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Synthesis, Structure, and Activity Evaluation of Two Silver(I) Complexes as *Helicobacter pylori* Urease Inhibitors[1]

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Abstract—Two silver(I) compounds, $[Ag(R,R\text{-hxn})](C_7H_4BrO_2) \cdot 2H_2O$ (**I**) (Chxn = 1,2-diaminocyclohexane) and $[Ag(C_5H_6N_2)_2]_2(C_8H_4O_4) \cdot 10H_2O$ (**II**), were synthesized and complex **I** was structurally characterized by X-ray crystallography. Compound **I** contains a *catena*-(*trans*-1,2-diaminocyclohexane) silver polycation ($[Ag(Chxn)]_\infty$) in a roughly linear fashion, while **II** possesses a linear-type silver monocation. Compounds **I** and **II** were evaluated for their inhibitory activities against *Helicobacter pylori* urease in vitro. Both were found to have strong inhibitory activities against *H. pylori* urease comparable to that of acetohydroxamic acid.

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INTRODUCTION

Urease, the first enzyme crystallized to be shown to possess two nickel ions, is produced by plants, fungi, yeast and bacteria, where it catalyzes the urea degradation to supply these organisms with a source of nitrogen for growth [1, 2]. Ureolytic activity of bacteria, such as *Klebsiella pneumoniae, Clostridium perfringens, Proteus mirabilis, Salmonella* sp., *Ureaplasma urealyticum*, and *Staphylococcus saprophytics* plays an important role in the pathogenesis of human and animal diseases [3, 4]. One of the most frequently studied bacterial urease is that from *Helicobacter pylori* (*H. pylori*) since it has been implicated in peptic ulcers and stomach cancer [5, 6]. Many urease inhibitors have been investigated in the past decades, like phosphorodiamidates, hydroxamic acid derivatives, and imidazoles [7], but part of them are prevented from using in vivo because of their toxicity or instability. Thus, it's of interest to control the activity of urease through the use of inhibitors with good bioavailability and low toxicity.

Our previous research reported that some silver(I) carboxylate complexes and silver(I) polymers with high cytotoxicity properties against carcinoma cells and inhibitory activities against xanthine oxidase and urease [8–11]. In this study, we designed and synthesized two silver(I) complexes, $[Ag(R,R\text{-Chxn})](C_7H_4BrO_2) \cdot 2H_2O$ (**I**) (Chxn = 1,2-diaminocyclohexane) and $[Ag(C_5H_6N_2)_2]_2(C_8H_4O_4) \cdot 10H_2O$ (**II**), and investigated their inhibitory activity against *H. pylori* urease.

[1] The article is published in the original.

EXPERIMENTAL

Materials and methods. Terephthalic acid, 4-aminopyridine, *p*-bromobenzoic acid, and *trans*-1,2-diaminocyclohexane were purchased from Aldrich and used without further purification. Elemental analyses for C, H, and N were carried out on a Perkin-Elmer 2400 analyzer. X-ray crystallography was carried out using a Bruker SMART APEX II CCD diffractometer. All chemicals and reagents used in current study were of analytical grade. Protease inhibitors (Complete mini EDTA-free) were purchased from Roche Diagnostics GmbH (Mannhein, Germany) and brucella broth was from Becton-Dickinson (Cockeysville, MD). Horse serum was from Hyclone (Utah, USA).

Synthesis of complex I was carried out by reaction of Ag_2O (0.5 mmol) with *p*-bromobenzoic acid (1.0 mmol) in aqueous ammonia (10 mL), and the mixture was stirred for 15 min until a clear solution was obtained. Then Chxn (1.0 mmol) was added to the above solution. The mixture was stirred at room temperature for 20 min to give a colorless clear solution. The resulting solution was kept in air for 7 days with ammonia gas escaping and the crystals of **I** were formed at the bottom of the vessel. Colorless crystals were collected and washed with water, and then dried in a vacuum desiccator with $CaCl_2$. The yield was 62%.

