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Copper (II) complexes of sterically hindered *o*-diphenol derivatives: synthesis, characterization and microbiological studies

Natalia V. Loginova[1]*, Tat'yana V. Koval'chuk[1], Rimma A. Zheldakova[2], Anna A. Chernyavskaya[1], Nikolai P. Osipovich[3], Gennady K. Glushonok[3], Henry I. Polozov[1], Victor L. Sorokin[1], Oleg I. Shadyro[1]

[1] *Faculty of Chemistry, Belarusian State University, Minsk 220050, Belarus*

[2] *Department of Microbiology, Belarusian State University, Minsk 220064, Belarus*

[3] *Research Institute for Physico-Chemical Problems, Belarusian State University, Minsk 220050, Belarus*

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Abstract: Cu (II) complexes with the sterically hindered diphenol derivatives 3,5-di(*tert*-butyl)-1,2-benzenediol (**I**), 4,6-di(*tert*-butyl)-1,2,3-benzenetriol (**II**) and the sulfur-containing 4,6-di(*tert*-butyl)-3-(2-hydroxyethylsulfanyl)-1,2-benzenediol (**III**) and 2-[4,6-di(*tert*-butyl)-2,3-dihydroxyphenylsulfanyl]acetic acid (**IV**) have been synthesized and characterized by elemental analysis, TG/DTA, FT-IR, ESR, XPS, XPD and conductivity measurements. Compounds **I**–**III** can coordinate in their singly deprotonated forms and act as bidentate ligands. These compounds yield Cu (II) complexes of the stoichiometry $Cu(L)_2$, which have square planar geometry ($g_{\parallel} > g_{\perp} > g_e$). Unlike them, compound **IV** behaves as a terdentate ligand, and its complex $Cu(L^{IV})_2$ has distorted octahedral geometry. According to ESR data, only the $Cu(L^{II})_2$ complex contains a very small amount of phenoxyl radicals. Antimicrobial activities of these ligands and their respective Cu (II) complexes have been determined with respect to Gram-positive and Gram-negative bacteria, as well as on yeasts. Their phytotoxic properties against *Chlorella vulgaris 157* were also examined.

Keywords: Copper (II) complexes, sterically hindered o-diphenols, spectroscopic study, antimicrobial activities, phytotoxic properties

1 Introduction

The synthesis and characterization of metal complexes with bioactive organic ligands to produce novel potential chemotherapeutic agents is rapidly developing. Of particular note is the pressing need for new antibacterials to replace those losing their effectiveness because of the fast development of microorganisms' resistance [1]. Thus, the discovery of new antimicrobial compounds or increasing the effectiveness of previously known drugs is important.

Derivatives of sterically hindered *o*-diphenols (SHD) have been found to be universal inhibitors of free-radical processes, retarding both the oxidation and the free-radical fragmentation reactions of important biomolecules (lipids, peptides, carbohydrates, vitamins etc.) [2–4]. Moreover, a sulfur-containing SHD derivative exhibits a high antiviral, neurotropic and nootropic activity [5–7], and many phenolic compounds have antibacterial activities [8–10]. In the presence of Cu (II) some phenolic compounds damage the cytoplasmic membrane, which is related to their bactericidal activity [11]. These data provide a good basis for attempts to use SHD as ligands in the syntheses of new bioactive metal complexes.

Copper's biomedical importance has been determined by studies of the chemistry of its bioactive complexes its pharmacologicy [12–29]. We have earlier reported the synthesis of an Ag (I) complex with one of the ligands, 2-[4,6-di(*tert*-butyl)-2,3-dihydroxyphenylsulfanyl]acetic acid. Since Ag (I) complexation leads to enhanced antimicrobial activity of this ligand [30], we were motivated to explore whether Cu (II) complexes of the SHD derivatives behave similarly.

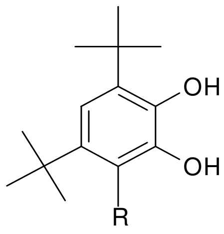
Ready substitution by an *S*-containing bioligand may lead to a broad spectrum of antimicrobial activity for some transition metal complexes with *O*-coordinated ligands [31, 32]. *S*-containing protein residues are potential target sites for the inhibition of bacterial and yeast growth by these metal complexes. Thus, the structure – antimicrobial activity correlation of Cu (II) complexes with O,O- and O,S-coordinated ligands is interesting.

Recently we have studied the interaction of a series of SHD derivatives with Cu (II), Zn (II), Ni (II) and Co (II) ions in aqueous ethanol solutions [33, 34]. Potentiometric investigation of the equilibria in systems containing these ions and SHD derivatives allowed us to recognize the complexes and to determine their composition and stability. On the basis of these data, procedures for synthesis and separation of individual solid metal complexes were developed.

As a part of our ongoing study of these metal complexes, we report herein the synthesis, characterization and antimicrobial activities of Cu (II) complexes with 3,5-di(*tert*-butyl)-1,2-benzenediol (**I**), 4,6-di(*tert*-butyl)-1,2,3-benzenetriol (**II**) and the sulfur-containing SHD derivatives 4,6-di(*tert*-butyl)-3-(2-hydroxyethylsulfanyl)-1,2-benzenediol (**III**), 2-[4,6-di(*tert*-butyl)-2,3-dihydroxyphenylsulfanyl]acetic acid (**IV**):

The solid Cu (II) complexes were characterized by means of elemental analysis, TG/DTA, FT-IR, ESR, and XPS. However, a full structural analysis could not be performed because no single crystals suitable for X-ray diffraction were obtained. Earlier

* E-mail: loginonv@bsu.by



I – R=H

II – R=OH

III – R=SCH_2CH_2OH

IV – R=SCH_2COOH

investigation showed that most of the metal complexes formed with sterically hindered diphenol and aminophenol derivatives containing one or two *tert*-butyl groups were hard to crystallize [30, 32–34]. Therefore, our determination of the coordination modes of the Cu (II) complexes was based on other physicochemical methods.

