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New Cobalt Complex with Dihydroxycoumarin: Synthesis and Kinetics of Its Redox-Activated Dissociation

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Abstract—A new redox-active cobalt(III) complex containing the 2-oxo-*2H*-chromene-6,7-diolate dianion and 4,4'-dimethoxy-2,2'-bipyridine as ligands is synthesized. The reduction of the synthesized complex with ascorbic acid in an inert atmosphere is studied in situ by NMR spectroscopy. The reduction is shown to result in the release of 6,7-dihydrocycoumarin acting as a model drug. This process has the first order with respect to the initial complex.

Keywards: in situ nuclear magnetic resonance spectroscopy, dihydroxycoumarin, cobalt complexes, redox-activated drug delivery

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INTRODUCTION

Malignant solid tumors are of serious problems in clinical practice since they are inefficiently amenable to radio- or chemotherapy because of hypoxia in tumor tissues [1, 2]. However, the differentiation of healthy and tumor tissues by the oxygen level provides the redox-activated delivery of drug molecules purposefully to tumor cells [3, 4]. In this case, the therapeutic efficiency of the drug enhances but their side effects decrease.

Metal complexes are actively studied as "molecular platforms" for the targeted delivery of anticancer drug molecules to tumor cells [5]. The cobalt complexes are of special interest from this point of view. Cobalt is a biogenic metal comprising vitamin B_{12}. The cobalt(III) complexes are distinguished by a sufficiently high stability constant providing their inertness when getting into the organism [6]. A correct choice of the ligands makes it possible to synthesize complexes, whose Co(III)/Co(II) redox potential lies in a potential range of biological intracellular reducing agents [7]. The cobalt(II) complexes formed by reduction are capable of eliminating one of the ligands [6]. Just this stage results in the release of a drug molecule. In addition, the reduction of the cobalt ion leads to a sharp change in its magnetic properties: the Co(III)/Co(II) transition is accompanied by the formation of the paramagnetic cobalt ion, which makes it possible to monitor drug release using MRT [8].

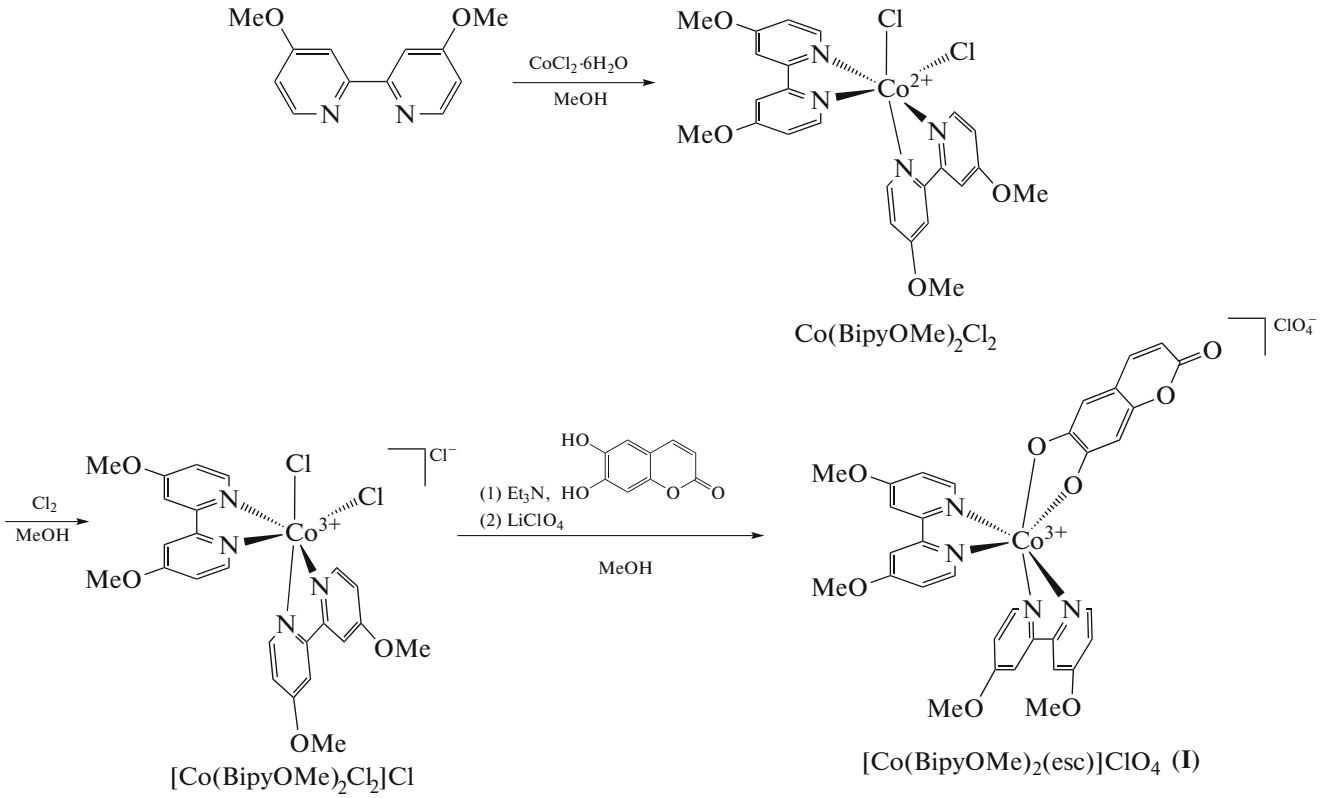
Numerous cobalt complexes with various ligands have been studied to date as potential "molecular platforms" for targeted drug delivery to tumor cells, and a number of encouraging results was obtained [9]. For example, the cobalt(III) complex containing two 2,2'-bipyridine molecules and 6,7-dihydroxycoumarin is cytotoxic against colon cancer cells under hypoxia conditions [7]. However, the strategy of redox activation has not been brought to the stage of clinic tests so far. One of the main problems is that in vitro results cannot be reproduced on in vivo models. Therefore, it is necessary to conduct systematic studies on establishing regularities of the redox activation for the further optimization of "molecular platforms" based on the cobalt complexes.

