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PHYSICO-CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF COPPER(II) COMPLEXES WITH 2-AMINO AND 2-METHYLBENZIMIDAZOLE DERIVATIVES

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Copper(II) chloride, in warm ethanolic solution, reacted with 2-amino and 2-methylbenzimidazole derivatives to give complexes of the formula $CuL_2Cl_2 \cdot nH_2O$, where L=1-benzyl-2-aminobenzimidazole, 1-(4-methylbenzyl)-2-aminobenzimidazole, 1-benzyl-2-methylbenzimidazole and 1-(4-methylbenzyl)-2-methylbenzimidazole; n=1 or 2). The complexes were characterized by elemental analysis of the metal, molar conductivity, magnetic susceptibility measurements and IR spectra. The molar conductivities of copper(II)complexes in dimethyl formamide (DMF) corresponding to a 1:1 type of electrolyte indicate that in all the complexes one of the coordinated chloride ions has been replaced by DMF molecule. The room temperature effective magnetic moments and IR data of the complexes suggest that all Cu(II) complexes have a tetrahedral configuration, which is realized by participation of the pyridine nitrogen of two organic ligand molecules and two chloride anions. The antimicrobial activity of the ligands and their complexes against Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus, Sarcina lutea and Saccharomyces cerevisiae was investigated. The effect of copper complexation on the ligand antimicrobial activity is discussed.

KEYWORDS: Benzimidazole; complexes; copper(II); physico-chemical characterization; antimicrobial activity

INTRODUCTION

The antimicrobial activities of imidazoles and benzimidazoles have long been established. Derivatives of these compounds are known for their antibacterial, antiviral and fungicidal activities (1-4). These groups of compounds are of wide interest because of their

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diverse biological activity and clinical applications. This ring system is present in numerous antiparasitic, antihelmintic and anti-inflammatory drugs. They are also inhibitors of photosynthesys, and some exhibit appreciable herbicidal activity (5-7). The success with these compounds stimulated the search for new biologically active derivatives. Many different benzimidazoles have such activities as analgetics, anticarcinogens, antihistaminic, sedatives, etc (8-10). Various benzimidazoles are effective inhibitors of the growth of lactobacilli, vaccinia virus, influenza virus and HIV-virus (11-12).

The coordination chemistry of benzimidazole and its derivatives has received considerable attention because of their biological significance and interesting spectral, magnetic and structural aspects. In view of previous observations (13-16) that the presence of metal ions considerably enhances the biological activity of organic molecules, we report the synthesis and study of copper(II) complexes with 2-amino and 2-methylbenzimidazole derivatives. The antimicrobial activity of these complexes have also been investigated and the effect of copper on the ligand antimicrobial activity is discussed.

EXPERIMENTAL

Reagents

All chemicals used to prepare the complexes were of analytical reagent grade, commercially available from different sources.

Synthesis of complexes

All the complexes were prepared following the same procedure. A solution of 5 mmol of $CuCl_2 \times 2H_2O$ in 10 cm^3 of EtOH was added to a solution of 10 mmol of the ligand (1-benzyl-2-aminobenzimidazole (L^1), 1-(4-methylbenzyl)-2-aminobenzimidazole (L^2), 1-benzyl-2-methylbenzimidazole (L^3), and 1-(4-methylbenzyl)-2-methylbenzimidazole (L^4)) in 10 cm^3 EtOH. The resulting mixture was refluxed for about 2 h and then cooled. The complexes were separated from the reaction mixture by filtration, washed with EtOH and dried *in vacuo* over $CaCl_2$. The yield of the complexes varied in the range of 40-45%.

Measurement methods

The copper content was determined by a chelatometric titration with EDTA. Magnetic susceptibility measurements were made at room temperature using an MSB-MKI magnetic susceptibility balance (Sherwood Scientific Ltd., Cambridge, England). Molar conductivies of freshly prepared 1×10^{-3} mol dm^{-3} solutions (DMF) were measured on a Jenway 4010 conductivity meter. Infrared spectra (KBr pellets) were recorded on an Infrared 457 Perkin-Elmer spectrophotometer.

Antimicrobial investigations

For these investigations the filter paper disc method was applied. Each of the investigated isolates of bacteria were seeded in the tubes with nutrient broth (NB). The seeded

NB (1 cm^3) were homogenized in the tubes with 9 cm^3 of melted (45°C) nutrient agar (NA). The homogenous suspension was poured into Petri dishes.

The discs of filter paper (diameter 5 mm) were ranged on cool. After cooling on the formed solid medium, 2×10^{-5} dm^3 of the investigated compounds were placed with micropipette. After incubation for 24 hours in a thermostat at 25-27°C, inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. Inhibition zone diameter over 8 mm indicates the tested compound is active against bacteria under investigation. Every test was done in three replications.

