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Synthesis, characterization and biological activity of organotitanium substituted heteropolytungstates

Xiao-Hong Wang,[a] Jing-Fu Liu,*[a] Ya-Guang Chen,[a] Qun Liu,[a] Ju-Tao Liu[a] and M. T. Pope[b]

[a] *Department of Chemistry, Northeast Normal University, Changchun, 130024, P.R. China*
[b] *Department of Chemistry, Georgetown University, Washington, D.C. 20057, USA*

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Eight new compounds α- and β-$M_xH_y[(CpTi)_3XW_9O_{37}]\cdot nH_2O$ ($M = K^+$, $x = 4$, $y = 3$; $M = NBu_4^+$, $x = 7$, $y = 0$; X = Si, Ge) have been synthesized from vacant heteropolytungstate precursors α-, β-$[XW_9O_{34}]^{10-}$ (X = Si, Ge) and Cp_2TiCl_2. The products were characterized by elemental analysis, IR, UV–VIS spectroscopy, ^{1}H NMR, ^{183}W NMR spectroscopy and polarography. ^{183}W NMR spectra of the complexes support the stoichiometry of the new heteropolyanions and the probable retention of the A-XW_9 units in water. The organotitanium substituted complexes showed promising activity in two human tumor cell lines *in vitro*.

The impetus for this work lies both in antiviral or antitumor activity and oxidations by the environmentally attractive oxidant H_2O_2 polyoxometalates (POMs). Heteropolyanions and isopolyanions have attracted attention in view of their potential application in catalysis,[1] and as antiviral and antitumoral agents.[2] Earlier work has demonstrated that the $[CpTi]^{3+}$ group can be incorporated into mono-lacunary Keggin or Dawson structures.[3–5] These complexes exhibited biological activity as a new potent class of anti-HIV agent,[6] and can be used as small individually distinguishable labels for conventional transmission electron microscopy.[5] However, little has been reported for $[CpTi]^{3+}$ trisubstituted polytungstates. The synthesis of $[(CpTiOH_2)_3P_2W_{18}O_{68}]^{12-}$ was reported a few years ago,[7] but no in depth characterization or investigation into its properties were given.

It has been known for some years that Cp_2TiCl_2 or complexes containing the $[CpTi]^{3+}$ group exhibit high antitumor activity, but their insolubility in water has restricted their application in chemotherapy. It is thus important to incorporate $[CpTi]^{3+}$ into soluble complexes for applications in chemotherapy. Owing to the instability of three-vacant heteropolytungstates and Cp_2TiCl_2 in aqueous solution, difficulties arise in preparing the $[CpTi]^{3+}$ trisubstituted heteropoly compounds. In this work we attempted to synthesize water-soluble $[(CpTi)_3XW_9O_{37}]^{n-}$ complex anion salts ($(CpTi)_3XW_9$) and investigate their antitumor activity.

This paper describes the synthesis and characterization of organotitanium trisubstituted heteropoly complexes derived from the lacunary α- and β-$[XW_9O_{34}]^{10-}$ anions (X = Si, Ge). MTT experiments on the resulting complexes show promising activity against Hela and SSMC-7721 human tumor cell lines.

Experimental

Starting materials

The compounds α-$Na_{10}[XW_9O_{34}]\cdot nH_2O$ (X = Si, Ge) and β-$Na_9H[XW_9O_{34}]\cdot 23H_2O$ (X = Si, Ge) were prepared according to ref. 8 while Cp_2TiCl_2 was prepared following ref. 9, and were identified by IR spectroscopy.

Physical measurements

^{1}H NMR spectra were recorded on a Bruker AC-80 spectrometer while ^{183}W NMR were recorded on a Unity-400 spectrometer. IR spectra were recorded on an Alpha Centauri FTIR spectrometer in the range 4000–200 cm^{-1} as KBr pellets. UV spectra were recorded for aqueous solutions on a Beckman Du-8D spectrometer. Polarograms were obtained using a 384B Polarographic Analyser.

W, Ti, Si and Ge contents were determined using a Leeman corporation inductively coupled plasma(ICP) emission spectrometer while C, N and H contents were determined using a PE-2400 analyser and K was determined by atomic absorption spectroscopy (PE-3030). Water contents were determined by thermogravimetry.

