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5-Arylazoniumuracil Dibromoaurates(I): X-ray Crystal Structure of Bis(6-amino-1,3-dimethyl-5-phenylazoniumuracil) Dibromoaurate Monobromide Monohydrate, $(DZH_2)_2(AuBr_2)Br \cdot H_2O$, and Growth Inhibition of HeLa Cells

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Abstract

The preparation of a series of gold(I) compounds of general formulae $(LH_2)(AuBr_2)$ and $(LH_2)_2(AuBr_2)Br$ (LH_2^+: 5-arylazoniumuracil cations) is described. The crystal structure of $(DZH_2)_2(AuBr_2)Br \cdot H_2O$ (DZH_2^+: 6-amino-1,3-dimethyl-5-phenylazoniumuracil cation) was determined. The crystals are triclinic; space group $P\bar{1}$; $a = 12.188(1)$, $b = 17.404(2)$, $c = 7.764(1)$ Å, $\alpha = 99.79(1)$, $\beta = 99.45(1)$, $\gamma = 86.94(1)^\circ$; $Z = 2$; $R = 0.030$ for 3593 observed reflections and 310 variable parameters. The structure is ionic, the asymmetric unit contains one $AuBr_2^-$ anion, two crystallographically independent DZH_2^+ cations, one isolated Br^- anion and one molecule of water of crystallization. In each DZH cation the additional proton is bonded to a nitrogen atom of the azo group. The growth inhibition of HeLa cells by these bromogold(I) salts has been tested.

Introduction

The 6-amino-1,3-dimethyl-5-phenylazouracil derivatives (hereafter referred to as LH) depicted in Fig. 1 can release a proton of the amino group to give the L^- anion. This anion is then liable to coordinate metal centres using the amino nitrogen atom and one of the nitrogen atoms of the azo group as donor sites [1]. However, the LH derivatives can also be protonated to yield the LH_2^+ 5-arylazoniumuracil cations.

In this paper, we report on compounds of general formulae $(LH_2)(AuBr_2)$ and $(LH_2)_2(AuBr_2)Br$ obtained through reduction of gold(III) to gold(I). Among them, the compound bis(6-amino-1,3-dimethyl-5-phenylazoniumuracil)dibromoaurate monobromide monohydrate, $(DZH_2)_2(AuBr_2)Br \cdot H_2O$, has been studied by X-ray crystallographic methods. Although the $AuBr_2^-$ ion has been known for a long time, crystallographic results on $AuBr_2^-$ salts remain rather sparse in the literature. Mainly, the mixed-valence species $[Au(S_2CNBu_2)_2](AuBr_2)$ [2] and $Rb_2(AuBr_2)(AuBr_4)$ [3] have been crystallographically characterized. Recently, the crystal structure of the simple salt $(n\text{-}Bu_4N)(AuBr_2)$ [4] has been reported and conducting ion radical salts with $AuBr_2^-$ as counter-ion have been described [5–8]. The crystallographic results indicate a linear coordination about the gold(I) centre, similar to the one observed in $K[Au(CN)_2]$, a salt which has been found to be active against P_{388} leukemia in mice [9].

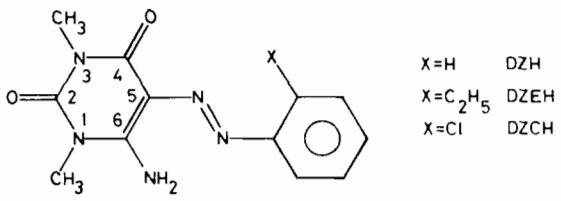

Fig. 1. Structural formulae of 6-amino-1,3-dimethyl-5-arylazouracil derivatives.

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In a previous work [10], the complex (DZH_2)-$(AuCl_2)\cdot 1{,}5H_2O$ was shown to have a high degree of growth inhibition of tumour cells *in vitro*, indicating that this complex would be active *in vivo*. From this reason, the analogous LH_2^+ salts quoted above have also been tested for growth inhibition of HeLa cells.

Experimental

6-Amino-1,3-dimethyl-5-phenylazouracil derivatives (LH, Fig. 1), were synthesized according to Todd *et al.* [11], using 6-amino-1,3-dimethyluracil and the corresponding aniline derivative as starting materials. After recrystallization from pyridine, the products were isolated as yellow needle-like crystals.

Preparation of the Gold(I) Compounds

Gold(I) compounds were prepared according to two different methods:

(i) An aqueous solution (10 ml) of $NaAuCl_4\cdot 2H_2O$ (2 mmol) and KBr (10 mmol) was added to a suspension of arylazouracil derivative (LH, 2 mmol) in hot ethanol (25 ml) containing HBr 1 N (2 ml). The mixture then was refluxed for 3 h. The resulting orange solution was allowed to stand at room temperature for two days. Red needles of the gold(III) compound $(LH_2)(AuBr_4)$ formed and were separated by filtration. The filtrate was kept standing for five more days. Orange crystals of the gold(I) compound $(LH_2)(AuBr_2)$ were thus obtained, filtered off, washed with ethanol and dried with diethyl ether.

