

[AU(DIEN)Cl]Cl₂: EXCHANGE PHENOMENA OBSERVED BY ¹H AND ¹³C NMR SPECTROSCOPY

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The solution behaviour of the square-planar gold(III) complex [Au(dien)Cl]Cl₂ (dien = 1,5-diamino-3-azapentane) was investigated by ¹H and ¹³C NMR spectroscopy. We have found that ¹H NMR spectra of [Au(dien)Cl]Cl₂ are characterised by exchange behaviour over the whole pH range, and some exchange effects are also seen in ¹³C NMR spectra of the deprotonated hydroxo derivative of the complex in alkaline solution. An exchange rate of > 378 s⁻¹ was determined from ¹H NMR spectra at pH* 2 (coalescence temperature 40 °C). In slightly acidic solutions of the complex, ¹H chemical shifts are in accordance with the known deprotonation of the central amine group of the complexed diethylenetriamine ligand. In ¹³C NMR spectra, two separate sets of resonances are observed for the chloro and the hydroxo complex of gold(III) diethylenetriamine. The hydroxo complex [Au(dien-H)OH]⁺ shows exchange effects in ¹³C NMR spectra. Variable temperature studies show the carbon atoms next to the central secondary amine to be inequivalent and each present in two different environments that are in intermediate to fast exchange on the NMR time-scale.

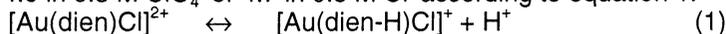
INTRODUCTION

Gold(III) has recently received much attention because of its possible involvement in the biological action/side-effects of anti-arthritis gold(I) drugs^{0,1}. It has been shown that gold(III) is a reactive metabolite of gold(I) in mice² and humans³. The oxidation of gold(I) to gold(III) has been demonstrated *in vitro* and *in vivo*^{4,5,6}.

The known gold (III) chelate complex [Au(dien)Cl]Cl₂ is of special interest because of its potential as a probe for gold(III) binding sites on biological molecules, *e.g.* nucleotides⁷ and peptides⁸.

Chlorodiethylenetriaminegold(III) chloride was first prepared in 1963⁹ and the crystal structure was solved in 1986¹⁰. In the solid state, the complex is pseudooctahedral with the nitrogens of the diethylenetriamine ligand occupying three of the equatorial coordination sites around gold(III) and one chlorine atom occupying the fourth coordination site in the plane. Two additional chlorine atoms are situated in axial positions with considerably longer Au-Cl bond lengths¹¹.

In aqueous solution, the complex [Au(dien)]³⁺ has been shown to take part in both acid-base and hydrolytic equilibria¹⁰. The complex loses a proton from one of the nitrogen ligands with a pK_a value¹⁰ of 4.0 in 0.5 M ClO₄⁻ or 4.7 in 0.5 M Cl⁻ according to equation 1.



It has been confirmed that deprotonation occurs from the central, secondary nitrogen in a single-crystal X-ray diffraction study in which both the crystal structures of [Au(dien)Cl]Cl[ClO₄] and [Au(dien-H)Cl][ClO₄] were solved¹¹.

In neutral to basic solution, the chloride at the fourth coordination site is replaced by hydroxide¹⁰ (equation 2).



We have used [Au(dien)Cl]Cl₂ in NMR studies of the interaction of gold(III) with peptides⁹. The complex has not yet been investigated in detail by NMR spectroscopy and therefore we have studied both ¹H NMR and ¹³C NMR spectra of aqueous solutions of the complex.

MATERIALS AND METHODS

Preparation of [Au(dien)Cl]Cl₂

The complex was prepared by the method of Baddley et al.¹⁰ (Found: C, 12.04; H, 3.03; N, 10.23. Calc. for C₄H₁₃AuCl₃N₃: C, 11.82; H, 3.22; N, 10.34%).

