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Conformational flexibility within the chelate rings of [Pt(en)(CBDCA-*O*,*O′*)], an analogue of the antitumour drug carboplatin: X-ray crystallographic and solid-state NMR studies

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The X-ray crystal structure of [Pt(en)(CBDCA-*O*,*O′*)] **1**, an analogue of the anticancer drug carboplatin, shows that platinum has an approximate square-planar coordination. The crystals are orthorhombic with space group *Pnma*. The Pt-CBDCA chelate ring adopts a flattened-boat conformation, similar to that found for carboplatin, and the Pt-ethylenediamine chelate ring exhibits both δ and λ conformations with equal populations. Ethylenediamine chelate ring inversion was observed by ^{13}C CP/MAS NMR spectroscopy. The exchange rate and the activation free energy ($\Delta G^{\ddagger}$) at 317 K were determined to be *ca.* 415 s^{-1} and 62 kJ mol^{-1}, respectively. The CBDCA ligand appears to have a direct effect on the dynamics of the en ring. Conformational flexibility of the CBDCA ring is also discussed. Such dynamic processes within chelated platinum complexes could play a role in the biological recognition of anticancer complexes.

Several side effects of cisplatin, such as nephro- and neurotoxicity, have led to the search for the second generation of platinum drugs that circumvent these problems. Such efforts have led to the discovery of carboplatin.[1] The replacement of the chloride ligand by chelated cyclobutane dicarboxylate maintains the high antitumour activity but greatly lowers the toxicity. There are a large number of clinical trials in progress on analogues that contain chelated diamine ligands and/or chelated dicarboxylates.[2] These may recognize biological targets such as DNA by a different mechanism from cisplatin. In particular, chelated complexes may not hydrolyse before reacting with DNA bases,[3] and initial second-sphere interactions may therefore direct the complex to selective regions of DNA. Outer-sphere recognition probably also plays an important role in dictating the target site of cisplatin.[4] It is notable that Natile and coworkers have elegantly illustrated that stereospecific substitution in diamino chelate rings can have a major influence on nucleotide recognition at the *trans* positions.[5] Conformational flexibility within the coordination sphere of platinum complexes is therefore likely to be an important feature in drug design.

In this work we have studied dynamic processes within the bis-chelated complex [Pt(en)(CBDCA)] **1** by X-ray crystallography and solid-state NMR spectroscopy, and compared its behaviour with that of carboplatin,[6,7] and some related Pt^{II} and Pd^{II} complexes.

Experimental

Cyclobutane-1,1′-dicarboxylic acid (H_2CBDCA) was purchased from Sigma, K_2PtCl_4, $LiOH \cdot H_2O$ and $AgNO_3$ from Johnson Matthey, ethylenediamine (en) and other chemicals from Aldrich. [Pt(en)Cl_2], [Pt(en)I_2], [Pd(en)$_2$][PdCl_4] and [Pt(CBDCA)(NH_3)$_2$] (carboplatin) were prepared according to literature methods.[8,9]

[Pt(en)(CBDCA)] **1** was prepared as follows. A suspension of [Pt(en)Cl_2] (127.8 mg, 0.39 mmol) and 2 mol equiv of $AgNO_3$ in H_2O (2 mL) was stirred for 24 h. The AgCl was filtered off. To the filtrate, H_2CBDCA (56.5 mg, 0.39 mmol) and 2 mol equiv of $LiOH \cdot H_2O$ were added (the pH of the solution was *ca.* 6.4). Crystals of **1** were obtained by slow evaporation of the above solution and used for the X-ray diffraction and solid-state NMR studies. Elemental analysis of **1**: Calcd (%) for $C_8H_{14}N_2O_4Pt$: C 24.19; H 3.55; N 7.05. Found: C 24.28; H 3.44; N 7.07.

X-Ray crystallography

The X-ray crystallographic analysis showed that crystals of complex **1** were orthorhombic with space group *Pnma*. Crystal data and refinement data are listed in Table 1.

