

Synthesis, characterization and antimicrobial evaluation of copper(II) complexes of 5-*tert*-butyl-pyrocatechin-derived Mannich bases

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Cu(II) complexes with 5-*tert*-butyl-3-(pyrrolidine-1-ilmethyl)-1,2-dihydroxybenzene (I), 5-*tert*-butyl-3-(piperidine-1-ilmethyl)-1,2-dihydroxybenzene (II), 5-*tert*-butyl-3-(azeno-1-ilmethyl)-1,2-dihydroxybenzene (III), 5-*tert*-butyl-3-(morpholine-1-ilmethyl)-1,2-dihydroxybenzene (IV), and 5-*tert*-butyl-3-(methylpiperazine-1-ilmethyl)-1,2-dihydroxybenzene (V) have been synthesized and characterized by means of elemental analysis, TG/DTA, FT-IR, ESR, UV-Vis spectroscopy and conductivity measurements. According to the data obtained, the compounds I–V coordinate in their singly deprotonated forms in an *O,N*-bidentate fashion. The synthesized complexes have the composition described by the general formula CuL_2 and are characterized by square planar geometry of their coordination cores CuO_2N_2 . The Cu(II) complexes have been screened for their antimicrobial activity on different species of pathogenic bacteria, fungi, and their antimicrobial potency has been discussed. A high antibacterial activity against *Mycobacterium smegmatis* and *Pseudomonas aeruginosa* has been found for the Cu(II) complexes synthesized.

Key words: Mannich bases, Cu(II) complexes, spectroscopic study, antibacterial activity

INTRODUCTION

In the last 25 years the incidence of microbial infection has increased on alarming levels over the world as a result of antimicrobial resistance. The health problem demands searching and synthesizing a new class of antimicrobial compounds effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regimen [1].

Mannich reaction is an important tool for synthesizing novel compounds. Mannich bases are physiologically reactive because of the basic function rendering the molecule soluble in aqueous solvents when it is transformed into aminium salt [2]. They find application as antitubercular, antimalarial, vasorelaxing, anticancer and analgesic drugs. Various phenolic derivatives have been reported to possess antioxidant, antifungal, antiviral, neurotropic and nootropic properties [2–8].

Copper has been known as the essential element participating in many biological processes, but its biomedical

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importance has been ascertained in the last decades due to the successful interaction between the chemistry of its bio-active complexes and pharmacological researches [9–20]. In this connection it is of interest to study various aspects of coordination chemistry of copper(II) interacting with Mannich bases derivatives and antimicrobial activity of the metal complexes synthesized.

We report herein the complexation, synthesis, characterization and antimicrobial activity of Cu(II) complexes with 5-*tert*-butyl-3-(pyrrolidine-1-ilmethyl)-1,2-dihydroxybenzene (I), 5-*tert*-butyl-3-(piperidine-1-ilmethyl)-1,2-dihydroxybenzene (II), 5-*tert*-butyl-3-(azepane-1-ilmethyl)-1,2-dihydroxybenzene (III), 5-*tert*-butyl-3-(morpholine-1-ilmethyl)-1,2-dihydroxybenzene (IV), and 5-*tert*-butyl-3-(methylpiperazine-1-ilmethyl)-1,2-dihydroxybenzene (V). The structure of the ligands is depicted in Fig. 1. The solid Cu(II)

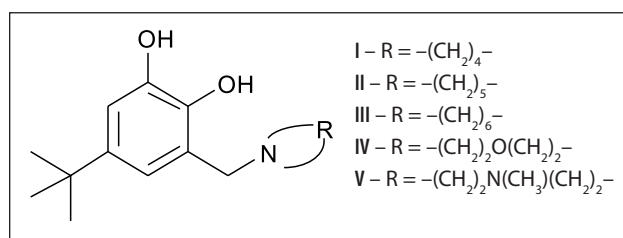


Fig. 1. Schematic representation of Mannich bases

complexes were characterized by means of elemental analysis, TG/DTA, FT-IR, ESR, UV-Vis spectroscopy, and conductivity measurements. However, a full structural analysis could not be performed because no single crystals suitable for X-ray diffraction studies were obtained. Therefore our notion of the coordination modes of the Cu(II) complexes was formed on the basis of findings provided by other physico-chemical methods and reported here. All the compounds synthesized were screened for the antibacterial and antifungal activity.

