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THERMAL, SPECTRAL AND BIOLOGICAL PROPERTIES OF Zn(II) COMPLEX COMPOUNDS WITH PHENAZONE

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The thermal decomposition of the complexes $Zn(form)_2 \cdot 2phen$ **(I)**, $Zn(ac)_2 \cdot 2phen$ **(II)**, $Zn(prop)_2 \cdot 2phen$ **(III)**, $Zn(but)_2 \cdot 2phen$ **(IV)**, where phen=phenazone, form=formiate, ac=acetate, prop=propionate, but=butyrate has been studied in air by TG/DTG and DTA methods. The possible mechanism of the thermal decomposition was proposed. The final product of thermal decomposition was ZnO. IR data show unidentate coordination of carboxylate group to Zn(II) ion. The complexes were tested against various strains of microorganisms and their efficiency decrease in the sequence yeasts>bacteria>filamentous fungi.

Keywords: *biological properties, IR spectra, phenazone, thermal properties, zinc(II) complexes*

Introduction

Carboxylatometal(II) complexes with N-donor ligands have been interesting from chemical and biological aspects for last decades. It is of interest to study interactions between metal ions and heterocyclic nitrogen compounds that are present in living systems and are used as medicaments. It is also known that heterocyclic compounds play significant role in biological systems that are a part of some vitamins and drugs [1].

The interaction of Zn(II) atom that has a key role in many biological processes is a subject of considerable interest. Zinc forms more than 300 metalloenzymes and its complexes have antimicrobial effect against bacteria, fungi and viruses [2, 3]. Zinc(II) complexes are interesting from chemical viewpoint and biological activity.

In recent years we have studied synthesis, spectral, thermal, structural, chromatographic and biological properties of several zinc(II) aliphatic [4–6] and aromatic carboxylates and their halogenoderivatives [7–9]. This paper describes the preparation of the zinc(II) complexes with phenazone and the study of their thermal, spectral and biological properties.

Experimental

The following chemicals of A. R. grade were used in the synthesis of the prepared complexes: HCOOH 98% (Lachema), CH_3COOH 98% (Lachema), CH_3CH_2COOH 99% (Lachema), $CH_3CH_2CH_2COOH$ 98% (Lachema), $ZnCO_3$ (Lachema), phenazone (Aldrich). Zinc carboxylates were prepared from zinc carbonate and appropriate carboxylic acid in molar ratio 1:2.

Syntheses

$Zn(form)_2 \cdot 2phen$ **(I)**

A water solution of 0.31 g zinc(II) formiate (0.002 mol) was added to a water solution of 0.753 g phenazone (0.004 mol) in molar ratio 1:2. The reaction mixture was stirred together during 30 min. The prepared complex was filtered off and reduced in volume in waterbath at 70°C. In a few days the white powder precipitated. Anal. calcd. for compound **(I)**: C 54.16; H 4.93; N 10.54; Zn 12.29%. Found: C 54.30; H 4.90; N 10.40; Zn 10.90%.

$Zn(ac)_2 \cdot 2phen$ **(II)**

The reaction mixture of a water solution containing 0.36 g (0.002 mol) zinc(II) acetate (0.002 mol) and 0.753 g phenazone (0.004 mol) was under continual

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stirring for 30 min. The mixture was filtered off and reduced in volume in waterbath at 80°C. In several days a white powder precipitated. Anal. calcd. for compound **(II)**: C 32.12; H 3.20; N 5.02; Zn 11.67%. Found: C 32.08; H 3.19; N 5.12; Zn 11.56%.

$Zn(prop)_2 \cdot 2phen$ **(III)**

A water solution of 0.753 g phenazone (0.004 mol) was added to a water solution of 0.423 g zinc(II) propionate (0.002 mol). The reaction mixture was stirred together for 30 min. Then it was filtered off and in waterbath. In several days white powder precipitated. Anal. calcd.: C 57.21; H 5.83; N 14.31; Zn 11.12%. Found: C 55.35; H 5.46; N 14.40; Zn 10.87%.

