Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

DNA modification by *cis*-diaminoplatinum(II) complexes with aminonitroxide ligands

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The mixed-ligand complexes *cis*-$Pt^{II}[R(CH_2)_nNH_2](NH_3)X_2$, where R = 2,2,6,6-tetramethyl-1-oxylpiperidin-4-yl, X_2 = ClI or Cl_2, n = 1 or 2, and binuclear complexes *trans*-3,4-bis[*cis*-ammine(iodochloro or dichloro)platinum(II)amino]-2,2,6,6-tetramethylpiperidin-1-oxyls were synthesized. The reactivity of the aminonitroxide complexes toward DNA, the destabilizing effect of the adducts on DNA structure, and the distribution of the Pt adducts along the DNA duplex were studied. The platination activity of the complexes is affected by the natures of both the leaving ligands X and the carrying amino ligands. The decrease in the platination activity of the complexes with an increase in the amino ligand sizes is probably caused by steric hindrance. The complexes that effectively platinate isolated DNA and cause a moderate destabilizition of DNA duplex possess high antitumor activities.

Key words: platinum(II) complexes, nitroxides, spin labels, DNA, platination, ESR spectroscopy, Cisplatin.

The key process in the inhibition of tumor growth by *cis*-diaminoplatinum(II) complexes is platination of DNA, which violates its replication.[1] The platination of DNA has been studied in detail by various methods, mainly, using Cisplatin.[2] Recently, we synthesized complexes **1**, **2**, $\mathbf{3_0}$ (*i.e.*, n = 0) and **4** with aminonitroxide ligands (L) and showed that some of them compare well with Cisplatin in antitumor activity[3—5] and are less toxic, presumably, due to the antioxidant properties of nitroxide radicals.[5] Since these complexes are paramagnetic, DNA platination can be studied by ESR, as we have done previously[4,6] for complexes **1** and **4**. The relationship between the structure and the antitumor activity of diaminoplatinum complexes is rather complicated and depends on many factors.[7] However, it is clear that a necessary condition is efficient binding of the complex to DNA in a cell.

The ligands X in diamino Pt^{II} complexes tend to be removed rather easily upon hydrolysis and/or replacement by nucleophilic groups of biomolecules. For this

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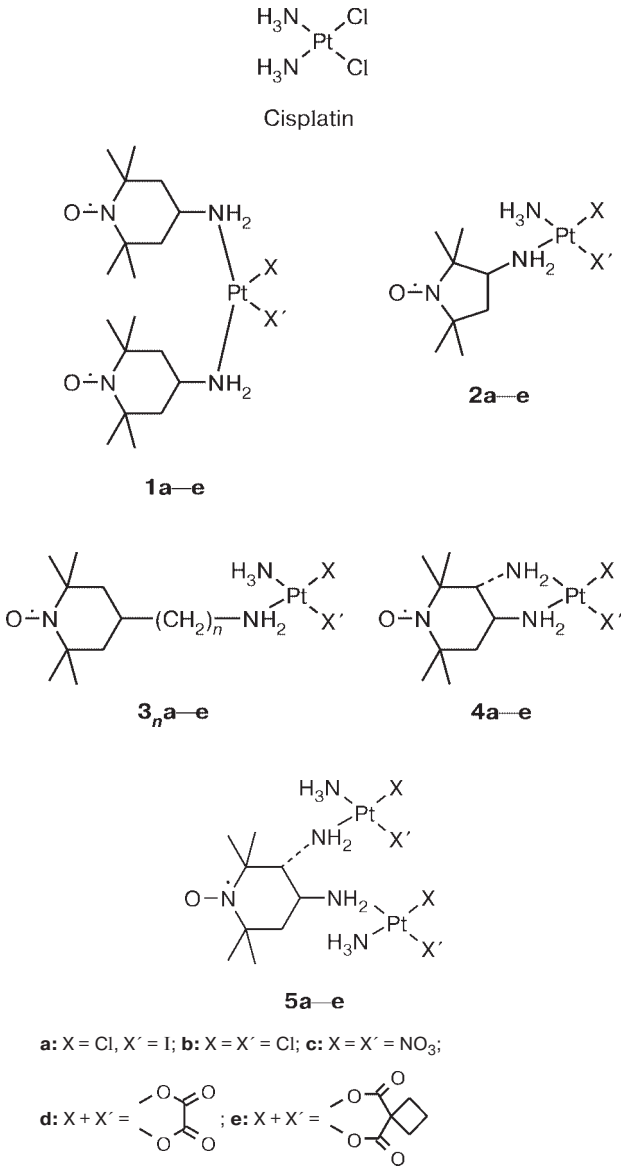

