ISSN 1070-3284, Russian Journal of Coordination Chemistry, 2013, Vol. 39, No. 11, pp. 777–786. © Pleiades Publishing, Ltd., 2013.
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# New Co(III) Dioximates with Hexafluorophosphate Ion as Stimulators of the Proteolytic Activity of the Micromycete *Fusarium gibbosum* CNMN FD 12

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Received May 16, 2013

**Abstract**—The coordination compounds $[Co(DH)_2(An)_2][PF_6]$ (**I**) and $[Co(NioxH)_2(Thio)_2][PF_6] \cdot 0.5DMF \cdot 0.5H_2O$ (**II**), where $DH^-$ and $NioxH^-$ are dimethylglyoxime and 1,2-cyclohexanedione dioxime monoanions, respectively; An is aniline; and Thio is thiourea, were synthesized. The composition and structure of the complexes were determined by elemental analysis, IR spectroscopy, and X-ray diffraction. Compounds **I** and **II** are ionic and consist of complex cations $[Co(DioxH)_2(A)_2]^+$, where DioxH is the α-dioxime residue, A is neutral organic molecule (aniline or thiourea), and $[PF_6]^-$ anions. The coordination polyhedra of the Co(III) complex cations are octahedra formed by the set of $N_6$ donor atoms of monodeprotonated $DH^-$ residues and two An molecules (in **I**) or by the $N_4S_2$ atoms of two $NioxH^-$ anions and two Thio molecules (in **II**). The formation of the crystal structure of **I** and **II** is largely determined by the $[PF_6]^-$ anions in which the fluorine atoms serve as acceptors in various hydrogen bonds. The compounds were tested as stimulators of biosynthesis of extracellular proteases of the micromycete *Fusarium gibbosum* CNMN FD 12. The introduction of the test complexes in optimized concentrations into the nutrition medium for cultivation of the producing strain enhances the biosynthesis of acid and neutral proteases by 63.6 and 92.5%, respectively.

**DOI:** 10.1134/S107032841311002X

## INTRODUCTION

The extension of the scope of applicability of enzyme preparations calls for studies aimed at elucidation of new ways for enhancing their biosynthetic properties. From this standpoint, of particular interest are chemical stimulators, including transition metal complexes [1] the minor nutrient elements of which are involved in the metabolic processes of the body where they perform qualitative and quantitative control over redox, hydrolytic, and other reactions, affecting directly or indirectly the enzyme activity [2].

A micronutrient vitally important for microorganisms is cobalt, which (along with other metal ions) functions as an activator of most kinases, synthetases, and aldolases and is present as a part of vitamin $B_{12}$ [3]. The recent studies carried out at the Institute of Microbiology and Biotechnology, Academy of Sciences of Moldova, show that cobalt-containing coordination compounds play an important role in the targeted synthesis of biologically active substances (carotenoids, phycobilins, cyanocobalamins, enzymes, and so on) produced by microorganisms of different taxonomic groups (cyanobacteria, algae, micromycetes) [1, 4–7]. It is known that some oximes are also involved in important metabolic processes that occur in the body [8] and cobalt dioximates can serve as models of important molecules such as vitamin $B_{12}$ [9, 10].

Analysis of the data of Cambridge Crystallographic Data Centre (CCDC) [11] revealed only one heterometallic Co(III) dioximate containing $[PF_6]^-$ anion [12]. Previously we synthesized and studied the complex $[Co(DH)_2(Thio)_2]_2F[PF_6]$ [13].

This communication describes the synthesis of new Co(III) dioximates, $[Co(DH)_2(An)_2][PF_6]$ (**I**) and $[Co(NioxH)_2(Thio)_2][PF_6] \cdot 0.5DMF \cdot 0.5H_2O$ (**II**), where $DH^-$ and $NioxH^-$ are dimethylglyoxime and 1,2-cyclohexanedione dioxime monoanions, respectively, An is aniline, and Thio is thiourea. The product composition and structure were determined by various physicochemical methods. The possibility of enhancement of the biosynthesis of proteolytic enzymes of the micromycete *Fisarium gibbosum* CNMN FD 12 by the synthesized cobalt complexes was studied.

## EXPERIMENTAL

**Synthesis of I.** Dimethylglyoxime (0.23 g, 0.002 mol) in methanol (20 mL) and aniline (0.23 mL,

0.0024 mol) were added to a mixture consisting of $Co(CH_3COO)_2 \cdot 4H_2O$ (0.25 g, 0.001 mol) and $KPF_6$ (0.2 g, 0.001 mol) in water (20 mL). The resulting solution was heated for 10–15 min. The hot solution was filtered and allowed to evaporate at room temperature. On slow evaporation, light brown plate crystals were formed. Yield ~30%. The compound was soluble in DMSO, DMF, methanol, and ethanol, less soluble in water, and insoluble in diethyl ether.

For $C_{20}H_{28}F_6N_6O_4PCo$

| | | | | |
|---|---|---|---|---|
| anal. calcd. (%): | Co, 9.50; | C, 38.72; | H, 4.55; | N, 13.55. |
| Found (%): | Co, 9.17; | C, 38.43; | H, 4.36; | N, 13.39. |

**Synthesis of II.** 1.2-Cyclohexanedione dioxime (0.28 g, 0,002 mol) in methanol (15 mL) and thiourea (0.15 g, 0.002 mol) were added to a mixture consisting of $Co(CH_3COO)_2 \cdot 4H_2O$ (0.25 g, 0.001 mol) and $KPF_6$ (0.2 g, 0.001 mol)) in water (20 mL). The resulting solution was heated for 10–15 min. The hot solution was filtered and allowed to evaporate at room temperature. On slow evaporation, brown plate crystals were formed. Yield ~35%. The compound was soluble in DMSO, DMF, methanol, and ethanol, less soluble in water, and insoluble in diethyl ether.

