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THE COPPER(II) DERIVATIVES OF S-(1,2-trans-DICHLORO-VINYL)-L-CYSTEINE AND RELATED COMPOUNDS

by

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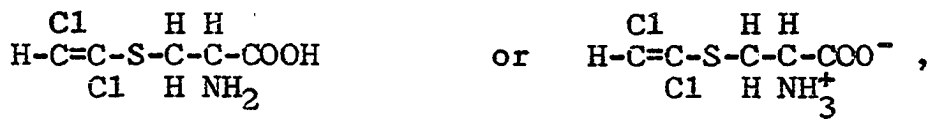
INTRODUCTION

S-(1,2-trans-dichlorovinyl)-L-cysteine has been reported by McKinney et al. (1) to be quite possibly the toxic factor in trichloroethylene-extracted soybean oil meal. The present investigation was undertaken to determine further some of the chemical properties of S-(1,2-trans-dichlorovinyl)-L-cysteine with the object of the ultimate correlation of these characteristics with the biological processes and thus an elucidation of the mechanism of the toxicity.

From a consideration of the structure of this S-derivative of L-cysteine:



it is evident that chelation of this compound with the metals can occur. The metals of physiological importance in the approximate order of decreasing abundance in the body are: calcium, potassium, sodium, magnesium, iron, manganese, copper, cobalt and zinc (2).

McKinney et al. (3) have reported the physiological result of the intake of the toxic factor as being an aplastic anemia, specifically a malfunctioning of the process of building new red blood cells among other effects. Thus the metals of immediate concern were those considered essential to this function, namely iron, cobalt and copper.

PREPARATION OF ORGANIC COMPOUNDS

Materials

Anhydrous liquid ammonia and 1,2,3-triketohydrindene hydrate (ninhydrin) were obtained from Matheson Coleman and Bell, East Rutherford, New Jersey. Samples of S-cyclopentane-L-cysteine and 1,2-bis-(S-L-cysteine)ethylene were generously contributed by Dr. J. C. Picken, Jr., Veterinary Medicine Research Institute, Ames, Iowa. L-cysteine, L-cysteine hydrochloride, L-cystine, and djenkolic acid were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and included lot analyses. Glycine, trichloroethylene, 1,2-dichloroethane, and β- mercaptopropionic acid were obtained from Eastman Organic Chemicals, Distillation Products Industries, Rochester, New York. S-ethyl-L-cysteine was obtained from the California Foundation for Biochemical Research, Los Angeles, California.

All other materials used were reagent grade chemicals wherever possible.

General Method of Preparation

The method of du Vigneaud (4) was applied to the preparation of the S-substituted derivatives of L-cysteine and β-mercaptopropionic acid. Sodium metal was dissolved in anhydrous liquid ammonia contained in a 1 liter borosilicate

glass filtering flask fitted with a drying tube containing anhydrous calcium chloride and with a 125 ml. conical flask connected to the top of the filtering flask by a large diameter rubber tubing to permit the introduction of solid materials with minimum exposure of the system to moist air. An amount of L-cysteine, L-cystine or β-mercaptopropionic acid equivalent to the sodium added was then introduced from the attached conical flask. The reaction mixture was magnetically stirred by a glass covered magnetic stirring bar, Teflon being attacked by the solution of metallic sodium in liquid ammonia. The S-containing acid was added until the blue color of the solution of sodium in liquid ammonia just disappeared. Then an equivalent amount of the desired chlorinated hydrocarbon, dissolved in a small amount of liquid ammonia, was slowly added with stirring. The reaction was allowed to continue for one-half hour after which time stirring was continued as the liquid ammonia was allowed to evaporate from the now open filtering flask. Dry air was introduced into the reaction vessel to hasten the evaporation of the liquid ammonia.

After the liquid ammonia had completely evaporated, water was added and the residual ammonia removed in vacuo. Acetic acid was added to lower the pH to about 5 at which pH the product precipitated. The product was then recrystallized

from water and dried under reduced pressure over anhydrous magnesium perchlorate.

The neutralization equivalent was determined by non-aqueous titration. The recrystallized product was dissolved in excess standard perchloric acid in glacial acetic acid containing acetic anhydride and titrated with anhydrous sodium acetate in glacial acetic acid. The end point was obtained potentiometrically and visually using methyl violet indicator. The perchloric acid was standardized against the sodium acetate solution which was in turn standardized against primary standard benzoic acid and primary standard potassium acid phthalate, both dissolved in glacial acetic acid. These end points were detected potentiometrically and visually by use of methyl violet indicator.

The acid dissociation constants were determined by aqueous titration. The product was dissolved in excess standard hydrochloric acid and titrated with standard sodium hydroxide. The reverse procedure was also carried out. The hydrochloric acid was standardized against primary standard tris-(hydroxymethyl)aminomethane using methyl purple indicator. The sodium hydroxide was standardized against primary standard potassium acid phthalate using phenolphthalein indicator.

To determine the absence of other possible substituted products, paper chromatograms were made. A water solution of

the product was spotted on Whatman no. 1 filter paper and dried. A solution of n-propanol-water, 70:30, was used as solvent in the descending chromatograms. The chromatograms were developed with 1,2,3-triketohydrindene hydrate using a 0.1 percent solution in water saturated n-butanol as a spray. The color was developed by heating at 105° for 10 minutes.

Preparation of Individual Compounds

S-(1,2-trans-dichlorovinyl)-L-cysteine (DCVC)

S-(1,2-trans-dichlorovinyl)-L-cysteine was prepared in the manner described by McKinney *et al.* (1). Sodium metal, 0.20 mole, was carefully added to 250-300 ml. of anhydrous liquid ammonia in a 1 liter borosilicate glass filtering flask. After the characteristic blue color had developed, 0.10 mole of dry L-cysteine or 0.05 mole of dry L-cystine was added gradually from the attached conical flask. The reaction vessel was placed in a glass crystallizing dish to permit application of ethyl alcohol to the outside of the flask to prevent frosting and allow inspection of the reaction mixture as the preparation progressed. The actual amount of amino acid added was adjusted to just remove the last traces of the blue sodium-liquid ammonia color. In most cases the resulting solution was clear with a slight pale yellow color. Some preparations resulted in a fine off-white suspension at

this stage which did not appear to adversely affect the remainder of the procedure. Distilled trichloroethylene, 0.10 mole in 25 ml. of anhydrous liquid ammonia, was carefully added with stirring, causing an off-white material to form in the clear solutions. The trichloroethylene was distilled before introduction into this reaction after washing with dilute hydrochloric acid to remove the ethanolamine stabilizer.

After addition of the trichloroethylene, the mixture was stirred for one-half hour. Dry air was then introduced to speed the evaporation of the liquid ammonia.

The slightly colored residue was readily dissolved in 300 ml. of water and freed from traces of ammonia in vacuo. The resulting solution was strongly basic. Glacial acetic acid was added to adjust the pH to about 5 using indicating pH paper. The resulting copious precipitate was diluted with an equal volume of 95 percent ethyl alcohol and chilled overnight in an ice bath. The precipitate was removed by filtration and dissolved in about 1000 ml. of water at 70°. Activated carbon, Norit A, was added to the hot solution which was filtered while hot. Again an equal volume of 95 percent ethyl alcohol was added and the solution chilled overnight. White, velvety, needle-like crystals were obtained which after one further water-ethyl alcohol recrystallization melted with decomposition at 156-157°. Careful evaporation of the mother

liquor produced further product which was similarly recrystallized with total yields of about 60-70 percent. The product was dried under reduced pressure over anhydrous magnesium perchlorate.

The experimental value for the neutralization equivalent was 216.0, the calculated value being 216.11. No elemental analyses were made. The acid dissociation constants obtained were: pK_1 2.44 (-COOH); pK_2 8.05 ($-NH_3^+$).

Paper chromatography showed the presence of only one spot sentitive to 1,2,3-triketohydrindene hydrate with an R_f value of 0.72, identical to that found by McKinney et al. (1). Some chromatograms showed another small spot due to L-cystine but this was removed from those preparations by use of a small amount of potassium cyanide in the crystallization process.

The solubility of the compound in water and water-ethyl alcohol has been reported (1). It was readily soluble in either acid or base, decomposing rather rapidly above pH 8.

1,2-bis-(L-cysteine)ethane

Metallic sodium, 0.40 mole, dry L-cysteine, 0.20 mole, and 1,2-dichloroethane, 0.10 mole, were added to 300 ml. of anhydrous liquid ammonia in the usual manner. After evaporation

of the liquid ammonia, dissolution of the slightly colored residue in 300 ml. of water, and removal of traces of ammonia in vacuo, the pH of the solution was lowered to 5 with glacial acetic acid yielding a slightly gray needle-like precipitate. The recrystallization was carried out as before using 1:3 water-ethyl alcohol solutions for crystallization. The product was separated by filtration and dried under reduced pressure over anhydrous magnesium perchlorate.

The neutralization equivalent determined experimentally was 135.3; the theoretical value was 134.1. No elemental analyses were made. The acid dissociation constants found were: pK_1 2.64 (-COOH); pK_2 3.15 (-COOH); pK_3 7.90 ($-NH_3^+$); pK_4 8.90 ($-NH_3^+$).

Paper chromatograms showed only one spot sensitive to 1,2,3-triketohydrindene hydrate.

S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid

Sodium metal, 0.20 mole, was added to 300 ml. of anhydrous liquid ammonia followed by 11.0 ml. (0.10 mole) of β-mercaptopropionic acid yielding a slightly gray, clear solution. Distilled trichloroethylene, 0.10 mole in 25 ml. of liquid ammonia, was slowly added giving a light brown residue in the reaction flask. The mixture was stirred for one hour. After evaporation of the liquid ammonia, the product was dissolved

in 250 ml. of water and traces of ammonia removed in vacuo. Diethyl ether, 200 ml., was added to extract the excess unreacted reagents and any other neutral materials. As the solution was highly basic, the desired product would be in the ionic form as the sodium or ammonium salt and thus not extractable. The aqueous phase was made acid with hydrochloric acid to a pH of about 2 to form the free acid of the desired product and again extracted with diethyl ether. The ether phase was evaporated yielding a brownish white residue. This residue was dissolved in water at 90°, treated with activated carbon (Norit A), filtered while hot and chilled in an ice bath overnight. A light fluffy white crystalline product was obtained which was dried over anhydrous magnesium perchlorate under reduced pressure.

This material melted sharply at 64°. The neutralization equivalent was determined in 1:1 ethyl alcohol-water solution with aqueous sodium hydroxide using phenolphthalein indicator. The calculated value of 201.04 compared favorably with the experimental value of 202.0. An elemental analysis gave the following results: C 30.23, H 3.05, S 15.22, Cl 36.69; theoretical: C 29.86, H 3.01, S 15.95, Cl 35.27.

This material was extremely difficult to isolate in pure form and exceptionally unstable, decomposing into an obnoxious liquid after about two months. Its low melting point (64°)

caused it to melt during the recrystallization attempts and on chilling often resulted in formation of a liquid-liquid system resulting in a noncrystalline product. Rapid chilling permitted isolation of a good crystalline product with a sharp melting point.

STUDIES OF THE EFFECT OF VARIOUS CATIONS OF PHYSIOLOGICAL IMPORTANCE ON THE S-SUBSTITUTED L-CYSTEINE DERIVATIVES

In view of the distinct possibility of complex formation being the mechanism whereby the S-(1,2-trans-dichlorovinyl)-L-cysteine was causing a malfunctioning of the physiological processes, the various cations known to be present in the body and particularly those known or suspected to be highly necessary to the functioning of the blood were investigated to determine which, if any, were affected by S-(1,2-trans-dichlorovinyl)-L-cysteine. The following metals were investigated: iron(II), iron(III), cobalt(II), nickel(II), manganese(II), zinc(II), cadmium(II), mercury(II), lead(II), chromium(III), aluminum, calcium, magnesium, strontium, silver, molybdenum(III), tin(II), copper(I) and copper(II). Only the latter, copper(II), was found, by the schemes employed, to exhibit any complex formation although silver and mercury(II) seemed to undergo some reaction. These latter two were not investigated further as they play no essential physiological role.

Amino acids and the S-substituted derivatives of cysteine exist in neutral solutions as zwitterions becoming completely protonated in strongly acid solutions and completely neutralized in strongly basic solutions. When neutral solutions of amino acids are allowed to react with cations, the release

of a proton, signified by a decrease in the pH of the solutions, is taken as an indication of complex formation. The absence of any complex formation will subsequently fail to cause a pH change if the solution of the amino acid and the metal cation are isohydric. This is particularly true where the complexes with amino acids are to take place through the amino group which in neutral solutions is protonated, the carboxylate group being free. Thus with the formation of a complex, the proton on the amino group is released to the solution thereby decreasing the pH of that solution. Simple salt formation involving the carboxylate group only would not result in any significant pH change; only complex formation involving the amino group would cause such a change. This change in pH, coupled with other observations, constituted the manner in which complex formation was investigated.

Experimental Procedures

Solutions of reagent grade nitrates, perchlorates, sulfates, or chlorides of the various metals investigated were made up to approximately 0.01 M. A 0.01 M solution of S-(1,2-trans-dichlorovinyl)-L-cysteine was prepared in water. Three methods for investigating the possibility of complex formation were utilized. These are extensions of those applied by Bjerrum (5) and Calvin and Melchior (6).

Method 1

The pH of the metal solution was adjusted with 0.01 N sodium hydroxide to that of the S-(1,2-trans-dichlorovinyl)-L-cysteine (about 5.0) where possible without precipitation of the metal as the hydroxide or the hydrated oxide. An aliquot of the metal ion solution was then titrated potentiometrically with the 0.01 M solution of S-(1,2-trans-dichlorovinyl)-L-cysteine to a ten fold excess. The failure of the pH to change more than about 0.3 pH unit was taken as indication of no complex formation by this method. This method was applied to copper(I), copper(II), calcium, manganese(II), zinc, iron-(II), magnesium, chromium(III), aluminum, cobalt(II), and silver. Figure 1 shows the results of this technique with a metal that forms a complex, copper(II), and a metal that fails to form a complex, iron(II), with S-(1,2-trans-dichlorovinyl)-L-cysteine.

