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THE COMPLEXES OF ANTIBIOTICS WITH TRACE METALS

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This article, basing on the available literature, summarizes the results of physiochemical research into the structure of complexes of trace metals with antibiotics. It presents the leading methods of instrumental analysis applied to determine the structure of complexes.

Keywords: ntibiotics, trace metals, complexes of trace metals, instrumental analysis

1. INTRODUCTION

The term antibiotics [Gr. *anti* - against, *bios* - life] refers to a group of organic natural chemical compounds produced by such microorganisms as: bacteria, actinobacteria, warious types of fungi and some plants and animal cells. Many substances isolated from these organisms are synthesized on an industrial scale as a whole (of synthetic origin, called chemiotherapeutics) or in part (of semisynthetic origin), [16]. The products of metabolism of actinobacteria constitute about 90% of natural antibiotics produced on an industrial scale [5].

The research into moulds has enabled the acquisition and consequently the synthesis and modification of the acquired compounds although their role in the life of microorganisms has not been explained [5]. From the medical point of view some of the most important metabolites secreted by the *Penicilium chrysogenum* Thom mould are penicillin G. and griseofulvin. Yet many other metabolites also have a wide range of action: antiseptic against *Staphylococcus aureus*, decomposing carbohydrates, oxidizing decanes, undecanes and hexadecanes from petroleum. The *Acremonium strictum* W.Gams mould

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produces cephalosporin, which has become the source compound for five generations and over 20 various derivatives of cephalosporins and the *Thamnidium elegans* Link mould produces an antibiotic inhibiting the development of mycobacteria [10]. A common feature of all antibiotics and chemiotherapeutics is their ability to inhibit the metabolism and multiplication - the basic life processes - of microorganisms.

The biosynthesis of antibiotics is based on several starting substances and depends on the environmental conditions. With both *Penicilium chrysogenum* and *Acremonium strictum* the substrates are acetyl-CoA, acetylserine, L-methionine, pyruvate, and 2-oxoglutarate of the Krebs cycle. These compounds are converted to three amino acids and subsequently to a tripeptide, which, as a result of enzyme action, produces the intermediate isopenicillin N. The *Penicilium* moulds convert it to penicillins, whereas the *Acremonium* moulds convert it to cephalosporins and cephamycins [5].

The growing drug resistance of microorganisms has resulted in the rapid development of pharmacology and chemistry. A lot of antibiotics have been found, which have been divided according to their chemical structure and the range of effect on microorganisms. With regard to structure the following can be identified: beta-lactams (penicillins, cephalosporins, monobactams, carbapenems, beta-lactamese inhibitors), tetracyclines, quinolones, aminoglycosides, peptide antibiotics, sulfonamides, linkosamides, chloramphenicol, macrolides, ansamycins.

Some of these groups turned out to be promising due to the possibility of synthesizing derivatives whose properties are stronger than those of the source substances, and which are more active, more water-soluble, more lasting, of better therapeutic index, broader application and limited adverse drug reaction.

Antibiotics differ from one another in terms of the way they effect microorganisms. Two mechanisms are, however, prevalent. One of them is based on the destruction or prevention of the production of the cell wall, which consequently leads to the loss of its contents by the cell. The other mechanism consists in the blocking of metabolic pathways by antibiotics - the inhibition of the process of creating proteins prevents the cell from developing and regenerating properly. The physiochemical properties vary and depend on a number of factors, such as pH, the functional groups in a molecule and its general structure [16].

Trace metals are elements including copper, manganese, iron, vanadium (or more precisely - vanadyl - VO^{2+}), chromium, zinc and cobalt. They are essential to the proper functioning of the organism. Their daily demand does not exceed 100 mg and their excess may cause numerous and serious side effects. The role of trace metals is most frequently to regulate metabolism because many of them constitute the important part of enzymes (zinc, molybdenum) or

vitamins (cobalt). They enable cellular respiration by supplying oxygen to tissues and cells (iron), are involved in the synthesis of neurotransmitters (copper) and protect from free radicals (manganese), [7]. Pure trace metals are toxic for the organism [8]. However, they enter the organism from food as complex compounds [7,12].

Many of the chemical compounds come in contact with antibiotics in human body, among them ions of trace metals. Effects of their synthesis can be harmful, neutral or curative and is dependent on their solubility, assimilation, polarity and chemical structure. Getting to know of that structure may be key to predicting the behavior of these substances in the organism, or even finding new ways of their usage. Knowing the most efficient methods of analyzing complex compounds will allow us to quickly and inexpensively research their previously mentioned attributes, witch in turn may be helpful in synthesizing next generation, more efficient and selective medicaments.

