SOME 3D-METALS DIOXIMATES– BIOSTIMULATORS FOR ENZYMES PRODUCTION OF INDUSTRIAL IMPORTANCE

Coropceanu Eduard

Institute of Chemistry, Academy of Sciences of Moldova – Chişinău, Moldova

Coropceanu Eduard, ecoropceanu@yahoo.com

Abstract: A range of cobalt(III), copper(II) and zinc coordination compounds with oxime ligands were synthesized. For obtained complexes the composition and structure were determined by modern physicochemical methods: elemental analysis, IR and UV-Vis spectroscopies, ^{1}H, ^{12}C, ^{19}F NMR, as well using the single crystal x-ray diffraction. After a conducted screening some complexes were selected which were used as stimulators of biosynthetic processes in some strains of fungi of *Aspergillus, Rhizopus, Penicillium, Fusarium* genus, for intensification of enzymogenesis (amylases, pectinases, lipases, proteases, cellulase, xylanases, etc..), biomass accumulation, shortening of time of technological process, as well as stabilizers of fungi activity in adverse conditions.
Key words: coordination compounds, stimulators, biosynthetic processes, fungi

Coordinative compounds of transition metals with chelating ligands occupy an important place in contemporary chemistry. This class also includes the dioximates of transition metals. The complexion capacity of α-dioximes with metals of *d*-type draws the researchers' attention not only from the perspective of the hemoglobin or vitamin B_{12} pattern synthesis [1], but also from one of a broad spectrum of synthetic, analytical and structural possibilities. Dioximates can be used as catalysts for industrial processes [2], stimulators of erythropoietin functions [3], anti-hypoxic preparations [4], preparations with antidote properties [5], basis to obtain new semiconducting materials [6], for the separation and purification of the metals that are complex generators etc.

The interest towards the complexes of 3*d* elements with α-dioximes is subject to their stereo-chemical and electronic structural diversity, presenting perspective objects to elucidate the general principles between composition, structure and properties. The varied structure of dioximates is due to different synthesis conditions (the pH of the solution, etc.), to the nature of axial ligands, to anions in the external sphere and other factors.

From the point of view of practical applicability of the dioximates of transition metals, there are now clearly outlined several directions in which the representatives of this class can successfully be used: elaboration of some artificial models of biological molecules of vital importance; elaboration of efficient bio-technologies to obtain enzyme preparations used in various branches of food and pharmaceutical industries, obtaining some compounds with useful medical properties, synthesizing materials with valuable physical properties (semiconductors, photo-luminescent materials); obtaining some systems to use in hydrogen production, etc.

Modern bio-technology pays a special attention to directed synthesis of bio-active substances by micro-organisms based on valuable fundamental studies with implications for medicine, micro-biological, chemical, pharmaceutical, cosmetic industries etc. Micro-organisms, due to specific features, as enhanced response to ambient changes, adaptive metabolism, short development cycle, are convenient objects for various researches. From

another point of view, organisms are recognized as advantageous economical sources for obtaining a wide range of important bio-active substances.

The ligands also play an important role in the manifestation of metal complexes properties as a part of their composition and, due to the wide set of donor atoms, create stable complexes with transition metal ions, different in composition, structure and properties. The biologically active substances, included as ligands in the composition of the metal complexes, essentially increase their efficiency [7, 8].

Previously, Co(III) dioximate with nicotinamide was tested in the treatment of hemolytic anemia and other diseases [3]. Co(III) dioximates can also be used as an antidote to extract various toxins (cyanide, phosphamide etc.) from the body [9].

There was performed the oriented synthesis of a series of dioximates of Co(III) with the general formula $[Co(DioxH)_2L_2]_nX \cdot nH_2O$, where DioxH is the monoanion of the dioxime: dimethylglyoxime (DH_2), · methylglioxime (MH_2), 1.2-cyclo-hexandiondioxime ($NioxH_2$), α-benzildioxime (DfH_2); L - thiocarbamide (Thio), pyridine (Py), aniline (An), nicotinamide (PP), sulphanilamide (Sam); X - $[BF_4]^-$, $[PF_6]^-$, $[SiF_6]^{2-}$, $[ZrF_6]^{2-}$, $[TiF_6]^{2-}$ etc., crystal structure and spectral characteristics of which have proven *trans*-octahedral configuration of the complexes. There have been studied the chemical and physical properties. It was stated that the anions in the external sphere and crystallization water molecules have an important role in the field of in the formation of the crystalline structure. The analyzed complexes were used as additives in the nutrition environment for some strains of fungi. The testing of the dioximates of cobalt(III) in the Laboratory of Enzymology of the Institute of Microbiology and Biotechnology of the ASM on the gender strains of *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium* showed that these complexes beneficially influence on the development of some biological processes of the respective fungi [10-14].