Method 2

The pH of the metal ion solution was again adjusted to that of the S-(1,2-trans-dichlorovinyl)-L-cysteine where possible without precipitation and an aliquot of the S-(1,2-trans-dichlorovinyl)-L-cysteine solution was titrated with the metal ion solution to a five-fold excess. Again a failure of the pH to change by greater than 0.3 pH unit was taken as indication of no complex formation. This method was applied to

Figure 1. Titration of metal ions with S-(1,2-trans-dichlorovinyl)-L-cysteine

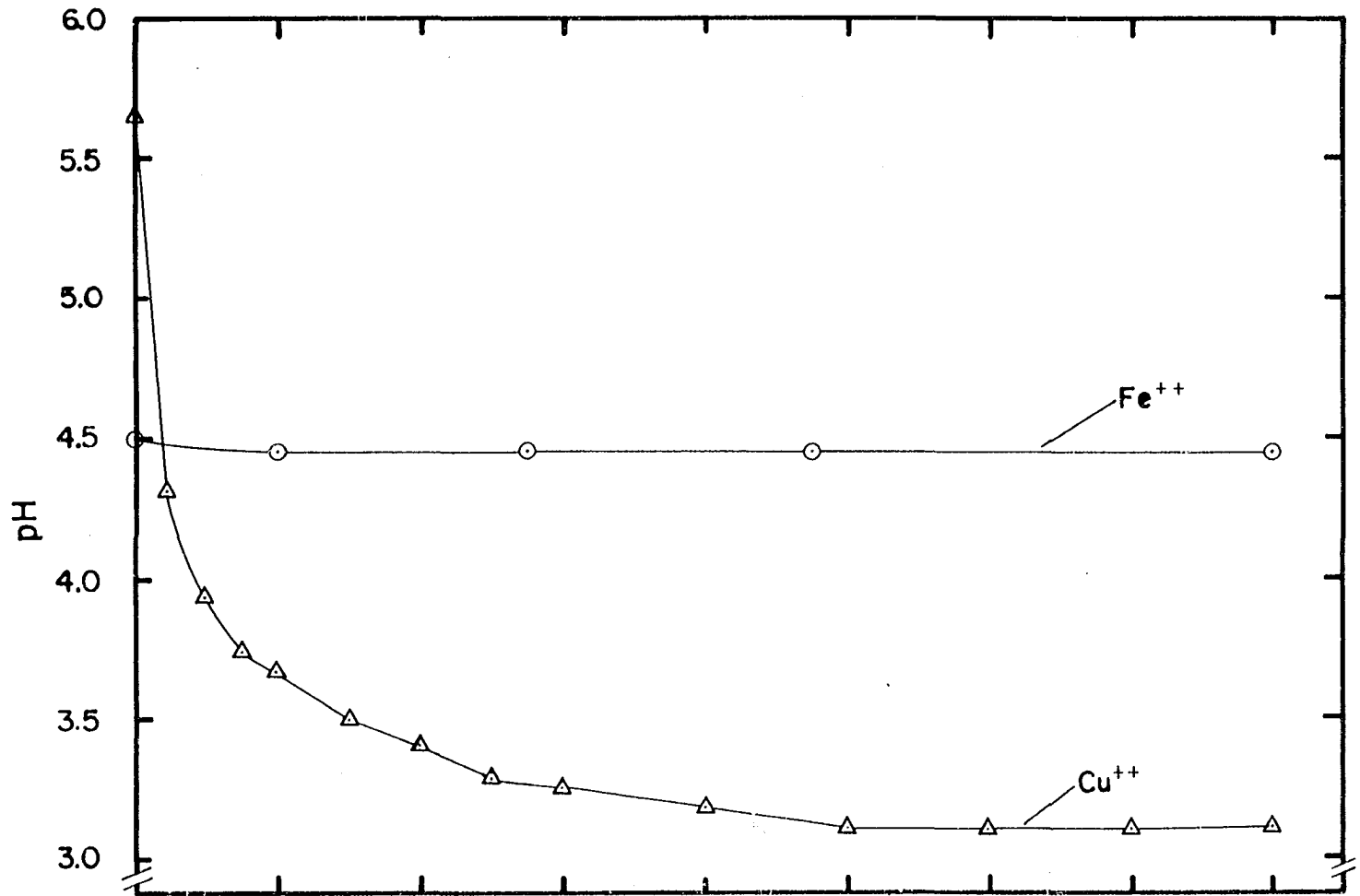

6.0
5.5
5.0
4.5
4.0
3.5
3.0
pH
Fe++
Cu++
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
VOLUME 0.01 M ORGANIC REAGENT (ml)

FIGURE 1.

those metals used in Method 1 above. Figure 2 shows the results of this technique with a metal that forms a complex, copper(II), and with a metal that fails to complex, magnesium.

Method 3

Where those metals investigated formed precipitates at the pH of the S-(1,2-trans-dichlorovinyl)-L-cysteine solution, the following scheme was used. The S-(1,2-trans-dichlorovinyl)-L-cysteine solution was adjusted to a pH of about 2 with a known amount of 0.1 N hydrochloric acid and equal aliquots of this solution were titrated with 0.01 N sodium hydroxide in the presence and absence of the metal ion solution, all solutions being isohydric at the start of the titration. The metal ion solution was also titrated with 0.01 N sodium hydroxide after addition of the same amount of 0.1 N hydrochloric acid as with the previous titrations. Plots of these titration curves were made on the same coordinates and the failure of the curve for the titration of the metal ion solution plus the S-(1,2-trans-dichlorovinyl)-L-cysteine plus the hydrochloric acid to deviate significantly from that curve obtained for the titration in the absence of metal ion was taken as evidence for no complex formation in the pH range investigated.

Figure 3 shows this type of result for copper(II) wherein complex formation takes place. The displacement to the right of the curve for copper(II) in the presence of S-(1,2-trans-

Figure 2. Titration of S-(1,2-trans-dichlorovinyl)-L-cysteine with metal ions

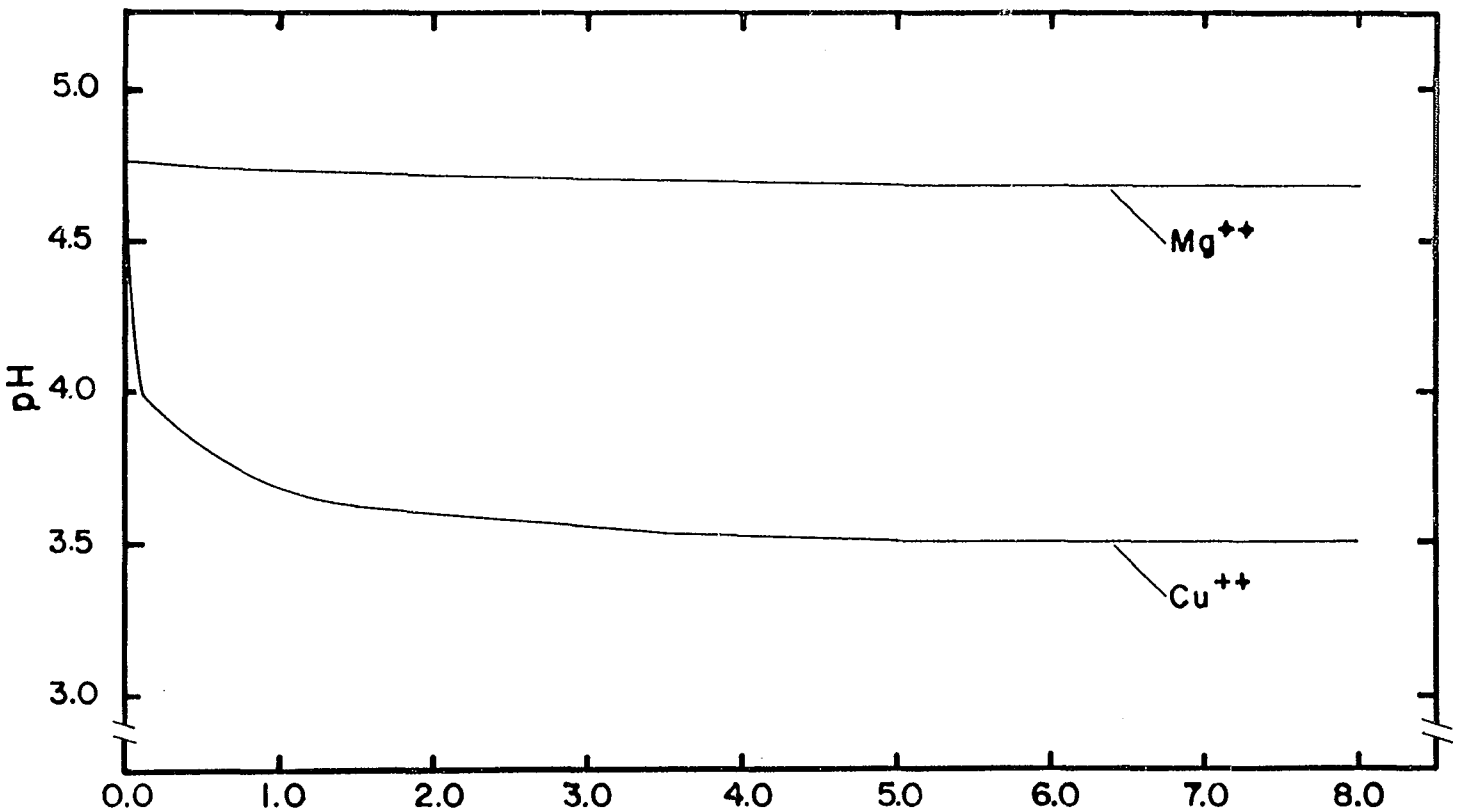


FIGURE 2. VOLUME 0.01 M METAL ION (ml)

Figure 3. Titration of S-(1,2-trans-dichlorovinyl)-L-cysteine (DCVC) with NaOH in presence and absence of a complexing metal ion, copper(II)

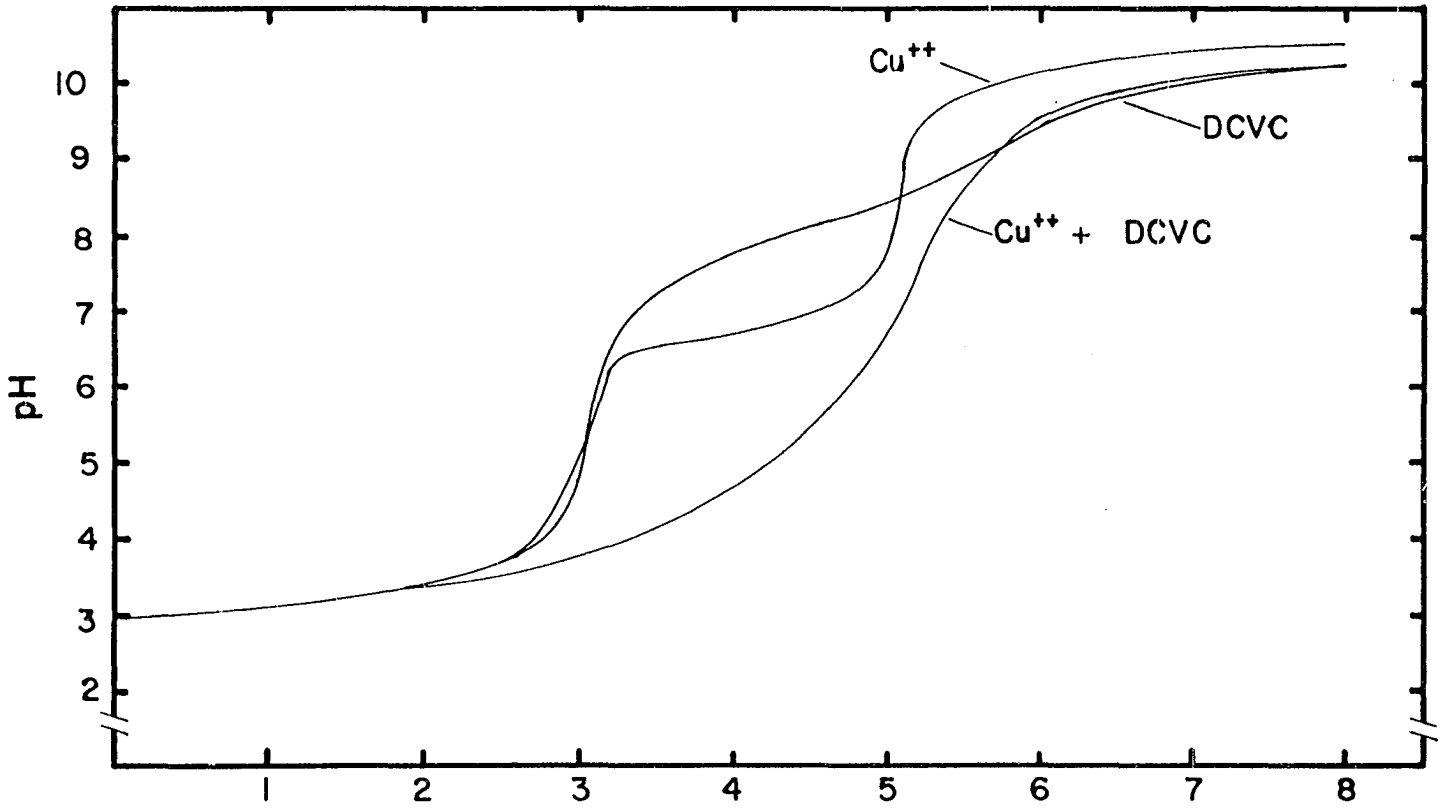


FIGURE 3 VOLUME 0.01 N NaOH (ml)

dichlorovinyl)-L-cysteine from that in the absence of the organic material is due to the hydrogen ion released from the S-(1,2-trans-dichlorovinyl)-L-cysteine on complexing with the copper(II). Figure 4 shows the results of this scheme for a metal that fails to complex with S-(1,2-trans-dichlorovinyl)-L-cysteine. There is no horizontal displacement of the curve obtained in the presence of the iron(III) ion.

This method was also applied to those metals investigated by Methods 1 and 2 above.

Similar methods were applied to the investigations concerning the other S-substituted L-cysteine compounds.

Figure 4. Titration of S-(1,2-trans-dichlorovinyl)-L-cysteine (DCVC) with NaOH in presence and absence of a noncomplexing metal ion, iron(III)

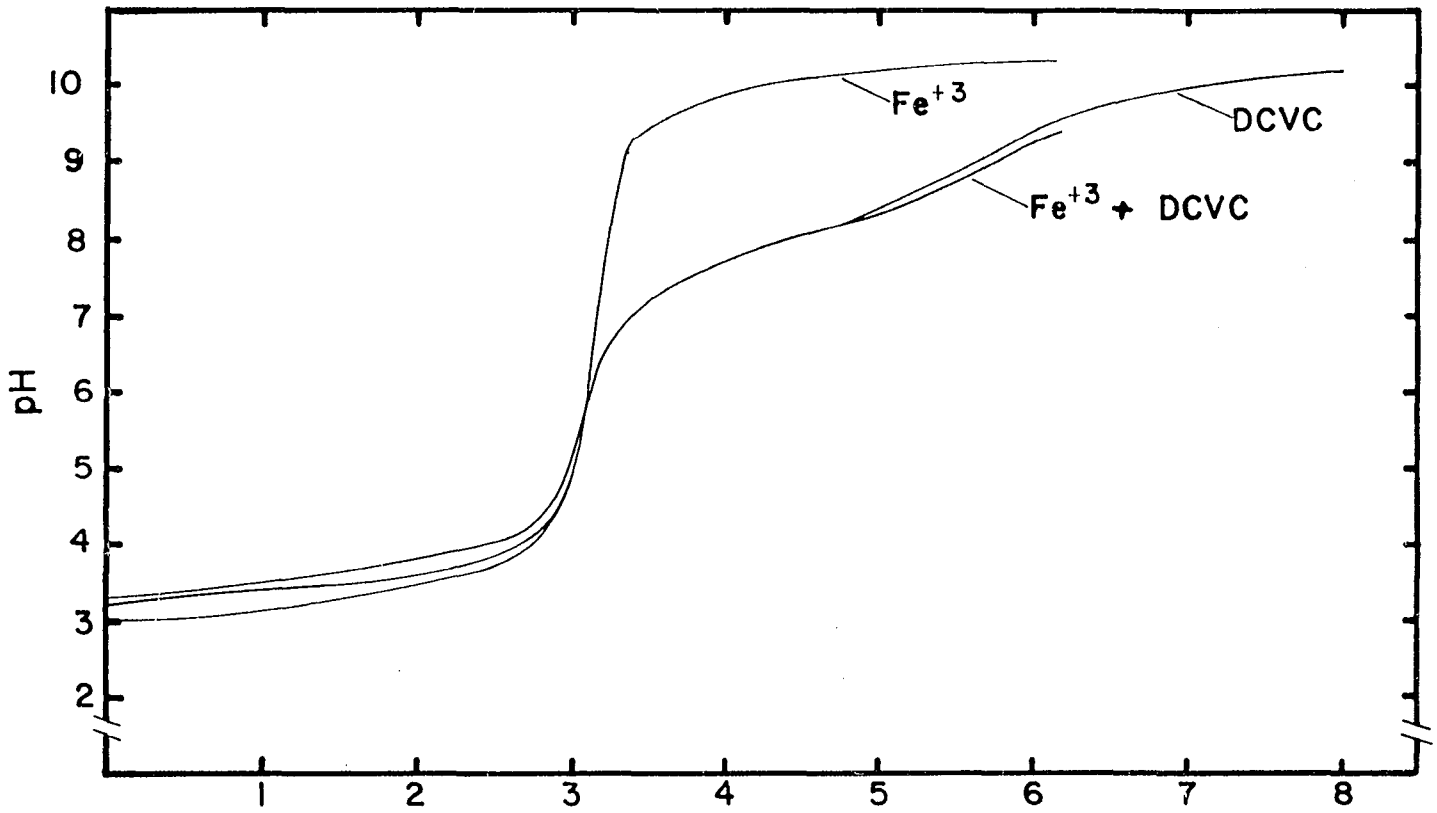


FIGURE 4 VOLUME 0.01 N NaOH (ml)

PREPARATION AND ANALYSIS OF COPPER(II) DERIVATIVES

The results of the investigations of the metals that might form complexes with S-(1,2-trans-dichlorovinyl)-L-cysteine and other S-substituted L-cysteine derivatives showed that of the metals normally found in the body, only copper(II) formed any detectable complexes. The indications for soluble complex formation involving silver and mercury(II) were not further investigated as they are not normally present in the body.

