[Chem. Pharm. Bull. 18(2) 368—373 (1970)]

UDC 547.468-386 : 546.562-386

Studies on the Sulfur-containing Chelating Agents. XXV.[1] Chelate Formation of Penicillamine and Its Related Compounds with Copper (II)

Yukio Sugiura and Hisashi Tanaka

Faculty of Pharmaceutical Sciences, Kyoto University[2]

(Received October 6, 1969)

Occurance of concurrent redox and complexation reaction between copper (II) and penicillamine, known as an excellent therapeutic agent for Wilson's disease, was investigated by both spectrophotometric and potentiometric methods. Mixing of excess copper (II) with penicillamine was produced a red-violet colored complex. This complex are characterized by much more intense absorption than those customarily found in cupric or cuprous complexes, and the complex was presumed as a mixed valence complex. In the presence of excess penicillamine, an yellow copper (I) complex was formed. These complexes were isolated from aqueous solution. It was found that the compounds which have strong excretion activity of copper, such as penicillamine and β-methyl-β-ethylcysteine, form stable red-violet complexes, while the compounds which are not effective for the excretion of copper, such as N-acetylpenicillamine and cysteine, do not form stable red-violet complexes.

In the previous papers,[1] the mode of the coordination, namely which functional groups of penicillamine coordinate to metal ion, were discussed through the stability constants, ultraviolet-visible spectra and proton magnetic resonance spectra. Although penicillamine has been used as a drug for the oral treatment of Wilson's disease which is caused by the abnormal metabolism of copper,[3] little has been known about the reaction of penicillamine with copper (II) from the following reasons. In general, the interaction of the sulfhydryl group with oxidizing metal ions, such as copper (II) and iron (III) is very complicated on account of the redox reaction between the ligand and the metal ion. In addition, in penicillamine, various modes of the coordination should be considered through three functional groups, namely sulfhydryl, amino and carboxyl groups. As the chelating ability is considered to have close connection with the biological activity of penicillamine, the detailed study on the reaction of penicillamine with copper (II) has been attempted by both the spectrophotometric and potentiometric methods. Recently it was proved that penicillamine forms copper (I) complex in which the ratio of the ligand to the metal is 1 to 1, by the polarographic method.[4]

Experimental

Materials——DL-Penicillamine was purchased from the Sigma Company and its related compounds, such as N-acetylpenicillamine, penicillamine methyl ester and β-methyl-β-ethylcysteine were obtained according to the method described in the previous paper.[1] The cupric ion solution was prepared from reagent grade cupric chloride and was standardized with EDTA, and cuprous ion solution was prepared by the reduction of cupric chloride solution with hydroxylamine hydrochloride. The pH of the solution

1) Part XXIV: Y. Sugiura, A. Yokoyama and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), submitted.
2) Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) *a*) N.P. Goldstein, R.V. Randall, J.B. Grass, J.W. Rosevear and W.F. McGuckin, *Neurology* (Minniap.), **12**, 231 (1962); *b*) B.S. Hartley and J.M. Walshe, *Lancet*, **11**, 434 (1963); *c*) D.A. Adams, R. Glodman, H. Maxwell and H. Latta, *Am. J. Med.*, **36**, 330 (1964); *d*) I.H. Scheinberg, *J. Chron. Dis.*, **1964**, 233; *e*) J.M. Walshe, *Clin. Sci.*, **26**, 401 (1964).
4) J.J. Wallon and A. Badinand, *Anal. Chim. Acta*, **42**, 445 (1968).

was adjusted to 6.2 with phosphate buffer. Carbonate free sodium hydroxide solution was prepared by the procedure described in the previous paper.[1] All the other reagents used were commercially available reagent grade materials.

Color Reaction of Penicillamine with Cupric Ion and Cuprous Ion——Cupric and cuprous ion solutions were added to 1% aqueous solution of penicillamine under a nitrogen atmosphore and the color changes of the solutions were observed at pH 6.2.

