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Urease Inhibition of Oxovanadium(V) Complexes with Hydrazone and Hydroxamate Ligands[1]

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Abstract—Two new oxovanadium(V) complexes, [VOL^1(SHA)] (**I**) and [VOL^2(BHA)] (**II**), were prepared by the reaction of [$VO(Acac)_2$] (Acac = acetylacetonate) with *N*'-(2-hydroxybenzylidene)isonicotinohydrazide (H_2L^1) and salicylhydroxamic acid (HSHA) and 4-chloro-*N*'-(2-hydroxy-3-methoxybenzylidene)benzohydrazide (H_2L^2) and benzohydroxamic acid (HBHA), respectively, in methanol. Crystal and molecular structures of the complexes were determined by elemental analysis, infrared spectra and single crystal X-ray diffraction (CIF file CCDC nos. 978238 (**I**) and 978392 (**II**)). The V atoms are in octahedral coordination. Thermal stability and the inhibition of urease of the complexes were studied.

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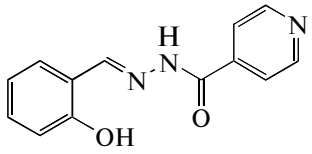
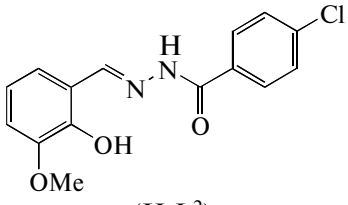
INTRODUCTION

Urease is a nickel containing enzyme, which has important negative effects on human, stockbreeding, and agriculture [1–4]. Control of the activity of urease through the use of inhibitors could counteract these negative effects. Metal complexes have been proved to be a kind of versatile enzyme inhibitors [5]. Among versatile metal complexes, those derived from hydrazones have been received particular attention in biological and medicinal chemistry [6–9]. In recent years, vanadium complexes have been reported to have interesting biological activities such as normalizing the high blood glucose levels and acting as models of haloperoxidases [10–12]. It is notable that Ara and coworkers reported that some binuclear vanadium(IV) complexes possess interesting urease inhibitory activities [13]. Aslam and co-workers reported that the Schiff bases of hydrazone type also possess urease inhibitory activities [14]. Recently, our research group has reported a few vanadium complexes with urease inhibitory activities [15, 16]. Considering the hydroxamic acids, such as acetylhydroxamic acid, benzohydroxamic acid, etc. have strong urease inhibitory activity [17–22], in the present paper, two new hydrazone oxovanadium(V) complexes, [VOL^1(SHA)] (**I**) and [VOL^2(BHA)] (**II**) (H_2L^1 = *N*'-(2-hydroxybenzylidene)isonicotinohydrazide, H_2L^2 = 4-chloro-*N*'-(2-hydroxy-3-methoxybenzylidene)benzohydrazide), bearing salicylhydroxamate (SHA) and benzohydroxamate (BHA) ligands, have been presented.



(H_2L^1)



(H_2L^2)

EXPERIMENTAL

Materials and measurements. Commercially available salicylaldehyde, 3-methoxysalicylaldehyde, isonicotinohydrazide, and 4-chlorobenzohydrazide were purchased from Aldrich and used without further purification. Protease inhibitor (Complete Mini EDTA-free) was purchased from Roche Diagnostics GmbH (Mannhein, Germany) and brucella broth was from Becton-Dickinson (Cockeysville, MD). Horse serum was obtained from Hyclone (Utah, America). Other solvents and reagents were made in China and used as received. H_2L^1 and H_2L^2 were prepared according to the literature method [23, 24]. C, H, and N elemental analyses were performed with a Perkin-Elmer elemental analyser. Infrared spectra were recorded on a Nicolet AVATAR 360 spectrometer as KBr pellets in the (4000–400) cm^{-1} region. Thermal

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stability analysis was performed on a PerkinElmer Pyris Diamond TG–DTA thermal analyses system.

Synthesis of I. A methanolic solution (10 mL) of $[VO(Acac)_2]$ (0.1 mmol, 26.5 mg) was added to a methanolic solution (10 mL) of H_2L^1 (0.1 mmol, 24.1 mg) and salicylhydroxamic acid (0.1 mmol, 15.3 mg) with stirring. The mixture was stirred for 30 min at room temperature to give a deep brown solution. The resulting solution was allowed to stand in air for a few days. Brown block-shaped crystals suitable for X-ray single crystal diffraction were formed at the bottom of the vessel. The isolated products were washed three times with cold ethanol and dried in air. The yield was 67%.

