Thermodynamics of Formation of Complexes of Copper(II) and Nickel(II) Ions with Glycylhistidine, β-Alanylhistidine, and Histidylglycine †

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Complexes formed between H^+, Cu^{2+}, *and* Ni^{2+} *and glycylhistidine, β-alanylhistidine, and histidylglycine have been studied potentiometrically at 25 °C and* $I = 0.10$M ($K[NO_3]$). The equilibria are very complicated but polynuclear species are insignificant below pH 10 in dilute solution (10^{-3}M). Suggestions are made as to the probable co-ordination sites as a function of pH.

THE results of a detailed potentiometric study of Cu^{2+} and Ni^{2+} complexes of a number of dipeptides containing only non-co-ordinating substituent groups have been presented in the preceding paper.[1] It was shown that the presence of bulky groups in the amino-half of the dipeptide had a marked effect on the stability of the $[CuL]^+$ complex (where HL is the neutral dipeptide) while they had little effect when substituted in the carboxyl half. With Cu^{2+} the most important complexed species was $[Cu(H_{-1}L)]$ in which the amide hydrogen ion was displaced to permit Cu^{2+}–amide nitrogen bonding. This species was predominant in the pH range 4—8 and was comparatively insensitive to the metal to ligand ratio. With Ni^{2+} the ionization of the amide proton was delayed until pH >8, the predominant species at low pH values being $[NiL]^+$ and $[NiL_2]$.

Dipeptides formed from amino-acids containing side-chains with potential donor centres are extremely important in biological systems. Among such dipeptides those containing histidine, His, are particularly interesting since the imidazole group in histidine contains a labile proton which ionizes in the intermediate pH region. We have therefore extended our study of the Cu^{2+} and Ni^{2+} complexes of dipeptides to include complexes with glycyl-L-histidine, Gly-His, β-alanyl-L-histidine (carnosine), Car, and L-histidyl glycine, His-Gly. These ligands were selected because they only introduce one additional donor centre compared to the simple dipeptides and this is incorporated in the amine half of the dipeptide in the last ligand and the carboxyl half in the other two. A comparison of Gly- and βAla-His demonstrates the effect of increasing ring size in complexes involving the amine half of the dipeptide (the more important half as far as bonding centres is concerned).

Complexes of His dipeptides have been studied by a number of workers but few thermodynamic results have been reported. In most cases the equilibria involved have been oversimplified. A recent review of co-ordination chemistry of imidazole complexes describes some of the work that has been carried out and highlights some of the problems and uncertainties.[2] The motivation for the present interest in His complexes is the finding that the His- residue plays a prominent role in the binding of metal ions to proteins. Histidyl dipeptides are better models for such protein binding sites than His itself and hence are particularly relevant. Carnosine (βAla-His) is found in animal muscle but cannot be a fragment of protein since these contain only α-amino-acid residues.

† No reprints available.

[1] G. Brookes and L. D. Pettit, preceding paper.

[2] R. J. Sundberg and R. B. Martin, *Chem. Rev.*, 1974, **74**, 471.

However, the additional methylene group provides valuable evidence in elucidation of the binding sites in related dipeptides.

The structure of a solid Cu^{2+}–Car complex has been determined.[3] It is a dimer with each Cu^{2+} bonded to an amino-nitrogen, a deprotonated amide nitrogen, and a carboxylate oxygen atom from one Car and an imidazole nitrogen from the neighbouring Car to give the $[Cu_2(H_{-2}L_2)]$ species. It has been argued that a dimer is also present in solution,[2] but until very recently no formation constants had been reported, the evidence being inferred from assorted observations. The most detailed study of Cu^{2+}–Car complexes is a ^1H n.m.r. study by Ihnat and Bersohn[4] who interpreted their results at high pH without introducing the dimeric complex. However, the data can be interpreted otherwise as pointed out by Sundberg and Martin.[2] Where formation constants have been reported, the models selected and the constants reported often differ considerably.[5,6]

The crystal structure of Cu^{2+}–Gly-His has also been determined by Freeman and his co-workers.[7] It is not a dimer (*cf.* Cu^{2+}–Car). Co-ordination is through the amino-, amide, and imidazole nitrogen atoms with a water molecule and a carboxyl group from a neighbouring ligand completing the co-ordination sphere. In solution it is thought that this bridging carboxyl–copper bond is absent,[5] but until very recently formation constants quoted were few and unreliable since they only partially described the system.

