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Synthesis, structural characterization, and antibacterial activity of Co (II)-complex of 2-acetoxy benzene carboxylic acid

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Abstract: Metal complexes are gaining more and more importance, especially in the design of drugs in coordination with metal. This has led to numerous studies on metal drug complexes that have shown to be effective in the treatment of various diseases caused by viruses as well as in the treatment of cancer. A cobalt(II) complex with the nonsteroidal anti-inflammatory drug 2-acetoxy benzene carboxylic acid was synthesized and characterized by physicochemical and spectroscopic techniques. Experimental data showed that the tested pharmaceutical active substance acts as a bidentate ligand through carbonyl oxygen of carboxyl and ester groups. Screening of antibacterial activity for complex samples was performed by well diffusion assay against six different bacterial strains (*E. coli, Staphylococcus aureus, Bacillus subtilis, Listeria monocytogenes, Salmonella Enteritidis and Pseudomonas aeruginosa)* and *Candida albicans*. Research has shown that the complex of 2-acetoxy benzene carboxylic acid with Co (II) ions shows a weak antibacterial and antifungal effect.

Keywords: 2-acetoxy benzene carboxylic acid. Drug-metal complex. Aspirin. Cobalt (II) complex.

1. Introduction

2-acetoxy benzene carboxylic acid is a pharmaceutically active compound and belongs to the most available and most used drugs. It acts as an analgesic, antipyretic, anti-inflammatory, and antirheumatic [1]. Alone or in combination with other drugs, 2-acetoxy benzene carboxylic acid is used today in the prevention of arteriosclerosis and intravascular thrombosis [2], the prevention of myocardial infarction [3], the treatment of cerebral apoplexy, the treatment of colon cancer [4], the treatment of preeclampsia, the reduction of dementia, the decrease in cataracts, and the control of diabetes. 2-acetoxy benzene carboxylic acid is better known as Aspirin, which is the registered name of the factory "Friedrich Bayer & Co." in whose laboratory the German chemist Felix Hofman first time synthesized 2-acetoxy benzene carboxylic acid or acetylsalicylic acid.

2-acetoxy benzene carboxylic acid is an acetyl derivative of salicylic acid. According to its structural characteristics, it belongs to the group of esters. The chemical structure of 2-acetoxy benzene carboxylic acid indicates the presence of an aromatic benzene ring, ester, and carboxyl groups in the molecule. (Fig.1)

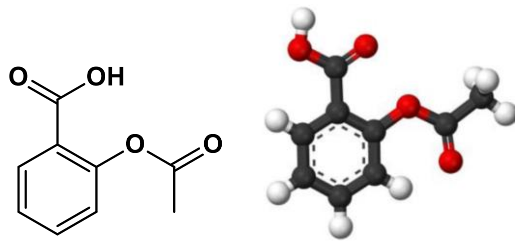

Figure 1. Molecular and 3D structure of 2-acetoxy benzene carboxylic acid

Coordination or complex compounds, in addition to being of fundamental importance for synthesis, structure, and reactivity research, also have an extensive practical application in industry as catalysts, dyes, and pigments, in medicine as metallo-drugs, chemotherapeutics, and contrast agents, in nanotechnology as precursors of semiconductor films and nanoparticles [5].

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Metal complexes are gaining more and more importance, especially in the design of drugs in coordination with metal. This has led to numerous studies on metal drug complexes that have been shown to be effective in the treatment of various diseases caused by viruses as well as in the treatment of cancer.

The coordination of FDA-approved drug of clinically verified drugs with metal ions can form a drug-metal complex (DMC), which can further grow into a drug-metal multiple-complex (DMMC) and drug-metal nano-aggregate (DMNA). As a next-generation drug, repositioning these materials can provide unique and enhanced biological activity and a high possibility for lab-to-market application [6].

