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# The Rates of Hydrolysis of Some Substituted Phenyl 2-Acetamido-2-deoxy-α- and -β-D-glucopyranosides

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The rates of hydrolysis of some substituted phenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides have been measured in acidic, neutral and alkaline aqueous solutions.

In acidic solution, the β-glycosides hydrolyze 2—6 times as fast as the corresponding α-glycosides, and the rates of hydrolysis of the β-glycosides exhibit almost linear $\rho$-$\sigma$ relationships ($\rho=-0.52$ at 61°, $-0.54$ at 78.2°) whereas those of the α-glycosides show seemingly convex $\rho$-$\sigma$ curves, and the tentative calculation on linear relationship shows small $\rho$ values ($\rho=-0.16$ at 61°, $-0.12$ at 68.9° and $-0.08$ at 78.2°). The kinetic parameters for the acid hydrolysis were calculated and discussed in connection with the participation of 2-acetamido group in intramolecular catalysis.

The hydrolysis of the glycosides at 120° in neutral buffer solution (pH 6.8) is facilitated by electron-withdrawing substituent ($\rho=+0.47$ for the α-glycosides, $+1.62$ for the β-glycosides). The pH-log $k_{obs}$ profile at 120° for the $p$-chlorophenyl β-glycoside indicates that the glycoside hydrolyzes at a spontaneous hydrolysis rate.

The rate of alkaline hydrolysis of the β-glycosides increases with $\sigma$($\rho=+1.69$ at 100°, $+1.65$ at 110°), but the α-glycosides are abnormally alkali-stable.

Some informations on the mechanism of action of hydrolase have been obtained by comparing the $\rho$-$\sigma$ relationship for the enzymatic hydrolysis with that for the acid and alkaline hydrolysis.[2] Substituted phenyl glycosides have often been used to elucidate the mechanism of enzymatic cleavage of the glycosidic bond, because the substituent on the benzene ring can be readily introduced. Several investigations have been reported on the kinetics of chemical hydrolysis of substituted phenyl 2-acetamido-2-deoxy-D-glucopyranosides,[3] but the number of the glycosides studied is limited and there is still a lack of information in reference to the kinetics of the hydrolysis.

In the present study, a series of substituted phenyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides were prepared, and the hydrolysis of the glycosides in acidic and alkaline solutions was compared in kinetic aspect with that of other glycosides, especially of substituted phenyl α- and β-D-glucopyranosides.

## Experimental

**Materials**——Some of the substituted phenyl 2-acetamido-2-deoxy-β-D-glucopyranosides were prepared by the König–Knorr reaction, condensation of acetochloroglucosamine with sodium phenolate. The β-glycosides crystallizing more readily were prepared from the mixture of α- and β-glycoside acetates obtained by fusion of glucosamine β-pentaacetate with an appropriate phenol in the presence of anhydrous zinc chloride. The α-glycoside acetates were separated from the filtrates of the β-glycoside acetates by column chromatography, or were prepared by the same reaction carried out at a higher temperature, as recommended by Weissmann[4] for the preparation of phenyl 2-acetamido-2-deoxy-α-D-glucopyranoside, with a higher yield than by the chromatography.

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[1] Location: *542, Miyama-cho, Funabashi-shi, Chiba.*

[2] *a*) R.L. Nath and H.N. Rydon, *Biochem. J.*, **57**, 1 (1954); *b*) S. Matsubara, *J. Biochem.* (Tokyo), **49**, 232 (1961); G. Lowe, G. Sheppard, M.L. Sinnott, and A. Williams, *Biochem. J.*, **104**, 893 (1967); T. Watabe and K. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **18**, 414 (1970).

[3] *a*) D. Piszkiewicz and T.C. Bruice, *J. Am. Chem. Soc.*, **89**, 6237, (1967); *b*) D. Piszkiewicz and T.C. Bruice, *ibid.*, **90**, 2156 (1968); *c*) D. Piszkiewicz and T.C. Bruice, *ibid.*, **90**, 5844 (1968).

[4] B. Weissmann, *J. Org. Chem.*, 31, 2505 (1966).

The present formulation of the α- and β-glycosides as anomeric pairs of pyranosides is supported by their optical rotation data: the pairs showed the values of 2A ($+64.4^\circ\times10^3$—$+68.6^\circ\times10^3$) and 2B($+63.7^\circ\times10^3$—$+66.3^\circ\times10^3$) reasonable when compared with the values[4] for phenyl 2-acetamido-2-deoxy-D-glucopyranosides: 2A($+63.8^\circ\times10^3$), 2B($+60.4^\circ\times10^3$).