(ii) An acetone/water solution (5:1, 25 ml) of $NaAuCl_4\cdot 2H_2O$ (2 mmol) was treated by an 8- to 10-fold excess of KBr. The initially red solution was heated at 60–70 °C for 1 h whereupon it turned colourless. The appropriate arylazouracil derivative (LH, 2 mmol) was then added and the mixture refluxed for 1 h. The resulting solution was placed in a refrigerator for two days. The orange crystals of $(LH_2)(AuBr_2)$ formed were filtered off, washed with ethanol and dried with diethyl ether.

Compounds of general formula $(LH_2)_2(AuBr_2)Br$ were obtained by the two methods described above but using a 1:2 Au:LH molar ratio.

X-ray Crystal Structure Determination of $(DZH_2)_2(AuBr_2)Br\cdot H_2O$

Crystal data are reported in Table 1. A block-like crystal (0.4 × 0.25 × 0.25 mm) was stuck at the end of a glass fiber and mounted on a CAD4 Enraf-Nonius diffractometer using the graphite-monochromatized Mo Kα radiation (λ = 0.71069 Å). The orientation matrix and the cell parameters were obtained by least-squares refinement of the setting angles of 25 reflections. The data collection was run in the ω–2θ scanning mode up to a Bragg angle $\theta = 25°$. Other relevant details of the data collection are listed in Table 1. The intensity of three standard reflections was monitored throughout the data collection; no significant variation was noticed. The usual Lorentz–polarization corrections were applied to the net intensities. Absorption corrections were calculated by the Gaussian integration method [13] using a 216-point grid.

The centrosymmetric space group $P\bar{1}$ was assumed on the basis of convincing N(Z) test [14]. The structure was solved by the Patterson method. Least-squares refinement was conducted using the block-

TABLE 1. Crystallographic data for $[(C_{12}H_{14}N_5O_2)^+]_2[AuBr_2^-]Br^-\cdot H_2O$

Crystal data	
Formula	$C_{24}H_{30}AuBr_3N_{10}O_5$
Molecular weight	975.3
Crystal system	triclinic
Space group	$P\bar{1}$
a	12.188(1) Å
b	17.404(2) Å
c	7.674(1) Å
α	99.79(1)°
β	99.45(1)°
γ	86.94(1)°
V	1581.8 Å^3
Z	2
$F(000)$	936
ρ_{exp}	2.05(2) g cm^{-3}
ρ_x	2.05 g cm^{-3}
μ(Mo Kα)	84.5 cm^{-1}
Data collection	
T	293 K
Radiation	Mo Kα, λKα = 0.71069 Å, graphite monochromator
Crystal detector distance	207 mm
Detector window	height = 4 mm, width = 4 mm
Take off angle	4.5°
Scan mode ω–2θ, maximum Bragg angle	25°
Scan width	(0.8 + 0.35 tg θ)° for omega angle
Values determining the scan speed	SIGPRE[a] = 0.5; SIGMA[a] = 0.015, VPRE[a] = 10°/min, TMAX[a] = 90 s
Conditions for refinement	
No. reflection for refinement of cell dimension	25
No. recorded reflections	5833 (excluding standards)
No. unique reflections	5550
No. utilized reflections	3593 [$I > 2\sigma(I)$]
No. refined parameters	310
Final reliability factors	
$R\Sigma\|k\|F_o\| - \|F_c\|\|/\Sigma k\|F_o\| = 0.030$	
$R_w = [\Sigma w(k\|F_o\| - \|F_c\|)^2/\Sigma wk^2F_o^2]^{1/2} = 0.034$ with $w = 1$	

[a]As defined in ref. 12.

diagonal approximation in the first stages and full-matrix in the final stages. Unit weights proved to be satisfactory. Scattering factors were taken from ref. 15 and anomalous dispersion corrections were applied to non-hydrogen atoms. Phenyl rings were treated as idealized rigid groups, with individual isotropic temperature factor for each carbon atom. Other non-hydrogen atoms were assigned anisotropic thermal parameters. All hydrogen atoms were located on a difference Fourier map. They were included in the structure factor calculations using idealized positions and an arbitrary isotropic temperature factor ($U = 0.07$ Å^2).