EXPERIMENTAL

Reagents and solutions

Chemicals were purchased from commercial sources and were used without further purification. The Mannich bases were prepared according to [21].

Apparatus and equipment

Previously we studied the complexation of Cu(II) ions with Mannich bases in water-ethanol solution, and stability constants of the complexes formed were calculated using the data of potentiometric titration obtained [22].

Infrared spectra of solids (the ligands I–V and the synthesized Cu(II) complexes thereof) were recorded at room temperature by the FT-IR Nicolet 380 spectrophotometer of the firm Thermo Electron Corporation, using the Smart Performer, in the wavelength range 4000–500 cm^{-1} .

Thermal analysis was performed with a device NETZSCH STA 449 C. TG/DTA measurements were run in nitrogen between 30 and 600 $^{\circ}\text{C}$ (10 $^{\circ}\text{C min}^{-1}$).

Elemental analyses were carried out according to the standard methods by the Microanalytical Laboratory, Physics Institute, National Academy of Sciences, Belarus. Copper determination was carried out using an atomic emission spectrometer with an inductively coupled plasma excitation source (Spectroflame Modula). A sample of a Cu(II) complex was decomposed on treatment with $\text{HNO}_3 + \text{H}_2\text{O}_2$ using a Milestone MLS-1200 Mega Microwave Digestion System. The complex having been decomposed, the content of metal in the resulting solution was determined.

Optical absorption spectra of solutions of the ligands and their metal complexes in acetonitrile were registered on a SPECORD S600 spectrophotometer in the wavelength range 200–1000 nm using a standard (1 cm) cell.

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 quoted in reference 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 $^{\circ}\text{C}$ using a TESLA BMS91 conductometer (cell constant 1.0).

Synthesis of the Cu(II) complexes with 5-*tert*-butyl-pyrocatechin-derived Mannich bases

Based on the data of potentiometric titration [22], the conditions were created to purposefully provide the preferential formation of the complex with Cu(II) : L = 1 : 2: a solution of Cu(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. The preparation followed a common procedure. A solution of 0.05 mmol $\text{Cu}(\text{CH}_3\text{COO})_2$ in 6 ml of water was added dropwise to a colorless solution of 0.1 mmol of I–V dissolved in 14 ml of ethanol (molar ratio Cu(II) : L = 1 : 2). The reaction mixture was stirred for 1.5 hours, the metal complex solution was left for several days to crystallize. The solid phase formed was collected on membrane filters (JG 0.2 μm), washed with ethanol and water, and dried *in vacuo* (yield > 70%).

Antimicrobial activity

Antibacterial activity of the compounds was estimated by a minimum inhibitory concentration (MIC, $\mu\text{g} \cdot \text{mL}^{-1}$) as described elsewhere [23]. The following test microorganisms (collection of Department of Microbiology, Belarusian State University) were used: *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus saprophyticus*, *Mycobacterium smegmatis*. The compounds being tested were dissolved in dimethyl sulfoxide (DMSO). A twofold serial dilution from 100 to 6.25 $\mu\text{g} \cdot \text{mL}^{-1}$ was used. The absence of microbial growth after an incubation period of 24 h at 37 $^{\circ}\text{C}$ for bacteria was taken to be a

criterion of effectiveness. In every case MIC was determined as the lowest concentration of the compound under study which inhibited the visible microbial growth, compared with the control system in which the microorganisms were grown in the absence of any test compound.

Antifungal activity of the compounds was tested against the following fungal species (the collection of the Department of Microbiology, the Belarusian State University): *Aspergillus niger*, *Alternaria alternata*, *Mucor* spp., *Botrytis cinerea*, *Monilia* spp., *Fusarium* spp., *Penicillium lividum*, *Sclerotinia sclerotiorum*. Compounds were dissolved in DMSO and diluted in the potato dextrose agar medium to yield working solutions of the test compounds with the concentration of $100 \mu\text{g} \cdot \text{mL}^{-1}$. Antifungal activity of the compounds was checked by the agar plate technique as described elsewhere [24]. The degree of inhibition of radial growth (*RI*) was calculated as follows [25]: $RI = 100(C-T)/C$ (%), 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.

