Hydrolysis Chemistry of the Metallocene Dichlorides $M(\eta^5\text{-}C_5H_5)_2Cl_2$, M = Ti, V, Zr. Aqueous Kinetics, Equilibria, and Mechanistic Implications for a New Class of Antitumor Agents

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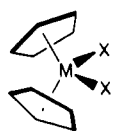
Abstract: This paper reports an integrated chemical/physicochemical investigation of the aqueous chemistry of Cp_2TiCl_2, Cp_2VCl_2, and Cp_2ZrCl_2, employing high-field FT NMR, chloride potentiometry, pH titrimetry, and electrical conductivity. Experimental conditions include those employed previously to study the hydrolysis of *cis*-dichlorodiammineplatinum(II) ("cisplatin") and those approximating physiological. In unbuffered aqueous 0.32 M KNO_3 solution at 37 °C, the order of decreasing hydrolytic stability of the M-(η^5-C_5H_5) bond in the Cp_2MCl_2 complexes (monitored by high-field FT NMR) was found to be V ($k_{initial} \leq 3.0 \times 10^{-3}$ h^{-1}) > Ti ($k_{initial}$ = 6.4 (1) × 10^{-3} h^{-1}) ≫ Zr ($k_{initial}$ = 3.8 (1) × 10^{-2} h^{-1}). Of the three complexes studied, only Cp_2VCl_2 was found to possess a stable M-(η^5-C_5H_5) bond at physiological pH. Addition of any of the Cp_2MCl_2 complexes to water (pure or 0.32 M KNO_3) results in rapid chloride ion dissociation with approximate half-lives for the loss of the second chloride (the first is too rapid to measure) of 50 min (M = Ti), 30 min (M = Zr), and 24 min (M = V) in contrast to the relatively slow chloride hydrolysis observed with cisplatin. Chloride dissociation is also more extensive than that in cisplatin. Equilibrium chloride ion concentration measurements indicate that the equilibrium constant (K_1) for the first Cp_2MCl_2 chloride dissociation is too large to measure, while K_2 = 4.2 (2.7) × 10^{-2}M (M = Ti) and 2.7 (1.2) × 10^{-3} M (M = V). Titrimetric studies indicate that the acidity of the Cp_2M^{2+}-bound water molecules is uniformly more acidic than the metal-bound water molecules of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (Ti, pK_a = 3.5 (5) and 4.35 (9); V, pK_a = 4.73 (3) and 5.15 (13)). Implications of these results for the observed biological activity of the Cp_2MCl_2 complexes are briefly discussed.

Over the past 5 years, Köpf and Köpf-Maier have shown that metallocene dihalides and bis(pseudo-halides)[1] of the constitution Cp_2MX_2(A), where Cp = η^5-C_5H_5, M = Ti, V, Nb, Mo,[2-5] X =



A

F, Cl, Br, I, NCS, and N_3,[6] are highly active against Ehrlich ascites tumor (EAT) cells, lymphoid leukemia L1210, and lymphocytic leukemia P388.[7,8] The Cp_2MX_2 compounds thus constitute a potent new class of organometallic antitumor agents. Analogies to the well-known antitumor agent *cis*-dichlorodiammineplatinum(II) ("cisplatin") and derivatives thereof[9,10] immediately come to mind since both classes of compounds possess metrically similar *cis*-MX_2 functionalities. That the carcinostatic activity of Cp_2MX_2 agents may be mechanistically similar to cisplatin is suggested by observations of inhibition of nucleic acid metabolism[11,12] and mitotic activity[13] as well as by evidence for the accumulation of the respective metals in the nuclear heterochromatin of the tumor cells.[13-15] Nevertheless, Pt(II) is a classic "soft acid" and the aforementioned M(IV) ions are classic "hard acids".[16] Thus, any strong chemical/mechanistic analogies between the cisplatin and Cp_2MX_2 classes of compounds must presently be regarded as speculative, especially in view of the paucity of basic, quantitative information on the chemistry of the latter compounds in aqueous solution.

A great deal is now known about the chemistry of cisplatin in dilute aqueous solution,[9,17] and indeed connections have been suggested between the facile, stepwise hydrolysis (chloride replacement) and certain aspects of the physiological activity. These equilibria are summarized in Figure 1. In contrast, what is known about Cp_2MX_2 aqueous chemistry is rather fragmentary and has been based largely upon qualitative solution spectroscopic studies and/or the nature of species which could be crystallized from various solutions.[18-27] In all cases, the conditions have been far

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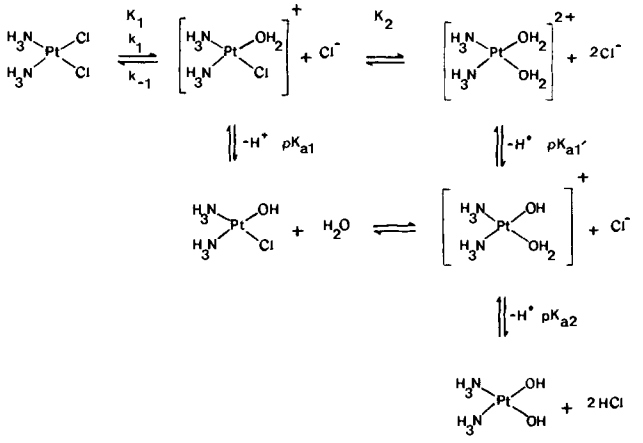

Figure 1. Hydrolysis equilibria of *cis*-$[Pt(NH_3)_2Cl_2]$. $K_1 = 4.37\ (13) \times 10^{-3}$ M (35 °C),[17b] $K_2 = 1.88\ (8) \times 10^{-3}$ M (35 °C),[17b] $k_1 \cong 7.6 \times 10^{-5}$ s^{-1} (35 °C),[28a] $k_{-1} \cong 1.0 \times 10^{-2}$ s^{-1} (25 °C),[24c] $pK_{a1} = 6.4$,[17d] $pK_{a1'} = 5.6$,[9b] $pK_{a2} = 7.3$.[9b]

from physiological. Clearly, any discussion of the mechanism of Cp_2MX_2 antitumor activity must be founded upon an accurate description of the species present in aqueous solution and the equilibria connecting them. To this end, we present here the results of an integrated chemical/physicochemical investigation employing high-field FT NMR, chloride potentiometry, pH titrimetry,[17b,28] and electrical conductivity of the aqueous chemistry of Cp_2TiCl_2, Cp_2VCl_2, and Cp_2ZrCl_2. The conditions include those approximating physiological (blood plasma[28b])/therapeutic[29] (~1 mM Cp_2MCl_2, ca. 103 mM NaCl, 37 °C, pH ≈7) and those employed to study the hydrolysis of cisplatin.[17,28] ^{1}H NMR studies have also been carried out in Me_2SO-d_6/H_2O(saline) mixtures buffered with $NaHCO_3$ (pH ~5.5) to simulate injection mixtures used for in vivo experiments.[6] The primary goal has been to establish the ease with which the η^5-cyclopentadienyl and chloride ligands undergo hydrolytic displacement and to better define the nature of the species present upon dissolution in aqueous media (surprisingly little information of this type is available for any early transition-metal organometallic compound). These data will serve as a foundation for subsequent studies of Cp_2MX_2–biomacromolecule interactions. The Cp_2ZrCl_2 compound is of comparative interest in the present context because, in contrast to Cp_2TiCl_2 and Cp_2VCl_2, it appears to have little or no carcinostatic activity.