TABLE 2. Non-hydrogen atom fractional coordinates and isotropic or equivalent temperature factors (Å^2 × 100) with e.s.d.s in parentheses

Atom	*x/a*	*y/b*	*z/c*	U_{eq}/U_{iso}
Au	−0.10959(3)	0.70566(2)	−0.22900(5)	5.34(2)
Br(1)	−0.25556(8)	0.77755(7)	−0.1034(2)	7.94(8)
Br(2)	0.04064(8)	0.63931(6)	−0.3530(1)	6.65(6)
Br(3)	0.15910(7)	0.98476(5)	0.3856(1)	5.23(5)
C(4)	0.2969(4)	0.8090(3)	0.1523(7)	4.5(2)
C(5)	0.3982(4)	0.8310(3)	0.1251(7)	5.7(2)
C(6)	0.4661(4)	0.7779(3)	0.0379(7)	6.0(2)
C(7)	0.4327(4)	0.7028(3)	−0.0222(7)	6.2(2)
C(8)	0.3314(4)	0.6808(3)	0.0050(7)	5.2(2)
C(9)	0.2635(4)	0.7339(3)	0.0922(7)	3.8(2)
N(10)	0.1632(5)	0.7071(4)	0.1226(8)	4.1(4)
N(11)	0.0933(5)	0.7545(4)	0.2011(8)	3.7(3)
C(12)	−0.0004(6)	0.7267(4)	0.229(1)	3.5(4)
C(13)	−0.0302(6)	0.6458(4)	0.182(1)	3.9(4)
N(14)	−0.1326(5)	0.6273(4)	0.2147(9)	4.3(4)
C(15)	−0.2072(7)	0.6813(5)	0.287(1)	4.6(5)
N(16)	−0.1727(5)	0.7592(4)	0.3358(8)	4.0(4)
C(17)	−0.0747(6)	0.7834(4)	0.309(1)	3.5(4)
N(18)	−0.0485(5)	0.8559(4)	0.3580(9)	4.3(4)
C(19)	−0.2555(6)	0.8169(5)	0.404(1)	5.6(5)
O(20)	−0.2961(5)	0.6638(4)	0.3079(9)	6.6(4)
C(21)	−0.1626(8)	0.5451(5)	0.177(1)	6.8(6)
O(22)	0.0314(5)	0.5950(3)	0.1196(8)	5.4(4)
C(23)	0.2778(4)	0.5709(3)	0.4178(8)	6.0(2)
C(24)	0.2677(4)	0.4936(3)	0.3449(8)	7.2(3)
C(25)	0.3510(4)	0.4549(3)	0.2625(8)	7.7(3)
C(26)	0.4444(4)	0.4935(3)	0.2531(8)	7.8(3)
C(27)	0.4544(4)	0.5708(3)	0.3260(8)	6.0(2)
C(28)	0.3711(4)	0.6095(3)	0.4083(8)	4.4(2)
N(29)	0.3874(5)	0.6882(4)	0.4771(8)	4.3(4)
N(30)	0.3143(5)	0.7323(4)	0.5529(8)	3.9(4)
C(31)	0.3384(6)	0.8062(4)	0.619(1)	3.9(4)
C(32)	0.4431(6)	0.8401(5)	0.610(1)	4.4(5)
N(33)	0.4591(5)	0.9159(4)	0.6904(9)	4.8(4)
C(34)	0.3804(7)	0.9625(5)	0.773(1)	5.0(5)
N(35)	0.2811(5)	0.9272(4)	0.7825(8)	4.2(4)
C(36)	0.2566(6)	0.8527(4)	0.707(1)	3.8(4)
N(37)	0.1624(5)	0.8243(4)	0.7170(9)	4.7(4)
C(38)	0.1974(7)	0.9767(5)	0.871(1)	5.7(6)
O(39)	0.3971(5)	1.0296(3)	0.8371(9)	6.6(4)
C(40)	0.5673(7)	0.9495(6)	0.693(2)	7.2(7)
O(41)	0.5163(5)	0.8031(3)	0.5377(8)	6.1(4)
O(42)	0.0242(7)	0.1063(4)	0.130(1)	8.8(6)

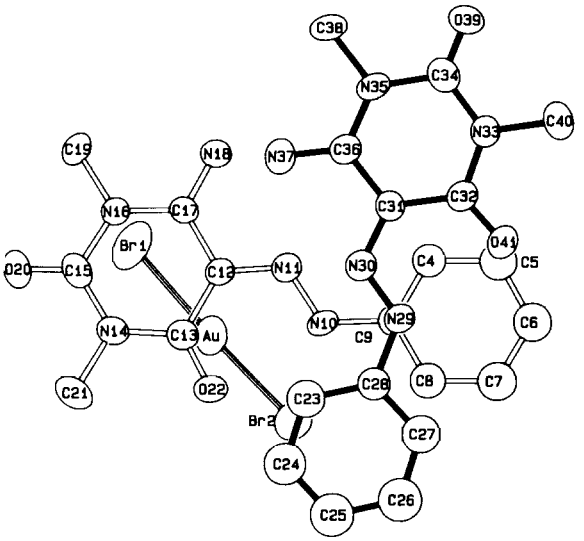

Fig. 2. Projection of the asymmetric unit (Br^- and H_2O omitted) onto the molecular plane. The two crystallographically independent $(C_{12}H_{14}N_5O_2)^+$ ions have been drawn, the first with white bonds and the second with black bonds. Hydrogen atoms have been omitted for clarity.

