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Heterobinuclear Cu(II)–L-Carnosine Complexes with Cd(II) or Zn(II) in Aqueous Solution*

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The heterobinuclear complexes of Cu(II)–L-carnosine (car) with Cd(II) or Zn(II) (M(II)) were studied in aqueous solution at $t = 37.0 \pm 0.1$ °C and $I = 0.15$ mol dm^{-3} ($K[NO_3]$). The three binary systems were investigated as well, in the same experimental conditions. The results pointed out that both Cd(II) and Zn(II) do not form binary species with dissociation of peptide hydrogen, while for Cu(II) the most relevant species is a dimeric complex in which the deprotonation of peptide group of L-carnosine very probably occurs. The heterobinuclear complexes that we evidenced in solution are of the type $[CuM(car)H_{-1}]^{2+}$ and $[CuM(car)H_{-2}]^{+}$ for both Cd(II) and Zn(II).

Introduction

The complex formation in solution of di- or tripeptides with metal ions was investigated to a some extent [1–6] in binary systems. Some ternary systems formed by a metal ion and two different ligands were also considered [7–9], while only the tripeptide glutathione was investigated as to the formation of ternary complexes in the presence of La(III), Zn(II) and Zn(II), Ca(II) [10]. The heterobinuclear complex formation may be relevant in solving analytical problems of multicomponent solutions and, sometimes, in the speciation of bio-fluids. In this connection we have considered some peptides which can also be seen as model ligands. Some data were reported in the literature [1–3] on the complex formation of metal ions with glycyl-L-histidine and β-alanyl-L-histidine (L-carnosine, car): they seem to indicate that the structural characteristics of the component aminoacids affect the stoichiometry and the stability of the species formed in solution.

X-ray [11] and e.s.r. [12] investigations have suggested that dimeric species are present in the system Cu(II)–L-carnosine, while only monomeric species are formed when Cu(II) and glycyl-L-histidine are considered [12]. Potentiometric data agree with these conclusions only for glycyl-L-histidine [1, 2]: for Cu(II)–L-carnosine there is disagreement as to the existence of a relevant dimeric species of the type $[Cu_2(car)_2H_{-2}]$, reported by Perrin *et al.* [1] and not evidenced by Pettit *et al.* [2] for whom the predominant complex in solution is $[Cu(car)H_{-1}]$.

Recently we reported [13] heterobinuclear Cu(II) complexes formed with L-histidine or histamine (L) and Cd(II), Zn(II) or Ni(II) (M) of the type $[CuML_2H_p]$ (with $p = 1, 0, -1, -2$). The dimeric structure of these heterobinuclear complexes seemed to be related to the existence of dimeric species of the type $[Cu_2L_2H_{-2}]$.

The aim of this work was the study of the behaviour of L-carnosine in the presence of two metal ions, namely Cu(II) and Cd(II) or Cu(II) and Zn(II). Before studying the heterobinuclear complexes, we also examined the binary systems since no information was found in the literature for Cd(II)–car complexes and there is disagreement as to the existence of dimeric $[Cu_2(car)_2H_{-2}]$ species [1, 2].

Experimental

L-carnosine was a Fluka product, the purity of which was checked by alkalimetric titrations.

In order to avoid the hydrolysis of dipeptide, a precisely weighed amount of L-carnosine was added to the solutions just before the beginning of the titrations. When necessary, the imidazole nitrogen and carboxylate group were protonated by addition of known amounts of $H[NO_3]$.

The stability of the dipeptide in solution with respect to hydrolysis was checked, in the absence of metal ions, by thin layer chromatography of solutions brought to $-\log c_H \cong 9.5$: no significant formation of β-alanine or L-histidine was found. The other reagents were prepared as described elsewhere [14–16].

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TABLE I. Values of Analytical Concentration for Alkalimetric Titrations of Binary and Ternary Complexes (mmol dm^{-3}).

