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A Kinetic Study of the Mutarotation of D-Xylose by the Polarographic Method[1]

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The polarographic behavior of D-xylose in several buffer solutions was investigated. The rate constants, k_1, k_2, k_{-1}, and k_{-2}, of the mutarotation of α-D-xylose, assumed as $\alpha \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \gamma \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} \beta$, were calculated by the use of Wiesner's method, but with a modified equation for the polarographic current. The overall mutarotation rate constant, k_0, which is defined by $k_0=(k_1k_{-2}+k_{-1}k_2)/(k_{-1}+k_{-2})$, was also determined. The rate constants, k_0, k_1, and k_2, were analyzed as a linear function of the buffer concentration. It was revealed that the catalytic effects of the buffer salts were mainly due to their basic components for all the buffer salts investigated. The Brönsted plots of k_1 and k_2 for all the basic components gave straight lines with the same slope of 0.42. The rate constants for several amines deviated from the slope; this deviation was ascribed to the steric hindrance caused by their large molecular sizes.

The mutarotation of reducing sugar in an aqueous solution is catalyzed by the use of acids and bases.[2-4] The measurements of the mutarotation velocity are usually made by polarimetry, NMR and other such methods, but the results are in most cases interpreted in terms of the mutarotation rate constant, k_0, defined by $k_0=k_\alpha+k_\beta$, where the reaction mechanism is assumed to be as in Eq. (1):

$$\alpha\text{-pyranose} \underset{k_\beta}{\overset{k_\alpha}{\rightleftharpoons}} \beta\text{-pyranose} \tag{1}$$

Los, Simpson, and Wiesner[5] have shown that, by the use of the polarographic method, four individual rate constants, k_1, k_2, k_{-1} and k_{-2}, of the mutarotation assumed as in Eq. (2):

$$\alpha\text{-pyranose} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \gamma\text{-form} \underset{k_2}{\overset{k_{-2}}{\rightleftharpoons}} \beta\text{-pyranose} \tag{2}$$

can be determined; here, the intermediate γ-form is assumed to be an electroactive free aldehyde form, and the polarographic current produced by a monosaccharide, such as D-glucose, is controlled by the kinetics of the formation of the γ-form from α- and β-pyranose. Experimental study of the isotope exchange of D-glucose C-1-^{18}O also supports the idea that the formation of the aldehydrol form is negligible in mutarotation kinetics.[2]

In this study, the individual rate constants of the base-catalyzed mutarotation of α-D-xylose are determined by the polarographic method; this method is based on Wiesner's assumption, but with a modified equation for the polarographic current. The results are discussed in view of the base-catalyzed reaction.

Expression for The Polarographic Current of D-*Xylose.* According to Los, Simpson, and Wiesner, the polarographic kinetic current, $\bar{i}_k$, of monosaccharide when it is present in two forms, *e. g.*, α- and β-pyranose at the concentrations of C_α and C_β, is given by:

$$\bar{i}_k = nF\bar{q}(D/(k_{-1}+k_{-2}))^{1/2}(k_1C_\alpha+k_2C_\beta) \tag{3}$$

where n denotes the number of electrons consumed in the electrolysis per molecule; F, the Faraday; $\bar{q}$, the mean electrode surface area, and D, the diffusion coefficient of the monosaccharide. Eq. (3) was first derived by assuming a reaction layer on the electrode surface, the thickness of which was given by:

$$\mu = (D/(k_{-1}+k_{-2}))^{1/2} \tag{4}$$

We ourselves have previously shown[6] that Eq. (3) can be derived by a rigorous mathematical procedure on the following assumption:

$$\tau(k_{-1}+k_1)\gg 1 \qquad \tau(k_{-2}+k_2)\gg 1, \tag{5}$$

τ being the drop time, and

$$K_\alpha = k_{-1}/k_1 \gg 1 \qquad K_\beta = k_{-2}/k_2 \gg 1, \tag{6}$$

while k_1/k_2 is not extremely different from unity. This is actually the case with D-glucose.[5] A similar argument has also been advanced by Paldus and Koutecký.[7]

As will be demonstrated later in this study, however, the rate constants for D-xylose are not so large as Eq. (5) predicts. Accordingly, a modified equation has to be used in analyzing the polarograpnic current of D-xylose.

By a close investigation of the rigorous mathematical solution for the polarographic kinetic current reported by Koutecký and Brdička,[8] and by assuming the reaction layer given by Eq. (4), we may derive a modified equation of the polarographic current:

$$\bar{i}_l = \bar{i}_k + \bar{i}_{corr} \tag{7}$$

with:

$$\bar{i}_{corr} = \frac{\bar{i}_d}{K_\alpha+K_\beta} \times \frac{1-\exp(-\tau(k_{-1}+k_{-2}))}{\tau(k_{-1}+k_{-2})} \times \frac{1.13}{1.13+(\tau(k_{-1}+k_{-2}))^{1/2}} \tag{8}$$

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[1] Presented at 17th. Ann. Symp. Polarography, Fukuoka, Oct. 16—18, 1971.

[2] W. Pigman and H. S. Isbell, "Advances in Carbohydrate Chemistry," Vol. 23, ed. by M. L. Wolfrom, and R. S. Tipson, Academic Press. London (1968), p. 11.

