Fig. 2.—Distribution of copper species (as % total copper) for system in which $[Cu]_T = 1 \times 10^{-4}$ M, $[DAS] = 4.984 \times 10^{-4}$ M: a, Cu^{2+}; b, $HLRLCu^+$; c, $^+CuLRLCu^+$; d, $(CuLRL)_2^*$; e, $Cu(LRLH)_2$.

methylene bridges. Dimers with structures analogous to this have been demonstrated cryoscopically for the copper(II) complexes of substituted disalicylidenebenzidine, disalicylidene-p-phenylenediamine, and disalicylidene-m-phenylenediamine.[11] The complex precipitated from neutral solutions of DAS containing copper(II) ion is believed to be di-μ-(2,7-diaminosuberato)-dicopper(II), which is the main species present in solution under the experimental conditions and which would be expected to be only sparingly soluble because of its non-ionic character. Chemical analysis is consistent with this structure, but its molecular weight could not be determined because of insolubility. The magnetic moment of 1.91, determined by the Gouy method, is typical of simple copper(II) complexes[12] and indicates the absence of Cu–Cu δ-bonding in this compound.

Polynuclear Complex Formation. II. Copper(II) with Cystine and Related Ligands

BY C. J. HAWKINS[1] AND D. D. PERRIN

In aqueous solution, cystine and copper(II) form mono- and polynuclear complexes. Stability constants for the polynuclear complexes are statistically related to the stepwise formation constants, K_1 and K_2, of the 1:1 and the 1:2 mononuclear complexes. At 20° and an ionic strength of 0.15, log K_1 is 7.00 and log K_2 is 6.72. These values are consistent with mono-(amino acid)-type bonding with a contribution from the disulfide group. A compound, believed to be the polynuclear complex, di-μ-cystinatodicopper(II), which is a major species over a wide range of conditions, has been isolated from solution and its magnetic moment has been measured. Complex formation between copper(II) ion and a number of ligands closely related to cystine also has been investigated. Stability constants of their copper(II) complexes at 20° and an ionic strength of 0.15 have been determined for bis-(2-aminoethyl) disulfide (BAED), 2,2'-dicarboxydiethyl disulfide (DDD), 3-(2-aminoethyldithio)-L-alanine (AEDA), and L-methionine. In all cases the sulfur atoms contribute to the coördination with copper(II), but polynuclear complex formation was not important under the experimental conditions examined.

Although it would be of some biochemical interest, no extensive and quantitative study of equilibria in the aqueous copper(II)–cystine system appears to have been made. This probably is due, in part, to the sparing solubilities of the ligand and the copper(II)–cystine complex and, in part, to the complicated nature of the problem. Kolthoff and Stricks[2] concluded from polarographic evidence that complex formation between copper(II) and cystine was not significant in ammoniacal solution. On the other hand, Hamaguchi and Kamemoto[3] explained polarographic observations by the slow formation of a mononuclear complex, in which

(as previously postulated by Rây and Bhaduri[4] and Scaife[5]) the two amino acid groupings are coördinated to the same metal ion. However, Leybold atomic models show that simultaneous attachment of both ends of the cystine anion to the same copper(II) ion is sterically impossible. This restriction also applies to the structurally related bis-(amino acid), 2,7-diaminosuberic acid, where it leads to both mono- and polynuclear copper(II) complex formation.[6]

In the present paper, equilibrium studies in aqueous solution between hydrated copper(II) ion and L-cystine are described, and the stability constants of the mono-

(1) Australian National University Scholar.
(2) I. M. Kolthoff and W. Stricks, *J. Am. Chem. Soc.*, **73**, 1728 (1951).
(3) H. Hamaguchi and Y. Kamemoto, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **81**, 346 (1960).

(4) P. Rây and A. Bhaduri, *J. Indian Chem. Soc.*, **27**, 297 (1950).
(5) J. F. Scaife, *Can. J. Biochem. Physiol.*, **37**, 1033 (1959).
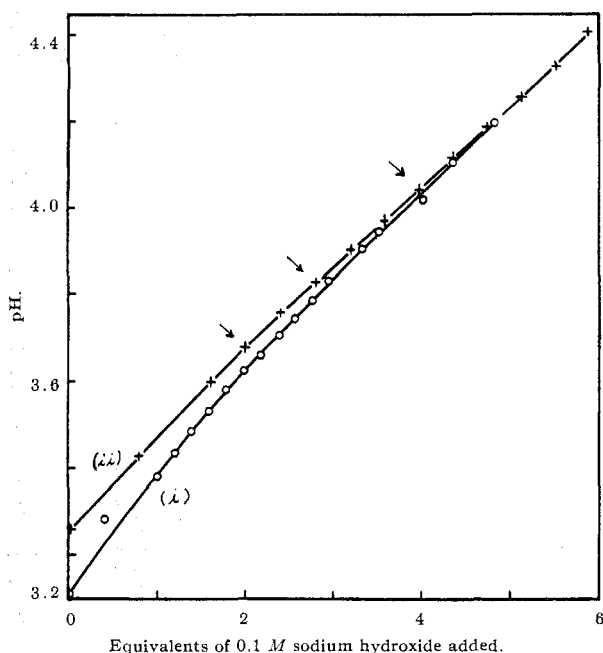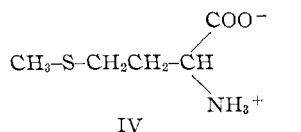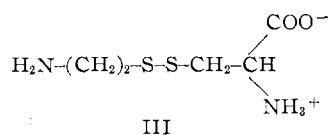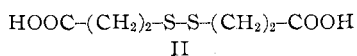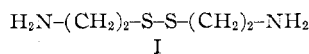(6) C. J. Hawkins and D. D. Perrin, *Inorg. Chem.*, **2**, 839 (1963).

