Kinetics and Mechanism for Reduction of Tetrabromoaurate(III) by Uridine [*]

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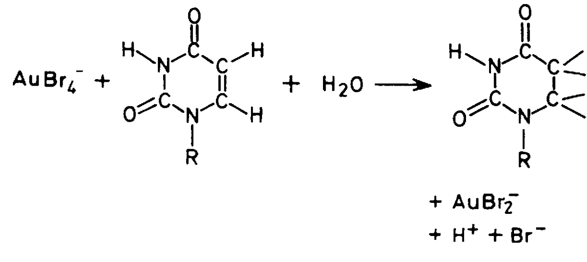
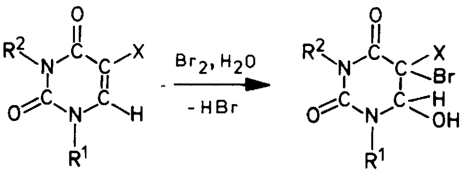
The reaction of tetrabromoaurate(III) with uridine in water occurs with a 1 : 1 stoicheiometry, producing gold(I) and 5-bromo-6-hydroxy-5,6-dihydrouridine. The reduction of $AuBr_4^-$, studied in the presence of uridine, bromide, and acid in excess with respect to gold, and in some cases also in the presence of added reaction products, obeys the rate expression $-d[AuBr_4^-]/dt = k_{obs.}[AuBr_4^-]/([Au]_T - [AuBr_4^-])$, where $[Au]_T$ is the total gold concentration ($[Au^{III}] + [Au^I]$). The rate $k_{obs.}$ is linearly dependent on the uridine concentration and invariant of the bromide and hydrogen-ion concentrations. The observed redox kinetics are consistent with a mechanism involving reductive elimination of bromine from tetrabromoaurate(III) followed by halogenation of the pyrimidine.

Studies on heavy-metal interactions with nucleic acid derivatives have been predominantly concerned with co-ordination.[1,2] Little information is available on the redox behaviour of these systems. This paper reports a study on the reduction of tetrabromoaurate(III) by uridine (1-β-D-ribofuranosyl-1*H*,3*H*-pyrimidine-2,4-dione) in water [equation (1), R = β-D-ribofuranosyl]. It has been shown previously that the reaction of $AuBr_4^-$ with cytosine (4-amino-1*H*-pyrimidin-2-one) in dimethyl sulphoxide leads to reduction of gold(III) to gold(I) and to formation of 5-bromocytosine.[3] Cytidine (4-amino-1-β-D-ribofuranosyl-1*H*-pyrimidin-2-one), uracil (1*H*,3*H*-pyrimidine-2,4-dione), and uridine react similarly in this solvent to give 5-bromopyrimidines.[4]



(1)

The direct halogenation of pyrimidine bases, nucleosides, and nucleotides has been studied extensively as these derivatives have important biological properties.[5] The mechanism of the reactions of bromine with uracil and methyl uracils in water has recently been described [equation (2): R^1 = H or CH_3; R^2 = H or CH_3; X = H or Br].[6] The active electrophile in these brominations is molecular bromine (Br_2) and the reactions are first order with respect to both the halogen and pyrimidine concentrations.



(2)

Experimental

Reagents.—The salt $K[AuBr_4]$ was prepared according to a literature method.[7] All other chemicals were reagent grade and were used as purchased. All solutions for u.v.–visible spectroscopic measurements were prepared from deionized and distilled water.

Measurements.—Proton n.m.r. spectra were recorded on Bruker WP 60 and WP 200 SY spectrometers. Chemical shifts were measured relative to internal HDO and referred to $SiMe_4$ by an independent measurement. U.v.–visible spectra were recorded on a Perkin-Elmer 576 ST spectrophotometer equipped with a thermostatted cell compartment. Kinetic runs were followed spectrophotometrically using a pair of cells with two compartments. The solutions in all compartments contained NaBr, H_2SO_4, and $NaNO_3$ at the same concentrations. The sample cell contained $K[AuBr_4]$ in one compartment and uridine in the other, both the complex and the nucleoside being twice as concentrated as in the reacting mixture. The solutions were prethermostatted and the reaction started by fast mixing of the solutions in the sample cell. An experiment was also carried out by mixing deaerated (with argon) solutions of the reactants. Some experiments were carried out by mixing a gold(III) solution with an exhausted solution from the reaction of uridine with either $K[AuBr_4]$ or Br_2, containing excess of nucleoside. Slow reactions were measured by recording the absorption spectra in the range 350—500 nm, whereas fast reactions were measured at a fixed wavelength (385 nm).

