Acknowledgments.—We wish to thank Mrs. J. Goodwin of this laboratory for the measurements of 60-Mc/sec spectra. We are especially indebted to Mr. Ross G. Pitcher of Varian Associates, Pittsburgh, Pennsylvania, and to Dr. L. D. Hall of the University of British Columbia, Vancouver, British Columbia, for the spin–spin decoupling experiments at 100 Mc/sec.

C-2 Oxyanion Participation in the Base-Catalyzed Cleavage of *p*-Nitrophenyl β-D-Galactopyranoside and *p*-Nitrophenyl α-D-Mannopyranoside[1]

ROBERT C. GASMAN[1] AND DONALD C. JOHNSON[2]

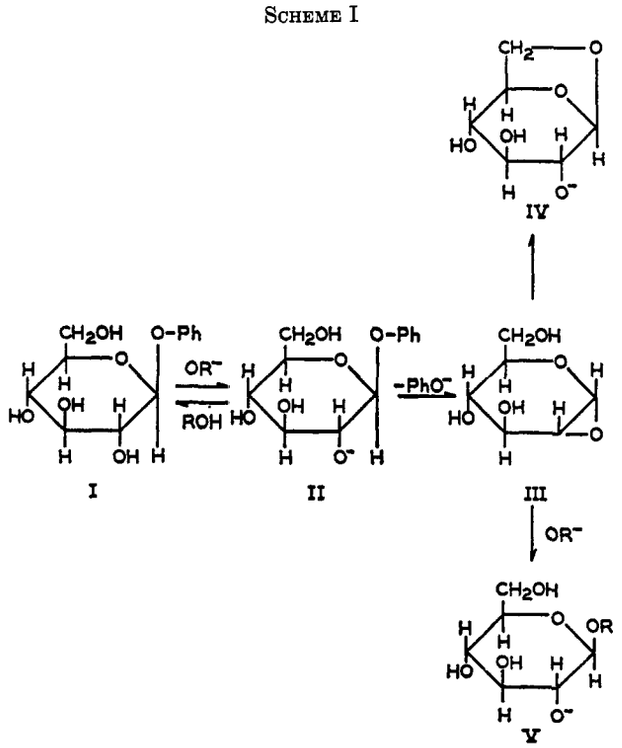
The Institute of Paper Chemistry, Appleton, Wisconsin 54911

Received December 27, 1965

p-Nitrophenyl 2-*O*-methyl-β-D-galactopyranoside and *p*-nitrophenyl 2-*O*-methyl-α-D-mannopyranoside were synthesized and their cleavage reactions were compared with those of *p*-nitrophenyl β-D-galactopyranoside and *p*-nitrophenyl α-D-mannopyranoside. The cleavage rates (35–55°) and products formed from these glycosides (all capable of a *trans*-diaxial orientation at C-1 and C-2) in methanolic and aqueous base solution were studied. Reaction of the glycosides in methanolic sodium methoxide resulted in over-all retention of configuration, whereas the 2-*O*-methylglycosides gave substantial amounts of *p*-nitroanisole and its reduction products (formed by secondary reaction with 2-*O*-methyl sugar). Kinetic studies included the effect of base concentration, the hydrogen isotope effect, ionic strength effects, and the effect of hydrogen peroxide. Blocking of the C-2 oxygen by a methyl group caused an enormous decrease in the rate of cleavage and a change in reaction pathway in both cases. The *p*-nitrophenyl glycosides react by neighboring-group participation of the C-2 oxyanion, whereas the 2-*O*-methylglycoside reactions proceed, at least in methanolic sodium methoxide, by bimolecular nucleophilic aromatic substitution.

Previous studies of the reaction of *para*-substituted phenyl D-glucosides[3] in basic solution (Scheme I) have shown that the β anomer reacts by a process which involves neighboring C-2 oxyanion participation.[4] A stereochemical requirement for operation of this process, axial disposition of the C-2 anion and the C-1 phenoxyl, may be fulfilled in the β-D-glucoside structure but not in the α. The structures of phenyl β-D-galactosides and phenyl α-D-mannosides also meet this stereochemical requirement. However, the information available concerning the β-galactosides is too limited to show whether or not the neighboring C-2 oxyanion process is important for their reactions as well.[4] No investigations specifically concerned with the role of the C-2 oxyanion in the reaction of phenyl α-D-mannosides have been reported.

SCHEME I



Previous investigators,[4,5] in considering reactions of *para*-substituted phenyl β-D-glucosides, have noted what appeared to be a trend toward an ionic mechanism as the electron-withdrawing character of the substituent increased. The basis for this observation was the downward trend in yield of 1,6-anhydro-β-D-glucopyranose toward 50% (*i.e.*, suggesting the anomeric carbon could be attacked equally well from either side of the ring as the electron-withdrawing character of the *para* substituent increased).

The primary purpose of this study, therefore, was to ascertain whether or not the neighboring-group process found to be important in the phenyl β-D-glucoside reactions extended to the reaction of other phenyl glycosides. A subordinate objective, however, was to determine whether or not incursion of an ionic mechanism was significant when the strong electron-withdrawing *p*-nitrophenyl group was the aglycon. To accomplish these purposes the rates and products formed in the reactions of the *p*-nitrophenyl glycosides and *p*-nitrophenyl 2-*O*-methylglycosides of β-galactose and α-mannose in methanolic and aqueous base solution were studied.

Results

Product Analysis.—Initial compositions of reaction mixture solutions, time, temperature of reaction, and

(1) A portion of a thesis submitted by R. C. Gasman in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy from Lawrence University, Appleton, Wis., Jan 1966.

(2) To whom inquiries should be addressed.

(3) All glycosides referred to herein are glycopyranosides.

(4) C. E. Ballou, *Advan. Carbohydrate Chem.*, **9**, 59 (1954).

(5) C. M. McCloskey and G. H. Coleman, *J. Org. Chem.*, **10**, 184 (1945).

TABLE I

SUMMARY OF PRODUCT ANALYSIS RESULTS

p-Nitrophenyl glycoside reactant (mole)	Reaction medium, composition	Reaction temp, °C	Time, hr	Major products	Identification	Quantity produced, %
β-D-Galactoside (0.010)	NaOMe–MeOH, 0.25 *N*, 300 ml, 0.025% water	65	12	Levogalactosan[a]	Mp, mmp, $[\alpha]^{25}D$	76.3
				Methyl β-D-galactoside	Mp, $[\alpha]^{25}D$, glpc[b]	7.9
				p-Nitroanisole	Glpc	
				p-Nitrophenol	Spectrophotometry	
α-D-Mannoside (0.010)	NaOMe–MeOH, 0.25 *N*, 300 ml, 0.025% water	65	51	Methyl α-D-mannoside	Mp, mmp, $[\alpha]^{25}D$	88.3[c]
				p-Nitrophenol	Glpc	88.2
				p-Nitroanisole	Glpc	
β-D-Galactoside (0.013)	NaOH–H_2O 0.25 *N*, 300 ml	55	25	Levogalactosan	Mp, $[\alpha]^{25}D$	46.5
				p-Nitrophenol	Glpc	88.2
				Galactose	Paper chromatog	
α-D-Mannoside (0.013)	NaOH–H_2O 0.25 *N*, 300 ml	55	2	*p*-Nitrophenol	Glpc	100.0
				Mannose	Paper chromatog	
2-*O*-Methyl-β-D-galactoside (0.010)	NaOMe–MeOH, 0.50 *N*, 400 ml, 0.017% water	65	198	*p*-Nitroanisole	Mp, mmp, infrared	13.3
				2-*O*-Methylgalactose	Paper chromatog	
				p-Azoxyanisole	Mp, mmp, infrared	
				p-Methoxyaniline	Glpc	
2-*O*-Methyl-α-D-mannoside (0.010)	NaOMe–MeOH, 0.50 *N*, 400 ml, 0.017% water	65	198	*p*-Nitroanisole	Mp, mmp, infrared	27.1
				2-*O*-Methylmannose	Paper chromatog	
				p-Azoxyanisole	Mp, mmp, infrared	
				p-Methoxyaniline	Glpc	
2-*O*-Methyl-β-D-galactoside (0.0003)	NaOH–H_2O, 1.037 *N*, 50 ml	55	120	*p*-Nitrophenol	Glpc	
2-*O*-Methyl-α-D-mannoside (0.0005)	NaOH–H_2O, 1.037 *N*, 50 ml	55	120	*p*-Nitrophenol	Glpc	

[a] 1,6-Anhydro-β-D-galactopyranose. [b] Gas–liquid partition chromatography. [c] Polarimetric yield estimate gives 85.5%.

yields of products based on reactants are reported in Table I.

