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Redox-active antifungal cobalt(II) and copper(II) complexes with sterically hindered *o*-aminophenol derivatives

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Abstract

Co(II) complexes with 4,6-di(*tert*-butyl)-2-aminophenol (HL^I) and 2-anilino-4,6-di(*tert*-butyl)phenol (HL^{II}) have been synthesized and characterized by means of physico-chemical methods. The compounds HL^I and HL^{II} coordinate in their singly deprotonated forms and behave as bidentate *O,N*-coordinated ligands; their low-spin Co(II) complexes are characterized by CoN_2O_2 coordination modes and square planar geometry. Both the free ligands and their Co(II) and Cu(II) complexes (we have produced and characterized the latter before) exhibit a pronounced antifungal activity against *Aspergillus niger*, *Fusarium* spp., *Mucor* spp., *Penicillium lividum*, *Botrytis cinerea*, *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Monilia* spp., which in a number of cases is comparable with that of Nystatin and Terbinafine or even higher. The reducing properties of the ligands and their metal(II) complexes, as well as their antifungal activities, were found to decrease in the order: $Cu(L^I)_2 > Cu(L^{II})_2 \geqslant Co(L^I)_2 > Co(L^{II})_2 > HL^I > HL^{II}$.

Keywords: *o*-Aminophenol derivatives; Co(II) and Cu(II) complexes; Spectroscopic study; Cyclic voltammetry; Antifungal activities

1. Introduction

Modern antifungal therapies include two main classes of compounds: polyene and azole drugs [1,2]. Despite the fact that it is *Candida* spp. and *Aspergillus* spp. that remain the main causative pathogens, the number of cases of systemic fungal infections due to strains of *Fusarium* spp., *Mucor* spp., *Scedosporium* spp. and others, which are resistant to the most widely used antifungal agents, is increasing [3]. In order to prevent this serious medical problem, the elaboration of new types of antifungal agents or the expansion of bioactivity of the previously known drugs is a pressing task [4,5]. The synthesis and characterization of metal complexes with organic bioactive ligands, in particular, of those with *o*-aminophenol derivatives, comprises a promising field of study in bioinorganic chemistry. Cell- and animal-based testing allowed to reveal a set of promising pharmacological properties for sterically hindered *o*-aminophenols, e.g. antioxidant, antihypoxic, anti-inflammatory and antiviral ones [6–10]. In particular, an antiherpetic medicine has been developed from 2-anilino-4,6-di(*tert*-butyl)phenol and is currently available [11,12]. The non-substituted *o*-aminophenol is known as Questiomycin B antibiotic with specific antifungal (antimycobacterial) activity [13]. However, alkylated *o*-aminophenol derivatives, particularly those with *tert*-butyl groups, are known to be substantially less toxic [14].

According to the results presented in a series of papers [15–20], sterically hindered *o*-aminophenol molecules are redox non-innocent when *O,N*-coordinated to some transition metal ions, and can exist in different protonation and oxidation forms (the *o*-aminophenol or *o*-iminosemiquinone) in coordination compounds. Thus, sterically

