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Influence of the Conformers of the Ligand 2-Methylamino-1-Phenylpropan-1-ol (HA) in the Complexes $(H_2A)_2[PdCl_4]$ on Their State in Solutions and Their Biological Activity

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Abstract—The systems Pd(II)–Cl^-–HA–H_2O (where HA are the conformers of 2-methylamino-1-phenylpropan-1-ol) were studied. The acid dissociation constants of the ligands HA and the resulting complexes were determined by pH-metric titration and mathematical modeling. The pH-distribution diagram of the complex species in the system containing different HA conformers was obtained. The complexes $(H_2A)_2[PdCl_4]$ were obtained and structurally characterized; their toxicity and radioprotective effects were estimated. The biological activity of the complexes was found to depend on both the ligand basicity and the hydrogen bonding system formed by different conformers of the ligand in the cation–anion complexes.

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A systematic investigation of the composition–structure–biological activity dependence for palladium compounds have revealed that some of its complexes can stabilize the cell membrane [1–5]. This has given impetus to a search for compounds with radioprotective activity among palladium complexes.

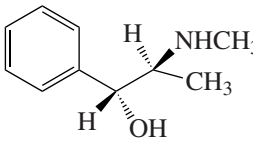
The class of cation–anion palladium complexes of the general formula $[NH_2R_1R_2][PdCl_4]$ with a radioprotective effect was first described in [6].

A test of the palladium acido complex $(l\text{-}C_{10}H_{16}NO)_2[PdCl_4]$ with protonated *l*-2-methylamino-1-phenylpropan-1-ol in the cation [7–11] for radioprotective activity has revealed that this complex can not only protect an organism from multiple exposure to low-intensity radiation but also influence its secondary immunodeficient states caused by the irradiation.

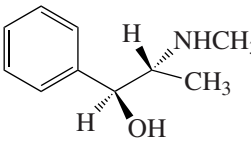
The possibility of forming cation–anion palladium complexes is determined by the tendency of a nitrogen- or hydroxo-containing ligand toward hydrogen bonding to the Cl atoms of the anion $[PdCl_4]^{2-}$. First of all, this tendency depends on the ligand basicity. According to [12], such ligands as secondary amines, piperazine, amino acids, etc. with deprotonation constants above 8.0 form sufficiently stable cation–anion complexes in aqueous solutions of hydrochloric acid. Weak bases (sulfanilamide and Novocain) are not protonated in concentrated HCl and the reaction product is always the aminate complex $[Am_nPdCl_2]$. A cation–anion complex for the above amines, as well as for strong reducing agents (e.g., hydrazine), can be obtained in nonaqueous media according to our procedure [12]. However, once dissolved even at pH 1–3, these complexes immediately undergo the Anderson rearrangement leading to an aminate complex.

Apart from the ligand basicity, the steric factor (relative positions of the Cl and H atoms and the OH groups) can also be important for hydrogen bonding in the complex $(H_2A)_2[PdCl_4]$ and the strength of the H bonds. When a ligand shows conformational isomerism, the resulting palladium complexes can differ in spatial structure and biological activity.

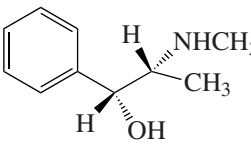
To elucidate these questions, here we used three forms of the ligand 2-methylamino-1-phenylpropan-1-ol ($C_{10}H_{15}NO$, HA):



(1*R*,2*S*)-2-methylamino-1-phenylpropan-1-ol (*l*-HA_1)



(1*S*,2*R*)-2-methylamino-1 phenylpropan-1-ol (*d*-HA_1)



(1*S*,2*S*)-2-methylamino-1-phenylpropan-1-ol (*d*-HA_2)

For the complexes $(l\text{-}H_2A_1)_2[PdCl_4]$ (**I**), $(d\text{-}H_2A_1)_2[PdCl_4]$ (**II**), and $(d\text{-}H_2A_2)_2[PdCl_4]$ (**III**), we determined the influence of the basicity and steric parameters of the ligand on the characteristics of the resulting complex species in aqueous solutions with pH varying from ~4 to 7 under the conditions that model the transformations of complexes **I**–**III** injected in animal organisms for determination of their biological activity. The complexes were injected intravenously as solutions in the physiological solution (aqueous 0.15 M NaCl).

