Journal of Thermal Analysis and Calorimetry, Vol. 72 (2003) 587–596

BIOLOGICAL AND PHYSICOCHEMICAL STUDY OF ZINC(II) PROPIONATE COMPLEXES WITH N-DONOR HETEROCYCLIC LIGANDS

K. Györyová [1*], *E. Szunyogová* [2], *J. Kovářová* [3], *D. Hudecová* [4], *D. Mudroňová* [5] *and E. Juhászová* [1]

[1]Department of Inorganic Chemistry, P. J. Šafarik University, Moyzesova 11, 041 54 Košice, Slovak Republic
[2]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 043 53 Košice, Slovak Republic
[3]Insitute of Macromolecular Chemistry AS CR, Heyrovského nám. 2, 162 06 Prague 6, Czech Republic
[4]Department of Biochemistry and Microbiology, Slovak Technical University, Radlinského 9, 812 37 Bratislava, Slovak Republic
[5]Department of Gnotobiology and Diseases of Young, Research Institute of Veterinary Medicine, Hlinkova 1/A, 040 01 Košice, Slovak Republic

Abstract

New zinc(II) propionate complexes $(CH_3CH_2COO)_2Zn \cdot L_n \cdot xH_2O$, where n=1–2, x=0 or 2, were prepared by reaction of zinc(II) propionate with heterocyclic ligands (L=theophylline, nicotinamide, methyl-3-pyridyl carbamate) and their thermal properties were studied. The prepared complex compounds were characterized by elemental analysis and IR spectra.

TG/DTG and DTA measurements of the prepared compounds were performed in the air atmosphere under dynamic conditions. The thermal decomposition can be characterized as a multi–step process. The first step is attributed to the elimination of water or N-donor ligand molecules. It is followed by the decomposition of propionate anion when diethyl ketone and carbon dioxide were released. Zinc oxide was found as a final product of the thermal decomposition of the complex compounds under study.

The volatile intermediate products of the thermal decomposition of zinc(II) propionate complexes were identified by IR-spectroscopy, qualitative chemical analyses and final solid product by X-ray powder diffraction method. Moreover, IR spectra suggest monodentate coordination of propionate anion to zinc. The complexes were tested against bacteria and filamentous fungi and show both antimicrobial activity and fungistatic effect towards pathogens as well as probiotic activity towards *Lactobacillus paracasei.*

Keywords: antibacterial and antifungal activity, infrared spectroscopy, methyl-3-pyridyl carbamate, N-donor ligands, nicotinamide, theophylline, thermal behaviour, zinc(II) propionate

* Author for correspondence: E-mail: gyoryova@kosice.upjs.sk

1388–6150/2003/ $ 20.00
Akadémiai Kiadó, Budapest

Introduction

Zinc is considered to be essential for many processes in living organisms. It is one of the most important trace elements in the body for its biological functions. Zinc is required as a catalytic component for more than 300 enzymes and as a structural constituent of many proteins, neuropeptides, hormones, hormone receptors and polynucleotides [1]. Its lack in an organism can cause serious harms and diseases [2].

Zinc(II) carboxylates form a part of coordination compounds that are studied from chemical and also biological viewpoints. Mészáros-Szécsényi *et al.* studied Zn(II), Co(II), Mn(II) and Cu(II) complexes with pyrazole based ligands and characterized these compounds by elemental analysis, FT-IR spectroscopy, thermal methods and molar conductivity measurements [3, 4]. Thermal and other properties were also characterized in 4,4'-bipyridinetrichloroacetato complexes of Mn(II), Ni(II) and Zn(II) [5]. Sofar, our scientific research has been focused on the study of syntheses, spectral, thermal, structural, chromatographic and biological properties of zinc(II) carboxylate complex compounds with N-donor ligands [6–9].

This paper presents results in the thermoanalytical study and biological activity of new zinc(II) propionate complexes with heterocyclic ligands such as theophylline (tph), nicotinamide (nad) and methyl-3-pyridyl carbamate (mpc).

