148. Stereoselective Formation of Ternary Copper(II) Complexes of (*S*)-Amino-acid Amides and (*R*)- or (*S*)-Amino Acids in Aqueous Solution

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Formation constants of ternary complexes of Cu^{II} with (*S*)-amino-acid amides (phenylalaninamide, prolinamide, and tryptophanamide) and (*R*)- or (*S*)-amino acids (valine, phenylalanine, proline, and tryptophan) were determined potentiometrically at 25° and $I = 0.1$ M (KCl). Significant stereoselectivity was found for the systems (*S*)-tryptophanamide/proline, (*S*)-prolinamide/tryptophan, and (*S*)-phenylalaninamide/proline, the diastereoisomeric complexes with 'homochiral' (*SS*) being more stable than with 'heterochiral' (*SR*) ligands. The stereoselectivity observed may be explained on the basis of repulsive interactions between the ligand side-chain residues. The present data on the stability of mixed complexes in solution allow to draw some conclusions on the mechanism of chiral discrimination of amino acids in HPLC (reversed-phase) using Cu^{II} complexes of (*S*)-amino-acid amides as selectors for ligand-exchange chromatography (LEC).

1. Introduction. – Several investigations on mixed complexes of transition-metal ions with amino acids or dipeptides and different ligands are reported in the literature both in solution and in the solid state because of their significance as models for metal-ion-assisted biological processes [1] [2]. The stabilities of these complexes were often explained on the basis of noncovalent interactions such as hydrophobic effects, aromatic ring stacking, H-bonding, or electrostatic ligand-ligand interactions [3].

Stereoselectivity in mixed complexes of Cu^{II} with α-amino acids has received little attention [4], and it appears to be essentially restricted to systems in which phenylalanine [5] [6], proline [7] [8], tryptophan [5–7], tyrosine [8] [9], and histidine [5] [6] are mutually involved, the effect being negligible, whenever one or both amino acids are aliphatic. Significant enantioselective effects were reported for Cu^{II} complexes of *N*-alkylated valine and proline [10], or histidine [5] with amino acids, although some data are not reliable (low precision) [10]. *N*-carboxymethyl derivatives of (*S*)-Val, Ser, Ileu, Ala, Asp, Glu are enantioselective towards (*R*)/(*S*)-Val, Thr, and Leu [11].

Diastereoisomeric mixed-complex formation has been considered to account for chiral discrimination of α-amino acids and derivatives in HPLC according to a mechanism of ligand exchange (LEC; Ligand-Exchange Chromatography) [12].

As a part of a general project aimed at studying enantioselective interactions of Cu^{II} complexes with amino acids [13], we reported that Cu^{II} complexes of (*S*)-amino-acid amides, added to the mobile phase, were able to give enantiomer separation of dansyl-amino acids [14] and unmodified amino acids [15] in HPLC (reversed phase). On the basis

of chromatographic parameters, it appears that the mechanism of chiral discrimination in the chromatographic system involves several equilibria: *i*) formation of mixed Cu^{II} complexes in aqueous solution, *ii*) formation of mixed complexes in the column stationary phase; *iii*) partition equilibria of the species between the aqueous and the stationary phase [16].

To evaluate the importance of the equilibria in aqueous solution on the overall discrimination mechanism, we have performed and report here a detailed potentiometric study of the formation constants of the ternary Cu^{II} complexes of (*S*)-amino-acid amides (phenylalaninamide (Phe-NH_2); prolinamide (Pro-NH_2); tryptophanamide (Trp-NH_2)) with (*R*)- or (*S*)-amino acids (Val, Phe, Pro, Trp).