The amount of DMSO in the medium was 1% and did not affect the growth of the microorganisms tested. There were three replicates for each dilution. Results were always verified in three separate experiments.

RESULTS AND DISCUSSION

Physico-chemical characterization

Using the synthetic procedure previously developed [26], complexes of Cu(II) with ligands I–V were separated in the

crystalline state. The elemental analyses data are given in Table 1. The results for the complexes Cu(II) with I–V are in agreement with the general formula CuL_2 . The substances produced are individual compounds as judged from the reproducible results of elemental analyses and X-ray powder diffraction patterns. All the complexes were insoluble in water, diethyl ether, nitromethane, and chloroform, but soluble in ethanol, acetonitrile, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, isopropanol, and acetone.

The low values of the molar conductivity in acetonitrile for all the complexes ($\Lambda_{\text{mol}} = 22.1\text{--}29.9 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) indicate their being essentially non-electrolytes in this solvent. Thus, the conductivity data suggest that the ligands I–V may be coordinated to the Cu(II) ion as uninegatively charged ligands.

Thermal analyses in nitrogen flow with identification of the final products by X-ray powder diffraction have shown all complexes to be anhydrous and unsolvated because their DTA curves lack any endothermic peaks over a wide range from 60 to 110 °C. A summary of the results is given in Table 2.

The complexes underwent decomposition within the temperature range of 100–600 °C. The complexes behaved similarly on thermal analysis and showed four stages of decomposition. The first two or three peaks generally occurred in the temperature range of 110–295 °C, presumably due to a loss of mixed fragments of the ligands. The next stage occurred in the temperature range of 250–440 °C and corresponded to the maximal weight loss. The last stage (365–550 °C) corresponded to a small weight loss and continued until decomposition of the organic ligands was complete, ultimately giving copper oxide as the residue [27, 28]. Above 550 °C pyrolytic decomposition of the ligands took place as usual. In all cases the final product was copper(II) oxide. The

Table 1. The results of chemical elemental analysis of Cu(II) complexes with ligands I–V

Complex	Brutto formula	Element content (calculated / found, %)			
		C	H	N	Cu
$\text{Cu(L}^{\text{I}})_2$	$\text{C}_{30}\text{H}_{44}\text{N}_2\text{O}_4\text{Cu}$	64.32/64.26	7.92/8.01	5.00/4.87	11.34/11.41
$\text{Cu(L}^{\text{II}})_2$	$\text{C}_{32}\text{H}_{48}\text{N}_2\text{O}_4\text{Cu}$	65.33/65.16	8.22/8.37	4.76/4.65	10.80/10.87
$\text{Cu(L}^{\text{III}})_2$	$\text{C}_{34}\text{H}_{52}\text{N}_2\text{O}_4\text{Cu}$	66.26/66.37	8.50/8.41	4.55/4.61	10.31/10.44
$\text{Cu(L}^{\text{IV}})_2$	$\text{C}_{30}\text{H}_{44}\text{N}_2\text{O}_6\text{Cu}$	60.84/60.99	7.49/7.60	4.73/4.66	10.73/10.81
$\text{Cu(L}^{\text{V}})_2$	$\text{C}_{32}\text{H}_{50}\text{N}_4\text{O}_4\text{Cu}$	62.16/62.25	8.15/8.09	9.06/8.99	10.28/10.17

Table 2. TG/DTA data for decomposition of Cu(II) complexes

Complex	TG range, °C	DTG, °C	Number of steps, <i>n</i>	Mass loss	Total mass loss	Assignment	Metallic residue
				Found / Calculated, %			
$\text{Cu(L}^{\text{I}})_2$	110–180, 190–250, 260–440, 445–550	145, 196, 233, 270, 495	5	86.49/85.80	86.49/85.80	Decomposition of 2HL ^I	CuO
$\text{Cu(L}^{\text{II}})_2$	125–190, 210–250, 260–390, 395–560	143, 238, 283, 493	4	86.99/86.48	86.99/86.48	Decomposition of 2HL ^{II}	CuO
$\text{Cu(L}^{\text{III}})_2$	110–140, 150–240, 250–380, 390–540	120, 206, 311, 470	4	86.76/87.09	86.76/87.09	Decomposition of 2HL ^{III}	CuO
$\text{Cu(L}^{\text{IV}})_2$	115–195, 220–295, 320–390, 400–530	148, 254, 360, 465	4	87.18/86.57	87.18/86.57	Decomposition of 2HL ^{IV}	CuO
$\text{Cu(L}^{\text{V}})_2$	110–155, 160–260, 265–350, 365–540	146, 174, 224, 254, 297, 470	6	86.31/87.14	86.31/87.14	Decomposition of 2HL ^V	CuO

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.

