ISOLATION AND STUDIES ON BIOLOGICAL ACTIVITIES OF MIXED LIGAND COMPLEXES OF COPPER(II) WITH 2.2'-BIPYRIDYL AND α-AMINO ACIDS

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ABSTRACT

The 1 : 1 : 1 copper(II)–2, 2'-bipyridyl (bipy)-α-amino acids [glycine (gly), α-alanine (ala) and β-phenylalanine (phe)] complexes have been prepared and isolated by chromatography and characterised by IR spectral studies and other methods. The biological activities of these complexes have been compared with the activities of copper(II) chloride, bipy, HCl and the binary complexes of copper(II) with bipy, gly and ala against different micro-organisms varying from gram-positive (*Streptococcus pyogenes*), gram-negative (*Escherichia coli* and *Proteus vulgaris*) to fungi (*Aspergillus tamarii* and *Penicillium frequentum*) and protozoa (*Euglena* and *Paramecium* species). The growth inhibition activity of ternary complexes has been found to be higher or comparable to other test compounds on gram-negative bacteria and protozoa and no inhibitory activity has been observed against gram-positive bacteria and fungi.

INTRODUCTION

THE bacteriostatic activities of 1, 10-phenanthroline and 2, 2'-bipyridyl (bipy) bases, their quaternary salts and metal complexes of identical or mixed ligands have been compared on gram-positive, gram-negative and acid fast organisms[1]. Dutta and De[2] reported the isolation of blue ternary complexes of copper(II) with bipy and glycine (gly)/α-alanine (ala) by crystallisation contaminated with shining green complex; however, in the present communication the preparation and isolation of ternary complexes of copper(II) with bipy and gly/ala/β-phenylalanine (phe) by column chromatography in pure state have been reported. These ternary complexes have been isolated to substantiate the results of pH-metric studies[3] and to test their biological activity against micro-organisms ranging from gram-positive (*Streptococcus pyogenes*), gram-negative (*Escherichia coli* and *Proteus vulgaris*) to fungi (*Aspergillus tamarii* and *Penicillium frequentum*) and protozoa (*Euglena* and *Paramecium* species).

EXPERIMENTAL

Copper(II) chloride, 2,2'-bipyridyl.HCl and α-amino acid (glycine, α-alanine or β-phenylalanine) in doubly distilled water were mixed in equimolar proportions. The mixture was neutralised with 0·1 M KOH solution and the final pH of the solution was adjusted to ~ 7·5. The blue crystals were isolated from the concentrated solution by evaporating at room temperature, which were then dissolved in the minimum quantity of MeOH and chromatographed over neutral Al_2O_3 with MeOH : Me_2CO (3 : 1) and water respectively.

1 : 1 : 1 Copper(II)-2,2'-bipyridyl-α-amino acid mixed ligand complexes

The MeOH: Me_2CO (3 : 1) elutions furnished blue crystalline product which was dissolved in MeOH and a blue amorphous solid was obtained by adding excess of ether, and the compound was dried in vacuum over anh. $CaCl_2$. The elemental analysis and other data are given in Table I.

1 : 1 Copper(II) 2,2'-bipyridyl complex

The water fractions gave green crystalline solid which was rectystallised from water and identified as copper(II)-2,2'-bipyridyl complex associated with chloride ions.

The 1 : 2 binary complexes of bipy, gly and ala with copper(II) were prepared in the usual manner.

Preparation of test solutions

Stock solutions of copper(II)chloride, 2, 2'-bipyridyl . HCl, bis(2,2'-bipyridyl) copper(II) chloride, bis(glycinato) copper(II), bis(α-alaninato) copper(II), glycinato (2,2'-bipyridyl) copper(II) chloride, α-alaninato (2, 2'-bipyridyl) copper(II) chloride and β-phenylalaninato (2,2'-bipyridyl) copper(II) chloride were prepared. The copper(II) chloride solution was standardized by titrating against standard EDTA.

