freeze–thaw cycles using Dry Ice–acetone. The tubes were placed in the bath and were successively withdrawn after appropriate intervals of time. The contents were cooled and diluted with 20 ml of acetone to stop the reaction and the rate of acid formation was determined by potentiometric titration of the aliquots with standard 0.003 *N* aqueous sodium hydroxide. The bath temperatures were controlled to ±0.03°.

Product Studies. The hydrolysis products of **6**-H, **7**-H, **8**-OCH_3, **8**-H, and **8**-CF_3 were examined by vpc. The ester (approximately 0.04 mmol) and a reference compound (*n*-tetradecane for **6**-H, *n*-tridecane for **7**-H, and *n*-octadecane for **8**-OCH_3, **8**-H, and **8**-CF_3) were dissolved in 14 ml of acetone, and 4 ml of 0.01 *N* aqueous sodium hydroxide and 2 ml of water were added, followed by enough 70% aqueous acetone to make the total volume 50 ml at 25°. The solution was heated in a glass bomb which was sealed under nitrogen after a degassing routine involving three freeze–thaw cycles using Dry Ice–acetone. After the specified time (10–12 half-lives), the contents were concentrated, made basic with dilute sodium bicarbonate solution and extracted with pentane. The combined pentane layers were washed with water. The pentane was distilled off and the residues were subjected directly to vpc analysis.

The results from **6**-H, **7**-H, and **8**-H are presented in Chart I. Yields of the oelfins and cyclopropane derivatives corresponding to **17** and **18** were 89 and 6% for **8**-OCH_3, and 78 and 17% for **8**-CF_3.

Mechanism of Hydrolysis of Benzoyl Glucose Acylals

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Abstract: The hydrolysis of 1-β-D-glucopyranosyl benzoate (I), 2,3-di-*O*-methyl-1-β-D-glucopyranosyl benzoate (II), and α- and β-2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl benzoate (III) has been studied in aqueous solution. At low and intermediate pH all three esters hydrolyze *via* spontaneous and acid-catalyzed paths (78°). The acid-catalyzed reaction has been characterized as an A-1 process proceeding through a glycosyl oxocarbonium ion. The data available are consistent with an SN1 mechanism for the spontaneous hydrolysis. At high pH (30°) multiphasic kinetics were observed for the hydrolysis of I and II. The multiphasic kinetics were interpreted in terms of HO^- catalyzed O → O benzoyl migration in competition with HO^- catalyzed hydrolysis (Schemes III and IV). For I, O → O benzoyl migration may occur from C_1 to C_2–OH and C_6–OH while for II acyl migration may only occur to C_6–OH. In the hydrolysis of IIIa,b where O → O benzoyl migration is not possible, multiphasic kinetics are not observed and hydrolysis is first order in $[HO^-]$. Hydrazinolysis of II and III is both nucleophilic and self general-base catalyzed nucleophilic in nature. When hydrazine serves as a nucleophile, O → O benzoyl group migration cannot compete with the displacement of the benzoyl group and multiphasic kinetics are not observed.

A number of enzyme-catalyzed glycosyl transfer reactions have been suggested to involve glycosyl enzyme intermediates in which the anomeric carbon of the glycosyl moiety is covalently linked to a carboxyl group (a glycosyl acylal).[2] A case in point is *E. coli* β-galactosidase.[3,4] The magnitude of the secondary α-deuterium kinetic isotope effects for hydrolysis and methanolysis of the galactosyl enzyme has been interpreted[5] in terms of an acylal in equilibrium with a minor mole fraction of an ion pair of carboxyl anion and sugar oxocarbonium ion. In the Phillips[6] mechanism for lysozyme, the carboxyl group of Asp-52 has been suggested to electrostatically stabilize a glycosyl oxocarbonium ion intermediate. A more reasonable alternative would involve formation of an acylal with Asp-52 which is in spontaneous equilibrium with a steady-state concentration of oxocarbonium ion.[7] Voet and Abeles isolated, by protein modification and denaturation, a covalent β-glucose–enzyme intermediate from sucrose phosphorylase.[8] Though the mode of covalent linkage of the glucosyl moiety was not shown, an acylal was considered. In the hydrolysis of the denatured glucosyl enzyme multiphasic kinetics were observed for glucose release. It occurred to us that multiphasic kinetics would be unique to the hydrolysis of a glycosyl acylal intermediate since the acyl group can transfer not only to solvent, but intramolecularly to the 2- and possibly the 5-hydroxyl groups of the glucose ring. Outside the COOH group there are no enzyme functional groups which, when bonded to the C_1 of a sugar, could migrate to other positions. Facile O → O acyl shifts of this nature are known in the hydrolysis of glycerol β-monoacetate[9] and pose a problem in the identification of the position of acylation in amino acyl RNA.[10] These considerations suggest that identification of the mode of attachment of a glycosyl moiety of a (denatured) glycosyl enzyme might be made on the basis of the kinetics of its hydrolysis.

We report herein the results of our study of the mechanism of hydrolysis of 1-β-D-glucopyranosyl benzoate (I), 2,3-di-*O*-methyl-β-D-glucopyranosyl benzoate (II), and α- and β-2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl benzoate (III). In previous studies, Fletcher and coworkers[11] prepared and examined the hydrolysis of several α-monosaccharide acylals. These studies were

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(2) See B. Capon, *Chem. Rev.*, **69**, 407 (1969), for a pertinent review of the literature.
(3) O. M. Viratelle, J. P. Tenu, J. Garnier, and J. Yon, *Biochem. Biophys. Res. Commun.*, **37**, 1036 (1969).
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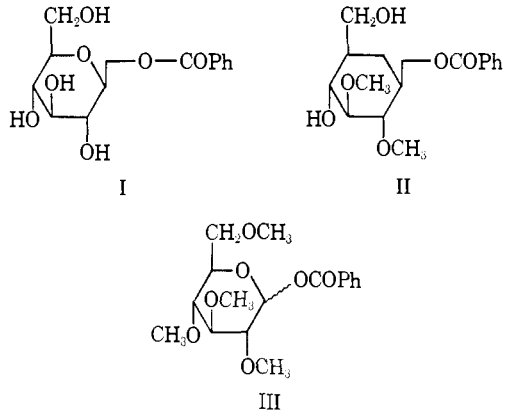

I II III

of a preparative nature and no quantitative rate data were obtained.

Experimental Section

1-β-D-Glucopyranosyl Benzoate (I). The method of Fletcher[12] was followed in the preparation of glucopyranosyl benzoate. The solid which was obtained was recrystallized from 0.01 *M* aqueous acetic acid: mp 183–184°, $[\alpha]^{30}_{589}$ −28.8° (*c* 0.500, H_2O); lit.[12] mp 191–192°, $[\alpha]^{20}_{D}$ −27° (*c* 0.45, H_2O).

Anal. Calcd for $C_{13}H_{16}O_7$: C, 54.93; H, 5.67. Found: C, 55.03; H, 5.80.

