Synthesis, structure, and antimetastatic activity of the *trans*-$[Pt(NC_5H_4C(O)NHC_2H_4ONO_2)_2Cl_2]$ complex

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The *trans*-$[Pt(NC_5H_4C(O)NHC_2H_4ONO_2)_2Cl_2]$ complex (2) was prepared by the reaction of nicorandyl (*N*-nitroethoxynicotinamide), which is widely used in cardiology, with K_2PtCl_4 in water. The structure of 2 was established by X-ray structural analysis. It was found that complex 2 exhibits high antitumor activity, in particular, antimetastatic activity, unlike the analogous Cu^{II} complex with bromine atoms.

Key words: platinum, complex with nicorandyl, antitumor activity.

Dichlorodiaminoplatinum(II) (*cis*-DDP) is used successfully in practical oncology, which gives impetus to an intensive search for new complexes of platinum with improved therapeutic properties.[1–3] In this connection, the aim of this work is to synthesize and study the structure and properties of a new platinum(II) complex containing nicorandyl (*N*-nitroethoxynicotinamide), which belongs to the group of nitrates used in the treatment of cardiovascular diseases.

It is known that in the course of metabolism, nitrates generate NO, which possesses wide-ranging biological activities.[4,5] In particular, there is evidenced that NO is a suppressor of angiogenesis, which, in turn, may be a potential target in the treatment of metastasis.[6] It was noted that NO can act as a potent effector in tumor growth.[7] Finally, experimental data are obtained that indicate that tumor growth and metastasis are inhibited by some vasodilators generating NO.[8]

Based on these data, we designed a new type of compounds whose pharmacological efficiency may be determined by the combined effect of platinum and NO generated by nicorandyl.

Results and Discussion

As part of systematic studies devoted to a search for new potent antitumor compounds, we have demonstrated that cardioactive nicorandyl reacts with mono- and binuclear metal fragments containing copper and rhodium atoms to form stable complexes. The structures of these complexes were established by X-ray structural analysis.[9] However, the paramagnetic monomer $[Cu(NC_5H_4C(O)NHC_2H_4ONO_2)_2Br_2]$ (1), which contains N-donor ligands in *trans* orientations, is inactive with respect to a number of experimental tumors. It is also known that, unlike the copper-containing compounds, the *cis*-diamine complexes of platinum(II) exhibit high antitumor activity owing to suppression of mitosis as a result of strong bonding between DNA and platinum through the replacement of *cis*-halide ligands and formation of N—H...O hydrogen bonds.[10,11] The geometry of the corresponding *trans*-complexes of platinum is unsuitable for complex formation with a DNA helix, and they are generally inactive although in some cases these compounds possess pronounced antitumor activity.[10,11] However, antimetastatic activity of this type of compounds is poorly known.

It was found that nicorandyl reacts with K_2PtCl_4 in water (the ratio of the reagents was 2 : 1) to form a yellow finely crystalline precipitate of *trans*-$[Pt(NC_5H_4C(O)NHC_2H_4ONO_2)_2Cl_2]$ (2) in 92% yield (Scheme 1).

Complex 2 is virtually insoluble in H_2O and moderately soluble in THF, alcohol, and DMF. The structure of compound 2 was established by X-ray structural analysis (Fig. 1, Table 1). The Cl atoms and coordinated nicorandyl molecules are in *trans* positions and form a typical planar-square environment around the Pt^{II} atom (the length of each Pt—Cl bond is 2.311(3) Å, and the length of each Pt—N bond is 2.019(8) Å). It should be noted that the formation of the *trans*-complex in this reaction is somewhat untypical because the analogous reaction with pyridine or alternative amines yielded the *cis*-compounds.[4] It can be suggested that in this case, the geometry of the complex is determined by

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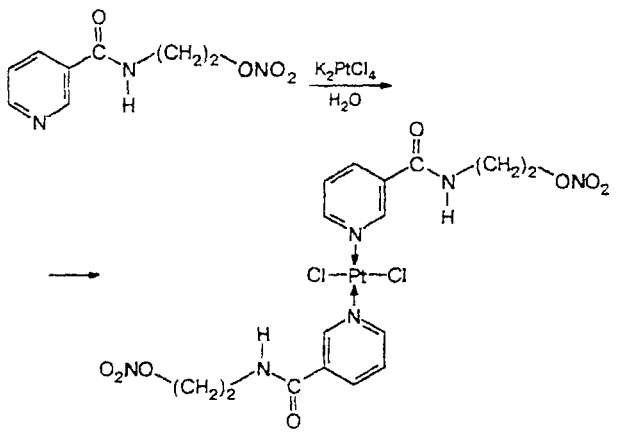
Scheme 1



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steric hindrances that occur in two nicorandyl molecules, which have bulky substituents and cannot occupy adjacent positions in the coordination environment around the Pt atom.