The solid derivatives of copper(II) with the various S-substituted L-cysteine compounds were prepared and analyzed. The copper(II) derivatives of glycine and S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid were also prepared.

General Method of Preparation

The derivatives of copper(II) and the S-substituted derivatives of L-cysteine were prepared in general by the addition of a slight excess of a solution of copper(II) nitrate to an acid solution of the organic acid. The pH was raised to about 4.5 with 1:3 ammonium hydroxide using a Beckman Model "G" pH meter. The solution was rapidly stirred, the precipitate allowed to settle, and twice washed with water by decantation. The precipitate was then dissolved in 1:3 nitric acid, filtered, and reprecipitated with 1:3 ammonium hydroxide at pH 4.5. After twice washing again with water, the precipi-

tate was separated by filtration with suction and dried under reduced pressure over anhydrous magnesium perchlorate. Some variations were found necessary and these are indicated under each individual compound.

Preparation of Individual Compounds

Copper(II) and S-(1,2-trans-dichlorovinyl)-L-cysteine

S-(1,2-trans-dichlorovinyl)-L-cysteine, 0.02 mole, was dissolved in 400 ml. of water at 70° and filtered to remove any undissolved material. Copper, 0.01 mole, as a solution of copper(II) nitrate in excess nitric acid, was added with the resulting clear blue solution having a pH of about 2. Dilute, 1:3, filtered ammonium hydroxide was added slowly with rapid stirring until the pH was raised to 4.5 where a velvety copper(II)-ammine blue precipitate was present. This solution was stirred overnight. The precipitate was washed by decantation three times and finally separated by filtration with suction and dried under reduced pressure over anhydrous magnesium perchlorate.

Copper(II) and S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid

Fresh copper(II) hydroxide was dissolved by adding 0.7 g. of S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid dissolved in 1:1 ethyl alcohol-water. The pH was adjusted to 4.9

with dilute, filtered ammonium hydroxide at which point a clean, green precipitate was present. After stirring overnight, the precipitate was washed twice with water by decantation and extracted into diethyl ether. The ether phase was evaporated after filtering, yielding a brilliant green crystalline product. This was dried under reduced pressure over anhydrous magnesium perchlorate.

Copper(II) and 1,2-bis-(S-L-cysteine)ethane

1,2-bis-(S-L-cysteine)ethane, 0.01 mole, was dissolved in 1 N hydrochloric acid and 0.01 mole of copper as copper(II) nitrate was added. The pH was raised to 4.5 with dilute filtered ammonium hydroxide yielding a velvety blue precipitate. This precipitate was redissolved in 30 ml. of 1 N hydrochloric acid, filtered, and reprecipitated with ammonium hydroxide as above. This precipitate was washed by decantation, filtered by suction, and dried under reduced pressure over anhydrous magnesium perchlorate. This compound tended to gain weight rapidly due to the absorption of water leading to the isolation of a second hydrate. The preparation as indicated above, led to the hemihydrate. This hemihydrate was hydrostated over a saturated solution of magnesium acetate tetrahydrate for 6 days and led to the isolation of the $2\frac{1}{2}$ hydrate. The relative humidity of this system is 66 percent at 20° (7).

Copper(II) and 1,2-bis-(S-L-cysteine)ethylene

1,2-bis-(S-L-cysteine)ethylene, 1.0 g. was dissolved in 1 N hydrochloric acid and excess copper as copper(II) nitrate was added. The pH was raised to 4.0 with dilute filtered ammonium hydroxide and the resulting light blue precipitate was washed twice by decantation, separated by filtration with suction, and dried under reduced pressure over anhydrous magnesium perchlorate.

Copper(II) and S-cyclopentane-L-cysteine

S-cyclopentane-L-cysteine was dissolved in 1 N hydrochloric acid and an equivalent amount of copper(II) nitrate was added. The pH was raised to 4.0 with dilute filtered ammonium hydroxide and the resulting soft blue precipitate was washed twice by decantation, filtered with suction, and dried under reduced pressure over anhydrous magnesium perchlorate.

Copper(II) and L-cysteine

An excess of L-cysteine hydrochloride was added to 250 ml. of 0.01 M copper(II) nitrate and the pH raised to 5.0 with dilute sodium hydroxide where the yellow-white precipitate formed on the addition of the L-cysteine hydrochloride dissolved. The pH was lowered to 3.0 with dilute, 1:3, nitric acid where a curdy, poorly filterable yellow-white precipitate formed. After washing twice by decantation, this ma-

terial was dried under reduced pressure over anhydrous calcium chloride. On exposure to air, the material rapidly turned nearly jet black.

This material was dissolved in dilute nitric acid where upon neutralization with dilute sodium hydroxide, the yellow-white precipitate again formed. After washing by decantation, filtering with suction and drying under reduced pressure over anhydrous magnesium perchlorate, exposure to air again caused an almost instantaneous transition to the black compound.

Harris (8) reported that an alkaline solution of white copper(I) cysteine darkens in air to give copper(II) cysteine however no solid compounds were prepared or analyzed. Albert (9) reported that in the presence of excess cysteine, copper-(II) yields a precipitate of copper(I) cysteine and cystine but again no solid compounds were isolated. Pirie (10) isolated copper(I) cysteine as a reddish-grey solid subject to air oxidation and darkening.

Attempts to prepare the copper(II) derivative of L-cysteine in an atmosphere of nitrogen failed to yield any homogenous product in the presence of excess copper(II) and yielded copper(II) cystine in the presence of excess cysteine followed by an excess of copper(II).

Copper(II) and bis-(S-L-cysteine)methane, (djenkolic acid)

Djenkolic acid was dissolved in dilute nitric acid, excess copper as the copper(II) nitrate was added and the pH raised to 4.5 with dilute filtered ammonium hydroxide. A light blue precipitate formed that was separated by filtration, redissolved in dilute nitric acid, filtered, reprecipitated with ammonium hydroxide, washed twice by decantation, filtered with suction, and dried under reduced pressure over anhydrous calcium chloride.

Copper(II) and S-ethyl-L-cysteine

An excess of copper(II) nitrate was added to a dilute nitric acid solution of S-ethyl-L-cysteine, the pH raised to 4.5 with dilute filtered ammonium hydroxide, the resulting light blue precipitate washed twice with water by decantation, filtered with suction, and dried under reduced pressure over anhydrous calcium chloride.

Copper(II) and α-aminoacetic acid, (glycine)

The method described by Sen *et al.* (11) was used. Copper(II) sulfate, 0.05 mole, was dissolved in 200 ml. of cold water. Sodium hydroxide, 0.41 mole in 20 ml. of water, was added. The fresh copper(II) hydroxide was filtered and washed free of excess alkali. It was then dissolved in a warm solution containing 3 g. of glycine, 0.04 mole, in 150 ml. of

water. The solution was warmed to 60° for 30 minutes. The cooled solution yielded bright blue needles which were recrystallized twice from water, separated by filtration with suction, and dried under reduced pressure over anhydrous magnesium perchlorate.

Copper(II) and L-cystine

L-cystine, 0.01 mole, was mixed with 0.011 mole copper(II) sulfate in 250 ml. water. Dilute nitric acid was added to a pH of 1 without solution of the cystine. The pH was raised to 4.5 with dilute filtered ammonium hydroxide and maintained there during the formation of the copper(II) complex. After the pH remained steady, the solution containing the velvety blue precipitate was stirred overnight, washed twice by decantation, filtered with suction, and dried under reduced pressure over anhydrous magnesium perchlorate.

Analysis of Copper Derivatives

As the central studies revolved around S-(1,2-trans-dichlorovinyl)-L-cysteine, a large amount of this material and its copper(II) complex was made. The copper in this complex and that formed with L-cystine was determined by electrodeposition after wet ashing with nitric acid. As only relatively small amounts of the other copper(II) compounds were available, copper was determined in them by the colorimetric method. The complexes were wet ashed with nitric acid, diluted to a

suitable volume, aliquots taken and the color developed with cuproine (2,2'-biquinoline) and extracted into isoamyl alcohol in the usual manner (12). Absorbance was read on a Beckman model "DU" spectrophotometer at a wavelength of 446 mμ and compared to a standard curve prepared in the same manner from a standard copper solution.

The analytical results obtained by this colorimetric method were substantiated by the Pregl microanalytical procedure for the determination of metals in organic compounds (13). Milligram samples of the complexes were decomposed with nitric and sulfuric acids in platinum boats in a Pregl combustion furnace and ignited to constant weight as the copper(II) oxide.

A summary of the analytical results is reported in Table 1.

Table 1. Summary of analytical results (Y = 1 organic residue)

Organic compound	Copper complex	Copper Theor. percent	Copper Exp. percent
S-(1,2-trans-dichloro-vinyl)-L-cysteine	CuY_2	12.87	12.85
S-(1,2-trans-dichloro-vinyl)-β-mercapto-propionic acid	CuY_2	13.71	13.77
1,2-bis-(S-L-cysteine)-ethane	$CuY.\frac{1}{2}H_2O$	18.76	18.81
	$CuY.2\frac{1}{2}H_2O$	16.95	17.09
1,2-bis-(S-L-cysteine)-ethylene	CuY	19.38	19.33

Table 1. (Continued)

Organic compound	Copper complex	Copper Theor.percent	Copper Exp.percent
S-cyclopentane-L-cysteine	CuY_2	14.30	14.21
L-cysteine	$CuY.H_2O$	31.51	31.30
Bis-(S-L-cysteine)methane (djenkolic acid)	CuY	20.12	19.93
S-ethyl-L-cysteine	CuY_2	17.68	17.65
α-aminoacetic acid (glycine)	$CuY_2.H_2O$	27.67	27.76
L-cystine	$CuY.\frac{1}{2}H_2O$	20.45	20.49

THE WATER SOLUBILITY OF THE COPPER(II) DERIVATIVE OF S-(1,2-trans-DICHLOROVINYL)-L-CYSTEINE AT VARIOUS pH VALUES

In view of the apparent low water solubility of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine it was decided to determine its water solubility at various acidities and in turn relate this solubility to the solubility product constant for the material.

Experimental Conditions

Buffer solutions of potassium acid phthalate with hydrochloric acid and sodium hydroxide were first used to cover the pH range 3 to 6. Tris-(hydroxymethyl)aminomethane with hydrochloric acid and sodium hydroxide was used to cover the pH range 6 to 9. However, it was found that both the phthalate and the tris-(hydroxymethyl)aminomethane systems complexed copper and thus the measured solubility was greater than would have been found had no complexing with the buffer system occurred. Insufficient data was available in the literature to adjust for these particular systems.

A sodium acetate buffer system was also used for the pH range 3 to 6 and a maleic acid system for the pH range 5.6 to 7.0. The complexing that occurs between copper(II) and both of these buffer systems could be taken into account in the calculations.

All solutions were maintained at an ionic strength of 0.05 and the temperature at which the solubilities were determined was 25 $\pm$ 0.05°. The solutions of the buffer and the solid copper(II) derivative were submerged in the constant temperature bath and vigorously stirred with a magnetic stirrer; five hours were necessary to assure equilibrium.

After the five hour equilibrating period, the solutions were filtered using suction through fine porous bottomed filtering crucibles and appropriate aliquots of the filtrate taken for subsequent analysis of the copper by the cuproine colorimetric method previously described (12). It was found that it was not necessary to wet ash the solutions prior to analysis. The copper found was taken as an initial measurement of the solubility. The values for the solubility as determined from the analysis for copper in the filtrates are given in Table 2.

Table 2. Solubilities of Copper(II) Bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate as determined by copper analysis without corrections for pH or buffer complexing

pH	Buffer system	Solubility (g/l x 10^2)
3.04	phthalate[a]	41.7
3.55	"	26.6
3.99	"	19.4
4.46	"	13.5
4.93	"	10.9
5.47	"	5.11

[a]Solubility values not used in subsequent calculations.

Table 2. (Continued)

pH	Buffer system	Solubility (g/l x 10^2)
3.28	acetate	24.1
4.10	"	9.24
5.00	"	3.57
6.00	"	1.16
6.33	"	0.847
5.60	maleate	2.93
6.00	"	2.06
6.43	"	1.35
7.06	"	0.715
6.00	THMAM[a,b]	1.30
6.51	"	1.47
6.93	"	1.87
7.43	"	2.40
7.93	"	4.43
8.39	"	8.89
8.85	"	18.8
9.15	"	37.6
5.75	water only	0.524

[b]Tris-(hydroxymethyl)aminomethane.

DERIVATION OF THE SOLUBILITY PRODUCT CONSTANT

The general equilibrium for the dissolving of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine is:

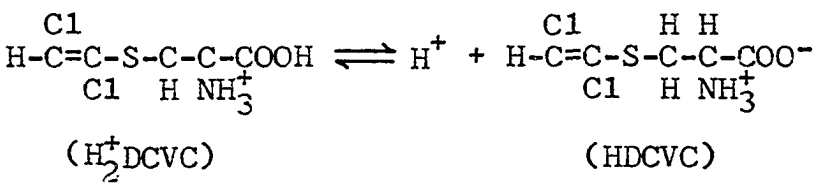
$$Cu(DCVC)_2 \rightleftharpoons Cu^{++} + 2DCVC^-,$$

where "$DCVC^-$" is the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion. As this is an anion of a weak acid, the solubility equilibrium would be affected by the pH of the solution, being displaced to the right as the pH was decreased and to the left as the pH was increased. As the solubility was determined at varying pH values, in order to achieve the true concentration of "$DCVC^-$" for use in the solubility product expression:

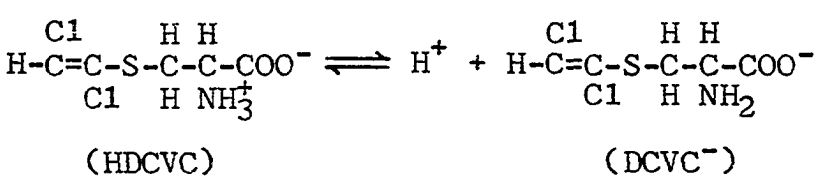
$$K_{sp} = [Cu^{++}][DCVC^-]^2,$$

account must be taken of the pH.

S-(1,2-trans-dichlorovinyl)-L-cysteine ionizes in the following fashion:



(H_2^+DCVC) (HDCVC)



(HDCVC) ($DCVC^-$)

The acid dissociation constants for the two reactions are:

$$K_1 = \frac{(H^+)(HDCVC)}{(H_2^+DCVC)} = 3.63 \times 10^{-3}; \quad pK_1 = 2.44$$

$$K_2 = \frac{(H^+)(DCVC^-)}{(HDCVC)} = 8.91 \times 10^{-9}; \quad pK_2 = 8.05,$$

where materials in parentheses are to be considered molar concentrations.