[3] H. S. Isbell and W. Pigman, "Advances in Carbohydrate Chemistry and Biochemistry," Vol. 24, ed. by M. L. Wolfrom, and R. S. Tipson, Academic Press. London. (1969), p. 13.

[4] B. Capon, *Chem. Rev.*, **69**, 407 (1969).

[5] J. M. Los, L. B. Simpson, and K. Wiesner, *J. Amer. Chem. Soc.*, **78**, 1564 (1956).

[6] M. Senda, *Rev. Polarogr.* (Kyoto), **6**, 95 (1958).

[7] J. Paldus and J. Koutecký, *Collect. Czech. Chem. Commun.*, **23**, 376 (1958).

[8] J. Koutecký and R. Brdička, *ibid.*, 12, 337 (1947).

and:

$$\bar{\imath}_d = \kappa(C_\alpha + C_\beta) \qquad \kappa\text{: Ilkovič constant,} \tag{9}$$

under the following conditions:

$$(\tau(k_{-1}+k_{-2}))^{1/2}/(K_\alpha + K_\beta) \ll 1 \tag{10}$$

and:

$$K_\alpha \gg 1, \qquad K_\beta \gg 1 \tag{11}$$

In these equations, $\bar{\imath}_k$ is defined by Eq. (3), $\bar{\imath}_{corr}$ is the correction term, and $\bar{\imath}_d$ is the diffusion current given by the Ilkovič equation, with the total bulk concentration of monosaccharide, being $C_\alpha + C_\beta$. Some details of the derivation of Eq. (7) are given in the Appendix.

As the mutarotation takes place in the bulk of the solution, the concentrations of α- and β-pyranose, C_α, and C_β, change with the time. For example, if we dissolve α-D-xylose in a solution at time $t=0$, C_α decreases with the time, whereas C_β increases with the time, in accordance with the following equation:[5]

$$C_\alpha = (C_\alpha)_{t\to\infty}(1+K\exp(-k_0 t)) \tag{12}$$

$$C_\beta = (C_\beta)_{t\to\infty}(1-\exp(-k_0 t)) \tag{13}$$

where:

$$k_0 = (k_1 k_{-2} + k_{-1} k_2)/(k_{-1}+k_{-2}), \tag{14}$$

$$K = (C_\beta/C_\alpha)_{t\to\infty} = (C_\beta)_{t\to\infty}/(C_\alpha)_{t\to\infty} = K_\beta/K_\alpha, \tag{15}$$

and:

$$C_\alpha + C_\beta = C_{total} = (C_\alpha)_{t=0}, \tag{16}$$

where the concentration of the intermediate γ-form is assumed to be negligibly small. That is, the polarographic current, which is given by Eq. (7) or Eq. (3), changes with the time. The combination of Eqs. (12) to (16) with Eq. (7) to analyze the polarographic current, that was recorded as a function of the time, makes it possible to compute the four rate constants, k_1, k_{-2}, k_{-1}, and k_2, in Eq. (2).

Experimental

Materials. The α-D-xylose was recrystallized from an aqueous solution;[10] $[\alpha]_D^{25} = +96$, mp 146 °C, mutarotation rate const. $k_0 = 1.30\times10^{-3}\ s^{-1}$ in water at 25±0.5 °C. All the other chemicals used were of a reagent grade. The solutions were prepared with bidistilled water. The buffer components were NaH_2PO_4–Na_2HPO_4, NH_3–NH_4Cl, Trys(hydroxymethyl)amino methane (TRIS), Tris(hydroxymethyl)methyl glycine (TRICINE), and N,N-bis(2-hydroxyethyl)glycine (BICINE). Potassium chloride was used to adjust the ionic strength to the desired values (usually 0.5).

Equipment. A Yanagimoto polaro-recorder type PA-103 was used for all the polarographic measurements. A Yanagimoto controlled-potential electrolyzer, type VE-3, was used for controlled-potential electrolysis, while the polarimetric measurements were carried out with a Yanagimoto polarimeter, type OR-20. The pH values were measured with a Hitachi Horiba M-5 pH meter.

An electrolysis cell (20 ml in capacity) was used; it was connected with a saturated calomel electrode (SCE) by means of an agar-gelatin bridge containing potassium chloride. All the experiments were carried out in a water thermostat controlled at 25±0.05 °C.

Method. A supporting electrolyte solution (15 ml) of the desired composition was freed of oxygen by passing a nitrogen stream through the solution. First, the residual current was recorded by means of the polarograph. Second, after the potential of the dropping mercury electrode has been set at −1.75 V *vs.* SCE, a weighed amount of α-D-xylose was dissolved in the deoxygenated electrolyte solution; the passing of the nitrogen stream through the solution was continued for about 30 seconds to complete the dissolution of the α-D-xylose. Then, the current intensity at −1.75 V *vs.* SCE was recorded as a function of the time. The recording of the current was continued until the current intensity reached a constant value. The nitrogen stream was passed over the solution during the measurement. Finally, after the mutarotation eqilibrium had been reached, a whole polarographic wave of the D-xylose was recorded. The current intensity was corrected for the residual current.

Results

General Polarographic Behavior of D-*Xylose.* The polarographic behavior of an equilibrated mixture of D-xylose was studied. At pH values lower than 9.5, no appreciable change in the polarogram of equilibrated solution of D-xylose was observed, at least not within two hours, at 25 °C. No appreciable change in specific rotation was observed, either. These results suggest that no hydrolysis, carbonyl amino reaction, or any complicated reaction of D-xylose occurs under the conditions used in the present experiment.