EXPERIMENTAL

We used $PdCl_2$ (high-purity grade), KCl (reagent grade), the ligands $l\text{-}HA_1$, $d\text{-}HA_1$, and $d\text{-}HA_2$ (Fluka); K_2PdCl_4 was prepared as described in [13]. Complex **I** was synthesized as described in [6].

Synthesis of complex II. The ligand $d\text{-}HA_1 \cdot HCl$ was dissolved in a minimum amount of water and acidified with HCl to pH 1. The resulting solution was filtered through a membrane filter (0.2 μm). Palladium dichloride (Pd : L = 1 : 2) was dissolved at 45°C in 4 N HCl and filtered through the same filter. The filtrate was added to the solution of the ligand at 45°C. The mixture was left for 15–20 h for crystallization. The precipitate that formed was filtered off and dried in air (atmospheric pressure, 60°C) and then *in vacuo*. The yield was 60%.

We failed to obtain complex **III** in the aforementioned way; so we carried out its synthesis in a nonaqueous medium.

Synthesis of complex III. The ligand $d\text{-}HA_2 \cdot HCl$ was suspended in dry benzene (80 ml). A suspension of bis(benzonitrile)palladium dichloride in benzene (20 ml) was added in portions to the stirred suspension of the ligand. The resulting mixture (Pd : L = 1 : 2) was stirred at room temperature for 24 h. The brown precipitate that formed was filtered off, washed with benzene and ether, and dried *in vacuo* over $CaCl_2$. The yield was 84%.

All the complexes obtained have the general formula $(C_{10}H_{16}NO)_2[PdCl_4]$). The analytical characteristics of complexes **I**–**III** are given in Table 1.

The palladium content of the complexes was determined gravimetrically. The chlorine content was determined by a volumetric method; the nitrogen content was determined on a standard analyzer. The IR spectra of complexes **I**–**III** were recorded on Bruker-JFS-113V and Bruker-JFS-45 FTIR spectrometers in the 50–4000 cm^{-1} range (suspensions in Vaseline or fluorinated oil and KBr (CsI) pellets).

The IR spectrum of complex **I** shows two bands at 3450 and 3399 cm^{-1} due to the stretching vibrations of the hydrogen-bonded OH groups (intramolecular hydrogen bond OH···Cl). In this range, the IR spectra of complexes **II** and **III** contain only one band (3392 and 3461 cm^{-1}, respectively). In the range of the metal–ligand stretching vibrations, the bands at 323, 332, and 329 cm^{-1} for complexes **I**, **II**, and **III**, respectively, are due to the Pd–Cl vibrations of the class E in the square planar anion$[PdCl_4]^{2-}$ with the local symmetry D_{4h}.

Table 1. Elemental analysis data for complexes **I–III**

Complex	Content (found/calculated), %		
	Pd	C	N
I	18.58/18.32	24.83/24.42	4.51/4.82
II	18.60/18.32	24.51/24.42	4.62/4.82
III	18.24/18.32	24.58/24.42	4.90/4.82

Procedure for pH-metric titration of solutions of the ligands and system [Pd(II)–Cl^-–HA–H_2O]. pH of the solutions were measured on an EV-74 ionometer with glass and silver-chloride electrodes to within ±0.05 pH units. The glass electrode was regularly calibrated by titrating 0.1 M HCl with a prepared alkali. The ionometer was adjusted against buffer solutions at 25°C. pH-Metric titration was carried out in a temperature-controlled (25°C) cell (V = 25 ml, ionic strength I = 0.1 (KCl)).

Carbonate-free KOH used for pH-metric titration was standardized against potassium biphthalate and succinic acid. The complexes were titrated with aqueous 0.0962 M KOH. The initial solutions were prepared by dissolving precisely weighed samples.

To determine the acid dissociation constants K_a of the ligands, we employed aqueous 0.005 M solutions of $l\text{-}HA_1 \cdot HCl$ and $d\text{-}HA_2 \cdot HCl$.

