Synthesis, Crystal Structure, Spectral and Thermal Characterization, and Antimicrobial Activity of $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$ (aepy = 2-aminoethylpyridine, HOr^{2-} = orotate)

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The copper(II) orotate complex with 2-aminoethylpyridine, $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$, was synthesized and characterized by means of elemental and thermal analysis, magnetic susceptibility, IR and UV/vis spectroscopic, single crystal X-ray diffraction, and antimicrobial activity studies. The complex crystallizes in the triclinic system, space group $P\bar{1}$, and the Cu(II) ion is five-coordinate with a distorted square-pyramidal coordination geometry. The aepy ligand and the orotate dianion behave as bidentate (*N,N′* and *N,*O_{acid}) chelating ligands. The crystal structure is stabilized by intermolecular O–H···O and N–H···O hydrogen bonds, and the orotate ligand exhibits a double hydrogen-bonding functionality. The new compound was found active against some gram (+)/(−) bacteria and yeast *Candida albicans* ATCC 10231, but there was no activity on *Aspergillus niger*.

Key words: Orotate Complex, Vitamin B13, 2-Aminoethylpyridine, Antimicrobial Activity

Introduction

Vitamin B13 (orotic acid, H_3Or) is an important uracil derivative as the effective precursor in the biosynthesis of uracil base of RNA in living organisms. In the last years, research in bioinorganic chemistry has revealed the important role of metal ions in most biological processes [1, 2]. Some metal orotate complexes have applications in curing certain syndromes involving metal ion and vitamin deficiencies and are promising as anticancer agents [3, 4]. It has also be reported that orotic acid and some of its derivatives have antimicrobial activity against microorganisms [5].

The coordination chemistry of orotic acid has found major interest. The orotate anions H_2Or^- and HOr^{2-} behave as a polyfunctional ligands and coordinate to metal ions by means of their pyrimidine nitrogen atoms and carbonyl or carboxylate oxygen atoms. The most common coordination mode of HOr^{2-} is ligation through a deprotonated nitrogen atom of the pyrimidine ring and a carboxylate oxygen atom so forming a five-membered chelate ring [6 – 11]. Recently, we have synthesized copper(II) orotate complexes with triethanolamine, $[Cu(HOr)(tea)] \cdot H_2O$ [9], monoethanolamine, $[Cu(HOr)(mea)_2] \cdot H_2O$ [8], 2,2′-bipyridine, $[Cu(HOr)(H_2O)(bipy)]$ [11], and ethylenediamine, $[Cu(HOr)(en)(H_2O)] \cdot H_2O$ [6]. X-Ray structures of other Cu(II) orotate complexes, $[Cu(HOr)(H_2O)_2]_n$ [12], $[Cu(HOr)(phen)]$ [13] and $[Cu(HOr)(NH_3)_2]$ [14] (phen = 1,10'-phenanthroline) were reported.

In this paper we present the synthesis, spectral and thermal characterization, crystal structure, and antimicrobial activity of a new aqua(2-aminoethylpyridine)-orotatocopper(II) hydrate complex **1**.

Results and Discussion

Preparation of the complex

The complex was prepared as described in the Experimental Section.

Magnetic susceptibility and UV/vis and IR spectrum

The Cu(II) complex **1** exhibits a magnetic moment value of 1.68 BM, which corresponds to one unpaired electron. The electronic spectrum of the Cu(II) complex in H_2O shows an absorption band at 564 nm (ε = 14 L mol^{-1}cm^{-1}), assigned to the $a_1 \rightarrow b_1$ *d-d* transition. The absorption bands below 350 nm are due to intra-ligand transitions. Selected IR bands of the complex are listed in the Experimental Section.

Thermal analysis

The thermal decomposition of complex **1** was followed up to 600 °C in a static air atmosphere (Fig. 1).

