Synthesis, crystal structure, and antitumor activity of the cadmium dichloride complex with semicarbazide

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The chelate cadmium dichloride complex with semicarbazide was synthesized. The use of this complex in the cytostatic combination therapy together with cyclophosphamide results in 67% survival of mice and a threefold increase in the average life span of survivors with P-388 leukemia.

Key words: cadmium dichloride, semicarbazide, complexes, antitumor activity, crystal structure.

In the middle of the 20th century, a research team in the USSR developed a method for the cancer treatment known as the semicarbazide-cadmium therapy or Kachugin´s method.[1] This method was successfully used for the treatment of patients in later stages of lung, intestinal, and breast cancer, melanoma, and some other cancer types.[2] However, for some reasons this method has not gained wide acceptance, although it has advantages, such as simplicity and practicality due to the use of moderately toxic semicarbazide. In Kachugin´s method, aqueous solutions of semicarbazide and moderately radioactive $^{113}CdCl_2$ are administered orally and successively according to a special procedure. Hence, it is of obvious interest to synthesize a cadmium complex with semicarbazide for the purpose of simplifying the administration of the above-mentioned compounds to a patient´s body and answering the question of whether this complex based on non-radioactive cadmium possesses antitumor activity.

The aims of the present study were to synthesize the cadmium dichloride complex with semicarbazide, determine its structure by X-ray diffraction, and investigate the antitumor activity against tumor strains of P-388 leukemia and Lewis carcinoma.

Semicarbazide is a bidentate ligand capable of forming chelate complexes with complex-forming metals. It would be expected that a cadmium complex with two or one five-membered rings will be formed. Actually, the chelate complex (**1**) with two five-membered rings is produced under mild conditions by mixing aqueous solutions of cadmium dichloride and semicarbazide. The structure of complex **1** was determined by elemental analysis, IR and ^1H NMR spectroscopy, and X-ray diffraction (Fig. 1). According to the X-ray diffraction data, complex **1** has a chelate structure. The cadmium atom is involved in two five-membered rings. The resulting complex has a *cis* structure. In accordance with the SAR-1 (structure-activity relationship) rule, this structure should provide higher antitumor activity compared to the corresponding *trans* complexes.[3,4] In complex **1**, the cadmium atom has the coordination number 6. The bond lengths in the ligands and the bond angles are given in Table 1. Molecules **1** form a molecular crystal (Fig. 2) *via* intermolecular van der Waals interactions and intermolecular O(2)...N(3) and O(1)...N(2) hydrogen bond (2.92 and 3.25 Å, respectively) represented by dashed lines in Fig. 2.

Experimental

Complex **1** was synthesized with the use of cadmium chloride hemi(pentahydrate) (GOST 4330-76) and semicarbazide hydrochloride (USToxic, m.p. 175—177 °C, 99% purity). The IR spectrum was recorded using an attenuated total internal

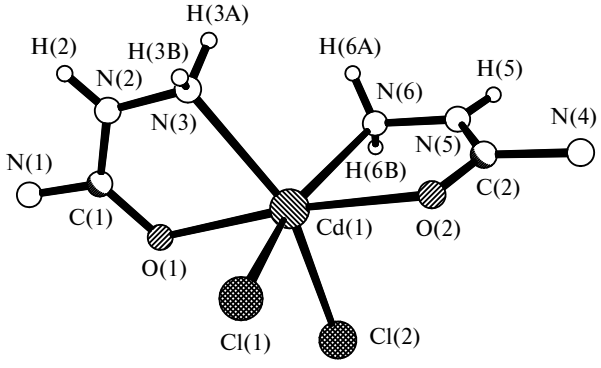

Fig. 1. Structure of complex **1**.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 9, pp. 1925—1928, September, 2011.

reflection (ATR) spectroscopic cell on a Perkin—Elmer Spectrum 100 instrument. The ^{1}H NMR spectrum was measured on a Bruker AM-500 spectrometer.

