# 165. Complex Formation Equilibria of L-Amino-Acid Amides with Copper(II) in Aqueous Solution

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Protonation and Cu(II) complexation equilibria of L-phenylalaninamide, $N^2$-methyl-L-phenylalaninamide, $N^2$,$N^2$-dimethyl-L-phenylalaninamide, L-valinamide, and L-prolinamide have been studied by potentiometry in aqueous solution. The formation constants of the species observed, $CuL^{2+}$, $CuL_2^{2+}$, $CuLH_{-1}^{+}$, $CuL_2H_{-1}^{+}$, and $CuL_2H_{-2}$, are discussed in relation to the structures of the ligands. Possible structures of bisamidato complexes are proposed on the ground of VIS and CD spectra. Since Cu(II) complexes of the present ligands (pH range 6–8) perform chiral resolution of dansyl- and unmodified amino acids in HPLC (reversed phase), it is relevant for the investigation of the resolution mechanism to know which are the species potentially involved in the recognition process.

**Introduction.** – Copper(II) complexes of L-amino acids have been extensively used as additives to the mobile phase for the chiral resolution of free or modified DL-amino acids in reversed-phase high-performance liquid chromatography (HPLC) [1]. L-Aspartyl- and L-prolylalkylamide complexed to Cu(II) [2] and Ni(II) [3], respectively, have also been used, and their enantioselectivity has been related to the alkyl chain length.

Independent of whether chiral recognition occurs in the stationary or in the mobile phase, it is generally assumed [4] to proceed *via* ligand exchange between the initial Cu(II) complex and the enantiomers, leading to formation of diastereoisomeric ternary complexes of different stability and/or affinity for the column.

In a general scheme aimed at studying the mechanism of chiral recognition, we have recently proposed tetradentate diamino-diamide-type ligands [5] [6] which give rise to Cu(II) complexes of different structures and stabilities. These complexes should undergo a slower rate of decomplexation and be more liable to apical or outer-sphere interaction with the enantiomers, rather than a simultaneous dechelation of two binding sites, as in the classical ligand-exchange mechanism. Moreover, we have also shown that Cu(II) complexes of simple L-amino-acid amides were able to perform excellent separation of dansyl-substituted [7] and free DL-amino acids [8] in reversed-phase HPLC.

With the purpose of characterizing the complexes which may be involved in the discrimination process, we have now investigated by potentiometry the equilibria of L-phenylalaninamide (Phe-$NH_2$), $N^2$-methyl-L-phenylalaninamide (Me-Phe-$NH_2$), $N^2$,$N^2$-

dimethyl-L-phenylalaninamide ($Me_2$-Phe-$NH_2$), L-valinamide (Val-$NH_2$), and L-prolinamide (Pro-$NH_2$) with $H^+$ and $Cu^{2+}$ in aqueous solution.

**Results and Discussion.** – Amide-group-containing ligands coordinate to divalent transition metals in aqueous solution through the carbonyl O-atom or through the deprotonated N-atom, according to the pH and to the presence of another ligating group useful for metal chelation [9]. There is a general agreement that, prior to deprotonation, the coordination site of the neutral amide group is the carbonyl O-atom, as recently confirmed by ESR data in solution [10].

As far as the amino-acid amides are concerned, the solution equilibria of glycinamide (Gly-$NH_2$) [11], alaninamide (Ala-$NH_2$) [12], serinamide (Ser-$NH_2$) [13], and tyrosinamide (Tyr-$NH_2$) [14] with Cu(II) have already been studied by potentiometry. Five species have been detected both for Gly-$NH_2$ and Ala-$NH_2$, *i.e.* $CuL^{2+}$, $CuL_2^{2+}$, $CuLH_{-1}^{+}$, $CuL_2H_{-1}^{+}$, and $CuL_2H_{-2}$, whereas a minor species, $CuLH_{-2}$, has been revealed for Ala-$NH_2$ only by spectrophotometry [15]. A dinuclear complex, $Cu_2L_2H_{-2}^{2+}$ has been claimed for Ser-$NH_2$, whereas, surprisingly, $CuL_2H_{-2}$ was lacking [13].

