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18. Motoichi Miyazaki, Tatsuo Senshu, and Zenzo Tamura :

Metal Complexes of D-Glucosamine and its Derivatives. VII.[*1] Metal Complexes of D-Glucosamінic Acid.

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As a part of the series of studies on metal complexes of D-glucosamine, D-glucosaminic acid (2-amino-2-deoxy-D-gluconic acid) was investigated on its nature of metal complex formation.

Recently, Gergely, *et al.*[1] reported the complex formation of glucosaminic acid with Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, and Fe^{2+}, and they studied the order of stability of these metal complexes by polarography and potentiometric titration. However, the stability constants of the complexes were not reported. Considering from the structure, glucosaminic acid can be supposed to be closely related to α-amino acid as Gergely, *et al.* cited and hence glucosaminic acid may have a fairly strong complex forming ability with metal ions. Moreover, poly OH groups of its molecule would participate in complex formation in solution.

In this paper, the pKa values of glucosaminic acid were determined by pH titration[2] and its complex formations with metal ions were investigated to obtain their stability constants.

Experimental

Materials——D-Glucosaminic acid was synthesized from D-glucosamine hydrochloride by the method of Hope and Kent,[3] and recrystallized from aqueous MeOH, needles, m.p. 250°(decomp.). *Anal.* Calcd. for $C_6H_{13}O_6N$: C, 36.92; H, 6.71; N, 7.18. Found : C, 36.60; H, 6.71; N, 7.03.

$Cu(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, $Ni(NO_3)_2$, $Cd(NO_3)_2$, $Co(NO_3)_2$, $Mn(NO_3)_2$, $Ca(NO_3)_2$ and $Mg(NO_3)_2$ were of analytical grade, and these stock solutions, $10^{-2}M$, were standardized with EDTA.

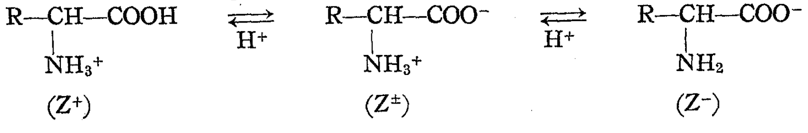
A carbonate-free $0.1M$ NaOH and $0.1M$ HNO_3 were used as titrants.

Apparatus and Procedure——Determination of pKa values of glucosaminic acid——The experimental method and apparatus used in this investigation were the same which had been reported in previous papers.[2] The temperature was kept at 30±0.5° and the ionic strength was held at 0.1 with KNO_3. The concentration of the solution of glucosaminic acid was the order of $10^{-3}M$. The sample solution was titrated with $0.1M$ HNO_3 or $0.1M$ NaOH in nitrogen atmosphere.

The pH of the solution was measured with a Toa Dempa pH meter Model HM-5A which was calibrated with the standard buffer solutions 4.01 and 6.86 at 25°.

Treatment of Data——Calculation of pKa values of glucosaminic acid was practiced as described below.

Generally, α-amino acids exist in three forms in solution, and the ratio of their amounts varies with hydrogen ion concentration.



$$\underset{\underset{NH_3^+}{|}}{R-CH-COOH} \quad \underset{H^+}{\overset{OH^-}{\rightleftarrows}} \quad \underset{\underset{NH_3^+}{|}}{R-CH-COO^-} \quad \underset{H^+}{\overset{OH^-}{\rightleftarrows}} \quad \underset{\underset{NH_2}{|}}{R-CH-COO^-}$$

(Z^+) $(Z^{\pm})$ (Z^-)

The apparent pKa values, pK_1' and pK_2' are calculated according to the following equations by the method of Akabori.[4]

[*1] Part Ⅵ : This Bulletin, **13**, 387 (1965).

[*2] Hongo, Tokyo (宮崎元一, 千秋達雄, 田村善蔵).

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Thus,

$$pK_1' = pH - \log\left(\frac{C}{C_1 - [H^+]} - 1\right)$$

$$pK_2' = pH - \log\left(\frac{C}{C_2 - [OH^-]} - 1\right)$$

where, K_1' : The apparent dissociation constant, $K'_1 = \frac{[H^+][Z^\pm]}{[Z^+]}$

K_2' : The apparent dissociation constant, $K'_2 = \frac{[H^+][Z^-]}{[Z^\pm]}$

C_1 : The total concentration of acid in solution

C_2 : The total concentration of base in solution

C : The total concentration of glucosaminic acid, $C = [Z^+] + [Z^\pm] + [Z^-]$

The stability constants of metal complexes were calculated according to the manner previously described⁽⁵⁾ referring to the method of Albert.⁽⁶⁾

Results and Discussion

The pKa Values of Glucosaminic Acid

Calculating from the pH titration data, the values for pK_1' and pK_2' of glucosaminic acid were determined as 2.01 and 9.06 respectively, while Gergely, *et al.* had reported 9.24 for pK_2.