Fig. 1.—Titration curves: (i) 1×10^{-3} M cupric perchlorate and 5.0×10^{-3} M DDD; (ii) 5×10^{-4} M cupric perchlorate and 2.506×10^{-3} M DDD. Arrows on curve (ii) indicate points referred to in Table II (ii).

and polynuclear species, and hence their concentrations under various conditions, are obtained. As a preliminary to this work, potentiometric studies on equilibria in aqueous solution between hydrated copper(II) ion and substances I–IV, which are closely related to cystine, also have been made.

$$H_2N-(CH_2)_2-S-S-(CH_2)_2-NH_2$$
$$I$$

$$HOOC-(CH_2)_2-S-S-(CH_2)_2-COOH$$
$$II$$

$$H_2N-(CH_2)_2-S-S-CH_2-\overset{\displaystyle COO^-}{\underset{\displaystyle NH_3^+}{CH}}$$
$$III$$

$$CH_3-S-CH_2CH_2-\overset{\displaystyle COO^-}{\underset{\displaystyle NH_3^+}{CH}}$$
$$IV$$

Experimental

Apparatus and Reagents.—Bis-(2-aminoethyl) disulfide (I) was prepared from 2-mercaptoethylamine,[7] isolated as the diperchlorate by adding excess concentrated perchloric acid to a cold alcoholic solution of the free base, and the salt was purified by recrystallization from ethanol to give colorless plates, m.p. 124° dec.

Anal. Calcd. for $C_4H_{14}O_8N_2Cl_2S_2$: C, 13.60; H, 4.00; N, 7.93. Found: C, 13.36; H, 3.96; N, 7.81.

2,2′-Dicarboxydiethyl disulfide (II) was prepared from 3-mercaptopropionic acid[8] and recrystallized from water to give colorless plates, m.p. 156°.

Anal. Calcd. for $C_6H_{10}S_2O_4$: C, 34.27; H, 4.79; S, 30.50. Found: C, 34.14; H, 4.91; S, 30.41.

(7) E. J. Mills and M. T. Bogert, *J. Am. Chem. Soc.*, **62**, 1173 (1940).

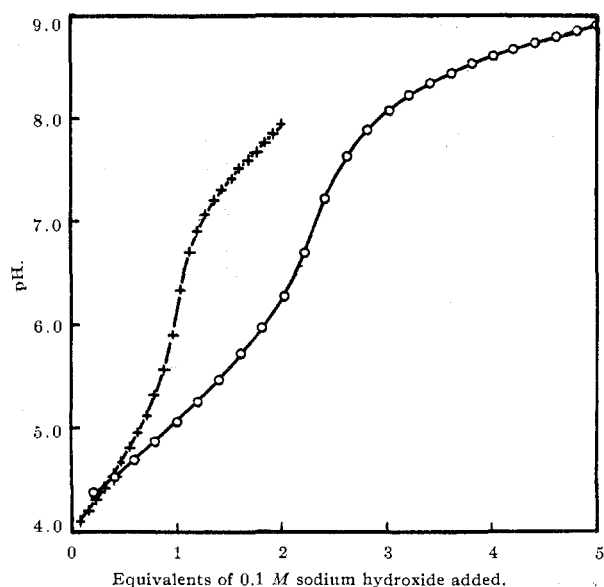(8) B. D. Westerman and W. C. Rose, *J. Biol. Chem.*, **75**, 533 (1927).

Fig. 2.—Titration curves: O, 2×10^{-4} M cupric perchlorate and 4.971×10^{-4} M AEDA hydrochloride; $+$, 5×10^{-4} M cupric perchlorate and 4.995×10^{-4} M AEDA hydrochloride.

3-(2-Aminoethyldithio)-L-alanine (III) was prepared as the hydrochloride according to the method of Schöberl and Gräfje[9] *via* the thiosulfinate ester of bis-(2-aminoethyl) disulfide. It was purified by dissolving the product in a minimum of water and fractionally precipitating the compound by adding acetone dropwise with stirring and chilling to give colorless crystals, m.p. 171–172°.

Anal. Calcd. for $C_5H_{13}O_2N_2ClS_2$: N, 12.03; S, 27.55. Found: N, 11.81; S, 27.47.

L-Methionine (IV) (California Corp. for Biochemical Research A grade reagent) was used without further purification. L-Cystine (British Drug Houses Ltd.) was purified by repeated solution in hydrochloric acid and reprecipitation by the dropwise addition of alkali. The final product was dried at 100° for 2 hr. and *in vacuo* for 16 hr. at room temperature. Other reagents, the apparatus, and the method of titration were as described in part I.[6] Titration curves have been plotted in Fig. 1 to 3.

Acid Dissociation Constants.—The pK_a values in Table I were determined by potentiometric titration and calculated using the complete Henderson–Hasselbalch equation. Overlapping pK_a values were obtained by a graphical method as in part I.[6] Because of the low solubility of cystine in water, values for pK_{a_1} and pK_{a_2} could not be obtained, and, to evaluate pK_{a_3} and pK_{a_4}, the amino acid was dissolved in two equivalents of alkali and back-titrated. Although the latter values probably are accurate only to within about ±0.10, because the solution, on back titration, was supersaturated, they agree within experimental error with literature values[10] of $pK_{a_3} = 8.02$ and $pK_{a_4} = 8.71$.