Metal complexes of antibiotics have shown promising antimicrobial activities against some resistant strains of bacteria by inhibiting the growth or killing the microorganisms. This phenomenon has a significant impact when pathogenic microorganisms develop continuous resistance to the available antibiotics via various cellular defense mechanisms. Therefore, searching for alternative drug molecules is essential to combat antimicrobial resistance microorganisms [7].

Transition metals can be coordinated with organic or biological molecules, creating complexes with altered pharmacological properties. Biometallic complexes with drugs are formed in the interaction of ligands with biometals via O-, N- and S-donor atoms of the corresponding functional groups (-OH, -COOH, -SH, -NH2), thereby improving the stability and overall biological importance of the molecule.

A versatile ligand in numerous compounds is the carboxyl group (RCOOH), which forms complexes of various coordination with its O-donor atoms and can act as a mono and bidentate ligand [8].

Cobalt is an indispensable trace element for the human body, and it can be found in both organic and inorganic forms. The organic form is a vital component of vitamin B12, serving a pivotal role in the synthesis of amino acids and specific proteins within the nerve cells, as well as in the production of neurotransmitters essential for the proper functioning of the body [9]. Vitamin B12 is a cofactor in DNA synthesis and the metabolism of fatty acids and amino acids [10]. Numerous studies have shown that cobalt complexes are of biological importance, and most structurally characterized ones show antiviral, antibacterial [11], antimicrobial [12], and antifungal [13] effects.

2. Materials and apparatus

2.1. Chemicals

In this study, the following chemicals were used for experimental work: 2-acetoxy-benzene carboxylic acid (acetyl-salicylic acid-ASA) (Thermoscientific), cobalt chloride hexahydrate (Semikem), Ethanol (Merck) and demineralized water. All used chemicals are of analytical grade.

2.2. Methods

2.2.1. Preparation of metal complex of 2-acetoxy benzene carboxylic acid

The complex was prepared by adding an aqueous metal chloride (0.01 mol) to an ethanolic solution of the ligand, 2-acetoxy benzene carboxylic acid (3.604 g, 0.02 mol). The mixture is refluxed in the flask with constant stirring for 3 hours at temperatures up to 60°C. A complex precipitate crystallizes from the solution, which is filtered off and dried for several days in a desiccator.

2.2.2. Methods of analysis of experimental samples

In this study, the following methods were used for the analysis and characterization of the obtained complex of 2-acetoxy benzene carboxylic acid with Co(II)-ion:

- Fourier transformation of infrared spectroscopy. Infrared spectroscopy is of great importance in organic analysis because it enables the detection of functional groups, examination of the type of chemical bonds, identification of the organic compound as a whole, monitoring of chemical reactions, and kinetics of chemical processes. Infrared spectra of experimental samples of 2-acetoxy benzene carboxylic acid were recorded on a Thermo Scientific Nicolet IS10 spectrophotometer at a resolution of 2 cm^{-1} and a range of 4000-400 cm^{-1}.
- UV/VIS spectrophotometric analysis. By applying this instrumental method, the 2-acetoxy benzene carboxylic acid complex with Co (II) ion was characterized, and the UV/VIS spectrophotometer Perkin Elmer Lambda 25 was used for the analysis. The absorbance was measured at a wavelength of 226 nm.
- Mass spectrometric analysis- The samples were analyzed using the high-resolution liquid chromatography method with a mass spectrometer, LC-MS/MS. Mass spectra were recorded using a system consisting of a system for direct sample introduction, HPLC Agilent 1200 Series with DAD detector, mass spectrometer with triple quadrupole Agilent Technologies 6420 Triple Quadrupole, and with electrospray ionization in positive and negative recording mode. The obtained samples were dissolved in methanol to a concentration of about 100 μg/mL and injected into the system with 50% methanol elution.
- Optical microscopy- Microscopic examinations

with a polarizing binocular microscope DM 2500P, brand Leica, at crossed nicols. Samples of 2-acetoxy benzene carboxylic acid metal complexes were treated with DMSO and left overnight at room temperature to crystallize. The 2-acetoxy benzene carboxylic acid complexes were transferred to a glass slide with tweezers, and microphotographs were then taken. Magnifications used: eyepiece 10x / 20, objectives 4x / 0.10 and 10x / 25. A microscope bulb's "white" light was used as a light source.

