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SPECTROSCOPIC AND THEORETICAL STUDY OF ERYTHROMYCIN AND AMPHOTERICIN B CATION COMPLEXES

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Abstract. In this article the ability of complex formation of erythromycin and the complexes of amphotericin B with Li^+, Na^+, K^+ cations were studied. Analysis of data obtained by the FT-IR measurement showed only small differences between the spectra of AmB complexes and the ligand. In order to illustrate the complex formed between the erythromycin and the cations Li^+, Na^+ and K^+ was performed the quantum-mechanical calculations. Analysis of the geometry complexes obtained indicates that the cations involved in coordination of the oxygen atoms of both of the OH groups and C=O, which is consistent with the data obtained from analysis of the FT-IR spectra. In similar manner, quantum-mechanical calculations for amphotericin B (AmB) and its complexes with metal cations. In this case, the difference calculated heat of formation (ΔHOF) complexes with Li^+, Na^+ and K^+ cations was -100.48 kJ/mol, -119.78 kJ/mol and -139.73 kJ/mol respectively.

Introduction. In the 70th century in the literature there is information about a new class of antibiotics, capable of delivering metal cations across biological membranes and more specifically across the mitochondrial membrane. The biological activity of these compounds is related to their natural ability to form complexes with metal ions and their transport through the lipid barrier which is a membrane, hence the name ionophores or ion carriers [1, 2]. The ability of binding molecules of ionophores, is based on their affinity, which matches the structure of the cavity of the host. The affinity is bigger, the more lasting connections arise [3]. The size of the cavity and ion beam determines the selectivity of ionophores. Of great importance in the process of selective molecular recognition also it has environmental pH, ionic strength and the kind of solvent [4, 5].

The first group of synthetic compounds, that was capable of complexing ions within its structure were cyclic ethers, synthesized by C. Pedersen and called crown ethers [6]. Pedersen received dibenzo-18-crown-6, a chemical that contributed to the formation of supramolecular chemistry. In 1987, a group of scientists C. Pedersen, Cram and Lehn received the Nobel Prize in chemistry.

Crown ethers are able to chelate cations of lanthanide, alkali metal or ammonium cation. Adjustment of ions into the cavity of the host (ionophore) depends largely on the size of their ionic radii, and the arrangement of oxygen atoms complexing cation. Due to the presence hydrocarbyl chains of molecules of crown ethers are readily soluble in organic solvents [7-9].

The mechanism of formation is a channel known to a lesser extent than the mechanism for moving the ions through the ionophoric carriers. Ionophoric antibiotics, using channel transport mechanism are also called pseudo-ionophores. Their operation is based on the incorporation of a hydrophobic structure biological membrane, forming a pore (often filled with water) allowing transmembrane ion transfer. This mechanism is less selective in relation to the carriers and may cause transport of undesirable substances [10].

Well-known ionophoric antibiotic, transporting the ion through the formed channel is gramicidin A, a compound isolated from the strains of *Bacillus brevis*. The structure of a linear combination of amino acids of the D and L configurations, staggered, one end and ethanolamine, and on the other a formyl group. The compound has a tendency to adopt the β-helix structure. When forming a channel which penetrates the membrane, the molecules organize themselves gramicidin A dimers of polypeptide chains, facing each N-terminally. Side chains of aminoacids are then routed outside the channel, allowing for better placement in a lipophilic membrane. Gramicidin channel is selective for monovalent cations and preferences transported ions arranged in an increasing number of $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$. There are also gramicidin B and C, in which the eleventh residue of L-tryptophan was changed respectively to L-phenylalanine and L-tyrosine [10-13].

Another ionophore antibiotic belonging to the group of non-cyclic carboxylic ionophore is salinomycin. It demonstrates the ability of complexing monovalent and bivalent cations, transporting them through biological membranes. Salinomycin affinity to cation arranged in series: $K^+ > Na^+ > Cs^+ > Sr^{2+} > > Ca^{2+} > Mg^{2+}$ [14-16].

The operation of this ionophore is similar to the aforementioned monensin. However, what sets this antibiotic, the possibility of selective destruction of breast cancer cells. This compound has the ability to destroy cancer stem cells and cancer cells resistant to apoptosis. Thus it can be used for antitumor therapy [17, 18].