For $C_{13}H_{22}N_2O_4BrAg$			
anal, calcd, %:	C, 34.08;	H, 4.84;	N, 6.11.
Found, %:	C, 34.00;	H, 4.98;	N, 6.03.

Synthesis of complex **II** was carried out according to our previous work [12].

Table 1. Crystallographic data and details of the experiment and refinement of the complexes **I**

Parameter	Value
	I
Molecular weight	458.11
Crystal system	Triclinic
Space group	$P\bar{1}$
Crystal size, mm	0.22 × 0.21 × 0.19
a, Å	7.1402(15)
b, Å	8.371(2)
c, Å	15.172(4)
α, deg	85.002(14)
β, deg	86.774(12)
γ, deg	65.932(11)
T, K	296(2)
V, Å^3	824.7(3)
Z	2
ρ_{calcd}, g cm^{-3}	1.845
F(000)	456
μ(MoK_α), mm^{-1}	3.660
θ Range, deg	2.7–25.7
Index ranges h, k, l	$-8 \le h \le 8$
	$-5 \le k \le 10$
	$-17 \le l \le 18$
Reflections collected	4673
Independent reflections	3200
Observed data, $I > 2\sigma(I)$	2239
Parameters	190
Goodness-of-fit on F^2	1.018
Final R indices ($I > 2\sigma(I)$)	$R_1 = 0.0456$; $wR_2 = 0.1184$
R indices, all data	$R_1 = 0.0729$; $wR_2 = 0.127$
Largest diff. peak and hole, e/Å^3	0.60 and −0.78

Crystal structure determinations. X-ray crystallographic data were collected on a Bruker SMART Apex II CCD diffractometer using graphite-monochromated MoK_α (λ = 0.71073 Å) radiation. The collected data were reduced using the SAINT program [13], and empirical absorption corrections were performed using the SADABS program [14]. The structure were solved by direct methods and refined against F^2 by full-matrix least-squares methods using the SHELXTL, version 5.1. All of the non-hydrogen atoms were refined anisotropically. All other hydrogen atoms were placed in geometrically ideal positions and constrained to ride on their parent atoms. The crystallographic data for the compounds are summarized in Table 1. Hydrogen-bonding interactions are given in Table 2.

Supplementary material for complex **I** has been deposited with the Cambridge Crystallographic Data Centre (no. 826191; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Measurement of inhibitory activity against urease. *H. 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 conditions (5% O_2, 10%) CO_2, and 85% N_2), as previously described [15].

The method of preparation of *H. pylori* urease was followed in [16]. Briefly, 50 mL broth cultures (2.0×10^8 CFU/mL) were centrifuged (5000 *g*, 4°C) to collect the bacteria, and after washing twice with phosphate-buffered saline (pH 7.4), the *H. pylori* precipitation was stored at −80°C. *H. pylori* was returned to room temperature, and after addition of 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 SephadexG-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 use in the experiment. The mixture, containing 25 μL (4 U) of *H. pylori* urease (100 mM HEPES, pH 6.8) and 25 μL of the test compound, was preincubated for 3 h at room temperature in a 96-well assay plate. After preincubation, 0.2 mL of 100 mM HEPES (pH 6.8) buffer containing 500 mM urea and 0.002% phenol red were added and incubated at 37°C. Urease activity was determined by measuring ammonia production using the indophenol method as described in [17].