For $C_{20}H_{15}N_4O_6V$

anal. calcd., %:	C, 52.4;	H, 3.3;	N, 12.2.
Found, %:	C, 52.2;	H, 3.3;	N, 12.3.

Synthesis of II. This complex was prepared according to the same method as that described for **I** with H_2L^1 replaced by H_2L^2 (0.1 mmol, 30.4 mg) and HSAH replaced by HBAH (0.1 mmol, 13.7 mg). The yield was 59%.

For $C_{22}H_{17}ClN_3O_6V$

anal. calcd., %:	C, 52.2;	H, 3.4;	N, 8.3.
Found, %:	C, 52.3;	H, 3.3;	N, 8.3.

X-ray crystallography. Diffraction intensities for complexes **I** and **II** were collected at 298(2) K using a Bruker D8 Venyture Photon diffractometer with MoK_α radiation (λ = 0.71073 Å). The collected data were reduced using the SAINT program [25], and multi-scan absorption corrections were performed using the SADABS program [26]. The structures were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL [27]. All of the non-hydrogen atoms were refined anisotropically. The amino H atoms in the structures were located from difference Fourier maps and refined isotropically, with N–H distances restrained to 0.90(1) Å. The remaining H atoms were placed in idealized positions and constrained to ride on their parent atoms. The crystallographic data for complexes **I** and **II** are summarized in Table 1. Selected bond lengths and angles are given in Table 2.

Supplementary material for structures **I** and **II** has been deposited with the Cambridge Crystallographic Data Centre (nos. 978238 (**I**) and 978392 (**II**); deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Urease inhibitory activity assay. *Helicobacter pylori* (ATCC 43504; American Type Culture Collection, Manassas, VA) was grown in brucella broth supplemented with 10% heat-inactivated horse serum for 24 h at 37°C under microaerobic condition (5% O_2, 10% CO_2, and 85% N_2). The method of preparation of *Helicobacter pylori* urease by Mao was followed [28]. Briefly, broth cultures (50 mL, 2.0×10^8 CFU mL^{-1}) were centrifuged (5000 *g*, 4°C) to collect the bacteria and after washing twice with phosphate-buffered saline (pH 7.4), the *Helicobacter pylori* precipitation was stored at –80°C. While the *Helicobacter pylori* was returned to room temperature, and mixed with 3 mL of distilled water and protease inhibitors, sonication was performed for 60 s. Following centrifugation (15000 *g*, 4°C), the supernatant was desalted through Sephadex-25 column (PD-10 columns, Amersham-Pharmacia Biotech, Uppsala, Sweden). The resultant crude urease solution was added to an equal volume of glycerol and stored at 4°C until used in the experiment. The mixture, containing 25 μL (4*U*) of *Helicobacter pylori* urease and 25 μL of the test compound, was pre-incubated for 3 h at room temperature in a 96-well assay plate. Urease activity was determined by measuring ammonia production by the indophenol method.

RESULTS AND DISCUSSION

Replacement of two acetylacetonate ligands of $[VO(Acac)_2]$ by hydrazone and hydroxamate ligands in methanol resulted in the formation of two structurally similar complexes. The complexes are soluble in DMF, DMSO, methanol, ethanol, and acetonitrile. Molar conductance of complexes **I** and **II** at the concentrations of 10^{-4} mol L^{-1} are 25 and 33 Ω^{-1} cm^2 mol^{-1}, respectively, indicating they are non-electrolytes [29].

The molecular structures and atom numbering schemes of complexes **I** and **II** are shown in Fig. 1, respectively. The V atoms in the complexes are in octahedral coordination with the three donor atoms of the hydrazone ligands and the hydroxy O atom of the hydroxamate ligand defining the equatorial plane and with one oxo O atom and the carbonyl O atom occupying the axial positions. The distances between atoms V(1) and O(6) are 1.58 Å in **I** and 1.59 Å in **II**, indicating they are typical V=O double bonds. The V(1)–O(4) bonds in the complexes are significantly longer than the other V–O bonds, yet, it is not uncommon for such complexes [30, 31]. The coordinate bond lengths in the complexes are comparable to each other and also similar to those observed in the mononuclear oxovanadium(V) complexes with octahedral coordination [32–34]. The angular distortion in the octahedral environment around V comes from the five- and six-membered chelate rings taken by the hydrazone ligands. For the same reason, the *trans* angles significantly deviate from the ideal values of 180°. Distortion of the octahedral coordination can be observed from the coordinate bond angles, ranging from 74.55(8)° to 106.92(8)° for the perpendicular angles and from 151.06(8)° to 167.96(9)° for the diagonal angles for **I**, and from 76.01(4)° to 102.92(5)° for the perpendicular