To determine the stability constants of the resulting complexes in the systems Pd(II)–Cl^-–HA–H_2O, we used aqueous 0.005 M solutions of $l\text{-}HA_1 \cdot HCl$ and $d\text{-}HA_2 \cdot HCl$ and aqueous 0.006 M K_2PdCl_4 (or a solution of $PdCl_2$ in aqueous KCl (0.15 M). The Pd : L ratio in the systems was always 1 : 2.

Data from pH-metric titration were processed by mathematical modeling with the AUTOEQUIL program [14].

Palladium complexes with different protonated conformers (the ligands HA in the cation) were tested for biological activity (acute toxicity and radiomodifying activity) with male mice of the line $F_1(CBA \times C_{57}B1)$.

Acute toxicity was estimated from the following parameters: $LD_{50/30}$ (lethal dose for 50% of test animals within 30 days) and MTD (maximum tolerable dose; in this case, $LD_{10/30}$).

Radiomodifying effect was determined from the dose reduction factor (DRF), which displays the tendency of a compound to lessen the consequences of the irradiation. To calculate DRF, $LD_{50/30}$ in the presence of a complex as a radioprotector is divided by $LD_{50/30}$ in a blank test. The doses of the complexes were 10 mg/kg

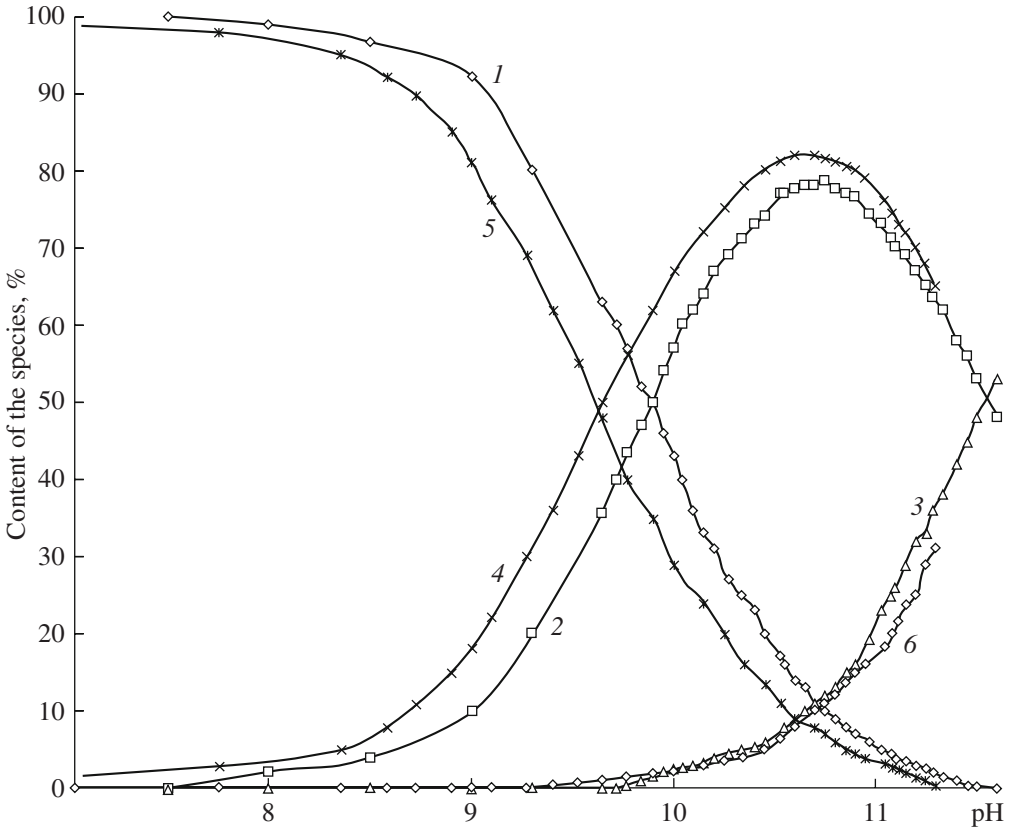


Fig. 1. Constitution diagrams of the ligands *l*-HA_1 and *d*-HA_2 in aqueous solutions at different pH values: (*1*) *l*-$H_2A_1^+$, (*2*) *l*-HA_1^0, (*3*) *l*-A_1^-, (*4*) *d*-HA_2^0, (*5*) *d*-$H_2A_2^+$, and (*6*) *d*-A_2^-.