For $C_{15.5}H_{30.5}F_6N_{8.5}O_5S_2PCo$

| | | | | |
|---|---|---|---|---|
| anal. calcd. (%): | Co, 8.61; | C, 27.22; | H, 4.49; | N, 17.41. |
| Found (%): | Co, 8.32; | C, 27.06; | H, 4.37; | N, 17.33. |

The composition and structure of the obtained complexes were established using elemental analysis, IR spectroscopy, and X-ray diffraction. IR a spectra were measured on a FT-IR spectrometer 100 in mineral oil in the 4000–400 $cm^{-1}$ range and on an ATR spectromreter in the 4000–650 $cm^{-1}$ range.

**X-ray diffraction.** The experimental data for **I** and **II** were measured at room temperature on a diffractometer with a CCD Xcalibur detector (Mo$K_\alpha$ radiation, graphite monochromator) for crystals shaped as brown plates. The structures were solved by the direct method and refined by the full-matrix least-squares method mainly by the anisotropic procedure for non-hydrogen atoms (SHELX-97) [14]. In **II**, three sites were found for atoms of the $PF_6^-$ anion with occupancies of 0.5, 0.3, and 0.2. The water hydrogen atoms in **II** were not determined; the structure was found to contain one $H_2O$ molecule with 0.25 occupancy and two molecules with 0.125 occupancies. The positions of other H atoms ion **I** and **II** were calculated geometrically and refined isotropically in the "rigid body" model with $U_{ef} = 1.2U_{eq}$ or $1.5U_{eq}$ of the corresponding O, N, and C atoms; in particular, the F and O(*w*) atoms in **II** were refined isotropically.

The crystal data and X-ray experiment details for **I** and **II** are summarized in Table 1, some interatomic distances and bond angles are in Table 2, and the geometric parameters of hydrogen bonds are in Table 3. The positional and thermal parameters for structures **I** and **II** are deposited in the CCDC (no. 919723 and no. 919724, respectively; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

**Biological assay.** A strain of the micromycete *Fusarium gibbosum* CNMN FD 12, a producer of acid and neutral proteases, served as the test subject [15].

The producer was cultivated in 0.5 L conical flasks containing 0.1 L of the nutrient medium of optimized composition (g/L): corn flour, 20.0; soy flour, 10.0; $CaCO_3$, 2.0, $(NH_4)_2SO_4$, 1.0. The initial pH of the medium was 6.25. This medium was used as the control in the tests. An aqueous suspension of spores of the 15-day culture grown on a wort agar medium (10% of the inoculated volume) with a density of $3 \times 10^6$ spores/mL served as the inoculum.

The complexes in concentrations of 5.0, 10.0, and 15.0 mg/L were introduced into the sterile nutrient medium as solutions simultaneously with the inoculum. The cultivation was accompanied by continuous stirring on a flask shaker at 180–200 rpm and 28–30°C; the cultivation time was 4–6 days.

The activities of acid (pH 3.6) and neutral (pH 7.4) proteases in the culture liquid were determined by the Wilstätter method based on determination of free carboxy groups formed upon hydrolysis of a 5% solution of gelatin. The amount of the enzyme that forms 1 mg of amine nitrogen in 1 h under the experimental conditions used was taken as the proteolytic activity unit [16].

## RESULTS AND DISCUSSION

The IR spectrum of **I** exhibits absorption bands at 2961 and 2829 ($CH_3$); 1567 (C=N); 1237 and 1086 ν(NO); 982 γ(OH); 734 γ(CNO), 511 and 434 $cm^{-1}$ ν(Co–N); bands confirming the presence of aniline molecules in the 1,6-coordinate: 3074 ν(C–H), 623 $cm^{-1}$ [γ(CCC) + γ(CNC)], and a band at 678 $cm^{-1}$ corresponding to vibrations of singly substituted aromatic rings δ(C–H).

In the IR spectrum of **II**, the absorption bands at 1562 ν(CN), 1220 $\nu_{as}$(NO), 1060 $\nu_s$(NO), 980 γ(OH), 725 γ(CNO), 535 $\nu_{as}$(Co–N), and 430 $cm^{-1}$ $\nu_s$(Co–N) can be assigned to the 1,2-cyclohexanedione dioxime monoanion, while the bands at 3312 $\nu_{as}$(NH), 3210 $\nu_s$(NH), 1615 $\delta(NH_2)$, 1060 [ν(CN) + ν(CS) + δ(HNC)], and 412 $cm^{-1}$ δ(NCS) attest to the presence of coordinated thiourea molecules in the complex.

Compounds **I** and **II** are ionic and consist of complex cations described as $[Co(DioxH)_2(A)_2]^+$ (DioxH is the α-dioxime residue, A are neutral aniline or thiourea molecules) and the $[PF_6]^-$ anions. The crystals of **II** also contain dimethylformamide and water solvent molecules. The unit cell of **I** has one independent $[Co(DH)_2(An)_2]^+$ cation, while the unit cell of **II** has two $[Co(NioxH)_2(Thio)_2]^+$ cations (A and B). All of