Preparation of complexes

α-$K_4H_3[(CpTi)_3SiW_9O_{37}]\cdot 12H_2O$ and α-$(NBu_4)_7[(CpTi)_3SiW_9O_{37}]$. A solution containing Cp_2TiCl_2 (1.245 g, 5 mmol), acetylacetone (6 mmol) and water (30 cm^3) was stirred for *ca.* 4 h to give a clear scarlet solution. Powdered α-$Na_{10}[SiW_9O_{34}]\cdot nH_2O$ (4.6 g, 1.7 mmol) was then added and after vigorous stirring for 1 h the solution turned from red to yellow. The undissolved residue was filtered off and KCl was added to the filtrate in small portions until no more precipitation was observed. The resulting yellow solid was recrystallized from hot water and dried under suction, giving a yield of 2.8 g (Calc. for α-$K_4H_3[(CpTi)_3SiW_9O_{37}]\cdot 12H_2O$: Si, 0.93; W, 55.42; Ti, 4.82; K, 5.22; C, 6.02; H_2O, 7.22. Found: Si, 0.87; W, 55.92; Ti, 4.90; K, 5.17; C, 6.10; H_2O, 7.28%).

$K_4H_3[(CpTi)_3SiW_9O_{37}]\cdot 12H_2O$ (1 g, 0.33 mmol) was dissolved in water (30 cm^3), and NBu_4Br (1 mmol) was added to give a yellow precipitate of the tetrabutylammonium salt which was recrystallized from warm acetonitrile. Yield 0.89 g (Calc. for α-$(NBu_4)_7[(CpTi)_3SiW_9O_{37}]$: C, 35.37; H, 6.20; N, 2.27; Si, 0.65; W, 38.43; Ti, 3.34. Found: C, 35.44; H, 6.23; N, 2.31; Si, 0.73; W, 38.11; Ti, 3.23%).

α-$K_4H_3[(CpTi)_3GeW_9O_{37}]\cdot 10H_2O$ and α-$(Bu_4N)_7[(CpTi)_3GeW_9O_{37}]\cdot H_2O$. $K_4H_3[(CpTi)_3GeW_9O_{37}]\cdot 10H_2O$ and $(NBu_4)_7[(CpTi)_3GeW_9O_{37}]\cdot H_2O$ were prepared analogously. Powdered α-$Na_{10}[GeW_9O_{34}]\cdot nH_2O$ (4.8 g, 1.7 mmol) was added to a solution of Cp_2TiCl_2 (5 mmol) and KCl was added to precipitate α-$K_4H_3[(CpTi)_3GeW_9O_{37}]\cdot 10H_2O$. Recrystallization from hot water gave a yellow powder. Yield: 2.6 g (Calc. for α-$K_4H_3[(CpTi)_3GeW_9O_{37}]\cdot 10H_2O$: C, 6.00; H, 0.60; Ge, 2.43; W, 61.35; Ti, 4.80; K, 5.20; H_2O, 6.02. Found: C, 6.10; H, 0.64; Ge, 2.51; W, 61.04; Ti, 4.89; K, 5.31; H_2O, 6.07%).

The preparation of α-$(NBu_4)_7[(CpTi)_3GeW_9O_{37}]\cdot H_2O$ was carried out similarly to that of α-$(NBu_4)_7[(CpTi)_3SiW_9O_{37}]$. Yield: 0.80 g (Calc. for α-$(NBu_4)_7[(CpTi)_3GeW_9O_{37}]\cdot H_2O$: C, 30.76; H, 6.11; N, 2.24; Ge, 1.67; W, 37.88; Ti, 3.29; H_2O, 0.42.

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Table 1 IR and UV spectra

Anion (Salt)	$\nu_{as(W-O_d)}/cm^{-1}$	$\nu_{as(W-O_b-W)}/cm^{-1}$	$\nu_{as(W-O_c-W)}/cm^{-1}$	$\nu_{as(X-O_a)}/cm^{-1}$	λ_{max}/nm
α-$(CpTi)_3SiW_9$ (K)	973	884	798, 771	920	200, 261
β-$(CpTi)_3SiW_9$ (K)	970	887	814, 778	913	202, 268
α-$(CpTi)_3GeW_9$ (K)	959	875	798, 759		201, 261
β-$(CpTi)_3GeW_9$ (K)	963	886	810, 753		203, 270
α-SiW_{12} (H)	980	894, 878	780	925	
β-SiW_{12} (H)	984	865	791	917	
α-GeW_{12} (H)	979	880	769	823	
β-GeW_{12} (H)	980	895	775	828	

Found: C, 30.97; H, 6.14; N, 2.31; Ge, 1.74; W, 38.11; Ti, 3.31; H_2O, 0.40%).