Since this study was completed and assessed, formation constants for the Cu^{2+} complexes of the ligands, studied at 37 °C and $I = 0.15$M, have been published by Agarwal and Perrin.[8] The models they select are, in most cases, very similar to those we propose, and, allowing for the different temperatures used, their results are close to ours.

EXPERIMENTAL

Dipeptides were obtained from the Sigma Chemical Co. as SIGMA grade.

Complex-formation constants were calculated from potentiometric-titration data with the aid of the MINIQUAD computer program[9] as described previously.[1]

RESULTS AND DISCUSSION

The behaviour of Cu^{2+} towards the dipeptides differed from that of Ni^{2+}. The results will therefore be considered separately. Calculated H^+ and Cu^{2+} complex-formation constants are given in Table 1, together with those recently reported by Agarwal and Perrin[8] and some earlier results. The convention used[1] was that accepted for describing the overall formation constants for reaction (1). Stepwise constants are referred to similarly,

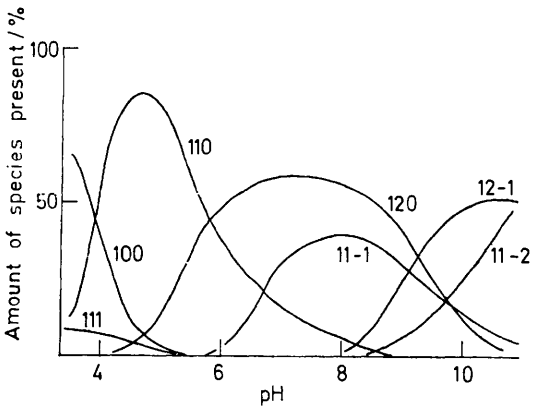
$$xM + yL + zH \xrightarrow{\beta_{xyz}} [M_xL_yH_z] \qquad (1)$$

e.g. K_{110}^{11-1} refers to reaction (2). The acid-association

$$[M(H_{-1}L)] + H \longrightarrow ML \qquad (2)$$

constants, K_{011}^{010}, refer to protonation of the amine nitrogen, K_{012}^{011} to protonation of the imidazole pyridine-like nitrogen, and K_{013}^{012} to protonation of the carboxyl group to give the species H_3L^{2+}. The values calculated are close to those reported by other workers,[5,6,8,10,11] and are readily explained in terms of electronic effects found in the component amino-acids.

Complexes with Cu^{2+}.—The major complex below pH 6 is the 110 species, rather than 11—1 found with simple dipeptides.[1] Also at low pH the monoprotonated 111 complex $[Cu(HL)]^{2+}$ is important and at higher pH values the 11—1 and 11—2 species become significant. A typical species distribution is shown in the Figure. In each case attempts were made to fit a large number of different models to the experimental data and the models quoted in Table 1 gave indisputably the best statistical fit. It is therefore reasonable to claim that all the complexed species are actually present in the equilibrium mixture, although there may be some uncertainty about the binuclear species 22—2 and 22—3. However, although they made a relatively small contribution in the pH range studied, their omission caused an appreciable decrease in the goodness of fit at high pH. They have therefore been included, particularly in view of other evidence for their existence.[3] The only significant difference between the models we propose and those of Agarwal and Perrin is the 22—2 dimeric complex of Car.[8] In all other cases our conclusions on the probable donor sites of the ligands are consistent with theirs.



Species-distribution plots for a 1 : 2 mixture of Cu^{2+} and His-Gly

Examination of the formation constants permits the following observations on the probable bonding sites.