**Table 1.** Crystallographic data, X-ray experiment details, and structure refinement parameters for **I** and **II**

| Parameter | Value | |
|---|---|---|
| | **I** | **II** |
| $M$ | 620.38 | 684.00 |
| System | Monoclinic | Triclinic |
| Space group | $P2_1/n$ | $P\bar{1}$ |
| Unit cell parameters | | |
| $a$, Å | 11.2656(4) | 8.3696(5) |
| $b$, Å | 6.4326(2) | 13.0655(9) |
| $c$, Å | 17.3050(5) | 15.4276(10) |
| α, deg | | 72.259(6) |
| β, deg | 98.751(3) | 78.323(5) |
| γ, deg | | 89.382(5) |
| $V$, Å$^3$ | 1239.44(7) | 1571.2(2) |
| $Z$ | 2 | 2 |
| $\rho_{calcd.}$, g/cm$^3$ | 1.662 | 1.446 |
| μ, mm$^{-1}$ | 0.843 | 0.804 |
| $F$(000) | 636 | 702 |
| Crystal size, mm | 0.22 × 0.1 × 0.04 | 0.4 × 0.12 × 0.09 |
| Region of θ, deg | 3.38–25.50 | 2.90–25.10 |
| Ranges of reflection indices | $-13 \le h \le 8$, $-7 \le k \le 4$, $-20 \le l \le 19$ | $-9 \le h \le 9$, $-15 \le k \le 15$, $-18 \le l \le 18$ |
| The number of measured/independent reflections ($R_{int}$) | 4152/2295 (0.0254) | 10471/5568 (0.0492) |
| Filling degree, % | 99.4 | 99.4 |
| The number of reflections with $I > 2\sigma(I)$ | 1791 | 3285 |
| The number of refined parameters | 177 | 415 |
| GOOF | 1.016 | 1.005 |
| $R$-factor ($I > 2\sigma(I)$) | $R_1 = 0.0435$, $wR_2 = 0.1085$ | $R_1 = 0.0873$, $wR_2 = 0.2361$ |
| $R$-factor (for the whole array) | $R_1 = 0.0603$, $wR_2 = 0.1198$ | $R_1 = 0.1319$, $wR_2 = 0.2543$ |
| $\Delta\rho_{max}$, $\Delta\rho_{min}$, $e$ Å$^{-3}$ | 0.518, –0.259 | 0.861, –0.599 |

the identified cations have $C_i$ symmetry; the $[PF_6]^-$ anion in **I** is $C_i$-symmetric, while the anion in **II** resides in a general position. The coordination polyhedra of the Co(III) complex cations are octahedra. In **I**, the octahedron is formed by the $N_6$ set of donor atoms of two monodeprotonated $DH^-$ residues and two An molecules, while in **II**, it is composed of the $N_4S_2$ atoms of two $NioxH^-$ and two Thio molecules (Fig. 1). The coordinated An and Thio molecules occur at the apical coordinates of the octahedra. The thiourea molecules in the complex cations A and B of **II** are identically arranged with respect to the equatorial plane: both Thio molecules occupy intermediate positions between the perpendicular and parallel arrangements. The dihedral angles between the $N_4$ and $SCN_2$ equatorial planes are 57.2° and 130.0° in A and B of **II**, respectively. This position of the Thio ligands in the complex cations is stabilized by intramolecular and intertmolecular hydrogen bonds (Table 3, Figs. 1b, 1c). The interatomic distances in the coordination polyhedra are as follows: Co–N(1), 1.886(2); Co–N(2), 1.916(2); and Co–N(3), 2.013(2) Å in **I**; Co–N(1), 1.890(5); Co–N(2), 1.892(4); and Co–S(1), 2.295(2) Å in complex A, **II**, and Co–N(1), 1.889(6); Co–N(2), 1.896(6); and Co–S(2), 2.280(2) Å in complex B, **II**, (Table 2). These interatomic distances do not differ from such distances in the fluorine-containing anions $[SiF_6]^{2-}$, $[ZrF_6]^{2-}$, $[AlF_6]^{3-}$, $[TiF_6]^{2-}$, $[SbF_6]^-$, $[BF_4]^-$ complexed with different α-dioximes ($DH^-$ and $NioxH^-$) and An and Thio molecules [17–26]. The equatorial planes of the coordination polyhedra of **I** and **II** accommodate two dioximate anions stabilized by intramolecular O–H···O hydrogen bonds, which join them into a stable pseudomacrocyclic system (Table 3, Fig. 1). The O···O interatomic distances of these H-bonds are 2.513(3) Å in **I** and 2.537(6) and 2.531(7) Å in cations A and B of **II**, respectively. The donor···acceptor