2,3-Di-*O*-methyl-4,6-*O*-benzylidene-D-glucopyranose. Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, 62.0 g (0.22 mol), prepared by the method of Capon,[13] in 350 ml of acetone, was stirred vigorously while 67 ml of dimethyl sulfate, 185 ml of 50% aqueous sodium hydroxide, and 100 ml of dimethyl sulfate were added sequentially and as rapidly as possible. After heating at reflux for 1 hr, 1.0 l. of water was added and the stirring was continued until the mixture had cooled. The crude solid was filtered, dried, and recrystallized from ether to give a white crystalline solid, mp 121–123°; lit.[13] mp 121–122°. The nmr and infrared spectra were consistent with the structure of methyl-2,3-di-*O*-methyl-4,6-*O*-benzylidene-α-D-glucopyranoside; nmr (CCl_4) τ 6.65 (singlet, 3 H), 6.60 (singlet, 3 H), 6.50 (singlet, 3 H), 4.65–6.80 (multiplet, 6 H), 5.30 (doublet, *J* = 3 Hz, 1 H), 4.55 (singlet, 1 H), 2.40–2.85 (multiplet, 5 H).

This solid, 47 g, was refluxed in 250 ml of 2 *N* HCl for 18 hr. After treatment with charcoal, the hot solution was filtered. On cooling, the aqueous mixture was washed with 2 × 50 ml of chloroform, neutralized by passage through an anion-exchange column (Dowex AG3-X4 HO^- form), and evaporated to dryness at reduced pressure. After drying for 18 hr under high vacuum, a viscous syrup was obtained: nmr (D_2O) τ 6.65 (singlet), 6.55 (singlet, 6 H), 6.05–7.04 (multiplet, 7 H), 5.50 (doublet, *J* = 8 Hz), 4.80 (doublet, *J* = 3 Hz, 1 H).

The dried syrup, dissolved in a minimum of acetonitrile, was added to 250 ml of benzaldehyde and 25 g of zinc chloride (freshly fused and powdered), and the mixture was stirred for 18 hr. Water, 750 ml, was added and the two phases were allowed to separate. The aqueous phase was extracted with 3 × 250 ml of benzene and the combined organic phases were concentrated. The excess benzaldehyde was distilled under high vacuum. The residue was dissolved in chloroform, filtered, and chromatographed on silica gel (10 g/g of product) using chloroform as eluent. After elution of the undistilled benzaldehyde, a white solid was obtained which was readily recrystallized from hexanes to yield 5.2 g of a crystalline material: mp 113–128°; $[\alpha]^{30}_{589}$ 1.84° (*c* 0.543, CH_3OH); nmr ($CDCl_3$) τ 6.46, 6.33 (singlets, 6 H), 7.17–5.50 (multiplet, 6 H), 5.27 (doublet, *J* = 8 Hz), 4.62 (doublet, *J* = 3 Hz, 1 H), 4.40 (singlet), 4.20 (broad singlet, 2 H), 2.55 (multiplet, 5 H).

2,3-Di-*O*-methyl-4,6-*O*-benzylidene-D-glucopyranose Benzoate. Benzoyl chloride, 2.6 g (18.5 mmol), was added to a solution of 5.2 g (17.6 mmol) of 2,3-di-*O*-methyl-4,6-*O*-benzylidene-D-glucose in 20 ml of pyridine. After 1.5 hr, the mixture was poured into ice–water and the solid was collected by filtration and dissolved in 150 ml of dichloromethane. The dichloromethane solution was washed with 5 ml each of 0.1 *N* HCl and water and evaporated to dryness. Recrystallization of the residual solid from methanol yielded white needles: mp 136–142°; $[\alpha]^{30}_{589}$ −50.0° (*c* 0.588, CH_3OH); ir (KBr) 1745 cm^{-1} (s); nmr ($CDCl_3$) τ 6.46 and 6.28 (singlets), 6.38 and 6.32 (singlets, 6 H), 6.62–5.50 (multiplet, 6 H), 4.42 and 4.38 (singlets, 1 H), 4.05 (doublet, *J* = 8 Hz), 3.46 (doublet, *J* = 3 Hz, 1 H), 2.5–1.8 (multiplet, 10 H).

2,3-Di-*O*-methyl-1-β-D-glucopyranose Benzoate (II). A solution of 1.2 g (2.3 mmol) of 2,3-di-*O*-methyl-4,6-*O*-benzylidene-D-glucopyranose benzoate in CH_3OH was boiled with charcoal and filtered hot, and hydrogenolysis was carried out in a Parr hydrogenator with 0.5 g of 10% palladium on charcoal at 30 psi. After filtration and evaporation of the solvent, the partially solid residue was recrystallized from benzene as rosettes: mp 123–124°; $[\alpha]^{30}_{589}$ −60.7° (*c* 0.626, CH_3OH); ir (KBr) 1735 (s), 1260 (s) cm^{-1}; nmr ($CDCl_3$) τ 6.25 and 6.40 (singlets, 6 H), 6.00–6.85 (multiplet, 8 H), 4.22 (doublet, *J* = 8 Hz, 1 H), 1.65–2.65 (multiplet, 5 H).

Anal. Calcd for $C_{15}H_{20}O_7$: C, 57.68; H, 6.46. Found: C, 57.59; H, 6.34.

α- and β-2,3,4,6-Tetra-*O*-methyl-D-glucopyranose Benzoate (III). Benzoyl chloride, 0.702 g (5.0 mmol), was added to a solution of 1.18 g of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose[14] (5.0 mmol) in 5 ml of pyridine. After 2 hr the mixture was added to ice–water, and the oil which separated was dissolved in chloroform. The chloroform solution was washed successively with 2 × 50 ml of 0.1 *N* HCl and 50 ml of water and evaporated to dryness. The residual oil was submitted to molecular distillation: bp 160–170° (0.35 mm); $[\alpha]^{30}_{589}$ 45.9° (*c* 0.626, $CHCl_3$) (lit.[15] $[\alpha]^{20}_{D}$ −27.55 (*c* 0.9, $CHCl_3$), β anomer; $[\alpha]^{20}_{D}$ 123.3 (*c* 0.6, $CHCl_3$), α-anomer); nmr (CCl_4) τ 7.0–6.06 (multiplet, 18 H), 5.74 (doublet, *J* = 8 Hz), 6.55 (doublet, *J* = 3 Hz, 1 H), 1.75–2.66 (multiplet, 2 H).

Anal. Calcd for $C_{17}H_{24}O_7$: C, 59.99; H, 7.11. Found: C, 61.04; H, 6.83.

Apparatus. All spectrophotometric kinetic measurements were made using either a Gilford Model 2000 spectrometer equipped with four thermospacers through which water at the desired temperature was circulated, a Cary 15 spectrophotometer equipped with the Radiometer autotitrator described by Maley and Bruice,[16] thermostated at 30.0 ± 0.1°, or a Cary 16 spectrophotometer thermostated at 30.0 ± 0.1°. Measurements of pH at high temperatures were made with a Metrohm EA 115 glass electrode and a salt bridge to a calomel electrode.

Optical rotations were measured using a Perkin-Elmer Model 141 polarimeter using 10-cm cells thermostated at 30.0 ± 0.1°. Infrared spectra were recorded on a Perkin-Elmer 137 spectrophotometer. Nmr spectra were recorded on a Varian T-60 spectrometer using TMS or DSS as internal standards.

The multiphasic kinetic curves were fitted using an EAI TR-20 analog computer. Hard copies of the curves were obtained using a Houston Instrument Model 2000 XY recorder interfaced to the computer output channels.

Kinetics. All kinetic measurements were made in aqueous solution at an ionic strength of 1.0 (KCl). Measurements were made in the alkaline region at 30°, and in the acidic region at 56.8, 60.3, 64.7, 70.0, 78.0, and 88°. All rates were determined by following decreasing absorbance at 240 nm. Stock solutions of the esters were prepared in methanol (*ca.* 5 mg/ml) and appropriate size aliquots of these solutions were added to the kinetic solution to produce an absorbance of ~0.8.