The purpose of this paper is to determine, based on literature data, the most efficient methods of identification of antibiotics-trace metals complexes.

2. RESEARCH METHODOLOGY REVIEW

The applied methodology consisted in the analyzing and comparing of the most frequently performed spectra and characteristic parameters of free antibiotics and their complexes with trace metals. The most frequently used methods were the following: the IR (infrared spectroscopy), UV-Vis (Ultraviolet-visible spectroscopy) spectrophotometry, ^{1}H NMR (Proton Nuclear Magnetic Resonance) and ^{13}C NMR (Carbon-13 Nuclear Magnetic Resonance) spectrometry, polarography, molecular modelling, crystallographic measurements, EXAFS (Extended X-Ray Absorption Fine Structure), mass spectrometry, EPR (Electron Paramagnetic Resonance) and the measurements of the magnetic moment [2, 3, 4, 11, 13].

3. ANALYSIS AND EVALUATION OF APPLIED METHODS' USEFULNESS

The IR spectrum of trace metal complexes with penicillins provides valuable information. The study of the presence of signals of free penicillin G and ampicillin and their complexes with copper allows one to clearly determine which functional groups are engaged in the coordinate bond. Table 1 confirms the research data. The lack of signal typical of lactam group testifies to the hydrolysis of the lactam ring, namely the decomposition of penicillin into the penicillic acid (Figure 1).

Table.1. IR spectrum data for benzilpenicillin (PenG) i ampicillin (Amp) and their binding with Cu(II) G* and Amp* stands for ligand of penicillic acid [13]

Functional group	ν [cm^{-1}] for PenG	ν [cm^{-1}] for $Cu(PenG)_2$	ν [cm^{-1}] for Amp	ν [cm^{-1}] for $Cu(Amp)_2$
C=O (lactam)	1780	no spectrum	1780	no spectrum
C=O (amide)	1680	1660	1680	1660

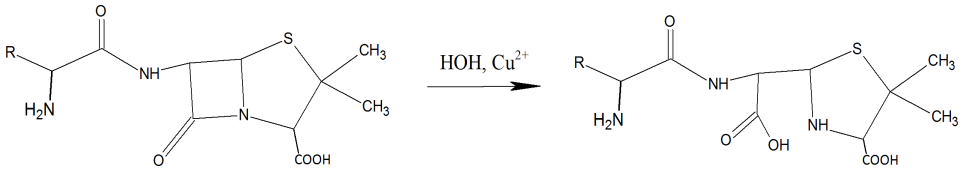

Fig. 1. Hydrolysis of penicillins to penicillic acid in the environment of copper ions [13]

The spectrophotometric study (UV-Vis) of penicillin solutions confirms their coordinative properties (Figure 2). For 1:1 solutions (ligand:metal) the maximum absorption is observed at 715-755 nm; with a portion of the ligand added to the solution the maximum is found at 318-366 nm (Figure 2). This is due to the precipitated sludge and confirms the creation of a new compound containing more ligands bound with the coordination centre. The minimum absorption for the compound is observed at 2:1 ligand:metal ratio [13].

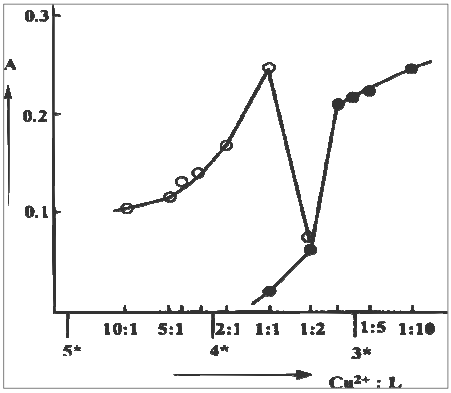

Fig. 2. Absorbance dependence for the copper-penicillin G complex for specific stoichiometric ratios. ● for λ 318 nm, ○ for λ 755 nm [13]