Several bis(amino-acid amidato) complexes of Ni(II), Cu(II), and Pd(II) have been synthesized and characterized spectroscopically (VIS, CD, ORD) [16] [17], but only one, $[Ni(Pro\text{-}NH)_2]\cdot 2H_2O$ by X-ray crystallography [18]. Recently, we have succeeded in the preparation and crystal-structure determinaton of $[Cu(Phe\text{-}NH)_2]$ [19], $[Cu(Pro\text{-}NH)_2]$, and $[Cu(Me_2\text{-}Phe\text{-}NH)_2(H_2O)]$ [20].

*Potentiometric Determinations.* The program SUPERQUAD [21] has been employed for the refinement of the trial equilibrium constants. The species initially tested were $CuL^{2+}$, $CuL_2^{2+}$, $CuLH_{-1}^{+}$, $CuL_2H_{-1}^{+}$, and $CuL_2H_{-2}$, namely those reported both for Gly-$NH_2$ [11] and Ala-$NH_2$ [12]. The exclusion of $CuLH_{-1}^{+}$ led to poorer fit (worse sample variance ($s^2$) and standard deviations of the constants), whereas the introduction in the

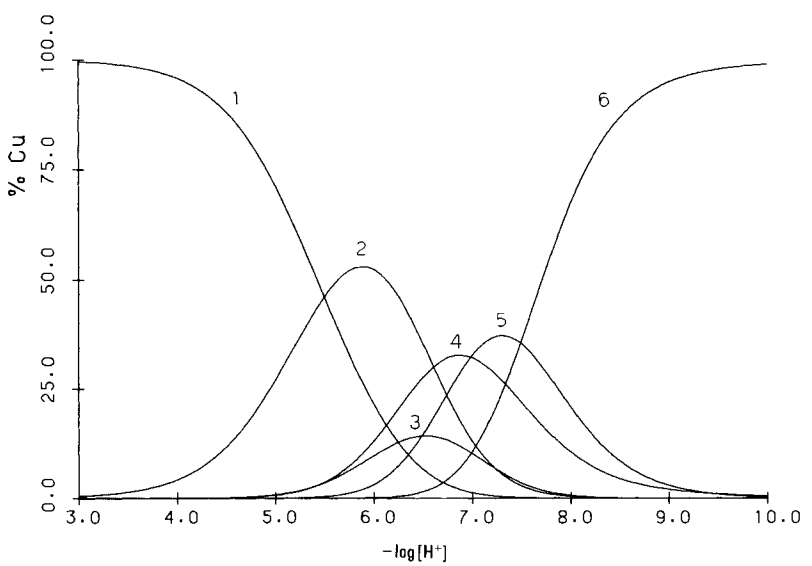


Fig. 1. *Species distribution for the Cu(II)/Pro-$NH_2$ system with* $c_{Cu} = 0.002$ M *and* $c_L = 0.004$ M *as a function of* $-\log[H^+]$. Curve 1, $Cu^{2+}$; 2, $CuL^{2+}$; 3, $CuL_2^{2+}$; 4, $CuLH_{-1}^{+}$; 5, $CuL_2H_{-1}^{+}$; 6, $CuL_2H_{-2}$.

