

Complexes cobalt(II), zinc(II) and copper(II) with some newly synthesized benzimidazole derivatives and their antibacterial activity

S. O. PODUNAVAC-KUZMANOVIĆ,^{a*} V. M. LEOVAC,^b N. U. PERIŠIĆ-JANJIĆ,^b J. ROGAN^c
and J. BALAŽ^d

^aFaculty of Technology, Bul. Cara Lazara 1, YU-21000 Novi Sad, ^bInstitute of Chemistry, Faculty of Science, Trg D. Obradovića 3, YU-21000 Novi Sad, ^cDepartment of General and Inorganic Chemistry, Faculty of Technology and Metallurgy, Karnegijeva 4, P.O.Box 494, YU-11001 Belgrade and ^dFaculty of Agriculture, Trg D. Obradovića 1, YU-21000 Novi Sad, Yugoslavia

(Received 21 October 1998, revised 15 January 1999)

The preparation and properties of some complexes of cobalt(II), zinc(II) and copper(II) with several newly synthesized benzimidazole derivatives (L) are reported. The complexes, of the general formula $[MCl_2L_2]$ ($M=Co(II), Zn(II)$) and $[CuCl_2L(H_2O)]$, have a tetrahedral structure. The complexes were characterized by elemental analysis, molar conductivity, magnetic susceptibility measurements, IR and absorption electronic spectra. The antibacterial activity of the benzimidazoles and their complexes was evaluated against *Erwinia carotovora* subsp. *carotovora* and *Erwinia amylovora*. The complexes were found to be more toxic than the ligands.

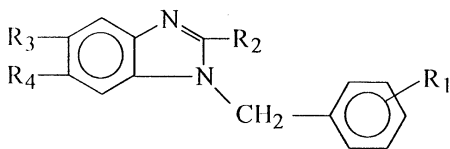
Key words: complexes, cobalt(II), zinc(II), copper(II), benzimidazole derivatives, physico-chemical characterization, biological activity.

Benzimidazole and its derivatives are of considerable importance because of their biological properties.^{1–6} Many different benzimidazoles have found commercial applications in the medical and agricultural fields where the derivatives have such activities as bacteriostats and bactericides, insecticides, fungicides, sedatives, anticarcinogens and phycopharmacological agents.^{7–12} The complexes of transition metal salts with benzimidazole derivatives have been extensively studied as models of some important biological molecules.^{13–17} The newly synthesized benzimidazole derivatives investigated in the present work possess good antibacterial and selective antifungal activities against different strains of bacteria and fung.¹⁸

In this paper we report the synthesis and some physico-chemical characteristics of complexes of three series of benzimidazoles (L) (Table I). The general formula of the complexes is $[MCl_2L_2]$ ($M=Co(II), Zn(II)$) and $[CuCl_2L(H_2O)]$. We also report the antibacterial activity of these complexes, the results of which are discussed in this communication.

* Author for correspondence

TABELA I. Structural formula of the ligands

				
Series I	R ₁	R ₂	R ₃	R ₄
I- <i>p</i> -CH ₃	<i>p</i> -CH ₃	H	CH ₃	CH ₃
I- <i>m</i> -OCH ₃	<i>m</i> -OCH ₃	H	CH ₃	CH ₃
Series II				
II- <i>m</i> -F	<i>m</i> -F	NH ₂	H	H
II- <i>m</i> -Cl	<i>m</i> -Cl	NH ₂	H	H
Series III				
III- <i>m</i> -CH ₃	<i>m</i> -CH ₃	NH ₂	CH ₃	CH ₃
III- <i>m</i> -F	<i>m</i> -F	NH ₂	CH ₃	CH ₃
III- <i>m</i> -Cl	<i>m</i> -Cl	NH ₂	CH ₃	CH ₃

EXPERIMENTAL

Reagents

Ligands were synthesized by Vlaović *et al.* according to a procedure described earlier.¹⁸ All chemicals used for the preparation of the complexes were of analytical reagent grade, commercially available from different sources.