TABLE I

ACID DISSOCIATION CONSTANTS AT 20° AND $\mu = 0.15$ (NaClO$_4$)

	pK_{a_1}	pK_{a_2}	pK_{a_3}	pK_{a_4}
Bis-(2-aminoethyl) disulfide (BAED)	8.82	9.16		
2,2′-Dicarboxydiethyl disulfide (DDD)	3.88	4.47		
3-(2-Aminoethyldithio)-L-alanine (AEDA)			8.28	9.30
L-Methionine	2.17	9.20		
	2.20[11]	9.34[11]		
L-Cystine			8.03	8.80

(9) A. Schöberl and H. Gräfje, *Liebigs Ann. Chem.*, **617**, 71 (1958).

(10) At 35° and $\mu = 0.1$; J. D. Greenstein, F. W. Klemperer, and J. Wyman, *J. Biol. Chem.*, **129**, 681 (1939).

(11) At 20° and $\mu = 0.01$; A. Albert, *Biochem. J.*, **47**, 531 (1950).

In compounds of the type $^+H_3N-R-CH\begin{smallmatrix}NH_3^+\\ \diagup \\ \diagdown \\ COO^-\end{smallmatrix}$, the highest pK_a

value is assigned to the basic amine group.[12] Hence, in AEDA, $pK_{a_1} = 9.30$ relates to the amine group and $pK_{a_2} = 8.28$ to the amino acid nitrogen.

Preparation of Copper(II)–Cystine Complex.—A blue, finely crystalline complex was prepared from copper sulfate and cystine by the method of Rây and Bhaduri.[4] It was extremely insoluble in all solvents that were tried, and it contained copper and cystine in an equimolar ratio.

Anal. Calcd. for $Cu(C_6H_{10}N_2S_2O_4)\cdot H_2O$: Cu, 19.87; C, 22.53; H, 3.78; N, 8.76; S, 20.05. Found: Cu, 19.75; C, 22.76; H, 3.69; N, 8.85; S, 20.19.

Discussion

Copper(II) and 2,2'-Dicarboxydiethyl Disulfide.—Leybold atomic models show that both ends of DDD (a complex-forming species of type HLRLH) are capable of simultaneous attachment to the same metal ion, and so, also, is the –S–S– group. Although the complexing ability of the latter, *per se*, probably is weak, the stabilizing effect associated with five- and six-membered chelate ring formation would be expected to favor the formation of the 1:1 complexes, V and VI, so that this ligand is not of the type discussed in part I.[6]
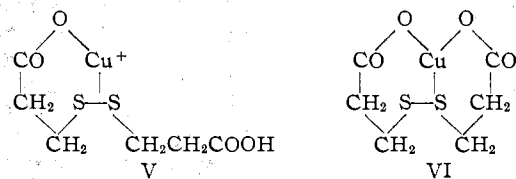
Because VI is a planar terdentate structure, formation of 1:2 complexes would not be expected to be very important. Relevant equilibria in acid solution would be

$$Cu^{2+} + HLRLH \rightleftharpoons HLRLCu^+ + H^+$$

$$Cu^{2+} + HLRLH \rightleftharpoons \overline{LRLCu} + 2H^+$$
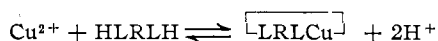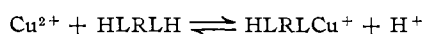
In the system where copper(II) ion is added to a solution of DDD and titrated with standard alkali, the equations for total concentrations of metal species, total concentrations of ligand species, and total charges become, respectively

$$[Cu]_T = [Cu^{2+}] + [HLRLCu^+] + [\overline{LRLCu}] \quad (1)$$

$$= [Cu^{2+}] + K_1[Cu^{2+}][HLRL^-] + K'K_{a_2}[Cu^{2+}][HLRL^-]/[H^+] \quad (2)$$

where K_1 and K' are the stability constants of HLRL-Cu^+ and $\overline{LRLCu}$

$$[HLRLH]_T = [HLRLH] + [HLRL^-] + [LRL^{2-}] + [HLRLCu^+] + [\overline{LRLCu}] \quad (3)$$

$$2[Cu]_T + [HLRL^-] + 2[LRL^{2-}] = [Na^+] + [H^+] + 2[Cu^{2+}] + [HLRLCu^+] \quad (4)$$

Hence, by subtracting eq. 1 from eq. 3 and rearranging

$$[Cu^{2+}] = a[HLRL^-] - ([HLRLH]_T - [Cu]_T) \quad (5)$$

(12) A. Albert, *Biochem. J*; **50**, 690 (1952).

where $a = 1 + [H^+]/K_{a_1} + K_{a_2}/[H^+]$. From eq. 1 to 5 it can be deduced that

$$aK_1[HLRL^-]^2 + \{(2a - b) - K_1([HLRLH]_T - [Cu]_T)\}[HLRL^-] + ([Na^+] + [H^+] - 2[HLRLH]_T) = 0 \quad (6)$$

where $b = 1 + 2K_{a_2}/[H^+]$.

Selecting two arbitrary points—one at the beginning, and the other at the end, of the titration (i) in Fig. 1—and using an iterative procedure to solve eq. 2, 5, and 6, K_1 and K' were evaluated. This value of $K_1 = 3.5 \times 10^2 \; M^{-1}$ then was used in eq. 5 and 6 to obtain $[Cu^{2+}]$ and $[HLRL^-]$ at each point in the titration, so that, by substitution in eq. 2, the corresponding values of K' could be calculated. The average over titration (i) was $K' = 1.04 \pm 0.12 \times 10^3 \; M^{-1}$, the maximum deviation being $0.23 \times 10^3 \; M^{-1}$. Arbitrarily selected points from titration (ii) (as indicated in Fig. 1) gave the values for K' listed in Table II (ii). These values agreed, within experimental error, with K' from titration (i). The consistency of K' over the two titrations, in which both the total concentration of metal and total concentration of ligand were different, strongly supports the above interpretation.