**Table 2.** Selected interatomic distances and bond angles in structures **I** and **II***

| In complex cations | | | |
|---|---|---|---|
| Bond | *d*, Å | | |
| | **I** | **II**, complex A | **II**, complex B |
| Co(1)–N(1) | 1.886(2) | 1.892(4) | 1.889(6) |
| Co(1)–N(2) | 1.916(2) | 1.891(5) | 1.895(6) |
| Co(1)–N(3)/S(1)/S(2) | 2.013(2) | 2.295(2) | 2.281(2) |
| N(1)–C(1) | 1.292(4) | 1.286(8) | 1.284(9) |
| N(1)–O(1) | 1.357(3) | 1.359(6) | 1.356(7) |
| N(2)–C(2) | 1.296(4) | 1.317(7) | 1.290(9) |
| N(2)–O(2) | 1.331(3) | 1.319(6) | 1.341(7) |
| C(1)–C(2) | 1.473(4) | 1.461(9) | 1.451(10) |
| C(1)–C(11) | 1.486(4) | 1.491(8) | 1.510(10) |
| C(2)–C(21)/C(14) | 1.485(4) | 1.484(9) | 1.485(10) |
| Angle | ω, deg | | |
| | **I** | **II**, complex A | **II**, complex B |
| N(1)CoN(2) | 81.11(10) | 81.7(2) | 80.7(3) |
| N(1)CoN(3)/S(1)/S(2) | 91.47(10) | 93.80(17) | 94.26(18) |
| N(2)CoN(3)/S(1)/S(2) | 90.23(10) | 86.69(17) | 87.11(18) |
| $N(1)CoN(2)^{\#1}/^{\#2}$ | 98.89(10) | 98.3(2) | 99.3(2) |
| $N(1)CoN(3)^{\#1}/S(1)^{\#1}/S(2)^{\#2}$ | 88.53(10) | 86.20(17) | 85.74(18) |
| $N(2)CoN(3)^{\#1}/S(1)^{\#1}/S(2)^{\#2}$ | 89.77(10) | 93.31(17) | 92.89(18) |
| C(1)N(1)O(1) | 119.3(2) | 118.8(5) | 119.4(6) |
| C(1)N(1)Co(1)/Co(2) | 117.2(2) | 116.7(4) | 116.2(5) |
| O(1)N(1)Co(1)/Co(2) | 123.49(19) | 124.3(4) | 124.3(5) |
| C(2)N(2)O(2) | 122.2(2) | 121.0(5) | 121.5(6) |
| C(2)N(2)Co(1)/Co(2) | 116.2(2) | 115.7(4) | 117.3(5) |
| O(2)N(2)Co(1)/Co(2) | 121.49(17) | 123.3(4) | 121.2(5) |
| N(1)C(1)C(2) | 112.9(3) | 113.1(5) | 114.1(6) |
| N(1)C(1)C(11) | 123.3(3) | 124.5(6) | 125.0(7) |
| C(2)C(1)C(11) | 123.7(3) | 122.4(6) | 120.8(7) |
| N(2)C(2)C(1) | 112.4(3) | 112.7(6) | 111.6(7) |
| N(2)C(2)C(21)/C(14) | 124.0(3) | 125.2(6) | 125.4(7) |
| C(1)C(2)C(21)/C(14) | 123.5(3) | 122.0(5) | 123.0(7) |
| In complex anions | | | |
| Bond | *d*, Å | | |
| | **I** | **II**, site I | **II**, site II (A) |
| P(1)–F(1) | 1.569(2) | 1.561(15) | 1.583(16) |
| P(1)–F(2) | 1.563(2) | 1.551(14) | 1.573(16) |
| P(1)–F(3) | 1.580(3) | 1.589(14) | 1.546(16) |
| P(1)–F(4) | | 1.570(14) | 1.535(17) |
| P(1)–F(5) | | 1.560(14) | 1.564(16) |
| P(1)–F(6) | | 1.589(14) | 1.563(16) |

**Table 2.** (Contd.)

| Angle | ω, deg | | |
|---|---|---|---|
| | **I** | **II**, site I | **II**, site II (A) |
| F(1)P(1)F(2) | 89.65(14) | 173.8(12) | 179.5(15) |
| F(1)P(1)F(3) | 91.16(16) | 87.9(10) | 90.4(11) |
| F(2)P(1)F(3) | 89.13(19) | 87.7(9) | 89.3(11) |
| F(1)P(1)F(1)$^{\#3}$/F(4) | 180 | 91.9(10) | 89.0(11) |
| F(1)P(1)F(2)$^{\#3}$/F(5) | 90.35(14) | 94.0(10) | 89.3(11) |
| F(1)P(1)F(3)$^{\#3}$/F(6) | 88.84(16) | 88.3(10) | 88.7(11) |
| F(2)P(1)F(1)$^{\#3}$/F(4) | | 92.3(10) | 91.3(11) |
| F(2)P(1)F(2)$^{\#3}$/F(5) | 180 | 90.6(10) | 93.7(13) |
| F(2)P(1)F(3)$^{\#3}$/F(6) | 90.87(19) | 87.4(9) | 92.2(11) |
| F(3)P(1)F(1)$^{\#3}$/F(4) | | 177.3(12) | 173.5(17) |
| F(3)P(1)F(2)$^{\#3}$/F(5) | | 92.8(10) | 92.8(13) |
| F(3)P(1)F(3)$^{\#3}$/F(6) | 180 | 90.9(9) | 81.3(17) |
| F(4)P(1)F(5) | | 89.9(9) | 90.2(12) |
| F(4)P(1)F(6) | | 86.5(9) | 91.7(11) |
| F(5)P(1)F(6) | | 175.7(12) | 173.7(16) |

* Symmetry codes for equivalent atoms:: $^{\#1}$ $-x+1, -y+1, -z$; $^{\#3}$ $-x, -y+1, -z$ (**I**); $^{\#1}$ $-x+1, -y+1, -z$; $^{\#2}$ $-x+1, -y+1, -z+1$ (**II**).

(N···O) interatomic distances in the intramolecular H-bonds N–H···O of **II** are 3.018(8) and 3.175(13) Å for complexes A and B, respectively. This type of architecture is typical of all *trans*-octahedral cobalt complexes with α-dioximes and thiourea [11, 21–26]. The interatomic distances and bond angles in $DH^-$, $NioxH^-$, An, and Thio of **I** and **II** do not differ from the values found previously in compounds containing these organic ligands and fluorine-containing anions [17–26].

The P–F bond lengths in the $[PF_6]^-$ – anions of **I** and **II** (1.563(2)–1.580(3) and 1.48(1)–1.58(2) Å, respectively) do not differ from those found in [12]. The $[PF_6]^-$ anion in **I** and **II** is outer-sphere and does not coordinate metal atoms, similarly to almost all fluorine-containing anions in cobalt dioximates [17–26].

The electrostatic interactions between the complex cations and $[PF_6]^-$ anions in the crystal structures of **I** and **II** are supplemented by intermolecular H-bonds, N–H···F and C–H···F (Table 3), which make a considerable contribution to the crystal packing.