Cephalosporins create complexes at various stoichiometric ratios, and the ligand co-ordinates the metal ion through various donor groups depending on pH. It all results closely from the molecule structure [4]. The study of the bonding of cefadroxil with metal ions has provided a lot of important information. These complexes are stable - as proved by the log K value (= 6 – 13) oraz ΔG (= 8.5 – 18.5 kcal/ mol·K). The IR spectrum of cefadroxil includes

a band at 1354 cm^{-1} attributed to the stretching vibrations of the C-N beta-lactam ring and the thiazolidine ring. There is no such band in the spectrum of the complexes, which indicates the participation of nitrogen atoms of these rings in the coordinate bond (Figure 3). The stretching vibrations of the C-O group coming from the beta-lactam ring of the cefadroxil molecule, which occur at 1759 cm^{-1}, are not found in the complex spectrum (Table 2). This is evidence of this group being engaged in the chelation of the metal ions. In the spectrum of the complex the bands for symmetrical stretching vibrations (COO) are shifted towards higher frequencies by about 30-50 cm^{-1} as compared with the spectrum of free cefadroxil. This points to the participation of the carboxyl group in the coordinate bond. Studies have also shown that the amide group is not engaged in the creation of the complex as its band does not undergo shifting [15].

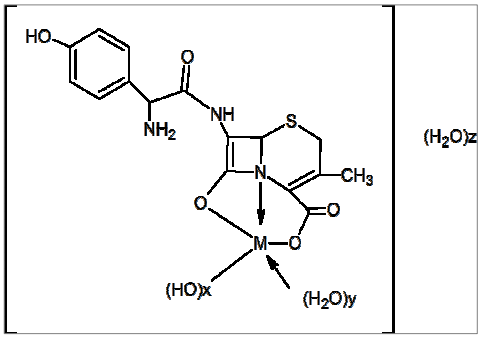

Fig. 3. The general structure of the cefadroxil-trace metal complex, where M stands for Fe(II) x=0 y=1 z=2,Ni(II) and Cu(II) x=1 y=0 z=1 [15]

Table 2. Selected bands of the IR spectrum attributed to cefadroxil and their complexes (L-ligand cefadroxil) within the wavelength of 4000-200 cm^{-1} [15]

Complex	ν (C-N) in the ring in [cm^{-1}]	ν (C=O) in the ring in [cm^{-1}]	ν_{sym} (COO) in [cm^{-1}]
[Fe(II) L(H_2O)] ·2H_2O	no band	no band	1261
[Ni(II)L(OH)]·H_2O	no band	no band	1236
[Cu(II) L(OH)]· H_2O	no band	no band	1234

The properties of the cefaclor-copper (II) complex were studied by means of spectrophotometry with the pH value set and maintained at 8.0 with the use of a pH meter. Cefaclor may behave like an acid and a base at the same time. It may also be a zwitterion. In the acidic environment its complex with copper (II) hydrolyzes into $Cu(CEF)^{+}$, and in the slightly basic environment a hydrocomplex Cu(OH)(CEF) is created, which is confirmed by the study of pH values (Figure 4). With the pH value over 9.0 the absorbance increases and the maximum shifts towards longer wave lengths - the $Cu(OH)_2$(CEF) complex is

created. With the pH over 10.7 the colour of solution turns intensly into yellow and orange, which probably results from the decomposition of cefaclor in the strongly basic environment. It was supposed that copper catalyzes the hydrolysis of cefaclor, but this hypothesis was not confirmed. The obtained results agree with the assumption that the metal ion is not engaged in the stabilization of the tetraedric complex created with the opening of the lactam ring. That confirms the prediction that the copper ion co-creates a complex outside the ring at pH = 8.0.

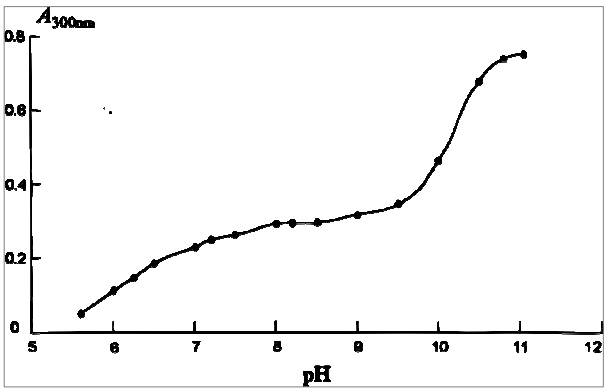

Fig. 4. Absorbance-pH dependence for the cefaclor-copper complex at 300 nm [3]

A UV-Vis spectrum was performed for the 400 - 282 nm range for copper salts (II), cefaclor and the complex of copper with cefaclor. For cefaclor the maximum absorption is found at 282 nm, whereas for the complex it occurred at 300 nm, which serves as confirmation of the creation of this complex (Figure 5), [3].