We also estimated the antimicrobial activities and phytotoxic properties of the Cu (II) complexes in comparison with those of the free ligands.

2 Experimental

2.1 Materials and methods

Chemicals were purchased from commercial sources and were used without further purification. The SHD derivatives were prepared according to literature methods [5, 7, 36–39]. The preparation of Cu (II) complexes is described below.

Infrared spectra of solids (ligands **I**–**IV** and their Cu (II) complexes) were recorded using a Spectrum 1000 spectrophotometer over the range 4000–400 cm^{-1} at room temperature, using Nujol mulls and polyethylene windows.

Thermal analysis was performed with a Derivatograph OD-103 MOM. TG/DTA measurements were in air between 20 and 450°C at 5 °C min^{-1}.

Elemental analyses were carried out according to standard methods by the Microanalytical Laboratory, Bioorganic Chemistry Institute, National Academy of Sciences, Belarus. Copper and sulfur determinations were carried out using an atomic emission spectrometer with an inductively coupled plasma excitation source (Spectroflame Modula). A Cu (II) complex sample was decomposed by treatment with HNO_3+H_2O_2 using a Milestone MLS 1200 Mega microwave digestion system. After the complex was decomposed, the contents of metal and sulfur in the resulting solution were determined.

XRD studies were made with an HZG 4A diffractometer ($Co_{K\alpha}$ radiation, MnO_2 filter). XPS studies were performed with an ES-2401 spectrometer. Sample surface etching with Ar^+ was employed. Core level energies were calibrated using the C 1s line with E_B=284.6 eV.

ESR spectra of polycrystalline samples were measured on an ERS-220 X-band spectrometer (9.45 GHz) at room temperature and at 77 K, using 100-kHz field modulation; g factors were determined relative to the standard marker DPPH (g=2.0036).

The molar conductance of 10^{-3} M solutions of the Cu (II) complexes in acetonitrile was measured at 20 °C using a TESLA BMS91 conductometer (cell constant 1.0).

2.2 Synthesis of the Cu (II) complexes with sterically hindered diphenol derivatives

Based on our previous findings [30, 31], preferential formation of the Cu (II):L=1:2 complex was achieved by adding a solution of Cu (II) salt in small portions to the ligand solution under continuous stirring, so that the complexation always took place with excess ligand present. The preparations followed a common procedure. A solution of 0.050 mmol $Cu(CH_3COO)_2 \cdot H_2O$ in 10 ml of water was added dropwise to a colorless solution of 0.100 mmol of **I**–**IV** dissolved in 10 ml of ethanol (molar ratio Cu (II):L=1:2). The reaction vessel was shielded from light and argon was bubbled through the solutions (pH≤6) during the synthesis to ensure the absence of oxygen and prevent the formation of *o*-semiquinones [35, 40]). Colored precipitates of Cu (II) complexes formed instantaneously. After 1.5 h stirring, they were collected on membrane filters (JG 0.2 μm), washed with ethanol and water, and dried *in vacuo* (yield>70 %).

Cu(L^I)$_2$. Dark blue. *Anal.* Calc. for $C_{28}H_{42}O_4Cu$: C, 66.50; H, 8.30; Cu, 12.55. Found: C, 66.47; H, 8.28; Cu, 12.49.

Cu(L^{II})$_2$. Cherry-brown. *Anal.* Calc. for $C_{28}H_{42}O_6Cu$: C, 62.54; H, 7.81; Cu, 11.80. Found: C, 62.51; H, 7.78; Cu, 11.76.

Cu(L^{III})$_2$. Brown. *Anal.* Calc. for $C_{32}H_{50}S_2O_6Cu$: C, 58.42; H, 7.59; S, 9.75; Cu, 9.64. Found: C, 58.40; H, 7.51;S, 9.72; Cu, 9.61.

Cu(L^{IV})$_2$. Green-brown. *Anal.* Calc. for $C_{32}H_{46}S_2O_8Cu$: C, 56.03; H, 6.71; S, 9.36; Cu, 9.25. Found: C, 55.98; H, 6.68; S, 9.34; Cu, 9.22.

2.3 Antimicrobial activities

Antimicrobial activities of the compounds were estimated by determining the minimum inhibitory concentration (MIC, μg/ml) as described elsewhere [41]. The following test microorganisms (collection of Department of Microbiology, Belarusian State University) were used: *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacilus subtilis*, *Sarcina lutea*, *Staphylococcus saprophyticus. Pichia pastoris, Lypomyces lipofer, Saccharomyces cerevisiae, Cryptococcus laurentiive, Candida utilis, Candida boidinii.*

Twofold serial dilutions in dimethyl sulfoxide (DMSO) from 250 to 8 μg·mL^{-1} were used. Absence of microbial growth after an incubation period of 24 h at 37 °C for bacteria or of 48 h at 30 °C for yeasts was the criterion of effectiveness. The MIC was the lowest concentration of the compound which inhibits visible microbial growth, when compared with the control system in which the microorganisms were grown in the absence of any test compound. The amount of DMSO in the medium was 1% and did not affect the growth of the microorganisms. There were three replicates for each dilution. Results were always verified in three separate experiments.