Intensity data were measured at room temperature on a Nonius CAD4 diffractometer in the ω/2θ scanning mode,

Table 1 Crystal data and structure refinement for [Pt(en)(CBDCA-*O*,*O′*)]

Empirical formula	$C_8H_{14}N_2O_4Pt$
Formula weight	397.30
Temperature	291 ± 2 K
Wavelength	1.54178 Å
Crystal system	Orthorhombic
Space group	*Pnma*
Unit cell dimensions	a = 8.660 (3) Å α = 90°
	b = 9.614 (3) Å β = 90°
	c = 12.713 (5) Å γ = 90°
Volume	1058.4 (6) $Å^3$
Z	4
Density (calculated)	2.493 g cm^{-3}
Absorption coefficient	24.825 mm^{-1}
F(000)	744
Crystal size	0.2 × 0.3 × 0.2 mm
θ range for data collection	5.77° to 71.87°
Index ranges	$0 \leqslant h \leqslant 10, 0 \leqslant k < 11, -15 \leqslant l \leqslant 15$
Reflections collected	2661
Independent reflections	1103 [R(int) = 0.1194]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1054/4/78
Goodness-of-fit on F^2	1.130
Final *R* indices [$I > 2\sigma(I)$]	$R1 = 0.0614$, $Wr = 0.1691$
R indices (all data)	$R1 = 0.0771$, $Wr2 = 0.2225$
Extinction coefficient	0.002 (1)
Largest diff. peak and hole	4.451 and −4.808 e $Å^{-3}$

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employing graphite monochromated CuKα radiation. Two asymmetric units of data were recorded to 72° θ. The crystals were weakly diffracting and suffered from statistical disorder, factors which affected the quality of the X-ray data measurements. The structure was solved using the heavy atom method, employing the program SHELXS-86.[10] In addition to the usual Lorentz and polarization corrections, absorption corrections were calculated using the DIFABS method.[11] The absorption corrections ranged from 0.678 to 1.832 with an average value of 0.964. The structure was refined by full-matrix least-squares on $|F^2|$ with SHELXL-93.[12] Anisotropic displacement parameters were used for the non-H atoms and isotropic ones for the H atoms in the geometrically fixed, riding mode. Refinement of the structure in the related space group $Pn2_1a$, was tried and proved to be unsatisfactory.

Solid-state NMR

^{13}C solid-state NMR spectra were recorded on a Bruker MSL-300 NMR spectrometer at 75 MHz, using cross polarization, proton decoupling and magic-angle spinning (CP/MAS). The sample was inserted into a 7 mm diameter sample rotor. Spinning speeds of 4.5 to 5.0 kHz were employed, and a 1 ms contact time was used. Acquisition times were 30–40 ms and the recycle time between scans was 3 to 8 s. Typically the 90° pulse length for ^{1}H was 4.5 ms and usually 2000 to 8000 scans were acquired. The chemical shifts are externally referenced to liquid TMS (0.0 ppm).

Solution NMR

Solution ^{13}C{^{1}H} NMR spectra were acquired on a JEOL GSX-270 NMR spectrometer at 67.5 MHz, using 16 K data points, relaxation delay of 2 s, and 4000 to 8000 transients. Tubes of 5 and 10 mm diameter were used. The chemical shifts are referenced internally to dioxane (67.3 ppm).

