Interactions of Dichloro-bis(η^5-cyclopentadienyl)titanium(IV) with Nucleosides

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Abstract

The interactions of dichloro-bis(η^5-cyclopentadienyl)titanium(IV) (titanocene dichloride, Cp_2TiCl_2) with nucleosides have been studied in methanolic solutions. Complexes of the general formula $[Cp_2Ti(Nucl)MeOH]Cl_2$ were isolated. The nucleoside complexes with one N(1)H ionizable imino proton (*i.e.* inosine and guanosine) undergo ionization in alkaline solution and complexes of the formula $[Cp_2Ti(Nucl{-}H^+)]Cl$ were isolated. All complexes have been characterized by elemental analyses and various spectroscopic techniques. In the first series of complexes, $[Cp_2Ti(Nucl)MeOH]Cl_2$, the nucleosides act as monodentate ligands with an intramolecular hydrogen bond between the coordinated methanol and the C6=O group, while in the second, $[Cp_2Ti(Nucl{-}H^+)]Cl$, they coordinate through both their N7 and O6 atoms.

Introduction

cis-Dichlorodiammineplatinum(II) was the first in a series of Pt(II) compounds to be recognized as an efficient therapeutic agent for the treatment of tumours in living organisms [1]. The antitumour action of platinum in the body was traced to chemical attack by the metal species on DNA molecules [2]. Since these discoveries the metallocene halides and bis(pseudohalides) of constitution Cp_2MX_2 (where $Cp = \eta^5\text{-}C_5H_5$; M = Ti, V, Nb, Mo; X = F, Cl, Br, I, NCS and N_3) have been proved to constitute a potent new class of organometallic antitumour agents [3–12].

In view of the structural similarities between Cp_2TiX_2 and *cis*-$Pt(NH_3)_2X_2$ (both possess the similar *cis*-MX_2 functionalities), it was proposed that the primary biological target for both classes of compounds is DNA [13, 14]. This proposal was supported by the fact that metal intracellular distribution in titanocene-treated cells is identical to that reported for the platinum complexes [15]. The inhibition of nucleic acid metabolism is also a common effect observed for both groups of agents [14].

The above findings led many research groups to investigate the reactions of the metallocene complexes Cp_2MX_2 with biomolecules and especially with the DNA constituents and related model compounds.

Thus Beauchamp and coworkers [16] reported the synthesis and crystal structure of chloro-bis(η^5-cyclopentadienyl)(purinato)titanium(IV), a model compound for the interaction of the antitumour titanocene dichloride molecule with DNA bases. The same group also reported the preparation and crystal structure of chloro-bis(η^5-cyclopentadienyl)(theophyllinato)titanium(III) [17], in which the N7/O6 chelation was unambiguously established. Marks and coworkers [18] also reported on the interactions of vanadocene dichloride with nucleotides and phosphoro esters.

In this paper we wish to report on the interactions of titanocene dichloride with nucleosides.

Results and Discussion

The interaction of titanocene dichloride with the nucleosides (Nucl) adenosine (Ado), guanosine (Guo), cytidine (Cyd) and inosine (Ino) in methanolic solution resulted in the formation of 1:1 complexes according to the equation:

$$Cp_2TiCl_2 + Nucl \xrightarrow{MeOH} [Cp_2Ti(Nucl)(MeOH)]Cl_2$$

The complexes behave as 1:2 electrolytes in methanol and this, together with the analytical results (Table I), fits well with the proposed formulation.

The nucleoside complexes with the N(1)H ionizable imino proton undergo further reaction in alkaline solution:

$$[Cp_2Ti(Nucl)(MeOH)]Cl_2 + KOH \longrightarrow [Cp_2Ti(Nucl{-}H^+)]Cl + KCl + H_2O$$

The formation of the first series of complexes seems to proceed via a two-step mechanism. Titano-

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TABLE I. Analytical[a] and Conductivity Data of the Complexes

