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Reactions of Auranofin ((1-Thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I)) in Aqueous Hydrochloric Acid

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Auranofin ((1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I)) is an orally active drug for the treatment of rheumatoid arthritis. Auranofin reacts with HCl in aqueous solution and in 50% methanol/water to form chloro(triethylphosphine)gold(I) ($ClAuPEt_3$) and with $ClAuPEt_3$ to form a thiolate-bridged dinuclear gold complex with two gold triethylphosphine moieties bound to a single thioglucose ligand ((μ-1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*:*S*)bis(triethylphosphine)digold(I) chloride). The thermodynamics and kinetics of these reactions have been studied in water and in 50% methanol/water. The equilibrium constants for the formation of $ClAuPEt_3$ are 4.6×10^{-4} M^{-1} in water and 2.0×10^{-3} M^{-1} in 50% methanol/water while the equilibrium constant for the formation of the thiolate-bridged digold complex is 1.2×10^3 (water), 1.3×10^2 (50% methanol/water), and 0.7×10^2 (95% methanol). The kinetics for the formation of the thiolate-bridged digold complex are too rapid to be observed by ordinary mixing techniques, but the chloride replacement of tetraacetylthioglucose could be studied via the stopped-flow method. For the reaction at 25 °C in 1.0 M Cl^-, $k_{obsd} = 385[H^+]$ in the mixed-solvent medium and $k_{obsd} = 210[H^+] + 4.3 \times 10^5[HTATG]$ in water in the presence of excess tetraacetylthioglucose.

Introduction

Complexes of gold(I) used in the treatment of rheumatoid arthritis are shown in Figure 1. The etiology of rheumatoid arthritis is not well-understood, and biological target site(s) responsible for the pharmacological activity of these gold(I) coordination compounds are not known. Implicit in recent reviews of the biologically active gold compounds[1-5] is the expectation that gold drugs may serve not only as a therapy for rheumatoid arthritis but also as a probe for discovering its cause. However, relatively little is known about the solution chemistry of gold(I) compounds, which is a major obstacle to achieving this goal.

Characteristic of these drugs as well as other gold(I) complexes is the tendency of the metal to coordinate two ligands in approximately linear coordination geometry.[1-5] Auranofin ((1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I)) is an orally active drug while Solganal (gold thioglucose) and Myochrysine (gold sodium thiomalate) are active only by injection. As suggested by the physical properties of their ligands, Solganal (with thioglucose ligands) and Myochrysine (with thiomalate ligands) are extremely water soluble whereas auranofin (with tetraacetylthioglucose and triethylphosphine ligands) is sparingly soluble in water. The reactions of auranofin are therefore more conveniently studied in mixed methanol/water solvent systems. However, experiments have been conducted in aqueous solution as well in this study so that the influence of the medium on ligand-exchange reactions of auranofin in aqueous HCl may be considered.

Model studies have recently been reported that quantitate ligand-exchange reactions of auranofin with biologically available ligands at physiologically relevant concentrations of drug and biomolecule.[6,7] Thiol-exchange reactions were observed. It is worth noting that monodentate thiol ligands such as bovine serum albumin (BSA) do not displace the phosphine moiety. A triple-label radioisotope methodology, which makes it possible to follow the fate of the gold ion of auranofin and both ligands in the same experiment, has been used to demonstrate that ligands are not displaced from auranofin or BSA-$AuPEt_3$ in the presence of a 1000-fold excess of NaCl (150 mM NaCl) at neutral pH.[6] Knowledge of ligand-exchange reactions of the metallodrugs in biological systems provides a basis for mechanism of action hypotheses. Knowledge of the reactivity of gold(I) complexes can provide critical insights into structure–activity relationships (SAR) and may possibly suggest a logical chemical basis for the synthesis of more effective compounds.[6]

Experimental Section

Auranofin ((1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*)(triethylphosphine)gold(I), Aur,[8] chloro(triethylphosphine)gold(I) ($ClAuPEt_3$),[8] and (μ-1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*:*S*)bis(triethylphosphine)digold(I) nitrate[9] ($DigoldNO_3$) were supplied in purified form by Smith Kline and French Laboratories. Tetraacetylthioglucose in the acid form (HTATG) was purchased from Aldrich Chemical Co. All other chemicals were reagent grade and supplied by Fisher Scientific Co.

Components of a 0.10 M auranofin and 0.10 M HCl mixture were isolated from 95% methanol/water 20 min after mixing.[10] This solvent was chosen because auranofin is much more soluble in methanol (10^{-1} M) than in water (10^{-4} M). The reaction components were identified by comparison of their TLC, NMR, and mass spectroscopic profiles with those of authentic samples of compounds of known structure.[10] Preparative silica gel TLC (5% acetone/CH_2Cl_2) was used to identify auranofin ($R_f = 0.5$) in the reaction mixture, along with tetraacetylthioglucose ($R_f = 0.7$), chloro(triethylphosphine)gold(I) ($R_f = 1.0$), and the thiolate digold complex with two gold centers (μ-1-thio-β-D-glucopyranose 2,3,4,6-tetraacetato-*S*:*S*)bis(triethylphosphine)digold(I) chloride ($R_f = 0.3$). Mass spectroscopic studies of aliquot mixtures did not reveal deacylated products.[10]

Standard absorption spectra (in 95% methanol) for the three gold-containing compounds identified above are illustrated in Figure 2. The maximum molar absorptivity for tetraacetylthioglucose is approximately 500 M^{-1} cm^{-1} (210 nm), whereas for auranofin $\epsilon_{220} = 5800$ M^{-1} cm^{-1}, for $ClAuPEt_3$ $\epsilon_{234} = 2200$ M^{-1} cm^{-1}, and for the thiolate digold complex $\epsilon_{242} = 7200$ M^{-1} cm^{-1}. Spectra were recorded with a Hewlett-Packard 8450A spectrophotometer, 1 cm cell path length, and were observed to differ only slightly in water and in methanol, as expected.