Results and Discussion

Crystal structure of [Pt(en)(CBDCA)] 1

The X-ray crystal structure of 1 is shown in Fig. 1(*a*) and (*b*). The atomic coordinates are listed in Table 2 and selected bond distances and angles of the molecule are shown in Table 3. As expected, the platinum atom is square-planar and coordinated to two bidentate ligands. The crystal contains the monomeric molecule, which lies across the crystallographic mirror perpendicular to the *b* axis. Platinum and the en ring atoms C6, C7, C8, C9 lie exactly in the mirror plane. The en ring is thus constrained to be exactly planar. Atoms O3, C4, O5 and N1 and their mirror-related counterparts complete the molecule, which thus exhibits mirror (C_s) symmetry. Atom C2 is disordered in two sites, C2A and C2B, with a distance of 0.77 Å apart, giving rise to two conformers, related by reflection across the mirror plane. The crystallographic space group is thus satisfied statistically by averaging the two conformers. The N1—C2A and C2A—C2B bond distances for the disordered en ring were constrained to the values shown in Table 3. The Pt-CBDCA chelate ring also possesses exact C_s symmetry in a flattened boat conformation. There are no unusual features in the bond lengths and angles of the molecule.

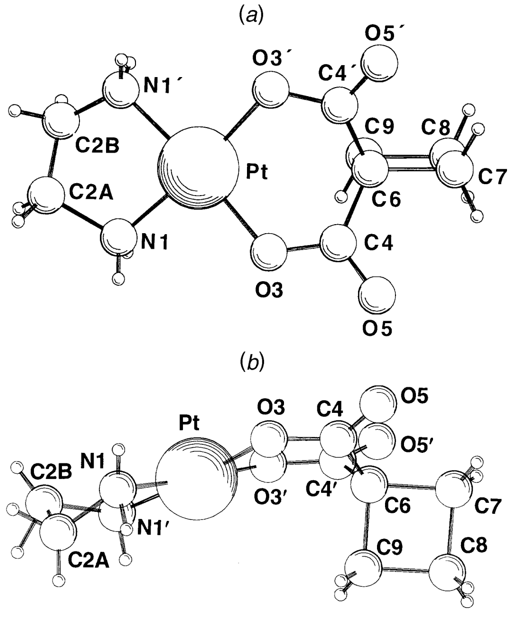

Fig. 1 View of [Pt(en)(CBDCA-*O*,*O*′)] 1, showing the numbering scheme with the H atoms as spheres of arbitrary diameter. (*a*) A view perpendicular to the least-squares plane; (*b*) view perpendicular to the CBDCA plane

Table 2 Atomic coordinates (× 10^4) for [Pt(en)(CBDCA-*O*,*O*′)]

	x	*y*	*z*
Pt	1149(1)	2500	435(1)
O3	1943(12)	4002(9)	−531(5)
O5	3316(13)	4710(13)	−1854(8)
N1	168(9)	3908(9)	1411(5)
C2A*	−1047(32)	3124(34)	2050(24)
C2B*	−291(25)	1806(19)	2365(15)
C4	3051(13)	3810(13)	−1188(7)
C6	4027(14)	2500	−1089(10)
C7	5463(15)	2500	−1814(11)
C8	6487(25)	2500	−825(14)
C9	5068(21)	2500	−104(14)

* Occupancy 0.5.

The Pt—N bond distance of 2.023(7) Å is very similar to values reported for other Pt-ethylenediamine compounds such as dichloroethylenediamineplatinum(II) (2.034 Å),[13] chloro(ethylenediamine){(−)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole}platinum(II) chloride (2.024 Å),[14] chloro-uracil-ethylenediamineplatinum(II) chloride (2.03 Å),[15] *trans*-*S*,*S*-[*N*,*N*′-bis(2-hydroxyethyl)ethylenediamine(oxalato-*O*,*O*′)-platinum(II)] (2.025 Å).

