SYNTHESIS, PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF 2-AMINOBENZIMIDAZOLE COMPLEXES WITH DIFFERENT METAL IONS

Sanja O. Podunavac-Kuzmanović, Dragoljub M. Cvetković and Ljiljana S. Vojinović

Complexes of 2-aminobenzimidazole (L) with nitrates of cobalt(II), nickel(II), copper (II), zinc(II) and silver(I) were synthesized. The molar ratio metal:ligand in the reaction of the complex formation was 1:2. It should be noticed, that the reaction of all the metal salts yielded bis(ligand) complexes of the general formula M(L)_2(NO_3)_2 \times nH_2O (M=Co, Ni, Cu, Zn or Ag; n=0, 1, 2 or 6). The complexes were characterized by elemental analysis of the metal, molar conductivity, magnetic susceptibility measurements and IR spectra. Co(II), Ni(II) and Cu(II) complexes behave as non-electrolytes, whilst Zn(II) and Ag(I) are 1:1 electrolytes. Cu(II) complex has a square-planar stereochemistry, Ag(I) complex is linear, whilst the Co(II), Ni(II) and Zn(II) complexes have a tetrahedral configuration. In all the complexes ligand is coordinated by participation of the pyridine nitrogen of the benzimidazole ring. The antimicrobial activity of the ligand and its complexes against Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus and Saccharomyces cerevisiae was investigated. The effect of metal on the ligand antimicrobial activity is discussed.

KEYWORDS: Benzimidazole; complexes; cobalt(II); nickel(II); copper(II), zinc(II); silver(I); physico-chemical characterization; antimicrobial activity

INTRODUCTION

Physiological activity and commercial applications of many benzimidazole derivatives have received much attention. Benzimidazole and its derivatives have different activities as they can act as bacteriostats or bactericides, fungicides, anticancerogens, etc (1–4). This ring system is present in numerous antiparasitic, antihelminthic and anti-inflammatory
drugs (5). Several thousands of analogs of imidazole and benzimidazole have been synthe-
sized and screened for pharmacological activity. Some of these compounds exhibited anti-
HIV activity (6,7). The complexes of transition metals with benzimidazole and related
ligands have been extensively studied as models of some important biological molecules
(8-12).

We have recently reported isolation, characterization and antimicrobial activities of
different metal complexes with benzimidazole derivatives (9-12). As an extension of our
previous studies, the objective of the present work was to synthesize and study physico-
chemical characteristics, as well as antimicrobial activity of 2-aminobenzimidazole comp-
lexes with nitrates of cobalt(II), nickel(II), copper(II), zinc(II) and silver(I). Antimicrobial
activities of the ligand and its complexes were evaluated against *Pseudomonas aerugino-
sa*, *Bacillus sp.*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*.

**EXPERIMENTAL**

**Reagents**

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

**Synthesis of complexes**

All the complexes were prepared following the same procedure. A solution of 5 mmol
of the metal nitrate in 10 cm³ of EtOH was added to a solution of 10 mmol of the ligand
(2-aminobenzimidazole (L)) in 10 cm³ of EtOH. The resulting mixture was boiled under
reflux on a water bath 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 CaCl₂. The
yield of the complexes varied in the range of 45-50%.

**Measurement methods**

The metal content was determined by using 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 conductivities of freshly prepared $1 \times 10^{-3}$ 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 inves-
tigated isolates of bacteria were seeded in the tubes with nutrient broth (NB). The seeded
NB (1 cm³) were homogenized in the tubes with 9 cm³ of melted (45°C) nutrient agar (NA).
The homogeneous 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, $2 \times 10^{-5}$ 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 5.5 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* and *Saccharomyces cerevisiae*. In parallel with antibacterial investigations of Co(II), Ni(II), Cu(II), Zn(II) and Ag(I) complexes, ligands were tested too, as well as the pure solvent. The concentration of each solution was $5 \times 10^{-2}$ mol dm$^{-3}$. Commercial DMF was used to dissolve the tested samples.

