Communications

Isolation, Reactivity, and Molecular Structure of $Pt_2\{\mu$-L-$SCH_2CH(COO)NHC(O)CH_3$-$S\}_2(bpy)_2$: Model of the Interaction of Platinum with Protein Sulfur Residues

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A limitation in the use of *cis*-platin as an antitumor drug is its concentration-dependent nephrotoxicity.[1,2] This toxicity is believed[3] to result from the binding of platinum to sulfur-functionalized protein residues. In order to probe the nature of this interaction, platinum amine complexes containing *N*-acetylcysteinato, accys, and glutathionato ligands have been the focus of several recent studies.[4-10] However, the structures of these products still have not been unambiguously determined.[11] As part of our investigation of agents for the release of platinum from protein cysteine residues,[12] we explored the reaction of *N*-acetyl-L-cysteine, L-Haccys, with $PtCl_2$(2,2′-bipyridine). We have isolated and purified a platinum accys complex in which the bipyridine ligand remains coordinated. We wish to report here the characterization of this complex, including a single-crystal X-ray structural determination. Additionally, we have found that the platinum–thiolato bonds of our cysteine residue complex are susceptible to cleavage through reaction with sodium diethyldithiocarbamate.

The title complex was prepared through the reaction of a suspension of $PtCl_2$(2,2′-bipyridine)[13] (**1**) (0.200 g, 4.7 mmol) and an equimolar amount of L-Haccys (0.076 g, 4.7 mmol) in 5 mL of water which was brought to pH 11 by treatment with aqueous 1 M KOH. The solution was stirred for 5 days at 25 °C and filtered. The filtrate was treated with 90 mL of diethyl ether and 80 mL of acetone. Upon 3 days of standing, light yellow, crystalline product arose from the dark brown oil which separated below the clear solution. The product, $Pt_2(\mu$-L-accys-$S)_2(bpy)_2$[14] (**2**), was isolated in 62% yield.

The molecular structure of **2** was determined through a single-crystal X-ray diffraction study.[15] A diagram of the obtained structure is seen in Figure 1. The heavy atom framework is identical to that which has been proposed by Appleton *et al.* on the basis of multinuclear NMR spectroscopic studies[8] for the species initially formed in the reaction between *cis*-$PtCl_2(NH_3)_2$ and model protein cysteine residue compounds, including GSH and Haccys. The reaction of $[Pt(dien)Cl]^+$ (dien = diethylenetriamine) and GSH has also been proposed to yield a diplatinum μ-thiolato complex.[7] The unusual syn orientation of the accys ligands probably is adopted in order to establish the observed hydrogen-bonding interactions with the solvate water molecules. Our results provide direct structural evidence of the dinuclear nature of the products arising from the reaction between platinum *cis*-amine complexes and model protein cysteine residue compounds.

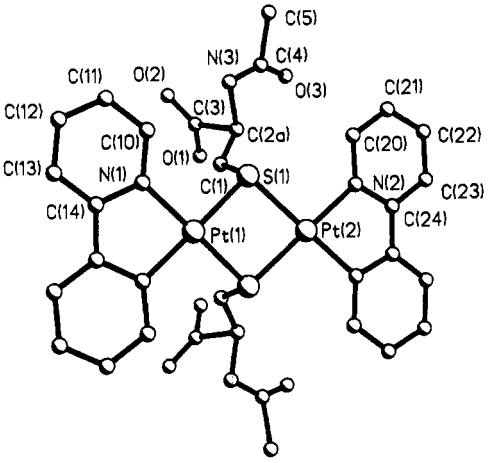

Figure 1. Projection of $Pt_2(\mu$-accys-$S)_2(bpy)_2$ (**2**), with thermal ellipsoids at arbitrary radii. Selected bond lengths (Å): Pt(1)–S(1), 2.312(5); Pt(2)–S(1), 2.303(5); Pt(1)–N(1), 2.01(1); Pt(2)–N(2), 2.01(1). The hydrogen atoms have been omitted for clarity.