The spectra of reaction mixtures that contain these compounds were reproduced as a weighted sum of standard spectra in the appropriate solvent (from 220 to 350 nm) by using the least-squares fitting program supplied by Hewlett-Packard.[11] The concentrations of the components of the reaction mixtures derived in this fashion yield equilibrium constants, as outlined below. These constants are reported to ±25%. Kinetics experiments were performed on a Dionex Model 110 stopped-flow

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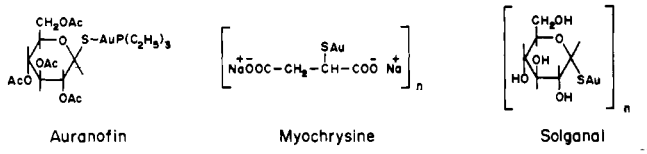

Figure 1. Gold(I) coordination compounds used in the treatment of rheumatoid arthritis. Myochrysine and Solganal are oligomeric compounds.[1-5]

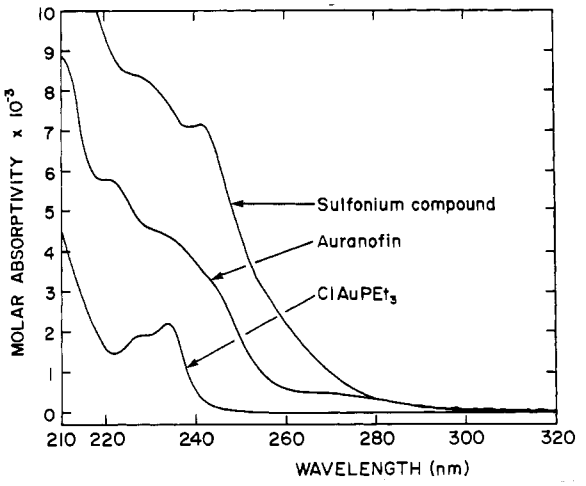

Figure 2. Standard absorption spectra in 95% methanol/water: (top) (μ-1-thio-β-D-glycopyranose 2,3,4,6-tetraacetato-*S*:*S*)bis(triethylphosphine)digold(I) nitrate with the absorbance of the nitrate counterion subtracted from the spectrum; (middle) auranofin; (bottom) chloro(triethylphosphine)gold(I).

apparatus interfaced to a Cromemco Z-2 microcomputer system with rate constants reported to ±15%.

Both the thermodynamic and kinetic studies use UV absorption spectroscopy to monitor reaction. Equilibrium constants for the reaction of auranofin with HCl derived from kinetics studies (in water and in 50% methanol) agree within experimental error with the equilibrium constants derived from analyses of the absorption spectra of reaction mixtures. The kinetics experiments are over within milliseconds. The absorption spectra of the reaction mixtures are established upon mixing and are stable for hours. These results imply that if deacylation of the tetraacetylthioglucose ligand of auranofin occurs, the deacylation process is either very fast or very slow. Since the absorption spectrum of auranofin is not sensitive to deacylation,[12] these studies do not establish whether deacylation of the TATG ligand of auranofin has occurred under the range of concentrations and solvent conditions considered.

Results

Absorption Spectra. Standard absorption spectra for the three gold(I) coordination compounds present in a 1:1 mole ratio of auranofin to HCl reaction mixture in 95% methanol are compared in Figure 2. The UV absorption bands observed for $ClAuPEt_3$ are characteristic of gold(I) compounds with a triethylphosphine ligand.[12,13] Additional broad bands are observed for gold(I) compounds containing a thiol ligand in addition to a triethylphosphine ligand. That is to say, charge-transfer bands are observed for gold compounds with phosphine and thiol ligands but not with chloride.[13,14] The absorption spectrum of the thiolate digold complex, in which two gold–triethylphosphine moieties are coordinated to a sulfur ligand, is characterized by an absorbance maximum at 242 nm, which is not observed in the spectrum of $ClAuPEt_3$ or auranofin or in the sum of the absorption spectra of the two compounds (Figure 2). These spectral differences make it possible to use UV spectroscopy to monitor the ligand-exchange reactions observed for auranofin in HCl.

Spectrophotometric Determination of Equilibrium Constants. Results of the spectrophotometric analysis of reaction mixtures

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Table I. Molar Concentrations of Components of the Reaction of Auranofin (7.7×10^{-5} M) with Aqueous HCl from a Spectrophotometric Analysis of the UV Spectrum from 220 to 350 nm

$[H^+] = [Cl^-]$, M	10^5[Aur], M	10^5[ClAuPEt$_3$], M	10^6[Digold$^+$], M	10^5[HTATG],[a] M
0.1	4.97	1.11	6.81	1.79
0.3	2.70	3.76	5.72	4.33
0.5	1.87	5.59	4.33	6.02
0.8	1.18	6.21	1.93	6.40
1.0	0.96	6.71	1.39	6.85

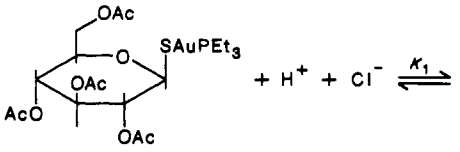
[a] [HTATG] = [ClAuPEt$_3$] + [Digold$^+$].

