STRUCTURE AND SOME BIOLOGICAL PROPERTIES OF Fe(III) COMPLEXES WITH NITROGEN-CONTAINING LIGANDS

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Abstract. Four coordination compounds of iron(III) with ligands based on hydrazine and sulfadiazine: $[Fe(dig)]Cl_3·2H_2O$ (**I**) (dig-semicarbazide diacetic acid dihydrazide), $[Fe(HL)SO_4]$ (**II**) (HL - sulfadiazine), $[Fe(H_2L^1)(H_2O)_2](NO_3)_3·5H_2O$ (**III**) (H_2L^1-2,6-diacetylpyridine bis(nicotinoylhydrazone) and $[Fe(H_2L^2)(H_2O)_2](NO_3)_3·1.5H_2O$ (**IV**) (H_2L^2 - 2,6-diacetylpyridine bis(isonicotinoylhydrazone) were synthesized. The spectroscopic and structure characterisation, as well as their biological properties, are presented. All tested coordination compounds, caused an inhibitory effect on the biosynthesis of hydrolases of the producer *Aspergillus niger* CNMN FD-10. The effect increased with the raise of the concentration from 5 mg/L to 15 mg/L. The complexes largely inhibited cellobiohydrolase and less- *β*-glucosidase. The inhibitory effect of the coordination compounds was quite similar in the case of endoglucanase and xylanase. Compound **I**, containing both chloride ions and dig, demonstrated a stronger inhibitory effect, while compound **III**, containing NO_3^- ions, showed the weakest inhibitory action.

Keywords: iron complexes, nitrogen-containing ligands, structure, biological properties.

Introduction

In the modern microbiology, investigation of the effects of external factors on the physiology of microorganisms remains an actual issue. Such studies allow solving the practical problems and implementation of the new technologies in biology and medicine.

The literature, in the last decades, denotes high physiological activity of the coordination compounds of transition metals on biological objects, including microorganisms [1-13]. Some authors note a significant role of these compounds in the directed synthesis of biologically active substances from microalgae and cyanobacteria, filamentous fungi and yeast [1-10,12].

Studies of the effects of coordination compounds on biosynthesis of extracellular hydrolases in filamentous fungi have been previously carried out [3-7,14]. It was established that these effects depend both on the properties of compounds (chemical composition, molecular structure, nature of the ligand and coordinated metal atom) and genus and species of the micromycetes strains, and specificity of the synthesized enzymes [3-7,14]. Some coordination compounds exhibit a selective action on the components of the synthesized enzyme complex. Thus, by adding the molybdenum complex with valine - $MoO_2(acac)_2Val$ (acac=acetylacetone, Val=valine) into the nutrient medium, the biosynthesis of *β*-glucosidase in micromycete *Penicillium expansum* CNMN FD 5C – producer of cellulases was enhanced by 69.4%, while the level of endoglucanase and cellobiohydrolase was not modified. Study of the influence of $(NH_4)_2VO_2Gly$ (Gly=glycine) on biosynthesis of cellulases of the same strain showed that the dioxovanadium complex increased the activity of all components of cellulase complex by 17.6-40.3% [14].

The targeted application of the coordination compounds of 3*d*-elements as regulators of the microbial synthesis of bioactive substances requires accumulation of factual material on the nature of their influence on all phases of the development of microorganisms and also identification of optimal conditions for their use. All of this information will ensure the resolution of some theoretical issues, such as elucidation of the mechanism of action of coordination compounds, establishing the relationship between the composition, structure and enzyme activity of the microorganisms, as well as the development of methods for their practical use. These objectives can be achieved by expanding both, the diversity of microorganisms and the involved in researches complexes.

Iron is one of the essential microelements for microorganisms. It is involved in the respiration in microorganisms, being also necessary for the protein, carbohydrate and lipid metabolism, including protein synthesis. Iron is crucial structure component of the cytochromes, which catalyze the process of electron transfer in the mitochondria. In living organisms redox system Fe^{3+}/Fe^{2+} plays an unique role [15,16].

This study was aimed at investigation of the effect of iron(III) complexes with various organic ligands derived from hydrazine and sulfadiazine on the biosynthesis of cellulases and xylanases at *Aspergillus niger* CNMN FD 10 micromycete.

Materials and methods
Chemistry

[Fe(dig)]Cl$_3$·2H$_2$O (**I**) was obtained by mixing the ethanolic solutions of FeCl$_3$·6H$_2$O (1.0g, 3.7 mmol in 50 mL ethanol) and digsemi (0.8 g, 3.7 mmol in 40 mL ethanol). The formed dark brown residue was separated by filtration, washed with ethanol and diethyl ether. The complex is soluble in water, less soluble in alcohol and diethyl ether. Yield- 75% [17,18].

[Fe(HL)SO$_4$] (**II**) was obtained by refluxing FeSO$_4$·7H$_2$O (2.0 g, 0.72 mmol) and sulfadiazine (0.22 g, 0.88 mmol) in methanol (20 mL) for 2 hours in a water bath. The brown precipitate was separated from the mother liquor by filtration, washed with methanol and diethyl ether. The complex is well soluble in methanol and DMF, less soluble in water and diethyl ether. Yield- 80% [13].

Methods for the synthesis of coordination compounds of iron with semicarbazide diacetic acid dihydrazide, sulfadiazine, 2,6-diacetylpyridine *bis*(nicotinoylhydrazone) and 2,6-diacetylpyridine bis(isonicotinoylhydrazone) have been developed. The complexes of iron(III) with Schiff bases on the basis of 2,6-diacetylpyridine were obtained in two ways: by condensation reactions of 2,6-diacetylpyridine with hydrazides of nicotinic and isonicotinic acid on the matrix of iron ions and by direct interaction of iron salt with the corresponding Schiff bases.

For determining the composition, spectral characteristics, molecular and crystal structures of the complexes elemental analysis, IR and Mössbauer spectroscopy and single crystal X-ray diffraction were applied. The composition and structure of the complexes **III** and **IV** were established by IR spectroscopy and X-ray diffraction and can be described by the following formulas: [Fe(H$_2$L^1)(H$_2$O)$_2$](NO$_3$)$_3$·5H$_2$O (**III**) and [Fe(H$_2$L^2)(H$_2$O)$_2$](NO$_3$)$_3$·1.5H$_2$O (**IV**).

IR spectroscopy

The IR spectra of the ligands and complexes were recorded on spectrophotometer FT-IR Perkin-Elmer "Spectrum 100" in the vaseline oil in the region 4000-400 cm^{-1} and ATR in the region 4000-650 cm^{-1}, and the absorption bands has been attributed according to the reference literature [23-26], (s. - strong, v.s. - very strong, m. – middle, w. – weak, sh. – shoulder, br. - broad).

Mössbauer spectroscopy

The ^{57}Fe-Mössbauer spectra were recorded with a common spectrometer in the constant-acceleration mode (MS4, Edina, USA) equipped with a ^{57}Co source in a rhodium matrix.

