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SUMMIT
SOFIA UNIVERSITY
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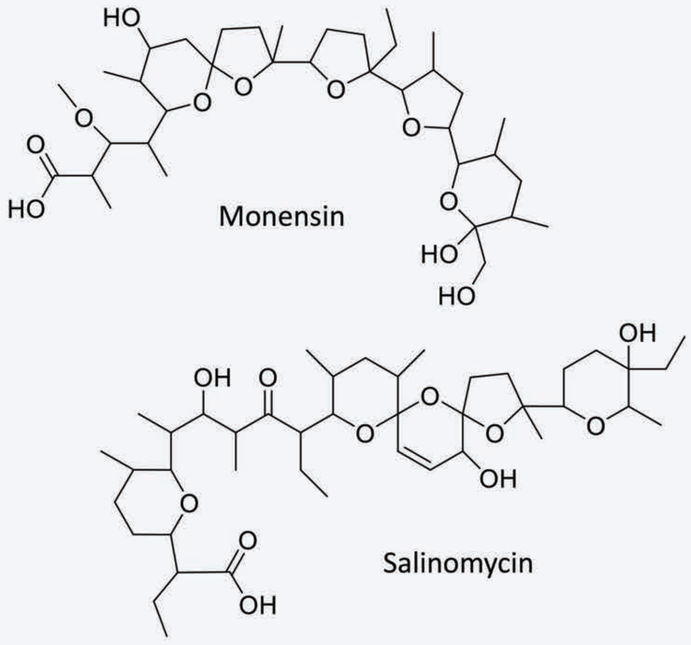
SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Research Group
Biocoordination and Bioanalytical Chemistry

Research Area
Chemistry Sciences

Polyether ionophores and their metal complexes



INTRODUCTION

The early history of polyether antibiotics dates back to 1951, when nigericin and X-537A (lasalocid A) were isolated from *Streptomyces* spp. These were found to exhibit activity against Gram-positive microorganisms and mycobacteria but were ineffective against Gram-negative bacteria and were not classified as polyether compounds at the time. Years later, in 1967, the structure of monensic acid (MonH) has been proved to be the first representative of polyether antibiotics, and its isolation, fermentation, chemical properties, anticoccidial activity, and mode of action have become known. Today, more than 120 natural polyether ionophores have been reported, and the main use of some of them is for the control of coccidiosis in agriculture. Statistics show that the most used antibiotics in veterinary medicine are lasalocid, monensin, salinomycin, narasin, and maduramycin.

PROJECT GUIDELINES

The main aim of the project is to study, through a combined experimental-theoretical approach, the complexation processes involving natural carboxylic polyethers and to build on the knowledge of the properties of their metal complexes.

Task 1. Evaluation of the metal ion - ionophore interaction in solution (the object of study will be metal cations in different oxidation states).

Task 2. Isolation and characterization of the observed complex species in the solid state.

Task 3. Evaluation of the biological activity of the characterized complexes.

METHODOLOGY

Experimental Strategy 1 - Studies in solution: The leading approach will be the use of circular dichroic spectroscopy, which is a method of exceptional importance (and so far without analogue) for assessing the complexation ability of the two antibiotics in a liquid medium.

Experimental Strategy 2 - Studies in solid phase: The entire set of physical methods used in coordination chemistry and proving the coordination mode of the antibiotics and the composition/structure of the coordination compounds will be applied.

Experimental strategy 3 - Biological activity of ionophores and their coordination compounds: The main activities through which the change in the biological activity of the newly synthesized compounds will be assessed include experiments at *in vitro* conditions with target objects - Gram-positive microorganisms and cell cultures.

Theoretical models: Obtaining reliable information about the behaviour in solution and calculating the spectral and macroscopic characteristics of antibiotics and their complexes require the use of molecular dynamic simulations with subsequent quantum chemical calculations. This approach allows for a molecular-level insight into the complexation processes in solution, which is of particular importance for the biological activity and will allow to draw conclusions about the structure-activity relationship.