One of the factors that substantially affects the therapeutic effect by drug delivery using "molecular platforms" is the reduction rate of the cobalt ion in the complex and the consecutive drug release. If the reduction is too slow, the complex may be at risk of withdrawing from the organism before a sufficient amount of the drug would be released to tumor cells. The substituents in the aromatic system of the ligands are known to affect the energy of molecular orbitals of the complexes and, as a consequence, their redox potential [10]. However, there are no systematic studies of the effect of substituent introduction into the 2,2'-bipyridine ring on the properties of the cobalt(III) complexes and the kinetics of drug release, although these studies could elucidate regularities

necessary for the rational design of the redox-active complexes for targeted drug delivery.

We have previously proposed an approach for in situ monitoring the redox activation of drugs in the cobalt(III) complexes by NMR spectroscopy. This allowed us to study the reduction with ascorbic acid of the heteroleptic cobalt(III) complexes containing 2,2'-bipyridine or 1,10-phenanthroline and 2-oxo-*2H*-chromene-6,7-diolate dianion as ligands [11] and to find that the reduction of the complex with phenanthroline is much faster.

In this work, we synthesized the new redox-active cobalt(III) complex $[Co(BipyOMe)_2(esc)]ClO_4$ (**I**) containing 4,4'-dimethoxy-2,2'-bipyridine and 2-oxo-2*H*-chromene-6,7-diolate dianion as ligands (Scheme 1) and studied its reduction with ascorbic acid by in situ NMR spectroscopy.



Scheme 1.

EXPERIMENTAL

The cobalt(III) complex $[Co(BipyOMe)_2Cl_2]Cl$, which was synthesized by the oxidation of the corresponding cobalt(II) complex with gaseous dichlorine [12], was used as the precursor for the synthesis of cobalt complex **I**. Dichlorine was prepared by the reaction of potassium permanganate with concentrated hydrochloric acid and dehydrated by passing through concentrated sulfuric acid [13]. Commercially available 4,4'-dimethoxy-2,2'-bipyridine (97%, Sigma-Aldrich), cobalt(II) chloride hexahydrate (98%, Sigma-Aldrich), 6,7-dihydroxycoumarin (98%, Sigma-Aldrich), lithium perchlorate (98%, Alfa Aesar), and triethylamine (99%, Sigma-Aldrich) were used as received.

Synthesis of complex $[Co(BipyOMe)_2Cl_2]Cl$. A solution of cobalt(II) chloride hexahydrate (0.84 mmol, 200 mg) in methanol (10 mL) was added to a solution of 4,4'-dimethoxy-2,2'-bipyridine (1.681 mmol, 363 mg) in methanol. The resulting mixture was refluxed for 2 h. The solution color changed from yellow to red-brown within this time. Then the reaction mixture was cooled to room temperature, and gaseous dichlorine (prepared by the reaction of potassium permanganate with concentrated sulfuric acid) was bubbled through the mixture. The target complex was formed as a crystalline green precipitate. The precipitate was filtered off, washed with ethanol, and dried in vacuo. The yield was 362 mg (72%).