Spectral Measurements——Deaerated solutions of cupric chloride and penicillamine obtained by bubbling with nitrogen gas were mixed, and the visible–ultraviolet spectra were measured by a Hitachi recording spectrophotometer model EPS–2. All absorbance measurements were made by a Shimadzu spectrophotometer model QV–50 at 22±0.5°. All reagents were dissolved in a phosphate buffer pH 6.2.

Potentiometric Titration——All titrations were carried out according to the procedure reported previously[1] at 22±0.5°.

Preparation of Copper Chelates——To an aqueous solution of cupric chloride (85 mg), penicillamine (100 mg) dissolved in buffer of pH 6.2 was added, and the mixture was concentrated *in vacuo* to yield violet precipitates. The precipitates were dissolved in a few drops of water, and acetone was added to yield violet crystals. This procedure was repeated twice to give the pure chelate. The product was filtered and washed with ethanol; yield 70 mg; decomp. p. 174—176°. *Anal.* Calcd. for $C_{10}H_{18}O_4N_2S_2Cu_32H_2O$: C, 23.05; H, 4.26; Cu, 36.09. Found: C, 23.49; H, 4.65; Cu, 36.58.

On evaporation of the filtrate crude colorless crystals were obtained. The product was recrystallized from water–ethanol; yield 35 mg; mp 205—206°. This product was found to be identical with penicillamine disulfide.[5]

To the solution of penicillamine (190 mg) dissolved in buffer of pH 6.2, aqueous solution of cupric chloride (20 mg) was added, with stirring. Pale yellow precipitate formed was allowed to stand for about an hour and then filtered and washed several times with distilled water–ethanol; yield 20 mg; decomp. p. 191—192°. *Anal.* Calcd. for $C_5H_{10}O_2NSCuH_2O$: C, 26.14; H, 5.26; Cu, 27.66. Found: C, 26.39; H, 5.36; Cu, 27.87.

Results and Discussion

The color reaction of penicillamine with cupric and cuprous ions are summarized in Table I.

Table I. Spot Test

Ligand	Metal ion	Color
SH[a]	Cu^{2+}	violet
SH	Cu^{+}	pale yellow
S–S[b]	Cu^{2+}	pale blue
S–S	Cu^{+}	colorless
SH	$Cu^{2+}+Cu^{+}$	violet
S–S	$Cu^{2+}+Cu^{+}$	pale blue
SH+S–S	Cu^{2+}	pale violet
SH+S–S	Cu^{+}	pale yellow

a) SH: penicillamine *b*) S–S: penicillamine disulfide

As seen in Table I, and as described in the experimental part, two kinds of complexes, namely red-violet and pale yellow complexes were observed in the pH range from 4 to 8. These two complexes are different each other in their ligand to metal ratios. The absorption spectra of the solutions in which penicillamine and cupric ion were mixed in the different ratio are shown in Fig. 1. When penicillamine was present in excess, pale yellow complex was produced and its absorption spectrum shows no absorption maximum in the visible region. On the other hand, when cupric ion was present in equivalence or in a little excess, the solution colored intensely in red–violet and in the absorption spectrum a characteristic broad peak was observed at 520 mμ with high extinction coefficient. The red–violet complex is presumed to be the mixed valence complex as observed in chlorocuprates (I, II) or copper (I, II)

5) H.M. Crooks, "The Chemistry of Penicillin," ed. by H.T. Clarke, J.R. Johnson and R. Robinson, Princeton University Press, Princeton, 1949, pp. 455—472.

thiomalates.[6] The intensificationof the absorption in the mixed valence metal complex is presumably due to a charge transfer mechanism. Recently, it has been reported by Day and Smith[7] that in the examples in which intermolecular charge transfer is encountered, direct overlap between donor and acceptor orbitals exists and the extinction coefficient of the order of about 10^3 is found. Similar suggestian was also pointed out by Hemmerich.[8] The plot of the absorbance at 520 mμ against the ratio of penicillamine to copper (II), the concentration of copper being set constant, is shown in Fig. 2.