The each glycoside used was prepared by the following procedures and their analytical data are shown in Table I.

**Table I.** Analytical Data of New Compounds of Substituted Phenyl 2-Acetamido-2-deoxy-D-glucopyranosides and Their Acetates

| | | | | | Analysis (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Substituent | Formula | mp[a] | Yield[b] | $[\alpha]_D^{20}$[c] | Calcd. | | | Found | | |
| | | | | | C | H | N | C | H | N |
| Substituted phenyl 3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosides | | | | | | | | | | |
| *p*-$CH_3O$ | $C_{21}H_{27}O_{10}N$ | 111 | 17 | +144 | 55.62 | 6.00 | 3.09 | 55.17 | 5.94 | 3.09 |
| *p*-$CH_3$ | $C_{21}H_{27}O_9N$ | 151.5 | 30 | +146 | 57.66 | 6.22 | 3.20 | 57.45 | 6.07 | 3.26 |
| *m*-$CH_3$ | $C_{21}H_{27}O_9N$ | 152 | 24 | +148 | 57.66 | 6.22 | 3.20 | 57.50 | 6.28 | 3.16 |
| *p*-Cl | $C_{20}H_{24}O_9NCl$ | 170.5 | 17 | +149 | 52.46 | 5.28 | 3.06 | 52.34 | 5.14 | 2.93 |
| *m*-Cl | $C_{20}H_{24}O_9NCl$ | 149 | 16 | +154 | 52.46 | 5.28 | 3.06 | 52.05 | 5.04 | 2.92 |
| Substituted phenyl 3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosides | | | | | | | | | | |
| *p*-$CH_3O$ | $C_{21}H_{27}O_{10}N$ | 191 | 13 | − 11.5 | 55.62 | 6.00 | 3.09 | 55.26 | 5.85 | 3.33 |
| *p*-$CH_3$ | $C_{21}H_{27}O_9N$ | 193 | 27 | − 12.3 | 57.66 | 6.22 | 3.20 | 57.42 | 6.08 | 3.07 |
| *m*-$CH_3$ | $C_{21}H_{27}O_9N$ | 194 | 38 | − 10.8 | 57.66 | 6.22 | 3.20 | 57.34 | 6.34 | 3.05 |
| *p*-Cl | $C_{20}H_{24}O_9NCl$ | 209 | 8 | − 10.5 | 52.46 | 5.28 | 3.06 | 52.58 | 5.36 | 2.83 |
| *m*-Cl | $C_{20}H_{24}O_9NCl$ | 202 | 11 | − 11.3 | 52.46 | 5.28 | 3.06 | 52.64 | 5.30 | 2.75 |
| Substituted phenyl 2-acetamido-2-deoxy-α-D-glucopyranosides | | | | | | | | | | |
| *p*-$CH_3O$ | $C_{15}H_{21}O_7N$ | 248 | 77 | +203 | 55.04 | 6.47 | 4.28 | 54.99 | 6.53 | 4.26 |
| *p*-$CH_3$ | $C_{15}H_{21}O_6N$ | 261 | 80 | +213 | 57.87 | 6.80 | 4.50 | 57.92 | 6.89 | 4.52 |
| *m*-$CH_3$ | $C_{15}H_{21}O_6N\cdot H_2O$ | 212 | 70 | +207 | 54.70 | 7.04 | 4.25 | 54.66 | 6.83 | 4.25 |
| *p*-Cl | $C_{14}H_{18}O_6NCl$ | 266(dec) | 74 | +200[d] | 50.68 | 5.47 | 4.22 | 50.72 | 5.41 | 3.97 |
| *m*-Cl | $C_{14}H_{18}O_6NCl\cdot H_2O$ | 213 | 80 | +194 | 48.07 | 5.76 | 4.00 | 48.36 | 5.65 | 3.77 |
| Substituted phenyl 2-acetamido-2-deoxy-β-D-glucopyranosides | | | | | | | | | | |
| *p*-$CH_3O$ | $C_{15}H_{21}O_7N\cdot H_2O$ | 243 | 83 | −6.0 | 52.17 | 6.71 | 4.06 | 52.34 | 6.81 | 4.28 |
| *p*-$CH_3$ | $C_{15}H_{21}O_6N\cdot H_2O$ | 235—236 | — | −4.5 | 54.70 | 7.04 | 4.25 | 54.96 | 7.28 | 4.47 |
| *m*-$CH_3$ | $C_{15}H_{21}O_6N\cdot H_2O$ | 228 | 84 | −3.0 | 54.70 | 7.04 | 4.25 | 54.80 | 7.03 | 4.45 |
| *p*-Cl | $C_{14}H_{18}O_6NCl\cdot H_2O$ | 236—237 | 66 | 0.0[e] | 48.07 | 5.76 | 4.01 | 48.04 | 5.66 | 3.89 |
| *m*-Cl | $C_{14}H_{18}O_6NCl\cdot\frac{1}{3}H_2O$ | 227 | 72 | 0.0 | 49.79 | 5.57 | 4.15 | 49.87 | 5.51 | 4.06 |