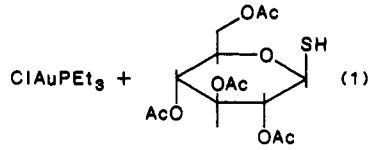
Table II. Equilibrium Constants Calculated from Data in Table I and Eq 3 and 4

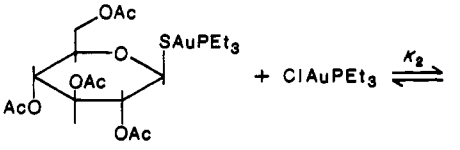
$[H^+] = [Cl^-]$, M	10^4K_1, M^{-1}	$10^{-3}K_2$
0.1	4.0	1.2
0.3	6.7	1.7
0.5	7.2	2.0
0.8	5.2	2.1
1.0	4.8	2.1

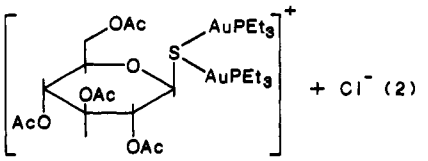
of a fixed concentration of auranofin (7.7×10^{-5} M) and 0.1, 0.3, 0.5, 0.8, and 1.0 M HCl in water at ambient temperature (25 °C) are summarized in Table I. The reaction was complete upon mixing, and no further change was observed in the absorption spectrum of the reaction mixture when monitored for 24 h after mixing.

The absorption spectra (from 220 to 350 nm) of the reaction mixtures were reproduced as a weighted sum of the standard spectra for the three gold-containing compounds identified as components of such a reaction mixture. The mathematical technique utilized is a weighted least-squares regression that produces a solution for as many simultaneous equations as there are data points within the wavelength range specified (220–350 nm). The percentages of each spectrum, specified as a standard, are added together to produce a spectrum that as closely as possible matches the spectrum of the mixture. These percentages then determine the concentration of the components in the mixture.[11] The analysis is consistent with the reaction scheme









$$K_1 = \frac{[ClAuPEt_3][HTATG]}{[Aur][H^+][Cl^-]} \quad (3)$$

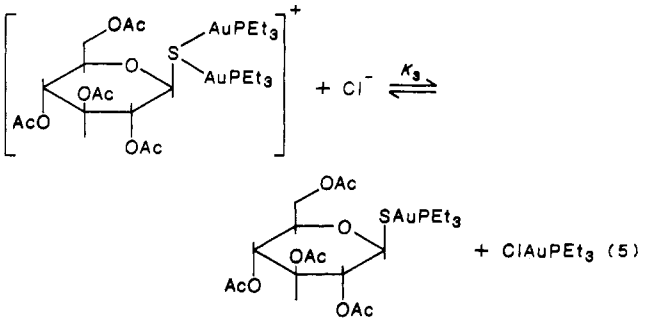
$$K_2 = \frac{[Digold^+][Cl^-]}{[Aur][ClAuPEt_3]} \quad (4)$$

Calculated equilibrium constants are given in Table II. The

reactions were repeated at 37 °C, and the equilibrium constants are the same (to two significant figures) at the higher temperture; at an ionic strength of 1.0 M (with NaCl), the average values of the equilibrium constants in water at 37 °C are $K_1 = 4.6 \times 10^{-4}$ M^{-1} and $K_2 = 2.0 \times 10^3$.

It is not necessary to invoke the second of the two coupled reactions in order to reproduce the spectra of analogous reaction mixtures in 50% methanol/water with [NaCl] = 1.0 M. The average value of K_1 in 50% methanol/water so obtained at 37 °C is 2.0×10^{-3}.

The results of this study are consistent with those obtained by utilizing a second approach to the study of the equilibrium. The equilibrium constant (K_3) for the reaction of sodium chloride with the thiolate digold complex (with nitrate as counterion) was determined:



$$K_3 = \frac{[\text{Aur}][\text{ClAuPEt}_3]}{[\text{Digold}^+][\text{Cl}^-]} \qquad (6)$$

The reaction was complete upon mixing, and no further change was observed in the absorption spectrum of the reaction mixture when monitored for 6 h. The least-squares analysis of the absorption spectrum of the reaction mixture, given the standard spectra of the reaction components proposed in eq 5, yields an equilibrium constant of $K_3 = 8.1 \times 10^{-4}$ in water at ambient temperature.

We note that K_3, the equilibrium constant for this reaction (eq 6), is the inverse of the equilibrium constant for the second of the two coupled reactions proposed for auranofin in HCl (eq 4). The determinations are in good agreement with $1/K_3 = 1/(8.1 \times 10^{-4}) = 1.2 \times 10^3$, approximately equal to the K_2 value obtained in the aqueous solvent system at constant ionic strength ($K_2 = 2.0 \times 10^3$). Equilibrium constants for the reaction of the thiolate digold complex with sodium chloride were obtained also in mixed solvent media: in 50% methanol/water $1/K_3 = 1/(7.8 \times 10^{-3}) = 1.3 \times 10^2$ and in 95% methanol/water $1/K_3 = 1/(1.4 \times 10^{-2}) = 0.71 \times 10^2$.

