Inorganic Chemistry Communications 14 (2011) 534–538

Contents lists available at ScienceDirect

Inorganic Chemistry Communications

journal homepage: www.elsevier.com/locate/inoche

A gold(III) complex and a tetrachloroaurate salt of the neuroepileptic drug gabapentin

Ahmed Shaikjee, Demetrius C. Levendis *, Helder M. Marques, Richard Mampa

Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, PO WITS, 2050, Johannesburg, South Africa

ARTICLE INFO

Article history:
Received 5 November 2010
Accepted 18 January 2011
Available online 25 January 2011

Keywords:
Gabapentin
Gold-complex
Neuroepileptic drug

ABSTRACT

Gabapentin (2-[1-(aminomethyl)cyclohexyl] acetic acid, Gp), a neuroepileptic drug, has been the subject of renewed interest in the past decade. In order to exploit the therapeutic potential of Gp several metal complexes of Gp have been investigated. In this paper we report on the preparation of a novel, unusually stable Au(III)–gabapentin complex, $[Au(Gp^0)Cl_3]$, with a Au(III)—N bond, and a second product, a gabapentin–$[AuCl_4]^-$ salt, both characterized by single-crystal X-ray diffraction and 2-dimensional ^{15}N–^1H NMR techniques. The crystal structure of $[Au(Gp^0)Cl_3]$ clearly shows the Au—N coordination (Au—N bond 2.043(2) Å) in the hydrogen bonded dimer. In the crystal, the cyclohexyl moiety is disordered over two positions, both having a chair conformation. In solution, the ^1H NMR of $[Au(Gp^0)Cl_3]$ in MeOD has resonances at 2.45, 2.51, 3.05 and 3.12 ppm, that suggest that $[Au(Gp^0)Cl_3]$ exists as two different isomers in solution, with the $—CH_2\text{-}NH_2\text{-}AuCl_3$ either axial or equatorial, and that on crystallization these isomers persist in the solid state.

The role of γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter [1] stimulated research into the synthesis of GABA analogs as potential central nervous system agents. One of these analogs is gabapentin [1-(aminomethyl) cyclohexane acetic acid, Gp] which is commercially available as Neurontin®. Gp is used as a neuroepileptic drug in the treatment of epilepsy [2] and more recently, neuropathic pain [3].

As active pharmaceutical ingredients (APIs) are frequently delivered to patients as capsules or tablets, the solid-state properties of the drug are important. Gp [4] can exist as different polymorphs [5,6], solvates under ambient conditions, including a novel hydrate [4], as a heptahydrate under high pressure [7], salts [8,9], co-crystals [10] or amorphous solids. Crystal growth and nucleation mechanisms that drive solid-state processes are still not well understood, as seen in the emergence [11] or disappearance [12] of new polymorphic forms as crystallization conditions are varied [11].

The first preparation and structural characterization of the Cu(II) and Zn(II) complexes of Gp were reported by Braga et al. [13] who used mechanochemical methods with the $ZnCl_2$ and $CuCl_2$ salts. In the complexes, both Zn(II) and Cu(II) are pseudo-tetrahedral and coordinate through the carboxylate oxygen of two gabapentin molecules, with two Cl^- ions occupying the other two sites. Zinc and copper were noted as being important metals in neurology. This motivated us to investigate other potentially beneficial metal complexes of gabapentin, particularly those metals that are thought to exhibit therapeutic potential [14,15].

Since Au(III) and Pt(II) are isoelectronic and isostructural there is considerable interest in the potential anti-tumor activity of Au(III) complexes. However, because of their poor kinetic and redox stabilities under physiological conditions, their use is problematic [16,17]. Coordination to polydentate ligands (en, dien, cyclam, terpy and phen) does improve matters [18], and these complexes (with Cl^- occupying the other coordination sites) do display significant anti-tumor activity against both cisplatin-sensitive and resistant human ovarian cell line A2780; however, cytotoxicity is also significant.

Square planar Au(III) complexes with di- and tripeptides are known, with coordination through some or all of N_δ of His, the N- and C-terminal amino and carboxylate groups and deprotonated amide N atoms [19–21,24]. Au(III) also coordinates to amino acids. Au(III) forms a bis–histidine complex with coordination through the amino group and a histidine N_δ [27]. It causes the deamination and subsequent decarboxylation of glycine with the formation of glyoxylic acid, NH_4^+, formic acid and CO_2, accompanied by the reduction of Au (III) to Au(0) [22]. This may be the reason why colloidal gold is often seen in the reactions of Au(III) with peptides [22]. Amino and carboxylate functionalities in alfalfa biomass reduce Au(III) to Au(0) through a Au(I) intermediate [23]. Amines, including Lys, complex with gold nanoparticles; the capping stabilizes the particles in solution and renders them water-dispersible [25]. Tyr, Arg and Gly-Tyr have been used as reductants of $[AuBr_4]^-$ to produce water-soluble dispersions of gold nanoparticles [26].