Table 1. Crystallographic data and refinement parameters for complexes **I** and **II**

Parameter	Value	
	I	**II**
Formula weight	458.3	505.8
Crystal color, habit	Brown, block	Brown, block
Crystal size, mm	0.27 × 0.27 × 0.22	0.20 × 0.20 × 0.18
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$
Unit cell parameters:		
a, Å	7.8899(9)	11.9883(4)
b, Å	19.693(2)	11.2681(4)
c, Å	13.065(2)	16.7981(6)
β, deg	96.663(2)	101.885(1)
V, Å^3	2016.4(4)	2220.5(1)
Z	4	4
ρ_{calcd}, g cm^{-3}	1.510	1.513
μ, mm^{-1}	0.538	0.612
F(000)	936	1032
Number of unique data	3762	4780
Number of observed data, $I > 2\sigma$, (I)	2742	4189
Number of parameters	284	302
Number of restraints	1	1
Final R indices, $I > 2\sigma$, (I)	$R_1 = 0.0385$, $wR_2 = 0.0917$	$R_1 = 0.0305$, $wR_2 = 0.0821$
R indices, all data	$R_1 = 0.0623$, $wR_2 = 0.1040$	$R_1 = 0.0361$, $wR_2 = 0.0868$
Goodness of fit on F^2	1.018	1.060

angles and from 153.78(5)° to 170.67(5)° for the diagonal angles for **II**. The displacement of the V atoms from the equatorial plane are 0.223(1) Å for **I** and 0.278(1) Å for **II**. The dihedral angle between the benzene ring and the pyridine ring of the hydrazone ligand in **I** is 2.2(3)°. The dihedral angle between the two benzene rings of the hydrazone ligand in **II** is 9.2(3)°. The crystal structures of the complexes are stabilized by intermolecular hydrogen bonds (Table 3, Fig. 2).

The sharp bands at 3290 cm^{-1} for **I** and 3333 cm^{-1} for **II** are assigned to the ν(N–H) vibrations of the hydroxamate ligands. Complexes **I** and **II** exhibit typical bands at 985 and 966 cm^{-1}, respectively, assigned to the V=O vibration. The bands due to ν(C=O) were absent in the complexes, but new C–O stretches appeared at 1245 cm^{-1} for **I** and 1251 cm^{-1} for **II**. This suggests occurrence of *keto*-imine tautomerization of the ligands during complexation. The intense ν(C=N) absorptions are observed at 1605 cm^{-1} for **I** and **II**. The carbonyl ν(C=O) vibrations related to the hydroxamate ligands are located at 1646 cm^{-1} for **I** and 1654 cm^{-1} for **II**. The weak peaks in the low wave numbers in the region (400–650) cm^{-1} may be attributed to V–O and V–N bonds in the complexes.

Differential thermal (DT) and thermal gravimetric analyses (TGA) were conducted to examine the stability of complexes **I** and **II** (Fig. 3). For **I**, the complex decomposed from 185 to 496°C, corresponding to the loss of the hydrazone and salicylhydroxamate ligands and the formation of V_2O_5. The total observed weight loss of 81.4% is close to the calculated value of 80.3%. For **II**, the complex decomposed from 180 to 490°C,

Table 2. Selected bond distances (Å) and angles (deg) for complexes **I** and **II**