In those cases where good first-order behavior was observed, the rate constants were calculated by a linear least-squares analysis of $\log [(A_\infty - A_0)/(A_\infty - A_t)] = -kt$ using an Olivetti-Underwood Programma 101 or the UCSB on-line interface to an IBM 360-75 computer. The analog computer programs required for the kinetic solution of Schemes III and IV are given in Schemes I and II. Analog computer programs, as presented, display curves I or II of Figures 1 and 2. The remaining curves can be generated and displayed by monitoring output of the respective integrator circuits and inverting the signal where necessary.

Results

The rate of hydrolysis of the glucopyranosyl benzoate esters I, II, and III at low pH is described by eq 1. The

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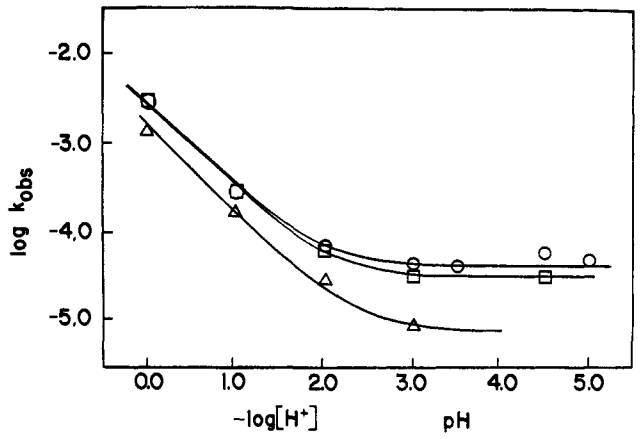
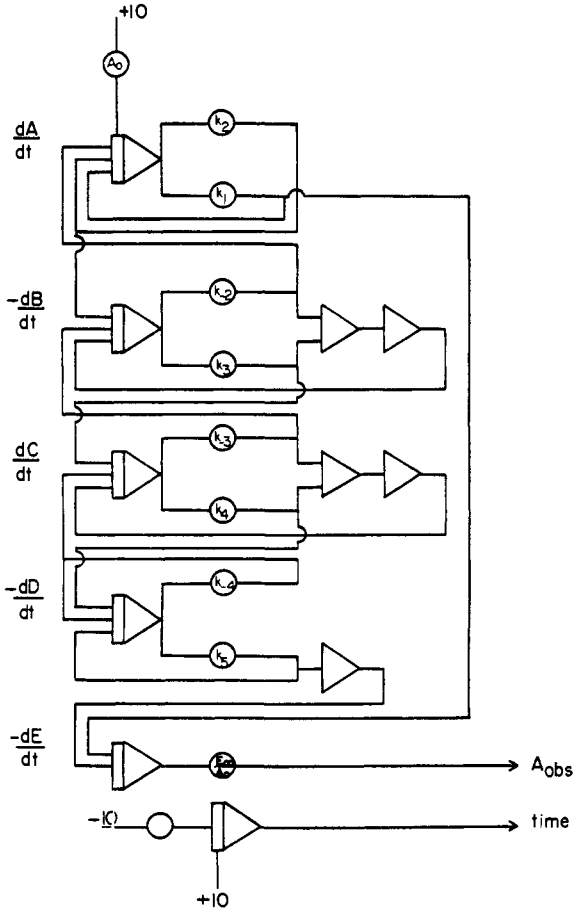


Figure 1. The pH-log k_{obsd} profile for the hydrolysis of I, II, and IIIa,b at 78.0°. The points are experimental and the curves are theoretical for eq 1. The rate constants are tabulated in Table I. I, ○; II, □; III, △.

Scheme I



$$k_{obsd} = k_0 + k_H a_H \quad (1)$$

pH dependence of the rate at 78° is illustrated in Figure 1 and the corresponding constants are presented in Table I. Catalysis of buffer species (acetate and formate) was not observed. At pH 5, the hydrolysis of I showed multiphasic character; the rate constant was determined using an analog computer, programmed as in Scheme I. The rate constant corresponds to k_1' of Scheme III. The remaining rate determinations exhibited good first-order kinetics. The plateau rate for III was too slow to determine conveniently and is not known accurately. The temperature dependence of the rate of acid-catalyzed hydrolysis of I, II, and III is shown in Figure 2, and the activation parameters derived from

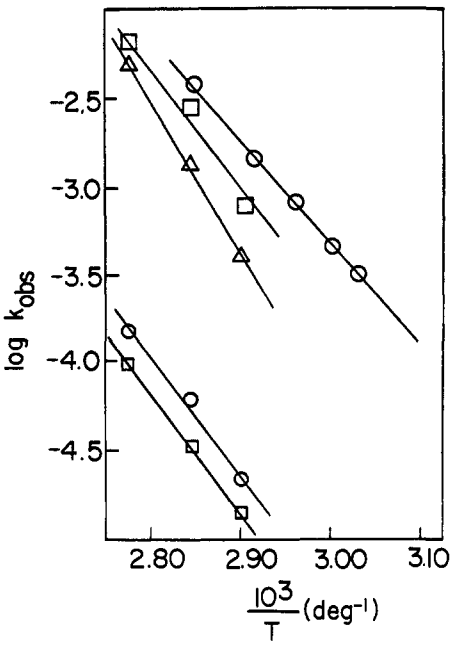


Figure 2. The Arrhenius plots for hydrolysis of I, II, and III at low pH. The upper lines are for hydrolysis at 1.0 *M* HCl and the lower lines are for hydrolysis at pH 4.50 in acetate buffers. I, ○; II, □; III, △.

Scheme II

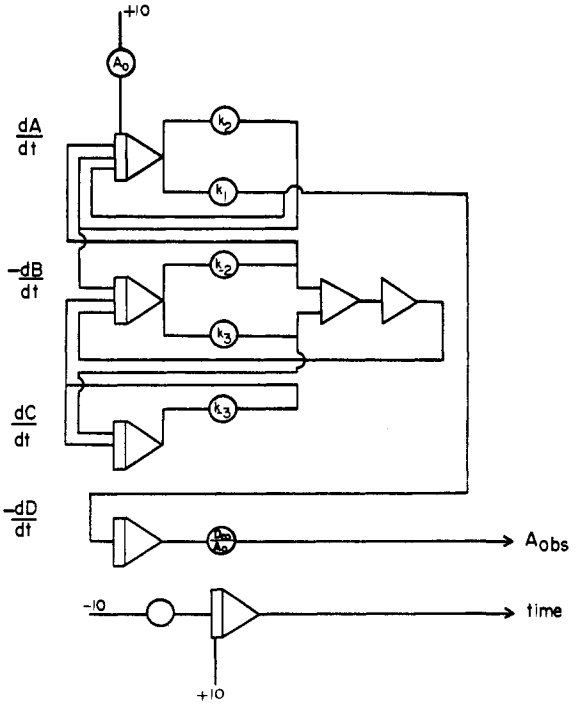


Table I. Rate Constants for Hydrolysis of I, II, and III at Low and Intermediate pH (78°; μ = 1.0 with KCl in H_2O)

	$10^3 k_H$, M^{-1} sec^{-1}	$10^5 k_0$, sec^{-1}
I	3.07	4.41
II	2.89	3.30
III	1.65	0.77