It will be seen that in aqueous solution the Cp_2MCl_2 systems display certain surprising similarities to, and marked differences from, cisplatin chemistry. Furthermore, the hydrolytic stability of the M–C_5H_5 linkage is markedly sensitive to the identity of M and, importantly, to the solution pH.

Experimental Section

Methods and Materials. All organometallic compounds were handled under prepurified nitrogen with use of standard Schlenk techniques. Organic solvents were thoroughly dried and deoxygenated in a manner appropriate to each. Water was doubly distilled, deionized (resistance ~ 17 M Ω^{-1} cm^{-1}), and thoroughly saturated with prepurified nitrogen; D_2O (Biorad, 99.87 mol % D) was saturated with prepurified nitrogen. The complexes Cp_2TiCl_2 and Cp_2VCl_2 were obtained from Strem Chemical Co. The former was recrystallized twice from THF while the latter was purified by anaerobic Soxhlet extraction with CH_2Cl_2 at 44 °C (under partial vacuum). The complex Cp_2ZrCl_2 was obtained from Sigma Chemical Co. and used as received. The purity of all metallocene dichlorides was checked by IR and/or ^{1}H NMR spectroscopy. Cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$) was obtained from Sigma Chemical Co. and was further purified by DMF recrystallization.[30] Purity was verified by IR and UV–vis spectroscopy. DSS (the sodium salt of 3-(trimethylsilyl)-1-propanesulfonic acid, $(CH_3)_3Si(CH_2)_3SO_3Na \cdot xH_2O$) was obtained from Aldrich Chemical Co. and used as received (>99% purity). NaOH solutions were freshly standardized by titration with KHP (potassium hydrogen phthalate) with phenolphthalein as the indicator. All other chemicals were reagent grade.

Physical Measurements. ^{1}H NMR spectra were recorded on a JEOL FX-270 FT instrument (16K data points) with use of homogated decoupling techniques for solvent (HDO) signal suppression in D_2O or the solvent suppression technique developed by Hore[31,32] for spectra taken in H_2O. All spectra were recorded with 45° flip angles (pulse width usually ~7.0 μs). Reported chemical shifts are referenced to HDO or DSS. Integration studies employed appropriate pulse delays (5.0 s); relative peak areas were established by cutting and weighing. Infrared spectra were recorded on Perkin-Elmer 599 or 283 spectrometers. Sample mulls were prepared in a glovebox with dry, degassed Nujol or Fluorolube and were studied between KBr plates in an air-tight, O-ring sealed holder.

Chloride concentrations during hydrolysis experiments were determined with an Orion 9417B solid-state chloride-sensitive electrode and an Orion double-junction reference electrode (using a 10% KNO_3 solution as the filling solution for the outer chamber).[33] Potentials were read with a Doric DS-100 digital multimeter (±0.01 mV readability). The chloride electrode was calibrated with stock solutions of KCl, about every 3 h, under conditions identical with those of the experiments (same temperature and ionic strength and saturating the solution with N_2) and allowed to come to thermal equilibrium in the solution for at least 15 min before each measurement was taken. Measurements of pH were performed with a Beckman SS-2 "Expandomatic" pH meter and a Broadley-Jones pH electrode having an internal reference (4 M KCl saturated with AgCl). Solution conductivity measurements were performed with a platinum electrode cell (Yellow Springs Instrument Co., Inc). connected to a Universal Impedance Measuring System (Electro Scientific Industries, Model 293). The cell constant (1.098 ± 0.029 cm^{-1} at 25.0 °C) was calibrated with a KCl solution. Experiments requiring temperature control were carried out in a constant temperature bath regulated (to within ±0.1 °C) with a Haake E2 temperature-control unit.

The high-resolution solid-state ^{13}C NMR spectrum of "Ti(η^5-$C_5H_5)_{0.31}O_{0.30}$(OH)" (**1**) was measured at a carbon frequency of 15.0 MHz on a JEOL FX 60QS spectrometer. High-power ^{1}H decoupling and magic angle spinning were employed. A bullet rotor made of Kel-F was used at a spinning speed of ~2.5 kHz. The sample was loaded in a glovebox under a dry nitrogen atmosphere. The insert of the rotor was coated with a light layer of high-vacuum silicone grease (Dow Corning) before being pressed into the outer part. The spectrum was externally referenced to liquid Me_4Si based on substitution of hexamethylbenzene (HMB) as secondary reference and assigning 132.3 and 16.9 ppm to the shifts of the aromatic and aliphatic carbons, respectively, of HMB to liquid Me_4Si. The proton 90° pulse width was 5 μs. The contact time for cross polarization was 3 ms, and the repetition time was 2 s. The magic angle was set by observing the ^{79}Br line of a small amount of dry KBr which was separated from the sample in the rotor by a thin Teflon disk. The sample weight was 110 mg; a satisfactory spectrum was obtained with 2640 scans.

Measurements of Cyclopentadiene Loss from Cp_2MX_2 Compounds. NMR samples were prepared by transferring the appropriate Cp_2MCl_2 compound from a tared (±0.01 mg), three-neck, 25-mL flask under an N_2 flush into a 10-mm NMR tube that had been charged with 3.00 mL of D_2O (or H_2O, with a 4-mm insert containing Me_2SO-d_6 for deuterium locking) previously saturated with N_2 for several minutes and KNO_3

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(~0.32 M) or NaCl (~0.10 M), as well as a known amount of DSS standard, if appropriate. ^{1}H NMR (270 MHz) spectra were then recorded at various time intervals, generally up to several days with the sample maintained at 37 °C. These C_5H_5D[34] (or C_5H_6) spectra were then compared to spectra (vs. DSS) of authentic samples of freshly cracked[35] cyclopentadiene in D_2O (or H_2O) (0.318 M in KNO_3 or 0.103 M in NaCl) at 37 °C. Control experiments established that cyclopentadiene is soluble to at least 4.0 mM in this solvent system; the empirical method of Lyman et al.[36] predicts a solubility of ca. 40 mM in pure water at 25 °C. Recording C_5H_6 spectra as a function of time and integration vs. the signal of the DSS standard established that cyclopentadiene proton exchange with D_2O has a half-life of 24 ± 6 h under these conditions (pH ~3).