- Melting point- An electrical device determines the melting temperature for metal complexes of 2-acetoxybenzene carboxylic acid, A.KRUSS Automatic Melting Point Meter – Semi-auto Version, for gradual heating, which contains a microscope that monitors the melting of crystals were performed in transmitted polarized light,

2.2.3. Evaluation of antibacterial activity

Screening of antibacterial activity for complex samples was performed by well diffusion assay against six different bacterial strains and *Candida albicans*. For this method reference bacterial strains were used: *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Salmonella* Enteritidis *and Pseudomonas aeruginosa*. The concentration of the tested complex samples was 0,1 mg/mL , DMSO as inert substance was used for complex dilutions.

Suspensions of 0.5 McFarland turbidity was made from overnight bacterial cultures. From the suspension, microorganisms were inoculated on the Mueller-Hinton agar plates (thickness of agar plates was 4 mm). Five wells (8 mm in diameter) were cut into the agar media with a sterilized cork borer. 100 μL of each complex was sterilely applied into wells. Agar plates were left at room temperature for 15 minutes, allowing the substance to diffuse into the agar. Plates were then incubated at 37°C/24 h. After the incubation period, the inhibitory zone for each strain and substance was measured.

2. Results and Discussion

Characteristic physical and analytical properties and significant IR spectral bands of the tested complex of 2-acetoxy benzene carboxylic acid with Co (II)- ion are presented in Table 1.

Table 1. Characteristic physical and analytical properties of the 2-acetoxy benzene carboxylic acid complex with Co (II)- ion.

Compound name	**2-acetoxy benzene carboxylic acid**	**$Co(Asp)_2Cl_2$**
Yield (%)	-	66,52
Compound color	white	purple-pink
Melting Point (°C)	138-140	119,2
IR (cm^{-1})	3411 (-OH); 1749 (C=O); 1679 (C=O); 1181 (C-O);	3230 (-OH); 1750 (C=O);1681 (C=O); 1293,1186 (C-O);754 (M-O);
λ_{max} (cm^{-1})	226	228

Figure 2. Synthesized complex of 2-acetoxy benzene carboxylic acid with Co (II) ion

The synthesized complex of 2-acetoxy benzene carboxylic acid with Co (II)- ion is characteristically colored (Fig. 2), probably due to charge transfer from the ligand to the metal and vice versa [14]. The value of the synthesized complex's melting point is lower than pure 2-acetoxy benzene carboxylic acid and is 119.2°C.

Analysis of the IR spectrum of the sample of 2-acetoxy benzene carboxylic acid with Co (II)-ion confirms the formation of the complex. The spectrum of pure 2-acetoxy benzene carboxylic acid shows an absorption band at 3411 cm^{-1} attributed to the –OH group. On the spectrum of the metal complex 2-acetoxy benzene carboxylic acid, the absorption bands originating from the –OH group are located at 3230 cm^{-1}. This hypsochromic shift of the stretching vibrational bands provides evidence that this group is one of the coordination sites of 2-acetoxy benzene carboxylic acid. In the spectrum of the metal complex, there is a bathochromic shift in the bands present in the spectrum of pure 2-acetoxy benzene carboxylic acid at 1749 cm^{-1} and 1679.0 cm^{-1}, which originate from the C=O group of the ester or carboxylic acid, so it can be concluded that C =O group also coordination site of 2-acetoxy benzene carboxylic acid [15].

Fig.3 shows the assumed structure of the complex Co(II)-2-acetoxy benzene carboxylic acid, where it can be seen that 2-acetoxy benzene carboxylic acid coordinates as a bidentate ligand through the carbonyl oxygen of the carboxyl and ester groups.