[3] H. C. Freeman and J. T. Szymanski, *Acta Cryst.*, 1967, **B22**, 406.
[4] M. Ihnat and R. Bersohn, *Biochemistry*, 1970, **9**, 4355.
[5] R. B. Martin and J. T. Edsall, *J. Amer. Chem. Soc.*, 1960, **82**, 1107.
[6] G. R. Lenz and A. E. Martell, *Biochemistry*, 1964, **3**, 750.
[7] J. F. Blount, K. A. Fraser, H. C. Freeman, J. T. Szymanski, and C. H. Wang, *Acta Cryst.*, 1967, **B22**, 396.
[8] R. P. Agarwal and D. P. Perrin, *J.C.S. Dalton*, 1975, 268.
[9] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.
[10] G. F. Bryce, R. W. Roeske, and F. R. N. Gurd, *J. Biol. Chem.*, 1965, **240**, 3837; 1966, **241**, 1072.
[11] H. Dobbie and W. Kermack, *Biochem. J.*, 1955, **59**, 246.

(i) The protonated complex of Car (111 species) was suggested by Lenz and Martell.[4] The formation constant for reaction (3), calculated from Table 1 (log K_{111}^{011} 4.51),

$$Cu^{2+} + HL \longrightarrow [Cu(HL)]^{2+} \quad (3)$$

is close to that for reaction of Cu^{2+} with imidazolylpropionic acid (4.56),[12] making it reasonable to assume that co-ordination is between the imidazole nitrogen and the carboxyl oxygen, the amino-nitrogen atom being protonated as in (A). The structure of the 111 His-Gly complex is less obvious. Comparison with the bonding centres in His complexes [13] is limited since the His carboxyl group is not present in the dipeptide. Assuming one of the donor nitrogen atoms is protonated, bonding must be between the amide oxygen atom and the non-protonated nitrogen giving two possible ' microcomponents ' for the 111 complex, (H) and (I). Intercomparison of the various stepwise constants suggests that (H) may be the more important component, particular because the chelate ring is smaller than in (I). Glycylhistidine did not form a $[Cu(HL)]^{2+}$ complex in detectable quantities.

(ii) The $[CuL]^{+}$ complexes (110) for all three dipeptides have higher stabilities than the corresponding complexes with simple dipeptides,[1] suggesting participation of the amine nitrogen and an imidazole nitrogen atom in the bonding scheme, presumably the pyridine-like rather than the pyrrole nitrogen.[2] With His-Gly a histamine mode of bonding, (J), would account for this higher stability. However, there is no such straightforward explanation for this additional stability with the other two dipeptides if the bonding centres are limited to the amine and imidazole nitrogen atoms and the carboxyl and amide oxygens. The only way in which co-ordination to two or more nitrogen atoms can be envisaged without destroying the planar *trans*-peptide bond is to assume ionization of the amide hydrogen before ionization of either the amine or imidazole hydrogen atoms, accompanied by metal–amide nitrogen (as opposed to amide oxygen) bonding. As a result two structures could contribute to the 110 species of Gly-His and Car, (B) and (C). The lower stability of the Car complex (log β_{110} 8.54) as compared to that for Gly-His (9.14) is a reflection of the greater stability associated with a five-membered chelate ring in (C) rather than a six-membered ring. If structures (B) or (C) are correct, proton displacement from the amide nitrogen takes place by pH 4. This is only a little lower (*ca.* 0.5 pH units) than the corresponding ionization in complexes of simple dipeptides and seems entirely reasonable. Further support for this early ionization of the amide proton is the delayed formation of the 11—1 species (in which copper-amide nitrogen bonding is generally accepted) until pH values well above those found for this reaction with simpler dipeptides. The importance of the 110 complexes in His dipeptides compared to the 11—1 complexes in simple dipeptides is therefore explained.