X-ray Crystallography

The unit cell parameters and the sets of reflections intensity were measured at 293 K on a Xcalibur E CCD diffractometer for crystals **III** and **IV** (MoK$_\alpha$ radiation, graphite monochromator). The structures of compounds **III** and **IV** were solved by the direct methods and refined by least squares in the anisotropic full matrix approximation for non-hydrogen atoms (SHELX-97) [19]. The C, N, and O-bound H atoms were placed in calculated positions and were treated using a riding model approximation with U_{iso}(H) = 1.2U_{eq}(C) or U_{iso}(H) = 1.5U_{eq}(O, C(CH$_3$). The crystallographic data and X-ray experiment details for structures **III** and **IV** are presented in Table 1, the selected interatomic distances are listed in Table 2 and the geometric parameters of hydrogen bonds are given in Table 3. Crystallographic data for **III** and **IV** have been deposited with the Cambridge Crystallographic Data Center, CCDC 1429807, 1429808. Copies of this information may be obtained from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1233-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

Table 1

Crystal data and structure refinement data for III and IV.

Parameter	Compound ***III***	Compound ***IV***
Formula	$C_{21}H_{33}FeN_{10}O_{18}$	$C_{21}H_{25}FeN_{10}O_{14.50}$
M	769.42	705.36
Crystal system	monoclinic	monoclinic
Space group	$P2_1/n$	$P2_1/c$
a, Å	13.0459(5)	13.4325(6)
b, Å	16.6677(4)	15.0766(5)
c, Å	15.2842(5)	15.3908(8)
β, °	100.730(3)°	107.895(5)
V, Å^3	3265.38(17)	2966.1(2)
Z	4	4
ρ_{calc}, g/cm^3	1.565	1.580
μ, mm^{-1}	0.555	0.595
$F(000)$	1596	1452
Crystal size, mm	0.35x0.2x0.1	0.2x0.15x0.15
θ range for data collection, °	2.91 – 25.50	3.04 – 25.50
Index ranges	$-11 \leq h \leq 15$	$-12 \leq h \leq 16$
	$-19 \leq k \leq 11$	$-16 \leq k \leq 18$
	$-18 \leq l \leq 11$	$-18 \leq l \leq 18$

Parameter	Compound **III**	Compound **IV**
Reflections collected/ Independent reflections	10714/6047 [R(int) = 0.0294]	10286/5472 [R(int) = 0.0476]
Reflections with $I>2\sigma(I)$	6047	5472
Parameters	451	424
GOOF	0.998	1.003
Final *R* indices ($I>2\sigma(I)$)	$R_1 = 0.0544$	$R_1 = 0.0631,$
	$wR_2 = 0.1221$	$wR_2 = 0.1415$
R indices (all data)	$R_1 = 0.1117$	$R_1 = 0.1083,$
	$wR_2 = 0.1453$	$wR_2 = 0.1628$
$\Delta\rho_{max}, \Delta\rho_{min}, e\times\text{Å}^{-3}$	0.553, −0.328	0.558, −0.370

Table 2

Bond lengths (Å) in coordination polihedra of III and IV.

Bond	Compound **III**	Compound **IV**
Fe(1)−O(1)	2.033(2)	2.087(3)
Fe(1)−O(2)	2.029(2)	2.043(3)
Fe(1)−O(1W)	2.036(2)	2.035(3)
Fe(1)−O(2W)	2.035(2)	2.028(3)
Fe(1)−N(3)	2.194(3)	2.176(3)
Fe(1)−N(4)	2.188(3)	2.203(3)
Fe(1)−N(5)	2.194(3)	2.187(3)

Table 3

Geometric parameters of hydrogen bonds for III and IV (Å and °).

D−H··A	d, Å			∠DHA,°	Symmetry transformations used to generate equivalent atoms
	D–H	H··A	D··A		
*Compound **III***					
O(1W)−H(1W1)··O(6A)	0.80	1.91	2.701(4)	173	*x, y, z*
O(1W)−H(2W1)··O(3W)	0.83	1.85	2.679(3)	179	*−x+1/2, y−1/2, −z+3/2*
O(2W)−H(1W2)··O(7W)	0.82	1.77	2.580(4)	169	*−x+3/2, y+1/2, −z+3/2*
O(2W)−H(2W2)··O(9A)	0.80	1.91	2.699(4)	170	*x, y, z*
N(1)−H(1A)··O(6W)	0.86	1.87	2.715(4)	170	*x, y, z*
N(7)−H(7A)··O(1A)	0.86	1.85	2.713(4)	177	*x, y, z*
N(7)−H(7A)··O(2A)	0.86	2.60	3.200(5)	128	*x, y, z*
O(3W)−H(1W3)··O(4A)	0.84	1.93	2.769(4)	173	*x, y, z*
O(3W)−H(2W3)··O(5W)	0.84	1.91	2.726(4)	162	*x, y, z*
O(4W)−H(1W4)··O(3W)	0.86	2.55	2.959(4)	110	*−x+1,−y+1,−z+1*
O(4W)−H(2W4)··O(9A)	0.84	2.04	2.855(4)	163	*x, y, z*
O(5W)−H(1W5)··O(8A)	0.84	2.25	2.962(4)	143	*−x+1,−y+1,−z+1*
O(5W)−H(1W5)··O(9A)	0.84	2.31	3.102(5)	158	*−x+1,−y+1,−z+1*
O(5W)−H(2W5)··O(1A)	0.85	1.93	2.777(4)	174	*x, y+1, z*
O(6W)−H(1W6)··O(3A)	0.83	2.24	2.968(5)	147	*x, y, z*
O(6W)−H(1W6)··O(2A)	0.83	2.26	3.033(5)	156	*x, y, z*
O(6W)−H(2W6)··O(4W)	0.80	2.03	2.737(4)	147	*−x+3/2, y−1/2, −z+3/2*
O(7W)−H(1W7)··O(6W)	0.80	2.26	3.054(5)	168	*x, y, z*
O(7W)−H(2W7)··O(8A)	0.85	2.20	3.013(5)	160	*x, y, z*
O(7W)−H(2W7)··O(7A)	0.85	2.27	2.952(5)	138	*x, y, z*
*Compound **IV***					
O(1W)−H(1W1)··O(3W)	1.02	1.68	2.568(9)	143	*x, y, z*
O(1W)−H(1W1)··O(4W)	1.02	1.73	2.649(11)	148	*x, y, z*
O(1W)−H(2W1)··O(1A)	0.93	1.76	2.683(4)	172	*x, y, z*