reason, these ligands are called leaving groups. The rate constants for hydrolysis of ligands X can differ[6] by a factor of 10^5 and, as a consequence, the biological effects of complexes differing only by ligands X are dissimilar. The amino ligands remain bound to platinum throughout the whole metabolism route until they reach the primary (regarding the antitumor activity) target, *viz.*, DNA molecule; thetefore, they are referred to as carrying ligands.[7,8]

The purpose of this work was to study the binding of new complexes to DNA using ESR and UV spectroscopy and, taking into account the previous results,[4,6] to summarize data on the influence of structures of aminonitroxide ligands on the efficiency of DNA platination and the properties of the resulting LPt^{II}—DNA adducts (L is an aminonitroxide). Using ESR spectroscopy, one can determine the degree of DNA platination, the mobility of the nitroxide radicals in the LPt^{II}—DNA adducts, and the distances between them. The degree of DNA destabilization due to the formation of adducts by complexes of various structures was studied by UV spectroscopy. These data could be useful for elucidating the relationship between the structure and activity of platinum complexes. In order to extend the structural series of the complexes, new mixed-ligand complexes $\mathbf{3_1}$, $\mathbf{3_2}$ (*i.e.*, n = 1, 2), and binuclear complex **5** were prepared.

Experimental

The platinum content in the complexes was determined by atomic-absorption spectroscopy using an AAS-3 spectrometer with an error of ±3 rel. %. IR spectra were recorded in the 400—4000 cm^{-1} range on a Specord IR-75 spectrometer in mineral oil, and ESR spectra were run at room temperature and at 77 K on an SE/X 2544 instrument with a microwave power of 0.3 mW and 0.32-mT modulations.

The initial 4-aminomethyl-, 4-(β-aminoethyl)-2,2,6,6-tetramethylpiperidin-1-oxyls (**6a**,**b**) and *trans*-3,4-diamino-2,2,6,6-tetramethylpiperidin-1-oxyl (**7**) were prepared by previously described procedures.[9—11]

***cis*-Ammine(4-aminomethyl-2,2,6,6-tetramethylpiperidin-1-oxyl)iodochloroplatinum(II) ($\mathbf{3_1a}$).** A solution of NaI (0.72 g, 4.8 mmol) in 0.4 mL of H_2O and a solution of 4-aminomethyl-2,2,6,6-tetramethylpiperidin-1-oxyl (0.62 g, 3.4 mmol) in 0.4 mL of H_2O were added successively with stirring at ~20 °C to a solution of $Na[Pt(NH_3)Cl_3]$ in 10 mL H_2O prepared from Cisplatin (1.00 g, 3.3 mmol).[12] The reaction mixture was stirred for 2 h at ~20 °C and kept in a refrigerator (~10 °C) for 10 h. The resulting precipitate was filtered off, thoroughly washed with cold water (4×2 mL), and dried in air to give 1.24 g (67%) of complex $\mathbf{3_1a}$, which was used in the synthesis of $\mathbf{3_1b}$. To prepare an analytical grade specimen $\mathbf{3_1a}$, the synthesis was carried out with $K[Pt(NH_3)Cl_3]\cdot 0.5H_2O$ isolated intermediately.[12] Complex $\mathbf{3_1a}$ represents small yellow crystals, m.p. 155—158 °C (decomp.). Found (%): C, 21.6; H, 4.41; N, 7.43; Pt, 34.1. $C_{10}H_{24}ClIN_3OPt$. Calculated (%): C, 21.47; H, 4.33; N, 7.51; Pt, 34.88. IR, ν/cm^{-1}: 1555, 1605, 3170, 3225, 3255 (NH_2, NH_3).

