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SYNTHESIS, STUDY OF THE STRUCTURE AND ACUTE TOXICITY OF THE COORDINATING COMPOUNDS OF Zn (II) WITH SUCCINIC AND PANTOTENIC ACIDS

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Abstract

There has been synthesized a new coordination compound Zn (II) with succinic and pantothenic acids. There have been studied elemental composition and some physicochemical properties of obtained complex. To establish purity and individuality, X-ray radiograph of the starting materials and the complex were obtained. The X-ray radiograph of the ligands differ sharply from those of the synthesized complex, which confirms its individuality and purity. It was found by IR spectroscopy, diffuse reflectance electronic spectra (DRES), and thermal analysis that succinic and homopantothenic acids are coordinated to the metal in a bidentate manner in a deprotonated form. The pharmaco-toxicological study of the complex compound showed that it belongs to low-toxic substances.

Keywords: *succinic acid, pantothenic acid, IR spectroscopy, X-ray phase and derivatographic analysis*

Introduction

It is known that chronic deficiency of the microelement of zinc leads to immunodeficiency. Zinc is an effective immune stimulant. Zinc is present in all internal organs, tissues, fluids, and many of the body's enzymatic systems. Zinc acts as a "secondary mediator" of immune cells and significantly shortens the period of inflammation. When a person catches a cold, a large amount of zinc is consumed in the first hours, and the need for it increases 6-8 times [1, 2]. Zinc has anti-inflammatory, antioxidant and antibacterial properties. Zinc reduces susceptibility to acute lower respiratory tract infections. Zinc ions stimulate the production of endogenous α- and γ-interferons, which also have antiviral effects, including the COVID-19 coronavirus.

Organic succinic acid is a component of every cell and is a powerful source of energy. It is also an antioxidant and protects the human body from toxins and free radicals.

Succinic acid protects cells from damage by toxins, improves microcirculation in tissues, stimulates protein synthesis, improves metabolism in the skin, increases immunity, eliminates swelling of soft tissues, improves skin tone, makes tissues more elastic, narrows pores, reduces pigmentation, regulates sebum production.

There are noted that with a lack of succinic acid, general health worsens, brain activity and performance decrease, chronic fatigue and memory loss. A decrease in immunity leads to frequent respiratory diseases, even a slight hypothermia or a minute contact with a sick person can provoke infection with an acute viral infection.

The main marker of succinic acid deficiency is skin condition. It loses its elasticity, dark circles and swelling appear under the eyes, the pores expand, and acne appears. Soft tissues become flabby, wrinkles are clearly visible [3].

Pantothenic acid gets its name from the Greek "pantothene", which means "everywhere", due to its extremely wide distribution. Vitamin B_5, entering the body, is converted into pantethine, which is part of the coenzyme - KoA, which plays an important role in the processes of oxidation and acetylation. Coenzyme A (KoA) is one of the few substances in the body involved in the metabolism of proteins, fats and carbohydrates.

Pantothenic acid is required for the metabolism of fats, carbohydrates, amino acids, the synthesis of vital fatty acids, cholesterol, histamine, acetylcholine, and hemoglobin.

Lack of pantothenic acid in the body leads to metabolic disorders, on the basis of which dermatitis, depigmentation and loss of hair, wool or feathers, cessation of growth, exhaustion, changes in the adrenal glands and nervous system, as well as disorders of coordination of movements, functions of the heart and kidneys, and stomach develop, intestines.

With a lack of pantothenic acid in the body, an excess of hydrochloric acid is formed in the stomach, which can cause the development of ulcerative and erosive diseases of the gastrointestinal tract [4].

Based on the foregoing, we have carried out a targeted synthesis of the coordination compound Zn (II), which has low toxicity and high biological activity with succinic and pantothenic acids.

Methods

Initial substances for the synthesis of complex compounds were used zinc nitrate salt, sodium hydroxide, succinic acid (SA), grade "clean" and calcium pantothenate (PTT) "pharmacopoeial" grade.

The analysis of the isolated compound for zinc content was carried out complexometric [5]. There was determined nitrogen by the Dumas micro-method [6], and the water content was determined gravimetrically. The melting point of the complex compound was determined in closed capillaries.

