Proc. Indian natn. Sci. Acad., **55**, A, No. 3, 1989, pp. 563–569.

FORMATION CONSTANTS AND ANTIMICROBIAL ACTIVITY STUDIES ON SOME TERNARY COMPLEXES OF COPPER(II) WITH ACETYLACETONE AND SALICYLIC ACIDS

Y ANJANEYULU *and* R PRABHAKARA RAO

Department of Chemistry, Nagarjuna University, Nagarjuna Nagar 522510, India

(*Received 27 April 1987; Accepted 17 May 1988*)

The formation constants of the ternary complexes of the type [CuAB] (where HA = acetylacetone and H_2B = Salicylic–, 5-chloro–, 3,5-dibromo–, 3,5-diiodo–, 3,5-dinitro–, thio–, and acetyl–, salicylic acids) are determined in ethanol–water (70 : 30 v/v solutions and I = 0.05M $NaClO_4$, 30°C with mass-balance equations using a computer programme written in Fortran IV. The antibacterial and antifungal activity of all these complexes are also studied. The difference in their toxic action against various bacteria and fungi has been explained on the basis of their formation constants and lipo solubility.

Key Words : Formation Constants; Antimicrobial; Cu(II); Acetylacetone; Salicylic Acid; Lipo Solubility; Antifungal

INTRODUCTION

SYNTHESIS and ESR studies on the ternary complexes of copper(II) with acetylacetone and salicylic acids were reported in our earlier communications.[1,2] In continuation of these studies, the complex formation equilibria of these ternary complexes are studied *p*H-metrically and an attempt has been made to correlate the antibacterial and antifungal activity of these complexes with their formation constants and lipophilic tendency. The results are discussed in this paper.

EXPERIMENTAL

The ligands used in this work were Fluka and BDH products of AnalaR quality. Copper(II) perchlorate solution was prepared by neutralising copper(II) carbonate with perchloric acid. The concentration of the copper(II) stock solution was determined by titrating with EDTA. Double distilled water and ethanol were used for the preparation of all the solutions.

The *p*H titrations were carried out at 30 °C and at an ionic strength of 0.05M $NaClO_4$ under nitrogen atmosphere using a digital *p*H meter (Systronics of India Ltd., Model 335, accuracy $\pm$0.01 *p*H unit) with a Toshniwal Moellor glass electrode. Due to the low solubility of these complexes in water, 70 per cent ethanol was used as a solvent. The electrode system was calibrated in terms of hydrogen ion concentration.[3]

Antimicrobial Activity

The solid binary and ternary complexes of copper(II) with acetylacetone and salicylic acids were synthesised in pure state by the procedures described earlier[1] and the antimicrobial activity of these complexes in DMF was studied *in vitro* by the serial dilution method against bacteria and by the paper disc method against fungi. The authentic stock cultures were supplied by the Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India. Peptone water and saline water were used for making the inoculum for bacteria and fungi (18h cultures) respectively. Nutrient broth and Sabouraud's dextrose agar were used as test media for bacteria and fungi respectively. The minimum inhibition concentration MIC (μg/ml) of the compounds against certain important human pathogenic bacteria and the average zone of inhibition (mm) of the compounds at 1000μg/ml against the fungi are given in Table III. All tests were carried out in triplicate.

Results and Discussion

The stability constant for the ternary systems were computed from the titrations in which the total concentrations of copper(II) (0.00098M) : ligand A : ligand B is kept at 1 : 1 : 1. The protonation constants for the free ligands A and B, and the stability constant data for the parent binary complexes of Cu(II) with A and B were estimated using Irving-Rossotti[4] technique at 30 °C and I = 0.05M $NaClO_4$ (Table I) and these values were held constant in the computer calculations of the ternary systems.

