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Interaction of Metal Ions with S-Alkylcysteines[1]

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S-2-Carbomethoxyethyl-L-cysteine was treated with aqueous solutions of various metal ions. Pyruvic acid, 3,3'-dithiodipropionic acid, dicopper(I) 3-mercaptopropionate, methanol, ammonium chloride and hydrogen chloride were the products with copper(II) chloride at 70°, while pyruvic acid and the metal mercaptides of 3-mercaptopropionic acid were formed with mercury(II) and palladium(II) chlorides. Decomposition was observed also with silver nitrate, but the products other than pyruvic acid were not identified. The chlorides of nickel(II), zinc(II), manganese(II), magnesium(II), iron(II), cobalt(II) and chromium(III) did not appear to cause decomposition. The decompositions of other S-alkylcysteine and cysteine derivatives were studied. Structural changes in the cysteine derivative were shown to affect the reaction. The decomposition effected by copper(II) is suggested as proceeding through an elimination reaction (at pH 1) of a chelate involving nitrogen and sulfur.

Introduction

Complexing of a metal ion with a sulfur atom may facilitate the breaking of a sulfur–carbon bond in a displacement reaction.[3] The thioether linkages (derived from cysteine) of cytochrome c are broken by the action of silver(I), mercury(I), mercury(II), lead(II), copper(II) and cadmium(II) ions to yield the porphyrin prosthetic group and a protein.[4]

Enzymic hydration of the double bond of α,β-unsaturated carboxylic acids has been suggested as involving a displacement reaction of a enzyme-S-group.[5]

Sulfur–carbon bonds may be broken in elimination reactions. The elimination of hydrogen sulfide or a mercaptan from cysteine, cystine and S-alkylcysteines in strongly basic solutions is an example.[6] These hydroxide-induced eliminations are reported to be catalyzed by lead(II) ions.

The only evidence for a possible elimination reaction of S-alkylcysteines in acidic media is in the decomposition of S-methyl-L-cysteine in boiling 18 N sulfuric acid, although the reaction was not necessarily interpreted as an elimination.[7] S-Methyl-L-cysteine sulfoxide undergoes what appears to be an elimination of the $CH_3S^+(OH)$ group in boiling 1 N hydrochloric acid.[8]

Extracts of seeds of *Albizzia julibrissin* Durazz. (silk tree) yield S-2-carboxyethyl-L-cysteine.[9] Its identification is said to mark the first-known occurrence of this amino acid in the vegetable kingdom. An enzyme from *Albizzia lopantha* Benth catalyzes the cleavage of this amino acid to 3-mercaptopropionic acid, pyruvic acid and am-

(1) Presented at the 135th Meeting of the American Chemical Society, Boston, Mass., April 9, 1959.

(2) Taken from the Ph.D. thesis of James R. Schaeffer, June, 1959.

(3) D. S. Tarbell and D. P. Harnish, *Chem. Revs.*, **49**, 1 (1951).

(4) K. G. Paul, *Acta Chem. Scand.*, **4**, 239 (1950).

(5) J. Stern and A. del Campillo, *J. Biol. Chem.*, **218**, 985 (1956).

(6) M. Bergmann and F. Stather, *Z. physiol. Chem.*, **152**, 189 (1926); H. T. Clarke and J. M. Inouye, *J. Biol. Chem.*, **94**, 541 (1931); **89**, 399 (1931); B. H. Nicolet, *J. Am. Chem. Soc.*, **53**, 3066 (1931); J. C. Andrews, *J. Biol. Chem.*, **80**, 191 (1928); **87**, 681 (1930); I. W. Stapleton and J. M. Swan, *Austral. J. Chem.*, **13**, 416 (1960).

(7) F. Challenger and H. D. Hollingworth, *J. Chem. Soc.*, **61** (1959).

(8) F. Ostermayer and D. S. Tarbell, *J. Am. Chem. Soc.*, **82**, 3752 (1960).

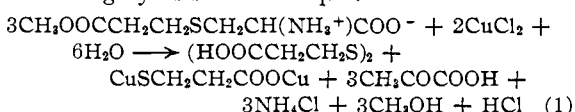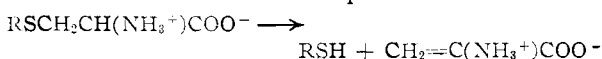(9) R. Gmelin, G. Strauss and G. Hasenmaier, *Z. Naturforsch.*, **13b**, 252 (1958).

monia. The presence or absence of metal ions or other cofactors was not reported.

There is no doubt that metal ions and cysteine derivatives are present in biological systems. The purpose of this work was to ascertain what kind of reactions metal ions could catalyze or effect on simple cysteine and cystine derivatives and what the nature of any metal–ion interaction with the cysteine derivatives might be. Conditions were neither as strongly basic[6] nor as strongly acidic[7] as conditions previously investigated.

Results and Discussion

Elimination of Sulfur-containing Fragment.— When S-2-carbomethoxyethyl-L-cysteine was treated with an equimolar amount of aqueous copper(II) chloride for 21 hours at 70°, 3,3'-dithiodipropionic acid, dicopper(I) 3-mercaptopropionate, pyruvic acid, ammonium chloride and methanol were produced. The stoichiometry was evaluated roughly as shown in eq. 1.

$$3CH_3OOCCH_2CH_2SCH_2CH(NH_3{}^+)COO^- + 2CuCl_2 +$$
$$6H_2O \longrightarrow (HOOCCH_2CH_2S)_2 +$$
$$CuSCH_2CH_2COOCu + 3CH_3COCOOH +$$
$$3NH_4Cl + 3CH_3OH + HCl \quad (1)$$

No 3-hydroxypropionic acid or acrylic acid (from a displacement of cysteine by water) or serine (from a displacement of 3-mercaptopropionic acid by water) could be detected.[10]

When the copper(II) chloride was added, the solution became acidic, pH 1. Relatively low yields of pyruvic acid were obtained, which may be explained by its copper-ion-catalyzed condensation and decarboxylation.[11] When the ratio of copper(II) chloride to substrate was decreased, the yield of 3,3'-dithiodipropionic acid was decreased. Since the copper(II) ions are consumed, the ions do not strictly play a catalytic role. However, the reaction probably is catalyzed by copper(II) ion, but a subsequent oxidation–reduction reaction removes it from the medium.[12] The formation of

(10) Serine was shown to be stable under the reaction conditions so that if it were formed it should have been detected.