2.4 Phytotoxic properties

The phytotoxic properties of the free ligands **I–IV** and their Cu (II) complexes against *Chlorella vulgaris 157* were tested as reported elsewhere [42].

3 Results and discussion

All the complexes yielded X-ray patterns which differed significantly from that of the ligands. The elemental analyses for the complexes of Cu (II) with **I–IV** are in agreement with the formula $Cu(L)_2$. The substances produced are individual compounds as judged from the reproducible elemental analyses and X-ray powder diffraction patterns.

The low solubility of most of these complexes in common organic solvents hinders their study in solution. All the complexes were insoluble in water, ethanol, diethyl ether, nitromethane and chloroform, and only $Cu(L^{I})_2$ and $Cu(L^{III})_2$ were soluble in acetonitrile. The low solubility of $Cu(L^{II})_2$ and $Cu(L^{IV})_2$ complexes in acetonitrile prevents their molar conductivities from being measured. The low values of the molar conductivity in acetonitrile for the complexes $Cu(L^{I})_2$ (Λ_{mol}=8.9 $\Omega^{-1}cm^2mol^{-1}$) and $Cu(L^{III})_2$ (Λ_{mol}=4.5 $\Omega^{-1}cm^2mol^{-1}$) indicate their being essentially non-electrolytes in this solvent [43]. Thus, the conductivity data suggest that the bidentate ligands, in particular **I** and **III**, may be coordinated to the Cu (II) ion as singly charged anions.

3.1 Thermal studies

Thermal analyses in flowing air followed by identification of the final products by X-ray powder diffraction has shown all complexes to be anhydrous and unsolvated, because their DTA curves lack any endothermic peaks over the range from 60 to 150 °C. A summary of the results is given in Table 1.

Table 1 TG/DTA data for decomposition of Cu (II) complexes.

Complex	Temperature (°C)	Process	% weight loss Found	Calc.
$Cu(L^{I})_2$	160–390	Exothermic	83.42	84.27
$Cu(L^{II})_2$	180–310	Exothermic	84.13	85.21
$Cu(L^{III})_2$	168–390	Exothermic	87.06	87.91
$Cu(L^{IV})_2$	190–410	Exothermic	87.58	88.41

These complexes undergo decomposition within the range 160–410 °C. Most of the complexes behaved very similarly on thermal analysis and showed two stages of decomposition, except for $Cu(L^{IV})_2$ (three stages). In the two-stage decomposition the first stage occurred in the temperature range of 200–280 °C, presumably due to a loss of ligand fragments [44]. The second stage occurred over the range 270–390 °C and continued until the complete pyrolysis of the organic ligands, ultimately leaving copper oxide. These two stages correspond to the maximal weight losses. In $Cu(L^{IV})_2$ the third stage (370–410 °C) corresponds to a small weight loss and is most likely due to decarboxylation of coordinated (deprotonated) carbonate groups [45]. Thus, the final pyrolysis was completed at 310, 390 and 410 °C respectively, giving CuO powder. Taking Cu $(L^I)_2$ as an example, the TGA curve shows that the maximum two-stage weight loss of 83.42 % from 160 to 390 °C corresponds to the loss of two ligand molecules from the Cu $(L^I)_2$ complex (Calc. 84.27 %): $Cu(L)_2 \rightarrow CuO$.

The agreement between the experimental and theoretical weight losses for the above processes confirms the formulas of the Cu (II) complexes; that is, TG/DTA data are consistent with the results of elemental analyses.

3.2 Infrared spectra

To specify the coordination modes in the Cu (II) complexes we used IR spectroscopy. In the spectrum of **I** there are two broad bands at 3461 and 3264 cm^{-1}, which indicate the presence of intermolecular hydrogen bonds involving hydroxyls [46]. A single narrow band at 3484 cm^{-1} present in the spectrum of the complex Cu $(L^I)_2$ confirms that the ligand is not fully deprotonated; it is believed that ligand **I** coordinates as a bidentate ligand in the anionic form HL^- after single deprotonation. The frequency of the aromatic ring mode (1595 cm^{-1}) shifted to 1577 cm^{-1} due to complexation. The absence of an intense C–O stretch at 1158 cm^{-1} in the spectrum of Cu $(L^I)_2$, and the presence of three bands at 1230–1140 cm^{-1} also confirm that hydroxyls are involved in **I** coordination with Cu (II).

The spectrum of **II** differs from that of $Cu(L^{II})_2$ by the presence of two narrow bands at 3540 and 3440 cm^{-1}, which correspond to the intermolecular hydrogen bond involving hydroxyls [46]. In the spectrum of $Cu(L^{II})_2$ there is only one broad band at 3387 cm^{-1} in that region which suggests that **II** coordinates in its singly deprotonated form in an O,O-bidentate fashion. The two C–O stretch bands at 1200–1097 cm^{-1} in the spectrum of **II** are shifted toward lower frequencies in the spectrum of $Cu(L^{II})_2$, indicating Cu (II) coordinated to hydroxyls of **II**. The strong band at 1463 cm^{-1} in the $Cu(L^{II})_2$ spectrum may be due to the radical-anionic ligand form [47] (see below).