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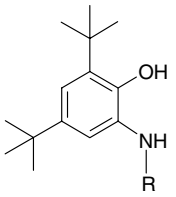
hindered *o*-aminophenols may be assumed to have great potential as ligands in synthesis of new bioactive metal complexes. As for the coordination chemistry of transition metal complexes formed by *O*,*N*-coordinated ligands of this sort, it is quite possible that synthetic difficulties have inhibited the study of metal complexes of sterically hindered *o*-aminophenols, since the attempts to isolate complexes of this type were often unsuccessful and resulted in the ligand being recovered without coordination [21,22]. This has prompted us to investigate the peculiarities of complexation of some di-*tert*-butylated *o*-aminophenolate ligands with transition metal ions [23,24]. Selecting Co(II) and Cu(II) complexes as the main subjects for this study, we had in mind that the antifungal effect of copper(II) and cobalt(II) ions had been known, their complexes still being under study in order to find effective antifungal agents [25–29]. The effect of some transition metal complexes on the structure of fungal and mammalian cell organelles has been studied [25,30–35]. In particular, when administered to some fungal strains, both metal-free 1,10-phenantroline derivatives and the their metal complexes inhibited respiration, reduced the levels of ergosterol in the membrane and altered cytochrome content [25,32–35]. In this connection it may be expected that metal complexes capable of participating in redox processes and of affecting electron-transport cell systems will be promising in the search for antifungal agents. Other papers should be also mentioned, their authors having shown that the bioactivity of metal complexes may be due to their redox effect on electron-transport cell systems, and that a correlation between *in vitro* potency and reduction potential is possible [36,37]. This encouraged us to investigate redox properties of the synthesized *o*-aminophenol derivatives and their Co(II) and Cu(II) complexes by cyclic voltammetry. In so doing we took into account that Co(II) and Cu(II) ions were able to take part in redox processes, thus affecting the properties of the ligands of the phenolic or quinoid types [38]. It was also interesting to find out whether there is any correlation between the antifungal activity of the compounds under study and their redox properties.

Since our earlier work had revealed that Ag(I) and Cu(II) complexation led to an enhancement of the antimicrobial activity of some sterically hindered phenolic derivatives [24,39–41], we were motivated to explore whether there was a similar trend in the case of other transition metal(II) complexes of these organic compounds. We report here the complexation of Co(II) ion with two sterically hindered *o*-aminophenols, namely, 4,6-di(*tert*-butyl)-2-aminophenol (HL^{I}, see the scheme below, R = H) and 2-anilino-4,6-di(*tert*-butyl)phenol (HL^{II}, R = Ph), in order to compare their coordinative behaviour in relation to Co(II) ion with the data obtained previously for their Cu(II) complexes [24] and to assess the influence of complexation on antifungal activities and redox properties of *o*-aminophenols. We have examined the possibility of the formation of *tert*-butyl-substituted *o*-aminophenolates of Cu(II) and Co(II), which may be less toxic than the oxidized (radical) forms of respective ligands [42,43].



HL^{I} – R=H

HL^{II} – R=Ph

2. Experimental

2.1. Materials and methods

Chemicals were purchased from commercial sources and were used without further purification. The two sterically hindered *o*-aminophenols and their Cu(II) complexes were prepared as described elsewhere [24,44,45]. The preparation of Co(II) complexes is described later.

Infrared spectra of solids were recorded with a Spectrum 1000 spectrophotometer in the wavelength range 4000–400 cm^{-1} at room temperature, using nujol mulls of the ligands and their complexes, and polyethylene windows. Thermal analysis was performed with a derivatograph OD-103 MOM. TG/DTA measurements were run in the air between 20 and 450 °C (5 °C min^{-1}). Elemental analyses were carried out according to the standard methods by Microanalytical Laboratory, Bioorganic Chemistry Institute, National Academy of Sciences, Belarus. Metal determination was carried out using an atomic emission spectrometer with an inductively coupled plasma excitation source (Spectroflame Modula). A sample of the Co(II) complex was decomposed on treatment with $HNO_3 + H_2O_2$ using Milestone mls 1200 mega microwave digestion system. After the complex had been decomposed, the content of the metal in the resulting solution was determined. XRD studies were made with an HZG 4 A diffractometer. The UV–Vis absorption spectra were measured in the 220–900 nm range with a Specord M500 spectrophotometer, using acetonitrile solutions of the compounds. Magnetic susceptibility was determined with the use of a Gouy balance at room temperature using $Hg[Co(SCN)_4]$ as a calibrant. ESR spectra of polycrystalline samples were measured with ERS-220 X-band spectrometer (9,45 GHz) at room temperature and at 77 K, using 100-kHz field modulation; *g* factors were quoted relative to the standard marker DPPH ($g = 2.0036$). The molar conductance of 10^{-3} mol L^{-1} solution of the metal(II) complex in acetonitrile was measured at 20 °C using a TESLA BMS91 conductometer (cell constant 1, 0). Electrochemical measurements were performed under dry nitrogen in a three-electrode two-compartment electrochemical cell using a glassy-carbon (GC) working electrode, Pt auxiliary electrode and Ag|AgCl|0.1 mol L^{-1} $(C_2H_5)_4NCl$ reference electrode. The supporting electrolyte was 0.1 mol L^{-1} $(C_2H_5)_4NClO_4$. The Ag|AgCl|0.1 mol L^{-1} $(C_2H_5)_4NCl$ ref-