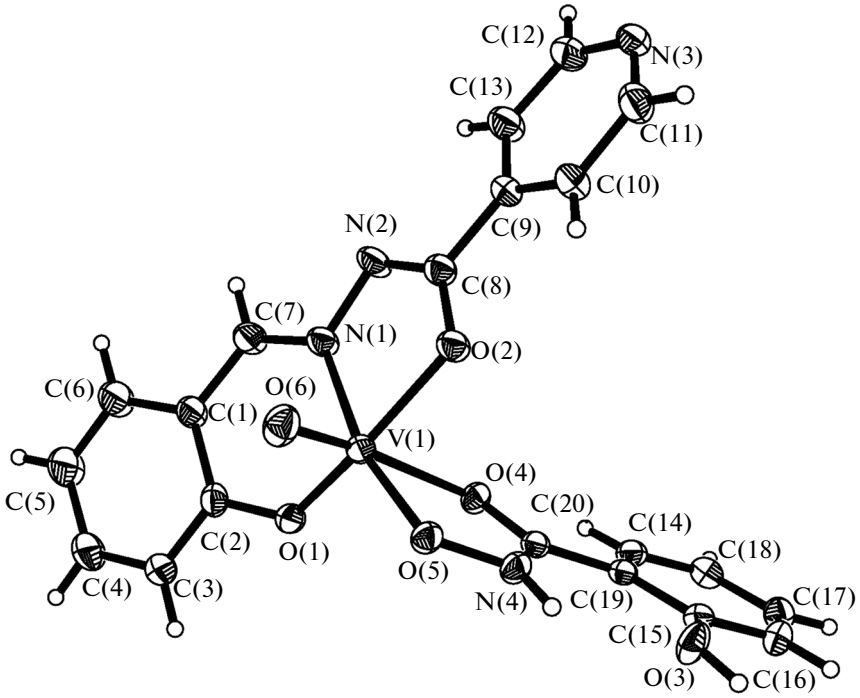
Bond	*d*, Å	Bond	*d*, Å
		I	
V(1)–O(1)	1.866(2)	V(1)–O(2)	1.968(2)
V(1)–O(4)	2.204(2)	V(1)–O(5)	1.873(2)
V(1)–O(6)	1.578(2)	V(1)–N(1)	2.085(2)
		II	
V(1)–O(1)	1.879(1)	V(1)–O(3)	1.941(1)
V(1)–O(4)	2.193(1)	V(1)–O(5)	1.865(1)
V(1)–O(6)	1.594(1)	V(1)–N(1)	2.086(1)
Angle	ω, deg	Angle	ω, deg
		I	
O(6)V(1)O(1)	100.0(1)	O(6)V(1)O(5)	92.47(9)
O(1)V(1)O(5)	106.92(8)	O(6)V(1)O(2)	100.2(1)
O(1)V(1)O(2)	151.06(8)	O(5)V(1)O(2)	92.71(8)
O(6)V(1)N(1)	94.6(1)	O(1)V(1)N(1)	83.35(8)
O(5)V(1)N(1)	166.34(8)	O(2)V(1)N(1)	74.55(8)
O(6)V(1)O(4)	167.96(9)	O(1)V(1)O(4)	84.03(8)
O(5)V(1)O(4)	75.49(7)	O(2)V(1)O(4)	80.50(7)
N(1)V(1)O(4)	97.15(8)		
		II	
O(5)V(1)O(6)	94.67(6)	O(6)V(1)O(1)	96.38(6)
O(5)V(1)O(1)	102.92(5)	O(6)V(1)O(3)	94.50(6)
O(5)V(1)O(3)	93.68(5)	O(1)V(1)O(3)	159.29(5)
O(6)V(1)N(1)	109.58(6)	O(5)V(1)N(1)	153.78(5)
O(1)V(1)N(1)	84.65(5)	O(3)V(1)N(1)	75.11(5)
O(6)V(1)O(4)	170.67(5)	O(5)V(1)O(4)	76.01(4)
O(1)V(1)O(4)	85.60(5)	O(3)V(1)O(4)	86.51(5)
N(1)V(1)O(4)	79.66(5)		

Table 3. Hydrogen bond distances (Å) and bond angles (deg) for complexes **I** and **II***

D–H···A	Distance, Å			Angle D–H···A, deg
	D–H	H···A	D···A	
		I		
O(3)–H(3)···N(3)^{i}	0.82	1.89	2.700(3)	172
		II		
N(3)–H(3)···O(1)^{ii}	0.90(1)	2.29(2)	3.005(2)	136(2)
N(3)–H(3)···O(2)^{ii}	0.90(1)	2.03(2)	2.809(2)	145(2)

* Symmetry codes: ^{i} $1-x, -1-y, -1-z$; ^{ii} $1-x, 2-y, -z$.