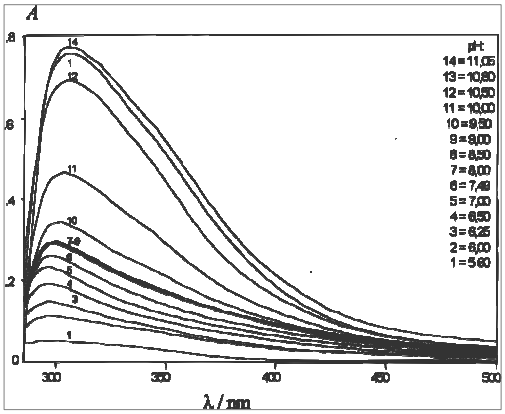

Fig. 5. The UV spectrum for the copper (II)-cefaclor complex in changing pH [3]

Aminoglycosides readily chelate metal ions, but for this to happen the condition must be met that there are four or more atoms of nitrogen (amine groups) present. Gentamicin includes 5 amine groups, only 3 of which have the capacity to coordinate, creating a 1:1 complex.

The ^1H NMR spectrum for the iron-gentamicin complex is a useful tool for analysing the structure of the compound (Figure 6).

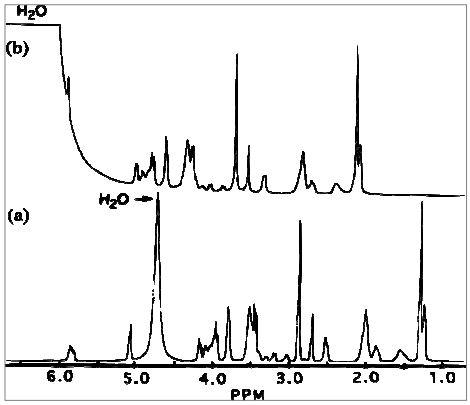

Fig. 6. The value of chemical shift for the complex (b) as compared with the free antibiotic (a) equals approx. 1 ppm [11]

It follows that the signal bandwidth in the spectrum of the complex approximates the signal bandwidth in the spectrum of free gentamicin. That has confirmed the hypothesis about the weak interaction of the ligand with the metal ion [11]. Molecular modelling indicates that the chelation of iron by one gentamicin molecule does not prevent attaching another gentamicin molecule. Hence, if there is a high concentration of the antibiotic, a 2:1 (antibiotic: metal ion) complex arises (Figure 7).

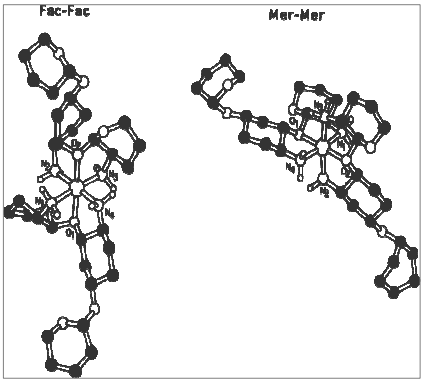

Fig. 7. Proposed structures for the 2:1 gentamicin:iron complex [11]

The research on aminoglycosides has shown that these antibiotics very effectively bind with copper (II) (kanamycin A even one hundred times as strongly as with other metals) in the environment of physiological pH [14]. Aminoglycosides are known for their oto-, hepato- and nephrotoxic effect on the human organism [16]. That may be caused by radicals, which appear in the presence of the complexes of these antibiotics with trace metals. Although these substances themselves do not have reduction-oxidation abilities and therefore require a special medium to participate in such processes, ions of transition metals Ni(II), Co(II), Zn(II) or Cu(II) seem to be ideally suited for that. Although the amount of 'free' copper in the human organism is quite small, during illness (tumour, inflammations) its levels increase and the creation of a complex becomes fully possible [14]. Peptide antibiotics have a great capacity to bind the ions of bivalent metals at the 1:1 ratio. It has been established that this affinity decreases in the series: $Cu^{2+} > Ni^{2+} > Co^{2+} \sim Zn^{2+} > Mn^{2+}$ [6]. It is thought that the bacitracin molecule chelates metal ions by means of the thiazolidine and imidazole ring of histidine. The engagement of the imidazole ring has been determined with the help of the ^{1}H NMR spectrum, on the basis of an equal shift of its value towards the lower range for both 2-CH and 4-CH and by analysing the ^{13}C NMR spectrum, at pH = 6-8. This theory needs confirmation by further studies. It has been established that the above-mentioned ions chelate with the sulphur atom of the thiazolidine ring and the carboxyl group of the glutamic acid (Figure 8). It follows from the EPR spectra that Mn^{2+} does not create complexes with the antibiotic below pH = 5.2. The Cu^{2+}- bacitracin complex, on the other hand, gives the EPR spectrum, which is consistent with the assumption that the complex has a distorted tetragonal geometry with two coordinated atoms of nitrogen and two atoms of oxygen. The EXAFS study indicates that in the coordination sphere of the metal there are three N-ligands and one ligand with an atom of oxygen, attributed most probably to the nitrogen of the terminal amine group of the imidazole ring of histidine, the thiazolidine nitrogen and the carboxyl group of the glutamic acid [6].