erence electrode was calibrated with ferricinium|ferrocene redox couple located at $E_{1/2} = +0.52$ V. Acetonitrile was used as a solvent.

2.2. Synthesis of the Co (II) complexes with sterically hindered o-aminophenol derivatives

Based on our previous findings [23], the conditions were created to purposefully provide the preferential formation of the Co(II) complexes, with the composition corresponding to the general formula CoL_2: a solution of Co(II) salt was added in small portions to the ligand solution under continuous stirring, so that the complexation always took place with the excess ligand present. A solution of 0.050 mmol $Co(CH_3COO)_2 \cdot 4H_2O$ in 10 mL of water was added dropwise to a colorless solution of 0.100 mmol of HL^I or HL^{II} dissolved in 10 mL of ethanol (molar ratio Co(II):HL = 1:2). As these ligands can be readily oxidized by oxygen, it is to be taken into account when producing their complexes in order to guard against formation of oxidized ligands (in particular, *o*-iminosemiquinones, as it was shown in some papers [15–21]). To this end, argon was bubbled through the solutions (pH $\leqslant$ 6) during the synthesis. Colored precipitates formed instantaneously. After 1.5–2 h stirring, they were collected on membrane filters (JG 0.2 μm), washed with ethanol and water, and dried *in vacuo* (yield > 70%). It should be specially emphasized that to separate individual $Co(L^I)_2$ complex, the precipitate must be more thoroughly washed with ethanol (5 × 50 mL) in order to eliminate uncoordinated ligand molecules which otherwise could be able to form adducts with the metal complex through hydrogen bonding.

$Co(L^I)_2$ complex. Grey-brown. *Anal.* Calc. for $C_{28}H_{44}N_2O_2Co$: C, 67.29; H, 8.88; N, 5.61; Co, 11.80. Found: C, 67.25; H, 8.83; N, 5.58; Co, 11.75%.

$Co(L^{II})_2$ complex. Blue. *Anal.* Calc. for $C_{40}H_{52}N_2O_2Co$: C, 73.69; H, 8.04; N, 4.30; Co, 9.05. Found: C, 73.61; H, 7.95; N, 4.23; Co, 8.96%.

2.3. Antifungal activities

Antifungal activities of the compounds were tested against the following fungal species (the collection of the Department of Microbiology, The Belarusian State University): *Aspergillus niger*, *Fusarium* spp., *Mucor* spp., *Penicillium lividum*, *Botrytis cinerea*, *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Monilia* spp. Compounds were dissolved in DMSO and diluted in potato dextrose agar medium to yield working solutions of the test compounds with the concentration of 100 μg mL^{-1}. The amount of DMSO in the medium was 1% and did not affect the growth of the tested microorganisms. Antifungal activities of the compounds against moulds and common plant pathogens were checked by the agar plate technique as described elsewhere [41]. The degree of inhibition of radial growth (RI) was calculated as follows [46]: $RI = 100(C - T)/C$ (%), where T is the mean value of the diameter of the fungal colonies in the presence of a given concentration of the compound tested, and C is the mean value of the diameter of the fungal colonies in the absence of the compound, measured under the same conditions.