A few examples of the experimental results on the dependence of the limiting current, $\bar{\imath}_l$, on the height of the mercury reservior, h, are shown in Table 1. The limiting current was inclined to increase slightly with an increase in the height of the mercury reservior. An analysis of the current-time (i-t) curve for single drop has shown that the slope of the log i *vs.* log t plot is 1.8/3, which is slightly smaller than the theoretical value, 2/3, which is expected for a purely kinetic current defined by Eq. (3). These results support

Table 1. Dependence of the limiting current $\bar{\imath}_l$ on the height of mercury reservior h

The concentration of D-xylose 0.066 M, Temp. 25 °C

h (cm)	$\bar{\imath}_l$ (cm)[a] Phosphate buff. 0.05 M, pH 6.99	TRIS buff. 0.05 M, pH 8.04	TRIS buff. 0.20 M, pH 8.04
62.4	2.3_6	4.4_0	4.8_1
72.4	2.4_7	4.5_2	5.0_1
82.4	2.4_5	4.6_0	5.0_2
c.f. $\bar{\imath}_l/\bar{\imath}_d$[b]	2.96×10^{-3}	5.67×10^{-3}	6.28×10^{-3}

a) Current sens. 0.4 μA/cm.

b) i_d: Hypothetical diffusion current of D-xylose.

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[9] Recently Nishihara and Matsuda have carried out more rigorous mathematical analysis of the problem by use of an electronic computer (Presented at 17th. Ann. Symp. Polarography, Fukuoka, Oct. 16—18, 1971). Preliminary examination showed that the errors resulted by use of Eqs. (7) to (9) did not exceed 8% for $(k\tau)^{1/2}<2$, under the condition (10) and (11).

[10] K. Anno and N. Seno, "Jikken Kagaku Koza", Vol. 23, ed. by S. Akabori and S. Funahashi, (in Japanese) Maruzen, Tokyo, (1957), p. 336.

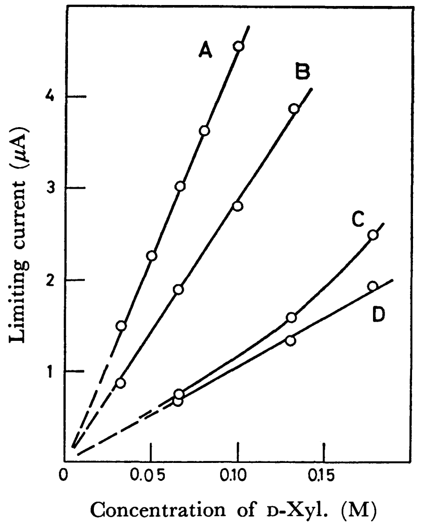

Fig. 1. Relationships between limiting current and the concentration of D-xylose. 25 °C, A: Ammonia buff. 0.100 M pH 8.79, B: TRIS buff. 0.020 M, pH 8.25, C: Phosphate buff. 0.005 M, pH 6.99, D: Phosphate buff. 0.070 M, pH 6.12.

the idea that the correction term, $\bar{i}_{\mathrm{corr}}$, in Eq. (7) should not be neglected in interpreting the kinetic current produced by D-xylose.

The limiting current is proportional to the D-xylose concentration in various supporting electrolyte solutions, as is shown by Curves A, B, and D in Fig. 1, but at a very low concentration of the buffer component a deviation from the linearity was observed, as is exemplified by Curve C in Fig. 1. Table 2 and Fig. 2 show the effects of the buffer concentration and the pH on the limiting current. In a basic solution, the limiting current increased exponentially with the pH. In an ammonia buffer solution with a pH of about 9, the limiting current decreased with a decrease in the concentration of the buffer component. On the contrary, in the phosphate buffer solution, the limiting current first decreased with a decrease in the concentration of the buffer component, but at a lower concentration (0.005 M) it began to increase and finally reached an unusually large value in an unbuffered solution. This last result might be explained by the autocatalytic effect of the hydroxy anion produced in company with the electro-reduction of D-xylose at the electrode surface. A simlar phenomenon has been reported by Brdička and his coworker[11–13] for the electro-reduction of formaldehyde at the dropping mercury electrode in an unbuffered solution.

TABLE 2. THE LIMITING CURRENT WITH VARIOUS BUFFER CONCENTRATIONS

Ammonia buff. pH 9.40 D-Xyl. 0.040 M	$\bar{i}_l$ (μA)	Phosphate buff. pH 6.99 D-Xyl. 0.066 M	$\bar{i}_l$ (μA)
0.533 M	4.01	0.100 M	1.06
0.268	3.44	0.050	0.92
0.133	3.03	0.010	0.68
0.067	2.78	0.005	0.67
0.033	2.68	0.001	1.22
		none	9.68

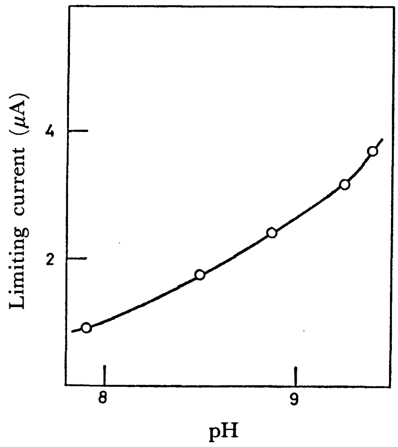

Fig. 2. The dependence of the limiting current on pH. D-xylose: 0.04 M, Ammonia buff.: 0.50 M, 25 °C.