(iii) Deprotonation of the 110 species of Gly-His and Car permits additional co-ordination through the amine (or imidazole) nitrogen atoms to form the very stable tridentate 11—1 species (D). It would be helpful to know the rate constants and activation energies for the 110 ⟶ 11—1 reactions. These parameters have been measured for the reaction between Cu^{2+} and Gly-Gly [14] and the slowness and large activation energy observed were explained as resulting from the structural rearrangement involving bond breakage which would occur if the structure of the $[CuL]^{+}$ complex is based on the Rabin model,[15] *i.e.* as in (4). If the 110 ⟶ 11—1 equilibrium between Cu^{2+} and Gly-His were found to be rapid with a low activation energy, the non-Rabin-type co-ordination proposed above for the 110 species

TABLE 1

Formation constants for the complexes $[Cu_x(H_zL_y)]$ at 25 °C and I = 0.10M ($K[NO_3]$). Standard deviations are given in parentheses

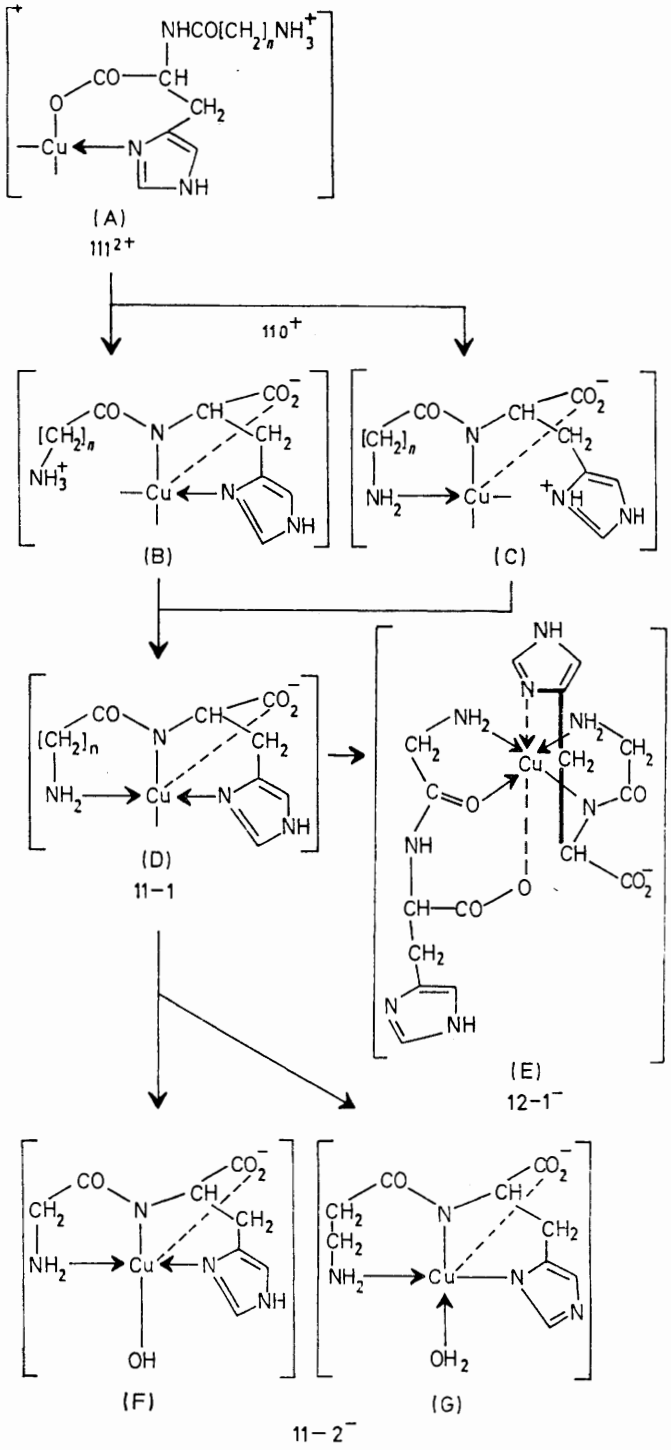
	Gly-His			Car [a]				His-Gly		
		b	*c*		*b*	*d*	*e*		*b*	*f*
log K_{011}^{010}	8.195(2)	7.97	8.24	9.466(3)	9.04	9.70	9.36	7.695(3)	7.15	7.50
log K_{012}^{011}	6.751(3)	6.61	6.77	6.835(4)	6.58	6.90	6.76	5.936(3)	5.39	5.58
log K_{013}^{012}	2.46(1)	2.66	2.66	2.60(1)	2.64	2.77		2.82(1)	2.32	2.96
log β_{xyz}										
111		12.25		13.98(1)	13.02			11.99(3)		
110	9.144(9)	8.68		8.52(1)	8.14	8.65	9.72	8.833(3)	8.02	
120	16.53(1)	15.41			14.4					
11—1	4.885(9)	4.54		2.92(1)	1.90			0.76(8)	1.72	
11—2	−4.840(3)	−4.94		−8.28(1)				−8.74(5)		
12—1	8.44(1)	7.68		5.37(4)	5.70			5.884(3)	5.66	
22—2	11.8(1)?				8.0				6.9	
22—3	2.2(1)?							4.7(1)?		
log K_{110}^{11-1} (pK_1')	4.26	4.14	4.00	5.60	6.24	5.55	5.14	8.07	6.30	6.01
log K_{11-1}^{11-2} (pK_2')	9.72	9.48	9.25?	11.20				9.49		10.6