* Corresponding author. Tel./fax: +27 11 7176742/7176749.
E-mail address: demetrius.levendis@wits.ac.za (D.C. Levendis).

1387-7003/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.inoche.2011.01.017

Thus, Au(III) has a fairly rich biological chemistry: it is redox-active; it can be coordinated by amino acids (especially Cys and Met) and proteins; it is able to deprotonate and bind to the amide N of peptides; and it is capable of cross-linking histidine imidazole rings [28].

Given the medical importance of GABA and related neurotransmitters on the one hand, and coordination complexes of Au(III) on the other, we have initiated a study into the complexes of Au(III) with a number of natural and synthetic neurotransmitters and their analogs. We report here the crystal structure of a Au(III) complex of Gp in which Au(III) is coordinated by Gp's amino group, and use NMR to indicate that this structure is likely to persist in solution. We report also the structure of the salt GpH[$AuCl_4$]. (See note [29(b)] for an explanation of the abbreviations used.)

The reaction of Gp with auric acid [29] leads to two distinct crystalline products, pale yellow needles (**I**) and orange blocks (**II**), which we have characterized crystallographically [30]. [Au(Gp^0)Cl_3] (**I**) was found to be a gold–Gp^0 complex involving an Au(III)—N bond in which the amine group has been deprotonated (Scheme 1(a)). GpH[$AuCl_4$] (**II**) is the salt (Scheme 1(b)).

[Au(Gp^0)Cl_3] (**I**) has two molecules in the asymmetric unit. One of the [Au(Gp^0)Cl_3] molecules is shown in Scheme 1(a). The two molecules in the asymmetric unit are hydrogen bonded *via* the carboxylic acid. The two [Au(Gp^0)Cl_3] molecules are almost identical but are not related by crystallographic symmetry. In one of the molecules there is an intramolecular hydrogen bond between N1 and O1 (2.903 Å) resulting in small conformational differences between the two molecules. The cyclohexyl of each of the Gp^0 molecules is disordered over two possible chair conformations with the methylamine group axial in one and equatorial in the other. This disorder can be correlated to the solution ^{1}H NMR spectra as discussed below. Diagrams showing the cyclohexane conformations in each of the disordered forms are given in Figs. 1 and 2 for the two molecules in the asymmetric unit.

The relative occupancies of each of the disordered positions of the cyclohexane chair for this molecule, axial or equatorial, are 0.67(1) and 0.33(1), respectively, as found by least squares refinement against the crystallographic data. The cyclohexyl in the second molecule in the asymmetric unit is similarly disordered over two positions with site occupancies of 0.70(1) and 0.30(1) for the axial and equatorial geometries, respectively.

The observation of two possible chair conformations in crystals of [Au(Gp^0)Cl_3] suggests that these conformations may not be significantly different in energy, resulting in an appreciable population of

