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SYNTHESIS AND BIOLOGICAL ACTIVITY OF COPPER(II) COORDINATION COMPOUNDS WITH 2-HYDROXY-1-NAPHTHALDEHYDE *N*(4)-ALLYL-3-THIOSEMICARBAZONE

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The paper presents the synthesis of the 2-hydroxy-1-naphthaldehyde *N*(4)-allyl-3-thiosemicarbazone (H_2L) and seven coordination compounds of copper with this ligand and heteroaromatic amines. The new obtained compounds were investigated by elemental analysis, IR spectroscopy and molar electric conductibility. For the synthesized compounds the antibacterial and antifungal activities *in vitro* were studied on a series of standard strains, such as *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231) and *Candida krusei* (ATCC 6258). The nature of heteroaromatic amines influences the activity of the coordination compounds that decreases in the following way: 3,4-Lut > 1,10-Phen > 2,2'-Bpy > Py > 4-Pic > 3-Pic. Almost all studied substances manifest high antioxidant activity towards $ABTS^{\bullet+}$ that exceeds the activity of Trolox which is used as standard antioxidant.

Keywords: *coordination compounds; 2-hydroxy-1-naphthaldehyde; thiosemicarbazones; antibacterial, antifungal and antioxidant activities.*

SINTEZA ȘI ACTIVITATEA BIOLOGICĂ A COMPUȘILOR COORDINATIVI AI CUPRULUI(II) CU *N*(4)-ALIL-3-TIOSEMICARBAZONA 2-HIDROXI-1-NAFTALDEHIDEI

Lucrarea conține descrierea sintezei *N*(4)-alil-3-tiosemicarbazonei 2-hidroxi-1-naftaldehidei (H_2L) și a șapte compuși coordinativi ai cuprului cu acest ligand și amine heteroaromatice. Compușii nou-obținuți au fost studiați cu ajutorul spectroscopiei IR, analizei elementale și al conductivității molare. Pentru compușii sintetizați a fost studiată *in vitro* activitatea antibacteriană și antifungică selectivă față de spectrul larg de tulpini standard de *Staphylococcus aureus (ATCC 25923), Bacillus cereus (ATCC 11778), Bacillus subtilis (ATCC 6633), Candida albicans (ATCC 10231)* și *Candida krusei (ATCC 6258).* Natura aminelor heteroaromatice influențează activitatea compușilor coordinativi și scade în felul următor: 3,4-Lut > 1,10-Phen > 2,2'-Bpy > Py > 4-Pic > 3-Pic. Metoda $ABTS^{\bullet+}$ a demonstrat că aproximativ toți compușii studiați manifestă o activitate antioxidantă mai pronunțată comparativ cu Trolox.

Cuvinte-cheie: *compuși coordinativi, 2-hidroxi-1-naftaldehidă, tiosemicarbazone, activitate antibacteriană, antifungică, antioxidativă.*

Introduction

Thiosemicarbazones represent an interesting class of ligands. They attract a significant attention due to their excellent complexation properties, variety of coordination modes and useful pharmacological properties such as anticancer, antimicrobial and antifungal properties [1-3]. Introduction of substituents at the *N*(4) position of thiosemicarbazones improves their solubility and coordinating ability and, as a result, increases biological activity. Due to the spread of resistant forms of microorganisms, the search for alternative and more efficient drugs is of great interest. Some 2-hydroxy-1-naphthaldehyde 4-substituted-3-thiosemicarbazones are already described in the scientific literature [4-7] and it was found that these compounds possess antimicrobial, anticancer, antioxidant, and anti-inflammatory activities.

The aim of this work is finding the conditions of synthesis, determination of the composition and physico-chemical properties of the copper coordination compounds with 2-hydroxy-1-naphthaldehyde *N*(4)-allyl-3-thiosemicarbazone and heteroaromatic amines.

Experimental

Materials and methods

N(4)-Allyl-3-thiosemicarbazide was synthesised by the reaction between allyl isothiocyanate and hydrazine hydrate [8]. 2-Hydroxy-1-naphthaldehyde (Sigma-Aldrich), and metal salts were used as received.

Infrared spectra of the compounds were recorded on a Bruker ALPHA FTIR spectrophotometer at room temperature in the range of 4000-400 cm^{-1}.