A fragment of crystal packing of the complex cations and anions in **I** is shown in Fig. 2. The complex cations $[Co(DH)_2(An)_2]^+$ are involved, through the amino groups of the coordinated An molecules, in intermolecular H-bonds, N(3)–H···O(2) ($-x+1, -y+2, -z$; N···O 3.019(3) Å), responsible for the formation of one-dimensional chain directed along the *y* axis of the crystal. The cation chains are additionally stabilized by intermolecular H-bonds, C(21)–H···O(1) ($x, y+1, z$; C(21)···O(1) 3.313(3), C(21)–H 0.96, H···O(1) 2.40 Å; CHO, 159°), while the N–H···F intermolecular H-bonds (N···F 3.063(4) Å) form layers along the *z* axis.

A fragment of crystal packing of the components of complex **II** is shown in Fig. 3. The complex cations $[Co(NioxH)_2(Thio)_2]^+$ (A and B) alternate thus forming chains along the *z* axis by means of intermolecular H-bonds, N(4*A*)–H···O(2*B*) ($-x+1, -y+1, -z+1$; N···O 2.870(8) Å) and N(3*B*)–H···O(1*A*) ($x, y, z$; N···O 2.870(9) Å). The chains are joined into layers by intermolecular H-bonds, N(4*B*)–H···F(1) ($-x+1, -y, -z+1$; N···F 3.03(2) Å), N(4*B*)–H···F(1) ($x, y, z$; N···F 3.15(3) Å), N(4*B*)–H···F(6) ($x, y, z$; N···F 3.11(3) Å), and C(14)–H(1)···F(4)/F(2*A*)/F(6*B*) ($-x+1, -y, -z+1$; C(14)···F 3.382(6)/3.409(5)/3.345(5), C(14)–H(1) 0.97, H(1)···F(4)/F(2*A*)/F(6*B*) 2.53/2.60/2.43 Å; CHF, 146°/141°/157°) in which fluorine atoms of $[PF_6]^-$ act as acceptors. The layers in crystal **II** are directed along the *x* axis (Fig. 3). The crystal cavities accommodate DMF molecules linked to the framework by intermolecular H-bonds, N(3*A*)–H···O(1) ($x, y+1, z$; N···O 2.850(10) Å) and N(4*A*)–H···O(1) ($x, y+1, z$; N···O 2.922(10) Å) in which acceptors are oxygen atoms and disordered water molecules connected by weak intermolecular H-bonds O(*w*)–H···F (O···F 3.108(10)–3.294(10 Å).

The other intermolecular contacts in structures **I** and **II** correspond to the sums of the van der Waals radii of the corresponding atoms.

Complexes of nutrient elements are of considerable interest for biotechnology. Due to their physiological activity, complexes of nutrient elements are studied by many biologists. Recent works demonstrate the pros-

**Table 3.** Geometric parameters of intra- and intermolecular hydrogen bonds in structures **I** and **II**

| D–H···A contact | Distance, Å | | | DHA angle | Coordinates of the A atoms |
|---|---|---|---|---|---|
| | D–H | H···A | D···A | | |
| | | | **I** | | |
| O(1)–H(1)···O(2) | 0.82 | 1.72 | 2.513(3) | 161 | $-x+1, -y+1, -z$ |
| N(3)–H(1)···O(2) | 0.90 | 2.12 | 3.019(3) | 172 | $-x+1, -y+2, -z$ |
| N(3)–H(2)···F(3) | 0.90 | 2.16 | 3.063(4) | 176 | $x, y, z$ |
| | | | **II** | | |
| O(1*A*)–H(1)···O(2*A*) | 0.82 | 1.75 | 2.537(6) | 159 | $-x+1, -y+1, -z$ |
| O(1*B*)–H(1)···O(2*B*) | 0.82 | 1.75 | 2.532(7) | 159 | $-x+1, -y+1, -z+1$ |
| N(3*A*)–H(1)···O(1) | 0.86 | 2.08 | 2.851(10) | 148 | $x, y+1, z$ |
| N(3*A*)–H(2)···O(2*A*) | 0.86 | 2.17 | 3.019(8) | 169 | $-x+1, -y+1, -z$ |
| N(4*A*)–H(1)···O(1) | 0.86 | 2.18 | 2.923(10) | 144 | $x, y+1, z$ |
| N(4*A*)–H(2)···O(2*B*) | 0.86 | 2.04 | 2.872(8) | 163 | $-x+1, -y+1, -z+1$ |
| N(3*B*)–H(1)···O(2A) | 0.86 | 2.15 | 2.871(9) | 141 | $x, y, z$ |
| N(3*B*)–H(2)···O(2*B*) | 0.86 | 2.36 | 3.179(13) | 160 | $-x+1, -y+1, -z+1$ |
| N(4*B*)–H(1)···F(3*A*) | 0.86 | 2.20 | 2.99(3) | 154 | $x, y, z$ |
| N(4*B*)–H(1)···F(5*B*) | 0.86 | 2.25 | 3.11(3) | 177 | $x, y, z$ |
| N(4*B*)–H(1)···F(6) | 0.86 | 2.45 | 3.10(2) | 133 | $x, y, z$ |
| N(4*B*)–H(1)···F(1) | 0.86 | 2.46 | 3.14(3) | 137 | $x, y, z$ |
| N(4*B*)–H(2)···F(1) | 0.86 | 2.25 | 2.98(2) | 143 | $-x+1, -y, -z+1$ |
| N(4*B*)–H(2)···F(3*A*) | 0.86 | 2.60 | 3.40(3) | 155 | $-x+1, -y, -z+1$ |