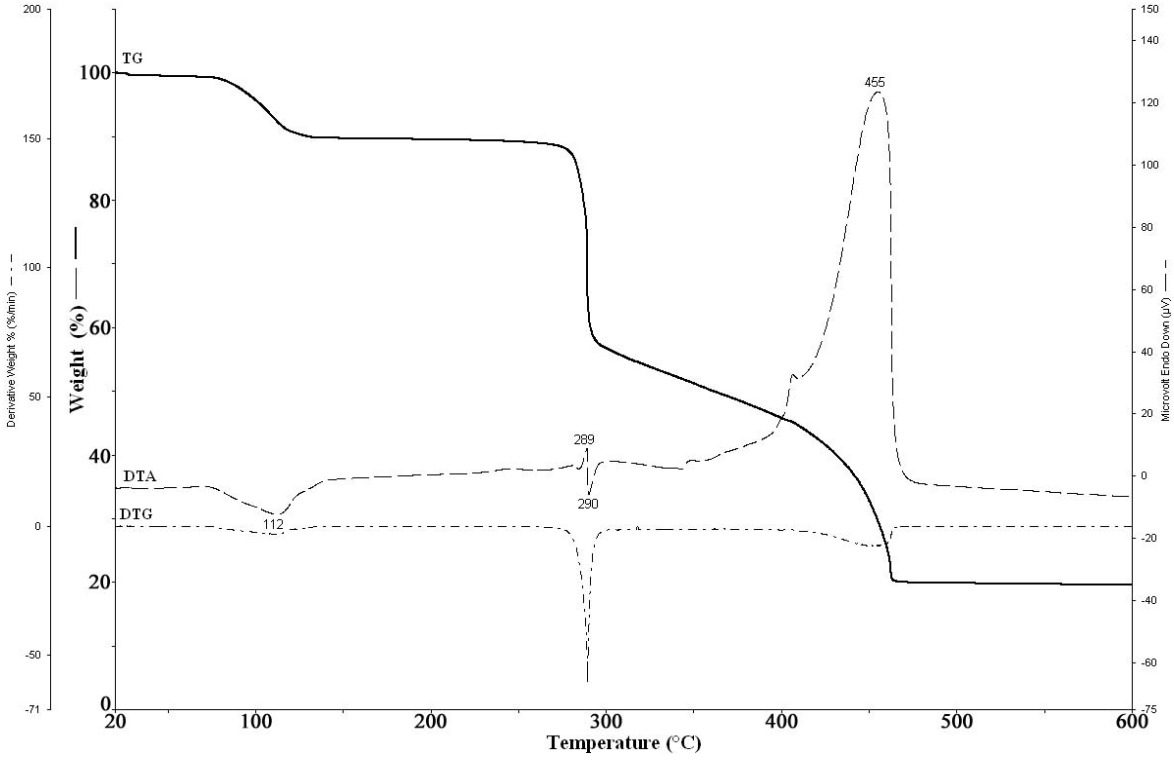

Fig. 1. TG, DTG and DTA curves of **1**.

The first stage between 99 and 137 °C is related to the release of both coordinated and crystal water molecules with an endothermic effect (DTA_{max} = 112 °C, mass loss found 10.22 %, calcd. 9.58 %). The second stage in the temperature range 257 – 296 °C has several consecutive steps related to the loss of the aepy ligand. The experimental mass loss of 32.41 % agrees well with the calculated mass loss of 32.51 %. The solid residue consisting of mainly Cu^{2+} HOr^{2-} decomposes between 297 and 472 °C with an extremely exothermic DTA peak at 455 °C to give a black end-product of CuO (found 80.03 %, calcd. 78.83 %).

Crystal structure

The details of the crystal structure solution are given in Table 1, selected bond lengths and angles and hydrogen bonding geometries in Tables 2 and 3, respectively. The crystal structure of the complex is presented in Fig. 2. The copper(II) ion is coordinated by one aqua, one aepy and one HOr^{2-} ligand, exhibiting a distorted square-pyramidal configuration of the CuN_3O_2-type. The aepy molecule acts as a bidentate donor through its two nitrogen atoms, creating a six-membered chelate ring, while the HOr^{2-} ligand is coordinated as a bidentate ligand through one nitrogen atom of the pyrimidine ring and one oxygen atom of the carboxylate group. The Cu–N_{aepy} bond lengths are 1.995(2) and 2.041(1) Å. The Cu–$N1_{HOr}$ and Cu–O_{HOr} bond lengths of 2.015(1) and 1.969(1) Å are comparable to those reported for related mononuclear copper(II) complexes of HOr^{2-} [6, 8, 9, 11, 13, 14]. The bond angle values for N(4)–Cu1–O(4) (156.80(8)°) and N(3)–Cu1–N(1) (171.15(6)°) indicate a significant distortion in the coordination polyhedron around the copper(II) ion.