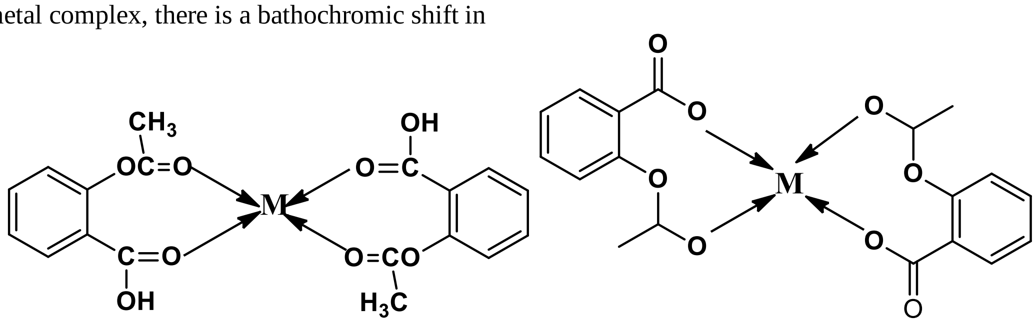

Figure 3. Chemical structure of metal complexes of 2-acetoxy benzene carboxylic acid

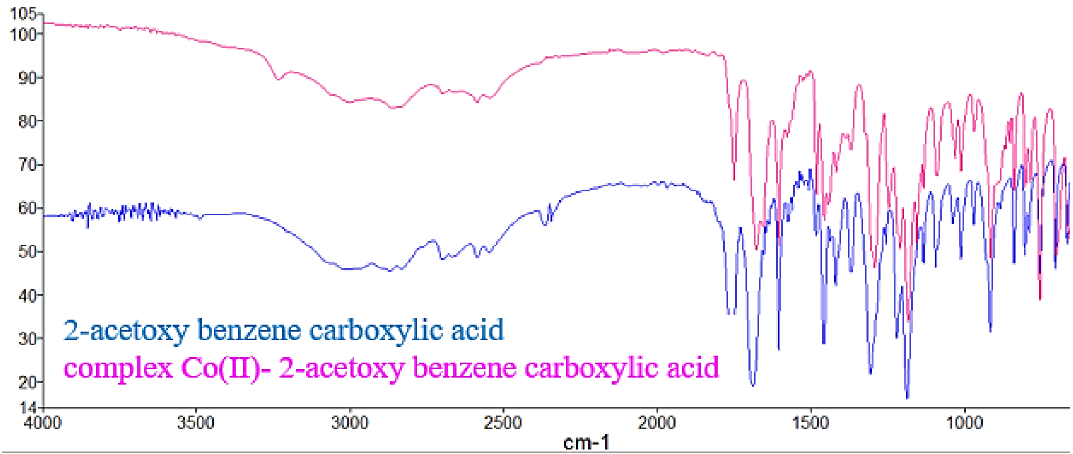

Figure 4. IR spectrum of 2-acetoxy benzene carboxylic acid with Co (II)- ion

The strong absorption bands at 1293 cm^{-1} in the spectra were attributed to the C-O stretching vibration of the carboxylic acid, while the intermediate bands located at 1181 cm^{-1} were attributed to the C-O stretching vibrations of the ester. The spectrum of the metal complex shows strong absorption bands in the range of 650-750 cm^{-1}, which are attributed to M-OH and M-O=C stretching bands of the metal complex, confirming the coordination through the oxygen of the acid group in the spectrum of the complex Co (II)-2-acetoxy benzene carboxylic acid [16] (Fig. 4)

The formation of the metal complex Co(II)-2-acetoxy benzene carboxylic acid was also confirmed by UV/VIS spectroscopy. The absorption maximum of pure 2-acetoxy benzene carboxylic acid occurs at 226 nm.

