MEMBRANE-PROTECTIVE PROPERTIES OF PALLADIUM (II) COMPLEXES CONTAINING ANTIOXIDANT FRAGMENT AS POTENTIAL RADIOPROTECTIVE AGENTS

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Abstract: *New complex compounds of palladium (II) with the biologically active ligand 2-ethyl-6 methyl-3-hydroxypyridine – mexidol were synthesized in acidic (pH=5.3) and slightly alkaline (pH=7.5) media, respectively, of the following composition: $(C_8H_{12}ON^+)_2[PdCl_4]$ – $(HL)_2[PdCl_4]$* ***(I)*** *and $[Pd(C_8H_{11}NO)_2Cl_2]$ – $[PdL_2Cl_2]$* ***(II)****. In complex* ***I****, two ligand molecules are protonated and occupy the outer coordination sphere as singly charged cations. In complex* ***II****, the ligands are coordinated monodentately via the nitrogen atom in the trans-position to each other. Complexes* ***I*** *and* ***II*** *were tested for radioprotective properties. The toxicity of complexes* ***I*** *and* ***II*** *is LD_{50} - 240 mg/kg and 256 mg/kg of animal weight, respectively. The biological test showed that complexes I and II, along with radioprotective properties, also have some antitumor activity.*
Key words*: palladium (II), mexidol, tetraacidoanion, hydrogen bond, coordination, ligand, denticity.*
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Introduction

The study of the nature and role of free radicals in biological processes has made significant advances over the past three decades. Free radicals are formed in the body as a result of the metabolism of oxygen dissolved in tissues, and the resulting active forms of oxygen cause oxidation of membrane lipids, proteins, polysaccharides, and nucleic acids [1, 2]. In recent years, the antioxidant drug Mexidol has been widely used in medical practice for the prevention and treatment of various pathological conditions. Mexidols (3-hydroxy-6-methyl-2-ethylpyridine) are derivatives of one substance - 3-oxypyridine, differing only in the acid in the salt. However, the acids cause differences in the pharmacological properties of these drugs, for example, differences in antihypoxic activity [3]. It seems logical to search for new drugs among complex salts of palladium (II) with 3-hydroxy-6-methyl-2-ethylpyridine (C_5H_2N-$R_1R_2R_3$, R_1-CH_3, R_2-C_2H_5, R_3-OH).

The 3-hydroxy-6-methyl-2-ethylpyridine, an inhibitor of free radical processes, is a membrane protector that also has antihypoxic and antioxidant properties [4, 5]. Compounds based on palladium with 3-hydroxy-6-methyl-2-ethylpyridine have not been found in the literature. Thus, the most pressing problem of radiation medicine remains the development of compounds with radio-modifying activity, the effect of which is manifested when introduced into the body both before and after ionizing radiation, as well as under conditions of chronic ionizing radiation in small doses. For the first time, we synthesized complex compounds of palladium with 3-hydroxy-6-methyl-2-ethylpyridine in the composition of $(HL)_2[PdCl_4]$ and $[PdL_2Cl_2]$ in the range of 5.3-5.7 pH-medium and various ratios of reactants (M:L). The structure of the complex $(C_8H_{12}ON^+)_2[PdCl_4]$ – $(HL)_2[PdCl_4]$ **(I)** was determined by X-ray diffraction [6].

Experimental part

The 3-hydroxy-6-methyl-2-ethylpyridine ligand was further purified from auxiliary substances by recrystallization.

IR - spectra were recorded on Thetmoscientific, Nicoletis 10 and Bruker IFS–113V spectrometers in vaseline or a suspension of fluorinated oils, and in the form of KBr tablets. Elemental analysis of non-metals was performed on a CHNS-O EMA 502 analyzer. The palladium content was determined by X-ray fluorescence on an EDX-7000 P spectrophotometer (SHIMADZU). Thermal behavior of the complex was studied on an STA 449 F3 Jupiter NETZSCH derivatograph at a heating rate of 10 deg/min. in air to 800°C. Diffraction patterns of the palladium complex and ligand samples were obtained on a DRON-8 diffractometer. The electrical conductivity of the complex was measured using a KEL-1M2 conductometer in water-alcohol solutions at 25^0C.

