Synthesis, structure, and antitumor properties of platinum(IV) complexes with aminonitroxyl radicals

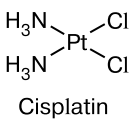
V. D. Sen´, V. V. Tkachev, L. M. Volkova, S. A. Goncharova, T. A. Raevskaya, and N. P. Konovalova*

Institute of Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. Fax: +7 (096) 514 3244. E-mail: senvd@icp.ac.ru

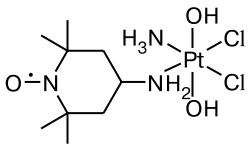
The platinum(IV) complexes *cis,trans,cis*-$Pt^{IV}(RNH_2)(NH_3)(OH)_2Cl_2$, where R is 2,2,6,6-tetramethyl-1-oxyl-4-piperidinyl (**1**) or 2,2,5,5-tetramethyl-1-oxyl-3-pyrrolidinyl (**2**), were prepared by oxidation of the corresponding *cis*-$Pt^{II}(RNH_2)(NH_3)Cl_2$ complexes with hydrogen peroxide. The reactions are catalyzed by tungstate salts, which makes it possible to carry out oxidation under mild conditions. The resulting complexes were characterized by elemental analysis, HPLC, and IR, UV, and ESR spectroscopy. The structure of complex **1** was established by X-ray diffraction analysis. Complex **1** exhibits the highest antitumor activity in an experimental tumor, *viz.*, in P388 leukemia. The resistance of the tumor to this complex developed much slower than that to Cisplatin.

Key words: platinum(IV) complexes, nitroxyl radicals, structure, antitumor activity, Cisplatin.

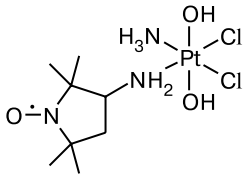
In recent years, platinum(IV) compounds have attracted attention as potential antitumor drugs along with *cis*-diamineplatinum(II) complexes, including Cisplatin as the parent compound. These complexes are powerful inhibitors of growth of tumor cells (including those resistant to Cisplatin) and possess moderate toxicity.[1] As part of continuing studies of platinum complexes with aminonitroxyl ligands,[2—4] we synthesized new platinum(IV) aminonitroxyl complexes **1** and **2**, established their structures, and studied their overall toxicity and antitumor activity, both in individual form and in combination with Cisplatin.



Cisplatin



1



2

The efficiency of an antitumor drug depends not only on the level of tumor cell damage but also on the rate at which a tumor cell population is adapted to the damaging action and acquires resistance to this action. In this connection, we performed a comparative determination of the rates of development of resistance to Cisplatin and complex **1**.

Experimental

The platinum content was determined by atomic absorption spectroscopy on an AAS-3 spectrometer, the accuracy of the determination was ±3 rel.%. The HPLC analysis was carried out on a Milikhrom chromatograph (2×64-mm column, Separon C18 (5 μm), detection at 220 or 240 nm) with the use of 12% aqueous MeOH containing KH_2PO_4 (0.05 mol) as the eluent. Under this conditions, the retention volumes (V_{ret}) were 750 μL for **1**, 540 μL for **2**, 970 and 510 μL for **3**·DMA (DMA is dimethylacetamide), 650 for **4**, and 220 μL for **5**. The IR spectra were recorded in the range of 400—4000 cm^{-1} on a Specord 75-IR spectrometer in Nujol. The electronic spectra were measured in the range of 200—800 nm on a Specord UV-VIS spectrophotometer. The ESR spectra were obtained at room temperature on a SE/X 2544 instrument at the microwave power of 2 mW and modulation amplitude of 0.32 mT.

The starting *cis*-ammine(4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl)dichloroplatinum(II) (**3**) and *cis*-ammine(3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl)dichloroplatinum(II) (**4**) were prepared according to a known procedure.[4] Complex **3** was used as the monosolvate with dimethylacetamide (**3**·DMA), which was obtained upon purification of **3** by precipitation from a solution in DMA with dry ether.

***e*-Ammine-*d*-(4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl)-*a,f*-dihydroxo-*b,c*-dichloroplatinum(IV) (1).** Potassium tungstate K_2WO_4 (3 mg) and H_2O_2 (0.5 mL, 12.4 mol kg^{-1}) were

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 403—408, February, 2003.

