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Redox-active metal(II) complexes of sterically hindered phenolic ligands: Antibacterial activity and reduction of cytochrome *c*. Part III. Copper(II) complexes of cycloaminomethyl derivatives of *o*-diphenols

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ABSTRACT

Redox-active copper(II) complexes of sterically hindered phenolic ligands have been synthesized using 5-*tert*-butyl-3-(pyrrolidinomethyl)-1,2-dihydroxybenzene (HL^{I}), 5-*tert*-butyl-3-(piperidinomethyl)-1,2-dihydroxybenzene (HL^{II}), 5-*tert*-butyl-3-(azepanylmethyl)-1,2-dihydroxybenzene (HL^{III}), 5-*tert*-butyl-3-(morpholinomethyl)-1,2-dihydroxybenzene (HL^{IV}), and 5-*tert*-butyl-3-(methylpiperazinomethyl)-1,2-dihydroxybenzene (HL^{V}). The novel compounds have been characterized by means of chemical and physico-chemical methods. The coordination core of these complexes is a square planar chromophore, $[CuO_2N_2]$, and the phenolic ligands coordinate in their monoanionic forms. The ligands and Cu(II) complexes have been screened for their antibacterial activity. The lowest MIC value (0.020 μmol ml^{-1}) has been found for $Cu(L^{III})_2$ and $Cu(L^{V})_2$ against *Mycobacterium smegmatis*, *Sarcina lutea* and *Staphylococcus aureus*, and this is comparable to the value for chloramphenicol. Their antibacterial activities were found to follow the order: (1) $HL^{I} > HL^{V} \geqslant HL^{III} \sim HL^{II} > HL^{IV}$; (2) $Cu(L^{III})_2 > Cu(L^{II})_2 \sim Cu(L^{I})_2 \sim Cu(L^{V})_2 > Cu(L^{IV})_2$; their reducing ability (determined electrochemically) followed the same order. The most bioactive complex, $Cu(L^{III})_2$, has the highest lipophilicity. A spectrophotometric investigation was carried out in order to estimate the rate of the reduction of bovine heart cytochrome *c* with the ligands and their Cu(II) complexes. HL^{I} and the complex $Cu(L^{III})_2$ have the highest reducing abilities (determined electrochemically), which are characterized by the highest Cyt *c* reduction rates respectively amongst the ligands and Cu(II) complexes.

1. Introduction

In earlier investigations, using the method of cyclic voltammetry, we have shown some mono- and di-substituted derivatives of sterically hindered phenolic ligands as well as their metal complexes to have a pronounced reducing ability, correlating with the antimicrobial activity and rate of reduction, of bovine heart cytochrome *c* (Cyt *c*) in a limited series of these compounds [1–4]. These results prompted us to extend our research to other sterically hindered phenolic ligands and their metal complexes in order to gain greater insight into what structural modifications of these compounds play an important role in their reducing ability and biological activity. For this purpose we chose the Mannich reaction because it is one of the main tools for the development of bioactive compounds [5]. The aim of modifying phenolic ligands was to produce novel redox-active ligands for complexation with transition metals and to further evaluate the changes in antibacterial activity and the rate of the reduction of Cyt *c* for both the phenolic ligands and their metal complexes.

Copper is known as an essential element, participating in many biological processes, and the role of Cu(II) complexation in enhancing the pharmacological profile of the antimicrobial activity of some drugs and bioactive compounds is known [6,7]. In this connection it is of interest to study various aspects of the coordination chemistry of Cu(II) ions interacting with phenolic ligands, and to investigate the physico-chemical and biological properties of the complexes synthesized.

In the present work, the reduction of Cyt *c* was investigated spectrophotometrically with five cycloaminomethyl derivatives of sterically hindered *o*-diphenols, namely 5-*tert*-butyl-3-(pyrro-

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lidinomethyl)-1,2-dihydroxybenzene (HL^I), 5-*tert*-butyl-3-(piperidinomethyl)-1,2-dihydroxybenzene (HL^{II}), 5-*tert*-butyl-3-(azepanylmethyl)-1,2-dihydroxybenzene (HL^{III}), 5-*tert*-butyl-3-(morpholinomethyl)-1,2-dihydroxybenzene (HL^{IV}) and 5-*tert*-butyl-3-(methylpiperazinomethyl)-1,2-dihydroxybenzene (HL^V) (see Section 2.2), as well as with their Cu(II) complexes. For the first time, the redox properties of the above-mentioned ligands and their Cu(II) complexes were determined electrochemically. As before [3,4], the results obtained are discussed in view of the presumed correlation between the capability of the compounds under study for reducing Cyt *c*, their antibacterial activity, redox properties, determined electrochemically, and lipophilicity.

2. Experimental

2.1. Materials and methods

All the reagents used for the synthesis of the cycloaminomethyl derivatives of sterically hindered *o*-diphenols HL^I–HL^V and their complexes are commercially available and were used without further purification. The synthesis of the above-mentioned ligands and their Cu(II) complexes was performed as described in Sections 2.2 and 2.3. The purity of the *o*-diphenol derivatives HL^I–HL^V was checked by Thin Layer Chromatography (TLC). Elemental analyses were carried out with a Vario EL (CHNS mode) instrument. Copper was determined using an atomic emission spectrometer with an inductively coupled plasma excitation source (Spectroflame Modula). Infrared spectra of solids were recorded with a Nicolet 380 spectrometer in the wavelength range 4000–400 cm^{-1} at room temperature, using «Smart Performer». Thermal analysis was performed with a Simultaneous Thermal Analyzer STA 449 C. X-ray Diffraction (XRD) analysis was carried out with an HZG 4A diffractometer (Co Kα radiation, MnO_2-filter). ESR (Electron Spin Resonance) spectra of polycrystalline samples were measured with an 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 2,2-diphenyl-1-picrylhydrazyl (DPPH). ^1H NMR spectra were recorded with a Varian Unit Plus 300 MHz spectrometer in $CDCl_3$; chemical shifts were reported in parts per million (ppm) relative to an internal standard of Me_4Si. Mass spectra (EI) were recorded on Shimadzu GCMS-QP 2010 Plus spectrometer. Ultraviolet–Visible (UV–Vis) absorption spectra were recorded with a SPECORD M500 spectrophotometer. The molar conductance of 10^{-3} mol l^{-1} solutions of the Cu(II) complexes in acetonitrile was measured at 20 °C using a TESLA BMS91 conductometer (cell constant 1.0). The lipophilicity test was made by determining the *n*-octanol/water partition coefficient (P_{ow}) [8]. 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} $(C_2H_5)_4NCl$ reference electrode was calibrated with the ferrocenium|ferrocene redox couple located at $E_{1/2}$ = +0.54 V. Anhydrous acetonitrile was used as the solvent. The $(C_2H_5)_4NClO_4$ and $(C_2H_5)_4NCl$ used to prepare solutions were dried respectively at 80 and 100 °C under vacuum for 3 h. Preparation of solutions and the subsequent filling of the electrochemical cells were carried out in a glove box under dry nitrogen.

