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Chemical and biological reactions of diacetato[2-(dimethylaminomethyl)-phenyl]gold(III), $[Au(O_2CMe)_2(dmamp)]$

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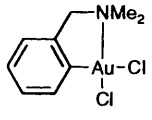
The gold(III) complex $[Au(O_2CMe)_2(dmamp)]$ [dmamp = 2-(dimethylaminomethyl)phenyl] is hydrolysed in wholly or partially aqueous solution. One acetate ligand, presumed to be that *trans* to the Au–C bond, exchanged with a water molecule rapidly on the NMR time-scale. At high concentrations of water a further hydrolysis step was also discernible, which involved the second acetate group while, in aged solutions, small amounts of a third species, possibly the second isomer of $[Au(O_2CMe)(dmamp)(H_2O)]^+$, was formed. The reaction of aqueous or dimethyl sulfoxide solutions of the gold(III) complex with various biological ligands was followed by NMR spectroscopy and a range of reactivities was found: caffeine and adenosine showed no reaction, L-cysteine, glutathione and adenine reacted quantitatively and guanosine and inosine showed partial reaction. In *in vitro* biological tests for antibacterial activity, $[Au(O_2CMe)_2(dmamp)]$ exhibited a potentially useful selectivity.

We have reported on the antibacterial and antitumour activity of $[AuCl_2(dmamp)]$ **1** [dmamp = 2-(dimethylaminomethyl)-phenyl] which showed some activity analogous to that of cisplatin.[1] The antibacterial selectivity was not high, and the application of this gold complex is inhibited by its negligible solubility in water. We have therefore sought a water-soluble derivative and the diacetato analogue **2** has proved very suitable. Initial studies suggested that it underwent ready hydrolysis, and we have now made a detailed study of this process in water and in mixed solvents. Since the complex shows interesting biological behaviour, some of which is summarised here, we have also examined its reactions with model biological molecules.



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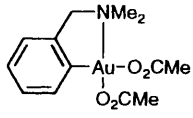

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Experimental

The complex $[Au(O_2CMe)_2(dmamp)]$ was synthesised as described earlier[2] (Found: C, 34.5; H, 3.9; Au, 44.2; N, 3.0. Calc. for $C_{13}H_{18}AuNO_4$: C, 34.7; H, 4.0; Au, 43.9; N, 3.1%). Hydrolysis reactions were studied by ^1H and ^{13}C NMR in D_2O and H_2O–$(CD_3)_2SO$, in the absence and presence of added acetate ion. Reactions with L-cysteine, glutathione and a selection of purines and nucleosides were also monitored by NMR in D_2O or $(CD_3)_2SO$. NMR measurements were made on a Brüker AC300 spectrometer at 300 (^1H) and 75 MHz (^{13}C), using 3000–6000 scans per spectrum (depending on solubility) and *ca.* 1 s scan^{-1}, at concentrations of 0.03–0.3 mol dm^{-3}; spectra were referenced to the solvent in each case. *In vitro* antimicrobial activity was assessed using *Staphylococcus aureus* NCTC 6571, *Enterococcus faecalis* NCTC 8727, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662. Minimum inhibitory concentration values were determined using the plate-dilution technique[3] with Iso-sensitest agar as the medium. An inoculum of 10^6 organisms cm^{-3} was applied with a multi-point inoculating device, and the plates were examined for microbial growth after incubation for 18 h at 37 °C. Ciprofloxacin was included as a control.

Comparative cytotoxicity was assessed[4] using the Chinese hamster ovary fibroblast cell line[5] obtained from the European Collection of Animal Cell Cultures. Cells were maintained in McCoy's 5a medium supplemented with 10% foetal bovine serum, 2 mmol dm^{-3} L-glutamine, 100 units cm^{-3} penicillin and 100 μg cm^{-3} streptomycin, at 37 °C under 5% CO_2. Cells were seeded onto 96-well microtitre plates at 5×10^4 cells cm^{-3} in 200 μl of medium. After pre-incubation (24 h), the cells were treated with 0.1, 1.0, 10.0 or 100 μg cm^{-3} of test compound for 2 h. The compound was then replaced with fresh medium and incubation continued for 48 h. Cell growth was assayed using the sulforhodamine B method.[6] Cell survival (%) was calculated relative to untreated control cells. Cisplatin and ciprofloxacin were used as controls.

Results and Discussion

Previous work on $[AuCl_2(dmamp)]$ was inhibited by its poor solubility, especially in water (0.25 mmol dm^{-3}). Replacement of the chloride ligands by acetate gave an enormous increase in solubility, to at least 300 mmol dm^{-3}. However, there was evidence of hydrolysis, which has now been studied by ^{13}C NMR.