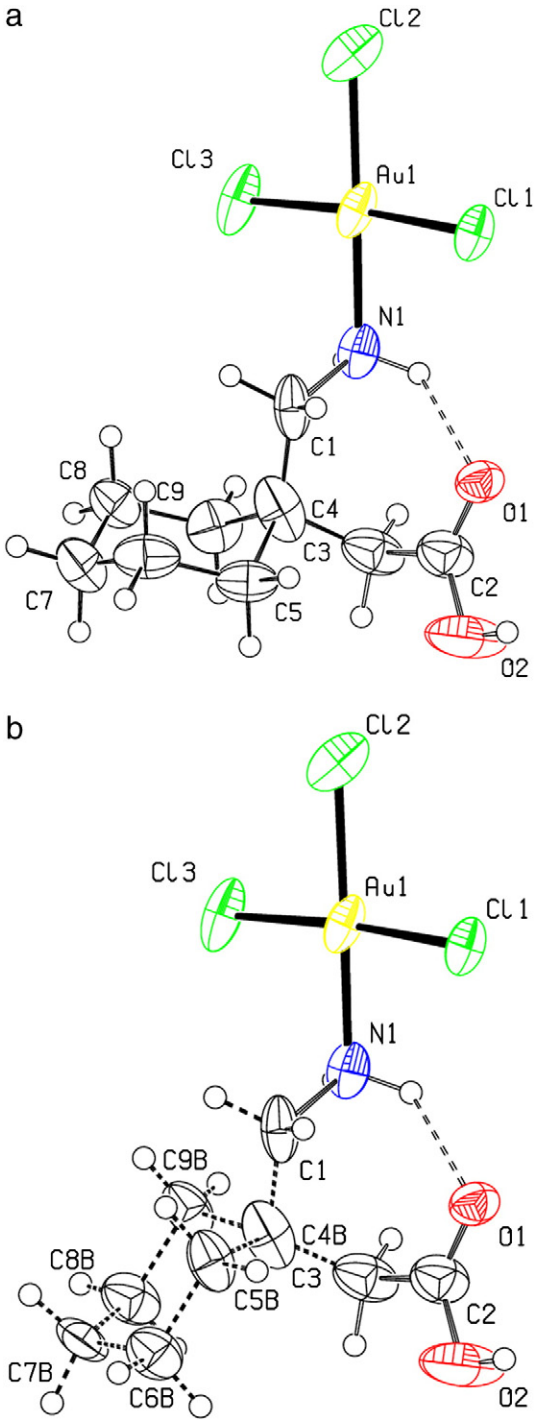
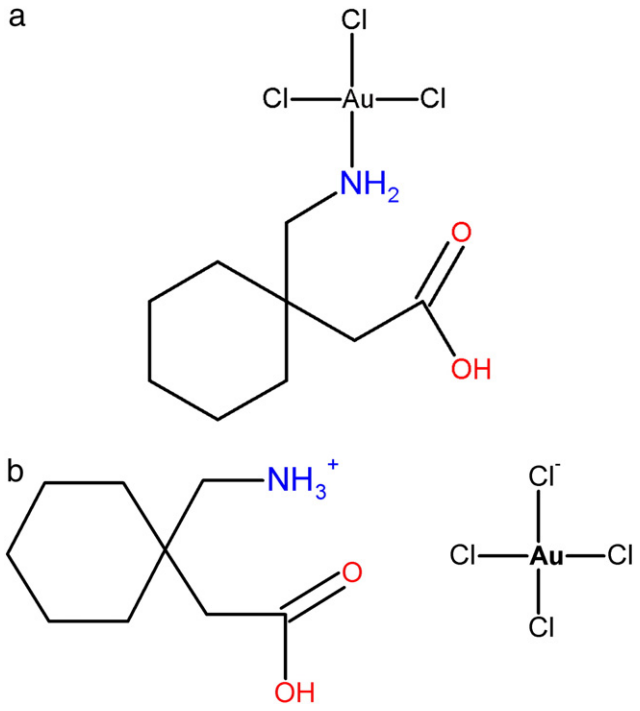


Fig. 1. (a) Molecular structure of the axial conformation of the —CH_2-NH_2-$AuCl_3$ moiety in [Au(Gp^0)Cl_3] showing the atom labeling scheme with 50% probability displacement ellipsoids. Dashed lines indicate N—H···O intramolecular hydrogen bonding interactions. (b) Molecular structure of the equatorial conformation of the —CH_2-NH_2-$AuCl_3$ moiety in [Au(Gp^0)Cl_3] showing the atom labeling scheme with 50% probability displacement ellipsoids. Open dashed lines indicate N—H···O intramolecular hydrogen bonding interactions. Solid dashed lines are covalent bonds indicating the alternative (equatorial) position of the Gp^0 cyclohexane.



Scheme 1. Schematic representation of (a) **I** [Au(Gp^0)Cl_3], the gold–gabapentin Au–N complex and (b) **II** GpH[$AuCl_4$] the gold–Gp salt.

both conformers in solution. One of these pairs is shown in Fig. 3. Crystallization may thus be kinetically controlled by nucleation events; moreover, packing forces may determine the conformation selected in the crystals. It is reasonable to assume that in the case of $[Au(Gp^0)Cl_3]$ both conformations of the cyclohexyl group exist in solution at room temperature, but they do not rapidly interconvert, possibly due to the intra-molecular $—NH_2–O=C^-$ and $NH_2–Cl$ hydrogen bonds. These two conformational forms of $[Au(Gp^0)Cl_3]$ are retained in the resulting disordered crystal, a consequence of the rapid precipitation of $[Au(Gp^0)Cl_3]$ due to the presence of the ethanol in the solvent. The persistence of the two conformations in a methanol solution was investigated further using ^1H and ^{15}N NMR as described later.

The orange salt, $GpH[AuCl_4]$, is shown in Fig. 4. The geometry of the methylamine group is equatorial with no apparent disorder. The geometry of the GpH cation in $GpH[AuCl_4]$ is similar to that observed in the GpH chloride salt [8,9]. The $[AuCl_4]^-$ ion has the typical square planar geometry.

In $GpH[AuCl_4]$ pairs of GpH cations are hydrogen bonded to each other *via* the protonated carboxylate groups. The negatively charged $[AuCl_4]^-$ ions occur in chains separating the GpH dimeric pairs (Fig. 5). This type of packing arrangement is similar to that found in several organic–inorganic hybrid materials in which layers of the organic moiety are separated by layers of inorganic anions.