a) uncorrected
b) calculated for pure products Yields of glycoside acetates refer to duplicate or triplicate preparations by the Helferich reaction. The yields of the β-glycoside acetates by the König–Knorr reaction are shown in experimental section.
c) Chloroform was the solvent for the glycoside acetates (2%) and water for the glycosides (0.45% for α-glycosides, 0.66% for β-glycosides, except *p*-chlorophenyl α- and β-glycosides).
d) determined in 0.14% aqueous solution
e) determined in 0.2% aqueous solution

**Substituted Phenyl 3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosides**——A mixture of 5 g of β-D-glucosamine pentaacetate, 6 g of an appropriate phenol and 1.8 g of anhydrous zinc chloride was fused in an open vessel at 153—154° for 20 min with frequent stirring. The reaction mixture was dissolved in 200 ml of chloroform and washed successively with ice water, cold 1N sodium hydroxide and ice water. The chloroform layer was dried over calcium chloride and evaporated to dryness under reduced pressure. The resulting dark colored residue was dissolved in a small amount of ethanol and the solution was subjected to column chromatography (carbon 10 g, celite 5 g) and the α-glycoside acetate was eluted with 200 ml of ethanol. Evaporation under reduced pressure and recrystallization from isopropanol gave the pure α-glycoside acetate.

**Substituted Phenyl 3,4,6-Tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosides**——(a) The condensation of acetochloroglucosamine with an appropriate phenol under the condition employed by Leaback[5]

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5) D.H. Leaback and P.G. Walker, *J. Chem. Soc.*, **1957**, 4754.

gave the β-glycoside acetates. The yields for the products shown in Table I were as follows in respect to the acetochloroglucosamine used: *p*-methoxyphenyl, 11.1%; *m*-tolyl, 22%; phenyl, 25%; *p*-chlorophenyl, 37%. (b) A mixture of 2 g of β-D-glucosamine pentaacetate, 2 g of an appropriate phenol and 0.5 g of anhydrous zinc chloride was heated at 125—130° in an oil bath for 45 min with stirring. The reaction mixture was treated as described for the preparation of the α-anomers, and the chloroform solution was decolorized with active carbon. Recrystallization from isopropanol gave the β-glycoside acetates. The filtrate of the β-glycoside acetate was evaporated to dryness and the residue was dissolved in a minimum quantity of benzene and applied to a column of magnesium silicate "Woelm." Elution with ethanol–benzene (1: 100) gave the α-anomer and elution with ethanol–benzene (2: 100) the β-anomer. Recrystallization from isopropanol gave the pure products.

**O-Deacetylation**——To a boiling solution of the glycoside acetate (1 g) in methanol–chloroform (1: 1, 20 ml) was added a freshly prepared solution of 0.1M sodium methoxide in methanol (0.5 ml), and immediately afterwards the solution was cooled with ice water. The solution was kept at room temperature overnight and the solvent was removed under reduced pressure. The residue was recrystallized from hot water.

**Rate Measurements**——The hydrolysis in 0.111M hydrochloric acid was carried out in a stoppered tube kept at constant temperature by refluxing chloroform (61.0°), the azeotropic mixture of benzene–*n*-hexane (68.9°) or ethanol–water (78.2°). The hydrolysis in 0.111M potassium hydroxide at 100° and 110°, and in 0.05M potassium phosphate buffer solution (pH 6.8 at room temperature) at 120°, was carried out in separated sealed tubes placed in a thermostat bath (±0.2°). Samples were removed at intervals and analyzed for the phenol liberated by the method of Folin and Ciocalteu,[6] except in the case of *p*-nitrophenyl glycosides, where the yellow color developed in glycine buffer was measured.[7]

The pseudo-first-order rate constant for the acid hydrolysis, $k_{\text{acid}}$, was obtained from $k=1/t \ln a/(a-x)$ by the method of least squares, where $a$ is the initial concentration of substrate and $x$ is the concentration of the phenol liberated at reaction time $t$. For the hydrolysis in alkaline and neutral solutions, the concentration of the phenol liberated $x$ was assumed to be expressed by a polynomial (of two degree), $x=b_0+b_1t+b_2t^2$ where $b_0$—$b_2$ are coefficients, and the initial pseudo-first-order reaction rate constant was obtained from the initial rate, $(dx/dt)_{t=o}$, which is equal to $b_1$. The calculation is rougher than that described by Gasman, *et al.*[8] where a polynomial of higher degree was used.

