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SUMMIT
SOFIA UNIVERSITY MARKING MOMENTUM FOR INNOVATION AND TECHNOLOGICAL TRANSFER

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

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Diverse chemistry of polyether ionophores

INTRODUCTION

Polyether ionophores (PIs) are a class of natural antibiotic compounds, primarily produced by *Streptomyces* and *Actinomadura* species, known for their ability to bind and transport metal cations across cellular membranes. They are widely used in poultry and cattle to control coccidiosis and act as growth promoters and antimucrobials.

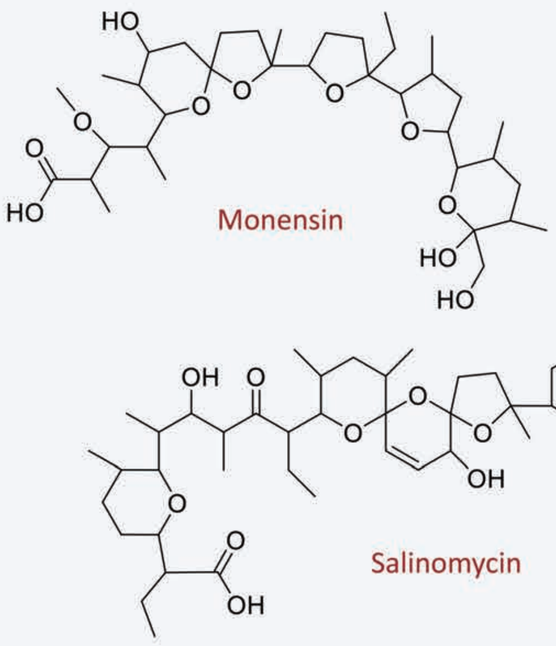
Key Characteristics and Functions:

Mechanism of action: PIs disrupt the intracellular cation balance by facilitating ion transport, which leads to osmotic changes, cellular swelling, and death of bacteria and parasites.

Antibacterial/antiparasitic activity: Highly effective against Gram-positive bacteria and protozoa, including drug-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).

Key examples: Monensin, lasalocid, salinomycin, narasin, maduramycin, and nanchangmycin.

Potential for human medicine: While toxicity has traditionally limited their use in humans, recent research suggests potential for repurposing them against resistant cancers and as antibacterial agents.



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 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.

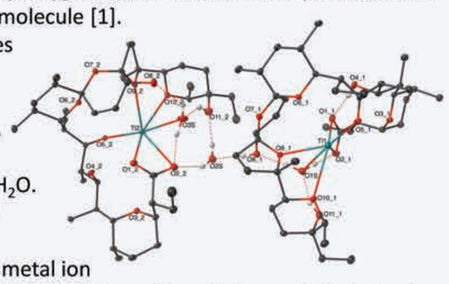
OUTCOME

Based on previous results and in addition to the tasks and strategies aforementioned, we expanded our research to study the ability of monensin to interact with ammonium ions, as well as the possibility of forming mixed-ligand complexes containing a metal ion, PI, and amino acid.

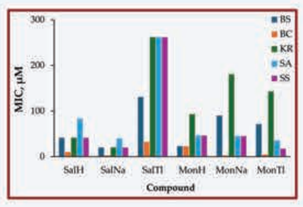
RESULTS

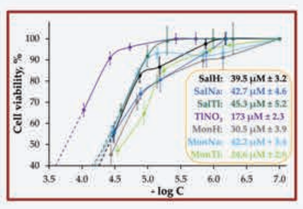
Crystal Structure and Properties of Thallium(I) Salinomycinate

The veterinary antibiotic salinomycin reacts with thallium(I) ion under basic conditions to form a mononuclear complex with very unsymmetric TlO_6 coordination, realized by oxygen donor atoms from pentadentate ligand monoanion and water molecule [1].



Every two coordination species (SalTl1 and SalTl2) are linked to each other by an additional water molecule, achieving the complex composition $[TlSal(H_2O)]\times0.5H_2O$. The *in vitro* antimicrobial and antitumor assays reveal that accommodation of the heavy metal ion in the hydrophilic cavity of the ligand reduces its activity most likely due to the formation of a stable complex that is unable to dissociate in the intracellular space and disrupt the metal homeostasis of the target bacterial strains and cancer cells. The observed results can serve as a starting point for further investigation of the properties of the polyether ionophore salinomycin under different experimental conditions.