Results

Reaction Products.—The ^1H n.m.r. spectrum of uridine (3.7×10^{-2} mol dm^{-3}) in D_2O shows doublets at δ 7.87 [H(6)], 5.89 [H(5)] $\{|J[H(5)-H(6)]| = 8.0$ Hz$\}$, and 5.90 [H(1′)], and a complex set of resonances at δ *ca.* 3.5—4.5 assigned to ribose protons H(2′)—H(5′).

The ^1H n.m.r. spectra of solutions of the nucleoside (2×10^{-2} or 6×10^{-2} mol dm^{-3}) in D_2O treated with bromine (molar ratio 1 : 2) and recorded after 15 min are consistent with the quantitative formation of 5-bromo-6-hydroxy-5,6-dihydrouridine (bromohydrin). The spectrum displays signals

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[*] 1-β-D-Ribofuranosyl-1*H*,3*H*-pyrimidine-2,4-dione.

of equal intensity centred at δ 5.8 [H(1′)], 5.5 [H(6)], and 4.5 [H(5)]. The relative chemical shift of the pyrimidine protons is assigned tentatively. The H(6) and H(5) resonances appear as a set of two major doublets at δ 5.49 and 4.55 {$|J[H(5)-H(6)]| = 2.5$ Hz} and two minor doublets (*ca.* 30% intensity) at δ 5.51 and 4.53 {$|J[H(5)-H(6)]| = 2.5$ Hz}. This pattern can be ascribed to the presence of two configurational isomers of the bromohydrin. The assignment of the spectrum is confirmed by decoupling experiments and by comparison of spectra obtained at 60 and 200 MHz. The spectrum in the H(5)–H(6) region is similar to that of 5-bromo-6-hydroxy-5,6-dihydrouracil in D_2O. Complex resonances observed at δ *ca.* 3.5—4.5 are assigned to ribose protons H(2′)—H(5′). The appearance and relative intensities of the H(1′)—H(5′) signals show that the ribose moiety is not involved in the redox reaction.

The ^1H n.m.r. spectra of equimolar mixtures of $K[AuBr_4]$ (2.4×10^{-2} or 3.6×10^{-2} mol dm^{-3}) and uridine in D_2O recorded after 6 d show quantitative bromination of the nucleoside to give over 90% 5-bromo-6-hydroxy-5,6-dihydrouridine. The main set of resonances observed is identical with the spectra of the Br_2–uridine mixtures. The presence of a signal at δ 8.34 shows also formation of a small amount of 5-bromouridine (5—10%). The chemical shifts of unreacted uridine and of bromination products do not change during the redox reaction.

Formation of metallic gold was observed in the reacting mixtures used for n.m.r. measurements, whereas no reduction to metal was observed in the dilute solutions used in the kinetic experiments. The redox reaction between $AuBr_4^-$ and uridine is accompanied by a decrease in absorbance in the range 350—500 nm, the ratios of optical densities at the different wavelengths remaining constant. When a solution containing 9.5×10^{-5} mol dm^{-3} $AuBr_4^-$, 8×10^{-5} mol dm^{-3} uridine, 5×10^{-2} mol dm^{-3} Br^-, and 10^{-2} mol dm^{-3} H^+ was allowed to react for 28 d the absorbance decreased to 16% of the initial value. A portion of this solution was treated with excess of bromine and then nitrogen was bubbled through it. The resulting spectrum was identical with that of the initial $AuBr_4^-$ solution. These results show that no reduction to metallic gold occurred and that the stoicheiometric ratio of gold : uridine is 1 : 1, corresponding to reduction of gold(III) to gold(I). The ^1H n.m.r. and u.v.–visible spectroscopic data rule out any appreciable formation of gold(III) complexes with uridine or with bromination products, in agreement with the low basicity of these pyrimidines. Formation of metal complexes of deprotonated pyrimidines is opposed by the acidity of the reacting mixtures. It can be inferred that also co-ordination at gold(I) does not occur with these ligands and that $AuBr_2^-$ is the gold(I) reaction product.