Greater emphasis was placed on analysis of products formed in methanolic sodium methoxide than in aqueous sodium hydroxide because, first, the same products may be formed in the aqueous medium by two different modes of cleavage and, second, these products often undergo further reaction. On the other hand, the products formed from reaction of the glycosides in methanolic sodium methoxide are not only more stable, but the nature of the products also denotes the point of bond cleavage.

The principal glyconic products of the reaction of *p*-nitrophenyl β-D-galactoside in methanolic sodium methoxide, 1,6-anhydro-β-D-galactopyranose and methyl β-D-galactoside, were formed in 76.3 and 7.9% yield, respectively. Reaction of *p*-nitrophenyl α-D-mannoside in the basic methanol gave methyl α-D-mannoside and *p*-nitrophenol in 88.3 (gas chromatography) and 88.2% (spectrophotometry) yield, respectively. A polarimetric estimate of the yield of methyl α-D-mannoside gave 85.5%. Although *p*-nitrophenol appeared to be the principal aglyconic product of both the galactoside and mannoside reactions, a small amount of *p*-nitroanisole (estimated to be less than 15%) was also found.

Although known standards were available for comparison, no evidence was found for the formation of methyl 2-*O*-methylglycosides and 1,6-anhydro-2-*O*-methyl sugars in the 2-*O*-methylglycoside–methanolic sodium methoxide reactions. Instead, *p*-nitroanisole and 2-*O*-methyl sugar were the primary reaction products. The amount of unreduced *p*-nitroanisole remaining in the 2-*O*-methylgalactoside and 2-*O*-methylmannoside reaction mixtures was 13.3 and 27.1%, respectively. In addition to *p*-nitroanisole, *p*-azoxyanisole and *p*-methoxyaniline were also found.

1,6-Anhydro sugar, the only stable glyconic reaction product among those formed in the glycoside–aqueous base reactions, was obtained in 46.5% yield from the galactoside–aqueous sodium hydroxide reaction.

Rates of Reaction.—The results of the present study and other work[6] have shown that *p*-nitrophenyl glycosides react with base in hydroxylic solvents to give *p*-nitrophenol anion, which is the only component of the reaction system which absorbs at 390–400 mμ. The appropriate rate law in terms of spectrophotometric absorbance is

$$dA/dt = \epsilon k_i[\text{NPGH}]^a \quad (1)$$

where dA/dt is the reaction rate, k_i the pseudo-first-order rate constant, and ϵ the extinction coefficient. It is often convenient to assume the reaction is first order ($a = 1.0$), relate glycoside concentration, [NPGH], to absorbance, A, and integrate eq 1. The spectrophotometric rate data for reaction of the compounds studied here failed to fit the integrated rate relation. The failure has been found to result from reduction of a portion of one primary reaction product, *p*-nitrophenol anion, by the other primary reaction product, sugar, to an absorbing substance which interferes with the use of the integrated first-order rate relation.

Therefore, initial pseudo-first-order rate constants, k_i, for reaction of the *p*-nitrophenyl glycosides at 35, 45, and 55° and the *p*-nitrophenyl 2-*O*-methylglycosides at 50 and 55° in aqueous sodium hydroxide were obtained from

$$[dA/dt]_0 = \epsilon k_i[\text{NPGH}]_0^a \quad (2)$$

The initial reaction rate, $[dA/dt]_0$, was obtained by evaluating the first derivative at time equal to zero of the relation for regression of spectrophotometrically

(6) J. A. Snyder and K. P. Link, *J. Am. Chem. Soc.*, **74**, 1883 (1952).

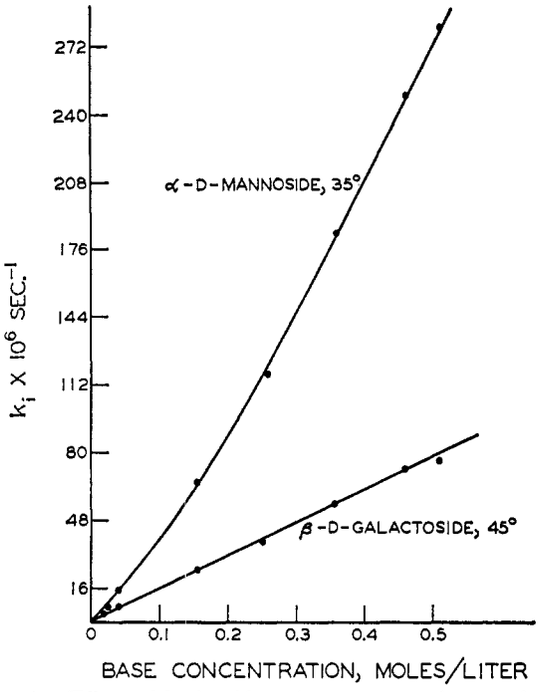


Figure 1.—Effect of hydroxide anion concentration on the rate of the glycoside reactions.

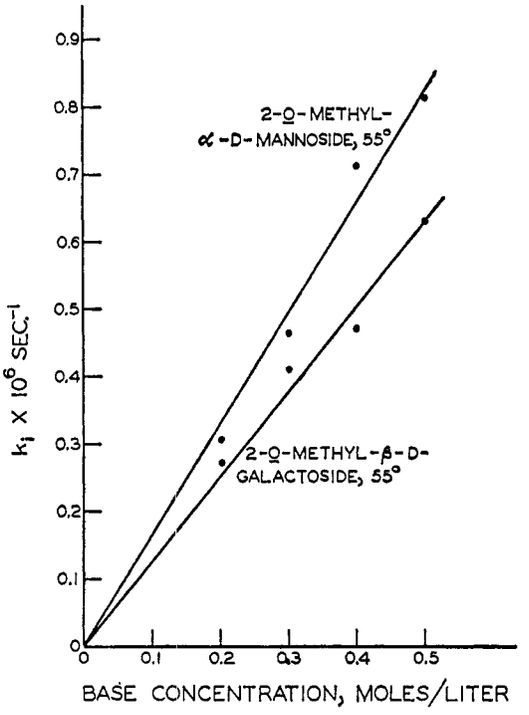


Figure 2.—Effect of hydroxide anion concentration on the rate of the 2-*O*-methylglycoside reactions.

measured absorbance, A, of the reaction system on reaction time, t

$$A = \sum_{i=0}^{n} B_i t^i \tag{3}$$

$$[dA/dt]_0 = \left[\sum_{i=1}^{n} i\, B_i t^{i-1}\right]_0 = B_1 \tag{4}$$

where B_i is the regression coefficient for the ith term. The number of terms in the regression necessary to represent the data adequately were determined by testing the significance of each term as it was added. The significance test used here amounts to Student's test of each regression coefficient against its standard error. Absorbance will be at least linearly related to time, t. Confidence limits for the regression coefficient of the t^2 term are calculated for the 90% level. If these limits do not include zero, it is concluded that introduction of the t^2 term significantly improves the fit of the representation of the data. The test is then repeated on subsequent powers, and the regression is fitted using only the significant terms.