* For correspondence.

TABLE I

Elemental analysis and other data of copper(II)–2,2′-bipyridyl-α-amino acid complexes

Compounds	Colour	M.P.s*	C obs.	C cal.	H obs.	H cal.	Cl obs.	Cl cal.	N obs.	N cal.	Cu obs.	Cu cal.
(A)	blue	d227–228°	43·62	43·77	3·55	3·64	10·64	10·79	12·69	12·86	19·54	19·30
(B)	blue	d230–231°	45·29	45·47	3·98	4·07	10·13	10·25	12·12	12·24	18·47	18·51
(C)	blue	d235°	54·19	54·40	4·19	4·29	8·28	8·47	9·91	10·02	15·30	15·15

* d = decomposition before melting.

Preparation of media

The biological experiments were performed in solid agar media for the bacteria and fungi. The standard milk agar, the violet red bile salt agar and potato dextrose agar were prepared to study the biological activity of the abovementioned compounds on *S. pyogenes* and *P. vulgaris*, *E. coli* and fungi (*A. tamarii* and *P. frequentum*) respectively. To obtain the isolated species, *Euglena* and *Paramecinm* were allowed to multiply in artificial media.

The experiments on bacteria and fungi were carried out in aseptic conditions using disc method. All the agar dishes were incubated at 37° C for bacteria and at 28° C for fungi for 48 hrs. After incubation, the growth of the microorganisms was measured. The observations have been recorded in Table III. Euglenae and Paramecia were treated with test solution of different concentrations for different periods and the observations are recorded in Table IV.

RESULTS AND DISCUSSION

The ternary complexes of copper(II) with 2,2′-bipyridyl and glycine/α-alanine/β-phenylalanine are characterized by their colour, solubility, melting points, elemental analysis and IR spectral studies. All the complexes are blue solids, soluble in polar solvents (H_2O, MeOH, EtOH) and decompose before melting at 227–228°, 230–231° and 235° C respectively.

The elemental analysis of these ternary complexes are in good agreement with the molecular formula : $CuC_{12}H_{12}ClN_3O_2$, $CuC_{18}H_{14}ClN_3O_2$ and $CuC_{10}H_{18}ClN_3O_2$ expected for glycinato (2,2′-bipyridyl) copper(II) chloride (A), α-alaninato (2,2′-bipyridyl) copper(II) chloride (B) and β-phenylalaninato (2,2′-bipyridyl) copper(II) chloride (C) respectively as recorded in Table I.

The important absorption peaks in the infrared spectra of these complexes are listed in Table II. The

TABLE II

Characteristic IR data of copper(II)-2, 2′-bipyridyl-α-amino acid complexes

(A)	(B)	(C)	Assignments
3430, 3270–3210	3450–3350, 3230–3200	3450–3320	N–H stretching
1640	1630	1602	C ··· O stretching
528	550	480	M–N stretching
418	..	415	M–O stretchnig
775, 732	775, 730	768, 730	Characteristic bands of 2,2′-bipyridyl ring

Compounds (A), (B) and (C) are the ternary complexes in Tables I and II.

formation of metal-nitrogen and metal-carboxylate bonding in the complexes is indicated[4–7] by the lowering of the N–H and C ··· O stretching vibrations. The metal-nitrogen (M–N) and metal-oxygen (M–O) stretching vibrations have been observed[8] below 550 cm^{-1}. The two strong peaks observed[9,10] at 770 and 730 cm^{-1} characterise the 2,2′-bipyridyl ring.

The biological activity of copper(II) chloride, 2,2′-bipyridyl . HCl, their binary and ternary chelates with α-amino acids and binary chelates of copper(II) with glycine and α-alanine on gram-positive (*S. pyogenes*), gram-negative (*E. coli* and *P. vulgaris*) microorganisms, fungi (*A. tamarii* and *P. frequentum*) and protozoa (*Euglena* and *Paramecium* species) has been recorded in Tables III and IV respectively.