Experimental

The following complexes were prepared: $Zn(prop)_2 \cdot 2H_2O$ (**I**), $Zn(prop)_2 \cdot tph$ (**II**), $Zn(prop)_2 \cdot 2nad$ (**III**) and $Zn(prop)_2 \cdot 2mpc$ (**IV**), where *prop*=$CH_3CH_2COO^-$. Theophylline, nicotinamide, methyl-3-pyridyl carbamate, zinc carbonate and propionic acid were products purchased from Aldrich and used without further purification.

Syntheses

Compound (**I**) was prepared by gradual addition of 4.2 cm^3 99% propionic acid (0.0558 mol) to a water solution of 3.5 g $ZnCO_3$ (0.0279 mol). Compounds **II**–**III** were prepared by dissolving the appropriate heterocycle ligand in water, followed by addition of water solution of zinc(II) propionate (**I**) in a molar ratio 2:1 (**II**) or 4:1 (**III**). The reaction mixtures were stirred for several minutes. Compound (**IV**) was prepared in hot methanolic solution of mpc added to a methanolic solution of zinc(II) propionate (**I**) in molar ratio 2:1 and refluxed for several hours. Then the reaction mixtures were reduced in volume in a water bath and left to stand at laboratory temperature. The complexes which formed were filtered off, washed with diethyl ether and dried over silica gel.

Instrumentation

C, H, N analyses were performed by Perkin Elmer 2400 CHN analyser. The content of zinc was determined complexometrically.

IR spectra were recorded with EXCALIBUR FTS 3000 MX FT-IR spectrophotometer in the region 4000–400 cm^{-1} using the diffusive reflection method.

TG/DTG and DTA curves were measured using Derivatograph and Perkin Elmer DSC 7/TGA 7 Thermoanalyser in air atmosphere under dynamic conditions (heating rate 10°C min^{-1}). Gaseous products of the thermal decomposition were collected and determined by IR spectra and methods of qualitative chemical analysis. Solid final product of the thermal decomposition was identified by X-ray powder diffraction analysis with MIKROMETA 2 (Czech Republic).

Antimicrobial activity

Zn(II) complexes (**I**–**IV**) and $ZnSO_4 \cdot 7H_2O$ were tested for their antimicrobial activity in a concentration 0.01 mol dm^{-3} under in vitro conditions against G^- bacteria - *Escherichia coli K88$^+$ent$^+$*, *Salmonella düsseldorf*, *Salmonella enterica* and G^+ bacteria - *Staphylococcus aureus*, *Lactobacillus paracasei*. Pathogenic bacteria were cultivated in PYG broth medium (5 g peptone for bacteriology, 5 g enzymatic caseine hydrolyzate, 10 g yeast extract, 10 g *D*(+)glucose/1000 cm^3, pH 7) and counts of cfu (colony forming units) were evaluated on PYG agar (composition as PYG broth + 18 g agar/1000 cm^3, pH 7) after 24 h cultivation and *L. paracasei* on MRS broth (Becton Dickinson, USA) after 48 h cultivation at 37°C in the atmosphere containing 80% CO_2 and 20% N_2.

Antifungal activity was tested against *Rhizopus oryzae*, *Altenaria alternata*, *Botrytis cinerea*, *Fusarium nivale*, *Trichoderma viride*, *Microsporum gypseum*. Fungi species were cultivated in SLA agar (composition – 60 g malt extract, 20 g agar, 1000 cm^3 distilled water, pH 6.5). Kinetic study of the fungal growth was evaluated by measuring of the colony size in regular time period. The results are presented in Table 4.

In a second approach the antibacterial and antifungal activity of compound (**IV**) was evaluated by microdilution method using G^+ bacteria *S. aureus* CCM 3935, G^- *E. coli* CCM 3988 and yeasts *Candida parapsilosis* in *L*-shaped tubes under vigorous shaking [10]. The efficiency of compound (**IV**) on filamentous fungi *A. alternata* CCMF–128, *B. cinerea* CCMF–16, *T. viride* CCMF 534, *M. gypseum*, *R. oryzae* and *F. nivale* was tested on solidified broth medium during static culturing. The concentration of compound (**IV**) used in all experiments ranged from 0.05–3 mmol dm^{-3}. The antimicrobial activity was characterized by IC_{50} values (concentration of compound which in comparison to the control inhibits the growth of microorganisms to 50%) and MIC values (minimal inhibitory concentration which inhibits microbial growth by 100%). The IC_{50} and MIC values were read from toxicity curves. 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 results are presented in Table 5.