The Titration of Glucosaminic Acid in the Presence of Metal Ion

In Figs. 1 and 2, the titration curves of glucosaminic acid and its mixture with metal ion are demonstrated. From the magnitude of the depression of the pH, copper complex seems to be the most stable among them. In zinc and cadmium complex

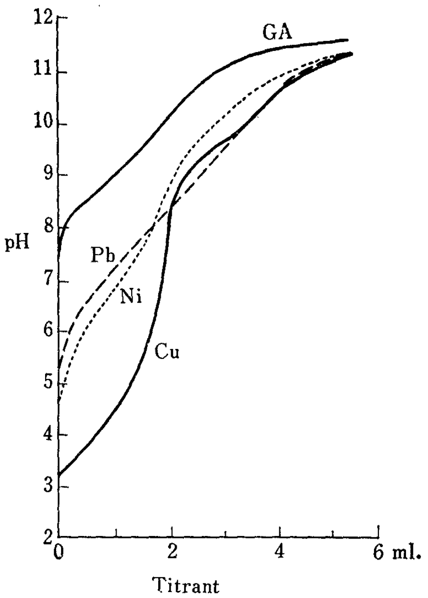

Fig. 1. Titration Curves of D-Glucosaminic Acid in the Presence of Respective Metal Ions

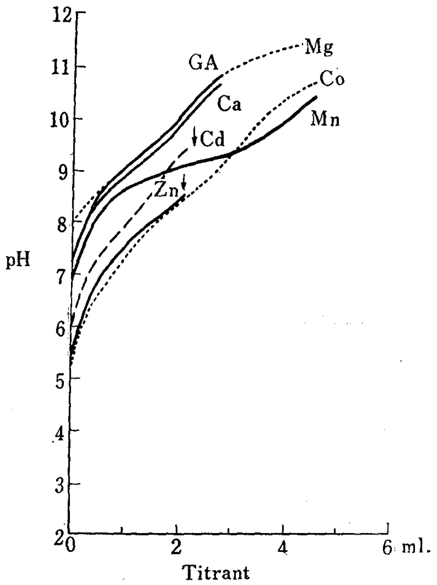

Fig. 2. Titration Curves of D-Glucosaminic Acid in the Presence of Respective Metal Ions

Molar ratio of D-glucosaminic acid to metal ion, 2:1.
Sample solution, 50 ml., $4 \times 10^{-3} M$ of D-glucosaminic acid, $2 \times 10^{-3} M$ of metal ion.
Temp. : 30±0.5°, $\mu = 0.1$ (KNO_3)
Titrant : 0.1M NaOH

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5) Z. Tamura, M. Miyazaki : This Bulletin, **13**, 333 (1965).
6) A. Albert : Biochem. J., **47**, 531 (1950).

systems, precipitations were perceived in the range from pH 8.5 to pH 9.5 and at about pH 9.5 respectively. These precipitations were supposed to be due to the formation of metal hydroxides or hydrolysis products of the complexes.

Therefore, the relative order of stability of metal complexes is supposed to be the following: Cu>Ni>Pb>Zn>Co>Cd>Mn>Ca, Mg. Except Pb complex, the order well agrees with that of Gergely, *et al.* determined by polarography: Cu>Ni>Zn>Co>Pb>Cd>Fe.

The Determinations of Stability Constants of Metal Complexes of Glucosaminic Acid

The applicability of Bjerrum's method for calculating the stability constants of these metal complexes were examined in the same manner reported previously.

As shown in Fig. 3, linear relations were observed between $\bar{n}/(\bar{n}\text{-}1)[L]$ and $(2\text{-}\bar{n})[L]/(\bar{n}\text{-}1)$ in copper complex system whose molar ratio of ligand to cupric ion was 2:1. The similar relations were also perceived with other metals.

The stability constants, log K_1 and log K_2, of metal complexes of glucosaminic acid were calculated as shown in Table I. Owing to the precipitations described above, log K_2 of zinc and cadmium complexes were not obtained. Furthermore, the stabilities of calcium and magnesium complexes were too small to obtain their values from the experimental data.