Continuation of Table 3

D−H··A	d, Å			∠DHA, °	Symmetry transformations used to generate equivalent atoms
	D–H	H··A	D··A		
Compound IV					
O(2W)−H(1W2)··O(3A)	0.89	1.82	2.669(4)	159	*x,−y+1/2, z+1/2*
O(2W)−H(1W2)··O(2A)	0.89	2.63	3.338(5)	138	*x,−y+1/2, z+1/2*
O(2W)−H(2W2)··O(1)	0.94	1.84	2.769(4)	171	*−x,−y,−z+2*
N(1)−H(1A)··O(5A)	0.86	1.91	2.759(5)	171	*x, y, z*
N(7)−H(7A)··O(9A)	0.86	1.97	2.805(6)	162	*x, y, z*
N(7)−H(7A)··O(8A)	0.86	2.27	2.994(7)	142	*x, y, z*

Microbiology

The effects of the synthesized coordination compounds on the biosynthesis of cellulases (cellobiohydrolase, endoglucanase, β-glucosidase) and xylanases at *Aspergillus niger* CNMN FD 10 micromycete have been investigated [20]. The method of submerged cultivation was applied. The producer was cultivated on the nutrient medium with the chosen composition (g/L): beet pulp - 20.0, KH_2PO_4 – 1.0, $CaCl_2 \cdot 2H_2O$ – 0.1, KCl – 0.1, $MgSO_4 \cdot 7H_2O$ – 0.3, $NaNO_3$ – 2.5, $FeCl_3$ – 0.01, H_2O – 1.0, pH 5.5-6.0, at 28-30ºC under constant stirring on shaker at 180-220 rpm. The duration of the cultivation was 6-9 days; during this period a maximum of biosynthesis of the aforementioned enzymes is observed. The complexes were added to nutrient medium in the concentrations of 5, 10 and 15 mg/L. The samples without the coordination compounds served as control.

Results and discussion
Chemistry

In compound **I** dig behaves like tridentate tripodal ligand. In the IR spectrum the complex absorption bands corresponding to stretching vibrations of NH and NH_2 groups are found in the region 3050-3500 cm^{-1}. However, the band of stretching vibrations of the bonds of amino group NH in the complex spectrum is shifted to longer wavelength region with ≈ 100 cm^{-1}, as compared with its position in the spectrum of the free dihydrazide, indicating on the presence of Me←NH_2 bond [21]. For dig, three bands are in the range of 1600-1700 cm^{-1} (1695, 1670 and 1635 cm^{-1}). Due to complexation, these bands are displaced by 30-50 cm^{-1} in the low frequency region, forming two strips (one wider), which suggests that the oxygen atoms of the carboxyl group are coordinated to the central atom [22].

The recorded at 300 and 80K Mössbauer spectra of [Fe(dig)]$Cl_3 \cdot 2H_2O$ (**I**) have been studied. They show a symmetrical doublet with the following parameters: at 300K IS = 0.69 mm/s and QS = 1.37 mm/s; at 80K IS = 0.79 mm/s and QS = 1.39 mm/s. The experimental value of the effective magnetic moment is equal to 6.04 MB (the pure spin value μ_{eff} for 5 unpaired electron is equal to 5.92 MB). The values of Mössbauer spectrum and μ_{eff} correspond to the metal oxidation state +3, *i.e.* iron is in the high-spin state. According to the value of the isomer shift and quadrupole splitting, the coordination environment of the central ion is nearly spherical, being completed by the donors of the organic ligand (O, NH, N) and two water molecules. The molar conductivity of the aqueous solution of the complex is 391.8 $ohm^{-1} \cdot cm^2 \cdot mol^{-1}$, which corresponds to four ions [17,18].

The IR spectrum of [Fe(HL)SO_4] (**II**) contains typical bands for the sulfadiazine: 3400-3200 (NH and NH_2 groups), 1600-1500 cm^{-1} (aromatic ring), 1320-1160 cm^{-1} (SO_2 group) [13].

[Fe(H_2L^1)(H_2O)$_2$](NO_3)$_3 \cdot 5H_2O$ (**III**), IR, ν(cm^{-1}): 3381 m., br., 3193 m., br., 3124 sh., 3085 m., 3063 m., 2988 m., br., 2750 m., br., 2650 m.,br., 2420 w., br., 2350 w.,br., 2106 w., 2006 w., 1752 w., 1737 w., 1630 m., 1617 sh., 1598w., 1566 m., 1533 s., 1507 m., 1395 v.s., 1380 v.s., 1357 v.s., 1313 v.s., 1272 sh., 1212 sh., 1165 s., 1148 m., 1108 m., 1075 w., 1056 s., 1039 s., 1020 m., 997 m., 978 sh., 958 w., 920 m., 885 w., 874 w., 820 s., 773 w., 752 w., 731 s., 702 m., 689 w., 689 m., 664 w., 621 m., 565 m., 554 sh., 533 s., 495 w., 445 s.

The IR spectrum of complex **III** at 3600-1900 cm^{-1} is almost identical with the spectrum of compound **IV**. The high-intensity band corresponding to ν(C=O), which in the spectrum of the non-coordinated ligand H_2L^1 appears at 1663 cm^{-1}, shifts to 1637 cm^{-1} in the spectrum of complex **III**, which proves that this group participates in the coordination to the metal atom. The absorption band at 1617 cm^{-1} can be assigned to the ν(CN) heterocycle, the bands at 1598, 1533 and 752 cm^{-1} - to the pyridine ring vibrations, and the band at 1566 cm^{-1} - to δ(NH) hydrazide fragment. The absorption bands associated with the NO_3^- anions vibrations, with different functions, could be observed at 1507, 1313 (the most intense band in the spectrum), 1300, 1039 and 997 cm^{-1}. The fact that the main bands (1313 and 1300 cm^{-1}), associated with fluctuations of NO_3^- anions vibrations of the complex, shift to shorter wavelengths, as compared to those for free NO_3^- anions (1380-1350 cm^{-1}), demonstrated that these anions are involved in the formation of various hydrogen bonds in the complex, which correspond by value of this frequencies to the monocoordinated and chelate-bidentate NO_3^- ions [23,26]. The band corresponding to ν(N-N) appears at 1165 cm^{-1}. The deformation vibrations δ(C-H) of the aromatic rings were found: planar vibrations at 1148, 1108 and 1020 cm^{-1}; non-planar vibrations at

752 cm^{-1} (three adjacent hydrogen atoms) and 820 cm^{-1} (isolated hydrogen atom) [23-25]. The stretching vibrations Fe-N are located at 688 and 678 cm^{-1}, and Fe-O - at 495 and 445 cm^{-1}.

[Fe(H$_2$L^2)(H$_2$O)$_2$](NO$_3$)$_3$·1.5H$_2$O (**IV**), IR, ν(cm^{-1}): 3522 m., br., 3151 w., 3117 w., 3080 m., 2990 m., br., 2894 m., br., 2793 m., br., 2762 m., br., 2652 m., br., 2581 m., br., 2452 m., br., 2342 sh., 1744 w., 1637 s., 1612 m., 1584 w., 1567 m., 1548 m., 1535 sh., 1498 m., 1409 s., 1379 sh., 1365 s., 1345 s., 1306 v.s., 1291 v.s., 1273 s., 1207 m., 1186 m., 1170 m., 1161 s., 1094 m., 1080 m., 1059 m., 1050 m., 1043 m., 1034 m., 1019 w., 1004 sh., 996 m., 913 m., 843 s., 827 s., 754 s., 722 w., 713 w., 691 m., 684 s., 651 w., 569 w., 556 w., 534 w., 500 w., 487 w., 442 m.