Compound	Ti (%)	Cl (%)	Λ_M (ohm^{-1} cm^2 mol^{-1})
$[Cp_2Ti(Ado)(MeOH)]Cl_2$	8.95(8.94)	13.20(12.95)	152(MeOH)
$[Cp_2Ti(Cyd)(MeOH)]Cl_2$	9.30(9.14)	13.20(13.55)	158(MeOH)
$[Cp_2Ti(Guo)(MeOH)]Cl_2$	8.70(8.49)	12.40(12.58)	160(MeOH)
$[Cp_2Ti(Guo-H^+)(MeOH)]Cl$	9.70(9.66)	7.35 (7.16)	65(DMF)
$[Cp_2Ti(Ino)(MeOH)]Cl_2$	8.80(8.72)	13.10(12.93)	165(MeOH)
$[Cp_2Ti(Ino-H^+)(MeOH)]Cl$	9.80(9.67)	7.55 (7.39)	68(DMF)

[a]The numbers in parentheses represent the calculated figures.

cene is first alcoholated and then one molecule of methanol is substituted by one nucleoside molecule:

$$[Cp_2Ti]Cl_2 + 2MeOH \longrightarrow [Cp_2Ti(MeOH)_2]Cl_2$$

$$[Cp_2Ti(MeOH)_2]Cl_2 + NuCl \longrightarrow [Cp_2Ti(Nucl)(MeOH)]Cl_2$$

Attempts to substitute both methanol molecules and prepare bis-nucleoside complexes were unsuccessful and this was also observed in the interactions of vanadocene dichloride with nucleotides and phosphoro esters [18].

The deprotonated complexes also behave as 1:1 electrolytes in DMF and this, together with the analytical results (Table I), fits well with the proposed formulation.

It is widely accepted that N7 is the preferred coordination site in guanosine, inosine and other 6-oxopurines [19]. This site in guanosine is believed to be the primary target for platinum antitumour complexes in cellular DNA [19, 20]. To explain this specificity, several models have been proposed, one of which assumes that initial metal binding to N7 is followed by deprotonation of the N(1)H imino proton and coordination of the O6 atom with a second coordination site on the metal, leading to an N7/O6 chelate. Although studies on model compounds have conclusively shown that such chelates can be formed with 6-thiopurines [21], evidence for chelation in 6-oxoligands is much less convincing. This problem was examined by Kistenmacher and coworkers [22, 23] by means of a series of copper complexes of theophylline. They confirmed N7 as the primary binding site and noticed that the O6 atom is generally hydrogen bonded with other ligands in the metal coordination sphere. The same behaviour has also been observed with other metal ions [24–27]. When hydrogen bonding ligands were not available, O6 was found to occupy an apical coordination site around copper, but the Cu–O6 distance (292 pm) was much longer than the Cu–N7 distance (~195 pm) [23]. Thus, even though this molecule can be described as a chelate in the sense that a ring exists, the two bonding interactions are hardly comparable. However, a genuine N7/O6 chelate complex was found in the crystal structure of bis-(η^5-cyclopentadienyl)(theophyllinato)titanium(III) in which the Ti–N7 and Ti–O6 distances (*ca.* 221 and 228 pm, respectively) are comparable and the angle O6–Ti–N7 is 79.6° [16].

In the first series of complexes of the present work, *i.e.* $[Cp_2Ti(Nucl)(MeOH)]Cl_2$ (where Nucl = Guo or Ino), the ν(C6=O) frequencies are lowered by about 20 cm^{-1} relative to the free nucleosides and this may be taken as an indication for the participation of the C6=O group in an intramolecular hydrogen bonding with the coordinated methanol molecule. This intramolecular hydrogen bonding is common when a second ligand capable of forming hydrogen bonds exists with an N7 coordinated purine nucleoside in the same metal [24–27 and refs. therein].

In the second series of complexes however, *i.e.* $[Cp_2Ti(Nucl-H^+)]Cl$, the ν(C6=O) are shifted to lower frequencies by about 75 cm^{-1} and this may be taken as a good indication for the C6=O keto oxygen involvement in coordination after N(1)H imino proton ionization [28–30]. Certainly, the double bond character of the C=O is also lowered when the oxygen interacts covalently with a metal without loss of the N(1)H imino proton [31]. Oxygen involvement in bonding, following deprotonation of the imino proton, has also been found in the crystal structure of *cis*-diammineplatinum a-pyridone blue [32], where both O^- and N atoms bridge two platinum atoms. Oxygen-Ag(I) interaction was also found by Kistenmacher *et al.* [33] in the crystal structure of (nitrato)(1-methylcytosine)silver(I).

