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Investigation of Vanadocene(IV) α-Amino Acid Complexes: Synthesis, Structure, and Behavior in Physiological Solutions, Human Plasma, and Blood

Jaromír Vinklárek,*,† Hana Paláčková,† Jan Honzíček,† Jana Holubová,† Michal Holčapek,‡ and Ivana Císařová§

Department of General and Inorganic Chemistry, University of Pardubice, nám. Čs. legií 565, 532 10 Pardubice, Czech Republic, Department of Analytical Chemistry, University of Pardubice, nám. Čs. legií 565, 532 10 Pardubice, Czech Republic, and Department of Inorganic Chemistry, Charles University, Hlavova 2030, 128 40 Prague 2, Czech Republic

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This work is focused on investigating the interaction of antitumor active metallocene vanadocene dichloride (Cp_2VCl_2) and amino acids in aqueous solution at physiological pH. Sixteen vanadocene amino acid complexes $[Cp_2V(aa)][X]$ (aa = gly, ala, val, leu, ile, phe, his, and trp; X = Cl, PF_6) were prepared and characterized on the basis of spectral measurements (EPR, MS, IR, Raman). Amino acids are coordinated to the vanadocene fragment through the oxygen atom of the carboxylic group and the nitrogen of the amino group, resulting in a five-membered chelate ring. Complexes $[Cp_2V(val)][PF_6]$ and $[Cp_2V(ile)][PF_6]$ have been characterized by X-ray structure analyses. It was evidenced that all prepared complexes are stable in both aqueous solutions with physiological pH and in therapeutic NaCl solutions. EPR spectra of vanadocene amino acid complexes in Krebs–Ringer solution in human blood plasma and in whole blood showed that these complexes react with the hydrogen carbonate anion present forming complex $Cp_2V(O_2CO)$.

Introduction

Bent metallocene complexes (Cp_2MCl_2; M = Ti, V, Nb, and Mo) attract considerable attention for their cytostatic activity.[1−9] In the last two decades, Cp_2TiCl_2 (TDC) was the most widely studied metallocene complex by biologists[10−19] and chemists.[20−28] As a result of this comprehensive research, TDC has advanced into phase II of the clinical trials.[29,30]

* To whom correspondence should be addressed. E-mail: jaromir.vinklarek@upce.cz.

† Department of General and Inorganic Chemistry, University of Pardubice.

‡ Department of Analytical Chemistry, University of Pardubice.

§ Charles University.

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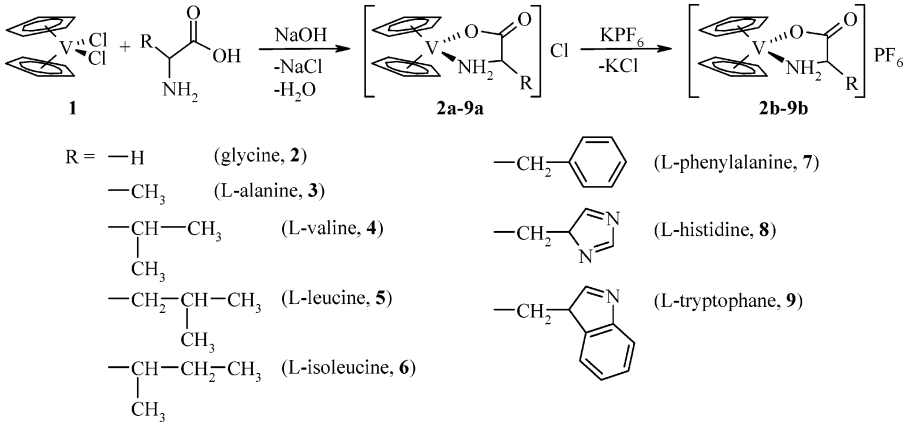
Scheme 1. Preparation of Amino Acid Complexes