The IR spectrum is characterized by broad absorption in the range of 3500-1900 cm^{-1}, the absorption bands of 3151 and 3117 cm^{-1} being distinguished on its background. These bands can be attributed to the stretching vibrations associated to OH and NH-groups, respectively. The intense band at 1637 cm^{-1} can be referred to a coordinated C=O group. The band of medium intensity at 1612 cm^{-1} can be assigned to the ν(CN) of the pyridine heterocycle and absorption bands at 1584, 1534 and 754 cm^{-1} – to the heterocycle vibrations itself. The deformation vibrations δ(NH) were found at 1567 cm^{-1}. The intense absorption band at 1409 cm^{-1} can be regarded as the result of interaction of ν(C=N) and ν(C=C) vibrations [23-25].

The most intense absorption bands of the spectrum are associated with the vibrations of NO$_3^-$ anion, which exerts various functions in the crystal lattice of the complex: 1498, 1306, 1291, 1043 and 996 cm^{-1}. Shift of the most intense absorption band of NO$_3^-$ (1291 cm^{-1}) to higher frequencies, in comparison with that of the free NO$_3^-$ anions (1380-1350 cm^{-1}), proves that these anions are involved in the formation of various hydrogen bonds in the complex and by the value is close to the frequency of mono coordinated and chelate-bidentate NO$_3^-$ anions [23-26]. The intense band at 1161 cm^{-1} refers to ν(N-N). The planar C-H deformation vibrations appear through the 1207, 1050 cm^{-1} (1,4-substituted aromatic ring) and 1171, 1080 and 1034 cm^{-1} (1,2,3-substituted aromatic ring) absorption bands. Non-planar C-H deformation vibrations of the aromatic rings appear at 827 cm^{-1} (for two adjacent hydrogen atoms) and 754 cm^{-1} (for three adjacent hydrogen atoms) [24,25]. The ν(Fe-N) oscillations are identified by bands at 692 and 685 cm^{-1} and ν(Fe-O)– by bands at 487 and 442 cm^{-1}, correspondingly.

The crystal and molecular structures of compounds **III** and **IV** were obtained by X-ray diffraction. It was established, that their crystals consists of complex mononuclear cations [Fe(H$_2$L^1)(H$_2$O)$_2$]$^{3+}$ and [Fe(H$_2$L^2)(H$_2$O)$_2$]$^{3+}$, NO$_3^-$ anions and solvated water molecules. The polyhedral coordination of Fe(III) consists of pentagonal bipyramids formed by N$_3$O$_2$ donor atoms set of pentadentate neutral ligands H$_2$L^1/H$_2$L^2 and two oxygen atoms of the coordinated H$_2$O molecules (Figures 1 and 2).
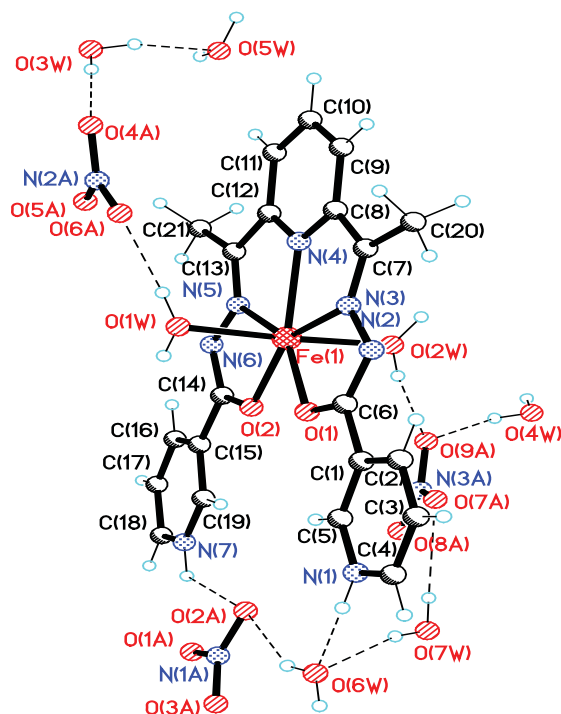
Figure 1. View of formula unit of III with numbering scheme.

The interatomic distances metal-ligand (H$_2$L^1/H$_2$L^2) in the equatorial plane are: Fe(1)−O(1) 2.033(2)/2.087(3) Å, Fe(1)−O(2) 2.029(2)/2.043(3) Å, Fe(1)−N(3) 2.194(3)/2.176(3) Å, Fe(1)−N(4) 2.188(3)/2.203(3) Å, Fe(1)−N(5) 2.194(3)/2.187(3) Å, while metal - apical atoms distances are − Fe(1)−O(1W) 2.036(2)/2.035(3) Å, Fe(1)−O(2W)

2.035(2)/ 2.028(3) Å (Table 2). The obtained values of the interatomic distances do not differ from those found in the complex diaqua-(2',2'''-(2,6-pyridinediyldiethylidyne)dioxamohydrazide)-iron(III) perchlorate hydrate [27,28]. In the Cambridge Structural Database 47 hits on structures of compounds of transition metals with ligands of this class are reported [29], mainly manganese or cobalt compounds, only 6 of them being iron compounds [27-33].

The analysis of the geometry of the ligands H_2L^1/H_2L^2 in **III** and **IV** shows that they are flat and four articulated pentametallocycles are formed as a result of their coordination to the metal in the equatorial plane. Two of the metallocycles are formed by the atoms OCNNFe, and two- by NCCNFe. Herewith, the values of dihedral angles between the formed metallocycles are in the range 1.1 – 5.7° in **III** and 0.4 – 2.2° in **IV**. In the same interval are the values of the dihedral angles between the coordinated atoms N_3O_2 of the ligand H_2L and the planes of the terminal pyridine rings, and between the terminal pyridine rings (2.9, 2.0, 5.3, 3.4° in **III** and 2.6, 3.0, 13.0, 13.5° in **IV**).

The analysis of positioning the hydrogen atoms from the ligands H_2L^1/H_2L^2 demonstrated the proton transfer from the N(2) and N(6) atom position to the N(1) and N(7) atoms. These conclusions are based both on the analysis of Fourier synthesis and geometrical considerations (distances N··O).