Synthesis of complexes

All the complexes are prepared following the same procedure. A solution of 1.25 mmol CoCl₂·6H₂O, ZnCl₂ or CuCl₂·2H₂O in 5 cm³ of EtOH was added to a solution of 2.5 mmol of the ligand in 5 cm³ 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.

Measurements methods

Elemental analysis was carried out by standard micromethods. Magnetic susceptibility measurements were made at room temperature using a MSB-MK1 magnetic susceptibility balance (Sherwood Scientific Ltd., Cambridge, England). Molar conductivities of freshly prepared 10⁻³ mol dm⁻³ solutions (DMF) were measured on a Jenway 4010 conductivity meter. The infrared spectra (KBr pellets) were recorded on an Infrared 457 Perkin-Elmer spectrophotometer. The electronic absorption spectra (UV-VIS) were taken on a Cary 219 spectrophotometer.

Antibacterial investigations

For these investigations the filter paper disc method was applied. The investigated isolates of bacteria were seeded in 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 suspensions were poured into Petri dishes.

The discs of filter paper (diameter 5 mm) were ranged on the cool medium. After cooling on the formed solid medium, $2 \times 10^{-5} \text{ dm}^3$ of the investigated compounds were applied using a micropipette. After incubation for 24 h in a thermostat at 25–27 °C, the inhibition (sterile) zone diameters (including disc) were measured and expressed in mm. An inhibition zone diameter over 8 mm indicates that the tested compound is active against the bacteria under investigation. Every test was done in triplicate.

The antibacterial activities of the investigated compounds were tested against two strains of bacteria (*Erwinia carotovora* subsp. *carotovora* and *Erwinia amylovora*). Parallel with the antibacterial investigations of the Cu(II), Zn(II) and Co(II) complexes, all ligands were also tested, as well as the pure solvent. The concentration of each solution was $5 \times 10^{-2} \text{ mol dm}^{-3}$. Commercial DMF was employed to dissolve the tested samples.

RESULTS AND DISCUSSION

The complexes were synthesized in the reaction of a warm ethanolic solution of the $\text{MCl}_2 \cdot n\text{H}_2\text{O}$ ($\text{M} = \text{Co(II)}, \text{Cu(II)}, \text{Zn(II)}$) with benzimidazole derivatives in a mole ratio 1:2. It should be noticed, that the reaction of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ yielded mono (ligand) complexes, even when the ligand was presented in a double excess.

TABLE II. Some physical characteristics and analytical data of the complexes

Complex	Colour	$\mu_{\text{eff}}(\mu_{\text{B}})$	λ_{M}^*	Found (Calcd.)%		
				M	C	H
$[\text{CoCl}_2(\text{L}_{\text{I-p-CH}_3})_2]$	blue	4.41	2.1	9.30 (9.36)	67.14 (64.78)	6.37 (6.72)
$[\text{CoCl}_2(\text{L}_{\text{II-m-Cl}})_2]$	blue	4.58	11.2	9.28 (9.14)	51.56 (52.11)	3.12 (3.72)
$[\text{CoCl}_2(\text{L}_{\text{III-m-F}})_2]$	blue	4.69	7.7	8.95 (8.82)	56.59 (57.49)	4.47 (4.79)
$[\text{CuCl}_2(\text{L}_{\text{I-p-CH}_3})\text{H}_2\text{O}]$	yellow	2.00	34.5	16.49 (16.53)	53.97 (53.06)	5.56 (4.68)
$[\text{CuCl}_2(\text{L}_{\text{II-m-F}})\text{H}_2\text{O}]$	brown	1.95	30.2	16.64 (16.92)	42.55 (44.75)	4.22 (3.48)
$[\text{CuCl}_2(\text{L}_{\text{III-m-CH}_3})\text{H}_2\text{O}]$	grean	1.83	17.3	15.87 (15.91)	51.40 (51.07)	5.32 (5.14)
$[\text{ZnCl}_2(\text{L}_{\text{I-m-OCH}_3})_2]$	white	diam.	5.8	9.82 (9.78)	55.17 (61.05)	5.22 (5.39)
$[\text{ZnCl}_2(\text{L}_{\text{II-m-Cl}})_2]$	white	diam.	4.3	9.60 (10.04)	48.30 (51.59)	3.89 (3.68)
$[\text{ZnCl}_2(\text{L}_{\text{III-m-Cl}})_2]$	rose	diam.	3.6	8.63 (9.25)	50.91 (54.30)	4.05 (4.52)