To specify the coordination modes in the Cu(II) complexes, we used IR spectroscopy. The IR parameters of the Cu(II) complexes are presented in Table 3. In the spectra of ligands I–IV there is a single band in the range of 3 409–3 375 cm^{-1} , indicating the presence of intermolecular hydrogen bonds involving phenolic hydroxyls [29]. The spectrum of V differs from the other spectra in that it demonstrates a broad band at 3 038 cm^{-1} , which corresponds to the intermolecular hydrogen bond involving hydroxyls [29–31]. In the spectra of Cu(II) complexes there is a broad band in the range 3 365–3 290 cm^{-1} ,

suggesting that phenolic hydroxyl groups participate in metal ion coordination. Besides, the frequencies of aromatic ring vibrations (1 609–1 594 cm^{-1} and 1 497–1 489 cm^{-1}) were found to be shifted to 1 584–1 553 cm^{-1} and 1 483–1 469 cm^{-1} due to the complexation. The change in the band intensity of C–O stretching vibrations and their shift into the lower frequency region in the spectra of Cu(II) complexes are also evidence in favour of the ligands I–V being coordinated to Cu(II) ions via oxygen atoms of phenolic hydroxyl groups. The shift of the bands at 1 220–1 020 cm^{-1} , assigned to C–N bond vibrations, to the lower frequency region in the spectra of these complexes suggests that the nitrogen is involved in the complexation. The bands in the range 540–501 cm^{-1} confirm that Cu–O and Cu–N bonds are formed [32].

Table 3. IR spectral assignments (cm^{-1}) for ligands I–V and their Cu(II) complexes

Compound	$\nu(-\text{OH})$	$\nu(\text{C}-\text{O})$	$\nu(\text{C}-\text{N})$	$\nu(\text{C}=\text{C})$	$\nu(\text{Cu}-\text{N})$	$\nu(\text{Cu}-\text{O})$
L ^I	3 409 w	1 320 m	1 120 m	1 608 w	–	–
		1 301 m	1 102 m	1 490 m		
		1 189 s				
Cu(L ^I) ₂	3 391 w	1 286 s	1 102 w	1 567 w	506 m	527 m
		1 261 s	1 062 m	1 481 s		
		1 201 w				
		1 164 w				
L ^{II}	3 403 m	1 326 w	1 098 m	1 609 w	–	–
		1 307 s	1 029 w	1 490 m		
		1 197 s				
		1 188 s				
Cu(L ^{II}) ₂	3 385 w	1 316 m	1 103 m	1 553 m	501 w	525 m
		1 283 m	1 037 m	1 483 m		
		1 237 m				
		1 154 w				
L ^{III}	3 403 m	1 331 w	1 119 w	1 606 w	–	–
		1 307 m	1 102 m	1 489 m		
		1 224 w	1 063 m			
		1 188 s				
Cu(L ^{III}) ₂	3 245w	1 304 w	1 104 w	1 560 s	506 w	538 m
		1 286 s	1 032 w	1 476 s		519 m
		1 224 s	1 016 w			
		1 174 w	1 004 w			
L ^{IV}	3 375 m	1 312 m	1 127 w	1 605 w	–	–
		1 300 s	1 071 m	1 493 m		
		1 228 m	1 031 m			
		1 192 m				
Cu(L ^{IV}) ₂	3 360 m	1 305 m	1 069 w	1 584 s	506 w	540 m
		1 216 m	1 032 w	1 483 m		522 m
		1 163 w	1 003 m			
		1 115 s				
L ^V	3 038 w	1 326 m	1 203 w	1 594 w	–	–
		1 299 w	1 082 w	1 497 w		
		1 168 m	1 050 w			
		1 135 m	1 036 w			
Cu(L ^V) ₂	3 350 m	1 307 m	1 194 m	1 555 m	501 w	530 m
		1 279 m	1 148 w	1 469 m		515 m
		1 218 m	1 029 w			