The copper that was found by the analytical scheme to determine the apparent solubility was also used to determine the total amount of S-(1,2-trans-dichlorovinyl)-L-cysteinate anion of all species by the relationship:

$$2(Cu^{++}) = (DCVC^-) = (\text{total of all species}).$$

For use in the solubility product expression, the actual concentration of S-(1,2-trans-dichlorovinyl)-L-cysteinate anion in the form "$DCVC^-$" was calculated as follows:

$$\text{Let: } \beta_1 = \frac{(\text{species desired})}{(\text{total of all species})} = \frac{(DCVC^-)}{(H_2^+DCVC)+(HDCVC)+(DCVC^-)}.$$

By use of the equations describing the acid dissociation of the material,

$$\beta_1 = \frac{K_1K_2}{(H^+)^2+K_1(H^+)+K_1K_2},$$

or on rearrangement for convenience in calculation:

$$\frac{1}{\beta_1} = \frac{(H^+)^2}{K_1K_2} + \frac{(H^+)}{K_2} + 1 .$$

Then:

$$(DCVC^-) = \beta_1 \text{ (total of all species)} = 2\beta_1(Cu^{++}).$$

When this value is substituted into the solubility product expression, it becomes:

$$K_{sp} = (Cu^{++}) [2\beta_1(Cu^{++})]^2 = 4\beta_1^2(Cu^{++})^3 .$$

The solubility product constants calculated, taking into consideration the affect of pH in the manner just derived, are tabulated in Table 3. Only solubilities determined in the acetate and maleate buffer systems were used for these and subsequent calculations.

The constantly increasing values as calculated in the above manner cannot be called solubility product constants in the true sense for to be constants, they must be invariant. It was found that all of the buffer systems used contained anions which complexed copper(II) to varying extents and as they were weak acid buffers, they would consequently have varying complexing ability dependent on pH. Insufficient values were available in the literature for the formation constants of the copper(II) phthalate system and none were available for the copper(II) tris-(hydroxymethyl)amino-

Table 3. Solubility product constants for Copper(II) Bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate corrected for pH

pH	Buffer system	K_{sp}	pK_{sp}
3.28	acetate	1.02×10^{-19}	18.99
4.10	acetate	3.16×10^{-19}	18.50
5.00	acetate	1.20×10^{-18}	17.92
6.00	acetate	4.07×10^{-18}	17.39
6.33	acetate	4.49×10^{-18}	17.35
5.60	maleate	1.04×10^{-17}	16.98
6.00	maleate	2.27×10^{-17}	16.64
6.43	maleate	4.46×10^{-17}	16.35
7.06	maleate	1.05×10^{-16}	15.98

methane system. Adequate values were available for the copper(II) acetate system and the copper(II) maleate system and the effect of this complexing tendency of the copper towards the buffer system anion and thus on the resulting solubility of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine was investigated.

In each case the tendency would be for the buffer system anion to complex the copper and thus shift the solubility reaction equilibrium to the right. In addition, as the pH of the solubility system was raised, the relative concentration

of the buffer system anion would also increase providing greater complexing capacity. This effect correlated well with the drift in values calculated for the solubility product constants reported in Table 3.

Corrections for Buffer System Anion Effect

In determining the solubility product constant for the copper(II) S-(1,2-trans-dichlorovinyl)-L-cysteinate system, it was necessary to have available for use in the mathematical expression describing the solubility product constant the concentration of the anion in the "DCVC$^-$" form and the concentration of free copper(II) arising only from the dissolution of the copper(II)-S-(1,2-trans-dichlorovinyl)-L-cysteine derivative. The concentration of "DCVC$^-$" has been calculated as a function of pH which is the only factor involved other than copper(II) ion. To obtain the free copper(II) ion concentration, it was necessary to take into consideration the complexing effect of the buffer system.

With the copper(II) acetate system, the following equilibria were considered:

$$CuOAc^+ \rightleftharpoons Cu^{++} + OAc^- \qquad K_1 = \frac{(Cu^{++})(OAc^-)}{(CuOAc^+)}$$

$$CuOAc_2 \rightleftharpoons CuOAc^+ + OAc^- \qquad K_2 = \frac{(CuOAc^+)(OAc^-)}{(CuOAc_2)}$$

$$CuOAc_3^- \rightleftharpoons CuOAc_2 + OAc^- \qquad K_3 = \frac{(CuOAc_2)(OAc^-)}{(CuOAc_3^-)}$$

$$CuOAc_4^{--} \rightleftharpoons CuOAc_3^- + OAc^- \qquad K_4 = \frac{(CuOAc_3^-)(OAc^-)}{(CuOAc_4^{--})}$$

where "OAc^-" is the acetate ion.

With the copper(II) maleate system the following equilibrium was considered:

$$CuMaleate \rightleftharpoons Cu^{++} + Maleate^{--} \qquad K = \frac{(Cu^{++})(Maleate^{--})}{(CuMaleate)} .$$

Ploquin (14) substantiates the use of only a 1:1 complex.

The dissociation constants used for these systems are indicated in Table 4. The value reported for the copper(II) maleate system was adjusted for activities in the manner reported by the authors.

Table 4. Dissociation constants for copper(II) acetate and copper(II) maleate

System	log K_1	log K_2	log K_3	log K_4	Ref.
Copper(II) acetate	-1.67	-0.98	-0.42	+0.19	15
	-1.62	-0.98			15
	-1.65	-1.0	-0.36		16
values used	-1.65	-0.99	-0.39	+0.19	
Copper(II) maleate	-2.90				17

The effect of pH on the concentration of free buffer anion was taken into account in a manner similar to that used for the S-(1,2-trans-dichlorovinyl)-L-cysteine and is expressed as follows:

$$\frac{1}{\beta_2} = \frac{(H^+)}{K} + 1 \text{ for the acetate buffer system and}$$

$$\frac{1}{\beta_3} = \frac{(H^+)^2}{K_1K_2} + \frac{(H^+)}{K_2} + 1 \text{ for the maleate buffer}$$

system, where the K's represent the respective acid dissociation constants for the buffer acid.

From the values of β_2 and β_3 it was possible to calculate the concentration of acetate ion and maleate ion, respectively, at the pH values of the corresponding solubility determinations, by the following relationship:

$$\text{(free anion)} = \beta\text{(total anion concentration)}.$$

The value used for the acid dissociation constant for acetic acid was 1.75×10^{-5} or $10^{-4.76}$ (7). The two acid dissociation constants for maleic acid vary with their source. Topp and Davies (18) give the pK values of 1.92 and 6.22, respectively, for the first and second acid dissociation constants. Other reported values are indicated in Table 5.

The values 1.92 and 6.22 were used in this work as well as 1.90 and 6.37 which are the average of all reported values.

Table 5. Acid dissociation constants for maleic acid

pK_1	pK_2	Reference
1.92	6.22	18
1.93	6.41	19
1.90	6.50	20
2.0	6.26	7
1.82	6.58	21

The concentration of free copper(II) ion was calculated from the values for the concentration of the free buffer anion and the respective dissociation constants of the copper(II) complexes with the buffer anion in a manner similar to that described for the copper(II)-S-(1,2-trans-dichlorovinyl)-L-cysteine system.

$$\text{Let } \beta_4 = \frac{\text{concentration of free copper}}{\text{concentration of copper in all forms}} .$$

For the acetate system:

$$\beta_4 = \frac{(Cu^{++})}{(Cu^{++})+(CuOAc^{+})+(CuOAc_2)+(CuOAc_3^{-})+(CuOAc_4^{--})}$$

and

$$\frac{1}{\beta_4} = \frac{(OAc^{-})^4}{K_1K_2K_3K_4} + \frac{(OAc^{-})^3}{K_1K_2K_3} + \frac{(OAc^{-})^2}{K_1K_2} + \frac{(OAc^{-})}{K_1} + 1 .$$

For the Maleate system:

$$\beta_4 = \frac{(Cu^{++})}{(Cu^{++}) + (CuMaleate)}$$

and

$$\frac{1}{\beta_4} = \frac{(Maleate^{--})}{K_1} + 1 .$$

For each system the concentration of free copper was calculated using the relationship:

$$(\text{free } Cu^{++}) = \beta_4(Cu^{++} \text{ in all forms}),$$

and this value was then used to calculate the concentration of the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion, both then being used to calculate the solubility product constant for copper(II) bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate. The calculated values for the solubility product constant are tabulated in Table 6.

The final expression for the solubility product constant taking into account pH effects and buffer system anion complexing effects becomes:

$$K_{sp} = 4\beta_1^2\beta_4^3(Cu^{++})^3 ,$$

where: (Cu^{++}) is the copper found by analysis,

β_1 is the correction for pH dependency of the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion, and

β_4 is the correction for the complexing of the copper by the anion of the respective buffer system used in the solubility determination.

Table 6. Solubility product constants for Copper(II) Bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate corrected for pH and buffer system anion complexing

pH	Buffer system	K_{sp}	pK_{sp}
3.28	Acetate	9.23×10^{-20}	19.03
4.10	"	1.40×10^{-19}	18.85
5.00	"	7.10×10^{-20}	19.15
6.00	"	5.69×10^{-20}	19.24
6.33	"	8.54×10^{-20}	19.07
	Average	8.91×10^{-20}	19.05
5.60	Maleate[a]	1.23×10^{-19}	18.91
6.00	"	6.94×10^{-20}	19.16
6.43	"	4.59×10^{-20}	19.34
7.06	"	5.07×10^{-20}	19.29
	Average	7.22×10^{-20}	19.14
5.60	Maleate[b]	2.41×10^{-19}	18.62
5.91	"	4.28×10^{-20}	19.37
6.00	"	1.24×10^{-19}	18.91
6.43	"	6.44×10^{-20}	19.19
7.06	"	5.41×10^{-20}	19.27
	Average	1.05×10^{-19}	18.98
5.76	Water[c]	1.24×10^{-19}	18.91

[a]Acid dissociation constant values used were 1.92 and 6.22.

[b]Acid dissociation constant values used were 1.90 and 6.39.

[c]Unbuffered; initial pH after boiling to expel carbon dioxide and cooling was 5.90, final value after equilibration was 5.62. Corrected for pH effect only.

COPPER(II) BIS-S-(1,2-trans-DICHLOROVINYL)-L-CYSTEINATE AND BOVINE BLOOD SERUM

Solubility in Bovine Blood Serum

In view of the low solubility of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine in water at a pH approaching that of the biological system, the possibility arose that the toxic affect of the S-(1,2-trans-dichlorovinyl)-L-cysteine might be due to a physical separation of the copper(II) from the normal physiological system as the insoluble copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine. In order to investigate this possibility, the solubility of the copper(II) derivative in bovine blood serum was determined.

Experimental procedure

Bovine blood serum was equilibrated with excess solid copper(II) bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate at 25° in a constant temperature bath with magnetic stirring for five hours in a manner duplicating the procedure used in determining its water solubility. The pH of the blood serum before equilibration was 7.9; after, it was 8.0.

After equilibration, the serum was filtered through a coarse and then medium porous bottomed filtering crucible. Considerable difficulty was experienced in this operation as

the serum tended to clog the porous plate. As it was the intent to follow the exact procedure utilized in the water solubility investigations, a centrifugation procedure was not used. A 50.0 ml. aliquot of the serum was wet ashed with 60 ml. of nitric acid and 20 ml. of perchloric acid and evaporated at fumes of perchloric acid to a volume of 5 ml. to reduce the amount of relatively insoluble ammonium perchlorate formed on neutralization. This solution was diluted to 50.0 ml. in a volumetric flask and suitable aliquots taken for copper analysis by the colorimetric cuproine method previously described (12). A parallel blank determination was made on the blood serum as well as on the reagents.

Results and conclusions

The solubility of copper(II) bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate in bovine blood serum was found to average 4.2 mg. of copper per 100 ml. of blood serum as compared to a normal copper level found of 70 μg. per 100 ml. This was equivalent to 32.6mg. of copper(II) bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate per 100 ml. of blood serum. The solubility of this material in water with adjustments to the same pH value as that of the blood serum was 0.026 mg. per 100 ml. of water. The results reported are the average of five determinations on three different samples of blood serum. Reproducible results were difficult to obtain primarily because of

the difficulties in filtering mentioned before and to natural differences in the blood serum samples themselves.

The increased solubility in blood serum as compared to comparable water solubility was thought to occur by one or more of the following possible schemes:

1. Formation of a soluble complex between copper(II) and serum buffers, proteins, or free amino acids.
2. Formation of a soluble complex between copper(II)-S-(1,2-trans-dichlorovinyl)-L-cysteine and serum protein.
3. Formation of a complex between S-(1,2-trans-dichlorovinyl)-L-cysteine and protein with copper free or more likely complexed with serum buffers or free amino acids in a soluble form.

Thus this system was subjected to further investigation using dialysis techniques.

Dialysis Investigations

In order to elucidate the scheme by which the solubility of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine was increased and acquire some insight into the possible situation of this L-cysteine derivative in the blood serum, dialysis techniques were employed. Blood serum, blood serum saturated with the copper(II) derivative, blood serum

containing copper(II) nitrate at the same copper level as that obtained by solution of the copper(II) derivative in the serum, and blood serum containing S-(1,2-trans-dichlorovinyl)-L-cysteine alone at an equivalent level were dialyzed against water and the serum samples before and after dialysis were analyzed for copper and the dialyzates were analyzed for copper and S-(1,2-trans-dichlorovinyl)-L-cysteine.

Experimental procedure

Bovine blood serum was equilibrated with excess solid copper(II) bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate with magnetic stirring at 25° in a constant temperature bath. Two 25.0 ml. samples of the filtered equilibrated serum, one 25.0 ml. sample of blood serum containing 3.1 mg. of copper added as the copper (II) nitrate, one 25.0 ml. sample of blood serum containing 5.1 mg. of S-(1,2-trans-dichlorovinyl)-L-cysteine and two 25.0 ml. samples of blood serum were each dialyzed against 250 ml. of deionized water. All samples were dialyzed for 24 hours at 25° with mechanical stirring provided by a synchronous clock motor rotating at 60 revolutions per minute driving a plastic paddle immersed in the serum samples.

After dialysis each sample was analyzed for copper by the colorimetric cuproine method after wet ashing with nitric and perchloric acids. Wet ashing the entire serum sample and

diluting to an appropriate volume accounted for any volume changes occurring on dialysis. The dialyzates were diluted to 250.0 ml. and aliquots evaporated to near dryness under reduced pressure over anhydrous calcium chloride and chromatographed along with pure S-(1,2-trans-dichlorovinyl)-L-cysteine with 70:30 n-propanol:water in the manner previously described for the S-substituted derivatives of L-cysteine. The remainder of the dialyzates were wet ashed with nitric and perchloric acids and analyzed for copper in the usual colorimetric cuproine method.

Results and conclusions

The results of the copper analyses showed that no copper dialyzed through the membrane from samples of unadulterated blood serum or samples that contained only S-(1,2-trans-dichlorovinyl)-L-cysteine. In those samples containing either the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine or copper(II) nitrate, less than 1 percent of the total copper dialyzed through the membrane. In addition, paper chromatograms showed that appreciable and essentially equivalent amounts of the S-(1,2-trans-dichlorovinyl)-L-cysteine dialyzed through the membrane whether it was introduced into the blood serum by the copper(II) derivative or alone. No quantitative estimate of the amount that dialyzed was determined. By comparison to known amounts of S-(1,2-

trans-dichlorovinyl)-L-cysteine chromatographed, more than just trace amounts dialyzed.

Although Albert (9) reports that glycine, with a formation constant of about $10^{15.5}$ (22, 23) can compete for copper against six times its weight of serum albumin, the level of other buffering materials, proteins, and free amino acids in the blood serum was sufficient to overcome the stronger complexing ability of the S-(1,2-trans-dichlorovinyl)-L-cysteine and strip the copper(II) away from the amino acid derivative to form a soluble but only slightly dialyzable copper complex system. Similarly, a relatively small amount of S-(1,2-trans-dichlorovinyl)-L-cysteine was present in this system in comparison to the glycine level described and thus cannot be correlated with Albert's work.

It was apparent that the copper was complexed with the native serum protein materials rather than dialyzable inorganic buffers and free amino acids, and that the S-(1,2-trans-dichlorovinyl)-L-cysteine was relatively uncomplexed under the laboratory conditions used and was thus capable of being dialyzed.

THE S-(1,2-trans-DICHLOROVINYL)-L-CYSTEINE ELECTRODE

The greater than thousand-fold increase in the solubility of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine in bovine blood serum as compared to that in water at the same pH, coupled with the results of the dialysis investigations, indicated that it would be desirable to contrive a method of determining the concentration of the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion in the biological system in such a manner that any sensitive equilibria involved would not be upset by the measuring scheme. Elemental or functional group analyses would fail due to obvious interferences from similar components present naturally in the blood serum system as well as the disrupting affect such methods would have on the equilibria. Similarly, chromatographic separations could not be relied upon to yield valid results.