The Pt—O distance [2.017(8) Å] is also consistent with those found in Pt-bicarboxylate complexes such as carbo-

Table 3 Selected bond length (Å) and angles (°) for [Pt(en)(CBDCA-*O*,*O*′)]

C6—C7	1.548(9)	C4—C6	1.522(14)
C7—C8	1.538(10)	C4—O5	1.232(14)
C8—C9	1.533(10)	Pt—N1	2.023(7)
C6—C9	1.543(9)	N1—C2A	1.48(3)
Pt—O3	2.017(8)	C2A—C2B	1.54(3)
O3—C4	1.285(13)		
C9—C6—C7	90.8(12)	C4′—C6—C4	111.7(11)
C8—C7—C6	88.7(13)	O5—C4—O3	119.0(11)
C9—C8—C7	91.5(13)	O5—C4—C6	122.3(10)
C8—C9—C6	89.0(13)		
		O3′—Pt—N1	174.7(4)
C4′—C6—C7	113.4(7)	O3—Pt—N1	92.2(3)
C4—C6—C7	113.4(7)		
C4′—C6—C9	113.0(7)	N1—Pt—N1′	84.0(5)
C4—C6—C7	113.0(7)	C2A—N1—Pt	106.6(12)
		C2B—C2A—N1	105(2)
O3′—Pt—O3	91.5(5)	N1′—C2B—C2A	107(2)
C4—O3—Pt	123.2(8)	C2B′—N1—Pt	108.2(9)
O3—C4—C6	118.7(9)		

Ring (Pt—O3—C4—C6—C4′—O3′) and ring (Pt—N1—C2A—C2B—N1′) are completed by the operation of *m* perpendicular to *b*.

platin [Pt(NH$_3$)$_2$(CBDCA-*O,O′*)] [(2.029 Å)[6] and (2.025 Å)[7]] *trans-S,S*-[*N,N′*-bis(2-hydroxyethyl)ethylenediamine(oxalato-*O,O′*)platinum(II)] (2.039 Å),[16] *cis*-1*R*,2*R*-cyclohexanediamine-*N,N′*-oxalatoplatinum(II) (2.01, 2.04 Å),[17] Pt(malonato-*O,O′*)-*cis*-1*R*,2*R*-cyclohexanediamine-*N*,*N′*-malonatoplatinum(II) (2.02 Å),[17] potassium *anti*-bis(2-methylmalonate)platinum(II) dihydrate (2.01, 2.00 Å),[18] and potassium dichloro-(oxalato)platinate(II) hydrate (2.04, 2.03 Å).[18]

The O3—Pt—O3′ angle [91.5(5)°] is typical for a six-membered chelate ring in a Pt^{II}-bicarboxylate complex.[6,7,17,18] The six-membered Pt-CBDCA ring adopts a flattened boat conformation which is similar to that observed in carboplatin crystals.[6,7] The N1—Pt—N1′ [84.0(5)°] angle has also a normal value for a five-membered ethylenediamine chelate ring. The Pt-en chelate ring exhibits both *d* and *l* twisted sites C2A and C2B in the alternative conformations (δ and λ), and both conformations were equally populated in the crystal, with N(1)—C—C—N(1′) torsion angles of 58.7(8)° (δ conformer) and −58.7(8)° (λ conformer), similar to those reported for other Pt-ethylenediamine complexes.[16]

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NMR studies of [Pt(en)(CBDCA)] 1

The $^{13}C\{^{1}H\}$ NMR solution spectrum of **1** at 298 K contains five signals that can be assigned to the ring carbons C8 (16.0 ppm), C7 and C9 (31.7 ppm), C6 (56.9 ppm), the carboxyl carbons C4 and C4′ (182.3 ppm) of CBDCA, and the methylene carbons C2A and C2B (48.7 ppm) of ethylenediamine. Similar ^{13}C chemical shifts for the CBDCA ligand have been reported for carboplatin.[6] Despite the inequivalence of the cyclobutane ring carbons in the solid-state structure of **1**, only one resonance was seen for C7 and C9, showing that rapid ring inversion occurs as for carboplatin.[6] Similarly, the δ and λ conformations of the Pt-ethylenediamine ring are in rapid exchange, only one set of signals being observed for the methylene carbons.