Reaction order, a, was obtained from a least-squares fit of initial rate measurements as a function of initial glycoside concentration $[NPGH]_0$, according to the logarithmic form of relation 2. All reactions appeared to be approximately first order with respect to glycoside and 2-*O*-methylglycoside (Table II).

TABLE II

KINETIC ORDER OF REACTION

p-Nitrophenyl D-glycoside	Base and solvent	Temp, °C	Glycoside reaction order	Base reaction order
β-Galactoside	OH^-, H_2O	35.06	0.987	0.998
		44.98	0.964	0.935
		55.04	0.956	0.857
	OMe^-, MeOH	35.03	0.981	0.794
α-Mannoside	OH^-, H_2O	35.06	1.04	1.12
		44.97	1.02	1.09
		55.04	1.01	1.05
	OMe^-, MeOH	35.03	1.15	1.01
2-*O*-Methyl-β-galactoside	OH^-, H_2O	50.05		0.753
		55.03	0.832	0.878
2-*O*-Methyl-α-mannoside	OH^-, H_2O	50.05		1.31
		55.03	0.981	1.11

Since the Lambert-Beer law relation was obeyed, the anion extinction coefficients, ϵ, for water, 1.79 ± 0.013 × 10^4, and methanol, 1.88 ± 0.008 × 10^4, were obtained by a least-squares fit of anion absorbance and concentration measurements to the relation.

Pseudo-first-order rate constants for reaction of the glycosides and 2-*O*-methylglycosides as a function of hydroxide ion concentration at constant ionic strength are presented graphically in Figures 1 and 2. All reactions appeared to be approximately first order with respect to base concentration. Rate constants for the mannoside reaction increase more rapidly than base concentration below 0.15 M, but are nearly proportional to base concentration thereafter. The galactoside and 2-*O*-methyl glycoside reaction rate constants appear to be nearly proportional to base concentration over the entire range studied.

Although differences between duplicate rate constant measurements for the *p*-nitrophenyl glycosides are usually no greater than 1–2%, differences for the substantially less reactive *p*-nitrophenyl 2-*O*-methylglycosides are ordinarily 2–4%.

Initial second-order rate constants, $(k_2)_i$, were obtained from a regression of pseudo-first-order rate constant on base concentration for the glycoside reactions at 35, 45, and 55° and for the 2-*O*-methylglycoside reactions at 50 and 55°. The second-order rate constants and the regression coefficients for significant terms are presented in Table III. Pseudo-first-order rate constants, k_i, for base concentrations, c, up to and including 0.50 N may be calculated from the regression coefficients, J_i, using relation 5.

$$k_i = J_1 c + J_2 c^2 \tag{5}$$

TABLE III

TEMPERATURE DEPENDENCE OF SECOND-ORDER RATE CONSTANTS AT CONSTANT IONIC STRENGTH IN AQUEOUS SODIUM HYDROXIDE

p-Nitrophenyl D-glycoside	$J_1 \times 10^{6}$ [a] $[(k_2)_i \times 10^{6}]$, l./mole sec	$J_2 \times 10^{6}$ [a]	Temp, °C
β-Galactoside	44.0		35.06
	153.0		44.98
	416.0		55.04
	10.58[b]	−4.45[b]	35.03
α-Mannoside	382.0	330.0	35.04
	1070.0	1080.0	44.97
	3090.0	1850.0	55.04
	9.80[b]		35.03
2-O-Methyl-β-galactoside	0.709	−0.322	50.05
	1.26		55.02
2-O-Methyl-α-mannoside	0.931		50.05
	1.65		55.02
β-Galactoside	45.2[c]		35.07

[a] Regression coefficient. [b] Methanolic sodium methoxide. [c] Measured at variable electrolyte concentration; perchlorate additions to maintain constant ionic strength were omitted.

Rate constants for reaction of the glycosides in sodium methoxide–methanol, sodium hydroperoxide–water, and sodium deuterioxide–deuterium oxide are presented in Tables III, IV, and V, respectively. The

TABLE IV

EFFECT OF HYDROPEROXIDE ANION ON REACTION RATE

p-Nitrophenyl D-glycoside	Equil O_2H^-,[a] M	Equil OH^-,[a] M	Measured av $k_i \times 10^{6}$, sec^{-1}	Estimated k_i[b] $\times 10^{6}$, sec^{-1}
β-Galactoside[c]	0.000	0.993	40.6 ± 1.3	...
	0.493	0.500	33.7 ± 0.2	22.0
	0.928	0.065	31.8 ± 0.5	2.86
α-Mannoside[c]	0.000	0.993	592.0 ± 10.0	...
	0.493	0.500	275.0 ± 11.0	274.0
	0.928	0.065	82.3 ± 5.8	25.2
2-O-Methyl-β-galactoside[d]	0.000	0.987	0.459 ± 0.08	...
	0.926	0.065	45.1 ± 2.0	0.046
2-O-Methyl-α-mannoside[d]	0.000	0.987	1.14 ± 1.0	...
	0.926	0.065	81.0 ± 7.5	0.061

[a] Estimated from data provided by Evans and Uri, *Trans Faraday Soc.*, **45**, 224 (1949). [b] Estimated from data presented in Table III. [c] Temperature 35.07 ± 0.01°. [d] Temperature 50.07 ± 0.03°.

TABLE V

HYDROGEN KINETIC ISOTOPE EFFECT

p-Nitrophenyl D-glycoside	Reaction medium	$k_i \times 10^{6}$, sec^{-1}	$k_i(D_2O)/k_i(H_2O)$
β-Galactoside	OH^-, 0.482 N, H_2O	73.7 ± 0.7[a]	1.35 ± 0.02
	OD^-, 0.482 N, D_2O	99.6 ± 0.5[b]	
α-Mannoside	OH^-, 0.482 N, H_2O	769.0 ± 6.0[a]	1.41 ± 0.01
	OD^-, 0.482 N, D_2O	1085.0 ± 5.0[b]	

[a] Estimated from regression relation and coefficients presented in Table III for 44.96°. [b] Based on two measurements at 44.96°.

effect of ionic strength on the glycoside reactions in methanolic and aqueous base solution was investigated briefly. The results are reported in Table VI.

Arrhenius activation energies for reaction of the glycosides and 2-O-methylglycosides in aqueous base solution were evaluated by least squares from initial

TABLE VI

SALT EFFECTS

p-Nitrophenyl D-glycoside	Base, M	ClO_4^-, M	$k_i \times 10^{6}$, sec^{-1}
β-Galactoside[a]	OH^-, 0.257	0.000	12.0 ± 0.5
	OH^-, 0.257	0.257	11.2 ± 0.1
β-Galactoside[a]	OMe^-, 0.248	0.000	2.31 ± 0.15
	OMe^-, 0.248	0.248	2.14 ± 0.15
α-Mannoside[a]	OMe^-, 0.248	0.000	2.12 ± 0.20
	OMe^-, 0.248	0.248	2.40 ± 0.20
2-O-Methyl-β-galactoside[b]	OH^-, 0.988	0.000	0.777 ± 0.001
	OH^-, 0.988	0.988	0.532 ± 0.041
2-O-Methyl-α-mannoside[b]	OH^-, 0.988	0.000	2.01 ± 0.04
	OH^-, 0.988	0.988	1.41 ± 0.01

[a] 35.03°. [b] 55.06°.

second-order rate constants using the logarithmic form of the Arrhenius relation. Thermodynamic activation functions at 55° from the absolute reaction rate theory calculated from the Arrhenius relation parameters are reported in Table VII.