## Result

The hydrolysis in 0.111M hydrochloric acid obeyed the pseudo-first-order kinetics, whereas the hydrolyses in 0.111M potassium hydroxide and in neutral buffer solution, pH 6.8 at 25°, deviated from the pseudo-first-order kinetics. The rate constant for the hydrolysis in alkaline and neutral buffer solutions was calculated, tentatively assuming that the reaction was of pseudo-first-order. The rate constant decreased with reaction time, with the exception of the *m*-chlorophenyl β-glycoside in neutral buffer solution, whose rate constant increased with time and the liberation of *m*-chlorophenol estimated by the method of Folin and Ciocalteu exceeded the theoretical amount calculated for the complete hydrolysis. The decrease in the rate constant can be ascribed to either the decomposition of the phenol liberated or the accumulation of a more stable product formed by a parallel reaction. The increase in the rate constant can be ascribed to the formation of a secondary product that is produced from the primary products, the glycon and the aglycon, giving more intensive color with the reagent. Initial pseudo-first-order reaction rate constants for the hydrolysis in alkaline solution, $k_{\text{alk}}$, and in neutral solution, $k_{\text{neu}}$, were therefore, estimated from the initial rate by a method similar to that described by Gasman.[8] The rate constants thus determined are shown in Table II and III.

## Discussion

### Acid Hydrolysis

As shown in Table II, the substituted phenyl 2-acetamido-2-deoxy-β-D-glucopyranosides hydrolyze in acidic solutions more rapidly than the α-anomers.

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6) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).

7) G.A. Levvy and J. Conchie, *Methods in Enzymology*, **8**, 577 (1968).

8) R.C. Gasman and D.C. Johnson, *J. Org. Chem.*, **31**, 1830 (1966).

Table II. Rate Constants and Kinetic Parameters of the Acid Hydrolysis of Some Substituted Phenyl 2-Acetamido-2-deoxy-α- and -β-D-glucopyranosides

| 2-Acetamido-2-deoxy-D-glucopyranoside ($\sigma$)[a] | α-Glycoside Temp. (°C) | α-Glycoside $10^6 k_{acid}$ (sec$^{-1}$) | α-Glycoside $\Delta H^{\neq b)}$ (kcal mole$^{-1}$) | α-Glycoside $\Delta S^{\neq b)}$ (eu) | β-Glycoside Temp. (°C) | β-Glycoside $10^6 k_{acid}$ (sec$^{-1}$) | β-Glycoside $\Delta H^{\neq}$ (kcal mole$^{-1}$) | β-Glycoside $\Delta S^{\neq}$ (eu) |
|---|---|---|---|---|---|---|---|---|
| *p*-Methoxyphenyl | 60.7 | 1.97 | 32.0 | 15.3 | 60.5 | 11.2 | 29.6 | 11.9 |
| (−0.268) | 67.8 | 4.15 | | | 76.8 | 93.7 | | |
| | 77.6 | 20.5 | | | | | | |
| *p*-Tolyl | 60.4 | 2.32 | 31.1 | 13.2 | 60.5 | 10.1 | 29.4 | 11.2 |
| (−0.170) | 67.8 | 6.68 | | | 76.6 | 81.8 | | |
| | 77.6 | 21.3 | | | | | | |
| *m*-Tolyl | 60.6 | 2.62 | 30.4 | 11.3 | 60.5 | 11.1 | 31.2 | 16.6 |
| (−0.069) | 67.9 | 6.92 | | | 76.9 | 106 | | |
| | 68.1 | 7.13 | | | | | | |
| | 77.0 | 23.3 | | | | | | |
| Phenyl | 60.0 | 2.43 | 31.0 | 13.2 | 60.9 | 7.56 | 30.2 | 12.8 |
| (0) | 60.0 | 2.30 | | | 76.8 | 62.5 | | |
| | 67.9 | 7.45 | | | | | | |
| | 76.8 | 23.3 | | | | | | |
| *p*-Chlorophenyl | 60.0 | 1.65 | 32.1 | 16.4 | 60.5 | 6.22 | 27.1 | 3.2 |
| (+0.227) | 67.8 | 5.03 | | | 76.9 | 42.9 | | |
| | 77.0 | 18.5 | | | | | | |
| *m*-Chlorophenyl | 60.0 | 1.40 | 33.0 | 18.3 | 60.0 | 5.10 | 26.7 | 1.8 |
| (+0.373) | 69.2 | 6.98 | | | 76.9 | 36.8 | | |
| | 76.9 | 15.9 | | | | | | |
| *p*-Nitrophenyl | | | | | 60.0 | 2.30 | | |
| | | | | | 76.8 | 30.7 | | |