C_{Cu}	C_{car}	C_{Zn}	C_{car}	C_{Cd}	C_{car}
3.0	3.0	5.0	5.0	4.0	4.0
4.0	4.0	3.0	6.0	4.0	8.0
5.0	5.0	3.0	9.0	3.0	9.0
6.0	6.0	4.0	12.0	4.0	12.0
3.0	6.0	3.0	12.0	3.0	12.0
3.5	7.0	15.0	3.0	15.0	3.0
4.0	8.0				
9.0	3.0				
8.0	4.0				

C_{Cu}	C_{Zn}	C_{car}	C_{Cu}	C_{Cd}	C_{car}
3.0	15.0	3.0	3.0	15.0	3.0
3.5	10.0	4.0	3.5	10.0	4.0
4.0	14.0	4.0	5.0	10.0	5.0
3.0	12.0	3.0	3.5	15.0	3.5
3.0	15.0	6.0	3.0	15.0	6.0
4.0	15.0	8.0	4.0	12.0	8.0

The study was done by taking potentiometric measurements of hydrogen ion concentration with a model E 600 METROHM potentiometer equipped by glass and saturated calomel electrodes supplied by the same firm, at t = 37.0 ± 0.1 °C and I = 0.15 mol dm^{-3} ($K[NO_3]$). The alkalimetric titrations were carried out in a stream of purified nitrogen. Temperature control was achieved by means of a liquid circulation from a thermostat. The standardization of glass electrode both in acidic and alkaline range was made by titrating nitric acid (4÷6 mmol dm^{-3}) with standard, carbonate free, K[OH].

When the contribution of a bivalent cation to ionic strength was highly significant (with bivalent cation in excess of the concentration of the ligand) the standardization was made in the presence of the bivalent cation as well, at the same concentration. The difference found in the value of E°, when C_{cd} = 15 mmol dm^{-3} is about 0.3–0.5 mV and becomes smaller if the excess concentration diminishes. Some experimental details for binary and ternary systems are collected in Table I (C's with subscript indicate analytical concentrations).

The equilibrium conditions and the stability of the ligand with respect to hydrolysis were controlled by back-titrations. The type of species present in solution and their stability constants were first evaluated graphically [17, 18] and then refined by two different least squares computer programs [17–19].

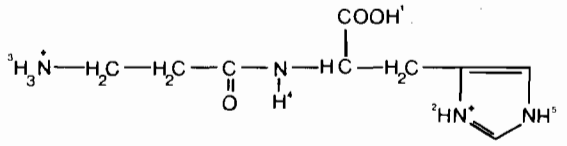
TABLE II. Values of Protonation Constants (as pK_i^H) of L-carnosine and log of Stability Constants of Zn(II) and Cd(II) Binary Complexes: t = 37.0 ± 0.1 °C, I = 0.15 mol dm^{-3} ($K[NO_3]$), standard deviations (σ) in parenthesis.

	a	b	c	d
pK_1^H	2.64(2)	2.64	2.60	–
pK_2^H	6.59(1)	6.58	6.835	6.75
pK_3^H	9.04(1)	9.04	9.466	9.32
	Zn(II)		Cd(II)	
	a	e	a	
$\log \beta_{0111}$	11.33(3)	11.22	11.32(2)	
$\log \beta_{0110}$	4.11(2)	3.86	3.03(1)	
$\log \beta_{0120}$	–	–	5.13(3)	

[a]This work. [b]Ref. [1]. [c]Ref. [2]. [d]Ref. [21]. [e]Ref. [3]. [c,d]Data obtained at t = 25 °C.

Results

The fully protonated form of L-carnosine (I) exhibits five hydrogen atoms (numbered in the formula (I)) which can dissociate. In the absence of



(I)

metal ions, up to $-\log c_H \cong 10$, there is the dissociation of three hydrogen ions per molecule, from carboxyl (1), imidazole (2) and amino (3) groups; the deprotonation of amide group (4) becomes important at $-\log c_H$ values where the hydrolysis of the dipeptide is relevant. The dissociation of pyrrole group (5) is normally not significant if metal ions are not present. Therefore we considered this ligand as H_3car^{2+} (or H_3A^{2+}), as did previously other authors [1, 2]. The values of the protonation constants, as pK_i^H, are listed in Table II and compared with some literature data.

In the presence of Cu(II) the dissociation of hydrogen ion exceeds the three equivalents per mol of ligand. Such excess may be probably related to the dissociation of the amide group, but the sole potentiometric measurements are not sufficient to distinguish whether the hydrogen ion in excess comes

from amide group, pyrrole group or a hydrolysis reaction.