Final positional parameters and equivalent or isotropic temperature factors for non-hydrogen atoms are given in Table 2. Atoms are labelled according to Fig. 2. Bond lengths and angles are given in Table 3. crystal packing is shown in Fig. 3. See also 'Supplementary Material'.

Physical Measurements

Microanalyses of C, H and N were performed with a Perkin-Elmer 240C analyzer. Gold was determined thermogravimetrically using a Mettler TG-50 thermobalance. Infrared spectra were recorded in the 4000–180 cm^{-1} range on a Perkin-Elmer 983-G spectrophotometer using KBr and polyethylene pellets. The equivalent conductance of the compounds was recorded at 20 °C in methanol solutions 3.9–4.0 × 10^{-4} M. Measurements were taken within 2 min from the preparation of the solution using a Radiometer CDM2f conductimeter. Analyses and conductivity data are given in Table 4.

Viability Assay

HeLa cells were seeded into Petri dishes at a density of 1×10^5 cells per dish in DMEM supplemented with 10% calf serum. After 24 h the gold(I) compound was added to the culture medium and the cells cultured for 24 h more. Dead cells were washed out and alive ones were trypsinized and counted to determine if the gold(I) compound was toxic. No toxic concentrations of drug were used in the subsequent growth inhibition assay. Each compound was

TABLE 3. Selected interatomic distances (Å) and bond angles (°) with e.s.d.s in parentheses

Au–Br(1)	2.361(1)	Br(1)–Au–Br(2)	177.34(6)
Au–Br(2)	2.359(1)	C(9)–N(10)–N(11)	120.8(5)
C(9)–N(10)	1.399(7)	N(10)–N(11)–C(12)	119.0(6)
N(10)–N(11)	1.301(8)	N(11)–C(12)–C(13)	124.4(7)
N(11)–C(12)	1.326(9)	N(11)–C(12)–C(17)	115.4(6)
C(12)–C(13)	1.444(9)	C(13)–C(12)–C(17)	120.2(6)
C(12)–C(17)	1.434(9)	C(12)–C(13)–N(14)	116.9(7)
C(13)–N(14)	1.376(9)	C(12)–C(13)–O(22)	122.8(7)
C(13)–O(22)	1.216(9)	N(14)–C(13)–O(22)	120.3(7)
N(14)–C(15)	1.383(9)	C(13)–N(14)–C(15)	124.0(6)
N(14)–C(21)	1.464(9)	C(13)–N(14)–C(21)	118.1(7)
C(15)–N(16)	1.411(9)	C(15)–N(14)–C(21)	117.9(7)
C(15)–O(20)	1.185(9)	N(14)–C(15)–N(16)	117.0(7)
N(16)–C(17)	1.346(9)	N(14)–C(15)–O(20)	122.2(7)
N(16)–C(19)	1.478(9)	N(16)–C(15)–O(20)	120.8(8)
C(17)–N(18)	1.294(9)	C(15)–N(16)–C(17)	123.7(6)
		C(15)–N(16)–C(19)	116.3(6)
		C(17)–N(16)–C(19)	119.7(6)
		C(12)–C(17)–N(16)	118.2(6)
		C(12)–C(17)–N(18)	121.6(6)
		N(16)–C(17)–N(18)	120.2(7)
C(28)–N(29)	1.395(8)	C(28)–N(29)–N(30)	122.9(6)
N(29)–N(30)	1.291(8)	N(29)–N(30)–C(31)	118.7(6)
N(30)–C(31)	1.329(9)	N(30)–C(31)–C(32)	123.3(7)
C(31)–C(32)	1.449(9)	N(30)–C(31)–C(36)	117.1(6)
C(31)–C(36)	1.432(9)	C(32)–C(31)–C(36)	119.6(7)
C(32)–N(33)	1.367(9)	C(31)–C(32)–N(33)	117.3(7)
C(32)–O(41)	1.226(9)	C(31)–C(32)–O(41)	122.5(7)
N(33)–C(34)	1.385(9)	N(33)–C(32)–O(41)	120.2(7)
N(33)–C(40)	1.467(9)	C(32)–N(33)–C(34)	124.1(7)
C(34)–N(35)	1.405(9)	C(32)–N(33)–C(40)	117.9(7)
C(34)–O(39)	1.202(9)	C(34)–N(33)–C(40)	118.0(7)
N(35)–C(36)	1.356(9)	N(33)–C(34)–N(35)	117.0(7)
N(35)–C(38)	1.476(9)	N(33)–C(34)–O(39)	121.7(7)
C(36)–N(37)	1.293(9)	N(35)–C(34)–O(39)	121.3(8)
		C(34)–N(35)–C(36)	123.5(6)
		C(34)–N(35)–C(38)	117.0(6)
		C(36)–N(35)–C(38)	119.4(6)
		C(31)–C(36)–N(35)	118.3(7)
		C(31)–C(36)–N(37)	121.3(7)
		N(35)–C(36)–N(37)	120.4(7)