*a*) Substituent constants in the Hammett equation.[9]

*b*) Parameters at 60° were calculated from $\Delta H^{\neq}=Ea-RT$, $\Delta S^{\neq}=(\Delta H^{\neq}-2.303\,RT\log kT/h+2.303\,RT\log k)/T$,[10] where $k=k_{obs}/h_0$. The $h_0$ value for 0.111 M hydrochloric acid used is 0.0992.[11]

Table III. Rate Constants of the Hydrolysis of Substituted Phenyl 2-Acetamido-2-deoxy-α- and -β-D-glucopyranosides in Neutral Solution and the Alkaline Hydrolysis of the β-Anomers

| 2-Acetamido-2-deoxy-D-glucopyranoside | $10^6 k_{neu}$[a] at 120° (sec$^{-1}$) α-Glycoside | $10^6 k_{neu}$[a] at 120° (sec$^{-1}$) β-Glycoside | $10^6 k_{alk}$[a] (sec$^{-1}$) β-Glycoside at 100° | $10^6 k_{alk}$[a] (sec$^{-1}$) β-Glycoside at 110° |
|---|---|---|---|---|
| *p*-Methoxyphenyl | 2.7 | 4.6 | 0.82 | 2.7 |
| *p*-Tolyl | 3.2 | 4.1 | 0.80 | 2.4 |
| *m*-Tolyl | 3.6 | 6.8 | 1.4 | 3.0 |
| Phenyl | 4.5 | 8.2 | 1.7 | 4.7 |
| *p*-Chlorophenyl | 5.0 | 21 | 4.4 | 12 |
| *m*-Chlorophenyl | 5.4 | 43 | 8.8 | 25 |

*a*) The initial pseudo-first-order reaction rate constants for the hydrolysis in neutral buffer solution (pH 6.8 at 25°), $k_{neu}$, and in 0.111 M potassium hydroxide, $k_{alk}$.

Generally, the mechanism[12] for acid hydrolysis of glycosides involves the fast process of protonation of the glycosidic oxygen atom to form the conjugate acid followed by the

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[9] J. Hine, "Physical Organic Chemistry," 2nd ed., Mcgraw-Hill Book Co., Inc., New York, 1962, p. 86.

[10] W.G. Overend, C.W. Rees, and J.S. Sequeira, *J. Chem. Soc.*, **1962**, 3429.

[11] L.P. Hammett and M.A. Paul, *J. Am. Chem. Soc.*, **56**, 827 (1934).

[12] *a*) M.S. Feather and J.F. Harris, *J. Org. Chem.*, **30**, 153 (1965); *b*) W.G. Overend, "The Carbohydrates," Vol. IA, 2nd ed., by W. Pigman, D. Horton, Academic Press, New York and London, 1972, p. 279; *c*) J.N. BeMiller, *Advances in Carbohydrate Chemistry*, **22**, 25 (1967).

rate-determining step of heterolysis to give a cyclic planar carbonium ion, which reacts with water to give the reducing sugar. Methyl β-D-glucopyranoside, which possesses an equatorial glycosidic bond in its stable conformer, C1, hydrolyzes more rapidly than the α-anomer having an axial glycosidic bond, and the reactivity in the former is ascribed to the higher free-energy of the equatorial glycosidic bond caused by a polar interaction between the equatorial methoxyl group and the ring oxygen atom. On the contrary, the α-glycoside with a bulky aglycon, as phenoxyl, hydrolyzes faster than the β-anomer because of the larger positive entropy of activation for the α-anomer, and it was suggested that the α-anomer is more highly oriented in the ground state than the β-anomer. According to Piszkiewicz and Bruice, 2-acetamido-2-deoxy-D-glucopyranosides with a bulky aglycon present a different situation, primarily because, in the β-glycoside, a steric repulsion between the aglycon and the bulky 2-acetamido group facilitates the change of the chair conformation to the half-chair conformation of the carbonium intermediate,[3c] enhancing the rate of acid hydrolysis, and secondarily because the intramolecular nucleophilic attack of C-1 by the neighbouring acetamido group provides spontaneous hydrolysis rate much greater than that provided by the 2-hydroxyl group of glucopyranosides. The pseudo-first-order rate constants $k_{obs}$ for the glycosides with a good leaving group such as nitrophenoxyl have been shown to be expressed as[3a,b]