TABLE VII

THERMODYNAMIC ACTIVATION FUNCTIONS FOR REACTION OF THE GLYCOSIDES AND 2-O-METHYLGLYCOSIDES AT 55.00 ± 0.07°

p-Nitrophenyl D-glycoside	ΔH^*, kcal/mole	ΔS^*, eu	ΔF^*, kcal/mole
β-Galactoside	21.9	−7.3	24.3
α-Mannoside	20.4	−8.1	23.1
2-O-Methyl-β-galactoside[a]	23.7	−13.0	28.1
2-O-Methyl-α-mannoside[a]	23.6	−13.0	28.0

[a] Based on initial second-order rate constants obtained at two temperatures.

Discussion

Reaction Products and Mechanism. The p-Nitrophenyl 2-O-Methylglycosides.—Inasmuch as nothing was known about the reaction of the 2-O-methylglycosides, the three mechanisms listed by Ballou[4] for the alkaline reaction of phenyl glycosides, namely, C-2 neighboring-group participation, SN1 and SN2 processes were considered prime initial hypotheses.

The products to be expected from reaction of the 2-O-methylglycosides in methanolic sodium methoxide by either of these three processes, methyl 2-O-methylglycosides, 1,6-anhydro-2-O-methyl sugar, and p-nitrophenol, were prepared or purchased for the purpose of standardizing qualitative paper and gas chromatographic analysis procedures. However, no evidence for the formation of these products was found (using the known standards) in the two p-nitrophenyl 2-O-methylglycoside–methanolic sodium methoxide reaction mixtures.

The finding of 2-O-methyl sugar and the isolation of p-nitroanisole from the reaction mixtures suggested instead a reaction process involving attack by the methoxide anion on C-1 of the benzene ring with displacement of the glycosyloxy group to yield p-nitroanisole and 2-O-methyl sugar.

The percentages of p-nitroanisole found in the 2-O-methylgalactoside and 2-O-methylmannoside reaction mixtures were 13.3 and 27.1%, respectively. Discovery of p-azoxyanisole and p-methoxyaniline in the reaction mixture suggested that a portion of the p-

TABLE VIII

REACTION PRODUCTS OF THE PHENYL GLYCOSIDES

Reactant	Reaction medium, compn	Reaction temp, °C	Time, hr	Major products[a]	Yield, %	Ref
β-D-Galactosides						
Phenyl	NaOH–H_2O, 1.3 *N*	100	9	Levogalactosan	91	*b*
	NaOMe–MeOH, 1.3 *N*	65	96	Levogalactosan		*c*
p-Nitrophenyl	NaOH–H_2O, 0.50 *N*	55	13	Levogalactosan	46.5	*d*
	NaOMe–MeOH, 0.25 *N*	65	36	Levogalactosan	76.3	*d*
				Methyl β-D-galactoside	7.9	*d*
α-D-Mannosides						
Phenyl	NaOH–H_2O, 1.3 *N*	100	336	Tar		*b*
	NaOMe–Me_2SO, 1.3 *N*	70	60	Methyl α-D-mannoside		*c*
p-Nitrophenyl	NaOMe–MeOH, 0.25 *N*	65	219	Methyl α-D-mannoside	88.3	*d*
β-D-Glucosides						
Phenyl	NaOH–H_2O, 1.3 *N*	100	9	Levoglucosan	88.0	*b*
	NaOMe–MeOH, 1.3 *N*	65	5	Recovered all reactant		*e*
p-Nitrophenyl	NaOH–H_2O, 1.3 *N*		3	Levoglucosan	60.0	*b*
β-D-Allosides						
Phenyl	NaOH–H_2O, 0.6 *N*	100	8	Levoallosan	54.0	*f*
	NaOMe–MeOH	65	53	Levoallosan		*c*

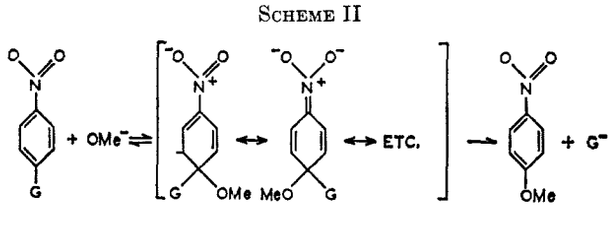
[a] The levoglycosans are the corresponding 1,6-anhydro-β-D-pyranoses. [b] Reference 11. [c] Reference 12. [d] The present study. [e] E. N. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Org. Chem.*, **10**, 194 (1945). [f] E. Zissis and N. K. Richtmyer, *ibid.*, **26**, 5244 (1961).

nitroanisole initially produced by glycosidic bond cleavage was reduced, presumably by the 2-*O*-methyl sugar. *p*-Nitroanisole has been reduced to *p*-azoxyanisole (67% conversion) by glucose in aqueous sodium hydroxide solution.[7]

Reagent grade *p*-nitroanisole and 2-*O*-methylgalactose reacted in methanolic sodium methoxide under conditions identical (except that reaction time was considerably shorter) with those used in the *p*-nitrophenyl 2-*O*-methylglycoside–methanolic sodium methoxide reaction. The finding of *p*-methoxyaniline in the reaction mixture supported the hypothesis that glycosidic cleavage was followed by partial reduction of the initial product, *p*-nitroanisole.[8]

On the basis of the foregoing information it is proposed that reaction of the *p*-nitrophenyl 2-*O*-methylglycosides in methanolic sodium methoxide proceeds by bimolecular nucleophilic attack of the methoxide ion on C-1 of the benzene ring as shown in Scheme II.

SCHEME II



The importance of the *p*-nitro substituent in facilitating nucleophilic aromatic substitution is well known,[9,10a] and it is possible that other *para*-substituted phenyl 2-*O*-methylglycosides would react by other mechanisms.

The *p*-Nitrophenyl Glycosides.—The product analysis results obtained in the present work and by others[11,12] for reaction of phenyl β-D-galactosides and phenyl α-D-mannosides in both methanolic sodium methoxide and aqueous sodium hydroxide are presented in Table VIII. The retention of configuration observed in the products formed from reaction of the glycosides in both media indicates participation by the neighboring C-2 oxyanion in the reaction of the phenyl β-D-galactosides and the α-D-mannosides as well as the phenyl β-D-glucosides.[13]

The results in Table VIII also show that 1,6-anhydro-β-D-glucopyranose (levoglucosan) is produced in greater quantity in the reaction of phenyl β-D-glucoside than in the reaction of *p*-nitrophenyl β-D-glucoside in aqueous sodium hydroxide. This was interpreted by McCloskey and Coleman[5] as a trend toward an SN1 mechanism as the electron-withdrawing character of the *para* substituent on the aglycon increased. This trend in results need not necessarily signify a change to an SN1 mechanism. Owing to the very high reactivity of *p*-nitrophenyl β-D-glucoside,[11,14] it seems certain that reaction of the *p*-nitrophenyl β-D-glucoside occurred at a lower temperature than phenyl β-D-glucoside. Intramolecular attack by the C-6 oxyanion on C-1 of the 1,2-anhydro intermediate formed in the neighboring-group reaction may be favored at higher temperatures while intermolecular attack on C-1 of the intermediate may be favored at lower temperatures.

The extent to which configuration is retained in the products of the *p*-nitrophenyl glycoside–methanolic

(7) H. W. Gailbraith, E. F. Degering, and E. F. Hitch, *J. Am. Chem. Soc.*, **73**, 1323 (1951).

(8) Since the nitro group is unusually effective as an activator for glycosidic bond cleavage, cleavage probably precedes reduction of the nitro group.

(9) P. R. Wells, *Chem. Rev.*, **63**, 171 (1963).