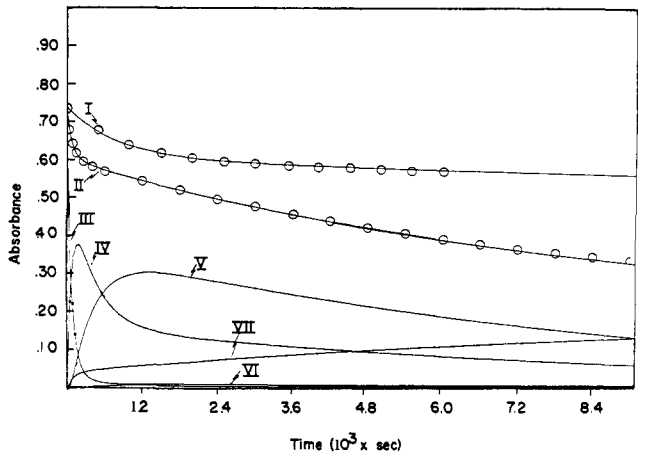


Figure 3. The time dependence of absorbance at 240 nm for the hydrolysis of I at pH 10.25 is indicated by the points. The time scale for curve I is one-tenth that indicated by the abscissa. Curves I and II are the theoretical fit to Scheme III obtained using the analog computer. Curves III through VII are theoretical curves representing the contribution to the observed absorbance at 240 nm by the various components of Scheme III. Curve III corresponds to A, curve IV to B, curve V to C, curve VI to D, and curve VII to product.

these data are tabulated in Table II. The rate of hydrolysis at 88° in 1.0 *M* DCl are given in Table III, as are the solvent isotope effects.

Table II. Activation Parameters for the Acid-Catalyzed and Spontaneous Hydrolysis of Glucose Acylals (25°; μ = 1.0 *M* with KCl in H_2O)

	$\Delta H^{\pm}$, kcal/mol[b]	$\Delta S^{\pm}$, eu[a,b]
k_H(I)	26.6 ± 0.2	6.4 ± 0.6
k_0(I)	27.7	0.3
k_H(II)	31.0	17.3
k_0(II)	28.1	0.7
k_H(III)	35.8	29.8

[a] Extrapolated to 25° from data obtained at higher temperatures. [b] Error limits for I are estimated from the standard deviation of the points from the least-squares slope of the Arrhenius plots.

Table III. Solvent Kinetic Isotope Effect in Acid-Catalyzed Hydrolysis (88°)

	Solvent[a]	$10^5 k_H$, M^{-1} sec^{-1}	k_{H_2O}/k_{D_2O}
I	H_2O	7.52	
	D_2O	13.2	0.568
II	H_2O	6.67	
	D_2O	15.9	0.420
III	H_2O	4.79	
	D_2O	9.95	0.481

[a] Solvent is 1.0 *M* in HCl or DCl, respectively.

The hydrolysis of the esters in the alkaline pH region occurred readily at 30.0°. Multiphasic kinetics were observed for the hydrolysis of I and II; see Figures 3 and 5. The observed time dependence of absorbance at 240 nm for the hydrolysis of I at pH 10.25 is shown in Figure 3. The simplest scheme to which the data could be successfully fitted was Scheme III; the curves of

Scheme III

$$\text{product} \xleftarrow{k_1'} \text{A} \underset{k_{-2}'}{\overset{k_2'}{\rightleftharpoons}} \text{B} \underset{k_{-3}'}{\overset{k_3'}{\rightleftharpoons}} \text{C} \underset{k_{-4}'}{\overset{k_4'}{\rightleftharpoons}} \text{D} \xrightarrow{k_5'} \text{product}$$

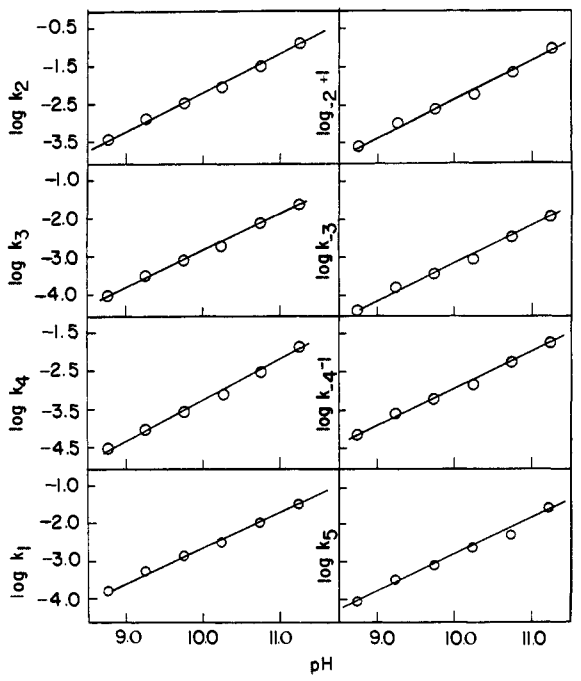


Figure 4. The pH dependence of the rate constants for hydrolysis of I obtained by using Scheme III to analyze the absorbance changes. The second-order rate constants derived from these data are tabulated in Table IV.

Figure 3 were generated by the analog computer using this scheme. The time dependence of absorbance for the hydrolysis of six pH values was fitted to Scheme III and the first-order rate constants, k_1' through k_5', obtained. Figure 4 shows that all rate constants are directly proportional to pH. The specific hydroxide

$$k_n' = k_n[\text{HO}^-] \qquad (2)$$

ion catalyzed rate constants, k_n, are tabulated in Table IV.

The hydrolysis of II at high pH also exhibited multiphasic kinetics which were fit to Scheme IV (Figure 5)

Scheme IV

$$\text{product} \xleftarrow{k_1'} \text{A} \underset{k_{-2}'}{\overset{k_2'}{\rightleftharpoons}} \text{B} \underset{k_{-3}'}{\overset{k_3'}{\rightleftharpoons}} \text{C}$$

by use of the analog computer. Constants k_n' followed eq 2 (Figure 6) to provide the value of k_n (Table IV) and were found to be directly proportional to pH.

Hydrolysis of the equimolar mixture of α- and β-D-tetra-*O*-methylglucopyranosyl benzoate (III) followed good first-order kinetics in the alkaline pH region. The observed rate constant could be described by eq 2, where k_1 is listed in Table IV. It can be shown, using the analog computer, that first-order kinetics will be observed for the reaction of a mixture of two compounds provided the ratio of their rate constants is less than 1.5. This must be the case with III.

Effects of Buffers in the Basic pH Range. Glycine (0.1–1.0 *M*, pH 9.5 and 9.9) and trifluoroethanol (0.1–1.0 *M*, pH 12.45) were found to have no observable effect on the rate of hydrolysis of I. At high free base concentrations ($\geq$0.4 *M*, pH 9.8), the solvolysis of II in the presence of glycine was first order. At lower free base concentrations multiphasic kinetics were observed and the ratio of A_∞/A_0 was larger than when the hydrolysis was carried out in the absence of buffers. Owing

Table IV. Hydroxide Ion Catalyzed Rate Constants for the Hydrolysis of Glucopyranosyl Benzoates (30.0 ± 0.1°, μ = 1.0 M with KCl in H_2O)

	k_1, M^{-1} sec^{-1}	k_2, M^{-1} sec^{-1}	k_{-2}, M^{-1} sec^{-1}	k_3, M^{-1} sec^{-1}	k_{-3}, M^{-1} sec^{-1}	k_4, M^{-1} sec^{-1}	k_{-4}, M^{-1} sec^{-1}	k_5, M^{-1} sec^{-1}
I	13.3 ± 0.35	52.7 ± 0.43	3.82 ± 0.43	9.60 ± 0.28	4.59 ± 0.17	2.81 ± 0.27	77.0 ± 0.25	11.6 ± 0.14
II	2.14 ± 0.15	2.16 ± 0.09	1.07 ± 0.19	2.79 ± 0.29	1.79 ± 0.6			
III	0.706 ± 0.004							