The individuality of the isolated complex was studied by comparing the X-ray radiograph of the starting substances and the complex compound, which were obtained on a Dron-UM-1 diffractometer with a Cu anticathode. IR spectra were recorded on an IRTracer-100 Fourier spectrometer (SHIMADZU CORP., Japan) complete with attachment frustrated total internal reflection (FTIR) MIRacle-10 with attachment a diamond / ZnSe prism (the spectral range on the wavenumber scale is 4000 ÷ 400 cm^{-1}; resolution - 4 cm^{-1}, sensitivity, signal/noise ratio - 60,000:1; scanning speed - 20 spectra per second). Thermal research was carried out on a derivatograph system F. Paulik, J. Paulik, L. Erdey firm "MOM" (Hungary).

There was studied acute toxicity of the complex compound on 30 white mice, weighing 19-21g, of mixed sex. An aqueous solution was prepared from the Zn(SA-2H)(PTT)·H_2O complex compound and intragastrically administered once to mice at doses of 500 mg / kg, 750 mg / kg, 1000 mg / kg, 1250 mg / kg, and 1500 mg / kg , which in volumes is 0,15 – 0,35 ml [7].

The animals were under continuous observation during the first hour, then under hourly observation during the first day of the experiment and once a day in the next 13 days of the experiment. As indicators of the functional state of animals, the general condition of the mice and their behavior, the intensity and nature of motor activity, the presence of seizures, coordination of movements, reaction to external stimuli and tone of skeletal muscles, appetite, body weight, the number and consistency of fecal masses were taken into account. During the experiment, the clinical state of the animals was monitored: the presence/absence of signs of poisoning, the time of their appearance, the death of mice.

All experimental animals were kept in standard conditions, on a common diet with free access to water and food [5]. After the end of the experiment, the average lethal doses (LD50) were determined [6].

Results

The synthesis was carried out according to the following procedure: a solution of 0.006 mol of sodium sulfate in 5 ml of water was added to a solution of 0.006 mol calcium salt of pantothenic acid in 10 ml water. The mixture was stirred for 2 hours. This formed a precipitate of calcium sulfate, which was filtered off. To the resulting uterine solution with stirring was added dropwise 0.006 mol of succinic acid and the nitrate salt of the metal dissolved in 5 ml water. This gave a colorless transparent solution. The resulting solution was evaporated to ¼ part of the initial volume, and precipitated with a fivefold amount of acetone. The formed precipitate was separated, washed with acetone and ether.

The reaction scheme is as follows:

a) $Ca(PTT-H)_2 + Na_2SO_4 \rightarrow 2Na(PTT-H) + CaSO_4\downarrow$

б) $Na(PTT-H) + Zn(NO_3)_2 + SA + H_2O \rightarrow Zn(SA-2H)(PTT)\cdot H_2O + NaNO_3 + HNO_3$

The X-ray radiograph of the ligands differ sharply from the current synthesized complex, which confirms its individuality and purity (Figure 1).

There were also studied the composition of the isolated compound was established by elemental analysis and some of its physicochemical properties (Table 1, 2).

Discussion

To establish the method of coordination of succinic and pantothenic acids, as well as, to a certain extent, the structure of the synthesized complex compounds, their IR absorption spectra were studied. Figure 2 shows the IR spectra of ligands and their complex compounds with zinc, and the frequencies (cm^{-1}) of some absorption bands in them are in Table 3.

A group of small bands was found in the IR spectrum of succinic acid. The highest frequency of them is assigned to υ (OH), and the remaining bands at 2925, 2629 and 2532 cm^{-1} to $υ_{as}$ (CH_2). The intense band at 1684 cm^{-1} is attributed to υ (C = O), and the band at 1306 cm^{-1} to mainly δ (OH) and the band at 1198 cm^{-1} to υ (C-O). These bands are characteristic of acid dimers. The bands at 1410 and 1175 cm^{-1} are responsible for scissor and fan vibrations of CH_2 (Figure 2).

The IR spectrum of pantothenic acid also contains bands characteristic of carboxylic acids: broad absorption at 2500-3000 cm-1, an intense band at 1720 cm-1 and bands at 1410, 1255 cm-1 (Fig. 3.3.1.1). The bands characteristic of secondary amides υ (NH), "amide-I, II, III", appear, as expected, in the region: 3100, 1646, 1545 and 1290 cm-1. The υ (C-O) bands of the primary and secondary alcohol groups were found at 1043 and 1082 cm-1, respectively. The υ (CH) bands and bending vibrations of the methyl and methylene groups appear at 2965, 2935, 2880, 1468, 1445, 1373, 1230, 1197, 1023 cm-1. Of these, the bands at 1230 and 1197 cm-1 are characteristic of the gem-dimethyl group.