1066-5285/03/5202-421 $25.00

added with stirring and cooling with ice to a thoroughly ground suspension of **3**·DMA (1.31 g, 2.42 mmol) in 10% EtOH (6 mL). After ~15 min, the color of the suspension changed from pink-orange to orange. The course of reaction was monitored by TLC (Silufol plates, MeCN—MeOH (2 : 1) as the eluent, R_f was 0.80 and 0.37 for **3** and **1**, respectively). Then the reaction mixture was stirred for 0.5 h, and the product was filtered off, washed with cold water (2×1 mL) and EtOH (2×2 mL), and dried in air. Complex **1** was obtained as orange crystals in a yield of 1.02 g (2.09 mmol, 86%). Crystallization from 10% EtOH afforded analytically pure complex **1**. According to the data from X-ray diffraction analysis (see below), complex **1** was obtained as a dihydrate. Crystals of **1** decomposed (turned dark) without melting at ≥190 °C. Vacuum drying ($3\cdot10^{-3}$ Torr, 56 °C, 1 h) was accompanied by crystal erosion. After vacuum drying, the elemental composition of complex **1** was most close to that of the monohydrate. Found (%): C, 21.78; H, 5.12; Cl, 14.06; N, 8.42; Pt, 38.2. $C_9H_{24}Cl_2N_3O_3Pt\cdot H_2O$. Calculated (%): C, 21.35; H, 5.18; Cl, 14.00; N, 8.30; Pt, 38.53. UV (H_2O), λ_{max}/nm (ε/L mol^{-1} cm^{-1}): 309 sh (550), 396 sh (60). IR of **1**·$2H_2O$ (Nujol), ν/cm^{-1}: 1598, 1614, 1630, 3330, 3410, 3490, 3607 (N—H, O—H, H_2O). ESR (H_2O): three lines, *g* factor was 2.0056, a_N = 1.697.

***e*-Ammine-*d*-(3-amino-2,2,5,5-tetramethylpyrrolidine-1-oxyl)-*a*,*f*-dihydroxo-*b*,*c*-dichloroplatinum(IV) (2)** was prepared analogously to complex **1** from compound **4** in 79% yield. The reaction time in the synthesis of **2** was longer (2.5 h) than that in the synthesis of **1** because of the lower rate of dissolution of complex **4** (see below). M.p. 185—187 °C (10% EtOH). For dried ($3\cdot10^{-3}$ Torr, 56 °C, 1 h) complex **2**, found (%): C, 19.90; H, 4.77; Cl, 15.12; N, 9.07; Pt, 41.15. $C_8H_{22}Cl_2N_3O_3Pt$. Calculated (%): C, 20.26; H, 4.68; Cl, 14.95; N, 8.86; Pt, 41.13. UV (H_2O), λ_{max}/nm (ε/L mol^{-1} cm^{-1}): 307 sh (550), 397 sh (73). IR (Nujol), ν/cm^{-1}: 1588, 3234, 3312, 3568 (N—H, O—H). ESR (H_2O): three lines, *g* factor was 2.0053, a_N = 1.581.

***cis*-Ammine(4-amino-2,2,6,6-tetramethylpiperidine)dichloroplatinum(II) (5).** A solution of NaI (0.8 g, 5.3 mmol) in H_2O (1 mL) and a solution of 4-amino-2,2,6,6-tetramethylpiperidine (0.67 g, 3.9 mmol) in H_2O (1 mL) were successively added with stirring to a solution of $Na[Pt(NH_3)Cl_3]$ in H_2O (15 mL), which was prepared from Cisplatin[5] (1.17 g, 3.9 mmol), at ~20 °C. The resulting suspension was stirred for 0.5 h and then kept in a refrigerator for 2 h. The precipitate was filtered off, washed with cold water (3×2 mL), and dried in air. *cis*-Ammine(4-amino-2,2,6,6-tetramethylpiperidine)iodochloroplatinum(II) was prepared in a yield of 1.27 g (61%). To transform the reaction product into the dichloro complex, it was suspended in water (15 mL) and dissolved by adding 2.4 *N* HNO_3 (1 mL). Then a solution of $AgNO_3$ (0.74 g, 4.4 mmol) in H_2O (1 mL) was added and the reaction mixture was stirred under conditions precluding exposure to light for 2 h. The precipitates of AgCl and AgI were separated by centrifugation and filtration through a dense filter. A 4 *M* KCl solution (2.7 mL) was added to the resulting yellow solution of *cis*-ammine(4-amino-2,2,6,6-tetramethylpiperidine)dinitratoplatinum(II). The reaction mixture was kept for 2 h, concentrated *in vacuo* to ~7 mL, and treated with a 30% aqueous NH_3 solution (1 mL) to isolate complex **5**. To brought crystallization of the product to completion, the reaction mixture was kept in a refrigerator for ~16 h. The resulting yellow crystals were filtered off and washed with cold water (2×2 mL) and EtOH. After drying, complex **5** was obtained in a yield of 0.48 g (28% with respect to the starting Cisplatin). Hydrochloride of complex **5** was crystallized from 10% EtOH as monohydrate **5**·HCl·H_2O, m.p. 240—243 °C (decomp.). Found (%): C, 21.37; H, 5.22; Cl, 20.96; N, 8.52; Pt, 38.3. $C_9H_{24}Cl_3N_3Pt\cdot H_2O$. Calculated (%): C, 21.89; H, 5.31; Cl, 21.54; N, 8.51; Pt, 39.51. IR of **5**·HCl·H_2O (Nujol), ν/cm^{-1}: 1582, 1622, 2460, 3070, 3165, 3260, 3400, 3453, 3510 (N—H, N^+—H, H_2O).

