REVIEW

By Prof. Adriana Georgieva Bakalova, PhD,
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Regarding: Doctor of Sciences thesis of Prof. Dr. Ivayla Nedialkova Pantcheva-Kadreva on "METAL COMPLEXES OF THE CARBOXILIC POLIETHERS MONENSIN AND SALINOMYCIN: STRUCTURE, PROPERTIES AND BIOLOGICAL ACTIVITY" for the award of the degree of Doctor of Science in the field of Higher Education **4. Natural Sciences, Mathematics and Computer Science**, professional field **4.2. Chemical Sciences**, **Analytical Chemistry**

The habilitation thesis of Prof. Dr. Ivayla Pancheva-Kadreva reflects the research in the field of chemistry of complex compounds for quite a long period of time, about 15 years. In it prof. Pancheva has managed to summarize the studies and research described in nine diploma theses and four PhD thesis. The most of the experimental work was funded by the Scientific Research Fund (DO-02-84/2008, KP-06-H29/3-2018).

To participate in the procedure for the award of the DSc degree in Higher Education **4. Natural Sciences, Mathematics and Informatics, professional field 4.2 Chemical Sciences, Analytical Chemistry**, prof. Pancheva-Kadreva has submitted 27 scientific publications in full text, including 21 articles in refereed and indexed journals, 4 - in chapters of monographs and 2 papers published in full text in proceedings of scientific conferences. A total of 204 independent citations (122 of them in the Scopus database) were noted for the publications used in this DSc procedure.

The habilitation thesis is written in 155 pages. It is illustrated with 59 figures, 30 tables and 5 Appendixes. It contains 5 sections: literature review, purpose, materials and methods, results and discussion. The bibliographic reference covers 188 literature sources.

Topical relevance

The habilitation thesis of Prof. Dr. Ivayla Pancheva-Kadreva is focused on the contemporary scientific and applied problems related to the synthesis of metal complexes of known and used in veterinary medicine drugs. In recent decades, this approach has been widely used to find and create new and effective drugs. Natural polyether ionophores are a class of antibiotics that have antibacterial and fungicidal properties and are used to treat coccidiosis. Therefore, many researchers have focused on the synthesis and investigation of new derivatives of monensin, salinomycin, lasalocid with improved antitumor activity, reduced toxicity and their use as antidotes in acute/chronic heavy metal poisoning.

The **literature review** is very thorough and includes a general characterization of polyether ionophore antibiotics, application and chemical modifications of two monovalent polyether ionophores - monensin and salinomycin.

Prof. Pancheva-Kadreva focuses on two ionophores for several reasons: monensin is an antibiotic widely used in veterinary medicine; salinomycin exhibits antiproliferative activity and at last but not at least both ionophores are produced in Bulgaria. Monensin is a sodium ionophore with a decreasing preference for alkaline ions in the order $Na^+ > K^+ > Rb^+ > Cs^+$. The complexes have general formula [MonM] and have been characterized by single crystal molecular X-ray diffraction. While salinomycin is considered to be a potassium ionophore and its metal complexes have not been studied by X-ray structure analysis due to their difficult crystallization. Attention has also been drawn to the use of monoensin and salinomycin for the treatment of coccidiosis (a contagious disease caused by parasites), a number of bacterial infections in animal husbandry, and their use as therapeutic agents in cancer.

Aim of the dissertation

Very impressive is the formulation of the conclusions from the literature review (in the form of what is known and what is not known about these two antibiotics), which guides the author to set and address the aims and objectives of the dissertation.

Materials and methods

The chapter describes the chemicals and reagents used for the synthesis and analysis of the new compounds, as well as the instrumentation used to demonstrate the structure and biological properties of the complexes. A wide range of contemporary analytical methods are used – such as elemental analyses, IR, UV-Vis, EPR, NMR, circular dichroism, thermogravimetric analysis, accelerated particle mass spectrometry and last but not at least, of course, X-ray structure analysis.

An interesting decision to present the thesis in a more compact form is that the syntheses for the preparation and isolation of the complex compounds, their physicochemical characterization as well as the antibacterial, cytotoxic and antitumor activity of the new compounds are not described.

Results and discussion

The synthesized and studied complexes of monensin and salinomycin were grouped into two groups, "classical" complexes in which the ligand coordinates bidentate to the metal ions in a “head-to-tail” mode, and "non-classical" complexes in which a different mode of binding of the antibiotics to the metal ions was observed.

In the first group of "classical" complexes, 32 complexes of monensin and salinomycin with alkaline earth and some transition metal ions of the second valence M^{2+} and some metal ions of the lanthanide line M^{3+} and Se^{4+} were obtained. The most of the complexes have been characterized by single crystal X-ray diffraction and their structural formulae in the solid state have been clearly demonstrated proved. Infrared spectra were also taken to confirm these structures. The complexes were also studied in solution by mass spectroscopy; NMR, EPR (in solid state and in solution). Fluorescence, transmission electron microscopy, thermal analysis, UV-Vis were also performed for some of the complexes to more fully characterize and prove the molecular and structural chemical formulas of the respective complex compounds.