***cis*-Bis(semicarbazide-O,N)dichlorocadmium (1).** Dry Na_2CO_3 (3.8 g, 35.85 mmol) was added with stirring to a solution of $H_2NCONHNH_2 \cdot HCl$ (8 g, 72 mmol) in water (20 mL) at ~20 °C. After the completion of gas evolution, a solution of $CdCl_2 \cdot 2.5\ H_2O$ (6.6 g, 28.9 mmol) in water (15 mL) was added. The reaction mixture was heated to 50 °C followed by the addition of methanol (50 mL). After cooling to ~20 °C, the mixture was kept in a refrigerator (+4 °C). After 12 h, the crystalline white precipitate was filtered off, successively washed with a cold mixture of water and methanol (1 : 1) and then with methanol, and dried in air. The yield of complex **1** was 9 g (75%), m.p. 154—155 °C. Found (%): C, 7.3; H, 1.8; Cl, 21.1; Cd, 33.6; N, 25.3. $C_2H_{10}Cl_2CdN_6O_2$. Calculated (%): C, 7.20; H, 3.02; Cl, 21.26; Cd, 33.71; N, 25.20. IR, ν/cm^{-1}: 3427.37, 3327.02, 3278.97, 3198.66, 2903.04, 2726.27, 1659.47, 1622.18, 1582.07, 1371.96, 1212.15, 1091.94, 953.45, 772.58, 705.66. ^{1}H NMR (DMSO-d_6), δ: 4.76 (br.s, 2 H, NHN<u>H</u>$_2$); 6.07 (s, 1 H, N<u>H</u>NH$_2$); 7.63 (br.s, 2 H, NH$_2$). Crystals suitable for X-ray diffraction were obtained by crystallization from aqueous methanol.

X-ray diffraction study of compound **1** was performed using a white single crystal of dimensions 0.5×0.4×0.45 mm at 293 K on a KM-4 diffractometer (Kuma Diffraction, Poland) (λ(Mo-Kα) = 0.71073 Å, ω/2θ-scanning technique). The crystallographic data and refinement statistics are as follows: molecular formula $C_2H_{10}Cl_2CdN_6O_2$, molecular weight 333.46, space group $P2(1)/c$, a = 9.630(2) Å, b = 12.643(3) Å, c = 8.330(2) Å, V = 1014.3(4) Å^3, Z = 6, d_{calc} = 2.538 g cm^{-3}, 1196 measured reflections, 981 reflections with $I > 2\sigma(I)$, 120 refined parameters, GOOF = 1.067, R_1 = 0.089. The structure was solved by direct methods.[5] The positional and thermal parameters of nonhydrogen atoms were refined by the full-matrix least-squares method with anisotropic displacement parameters.[5,6] The hydrogen atoms were positioned geometrically and refined using a riding model. The interatomic distances and bond angles are given in Table 1. The atomic coordinates are listed in Table 2.

Table 1. Bond lengths (d) and bond angles (ω) in the structure of **1**

Bond	d/Å	Angle	ω/deg
Cd(1)—O(2)	2.33(2)	O(2)—Cd(1)—O(1)	173.6(4)
Cd(1)—O(1)	2.32(2)	O(2)—Cd(1)—N(6)	70.6(6)
Cd(1)—N(6)	2.37(2)	O(1)—Cd(1)—N(6)	114.3(7)
Cd(1)—N(3)	2.42(2)	O(2)—Cd(1)—N(3)	113.4(6)
Cd(1)—Cl(2)	2.570(5)	O(1)—Cd(1)—N(3)	71.2(7)
Cd(1)—Cl(1)	2.589(8)	N(6)—Cd(1)—N(3)	93.8(5)
O(1)—C(1)	1.29(3)	O(2)—Cd(1)—Cl(2)	90.6(4)
O(2)—C(2)	1.21(4)	O(1)—Cd(1)—Cl(2)	85.1(4)
C(1)—N(1)	1.27(4)	N(6)—Cd(1)—Cl(2)	92.3(5)
C(1)—N(2)	1.32(4)	N(3)—Cd(1)—Cl(2)	155.9(6)
C(2)—N(5)	1.33(4)	O(2)—Cd(1)—Cl(1)	84.7(5)
C(2)—N(4)	1.45(3)	O(1)—Cd(1)—Cl(1)	90.9(6)
N(2)—N(3)	1.41(3)	N(6)—Cd(1)—Cl(1)	154.4(5)
N(5)—N(6)	1.40(3)	N(3)—Cd(1)—Cl(1)	89.9(6)
		Cl(2)—Cd(1)—Cl(1)	94.67(17)
		C(1)—O(1)—Cd(1)	115.4(18)
		C(2)—O(2)—Cd(1)	116.4(17)
		N(1)—C(1)—O(1)	121(3)
		N(1)—C(1)—N(2)	116(2)
		O(1)—C(1)—N(2)	123(3)
		O(2)—C(2)—N(5)	122.3(19)
		O(2)—C(2)—N(4)	119(3)