RESULTS AND DISCUSSION

Crystal structure of complex **I** was shown in Fig. 1. Compound **I** consists of a *catena*-(*trans*-1,2-diaminocyclohexane) silver polycation ($[Ag(Chxn)]_\infty$), the *p*-bromobenzoate counter-anions and some lattice water molecules. In the $[Ag(Chxn)]_\infty$ polycation, Ag^+ cation is coordinated to two amino N atoms of two Chxn ligands in a roughly linear fashion, with the Ag–N distances of 2.138(5) and 2.143(4) Å. The NAgN angle is 174.1(2)°, which is in good agreement with that ranging from 156.46(10)° to 177.14(18)° observed in analogous $[Ag(Chxn)]_\infty$ polycations [18]. Distances between Ag cations in two adjacent $[Ag(Chxn)]_\infty$ strands amount to 4.372 Å (Fig. 2). This shows no chemically relevant intermetal contacts are present in **I**. Interestingly, the weak N–H···O hydrogen bonds between the Chxn ligands and the lattice water molecules with the N···O distances ranging from

Table 2. Hydrogen-bonding interactions of **I**

Contact D–H···A	Distance, Å			Angle DHA, deg
	D–H	H···A	D···A	
N(1)–H(1)···O(3)	0.90	2.24	3.023(8)	146
N(1)–H(1)···O(4)	0.90	2.54	2.926(9)	107
N(2)–H(2)···O(3)	0.90	2.20	3.104(7)	177
O(3)–H(3)···O(4)	0.85	2.16	3.006(7)	174
O(3)–H(3)···O(1)	0.85	2.12	2.789(6)	135
O(4)–H(4)···O(2)	0.85	1.94	2.768(8)	163
O(4)–H(4)···O(1)	0.85	1.94	2.740(7)	157
O(4)–H(4)···O(2)	0.85	2.59	3.330(8)	146

Table 3. Inhibition of *H. pylori* urease by the title compounds, *p*-bromobenzoic acid, terephthalic acid and Ag^+ cation

Tested materials	IC_{50} (μM)
Terephthalic acid	>100
p-Bromobenzoic acid	>100
Ag^+	0.76 ± 0.08
Complex **I**	0.80 ± .03
Complex **II**	0.12 ± 0.02
Acetohydroxamic acid	36.47 ± 0.19

2.926(9) to 3.104(7) Å increase the dimensionality of the solids from chain polymers to layer structures. There are a great number of weak O–H···O hydrogen bonds between the lattice water molecules with the O···O distance of 3.006(7) Å, and between the lattice water molecules and the deprotonated carboxylate of the *p*-bromobenzoate anions with O···O distances ranging from 2.768(8) to 3.330(8) Å, to generate the one-dimensional (1D) chains (Fig. 3, Table 2). This enhances the structural stability of compound **I**.

The crystal structure of complex **II** is shown in Fig. 4, which has been reported in our previous work [12].

The abilities against urease of *p*-bromobenzoic acid, terephthalic acid, Ag^+, compounds **I** and **II** had been studied by the IC_{50} values of the material (25 μL, 100 μg) tested against *H. pylori* urease (25 μL, 10 kU/L) using urea (500 mM) in HEPES buffer (0.2 mL, 100 mM; pH 6.8). On reaction with *H. pylori* urease in the presence of phenol red, *p*-bromobenzoic acid and terephthalic acid as enzyme inhibitors had no ability to inhibit urease (IC_{50} > 100 μM). This indicated terephthalic acid had less influence on the activity of *H. pylori* urease. Under the same condition, both the two complexes had much stronger urease inhibitory activities with the IC_{50} values of 0.8 μM for **I** and 0.12 μM for **II**, respectively, as shown in Table 3. Ag^+ cation also showed potent urease inhibitory activities, compared with that of the standard inhibitor acetohydroxamic acid which had IC_{50} values of 36.47 μM. The results are agree with those reported previously [19, 20].