(11) A. Schellenberger and R. Selke, *J. prakt. Chem.*, **8**, 379 (1959).

(12) Attempts were made to follow the reaction of S-alkylcysteines and analogs by spectrophotometric and conductometric methods. Apparently because of the complexity of the reaction, no useful data were obtained. Part of the time the reaction mixture was heterogeneous.

3,3'-dithiodipropionic acid and dicopper(I) 3-mercaptopropionate may be explained by oxidation of the mercapto acid with copper(II) ions. The dicuprous salt precipitated only after copper(II) ions were removed by an ion-exchange column. This salt was not especially stable and tended to become green because of oxidation of the copper(I). It has been reported that the reaction of copper(II) ions with mercaptans in the absence of oxygen leads to copper(I) mercaptides.[13]
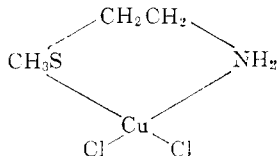
Copper(II) ions appear to be responsible for the decomposition since S-2-carbomethoxyethyl-L-cysteine undergoes no change other than hydrolysis of the ester group when heated for 21 hours at 80° at pH 1.[14] S-Methyl-L-cysteine is stable to boiling 6 N hydrochloric acid.[7] The ester group was always hydrolyzed during the reaction.

The type of decomposition shown by eq. 1 may be general for S-alkylcysteine derivatives since S-methyl-L-cysteine and S-2-carboxyethyl-L-cysteine are decomposed also by copper(II) chloride under the same conditions.

The reaction appears to be a copper-ion-catalyzed elimination of a mercaptide ion. The intermediate 2-aminoacrylic acid, which has been postulated in other types of reactions,[6,8,15] would be expected to hydrolyze to pyruvic acid and ammonium chloride via the tautomeric imino compound.

$$RSCH_2CH(NH_3{}^+)COO^- \longrightarrow$$
$$RSH + CH_2{=}C(NH_3{}^+)COO^-$$

Both the amino and carboxyl groups seem necessary for the copper-ion-induced decomposition because neither 3,3'-thiodipropionic acid nor S-methyl-2-aminoethyl sulfide underwent reaction. The latter formed a stable green solid with copper(II) chloride whose elementary analysis agreed with that of a chelate of the structure

Its formation constant and thermodynamic quantities have been reported previously but not its analysis.[16]

S-2-Carbomethoxyethylhomocysteine does not decompose when treated with copper(II) chloride under the same conditions that caused decomposition of the cysteine derivative. In this compound a β-elimination of the hydrogen (alpha to
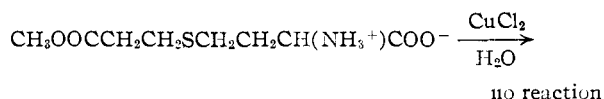
(13) P. Klason, Ber., 20, 3412 (1887); L. J. Harris, Biochem. J., 16, 739 (1922); W. E. Duncan, E. Ott and E. E. Reid, Ind. Eng. Chem., 23, 381 (1931); I. M. Kolthoff and W. Stricks, J. Am. Chem. Soc., 73, 1728 (1951); I. M. Klotz, G. H. Czerlinski and H. A. Fiess, ibid., 80, 2920 (1958); L. Robert, E. Neiadas and C. Charransol, Compt. rend. soc. biol., 152, 1663 (1958); E. Neiadas and L. Robert, Compt. rend., 246, 2543 (1958).

(14) Palladium(II) and mercury(II) ions also effect decomposition. Palladium(II) chloride gave pyruvic acid (36%) and palladium di-3-mercaptopropionic acid, and mercury(II) chloride gave pyruvic acid and S-chloromercuri-3-mercaptopropionic acid.

(15) D. E. Metzler and E. E. Snell, J. Biol. Chem., 198, 353 (1952); W. E. Parham and J. M. Wilbur, Jr., J. Am. Chem. Soc., 81, 6071 (1959).

(16) E. Gonick, W. C. Fernelius and B. E. Douglas, ibid., 76, 4671 (1954); G. H. McIntyre, Jr., B. P. Block and W. C. Fernelius, ibid., 81, 529 (1959).
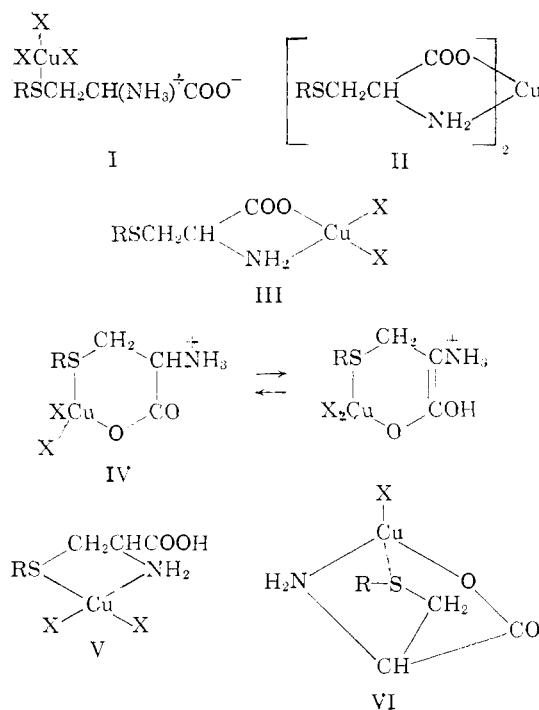
the carboxyl and amino groups) and mercaptide ion cannot occur.

$$CH_3OOCCH_2CH_2SCH_2CH_2CH(NH_3{}^+)COO^- \xrightarrow[\text{H}_2\text{O}]{\text{CuCl}_2}$$

no reaction

Cysteine or cystine gives pyruvic acid and copper(II) sulfide when heated with copper(II) chloride at 70° for 18 hours. Since no copper sulfide was detected in the decomposition reactions of S-2-carbomethoxyethyl-L-cysteine, cysteine and cystine probably were not intermediates. (Cysteine apparently is eliminated from S-3-ketopentyl-L-cysteine by the action of aqueous copper(II) chloride since considerable copper(II) sulfide was formed. This probably reflects the greater acidity of the hydrogen alpha to the carbonyl group over that of the hydrogen alpha to the carboxyl and amino groups.)