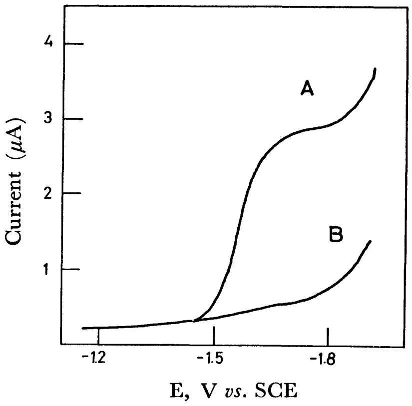

Fig. 3. Controlled potential electrolysis of D-xylose at mercury cathode −1.8 V *vs.* SCE. Ammonia buff.: 1.0 M, pH 9.5, 25 °C. A: 0.05 M D-xylose, B: After about nine hours of electrolysis.

A controlled potential electrolysis of D-xylose was carried out at the mercury-pool cathode in an ammonia buffer solution with a pH of 9.5. After about nine hours of electrolysis at −1.8 V *vs.* SCE, the kinetic wave of D-xylose disappeared almost completely (Fig. 3). During the electrolysis, the generation of bubbles was observed on the mercury-cathode surface. This may be attributed to the evolution of hydrogen, which occurs parallel with the reduction of D-xylose. The electrolyzed solution was paper-chromatographed,[14] and the spot of D-xylitol was identified on the chromatogram. Provided that the number of electrons consumed per molecule of D-xylose is 2, calculation showed that about two thirds of the total electricity was consumed by the reduction of the D-xylose and one third, by the reduction of the hydrogen

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11) R. Brdička, *Collect. Czech. Chem. Commun.*, **20**, 387 (1955).

12) R. Brdička, *Z. Electrochem.*, **59**, 787 (1955).

13) R. Brdička and L. Němec, *Rev. Polarogr.* (Kyoto), **11**, 5 (1963).

14) Ref. 10) p. 392.

Table 3. Buffer solutions
The concentration of D-xylose 0.132—0.066 M
Temp. 25 °C, Ionic strength 0.5

Buffer	Base Component	pK_A	Concentration (mol/l)	pH
Phosphate	HPO_4^{2-}	7.2	0.03—0.10	6.12—6.99
TRICINE	$(CH_2OH)_3\text{-}CNH$ $-OOCCH_2$	8.1	0.02—0.20	7.88—8.42
TRIS	$(CH_2OH)_3\text{-}CNH_2$	8.2	0.02—0.20	8.04—8.42
BICINE	$(C_2H_4OH)_2\text{-}N$ $-OOCCH_2$	8.3	0.02—0.15	8.10—8.92
Ammonia	NH_3	9.2	0.05—0.50	8.16—8.59

Table 4. The overall and individual rate constants in various buffer solutions

	Phosphate 0.05 M pH 6.99	TRICINE 0.10 M pH 8.22	TRIS 0.10 M pH 8.27	BICINE 0.10 M pH 8.29	Ammonia 0.10 M pH 8.59
k_0 s^{-1}	9.1×10^{-3}	5.3×10^{-3}	6.5×10^{-3}	3.4×10^{-3}	8.7×10^{-3}
k_1 s^{-1}	16.2×10^{-3}	8.1×10^{-3}	9.9×10^{-3}	4.5×10^{-3}	15.9×10^{-3}
k_2 s^{-1}	5.0×10^{-3}	3.2×10^{-3}	3.8×10^{-3}	2.2×10^{-3}	4.7×10^{-3}
k_{-1} s^{-1}	15.2	0.9	1.3	0.4	2.1
k_{-2} s^{-1}	8.6	0.7	0.9	0.4	1.1
$K_\alpha+K_\beta$	2.7×10^3	3.3×10^2	3.7×10^2	2.7×10^2	3.7×10^2

ion, in the controlled-potential electrolysis on the mercury-pool electrode at −1.8 V.

Determination of Mutarotation Rate-Constants of α-D-Xylose. The composition and pH's of the buffer solution in which the mutarotation rate-constants were determined are summarized in Table 3. These conditions have been selected to avoid complexities arising from too high a basisity or too low a buffer concentration, as has been described above. Too high a concentration of buffer components resulted in an increase in the final ascending of the base current and made accurate measurement of the limiting current difficult.

The experimental results were analyzed by the use of the theoretical equations given above, and the individual rate constants, k_1, k_2, k_{-1}, and k_{-2}, were computed. In applying Eqs. (7), (8) and (4), a successive approximation method was employed. The computation was carried out by the use of an electronic computer, FACOM 230—60 (Kyoto University). The diffusion coefficient, D, was assumed to be 6.58×10^{-6} cm^2 s^{-1},[15] and the equilibrium constant, K (defined by Eq. (15)), to be 1.873.[2]

Examples of the four individual rate constants, k_1, k_2, k_{-1}, and k_{-2}, and the overall rate-constant, k_0, defined by Eqs. (2) and (14), are shown in Table 4. In general, the backword or ring-closing rate constants, k_{-1} and k_{-2}, could be determined with only a poor precision. Accordingly, a detailed analysis of the rate constants was made with the forward or ring-opening rate constants, k_1 and k_2, and the overall rate constant, k_0.