The crystal water molecules form intermolecular hydrogen bonds with the carboxyl atom O3 of HOr^{2-} and the amine hydrogen atom (N-H4B) of the eapy molecule with a pattern $R_4^4(8)$ in Etter's notation [16]. The orotate ligand is hydrogen-bonded to a symmetry-related orotate ligand through N2–H2···O1^{i} and N2^{i}–H2^{i}···O1 forming a DA:AD dimer ($R_2^2(8)$ with i = $1-x$, $1-y$, $-z$) [16]. There are also H-bonds between the aqua ligand and the carbonyl oxygen atom O2 of the orotate ligand. Furthermore, there are weak π-π interactions between the pyrimidine and

Table 1. Crystal data and structure refinement parameters for **1**.

Empirical formula	$C_{12}H_{16}CuN_4O_6 \cdot H_2O$
Formula weight	393.84
Temperature, K	296
Wavelength, Å	0.71069
Crystal system	triclinic
Space group	$P\bar{1}$
a, Å	8.1661(7)
b, Å	8.9797(8)
c, Å	10.8656(10)
α, deg	91.708(8)
β, deg	107.332(7)
γ, deg	105.050(7)
V, Å^3	729.52(11)
Z	2
Absorption coefficient, mm^{-1}	1.54
D_{calc}, mg m^{-3}	1.710
Crystal size, mm^3	$0.72 \times 0.37 \times 0.13$
Θ Range for data collection, deg	2.72 – 27.8
Measured reflections	10950
Independent reflections	3387
Absorption correction	integration
Refinement method	Full-matrix least-squares on F^2
$R1/wR2[F^2 \geq 2\sigma(F^2)]$	0.027/0.070
$R1$ (all data)	0.050
Goodness-of-fit on F^2	1.08
Largest diff. peak and hole, e Å^{-3}	0.30/−0.45

Table 2. Selected bond lengths (Å) and bond angles (deg.) for **1**.

Bond lengths:			
O2–C2	1.2428 (19)	N1–Cu1	2.0146 (13)
O5–Cu1	2.3255 (15)	O4–Cu1	1.9689 (13)
O3–C5	1.232 (2)	Cu1–N4	1.9951 (17)
C4–C5	1.510 (2)	Cu1–N3	2.0410 (14)
C1–O1	1.236 (2)	N4–C12	1.463 (2)
Bond angles:			
C1–N1–Cu1	129.05 (11)	N4–Cu1–N3	94.00 (6)
C4–N1–Cu1	112.68 (10)	N1–Cu1–N3	171.15 (6)
C5–O4–Cu1	116.88 (11)	O4–Cu1–O5	100.51 (6)
O4–Cu1–N4	156.80 (8)	N4–Cu1–O5	102.33 (7)
O4–Cu1–N1	81.27 (5)	N1–Cu1–O5	91.98 (6)
N4–Cu1–N1	93.90 (6)	N3–Cu1–O5	90.21 (6)
O4–Cu1–N3	89.90 (6)		

Table 3. Hydrogen bonding interactions (Å, deg.) in **1**[a].

D–H···A	d(D−H)	d(H···A)	d(D···A)	∠(DHA)
O5–H5B···O2^{i}	0.73 (3)	2.11 (3)	2.8266 (19)	170 (3)
O5–H5A···O2^{ii}	0.77 (3)	2.02 (3)	2.787 (2)	174 (3)
N2–H2N···O1^{iii}	0.85 (2)	1.96 (3)	2.8029 (19)	174 (2)
N4–H4B···O6^{iv}	0.93 (3)	2.14 (3)	3.029 (3)	159 (2)
O6–H6A···O3	0.82 (4)	2.03 (4)	2.826 (3)	165 (3)
C6–H6C···O4	0.91 (2)	2.21 (2)	2.810 (2)	122 (2)
N4–H4A···O1	0.79 (3)	2.26 (3)	2.805 (2)	126 (2)

[a] Symmetry codes: [i] $x, y-1, z$; [ii] $-x+2, -y+1, -z$; [iii] $-x+1, -y+1, -z$; [iv] $x-1, y, z$.