***cis*-Ammine(4-aminomethyl-2,2,6,6-tetramethylpiperidin-1-oxyl)dichloroplatinum(II) ($\mathbf{3_1b}$).** Silver nitrate (220 mg, 1.29 mmol) was added to a suspension of complex $\mathbf{3_1a}$ (0.402 g, 0.72 mmol) in 10 mL of water, and the mixture was stirred with a magnetic stirrer for 12 h in the dark. The precipitated silver halides were thoroughly separated by centrifuging and filtering through a dense glass filter to give a solution of orange-colored dinitrato complex $\mathbf{3_1c}$, which did not contain $AgNO_3$.[5] A solution of KCl (0.30 g) in 1 mL of water was added with stirring to the solution of $\mathbf{3_1c}$ and the mixture was allowed to stand for 12 h at ~20 °C. The reaction solution was concentrated to ~3 mL and the orange crystals thus formed were filtered off, washed with cold water and EtOH, and dried *in vacuo* to give 195 mg (58%) of complex $\mathbf{3_1b}$, m.p. 172—174 °C (decomp.). Found (%): C, 25.9; H, 5.37; N, 8.83; Pt, 40.9. $C_{10}H_{24}Cl_2N_3OPt$. Calculated (%): C, 25.69; H, 5.18; N, 8.99; Pt, 41.74. IR, ν/cm^{-1}: 1557, 1630, 3178, 3230 (NH_2, NH_3).

***cis*-Ammine[4-(β-aminoethyl)-2,2,6,6-tetramethylpiperidin-1-oxyl]iodochloroplatinum(II) ($\mathbf{3_2a}$)** was prepared similarly to

3_1a, yield 74%, m.p. 145—148 °C (decomp.). Found (%): C, 23.3; H, 4.69; N, 7.53; Pt, 33.2. $C_{11}H_{26}ClIN_3OPt$. Calculated (%): C, 23.03; H, 4.57; N, 7.33; Pt, 34.02. IR, ν/cm^{-1}: 1560, 1609, 1627, 3190, 3245 (NH_2, NH_3).

***trans*-3,4-Bis(*cis*-ammineiodochloroplatinum(II)amino)-2,2,6,6-tetramethylpiperidin-1-oxyl (5a).** A solution (0.42 g, 2.55 mmol) of KI in 3 mL of water was added with stirring at ~20 °C to a solution of $K[Pt(NH_3)Cl_3]\cdot 0.5H_2O$ (313 mg, 0.85 mmol) in 4 mL of H_2O, and then a solution of *trans*-3,4-diamino-2,2,6,6-tetramethylpiperidin-1-oxyl (85 mg, 0.45 mmol) in 2 mL of water was added dropwise over a period of 1 h. The reaction mixture was allowed to stand for 3 h at ~20 °C, the precipitated crystals were filtered off, washed successively with water, EtOH, and Et_2O, and dried *in vacuo* to give 290 mg (73%) of complex **5a** as a red-brown finely crystalline material, which decomposes (grows dark) without melting at a temperature of ≥200 °C. Found (%): C, 11.3; H, 2.89; N, 7.53; Pt, 41.2. $C_9H_{26}Cl_2I_2N_5OPt_2$. Calculated (%): C, 11.56; H, 2.80; N, 7.49; Pt, 41.72. IR, ν/cm^{-1}: 1550, 1608, 3162, 3215 (NH_2, NH_3).

***trans*-3,4-Bis(*cis*-amminedichloroplatinum(II)amino)-2,2,6,6-tetramethylpiperidin-1-oxyl (5b).** Silver nitrate (242 mg, 1.42 mmol) was added to a triturated suspension of complex **5a** (350 mg, 0.37 mmol) in 8 mL of water and the mixture was stirred with a magnetic stirrer for 16 h in the dark. The precipitated silver halides were thoroughly separated by centrifuging and filtering through a dense glass filter to give a solution of orange-colored complex **5c**, which did not contain $AgNO_3$. A solution of KCl (0.23 g, 3 mmol) in 1 mL of water was added with stirring to a solution of complex **5c** and the mixture was allowed to stand for 12 h at ~20 °C. The orange crystals were filtered off, washed with cold water and EtOH, and dried *in vacuo* to give 110 mg (40%) of complex **5b**, which decomposes (grows dark) without melting at a temperature of ≥210 °C. Found (%): C, 14.8; H, 3.59; N, 9.53; Pt, 50.6. $C_9H_{26}Cl_4N_5OPt_2$. Calculated (%): C, 14.37; H, 3.48; N, 9.31; Pt, 51.86. IR, ν/cm^{-1}: 1556, 1627, 3165, 3238, 3265 (NH_2, NH_3).

