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CONFORMATIONAL FLEXIBILITY AND ITS POTENTIAL INFLUENCE ON METABOLIC REACTIONS FOR THE ANTI-TUMOUR COMPOUND *a*-AMMINE-*bd*-BIS(BUTYRATO)-*cf*-DICHLORO-*e*-CYCLOHEXYLAMINE PLATINUM(IV)

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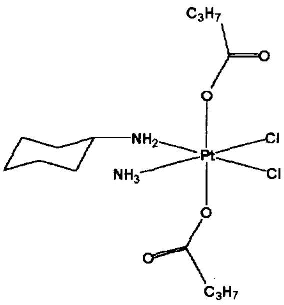
Abstract

The crystal structure of the experimental anti-tumour compound *a*-ammine-*bd*-bis(butyrato)-*cf*-dichloro-*e*-cyclohexylamine platinum(IV) is reported. There are four independent molecules in the crystallographic asymmetric unit, which show two distinct conformations about the carboxylate linkage to the platinum atom. Implications for metabolic activation are discussed and the potential for base-catalysed solvolysis of platinum(IV) amine complexes is demonstrated.



The title compound ("JM221") is one of the more active in a series of platinum(IV) ammine/amine dicarboxylates that have been developed as third-generation orally-active anti-cancer agents, showing selective cytotoxicity to cis-platinum-resistant human ovarian tumour cell lines and anti-tumour activity to these tumours in rodents[1-4]. The metabolism of JM221 is expected to involve both substitution and reduction pathways, as has been observed for the related acetate complex JM216[5]. Substitution in platinum(IV) complexes is very slow in the absence of catalysis so the identification of substitution products as metabolites of these complexes suggests that some metabolism does occur, possibly involving base attack on the amine ligands[6] activating the complex to substitution of the chloride ligands. Due to the lack of reactivity between platinum(IV) complexes and DNA it is believed that reduction to platinum(II) precedes the formation of the active lesion[7]. Reduction of JM221 results in loss of the carboxylate groups[8] and the more reactive platinum(II) species can then bind to DNA, forming intrastrand and interstrand cross-links with guanosine residues. The asymmetry of the mixed amine complex can lead to two orientational isomers of the platinum(II)-DNA adduct[9], which could have implications for the anti-tumour activity of JM221.

We have undertaken a crystallographic analysis of JM221 in order to examine structural features which may influence its reactivity and hence provide further understanding of its metabolic behaviour. We show here that JM221 itself shows conformational isomerism, with two distinct conformers present in the crystal structure. The crystal structure of the related compound JM216 has recently been reported[10].

Methods

Synthesis

a-ammine-*bd*-bis(butyrato)-*cf*-dichloro-*e*-cyclohexylamine platinum(IV) (JM221), *cis,trans,cis*-[$PtCl_2(O_2CC_3H_7)_2NH_3(c\text{-}C_6H_{11}NH_2)$]. This was prepared by reaction of *cis,trans,cis*-[$PtCl_2(OH)_2NH_3(c\text{-}C_6H_{11}NH_2)$] with butyric anhydride as previously described[8,11].

af-bis(butyrato)-*b*-ammine-*d*-chloro-*c*-cyclohexylamine-*e*-methoxo platinum(IV) (**2**), *cis,trans,cis* [$PtCl(OMe)(O_2CC_3H_7)_2NH_3(c\text{-}C_6H_{11}NH_2)$].

To a solution of sodium methoxide in methanol (0.1M, 36ml) was added JM221 (2g, 3.6mmol). The solution was stirred at ambient temperature for 3 days and then filtered. The filtrate was evaporated to dryness under reduced pressure and extracted with ethanol. The extract was evaporated and the extraction repeated. Evaporation of the second extract gave an oil which crystallised on standing. The product was collected by filtration and washed with ethanol and diethyl ether. Yield 1.0g (50%). IR 539, 531 (Pt-O), 353 (Pt-Cl) cm^{-1}. ^{1}H NMR ($CDCl_3$) Pt-OCH_3 δ 2.92, ^3J(Pt-H) = 18 Hz. Anal. $C_{15}H_{33}N_2ClO_5Pt$ requires C 32.63, H 6.03, N 5.08, Cl 6.44%; found C 32.69, H 5.82, N 4.88, Cl 6.64%.

Crystallography

Pale-yellow crystals of JM221 were obtained as very thin plates by recrystallisation from aqueous ethanol. These rapidly decayed at room temperature unless kept under solvent. Cell dimensions are: a =37.49(4), b = 8.42(2), c = 27.04(3) Å, with the space group being $Pna2_1$. This size of unit cell corresponds to four independent molecules of JM221 in the asymmetric unit. Intensity data were collected for a single crystal cooled to -120°C, to a maximum θ = 55° using a Rigaku AFC5R diffractometer and a rotating-anode generator with Cu-K_α graphite-monochromated radiation. A total of 5471 unique reflections were measured, of which 4879 had significant intensity ($I \geq 2.0\sigma I$). There was 3.5% decay during the data collection, which was corrected for by a linear decay function. The structure was solved by direct methods[12] to locate the four platinum atoms followed by several rounds of electron-density map calculations to locate the 94 other atoms (including solvent). It was refined by full-matrix least-squares techniques[13] to a final R factor of 0.0807 for the 4879 significant reflections. This relatively high R factor is most probably due to the high thermal motion of the terminal carbon atoms on the butyl side-chains, as well as crystal decay during data collection. Attempts to refine in the alternative centrosymmetric space group Pnma (with two molecules in the asymmetric unit) were unsuccessful, with the lowest R factor achieved being 0.13. Examination of the structure showed that the terminal atoms of the butyl side-chains were not symmetrically-related, therby breaking the symmetry required in the Pnma space group assignment. The positions of hydrogen atoms were generated by geometric considerations and their contributions were included in the final structure-factor calculations. An analytical absorption correction was applied to the data. Coordinates and derived geometric parameters have been deposited at the Cambridge Crystallographic Data Centre.