(a)



(b)

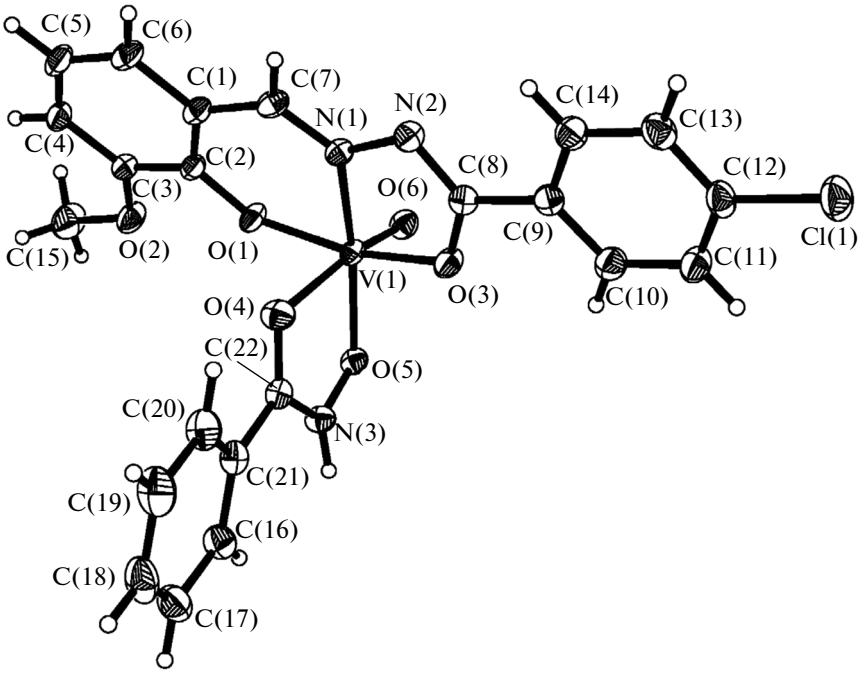


Fig. 1. ORTEP plot of the crystal structure of **I** (a) and **II** (b). Displacement ellipsoids of non-hydrogen atoms are drawn at the 30% probability level.