RESULTS AND DISCUSSION

The elemental analysis of complexes, magnetic moments and molar conductivity data are summarized in Table 1.

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

<table>
<thead>
<tr>
<th>Complex</th>
<th>Colour</th>
<th>$\mu_{\text{eff}} (\mu_B)$</th>
<th>$\lambda$ M$^{-1}$</th>
<th>Metal % Found (Calcd.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(L)$_2$(NO$_3$)$_2$ · 6H$_2$O</td>
<td>blue</td>
<td>4.58</td>
<td>13.4$^a$</td>
<td>10.27 (10.58)</td>
</tr>
<tr>
<td>Ni(L)$_2$(NO$_3$)$_2$ · H$_2$O</td>
<td>violet</td>
<td>3.35</td>
<td>16.2$^a$</td>
<td>12.14 (12.58)</td>
</tr>
<tr>
<td>Cu(L)$_2$(NO$_3$)$_2$</td>
<td>blue</td>
<td>1.83</td>
<td>15.6$^a$</td>
<td>13.89 (14.01)</td>
</tr>
<tr>
<td>Zn(L)$_2$(NO$_3$)$_2$ · 2H$_2$O</td>
<td>white diam.</td>
<td>86.1$^b$</td>
<td></td>
<td>13.02 (13.30)</td>
</tr>
<tr>
<td>Ag(L)$_2$NO$_3$</td>
<td>light brown diam.</td>
<td>88.7$^b$</td>
<td></td>
<td>24.32 (24.75)</td>
</tr>
</tbody>
</table>

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

The complexes were synthesized in the reaction of warm ethanolic solution of the Me(NO$_3$)$_2$ · nH$_2$O (M = Co(II), Ni(II), Cu(II), Zn(II) and Ag(I)) with 2-aminobenzimidazole (L) in a mole ratio 1:2. It should be noticed that the reaction of all the metal ions yielded bis(ligand) complexes. All the complexes are insoluble or sparingly soluble in common organic solvents such as alcohols or acetone, but highly soluble in dimethylformamide and dimethylsulphoxide.

The molar conductivity values of the Co(II), Ni(II) and Cu(II) complexes (Table 1) indicate that these complexes behave as non-electrolytes. The molar conductivity of Zn(II) complex in DMF solutions fall in the range of 65-90 Scm$^{-1}$mol$^{-1}$, corresponding to a 1:1 type of electrolyte (13). It indicates that one coordinated nitrate anion in the Zn(II) complex has been replaced by solvent molecule. The molar conductivity of the Ag(I) complex in methanol coresponds to a 1:1 type of electrolyte.

**Magnetic properties**

An indication of the most probable stereochemistry of the synthesized Co(II) complex is given by its colour and magnetic moment. Namely, blue cobalt(II) complexes usu-
ally have a tetrahedral configuration. The magnetic value of cobalt(II) complex (Table 1) are in the expected range (\(\mu_{\text{eff}}=4.2-4.7\mu_B\)) for a tetrahedral stereochemistry. The room temperature effective magnetic moment for Ni(II) complex suggests tetrahedral configuration, too, whilst the \(\mu_{\text{eff}}\) value for Cu (II) complex supports square-planar geometry (14). Zinc(II) and Ag(I) complexes are diamagnetic so the complex of zinc is tetrahedral, whilst silver complex is linear.

Infrared spectra

The infrared spectrum of the ligand exhibits the bands at 3450-3330 cm\(^{-1}\) and ca. 1650 cm\(^{-1}\), assigned to \(\nu\) (NH\(_2\)) and \(\delta\) (NH\(_2\)) of the benzimidazole ring, respectively (15). The band appearing at about 1560 cm\(^{-1}\) may be assigned to \(\tau\eta\) \(\nu\) (C=N) vibrations. The infrared spectra of the investigated complexes are similar to those of the corresponding ligands.