Table 4. The main parameters of ESR spectra of Cu(II) complexes

Complex	g_{\parallel}	g_{\perp}	G	ΔH
Cu(L ^I) ₂	2.290	2.062	4.8	175
Cu(L ^{II}) ₂	2.290	2.062	4.8	90
Cu(L ^{III}) ₂	2.300	2.050	6.2	180
Cu(L ^{IV}) ₂	2.276	2.062	4.6	125
Cu(L ^V) ₂	2.280	2.056	5.2	125

The solid state ESR spectra of all the complexes were recorded both at room temperature and at 77 K. The ESR parameters of the Cu (II) complexes are presented in Table 4.

The ESR spectra of the Cu(II) complexes in the polycrystalline state (77 K) are typical axial ones (Fig. 2) with well-defined g_{\parallel} and g_{\perp} features respectively at the values ~ 2.29 and 2.06. It should be noted that 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. Hyperfine structure from the central ion (⁶³Cu and ⁶⁵Cu, $I = 3/2$) on the line of parallel orientation is observed for all the complexes, and that on the line of perpendicular orientation is observed only for Cu(L^{II})₂ and Cu(L^{III})₂ complexes. From Table 4 it is seen that the spin Hamiltonian parameters of the complexes at hand are close, thus, the coordination cores of the complexes obtained are closely allied structurally.

The geometric parameter G , which is a measure of the exchange interaction between copper (II) centers in the polycrystalline compound, is calculated using the equation $G = (g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)$ [33, 34]. If $G > 4$, exchange interaction is negligible, whereas a considerable exchange interaction occurs for $G < 4$. The geometric parameter G for all the complexes appears to be greater than 4, which shows that there are no magnetic exchange interactions between the copper (II) centers [34, 35].

The solid-state ESR spectra of all these complexes at 77 K are quite similar and exhibit axially symmetric g -tensor parameters 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 stereochemistry [36, 37]. Spin Hamiltonian parameters are known to be affected by the composition and structure (*cis*, *trans*) of the coordination core, by the nature of the remote substitute as well as by the charge of the chelate complex.

The g_{\parallel} values for the coordination sphere are in a narrow range depending on its composition and have the following values [CuO₄] – 2.26–2.30; [*trans*-CuN₂O₂] – 2.220–2.200; [*cis*-CuN₂O₂] – 2.190–2.180; [CuN₄] – 2.15–2.17; [CuN₄]²⁺ – 2.26–2.28. The values of g -tensor components are generally increased upon the coordination core being distorted [38]. A noticeable growth in g_{\parallel} in the spectra of the Cu(II) complexes synthesized as compared to the above-mentioned g_{\parallel} value for the undistorted square planar core can be due to distortion (in varying degrees) of the coordination core in these complexes. It is evident that the

undistorted square planar *cis* configuration of the coordination core cannot be realized in Cu(II) complexes with the *tert*-butyl groups and cyclic substituents at the nitrogen atom. According to [38], in case of tetrahedral distortion of the coordination core [*trans*-CuN₂O₂] the g_{\parallel} value can grow up to 2.28–2.32, and for [*cis*-CuN₂O₂] no higher than up to 2.24. In this connection it can be suggested that Cu(II) ions in these complexes form the chromophore [*trans*-CuN₂O₂], the ground state $d_{x^2-y^2}$ being characteristic for the metal ion [34]. Thus, the Cu(II) ion is in a distorted square planar environment formed by *N,O*-coordinating ligands, which agrees with the data obtained by other physico-chemical