There are strong bands at 3536 and 3339 cm^{-1} in the spectrum of ligand **III,** while in that of $Cu(L^{III})_2$ a band at 3399 cm^{-1} is present, characteristic of the bidentate ligand **III** bound to Cu (II) in the anionic HL^- form. The shift of the C–S bands at 840–815 cm^{-1} toward lower wavenumbers in the spectrum of this complex suggests that the sulfur is involved in the complexation.

The spectrum of **IV** differs from that of $Cu(L^{IV})_2$ by the presence of three bands at 3545, 3447 and 3449 cm^{-1}, which correspond to the intermolecular hydrogen bond of hydroxyl. In the spectrum of $Cu(L^{IV})_2$ only one broad band at 3377 cm^{-1} is present. In the spectrum of **IV** the C=O stretch is observed at 1690 cm^{-1}. It is absent in the spectrum of $Cu(L^{IV})_2$, but new bands at 1550 and 1600 cm^{-1} appear, characteristic of $–COO^-$ [46]. The shift of the carboxyl stretch indicates that the hydrogen ions in two coordinated ligand molecules have been substituted by Cu (II). Cu (II) may be bound to the sulfur atom of the ligand as well. In contrast to the spectrum of **IV** where a strong narrow C–S stretch is located at 1030 cm^{-1}, the corresponding band for $Cu(L^{IV})_2$ is much weaker and shifted to 1000 cm^{-1}.

It should be noted that in the spectra of all the complexes under study there are Cu–O stretching bands in the region of 470–580 cm^{-1}. As Cu–S stretching usually occurs below 400 cm^{-1}, they could not be observed with the instrument employed.

3.3 ESR spectra

The ESR parameters of the Cu (II) complexes are presented in Table 2. The ESR spectra of complexes $Cu(L^{I})_2$ and $Cu(L^{II})_2$ at room temperature are not observed, but all the complexes show spectra at 77 K. The spectra of complexes $Cu(L^{III})_2$ and $Cu(L^{IV})_2$ are virtually identical at room temperature and at 77 K.

The ESR spectra of polycrystalline (77 K) $Cu(L^{I})_2$, $Cu(L^{II})_2$ and $Cu(L^{III})_2$ are typical axial spectra (Fig. 1) with $g_{\parallel}$ and $g_{\perp}$ features at values about 2.28-2.30 and 2.03-2.04. The variations in the $g_{\parallel}$ and $g_{\perp}$ values indicate that the geometry of the compounds in the solid state is affected by the coordinating counter ions.

Table 2 ESR parameters of Cu (II) complexes.

Complex	$A_{\parallel}$	$g_{\parallel}$	$g_{\perp}$	$g(R)$	g_1	g_2	g_3	G
$Cu(L^{I})_2$	178	2.285	2.0278	-	-	-	-	11.09
$Cu(L^{II})_2$	171	2,278	2.0331	2.0043	-	-	-	8.95
$Cu(L^{III})_2$	145	2.297	2.0407	-	-	-	-	7.67
$Cu(L^{IV})_2$	-	-	-	-	2.230	2.111	2.0228	-

The geometric parameter G, which is a measure of the exchange interaction between copper (II) centers, is calculated using the equation $G=(g_{\parallel}-2.0023)/(g_{\perp}-2.0023)$ [48]. If $G>4$, exchange interaction is negligible, whereas a considerable exchange interaction occurs for $G<4$. G for the complexes $Cu(L^{I})_2$, $Cu(L^{II})_2$ and $Cu(L^{III})_2$ appears to be greater than 4, which shows that there are no magnetic exchange interactions between the copper (II) centers [49]. The spectra of these three complexes are quite similar and exhibit an axially symmetric g-tensor with $g_{\parallel} > g_{\perp} > 2.0023$. These data indicate that the copper site has a $d_{x^2-y^2}$ ground state characteristic of square planar geometry [50, 51]. This structure agrees with the elemental analyses.

The ESR spectrum of $Cu(L^{IV})_2$ shows apparent rhombic symmetry ($g_1 = 2.230$; $g_2 = 2.111$; $g_3 = 2.0228$), which indicates partial mixing of the d_z^2 orbital with the $d_{x^2-y^2}$ orbital [52]. Although it is impossible to obtain accurate molecular values from polycrystalline ESR spectra, it is possible to distinguish unambiguously between $d_{x^2-y^2}$ and d_z^2 as ground states. Thus, the observed g-values ($g_{\parallel}>g_{\perp}>0$) and the value of $R=(g_2-g_1)/(g_3-g_2)$ of 0.74 suggest a predominantly $d_{x^2-y^2}$ ground state [49].

According to [53], $g_{\|}$ values less than 2.3 indicate considerable covalent character in the M–L bonds, and values greater than 2.3 indicate ionic bonding. The $g_{\|}$ values of the Cu (II) complexes were found to be less than 2.3, suggesting considerable covalent character in the M–L bonds. The smaller $A_{\|}$ value for Cu(L^{III})$_2$ has been attributed to the high covalency of the Cu–S bonding [54].