Table II
Copper(II) and 2,2'-Dicarboxydiethyl Disulfide[a]

$[Cu^{2+}]$ $\times 10^4, M$	$[HLRL^-]$ $\times 10^4, M$	K' $\times 10^{-3}, M^{-1}$	$[Cu^{2+}]$ $\times 10^4, M$	$[HLRL^-]$ $\times 10^4, M$	K' $\times 10^{-3}, M^{-1}$
(i) $[Cu]_T = 1.0 \times 10^{-3} M$, $[HLRLH]_T = 5.0 \times 10^{-3} M$					
6.942	9.898	1.14	5.168	16.166	1.21
6.769	10.818	(0.93)	4.974	16.819	1.21
6.547	11.670	0.93	4.913	17.583	1.03
6.270	12.434	1.08	4.563	18.633	1.06
6.107	13.341	0.95	4.473	19.160	0.98
5.886	14.135	0.97	4.301	19.993	0.86
5.542	14.691	1.27	3.861	20.453	0.99
5.387	15.497	1.17	3.644	20.678	(0.93)
(ii) $[Cu]_T = 5.0 \times 10^{-4} M$, $[HLRLH]_T = 2.506 \times 10^{-3} M$					
3.546	8.110	0.93	2.726	10.600	1.23
3.157	9.312	1.24			

[a] Calculated from titration curves given in Fig. 1, taking $K_1 = 3.5 \times 10^2 \; M^{-1}$.

Among related ligands, a linear relationship commonly exists between the acid dissociation constants of the ligands and the stability constants of the metal complexes they form. The value of $\log K_1 = 2.54$ for the copper(II) complex with the carboxylic acid, DDD, ($pK_a = 3.88$) is much greater than would be expected for a simple carboxylic acid of similar pK_a ($\log K_1 \approx 1.4$).[13,14] The additional stabilization is attributed to the contribution the disulfide group makes to the coordination; a similar effect is found for the sulfide group in the copper(II) complex with ethylthioacetic acid, where $\log K_1 = 2.56$ and $pK_a = 3.61$.[15]

The enhanced stability associated with chelate ring formation is almost entirely confined to rings containing

(13) S. Fronaeus, "Komplexsystem hos koppar," Gleerupska-Universitets-Bokhandeln, Lund, 1948.
(14) D. D. Perrin, *Nature*, **191**, 253 (1961).
(15) At 20° and $\mu = 1.0$; A. Sandell, *Acta Chem. Scand.*, **15**, 190 (1961).
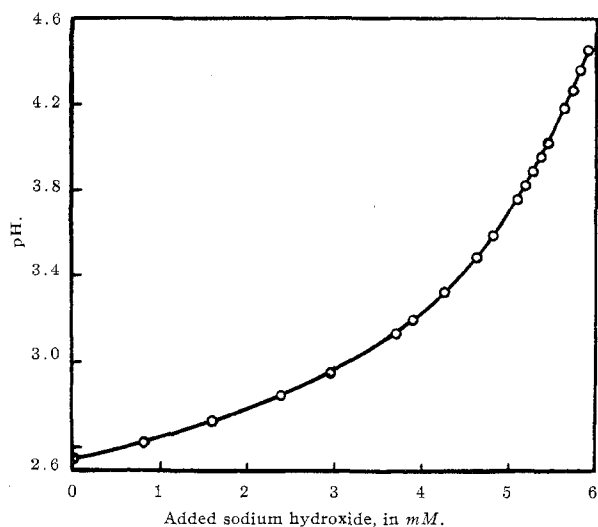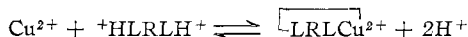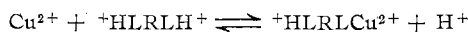
Fig. 3.—Titration curve: 1×10^{-3} M cupric perchlorate, 5×10^{-3} M methionine, and 5×10^{-3} M perchloric acid.

five or six members; it would certainly not be expected to operate in complexes coördinated only through the two carboxyl groups of DDD where, in this case, formation of an 11-membered chelate ring would be required. On the other hand, bonding of the metal through the –S–S– linkage as well as the carboxyls would give a terdentate structure containing two six-membered chelate rings and it is suggested that the complex, $\overline{\text{LRLCu}}^-$, has this structure. Similar terdentate behavior has been postulated by Sandell[15] and Tichane and Bennett[16] for the 1,1'-dicarboxydimethyl sulfide–copper(II) complex. Likewise, the disulfide group in BAED is thought to contribute to complex formation comparable with V and VI. Because, in both DDD and BAED the three donor groups are able to coördinate with the same metal ion, polynuclear complex formation would not be expected.

Copper(II) and Bis-(2-aminoethyl) Disulfide.—In BAED (which is a ligand of the type LRL) both amine groups can bond to the same metal ion. For reasons similar to those discussed for DDD, the important equilibria with copper(II) in acid solution can be assumed to be

$$\text{Cu}^{2+} + {}^+\text{HLRLH}^+ \rightleftharpoons {}^+\text{HLRLCu}^{2+} + \text{H}^+$$

$$\text{Cu}^{2+} + {}^+\text{HLRLH}^+ \rightleftharpoons \overline{\text{LRLCu}}^{2+} + 2\text{H}^+$$

The sparing solubility of the complex limits the accessible range of experimental conditions. Taking two arbitrarily selected points—one at the beginning, and the other at the end, of the titration in which the metal:ligand ratio was 1:5—the corresponding equations give K_1 and K' as 6.15×10^3 M^{-1} and 5.02×10^6 M^{-1}, respectively, where

$$K_1 = [{}^+\text{HLRLCu}^{2+}]/[\text{Cu}^{2+}][\text{LRLH}^+]$$

and

$$K' = [\overline{\text{LRLCu}}^{2+}]/[\text{Cu}^{2+}][\text{LRL}]$$

Values of K' at other points in the titration (obtained using $K_1 = 6.15 \times 10^3$ M^{-1}) are included in Table III. The average over the titration was $K' = 4.97 \pm 0.10 \times 10^6$ M^{-1}, the maximum deviation being 0.16×10^6 M^{-1}.