Recently, the strong interest in Cp_2VCl_2 (VDC, **1**)[31−37] and Cp_2MoCl_2 (MDC)[38−40] has reappeared because these have some suitable properties such as the greater stability of the Cp_2M moiety.[41] When various metallocenes are compared, there are some distinct differences in both the chemistry of the components of DNA and the cytostatic activity.[42] Although TDC and MDC form stable complexes with alkylated nucleobases and nucleotides,[43−48] no such complexes were found for VDC (**1**).[49] Some studies indicate that the cytostatic activity of bent metallocenes should be ascribed to the inhibition of the DNA processing enzymes such as proteinkinase C[50] and topoisomerase II.[50,51] Therefore, detailed knowledge of the interaction between metallocenes and essential α-amino acids (components of enzymes) is necessary to gain an understanding of the mechanism of cytostatic action at the molecular level. Although titanocene[52,53] and molybdenocene[54−58] derivatives of amino acids were known for a long time, the preliminary work on vanadocene derivatives with the simplest amino acids has been performed only recently.[59]

The aim of this study is to investigate the behavior of vanadocene complexes in water, physiological solutions, human blood plasma, and whole human blood. These observations result in a considerable view of the complexes that are formed before and after metallocene drug applications.

Results and Discussion

Syntheses of α-Amino Acid Complexes. Vanadocene complexes **2a**−**9a** are formed in water by the reaction of vanadocene dichloride (**1**) with an appropriate α-amino acid after neutralization by sodium hydroxide (Scheme 1). The solid powder samples of the amino acid complexes were obtained after the evaporation of the solvent and the crystallization from the acetone−methanol mixture or by the method described previously.[59]

When EPR spectra of the reaction mixture and the methanol solution of the pure product were compared, no differences were observed. Therefore, we can say that only amino acid complexes **2a**−**9a** are present in the reaction mixture. Complexes **2a**−**9a** are extremely soluble in water and methanol. They are almost insoluble in organic solvents (CH_2Cl_2, $CHCl_3$, hexane, acetone, benzene, and toluene).

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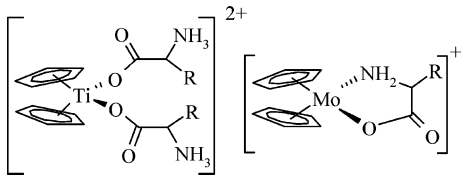

Figure 1. Bonding of amino acids to the metallocene(IV) moiety.

The Cl^- ions can be replaced by a reaction with KPF_6 in water resulting in corresponding PF_6^- salts **2b**−**9b**. In contrast to the chloride complexes (**2a**−**9a**), the PF_6^- complexes (**2b**−**9b**) are much less solubile both in water and methanol, probably because of a large anion effect.

Two bonding modes of the amino acid to bent metallocene are known (Figure 1). The first one is realized exclusively via the oxygen atom of the carboxylic groups of two amino acid molecules. This bonding was observed in the case of titanocene complexes.[52,53,60] The more common chelate bonding mode, realized via both the oxygen of the carboxylic group and the nitrogen of the amino function, was previously found for a majority of molybdenocene derivatives.[54,55] Therefore, the prepared amino acid vanadocene complexes (**2**−**9**) were studied by means of spectroscopic techniques with a view to determine the bonding mode of the amino acid.

IR and Raman Spectroscopy. The integrity of the bent vanadocene moiety $[Cp_2V]^{2+}$ in compounds **2**−**9** was accomplished on the basis of vibrational spectra. The presence of two η^5-bonded cyclopentadienyl rings is evident from medium to strong absorptions at ν_a(C−H) (IR: ∼3140 cm^{-1}), ν_s(C−H) (IR: ∼3096 cm^{-1}, Raman: ∼3105 cm^{-1}), ν_s(C−C) (Raman: ∼1132 cm^{-1}), and δ(C−H) (IR: ∼840 cm^{-1}).[61] The very strong Raman band at ∼285 cm^{-1} (Cp ring tilting) is typical for bent metallocenes.[62]

Intensive bands of the NH_2 stretching vibrations ($\nu_a(NH_2)$ ∼3345 cm^{-1}, $\nu_s(NH_2)$ ∼3210 cm^{-1}) as well as the band of the COO vibration (ν_a(COO) ∼1640 cm^{-1}) in IR spectra of complexes **2**−**9** were observed. This indicates that the amino acid could be bonded through both amino and carboxylic groups. This presumption was confirmed by a comparison with the vibrational spectra of α-amino acid compounds containing the free NH_2 function. Similar observations were reported for other chelate complexes of α-amino acids.[63]

EPR Spectroscopy. From recently published works, it is known that changes in hyperfine coupling (HFC) tensors reflect the different coordination environments around the vanadium atom.[64,65] These HFC differences are often observed both in isotropic EPR spectra (measured in solution) and anisotropic EPR spectra (measured in solid state).