$Zn(but)_2 \cdot 2phen$ **(IV)**

The reaction mixture of a water solution containing 0.479 g zinc(II) butyrate (0.002 mol) and 0.753 g phenazone (0.004 mol) was under continual stirring for 30 min. The mixture was filtered off and reduced in volume in waterbath at 70°C. In several days a white powder precipitated. Anal. calcd. for compound **(IV)**: C 58.51; H 6.22; N 9.10; Zn 10.61%. Found: C 58.29; H 6.40; N 9.25; Zn 9.72%.

Instrumentation

C, H, N analyses were performed using a Perkin Elmer 2400 CHN analyser. Zinc content was determined complexometrically.

TG/DTG and DTA measurements were carried out using a Perkin Elmer DSC7/TGA7 thermoanalyser in air atmosphere, heating rate 10°C min^{-1} and sample mass 10 mg.

IR spectra were recorded on a SPECORD M-80 spectrophotometer in the range 4000–400 cm^{-1} using KBr pellets.

Antimicrobial activity

The antibacterial activity of the prepared Zn(II) compounds was evaluated by a micro-dilution [10] method using G^+ bacteria *Staphylococcus aureus* CCM 3953, G^- bacteria *Escherichia coli* CCM 3988 (both from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic) and by macrodilution method in L-shape tubes using the yeasts *Candida parapsilosis* (purchased from the Laboratory of Medical Mycology, Postgraduate Medical Institute, Bratislava, Slovakia) under vigorous shaking [11]. The efficiency of prepared derivatives on filamentous fungi *Rhizopus oryzae*, *Alternaria alternata*, *Fusarium nivale* (from the Collection of Microorganisms of Department of Biochemistry and Microbiology, Faculty of Chemical and Food Technology STU, Bratislava, Slovakia), *Botrytis cinerea* CCM F-16, *Trichoderma viride* CCM F-534 (both from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic) and *Microsporum gypseum* (from the Laboratory of Medical Mycology, Postgraduate Medical Institute, Bratislava, Slovakia) was observed by macro-dilution technique on solidified broth medium during static culturing [12]. Chromatographically pure compounds were dissolved in dimethylsulfoxide (DMSO); its final concentration never exceeded 1.0 vol% in either control or treated samples. Concentration of tested Zn(II) compounds was in the range of 0.01–3.00 mmol dm^{-3} in all experiments.

The antimicrobial activity was characterized by the IC_{50} values (concentration of a compound which in comparison to the control inhibits the growth of microorganisms to 50%) and MIC values (minimal inhibitory concentration of a derivative which inhibits microbial growth by 100%). The IC_{50} and MIC values were read from toxicity curves. MIC experiments on subculture dishes were used to assess the minimal microbicidal concentration (MMC). Subcultures were prepared separately in Petri dishes containing competent agar medium and incubated at 30°C for 48 h (bacteria, yeasts) and at 25°C for 96 h (filamentous fungi). The MMC value was taken as the lowest concentration, which showed no visible growth of microbial colonies in the subculture dishes.

Results and discussion

Thermal behaviour

$Zn(form)_2 \cdot 2phen$ **(I)**

The TG/DTG and DTA curves of compound **(I)** are given in Fig. 1. The TG curve indicates that it is thermally stable up to 120°C. Above this temperature 2 mol of phenazone are released in an endothermic effect with a minimum on the DTA curve at 280°C. In the next step of thermal decomposition the compound loses formaldehyde and carbone dioxide, accompanied by an endothermic effect at 330°C on the DTA curve. The final product was ZnO. The following mechanism of thermal decomposition was proposed:

$$Zn(HCOO)_2 \cdot 2phen \rightarrow 2phen + CH_2O + CO_2 + ZnO$$

$Zn(ac)_2 \cdot 2phen$ **(II)**

The TG curve of compound **(II)** indicates that the complex is stable up to 160°C as given in Fig. 2. Then two mol of phenazone are released in one step followed by elimination of acetone and carbon dioxide