X-ray diffraction study of complex 1. The X-ray diffraction data (8088 independent reflections) were collected in the independent region of the reciprocal space ($2\theta \le 80°$) by the ω/2θ scan technique from an isometric single crystal of poor quality and of an arbitrary shape ~0.03 mm in radius. After exclusion of

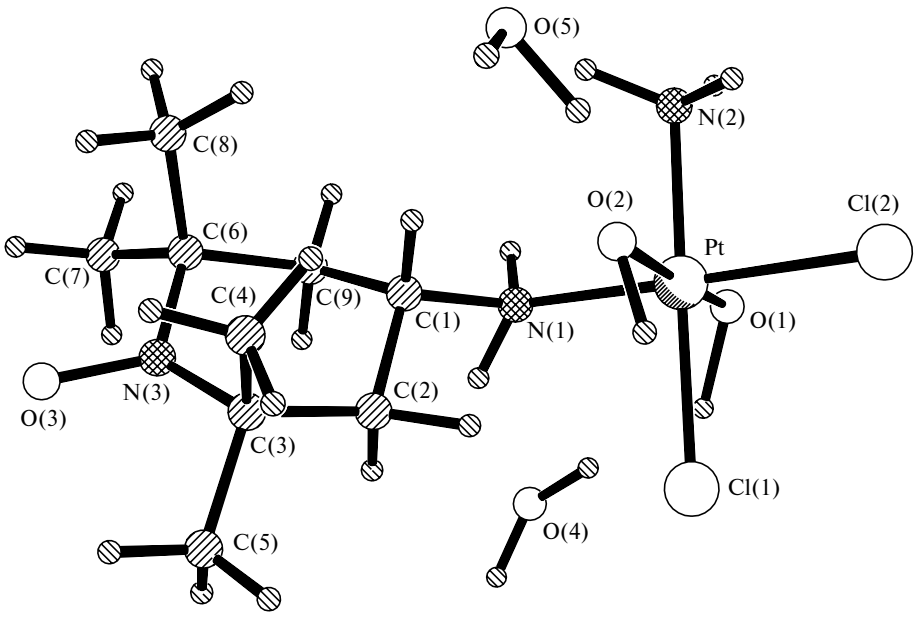


Fig. 1. Molecular structure of the dihydrate of complex **1**.

systematic absences, the X-ray data set contained 4498 measured reflections with $I > 2\sigma(I)$; a KM-4 kappa-geometry diffractometer (Kuma-Diffraction, Poland), Mo-Kα radiation. The principal crystallographic data are as follows: $C_9H_{24}Cl_2N_3O_3Pt \cdot 2H_2O$; $a = 11.710(3)$ Å, $b = 9.797(3)$ Å, $c = 7.913(3)$ Å, $\alpha = 77.08(4)°$, $\beta = 92.29(4)°$, $\gamma = 100.81(3)°$, $V = 869.1(5)$ Å^3, $M = 524.39$, $d_{calc} = 2.26$ g cm^{-3}, space group $P1$, $Z = 2$. The structure was solved by direct methods and refined by the full-matrix least-square method based on F^2 with anisotropic thermal parameters for nonhydrogen atoms using the SHELXL97 program package. The positions of all H atoms were calculated geometrically and were not refined taking into account the poor quality of the single crystal used. The final R factor was 6.7%, the good-of-fitness was 1.080. The molecular structure of **1** is shown in Fig. 1. The principal geometric parameters of **1** are given in Tables 1 and 2.