To determine whether the $[Au(Gp^0)Cl_3]$ complex is retained in solution the CD_3OD ^1H and ^{15}N NMR spectra of Gp, the Gp–gold complex $[Au(Gp^0)Cl_3]$ and the gold salt, $GpH[AuCl_4]$ were recorded [31]. At room temperature the two cyclohexyl conformers of pure Gp inter-convert rapidly on the NMR timescale and only one signal is observed for each of the aminomethyl (2.880 δ) and carboxymethyl (2.448 δ) protons. The methylene signals associated with $—CH_2\text{-}NH_3^+$ and $CH_2\text{-}COOH$ were unequivocally assigned by using 2-dimensional ^1H $\{^{15}N\}$ NMR. These are in agreement with previous observations made by Ananda et al. [8], who also found that two distinct resonances are observed for each of the methylene groups in Gp on cooling to −80 °C. They attribute this observation to an equilibrium between the axial and equatorial conformations of the methylamine groups in solution at low temperature.

The only significant differences apparent in the Gp, $[Au(Gp^0)Cl_3]$ and $GpH[AuCl_4]$ NMR spectra relate to the methylene groups, and so only these peaks are discussed further (see Fig. 6 and Supplementary material).

$[Au(Gp^0)Cl_3]$ in solution shows four sharp singlets. In view of the nature of the disordered crystals obtained from this solution and the observations made by Ananda et al. for pure Gp [8], it would be reasonable to assume that two of these peaks (at 2.513 δ and 3.048 δ) correspond to the chair conformation of cyclohexyl with the methylamine group in the equatorial position, and the other two peaks (at 2.473 δ and 3.199 δ) correspond to the chair conformation of cyclohexane with the methylamine group in the axial position. The peaks at 2.513 δ and 3.048 δ integrate for 1.27 protons each. The peaks at 2.473 δ and 3.199 δ integrate for 0.67 protons each. The peak

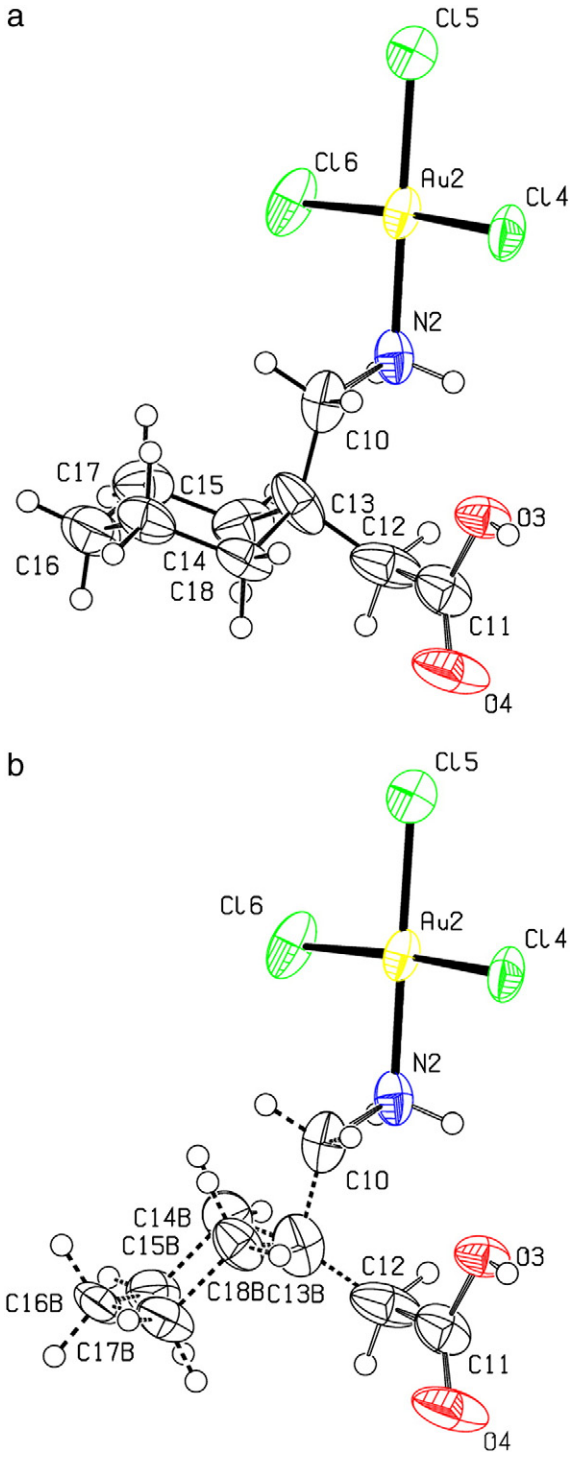


Fig. 2. (a) Molecular structure of the equatorial conformation of the second molecule in the asymmetric unit in $[Au(Gp^0)Cl_3]$ showing the atom labeling scheme with 50% probability displacement ellipsoids. (b) Molecular structure of the axial conformation of the second molecule in the asymmetric unit in $[Au(Gp^0)Cl_3]$ showing the atom labeling scheme with 50% probability displacement ellipsoids. Solid dashed lines are covalent bonds indicating the alternative (axial) position of the Gp^0 cyclohexane.