Results and discussion

The structure analysis has shown that the crystallographic asymmetric unit of JM221 contains four independent molecules of JM221 together with two molecules of ethanol solvent. The four molecules exist as two pseudo-enantiomorphically equivalent pairs, 1 and 3, and 2 and 4; exact equivalence between the two members of a pair is not achieved (which would result in a higher-symmetry space group) since one has slightly differing alkyl chain conformations compared to the other, thus breaking exact inversion symmetry. (Refinement in the alternative centrosymmetric space group Pnma, which would have just two molecules in the asymmetric unit, was unsuccessful). All four molecules in the present structure have the same overall structure of square planar chlorine and nitrogen ligands, with the carboxylato groups being axial. The cyclohexane groups all adopt a chair conformation. Each has an asymmetric orientation with respect to the two axial groups, so that one is more sterically hindered, and consequently may be significantly less susceptible to metabolic attack. An identical arrangement has been observed in the crystal structure of JM216[6].

Each molecule within a pair differs from the other primarily due to differences in orientation of the "upper", less sterically hindered of the two axial carboxylate substituents (Figure 2 and Table 1). Molecules 1 and 3 have this group oriented so that the carbonyl oxygen atom O3 is oriented away from nitrogen N1. Both O3 and O4 form an intramolecular hydrogen bond with the amine nitrogen N2. By contrast, molecules 2 and 4 have this upper carboxylate group rotated by ca 75° about the Pt-O1 bond, so that now carbonyl O3 is oriented mid-way between the two nitrogen atoms N1 and N2, with both N ... O distances in standard hydrogen-bonding ranges. Thus, molecules 2 and 4 (and JM216) each have three intramolecular hydrogen bonds compared to the two in molecules 1 and 3.

There has been little work to date on the mechanisms of the metabolism of platinum(IV) complexes. Substitution reactions of platinum(IV) complexes have been largely ignored and though base-catalysed hydrolysis has been extensively studied for cobalt(III) amine complexes there have been few reports of corresponding studies of platinum(IV) complexes[6]. The dissociative conjugate-base mechanism for these reactions involves initial proton removal from an amine ligand generating a substitutionally labile amido species.

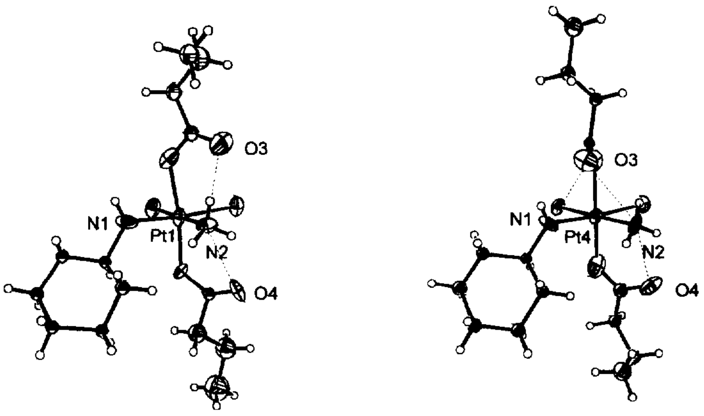


Fig 1. ORTEP[14] plots of molecules 1 and 4 in the asymmetric unit of JM221. Hydrogen bonds are shown as dotted lines.

We have found that platinum(IV) complexes such as JM221 can readily undergo this type of reaction. Using methoxide as base the chloride ligands can be readily replaced by solvent. In methanol this leads to formation of a methoxo complex **2** which has been characterised by IR and ^{1}H NMR spectroscopy (see Methods above) and crystallographic analysis[15]. Substitution of the chloride ligand *trans* to cyclohexylamine occurs in preference to that *trans* to ammine.

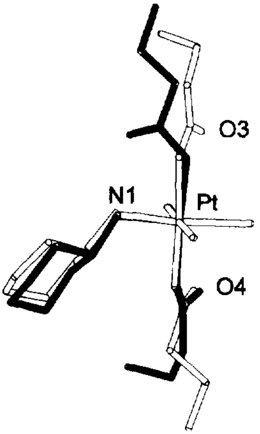


Fig 2. Superposition of molecules 1 and 4 (with filled-in bonds), showing the differences in orientation of the upper butyrato group

Table 1. *Selected structural features of JM221, compared to those of JM 216*

	JM221 molecules	1	2	3	4	JM216
Torsion angles: (in °)	N1-Pt-O1-C7	121.3	-50.9	-116.2	45.1	-52.3
	N1-Pt-O2-C11	-120.9	119.6	129.4	-118.1	125.9
Distances: (in Å)	N1 ... O3	4.16	2.87	4.31	2.85	2.86
	N2 ... O3	2.70	2.94	2.76	2.94	2.84
	N2 ... O4	2.85	2.67	2.88	2.76	2.78

The multiple hydrogen bonding observed in the crystal structure, in particular the additional strong hydrogen bond seen in molecules 2 and 4 between the axial ligand and the ammine nitrogen, could be influential in stabilising the ammine protons with respect to removal by base, slowing metabolism by substitution reactions and also providing stabilisation to metabolic reduction to more reactive platinum(II) species.

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