Molecules **2007**, *12*, 782-796

molecules

ISSN 1420-3049

http://www.mdpi.org

Full Paper

Complexes of $3d^n$ Metal Ions with Thiosemicarbazones: Synthesis and Antimicrobial Activity

Tudor Rosu [1,*], Aurelian Gulea [2], Anca Nicolae [3] and Rodica Georgescu [4]

[1] Inorganic Chemistry Department, University of Bucharest, 23 Dumbrava Rosie Street, sector 2, Bucharest, Romania

[2] Faculty of Chemistry, Inorganic Chemistry Department, USM-R. Moldavia; E-mail: dociu1946@yahoo.com

[3] Organic Chemistry Department, University of Bucharest, 90 – 92 Panduri Bd., sector 4, Bucharest, Romania; E-mail: nicolae.anca@unibuc.ro

[4] Nuclear Physics and Engineering Institute "Horia Hulubei", Bucharest, Romania; E-mail: r_georgescu@irasm.ro

* Author to whom correspondence should be addressed; E-mails: t_rosu0101@yahoo.com or rosu.tudor@unibuc.ro

Received: 27 February 2007; in revised form: 5 April 2007 / Accepted: 12 April 2007 / Published: 17 April 2007

Abstract: The chelating behavior of the thiosemicarbazone derivatives of 2-hydroxy-8-R-tricyclo[7.3.1.0.^{2,7}]tridecane-13-one (where R = H, CH_3, C_6H_5) towards Co(II), Ni(II) and Cu(II) has been investigated by elemental analysis, molar conductivity measurements, UV-VIS, IR, ESR spectroscopy and thermal studies. It was deduced from the experiments performed that the ligands coordinate to metal ions in different ways – neutral bidentate or mononegative bidentate – depending on the nature of R. Also, if metal acetates are used instead of metal chlorides, the ligands coordinate in a mononegative bidentate fashion, regardless of the nature of R or the thiosemicarbazone type ligand. The antimicrobial activity of the ligands and of the complexes towards samples of *Acinetobacter boumanii, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* was determined.

Keywords: Thiosemicarbazone complexes, copper complexes, thiosemicarbazones, antimicrobial activity

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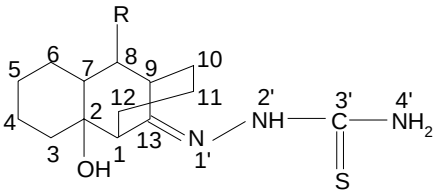
Introduction

Thiosemicarbazones are known for their capacity to act as polydentate ligands, as well as for their biological activity. Collins *et al*. have reported the correlation between structure and anti-mycobacterial activity in a series of 2-acetylpyridine thiosemicarbazones [1]. In many cases, by coordination to different transition metal ions that can be found in biological systems, it is possible to obtain complexes that are more efficient drugs than the corresponding free ligands [2a,b,c,d,e].

Copper(II) complexes possess a wide range of biological activity and are among the most potent antiviral, antitumor and anti-inflammatory agents. For example, a copper(II) complex of 2-formylpyridine thiosemicarbazone has been shown to inhibit the RNA-dependent DNA polymerases and the transforming ability of Rous sarcoma virus (RSV) [3]. In addition, copper(II) complexes of 2-acetylpyridine thiosemicarbazones are active anti-malarial agents [4]. They possess strong antineoplastic activity against a number of transplantable tumors, spontaneous murine tumors and human tumors. The mechanism of their antitumor action is thought to involve either inhibition of the enzyme ribonucleotide reductase, an obligatory enzyme in DNA synthesis [5-7], or creation of lesions in DNA strands [8]. The antifungal activity of Ni(II), Cu(II) and Zn(II) complexes with 5-nitro-2-furfural thiosemicarbazone has been demonstrated [9,10]. In addition, Agarwal *et al*. reported in 2006 the synthesis, magnetospectral, antibacterial, and antifungal properties of Cu(II) complexes of 4[N-(benzylidene)amino]-, 4[N-(4-methoxybenzylidene)amino]-, 4[N-(4-dimethylaminobenzylidene) amino]- and 4[N(cinnamalidene)amino] antipyrine thiosemicarbazone [11].

This paper describes the synthesis and characterization of some complexes of transition metal ions Co(II), Ni(II) and Cu(II) with a series of thiosemicarbazone derivatives 2-hydroxy-8-R-tricyclo-[7.3.1.0.^{2,7}]tridecane-13-one (R = H, CH_3, C_6H_5).

Figure 1. The structures of the ligands (L_H, L_{CH3}, L_{C6H5}).



R = H ,CH_3 , C_6H_5

Characterization of the newly prepared complexes was accomplished by IR, UV-VIS and ESR spectroscopy, thermal analysis and molar electrical conductivity measurements. From the experimental data it was inferred that depending on the nature of R, the ligand coordinates to metal ions in different ways: neutral bidentate or mononegative bidentate. Also, if metal acetates are used instead of metal chlorides, then the ligands coordinate in a mononegative bidentate fashion, regardless of the nature of R or type of thiosemicarbazone ligand. In addition, taking into consideration the use of copper complexes in the treatment of some diseases, mentioned above, we tested the antimicrobial activity of the prepared combinations using strains of *Acinetobacter boumanii, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* isolated from different pathological products obtained from patients with infections associated with the use of cardiovascular prosthetic devices.

