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Formation of a Cationic Gold(I) Complex and Disulfide by Oxidation of the Antiarthritic Gold Drug Auranofin

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The mechanism of action of auranofin, an antiarthritic gold(I) drug, is unknown, but several studies suggest that oxidation may be important for its biochemical effect. Bulk electrolysis studies on auranofin [$(Et_3P)Au(TATG)$; TATG = 2,3,4,6-tetraacetyl-1-thio-D-glucopyranosato] at +1.2 and +1.6 V versus Ag/AgCl in 0.1 M Bu_4NBF_4/CH_2Cl_2 results in *n* values of 0.5 and >2 electrons, respectively. Oxidation of auranofin with the mild oxidant, Cp_2Fe^+, results in formation of disulfide and a digold(I) cation with a bridging thiolate ligand, $[(Et_3PAu)_2(\mu\text{-TATG})]^+$ (**1**). The X-ray structure of the PMe_3 analogue, $[(Me_3PAu)_2(\mu\text{-TATG})](NO_3)$ (**2**), is reported. Compound **2** forms a tetranuclear cluster containing an almost perfect square of four gold atoms with Au···Au distances averaging 3.14 Å. The complex crystallizes in the tetragonal space group $P4_22_12$ with cell constants $a = 26.1758(6)$ Å, $b = 26.1758(6)$ Å, $c = 9.7781(3)$ Å, $\alpha = \beta = \gamma = 90°$, $V = 6699.7(3)$ Å^3, $Z = 4$, R1 = 0.0644, and wR2 = 0.1152. A mechanism for oxidation of auranofin and possible biological implications are discussed.

While the mechanism of the medicinal activity of gold drugs remains elusive, there is evidence to suggest that redox pathways could be involved.[1,2] The oxidative pathologies that have been noted for rheumatoid arthritis and the ease by which gold(I) thiolates undergo oxidation suggest several possible redox roles in the anti-inflammatory response and/or in deleterious secondary reactions.[2,3] This range of potential biological activity points to the need to better understand the reactivity of gold sulfur complexes in order to establish the possible mechanistic pathways, as well as to suggest pathways that are likely.

Earlier work in our laboratory established that phosphine gold(I) complexes containing terminally bonded aromatic thiolate ligands undergo sulfur based oxidation at ca. +0.7 to +0.9 V producing disulfide and multinuclear cationic gold(I) clusters with bridging thiolate ligands.[4,5] Furthermore, it was demonstrated that these gold(I) μ-thiolate clusters oxidize at significantly higher potentials. Cyclic voltammetry studies of auranofin in 0.1 M Bu_4NBF_4/CH_2Cl_2 using Pt working and auxiliary electrodes show two irreversible oxidation processes occurring at +1.1 and +1.6 V versus Ag/AgCl.[6] The electrochemical response is sensitive to adsorption effects and the nature of the electrolyte solution (i.e., BF_4^- vs PF_6^- counterions). In previous electrochemical studies on a series of phosphine gold(I) thiolate complexes, the first oxidation was assigned as sulfur based.[4] This assignment is also consistent with electronic structure studies, which assigned the HOMO as primarily sulfur in character.[7]

In research at Smith, Kline & French Laboratories leading up to FDA approval of auranofin, several researchers proposed cationic gold(I) complexes as reactivity products of auranofin. Hill and Elder et al. obtained preliminary data on the solid state structure of $[(Et_3PAu)_2(\mu\text{-TATG})]_2(NO_3)_2$, in which pairs of gold atoms coordinate to a bridging

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thiolate.[8] The quality of the data was not high enough to completely solve the structure, so it was never published. However, assay in a rat arthritis model showed the cationic complex to have activity similar to auranofin at 10 mg Au/Kg. Hemple et al. studied the effect of aqueous HCl on auranofin to mimic its behavior in stomach acid. The ionic complex, $[(Et_3PAu)_2(\mu\text{-TATG})]Cl$, was proposed as one of the products. However, trials to isolate the ionic structure resulted only in $(Et_3P)Au(TATG)$ and Et_3PAuCl.[9] It was with these perspectives that we sought to determine the fate of auranofin upon oxidation.

Bulk electrolysis experiments on auranofin at +1.2 V versus Ag/AgCl in 0.1 M Bu_4NBF_4/CH_2Cl_2 result in $n = 0.5$.[10] Completion of electrolysis was checked by cyclic voltammetry, which confirmed the disappearance of the first oxidation wave in the electrolysis product solutions. The second oxidation peak at approximately +1.6 V is still present. Bulk electrolyses of auranofin at +1.6 V yield n values greater than 2. This is consistent with gold based oxidation, but the oxidation products at the higher potential have not yet been determined.