The estimation of high toxicity of complex **2** demonstrated that the newly synthesized compound exhibits substantially lower total toxicity than the known cisplatin.

Preparation	LD_{100}	LD_{50}	MDP
Cisplatin	16.0	12.0	8.0
Complex **2**	40.0	36.0	30.0

The experimental data on biological activity of compound **2** (studied with the use of two transplanted metastizing tumors, Tables 2 and 3) demonstrated that hepatometastases of the tumor ACACOL (adenocarcinoma of colon) is more sensitive to both preparations under study than Lewis carcinoma of lungs. In the first case, the indices of inhibition were 81—90%. It should be noted that of the newly synthesized *trans*-complex of platinum possesses antimetastatic activity comparable to the reference preparation *cis*-DDP (cisplatin). However, taking into account the lower total toxicity of complex **2**, it is worthwile searching further for active compounds of this series.

Table 1. Principal bond lengths (d) and bond angles (ω) in the molecule of complex **2**

Bond	d/Å	Bond	d/Å
Pt(1)—Cl(1)	2.311(3)	O(4)—N(3)	1.210(13)
Pt(1)—N(1)	2.019(8)	N(1)—C(1)	1.315(12)
O(1)—C(6)	1.231(10)	N(1)—C(5)	1.348(12)
O(2)—N(3)	1.388(13)	N(2)—C(6)	1.369(11)
O(2)—C(8)	1.461(13)	N(2)—C(7)	1.450(13)
O(3)—N(3)	1.182(14)	N(2)—H(1n)	1.027(22)

Angle	ω/deg	Angle	ω/deg
Cl(1)—Pt(1)—N(1)	90.8(2)	O(2)—N(3)—O(3)	118.2(10)
Cl(1)—Pt(1)—Cl(1a)	180.0(1)	O(2)—N(3)—O(4)	113.7(9)
N(1)—Pt(1)—Cl(1a)	89.2(2)	O(3)—N(3)—O(4)	128.1(11)
N(1)—Pt(1)—N(1a)	180.0(1)	N(1)—C(1)—C(2)	124.3(8)
N(3)—O(2)—C(8)	115.0(8)	N(1)—C(1)—H(11)	124.7(21)
Pt(1)—N(1)—C(1)	122.7(6)	N(1)—C(5)—C(4)	122.1(9)
Pt(1)—N(1)—C(5)	119.2(6)	N(1)—C(5)—H(51)	123.3(21)
C(1)—N(1)—C(5)	118.0(8)	O(1)—C(6)—N(2)	121.2(8)
C(6)—N(2)—C(7)	122.7(7)	O(1)—C(6)—C(2)	123.1(8)
C(6)—N(2)—H(1n)	114.1(20)	N(2)—C(6)—C(2)	115.7(7)
C(7)—N(2)—H(1n)	123.3(19)	N(2)—C(7)—C(8)	112.6(9)
		O(2)—C(8)—C(7)	105.1(8)

Experimental

Nicorandyl was prepared according to a procedure reported previously.[9] The IR spectra were recorded on a Specord-75IR instrument as Nujol mulls.