3. Results and discussion

3.1. Physico-chemical characterization

The stoichiometry of the solid complexes was confirmed by elemental analyses of the isolated chelates. The data show satisfactory agreement with the proposed formulae CoL_2. The complexes demonstrated X-ray patterns of their own, which were significantly different from those of the respective ligands. However, a full structural analysis could not be performed, no crystals suitable for single-crystal X-ray analysis having been obtained, which is not uncommon for compounds with sterically hindered ligands [22]. It is noteworthy that a similar problem when carrying out crystallization of metal complexes of aminophenol derivatives is described in [21,47]. Because of the well-known difficulties in direct X-ray investigations of these complexes, the geometrical arrangement of the ligating atoms in the Co(II) complex has been investigated by several spectroscopic and magnetochemical methods.

Thermal analysis in air flow with identification of the final products by X-ray powder diffraction has shown $Co(L^I)_2$ and $Co(L^{II})_2$ complexes to be anhydrous and unsolvated. No weight loss was observed until decomposition which began about 185 °C (for $Co(L^I)_2$) or 200 °C (for $Co(L^{II})_2$), with exothermic peaks at 190 °C (for $Co(L^I)_2$) or 210 °C (for $Co(L^{II})_2$) without any noticeable weight loss, and at 360 °C (for both complexes), ultimately leaving CoO as the residue. The maximal weight loss (83.72% and 84.1%, respectively, for $Co(L^I)_2$ and $Co(L^{II})_2$) corresponds to the loss of two ligand molecules (Calc. 84.99% and 85.2%, respectively, for $Co(L^I)_2$ and $Co(L^{II})_2$). The agreement between the experimental and theoretical weight losses for the above processes confirms the above-mentioned general formula CoL_2.

The Co(II) complexes are soluble in acetonitrile, acetone, dimethyl formamide, dimethyl sulfoxide, insoluble in nitromethane and ethanol. The conductivity data for the complexes $Co(L^I)_2$ ($\Lambda_{mol} = 11.4\ \Omega^{-1}\ cm^2\ mol^{-1}$) and $Co(L^{II})_2$ ($\Lambda_{mol} = 5.8\ \Omega^{-1}\ cm^2\ mol^{-1}$) indicate their being essentially non-electrolytes in acetonitrile [48], and suggest that the bidentate ligands HL^I and HL^{II} may be coordinated to Co(II) ion as uninegatively charged ligands.

To specify the coordination modes in the Co(II) complexes, we used IR spectroscopy (Table 1).

The IR spectra of the Co(II) complexes as against those of the free ligands are distinguished by the following features: (i) the bands corresponding to ν(OH) vibrations in the free ligands disappear, suggesting the participation of the *o*-OH in complex formation through proton displacement; (ii) the bands at 1230–1140 cm^{-1} assigned to C–I stretching vibrations are shifted towards lower wave num-

Table 1
IR spectral assignments (cm^{-1}) of the ligands and Co(II) complexes

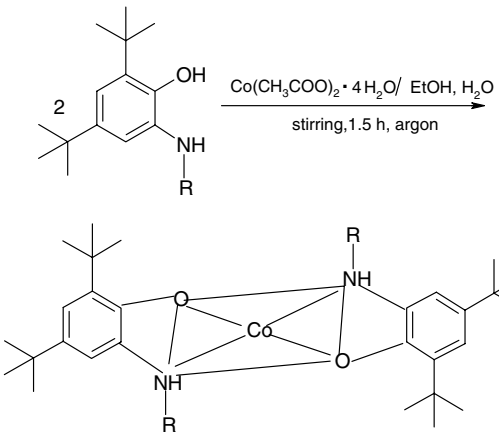
Compound	ν(OH)	ν(C–O)	ν(C–N)	ν(C=C)	ν(N–H)	ν(Co–N)	ν(Co–O)
HL^I	3290m	1235s	1292w	1593m	3366m		
		1204m					
		1142w					
$Co(L^I)_2$		1180s	1300m	1556m	3340w	469w	570w
		1091m				435w	539m
		1044m					514m
		1021m					
HL^{II}	3585m	1202m	1311s	1599s	3352m		
		1150w		1495s			
$Co(L^{II})_2$		1144s	1302m	1537m	3334w	469w	571w
		1106s				424w	545w
		1074w					513m