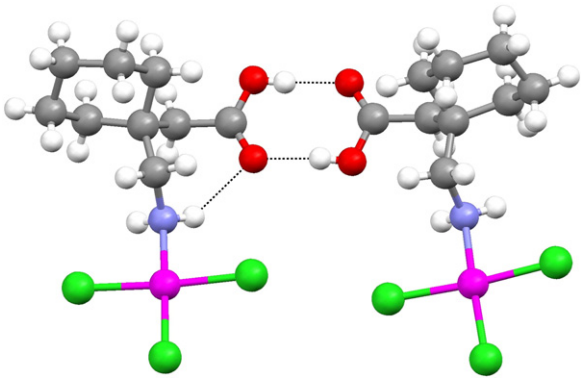


Fig. 3. The two molecules of gold(III) gabapentin complex $[Au(Gp^0)Cl_3]$ in the asymmetric unit with only one of the disordered cyclohexane rings shown. Dotted lines indicate N—H···O intramolecular or O—H···O intermolecular hydrogen bonding interactions and also indicate N···H·O and C···H.O intermolecular hydrogen bonds which form R_2^2 [8] graph-set motifs.

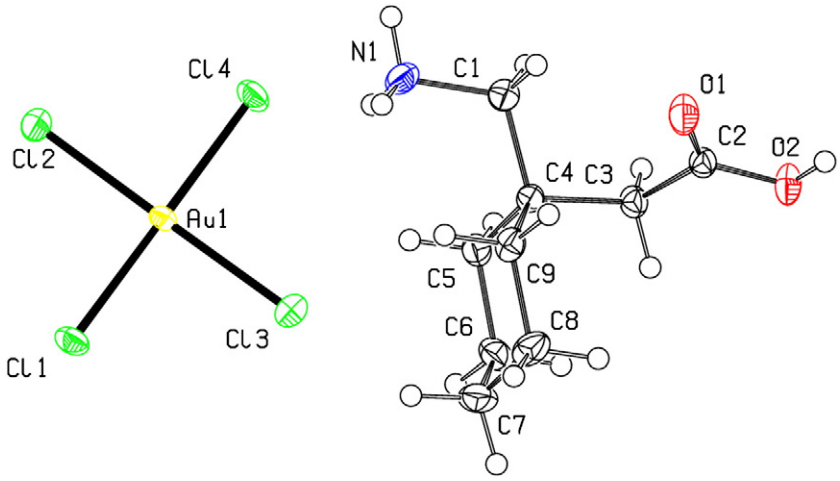


Fig. 4. Molecular structure of GpH[$AuCl_4$] showing the atom labeling scheme with 50% probability displacement ellipsoids.

integration is in reasonable agreement with the site occupancies of the two conformers observed crystallographically. We tentatively conclude that both of the chair conformations are present in solution at 293 K, stabilized at room temperature by the intramolecular hydrogen bonds. However, we cannot rule out the possibility that some of the [Au(Gp^0)Cl_3] complex dissociates to form the GpH[$AuCl_4$] salt on dissolving in methanol since the ^{1}H NMR spectrum of the salt peaks (2.513 δ and 3.055 δ) are close to those of the central peaks of the [Au(Gp^0)Cl_3] spectrum. The deduction that both axial and equatorial conformations exist in solution, together with our observation that under certain conditions [Au(Gp^0)Cl_3] is reduced to metallic gold and as yet unidentified products, is under investigation and will be reported elsewhere.

This work has shown, for the first time, the formation of a stable complex between Au(III) and Gp in the solid state, in which the Au(III) is coordinated to the amino nitrogen. This complex persists in solution. Thus an intermediate in the oxidation of gabapentin by Au (III) has been trapped as a result of rapid crystallization. The [Au(Gp^0)Cl_3] complex could have important biological and pharmaceutical significance.

Acknowledgements

The University of the Witwatersrand, Johannesburg, and the National Research Foundation, Pretoria, is acknowledged for funding; Dr Manuel Fernandes is also acknowledged for X-ray data collection and advice on the disorder refinements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.inoche.2011.01.017.