Table 1. *Logarithms of Protonation and Cu(II)-Complex Formation Constants* ($\beta_{pqr} = [Cu_pL_qH_r]/[Cu]^p[L]^q[H]^r$) *for* L-*Phenylalaninamide* (Phe-$NH_2$), $N^2$-*Methyl*-L-*phenylalaninamide* (Me-Phe-$NH_2$), $N^2$,$N^2$-*Dimethyl*-L-*phenylalaninamide* ($Me_2$-Phe-$NH_2$), L-*Valinamide* (Val-$NH_2$), L-*Prolinamide* (Pro-$NH_2$) *at 25° and* I = *0.1* M (KCl). Standard deviations are given in parentheses. Data for glycinamide (Gly-$NH_2$) and L-alaninamide (Ala-$NH_2$) are taken from the literature.

| Ligand | $HL^+$ | $CuL^{2+}$ | $CuL_2^{2+}$ | $CuLH_{-1}^+$ | $CuL_2H_{-1}^+$ | $CuL_2H_{-2}$ | $CuLH_{-2}$ | $s^2$[a]) | $n$[a]) |
|---|---|---|---|---|---|---|---|---|---|
| Phe-$NH_2$ | 7.26(1) | – | – | – | – | – | – | 2.72 | 112 |
| | | 4.42(1) | 7.84(2) | −2.08(3) | 1.90(2) | −5.46(2) | – | 1.59 | 187 |
| Me-Phe-$NH_2$ | 7.38(1) | – | – | – | – | – | – | 1.37 | 202 |
| | – | 3.79(1) | 6.75(6) | −2.60(3) | 0.82(1) | −6.10(1) | – | 0.57 | 243 |
| $Me_2$-Phe-$NH_2$ | 6.88(1) | – | – | – | – | – | – | 0.66 | 210 |
| Val-$NH_2$ | 7.72(1) | – | – | – | – | – | – | 1.00 | 111 |
| | – | 4.55(1) | 8.14(5) | −1.99(1) | 1.82(2) | −5.66(2) | – | 2.37 | 178 |
| Pro-$NH_2$ | 8.69(1) | – | – | – | – | – | – | 4.79 | 114 |
| | – | 5.74(1) | 10.36(3) | −0.86(2) | 3.87(2) | −3.62(1) | – | 2.13 | 214 |
| Gly-$NH_2$[b]) | 7.96(1) | – | – | – | – | – | – | – | – |
| | – | 5.22(1) | 9.58(1) | −1.57(1) | 2.63(1) | −5.54(1) | – | – | – |
| Ala-$NH_2$[c]) | 8.18(1) | – | – | – | – | – | – | – | – |
| | – | 5.07(2) | 8.99(2) | −2.15(4) | 2.02(2) | −6.14(2) | – | – | – |
| Ala-$NH_2$[d]) | – | 5.07(1) | 8.93(1) | −2.14(1) | 1.95(1) | −6.19(1) | −10.87(5) | – | – |

[a]) $s^2 = \Sigma w_i (E_i^{obs} - E_i^{calc})^2/(n-m)$ = sample variance; $w_i = 1/\sigma_i^2$, where $\sigma_i$ is the expected error on each experimental observation ($E_i$); $n$ = number of observations; $m$ = number of parameters refined.

[b]) From [11], $I = 0.1$M ($KNO_3$).

[c]) From [12], $I = 0.5$M (KCl), by potentiometry.

[d]) From [15], $I = 0.5$M (KCl), by spectrophotometry.

Table 2. *Stepwise Equilibrium Constants* (log *K*) *for Cu(II) Complexes of* L-*Phenylalaninamide* (Phe-$NH_2$), $N^2$-*Methyl*-L-*phenylalaninamide* (Me-Phe-$NH_2$), L-*Valinamide* (Val-$NH_2$), L-*Prolinamide* (Pro-$NH_2$) *at 25° and* I = *0.1* M (KCl). Data for glycinamide (Gly-$NH_2$) and L-alaninamide (Ala-$NH_2$) are taken from the literature.