Determination of toxicity and antitumor activity. The complexes were injected into animals intraperitoneally as aqueous solutions. The total toxicity (LD_{50}) of the compounds was tested in BDF_1 mice upon a single injection. The antitumor activity was examined in an experimental tumor, *viz.*, in P388 leukemia. The transplantation of tumors was carried out according to a standard procedure.[6] The activity of the complexes was evaluated from an increase in the median life span (ILS) of treated animals compared to control animals: ILS (%) = 100(T/C – 1), where T and C are the median life span (days) of treated and control animals, respectively. The cured animals (remained alive for ≥60 days) were considered separately.

Development of drug resistance. The development of P388 leukemia resistance to complex **1** and Cisplatin was studied in mice (six—ten animals per group). The resistance was induced by the successive transplantation of tumor cells from animals treated with complex **1** (strain P388/**1**) or Cisplatin (P388/CP), which were used in equitoxic doses. In the series of successive transplantation generations, the doses of the drugs were gradually increased from 1.5 to 15 mg kg^{-1} for complex **1** and from 0.4 to 4 mg kg^{-1} for Cisplatin. Both drugs were injected 1, 5, and 9 days after the tumor transplantation. At each generation, the sensitivity of each strain to the drug-inductor used in the optimum dose (15 mg kg^{-1} for **1** and 4 mg kg^{-1} for Cisplatin) was tested. The activities of the complexes were evaluated from ILS, T = 60 days being assumed for animals, whose life span was ≥60 days.

Table 1. Bond lengths (d) in molecule **1**

Bond	d/Å	Bond	d/Å
Pt—O(1)	1.997(7)	N(3)—C(6)	1.501(12)
Pt—O(2)	2.005(6)	C(1)—C(9)	1.511(11)
Pt—N(1)	2.055(6)	C(1)—C(2)	1.518(11)
Pt—N(2)	2.021(8)	C(2)—C(3)	1.510(11)
Pt—Cl(1)	2.306(2)	C(3)—C(4)	1.522(15)
Pt—Cl(2)	2.315(3)	C(3)—C(5)	1.538(14)
O(3)—N(3)	1.281(9)	C(6)—C(7)	1.508(14)
N(1)—C(1)	1.489(9)	C(6)—C(9)	1.534(12)
N(3)—C(3)	1.473(11)	C(6)—C(8)	1.535(14)

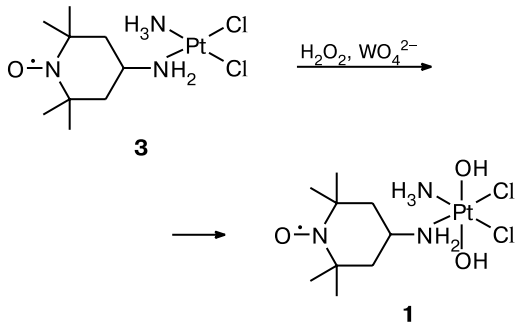
Table 2. Bond angles (ω) in molecule **1**

Angle	ω/deg	Angle	ω/deg
O(2)—Pt—O(1)	174.2(3)	C(3)—N(3)—C(6)	123.0(7)
O(2)—Pt—N(2)	86.4(4)	N(1)—C(1)—C(9)	107.4(6)
O(1)—Pt—N(2)	88.7(4)	N(1)—C(1)—C(2)	110.6(6)
O(2)—Pt—N(1)	93.3(3)	C(9)—C(1)—C(2)	108.4(7)
O(1)—Pt—N(1)	83.5(3)	C(3)—C(2)—C(1)	113.3(7)
N(1)—Pt—N(2)	91.2(3)	N(3)—C(3)—C(2)	110.1(7)
O(2)—Pt—Cl(1)	94.0(3)	N(3)—C(3)—C(4)	109.5(8)
O(1)—Pt—Cl(1)	90.9(2)	C(2)—C(3)—C(4)	111.9(8)
N(2)—Pt—Cl(1)	179.2(3)	N(3)—C(3)—C(5)	105.7(8)
N(1)—Pt—Cl(1)	89.5(2)	C(2)—C(3)—C(5)	108.8(8)
O(2)—Pt—Cl(2)	90.2(3)	C(4)—C(3)—C(5)	110.6(9)
O(1)—Pt—Cl(2)	92.8(2)	N(3)—C(6)—C(7)	108.1(8)
N(2)—Pt—Cl(2)	87.3(3)	N(3)—C(6)—C(9)	107.6(6)
N(1)—Pt—Cl(2)	176.0(2)	C(7)—C(6)—C(9)	110.1(8)
Cl(1)—Pt—Cl(2)	92.0(1)	N(3)—C(6)—C(8)	109.2(8)
C(1)—N(1)—Pt	120.2(5)	C(7)—C(6)—C(8)	110.2(9)
O(3)—N(3)—C(3)	117.5(7)	C(9)—C(6)—C(8)	111.5(8)
O(3)—N(3)—C(6)	115.7(7)	C(1)—C(9)—C(6)	113.9(7)