for mice exposed five times to γ-radiation (time interval between the exposures was 24 h). The complexes were injected intraperitoneally 15 min after the irradiation.[1]

RESULTS AND DISCUSSION

To determine the acid dissociation constants of the ligands HA (*l*-HA_1 and *d*-HA_2), we carried out pH-metric titration of aqueous 0.005 M solutions of *l*-$HA_1 \cdot HCl$ and *d*-$HA_2 \cdot HCl$ with aqueous 0.0962 M KOH as described above. We considered the following equilibria:

$$H^+ + A^- = AH^0, \quad \beta_1 = [HA]/[H^+]\,[A^-];$$

$$2H^+ + A^- = H_2A^+, \quad \beta_2 = [H_2A^+]/[H^+]^2\,[A^-].$$

Taking β_1 and β_2 to be the protonation constants, we obtain pK_1 and pK_2:

$$pK_1 = \log\beta_2 - \log\beta_1, \quad pK_2 = \log\beta_2.$$

The constants $\log\beta_n$ were unknown variables and the independent (basic) components of the matrix were the species H^+, L^-, OH^-, HA, and H_2A^+. Our calculations involved successive steps of mathematical modeling as described in [14, 15]. The constitution diagrams of the ligands *l*-HA_1 and *d*-HA_2 at different pH values of solutions are shown in Fig. 1. According to the data obtained, pK_a of *l*-HA_1 is 9.87 ± 0.08 (pK_{a1}) and 11.54 ± 0.08 (pK_{a2}) and pK_a of *d*-HA_2 is 9.657 ± 0.04 (pK_{a1}), and 11.60 11.60 ± 0.06 (pK_{a2}).

The pK_{a1} value of *l*-HA_1 is higher by 0.2 logarithmic units than that of *d*-HA_2, which corresponds to the weaker acid properties of the cation *l*-$H_2A_1^+$ and will inevitably affect the strength of the hydrogen bonds in complex **I** compared to complex **III**. The pK_{a2} values of *l*-HA_1 and *d*-HA_2 are identical to within the experimental error.

It should be noted that $pK_{a1} = 9.87$ obtained for *l*-HA_1 is in good agreement with the literature data (at 15–25°C, $pK_{a1} = 9.75$ [16], 9.89, and 9.58 [17]). The literature data for the other constants of *l*-HA_1 and *d*-HA_2 are unavailable.

[1] Biological tests were carried out by the research group headed by I.D. Treshchalin at the Gauze Research Institute of New Antibiotics of the Russian Academy of Medical Sciences.)