Results and Discussion

Synthesis

The ligands L_H, L_{CH_3} and $L_{C_6H_5}$ used in this work were obtained by the method described elsewhere [12]. The $[M(L_H)_2]Cl_2$, $[M(L_R)_2]$ complexes (M = Cu(II), Ni(II), Co(II); R = CH_3, C_6H_5) and $[ML_R(H_2O)_2ac]$ complexes (M = Cu(II), Ni(II), Co(II); R = H, CH_3, C_6H_5) were prepared by reaction of the corresponding ligands with methanol solutions of a suitable M(II) salt.

Properties

The molar conductivity values ($\Omega^{-1}\cdot cm^2\cdot mol^{-1}$) of the complexes in nitrobenzene $[M(L_H)_2]Cl_2$ (55.7 for $[Cu(L_H)_2]Cl_2$, 53.9 for $[Co(L_H)_2]Cl_2$ and 58.4 for $[Ni(L_H)_2]Cl_2$, respectively) indicate that these complexes are 1:2 type electrolytes. The complexes display low solubility in chloroform, acetone and water, but they are soluble in methanol and dimethylformamide (DMF). The color of aqueous solutions changes after some 10 minutes, indicating that the complexes decompose in water. The molar conductibility values below 10 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ for the other complexes, in nitrobenzene, indicate that these complexes are non-electrolytes.

Thermal Decomposition

The complexes of this study were investigated by thermogravimetry. The TG and DTG curves for all the complex combinations are similar, with the exception of the loss of weight temperatures. The decomposition takes place in two steps for $[M(L_H)_2]Cl_2$, $[M(L_{CH3})_2]$ and $[M(L_{C6H5})_2]$ type complexes (220 – 450 and 430 – 690°C) and in three steps for $[ML_R(H_2O)_2ac]$ type complexes (112 – 153, 174 – 357 and 452 – 697°C).

The weight loss between 112 – 153°C is due to the loss of two water molecules per molecule of complex. Experimental data for the thermogravimetric analyses are presented in Table 1. The final residues were analyzed by IR spectroscopy and identified as CuO, Co_2O_3 and NiO, and the %M corresponded to the calculated one.

IR Spectra

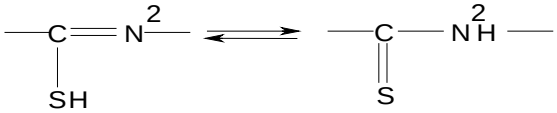
Relevant IR data are given in Table 2. The IR spectra of the ligand L_H shows an absorption band due to $\nu_{C=S}$ at 740 cm^{-1}, associated with the $\nu_{C=S} + \nu_{C=N}$ absorption band at 1291 cm^{-1}, both of medium intensity. In the IR spectra of $[M(L_H)_2]$, the specific band for $\nu_{C=S}$ has a negative shift associated with a positive shift of the absorption band for $\nu_{C=S}+\nu_{C=N}$. This suggests that the ligand coordinates through the thiocarbonyl sulfur. The $\nu_{C=N}{}^{1'}$ frequency values decreased and the values of the $\nu_{N}{}^{1'}{}_{-N}{}^{2'}$ frequencies increased in the IR spectra of the complexes, compared with the values of the ligand spectrum, without a significant change in the $\nu_{N}{}^{2'}{}_{H}$ frequencies, suggesting that the ligand L_H coordinates to metal ions through the $N^{1'}$ atom.

Table 1: Thermogravimetric analysis data of the prepared complex combinations.

Compound	Temperature range (°C)	Eliminated fragment	Weight loss %
$[M(L_H)_2]Cl_2$	220-355	Cl_2+L_H	49.50-51.00
	430-680	L_H	39.00-41.00
	680	MO	10.70-12.50
$M(L_{CH3})_2]$	240-420	L_{CH3}	44.00-46.00
	450-685	L_{CH3}	44.00-45.00
	685	MO	11.00-13.00
$[M(L_{C6H5})_2]$	248-450	L_{C6H5}	45.93-46.00
	453-690	L_{C6H5}	45.93-46.20
	690	MO	9.50-10.25
$[M(L_H)(H_2O)_2ac]$	112-137	H_2O	7.50-8.50
	174-346	Ac	12.93-13.70
	452-657	L_H	63.00-64.23
	657	MO	16.90-18.45
$[M(L_{CH3})(H_2O)_2ac]$	115-145	H_2O	7.40-8.50
	180-354	Ac	12.65-13.47
	463-668	L_{CH3}	64.10-65.23
	668	MO	16.43-17.85
$[M(L_{C6H5})(H_2O)_2ac]$	119-153	H_2O	6.73-7.55
	182-357	Ac	11.00-12.32
	467-697	L_{C6H5}	69.86-72.13
	697	MO	14.20-15.69

In the IR spectra of the $[M(L_{CH3})_2]$ and $[M(L_{C6H5})_2]$ complexes, the $\nu_N{}^{2'}{}_H$ band disappears and the $\nu_{C=N}{}^{1'}$ and $\nu_N{}^{1'}{}_{-N}{}^{2'}$ bands act in the same way as in the $[M(L_H)_2]Cl_2$ complexes. Also, the $\nu_{C=S}$ band and the associated $\nu_{C=S} + \nu_{C=N}$ band disappeared and ν_{C-S} band appears in the 547-600 cm^{-1} range. This behavior confirms the coordination of ligand by sulfur atoms in the deprotonated thioenolic form. The ligand L_H coordinates differently to metallic ions, as compared to the L_{CH3} and L_{C6H5} ligands. This behavior is probably due to the influence of the R radical from the ligand L_R upon the unstable electronic pair of sulfur atom in the presence of the adjacent H atom, making possible the thioketo-thioenol equilibrium shown in Figure 2.