In the crystal of compound **III** the complex cation $[Fe(H_2L^1)(H_2O)_2]^{3+}$ is bound to anions (NO_3^-) through the hydrogen bonds N(7)–H··O(1A), O(1W)–H··O(6A) and N(7)–H··O(1A), O(1W)–H··O(6A) (Figure 3, Table 3). The molecules of the water of crystallization involved in hydrogen bonds both as the donor and the acceptor, contribute to the formation of a complex system of bonds that connect the components in the crystal. The 3D network from the crystal is also determined by the participation of O atoms from the inorganic NO_3^- anions to the formation of hydrogen bonds as the acceptor.
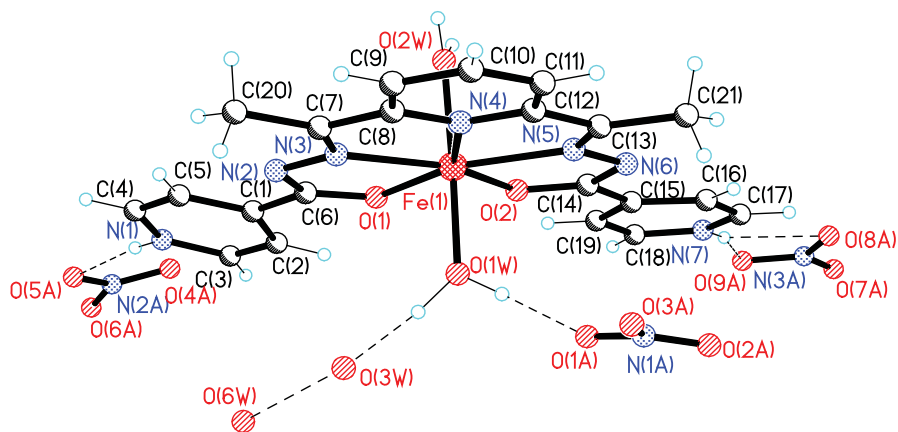

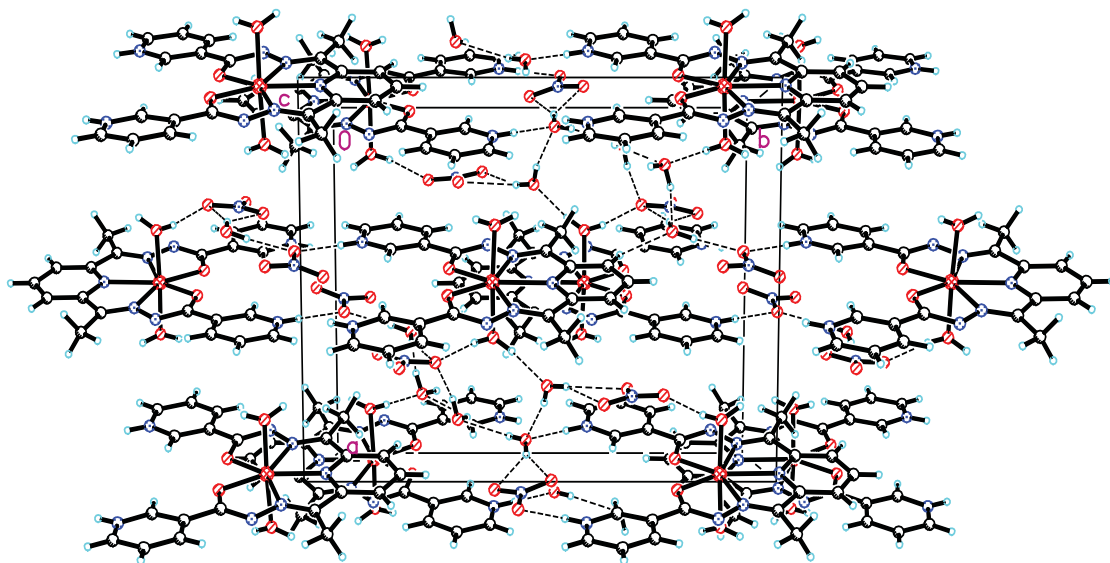**Figure 2. View of formula unit of IV with numbering scheme.**

Figure 3. A fragment of crystal packing in III.

In the crystal of **IV** the complex cation $[Fe(H_2L^2)(H_2O)_2]^{3+}$ is connected to the anion (NO_3^-) through the hydrogen bonds N(1)–H··O(5A), N(7)–H··O(8A), N(7)–H··O(9A), O(1W)–H··O(1A), O(2W)–H··O(2A)* and O(2W)–H··O(3A)* (Figure 4, Table 3). In addition, the complex cations are bound to each other through the hydrogen bonds of the type O(2W)–H··O(2)*. The molecules of the water of crystallization involved in hydrogen bonds both as the donor and the acceptor, contribute to the formation of a complex system of bonds that connect the components in the crystal. The 3D network from the crystal is also determined by the participation of O atoms from the inorganic NO_3^- anions to the formation of hydrogen bonds as the acceptor. In the crystal, centrosymmetric clusters of four molecules of water of crystallization and two NO_3^- anions (N(3A), O(3W) and O(6W)) can be seen.
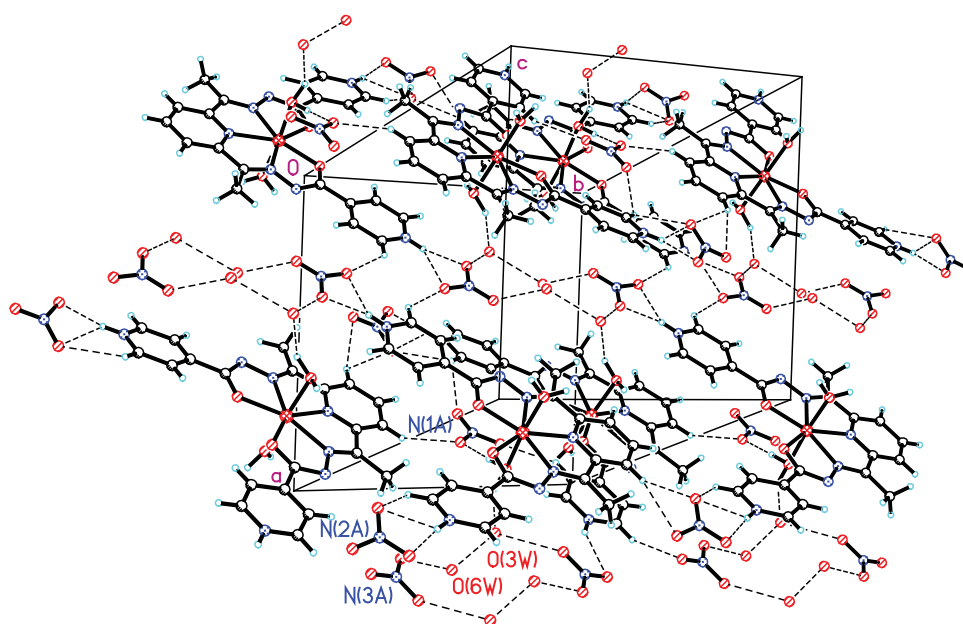

Figure 4. A fragment of crystal packing in IV.