An upward shift (5-10 cm\(^{-1}\)) of \(\nu\) (C=N) in the IR spectra of the complexes as compared to their values for the free ligand, suggests coordination through the pyridine nitrogen of 2-aminobenzimidazole (16). The bands due to \(\nu\) (NH\(_2\)) and \(\delta\) (NH\(_2\)) in the complexes are shifted to lower frequency. These shifts may be indicative of the presence hydrogen bonding between the –NH\(_2\) group of the ligand and water molecules. The results indicate the presence of two types of nitrate: coordinated nitrate anions and free nitrate anions in synthesized complexes. The peak at 1030 cm\(^{-1}\) is attributed to N-O stretching vibrations of the coordinated nitrate groups. A very strong band corresponding to NO\(_3\) stretching of free nitrate anions is observed at 1378 cm\(^{-1}\). 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 copper(II) complex has a square-planar stereochemistry, silver(I) complex is linear, whilst the cobalt(II), nickel(II) and zinc(II) complexes have a tetrahedral configuration. In all the complexes (except silver complex), configurations are realized by coordination of the two organic ligand molecules through the pyridine nitrogen, nitrate anions or molecules of water. In the case of silver complex, water molecules and nitrate anions are not included in coordination.

Antimicrobial investigations

In this work, 2-aminobenzimidazole and its complexes were screened for their \textit{in vitro} antimicrobial activity against the four microbial isolates. The antimicrobial activities of the compounds were tested by the agar disc-diffusion method. The results of these studies are summarized in Table 2.

As can be seen from Table 2, the majority of the investigated compounds displayed \textit{in vitro} antimicrobial activity against very persistent microorganisms. The starting ligand (2-aminobenzimidazole), as well as its silver complex showed no antimicrobial activity. However, some metal ions (Co, Cu, Zn) coordinated with ligand strongly increased the general antimicrobial activity of the 2-aminobenzimidazole. In the case of gram-negative isolate \textit{Pseudomonas aeruginosa} only copper(II) and zinc(II) complexes exhibited antibacterial activity. Gram-positive bacteria \textit{Bacillus species} was persistent only in the case
of nickel(II) and silver(I) complexes. In the case of Staphylococcus aureus nickel(II) and copper(II) complexes exhibited very low inhibitory activity, whilst cobalt(II) and zinc(II) complexes were highly active against the same bacteria. Also, Ni(II) and Cu(II) complexes had no activity against yeast Saccharomyces cerevisiae, but Co(II) and Zn(II) complexes were very active. Of the complexes, the most active compound was the one containing zinc(II), which exhibited high inhibitory activity against all the tested isolates. Cobalt(II) complex was also highly active, except in the case of gram-negative bacteria. Comparing the activities of the tested compounds, it can be seen that some of the complexes were more active than the starting ligand. In view of the structural formula of the complexes that exhibited antimicrobial activity, it can be thought that metal may play a significant role. This can be explained in terms of chelation theory, which states that a decrease in the polarizability of the metal can enhance the lipophilicity of the complexes (12).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Microbial isolates tested</th>
<th>P. aeruginosa</th>
<th>B. species</th>
<th>S. aureus</th>
<th>S. cerevisiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td></td>
<td>∅</td>
<td>∅</td>
<td>∅</td>
<td>∅</td>
</tr>
<tr>
<td>Co(L)2(NO3)2 × 6H2O</td>
<td></td>
<td>∅</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ni(L)2(NO3)2 × H2O</td>
<td></td>
<td>∅</td>
<td>∅</td>
<td>+/−</td>
<td>∅</td>
</tr>
<tr>
<td>Cu(L)2(NO3)2</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>+/−</td>
<td>∅</td>
</tr>
<tr>
<td>Zn(L)2(NO3)2 × 2H2O</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Ag(L)2NO3</td>
<td></td>
<td>∅</td>
<td>∅</td>
<td>∅</td>
<td>∅</td>
</tr>
</tbody>
</table>