Figure 2.



In the IR spectra of $[ML_R(H_2O)_2ac]$ complexes the $\nu_{C=N}{}^{1'}$, $\nu_N{}^{1'}{}_{-N}{}^{2'}$, $\nu_N{}^{2'}{}_H$, $\nu_N{}^{4'}{}_H$, $\nu_{C=S}$, $\nu_{C=S}$, $\nu_{C=N}$, $\nu_{C=N}{}^{2'}$ and ν_{C-S} frequencies display almost the same behavior as in the spectra of the $[M(L_{CH3s})_2]$ and $[M(L_{C6H5})_2]$ complexes. Additionally, two absorption bands in the 1431-1469 cm^{-1} and 1509-1532 cm^{-1}

ranges, assigned to $\nu_{c=o(s)}$ and $\nu_{c=o(as)}$ frequencies, confirm the bidentate nature of coordinated acetate [13, 14]. In addition, a band appears in the 805-913 cm^{-1} range, which can be attributed to the ρ_r vibration mode specific to water molecules coordinated to metal ions [13, 14].

Table 2. IR spectral data for the ligands and their complexes $(cm)^{-1}$.

Compound	$\nu_{C=N}^{1'}$	$\nu_{N}^{1'}{}_{-N}^{2'}$	$\nu_{N}^{2'}{}_{H}$	$\nu_{N}^{4'}{}_{H}$	$\nu_{C=S}$; $\nu_{C=S}$ + $\nu_{C=N}$	$\nu_{C=O(as)}$ $\nu_{C=O(s)}$	$\nu_{C=N}^{2'}$	ν_{C-S}	ν_{C-O}	ν_{OH}
$\mathbf{L_H}$	1599	1016	3230	3319	740 1291	-	-	-	-	3426 1144
$[M(L_H)_2]Cl_2$										
$(CuC_{28}H_{48}N_6O_2S_2)Cl_2$	1556	1056	3232	3322	712 1315	-	-	-	-	3424 1143
$(CoC_{28}H_{48}N_6O_2S_2)Cl_2$	1564	1060	3229	3318	716 1320	-	-	-	-	3425 1142
$(NiC_{28}H_{48}N_6O_2S_2)Cl_2$	1560	1058	3233	3320	718 1317	-	-	-	-	3420 1140
$\mathbf{L_{CH3}}$	1621	1060	3213	3330	782 1296	-	-	-	-	3425 1140
$[M(L_{CH3})_2]$										
$Cu(C_{30}H_{50}N_6O_2S_2)$	1580	1094	-	3329	-	-	1595	547	-	3422 1142
$Co(C_{30}H_{50}N_6O_2S_2)$	1578	1103	-	3332	-	-	1598	562	-	3420 1138
$Ni(C_{30}H_{50}N_6O_2S_2)$	1575	1098	-	3328	-	-	1593	554	-	3424 1138
$\mathbf{L_{C6H5}}$	1633	1075	3191	3338	798 1302	-	-	-	-	3423 1132
$[M(L_{C6H5})_2]$										
$Cu(C_{40}H_{54}N_6S_2)$	1591	1108	-	3340	-	-	1615	590	-	3430 1138
$Co(C_{40}H_{54}N_6O_2S_2)$	1593	1113	-	3336	-	-	1619	601	-	3426 1130
$Ni(C_{40}H_{54}N_6O_2S_2)$	1586	1109	-	3340	-	-	1613	595	-	3428 1136
$[ML_H(H_2O)_2ac]$										
$Cu(C_{16}H_{30}N_3O_5S)$	1559	1047	-	3325	-	1532 1469	1586	552	-	3418 1137
$Co(C_{16}H_{30}N_3O_5S)$	1568	1050	-	3321	-	1526 1452	1589	571	-	3419 1138
$Ni(C_{16}H_{30}N_3O_5S)$	1565	1049	-	3323	-	1517 1448	1581	564	-	3415 1139

Table 2. Cont.

Compound	$\nu_{C=N}^{1'}$	$\nu_{N}^{1'}{}_{-N}^{2'}$	$\nu_{N}^{2'}{}_{H}$	$\nu_{N}^{4'}{}_{H}$	$\nu_{C=S}$; $\nu_{C=S}$ + $\nu_{C=N}$	$\nu_{C=O(as)}$ $\nu_{C=O(s)}$	$\nu_{C=N}^{2'}$	ν_{C-S}	ν_{C-O}	ν_{OH}
[$ML_{CH3}(H_2O)_2$ac]										
Cu($C_{17}H_{32}N_3O_5S$)	1572	1078	-	3332	-	1528 1462	1603	562	-	3420 1141
Co($C_{17}H_{32}N_3O_5S$)	1569	1087	-	3336	-	1521 1448	1598	567	-	3419 1139
Ni($C_{17}H_{32}N_3O_5S$)	1567	1082	-	3334	-	1514 1437	1592	561	-	3422 1137
[$ML_{C6H5}(H_2O)_2$ac] Cu($C_{22}H_{34}N_3O_5S$)	1583	1098	-	3342	-	1522 1458	1608	583	-	3425 1135
Co($C_{22}H_{34}N_3O_5S$)	1580	1101	-	3339	-	1518 1442	1602	594	-	3430 1134
Ni($C_{22}H_{34}N_3O_5S$)	1578	1097	-	3341	-	1509 1431	1595	580	-	3428 1137

Electronic spectra

The electronic spectroscopy data for the complexes in solid state are presented in Table 3. The values of the electronic transitions specific for the complex combinations [Cu(L_R)] are very close (±30 cm^{-1}). For [$CoL_R(H_2O)_2$ac] and [$NiL_R(H_2O)_2$ac] complexes, the values of the B, β and Dq parameters are also very close. These values indicate a similar ligand field strength. The electronic transitions of [$ML_R(H_2O)_2$ac] complexes suggest an octahedral geometry around the metal ion.