β-$K_4H_3[(CpTi)_3XW_9O_{37}]\cdot nH_2O$ and β-$(NBu_4)_7[(CpTi)_3XW_9O_{37}]$ (X = Si, Ge). These salts were prepared analogously using the same quantities as above.

Yield of β-$K_4H_3[(CpTi)_3SiW_9O_{37}]\cdot 11H_2O$, 2.9 g (Calc.: C, 6.06; H, 0.61; Si, 0.94; W, 55.72; Ti, 4.84; K, 5.25; H_2O, 6.66. Found: C, 6.11; H, 0.58; Si, 0.89; W, 56.23; Ti, 4.89; K, 5.17; H_2O, 6.73%).

Yield of β-$K_4H_3[(CpTi)_3GeW_9O_{37}]\cdot 10H_2O$, 2.3 g (Calc.: C, 6.00; H, 0.60; Ge, 2.43; W, 61.35; Ti, 4.80; K, 5.20; H_2O, 6.02. Found: C, 6.08; H, 0.63; Ge, 2.54; W, 62.00; Ti, 4.89; K, 5.34; H_2O, 6.04%).

Yield of β-$(NBu_4)_7[(CpTi)_3SiW_9O_{37}]$, 0.91 g (Calc.: C, 35.37; H, 6.20; N, 2.27; Si, 0.65; W, 38.43; Ti, 3.34. Found: C, 35.52; H, 6.24; N, 2.30; Si, 0.71; W, 38.00; Ti, 3.25%).

Yield of β-$(NBu_4)_7[(CpTi)_3GeW_9O_{37}]$, 0.78 g (Calc.: C, 35.00; H, 5.81; N, 2.25; Ge, 1.68: W, 38.03; Ti, 3.31. Found: C, 34.89; H, 5.79; N, 2.24; Ge, 1.73; W, 38.34; Ti, 3.28%).

Antitumor activity studies

The antitumor activity of POMs on two human cancer cells was tested by the MTT experiment described below.

MTT, 3-[4,5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide, also known as thiazolyl blue, is a dye which can accept a hydrogen atom. Surviving tumor cells are able to reduce the yellow MTT to an insoluble blue formazan in water whereas dead tumor cells do not possess this capability. The formazan product is dissolved in DMSO and then determined colorimetrically with a Microplate Reader (490 nm).

Subcultured Hela and SSMC-7721 cells were suspended in 0.25% trypsin. The cell suspension (*ca.* 10^5–10^6 cells mL^{-1}) was added to a 96 well plate (100 μL per well) and incubated at 37 °C in a 5% CO_2 incubator for 24 h. 100 μL samples containing POMs were then added. After 72 h, 20 μL MTT solution (5 mg mL^{-1} in 0.01 M PBS (phosphate buffer solution)) was added and the mixture allowed to incubate for 4 h. The supernatant was removed and DMSO (150 μL) added. The resulting mixture was shaken for 10 min at room temperature and colorimetric analysis was used to examine the cell survival rate and the effective cell 50% lethal concentrations (IC_{50}) were calculated by a statistical method.

The samples containing POMs were obtained by dissolving the POMs in PBS, autoclaving and diluting by a RPMI 1640 medium with water to a final concentration of 100, 10 or 1 μg mL^{-1}.

Results and discussion

Preparation of POMs

In a previous report,[7] the synthesis of $K_7Na_2[(CpTiOH_2)_3P_2W_{18}O_{68}]\cdot 15H_2O$ was carried out by the reaction of $CpTiCl_3$ with $Na_8HPW_9O_{34}\cdot xH_2O$ in water, but only a low yield of product was obtained. On changing the Ti precursor to $[Cp_2Ti(acac)]^+$, α- and β-$[(CpTi)_3XW_9O_{37}]^{7-}$ anions were formed in high yields according to the overall process outlined in eqns. (1) and (2).

Table 2 ^1H and ^{183}W NMR spectra[a]

Anion	$\delta(^1H)$	$\delta(^{183}W)$
α-$(CpTi)_3(SiW_9)$	6.57	−92.78(2),[b] −129.11(1)
β-$(CpTi)_3(SiW_9)$	6.57	−103.64(2), −147.96(1)
α-$(CpTi)_3(GeW_9)$	6.61	−49.87(2), −122.83(1)
β-$(CpTi)_3(GeW_9)$	6.59	−67.22(2), −112.37(1)

[a] For the NBu_4 salts dissolved in CD_3CN. [b] Relative intensities in parentheses.