By comparing the spectrum of pure 2-acetoxy benzene carboxylic acid with the spectrum of the metal complex sample, differences are visible, which indicates the coordinative bond of the metal and 2-acetoxy benzene carboxylic acid as an O-donor ligand.

The spectrum of the metal complex (Fig. 5) shows an absorption maximum at 228 nm, that is, shifted towards longer wavelengths, corresponding to a bathochromic or red shift.

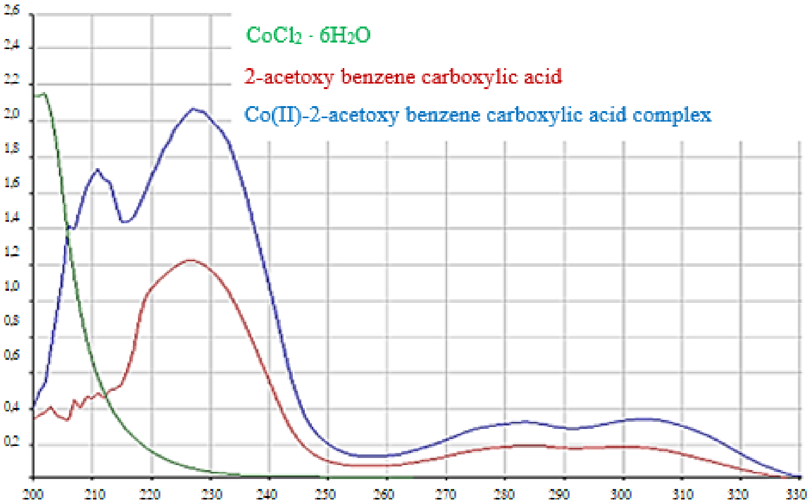

Figure 5. The absorption spectrum of 2-acetoxy benzene carboxylic acid with Co (II)- ion

The mass spectroscopy method was used to analyze and confirm the obtained metal complexes of 2-acetoxy benzene carboxylic acid. Fig 6. shows the mass spectrum of the resulting complex Co(II)-2-acetoxy benzene carboxylic acid in positive ionization. The spectrum of the resulting complex shows a peak of low intensity at m/z= 598, which corresponds to the molecular mass of the resulting complex Co(II)-2-acetoxy benzene carboxylic acid in a ratio of 2:1 in favor of the 2-acetoxy benzene carboxylic acid molecule. There are peaks at m/z= 575 and m/z= 450 that arise from the fragmented ion of the resulting complex. By extracting one molecule of 2-acetoxy benzene carboxylic acid, a peak at m/z = 418 is obtained. As in the mass spectrum obtained for the Cu(II)-2-acetoxy benzene carboxylic acid complex, there are peaks at m/z = 221 and m/z = 203, which are assumed to originate from the present impurities $(ASA + Na)^+$ and $(ASA + K)^+$. The peaks formed at m/z = 171, m/z = 153, and m/z = 139 are probably molecular ions formed due to the fragmentation of 2-acetoxy benzene carboxylic acid. Low-intensity peaks at m/z = 655, m/z = 687, and m/z = 833 probably arise during the electrospray ionization.

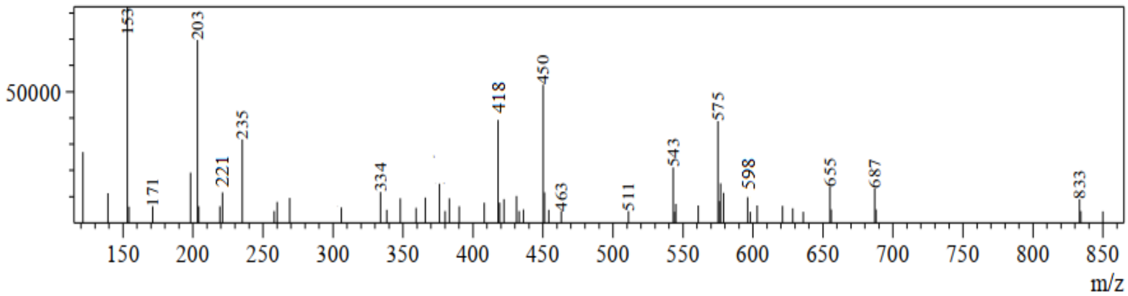

Figure 6. Mass spectrum of the Co(II)2-acetoxy benzene carboxylic acid complex in positive ionization