2.2. Synthesis of the ligands – cycloaminomethyl derivatives of *o*-diphenols (HL^I–HL^V)

The compounds HL^I–HL^V were synthesized according to the methodology described in the literature [9]. 4-*Tert*-butylcatechol HL (Sigma) (10 mmol), paraformaldehyde (10 mmol), a secondary amine (pyrrolidine, piperidine, hexamethylene amine, morpholine or methylpiperazine) (11 mmol) and isopropyl alcohol (20 ml) were mixed, and the mixture was refluxed for 2–4 h. Then the solution was boiled dry under vacuum, and the residue was recrystallized from petroleum ether (or ethyl acetate) and dried under vacuum.

2.2.1. 5-Tert-butyl-3-(pyrrolidinomethyl)-1,2-dihydroxybenzene (HL^I)

Yield: 72%; M.p.: 132–134 °C. M.wt.: 249.35. *Anal.* Calc. for $C_{15}H_{23}O_2N$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.17; H, 9.18; N, 5.57%. Mass spectrum (*m/z*, *I*%): 249.25 (M^+, 27), 234.20 (M–CH_3, 14). Prominent IR absorption bands (ν, cm^{-1}): 3409w (O–H), 1608w and 1490m (C=C arom), 1320m, 1301m and 1189s (C–O), 1120m and 1102m (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 7.70 [s, 2H, 2OH], 6.89 [dd, 1H, aromatic H, *J* 0.4, 2.3 Hz], 6.52 [m, 1H, aromatic H], 3.82 [s, 2H, CH_2], 2.67 [m, 4H, $N(CH_2)_2$], 1.87 [m, 4H, $(CH_2)_2$], 1.28 [s, 9H, Me_3C].

2.2.2. 5-Tert-butyl-3-(piperidinomethyl)-1,2-dihydroxybenzene (HL^{II})

Yield: 87%; M.p.: 164–166 °C. M.wt.: 263.38. *Anal.* Calc. for $C_{16}H_{25}O_2N$: C, 72.96; H, 9.57; N, 5.32. Found: C, 73.09; H, 9.79; N, 5.29%. Mass spectrum (*m/z*, *I*%): 263.25 (M^+, 24), 248.20 (M–CH_3, 16). Prominent IR absorption bands (ν, cm^{-1}): 3403 m (O–H), 1609w and 1490m (C=C arom), 1326w, 1307s, 1197s and 1188s (C–O), 1098m and 1029w (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 7.10 [br s, 2H, 2OH], 6.61 [m, 1H, aromatic H], 6.45 [m, 1H, aromatic H], 3.75 [s, 2H, CH_2], 2.60 [m, 4H, $N(CH_2)_2$], 1.67 [m, 6H, $(CH_2)_3$], 1.35 [s, 9H, Me_3C].

2.2.3. 5-Tert-butyl-3-(azepanylmethyl)-1,2-dihydroxybenzene (HL^{III})

Yield: 88%; M.p.: 160–162 °C. M.wt.: 277.41. *Anal.* Calc. for $C_{17}H_{27}O_2N$: C, 73.60; H, 9.81; N, 5.05. Found: C, 73.52; H, 9.64; N, 4.97%. Mass spectrum (*m/z*, *I*%): 277.40 (M^+, 30), 262.35 (M–CH_3, 14). Prominent IR absorption bands (ν, cm^{-1}): 3403 m (O–H), 1606w and 1489 m (C=C arom), 1331w, 1307m, 1224w and 1188s (C–O), 1119w, 1102m and 1063m (C–N). ^1H NMR δ_H(100 - MHz, $CDCl_3$): 7.20 [br s, 2H, 2OH], 6.77 [d, 1H, aromatic H, *J* 2.3 Hz], 6.55 [d, 1H, aromatic H, *J* 2.3 Hz], 3.78 [s, 2H, CH_2], 2.53 [m, 4H, $N(CH_2)_2$], 1.67 [m, 8H, $(CH_2)_4$], 1.27 [s, 9H, Me_3C].

2.2.4. 5-Tert-butyl-3-(morpholinomethyl)-1,2-dihydroxybenzene (HL^{IV})

Yield: 85%; M.p.: 150–152 °C. M.wt.: 265.35. *Anal.* Calc. for $C_{15}H_{23}O_3N$: C, 67.89; H, 8.74; N, 5.28. Found: C, 68.03; H, 8.92; N, 5.17%. Mass spectrum (*m/z*, *I*%): 265.15 (M^+, 49), 250.20 (M–CH_3, 12). Prominent IR absorption bands (ν, cm^{-1}): 3375m (O–H), 1605w and 1493m (C=C arom), 1312m, 1300s, 1228m, 1192m and 1107s (C–O), 1127w, 1071m and 1031m (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 6.77 [d, 1H, *J* 2.3 Hz, aromatic H], 6.56 [d, 1H, *J* 2.3 Hz, aromatic H], 3.71 [m, 4H, $O(CH_2)_2$], 3.66 [c, 2H, CH_2], 2.55 [m, 4H, $N(CH_2)_2$], 1.23 [s, 9H, Me_3C].

2.2.5. 5-Tert-butyl-3-(methylpiperazinomethyl)-1,2-dihydroxybenzene (HL^V)

Yield: 84%; M.p.: 157–158 °C. M.wt.: 278.39. *Anal.* Calc. for $C_{16}H_{26}O_2N_2$: C, 69.03; H, 9.41; N, 10.06. Found: C, 68.94; H, 9.27; N, 9.84%. Mass spectrum (*m/z*, *I*%): 278.35 (M^+, 20), 263.30 (M–CH_3, 4). Prominent IR absorption bands (ν, cm^{-1}): 3038w (O–H), 1594w and 1497w (C=C arom), 1326m, 1299w, 1168m and 1135m (C–O), 1203w, 1082w, 1050w and 1036w (C–N). ^1H NMR δ_H(100 MHz, $CDCl_3$): 6.89 [d, 1H, aromatic H, *J* 2.4 Hz,], 6.82 [br s, 2H, 2OH], 6.52 [d, 1H, aromatic H, *J* 2.4 Hz,], 3.70 [s, 2H, CH_2], 2.55 [m, 8H], 2.32 [s, 3H, NMe], 1.26 [s, 9H, Me_3C].

2.3. Synthesis of the Cu(II) complexes with cycloaminomethyl derivatives of o-diphenols

Based on the data of potentiometric titrations in a water–ethanol (1:1) solution [10], the conditions were created to purposefully provide the preferential formation of complexes with a Cu(II):L ratio of 1:2. A solution of 0.05 mmol of $Cu(CH_3COO)_2$ in 6 ml of water was added dropwise to a colorless solution of 0.1 mmol of the compounds HL, HL^I–HL^V dissolved in 14 ml of ethanol (molar ratio Cu(II):L = 1:2). The reaction mixture was stirred for 1.5 h, after which the metal complex solution was left for several days to precipitate. The solid phase that formed was collected on membrane filters (JG 0.2 μm), washed with ethanol and water, and dried *in vacuo* (yield > 70%). We tried the following methods of growing single crystals: (i) slow evaporation of acetonitrile, chloroform, 70% aqueous ethanol or 70% aqueous acetone solutions of metal complex at room temperature in the dark; (ii) recrystallization of the powdery material from acetonitrile, 2-propanol or chloroform; (iii) a small portion (0.02 g) of the complex was anaerobically redissolved in 2.5 ml of acetonitrile (closed system vapor diffusion method), and carefully overlaid with 25 ml of anhydrous ether and left in a refrigerator. Unfortunately, none of these methods gave crystals suitable for X-ray diffraction studies.

