and 1,10-phenanthroline; L = solvent molecules; X = Cl, Br).[2,3] The latter family of compounds is of particular interest due to their improved anticancer activity against different tumor lines (e.g., human oral carcinoma KB) as compared to their $Rh_2(\mu\text{-}O_2CR)_4L_2$ counterparts (L = solvent molucules).[3] A perusal of the literature reveals a scarcity of information vis-à-vis the cellular metabolism of these dirhodium compounds, but it is known from preliminary studies that DNA replication is significantly inhibited and that protein synthesis is slightly affected.[4]

Work carried out in our laboratories over the past 10 years has confirmed that dirhodium compounds react with nucleic acids to yield unprecedented adducts that involve the use of the purine nucleobases as bridging ligands.[5] In this current report, we now turn to another aspect of the possible metabolic fate of the metal complexes, namely the formation of products with S-containing biomolecules. Several brief reports on the reactivity of dirhodium carboxylate compounds with cysteine have appeared in the literature, but the results are preliminary and even contradictory.[4,6] Obviously the topic merits further investigation if we are to advance toward an understanding of the real biological targets of dirhodium compounds.[7]

In this paper, reactivity studies of $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2(CH_3CN)_2][PF_6]_2$ (**1**) with the model ligand 2-aminothiophenol are reported.[8] This work was carried out with the more general aim of gaining structural information on the possible reaction products of these dirhodium compounds with sulfuhydryl-containing amino acids and peptides such as cysteine and glutathione (GSH), depicted in Chart 1.

Experimental Section

Materials and Methods. Acetonitrile and ethanol were dried over 3 and 4 Å molecular sieves, respectively, and distilled under a nitrogen atmosphere prior to use. Toluene was freshly distilled from Na/K before use. The starting materials $[Rh_2(\mu\text{-}O_2CCH_3)_2(bpy)_2(CH_3CN)_2][BF_4]_2$ and $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2(CH_3CN)_2][PF_6]_2$ were prepared by literature methods.[9] The reagent 2-aminothiophenol was purchased from Aldrich and used as received.

Physical Measurements. The ^{1}H NMR spectrum of **2** was recorded on a Unity-300 NMR spectrometer. Infrared spectral measurements were performed on a Nicolet Nexus 470 FTIR

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(8) Reactions of **1** with thiol-containing biomolecules, such as cysteine and glutathione, are also being performed in our laboratory. These products are still under investigation, but preliminary results indicate that a reduction is occurring under anaerobic conditions. The current model chemistry led to more tractable products and allowed for the structural determination of products in the presence of oxygen.

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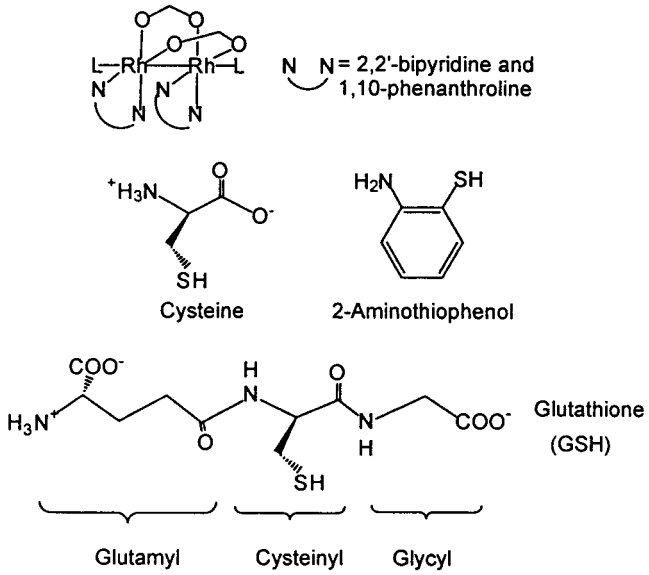
Chart 1



spectrometer on samples suspended in Nujol mulls between KBr plates. The electronic spectrum of **2** was recorded in CH_3CN on a Shimadzu UV-1601 spectrophotometer.

