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Studies on the Sulfur-containing Chelating Agents. XXIV.[1] Acid Dissociation and Chelate Formation of Penicillamine

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This study was initiated to study on the relationship between the chelate formation and the detoxication activity of penicillamine against heavy metal poisoning. The equilibrium of acid dissociation and the mode of the coordination of metal chelates were investigated by means of potentiometric titration, ultraviolet–visible spectra and proton magnetic resonance spectra. The equilibrium constants among various chemical species of penicillamine in aqueous solution were determined and the results were comparable with those in cysteine. The mode of coordination between penicillamine and metal ions was deduced through the comparison of the formation constants and the absorption spectra of the metal chelates of penicillamine with those of the related compounds. In the chelates produced from penicillamine with Co^{2+}, Zn^{2+} and Ni^{2+}, the coordination occurs through sulfur and nitrogen atoms while the carboxyl group remains off in coordination, and in the chelates produced from penicillamine with Hg^{2+}, Cd^{2+} and Pb^{2+}, sulfur, nitrogen and also oxygen atoms contribute to the coordination. The investigation by proton magnetic resonance spectroscopy in an aqueous solution gave more reliable information for the coordination of carboxyl group in penicillamine toward Hg^{2+}, Cd^{2+} and Pb^{2+}.

Penicillamine (β,β-dimethylcysteine) has been known as a degradation product of penicillin.[3] Recently, increasing interest is being paid to its use as an antidote in heavy metal poisoning,[4] and in practice it has been used as a therapeutic agent for Wilson's disease[5] which is caused by the abnormal metabolism of copper. In this connection, we picked up penicillamine as one of the biologically interesting chelating agents, in the series of the study on the sulfur–containing chelating agents which we have conducted.

Although the stability constants of its metal chelates have been determined potentiometrically or polarographically by several workers in recent years,[6] the information on the structure and the mode of the coordination has not been obtained, and little attention has been paid to the relationship between the chelate formation and the detoxication activity. We planned the extensive study on the chelate formation of penicillamine and already reported the application of penicillamine for the radiochemical determination of several kinds of metals, by the utilization of its chelating ability.[7] Penicillamine has three coordination groups, namely sulfhydryl, amino, and carboxyl groups, and the acid dissociation of penicillamine and the mode of the coordination in the metal chelates would have important effects on the biological

1) Part XXIII: A. Yokoyama, S. Kawanishi and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 363 (1970).
2) Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) E.P. Abraham, E. Chain, W. Baker and P. Robinson, *Nature*, **151**, 107 (1943).
4) *a*) H.V. Aposhian, *Science*, **128**, 93 (1958); *b*) V. Nigrovic, *Arzneimittel-Forsch.*, **13**, 787 (1963); *c*) A. Goldberg, J. Smith and A. Lochhead, *Brit. Med. J.*, **1**, 1270 (1963); *d*) E.J. Eyring and E.P. Engleman, *Arthritis Rheumat.*, **6**, 216 (1963).
5) *a*) J.M. Walshe, *Lancet*, **1**, 25 (1956); *b*) J.M. Walshe, *Am. J. Med.*, **21**, 487 (1956); *c*) S. Orilly and W. Bank, *Nature*, **212**, 1597 (1966).
6) *a*) E.J. Kuchinskas and Y. Rosen, *Arch. Biochem. Biophys.*, **97**, 370 (1962); *b*) D.A. Doornbos and J.S. Faber, *Pharm. Weekblad*, **99**, 289 (1964); *c*) I.H. Suffet and W.C. Rurdy, *J. Electroanal. Chem.*, **11**, 302 (1966); *d*) D.D. Perrin and I.G. Sayce, *J. Chem. Soc.* (A), **1968**, 53.
7) *a*) H. Tanaka, Y. Sugiura, K. Seki, K. Sakaguchi and A. Yokoyama, *Bunseki Kagaku*, **17**, 1309 (1968); *b*) H. Tanaka, Y. Sugiura and A. Yokoyama, *Bunseki Kagaku*, **17**, 1424 (1968).

activity of this ligand. This paper deals with the acid dissociation of penicillamine and the mode of the coordination, namely which functional groups of its molecule contribute to the chelate formation.

Experimental

Materials——DL-Penicillamine and L-penicillamine hydrochloride were purchased from Sigma Company. Penicillamine methyl ester hydrochloride was prepared by the treatment of penicillamine in absolute methanol with dry hydrogen chloride.[8] The product was recrystallized from methanol–ether mixture. N-Acetylpenicillamine was obtained by the treatment of penicillamine with the equivalent amount of sodium hydroxide and acetic anhydride, and the product was recrystallized from water.[9]

S-Methylpenicillamine was prepared by the action of methyl iodide with penicillamine.[10] β-Methyl-β-ethylcysteine was synthesized according to the method of Abbot Company,[9] and β-mercaptoisovaleric acid was obtained by the action of hydrogen sulfide with β,β-dimethylacrylic acid.[11] Metal ion solutions prepared from reagent grade metal salts were standardized with EDTA. Carbonate-free potassium hydroxide solution was prepared by the procedure described by Armstrong[12] and was standardized by the titration with potassium hydrogen phthalate. All the other reagents used were commercially available reagent grade materials. Deionized water was used throughout the experiment.

