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Copper(II) Complexes of 1-Alkylamidino-O-alkylurea (alkyl=methyl or ethyl) and Its Growth Inhibition Activity Against *Ceratocystis paradoxa*

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Copper(II) complexes of the type $[Cu(1\text{-}AAAUH)_2]X_2$ where 1-AAAUH = 1-alkylamidino-O-alkylurea, X = Cl, Br, I, have been synthesized and characterized by elemental analyses, magnetic moments, electronic absorption, IR and EPR spectroscopy. EPR spectra of two complexes $[Cu(II)(1\text{-methylamidino-O-methylurea})_2]Cl_2$ and $[Cu(II)(1\text{-ethylamidino-O-methylurea})_2]Cl_2$ were recorded in solid state and in DMF and bonding parameters α^2, β^2 and γ^2 were calculated. The orbital factors K_{II} and $K_{\perp}$ were also calculated. The trend $K_{II} < K_{\perp}$ indicates the presence of significant in plane π-bonding. The *bis*(1-alkylamidino-O-alkylurea)copper(II) complexes show growth inhibitory against pathogenic fungus *Ceratocystis paradoxa*.

Key Words: Copper(II) complexes, *C. paradoxa*, Antibacterial, Antifungal, EPR, Ethyldicyandiamide, Methyldicyandiamide.

INTRODUCTION

Ananas comosus (pineapple) is one of the major commercial fruit crops grown in Manipur. Without preservative measures the ripe pineapples can not be stored beyond 10-12 d due to infection by fungal pathogens *Ceratocystis paradoxa*. During the peak fruiting season (June-July), the warm and humid climate favours the rapid growth of the spoilage organisms and hastens deterioration of the fruits. In recent years attention had been focused to control the post harvest fruit loss by adopting different methods.

The shelf-life of pineapples could be considerably improved by treatment with a combination of relatively low doses of γ-rays which did not produce any undesirable effect and cold storage[1]. The *in vitro* study showed that γ-rays inhibited radial growth and conidial germination of *C. paradoxa*[2]. Some plant extracts and benomyl [methyl-(1-butyl-carbamoyl)benzimidazol-2-yl carbamate], a systemic fungicide also known as benlate could control the post-harvest disease of pineapples caused by *C. paradoxa*[3].

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In our previous investigation of *bis*(1-*n*-butylamidino-O-alkylurea)copper(II) chloride complexes[4] to biological activities in terms of the antibacterial and antifungal properties, the complexes show growth inhibitory activity against pathogenic fungi, *viz.*, *Ceratocystis paradoxa*, *Aspergillus niger, Trichoderma koningii* and pathogenic bacteria, *viz.*, *Bacillus* sps. In continuation of such work, the study of copper(II) complexes of 1-methyl/ethyl amidino-O-methyl/ethylurea which have versatile hydrogen-bonding capacity and the complexes show growth inhibitory against pathogenic fungus *Ceratocystis paradoxa* have been reported.

EXPERIMENTAL

Methyldicyandiamide and ethyldicyandiamide was prepared by following published procedure[5].

Preparation by the complexes: *Bis*(1-methylamidino-O-methylurea)copper(II) chloride.

Bis(1-methylamidino-O-methylurea)copper(II) chloride was prepared by refluxing cupric chloride dihydrate (1.7 g) and methyldicyandiamide (2 g) on a steam bath for 3 h. The light blue clour complex was filtered off immediately after keeping overnight in a refrigerator. The compound was recrystallized from methanol and dried in air.

Bis**(1-methylamidino-O-ethylurea)copper(II)chloride:** This compound was prepared by refluxing cupric chloride dihydrate (0.85 g) and methyldicyandiamide (1 g) in ethanol for 4 h. The complexes was recrystallized from ethanol and dried in air.

The corresponding iodide and bromide complexes was prepared by following the published procedure[6]. The analytical, magnetic susceptibility and electronic data are presented in Table-1.

The filter paper disc method, based on diffusion capacity of test compound through an agar medium was used for screening the antimicrobial property of the complexes against test organism[7]. The antifungal activity of the following 5 complexes were carried out:

[Cu(1-methylamidino-O-methylurea)]copper(II) bromide.
[Cu(1-methylamidino-O-ethylurea)]copper(II) iodide.
[Cu(1-methylamidino-O-methylurea)]copper(II) iodide.
[Cu(1-ethylamidino-O-ethylurea)]copper(II) iodide.
[Cu(1-ethylamidino-O-methylurea)]copper(II) iodide.

