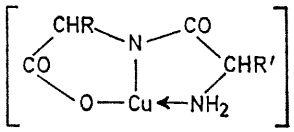
Stereoselective Complex Formation between Simple Dipeptides and Hydrogen and Copper(II) Ions †

By **Glenn Brookes** and **Leslie D. Pettit,*** Department of Inorganic and Structural Chemistry, The University, Leeds LS2 9JT

Formation constants for the various complexes formed between H^+ and Cu^{2+} and some dipeptides containing amino-acid residues of differing optical hands (Gly-Val, Gly-Phe, and Leu-Leu) have been measured at 25 °C and $I = 0.10$M ($K[NO_3]$). There is no stereoselectivity when only one of the amino-acids is optically active. When both residues contain asymmetric centres there is no significant stereoselectivity in the complexes formed prior to ionization of the amide hydrogen ion, but it is significant once ionization has taken place. Dipeptide complexes containing amino-acid residues of the same chirality {*e.g.* [Cu(L-Leu-L-Leu)]} are more stable than those formed by *racemic* dipeptides {*e.g.* [Cu(L-Leu-D-Leu)]}.

STEREOSELECTIVITY in the formation of bis complexes of Cu^{2+} with simple bidentate amino-acids is very rare, although it has been shown to be present in some ternary complexes in which one of the ligands around the Cu^{2+} ion is histidine, His.[1,2] Dipeptides (HL) contain two amino-acid residues and therefore their metal mono complexes can be regarded, very superficially, as approximating to a bis complex of the component amino-acids. However, the potential donor centres are modified significantly by the formation of the peptide bond which limits the number of atoms able to act simultaneously as donor atoms to three (assuming there are no donor atoms in the amino-acid side chains). In the presence of Cu^{2+} at intermediate pH values the proton on the amide nitrogen atom ionizes to allow the formation of a metal–nitrogen bond. This nitrogen atom will be planar and will impose stereochemical restraints on the two chelate rings present in the resulting neutral complex, $[Cu(H_{-1}L)]$. As



(I)

a result it is likely that the increased intramolecular forces may be great enough to cause a significant difference in stability between complexes of simple dipeptides which contain different combinations of optical hands of their component amino-acids, but are otherwise identical. Such stereoselectivity is possible even though there is no detectable stereoselectivity in the analogous bis(amino-acid) complexes. Until now there has been no evidence for such stereoselectivity, although another type of stereoselectivity has been reported in simple dipeptide complexes (*e.g.* glycyl-L-valine, Gly-Val) of Co^{III} where two different isomers, formed in unequal amounts, were detected by ^1H n.m.r. measurements.[3] These isomers correspond to the Δ and Λ configurations found in octahedral co-ordination.

The acid dissociation constants of a number of dipeptides prepared from amino-acids of different optical hands have been measured by Ellenbogen [4] and by Li *et al.*[5] They concluded that the acidity of the carboxyl proton was greatest for dipeptides containing amino-acid residues of different optical hands, whereas the acidity of the amino-proton was greatest for those of the same optical hand. The reason suggested for this stereoselectivity was differences in the ease of folding and unfolding the dipeptide as the optical configuration was changed from LL to LD. On the basis of molecular models it is clear that the racemic dipeptide (LD or DL) will fold more easily than the one with identical optical hands (LL or DD) if the arrangement about the peptide bond is *trans*. Hence the carboxyl group of the LD (or DL) isomer is expected to ionize more readily than the corresponding LL or DD proton since the folded conformation of the dipeptide would be stabilized by electrostatic interaction between the ionized CO_2^- and protonated NH_3^+ groups. By the same reasoning, ionization of the NH_3^+ proton would be delayed in the LD (or DL) dipeptide. Hence the experimental results have a simple conformational explanation. This explanation is valid only if co-ordination around the peptide linkage is *trans*; in fact, if it were *cis* the opposite stereoselectivity would be expected. The *trans* configuration was favoured by Ellenbogen as a result of thermodynamic studies at different temperatures.[4]

The metal complexes of some isomeric dipeptides have been studied [5] and the results indicate that the stability of the metal complexes is affected by the optical configuration. However, the Cu^{2+} complexes were not studied and the constants calculated were based on a model for the system which is now unacceptable: for example, the complex species [ML] and $[ML_2]$ were considered while the complex in which the peptide nitrogen is deprotonated, $[M(H_{-1}L)]$, was neglected.

We have completed a study of the H^+ and Cu^{2+} complexes of a selection of dipeptides, prepared from simple bidentate amino-acids of different optical hands. The ligands studied fell into two classes. The first class included Gly-D- and Gly-L-Val and Gly-D- and Gly-L-Phe (Phe = phenylalanine). Only one amino-acid residue is optically active and the substituent groups are large but non-co-ordinating, one being aliphatic and the other

† No reprints available.

[1] V. A. Davankov and P. R. Mitchell, *J.C.S. Dalton*, 1972, 1012; J. H. Ritsma, Thesis, Groningen, 1973.