Kinetics Studies. The kinetics studies of reaction 1 reported here were conducted at chloride concentrations sufficient to suppress reaction 2 so that, unless otherwise noted, the latter reaction contributes less than 10% of the total spectral change observed. The kinetics of reaction 1, which fall in the millisecond time range under the conditions of our experiments, have been investigated in water and in 50% methanol/water by using a stopped-flow kinetics technique.

Kinetics studies of reaction 2 were attempted in water and in 50% methanol, but the total spectral change associated with the reaction of auranofin with $ClAuPEt_3$ was complete within the mixing time of the stopped-flow instrument (determined as being about 1.5 ms). Two strategies were tried: 75 μM auranofin was mixed with an equivalent concentration of $ClAuPEt_3$; alternatively, 50 μM sulfonium nitrate was mixed with NaCl varying from 0.01 to 1.0 M (cf. eq 5).

Mixed-Solvent Kinetics. Description of the kinetic work begins with a consideration of the reactions in a 50% methanol/water (v/v) mixture. At 1.0 M Cl^-, reaction 1 goes to ≥97% completion at $[H^+] \geq 0.1$ M and [Aur] ≃ 20 μM. At this high chloride concentration very little thiolate-bridged digold complex is formed and, as anticipated, the spectrum of the gold product is that of $ClAuPEt_3$. The rate shows a linear dependence on $[H^+]$ (cf. Figure 3) in rapid-mixing experiments under these experimental conditions at 25 °C. An electrolyte alternative to NaCl is required to maintain the ionic strength at 1.0 M in order to determine the dependence of the rate on chloride ion. The surrogate anion should not have an appreciable absorption at 220 nm, the wavelength at which the reaction kinetics were monitored, and preferably should be a weak Lewis base. Perchlorate ion proved ideal. The observed rate constant for the reaction of HCl with auranofin in the presence of perchlorate is also shown in Figure 3. The reaction is much slower than in the presence of chloride, and as expected from equilibrium considerations, the product absorbs at 242 nm, the absorption maximum of the sulfonium ion. Additional studies (vide supra) have shown that reaction 2 is much faster than reaction 1. Thus, the *kinetics being observed* for the acid degradation of auranofin is for the replacement of the tetraacetylthioglucose ligand both in the presence of perchlorate and in the presence of chloride but is followed by rapid conversion to the thiolate digold complex in the former medium. The results at 25 °C in 1.0 M Cl^- yield $k_{obsd} = 385[H^+]$, while in 1.0 M ClO_4^-, $k_{obsd} = 2.6[H^+] + 0.6$. This two-term rate law for ClO_4^-, may reflect a small medium effect but, regardless, the main observation is that ligand replacement is much slower in a perchlorate medium than in one rich in chloride.

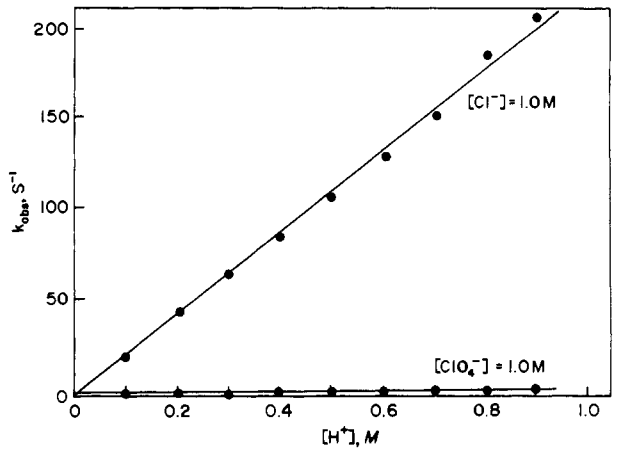


Figure 3. Kinetics of the acid degradation of auranofin (reaction 1) in 50% methanol/water with 1.0 M chloride and 1.0 M perchlorate.

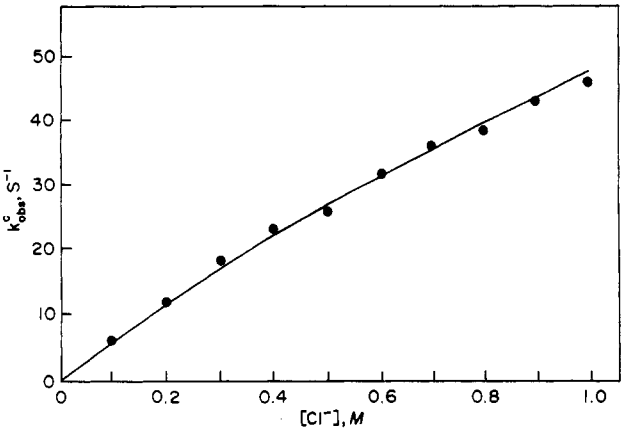


Figure 4. Kinetics of the acid degradation of auranofin (reaction 1) in 50% methanol/water as a function of chloride concentration. The ionic strength is adjusted to 1.0 M with sodium perchlorate, and $T = 16.4$ °C.