$$k_{\text{obs}} = k_{\text{H}}a_{\text{H}} + k_0 + k_{\text{OH}}K_w/a_{\text{H}}$$

where $a_{\text{H}}$ is the activity of hydrogen ion, $k_{\text{H}}$ is the second-order rate constant for specific acid catalyzed hydrolysis, $k_{\text{OH}}$ is the second-order rate constant for specific base catalyzed hydrolysis, $K_W$ is the dissociation constant of water and $k_0$ is the first-order rate constant for spontaneous hydrolysis. The values of $k_0$ at 78.2° reported by Piszkiewicz and Bruice for *o*- and *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside are $8.72\times10^{-3}$ and $1.03\times10^{-3}$ min$^{-1}$, respectively.

In neutral solution, the substituted phenyl 2-acetamido-2-deoxy-β-D-glucopyranosides as well as the α-anomers were stable at lower temperatures unlike the nitrophenyl β-glycosides, but hydrolyzed at a higher temperature as shown in Table III. The values of $k_{\text{neu}}$ at 120° and the ρ-σ plot are shown in Table III and Fig. 3, respectively. The hydrolysis in neutral solution suggested the occurrence of spontaneous hydrolysis. The values for the Hammett reaction constant ρ, for the α- and β-glycosides are $+0.47\pm0.072$ (standard error) and $+1.62\pm0.16$, respectively. Fig. 1 shows the pH-log $k_{\text{obs}}$ profile for the *p*-chlorophenyl β-glycoside which indicates that the glycoside hydrolyzes at the spontaneous hydrolysis rate, though of much lesser magnitude. The pH values shown in Fig. 1 were measured at room temperature and, therefore, exhibit only rough values at 120°. Even if all the glycosides hydrolyze at the spontaneous hydrolysis rate, the value for $k_0$ should not exceed that for $k_{\text{neu}}$, and consequently should be much smaller than the value for $k_{\text{acid}}$. Therefore, $k_{\text{acid}}$ can stand for $k_{\text{H}}a_{\text{H}}$.

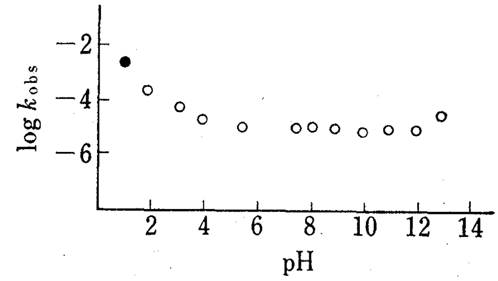

Fig. 1. pH-log $k_{\text{obs}}$ Profile for the Hydrolysis of *p*-Chlorophenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside at 120°

Buffers employed were glycine-hydrochloride (pH 2.0 to 3.0), sodium acetate-acetic acid (pH 4.0 to 5.5), tris-hydrochloride (pH 7.0 to 9.0), glycine-sodium hydroxide (pH 10.0 to 12.0). $\mu=0.1$.
○: experimental at 120°
●: a value calculated from the data in Table II

In the present study the β-glycosides hydrolyzed 2—6 times as fast as the α-glycosides. About three fold enhancement of $k_{\text{H}}$ for the 2-acetamido-2-deoxy-β-D-glucopyranosides was observed when compared with the values of $k_{\text{H}}$ for the corresponding β-glucosides.[2a] The explanation of the enhancement is afforded by the steric repulsion between the bulky 2-acetamido and the aryloxyl group as described above. On the contrary, replacing the 2-hydroxyl group in the glucopyranosides by an acetamido group diminished the value of $k_{\text{H}}$ by 1/2—3/4;

the bulky groups at C-1 and C-2 are presumed to hinder the change in conformation to the half-chair conformation of the carbonium intermediate.