(10) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill Book Co., Inc., New York, N. Y., 1962: (a) pp 387–394; (b) pp 112–114.

(11) E. N. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 3 (1943).

(12) E. Zissis and N. K. Richtmyer, *J. Org. Chem.*, **30**, 462 (1965).

(13) Zissis and Richtmyer[12] have pointed out that their evidence for the presence of methyl α-D-mannoside in the phenyl α-D-mannoside–methanolic sodium methoxide reaction mixture is based only on paper chromatographic mobility.

(14) A. Dyfverman and B. Lindberg, *Acta Chem. Scand.*, **4**, 878 (1950).

sodium methoxide reaction suggests the SN1 process is not important in the reactions of the glycosides studied in this work.

The presence of small amounts of *p*-nitroanisole (less than 15%) in both *p*-nitrophenyl glycoside–methanolic sodium methoxide reaction mixtures indicates incursion of a bimolecular nucleophilic attack on C-1 of the benzene ring identical with the mode of reaction found for the *p*-nitrophenyl 2-*O*-methylglycosides. Second-order rate constant values, presented in Table III, show that reaction of the glycosides is considerably slower in methanolic sodium methoxide than in aqueous sodium hydroxide.[15] The over-all activation energy for the neighboring-group participation process in methanolic sodium methoxide may be great enough that the nucleophilic aromatic substitution reaction route is competitive.

Hydrogen Kinetic Isotope Effect.—The base-catalyzed neighboring-group process for reaction of the

$$\text{NPGH} + \text{OR}^- \rightleftharpoons \text{NPG}^- + \text{ROH} \quad \text{(a)}$$

$$\text{NPG}^- \longrightarrow \text{products (1,2-anhydro sugar, NP}^-) \quad \text{(b)}$$

glycosides, NPGH, with base, OR^-, may be represented by reactions a and b where NPG^- and ROH are the glycoside conjugate base and solvent, respectively. The hydrogen kinetic isotope effect, $k_i(D_2O)/k_i(H_2O)$, the ratio of rate constants for reaction in deuterium oxide and in water,[16] has been used to determine which step is rate controlling in base-catalyzed reactions. If step a is rate controlling the reaction is found to proceed less rapidly, $k_i(D_2O)/k_i(H_2O) < 1$, in deuterium oxide than in water. On the other hand, if step b is rate controlling, the reaction is found to proceed more rapidly, $k_i(D_2O)/k_i(H_2O) > 1$, in deuterium oxide than in water.

The data in Table V show the isotope effect, $k_i(D_2O)/k_i(H_2O)$, for the galactoside reaction is 1.35, while for the mannoside reaction it is 1.41. According to the hydrogen kinetic isotope criterion this indicates that step b is rate controlling in both reactions. Furthermore, proton transfers between atoms with unshared electron pairs have rate constants of the order 10^{11} to 10^{-1} l./mole sec at 25°.[10b] Since the measured rate constants for the glycoside reactions are of the order 10^{-4} to 10^{-5} l./mole sec at 35°, the reaction rate is probably not determined by step a which is a proton-transfer reaction.

Reaction Order.—Dyfverman and Lindberg[14] studied the dependence of k_i on base concentration, $[OR^-]$, for the reaction of *p*-chlorophenyl β-D-glucoside in aqueous sodium hydroxide at 100°. Their results indicated the reaction was 0.044 order[17] with respect to base. A line through their data points does not pass through the origin as required for a second-order reaction.

The dependence of k_i on $[OR^-]$ found for reaction of the *p*-nitrophenyl glycosides in aqueous sodium hydroxide at 45 and 35° is shown in Figures 1 and 2. In contrast to the results obtained earlier,[14] the data points obtained in the present study form a smooth curve which passes through the origin.

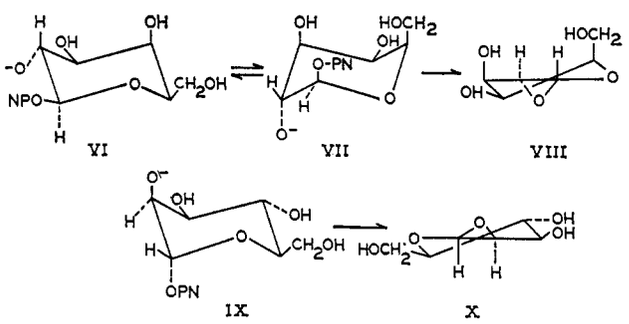
Values for reaction order with respect to glycoside and with respect to base are presented in Table II. The values are in good agreement with the requirement for a base-catalyzed process that the reaction be kinetically first order with respect to both glycoside and base. Despite precautions taken to maintain constant ionic strength in all glycoside and 2-*O*-methylglycoside reactions by addition of the weakly nucleophilic perchlorate anion, appreciable deviations of certain base reaction order values from unity (greater than 0.1) occur. Variation in the activity coefficients of the reacting species with base concentration appears to be responsible for the deviations.

The reaction order values for the 2-*O*-methylglycoside reactions given in Table II indicate the reaction was approximately first order with respect to glycoside and base as required for reaction by bimolecular nucleophilic aromatic substitution. The significant deviations of several reaction order values from unity is imputed primarily to the low precision (difference between duplicates is 2–4%) of the initial pseudo-first-order rate constant data.

Previous investigators,[4,5] as mentioned earlier, have noted what appeared to be a trend toward an SN1 mechanism in the reaction of phenyl glycosides as the electron-withdrawing character of the *para* substituent increased. The over-all second-order kinetics found in this study for reaction of both phenyl glycosides and phenyl 2-*O*-methylglycosides substituted with the strong electron-withdrawing *p*-nitro group indicates an SN1 process is not involved in these reactions even in cases where C-2 oxyanion participation is precluded.

Activation Enthalpy and Entropy.—Since neighboring-group participation requires a coplanar arrangement of the atomic centers involved,[18] the galactoside must react through its less stable "alternative" conformation (VII, Scheme III), whereas the mannoside can

SCHEME III



react through its more stable "normal" conformation IX.[19] Development of a mutual interaction between the C-3 hydroxyl, the C-1 phenoxyl, and the C-5 hydroxymethyl group on the galactoside molecule accompanies the change of VI to VII. No similar interaction develops in the mannoside reaction (compare structures VII and IX). The ratio of second-order rate constants, k_M/k_G, at 55° is 7.4. The primary reason for this difference may well be the much higher concentration of the reactive conformer of the mannoside as compared to the galactoside.

(15) A difference in the acid dissociation constants for the C-2 hydroxyl may be responsible for the glycoside reactivity difference in the two solvents.

(16) K. Wiberg, *Chem. Rev.*, **55**, 713 (1955).

(17) Calculated from reported polarimetric rate data.[14]

(18) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Holt, Rinehart and Winston, Inc., New York, N. Y., 1959, pp 561, 562.

(19) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," John Wiley and Sons, Inc., New York, N. Y., 1965, pp 363–365.

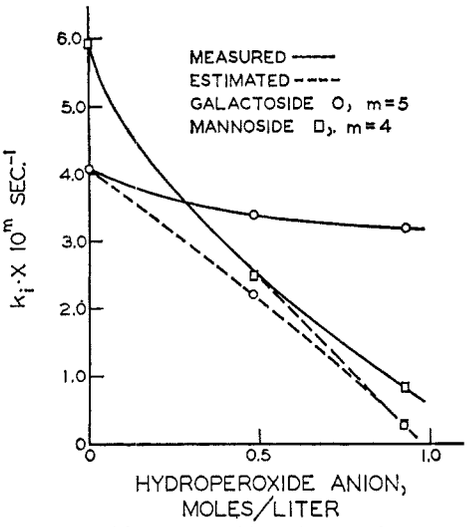


Figure 3.—Effect of hydroperoxide anion on the reaction of the glycosides.