When passing from the spectra of ligands to the spectrum of the mixed-ligand zinc complex, the bands of the carboxyl group disappear and two intense bands of the carboxylate group appear at

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1541 and 1388 cm^{-1}, assigned to $\upsilon_{as}(COO)$ and $\upsilon_s(COO)$, respectively (Table 3). The "amide-I" band undergoes a low-frequency shift by 63cm^{-1}, and the "amide-II and III" bands undergo a high-frequency shift by 3cm^{-1} and 58cm^{-1}, respectively. This is probably due to the migration of the cleaved proton of the carboxyl group to the nitrogen of the secondary amide, which is confirmed by the appearance of the $\upsilon(\overset{+}{N}H_2)$ bands in the spectra of the complexes. This, of course, excludes the participation of the nitrogen atom of the secondary amide in coordination with the metal ion. Consequently, in the considered mixed-ligand complex, pantothenic acid is coordinated to the metal with the participation of the oxygen of the carboxylato group. Taking into account the revealed great tendency of succinic acid to coordinate tetradentate in the doubly deprotonated state, it can be assumed that in this case there is also bidentate coordination of the carboxylato group of the ligands to the metal.

Metal environment in the compound is in good agreement with the presence in its IR spectrum of the υ (Zn-O) band at 436 cm^{-1}.

On the derivatogram DTA curve Zn(SA-2H)(PTT)·H_2O, several endothermic effects are noted at 125, 202, 392, 490, 580° C and a multitude of exothermic effects. The appearance of the first endothermic effect is consistent with the removal of water molecules.

Based on the above, the considered complex can be attributed to the following polymer structure (figure 4).

Experiments on the study of acute toxicity showed that after a single intragastric administration of a complex compound at a dose of 500 mg/kg, the general condition of the mice was stable and no visible changes were observed in their behavior. Coordination of movements and tone of skeletal muscles - normal, no seizures were observed, reaction to tactile, pain, sound and light stimuli - pronounced, condition of hair and skin - good, no changes, color of mucous membranes and pupil size - without deviations from the norm, appetite - pronounced, water consumption did not increase, body weight was unchanged, the amount and consistency of fecal masses were normal. There were no observed the death of mice within 14 days.

With the introduction of the complex compound Zn(SA-2H)(PTT)·H_2O at a dose of 750 mg/kg, the mice developed lethargy, lack of mobility, and the death of 1 mice was observed.

When the complex compound was administered at a dose of 1000 mg/kg, the experimental animals experienced respiratory depression, coordination of movements was impaired, and motor activity was reduced. The animals reacted poorly to light, sound, tactical and painful stimuli, food and water consumption decreased significantly. In this group, 3 individuals died.

When a dose of 1250 mg/kg was administered, the animals assumed a lateral position, did not respond to external stimuli, and a rapid heart rate was observed. In this group, 4 individuals died.

The administration of a dose of 1500 mg/kg caused the total death of the animals immediately after the administration of the complex compound.

In the following days, observation of the animals showed that as the signs of intoxication decreased, the state of the surviving animals returned to normal by the end of the experiment. Animals do not refuse food and water, react to external stimuli, diuresis, consistency and amount of fecal matter are normal.

LD_{50} of the Zn complex compound Zn(SA-2H)(PTT)·H_2O was 935.1 (584.7 ÷ 1293.4) mg/kg.

The results of the experiment are shown in table 4.

Succinic and pantothenic acids are well compatible in the coordination sphere of Zn (II). In the complex under consideration, pantothenic acid is coordinated to zinc with the participation of the oxygen of the carboxylato group. Taking into account the composition of the Zn (II) complex, as well as the revealed high tendency of succinic acid to coordinate tetradentate in the doubly deprotonated state, it can be assumed that in this case there is bidentate coordination of the carboxylato group of the ligands to the metal. According to the derivatographic study of the compound Zn(SA-2H)(PTT)·H_2O, the water molecules in it are removed with one endothermic effect and are outer-sphere. The data obtained show that the LD_{50} of the complex compound was 935.1 (584.7 ÷ 1293.4) mg / kg and belongs to low-toxic substances.