Hydrolysis of $[Au(O_2CMe)_2(dmamp)]$

In $CDCl_3$ solution, the ^1H and ^{13}C NMR spectra of $[Au(O_2CMe)_2(dmamp)]$ are as expected,[1] except for slight broadening of one of the two pairs of acetate signals [Fig. 1(*a*), Tables 1 and 2]. The ^{13}C NMR spectrum in $(CD_3)_2SO$ is very similar in shape, although the chemical shifts are rather different [Fig. 1(*b*), Table 1]. However, in aqueous solution, the broadening of one set of acetate signals is so great that the peaks can barely be discerned [Fig. 1(*c*)]. Similar broadening is observed in methanolic solution. The broadening is best explained on the assumption that one acetate ligand is undergoing exchange at a rate which is comparable to the NMR time-scale. Addition of sodium acetate to the aqueous solution [0.5–2.0 mol mol(Au)$^{-1}$] increases the intensity of the broadened acetate signals, and sharpens them considerably,

confirming that the exchange involves free acetate in solution. The broadening seen in $CDCl_3$ and $(CD_3)_2SO$ solutions is therefore attributed to exchange with traces of water.

The aqueous solution spectra show the presence of a second set of signals corresponding to the dmamp ligand, but no additional acetate signals. In some solutions there were indications of a third species, which did give an extra set of acetate signals. In order to simplify the spectra, it was decided to investigate solutions in water-miscible organic solvents containing controlled quantities of water. Dry acetonitrile gave a good, sharp, single spectrum [Fig. 2(*a*)] and proved suitable for the water-addition experiments.

In acetonitrile containing increasing amounts of water (from 1:1 to 50:1 with respect to the complex) only a single major set of signals is seen (Fig. 2). Most of the peaks are sharp, with the

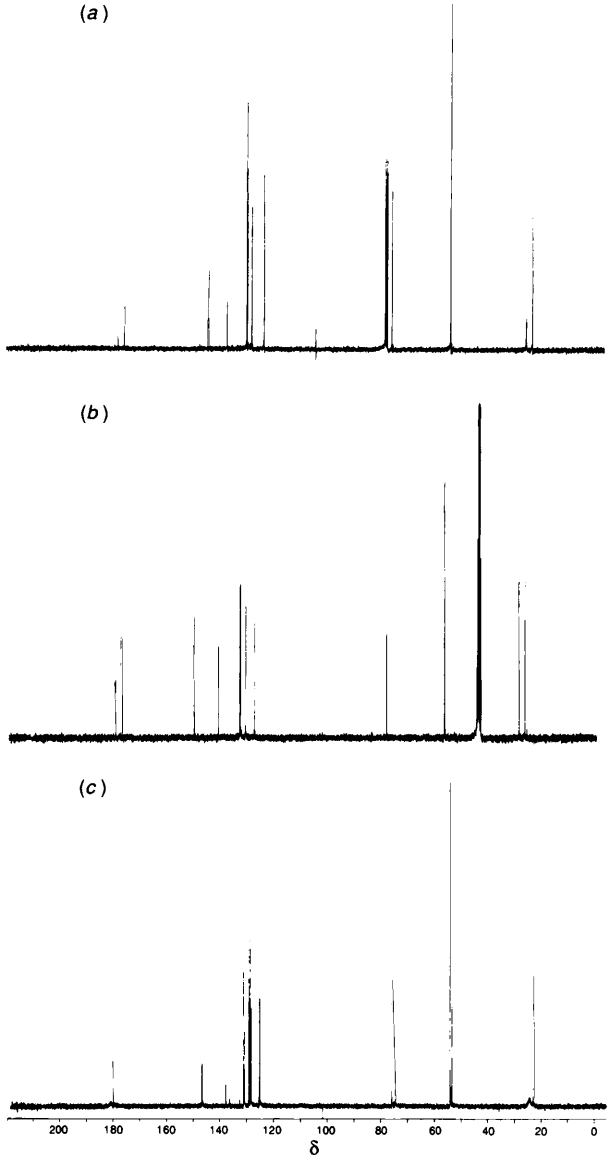


Fig. 1 Carbon-13 NMR spectra of $[Au(O_2CMe)_2(dmamp)]$ in (*a*) $CDCl_3$, (*b*) $(CD_3)_2SO$ and (*c*) D_2O at *ca.* 0.03 mol dm^{-3}. The signals at *ca.* δ 101 are spurious