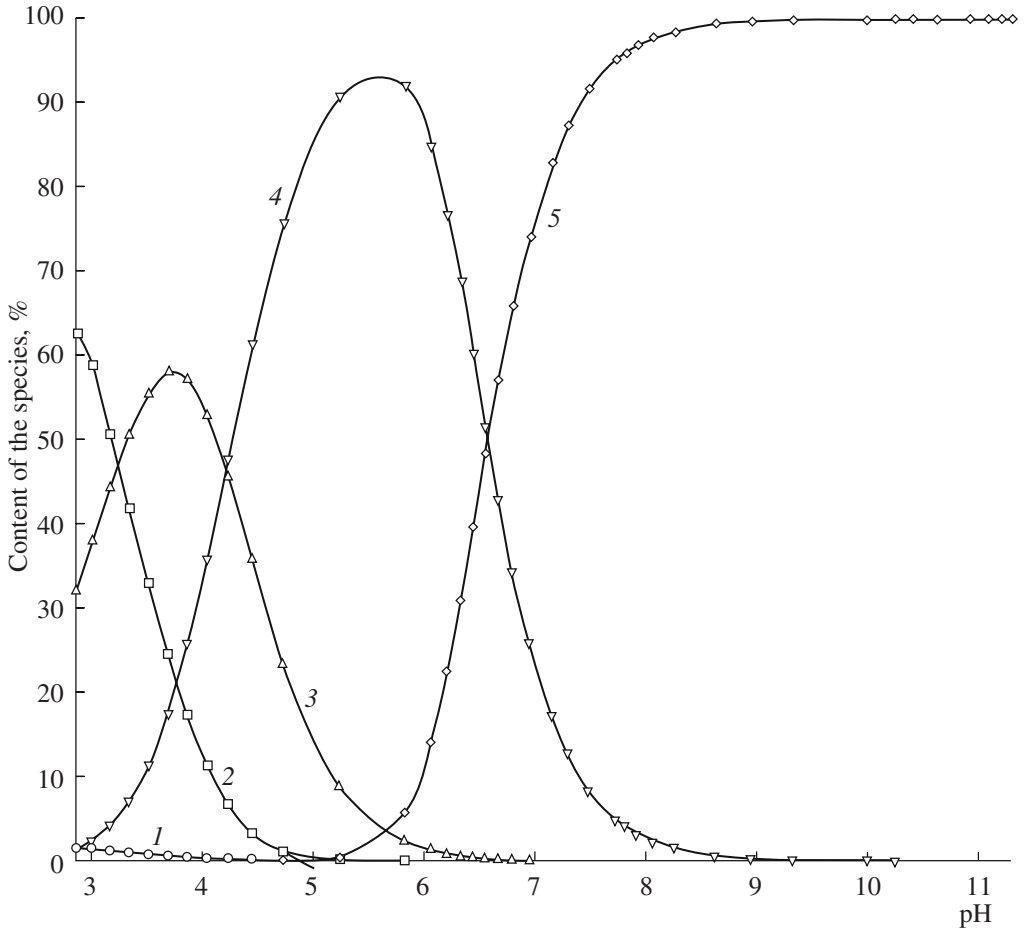


Fig. 2. Distribution of the complex species in the system Pd(II)–*l*-HA_1–Cl^-–H_2O: (*1*) $PdCl_3^-$, (*2*) $PdCl_4^{2-}$, (*3*) $Pd(l\text{-}HA_1)Cl_2^0$, (*4*) $Pd(l\text{-}A_1)Cl_2^-$, and (*5*) $Pd(l\text{-}A_1)_2^0$.

To study the system Pd(II)–HA–Cl^-–H_2O, we carried out pH-metric titration of mixtures of $PdCl_2$ and *l*-HA_1 (system **A**) or $PdCl_2$ and *d*-HA_2 (system **B**) in the ratio 1 : 2. The initial solutions for titration ($CPd^+ = 4 \times 10^{-3}$ M, $CH_2A^+ = 8 \times 10^{-3}$ M, and $CCl^- = 1.124$ M) were titrated with 0.0871 M KOH.

Distribution diagrams of the complex species in solutions, with isolation of the major species and determination of their constants of formation β, were plotted separately for each case with the same molecular matrix. The starting model included four independent components (H^+, Cl^-, Pd(II), *l*-HA_1 or *d*-HA_2), OH^-, both protonated species *l*-$H_2A_1^+$ or *d*-$H_2A_2^+$, palladium mono- and dihydroxo complexes $[PdOH]^+$ and $Pd(OH)_2$, and chloride complexes $PdCl_n$ ($n = 2$–4). We ignored the monochloro complex (because of a great excess of chloride ions) and the chlorohydroxo complexes found in the system Pd(II)–Cl^-–H_2O [15], from which the OH^- group can be displaced in the presence of a nitrogen-containing ligand.

The distribution diagrams of the complex species in systems **A** and **B** (Figs. 2, 3) show that some or other complexes dominate differently at different pH values. This can be seen better in Fig. 4.