It is clear that the values of the activation entropy and enthalpy functions for the glycoside and 2-*O*-methylglycoside reactions fall into two groups. The significantly lower reactivity of the 2-*O*-methylglycosides compared with the glycosides is reflected in both enthalpy and entropy of activation.[20] These differences in activation function values for the two pairs of glycosides indicate their reactions in aqueous sodium hydroxide proceed by different pathways. This conclusion has already been drawn for their reactions in methanolic sodium methoxide from consideration of the products formed in the glycoside and 2-*O*-methylglycoside reactions in that medium.

The activation enthalpy for the galactoside reaction, 21.9 kcal/mole, was obtained in this study from second-order rate constants measured at 35, 45, and 55°. Snyder and Link[6] report an activation enthalpy of 25.9 kcal/mole for this reaction based on pseudo-first-order rate constants measured at 45 and 65°. Pseudo-first-order rate constants and second-order rate constants for base-catalyzed processes are seldom linearly related through catalyst concentration.[21] Thus, a discrepancy between activation enthalpy estimates obtained from the two different kinds of rate constants is not surprising.

Reaction in Alkaline Peroxide.—A method for distinguishing between reaction by base catalysis and bimolecular nucleophilic substitution has been described by Pearson and Edgington.[22] The method utilizes the great difference between the effectiveness of the hydroperoxide anion and that of the hydroxide anion as nucleophiles and as bases. Pearson and Edgington[22] found replacement of the hydroxide anion by the hydroperoxide anion in reactions which proceed by bimolecular nucleophilic substitution processes[23] increased the reaction rate by factors as large at 10^4. On the other hand, replacement was found to reduce the reaction rate of base-catalyzed processes such as the cyclization of ethylenechlorohydrin in aqueous sodium hydroxide.

The effect of hydroxide anion replacement on the reaction rate of the glycosides and 2-*O*-methylglycosides in aqueous sodium hydroxide is shown in Table IV. In Figure 3, a graphic illustration of the effect of replacement on the glycoside reactions is presented. The decrease in reaction rate effected by reducing the hydroxide anion concentration with addition of hydrogen peroxide further supports the hypothesis that the reaction proceeds by a base-catalyzed process.

An estimate of the rate constant for the reaction of the glycosides and 2-*O*-methylglycosides with the hydroxide anion component of the alkaline peroxide system was obtained from calculated equilibrium hydroxide anion concentration and the regression coefficients for the dependence of k_i on OH^- given in Table III. Comparison of the variation in the measured (solid line) and estimated (dashed line) rate constants with hydroperoxide anion concentration in Figure 3 suggests reaction of the glycosides with hydroperoxide anion. Since the catalytic effect of the hydroperoxide anion would be negligibly small compared to the effect of the hydroxide anion,[25] glycoside reaction with hydroperoxide anion may involve attack by the anion on the benzene ring to displace the glycosidic moiety by aromatic nucleophilic substitution.[26]

The sharply contrasting behavior of the glycosides and the 2-*O*-methylglycosides in alkaline peroxide clearly indicates that the reaction of the two pairs of compounds proceed by different pathways. The reaction of the 2-*O*-methylglycosides with hydroperoxide anion is nearly 100 times more rapid than their reaction with hydroxide anion. The reaction rate enhancement for the 2-*O*-methylglycosides effected by introduction of the hydroperoxide anion is in agreement with the hypothesis that their reaction proceeds by nucleophilic aromatic substitution.

However, if the reactions of the compounds considered here proceed entirely or in part by nucleophilic aromatic substitution, then instead of reaction leading to the formation of a single absorbing product, *p*-nitrophenolate anion, a mixture of absorbing products, *p*-nitrophenyl peroxide and *p*-nitrophenolate anions, may result. Although formation of the anion of *p*-nitrophenyl hydroperoxide undoubtedly occurs in these reactions, the rate of decomposition of the anion to yield the *p*-nitrophenolate anion is probably far greater than the

(20) The ratios of the second-order rate constants at 55° (see Table III) indicate that the galactoside is approximately 330 times more reactive than the 2-*O*-methylgalactoside, whereas the mannoside appears to be 1870 times more reactive than the 2-*O*-methylmannoside. Greater significance is attached to the change in the character of the reaction process resulting from methylation of the C-2 hydroxyl than to the large reduction in reaction rate effected by the methylation. The magnitude of the anchimeric assistance (driving force afforded by neighboring-group participation) provided by the C-2 oxyanion, however, cannot be less than the rate enhancements found in going from the 2-*O*-methylglycosides to the glycosides.

(21) R. P. Bell, "Acid-Base Catalysis," Oxford University Press, London, 1941, Chapter 2.

(22) R. G. Pearson and D. N. Edgington, *J. Am. Chem. Soc.*, **84**, 4607 (1962).

(23) They observed this behavior for aliphatic nucleophilic S$_N$2 type substitutions, but it is reasonable to expect aromatic nucleophilic substitution processes would exhibit similar behavior.[24]

(24) J. F. Bunnett, *Quart. Rev.* (London), **12**, 1 (1958).

(25) The base catalysis effects of the hydroperoxide and hydroxide anions should be inversely related to the acid dissociation constants of hydrogen peroxide and water through the Brønsted catalysis law. An estimate of the ratio of the rates of catalysis due to hydroperoxide and hydroxide can be obtained using the estimated equilibrium concentrations of hydroxide and hydroperoxide.

$$\frac{\text{rate}_{O_2H^-}}{\text{rate}_{OH^-}} = \frac{K_{H_2O}\,[O_2H^-]_{equil}}{K_{H_2O_2}[OH^-]_{equil}} = \frac{1.005 \times 10^{-14}0.928}{2.24 \times 10^{-12}0.065} = 0.064$$

(26) This could also indicate incursion of S$_N$2 attack on the anomeric carbon.

rate of reaction of the glycosides and 2-*O*-methylglycosides.[27,28]

Salt Effects.—The data in Table VI show that salt effects, if they do occur in the glycoside reactions, are not much larger than the experimental error normally associated with the initial pseudo-first-order rate constants obtained in this study. In a word, salt effects appear to be absent. A further example of the absence of a salt effect is provided by values reported in Table III for the initial second-order rate constants for reaction of *p*-nitrophenyl β-D-galactoside in aqueous sodium hydroxide at 35°. These rate constants measured with perchlorate added to maintain constant ionic strength and without added perchlorate are 44.0×10^{-6} and 45.2×10^{-6} l./mole sec, respectively. This difference in rate constants is essentially within the uncertainty of the measurements.

The negligible effect of changes in medium ionic strength on the glycoside reaction rates is in agreement with the Debye–Hückel theory prediction for reaction of a neutral molecule with an ion in a base-catalyzed process.[29] The agreement, however, is very likely fortuitous since changes in the activity coefficient of the hydroxide ion cannot be adequately represented by theory at the electrolyte levels (*ca.* 0.5 *M*) used in this work. Moreover, the activity coefficient of the neutral glycoside molecule (also that of the activated complex) may be affected by ionic strength changes at these electrolyte levels. Therefore, it is probable that the negligible salt effects found result from some cancelling out of changes in the individual reacting species' activity coefficients with changing ionic strength.

On the other hand, Table VI shows that addition of approximately 1.0 *N* sodium perchlorate reduces the pseudo-first-order rate constant for reaction of 2-*O*-methyl-β-D-galactoside by 39% and the rate constant for reaction of 2-*O*-methyl-α-D-mannoside by 35%.

Reactions proceeding by nucleophilic aromatic substitution would be expected to exhibit a negative salt effect. Dispersal of charge in the activated complex for bimolecular nucleophilic substitutions is inhibited by increases in the ionic strength of the reaction medium.

