Brief Communications

Synthesis, structure, and biological activity of the *cis*-[4-amino-2,2,6,6-tetramethylpiperidine-*N*,*N*´]dichloropalladium(II) complex

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A previously unknown palladium complex was synthesized by the reaction of 4-amino-2,2,6,6-tetramethylpiperidine with $PdCl_2$. The X-ray diffraction study showed that the complex formed by 4-amino-2,2,6,6-tetramethylpiperidine with $PdCl_2$ is a chelate, in which the bidentate ligand adopts a boat conformation with the nitrogen atoms occupying *cis* positions at the palladium atom. The resulting *cis*-[4-amino-2,2,6,6-tetramethylpiperidine-*N*,*N*´]dichloropalladium(II) complex exhibits strong antimetastatic activity against experimental B16 melanoma at moderate toxicity.

Key words: synthesis, complexation with $PdCl_2$, metal complex, *cis*-[4-amino-2,2,6,6-tetramethylpiperidine-*N*,*N*´]dichloropalladium(II), chelate, X-ray diffraction study, antimetastatic activity, toxicity.

Earlier,[1—3] we have described complexes synthesized from $PtCl_2$ or $PdCl_2$ and substituted amides of pyridinecarboxylic acids as ligands and revealed some characteristic features of the complex formation reactions. Subsequently, we have used these ligands to synthesize complexes with platinum tetrachloride,[4,5] which have *cis*-stereochemistry, as was shown by X-ray diffraction analysis. While these complexes are low-toxic (197—1000 mg kg^{-1}) and do not cause myelosuppression, they inhibit the experimental metastasis of Lewis lung carcinoma and B16 melanoma by 96—98%. In addition, it was experimentally shown that the synthesized complexes act on Ca^{2+}-Mg^{2+}-dependent ATPase of the sarcoplasmic reticulum, thus inhibiting the calcium transport across biological membranes.[6] This leads to the disturbance of the normal ratio of calcium ions on the extra- and intracellular membrane surfaces followed by the inhibition of the aggregation of platelets (a critical step in the metastatic process) and their binding to metastatic cells, which results in the loss of the cell adhesion to blood-vessel walls. However, these compounds cannot be

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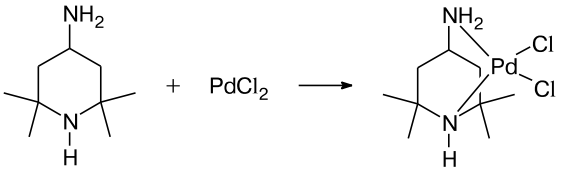
recommended for the application to biological objects because of their low solubility in water. Hence, a search for new highly efficient complexes, which are readily soluble in water and have high activity, is a topical problem in the chemistry of antitumor and antimetastatic drugs. In continuation of our studies on the complexation in a series of substituted pyridines, it was of interest to investigate the complex formation reaction of palladium dichloride with 4-amino-2,2,6,6-tetramethylpiperidine, because piperidines are physiologically active compounds and can actively participate in metabolic processes.

The aim of the present study is to synthesize a previously unknown water-soluble palladium complex with the use of 4-amino-2,2,6,6-tetramethylpiperidine as the ligand, which is supposed to have *cis*-stereochemistry, and to determine the molecular structure of the resulting complex. It is known that complexes with *cis*-stereochemistry have higher therapeutic activity compared to those with *trans*-geometry.[7]

Results and Discussion

It was expected that the use of 4-amino-2,2,6,6-tetramethylpiperidine as the ligand in the reaction with $PdCl_2$ would make it possible to prepare complex **1** with *cis*-stereochemistry (Scheme 1).

Scheme 1



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The X-ray diffraction study confirmed that compound **1** has the expected (see Scheme 1) molecular structure (Fig. 1).

Compound **1** is a molecular complex, in which the Pd^{II} atom is *cis*-coordinated by two chlorine atoms and two nitrogen atoms of the 4-amino-2,2,6,6-tetramethylpiperidine molecule acting as a bidentate ligand (see Fig. 1). In complex **1**, the ligand adopts a boat conformation with positions 1 and 4 of the heterocycle being in close proximity. The resulting chelate forms the 6,6,7,7-tetramethyl-1,3-diazo-2-palladabicylco[2.2.2]octane framework. We do not report the geometric parameters of complex **1** since it is unreasonable to discuss them because of the low quality of the crystals.

Table 1. Biological activity of preparations

Preparation	LD_{100}	LD_{50}	MTD/mg kg^{-1}
Cisplatin	16	12.5	8.0
Complex **1**	300	250	200

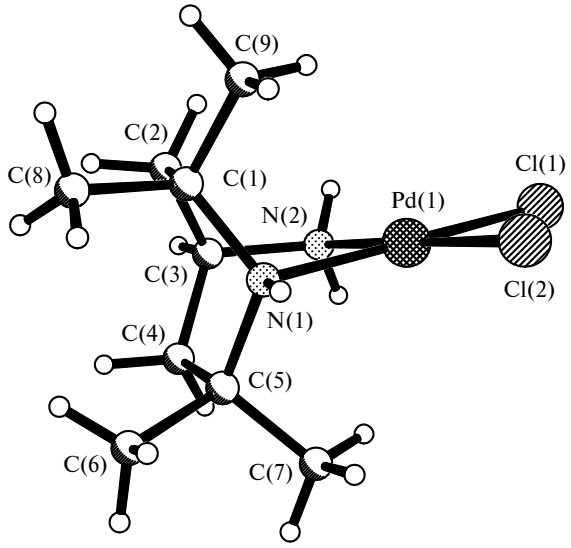

Fig. 1. Molecular structure of complex **1**.