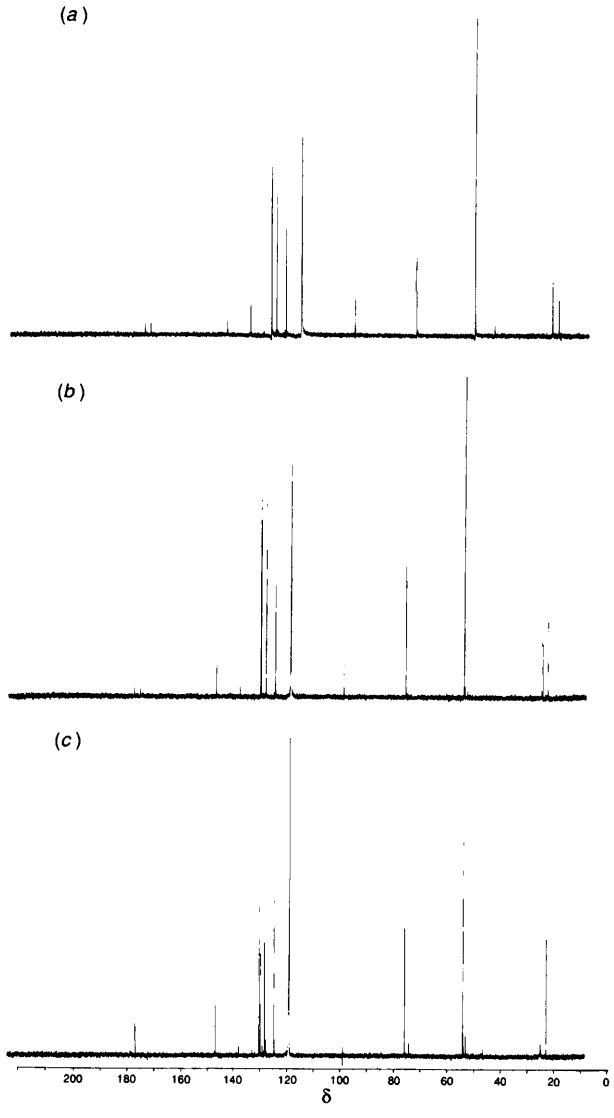


Fig. 2 Carbon-13 NMR spectra of $[Au(O_2CMe)_2(dmamp)]$ in CD_3CN with added water. Molar ratios H_2O:Au are (*a*) 0:1, (*b*) 1:1 and (*c*) 20:1 and the total gold concentration is *ca.* 0.03 mol dm^{-3}

Table 1 Carbon-13 NMR spectroscopic data (δ) for $[Au(O_2CMe)_2(dmamp)]$ in various solvents

Solvent	Aromatic carbon atoms	CH_2	$N(CH_3)_2$	CH_3CO_2	CH_3CO_2
$CDCl_3$	143.5, 136.6, 129.0, 128.6, 127.3, 122.8	75.1	53.2	24.6, 22.2 (br)	177.6, 175.3 (br)
$(CD_3)_2SO$	149.5, 140.3, 132.4, 132.2, 130.3, 127.0	77.7	56.3	28.3, 26.0	178.9, 177.0
D_2O	146.3, 137.4, 130.8, 128.9, 128.4, 124.9	75.7	54.0	24.2 (br), 22.6	181.0 (br), 179.0
CD_3CN	145.4, 136.5, 128.7, 128.6, 126.7, 123.2	74.5	52.6	23.6, 21.3	176.0, 173.9

Table 2 Proton NMR spectroscopic data (δ) for $[Au(O_2CMe)_2(dmamp)]$ in various solvents

Solvent	Aromatic hydrogen atoms	CH_2	$N(CH_3)_2$	CH_3CO_2
$CDCl_3$	7.13 (m), 6.97 (m)*	4.20 (s)	3.10 (s)	2.08 (s), 1.98 (s)
$(CD_3)_2SO$	7.32 (t), 7.21 (d), 7.12 (t), 6.91 (d)	4.53 (s)	3.03 (s)	2.01 (s), 1.87 (s)
D_2O	7.37 (t), 7.19 (m)*, 6.92 (d)	4.52 (s)	3.19 (s)	2.22 (s), 2.18 (s)

* Individual proton signals not well resolved.