Table I. Stability Constants of Metal Complexes of D-Glucosaminic Acid

Metal ion	Stability constants log K_1	log K_2	log K_1K_2	Metal ion	Stability constants log K_1	log K_2	log K_1K_2
Cu^{2+}	8.0	6.6	14.6	Co^{2+}	4.5	3.9	8.4
Ni^{2+}	5.6	4.4	10.0	Cd^{2+}	4.2	—	—
Pb^{2+}	5.0	4.4	9.4	Mn^{2+}	3.2	3.5	6.7
Zn^{2+}	4.9	—	—				

Ligand to metal=2:1, $C_M=2\times10^{-3}M$, 30±0.5°, $\mu=0.1(KNO_3)$

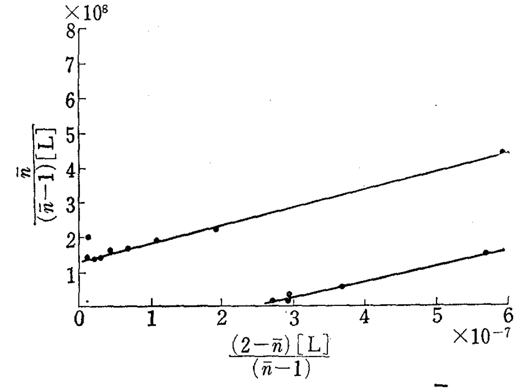

Fig. 3. Relation between $\frac{\bar{n}}{(\bar{n}-1)[L]}$ and $\frac{(2-\bar{n})[L]}{(\bar{n}-1)}$ in Copper Complexes of D-Glucosaminic Acid

$4\times10^{-3}M$ of D-glucosaminic acid, $2\times10^{-3}M$ of cupric ion.

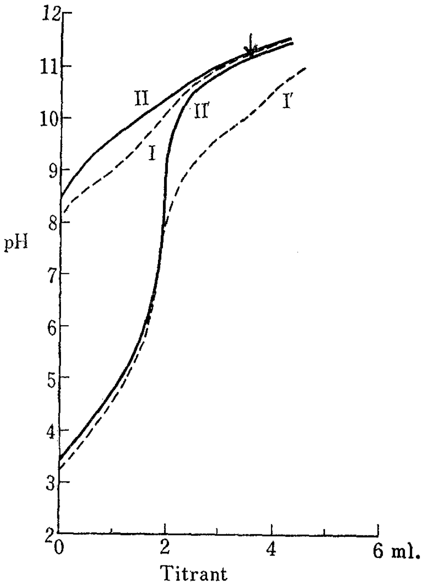

Fig. 4. Titration Curves of D-Glucosaminic Acid and Glycine in the Presence of Cupric Ion

I : D-glucosaminic acid
I' : D-glucosaminic acid+cupric ion
II : glycine
II' : glycine+cupric ion
Ligand to cupric ion, 2:1, ligand concentration: $4\times10^{-3}M$
Temp.: 30±0.5°, $\mu=0.1$ (KNO_3)
Titrant: $0.1M$ NaOH

The Comparison of Copper Complex Formation of Glucosaminic Acid with That of Glycine

In order to investigate the role of OH groups of glucosaminic acid in copper complex formation, its titration curves in the presence and in the absence of cupric ion were compared with those of glycine under the same conditions. Fig. 4 shows that the titration curves of both amino acids are similar except in the alkaline region of their copper complex where pH depression occurs only with glucosaminic acid. Moreover, a precipitation of hydroxide or hydrolysis products of complex is observed only in the glycine solution.

These results suggest the contribution of OH groups of glucosaminic acid in alkaline regions to the complex formation by their dissociation and coordination to copper.

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Summary

Stability constants, log K_1 and log K_2, of complexes of D-glucosaminic acid with Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, and Mn^{2+} were determined by pH titration method. Copper complexes were found to be the most stable and the contribution of OH groups of glucosaminic acid to the complex formation was observed.

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19. Motoichi Miyazaki, Toshio Imanari, Tamiko Kunugi, and Zenzo Tamura: Gas Chromatography of Copper (II) and Nickel (II) Chelates of Some β-Ketoimine Derivatives of 2,4-Pentanedione and Salicylaldehyde.[*1]

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Recently, gas chromatography has been applied to various volatile metal chelates of 2,4-pentanedione and of its fluoro derivatives, and many investigators[1~10] have suggested that gas chromatography would be very useful for the separation of metal chelates or the micro analysis of metals. However, the study of chelates of other types of ligand than 2,4-pentanedione and its fluoro-derivatives have not been reported.

[*1] A preliminary report was presented at the 84th Annual Meeting of Pharmaceutical Society of Japan in Tokyo (April, 1964).

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