Rh(η^2-C_6H_6NS)(η^1-C_6H_6NS)$_2$(phen), 2. To a solution of 27 mg of $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2(CH_3CN)_2][PF_6]_2$ (0.025 mmol) in 15 mL of CH_3OH, was added dropwise a solution of C_6H_7NS (0.253 mmol) in 5 mL of CH_3CN. The resulting dark brown mixture was allowed to stir at room temperature for 5 min after which time a stream of dry O_2(g) was bubbled through the dark brown solution for a period of 15 min. The reaction was stirred at room temperature for 15 h, during which time the dark brown solution slowly turned yellow-orange with concomitant formation of a finely divided yellow precipitate. The precipitate was removed by filtration in air, and crystals suitable for X-ray diffraction analysis were obtained by dissolving the crude product in ethanol and layering it with toluene. Thin yellow platelets were obtained after 2 days. Yield = 45%. UV−Vis (nm): 395 and 472 (sh). IR (cm^{-1}): 683.3, 846.0. ^{1}H NMR (δ; ppm): 4.74 (NH_2); 5.19, 5.31, 5.43 (*m*, 2-aminothiophenol); 5.67, 5.93, 6.25 (*m*, 2-aminothiophenol); 6.51, 6.74, 7.05, 7.14 (*m*, phen).

X-ray Structural Studies of 2. A yellow platelet crystal of **2** ($0.21 \times 0.16 \times 0.05$ mm^3) was secured on a glass fiber with Dow-Corning grease. Data were collected at 110 ± 1 K on a Bruker SMART CCD area detector diffractometer equipped with graphite monochromated Mo Kα radiation ($\lambda_\alpha = 0.710\ 69$ Å; Table 1). The frames were integrated in the Bruker SAINT software package,[10] and the data were corrected for absorption using the SADABS program.[11] The structure was solved by direct methods (SIR97)[12] and refined by full-matrix least-squares calculations on F^2 (SHELXL-97).[13] Non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located in the difference maps and refined isotropically with the exception of those on the dangling aminothiophenol group which were introduced in calculated positions. The amino group on the dangling aminothiophenol ligand is disordered and was modeled at 50% in two equivalent positions on opposite sides of the phenyl ring.

Table 1. Summary of Important Crystal Parameters for $Rh^{III}(\eta^2\text{-}C_6H_6NS)(\eta^1\text{-}C_6H_6NS)_2$(phen) (**2**)

empirical formula	$C_{30}H_{26}N_5RhS_3$
fw	655.65
temp	110 K
wavelength	0.710 69 Å
space group	$P\bar{1}$
a	7.097(5) Å
b	12.744(5) Å
c	15.429(5) Å
α	75.400(5)°
β	87.600(5)°
γ	86.200(5)°
V, *Z*	1347.0(12) Å^3, 2
D_{calcd}	1.617 Mg/m^3
μ_{Mo}	8.98 cm^{-1}
cryst size	49 × 159 × 210 *m*m
reflns collected	12 293
independ reflns	6392 [R_{int} = 0.0899]
data/restraints/params	6392/0/446
goodness-of-fit on F^2	0.969
obsd reflns [$I > 4\sigma(I)$]	6392
final *R* indices [$I > 4\sigma(I)$]	R1 = 0.0635, wR2[a] = 0.1534
R indices (all data)	R1 = 0.0798, wR2[a] = 0.1628

[a] $R1 = \sum[(F_o - F_c)]/\sum(F_o)$. $wR2 = \{\sum[w(F_o^2 - F_c^2)^2/\sum[w(F_o^2)^2]\}^{1/2}$. $w = 1/[\sigma^2(F_o^2) + (0.0990P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$.

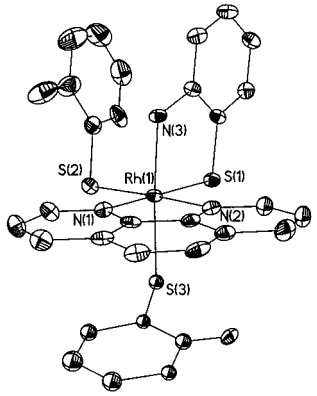


Figure 1. Thermal ellipsoid plot of Rh(η^2-C_6H_6NS)(η^1-C_6H_6NS)$_2$(phen) (**2**) at the 50% probability level with unique atom labels.