Sodium diethyldithiocarbamate, Na(ddtc), has been found[3] to reduce the nephrotoxicity resulting from *cis*-platin therapy and is believed[3,16] to act by removing platinum bound to protein sulfur sites. Recently, the model complex $[Pt_2(dien)_2(\mu$-GS-Me)$]^{3+}$ was found to react with Na(ddtc) to produce $Pt(ddtc)_2$ while $[Pt_2(dien)_2(\mu$-GS)$]^{2+}$ was found to be unreactive in parallel experiments.[17] From these results it was concluded that Na-

(1) Krakoff, I. H. *Cancer Treat. Rep.* **1979**, *63*, 1523–1525.
(2) Dentno, M.; Luft, F. C.; Yum, N. M.; Williams, S. D.; Einhorn, L. H. *Cancer* **1978**, *41*, 1274–1281.
(3) Borch, R. F.; Pleasants, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 6611–6614.
(4) Odenheimer, B.; Wolf, W. *Inorg. Chim. Acta* **1982**, *66*, L41–L43.
(5) Dedon, P. C.; Borch, R. F. *Biochem. Pharmacol.* **1987**, *36*, 1955–1964.
(6) Corden, B. J. *Inorg. Chim. Acta* **1987**, *137*, 125–130.
(7) Lempers, E. L. M.; Inagaki, K.; Reedijk, J. *Inorg. Chim. Acta* **1988**, *152*, 201–207.
(8) Appleton, T. G.; Connor, J. W.; Hall, J. R.; Prenzler, P. D. *Inorg. Chem.* **1989**, *28*, 2030–2037.
(9) Berners-Price, S. J.; Kuchel, P. W. *J. Inorg. Biochem.* **1990**, *38*, 305–325.
(10) Djuran, M. I.; Lempers, E. L. M.; Reedijk, J. *Inorg. Chem.* **1991**, *30*, 2648–2652.
(11) Lempers, E. L. M.; Reedijk, J. *Adv. Inorg. Chem.* **1991**, *37*, 175–217.
(12) Wyatt, K. S.; Harrison, K. N.; Jensen, C. M. *Inorg. Chem.* **1992**, *31*, 3867–3868.
(13) The complex was synthesized by refluxing K_2PtCl_4 (2.00 g, 4.81 mmol) with 1 equivalent of 2,2′-bipyridine (0.754 g, 4.81 mmol) in 100 mL of water for 0.5 h. The resulting orange precipitate was isolated by filtration, washed with diethyl ether, and vacuum-dried (1.760 g, 86% yield).
(14) ^1H NMR (500 MHz, D_2O), δ: 8.75 (br s), 8.62 (br s), 8.28 (m), 8.12 (m), 7.82 (m), 7.76 (m) (8H, aromatic); 4.32 (br m, CH_2CH(NHC(O)OMe)(C(O)OH)); 3.59, 3.20 (br m, SCH_2); 1.69 (s, $COCH_3$). $^{13}C\{^1H\}$ NMR (126 MHz D_2O), δ: 175.0 (COOH); 174.4 (COMe); 155.9, 148.0, 143.8, 130.1, 126.0 (aromatic); 56.0 (CH_2CH(NHC(O)OMe))(C(O)OH)); 38.1 (SCH_2); 22.7 ($COCH_3$). ^{195}Pt NMR (107.2 MHz D_2O), δ: −2909.
(15) Single crystals suitable for X-ray diffraction were obtained by slow evaporation of an acetone/diethyl ether/water solution of **2**. Crystallographic data for **2**·5.5H_2O: monoclinic $C2/m$, $Z = 4$ (0.5 molecule of **2** and 2.75 water solvates per asymmetric unit), $a = 19.491(8)$ Å, $b = 19.266(6)$ Å, $c = 11.494(4)$ Å, $\beta = 102.88(3)°$, $V = 4208(3)$ Å^3, $\rho_{calc} = 2.60$ g/cm^3; $\mu = 10.44$ cm^{-1}; Nicolet P3 diffractometer, Mo Kα radiation ($\lambda = 0.710\,73$ Å); 2369 independent reflections with $4° < 2\theta < 42$ collected, 1925 reflections used in refinement with $I > 3\sigma(I)$; $R = 0.063$, $R_w = 0.093$, GOF = 3.14. The β-carbon of the accys ligand and two of the water solvates were found to be thermally disordered.
(16) Gonias, S. L.; Oakley, A. C.; Walther, P. J.; Salvatore, S. V. *Cancer Res.* **1984**, *44*, 5764–5770.
(17) Lempers, E. L. M.; Reedijk, J. *Inorg. Chem.* **1990**, *29*, 217–222.