Conclusions

The results of this study may be examined with respect to C-2 oxyanion participation and the glycoside reactions from two viewpoints.

First, the change in the character of the reaction process which results from methylation of the glycoside C-2 hydroxyl clearly indicates C-2 oxyanion participation for their reaction in basic solution. Evidence for this change in reaction character comes from consideration of differences in the glycoside and 2-*O*-methylglycoside reactions with respect to the nature of the products, the thermodynamic activation functions, and the behavior in the presence of hydroperoxide anion and in the presence of inert salts.

Second, the results *per se* indicate the glycoside reactions proceed by a base-catalyzed, neighboring-group process. These results include the observed retention of configuration in the reaction products, over-all second-order kinetics, and reduction in reaction rate effected by replacement of hydroxide by hydroperoxide anion.

The magnitude of the hydrogen kinetic isotope effect found for the glycosides suggests their reaction is a specific base-catalyzed process.

The alternatives to phenyl glycoside reaction by C-2 oxyanion participation, the SN1 and SN2 processes,[4] apparently are supplanted by bimolecular nucelophilic aromatic substitution in the case of the *p*-nitrophenyl glycosides.

The strong electron-withdrawing character of the nitro group has provided the *p*-nitrophenyl 2-*O*-methyl glycosides with a reaction pathway of lower energy than that required for participation by the C-2 methoxyl group. The pathway here is again bimolecular nucleophilic aromatic substitution.

Experimental Section[3,30]

2-O-Methyl Ethers of Galactose and Mannose.—Reaction of phenyl D-galactoside tetraacetate (80 g, 0.17 mole) in 2.17 *N* aqueous potassium hydroxide (1500 ml) gave 1,6-anhydro-β-D-galactopyranose. After neutralization, the salts which precipitated during concentration of the reaction solution were removed by filtration. The mixture of remaining salts and product was dried by repeated concentration with absolute ethanol (four 75-ml portions) and taken up in very dry ethanol (75–100 ml, dried by distillation from magnesium ethoxide), and the ethanol solution was added slowly with stirring to dry acetone (700 ml, dried by shaking with Drierite, followed by distillation) containing concentrated sulfuric acid (4 ml). Anhydrous copper sulfate (50 g) was added to the mixture which was stirred for 24 hr at room temperature.

The copper sulfate was removed by filtration, and the filtrate was neutralized by shaking with a large excess of solid calcium hydroxide. After the hydroxide had been filtered off, the acetone filtrate was concentrated to dryness *in vacuo*, and the crystalline residue was recrystallized from absolute ethanol to yield 3,4-*O*-isopropylidene-1,6-anhydro-β-D-galactopyranose (51%).

2-*O*-Methylgalactose was obtained by methylation of the C-2 hydroxyl of 3,4-*O*-isopropylidene-1,6-anhydro-β-D-galactopyranose[31,32] with dimethyl sulfate in acetone[33] and acid hydrolysis of the resulting 2-*O*-methyl acetal.[32]

The C-2 hydroxyl of 3,4:5,6-di-*O*-isopropylidenemannose dimethyl dithioacetal[34,35] was also methylated by the dimethyl sulfate procedure.[33] 2-*O*-Methylmannose was then obtained by removal of the acetal and thioacetal groups with acid hydrolysis.[36]

***p*-Nitrophenyl β-D-Glycosides of Galactose and 2-*O*-Methylgalactose.**—Galactose and 2-*O*-methylgalactose were acetylated following the procedure of Fernez and Stoffyn.[31]

1,3,4,6-Tetra-*O*-acetyl-2-*O*-methyl-D-galactose, which has not previously been reported, gave mp 133–134°, $[\alpha]^{25}D$ 26.8° (*c* 1.05, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{22}O_{10}$: C, 49.71; H, 6.14; OCH_3, 8.56. Found: C, 50.01; H, 6.05; OCH_3, 8.86.

Galactosyl bromides were prepared by treating the sugar acetates with hydrogen bromide in glacial acetic acid.[31]

3,4,6-Tri-*O*-acetyl-2-*O*-methyl-α-D-galactosyl bromide gave mp 134–135°, $[\alpha]^{25}D$ 218° (*c* 1.07, $CHCl_3$). *Anal.* Calcd for $C_{13}H_{19}BrO_8$: C, 40.74; H, 5.01; Br, 20.85. Found: C, 40.85; H, 4.97; Br, 20.65.

The *p*-nitrophenyl glycoside acetates were obtained by treating the galactosyl bromides with sodium *p*-nitrophenoxide in aqueous acetone.[37]

(27) A. G. Davies, "Organic Peroxides," Butterworth and Co. (Publishers) Ltd., London, 1961, p 108.

(28) C. Walling and S. A. Buckler, *J. Am. Chem. Soc.*, **77**, 6032 (1955).

(29) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, p 150.

(30) All melting points are uncorrected. Microanalyses were performed by Celler Laboratories, Charleston, W. Va.

(31) A. Fernez and P. J. Stoffyn, *Tetrahedron*, **6**, 139 (1959).

(32) D. McCreath, F. Smith, E. G. Cox, and A. I. Wagstaff, *J. Chem. Soc.*, 387 (1939).

(33) W. L. Glen, G. S. Myers, and G. A. Grant, *ibid.*, 2568 (1951).

(34) H. Zinner, *Ber.*, **84**, 780 (1951).

(35) E. J. C. Curtis and J. K. N. Jones, *Can. J. Chem.*, **38**, 890 (1960).

(36) E. Pacsu and S. M. Trister, *J. Am. Chem. Soc.*, **63**, 925 (1941).

(37) W. F. Goebel and O. T. Avery, *J. Exptl. Med.*, **50**, 521 (1929).

p-Nitrophenyl 2-*O*-methyl-β-D-galactoside triacetate gave mp 146–147°, $[\alpha]^{25}D$ −32.4° (*c* 0.896, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{23}NO_{11}$: C, 51.69; H, 5.26; N, 3.17; OCH_3, 7.03. Found: C, 51.83; H, 5.27; N, 3.22; OCH_3, 7.11.

Glycoside acetates were deacetylated with a catalytic amount of sodium methoxide in dry methanol.[38] The glycosides were purified by recrystallization to constant melting point and specific rotation.

p-Nitrophenyl β-D-galactoside gave mp 177–178°, $[\alpha]^{25}D$ −84.0° (*c* 0.804, H_2O);[39] lit.[37] mp 181–182°, $[\alpha]^{25}D$ −86° (*c* 0.98, H_2O). *p*-Nitrophenyl 2-*O*-methyl-β-D-galactoside gave mp 168.5–169°, $[\alpha]^{25}D$ −82.1° (*c* 0.817, H_2O). *Anal.* Calcd for $C_{13}H_{17}NO_8$: C, 49.52; H, 5.45; N, 4.44; OCH_3, 9.84. Found: C, 49.42; H, 5.49; N, 4.29; OCH_3, 9.7.

p-Nitrophenyl α-D-Glycosides of Mannose and 2-O-Methylmannose.—Mannose and 2-*O*-methylmannose were acetylated following the procedure of Fernez and Stoffyn.[31]

The acetylated mannose was fused with *p*-nitrophenol at 160° in the presence of a catalytic amount of anhydrous zinc chloride following the Helferich method.[38,40]

p-Nitrophenyl 2-*O*-methyl-α-D-mannoside triacetate gave mp 123–124°, $[\alpha]^{25}D$ 129° (*c* 1.7 $CHCl_3$). *Anal.* Calcd for $C_{19}H_{23}NO_{11}$: C, 51.69; H, 5.26; N, 3.17; OCH_3, 7.03. Found: C, 52.21; H, 5.71; N, 3.34; OCH_3, 7.11.