The stability constants of the binary and ternary complexes were expressed in the most general form

$$\beta_{nn'pq} = \frac{c_{[Cu_nM_{n'}A_qH_p]}}{c_{Cu}^n c_M^{n'} c_A^q c_H^p}$$

where M = Zn(II) or Cd(II) and c s with subscript indicate concentrations at equilibrium. In the study of the binary systems we considered all the stoichiometric ratios between the reagents ($C_{Cu} \leqslant C_A$ and $C_M \leqslant C_A$) because of the experimental conditions needed in order to obtain a larger amount of heterobinuclear species (excess of M(II)) and for the possible formation of homobinuclear complexes.

Binary Systems

Zn(II), Cd(II)

The formation of binary complexes Zn(II)–car and Cd(II)–car was at first studied by alkalimetric titrations with stoichiometric rations $C_A/C_M \geqslant 1$. In solutions containing Zn(II), even with an excess of ligand, equilibrium was not found above $-\log c_H \cong 7$. The treatment of our experimental data both with graphical method and numerical refinement confirmed the presence in solution of the same species previously reported by Perrin *et al.* [3], namely $[Zn(car)H]^{2+}$ and $[Zn(car)]^+$. For Cd(II) ion, besides the complexes found for Zn(II), the complex $[Cd(car)_2]$ was also evidenced. The titration curves obtained in the presence of an excess of metal ion were then calculated with the values of the stability constants previously refined for ratios $C_A/C_M \geqslant 1$. Both for Zn(II) and Cd(II) the mean error between the experimental and calculated C_{OH} values was <0.5%. This result also confirms that the glass electrode calibration is reliable even in the presence of an excess of metal ion.

In Table II are listed the values of the stability constants calculated by considering all the titration curves at the same time ($C_A/C_M \geqslant 1$). In the same Table the values of Perrin *et al.* [3] are also collected: the log β_{0111} are in good agreement, while our log β_{0110} is slightly higher.

Cu(II)

Preliminary data obtained for Cu(II) with reagent ratios $C_A/C_{Cu} \geqslant 1$ were refined by numerical approximation using as starting values those found by Perrin *et al.* [1] in the same experimental conditions and by Pettit *et al.* [2] for different temperature and ionic strength. Both the type of species present in solution and the values of the stability constants agree with the data of Perrin, but this set of constants does not allow a correct calculation of the titration curves when $C_A/C_{Cu} < 1$. The analysis of the

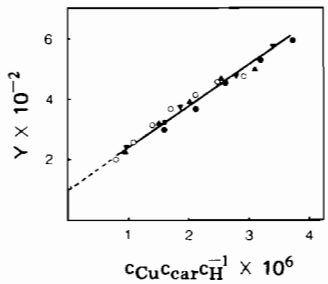


Fig. 1. Values of Y *vs.* $X = C_{Cu}C_{car}C_H^{-1}$ for Cu(II)–car system. • C_{Cu} = 3.00; C_{car} = 3.00. ▲ C_{Cu} = 4.00; C_{car} = 4.00. △ C_{Cu} = 5.00; C_{car} = 5.00. ○ C_{Cu} = 6.00; C_{car} = 6.00. (The concentrations are expressed in mmol dm^{-3}).

distribution curves *vs.* $-\log c_H$ shows that the dimeric species 202–2 is negligible up to $-\log c_H = 4.8$ for all C_A/C_{Cu} ratios and moreover it comes out from a preliminary treatment of the data that species with the ratio $C_A/C_{Cu} < 1$ must be also taken into account.

The numerical refinement of the data up to $-\log c_H \cong 4.8$ confirms that, in addition to the species $[Cu(car)H]^{2+}$ and $[Cu(car)]^+$, a complex of the type $[Cu_2(car)H_{-1}]^{2+}$ is probably formed in solution.

In order to verify whether the complexation scheme initially supposed for concentration ratios $C_A/C_{Cu} \geqslant 1$ was modified or not by the presence of the complex $[Cu_2(car)H_{-1}]^{2+}$ the data for ratios $C_A/C_{Cu} = 1$ were graphically analyzed by using the approximate values of log β_{1011}, log β_{1010} and log β_{201-1} already determined.