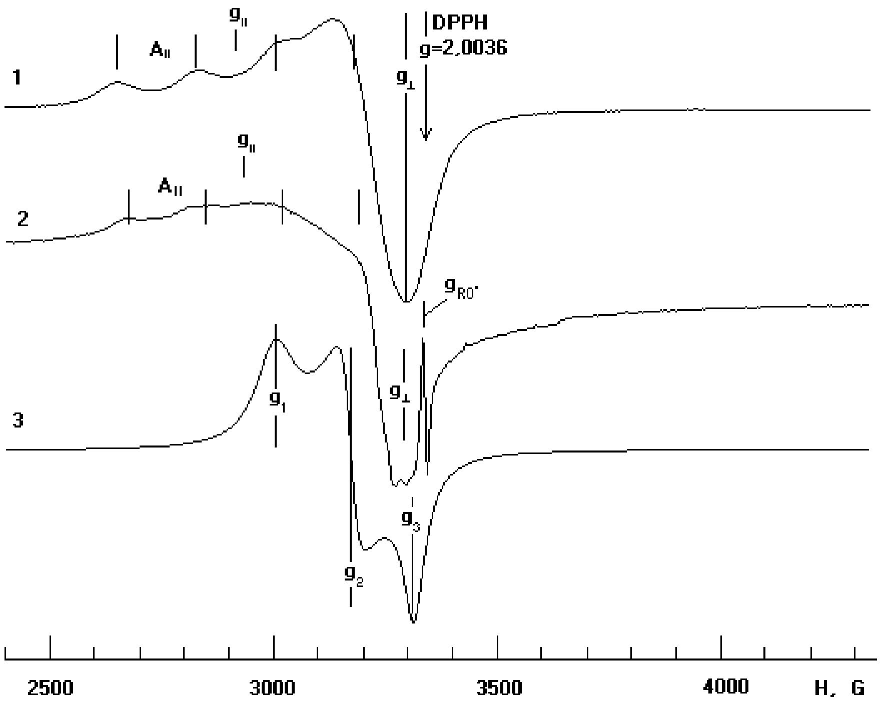


Fig. 1 ESR spectra of polycrystalline Cu(L^{I})$_2$ (1), Cu(L^{II})$_2$ (2) and Cu(L^{IV})$_2$ (3) at 77 K.

It should be noted that the ESR spectrum of Cu(L^{II})$_2$ differs from those of Cu(L^{I})$_2$, Cu(L^{III})$_2$ and Cu(L^{IV})$_2$ by the presence of a narrow singlet with g near 2 (Table 2). On the basis of g values (respectively 2.0043 and 2.0045), it may be suggested that they belong to phenoxyl radicals. In the free crystalline ligands no radicals are detected. The concentration of phenoxyl radicals in Cu(L^{II})$_2$ is about 4-8·10^{15} g^{-1}. They may form in solution during the synthesis and become incorporated into the complexes when the latter are precipitated. Our previous electrochemical study [55] showed that phenoxyl radicals could be formed because ligand **II** has a significantly higher reducing power than the others. The absence of stabilized radicals in Cu(L^{I})$_2$, Cu(L^{III})$_2$ and Cu(L^{IV})$_2$ suggests that redox reactions during complexation depend on the ligand and reaction conditions [40, 56].

3.4 X-ray photoelectron spectra

Because Cu(L^{I})$_2$ and Cu(L^{II})$_2$ decompose under the experimental conditions, their spectra could not be obtained.

In $Cu(L^{III})_2$ the Cu 2p spectrum is spin split into two peaks at 934.7 and 933.3 eV (Fig. 2e). In Cu (II) compounds Cu 2p energy is 934–935.5 eV; for Cu (I) it is 932.1 eV [57]. The higher binding energies observed suggest that the copper atoms are doubly charged.