Table 3 Carbon-13 NMR spectroscopic data (δ) for $[Au(dmamp)(H_2O)_2]^{2+}$ in various solvents

Solvent	Aromatic carbon atoms	CH_2	$N(CH_3)_2$
CD_3CN–H_2O (H_2O:Au = 20:1)	*a*, *a*, 130.9, 129.9, 128.7, 125.6	75.2	44.0
$CDCl_3$	147.9, *a*, 129.2, 127.8, 127.0, 123.0	74.7	52.8
D_2O [b]	146.5, 136.0, 130.5, 128.6, 128.0, 125.0	74.4 (vbr)	53.3 (vbr)

[a] Obscured or too weak to detect. [b] Signals for second isomer of $[Au(dmamp)(O_2CMe)(H_2O)]$: *a*,*a* (vbr), 132.3, 130.6, 128.7, 125.3 (aromatic carbon atoms); 75.0 (CH_2); 53.6 (vbr) [$N(CH_3)_2$]; 24.2 (CH_3CO_2); 181.0 (CH_3CO_2).

Table 4 Relative amounts[a] of hydrolysis products in acetonitrile–water[b]

H_2O:Au molar ratio	$[Au(X)(O_2CMe)(dmamp)]$ (X = O_2CMe or H_2O)	$[Au(dmamp)(H_2O)_2]^{2+}$
0:1	98.3	1.7
10:1	95.6	4.2
20:1	92.9	7.1
50:1[c]	87.8	9.2
ca. 15 000:1[d]	53.3	46.7

[a] Estimated from ^{13}C NMR intensities. [b] 78:22 D_2O:H_2O, *ca.* 30 mmol(Au) dm^{-3}. [c] $[Au(dmamp)(O_2CMe)(H_2O)]^+$ (second isomer) 3.0. [d] 100% water.

exception of the CH_3 and CO_2 peaks of the acetate group mentioned above. These peaks become broader as the water content increases, indicating that this acetate group is involved in an exchange process (1). One acetate group is evidently much

$$[Au(O_2CMe)_2(dmamp)] + H_2O \rightleftharpoons [Au(O_2CMe)(dmamp)(H_2O)]^+ + MeCO_2^- \quad (1)$$

more labile than the other. On the basis of the *trans* effect, this would be expected to be that *trans* to the Au–C bond, since a carbanion has a stronger *trans*-labilising effect than an amine group. The fact that the peaks for the exchanging acetate group are broadened more than the remaining peaks of the spectrum is because the extent of broadening depends inversely on the difference in chemical-shift frequencies between the two forms in equilibrium, relative to the rate of exchange.[7] The chemical shift differences are far less for the dmamp ligand and the one acetate which remains co-ordinated than for the acetate which exchanges between the bound and free forms.

At H_2O:Au ratios of 10:1 and above, additional sets of peaks appear. Their intensity was always small, but increased with increasing water content (Tables 3 and 4). The same species can be detected in $CDCl_3$ and $(CD_3)_2SO$, again presumed to be due to adventitious water. However, no additional acetate peaks are seen in any of these spectra. Unless there are accidental coincidences, this indicates the presence of a species which contains the dmamp ligand but no acetate, *i.e.* the diaqua complex [equation (2)]. A rough indication of the amounts of

$$[Au(O_2CMe)(dmamp)(H_2O)]^+ + H_2O \rightleftharpoons [Au(dmamp)(H_2O)_2]^{2+} + MeCO_2^- \quad (2)$$

the mono- and di-aqua complexes was made by measuring the relative heights of corresponding peaks; in pure water (*ca.* 0.03 mol dm^{-3}) they were about 2.5:1 and the ratio decreased to about 1.6:1 on addition of 2 molar equivalents of sodium acetate. It is, of course, likely that the mono- and the di-aqua complexes are both in equilibrium with their deprotonated forms, $[Au(O_2CMe)(dmamp)(OH)]$ and $[Au(dmamp)(H_2O)(OH)]^+$ or $[Au(dmamp)(OH)_2]$ respectively.

The pH of the aqueous solution is about 4.0, and is unchanged by the addition of extra acetate ion; this value is that expected for an $MeCO_2H$–$MeCO_2^-$ buffer system. The aqueous solution without added acetate also exhibited considerable electrical conductivity [270–340 S mol(Au)$^{-1}$ at concentrations of 7.3–3.2 mmol dm^{-3}].

In some of the aged aqueous solutions, a third set of dmamp NMR signals was seen, of low intensity (see Table 4), together with a pair of acetate signals very close to those of the principal non-exchanging acetate (Table 3). No definite identification can be made, but it seems reasonable to suggest that this could be the second isomer of the monoaqua monoacetate complex, $[Au(O_2CMe)(dmamp)(H_2O)]^+$, or its deprotonated form, in which the co-ordinated water molecule (hydroxide ion) is *trans* to the Au–N bond. In substitution reactions of $[AuCl_2(dmamp)]$, the incoming ligand is always found in the position *trans* to the Au–N bond,[4] even though the initial substitution is expected to occur *trans* to Au–C. Substitution must therefore be followed by an isomerisation. Rapid exchange of the co-ordinated water molecule with solvent water is expected for this isomer, but would not be detected.

