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Synthesis and biological evaluation of copper (II) complexes of sterically hindered *o*-aminophenol derivatives as antimicrobial agents

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Abstract—Cu(II) complexes with 4,6-di(*tert*-butyl)-2-aminophenol (**I**) and 2-anilino-4,6-di(*tert*-butyl)phenol (**II**) have been synthesized and characterized by means of elemental analysis, TG/DTA, FT-IR, UV–vis, ESR, and conductance measurements. The compounds **I** and **II** can coordinate in their singly deprotonated forms and behave as bidentate O,N-coordinated ligands; their CuL_2 complexes are characterized by CuN_2O_2 coordination modes and square planar geometry. In vitro antimicrobial screening against Gram-positive and Gram-negative bacteria, yeasts, and moulds indicated that the compound **I** and its Cu(II) complex were more active than Questiomycin B, the compound **II**, and its Cu(II) complex.

Although several classes of antimicrobial compounds are presently available, microorganisms' resistance to these drugs constantly emerges. In order to prevent this serious medical problem, the elaboration of new types of antibacterial agents or the expansion of bioactivity of the previously known drugs is a very actual task.[1] The synthesis and characterization of metal complexes with organic bioactive ligands is one of the promising fields for the search, in particular, of metal complexes with derivatives of sterically hindered *o*-aminophenols. Cell- and animal-based testing allowed to reveal a set of promising pharmacological properties for sterically hindered *o*-aminophenols, for example, antioxidant, antihypoxic, anti-inflammatory and antiviral properties.[2] In particular, an antiherpetic medicine has been developed from 2-anilino-4,6-di(*tert*-butyl)phenol and is currently available.[3] Non-substituted *o*-aminophenol is known as Questiomycin B antibiotic with specific antifungal (antimycobacterial) activity.[4] However, alkylated *o*-aminophenol derivatives, particularly those which contain *tert*-butyl groups, are known to be substantially less toxic.[5] On the other hand, complexation with metals is known to enhance the pharmacological activity of drugs due perhaps to resulting higher liposolubilities leading to greater intracellular accumulations.[6] In some cases, metal complexes are less toxic than the parent drugs.[6d] These data provide a good platform for attempts to use sterically hindered *o*-aminophenols as ligands in synthesis of new bioactive metal complexes.

According to the results presented in a series of papers,[7] sterically hindered *o*-aminophenol molecules are redox noninnocent when O,N-coordinated to some transition metal ions, and can exist in different protonation and oxidation forms (the *o*-aminophenol or *o*-iminosemiquinone) in coordination compounds.

As for the coordination chemistry of transitional metal complexes formed by O,N-coordinated ligands of this sort, it is quite possible that synthetic difficulties have inhibited the study of complexes of that kind, since the attempts to isolate metal complexes with ligands of this type were often unsuccessful and resulted in recovering

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of the ligand without coordination.[8] This has prompted us to investigate the peculiarities of complexation of some di-*tert*-butylated *o*-aminophenolate ligands with transition metal ions. In the course of these studies, we have investigated the acid-base properties and the interaction of a series of sterically hindered *o*-diphenols with transition metal ions in aqueous ethanol solutions.[9] Selecting Cu(II) complexes as the main subjects for this study, we had in mind that this biometal is vital to human as a component of many enzymes and metalloproteins, and is able to take part in redox processes, thus affecting the properties of the ligands of the phenolic or quinoid types.[10] In our studies the experimental data on the quantitative aspects of complexation equilibria for Cu(II) ions forming complexes with the above-mentioned ligands in aqueous ethanol solutions were obtained from potentiometric titration, thus allowing us to select the best conditions for synthesizing ML_2 complexes (where M = metal (II) ion; L = ligand). It has been established that the modification of the ligand molecule may drastically change the coordination equilibria and stabilities of the respective species.[9] In order to limit the amount of information for this paper, we have chosen only two sterically hindered *o*-aminophenols which were used to synthesize copper (II) complexes. We report herein the synthesis, characterization, and antimicrobial activities of Cu(II) complexes with 4,6-di(*tert*-butyl)-2-aminophenol (**I**, see the scheme above R = H) and 2-anilino-4,6-di(*tert*-butyl)phenol (**II**, R = Ph). We also estimated the antimicrobial activities of *o*-aminophenol (**III**) as compared with those of above-mentioned compounds.