An electrode system, sensitive to the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion concentration similar to that used for chloride ion (24) or oxalate or calcium ions (25) was considered the best possible approach and would least disturb the biological equilibria involved. Such an electrode would consist of a copper wire coated with a layer of the copper(II) complex of S-(1,2-trans-dichlorovinyl)-L-cysteine. Then, relying on the low solubility of this complex, the potential measured would be a function of the anionic concentra-

tion of the copper(II) complex much in the same fashion as with the Ag:AgCl electrode system (24).

Experimental Procedures

The following methods were used in the attempts to prepare such an electrode. In all cases the anode of the electrodeposition system was an 18 gauge copper wire pretreated in various ways except in one case where a platinum electrode was used; a platinum gauze served as the cathode. The electrolyte consisted of a 0.01 M solution of S-(1,2-trans-dichlorovinyl)-L-cysteine adjusted to pH 7 with sodium hydroxide where the solubility of the copper(II) complex was the lowest and the anionic concentration of the amino acid derivative was the greatest. In each method used, another electrolyte was utilized containing sodium perchlorate to reduce the internal resistance of the electrolyte.

With the copper wire as the anode, the general sequence of events would involve the slow dissolution of the copper wire at the surface followed by the simultaneous combination with the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion to form a layer of insoluble copper(II) complex on the surface of the anode. The anode was slowly rotated at about 60 revolutions per minute. Current was supplied by 6-8 volt lead storage batteries through suitable resistances.

Method 1

The copper anode was cleaned in 1:1 nitric acid and the electrolysis carried out at 4 and 10 milliampere for 1 hour and for 5 hours.

Method 2

An 18 gauge platinum wire was copper plated by electrodeposition and used as the anode under the conditions of Method 1.

Method 3

The copper wire anode was copper plated prior to use as the anode under the conditions of Method 1.

Method 4

The copper wire anode was annealed at 700° for 1 hour prior to use in the electrolysis to remove any strains developed during its manufacture by extrusion. After annealing, it was cleaned with 1:1 nitric acid and used as the anode under the conditions of Methods 1 and 3 above.

All of the resulting deposits had good characteristic color but a definite tendency for localized buildup on the anode wire. The deposit would start to form at one or two spots on the anode and enlarge from there. Regardless of the

time allowed for deposition or the current used, bare areas of the copper anode remained between areas deposited to a thickness of 1 to 2 mm. These thick deposits, after drying in air, were easily removed with a camel's-hair brush leaving an uneven deposit.

In an effort to inhibit this localized buildup an interrupter in the form of a cam-activated microswitch was included in the plating circuit. The circuit was interrupted at rates of 90 to 160 times per minute as the previously described methods were again applied. No improvement in the character of the deposit was obtained.

A further attempt was made to improve the character of the deposit by the use of a reverse plating system. The applied potentials were adjusted such that the copper wire served as the anode for time periods varying from 7 to 30 seconds at 5 milliampere while the platinum gauze became the anode for periods of time from 1 to 5 seconds at currents of up to 40 milliampere. The net result with each attempt was a deposition of the copper(II) complex on the copper wire anode. This system was applied in the previously described methods with and without the interrupting mechanism in the plating circuit without any improvement in the character of the deposit. All efforts to deposit an even solid coating of the copper(II) complex of S-(1,2-trans-dichlorovinyl)-L-cysteine failed, bare

copper wire being readily evident in all cases and a loose deposit formed during extended depositions that could be readily brushed off with a camel's-hair brush.

INFRARED STUDIES OF THE S-SUBSTITUTED DERIVATIVES OF L-CYSTEINE AND THEIR COPPER(II) COMPLEXES

Amino acids, their salts and complexes exhibit characteristic infrared absorptions in the general regions 2.85 to 3.5 μ, 4.25 to 5.0 μ and 5.7 to 6.7 μ. The general assignments are as follows: 2.85 to 3.9 μ for the NH, OH, CH and SH stretching vibrations, 4.25 to 5.0 μ generally present but incompletely assigned absorptions usually attributed to NH vibrations, 5.7 to 6.7 μ for the C-O stretching vibration, CO_2^- antisymmetric vibrations, NH_3^+ symmetric and antisymmetric and NH_2 bending vibrations.

Glycine, as the zwitterion and the copper(II) complex, previously reported by Sen *et al.* (11), was used as a starting point for the systematic study of the infrared spectra of the compounds prepared in this work in an attempt to confirm the points of attachment of the copper(II) ion to the organic molecule. Fuson *et al.* (26) and Bellamy (27) report that the presence of a sulfur atom in the amino acids has no apparent affect on the spectra in the 3 to 7 μ region.

All spectra were made on the Perkin-Elmer Model 21 Double Beam Infrared Spectrophotometer using sodium chloride optics and potassium bromide discs. This method yielded a spectrum for the copper(II) glycine complex that was identical to that reported by Sen using the same scheme. The spectrum for

S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid was made in a nujol mull.

The assignments reported for the absorptions noted in the infrared spectra of the amino acids, their salts, complexes and derivatives are cataloged below generally according to wavelength region and including the experimental results obtained with those compounds investigated in this work. The infrared spectra obtained are reported in Figures 5 through 26.

The 3 μ Region

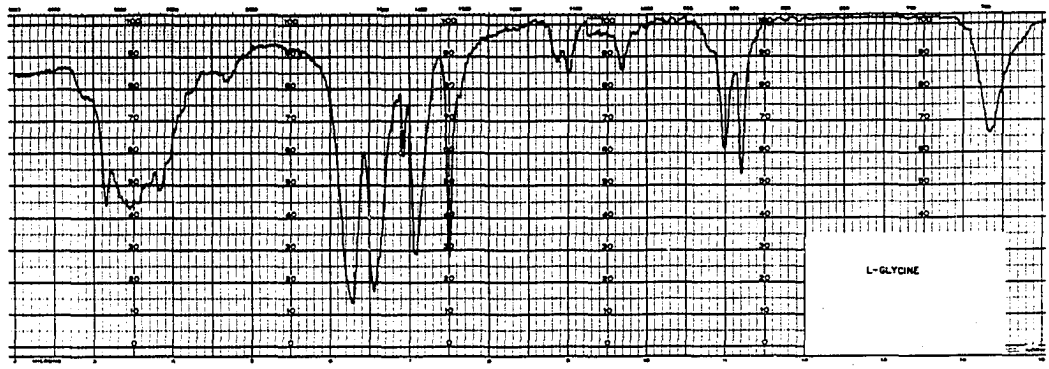
The 3.1 to 3.9 μ region of the amino acid infrared absorption spectra contains the NH, CH, OH, and where present, the SH stretching vibrations. No normal NH stretching vibrations are found in the usual 2.85 to 3.03 μ region according to Bellamy (27) due to intermolecular hydrogen bonding. Svatos et al. (28) reports that this 3.1 to 3.9 μ region is irregular, broadly blurred and poorly resolved, especially in the solid state, for the same reason. Such intermolecular bonding would exist through the nitrogen of one molecule being bonded to the nitrogen or carboxyl oxygen of another molecule through a hydrogen bridge; similarly for the oxygen on the carboxyl group possibly being hydrogen bonded to a third molecule. With salt formation the normal 2.85 to 3.03 μ absorptions return due to the presence of the NH_2 group unless chelation occurs (27). On chelation, these absorptions due to NH stretching vibrations are better defined

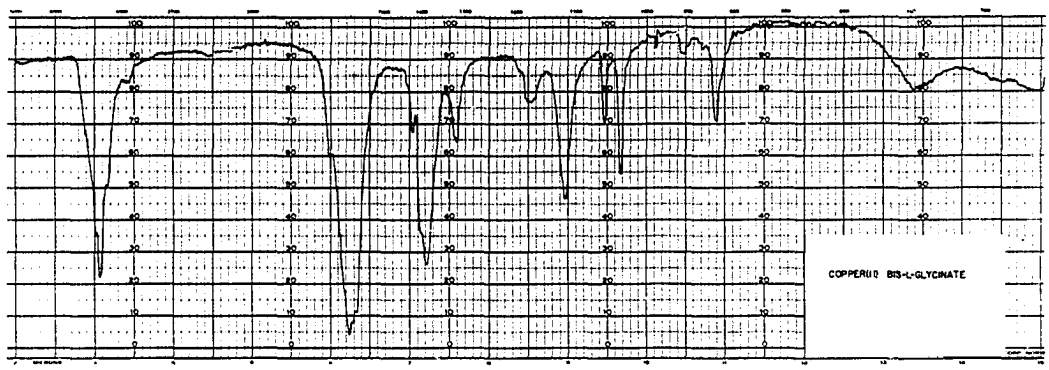
Figure 5. Infrared spectrum of glycine

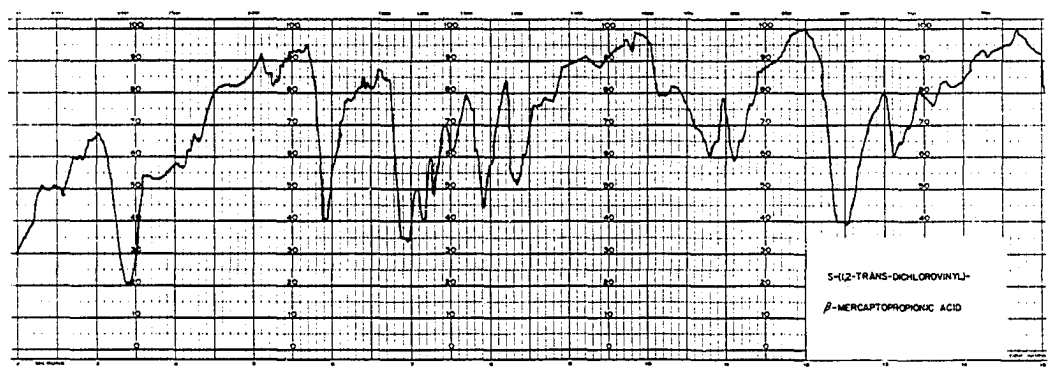
Figure 6. Infrared spectrum of copper(II) bis-glycinate

Figure 7. Infrared spectrum of S-(1,2-trans-dichlorovinyl)-β mercaptopropionic acid

Figure 8. Infrared spectrum of copper(II) bis-S-(1,2-trans-dichlorovinyl)-β-mercaptopropionate


L-GLYCINE


COPPER(II) BIS-L-GLYCINATE


S-(1,2-TRANS-DICHLOROVINYL)-
β-MERCAPTOPROPIONIC ACID

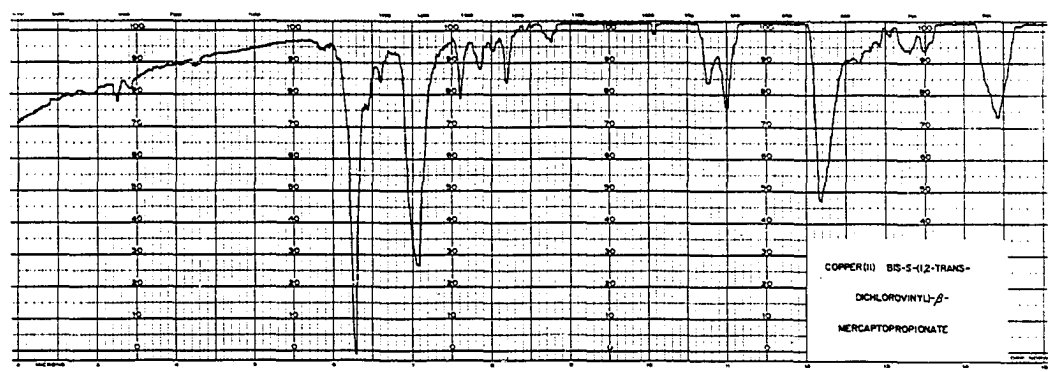
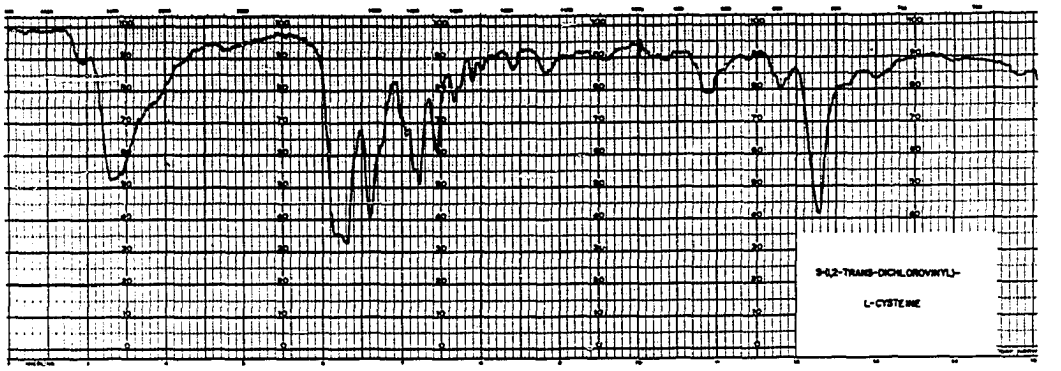

COPPER(II) BIS-S-(1,2-TRANS-
DICHLOROVINYL)-β-
MERCAPTOPROPIONATE

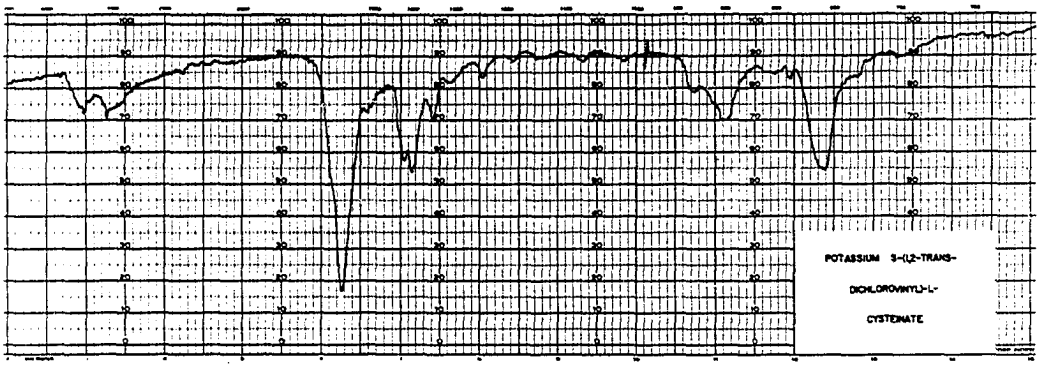
Figure 9. Infrared spectrum of S-(1,2-trans-dichlorovinyl)-L-cysteine

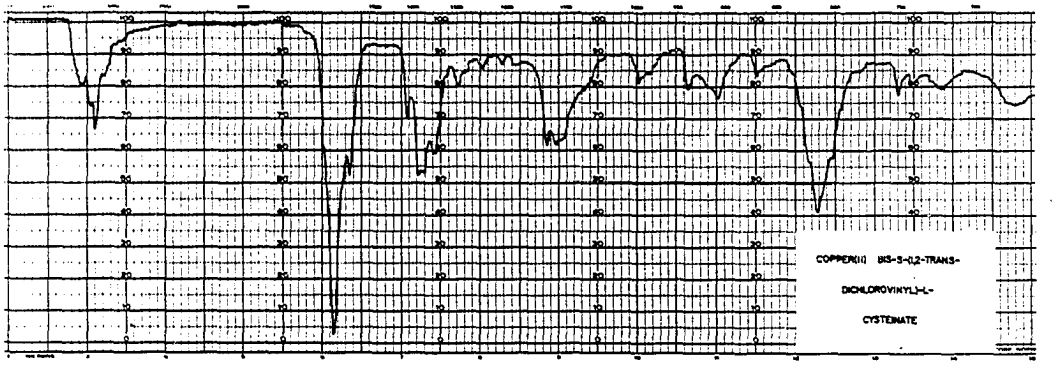
Figure 10. Infrared spectrum of potassium S-(1,2-trans-dichlorovinyl)-L-cysteinate