One of the better-known carboxylic ionophore antibiotics is lasalocid. Despite the many desirable properties, characterized by high toxicity. Receiving lasalocid acid derivatives, can lead to a reduction of its harmfulness, and thus increase its use [19]. Ionophore lasalocid is isolated by Berger from a strain of Streptomyces lasaliensis in the form of sodium salt [20]. The structure (Figure 1) defined by Westley and co-workers in 1970 [21]. Lasalocid belongs to linear molecules. Its structure there are two rings: tetra-hydropyran and tetrahydrofuran, and the carbon chain of the aromatic ring of salicylic acid. Furthermore, the molecule contains three hydroxyl groups and one carboxyl [14].

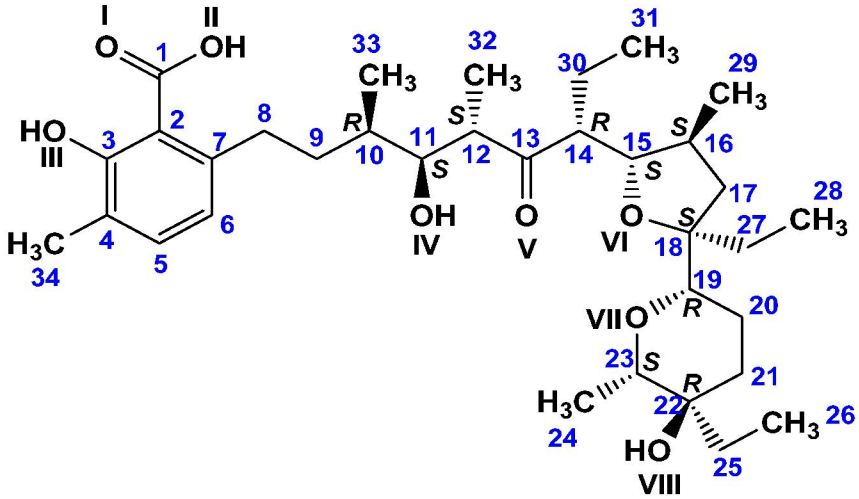

Figure 1 – The structure of lasalocid

Macrolides are a another group of antibiotics of bacteriostatic activity. The name comes from the words macro (large) and oligo (lactone), as the molecules of these antibiotics have a 12-16 atom lactone core. Erythromycin is a well known natural macrolide antibiotic obtained from Streptomyces erythreus [22]. Erythromycin belongs to polyhydroxylactones, molecule is containing two sugar rings and the aglyconic fragment (Figure 2).

Amphotericin B (Figure 3) is a natural antibiotic first isolated in 1955 from the gram-positive bacteria Streptomyces nodosus. Amphotericin B has a wide range of antimycotic activity, it is effective against prostatic hypertrophy and hypercholesterolemia and is also used as fungicide in infections of AIDS patients. It is produced selled by many firms under the commercial names of Fungillin, Amphotericin B, Amphozone, Ampho-Moronal, Wypicil and Ampho-Moronal V.