The ^1H NMR bands in the aromatic proton region are very useful in assigning the coordination sites of the nucleosides and are given in Table II.

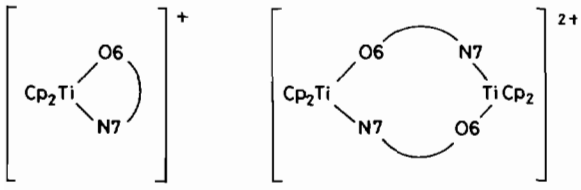
All complexes show a very strong band around 6.6 ppm, assigned to proton resonance of the cyclopentadienyl moieties.

TABLE II. ^{1}H NMR Chemical Shifts of the Complexes in DMSO-d_6 (ppm)

Compound	NH_2	NH	H2	H5	H6	H7	H1	H (η^5-Cp)
Adenosine	7.37		8.15			8.36	5.90 5.80	
$[Cp_2Ti(Ado)(MeOH)]Cl_2$	7.88		8.32			8.95	6.31 6.23	6.65
Cytidine	7.15			5.75 5.65	7.85 7.80		6.24 6.17	
$[Cp_2Ti(Cyd)(MeOH)]Cl_2$	8.42 7.62			6.20 6.12	8.25 8.14		6.26 6.24	6.58
Guanosine	6.40	10.60				7.85	5.74 5.68	
$[Cp_2Ti(Guo)(MeOH)]Cl_2$	6.70	11.02				8.51	5.85 5.78	6.63
$[Cp_2Ti(Guo-H^+)]Cl$	6.75					8.53	5.87 5.80	6.61
Inosine		12.35	8.15			8.25	6.03 5.93	
$[Cp_2Ti(Ino)(MeOH)]Cl_2$		12.40	8.22			8.85	6.24 6.17	6.55
$[Cp_2Ti(Ino-H^+)]Cl$			8.35			8.88	6.26 6.19	6.58

The complex $[Cp_2Ti(Cyd)(MeOH)]Cl_2$, besides the cyclopentadienyl resonance, shows three doublets in the aromatic proton region [8.42, 7.62 ppm (NH_2); 8.25, 8.14 ppm (H6); 6.20, 6.12 ppm (H5)]. Both H5 and H6 are shifted downfield, with the larger shift for H5. This indicates that H5 is closer to the coordination site on the ligand, probably the N3 atom [34–36 and refs. therein]. Further evidence for the participation of the N3 atom in coordination comes from the NH_2 resonance, which appears as a doublet due to the hindered rotation of the C–NH_2 bond as a result of the N3 coordination [34, 36 and refs. therein].

In the complexes $[Cp_2Ti(Guo)(MeOH)]Cl_2$ and $[Cp_2Ti(Guo-H^+)]Cl$ the H8 resonance is shifted by 0.66 and 0.68 ppm, respectively, downfield relative to free guanosine and this is good evidence that the N7 atom participates in coordination in both complexes. The complexes $[Cp_2Ti(Ino)(MeOH)]Cl_2$ and $[Cp_2Ti(Ino-H^+)]Cl$ show two resonances at 8.85 and 8.88 ppm for the former and 8.35 and 8.78 ppm for the latter, assigned to H2 and H8 respectively. The downfield shift of the H8 resonance by 0.60 ppm in the first and by 0.63 ppm in the second complex is comparable to that found in other similar cases [34–37] and may be taken as an indication of the N7 coordination of inosine to titanium in both complexes. These observations together with the IR data (Table II) suggest that inosine and guanosine act as bidentate ligands in the complexes $[Cp_2Ti(Nucl-H^+)]Cl$ through both their N7 and O6 atoms, either in a chelate (**I**), or a dimeric (**II**) structure:



I **II**

Finally the complex $[Cp_2Ti(Ado)(MeOH)]Cl_2$ shows two resonances at 8.32 and 8.95 ppm, assigned to H2 and H8 respectively. Since H8 shifts downfield by 0.59 ppm while H2 is shifted by only 0.17 ppm relative to free adenosine, it is concluded that the N7 atom is the only binding site in the above complex [38].