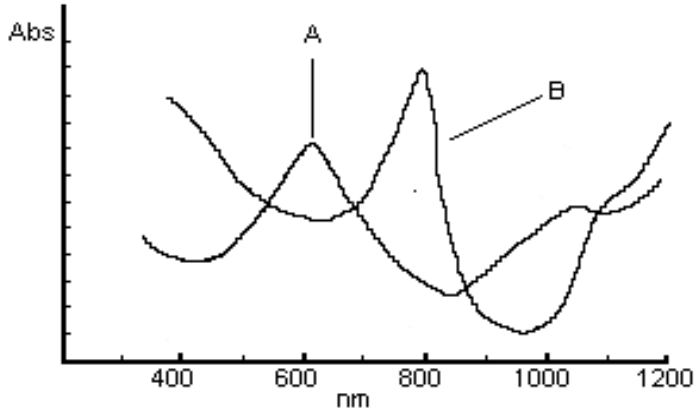
Table 3. Electronic spectral data for complexes in solid state (cm^{-1}).

Compound	**Transitions d - d**			**B**	**β**	**D_q**	**Geometry**
[Cu(L_H)$_2$] Cl_2	$^2B_2 \rightarrow {}^2A_1$ 13710			-	-	-	Pseudo-tetrahedral
[Cu(L_{CH3})$_2$] **[Cu(L_{C6H5})$_2$]**	12630						Pseudo-tetrahedral
[Co(L_H)$_2$] Cl_2	- -	$^4A_2 \rightarrow {}^4T_1$ 9340	$^4A_2 \rightarrow {}^4T_1$(P) 16445	919	0,947	- -	Pseudo-tetrahedral
[Co(L_{CH3})$_2$] **[Co(L_{C6H5})$_2$]**	-	9090	13030	808	0,833	-	Pseudo-tetrahedral
[Ni(L_H)$_2$] Cl_2	ν_1 12320	ν_2 22480	ν_3 25790				Sq - pl
[Ni(L_{CH3})$_2$] **[Ni(L_{C6H5})$_2$]**	11650	21200	24350				Sq - pl
[Cu$L_R(H_2O)_2$ac]	$xy \rightarrow x^2 - y^2$ 12450	$xz, yz \rightarrow x^2 - y^2$ 16640	- -	-	-	-	O_h
[Co$L_R(H_2O)_2$ac]	$^4T_{1g} \rightarrow {}^4T_{2g}$ 10020	$^4T_{1g} \rightarrow {}^4A_{2g}$ -	$^4T_{1g} \rightarrow {}^4T_{1g}$(P) 20830	887	0,914	1252	O_h
[Ni$L_R(H_2O)_2$ac]	$^3A_{2g} \rightarrow {}^3T_{2g}$ 10210	$^3A_{2g} \rightarrow {}^3T_{1g}$ 18330	$^3A_{2g} \rightarrow {}^4T_{1g}$(P) 27140	991	0,952	1022	O_h

The same phenomenon may be seen for the $[Cu(L_R)]Cl_2$ complexes. A minor change in electronic transition values can be seen also for Co (II) and Ni (II) complexes. This behavior proves that the ligand field strength is essentially the same for this type of complexes with the same metal ions, regardless of the nature of R. The inter-electronic repulsion values could be calculated only for some of the complexes prepared, using the d-d transition energy values. The values of the B and β parameters indicate a covalent bond between M-L in $[Co(L_R)_2]$ complexes, compared to $[Co(L_R)_2]Cl_2$ complexes.

For $[M(L_R)_2]$ and $[M(L_R)_2]Cl_2$ (M=Cu, Co) complexes, the energy values of d-d transitions suggest a distorted tetrahedral arrangement for the metal ion, and a square-planar arrangement for $[Ni(L_R)_2]$ and $[Ni(L_R)_2]Cl_2$ complexes [10,13,15]. Figure 3 shows the electronic spectrum for $[Co(L_R)_2]Cl_2$, (A) and $[Co(L_R)_2]$, (B) complexes.

Figure 3. Electronic spectra of $Co(L_H)_2]Cl_2$, (A) and $[Co(L_R)_2]$, (B) complexes.



ESR Spectra

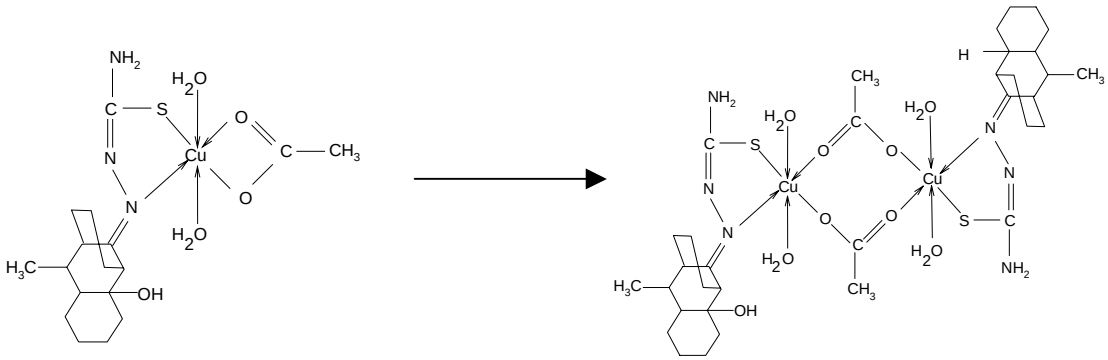
X-band ESR spectra for Cu(II) complexes obtained from $CuCl_2$ and L_R were recorded using methanol solutions at 77K. The values of the {g} tensor confirm the uniaxial symmetry that corresponds to the following sequence: $g_z>g_y$; $g_z>g_x$ [16]. The g values for $[Cu(L_R)_2]^n$ complexes, where n = +2, for R=H and n = 0, for R=CH_3, C_6H_5 are presented in Table 4 .