pects of using these complexes as stimulators and bioregulators of the synthesis of secondary metabolites in microorganisms of different taxonomic groups. The stimulating effect of some dioxime complexes on the biosynthesis of extracellular hydrolases (amylases, lipases, cellulases) was elucidated. For instance, cobalt(III) dioximates, $[Co(DH)_2(Thio)_2]_3F[SiF_6] \cdot 1.5H_2O$, $[Co(DH)_2(Thio)_2]_2[SiF_6] \cdot 3H_2O$, and $[Co(DH)_2(Thio)_2][BF_4] \cdot 3H_2O$, are effective stimulators of the hydrolytic activity of the strains *Rhizopus arrhizus* F 67 (pectinase producer) and *Aspergillus niger 33* CNMN FD 06A (amylase producer), which enhance the enzyme biosynthesis by 97.1–115.3% and 26.3–42.6%, respectively, and shorten the biological cycle of the pectinase producer by 24 h. The addition of $[Co(DH)_2(An)_2]_2[TiF_6] \cdot 3H_2O$, $[Co(NioxH)_2(Sam)_2]_2[TiF_6] \cdot 3H_2O$, or $[Co(NioxH)_2(An)_2]_2[TiF_6] \cdot 3H_2O$ (5–10 mg/L) into the nutrient medium of the micromycete *A. niger* 33-19 CNMN FD 02A shortens the strain cultivation cycle by 24–48 h and increases the amylolytic activity by 23–64% [5–7, 27, 28].

Many proteases are metalloenzymes requiring metal ions to stabilize the molecular structure and to exhibit the catalytic activity. Most of metalloproteases contain zinc ions in the active site. However, some proteases contain one or two cobalt or magnesium ions. Cobalt-dependent methionine aminopeptidase was detected in *Escherichia coli* [29].

Experimental studies carried out at international research centers demonstrated that zinc ions in the protease active site can be replaced by other metal ions (Co, Mn, Cu) with the enzyme catalytic activity remaining intact; this is widely used, in particular, to study the structures of zinc-containing enzymes. For example, for the protease synthesized by *Staphylococcus*, it was found that $Zn^{2+}$ ions can be replaced by cobalt ions. Co-substituted enzymes are good models of natural enzymes; their activity is often similar to that of zinc-containing enzymes but the spectrum of the enzymatic activity may change [30, 31].

In view of the fact that proteases are widely used in some branches of industry, i.e., food, textile, and leather industries, in the production of detergents, in medicine, and in pharmacology, study of the effect of cobalt dioxime complexes on the biosynthesis of extracellular proteases of the micromycete *Fusarium gibbosum* CNMN FD 12 is of both scientific and practical interest [32].

The investigations showed that the test complexes have a stimulating effect on the proteolytic activity of the micromycete *Fusarium gibbosum* CNMN FD 12; the magnitude of the effect varies depending on the concentration used (Table 4).

The activity maxima of acid and neutral proteases in test and control runs coincided in time, being observed on the 5th day of producer cultivation.