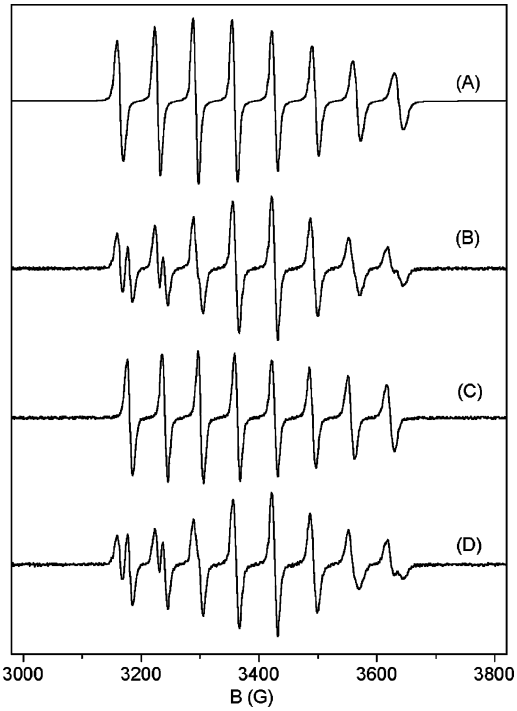

Figure 2. EPR spectra of complex **2a** (ν_0 = 9.46 GHz) in methanol solution (A), in the Krebs−Ringer solution immediately after dissolution (B), in the Krebs−Ringer solution 24 h after dissolution (C), and in whole blood (D).

Table 1. Experimental HFC Tensors (10^{-4} cm^{-1}) and g Tensors of Amino Acid Complexes

	A_{iso}	g_{iso}	A_x	g_x	A_y	g_y	A_z	g_z
2a	62.6	1.986	76.8	1.989	102.7	1.966	7.4	2.003
2b	62.9	1.986	76.8	1.982	102.7	1.969	8.2	2.007
3a	62.5	1.986	77.2	1.986	102.2	1.967	7.1	2.005
3b	62.7	1.985	77.3	1.977	102.2	1.968	7.7	2.010
4a	62.2	1.988	76.6	1.989	102.3	1.967	6.7	2.008
4b	62.3	1.983	76.6	1.979	102.3	1.970	7.3	2.000
5a	62.7	1.989	76.6	1.989	102.5	1.965	7.8	2.013
5b	62.7	1.983	76.7	1.982	102.7	1.969	7.9	1.998
6a	62.5	1.987	76.9	1.981	102.4	1.965	7.0	2.015
6b	62.7	1.985	77.1	1.980	102.7	1.964	7.2	2.011
7a	62.7	1.986	77.7	1.986	102.5	1.964	6.7	2.008
7b	62.5	1.981	76.4	1.979	102.1	1.960	8.0	2.004
8a	62.6	1.985	77.7	1.988	102.6	1.966	6.8	2.001
8b	62.3	1.980	77.3	1.975	102.0	1.965	6.7	2.000
9a	62.6	1.986	76.9	1.991	102.5	1.966	7.6	2.001
9b	62.6	1.985	76.9	1.970	101.4	1.958	7.6	2.027