Table 4. The g values determined from ESR spectra.

Compound	g_x	g_y	g_z
$[Cu(L_H)_2]Cl_2$	2.054	2.107	2.211
$[Cu(L_{CH3})_2]$	2.035	2.115	2.203
$[Cu(L_{C6H5})_2]$	2.040	2.116	2.210

If a buffer solution of acetic acid-sodium acetate is added to a methanol solution of $[CuL_{CH3}(H_2O)_2ac]$, a brown precipitate can then be obtained. A strong band around 16230 cm^{-1} specific to octahedral Cu^{2+} characterizes the electronic spectra of this compound. Combining this experimental data with elemental analysis and IR spectra, the reaction mechanism of Scheme 1 can be proposed:

Scheme 1.



If the brown precipitate is dissolved in pyridine, the resulting brown solution becomes blue-green after 24 hours. ESR spectra of the two solutions recorded at 77K (Figure 4) suggest a transformation of compound **1** into compound **2**, as shown in Scheme 2 [17, 18].

Scheme 2.

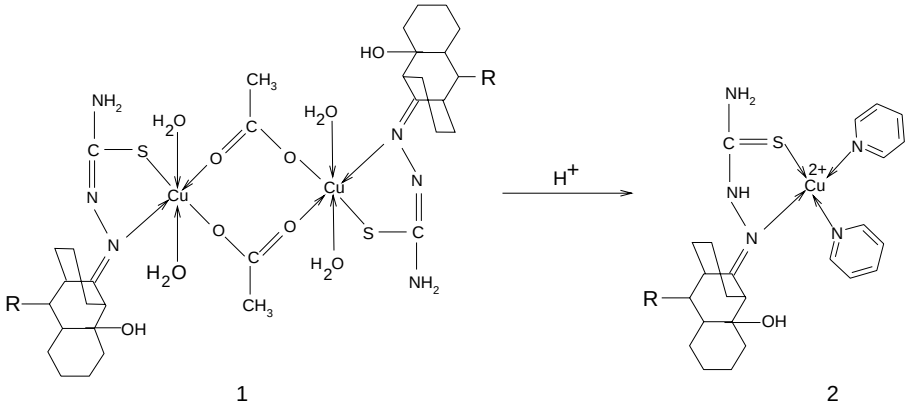
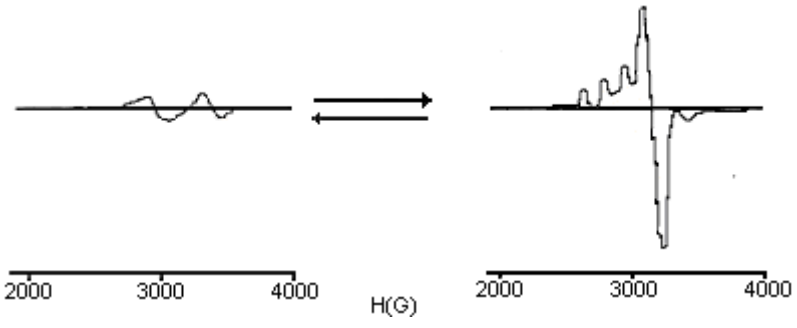
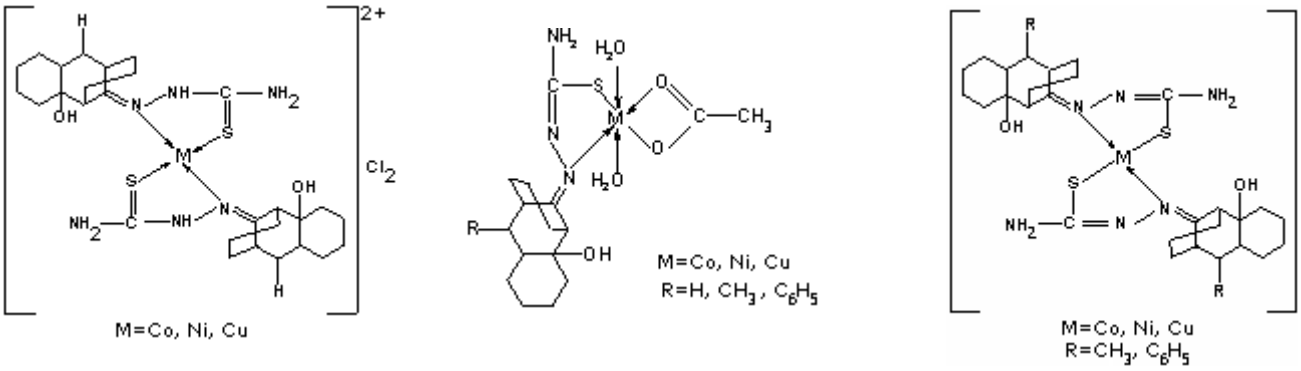


Figure 4. ESR for complexes **1** and **2** in pyridine at 77K.