For Cp_2TiCl_2 and Cp_2ZrCl_2, the percentage loss of metal-bound Cp was calculated by integrating the liberated cyclopentadiene (C_5H_5D[34] or C_5H_6) δ 2.91 resonance vs. the remaining metal-bound η^5-C_5H_5 signals and/or the closest DSS signal. In the case of paramagnetic Cp_2VCl_2, the metal-bound η^5-C_5H_5 resonances were too broad for accurate integration, so the intensity of the C_5H_5D signal (liberated from a known quantity of Cp_2VCl_2) was compared to the signal from a known quantity of DSS. Experiments carried out at near neutral pH were buffered by addition of NaOH (or $NaHCO_3$) over time.

Measurement of Chloride Loss from Cp_2MCl_2 Compounds. Rates and Equilibria. Samples were prepared by transferring the appropriate, finely ground complex from a tared (±0.01 mg), three-neck, 25-mL flask under an N_2 flush into a five-neck, 100-mL flask charged with 40.00 mL of a 0.318 M KNO_3 solution that had been saturated with N_2 for at least 10 min. The flask containing this mixture was lowered into a constant temperature bath and N_2 saturation (gentle bubbling) along with magnetic stirring were maintained throughout the course of the experiment. Control experiments established that free chloride was not lost in the form of HCl gas. Also, other control experiments carried out with the exclusion of laboratory light established that there were no photochemical effects in these systems under the conditions of our study. Measurements of the chloride concentration using the Orion chloride-sensitive electrode were not initiated until Cp_2MCl_2 dissolution was complete. This time varied from 10 to 60 min, depending on the identity of M and the concentration of Cp_2MCl_2. Measurements of chloride were made as a function of time, and in most cases, equilibrium was reached within 3 to 5 h. All equilibrium chloride measurements were made 5–10 h from the time of mixing, over a 10–15-fold concentration range of Cp_2MCl_2. Error estimates in chloride concentration were derived from 6–10 experiments for each Cp_2MCl_2 complex, based on errors in the concentration of Cp_2MCl_2 and the slope and y intercept of the chloride calibration line, using standard error propagation methods.[37,38] Several comparison experiments were also carried out with cisplatin (equilibrium measurement carried out after 1 week from time of mixing).

Titration of Coordinated Water. The hydrolysis of the Cp_2MCl_2 compounds was also investigated titrimetrically.[17b,28] Solutions prepared as described in the foregoing section were titrated under nitrogen with a standardized NaOH solution, using a pH electrode to determine the end point. Identical samples minus the Cp_2MCl_2 compound were employed as blanks.

Conductivity Measurements. Solutions of Cp_2TiCl_2 in Me_2SO and pure water were prepared as described above. The equivalent conductance, Ω_e, was measured as a function of time and concentration; it was calculated as shown in eq 1,[39] where K is the conductivity cell constant

$$\Omega_e = 1000(K)/R_{corr}C \quad (1)$$

(found to be 1.098 ± 0.029 cm^{-1} at 25.0 °C for KCl in pure water), $R_{corr} = (R_{soln}^{-1} - R_{solv}^{-1})^{-1}$ = corrected AC resistance measured at 1 KHz,[40] and C is the concentration of Cp_2TiCl_2.

Isolation and Characterization of Cp_2TiCl_2 Hydrolysis Products. "Ti(η^5-C_5H_5)$_{0.31}$O$_{0.30}$(OH)" (1). A weighed quantity of Cp_2TiCl_2 (0.4374 g, 1.76 mmol) was dissolved under nitrogen in doubly distilled deionized water (100 mL) at room temperature. The pH of the solution was then raised to 7.40 by dropwise addition of an NaOH solution (ca. 0.6 M). A light yellow precipitate formed immediately. The mixture was filtered after being stirred about 2 h. The resulting yellow solid was then washed with 3 × 5 mL of ice-cold water, 4 × 5 mL of ice-cold methanol, and 4 × 5 mL of pentane and dried in vacuo to yield ~194 mg (~100% yield based on Ti) of **1**. IR data (cm^{-1}): 3100 w, 1440 s, 1260 m, 1015 s, 930–760 v br, 550 br. Solid state ^{13}C NMR: singlet at δ 115.3 ($\nu_{1/2}$ ~ 75 Hz). Anal. (Dornis and Kolbe, under Ar) Calcd for Ti(η^5-C_5H_5)$_{0.31}$O$_{0.30}$(OH): C, 20.70; H, 2.84; Ti, 53.31; Cl, 0.0. Found: C, 20.70; H, 2.93; Ti, 53.50; Cl, <0.1. Melting point: ~259 °C dec.

A similar experiment with Cp_2VCl_2 failed to result in a precipitate.

"Ti(η^5-C_5H_5)$_{1.66}$O$_{4.5}$(OH)$_{4.4}$" (2). A weighed quantity of Cp_2TiCl_2 (0.5122 g, 2.06 mmol) was dissolved in Me_2SO (~15 mL) at room temperature. The solution was then diluted with saline and the pH of the solution was raised to 5.50 by addition of $NaHCO_3$ (ca. 0.1 M) to give a final volume of ~150 mL. A light yellow precipitate formed immediately and was isolated directly after formation. The resulting yellow solid was then washed with 4 × 5 mL of ice-cold water, 4 × 5 mL of ice-cold methanol, and 4 × 5–10 mL of pentane and dried in vacuo to yield ~253 mg (~40% yield based on Ti) of **2**. IR data are the same as those for **1**. Anal. (Dornis and Kolbe, under Ar) Calcd for Ti(η^5-C_5H_5)$_{1.66}$O$_{4.5}$(OH)$_{4.4}$: C, 32.91; H, 4.20; Ti, 15.83; Cl, 0.0. Found: C, 32.33; H, 3.44; Ti, 15.38; Cl, <0.1.

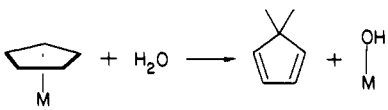
Table I. Initial Rate of Cyclopentadiene Loss (C_5H_5D or C_5H_6) from Cp_2MCl_2 Solutions in D_2O (or H_2O) at 37.0 °C

M	k_{obsd},[a] h^{-1}	k_{corr},[b] h^{-1}	solution composition
Ti	1.22 (2) × 10^{-2}	6.4 (1.0) × 10^{-3}	0.318 M KNO_3 (D_2O)
	6.1 (6) × 10^{-3}	1.2 (3) × 10^{-2}	0.103 M NaCl (D_2O)
	6.8 (0.6) × 10^{-3}	n.a.	0.318 M KNO_3, pH 7.5
	1.53 (0.20) × 10^{-2}	1.01 (0.16) × 10^{-2}	Me_2SO-d_6/saline, unbuffered
V	≲3.0 × 10^{-3}	≲3.0 × 10^{-3}	0.318 M KNO_3 (D_2O)
	≲3.0 × 10^{-3}	≲3.0 × 10^{-3}	0.103 M NaCl (D_2O)
Zr	4.92 (2) × 10^{-2}	3.8 (1.0) × 10^{-2}	0.318 M KNO_3 (D_2O)
	5.4 (6) × 10^{-2}	3.8 (8) × 10^{-2}	0.103 M NaCl (D_2O)

[a] From a least-squares fit to a ln ([C_5H_5D]) vs. time plot. [b] See ref 41. n.a. = not applicable.