[2] G. Brookes and L. D. Pettit, *J.C.S. Chem. Comm.*, 1974, 813.

[3] G. Stadherr and R. B. Martin, *Inorg. Chem.*, 1973, **12**, 1810.

[4] E. Ellenbogen, *J. Amer. Chem. Soc.*, 1956, **78**, 369.

[5] N. C. Li, G. W. Miller, N. Solony, and B. T. Gillis, *J. Amer. Chem. Soc.*, 1960, **82**, 3737.

aromatic. The second class included the four isomeric forms of leucyl-leucine (Leu-Leu), *i.e.* DD, LL, DL, and LD. Of these the first pair are enantiomeric, as are the second pair, while the two pairs are diastereoisomeric.

EXPERIMENTAL

The dipeptides were obtained from the Sigma Chemical Co. (SIGMA grade). The experimental procedure for measuring the formation constants is described elsewhere.[6]

RESULTS AND DISCUSSION

Calculated formation constants for the hydrogen-ion and copper complexes are given in Table 1. The constants quoted are overall formation constants for reaction (1). The value for β_{11-1} refers to reaction (2) in which a copper–peptide-nitrogen bond is formed to give two approximately planar five-membered chelate rings.

$$x\mathrm{Cu}^{2+} + y\ \mathrm{L}^- + z\mathrm{H}^+ \xrightarrow{\beta_{xyz}} [\mathrm{Cu}_x\mathrm{L}_y\mathrm{H}_z] \qquad (1)$$

$$\mathrm{Cu}^{2+} + \mathrm{NH_2{\cdot}CHR'{\cdot}CO{\cdot}NH{\cdot}CHR{\cdot}CO_2^-} \longrightarrow (\mathrm{I}) + \mathrm{H}^+ \qquad (2)$$

Examination of the formation constants demonstrates that there are no significant stereoselective effects in any of the complexes of the glycyl dipeptides. These contain only one asymmetric centre and would not, therefore, be expected to be demonstrably different. Such differences as were found are associated with complex species which are only minor components of the equilibrium mixture and therefore the constants have large standard deviations making the differences of no significance.

As expected both ionizable hydrogen ions of the Gly-Phe dipeptides are more acidic than those of the Val analogues. However, in spite of this, the log β_{110} values for the former are slightly greater, and the log β_{11-1} values are considerably greater than for the Gly-Val analogues. This is possibly the result of weak interaction betwee Cu^{2+} and the phenyl ring.[7] If this reaction is in the form of a nucleophilic attack by the copper, it would be expected to be enhanced as the electronegativity of the copper is reduced. This is the case when comparing copper in the 110 and 11—1 species and provides an explanation of the increased relative stability of the 11—1 species. It is therefore probable that this is a source of stereoselectivity in dipeptides of Phe containing two asymmetric centres. Stereoselectivity resulting from this type of interaction has been detected in bis(*N*-benzylprolinato)copper complexes.[8]

Results for the various isomers of Leu-Leu demonstrate clearly that stereoselective effects are present in their copper complexes. Complexes of the LL and DD isomers are, as expected, of the same stability, within experimental error (3σ), as are the complexes of the LD and DL isomers. The inclusion of all four isomers in the study and the expected pairing of results gives increased confidence in the magnitude of the difference between the results for the *racemic* (DL or LD) and optically homogeneous (LL or DD) dipeptides. The formation constants of the 110 and 12—1 complexes are not significantly different. The differences for the other complexes are in Table 2.

Stereoselectivity in the hydrogen-ion complex-formation constants is entirely consistent with that first reported by Ellenbogen.[4] The overall formation constants for the fully protonated dipeptides (H_2L^+), log β_{012}, show no significant stereoselectivity demonstrating that the origin of the stereoselectivity in the stepwise constants must lie in the structure of the $HL^{\pm}$ zwitterion. The stabilization of the *racemic* monoprotonated complex (0.36 log units) compares with that found for leucyltyrosine (Leu-Tyr) (0.54 log units) and alanylalanine (Ala-Ala) (0.16) [5] and is satisfied by the same explanation in terms of electrostatic attraction between the NH_3^+ and CO_2^- groups in the folded conformation.