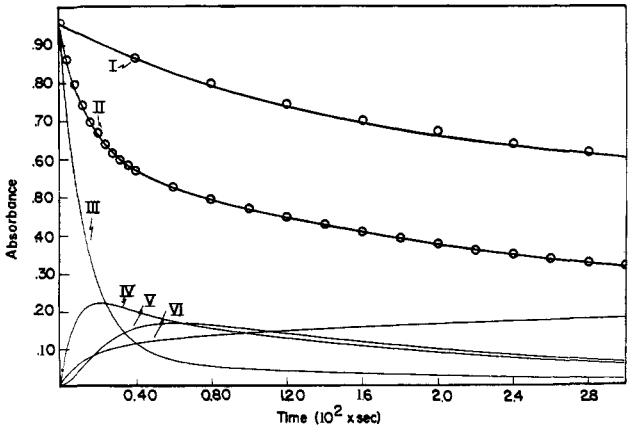


Figure 5. The time dependence of absorbance at 240 nm for the hydrolysis of II at pH 11.02 is indicated by the points. The time scale for curve I is one-tenth that indicated by the abscissa. Curves I and II are the theoretical fit to Scheme IV obtained using an analog computer. Curves III through VI are theoretical curves representing the contribution to the observed absorbance at 240 nm by the various components of Scheme III. Curve III corresponds to A, curve IV to B, curve V to C, and curve VI to product.

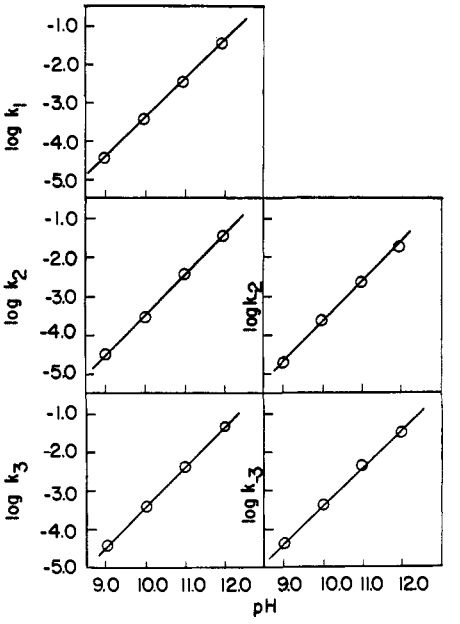


Figure 6. The pH dependence of the rate constants for the hydrolysis of II obtained by using Scheme IV to analyze the absorbance changes. The second-order rate constants derived from the data are tabulated in Table IV.

to the complex nature of the reaction of glycine with II, no attempt was made to carry out a quantitative analysis. The reaction of glycine (0.1–0.6 M) with III produced small increases in the rate of solvolysis.

Hydrazine was the only buffer species examined which had an appreciable effect on the rates of disappearance of each of the esters. At all concentrations and pH's investigated, the reaction of hydrazine with I and II followed pseudo-first-order kinetics. The observed rate constants were found to obey eq 3. The depen-

$$k_{obsd} = k_N \frac{K_a[B_T]}{a_H + K_a} + \left[k_{gb}\left(\frac{K_a}{a_H + K_a}\right) + k_{ga}\frac{a_H K_a}{(a_H + K_a)^2}\right][B_T]^2 \quad (3)$$

dence of k_{obsd} on buffer concentration is illustrated in Figure 7 for the hydrazinolysis of II. The rate constants for hydrazinolysis of I and II are given in Table V.

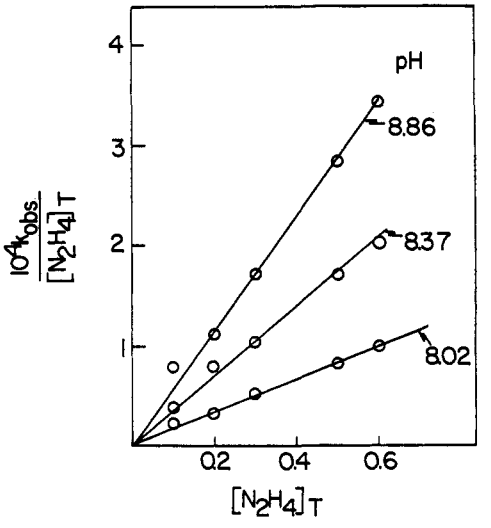


Figure 7. Second-order dependence on the concentration of hydrazine for the rate of hydrazinolysis of II.

Table V. Rate Constants for Hydrazinolysis of Glucopyranosyl Benzoates (30.0 ± 0.1°, μ = 1.0 M with KCl in H_2O)

	10^3k_N, M^{-1} sec^{-1}	10^3k_{gb}, M^{-2} sec^{-1}	10^3k_{ga}, M^{-2} sec^{-1}
I	0.612 ± 0.007	74.2 ± 1.1	3.15
II	2.39 ± 0.03	79.7 ± 3.1	1.03
III[a]	0.25	44.1	13.3
	0.94	11.6	0.99

[a] The rate constants derived for III are not very accurate owing to the difficulty in obtaining accurate first-order rate constants on a mixture of two compounds.

The reaction of III in the presence of hydrazine exhibited biphasic kinetics at several of the hydrazine concentrations investigated. The rate constants were obtained by fitting the biphasic curves using the analog computer programmed to solve eq 4. The observed

$$d(\text{product})/dt = k_\alpha[\alpha\text{-III}] + k_\beta[\beta\text{-III}] \quad (4)$$

rate constants were analyzed using eq 3, and the derived

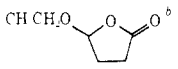
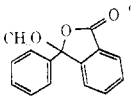
rate constants are listed in Table V. The rate constants in Table V were determined from measurements made at three pH's between 8.0 and 9.0 (0.1–0.6 *M* hydrazine) for five concentrations at each pH.

Discussion

The hydrolysis of the acylals (I, II, and III) at low pH is quite slow. At higher temperatures two distinct processes are observable. Below pH 3, the hydrolysis is hydrogen ion catalyzed (k_H) and above pH 3 spontaneous (k_0) (Figure 1). Since buffer acids are not catalysts and the kinetic deuterium solvent isotope effect ($k_H^{H_2O}/k_D^{D_2O}$) = 0.4–0.6, k_H represents specific acid catalysis. Two mechanisms for specific acid catalysis must be considered; one in which solvent plays no nucleophilic role (unimolecular or A-1) and one in which solvent molecules participate (bimolecular or A-2). Several parameters have been used to distinguish between these mechanisms.[17-20] Kinetic solvent isotope effects and activation parameters[21-23] have been employed in the present study.