Results and Discussion

Synthesis and structures of the complexes. The Pt^{II} complexes were oxidized with an excess of H_2O_2 under relatively drastic conditions (70 °C, ≥2 h) according to a known procedure.[5] We found that the reactions were substantially accelerated in the presence of catalytic amounts of tungstate salts, for example, of K_2WO_4 (Scheme 1). When complexes **3** and **4** were taken at the initial concentration of $(3—5) \cdot 10^{-3}$ mol L^{-1} ($[K_2WO_4] = 10^{-3}$ mol L^{-1} and $[H_2O_2] = 0.5$ mol L^{-1}), only peaks of products **1** and **2** were detected by HPLC already within 5 min after the beginning of the reaction even on cooling in an ice bath. Under analogous conditions but in the absence of the catalyst at ~20 °C, the time of half-transformation was ~1 h. Hence, preparative oxidation in the presence of K_2WO_4 (~10^{-3} mol L^{-1}) under mild conditions (0—20 °C) is limited only by the rate of dissolution of a suspension of the starting Pt^{II} complex, complex **4** being dissolved several times more slowly than complex **3**.

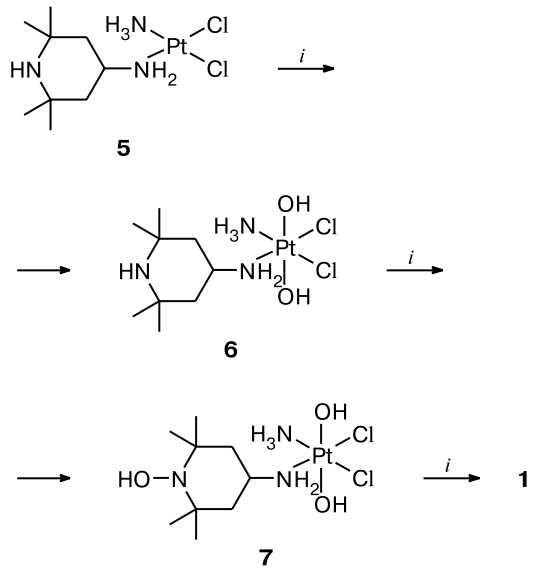
Scheme 1



Taking into account the data on tungstate-catalyzed oxidation of piperidines to piperidineoxyls,[7] it was of in-

terest to study the possibility of the preparation of complex **1** by one-step oxidation of aminopiperidine complex **5** (Scheme 2).

Scheme 2



i. H_2O_2, WO_4^{2-}

The course of oxidation of complex **5** was monitored by HPLC (Fig. 2). Within 5 min after the beginning of the reaction, the peak of the starting complex **5** with V_{ret} = 220 μL was not detected in the reaction mixture; instead, an HPLC peak with V_{ret} = 250 μL appeared, which, apparently, corresponded to complex **6**. Both peaks can be observed in the absence of K_2WO_4 in the reaction mixture. Then the intensity of the peak with V_{ret} = 250 μL decreased and two new peaks with V_{ret} = 570 and 750 μL appeared. Based on the retention volumes, these peaks were identified as intermediate complex **7** and target complex **1**, respectively. For the purpose of identification, complex **7** was prepared independently by reduction of complex **1** with alcohol in an acidic medium[7] (20 mg of **1** in 0.1 mL of 2 *N* HCl in alcohol, ~20 °C, 1 h). The concentration of complex **7** increased during ~2 h, reached ~10% of the concentration of the starting complex, and then decreased. It appeared that the concentration of product **1** also went through a maximum after 4—5 h (~35% of the concentration of the starting complex **5**). In this process, the pH of the reaction mixture was changed from ~10 to ~8 during 4 h resulting in the termination of oxidation of the N—H group of piperidine due to its protonation.[7] After a subsequent increase in pH of the reaction mixture by adding a solution of K_2CO_3 (see Fig. 2, curves *2*, *4*, and *6*), intermediate **7** appeared again in a substantial concentration, and the concentration of product **1** reached ~45% with respect to the concentration of