Results and discussion

The data of chemical analyses are reported in Table 1. Calculated data are in a good agreement with experimental values.

Table 1 Chemical analyses

Compounds	C/%		H/%		N/%		Zn/%	
	theor.	exp.	theor.	exp.	theor.	exp.	theor.	exp.
(**I**)	29.11	29.07	5.70	5.64	0.00	0.00	26.43	26.25
(**II**)	39.85	39.94	4.63	4.71	14.31	14.15	16.70	15.42
(**III**)	47.45	47.36	4.87	4.90	12.30	12.22	14.35	13.99
(**IV**)	46.58	46.62	5.08	5.03	10.86	10.80	12.67	12.42

IR spectra

The characteristic absorption bands of IR spectra for compounds (**I**–**IV**) are reported in Table 2. The presence of the crystallization water in the complexes was confirmed by stretching ν(O–H) and deformation δ(O–H) vibrations at 3500 and 1650 cm^{-1}. The magnitude of Δ_{COO^-} (calculated from IR spectra), $\Delta_{COO^-} = \nu_{as}(COO^-) - \nu_s(COO^-)$, the criterion of the coordination mode of the carboxylate ion to the central atom is in the range of 340–210 cm^{-1}, which means a monodentate coordination of the propionate group to zinc [11]. The other characteristic absorption bands are in a good accordance with literature data [12].

Table 2 Characteristic absorption bands (ν/cm^{-1}) in infrared spectra

Assignment	(**I**)	(**II**)	(**III**)	(**IV**)
$\nu(O–H)_{H_2O}$	3500	–	–	–
$\delta(O–H)_{H_2O}$	1650	–	–	–
ν(N–H)	–	3100	3400	–
$\nu(N–CH_3)$	–	1170	–	–
ν(C=O)	1540	1680	1710	1700
$\nu_{as}(COO^-)$	1620	1610	1620	1600
$\nu_s(COO^-)$	1400	1300	1400	1390
Δ_{COO^-}	220	310	220	210

Thermoanalytical measurements

Thermoanalytical data of the prepared compounds are given in Table 3. The mechanism of the thermal decomposition was determined on the basis of the results of IR-spectroscopy of the volatile products ($\nu CO_{(ketone)}$=1713 cm^{-1}, $\nu(C–H)_{CH_3^-}$ =2980 cm^{-1}; $\nu_{as\ CO_2} = 2349\ cm^{-1}$, $\nu_{s\ CO_2} = 1342\ cm^{-1}$, $\delta_{CO_2} = 667\ cm^{-1}$) and chemical analysis.

Table 3 Thermoanalytical data

Complex	DTA peak/effect/°C	Mass loss/mg mmol^{-1}		Loss of
		exp.	calc.	
	220/endo	19.80	18.02	H_2O
(**I**)	380, 620/endo	130.90	104.00	H_2O, $(C_2H_5)_2CO$, CO_2
	900	84.16	81.38	ZnO
	160/endo	181.40	180.17	tph
(**II**)	350/endo	129.60	130.00	$(C_2H_5)_2CO$, CO_2
	900	81.17	81.38	ZnO
	280/endo	246.57	246.26	2nad
(**III**)	420, 510/endo, exo	127.85	130.00	$(C_2H_5)_2CO$, CO_2
	900	82.19	81.38	ZnO
	250/endo	305.10	304.40	2mpc
(**IV**)	410/endo	128.70	130.00	$(C_2H_5)_2CO$, CO_2
	900	82.64	81.38	ZnO

$Zn(prop)_2 \cdot 2H_2O$ (**I**)

The TG/DTG and DTA curves of compound (**I**) are depicted in Fig. 1. The first step of the thermal decomposition belongs to the release of one water molecule. The DTA curve displays as the next step two endothermic peaks with the minima at 380 and 620°C. The previous peak corresponds to the elimination of the second water molecule, and diethyl ketone. At 620°C carbon dioxide is released. ZnO was found as a fi-