Platination of DNA. Platination was carried out using fragmented DNA from the bovine spleen (Reakhim, Russia) with a mean molecular mass of ~$1.65\cdot 10^6$ (~2500 nucleotide pairs). The DNA concentration was determined from the optical density of the sample at λ = 260 nm, using the extinction coefficient ε = $6.6\cdot 10^3$ L mol^{-1} cm^{-1}. The platination of DNA was studied for sufficiently water-soluble complexes (≥0.3 mg mL^{-1}). The platination was carried out in a 0.01 *M* solution of $NaHCO_3$ for 24 h at 37 °C; the initial r_{in} = [complex]/[nucleotide] molar ratios ranged from 0.001 to 0.3. After separation of the unreacted complex,[4,6] the degree of modification r = [bound LPt^{II}]/[nucleotide] was determined by ESR on the basis of the >N—O$^{\bullet}$ content or by atomic-absorption spectroscopy on the basis of the platinum content. The results of both methods coincided to within experimental errors (5—20%).

The melting point (T_m) and the melting range (ΔT) for the initial and platinated DNA were found as described previously.[6]

Results and Discussion

Synthesis and structure of complexes. Complexes **3_1a** and **3_2a** were synthesized from the Cisplatin *via* the intermediate $Na[Pt(NH_3)Cl_3]$ complex,[12] which was treated without isolation, successively with NaI and aminonitroxides **6a** and **6b**, respectively. Binuclear complex **5a** was prepared in a good yield and with good purity in a similar way from the isolated $K[Pt(NH_3)Cl_3]\cdot 0.5H_2O$ [12] and 0.5 equiv. of radical **7**. The syntheses of intermediate dinitrato complexes **3_1c** and **5c** and dichloro complexes **3_1b** and **5b** were carried out as described previously.[5]

Complexes **3_na**,**b** and **5a**,**b** are crystalline materials, which decompose on melting. Iodine-containing complexes **3_1a**, **3_2a**, and **5a** are poorly soluble in water (≤0.1 mg mL^{-1}); the solubility of dichloro complexes **3_1b** and **5b** is ~0.4 mg mL^{-1}, and **3_1c**, **3_2c**, and **5c** cannot be isolated from aqueous solutions even at a concentration of ~40 mg mL^{-1}.

The new complexes were identified as *cis*-isomers judging from stronger *trans*-effect of the halide ligands upon inclusion of the N-ligand in the Pt^{II} coordination sphere[13] and by analogy with complex **2b**, for which the *cis*-structure was established by X-ray diffraction analysis.[5] Complexes **3_1a**, **3_2a**, and **5a** are, apparently, isomer mixtures regarding the positions of ligands X and X´. Complexes **4** and **5**, prepared from diamino ligand **7**, contain *trans*-arranged amino groups, as indicated by the X-ray diffraction data for one of the bis-sulfamide diastereomers, prepared by the reaction of **7** with (1*S*)-(+)-camphorsulfonyl chloride.[14] The bands at 3160—3265 and 1550—1630 cm^{-1} in the IR spectra of **3_1a**,**b**, **3_2a**, and **5a**,**b** correspond to the stretching and bending vibrations, respectively, of the NH_2 and NH_3 groups. The ESR spectra of dilute aqueous solutions of the complexes comprise three lines, which is consistent with the monoradical structures of these compounds. The HFC constants at nitrogen (a_N = 1.69 mT) and the *g*-factors for complexes **3** and **5** (2.0056) are equal and are typical of aqueous solutions of six-membered nitroxides. One more piece of evidence for the binuclear structure of **5b** is DNA stabilization upon the formation of adducts with **5b** (see below), unlike the situation with complexes **1**—**4**.

The relationship between the structure of complexes and their activity in adduct formation with DNA. According to present-day data,[2] the antitumor activity of diamino Pt^{II} complexes is due to the bidentate binding of platinum to DNA through the formal displacement of the ligands X mainly by the N(7) atoms of purine bases. In the case of mononuclear complexes (for example, Cisplatin, complexes **1**—**4**), up to 80—90% of the adducts are 1,2-intrastrand cross-links either between neighboring guanines or between adenine and guanine in ~2 : 1 ratio. The rest are 1,3-intrastrand cross-links (~10%), monodentate adducts (~3%), and interstrand cross-links (~1%). The data for model oligonucleotides obtained by various methods show[2] that the 1,2-cross-linking of neighboring guanines induces bending of the helix by 40—80°

toward the major groove and deteriorates the coplanarity of the guanine rings, so that the angle between the planes of the platinated bases is ~50°. In the case of binuclear complexes such as **5**, which also exhibit high antitumor activities, the fraction of inter-strand cross-links reaches[15] ~70%.