| | Phe-$NH_2$ | Me-Phe-$NH_2$ | Val-$NH_2$ | Pro-$NH_2$ | Gly-$NH_2$[a]) | Ala-$NH_2$[b]) |
|---|---|---|---|---|---|---|
| $Cu^{2+} + L \rightleftharpoons CuL^{2+}$ | 4.42 | 3.79 | 4.55 | 5.74 | 5.22 | 5.07 |
| $CuL^{2+} + L \rightleftharpoons CuL_2^{2+}$ | 3.42 | 2.96 | 3.59 | 4.62 | 4.36 | 3.92 |
| $CuL^{2+} \rightleftharpoons CuLH_{-1}^+ + H^+$ | −6.50 | −6.39 | −6.54 | −6.60 | −6.79 | −7.22 |
| $CuL_2^{2+} \rightleftharpoons CuL_2H_{-1}^+ + H^+$ | −5.94 | −5.93 | −6.32 | −6.49 | −6.95 | −6.97 |
| $CuL_2H_{-1}^+ \rightleftharpoons CuL_2H_{-2} + H^+$ | −7.36 | −6.92 | −7.48 | −7.49 | −8.17 | −8.16 |

[a]) From [11], $I = 0.1$M ($KNO_3$).

[b]) From [15], $I = 0.1$M (KCl).

preceding model of $CuLH_{-2}$, $Cu_2L_2H_{-2}^{2+}$, and $CuL_2H_{-3}^-$, one at a time, led to their rejection. Therefore, the present ligands give rise to the same complexes as formed by Gly-$NH_2$ and Ala-$NH_2$.

Protonation and cumulative formation constants of the Cu(II) complexes obtained for Phe-$NH_2$, Me-Phe-$NH_2$, $Me_2$-Phe-$NH_2$, Val-$NH_2$, and Pro-$NH_2$ at 25° and $I = 0.1$M (KCl) are reported in *Table 1*. An example of a species-distribution diagram for Pro-$NH_2$ is presented in *Fig. 1*. Analogies and differences between the amino-acid amides considered appear from the analysis of the stepwise equilibrium constants reported in *Table 2*.

The log $K$ values obtained for the equilibrium

$$Cu^{2+} + L \rightleftharpoons CuL^{2+} \quad (1)$$

(4.42 for Phe-$NH_2$, 4.55 for Val-$NH_2$, 5.74 for Pro-$NH_2$) are consistent with the electronic effect of the amino-acid side chain and follow the same trend of the corresponding dipeptides [22] (Phe-Gly, 4.66; Val-Gly, 4.87; Pro-Gly, 6.39) which are known to form chelates through N-atom and amide O-atom in acidic solution [1]. The lower stability of $Cu(Me\text{-}Phe\text{-}NH_2)^{2+}$ (3.79) relative to $Cu(Phe\text{-}NH_2)^{2+}$ (4.42) follows the same trend observed for $Cu(Me\text{-}Phe)^+$ (7.12) [23] *vs.* $Cu(Phe)^+$ (7.90) [22] and for $Cu(Me\text{-}Gly\text{-}Gly)^+$ (5.32) *vs.* $Cu(Gly\text{-}Gly)^+$ (5.55) [24]. More remarkable is the decrease of the formation constant of $Cu(Me\text{-}Phe\text{-}NH_2)^{2+}$ relative to the other secondary amine, Pro-$NH_2$: in the former, the lone pair of the N-atom is partially shielded by the Me group during the inversion process and is, therefore, less available to complexation. Moreover, the Me group could provide hindrance to solvation, thus destabilizing the charged complex. Accordingly, when two Me groups are present at N(2) as in $Me_2$-Phe-$NH_2$, no complexation is observed. This indicates that the disubstituted amine moiety is not an effective anchoring group in promoting chelation to the amide O-atom and keeping copper ions in solution. On the other hand, we obtained crystals of $[Cu(Me_2\text{-}Phe\text{-}NH)_2(H_2O)]$ from $MeOH/H_2O$ solutions [20].