bers as compared to their positions in the spectra of the ligands; (iii) the positions of the bands assigned to N–H (3352 and 3366 cm^{-1}) and C–N (1292 and 1311 cm^{-1}) stretching vibrations are changed, the presence of the weak bands 3340 and 3334 cm^{-1} in the spectra of the complexes indicating the participation of NH group in coordination without deprotonation; and (iv) bands of moderate intensity appear at 571–424 cm^{-1}, which can be assigned to ν(Co–N) and ν(Co–O). The above-listed facts suggest that NH-groups and deprotonated hydroxyls take part in forming the CoN_2O_2 coordination cores in the Co(II) complexes [49].

The electronic absorption spectra of the free ligands in acetonitrile show characteristic intraligand transition bands at 225 and 280 nm [21,50]. The spectra of the Co(II) complexes in acetonitrile solution exhibit absorption bands in the high-energy region, which can be assigned to intraligand transitions: 230–240 nm ($\log\varepsilon = 4.3$–4.4) and 280–282 nm ($\log\varepsilon = 4.1$–4.2). The bands located in the 305–430 nm region can be attributed to the ligand-to-cobalt(II) charge transfer transitions $N(\sigma) \rightarrow Co^{II}$ and $O_{phenolate} \rightarrow Co^{II}$: respectively, 305 nm ($\log\varepsilon = 4.1$) and 395 ($\log\varepsilon = 3.9$), 430 nm ($\log\varepsilon = 3.7$) [50,51]. The new absorption bands that appeared upwards of 500 nm can be imputed to the d–d transitions of the $Co(L^I)_2$ and $Co(L^{II})_2$ complexes: respectively, 520 nm ($\log\varepsilon = 3.65$) and 640 nm ($\log\varepsilon = 3.69$), characteristic of the low-spin, square planar Co(II) complexes [47,50–58]. This is also corroborated by the effective magnetic moment values: $\mu_{eff} = 2.38$ BM for the $Co(L^I)_2$ complex and $\mu_{eff} = 2.58$ BM $Co(L^{II})_2$ complex, expected for the low-spin square planar geometry around Co(II) ions [47,51–56].

The ESR spectra of Co(II) complexes were obtained in the solid (polycrystalline) state at 77 K and room temperature in order to confirm the geometry of the coordination cores. These spectra are typical axial ones with $g_{\|} = 1.96$ and $g_{\perp} = 2.01$ (for the $Co(L^I)_2$ complex) and $g_{\|} = 1.98$ and $g_{\perp} = 2.07$ (for the $Co(L^{II})_2$ complex), characteristic of low-spin, square-planar Co(II) complexes [47,51,54,55,59–61]. No signal of stabilized radicals present in the ESR spectrum at 77 K, as well as the ν(C=O) and ν(C=N) stretching vibrations lacking in the IR spectrum of Co(II) complex, respectively, in the ranges of 1400–1500 and 1690–1640 cm^{-1}, confirm the phenolate character of the ligand.

In the light of the physico-chemical data and elemental analysis results the mode of bonding in the Co(II) complex can be represented schematically as shown below:



This coordination core agrees with the data obtained by physico-chemical methods for the Cu(II) complex investigated previously [24], where the copper site has the square planar stereochemistry. It should be particularly emphasized that any departure from the specified conditions of the synthesis makes the possibility of separating the individual complex problematic, the free ligands in their oxidized forms being generally obtained in abundance along with it.