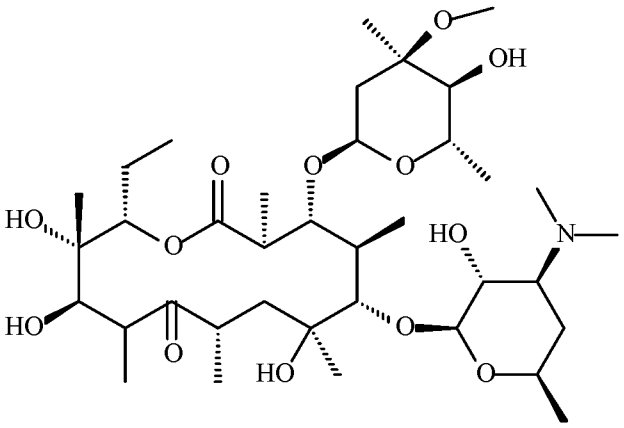

Figure 2 – Erytromycin

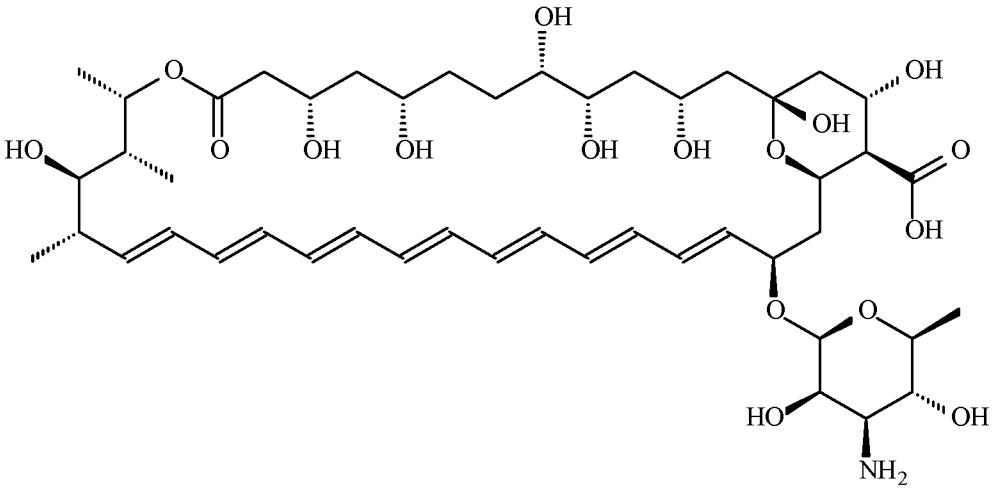

Figure 3 – Amphotericin B

The physico-chemical properties of this antibiotic are related to its structure which contains two main elements: the alkyl chain with hydroxyl groups and the chain of coupled double bonds. The rigid olefin chain (Figure 3) prevents the molecule to coil up [fold up] in the process of ion complexation, which is so characteristic of ionophores such as lasalocid acid or monensin [22].

Nowadays the scientists of M.Auezov South Kazakhstan State University and Adam Mickiewicz University in Poznan began scientific researches by the investigatin of synthesis of biological and chemical activity of functionalized ionophores in the frame of Grant No 68-10 from 12.02.2015.

In this work we present results of research of spectroscopic and theoretical study of erythromycin and amphotericin B cation complexes. In order to illustrate the complex formed between the erythromycin and the cations Li^+, Na^+ and K^+ was performed the quantum-mechanical calculations.

Experimental

Erythromycin and amphotericin B was purchased in pure form. The structure was also confirmed by NMR analysis.

For preparations of the complexes the following compounds were used: $LiClO_4$, $NaClO_4$, $KClO_4$, (Aldrich), acetonitrile (Fluka) additionally dried under vacuum. The solutions of the complexes were obtained by dissolving of the respective salts and ligands in acetonitrile at the 5:1 ratio. Acetonitrile was of spectroscopic grade and was dried over a 3Å molecular sieve. All preparations and transfers of solutions were carried out in a carefully dried glovebox under nitrogen atmosphere.

FT-IR measurements. All samples were measured in 200 mg KBr tabs. The mass of samples was 1 mg per tab. The FT–IR spectra were recorded, using a IFS 66/s FT-IR spectrophotometer from Bruker, equipped with an MCT detector (125 scans, resolution 2 cm^{-1}).

Calculation procedure. Semi-empirical calculations (PM6) of the heat of formation (HOF) and the geometric optimization were performed using the Scigress 2.1.0 program.

Results and discussion

The ability of complex formation of erythromycin with Li^+, Na^+, K^+ cations was studied. Analysis of FT-IR showed involvement in the formation of complexes, not only the hydroxyl groups, but the groups C=O, both ketone and lactone. In-band assigned to the vibration ν(OH) in the spectrum of the free ligand can be distinguished three maxima at 3525 cm^{-1}, 3473 cm^{-1} and 3409 cm^{-1}, demonstrating the involvement of hydroxyl groups in the hydrogen bonds of varying strength. The spectra of complexes in this range showed only a single wide band at 3486 cm^{-1} for the Na^+ and K^+ complexes and 3430 cm^{-1} for Li^+ complex. In the range of the band assigned to the ν(OH) in the spectra of free ligand we noticed two maximaat 1736 cm^{-1} and 1715 cm^{-1}, in the spectra of complexes this two bands are sha red giving one band with maximum at 1717 cm^{-1}, 1721 cm^{-1} and 1730 cm^{-1} for Li^+, Na^+, K^+, respectively. It has been proved in this way, the equal involvement of groups OH and C=O formation of complexes, which may indicate the presence of cation fluctuations in the molecule.