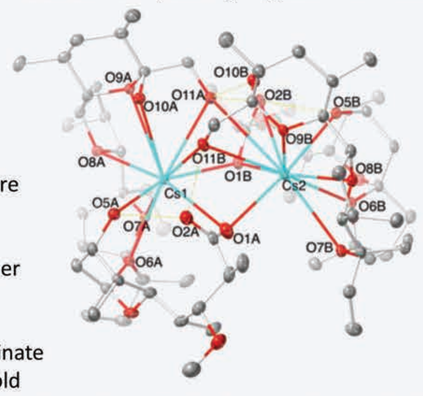
Head of the research group
Prof. Ivayla Pantcheva

Members of the group
Prof. Vasil Atanasov // Dr. Silviya Stoyanova
Dr. Ivo Ivanov // Dr. Yana Goranova
PhD student Nikolay Petkov
Stiliyan Kolev // Miroslav Boyadzhiev
Nikita Bozhilova

RESULTS

Unusual Caesium Monensinate A: Crystallographic Evidence for the Formation of Dinuclear Coordination Species in the Solid State

The polyether ionophore monensin A (MonH), applied as silver monensinate, reacts with caesium cations to form a dinuclear complex $[Mon_2Cs_2]$ the structure of which has been solved by single-crystal X-ray diffraction [1]. Two Cs^+ ions are located in the hydrophilic cage of two ligand anions, achieving coordination number eight. In addition, the metal cations are bridged by two functional groups of monensinate A, completing the inner tenfold coordination sphere. NMR studies show that the dinuclear complex dissociates to its mononuclear counterparts in methanol solutions. Further molecular dynamics theoretical modelling of the interaction of monensinate A with alkali metal ions reveals the effect of solvent polarity on the zipping ability of the ligand. Thus, in methanol, used as an explicit solvent, potassium and rubidium cations fully occupy the cavity of the ligand, whereas the sodium monensinate exists in an "open" form, with Na^+ ions still interacting with the monodentate carboxylate group. The replacement of methanol by the less polar chloroform induces the folding of monensinate A and the formation of "closed" structures with all group 1 metal cations. The obtained data explain the specifics in the behaviour of monensinate A caused by the environment, e. g., physical state or solvent.

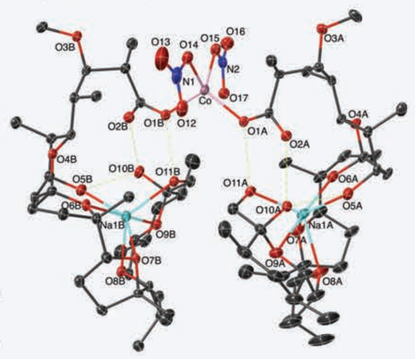


RESULTS

Cobalt(II) and Manganese(II) Complexes of Sodium Monensinate A Bearing Nitrate Co-Ligands

The crystal structures and properties of Co(II) and Mn(II) complexes of sodium monensinate (MonNa) derived from the reaction of MonNa with cobalt or manganese dinitrates are presented [2].

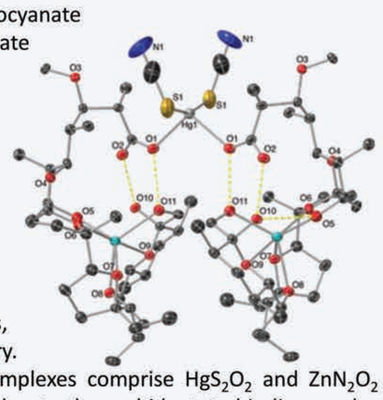
The newly obtained coordination compounds have the same composition $[M(MonNa)_2(NO_3)_2]$ but the transition metal ions are placed in a different environment. The two nitrate ligands behave mono- or bidentately bound in the Co(II)- and Mn(II)-containing species, respectively, while the monensinate ligands act in a similar manner through their monodentate carboxylate functions. The formed CoO_4 and MnO_6 units determine the geometry of the corresponding inner coordination cores of the complexes as a tetrahedron in the case of Co(II), and as a strongly distorted octahedral structure in Mn(II) species. The effect of inorganic anions on the antibacterial performance of sodium monensinate appears to be negligible, while the presence of Co(II) or Mn(II) cations preserves or enhances the activity of unmodified MonNa, which differentially affects the growth of *Bacillus subtilis*, *Bacillus cereus, Kocuria rhizophila, Staphilococcus aureus*, and *Staphilococcus saprophyticus* strains.