Many scientists have conducted research into the complexes of trace metals with quinolones because the presence of carboxyl and carbonyl groups ensures good chelation and the creation of relatively small compounds [16]. The proposed method of the complexation of metal ions postulates interaction between the 4-oxo and the neighbouring hydroxyl group, which are a constant and indispensable element ensuring that quinolone antibiotics have bacteriostatic abilities.

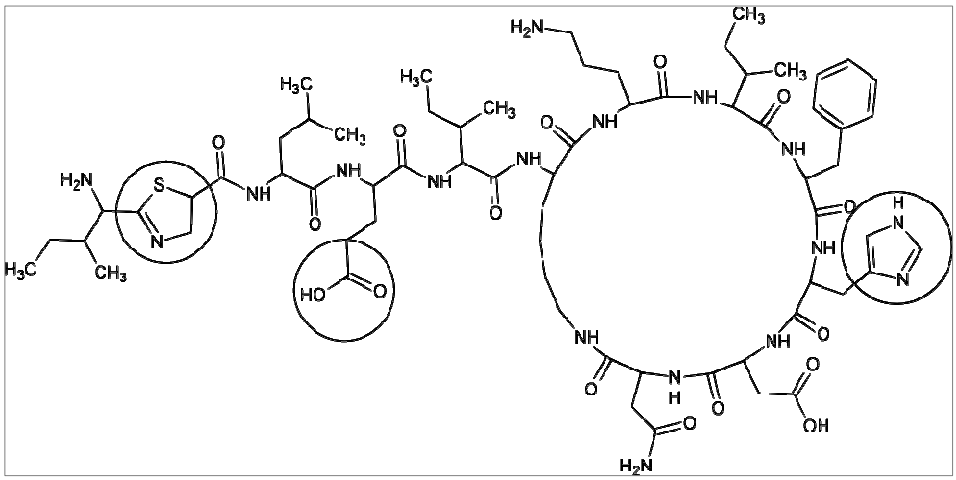

Fig. 8. Bacitracin with the groups engaged in the chelation of ions of trace metals [6]

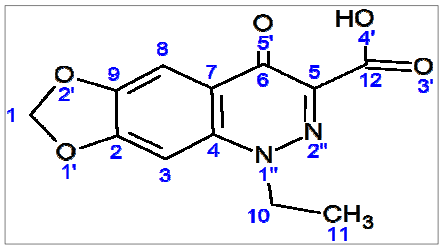

Fig. 9. Cinoxacin. The presence of the 5-oxo and carboxyl group ensures excellent chelating properties [16]

Another example that confirms the method of chelation of metal ions by quinolones is the complex $[Cu(hsm)(nal)H_2O]Cl \cdot 3H_2O$ (hsm - histamine, nal - nalidixic acid), in which one can observe d-d transitions at 625 nm, attributed to the complexes of square based pyramidal geometry including two coordinated nitrogen atoms. In the IR spectrum one can observe broad bands between 1634 and 1501 cm^{-1} attributed to the stretching vibrations including both the functional group COO and CO. It serves as further confirmation of the interaction between the metal ion and the carbonyl (5-oxo) and the carboxyl group within a molecule of this antibiotic (Figure 9), [1]. Another case confirming the structure of the complexes of metal ions with quinolones is the $[Cu(hsm)(cnx)NO_3] \cdot H_2O$ complex (where hsm stands for histamine, and cnx for cinoxacin - Drawing 4). It acquires the geometry of a distorted square based pyramid - the crystallographic analysis serves as confirmation (Figure 10, Tab. 3), [2].