The effect of the structure of Pt^{II} nitroxide complexes on their activity toward the formation of DNA adducts was studied for the initial ratio of the reactants $r_{in} = 0.1$ (see Experimental). The degrees of modification r obtained for various complexes correspond to the x-coordinates of the points in Fig. 1. The y-coordinates of these points show the specific destabilization δT_m of the DNA duplex, which corresponds to the decrease in the DNA melting point (in °C) induced by the formation of one adduct per every 100 nucleotides. This value was calculated using the formula

$$\delta T_m = (T_m' - T_m)/100r,$$

where T_m and T_m' are the melting points of the initial and platinated DNA, respectively (the melting curves were published previously[6]). The r value depends on the natures of both the leaving ligand X and the carrying amino ligand. For complexes containing identical carrying ligands, the difference between platination activities is determined by the rate of hydrolysis of the leaving ligand X. However, there exists evidence that complexes stable against hydrolysis, such as 1,1-cyclobutanedicarboxylates of Pt^{II}, can react with DNA, bypassing the intermediate step of hydrolysis.[6,16] Easily hydrolyzable complex **4c**, which is converted almost completely into a reactive aqua complex during dissolution,[6] exhibits the highest platinating activity. The retardation of hydrolysis on passing to dichloro, oxalato-, or 1,1-cyclobutanedicarboxylato complexes is reflected in the decrease in the number of DNA adducts formed. The platinating activity of **4c** can decrease if the reaction mixture contains anions capable of forming Pt^{II} complexes stable to hydrolysis. Thus the number of DNA adducts formed by complex **4c** in a citrate buffer (point **4c** (c.b.), see Fig. 1) is about half that formed in a bicarbonate buffer (point **4c**), all other factors being the same.

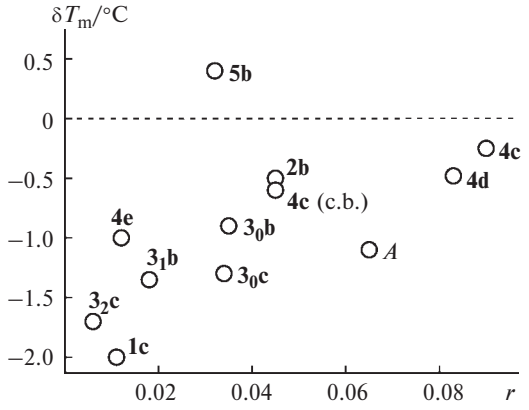

Fig. 1. Relationship between the platinating activity (r) and specific destabilization of the DNA duplex (δT_m) for various platinum complexes. The platination of DNA was carried out in 0.01 M $NaHCO_3$ over a period of 24 h at 37 °C at the initial molar ratio $r_{in} = 0.1$. Point **4c** (c.b.) corresponds to platination in a 0.01 M citrate buffer, pH 7, A is Cisplatin.

When the carrying amino ligands are rather large, they exert a crucial influence on the platinating activity of the complexes. Apparently, both the volume and the linear size of the ligands are significant, because the bulky biradical complex **1c** and complex $\mathbf{3_2c}$, in which the amino group is separated from the piperidine ring by an ethylene bridge are characterized by close r values, and these values are 10—15 times lower than those for complex **4c**.

The plot presented in Fig. 1 indicates that the DNA adducts formed by the most inert complexes are characterized by the highest specific destabilization δT_m of the DNA duplex, whereas the least destabilization is induced by the adducts formed by reactive complexes **4c**,**d**. Complexes **2b** and $\mathbf{3_0b}$, which show a high antitumor activity,[5] platinate DNA only 1.5—2 times less efficient than Cisplatin, and their adducts, despite the large bulk of the ligands, destabilize DNA to an even smaller extent than the Cisplatin adducts.