As far as the species $CuLH^+_{-1}$ is concerned, the log $K$ values of the equilibrium

$$CuL^{2+} \rightleftharpoons CuLH^+_{-1} + H^+ \quad (2)$$

are practically the same for Phe-$NH_2$, Val-$NH_2$, and Pro-$NH_2$ and close to those of Gly-$NH_2$ and Ala-$NH_2$. This behaviour is present also in Cu(II)-dipeptide complexes [22] where, apart from the additional contribution of the carboxylato group, the log $K$ variation is very similar (Phe-Gly, $-3.39$; Val-Gly, $-3.74$; Pro-Gly, $-3.95$; Ala-Gly, $-4.07$; Gly-Gly, $-3.95$). The presence of the Me group at N(2) in Me-Phe-$NH_2$ does not affect as much the deprotonation of the amide which occurs with a constant ($-6.39$) not dissimilar from Phe-$NH_2$ ($-6.50$), analogously to what was observed for Gly-Gly (3.95) and Me-Gly-Gly, ( = sarcosylglycine, $-3.96$) [24].

The deprotonation of one amide group from $CuL_2^{2+}$ according to the equilibrium

$$CuL_2^{2+} \rightleftharpoons CuL_2H^+_{-1} + H^+ \quad (3)$$

appears to be more favourable for Phe-$NH_2$ (log $K = -5.94$) and for Me-Phe-$NH_2$ ($-5.93$) than for other ligands (Val-$NH_2$, $-6.32$; Pro-$NH_2$, $-6.49$; Ala-$NH_2$, $-6.97$; Gly-$NH_2$, $-6.95$). This may be due either to the electronic withdrawing effect of the phenyl ring or to its particular position. Actually, as it has been shown in the crystal structure of $[Cu(Phe\text{-}NH)_2]$ [19], also in solution a phenyl ring could be tilted above the coordination plane, thus increasing the electronegativity of the metal atom *via* electron transfer from $Cu^{2+}$ to vacant aromatic $\pi^*$ orbitals and favouring the amide deprotonation. Cu(II)-phenyl ring interactions in solution have been claimed in Cu(II) ternary systems, though their effect on the properties of the complexes have not been explored in detail [25].

Finally, the equilibrium

$$CuL_2H^+_{-1} \rightleftharpoons CuL_2H_{-2} + H^+ \quad (4)$$

is favoured for Me-Phe-$NH_2$. Actually, $CuL_2H_{-2}$ reaches a nearly square planar geometry: the presence of the Me group at N(2) in the equatorial position does not hinder such a geometry and may provide a more efficient electron-donating group for coordination. In contrast, in Cu(Pro-$NH)_2$ the rigid cycloalkyl substituent is most probably in the axial position, thus destabilizing the complex. Evidence for this assumption has been provided by CD measurements (see below).

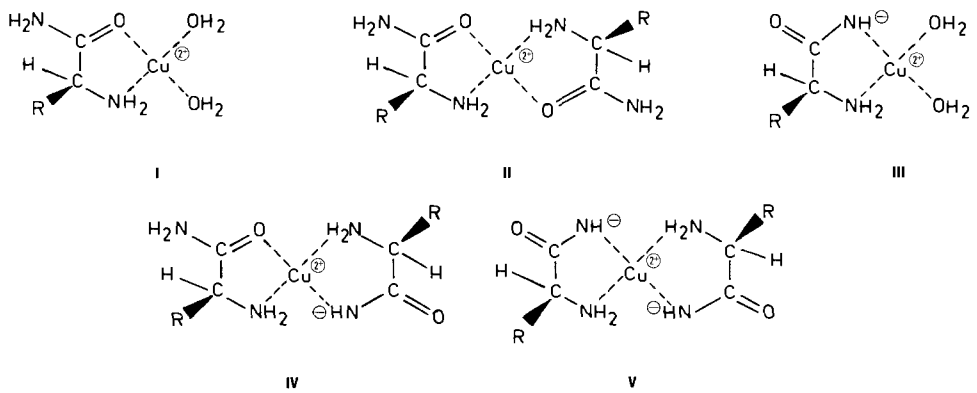

Fig. 2. *Schematic structures of the complexes* $CuL^{2+}$ (**I**), $CuL_2^{2+}$ (**II**), $CuLH^+_{-1}$ (**III**), $CuL_2H^+_{-1}$ (**IV**), *and* $CuL_2H_{-2}$ (**V**)

In *Fig. 2* are presented the schematic structures of the complexes formed by the present ligands: $CuL^{2+}$ (**I**), $CuL_2^{2+}$ (**II**), $CuLH^+_{-1}$ (**III**), $CuL_2H^+_{-1}$ (**IV**), and $CuL_2H_{-2}$ (**V**).