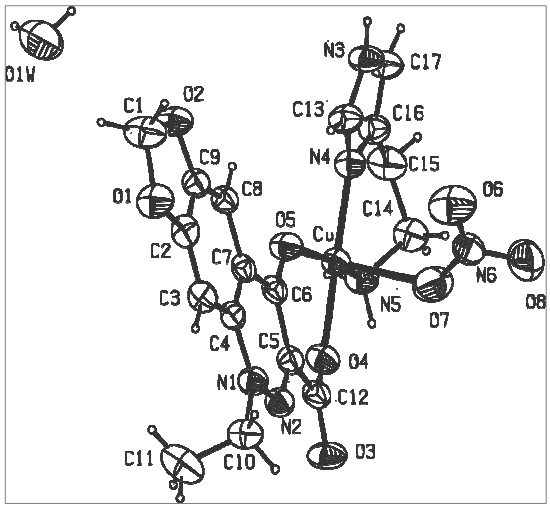

Fig. 10. The crystal structure of the complex with the particular atoms marked [Cu(hsm)(cnx)NO3] ·H2O [2]

Table 3: Comparison of bond length in free cinoxacin and its complex with copper (II) based upon crystallographic research [2]

Carbon bond in antibiotic	Bond length in free antibiotic	Bond length in complex of antibiotic	Type of change in bond
C(5) - C(6)	1.451	1.442	shortening
C(6) - O(5')	1.248	1.270	lengthening
C(12) - O(4')	1.316	1.269	shortening
C(5) - C(12)	1.503	1.511	lengthening

4. SUMMARY

In this study the most frequent methods of instrumental analysis applied to identify complexes of trace metals with antibiotics have been selected from the available literature and their accuracy has been compared. The methods used for identification and analyzing the results have been expounded with reference to particular groups of antibiotics. Also, available information has been collected on the physicochemical properties of the complexes. This knowledge is indispensable to make predictions about the behaviour of molecules in the organism and to create new, lasting and effective antibiotics.

Each method of analysis has it's pros and cons. In IR and UV-Vis spectroscopy the ease of sample preparation, result interpretation and inexpensive procedure is offset by the fact that not all substancs can be

examined. The EPR method only allows the study of trace metals with unpaired electrons. ^{1}H NMR and ^{13}C NMR methods are costly, altough they provide useful information about structure.

In case of complicated structure compounds mass spectrometry defines their mass but is otherwise difficult to interpret. The EXAFS method does not give sufficient information about the structure, and is only limited to defining distance between atoms, furthermore it is used to examine amorphic materials. Polarography provides information about ion stability and concentration, but it is hazardous for scientists' health, there is a possibility of sample contamination and interferation with electrolytes. Cristallographic analysis are costly, the studies themselves are time consuming and in case of complicated structure compounds is difficult to interpret. Howevere it provides us with greatest amount of information about examined substance structure.

Each of the presented methods has in its own way confirmed or negated the hypothetical structure of the complex. The preferred method was IR and UV-Vis spectroscopy. It is most probably due to the ease it ensures in preparing the results and the relatively low cost of measurements as compared with f. ex. EPR, polarography or magnetic methods.

5. CONCLUSIONS

This study, based on the literature data, summarizes the results of physiochemical research on the structure of complexes of trace metals with antibiotics. These compounds have been studied with nearly all known groups of antibiotics as they contain heteroatoms and as such have excellent donor properties. Their structure is not always easy to interpret. No single method allows us to clearly specify the structure of the examined compound. Using multiple analysis methods gives better results. Spectroscopic and crystallographic methods are prefered by scientists. The knowledge of a structure, the methods of its synthesis and its effect on the human organism allows for the creation of a new class of substances. Its extended effect on microorganisms may result in their reduced drug resistance.

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KOMPLEKSY ANTYBIOTYKÓW Z METALAMI ŚLADOWYMI

S t r e s z c z e n i e

W artykule, na podstawie danych pochodzących z pismiennictwa, zestawiono wyniki badań fizykochemicznych nad strukturą kompleksów antybiotyków z metalami śladowymi. Przedstawiono wiodące metody analizy instrumentalnej służące do określania struktury kompleksów.

Słowa kluczowe: antybiotyki, metale śladowe, kompleksy metali śladowych, analiza instrumentalna