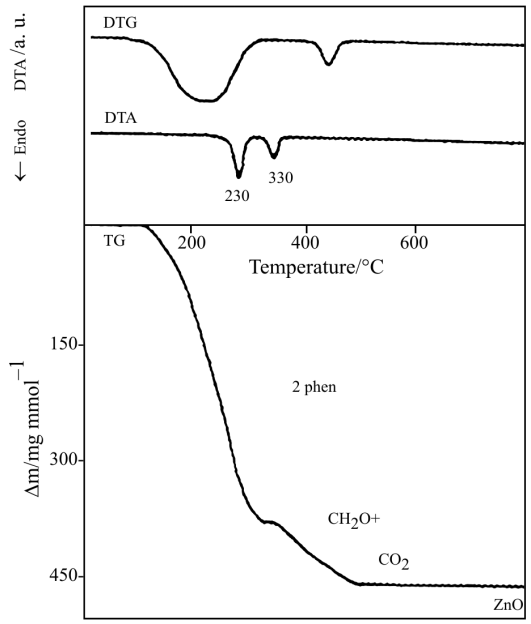

Fig. 1 TG/DTG and DTA curves of $Zn(form)_2 \cdot 2phen$ **(I)**

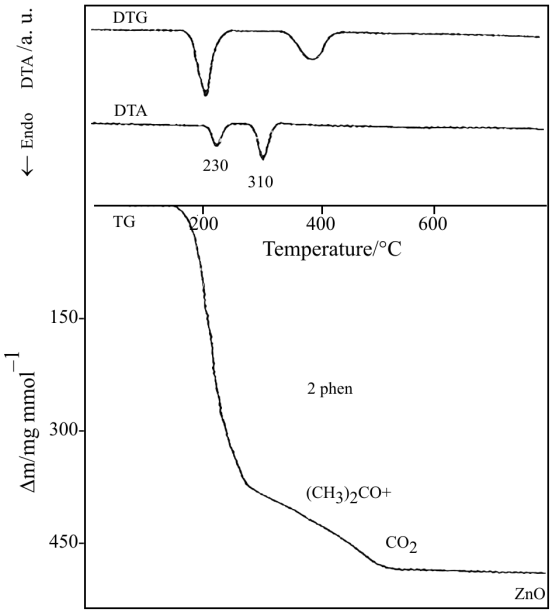

Fig. 2 TG/DTG and DTA curves of $Zn(ac)_2 \cdot 2phen$ **(II)**

in the next step. The final product was ZnO. The most probable scheme of the thermal decomposition is here:

$$Zn(CH_3COO)_2 \cdot 2phen \rightarrow 2phen + (CH_3)_2CO + \\ + CO_2 + ZnO$$

$Zn(prop)_2 \cdot 2phen$ **(III)**

The TG curve displays that compound **(III)** is thermally stable up to 140°C as given in Fig. 3. Above this temperature, the elimination of neutral ligand begins with a minimum on the DTA curve at 220°C in an endothermic effect. In next step, diethyl ketone and carbon dioxide are released at 320°C as it is displayed on the DTA curve. The final product was ZnO. The scheme of possible thermal decomposition is here:

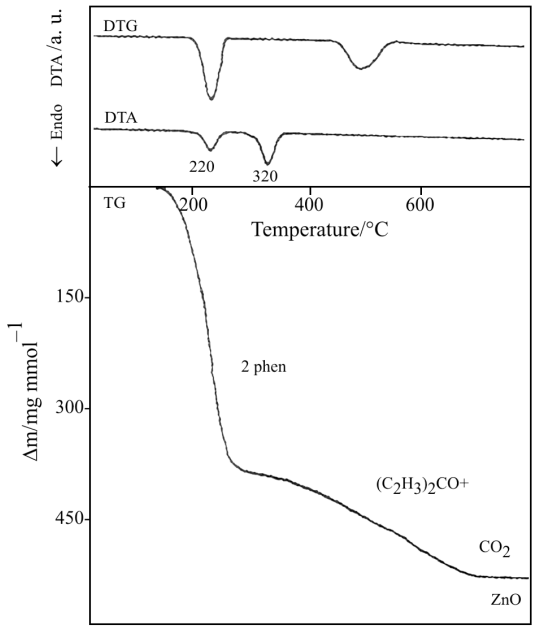

Fig. 3 TG/DTG and DTA curves of $Zn(prop)_2 \cdot 2phen$ **(III)**

$$Zn(CH_3CH_2COO)_2 \cdot 2phen \rightarrow 2phen + (C_2H_5)_2CO + \\ + CO_2 + ZnO$$