TABLE III

COPPER(II) AND BIS-(2-AMINOETHYL) DISULFIDE

$[\text{Cu}]_\text{T} = 5.0 \times 10^{-4}$ M, $[\text{LRL}]_\text{T} = 2.505 \times 10^{-3}$ M, K_1 taken $= 6.15 \times 10^3$ M^{-1}

[NaOH] $\times 10^4$, M	pH	[Cu²⁻] $\times 10^4$, M	[LRLH⁺] $\times 10^3$, M	K' $\times 10^{-6}$, M^{-1}
1.011	6.45	4.410	0.905	(5.02)
1.213	6.50	4.302	1.010	5.13
1.415	6.55	4.196	1.128	5.00
1.617	6.59	4.089	1.230	5.04
1.818	6.63	3.983	1.342	4.95
2.020	6.67	3.878	1.448	4.90
2.423	6.75	3.671	1.645	4.90
2.826	6.78	3.467	1.850	4.83
3.228	6.83	3.267	2.031	4.90
3.630	6.87	3.068	2.205	(5.02)

The claim by Knoblock and Purdy[17] that BAED does not form a complex with copper(II) at pH 7.4 is not consistent with the present experimental results.

Copper(II) and 3-(2-Aminoethyldithio)-L-alanine.— For reasons discussed below, the basic amino acid AEDA (type HLRL) is believed to form simple amino acid type complexes with copper(II). The corresponding stability constants, K_1 and β_2, have been calculated by the graphical method of Irving and Rossotti[18] based on the equation

$$\frac{\bar{n}}{(1 - \bar{n})[{}^-\text{LRLH}^+]} = K_1 + \frac{\beta_2(2 - \bar{n})[{}^-\text{LRLH}^+]}{(1 - \bar{n})} \quad (7)$$

where $\bar{n}$ is defined as the average number of ligand molecules bound by each metal ion and $[{}^-\text{LRLH}^+]$ is the concentration of free complexing species in which the amine, but not the amino acid nitrogen, is protonated. The linear plot of eq. 7 for titrations of acid solutions in which the metal:ligand molar ratios were 2:5 and 1:1 is shown in Fig. 4. The intercept and slope give log $K_1 = 7.08$ and log $\beta_2 = 13.80$.

AEDA has four different groups potentially capable of complex formation with copper(II). These comprise the amine, the disulfide, and the amino and carboxyl groups of the amino acid. However, the experimental results were unable to satisfy equations derived on the assumption that both the amine and amino acid groups (with or without any contribution from the disulfide) were attached to the same copper ion. If only one of these groups was involved, the much greater complex-forming ability of an amino acid grouping than of a simple amine[19] would make the former the more likely binding site in copper complexes with AEDA. It also appears probable, especially from results with DDD, BAED, and methionine, that the

(16) R. M. Tichane and W. E. Bennett, *J. Am. Chem. Soc.*, **79**, 1293 (1957).

(17) E. C. Knoblock and W. C. Purdy, *J. Electroanal. Chem.*, **2**, 493 (1961).

(18) H. Irving and H. S. Rossotti, *J. Chem. Soc.*, 3397 (1953).
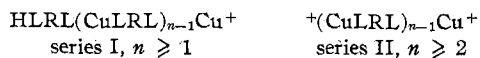
(19) J. Bjerrum, G. Schwarzenbach, and L. G. Sillén, "Stability Constants, Part I: Organic Ligands," Spec. Publ. No. 6, The Chemical Society, London, 1957.

disulfide group would be coördinated. Results from Fig. 4, calculated on the assumption that the amino acid grouping, but not the terminal amine group, is involved in complex formation, show that in acid solutions different ligand:metal ratios lead to constant values of log K_1 and log β_2. In neutral and alkaline solutions the amine group can no longer be taken to be completely protonated nor can it be assumed that the dissociation constant of this group is the same as in non-complexed AEDA. However, the titration curve in Fig. 2 is reproduced quantitatively using the above values of log K_1 and log β_2 if $pK_a = 8.2$ is assigned to this amine group in the copper–AEDA complexes. This decrease of 1.1 from the pK_a value in AEDA is composite: it is due to differences in inductive effects in AEDA and its copper complexes and also includes any tendency of the amine group to coördinate to the copper ion.

Copper(II) and L-Methionine.—The stability constants, log K_1 and log β_2, for the methionine–copper(II) system, obtained from the titration data by the Irving and Rossotti method,[18] were found to be 8.00 and 15.23, respectively; the plot of eq. 7 is shown in Fig. 5. Log β_2 agrees with values previously determined under different conditions.[19]

Comparison of the stability constants for the copper-(II) complexes of methionine with those of simple amino acids indicates that the sulfur atom contributes to the coördination in the methionine complexes. Thus, in spite of a drop of 0.65 unit in the pK_a of methionine relative to glycine, the stability constants of their copper complexes are similar (log $K_1 = 8.00$, log $\beta_2 = 15.23$, $pK_a = 9.20$; cf. glycine,[20] log $K_1 = 8.12$, log $\beta_2 = 15.03$, $pK_a = 9.85$). In the 1:2 complex the sulfur atoms probably occupy remote positions in a typically distorted octahedral structure such as is commonly found in copper(II) complexes.[21]

Copper(II) and L-Cystine.—From their structural resemblance, cystine and DAS would be expected to form similar series of complexes with copper(II) ion. These comprise the linear polynuclear complexes of series I, II, and III and one series of cyclic complexes IV.[6] The general formulas of these complexes are

HLRL(CuLRL)$_{n-1}$Cu$^+$ $^+$(CuLRL)$_{n-1}$Cu$^+$
  series I, $n \geqslant 1$ series II, $n \geqslant 2$

HLRL(CuLRL)$_n$H $^{-}$LRL(CuLRL)$_{n-1}$Cu^{-}
  series III, $n \geqslant 1$ series IV, $n \geqslant 2$

where HLRLH represents neutral (including zwitterionic) cystine.