Kinetics.—All kinetic experiments were carried out at 25 °C in the presence of added H_2SO_4 and NaBr. The ionic strength was maintained at 1.1 mol dm^{-3} with $NaNO_3$. Under the experimental conditions, $AuBr_4^-$ is stable and its spectrum in the region 350—500 nm obeys the Lambert-Beer law. It is not appreciably affected by changes in $[H_2SO_4]$, [NaBr], and $[NaNO_3]$. Large excesses of acid, bromide, and uridine compared to gold were used in order to keep the concentration of these species constant during each kinetic run. The hydrogen-ion and bromide-ion concentrations used were sufficiently large to avoid any significant hydrolysis of $AuBr_4^-$, according to literature data.[8] At the pH values used uridine is in its neutral diketo-form: pK_a of uridine, 9.2; pK_a of protonated uridine, <0.5.[1,9] The degree of auto-association of the nucleoside is small: from reported equilibrium constants,[10] uridine is calculated to be 94% monomer at the highest concentration used (5×10^{-2} mol dm^{-3}) and, for example, 98.5% monomer at 2×10^{-2} mol dm^{-3}. No allowance for autoassociation has been made in the calculation of rates.

Kinetic data for reaction (1) were found to fit the rate law (3) where $[Au]_T$ is the total gold concentration ($[Au^{III}]$ +

$$[Au]_T\ln[AuBr_4^-] - [AuBr_4^-] = [Au]_T\ln[Au^{III}]_0 - [Au^{III}]_0 - k_{obs.}t \quad (3)$$

$[Au^I]$) and $[Au^{III}]_0$ is the starting concentration of tetrabromoaurate(III). The concentrations of $AuBr_4^-$ were estimated from the absorbance at 385 nm and the rate constants, $k_{obs.}$, were calculated from plots of ($[Au]_T\ln[AuBr_4^-] - [AuBr_4^-]$) *vs.* *t* by least-squares analysis. In all cases, linear plots from equation (3) were obtained for greater than 80% completion of reaction.

Values of $k_{obs.}$ obtained for different concentrations are reported in the Table. Duplicate runs showed that the kinetics were reproducible within 10% and independent of ageing of the solutions of the reactants (*e.g.* 15 min to 1 d).

In addition to the experiments quoted in the Table, an experiment was carried out under argon using the following concentrations: 1.1×10^{-4} mol dm^{-3} $AuBr_4^-$, 2×10^{-2} mol dm^{-3} H^+, 10^{-1} mol dm^{-3} Br^-, 2×10^{-2} mol dm^{-3} uridine, and ionic strength, $I = 1.1$ mol dm^{-3}. The observed rate, $k_{obs.} = 5.5 \times 10^{-8}$ mol dm^{-3} s^{-1}, is close to the value obtained from an experiment carried out with the same concentrations but in air, showing that molecular oxygen is not involved in the redox mechanism. An experiment performed at $I = 4 \times 10^{-2}$ mol dm^{-3}, 1.1×10^{-4} mol dm^{-3} $AuBr_4^-$, 2×10^{-2} mol dm^{-3} H^+, 10^{-2} mol dm^{-3} Br^-, and 5×10^{-2} mol dm^{-3} uridine gave a $k_{obs.}$ value of 8.8×10^{-8} mol dm^{-3} s^{-1}.

Discussion

Spectroscopic analysis of the system tetrabromoaurate(III)–uridine in water shows that reaction (1) occurs. Metal co-ordination of the nucleoside or oxidation of the ribose moiety are not observed. The formation of two isomers of 5-bromo-6-hydroxy-5,6-dihydrouridine (bromohydrin) can be ascribed to occurrence of both *cis* and *trans* addition of Br and OH to the C(5)=C(6) double bond. 5-Bromouridine is also formed in small amount, probably through elimination of water from the bromohydrin.[5] The formation of metallic gold observed under the conditions of the n.m.r. measurements ($[Au^{III}]$ *ca.* 10^{-2} mol dm^{-3}) is accounted for by disproportionation of gold(I) produced by reaction (1), according to equation (4), for which the equilibrium constant has been reported

$$3AuBr_2^- \rightleftharpoons AuBr_4^- + 2Au + 2Br^- \quad (4)$$

to be 10^{-5}.[11] Lack of formation of metallic gold from reacting mixtures in the kinetic experiments, even where expected on the basis of equilibrium (4), can be ascribed to the slowness of this reaction at low gold(I) concentrations.

The rate of reaction (1) is independent of bromide and hydrogen-ion concentration. Half-times of the redox reaction are found to be linearly dependent on the inverse of the uridine concentration, indicating a first-order dependence on the nucleoside, and linearly dependent on the initial concentration of tetrabromoaurate(III), suggesting retardation by products. Reactions carried out in the presence of exhausted gold–uridine mixtures are found to be slower than corresponding reactions carried out without addition of products. As addition of the bromohydrin to the reacting mixtures does not produce any significant change in rate, it is concluded that gold(I) is responsible for the retardation.