The antimicrobial activities of the investigated compounds were tested against *Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus, Sarcina lutea* and *Saccharomyces cerevisiae*. In parallel with antimicrobial investigations of Cu(II) complexes, all ligands were tested too, as well as the pure solvent. The concentration of each solution was 5×10^{-2} mol dm^{-3}. Commercial DMF was employed to dissolve the tested samples.

RESULTS AND DISCUSSION

The elemental analysis of complexes, magnetic moments and molar conductivity data are summarized in Table 1. The complexes were synthesized in the reaction of warm ethanolic solution of the $CuCl_2 \times 2H_2O$ with L^1, L^2, L^3 or L^4 in a mole ratio 1 : 2. It should be noticed that the reaction of the copper ions yielded bis(ligand) complexes.

All the complexes are insoluble in most common organic solvents. They are highly soluble in DMF and dimethyl sulphoxide (DMSO), somewhat less soluble in MeOH and EtOH.

Table 1. Some physical characteristics and analytical data of the complexes

Complex	Colour	μ_{eff} (μ_B)	λ_M *	Metal (%) Found (Calcd.)
$Cu(L^1)_2Cl_2 \times H_2O$	yellow-green	1.83	84.6	10.47 (10.62)
$Cu(L^2)_2Cl_2 \times 2H_2O$	yellow-green	1.81	80.2	9.40 (9.86)
$Cu(L^3)_2Cl_2 \times H_2O$	brown	1.79	76.2	10.29 (10.65)
$Cu(L^4)_2Cl_2 \times 2H_2O$	dark-red	1.89	74.7	9.95 (9.89)

* In DMF, 1 mmol dm^{-3} solution at 25° C; in S cm^2 mol^{-1}

The molar conductivities of copper(II) complexes in DMFsolutions fall in the range of 74-85 Scm^2mol^{-1} corresponding to a 1:1 type of electrolyte (65-90 Scm^2mol^{-1}) (17). This indicates that in DMF solutions one of the coordinated chloride ions has been replaced by solvent molecule.

Magnetic properties

The room temperature effective magnetic moments of the copper(II) complexes are in the range of 1.73-2.20μ_B, which corresponds to one unpaired electron typical of the monomeric d^9 system (18).

Infrared spectra

The band appearing at about 1550 cm^{-1}, for all the ligands, may be assigned to the ν (C=N) vibrations (19,20). Substituted phenyl group shows ring vibrations at 1485 and 740 cm^{-1}. The IR of the ligands L^1 and L^2 exhibit the bands at 3450-3330 cm^{-1} and ca. 1650 cm^{-1}, assigned to ν (NH_2) and δ (NH_2) of the benzimidazole ring, respectively (20). The IR of the investigated complexes are similar to those of the corresponding ligands.

An upward shift (5-15 cm^{-1}) of ν(C=N) in the IR spectra of the complexes as compared to theirs values in the free ligands, suggests coordination through pyridine nitrogen of benzimidazoles. The $Cu(L^1)_2Cl_2 \times H_2O$ and $Cu(L^2)_2 Cl_2 \times 2H_2O$ bands due to ν (NH_2) and δ (NH_2) are shifted to lower frequency. These shifts may be indicative of hydrogen bonding formed between – NH_2 group from the ligand and water molecule. The other bands in the spectrum of each complex are similar to those in the corresponding ligand spectrum except for slight shifts in their positions and changes in their intensities due to coordination.

The presented results (molar conductivities, magnetic moments and IR spectra) suggest that all the copper(II) complexes have a tetrahedral configuration, which is realized by coordination of the two organic ligand molecules through the pyridine nitrogen and chloride anions or water molecules.

Antimicrobial investigationsin

All the ligands and their copper(II) complexes were screened for their antimicrobial activities against *Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus, Sarcina lutea* and *Saccharomyces cerevisiae.* The relevant data are presented in Table 2.

Table 2. Results of *in vitro* antimicrobial activity of the tested compounds

Compound	*P. aeruginosa*	*B. species*	*S. aureus*	*S. lutea*	*S. cerevisiae*
L^1	+	++	++	++	∅
$Cu(L^1)_2Cl_2 \times H_2O$	+++	+++	+	+++	∅
L^2	++	++++	++++	++++	+++
$Cu(L^2)_2Cl_2 \times 2H_2O$	+++	+++	+++	+++	++
L^3	+	+	+	++	∅
$Cu(L^3)_2Cl_2 \times H_2O$	+++	+++	+	+++	∅
L^4	++	+++	+++	+++	∅
$Cu(L^4)_2Cl_2 \times 2H_2O$	+++	+++	++	++	∅

Key to simbols:
Very highly active ++++, Highly active +++, Moderately active ++, Slightly active +, Inactive ∅