A suitable linear combination of the mass balance equations allows the calculation of c_{Cu}, c_A and a function Y [17, 18]

$$Y = \frac{C_{car} - c_{Cu}c_{car}(\beta_{1011}\, c_H + \beta_{1010} + c_{Cu}c_H^{-1}\beta_{201-1})}{c_{Cu}c_{car}c_H^{-1}} =$$

$$= \beta_{101-1} + \beta_{202-2}c_{Cu}c_{car}c_H^{-1}$$

The experimental values of Y plotted *vs.* $X = c_{Cu}c_{car}c_H^{-1}$ (Fig. 1) are arranged on a straight line with positive slope and intercept, so we can conclude that even in the presence of the species $[Cu_2(car)H_{-1}]^{2+}$ it seems likely to suppose the existence of a dimeric complex involving the dissociation of the peptide hydrogen.

The stability constants of the different complexes were refined both by a least squares computer program based on $\bar{z}$ function [18] and by MINIQUAD 76A [19] and the results agree very well with each other. The values are collected in Table III with their standard deviations; by the set of these stability constants the mean error on C_{OH} (base added for each point of titration curves) is always less than

TABLE III. Values of log of Stability Constants of Cu(II)–L-carnosine Complexes and of Heterobinuclear Ternary Complexes. t = 37.0 ± 0.1 °C, I = 0.15 mol dm^{-3} ($K[NO_3]$), standard deviations (σ) in parenthesis.

	Cu(II)		
	a	b	c
$\log \beta_{1011}$	12.65(3)	13.02	13.98
$\log \beta_{1010}$	8.12(1)	8.14	8.52
$\log \beta_{101-1}$	1.9(1)	1.90	2.92
$\log \beta_{201-1}$	5.34(2)	–	–
$\log \beta_{202-2}$	7.93(1)	7.96	–
$\log \beta_{2020}$	–	18.56	–
$\log \beta_{1021}$	20.28(5)	20.25	–
$\log \beta_{1020}$	14.05(4)	14.39	–
$\log \beta_{102-1}$	5.23(4)	5.70	5.37
$\log \beta_{101-2}$	–8.25(5)	–	–8.28
$\log \beta_{202-3}$	2.42(4)	–	–
$\log K^{Cu}_{Cu(CuAH_{-1})}$	3.44	–	–
	Cu(II)–Zn(II)/Cu(II)–Cd(II)		
	a		a
$\log \beta_{111-1}$	4.42(3)		4.47(3)
$\log \beta_{111-2}$	–2.46(4)		–3.43(5)
$\log K^{M}_{M(CuAH_{-1})}$	2.52		2.57

$$K^{Cu}_{Cu(CuAH_{-1})} = \frac{c[Cu_2AH_{-1}]}{c_{Cu}c[CuAH_{-1}]}$$

$$K^{M}_{M(CuAH_{-1})} = \frac{c[CuMAH_{-1}]}{c_{M}c[CuAH_{-1}]}$$

[a]This work. [b]Ref. [1], 25 °C and 0.1 mol dm^{-3} ($K[NO_3]$). [c]Ref. [2].

±0.5%. In Fig. 2 are drawn the distribution curves for ratios C_A/C_{Cu} = 1(a) and C_A/C_{Cu} = 2(b): the most relevant species in solution is $[Cu_2(car)_2H_{-2}]$, while the complex $[Cu_2(car)H_{-1}]^{2+}$ is important only at the beginning of the titration, in particular for a ratio C_A/C_{Cu} = 1.

This behaviour is consistent with the results found by our preliminary investigations and by Perrin *et al.* [1] according to which a good approximation can be obtained even if we neglect the species $[Cu_2(car)H_{-1}]^{2+}$. Our set of stability constants is generally in good agreement with that of Perrin *et al.* [1], specially for the most relevant species: the species $[Cu_2(car)_2]^{2+}$ which we have not found, seems to be not very important in the overall equilibrium, while the complexes $[Cu(car)H_{-2}]^-$ and $[Cu_2(car)_2H_{-3}]^-$ are quite negligible in the $-\log c_H$ range investigated by the quoted authors.