At pH 3–3.5, system **A** contains the anions $[PdCl_4]^{2-}$ and the protonated ligand *l*-$H_2A_1^+$. However, at these pH values, the cation is deprotonated, the coordination sphere of palladium in $[PdCl_4]^{2-}$ loses a Cl atom, and palladium coordinates the ligand through the N atom to form the monoaminate complex $[Pd(l\text{-}HA_1)Cl_2]^0$. Deprotonation of the cation *d*-$H_2A_2^+$ in system **B** at pH 3.5–4.0 also leads to the aminate complex $[Pd(d\text{-}HA_2)Cl_3]^-$. The contents of $[Pd(l\text{-}HA_1)Cl_2]^0$ and $[Pd(d\text{-}HA_2)Cl_3]^-$ reach the highest values at pH 4 and > 4.5, respectively.

With an increase in pH (> 5), the deprotonation of the OH groups in both systems **A** and **B** gives rise to the similar complex species $[Pd(l\text{-}A_1)Cl_2]^-$ and $[Pd(d\text{-}A_2)Cl_2]^-$, in which the ligands are coordinated through the N and O

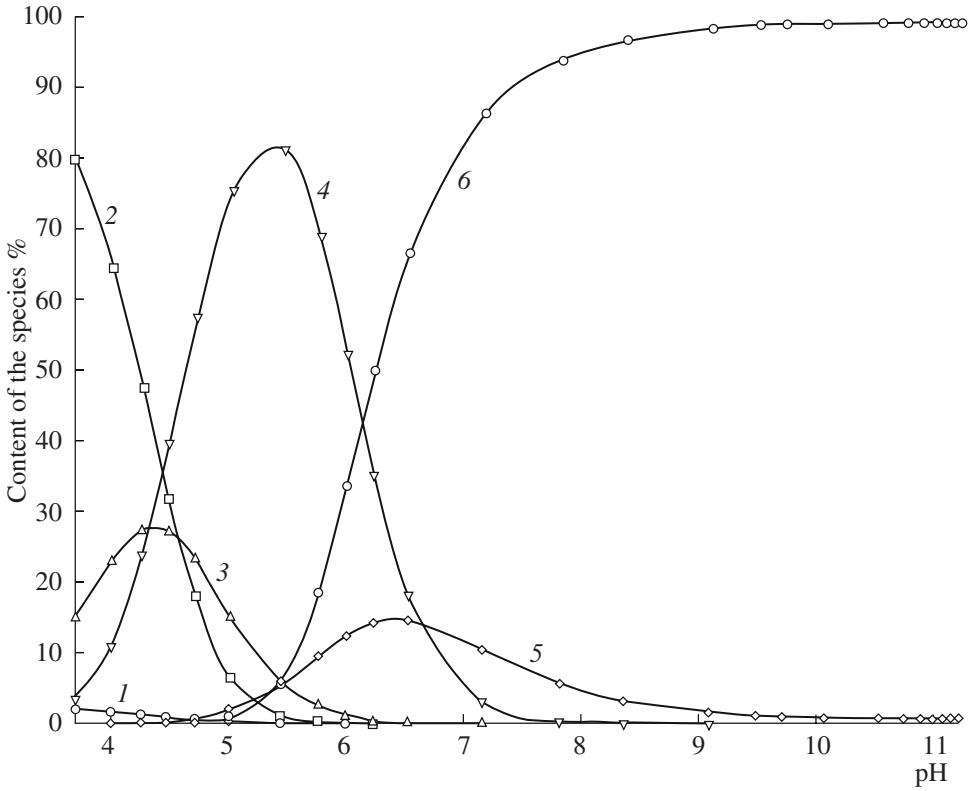


Fig. 3. Distribution of the complex species in the system Pd(II)–*d*-HA_2–Cl^-–H_2O: (*1*) $PdCl_3^-$, (*2*) $PdCl_4^{2-}$, (*3*) Pd(*d*-HA_2)Cl_3^-, (*4*) Pd(*d*-A_2)Cl_2^-, (*5*) Pd(*d*-A_2)Cl$(OH)^-$, and (*6*) Pd(*d*-A_2)$_2^0$.