Under the experimental conditions, the concentrations of the species HLRLH$_2^+$ and $^+$H$_2$LRLH$_2^+$ were assumed to be insignificant. The method of calculation described in part I[6] then enabled values of K_1 and K_2 to be obtained for the formation constants of the mononuclear species HLRLCu$^+$ and Cu(LRL)$_2$, respectively, from the titration data in Table IV.

(20) At 20° and $\mu = 0.1$; H. Irving, R. J. P. Williams, D. J. Ferrett, and A. E. Williams, *J. Chem. Soc.*, 3498 (1954).
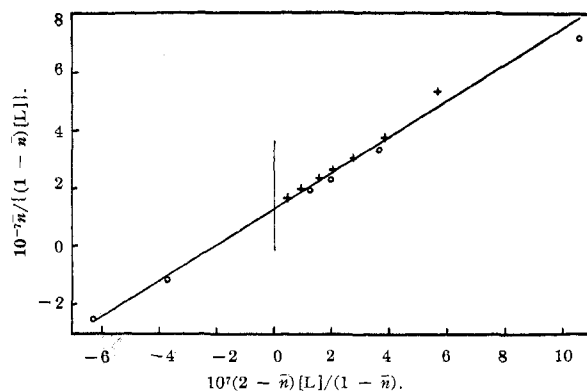(21) L. E. Orgel and J. D. Dunicz, *Nature*, **179**, 462 (1957).

Fig. 4.—Evaluation of K_1 and β_2 for AEDA–copper(II) complexes by the graphical method.

TABLE IV

TITRATIONS OF COPPER PERCHLORATE AND CYSTINE WITH 0.1 M SODIUM HYDROXIDE AND COMPUTED CONCENTRATIONS OF [Cu^{2+}] AND [HLRL$^-$]

pH	[NaOH] × 10^4, M	[Cu^{2+}] × 10^5, M	[HLRL$^-$] × 10^8, M	pH	[NaOH] × 10^4, M	[Cu^{2+}] × 10^5, M	[HLRL$^-$] × 10^8, M
(i)	1 × 10^{-4} M Cu(ClO$_4$)$_2$ and 2.996 × 10^{-4} M cystine						
4.14	0.0000	4.970	2.775	4.42	1.0110	2.150	4.669
4.19	.2023	4.455	3.012	4.50	1.2129	1.637	5.415
4.24	.4046	3.893	3.299	4.58	1.4148	1.177	6.440
4.29	.6068	3.312	3.650	4.69	1.6166	0.751	8.123
4.36	.8090	2.693	4.122	4.85	1.8183	0.390	11.389
(ii)	2 × 10^{-4} M Cu(ClO$_4$)$_2$ and 4 × 10^{-4} M cystine						
3.94	0.0000	11.970	2.218	4.26	2.0200	5.427	3.659
3.97	.2023	11.330	2.327	4.30	2.2215	4.780	3.907
4.00	.4046	10.670	2.441	4.34	2.4230	4.117	4.214
4.02	.6068	10.120	2.538	4.39	2.6244	3.524	4.555
4.05	.8090	9.466	2.658	4.44	2.8257	2.894	5.027
4.08	1.0110	8.822	2.783	4.50	3.0269	2.307	5.619
4.11	1.2129	8.186	2.914	4.57	3.2281	1.756	6.434
4.145	1.4148	7.469	3.076	4.66	3.4291	1.244	7.633
4.18	1.6166	6.782	3.460	4.76	3.6301	0.800	9.510
4.22	1.8183	6.041	3.659				
(iii)	3 × 10^{-4} M Cu(ClO$_4$)$_2$ and 2.990 × 10^{-4} M cystine						
3.90	0.0000	21.59	1.364	4.35	3.2281	10.34	1.845
3.95	0.4046	20.33	1.440	4.42	3.6301	9.00	1.883
4.00	0.8090	18.89	1.517	4.51	4.0319	7.57	1.922
4.06	1.2129	17.43	1.586	4.60	4.4333	6.21	1.955
4.11	1.6166	15.98	1.649	4.70	4.8344	5.02	1.982
4.17	2.0200	14.31	1.713	4.84	5.2352	3.75	2.006
4.22	2.4230	13.21	1.752	5.03	5.6356	2.48	2.022
4.28	2.8257	11.83	1.799				

Two arbitrarily selected points in the titration of a solution containing copper and cystine in a molar ratio of 1:3 gave $K_1 = 1.0 \times 10^7$ M^{-1} and $K_2 = 5.3 \times 10^6$ M^{-1}. These constants were inserted into a suitable program for an IBM 1620 digital computer so that the concentrations of free metal ion and free complexing species could be computed[6] at each point in a number of titrations in which the metal:ligand molar ratio, the total copper concentration, and the total ligand concentrations were varied. The values obtained are listed in Table IV.