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

The results of the elemental analyses of the complexes, as well as the magnetic moment and molar conductance data are summarized in Table II. All the complexes

are insoluble in most common organic solvents. They are highly soluble in DMF, somewhat less soluble in MeOH and EtOH.

The molar conductances of the cobalt(II) and zinc(II) complexes in DMF solutions fall in the range 2.1–11.2 S cm² mol⁻¹. These values indicate that the complexes behave as non-electrolytes in DMF.¹⁹ The molar conductance values of the copper(II) complexes, compared with the values of non-electrolytes, are higher (17.3–34.5 S cm² mol⁻¹). These values are considerably less than the molar conductances of 1:1 type electrolytes ($\lambda_M = 65\text{--}90$ S cm² mol⁻¹),¹⁹ which indicates the partial substitution of coordinated chloride ions with solvent molecules.

Magnetic properties

An indication of the most probable geometric configuration of the synthesized Co(II) complexes is given by their colour and magnetic moments (Table II). Namely, blue cobalt(II) complexes usually have a tetrahedral configuration. The magnetic moment values (Table II) of the cobalt(II) complexes are in the expected range (4.2–4.7 μ_B) for tetrahedral stereochemistry.²⁰

The room temperature effective magnetic moments of the copper(II) complexes are in the range of 1.73–2.20 μ_B , which corresponds to one unpaired electron typical for tetrahedral geometry.²¹

Electronic spectra

The electronic spectra of the investigated complexes and those of the corresponding ligands were recorded in DMF (Table III). The cobalt(II) complexes retained their blue colour in solution, the zinc(II) complexes are uncoloured, but all the different coloured copper(II) complexes are yellow in DMF solution.

TABLE III. Electronic spectral data of the complexes

Complex	Absorption maxima [kK] [*]	(ϵ_{\max})[dm ² mol ⁻¹ cm ⁻¹]
[CoCl ₂ (L _{I-p-CH₃}) ₂]	35.7sh (1110) 34.5 (1210) 16.4sh (280)	15.9 (320) 15.3sh (310)
[CoCl ₂ (L _{II-m-Cl}) ₂]	35.7sh (840) 33.9 (1040) 16.2sh (360)	15.9 (380) 15.4sh (360)
[CoCl ₂ (L _{III-m-F}) ₂]	35.7sh (920) 33.3 (1140) 17.1sh (260)	16.1 (470) 15.4sh (470)
[CuCl ₂ (L _{I-p-CH₃})H ₂ O]	35.7sh (385) 34.0 (475) 28.1 (240)	23.8sh (100)
[CuCl ₂ (L _{III-m-F})H ₂ O]	35.7sh (525) 33.6 (630) 28.2 (275)	23.5sh (130)
[CuCl ₂ (L _{II-m-CH₃})H ₂ O]	35.8sh (480) 33.8 (690) 28.6 (310)	26.7 sh (200)
[ZnCl ₂ (L _{I-m-OCH₃}) ₂]	35.7sh (375) 34.5 (430)	
[ZnCl ₂ (L _{II-m-Cl}) ₂]	35.7 sh (500) 34.0 (590)	
[ZnCl ₂ (L _{III-m-Cl}) ₂]	35.7 sh (455) 33.6 (560)	

^{*} sh = shoulder, kK = 1000 cm⁻¹

The spectra of the ligands show two intense bands in the UV region around 40 and 35 kK (splitted); the former may be assigned to the imidazole moiety and the latter to the benzene ring of the ligand.²² The splitting of the second band may be due to a lowering of the symmetry of the benzene ring.