Structural and compositional complexity, the metal presence as central atom shows the perspective of using the coordinative compounds as stimulators and regulators of biological processes in the cell of microorganisms. The inclusion of several micro-elements (eg: Co, Si, F; Co, P, F; Co, B, F and Co, Ti, F) in the composition of the same complex contributes to the manifestation of the bio-stimulating properties of the respective dioximates. Cobalt is a necessary element for the biological systems. Other elements are equally necessary for organisms. Boron, for example, participates in regulating membrane functions, biosynthesis of nucleic acids. Titanium is an indispensable element for many organisms, with vital functions: increases erythropoiesis, and catalyzes the synthesis of hemoglobin, imunogenesis [15]. Complexes of titanium are not only stimulating agents of phagocytosis, but also substances that activate cellular and humoral immune reactions. Titanium chelates, as we know, affect the reproduction function of some animal species. Experimentally, it has been proved that titanium is an element that is easily removed from the body and does not present danger to get accumulated in large quantities. Moreover, being considered a bio-compatible element it is used in medicine to join fractured bone tissue.

The synthesis of tested complexes was conducted in an aqua-methanol medium. As a result of the analysis of the IR spectra for thiocarbamide there were observed some changes in the values of the absorption bands of valence and deformation vibrations related to the respective oscillation values in the spectrum of free thiocarbamide. The frequencies $\nu_{as}(NH)$=3310-3370, $\nu_s(NH)$=3210-3240, $\delta(NH_2)$=1615-1625, $\delta(NCS)$=412-415 cm^{-1} indicate the presence of thiocarbamide coordinated to the metal.

In the IR spectra of the complexes that contain pyridine there are manifested the characteristic bands to the *trans*-dioximates Co (III): ν(C=N)=1556-1588 and ~1455 cm^{-1}, the bands of deformation vibrations δ(C–H)=676-765 cm^{-1} of the mono-substituted aromatic ring, ν(Co-N)=510-520 and 460-465 cm^{-1}. The valence frequencies changes ν(C-H)=3120-3230 cm^{-1} and ν(C=C)=1610-1615 cm^{-1} confirm the coordination of pyridine molecules to the metal. These two bands are shifted to higher values compared to their position in the spectrum of uncoordinated pyridine.

The dimethylglyoxime monoanione in complexes is confirmed by the bands ν(CN)=1560–1580, ν_{as}(NO)=1230–1245, ν_{s}(NO)=1080–1095, δ(OHO)=1825–1750, γ(OH)=970–985, γ(CNO)=730–745, ν_{as}(Co-N)=505-525 and ν_{s}(Co-N)=425-440 cm^{-1}. The coordination of the dioximes to the central atom is proved by the appearance of new bands or by intensity changing of the existing bands in the spectrum. For example, the band that appears in the region 1220-1240 cm^{-1} refers to ν (NO) of ionized N-OH group of the dioxime. This band does not appear in the spectrum of the free dioxime. The intensity decrease of the band γ(OH)=970-985 cm^{-1} and the appearance of a weak band δ(OHO)=1825-1750 cm^{-1} proves the deprotoning of dioximes during coordination and the formation of intra-molecular hydrogen bonds of O -H·O type in the equatorial plane.

It was studied the influence of some dioximates of cobalt(III) on the strains of fungi of the genera *Aspergillus, Rhizopus, Penicillium, Fusarium.* In parallel it was tasted the influence of the complexes and their parts (original salt, dioxime, neutral ligands) on the bio-synthetic processes. It was found that the influence of the complexes is higher, a fact which points to the possibility of using them as bio-stimulators of the enzymogenetic processes and bio-mass accumulation.