*Spectroscopic Data.* As shown in *Fig. 1,* several species are present in the pH range examined. Therefore, only the $CuL_2H_{-2}$ complex can be easily characterized by VIS spectroscopy at pH $\approx$ 10. Absorption maxima and extinction coefficients measured for Phe-$NH_2$ ($\lambda_{max}$ 520 nm ($\varepsilon$ = 58 l $mol^{-1}$ $cm^{-1}$)), Val-$NH_2$ (513 (58)), Pro-$NH_2$ (513 (79)) are in agreement with those reported in [17]. Accordingly, the species Cu(Me-Phe-$NH)_2$ shows an absorption maximum at 504 nm ($\varepsilon$ = 82) consistent with a *trans*-diaminodiamidato-type square planar coordination [9].

The CD spectra of Cu(Phe-$NH)_2$, Cu(Val-$NH)_2$, Cu(Me-Phe-$NH)_2$, and Cu(Pro-$NH)_2$ show a negative band in the region 540–560 nm (*Fig. 3*), mainly consistent with the disposition of the substituent at C(2) rather than with the puckering of the chelate rings which are nearly planar as suggested by *Komorita et al.* [17]. A second band, negative for Cu(Pro-$NH)_2$ and positive for Cu(Me-Phe-$NH)_2$ is present at 465 and 453 nm, respectively, accounting for the formation of a second centre of asymmetry on the coordinated alkylamino group. The different sign observed could be explained by the assumption that in Cu(Pro-$NH)_2$, the cycloalkyl substituents are enforced in a pseudoaxial position, whereas the Me groups of Cu(Me-Phe-$NH)_2$ can be assessed in a more favourable equatorial position.

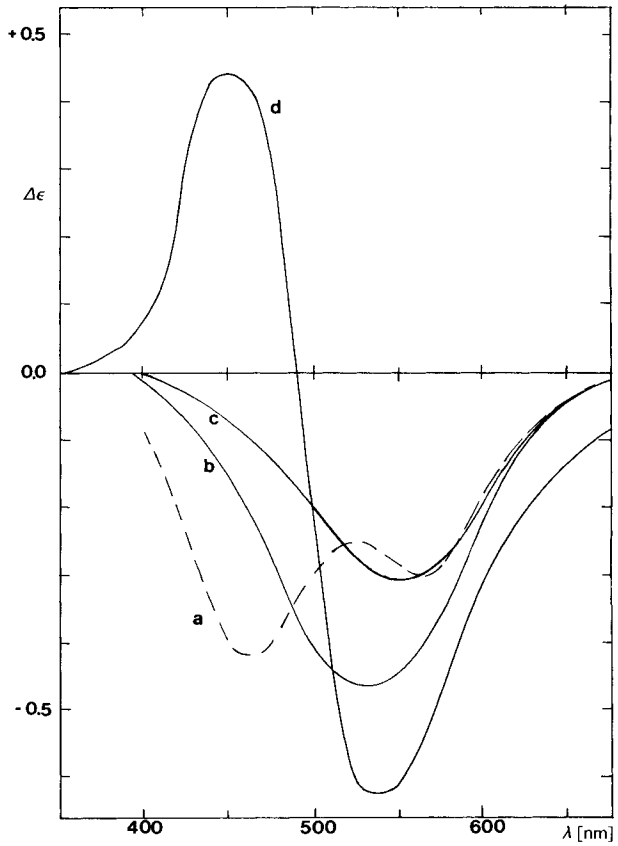


Fig. 3. *CD spectra of* $CuL_2H_{-2}$ *complexes of Pro-*$NH_2$ (a), *Val-*$NH_2$ (b), *Phe-*$NH_2$ (c), *and Me-Phe-*$NH_2$ (d)