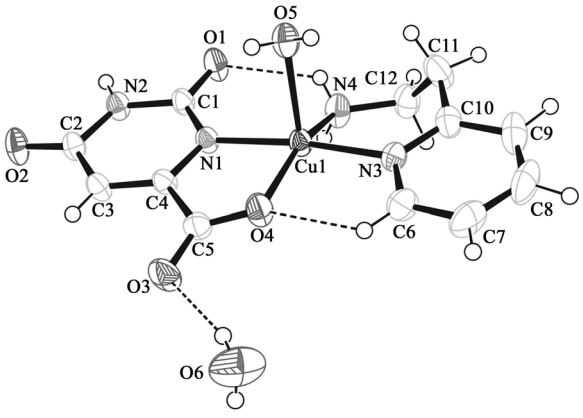

Fig. 2. Molecular structure of **1**, showing the atom labeling scheme and displacement ellipsoids at the 50 % probability level.

pyridine rings [CgB···CgA^{ii} 3.685 Å (ii): $x, y-1, z$] as well as the pyrimidine rings of neighboring HOr^{2-} ligands [CgA···CgA^{iii} 3.809 Å, (iii): $2-x, 1-y, -z$]. Thus, individual molecules are held together by hydrogen bonds and aromatic π-π stacking interactions, forming a structure with three-dimensional character (Fig. 3).

Antimicrobial activity

The *in vitro* antimicrobial activity of $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$ was tested using the disc diffusion method. The results were compared to those of $[Cu(H_2Or)_2(H_2O)_3]$ [17] and orotic acid. Orotic acid, $[Cu(H_2Or)_2(H_2O)_3]$ and $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$ show different antimicrobial activities against test strains. As can clearly be seen from Table 4, it appears that the activity of the tested complexes increases with concentration with exception of *A. niger*. It is a well-known fact that the concentration plays a vital role in increasing the degree of inhibition. While orotic acid, $[Cu(H_2Or)_2(H_2O)_3]$ and $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$ did not show any activity on a mould of *A. niger*, there was antimicrobial activity on *C. albicans* ATCC 10231.

When the results obtained are compared to those of $[Cu(H_2Or)_2(H_2O)_3]$ and orotic acid, $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$ appears to have the lower inhibitory effect on gram (+), gram (−) and *C. albicans* ATCC 10231, but the results also show that the complexes have an enhanced activity compared to orotic acid itself. Orotic acid and metal complexes have been demonstrated to possess antibacterial and antifungal properties [5].

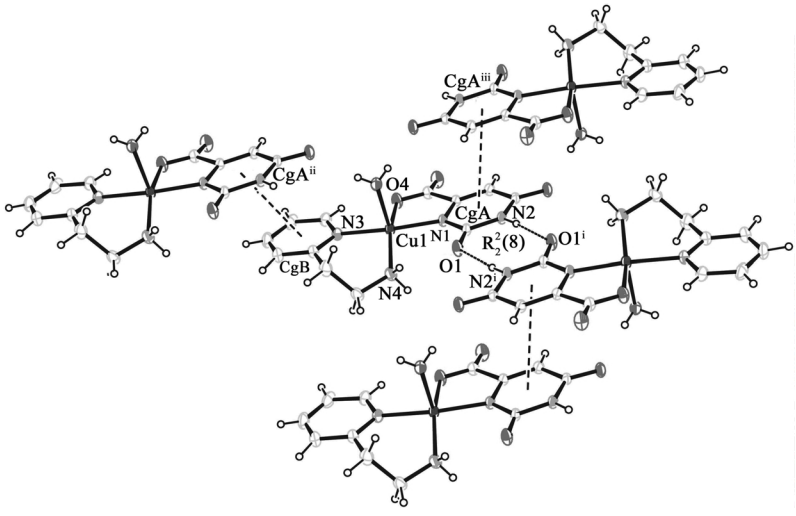

Fig. 3. Hydrogen bonding interactions (DA:AD dimer pairs) and π-π interactions in the crystal structure of **1**.

Table 4. Antimicrobial activity of orotic acid, $[Cu(H_2Or)_2(H_2O)_3]$ and $[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$[a].

	Gram (−)			Gram (+)		Eucaryot	
	E. coli	*C. freundii*	*P. putida*	*B. cereus*	*S. aureus*	*A. niger*	*C. albicans*
Orotic acid							
400 μg	7	8	7	–	–	–	–
1000 μg	9	10	9	8	8	–	7
1500 μg	10	11	10	10	9	–	9
$[Cu(H_2Or)_2(H_2O)_3]$							
400 μg	11	10	–	8 (21[b])	7 (16[b])	–	7
1000 μg	15	15	9	10 (27[c])	11 (20[c])	–	11
1500 μg	18	18	16	13 (30[d])	12 (22[c])	–	12
$[Cu(HOr)(aepy)(H_2O)] \cdot H_2O$							
400 μg	8	8	7	(16[b])	(16[b])	–	7
1000 μg	12	10	9	7 (23[c])	8 (18[b])	–	9
1500 μg	16	13	12	9 (30[d])	10(20[c])	–	11
(DMSO)							
50 μL	–	–	–	–	–	–	–

[a] Clear zone diameter outside parentheses, cloudy zone diameter in parentheses (values in mm); –: no activity observed; [b] very cloudy; [c] moderately cloudy; [d] poorly cloudy.