TABLE III

Biological activity of copper(II) chloride, 2,2′-bipyridityl.HCl, binary and ternary complexes with the base and α-amino acids on bacteria and fungi

Compounds Nos.***	Molar conc. M	*S. pyogenes*	*E. coli*	*P. vulgaris*	*A. tamarii*	*P. frequentum*
1	0·04	×	+ (3mm)*	−	−	−
	0·004	×	−	−	−	−
	0·0004	×	×	×	−	−
	0·00004	×	−	−		
2	0·04	−	+ (4mm)*	+ (7mm)* ×	+ (8mm)** ×	+(10mm)** ×
	0·004	×	−	−	−	−
	0·0004	×	−	−	−	−
	0·00004	×	−	−		
3	0·04	−	+ (3mm)*	+ (14mm)*	−	+ (12mm)**
	0·004	× (10mm)	−	−	−	−
	0·0004	× (15mm)	−	−	−	−
	0·00004	× (12mm)	−	−		
4	0·04	−	+ (2mm)* ×	−	−	−
	0·004	×	−	−	−	−
	0·0004	×	−	−	−	−
	0·00004	×	−	−		
5	0·04	−	+ (3mm)* ×	−	−	−
	0·004	×	−	−	−	−
	0·0004	×	−	−	−	−
	0·00004	×	−	−		
6	0·04	−	+ (1mm)* ×	+ (10mm)**	−	×
	0·004	×	−	−	−	−
	0·0004	×	−	−	−	−
	0·00004	×	−	−		
7	0·04	−	+ (3mm)* ×	+ (12mm)**	−	×
	0·004	−	−	−	−	−
	0·0004	×	−	−	−	−
	0·00004	×	−	−		
8	0·04	−	+ (5mm)* ×	+ (13mm)*	−	−
	0·004	−	−	−	−	−
	0·0004	×	−	−	−	−
	0·00004	×	−	−		

\+ growth inhibition * = growth inhibition zone

** = incomplete growth inhibition zone. × = growth stimulation.

− = neither inhibition nor stimulation in growth.

*** 1. Copper(II) chloride. 2.2, 2′-bipyridyl . HCl. 3. bis (2,2′-bipyridyl) copper(II) chloride. 4. bis (glycinato) copper(II). 5. bis (α-alaninato) copper(II). 6. glycinato (2,2′-bipyridyl) copper(II) chloride. 7. α-alaninato (2,2′-bipyridyl) copper(II) chloride. 8. β-phenylalaninato (2,2′-bipyridyl) copper(II) chloride.

TABLE IV

Biological activity of copper(II) chloride, 2,2′-bipyridyl HCl, binary and ternary copper(II) complexes with the base and α-amino acids on protozoa

Compound Nos.*	Molar conc. M	Time** to kill the protozoa	
		Euglena	*Paramecium*
1	0·01	15 m	15 m
	0·004	15 m	15 m
	0·002	1 h	30 m
	0·001	24 h	24 h
2	0·01	30 m	15 m
	0·004	1 h	1 h
	0·002	24 h	24 h
	0·001	24 h	24 h
3	0·01	30 m	15 m
	0·004	1 h	30 m
	0·002	24 h	24 h
	0·001	24 h	24 h
4	0·01	30 m	30 m
	0·004	1 h	1 h
	0·002	24 h	24 h
	0·001	24 h	24 h
5	0·01	30 m	30 m
	0·004	1 h	1 h
	0·002	24 h	24 h
	0·001	24 h	24 h
6	0·01	30 m	15 m
	0·004	30 m	30 m
	0·002	24 h	24 h
	0·001	24 h	24 h
7	0·01	30 m	15 m
	0·004	1 h	30 m
	0·002	24 h	24 h
	0·001	24 h	24 h
8	0·01	15 m	15 m
	0·004	30 m	15 m
	0·002	1 h	1 h
	0·001	24 h	24 h

* Name and order of the compounds are as mentioned in Table III.