Microbiology

The influence of iron(III) coordination compounds with nitrogen-containing ligands on the enzyme activity of *Aspergillus niger* CNMN FD 10 micromycete is presented in Table 4. Each type of enzyme reacted differently to the addition of the coordination compounds into the nutrient medium.

Cellobiohydrolase is an enzyme acting on the key stages of the hydrolysis of cellulose and releasing cellobiose from the non-reducing ends of the chains. In the control samples, without complexes, on the 6th day of cultivation of the producer, the activity of the enzyme was 0.14 U/mL. The maximal activity of cellobiohydrolase was observed on the 7th day of cultivation, constituting 0.18 U/mL. Coordination complexes have demonstrated an obvious inhibitory effect on the biosynthesis of cellobiohydrolase. Particularly, the inhibition increased with the raise of the concentration of compounds from 5 mg/L to 15 mg/L. Thus, on the day with the maximum biosynthesis of cellobiohydrolase (the 7th day), using compounds **II, III** and **IV** at the concentration of 5 mg/L, the enzyme activity in experimental samples constituted 0.16-0.17 U/mL, which was 5.88-12.5% lower than in the control samples (0.18 U/mL). With the augment of the concentration to 15 mg/L the activity of cellobiohydrolase decreased to 0.11-0.12 U/mL, *i.e.* it was 39-40% lower, as compared to the control. Compound **I** showed the greatest inhibitory effect, reducing the enzyme activity to zero in the concentration of 15 mg/L. On the next days (8th and 9th) the inhibitory effect was maintained.

β-Glucosidase is an enzyme acting at the final stages of cellulose hydrolysis, which cleaves cellobiose (formed after the action of cellobiohydrolase) to glucose. This enzyme reacted less to the addition of coordination compounds in the growth medium, in comparison with cellobiohydrolase.

In the control samples the activity of *β*-glucosidase on the 6th day of cultivation was 2.47 U/mL. The maximal activity of *β*-glucosidase was observed on the 7th day, being 2.80 U/mL. On the 8th day the enzyme activity decreased insignificantly till 2.59 U/mL. On the 9th day a sharp decrease in activity of *β*-glucosidase to 1.69 U/mL was observed.

Table 4

The effect of iron(III) coordination compounds on the enzyme activity (U/mL) of *Aspergillus niger* CNMN FD 10 micromycete.

Compound	Conc., mg/L	Cellobiohydrolase				β-Glucosidase				Endoglucanase				Xylanase			
		6th day	7th day	8th day	9th day	6th day	7th day	8th day	9th day	6th day	7th day	8th day	9th day	6th day	7th day	8th day	9th day
[Fe(dig)]Cl$_3$·2H$_2$O **(I)**	5	0.08	0.07	0.0	0.03	2.23	2.36	2.09	1.47	4.08	4.41	4.04	3.91	50.59	52.24	56.16	32.64
	10	0.05	0.0	0.0	0.02	2.08	1.67	2.06	1.36	3.34	4.57	3.51	3.59	47.87	46.78	56.57	32.64
	15	-	0.0	0.0	0.0	1.75	1.82	1.45	1.33	2.81	2.45	3.18	2.20	29.92	29.38	37.53	31.55
[Fe(HL¹)SO$_4$] **(II)**	5	0.11	0.16	0.06	0.04	2.28	2.55	2.53	1.63	3.92	4.89	5.06	4.89	52.22	65.28	71.80	39.17
	10	0.07	0.15	0.06	0.03	2.21	2.42	2.55	1.41	4.41	4.73	4.89	3.91	45.69	52.22	52.22	39.17
	15	0.065	0.12	0.01	0.08	2.21	2.39	2.28	1.30	4.08	3.91	4.24	3.67	53.31	41.34	50.05	34.82
[Fe(H$_2$L¹)(H$_2$O)$_2$](NO$_3$)$_3$·5H$_2$O **(III)**	5	0.11	0.16	0.07	0.03	2.28	2.66	2.43	1.55	4.73	5.06	5.38	4.49	35.90	69.63	50.05	39.17
	10	0.05	0.11	0.01	0.06	2.23	2.51	2.40	1.41	4.73	5.06	5.14	4.24	31.55	68.54	46.78	27.20
	15	0.03	0.11	0.00	0.01	2.21	2.55	2.28	1.25	4.08	4.89	5.14	3.51	29.38	65.28	38.08	26.12
[Fe(H$_2$L²)(H$_2$O)$_2$](NO$_3$)$_3$·1.5H$_2$O **(IV)**	5	0.07	0.17	0.07	0.07	2.43	2.55	2.47	1.63	3.92	4.57	4.73	4.41	29.38	65.28	65.28	22.85
	10	0.05	0.11	0.07	0.07	2.43	2.51	2.43	1.63	3.75	4.41	4.73	4.08	21.76	60.92	54.40	21.76
	15	0.04	0.12	0.07	0.06	2.36	2.39	2.28	1.36	3.42	3.67	4.08	3.67	28.29	45.69	42.43	21.76
Control	-	0.14	0.18	0.10	0.10	2.47	2.80	2.59	1.69	4.98	5.55	5.66	5.55	53.31	78.33	77.25	43.88

The use of coordination compounds at the lowest concentration (5 mg/L) showed the *β*-glucosidase activity of 2.23-2.43 U/mL (on the 6th day), 2.36-2.66 U/mL (on the 7th day), 2.09-2.53 U/mL (on the 8th day) and 1.47 - 1.63 U/mL (on the 9th day). Comparison with the control samples revealed that the inhibitory effect was not significant. With the elevation of the concentration of compounds to 15 mg/L, the activity of *β*-glucosidase decreased more, however, the enzyme activity of compounds **II**, **III** and **IV** did not exceed 20%. The greatest inhibitory effect both on the biosynthesis of *β*-glucosidase and cellobiohydrolase was shown by the iron complex **I**, containing chloride ions and dig-ligand. At the concentration of 15 mg/L the activity of *β*-glucosidase constituted: 1.75 U/mL (control - 2.47 U/mL) – on the 6th day, 1.82 U/mL (control - 2.80 U/mL) – on the 7th day; 1.45 U/mL (control - 2.59 U/mL) – on the 8th day, which was 41.14%; 53.85% and 78.62% lower than the activity in the control samples, respectively.

Endoglucanases are enzymes that hydrolyze cellodextrins and cellulose derivatives, such as CMC (carboxymethyl cellulose). In the control samples the activity of endoglucanase varied insignificantly– 5.55 - 5.66 U/mL on the 7th-9th days of cultivation with maximal activity on the 8th day. As in the case of cellobiohydrolase and β-glucosidase, all tested complexes demonstrated an inhibitory effect, which enhanced with the increase of the concentration of compounds, regardless of the culture period. Thus, in the day of the maximal activity of the endoglucanase (the 8th day) it decreased depending on the complex and the applied concentration (from 5 mg/L to 15 mg/L) from 5.38 U/mL to 3.18 U/mL. The lowest inhibitory effect was noted in the presence of compound **III**: the enzyme activity decreased from 5.38 U/mL to 5.14 U/mL. The highest inhibitory effect was registered in the presence of compound **I**: the enzyme activity dropped from 4.04 U/mL to 3.18 U/mL.