NMR Spectroscopy

For ¹³C NMR studies, a 50 mM solution of [Au(dien)Cl]Cl₂ in D₂O was prepared with sodium 3-(trimethylsilyl)tetradeuteriopropionate (TSP-d₄) as internal reference. The pH of the solution was adjusted using NaOD and DNO₃ solutions. Measurements of pH were made using an Aldrich micro combination electrode and Corning 240 meter, calibrated with Aldrich buffer solutions. Readings of the pH meter for D₂O solutions were not corrected for deuterium isotope effects and are designated as pH* values. Broad-band proton decoupled ¹³C-{¹H} NMR spectra were accumulated overnight at 67.8 MHz on a JEOL GSX270 spectrometer and typically processed using an exponential function equivalent to a line-broadening of 5 Hz. The solution was kept frozen between experiments.

For ¹H NMR studies, 10 mM solutions of [Au(dien)Cl]Cl₂ in D₂O with dioxane as internal reference (δ 3.768 relative to TSP-d₄ at 295 K) were used and spectra accumulated at 270 MHz. Spectra were typically processed using an exponential function equivalent to a line-broadening of 0.4 Hz. Typical pulsing conditions were: spectral width 3.2 KHz, pulse width 45°, relaxation delay 2.2 s, 16 K datapoints, 128 transients. The pH titration curves were fitted to a modified form of the Hill equation¹² using the program KALEIDAGRAPH¹³ on an Apple Macintosh computer.

A 100 mM solution of [Au(dien)Cl]Cl₂ in 0.6 ml D₂O was used for the 2D [¹H, ¹³C] HSQC NMR experiment. The pH of the solution was adjusted to 1.5 using 1 M DCl. The 2D [¹H, ¹³C] HSQC NMR spectrum was recorded at 298 K using the sequence of Stonehouse et al.¹⁴ on a Varian INOVA 600 spectrometer (¹H 600 MHz, ¹³C 150.8 MHz). The ¹³C-spins were decoupled by irradiation with the GARP-1 sequence (optimized for ¹J(C, H) = 140 Hz) during acquisition. Water suppression was achieved by pulsed-field gradients. Typically, 128 scans were acquired for each of 64 increments of *t*₁ and the final resolution was 4 Hz/point for the F2 dimension and 8 Hz/point for the F1 dimension.

RESULTS**¹³C NMR Studies**

Figure 1 shows the numbering system used for the carbon atoms in the complex [Au(dien)Cl]²⁺.

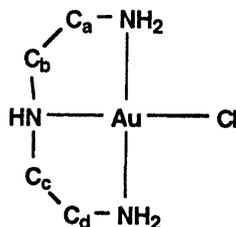


Figure 1: Numbering system for the carbon atoms in [Au(dien)Cl]²⁺

Proton-decoupled ¹³C NMR spectra of [Au(dien)Cl]Cl₂ at selected pH* values are shown in Figure 2 and a plot of chemical shift values of the various ¹³C resonances versus pH* is shown in Figure 3. Up to pH* 5.2, only two ¹³C signals were observed indicating that carbon atoms C_a and C_d plus C_b and C_c are equivalent. The more downfield of the two resonances (peak 1) was assigned to the equivalent carbon atoms C_b and C_c next to the secondary amine. Peak 2 was assigned to the equivalent carbon atoms C_a and C_d next to the primary amine groups. This was based on the previous assignment of resonances for the palladium(II)-diethylenetriamine complex on the basis of chemical shift data¹⁵ for CH₃NHCH₂CH₂NHCH₃ and NH₂CH₂CH₂NH₂ and is in agreement with some previously reported carbon-13 data for the complex¹⁶. Both the resonances were pH dependent with peak 1 showing a downfield shift of 4.9 ppm and peak 2 an upfield shift of 1.5 ppm upon pH increase from pH* 3.0 to pH* 6.5 (Figures 2 and 3). The shifts were fitted to a pK_a value of 4.74 ± 0.04 and attributed to the deprotonation of the central secondary amine group as suggested by Baddley et al.¹⁰ and confirmed by X-ray crystallographic studies¹².