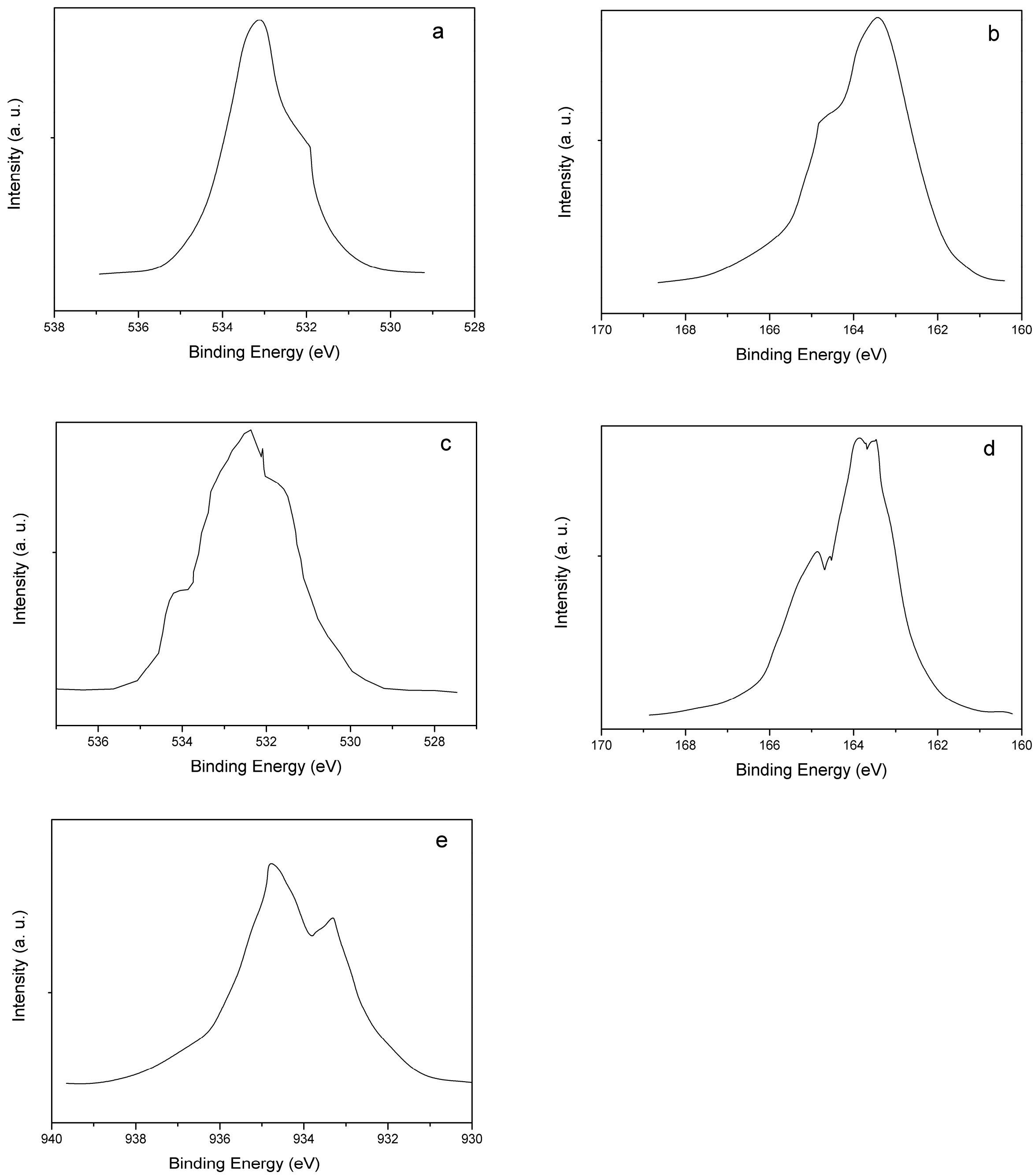


Fig. 2 X-ray photoelectron spectra: (a) O 1s spectrum of free **III** ligand; (b) S 2p spectrum of free **III** ligand; (c) O 1s spectrum of $Cu(L^{III})_2$; (d) S 2p spectrum of $Cu(L^{III})_2$; (e) Cu 2p spectrum of $Cu(L^{III})_2$.

The S 2p spectrum of $Cu(L^{III})_2$ has three peaks at 164.9, 163.9 and 163.6 eV (Fig. 2d). The values in the parent ligand are 163.4 and 164.5 eV (Fig. 2b). The increased binding energy in $Cu(L^{III})_2$ and the additional peak may indicate S coordination.