The evidence indicates (1) that no displacement of a mercaptide group by water occurs; (2) that copper(II) ions are involved; (3) that both the amino and carboxyl groups are necessary, probably to activate the α-hydrogen; (4) that the decomposition is a β-elimination reaction; (5) that the carbon–sulfur bond to the amino acid residue is broken.

Possible Intermediates.—Coördination of copper(II) ions with one or more of the various donor groups in the S-alkylcysteine derivatives may provide the driving force for the elimination reaction. Structures for possible copper-containing reactive intermediates are I-VI.
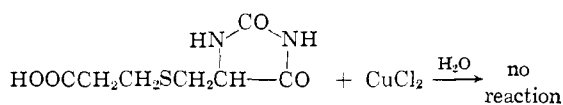

The complex I is not a likely intermediate because the hydrochloride of S-2-carbomethoxyethyl-L-cysteine does not decompose when heated at 70° for 21 hours with aqueous copper(II) chloride. The electron-withdrawing ammonium and

carboxyl groups would activate the α-hydrogen toward removal by a base. The complexing of copper(II) with the sulfur might be expected to transform the RS–group into a good leaving group; hence, inertness implies that the complexing with copper is unimportant. Cystine betaine is stable to aqueous copper(II) chloride, whereas cystine,

$$\overset{+\text{N(CH}_3)_3}{|}$$
$$(^-\text{OOCCHCH}_2\text{S})_2 + \text{CuCl}_2 \xrightarrow{\text{H}_2\text{O}} \text{no reaction}$$
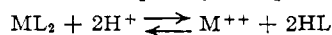
itself, is not. The possibility that a positive charge on nitrogen prevents the complexing of copper(II) does not seem likely since the hydantoin of S-2-carboxyethyl-L-cysteine does not decompose in the presence of copper(II) ions. Acylation of the nitrogen of cysteine, which probably increases the acidity of the α-hydrogen, has been reported to increase the elimination of hydrogen sulfide by hydroxide ion.[17]

$$\text{HN}\overset{\overset{\text{CO}}{\diagup\,\diagdown}}{}\text{NH}$$
$$\text{HOOCCH}_2\text{CH}_2\text{SCH}_2\overset{|}{\text{CH}}\text{---}\overset{|}{\text{CO}} + \text{CuCl}_2 \xrightarrow{\text{H}_2\text{O}} \overset{\text{no}}{\text{reaction}}$$

The drop in pH and formation of an insoluble precipitate when S-2-carbomethoxyethyl-L-cysteine and aqueous copper(II) chloride were mixed implies the formation of a chelate.[18] The blue solid is believed to be a copper(II) chelate with two moles of S-2-carbomethoxyethyl-L-cysteine; the structure II is assigned because of its analysis and the similarity of its infrared spectrum to that of 2:1 amino acid–metal ion chelates.[19]

Structure II does not seem to be directly involved in the decomposition reaction since it is stable in water (neutral pH) at 100° for 20 hours. It does yield 10% pyruvic acid when heated at 70° for 21 hours at pH 1. Protons can compete with copper(II) ions for the ligand. If a value of 10^{10} is taken for the second association constant of the ligand for a proton and a value of 10^{-16} taken for the product of the dissociation constants for 2:1 and 1:1 copper(II)–amino acid chelates,[20] the equilibrium constant in dilute solution for the reaction is 10^4 so that at pH 1 the chelate would be dissociated almost completely at equilibrium. The

$$\text{ML}_2 + 2\text{H}^+ \rightleftarrows \text{M}^{++} + 2\text{HL}$$

2:1 chelate, however, has a low solubility in water and its dissociation as actually observed is slow.

The blue chelate II is always formed because of its low solubility. Since II appears unreactive, it may be converted into some other structure, III, IV, V or VI, which is reactive. Obviously, an equilibrium between II and III exists. The sodium salt of S-2-carbomethoxyethyl-L-cysteine when

treated with aqueous copper(II) chloride gives II and a deep blue solution which presumably contains some of the 1:1 chelate III. This blue solution was stable when heated at 70° for 21 hours.

Structure III might decompose by coördination of a second copper (II) ion with sulfur followed by elimination of RSCu$^+$, the α-hydrogen being made more acidic by the chelation. This is not believed to occur because in the experiment mentioned above, with the sodium salt of the substrate, excess copper(II) ions were present, yet no decomposition occurred.[21]

Chelates of copper(II) ion with oxygen and sulfur as in IV and with nitrogen and sulfur as in V and with all three donor groups as in VI are possible reactive intermediates. In both cases the acidity of the α-hydrogen of the amino acid is increased by either the ammonium group or the carboxyl group. Coördination of copper(II) ions with sulfur might weaken the bond between sulfur and carbon. Copper(II) ion usually prefers to coördinate with basic nitrogen over oxygen which would favor V over IV.[22]

Chelates of cobalt(II) and cobalt(III) with cysteine involving oxygen and sulfur and nitrogen and sulfur have been reported.[23] Zinc(II) ion coördinates with the nitrogen and the sulfur of cysteine but with the oxygen and nitrogen of methionine.[24] It has been stated that a sulfur–copper–nitrogen complex should be stronger than either a sulfur–copper–oxygen or a nitrogen–copper–oxygen complex.[25] Chelates of 3-mercaptopropionic acid which are six-membered rings analogous to IV are not as likely to form as chelates involving five-membered rings.[26] If chelate IV were a reactive intermediate it should be possible to form a similar structure with cystine betaine which, as has been shown, is stable to aqueous copper(II) ions. This evidence favors V over IV.