RESULTS

Heteronuclear Complexes of Hg(II) and Zn(II) with Sodium Monensinate as a Ligand

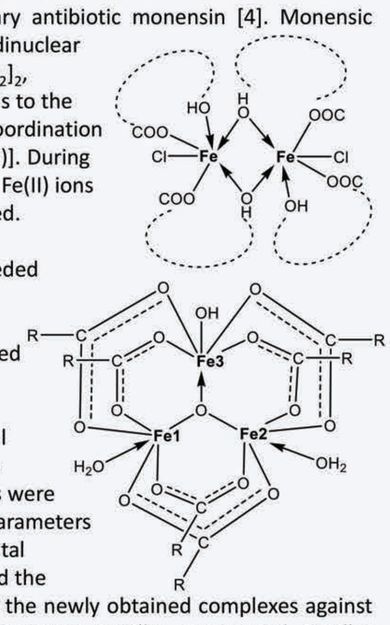
The commercial veterinary antibiotic sodium monensinate (MonNa) binds mercury(II) or zinc(II) cations as thiocyanate $[Hg(MonNa)_2(SCN)_2]$ or isothiocyanate $[Zn(MonNa)_2(NCS)_2]$ neutral coordination compounds [3]. The structure and physicochemical properties of the complexes were evaluated by the methods of single crystal and/or powder X-ray diffraction, infrared, nuclear magnetic resonance, X-ray photoelectron spectroscopies, and electrospray-mass spectrometry. The primary cores of the two complexes comprise HgS_2O_2 and ZnN_2O_2 coordination motifs, respectively, due to the ambidentate binding modes of the SCN–ligands. The directly bound oxygen atoms originate from the carboxylate function of the parent antibiotic. Sodium cations remain in the hydrophilic cavity of monensin and cannot be replaced by the competing divalent metal ions. Zinc(II) binding does not influence the monensin efficacy in the case of *Bacillus cereus* and *Staphylococcus aureus* whereas the antimicrobial assay reveals the potential of Zn(II) complex species as a therapeutic candidate for the treatment of infections caused by *Bacillus subtilis*, *Kocuria rhizophila*, and *Staphylococcus saprophyticus.*



RESULTS

Synthesis, Spectral Characterization, and Structural Modelling of Di- and Trinuclear Iron(III) Monensinates with Different Bridging Patterns