Apparatus and Procedure——On the titration vessel, 5 ml of 1M potassium nitrate, 1 ml of 0.1M metal ion solution and 10 ml of 2.0×10^{-2}M ligand were added, and the total volume was adjusted to 50 ml by adding water. The titration was carried out with 0.1N carbonate-free potassium hydroxide solution in nitrogen atmosphere. All potentiometric measurements were made at $22\pm0.1°$. A Metrohm syring burette of 10 ml was used with calibration up to 0.01 ml. The pH measurements were made with a Metrohm pH meter, model E 300-B, equipped with an external electrode.

The spectrophotometric measurements were carried out in an aqueous solution of pH range from 6 to 12, with a Hitachi recording spectrophotometer model EPS-2. The pH of the solution was adjusted with borate buffer.

Proton magnetic resonance spectra were measured at 60 Mc with a Varian Associates Model A-60 spectrometer. The spectra for penicillamine and its mercury(II), lead(II), cadmium(II), zinc(II) and nickel(II) chelates in aqueous medium were measured in concentration of 0.1M and 0.5—1.0% solution of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as an internal standard. The pH of the solution was adjusted with 0.1N hydrochloric acid and 0.1N sodium hydroxide.

Calculations——The macro acid dissociation constants (K_1,K_2 and K_3) of ligands were calculated by the following equation,[12a]

$$pK_a = pH + \log\frac{C-[KOH]-\{H^+\}+\{OH^-\}}{[KOH]+\{H^+\}-\{OH^-\}}$$

where C is the total concentration of the ligand and [KOH] is the concentration of potassium hydroxide added. The value of the hydrogen ion activity$\{H^+\}$ was determined from the reading of the pH meter and the hydroxyl ion activity$\{OH^-\}$ was calculated from the value of $k_w(0.796\times10^{-14})$[12a], hence K_a is the compositive constant at $\mu=0.10(KNO_3)$ and $t=22°$.

The stability constants (k_1 and k_2) were calculated by the adaptation of Bjerrum's method,[13] modified by Li, Grawron and Bascuas.[14] We used the following equations to calculate the concentration of the free ligand ($[A^{2-}]$) and the average number of ligands bound per metal ion ($\bar{n}$).

$$[A^{2-}]=\frac{C-[KOH]-\{H^+\}+\{OH^-\}}{P}$$

$$\bar{n}=\frac{C-Q[A^{2-}]}{M}$$

8) Merck & Co., Inc., Brit. Patent 622298 (1949) [*C.A.*, **43**, 7039 (1949)].
9) 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.
10) K. Savard, E.M. Richardson and G.A. Grant, *Can. J. Research*, **24B**, 28 (1946).
11) O. Süs, *Ann.*, **559**, 92 (1948).
12) *a*) A. Albert and E.P. Serjeant, "Ionization Constants of Acids and Bases," Methuen, London, 1962, pp. 16—42; *b*) D.M.G. Armstrong, *Chem. Ind.* (London), **1955**, 1405.
13) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Sons, Copenhagen, 1941.
14) N.C. Li, O. Grawron and G. Bascuas, *J. Am. Chem. Soc.*, **76**, 225 (1954).

In these equations, M is the total concentration of metal, and P and Q are the terms defined by the following equations,

$$P=\frac{\{H^+\}}{K_a}+\frac{2\{H^+\}^2}{K_aK_a'}$$

$$Q=\frac{\{H^+\}}{K_a}+\frac{\{H^+\}^2}{K_aK_a'}+1$$

where K_a and K'_a are the macro acid dissociation constants of the ligand, refferring K_a to the higher constant. The preliminary values of the stepwise stability constants (k_1 and k_2) were given from the values

