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45. Zenzo Tamura, Motoichi Miyazaki, and Takamasa Suzuki: Metal Complexes of D-Glucosamine and its Derivatives. III.*1 Determinations of Acid Dissociation Constants of D-Glucosamine and its Three O-Methyl Derivatives.*2

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D-Glucosamine (2-amino-deoxy-D-glucose) (I), a component of biologically significant materials, has a tendency to form metal complexes owing to its amino and hydroxyl groups, but very few studies have been reported concerning their metal complex formation. Moreover, any quantitative understanding as to the nature of these complexes has never been obtained.

A series of the present works deal with an investigation on the complexes of I and its modifications with some metallic ions formed under biologically similar conditions.

This paper is concerned with the determinations of pKa values of I and its three O-methyl derivatives; methyl-β-D-glucosaminide (II), 3-O-methyl-D-glucosamine (III) and 3,4,6-tri-O-methyl-D-glucosamine (IV) by pH titration method.

Since these compounds had aldehyde groups except II and they might be decomposed with alkali in aqueous solution, the stability of I against alkali was examined by the improved Fehling method[1] preceding to the pH titration, representing these compounds.

Stability of I being checked, pH titrations were carried out to determine pKa values.

Experimental

D-Glucosamine (I)——Hydrochloride of I was supplied through the courtesy of the Chugai Pharmaceutical Co., Ltd., and it was recrystallized from aqueous MeOH and dessoicated over $CaCl_2$ *in vacuo*. m.p. 160° (decomp.). *Anal.* Calcd. for $C_6H_{13}O_5N \cdot HCl$: C, 33.42; H, 6.54; N, 6.50. Found: C, 33.49; H, 6.25; N, 6.63.

Methyl-β-D-glucosaminide (II)——Its hydrochloride was synthesized from I by the method of Viscontini and Meier.[2] m.p. 189° (decomp.). *Anal.* Calcd. for $C_7H_{15}O_5N \cdot HCl$: C, 36.63; H, 6.97; N, 6.10. Found: C, 36.61; H, 7.29; N, 6.14.

3-O-Methyl-D-glucosamine (III)——Its hydrochloride was synthesized from I by the method of Neuberger.[3] m.p. 215° (decomp.). *Anal.* Calcd. for $C_7H_{15}O_5N \cdot HCl$: C, 36.63; H, 6.97; N, 6.10. Found: C, 36.34; H, 7.13; N, 6.01.

3,4,6-Tri-O-methyl-D-glucosamine (IV)——Its hydrochloride was synthesized by the method of Neuberger.[4] m.p. 215° (decomp.). *Anal.* Calcd. for $C_9H_{19}O_5N \cdot HCl$: C, 41.94; H, 7.82; N, 5.43. Found: C, 42.05; H, 7.96; N, 5.29.

Apparatus and Procedure

Examination of Stability of I——Four kinds of sample solution of I in $10^{-3}M$, being adjusted to pH 5.80, 7.40, 8.15 and 10.77 respectively with sodium hydroxide, were maintained at $30 \pm 2°$ for one hour and the effect of pH change on aldehyde content of I was examined. The determination was carried out by the improved Fehling method,[1] and the effect of time elapse on standing of I at pH 8.2 was also examined by the same procedure.

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*1 Part I and II: Z. Tamura, M. Miyazaki: Japan Analyst, **12**, 470, 561 (1963).

*2 Presented before the 82nd Annual Meeting for the Pharmaceutical Society of Japan, Shizuoka, November, 1962.

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1) Z. Tamura, M. Miyazaki: Japan Analyst, **12**, 470, 561 (1963).
2) M. Viscontini, J. Meier: Helv. Chim. Acta, **35**, 807 (1952).
3) A. Neuberger: J. Chem. Soc., **1941**, 50.
4) *Idem*: *Ibid.*, **1940**, 29.

Determinations of pKa Values——The experimental method used in this investigation is the pH titration in aqueous solution by a standard base. The temperature was regulated at 30±0.5°, and the ionic strength was maintained relatively constant by using a medium of 0.10*M* sodium nitrate. Fifty ml. of $10^{-3}M$ sample solution was titrated with 0.1 *M* carbonate-free sodium hydroxide in nitrogen atmosphere by means of 10 ml. semi-micro burette, and the pH of the solution was measured with a Toa Dempa pH meter, Model HM-5A equipped with both of a glass and a calomel electrodes. The pH meter was calibrated in advance by the standard buffer solutions, 4.01 and 6.86 at 25°.

The Treatment of Data——The calculation of pKa values was practiced by an application of Schwarzenbach's method,[5] assuming that the materials were all mono basic acids. The validity of this assumption was ascertained by comparing the experimental curve with the one calculated theoretically.

The equations of Schwarzenbach's are as follows :

$$\sum_{j=0}^{m}(g-j)[H^+]\bar{K}^H_{Hjz}=0 \qquad (1)$$

when $m=1$, (1) becomes (2)

$$g+(g-1)[H^+]K^H_{Hz}=0 \qquad (2)$$

and

$$g=(m-a)+\frac{[OH^-]-[H^+]}{[Z_t]} \qquad (3)$$

where K^H_{Hz} : acid dissociation constant. (=Ka)
m : the maximum number of proton which is liberated.
g : the real degree of neutralization.
a : the apparent degree of neutralization.
$[Z_t]$: the total concentration of ligand.