2.3.1. $Cu(L^I)_2$

Beige. Yield: 73–75%. M.wt.: 559.70. *Anal.* Calc. for $C_{30}H_{44}N_2O_4Cu$: C, 64.32; H, 7.92; N, 5.00; Cu, 11.34. Found: C, 64.26; H, 8.01; N, 4.87; Cu, 11.41%.

2.3.2. $Cu(L^{II})_2$. Deep brown

Yield: 70–73%. M.wt.: 587.79. *Anal.* Calc. for $C_{32}H_{48}N_2O_4Cu$: C, 65.33; H, 8.22; N, 4.76; Cu, 10.80. Found: C, 65.16; H, 8.37; N, 4.65; Cu, 10.87%.

2.3.3. $Cu(L^{III})_2$. Greyish-yellow

Yield: 70–73%. M.wt.: 612.76. *Anal.* Calc. for $C_{34}H_{52}N_2O_4Cu$: C, 66.26; H, 8.50; N, 4.55; Cu, 10.31. Found: C, 66.37; H, 8.41; N, 4.61; Cu, 10.44%.

2.3.4. $Cu(L^{IV})_2$. Beige

Yield: 70–72%. M.wt.: 591.72. *Anal.* Calc. for $C_{30}H_{44}N_2O_6Cu$: C, 60.84; H, 7.49; N, 4.73; Cu, 10.73. Found: C, 60.99; H, 7.60; N, 4.66; Cu, 10.81%.

2.3.5. $Cu(L^V)_2$. Deep brown

Yield: 78–80%. M.wt.: 617.76. *Anal.* Calc. for $C_{32}H_{50}N_4O_4Cu$: C, 62.16; H, 8.15; N, 9.06; Cu, 10.28. Found: C, 62.25; H, 8.09; N, 8.99; Cu, 10.17%.

2.3.6. $Cu(L)_2$

Analytical data for this Cu(II) complex with the ligand HL (Fig. 1) are given in Ref. [11].

2.4. Physico-chemical characterization of the Cu(II) complexes

2.4.1. $Cu(L^I)_2$

Molar conductivity (in acetonitrile): $\Lambda_{mol} = 22.1\ \Omega^{-1}\ cm^2\ mol^{-1}$. Thermogravimetric/differential thermal analysis (TG/DTA) data: no weight loss was observed until decomposition, which began at about 110 °C, with five endothermic peaks at 145, 196, 233, 270 and 495 °C, ultimately leaving CuO as the residue. The maximal weight loss of 86.49% corresponds to the loss of two ligand molecules in the $Cu(L^I)_2$ complex (Calc. 85.80%). Prominent IR absorption bands (ν, cm^{-1}): 3391w, br (O–H), 1567w and 1481s, sh (C=C arom), 1286s, sh, 1261s, 1201w and 1164w (C–O), 1102w and 1062m, sh (C–N), 527m (Cu–O), 506m (Cu–N). ESR parameters (77 K): $g_{||} = 2.290$, $g_{\perp} = 2.062$, $A_{||} = 113$ Gs. UV–Vis data (acetonitrile) (λ_{max}, nm ($\log\varepsilon$)): 475 (2.87), 340sh (3.51), 305sh (3.77), 282 (3.85), 224 (4.13).

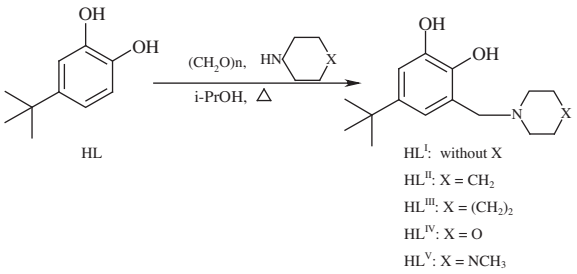

Fig. 1. Reagents and conditions for the synthesis of compounds HL^I–HL^V.

2.4.2. $Cu(L^{II})_2$

Molar conductivity (in acetonitrile): $\Lambda_{mol} = 28.2\ \Omega^{-1}\ cm^2\ mol^{-1}$. Thermogravimetric/differential thermal analysis (TG/DTA) data: no weight loss was observed until decomposition, which began at about 125 °C, with four endothermic peaks at 143, 238, 283 and 493 °C, ultimately leaving CuO as the residue. The maximal weight loss of 86.99% corresponds to the loss of two ligand molecules in the $Cu(L^{II})_2$ complex (Calc. 86.48%). Prominent IR absorption bands (ν, cm^{-1}): 3385w, br (O–H), 1553m, sh and 1483m (C=C arom), 1316m, 1283m, 1237m, sh and 1154w (C–O), 1103m and 1037m, br (C–N), 525m, sh (Cu–O), 501w (Cu–N). ESR parameters (77 K): $g_{||} = 2.290$, $g_{\perp} = 2.062$, $A_{||} = 120$ Gs. UV–Vis data (acetonitrile) (λ_{max}, nm ($\log\varepsilon$)): 505 (2.57), 410sh (3.07), 340sh (3.51), 282 (3.87), 225 (4.12).

2.4.3. $Cu(L^{III})_2$

Molar conductivity (in acetonitrile): $\Lambda_{mol} = 29.9\ \Omega^{-1}\ cm^2\ mol^{-1}$. Thermogravimetric/differential thermal analysis (TG/DTA) data: no weight loss was observed until decomposition, which began at about 110 °C, with four endothermic peaks at 120, 206, 311 and 470 °C, ultimately leaving CuO as the residue. The maximal weight loss of 86.76% corresponds to the loss of two ligand molecules in the $Cu(L^{III})_2$ complex (Calc. 87.09%). Prominent IR absorption bands (ν, cm^{-1}): 3245w, br (O–H), 1560s, br and 1476s (C=C arom), 1304w, 1286s, sh, 1224s and 1174w (C–O), 1104w, 1032w, 1016w and 1004w, sh (C–N), 538m and 519m, sh (Cu–O), 506w (Cu–N). ESR parameters (77 K): $g_{||} = 2.300$, $g_{\perp} = 2.050$, $A_{||} = 124$ Gs. UV–Vis data (acetonitrile) (λ_{max}, nm ($\log\varepsilon$)): 470 (2.93), 355sh (3.89), 302 (3.85), 292 (3.85), 224 (4.15).

2.4.4. $Cu(L^{IV})_2$

Molar conductivity (in acetonitrile): $\Lambda_{mol} = 25.1\ \Omega^{-1}\ cm^2\ mol^{-1}$. Thermogravimetric/differential thermal analysis (TG/DTA) data: no weight loss was observed until decomposition, which began at about 115 °C, with two exothermic peaks at 148 and 254 °C, and two endothermic peaks at 360 and 465 °C, ultimately leaving CuO as the residue. The maximal weight loss of 87.18% corresponds to the loss of two ligand molecules in the $Cu(L^{IV})_2$ complex (Calc. 86.57%). Prominent IR absorption bands (ν, cm^{-1}): 3360m, br (O–H), 1584s, br and 1483m, sh (C=C arom), 1305m, 1216m, 1163w and 1115s, sh (C–O), 1069w, 1032w and 1003m (C–N), 540m and 522m (Cu–O), 506w (Cu–N). ESR parameters (77 K): $g_{||} = 2.276$, $g_{\perp} = 2.062$, $A_{||} = 128$ Gs. UV–Vis data (acetonitrile) (λ_{max}, nm ($\log\varepsilon$)): 510 (2.55), 340sh (3.47), 305sh (3.61), 278 (3.84), 224 (4.12).