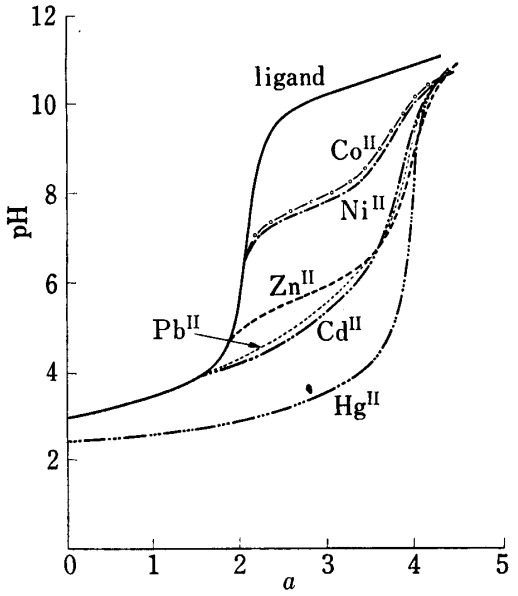

Fig. 1. Titration Curves of N-Acetylpenicillamine with Metals; Ligand (4.0×10^{-3}M) to Metal Ratio=2:1

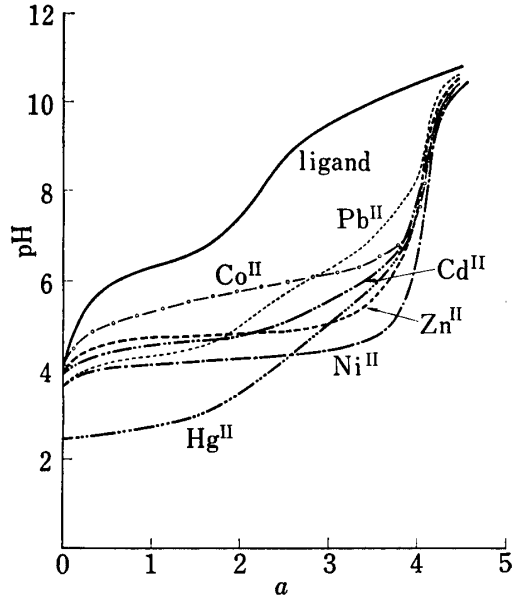

Fig. 2. Titration Curves of DL-Penicillamine Methyl Ester Hydrochloride with Metals; Ligand (4.0×10^{-3}M) to Metal Ratio=2:1

a: moles of base per metal

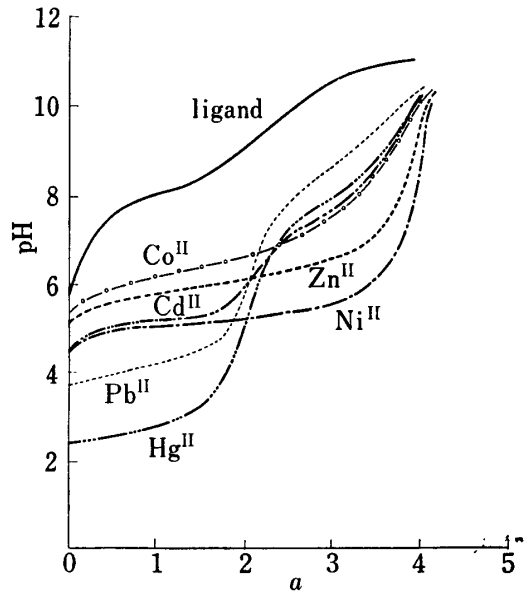

Fig. 3. Titration Curves of DL-Penicillamine with Metals; Ligand (4.0×10^{-3}M) to Metal Ratio=2:1

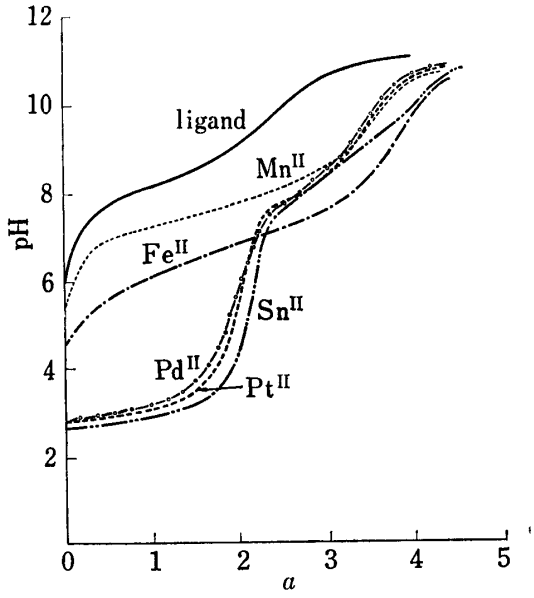

Fig. 4. Titration Curves of DL-Penicillamine with Metals; Ligand (4.0×10^{-3}M) to Metal Ratio=2:1

a: moles of base per metal

of pA at $\bar{n}=0.5$ and $\bar{n}=1.5$ on the formation curves obtained by plotting $\bar{n}$ against pA, namely log $1/[A^{2-}]$. By the use of successive approximation method by Bjerrum, the final stability constants of the chelates were determined. The stability constant of mercury chelate which could not be accurately determined by the above-mentioned method was approximately calculated with algebraic method deviced by Chaberek and Martell.[15]

Results and Discussion

The titration curves are presented in Figs. 1, 2, 3, 4, and 5, and the formation curves are shown in Fig. 6, 7 and 8.