Tetrahedral complexes of copper(II) are rather rare, being formed only if there is great steric strain or undue electrostatic interaction in a square planar complex.[27] Attempts to construct Courtauld or Stuart–Briegleb models of VI failed; it

(17) M. Bergmann and F. Stather, Z. physiol. Chem., **152**, 189 (1926); E. Brand and M. Sandberg, J. Biol. Chem., **70**, 381 (1926); B. H. Nicolet, ibid., **88**, 403 (1930); J. C. Andrews and K. C. Andrews, ibid., **102**, 253 (1933); J. S. Fruton and H. T. Clarke, ibid., **106**, 667 (1934).

(18) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice–Hall, Inc., New York, N. Y., 1952, p. 39.

(19) S. Mizushima, D. N. Sen, C. Curran and J. V. Quagliano, J. Am. Chem. Soc., **77**, 211 (1955); A. J. Saraceno, I. Nakagawa, S. Mizushima, C. Curran and J. V. Quagliano, ibid., **80**, 5018 (1958); A. Rosenberg, Acta Chem. Scand., **10**, 840 (1956).

(20) Rounded values for the constants were taken from ref. 18, Appendix I.

(21) A referee has suggested that perhaps a proton coördinates with the sulfur in III to facilitate the departure of RS–. Sulfur probably coördinates more strongly with copper(II) ion than with a proton. In the experiment with the sodium salt of S-2-carbomethoxyethyl-L-cysteine, there were excess copper(II) ions present. Since there was no decomposition of this solution, presumably containing III and copper(II) ions, it does not seem likely that a proton could accomplish what copper(II) could not. There seems to be no direct evidence for the greater tendency of copper(II) ions over protons to coördinate with sulfur in a sulfide: the solubility of copper(II) chloride in dimethyl and diethyl sulfides [A. Weiner, Z. anorg. Chem., **15**, 8, 25 (1897)] as compared with the relative lack of basicity of sulfur to protons [R. L. Burwell, Chem. Revs., **54**, 615 (1954)] may be pertinent. One also might recall the easy precipitation of copper(II) sulfide from solutions of non-oxidizing acids.

(22) R. N. Keller and R. W. Perry in J. C. Bailar, Jr., "Chemistry of the Coördination Compounds," Reinhold Publishing Corp., New York, N. Y., 1956, p. 174 ff.; S. Ahrland, J. Chatt and N. R. Davies, Quart. Revs., **12**, 265 (1958).

(23) R. G. Neville and G. Gorin, J. Am. Chem. Soc., **78**, 4893 (1956); R. G. Neville, ibid., **79**, 518 (1957); G. Gorin, J. E. Spessard, G. A. Wessler and J. P. Oliver, ibid., **81**, 319 (1959).

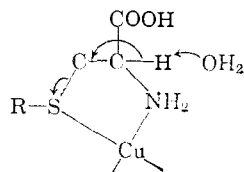(24) N. C. Li and R. A. Manning, ibid., **77**, 5225 (1955).

(25) A. H. Corwin in W. D. McElroy and B. Glass, "Copper Metabolism," The Johns Hopkins Press, Baltimore, Md., 1950, p. 16.

(26) Q. Fernando and H. Freiser, J. Am. Chem. Soc., **80**, 4928 (1958).

(27) F. Lions and K. V. Martin, ibid., **79**, 1273 (1957).

was evident that there would be considerable strain in VI, whereas models of the square planar complexes were relatively unstrained. To counterbalance the difficulty of its formation, VI would have to be unusually reactive.

It seems most reasonable to regard the reaction as an elimination reaction of V in which water functions as the base. An ionization of the S–C bond to yield a primary carbonium ion seems energetically unlikely, although such an intermediate has been suggested in the decomposition of methyl S-3-methylthiopropionate in boiling 6 N hydrochloric acid.[7]

At high pH the elimination reaction can proceed without the intervention of copper(II) ions,[6] but at low pH the lower basicity of the active base, water, must be compensated by an increased lability of the group which is eliminated. It is important that the formation of this reactive leaving group apparently depends on the ability of the substrate to chelate with the metal ion: when the chelating ability is absent, there is no decomposition. It should be pointed out that the chelation of copper-(II) ion as shown in V not only weakens the C–S bond but also may increase the acidity of the α-hydrogen which is removed by water

Experimental

Microanalyses are by Galbraith Laboratories, Knoxville, Tenn., and Midwest Microtech Laboratories, Skokie, Ill.

Melting points are uncorrected. Infrared spectra were measured on a Perkin-Elmer model 21 instrument with sodium chloride optics.

Preparation of Sulfides.—S-Methyl-L-cysteine,[28] S-2-carboxyethyl-L-cysteine,[29] 3,3'-thiodipropionic acid,[29] S-methyl-2-aminoethyl sulfide[30] and cystine betaine[31] were prepared according to previously described methods. Cystine betaine had absorption in the infrared at 1635 cm.$^{-1}$ (ionized carboxyl group) which is absent in the infrared spectrum of cysteine ethyl ester hydrochloride.

S-2-Carbomethoxyethyl-L-cysteine.—To 1.20 g. (0.01 mole) of cysteine partially dissolved in 7.5 ml. of water, 0.80 g. (0.01 mole) of methyl acrylate and 2 drops of 40% Triton B were added. The reaction mixture was shaken for 2 minutes, during which time it solidified. The white solid was collected by suction filtration and recrystallized from 2 ml. of hot water. The solution yielded 0.4 g. of white material, m.p. approximately 180°. Upon reduction of the volume of the filtrate to 1 ml., another 0.30 g. was obtained. m.p. approximately 180°. The total yield was 0.70 g. (34%). A peak at 1740 cm.$^{-1}$ characteristic of ester carbonyl was evident in the infrared spectrum (KBr).

Anal. Calcd. for $C_7H_{13}O_4NS$: C, 40.56; H, 6.32; S, 15.47. Found: C, 40.69, 40.44; H, 6.34, 6.39; S, 15.48.

S-2-Carboxyethyl-L-cysteine (2-Amino-3,3'-thiodipropionic Acid) Hydrochloride.—To 1.0 g. (0.05 mole) of S-2-Carboxyethyl-L-cysteine 14 ml. of concentrated hydrochloric acid was added. After several hours on a steambath the water was removed under vacuum. The oily residue crystallized while standing at room temperature

at 0.1 mm., m.p. 135–140°, $[\alpha^{24}D]$ −8.51° (the free base in 1 N HCl)[32] (lit.[29] $[\alpha]^{16}D$ −8.19°). The infrared spectrum (KBr) has a band at 1725 cm.$^{-1}$ (acid carbonyl) and no bands in the amino-acid region. The hydrochloride was subjected to paper chromatography. Water-saturated butanol and formic acid (95:5, v.:v.) was the developing solvent and ninhydrin the indicator. Only one spot corresponding to the amino acid was observed.