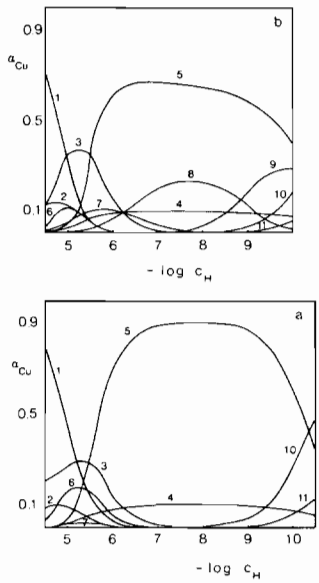


Fig. 2. Distribution of the species, as α_{Cu} *vs.* $-\log c_H$ for Cu(II)–car system. a) C_{Cu} = 4.00; C_{car} = 4.00. b) C_{Cu} = 3.00; C_{car} = 6.00. (The concentrations are expressed in mmol dm^{-3}). 1) Cu^{2+}; 2) $[Cu(car)H]^{2+}$; 3) $[Cu(car)]^+$; 4) $[Cu(car)H_{-1}]$; 5) $[Cu_2(car)_2H_{-2}]$; 6) $[Cu_2(car)H_{-1}]^{2+}$; 7) $[Cu(car)_2H]^+$; 8) $[Cu(car)_2]$; 9) $[Cu(car)_2H_{-1}]^-$; 10) $[Cu_2(car)_2H_{-3}]^-$; 11) $[Cu(car)H_{-2}]^-$.

Ternary Systems

The formation of ternary species can be evidenced by comparing the experimental titration curves in the presence of the three reagents with the curves calculated assuming that only binary complexes are formed in solution. For heterobinuclear complexes we obtained the best experimental conditions using an excess of the metal ion which forms weaker complexes with the ligand considered. Some experimental details for the titrations of ternary systems are collected in Table I. The excess concentrations of Cd(II) or Zn(II) are the same as those we considered in the study of binary systems.

In Table IV are reported the Δ % values (percent difference between experimental and calculated C_{OH}) both for Cu(II)–Cd(II) and Cu(II)–Zn(II). While Δ % is quite negligible in the initial part of the titration curve, it takes significant values with increasing $-\log c_H$ and also shows a maximum.

According to what has been previously observed in the calculation of the titration curves of binary systems, we can consider as meaningful, an error in the value of C_{OH} higher than 1%: this condition and the systematic deviation of C_{OH} at the same $-\log c_H$ value (the corresponding error between experimental and calculated $-\log c_H$ values, for the same C_{OH}, is in the range 0.05–0.25), may indicate the presence in solution, of ternary heterobinuclear species. Both the graphical method [17, 18] and the

TABLE IV. Percent Difference (Δ %) between Experimental and Calculated C_{OH}.[a]

$-\log c_H$	Cd(II)		
	$C_{OH_{calc}}$	$C_{OH_{exp}}$	Δ %
4.0	2.95	2.96	0.33
4.5	3.71	3.75	1.06
5.0	5.77	6.14	6.02
5.5	8.77	9.32	5.95
6.0	10.94	11.19	2.23
6.5	11.70	11.84	1.18
7.0	11.91	12.09	1.48
7.5	11.98	12.42	3.54
$-\log c_H$	Zn(II)		
	$C_{OH_{calc}}$	$C_{OH_{exp}}$	Δ %
4.0	2.95	2.95	–
4.5	3.71	3.77	1.59
5.0	5.77	6.12	5.71
5.5	8.77	9.30	5.69
6.0	10.94	11.26	2.84
6.5	11.70	12.15	3.70
7.0	11.91	13.01	8.45

[a] $C_{Cu} = C_A = 3.00$; $C_M = 15.00$; $C_H = 9.00$ (M = Cd(II) or Zn(II)); concentrations in mmol dm^{-3}.

numerical refinement [17–19] showed that the experimental data can be well interpreted by considering the complexes $[CuM(car)H_{-1}]^{2+}$ and $[CuM(car)H_{-2}]^{+}$. The values of the stability constants are listed in Table III. In Table V the formation percentages (referred to Cu(II)) are collected for the most relevant species, when $C_{Cu} = C_{car} = 3.00$ and $C_{Cd} = 15.0$ or $C_{Zn} = 15.0$.