Results and Discussion. – *Cu^{II} Binary Complexation Equilibria.* Protonation and Cu^{II} complexation by (*S*)-tryptophanamide has been examined in this work, whereas Cu^{II}-complex formation constants for (*S*)-prolinamide and (*S*)-phenylalaninamide were reported by us in a previous work [17]. The potentiometric data, processed by the program SUPERQUAD [18], well fitted the chemical model containing the species found for most of amino-acid amides already studied [17] [19], *i.e.*, $[CuL]^{2+}$, $[CuL_2]^{2+}$, $[CuLH_{-1}]^{+}$, $[CuL_2H_{-1}]^{+}$, $[CuL_2H_{-2}]$. In addition, other species like $[CuLH_{-2}]$, $[Cu_2L_2H_{-2}]^{2+}$, and $[CuL_2H_{-3}]^{-}$ were considered first, but were then rejected. Protonation and Cu^{II}-complex formation constants of the amides examined are reported in *Table 1*.

Table 1. *Logarithms of Protonation and Cu^{II} Complex-Formation Constants ($\beta_{pqr} = [Cu_pL_qH_r]/[Cu]^p[L]^q[H]^r$) of (S)-Tryptophanamide (Trp-NH_2), (S)-Phenylalaninamide (Phe-NH_2), and (S)-Prolinamide (Pro-NH_2).* $T = 25°$, $I = 0.1$M (KCl). Standard devations are given in parentheses.

	Trp-NH_2[a])	Phe-NH_2[b])	Pro-NH_2[b])
HL^+	7.49(1)	7.26(1)	8.69(1)
$[CuL]^{2+}$	4.70(1)	4.42(1)	5.74(1)
$[CuL_2]^{2+}$	8.86(1)	7.84(2)	10.36(3)
$[CuLH_{-1}]^{+}$	−1.99(4)	−2.08(3)	−0.86(2)
$[CuL_2H_{-1}]^{+}$	2.73(1)	1.90(2)	3.87(2)
$[CuL_2H_{-2}]$	−4.93(1)	−5.46(2)	−3.62(1)

[a]) This work. [b]) From [17].

To examine the chemical model obtained by potentiometry for the Cu^{II}/Trp-NH_2 system and to characterize the complex species, a spectrophotometric study in the visible region (400–790 nm) was carried out. Spectral data were processed by the program SQUAD [20], and the absorption coefficients of the species (ε) were calculated at various wavelengths, by using the formation constants listed in *Table 1*. The λ_{max} and the corresponding ε values obtained for the complexes were consistent with those reported for Cu^{II}/(*S*)-Ala-NH_2 [21]: $[CuL]^{2+}$ ($\lambda = 764$ nm, $\varepsilon = 51$ $M^{-1}cm^{-1}$), $[CuL_2]^{2+}$ (650, 56), $[CuLH_{-1}]^{+}$ (643, 43), $[CuL_2H_{-1}]^{+}$ (582, 54), $[CuL_2H_{-2}]$ (516, 56). These results confirm the previous hypothesis about the coordination modes of Cu^{II} with amino-acid amides in solution [17] [19].

As far as the constants of the equilibria of Cu^{II} with the amino acids examined are concerned, literature values [22] concerning (*S*)-enantiomers were used for the calculations of the ternary systems (*Table 2*).

Table 2. *Literature Values* (from [22]) *for Protonation and Cu^{II} Complex-Formation Constants* ($\log\beta_{pqr}$) *of (S)-Amino Acids, Val, Phe, Pro, and Trp Used in the Calculations. $T = 25°$, $I = 0.1$M.*

	(*S*)-Val	(*S*)-Pro	(*S*)-Phe	(*S*)-Trp
HA	9.49(3)	10.41(10)	9.09(4)	9.32(5)
H_2A^+	11.75(2)	12.30(10)	11.26(4)	11.67(5)
$[CuA]^+$	8.09(4)	8.84(2)	7.90(4)	8.25(3)
$[CuA_2]$	14.90(10)	16.36(8)	14.80(10)	15.40(10)

Ternary Systems Cu^{II}/(S)-Amino-acid Amide/(R)- or (S)-Amino Acid. With the assumption that, in the ternary complexes, Cu^{II} presents the same coordination number and geometry as in its bis-complexes with amino acids and amino-acid amides, only the species $[CuLA]^+$ and $[CuLH_{-1}A]$ were considered in the calculations. The results are shown in *Tables 3–5*. The distribution diagrams for Cu^{II}/(*S*)-Trp-NH_2/(*R*)-Pro and

Table 3. *Formation Constants* ($\log\beta_{pqrs}$; $\beta_{pqrs} = [Cu_pL_qA_rH_s]/[Cu]^p[L]^q[A]^r[H]^s$) *of the Ternary Cu^{II} Complexes of (S)-Pro-NH_2 with (R)- or (S)-Amino Acids. $T = 25°$ and $I = 0.1$M (KCl).* Standard deviations are given in parentheses. L = Amino-acid amide, A^- = aminoacidato.