Amino acids complexes **2**−**9** dissolved in methanol give simple eight-line EPR spectra corresponding to one paramagnetic species (e.g., Figure 2, spectrum A). The anisotropic spectra were obtained from frozen methanol solutions. The HFC tensors of amino acid complexes **2**−**9** were in a very narrow range ($A_{iso} = 62.6 \pm 0.2 \times 10^{-4}$ cm^{-1}, $A_x = 77.0 \pm 0.4 \times 10^{-4}$ cm^{-1}, $A_y = 102.4 \pm 0.3 \times 10^{-4}$ cm^{-1}, and $A_z = 7.3 \pm 0.5 \times 10^{-4}$ cm^{-1}; see Table 1). Replacing Cl^- with PF_6^- as well as using different amino acids does not affect the EPR parameters. From this uniformity, it is evident that the same coordination around the central metal takes place. On the basis of EPR and particularly, the X-ray diffraction analyses (see below) of **4b** and **6b**, the chelate bonding mode of the amino acid was established for complexes **2**−**9**. Additionally, the complexes with two monodentate amino acids (bonded via the oxygen of the

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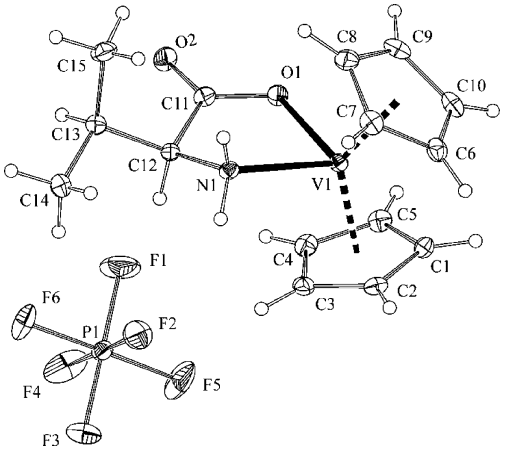

Figure 3. ORTEP drawing of the molecular structure of $[Cp_2V(val)][PF_6]$ (**4b**) with atom numbering (ellipsoids: 30% probability).

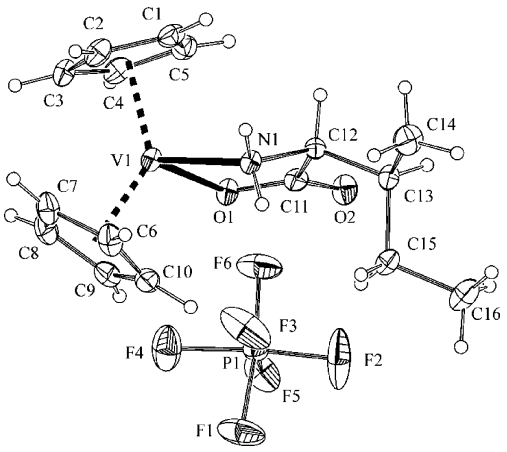

Figure 4. ORTEP drawing of the molecular structure of $[Cp_2V(ile)][PF_6]$ (**6b**) with atom numbering (ellipsoids: 30% probability).

carboxylic group) should give an A_{iso} value in the range that is typical for complexes with monocarboxylic acids ($\sim 75 \times 10^{-4}$ cm^{-1}).[59,65]

The preparation of complexes with two amino acid ligands was attempted by using a large excess of amino acid (1:10). The EPR spectra measured after neutralization as well as the EPR spectra measured after dissolving the products in methanol correspond to complexes **2a**−**9a**. Thus, it is evident that no other type of complex is formed.

Mass Spectrometry. The base peaks of the first-order positive-ion ESI mass spectra of compounds **2a**−**9a** and **2b**−**9b** are the $[M-Cl]^+$ and $[M-PF_6]^+$ ions, respectively. Tandem mass spectra of $[M-Cl]^+$ also provide common features because the $[Cp_2V]^+$ ion at *m*/*z* 181 is the base peak or the second most abundant peak in all cases. The neutral losses observed in MS/MS spectra are C_5H_6 (Δ *m*/*z* 66), CO_2 (44), CO (28), and NH_3 (17). The fragmentation behavior is in accordance with expected structures.