Experiments were conducted at a constant $[H^+] = 0.2$ M and $\mu = 1.0$ M but with varying concentrations of NaCl and $NaClO_4$. Kinetic measurements were made at four different temperatures over a 15 °C range. Figure 4 shows the results obtained at 16.4 °C as an example. The value of k_{obsd} has been corrected for the non-chloride-mediated auranofin degradation as determined with perchlorate at the same temperature. At all temperatures, the plot of k_{obsd} vs $[Cl^-]$ shows typical "saturation" behavior, i.e., decreasing dependence of the rate on $[Cl^-]$ as $[Cl^-]$ increases.

Table III. Kinetic Results for the Reaction of Tetraacetylthioglucose with $ClAuPEt_3$ in a 50% Methanol/Water Mixture (T = 25 °C, μ = 1.0 M (Cl^-), [$ClAuPEt_3$] = 10 μM)

$[H^+]_0$, M	$10^4[TATG]_0$, M	k_{obsd}, s^{-1}	k_{calcd}, s^{-1} [a]
5.0×10^{-4}	1.0	14.0	13.3
1.0×10^{-3}	1.0	10.4	10.9
5.0×10^{-3}	1.0	9.5	10.1
1.0×10^{-2}	1.0	11.3	11.6
5.0×10^{-4}	2.0	30.8	26.3
1.0×10^{-3}	2.0	21.7	21.3
5.0×10^{-3}	2.0	17.5	18.4
1.0×10^{-2}	2.0	20.6	19.7

[a] See eq 12.

Table IV. Kinetic Results in Aqueous Media for the Reaction of Auranofin with Acid (μ = 1.0 M, [Aur] = 18.5 μM)

$[H^+]_0$, M	$[Cl^-]_0$, M	$[HTATG]_0$, M	T, °C	k_{obsd}, s^{-1}
0.10	1.0	1.6×10^{-4}	25	90.6
0.20	1.0	1.6×10^{-4}	25	103
0.30	1.0	1.6×10^{-4}	25	123
0.40	1.0	1.6×10^{-4}	25	147
0.50	1.0	1.6×10^{-4}	25	173
0.20	1.0	8.0×10^{-5}	25	67.8
0.20	1.0	1.0×10^{-4}	25	91.7
0.20	1.0	1.4×10^{-4}	25	108
0.20	1.0	1.5×10^{-4}	25	102, 104
0.20	0.50	1.5×10^{-4}	25	69.2
0.20	0.60	1.5×10^{-4}	25	77.9
0.20	0.70	1.5×10^{-4}	25	85.9
0.20	0.90	1.5×10^{-4}	25	94.7
0.10	1.0	1.5×10^{-4}	17	62.6
			36	126
0.20	1.0	1.5×10^{-4}	17	76.1
			36	165
0.30	1.0	1.5×10^{-4}	17	90.8
			36	187
0.50	1.0	1.5×10^{-4}	17	100
			36	237

Double-reciprocal plots ($1/k_{obsd}$ vs $1/[Cl^-]$ are linear, leading to an expression of the form

$$k^c_{obsd} = \frac{a[H^+][Cl^-]}{1 + b[Cl^-]} \quad (7)$$

with a = 308 (16.4 °C), 388 (21.0 °C), 444 (25.0 °C), 714 (31.0 °C) and b = 0.35 (16.4 °C), 0.33 (21.0 °C), 0.30 (25.0 °C), 0.30 (31.0 °C). The values at 25 °C lead to $k_{obsd} = 342[H^+]$ at $[Cl^-]$ = 1.0 M in good agreement with the results of experiments described earlier. The smooth curve drawn in Figure 4 is based upon the parameters given above at 16.4 °C.

The reverse of reaction 1 was also considered in the same solvent system by mixing either 100 or 200 μM tetraacetylthioglucose with 10 μM $ClAuPEt_3$ in a 1.0 M Cl^- medium. In the *absence of added acid*, the reaction to form auranofin is too fast to be monitored by stopped-flow methods. However, in the presence of low concentrations of HCl kinetic data could be obtained with the stopped-flow method. The results are summarized in Table III and compared with calculated rate constants derived from a mechanism proposed below.

Aqueous Kinetics. In contrast to the mixed methanol/water solvent system, the reaction of auranofin with acid does not proceed to completion in water under the range of experimental conditions needed for an extensive kinetic study. Excess tetraacetylthioglucose was added to the reaction mixtures, and the kinetic profiles were first order for over 3 half-lives in the presence of excess H^+, Cl^-, and HTATG.

The kinetic studies summarized in Table IV demonstrate that k_{obsd} depends directly on $[H^+]$, $[Cl^-]$, and [HTATG]:

$$k_{obsd} = k_1[H^+][Cl^-] + k_{-1}[HTATG] \quad (8)$$

A plot of $k_{obsd}/[HTATG]$ versus $[H^+][Cl^-]/[HTATG]$ is shown in Figure 5. The data for the various experiments fall close to the same straight line with a minor but systematic deviation for the $[Cl^-]$ variation.