tested at least three times at a given concentration. Results were expressed as a total number of cells recovered from the drug treatment. Since gold(I) compounds were dissolved in DMSO, controls with DMSO at the same dilution as the gold(I) compounds (2 and 10 μl per 2 ml of medium) and without DMSO were carried out in parallel.

Growth Inhibition Assay

Fifty drug-treated cells were seeded into 30-mm Petri dishes in DMEM plus 10% calf serum and allowed to form isolated clones during a period of 12 days. At the end of this time, cells were fixed and stained with crystal violet. Clones obtained were classified in three types, according to their size: >1 mm (big), <1 mm (small), and isolated cells corresponding to cells that were not able to proliferate to form clones. The amount of size-classed clones was expressed as a percentage of the total amount per dish.

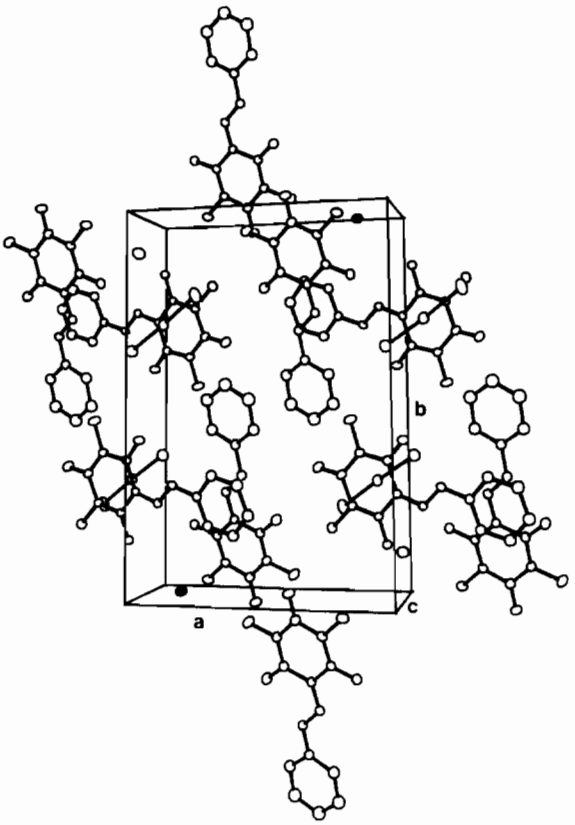

Fig. 3. Perspective view of the molecular packing. Black isolated atoms indicate Br^- ions, white isolated atoms indicate water molecules.

Results and Discussion

Air stable orange compounds of general formulae $(LH_2)(AuBr_2)\cdot H_2O$ (LH = DZH, DZEH) and $(LH_2)_2(AuBr_2)Br\cdot H_2O$ (LH = DZH, DZEH, DZCH) are obtained in good yield from $NaAuCl_4$ and the corresponding arylazouracil derivatives LH. The conductivity measurements indicate that the compounds $(LH_2)(AuBr_2)\cdot H_2O$ are 1:1 electrolytes while the compounds $(LH_2)_2(AuBr_2)Br\cdot H_2O$ are 2:1:1 electrolytes [16] and thus can be considered as salts formed by the arylazoniumuracil cation LH_2^+ and the $AuBr_2^-$ and (or not) Br^- anions. Two chemical processes have been achieved: reduction of gold(III) to gold(I) and protonation of the arylazouracil derivative, LH.