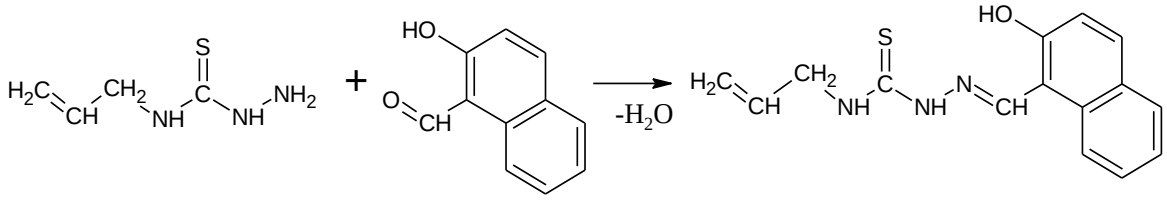
The determination of copper content in the synthesised coordination compounds, using titration methods, was performed similarly to the literature procedures [9-10].

The melting point of the pro-ligand was measured using capillary method [11].

The molar conductivity values were determined in 10mM dimethylformamide solutions using slide wire bridge R-38.

Synthesis of the 2-hydroxy-1-naphthaldehyde* N*(4)-allyl-3-thiosemicarbazone (H_2L)

2-Hydroxy-1-naphthaldehyde *N*(4)-allyl-3-thiosemicarbazone (H_2L) **(Scheme 1)** was prepared according to a modification of the procedure described in the literature [5].



Scheme 1. Synthesis of the 2-hydroxy-1-naphthaldehyde *N*(4)-allyl-3-thiosemicarbazone.

4-Allyl-3-thiosemicarbazide (1.31 g, 10 mmol) was dissolved in 20 mL of ethanol with constant stirring. After that, the ethanolic solution (15 mL) of 2-hydroxy-1-naphthaldehyde (1.72 g, 10 mmol) was added and the mixture was stirred at 80^0C for 2 hours. Upon cooling, a yellow crystalline product began to separate. This was collected by filtration, washed with ethanol and dried *in vacuo*.

Yellow solid. Yield: 82%; m.p.: 200-202^0C; FW: 172.18 g/mol;

Main IR peaks (cm^{-1}): ν(OH) 3420, ν(NH) 3184, 3079, ν(C=C allyl) 1645, ν(C=N^1) 1621, ν(C=S) 1329, ν(C–O) 1219.

Synthesis of coordination compounds

The complex **I** was obtained by stirring a hot solution of **H_2L** in ethanol with monohydrate of copper acetate in 1:1 molar ratio. The brown precipitate has formed during stirring. It was filtered and allowed to dry at room temperature. The complexes **II-VII** were obtained by dissolving the resulting complex **I** in ethanol and the corresponding heteroaromatic amine was added to the reaction mixture: 1,10-phenanthroline **(II)**, 2,2'-bipyridyl **(III)**, pyridine **(IV)**, 3-methylpyridine **(V)**, 4-methylpyridine (**VI**), 3,4-dimethylpyridine (**VII**). The resulting precipitates were filtered off, washed with a small amount of ethanol and dried.

Biological studies

Antibacterial bioassay

The antimicrobial activities of the pro-ligand and complexes were evaluated *in vitro* against *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633) standard strains. Determination of the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) was done using the serial dilutions in liquid broth method [12-13]. The tested substances were dissolved in DMSO in concentration of 10 mg/mL. The next dilutions were made using 2% of peptonate bullion.

Antifungal bioassay

Antimycotic properties of the synthesised substances were investigated *in vitro* on laboratory stems of *Candida albicans* (ATCC 10231) and *Candida krusei* (ATCC 6258). The activity was determined in liquid Sabouraud nutritive medium (pH 6.8). The inoculates were prepared from fungi stems which were harvested during 3–7 days. Their concentration in suspension is $(2–4)\cdot10^6$ colonies forming units/mh. Sowings for levures and micelles were incubated at 37^0C during 7 and 14 days, respectively.

Antioxidant activity

The antioxidant activity by the $ABTS^{\bullet+}$ method was assessed according to the method described by Re et al. [14] with modifications. The $ABTS^{\bullet+}$ radical cation was formed through the reaction of ABTS solution 7 mM with potassium persulfate solution 140 mM, incubated at 25°C in the dark for 12–16 hours. Once formed, the $ABTS^{\bullet+}$ solution was diluted with acetate buffered saline (0.02M, pH 6.5) to give an absorbance of 0.7 ± 0.01 at 734 nm. Solutions in DMSO of Trolox, **H_2L** and complexes **I-VII** were prepared. Then, 20 µl of each dilution was transferred to a 96-well microtiter plate and 180 µl of working solution of $ABTS^{\bullet+}$ was dispensed with the dispense module of hybrid reader (BioTek) and shaken for 15 s. After 30 min of incubation was measured

the decrease in absorbance at 734 nm. Blank samples do not contain $ABTS^{\bullet+}$. The decrease in absorbance is expressed as % inhibition and is calculated according to the following formula:

$$((Abs_{control}–Abs_{sample})/Abs_{control})\cdot 100$$

Results and discussion

The thiosemicarbazone **H_2L** and seven new metal complexes were synthesised in ethanol in good yield. Complex **I** was prepared by the direct reaction between the pro-ligand **H_2L** and the copper acetate monohydrate, the rest were prepared by the direct reaction between complex **I** and heteroaromatic amines. The obtained coordination compounds are microcrystalline solids and are stable in air. The elemental analysis on copper suggest the general formula $Cu(H_2O)(L)$ and Cu(A)(L) (L=1,10-phenanthroline (1,10-Phen), 2,2'-bipyridyl (2,2'-Bpy), pyridine (Py), 3-methylpyridine (3-Pic), 4-methylpyridine (4-Pic), 3,4-dimethylpyridine (3,4-Lut)).

Table 1

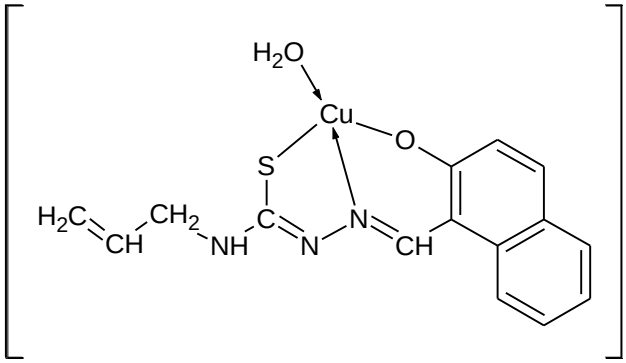
Physical and analytical data of the metal complexes (I-VII)

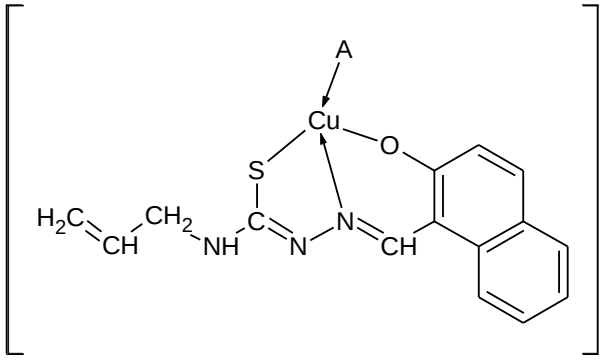
No	Compound	Formula	η^a, %	Found / calculated, metal %	λ^b
I	$Cu(H_2O)(L)$	$C_{15}H_{15}CuN_3O_2S$	78	17.23/17.41	11
II	Cu(1,10-Phen)(L)	$C_{27}H_{21}CuN_5OS$	71	11.64/12.06	10
III	Cu(2,2'-Bpy)(L)	$C_{25}H_{21}CuN_5OS$	80	12.44/12.63	9
IV	Cu(Py)(L)	$C_{20}H_{18}CuN_4OS$	80	14.80/14.92	11
V	Cu(3-Pic)(L)	$C_{21}H_{20}CuN_4OS$	87	14.50/14.44	11
VI	Cu(4-Pic)(L)	$C_{21}H_{20}CuN_4OS$	86	14.31/14.44	14
VII	Cu(3,4-Lut)(L)	$C_{22}H_{22}CuN_4OS$	88	13.90/14.00	11

a – yield; b – molar conductivity in dimethylformamide at room temperature, $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$.

The molar conductivity values of the synthesized complexes **I-VII** are in the range 9 - 14 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ that indicates that complexes **I-VII** are non-electrolytes.

The thiosemicarbazone **H_2L** coordinates as a double-deprotonated tridentate ligand with ONS-set of donor atoms. Type of the ligand coordination with the central ions was elucidated from comparative analysis of IR spectra of complexes **(I-VII)** and the pro-ligand **H_2L**. It coordinates to the central ions by deprotonated phenolic oxygen atom, azomethinic nitrogen and sulphur in the thiol form atoms forming five- and six-membered metallacycles. The proposed distribution of chemical bonds in the coordination compounds is shown in scheme 2.





A= Py, 3-Pic, 4-Pic, 3,4-Lut.