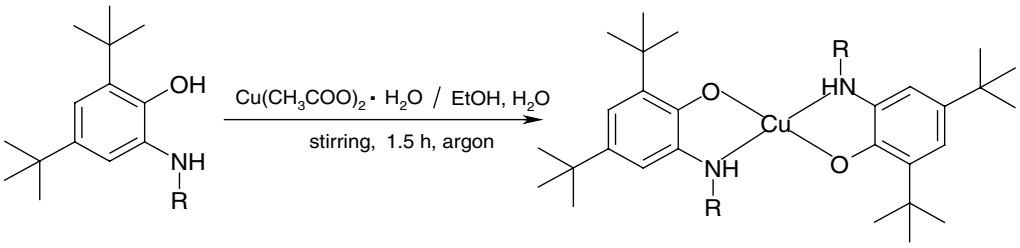
Bis(*o*-iminobenzosemiquinonato)copper (II) complex, which is formed upon the interaction of Cu(II) ions with ligand **II**, has been reported in the paper,[7b] where the neutral square planar Cu(II) complex of the stoichiometry ML_2 (L = *o*-iminosemiquinone) has been shown to be a diradical with a singlet ground state. In order to increase understanding of transition metal (II) interactions with sterically hindered *o*-aminophenols, we have examined the possibility of the formation of *tert*-butyl-substituted *o*-aminophenolates in the aforesaid systems, which may be less toxic than the redox (radical) forms of respective ligands.[11]

Two sterically hindered *o*-aminophenols were prepared as described elsewhere.[2e,12] Based on our previous findings,[9] the conditions were created to purposefully provide the preferential formation of the complex with M (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. 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** or **II** dissolved in 10 ml of ethanol (molar ratio M(II):L=1:2). As these ligands can be readily oxidized by oxygen, it is to be taken into account when producing their complexes with transition metal ions, especially with copper (II), in order to prevent the production of oxidized forms of ligands (in particular, *o*-iminosemiquinones, as it was shown in[7,8a]). Because of this, argon was bubbled through the solutions (pH $\leqslant$6) during the synthesis to ensure the absence of oxygen. 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 > 70–80%).

The solid products resulting from the interaction of Cu(II) ions with the ligands **I** and **II** were well characterized by means of elemental analysis, TG/DTA, FT-IR, UV–vis, ESR and conductance measurements.[13,14] The complexes demonstrated X-ray patterns of their own, differing significantly from those of respective ligands. However, a full structural analysis could not be performed, because no crystals suitable for single-crystal X-ray analysis were obtained. It has been reported that some metal complexes with sterically hindered ligands were hard or impossible to crystallize.[8] In order to obtain some structural information in solid state of the complexes, IR, electronic absorption, and ESR spectra have been examined.

The elemental analysis data for the complexes Cu(II) with ligands **I** and **II** are in agreement with the general formula ML_2.[13,14] Thermal analysis in air flow with identification of the final products by X-ray powder diffraction has shown all the complexes to be anhydrous and unsolvated.[13,14] The agreement between the experimental and theoretical weight losses for the above processes confirms the above-mentioned general formula of the Cu(II) complexes. The conductivity data indicate their being essentially non-electrolytes in acetonitrile,[15] and suggest that the bidentate ligands **I** and **II** may be coordinated to Cu(II) ions as uninegatively charged ligands.[13,14]

IR spectra of Cu(II) complexes as against those of free **I** and **II** ligands are distinguished by the following features:[13,14] (i) the bands corresponding to ν(OH) vibrations in the free **I** and **II** ligands (respectively, at 3366 and 3552 cm^{-1}) disappear, suggesting that the ligands lose the hydroxyl proton on coordination; (ii) the bands at 1230–1140 cm^{-1} assigned to C–O stretching vibrations are shifted toward lower wave numbers as compared to their positions in the spectra of respective ligands; (iii) the position of the bands assigned to N–H and C–N stretching vibrations is changed; (iv) bands of moderate intensity appear at 580–420 cm^{-1}, which can be assigned to ν(Cu–N) and ν(Cu–O). The above-listed facts suggest that protonated amino groups and deprotonated hydroxyls take part in forming the CuN_2O_2 coordination core in Cu(II) complexes.[16] The UV–vis spectra of the complexes in the solid state exhibit absorption bands in the high-energy region at 300–400 nm which can be assigned to intra-ligand bands and the ligand to copper (II) charge transfer transitions.[17a] The absorption band attributable to a d–d transition in the spectra of Cu(II) complexes is observed as a broad shoulder centered at about 535 nm which is due to the square planar CuN_2O_2 chromophore.[17] In acetonitrile solution the spectra of Cu(II) complexes are similar to those observed in the solid state, indicating that the structures of the complexes are retained in solution.