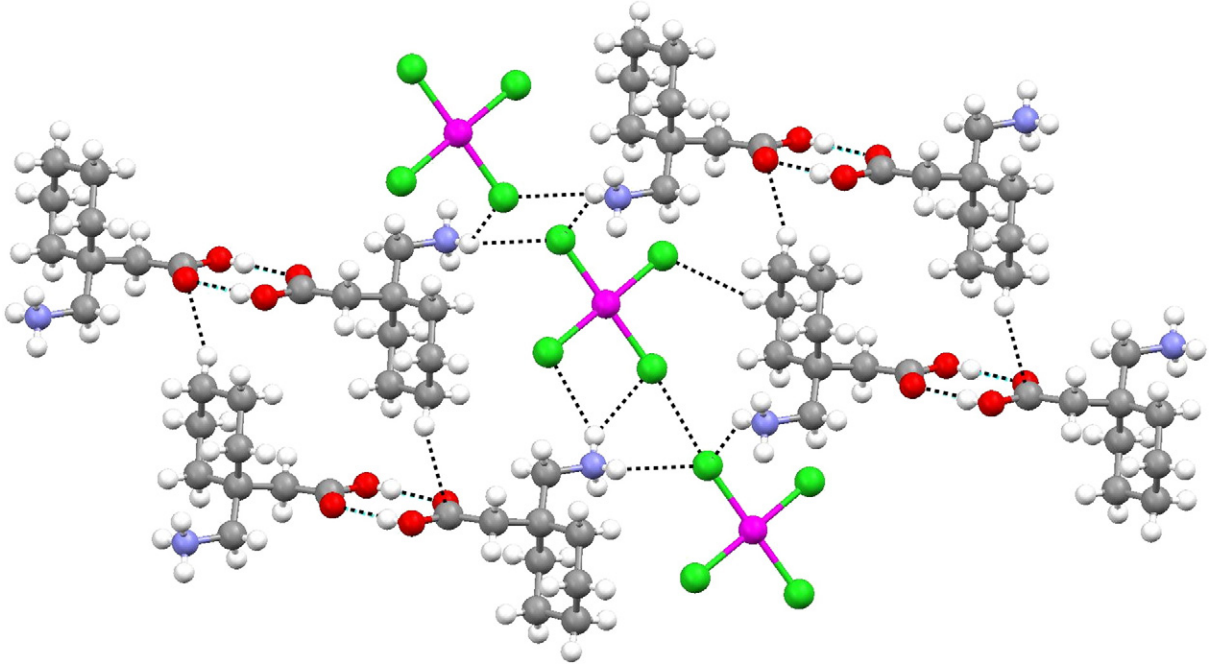


Fig. 5. Packing diagram of the GpH[$AuCl_4$] salt showing layers of hydrogen bonded GpH molecules separated by chains of Cl···Cl interacting $AuCl_4^-$ ions. Dotted lines indicate N—H···Cl, O—H···O or C—H···O intermolecular interactions and also indicate N···H·O and C···H·O intermolecular hydrogen bonds which form R_2^2 [8] graph-set motifs.

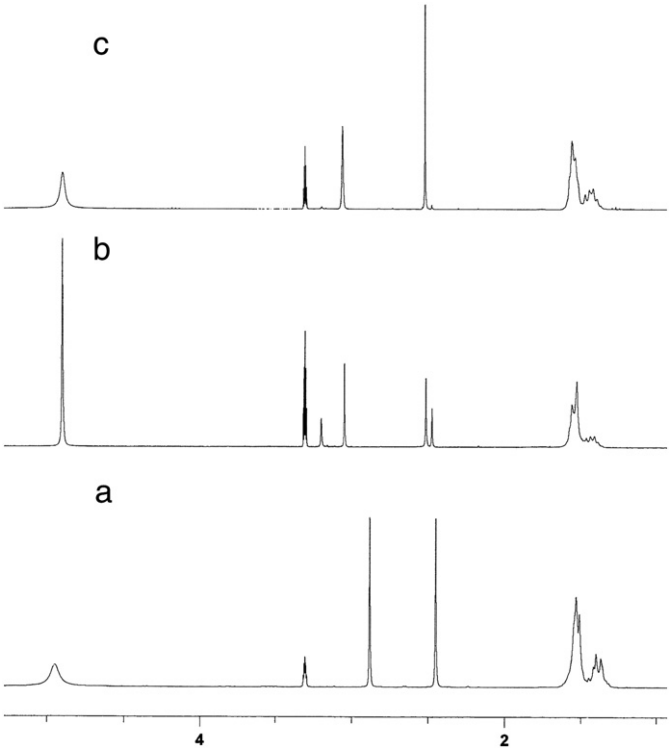


Fig. 6. The ^{1}H NMR of (a) Gp, (b) the [Au(Gp^0)Cl$_3$] complex (c) the GpH[AuCl$_4$] salt in CD$_3$OD.