It is evident that all the ligands and their copper(II) complexes are active against gram-positive and gram-negative bacteria. Only compound L^2 and its complex showed activity against all of the standard test organisms, including yeast and gram-positive and gram-negative bacteria. The basic antimicrobial activity of 2-amino and 2-methylbenzimidazole derivatives is produced by the presence of benzimidazole ring as a known pharmacophore. Antimicrobial results shows that amino group as substituent at the position 2 in-

creased the general antimicrobial activity of the relevant benzimidazoles (21). If the benzimidazole nucleus was replaced with a substituted benzyl group at the N1 atom, the antimicrobial activity was increased, particularly in the presence of *meta* or *para* substituents (21). The proof of this is that the most active compound was 1-(4-methylbenzyl)-2-aminobenzimidazole (L^2), as well as its copper(II) complex (Table 2). In the case of *Saccharomyces cerevisiae* 4-methyl substituted benzyl group at the N1 atom highly increased the general antimicrobial activity of the relevant tested benzimidazoles. On the other hand, its Cu(II) complex showed medium activity. The other tested ligands and complexes had no activity against yeast *Saccharomyces cerevisiae.* Comparing the activities of the tested ligands and their complexes, it was found that some of the complexes were more active than starting ligands. The copper(II) complexes were more active than ligands against *Pseudomonas aeruginosa*, but less active than the ligand itself against *Staphylococcus aureus* and *Sarcina lutea* (except for $Cu(L^1)_2Cl_2 \times H_2O$ and complexes activity, substituted ligands and copper moiety may play a role in the antimicrobial activity.

CONCLUSIONS

With copper(II) 2-amino- and 2-methylbenzimidazole formed complexes of the general formula $CuL_2Cl_2 \times nH_2O$, where L=1-benzyl-2-aminobenzimidazole, 1-(4-methylbenzyl)-2-aminobenzimidazole, 1-benzyl-2-methylbenzimidazole and 1-(4-methylbenzyl)-2-methylbenzimidazole; n=1 or 2). The molar conductivities of copper(II) complexes in DMF solutions are in the range corresponding to a 1:1 type of electrolyte. This indicates that in all the complexes one of the coordinated chloride ions has been replaced by DMF molecule. All the complexes are tetrahedral, the configuration being realized by coordination of the pyridine nitrogen of the two organic ligand molecules and two chloride anions. The results of antibacterial investigations indicate that all the ligands and their copper(II) complexes are active against gram-positive and gram-negative bacteria. Only compound L^2 and its complex showed activity against all of the standard test organisms, including yeast and gram-positive and gram-negative bacteria. The most active compound was 1-(4-methylbenzyl)-2-aminobenzimidazole (L^2), as well as its copper(II) complex. Comparing the activities of the tested ligands and their complexes, it was found that some of the complexes were more active than the starting ligands. The copper(II) complexes were more active than ligands against *Pseudomonas aeruginosa*, but less active than the ligand against *Staphylococcus aureus* and *Sarcina lutea* (except $Cu(L^1)_2Cl_2 \times H_2O$ and $Cu(L^3)_2Cl_2 \times H_2O$). Consideration of the structural formula of the compounds that exhibit antimicrobial activity, substituted ligands and copper moiety may play a role in the antimicrobial activity.

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ФИЗИЧКО-ХЕМИЈСКА КАРАКТЕРИЗАЦИЈА И АНТИМИКРОБНА АКТИВНОСТ БАКАР(II) КОМПЛЕКСА СА 2-АМИНО И 2-МЕТИЛ ДЕРИВАТИМА БЕНЗИМИДАЗОЛА

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Хлорид бакра(II) у топлим етанолним растворима реагује са 2-амино и 2-метил дериватима бензимидазола дајући комплексе типа $CuL_2Cl_2 \times nH_2O$ (L=1-бензил-2-аминобензимидазол, 1-(4-метилбензил)-2-аминобензимидазол, 1-бензил-2-метилбензимидазол и 1-(4-метилбензил)-2-метилбензимидазол; n=1 или 2). Комплекси су окарактерисани елементарном анализом (метал), магнетним и кондуктометријским мерењима и IR спектрима. На основу вредности моларних проводљивости комплекса бакра(II) у ДМФ раствору карактеристичних за комплексе електролита типа 1:1 изводи се закључак да је један од координованих хлоридних анјона замењен молекулом растварача. На основу вредности магнетних момената и IR спектара изолованих комплекса бакра(II) може се претпоставити да испитивани комплекси имају

тетраедарску структуру која се реализује монодентатном координацијом два молекула лиганда преко пиридинског атома азота и координацијом два хлоридна јона. Испитана је антимикробна активност лиганада као и њихових комплекса на *Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus, Sarcina lutea* и *Saccharomyces cerevisiae.* Продискутован је утицај комплексирања бакра на антимикробну активност лиганада.

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