Results

The initial focus of this investigation was to characterize Cp_2MX_2 species present in aqueous solutions by first assessing the hydrolytic lability of the η^5-C_5H_5 (e.g., eq 2) and chloride (e.g., eq 3 and 4) ligands. Experiment design included both physiological and nonphysiological conditions as well as control of ionic



(2)

$$Cp_2MCl_2 + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Cp_2M(H_2O)Cl^+ + Cl^- \quad (3)$$

$$Cp_2M(H_2O)Cl^+ + H_2O \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Cp_2M(H_2O)_2^{2+} + Cl^- \quad (4)$$

(34) (a) ^{1}H NMR data: δ 2.91 (multiplet), 6.62 (multiplet), 6.57 (multiplet). Under the conditions of these experiments, the half-life for suprafacial, sigmatropic 1,5-hydrogen rearrangement is expected to be on the order of minutes.[34b,c] Thus, the deuterium atom is expected to be scrambled among all three skeletal positions; within experimental error, the relative intensities of the three ^{1}H signals were found to be identical. (b) Roth, W. *Tetrahedron Lett.* **1964**, 1009–1013. (c) McLean, S.; Haynes, P. *Tetrahedron* **1965**, *21*, 2329–2342.

(35) Conditions: Dicyclopentadiene (Aldrich Chemical Co.) is heated to 150–160 °C for several hours under partial vacuum. The monomer is collected on a Vigreux column and is stored at −30 °C. See: Barton, D., Ollis, W. D., Eds. "Comprehensive Organic Chemistry"; Pergamon Press: Oxford, 1979; Vol. 1, p 179.

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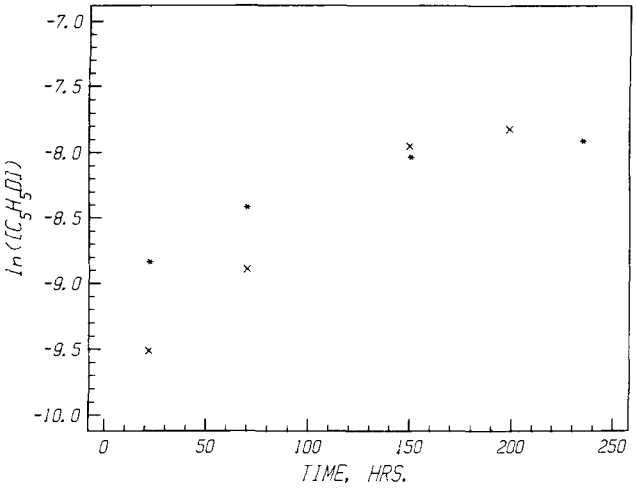


Figure 2. ln ($[C_5H_5D]$) vs. time plot for Cp_2TiCl_2 hydrolysis in D_2O at 37.0 °C followed by ^1H NMR. (×) $[Cp_2TiCl_2]_0 \cong 0.870$ mM in 0.318 M KNO_3, (*) $[Cp_2TiCl_2]_0 \cong 0.977$ mM in 0.103 M NaCl. In both cases, cyclopentadiene/Ti ≈0.5 after 200 h.

strength (0.32 M KNO_3[17,28]) and efforts to draw comparison to cisplatin.[17,28] The integrity of the $M(\eta^5\text{-}C_5H_5)_2$ framework (75% of the formal coordination sphere) was investigated first.

Cyclopentadienyl Protonolysis. The appearance of free cyclopentadiene was monitored for Cp_2MCl_2 solutions by high-field ^1H NMR. Appropriate account was taken of the fact that the anticipated product in D_2O is C_5H_5D,[34] and controls were carried out to measure the rate of proton exchange with solvent deuterons.[41] Numerical data are summarized in Table I. In the case of Cp_2TiCl_2 in KNO_3/D_2O solutions, significant amounts of cyclopentadiene are only detected after many hours. The appearance of free cyclopentadiene initially follows approximate first-order kinetics (e.g., Figure 2) and the half-life for Cp_2TiCl_2 ring loss by this process is 57.0 ± 0.9 h at 37 °C. After 200 h, cyclopentadiene/Ti ~ 0.5. No new $\eta^5\text{-}C_5H_5$ ^1H resonances are detected over the course of several days (Figure 3A), and NMR verification with an authentic sample of $[CpTi(Cl)O]_3$[19a] gave no evidence for detectable quantities of this complex.[42] Thus, the concentration of oligomeric products appears to be negligible under these conditions. Similar kinetic and product results were obtained for Cp_2TiCl_2 in KNO_3/H_2O solutions. Interestingly, when the chloride concentration of the Cp_2TiCl_2 NMR samples is increased to the level in blood plasma (0.103 M NaCl), the rate of ring protonolysis is not greatly affected. At 37 °C, we estimate that $t_{1/2} = 114 \pm 11$ h.

In comparison to Cp_2TiCl_2, Cp_2VCl_2 is even more resistant to $\eta^5\text{-}C_5H_5$ ring protonolysis. At 37 °C without added chloride (0.32 M KNO_3), we estimate the $t_{1/2}$ for this process must be greater than 10 days. After 26 days, cyclopentadiene/V ≈0.02. Experiments were also conducted with solutions 0.103 M in NaCl; there was no detectable (<1%) ring loss after 1 week at 37 °C. In contrast to the titanium and vanadium complexes, Cp_2ZrCl_2 suffers rapid $\eta^5\text{-}C_5H_5$ hydrolysis, with a half-life of 14.1 ± 0.6 h at 37 °C (0.318 M KNO_3). In 0.103 M NaCl, the estimated half-life is 12.7 ± 1.4 h. As can be seen in Figure 3B, a multitude of $\eta^5\text{-}C_5H_5$ resonances is observable after several hours.