During the calculation of pKa values, $\gamma=0.78$[6] and $K_{H_2O}=1.89\times10^{-14}$[7] were used for the activity coefficient of proton and the ionic product of water at 30°, respectively.

Results and Discussion

The Stability of I

The stability of I was examined by the improved Fehling method as described in this paper.

From the results of determination of aldehyde content, the experimental values which were obtained at a pH 5.80, 7.40, and 8.15 were found to be the same within experimental errors and coincided well with the theoretical amount calculated which was considered to be present in sample solutions. However, only in the case of pH 10.77, a slightly smaller value of aldehyde content was observed, and it might be considered that the aldehyde group would be influenced by hydroxide ion in such a stronger alkaline region.

Furthermore, the effect of time elapse against aldehyde group on keep standing I at pH 8.2 was studied with the same sample solutions by determining aldehyde content of I, and it was found that any changes of the experimental values was not observed within one hour.

Considering from these results, it may therefore be inferred that the aldehyde group of I will be kept intact, and hence, I will be stabilized in

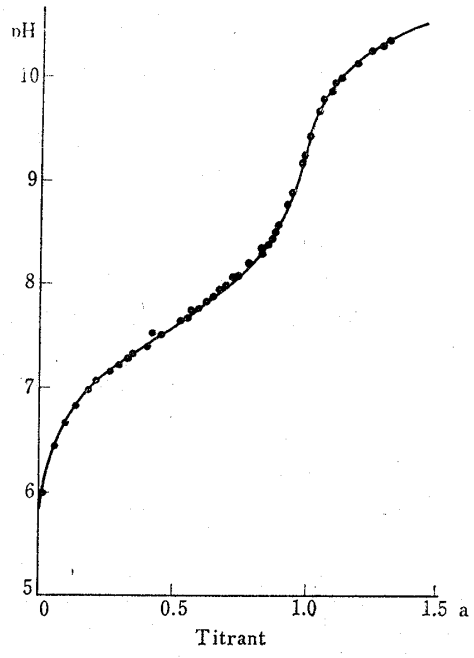

Fig. 1. Titration Curve of D-Glucosamine (I)
concentration : (I) (hydrochloride) $1.0315\times10^{-3}M$, temp. : 30±0.5°, ionic strength : $\mu=0.1(KNO_3)$, titrant : 0.1*M* NaOH
a=moles of base added per mole of I
• experimental point
—— theoretical line

5) G. Schwarzenbach : Helv. Chim. Acta, 33, 9479 (1950).
6) S. Chaberek, A. E. Martell : J. Am. Chem. Soc., **74**, 5057 (1952).
7) H. Yoshimura : "Theory and Measurement of pH" Appendix (1954). Maruzen Co., Ltd., Tokyo.

the solution until its hydrogen ion concentration reaches to the value of about pH 8 within one hour.

Thus, in determining pKa of I, II, III, and IV, calculations were made referring to the titration data which were obtained in the region from the pH at the beginning of the titration to about pH 8.

The Determinations of pKa

The titration curve of I is illustrated in Fig. 1. The curve has a buffering region in its shape and a relatively steep inflexion at a=1. The portion of the curve corresponding to the value from 0 to 1 indicates the neutralization of one mole of proton present in per mole of I during the titration. As to the titrations of II, III, and IV, similar curves to that of I were obtained.

From the above observations, such an assumption was made in order to calculate their pKa values that they were all mono basic acid and dissociated in aqueous solution according to the following equation :

$$S\text{-}\overset{+}{N}H_3 \overset{Ka}{\rightleftarrows} S\text{-}NH_2 + H^+ \qquad Ka=\frac{[S\text{-}NH_2][H^+]}{[S\text{-}NH_3^+]}$$

where, S stands for the sugar moiety of these compounds and Ka shows the acid dissociation constant.

In order to verify the assumption, an examination was attempted with I, representing these four compounds.

The titration curve of I was compared with its theoretical curve calculated from the pKa determined experimentally.

The results are shown in Fig. 1 and it was observed that these two curves agreed well and that the assumption mentioned previously was valid for I under the experimental conditions.

Therefore, it may be conceivable that all of these four compounds behave as mono basic acid during the titration.

As pKa values are shown in Table I, an introduction of methyl groups into D-glucosamine molecule seems to increase the acidity of this type of compound, and therefore, smaller pKa values than that of I are obtained. The mechanism of the effect of methyl groups has not been clarified at present.

TABLE I. pKa Values of Four D-Glucosamine Compounds
temp.=30±0.5°; μ=0.1 (KNO_3); pH range about 7.0~8.6

Material	pKa	Material	pKa
D-Glucosamine (I)	7.47	3-O-Methyl-D-glucosamine (III)	7.10
Methyl-β-D-glucosaminide (II)	7.15	3,4,6-Tri-O-methyl-D-glucosamine (IV)	6.92

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Summary

The acid dissociation constants of D-glucosamine (I) and its three O-methyl derivatives were determined by titration method. The stability of I was also studied representing these compounds by the improved Fehling method preceding to the pH titration, and I was found to be stable in aqueous solution under experimental conditions. From the analysis of the titration data, I could be verified to behave as a mono basic acid by the comparison of the experimental titration curve with the one theoretically calculated. Finally, the pKa values of these four compounds were calculated.

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