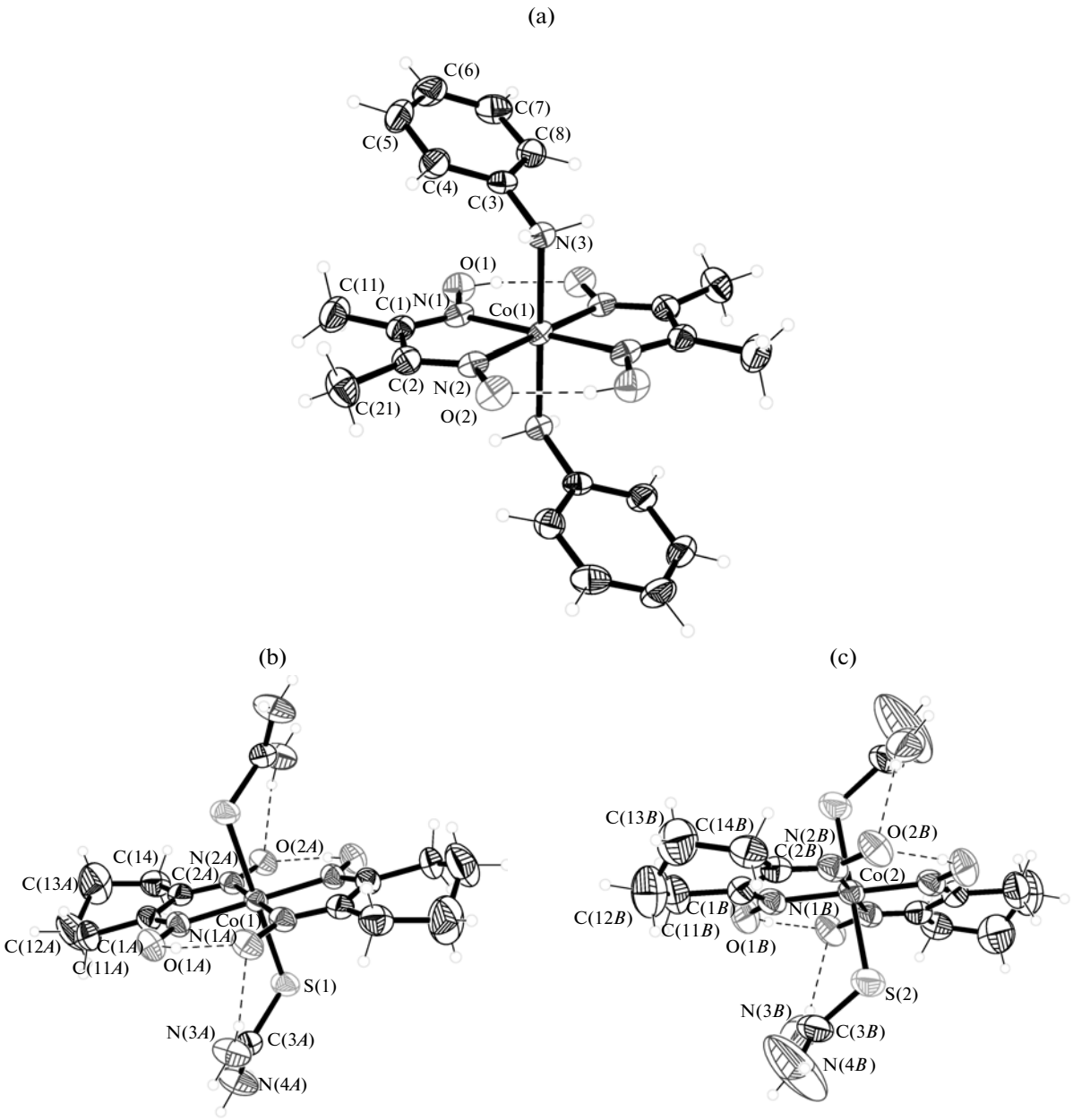


**Fig. 1.** Structure of complex cations in (a) **I** and (b), (c) **II**.

When the dimethylglyoxime complex with aniline and $[PF_6]^-$ (complex **I**) is introduced into the nutrient medium for micromycete cultivation, the activity of acid proteases is 3.108–4.116 U/mL versus 2.772 U/mL observed in the control run. The activity increase is 12.1–48.5%, the maximum being observed at the complex concentration of 10 mg/L (Fig. 4).

Neutral proteases have activity of 3.948–6.468 U/mL with the highest level corresponding to 10.0 mg/L concentration of the complex. The activity increase was 17.5–92.5%. Note that on the 4th day of strain cultivation in the presence of this complex (5 mg/L), the activity of the neutral proteases was 10% higher than the maximum activity in the control achieved on the 5th day of cultivation (the day of maximum effect in the control).

When micromycete is cultivated in the presence of complex **II**, the activity of acid proteases is 3.192–4.53 U/mL, being higher than the activity of the control by 15.2–63.6%. The highest activity is achieved when the amount of the complex introduced is 5 mg/L. In the case of neutral proteases, the activity in the test run is 5.376–6.30 U/mL, which is 50.0–87.5% higher than the control activity. The highest activity is also observed when the complex concentration is 5 mg/L.

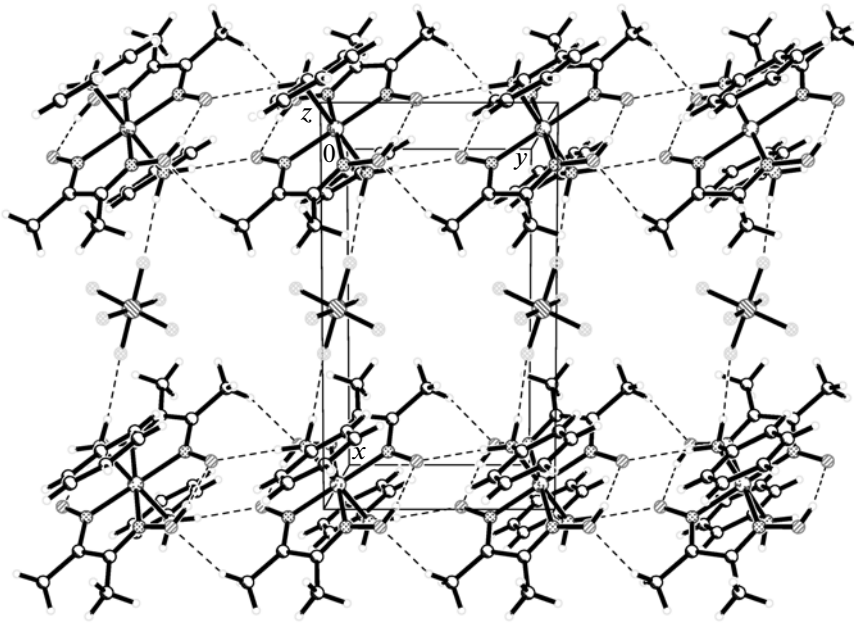


**Fig. 2.** Formation of chains of complex cations in **I** and their joining by the $[PF_6]^-$ anion.

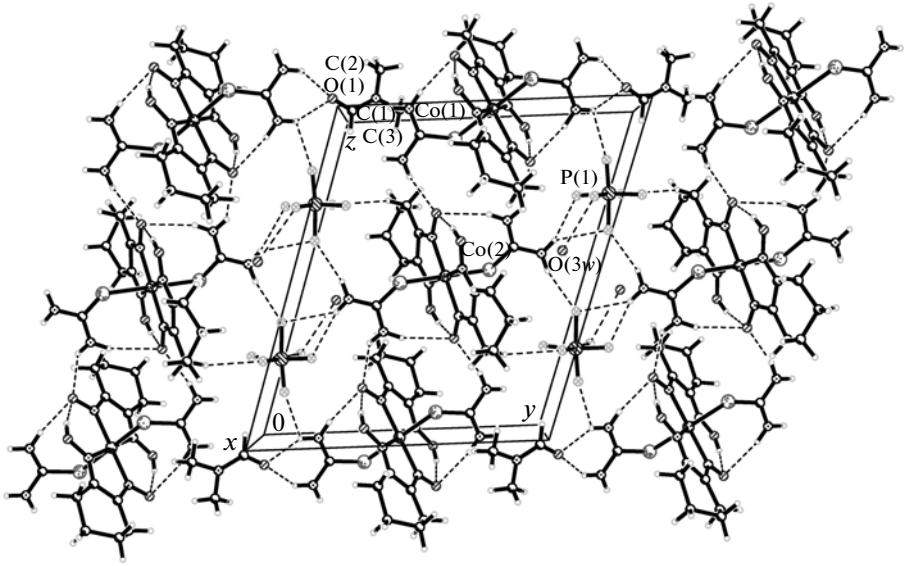


**Fig. 3.** Fragment of component packing in **II**. Formation of chains of alternating complex cations A and B in **II** and their joining by the $[PF_6]^-$ anions (only one position is shown for $[PF_6]^-$ and water molecules).