C. paradoxa culture, procured from Department of Life Sciences, Manipur University was maintained in Potato Dextrose Agar (PDA).

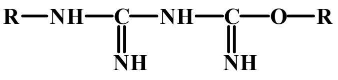
Potato dextrose agar was prepared as follows: An agar (20 g) was dissolved in 500 mL water by heating. Potato extract was prepared by steaming 200 g of sliced potatoes in 500 mL distilled water and then filtered through muslin cloth. To this extract was added 20 g dextrose. Then this extract was mixed with agar solution and adjusted the volume to 1000 mL with distilled water and sterilized in the autoclave and cooled to *ca.* 60 ºC and poured into the sterilized plates.

The 72 h old fungal spores were harvested into phosphate buffered saline and swabbed on to PDA plates with the help of an 'L' shape glass rod. The density of the spores at the time of inoculation is *ca.* 10^6 spores/mL. The metal complexes to be tested were dissolved in distilled water to a final concentration of 0.5 and 1 % and soaked in filter paper discs of 5 mm diameter and 1 mm thickness. These filter papers were found to hold a quantity of 0.01 mL solution. These discs were placed on the already seeded plates and incubated at 28 ± 2 ºC for 72 h. A clearing zone around the disc indicated the inhibitory activity of the complexes on the test organism.

Microanalyses were carried out on a carbon, hydrogen, nitrogen analyzer Perkin-Elmer 240C model; IR spectra were recorded on KBr disks on a Shimadzu FT-IR-8400S, whereas solutions spectra were recorded on a Beckmann DU-640 spectrophotometer. EPR experiments were conducted using a Bruker ESP-300 spectrometer operated at X-band frequency (9.5 GHz) with 100 KHz field modulation. DPPH was used as a field marker. The room temperature magnetic moments (μ_{eff}) were measured using a PAR vibrating sample magnetometer (VSM) model 155. Molar conductance in MeOH was measured at room temperature on an Elico conductivity bridge type Cm-82T.

RESULTS AND DISCUSSION

Methyldicyandiamide and ethyldicyandiamide have strong nitrile (C=N) band at 2145 and 2155 cm^{-1}, respectively. The IR spectra of the complexes show the absence of these bands and have a very strong ν_{asy}(C-O-C) stretch at *ca.* 1225-1205 cm^{-1} and ν_{sym}(C-O-C) at *ca.* 972-965 cm^{-1}. The IR spectra of the ligands have a band at *ca.* 1610 cm^{-1} for the alkylamidino(azomethine) C=N stretching. The appearance of a strong band at *ca.* 1585 cm^{-1} and a band at *ca.* 1662 cm^{-1} have been assigned for ν(C=N) of the alkylamidino part and for ν(C=N) of the N=C-O-C fragment of 1-methyl/ethylamidino-O-methyl/ethylurea (**I**)[4,8].



$$R{-}NH{-}\underset{\overset{\|}{NH}}{C}{-}NH{-}\underset{\overset{\|}{NH}}{C}{-}O{-}R$$

R = methyl or ethyl

(**I**)

The electronic spectra of the complexes suggest the square planar geometry having CuN_4 chromophore and the room temperature magnetic susceptibility of the complexes are close to the spin-only value for a d^9 configuration. The conductance measurements in DMF of the complexes suggest that the complexes are 1:2 electrolytes[9].

The EPR spectra of the two copper(II) complexes in the solid state showed 2 g values *i.e.*, g_{II} ~ 2.1991 and $g_{\perp}$ ~ 2.0517 at room temperature and 77 K. These features are characteristics of copper(II) at an axial site with an unpaired electron

TABLE-1
ANALYTICAL DATA, MAGNETIC MOMENT, MOLAR CONDUCTIVITIES AND ELECTRONIC SPECTRAL DATA OF SOLID BIS(1-ALKYLAMIDINO-O-ALKYLUREA)COPPER(II)COMPLEXES.