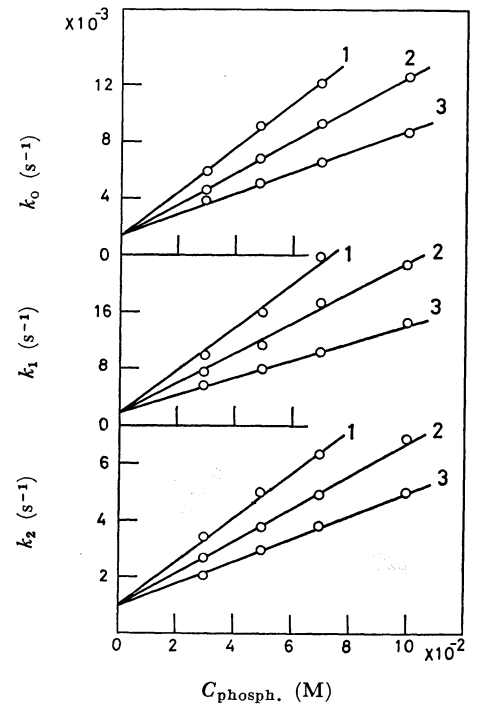

Fig. 4. The rate constants as a function of the concentration of phosphate buffer, C_{phosph}. 25 °C, 1: pH 6.99, 2: pH 6.59, 3: pH 6.12.

At a constant pH, the rate constants, k_0, k_1, and k_2, increased linearly to the buffer concentration. Some representative results are shown in Fig. 4 and Fig. 5.

Generally, the rate constant of a catalyzed reaction can be expressed as follows:[3]

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[15] L. Friedman and P. G. Carpenter, *J. Amer. Chem. Soc.*, **61**, 1745 (1939).

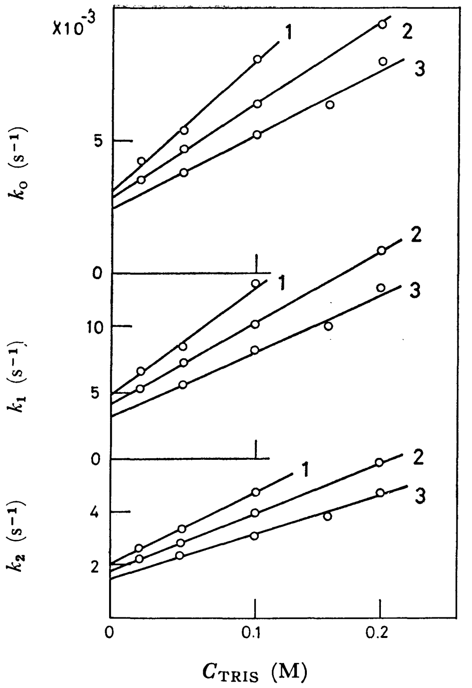

Fig. 5. The rate constants as a function of the concentration of TRIS buffer, C_{TRIS}. 25 °C, 1: pH 8.42, 2: pH 8.27, 3: pH 8.04.

$$k_i = k_{i,w} + k_{i,OH}(OH^-) + k_{i,A}(A) + k_{i,B}(B) \qquad (17)$$

$$i=0,\ 1 \text{ and } 2$$

where $k_{i,w}$ represents the catalytic constant of the solvent molecule (water in our case), where $k_{i,OH}$, $k_{i,A}$, and $k_{i,B}$, represent the catalytic coefficients of the catalysts indicated by the subscripts, and where the symbols in brackets represent the concentrations (activities) of the catalysts, OH^-, A, and B, being the hydroxyl ion, acid, and the base of buffer components. In this expression, the concentration of the hydrogen ion is neglected because the measurements were made in neutral or basic solutions. The contribution of the D-xylose anion is also neglected. The significance of this approximation will be discussed later.

When the rate constant is plotted against the sum of the concentrations of the buffer components, $C_{buff}=(A)+(B)$, at a given pH, a straight line is obtained; the slope of this line is $(k_{i,A}M+k_{i,B})/(1+M)$, M being $(A)/(B)$, and the intercept on the k_i-axis is $k_{i,w}+k_{i,OH}$ (OH^-). A plot of this intercept against (OH^-) will give a straight line, from which $k_{i,OH}$ (slope) and $k_{i,w}$ (intercept) can be obtained. Fig. 4 shows a plot of k_i *vs.* C_{buff} for phosphate buffer solutions of pH 6.12, 6.59, and 6.99. Three straight lines with different slopes are obtained, but their intercepts on the k_i-axis coincide within the limits of experimental error. These results suggest that $k_{i,OH}(OH^-)$ is negligible in comparison with $k_{i,w}$ in such neutral solutions. Fig. 5 shows the same plot for the TRIS buffer; straight lines with different slopes and different intercepts on the k_i-axis are given for each solution at a given pH. Similar results were obtained for all the other basic buffers. The intercept values thus obtained from the k_i *vs.* C_{buff} curves are plotted against (OH^-) in Fig. 6. In accordance with theoretical considerations, all the data are distributed along the regression line, from which $k_{i,w}$ and $k_{i,OH}$ for overall as well as individual reaction rates are determined. The $k_{i,w}$ value obtained in this way is in good agreement with those obtained from the intercepts in Fig. 4 (*e.g.* $k_{o,w}=1.3\times10^{-3}\,s^{-1}$ from Fig. 6 and $k_{o,w}=1.4\times10^{-3}\,s^{-1}$ from Fig. 4).