Synthesis of the complex

$[Pd(C_8H_{11}NO)_2Cl_2]$ – $[PdL_2Cl_2]$ (II). The complex palladium salt – $K_2[PdCl_4]$ weighing 0.41 g (1.26 mmol) is dissolved in 15 ml of water and heated to 45 °C. 2-Ethyl-6-methyl-3-hydroxypyridine weighing 0.34 g (2.48 mmol) is also dissolved in 15 ml of water at 50 °C. Then both hot reaction solutions are mixed with vigorous stirring (pH = 7.5). The reaction mixture is stirred for 1.5 hours at 60 °C. Then the reaction mixture is transferred to a porcelain cup and slowly evaporated in a water bath at 40 °C to a small volume. Upon cooling after three days, yellowish-brown needle-shaped crystals precipitate from the mother liquor. The crystals of the complex $[PdL_2Cl_2]$ are too small, so X-ray structural analysis was not performed for the complex. The precipitate is filtered and washed with ethanol, then with diethyl ether. The crystals are dried first in air, then in a vacuum to constant weight. Yield was 0.50 g (88% of the introduced palladium). The substance is highly soluble in water.

Results and discussion

The results of elemental analysis of the $[PdL_2Cl_2]$ (II) complex are presented in Table 1.

Table 1. Results of elemental analysis of complex **II**

Complex	Pd		N		Cl		C		H	
	Fou.	Calc.	Fou.	Calc.	Fou.	Calc.	Fou.	Calc.	Fou.	Calc.
$[PdL_2Cl_2]$	23.79	23.56	6.31	6.20	15.86	15.70	42.70	42.56	4.98	4.87

The value of molar electrical conductivity of the solution (μ) of complex **I** at a concentration of $1\cdot10^{-3}$ mol/dm^3 equal to 224.7 $Ohm^{-1}sm^2mol^{-1}$ indicates that compound **I** is a triionic electrolyte. For comparison, the electrical conductivity of the triionic electrolyte $BaCl_2$ was determined, for which the molar electrical conductivity was 223.1 $Ohm^{-1}sm^2mol^{-1}$. The molar electrical conductivity of complex **II** in an aqueous-alcoholic solution at a condensation of $1\cdot10^{-3}$ M showed a value of 38.5 $Ohm^{-1}sm^2mol^{-1}$, which confirms the non-electrolyte nature of the complex. To establish the purity and individuality of the obtained complexes, their X-ray diffraction patterns were taken. The ligand diffraction patterns differ sharply from the synthesized complexes, which confirm their individuality and purity (Fig. 1).

To identify the obtained compounds, the IR- spectra of the initial palladium salts, ligand and synthesized new complexes were recorded, then a comparative and appropriate assignment of absorption bands in the IR- spectra was made. Comparison of the IR- spectra of the free ligand and synthesized complexes **I** and **II** allows us to unambiguously determine the structure and mode of coordination of the ligands in them. In the IR spectrum of the

uncoordinated ligand, the existing broad absorption band at 3444 sm^{-1} is assigned to the free phenolic O-H group. The observed asymmetric absorption band of the stretching vibration at 1303 sm^{-1} belongs to the C–O group [7]. During complex formation due to hydrogen bonds, this band decreases to 1620 sm^{-1}, which is accompanied by a decrease in the band intensity [8].

The IR -spectra of the complexes and ligands in the range of 1500-4000 sm^{-1} are different, which allows us to identify the spectral changes that occur during the formation of the complex from the IR spectra. For the ligands, IR bands are observed that are characteristic of hydrochloric acid salts of these ligands with a hydrogen bond of the H…Cl type, for which the. IR band at 2470 sm^{-1} is characteristic.