The oxidation products at the lower potential were characterized by employing the one-electron oxidant ferrocenium.[11] Reaction of auranofin with one-half molar equivalent of ferrocenium yields ferrocene, disulfide, and a cationic gold(I) complex according to eq 1.[12]

$$2(R_3P)Au(TATG) + Cp_2Fe^+ \rightarrow Cp_2Fe + 0.5(TATG)_2 + [(R_3PAu)_2(\mu\text{-TATG})]^+ \quad (1)$$

$$R = CH_3CH_2\ (\mathbf{1});\ CH_3\ (\mathbf{2})$$

Cyclic voltammetry measurements on the crude product following complete oxidation of auranofin using Cp_2Fe^+ showed a reversible wave due to Cp_2Fe and an irreversible peak at +1.65 V.[13] Ferrocene and the disulfide, $(TATG)_2$, were identified in the crude reaction mixture by ^1H NMR.[14,15] The cationic gold complex was isolated as the PF_6^- salt after recrystallization from CH_2Cl_2/Et_2O. The PMe_3 derivative of auranofin can also be oxidized by Cp_2Fe^+ in an identical fashion (eq 1).

Numerous attempts to grow X-ray quality crystals of the PEt_3 derivative (**1**) were unsuccessful. These attempts included changing the counteranion (PF_6^-, NO_3^-, $CF_3SO_3^-$, BF_4^-) and using a variety of solvent combinations. Finally, X-ray quality crystals of the PMe_3 derivative (**2**) were obtained by using the combination of the NO_3^- counteranion and the solvent mixture CH_2Cl_2/Et_2O (1:3).

Crystals of $[(Me_3PAu)_2(\mu\text{-TATG})]_2(NO_3)_2$ (dimer of **2**) were obtained as colorless needles.[16] The complex crystallizes in the tetragonal space group $P4_22_12$.[17] The molecular and crystallographic symmetries coincide running through the center of the cation and anions (N1 and N2 of the nitrate ions lie on special positions). The oxygen atoms in NO_3^- exhibit disorder, and an unknown solvent, presumably a highly disordered Et_2O, or multiple H_2O molecules, is also present in the crystalline lattice. The molecular structure (NO_3^- omitted) is shown as an ORTEP drawing (Figure 1a) and a ball-and-stick representation (Figure 1b). Selected bond lengths and angles are listed in the figure caption.

The cationic digold complex, **2**, is associated into dimers via intermolecular Au···Au interactions between monomers. The structure consists of nearly linear two-coordinate gold(I) atoms (P−Au−S angles average 176°). Each thiolate ligand bridges two gold(I) atoms with an acute angle of approximately 83°, and the four gold atoms form an almost

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(10) Bulk electrolyses of auranofin at +1.2 V vs Ag/AgCl in 0.1 M Bu_4NBF_4/CH_2Cl_2 resulted in 0.49 ± 0.12 electrons being passed on the basis of 9 experiments: 0.44, 0.65, 0.25, 0.6, 0.54, 0.54, 0.49, 0.38, and 0.61.

(11) Electrochemical and chemical oxidation experiments were also carried out in CH_3CN solution with very similar results. The cyclic voltammogram of auranofin and the NMR spectra of the cationic gold(I) complex appear to be more sensitive to the nature of the anion than the solvent.

(12) To 500 mg (0.73 mmol) of auranofin dissolved in 100 mL of CH_2Cl_2 under N_2 was added 122 mg (0.36 mmol) of $(Cp_2Fe)PF_6$ (1:0.5). Stirring continued for 24 h until the reaction mixture changed from blue (ferrocenium) to yellow (ferrocene). The solvent was evaporated in vacuo, and the residue was washed with ether (3×) to remove Cp_2Fe. The off-white solid was recrystallized by dissolving in 3 mL of CH_2Cl_2, followed by addition of ether or hexane. Reaction of $(Me_3P)Au(TATG)$ with $(Cp_2Fe)PF_6$ proceeds in a similar fashion. Analytically pure samples using different counteranions were prepared as described in ref 20.

(13) Complex **1** oxidizes irreversibly at ca. +1.65 V, and the disulfide, $(TATG)_2$, also exhibits an irreversible oxidation in this region.

(14) The resonances for the ring protons (H1−H6) on the thioglucose ligand in auranofin and related structures have been unambiguously assigned.[15] Monitoring the resonance shift of H1 is an efficient approach for detecting coordination to gold(I) or oxidation of sulfur. Thus formation of the disulfide, $(TATG)_2$, is confirmed by the appearance of a doublet at 4.65 ppm for H1 (in $CDCl_3$). For comparison, this proton appears as a doublet at 5.14 ppm for auranofin and at 5.25 ppm for **1**.

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(16) A reviewer has questioned the existence of the tetranuclear structure in dilute solution. This question remains unresolved. However, in related chemistry, oxidation of $dppe(AuSR')_2$ results in formation of $[(dppe)Au_2(\mu\text{-}SR')]_2^{2+}$ in which dppe and thiolate bridge between two *different* gold atoms. In this case, it is likely that the tetranuclear structure is present in solution.