Table 3 Polarographic half-wave potentials for the K salts dissolved in buffer

Anions	E_I/V	E_{II}/V	E_{III}/V
α-$(CpTi)_3SiW_9O_{37}{}^{7-}$	−0.526	−0.808(4)	
β-$(CpTi)_3SiW_9O_{37}{}^{7-}$	−0.524	−0.732(2)	−0.912(2)
α-$(CpTi)_3GeW_9O_{37}{}^{7-}$	−0.460	−0.646(2)	−0.816(2)
β-$(CpTi)_3GeW_9O_{37}{}^{7-}$	−0.463	−0.668(2)	−0.847(2)

$$Cp_2TiCl_2 + Hacac \longrightarrow [Cp_2Ti(acac)]^+ + H^+ + 2Cl^- \quad (1)$$

$$3[Cp_2Ti(acac)]^+ + XW_9O_{34}{}^{10-} \longrightarrow [(CpTi)_3XW_9O_{37}]^{7-} \quad (2)$$

IR and UV spectra

Observed data and tentative assignments of the main IR and UV bands of the complexes and their precursors are given in Table 1.

Like $XW_{12}O_{40}{}^{4-}$ (XW_{12}), the IR spectra of the substituted heteropolyanions retain the four characteristic peaks $\nu_{as(W-O_d)}$ (O_d, terminal oxygen), $\nu_{as(W-O_b-W)}$, $\nu_{as(W-O_c-W)}$ (O_b, O_c, bridging oxygen) and $\nu_{as(X-O_a)}$ (O_a, central oxygen), indicating that the new complexes retain the basic framework of the Keggin structure. However, the terminal oxygen vibrations for $[(CpTi)_3XW_9O_{37}]^{7-}$ are shifted to lower energy relative to the terminal oxygen vibrations for $XW_{12}O_{40}{}^{4-}$ owing to an increase of negative charge on the polyanion. Additionally, $\nu_{as(W-O_c-W)}$ peaks of the complexes are split probably as a result of a change of anion symmetry, similar to that found for other trisubstituted Keggin anions.[10]

The IR spectra display a single, sharp absorption at 1468–1469 cm^{-1} in the 1300–1500 cm^{-1} region characteristic of the C–C stretch for a η^5-C_5H_5 ligand bonded to Ti^4 while bands at 3032, 3038 cm^{-1} in the 3030–3040 cm^{-1} region arise from the C–H stretches of η^5-C_5H_5.

The UV spectra of the α- and β-$[(CpTi)_3XW_9O_{37}]^{7-}$ anions show two peaks at *ca.* 200 and 261 or 268 nm. The higher energy band can be assigned to a $O_{b/c}\rightarrow W$ charge-transfer transition, while the lower energy band is characteristic of 12-heteropolytungstates and is attributed to $O_d\rightarrow W$ charge-transfer.

^1H and ^{183}W NMR spectra

^1H and ^{183}W NMR spectra are listed in Table 2.

The ^1H NMR spectra of the complexes all display an η^5-C_5H_5 resonance at *ca.* δ 6.60.

Table 4 Inhibitory effect of the complexes on two human tumor cell lines *in vitro*[a]

Anion	Dose/ μg L^{-1}	SSMC-7721 Inhibitory effect (%)	SSMC-7721 IC$_{50}$[b]/ μg L^{-1}	Hela Inhibitory effect (%)	Hela IC$_{50}$/ μg L^{-1}
α-[(CpTi)$_3$SiW$_9$O$_{37}$]$^{7-}$	100	100	21.6	100	38.7
	10	23.5		13.2	
	1	2.4		1.1	
β-[(CpTi)$_3$SiW$_9$O$_{37}$]$^{7-}$	100	100	18.6	100	33.6
	10	27.8		15.9	
	1	2.6		1.5	
β-[(CpTi)$_3$GeW$_9$O$_{37}$]$^{7-}$	100	100	23.7	100	25.6
	10	20.4		19.4	
	1	2.2		2.0	
α-[(CpTi)$_3$GeW$_9$O$_{37}$]$^{7-}$	100	100	11.2	100	21.3
	10	48.0		24.1	
	1	6.4		2.3	

[a] For the potassium salts dissolved in water. [b] The 50% inhibitory concentration (IC$_{50}$) is defined as the concentration which suppresses tumor cells by 50%.