The formation constants of ternary complexes (β_{MAB} = [MAB]/[M][A][B]) with HA and H_2B are calculated using appropriate mass-balance equations and solving the corresponding cubic equations.[5] A computer programme is developed

Table I

Acid dissociation constants of the ligands and formation constants of binary complexes of copper (II)

(I = 0.05M $NaClO_4$, 30 °C)

Name of the ligand	pK_1	pK_2	log K^{Cu}_{CuB}	log $K^{CuB}_{CuB_2}$
acetylacetone	9.80		*10.30	**8.65
salicylic acid	3.20	13.25	9.90	7.75
5-chlorosalicylic acid	2.70	12.20	9.70	7.45
3,5-dibromosalicylic acid	2.55	10.40	9.30	7.10
3,5-diiodosalicylic acid	3.80	11.25	9.20	6.90
3,5-dinitrosalicylic acid	1.55	7.70	5.05	2.45
thiosalicylic acid	5.40	9.50	9.10	7.25
acetylsalicylic acid	4.60		4.00	3.10

*log K^{Cu}_{CuA} **log $K^{CuA}_{CuA_2}$

written in Fortran IV to refine the stability constants of binary complexes and to calculate the formation constants of ternary complexes and the concentrations of various complex species present in the solution at each point on the titration. The calculations were executed with an IBM computer. The calculations were restricted to *p*H values below 8, in order to avoid complications due to the hydrolysis of various complex species at higher *p*H. The results obtained are listed in Table II. The charges for all the complex species reported in this paper are omitted for clarity.

TABLE II

Stability constants of ternary complexes of copper (II) with acetylacetone (A) and ligand (B) systems

(I = 0.05M $NaClO_4$, 30 °C)

Ligand (B)	log β_{CuAB}	Δ log K	log X
salicylic acid	20.00	— 0.20	3.40
5-chlorosalicylic acid	19.50	— 0.50	2.95
3, 5-dibromosalicylic acid	18.85	— 0.75	2.35
3, 5-diiodosalicylic acid	18.75	— 0.75	2.50
3, 5-dinitrosalicylic acid	15.10	— 0.25	3.70
thiosalicylic acid	19.30	— 0.10	3.30
acetylsalicylic acid	13.15	— 1.15	0.25

Distribution of the Complex Species for the Ternary System-(Acetylacetonato)-(Salicylato) Copper(II)

The typical distribution of the various complex species formed in the solution (as a percentage of the total metal ion concentration) with respect to *p*H when the concentration of Cu(II) : acetylacetone : salicylic acid is maintained at 1 : 1 : 1 is shown in Fig 1. These investigations revealed that the 1 : 1 Cu(II)–acetylacetone forms to an extent of 90 per cent around *p*H 3.5 and its concentration gradualy decreases with increase in *p*H, whereas the corresponding 1 : 2 complex exists to an extent of 10 per cent in the *p*H range 4.5 to 5.5. Similarly, the 1 : 1 Cu (II)-salicylate forms to an extent of 8 per cent in *p*H range 5 to 6 whereas the corresponding 1 : 2 complex exists to an extent of 13 per cent. The ternary complex formation starts at *p*H 3.5 and increases with increase in the *p*H and reaches to 70 per cent at *p*H 6.5. The species distribution diagram with *p*H for the other systems also show similar qualitative features as observed in Fig 1.

In order to characterise the stability of ternary complex species in relation to that of the parent binary complexes, the values of Δ log K, the difference in stability between the binary and ternary complexes and log X, the disproportionation constant, were determined (Table II) for all the ternary systems. From statistical considerations, it was shown that considerably less negative values of Δ log K and more positive values of log X indicate greater stabilities of the ternary complexes relative to the binary analogues.

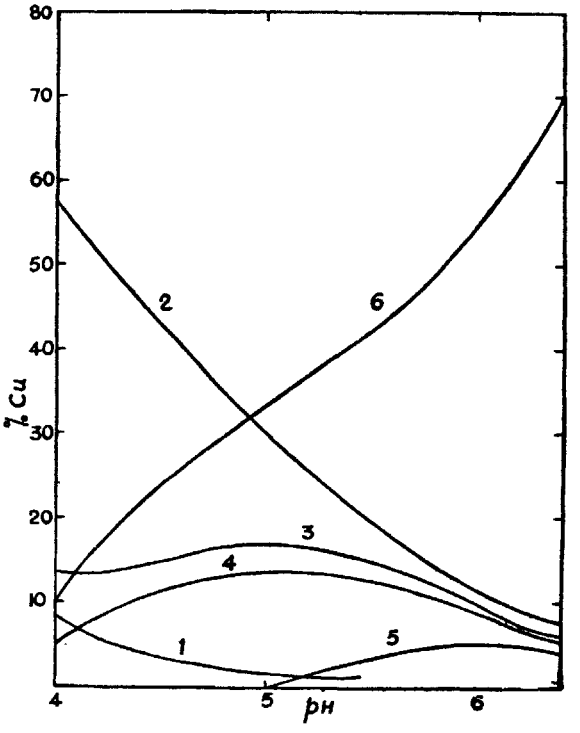

FIG 1 Distribution of complex species for Cu(II)-acetylacetone (A)—salicylic acid (B) system 1. Cu, 2. CuA, 3. CuA_2, 4. CuB, 5. CuB_2, 6. CuAB.