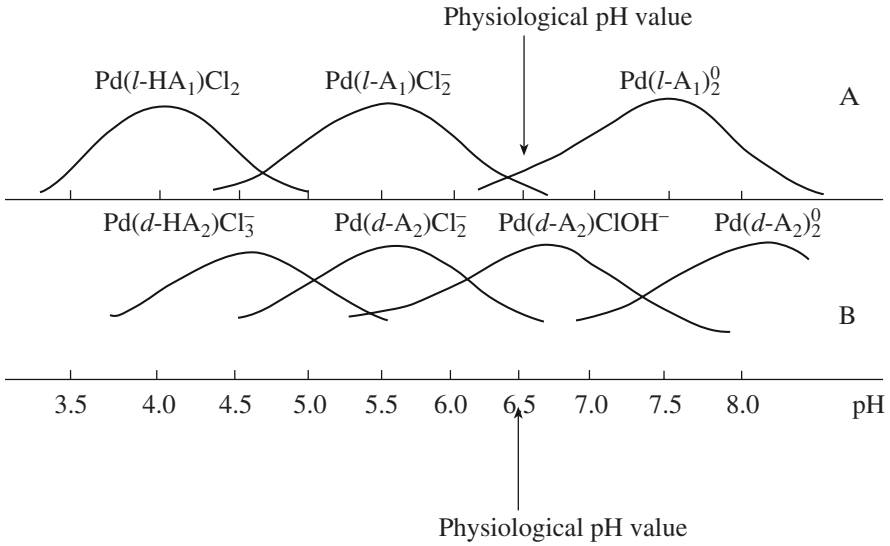


Fig. 4. Successive formation of the complex species with an increase in pH for the systems Pd(II)–*l*-HA_1–Cl^-–H_2O (A) and Pd(II)–*d*-HA_2 –Cl^-–H_2O (B).

Table 2. Acute toxicity of the complexe $(H_2A)_2[PdCl_4]$

Complex	Way of injection	$LD_{50/30}$, mg/kg	MTD ($LD_{10/30}$), mg/kg
I	Intraperitoneal	245 (285–205)	180
	Intravenous	140 (155–125)	110
II	Intravenous	140 (155–125)	110
III	Intravenous	100 (109.5–91.5)	75

atoms ($\beta = 29.8 \pm 0.1$ and 28.8 ± 0.1, respectively). The lower constant of formation of $[Pd(d\text{-}A_2)Cl_2]^-$ suggests its lower stability. Indeed, with a further increase in pH, the complex species differ. The complex $[Pd(l\text{-}A_1)Cl_2]^-$ remains unchanged, while $[Pd(l\text{-}A_1)Cl_2]^-$ is transformed into the complex species $[Pd(d\text{-}A_2)ClOH]^-$ with $\beta = 21.2 \pm 0.2$. At pH > 6.5, both ligands form similar complexes $[Pd(l\text{-}A_1)_2]^0$ and $[Pd(d\text{-}A_2)_2]^0$ with close β values (21.7 ± 0.2 and 21.2 ± 0.2, respectively).

Thus, the results obtained suggest that the hydrogen bonds in the complexes $(H_2A)_2[PdCl_4]$ affect the acid-basic forms of the ligand. For instance, the free ligands l-HA_1 or d-HA_2 are deprotonated to give the species A_1^- and A_2^- and this process is most intense at pH > 10. For the coordinated ligands, the strongest deprotonation occurs at pH > 5. The complexes $[Pd(l\text{-}A_1)Cl_2]^-$ and $[Pd(d\text{-}A_2)Cl_2]^-$ also have different stabilities in solution, which is evidently due to hydrogen bonding systems because of different positions in the ligand conformers of the groups forming hydrogen bonds. This is reflected by their different constants of formation. The complex $[Pd(l\text{-}A_1)Cl_2]^-$ remains intact almost to the physiological pH value (when its biological activity should appear), while $[Pd(d\text{-}A_2)Cl_2]^-$ is hydrolyzed even at pH 5.5 to form the more reactive complex species $[Pd(d\text{-}A_2)ClOH]^-$ capable of reacting with fragments of a biological medium (amino acids, peptides, etc.). In turn, this can result in the loss of its specific activity and increase its toxicity.