The solid-state ^{13}C CP/MAS NMR spectrum of complex **1** at 297 K is shown in Fig. 2(*a*). Now two broad signals are observed at 49.6 and 50.3 ppm, which are assignable to the en CH$_2$ carbons. On increasing the temperature, the two signals became closer, began to merge at 317 K, and gave rise to a single sharp signal at 390 K [Fig. 2(*b*), peak **c**]. Based on the assumption that the two carbon signals are well separated at 297 K ($\Delta\upsilon_{1/2} = 186$ Hz), and two-site exchange with equal populations,[19] the exchange rate constant was determined to be 415 s^{-1}. At the coalescence temperature of *ca.* 317 K, the activation energy $\Delta G^{\ddagger}$ was calculated to be 62 kJ mol^{-1}. The energy barriers for this conformational change in an unsubstituted five-membered chelate ring, with a metal–nitrogen bond length of 2.0 Å, have been estimated by strain-energy minimization calculations to be 20 kJ mol^{-1} in solution.[20] In the solid state, the energy barrier would be expected to be much higher.

Because there are very limited solid-state NMR data available for ethylenediamine complexes, we also studied the ^{13}C NMR spectra of two other platinum(II) and palladium(II) ethylenediamine complexes at 297 K. Only one signal was observed for the two ring carbons of both [Pt(en)I$_2$] (50.1 ppm) and [Pd(en)$_2$][PdCl$_4$] (46.5 ppm). We have recently reported solid-state NMR data for the outer-sphere macrochelate complexes [Pt(en)(5′-GMP)$_2$] (49.3 ppm) and [Pd(en)(5′-GMP)$_2$] (48.9 ppm), where again the ethylenediamine ligand also gave rise to only one signal.[21] Therefore it appears that the slower exchange rate in complex **1** is due to the presence of the CBDCA chelate ring.

The other five major peaks observed in the solid-state ^{13}C NMR spectrum of **1** [Fig 2(*a*)] can be assigned to C8 (16.8 ppm), C6 (56.0 ppm), C7 (27.5 ppm), C9 (35.9 ppm) and C4 and C4′ (181.3 ppm) of the CBDCA ligand. A series of minor signals were also observed [denoted by * in Fig. 2(*a*)]. These signals decreased in intensity or disappeared at 390 K [Fig. 2(*b*)]. From their chemical shifts and temperature dependences, they can tentatively be assigned to a minor alternative conformation of the CBDCA ligand.

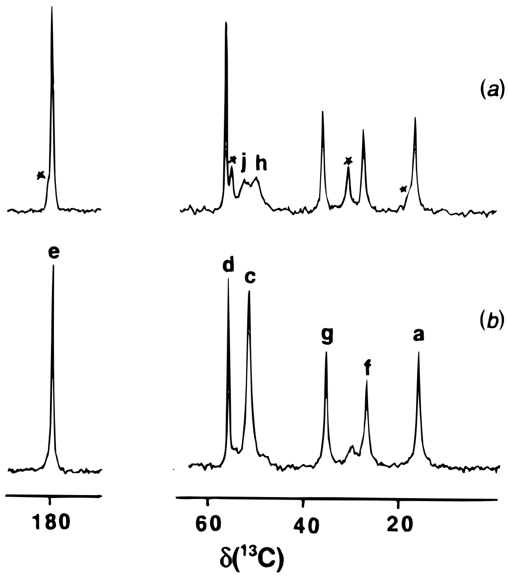

Fig. 2 The ^{13}C CP/MAS spectra of [Pt(en)(CBDCA-*O,O′*)] **1** at (*a*) 297 K and (*b*) 390 K. Peaks **h** and **j** are assigned to the CH$_2$ groups of ethylenediamine and coalesce to give peak **c**; other assignments are: **a**, C8; **f** and **g**, C7 and C9; **d**, C6; **e**, C4 and C4′. The * denotes minor peaks which may due to a different conformation of the CBDCA ring (see text)

Further support for the presence of conformational flexibility within the CBDCA ligand of **1** comes from the observation that the cyclobutane ring was not perfectly located in the X-ray diffraction electron density map. Similarly, in crystalline carboplatin the CBDCA ring also exhibits substantial motion at room temperature, which has been related to the dynamic puckering between two conformations.[7] The potential barrier was estimated to be below 6 kJ mol^{-1}.