∅ - no activity; +/− - very low inhibitory activity; + - low inhibitory activity; +++ - high inhibitory activity

**CONCLUSIONS**

The ligand, 2-aminobenzimidazole (L), formed with cobalt(II), nickel(II), copper(II), zinc(II) and silver(I) the complexes of the general formula M(L)2(NO3)2×nH2O (M=Co, Ni, Cu, Zn or Ag; n=0, 1, 2 or 6). The complexes were synthesized and characterized by elemental analysis of the metal, molar conductivity, magnetic susceptibility measurements and IR spectra. Co(II), Ni(II) and Cu(II) complexes behave as non-electrolytes, whilst Zn(II) and Ag(I) are 1:1 electrolytes. Cu(II) complex have a square-planar stereochemistry, Ag(I) complex is linear, whilst the Co(II), Ni(II) and Zn(II) complexes have a tetrahedral configuration. In all the complexes (except silver complex), configuration is realized by coordination of the pyridine nitrogen, nitrate anions or molecules of water. In the case of silver complex, water molecules and nitrate anions are not included in coordination. The results of antimicrobial investigations indicate that the starting ligand (2-aminobenzimidazole), as well as the silver complex, showed no antimicrobial activity. However, some metal ions (Co2+, Cu2+, Zn2+) coordinated with the ligand, strongly increased the general antimicrobial activity of the 2-aminobenzimidazole. Of the complexes, the most active compound was the one containing zinc(II), which exhibited high inhibitory activity against all the tested isolates. Cobalt(II) complex was also highly active,
except in the case of gram-negative bacteria. In view, of the structural formula of the complexes that exhibit antimicrobial activity, metal moiety may play a significant role in the antimicrobial activity.

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REFERENCES


СИНТЕЗА, ФИЗИЧКО-ХЕМИЈСКА КАРАКТЕРИЗАЦИЈА И БИОЛОШКА АКТИВНОСТ КОМПЛЕКСА 2-АМИНОБЕНЗИМИДАЗОЛА СА РАЗЛИЧИТИМ ЈОНИМА МЕТАЛА

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Синтетисани су комплекси 2-аминоbensimidазола (L) са нитратима кобалта(II), никла(II), бакра(II), цинка(II) и сребра(I). Комплекси се добијају реакцијом топлих етанолних раствора лиганда са нитратима метала у молском односу 2:1, дајући комплексе типа M(L)_2(NO_3)_2·nH_2O (M=Co, Ni, Cu, Zn или Ag; n=0, 1, 2 или 6). Комплекси су окarakтерисани елементарном анализом (метал), магнетним и кондуктометријским мерењима и IR спектрима. Врло ниске вредности моларне проводљивости за комплексе кобалта(II), никла(II) и бакра(II) у ДМФ указују на неелектролитне карактеристике раствора комплекса. На основу вредности моларних проводљивости за комплексе цинка(II) и сребра(I) изводи се закључак да су то електролити типа 1:1. На основу вредности магнетних момента и IR спектара изолованих комплекса кобалта(II), никла(II) и цинка(II) може се претпоставити да испитивани комплекси имају тетраедарску структуру. За комплексе бакра(II) претпостав-
љена је квадратно-планарна структура, док су комплекси сребра(I) линеарни. Стереохемија свих синтетисаних комплекса (изузев комплекса сребра) реализује се моноидентатном координацијом два молекула лиганда преко пиридинског атома азота и координацијом нитратног јона или молекула воде. У случају сребра(I) комплекса нитратни јони, као и молекули воде не учествују у координацији. Испитана је антимикробна активност лиганда и његових комплекса на четири врсте микроорганизама: Pseudomonas aeruginosa, Bacillus sp., Staphylococcus aureus и Saccharomyces cerevisiae. Дискутован је утицај метала на повећање антимикробне активности лиганда.

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