On the basis of the above observations the reaction mechan-

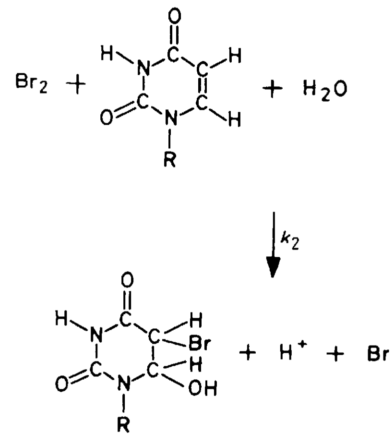
Table. Rate constants for reduction of $AuBr_4^-$ by uridine

$10^4[AuBr_4^-]$ / mol dm^{-3}	$10^3[H^+]$ / mol dm^{-3}	$[Br^-]$ / mol dm^{-3}	10^3[uridine] / mol dm^{-3}	$10^7k_{obs.}$ / mol dm^{-3} s^{-1}	$10^6k_{obs.}$/[uridine] / s^{-1}
0.52	20	0.10	26	0.57	2.2
0.99	100	0.10	50	1.0	2.0
0.97	20	0.10	50	1.2	2.4
0.95	2	0.10	50	1.1	2.2
1.01	20	0.10	2	0.054	2.7
1.06	20	1.0	50	1.1	2.2
1.03	20	0.01	50	1.1	2.2
0.94	100	0.10	2	0.035	1.8
1.06	20	0.10	20	0.48	2.4
1.08	20	0.10	5	0.13	2.6
1.03	100	0.10	10	0.23	2.3
1.00	8	0.01	25	0.56	2.2
2.09	20	1.00	50	1.2	2.4
5.09	20	1.00	50	1.1	2.2
2.07	20	1.00	25 [a]	0.60	2.4
1.10	20	0.10	49 [b]	0.98	2.0
1.06	20	0.10	50 [c]	0.89	1.8
2.07	20	1.00	25 [d]	0.49	2.0

[a–d] Experiments carried out in the presence of added reaction products: 10^{-4} mol dm^{-3} bromohydrin, 2.7×10^{-3} mol dm^{-3} bromohydrin, 0.9×10^{-4} mol dm^{-3} gold(I) and bromohydrin, and 10^{-4} mol dm^{-3} gold(I) and bromohydrin, respectively.

ism in equations (5) and (6) can be proposed, involving reductive elimination of bromine from tetrabromoaurate(III) followed by halogenation of the pyrimidine. Reductive elimination from $AuBr_4^-$ (k_1) and oxidative addition to

$$AuBr_4^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} AuBr_2^- + Br_2 \qquad (5)$$



(6)

$AuBr_2^-$ (k_{-1}) are assumed to be first-order and second-order reactions, respectively. Using the steady-state approximation for Br_2, rate law (7) is obtained. By assuming $k_{-1}[AuBr_2^-] \gg$

$$-\frac{d[AuBr_4^-]}{dt} = \frac{k_1k_2[AuBr_4^-][\text{uridine}]}{k_{-1}[AuBr_2^-] + k_2[\text{uridine}]} \qquad (7)$$

k_2[uridine] as required by the observed first-order dependence upon the nucleoside concentration, equation (7) reduces to (8) where $[Au]_T$ is the total gold concentration ($[Au^{III}]$ + $[Au^I]$). Integration of equation (8) leads to a rate expression identical with the experimental rate law (3), where the rate constant is given by (9). Dividing $k_{obs.}$ values by the uridine

$$-\frac{d[AuBr_4^-]}{dt} = \frac{k_1k_2}{k_{-1}} \cdot \frac{[AuBr_4^-][\text{uridine}]}{[Au]_T - [AuBr_4^-]} \qquad (8)$$

$$k_{obs.} = (k_1k_2/k_{-1})\,[\text{uridine}] \qquad (9)$$

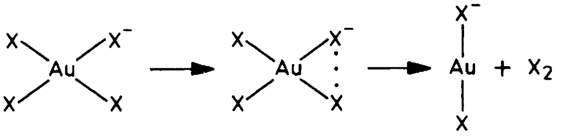
concentration gives the k_1k_2/k_{-1} values reported in the Table. These values are reasonably consistent over a wide range of initial concentrations and give a mean value of $(2.2 \pm 0.1) \times 10^{-6}$ s^{-1}. The value of $k_{obs.}$/[uridine] obtained at $I = 4 \times 10^{-2}$ mol dm^{-3}, 1.8×10^{-6} s^{-1}, indicates no significant ionic strength effect in agreement with the proposed mechanism. The lack of any acid dependence of $k_{obs.}$ is in accord with the fact that in the pH range used bromination through deprotonated base does not occur for uracils having a substituent at N(1).[6]