$Zn(but)_2 \cdot 2phen$ **(IV)**

The TG/DTG and DTA curves of compound **(IV)** are depicted in Fig. 4. The thermal decomposition begins with the release of 2 mol phenazone with an endothermic peak at 220°C. Then it is followed by pyrolysis of butyrate anion attributing to the elliminination of dipropylketone and carbon dioxide. It is displayed on the DTA curve as an endothermic effect with a minimum at 340°C. ZnO was found as a final product. The scheme of possible thermal decomposition is here:

$$Zn(CH_3CH_2CH_2COO)_2 \cdot 2phen \rightarrow 2phen + \\ + (C_3H_7)_2CO + CO_2 + ZnO$$

The study of thermal behaviour of the prepared compounds suggest that the formation of intermediates is various and depends on the length of a carboxylic chain. The presence of intermediates and volatiles products were confirmed by IR spectra ($\upsilon_{CO_{(ketone)}}$=1715 cm^{-1}, $\upsilon_{(C-H)_{CH_3}}$=2980 cm^{-1}; $\upsilon_{as(CO_2)}$= 2350 cm^{-1}, $\upsilon_{s(CO_2)}$=1342 cm^{-1}, δ_{CO_2}=670 cm^{-1}) and methods of qualitative chemical analyses.

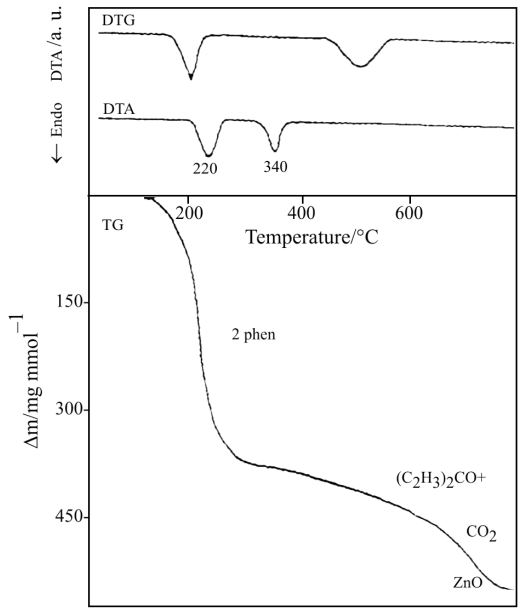

Fig. 4 TG/DTG and DTA curves of Zn(but)$_2$·2phen **(IV)**

It is proposed that the different thermal stability is caused by the presence of various carboxylate anion and N-donor ligand.

IR spectra

The absorption bands of carbonyle group occurred in the range 1700–1680 cm^{-1}. Calculated form IR spectra the magnitude of Δ_{COO} was determined, where $\Delta_{COO}=\nu_{as(COO^-)}-\nu_{s(COO^-)}$. It is used as a criterion how carboxylate groups are coordinated to metal ions [13]. Calculations gave values of Δ_{COO} in the range 340–200 cm^{-1}. The values of Δ_{COO}, the three bands in the range 920–740 cm^{-1} and a strong absorption band around 540 cm^{-1} confirm unidentate mode of carboxylate to Zn^{2+} ion for compounds **I–IV**. The other characteristic absorption bands of the prepared complexes are reported in Table 1. The position of all found bands is in a good accordance with literature data [14].