The complexes of amphotericin B with Li^+, Na^+, K^+ cations was obtained and proved by ESI-MS analysis. Analysis of data obtained by the FT-IR measurement showed only small differences between the spectra of AmB complexes and the ligand. They failed to thus demonstrate the formation of aggregates AmB with metal cations in nonpolar solvents.

In order to illustrate the complex formed between the erythromycin and the cations Li^+, Na^+ and K^+ was performed the quantum-mechanical calculations. Whenever the structure of the molecule or complex has been pre-calculated by the method of molecular mechanics (MM2), and then calculating the energy and the geometry using the semi-empirical methods (PM6) (Figure 4).

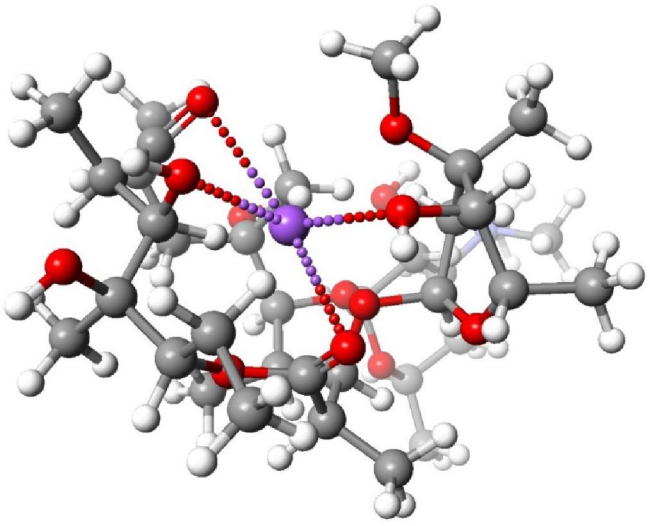

Figure 4 – The calculated structure of the complex of erythromycin with Na^+ cation

The calculated differences in heat of formation (ΔHOF) complexes with Li^+, Na^+ and K^+ cations was -374.05 kJ/mol, -467.04 kJ/mol and -484.20 kJ/mol, respectively. On this basis, the best energy is the formation of complexes by erythromycin with potassium cations, followed by sodium and lithium. Analysis of the geometry complexes obtained indicates that the cations involved in coordination of the oxygen atoms of both of the OH groups and C=O, which is consistent with the data obtained from analysis of the FT-IR spectra.

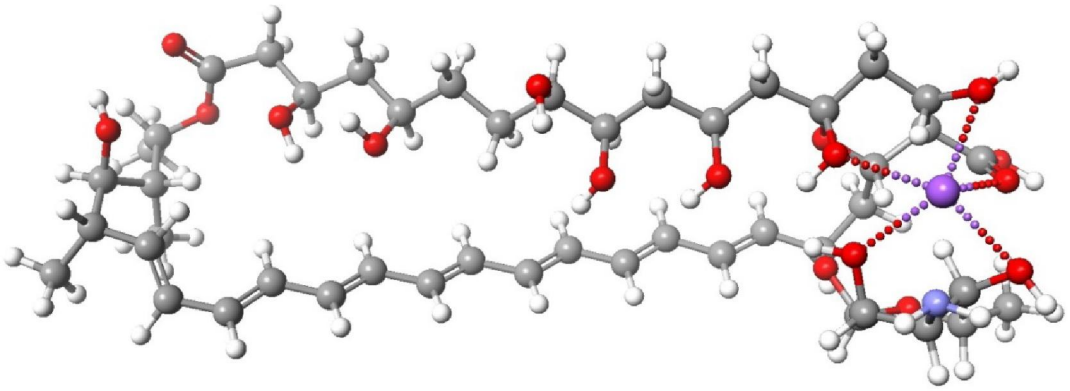

Figure 5 – The calculated structure of the complex of amphotericin B with Na^+ cation