The biological activity of complex **1** was tested in mice at the Laboratory of Experimental Tumor Chemotherapy of the Institute of Problems of Chemical Physics of the Russian Academy of Sciences. Complex **1** was shown to have strong antimetastatic activity against experimental metastatic B16 melanoma (the index of metastasis inhibition (IMI) is 89%) at moderate toxicity (Table 1). For comparison, IMI for cisplatin, which is the chemotherapy drug widely used for the cancer treatment, is 92%.

To summarize, we synthesized new complex **1** starting from palladium dichloride and 4-amino-2,2,6,6-tetramethylpiperidine. Moderate toxicity, fairly good solubility in water, and strong antimetastatic activity of complex **1** suggest that this compound is a promising target for extensive testing in biological materials.

Experimental

The IR spectrum was recorded on a Perkin-Elmer Spectrum-100 instrument. The ^1H NMR spectra were measured on a Bruker-500 spectrophotometer.

Synthesis of *cis*-[4-amino-2,2,6,6-tetramethylpiperidine-*N*,*N*′]dichloropalladium(II). 4-Amino-2,2,6,6-tetramethylpiperidine (0.44 g, 2.8 mmol) was added with stirring to a suspension of $PdCl_2$ (0.5 g, 2.8 mmol) in water (30 mL) at ~20 °C. The reaction mixture was stirred until $PdCl_2$ was completely dissolved (~24 h) and then concentrated in a fume hood using air blow-off until the precipitation of crystals started. After 10—12 h, the pale-brown precipitate was filtered off, washed with a small amount of ice water and ethanol, and dried in air. Complex **1** was obtained in a yield of 0.8 g (80%), m.p. 174 °C (decomp).

Found (%) : C, 32.58; H, 5.71; N, 8.34; Pd, 32.43; Cl, 20.68. $C_9H_{20}N_2PdCl_2$. Calculated (%): C, 32.5; H, 5.76; N, 8.42; Pd, 31.99; Cl, 21.33. IR, ν/cm^{-1}: 703 (w), 788 (m), 866 (m), 891 (m), 931 (s), 968 (s), 1018 (s), 1050 (s), 1068 (s), 1087 (s), 1160 (s), 1182 (s), 1221 (s), 1222 (s), 1269 (m), 1293 (w), 1307 (m), 1348 (m), 1363 (m), 1380 (s), 1392 (s), 1443 (s), 1475 (s), 1588 (s), 1609 (s), 2110 (w), 2468 (s), 2594 (s), 2652 (m), 2759 (s), 2830 (m), 2925 (w), 2946 (w), 3010 (m), 3184 (s), 3377 (s). ^{1}H NMR (DMSO-d_6): 1.35 (s, 6 H, CH_3); 1.41 (s, 6 H, CH_3); 1.51 (m, 2 H, CH_2); 2.23 (m, 2 H, $-CH_2-$); 3.19 (m, 1 H, CH); 7.94 (br.s, 2 H, NH_2); 9.26 (br.s, 1 H, NH). The sample for the X-ray diffraction study was obtained by the crystallization from a mixture of acetonitrile and water in a ratio of 1 : 2.5.

X-ray diffraction study of complex 1. The unit cell parameters for compound **1** were measured and the three-dimensional X-ray diffraction data set was collected on a KM-4 four-circle diffractometer (KUMA-Diffraction, Poland) from a poor-quality crystal with dimensions of 0.15×0.1×0.1 mm. Compound **1** crystallizes in the monoclinic system: $C_9H_{20}N_2PdCl_2$, M_r = 333.57, a = 12.814(3) Å, b = 11.519(2) Å, c = 17.803(4) Å, β = 91.97(3)°, V = 2626(1) Å^3, d = 1.687 g cm^{-3}, λ = 1.5418 Å, space group $C2/c$, Z = 8, μ = 14.88 mm^{-1}.

A total of 1591 reflections with $F_0 > 4\sigma(F_0)$ were measured for compound **1** in the angle range $3.5 < \theta < 80.5°$ using the ω/2θ-scan technique. The structure was solved by direct methods using the SHELXL-97 program package.[8] The coordinates of nonhydrogen atoms were refined by the full-matrix least-squares method with anisotropic displacement parameters. The hydrogen atoms were positioned geometrically and refined using a riding model. The final R factor is 0.075.

Biological assays. Experiments were carried out in BDF_1 hybrid mice with a weight of 22—24 g. Each group included 8—10 animals. The inoculum size for experimental B16 melanoma was $5 \cdot 10^6$ tumor cells. The B16 melanoma cells were injected subcutaneously. The B16 melanoma-bearing mice were sacrificed 24 days after the transplantation of tumor cells. The number of lung metastases was determined, and then the index of metastasis inhibition, which is an indicator of the antimetastatic activity of the complex, was calculated according to the equation

$$\mathrm{IMI} = \frac{(A_C B_C - AB)}{(A_C B_C)} 100\%$$

where A_C and A are the frequencies of metastasis in the control and test groups, respectively, B_C and B are the average numbers of metastases in the control and test groups, respectively. The preparations were administered intraperitoneally as aqueous solutions.

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