Crystal Structures of $[Cp_2V(val)][PF_6]$ (4b) and $[Cp_2V(ile)][PF_6]$ (6b). Figures 3 and 4 show the molecular structures of complexes **4b** and **6b**, respectively. The important structural parameters are listed in the Table 2. The cationic part in complexes **4b** and **6b** has a typical bent metallocene structure in which the nitrogen and oxygen atoms of the amino acid and two η^5-bonded Cp rings occupy

Table 2. Selected Bond Lengths (Å) and Bond Angles (deg) of Complex **4b** and **6b**

	4b	**6b**
V−Cg1[a]	1.9675(12)	1.966(2)
V−Cg2[b]	1.9630(13)	1.965(2)
Cg1−V−Cg2	133.48(5)	133.16(9)
V−N1	2.148(2)	2.142(3)
V−O1	2.004(16)	2.010(3)
N1−V−O1	77.45(7)	76.72(12)
Pr1−Pr2[c]	46.45(16)	46.7(3)

[a] Cg1 = centroid of atoms C1−C5. [b] Cg2 = centroid of atoms C6−C10. [c] Pr1 = plane defined by atoms C1−C5; Pr2 = plane defined by atoms C6−C10.

the pseudotetrahedral coordination sites around the vanadium(IV) center. Cg−V distances (1.963−1.968 Å) and Cg−V−Cg angles (∼133°) are comparable with those of the other vanadocene(IV) complexes (Cg−V = 1.96−1.97 Å; Cg−V−Cg = 131−135°).[34,65,66] The V−O bond lengths (2.004(16) Å for **4b** and 2.010(3) Å for **6b**) are close to the values found in vanadocene complexes with mono and dicarboxylic acids (2.02−2.04 Å).[65,67] Neighboring cationic units are connected through intermolecular hydrogen bonds between the proton of the amino group and the carbonyl oxygen of the carboxylic group forming a chain of molecules along the *a* axis (**4b**: N(1)···O(2) 2.959(3) Å, N(1)−H(1B)···O(2) 170(3)°; **6b**: N(1)···O(2) 2.957(5) Å, N(1)−H(1B)···O(2) 174°).

Behavior of Complexes 2a−9a in Aqueous Solutions at Various pH. Detailed knowledge of the behavior of prepared complexes in aqueous media has elemental importance because antitumor drugs are in contact with water both when they are dissolving in a therapeutic solution and after they enter into the organism.

On the basis of EPR spectroscopic measurements, it was confirmed that in the water solution amino acids complexes **5a**−**9a** behave in the same way as was described previously for compounds **2a**−**4a**.[59] Such compounds are stable at a pH range of 5−10 for 3 days after dissolving. In the more acidic solutions (pH 3.5−5), an equilibrium between the appropriate amino acid complex and the aqua complex $[Cp_2V(H_2O)_2]^{2+}$ (**10**; $A_{iso} = 74.3 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.983$)[68] was observed. At pH lower than 3.5 only complex **10** was detected by EPR spectroscopy. The conversion of the amino acid complexes into complex **10** is a reversible reaction.

Degradation of amino acid complexes into an aqua complex and a free amino acid was subsequently confirmed by positive nihydrine reaction. Ninhydrine reacts with a free (nonbonded) amino group of an amino acid to form a colored compound, which was detected by UV−Vis spectroscopy ($\lambda_{max} = 570$ nm).

Complexes **2a**−**9a** are not stable in basic solutions. At pH values higher than 10 some EPR silent vanadium compounds are formed. This is caused by the degradation

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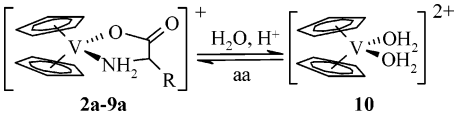
Scheme 2. Behavior of Complexes **2a**−**9a** in Acidic Solution[a]



[a] Labeled as in Scheme 1.

of amino acid complexes, which is accompanied by the irreversible evolution of cyclopentadiene.

The results obtained from the study of the behavior of these complexes in aqueous solutions at different pH were applied to more complex systems (see below).

Behavior of Complexes 2a−9a in Physiological Solutions. The behavior of complex **2a** in physiological sodium chloride solution (0.9% NaCl) and in Krebs−Ringer solution was studied by EPR spectroscopy. The sodium chloride solution (0.9% NaCl) is the simplest physiological solution, and it is isotonic with blood plasma. Therefore, it can be used as a therapeutic or an application solution. The Krebs−Ringer solution is consistent with the ionic composition and the pH of human plasma. Consequently, the interactions of prepared complexes with Krebs−Ringer solution components are analogous to conditions in vivo but without the effects of the organic compounds contained in blood or plasma (proteins, fats, saccharides, etc.).