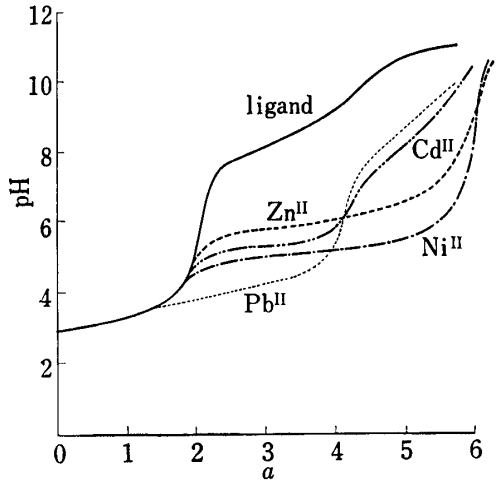

Fig. 5. Titration Curves of L-Penicillamine Hydrochloride with Metals; Ligand (4.0×10^{-3}M) to Metal Ratio=2:1

a: moles of base per metal

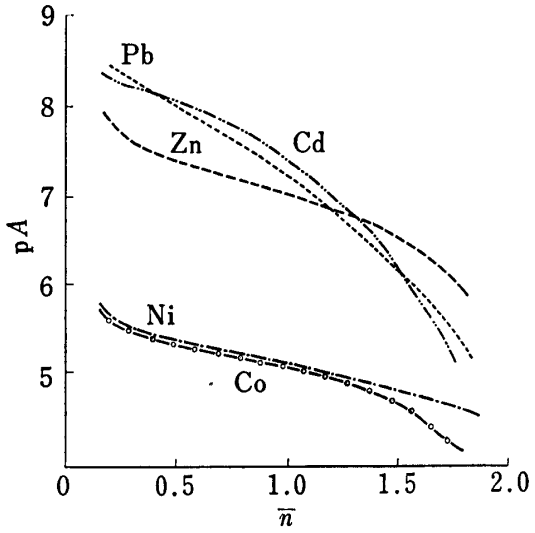

Fig. 6. Formation Curves of N-Acetyl-penicillamine with Metals

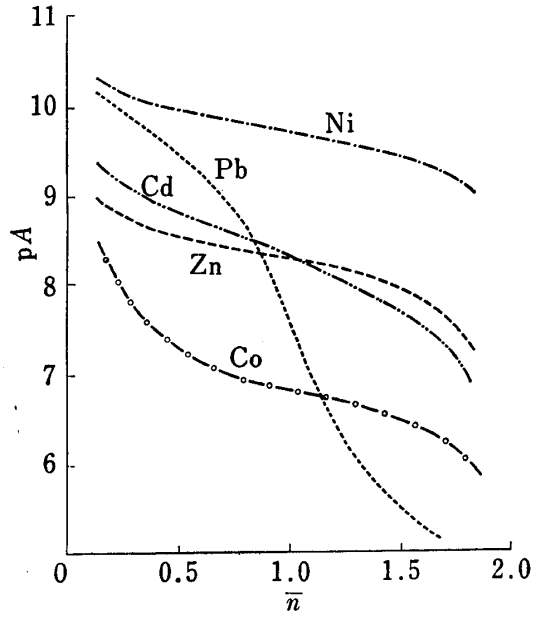

Fig. 7. Formation Curves of Penicillamine Methyl Ester with Metals

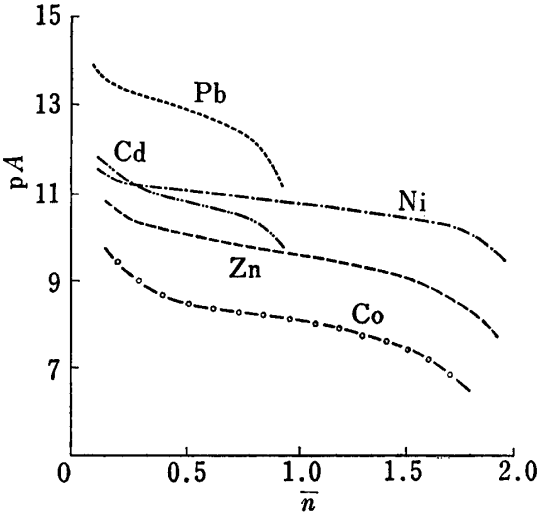

Fig. 8. Formation Curves of DL-Penicill-amine with Metals

15) S. Chaberek and A.E. Martell, *J. Am. Chem. Soc.*, **74**, 5052 (1952).