S-2-Carboxyethyl-L-cysteine Hydantoin.—A modified procedure of Hess was used to prepare the hydantoin.[33] To 2.0 g. (0.01 mole) of S-2-carbomethoxyethyl-L-cysteine dissolved in 15 ml. of boiling water, 0.85 g. (0.01 mole) of potassium cyanate was added. After the solution was boiled for 20 minutes, 25 ml. of 10% hydrochloric acid was added. The solution was refluxed for 2 hours and cooled. An oily liquid remained after removal of the water by heating under water-aspirator vacuum. When 50 ml. of acetone was added, 1.0 g. of potassium chloride was precipitated. The acetone solution was evaporated and 2.0 g. of a tan solid was obtained. The solid was dissolved in boiling acetone, treated with charcoal, filtered, and the acetone evaporated to yield a substance, m.p. 133.5–136°. The infrared spectrum (KBr) has a broad band at 1695 cm.$^{-1}$ (acid carbonyl) and a shoulder at 1765 cm.$^{-1}$ and no bands in the amino acid region.

Anal. Calcd. for $C_7H_{10}O_4N_2S$: C, 38.52; H, 4.61. Found: C, 38.71; H, 4.86.

S-2-Carbomethoxyethyl-L-homocysteine.—The amino acid was prepared using the method described for the preparation of S-2-carbomethoxyethyl-L-cystine. To 3.0 g. (0.022 mole) of L-homocysteine dissolved in 40 ml. of water, 2.0 g. (0.025 mole) of methyl acrylate and 2 drops of 40% Triton B were added. The mixture solidified after 30 minutes. The white solid (4.0 g., 82%) was collected by suction filtration and was recrystallized from 95% methanol, m.p. 230–231.5°, $[\alpha]^{24}D$ −1.63° (1 N HCl).[32] The infrared spectrum (KBr) has bands at 1740 cm.$^{-1}$ (ester carbonyl) and at 1625, 1575 and 1515 cm.$^{-1}$ (amino acid).

Anal. Calcd. for $C_8H_{15}O_4NS$: C, 43.42; H, 6.83; S, 14.49. Found: C, 43.75; H, 6.85; S, 14.55.

S-3-Ketopentyl-L-cysteine.—Cysteine (6.0 g., 0.05 mole) and methyl vinyl ketone (3.5 g., 0.05 mole) were dissolved in 50 ml. of water; the solution was made homogeneous by the addition of 10 ml. of methanol. Triton B (2 drops, 40%) was added and the reaction mixture shaken for 3 days. The solvent was removed at room temperature *in vacuo*, and the solid remaining was dissolved in 30 ml. of water. The solution was treated with decolorizing charcoal, and the product (7.0 g., 74%, m.p. 170–173° dec.) was precipitated by the addition of acetone. The ketone was purified by two more precipitations. Its infrared spectrum has bands at 1725 cm.$^{-1}$ (ketone carbonyl) and at 1625, 1590 and 1492 cm.$^{-1}$ (amino acid).

Anal. Calcd. for $C_7H_{13}O_3NS$: C, 43.96; H, 6.85. Found: C, 43.72; H, 6.82.

Reaction of S-2-Carbomethoxyethyl-L-cysteine with Copper(II) Chloride.—In 40 ml. of water, $CuCl_2 \cdot 2H_2O$ (5.8 g., 0.034 mole) was added to S-2-carbomethoxyethyl-L-cysteine (7.0 g., 0.034 mole). A blue solid formed and the pH became 1 (pH meter). The heterogeneous mixture was heated 21 hours at 80°, during which the blue solid slowly dissolved giving a blue solution which later turned green. The hot solution was filtered, cooled, and placed in the refrigerator. The 3,3'-dithiodipropionic acid, 1.7 g. (72%), was collected; and the filtrate run through an ion-exchange column filled with 150 ml. of wet IR 120H (Rohm and Haas Co.). The total volume of water passed through the column was 200 ml. As the liquid dropped from the column a tan precipitate formed. The solution was placed in the refrigerator for 2 days and the precipitate (0.71 g.) collected. The tan solid became green on drying. The resin was removed from the column and washed with 200 ml. of water in several small portions. The washings contained an additional 0.48 g. of tan solid which turned green on drying. The filtrate (original 200 ml. plus washings) obtained by collection of the solid was filtered and evaporated (1 mm.) at room temperature to 40 ml. To this clear solution, 1.8 g. of 2,4-dinitrophenylhydrazine dissolved in alcoholic sulfuric acid was added and the precipitate which

(28) V. du Vigneaud, H. S. Loring and H. Craft, *J. Biol. Chem.*, **105**, 481 (1934).

(29) A. Schoberl and A. Wagner, *Chem. Ber.*, **80**, 379 (1947).

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

(31) M. Schubert, *J. Biol. Chem.*, **111**, 676 (1935).

(32) The optical rotation was kindly done by Miss Phyllis Fine.

(33) W. Hess, *J. Am. Chem. Soc.*, **56**, 1421 (1934).

formed was collected. The 2,4-dinitrophenylhydrazone (2.0 g., 21%) was recrystallized twice from hot ethanol; m.p. 209.5–212°, mixed m.p. with authentic pyruvic acid 2,4-dinitrophenylhydrazone, 210–212°. The infrared spectrum (KBr) of the green solid has a small peak at 1725 cm.$^{-1}$ (acid carbonyl) and a broad peak centered at 1575 cm.$^{-1}$ (carboxylate anion).

Anal. Calcd. for green solid, CuSCH₂CH₂COOCu: C, 15.58; H, 1.74; S, 13.86. For CuSCH₂CH₂COOCu·H₂O: C, 14.45; H, 2.43; S, 12.86. Found: C, 14.78; H, 2.87; S, 12.34.