The parameters $\Delta \log K$ and $\log X$ for the [CuAB] complexes are determined by equations (1) to (4)

$$[CuA] + [CuB] \rightleftharpoons [CuAB] + Cu \qquad ...(1)$$

$$\Delta \log K = \log {}^{\beta}CuAB - (\log {}^{\beta}CuA + \log {}^{\beta}CuB) \qquad ...(2)$$

$$[CuA_2] + [CuB_2] \rightleftharpoons 2\,[CuAB]$$

$$^{X}CuAB = [CuAB]^2/[CuA_2[[CuB_2] \qquad ...(3)$$

$$\log X = 2 \log {}^{\beta}CuAB - (\log {}^{\beta}CuA_2 + \log {}^{\beta}CuB_2) \qquad ...(4)$$

The $\log X$ values in Table II for all the ternary systems are significantly more positive than those expected on statistical ground indicating considerable interaction of the ligands through the Cu(II) ion in ternary complex formation. However, the $\Delta \log K$ values do not deviate much from the statistical values.[6] The low $\Delta \log K$ value in the case of acetylsalicylic acid may be most likely due to the steric hindrance in ternary complex formation. From the data given in Table II, it can be seen that the order of stability constants $\log {}^{\beta}CuAB$ for the mixed complexes is

salicylic–, > 5-chloro–, > thio–, > 3,5-dibromo–, > 3,5-diiodo–, > 3,5-dinitro–, > acetyl

Stability Constants and Antimicrobial Activity

The antibacterial and antifungal activity of bis(salicylato)Cu(II) bis(acetylacetonato) Cu(II) and the ternary complexes of Cu(II) with acetylacetone and salicylic acids are systematically studied against certain important human pathogenic bacteria and fungi. To understand the lipophilic tendency of these complexes, their distribution between chloroform and 7.4 *p*H phosphate buffer, (which is considered as a good biological model[7,8] to understand the lipid solubility) is determined and is expressed in terms of log *D* values (Table III). These investigations revealed that salicylic acid, substituted salicylic acids and their Cu(II) complexes do not possess any activity against the growth of the bacteria and fungi studied even at relatively high concentrations (for bacteria > 500ppm and fungi > 2000μg/ml) which may be due to their lower lipid solubility. Acetylacetone and its binary Cu(II) chelate are found to possess low activity even though their partition in chloroform is high, while the ternary complexes of Cu(II) with acetylacetone and salicylic acids in general are found to possess high toxic activity than the binary complexes. The enhancement in activity of the ternary complexes over the binary complexes become more pronounced when compared in terms of the molarity of the complexes than in ppm.

Our earlier investigations gave evidence for the possibility of two types of mechanisms for the toxic action exhibited by these metal chelates against the growth of the bacteria and fungi.

(*a*) Thiols, which are the vital constitugents in the living cells, having reduction potential for the disulphide form at or below 200mV, may be oxidised by copper complexes as per the redox reaction

$$2RSH + Cu(A)(B) \rightleftharpoons Cu\,(I)\,SR + \tfrac{1}{2}RSSR + A + B$$

which restricts the growth of the organisms in the presence of these complexes.

(*b*) The neutral complex first penetrate the cell and at the site of action may undergo dissociation[9] into corresponding 1 : 1 binary complexes as shown below

$$2CuAB \rightleftharpoons Cu(A) + Cu(B) + A + B$$

and these coordinatively unsaturated species become toxic entities by combining with and blocking the metal binding sites of enzymes.

Thus, in the mixed complexes, in addition to 1 : 1 copper-acetylacetone, the 1 : 1 copper-salicylate may also be acting as a toxic agent which increases their activity. The 1 : 1 copper-salicylate is better transported to the site of action as mixed complex than the binary copper–salicylate complexes. The binary copper salicylates are less toxic as due to their lower lipid solubility and considerable amount of 1 : 1 copper-salicylate complexes cannot reach the side of action. As per both the above mechanisms the toxic activity of the complexes will be controlled by their stability.