^{1}H NMR (CD_3OD; 300 MHz; δ, ppm): 9.63 (d, J = 6.9 Hz, 2H, C*H*), 8.34 (d, J = 2.6 Hz, 2H, C*H*), 8.16 (d, J = 2.6 Hz, 2H, C*H*), 7.64 (dd, J = 6.9, 2.6 Hz, 2H, C*H*), 7.21–6.92 (m, 4H, C*H*), 4.21 (s, 6H, OCH_3), 3.98 (s, 6H, OCH_3).

MS (ESI), *m/z*: $[Co(BipyOMe)_2Cl_2]^+$, calculated 561.1, found 560.9.

Synthesis of complex $[Co(BipyOMe)_2(esc)]ClO_4$ (I). A solution of 6,7-dihydroxycoumarin (0.5 mmol, 89 mg) and triethylamine (1 mmol, 10.2 mg, 139 μL) in methanol (10 mL) were added to a solution of $[Co(BipyOMe)_2Cl_2]Cl$ (0.5 mmol, 299 mg) in methanol (15 mL). The resulting mixture was refluxed for 3 h and then cooled to room temperature. A solution of lithium perchlorate (1.25 mmol, 133 mg) in methanol (5 mL) was added, and the mixture was stirred for 30 min on cooling in a water bath to crystallize the target complex. The formed green precipitate was filtered off, washed with isopropanol and diethyl ether, and dried under reduced pressure. The yield was 249 mg (65%).

^{1}H NMR (DMSO-d_6; 400 MHz; δ, ppm): 8.59 (br.s, 2H, C*H*), 8.50 (dd, J = 42.0, 6.6 Hz, 2H, C*H*), 8.49 (d, J = 6.8 Hz, 2H, C*H*), 7.65–7.59 (m, 2H, C*H*), 7.57 (d, J = 9.3 Hz, 1H, C*H*CHCOO), 7.32 (dd, J = 33.9, 6.4 Hz, 2H, C*H*), 7.17–7.10 (m, 2H, C*H*), 6.55 (s, 1H, C*H*), 6.41 (s, 1H, C*H*), 5.82 (d, J = 9.3 Hz, 1H, CHC*H*COO), 4.12 (s, 6H, OCH_3), 4.02 (s, 6H, OCH_3).

^{13}C NMR (DMSO-d_6; 101 MHz; δ, ppm): 170.07, 170.00, 169.47, 169.45, 167.89, 161.83, 158.77, 157.88, 157.84, 157.82, 152.54, 152.48, 150.63, 150.61, 149.25, 144.69, 114.79, 114.76, 114.66, 112.47, 112.46, 111.50, 110.01, 109.49, 107.58, 102.45, 57.81, 57.73.

MS (ESI), *m/z*: $[Co(BipyOMe)_2(esc)]^+$, calculated 667.1, found 667.3.

^{1}H and ^{13}C NMR spectra of the cobalt complexes were recorded for solutions in deuterated methanol and dimethyl sulfoxide on Bruker Avance 300 and Varian Inova 400 NMR spectrometers with the working frequencies for protons 300.15 and 400.13 MHz, respectively. The chemical shifts were determined relatively to signals of residual protons of the solvents (^{1}H 2.50 ppm, ^{13}C 39.52 ppm for DMSO-d_6; ^{1}H 3.31 ppm, ^{13}C 49.0 ppm for CD_3OD).

Mass spectrometry of the cobalt complexes and their reduction products was carried out on an LCMS-2020 liquid chromatograph combined with a mass spectrometer (Shimadzu, Japan) with electrospray ionization and a quadrupole detector (detection of positive and negative ions with *m/z* in a range of 50–2000). The electrospray voltage was 4.5 kV, and the temperatures of the desolvation line and heating block were 250 and 400°C, respectively. Nitrogen (99.5%) was used as the spraying and drying gas, and acetonitrile (99.9+%, ChemLab) with a flow rate of 0.4 mL/min served as the mobile phase. The volume of the analyzed sample was 0.5 μL.

In situ NMR spectroscopy. Complex **I** (10 μmol, 7.7 mg), ascorbic acid (20 μmol, 3.6 mg), CD_3CN (550 μL) and dibromomethane (3 μL) (internal standard) were placed in a tube with a screw top and septa. Then the tube was frozen in liquid nitrogen, evacuated, and filled with argon.