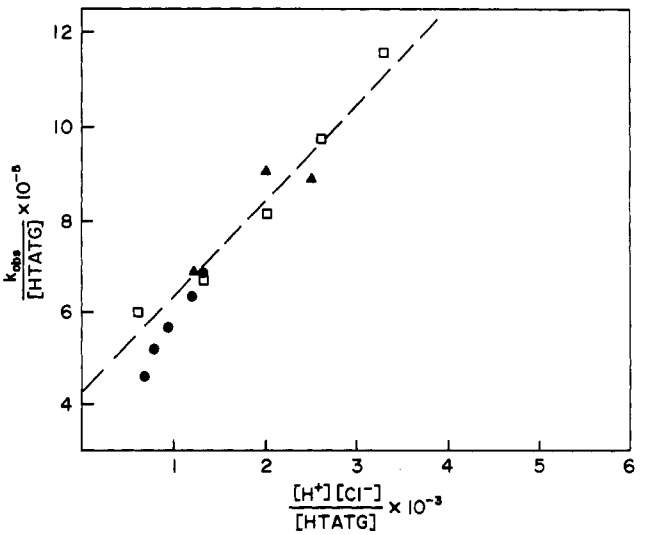


Figure 5. Kinetics of the acid degradation of auranofin (reaction 1) in water: (●) chloride variation, (□) H^+ variation; (▲) HTATG variation. The dashed line ignores the chloride variation. The ionic strength is adjusted to 1.0 M with sodium perchlorate, and T = 25 °C.

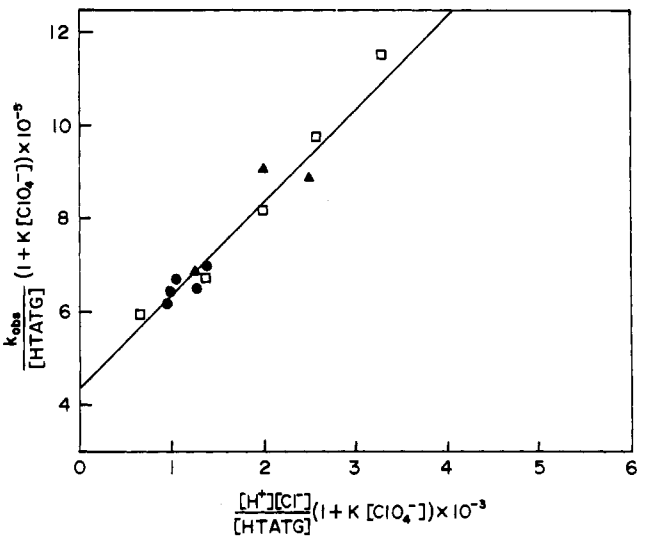


Figure 6. Kinetics of the acid degradation of auranofin (reaction 1) in water after "correction" for the influence of perchlorate on the reaction. The ionic strength is adjusted to 1.0 M with sodium perchlorate, and T = 25 °C. The best straight line for all the data is shown.

The reaction of auranofin with acid is relatively slow in 1.0 M ClO_4^- at 25 °C; $k_{obsd} = 14.3[H^+] + 1.83$. (No HTATG was added since the reaction is complete under these conditions.) There may be a small medium effect on the observed kinetics in aqueous solution arising from the replacement of Cl^- by ClO_4^-; the Cl^- dependence in aqueous solution is not of a saturation type observed in the mixed-solvent system but rather a rate enhancement. We have corrected for this small but systematic trend in a formal sense by assuming that the effect of ClO_4^- is primarily on the reverse process of reaction 1 (k_{-1}) which is not observed in the mixed-solvent medium, where reaction 1 goes to completion. A term of the form $1 + K[ClO_4^-]$ with K = 0.64 is introduced in the kinetics expression to correct for this effect. The "corrected" data are shown in Figure 6; in no case does k_{calcd} differ from k_{obsd} by more than 3.7%. The slope of the line (k_1) equals 210 M^{-2} s^{-1}, and the intercept (k_{-1}) equals 4.3×10^5 M^{-1} s^{-1}. These values lead to an equilibrium constant for reaction 1 in water based purely on kinetic measurements of 4.9×10^{-4} M^{-1} at 25 °C.

Activation parameters for reaction 1 were determined in aqueous solution. The data used to determine the activation parameters are given in Table IV. It is found that for k_1 E_a =

26 kJ/mol and $\Delta S^{\ddagger} = -125$ J/(deg mol), while for k_{-1} $E_a = 31$ kJ/mol and $\Delta S^{\ddagger} = -48$ J/(deg mol).

Discussion

The results of the kinetics experiments strongly suggest that the mechanism of the reaction of auranofin with HCl in the mixed methanol/water system is primarily associative in nature. It is proposed that the stoichiometric mechanism is likely to begin with a rapid attack on the gold site by chloride ion to form a three-coordinate complex followed by a rate-limiting proton-assisted scission of the gold–sulfur bond:

$$\text{(i)}\ Cl^- + TATG{-}AuPEt_3 \underset{K_0}{\overset{\text{fast}}{\rightleftharpoons}} TATG{-}Au(PEt_3)Cl \qquad (9)$$

$$\text{(ii)}\ TATG{-}Au(PEt_3)Cl + H^+ \xrightarrow{k_0} HTATG + ClAuPEt_3$$

$$k^c_{obsd} = \frac{k_0 K_0 [H^+][Cl^-]}{1 + K_0[Cl^-]} \qquad (10)$$

This series of steps would account for the linear dependence of k_{obsd} on $[H^+]$ as well as the saturation profile obtained in k_{obsd} versus $[Cl^-]$ plots (cf. Figure 4).