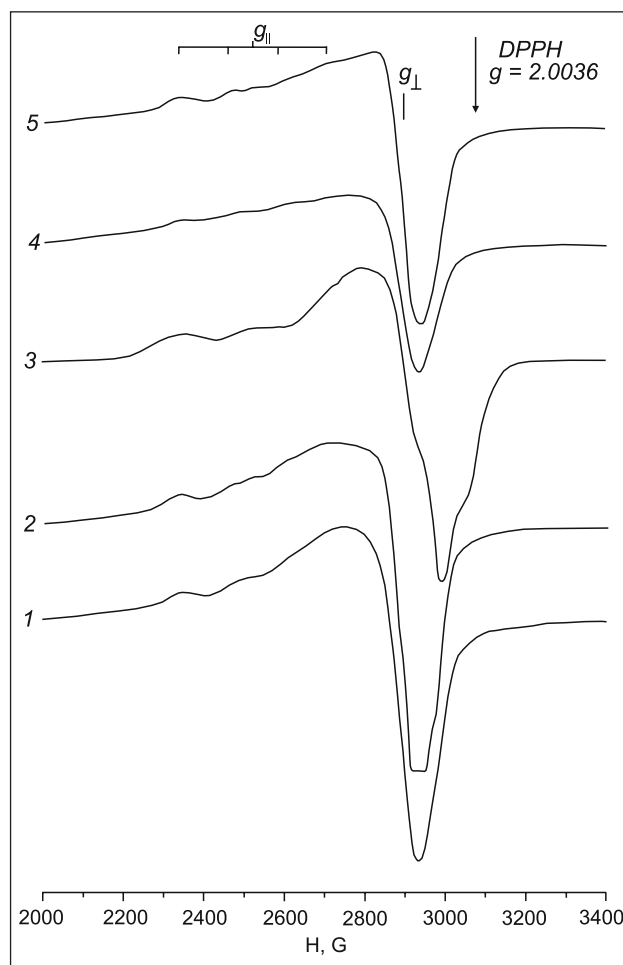


Fig. 2. ESR spectra of compounds Cu(L^I)₂ (1), Cu(L^{II})₂ (2), Cu(L^{III})₂ (3), Cu(L^{IV})₂ (4) and Cu(L^V)₂ (5) in the polycrystalline state at 77 K

Table 5. UV-VIS spectrum assignments for Cu(II) complexes and their coordination cores

Complex	Chromophore	Absorption bands, λ_{\max} , nm	Coordination polyhedron
$\text{Cu(L}^{\text{I}}\text{)}_2$		440–570 (<i>d-d</i>)	
$\text{Cu(L}^{\text{II}}\text{)}_2$		330–440 ($\text{O}_{\text{phen,H}_2\text{O}} \rightarrow \text{Cu}^{\text{II}}$ LMCT)	
$\text{Cu(L}^{\text{III}}\text{)}_2$	CuN_2O_2	300–370 ($\text{N}(\sigma) \rightarrow \text{Cu}^{\text{II}}$ LMCT)	Square planar
$\text{Cu(L}^{\text{IV}}\text{)}_2$		225, 280–285 (ILA)	
$\text{Cu(L}^{\text{V}}\text{)}_2$			

methods. According to [39], g_{\parallel} values less than 2.3 indicate a considerably covalent character of M–L bonds, and those greater than 2.3 are indicative of the ionic one. The g_{\parallel} values of the Cu(II) complexes were found to be less than 2.3, which allows one to assume a considerably covalent character of the M–L bonds.

Geometry of coordination cores of Cu(II) complexes with ligands I–V was determined on the basis of analysis of electronic absorption spectra. In general, the spectra under study are typical for low-spin Cu(II) complexes with strong-field ligands. Electronic absorption spectra of Cu(II) complexes, their main characteristics summarized in Table 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 absorption (ILA) [40].

Compounds I–V are characterized by an absorption band in the region 225–285 nm present in electronic absorption spectra. In the spectra of the complexes there are absorption maxima in the UV region (225–285 nm) which belong to ILA.

When specifying the spectral position of the bands LMCT and *d-d* transitions, we took into account that in the strong field of the ligands I–V coupling of spins in the Cu(II) ion occurs, as supported by ESR data. 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 (CuL_2) shape of their chromophores CuN_2O_2 . The maxima in the regions 300–370 nm and 330–440 nm appearing in the spectra as a result of complexation with Cu(II) ions suggest the presence of LMCT transitions: $\text{N}(\sigma) \rightarrow \text{Cu}^{\text{II}}$ and $\text{O}_{\text{phen}} \rightarrow \text{Cu}^{\text{II}}$ [40, 41]. The structure of the complexes is depicted in Fig. 3.