2.4.5. $Cu(L^V)_2$

Molar conductivity (in acetonitrile): Λ_{mol} = 26.9 Ω^{-1} cm^2 mol^{-1}. Thermogravimetric/differential thermal analysis (TG/DTA) data: no weight loss was observed until decomposition which began at about 110 °C, with three exothermic peaks at 146, 174 and 224 °C and three endothermic peaks at 254, 297 and 470 °C, ultimately leaving CuO as the residue. The maximal weight loss of 86.31% corresponds to the loss of two ligand molecules in the $Cu(L^V)_2$ complex (Calc. 87.14%). Prominent IR absorption bands (ν, cm^{-1}): 3350m, br (O–H), 1555m, br and 1469m (C=C arom), 1307m, 1279m, br and 1218m (C–O), 1194m, 1148w and 1029m, sh (C–N), 530m and 515m (Cu–O), 501w (Cu–N). ESR parameters (77 K): $g_{||}$ = 2.280, $g_{\perp}$ = 2.056, $A_{||}$ = 118 Gs. UV–Vis data (acetonitrile) (λ_{max}, nm ($\log\varepsilon$)): 500 (2.71), 405sh (3.01), 340sh (3.28), 277 (3.82), 224 (4.07).

2.4.6. $Cu(L)_2$

For physico-chemical characteristics of this Cu(II) complex with the ligand HL (Fig. 1) see Ref. [11].

2.5. Biological assays

The following test microorganisms (collection of the Department of Microbiology, Belarusian State University) were used: *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Salmonella typhimurium*, *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus saprophyticus*, *Staphylococcus aureu* and *Mycobacterium smegmatis.* The antibacterial (bacteriostatic) activity of the compounds was tested *in vitro* using the twofold serial dilutions in liquid broth method for determination of a minimum inhibitory concentration (MIC) [12,13]. The microorganisms were subcultured for testing in Mueller–Hinton broth (Merck) that contains 300 g of beef infusion, 17.5 g of acid hydrolysate of casein, 1.5 g of starch and 1 l of distilled water (pH 7.4 ± 0.2). The composition of this medium provides favorable conditions for growing bacteria cultures and does not contain any substances that can destroy the ligands synthesized and their metal complexes during testing in the incubation medium, as was shown spectrophotometrically in the preliminary examination. The cells were suspended, according to the McFarland protocol, in saline solution to produce a suspension of about 10^6 CFU/ml (colony-forming units per ml). Because all the compounds tested are insoluble in water, they were dissolved, according to standard recommendations [13,14], in a small volume of an organic solvent (dimethyl sulfoxide or acetonitrile), which is well miscible with the aqueous nutrient medium on dilution and does not react with the compounds under test. This solvent was used for making stock solutions (3.2 mg ml^{-1}) of the test compounds. Stock solutions can be further diluted with broth. Serial dilutions of stock solutions were carried out in test tubes to certain concentrations. The concentrations of the compounds under test, evaluated in Mueller–Hinton broth, ranged from 200 to 3.1 μg ml^{-1}. A specified volume of a 24 h old inoculum was added to each tube. The organic solvent (dimethyl sulfoxide or acetonitrile) at the final concentration (1%) in the nutrient medium did not affect the growth of the test microorganisms. The incubation was carried out at 37 °C for 24 h, and the optical density (OD 600) was determined for bacterial cultures in the presence and absence of the compounds tested. The contents of the test tubes in which the concentration of these compounds was sufficient to suppress the microbial growth remained clear, while their turbidity was evidence for the presence of bacteria. The MIC, defined as the lowest concentration of the test compound which inhibits the visible microbial growth, was determined after an incubation period. The MIC values are given in μg ml^{-1}, according to the CLSI reference [14], as well as in μmol ml^{-1} to reveal a correlation between the antibacterial activity and the reducing ability of the compounds. Tests using dimethyl sulfoxide (or acetonitrile) as a negative control were carried out in parallel. The results were always verified in three separate experiments.

Bovine heart cytochrome *c* (Sigma) was used. Spectrophotometric experiments were performed with a Shimadzu UV-1202 spectrophotometer using a quartz cuvette with a 1 cm optical path. The Cyt *c* concentration was determined by its interaction with excess sodium dithionite, using the absorption coefficient ε_{550} = 21 $mmol^{-1}$ l cm^{-1} [15]. Ar-saturated acetonitrile solutions of the ligands and the Cu(II) complexes under study and Cyt *c* (7 μmol l^{-1}) were used. The experiments were performed in 10 mmol l^{-1} sodium phosphate buffer (pH 7.4) at 20 °C. Aliquots of the compounds under study were added to the Cyt *c* solution up to a final concentration of 35 μmol l^{-1}. The initial rate of Cyt *c* reduction (ν) was evaluated by the slope of the kinetic curve A_{550} vs. time according to Ref. [16]. The characteristic absorption bands at 550 nm, appearing when the ligands or their metal complexes are added to the solution of the oxidized Cyt *c*, bear witness to the ability of the phenolic compounds to reduce Cyt *c in vitro* [17,18]. The results were confirmed in three independent experiments.

3. Results and discussion

3.1. Physico-chemical characterization

Cu(II) complexes with the ligands HL^I–HL^V were synthesized in the amorphous state as judged from the reproducible results of diffuse X-ray diffraction patterns. As described in Section 2.3, repeated attempts to produce crystalline specimens failed. The general formula CuL_2 has been established on the basis of the elemental analysis data for the Cu(II) complexes (Sections 2.3.1–2.3.5).

The Cu(II) complexes are insoluble in water, diethyl ether and chloroform, but soluble in ethanol, acetone, acetonitrile, tetrahydrofuran, dimethyl sulfoxide and dimethylformamide. The values of the molar conductivity in acetonitrile for all these complexes (Λ_{mol} = 22.1–29.9 Ω^{-1} cm^2 mol^{-1}) suggest that the cycloaminomethyl derivatives of the *o*-diphenols HL^I–HL^V are coordinated to the Cu(II) ion as monoanionic species of the ligands, since the above-mentioned data indicate their being essentially non-electrolytes [19].

Thermal analysis in a nitrogen flow, with identification of the final products by X-ray powder diffraction, has shown all the complexes to be anhydrous and unsolvated, because their DTA curves lack any endothermic peaks over the range from 60 to 110 °C. A summary of the thermoanalytical results is given in Sections 2.4.1–2.4.5. The complexes behaved similarly on thermal analysis and showed four stages of decomposition. The agreement between the experimental and theoretical weight losses for the above processes confirms the formulas of the Cu(II) complexes, that is the TG/DTA data are consistent with the results of the elemental analyses.