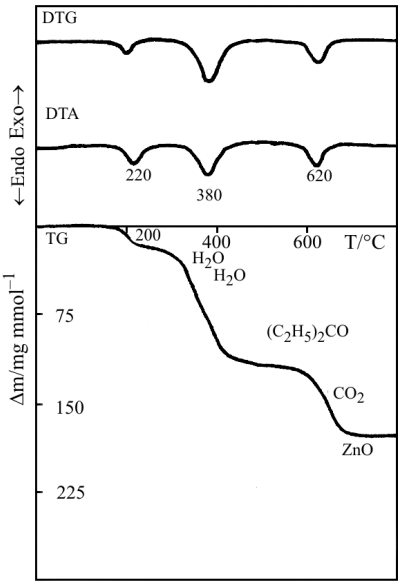

Fig. 1 Thermal decomposition of $Zn(prop)_2 \cdot 2H_2O$

nal product. The following reaction was proposed for the mechanism of the thermal decomposition:

$$Zn(prop)_2 \cdot 2H_2O \rightarrow 2H_2O + (C_2H_5)_2CO + CO_2 + ZnO$$

$Zn(prop)_2 \cdot tph$ (**II**)

The thermal decomposition of compound (**II**) (Fig. 2) starts with the elimination of tph molecule as it is displayed on the DTA curve with a minimum at 160°C. It is followed by pyrolysis of propionate anion that is attributed to the release of diethyl ketone and carbon dioxide in an endothermic process with a peak minimum at 350°C on the DTA curve. As a final product, ZnO was found. The mechanism of the thermal decomposition can be proposed in this way:

$$Zn(prop)_2 \cdot tph \rightarrow tph + (C_2H_5)_2CO + CO_2 + ZnO$$

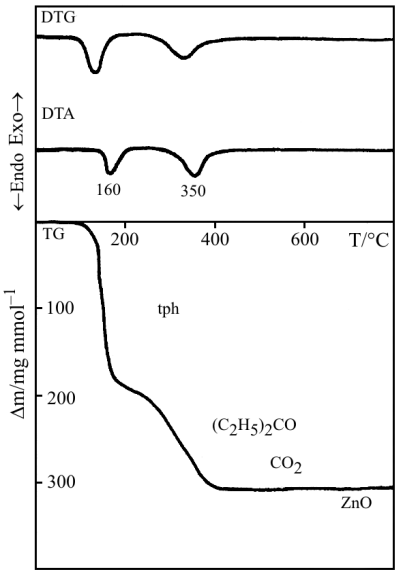

Fig. 2 Thermal decomposition of $Zn(prop)_2 \cdot tph$

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

The first step of the thermal decomposition is the release of nad molecules with an endothermic peak at 280°C on the DTA curve (Fig. 3). Then carboxylate anion begins to decompose with the elimination of diethyl ketone and carbon dioxide. The DTA curve shows an endothermic effect at 420 and an exothermic effect at 510°C. The solid final product was ZnO. The mechanism of the thermal decomposition can be proposed by the following equation:

$$Zn(prop)_2 \cdot 2nad \rightarrow 2nad + (C_2H_5)_2CO + CO_2 + ZnO$$

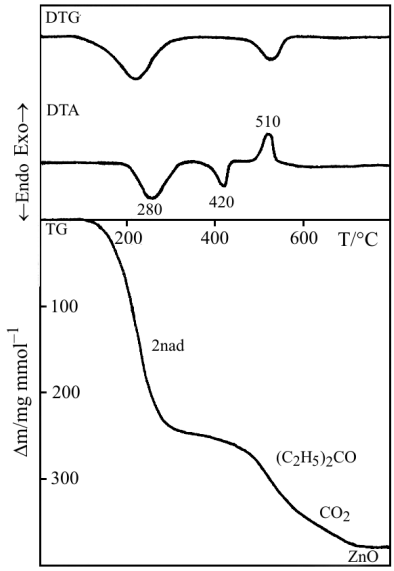

Fig. 3 Thermal decomposition of $Zn(prop)_2 \cdot 2nad$

$Zn(prop)_2 \cdot 2mpc$ (**IV**)

The TG/DTG and DTA curves of compound (**IV**) are given in Fig. 4. The TG curve indicates that (**IV**) is thermally stable up to 90°C. At higher temperature, 2 molecules of mpc are released with an endothermic peak at 250°C. It is followed by pyrolysis of propionate anion that corresponds to the mass loss of diethyl ketone and carbon dioxide. The DTA