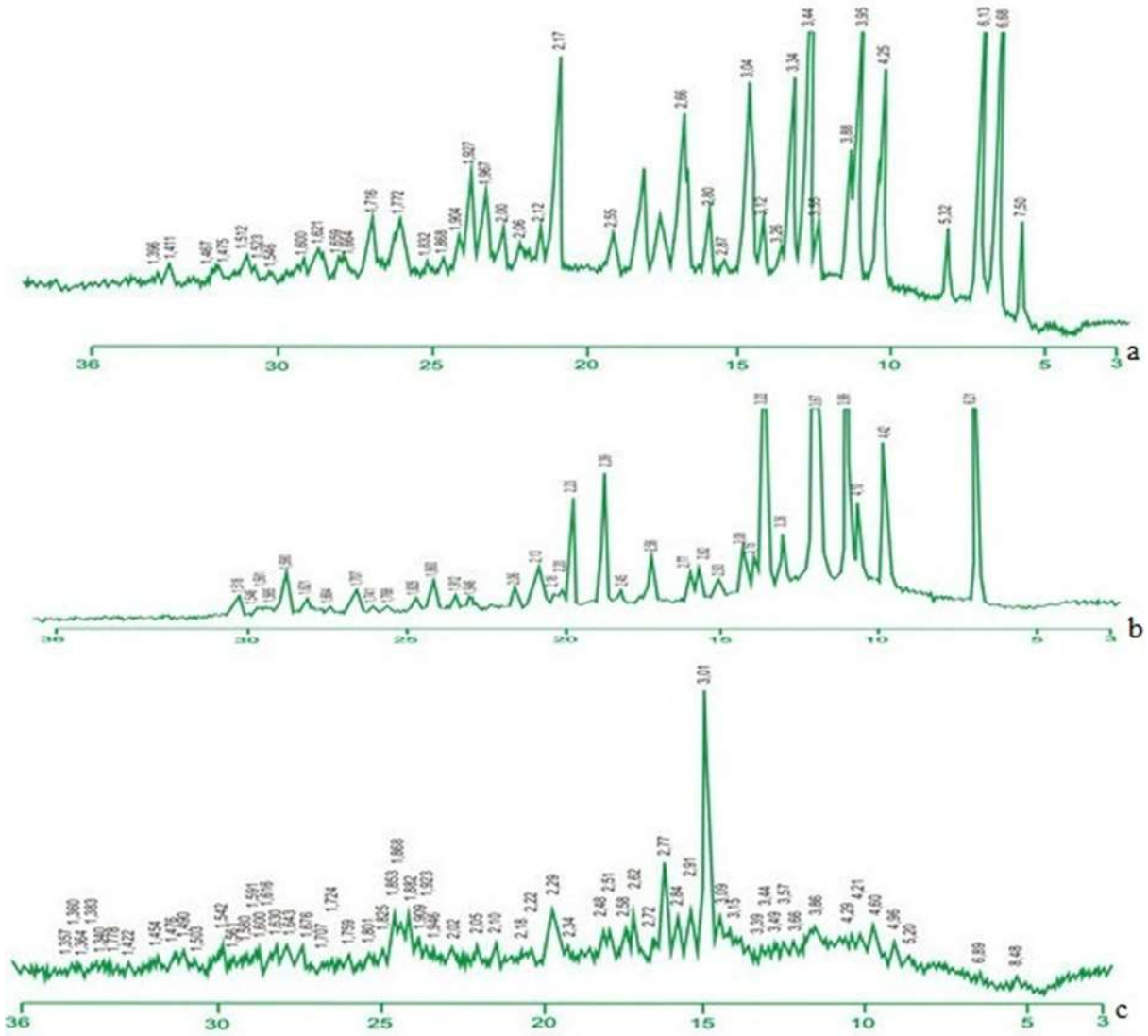

Fig. 1. X-ray diffraction patterns of ligand-L(a) and complexes $(HL)_2[PdCl_4]$(b), $[Pd(L)_2Cl_2]$(c)

The doublet band at 3340/3300 sm^{-1} is due to the intramolecular hydrogen bond OH ... Cl. The band at 2470 sm^{-1}, related to the hydrogen bond, disappears in the IR -spectra of the complexes NH...Cl and bands of the R cation with frequencies of 2860-2880 sm^{-1} and 1562-1570 sm^{-1} (δNH^+) appear. The intense doublet band 3404-3460/3380-3408 sm^{-1} refers to the stretching vibration of the OH group participating in the intramolecular hydrogen bond HO...Cl with the chlorine atoms of the acidoanion. The complex nature of the IR spectra of the complexes in the region of 2300-3400 sm^{-1} indicates the existence of both intermolecular and intramolecular hydrogen bonds NH...Cl in these compounds. Thus, according to the IR spectroscopy data, there are two types of hydrogen bonds in the complex: intramolecular hydrogen bond NH...Cl, OH...Cl and intermolecular hydrogen bond NH...Cl. The

presence of hydrogen bonds in the complex has also been demonstrated by X-ray structural analysis [6].