3.2. Cyclic voltammetry

Redox properties of the compounds were examined by cyclic voltammetry. Cyclic voltammogram of the ligand HL^I at positive potential shows two anodic peaks at 0.58, 1.72 V, and only one cathodic peak at 0.495 V on the reverse scan (Fig. 1a, solid line). For the ligand HL^{II} a dou-

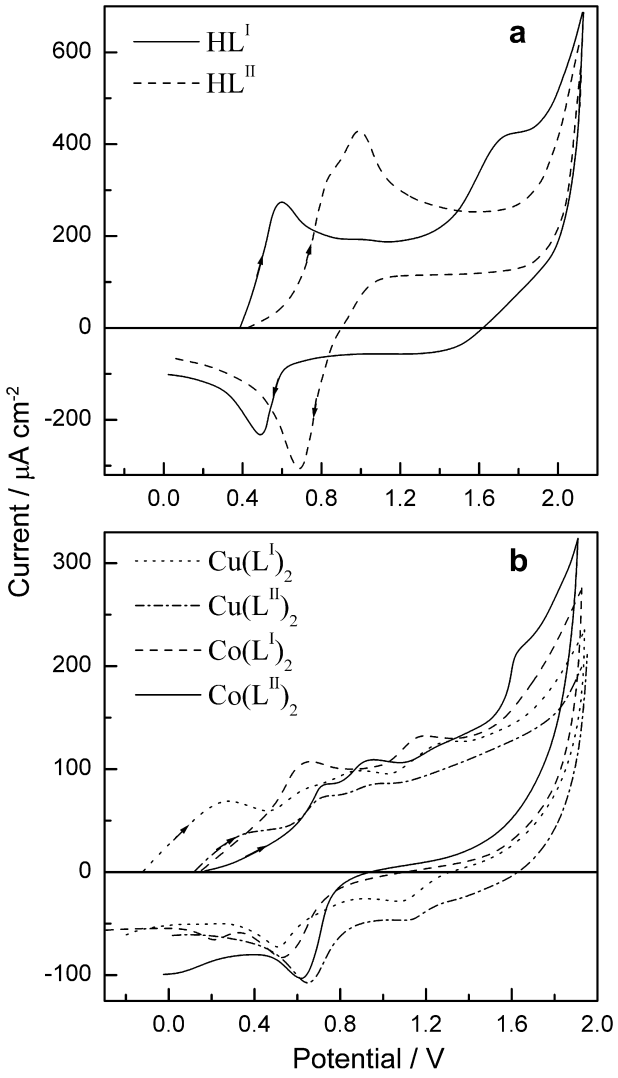


Fig. 1. Cyclic voltammograms (50 mV/s) of the ligands (1.36 mmol L^{-1}) HL^I (solid line), HL^{II} (dashed line) (a) and metal complexes (0.68 mmol L^{-1}) $Cu(L^I)_2$ (dotted line), $Cu(L^{II})_2$ (dashed-dotted line), $Co(L^I)_2$ (dashed line), $Co(L^{II})_2$ (solid line) (b) in 0.1 mol L^{-1} $(C_2H_5)_4NClO_4$ acetonitrile solution on glassy-carbon electrode. (The potentials were measured against Ag|AgCl|0.1 mol dot L^{-1} $(C_2H_5)_4NCl$ reference electrode.)

ble anodic peak is observed at 0.85–0.98 V (is not two overlapping peaks), and a cathodic peak (0.70 V) on the reverse scan (Fig. 1a, dashed line). On cathodic polarization (down to −1.8 V) for HL^I and HL^{II} no peaks are observed. Controlled potential electrolysis carried out for HL^{II} at 1.2 V consumed two electron per molecule. The UV–Vis spectra of the oxidized HL^{II} solution show two broad absorption bands at 400 and 500 nm. These findings agree with the results of electrochemical investigation of HL^{II} compound [16,21], according to which HL^{II} undergoes 2 e-oxidative transformation to give iminobenzoquinone. Controlled potential coulometry for HL^I shows two 1 e-oxidative processes corresponding to anodic peaks at 0.58 and 1.72 V. On the basis of the data on oxidation of *o*-aminophenols [15–19,21] it may be suggested that HL^I is successively oxidized to iminosemiquinone and iminobenzoquinone.