Table 2. Coordinates of nonhydrogen atoms

Atom	x	y	z
Cd(1)	0.2506(3)	−0.00008(13)	0.0429(1)
Cl(1)	0.0691(8)	−0.0600(4)	0.2533(9)
Cl(2)	0.4290(8)	0.0619(4)	0.2522(8)
O(1)	0.363(2)	−0.1621(13)	0.060(2)
O(2)	0.137(2)	0.163(1)	0.057(2)
C(1)	0.315(3)	−0.235(2)	−0.032(3)
C(2)	0.180(3)	0.2318(18)	−0.031(3)
N(1)	0.365(3)	−0.3287(17)	−0.029(3)
N(2)	0.216(3)	−0.2190(14)	−0.139(3)
N(3)	0.150(3)	−0.1198(13)	−0.151(3)
N(4)	0.127(4)	0.3382(17)	−0.013(3)
N(5)	0.281(3)	0.2144(14)	−0.138(4)
N(6)	0.341(2)	0.1147(13)	−0.156(2)

Study of antitumor activity. The investigation of the acute toxicity of compound **1** performed in the Laboratory of Experimental Tumor Chemotherapy in the Institute of Problems of Chemical Physics of the Russian Academy of Sciences showed that the complex has high toxicity (LD_{50} = 18.5 mg kg^{-1}) comparable to that of cisplatin (LD_{50} = 12.5 mg kg^{-1}).

The antitumor activity of complex **1** was investigated using the experimental P-388 leukemia and Lewis lung carcinoma models. The experiments were performed on the BDF_1 line of mice with a weight of 22—24 g. Each group included 8—10 animals. The inoculum size for experimental Lewis lung carcinoma and P-388 leukemia was 10^6 and $5 \cdot 10^6$ tumor cells, respectively. By itself, complex **1** did not exhibit antitumor activity (Table 3) against P-388 leukemia. Hence, complex **1** was used in cytostatic combination therapy togehter with cyclophosphane.

Results and Discussion

The data on antitumor activity are given in Table 3. It can be seen that the cytostatic combination therapy (complex **1** combined with cyclophosphane) results in 67% survival of mice. It should be noted that an increase in the average life span (ILS) of 33% survivors is 362% compared to mice in the control group, which can be apparently attributed to the strongest synergistic effect of the combined use of these compounds in the combination therapy.

The data on the inhibition of Lewis lung carcinoma growth by complex **1** are presented in Fig. 3. The antitumor efficacy of complex **1** was determined according to a procedure described earlier.[7] It should be noted that the antitumor activity of complex **1** against Lewis lung carcinoma is only at the 30% level.