	Val		Pro		Phe		Trp	
	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)
$[CuLA]^+$	12.97(2)	13.02(1)	13.63(2)	13.65(2)	12.94(1)	12.99(1)	13.53(2)	13.70(1)
$[CuLH_{-1}A]$	5.94(1)	6.05(1)	6.47(2)	6.51(1)	6.16(1)	6.23(1)	6.66(1)	6.95(1)
s^{2a})	0.58	0.36	1.56	1.19	3.80	2.46	0.67	0.01
n^a)	180	185	216	262	326	361	226	130

a) $s^2 = \Sigma w_i(E_i^o - E_i^c)^2/(n-m)$ = sample variance; $w_i = 1/\sigma_i^2$, where σ_i is the expected error on each experimental *e.m.f.* value (E_i^o); n = number of observations; m = number of parameters refined.

Table 4. *Formation Constants* ($\log\beta_{pqrs}$) *of the Ternary Cu^{II} Complexes of (S)-Phe-NH_2 with (R)- or (S)-Amino Acids. $T = 25°$ and $I = 0.1$M (KCl).* Standard deviations are given in parentheses.

	Val		Pro		Phe		Trp	
	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)
$[CuLA]^+$	11.93(1)	11.88(1)	12.55(1)	12.65(1)	11.73(2)	11.71(3)	12.39(1)	12.42(1)
$[CuLH_{-1}A]$	4.99(1)	4.91(1)	5.58(1)	5.76(1)	5.03(1)	5.01(2)	5.65(1)	5.67(1)
s^{2a})	0.72	0.83	0.69	0.64	0.37	0.58	1.59	1.35
n^a)	323	259	190	246	159	151	201	243

a) *Cf. Footnote* to *Table 3.*

Table 5. *Formation Constants* ($\log\beta_{pqrs}$) *of the Ternary Cu^{II} Complexes of (S)-Trp-NH_2 with (R)- or (S)-Amino Acids. $T = 25°$ and $I = 0.1$M (KCl).* Standard deviations are given in parentheses.

	Val		Pro		Phe		Trp	
	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)	(*R*)	(*S*)
$[CuLA]^+$	12.36(1)	12.35(1)	12.88(2)	13.27(1)	12.29(1)	12.30(1)	12.80(1)	12.81(1)
$[CuLH_{-1}A]$	5.33(1)	5.28(1)	5.85(2)	6.20(1)	5.33(1)	5.33(1)	5.89(1)	5.93(1)
s^{2a})	0.55	1.44	4.10	3.39	1.02	1.74	0.86	1.49
n^a)	196	233	265	314	232	260	316	249