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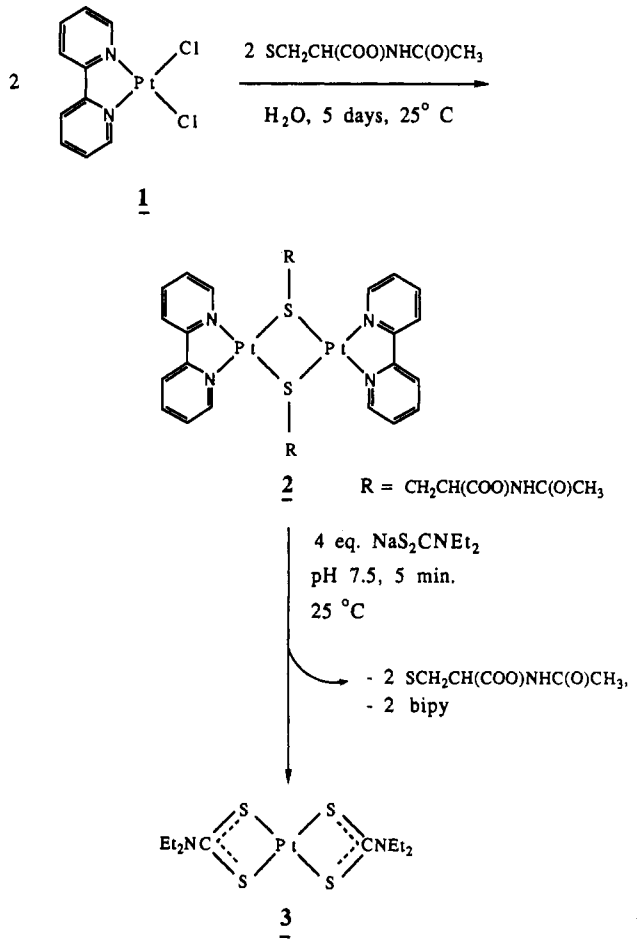
Scheme I



(ddtc) is effective in removing platinum only from methionine sites and not cysteine sites in proteins. We have explored the reaction of **2** with Na(ddtc). As seen in Scheme I, addition of 4 equiv of Na(ddtc) (0.044 g, 0.194 mmol) to a solution of **2** (0.050 g, 0.049 mmol) in 10 mL of water buffered at pH 7.5 at 25 °C results in the precipitation of $Pt(ddtc)_2$[18] (**3**) in 96% yield within 5 min. Although the reactivity between the model complexes and Na(ddtc) should be influenced by the differing trans effect of the aromatic chelating amine ligand in our complex and that of the chelating aliphatic dien ligand, the contrast in the observed reactivities is quite surprising. Explanation of the differing reactivities awaits isolation and structural characterization of a platinum cysteine residue complex containing aliphatic amine ligands and determination of its reactivity with Na(ddtc).

It has not been established whether platinum binding to protein cysteine residues involves μ-thiolato interactions. Our results clearly demonstrate the preferential formation of diplatinum μ-S amino acid species. However, the likelihood of platinum dimer formation *in vivo* can be questioned in view of the low *in vivo* platinum concentrations resulting from therapeutic doses of *cis*-platin. Thus it is uncertain whether the structure which we have elucidated for **2** models the binding of platinum to cysteine sites in proteins. However, our study does unambiguously establish the structure of the products obtained in the reaction of platinum amine chlorides with Haccys. Furthermore this work suggests that, contrary to the indications of earlier studies, Na(ddtc) might be somewhat effective in releasing platinum from cysteine sites in proteins.

Note Added in Proof. Since submission of this manuscript, we have found that the ethylenediamine analog of **2** undergoes a similar rapid reaction with Na(ddtc).

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Supplementary Material Available: Tables of crystal data, thermal parameters, bond distances, bond angles, and atom coordinates for $Pt_2(\mu$-L-accys-*S*$)_2(bpy)_2$, $2{\cdot}5.5H_2O$ (6 pages). Ordering information is given on any current masthead page.

(18) ^{1}H NMR (500 MHz, CD_2Cl_2), δ: 3.57 (q, J_{H-H} = 7.2 Hz, CH_2); 1.28 (t, J_{H-H} = 7.2 Hz, CH_3). $^{13}C\{^1H\}$ NMR (126 MHz CD_2Cl_2), δ: 210.6 (S_2C); 44.4 (CH_2); 12.4 (CH_3).