Because of the amorphous state of the complexes synthesized, we used several spectroscopic methods to specify the coordination modes in the Cu(II) complexes. The IR parameters of the Cu(II) complexes are presented in Sections 2.4.1–2.4.5. In the spectra of HL^I–HL^V (Sections 2.2.1–2.2.5) there is a single band in the range 3409–3375 cm^{-1}, indicating the presence of intermolecular hydrogen bonds involving phenolic hydroxyls [20]. The spectrum of HL^V differs from the other spectra in that it demonstrates a broad band at 3038 cm^{-1}, which corresponds to an intermolecular hydrogen bond involving hydroxyl groups [20,21]. The shift of this band to the 3365–3290 cm^{-1} region in the spectra of Cu(II) complexes suggests that the phenolic hydroxyl groups participate in metal ion

coordination. Besides, the frequencies of the aromatic ring vibrations (1609–1594 and 1497–1489 cm^{-1}) were found to be shifted to 1584–1553 and 1483–1469 cm^{-1} due to the complexation. The change in the band intensity of the C–O stretching vibrations and their shift to the lower frequency region in the spectra of the Cu(II) complexes are also evidence in favor of the ligands HL^{I}–HL^{V} being coordinated to the Cu(II) ions via the oxygen atoms of the phenolic hydroxyl groups. The shift of the bands at 1220–1020 cm^{-1}, assigned to C–N bond vibrations, to the lower frequency region in the spectra of these complexes suggests that the nitrogen atom is involved in the complexation. It should be noted that in the spectra of all these complexes there are bands in the region 540–501 cm^{-1}, which may be assigned to the stretching vibrations of Cu–O and Cu–N bonds [20].

The solid state ESR spectra of all the complexes were recorded both at room temperature and at 77 K, and the ESR parameters are presented in Sections 2.4.1–2.4.5. A representative spectrum for one of the Cu(II) complexes in solid state at 77 K is shown in Fig. 2.

At room temperature the spectra look virtually the same as at liquid nitrogen temperature (77 K), and it is just the signal intensity that is significantly lower. A hyperfine structure is observed for all the complexes. The solid state ESR spectra of all these complexes at 77 K are quite similar and exhibit axially symmetric *g*-tensor parameters, with $g_{||} > g_{\perp} > 2.0023$. The $g_{||}$, $g_{\perp}$ and $A_{||}$ values were calculated from the spectra using the DPPH free radical as the '*g*' marker. The spin Hamiltonian parameters of the complexes do not differ much (Sections 2.4.1–2.4.5), thus their coordination cores are similar. Because of the steric effects produced by bulky groups of the cycloaminomethyl derivatives of the sterically hindered *o*-diphenols, in their Cu(II) complexes there is a significant distortion of their square-planar coordination core. According to [22], $g_{||}$ values less than 2.3 indicate considerably covalent character of M–L bonds, and those greater than 2.3 are indicative of ionic character. The $g_{||}$ values of the Cu(II) complexes were found to be less than 2.3. Applying this criterion, a covalent character of the M–L bonds in the Cu(II) complexes under study can be predicted. The geometric parameter $G = (g_{||} - 2)/(g_{\perp} - 2)$, which is a measure of the exchange interaction between copper centers in solid complexes [23], appears to be greater than 4 for all the compounds: 4.8 (for $Cu(L^{I})_2$), 4.8 (for $Cu(L^{II})_2$), 6.2 (for $Cu(L^{III})_2$), 4.6 (for $Cu(L^{IV})_2$) and 5.2 (for $Cu(L^{V})_2$). The calculated *G* values show that the exchange interaction between the copper centers is negligible [23]. Thus, all the Cu(II) complexes, with the trend $g_{||} > g_{\perp} > g_e$ and a *G* value falling within the above-mentioned range, are consistent with a $d_{x^2-y^2}$ ground state [24–27], and the Cu(II) ion being in a distorted square planar environment formed by *N,O*-coordinating ligands, which agrees with the data obtained by other physico-chemical methods.

The geometry of coordination cores of the Cu(II) complexes with HL^{I}–HL^{V} was determined on the basis of analysis of the electronic absorption spectra. The electronic absorption spectra of the Cu(II) complexes, the main characteristics of which are summarized in Sections 2.3.1–2.3.5, generally include crystal field transitions (*d–d*) and charge transfer transitions involving orbitals of a ligand and the metal (LMCT), as well as intraligand absorptions (ILA) [28]. In the spectra of the Cu(II) complexes there are absorption maxima in the UV region (225–285 nm) which belong to ILA. A band in the region 440–570 nm in the spectra of all the Cu(II) complexes under study may be indicative of the square planar shape of their chromophores $[CuN_2O_2]$ [28,29]. The maxima in the regions 300–370 and 330–440 nm, appearing in the spectra as a result of complexation with the Cu(II) ions, suggest the presence of LMCT transitions: $N(\sigma) \rightarrow Cu^{II}$ and $O_{phen} \rightarrow Cu^{II}$ [28,29].

In the light of the physico-chemical characterization, the general mode of the ligating atoms in the Cu(II) complexes can be represented as shown in Fig. 3.

3.2. Electrochemical studies

On the basis of our previous data, it is safe to assume that the redox properties of the phenolic derivatives under study and their metal complexes can significantly affect their bioactivity. This provided a reason to carry out an electrochemical investigation of the redox properties of the compounds HL^{I}–HL^{V} and their Cu(II) complexes; the experimental data of which are presented in Table 1 and Fig. 4. These ligands belong to *o*-diphenol derivatives and readily undergo electrochemical oxidation reactions. Differing from each other only by the substituent in position 3, these compounds have similar electrochemical properties: the potential values of the peaks are in a narrow range (Table 1 and Fig. 4).

In voltammograms of the compounds HL^{I}–HL^{V} on an anodic potential scan there are two peaks in the ranges 0.37–0.56 and 0.90–1.32 V, with cathodic counterparts on the reverse scan corresponding to reduction of oxidation products in the ranges 0.14–0.32 and 0.71–1.11 V.

Controlled electrolysis of solutions of these compounds demonstrated that at potentials corresponding to the peaks at 0.37–0.56 and 0.90–1.32 V the compounds HL^{I}–HL^{V} undergo successive one-electron oxidation processes to form *o*-benzosemiquinones and *o*-benzoquinones, as confirmed by their absorption spectra (λ_{max} = 380–400 nm). Cathodic peaks in the ranges 0.14–0.32 and 0.71–1.11 V correspond to their reduction on the reverse scan. As we might expect, no reduction of these compounds is observed under cathodic polarization (down to −2.0 V).

Depending on the peculiarities of their redox properties, the Cu(II) complexes of *o*-diphenol derivatives with cycloaminomethyl substituents can be arbitrarily divided into two groups: (i) $Cu(L^{I})_2$ and $Cu(L^{II})_2$; (ii) $Cu(L^{III})_2$–$Cu(L^{V})_2$. $Cu(L^{I})_2$ and $Cu(L^{II})_2$ show a lower reducing ability as compared with that of respective ligands (Table 1).

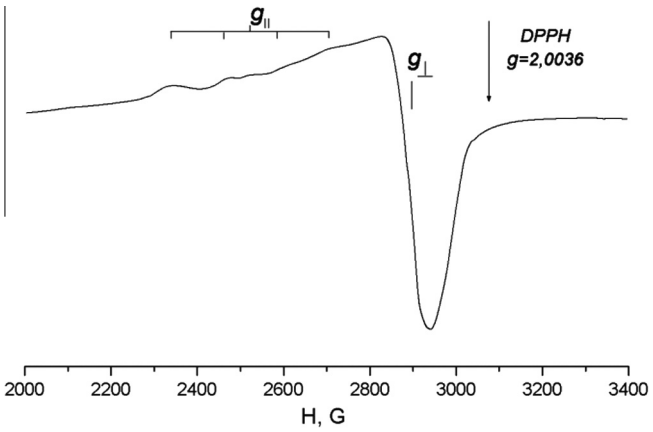

Fig. 2. ESR spectrum of the complex $Cu(L^{V})_2$ in the solid state at 77 K.