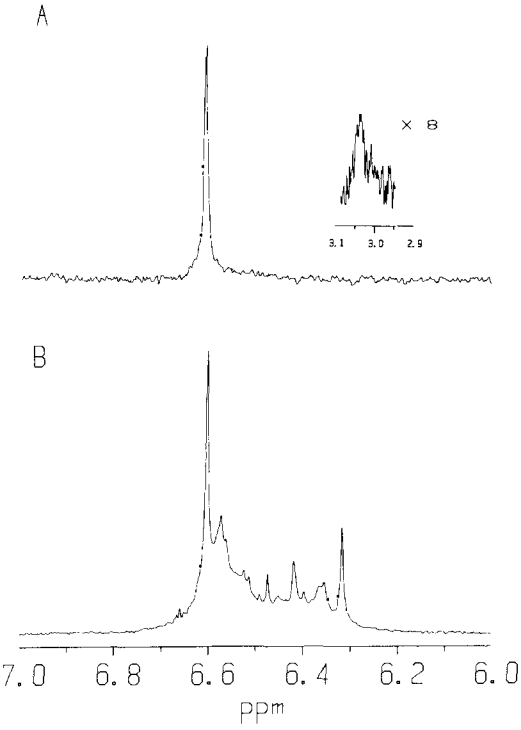


Figure 3. ^1H NMR (270 MHz) spectra of (A) Cp_2TiCl_2 in 0.103 M NaCl (D_2O) at 37.0 °C after ~70 h, and (B) Cp_2ZrCl_2 in 0.318 M KNO_3 (D_2O) at 25.0 °C after ~3 h. **Inset** in part A is ×8.

A series of experiments was also carried out at physiological pH. Pilot experiments in D_2O indicated that the amount of free cyclopentadiene released from these complexes could not be measured, since cyclopentadiene was found to undergo rapid deuterium exchange near neutral pH. Since a superior method for water solvent suppression had been reported by Hore[31,32] at this time, experiments were carried out in H_2O with use of this technique. The initial rate of ring loss measured for Cp_2TiCl_2 at pH ~7.5 in 0.318 M KNO_3 was $(6.8 \pm 0.6) \times 10^{-3}$ h^{-1}. This value is identical (within experimental error) with the initial rate measured for Cp_2TiCl_2 in unbuffered 0.318 M KNO_3 (in D_2O) of $(6.4 \pm 1.0) \times 10^{-3}$ h^{-1}, after correcting for the rate of deuterium exchange.[41] Although the rates are similar in these two cases, the first data point taken for Cp_2TiCl_2 at pH ~7.5 already indicated a $[C_5H_6]/[Ti]$ ratio of ≳1.0, and the ratio eventually approached a limiting value of 2.0. In contrast, a limiting ratio of ~0.5 was approached in the case of Cp_2TiCl_2 in unbuffered solution (pH ≈3). These observations are consistent with the empirical formulation assigned to the isolated hydrolysis product at pH ≈7.6 (**1**, vide infra).

Finally, some studies were carried out in $Me_2SO\text{-}d_6/H_2O$(saline) solutions.[6] In unbuffered solutions, the initial rate of ring loss for Cp_2TiCl_2 was found to be $(1.01 \pm 0.16) \times 10^{-2}$ h^{-1}, after correcting for the rate of deuterium exchange of C_5H_6 with aqueous $Me_2SO\text{-}d_6$ (measured in a control experiment).[41] When the pH of a freshly prepared solution of this complex was adjusted to ~5.5 with $NaHCO_3$, a precipitate formed immediately. Analysis of the supernatant by ^1H NMR indicated the presence of only free cyclopentadiene, with no evidence of $\eta^5\text{-}C_5H_5$ resonances. Analysis by IR and elemental analysis (see Experimental Section for details) of the resulting precipitate (**2**) prepared on a larger scale indicated an empirical formula of "$Ti(\eta^5\text{-}C_5H_5)_{1.66}O_{4.5}(OH)_{4.4}$". This formulation indicates a loss of ap-

(41) The process of metal-($\eta^5\text{-}C_5H_5$) cleavage (k_1) followed by deuterium exchange of the expelled cyclopentadiene (k_2) was treated as two consecutive, first-order reactions (A $\xrightarrow{k_1}$ B $\xrightarrow{k_2}$ C). With $[A]_0$, k_2 (estimated by the rate of deuterium exchange of C_5H_6 in D_2O or $Me_2SO\text{-}d_6$) and [B] as a function of time all known, a best value for k_1 was estimated by fitting the measured [B] at a given time to the expression

$$-\frac{dB}{dt} = \frac{[A]_0 k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t})$$

(see: Moore, J. W.; Pearson, R. G. "Kinetics and Mechanisms"; 3rd ed.; John Wiley & Sons: New York, 1981; p 290) through iterative calculations, using the observed, overall rate constant as a first estimate of k_1. A straightforward computer program in BASIC was used to accomplish this. The procedure was carried out for a variety of [B],t pairs, so that the final estimate used for k_1 was obtained by averaging the set of k_1 values obtained from the computer fit from each of the [B],t pairs. A check on the reliability of this final estimate for k_1 was achieved by estimating [B] at any time and comparing the result to the [B] actually measured. In all cases, the estimated [B] was within ~10–15% of that measured.

(42) The ^1H NMR of $(CpTiClO)_3$ in D_2O shows a singlet at δ 6.42, whereas Cp_2TiCl_2 in D_2O shows a singlet of δ 6.62. No resonances were observed at δ 6.42 during the course of the experiment with Cp_2TiCl_2 (~10 days).

Table II. Rate of Second Chloride Ion Loss from Cp_2MCl_2 in 0.318 M KNO_3 Solutions at 37.0 °C (Concentrations Range from ~1.0 to 3.5 mM)

M	k, h^{-1}
Ti	0.84 (0.14)
Zr	1.31 (0.15)
V	1.73 (0.18)

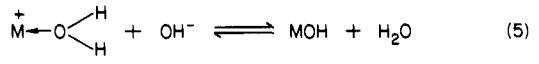
Table III. Equilibrium Constant Data for Chloride Hydrolysis in Cisplatin, Cp_2TiCl_2, and Cp_2VCl_2

compound	K_1, M	K_2, M
cis-$Pt(NH_3)_2Cl_2$[a]	4.37 (13) × 10^{-3}	1.88 (8) × 10^{-3}
Cp_2TiCl_2[b]	*c*	4.2 (2.7) × 10^{-2}
Cp_2VCl_2[b]	*c*	2.7 (1.2) × 10^{-3}

[a] At 35 °C, 0.318 M KNO_3. Reference 17b. [b] At 37 °C, 0.318 M KNO_3. [c] Too large to measure; see text for explanation.

proximately 0.33 equiv of cyclopentadienide per metal. In contrast, when the pH of a freshly prepared Me_2SO-d_6/saline solution of Cp_2VCl_2 was adjusted to ~6.4, no precipitate formed. Interestingly, analysis of the solution soon after addition of $NaHCO_3$ (about 1.5 h; solutions of similar composition were used in in vivo studies[6] immediately after addition of $NaHCO_3$) by ^1H NMR indicated that only ~3.0% free cyclopentadiene had been released (based on equivalents of Cp_2VCl_2).

Chloride Hydrolysis. In the case of cisplatin, the equilibria shown in Figure 1 were deduced primarily by titration of the acidic bound water molecules of the hydrolysis products (eq 5). Such



(5)

an analysis of pH vs. titer also yields the pK_a values for the coordinated water molecules and is, in principle, sensitive to the formation of μ-OH and μ-O polynuclear species.[28b] However, since this approach does not measure free chloride directly, there exists the possibility that certain equilibria may be misconstrued (i.e., titration of bound water without chloride loss). Also, such an approach is only viable when the equilibration time is slow compared to the titration time.