Experimental

Materials and Methods

The nucleosides and titanocene dichloride were purchased from Fluka A.G. and were used without further purification.

The IR spectra were recorded on a Jasko spectrophotometer as KBr pellets. ^{1}H NMR spectra were obtained on a Varian T-60 high resolution spectrometer, with tetramethylsilane as internal reference.

Preparation of the Complexes

$[Cp_2Ti(Nucl)(MeOH)]Cl_2$ (I)

Dichloro-bis(η^5-cyclopentadienyl)titanium(IV) (titanocene dichloride, Cp_2TiCl_2) 1 mmol (0.249 g) and 1 mmol of each of the nucleosides adenosine (Ado), cytidine (Cyd), guanosine (Guo) and inosine (Ino) were suspended in 200 ml methanol and stirred for 4 h at room temperature. The mixture was then filtered and the filtrate evaporated to a small volume (*ca.* 10 ml). After cooling in an ice bath, the precipitate was filtered and dried at 50 °C under vacuum. The yield was in the range of 55 to 60%.

$[Cp_2Ti(Nucl-H^+)]Cl$ (II)

Aliquots (1 mmol) of each of the complexes $[Cp_2Ti(Nucl)(MeOH)]Cl_2$ (Nucl = Guo ir Ino) were dissolved in 10 ml DMF and to that was added 1 mmol KOH dissolved in 5 ml absolute ethanol. The mixture was stirred for 2 h and the precipitated KCl was filtered. The compound was then precipitat-

ed with excess ether and dried at 50 °C under vacuum. The yield was in the range of 50 to 55%.