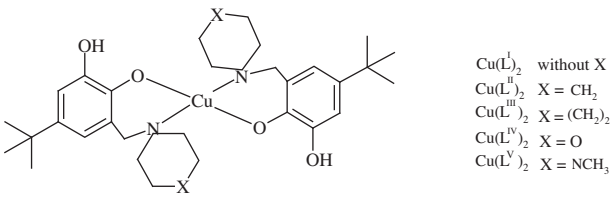

Fig. 3. The plausible coordination mode of the Cu(II) complexes.

Table 1
Cyclic voltammetry data (anodic scan) for HL^{I}–HL^{V} and their Cu(II) complexes.

Compound	Processes under anodic polarization						Processes under cathodic polarization			
	E^1_{pa}, V	E^1_{pc}, V	$E^1_{1/2}$, V*	E^2_{pa}, V	E^2_{pc}, V	$E^2_{1/2}$, V	E^1_{pc}, V	E^1_{pa}, V	E^2_{pc}, V	E^2_{pa}, V
HL^{I}	0.37	0.19	0.28	1.22	0.78	1.00	–	–	–	–
$Cu(L^{I})_2$	1.09	0.13	0.61	1.50	1.12	1.31	−0.71	−0.12	–	–
HL^{II}	0.45	0.24	0.35	1.30	0.71	1.01	–	–	–	–
$Cu(L^{II})_2$	1.20	0.16	0.59	1.55	1.10	1.33	−0.71	−0.14	−1.06	−0.07
HL^{III}	0.43	0.26	0.35	1.32	0.78	1.05	–	–	–	–
$Cu(L^{III})_2$	0.53	0.20	0.37	1.30	1.13	1.22	–	–	−1.55	−0.13
HL^{IV}	0.56	0.32	0.44	1.28	1.08	1.18	–	–	–	–
$Cu(L^{IV})_2$	1.38	0.18	0.78	–	–	–	−0.82	−0.15	−1.83	−0.20
HL^{V}	0.45	0.14	0.30	0.91	0.44	0.68	–	–	–	–
$Cu(L^{V})_2$	1.10	0.17	0.64	1.64**			−0.75	–	−1.23	−0.11
$Cu(L)_2$	1.33	1.11	1.22	–	–	–	−0.27	0.07	–	–

* The formal potential of the redox system $E^1_{1/2}$, used as a criterion of reducing ability (according to [30]), was calculated as the average potential of the peaks found by the cyclic voltammetry method: $E^1_{1/2} = (E_{pa}{}^1 + E_{pc}{}^1)/2$).
** Irreversible process.

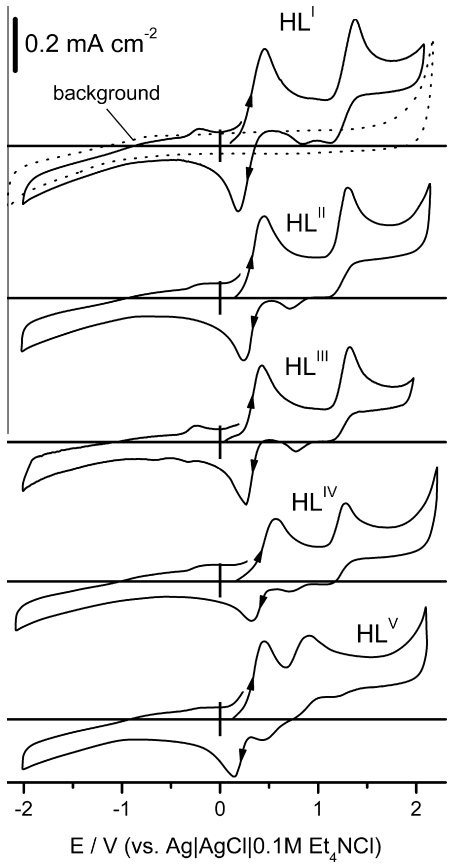

Fig. 4. Cyclic voltammograms (50 mV/s) of HL^{I}–HL^{V} (1.36 mmol l^{-1}) in 0.1 mol l^{-1} $(C_2H_5)_4NClO_4$ acetonitrile solution on glassy-carbon electrode and background cyclic voltammogram of a glassy-carbon electrode (*dotted line*).

On cathodic polarization, beginning from about −0.5 V for $Cu(L^{I})_2$ and $Cu(L^{II})_2$, copper is reduced, its oxidation peak on the reverse scan being observed at about −0.12 V. It should be noted that on cathodic polarization two current waves are characteristic of $Cu(L^{II})_2$, which may be related to the realization of a two-step reduction of Cu(II) to Cu(I) and then to Cu(0). For $Cu(L^{I})_2$ the second reduction step possibly lies beyond the accessible potential range.

In contrast to $Cu(L^{I})_2$ and $Cu(L^{II})_2$, the complexes $Cu(L^{III})_2$, $Cu(L^{IV})_2$ and $Cu(L^{V})_2$ are readily oxidized electrochemically and demonstrate pronounced reducing properties (Table 1). Nevertheless, they are also weaker reductants than their respective ligands. Upon cathodic polarization one (for $Cu(L^{III})_2$) or two (for $Cu(L^{IV})_2$ and $Cu(L^{V})_2$) peaks related to copper reduction are observed in voltammograms. As noted above, they may be related to a two-step Cu(II) reduction. An anodic peak in the potential range −0.10 to −0.20 V on reverse scan corresponds to oxidation of the reduction products of $Cu(L^{III})_2$, $Cu(L^{IV})_2$ and $Cu(L^{V})_2$, which is similar to the behavior of the first group of complexes.

Thus, the electrochemical investigation substantiated the results of the spectroscopic one, according to which the coordination cores of $Cu(L^{I})_2$, $Cu(L^{II})_2$, $Cu(L^{III})_2$, $Cu(L^{IV})_2$ and $Cu(L^{V})_2$ are formed by Cu(II) ions and the ligands in the monoanionic form. Furthermore, it was found that the complexes rank below the ligands in reducing ability, with the $E^1_{1/2}$ values being close both for the phenolic ligands (from 0.59 to 0.78 V) and for the Cu(II) complexes (from 0.28 to 0.44 V), but with $Cu(L^{III})_2$ showing a reducing ability comparable to that of the ligands.