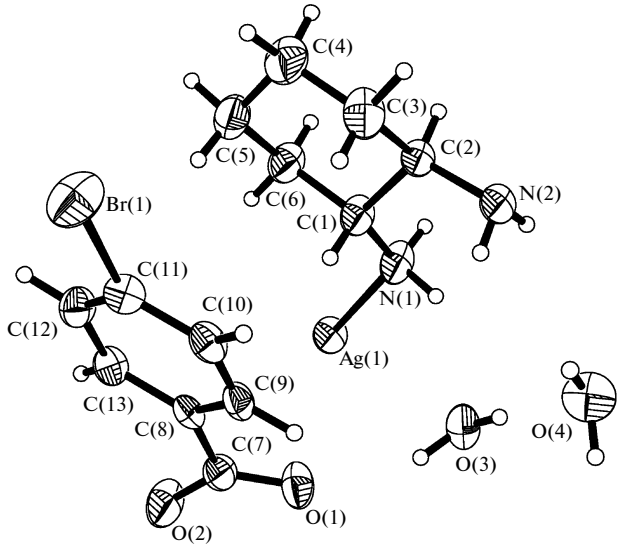

Fig. 1. Molecular structure of compound **I** in the solid state with atom numbering scheme. Thermal ellipsoids are drawn at 30% probability.

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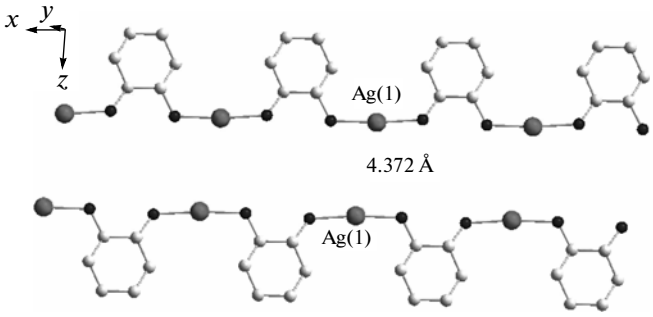

Fig. 2. Parallel $[Ag(Chxn)]_\infty$ polycation chains in **I**.

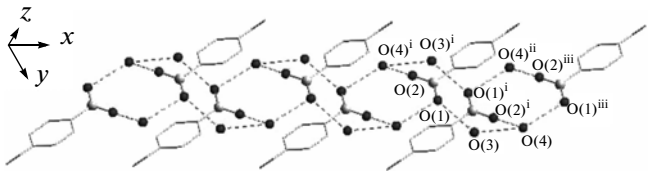

Fig. 3. A one-dimensional (1D) chain assembled via O–H ··· O hydrogen bonds in the crystal structure of **I** (symmetry codes: [i] $1-x, 1-y, 1-z$; [ii] $2-x, 1-y, 1-z$; [iii] $1+x, y, z$).

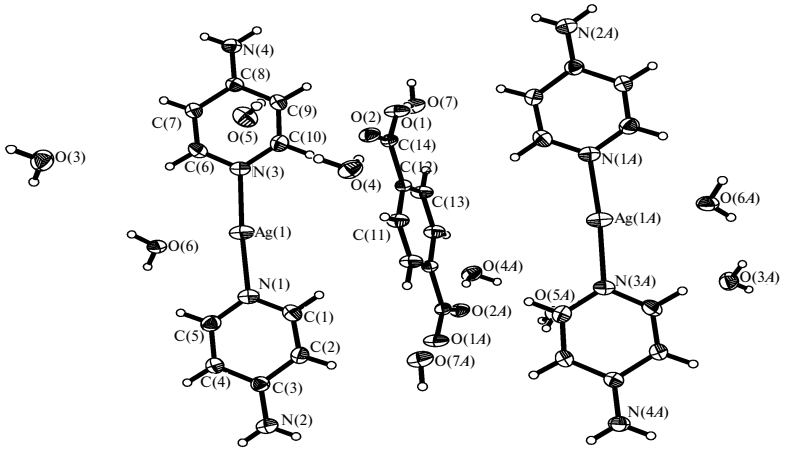

Fig. 4. An ORTEP diagram showing molecular structure of **II**. Thermal ellipsoids are shown at 30% probability level (symmetry code: (*A*) $1-x, 1-y, -z$).

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