a) *Cf. Footnote* to *Table 3.*

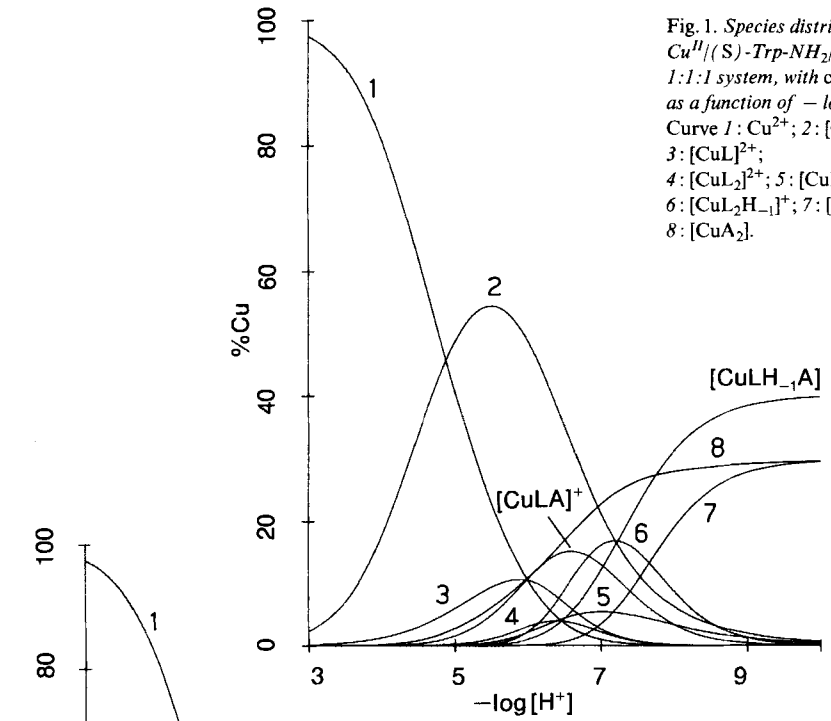

Fig. 1. *Species distribution for the* Cu^{II}*/(S)-Trp-*NH_2*/(R)-Pro 1:1:1 system, with* $c_{Cu} = 0.001$ M, *as a function of* $-\log[H^+]$. Curve *1*: Cu^{2+}; *2*: $[CuA]^+$; *3*: $[CuL]^{2+}$; *4*: $[CuL_2]^{2+}$; *5*: $[CuLH_{-1}]^+$; *6*: $[CuL_2H_{-1}]^+$; *7*: $[CuL_2H_{-2}]$; *8*: $[CuA_2]$.

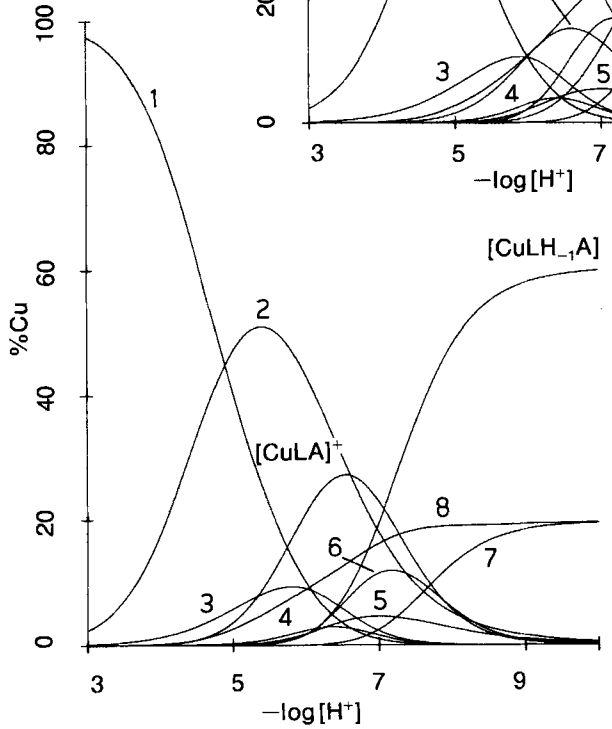

Fig. 2. *Species distribution for the* Cu^{II}*/(S)-Trp-*NH_2*/(S)-Pro 1:1:1 system, with* $c_{Cu} = 0.001$ M, *as a function of* $-\log[H^+]$. Curve *1*: Cu^{2+}; *2*: $[CuA]^+$; *3*: $[CuL]^{2+}$; *4*: $[CuL_2]^{2+}$; *5*: $[CuLH_{-1}]^+$; *6*: $[CuL_2H_{-1}]^+$; *7*: $[CuL_2H_{-2}]$; *8*: $[CuA_2]$.

Cu^{II}/(*S*)-Trp-NH_2/(*S*)-Pro systems as a function of pH are reported in *Figs. 1* and *2*, respectively.