Figure 11. Infrared spectrum of copper(II) bis-S-(1,2-trans-dichlorovinyl)-L-cysteinate

Figure 12. Infrared spectrum of L-cysteine


S-(1,2-TRANS-DICHLOROVINYL)-
L-CYSTEINE


POTASSIUM S-(1,2-TRANS-
DICHLOROVINYL)-L-
CYSTEINATE


COPPER(II) BIS-S-(1,2-TRANS-
DICHLOROVINYL)-L-
CYSTEINATE

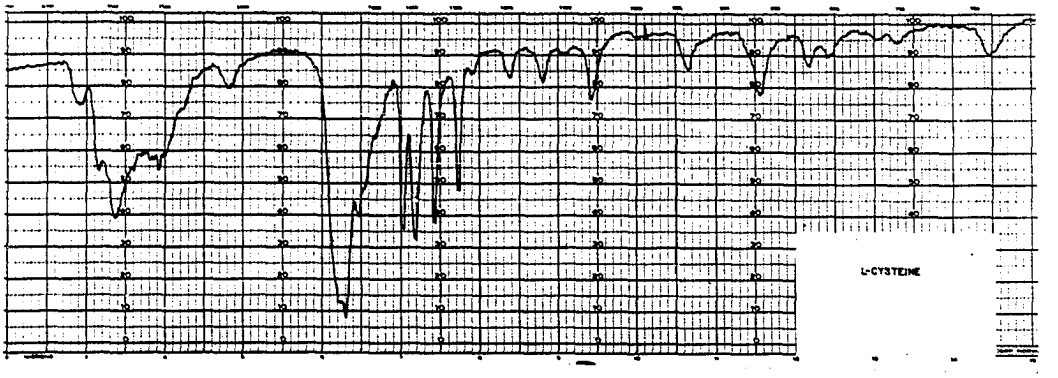
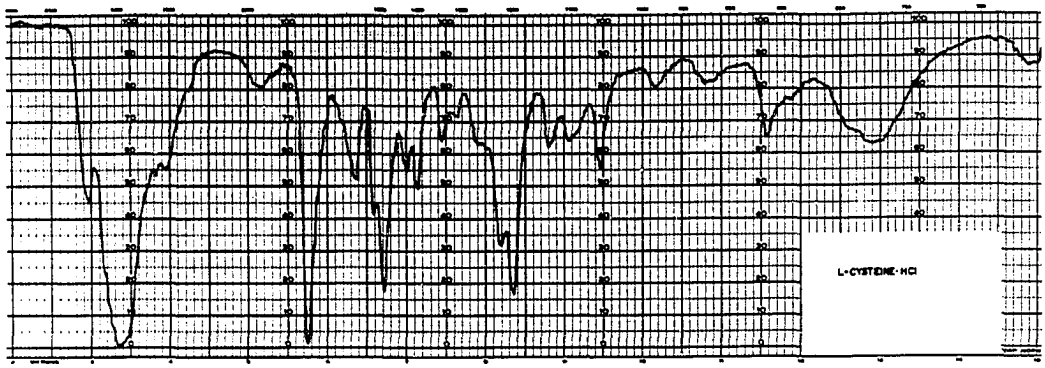

L-CYSTEINE

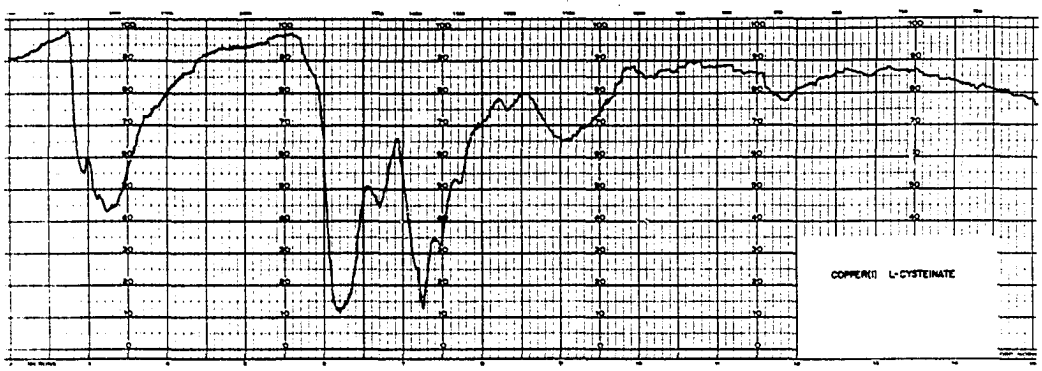
Figure 13. Infrared spectrum of L-cysteine hydrochloride

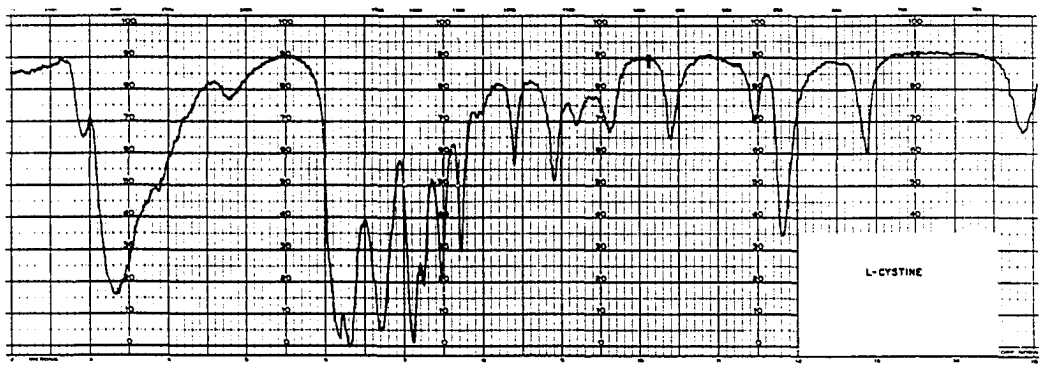
Figure 14. Infrared spectrum of copper(I) cysteinate

Figure 15. Infrared spectrum of L-cystine

Figure 16. Infrared spectrum of copper(II) L-cystinate


L-CYSTEINE·HCl


COPPER(I) L-CYSTEINATE


L-CYSTINE

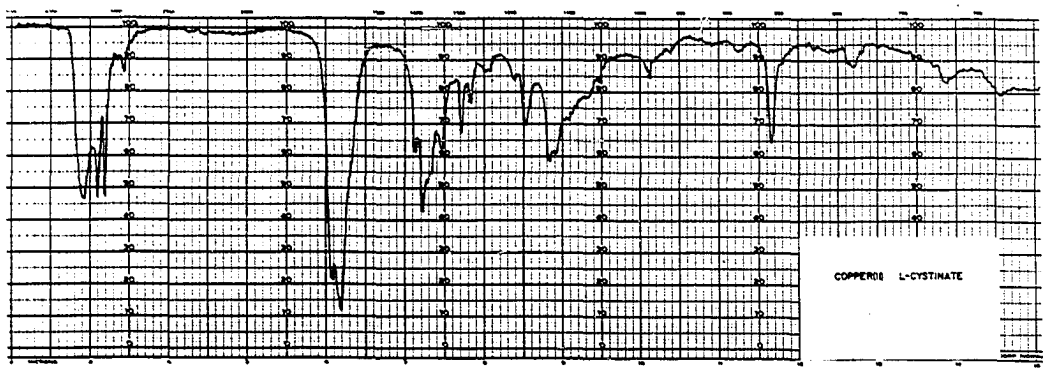
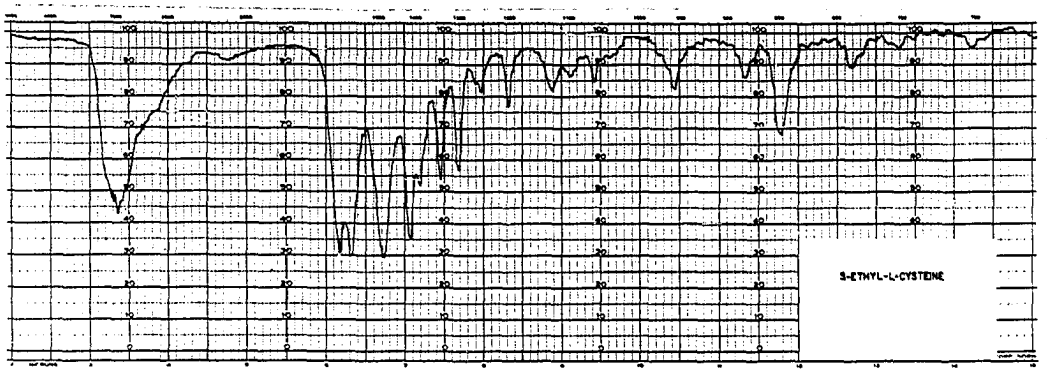

COPPER(II) L-CYSTINATE

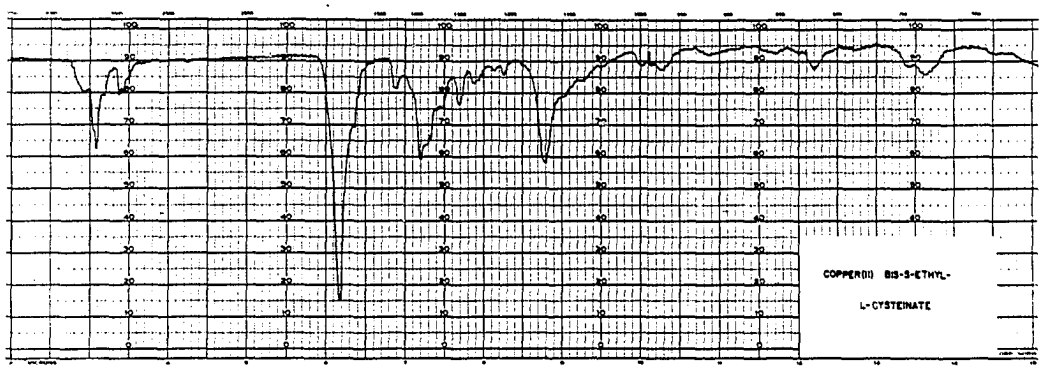
Figure 17. Infrared spectrum of S-ethyl-L-cysteine

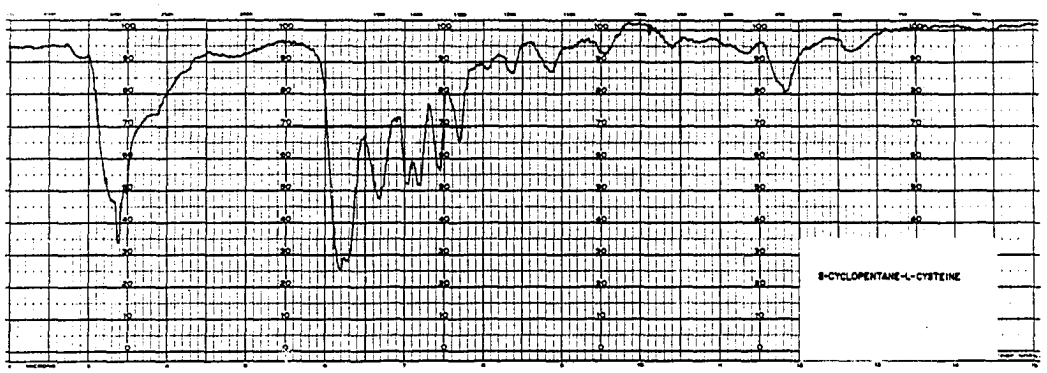
Figure 18. Infrared spectrum of copper(II) bis-S-ethyl-L-cysteinate

Figure 19. Infrared spectrum of S-cyclopentane-L-cysteine

Figure 20. Infrared spectrum of copper(II) bis-S-cyclopentane-L-cysteinate


S-ETHYL-L-CYSTEINE


COPPER(II) BIS-S-ETHYL-
L-CYSTEINATE


S-CYCLOPENTANE-L-CYSTEINE

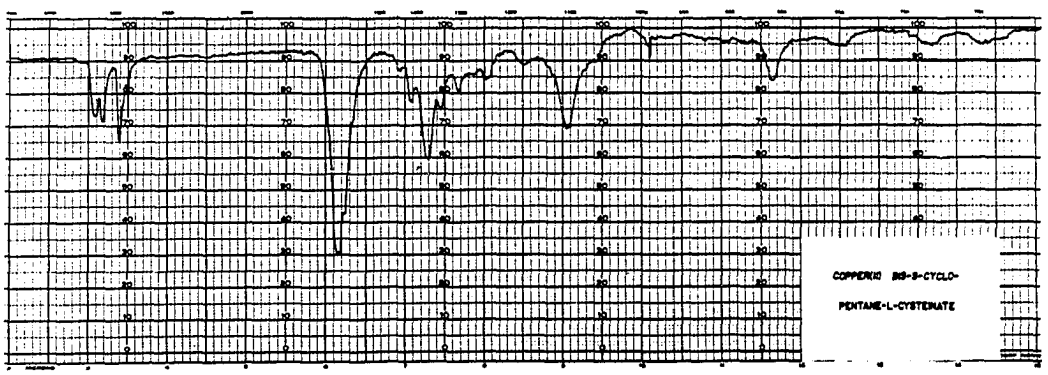
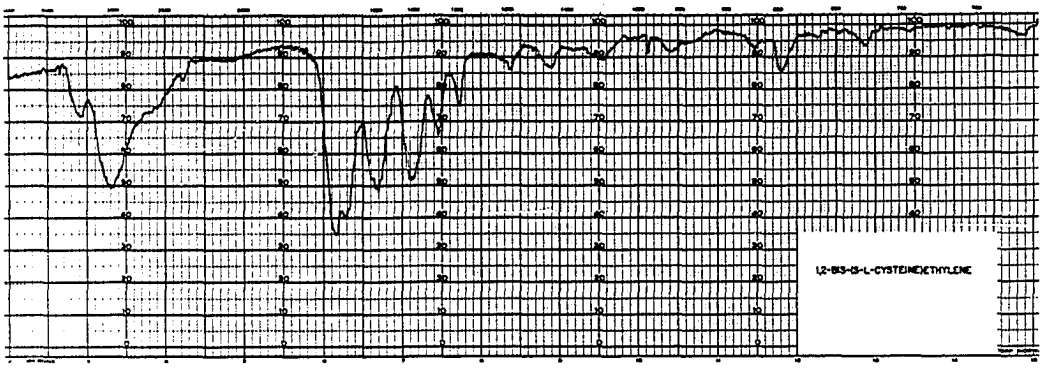

COPPER(II) BIS-S-CYCLO-
PENTANE-L-CYSTEINATE

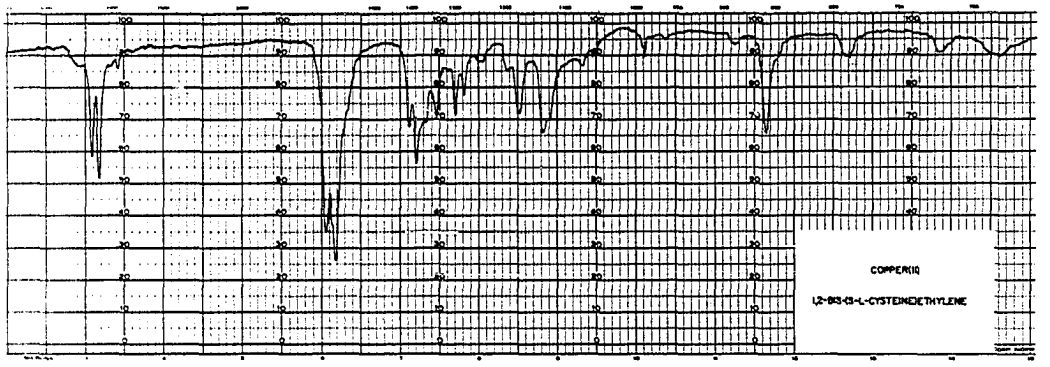
Figure 21. Infrared spectrum of 1,2-bis-(S-L-cysteine)-ethylene