The ESR spectrum of a frozen solution of compound **2**, recorded at 77 K, shows that the dinuclear complex 1 dissociates to form a mononuclear species with $g_{\perp}$ =2.08, $g_{//}$ =2.25 and $A_{//}$ =155 Gauss. The precise structure of this mononuclear species is still unknown, but the $g_{//}$ and $A_{//}$ values indicate that three nitrogens are probably coordinated to the Cu(II) ion [19]. From the experimental data, different spatial arrangements can be proposed for the prepared complexes (Figure 5).

Figure 5.



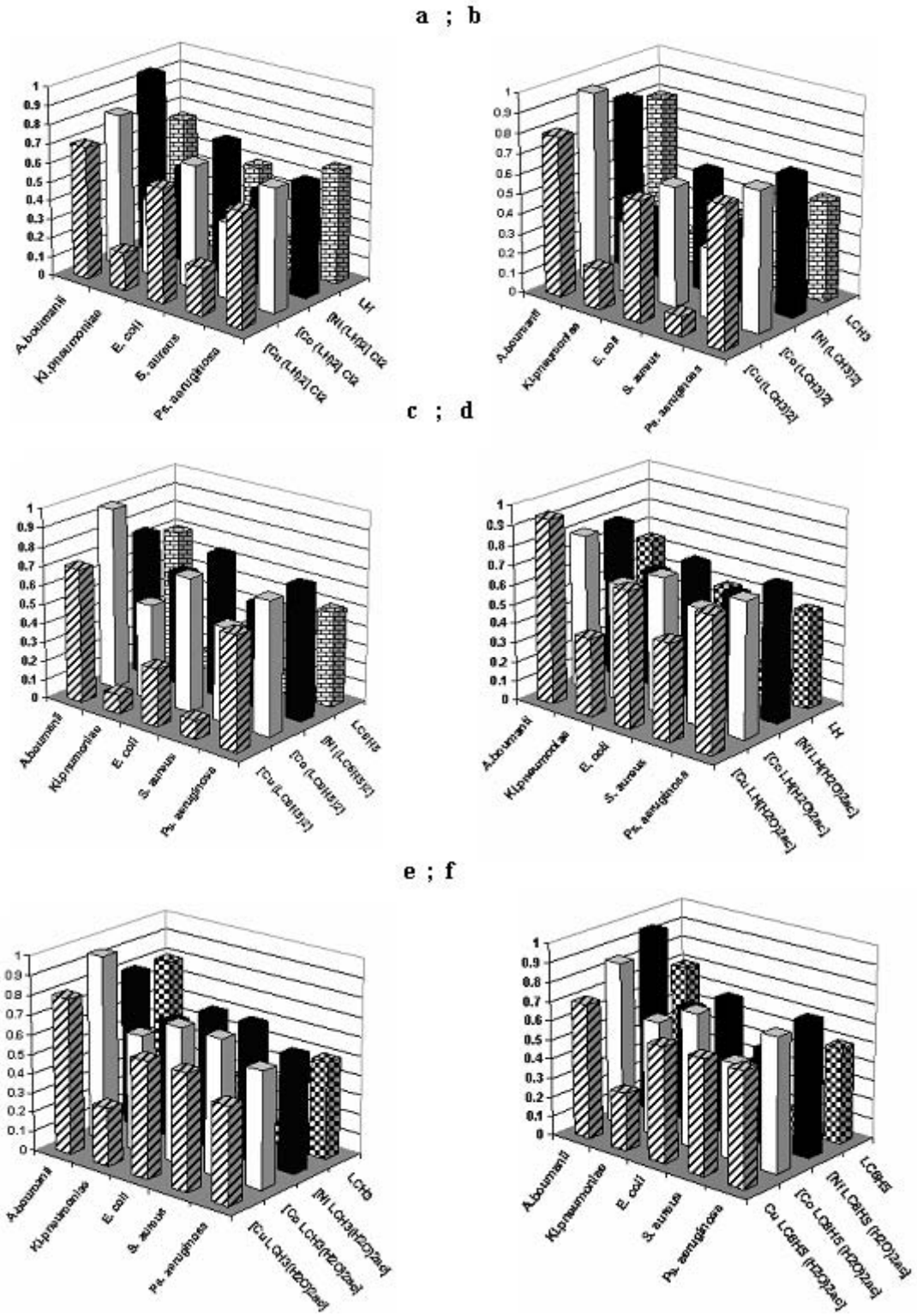
It was found that the ligand with R=H is coordinated in a bidentate neutral mode, while the ligand containing R=CH_3, C_6H_5 is coordinated in a bidentate mononegative way. The presence of an electron donating group (CH_3 or C_6H_5) changes the charge density and influences the coordination mode of the ligand to metal ion. In the case of M(II) acetates, the ligand coordinates in a bidentate mononegative way, no matter the nature of R.

Antimicrobial activity assays

The antimicrobial activities of the complexes and ligands were screened by adapted qualitative, diffusimetric methods (i.e. distribution of the tested solutions on filter paper disks, in agar wells or in spots on solid media that have been inoculated with test microbial strains) and quantitative methods based on serial two-fold dilutions of the tested compounds in order to establish the corresponding Minimal Inhibitory Concentrations (MIC). Five bacterial strains, i.e. *Acinetobacter boumanii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, freshly isolated from patients with infections associated with cardiovascular devices from different clinical sources and identified by conventional methods were cultivated on solid media and incubated at 37°C for 24 hours prior to testing.