Reactions with biological ligands

Solutions of $[Au(O_2CMe)_2(dmamp)]$ were treated with equimolar amounts of L-cysteine, glutathione, caffeine, guanosine, adenine, adenosine or inosine and the ^{13}C NMR spectra examined. It was possible to obtain good spectra from aqueous solutions containing L-cysteine, glutathione or caffeine, but adenine and the nucleosides were insufficiently soluble. The latter were therefore examined in $(CD_3)_2SO$. Attempts were also made to use guanine, but good spectra could not be obtained in either solvent. No attempts were made to control the pH of the solutions which, as shown above, are effectively self-buffering. In the case of guanosine, inosine and caffeine, signals for the free nucleosides were seen and showed no change in chemical shift from solutions of the pure materials. It is therefore assumed that co-ordination chemical shifts can be safely calculated in all cases from the spectra of the pure components.

L-Cysteine. For L-cysteine quantitative reaction occurred, and the signals of the diacetate complex and L-cysteine were replaced by a new single set (Table 5). Only one set of acetate resonances was observed, at a position consistent with free acetate (δ 21.5 and 177.9), suggesting that the cysteine was bound as a bidentate ligand. To establish the mode of binding, the co-ordination chemical shifts [$\Delta\delta$ = δ(complex) − δ(ligand)] of the signals for the three carbon atoms of L-cysteine were determined (Table 5). All were positive, but the carboxyl carbon showed a much smaller value than the other two carbon atoms. It is therefore likely that cysteine is bound to gold through the sulfur and nitrogen, as would be expected for co-ordination to a soft metal. The simplicity of the spectrum indicates that only one isomer is produced, but insufficient data are available to identify which it is. However, the thermodynamically stable form is expected to be that in which the two softest donors, carbon and sulfur, are mutually *cis*. The one complication in the spectrum is the doubling of the methyl signals for the NMe_2 group, which is presumably due to the lack of a plane of symmetry through the five-membered chelate ring formed by the L-cysteine. The methyl groups are therefore not equivalent. All other carbon atoms of the dmamp ligand lie

Table 5 Carbon-13 NMR chemical shifts (δ) for L-cysteine before and after reaction with $[Au(O_2CMe)_2(dmamp)]$ in D_2O[a]

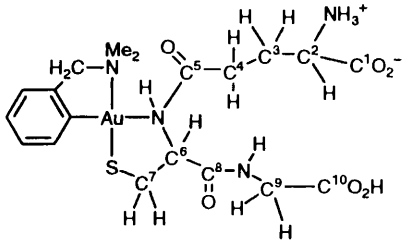
	$SCH_2C(NH_2)CO_2$	$SCH_2C(NH_2)CO_2$	$SCH_2C(NH_2)CO_2$
L-Cysteine	25.9	56.9	173.5
L-Cysteine + $[Au(O_2CMe)_2(dmamp)]$	35.9	63.3	175.9
Δδ[b]	+10.0	+6.4	+2.4

[a] Acetate signals were found at δ 22.6, 24.2 and δ 179.0, 181.0 before reaction and δ 21.5 and 177.9 after. Signals for the dmamp ligand are at δ 148.0, 141.8, 132.9, 129.5, 128.8, 125.9, 72.7, 52.2 and 52.0. [b] Δδ = δ(complex) − δ(ligand).

Table 6 Carbon-13 NMR chemical shifts (δ) for glutathione[a] before and after reaction with $[Au(O_2CMe)_2(dmamp)]$ in D_2O[b]

	C^2	C^3	C^4	C^6	C^7	C^9	C^1, C^5, C^8, C^{10}
Glutathione	54.7	26.9	32.1	56.6	26.4	42.5	173.4, 174.5, 174.6, 175.8
Glutathione + $[Au(O_2CMe)_2(dmamp)]$	54.9	27.0	32.2, 32.3	60.3, 60.4	36.8	43.5, 43.7, 43.8	172.1, 172.3, 174.7, 175.6, 175.8, 176.1
Δδ	+0.2	+0.1	+0.1, +0.2	+3.7, +3.8	+10.4	+1.0, +1.2, +1.3	Mostly < 1–2 ppm one ≥ +7.3

[a] Glutathione assignments according to ref. 8. [b] Acetate signals were found at δ 26.0, 28.3 and δ 176.7, 178.4 before reaction and δ 21.7 and 178.2 after.