Absorption in the region of 1260 and 1320 sm^{-1} is related to in-plane deformation vibrations of the free phenolic O–H group [7,8]. The out-of-plane deformation O–H group is located below 700 sm^{-1}. These facts indicate that the phenolic hydroxyl group of the ligands does not participate in the coordination with palladium (Fig. 2 and 3). During complex formation in an acidic medium, the pyridine nitrogen atom of the ligand is protonated and occupies the outer sphere as a singly charged ion. This is evidenced by the absorption bands at 3321 sm^{-1} [7, 8].

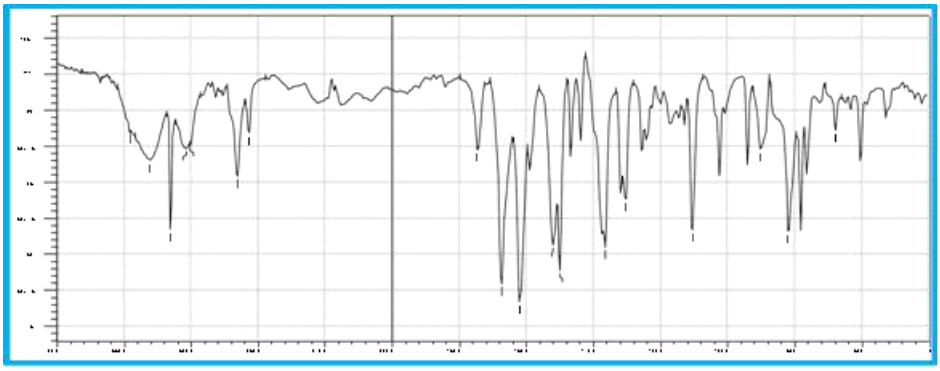

Fig.2. IR spectrum of the ligand – L

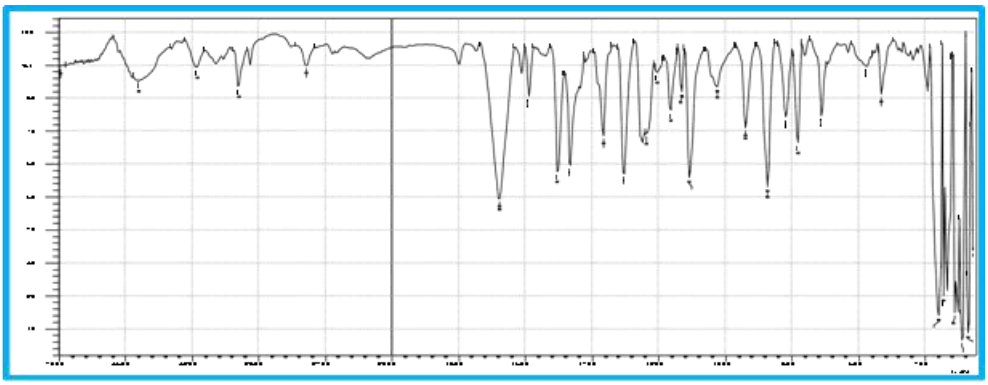

Fig. 3. IR spectrum of the $[Pd(L)_2Cl_2]$ complex.

For complex **II**, the observed changes in the region of stretching (1678–1203 sm^{-1}) and stretching-deformation (1107–875 sm^{-1}) vibrations of the pyridine ring in the IR spectra indicate its participation in the formation of the coordination bond. In particular, the redistribution of electron density during complex formation leads to an increase in the frequencies of stretching vibrations of the bonds (C=N) of the pyridine ring by an average of 10–40 sm^{-1}. In the IR -spectrum of complex compound II, the stretching vibrations of the $\nu_{Pd–N}$ and $\nu_{Pd–Cl}$ bonds are observed as unsplit bands at 358–347 and 312–282 sm^{-1}, respectively, which indicates the trans structure of this complex [9, 10].