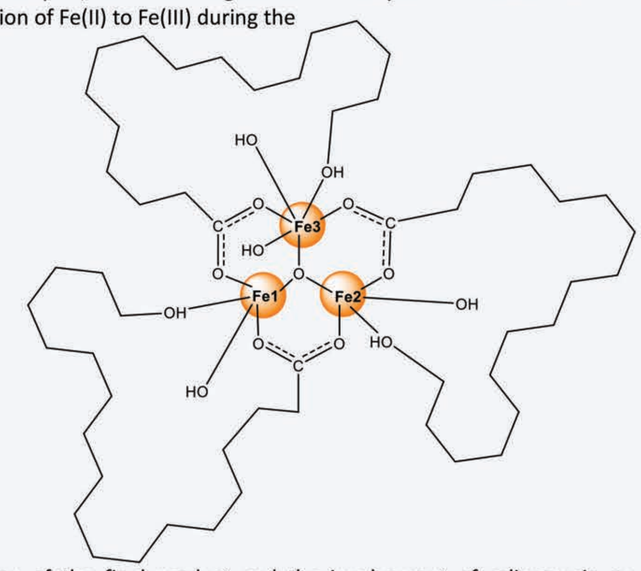
We report the solid-state isolation and structural characterization of novel iron(III) complexes of the veterinary antibiotic monensin [4]. Monensic acid ($MonH \times H_2O$) forms with $FeCl_3$ dinuclear complex of composition $[FeCl(Mon)_2]_2$, while its interaction with $FeSO_4$ leads to the isolation of a triangular oxo-ferric coordination species $[Fe_3O(Mon \times H_2O)_6(H_2O)_2(OH)]$. During the last procedure, oxidation of the Fe(II) ions by atmospheric oxygen was observed. In the presence of organic bases, both complexation reactions proceeded to successfully deprotonate the carboxylic function of the ligand. Iron(III) complexes were characterized by IR, EPR, NMR, and Mössbauer spectroscopies as well as with thermal (TG-DTA/MS) and elemental analyses. In addition, the structures of the two coordination compounds were modelled and selected calculated parameters were compared with the experimental results. The biological assay revealed the enhanced antibacterial potential of the newly obtained complexes against the Gram-positive aerobic microorganisms *Bacillus cereus* and *Bacillus subtilis*.



RESULTS

Fe(III) Oxo-Complex Containing Salinomycinate and Hydroxo Ligands

The properties of a trinuclear ferric oxo-complex derived from the reaction of ionophorous antibiotic salinomycinic acid with Fe(II) ions are discussed [5]. The coordination species were characterized by IR, EPR and Mössbauer spectroscopies, and thermogravimetric analysis. The data reveal the oxidation of Fe(II) to Fe(III) during the isolation of the final product and the involvement of salinomycin as a monoanion bridging metal ions. The effect of Fe(III) ions on the antibacterial efficacy of the parent ligand was assessed towards a panel of three Gram-positive microorganisms: *Bacillus subtilis, Bacillus cereus* and *Kocuria rhizophila*.



FUTURE WORK

Thallium(I) salinomycinate (manuscript in progress)

Ammonium monensinate (under evaluation)

Lanthanide complexes of monensin and salinomyin (under evaluation)

1. Petkov, N., Ivanova, A., Nedyalkova, M., Simova, S., Dorkov, P., Ugrinov, A., Pantcheva, I. **Chem. – Eur. J. (2025)** http://dx.doi.org/10.1002/chem.202404453

2. Petkov, N., Boyadzhiev, M., Bozhilova, N., Encheva, E., Dorkov, P., Ugrinov, A., Pantcheva, I. **Int. J. Mol. Sci. 25(22) (2024) 12129** https://doi.org/10.3390/ijms252212129

3. Pantcheva, I., Petkov, N., Encheva, E., Kolev, S., Simova, S., Tsanev, A., Dorkov, P., Ugrinov, A. Heteronuclear Complexes of Hg(II) and Zn(II) with sodium monensinate as a ligand. **Molecules 29(13) (2024) 3106** https://doi.org/10.3390/molecules29133106

4. Petkov, N., Tadjer, A., Simova, S., Cherkezova-Zheleva, Z., Paneva, D., Stoyanova, R., Kukeva, R., Dorkov, P., Pantcheva, I. **Inorganics 12(4) (2024) 114** https://doi.org/10.3390/inorganics12040114

5. Petkov, N. N., Pantcheva, I. N., Kukeva, R. R., Paneva, D. G., Cherkezova-Zheleva, Z. P., Dorkov, P. D. **Bulg. Chem. Comm. 56, SI C (2024) 83-88** https://doi.org/10.34049/bcc.56.C.SI-8

Contract BG-RRP-2.004-0008 for the financing of project "Sofia University - Marking Momentum for Innovation and Technological Transfer" under pillar 2 "Establishing a network of research higher education institutions in Bulgaria", component "Innovative Bulgaria" from National Recovery and Resilience Plan part of the program to accelerate economic recovery and transformation through science and innovation