Salomaa[23a] studied the temperature dependence of the acid-catalyzed reactions of methoxymethyl formate, methoxymethyl acetate, and ethoxymethyl acetate. The value of $\Delta S^{\pm}$ was in good agreement for an A-1 mechanism for the latter two compounds. A competition between the A-1 and A-2 mechanisms was established for the hydrolysis of methoxymethyl formate.[24] Thus, a plot of log k_{obsd} *vs.* $1/T$ for methoxymethyl formate was distinctly curved.[23a] Salomaa[23a] analyzed the data in terms of two mechanisms and the $\Delta S^{\pm}$ values obtained from his analysis are presented in Table VI. Weeks and coworkers[22] have found

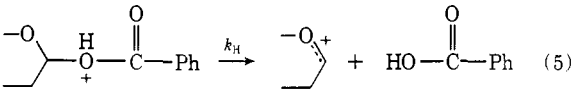
Table VI. Entropy of Activation and Kinetic Solvent Isotope Effect for the Acid-Catalyzed Hydrolysis of Various Acylals

Substrate	$\Delta S^{\pm}$, eu	k_{H_2O}/k_{D_2O}	Mechanism
CH_3OCH_2OCHO[a]	+1.7		A_1
	−12.7		A_2
$CH_3OCH_2OCOCH_3$[a]	+4.1		A_1
$CH_3CH_2OCH_2OCOCH_3$[a]	+5.2		A_1
	−6.9	0.42	A_1
	−19.4	0.50	A_2

[a] Calculated from data presented in ref 23a. [b] Reference 21. [c] Reference 22.

$\Delta S^{\pm}$ for the acid-catalyzed hydrolysis of methyl pseudo-2-benzoylbenzoate to be −19.4 eu in agreement with an A-2 mechanism (Table VI). Finally, Fife has concluded[21] that the acid-catalyzed hydrolysis of γ-ethoxy-γ-butyrolactone occurs by an A-1 mechanism. The value for $\Delta S^{\pm}$ was slightly negative (Table VI) but was deemed not unreasonable due to the restricted rotation about the bond being broken in the transition state. The kinetic solvent isotope effects are also included in Table VI, where available. Comparison of the data of Tables II, III, and VI indicates that the acid-catalyzed hydrolysis of I, II, and III occurs by an A-1 mechanism.

Since alkyl benzoate esters (or esters in general) are not subject to AAL1 hydrolysis, the A-1 hydrolysis of the acylals of this study must proceed *via* ionization to benzoic acid and an oxocarbonium ion. The specific acid-catalyzed hydrolysis of glycosides represents much



(5)

the same process.[25]

The rate constants obtained for the hydrolysis of I and II were independent of pH (3.0 to 5.0) and buffer (to 0.5 *M*). At least three kinetically equivalent mechanisms can be envisioned for spontaneous hydrolysis (k_0). A specific acid–specific base catalyzed mechanism can be readily ruled out since it would demand a rate constant for attack of HO^- upon the protonated acylal which would exceed the diffusion-controlled limit when a conservative estimate[26] for the pK_a of the protonated acylal is employed. Direct displacement by H_2O can be discarded since the rate constants are independent of the concentration of added nucleophile even though acetate and formate are much better nucleophiles than water and the entropies of activation indicate that water is not involved in the transition state (compare Table II and Table VII). The log k_{obsd} *vs.* pH profile for III (Figure 1) was fitted to the theoretical curve by assuming a pH independent rate constant of 0.77×10^{-5} sec^{-1}. The most reasonable mechanism is that of SN1 heterolysis to oxocarbonium ion and benzoate anion. Fife[21] has made similar observations for the pH-independent hydrolysis of γ-ethoxy-γ-butyrolactone. In addition, the activation parameters derived for the spontaneous hydrolysis of I and II are similar to those in the literature for SN1 reactions of several benzoate esters.

The observation of a pH independent hydrolysis of acylals is not new. Fife[21] found the rate of hydrolysis of γ-ethoxy-γ-butyrolactone to be pH independent when $5 \leqslant pH \leqslant 9$. Despite the large negative entropy of activation, −18.5 eu, the uncatalyzed opening to a zwitterion intermediate was indicated by solvent effects and the kinetic solvent isotope effect. In a recent study Hussain and coworkers[28] have found that the rates of hydrolysis of 2-pyranyl benzoate, 1-ethoxy-1-ethyl benzoate, and 1-ethoxy-1-ethylaspirin are independent of pH between 2.0 and 12.0. The effects of mixed solvents and the kinetic solvent isotope effect were claimed to be consistent with an SN1 solvolysis of these compounds.[28] Thus, the pH independent hydrolysis of the

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(26) Bunton and DeWolfe[27] have estimated the pK_a for dimethyl acetal to be −5.4. The pK_a for a protonated acylal would undoubtedly be lower than this value owing to the inductive effect of the benzoyl group.
(27) C. A. Bunton and R. H. DeWolfe, *J. Org. Chem.*, 30, 1371 (1965).
(28) A. Hussain, M. Yamasaki, and J. E. Truelove, *J. Pharm. Sci.*, in press. We would like to thank Dr. Hussain for communicating these results prior to publication.

acylals so far studied seems to be consistent with the SN1 mechanism. Such a finding is not surprising. Many examples can be found in the literature where the hydrolysis of benzoate esters yields a stable carbonium ion intermediate.[29,30] The oxocarbonium ions generated from acylals are stabilized by the neighboring C_5 oxygen and would be expected to be relatively easily formed under conditions where little catalysis can be expected.

The hydrolyses of I, II, and III at pH >7.0 are much more facile than hydrolysis at lower pH values. The reactions of I and II are substantially more complicated than at lower pH's as indicated by multiphasic kinetics at constant pH (see Results). The results will be discussed first in terms of elucidating the chemistry and then in terms of the mechanisms of various processes.

The observation of multiphasic kinetics is often an indication that two or more substances are reacting with different rate constants. Substantial deviation from first-order kinetics is observed when the ratio of the rate constants for two compounds $\geqslant 3.0$. The following observations were used to eliminate the possibility that hydrolysis of a mixture (*e.g.*, α and β isomers) was being observed. For I, the specific rotation at the sodium D-line was constant after several recrystallizations and did not change with time in slightly acidic solutions, $[\alpha]^{30}_{589}$ −28.8. Rotations at several wavelengths, 578, 546, 436, and 365 nm, likewise remained constant. The melting point was sharp at 183–184°. Fletcher[12] and Zervas[31] have reported $[\alpha]^{20}D$ −27° and mp 191–192° for 1-β-D-glucopyranosyl benzoate. The fact that the rotation remained constant with time and repeated recrystallization indicated that the benzoyl substituent must be bound to the C_1 oxygen. The nmr spectrum served to establish that only the β anomer was present. An nmr spectrum of I in DMSO-d_6 had only one doublet in the region where the anomeric proton signals are to be found, τ 4.19, J = 7 Hz. Addition of benzene did not alter the spectrum. The nmr spectra of 4,6-*O*-benzylidene-D-glucose benzoate and 2,3-di-*O*-methyl-4,6-*O*-benzylidene-D-glucose benzoate both exhibited a pair of doublets in this region, J = 7 and J = 3 Hz, assignable to the β and α anomers, respectively. Thus, the substance which exhibited multiphasic kinetics must have been a single isomer and the unusual behavior must be inherent to the reaction.

One possibility for the multiphasic kinetics is that rearrangement to a nonreactive species is competitive with hydrolysis. This nonreactive species must then hydrolyze very slowly, or undergo a rate-determining rearrangement to the original reactive ester. In support of this hypothesis, Fletcher and coworkers[11] have observed that various acylals of α-D-glucopyranose and α-D-ribofuranose readily undergo acyl transfer in slightly alkaline solution. These workers[11] claimed that the β anomers were stable under these conditions.