In the case of the Cp_2MCl_2 complexes, chloride hydrolysis was studied not only by the aforementioned titrimetric procedure but also by using a chloride-sensitive electrode. In addition, the chloride-sensitive electrode technique was applied for the first time to the reference compound for this study, cisplatin. The results of the chloride analyses are set out in Tables II and III.

Addition of any of the Cp_2MCl_2 complexes to water (pure or 0.32 M in KNO_3) results in a rapid increase in the chloride ion concentration of the solution. Indeed, by the time that Cp_2MCl_2 dissolution is complete, $[Cl^-]/[M] > 1.0$, and it seems likely that ionization (e.g., eq 3) is closely connected with the dissolution process. The initial portions of the free chloride concentration vs. time plots were found to be approximately linear (see, for example, Figure 4). Fitting a least-squares line to the initial data points after dissolution allows a rough estimation of the half-lives for loss of the second Cp_2MCl_2 chloride ion as equilibrium is approached. This information is set out in Table II. In the time required for chloride loss to reach approximate equilibrium, the concurrent CpH loss is rather minor: less than 7% for M = Ti and less than 1% for M = V. For this reason, ring protonolysis will be ignored in the ensuing analyses of chloride equilibria.

The extensive $[Cl^-]_{equilibrium}$ data for Cp_2TiCl_2 and Cp_2VCl_2 as a function of concentration in unbuffered solutions were first analyzed by assuming a cisplatin model (eq 3) and 4). A variety of numerical approaches failed to yield self-consistent values of K_1 (eq 3) and K_2 (eq 4) over the entire concentration range; it soon became apparent (not surprisingly) that the combined values of K_1 and K_2 for the Cp_2MCl_2 compounds are far too large for such an approach. For example, while equilibrium values of $[Cl^-]/[metal]$ are in the neighborhood of 0.8–1.2 for cisplatin over a fairly large concentration range, $[Cl^-]/[metal]$ for the Cp_2MCl_2 compounds is invariably between 1.5 and 1.9 and relatively in-

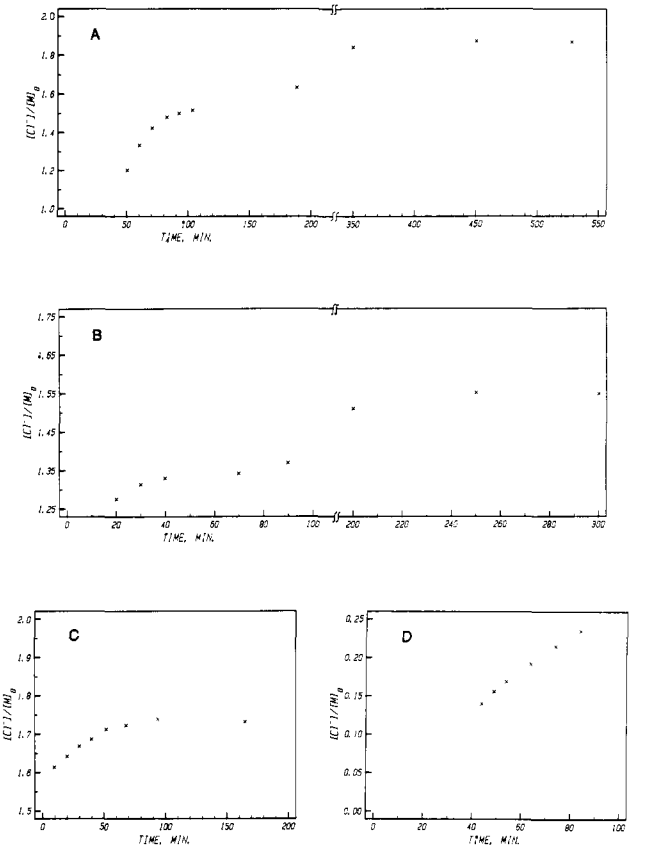


Figure 4. Concentration of free chloride/concentration metal vs. time (from time of mixing) plot for (A) Cp_2TiCl_2, (B) Cp_2VCl_2, (C) Cp_2ZrCl_2, and (D) *cis*-$[Pt(NH_3)_2Cl_2]$. All solutions are 0.318 M in KNO_3 and at 37 °C.

sensitive to concentration. The Cp_2MCl_2 data could, however, be fit satisfactorily (i.e., the experimental $[Cl^-]$ values could be predicted to within 10% over the entire concentration range) to a simple model where $K_1 \gg K_2$ and where significant quantities of oligomers are not present (vide infra). Data and standard deviations are set out in Table III and are compared to K_1 and K_2 reported[17b] for cisplatin by titration of bound water. As a check on the latter data, an equilibrium $[Cl^-]$ measurement was performed on a 0.9983 mM cisplatin solution and compared to the value predicted by the titrimetric data of Martin.[17b] Our value of $[Cl^-]/[cis\text{-}Pt(NH_3)_2Cl_2] = 0.866$ compares favorably with the value of 0.928 predicted from Martin's equilibrium constants.

In the course of obtaining ancillary Cp_2MCl_2 $[Cl^-]$ data via pH titrations, it was discovered that base both accelerates the hydrolysis process and, not surprisingly (cf., eq 3–5), shifts the chloride dissociation equilibria substantially to the right. Thus, for both Cp_2TiCl_2 and Cp_2VCl_2, 2.04 ± 0.06 equiv of NaOH were consumed in reaching the end point of the titration (in ca. 15 min). Chloride-sensitive electrode measurements, made after the addition of 2.0 equiv of base, verified these observations—the equilibria were considerably further to the right ($[Cl^-]/[metal]$ approached 2.0) and were attained within a few minutes. The consumption of ca. 2.0 equiv of base in the titration argues[28b] that the formation of oligomers is minimal under these conditions.

The above titrimetric experiments were also useful for estimating the acidity of the Cp_2M^{2+}-bound water molecules. pK_a values were obtained by using the method of Martin.[43] Although not important for Cp_2VCl_2, the NMR results indicate that CpH loss from Cp_2TiCl_2 is enhanced at elevated pH values (vide supra). Although the effect of such processes on the Cp_2TiCl_2 titrations (and derived pK_a parameters) is difficult to assess quantitatively, the rapidity with which the titrations were carried out (ca. 15 min) and the fact that they were stoichiometric in base (2.0 equiv/Ti)

(43) Martin, R. B. *J. Phys. Chem.* **1971**, *75*, 2657–2661.