References

[1] N.G. Bowery, Annu. Rev. Pharmacol. Toxicol. 33 (1993) 109.
[2] C.P. Taylor, in: D.W. Chadwick (Ed.), New Trends in Epilepsy Management: the Role of Gabapentin, 198, Royal Society of Medicine Services Ltd, London, 1993, pp. 13–40.
[3] L. Magnus, Nonepileptic uses of gabapentin, Epilepsia 40 (1999) S66–S72.
[4] (a) J. Ibers, Acta Crystallogr. Sect. C: Cryst. Struct. Commun. 57 (2001) 641–643; (b) P.G. Vasudev, S. Aravinda, K. Ananda, S.D. Veena, K. Nagarajan, N. Shamala, P. Balaram, Chem. Biol. Drug Des. 73 (2009) 83–96.
[5] D. Braga, F. Grepioni, L. Maini, K. Rubini, M. Polito, R. Brescello, L. Cotarca, M.T. Duarte, V. Andre, M.F.M. Piedade, New J. Chem. 32 (2008) 1788–1795.
[6] H.A. Reece, D.C. Levendis, Acta crystallogr, Sect. C: Cryst. Struct. Commun. 64 (2008) O105–O108.
[7] F.P.A. Fabbiani, D.C. Levendis, G. Buth, W.F. Kuhs, N. Shankland, H. Sowa, Cryst. Eng. Commun. 12 (2010) 2354–2360.
[8] K. Ananda, S. Aravinda, P.G. Vasudev, K.M.P. Raja, H. Sivaramakrishnan, K. Nagarajan, N. Shamala, P. Balaram, Curr. Sci. 85 (2003) 1002–1011.
[9] J.P. Jasinski, R.J. Butcher, H.S. Yathirajan, L. Mallesha, K.N. Mohana, B. Narayana, J. Chem. Crystallogr. 39 (2009) 777–780.
[10] (a) M. Wenger, J. Bernstein, Cryst. Growth Des. 8 (2008) 1595–1598; (b) L.S. Reddy, S.J. Bethune, J.W. Kampf, N. Rodríguez-Hornedo, Cryst. Growth Des. 9 (2009) 378–385.
[11] G.M. Day, A.V. Trask, W.D.S. Motherwell, W. Jones, Chem. Commun. (2006) 54–56.
[12] J.D. Dunitz, J. Bernstein, Acc. Res. 28 (1995) 193–200.
[13] D. Braga, F. Grepioni, L. Maini, R. Brescello, L. Cotarca, Cryst. Eng. Commun. 10 (2008) 469–471.
[14] R. Bau, A. Schreiber, T. Metzenthin, R.S. Lu, F. Lutz, W.T. Klooster, T.F. Koetzle, H. Seim, H.P. Kleber, F. Brewer, S. Englard, J. Am, Chem. Soc. 119 (1997) 12055–12060.
[15] I. Ott, Coord. Chem. Rev. 253 (2009) 1670–1681.
[16] R.V. Parish, B.P. Howe, J.P. Wright, J. Mack, R.G. Pritchard, R.G. Buckley, A.M. Elsome, S.P. Fricker, Inorg. Chem. 35 (1996) 1659–1666.
[17] P. Calamai, A. Guerri, L. Messori, P. Orioli, G.P. Speroni, Inorg. Chim. Acta 285 (1999) 309–312.
[18] L. Messori, F. Abbate, G. Marcon, P. Orioli, M. Fontani, E. Mini, T. Mazzei, S. Carotti, T. O'Connell, P. Zanello, J. Med. Chem. 43 (2000) 3541–3548.
[19] M. Wienken, B. Lippert, E. Zangrando, L. Randaccio, Inorg. Chem. 31 (1992) 1983–1985.
[20] S.L. Best, T.K. Chattopadhyay, M.I. Djuran, R.A. Palmer, P.J. Sadler, I. Sovago, K. Varnagy, J. Chem. Soc. Dalton Trans. (1997) 2587–2596.
[21] T. Kolev, B.B. Koleva, S.Y. Zareva, M. Spiteller, Inorg. Chim. Acta 359 (2006) 4367–4376.
[22] (a) J. Zou, Z.J. Guo, J.A. Parkinson, Y. Chen, P.J. Sadler, Chem. Commun. (1999) 1359–1360; (b) P.K. Sen, N. Gani, J.K. Midya, B. Pal, Transit. Met. Chem. 33 (2008) 229–236.
[23] J.L. Gardea-Torresdey, K.J. Tiemann, J.G. Parsons, G. Gamez, I. Herrera, M. Jose-Yacaman, Microchem. J. 71 (2002) 193–204.
[24] B.B. Ivanova, J. Coord. Chem. 58 (2005) 587–593.
[25] P.R. Selvakannan, S. Mandal, S. Phadtare, R. Pasricha, M. Sastry, Langmuir 19 (2003) 3545–3549.
[26] S.K. Bhargava, J.M. Booth, S. Agrawal, P. Coloe, G. Kar, Langmuir 21 (2005) 5949–5956.
[27] J.A. Cuadrado, W.X. Zhang, W. Hang, V. Majidi, J. Environ. Monit. 2 (2000) 355–359.
[28] S.L. Best, P.J. Sadler, Gold Bull. 29 (1996) 87–93.