Results

The salt $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2][PF_6]_2$ reacts with excess 2-aminothiophenol in the presence of O_2 to yield the neutral compound $Rh^{III}(\eta^2\text{-}C_6H_6NS)(\eta^1\text{-}C_6H_6NS)_2$(phen) (**2**) as determined by X-ray crystallographic methods. The molecular structure consists of an octahedral Rh^{III} center surrounded by one phenanthroline ligand and three 2-aminothiophenolate ligands (Figure 1) in a facial arrangement. One 2-aminothiophenol molecule acts as a N−S chelate to the metal center, while the other two are bound in a monodentate fashion through the thiolate groups. The trans influence exerted by the thiolate groups[14] leads to longer Rh−N distances in compound **2**, as compared to the Rh−N distances in $[Rh_2(\mu\text{-}O_2CCH_3)_2(N{-}N)_2]^{2+}$ complexes, whose average

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(11) Sheldrick, G. M. *SADABS, Siemens Area Detector Absorption (and Other) Correction*; University of Göttingen: Göttingen, Germany, 1998.
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Rh−N distances are in the range 1.9−2.0 Å.[9,15] The combination of the disparate M−L distances (Rh−S = 2.31−2.38 Å versus Rh−N = 2.09−2.11 Å) and the bite angle of both chelating ligands (∼79°) leads to a distorted octahedral environment for the metal center.

Characterization of compound **2** in solution was achieved by ^{1}H NMR spectroscopy in CD_3CN. The aromatic phenanthroline protons appear at δ = 6.51, 6.74, 7.05, and 7.14 ppm, whereas the resonances at δ = 5.19, 5.31, 5.43, 5.67, 5.93, and 6.25 ppm are assigned to the 2-aminothiophenolate ligands. The assignment of the resonances was made according to the multiplicities and chemical shifts of the free ligands. The broad resonance at 4.74 ppm is attributed to the amino group which is shifted downfield by 0.67 ppm from the free 2-aminothiophenol ligand (4.07 ppm).

Discussion

The main finding of this study is that $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2]^{2+}$ reacts with 2-aminothiophenol to form a stable Rh^{III} compound in the presence of O_2. As stated earlier, the ligand 2-aminothiophenol was used as a model for the chemistry of reactive thiol groups such as those found in amino acids and glutathione. Although the mechanistic pathway remains unclear, a feasible hypothesis is that the reaction occurs by a two-step pathway, which first involves coordination of the 2-aminothiophenol ligands to the Rh_2 core followed by oxidation of the two Rh^{II} centers to Rh^{III}. The resulting dinuclear $Rh_2^{III,III}$ with no metal−metal bond is expected to be susceptible to cleavage reactions upon further addition of 2-aminothiophenol. Support for this proposed pathway was obtained from the analogous reaction of $[Rh_2(\mu\text{-}O_2CCH_3)_2(bpy)_2][BF_4]_2$ (bpy = 2,2′-bipyridine) with 4 equiv of 2-aminothiophenol in air, which led to crystals at low temperature. Although the poor quality of the crystals does not allow for a detailed structural analysis to be made, the important aspects of the structure can nevertheless be discerned. The X-ray data show that two different cations, $[Rh_2(\mu\text{-}C_6H_6NS)_2(C_6H_6NS)_2(bpy)_2]^{2+}$ (**3a**) and $[Rh^{III}(C_6H_6NS)_2(bpy)]^+$ (**3b**), are cocrystallized in the same unit cell.[16] Plots of the coordination environment of these two species are depicted in Figure 2.

The dinuclear cation **3a** contains a planar Rh_2S_2 core with Rh−S distances in the range 2.3−2.4 Å; these data are in accord with distances reported for other dirhodium complexes that contain bridging thiolate ligands (Rh−S = 2.38−2.39 Å in $[RhCl(H)(\mu\text{-}SH)(PPh_3)_2]_2\cdot 2CH_2Cl_2$).[17] In addition to the two $(\mu\text{-}S)^-$ bridges which also act as chelating ligands through the amino groups, the metal is coordinated to a bpy ligand and to a S atom from a dangling 2-aminothiophenolate anion. The distance between the two metal centers is 3.51(3) Å, which is outside the normal range of Rh^{II}−Rh^{II} single bond distances (∼2.5−2.7 Å). Moreover, the structure of $CpRh^{II}(\mu\text{-}SR)_2Rh^{II}Cp$ was reported to have a much shorter Rh−Rh distance, *viz.*, 2.64 Å.[18] On the basis of these considerations, the assignment of the Rh oxidation state in **3a** as Rh^{III} appears to be justified.