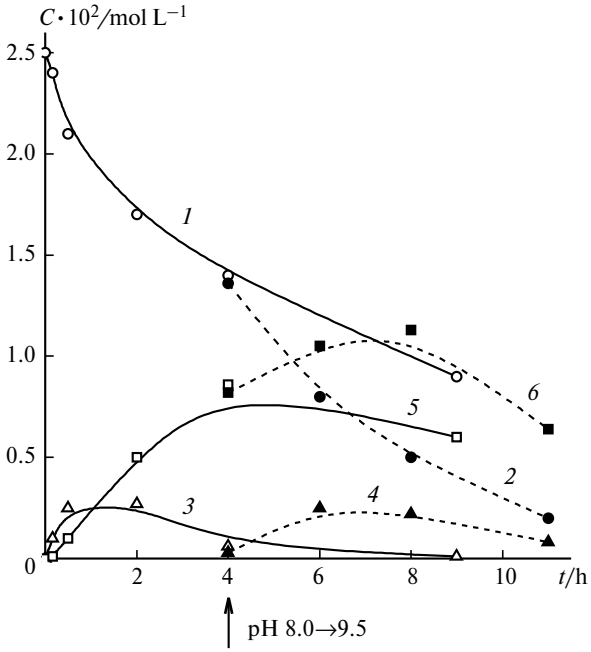


Fig. 2. Changes in the concentrations of complexes **6** (*1*, *2*) and **7** (*3*, *4*) and the final product **1** (*5*, *6*) in oxidation of complex **5** at ~20 °C. The initial concentrations: $[\mathbf{5}]_0 = 2.5 \cdot 10^{-2}$ mol L^{-1}, $[H_2O_2]_0$ = 0.5 mol L^{-1}, $[K_2WO_4]_0 = 1.7 \cdot 10^{-3}$ mol L^{-1}, and [Trilon B]$_0 = 2 \cdot 10^{-3}$ mol L^{-1}. In the parallel experiment, pH of the reaction mixture was increased from 8 to ~9.5 within 4 h after the beginning of the reaction by adding a concentrated solution of K_2CO_3 to $[K_2CO_3] = 5 \cdot 10^{-2}$ mol L^{-1} (curves *2*, *4*, and *6*).

the starting complex and then decreased again. The total concentration of complexes **6**, **7**, and **1** rapidly decreased compared to the starting concentration of complex **5**, which indicates that side reactions proceeded. Apparently, hydrolysis of the Cl ligands took place as such a side reaction at high pH. Hydrolysis afforded hydroxo and aqua complexes.[8] Due to acid-base equilibria and formation of labile complexes with the components of the buffer, these complexes did not show peaks in HPLC at pH of the eluent of ~5 and were detected only from an increase in the background absorption.

It is known[7] that the catalytic action of the WO_4^{2-} anions consists in their ability to form the pertungstate anions WO_n^{2-} (n = 5—8), which are more efficient oxidants than H_2O_2. One would expect that the yield of complex **1** would be increased in the presence of higher concentrations of the catalyst. However, it appeared that a fivefold increase in $[K_2WO_4]$ led to only a slight increase (by 5—10%) in the maximum concentration of product **1** in the reaction mixture. This may be associated with the involvement of WO_n^{2-} in undesirable hydrolysis of the complexes. The results of our study provide evidence that direct oxidation of complex **5** to complex **1** cannot be used as an efficient alternative of oxidation of complex **3** in the preparative synthesis of complex **1**.