Complex *trans*-[Pt(NC$_5$H$_4$C(O)NHC$_2$H$_4$ONO$_2$)$_2$Cl$_2$] (2). An aqueous solution of nicorandyl (0.84 g, 4 mmol) was added to a solution of K_2PtCl_4 (0.83 g, 2 mmol) in water (25 mL). The reaction mixture was warmed to 40—50 °C. After several minutes, a finely crystalline "oiled" precipitate formed. The precipitate was separated by decantation, washed on a filter successively with water, cooled (−10 °C) alcohol, and ether, and dried in air. The yield was 1.27 g (1.85 mmol, 92%). Found (%): C, 27.84; H, 2.74; N, 12.56. $C_{16}H_{18}Cl_2N_6O_8Pt$. Calculated (%): C, 27.90; H, 2.62; N, 12.21. IR, ν/cm^{-1}: 576 w, 660 w, 688 m, 720 m, 740 m, 820 m, 856 m, 900 m, 1000 m, 1060 w, 1102 m, 1160 w, 1180 w, 1278 s, 1304 m, 1362 m, 1374 m, 1422 m, 1450 s, 1540 s, 1548 m, 1620 s, 1650 s, 1670 s, 1726 w, 3040 m, 3270 m. Crystals suitable for X-ray structural analysis were prepared by slow evaporation of a solution of compound **2** in a 1 : 1 THF—H_2O mixture.

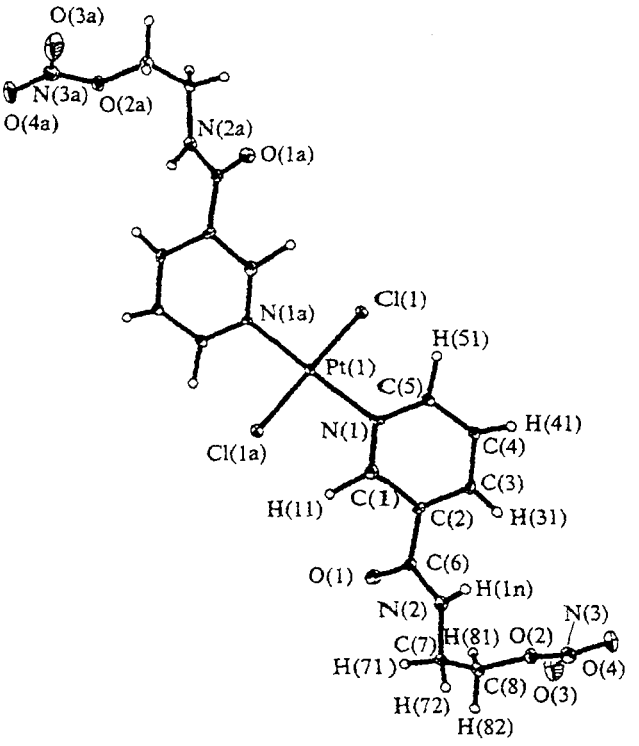

Fig. 1. Structure of complex **2**.

Table 2. Comparative antimetastatic activity of complex **2** and cisplatin (hepatometastases of the ACACOL tumor)

Preparation	Dose /mg kg^{-1}	Mode of introduction[a]	n_m/n_{tot} [b]	m_{av} [c]	$P < 0.05$	IIM (%)[d]
Complex **2**	6.0	2—8	8/13	1.1±0.4	0.0001	90
Cisplatin	2.0	2—8	6/6	1.3±0.2	0.0001	81
Complex **2**	12.0	2, 4, 6, 8, 10	5/6	1.3±0.3	0.0001	93
Cisplatin	4.0	2, 4, 6, 8, 10	3/7	0.4±0.2	0.0001	99
R.gr.[e]	—	—	13/13	7.0±0.4	—	—

[a] Time (days) after transplantation.
[b] n_m is the number of animals with metastases, and n_{tot} is the total number of animals.
[c] m_{av} is the average number of metastases per mouse.
[d] Index of inhibition of metastases.
[e] Reference group.

Table 3. Comparative antimetastatic activity of complex **2** and cisplatin (Lewis carcinoma of lungs)

Preparation	Dose /mg kg^{-1}	Mode of introduction[a]	n_m/n_{tot} [b]	m_{av} [c]	$P < 0.05$	IIM (%)[d]
Complex **2**	6.0	2—8	10/10	5.3±0.4	0.0001	59
Cisplatin	2.0	2—8	6/6	2.0±0.4	0.0001	85
Complex **2**	12.0	2, 4, 6, 8, 10, 12, 14	10/10	4.0±0.5	0.0001	69
Cisplatin	4.0	2, 4, 6, 8, 10, 12, 14	6/6	3.5±0.9	0.0001	73

[a] Time (days) after transplantation.
[b,c,d] For notations, see Table 2.