The enantioselective effect may be expressed quantitatively by the difference between the formation constant of the diastereoisomeric ternary complex formed by the (*S*)-amide with the (*R*)- ($\log\beta_{SR}$) and with the (*S*)-amino acid ($\log\beta_{SS}$):

Table 6. *Enantioselectivity, Expressed as* $\Delta\log\beta = \log\beta_{SR} - \log\beta_{SS}$, *in the Formation of* Cu^{II} *Ternary Complexes of (S)-Amino-acid Amides with (R)- or (S)-Amino Acids.* $T = 25°$ and $I = 0.1$ M (KCl). Standard deviations are given in parentheses[a]).

		Pro-NH_2	Phe-NH_2	Trp-NH_2
Val	$[CuLA]^+$	−0.05(2)	0.05(1)	−0.01(1)
	$[CuLH_{-1}A]$	−0.11(1)	0.08(1)	0.05(1)
Pro	$[CuLA]^+$	−0.02(3)	−0.10(1)	−0.39(2)
	$[CuLH_{-1}A]$	−0.04(2)	−0.18(1)	−0.35(2)
Phe	$[CuLA]^+$	−0.05(1)	0.02(4)	−0.01(1)
	$[CuLH_{-1}A]$	−0.07(1)	0.02(2)	0.00(1)
Trp	$[CuLA]^+$	−0.17(3)	−0.03(1)	−0.01(1)
	$[CuLH_{-1}A]$	−0.29(1)	−0.02(1)	−0.04(1)

[a]) $\sigma(\Delta\log\beta) = [\sigma^2(\log\beta_{SR}) + \sigma^2(\log\beta_{SS})]^{1/2}$.

$$\Delta\log\beta = \log\beta_{SR} - \log\beta_{SS}$$

$\Delta\log\beta$ values calculated for the ligands examined (*Table 6*) show significant stereoselectivity in the systems (*S*)-Trp-NH_2/Pro, (*S*)-Pro-NH_2/Trp, and (*S*)-Phe-NH_2/Pro, both for the species $[CuLA]^+$ and $[CuLH_{-1}A]$. Interestingly, there are two common features of these complexes: *i*) (*S*)-Pro or (*S*)-Pro-NH_2 are always involved together with different aromatic amides or amino acids, respectively; *ii*) the species (both $[CuLA]^+$ and $[CuLH_{-1}A]$) with 'homochiral' ligands (*SS*) are always more stable than the corresponding ones with 'heterochiral' ligands (*SR*).

Both possible *cis*- and *trans*-configurations must be considered for the structures of the species $[CuLH_{-1}A]$ (*Fig. 3*).

On the basis of molecular models, the (*SR*)-*cis*-isomers turn out to be sterically hindered at least in some conformers, thus appearing less stable than the (*SS*)-*cis*-isomers, in which the two residues are on the opposite sides of the Cu coordination plane. On the contrary, the *trans*-conformers of both diastereoisomeric species (*SS*) and (*SR*) do not present any repulsive interaction. On the other hand, the higher stability constants for the (*SS*)-species cannot be due to the hydrophobic interactions between the amide/amino acid side chains, considering that (*S*)-Pro is always involved, which has one of the lowest *Hansch* coefficients ($\pi = 0.70$) observed for neutral amino acids [23].

The enantioselectivities presented by the complex $[CuLH_{-1}A]$ in the systems (*S*)-Trp-NH_2/Pro ($\Delta\log\beta = -0.35$), (*S*)-Pro-NH_2/Trp (−0.29), and (*S*)-Pro-NH_2/Val (−0.11) are consistent with those observed with the complex [CuAA'] of the corresponding amino acids, *i.e.*, Trp/Pro (−0.26) [7] and Val/Pro (−0.08) [9], but they are higher.

$\Delta\log\beta$ value is small and negative for (*S*)-Pro-NH_2/Val and (*S*)-Pro-NH_2/Phe, small and positive for (*S*)-Phe-NH_2/Val and (*S*)-Trp-NH_2/Val, whereas it is negligible or zero in the remaining systems.

It is worth mentioning that there is no stereoselectivity, when both the amide and the amino acid are aromatic, thus excluding stacking interactions in the systems. Only repulsive interactions due to the rigid pyrrolidine ring appear to be responsible for stereoselectivity of Cu^{II} complexes in aqueous solution.