Table IV. pK_a Data for Water Molecules Bound to *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ and $Cp_2M(H_2O)_2^{2+}$ Complexes

complex	pK_{a1}	pK_{a2}
cis-$Pt(NH_3)_2(H_2O)_2^{2+}$ [a]	5.6	7.3
$Cp_2Ti(H_2O)_2^{2+}$ [b]	3.51 (5)	4.35 (9)
$Cp_2V(H_2O)_2^{2+}$ [b]	4.73 (3)	5.15 (13)

[a] At 20 °C. Jensen, K. A. *Z. Anorg. Allg. Chem.* **1939**, *242*, 87–91. [b] At 37 °C, 0.318 M KNO_3.

argue that the effect should be minor. The results are summarized and compared to cisplatin in Table IV. That the coordinated water molecules are quite acidic for the present compounds is consistent with being bound to a highly charged metal center. Burgess[44] cites the pK_a of water molecules bound to aqueous Ti(IV) to be in the range −4 to 3.0.

Electrical Conductivity of Cp_2TiCl_2 Solutions. If a compound completely dissociates in the solvent and is a binary electrolyte, then Ω_e and C will obey the Onsager equation:[45]

$$\Omega_e = \Omega_0 - (a\Omega_0 + b)C^{1/2} = \Omega_0 - SC^{1/2} \quad (6)$$

where Ω_0 is the equivalent conductance at infinite dilution and S is the "Onsager slope" which reflects the mobilities of ions in solution. For conductivity measurements made on Cp_2TiCl_2 dissolved in dry Me_2SO, Ω_0 was found to be 24.5 ± 1.0 Ω^{-1} equiv^{-1} cm^{-1}. This value falls within the range of 23–42 Ω^{-1} cm^{-1} equiv^{-1} cited for a noncomplex 1:1 electrolyte in Me_2SO.[46]

Attempts to identify the Cp_2TiCl_2 electrolyte type in aqueous solution, using the method of Feltham and Hayter,[47,48] failed, for the Onsager slope determined in H_2O did not correlate with a unique electrolyte type. This could be attributed to the fact that a mixture of 1:1 and 2:1 electrolytes is expected to exist in Cp_2MCl_2 aqueous solutions (see Chloride Hydrolysis Section and Figure 1).

Isolation of Cp_2TiCl_2 Hydrolysis Products. Since it was noted that precipitation occurs when base is added to aqueous Cp_2TiCl_2 solutions, efforts were made to identify the precipitate. Preparative scale reactions (see Experimental Section for details) were employed. On the basis of solid-state ^{13}C NMR, IR, and elemental analysis, the empirical formula "$Ti(\eta^5\text{-}C_5H_5)_{0.31}O_{0.30}(OH)$" represents the simplest formulation for **1**. The stoichiometries of **1** and **2** are consistent with our and the observations of others[49] that Cp_2Ti^{IV} ring loss becomes more facile at higher pH values. The IR spectra of **1** and **2**,[50] as well as the solid-state ^{13}C CPMAS NMR spectrum of **1** (single resonance at δ 115.3), indicate the presence of oligomeric, μ-OH or μ-O complexes (Ti–O–Ti stretch similar to that reported for $[(\eta^5\text{-}C_5H_5)TiClO]_4$[19a]) with magnetically equivalent η^5-C_5H_5 ligands. The η^5-C_5H_5 chemical shift of **1** is comparable to that found for Cp_2TiCl_2 in the solid state (~116 ppm).[51]

In contrast to the Cp_2TiCl_2 result, it was found that pH adjustment of aqueous solutions of Cp_2VCl_2 up to pH 8 with NaOH did not cause precipitation.

Discussion

This work provides the most complete picture to data of the fate of the Cp_2TiCl_2 and Cp_2VCl_2 antitumor agents in aqueous solution. This information, particularly with regard to the hydrolytic lability of the M–(η^5-C_5H_5) and M–Cl bonds, should serve as a foundation for understanding the chemical behavior of these complexes in biological systems. In the case of Cp_2TiCl_2, it is clear that the Cp_2Ti^{2+} framework is not intact for very long at physiological pH. This result considerably complicates theories that metal-bound cyclopentadienide acts as a carrier ligand into the cell.[13] However, the reactivity of the Cp_2M^{2+} framework under physiological conditions in the presence of serum components is still unknown.

Cyclopentadienyl Protonolysis. The observed lability of the M–C_5H_5 bond in unbuffered Cp_2MCl_2 solutions can be correlated with, among other factors, the effective eight-coordinate ionic radius of M: V(IV), 0.72 Å; Ti(IV), 0.74 Å; and Zr(IV), 0.84 Å.[52] It is reasonable that larger and less coordinatively saturated metal coordination spheres will facilitiate hydrolytic cyclopentadienyl attack. The present results show that the M–C_5H_5 bond in the antitumor active compounds Cp_2TiCl_2 and Cp_2VCl_2 is expected to be stable over a period of days in unbuffered, low-pH solutions having the chloride ion concentration present in human plasma (103 mM). The behavior of Cp_2ZrCl_2 is clearly different, and ring protonolysis is rapid in unbuffered solutions. In contrast to low-pH results, Cp_2TiCl_2 ring hydrolysis is rather extensive at pH values approaching physiological and most of the compound precipitates as **1** with the loss of ca. 1.69 equiv of cyclopentadiene per titanium. Protonolysis of the first cyclopentadienyl ring is very rapid, while loss of the second proceeds at a rate comparable to the initial ring loss in unbuffered solutions. Of the three metallocene dihalides investigated, only Cp_2VCl_2 is appreciably stable to M–C_5H_5 protonolysis at physiological pH values. In regard to therapeutic media, we find that the M–C_5H_5 bond of Cp_2TiCl_2 is labile in Me_2SO/saline solutions buffered to pH ~5.5 with $NaHCO_3$ (precipitating **2**), while that of Cp_2VCl_2 is far more stable. These results may help to rationalize in vivo observations[6] that Cp_2TiCl_2 induces chemical peritonitis while Cp_2VCl_2 does not.

Chloride Hydrolysis. The kinetics of the chloride hydrolysis of the Cp_2MCl_2 compounds exhibit some striking differences from those of cisplatin. Using our data, we estimate the half-lives for the first and second hydrolysis steps for cisplatin to be ~154 ± 9 and ~890 min, respectively, at 37 °C. The half-lives for the first hydrolysis step of the Cp_2MCl_2 complexes are so short that they cannot be measured by chloride potentiometry, and the half-lives for the second chloride displacement by water are ca. 20–40-fold less than that measured for cisplatin (see Table II). The Cp_2TiCl_2 half-life is ca. twice that of Cp_2VCl_2.

The differences between the thermodynamics of the chloride hydrolysis of cisplatin and the Cp_2MCl_2 complexes are consistent with such qualitative correlations as HSAB[16] theory as well as oxophilicity. The equilibrium constant for the first hydrolysis step for the Cp_2MCl_2 complexes is too large to be estimated by our technique, whereas K_1 for cisplatin is 4.37 (13) × 10^{-3} M.[17b] The K_2 of Cp_2TiCl_2 is about 22 times greater than that of cisplatin and about 16 times greater than that of Cp_2VCl_2. The free energy for the second hydrolysis step for Cp_2TiCl_2 is thus approximately 1.9 kcal/mol more favorable than that for cisplatin. Equilibrium studies were not carried out with Cp_2ZrCl_2, since the Zr–C_5H_5 bond is so labile in aqueous solution that the aforementioned model for the hydrolysis equilibria could not be applied.