The ^{1}H NMR spectrum of the initial mixture was recorded at 40°C on a Bruker Avance 300 spectrometer with a working frequency for protons of 300.15 MHz. Chemical shifts (δ, ppm) were determined relatively to the residual signal of the solvent (^{1}H 1.94 ppm for CD_3CN). The following detection parameters were used: spectral range 150 ppm, detection time 0.2 s, relaxation delay 0.6 s, pulse duration 9.5 μs, and acquisition number 32. The obtained free induction decays were processed to increase the signal/noise ratio by exponential weighing with the coefficient to 1. Then D_2O (150 μL) was syringed through the septa, and the mixture was shaken to the complete dissolution of ascorbic acid. The further detection of the NMR spectra was performed at an interval of 2 min within 40 min at 40°C using the same parameters as those for recording the spectrum of the initial mixture. The conversion rate was estimated from the initial complex consumption. The content of the complex in the mixture (in % of the initial amount) was calculated from the ratio of the integral intensity of the signal from the dibromomethane protons (5.09 ppm) to the integral intensity of the multiplet signal (8.57–8.68 ppm) chosen due to integration convenience, since this signal is observed during the whole reduction and is not overlapped with other signals.

RESULTS AND DISCUSSION

The cobalt(III) complex $[Co(BipyOMe)_2(esc)]ClO_4$ (**I**) was synthesized in several stages (Scheme 1). At the first stage, 4,4'-dimethoxy-2,2'-bipyridine was introduced into complex formation with cobalt(II) chloride hexahydrate [12]. The resulting cobalt(II) complex $[Co(BipyOMe)_2Cl_2]$ was oxidized with gaseous dichlorine generated by the reaction of potassium permanganate with concentrated hydrochloric acid [13] to form the cobalt(III) complex $[Co(BipyOMe)_2Cl_2]Cl$. The subsequent reaction of $[Co(BipyOMe)_2Cl_2]Cl$ with 6,7-dihydroxycoumarin in the presence of triethylamine and lithium perchlorate afforded the target complex $[Co(BipyOMe)_2(esc)]ClO_4$ (**I**), which was characterized by mass spectrometry (Fig. 1) and ^{1}H and ^{13}C NMR spectroscopy (Figs. 2 and 3).

The earlier developed approach [11] with some changes was applied to study in situ the reduction of complex **I** by NMR spectroscopy. An internal stan-

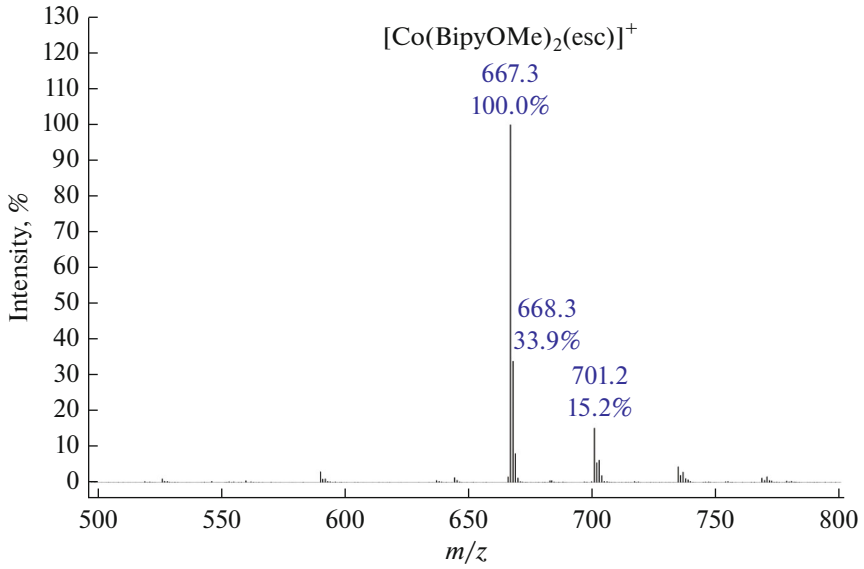


Fig. 1. Mass spectrum of complex **I** detected for positive ions using electrospray ionization.