Several schemes were considered as likely possibilities to which the time dependence of the absorbance might be fitted. For each scheme the analog computer was used to simulate the time dependence of the absorbance. The following minimal assumptions were made: (1) the products of acyl transfer (rearranged benzoate esters) have the same molar absorptivity as I at 240 nm; (2) *at infinite time,* the only species absorbing at 240 nm is the benzoate anion; (3) hydrolysis occurs only in those isomers where the benzoyl group is bonded to the C_1 oxygen. This last assumption provides the simplification necessary to obtain a unique fit of the data. The validity of this assumption is discussed more thoroughly in what follows.

As noted in the Results, Scheme III is the simplest scheme for which a good fit to the data could be obtained. A fit was deemed acceptable only when the theoretical curve passed within 0.005 absorbance unit of every point. In most cases the fits obtained were much better than these limits. The computer-generated time dependences of the absorbance, represented by curves I and II of Figure 3, were found to be very sensitive to the variable voltages representing the rate constants k_1, k_2, k_{-2}, k_4, k_{-4}, and k_5. For example, a change of 10% in one of the settings produced a noticeable deviation from an acceptable fit to the data. Furthermore, if a second attempt was made to fit the data, the voltages obtained for the various rate constants differed by less than 10%. The curve fitting process is thus highly reproducible giving the same rate constants each time the curve is fitted. The high reproducibility must be attributed to the fact that each rate constant makes its primary effect felt in only one portion of the multiphasic curve. The curve fitting process was quite insensitive to the voltages corresponding to k_3 and k_{-3} provided $k_3 \approx 2k_{-3}$. The ratio, k_3/k_{-3}, is approximately equal to the anomeric equilibrium constant for D-glucopyranose.[32]

The kinetic scheme employed for the fitting of the experimental data can be easily related to known reactions of glucose and glucose esters. In analogy with the established rapid benzoyl transfers observed by Fletcher for the α anomer of I,[11] the rearrangement of A to B in Scheme III is consistent with the benzoyl transfer shown in Scheme V. Transfer of the benzoyl group to both the C_2 and C_6 oxygens must be considered as likely possibilities. Thus for I, the observed initial rapid decrease in absorbance at 240 nm (curve II of Figure 3) is associated with the rapid alkaline hydrolysis of the unrearranged substrate. The competing benzoyl transfer reaction yields the unreactive 2- and/or 6-glucopyranose benzoates. The magnitude of the initial rapid decrease in absorbance associated with the disappearance of I is controlled by the rate constants, k_1 and k_2. Thus, k_1 and k_2 are used to obtain the theoretical fit to this portion of curve II. As can be seen from curve III of Scheme I, the substrate, I, remains at a low steady state concentration throughout the reaction even though it disappears very rapidly in the initial stages of the reaction. Curve IV indicates a rapid buildup in concentration of the rearranged esters. The fitting of curve II is controlled primarily by the value of k_{-2} at these intermediate times. In addition, transfer of the benzoyl group to the C_3 and C_4 hydroxyl groups is probably occurring[33] but cannot be detected spectrally. The relatively rapid decrease in curve IV and the corresponding increase in curve V are attributed to anomeriza-

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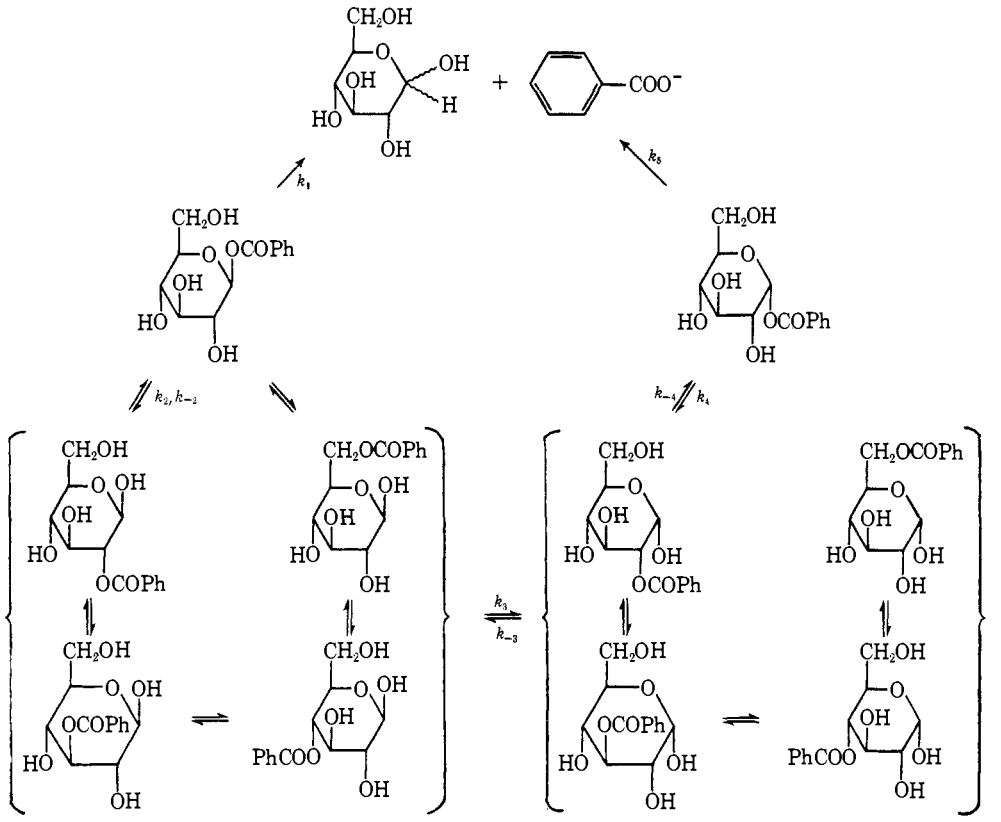
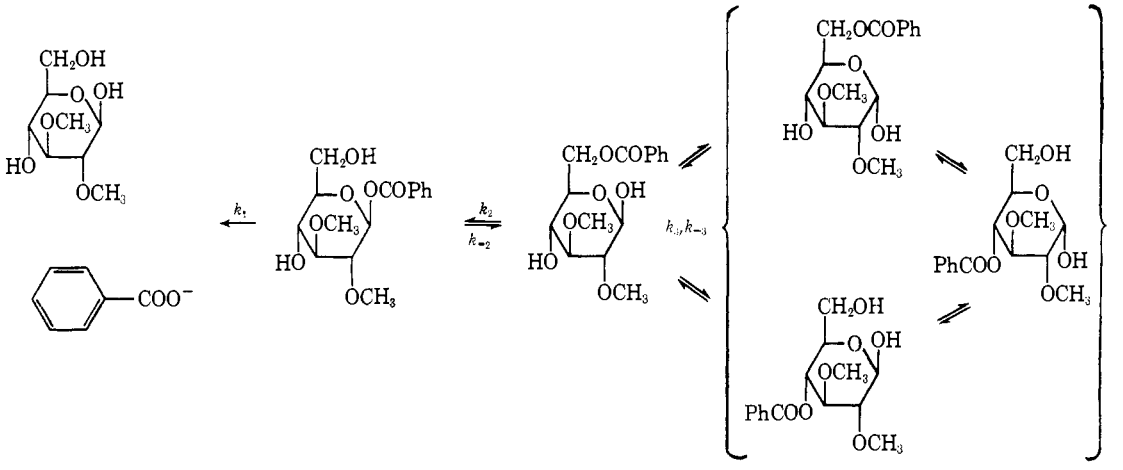
Scheme V



Scheme VI



tion, yielding a complex mixture of monobenzoates. Thus, as curves IV and V approach the equilibrium ratio, it is quite likely that an equilibrium mixture of 2-, 3-, 4- and 6-glucopyranosyl benzoates composes the reaction mixture; see Scheme V. This postulation is supported by the observed polarimetric rate constant, found to be approximately equal to the sum of $k_1 + k_2$ [0.1 *M*, 1,4-diazabicyclo[2.2.2]octane, pH 9.0]. The fitting of curve II to the observed absorbances at long times is controlled by the rate constants k_4, k_{-4}, and k_5. These rate constants determine the rate of formation of the α anomer of I and its subsequent hydrolysis. It can be seen that the concentration of this isomer remains at a low stationary state level throughout the reaction. Curve VII represents the increasing absorbance due to the benzoate anion. Since the molar absorptivity of benzoate anion is much smaller than for the esters at 240 nm, the overall change in absorbance is smaller than observed for the combined absorbance change for the esters, curve II. The high reproducibility of the rate constants for fitting the data to Scheme III is now readily understood. Each section of the absorbance *vs.* time curve is controlled by a different combination of rate constants which regulate the concentration of each compound or group of compounds.