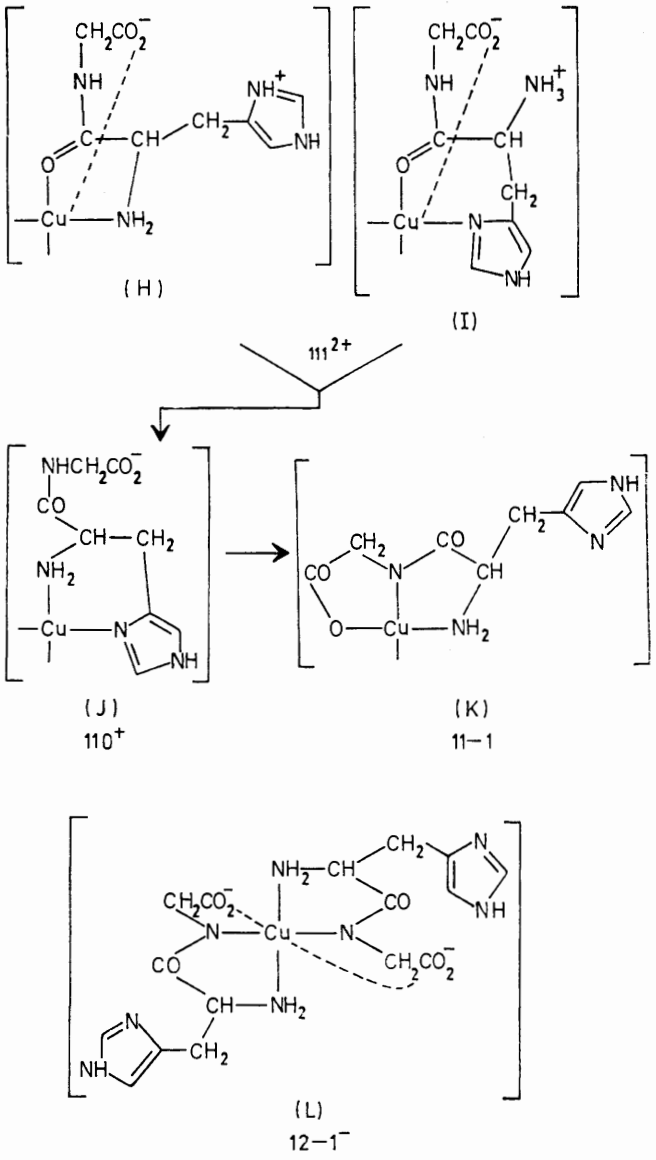
[a] Other constants calculated in this study were log β_{112} = 18.78(5) and log β_{122} = 26.8(1). [b] Ref. 8, 37 °C. [c] Refs. 5 and 10. [d] Ref. 11. [e] Ref. 6. [f] Ref. 10.