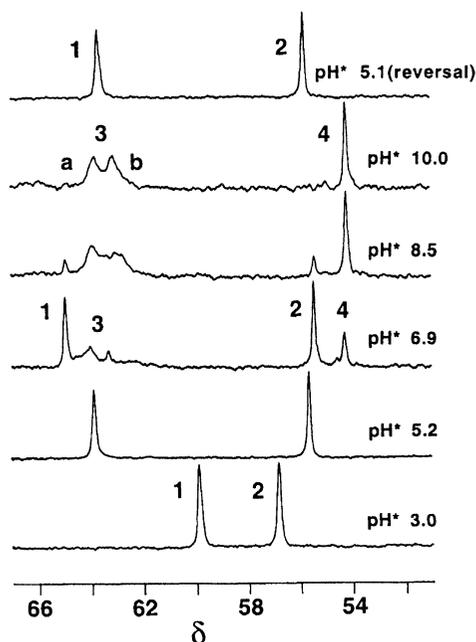


Figure 2: 67.8 MHz $^{13}\text{C}\{-^1\text{H}\}$ NMR spectra of 50 mM $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O at various pH^* values, the spectrum of a sample after reversal from $\text{pH}^* 10.0$ to $\text{pH}^* 5.1$ is also shown

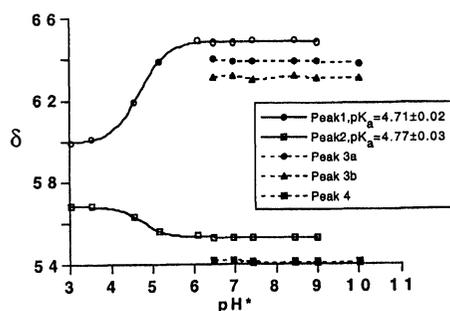


Figure 3: ^{13}C chemical shift values versus pH^* for $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O ; the curves for peaks 1 and 2 are computer best-fits

In spectra recorded at $\text{pH}^* 6.1$ and higher, new resonances (two broad peaks 3a, 3b and one sharp peak 4) appeared and increased in intensity with increasing pH until they were the only resonances observed at $\text{pH}^* 10.0$. From previous studies¹⁰, the complex would be expected to be fully hydrolysed by $\text{pH} 10$, so that the new peaks appearing at $\text{pH}^* \geq 6.1$ were assigned to the deprotonated hydroxo derivative $[\text{Au}(\text{dien-H})\text{OH}]^+$. From their chemical shift values, the downfield (broad) resonances (peaks 3a and 3b) were again assigned to the carbon atoms C_b and C_c next to the secondary, central amine, while the upfield, sharp peak (peak 4) was assigned to the carbon atoms C_a and C_d next to the primary amines. The changes in the spectra of $[\text{Au}(\text{dien})\text{Cl}]^{2+}$ were reversible as lowering the pH from $\text{pH}^* 10.0$ to $\text{pH}^* 5.1$ yielded the same spectrum as before the pH increase.

Increasing the temperature of a solution of $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O , $\text{pH}^* 9.9$ from $22\text{ }^\circ\text{C}$ to $60\text{ }^\circ\text{C}$ led to a clear sharpening of peaks 3a and 3b in $^{13}\text{C}\{-^1\text{H}\}$ NMR spectra (Figure 4). Temperatures higher than $60\text{ }^\circ\text{C}$ could not be employed due to the instability of the complex.