A direct pH titration of penicillamine with alkali gave two "macroscopic" pK values, namely pK_2=8.09 and pK_3=10.55. These values can not be simply assigned to the dissociation of each group, various forms in solution being considered. The equilibrium may be formulated in terms of set of the microscopic constants, K_{12}, K_{13}, K_{123} and K_{132}, according to Fig. 9. These microscopic constants are related to the macroscopic ones by the following equations.

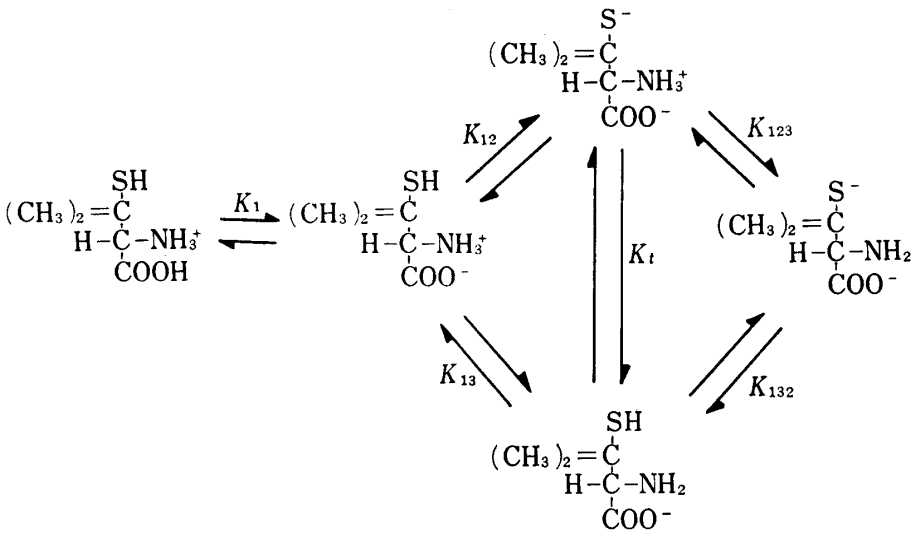

Fig. 9. Scheme of Ionization Equilibrium of Penicillamine

$$K_2=K_{12}+K_{13} \quad (1)$$

$$1/K_3=1/K_{123}+1/K_{132} \quad (2)$$

$$K_t=K_{12}/K_{13}=K_{132}/K_{123} \quad (3)$$

The microscopic constants were calculated by the use of the equations 1, 2 and 3. In the calcutation, the value determined for the proton ionization from the NH_3^+ group of S-methylpenicillamine was used expediently as the value of K_{13}, with a assumption that methylation of the mercapto group of penicillamine would not give large effect on the acidity of its NH_3^+ group.

The microscopic constants are presented in Table I and are compared with the data in cysteine determined by Wrathall, *et al.*[16] The dissociation constants of the derivatives of penicillamine are shown in Table II.

Table I. Micro Acid Dissociation Constants of Penicillamine

	pK_{12}	pK_{13}	pK_{123}	pK_{132}	K_t
Penicillamine	8.32	8.48	10.31	10.17	1.4
Cysteine[16]	8.58	8.63	10.32	10.25	1.2

Table II. Acid Dissociation Constants of Penicillamine and Its Derivatives

	pK_1	pK_2	pK_3
Penicillamine	<2.5	8.09	10.55
S-Methylpenicillamine	<2.5	8.48	—
Penicillamine methyl ester	—	6.30	9.17
N-Acetylpenicillamine	3.28	—	10.26

16) D.P. Wrathall, R.M. Izatt and J.J. Christensen, *J. Am. Chem. Soc.*, **86**, 4779 (1964).

The mode of the coordination in penicillamine will be deduced through the comparison of the stability constants of penicillamine metal chelates with those of the metal chelates of N-acetylpenicillamine and penicillamine methyl ester.

The stability constants of cobalt, nickel, zinc, cadmium, lead and mercury chelates of the above-mentioned ligands were determined potentiometrically and the values calculated from Fig. 1, 2, 3 and 5 are shown in Table III together with those of the chelates of valine.[17]

On the binding of penicillamine with metal ions, four modes of the coordination can be considered, depending on the combinations of the coordinating groups, namely (a) $-NH_2$ and COO^-, (b) $-S^-$ and COO^-, (c) $-S^-$ and NH_2 and (d) $-S^-$, $-NH_2$ and COO^-.

Li and Manning determined the stability constants of the zinc chelate of cysteine, and it was compared with those of glycine, mercaptoacetic acid, mercaptoethylamine, in which the coordinations between nitrogen and oxygen, sulfur and oxygen, and sulfur and nitrogen are considered respectively, and the mode of the coordination in cysteine–zinc chelate could be concluded as sulfur–nitrogen coordination,[18] because the stability constant of zinc–cysteine is very close to that of zinc–mercaptoethylamine.