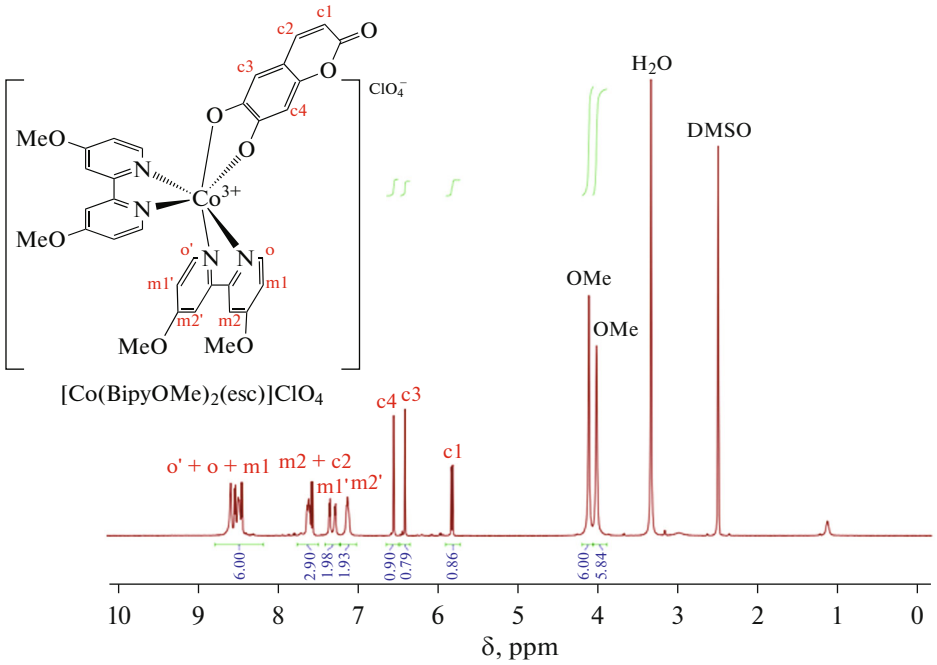


Fig. 2. ^{1}H NMR spectrum of complex **I** in a DMSO-d_6 solution at room temperature.

dard was used to enhance the accuracy of the estimation of the content of the complex. Dibromomethane was chosen as this standard, since its ^{1}H NMR spectrum exhibits one singlet about 5 ppm, which is not overlapped with signals of protons of the studied complex. A weighed sample of the cobalt(III) complex, ascorbic acid (2 equiv), acetonitrile-d_3, and dibromomethane were placed in a tube with a screw top and septa. The tube was frozen in liquid nitrogen, evacuated, and filled with argon. The ^{1}H NMR spectrum of the initial mixture was detected at 40°C. Then deuterated water was syringed to the tube through the septa, and the ^{1}H NMR spectra were recorded at 40°C. Upon the addition of water, ascorbic acid passed to the solution and initiated the reduction. The assumed reduction products are presented in Scheme 2.