X-ray structural study of complex 2. At 22 °C, crystals of **2** are monoclinic, a = 11.510(3) Å, b = 5.003(2) Å, c = 22.454(6) Å, β = 91.04(2)°, V = 1292.8(6) Å^3, space group $P2_1/c$. The intensity data (1625 independent reflections with $I > 4\sigma$) were collected on a Siemens R3v/m diffractometer (Mo-Kα radiation, λ = 0.71073 Å). The structure was solved by the heavy-atom method and refined in anisotropic approximation for all the nonhydrogen atoms. The positions of the hydrogen atoms were located from difference Fourier synthesis and refined isotropically (R = 0.046, R_w = 0.059). Absorption correction (μ = 56.82 cm^{-1}) was applied at the stage of isotropic refinement using the DIFABS program.[13] All calculations were carried out using the SHELXTL program package.[14] The atomic coordinates and equivalent isotropic thermal parameters are given in Table 4.

The biological activity of complex 2 was studied using two transplanted metastazing tumors. In all assays, cisplatin was used as a reference preparation. Experimental hepatometastases were induced by intrasplenetic inoculation of 10^6 tumor cells of ACACOL (adenocarcinoma of colon) to mice of the Balb/C line. Within 21 day after transplantation, the mice were killed, their livers were taken, and the number of animals with metastases (the frequency of metastazing) and the average number of metastases per mouse (the intensity of metastazing) were determined. The index of inhibition of metastases (IIM (%)) was an estimate, which takes into account both characteristics. The index was calculated by the following equation:

$$\text{IIM} = (A_R B_R - AB)/(A_R B_R),$$

where A_R and A are the frequencies of metastases in the reference and test groups, respectively; and B_R and B are the

Table 4. Atomic coordinates (×10^4) and equivalent isotropic thermal parameters (U_{eq}×10^3) for complex **2**

Atom	x	y	z	U_{eq}/Å^2
Pt(1)	0	1/2	1/2	20(1)
Cl(1)	−1287(2)	4707(4)	4201(1)	29(1)
O(1)	4576(6)	5387(11)	4239(4)	33(1)
O(2)	6234(6)	11383(14)	3159(4)	32(1)
O(3)	6739(11)	9319(17)	2334(5)	73(2)
O(4)	5980(8)	13244(15)	2290(4)	55(2)
N(1)	1086(6)	7278(14)	4519(4)	21(1)
N(2)	4997(8)	9799(13)	4166(4)	26(1)
N(3)	6335(8)	11267(18)	2544(5)	40(2)
C(1)	2216(8)	6888(16)	4516(5)	25(2)
C(2)	2997(7)	8440(15)	4191(4)	18(2)
C(3)	2527(9)	10585(17)	3865(5)	27(2)
C(4)	1356(8)	11030(17)	3870(5)	24(2)
C(5)	651(9)	9354(16)	4204(5)	23(2)
C(6)	4232(8)	7713(16)	4204(5)	23(2)
C(7)	6238(9)	9413(16)	4110(5)	27(2)
C(8)	6587(9)	8960(20)	3477(6)	32(2)
H(1n)	4615(30)	11650(32)	4189(27)	46(3)
H(11)	2711(31)	5162(31)	4783(28)	44(3)
H(31)	3060(30)	11532(32)	3662(28)	50(3)
H(41)	1200(30)	12849(32)	3703(28)	46(3)
H(51)	−277(31)	9693(31)	4134(29)	51(3)
H(71)	6376(30)	7648(32)	4308(28)	45(3)
H(72)	6700(30)	10805(32)	4328(28)	50(3)
H(81)	6331(30)	7640(32)	3266(28)	47(3)
H(82)	7475(30)	8697(32)	3470(28)	47(3)

average number of metastases in the reference and test groups, respectively. Two doses and two modes of introduction were studied (see Table 2). Preparations were introduced intraperitoneally.

Antimetastatic activity of the new platinum complex was also studied using a metastazing experimental tumor, Lewis carcinoma of lungs (LL). The tumor was inoculated subcutaneously to BDF_1 hybrid mice. The inoculum contained 10^6 tumor cells. Within 26 days after transplantation, lungs were taken, and the number of metastatic colonies was determined. The results of assays are given in Table 3.

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