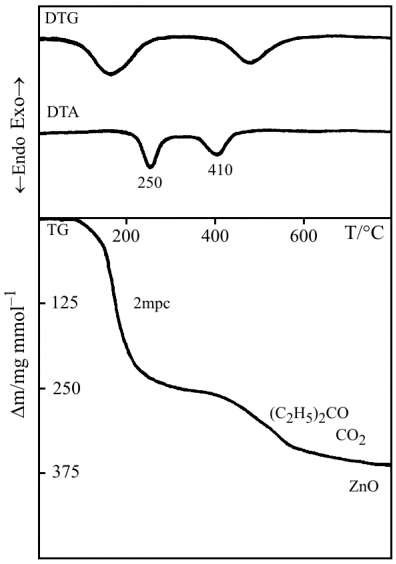

Fig. 4 Thermal decomposition of $Zn(prop)_2 \cdot 2mpc$

Table 4 Antibacterial activity of compounds (**I**–**III**)

Complex	Pathogens									Probioticum		
	G^-						G^+			G^+		
	E. coli			*S. düsseldorf*			*S. aureus*			*L. paracasei*		
	cfu	log(cfu)	Δlog	cfu	log(cfu)	Δlog	cfu	log(cfu)	Δlog	cfu	log(cfu)	Δlog
(**I**)	$8.1\cdot10^5$	5.908	2.9	$1.6\cdot10^6$	6.204	2.6	$8.7\cdot10^7$	7.940	0.6	$4.1\cdot10^8$	8.613	0.2
(**II**)	$4.5\cdot10^5$	5.653	3.1	$8.0\cdot10^3$	3.903	4.9	$1.0\cdot10^7$	7.000	1.6	$4.1\cdot10^8$	8.613	0.2
(**III**)	$1.9\cdot10^4$	4.279	4.9	$2.0\cdot10^1$	1.301	7.5	$2.8\cdot10^6$	6.447	2.1	$3.4\cdot10^8$	8.531	0.3
control	cfu=$6.1\cdot10^8$			cfu=$6.7\cdot10^8$			cfu=$3.7\cdot10^8$			cfu=$6.6\cdot10^8$		
medium	log(cfu)=8.786			log(cfu)=8.830			log(cfu)=8.568			log(cfu)=8.820		

cfu=colony forming units
$\Delta\log=\log(\text{cfu})_{\text{medium}}-\log(\text{cfu})_{\text{complex}}$

Table 5 Antimicrobial activity of compound (**IV**)

Complex	Yeasts		Bacteria				Filamentous fungi											
	C. parapsilosis		*S. aureus*		*E. coli*		*R. oryzae*		*A. alternata*		*B. cinerea*		*F. nivale*		*T. viride*		*M. gypseum*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
(**IV**)	0.2	2^c	2.1	>3	0.6	>3	1.6	3^s	2	3^s	1.2	>3	>3	>3	>3	>3	1.7	>3

^{s}=mirobistatical effect
^{c}=microbicidal effect

curve displays this as an endothermic effect with a minimum at 410°C. ZnO was a final product. The scheme of the thermal decomposition can be proposed like this:

$$Zn(prop)_2 \cdot 2mpc \rightarrow 2mpc + (C_2H_5)_2CO + CO_2 + ZnO$$

Biological properties

Microbiological studies of compounds (**I**–**III**) reported in Table 4 show a strong antimicrobial activity against G^- pathogens *Escherichia coli* and *Salmonella düsseldorf* and an average activity against G^+ pathogenic bacteria *Staphyloccocus aureus*. Because similar carboxylate complexes with sodium ion showed no activity to colony forming units (cfu) of G^- and G^+ bacteria, zinc ion can be assumed as responsible for the observed antibacterial activity [13]. The most active compounds are (**II**) and (**III**), where the value of difference between $\log(cfu)_{medium}$ and $\log(cfu)_{complex}$ is from 3.1 to 7.5. The compounds comprise nad and tph to which the bacteria are sensitive. On the other hand compounds (**I**–**III**) do not inhibit the growth of the probiotic G^+ bacteria *Lactobacillus paracasei* and so they can be used as potentional components of probiotics.