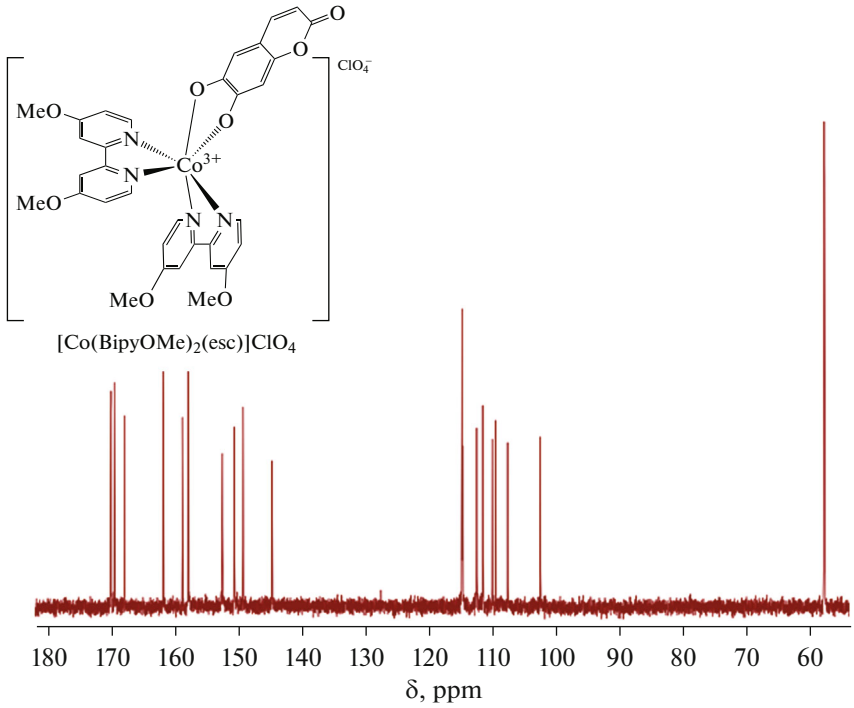
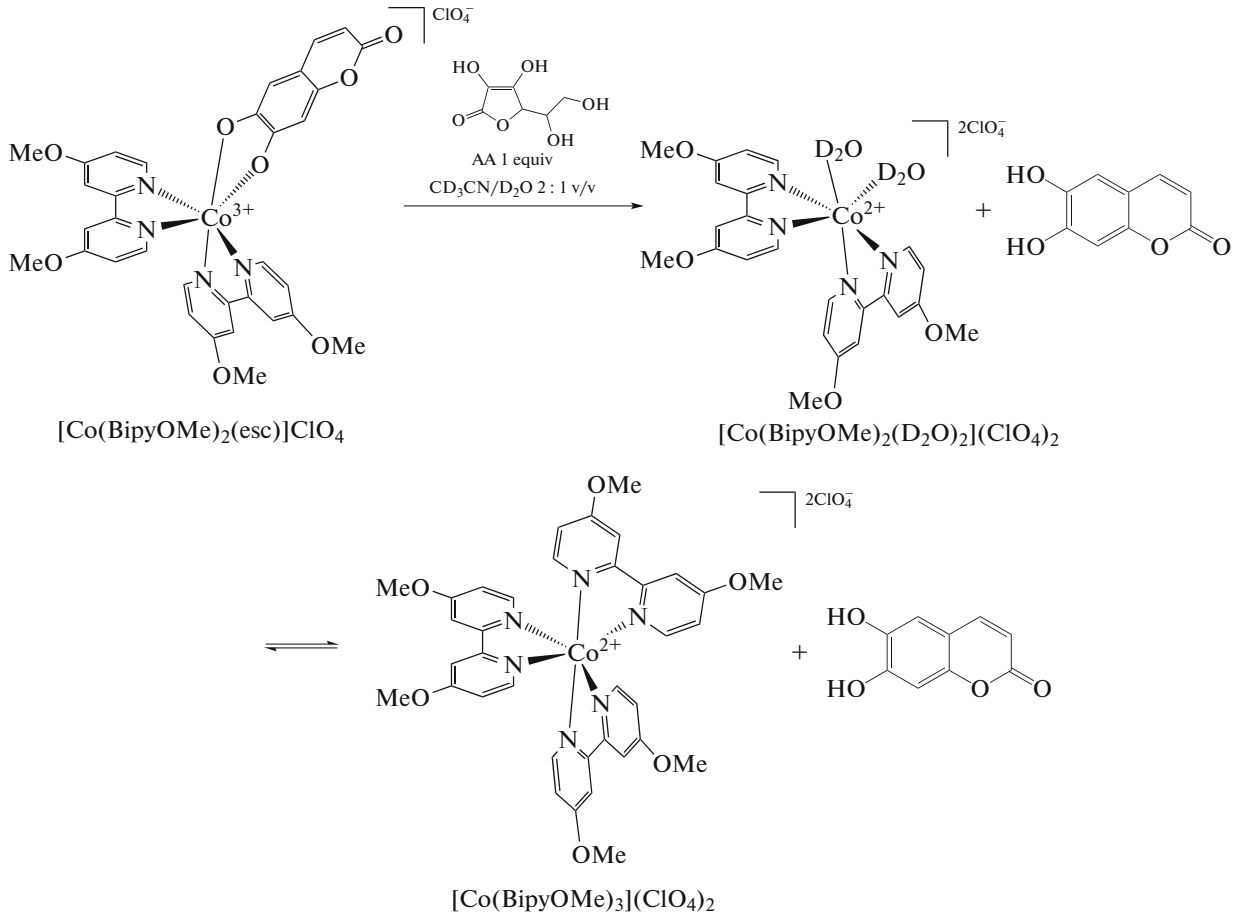


Fig. 3. ^{13}C NMR spectrum of complex **I** in a DMSO-d_6 solution at room temperature.



Scheme 2.