Similarly to **I**, complex **II** accelerates the protease biosynthesis. On the 4th day of cultivation of the producer, the activity of acid proteases grown in the presence of **II** was 2.688 U/mL (15 mg/L of the complex) and the activity of neutral proteases was 3.612 U/mL (1 mg/L of the complex), i.e., the activity levels in test runs are nearly equivalent to the activity of the control run observed on the 5th day: 2.772 and 3.360 U/mL, respectively.

The maximum of enzyme biosynthesis appearing 24 h earlier in the case where the nutrient medium contains the complexes points to intensification of all phases of development of the microorganism.

**Table 4.** Change of the proteolytic activity of the micromycete *Fusarium gibbosum* CNMN FD 12 under the action of cobalt complexes with dioximes

| Complex | Conc., mg/L | Activity of acid proteases, U/mL | | | Activity of neutral proteases, U/mL | | |
|---|---|---|---|---|---|---|---|
| | | 4th day | 5th day | 6th day | 4th day | 5th day | 6th day |
| **I** | 5 | 0.336 ± 0.01 | 3.15 ± 0.04 | 0.420 ± 0.04 | 3.696 ± 0.07 | 5.208 ± 0.07 | 2.352 ± 0.04 |
| | 10 | 0.42 ± 0.04 | 4.116 ± 0.04 | 0.504 ± 0.04 | 2.772 ± 0.04 | 6.468± 0.04 | 0.504 ± 0.05 |
| | 15 | 0.756 ± 0.07 | 3.108 ± 0.04 | 0.392 ± 0.02 | 2.856 ± 0.07 | 3.948 ± 0.07 | 0.392 ± 0.02 |
| **II** | 5 | 0.756 ± 0.04 | 4.53 ± 0.03 | 1.596 ± 0.04 | 3.612 ± 0.04 | 6.30 ± 0.04 | 2.52 ± 0.07 |
| | 10 | 1.512 ± 0.01 | 3.528 ± 0.04 | 1.512 ± 0.04 | 3.024 ± 0.07 | 5.04 ± 0.08 | 3.864 ± 0.04 |
| | 15 | 2.688 ± 0.04 | 3.192 ± 0.07 | 0.420 ± 0.04 | 3.108 ± 0.01 | 5.376 ± 0.08 | 1.932 ± 0.07 |
| Control | | 0.504 ± 0.04 | 2.772 ± 0.04 | 1.176 ± 0.07 | 1.26 ± 0.01 | 3.36 ± 0.07 | 2.52 ± 0.04 |

Thus, the results indicate that Co(III) dioximates with hexafluorophosphate ions can serve as stimulators of enzyme formation of the micromycete *Fusarium gibbosum* CNMN FD 12. The testing of $[Co(DH)_2(An)_2][PF_6]$ and $[Co(NioxH)_2(Thio)_2][PF_6] \cdot 0.5DMF \cdot 0.5H_2O$, carried out for optimal concentrations of 10 and 5 mg/L, respectively, demonstrated that they increase the activities of both acid proteases by 48.5% (compound **I**) and 63.6% (compound **II**) and neutral proteases by 92.5% (compound **I**) and 87.5% (compound **II**) by accelerating their biosynthesis. Thus, enzymatic preparations can be produced over shorter periods of time and with less energy expenditure.

## ACKNOWLEDGMENTS

This work was partially supported by project no. 12.819.18.13A "New Methods of Production of Proteolytic Preparations with Different Purification Degrees from Micromycetes."

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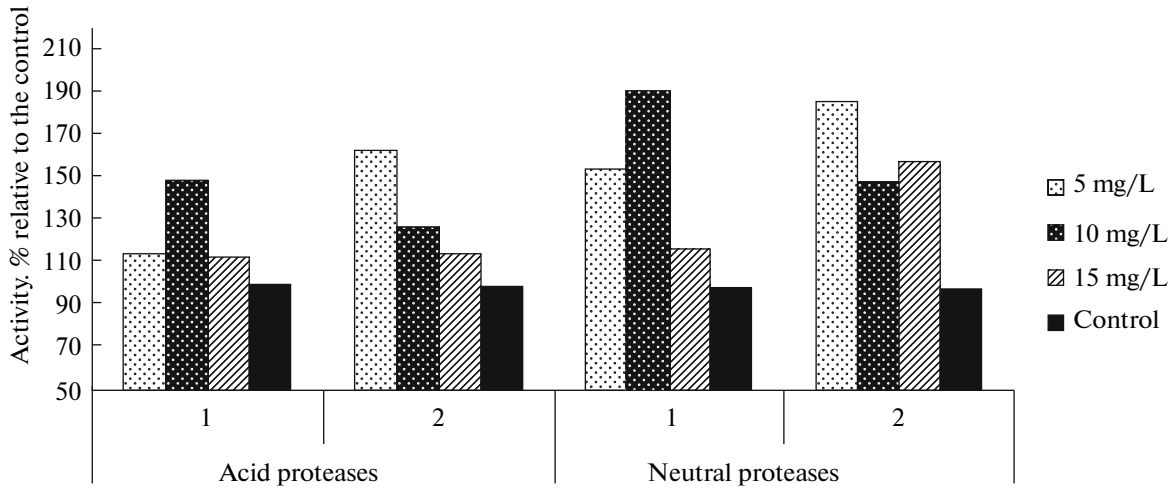


**Fig. 4.** Effect of dioxime complexes (**I**, $[Co(DH)_2(An)_2][PF_6]$ and **II**, $[Co(NioxH)_2(Thio)_2][PF_6] \cdot 0.5DMF \cdot 0.5H_2O$) on the activity of acid and neutral proteases of *Fusarium gibbosum* (on the 5th day of cultivation).

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*Translated by Z. Svitanko*