Complex **2a** does not interact with physiological sodium chloride solutions. The EPR spectrum is stable for days in such physiological solutions without the appearance of a signal from any of the other compounds. Consequently, the behavior of **2a** corresponds to its behavior in pure water at neutral pH.

Complex **2a** dissolved in Krebs−Ringer solution gives an EPR spectrum that is a superposition of the signals of two paramagnetic species (Figure 2, spectrum B). The first one was identified as **2a**, whereas the second was carbonate complex $Cp_2V(O_2CO)$ (**11**) ($A_{iso} = 58.4 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.980$). Compound **11** was previously described as a product of the interaction of vanadocene dichloride with an aqueous solution of hydrogencarbonate as well as with a Krebs−Ringer solution.[69]

The decreasing EPR signal intensity of complex **2a** and increasing signal intensity of complex **11** was found during the time-dependent EPR measurements. After 24 h, only the signal of **11** was observed in EPR (Figure 2, spectrum C). This spectrum did not change in intensity, number of bands, and character for the next 8 h. The presence of a noncoordinated amino acid was proved by a ninhydrine test similar to that described above.

Further measurements showed that complexes **3a**−**9a** have the same behavior in both physiological NaCl solutions and in Krebs−Ringer solutions as that of complex **2a** (Scheme 3). The behavior of vanadocene dichloride (**1**) in Krebs−Ringer physiological solutions was described previously.[69] In this case, only one EPR-active complex was detected and identified as **11**.

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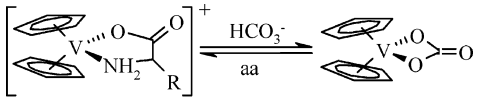
Scheme 3. Behavior of Complexes **2a**−**9a** in Krebs−Ringer Solution[a]



[a] Labeled as in Scheme 1.

Behavior of Complexes 1 and 2a−9a in Human Blood Plasma. Vanadocene dichloride (**1**) dissolved in blood plasma gives a single eight-line EPR spectrum ($A_{iso} = 58.0 \times 10^{-4}$ cm^{-1}, $g_{iso} = 1.989$) of complex **11**. Compound **11** is stable in such solutions for 24 h.

The behavior of complexes **2a**−**9a** in plasma is different from that of complex **1**. On the basis of EPR spectra, it was found that after dissolution, two paramagnetic species are present. They were identified as starting amino acid compounds (**2a**−**9a**) and carbonate complex **11**. After 24 h, a majority of complex **11** was observed in the EPR spectrum. Vanadocene complexes **1** and **2a**−**9a** show approximately the same behavior in plasma as was that in the Krebs−Ringer solution. Thus, an interaction with the organic components of human blood cannot be presumed.

Behavior of Complexes 1 and 2a−9a in Human Whole Blood. Vanadocene dichloride (**1**) shows the same behavior in both whole blood and plasma. In its EPR spectrum, only one paramagnetic species corresponding to carbonate complex **11** was observed.[69] The intensity or the pattern of this spectrum did not change 24 h after dissolving.

Amino acid complexes **2a**−**9a** show higher stability in whole blood than in blood plasma. Its EPR spectra contain signals of two paramagnetic species (Figure 2, spectrum D). The first of these was identified as an appropriate amino acid complex and the second one as carbonate complex **11**. However, these EPR spectra of compounds **2a**−**9a** in whole blood are stationary for 24 h.

Conclusions

In this study, an interaction of vanadocene dichloride with α-amino acids in an aqueous environment was unambiguously evidenced by means of EPR spectroscopy. The amino acid complexes formed $[Cp_2V(aa)][X]$ (aa = gly, ala, val, leu, ile, phe, his, and trp; X = Cl, PF_6) were isolated and characterized by spectroscopic techniques, and the structures of compounds $[Cp_2V(val)][PF_6]$ (**4b**) and $[Cp_2V(ile)][PF_6]$ (**6b**) were determined by X-ray diffraction analysis. All amino acids are coordinated to the vanadocene fragment through one oxygen atom of the carboxylic group and a nitrogen atom of the amino function. No other type of amino acid interaction was observed.