TABLE III

Antimicrobial activity, formation constant and distribution data of the ternary complexes of copper (II)

Name of the compound	log *D*	log β_{CuAB}	log K^{Cu}_{CuB}	antifungal activity (mm) (average zone of inhibition)				antibacterial activity MIC (μg/ml)				
				a	*b*	*c*	*d*	*A*	*B*	*C*	*D*	*E*
1	+ 0.60	20.00	9.90	13	15	14	13	3.1	6.2	6.2	12.5	12.5
2	+ 0.18	19.50	9.70	10	10	10	10	6.2	12 5	12.5	25	25
3	+ 0.37	18.85	9.30	10	10	10	10	6.2	12.5	12.5	25	25
4	+ 0.48	18.75	9.20	11	10	10	10	6.2	12.5	12.5	25	25
5	− 0.76	15 10	5.05	16	18	15	16	1.6	6.2	3.1	3.1	6.2
6	− 0.51	19.3	9.10	—	7	8	8	25	50	25	50	50
7	− 0.47	13.15	4.00	—	—	—	—	50	100	50	100	100

Compound : 1. (acetylacetonato) (salicylato) Cu (II)., 2. (acetylacetonato) (5-chlorosalicylato) Cu (II).
3. (acetylacetonato) (3,5-dibromosalicylato) Cu(II)., 4. (acetylacetonato) (3, 5-diiodosalicylato) Cu(II).
5. (acetylacetonato) (3,5-dinitrosalicylato) Cu(II)., 6. (acetylacetonato) (3 thiosalicylato) Cu(II).
7. (acetylacetonato) (acetylsalicylato)Cu(II).

Fungi : *a. Pencillium spp*; *b. Aspergillus niger*; *c. Aspergillus fumigatus*; *d. Candida salbicans*;

Bactaria : *A. Staphylococcus albus*; *B. Staphylococcus aureus*; *C. Pseudomonas pyogenes*; *D. Vibrio cholorae*; *E. Shegalla sonnei*;

If we examine the log $^{\beta}$CuAB, log K^{Cu}_{CuB} and log D values of these complexes and their corresponding antimicrobial activity, it can be seen that the antimicrobial activity of these complexes is dependent on both formation constant and liposolubility. As can be seen from Table III, except in the case of (acetylacetonato) (3,5-dinitrosalicylato) Cu(II), that the complexes with the same value of log $^{\beta}$CuAB + log D or log K^{Cu}_{CuB} + log D have equal toxic activity against many microorgansims indicating that both these factors control the activity. (Acetylacetonato) (3,5-dinitrosalicylato) Cu(II) has shown exceptionally high activity with all the organisms studied, and similar exceptional behaviour is also made in the case of ternary complexes of Cu(II) with 8-hydroxyquinoline and 3,5-dinitrosalicylic acid. These investigations further confirm that chelation alone is not sufficient for enhancing the antimicrobial activity but it must be accompanied by lipid solubility also.

Acknowledgements

One of us (R P Rao) is thankful to the University Gvants Commission, New Delhi for the award of Research Associateship.

References

1 Y Anjaneyulu and R Prabhakara Rao *Synth React inorg metal-org Chem* **16** (1986) 257
2 Y Anjaneyulu, N V S, Rao R Prabhakara Rao and V G K M Pisipati *Proc Indian Acad Sci (Chem Sci)* **97** (1986) 177
3 L G Van Uitert and W C Fernelius *J Am chem Soc* **75** (1953) 451 3862
4 H M Iriving and H S Rossotti *J chem Soc* (1953) 3397
5 M T Beck *Chemistry of Complex Equilibria* Van Nostrand Reinhold Company London (1970) 70-73
6 H Sigel *Angew Chem* **14** (1975) 394
7 A Albert, C W Rees and A J H Tomlindon *Rec Trev Chim* **75** (1956) 394
8 A B Durkee *Agric Food Chem* **6** (1958) 194
9 H Gershon, S G Stephen and David Olney *Contr Boyce Thompson Inst* **24** (1969) 167