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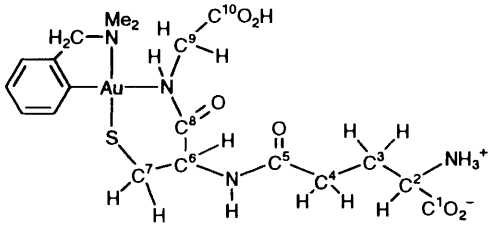

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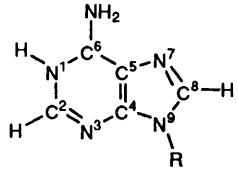
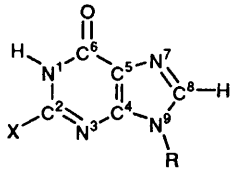
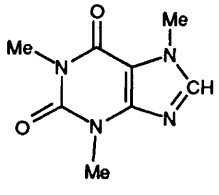


	R
Adenine	H
Adenosine	ribose



	R	X
Guanosine	ribose	NH_2
Inosine	ribose	H



Caffeine

in the co-ordination plane of the gold, and are unaffected by this asymmetry.

Glutathione. Reaction of glutathione with $[Au(dmamp)(O_2CMe)_2]$ also results in displacement of both acetate ligands and the formation of a single, chelated complex (Table 6). The Δδ values again indicate that the thiol group is co-ordinated (large positive Δδ for C^7, substantially positive Δδ for C^6). It might be expected that a five-membered ring would be formed by co-ordination of the imido nitrogen atom of the glycine residue (structure **I**). However, although the individual carbonyl signals cannot be unambiguously assigned, it is clear that only one undergoes a substantial co-ordination shift (at least +7.3 ppm). This is more compatible with structure **II**, in which C^8 is part of the chelate ring, and the other carbonyl groups are well separated from the gold atom. This is supported by the ^1H NMR spectrum, in which the protons attached to C^9 undergo the greatest co-ordination shift (−0.4 ppm, others 0 to −0.2 ppm).

It is also noticeable that most of the signals of glutathione are doubled, as are those of the dmamp ligand. The C^1 (dmamp) signal is single and at a chemical shift very similar to that for the L-cysteine adduct (δ 147.0), showing that there is only a single co-ordination isomer present, with the same geometry as for L-cysteine (S *trans* to NMe_2). The complexity of the remainder of the spectrum must be due to the presence of two asymmetric centres in the chelate ring (C^6 and the nitrogen atom). This may also account for there being three signals at the C^9 position.

Nucleosides. The ^{13}C NMR spectra of a 1:1 mixture of adenosine and $[Au(O_2CMe)_2(dmamp)]$ gave a single set of signals for the heterocyclic rings, essentially unshifted from the positions of the free nucleoside (Table 7). Some additional signals were found for the gold complex (those of the methyl and methylene groups are reasonably readily discernible), suggesting that some decomposition had occurred, rather than adduct formation. For guanosine and inosine it was clear that incomplete reactions were occurring, but the relative intensities of the two principal sets of signals suggested that about 60% of the gold was present as the new complex. In each case a second set of acetate signals is seen (two new pairs), indicating monodentate co-ordination by the nucleosides.

For guanosine, Δδ is substantially positive for C^6 and C^2, modestly positive for C^5 and C^8 and small and negative for C^4. Binding through either N^1 or the NH_2 group is indicated, although this contrasts with data for $AuMe_2^+$ and for cisplatin.[9,10] Inosine shows a large positive Δδ for C^2 and C^6 and a small negative Δδ for C^4. In this case, the chemical shifts for C^2 and C^4 are reversed by co-ordination, but C^2 can be recognised from the DEPT (distortionless enhancements by

Table 7 Carbon-13 NMR data for adenine and the nucleosides before and after reaction with $[Au(O_2CMe)_2(dmamp)]$ in $(CD_3)_2SO$[a]