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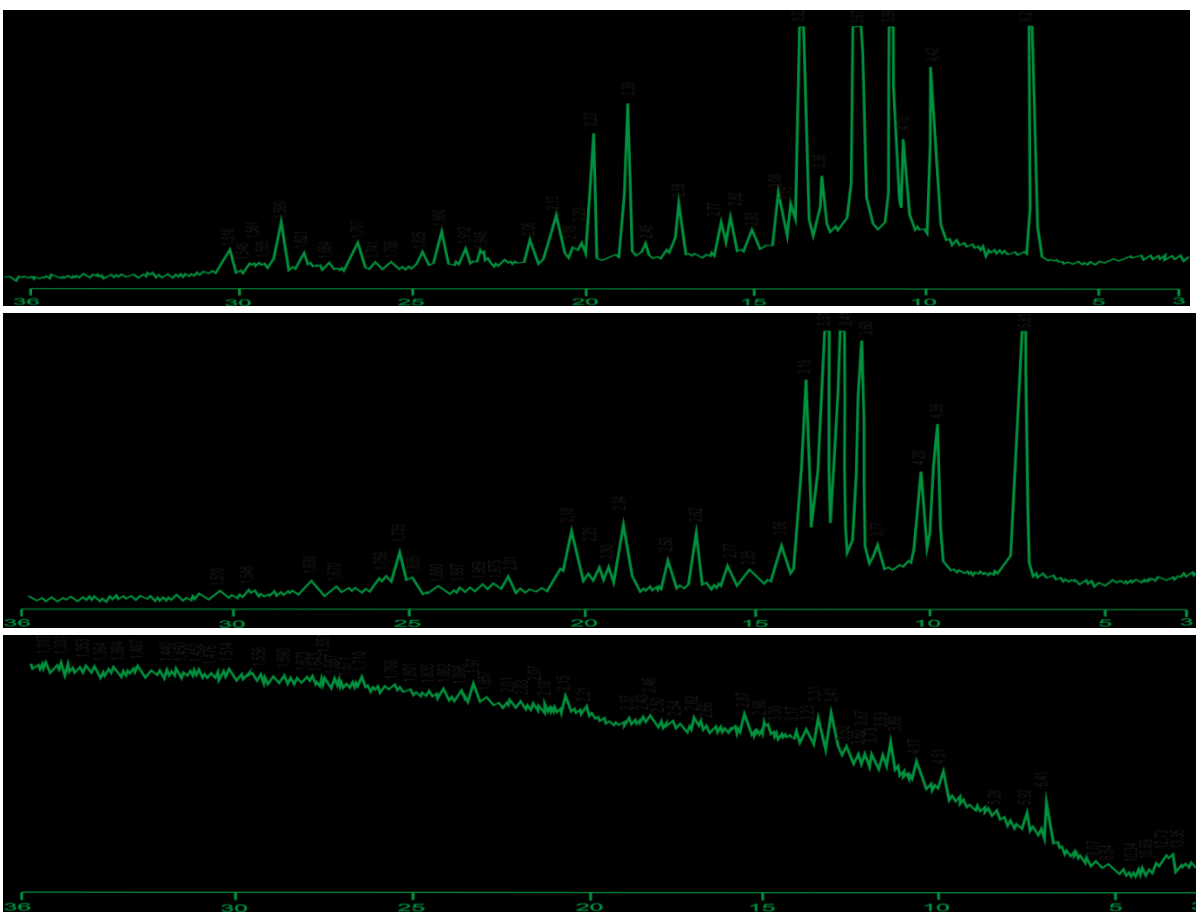
Compound	Found, %			Calculated, %		
	M	N	H_2O	M	N	H_2O
Zn(SA-2H)(PTT)·H_2O	14,97	3,20	10,30	14,67	3,14	10,09

Table 1. Elemental composition of the isolated complex compound

Compound	Colour	Temp, °C	Solubility, g/100g water
Zn(SA-2H)(PTT)·H_2O	white	152	10,30

Table 2. Some physicochemical properties of the isolated complex compound



a b c

Figure 1. X-ray radiograph of PTT (a), SA (b), Zn(SA-2H)(PTT)·H_2O (c)