$Cu(L^{I})2$, R=H; $Cu(L^{II})2$, R=Ph

In agreement with the proposed coordination core in Cu(II) complexes, the solid-state ESR spectra of the both Cu(II) complexes at 77 K exhibit an axially symmetric *g*-tensor parameter with $g_{\parallel} > g_{\perp} > 2.0023$.[13] These data indicate that the copper site has a $d_{x^2-y^2}$ ground state characteristic of square planar stereochemistry.[18] No signal of stabilized radicals present in ESR spectra, as well as the *ν*(C=O) and *ν*(C=N) stretching vibrations lacking in IR spectra of Cu(II) complexes, respectively, in the ranges of 1400–1500 and 1690–1640 cm^{-1}, confirms the phenolate character of the ligands. In the light of the physico-chemical data and elemental analysis results the mode of bonding in the Cu(II) complexes can be represented schematically as shown above.

The possibility of separating the respective individual complexes becomes conjectural upon changing the conditions of the synthesis, free ligands in oxidized forms being generally obtained in abundance along with them.

Antimicrobial activities of the compounds were estimated by a minimum inhibitory concentration (MIC, μg/ml) in the usual fashion.[19] The test microorganisms (the collection of Department of Microbiology, Belarusian State University) are listed in Table 1. Standard antifungal agent like Questiomycin B (**III**) was also screened under the same conditions for comparison.

Compounds **II**, **III** and $Cu(L^{II})_2$ complex virtually lack any noticeable antimicrobial activities: a detectable growth inhibition is observed at concentrations above 50 μg mL^{-1}; and it was only the compound **III** that showed a moderate activity against *Bacillus subtilis*.

Evaluating the antimicrobial activities of the ligand **I** and $Cu(L^{I})_2$ complex, we can note that they also demonstrated a low inhibiting ability toward Gram-negative bacteria: MIC > 50 μg mL^{-1}. The results of estimating their antimicrobial activities against Gram-positive bacteria are informative enough. First, Gram-positive bacteria are more sensitive to $Cu(L^{I})_2$ complex and give no growth if concentrations of the latter are above 3.125–6.25 μg mL^{-1}. Second, the complexation with Cu(II) ions can somewhat increase the activities of the ligand **I** against Gram-positive bacteria. It is well known that the bacterial cell wall is a good target for antimicrobial agents, metal complexes among them. In general, we have revealed a significant difference of MIC for the ligand **I** and its Cu(II) complex under study against Gram-positive and Gram-negative bacteria. The latter finding is accounted for by the fact that the first barrier capable of limiting antimicrobial activities is the outer membrane of Gram-negative bacteria. This fact is widely known and referred to as 'intrinsic resistance' of Gram-negative bacteria.

However, we could not reveal any clear differences in activities of the compounds in hand against yeasts and moulds. In particular, $Cu(L^{I})_2$ complex is more active against *Pichia pastoris*, *Lypomyces lipofer*, *Saccharomyces cerevisiae*, and *Hansenula* sp. than the respective igand **I**. Furthermore, Cu(II) ions present may decrease the toxic effect of the ligand **I** against *Cryptococcus laur-*

Table 1. Antimicrobial activities of the compounds tested as evaluated by the minimum inhibitory concentration (MIC, μg mL^{-1})

Test organisms	**I**	$Cu(L^{I})_2$	**II**	$Cu(L^{II})_2$	**III**
Pseudomonas aeruginosa	>50	>50	>50	>50	>50
Serratia marcescens	>50	>50	>50	>50	>50
Escherichia coli	>50	>50	>50	>50	>50
Bacilus subtilis	25	6.25	>50	>50	>25
Sarcina lutea	12.5	3.125	>50	>50	—
Staphylococcus saprophyticus	12.5	3.125	>50	>50	>50
Candida utilis	>50	50	>50	>50	>50
Candida albicans	>50	>50	>50	>50	>50
Candida boidinii	>50	>50	>50	>50	>50
Pichia pastoris	6.25	3.125	>50	>50	>50
Cryptococcus laurentiive	6.25	12.5	>50	>50	—
Lypomyces lipofer	6.25	3.125	>50	>50	—
Hansenula sp.	6.25	3.125	>50	>50	>50
Saccharomyces cerevisiae	6.25	3.125	>50	>50	>50
Rhodotorula rubra	50	>50	>50	>50	—
Aspergilus niger	>50	>50	>50	>50	>50

entiive. At the same time the ligand **I** does not show any appreciable activity against *Aspergilus niger*, *Rhodotorula rubra*, *Candida utilis*, *Candida albicans*, and *Candida boidinii* (MIC > 50 μg mL^{-1}), the complexation of this ligand with Cu(II) ions leaves MIC virtually unaffected.

Estimating the effect of complexation with Cu(II) ions on the antimicrobial activities of the test derivatives of sterically hindered *o*-aminophenols, we may assume that one of the likely reasons for the different activities of the complexes is related to the nature of the substitute in the ring and to a substitute being present at the nitrogen atom of the amino group. However, further experiments should be performed to bring this issue to a close.