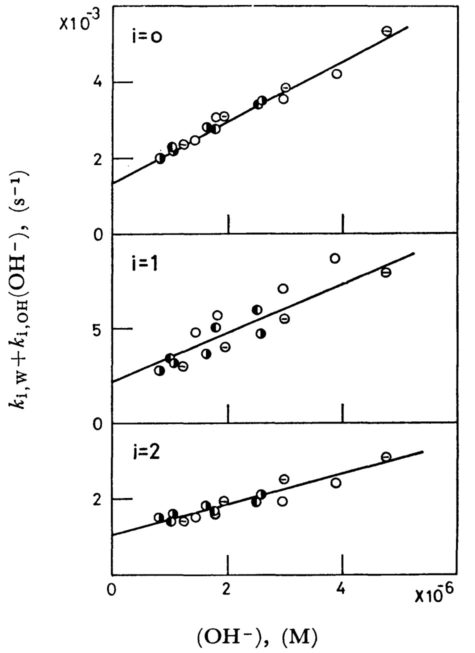

Fig. 6. The plot of $k_{i,w}-k_{i,OH}(OH^-)$ against the concentration of hydroxy ion, (OH^-).
○: NH_3, ◐: TRIS, ◑: TRICINE, ⊖: BICINE

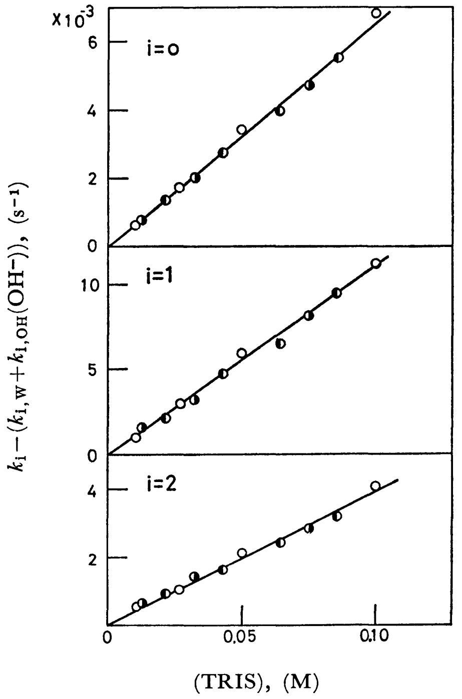

Fig. 7. The plot of $k_i-(k_{i,w}+k_{i,OH}(OH^-))$ against the concentration of the base conponent of TRIS buffer. (TRIS).
○: $M=1$, ◑: $M=4/6$, ◐: $M=6/4$.

Figure 7 shows a plot of $k_i-(k_{i,w}+k_{i,OH}(OH^-))$ against (B) for the TRIS buffer. A straight line with a slope of $k_{1,B}+M\times K_{1,A}$ can be expected from each for a given M. As may be seen from the figure, however, all the points lie on a single straight line, irrespective of the M values. This suggests that $k_{1,B}\gg M\times k_{1,A}$ *i. e.*, that $k_{1,A}$ is negligibly small compared with $k_{1,B}$ for the buffer components examined. Similar results were obtained for all the other basic buffers examined.

Discussion

The values of the catalytic rate coefficients are summarized in Table 5. The coefficients, $k_{1,B}$'s, for B=TRIS, TRICINE, and BICINE are those for the base components of the amine buffers. The water molecule is both an acid and a base, but for the mutarotation of D-glucose in a neutral solution, the basic catalytic function is supposed to predominate over the acid one.[3] For the mutarotation of D-xylose, the same argument is justified on the basis of the Brönsted plot (Fig. 8). The HPO_4^{2-} and $H_2PO_4^-$ ions are also bifunctional, but in a neutral buffer solution the former ion functions as a base, and the latter, as an acid. Accordingly, their catalytic activities, as observed in a neutral solution, should in the first place be basic for HPO_4^{2-} and acidic for $H_2PO_4^-$. It may be seen in Table 5 that, except for the rate coefficients for $H_2PO_4^-$, the rate coefficients, $k_{1,B}$, for the $\alpha\rightarrow\gamma$ reaction are always larger than the $k_{2,B}$ for $\beta\rightarrow\gamma$, where B is a base catalyst. In Fig. 8, log $k_{1,B}$ and log $k_{2,B}$ are plotted against the pK_A value of the base catalyst, B. The rate coefficients for the water molecule, assumed to be a base catalyst, were calculated by means of $k_{i,w}/55.6$ M and plotted against pK_A= $-\log 55.6$.[3] The rate coefficients for H_2O, HPO_4^{2-}, NH_3 and OH^- are on a straight line with a slope of 0.42. The value of this slope seems reasonable in view of the value, 0.40 or 0.34, observed for the overall mutarotation rate constants of D-glucose.[3] The rate coefficients for the base components of TRIS, TRICINE, and BICINE are smaller than the theoretical values expected from the Brönsted plot and their pK's, and become smaller with an increase in their molecular size. These results should be attributed to the steric hindrance effect in the catalytic mutarotation reaction.[16,17] Smith[18] and Los and Simpson[19,20] have pointed out that the catalytic effect caused by sugar anion can generally not be neglected. According to these authors, the rate coefficient for (OH^-) in Eq. (17) should be rewritten as:

$$k_{i,OH} = k'_{i,OH} + k_{i,xyl}(\text{xyl})K_{xyl}/K_w$$

where k'_{OH} and $k_{i,xyl}$ are the true rate coefficients for OH^- and the xylosate anion respectively, where (xyl) is the concentration of D-xylose where $K_{xyl}(=10^{-12.3})$[21] is the dissosiation constant of the Xylose$\rightleftharpoons$Xylosate Anion+H^+ reaction, and where K_w is the ionic product of water. Appling the Brönsted rule (Fig. 8) to D-xylose, we obtain $k_{1,xyl}\sim 30\ s^{-1}$ l/mol. In our experiments, (xyl) was 0.06 M, so that $k_{1,xyl}$ (xyl) $K_{xyl}/K_w\sim$ 90 s^{-1} l/mol, which is approximately 8% or less of $k_{1,OH}$. Accordingly, the effect by the xylosate anion may be considered to be of secondary significance in our case. In reality, no appreciable experimental indication was given of the significant contribution of the xylosate anion to catalysis, such as a nonlinear dependence of the polarographic current on (xyl).

Table 5. The overall and the forward rate coefficients of the mutarotation of D-xylose (Temp. 25 °C)

	i		
	o	1	2
$k_{i,w}$ (s^{-1})	1.4×10^{-3} a)	2.4×10^{-3}	1.0×10^{-3}
$k_{i,H_2PO_4^-}$ (s^{-1} l mol^{-1})	0.2×10^{-2}	0.3×10^{-2}	1.7×10^{-2}
$k_{i,HPO_4^{2-}}$ (s^{-1} l mol^{-1})	2.1×10^{-1}	4.1×10^{-1}	1.0×10^{-1}
$k_{i,TRICINE}$ (s^{-1} l mol^{-1})	4.2×10^{-2}	7.7×10^{-2}	2.4×10^{-2}
$k_{i,TRIS}$ (s^{-1} l mol^{-1})	6.7×10^{-2}	1.1×10^{-1}	4.2×10^{-2}
$k_{i,BICINE}$ (s^{-1} l mol^{-1})	1.0×10^{-2}	2.0×10^{-2}	7.5×10^{-3}
k_{i,NH_3} (s^{-1} l mol^{-1})	4.7×10^{-1}	7.6×10^{-1}	2.5×10^{-1}
$k_{i,OH}$ (s^{-1} l mol^{-1})	8.0×10^{2}	1.2×10^{3}	4.0×10^{2}

a) $1.3\times10^{-3}s^{-1}$ was obtained by polarimetry.

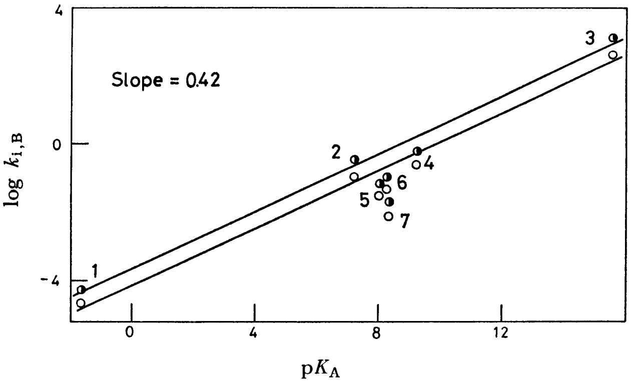

Fig. 8. Brönsted plot for the mutarotation of D-xylose. ◑: k_1, ○: k_2, 1: H_2O, 2: HPO_4^{2-} 3: OH^-, 4: NH_3, 5: TRICINE, 6: TRIS, 7: BICINE.

The equilibrium constants of the γ-form to the α- and β-pyranose forms, $K_\alpha+K_\beta$, are given in Table 4. These values were found to change slightly with the change in the buffer concentration; for example, $K_\alpha+K_\beta$ for the TRIS buffer changed 4.5×10^2 to 2.5×10^2 with the change in the buffer concentration from 0.20 to 0.02 M, while $K_\alpha+K_\beta$ for the phosphate buffer changed from 4.2×10^3 to 2.1×10^3 with the change in the buffer concentration from 0.10 to 0.03 M. A similar downward trend of $K_\alpha+K_\beta$ with a decrease in the buffer concentration was also reported by previous authors.[5] The relatively large difference between the $K_\alpha+K_\beta$ values obtained from the phosphate buffer and the amine buffers should also be noted. These results suggest a further complicated structure of the intermediate form in the reaction mechanism of the mutarotation of monosaccharide. Further studies on the mechanism of mutarotation for monosaccharide are now in progress in this laboratory and will be

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presented in another paper.[22)]