[12] A. Chakrovorty and F. A. Cotton, *J. Phys. Chem.*, 1963, **67**, 2878.
[13] D. R. Williams, *J.C.S. Dalton*, 1972, 790.
[14] R. F. Pasternack, M. Angwin, and E. Gibbs, *J. Amer. Chem. Soc.*, 1970, **92**, 5878.
[15] R. B. Rabin, *Trans. Faraday Soc.*, 1956, **52**, 1130.

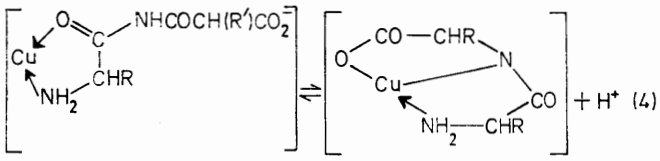
would be supported. *X*-Ray diffraction has confirmed that in the solid state Gly-His is bound to Cu^{2+} in the 11—1 complex as in (D).[7]

Deprotonation of the 110 species of His-Gly (as represented by log $K_{110}^{11\text{-}1}$, pK_1') produces a 11—1 complex of stability comparable to that formed by the simple dipeptides,[1] suggesting comparable bonding. It is impossible for all three nitrogen donors of His-Gly to lie in the same co-ordination plane. The suggested structure is therefore (K) in which the rigid planar rings do not allow the imidazole nitrogen atom to move effectively into an apical bonding position, making it non- or only weakly co-ordinating.





(iv) Glycylhistidine forms a comparatively stable bis complex, 12—1, suggesting additional bonding to that proposed for other comparable dipeptide complexes. Bis complexes have not been reported in previous studies (summarized in Table 1) but they are clearly important in the presence of excess of dipeptide. Glycylhistidine is able to co-ordinate facially in a tridentate fashion with formation of a Cu–N (imidazole) bond along the *z* axis of



the Cu^{2+}. A possible structure with this additional bonding is (E) and this could account for the increased stability found. Carnosine could bond in this way but one of the chelate rings would be larger and so the stability reduced, as is actually found. The 12—1 bis complex of His-Gly is also markedly weaker than that of Gly-His. A likely structure for the His-Gly complex is (L). There is now no appreciable apical bonding from nitrogen atoms although a carboxyl group could take this position. Bonding in the co-ordination plane is to two amino-, one imidazole, and one amide nitrogen atom.

(v) The $[Cu(H_{-1}L)]$ (11—1) species can lose a second proton to form the 11—2 complex, the ionization reaction being represented in Table 1 by pK_2' ($\log K_{11\text{-}1}^{11\text{-}2}$). Values of pK_2' for Gly-His and His-Gly (*ca.* 9.5) are similar to those for the simple dipeptides (9—9.5) suggesting a similar reaction, *i.e.* the displacement of a proton from a co-ordinated water molecule to give the complex $[Cu(H_{-1}L)(OH)]$.[1] This ionization has been attributed to such a hydrolysis reaction [10] or to ionization of a pyrrole-type proton from the imidazole ring.[5,16] This latter reaction was proposed as a result of spectrophotometric studies, in particular the colour change found is the analogous Ni^{2+} complexes. On the basis of formation constants we favour hydrolysis of co-ordinated water to give the 11—2 complex of Gly-His with the structure (F). However, the spectroscopic evidence for pyrrole ionization,[15] although at a surprisingly low pH, is strong and it is possible that both reactions are contributing to the overall value for $\beta_{11\text{-}2}$. It is unlikely that the hydrolysis reaction taking place in this pH region with simple dipeptides is completely absent. The value of pK_2' for Car (11—2) is markedly higher than that for the other two dipeptides and is more compatible with ionization of a pyrrole proton. The pK value for this reaction has been estimated at 14.2 [17] and it is reasonable to expect some lowering of the basicity under the influence of the increased bonding interactions to give structure (G). The absence of a dissociation at pK 9 due to hydrolysis of a co-ordinated water molecule [as suggested with Gly-His, (F)] is not explained. It could be the result of the larger chelate ring in Car complexes, but it is surprising if such steric factors can prevent co-ordination of a water molecule or, if it did co-ordinate, delay its hydrolysis to pH >11.