Table 1 Characteristic absorption bands in the IR spectra of the prepared complexes [ν(cm^{-1})]

Assignment/compound	**(I)**	**(II)**	**(III)**	**(IV)**
$\nu_{(C=O)}$	1680	1700	1700	1690
$\nu_{as(COO^-)}$	1640	1600	1640	1650
$\nu_{s(COO^-)}$	1400	1400	1300	1400
Δ_{COO}	240	200	340	250
$\nu_{(C-H)_{ph}}$	3050	3080	3090	3070
$\delta_{(C-H)_{ph}}$	1400–1000	1400–1000	1380–1000	1390–1000
$\gamma_{(C-H)_{ph}}$	750–600	750–580	800–550	800–580
$\nu_{(C=C)_{ph}}$	1550	1560	1560	1570
$\nu_{(C-C)_{ph}}$	1520	1510	1510	1500
$\nu_{(C-C)_{pyr}}$	1600–1550	1590–1515	1600–1510	1600–1570

ph – phenyl, pyr – pyrazole

Table 2 Antimicrobial activity of Zn(II) complexes characterized by numerical values of IC_{50} and MIC (mmol dm^{-3})

Complex	Bacteria				Yeasts				Filamentous fungi					
	S. aureus		*E. coli*		*C. parapsilosis*		*R. oryzae*		*A. alternata*		*B. cinerea*		*F. nivale*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
(I)	1.39	2^s	1.89	3^s	0.01	3^s	1.86	>3	1.94	>3	2.66	>3	>3	>3
(II)	1.39	2^c	2.35	>3	0.01	2^s	2.82	>3	2.40	>3	>3	>3	>3	>3
(III)	1.23	2^c	1.42	3^s	0.01	>3	1.80	3^s	1.53	3^s	2.1	>3	>3	>3
(IV)	1.34	2^c	2.82	>3	0.01	2^s	1.95	>3	0.86	2^s	1.53	>3	>3	>3
(V)	>3	>3	>3	>3	1.79	>3	>3	>3	>3	>3	>3	>3	>3	>3

(I) – Zn(form)$_2$·2phen, **(II)** – Zn(ac)$_2$·2phen, **(III)** – Zn(prop)$_2$·2phen, **(IV)** – Zn(but)$_2$·2phen, **(V)** – phenazone, ^{s} – microbistatical effect, ^{c} – microbicidal effect

Biological properties

Results of the quantitative determination of antimicrobial activity, characterized by IC_{50} and MIC values, are presented in Table 2. The compounds tested differ in their bioactivities against bacteria, yeasts and filamentous fungi; the bioactivities decrease in the sequence yeasts>bacteria>filamentous fungi.

The compound **(III)** showed the highest antibacterial activity against G^+ *Staphylococcus aureus* (IC_{50}=1.23 and 1.27 mmol dm^{-3}, respectively; MIC=2 mmol dm^{-3}) with bactericide effect. The effect of the Zn(II) compounds against G^- *Escherichia coli* was considerable lower. Compound **(III)** has shown again the highest antibacterial activity, its IC_{50}=1.42 mmol dm^{-3}. Total growth inhibition of G^- *E. coli* was obtained by compound **(III)** at the concentration of 3 mmol dm^{-3} with bacteristatic effect.

The highest inhibition effect against *Candida parapsilosis* was observed with compounds **(II)** and **(IV)**. Total growth inhibition of yeasts was obtained by both compounds at the concentration of 2 mmol dm^{-3}, with fungistatic effect.

The compound **(III)** showed the highest inhibition effect on *Rhizopus oryzae* – IC_{50}=1.8 mmol dm^{-3}, MIC=3 mmol dm^{-3}, with fungistatic effect on the fungal spores. Only two compounds **(III)** and **(IV)** with IC_{50}=1.53 and 0.86 mmol dm^{-3}, respectively; MIC=3 and 2 mmol dm^{-3}, respectively with fungistatic effect on the fungal spores, were significantly active against phytopathogen *Alternaria alternata*. Zn(II) compounds did not affect the growth of *Fusarium nivale* and *Trichoderma viride* (IC_{50}>3 mmol dm^{-3}. Antifungal efficiency of the compounds tested filamentous fungi decreases in the order *A. alternata>R. oryzae>B. cinerea> M. gypseum>F. nivale=T. viride*.

N-heterocyclic ligand phenazone **(V)** not affected the growth of all model bacteria, yeasts and filamentous fungi, its IC_{50}>3 mmol dm^{-3}.

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