The results obtained on the basis of the influence testing of the dioximates Co(III) on physiological processes of some strains of micromycete allow concluding that in the series of the analyzed complexes it can be selected:

- bio-stimulators of enzymogenetic processes of fungi (eg: the complex $[Co(DH)_2(Thio)_2]_2[SiF_6]\cdot 3H_2O$ increases pectolytic activity of the fungi *Rhizopus Arrhizus* with 184.08% if compared to the control sample);
- catalysts for biomass accumulation processes (for example: at the introduction of the compound $[Co(DH)_2(Thio)_2][BF_4]\cdot 3H_2O$ with a concentration of 5-10 mg/l in· the cultivation medium of the fungi strain of *Aspergillus niger 412* it was recorded a double increase of the biomass productivity - with 188.3-197.07% if compared to the control sample);
- stabilizers of the vital bio-chemical processes in unfavorable conditions (for example, in unfavorable thermal conditions, introducing complex $[Co(DH)_2(Py)_2]BF_4$ in the nutritional medium of fungi *Aspergillus niger 412* increased bio-mass accumulation process with 132.93% if compared to the control sample);
- accelerators of the biological development of micro-organisms, reducing the technological cycle with 24-48 hours, that is, to ~ 30% of the entire cycle length (when introducing the complex $[Co(DH)_2(An)_2]_2[TiF_6]$ in the nourishing medium of the micromycete *Aspergillus niger* 33-19 CNMN FD 02A), a fact that presents economic interest.

When using copper and zinc dioximic compounds as additives in the nourishing media of some strains of fungi it has also been established an intensification of the bio-synthetic processes.

The effect of metal complexes varies according to the origin of micro-organism, type of the synthesized enzymatic system, the composition and concentration of the

compound [10-14]. Summarizing the obtained results, we can conclude that the tested dioximates show properties of stimuli (bio-mass accumulation, increased enzymatic activity), stabilize the bio-synthetic processes in unfavorable conditions of activity, reduce the technological cycle. All these properties are influenced by the presence of the ion Co^{3+} as a generating complex, as well as by the nature of the ligands in the internal sphere and that of the anion in the external one. These experimental results create favorable conditions for the development of economically advantageous technologies of cultivation some types of strains of fungi of industrial importance.

Bibliografie:

1. Brescian-Pahor N. et al.//Organocobalt B_{12} models: axial ligand effects on the structural and coordination chemistry of cobaloximes//Coord. Chem. Rev. 1985. V.63. P. 1.
2. Рогачев Б.Г., Хиндекель М.Л. // Изв. АН СССР. 1969. № 1. 141.
3. Матковский К.Л. и др.//Изв. АН МССР. Сер. биол. и хим. наук. 1969. N4. С. 3.
4. Матковский К.Л. // Изв. АН МССР. 1971. №5. С. 88.
5. Матковский К.Л., Болога О.А. // Глобус науки. 2006. Т. 6. С. 34.
6. Thomas T.W., Underhill A.E. // Chem. Commun. 1969. V.13. P. 725.
7. Парпиев Н.А. и др. Координационные соединения металлов с лекарственными препаратами. Ташкент: ФАН. 1982. 138 с.
8. Азизов М.А. О комплексных соединениях микроэлементов с биоактивными веществами. Ташкент. 1963. 245 с.
9. Сасинович Л. и др.//Изв. АН МССР. Сер. биол. и хим. наук. 1977. №1. С. 85.
10. Десятник А.А. и др.// Использование диметилглиоксиматов Co(III) при биосинтезе пектиназ Rhizopus arrhizus// Коорд. химия. 2002. Т.28, №2. С. 144.
11. Боурош П.Н и др.//. Супрамолекулярная организация структуры кристаллов соединения $[Co(DH)_2(PP)_2][BF_4]\cdot 2H_2O$ и его биологические свойства // Коорд. химия. 2009. Т. 35. Nr.10. С. 761.
12. Coropceanu E. et al. The synthesis and the study of the biological activity of some cobalt(III) dioximates with fluorine // Chem. Journ. of Mold. 2008. V.3. N.2. P. 70.
13. Gărbălău N. V. et al. Fluorura-hexafluorofosfat-bis[di(tiocarbamid)bis-(dimetilglioximato)cobalt(III)], care posedă proprietăți de biostimulator//Brevet de invenție MD 2833. 2005.
14. Deseatnic A. et al. Mediu nutritiv pentru cultivarea tulpinii de fungi *Aspergillus niger 33-19* CNMN FD 02A. Brevet de invenție MD 3943. 2009.
15. Ершов Ю.А. и др. Общая химия. Биофизическая химия. Химия биогенных элементов. Москва: Высшая школа. 1993. 560 с.