Thus, our investigation showed that we had managed to avoid oxidation of the ligands upon their complexation with Cu(II) ions. We synthesized Cu(II) complexes with two derivatives of sterically hindered *o*-aminophenols, which seemed to be promising for studying their antimicrobial activities. It should be emphasized that the antimicrobial tests of the ligands and their Cu(II) complexes were first performed here. Antimicrobial screening in vitro indicated that the compound I and its Cu(II) complex were more active than Questiomycin B antibiotic tested. These facts may be of interest in designing new drugs.

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13. Physical and spectral characteristics of $Cu(L^{I})_2$ complex. Anal. Calcd for $C_{28}H_{44}N_2O_2Cu$: C, 66.68; H, 8.79; N, 5.56; Cu, 12.61. Found: C, 66.65; H, 8.77; N, 5.53; Cu, 12.57. The gray-green powder is soluble in acetonitrile, ethanol, acetone, dimethylformamide, and partly in nitromethane. TG/DTA data: no weight loss was observed until decomposition, which began at about 175 °C with exothermic peaks at 180 °C (without any noticeable weight loss) and at 360 °C, ultimately leaving CuO as the residue. The maximal weight loss of 83.52% corresponds to the loss of two ligand molecules in the $Cu(L^{I})_2$ complex (calcd 84.21%). Prominent IR bands (nujol mull) (cm^{-1}): 424w ν(Cu–N), 516w ν(Cu–O), 583 m ν(Cu–O), 656 m ν(N–H), 812 s ν(N–H), 891 m, 1024 m, 1069 s ν(C–O), 1207 s ν(C–O), 1245 m, 1275 m ν(C–N), 1576 m ν(C–H), 1653 s ν(C–N), 2344w, 2363w, 3066w ν(C–H), 3295 m ν(N–H). Molar conductivity (in acetonitrile): $\Lambda_{mol} = 10.6\ \Omega^{-1}cm^2\ mol^{-1}$. UV–vis data (nujol mull) (λ_{max}, nm): 535sh, 400, 300; UV–vis data (acetonitrile) (λ_{max}, nm): 530sh, 395, 295. ESR data: $g_{\|} = 2.32$; $g_{\perp} = 2.06$.
14. Physical and spectral characteristics of $Cu(L^{II})_2$ complex. Anal. Calcd for $C_{40}H_{52}N_2O_2Cu$: C, 73.13; H, 7.92; N, 4.27; Cu, 9.68. Found: C, 73.10; H, 7.88; N, 4.24; Cu, 9.65. The green powder is soluble in acetonitrile, acetone, dimethylformamide, insoluble in nitromethane and ethanol. TG/DTA data: no weight loss was observed until decomposition, which began at about 200 °C with exothermic peaks at 215 °C (without any noticeable weight loss) and at 370 °C, ultimately leaving CuO as the residue. The maximal weight loss of 86.97% corresponds to the loss of two ligand molecules in the $Cu(L^{II})_2$ complex (calcd 87.89%). Prominent IR bands (nujol mull) (cm^{-1}): 450w ν(Cu–N), 540w ν(Cu–O), 597w, 701m, 763w, 834m, 993m, 1027m, 1105w ν(Cu–O), 1178w ν(C–O), 1203 m ν(C–O), 1257m, 1299w ν(C–N), 1334m, 1578m ν(C–H), 3332m ν(N–H). Molar conductivity (in acetonitrile): $\Lambda_{mol} = 4.1\ \Omega^{-1}\ cm^2\ mol^{-1}$. UV–vis data (nujol mull) (λ_{max}, nm): 540sh, 400, 310; UV–vis data (acetonitrile) (λ_{max}, nm): 535sh, 400, 305. ESR data: $g_{\|} = 2.27$; $g_{\perp} = 2.07$.
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19. Pershin, G. N., Ed.; *Methods of the experimental chemotherapy*; Meditsina: Moskwa, 1971, p 103 (in Russian). The evaluation of the inhibitory effect of the ligands and their complexes on the microbial growth was carried out by the twofold serial dilution method. Mueller Hinton broth and Sabouraud liquid medium were employed as culture media for bacteria and fungi, respectively. The compounds were dissolved in dimethylsulfoxide (DMSO) and tested at concentrations ranging from 3.125. to 100 $\mu g\,mL^{-1}$. Test inoculum of 5×10^4 bacteria/mL and 10^3 yeasts or spores/mL was applied. The 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 taken to be a criterion of effectiveness. In every case MIC was determined as the lowest concentration of the compound under study inhibiting the visible microbial growth as 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 tested. There were three replicates for each dilution. Results were always verified in three separate experiments.