[29] (a) Suitable crystals for X-ray diffraction were obtained by the dissolution of equimolar amounts of Gp (0.1711 g gabapentin, 1.0 mmol) and auric acid (0.3938 g of AuCl$_3$.6H$_2$O, 1.0 mmol) in a mixture of 1:1 distilled water (5.0 ml) and ethanol (5.0 ml) in a sample vial (40mmx10mm diameter) at 293 K. The pale yellow crystals appear within 1–2 days and can be filtered off. On allowing the solution to stand longer, for up to two weeks, the orange form crystallizes concomitantly with the pale yellow crystals. These were separated by hand under an optical microscope.(b) A note on the abbreviations used: by Gb we mean the common zwitterionic form; by Gb^0 we mean the neutral form; by GbH we mean the cationic form. Therefore the abbreviation [Au(Gb^0)Cl$_3$] refers to the Au(III) complex with the neutral Gp^0 (as shown in Scheme 1(a)) and GpH[AuCl$_4$] refers to the salt as shown in Scheme 1.(b).
[30] CCDC 799398 & 799399 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Crystal data for I. CCDC deposition number 799398. Empirical formula $C_9H_{17}AuCl_3NO_2$, $M_r = 474.55$, monoclinic space group $P2_1/n$, $a = 14.8729(3)$, $b = 7.0060(1)$, $c = 26.4622(5)$ Å, $\beta = 93.355(1)°$, $V = 2752.62(9)$ Å^3, $T = 173(2)$ K, $Z = 8$, $D_c = 2.290$ Mg/m^3, μ (Mo K_α) $= 11.257$ mm^{-1}, $F(000) = 1792$, 42829 reflections measured, 5978 unique [R(int) = 0.083] which were used in all calculations. Refinement converged as a final $R_1 = 0.0376$ ($wR_2 = 0.0896$) for 5978 observed reflections [$I > 2\sigma(I)$]. Crystal data for II. CCDC deposition number 799399. $C_9H_{18}AuCl_4NO_2$, $M_r = 511.01$, triclinic space group P-1, $a = 6.6890(2)$, $b = 7.6819(2)$, $c = 14.4134(3)$ Å, $\alpha = 79.526(2)°$, $\beta = 89.864(1)°$, $\gamma = 81.248(2)°$, $V = 719.56(3)$ Å^3, $T = 173(2)$ K, $Z = 2$, $D_c = 2.359$ Mg/m^3, μ (Mo K_α) $= 10.954$ mm^{-1}, $F(000) = 484$, 15456 reflections measured, 3458 [R(int) = 0.0512], which were used in all calculations. Refinement converged as a final $R_1 = 0.0208$ ($wR_2 = 0.0540$) for 3458 observed reflections [$I > 2\sigma(I)$]. Crystal data were measured on a Bruker APEXII CCD diffractometer using the ω scan mode with Mo K_α radiation, $\lambda = 0.71073$ Å, and corrected for absorption using the face indexed integration method. The structures were solved by direct methods and refined anisotropically on F^2 (G.M. Sheldrick, Acta Crystallogr. A 64 (2008) 112). Details of the crystallographic data for [Au(Gp^0)Cl$_3$] and GpH[AuCl$_4$] are supplied in Tables 1(a–b) and 2(a–b) in Appendix 1.
[31] The 1H- and 2-dimensional 1H-^{15}N solution NMR spectra were recorded using a BRUKER Avance 300 (1H, 300.13 MHz) and a BRUKER AMX 400 MHz spectrometer (operating at 400.13 MHz (1H) and 40.55 MHz (^{15}N)) respectively. Spectra were recorded in methanol (CD3OD) solution and are referenced with respect to internal tetramethylsilane. 1H-NMR(CD3OD), δ, ppm): (i) Gabapentin: 4.94 (s, 1H), 2.88 (s,1H), 2.45 (s, 1H), 1.41 (m, cyclohexyl-H); (ii) [Au(Gp^0)Cl$_3$] complex: 4.90 (s, 1H), 3.20 (s, 0.69H), 3.05 (s, 1.26H), 2.51 (s, 1.27H), 2.47 (s, 0.67H),1.47 (m, cyclohexyl-H); (iii) GpH[AuCl4] salt: 4.90 (s, 1H), 3.06 (s, 1H), 2.51 (s, 1H), 1.44 (m, cyclohexyl-H). Two-dimensional ^{15}N–^{1}H spectra were obtained using the pulse sequence of Bax, Griffey and Hawkins [J. Magn. Reson., 55 (1983) 310.]. Natural abundant nitrogen-15 spectra were recorded with the delay time based on $^1J(^{15}N–^1H) = 10.5$ Hz.