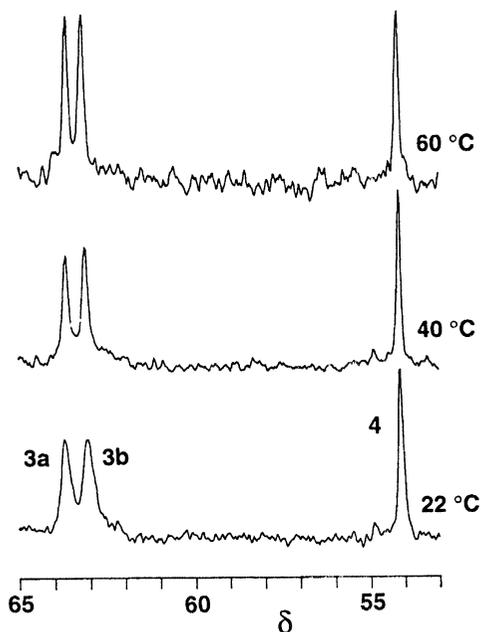


Figure 4: 67.8 MHz $^{13}\text{C}\{-^1\text{H}\}$ NMR spectra of 50 mM $[\text{Au}(\text{dien-H})(\text{OH})]^+$ in D_2O , pH^* 9.9, at various temperatures

^1H NMR Studies

^1H NMR spectra of $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O were characterized by exchange processes over the whole pH range (Figure 5). In the pH^* range 3.0 to 5.9, only two broad peaks were seen which showed pH-dependent shifts. Peak 1 ($\Delta\delta = 1.09$ ppm in the pH^* range 3.0 to 7.0) was broad over the whole investigated pH range. Peak 2 ($\Delta\delta = 0.27$ ppm in the pH^* range 3.0 to 7.0) was broad at low pH values but turned into a relatively sharp triplet in spectra of pH^* 4 and above. The pH-dependent shifts of those peaks were again attributed to the deprotonation of the central secondary amine group. Peak 1 was assigned to the methylene protons of C_b and C_c because of its large pH-dependent shift upon deprotonation of the central amine; peak 2 with a smaller shift difference was assigned to the protons attached to C_a and C_d .

An increase of the temperature from 22 °C to 60 °C of a solution of $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O at pH^* 3.2 resulted in the appearance of two triplets in place of the very broad resonances seen at this pH^* value at 22 °C (Figure 6). Upon lowering the pH^* to 2.0 at 22 °C, both peak 1 and peak 2 split into two broad resonances (peaks 1a, 1b and 2a, 2b respectively, Figure 7). Upon temperature increase, those peaks broadened and coalesced (Figure 7) with coalescence temperatures of ca. 30 °C for peak 2 and ca. 40 °C for peak 1. The calculated exchange rates at these temperatures from the chemical shift difference at 22 °C were $> 118 \text{ s}^{-1}$ for peak 2 and $> 378 \text{ s}^{-1}$ for peak 1.

At pH^* values of 7.0 and above, additional ^1H NMR resonances were observed. Contrary to the ^{13}C spectra, where a complete set of new peaks appeared for the hydroxo species at higher pH values, the difference between chloro and hydroxo species was not as clear in ^1H NMR spectra. Peak 1 was observable as a broad resonance up to pH^* 12.1 and must therefore represent protons in the chloro- as well as the hydroxo species. Two very broad resonances (peaks 3a and 3b) appeared on either side of peak 1 in spectra between pH^* 7.0 and 10.9 (Figure 5). New resonances also appeared around peak 2 at pH^* values of 7.9 and higher (peak 4). They were not of equal intensity and were difficult to follow.

Variable temperature experiments of a solution at pH^* 9.9 (Figure 8) showed peak 1 sharpening to form a still broad triplet at higher temperatures, while the very broad peaks 3a and 3b broadened and coalesced at ca. 40 °C. The exchange rate at this temperature was $> 443 \text{ s}^{-1}$ from the chemical shift difference at 22 °C. At the same pH^* value, the resonances for the methylene protons of C_a and C_d (peaks 2 and 4) broadened upon temperature increase.