The qualitative screening results demonstrated that the three examined diffusion methods exhibited different sensitivities in detecting the antimicrobial potential of the tested compounds, with the most efficient one for different bacterial strains proving to be the spot method. The results of the quantitative assays (Figure 6) showed that the tested compounds $[M(L_H)_2]Cl_2$ proved to be active on *Klebsiella pneumoniae* and on *Staphylococcus aureus*, with the complex $[Cu(L_H)_2]Cl_2$ and the L_H ligand being the most active ones. The complexes of the $[M(L_{CH3})_2]$ type also show activity towards the same bacteria, and moreover, the L_{CH3} ligand is active on *Escherichia coli* too. Among the compounds of the $[M(L_{C6H5})_2]$ class, the complex $[Cu(L_{C6H5})_2]$ and the L_{C6H5} ligand show a pronounced activity towards *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli.* From the compounds of the $[ML_R(H_2O)_2ac]$ class, only the $[CuL_R(H_2O)_2ac]$ complex shows activity towards *Klebsiella pneumoniae*, whereas the rest of the complexes present very low activities against the other bacterial strains.

Figure 6. Graphical representation of the MIC values (mg/mL) of the tested compounds towards different bacterial strains.



Conclusions

The geometry of the studied complexes is influenced by the nature of ligand, the nature of R, which determines a variation of charge density at the coordination site and by the nature of the metal salts used in their preparation.

The sensitivity spectrum of the microbial strains towards the ligands and the corresponding complexes was determined by qualitative and quantitative methods and the following conclusions were reached:

- the qualitative anti-microbial activity screening results of the tested compounds proved that the most efficient method is the spot method;
- the quantitative anti-microbial activity test results proved that both the ligands and the complex combinations have specific anti-microbial activity, depending on the microbial species tested;
- the complex combinations of the $[ML_R\ (H_2O)_2ac]$ type, except for the $[CuL_R\ (H_2O)_2ac]$ complex, show very low activity towards the tested bacterial strains.

Experimental

General

The metallic ion contents were determined by atomic absorption spectroscopy with a Carl Zeiss Jena AAS 1N spectrometer; Cl was determined by a gravimetric method; C, H, and N were analyzed with a Carlo Erba elemental analyzer. The prepared complexes and their elemental analysis are given in Table 5. Elemental analyses were performed after drying the complexes at 105 °C. The molar conductivity was determined with CONSORT–C533 conductometer. Electronic spectra were recorded by the diffuse-reflectance technique, using MgO as diluting matrix, on a JASCO V-550 spectrophotometer. IR spectra were recorded with a BioRad FTS 135 spectrophotometer in the 4000-400 cm^{-1} region using KBr pellets. All the complexes were studied by thermogravimety (TG) in static air atmosphere, with a sample heating rate of 10 °C/min. using a DuPont 2000 ATG thermo balance. ESR spectra were recorded on polycrystalline powders and solutions at room temperature and 77K with an ART-6, model IFA-Bucharest, X-band spectrometer (9.01 GHz) connected to a PC equipped with a 100KHz field modulation unit. The required chemicals were purchased from Merck and Chimopar Bucharest and all manipulations were performed using materials as received. The ligands L_H (m.p. 183-184 ^oC), L_{CH3} (m.p. 194-195 ^oC), L_{C6H5} (m.p. 204-205 ^oC) were obtained by the method described elsewhere [12].

Synthesis of $[M(L_H)_2]Cl_2$ and $[M(L_R)_2]$ complexes; M = Cu(II), Ni(II), Co(II); L= 2-hydroxy-8-R-tricyclo[7.3.1.0.^{2,7}]tridecane-13-one-thiosemicarbazone; R = CH_3, C_6H_5

An alcoholic solution of ligand L_H (2 mmol in 20 mL) or L_R was added to an alcoholic solution of metal chloride (1 mmol in 20 mL). The resulted solution was heated at 60^{o}C with vigorous stirring for 3-4 hours. After cooling to room temperature and allowing the solution to stand in dark for 10-12 hours, precipitates of different colors were formed, which were filtered, washed with alcohol to remove the residue and vacuum dried.

Synthesis of [$ML_R(H_2O)_2ac$] complexes; M = Cu(II), Ni(II), Co(II)

To an alcoholic solution of L_R (1 mmol in 50 mL) an alcoholic solution of a metal acetate (1 mmol in 30 mL) was added. The [$ML_R(H_2O)_2ac$] complexes with different colors were obtained following the same procedure described earlier. All these final products were obtained as microcrystalline powders. If a buffer solution of acetic acid-sodium acetate of pH=3.5 (15 mL) is added to a methanol solution of [$Cu(L_R)_2$] complex (2 mmol in 25 mL) then a light brown precipitate can be obtained, which corresponds to the molecular formula [$Cu_2(L_R)_2(H_2O)_4(ac)_2$].

Table 5. Elemental analyses.