Thermogravimetric study of the obtained complexes **I** and **II** showed that the decomposition of the complexes, depending on their composition and structure, occurs differently. The heating curve of the compound $(HL)_2[PdCl_4]$ (Fig. 5a) shows endothermic effects at 122, 197 and 342 ^{0}C, as well as many exothermic effects. At the first stage (weight loss 16.0%), two HL molecules are split off (calculated value 16.6%). At the second stage, four chlorine atoms are split off from the $[PdCl_4]^{2-}$ anion (weight loss 45.9%, calculated 49.4%).

The first endothermic effect corresponds to the removal of two ligand molecules and a chlorine atom. At this temperature, dehydrohalogenation with migration into the inner sphere of the ligand does not occur. On the derivatogram (DTA) curve of the compound $[Pd(L)_2Cl_2]$ (Fig. 4b), only two endothermic effects are noted at 190 and 208^0 C, as well as many exothermic effects. The appearance of the

first endothermic effect is consistent with the removal of two ligand molecules and a chlorine atom. The data of derivatographic analysis of the studied compounds showed that the complexes decompose without melting and the final product of thermolysis is metallic palladium [11].

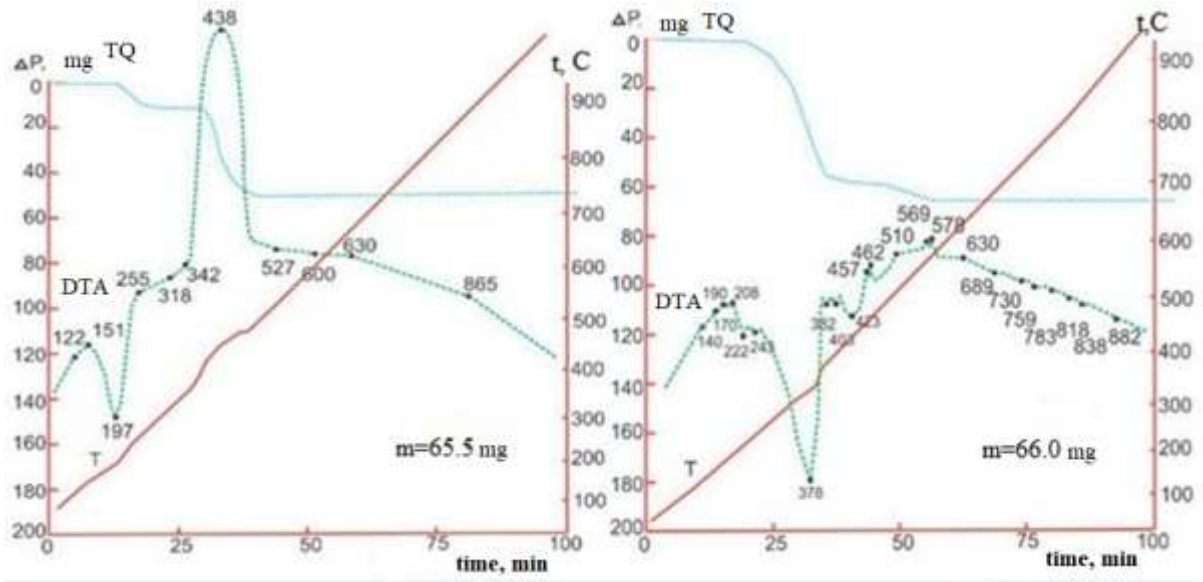

Fig. 4. Derivatograms of $(HL)_2[PdCl_4]$ (a), $[PdL_2Cl_2]$ (b)

Thus, the results of IR spectroscopy, X-ray diffraction, elemental analysis and molar conductivity show that the interaction of 2-ethyl-6-methyl-3-hydroxypyridine with palladium (II) derivatives in acidic medium results in the formation of a tetraacidoanion – $[PdCl_4]^{2-}$, while the pyridine nitrogen atom of mexidol is protonated and occupies the outer coordination sphere as a single-charged cation. In a neutral medium, the $[Pd(L)_2Cl_2]$ complex is formed, in which two ligand molecules are coordinated by nitrogen atoms in the trans position to each other.

The radioprotective properties of complexes **I** and **II** were studied in inbred male $C_{57}B1$ mice weighing 18–24 g with intravenous administration at doses of 1/8 and 1/2 of the LD_{50} value 15–20 min before single irradiation and 15–20 min after irradiation with each fraction with three-time daily irradiation.