As seen in Table III, the stability constants of penicillamine chelates are closer to those of penicillamine methyl ester in which the metal is bound between $-S^-$ and NH_2 than those of N-acetylpenicillamine and valine in which the metal is bound between $-S^-$ and COO^-, and NH_2 and COO^- respectively.

Table III. Stability Constants of Divalent Metal Chelates of Penicillamine and Its Derivatives

Metal \ Ligand		Valine[17]	N-Acetyl-penicillamine	Penicillamine methyl ester	DL-Penicill-amine	D-Penicill-amine[6b]	L-Penicill-amine
Co^{II}	$\log k_1$	4.57	5.60	7.09	8.38	9.04	
	$\log k_2$	3.67	4.59	6.76	7.71	8.08	
Ni^{II}	$\log k_1$	5.37	5.65	9.95	11.20	11.17	11.62
	$\log k_2$	4.16	4.93	9.40	10.68	12.08	11.04
Zn^{II}	$\log k_1$	5.00	7.61	8.82	9.95	10.16	10.12
	$\log k_2$	4.10	6.54	8.08	9.12	9.53	9.07
Cd^{II}	$\log k_1$		*a*	9.09	10.92		10.96
	$\log k_2$			7.33			
Pb^{II}	$\log k_1$		*a*	9.41	12.88	13.48	13.18
	$\log k_2$			5.82		3.88	
Hg^{II}	$\log k_1$			14.2	16.4		
	$\log k_2$						

a: The value cannot be accurately calculated as the formation curve is unsymmetrical.

As can be seen in Fig. 3 and 5, the titration curves of the ligand-to-metal molar ratio 2:1, in the cases of Co^{2+}, Zn^{2+} and Ni^{2+}, gave inflections at $a=4$, indicating the formations of 2:1 chelates, whereas, in the titrations with Hg^{2+}, Cd^{2+} and Pb^{2+} inflections were observed at $a=2$. In addition, in the titrations of 1:1 molar ratio, inflections were observed at $a=2$. These facts indicate that the ligand probably behaves as a terdentate ligand in these chelates.

In view of the above facts the following conclusion can be reasonablly derived. In the chelates produced from penicillamine with Co^{2+}, Zn^{2+} and Ni^{2+}, the coordination occurs through sulfur and nitrogen atoms while the carboxyl group remains off in coordination, and in the chelates produced from penicillamine with Hg^{2+}, Cd^{2+} and Pb^{2+}, sulfur, nitrogen and also oxygen atoms contribute to the coordination. As shown in Fig. 4, in the cases of Pd^{2+}, Pt^{2+} and Sn^{2+}, similar titration curves were obtained to the cases of Hg^{2+}, Cd^{2+} and Pb^{2+}.

17) L.E. Maley and D.P. Mellor, *Nature*, **165**, 453 (1950).
18) N.C. Li and R.A. Manning, *J. Am. Chem. Soc.*, **77**, 5225 (1955).

As shown in Fig. 10, the differences in the stability constants between penicillamine chelates and penicillamine methyl ester chelates are considerably greater in mercury, cadmium and lead chelates than in cobalt, zinc and nickel chelates.

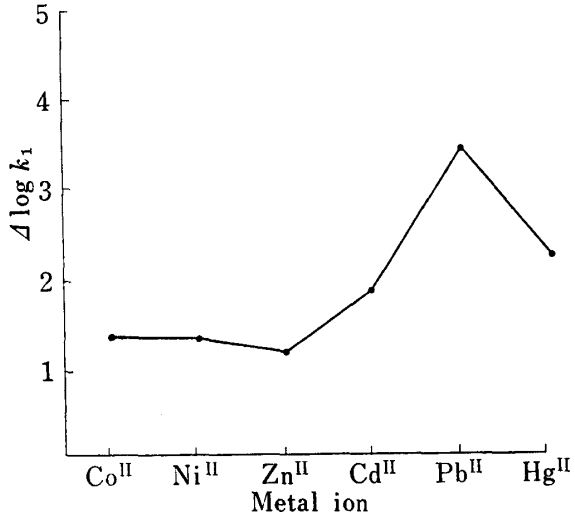

Fig. 10. Differences in Stability Constants between Penicillamine Chelates and Penicillamine Methyl Ester Chelates

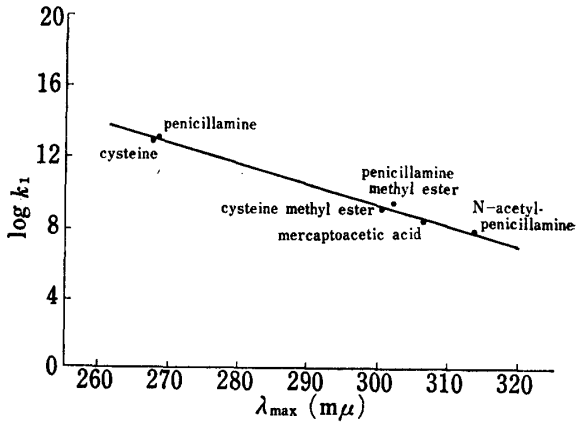

Fig. 11. Correlation of Stability Constants to Wavelengths of Absorption Maximum in Lead Chelates