In the first synthesis described above (i) the gold(I) compounds are obtained using the bromide

TABLE 4. Analytical data and physical properties of gold(I) salts

	C (%)		H (%)		N (%)		Au (%)		Yield (%)	Λ ($cm^2\ \Omega^{-1}\ mol^{-1}$)	Concentration $\times 10^4$ (M)	Description
	calc.	found	calc.	found	calc.	found	calc.	found				
$(DZH_2)_2(AuBr_2)Br \cdot H_2O$	29.55	29.84	3.08	3.15	14.37	14.40	20.20	19.60	76	190	4.00	orange crystals
$(DZH_2)(AuBr_2) \cdot H_2O$	22.68	22.46	2.52	2.60	11.03	11.02	31.02	31.06	51	102	3.87	orange crystals
$(DZEH_2)_2(AuBr_2)Br \cdot H_2O$	32.04	31.88	3.63	3.37	13.38	12.93	18.81	19.03	46	177	3.95	orange powder
$(DZEH_2)_2(AuBr_2) \cdot H_2O$	25.35	25.44	3.02	2.95	10.56	10.69	29.71	29.88	72	94	3.89	orange crystals
$(DZCH_2)(AuBr_2) \cdot H_2O$	21.52	21.45	2.24	1.82	10.46	10.62	29.42	29.73	80	106	4.01	orange powder

ion as a reductor. It is well known that dissolving large amounts of $K(AuBr_4)$ in a bromide solution results in the formation of Br_2 [17]. The mechanism proposed for the reaction [18] is as follows

$$Br-Au(Br)_2-Br^- + Br^- \longrightarrow Br-Au(Br)_2-Br\ldots Br^{2-} \longrightarrow AuBr_2^- + Br_2 + Br^-$$

However, in this case the reduction of $AuBr_4^-$ is not complete and $(LH_2)(AuBr_4)$ salts are also obtained. The other method (ii) uses acetone as reducing agent [19], according to the following reaction

$$NaAuBr_4 + Me_2C{=}O \longrightarrow Na(AuBr_2) + BrCH_2COMe + HBr$$

$$Na(AuBr_2) \xrightarrow{LH} (LH_2)(AuBr_2)$$

$$Na(AuBr_2) \xrightarrow{2LH,\ HBr} (LH_2)_2(AuBr_2)Br$$

The protonation reaction raises the question of the protonation site. Infrared spectra of salts obtained (Table 5) show that the N=N stretching band is shifted (by *c.* 75 cm^{-1}) to lower wavenumber as compared with the corresponding unprotonated derivatives LH. This indicates that a nitrogen atom of the azo group is the protonation site. This shift has also been observed in the complex $(DZH_2)(AuCl_2) \cdot 1.5H_2O$ for which the X-ray crystal structure determination indicates that the protonation site is also a nitrogen atom of the azo group [10].

The Au–Br stretching bands appear at frequencies similar to those observed for related compounds containing the linear $AuBr_2^-$ anion [19]. No significant difference is noticed from one salt to another (Table 5).

TABLE 5. Infrared data for gold(I) compound (cm^{-1})

Compound	ν(N=N)	ν(Au–Br)
DZH	1522	
$(DZH_2)_2(AuBr_2)Br \cdot H_2O$	1447	252
$(DZH_2)(AuBr_2) \cdot H_2O$	1446	250
DZEH	1523	
$(DZEH_2)_2(AuBr_2)Br \cdot H_2O$	1450	250
$(DZEH_2)(AuBr_2) \cdot H_2O$	1450	245
DZCH	1529	
$(DZCH_2)(AuBr_2) \cdot H_2O$	1455	245

Crystal and Molecular Structure of $(DZH_2)_2$-$(AuBr_2)Br \cdot H_2O$

To support the above discussion, the X-ray crystallographic study of the title compound has been carried out. The structure is ionic, the asymmetric unit contains one $AuBr_2^-$ anion, two crystallographically independent DZH_2^+ cations (Fig. 2), one isolated Br^- anion and one molecule of water of crystallization.

The presence of the isolated linear $AuBr_2^-$ group confirms that Au(III) has been reduced to Au(I). However, the usually linear $AuBr_2^-$ anion is slightly bended with a Br–Au–Br angle of 177.34(6)°. There is no obvious theoretical reason and crystal packing only can be invoked to explain this distortion. In the asymmetric unit, the $AuBr_2^-$ group lies almost parallel to the plane of the pyrimidine ring at a mean distance of 3.4 Å.

It can be seen from Table 3 that the two independent DZH_2^+ cations are quite similar. Careful analysis of Fourier maps showed that the protonation of the originally neutral molecules takes place at the diazo N(10) atom for one molecule and N(29) for the other one. This nitrogen atom has also been shown to be one of the coordination sites of the DZ^- anion in the complexes $Cu(DZ)_2 \cdot DMSO$, $Pd(DZ)_2$ and Au(DZC)-Cl_2 [1] and the protonation site in the complex $(DZH_2)(AuCl_2) \cdot 1.5H_2O$.

Both DZH_2^+ cations are essentially planar indicating extended electronic delocalization. In the crystal structure, these cations occur in pairs with a mean distance of 3.2 Å between the two almost parallel molecular planes. However, Fig. 2 shows that, within such pairs, there is no significant overlap of the π-electron systems. The deviations of atoms from the least-squares planes of the pyrimidine rings are given in Table 6 together with relevant dihedral angles.