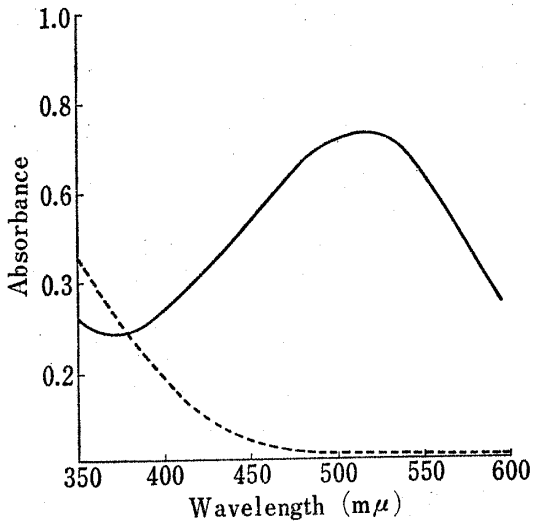

Fig. 1. Absorption Spectra of Copper Complexes of Penicillamine

——: 1.0×10^{-3}M of copper (II) and 1.4×10^{-3}M of penicillamine
------: 5.0×10^{-4}M of copper (II) and 1.0×10^{-2}M of penicillamine
pH 6.2

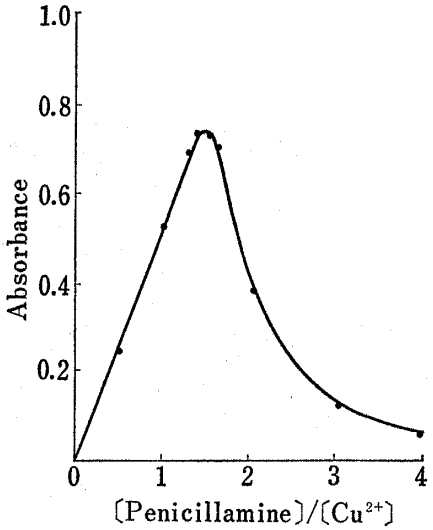

Fig. 2. Change in Absorbance at 520 mμ with Ratio of Penicillamine to Copper

concentration of copper(II): 1.0×10^{-3}M
pH 6.2

As indicated in Fig. 2, the absorbance at 520 mμ linearly increases until the ratio reaches about 1.35, but beyond this ratio it decreases. Fig. 3 was obtained from the similar experiments, the concentration of penicillamine being set constant. Below the ratio of 0.5 the solution is slightly colored, but the intensity at 520 mμ rises very sharply until the ratio reaches about 0.75 and the absorbance becomes constant after the ratio reaches to 0.75.

The titrations were carried out with decarbonated alkali solution in the molar ratios of penicillamine to copper, 1:1, 1.3:1, 1.6:1, 1.8:1 and 2:1.

As can be seen in Fig. 4, the titration curve of 1:1 molar ratio has an inflection at $a=1$, while the curves of the other molar ratios give the inflection at $a=2$. Among all titration curves, when the molar ratio is 1.3:1 the pH inflection was most sharp, and these observations coincide to the results of absorption spectra measurement. On the basis of these data, it seemes reasonable to assume that the red–violet complex is formed by the reaction illustrated in Fig. 5.

Penicillamine is partially oxidized to disulfide by initial copper (II) ion and then the yellow complex is formed by the reaction of penicillamine with simultaneously yielded cuprous ion. Further, in the presence of enough copper (II) ion for equation 4, the red–violet complex, namely mixed valence complex, is formed. It can therefore be presumed that in the

6) I.M. Klotz and G.H. Czerlinski, *J. Am. Chem. Soc.*, **80**, 2920 (1958).
7) P. Day and D.W. Smith, *J. Chem. Soc.* (A), **1967**, 1045.
8) P. Hemmerich, "The Biochemistry of Copper," ed. by J. Peisach, P. Aisen and W. Blumberg, Academic Press, Inc., New York, 1966, pp. 15—34.