Similar results were obtained for xylanase, enzyme that decomposes hemicellulose, one of the major components of plant cell walls. In the control samples the maximal activity of the xylanase was on the 7th and 8th days, constituting 78.33 U/mL and 77.25 U/mL, respectively. On the 9th day xylanase activity decreased to 43.88 U/mL. Similarly to the afore-discussed enzymes, the inhibitory effect of all studied coordination compounds raised with the increase of the concentration, regardless of the culture period. For example, on the 7th day of cultivation, xylanase activity decreased, depending on the composition of the complex and the concentration (from 5 mg/L to 15 mg/L) from 69.63 U/mL to 41.34 U/mL, as compared to the activity of xylanase in the control samples of 78.33 U/mL. As in the case of the endoglucanase, the weakest inhibitory effect was shown by complex **III** and the greatest- at the use of complex **I**.

The properties of the investigated iron(III) coordination compounds with different ligands (especially, complex **I**) to inhibit the biosynthesis of cellulases and xylanase at *Aspergillus niger* CNMN FD 10 micromycete suggests the possibility of their use in plant protection, namely in the diseases caused by microscopic fungi of the genus *Aspergillus*.

Conclusions

Iron(III) complexes with Schiff bases with 2,6-diacetylpyridine-bis(nicotinoylhydrazone) and 2,6-diacetylpyridine-bis(isonicotinoylhydrazone) and complexes with semicarbazide diacetic acid dihydrazide and sulfadiazine, have demonstrated biologic activity. The Schiff bases coordinate to iron(III) as pentadentate ligands, forming coordination compounds with pentagonal bipyramidal molecular structure, their base being composed of neutral molecule of the Schiff base that are coordinated *via* the set of the N_3O_2 donor atoms, and the water molecules localized on its peaks.

All tested Fe(III) complexes with nitrogen-containing ligands, caused an inhibitory effect on the biosynthesis of hydrolases at *Aspergillus niger* CNMN FD 10, which increased with the raise of concentration.

Coordination compounds showed different effects on the biosynthesis of the enzymes synthesized by *Aspergillus niger* CNMN FD 10 micromycete, due to the different specificity of action of these hydrolases: complexes largely inhibited cellobiohydrolase and less - *β*-glucosidase. The inhibitory effect on the endoglucanase and xylanase was approximately the same.

The greatest inhibitory effect was demonstrated by [Fe(dig)]Cl$_3$·2H$_2$O (**I**), containing chloride ion and semicarbazide diacetic acid dihydrazide, the lowest inhibitory effect was shown by [Fe(H$_2$L^1)(H$_2$O)$_2$](NO$_3$)$_3$·5H$_2$O (**III**), containing the Schiff base 2,6-diacetylpyridine bis(nicotinoylhydrazone) and NO$_3^-$ anions.

References
1. Ciloci, A.; Bivol, C.; Stratan, M.; Reva, V., Clapco, S.; Tiurin, J.; Rija, A.; Coropceanu, E. The effect of [Co(DH)$_2$(An)$_2$]$_2$[TiF$_6$]·3H$_2$O coordination compound on polypeptide spectrum of amylolytic preparation from *Aspergillus niger* 33-19 CNMN FD 02a strain. The Annals of Oradea University, Biology Fascicle, 2012, 19(2), pp. 128-132.
2. Clapco, S.; Bivol, C.; Ciloci, A.; Stratan, M.; Coropceanu, E.; Tiurin, J.; Rija, A.; Labliuc, S.; Bulhac, I. The effect of some metal complexes of oxime ligands on proteolytic activity of *Fusarium gibbosum* CNMN FD 12 strain. The Annals of Oradea University, Biology Fascicle, 2013, 20(1), pp. 53-58.

3. Deseatnic-Ciloci, A.; Tiurina, J.; Ciapurina, L.; Turta, C.; Bivol, C.; Clapco, S.; Dvornina, E.; Labliuc, S. Coordination compounds of copper with aminoacids as potential stimulators of micromycetes extracellular hydrolases biosynthesis. Priorities of Chemistry for a Sustainable Development, PRIOCHEM, Bucharest, Romania, 2013, pp. 119-121.

4. Bourosh, P.; Coropceanu, E.; Ciloci, A.; Clapco, S.; Bologa, O.; Bivol, C.; Tiurina, J.; Bulhac, I. New Co(III) dioximates with hexafluorophosphate ion as stimulators of the proteolytic activity of the micromycete *Fusarium gibbosum* CNMN FD 12. Russian Journal of Coordination Chemistry, 2013, 39(11), pp. 777-786.

5. Coropceanu, E.; Deseatnic, A.; Stratan, M.; Rija, A.; Bologa, O.; Tiurin, J.; Labliuc, S.; Clapco, S.; Bulhac, I. The study of the biological activity of some cobalt(III) dioximates with fluorine containing anions. Chemistry Journal of Moldova, 2008, 3(2), pp. 70-80.

6. Dulcevscaia, G.; Kravtsov, V.; Macaev, F.; Duca, Gh.; Stingachi, E.; Pogrebnoi, S.; Boldescu, V.; Clapco, S.; Tiurina, J.; Deseatnic-Ciloci, A.; Lipkowski, J.; Liu, S.-X.; Decurtins, S.; Baca, S. New copper(II) complexes with isoconazole: synthesis, structures and biological properties. Polyhedron, 2013, 52, pp. 106-114.

7. Bivol, C.; Lazarescu, A.; Turta, C.; Rudic, V. Action of some coordinative compounds of Fe(III) with α-aminoacids on photosynthetical pigments content in microalgae *Dunaliella salina* CVNMN-AV-01. Journal of Academy of Sciences of Moldova. Life sciences, 2012, 3(317), pp. 149-154. (in Romanian).

8. Rudic, V.; Cepoi, L.; Rudi, L.; Miscu, V.; Chiriac, T.; Cojocari, A.; Lozan, V.; Coropceanu, E.; Bologa, O. Influence of coordinative compounds of cobalt with dioximates on biosynthetical processes in red alga *Porphiridium cruentum*. Journal of Academy of Sciences of Moldova. Life sciences, 2012, 1(316). pp. 144-151. (in Romanian).

9. Rudi, L.; Cepoi, L.; Miscu, V.; Chiriac, T.; Valuta A.; Codreanu, S.; Sadovnic, D.; Rudic, V. Biomass and carotenoid production by green alga Haematococcus pluvialis during the life cycle under the influence of Co(II) Schiff base complexes. Journal of Academy of Sciences of Moldova. Life sciences, 2014, 2(323), pp. 163-171. (in Romanian).