(17) Crystallographic data for **2**: $C_{40}H_{74}Au_4N_2O_{24}P_4S_2$, MW = 1942.88, T = 150(2) K, λ = 0.71073 Å, tetragonal, space group $P4_22_12$, a = 26.1758(6) Å, b = 26.1758(6) Å, c = 9.7781(3) Å, $\alpha = \beta = \gamma = 90°$, V = 6699.7(3) Å^3, Z = 4, D_c = 1.926 Mg/m^3, μ = 8.957 mm^{-1}, and $F(000)$ = 3712. Data were collected on a Siemens SMART 1K CCD diffractometer. A total of 44007 reflections were collected in the θ range 2.46−28.31° of which 8321 were unique (R_{int} = 0.1028). An absorption correction, based on the multiscan technique and beam corrections, was applied using SADABS (transmission correction: min 0.1408, max 0.5728). The structure was solved by a combination of the Patterson method using SHELXTL v5.1 and the difference Fourier technique and refined by full-matrix least-squares on F^2. Refinement converged with crystallographic agreement factors of R1 = 0.0644, wR2 = 0.1152, for all 6114 reflections with $I \geq 2\sigma(I)$ (R1 = 0.0958, wR2 = 0.1302 for all data) and GOF = 1.099.

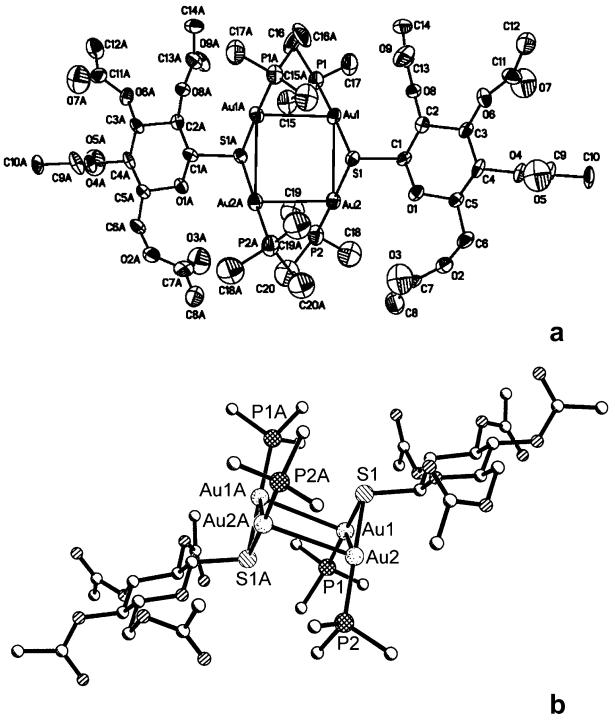

Figure 1. Dicationic structure of **2** with hydrogen atoms and NO_3^- anions omitted for clarity. (a) Thermal ellipsoid representation (50%) looking down on the gold square and (b) ball-and-stick representation shown as a side view. Selected bond lengths (Å) and angles (deg): Au(1)−P(1) 2.259(4); Au(1)−S(1) 2.334(3); Au(1)−Au(2) 3.106(7); Au(1)−Au(1A) 3.171(11); Au(2)−P(2) 2.270(4); Au(2)−S(1) 2.355(3); Au(2)−Au(2A) 3.144(12); P(1)−Au(1)−S(1) 176.94(14); Au(2)−Au(1)−Au(1A) 89.74(2); P(2)−Au-(2)−S(1) 175.1(2); Au(1)−Au(2)−Au(2A) 90.23(2); Au(1)−S(1)−Au(2) 82.96(10).

perfect square.[18] The thiolate-bridged Au(1)···Au(2) distance (3.11 Å) is slightly shorter than the nonbridged Au···Au distances (3.17 and 3.14 Å). The thioglucose ligand adopts a chair configuration with one ligand above and the other one below the plane of four gold atoms (Figure 1b).