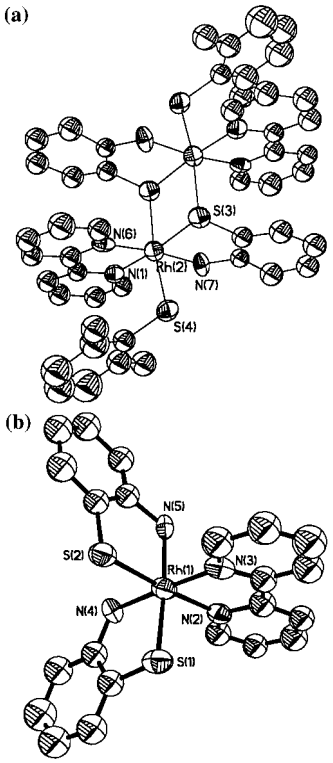

Figure 2. Thermal ellipsoid plots of (a, top) $[Rh_2(\mu\text{-}C_6H_6NS)_2(C_6H_6NS)_2(bpy)_2]^{2+}$ (**3a**) and (b, bottom) $[Rh^{III}(C_6H_6NS)_2(bpy)]^+$ (**3b**) at the 50% probability level with unique atom labels.

The mononuclear compound **3b** consists of a distorted octahedral Rh^{III} ion surrounded by three chelating ligands, namely, two 2-aminothiophenol molecules in the thiolate form and one 2,2′-bipyridine ligand. The Rh−N and Rh−S ligand distances in **3b** are analogous to those found in complex **2**. The absence of dangling 2-aminothiophenolate ligands in **3b** versus **2** is attributed to the fact that excess 2-aminothiophenol was used in the synthesis of **2**.

Concluding Remarks

It is important to understand how anticancer active dirhodium compounds react with thiol-containing biomolecules if one is to gain a full understanding of possible biological targets. This study provides structural information about the possible fate of the S−H group in such reactions. The main reaction reported here is the formation of $Rh^{III}(\eta^2\text{-}C_6H_6NS)(\eta^1\text{-}C_6H_6NS)_2(phen)$ (**2**) from the reaction of $[Rh_2(\mu\text{-}O_2CCH_3)_2(phen)_2(CH_3CN)_2]^{2+}$ with excess 2-amino-

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(16) Crystal data for **3**. Triclinic space group $P\bar{1}$, a = 13.727(3) Å, b = 14.370 Å, c = 15.985 Å; α = 71.04(3)°, β = 84.26(3)°, γ = 88.83(3)°; V = 2966.9(11) Å^3; T = 110(2) K; Z = 2. Graphite monochromatized Mo Kα radiation (λ_α = 0.710 69 Å), Bruker CCD diffractometer, 0.25 × 0.35 × 0.50 mm^3, 15 221 reflections measured, 11 610 of which were unique (R_{int} = 0.1191). The structure was solved by direct methods (SIR97) followed by Fourier synthesis and refined on F2 (SHELX-97). The final refinement gave $R(F^2)$ = 0.1326 and wR2(F^2) = 0.3045, by using 4130 reflections per 411 parameters ($I > 4\sigma$).

(17) Mueting, A. M.; Boyle, P.; Pignolet, L. H. *Inorg. Chem.* **1984**, *23*, 44.

(18) Connelly, N. G.; Johnson, G. A.; Kelly, B. A.; Woodward, P. *J. Chem. Soc., Chem. Commun.* **1977**, 436.

thiophenol in the presence of oxygen. One plausible reaction pathway to this product is the formation of a $Rh_2^{II/II}$ intermediate with 2-aminothiophenol bridging ligands. The proposed Rh^{II} intermediate, with the electron-rich sulfur donor ligands, would be easier to oxidize than the parent compound, which is indefinitely stable in air. Efforts to isolate the Rh^{II} intermediate(s) by carrying out the reaction under anaerobic conditions have been unsuccessful due primarily to the fact that the products are not very tractable. Some insight into intermediates was obtained, however, by low-temperature crystallization of the product from reactions of $[Rh_2(\mu\text{-}O_2CCH_3)_2(bpy)_2(CH_3CN)_2]^{2+}$ with 4 equiv of 2-aminothiophenol, which led to the isolation of $[Rh_2^{III,III}(\mu\text{-}C_6H_6NS)_2(C_6H_6NS)_2(bpy)_2]^{2+}$ (**3a**) and $[Rh^{III}(C_6H_6NS)_2(bpy)]^{+}$ (**3b**) as cocrystallized cations in the same unit cell. The existence of $[Rh_2^{III,III}(\mu\text{-}C_6H_6NS)_2(C_6H_6NS)_2(bpy)_2]^{2+}$ (**3a**) hints that the reaction proceeds via stable dinuclear intermediates before cleavage to Rh^{III} mononuclear species occurs.

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Supporting Information Available: X-ray crystallographic files for **2** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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