The degree of DNA destabilization can also depend on the orientation of the amino ligands in the adducts relative to the axis of the DNA helix. It is clear that the orientations are different for mono and diamino ligands. In addition, racemic complexes **2**, **4**, and **5** are expected to produce two diastereomeric adducts each. In the two diastereomeric adducts formed by the Pt^{II}(*trans*-DACH)X_2 complexes (DACH is 1,2-diaminocyclohexane), the cyclohexane rings are arranged at right angle to the axis of the DNA helix; in the opinion of the authors of previous publications,[17,18] this creates the lowest steric interaction. It follows from Fig. 1 that the adducts of complexes **4** having a similar structure cause the least pronounced destabilization of the DNA molecule. Much greater distortions of DNA are induced by the diastereomer of the Pt^{II}(*cis*-DACH) adduct in which the cyclohexane ring is arranged along the axis of the DNA helix.[18] Since complexes **1**—**3** form adducts in which the rather bulky amino ligands are arranged along the DNA major groove,[19] the observed higher destabilizing influence of these adducts (see Fig. 1) can be reasonably attributed to the enhanced steric hindrance.

Unlike complexes **1**—**4**, adducts formed by binuclear complex **5b** induce an increase in the DNA melting point (see Fig. 1). This does not mean that these adducts do not disturb the DNA structure. The effect is due to the predominant (up to 70%)[15] formation of interstrand cross-

links. For point **5b** in Fig. 1 ($r = 0.032$), provided that two nucleotides are cross-linked, the length of the DNA fragment is 2500 pairs of nucleotides, and the proportion of the interstrand cross-links is 70%, one can calculate the average number of cross-links per DNA fragment, which would be $0.032 \cdot 2500 \cdot 0.7 \cdot 2/2 = 56$. Thus covalent cross-linking of DNA strands prevents thermal destruction of the duplex.

The positions of the LPt^{II} fragments in DNA. The differences in the reactivity of complexes caused by different rates of displacement of the ligands X and by the influence of the amino ligand bulk can also be manifested as different selectivities of DNA platination. In the case of selective binding, one should expect nonuniform distribution of the adducts along the DNA strand. In terms of the helix—coil transition theory in double-stranded polynucleotides,[20] slight destabilization of the polymeric DNA is expected when local defects of the secondary structure are concentrated in particular sections of the molecule, whereas a uniform distribution of the defects would entail a more pronounced destabilizing effect. It is reasonable to assume that the adducts formed from low-reactivity complexes would be arranged more uniformly due to steric restrictions and, therefore, they would cause a more pronounced DNA destabilization. Yet another factor that may influence the degree of DNA destabilization is orientation of the amino ligands in adducts relative to the axis of the DNA helix. The ESR spectra of a DNA with a high degree of modification ($r > 0.05$) recorded at 77 K show a pattern typical of closely spaced nitroxide groups that undergo magnetic dipole-dipole coupling. The ESR spectral pattern should depend on the positions of the adducts in DNA. The shape of the ESR line of nitroxides observed at 77 K can be characterized by the parameter d_1/d, which is the ratio of the sum of the amplitudes of the terminal peaks to the amplitude of the central peak.[21] This parameter is rather sensitive to magnetic interaction. In the case of two interacting radicals, the distance L between them can be determined from the d_1/d value using the relation[21]

$$L = 0.93 + 0.077/[d_1/d - (d_1/d)_0] \text{ (nm)}, \qquad (1)$$

where the subscript "0" refers to the case of zero interaction between the nitroxides. The $(d_1/d)_0$ value is determined by measuring the d_1/d values for low modification degrees. In our case, $(d_1/d)_0 = 0.45$.

The d_1/d values for DNA modified by various complexes are shown in Fig. 2 as points for different modification degrees r. The continuous line represents the theoretical dependence of d_1/d on r calculated by the Monte Carlo method in terms of a model assuming nonselective (random) arrangement of adducts in the DNA molecule. The addition of $2rN$ complexes to a DNA molecule comprising N nucleotide pairs was simulated on a computer.