3.3. Biological evaluation

3.3.1. Antibacterial activity

Continuing our previous biological evaluation of sterically hindered *o*-diphenol derivatives and their metal complexes, we have carried out a microbiological investigation of *o*-diphenol derivatives with cycloaminomethyl substituents and their Cu(II) complexes to assess how modifying the composition and structure of the phenolic ligands affects the antibacterial activity of these newly synthesized compounds. MIC values of HL^{I}-HL^{V} and their Cu(II) complexes are listed in Table 2. Commonly used antibiotics (streptomycin, tetracycline and chloramphenicol) were tested as positive controls. These compounds were found to have various antibacterial activities (MIC ~12.5–100 μg ml^{-1}) against the Gram-negative bacteria *Pseudomonas aeruginosa*, *Serratia marcescens*, *Salmonella typhimurium* and *Escherichia coli*. The lowest MIC value (12.5 μg ml^{-1} or 0.021 μmol ml^{-1}) of $Cu(L^{II})_2$ for *Pseudomonas aeruginosa*, is comparable with that of chloramphenicol (12.5 μg ml^{-1} or 0.039 μmol ml^{-1}) and is significantly lower than that of streptomycin (100 μg ml^{-1} or 0.172 μmol ml^{-1}). Gram-positive bacteria (*Staphylococcus saprophiticus*, *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus aureus* and *Mycobacterium smegmatis*) are more sensitive to these compounds than Gram-negative ones (Table 2). On the whole, the Cu(II) complexes are characterized by a comparable inhibiting action against the Gram-positive bacteria tested (Table 2).The lowest MIC value (12.5 μg ml^{-1} or 0.020 μmol ml^{-1}), found for $Cu(L^{III})_2$ and $Cu(L^{V})_2$ against *Mycobacterium smegmatis, Sarcina lutea* and *Staphylococcus aureus*, is comparable respectively to those of chloramphenicol and streptomycin (for *Sarcina lutea*).

Table 2
Antibacterial activity of HL^{I}–HL^{V} and their Cu(II) complexes, evaluated by their minimum inhibitory concentration.*

Compound	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Salmonella typhimurium*	*Escherichia coli*	*Bacillus subtilis*	*Sarcina lutea*	*Staphylococcus saprophyticus*	*Staphylococcus aureus*	*Mycobacterium smegmatis*
HL^{I}	100 (0.401)	100(0.401)	100 (0.401)	100 (0.401)	100 (0.401)	50 (0.201)	50 (0.201)	50 (0.201)	50 (0.201)
$Cu(L^{I})_2$	100 (0.179)	100 (0.179)	100 (0.179)	100 (0.179)	25 (0.045)	25 (0.045)	25 (0.045)	25 (0.045)	25 (0.045)
HL^{II}	100 (0.380)	100 (0.380)	100 (0.380)	100 (0.380)	100 (0.380)	100 (0.380)	100 (0.380)	50 (0.190)	100 (0.380)
$Cu(L^{II})_2$	12.5 (0.021)	100 (0.170)	50 (0.085)	100 (0.170)	25 (0.043)	25 (0.043)	25 (0.043)	25 (0.043)	25 (0.043)
HL^{III}	100 (0.363)	100 (0.363)	100 (0.363)	100 (0.363)	100 (0.363)	100 (0.363)	100 (0.363)	100 (0.363)	100 (0.363)
$Cu(L^{III})_2$	100 (0.163)	100 (0.163)	100 (0.163)	100 (0.163)	25 (0.041)	12.5 (0.020)	25 (0.041)	12.5 (0.020)	12.5 (0.020)
HL^{IV}	200 (0.754)	200 (0.754)	200 (0.754)	200 (0.754)	200 (0.754)	200 (0.754)	200 (0.754)	200 (0.754)	200 (0.754)
$Cu(L^{IV})_2$	25 (0.042)	100 (0.169)	100 (0.169)	25 (0.042)	50 (0.084)	50 (0.084)	50 (0.084)	50 (0.084)	50 (0.084)
HL^{V}	100 (0.359)	100 (0.359)	100 (0.359)	100 (0.359)	100 (0.359)	100 (0.359)	100 (0.359)	100 (0.359)	100 (0.359)
$Cu(L^{V})_2$	100 (0.162)	100 (0.162)	100 (0.162)	100 (0.162)	50 (0.081)	12.5 (0.020)	25 (0.041)	50 (0.082)	12.5 (0.020)
$Cu(L)_2$	100 (0.337)	100 (0.337)	100 (0.337)	100 (0.337)	25 (0.169)	12.5 (0.084)	12.5 (0.084)	25 (0.169)	12.5 (0.084)
Streptomycin	>100 (0.172)	6.2 (0.011)	12.5 (0.021)	3.1 (0.005)	6.2 (0.011)	12.5 (0.021)	6.2 (0.011)	6.2 (0.011)	3.1 (0.005)
Tetracycline	–	–	6.2 (0.014)	3.1 (0.007)	6.2 (0.014)	6.2 (0.014)	6.2 (0.014)	3.1 (0.007)	–
Chloramphenicol	12.5 (0.039)	–	6.2 (0.019)	6.2 (0.019)	3.1 (0.009)	–	6.2 (0.019)	6.2 (0.019)	12.5 (0.039)

* MIC values are given in μg ml^{-1} (in parentheses – MIC, μmol ml^{-1}).

Table 3
Octanol/water partition coefficients ($\log P_{ow}$) of HL^{I}–HL^{V} and their Cu(II) complexes.

Compound	$\log P_{ow}$	Compound	$\log P_{ow}$
HL^{I}	2.00 ± 0.14	HL^{IV}	1.65 ± 0.16
$Cu(L^{I})_2$	2.40 ± 0.11	$Cu(L^{IV})_2$	2.76 ± 0.10
HL^{II}	2.61 ± 0.10	HL^{V}	1.15 ± 0.18
$Cu(L^{II})_2$	3.10 ± 0.09	$Cu(L^{V})_2$	2.15 ± 0.13
HL^{III}	3.58 ± 0.08	HL	1.82 ± 0.15
$Cu(L^{III})_2$	4.40 ± 0.07	$Cu(L)_2$	2.30 ± 0.12

Table 4
Rates of reduction of Cyt *c* (v) with HL^{I}–HL^{V} and their Cu(II) complexes.

Compound	v^* (nmol min^{-1})	Compound	v (nmol min^{-1})
HL^{I}	6.5	$Cu(L^{III})_2$	2.3
$Cu(L^{I})_2$	1.1	HL^{IV}	3.3
HL^{II}	3.3	$Cu(L^{IV})_2$	0.9
$Cu(L^{II})_2$	1.0	HL^{V}	3.5
HL^{III}	3.5	$Cu(L^{V})_2$	1.1

* The absolute error does not exceed 0.1 nmol min^{-1}.

Microbiological tests show that the majority of the Cu(II) complexes are more active than the respective *o*-diphenol derivatives with cycloaminomethyl substituents, being another testimony to complexation being able to enhance the action of bioactive organic compounds [7]. These effects can be due to the higher lipophilicity of the complexes (Table 3). Changing the lipophilicity is likely to result in bringing down the solubility and permeability of cell barriers, which in turn enhances the bioavailability of biocides.

It has been revealed that the starting Cu(II) acetate does not act against bacteria, up to a dose of 200 μg ml^{-1}, that is the antibacterial activity of the complexes synthesized does not correlate with the toxicity of the Cu(II) ions to the bacteria.