From this fact, it may be concluded that the participation of the carboxyl group in the chelation contributes greatly to the higher stability in mercury, cadmium and lead chelates, whereas in cobalt, zinc and nickel chelates, carboxyl group does not participate in the chelation and their stabilities are lower.

The lower stability in the chelates of penicillamine methyl ester than that of penicillamine chelates can be explained by the smaller pK values of penicillamine methyl ester than those of other ligands as shown in Table II.

In addition, in an attempt to discuss the mode of the coordination in penicillamine metal chelates, the absorption spectra of nickel and lead chelates of penicillamine in ultraviolet and visible region were investigated in comparison with those of the chelates of various model compounds in which the mode of coordination can be revealed. Absorption maxima and extinction coefficients of nickel and lead chelates determined in ultraviolet and visible region are listed in Table IV.

Table IV. Spectral Data for Nickel and Lead Chelates

Ligand	Nickel chelate		Lead chelate	
	Absorption max (mμ)	Extinction coefficient ($\times 10^2$)	Absorption max (mμ)	Extinction coefficient ($\times 10^3$)
Penicillamine	467	1.3	268	4.4
Cysteine	467	1.3	267	4.1
β-Methyl-β-ethylcysteine	467	1.4	268	4.5
Valine	—	—	—	—
S-Methylpenicillamine	—	—	—	—
N-Acetylpenicillamine	518	2.9	313	3.9
N-Acetyl-β-methyl-β-ethylcysteine	520	2.9	311	4.0
β-Mercaptoisovaleric acid	520	3.1	313	4.1
2-Mercaptoethylamine	470	1.1	301	4.0
Cysteine methyl ester	468	1.2	300	3.7
Penicillamine methyl ester	468	1.3	302	4.0

In nickel chelates of sulfur–oxygen coordination ligands such as N-acetylpenicillamine, β-mercaptoisovarelic acid, and sulfur–nitrogen coordination ligands such as 2-mercaptoethylamine, penicillamine methyl ester, the absorption maxima and extinction coefficient were found to be about 520 mμ and 300, 470 mμ and 120, respectively.

As seen in Table IV the spectrum in penicillamine–nickel chelate was found to be similar to those of sulfur–nitrogen coordination ligands and hence the mode of the coordination in penicillamine–nickel chelate can be formulated as sulfur–nitrogen coordination. While in the lead chelates of sulfur–oxygen coordination ligands and sulfur–nitrogen coordination ligands, the absorption maxima were found 310 mμ and 300 mμ, respectively, whereas an absorption maxima was observed at 268 mμ in penicillamine lead chelate. The coordination through sulfhydryl, amino and carboxyl groups can therefore be presumed in penicillamine lead chelate. Above mentioned observations indicate that the penicillamine act probably as bidentate ligand toward nickel and terdentate ligand toward lead.

The relationship between the wavelength of absorption maxima of lead chelates of penicillamine and its related compounds and their formation constants gives a straight line as shown in Fig. 11.

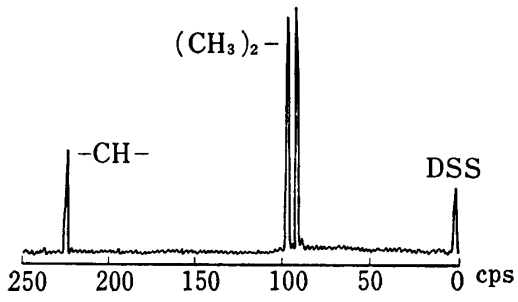

Fig. 12. Proton Magnetic Resonance Spectrum of Penicillamine at pH 2.5

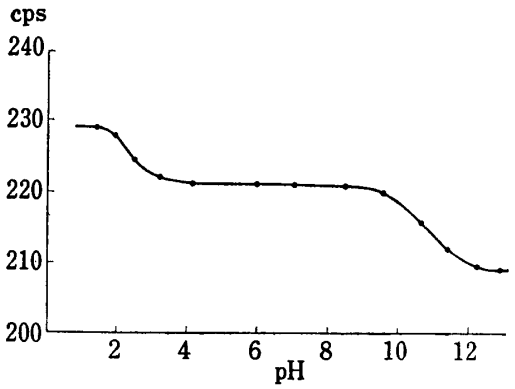

Fig. 13. Effect of pH on Chemical Shift of Methine Proton of Penicillamine in Aqueous Solution