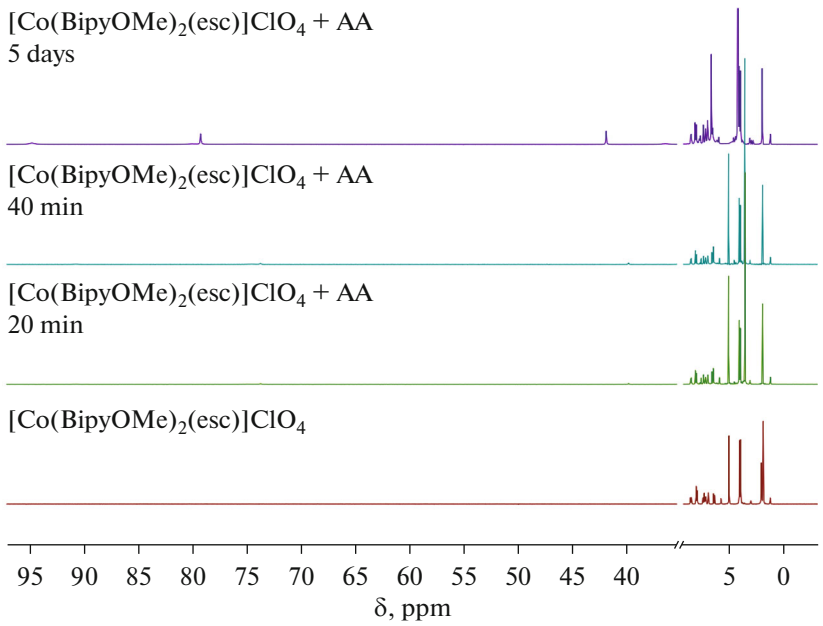


Fig. 4. Dynamics of changing ^{1}H NMR spectrum with time during the reduction of complex **I** with ascorbic acid in an argon atmosphere in a mixture of acetonitrile-d_3 and deuterated water (3.7 : 1, vol/vol).

The ^{1}H NMR spectra illustrating the dynamics of reduction of complex **I** with ascorbic acid are shown in Fig. 4. The diamagnetic (from 0 to 10 ppm) and paramagnetic (from 15 to 120 ppm) ranges can be distinguished in the spectra. The first range contains signals of the initial complex, ascorbic acid, its oxidation products, and free 6,7-dihydroxycoumarin, and signals of the formed cobalt(II) complexes are observed in the second range. As the reaction occurs, the signal intensity in the diamagnetic range decreases and that in the paramagnetic range, on the contrary, increases. It is seen that the reaction is rather slow, since the intense signals in the paramagnetic range appear only in 5 days. The number of signals in the paramagnetic range and their chemical shift and integral intensity correspond to the $[Co(BipyOMe)_3]^{2+}$ complex, which is additionally confirmed by the mass spectrometric analysis of the reduction products. The mass spectrum of the reaction mixture shown in Fig. 5 exhibits intense signals with *m/z* 353.8, 590.2, and 526.2 assigned to $[Co(BipyOMe)_3]^{2+}$, $[Co(BipyOMe)_2(ClO_4)]^+$, and $[Co(BipyOMe)_2Cl]^+$ ions. The appearance of adducts with the chloride anion can be related to the incomplete replacement of the chloride ion by perchlorate in the synthesis of complex **I** from $[Co(BipyOMe)_2Cl_2]Cl$. The mass spectrum of the reaction mixture detected for positive ions also contains a signal with *m/z* 680.3, which can presumably be attributed to the cobalt(II) complex with the oxidized form of ascorbic acid as one of the ligands (Fig. 5). Thus, the cobalt(III) complex $[Co(BipyOMe)_2(esc)]ClO_4$ is reduced with the formation of the cobalt(II) complex $[Co(BipyOMe)_3](ClO_4)_2$ and is accompanied by the release of the 6,7-dihydroxycoumarin molecule. An analogous reduction mechanism was observed for the cobalt(III) complexes with 6,7-dihydroxycoumarin containing bipyridine or phenanthroline instead of the 4,4'-dimethoxy-2,2'-bipyridine ligand.

The kinetic curve for the reduction of complex **I** with ascorbic acid in an argon atmosphere obtained in situ by the NMR spectroscopy data is shown in Fig. 6. It is seen that the first 10 min correspond to the induction period of the reaction where the concentration of the initial complex remained unchanged. Then a slow decrease in the amount of the initial complex was observed. The conversion was ~60% in 40 min after the addition of ascorbic acid. The dependence of the natural logarithm of the concentration of the initial complex on time is approximated by a straight line with the reliability coefficient close to unity (Fig. 6). The linear approximation indicates in favor of the first order of the rate-determining step of the reduction of the complex. It is most probably that this step is the release of the 6,7-dihydroxycoumarin molecule. The reduction rate constant of complex **I** with ascorbic acid in an inert atmosphere at 40°C was estimated from the slope ratio of the straight line shown in Fig. 6 and amounted to 5×10^{-4} s^{-1}.