In these calculations only the equations for total metal and total complexing species are used, so that the total charge equation provides an independent check
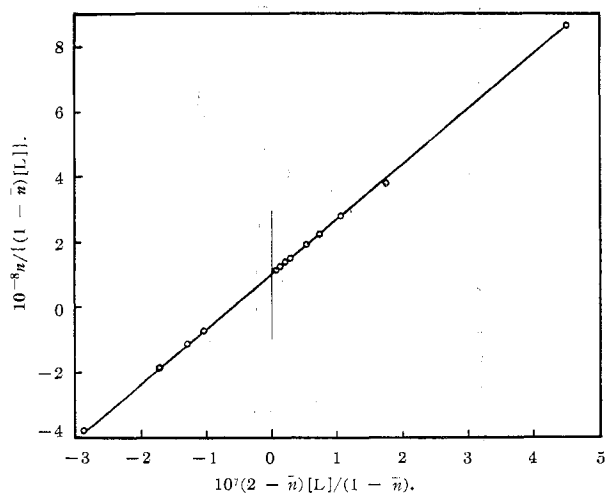
Fig. 5.—Evaluation of K_1 and β_2 for methionine–copper(II) complexes by the graphical method.

on the inherent assumptions. The average deviation from the known values of $2[\text{Cu}]_\text{T}$ of values calculated using the results in Table IV and this equation was less than $\pm 1\%$ for points where copper(II) was up to 90% complexed. This range represents an uncertainty of a little less than ± 0.05 in log K_1 and log K_2. The largest deviations, 2.1, 3.5, and 4.5%, occurred at the end of the 1:1 titration. This agreement strongly suggests that the equilibria in the cystine–copper(II) system are correctly represented by a statistical distribution of complexes of the types postulated.

The close similarity of the stability constants for the mononuclear complexes of cystine and copper(II) with those of 3-(2-aminoethyldithio)-L-alanine (AEDA) indicates that cystine forms its complexes through the same groups as the structurally related AEDA.

TABLE V

OVER-ALL STABILITY CONSTANTS AT 20° AND μ = 0.15

Ligand	pK_a	log K_1	log β_2
DAS	9.23	8.03	14.20
Methionine	9.20	8.00	15.23
AEDA	8.28	7.08	13.80
Cystine	8.03	7.00	13.72

Thus, in its mononuclear complexes, cystine is coordinated to copper through one of the amino acid groups and also through the disulfide group. The ratios, $K_1/K_2 = 2$, for the copper complexes of cystine and AEDA, and $K_1/K_2 = 6$, for the methionine–copper(II) system, are much lower than that for the DAS $(K_1/K_2 \approx 70)^6$ and other amino acid copper(II) complexes.[19] These differences probably are due to the bonding of the sulfur atoms. The coördination to sulfur would be greater in the 1:2 than in the 1:1 complexes, so that K_2 would be increased relative to K_1. This is a consequence of the reduction in net positive charge on the copper ion with progressive coördination of electron-donating amino and carboxyl groups, thereby increasing the tendency of d-electrons on copper(II) to be donated into vacant d-orbitals of sulfur atoms ("back-bonding").

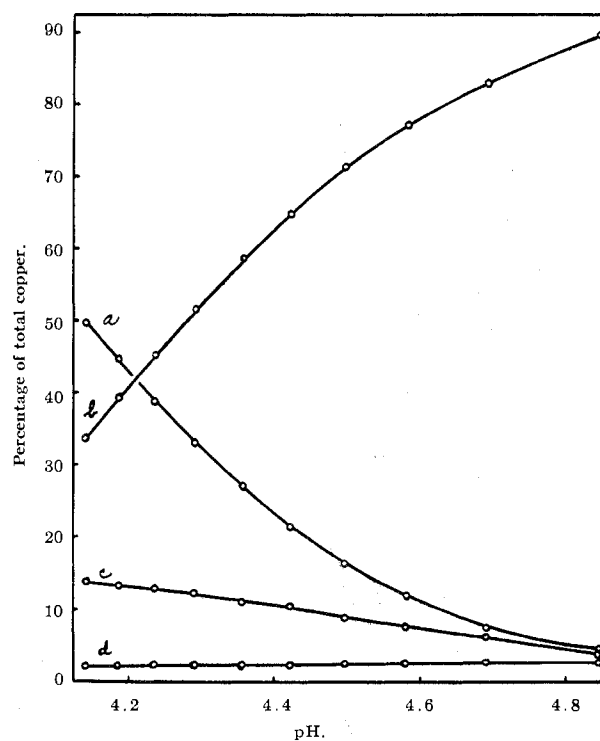In the same way as for DAS, and using the cor-

Fig. 6.—Distribution of copper species (as % total copper) for the system where $[\text{Cu}]_\text{T} = 1 \times 10^{-4}$ M, [cystine] = 2.996×10^{-4} M: a, Cu^{2+}; b, $(\text{CuLRL})_2^*$; c, HLRLCu^+; d, $\text{Cu}(\text{LRLH})_2$.

responding equations,[6] over-all stability constants of any members of the copper complexes represented by series I to IV can be obtained from K_1, K_2, and K_{a_3}. Values for the more important species are given in Table VI.

TABLE VI

OVER-ALL STABILITY CONSTANTS OF CYSTINE–COPPER(II) COMPLEXES AT 20° AND μ = 0.15

Species	Products	Constant
HLRLCu$^+$	Cu^{2+} + HLRL$^-$	log $\beta_{1:1}$ = 7.00
HLRLCuLRLCu$^+$	2Cu^{2+} + HLRL$^-$ + LRL^{2-}	log $\beta_{2:2}$ = 21.33
HLRLCuLRLH	Cu^{2+} + 2HLRL$^-$	log $\beta_{1:2}$ = 13.72
HLRLCuLRLCuLRLH	2Cu^{2+} + 2HLRL$^-$ + LRL^{2-}	log $\beta_{2:3}$ = 28.05
$^+$CuLRLCu$^+$	2Cu^{2+} + LRL^{2-}	log $\beta_{2:1}$ = 14.00
(CuLRL)$_2^a$	2Cu^{2+} + 2LRL^{2-}	log $\beta_{2:2}^*$ = 28.05

^{a} Ring-type complex, $n = 2$ in series IV.