The involvement of a coordination number greater than 2 for the gold(I) complex in this mechanism is not without precedent. Mössbauer experiments provide evidence for stable complexes of gold(I) in which the coordination number is greater than 2,[15] and the crystal structure of a four-coordinate tetrahedral gold(I) compound has recently been reported.[16,17] Furthermore, NMR data also support associative thiol exchange at the gold(I) center in solution.[18]

The experimental results can be used to quantitate the proposed mechanism. A comparison of eq 7 with eq 10 leads to k_0 (M^{-1} s^{-1}) = 800 (16.4 °C), 1180 (21.0 °C), 1480 (25.0 °C), and 2380 (31.0 °C), while K_0 (M^{-1}) = 0.35 (16.4 °C), 0.33 (21.0 °C), 0.30 (25.0 °C), and 0.30 (31.0 °C). From these results we estimate that $\Delta H^{\circ} \approx -8$ kJ/mol and $\Delta S^{\circ} \approx -40$ J/(deg mol) for the fast preequilibrium step of the proposed mechanism. $E_a = 49$ kJ/mol and $\Delta S^{\ddagger} = -30$ J/(deg mol) for the rate-determining step.

The activation parameters in water are similar to those in the mixed-solvent system (i.e. relatively small activation energies and substantial negative entropies), suggesting that similar mechanisms are operating in both solvent media. It should be noted that $\Delta E_a = 5$ kJ/mol for reaction 1 in water, which indicates a very small dependence of K_1 upon temperature, in agreement with the similarity in the equilibrium constants determined spectrophotometrically for this reaction at ambient temperature and at 37 °C in a separate experiment. We calculate from the *kinetic* results that $K_1 = 4.5 \times 10^{-4}$ M^{-1} at 37 °C and $\mu = 1.0$ as compared to the value of 4.6×10^{-4} M^{-1} obtained at the same temperature and ionic strength using spectrophotometric techniques (vide supra).

At very low concentrations of acid yet another pH dependence in k_{obsd} emerges. Under these conditions, the acid/base equilibrium of tetraacetylthioglucose must be considered:

$$Aur + H^+ + Cl^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} HTATG + ClAuPEt_3 \overset{K_a}{\rightleftharpoons}$$

$$TATG^- + ClAuPEt_3 \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} Aur + Cl^- \qquad (11)$$

Under pseudo-first-order conditions ($[H^+]$, $[Cl^-]$ and total tetraacetylthioglucose ($[TATG]_0$) in large excess)

$$k_{obsd} = \frac{(k_1[H^+] + k'_1)[Cl^-]}{1 + K_0[Cl^-]} + \frac{(k_{-1} + k'_{-1}K_a/[H^+])[TATG]_0}{1 + K_a/[H^+]} \qquad (12)$$

Table V. Summary of the Reaction of 10^{-5} M Auranofin in Water with 0.1 M Chloride As the pH Varies

pH	% degradn	half-life of reacn, s	pH	% degradn	half-life of reacn, s
1.0	50	0.3	3.0	7	33.0
2.0	20	3.3	4.0	2	333.0

The pK_a of HTATG was determined as 7.17 in a 50% methanol/water mixture, and given that $k_1 = 444$ M^{-2} s^{-1} as determined at low pH (cf. eq 10), $K_0 = 0.30$ M^{-1}; we obtain $k_{-1} = 7.8 \times 10^4$ M^{-1} s^{-1}, $k'_1 = 0.15$ M^{-1} s^{-1}, and $k'_{-1} = 3.8 \times 10^8$ M^{-1} s^{-1}. Values of k_{calcd} in Table III were determined from these parameters and eq 12.

The acid degradation of auranofin is considerably slower in the presence of excess perchlorate than in the presence of excess chloride in water and in 50% methanol/water. This observation supports the role suggested for a good nucleophile in the acid degradation of auranofin. The major product of reaction in the presence of excess chloride is $ClAuPEt_3$. The major product in the presence of excess perchlorate is the thiolate digold complex.

We note that the kinetics of acid degradation in the presence of excess perchlorate is faster in water than in the mixed-solvent system. The reaction would be expected to be comparable in rate in the less polar, lower dielectric medium (50% methanol) if ClO_4^- serves as the nucleophile in this reaction. However, if water is the nucleophile, the reaction would be expected to be slower in 50% methanol, as observed.

The importance of both *entering and leaving groups* in substitution kinetics at the gold(I) site is documented by our results. The rate constant (50% methanol) for substitution of the chloride of $ClAuPEt_3$ by $TATG^-$ (3.8×10^8 M^{-1} s^{-1}) is 5000 times faster than for HTATG (7.8×10^4 M^{-1} s^{-1}). The substitution of the chloride of $ClAuPEt_3$ by auranofin (i.e., reaction 2) has a rate constant greater than 10^6 M^{-1} s^{-1}. The displacement of a ligand coordinated to gold(I) by chloride was also observed. The displacement of $TATG^-$ in auranofin by chloride in a low-acidity medium has a rate constant (50% methanol) of 0.15 M^{-1} s^{-1} while the displacement of pyridine in $(py)AuPEt_3^+$ by chloride is too fast to measure by stopped-flow methods[19] and thus has a rate constant greater than 10^6 M^{-1} s^{-1}.