*Chromatographic Data.* Enantiomeric separation of dansyl-substituted and unmodified DL-amino acids by HPLC was performed on a $C_{18}$ column [7] [8], using as eluent aqueous solutions of the different L-amino-acid amides and Cu(II) acetate in a molar ratio 2:1 ($c_{Cu}$ = 1 mM) within a pH range 6–8. The best enantioselective separation factors ($\alpha$) were obtained with Me-Phe-$NH_2$, Phe-$NH_2$, and Val-$NH_2$, whereas with Pro-$NH_2$ only apolar DL-amino acids were resolved. We believe that the discrimination process essentially occurs on the column where the initial complex is adsorbed. The choice of the pH takes into account also the solutes to be separated: thus, dansyl-DL-amino acids are better separated at high pH (7–7.5), the deprotonation of the sulfonamide favouring the diastereoisomeric ternary complex, whereas unmodified DL-amino acids require variable pH conditions according to the nature of the DL-amino-acid side chain.

In conclusion, practically every complex species present in the pH range indicated is potentially enantioselective. However, the mechanism of resolution is very complicated and involves many factors which will be discussed elsewhere.

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## Experimental Part

*General.* $[\alpha]_D$: at $\lambda = 546$ nm, *Rudolph* research polarimeter *III*; 10-cm cell. IR (KBr): *Perkin-Elmer-298* spectrophotometer; in $cm^{-1}$. UV/VIS: *Jasco-Uvidec-505* apparatus. CD: *Jasco-500 A* spectropolarimeter equipped with *DP 500 N*. $^1$H- and $^{13}$C-NMR: *Varian* instruments *EM 360* and *XL-100*; chemical shifts in $\delta$[ppm] relative to TMS as an internal standard.

*Reagents.* L-Phenylalaninamide, L-valinamide, and L-prolinamide hydrochlorides were obtained from *Sigma Chemical Co.*, and $N^2$-methyl-L-phenylalaninamide and $N^2$,$N^2$-dimethyl-L-phenylalaninamide hydrochlorides were synthesized as described below. The ligands were dried over $P_4O_{10}$ *in vacuo* and their purity checked potentiometrically. KOH and HCl solns. were prepared by diluting *Merck Titrisol* ampoules. KOH soln. titre was checked against potassium hydrogen phthalate (*C. Erba*, dried at 120°). $CuCl_2 \cdot 2H_2O$ (*C. Erba*) was employed for the preparation of a stock soln. which was analysed by EDTA.

$N^2$-*Methyl-L-phenylalaninamide Hydrochloride* (Me-Phe-$NH_2 \cdot$HCl) was synthesized from L-phenylalaninamide hydrochloride by reaction with benzaldehyde, reduction with $NaBH_3CN$, and subsequent methylation with formaldehyde in HCOOH according to the *Clarke-Eschweiler* procedure [26]. The $N^2$-benzyl-$N^2$-methylphenylalaninamide was purified by crystallization and deprotected to the title compound by hydrogenolysis in the presence of 10% Pd/C at 40° for 3 h. The soln. was filtered, acidified to pH 2–3 with HCl/MeOH, evaporated to small bulk under vacuum, and recrystallized from MeOH/acetone. Yield 60%. M.p. 200° (dec.). $[\alpha]_D^{25} = +42.78$ (95% EtOH, $c = 1$). IR: 3320–3280, 3250, 1700, 1640, 1580, 800–700. $^1$H-NMR (as amine in $CDCl_3$): 1.76 (br. *s*, $CH_3NH$); 2.31 (*s*, $CH_3$); 2.73 (*m*, 1 H–C(3)); 3.13–3.30 (*m*, H–C(2), 1 H–C(3)); 5.70 (br. *s*, $CONH_2$); 7.20–7.40 (*m*, 5 arom. H). $^{13}$C-NMR ($CDCl_3$): 35.4 ($CH_3$); 39.2 (C(3)); 66.15 (C(2)); 126.9, 128.8, 129.1 (arom. CH); 140.8 (arom. C); 177.0 (CO). MS: 179 (traces, $M^+ - 1$), 134 (100), 119 (18), 91 (19), 87 (53), 77 (7), 65 (9).