The complexes exhibit very intense bands in the UV region and weak broad bands in the visible region (except the Zn(II) complexes). The positions and features of the bands in the UV region are nearly the same as those of the ligand bands.

The electronic absorption spectra of the cobalt(II) complexes show a broad band in the 15.2–17.1 kK region. The visible spectrum is dominated by the highest energy $^4A_2 \rightarrow ^4T_1(P)$ transition,^{20,23} typical for tetrahedral cobalt(II) complexes. The high molar extinction coefficients of the cobalt(II) complexes and their blue colour suggest the tetrahedral configuration is retained in solution.²³

The electronic absorption spectra of the copper(II) complexes show a broad absorption band about 28 kK and a shoulder around 23–26 kK. The broad band at 28 kK overlaps with the intense band of the ligand at about 35 kK.²⁴

The electronic spectra of the zinc(II) complexes exhibit very intense bands in the UV region around 35 kK, the same as those of the ligand bands.

Infrared spectra

The infrared spectra of the ligands exhibit a band at 3450–3330 cm^{-1} and ca. 1650 cm^{-1} , assigned to $\nu(\text{NH}_2)$ and $\delta(\text{NH}_2)$ of the benzimidazole ring, respectively²⁵ (series II and III). The band appearing at about 1550 cm^{-1} , for all the ligands, may be assigned to $\nu(\text{C}=\text{N})$ vibrations.^{24,25} The substituted phenyl group shows ring vibrations at 1485 and 740 cm^{-1} . The infrared spectra of the complexes investigated are similar to those of the corresponding ligands.

An upward shift (5–15 cm^{-1}) of $\nu(\text{C}=\text{N})$ band in the IR spectra of the complexes as compared to its value in the free ligand, suggests coordination through the pyridine nitrogen of the benzimidazoles.²⁴ The bands due to $\nu(\text{NH}_2)$ and $\delta(\text{NH}_2)$ in the complexes are shifted to lower frequency in all the complexes. These shifts may be indicative of the presence of hydrogen bonding.^{26,27} In the case of $[\text{CuCl}_2\text{L}(\text{H}_2\text{O})]$, hydrogen bonds may be formed between the NH_2 group of the ligands and the coordinated 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, electronic and IR spectra) suggest that the Co(II) and Zn(II) complexes are tetrahedral, which is realized by participation of the pyridine nitrogen of two organic ligand molecules and two chloride anions, typical for these classes of organic ligands.^{28,29} In the case of the Cu(II) complexes, two types of geometries of tetracoordinated copper(II) are possible: the tetrahedral and square-planar.³⁰ From the presented results, it was not

possible to determine the exact geometry. We concluded that the real structure is between the tetrahedral and square-planar geometries. This configuration is realized by the participation of the pyridine nitrogen of one organic ligand molecule, two chloride anions and one molecule of water.

Antibacterial activities

Some recent papers^{18,31,32} dealing with the antibacterial, antiviral, antihistaminic and fungicidal activity of benzimidazoles encouraged us to investigate the biological activity of the complexes synthesized in the present work. The antibacterial activity of these complexes was tested against phytopathogenic strains of bacteria in order to obtain new potent formulations for plant protection. All the complexes were screened for their antibacterial activities against *Erwinia carotovora* subsp. *carotovora* and *Erwinia amylovora*. The relevant data are presented in Table IV.

TABLE IV. Antibacterial activity of the benzimidazole derivatives and their complexes

Compound	Inhibition zone diameter (mm)	
	<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	<i>Erwinia amylovora</i>
Ligands		
I- <i>p</i> -CH ₃	0	0
I- <i>m</i> -OCH ₃	0	0
II- <i>m</i> -Cl	16	22
II- <i>m</i> -F	15	23
III- <i>m</i> -CH ₃	13	17
III- <i>m</i> -Cl	10	9
III- <i>m</i> -F	8	11
Complexes		
[CoCl ₂ (L _{I-<i>p</i>-CH₃}) ₂]	0	0
[CoCl ₂ (L _{II-<i>m</i>-Cl}) ₂]	20	25
[CoCl ₂ (L _{III-<i>m</i>-F}) ₂]	12	15
[CuCl ₂ (L _{I-<i>p</i>-CH₃})H ₂ O]	0	0
[CuCl ₂ (L _{II-<i>m</i>-F})H ₂ O]	22	30
[CuCl ₂ (L _{III-<i>m</i>-CH₃})H ₂ O]	17	17
[ZnCl ₂ (L _{I-<i>m</i>-OCH₃}) ₂]	0	0
[ZnCl ₂ (L _{II-<i>m</i>-Cl}) ₂]	18	23
[ZnCl ₂ (L _{III-<i>m</i>-Cl}) ₂]	12	16