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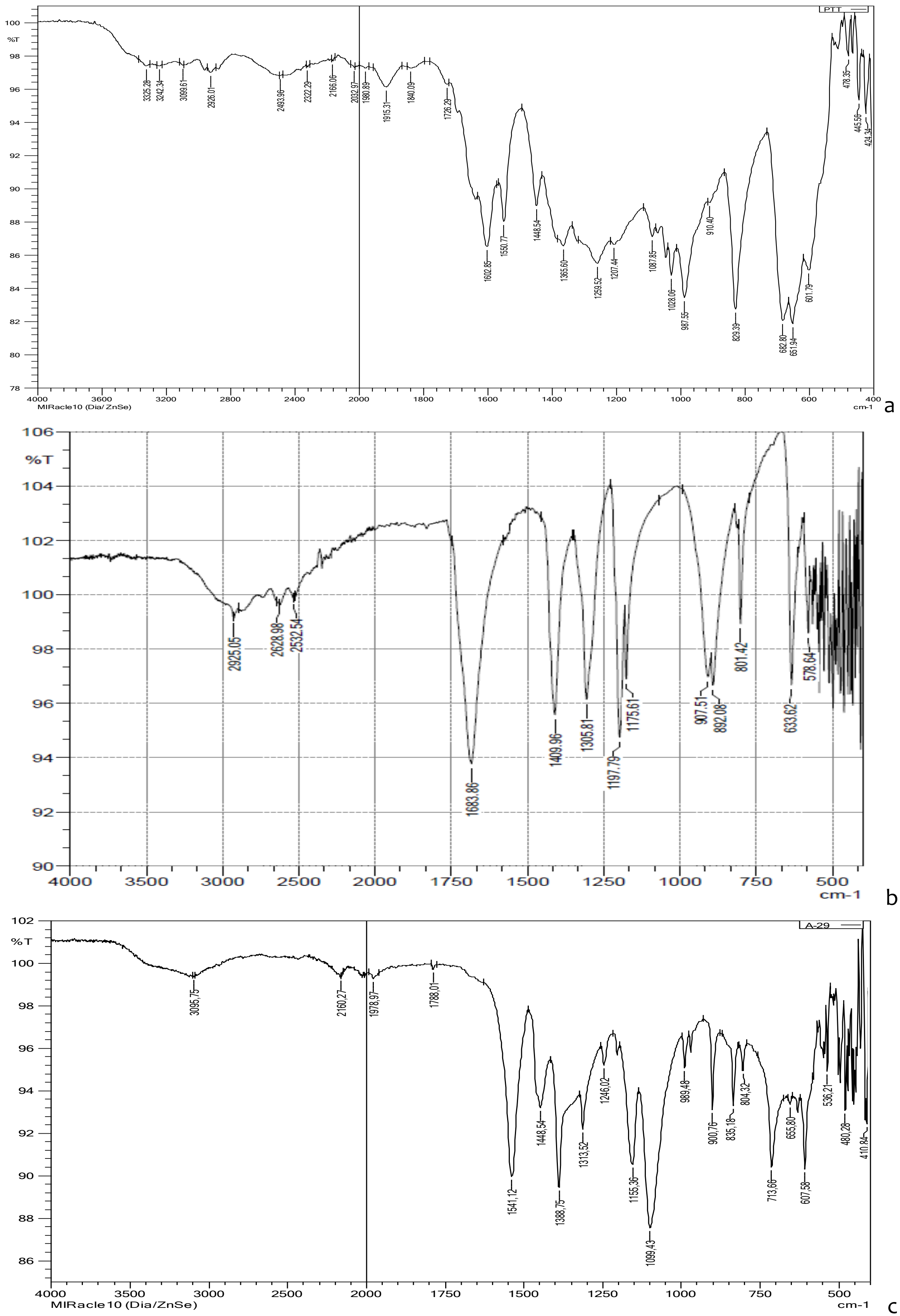

Figure 2. IR spectra PTT (a), SA(b), Zn(SA-2H)(PTT)·H_2O (c)