Complexes **1** and **2** were obtained as yellow crystalline compounds, whose solubilities in water were ~10 and ~7 mg mL^{-1}, respectively. The structures of these complexes were confirmed by elemental analysis and spectroscopic data (see the Experimental section). The structure of complex **1** was established by X-ray diffraction analysis (see Fig. 1). In the octahedral environment about the Pt^{IV} atom, the pairs of the nitrogen and chlorine atoms are located in a single plane in the *cis* positions, and the O(1) and O(2) atoms are located in the *trans* positions. The bond lengths and bond angles (see Tables 1 and 2) in the coordination sphere of the Pt atom are close to the corresponding parameters for *e*-ammine-*d*-cyclohexylamine-*a*,*f*-bis(acetoxy)-*b*,*c*-dichloroplatinum(IV).[9] The piperidine ring of the nitroxyl ligand adopts a chair configuration. The angle between the C(2)C(3)C(6)C(9) and C(1)C(2)C(9) planes is 52.1°. The angle between the C(2)C(3)C(6)C(9) and C(3)C(6)N(3) planes is 39.5°. Two water molecules, which are not involved in the coordination sphere about the platinum atom, form hydrogen bonds with the O(1), O(2), and N(2) atoms, thus linking the molecules into a three-dimensional framework.

Toxicity and antitumor activity of the complexes. The data on the total toxicity and antileukemic activity of the complexes are given in Table 3. The toxicity of Pt^{IV} complexes **1** and **2** is approximately half as large as that of Pt^{II} complexes **3** and **4**,[4] the piperidine-1-oxyl derivatives being more toxic than their pyrrolidine-1-oxyl analogs in both cases. Conceivably, the latter fact is associated with the difference in the redox properties of piperidine- and pyrrolidine-1-oxyls and, as a consequence, with the differences in metabolism of these drugs.[4]

The most substantial antileukemic effect was observed for complex **1**, which cured four of six test animals (animals that remained alive for more than 60 days were considered as cured animals), and the life span of the remaining two animals was increased, on the average, by 270%.

Table 3. Toxicity and antileukemic (P388) activity of complexes **1**·$2H_2O$ and **2**[a]

Complex	LD_{50}[b]/mg kg^{-1} (mmol kg^{-1})	Single dose /mg kg^{-1}	ILS[c] (%)
1·$2H_2O$	27 (0.052)	9	270 (4/6)
2	45 (0.095)	15	133 (0/6)
Cisplatin	12 (0.040)	4	169 (1/6)

[a] The compounds were injected into animals intraperitoneally 1, 5, 9, 13, and 17 days after the tumor transplantation.
[b] The dose that caused the death of 50% of mice.
[c] The median life span of animals in the control group was 11.2 days. The number of cured animals (they remained alive for more than 60 days) and the total number of animals in the group are given in parentheses in the numerator and denominator, respectively.

Table 4. Enhancement of the antitumor effect in the case of the combined use of Cisplatin and complex **1** in the treatment of P388 leukemia

Complex	Single dose[a] /mg kg^{-1}	ILS (%)	Animals remained alive (%)
Cisplatin	0.6	182	0
1·$2H_2O$	1.4	118	0
Cisplatin + **1**·$2H_2O$	0.6 + 1.4	—	100

[a] The compounds were injected into animals intraperitoneally 1—7 days after the tumor transplantation.

In this test, the activity of complex **1** was much higher than the activities of complex **2** and Cisplatin (see Table 3).

Interesting results were obtained in the study of the combined action of Cisplatin and complex **1** on P388 leukemia. In these assays, very low doses of the drugs (1/20 of LD_{50}) were used. The separate use of the drugs in these doses led to low ILS, whereas their combined application resulted in the synergism and 100% cure of animals (Table 4).

A very important point is that the rate of development of P388 leukemia resistance to Cisplatin differed substantially from that to complex **1** (Fig. 3). As can be seen from