(vi) While the inclusion of binuclear species caused a marked improvement in the fit between experimental and calculated quantities at high pH, they were never important species below pH 10. What is more the only comparatively important complex was not a true dimer but a $[Cu_2(H_{-3}L_2)]$ species, and this could not be detected reliably with Car. Binuclear species must undoubtedly be present in solution since crystals have been isolated.[3] However, we found no evidence for suggestions that such a species is important in solution [2,8] and obtained results in agreement with Ihnat and Bersohn's interpretation of their ^1H n.m.r. data.[4] The solid dimer reported, the 22—2 species, would have zero charge and so would be likely to precipitate from a solution of relatively low concentration. In the 22—2 crystal the copper ions were bridged by dipeptide molecules; in the 22—3 species it is likely that hydroxide bridges are involved since the complex only forms at high pH. Circular-dichroism and spectroscopic data in favour of a dimeric structure for the Car complex [17] received no support from our potentiometric study, although it is possible that polynuclear species may be more important in the more concentrated solutions required for spectral study. Agarwal and Perrin concluded that the dimer $[Cu_2(H_{-2}L_2)]$ was the major species in Cu^{2+}–Car mixtures at pH >6.[8] It is difficult to suggest an explanation for such contradictory results, unless they are a result of the trial models and minimization technique employed. All our attempts to retain such a dimeric species in our minimizations failed and on all statistical evidence we obtained entirely acceptable fits without its inclusion. The 220 species detected by Agarwal and Perrin are, at first sight, unlikely.

A representative species-distribution plot, for the Cu^{2+}–His-Gly system, is shown in the Figure.

Complexes with Ni^{2+}.—The only formation constants for the Ni^{2+}–dipeptide complexes that have been reported are some early and incomplete results for Gly-His and Car.[5,6,10] One important aspect of the reaction between Ni^{2+} and Gly-His is the colour change from blue to yellow on addition of a fourth equivalent of alkali in the pH region 9—10. This has been studied spectrophotometrically and assigned to ionization of the pyrrole proton from the imidazole ring with subsequent rearrangement to square-planar co-ordination,[10] and possible formation of a tetramer.[16] By analogy it was suggested that a similar tetramer may form with Cu^{2+}.

We found that titrations of 1 : 1 Ni^{2+}: ligand mixtures were slow to reach equilibrium in the intermediate pH range. All titrations, therefore, were carried out on 1 : 3 and 1 : 5 mixtures when equilibrium was established comparatively rapidly and reproducibly. Calculated formation constants, together with values previously reported, are given in Table 2. Two models fitted the Ni^{2+}–Gly-His data equally well, making it impossible to distinguish between them on statistical grounds, the pairs of complexes $[Ni(H_{-2}L)]$ and $[Ni(H_{-2}L_2)]$ and $[Ni(H_{-3}L)]$ and $[Ni(H_{-3}L_2)]$ simulating one another effectively in all calculations. Fortunately they are comparatively minor species at all but high pH values and, on the basis of the work of Martin and his co-workers,[16,18] we propose model (A) as a partial solution since this accounts most satisfactorily for the change in co-ordination at high pH. The two models gave identical results up to the addition of a fourth equivalent of alkali. The most important species by far is the 11—1 complex which, under the conditions used, is the major species between

[16] R. J. Morris and R. B. Martin, *J. Inorg. Nuclear Chem.*, 1971, **33**, 2913.

[17] H. Walba and R. Isensee, *J. Amer. Chem. Soc.*, 1955, **77**, 5488; G. Yagil, *Tetrahedron*, 1967, **23**, 2855.