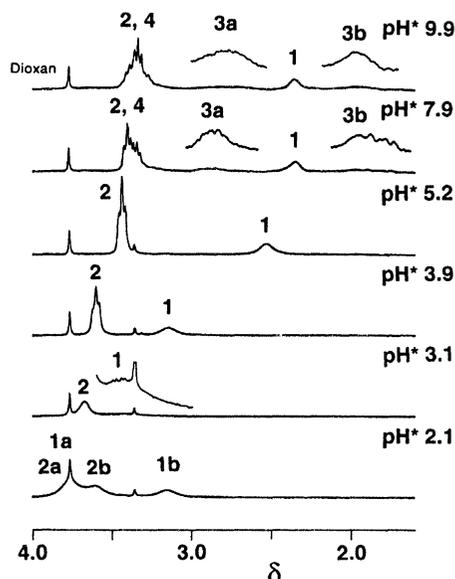


Figure 5: 270 MHz ^1H NMR spectra of 10 mM $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O at various pH^* values

$[\text{H},^{13}\text{C}]$ NMR Spectroscopy

The 2D HSQC NMR spectrum of $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ at pH^* 1.5 showed four cross-peaks at 3.90/58.30, 3.64/58.30, 3.89/61.05 and 3.20/61.05 ppm (Figure 9). The former two cross-peaks can be assigned to the two $-\text{CH}_2-\text{NH}_2$ groups (C_a and C_d) and the latter two to the $-\text{NHCH}_2$ (C_b and C_c) groups of the dien ligand. Only two sets of carbon resonances were observed for the dien ligand at pH^* 1.5. It is notable that the two protons of NHCH_2 had a ^1H chemical shift difference of *ca.* 0.7 ppm.

DISCUSSION

The pK_a value of 4.74 ± 0.04 (in D_2O , 22 °C, $I \rightarrow 0$) determined in the present study for the deprotonation of the central, secondary amine group is in a similar range to the one determined by Baddley et al.¹⁰ ($\text{pK}_a = 4.0$ in H_2O , 25 °C, $I = 0.5$ with ClO_4^-). The difference between the two values is likely to be due to the different conditions, *i.e.* a combination of different ionic strength and deuterium isotope effect. The largest ^{13}C shifts are seen on the carbon atoms C_b and C_c next to the deprotonating group. This was also found in the complex $[\text{Pt}(\text{dien})(\text{Me}_2\text{SO})]^{2+}$, in which the central amine group deprotonates with a pK_a value¹⁷ of 11.94. The two-dimensional $[\text{H},^{13}\text{C}]$ HSQC NMR experiment confirms the assignment of the respective ^1H and ^{13}C resonances in the one-dimensional spectra.

Exchange processes in the hydroxo species $[\text{Au}(\text{dien-H})(\text{OH})]^+$ account for the broad appearance of peaks 3a and 3b in the ^{13}C spectra (Figure 2). Upon increase in temperature from 22 °C to 60 °C (Figure 4), the two broad peaks become sharper. The sharpening of the resonances suggests that the two resonances are not exchanging with each other, otherwise signals would be expected to become broader at higher temperatures and eventually coalesce to a single peak. In contrast to this, an increasing sharpening of the peaks is observed that can only be explained by assuming that each of the two broad peaks is already an average of two resonances in intermediate to fast exchange on the NMR time-scale. The carbon atoms next to the terminal amines, C_a and C_d stay equivalent under the conditions used. This behaviour suggests that the carbon atoms C_b and C_c next to the central amine in $[\text{Au}(\text{dien-H})]^{2+}$ (peaks 3a and 3b) are inequivalent in the hydroxo species and that each of the inequivalent carbon atoms C_b and C_c is present in two different environments that are in intermediate to fast exchange on the NMR time-scale. The different environments may be due to different conformations of the five-membered chelate rings which exist in two enantiomeric forms (λ and δ) with the interconversion between these structures proceeding via an envelope conformation¹⁸.