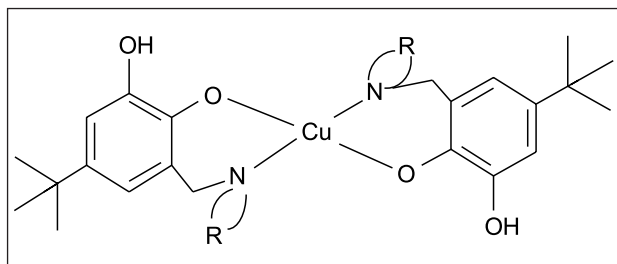


Fig. 3. Schematic representation of the coordination polyhedron of Cu(II) complexes

Antimicrobial activity

To evaluate antimicrobial activity of the ligands I–V and their Cu(II) complexes against bacteria (Gram-negative and Gram-positive) and fungi, their primary pharmacologic screening was carried out, and its main results are summarized in Tables 6, 7. Commonly used antibiotics (streptomycin, tetracycline, chloramphenicol, nistatin and terbinafine) were tested as positive controls.

The compounds I–V and their metal complexes were found to have a moderate inhibition activity ($\text{MIC} \sim 100 \mu\text{g} \cdot \text{mL}^{-1}$) against the Gram-negative bacteria *Pseudomonas aeruginosa*, *Serratia marcescens* and *Escherichia coli* (Table 6). The highest activity ($\text{MIC} = 12.5 \mu\text{g} \cdot \text{mL}^{-1}$) is characteristic of $\text{Cu(L}^{\text{IV}}\text{)}_2$ against *Pseudomonas aeruginosa*, which is comparable with those of chloramphenicol. Gram-positive bacteria *Staphylococcus saprophyticus*, *Bacillus subtilis*, *Sarcina lutea* are more sensitive to Mannich bases I–V and their metal complexes than Gram-negative ones, and do not grow when concentrations of these

Table 6. Antibacterial activity of the free ligands I–V and their Cu(II) complexes evaluated by the minimum inhibitory concentration (MIC, $\mu\text{g} \cdot \text{mL}^{-1}$)

Compound	<i>P. aeruginosa</i>	<i>S. marcescens</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. lutea</i>	<i>S. saprophyticus</i>	<i>M. smegmatis</i>
L^{I}	>100	>100	>100	100	100	25	25
$\text{Cu(L}^{\text{I}}\text{)}_2$	100	100	100	25	25	25	25
L^{II}	100	>100	>100	>100	>100	>100	100
$\text{Cu(L}^{\text{II}}\text{)}_2$	25	>100	>100	25	50	50	25
L^{III}	>100	>100	>100	>100	>100	50	100
$\text{Cu(L}^{\text{III}}\text{)}_2$	100	100	>100	50	50	50	100
L^{IV}	100	100	50	100	100	100	50
$\text{Cu(L}^{\text{IV}}\text{)}_2$	12.5	100	25	50	25	100	25
L^{V}	100	100	100	100	100	100	100
$\text{Cu(L}^{\text{V}}\text{)}_2$	100	100	100	50	25	100	12.5
Streptomycin	>100	6.2	3.1	6.2	12.5	6.2	3.1
Tetracycline	–	–	3.1	6.2	6.2	6.2	–
Chloramphenicol	12.5	–	6.2	3.1	–	6.2	12.5

Table 7. Antifungal activities of the free ligands I–V and their Cu(II) complexes expressed as the radial inhibition of mycelial growth (RI, %)

Compound	<i>Aspergillus niger</i>	<i>Fusarium spp.</i>	<i>Mucor spp.</i>	<i>Penicillium lividum</i>	<i>Botrytis cinerea</i>	<i>Monilia spp.</i>	<i>Sclerotinia sclerotiorum</i>	<i>Alternaria alternata</i>
L ^I	60	80	0	0	70	15	60	60
Cu(L ^I) ₂	90	100	0	0	100	25	90	90
L ^{II}	40	100	50	90	50	20	50	75
Cu(L ^{II}) ₂	55	100	60	95	100	50	100	100
L ^{III}	55	30	30	90	40	50	90	100
Cu(L ^{III}) ₂	70	80	50	100	60	50	100	100
L ^{IV}	100	100	50	40	70	40	100	90
Cu(L ^{IV}) ₂	100	100	50	40	70	40	100	95
L ^V	100	100	50	40	90	40	95	100
Cu(L ^V) ₂	100	100	50	40	100	40	100	100
Nystatin	80	90	100	90	60	50	50	50
Terbinafine	100	80	25	40	60	50	40	60

compounds exceed 25 µg mL⁻¹. Metal complexes Cu(L^I)₂ and Cu(L^V)₂ were found to be the most active against the majority of the Gram-positive bacteria tested. The highest activity (MIC = 12.5 µg · mL⁻¹) is characteristic of Cu(L^V)₂ against *Mycobacterium smegmatis*, which is comparable with those of chloramphenicol.