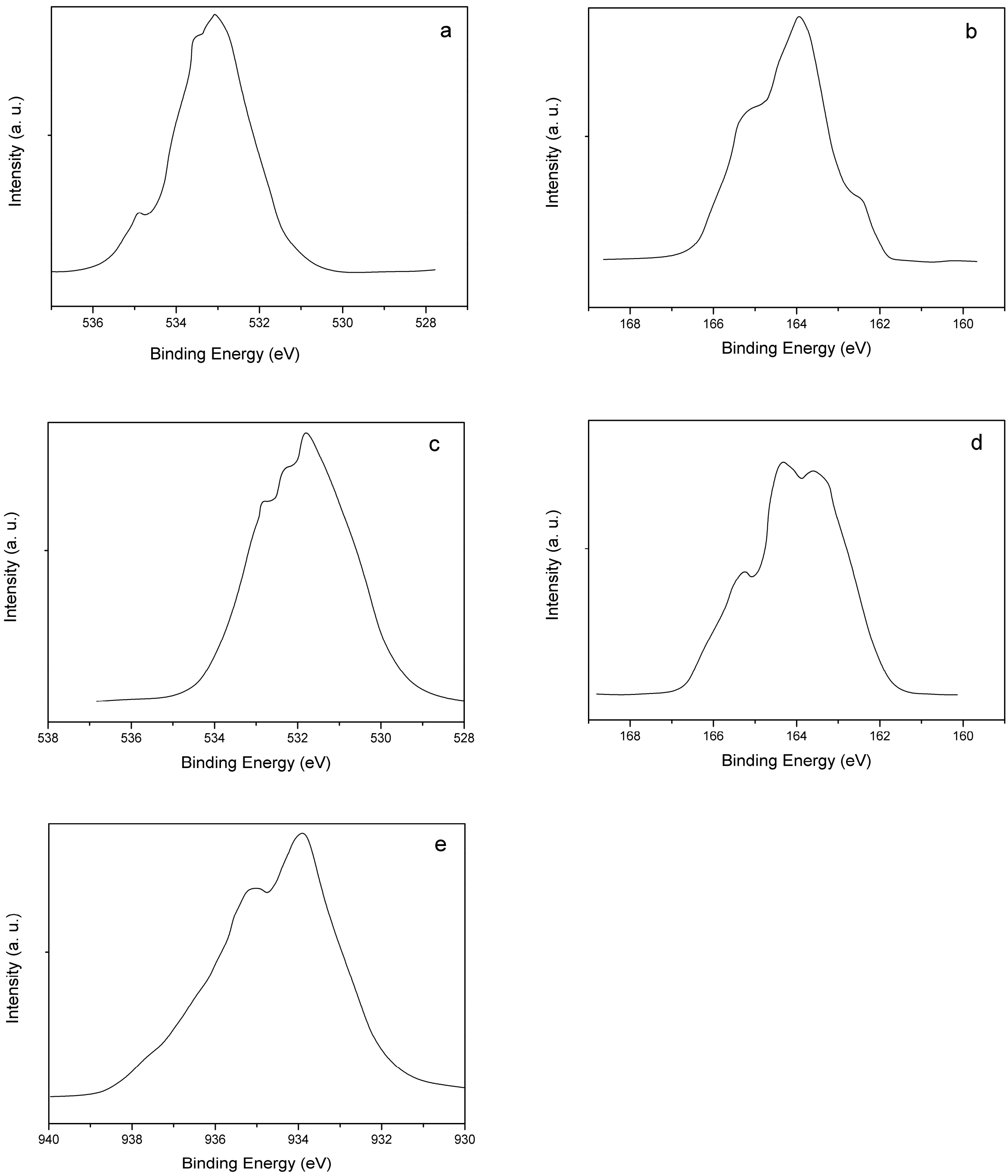


Fig. 3 X-ray photoelectron spectra: (a) O 1s spectrum of free ligand **IV**; (b) S 2p spectrum of free ligand **IV**; (c) O 1s spectrum of $Cu(L^{IV})_2$; (d) S 2p spectrum of $Cu(L^{IV})_2$; (e) Cu 2p spectrum of $Cu(L^{IV})_2$.

The O 1s binding energies in free ligand **III** are 533.1 and 532.2 eV (Fig. 2a). The O

1s of $Cu(L^{III})_2$ is spin split into three peaks at 533.9, 532.7 and 531.7 eV (Fig. 2c). Thus, the phenolic hydroxyl O 1s binding energy increases due to its coordination to Cu (II). An additional peak at 531.7 eV is due to the singly deprotonated form of ligand **III** on complexation [57].

In the case of $Cu(L^{IV})_2$ the Cu 2p is spin split into two distinct peaks at 935.4 and 934.4 eV (Fig. 3e), suggesting that the copper atoms are doubly charged.

The S 2p spectrum of $Cu(L^{IV})_2$ has three distinct peaks at 165.7, 164.7 and 164.0 eV (Fig. 3d). The S 2p binding energies in free ligand **IV** are 165.1, 164.0 and 162.6 eV (Fig. 3b). The higher binding energies and decreased electron density may indicate that the S atom is in the $Cu(L^{IV})_2$ coordination sphere.

In free ligand **IV** the O 1s binding energies are 534.9, 533.6 and 533.1 eV (Fig. 3a). The O 1s spectrum of $Cu(L^{IV})_2$ contains three distinct peaks at 533.3, 532.7 and 532.2 eV (Fig. 3c). The increase in binding energy from 532.2 to 533.1 eV suggests electron donation by the hydroxyl. The decrease in binding energy from 534.9 to 533.3 eV and from 533.6 to 532.7 eV may be due to carboxylate anion formation [57].

3.5 Antimicrobial activities

Bacteria are first used to discover a compound's biological effect; the next stages include eukaryotic cells (of plant and animal origin). This scheme was used in our investigation of the biological activities of Cu (II) - SHD complexes. Table 3 lists the antimicrobial activities of the Cu (II) complexes and of the free ligands, as estimated by the bactericidal MIC ($\mu g \cdot mL^{-1}$). It should be emphasized that antimicrobial tests of these compounds were first performed here.

We note that **I**–**IV** demonstrated a low inhibiting ability toward Gram-negative bacteria: MIC>250 $\mu g \cdot mL^{-1}$. For the bacteria tested, $Cu(L^{I})_2$ showed moderate activities, its MIC being 125 $\mu g \cdot mL^{-1}$. $Cu(L^{II})_2$ does not inhibit growth of *Pseudomonas aeruginosa*, which agrees with the literature showing them to be highly resistant to a wide variety of antimicrobial compounds, presenting a problem of great concern to modern medicine. In general, complexation with Cu (II) ions increases the activities of **I**, **II** against Gram-negative bacteria somewhat.

Ligands **III**, **IV** and their Cu (II) complexes lack virtually any antibacterial activities: detectable growth inhibition is observed only at concentrations above 500 $\mu g \cdot mL^{-1}$.

The estimated antimicrobial activities against Gram-positive bacteria are informative. First, Gram-positive bacteria are more sensitive than Gram-negative to the free ligands and give no growth if concentrations are above 3–25 $\mu g \cdot mL^{-1}$. Second, Cu (II) ions present may substantially decrease the toxic effect of the ligands against Gram-positive bacteria. Often enough the same MIC values are found for a free ligand and its Cu (II) complex, for instance, in the case of **I**, **II** and their complexes against *Staphylococcus saprophyticus* or **II**, **III** and their complexes against *Sarcina lutea*.

The bacterial cell wall is a good target for antimicrobial agents, metal complexes among them. The Gram-positive bacterial cell wall is primarily made up of peptidoglycan

Table 3 Antimicrobial activities of the free ligands **I–IV** and their Cu (II) complexes, evaluated by minimum inhibitory concentration (MIC, μg·mL^{-1}).

Compound	*Pseudomonas aeruginosa*	*Serratia marcescens*	*Escherichia coli*	Bacillus subtilis	*Sarcina lutea*	*Staphylococcus saprophyticus*
L^{I}	>250	>250	>250	25	<3.1	25
$Cu(L^{I})_2$	125	125	125	50	12.5	25
L^{II}	>250	>250	>250	25	<3.1	25
$Cu(L^{II})_2$	>250	125	125	25	<3.1	25
L^{III}	>500	>500	>500	6.2	6.2	25
$Cu(L^{III})_2$	>500	>500	>500	12.5	6.2	12.5
L^{IV}	>500	>500	>500	25	<3.1	12.5
$Cu(L^{IV})_2$	>500	>500	>500	25	12.5	6.2
Compound	*Cryptococcus laurentive*	*Lypomyces lipofer*	*Pichia pastoris*	*Candida boidinii*	*Candida utilis*	*Saccharomyces cerevisiae*
L^{I}	62.5	<8	31.25	<8	<8	62.5
$Cu(L^{I})_2$	>125	62.5	62,5	31.25	16	31.25
L^{II}	31.25	16	<8	31.25	<8	16
$Cu(L^{II})_2$	62.5	31.25	<8	62.5	16	31.25
L^{III}	16	16	8	>250	>250	16
$Cu(L^{III})_2$	62.5	16	8	62.5	62.5	16
L^{IV}	125	16	62.5	250	125	31.25
$Cu(L^{IV})_2$	62.5	16	31.25	62.5	125	62.5