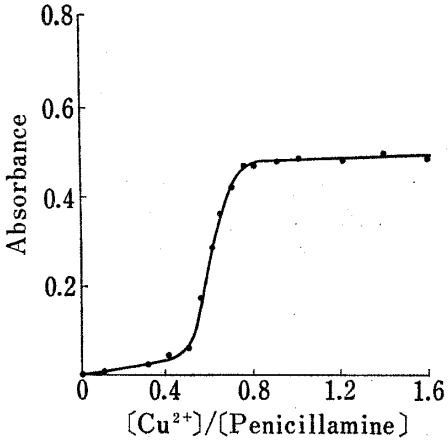

Fig. 3. Change in Absorbance at 520 mμ with Ratio of Copper to Penicillamine

concentration of penicillamine: 1.0×10^{-3}M
pH 6.2

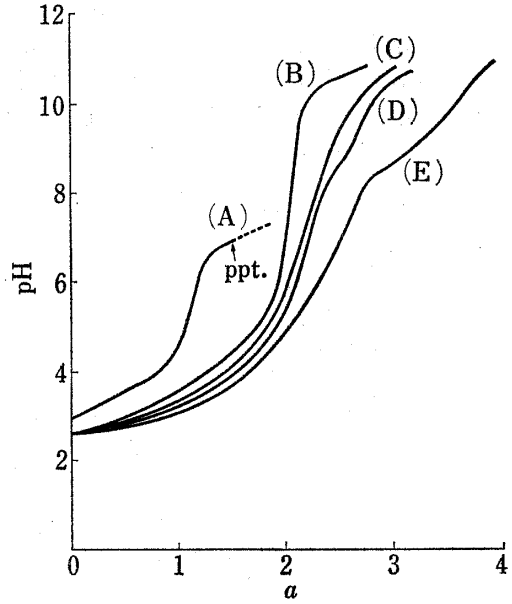

Fig. 4. Titration Curves of Penicillamine with Cupric Chloride

ligand to metal ratio
(A): 1.0:1.0 (B): 1.3:1.0 (C): 1.5:1.0
(D): 1.8:1.0 (E): 2.0:1.0
a: moles of base per metal