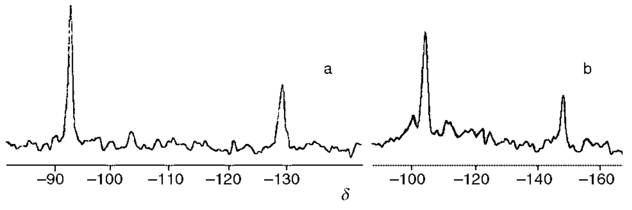

Fig. 1 ^{183}W NMR spectra of α-(CpTi)$_3$SiW$_9$ (a) and β-(CpTi)$_3$SiW$_9$ (b).

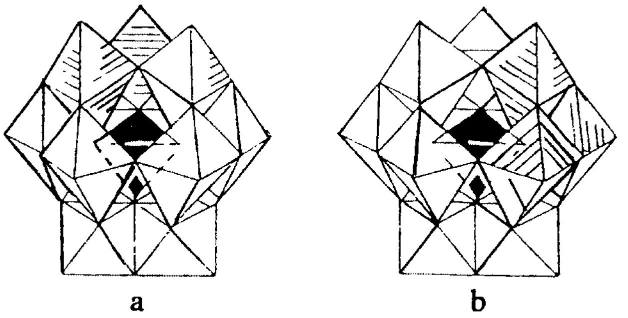

Fig. 2 Polyhedral representation of A-β-XW$_9$(CpTi)$_3$ (a) and B-β-XW$_9$(CpTi)$_3$ (b).

The ^{183}W NMR spectra (Fig. 1) of NBu$_4$ salts of the α- and β-(CpTi)$_3$XW$_9$ anions show two signals of relative intensity 2:1, indicating that there are two types of coordination environment of tungsten in the α- and β-(CpTi)$_3$XW$_9$ anions, corresponding to six and three W centers. This fact supports the stoichiometry of the new heteropolyanions and the probable retention of the A-XW$_9$ unit (Fig. 2).

In such an arrangement, the three substituted [CpTi]$^{3+}$ groups occupy the three vacant sites of the parent cluster in corner-sharing CpTiO$_5$ octahedra.

When three CpTi groups occupy the three vacant sites of α- or β-enneatungstosilicates, two co-ordination possibilities arise, *i.e.*, the arrangement (a) of a triad of corner-sharing CpTiO$_5$ octahedra, showing two non-equivalent tungsten environments (Fig. 2(a)), or an arrangement (b) of a triad of edge-sharing octahedra, which has five non-equivalent tungsten environments (Fig. 2(b)). Clearly, the two-line ^{183}W NMR spectra confirm that the complexes have a-type α- or β-Keggin structures.

Polarography

Polarographic $E_{1/2}$ values were determined at 298 K in 1 mol dm^{-3} acetate buffer solution at pH = 4.7 with a saturated calomel reference electrode and dropping mercury working electrodes. The $E_{1/2}$ data for the complexes in Table 3 show the following characteristics: (1) the reduction processes of α- and β-(CpTi)$_3$XW$_9$ anions involve both the reduction of titanium and tungsten with the first peak (E_I) assigned to the reduction of Ti^{IV} to Ti^{III}. (2) Reduction of W^{VI} of the anions shows one-step four-electron (α-(CpTi)$_3$SiW$_9$), or two-step, two-electron (α,β-(CpTi)$_3$GeW$_9$ and β-(CpTi)$_3$SiW$_9$) reduction process according to analysis of each of the polarographic waves.

Antitumor activity

Antitumor activity data for the complexes were determined by the MTT method as summarized in Table 4.

From Table 4, it is seen that the complexes show high antitumor activity. The degree of inhibition varies for the different lacunary heteropolyanions. The inhibitory effect of the β-isomer, β-(CpTi)$_3$SiW$_9$, is higher than that of α-isomer, α-(CpTi)$_3$SiW$_9$, however, the inhibitory effect of the tungstogermanates is reversed to that of tungstosilicates, the IC$_{50}$ value of α-(CpTi)$_3$GeW$_9$ being higher than that of β-(CpTi)$_3$GeW$_9$. The degree of antitumor activity of the complexes may be related to the order of the redox potentials of the polyanions.

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