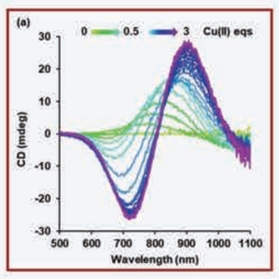


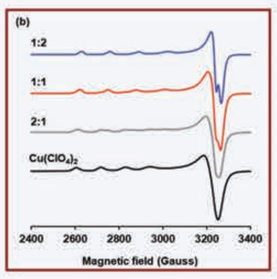


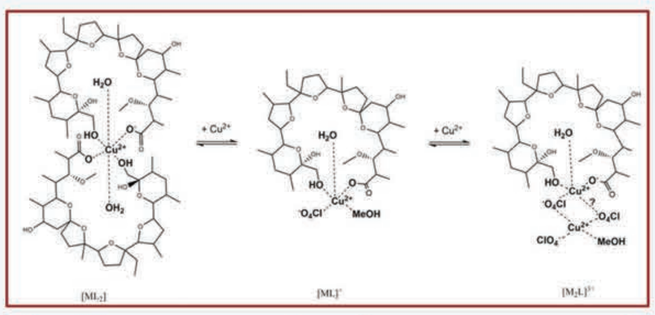
RESULTS

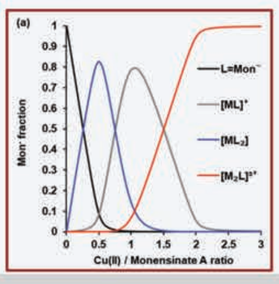
Copper(II), a Peculiar Metal Ion for Complexation with Monensin A Ionophore

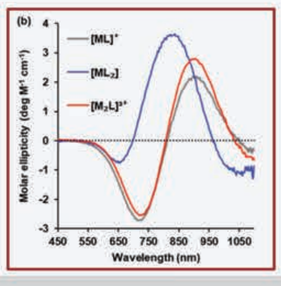
The coordination ability of the polyether ionophorous antibiotic monensin A to bind Cu(II) ions was evaluated in methanolic solutions by a set of complementary spectroscopic methods and mass-spectrometry [2]. The VIS- and NIR-CD spectroscopy evidenced the distinctive character of Cu(II) forming three different chiral species which depend on metal-to-ligand molar ratio. Thus, the monensinate A anion is bound in a neutral bis $[CuL_2]$ complex at copper(II) deficit, while at comparable reactant concentrations the mono $[CuL]^+$ species prevailed, which proved to be at least two orders of magnitude more stable than those formed with Ni(II), Co(II) or Zn(II). Due to the increased stability of the $[CuL]^+$ complex Cu(II) may become a good candidate for a competing agent with the native sodium ion. The observed species at these reaction conditions corroborate well our previous solution studies using monensinate A and colored Co(II), Ni(II) or colorless Mg(II), Ca(II), Zn(II) metal cations. Notably, further increase of Cu(II) in the system led to the formation of dinuclear $[Cu_2L]^{3+}$ complex not observed before. The two metal centers are most likely linked to each other by at least one counter ion originating from the added copper(II) salt ($Cu(ClO_4)_2$ or $CuCl_2$). The existence of the dimer in solution was confirmed by EPR spectroscopy. A peculiar property of this complex is that it contains two magnetically independent copper(II) centers, one with a similar coordination environment to $[CuL]^+$ and the second – to the free metal salt. The presence of the dinuclear species was further checked by ESI-MS, becoming the first example of dinuclear monensinate A construct bearing divalent metal cations.