Compound	**%H exp. (calc)**	**%C exp. (calc)**	**%N exp. (calc)**	**%M exp. (calc)**	**%Cl exp. (calc)**	**Color**	**Dec. Point (^{0}C)**	**Yield (%)**
[$M(L_H)_2$]Cl_2								
($CuC_{28}H_{48}N_6O_2S_2$)Cl_2	6.31	48.66	12.25	9.22	10.03	light-	218	81
(698.5)	(6.87)	(48.10)	(12.02)	(9.11)	(10.16)	green		
($CoC_{28}H_{48}N_6O_2S_2$)Cl_2	6.25	48.85	12.43	8.63	10.10	dark-	245	79
(694)	(6.91)	(48.41)	(12.10)	(8.50)	(10.23)	green		
($NiC_{28}H_{48}N_6O_2S_2$)Cl_2	6.40	48.80	12.50	8.42	10.18	brown	231	85
(694)	(6.91)	(48.41)	(12.10)	(8.50)	(10.23)			
[$M(L_{CH3})_2$]								
$Cu(C_{30}H_{50}N_6O_2S_2)$	7.30	55.40	12.56	9.62		light-	239	67
(653.5)	(7.65)	(55.08)	(12.85)	(9.71)		green		
$Co(C_{30}H_{50}N_6O_2S_2)$	7.28	55.87	12.72	8.90		dark-	261	72
(649)	(7.70)	(55.46)	(12.94)	(9.09)		green		
$Ni(C_{30}H_{50}N_6O_2S_2)$	7.31	55.73	12.67	8.89		brown	259	75
(649)	(7.70)	(55.46)	(12.94)	(9.09)				
[$M(L_{C6H5})_2$]								
$Cu(C_{40}H_{54}N_6O_2\ S_2)$	6.58	62.18	10.48	8.00		light-	245	63
(777.5)	(6.94)	(61.76)	(10.80)	(8.16)		green		
$Co(C_{40}H_{54}N_6O_2S_2)$	6.54	62.48	10.53	7.50		dark-	263	62
(773)	(6.98)	(62.09)	(10.86)	(7.63)		green		
$Ni(C_{40}H_{54}N_6O_2S_2)$	6.57	62.57	10.66	7.52		brown	260	68
(773)	(6.98)	(62.09)	(10.86)	(7.63)				

Table 5. Cont.

Compound	**%H exp. (calc)**	**%C exp. (calc)**	**%N exp. (calc)**	**%M exp. (calc)**	**%Cl exp. (calc)**	**Color**	**Dec. Point (^{0}C)**	**Yield (%)**
[$ML_H(H_2O)_2ac$]								
$Cu(C_{16}H_{30}N_3O_5S)$	6.59	43.91	9.68	14.27		dark-	179	87
(439.5)	(6.83)	(43.68)	(9.55)	(14.44)		green		
$Co(C_{16}H_{30}N_3O_5S)$	6.47	44.58	9.38	13.40		dark-	163	79
(435)	(6.89)	(44.13)	(9.65)	(13.56)		green		
$Ni(C_{16}H_{30}N_3O_5S)$	6.63	44.52	9.43	13.39		green	197	81
(435)	(6.89)	(44.13)	(9.65)	(13.56)				
[$ML_{CH3}(H_2O)_2ac$]								
$Cu(C_{17}H_{32}N_3O_5S)$	6.80	45.31	9.51	13.87		green	198	86
(453.5)	(7.05)	(44.98)	(9.26)	(14.00)				
$Co(C_{17}H_{32}N_3O_5S)$	6.84	45.94	9.07	13.00		dark-	198	83
(449)	(7.12)	(45.43)	(9.35)	(13.14)		green		
$Ni(C_{17}H_{32}N_3O_5S)$	6.75	45.87	9.11	12.98		green	208	85
(449)	(7.12)	(45.43)	(9.35)	(13.14)				
[$ML_{C6H5}(H_2O)_2ac$]						green		
$Cu(C_{22}H_{34}N_3O_5S)$	6.27	51.63	8.37	12.02			194	73
(515.5)	(6.59)	(51.21)	(8.14)	(12.13)				
$Co(C_{22}H_{34}N_3O_5S)$	6.32	51.97	8.00	11.37		dark-	185	79
(511)	(6.65)	(51.66)	(8.21)	(11.54)		green		
$Ni(C_{22}H_{34}N_3O_5S)$	6.37	51.86	7.93	11.45		green	202	82
(511)	(6.65)	(51.66)	(8.21)	(11.54)				

Biological assays

In order to determine the antimicrobial activity, the working technique used was similar to that described in [20]. Thus, the fresh cultures obtained from clinical isolates were suspended in sterile saline and adjusted to a standard density of 0.5 MacFarland. The microbial suspensions were plated on solid Mueller Hinton medium and solutions of the test compounds (10 μL) prepared in DMF (1 mg/mL) were added on filter paper disks, in agar wells or as spots. Concomitantly, the disks were impregnated with the same concentration of gentamycin, which was used as reference standard for reporting the antibiotic sensitivity. The plates were incubated at 37 °C for 24 hours. During incubation, the tested compounds diffused around the test area creating a concentration gradient. The antimicrobial activity was recorded as any area of microbial growth inhibition that occurred in the diffusion area. The quantitative antimicrobial activity assay was performed by the two-fold serial microdilution method in liquid medium (nutrient broth for bacterial and liquid YPG for fungal strains). Serial two-fold dilutions of a stock solution in DMF (from 1000 to 62.5 μg/mL) were performed in 60 multi-well plates, in a total volume of 200 μL medium and a standard microbial suspension (50 μL) was added in each well. After 20-24 hours, the plates are examined visually for evidence of bacterial growth. Results are

recorded as minimum inhibitory concentrations (MIC) at the lowest concentration of the tested compound that completely inhibited microbial growth.

Acknowledgements

The authors would like to thank the Organic Chemistry Department - ICECHIM, Bucharest for microanalysis and LCS-Bucharest, Romania, for antimicrobial activity assays.

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Sample availability: Available from MDPI. See the supporting information.