From standard electrode potentials of the systems $AuBr_4^-$–$AuBr_2^-$ ($E^\ominus = 0.81$ [11] or 0.825 V [12]) and Br_2–Br^-,[12] values of the equilibrium constant $K = 3.4 \times 10^{-10}$ or 1.1×10^{-9} mol dm^{-3} are calculated for reaction (5). Substitution of K for k_1/k_{-1} in equation (9) gives values of $k_2 = 6.5 \times 10^3$ or 2×10^3 dm^3 mol^{-1} s^{-1}. For reactions of bromine with uracil and methyluracils in water [equation (2)], second-order rate constants in the range $k_2 = 5 \times 10^4$ (uracil) to 10^5 dm^3 mol^{-1} s^{-1} (1,3-dimethyluracil) have been reported.[6] The calculated k_2 values for reaction (1) are similar to k_2 values for uracils and confirm the proposed mechanism [equations (5) and (6)]. This mechanism is also consistent with the observation that the same ratio of bromohydrin isomers is obtained from reaction of uridine with either Br_2 or $AuBr_4^-$. Comparison of k_2 values indicates that the reactivity of uracil toward Br_2 is one order of magnitude greater than that of uridine. Bromination of uracil by $AuBr_4^-$ is found to be faster than bromination of uridine.[4]

The reaction of $AuBr_2^-$ with Br_2, assumed to be first order with respect to both gold(I) and bromine concentrations in the present mechanism, cannot be studied directly as the rates are too rapid to be measured with the stopped-flow technique. This finding is consistent with $k_{-1}[AuBr_2^-] \gg k_2$[uridine], if not proving it.

Reductive elimination of Br_2 from $AuBr_4^-$ [equation (5),

k_1] has been assumed to be a first-order reaction. However, a unimolecular mechanism of the type (10) (X = halogen) is considered unlikely.[13] An alternative mechanism for reductive elimination of bromine involves bromide ion as reductant [equation (11)]. An intermolecular mechanism of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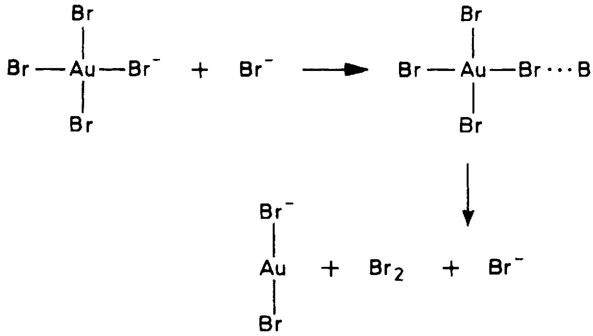
(11)

this type has been proposed for reduction of tetrachloro- and tetrabromo-aurate(III) by iodide.[13] Although solutions of $AuBr_4^-$ and Br^- at the concentrations used for the kinetic experiments are stable, it is known that dissolving large amounts of K[$AuBr_4$] in bromide solution results in the formation of Br_2.[14] Mechanism (11) requires the first-order rate constant k_1 in equation (8) to be replaced by a pseudo-first-order rate constant $k_1 = k_1'[Br^-]$. However, if the rate of the forward reaction in pre-equilibrium (5) depends on the bromide-ion concentration, the rate of the back reaction (*i.e.* the oxidative addition) must also depend in the same way on [Br^-] and the rate constant k_{-1} in equation (8) must be replaced by a pseudo-first-order rate constant $k_{-1} = k_{-1}'[Br^-]$. Therefore, the rate law of reaction (1) is independent of the bromide-ion concentration also in this case. A possible stage of the oxidative-addition reaction involving Br^- is the formation of tribromide ion (Br_3^-) as the active species toward gold(I). It is noted that I_3^- has been found to react much more rapidly than I_2 with $Au(CN)_2^-$ to give [$Au(CN)_2I_2$]$^-$.[15]

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Received 28*th March* 1983; *Paper* 3/499