** m = minutes, h = hours.

Note : In active protozoa, free from test compounds, after keeping in fresh nutrient medium for twenty-four hours were found to be dead.

The activities of these compounds reveal that each class of these compounds shows the highest inhibitory activity against gram-negative organisms. The gram-positive organism has been found to be resistant towards all the compounds. All the compounds, more or less, stimulate the growth of *S. pyogenes* at 0·004 M or lower concentrations. The growth stimulating action among 2,2′-bipyridyl . HCl and its copper(II) derivatives against *S. pyogenes* has been found to be more pronounced specially that of bis (2,2′-bipyridyl) copper(II) chloride. The trend of growth inhibition has been observed in case of mixed ligand derivatives against *E. coli* and incomplete inhibition is clearly shown by these complexes in the case of *P. vulgaris*. The binary complex of 2,2′-bipyridyl indicates large inhibition among all the test compounds against *P. vulgaris* at 0·04 M concentration and 2,2′-bipyridyl . HCl at the same concentration against the same organism shows a clear zone of inhibition followed by growth stimulation, however, against *E. coli* only a small inhibition zone has been observed. The comparative study of activity of these compounds shows that except β-phenylalaninato (2,2′-bipyridyl) copper(II) chloride, all the mixed ligand complexes have been found to be less effective than the base salt and its binary complex. Copper(II) chloride itself gave no indication to inhibit the growth of *P. vulgaris*. It rather stimulates the growth of *S. pyogenes* and a very slight growth inhibiting tendency has been shown against *E. coli*. The binary complexes of α-amino acids more or less enhance the growth in *S. pyogenes* at all concentrations and an incomplete inhibition of low magnitude followed by the growth stimulation has been observed in *E. coli* at 0·04 M concentration. Neither inhibition nor stimulation to growth has been observed in the case of *P. vulgaris* at any concentration.

It is evident from the observation that only 2, 2′-bipyridyl salt and its binary copper(II) complex are found to be growth inhibitors. 2,2′-bipyridyl salt is most active at 0·04 M concentration against *A. tamarii* and *P. frequentum* species. At higher dilutions the activity has been found totally absent. 2,2′-bipyridyl . HCl shows incomplete inhibition zone in both the species. Bis (2, 2′-bipyridyl) copper(II) chloride shows a broad range of incomplete inhibition only in *P. frequentum* and no sign of inhibition has been observed in *A. tamarii* at 0·04 M concentration. None of the mixed ligand complexes or copper(II) chloride and copper(II) chelates of α-amino acids show any activity within the range of 0·04 M to 0·0004 M concentration against any of the species.

The activity of these compounds noted against protozoa reveals that *Paramecium* is more susceptible than *Euglena*. All the compounds are able to kill *Paramecia* comparatively in a shorter period. Copper(II)

chloride itself has been found to be more effective than the rest of the compounds. The mixed ligand complexes are found to be less effective than the base monohydrochloride and its binary complex. All the compounds are found to be potent to kill the protozoa upto 0·001 M concentration within 24 hrs and higher concentration has proved to be more effective in shorter period.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF *a*, *a'*-THIOBISFORMAMIDINE DERIVATIVES: PART II

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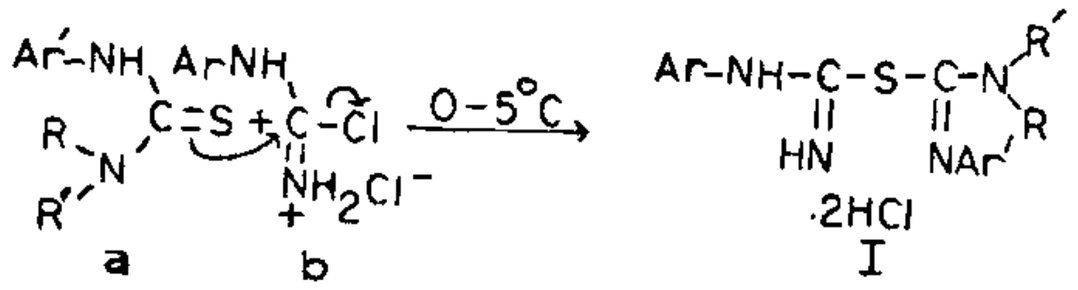
ABSTRACT