Conclusions

An oral dose of auranofin translates to a maximum concentration of 9 mg/L = 1.3×10^{-5} M in the aqueous phase of the stomach. The pH of the stomach varies and can assume a range of acidic pH values spanning the spectrum from pH 1 to neutral pH. Effects of the reaction of 10^{-5} M auranofin with 0.1 M chloride in aqueous HCl are calculated as a function of pH in Table V. Also included in Table V is the half-life of reaction ($t_{1/2} = 0.693/k_{obsd}$). The reaction is faster and more extensive at lower pH. However, under the most acidic condition characteristic of the stomach (pH 1), approximately 50% of the gold–sulfur bonds remain intact at equilibrium. The reaction is over within the mixing time of the reactants. Under less acidic conditions (pH 3), 90% of the gold–sulfur bonds remain intact and the reaction is over in less than 2 min. At 0.1 M chloride, the chloride concentration characteristic of the stomach and of the blood, formation of the thiolate digold complex is suppressed (cf. eq 2).

Previous studies[6,7] have demonstrated that auranofin can undergo facile thiol-exchange reactions with biological ligands without affecting the coordination of the phosphine ligand. This study demonstrates that an acidic medium, like the stomach, enhances the reactivity of the thiol ligand of auranofin. These results support a hypothesis that the thiol ligand of auranofin exchanges with biological ligands, most likely thiol ligands, in the biological system.[6,8,21] This hypothesis is consistent with the

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similarity in biological activity observed for thiosugar analogues of auranofin in an animal model for rheumatoid arthritis.[8]

Biological studies in animal models for rheumatoid arthritis have demonstrated that oral absorption is characteristic of gold(I) compounds in which at least one ligand is a phosphine.[20] Oral absorption is demonstrated by atomic absorption measurements, which quantitate the concentration of gold in the serum following oral administration. This observation is consistent with the lipophilicity of the phosphine ligand, since lipophilicity facilitates membrane diffusion. The results of our study are consistent, as a model study representing the much more complicated biological system, with this biologically derived correlation between oral absorption and chemical structure.

Relatively few kinetics studies of gold(I) compounds have been previously reported,[4,22] and there are none in which the medicinally important phosphine ligand is present. It should be noted that the phosphine ligand stabilizes the +1 oxidation state of gold in aqueous solution.[4] The oxidation–reduction chemistry of this class of compounds remains largely unexplored[8] but may be an important factor in the biological effects of these compounds.[23-25]

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Oxidation of Cobalt(II) Amine Complexes to Mononuclear Cobalt(III) Complexes by Dioxygen

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The mechanism of the reaction of (ethylenediamine)(diethylenetriamine)cobalt(II) complexes with dioxygen to give the mononuclear cobalt(III) compound has been investigated by using ^{59}Co NMR. The use of this method enables the concentrations of the initially formed peroxo compounds and of the mononuclear cobalt(III) complexes to be monitored concurrently. It is concluded that the peroxo compounds are intermediates in the formation of the mononuclear cobalt(III) complex. The suggested mechanism involves reduction of the peroxo complex by cobalt(II). This reaction proceeds significantly faster for one isomer of the peroxo complex than for the other.

Introduction

The preparation of cobalt(III) amine complexes by oxidation of the corresponding Co(II) compounds with air has been well-known for many years. It is a standard experiment in most undergraduate inorganic laboratory courses. In spite of this familiarity there are still uncertainties regarding the mechanisms by which the Co(III) compounds are formed. Fallab and Mitchell[1] have discussed the problems in this area in a recent review.

The initial products of the reaction have been well characterized as (μ-peroxo)dicobaltate(III) complexes. These compounds are of limited stability and eventually decompose, in a manner that is usually described as "irreversible", to give mononuclear Co(III) complexes. The half-life for this decomposition varies from less than a second to several days depending on the nature of the ligands. The mechanism of this step is uncertain. It is of course not necessarily true that it will be the same for all ligands.

The simplest mechanism would be to form the peroxo complex from Co(II) and dioxygen and then to hydrolyze this complex to give the Co(III) complex and hydrogen peroxide. This implies that hydrogen peroxide should be formed in stoichiometric quantities. In two cases this has been observed. The first involves the cyano complex[2] and the second the trans dinitrobis(ethylenediamine) complex.[3] In these two cases the decomposition occurs under acid conditions (pH ~1). Most other cobalt peroxo complexes decompose to Co(II) and dioxygen under these conditions and only give the Co(III) complex in neutral or alkaline solution. Shibahara et al.[3] have discussed the role of strong-field ligands in favoring hydrolysis to Co(III) and hydrogen peroxide. Although there have been claims to the contrary, it now seems to be accepted that hydrogen peroxide is not present during the irreversible decomposition of cobalt peroxo complexes to the Co(III) compounds except for the above complexes under strongly acid conditions.[1] An alternative mechanism is therefore required.

There are several possibilities. Hydrogen peroxide may indeed be formed but catalytically decomposes immediately to dioxygen and water. A number of salts and complexes of cobalt are known to catalyze this reaction.[4] This would require the release of a stoichiometric quantity of oxygen, and although, in some cases, oxygen has been reported as a product, the evidence indicates that it is not formed in stoichiometric quantities.[1] In such instances it becomes questionable whether the peroxo compounds act as intermediates in the formation of the Co(III) complexes. Thus Stadtherr et al.[5] suggest: "The simplest interpretation of those systems in which no hydrogen peroxide can be detected nor oxygen reemitted upon formation of mononuclear Co(III) complexes is that these complexes are not formed from the binuclear peroxocobalt(III) complexes. The peroxo complexes in these cases are unreactive species formed in a side reaction." It has been suggested that reduction of the peroxide moiety to water is accompanied by oxidation of the ligands.[6,7] This mechanism seems to be

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