The difficulty of interpreting the electrochemical data for metal complexes of sterically hindered *o*-aminophenol derivatives is caused by the fact that, as noted above, in coordination compounds ligands of this kind can exist in different protonation and redox forms (*o*-aminophenol or *o*-iminosemiquinone). It should be emphasized that the electrochemical properties of the ligands and metal complexes have been studied mainly to compare their reducing power rather than to monitor spectral and structural changes accompanying an electron transfer. The assignment of the redox processes was facilitated by comparison with the literature [16,21,62].

On voltammograms of Cu(II) complexes in the range from −0.1 to +2.1 V anodic peaks are observed: at 0.27, 0.61, 0.89, 1.27 V for $Cu(L^I)_2$ (Fig. 1b, dotted line) and 0.4, 0.7, 0.96 V for $Cu(L^{II})_2$ (Fig. 1b, dashed-dotted line). Cathodic peaks are registered on the reverse scan: at 0.14, 0.51, 1.07 V for $Cu(L^I)_2$ and 0.25, 0.65, 1.12 V for $Cu(L^{II})_2$. Two peaks, at 0.14 and 0.25 V, for Cu(II) complexes are observed in voltammograms on polarization to about 0.6–1.0 V; upon more anodic polarization on the reverse scan they are obscured by the peaks at 0.51 and 0.65 V. On anodic polarization two peaks are observed at 0.65 and 1.19 V for $Co(L^I)_2$ complex, and three peaks are observed at 0.71, 0.94 and 1.63 V for $Co(L^{II})_2$, with cathodic ones on the reverse scan at 0.54, 0.21 V for $Co(L^I)_2$ and 0.62 V for $Co(L^{II})_2$) (Fig. 1b, dashed and solid lines). No prominent cathodic processes are observed on cathodic polarization from the open circuit potential down to −1.8 V.

The cyclic voltammograms of $Cu(L^I)_2$ and $Cu(L^{II})_2$ complexes are more or less similar in their shape, voltammogram of $Cu(L^{II})_2$ being shifted by 0.15–0.20 V into cathodic region because of the influence of phenyl substitute in aminogroup (Fig. 1b). A similar regularity can be observed also for voltammograms of $Co(L^I)_2$ and $Co(L^{II})_2$ complexes. A comparison between cyclic voltammograms of $Cu(L^I)_2$ and $Co(L^I)_2$ complexes shows that common peaks are present at ~0.65 V, and a comparison between voltammograms of $Cu(L^{II})_2$ and $Co(L^{II})_2$ complexes shows that there are common peaks at 0.9 V, corresponding to oxidation of the ligands L^I and L^{II}, as well as cathodic peaks at ~0.5 V (for the complexes of the ligand L^I) and at ~0.65 V (for those of the ligand L^{II}), relating, respectively, to the reduction of the products of L^I and L^{II} oxidation on the reverse scan.

On the basis of the above findings obtained by cyclic voltammetry, and taking into account the data of [62], it may be suggested that Cu(II) and Co(II) ions take part in a sequence of electron transfers; otherwise, whatever the metal (II) ion nature, the redox potentials of the complexes would have differed only slightly in a series of ligands. A comparison between the redox properties of the ligands and those of their metal(II) complexes shows that their reducing ability decreases in the following order: $Cu(L^I)_2 > Cu(L^{II})_2 \geqslant Co(L^I)_2 > Co(L^{II})_2 > HL^I > HL^{II}$ (Fig. 1).