$N^2$,$N^2$-*Dimethyl-L-phenylalaninamide Hydrochloride* ($Me_2$-Phe-$NH_2 \cdot$HCl). L-Phenylalaninamide hydrochloride (50 mmol) and formaldehyde (40% aq. soln.; 50 mmol) in MeOH were stirred in the presence of 10% Pd/C (2 g) under $H_2$ at 40° for 3 h. The soln. was filtered, acidified to pH 2–3 with HCl/(MeOH, evaporated to small bulk under vacuum, and recrystallized from MeOH/acetone. Yield 85%. M.p. 224–226°, $[\alpha]_D^{25} = +46.74$ (MeOH, $c = 1$). IR: 3300–3280, 2000, 1670, 1600, 760–650. $^1$H-NMR (as amine in $CDCl_3$): 2.40 (*s*, 2 $CH_3$); 2.80–3.37 (*m*, H–C(2), 2 H–C(3)); 5.80 (br. *s*, 1H, $CONH_2$); 6.70 (br. *s*, 1H, $CONH_2$); 7.30 (*s*, 5 arom. H). $^{13}$C-NMR ($CDCl_3$): 33.6 (C(3)); 43.0 ($CH_3$); 71.3 (C(2)); 126.7, 129.1 (arom. CH); 139.2 (arom. C); 175.8 (CO). MS: 192 (traces, $M^+ - 1$), 148 (100), 133 (19), 101 (41), 91 (13), 77 (7), 65 (5).

*Potentiometric Measurements.* All experiments were performed at 25±0.1° and $I = 0.1$M (KCl) under $N_2$. Appropriate aliquots of soln. of the ligands, prepared by weight, were titrated with standard KOH (0.1M KCl) in the absence and in the presence of Cu(II). Freshly boiled bidistilled $H_2O$ was used throughout.

The protonation constants of the ligands were obtained by titrating three 50-ml samples (0.005–0.008M) of each ligand. For the complex formation constants, five or six titrations were performed with different ligand/metal ratios (1.5 up to 4), $c_{Cu}$ ranging from 0.001 to 0.003M.

Potentiometric titrations were carried out with a fully automatic apparatus equipped with *Radiometer PHM64* digital voltmeter and 5 ml *Metrohm Dosimat E535* motor burette, both controlled by an *Apple IIe* personal computer [27] [28]. The electrodic chain (*G 202 B Radiometer* glass electrode and KCl-sat. calomel *E 7786 Ingold* electrode) was calibrated in terms of $[H^+]$ by the *Gran* method [29] by titrating 0.4–0.5 mmol of HCl in a starting volume of 50 ml with KOH soln. (*ca.* 0.2M). The mean value of the ionic product of $H_2O$ ($pK_W$), as evaluated from the alkaline part of the *Gran* plot, is 13.77(1).

*CD Spectra.* CD spectra were obtained at pH ≈ 10 with a $CuL_2H_{-2}$ complex concentration of 1 mM for the 700–350-nm region and of 0.2 mM for the UV region (350–200 nm).

*Calculations.* The stability constants were refined by the program SUPERQUAD [11] on a *Cray X-MP/12* computer of *CINECA*, Bologna. The program calculates the weighted squares of the residuals between observed and calculated *e.m.f.* values. The weighting of the experimental observations takes into account the errors of both *e.m.f.* and titrant volume that were estimated as 0.2 mV and 0.008 ml, respectively. The protonation and complexation constants were refined from separate sets of data, each group of titrations being treated in a unique batch.

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