A similar analysis of the multiphasic absorbance *vs.* time curves obtained in hydrolysis of II was carried out using Scheme IV, and the corresponding chemical interconversions are outlined in Scheme VI. The detailed analysis of the various competing reactions is similar to that outlined above for I. Competing with the initial rapid hydrolysis of II is the benzoyl transfer to the C_6 oxygen with rate constant k_2. Subsequent anomerization or benzoyl transfer to the C_4 oxygen yields an unreactive intermediate which causes the multiphasic kinetic behavior. Reversal of each of these re-

arrangements with rate constants k_{-3} and k_{-2} maintains a slowly decreasing concentration of II which is slowly hydrolyzed at longer times.

It is necessary to examine the validity of assumption 3 used to obtain the theoretical curves of Figures 3 and 5. The assumption was based on the fact that the rate of hydrolysis of esters has been shown to be proportional to the pK_a of the leaving group.[34] The pK_a for the hemiacetal has been estimated to be 14.0 by Pocker.[35] The pK_a of the primary and secondary glucosyl hydroxyl groups is expected to be approximately 15.0, as found by Long[36] for ethylene glycol. The rate of hydrolysis of I at long reaction times is given by eq 6, if

$$\mathrm{d(product)}/dt = k_1[\mathrm{A}][\mathrm{HO^-}] + k_5[\mathrm{D}][\mathrm{HO^-}] \quad (6)$$

this assumption is correct. If, on the other hand, hydrolysis of the intermediates B and C also gives rise to product, the appropriate rate equation is given by eq 7.

$$\mathrm{d(product)}/dt = k_1[\mathrm{A}][\mathrm{HO^-}] + k_5[\mathrm{D}][\mathrm{HO^-}] + k_6[\mathrm{B}][\mathrm{HO^-}] + k_7[\mathrm{C}][\mathrm{HO^-}] \quad (7)$$

Equations 6 and 7 can be simplified if it is assumed that $k_1 \approx k_5$ and $k_6 \approx k_7$. The ratio ([B] + [C])/([A] + [D]) can readily be obtained from the curves of Figure 3. Making these substitutions and eliminating [HO⁻], eq 6 and 7 simplify to eq 8 and 9. It can be estimated from

$$\mathrm{d(product)}/dt = 2k_1[\mathrm{A}] \quad (8)$$

$$\mathrm{d(product)}/dt = 2k_1[\mathrm{A}] + 50k_6[\mathrm{A}] \quad (9)$$

eq 8 that a significant proportion of the product can be derived from B and C of Scheme III if $k_1 \approx 25k_6$. Quantitative data for the rates of hydrolysis of benzoate esters of polyhydroxylic alcohols are lacking. As a result, hydrolysis of B and C of Scheme III cannot be ruled out unequivocally.

The rate constants (Table IV) obtained by fitting the experimental absorbance–time data, using Schemes III and IV, were directly proportional to the hydroxide ion concentration (see Figures 4 and 6). The hydroxide ion catalyzed rate constants are tabulated in Table IV. It is evident from Table IV that the rate constants for comparable processes are of the same magnitude for each of the esters. The differences are slightly larger than would be anticipated from the difference in the substituent constants, σ^*, alone. It appears that the neighboring hydroxyl groups are providing a small amount of catalysis. Considerable discussion of the mechanism of neighboring hydroxyl group assistance in ester hydrolysis can be found in the literature.[10] The rate enhancements obtained by neighboring hydroxyl groups participating are known to be small.[37-39] Bruice and Fife[38] showed that both cis and trans 1,2-diol monoacetates in five-membered rings are hydrolyzed six to ten times faster than the methoxyl-substituted compound. The trans monoacetate hydrolyzed slightly slower than the cis isomer. Kupchan[37,40] observed rate enhancements of the same magnitude for cis-1,2 and cis-1,3-diaxial diol monoacetates in six-membered rings. The trans 1,3-diol monoacetates were ineffective owing to the restrictive stereochemistry.[37] The rate enhancements observed for I and II are similar to those noted above. The C_2 hydroxyl group appears to be a more effective catalyst than the C_6 hydroxyl group (compare I and II), probably because of a more favorable stereochemistry. The C_6 hydroxyl group must provide catalysis since the rate of hydrolysis of II is substantially greater than that of III. The data of Figures 4 and 6 indicate that acyl transfer, the steps controlled by k_2, k_{-2}, k_4, and k_{-4}, is also catalyzed by hydroxide ion as would be anticipated for an O → O acyl shift (*i.e.*, direct intramolecular nucleophilic attack of the conjugate base of the substrate).

With the exception of hydrazine, the complexity of the hydrolysis reaction makes the study of the effects of buffer species difficult (see Results). The reaction of hydrazine with I, II, and III differs significantly from the other buffers examined. The multiphasic kinetic behavior observed during the hydrolysis of I and II was replaced by a pseudo-first-order reaction. The rate constants and the final absorbances at 240 nm were significantly greater than those observed for hydrolysis at the same pH. These observations can be explained in terms of the established enhanced reactivity of hydrazine in several reactions which has been attributed to the "α effect."[41] Thus, in the present study, hydrazine reacts at the benzoyl carbonyl carbon at a rate which exceeds that for hydrolysis or rearrangement. First-order kinetics are expected if the aminolysis reaction is much faster than the O → O benzoyl transfer and the higher final absorbance is associated with the amide product. For each of the esters investigated, second-order dependence on hydrazine was found as anticipated for self general base catalyzed nucleophilic displacement on an ester bond.[42]

It is worthwhile noting the similarities of the results of this study for I and II and the behavior observed by Voet and Abeles[8] for the glucosyl–enzyme intermediate from sucrose phosphorylase. Thus, multiphasic kinetics were observed for the release of glucose from the periodate modified glucosyl–enzyme complex, and from the urea denatured glucosyl–enzyme complex. The rate of release of glucose from glucosyl peptides, obtained from a pepsin digest, was enhanced significantly in the presence of *N*-methylhydroxylamine, an "α-effect" nucleophile. The peptide fragments and the modified glucosyl–enzyme complex were increasingly unstable with increasing [HO⁻]. Each of these properties was observed in the base-catalyzed hydrolysis of I. The similarities in behavior noted above provide support for the postulation[8] of an acylal intermediate in the mechanism of sucrose phosphorylase.

Acknowledgment. This work was supported by a grant from the National Institutes of Health.

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