	C^2	C^4	C^5	C^6	C^8	dmamp CH_2	$N(CH_3)_2$	CH_3CO_2[b]	CH_3CO_2[b]	% Reaction
Adenine	156.2	154.0	122.3	159.6	142.7	77.1	56.3	28.3, 26.0	177.0, 178.9	
Adenine + $[Au(O_2CMe)_2(dmamp)]$	157.4	155.1	127.4	159.9	148.2	81.0	55.7	28.2, 25.3	180.7, 176.2	100
Δδ	+1.2	+1.1	+5.1	0.3	+5.5	+2.9	−0.6	−0.1, −0.7	+3.7, −2.3	
Adenosine	156.3	152.9	123.3	160.0	143.8	77.1	56.3	28.2, 26.0	177.0, 178.9	
Adenosine + $[Au(O_2CMe)_2(dmamp)]$	156.2	152.9	123.2	160.0	143.7	76.4, 76.0	56.6 to 54.6	28.5, 25.8	176.7, 178.4	0
Δδ	−0.1	0.0	−0.1	0.0	−0.1	−0.5, −1.1	0.3 to −1.7	+0.3, +0.2	−0.3, −0.5	
Guanosine	157.6	155.3	120.6	160.8	139.6	77.1	56.3	26.0, 28.3	177.0, 178.9	
Guanosine + $[Au(O_2CMe)_2(dmamp)]$	160.2	155.8	121.6	165.1	≈139	77.0	55.5, 55.7	25.3, 28.2	180.5, 176.2	60
Δδ	+2.6	+0.5	+1.0	+4.3	≈−0.5	−0.1	−0.8, −1.0	−0.7, −0.1	+3.5, −2.5	
Inosine	149.8	152.1	128.3	160.5	142.7	77.1	56.3	26.0, 28.3	177.0, 178.9	
Inosine + $[Au(O_2CMe)_2(dmamp)]$	155.8	151.9	≈130.3	163.1	142.3	77.1	55.6, 55.9	25.1, 28.3	176.0, 179.4	60
Δδ	+0.6	−0.2	≈+2.0	+2.6	−0.4	0	−0.2, −0.5	0, −0.9	+2.4, −2.7	

[a] For the mixtures, only the new signals are listed. [b] Acetate signals were assigned on the basis that the free acetate would have constant chemical shifts (δ 28.2, 176.2) and replaced the labile acetate (δ 28.3, 178.9).

Table 8 Antibacterial minimum inhibitory concentrations (μg cm^{-3})*

Organism	*Staphylococcus aureus*	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*
$[Au(O_2CMe)_2(dmamp)]$	0.25–1.0 (0.56–2.2)	0.25–1.0 (0.56–2.2)	2.5–10 (5.6–22.3)	50–100 (111–223)
Ciprofloxacin	< 0.25 (< 0.65)	1.0–2.5 (2.6–6.5)	< 0.25 (< 0.65)	< 0.25 (< 0.65)

* Range between the highest concentration allowing growth and the lowest inhibiting growth.[11] Figures in parentheses are concentrations in μmol dm^{-3}.

Table 9 Comparative toxicity against Chinese hamster ovary cells (% cell survival)[a]

Concentration (μg cm^{-3})[b]	100	10	1	0.1
$[Au(O_2CMe)_2(dmamp)]$	0.4 ± 2	96.1 ± 0.4	97.9 ± 1.9	99.5 ± 0.5
Cisplatin	8.8 ± 0.7	87.9 ± 4.4	97.9 ± 2.9	101.9 ± 2.1
Ciprofloxacin	89.7 ± 1.7	102.8 ± 2.3	102.0 ± 2.8	102.8 ± 3.1

[a] Mean of three tests for each compound and each concentration. [b] Concentrations (μmol dm^{-3}) are: $[Au(O_2CMe)_2(dmamp)]$ 220, 22, 2.2 and 0.22; cisplatin 330, 33, 3.3 and 0.33; ciprofloxacin 260, 26, 2.6 and 0.26.

polarisation transfer) spectrum. It is also curious that only a single signal is seen for these carbon atoms although most of the others are at least duplicated. The C^5 signal is difficult to distinguish from the dmamp signals, but shows a small positive Δδ. These data are best interpreted by co-ordination through N^1 or N^3.

It may be that the normal co-ordination mode, through N^7, is discouraged by steric interaction between the ribose unit and the gold complex, since both nucleosides co-ordinate through groups away from the ribose.

Purines. The mixture containing adenine gave a single set of resonances with different chemical shifts from those of the pure components. Since two pairs of acetate signals are seen monodentate binding is occurring. Large positive co-ordination chemical shifts were seen for C^5 and C^8 (Table 7), suggesting co-ordination by N^7 of the imidazole ring. Similar co-ordination was proposed from ^1H NMR spectra for the $AuMe_2$–adenosine complex.[9] Since the co-ordinated acetate group (see Table 7 for the assignment) shows no sign of exchange with water or the free acetate ion, it presumably occupies the position *trans* to the dmamp-amine group. Any exchange involving the adenine must be relatively slow.

The spectra of mixtures of the gold complex and caffeine gave no evidence for the formation of new complexes in either aqueous or chloroform solution.

Biological studies

Minimum inhibitory concentration data showing the effect of $[Au(O_2CMe)_2(dmamp)]$ on a range of bacteria are given in Table 8. The complex shows broad-spectrum antibacterial activity with some specificity towards the Gram-positive organisms *Staphylococcus aureus* and *Enterococcus faecalis*. In view of this promising selectivity, cytotoxicity was also assessed relative to Chinese hamster ovary cells (Table 9). The gold complex showed cytotoxicity similar to cisplatin, and both complexes are more cytotoxic than ciprofloxacin (a quinolone antibiotic).