Compound	υ(C=O), υ(COO)	$\upsilon(\overset{+}{N}H_2)$	"Amide - I"(υ(C=O))	" Amide - II"(δ(NH)+ υ(CN))	" Amide - III"(υ(CN)+δ(NH))	υ(M-O)
SA	1684	-	-	-	-	-
PTT	1712	-	1633	1537	1255	-
Zn(SA-2H)(PTT)·H_2O	1541, 1388	3320, 3097	1550	Per.	1313	410

Table 3. Frequencies (cm-1) of some absorption bands of the IR spectra of succinic, pantothenic acids and their mixed-ligand complex with zinc

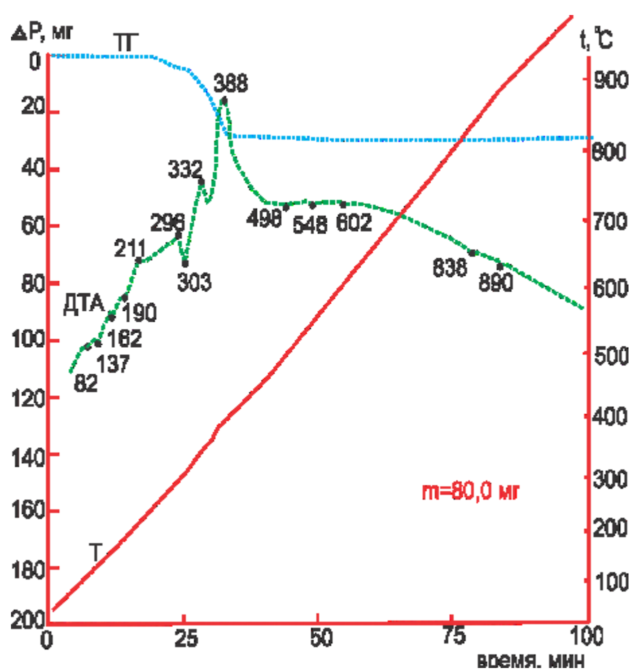

Figure 3. Derivatogram Zn(SA-2H)(PTT)·H_2O

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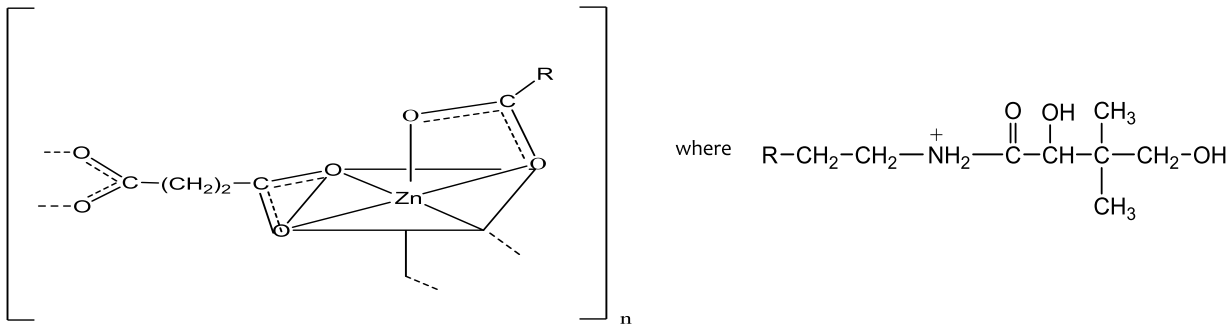
R
C
O
O
O
C
(CH2)2
C
O
O
Zn
O
n
where
R–CH2–CH2–NH2–C–CH–C–CH2–OH
+
O
OH
CH3
CH3

Figure 4. Structure of coordination compound Zn(SA-2H)(PTT)·H_2O

№ live	weight, gr	dose mg / kg	route of administration	lethal excretion
1	20			No
2	19			No
3	21			No
4	19	500	survived	No
5	19			No
6	20			No
1	19			No
2	19			No
3	20		survived	death
4	19	750		No
5	20			No
6	19			No
1	21			No
2	20			No
3	19	1000	survived	death
4	19			No
5	21			death
6	20			death
1	21			death
2	21			No
3	20			No
4	19	1250	survived	death
5	21			death
6	20			death
1	20			death
2	19			death
3	20			death
4	20	1500	survived	death
5	19			death
6	20			death
LD_{50}		935,1 (584,7÷1293,4) mg / kg		

Table 4. Determination of acute toxicity (LD50) of a complex compound

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