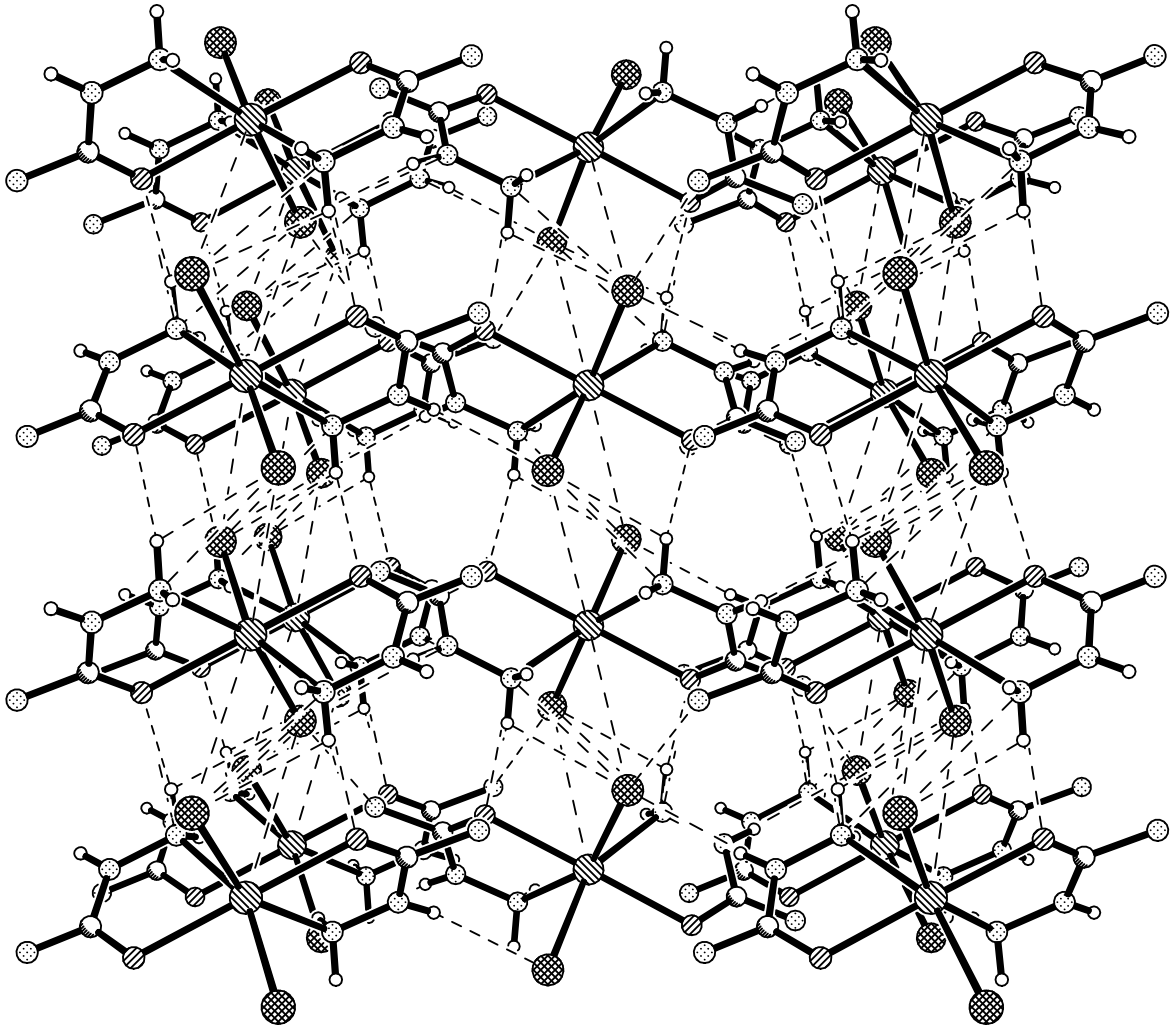

Fig. 2. Fragment of the crystal structure of compound **1**.

It is known that there is a direct relationship between the inhibition of calcium ion transport across biological membranes and the inhibition of metastasis (index of metastasis inhibition).[7] This correlation has been found earlier in studies of the effect of platinum tetrachloride complexes with a series of pyridinecarboxylic acids, which were synthesized by our research group,[8] on Ca^{2+}—Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum isolated from white muscles of rabbit hind limbs.[9] It was shown that Pt^{IV} complexes with the above-mentioned ligands, including ligands containing groups capable of generating NO in the course of the metabolism, act on Ca^{2+}-Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum, thus inhibiting the calcium transport across biological membranes. This leads to the disturbance of the normal ratio of calcium ions on the extra- and intracellular membrane surfaces followed by the disturbance of the aggregation of thrombocytes (an important step in the metastasis) and their binding to metastatic cells, which results in the loss of the cell adhesion of the latter to blood-vessel walls. Studies performed by the Group for Biological Assay in

Table 3. Efficacy of the combined use of cyclophosphane and complex **1** on P-388 leukemia*

Agent	Dose /mg kg^{-1}	Time of administration/days	Number of survivors animals**	ILS
			%	
Cyclophosphane	30.0	1, 6	33.0	319.0
Complex **1**	6.0	1—6	0	8.0
Cyclophosphane	30.0	1, 6		
+	+	+	67.0	362
complex **1**	6.0	1—6		

* $P \le 0.005$.

** The survivors are animals that were alive on day 60 after the tumor transplantation.

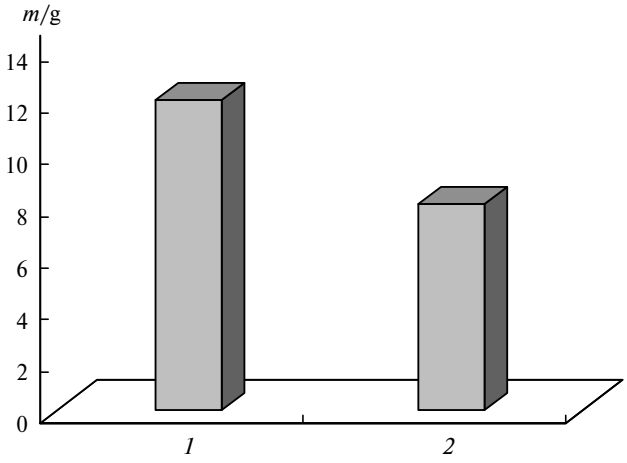

Fig. 3. Inhibition of Lewis lung carcinoma growth by complex **1**: *1*, control; *2*, complex **1**; *m* is the tumor weight. The time of administration: 2nd, 4th, 6th, 8th, and 10th days; the dose is 1.6 mg kg^{-1}.

the Institute of Problems of Chemical Physics of the Russian Academy of Sciences according to the same procedure showed that the action of complex **1** on the Ca^{2+}—Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum results in the 69% inhibition of the calcium transport across biological membranes. Based on the above data, it would be expected that complex **1** will exhibit antimetastatic activity against solid tumors.

We thank G. V. Strukov (Institute of Solid State Physics, Russian Academy of Sciences) for valuable discussion of the paper.

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Received March 4, 2011;
in revised form June 29, 2011