The two additional protons on the N(10) and N(29) atoms are involved in intramolecular hydrogen bonds N(10)–H(48)···O(22) and N(29)–H(62)···O(41) (N–H···O angles and N···O and H···O distances are *c.* 132°, 2.59, 1.85 Å and 135°, 2.54, 1.78 Å, respectively), contributing to the rigidity of the planar system. Coplanarity of the pyrimidine and phenyl rings has also been observed in the complex $(DZH_2)(AuCl_2) \cdot 1.5H_2O$.

The molecule of water of crystallization is presumably loosely tied to one of the DZH_2^+ cations through hydrogen bonding: N(37)···O(42) = 2.85 Å, H(70)···O(42) = 1.93 Å and N(37)–H(70)···O(42) = 163°.

Bond lengths and angles compare quite well with those previously obtained for other complexes of DZH [1, 10]. Each nitrogen atom has a planar environment indicating that the lone pair is involved in the bonding pattern. In the pyrimidine rings all C–C and C–N distances are intermediate between single and double bond lengths as expected from electronic delocalization. C···N bond lengths range from 1.293 to 1.411 Å indicating some variation of bond multiplicity. The C(17)–N(18) and C(36)–N(37) bonds are strikingly short (1.294 and 1.293 Å, respectively). However straight molecular mechanics calculations [20], performed using the amine type for N(18) and N(37) atoms failed to explain such a feature. Further theoretical calculations will be carried out using more sophisticated models.

TABLE 6. Deviations (Å) of atoms from the least-squares planes of the pyrimidine rings, dihedral angles (°) between planes and relevant torsional angles (°)

Plane 1		Plane 2	
Atoms defining the plane	Deviations	Atoms defining the plane	Deviations
C(12)	0.013	C(31)	0.001
C(13)	−0.006	C(32)	0.002
N(14)	−0.009	N(33)	−0.012
C(15)	0.017	C(34)	0.018
N(16)	−0.011	N(35)	−0.016
C(17)	−0.004	C(36)	0.006
Other atoms	Deviations	Other atoms	Deviations
N(18)	−0.035	N(37)	0.003
C(19)	0.089	C(38)	0.010
O(20)	0.057	O(39)	0.043
C(21)	−0.097	C(40)	−0.096
O(22)	−0.040	O(41)	−0.006
N(11)	0.058	N(30)	−0.039
N(10)	0.126	N(29)	−0.080
C(9)	0.165	C(28)	−0.162
C(8)	0.269	C(27)	−0.149
C(7)	0.256	C(26)	−0.211
C(6)	0.140	C(25)	−0.286
C(5)	0.036	C(24)	−0.300
C(4)	0.049	C(23)	−0.238
Dihedral angles			
(1)–Φ1	5.3	(2)–Φ2	3.4
(1)–(2)	7.6	Φ1–Φ2	0.9
Torsional angles			
C(9)–N(10)–N(11)–C(12)		−179.5	
C(28)–N(29)–N(30)–C(31)		178.2	

Growth Inhibition of HeLa Cells

Table 7 lists the percentage of growth inhibition of HeLa cells (human tumor cervix) by the bromogold(I) salts. The data indicate that at a concentration of 10 μM these gold(I) compounds are poor growth inhibitors since the reduction in the number of colonies formed is lesser than 50% relative to controls [21, 22]. Furthermore, all these compounds are weaker growth inhibitors than the analogous chlorogold(I) complex and the gold(III) complexes

TABLE 7. Growth inhibition of HeLa cells by gold(I) compounds[a]

Compounds	Concentration (μM)	Viability cells $\times 10^4$	Growth inhibition	
			size	(%)
$(DZEH_2)(AuBr_2)\cdot H_2O$	10	13.5 ± 3	small	51
			big	41
			isolated cells	8
	50	13 ± 0.5	small	56
			big	14
			isolated cells	30
$(DZH_2)_2(AuBr_2)Br\cdot H_2O$	10	10.13 ± 1.37	small	10
			big	80
			isolated cells	10
	50	10.12 ± 2.88	small	10
			big	0
			isolated cells	90
$(DZCH_2)(AuBr_2)\cdot H_2O$	10	13.3 ± 0.87	small	25
			big	65
			isolated cells	10
	50	8 ± 1.12	small	25
			big	50
			isolated cells	25
$(DZH_2)(AuBr_2)\cdot H_2O$	10	13.75 ± 1.75	small	22
			big	70
			isolated cells	8
	50	12.37 ± 0.37	small	42
			big	28
			isolated cells	30
Control		15.8 ± 1.46	small	39
			big	60
			isolated cells	<1
Control + DMSO (10 μl)		18.3 ± 3.9	small	40
			big	59
			isolated cells	<1

[a]All compounds are stable in the assay medium (pH = 7.2).