The antibacterial activity of the novel *o*-diphenol derivatives with cycloaminomethyl substituents and their Cu(II) complexes may be characterized as follows: (i) HL^{I}–HL^{V} show virtually the same low inhibiting action; (ii) the majority of the Cu(II) complexes also almost do not differ in their bioactivity, but the complex $Cu(L^{III})_2$, with the highest reducing ability and the highest lipophilicity among these complexes, is the most active one against bacteria (Tables 2 and 3). This result could be expected considering our previous data [1–4] about the correlation between redox properties of *o*-diphenol derivatives and their antibacterial activity. The MIC (μmol ml^{-1}) values of the compounds were found to follow the order (Table 2): (i) $HL^{I} > HL^{V} \geqslant HL^{III} \sim HL^{II} > HL^{IV}$; (ii) $Cu(L^{III})_2 > Cu(L^{II})_2 \sim Cu(L^{I})_2 \sim Cu(L^{V})_2 > Cu(L^{IV})_2$; their reducing ability (determined electrochemically) followed the same order (Table 1). Thus, introduction of cycloaminomethyl substituents into the molecule of the sterically hindered *o*-diphenol HL resulted in the level of antibacterial activity (see MIC, μmol ml^{-1}) becoming slightly higher as compared to that of the complex $Cu(L)_2$ with 4-*tert*-butylcatechol HL (Fig. 1, Table 2). This change in activity may be related to the fact that the ligand modification had a pronounced effect on some physico-chemical characteristics of the novel Cu(II) complexes: their reducing ability increased (Table 1) and the lipophilicity of the most active complexes, $Cu(L^{III})_2$ and $Cu(L^{II})_2$, also became higher (Table 3).

3.3.2. Reduction of cytochrome c

The results of the spectrophotometric investigation of the redox interaction of the oxidized form of Cyt *c* with HL^{I}–HL^{V} and their Cu(II) complexes are given in Table 4. As in our previous publications [1–4], when discussing experimental evidence, we take into account the findings presented in [17,18,31] as well as the results of the electrochemical investigation (see Section 3.2), which allow us to suggest a possible route of oxidation for the compounds under study: different phenolic derivatives transfer electrons in an outer-sphere process involving the exposed heme edge surrounded by positively charged amino acid residues; aromatic redox active amino acid residues also can be potential participants of the electron transfer. Oxidation of *o*-diphenol derivatives with cycloaminomethyl substituents *in vitro* under anaerobic conditions can include two successive one-electron steps of oxidation of their ionic forms to yield *o*-benzoquinones on interaction with Cyt *c* via intermediate *o*-benzosemiquinone formation (Fig. 5).

It was found that the rate of Cyt *c* reduction with HL^{II}–HL^{V} is lower as compared to compound HL^{I} (Table 4), which may be due to its higher reducing ability, determined electrochemically (Table 1). Comparable rates of Cyt *c* reduction with HL^{II}–HL^{V} can be due first of all to similar reducing abilities of these compounds: $E^{1}_{1/2}$ is found to be in the range 0.3–0.4 V (Table 1).

All the Cu(II) complexes reduce Cyt *c* at a rate several times lower than that of the redox process involving HL^{I}–HL^{V} (Table 4). Among the Cu(II) complexes it is the complex $Cu(L^{III})_2$ that shows the highest rate of Cyt *c* reduction. According to the electrochemical data, this complex is the most active reducing agent in the series of Cu(II) complexes (Table 1). It is known [17] that metal complexes can interact with Cyt *c* both in their molecular form and via the phenolate ligand, formed upon their dissociation. The rate of Cyt *c* reduction with the rest of the Cu(II) complexes varies in a very narrow range (0.9–1.1 nmol min^{-1}) and correlates with their reducing ability determined electrochemically, which also is characterized by a narrow range of $E^{1}_{1/2}$ values (0.60–0.78 V). Furthermore, the comparable antibacterial activity of these Cu(II)

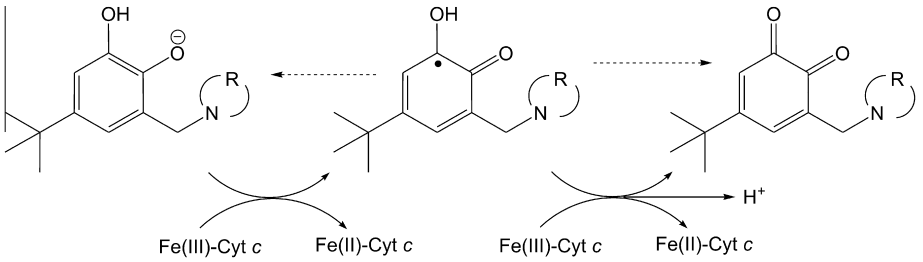

Fig. 5. Scheme of the reduction of Cyt *c* with the anion form of the phenolic ligands; Fe(III)-Cyt *c* and Fe(II)-Cyt *c* are respectively the oxidized and reduced forms of Cyt *c* (disproportionation of the ligand is depicted by a dashed line) [17].

complexes should be noted. In summary, the sequences characterizing the decrease of the rates of Cyt *c* reduction with the ligands and their Cu(II) complexes are the same as those characterizing their antibacterial activity and reducing ability:

(1) $HL^{I} > HL^{V} \sim HL^{III} \geqslant HL^{II} \sim HL^{IV}$; (2) $Cu(L^{III})_2 > Cu(L^{II})_2 \sim Cu(L^{I})_2 \sim Cu(L^{V})_2 \geqslant Cu(L^{IV})_2$.

4. Conclusions

Novel redox-active Cu(II) complexes with cycloaminomethyl derivatives of *o*-diphenols were synthesized and isolated in the amorphous state. These complexes were characterized by means of analytical, thermochemical, spectral and electrochemical methods. According to the data obtained, the ligands coordinate in their monoanionic forms in an *O,N*-bidentate fashion. The Cu(II) complexes are characterized by a distorted square planar geometry of their CuO_2N_2 coordination cores. The cycloaminomethyl derivatives of *o*-diphenols and their Cu(II) complexes have been screened for their antimicrobial activity against different species of pathogenic bacteria. All the Cu(II) complexes are more lipophilic and more bioactive than the respective ligands. The complexes were found to have a low inhibition activity against Gram-negative bacteria, while Gram-positive ones are more sensitive to these compounds. The lowest MIC value (12.5 μg ml^{-1} or 0.020 μmol ml^{-1}), found for $Cu(L^{III})_2$ against *Mycobacterium smegmatis*, *Sarcina lutea* and *Staphylococcus aureus*, is comparable respectively to those of chloramphenicol (12.5 μg ml^{-1} or 0.039 μmol ml^{-1}) and streptomycin for *Sarcina lutea* (12.5 μg ml^{-1} or 0.021 μmol ml^{-1}). The antibacterial activity of the compounds was found to follow the order: (1) $HL^{I} > HL^{V} \geqslant HL^{III} \sim HL^{II} > HL^{IV}$; (2) $Cu(L^{III})_2 > Cu(L^{II})_2 \sim Cu(L^{I})_2 \sim Cu(L^{V})_2 > Cu(L^{IV})_2$; their reducing ability (determined electrochemically) followed the same order. Moreover, the most bioactive complex, $Cu(L^{III})_2$, has the highest lipophilicity. It is the ligand HL^{I} and the complex $Cu(L^{III})_2$ with the highest reducing ability (determined electrochemically) that are characterized by the highest Cyt *c* reduction rate respectively among the ligands and Cu(II) complexes. Correspondingly the rate of Cyt *c* reduction for the rest of the Cu(II) complexes as well as their antibacterial activity, lipophilicity and reducing ability (determined electrochemically) vary in a very narrow range.

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