References

1 B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansur, *Nature (London), 222,* 385 (1969).
2 T. G. Spiro (ed.), in 'Nucleic Acid–Metal Ion Interactions', 'Metal Ions in Biology Series', Vol. 1, 1980; J. P. Macquet and J. L. Butour, *Biochemie, 60,* 901 (1978).
3 H. Köpf and P. Köpf-Maier, *Angew. Chem., Int. Ed. Engl., 18,* 477 (1979).
4 P. Köpf-Maier, B. Hesse and H. Köpf, *J. Cancer Res. Clin. Oncol., 96,* 43 (1980).
5 P. Köpf-Maier and H. Köpf, *Z. Naturforsch., Teil B, 34,* 805 (1979).
6 P. Köpf-Maier, M. Leitner and H. Köpf, *J. Inorg. Nucl. Chem., 42,* 1789 (1980).
7 P, Köpf-Maier, M. Leitner, R. Voigtländer and H. Köpf, *Z. Naturforsch., Teil C, 34,* 1174 (1979).
8 P. Köpf-Maier, B. Hesse, R. Voigtländer and H. Köpf, *J. Cancer Res., Clin. Oncol., 97,* 31 (1980).
9 P. Köpf-Maier, W. Wagner, B. Hesse and H. Köpf, *Eur. J. Cancer, 17,* 665 (1981).
10 R. Köpf-Maier, W. Wagner and H. Köpf, *Cancer Chemother. Pharmacol., 5,* 237 (1985).
11 P. Köpf-Maier, A. Moormann and H. Köpf, *Eur. J. Cancer Clin. Oncol., 21,* 853 (1985).
12 J. H. Toney, L. N. Rao, M. S. Murthy and T. J. Marks, *Breast Cancer Res. Treat., 6,* 185 (1985); M. S. Murthy, J. H. Toney, L. N. Rao, L. Y. Kuo and T. J. Marks, *Proc. Am. Assoc. Cancer Res., 27,* 279 (1986).
13 P. Köpf-Maier and H. Köpf, *Naturwissenschaften, 67,* 272 (1981).
14 P. Köpf-Maier, W. Wagner and H. Köpf, *Naturwissenschaften, 68,* 272 (1981).
15 P. Köpf-Maier and D. Krahl, *Naturwissenschaften, 68,* 273 (1981).
16 A. L. Beauchamp, D. Cozak and A. Mardhy, *Inorg. Chim. Acta, 92,* 191 (1984).
17 D. Cozak, A. Mardhy, M. J. Oliver and A. Beauchamp, *Inorg. Chem., 25,* 2600 (1986).
18 J. H. Toney, C. P. Brock and T. J. Marks, *J. Am. Chem. Soc., 108,* 7263 (1986).
19 L. G. Marzilli, T. J. Kistenmacher and G. L. Eichhorn, *Metal Ions Biol., 1,* 179 (1980).
20 J. K. Barton and S. J. Lippard, *Metal Ions Biol., 1,* 31 (1980).
21 H. I. Heitner and S. J. Lippard, *Inorg. Chem., 13,* 815 (1974); E. Sletten and A. Apeland, *Acta Crystallogr., Sect B, 31,* 2019 (1975).
22 T. Sorrell, L. G. Marzilli and T. J. Kistenmacher, *J. Am. Chem. Soc., 98,* 2181 (1976); D. J. Szalda, T. J. Kistenmacher and L. G. Marzilli, *Inorg. Chem., 15,* 2783 (1976); T. J. Kistenmacher, D. J. Szalda, C. C. Chiang, M. Rossi and L. G. Marzilli, *Inorg. Chem., 14,* 1886 (1975).
23 D. J. Szalda, T. J. Kistenmacher and L. G. Marzilli, *J. Am. Chem. Soc., 98,* 8371 (1976).
24 T. J. Kistenmacher, *Acta Crystallogr. Sect. B, 31,* 85 (1975); T. J. Kistenmacher and D. J. Szalda, *Acta Crystallogr., Sect. B, 31,* 90 (1975).
25 K. Caldwell, G. B. Deacon, B. M. Gatehouse, S. C. Lee and A. J. Candy, *Acta Crystallogr., Sect. C, 40,* 1533 (1984); A. R. Norris, S. E. Taylor, E. Buncel, F. Belanger-Gariepy and A. L. Beauchamp, *Inorg. Chim. Acta, 92,* 271 (1984).
26 E. Buncel, R. Kumar, A. R. Norris and A. L. Beauchamp, *Can. J. Chem., 63,* 2575 (1985).
27 J. R. Perno, D. Kwikel and T. G. Spiro, *Inorg. Chem., 26,* 400 (1987).
28 M. Ogawa and T. Sakaguhi, *Chem. Pharm. Bull., 19,* 1650 (1971).
29 A. T. Tu and M. J. Heller, *Metal Ions Biol. Systems., 1,* 1 (1974).
30 J. Dehand and J. Jordanov, *J. Chem. Soc., Chem. Commun.,* 598 (1976).
31 A. J. Canty and R. S. Tobias, *Inorg. Chem., 18,* 413 (1979).
32 J. K. Barton, H. N. Rabinowith, D. J. Szalda and S. J. Lippard, *J. Am. Chem. Soc., 99,* 2827 (1977).
33 T. J. Kistenmacher, M. Rossi and L. G. Marzilli, *Inorg. Chem., 18,* 240 (1979).
34 M. M. Singh, Y. Rossopoulos and W. Beck, *Chem. Ber., 116,* 1364 (1983).
36 G. Pneumatikakis, *Polyhedron, 3,* 9 (1984).
36 G. Pneumatikakis, J. Markopoulos and A. Yannopoulos, *Inorg. Chim. Acta, 136,* L25 (1987).
37 G. Pneumatikakis, N. Hadjiliadis and T. Theophanides, *Inorg. Chem., 17,* 915 (1978); N. Hadjiliadis and G. Pneumatikakis, *J. Chem. Soc. Dalton Trans.,* 1691 (1978).
38 G. Pneumatikakis and N. Hadjiliadis, *J. Chem. Soc. Dalton Trans.,* 596 (1979).