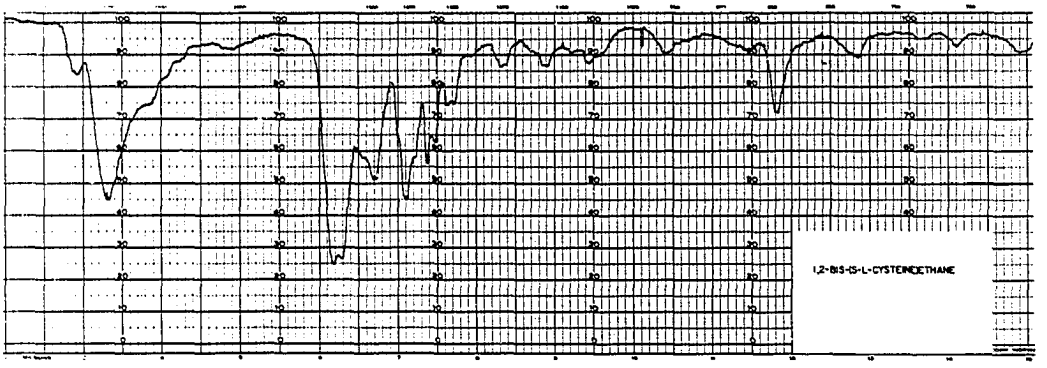
Figure 22. Infrared spectrum of copper(II) 1,2-bis-(S-L-cysteine) ethylene

Figure 23. Infrared spectrum of 1,2-bis-(S-L-cysteine)ethane

Figure 24. Infrared spectrum of copper(II) 1,2-bis-(S-L-cysteine)ethane


1,2-BIS-(S-L-CYSTEINE)ETHYLENE


COPPER(II)
1,2-BIS-(S-L-CYSTEINE)ETHYLENE


1,2-BIS-(S-L-CYSTEINE)ETHANE

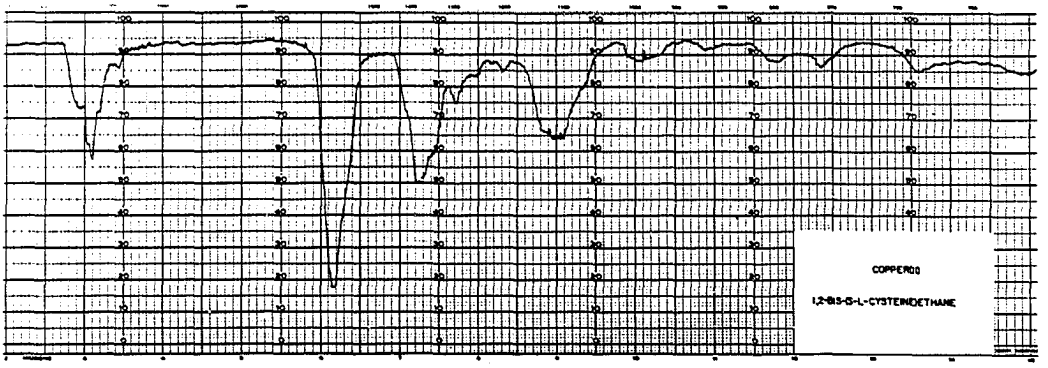
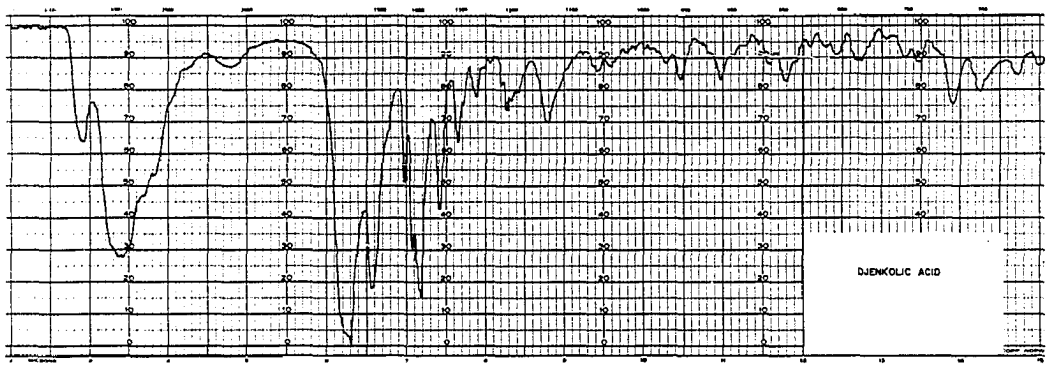
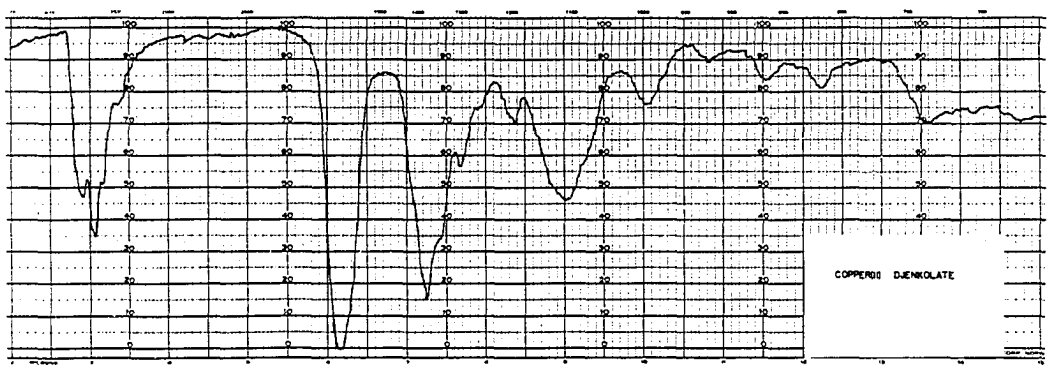

COPPER(II)
1,2-BIS-(S-L-CYSTEINE)ETHANE

Figure 25. Infrared spectrum of djenkolic acid

Figure 26. Infrared spectrum of copper(II) djenkolate


DJENKOLIC ACID


COPPER(II) DJENKOLATE

and simultaneously intensified. In addition, the NH stretching vibration frequency at about 3.0 μ occurring in the metal salts of amino acids splits into two or more absorptions at higher wavelengths on the formation of metal complexes through the amino nitrogens (29). Potassium glycinate has two absorptions at 2.96 and 2.98 μ of almost equal intensities whereas the copper(II) complex shows three well defined absorptions at 3.0, 3.07 and 3.17 μ, all of which are more intense and thus confirm the presence of a nitrogen to metal covalent bond (28). The glycine zwitterion itself absorbs only at 3.16 μ.

In the infrared spectra obtained for the compounds and their copper(II) complexes investigated in this work there were no absorptions of medium intensity in the 2.85 to 3.03 μ region. Some very weak absorptions were noted but they were consistently weak and unvarying on complex formation. In all of the amino acid compounds investigated there was a distinct muddling of the 3.1 to 3.5 μ region in the free acid. This area became distinctly better defined and better resolved upon formation of the copper(II) complex with two or three distinct bands emerging from the previously confused area. The distinct absorptions arising on complex formation were within the 3.0 to 3.2 μ region and are indicative of complex formation between copper(II) and the nitrogen of the amino acid derivatives. These characteristics concur well with those reported by Svatos *et al.* (28). Similarly these absorptions are increased in intensity with re-

spect to the CH absorptions at about 3.4 μ. The potassium salt of S-(1,2-trans-dichlorovinyl)-L-cysteine shows one absorption at 2.98 μ and the copper(II) complex of this same compound exhibits three absorptions at 2.96, 3.03 and 3.10 μ. The zwitterion form has a weak absorption at 3.17 μ and a strong absorption at 3.29 μ. Thus the transition of absorbancies noted in this series, which parallels that found in the present work and reported for the glycine series (28) also indicates that a covalent bond exists between the copper and the nitrogen of the amino acid derivative.

The SH stretching vibration found to occur only in L-cysteine at 3.92 μ disappeared on formation of the copper(I) derivative indicating attachment of the copper(I) through the sulfhydryl sulfur. This site concurs with that reported by Pirie (10).

The 4 μ Region

In the 4.25 to 5.0 μ region there exist two absorptions, one at about 4.25 μ which is unassigned. In the compounds examined in this work, a very weak absorption was found at about 4.25 μ in all of the compounds occurring as a shoulder on the broad and poorly defined absorption in the 3 μ region of the free acids. It remained weak but was better defined in the copper(II) complexes.

The second absorption was at about 4.7 μ and is assigned

the NH stretching vibration of the NH_3^+ group by Koegel et al. (30). This absorption is absent in the NH_2^+ type amino acids as reported by Randall et al. (31). With the compounds examined in this study this weak absorption existed in all of the compounds except 1,2-bis-(S-L-cysteine)ethylene. Where present in the free acid, this band disappeared in all cases upon formation of a metal salt or complex which, significantly, has not been reported. The disappearance of this absorption would follow from the loss of the hydrogen ion of the primary amino group to form the NH_2 group in the presence of the metal ion.

The 5 to 7 μ Region

The region 5.7 to 6.7 μ shows three principal absorptions for all amino acids. The hydrohalides show a strong absorption in the 5.7 to 5.8 μ region assigned to the carbonyl stretching vibration of the unionized carboxyl group (27, 31). This appears at a somewhat lower wavelength than that usually assigned to carboxylic acids which usually absorb in the 5.7 to 5.9 μ region, but this shift is associated with the influence of the amino group. The spectra obtained in this work for L-cysteine hydrochloride showed a very strong absorption at 5.75 μ. The spectra for the S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid showed a strong absorption at 5.90 μ which was assigned to the acid carbonyl vibration and was at a higher wavelength than for the corresponding amino acids but at the proper wavelength for normal carboxylic acids.

The hydrohalides have a second weak absorption at about 6.1 to 6.2 μ assigned to the NH_3^+ symmetric vibration (27). In this same range Fuson et al. (26) and Randall et al. (31) assign the strong absorption found to the NH_3^+ bending vibration. This absorption is sometimes called the Amino Acid I absorption. In the L-cysteine hydrochloride examined, a strong absorption at 6.36 μ was found and was assigned to the NH_3^+ vibration. Randall et al. (31) reports the carbonyl and NH_3^+ vibration absorptions as being at 5.75 and 6.35 μ, respectively, agreeing with those values found in this work.

A third absorption was reported in the region 6.45 to 6.72 μ and is variously assigned as the NH deformation vibration (30), the NH_3^+ antisymmetrical vibration (27), or simply the unassigned Amino Acid II absorption (26) of amino acid hydrohalides (31). Experimentally obtained absorptions were found for L-cysteine hydrochloride, the only hydrohalide examined, at 6.58 and 6.71 μ which are assigned as the Amino Acid II absorption in view of the inconclusiveness of the literature assignments.

The amino acid zwitterion itself is characterized by the absence of the 5.7 to 5.8 μ absorption, being shifted instead to the region 6.25 to 6.45 μ and assigned as the antisymmetrical ionized carboxyl vibration (27, 30, 31, 32). The range for the amino acid zwitterion as reported by Randall et al. (31) is

from 6.1 to 6.4 μ and for this same carboxylate vibration of the metallic salts, 6.2 to 6.35 μ. These absorptions are often unresolved from the NH_3^+ or NH_2 absorptions thus contributing to the broadness of this absorption. Randall *et al.* (31) also reports that in all cases the absorption due to the NH_3^+ is weaker than that due to the CO_2^- and is variable in intensity.

For the compounds examined in the work a generally broad and rather poorly resolved absorption band was found in this region. The L-cysteine zwitterion showed absorptions at 6.23 and 6.31 μ assigned to the NH_3^+ and CO_2^- vibrations, respectively. On formation of the copper(II) salt of S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid, the carbonyl band at 5.90 μ disappears and the strong carboxylate ion band appears at 6.29 μ indicating that normal ionic salt formation has taken place. In all the other compounds investigated the CO_2^- vibration is preserved in the region 6.15 to 6.36 μ with no appreciable shift on formation of the copper(II) complex agreeing with the glycine series reported by Sen *et al.* (11). Thus the bonding between copper(II) and the amino acid derivative is ionic in nature at the carboxylate site, for coordinate covalent bonding would have resulted in the disappearance of this band and more dramatically the appearance of the carbonyl band in the 5.7 to 5.8 μ region which did not occur. The NH_3^+ vibration and the NH_2 vibrations were found in the free acid or zwitterion form of the compounds investigated and contributed to the general broad-

ness of the absorptions within this region. On the formation of the copper(II) complexes, the NH_3^+ absorption at about 6.1 to 6.2 μ generally disappeared. This disappearance was difficult to confirm due to the prevailing broadness of the absorptions present. The NH_2 bending vibration was retained, strengthened and shifted to about 6.3 μ concurring with Penland et al. (32).

The absorption at about 6.45 to 6.72 μ variously assigned was found in all compounds containing the amino group and similarly disappeared on formation of the salt or metal complex. Thus the assignment given this absorption by Bellamy (27) as being due to the NH_3^+ antisymmetrical vibration seems correct. It should not, however, be assigned as the amino acid hydrochloride band as has been reported (31).

Duval and Lecomte (33) report a second weak absorption in the copper(II) complex of glycine at 6.13 μ which they assign to the cyclic structure between the amino nitrogen and the carbonyl oxygen of a single amino acid molecule. A weak absorption at this wavelength was found in the spectra of this same compound during the present work.

Beyond the 7 μ Region

Beyond 7 μ the assignments previously reported are scattered principally due to the presence in this region of the skeletal vibrations which vary unpredictably from compound to

compound and the infrared spectra reflect this. Duval and Lecomte (33) assign an absorption by glycine at 7.1 to 7.15 μ to the CO_2^- group as it disappears in the hydrochloride. They similarly assign an absorption at 7.35 μ in glycine and 7.32 μ in the copper(II) complex to the CO_2^- symmetrical vibration. Fuson et al. (26) assign an absorption at 7.15 μ to the CO_2^- group in the S-substituted derivatives of cysteine. Koegel et al. (30) make a similar assignment at about 7.1 μ for the fifty amino acids they studied. All are reported as strong, sharp absorptions. Part of this region is included in the 6.85 to 7.25 μ region for absorption due to CH_2 bending and deformation vibrations reported by Randall et al. (31) and Sen et al. (11).

In the compounds examined in this work an absorption of medium to strong intensity at about 7.1 to 7.2 μ appeared in all of the compounds and can be assigned to the CO_2^- group. However, a band at 7.15 μ was found for the spectra of L-cysteine hydrochloride and is also reported by Randall et al. (31).

Bellamy (27) reports a further absorption of medium intensity in the region 7.3 to 7.7 μ which he assigns to the NH_3^+ symmetrical deformation vibration which is retained in derivatives. No indication of the types nor locations of these derivatives is made. From the spectra obtained in this work this assignment seems questionable as this band has been obtained consistently between 7.4 and 7.7 μ and remains, although some-

what weaker, in the copper(II) derivatives. However, the CS stretching vibration also occurs in this region and may account for some of the absorptions noted in this region.

The unassigned absorption at about 8.4 μ reported by Fuson et al. (26) for all of the S-substituted amino acids examined was also found in all of the amino acid compounds examined in this work with the exception of glycine. The absorption of medium intensity found in the spectrum of the copper(II) complex of glycine is probably due to a shifted nitrogen vibration as the NH_3^+ bend or rock is in this region, 8.0 to 9.1 μ, and Duval and Lecomte (33) assign an absorption at about 9.0 μ in the copper(II) complex of glycine to CH_2 or NH_2 bending vibrations. An absorption at 8.96 μ was found in the spectra of the copper(II) complex of glycine examined.