(as much as 90 %, but ∼10 % in Gram-negative bacteria) [58]. The cell walls of Gram-negative bacteria are more complex due to the presence of an outer membrane in addition to a thin peptidoglycan layer [59]. Nevertheless, the overall functional group chemistry of both classes of bacterial surfaces are identical [60]; differences in minor components and structure may exist.

In general, we have shown a significant difference in MIC against Gram-positive and Gram-negative bacteria for all the compounds studied. This behavior is common and referred to as “intrinsic resistance” of Gram-negative bacteria [61]; their outer membrane acts as a barrier to antimicrobial activity.

The effects of the compounds on yeasts are somewhat similar to those against Gram-positive bacteria. Free ligands are generally more toxic than their Cu (II) complexes, but we could not demonstrate any clear differences in activities of these compounds against yeasts (Table 3). In most cases complexation of ligands with Cu (II) ions doubles the MIC value or leaves it virtually unaffected. Nevertheless, most of the compounds exert a pronounced antimicrobial effect on the yeast strains tested, and ligands **II** and **III**, as well as their Cu (II) complexes, are active against *Pichia pastoris* in low concentration (8 μg·mL^{-1}). The same high activity is also characteristic of **I** against *Candida utilis, Candida boidinii* and *Lypomyces lipofer*. At the same time the free ligand **III** shows no appreciable activity against *Candida utilis* or *Candida boidinii* (MIC>125 μg·mL^{-1}), while its Cu (II) complex exerts a pronounced antimicrobial effect (MIC=62.5 μg·mL^{-1}). According to [62], a better membrane penetrating ability explains the frequently observed cases when complexes are more effective against the yeasts than the free ligands. It has also been supposed [62] that the reduced activity of the compounds against yeasts can be attributed to their inability to form hydrogen bonds with cell constituents.

We also investigated the phytotoxic activity of free ligands and their complexes against a culture of the green algae *Chlorella vulgaris 157*, which may be regarded as a model eukaryotic organism. We investigated the concentration range from 250 to 32 μg·mL^{-1}. Ligand **I** and its Cu (II) complex were found to inhibit *Chlorella* growth at the same concentrations (250 μg·mL^{-1}), the areas of the inhibited growth being of the same size. Cu(L^{III})$_2$ complex also inhibited *Chlorella* growth. There were no areas of inhibited growth at all for ligand **IV** or its Cu (II) complex at a concentration of 250 μg×mL^{-1}.

Unfortunately, a general correlation between chemical structure and bioactivity cannot be drawn. Estimating the effect of Cu (II) complexation on the antimicrobial activities of sterically hindered *o*-diphenols, we hypothesize that one reason for the different activities of the complexes is their lower solubility in non-aqueous solvents (compared to those of the free ligands) and to the peculiarities of their coordination spheres. However, further experiments should be performed to resolve the question.

4 Conclusion

We have synthesized Cu (II) complexes with some sterically hindered *o*-diphenols, which seemed promising for their antimicrobial activities. Compounds **I–III** can coordinate in their singly deprotonated forms and act as bidentate ligands. These compounds yield Cu (II) complexes of Cu(L)$_2$ stoichiometry, with square planar geometry. Unlike them, **IV** behaves as a terdentate ligand, and its Cu(L^{IV})$_2$ complex has distorted octahedral geometry. Our investigation showed that we avoided oxidation of **I**, **III** and **IV** on complexation with Cu (II) ions, and it was only for **II** that a very small amount of stabilized phenoxyl radicals were formed.

For all the compounds investigated, we found a significant difference in MIC against Gram-positive and Gram-negative bacteria. **I**–**IV** and their Cu (II) complexes demonstrated a low inhibiting ability toward Gram-negative bacteria. Gram-positive bacteria are more sensitive to the ligands and their Cu (II) complexes, and give no growth if concentrations of the latter are above 3–25 μg·mL^{-1} Often the same MIC values are found for a free ligand and its Cu (II) complex. Complexation with Cu (II) ions increases the activities of **III**, **IV** against *Staphylococcus saprophyticus* somewhat. On the other hand, Cu (II) ions decrease the activities of the ligands **I** and **III** against *Bacillus subtilis* and the activities of the ligands **I**, **IV** against *Sarcina lutea*. In most cases complexation of ligands with Cu (II) ions doubles the MIC value against yeasts or leaves it virtually unaffected. Complexation of the ligand **III** with Cu (II) results in an enhancement of the activity of the former against *Candida utilis* and *Candida boidinii*. It should be noted that the presence of phenoxyl radicals in $Cu(L^{II})_2$ had no marked enhancing effect on the antimicrobial activities of the latter compared with that of the parent ligand **II**.

In general, most of the compounds tested show broad spectra of antimicrobial activity. This may be of interest in the design of new drugs.

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