Compound	Complex	Colour	m.p. (ºC) / Yield (%)	Analytical found (calcd.) %				Conductance in MeOH at 25 ºC		λ_{max} (cm^{-1}) in methanol	μ_{eff} (BM)
				Cu	N	Anion	H_2O	Conc. (mm)	ΛM (Ω^{-1} cm^2 mol^{-1})		
[Cu(MAMUH)$_2$]Cl$_2$.H$_2$O	1	Violet	165 (80)	15.3 (15.39)	27.1 (27.15)	17.18 (17.21)	4.30 (4.36)	2	160	18100	1.88
[Cu(MAEUH)$_2$]Cl$_2$	2	Violet	170 (70)	15.0 (15.03)	26.4 (26.51)	17.0 (16.8)	–	2	155	18200	1.92
[Cu(MAMUH)$_2$]Br$_2$	3	Light violet	159 (65)	13.1 (13.14)	23.1 (23.17)	33.2 (33.06)	–	2	140	18240	1.84
[Cu(MAMUH)$_2$]I$_2$	4	Light violet	170 (55)	10.9 (10.99)	19.3 (19.39)	43.9 (43.98)	–	2	128	17959	1.79
[Cu(MAEUH)$_2$]I$_2$.H$_2$O	5	Red violet	156 (50)	10.2 (10.18)	17.9 (17.96)	40.6 (40.73)	2.90 (2.88)	2	120	18600	1.82
[Cu(EAMUH)$_2$]I$_2$	6	Light violet	180 (60)	10.4 (10.48)	18.4 (18.49)	41.9 (41.95)	–	2	125	18004	1.90
[Cu(EAMUH)$_2$]I$_2$	7	Light violet	160 (60)	9.98 (10.02)	17.6 (17.68)	40.1 (40.09)	–	2	110	17901	1.89

MAMUH = 1-Methylamidino-O-methylurea; MAEUH = 1-Methylamidino-O-ethylurea; EAMUH = 1-Ethylamidino-O-methylurea; EAEUH = 1-Ethylamidino-O-ethylurea. *Calculated values are given inside the perenthesis.

TABLE-2
EPR AND BONDING PARAMETERS OF *BIS*(1-ALKYLAMIDINO-O-METYLUREA)COPPER(II) CHLORIDE IN DMF

Complex	Temp.	λ_{max} (cm^{-1})	A_{II} (G)	$A_{\perp}$ (G)	A_{iso} (G)	g_{II}	$g_{\perp}$	g_{iso}	α^2	β^2	γ^2	K	K_{II}	$K_{\perp}$	G
[Cu(MAMUH)$_2$Cl$_2$	300	18400			85.00										
	77		198	28	84.67	2.1991	2.0517	2.1008	0.8079	0.9150	0.9169	0.3646	0.7393	0.7408	3.8510
[Cu(EAMUH)$_2$Cl$_2$	300	18320			80.00										
	77		196	30	85.30	2.1994	2.0520	2.1011	0.8028	0.9202	0.9236	0.3648	0.7383	0.7415	3.8346

in the $d_{x^2-y^2}$ orbital. The g values are closer to those reported for 4 coordinate planar complexes at room temperature, suggesting that these complexes also posses a near square planar geometry[10,11]. The EPR parameters g_{II}, $g_{\perp}$, A_{II}^{Cu}, $A_{\perp}^{Cu}$ were measured from the spectra of magnetically diluted copper complexes in frozen DMF solutions at 77 K. The absence of a half-field signal ($\Delta M_s = \pm 2$) at *ca.* 1600 G and the values of the parameters indicated that the complexes exist as mononuclear species in DMF.

The EPR parameters and *d-d* transition energies were used to evaluate the bonding parameters α^2, β^2 and γ^2, which may be expanded as measures of the covalency of the in-plane σ bonds and in-plane and out-of-plane π-bonds, respectively. The in-plane σ-bonding parameter α^2 was calculated using the expression[4]:

$$\alpha^2 = -\left(\frac{A_{II}}{0.036}\right) + (g_{II} - 2.0023) + \frac{3}{7}(g_{\perp} - 2.0023) + 0.04$$

The orbital reduction factors K_{II} and $K_{\perp}$ were estimated from the expressions[4].

$$K_{II}^2 = (g_{II} - 2.0023)E_{d\text{-}d}/8\lambda_o,$$

$$K_{\perp}^2 = (g_{II} - 2.0023)E_{d\text{-}d}/2\lambda_o$$

where $K_{II} = \alpha^2\beta^2$, $K_{\perp} = \alpha^2\gamma^2$ and λ_o represents the one electron spin-orbit coupling constant for the free ion, equal to -828 cm^{-1}. In case of a pure σ-bonding $K_{II} \approx K_{\perp}$ *ca.* 0.77, whereas $K_{II} < K_{\perp}$ implies considerable in-plane π-bonding while for out-of-plane π-bonding $K_{II} > K_{\perp}$.