The fact that the minimum inhibitory concentration values of $[Au(O_2CMe)_2(dmamp)]$ for the Gram-positive bacteria tested are an order of magnitude lower than the cytotoxic concentrations against the Chinese hamster ovary cells indicates a potentially useful *in vitro* selectivity for these organisms. This is

in marked contrast to $[AuCl_2(dmamp)]$ which had much poorer discrimination.[1] Comparisons with the behaviour of other gold complexes is delayed until the presentation of more detailed and additional biological tests on $[Au(O_2CMe)_2(dmamp)]$ and its analogues.[12]

Conclusion

We had postulated that, as a consequence of its formal resemblance to cisplatin, $[AuCl_2(dmamp)]$ might show analogous biological activity.[1] The gold complex did indeed exhibit some antimicrobial and cytotoxic properties, but full evaluation and exploitation was inhibited by poor aqueous solubility. The diacetate complex, $[Au(O_2CMe)_2(dmamp)]$, has much greater solubility and, like cisplatin, undergoes considerable hydrolysis in aqueous solution. One acetate group is replaced readily and a second less readily. This observation increases the analogy with cisplatin, since the latter is known to hydrolyse within cells, and the binding of platinum(II) to DNA occurs much more readily after this hydrolysis.[13]

The similarity is far from complete, however. The binding of cisplatin to DNA is principally through the N^7 atoms of two adjacent guanine groups, a process which may be aided by hydrogen bonding between the ammine groups and the phosphate residue in DNA.[13] Such hydrogen bonding is clearly impossible for dmamp complexes. Furthermore, the NMR studies of model reactions of $[Au(O_2CMe)_2(dmamp)]$ with biological ligands show a marked preference for S-donor ligands (cysteine, glutathione). The N-donor nucleosides did not give quantitative binding and appeared to bind *via* N^1, N^3 or NH_2 rather than N^7. In addition, the purine caffeine did not bind to $[Au(O_2CMe)_2(dmamp)]$, but there was evidence of quantitative co-ordination, *via* N^7, for adenine.

Thus, although both cisplatin and $[Au(O_2CMe)_2(dmamp)]$ display interesting *in vitro* antitumour activity, and the latter demonstrates promising selectivity for Gram-positive bacteria and with respect to mammalian Chinese hamster ovary cells, the mode of action of these two complexes is almost certainly different.

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References

1 R. V. Parish, B. P. Howe, J. P. Wright, J. Mack, R. G. Pritchard, R. G. Buckley, A. M. Elsome and S. P. Fricker, *Inorg. Chem.*, in the press.
2 J. Vicente, M. T. Chichote and M. D. Bermúdez, *J. Organomet. Chem.*, 1984, **268**, 191.
3 J. A. Washington and A. L. Barry, *Manual of Clinical Microbiology*, eds. E. H. Lennette, E. H. Spaulding and J. P. Traunt, American Society of Microbiology, Washington DC, 2nd edn., 1974, p. 410.
4 S. P. Fricker, *Toxicol. In Vitro*, 1994, **8**, 821.
5 T. T. Puck, J. Cieciura and R. Robinson, *J. Exp. Med.*, 1958, **108**, 945.
6 J. D. Higgins III, L. Neely and S. Fricker, *J. Inorg. Biochem.*, 1993, **49**, 149.
7 J. Sandström, *Dynamic NMR Spectroscopy*, Academic Press, New York, 1982.
8 A. Anvarhusein, A. Isab and P. J. Sadler, *J. Chem. Soc., Dalton Trans.*, 1982, 135.
9 Y. Mizuno and S. Komiya, *Inorg. Chim. Acta*, 1986, **125**, L13.
10 S. K. Miller and L. G. Marzilli, *Inorg. Chem.*, 1985, **24**, 2421.
11 A. M. Elsome, W. Brumfit, J. M. T. Hamilton-Miller, P. D. Savage, R. O. King and S. P. Fricker, *Abstr. 31st Int. Conf. Antimicrobial Agents and Chemotherapy*, American Society of Microbiology, 1991, A387.
12 R. G. Buckley, A. M. Elsome, S. P. Fricker, G. R. Henderson, B. R. C. Theobald, L. Kelland, B. P. Howe and R. V. Parish, unpublished work.
13 E. L. M. Lempers and J. Reedijk, *Adv. Inorg. Chem.*, 1991, **37**, 175.

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