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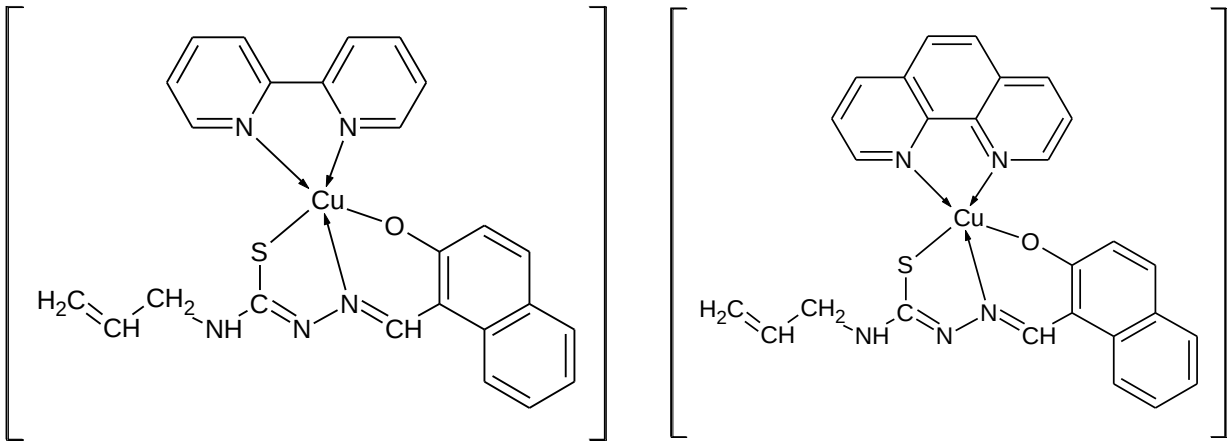

Scheme 2. Proposed distribution of chemical bonds in the metal complexes.

For the synthesised compounds the antibacterial and antifungal activities *in vitro* were studied on a series of standard strains. The study of antibacterial and antifungal activities **(Table 2)** showed that **H_2L** and its coordination compounds possess bacteriostatic and bactericidal activities. The activity of the synthesised compounds towards fungi is less pronounced than towards Gram-positive bacteria.

Table 2

The minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) (µg/mL)

Compound	*Staphylococcus aureus* ATCC 25923		*Bacillus cereus* ATCC 11778		*Bacillus subtilis* ATCC 6633		*Candida albicans* ATCC 10231		*Candida krusei* ATCC 6258	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
H_2L	500.0	1000	15.62	31.25	15.63	31.25	500.0	>1000	500.0	>1000
I	0.9766	1.953	0.9766	1.953	0.4883	0.9766	500.0	>1000	500.0	>1000
II	0.1221	0.2441	0.9766	1.953	0.4883	0.9766	250.0	500.0	125.0	250.0
III	0.2441	0.4883	1.953	3.906	0.9766	1.953	125.0	250.0	62.50	125.0
IV	0.4882	0.9765	0.4882	0.9765	-[a]	-[a]	250.0	500.0	250.0	500.0
V	0.9765	1.953	1.953	3.906	-[a]	-[a]	500.0	>1000	250.0	500.0
VI	0.4883	0.9766	1.953	3.906	0.9766	1.953	125.0	250.0	125.0	250.0
VII	0.2441	0.4883	0.4883	0.9766	0.4883	0.9766	250.0	500.0	62.50	125.0

a - not studied.

The **H_2L** and coordination compounds show selective antimicrobial and antifungal activity towards a series of standard strains *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231) and *Candida krusei* (ATCC 6258) in the range of concentration 0,1221-1000 µg/mL. It was found that the copper complex with 3,4-dimethylpyridine is one of the most active. The comparison of antibacterial and antifungal activities of these compounds against the selected types of bacteria indicates that the nature of heteroaromatic amines influences on the activity of the coordination compounds and that decreases in the following way 3,4-Lut > 1,10-Phen > 2,2'-Bpy > Py > 4-Pic > 3-Pic.

The antioxidant properties of the synthesised compounds were studied using the $ABTS^{\bullet+}$ method (**Table 3**). Almost all studied substances manifest higher antioxidant activity than Trolox, which is used in medical practice as standard antioxidant. Compound **VII** shows the best antioxidant activity. The activity of coordination compounds depends on the nature of heteroaromatic amines and decreases in the following way 3,4-Lut > Py > 2,2'-Bpy > 3-Pic > 1,10-Phen > 4-Pic.

Table 3

IC_{50} values of the synthesised substances towards $ABTS^{\bullet+}$ radical cation

Compound	IC_{50}, μM
H_2L	12.24
I	18.88
II	33.69
III	11.56
IV	9.53
V	15.41
VI	>100
VII	8.90

Conclusions

In this work 2-hydroxy-1-naphthaldehyde *N*(4)-allyl-3-thiosemicarbazone was synthesised and was used for synthesis of seven coordination compounds of copper. These compounds were studied using IR spectroscopy, elemental analysis and molar conductivity. It was determined, that the coordination compounds show antibacterial and antifungal activities in the range of concentration 0.1221-1000 μg/mL. Almost all studied substances manifest high antioxidant activity towards $ABTS^{\bullet+}$ that exceeds the activity of Trolox which is used as standard antioxidant.

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