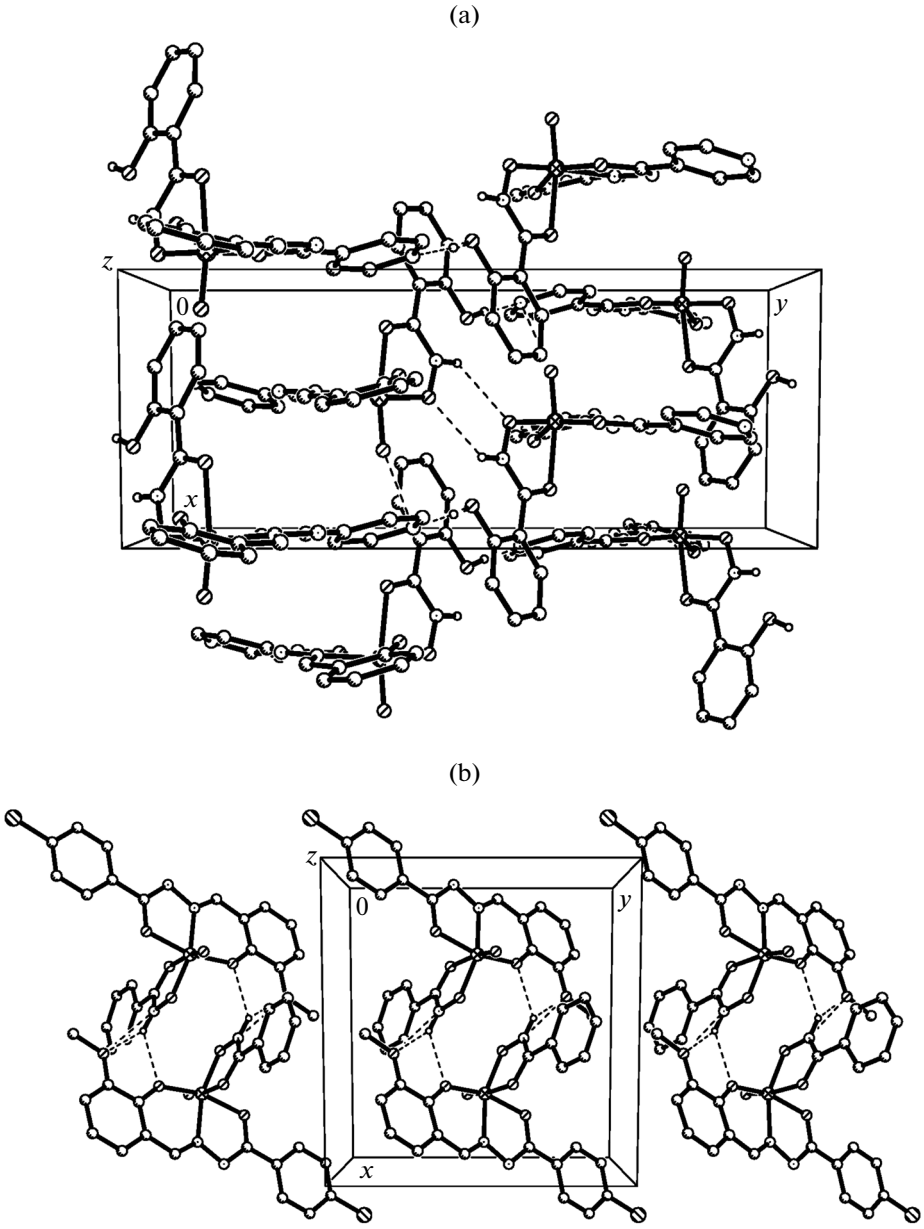


Fig. 2. Packing of molecules for compound **I**. Hydrogen bonds are shown as dashed lines.

corresponding to the loss of the hydrazone and benzohydroxamate ligands and the formation of V_2O_5. The total observed weight loss of 83.7% is close to the calculated value of 82.0%.

The results of the urease inhibition are summarized in Table 4. Compared with the reference inhibitor acetohydroxamic acid (AHA), the free hydrazones and the vanadyl sulfate have very weak interactions against the urease. Complexes **I** and **II** at concentration of 100 μmol L^{-1} have urease inhibitory activities with percent inhibition of (26.5 ± 1.6) and (55.9 ± 2.7), respectively, and with IC_{50} value of 66.3 μmol L^{-1} for **II**. It is clear that the activity of **II** is stronger than that

Table 4. Inhibition of urease by the tested materials

Tested materials*	Percent inhibition	IC_{50}, μmol L^{-1}
I	26.5 ± 1.6	
II	55.9 ± 2.7	66.3
H_2L^1	8.5 ± 1.8	
H_2L^2	17.7 ± 3.1	
Vanadyl sulfate	26.5 ± 1.9	220
AHA	88.2 ± 4.0	37.5

* Concentration of the tested material is 100 μmol L^{-1}.

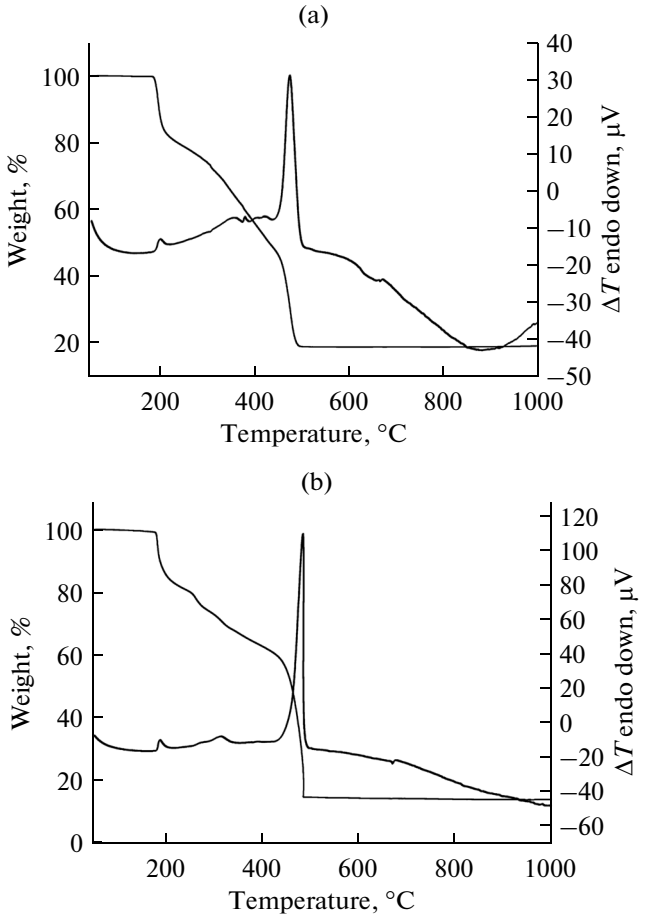


Fig. 3. DT–TGA curve of **I** (a) and **II** (b).

of **I**, but both of them are weaker than AHA. Even though, it is important for the exploration of novel urease inhibitors with vanadium complexes, since such compounds are prospective biological materials.

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