$Au(DZ)Cl_2$ and $Au(DZC)Cl_2$ [10]. At a concentration of 50 μM only the compounds $(DZEH_2)(AuBr_2)\cdot H_2O$ and $(DZH_2)_2(AuBr_2)Br\cdot H_2O$ inhibit reasonably well, especially the first one with a growth inhibition of 100%. However, as the concentration of gold compound required to reduce the number of colonies by 50% relative to control, IC_{50}, seems to be greater than 20 μM, both complexes must be marginally active *in vivo* [21, 22]. In spite of this, work is in progress to further characterize the anticancer properties of our complexes *in vivo*, since some gold complexes inactive *in vitro*, exceptionally to the general rule, have proved to be active *in vivo* [21].

Supplementary Material

Components of the anisotropic temperature factors, hydrogen atom positional parameters and observed and calculated structure factor amplitudes are available from the authors on request.

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References

1 J. Ruiz, *Thesis*, UNED, Madrid, 1988.
2 P. T. Beurkens, H. J. A. Blaauw, J. A. Cras and J. J. Steggerda, *Inorg. Chem.*, 7 (1968) 805.

3 J. Strähle, J. Gelinek and M. Kölmel, *Z. Anorg. Allg. Chem., 456* (1979) 241.
4 P. Braunstein, A. Müller and H. Bögge, *Inorg. Chem., 25* (1986) 2104.
5 M. A. Beno, M. A. Firestone, P. C. W. Leung, L. M. Sowa, H. H. Wang, J. M. Williams and M. H. Whangbo, *Solid State Commun., 57* (1986) 735.
6 (a) R. Kato, H. Kobayashi and A. Kobayashi, *Chem. Lett., 5* (1986) 785; (b) T. Mori, F. Sakai, G. Saito and H. Inokuchi, *Chem. Lett., 6* (1986) 1037; (c) *9* (1986) 1589; (d) R. Kato, H. Kobayashi, A. Kobayashi, T. Mori and H. Inokuchi, *Chem. Lett., 2* (1987) 277; (e) R. Kato, H. Kobayashi and A. Kobayashi, *Synth. Met., 19* (1987) 629.
7 (a) E. Amberger, K. Polborn and H. Fuchs, *Angew. Chem., 98* (1986) 749; (b) E. Amberger, H. Fuchs and K. Polborn, *Angew. Chem., 98* (1986) 751.
8 M. Kurmoo, D. R. Talham, P. Day, I. D. Parker, R. H. Friend, A. M. Stringer and J. A. K. Howard, *Solid State Commun., 61* (1987) 459.
9 K. C. Dash and H. Schmidbaur, in H. Sigel (ed.), *Metal Ions in Biological Systems,* Vol. 14, Marcel Dekker, New York, 1982, p. 179.
10 R. Kivekäs, E. Colacio, J. Ruiz, J. D. López and P. León, *Inorg. Chim. Acta, 159* (1989) 103.
11 B. Lythgoe, A. R. Todd and A. Topham, *J. Chem. Soc.,* (1944) 315.
12 A. Mosset, J.-J. Bonnet and J. Galy, *Acta Crystallogr., Sect. B, 33* (1977) 2639.
13 P. Coppens, L. Leiserowitz and D. Rabinovich, *Acta Crystallogr., 18* (1965) 1035.
14 E. R. Howells, D. C. Phillips and D. Rogers, *Acta Crystallogr., 3* (1950) 210.
15 *International Tables for X-ray Crystallography,* Vol. 4, Kynoch Press, Birmingham, 1974.
16 W. J. Geary, *Coord. Chem. Rev., 7* (1971) 81.
17 Y. T. Fanchiang, *Inorg. Chem., 21* (1982) 2344.
18 R. Ettore, *J. Chem. Soc., Dalton Trans.,* (1983) 2329.
19 P. Braunstein and R. J. H. Clark, *J. Chem. Soc., Dalton Trans.,* (1973) 1845.
20 M. Saunders and R. Jarret, *MPMI,* program derived from N.L. Allinger's MM2 and MMP1, QCPE nos. 395 and 318, Indiana State University.
21 C. K. Mirabelli, R. K. Johnson, D. T. Hill, L. F. Faucette, G. R. Girard, G. Y. Kuo, C. M. Sung and S. T. Crooke, *J. Med. Chem., 29* (1986) 218.
22 C. K. Mirabelli, D. T. Hill, L. F. Faucette, F. L. McCabe, G. R. Girard, D. B. Bryan, B. M. Sutton, J. O. L. Barkus, S. T. Crooke and R. K. Johnson, *J. Med. Chem., 30* (1987) 2181.