Discussion and Conclusions

Our results on binary Cu(II)–car system confirm the existence in solution of the dimeric complex $[Cu_2(\mathbf{car})_2H_{-2}]$ previously reported by Perrin *et al.* [1]: the relevance of this dimeric species was also emphasized by an e.s.r. investigation on frozen aqueous solutions [12]. In the same paper the authors pointed out that with C_A/C_{Cu} ranging from 100 to 1000, four L-carnosine molecules are bonded, via the imidazole nitrogen, to each Cu(II) ion. The solid state investigation, by X-ray diffraction [11], showed that in the dimeric complex Cu(II) ions are bound to L-carnosine via amino, imidazole and amide (peptide) nitrogens. The dissociation of the amide hydrogen is typical for peptide compounds: Pettit *et al.* [4] suggested that, depending on $-\log c_H$ values, the interaction of peptide group with

TABLE V. Percentages of Cu^{2+} Ion Present in Heterobinuclear Complexes ($C_{Cu} = C_A = 3.00$; $C_M = 15.00$ mmol dm^{-3}; M = Cd(II) or Zn(II)).

	$-\log c_H$	Cu^{2+}	$[CuAH]^{2+}$	$[CuA]^{+}$	$[CuAH_{-1}]$	$[Cu_2A_2H_{-2}]$	$[Cu_2AH_{-1}]^{2+}$	$[CuMAH_{-1}]^{2+}$	$[CuMAH_{-2}]^{+}$
Cd(II)	4.5	84.2	6.8	6.3	0.1	–	1.7	0.7	–
	5.0	48.9	7.4	21.9	1.3	1.4	10.7	7.2	–
	5.5	16.0	2.5	23.6	4.5	16.4	11.9	23.7	–
	6.0	4.0	0.4	11.3	6.8	37.3	4.5	35.0	0.4
	6.5	1.0	–	4.0	7.6	45.9	1.3	38.5	1.5
	7.0	0.3	–	1.3	7.7	46.8	0.4	38.7	4.9
	7.5	–	–	0.4	7.2	41.8	0.1	36.0	14.4
Zn(II)	4.5	84.2	6.8	6.4	0.1	–	1.7	0.6	–
	5.0	49.1	7.5	22.1	1.3	1.4	10.8	6.5	–
	5.5	16.1	2.6	24.0	4.6	16.9	12.2	21.6	1.0
	6.0	4.0	0.4	11.3	6.8	37.2	4.5	31.3	4.1
	6.5	1.1	–	3.7	7.2	41.1	1.3	32.2	13.4
	7.0	0.3	–	1.0	6.2	30.3	0.3	26.7	35.1

Cu(II) occurs first by coordination to amide oxygen, while at higher $-\log c_H$ values the complexes are stabilized by replacement of the amide hydrogen. For peptides which have no coordinating group in the side chain, dimeric species were not found. The imidazole residue of L-carnosine increases the number of potentially coordinating groups but this condition is not sufficient for the formation of a dimer. The e.s.r. data have suggested that the dimension of chelation ring are also important, since both glycyl-L-histidine and L-homocarnosine do not form dimeric complexes [12].

Our data also suggest the presence of the species $[Cu_2(car)H_{-1}]^{2+}$ which was not found either with L-histidine or histamine [20]; the number of potentially donor groups allows the addition of a second Cu(II) ion if the two coordination centers are sufficiently apart.

The formation of the species $[Cu_2(car)H_{-1}]^{2+}$ might occur with the addition of a Cu(II) ion to the complex $[Cu(car)H_{-1}]$. Previous findings [1, 2] indicate that in this monomeric species there is an hexatomic chelation ring, including the terminal amino group and the amide nitrogen; moreover Pettit *et al.* [2] suggested an extra-stabilization of this complex by a second heptaatomic chelation ring, including also the imidazole nitrogen.

Our results do not agree with Pettit *et al.* as to the particular stabilization of $[Cu(car)H_{-1}]$, and it looks likely that the imidazole nitrogen is available for the coordination of the second Cu(II) ion. The same hypothesis may be put forward for Zn(II) or Cd(II) binding in heterobinuclear complexes: Zn(II) and Cd(II) do not form species in which the amide group is deprotonated, so it is very unlikely that they interact with the peptide group of the ligand in heterobinuclear species.