Similarly, from Table IV, the concentrations of any of these complexes can be calculated throughout the titrations and hence their individual significance in the system can be assessed. The concentrations of the principal species, expressed as percentage of total copper, are plotted against pH in Fig. 6 for the 1:3 case. Results for copper:ligand ratios of 1:1 and 1:2 followed similar trends. The species Cu^{2+}, HLRLCu$^+$, Cu(LRLH)$_2$, and (CuLRL)$_2^*$ account for more than 97% of all the copper present.

The mononuclear complex, HLRLCu$^+$, and, surprisingly, the straight-chain dimer, $^+$CuLRLCu$^+$, are less important than they were in the DAS–copper(II) system. However, as in the DAS case, the binuclear complex, (CuLRL)$_2^*$, is the major species, especially over the second half of each titration. This complex is postulated to be di-μ-cystinatodicopper(II) and to have

the structure VII.

VII
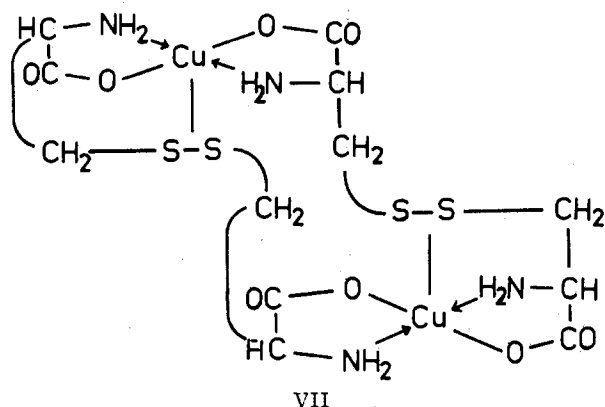
In this structure, each copper forms part of three five-membered rings while at the same time both coppers are linked in a multi-membered ring. The blue, finely crystalline complex precipitated from neutral solutions of cystine containing copper(II) ion is believed to be VII, which is the form in which most of the copper is present in solution under the experimental conditions; this species would be expected to be only sparingly soluble because of its non-ionic character. Chemical analysis is consistent with this structure, but its molecular weight could not be determined because of insolubility. The magnetic moment of 1.91, determined by the Gouy method, is identical with that for the corresponding DAS complex,[6] again indicating the absence of Cu–Cu δ-bonding. Leybold atomic models confirm that structure VII is possible, but provide no information about the stereochemistry of the bond between the copper and the –S–S– groups. Appreciable vertical movement can occur for the –S–S– group when it is located above a copper atom, and sufficient lateral displacement is possible that bonding could be through either sulfur atom or through a π-bond involving one of their shared orbitals.

Acknowledgments.—The authors thank Miss Elizabeth Reid for the computer program and operation and Dr. N. Gill for use of the magnetic balance.

Some Transition Metal Complexes of Ethylenethiourea.   I.   Cobalt(II)[1]

BY RICHARD L. CARLIN AND SMITH L. HOLT, JR.

A series of compounds involving ethylenethiourea coördinated to the cobalt ion in the halides and perchlorate has been prepared and characterized. Tetrahedral stereochemistry is found in each case. This conclusion is based on the careful measurement and analysis of the visible spectra and magnetic susceptibilities. The complex derived from cobalt acetate and ethylenethiourea is probably octahedral. Ethylenethiourea occurs at the very low end of the nephelauxetic series.

Cotton, Goodgame, and Goodgame[2] have shown how the stereochemistry of cobalt(II) may be characterized by a careful study of the electronic spectra and paramagnetic susceptibilities of a series of similar compounds. Valuable data are obtained for the electronic parameters of the tetrahedral metal ion, while useful information is also gained concerning the ligands. Thus, the order of the various ligands in the spectrochemical and nephelauxetic series has been obtained, as well as information conerning the variation in spin-orbit coupling constants in the complexes. Knowledge concerning the solvent effects on the spectra may also be obtained in this manner.

Little information, however, has been included in previous studies of the first row metal ions on sulfur as a donor atom. Sulfur is frequently less effective than its congener, oxygen, in its ability to function as a donor to first row metal ions. This is, of course, only a relative matter; witness the many sulfide precipitates,

as well as the much greater stability of compounds formed by thioethers compared to ethers.

Nardelli and co-workers[3] have recently prepared several complexes of ethylenethiourea (2-imidazolidinethione, etu) with the chlorides of iron(II), nickel, and cobalt. Their work consisted of preparation, analysis, and characterization by X-ray crystallographic techniques; their results showed that sulfur was the donor atom. Neither the spectra nor the magnetic susceptibilities of any of these compounds were reported. Pursuant to our interest in sulfur as a donor atom, we have prepared the same complexes as well as those resulting from the use of other salts of the metals and have measured the electronic parameters characterizing ethylenethiourea as a ligand. In this paper we report our work on cobalt(II) and we shall report later on the complexes of other metal ions.

Experimental[4]

The earlier report[3] of the preparation of CoCl₂·2etu involved

(1) Presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1962.

(2) F. A. Cotton, D. M. L. Goodgame, and M. Goodgame, *J. Am. Chem. Soc.*, **83**, 4690 (1961), and earlier papers.

(3) M. Nardelli, I. Chierici, and A. Braibanti, *Gazz. chim. ital.*, **88**, 37 (1958).

(4) Microanalyses are by Schwarzkopf Microanalytical Laboratory.