Scheme 1 illustrates a possible mechanism for the first oxidation process that accounts for the *n* value (0.5) and the observed products.[19] Similar results have been shown for other phosphine gold(I) thiolate complexes.[5] The LAu^+ species is probably solvated and/or associated with an anion, BF_4^- in the case of bulk electrolysis experiments. Support

Scheme 1 [a]

$$\begin{aligned} LAuSR &\xrightarrow{-1e^-} LAuSR^+ \\ LAuSR^+ &\longrightarrow LAu^+ + RS^\cdot \\ RS^\cdot &\longrightarrow {}^1/_2\,RSSR \\ LAu^+ + LAuSR &\longrightarrow [(LAu)_2(\mu\text{-}SR)]^+ \\ \hline 2\,LAuSR &\xrightarrow{-1e^-} {}^1/_2\,RSSR + [(LAu)_2(\mu\text{-}SR)]^+ \end{aligned}$$

[a] L = Et_3P, Me_3P; SR = TATG.

for this mechanism is provided by experiments in which **1** and **2** are independently prepared by addition of $R_3PAu^+X^-$ to R_3PAu(TATG) in CH_2Cl_2 solution.[20]

Oxidation of auranofin by Cp_2Fe^+ is noteworthy because ferrocenium generally behaves as an outer sphere electron transfer reagent and it has a reversible redox couple in 0.1 M Bu_4NPF_6/CH_2Cl_2 at + 0.46 V versus SCE.[21] This result suggests that the true redox potential of auranofin is much lower than +1.1 V. Thus, oxidation of auranofin could occur more easily (i.e., at lower potentials) than previously appreciated. The mechanism of oxidation of gold(I) thiolates by Cp_2Fe^+ and other one-electron oxidants is currently under investigation.

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Supporting Information Available: Crystal structure data, bond angles and distances, and atomic coordinates and equivalent isotropic displacement parameters are available for [$(Me_3PAu)_2$(*μ*-TATG)](NO_3) (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) For other examples of this structural motif see: (a) Wang, S.; Fackler, J. P., Jr. *Inorg. Chem.* **1990**, *29*, 4404−4407. (b) Sladek, A.; Schneider, W.; Angermaier, K.; Bauer, A.; Schmidbaur, H. *Z. Naturforsch., B: Chem. Sci.* **1996**, *51*, 765−772. (c) Lopez-de-Lurzuriaga, J. M.; Sladek, A.; Schneider, W.; Schmidbaur, H. *Chem Ber./Recl.* **1997**, *130*, 641−646.

(19) Alternative mechanisms can be proposed that do not involve free thiyl radicals, e.g., $2R_3PAuSR^+ \rightleftharpoons 2R_3PAu^+ + RSSR$. Evidence for the existence of the thiyl radical is currently under study.

(20) Independent syntheses of [$(R_3PAu)_2$(TATG)]X (R = Et, Me; X = PF_6^-, NO_3^-, $CF_3SO_3^-$, BF_4^-) follow: AgX (1 mmol) was dissolved in 10 mL of CH_3CN or C_2H_5OH and was added slowly to 1 mmol of Et_3PAuCl or Me_3PAuCl dissolved in 10 mL of CH_2Cl_2 at 0 °C. The mixture was stirred in the dark at 0 °C for 30 min and then filtered through Celite 545. The filtrate was reduced to 3 mL in vacuo, and ether was added to form a white precipitate of R_3PAuX which was filtered and washed with ether. To 1 mmol of (R_3P)Au(TATG) dissolved in 10 mL of CH_2Cl_2 was added 1 mmol of R_3PAuX dissolved in 10 mL of CH_2Cl_2, and the mixture was stirred for 30 min at 0 °C. The mixture was reduced to 5 mL in vacuo, and ether was added to give an off-white precipitate. The sample was recrystallized by diffusion of ether into CH_2Cl_2 and dried over P_2O_5 in vacuo for 24 h. Anal. Calcd for [$(Et_3PAu)_2$(*μ*-TATG)]$_2(NO_3)_2$ ($C_{52}H_{98}Au_4N_2O_{24}P_4S_2$): C 29.58; H 4.68. Found, C 29.14; H 4.60. Mass spectroscopy (ESI): *m/e* 993.2 (993.6 calcd for [$(Et_3PAu)_2$(TATG)]$^+$). ^{1}H NMR (300 MHz; $CDCl_3$): 1.2 (18H, dt, PCH_2CH_3), 1.85−2.1 (24H, m, 4OAc + 6PCH_2CH$_3$), 4.0 (1H, m, H5), 4.25−4.30 (2H, dd, H6), 5.0−5.15 (3H, m, H2−H4), 5.50 (1H, d, H1). ^{31}P {^{1}H}NMR (300 MHz, $CDCl_3$): 36.5 ppm. Anal. Calcd for [$(Me_3PAu)_2$(TATG)]$_2(NO_3)_2$·Et_2O ($C_{44}H_{84}Au_4N_2O_{25}P_4S_2$): C 26.20; H 4.20. Found, C 26.13; H 4.01. ^{1}H NMR (300 MHz; $CDCl_3$): 1.2 (18H, d, PCH_3), 2.0−2.1 (12H, 4s, 4OAc), 3.9 (1H, m, H5), 4.20−4.25 (2H, dd, H6), 5.0−5.2 (3H, m, H2−H4), 5.35 (1H, d, H1). ^{31}P {^{1}H}NMR (300 MHz, $CDCl_3$): −0.17 ppm.

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