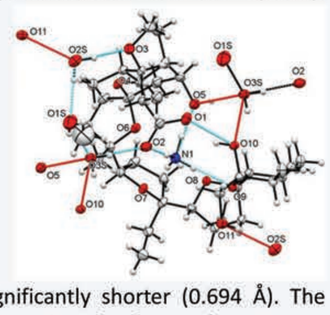




RESULTS

Ammonium Monensinate A

At the present we report the formation of monensinate host-guest complex containing ammonium ions ($MonNH_4$) [3]. The polyether chain of monensinate A ligand folds into a pseudocyclic structure through interaction between the "head" carboxylate atom O1 and "tail" hydroxyl group O10-H10. Most of the oxygen atoms (O2, O5-O9) are oriented inwards to shape a hydrophilic cavity that encapsulates the ammonium cation, involved as a donor in the formation of hydrogen bonds with O2 (NH1A), O5 (NH1D), and O9 (NH1C). The distances between the nitrogen and protons 1A, 1C and 1D are of order of 0.934-1.096 Å at the expense of the fourth bond NH1B, which length is significantly shorter (0.694 Å). The crystal structure of the title compound contains also three solvates – one methanol (1S) and two water (2S, 3S) molecules, which participate in weak interactions with ammonium monensinate, and additionally determine its packing. On an intramolecular level, methanol is playing a dual role serving both as a donor (O1SH1S···O3S) and as an acceptor (O2SH2SA···O1S) towards the two waters molecules. Waters 2S and 3S are donors in their interaction with the methoxy O3 (O2SH2SB···O3) and carboxylate O2 (O3SH3SA···O2) atoms. The packing of $MonNH_4$ is achieved by a network of H-bonds formed between the two water molecules 2S/3S and second discrete unit through the interactions O11H11···O2S, O3SH3SB···O10, O5H5···O3S. In summary, the ammonium monensinate A exists in a 3D layered structure, supported by eight intra- and three intermolecular H-bonds. The participation of carboxylate atoms O1 and O2 in one and two weak interactions, respectively, reflects the C-O bond distances, with C1-O1 (1.256 Å) being slightly shorter than C1-O2 (1.268 Å).



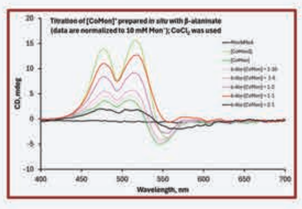
RESULTS

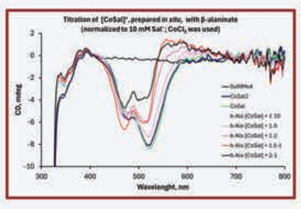
Ternary Co(II) complexes of PIs with amino acids

The knowledge on coordination chemistry of monensinate A with divalent metal ions reveals the formation at least of two types of metal complexes in methanolic solution – bis $[M(Mon)_2(H_2O)_2]$ and mono $[MMon]^+$. The first species are neutral, and their structure is solved by single crystal X-ray diffraction analysis (SCXRD). The second complexes are detectable by circular dichroic (CD) spectroscopy, but the structure is still unsolved due to difficulties with their isolation in solid state. To compensate the charge of $[MMon]^+$, we monitored the behaviour of [CoMon] in the presence of increasing amount of two deprotonated achiral amino acids. For the sake of comparison, the analogous salinomycinate system was also studied.

System I: [CoMon] (IA) / [CoSal] (IB) and β-alaninate

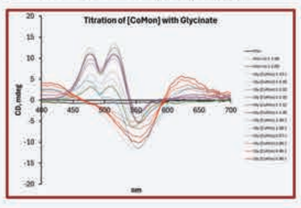
The data disclose the formation of ternary Co(II) complexes of suggested composition [Co(PI)(β-Ala)] (red spectra). Further addition of amino acid seems to undergo the formation of $[Co(\beta\text{-}Ala)_2]$, which is optically inactive.

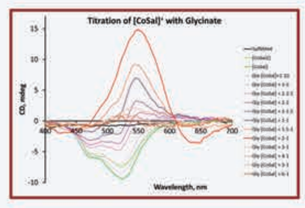




System II: [CoMon] (IIA) / [CoSal] (IIB) and glycinate

The behaviour of the two Co(II) systems in the presence of glycinate differs: while the ternary complex of monensinate is stable in the presence of an excess of the amino acid, that of salinomycinate forms exclusively at 2-1 molar ratio and further increase of glycinate amount shifts the equilibrium towards $[Co(Gly)_2]$.





The data obtained are promising and point to enriching the coordination chemistry of polyether ionophorous antibiotics [4].

1. Petkov, N., Dorkov, P., Ugrinov, A., Encheva, E., Abrashev, M., Zasheva, D., Daneva, T., Pantcheva, I.N. **Intern. J. Mol. Sci. 26(13)** (2025) 6504. https://doi.org/10.3390/ijms26136504

2. Kis, M. L., Hajdu, B., Jakusch, T., Kele, Z., Dorkov, P., Kukeva, R., Pantcheva, I., Gyurcsik, B. **Chem. – Eur. J. (2026) e02908**. https://doi.org/10.1002/chem.202502908

3. Petkov, N., Ivanova, A., Simova, S., Dorkov, P., Ugrinov, A., Pantcheva, I. Ammonium monensinate A – crystal structure, solution chemistry and biological performance (manuscript under preparation)

4. Pantcheva, I. Co(II) and Cu(II) ternary complexes of polyether ionophores with achiral amino acids (research in progress)

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