From the data, it is evident that ligands belonging to the series II and III and their complexes are active against both of the bacteria under investigations. In the case of the ligands, the most active compounds are from series II. Benzimidazoles from the series III come next in order of activity. It was found that compounds of series I are not effective against the investigated bacteria.

From the results, it can be concluded that the basic antibacterial activity of the benzimidazoles is due to the presence of an amino group at position 2 of the benzimidazole ring. At the same time, methyl groups at the 5 or 6 position decreases the general antibacterial activity of the relevant benzimidazoles.

On comparing the biological activity of the ligands and their complexes, it was found that the complexes are more effective against both of the bacteria.^{26,33} The higher activity of the complexes, as compared to the free ligands, can be understood in terms of the chelation theory.³⁴ This theory explains that a decrease in the polarizability of the metal could enhance the lipophilicity of the complexes.

Of the complexes, the most active compounds are those containing copper(II). This may be attributed to the high biological activity of free copper(II) ions.

From the present investigations, it can be concluded that the newly synthesized benzimidazoles and their complexes could find application as various pharmaceuticals and for plant protection.

ИЗВОД

КОБАЛТ(II), ЦИНК(II) И БАКАР(II) КОМПЛЕКСИ СА НОВОСИНТЕТИСАНИМ ДЕРИВАТИМА БЕНЗИМИДАЗОЛА

С. О. ПОДУНАВАЦ-КУЗМАНОВИЋ^а, В. М. ЛЕОВАЦ^б, Н. У. ПЕРИШИЋ-ЈАЊИЋ^б, Ј. РОГАН^в и Ј. БАЛАЖ^а

^аТехнолошки факултет, Булевар Цара Лазара 1, 21000 Нови Сад, ^бИнститут за хемију, ПМФ, Трг Досијеја Обрадовића 3, 21000 Нови Сад, ^вТехнолошко-металуршки факултет, Карнегијева 4, 11001 Београд и ^дПољопривредни факултет, Трг Досијеја Обрадовића 1, 21000 Нови Сад

Описана је синтеза тетраедарских комплекса кобалта(II), цинка(II) и бакра(II) са новосинтетисаним дериватима бензимидазола опште формуле $[MCl_2L_2]$ ($M=Co(II), Zn(II)$) и $[CuCl_2L(H_2O)]$. Комплекси су окарактерисани елементарном анализом, магнетним и кондуктометријским мерењима, IR и електронским апсорпционим спектрима. Испитана је антибактеријска активност деривата бензимидазола и њихових комплекса на бактерије *Erwinia carotovora* subsp. *carotovora* и *Erwinia amylovora*. Нађено је да комплекси показују већу биолошку активност од самих лиганата.

(Примљено 21. октобра 1998, ревидирано 18. јануара 1999)