The reaction mixture was subjected to paper chromatography on Whatman No. 1 paper. Water-saturated butanol-formic acid (95.5, v.:v.) was the developing solvent, and ninhydrin, the indicator. The only amino acids detected in the reaction mixture were S-2-carbomethoxyethyl-L-cysteine and S-2-carboxyethyl-L-cysteine.

Reaction of Various Molar Ratios of Copper(II) Chloride with S-2-Carbomethoxyethyl-L-cysteine.—To 5 ml. of water in each of 5 test-tubes 1.0 g. of S-2-carbomethoxyethyl-L-cysteine was added. After the ester had dissolved, the required amount of copper(II) chloride was added to prepare solutions that were 0.10, 0.50, 1.0 and 4.0 molar in copper(II). The tubes were heated for 21 hours at 70° and allowed to stand at room temperature for 2 days. The solutions were heated, filtered, and the disulfide collected when the solution cooled. As the ratio of copper(II) chloride increased from 0.1, 0.50, to 1.0 the yield of 3,3′-dithiodipropionic acid increased from 0, 64 to 85%. When the ratio of copper(II) chloride to amino acid was increased from 4:1 the yield of 3,3′-dithiodipropionic acid dropped to 0% since excess copper(II) chloride reacts with 3,3′-dithiodipropionic acid to give 2-carboxyethanesulfonic acid and di-cuprous-3-mercaptopropionate.[34]

Acid Hydrolysis of S-2-Carbomethoxyethyl-L-cysteine.—A solution (0.50 g., 0.002 mole) in 20 ml. of water was acidified with concentrated hydrochloric acid to pH 1. The solution was heated 21 hours on a steam-bath and the reaction mixture was subjected to paper chromatography. The developing solvent was water-saturated butanol-formic acid (95:5, v.:v.) and ninhydrin was used to identify the amino acids. Two spots found for the hydrolysis mixture were shown to be starting material and S-2-carboxyethyl-L-cysteine by comparison with spots for the known compounds. A 2,4-dinitrophenylhydrazine test on the reaction mixture was negative.

Reaction of Palladium(II) Chloride with S-2-Carbomethoxyethyl-L-Cysteine.—A solution of S-2-carbomethoxyethyl-L-cysteine (0.5 g., 0.002 mole) and PdCl₂·2H₂O (0.26 g., 0.001 mole) in 3 ml. of water was heated during which a black-brown solid formed containing palladium. From the filtrate pyruvic acid 2,4-dinitrophenylhydrazone (0.25 g., 36%) could be obtained. The 2,4-dinitrophenylhydrazone was recrystallized from hot alcohol; m.p. 212–213°. A few drops of the reaction mixture were added to 1 ml. of basic hydroxylamine solution. After the solution stood for 15 minutes it was paper chromatographed using water-saturated butanol–formic acid (95:5, v.:v.) as the developing solvent and 5% ferric chloride solution as the indicator.[35] The only ester present in the reaction mixture was S-2-carbomethoxyethyl-L-cysteine. A few drops of the reaction mixture were added to 0.10 N sodium hydroxide and the solution was chromatographed using butanol–1.5 N ammonium hydroxide (1:1, v.:v.) as the developing solvent and 0.14% brom phenol blue as the indicator. By comparison with chromatographic behavior of the sodium salts of pyruvic acid, acrylic acid and 3-hydroxypropionic acid, only pyruvic acid was shown to be present in the reaction mixture. The palladium-containing solid which formed during the reaction period had a band at 1725 cm.$^{-1}$ (acid carbonyl) in the infrared spectrum (KBr)

Anal.[36] Calcd. for Pd(SCH₂CH₂COOH)₂: Pd, 33.69. For ClPdSCH₂CH₂COOH: Pd, 42.97. For PdS: Pd, 70.79. Found: Pd, 33.58, 34.45.

Reaction of Mercury(II) Chloride with S-2-Carbomethoxyethyl-L-cysteine.—A solution of S-2-carbomethoxyethyl-L-cysteine (2.0 g., 0.009 mole) and HgCl₂ (2.7 g., 0.009 mole) in 10 ml. of water was heated for 30 hours at 70° in a sealed tube. A brown solid, 1.5 g., was formed during the 30-hour heating period. On treatment of the filtrate with 2,4-dinitrophenylhydrazine dissolved in alcoholic sulfuric acid, 0.30 g. (11%) of pyruvic acid 2,4-dinitrophenylhydrazone was obtained. The 2,4-dinitrophenylhydrazone was recrystallized from hot alcohol; m.p. 211–213°, mixed m.p. with pyruvic acid 2,4-dinitrophenylhydrazone, 210–212°. The infrared spectrum of the brown solid has a band at 1725 cm.$^{-1}$ (acid carbonyl).

The reaction was carried out again in the same manner with the exception that 40 ml. of water was used. All the mercuric chloride was dissolved before addition of the sulfide. The reaction time was extended to 48 hours. The mercaptide which formed in this case was light yellow. A few drops of the reaction mixture were added to 0.10 M sodium hydroxide and the solution was paper chromatographed using butanol–1.5 N ammonium hydroxide as the developing solvent and 0.14% brom phenol blue as the indicator. By comparison of the chromatographic behavior of the reaction mixture with the sodium salts of pyruvic acid, acrylic acid and 3-hydroxypropionic acid, only pyruvic acid was shown to be present in the reaction mixture.

Anal.[37] Calcd. for yellow solid ClHgSCH₂CH₂COOH: Hg, 58.79. Found: Hg, 59.65.

Reaction of S-Methyl-L-cysteine with Copper(II) Chloride.—A solution of S-methyl-L-cysteine (0.52 g., 0.0038 mole) and CuCl₂·2H₂O (0.65 g., 0.0038 mole) in 5 ml. of water was heated 21 hours at 70° and pH 1 in a sealed tube. A yellow solid, 0.10 g. (72%), which formed during the reaction was collected and dissolved in concentrated hydrochloric acid. Methyl mercaptan was liberated. When the acidic solution was made basic with sodium hydroxide, yellow cuprous hydroxide precipitated. To the filtrate 2,4-dinitrophenylhydrazine (1.0 g.) dissolved in alcoholic sulfuric acid was added; and the pyruvic acid 2,4-dinitrophenylhydrazone which precipitated, 0.17 g. (16%), m.p. 196–205°, was recrystallized from hot alcohol; m.p. 210–212°, mixed m.p. with an authentic sample, 209–212°.