In the second group of "non-classical" complexes, mononuclear monocomplexes of monensin with Hg^{2+}, of salinomycin with Pb^{2+}, mononuclear monocomplexes of monensim and of salinomycin with Ce^{4+}, mixed metal complexes of sodium monensin with Co^{2+}, Mn^{2+} and Cu^{2+} are considered.

The complex of Hg^{2+} with monoenzyme was studied by X-ray structure analysis and its structurural formula [HgMon(H_2O)] was proved unambiguously. For the complex of Pb^{2+} with salinomycin, different methods of physicochemical analysis were used and the most probable chemical formula was proposed.

Complexes of monenzyme and salinomycin with Ce^{4+} of the species [$CeL(NO_3)_2(OH)$] have been isolated and characterized and compared with those considered to be "classical" complexes of the same metal ion with another general formula [$CeL_2(OH)_2$]. In the case of so-called "non-classical" complexes, the main difference is that they contain two nitrate anions directly bound to the metal ion.

From the point of view of the chemistry of complex compounds, the so-called "mixed-metal complexes" of monensin sodium with various divalent metal ions of coordination number four are of interest. In the structure of these compounds, two molecules of monensin sodium salt bind monodentally to the complexing agent, and the other two chemical bonds are made by the chloride anions of the parent metal salts. The neutral complexes of Mn^{2+} and Co^{2+} crystallize with one molecule of water and that of Cu^{2+} with a molecule of acetonitrile. The difference in the structure of the three complexes is that in the first two the metal ions bind to the donor atoms, forming a slightly distorted tetrahedral geometry, whereas in the Cu^{2+} complex the coordination polyhedron is square-planar. The sodium ion remains in the monensin cavity and cannot be displaced by the corresponding metal ion.

After isolating and proving the molecular and structural formulas of the new complexes, Prof. Pancheva-Kadreva investigated the coordination ability of ligands with different metal ions in solution using circular dichroism spectroscopy (CD) and theoretical models. It was found that circular dichroism (CD) can be applied to study the properties of only monensin as a chiral molecule and is inapplicable to salinomycin. The formation of positively charged coordination particles in methanol solutions, one with M^{2+} and two with M^{3+}, was demonstrated for the first time by this method.

To evaluate the incorporation of different metal ions into the structure of polyether ionophores, their antibacterial activity against gram positive and gram negative microorganisms was investigated. The minimum inhibitory concentration (the lowest concentration at which no visible growth of the strains was observed) was used to show the inhibition of bacterial growth. Metal ions were found to potentiate the ability to inhibit visible bacterial growth, retain the activity of the starting ligand and inhibit the action of ionophores.

Monoenzyme complexes with different metal ions have been screened for cytotoxic activity *in vitro* on a panel of human tumor cell lines of various origins, as well as on the non-tumor line Lep3 of human origin.

Some of the complexes of monensin and salinomycin with biometal ions have been studied *in vivo* on white male mice. The most toxic were the complexes of salinomycin with Mg^{2+} and Ca^{2+}. This is the reason to investigate their effect on surviving animals by further biochemical analyses. The results obtained showed that three days treatment with the studied salinomycin complexes did not affect the liver and kidney functions of the experimental animals.

In the **conclusions** prof. Pancheva seeks to summarize everything described in the dissertation and to bring out the most significant regularities found during the development of the dissertation.

The **achievements** are of fundamental nature. The results obtained are promising and a more in-depth study of the complexes of monensin and salinomycin with different metal ions on a larger number of laboratory animals could lead to the creation of effective and selective chemotherapeutics.

The DSc thesis is written very concisely, without unnecessary details, with summaries after each section, which shows the personal involvement of prof. Pancheva in every stage of the synthesis, characterization, and investigation of the compounds. A wide range of instrumental methods of analysis and investigation is used. Orginal results were obtained. The generalizations are correct and logical. Exceptional precision was shown in interpreting the results obtained.

The **abstract** has been prepared as required and correctly reflects the main points and scientific contributions of the thesis.

I have no principal **criticisms** of the research, characterization and biological activity of the new complex compounds.

In **conclusion**, in terms of topicality, volume and quality, the presented DSs thesis and the published (related) articles fully meet the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation and the Regulations for the Development of Academic Staff of Sofia University "St. Kliment Ohridski". Furthermore, I consider that prof. Pancheva is a well-established researcher with a high professional level of scientific work and results that represent a definite scientific achievement, which provides me reason to give a positive assessment and to confidently recommend the Scientific Jury to vote positively for the award of the degree of Doctor of Science to Prof. Dr. Ivayla Pancheva-Kadreva in the professional field **4.2. Chemical sciences, scientific specialty "Analytical chemistry".**

19.02.2024
Sofia

Reviewer:
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