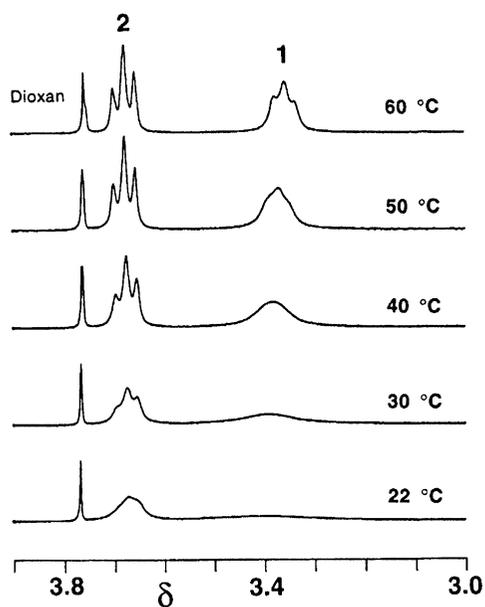


Figure 6: 270 MHz ¹H NMR spectra of 10 mM [Au(dien)Cl]Cl₂ in D₂O, pH* 3.2, at various temperatures

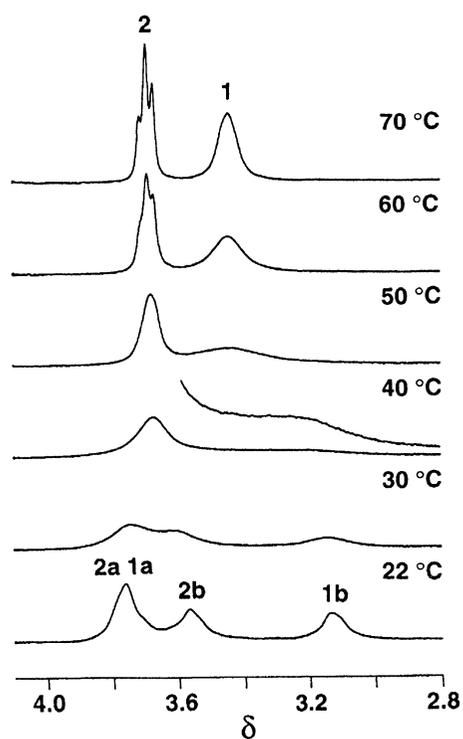


Figure 7: 270 MHz ¹H NMR spectra of 10 mM [Au(dien)Cl]Cl₂ in D₂O, pH* 2.0, at various temperatures

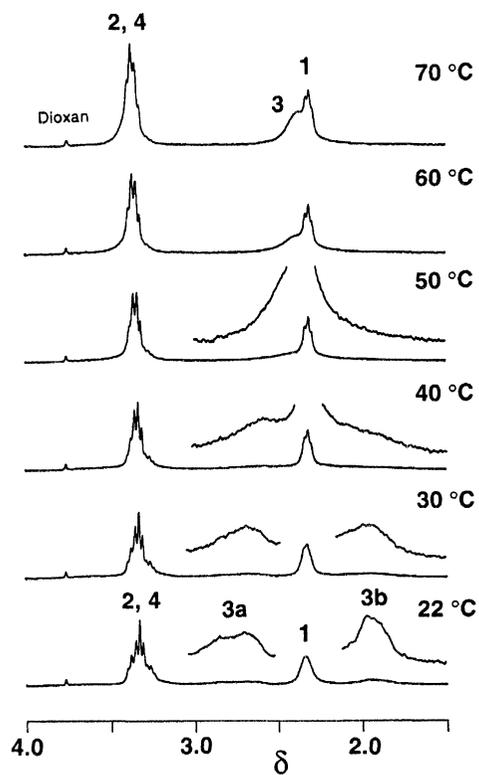


Figure 8: 270 MHz ^1H NMR spectra of 10 mM $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O , $\text{pH}^* 9.9$, at various temperatures