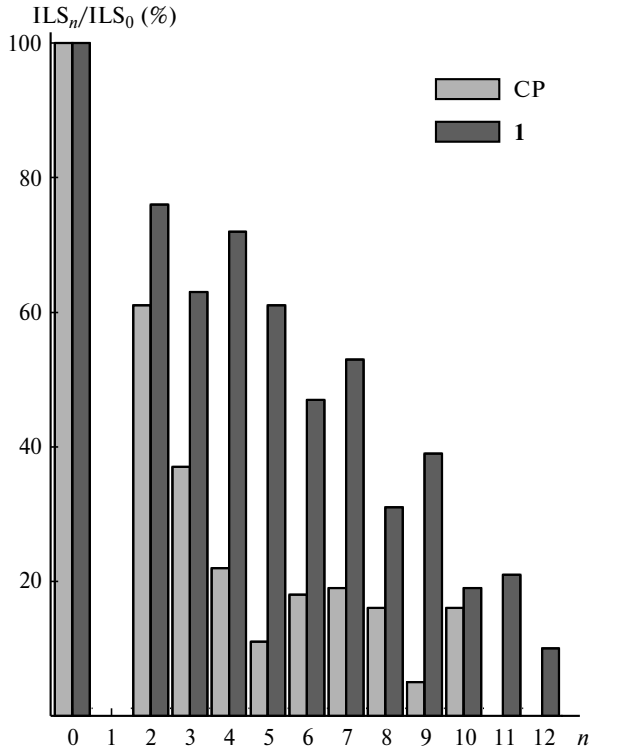


Fig. 3. Decrease in the sensitivity of P388 leukemia to Cisplatin (CP) and complex **1** in the series of successive transplantation generations *n*. For the zeroth generation, the sensitivity of P388 leukemia to the drugs was taken as 100%.

the data in Fig. 3, P388 leukemia cells rapidly developed resistance to Cisplatin. The sensitivity of the tumor to the drug was decreased by 40% even at the second transplantation generation. At the third generation, the sensitivity decreased further by 20%. Compared to the parent tumor, the sensitivity to Cisplatin is decreased by a factor of five at the fourth generation. Such a tumor is commonly considered as a resistant tumor. The sensitivity to complex **1** decreased much more slowly and was reduced by only 25% at the fourth generation. The full resistance of P388 leukemia to complex **1** was achieved only at the tenth generation, *i.e.*, 2.5 times more slowly than that to Cisplatin.

To summarize, platinum(IV) complexes with the aminonitroxyl radicals were prepared and characterized for the first time. These complexes possess high activity against the experimental tumor, *viz.*, P388 leukemia. The advantages of complex **1** over Cisplatin are its higher antitumor activity and a much more slow rate of the development of drug resistance. The combined use of low doses of complex **1** and Cisplatin resulted in the mutual enhancement of the antitumor action of the drugs and led to 100% cure of animals.

This study was financially supported by the International Science and Technology Center (ISTC, Grant 1550).

References

1. L. R. Kelland, in *Uses of Inorganic Chemistry in Medicine*, Ed. N. P. Farrell, Springer, Berlin, 1999, 109.
2. V. D. Sen´, V. A. Golubev, L. M. Volkova, and N. P. Konovalova, *J. Inorg. Biochem.*, 1996, **64**, 69.
3. V. D. Sen´, A. V. Kulikov, A. V. Shugalii, and N. P. Konovalova, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 1640 [*Russ. Chem. Bull.*, 1998, **47**, 1598 (Engl. Transl.)].
4. V. D. Sen´, N. A. Rukina, V. V. Tkachev, A. V. Pis´menskii, L. M. Volkova, S. A. Goncharova, T. A. Raevskaya, A. G. Tikhomirov, L. B. Gorbacheva, and N. P. Konovalova, *Izv. Akad. Nauk, Ser. Khim.*, 2000, 1624 [*Russ. Chem. Bull.*, *Int. Ed.,* 2000, **49**, 1613].
5. C. M. Giandomenico, M. J. Abrams, B. A. Murrer, J. F. Vollano, M. I. Rheinheimer, S. B. Wyer, G. E. Bossard, and J. D. Higgins, *Inorg. Chem.*, 1995, **34**, 1015.
6. *Eksperimental´naya otsenka protivoopukholevykh preparatov v SSSR i SShA* [*Experimental Evaluation of Antitumor Drugs in the USSR and USA*], Eds. Z. Sof´ina, A. Syrkin, A. Goldin, and A. Klein, Meditsina, Moscow, 1980, 296 pp. (in Russian).
7. V. D. Sen´, V. A. Golubev, and N. N. Efremova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1982, 61 [*Bull. Acad. Sci. USSR, Div. Chem. Sci.*, 1982, **41** (Engl. Transl.)].
8. A. V. Shugalii, A. V. Kulikov, M. V. Lichina, V. A. Golubev, and V. D. Sen´, *J. Inorg. Biochem.*, 1998, **69**, 67.
9. S. Neidle, C. F. Snook, B. A. Murrer, and C. F. J. Barnard, *Acta Crystallogr., Sect. C*, 1995, **51**, 822.

Received August 1, 2002