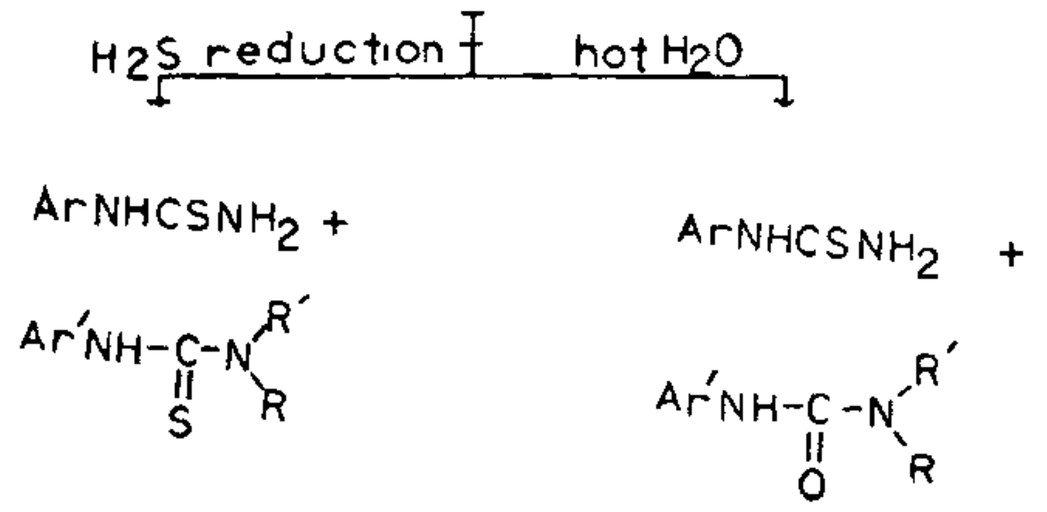
A series of N^1,N^4 diaryl/aryl alkyl-N^3,N^3 dialkyl/N^3 alkyl-*a*,*a'* thiobisformamidine salts have been prepared by the condensation of *a*-chloroarylformamidines with 1-aryl-3 alkyl/3,3 dialkyl thioureas in ether-acetone medium at 0–5° C. Their local anaesthetic, antifungal, larvicidal activities and chemical behaviour have been studied.

INTRODUCTION

INTERACTION of *a*-chloroarylformamidine hydrochlorides[1,2] with monosubstituted aryl[3], alkyl[4] or acylthioureas[5] has been investigated. The present study of the reaction with 1, 3 arylalkyl or 1, 3, 3-aryldialkylthioureas was undertaken to see the effect of substitution on the course of reaction and also to evaluate the biological potency of newly formed compounds.

At lower temperatures (0–5°) *a*-chloroarylformamidine hydrochlorides reacted with 1-aryl-3, 3-dialkyl/3-alkyl-thioureas in ether-acetone medium to afford corresponding *a*, *a'*-thiobisformamidine salts (I), as shown in scheme 1.





SCHEME I

The structure of I was confirmed on the basis of decomposition products and analysis of their picrates. On boiling with water compound (I) decomposed into mono-substituted arylthiourea corresponding to the *a*-chloroarylformamidine hydrochloride used and di- or tri-substituted arylalkyl urea corresponding to the thiourea used. Reduction of I with hydrogen sulphide in alcoholic ammonia gave arylthiourea and di- or tri-substituted arylalkylthioureas.