Thus, we synthesized the new redox-active cobalt(III) complex $[Co(BipyOMe)_2(esc)]ClO_4$ containing the 2-oxo-*2H*-chromene-6,7-diolate dianion as one of the ligands. The reduction of the synthesized complex with ascorbic acid in an inert atmosphere was studied in situ by NMR spectroscopy. The release of

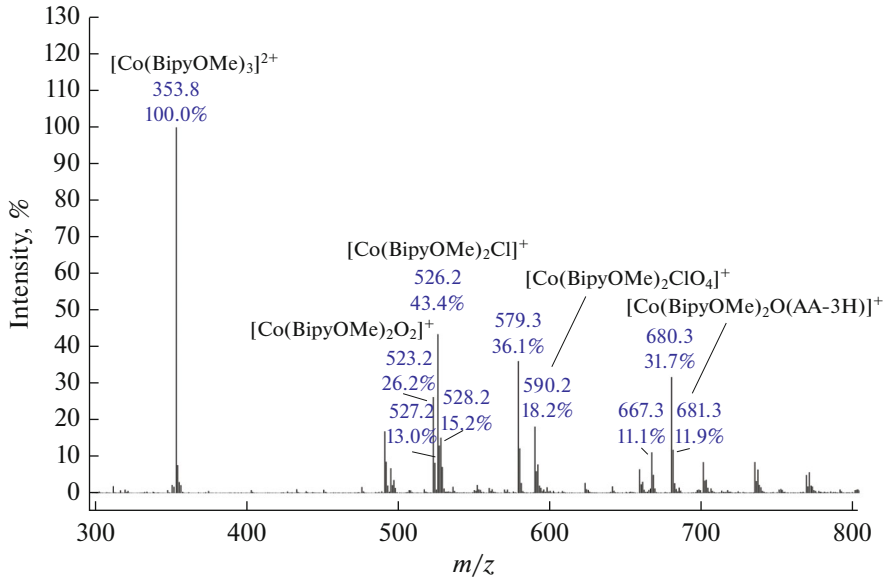


Fig. 5. Mass spectrum of the reduction products of complex **I** with ascorbic acid detected for positive ions.

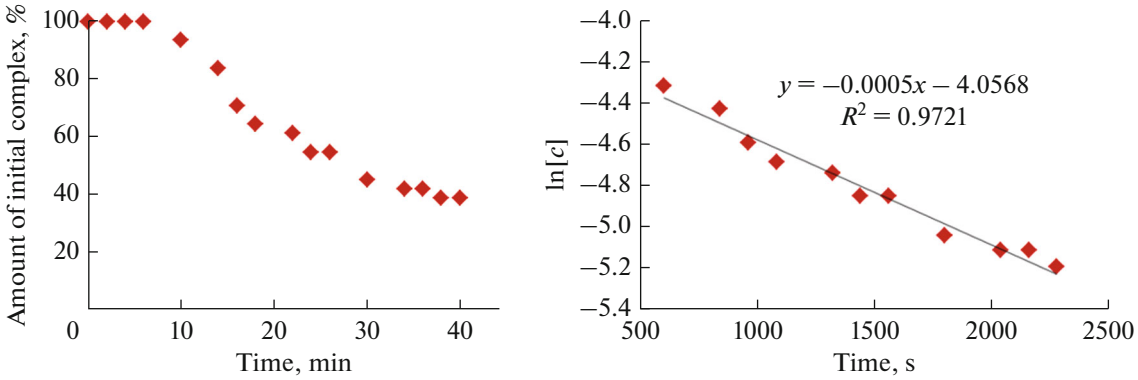


Fig. 6. (left) Kinetic curve of the consumption of complex **I** when reduced with ascorbic acid in argon and (right) the dependence of the logarithm of the concentration of complex **I** ln[*c*] on the reaction time.

6,7-dihydroxycoumarin and formation of the cobalt(II) complex $[Co(BipyOMe)_3]^{2+}A^{2-}$ were shown to occur during the reduction. The rate-determining step of the reduction of the studied complex has the first order, and the rate constant of the process is only 5×10^{-4} s^{-1}. The low rate constant indicates that the molecular structure of the studied complex should be optimized to impart it the properties providing the fast redox-activated drug delivery to tumor cells.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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