The glycosides, obtained by methoxide anion catalyzed deactylation of the acetates,[38] were purified by recrystallization to constant melting point and specific rotation.

p-Nitrophenyl α-D-mannoside gave mp 181–182°, $[\alpha]^{25}D$ 155° (*c* 0.821, H_2O); lit.[38] mp 183–184°, $[\alpha]^{25}D$ 145° (*c* 0.20, H_2O). *p*-Nitrophenyl 2-*O*-methyl-α-D-mannoside gave mp 153–154°, $[\alpha]^{25}D$ 114° (*c* 0.838, H_2O). *Anal.* Calcd for $C_{13}H_{17}NO_8$: C, 49.52; H, 5.45; N, 4.44; OCH_3, 9.84. Found: C, 49.36; H, 5.47; N, 4.54; OCH_3, 9.3.

Rate Measurements.—Reactant solutions were prepared from carbon dioxide free distilled water using pure samples of glycosides and 2-*O*-methyl glycosides and analytical grade inorganic chemicals. Sodium carbonate, which is insoluble in 1:1 w/w sodium hydroxide–water solution, was eliminated by centrifugation. Reactant base solutions were prepared by diluting this concentrate. Methanol reactant solutions were prepared from solvent dried by distillation from magnesium methoxide. Sodium hydroperoxide solutions were prepared just before use by mixing standard aqueous solutions of hydrogen peroxide and sodium hydroxide. The solutions contained $2 \times 10^{-4} M$ disodium dihydrogen ethylenediaminetetraacetic acid dihydrate to inhibit decomposition of the hydroperoxide. All possible precautions were taken to avoid contaminating the 99.77% deuterium oxide reactant solutions with ordinary water. Calculations showed no significant change in the deuterium content of solvent had occurred as a result of addition of the solutes, glycoside, and base.

Ten-milliliter aliquots of separate solutions, (1) base (0.08–1.00 *N*) and inert salt (to maintain constant ionic strength) and (2) glycoside or 2-*O*-methylglycoside (0.0001–0.0016 *M*), prepared at 25° were transferred to separate thermostated glass tubes, which were then stoppered. After a 30-min equilibration period, the base solution tube was removed from the thermostat, wiped dry, and placed within the tube containing the glycoside solution. The reactant solutions were mixed by breaking the base solution tube after an additional 15-min equilibration and an aliquot (4 ml) was transferred to a spectrophotometer cell. A continuous record of the increase in absorbance of the reacting solution as a function of time was obtained with a Cary Model 15 spectrophotometer. Time lapse between mixing reactant solutions and commencement of absorbance measurements seldom exceeded 25–35 sec. Water from a thermostat was circulated through the walls of the cell compartment and thermostatable cell-jacket accessory. Thermometers were located so as to measure the temperature of the circulating stream immediately entering and leaving the cell compartment. The cell or reaction temperature was taken to be the arithmetic average of the temperatures of the entering and leaving water streams. If a noticeable temperature change (greater than 0.05°) occurred while a reaction was followed, the data were discarded.

The glycoside or 2-*O*-methylglycoside reactants were found not to interfere in the determination of *p*-nitrophenol anion concentration at 401 mμ in water and 393 mμ in methanol.

Product Analysis.—The reaction of *p*-nitrophenyl glycosides and *p*-nitrophenyl 2-*O*-methylglycosides in methanolic sodium methoxide or aqueous sodium hydroxide were allowed to proceed essentially to completion at constant temperature. Normally, separate reaction mixtures were used for (1) product isolation and identification and (2) yield determinations.

Reactions in methanolic sodium methoxide were allowed to proceed under reflux in the absence of moisture.[41] After cooling, methanol was removed *in vacuo* (bath temperature 35°), and the syrupy residues were taken up in 100 ml of water.

Aqueous solutions of the methanolic sodium methoxide and aqueous sodium hydroxide reaction mixtures were passed through a column of Amberlite IR-120 cation-exchange resin to remove the sodium ion. The weakly acidic aqueous eluates from the ion-exchange resin were concentrated to approximately 100 ml and extracted with either ether or benzene in a continuous operation for 24 hr to remove aromatic reaction products. The extract solutions were dried over anhydrous sodium sulfate, concentrated to a small volume, and set aside for subsequent analysis.

The extracted aqueous solution was passed through a column of Amberlite IR-45, weakly basic, anion-exchange resin, to remove the remaining organic acids. Eluates from the resin column were concentrated to a small volume, which was saved for later analysis.

Reaction products were isolated by crystallization, preparative paper chromatography, or in some instances by steam distillation, and identified by comparing their physical properties with those of pure knowns.[42] Minor reaction products were identified by comparing their gas chromatographic retention times with those of pure knowns.

All gas chromatographic analyses were conducted with an Aerograph Hi Fi Model A 600 B gas chromatograph equipped with a hydrogen-flame ionization detector. Two columns were used for the quantitative analyses. The first has already been described.[44] The second consisted of 76 cm of a 1:1 v/v mixture of 20% w/w Apiezon M grease on Chromosorb W, 60–80 mesh, and 20% w/w butanediol succinate polyester on Chromosorb W, 60–80 mesh. All packings were housed in a 0.32-cm-i.d. stainless steel shell. Prepurified grade nitrogen was the carrier gas and the flow rate was 72.0 cc/min (measured at room temperature).

The amounts of reaction products formed were determined by a quantitative gas chromatographic procedure calibrated with an internal standard. All reaction products except the methyl glycosides could be determined without prior derivatization. It was found that the methyl glycosides, which had to be acetylated prior to gas chromatography,[45] could be quantitatively converted to the acetates by the pyridine–acetic anhydride procedure of Fernez and Stoffyn.[31]

Jones and Perry[44] reported a successful separation of anomeric methyl glycosides with column 1. Their success, however, could not be duplicated. When alcoholic solutions of pure methyl glycopyranosides were injected onto column 1, they appeared to decompose. No decomposition was observed when acetylated glycosides or unacetylated 1,6-anhydroglycopyranoses were gas chromatographed.

Generally, when at least two of the five hydroxyls in the pyranose ring form of the aldohexoses were blocked, the derivatized sugars were sufficiently volatile for chromatography on polar liquids, *e.g.*, butanediol succinate polyester. Thus, good separations of not only the anomeric methyl glycoside tetraacetates, but also of the unacetylated anomeric methyl 2-*O*-methylmannosides were obtained with column 2.

Acknowledgment.—The authors are grateful to Drs. D. G. Williams and N. S. Thompson for their suggestions.

(38) J. Conchie and G. A. Levvy, *Methods Carbohydrate Chem.*, **2**, 345 (1963).

(39) Water was the final recrystallization solvent for all *p*-nitrophenyl glycosides.

(40) B. Helferich and E. Schmitz-Hillebrecht, *Ber.*, **66**, 378 (1933).

(41) The solvent was dried by distillation from magnesium methoxide and contained less than 0.03% water as measured by the Karl Fischer method.

(42) The methyl glycosides and methyl 2-*O*-methylglycosides were prepared by the Fischer method.[43] The 1,6-anhydro sugars of galactose and 2-*O*-methylgalactose were obtained in the course of preparing 2-*O*-methylgalactose. 1,6-Anhydro-β-D-mannopyranose was a gift of N. K. Richtmyer.

(43) J. K. Dale and C. S. Hudson, *J. Am. Chem. Soc.*, **52**, 2534 (1930).

(44) H. G. Jones and M. B. Perry, *Can. J. Chem.*, **40**, 1339 (1962).

(45) Methyl glycoside acetate samples were prepared for use in calibrating the quantitative gas chromatography analytical procedure.