Our data on the toxicity and radioprotective activity of the palladium complexes obtained are consistent with the aforesaid assumptions. Data on acute toxicity of complexes **I**–**III** are summarized in Table 2; data on their radiomodifying effect are given in Table 3. According to Tables 2 and 3, complex **II** is as toxic as the basic complex **I** ($LD_{50/30}$ and MTD ($LD_{10/30}$)), while complex **III** is more toxic than complex **I**.

Our data on the radioprotective activity of complexes **I**–**III** suggest a substantial influence of the ligand conformation on the biological activity of the complex. For instance, complex **I** with l-HA_1 exhibits a higher radioprotective effect than complexes **II** and **III**. The highest DRF value (1.47) was provided by complex **I**. The average single radiation dose that causes the death of 50% of test animals was 4.55 Gy for complex **I** and 3.26 Gy for complex **III**.

Our data on the influence of the ligand conformation on the biological activity become clear in terms of restructuration of the hydrogen bonds in complexes **I** and **III** when passing from l-HA_1 to d-HA_2.

According to X-ray diffraction data for complex **I** [18], its crystal consists of the anions $[PdCl_4]^{2-}$ and the cations l-$H_2A_1^+$ (Fig. 5). In the cation l-$H_2A_1^+$, the CH_3 groups at the N and C(8) atoms are in the *gauche*-con-

Table 3. Radiomodifying effect of the complexes $(H_2A)_2[PdCl_4]$

Complex	Irradiation dose per fraction, Gy	Total irradiation dose, Gy	Survival rate, %	$LD_{50/30}$, Gy	DRF
Control test	2.0	10	100	2.8	1.0
	2.5	12.5	80		
	3.0	15.0	30		
Complex **I** + Irradiation	2.5	12.5	100	4.55	1.47
	3.0	15.0	80		
	3.5	17.5	30		
Complex **II** + Irradiation	2.5	12.5	100	3.24	1.16
	3.0	15.0	80		
	3.5	17.5	10		
Complex **III** + Irradiation	2.5	12.5	100	3.26	1.30
	3.0	15.0	70		
	3.5	17.5	30		

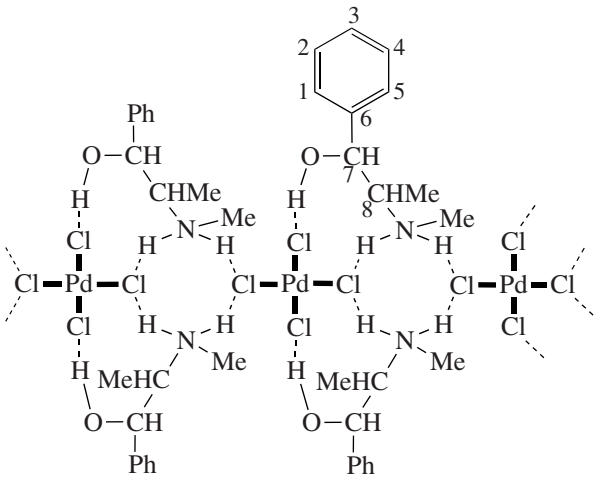


Fig. 5. Structure **I**.

formation with respect to the N–C(8) bond, the N atom and the OH^- group are in the *gauche*-conformation with respect to the C(7)–C(8) bond, and the N atom and the C(1)···C(6) atoms of the benzene ring are in the *trans*-conformation with respect to the same bond. This conformation of the cation is favorable for the formation of a complex system of hydrogen bonds linking the cations and anions. The cation contains three "active" protons involved in hydrogen bonding to the coordinated Cl atoms of the anions. The fragments ···H–N–H··· of a pair of cations serve to unite the anions into infinite chains along the axes of these anions. In each pair, the OH^- groups point to the symmetrically attached Cl atoms (Cl(3) and Cl(3)') of the same anion, thus forming the corresponding hydrogen bonds.

The ligand *d*-HA_2 with a different spatial conformation forms a different system of hydrogen bonds; so complex **III** differs in stability (and, consequently, in the ability to interact with biological systems) from complex **I**.

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