REFERENCES

1. K. H. Gibson, R. Wood, A. A. Oldham, J. S. Major, *Bioorg. Med. Chem. Let.* **3** (1993) 1019
2. I. Yalcin, E. Sener, *Int. J. Pharm.* **98** (1993) 1
3. D. L. Brandon, R. G. Binder, R. E. Wilson, W. C. Montague, *J. Agr. Food Chem.* **41** (1993) 996
4. R. R. Tidwell, S. K. Jones, N. A. Naimen, L. C. Berger, W. B. Brake, C. C. Dykstra, J. E. Hall, *Antimicrob. Agents Chemother.* **37** (1993) 1713
5. L. Mishra, V. K. Singh, N. K. Dubey, A. K. Mishra, *Biosci. Biotech. Biochem.* **57** (1993) 989
6. H. S. Gunes, G. Cosar, *Arzn. Forch. Drug. Res.* **42-2** (1992) 1045
7. M. Tuncbilek, H. Goker, R. Ertan, R. Eryigit, E. Kendi, N. Atlantar, *Arch. Pharm.* **330** (1997) 372
8. I. Oren, O. Temiz, I. Yalcin, E. Sener, A. Akin, N. Ucatürk, *Arzn. Forch. Drug. Res.* **47** (1997) 1393
9. A. Sparatore, F. Novelli, F. Sparatore, *Farmaco* **52** (1997) 509

10. H. Kucukbay, B. Durmaz, *Arzn. Forch. Drug. Res.* **47** (1997) 667
11. M. R. Cuberes, M. Contijoch, C. Calvet, J. Alegre, J. R. Quantana, J. Frigola, *Chem. Pharm. Bull.* **45** (1997) 1287
12. K. S. Gudmudsson, J. C. Drach, L. L. Wotring, L. B. Townsend, *J. Med. Chem.* **40**(1997) 785
13. L. Mishra, V. K. Singh, *Synth. React. Inorg. Met.- Org. Chem.* **24** (1994) 95
14. L. Mishra, V. K. Singh, *Indian J. Chem. Sect. A* **32** (1993) 446
15. V. Alexander, *Inorg. Chim. Acta* **204** (1993) 109
16. F. Kratz, B. Nuber, J. Weiss, B. K. Keoler, *Polyhedron* **11** (1992) 487
17. V. K. Revankar, V. B. Mahale, *Indian. J. Chem. Sect. A.* **28** (1989) 683
18. Đ. Vlaović, J. Čanadanović-Brunet, J. Balaž, I. Jurnaić, D. Đoković, K. Mackenzie, *Biosci. Biotech. Biochem.* **56** (1992) 199
19. W. J. Geary, *Coord. Chem. Rev.* **7** (1971) 81
20. M. Goodgame, F. A. Cotton, *J. Am. Chem. Soc.* **84** (1962) 1543
21. F. E. Mabbs, D. J. Machin, *Magnetism and Transition Metal Complexes*, Chapman Hall, London (1973)
22. C. N. Rao, *Ultra-violet and Visible Spectroscopy*, 3rd ed., Butterworth and Co. Ltd., (1975)
23. F. A. Cotton, G. Wilkinson, *Advanced Inorganic Chemistry*, Wiley, New York (1989)
24. V. H. Arali, V. K. Revankar, V. B. Mahale, P. J. Kulkarni, *Transition Met. Chem.* **19** (1994) 57
25. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, Wiley, New York (1986)
26. S. B. Balaji, N. M. Nanje Gowda, *Indian J. Chem.* **31** (1992) 463
27. L. Mishra, Y. K. Singh, *Indian J. Chem.* **32 A** (1993) 446
28. F. Mani, G. Scapacci, *Inorg. Chim. Acta* **16** (1976) 163
29. J. B. Hodgson, G. C. Percy, D. A. Thornton, *J. Mol. Struct.* **66** (1980) 75
30. B. J. Hathaway, "Copper", *Comprehensive Coordination Chemistry*, G. Wilkinson, R. D. Gillard, J. Mc. Cleverty, Eds., Pergamon Press, Oxford (1987)
31. G. A. Mokrushina, S. K. Kotovskaya, G. N. Turienkova, V. I. Iliencko, V. G. Platonov, I. V. Kiselieva, *Khim. pharm. zh.* (1988) 195
32. N. Noyanalpan, E. Bercin, P. Cakar, V. Abbasoglu, *Gazi. Univ. Eczacilik Fak. Derg.* **4** (1987) 33
33. R. K. Parashar, R. C. Sharma, *Inorg. Chim. Acta* **151** (1988) 201
34. R. S. Srivastava, *Inorg. Chim. Acta* **56** (1981) 65.