Fig. 2 shows the $\rho$-$\sigma$ relationships for the acid hydrolysis of the $\alpha$- and the $\beta$-glycosides. The $\rho$-$\sigma$ relationships for the $\beta$-glycosides were shown to be almost linear with the points of the *m*-tolyl and the *p*-nitrophenyl $\beta$-glycoside considerably deviating from the linearity. The $k_{obs}$ at 78.2° for the phenyl $\beta$-glycoside, $7.47\times10^{-5}$ sec$^{-1}$, agrees with the value $7.39\times10^{-5}$ calculated from the data given by Piszkiewicz, *et al.*,[3a,b] while $2.97\times10^{-5}$ obtained for the *p*-nitrophenyl $\beta$-glycoside is smaller than $3.68\times10^{-5}$ calculated from the same report. With the published data, the point deviates further from the linearity. The low negative values of $\rho$, $-0.52\pm0.022$ at 61° and $-0.54\pm0.039$ at 78.2°, are comparable to the low negative value, $-0.66$, for the acid hydrolysis of various substituted phenyl $\beta$-D-glucopyranosides.[2a] The low value of $\rho$ has been explained by Hall, *et al.*[13] as resulting from the mutual partial cancellation of two opposing effects of the electron-withdrawing substituent, one decreasing the equilibrium concentration of the conjugate acid, and the other facilitating the heterolysis of the conjugate acid. Piszkiewicz, *et al.*[3b] reported the $\rho$ value of $-0.11$ for the hydrolysis of 2-acetamido-2-deoxy-$\beta$-D-glucopyranosides having an aglycon such as *o*- and *p*-nitrophenyl, *o*-methoxycarbonylphenyl, phenyl and naphtyl groups. This value is much smaller than that observed in the present study. This difference is presumed to arise from the fact that the glycosides used by Piszkiewicz, *et al.* for the study on the intramolecular catalysis involved the different series of substituents. The *ortho*-substituted compounds, for example, are believed to exhibit an anomaly in their reactivities.

Unlike the $\beta$-glycosides, the acid hydrolysis of the $\alpha$-glycosides shows $\rho$-$\sigma$ relationships of seemingly convex curves at three different temperatures. A $\rho$-$\sigma$ relationship that is convex

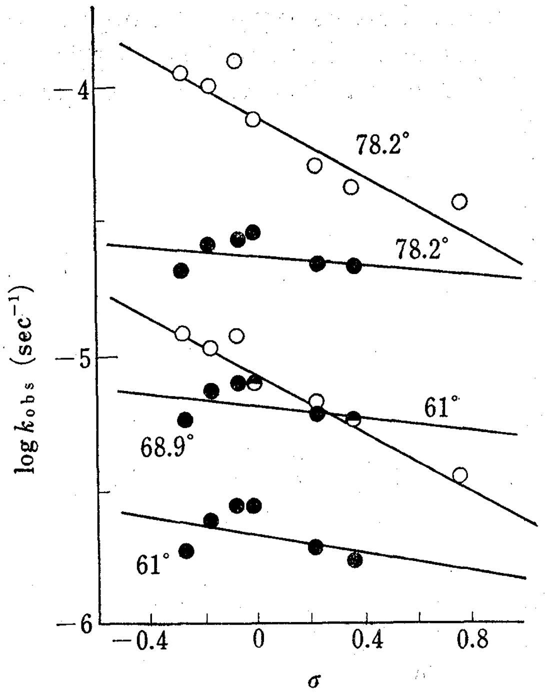

Fig. 2. Hammett Plots for the Acid Hydrolysis of Substituted Phenyl 2-Acetamido-2-deoxy-$\alpha$-, and -$\beta$-D-glucopyranosides in 0.111 M Hydrochloric Acid

$\alpha$-glycoside (●), $\beta$-glycoside (○)

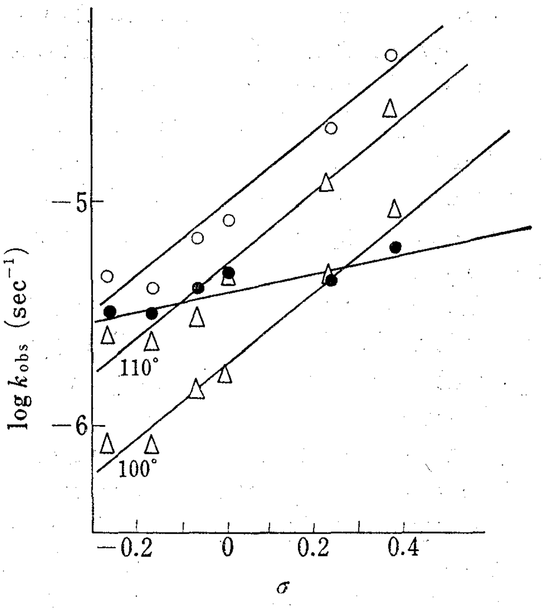

Fig. 3. Hammett Plots for the Hydrolysis of Substituted Phenyl 2-Acetamido-2-deoxy-$\alpha$- and -$\beta$-D-glucopyranosides in Alkaline and Neutral Solutions