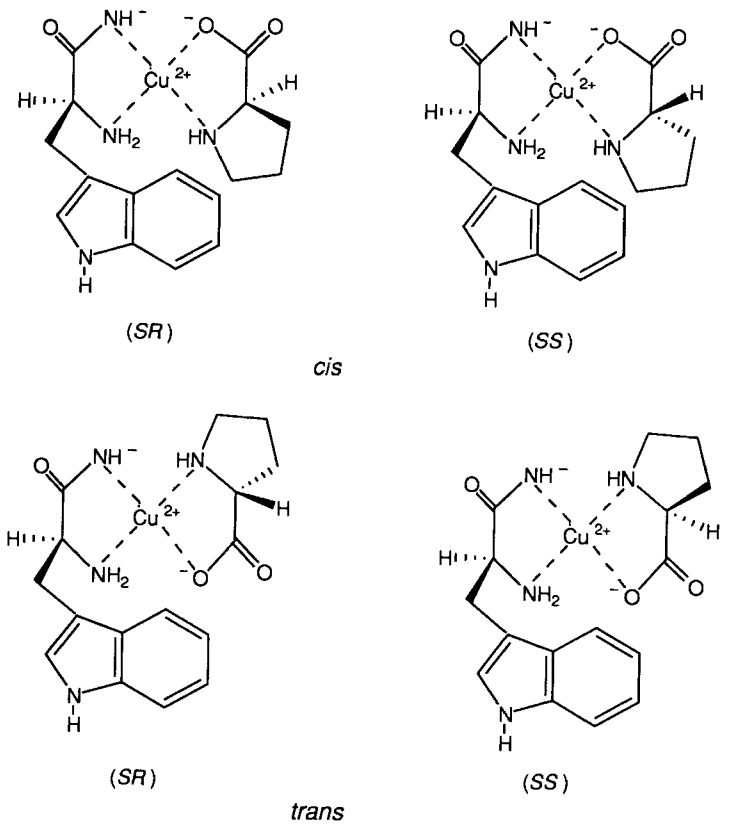

Fig. 3. *Schematic structures of the* cis- *and* trans-*isomers of the ternary complex [CuLH$_{-1}$A] for the system* Cu^{II}/(S)-*Trp-NH$_2$*/(R)- *or* (S)-*Pro.*

Indeed, very good enantioselectivity factors (α) were observed in HPLC when using (*S*)-Pro-NH_2 as selector ($\alpha = 3.05$ for Val), as well as for (*S*)-Phe-NH_2 and (*S*)-Trp-NH_2 [15]. Indeed, with (*S*)-Trp-NH_2, it was possible to demonstrate for the first time that a ligand-exchange mechanism occurs in the chromatographic system. As Cu^{II} is a fluorescence quencher, in the binary complex bis((*S*)-tryptophanamidato)copper(II), fluorescence was almost completely quenched. When using this complex as eluent and a fluorescence detector, a unique signal was observed with the same retention time for all (*R*)- and (*S*)-amino acids, corresponding to free (*S*)-Trp-NH_2. The mixed Cu complexes, being nonfluorescent, were not detectable under the conditions used. Indeed, fluorescence was induced by displacement of one (*S*)-Trp-NH_2 ligand from the initial binary complex, according to

$$[Cu(Trp\text{-}NH_2)_2] + A^- \rightleftharpoons [Cu(Trp\text{-}NH_2)A]^- + Trp\text{-}NH_2$$

Moreover, when a UV detector was used at 254 nm, three signals were obtained, one corresponding to (*S*)-Trp-NH_2 and the other two to the diastereoisomeric ternary complexes [15].

However, the amino-acid elution order observed ($(R) < (S)$) is in contrast with the stabilities of the mixed complexes reported here ($(SR) < (SS)$), supporting the assumption that the equilibria of ligand exchange occurring in aqueous solution do not exclusively account for the overall discriminative process, but that the interactions of the mixed complexes with the reversed-phase column are the most relevant. Moreover, it is reasonable to assume that both *cis*- and *trans*-mixed species can be formed in solution, and that the more retained species, protruding the side chains of both the amide and the amino acid into the stationary phase, are the *trans*-complexes.