It was evidenced that prepared chelate complexes **2a**−**9a** are stable in aqueous solutions with physiological pH and in therapeutic NaCl solutions. Dissolving of amino acid complexes (**2**−**9**) in aqueous media does not lead to a decrease in pH, as can be observed in the case of vanadocene dichloride (**1**). Additionally, the amino acids evolved during the decomposition of the complexes and are biologically acceptable. From these aspects, vanadocene amino acid complexes could be used as a novel potent antitumor agent.

For the description of possible behavior in vivo, the stabilities of complexes **2a**−**9a** in a Krebs−Ringer solution, in human blood plasma, and in whole blood were studied. Experiments detected that all complexes **2a**−**9a** react with the hydrogencarbonate anion present to form complex $Cp_2V(O_2CO)$ (**11**). While in the Krebs−Ringer solution and in blood plasma, all amino acid complexes are completely transformed to complex **11** over several hours, and in whole blood, both starting amino acid complexes (**2a**−**9a**) and carbonate complex (**11**) are present for 24 h. Although the behavior of **2a**−**9a** in Krebs−Ringer solution and blood plasma is somewhat different, we did not observe any new EPR active species as products of the interaction with the organic components of plasma or whole blood.

The phenomenon of the partial dissociation of the five-membered chelate ring of amino acid is an important aspect for the preservation of the cytostatic activity of vanadocene complexes. It is of importance that both amino acid complexes **2**−**9** and carbonate complex **11** preserved a vanadocene fragment $[Cp_2V]^{2+}$, which is presumably responsible for the antitumor activity of vanadocene complexes.

EPR spectroscopic investigations in complex biological systems such as blood plasma or whole blood, give a novel view of the chemistry of antitumor active metallocene complexes. Unfortunately, nuclear magnetic resonance experiments are not suitable for comparable studies of diamagnetic titanocene(IV) and molybdenocene(IV) compounds because of the lower sensitivities of ^{47,49}Ti NMR and ^{95}Mo NMR spectroscopy, respectively.

Experimental Section

Methods and Materials. All operations were performed under argon by using conventional Schlenk-line techniques. The solvents were purified and deoxygenated by standard methods. Water was deionized, double distilled, and saturated with argon. Carbonate-free sodium hydroxide was prepared by slow dissolution of sodium in water. The α-amino acids glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-histidine, and L-tryptophane (Fluka) were used without further purification.

Krebs−Ringer solution (6.9 g NaCl, 3.36 g KCl, 0.28 g $CaCl_2$, 0.16 g KH_2PO_4, 0.14 g $MgSO_4$ and 2.1 g $NaHCO_3$ per liter of redistilled water, pH 7.4) and therapeutic saline solution (0.9% NaCl) were used as solutions. All chemicals were of analytical grade. Deionized redistilled water was used in all experiments. Both human blood plasma and blood were stabilized by heparine (standard tubes Vacuette, greiner bio-one). Stabilization by another anticoagulant such as citrate and EDTA was not useful because these agents degrade the vanadocene complexes.

Complexes **1**,[70] and **2**−**4**[59] were prepared by the published methods. Syntheses of compounds **5a** and **5b** are outlined in detail as an example of the general methodology for the synthesis of compounds used here. Synthetic details of all new compounds, spectroscopic, and analytic data are available in the Supporting Information.

Synthesis of $[Cp_2V(leu)]Cl$ (5a). After dissolving compound **1** (0.5 g, 1.98 mmol) in 20 mL of water, L-leucine (0.26 g, 1.98 mmol) was added to the solution. Then, the reaction mixture was neutralized by 3.96 mL of carbonate-free NaOH ($c = 0.5$ mol·L^{-1}), the solvent was removed in vacuo, and the solid residue was crystallized from the acetone−methanol mixture. The green powder was then washed with 10 mL of acetone and dried in vacuo. Yield: 0.57 g (1.64 mmol, 83%).