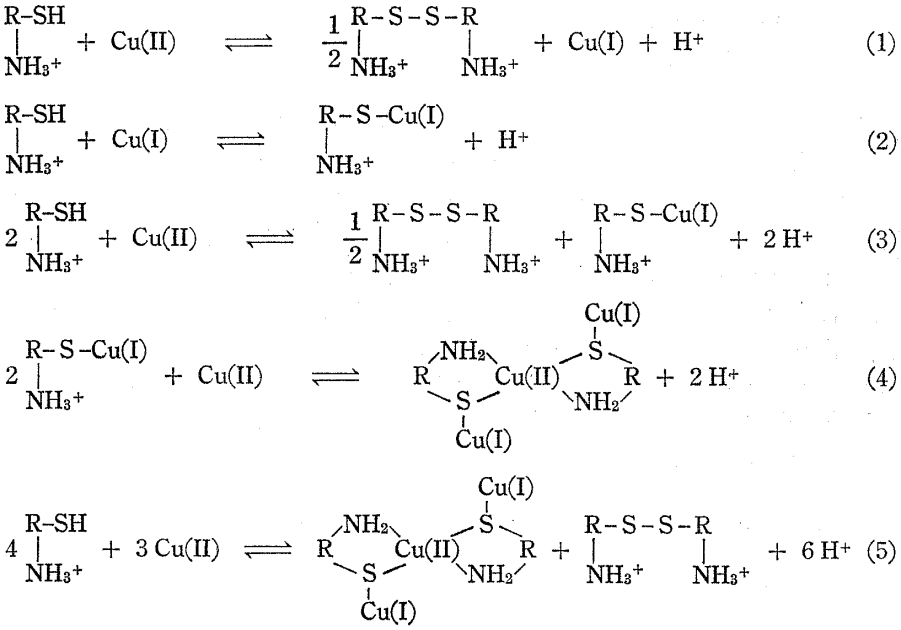

Fig. 5. Probable Formation Mechanism of Red-violet Complex

reaction of copper (II) ion with penicillamine the net result is finally concluded as equation (5). The yellow complex is formed according to equation (3) when penicillamine added to copper (II) ion is in large excess, because the equation (4) does not proceed in this condition. In an effort to confirm the mechanism above-mentioned, the red-violet and yellow complexes were isolated from the reaction solutions containing penicillamine and copper (II) ion in the molar ratios of 1.35 and 15.0, respectively, according to a manner described in the experimental part.

The infrared absorption spectrum of the red-violet complex isolated is shown in Fig. 6. As seen in Fig. 6, the stretching band of the mercapto group near 2500 cm^{-1} was not observed

and a band of coordinated amino group was observed at 3400 cm^{-1}, while in penicillamine and the yellow complex a band of ammonium ion was observed at 3100 cm^{-1} instead of the band of amino group. This fact supports that the mercapto and amino groups participate to the coordination in the mixed valence complex, and also that the nitrogen atom does not participate to the coordination in the yellow complex.

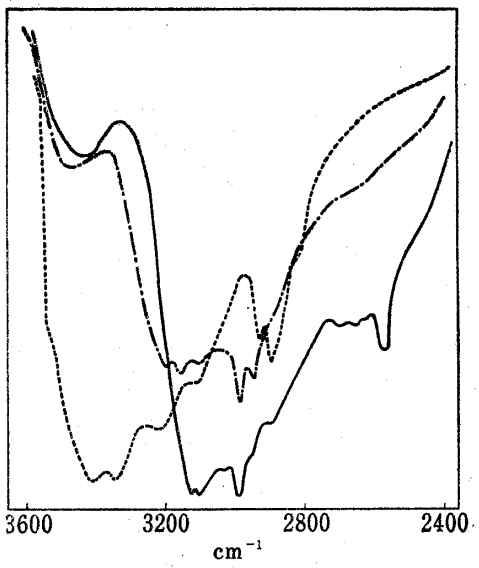

Fig. 6. Infrared Spectra (in Nujol)

————: penicillamine
·····: red–violet complex
— — — —: yellow complex

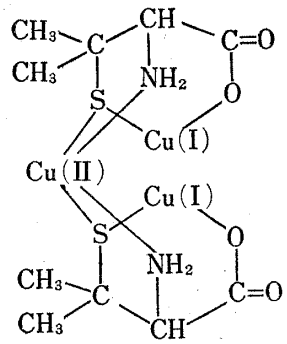

Fig. 7. Structure of Red–violet Complex

On the basis of these results the structure of the red–violet complex may be formulated as shown in Fig. 7. This constitution was found to be free from steric hindrance and stabillized by two methyl groups in penicillamine, from the molecular model of the complex.

The similar red–violet complex was also formed by mixing of cupric ion with β-methyl-β-ethylcysteine, whereas cysteine which is similar in functional groups to penicillamine does not produce it. It is therefore expected that the little difference of structure in the related compounds of penicillamine may give large effect on the reaction with copper (II) ion. Thus the complex formation with copper (II) ion and various related compounds of penicillamine such as shown in Table II were also studied by the ultraviolet and visible absorption spectra in the neutral aqueous solution.

Table II. Penicillamine and Its Related Compounds

I	penicillamine	β-methyl-β-ethylcysteine
II	N-acetylpenicillamine	N-formylpenicillamine
	penicillamine methyl ester	N-acetyl-β-methyl-β-ethylcysteine
	β-mercaptoisovaleric acid	α-amino-β-mercaptoisobutane
III	cysteine N-acetylcysteine	α-amino-β-mercaptopropane
	2-mercaptoethylamine	α-mercaptopropionyl glycine
IV	valine S-methylpenicillamine	penicillamine disulfide

The absorption spectra and the stability of the color of the red–violet complexes formed are shown in Fig. 8 and 9, respectively.