The antimicrobial activity of compound (**IV**) is summarized in Table 5. The highest antimicrobial effects of derivative (**IV**) were found against G^+ bacteria *S. aureus* (IC_{50}=0.2 and MIC=2 mmol dm^{-3}) with bactericide effect and yeasts *C. parapsilosis* (IC_{50}=0.6 mmol dm^{-3}). The most sensitive filamentous fungi to compound (**IV**) were *R. oryzae* and *A. alternata* (MIC=3 mmol dm^{-3} fungistatic effect). Compound (**IV**) did not affect the growth of *F. nivale* and *T. viride* (IC_{50}>3 mmol dm^{-3}).

Conclusions

We have found that zinc(II) propionate complexes (**II**–**IV**) start to decompose with the release of neutral ligand molecules. Compound (**I**) begins to decompose with the dehydration process. Diethyl ketone and carbon dioxide were found as volatile intermediate products of the thermal decomposition. ZnO was found as a final solid product.

The comparison of thermal stability of zinc(II) propionates with earlier prepared zinc(II) butyrates and isobutyrates shows that zinc(II) propionates are less stable compounds [14]. The thermal stability is increasing in the following order:

Compound	(**II**) <	(**I**) <	(**IV**) <	(**III**)
DTA peak °C	160 endo	220 endo	250 endo	280 endo

Antimicrobial effect of the prepared compounds depends on the type of bacteria (G^+, G^-). Zn(II) complexes were more active against G^- bacteria that can be caused by different cell wall which is in G^- thinner and chemically less resistant. The most effective inhibitors against pathogenic bacteria were complexes with nad and tph. Zn(II) complexes can be used for potentiating the efficiency of probiotics because the inhibition of the compounds was very low against *L. paracasei*. Antifungal properties of the complex (**IV**) was confirmed, too. The most sensitive fungi to compound (**IV**) were *R. oryzae* and *A. alternata* (MIC=3 mmol dm^{-3}).

This work was supported by the Slovak Ministry of Education project VEGA No. 1/9247/02. This financial support is gratefully acknowledged.

References

1 M. J. Kendrick, M. T. May, M. J. Plishka and K. D. Robinson, Metals in Biological System, Ellis Horwood, London 1992.
2 N. Fabris and E. Mocchegiani, Aging Clin. Exp. Res., 7 (1995) 77.
3 K. Mészáros-Szécsényi, E. Z. Ivegeš, V. M. Leovac, A. Kovács, G. Pokol and Z. K. Jacimovic, J. Therm. Anal. Cal., 56 (1999) 493.
4 K. Mészáros-Szécsényi, V. M. Leovac, Z. K. Jacimovic, V. I. Cesljevic, A. Kovács, G. Pokol and S. Gal, J. Therm. Anal. Cal., 63 (2001) 723.
5 D. Czakis-Sulikowska, A. Czylkolwska and A. Malinowska, J. Therm. Anal. Cal., 67 (2002) 667.
6 K. Györyová, J. Kovářová, M. Melník and E. Andogová, J. Therm. Anal. Cal., 56 (1999) 503.
7 V. Zeleňák, K. Györyová and E. Andogová, Thermochim. Acta, 354 (2000) 81.
8 K. Györyová, J. Kovářová, E. Andogová, V. Zeleňák and F. A. Nour El-Dien, J. Therm. Anal. Cal., 67 (2002) 119.
9 A. Oriňák, K. Györyová, E. Matisová, J. Šlesárová, J. Skoršepa and M. Ganajová, J. Chrom. A, 660 (1994) 67.
10 D. Hudecová, S. Jantová, M. Melník and M. Uher, Folia Microbiol., 41 (1996) 473.
11 K. Nakomoto, Infrared and Raman Spectra of Inorganic Compounds, John Wiley, New York 1986.
12 J. P. Graselli and W. M. Ritchey, Atlas of Spectral Data and Physical Constants for Organic Compounds, Press, Ohio 1975.
13 M. Melnik, M. Onderová and M. Hoľko, Inorg. Chim. Acta, 67 (1982) 117.
14 K. Györyová, V. Balek and J. Kovářová, Thermochim. Acta, 269/270 (1995) 425.