[18] E. W. Wilson, M. H. Kasperian, and R. B. Martin, *J. Amer. Chem. Soc.*, 1970, **92**, 5365.

pH 6 and 10. At low pH the 111 species is important and by pH 9 the 12—1 species is becoming significant. Examinations were not made much above pH 10 and, because of the slowness of the reaction, the 1 : 1 mixtures used in the study of possible tetramers, $[\{NiL\}_4]^{4-}$, were not used.[16]

The protonated 111 complex of Gly-His (log K_{111}^{011} 2.87) is presumably bonded through the imidazole nitrogen atom and the amide oxygen. While the mono complex 110 was apparently too weak to be detected with certainty, the 120 and 130 complexes were comparatively stable, if only minor species. The high stability of the 11—1 species indicates ionization of the amide proton of the dipeptide molecule (*cf.* the complexes with Cu^{2+}) to allow bonding through the three nitrogen-donor centres similar to that with Cu^{2+} in structure (D). The two proton-ionization reactions required to give the species 11—2 and 11—3 [assuming model (A) is correct] take place at a higher pH than previously expected. One (probably pK_2') is assumed to correspond to the ionization of a pyrrole-type proton to form a new Ni–N bond, although this is not possible in a simple complex without breaking an existing Ni–imidazole bond [*cf.* structure (G)]. If this is the case, pK_3' probably corresponds to hydrolysis of a co-ordinated water molecule. If the proton-ionization reaction represented by pK_2' produces a complex in which all four ligand nitrogen atoms are co-ordinated, it is difficult to conceive of a structure without resorting to polynuclear complex formation since it is impossible for both nitrogen atoms of the imidazole ring to co-ordinate to the same metal atom. Hence it is possible that model (A) is only a partial solution to a far more complicated equilibrium, involving some polynuclear complex formation.

The equilibria with carnosine were less complicated over the pH range studied. Complexes up to the 11—1 species are assumed to be comparable to their Gly-His analogues and the absence of the complications found with the latter ligand are probably the result of a slightly lower maximum pH in the potentiometric-titration data.

With Ni^{2+}, His-Gly behaved very differently to Gly-His and Car. Under the experimental conditions used only two complexes (the 110 and 120 species) were required to fit the experimental data extremely well right up to pH 9.8. Nickel(II), unlike Cu^{2+}, appears unable to promote ionization of the proton on the amide nitrogen atom of His-Gly in structure (K). As a result there is virtually 100% formation of the $[NiL_2]$ complex (120) at pH 9. The bonding is therefore likely to be similar to that in histamine in both complexes, *i.e.* similar to that with Cu^{2+} in structure (J).

TABLE 2

Formation constants for the complexes $[Ni_x(H_zL_y)]$ at 25 °C and I = 0.10M ($K[NO_3]$). Standard deviations are given in parentheses

	Gly-His		Car			His-Gly
log β_{xyz}	(A)	(B)				
111		11.07(2)	12.29(1)			
121		15.84(5)				
110		3.9(2)?	4.30(2)	2.80 [a]	5.42 [b]	6.844(6)
120		8.82(6)		4.90 [a]		12.386(8)
130		11.57(6)		6.5 [a]		
11—1		−1.502(9)	−3.152(9)			
12—1		0.92(3)	−1.04(5)			
11—2	−12.48(1)					
11—3	−23.71(4)					
12—2		−9.49(1)				
12—3		−20.62(3)				
pK_1' (log K_{110}^{11-1})	5.5?		7.45	7.35 [a]		
pK_2' (log K_{11-1}^{11-2})		10.98		8.40 [a]		
pK_3' (log K_{11-2}^{11-3})		11.23				

[a] Ref. 5. [b] Ref. 6.

With Ni(II), His-Gly behaved very differently to Gly-His and Car. Under the experimental conditions used only two complexes (the 110 and 120 species) were required to fit the experimental data extremely well right up to pH 9.8. Nickel(II), unlike Cu^{2+}, appears unable to promote ionization of the proton on the amide nitrogen atom of His-Gly in structure (K). As a result there is virtually 100% formation of the $[NiL_2]$ complex (120) at pH 9. The bonding is therefore likely to be similar to that in histamine in both complexes, *i.e.* similar to that with Cu^{2+} in structure (J).

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