Antifungal activity of the ligands I–V and their metal complexes against the following fungi strains: *Aspergillus niger*, *Alternaria alternata*, *Mucor spp.*, *Botrytis cinerea*, *Monilia spp.*, *Fusarium spp.*, *Penicillium lividum*, *Sclerotinia sclerotiorum* was evaluated (Table 7). The current interest in plant pathogenic fungi is caused not only by the search for means of fighting plant diseases but also by the fact that these microorganisms are a health hazard producing allergy, systemic mycoses [42, 43]. In this connection searching for new effective agents of fighting mold is a burning problem of practical mycology. Most of the compounds (both the free ligands and the complexes) exert a pronounced antifungal activity (RI ≥ 70%), which in a number of cases is comparable with that of nystatin and terbinafine or even higher. A high antifungal activity of the ligands I, IV, V and their Cu(II) complexes (RI = 70–100%) is characteristic of such fungi cultures as *Alternaria alternata*, *Fusarium spp.*, *Aspergillus niger* and *Sclerotinia sclerotiorum*.

These observations show that the majority of the compounds are more active than the respective Mannich bases. In some cases both Mannich bases and their complexes are similarly active against bacteria and fungi. Chelation may enhance or suppress the biochemical potential of bioactive organic species [44]. The higher activity of the metal complexes may be due to the effect of metal ions on the normal cell membrane. Metal chelates bear polar and nonpolar properties together; this makes them suitable for permeation to the cells and tissues. Changing hydrophilicity and lipophilicity is likely to result in bringing down the solubility and permeability of cell barriers, which in turn enhances the bioavailability of chemotherapeutics, on the one hand, and potentiality, on the other hand.

CONCLUSIONS

The complexation of 5-*tert*-butyl-pyrocatechin-derived Mannich bases with Cu(II) ions was studied in water-ethanol solution. It was shown that complexes of the composition Cu(II)/ligand = 1 : 2 were formed, their overall stability constants varying in the range 4.3 · 10¹³–1.5 · 10¹⁶ were determined. Five novel Cu(II) complexes with Mannich bases were separated in the crystalline state. The complexes synthesized were characterized by means of elemental analysis, TG/DTA, FT-IR, ESR, UV-Vis spectroscopy, and conductivity measurements. According to the data obtained, the Mannich bases coordinate in their singly deprotonated forms in an *O,N*-bidentate fashion. The complexes synthesized have the composition described by the general formula CuL₂ and are characterized by square planar geometry of their coordination cores CuO₂N₂. The Cu(II) complexes have been screened for their antimicrobial activity on different species of pathogenic bacteria and fungi. The Mannich bases and their metal complexes were found to have a moderate inhibition activity against Gram-negative bacteria, while Gram-positive ones are more sensitive to Mannich bases and their metal complexes. A high level of activity of all the complexes under study against *Sarcina lutea* and *Mycobacterium smegmatis* should be noted. In general, most of the compounds tested show broad spectra of antifungal activity, which in a number of cases is higher than that for antifungal antibiotics. These facts may be of interest in the design of new effective drugs and agents active against plant pathogenic fungi. The results obtained show that the majority of the metal complexes investigated are more active than the respective Mannich bases.

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**VARIO(II) KOMPLEKSŲ SU 5-TERT-BUTILPIRO-
KATECHINO MANIČHO BAZĖMIS SINTEZĖ,
CHARAKTERIZAVIMAS IR ANTIMIKROBINIO
AKTYVUMO ĮVERTINIMAS**

Santrauka

Susintetinti ir charakterizuoti nauji Cu(II) kompleksai su organiniais ligandais. Tirtas šių kompleksų antimikrobinis aktyvumas. Nustatytas aukštas šių kompleksų aktyvumas prieš *Mycobacterium smegmatis* ir *Pseudomonas aeruginosa*.