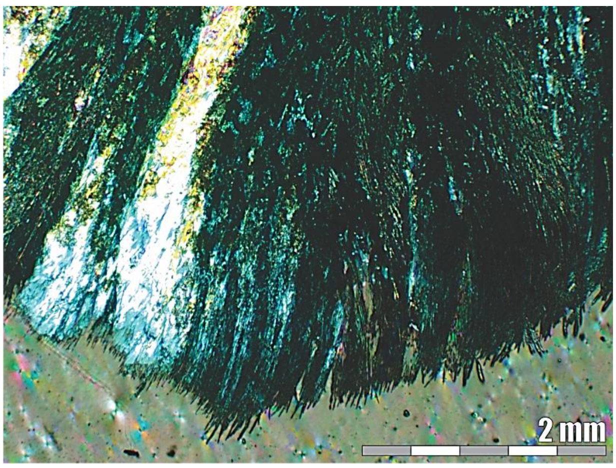

Figure 7. Morphological appearance of the 2-acetoxy benzene carboxylic acid complex with $CoCl_2$

Studies on the growth of aromatic carboxylic acid crystals and morphological control have significant pharmaceutical importance, and many of these compounds are used as analgesics and anti-inflammatory drugs. The morphology and crystal structure of the Co(II)-2-acetoxy benzene carboxylic acid complex is shown in the Figure. Crystals of the 2-acetoxy benzene carboxylic acid complex with cobalt (II) chloride (Fig. 7) appear in radial-radial forms, that is, as articulated-pinnate. The crystal size ranges from 3 to 6 mm.

The results of testing the antibacterial and antimicrobial activity of the synthesized complex Co(II)-2-acetoxy benzene carboxylic acid are shown in Table 2.

Table 2. Result of antibacterial and antifungal test.

Reference strain	**Reference number**	**Inhibition zones and sensitivity of analyzed Co(II)2-acetoxy benzene carboxylic acid complex***
Bacillus subtilis	WDCM00003	15
Candida albicans	WDCM00054	13
E. coli	WDCM00012	-
Pseudomonas aeruginosa	WDCM00025	12
Listeria monocytogenes	WDCM00109	14
Staphylococcus aureus	WDCM00034	12
Salmonella Enteritidis	WDCM00030	11

*The inhibition zones were reported in millimeters (mm)

Research has shown that *E. coli* strains did not show any inhibition by the diffusion method in agar wells. In contrast, *S. Enteritidis* and *Pseudomonas aeruginosa* showed a mild inhibitory effect for the examined complex. Co(II) complex of 2-acetoxy benzene carboxylic acid was more effective against *Listeria monocytogenes* and *Candida albicans*. *Bacillus subtilis* is the strain with the highest sensitivity to the tested complex and an inhibition zone of 15 mm.

3. Conclusion

Structural characterization was performed Using FTIR, MS, UV/VIS, and Optical Spectroscopy, and the formation of a complex between 2-acetoxy benzene carboxylic acid molecules and Co (II) ions was proven. The obtained complexes of 2-acetoxy benzene carboxylic acid with metal are colored, resulting from charge transfer from the ligand to the metal and vice versa.

The structural characterization of the investigated metal complexes of 2-acetoxy benzene carboxylic acid indicates that 2-acetoxy benzene carboxylic acid coordinates as a bidentate ligand through the carbonyl oxygen of the carboxyl and ester groups. The 2-acetoxy benzene carboxylic acid complex with Co(II) ions shows a weak antibacterial effect against different bacterial strains.

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