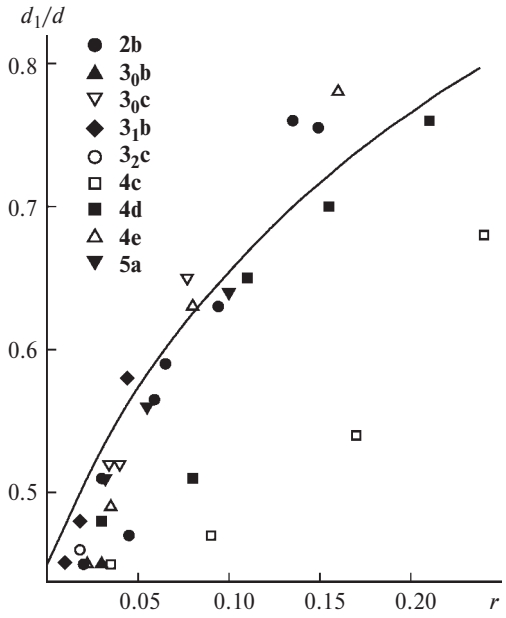

Fig. 2. ESR parameter d_1/d *vs.* degree of modification r for various complexes. The dots are experimental data and the curve is the theoretical dependence obtained by the Monte Carlo method for $b = 0.6$ nm (see the text). The errors in determination of the d_1/d parameter do not exceed ±0.02. The ESR spectra were recorded at 77 K for magnetic field modulation of 0.32 mT and a microwave power of 0.3 mW.

The next step was to determine the probability (p_n) that no adducts are present between a distinguished (zero) nucleotide and an nth nucleotide pair. The d_1/d value was calculated using the relation

$$\frac{d_1}{d} = \left(\frac{d_1}{d}\right)_0 + \sum_n p_n \frac{0.77}{L_n - 0.93}, \qquad (2)$$

where L_n is the distance between the nitroxide groups of two adducts attached to the distinguished nucleotide pair and to the nth nucleotide pair. Equation (2) is derived by averaging the d_1/d values found by relation (1) over the distances L. The L_n distances were calculated from the Cartesian coordinates of the nitroxide groups assuming that they are located at distance b from the axis of the DNA molecule (Fig. 3). If the coordinate system is oriented in such a way that the Z axis is directed along the DNA axis and the nitroxide group of the distinguished adduct is located in the X axis, the coordinates of the nitroxide groups are $b\cos\varphi$, $b\sin\varphi$, and h. For the distinguished adduct $\varphi = 0$, $h = 0$; for the adduct attached to the nth nucleotide of the same strand as the distinguished adduct, $\varphi = 36n$ (deg), $h = 0.34n$ (nm), while for the complementary strand, $\varphi = 143 + 36n$, $h = 0.34n$ (nm). The φ and h values corresponding to the B-form of DNA were used. It is known for this form that the distance between the neighboring nucleotide pairs is 0.34 nm and

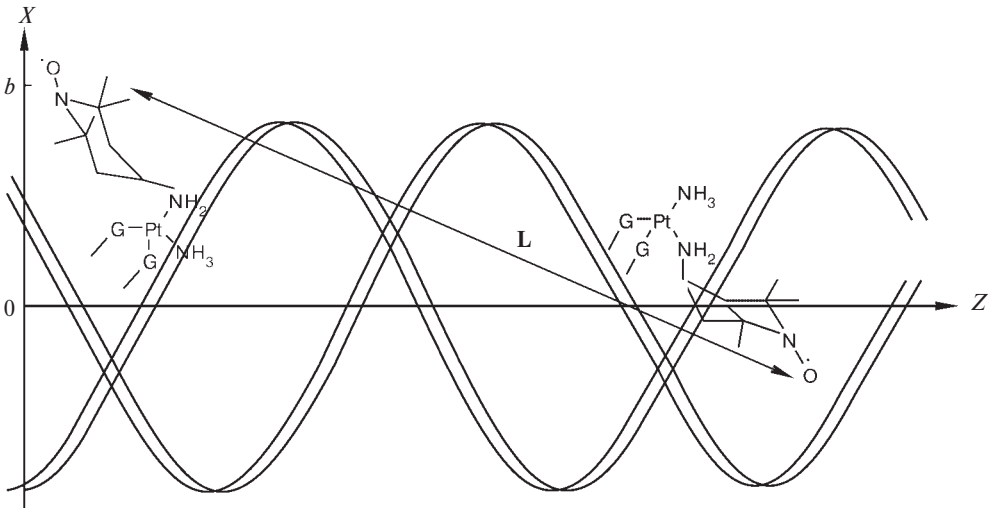

Fig. 3. Probable arrangement of the platinum nitroxide fragments for binding to neighboring bases within one DNA strand.