It seems more probable that there is a reaction between the complex $[Cu(car)H_{-1}]$ and heteroion

$$[Cu(car)H_{-1}] + M^{2+} \rightleftharpoons [CuM(car)H_{-1}]^{2+}$$

The equilibrium constant of the above reaction should be not very different from that reported for the interaction between metal ions and imidazole [21]. The value of stability constants for hetero- or homobinuclear species, according to our hypothesis, may be estimated by

$$\beta_{111-1} = \beta_{101-1} \cdot \beta^{M}_{MIm}$$
$$\beta_{201-1} = \beta_{101-1} \cdot \beta^{Cu}_{CuIm} \qquad (1)$$

where Im = imidazole. The approximate values of stability constants, for imidazole complexes, which can be calculated at 37.0 °C and I_c = 0.15 mol dm^{-3} (K[NO_3]) by literature data [21] are $\log \beta^{Cd}_{CdIm} \cong$ 2.69, $\log \beta^{Zn}_{ZnIm} \cong 2.46$ and $\log \beta^{Cu}_{CuIm} \cong 3.97$. By these values we obtain, from (1), $\log \beta_{111-1} \cong 4.6$ for Cd(II), $\log \beta_{111-1} \cong 4.4$ for Zn(II) and $\log \beta_{201-1} \cong 5.9$. The agreement between the experimental (Table III) and estimated values is good for Cd(II) and Zn(II); in fact the difference is lower than the uncertainty on $\log \beta_{101-1}$. For Cu(II), the estimated value is significantly higher and suggests that a heteroion such as Cd(II) or Zn(II) is favoured in bonding formation with $[Cu(car)H_{-1}]$ species, if our hypothesis on the reaction of heterobinuclear complex formation is valid.

Both with Cd(II) and Zn(II) our data are consistent with the presence in solution (at higher $-\log c_H$ values) of a complex $[CuM(car)H_{-2}]^{+}$; the values of the stability constants, significantly higher for Zn(II), rather suggest the existence of a hydrolytic equilibrium than the dissociation of =NH pyrrole group of histidyl residue.

References

1 R. P. Agarwal and D. D. Perrin, *J Chem. Soc. Dalton,* 268 (1975).
2 G. Brookes and L. D. Pettit, *J. Chem. Soc. Dalton,* 2112 (1975).
3 R. P. Agarwal and D. D. Perrin, *J. Chem. Soc. Dalton,* 1046 (1975).
4 G. Brookes and L. D. Pettit, *J. Chem. Soc. Dalton,* 2106 (1975).
5 A. Gergely and I. Nagypal, *J. Chem. Soc. Dalton,* 1104 (1977).
6 R. P. Agarwal and D. D. Perrin, *J. Chem. Soc. Dalton,* 53 (1977).
7 H. Sigel, *Inorg. Chem., 14,* 1535 (1975).
8 I. Nagypal and A. Gergely, *J. Chem. Soc. Dalton,* 1109 (1977).
9 H. Sigel, C. F. Naumann, B. Prijs, D. B. Cormick and M. C. Falk, *Inorg. Chem., 16,* 790 (1977).
10 M. L. D. Touche and D. R. Williams, *J. Chem. Soc. Dalton,* 1355 (1976).
11 H. C. Freeman and J. T. Szymanski, *Acta Crystallogr., 22,* 406 (1967).
12 C. E. Brown, W. E. Antholine and W. Froncisz, *J. Chem. Soc. Dalton,* 590 (1980).
13 P. Amico, G. Arena, P. G. Daniele, G. Ostacoli, E. Rizzarelli and S. Sammartano, *Inorg. Chem., 20,* 772 (1981).
14 G. Ostacoli, P. G. Daniele and A. Vanni, *Ann. Chim., 63,* 815 (1973).
15 P. G. Daniele, G. Ostacoli and V. Zelano, *Ann. Chim., 65,* 455 (1975).
16 P. G. Daniele, P, Amico and G. Ostacoli, *Ann. Chim. 69,* 61 (1979).
17 G. Ostacoli, P. G. Daniele and A. Vanni, *Ann. Chim., 65,* 197 (1975).
18 P. G. Daniele, G. Ostacoli and A. Vanni, *Atti Acc. Sci. Torino, 109,* 547 (1974–75).
19 P. Gans, A. Sabatini and A. Vacca, *Inorg. Chim. Acta, 18,* 237 (1976).
20 P. G. Daniele and G. Ostacoli, *Ann. Chimica, 66,* 387 (1976).
21 R. M. Smith and A. E. Martell, 'Critical Stability Constants', Vol. 2, Plenum Press, London–New York, 1975.