Also α^2, β^2 and γ^2 are much less than 1 (the value for 100 % ionic bonding) which indicate for both strong in-plane σ and in-plane π-bonding.

In the complexes under study it is observed that $K_{II} < K_{\perp}$ indicating the presence of significant in-plane π-bonding and α^2, β^2 and γ^2 values evaluated (Table-2).

Five copper(II) complexes and $CuCl_2.2H_2O$ salt were tested for their antifungal activity. The effect of the copper(II) complexes on *C. paradoxa* is presented in Fig. 1.

The test solutions at two concentrations 0.5 and 1 % were used for antifungal study. The control (distilled water) possesses absolutely no inhibitory activity in the test organism. Similarly all the test chemicals at a concentration of 0.5 % show no inhibition zone but they show inhibition zone at 1 % concentration. The inhibition of the $CuCl_2.2H_2O$ and the synthesized metal complexes are in the order $[Cu(1\text{-ethylamidino-O-ethylurea})_2]I_2$ > $[Cu(1\text{-ethylamidino-O-methylurea})_2]I_2$ > $[Cu(1\text{-methylamidino-O-ethylurea})_2]I_2$ > $[Cu(1\text{-methylamidino-O-methylurea})_2]I_2$ > $[Cu(1\text{-methylamidino-O-methylurea})_2]Br_2$ > $CuCl_2.2H_2O$. The $CuCl_2.2H_2O$ is least resistant to this fungus when compared to the complexes. The antifungal activity of the synthesized Cu(II)-complexes depends on the size of the alkyl groups present and the concentration of the test solution used. These Cu(II) complexes have greater inhibitory activity on *C. paradoxa* compared to their corresponding metal salt ($CuCl_2.2H_2O$). This may probably be due to the extensive conjugation effects observed in the metal complexes than the metal salts.

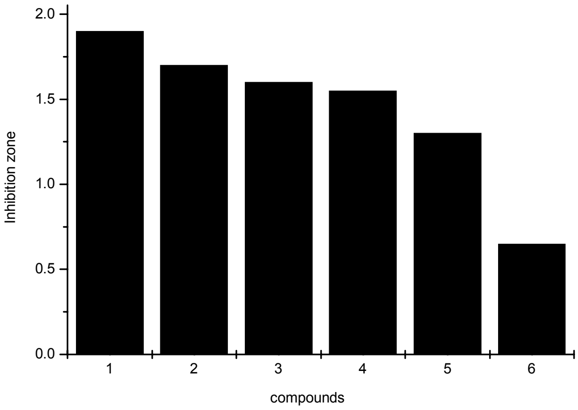


Fig. 1. Effect of $CuCl_2.2H_2O$ and its complexes on *Ceratocystis paradoxa*. 1 = $[Cu(1\text{-ethylamidino-O-ethylurea})_2]I_2$; 2 = $[Cu(1\text{-ethylamidino-O-methylurea})_2]I_2$; 3 = $[Cu(1\text{-methylamidino-O-ethylurea})_2]I_2H_2O$; 4 = $[Cu(1\text{-methylamidino-O-methylurea})_2]I_2$; 5 = $[Cu(1\text{-methylamidino-O-methylurea})_2]Br_2$; 6 = $CuCl_2.2H_2O$

Since these Cu(II) complexes have inhibitory activities against the test fungus, further investigations are needed whether it can be used as fungicide or not.

Conclusion

Seven copper(II) complexes of 1-alkylamidino-O-alkylurea where alkyl = methyl or ethyl have been synthesized and characterized. The EPR spectra of two complexes $[Cu(1\text{-methylamidino-O-methylurea})_2]Cl_2$ and $Cu(1\text{-ethylamidino-O-methylurea})_2]Cl_2$ suggest square planar geometry having CuN_4 chromophore. From the EPR data, the presence of significant in-plane π-bonding has been suggested.

The tested complexes showed growth inhibition against *C. paradoxa* (the causal organism of the soft rot disease of pineapple).

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