An electrolytic analysis of the yellow solid for copper was carried out.[38] It may have been contaminated with copper-(II) mercaptide or with the disulfide.

Anal. Calcd. for CH₃SCu: Cu, 57.45. Found: Cu, 51.80, 54.85, 52.00, 52.94.

Reaction of S-2-Carboxyethyl-L-cysteine with Copper(II) Chloride.—S-2-Carboxyethyl-L-cysteine (2.0 g., 0.013 mole) and CuCl₂·2H₂O (1.2 g., 0.007 mole) in 10 ml. of water in a sealed tube were heated for 21 hours at 70° and pH 1. Upon completion of the reaction the 3,3′-dithiodipropionic acid (0.23 g., 34%) which formed on cooling was collected. It was recrystallized from hot water; m.p. 153–155°, mixed m.p. with authentic 3,3′-dithiodipropionic acid, 153–155°. Pyruvic acid, 0.42 g. (15%), was isolated as its 2,4-dinitrophenylhydrazone, m.p. 212–213°, mixed with a sample of authentic pyruvic acid 2,4-dinitrophenylhydrazone, 210–213°.

Treatment of 3,3′-Thiodipropionic Acid with Copper(II) Chloride.—A solution of 3,3′-thiodipropionic acid (0.60 g., 0.003 mole) and CuCl₂·2H₂O (0.58 g., 0.003 mole) in 4 ml. of water was heated for 21 hours at 70° in a sealed tube. The 3,3′-thiodipropionic acid (0.30 g.) was recovered unchanged and after a recrystallization from water did not depress the melting point of an authentic sample of acid, m.p. 127–129°, mixed m.p. 127–129°. An additional 0.10 g. of acid was obtained after ion exchange of the filtrate. There was no evidence for any other product.

Methyl-2-aminoethyl Sulfide–Copper(II).—To 1 ml. of 50% aqueous alcohol, 0.20 g. (0.0021 mole) of S-methyl-2-aminoethyl sulfide, 3 ml. of 50% ethanol containing 0.37 g. (0.0022 mole) of CuCl₂·2H₂O and 1 ml. of 50% ethanol were added. The solution became dark blue, and a solid gradually precipitated. After the solution stood for several hours, 0.38 g. (78%) of a green substance was obtained, m.p. 124–124.5°.

Anal. Calcd. for C₃H₉NSCl₂Cu: C, 15.96; H, 4.02. Found: C, 15.59; H, 4.07.

(34) P. W. Preisler and D. E. Preisler, *J. Am. Chem. Soc.*, **54**, 2984 (1932).

(35) R. Block, R. Le Strange and G. Zweig, "Paper Chromatography," Academic Press, Inc., New York, N. Y., 1952, p. 96.

(36) W. F. Hillebrand and G. E. F. Lundell, "Applied Inorganic Analysis," John Wiley and Sons, Inc., New York, N. Y., 1929, p. 281.

(37) Reference 36, p. 174

(38) Reference 36, p. 197.

When equimolar aqueous solutions of S-methyl-2-amino-ethyl sulfide and copper(II) chloride were heated for 21 hours at 70°, no decomposition occurred; only the chelate (14%) was formed.

Treatment of S-2-Carbomethoxyethylhomocysteine with Copper(II) Chloride.—The amino acid (1.0 g., 0.005 mole) was dissolved in 5 ml. of water and $CuCl_2 \cdot 2H_2O$ (0.76 g., 0.005 mole) added. The resulting blue solution was heated 21 hours at 70°. During the heating the solution turned green. The solution was filtered and passed through an ion exchange column of IR 120 H. Neither 3,3'-dithio-dipropionic acid nor a 2,4-dinitrophenylhydrazone could be isolated. Approximately 20% of pure starting material was obtained after several recrystallizations from hot 95% ethanol.

Reaction of Copper(II) Chloride with Cysteine.—A solution of cysteine (1.2 g., 0.009 mole) and $CuCl_2 \cdot 2H_2O$ (1.7 g., 0.009 mole) in 10 ml. of water was heated 18 hours at 70°; during this time 0.88 g. (93%) of copper sulfide formed. On treatment of the filtrate with 2,4-dinitrophenylhydrazine dissolved in alcoholic sulfuric acid, 0.80 g. (28%) of pyruvic acid 2,4-dinitrophenylhydrazone formed, m.p. 208–210°. The 2,4-dinitrophenylhydrazone was recrystallized from hot alcohol; m.p. 209–212°, mixed m.p. with authentic pyruvic acid 2,4-dinitrophenylhydrazone, 208–210°.

Reaction of Copper(II) Chloride with Cystine.—The reaction was conducted under the same conditions as described for the cysteine decomposition. Copper sulfide was obtained in almost quantitative yield. Pyruvic acid was isolated as the 2,4-dinitrophenylhydrazone in roughly the same yield as in the cysteine decomposition reaction.

Reaction of Copper(II) Chloride with S-3-ketopentyl-L-cysteine.—A solution of S-3-ketopentyl-L-cysteine (1.9 g., 0.01 mole) and $CuCl_2 \cdot 2H_2O$ (2.5 g., 0.014 mole) were heated 21 hours at 70°. During this time 1.0 g. of CuS precipitated. The reaction mixture was treated with 2,4-dinitrophenylhydrazine dissolved in alcoholic sulfuric acid and two 2,4-dinitrophenylhydrazones were separated on a column of Florisil (ethanol–toluene (1:5, v.:v.) solvent). These were possibly the 2,4-dinitrophenylhydrazones of pyruvic acid and methyl vinyl ketone. Attempts to elute them with methanol–toluene resulted in their decomposition.