$\beta$-Glycoside: in 0.05M phosphate buffer (pH 6.8 at 25°) (○) at 120°, in 0.111M potassium hydroxide (△) at 100° and 110°
$\alpha$-Glycoside: in 0.05M phosphate buffer (●) at 120°

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[13] A.N. Hall, S. Hollingshead, and H.N. Rydon, *J. Chem. Soc.*, **1961**, 4290.

upward indicates[14)] the reaction to be a multi-step reaction which has never been considered in the hydrolytic mechanism of glycoside. However, the examination of the linear $\rho$-$\sigma$ relationship reported by Hall, *et al.*[13)] for the acid hydrolysis of the substituted phenyl $\alpha$-D-glucopyranosides reveals that the deviation magnitude of the points from linearity in their study is comparable to that in the present study on the 2-acetamido-2-deoxy-$\alpha$-D-glucopyranosides. The values of $\rho$ were calculated, tentatively assuming the linear $\rho$-$\sigma$ relationship, to be $-0.16 \pm 0.068$ at 61°, $-0.12 \pm 0.059$ at 68.9° and $-0.08 \pm 0.049$ at 78.2°. Hall, *et al.*[13)] reported the vanishingly small value of $\rho$, $-0.006$, for the acid hydrolysis of the substituted phenyl $\alpha$-D-glucopyranosides.

Kinetic parameters were calculated assuming that the values of $k_{\text{acid}}$ stand for the values of $k_{\text{H}}a_{\text{H}}$, except for the *p*-nitrophenyl $\beta$-glycoside which hydrolyzed at the rates of both specific acid hydrolysis and spontaneous hydrolysis. Inspection of Table II indicates that the value for enthalpy of activation, $\Delta H^{\neq}$, for the $\beta$-glycoside is less by 1—2 kcal/mole than for the corresponding $\alpha$-glycoside while the value for entropy of activation $\Delta S^{\neq}$, for the $\beta$-glycoside is 2—15 eu less than for the corresponding $\alpha$-glycoside, with the exception of the pair of *m*-tolyl glycosides. This difference is noticeable in the *p*- and *m*-chlorophenyl glycosides. This finding suggests the possible participation of an intramolecular attack of C-1 of the conjugate acid by the 2-acetamido group in the acid hydrolysis of the *p*- and the *m*-chlorophenyl $\beta$-glycosides, where the orientation of the catalytic 2-acetamido group should decrease the value for $\Delta S^{\neq}$.

### Hydrolysis in Alkaline Solution

Fig. 3 shows that the hydrolysis of the $\beta$-glycosides is under electronic control in alkaline solution more than in acidic solution. The $\rho$ value for the alkaline hydrolysis is $1.69 \pm 0.126$ at 100° and $1.65 \pm 0.205$ at 110° and considerably smaller than that for the substituted phenyl $\beta$-D-glucopyranosides,[2a,13)] $+2.48$. On the other hand, the $\alpha$-glycosides were extremely resistant against alkaline hydrolysis.

Analogously to the base catalyzed hydrolysis of phenyl hexopyranosides,[15)] there are three possible mechanisms for the alkaline hydrolysis of the substituted phenyl 2-acetamido-2-deoxy-$\alpha$- and $\beta$-D-glucopyranosides: (a) the mechanism analogous to C-2 oxyanion participation in which a proton is abstracted from 2-acetamido nitrogen,[3a)] (b) C-6 oxyanion participation proposed for the base catalyzed hydrolysis of phenyl $\alpha$-D-glucopyranoside and (c) the ionic dissociation mechanism proposed for the base catalyzed hydrolysis of phenyl $\beta$-D-mannopyranoside. The greater reactivity of the $\beta$-glycosides studied indicates that the mechanism (a) is believed to be operative in the alkaline hydrolysis of the $\beta$-glycosides, the 1,2-*trans* glycosides.

The experiment on the *p*-chlorophenyl $\alpha$-glycoside showed that the liberation of the aglycon was not observed even after treatment with 0.111 M potassium hydroxide at 120° for 96 hr whereas this glycoside hydrolyzed at a considerably high rate in the neutral buffer solution at the same temperature. This anomaly suggests that the treatment with alkali converts the $\alpha$-glycoside into an alkali-stable substance.

A calculation from the data in Table III showed that 36—40% of the $k_{alk}$ value is attributable to the spontaneous hydrolysis. Therefore kinetic parameters were not calculated.

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14) B.M. Anderson and W.P. Jencks, *J. Am. Chem. Soc.*, **82**, 1773 (1960).

15) C.E. Ballou, *Advances in Carbohydrate Chemistry*, **9**, 59 (1954).