The following interesting characters were drawn in Fig. 8 and 9 concerning the reaction of complex formation in connection with the chemical structure of the ligands.

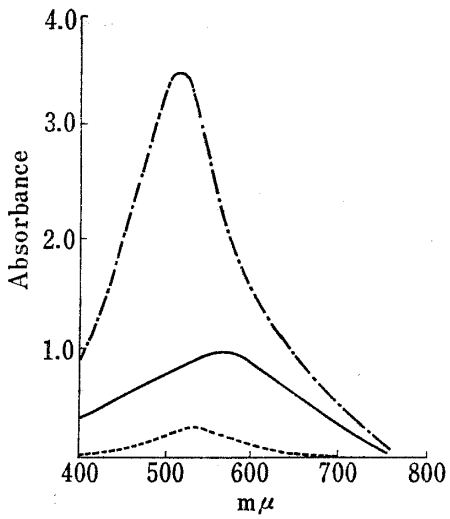

Fig. 8. Absorption Spectra of Copper Complexes

——— : penicillamine
———— : N-acetylpenicillamine
---------- : 2-mercaptoethylamine
concentration of copper(II): 8.0×10^{-3}M
concentration of thiol compounds: 4.0×10^{-3}M
pH 6.2

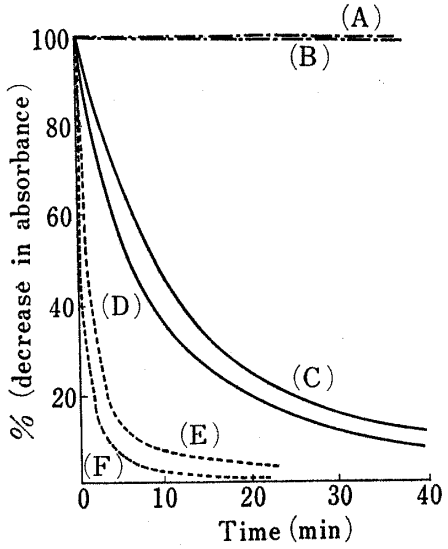

Fig. 9. Stability of Color of Red-violet Complexes

ligand
(A): β-methyl-β-ethylcysteine
(B): penicillamine
(C): N-acetylpenicillamine
(D): N-formylpenicillamine
(E): 2-mercaptoethylamine
(F): α-amino-β-mercaptopropane

The compounds shown in groups I and II in Table II, which have two substituent groups on the β-carbon atom of cysteine, show the evident tendency to form far more stable red-violet complex which may be the mixed valence complex than the compounds shown in group III.

In the compounds shown in group I such as β-methyl-β-ethylcysteine and penicillamine, which have three coordinating groups, namely mercapto, amino and carboxyl groups, form more stable red-violet complex than the compounds shown in group II which have two coordinating groups, namely mercapto and amino or mercapto and carboxyl groups.

Taking into account the following facts in their biochemical behaviors the above-mentioned results are particularly interesting. As the number of the substituents on the β-carbon atom of cysteine increases, the degradation of the cysteine molecule by L- and D-amino acid oxidases decreases.[9] In addition, the correlation between the activities of penicillamine and the related compounds in stimulating the excretion of copper in urine[10] and their chelating abilities with copper was investigated. The compounds which have strong activity for the excretion of copper, such as penicillamine and β-methyl-β-ethylcysteine, form stable mixed valence complexes, while the compounds which do not enhance the excretion of copper in urine, such as N-acetylpenicillamine, cysteine and penicillamine disulfide, do not form stable mixed valence complex. These results may suggest that the mixed valence complex formation may be an important form in the course of the elimination of copper with penicillamine.

9) H.V. Aposhian, "Metal-Binding in Medicine," ed. by M.J. Seven, J.B. Lippincott Company, Philadelphia, 1960, pp. 290—295.
10) H.V. Aposhian, *Federation Proc.*, **20**, 185 (1961).