a full convolution contains 10 nucleotide pairs. The angle between the positions of nucleosides from different chains (with the same coordinate Z) amounts to 143°. This angle is found as $360 \cdot 1.15/(1.15 + 1.75) = 143$, where 1.15 and 1.75 nm are the widths of the minor and major grooves, respectively, between the DNA phosphate chains.[22] It was assumed that, due to steric restrictions, the adducts in one strand are separated by at least one nucleotide. The probabilities p_n can also be found using the relation $p_n = 0.5(1 - r)^{n-1}r/0.88$; the coefficient 0.88 is the sum of all p_n values; it differs from unity because for an isolated strand, $p_1 = 0$ due to steric restrictions. For the simplified model considered, the theoretical dependence of d_1/d on r (the curve in Fig. 2) describes adequately the data for most of the complexes with $b = 0.6$ nm. Taking into account the X-ray diffraction data[5] for **2b**, the distance between the N(7) atom of guanine and the nitroxide group of the adduct is ~1 nm. The shorter theoretical b value may be due to the fact that the angle between nitroxide and the axis of the DNA helix differs from 90° and/or to structure distortions, resulting in proximity of the neighboring nitroxide groups. The results for complex **4c** do not fit the theoretical dependence of d_1/d on r (see Fig. 2). The data for highly reactive **4c** could be interpreted by assuming binding to peripheral (*e.g.*, phosphate) groups of DNA. However, this interpretation does not seem convincing in view of the expected lability of such adducts.

The coincidence of the theoretical and experimental dependences of d_1/d on r for most of the complexes attests to the absence of selective platination of DNA for $r \geq 0.05$. The insufficient sensitivity of the ESR method precludes determination of the distances between the radicals and, hence, the possible selectivity of DNA platination for the low degrees of modification attainable *in vivo*.

In the adducts of complexes $\mathbf{3_1}$ and $\mathbf{3_2}$, the distance between the Pt atom and the piperidinoxyl molecule increases by one or two methylene groups with respect to that in the adducts formed by complexes $\mathbf{3_0}$. According to ESR, this results in a sharp increase in the radical mobility (Fig. 4). Apparently, this is associated with the partial exit of the piperidine ring from the relatively shallow major DNA groove and with an increase in its rotational mobility.

Thus, the study of new complexes **1**—**5** made it possible to elucidate the dependence of the efficiency of their covalent binding to DNA on the nature of leaving groups X and the carrying aminonitroxide ligands and to identify the dependence of the degree of destabilization of the modified DNA on the structure of the adducts. Complexes **2**, $\mathbf{3_0}$, and **4** are comparable with Cisplatin regarding the efficiency of binding to DNA and the degree of DNA destabilization. The presence of two bulky amino ligands (as in complexes **1**) or an increase in the bulk of one ligand (as in complexes $\mathbf{3_2}$) decreases the

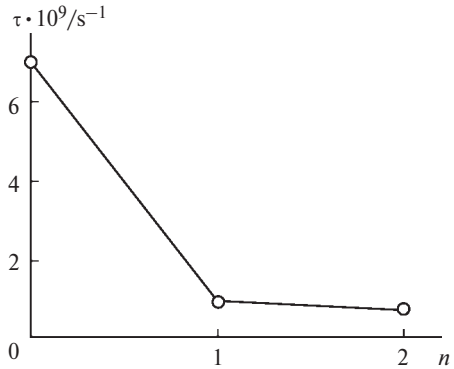

Fig. 4. Rotational correlation time for piperidinoxyls (τ) in the DNA modified with complexes $\mathbf{3_0}$, $\mathbf{3_1}$, and $\mathbf{3_2}$, *vs.* the length of the $(CH_2)_n$ bridge.

platinating activity, apparently, due to steric hindrance. The destabilizing effect measured based on the DNA melting point increases with an increase in the linear dimensions and the volume of the amino ligands. According to ESR data, when $r \geq 0.05$, the adducts are uniformly distributed along the DNA strand, which does not rule out the presence of selectivity at lower degrees of platination. With allowance for published data,[3—5] it can be concluded from the biological activities of complexes **1**—**4** that high antitumor activity is characteristic of complexes that efficiently platinate an isolated DNA and, simultaneously, entail a moderate destabilization of the DNA duplex.

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