Synthesis of $[Cp_2V(leu)]PF_6$ (5b). After dissolving complex **5a** (0.2 g, 0.58 mmol) in 2 mL of water, 1 mL of saturated KPF_6 solution was added. The mixture was stirred for 10 min. Then the precipitate was filtered on the frit, washed with 2 mL of cold water, and dried in vacuo. Yield: 0.15 g (0.33 mmol, 57%).

Table 3. Crystal Data of **4b** and **6b**: Measurement and Refinement Details[a]

compound	**4b**	**6b**
formula	$C_{15}H_{20}NO_2V, F_6P$	$C_{16}H_{22}NO_2V, F_6P$
crystal system	monoclinic	monoclinic
space group	$P2_1$ (No. 4)	$P2_1$ (No. 4)
a (Å)	6.1600(2)	6.27500(10)
b (Å)	15.4740(4)	15.8800(4)
c (Å)	9.7220(3)	9.8940(3)
β (deg)	108.1800(15)	105.7680(16)
Z	2	2
V (Å^3)	880.44(5)	948.81(4)
D_c (g cm^{-3})	1.668	1.597
crystal size (mm)	0.4 × 0.3 × 0.25	0.36 × 0.3 × 0.25
color	green	green
shape	plate	plate
μ (mm^{-1})	0.724	0.675
h range	−8, 8	−8, 8
k range	−19, 20	−20, 20
l range	−12, 12	−12, 12
reflections measured	12 060	13 625
independent (R_{int}[a])	4005 (0.033)	4311 (0.033)
observed [$I>2\sigma(I)$]	3811	4134
no. of parameters	245	246
GOF[b]	1.038	1.033
R[c], wR[c]	0.0319, 0.0744	0.0555, 0.1570
$\Delta\rho$ (eÅ^{-3})	0.631,−0.363	1.520, −0.668

[a] $R_{int} = \Sigma|F_o^2 - F_{o,mean}^2|/\Sigma F_o^2$. [b] GOF $= [\Sigma(w(F_o^2 - F_c^2)^2)/(N_{diffrs} - N_{params})]^{1/2}$ for all data. [c] $R(F) = \Sigma||F_o| - |F_c||/\Sigma|F_o|$for observed data, $wR(F^2) = [\Sigma(w(F_o^2 - F_c^2)^2)/(\Sigma w(F_o^2)^2)]^{1/2}$ for all data.

X-ray Crystallography. The X-ray data for **4b** and **6b** were obtained at 150 K using an Oxford Cryostream low-temperature device on a Nonius KappaCCD diffractometer with Mo Kα radiation ($\lambda = 0.71073$ Å), a graphite monochromator, and the φ and ω scan mode. Data reductions were performed with DENZO-SMN.[71] The absorption was neglected. Structures were solved by direct methods (Sir92)[72] and refined by full matrix least-squares on the basis of F^2 (SHELXL97).[73] Crystal data are summarized in Table 3. Hydrogen atoms on carbon atoms in all of the structures were calculated into ideal positions, riding during refinement on the respective pivot atom. The isotropic displacement parameters of these hydrogen atoms were set to 1.2 × U_{eq} of the attached atom or 1.5 × U_{eq} for the methyl moiety. Hydrogen atoms of the amino moiety were found on difference Fourier maps and refined either isotropically (**4b**), riding during refinement on nitrogen atom (**6b**) with displacement parameter set to 1.2 × U_{eq} (N).

The anions PF_6^- in **4b** and **6b** structures are poorly ordered, acquiring large displacement parameters and leaving the high

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residuals on difference Fourier maps. However vanadocene moieties remain unaffected, affording the satisfactory resolution of their structures.

Full crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC **270100** and **270101** for **4b** and **6b**, respectively). Copies of the data can be obtained free of charge upon request from CCDC, 12, Union Road, Cambridge CB2 1EZ, U.K. (fax: (+44) 1223−336−033; e-mail: deposit@ccdc.cam.ac.uk).

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Supporting Information Available: Crystallographic details for $[Cp_2V(val)]PF_6$ (**4b**) and $[Cp_2V(ile)]PF_6$ (**6b**) in CIF format, experimental procedures, and spectroscopic and analytical details. This material is available free of charge via the Internet at http://pubs.acs.org.

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