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Appendix

The expression for the instantaneous limiting current, $i(t)$, of an electrode reaction with a preceding chemical reaction;

$$\underset{\text{(chem)}}{\mathrm{A} \underset{k_\mathrm{b}}{\overset{k_\mathrm{f}}{\rightleftharpoons}} \mathrm{Ox}} \underset{\text{(el.)}}{\xrightarrow{ne}} \mathrm{Red}, \tag{1'}$$

at a stantionary plane electrode has been given by:[8)]

$$\frac{i(t)}{nFq} = \frac{D^{1/2}C^*}{\pi^{1/2}(K^2-1)}\left[\frac{K\exp(-lt)-1}{t^{1/2}} + \frac{K^2(\pi k_\mathrm{f})^{1/2}}{(K-1)^{1/2}} \times \exp\left(\frac{k_\mathrm{f}t}{K-1}\right)\left\{\mathrm{erf}\left(K\left(\frac{k_\mathrm{f}t}{K-1}\right)^{1/2}\right) - \mathrm{erf}\left(\left(\frac{k_\mathrm{f}t}{K-1}\right)^{1/2}\right)\right\}\right], \tag{2'}$$

where n indicates the number of electrons involved in the electrode reaction; q, the electrode surface area; D, the diffusion coefficient of the depolarizer; C^*, the sum of the concentrations of A and Ox; $K=k_\mathrm{b}/k_\mathrm{f}$, and $l=k_\mathrm{f}+k_\mathrm{b}$.

When $K \gg 1$ and when lt is in the order of unity or less, so that $(k_\mathrm{f}t/K)^{1/2} \simeq (lt)^{1/2}/K$, $K\exp(-lt) \gg 1$, $\mathrm{erfc}((lt)^{1/2}/K) \simeq 1$, and $\exp(lt/K^2) \simeq 1$, Eq. (2′) may be reduced in a good approximation to:

$$\frac{i(t)}{nFq} = \frac{D^{1/2}C^* l^{1/2}}{K} + \frac{D^{1/2}C^*}{K(\pi t)^{1/2}} \times \exp(-lt) \times \{1-(\pi lt)^{1/2}\exp(lt)\mathrm{erfc}((lt)^{1/2})\} \tag{3'}$$

Accordingly, the mean limiting current, $\bar{\imath}_\mathrm{l}$, at the dropping mercury electrode is given by:

$$\bar{\imath}_\mathrm{l} = \frac{1}{\tau}\int_0^\tau nFq\frac{D^{1/2}C^* l^{1/2}}{K}\mathrm{d}t + \frac{1}{\tau}\int_0^\tau nFq\frac{D^{1/2}C^*}{K(\pi t)^{1/2}} \times \exp(-lt)\{1-(\pi lt)^{1/2}\exp(lt)\mathrm{erfc}((lt)^{1/2})\}\mathrm{d}t \tag{4'}$$

The second term on the right-hand side of Eq. (4′) could be given in the first approximation for a smaller value of $l\tau$ by:

$$g_2(\tau) = \frac{1-\exp(-l\tau)}{l\tau}\left[\frac{1}{\tau}\int_0^\tau nFq\frac{D^{1/2}C^*}{K(\pi t)^{1/2}}\mathrm{d}t - \frac{1}{\tau}\int_0^\tau nFq\frac{D^{1/2}C^*}{K}l^{1/2}\exp(lt)\mathrm{erfc}((lt)^{1/2})\mathrm{d}t\right] \tag{5'}$$

Furthermore, according to Matsuda[23)] or Koutecký,[24)] the second term in the brackets on the right-hand side of Eq. (5′) should be replaced by $(\bar{\imath}_\mathrm{d}/K)/(1+1.13(l\tau)^{-1/2})$ for the case of the dropping mercury electrode. In conclusion, the mean limiting current, $\bar{\imath}_\mathrm{l}$, at the dropping mercury electrode may be given by:

$$\bar{\imath}_\mathrm{l} = 0.81\frac{(l\tau)^{1/2}}{K}\bar{\imath}_\mathrm{d} + \frac{\bar{\imath}_\mathrm{d}}{K} \times \frac{1-\exp(-l\tau)}{l\tau} \times \left(1-\frac{1}{1+1.13(l\tau)^{-1/2}}\right) \tag{6'}$$

where $\bar{\imath}_\mathrm{d}$ is the diffusion current given by the Ilkovič equation with the total bulk concentration, C^*.

If we proceed with the reaction scheme (2), $K_\alpha+K_\beta$ and $k_{-1}+k_{-2}$ should be substituted for K and l respectively.[6)] we thus obtain Eq. (7′):

$$\bar{\imath}_\mathrm{l} = 0.81\frac{(\tau(k_{-1}+k_{-2}))^{1/2}}{K_\alpha+K_\beta}\bar{\imath}_\mathrm{d} + \frac{\bar{\imath}_\mathrm{d}}{K_\alpha+K_\beta} \times \frac{1-\exp(-\tau(k_{-1}+k_{-2}))}{\tau(k_{-1}+k_{-2})} \times \left(1-\frac{1}{1+1.13(\tau(k_{-1}+k_{-2}))^{-1/2}}\right) \tag{7'}$$

The first term on the right-hand side of Eq. (7′) corresponds to $\bar{\imath}_\mathrm{k}$ in Eq. (7), and the second term, to $\bar{\imath}_\mathrm{corr}$. It can easily be seen that $\bar{\imath}_\mathrm{l}$ is reduced to $\bar{\imath}_\mathrm{k}$ when $(k_{-1}+k_{-2}) \gg 1$, whereas $\bar{\imath}_\mathrm{l}$ is reduced to $\bar{\imath}_\mathrm{d}/(K_\alpha+K_\beta)$ when $\tau(k_{-1}+k_{-2})$ becomes zero.[9)]

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