The combined Cp_2MCl_2 chloride potentiometry and bound water titrimetric results (Tables II–IV) reveal interesting quantitative facts about M–Cl integrity under physiological conditions. Unlike mM cisplatin, where all six complexes pictured in Figure 1 are present to some extent at pH ≈7, and where endocytosis from plasma ([Cl^-] = 103 mM) to intracellular regions ([Cl^-] ≈ 4 mM) is expected to result in very substantial (although not necessarily rapid) Pt–Cl hydrolysis, the behavior of the Cp_2MCl_2 complexes is predicted to be quite different. Not only is rapid, essentially quantitative chloride loss predicted even in plasma, but the predominant form of the hydrolysis products will be as hydroxo rather than as aquo complexes. Thus, for Cp_2VCl_2, the only member of the present series for which cyclopentadienyl ligation is likely to remain intact, analogy to Figure 1 predicts the predominant species in plasma to be $Cp_2V(OH)_2$.

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Conclusions

This study presents a quantitative physicochemical analysis of the aqueous chemistry of the Cp_2MCl_2 compounds (M = Ti, V, Zr). The chloride hydrolysis of these compounds has been shown to be both more rapid and more extensive than that of the reference compound for this study, cisplatin, although the data obtained from both classes of compounds can be analyzed with essentially the same equilibrium model. A key difference between the hydrolysis behavior of these two classes of compounds is that for the Cp_2MCl_2 complexes, another concurrent process, namely loss of cyclopentadienide, can become important depending upon the nature of M and the solution pH. In the case where M = Ti, ring loss is extensive at physiological pH, while it is not observed with Cp_2VCl_2. Since both of these compounds are antitumor active, any discussion of their mechanism of action must take this observation into account. It is suggested that, because of the structural integrity of the Cp_2V^{2+} framework under physiological conditions, Cp_2VCl_2 should be the compound of choice for any future studies involving Cp_2MCl_2–biomolecule interactions.

Acknowledgment. We are grateful to National Science Foundation (CHE8306255) and to Sigma Xi (Grant-in-Aid award to J.H.T.) for support of this research. We also thank Dr. Paul J. Toscano for assistance in recording the CPMAS ^{13}C NMR spectrum of **1**, Ray Wieboldt for assistance in developing the methodology for H_2O solvent suppression in the ^1H NMR experiments, and Scott W. Campbell for assistance in the numerical analyses.

Registry No. $Cp_2Ti(H_2O)_2^{2+}$, 75576-50-0; $Cp_2V(H_2O)_2^{2+}$, 93895-89-7; Cp_2TiCl_2, 1271-19-8; Cp_2VCl_2, 12083-48-6; Cp_2ZrCl_2, 1291-32-3.

Metastable Fe/S Clusters. The Synthesis, Electronic Structure, and Transformations of the $[Fe_6S_6(L)_6]^{3-}$ Clusters (L = Cl^-, Br^-, I^-, RS^-, RO^-) and the Structure of $[(C_2H_5)_4N]_3[Fe_6S_6Cl_6]$

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Abstract: A series of new novel $[Fe_6S_6(L)_6]^{3-}$ clusters (L = Cl, Br, I, SC_6H_4-*p*-CH_3, OC_6H_4-*p*-CH_3) have been synthesized and characterized as their Et_4N^+ salts. The crystal structure of $(Et_4N)_3Fe_6S_6Cl_6 \cdot CH_3CN$ (I) is described in detail. The latter crystallizes in the $C2/c$ space group with cell constants a = 20.092 (5) Å, b = 17.937 (6) Å, c = 13.790 (4) Å, β = 91.33 (2)°, Z = 4, and V = 4968 Å^3. The structure was solved by conventional methods from 2604 reflections and was refined by full-matrix least-squares techniques (206 parameters) to a final R value of 0.047. The anion in I contains the new $[Fe_6S_6]^{3+}$ distorted hexagonal prismatic core which consists of alternating tetrahedral Fe and triply bridging S atoms. Three of the Fe coordination sites are occupied by core sulfide atoms while the fourth coordination site is filled by the terminal chloride ligands. There are two sets of Fe···Fe distances and Fe–S–Fe angles in the Fe_6S_6 core with mean values of 2.765 (3) Å, 3.790 (8) Å and 74.7 (1)°, 113.2 (3)°, respectively. The $[Fe_6S_6]^{3+}$ core appears to be a metastable entity and is easily transformed, upon heating, to the thermodynamically more stable $[Fe_4S_4]^{2+}$ core. The chemical properties and electronic spectra of these clusters are reported. The clusters in CH_2Cl_2 solution display a reversible 3-/2- couple and an irreversible 3-/4- couple. Zero field and magnetically perturbed Mössbauer spectra are reported for all clusters. Their isomer shift and quadrupole splitting values are quite similar to those in the corresponding $(Fe_4S_4(L)_4)^{2-}$ cubane clusters. The $(Fe_6S_6(L)_6)^{3-}$ prismane clusters exhibit characteristic electron paramagnetic resonance (EPR) spectra (9 K) indicative of $S = {}^1/_2$ ground states. The Mössbauer and EPR results, in conjunction with magnetic susceptibility data in the 1.5–300 K temperature range for I, also are consistent with an $S = {}^1/_2$ ground state. The biological implications of the $[Fe_6S_6]$ cores are discussed.

Synthetic analogues for the $(Fe_4S_4(Cys)_4)^{2-,3-}$, $(Fe_2S_2(Cys)_4)^{2-}$, and $(Fe(Cys)_4)^{2-,1-}$ active sites in the non-heme iron proteins (NHIP) have been synthesized and structurally characterized.[1-3] The analogue complexes contain aliphatic or aromatic thiolate terminal ligands in place of the cysteinyl residues, and their syntheses can be accomplished by various procedures. The "spontaneous self-assembly" synthesis[4] of the $(Fe_4S_4(SR)_4)^{2-}$ and $(Fe_2S_2(SR)_4)^{2-}$ clusters from mixtures of appropriate reactants occurs readily and its success is based on the premise that "... clusters derived from substitutionally labile iron(II,III) reactants form as a consequence of being the thermodynamically most stable, soluble reaction products".[4]

More recently spontaneous self-assembly reactions have been employed successfully in the synthesis of interesting clusters with less direct biological relevance which include the $(Fe_6S_9(SR)_2)^{4-}$,[5] $(Fe_3S_4(SR)_4)^{3-}$,[6] and $(Fe_6(S)_8(PEt_3)_6)^{2+}$[7] ions. In these complex

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