Among the copper complexes, only those with copper–amide-nitrogen bonds showed stereoselectivity. Hence stereoselectivity is insignificant at low pH (below 4.5) when the major species is the 110 complex, but is important in the biological pH region when the 11—1

TABLE 1

Overall formation constants for the species $[Cu_xL_yH_z]$ at 25 °C and $I = 0.10$M(K[NO_3]). Standard deviations are given in parentheses

Dipeptide	log K_{011}^{010}	log K_{012}^{011}	log β_{110}	log β_{12-1}	log β_{11-1}	log β_{11-2}	log β_{22-3}
Gly-D-Val [a]	8.232(4)	3.184(5)	5.73(1)	4.38(2)	1.102(2)	−8.20(1)	−4.18(7)
Gly-L-Val [b,c]	8.233(3)	3.154(5)	5.77(1)	4.48(1)	1.122(1)	−8.17(1)	−4.34(10)
Gly-D-Phe	8.166(3)	2.969(4)	5.90(2)	5.20(1)	1.948(2)	−7.424(5)	−2.94(6)
Gly-L-Phe [c]	8.157(3)	2.987(4)	5.82(4)	5.23(4)	1.934(4)	−7.45(1)	−3.06(10)
D-Leu-D-Leu	7.895(8)	3.525(10)	5.39(3)	4.49(4)	1.351(6)	−7.84(1)	
L-Leu-L-Leu [c]	7.911(5)	3.455(5)	5.24(2)	4.46(2)	1.378(1)	−7.816(3)	
D-Leu-L-Leu	8.251(8)	3.129(9)	5.23(2)	4.38(2)	0.636(2)	−8.535(7)	
L-Leu-D-Leu	8.279(8)	3.166(9)	5.20(3)	4.36(4)	0.628(2)	−8.556(8)	

[a] log $\beta_{120} = 11.07(6)$. [b] log $\beta_{120} = 11.25(5)$. [c] From ref. 6.

TABLE 2

Stereoselectivity in the copper complexes of Leu-Leu

$\Delta \log \beta$ (or K) $= 0.5\ [\log \beta_{LL} + \log \beta_{DD} - (\log \beta_{LD} + \log \beta_{DL})]$

$\Delta \log K_{011}^{010}$	−0.36
$\Delta \log K_{012}^{011}$	+0.34
$\Delta \log \beta_{11-1}$	+0.73
$\Delta \log \beta_{11-2}$	+0.72

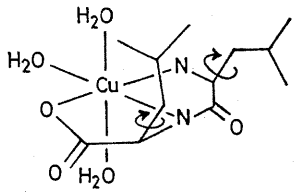
[6] G. Brookes and L. D. Pettit, *J.C.S. Dalton*, 1975, 2106.

[7] D. Van der Helm and C. E. Tatsch, *Acta Cryst.*, 1972, **B28**, 2307.

[8] G. G. Aleksandrov, Yu. T. Struchkov, A. A. Kurganov, S. V. Rogozhin, and V. A. Davankov, *J.C.S. Chem. Comm.*, 1972, 1328.

species predominates. If the stereoselectivity found (*i.e.* LL > DL) is a general phenomenon with other dipeptides it could have an important biological significance.

The 11—1 complex of Leu-Leu may be represented as in (II). Since the two chelate rings are effectively planar there were will be no distinction between axial and equa-



(II)

torial substituents on the asymmetric carbon atoms, and any electronic effects operating within the molecule would be equally important above and below the equatorial plane. Rotation of the bulky side chains about the bonds indicated would cause mutual hindrance in the LL (or DD) complexes but not in the LD (or DL) species. This would favour stabilization opposite to that found. However, rotation would also tend to dehydrate the copper of water molecules co-ordinated along the *z* axis. The *racemic* LD (or DL) isomers permit interference with both water molecules whereas only one is destabilized in the LL (or DD) complex. Hence differences in steric interference between the peptide side chains and co-ordinated water could account for preferential formation of the optically homogeneous dipeptide complex.

This argument suggests that there should be stereoselectivity in the binary amino-acid complexes, $[Cu(Leu)_2]$. We have studied the systems in detail and can find no significant differences in titrations of Cu^{2+} with L- and DL-Leu. This absence of significant stereoselectivity may be the result of either: (*i*) the more flexible chelate rings formed by amino-acids compared to those in dipeptide complexes (they could allow more tolerance to steric interactions between co-ordinated water molecules and the amino-acid side chains); (*ii*) the shorter copper–amide-nitrogen bond length in the dipeptide complexes (this would permit the bulky side chains to interact more strongly with axially co-ordinated water molecules); and (*iii*) the possibility of *cis–trans* isomerism in the bis(amino-acid) complexes. (This would help to minimize steric interactions and self-compensating changes in the component microconstants may not have much influence on the overall macroconstant. Such isomerism is clearly impossible in the dipeptide complexes.)

Deprotonation of the 11—1 complex to give 11—2 species is believed to involve hydrolysis of a co-ordinated water molecule to form the hydroxo-complex $[Cu(H_{-1}L)(OH)]^-$. It may be assumed that the water molecule involved occupies the fourth site in the co-ordination plane.[6] If the origin of the stereoselectivity in the 11—1 species is as suggested above, the 11—2 species should show an almost identical stereoselectivity. This is what was found (Table 2).

Attempts to detect stereoselectivity in Ni^{2+} complexes of Leu-Leu dipeptides proved ineffective as a result of early precipitation and poor reproducibility in the titration data. These problems with Ni^{2+} were more marked than when other dipeptides were used.[6]

[5/775 *Received, 25th April,* 1975]