10. Valuta, A.; Cepoi, L.; Rudi, L.; Bulhac, I.; Bourosh, P.; Bologa, O. Phycobiliprotein accumulation in cyanobacterium *Nostoc linckia* and modification of antioxidant activity. The Annals of Oradea University, Biology Fascicle, 2015, 22(1), pp. 13-19.

11. Ciobanica, O.; Bourosh, P.; Bologa, O.; Bulhac, I.; Lozan, V.; Shofransky, V. Synthesis and crystal structure of a new Fe(ii) α-dioximate with triazine. Chemistry Journal of Moldova, 2013, 8(1), pp. 78-82.

12. Usatii, A.; Topala, L.; Chirita, E. Calcatiniuc, A., Borisova, T. Productivity, lipidogenesis and carotinogensis of yeast *Rhodotorula gracilis* CNM-YS-III/20 at the cultivation in presence of coordinative compounds of Mo(IY), Cr(III), Co(III), V(V). Journal of Academy of Sciences of Moldova. Life sciences, 2003, 1, pp. 99-103. (in Romanian).

13. Vomisescu, C.; Bourosh, P.; Kravtsov, V.; Dragancea, D. Nickel(II) complex derived from 2-hydroxy-3-methoxybenzaldehyde semicarbazone and 2,2'-bipyridine. Chemistry Journal of Moldova, 2013, 8(2), pp. 78-82.

14. Ciloci, A.; Tiurina, J.; Bologa, O.; Clapco, S.; Labliuc, S.; Pasha, L.; Coropceanu, E.; Rija, A. Coordinative compounds of Mo and V as possible stimulators of biosynthesis of exocellular cellulases of micromycete *Penicillium expansum* CNMN FD 05 C. Journal of Academy of Sciences of Moldova. Life sciences, 2012, 3(318), pp. 158-165. (in Russian).

15. Lemenovschi, D.A. Metalic composition in nature. Soros Educational Journal, 1997, 9, pp. 48-54. (in Russian).

16. Ershov, Iu.A.; Pletneova, T.V. The chemistry of biogenic elements. Vysshaja shkola: Moscow, 1993, 560 p. (in Russian).

17. Verejan, A.V. Coordination compounds of transition metals with semicarbazide diacetic acid and its derivatives. Ph.D. Thesis, A.V. Bogatskii Physico-Chemical Institute, Ukrainian Academy of Sciences, Odessa, Ukraine, 1991. (in Russian).

18. Gerbeleu, N.V.; Verejan, A.V.; Byrca, M.C.; Turta, C.I.; Lozan, V.I.; Bologa, O.A. Mössbauer spectra of coordination compounds of iron(III) with semicarbazide diacetic acid and its dihydrazide. Proceedings of the VIth Conference on Spectroscopy of Coordination Compounds, Krasnodar, Russia, 1990, p. 170. (in Russian).

19. Sheldrick, G.M. A short history of SHELX. Acta Crystallographica, 2008, A64 (1), pp. 112-122.

20. Ciloci, A.; Tiurina, J.; Clapco, S.; Labliuc, S.; Stratan, M.; Grumeza, M. Fungal strain *Aspergillus niger* – producer of cellulozolytic and xylanolytic enzymes. MD Patent, 2010, No. 4072. (in Romanian).

21. Haritonov, Iu.Y.; Machoshvili, R.I.; Gogorishvili, P.V.; Shamilishvili, O.H. Investigation of metal coordinative complexes with oxalyl dihydrazide by IR spectroscopy. Russian Journal of Inorganic Chemistry, 1972, 17(11), pp. 2992-2995. (in Russian).

22. Haritonov, Iu.Y.; Machoshvili, R.I.; Goeva, L.V.; Scelokov, R.N. Metal coordinative compounds with diformyl hydrazine. Russian Journal of Coordinative Chemistry, 1975, 1(3), pp. 333-341. (in Russian).

23. Nakamoto, K. IR spectra and Raman spectra of inorganic and organic coordinative compounds. Mir: Moscow, 1991, 536 p. (in Russian).

24. Nakanisi, K. Infrared spectra and structure of organic compounds. Mir: Moscow, 1965, 216 p. (in Russian).

25. Bellami, L. Infrared spectra of complex molecules. IL: Moscow, 1963, 590 p. (in Russian).

26. Tarasevici, B.N. Infrared spectra of basic class of organic compounds. Reference materials. Moscow, 2012, 54 p. (in Russian).

27. Andjelkovic, K.; Bacchi, A.; Pelizzi, G.; Jeremic, D.; Ivanovic-Burmazovic, I. Diaqua-(2',2'''-(2,6-pyridinediyldiethylidyne)dioxamohydrazide)-iron(III) perchlorate hydrate WUTPIK. Journal of Coordination Chemistry, 2002, 55, pp. 1385-1392.

28. Palenik, G.J.; Wester, D.W.; Rychlewska, U.; Palenik, R.C. Pentagonal-Bipyramidal Complexes. Synthesis and crystal structures of Diaqua[2,6-diacetylpyridine bis(semicarbazone)]chromium(III) hydroxide dinitrate hydrate and Dichloro[2,6-diacetilpyridine bis(semicarbazone)]iron(III) chloride dehydrate. Inorganic Chemistry, 1976, 15(8), pp. 1814-1819.

29. Allen, F.H. Cambridge structural database. Acta Crystallographica, 2002, 58B(3-1), pp. 380-388.

30. Palenik, G.J.; Wester, D.W. Pentagonal-Bipyramidal complexes. Crystal and molecular structures of chloroaqua(2,6-diacetylpyridine bis(semicarbazone))manganese(II),-iron(II),-cobalt(II), and –zinc(II) chloride dehydrates. Inorganic Chemistry, 1978, 17(4), pp. 864-870.

31. Bonardi, A.; Carini, C.; Merlo, C.; Pelizzi, C.; Pelizzi, G.; Tarasconi, P.; Vitali, F.; Cavatorta F. Synthesis, spectroscopic and structural characterization of mono- and bi-nuclear iron(II) complexes with 2,6-diacetylpyridine bis(acylhydrazones). Journal of the Chemical Society. Dalton Transactions, 1990, pp. 2771-2777.

32. Gao-Feng, Liu; Filipovic, M.; Heinemann, F.W.; Ivanovic-Burmazovic, I. Diaqua-(2,6-diacetylpyridinebis(semiox amazide)-N,N',N'',O,O')-iron(II) dinitrate monohydrate TIRFAC. Inorganic Chemistry, 2007, 46, pp. 8825-8835.

33. Bar, A.K.; Pichon, C.; Gogoi, N.; Duhayon, C.; Ramasesha, S.; Sutter J. Single-ion magnet behavior of heptacoordinated Fe(II) complexes: on the importance of supramolecular organization. Chemical Communications, 2015, 51(17), pp. 3616-3619.