We recorded the solid-state ^{13}C CP/MAS NMR spectrum of crystalline carboplatin at 298 K, but only five signals were observed at 16.4, 28.1, 35.3, 56.5 and 182.0 ppm, which can be assigned to the five magnetically non-equivalent types of carbon atoms in the CBDCA ligand (four cyclobutane resonances, one peak for the two carboxylates). The shifts are very similar to those of **1** [Fig. 2(*a*)]. Since no minor signals were observed in the spectrum, it can be concluded that the energy barrier for the dynamic puckering is lower than for **1**.

Taken together, it seems clear that two chelate rings in bis(chelated)-Pt^{II} complexes exhibit interactive conformational dynamics. Such effects may be important to molecular recognition processes involving platinum drugs and may be relevant to the design of anticancer drugs.

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References

1 *Platinum and Other Metal Coordination Compounds in Cancer Chemotherapy 2*, ed. H. M. Pinedo and J. H. Schornagel, Plenum, New York, 1996.

2 J. Reedijk, *J. Chem. Soc., Chem. Commun.*, 1996, 801.

3 U. Frey, J. D. Ranford and P. J. Sadler, *Inorg. Chem.*, 1993, **32**, 1333.

4 S. K. C. Elmroth and S. J. Lippard, *Inorg. Chem.*, 1995, **34**, 5234.

5 D. Kiser, F. P. Intini, Y. H. Xu, G. Natile and L. G. Marzilli, *Inorg. Chem.*, 1994, **33**, 4149.

6 S. Neidle, I. M. Ismail and P. J. Sadler, *J. Inorg. Biochem.*, 1980, **13**, 205.

7 B. Beagley, D. W. J. Cruickshank, C. A. McAuliffe, R. G. Pritchard and A. M. Zaki, *J. Mol. Struct.*, 1985, **130**, 97.

8 G. L. Johnson, *Inorg. Synth.*, 1966, **8**, 242.

9 R. C. Harrison, C. A. McAuliffe and A. M. Zaki, *Inorg. Chim. Acta*, 1980, **46**, 205.

10 G. M. Sheldrick, *SHELXS-86*, University of Göttingen, Germany, 1986.

11 N. Walker and D. Stuart, *Acta Crystallogr., Sect. A*, 1983, **39**, 158.

12 G. M. Sheldrick, *SHELXL-93*, University of Göttingen, Germany, 1993.

13 L. T. Ellis and T. W. Hambley, *Acta Crystallogr., Sect. C*, 1994, **50**, 1888.

14 G. M. Arvanitis, M. E. Berardini, G. N. Parkinson and B. S. Schneider, *Acta Crystallogr., Sect. C*, 1993, **49**, 1246.

15 R. Faggiani, B. Lippert and C. J. L. Lock, *Inorg. Chem.* 1980, **19**, 295.

16 Q. Xu, A. R. Khokhar and J. L. Bear, *Inorg. Chim. Acta*, 1990, **178**, 107.

17 M. A. Bruck, R. Bau, M. Boji, K. Inagaki and Y. Kidani, *Inorg. Chim. Acta*, 1984, **92**, 279.

18 S. O. Dunham, R. D. Larsen and E. H. Abbott, *Inorg. Chem.*, 1991, **30**, 4328.

19 H. Friebolin, *Basic One- and Two-Dimensional NMR Spectroscopy*, VCH, Weinheim, 1991, pp. 267–274.

20 C. J. Hawkins and J. A. Palmer, *Coord. Chem. Rev.*, 1982, **44**, 1.

21 K. J. Barnham, C. J. Bauer, M. I. Djuran, M. A. Mazid, T. Rau and P. J. Sadler, *Inorg. Chem.*, 1995, **34**, 2826.

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