The absorption at about 8.2 μ reported by Bellamy (27) as being attributable to the unionized COOH group and as either the C-O stretching or C-OH bending vibration, was not found in any of the compounds investigated. However, an absorption at about 8.3 μ was found for the S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid and shifted to 8.18 μ as a strong band in the copper(II) salt. Thus a more proper assignment might be to the unionized salt rather than to the unionized acid with the actual assignment being the C-O stretching vibration.

The assignment of the absorption at 9.0 μ to the CH_2 or

NH_2 bending vibration in the copper(II) complex of glycine is felt to be solely due to the CH_2 bending, for all of the compounds examined showed a weak absorption in the range 8.8 to 9.0 μ that was not significantly changed on formation of the copper(II) complex.

A similar assignment is made in the 10 to 11.1 μ region for the NH_3^+ bend or rock by Bellamy (27). Duval and Lecomte (33) assign an absorption in the range 10.5 to 12.5 μ but most commonly around 11 μ to the CO_2^- deformation vibration which is found in both glycine and its copper(II) complex. These bands were found at varying wavelengths in the compounds examined in this work but with the C-Cl rocking and stretching vibrations, CO_2^- deformation vibrations, and the NH_3^+ bending or rocking vibrations reported in this region, no firm assignment to the absorptions found experimentally can be made. The bands found were present but somewhat diminished on formation of the copper(II) complexes but no correlations within the series of S-substituted L-cysteine derivatives and their copper(II) complexes could be made. Also in this region are the skeletal vibrations that make assignments difficult.

The absorptions due to the C-S or C-S-C stretching in the 14.4 to 15.4 μ region are reported by Bellamy (27) as being weak and normally well masked by skeletal vibrations and of little value for identification purposes. The CH_3S absorbs at

about 14.2 to 14.6 μ, the RCH_2S at about 15.15 to 15.5 μ and the RR'CHS at greater than 15.5 μ (27).

Svatos et al. (28) reports that the N-metal stretching frequency of copper(II) salicylaldimine lies at about 450 cm^{-1} (22.2 μ) and the O-metal stretching frequency lies at about 365 cm^{-1} (27.4 μ) for the same compound. Both of these lie in the cesium bromide region and were beyond the range of the instruments available for this study. It may be presumed from this report and atomic weight considerations that the N- and O-metal stretching frequencies for the compounds of interest in this present work would lie in approximately the same region.

THE PROPOSED STRUCTURES FOR THE COPPER(II) COMPLEXES OF THE S-SUBSTITUTED L-CYSTEINE DERIVATIVES

The selectivity for copper(II) exhibited by S-(1,2-trans-dichlorovinyl)-L-cysteine and its related S-substituted L-cysteine derivatives was of particular interest in view of past work by other investigators of metal-cysteine complexes. In cysteine itself there are three possible bonding sites - the sulfur by ionic bonding with the removal of the hydrogen or by covalent bonding without the removal of the hydrogen but more likely the former, the amino group with covalent bonding, and the carboxylate group with ionic bonding. No differences exist in the cases of the S-substituted derivatives of cysteine other than the removal of the possibility of ionic bonding through the sulfur.

Copper(II) is almost exclusively tetracoordinate being capable of accommodating a combination of ionic and covalent bonds to satisfy its normal coordination number of four. Formation of hexaco-ordinate compounds with copper(II) is relatively rare and the compounds usually unstable. Thus in both cysteine and the S-substituted derivatives three bonding sites per molecule are available and with the formation of two to one complexes between the amino acids and copper, six bonding sites are available for one copper(II) ion. Which sites are actually involved has been the subject of considerable work.

Schubert (34) has reported that cysteine complexes with cobalt(II) through the carboxylate group and the sulfur with the amino group free, justified by comparative analysis with similar organic materials. The ultraviolet spectra of the cobalt(II)-thioglycolic acid and the cobalt(II)-cysteine complexes are very similar and thus the bonding, which in the thioglycolic acid is definitely through the carboxylate and the sulfur, must be similar. The cobalt(II)-cysteine complex easily forms crystalline hydrochlorides and picrates through the amino group thus indicating its freedom in the cobalt(II)-cysteine complex. With cobalt(II)-alanine, two complexes are found - one in strong base with bonding through the carboxylate and the amino group, the other in acid solution with bonding through the carboxylate only. This is accompanied by a distinct color change from blue in basic solution, due to the coordinated amino group, to a much lighter color in acid solution where the amino group is protonated and thus not involved in complex formation. In the cobalt(II)-cysteine complex, the same color is noted in both basic and acid solution up to 6 M. The same ultraviolet spectra was found throughout this pH range; thus the amino group was considered to be free in this complex.

Such a thesis was substantiated by the early work of Michaelis and Barron (35). In their work they studied cobalt(II), cobalt(III), iron(II), iron(III) and nickel(II), all of which gave distinct color reactions with cysteine which were

functions of the oxidation state of the metal involved as well as of the cysteine-cystine system. They stated "whereas cysteine esterified at the -SH group shows no color reaction with any metal salt at all" indicating that bonding in such cases occurs through the sulfur when it is free or protonated but when deactivated by the presence of an organic derivative on the sulfur, no such reaction can occur. They failed, unfortunately, to investigate copper(II) with the esterified compounds.

Harris (8) reported that cysteine formed colored complexes with iron(III), manganese(III), copper(II), copper(I), cobalt-(II), nickel(II), tin(II), tin(IV), mercury(II) and bismuth-(III).

Contrary to these theses stating that cysteine bonds through the sulfur and the carboxylate group, Albert (9) reported that with cysteine hydrochloride, three hydrogens are lost during the formation of complexes with copper(II), nickel-(II), and cobalt(II) with the resulting bonds to the cysteine being through the amino group and the sulfur, the carboxylate group being free and ionized. This they concluded from the observation that the titration curve of the amino acid with sodium hydroxide in the presence or absence of metal ion was invariant over that portion attributed to the carboxylate group being titrated.

White et al. (36) reported that soluble complexes of cysteine with nickel(II) are formed through the amino group and the sulfur. In the case of copper(II), a soluble 1:1 complex was formed with oxidized glutathione with the bonding through the two amino and two carboxylate groups. As their work showed a 1:1 complex also being formed with cystine (oxidized cysteine), they postulated that this was also a bonding through the amino and carboxylate groups with the sulfurs not involved in the bonding.

Li and Manning (37) substantiate the bonding of cysteine to metals through the sulfur and amino group for zinc(II) by comparison of formation constants of zinc(II)-cysteine (pK = 18.70) and zinc(II)-mercaptoacetic acid (pK = 14.41, bonding through the S^- and the CO_2^-) and zinc(II)-mercaptoethylamine (pK = 18.74, bonding through the S^- and the NH_2). A better comparison, taking into account the chelate ring size, would have been β-mercaptopropionic acid rather than mercaptoacetic acid. Six-membered rings are considered more stable thus enhancing the value of the formation constant to where it might have approached that of the zinc(II)-cysteine system.

They similarly showed by comparison of the formation constants for zinc(II)-glycine (pK = 9.94) and zinc(II)-methionine (pK = 8.42) that the bonding is through the amino and carboxylate groups only, with the sulfur not involved.

These theses concerning the location of the bonding in metal-cysteine complexes were confirmed by the present investigation. In the S-(1,2-trans-dichlorovinyl)-L-cysteine, the sulfur was blocked by the dichlorovinyl group and only the carboxylate and amino groups were available for complex formation. Thus the lack of any detectable complex formation for the metal ions investigated, other than copper(II), is substantiated. When this sulfur group is free or protonated, it may well be the site of bonding rather than the amino group.

An examination of the work reported by Benesch and Benesch (38) regarding the acid strength of the -SH group in cysteine and related compounds shows that there is little difference between the acid strengths of the -SH group and the protonated amino group. The pK of the -SH group in conjunction with the NH_3^+ group is 8.53 while the pK of the NH_3^+ group with the -SH group present is 8.86. The pK of the -SH group with the NH_2 group present is 10.03 and the pK of the NH_3^+ group with the S^- ion present is 10.36. Thus from an acid-base consideration, there is little distinction between the two groups with the -SH group considered the more acidic but by only 0.33 pK units and other considerations determine the site of bonding when both sites, the -SH and the amino group, are available.

A general statement can be made that if the -SH, the amino group and the carboxylate group are all available for bonding,

bonding in such amino acids will involve the sulfur, the amino group and then the carboxylate group in that order. The absence of any one of these will lead to utilization of the other two as necessary to satisfy the coordination number of the concerned metal ion. The absence of a free sulfur, however, may well lead to the formation of no complex at all or at best a very weak complex depending on the metal ion involved and the size and character of the S-substituted group.

To further substantiate the utilization of the amino group in the copper(II) complex of S-(1,2-trans-dichlorovinyl)-L-cysteine and the other related S-substituted L-cysteine compounds, the homolog of S-(1,2-trans-dichlorovinyl)-L-cysteine without the amino group was prepared. The copper(II) derivative of this, S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid, was a bright green in contrast to the velvety blue of the copper(II) complex of the amino acid derivative. This color was attributed to the amino group as the complex was isolated in the anhydrous form. That S-(1,2-trans-dichlorovinyl)-L-cysteine was incapable of forming the hydrochloride was shown by McKinney et al. (1), and precluded the possibility of following up Schubert's work with the preparation of the hydrochloride of the copper(II) complex.

Molecular models were constructed to aid in justifying the proposed structure of these complexes involving ionic bonding

to the carboxylate group and covalent bonding to the nitrogen. With two molecules of S-(1,2-trans-dichlorovinyl)-L-cysteine coordinated in this manner in a planar configuration about a central copper(II) ion, the sulfur atoms were located above and below the metal ion but not in a vertical line to the plane of the coordinated atoms. With the central metal ion bonded ionically to the carboxylate and covalently to the sulfur atoms in a planar configuration, the nitrogen atoms were well removed from the central metal ion. As they are required to justify the colors of the complexes and are shown to be involved by the results of the infrared studies, this structure seemed unlikely. If covalent bonding was permitted in a planar configuration involving the nitrogen atoms and the sulfur atoms, the carboxylates were not involved and structurally, at least, this was a possible form. However, in view of the above discussion, this seemed to be unlikely.

That the nitrogen atoms are involved by covalent bonding was confirmed by the colors and the interpretations of the infrared spectra. That the sulfur atoms were not involved was determined from the above discussion of the reactivities of S-substituted cysteine compounds. Thus the carboxylate must be involved both to satisfy the electrical charge of the resulting complex as well as the coordination number of the copper(II) ion. This bond was ionic in nature in view of the charge con-

siderations and the conclusions derived from the infrared spectra. Real confirmation of this required extension of the infrared investigations into the cesium bromide region to elucidate the metal-oxygen, metal-nitrogen and metal-sulfur vibrations which was beyond the capabilities of the instruments available for this study.

SUMMARY

S-(1,2-trans-dichlorovinyl)-L-cysteine has been reported as the possible toxic factor in trichloroethylene-extracted soybean oil meal (1) and the cause of fatal aplastic anemia in calves (3). The present work was undertaken to study further some of the chemical characteristics of this compound.

Investigations into the reactivity of S-(1,2-trans-dichlorovinyl)-L-cysteine towards the metal ions normally found in physiological systems showed a reaction only with copper(II). The method used to detect any compound formation involved titrating dilute solutions of S-(1,2-trans-dichlorovinyl)-L-cysteine with an isohydric solution of the metal ion. A resulting change in pH of greater than 0.3 pH units was considered indicative of compound formation. The reverse titration was also employed, the pH range studied in both cases being 3 to 5. The methods of Bjerrum (5) and Calvin and Melchior (6) were also used in which titrations with dilute standard alkali are made of a metal ion solution and of the organic reagent singly and together in the presence of a known and constant amount of dilute acid. The pH range studied by this method was 2 to 9. Any horizontal displacement of the titration curve of the metal ion plus amino acid due to the displacement of a proton from the amino acid is taken as indication of the formation of a complex. The application of these methods indicated no reactivity between S-(1,2-trans-dichlorovinyl)-L-cysteine and

copper(I), iron(II), iron(III), manganese(II), chromium(III), cobalt(II), nickel(II), molybdenum(III), tin(II), lead(II), calcium, magnesium, strontium, zinc, cadmium, or aluminum. Some reaction was noted with silver and with mercury(II) but was not investigated further as these cations are not of normal physiological interest. Thus the specificity of S-(1,2-trans-dichlorovinyl)-L-cysteine towards copper(II) was significant.

Copper(II) and S-(1,2-trans-dichlorovinyl)-L-cysteine unite in the ratio of one copper atom to two molecules of the amino acid. The compound formed is pale blue in color and extremely insoluble in water. The solubility of this compound was determined in acetate and maleate buffer systems from pH 3 to 7. Not only is the solubility dependent on pH, as would be expected for the insoluble metal derivative of a weak acid, but also on the buffer system employed owing to competitive complexing of the copper(II) by the anion of the buffer system. An expression for the solubility product constant was derived taking into account this pH and buffer system dependency. The final expression for the solubility product constant is:

$$K_{sp} = 4\beta_1^2\beta_4^3[Cu^{++}]^3,$$

where: $[Cu^{++}]$ is the total dissolved copper found by analysis,

β_1 is the correction for pH dependency of the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion, and

β_4 is the correction for the complexing of copper(II) by the anion of the respective buffer system used in the solubility determination also corrected for pH dependency.

The reported value for the solubility product constant is 9.70×10^{-20} at 25°.

The solubility of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine in bovine blood serum was determined to be 32.6 mg. per 100 ml. of serum compared to 0.026 mg. per 100 ml. of water corrected to the physiological pH and both at 25°. Thus the toxicity of this S-substituted L-cysteine derivative is not likely caused by a physical removal of the physiological copper as the insoluble copper(II)-S-(1,2-trans-dichlorovinyl)-L-cysteine compound.

Dialysis studies indicated that S-(1,2-trans-dichlorovinyl)-L-cysteine present in bovine blood serum was dialyzable against water whether introduced alone or as the copper(II) derivative. However, less than one percent of the total copper present in the serum, native copper plus copper introduced into the serum as the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine or as copper(II) nitrate at the same copper level, was dialyzable against water and in equivalent amounts. Thus the S-(1,2-trans-dichlorovinyl)-L-cysteine appeared not to be combined with copper(II) under the laboratory conditions imposed, the copper(II) being united preferentially with the

native serum proteins.

Unsuccessful attempts were made to prepare a copper-S-(1,2-trans-dichlorovinyl)-L-cysteine electrode by electrodeposition of the copper(II) derivative of S-(1,2-trans-dichlorovinyl)-L-cysteine on a copper wire. It was hoped that such an electrode might be responsive to the S-(1,2-trans-dichlorovinyl)-L-cysteinate anion.

In addition to S-(1,2-trans-dichlorovinyl)-L-cysteine, 1,2-bis-(S-L-cysteine)ethane and S-(1,2-trans-dichlorovinyl)-β-mercaptopropionic acid were prepared, the latter being the homolog of S-(1,2-trans-dichlorovinyl)-L-cysteine without the amino group. The copper(II) derivatives of these compounds and of 1,2-bis-(S-L-cysteine)ethylene, L-cystine, djenkolic acid, glycine, and S-cyclopentane-L-cysteine, as well as the copper(I) derivative of L-cysteine, were prepared and analyzed.

The infrared absorption spectra of these copper derivatives and of their parent organic acids were obtained and are reported. With glycine and the S-substituted L-cysteine derivatives, the intensification and shift to higher wavelengths of the NH stretching vibrations in the 3 μ region on formation of the copper(II) derivatives indicated compound formation by covalent bonding through the amino group and accounted for the light blue colors of the resulting copper(II) compounds. The

preservation of the carboxylate vibration absorption at about 6.2 μ without any significant shift on formation of the copper-(II) derivatives indicated ionic bonding of copper(II) to the carboxylate group. With L-cysteine, the only isolable copper compound was the black 1:1 copper(I) derivative with bonding taking place only through the sulfur atom as confirmed by the disappearance of the SH vibration absorption at 3.92 μ. The conclusions derived from the results of the infrared investigations were substantiated by the use of molecular models and correlations from reported work on the reactivities of other related compounds toward metal ions.

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"When all is done, the help of good
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Bacon

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