In similar manner, quantum-mechanical calculations for amphotericin B (AmB) and its complexes with metal cations. In this case, the difference calculated heat of formation (ΔHOF) complexes with Li^+, Na^+ and K^+ cations was -100.48 kJ/mol, -119.78 kJ/mol and -139.73 kJ/mol respectively. It has been found that the energetically most favorable is the formation of complexes with cations potassium and then sodium and lithium, as well as with erythromycin. Clearly, however, lower values of ΔHOF, indicates poorer ability of amphotericin B to form complexes with cations of these metals, which is consistent with the spectroscopic observations. Analysis of complex geometry shows involvement in coordinating cations only oxygen atoms from groups with pyranose rings in the "head" of the molecule (Figure 5). The analysis of the geometry of this molecule that the involvement of oxygen atoms of the OH groups of the alkyl chain is prevented by a chain of conjugated double bonds. It was attempted to calculate the structure of the aggregate of some molecules AmB with potassium cations, which was observed in aqueous solutions. The calculations did not give satisfactory results because, it was unable to calculate the energetically favorable structures. Probably to form such structures, it is necessary the environment of polar solvent such as water. Calculations were performed for the isolated molecules in a vacuum, these conditions reflect well the behavior of molecules only in nonpolar solvents.

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ЭРИТРОМИЦИН ЖӘНЕ АМФОТЕРИЦИН В-ның КАТИОНДЫ КОМПЛЕКСТЕРІН СПЕКТРОСКОПИЯЛЫҚ ЖӘНЕ ТЕОРИЯЛЫҚ ЗЕРТТЕУ

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Тірек сөздер: антибиотиктер, эритромицин, амфотерицин В, квантты-механикалық есептеулер.

Аннотация. Мақалада эритромицин мен амфотерицин В-ның Li^+, Na^+, K^+ катиондарымен комплекс түзу мүмкіндіктері зерттелген. FT-IR өлшулері кезінде алынған мәліметтерді талдау, AmB комплекстері мен лигандалары спектрлерінде аздаған ғана айырмашылықтарды көрсетті. Эритромицин мен Li^+, Na^+ и K^+ катиондары арасында түзілген комплекстерді түсіндіру үшін кватты-механикалық есептеулер жүргізілді. Алынған комплекстердің геометриясын талдау, катиондар -ОН және С=О топтарының оттегі атомдарын бағыттауға қатысатындығын көрсетті және бұл ИҚ-спектрлерді талдау кезінде алған мәліметтермен толықтай сәйкестенеді. Осыған ұқсас, кватты-механикалық есептеулер амфотерицин В (AmB) және оның комплекстерінің металл катиондарымен үшін де жүргізілді. Бұл жағдайда, комплекстердің Li^+, Na^+ и K^+ катиондарымен есептелген жылу түзу айырмашылығы, сәйкесінше, -100,48 кДж/моль, -119,78 кДж/моль и -139,73 кДж/моль құрады.

СПЕКТРОСКОПИЧЕСКОЕ И ТЕОРЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ КАТИОННЫХ КОМПЛЕКСОВ ЭРИТРОМИЦИНА И АМФОТЕРИЦИНА В

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Ключевые слова: антибиотики, эритромицин, амфотерицин В, квантово-механические расчеты.

Аннотация. В статье была исследована возможность образования комплекса эритромицина и комплексы амфотерицина В с катионами Li+, Na+, K+. Анализ данных, полученных при измерении FT-IR, показал лишь небольшие различия в спектрах AmB комплексов и лигандов. Для пояснения комплекса, образованного между эритромицином и катионами Li^+, Na^+ и K^+ , произвели квантово-механические расчеты. Анализ гео-метрии полученных комплексов показывает, что катионы участвуют в координации атомов кислорода групп -ОН и С=О, что согласуется с данными, полученными из анализа спектров ИК-спектров. Аналогичным образом квантово-механические расчеты были произведены для амфотерицина В (AmB) и его комплексов с катионами металлов. В этом случае разница рассчитанной теплоты образования (ΔHOF) комплексов с катио-нами Li +, Na + и K + составили -100,48 кДж/моль, -119,78 кДж/моль и -139,73 кДж/моль соответственно.

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