Experimental Section

Preparation of the complex

A solution of aepy (0.61 g, 5 mmol) in water (10 mL) was added dropwise with stirring to a mixture of orotic acid monohydrate (0.87 g, 5 mmol) and $Cu(CH_3COO)_2$ (0.45 g, 2.5 mmol) in distilled water (20 mL). The solution was heated to 50 °C in a temperature-controlled bath and stirred for 2 h at 50 °C. Then the reaction mixture was cooled to r. t. The blue crystals were filtered, washed with 10 mL each of cool distilled water and acetone, and dried in air. – IR (KBr pellet): ν = 3514 (OH), 3377 – 3284 (NH_2), 3110 (NH), 1646 (C=O_{acid}+$C_{(2)}$=O), 1632 (C=C+ν_{ring}) cm^{-1}. – $C_{12}H_8CuN_4O_7$ (393.84): calcd. C 36.66, H 4.63, N 14.25; found C 36.60, H 4.61, N 14.23.

Materials and measurements

All chemicals were purchased as analytical grade. The IR spectrum was obtained with a Bruker Tensor 27 FT-IR spectrometer using a KBr pellet for the 4000 – 400 cm^{-1} range. Elemental analyses for C, H and N were performed using a Carlo Erba 1106 microanalyzer. The magnetic susceptibility measurement at r. t. was performed using a Sherwood Scientific MXI model Gouy magnetic balance. The UV/vis spectrum was obtained for an aqueous solution of the complex (10^{-3} M) with a Unicam UV2 spectrometer in the range 900 – 190 nm. A Perkin Elmer Diamond TG/DTA thermal analyzer was used to record simultaneous TG, DTG and DTA curves in static air atmosphere at a heating rate of 10 °C min^{-1} in the temperature range 20 – 1000 °C using platinum crucibles.

Crystallographic analysis

Single crystal X-ray data were collected on a Stoe X-AREA diffractometer using monochromated MoK_α radiation at 296 K. The structure was solved by Direct Methods and completed by Fourier methods. The program used

for cell refinement was Stoe X-AREA [18], for solving and refining the structure SHELXS/L-97 [19]. Molecular graphics: ORTEP-III for Windows [20]. Software used to prepare material for publication: WINGX [21] publication routines. Further details concerning data collection and refinement are given in Table 1.

Antimicrobial activity studies

The antimicrobial activities were tested against standard strains of Gram positive (*Staphylococcus aureus* ATCC 6535, *Bacillus cereus* ATCC 7064) and Gram negative (*Escherichia coli* W3110, *Citrobacter freundii* NRRLB 2643, *Pseudomonas putida* NRRLB 13) bacteria and yeast (*Candida albicans* ATCC 10231) and mould (*Aspergillus niger*) using the disk diffusion method. Antimicrobial studies were performed according to broth dilution [one gram (+) and one gram (−)] and agar diffusion methods.

Agar diffusion experiments were based on the obtained MIC values by dilution methods. Stock solutions of $[Cu(H_2Or)_2(H_2O)_3]$ and the new complex were prepared in dimethylsulfoxide (DMSO, Merck) in concentrations needed for experiments. Nutrient Agar (Merck) plates were prepared and dried for 5 d at r. t. All the microorganisms were grown in Nutrient Broth (Merck) at 37 °C for 24 h. Test strains were spread on solid nutrient agar surfaces by using a sterile glass rod. The final inoculation (inoculums) was approximately 10^5 cfu mL^{-1}. Absorbent paper discs of 6 mm were placed on agar surface and impregnated with known concentrations (400, 1000 and 1500 μg for each disc) determined previously by MIC tests. To ensure that the solvent had no effect on bacterial growth, a control test was performed with a test medium supplemented with DMSO following the same procedures as used in the experiments. Incubation was done at 37 °C for 24 h after inoculums. Antimicrobial activity was indicated by the presence of clear inhibition zones and cloudiness around the discs, the diameters being measured in millimeters. All tests were repeated three times and average data taken as final result.

Supplementary material

CCDC 647824 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

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