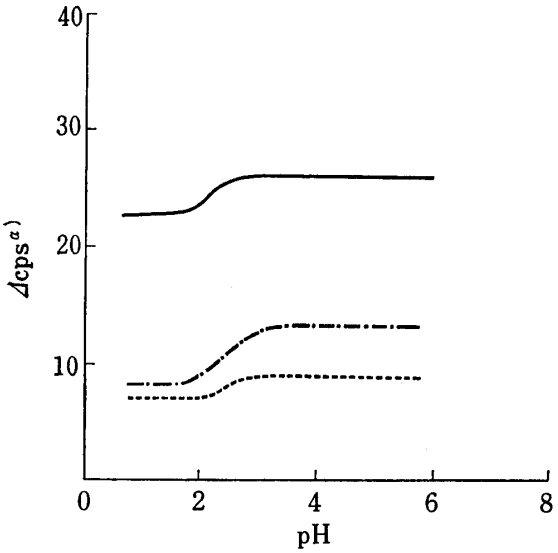

Fig. 14. Effect of pH on Chemical Shifts of Methine Proton of Penicillamine Chelates

——: mercury chelate —·—·—: lead chelate
------: cadmium chelate
a) values indicate the difference in chemical shift between penicillamine and its metal chelates

The investigation by proton magnetic resonance (PMR) spectroscopy in aqueous solution was expected to give more satisfactory information for the coordination of carboxyl group in penicillamine toward Hg^{2+}, Pb^{2+} and Cd^{2+}. PMR spectrum of penicillamine in aqueous solution at pH 2.5 is presented in Fig. 12. Only proton resonance for the hydrogen atoms bound to carbon atoms could be observed. The pH dependence of the chemical shift of the methine proton of penicillamine in aqueous solution is shown in Fig. 13.

The chemical shift of the methine proton are greatly influenced by pH only in the range of pH 2 to 3 and 9 to 12, wher ecarboxyl groups, protonated amino group and sulfhydryl group dissociate respectively. The PMR spectra of the solutions containing Hg^{2+}, Pb^{2+} and Cd^{2+} with the ratio to penicillamine 1 to 1 were examined over the pH

range from 0.5 to 6 in aqueous solution, and they were compared with that of penicillamine. In the presence of above-mentioned metal ion, the shift to lower field of the signal of the methine proton was observed and the remarkable inflections at pH 2 to 3 seen in Fig. 14 may be explained by the binding of the carboxyl group of penicillamine toward Hg^{2+}, Cd^{2+} and Pb^{2+}.

Furthermore, the degree of the change in the chemical shift observed on the curves at pH 2 to 3 decreases in order of $Pb^{2+}>Hg^{2+}>Cd^{2+}$. This order is in good agreement with the order of $\Delta\log k_1$ shown in Fig. 10. This order may be considered as the order in the affinity of the metal to the carboxyl group of penicillamine.

While, in the nickel and zinc chelates, their PMR spectra coincided to those of penicillamine at pH 2 to 3, and hence the coordination from the carboxyl group toward these ions can be negligible.

It was impossible to investigate the reaction of Hg^{2+} and Pb^{2+} in alkaline medium by PMR, because of the formation of the precipitate.

Judging from the above-mentioned results, it is reasonable to conclude that the mode of the coordination between penicillamine and metal in mercury(II), cadmium(II), lead(II), palladium(II), tin(II) and platinum(II) chelates is terdentate sulfur-nitrogen-oxygen type, and in other metals, that is bidentate sulfur-nitrogen type. It is of great interest that penicillamine especially tends to act as a terdentate ligand toward the metal ions with the larger atomic number such as mercury and lead, and also that penicillamine is especially effective to those metals for their excretion and detoxication, and not so effective to the metal which coordinates with penicillamine in sulfur-nitrogen bidentate type.[19]

It has been known that antidotal effect of penicillamine is remarkably different from each other between their optical isomers,[20] but their metal-binding activity is not different as shown in Table III. The non-effectiveness of L-penicillamine for metal poisoning is not explained by its chelating ability, but by the combination with pyridoxal-5-phosphate to form a thiazolidene carboxylic acid.[21]

19) B. Pommey, *Therapie*, **22**, 739 (1967).
20) H.V. Aposhian, *Federation Proc.*, **20**, 185 (1961).
21) *a*) E.J. Kuchinskas, A. Horvath and V. Vigneaud, *Arch. Biochem. Biophys.*, **68**, 69 (1957); *b*) V. Vigneaud, E.J. Kuchinskas and A. Horvath, *Arch. Biochem. Biophys.*, **69**, 130 (1957); *c*) P. Holzt and D. Plam, *Pharmacol. Rev.*, **16**, 113 (1964).