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Experimental Part

Reagents. (*S*)-Phenylalaninamide, (*S*)-prolinamide, and (*S*)-tryptophanamide hydrochlorides (*Sigma*), (*R*)- and (*S*)-valine, (*R*)- and (*S*)-phenylalanine, (*R*)- and (*S*)-proline, and (*R*)- and (*S*)-tryptophan (*Fluka*) were all of high purity, and they were used without further purification. The elemental analysis (C, H, N) of all the ligands gave acceptable results. Their purity was checked by means of potentiometric titrations with KOH soln. The ligands were dried over P_4O_{10} *in vacuo*, and stock soln. (*ca.* 0.02M) were prepared by weight and used within 2–3 days. KOH and HCl solns. (*ca.* 0.2M) were prepared by diluting concentrated *Merck Titrisol* ampoules. The concentration of the KOH soln. was determined potentiometrically by titration against potassium hydrogen phthalate (*Merck*, dried at 120°), and the titre of the HCl solns. was derived from potentiometric titrations with KOH. $CuCl_2 \cdot 2H_2O$ (*C. Erba*) was employed for the preparation of a stock soln. (0.01701M) which was analyzed by EDTA. All solns. were prepared with freshly boiled bidistilled H_2O.

Potentiometric Measurements. The experiments were carried out at 25 ± 0.1° and $I = 0.1$M (KCl) under N_2 stream previously saturated with water vapor in 0.1M KCl soln. Potentiometric titrations were performed with an automatic apparatus equipped with radiometer *PHM64* digital voltmeter and 5-ml *Metrohm 655 Dosimat* motor burette, both controlled by an *Apple IIe* PC. The electrode couple (*Ingold B2905* glass and KCl-sat. calomel *E7786 Ingold* electrodes) was calibrated in terms of $[H^+]$ by titrating HCl solns. (0.004M) in a starting volume of 50 ml with standard KOH solns. The PC program BEATRIX [24], based on the *Gran* method [25], was used to calculate V_e (equivalence volume), E° (electrodic chain standard potential), and pK_w (13.77(1)).

Appropriate aliquots of the soln. of the ligands, Cu^{II}, and HCl were added in the cell, and the volume was adjusted to 50 (80) ml with H_2O.

The protonation constant of (*S*)-Trp-NH_2 was determined by alkalimetric titration of four samples (0.005–0.008M) of the ligand. For the Cu^{II}/(*S*)-Trp-NH_2 equilibria, six titrations were performed with various ligand/metal ratios (1.5:1 to 5:1), c_{Cu} ranging from 0.001 to 0.002M, whereas, for each of the ternary systems considered, 4–6 titrations were carried out with Cu/L/A ratios of 1:1:1 and 1:2:1 ($c_{Cu} = 0.001$–0.002M). The pH range explored was between 3 and 10, except for (*S*)-Trp-NH_2/(*R*)-Trp system, because, at pH of *ca.* 8.6, a precipitate was formed.

Spectrophotometric Measurements. The absorption spectra were recorded on a *Kontron Uvikon 860* spectrophotometer using a 0.1M KCl soln. as reference. Matched quarz cells of 4-cm pathlength were employed. Aliquots of the potentiometric solns. were taken at ten prefixed pH values with a syringe and transferred into the cuvette, and the spectra recorded between 400 and 790 nm at 2-nm intervals. The absorption values (λ_{max}, A) obtained were transmitted to a *IBM* PC by a communication routine and then converted to the SQUAD [20] format.

Calculations. The stability constants were calculated by the computer program SUPERQUAD [18], employing the sum of the weighted squares of the residuals between observed and calculated *e.m.f.* values as the optimization function. The weighting of the exper. 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. Trial $\log\beta$ values for the ternary complexes were refined, while the constants pertaining ligand protonation and binary Cu^{II} complexation were fixed. For each system, the data from different titrations were treated in a unique batch.

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