Treatment of S-2-Carboxyethyl-L-cysteine Hydrochloride with Copper(II) Chloride.—A green solution (pH 1) of the hydrochloride (1.0 g., 0.004 mole) and $CuCl_2 \cdot 2H_2O$ (0.70 g., 0.004 mole) was heated 21 hours at 70° without change in appearance. Copper(II) ions were precipitated with sodium sulfide. After acidification of the solution with concentrated hydrochloric acid, the water was removed under vacuum. A green solid (0.90 g.) was obtained. The color was removed by a further treatment with sodium sulfide. The hydrochloride, 0.79 g. (79%), was identified by paper chromatography. There was no evidence of any other product.

Treatment of Cystine Betaine with Copper(II) Chloride.—A solution of cystine betaine (1.0 g., 0.003 mole) and $CuCl_2 \cdot 2H_2O$ (0.61 g., 0.003 mole) in 5 ml. of water was heated in a sealed tube for 21 hours at 70°. Afterward, cupric ion was removed by bubbling hydrogen sulfide through the solution. The formation of copper sulfide during the heating period was not observed. Iodine was added to the solution until it remained slightly orange (after shaking) when excess iodine was added to the solution. The mixture was treated with an equivalent of flavianic acid (2,4-dinitro-1-naphthol-7-sulfonic acid). Approximately 60% of the starting material was obtained as the flavianate, m.p. 231–232.5°, mixed m.p. with an authentic sample of cystine betaine, 229–230°. There was no evidence for any reaction.

Treatment of S-2-Carboxyethyl-L-cysteine Hydantoin with Copper(II) Chloride.—A solution of S-2-carboxyethyl-L-cysteine hydantoin (1.0 g., 0.005 mole) and $CuCl_2 \cdot 2H_2O$ (0.78 g., 0.005 mole) in 10 ml. of 50% methanol was heated for 21 hours at 70°. The original light blue solution did not change color on heating. When the solution was evapo

rated to 3 ml., after removal of the copper by hydrogen sulfide, approximately 50% of the hydantoin was recovered (after purification). Attempts to isolate 3,3'-dithiodipropionic acid or a 2,4-dinitrophenylhydrazone were unsuccessful. The methanol did not interfere with the decomposition since S-2-carbomethoxyethyl-L-cysteine decomposed to pyruvic acid when heated with $CuCl_2 \cdot 2H_2O$ in 50% methanol.

Di-[S-2-carbomethoxyethyl-L-cysteine]Copper(II).— To 0.50 g. (0.0024 mole) of S-2-carbomethoxyethyl-L-cysteine dissolved in 6 ml. of water was added 0.41 g. (0.0024 mole) of $CuCl_2 \cdot 2H_2O$. The blue solid was collected after 30 minutes, 0.14 g. (24%), m.p. 225–225.5° dec.; infrared spectrum (KBr): 676, 692, 733, 807, 828, 837, 890, 926, 980, 1011, 1072, 1125–1195, 1250, 1308, 1340–1365, 1390, 1419, 1435, 1566, 1594, 1640, 1732, 2920, 3140, 3210, 3280, 3430 cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{24}O_8N_2S_2Cu$: C, 35.32; H, 5.09; S, 13.47. Found: C, 35.57; H, 5.26; S, 13.88.

The infrared spectrum of the chelate has bands similar to the 2:1 amino acid–copper chelates of S-methylcysteine, glycine (infrared spectrum (KBr): 670, 743, 920, 957, 1035, 1058, 1116, 1178, 1196, 1321, 1366–1408, 1422, 1538–1641, 1674, 2910, 3080–3450 cm.$^{-1}$) and the literature values[19] for the 2:1 glycine–copper chelate in the regions around 3300, 3250, and 3150 (NH stretching) and 1600 cm.$^{-1}$ (carboxylate anion).

Stability of S-2-Carbomethoxyethyl-L-cysteine Copper(II) in Water.—The chelate (0.1 g.) was dissolved at 100° in 50 ml. of water. The solution was heated 21 hours at 100° and cooled. The chelate, 0.10 g., was recovered unchanged, m.p. 225–225.5°.

Decomposition of Di-[S-2-carbomethoxyethyl-L-cysteine]-Copper(II) in Hydrochloric Acid.—A suspension of 0.30 g. of di-[S-2-carbomethoxyethyl-L-cysteine]copper-(II) in 30 ml. of water in a test-tube was adjusted to pH 1. The tube was sealed and heated at 70° for 21 hours. The undissolved blue complex slowly turned brown during the heating and some of the chelate dissolved since the solution above the complex became blue. After unreacted material was removed by filtration, the 3,3'-dithiodipropionic acid was isolated after reduction of the volume. The filtrate was treated with 2,4-dinitrophenylhydrazine dissolved in alcoholic sulfuric acid. Pyruvic acid 2,4-dinitrophenylhydrazone was collected, 0.036 g., m.p. 210–212°.

Treatment of Sodium S-2-Carbomethoxyethyl-L-cysteine with Copper(II) Chloride.—To a solution of S-2-carbomethoxyethyl-L-cysteine (1.1 g., 0.005 mole) in 50 ml. of water was added 5 ml. of 1 N sodium hydroxide followed by 0.85 g. (0.005 mole) of $CuCl_2 \cdot 2H_2O$. The water-insoluble 2:1 copper chelate (50%) was removed and the reaction heated for 21 hours at 70°. The blue solution did not change color during the heating period. Neither 3,3'-dithiodipropionic acid nor pyruvic acid could be detected.

Reaction of Serine with Copper(II) Chloride.—A solution of serine (1.0 g., 0.01 mole) and $CuCl_2 \cdot 2H_2O$ (1.6 g., 0.01 mole) was heated for 21 hours at 70°. On addition of the $CuCl_2 \cdot 2H_2O$, the solution became blue-green (pH 1). A small amount of gray solid formed during the 21-hour period. A 2,4-dinitrophenylhydrazone test for a carbonyl compound was negative. After addition of hydrogen sulfide, the copper sulfide was removed by filtration. The water was removed from the filtrate *in vacuo*. An oily residue was obtained which solidified on standing 24 hours at 1 mm. pressure. The solid contained nitrogen and chloride ion. It gave an immediate precipitate with acidic silver nitrate. The solid was subjected to paper chromatography using water-saturated butanol–formic acid (95:5, v.:v.) as the developing solvent and ninhydrin as the indicator. The material proved to be identical with authentic serine in its behavior on paper chromatography.