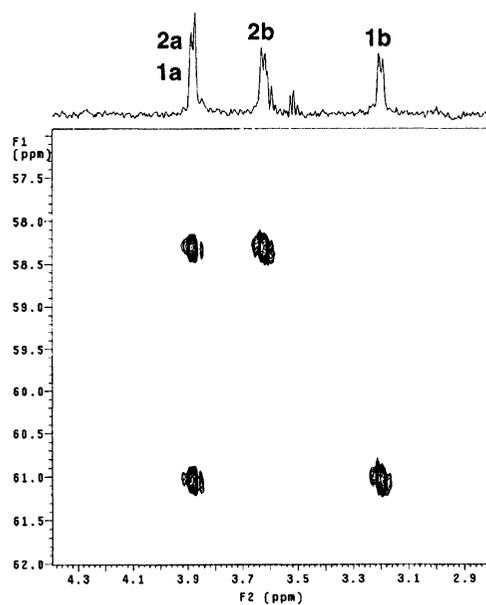


Figure 9: 2D $[^1\text{H}, ^{13}\text{C}]$ HSQC NMR spectrum of 100 mM $[\text{Au}(\text{dien})\text{Cl}]\text{Cl}_2$ in D_2O , $\text{pH}^* 1.5$

However, the interconversion is usually believed to be too rapid to be observed¹⁹. Various explanations are possible for the inequivalence of C_b and C_c in the hydroxo-species. It might be due to the presence of a hydroxo-bridged dimer. The crystal structure of dimethylgold(III) hydroxide, a hydroxo-bridged tetrameric gold(III) complex, has been reported²⁰ and hydroxo-bridged complexes are well known for palladium(II) and platinum(II) complexes. Restricted rotation in a possible dimer could account for the inequivalence of the carbon atoms. Another possibility is hydrogen-bonding with axial hydroxide substituents around gold(III) which could render the molecule less symmetrical. It must be noted that the carbon atoms are equivalent in the chloro species [Au(dien-H)Cl]⁺. In this complex, the axial substituents are chloride ions and they are thought to be replaced by hydroxide upon increasing the pH of the solution¹⁰.

Proton NMR spectra of [Au(dien)Cl]Cl₂ show exchange-broadening over the whole pH range, *i.e.* both chloro and hydroxo complexes are affected. The broadening at low and at higher pH is likely to be caused by different mechanisms. At low pH (pH* 2.0, Figure 7), broadening could be explained most easily by a ring-opening and -closing equilibrium involving protonation of one of the terminal NH₂ groups. An equilibrium between unprotonated ring-closed and protonated ring-opened species of [Au(dien)Cl]²⁺ has been suggested to occur in acidic solution on the basis of kinetic studies²¹. Ring opening at one end of the molecule in [Au(dien)Cl]Cl₂ would render the methylene groups of the two five-membered chelate rings inequivalent and broadening would occur if the exchange between ring-opened and ring-closed species was at an intermediate rate on the NMR time-scale. As the H⁺ concentration decreases, ring-opening would be suppressed, consistent with the sharpening of the resonances for the methylene protons of C_a and C_d at pH* > 3 (peak 2 in Figure 5). However, the observation of only two carbon resonances at pH* 1.5 suggests that no ring-opening reaction occurs at that pH* value. The inequivalence of the ¹H resonances in strongly acidic solution must therefore be due to a different mechanism, possibly again an interconversion of different conformations of the five-membered chelate rings.

The exchange rates calculated from ¹H NMR temperature studies at pH* 2 and 10 are estimates because of the broadness of the resonances at the lowest temperatures studied. The maximum chemical shift difference of the exchanging protons in the two environments might be larger and the exchange rates are therefore lower limits.

Additional resonances in ¹H NMR spectra of [Au(dien)Cl]Cl₂ (peaks 3a, 3b and 4, Figure 5) occur above pH* 7 in solutions of the complex alone, but not in spectra of a mixture of [Au(III)(Gly-Gly-L-His-H₂)]⁺ and the complex, in which the major species between pH* 6 and 12 is the bridged imidazole complex⁹. Therefore, those additional resonances are a characteristic of the hydroxo complex, possibly due to a hydroxo-bridged complex, rather than a feature of the diethylenetriamine ligand.

We have shown in this work that the seemingly simple complex of gold(III) with diethylenetriamine exhibits an extremely complex solution behaviour. More work is certainly needed to unravel the basis of the various exchange processes observed in [Au(dien)]³⁺.

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