Irradiation was carried out with ^{137}Cs γ-rays (power 1.88–1.75 rad/s) on the IGUR setup. A single irradiation of animals was performed at a dose of 8.2 Gy, the scale of increasing radiation doses with three-time daily fractional exposure included doses of 2.5, 3.0, and 3.5 Gy [12,13].

The animals of the experimental groups were injected intravenously into the tail vein with a 0.45% solution of the complexes in isotonic sodium chloride solution at doses of 1/8 and 1/2 of the LD_{50} value (for **I** LD_{50} = 240 mg/kg, for **II** LD_{50} = 256 mg/kg). The animals of the corresponding irradiation control groups were injected intravenously with the physiological solution in a volume of 0.3 ml per mouse. The animals of the biological control group were not exposed to irradiation, the studied complexes, or the solvent. Each experimental group of animals included 12 to 24 individuals, including an irradiation control group and a biological control group. The animals were observed for 30 days. The condition and behavior of the animals were monitored, the number of dead animals and the time of their death were noted. The survival rate of animals was determined as a percentage on the 30th day of observation.

Complexes **I** and **II** are characterized by moderate toxicity (class 3 toxicity) and high antitumor and radioprotective activity. When **I** and **II** were applied to Lewis lung carcinoma (LLC), statistically significant antitumor and antimetastatic effects were observed, exceeding the effect of cisplatin: when using **I** and **II**, LLC growth inhibition reached 64.0%, and in the

case of cisplatin - 48.0%. Suppression of LLC metastasis activity in the case of I was 92.5%, for **II** - 77.9%, compared with 69.0% in the case of cisplatin. It was established that **I** exhibits a pronounced radioprotective effect. With a single irradiation of animals at a dose of 8.2 Gy, their survival was 59.7% versus 4.5% in the control. With fractional irradiation of animals in increasing doses of 2.5, 3 and 3.5 Gy, their survival was 78.0% versus 33.0% in the control. Complexes **I** and **II** have radio-modifying activity when introduced into the body both before and after ionizing radiation, as well as under conditions of chronic ionizing radiation in small doses. Mexidamole is excreted from the body with urine 5-8 hours after intravenous administration.

It has been established that mexidamole, along with radioprotective properties, also has some antitumor activity, which is very important in radiation therapy. The combination of pronounced radioprotective properties and antitumor activity characterizes mexidamole from the best side.

Complex-**II** $[PdL_2Cl_2]$ are of considerable interest from the point of view of studying their cytotoxic properties. For a long time, researchers engaged in the search for new antitumor drugs neglected palladium compounds, which is explained by its tendency to form complexes of *trans*-structure and greater lability of products compared to platinum analogs. However, today it is known that the trans-structure of the complex is an obstacle to the manifestation of antiproliferative activity, and the rate of substitution reactions in palladium complexes is successfully reduced by introducing bulky ligands into their composition, for example, heterocyclic amines, which create steric hindrances for nucleophilic attack, as well as chelating ligands, which contributes to the formation of thermodynamically stable products [14, 15]. The $[PdL_2Cl_2]$ complex synthesized by us has good solubility in aqueous media and has a trans-configuration and thermodynamic stability, which is of particular interest from the point of view of biological activity. The obtained data indicate the possibility of using complexes **I** and **II** as a radioprotector, as well as in the treatment of malignant neoplasms.

Conclusion

The data of IR spectroscopy, elemental analysis and molar conductivity showed that, when mexidol interacts with palladium dichloride, either a neutral complex $[PdL_2Cl_2]$ or a tetraacidoanion - $[PdCl_4]^{2-}$ is formed depending on the pH of the medium. In this case, the ligands in the neutral complex are coordinated via the nitrogen atom of the pyridine ring in the trans position, and in the second case, the pyridine nitrogen atom of mexidol is protonated in an acidic medium and occupies the outer coordination sphere as a single-charged cation. The obtained 2-ethyl-6-methyl-3-hydroxypyridine tetrachloropalladium dichloride mexidanole and *bis*-2-ethyl-6-methyl-3-hydroxypyridine palladium dichloride, along with radioprotective properties, also have some antitumor activity.

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