Table 2
Antifungal activities of the compounds tested as evaluated by radial inhibition of mycelial growth (RI, %)

Compound	RI (%)							
	Alternaria alternata	*Sclerotinia sclerotiorum*	*Monilia* spp.	*Aspergillus niger*	*Fusarium* spp.	*Mucor* spp.	*Penicillium lividum*	*Botrytis cinerea*
HL^{I}	60	100	70	55	70	50	40	60
$Cu(L^{I})_2$	100	100	100	65	100	100	80	100
$Co(L^{I})_2$	90	100	55	60	90	80	55	90
HL^{II}	50	90	30	40	60	30	30	50
$Cu(L^{II})_2$	90	100	70	55	90	70	55	100
$Co(L^{II})_2$	90	100	50	50	80	60	50	90
$Cu(CH_3COO)_2$	0	0	0	0	0	0	0	0
$Co(CH_3COO)_2$	0	0	0	0	0	0	0	0
Nistatin	40	50	40	70	90	50	90	60
Terbinafine	50	50	50	100	80	50	60	60
Questiomycin B	40	50	50	30	40	50	40	30

3.3. Antifungal activities

The test microorganisms (the collection of the Department of Microbiology, the Belarusian State University) are listed in Table 1. Inhibition values of the free ligands and their Co(II) and Cu(II) complexes are listed in Table 1. Commonly used antifungal drugs Nystatin, Terbinafine and Questiomycin B were tested as positive controls. The effect of the starting metal(II) acetates is also reported. It should be emphasized that the antifungal tests of these ligands and their Co(II) and Cu(II) complexes were first performed here.

It was found that the inhibitory effects of HL^{I} and HL^{II} ligands and their metal(II) complexes differed with the species of fungi. All of them demonstrate the highest inhibitory properties against *S. sclerotiorum*. A like high activity against *Fusarium* spp., *A. alternata* and *B. cinerea* is characteristic of Co(II) and Cu(II) complexes (Table 1). And it was only $Cu(L^{I})_2$ that showed the highest antifungal activity against *Mucor* spp. and *Monilia* spp. Both the free ligands and Co(II) and Cu(II) complexes exhibit pronounced antifungal activities against some strains, which in a number of cases were comparable with those of Nystatin and Terbinafine or even higher (Table 2).

Antifungal screening *in vitro* indicated that in the cases mentioned above a synergistic effect is present, the antifungal activities of the metal(II) complexes being higher than those of both the ligand and the starting metal salts. This provides reason to believe that antifungal activities of the metal(II) complexes synthesized do not correlate with the toxicity of the metal(II) ions against the fungi tested. It is noteworthy that the two sterically hindered derivatives of *o*-aminophenol synthesized and their metal(II) complexes were more active than Questiomycin B antibiotic tested. In this connection, when estimating the effect of modification of the molecule of *o*-aminophenol by introducing substitutes (two *tert*-butyl ones into the ring and a phenyl one into aminogroup) and complexation with Cu(II) and Co(II) ions on the antifungal activities of the compounds tested, we may suggest that one of the likely reasons for their different activities is related to the nature of the substitute in the ring and to a substitute being present at the nitrogen atom of the amino group. Moreover, the antifungal activities of the complexes exhibit some metal-ion-dependency. By and large the inhibitory effects of the compounds examined decrease in the order: $Cu(L^{I})_2 > Cu(L^{II})_2 \geqslant Co(L^{I})_2 > Co(L^{II})_2 > HL^{I} > HL^{II}$ (Table 1), and their reducing ability declines in the same order, as it was shown above. These data are in accordance with the results of the studies [32–35], where the influence of redox properties of the metal complexes on their antifungal effect was found, and it was shown that this effect appears to be mediated through the disruption of mitochondrial function and uncoupling respiration. These facts may be of interest in designing new antifungal drugs, since this effect is different to the mode of action of the conventional azole and polyene drugs.

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