A slow evolution of gas started at 205° (internal temperature). The melt was heated for 30 min. at 210–220°, at which temperature the decarboxylation proceeded fast. Distillation gave then 3.8 g. (26%) of the nitrile, boiling at 135° (25 mm.), $n^{23}D$ 1.5955 (60% of *trans*).

The decarboxylation of the acid was repeated in the presence of 0.2 g. of cuprous chloride. The decarboxylation started at 190° (internal temperature) and was finished after 10 min. at 210°. Distillation then gave 10.3 g. (69%) of the nitrile boiling at 135° (20 mm), $n^{23}D$ 1.5919 (35% *trans*-nitrile).

The Rates of Acid Hydrolysis of the Phenyl β-D-Glucopyranosiduronic Acids and Phenyl β-D-Glucopyranosides of Phenol, *p*-Cresol, and *p*-Chlorophenol

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The effects of the electron affinity of the aglycon group on the rates of acid hydrolysis of phenyl β-D-glucopyranosiduronic acids and phenyl β-D-glucopyranosides were studied. The aglycon groups were *p*-cresyl, phenyl, and *p*-chlorophenyl, and the rates were determined at 50–60° in 2.00–20.0 wt. % sulfuric acid. Linear correlations between the logarithms of the first-order rate constants and the Hammett acidity function were found for both series. The large positive entropies of activation were essentially the same for both series (+10 cal. per °K. per mole) so that the greater free energies of activation of the phenyl β-D-glucopyranosiduronic acids (2.0 kcal. per mole greater) were due to greater enthalpies of activation. The Hammett ρ-values for the phenyl β-D-glucopyranosides and β-D-glucopyranosiduronic acids were −0.48 ± 0.04 and −0.09 ± 0.05, respectively. These results are most consistent with the interpretation that the two series hydrolyzed *via* the rapid protonation of the glycosidic oxygen followed by slow heterolysis of the glycosyl oxygen bond. The stabilizing effect of the C-5 carboxyl group compared to the hydroxymethyl group was due to a greater inductive effect of the latter. The nature of the inductive effect is discussed.

Of recent interest has been the stabilizing effect produced when a C-5 hydroxymethyl group of a glycopyranoside is replaced by a carboxyl group (carboxyl stabilizing effect).[2-8] While the carboxyl stabilizing effect seems well-established, the nature of the effect is not well-understood. Two explanations have been offered. The first of these is the inductive effect hypothesis which proposes that the hydrolysis takes place *via* a cyclic mechanism [A-1 (A) mechanism], and that the carboxyl stabilizing effect is due to the greater inductive effect of the carboxyl group.[3-9] The proposed cyclic mechanism of acid-catalyzed glycoside hydrolysis[10-12] is shown in Fig. 1.

More recently, a second explanation has been offered based on work on the acid hydrolysis of methyl uronosides.[2,7] It was suggested that replacement of the C-5 hydroxymethyl group with a carboxyl group causes an undefined change in the reaction mechanism.

For the most part, kinetic studies of the carboxyl stabilizing effect have been confined to the effect of temperature on the acid hydrolysis rates of the uronosides and their corresponding C-5 hydroxymethyl glycosides at a given acid concentration.[2,6,7] In some cases, these rates were determined only at a single temperature and acid concentration.[3,8] A better understanding of the carboxyl stabilizing effect would be gained through an investigation of the effect of the electron affinity of the aglycon group as well as the effects of temperature and acid concentration. The β-D-glucopyranosiduronic acids (β-D-glucuronides) and β-D-glucopyranosides (β-D-glucosides) of phenol, *p*-chlorophenol, and *p*-cresol were chosen for this purpose.

Results

Figure 2 shows plots of the first-order rate constants of phenyl β-D-glucuronide *vs.* the hydronium ion concentration. The hydronium ion concentrations were calculated from the ionization of aqueous sulfuric

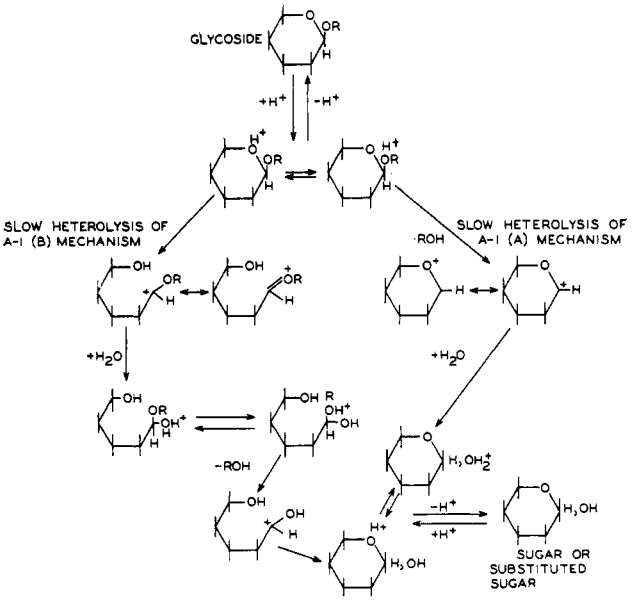

Fig. 1.—Proposed mechanisms of acid-catalyzed glycoside hydrolysis.

(1) A portion of a thesis submitted in partial fulfillment of the requirements of The Institute of Paper Chemistry for the degree of Doctor of Philosophy from Lawrence College, Appleton, Wis., June, 1963.

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TABLE I

COEFFICIENTS FOR THE RATE-CONSTANT ISOTHERMS OF PHENYL β-D-GLUCURONIDES AND PHENYL β-D-GLUCOSIDES

Glycoside	Temp., ±0.05°C.	Isotherm coefficients[a] B	D	F
Phenyl β-D-glucuronide	50.45	13.34	0.9341	0.06984
	55.15	28.36	0.7967	0.3168
	59.90	56.97	3.805	0.3275
p-Chlorophenyl β-D-glucuronide	50.45	12.14	0.6564	0.06738
	55.15	25.21	0.3303	0.2712
	59.90	51.78	2.663	0.3792
p-Cresyl β-D-glucuronide	50.45	12.49	1.136	0.02422
	55.15	25.82	1.924	0.1393
	59.90	53.50	4.617	0.1653
Phenyl β-D-glucoside	50.10	279.4	82.37	−4.670
	55.00	571.3	151.3	−6.746
	59.95	1182.	250.2	4.544
p-Chlorophenyl β-D-glucoside	50.10	203.1	63.33	−3.466
	55.00	410.1	113.7	−5.358
	59.95	803.0	334.7	−36.43
p-Cresyl β-D-glucoside	50.10	301.4	111.6	−10.58
	55.00	655.4	202.8	−17.83
	59.95	1266	494.9	−60.40

[a] $k' = B[H_3O^+] + D[H_3O^+]^3 + F[H_3O^+]^5$ where k' is the first-order rate constant (min.$^{-1}$ × 10^6), $[H_3O^+]$ is the hydronium ion concentration (moles per liter), and B, D, F are isotherm coefficients.

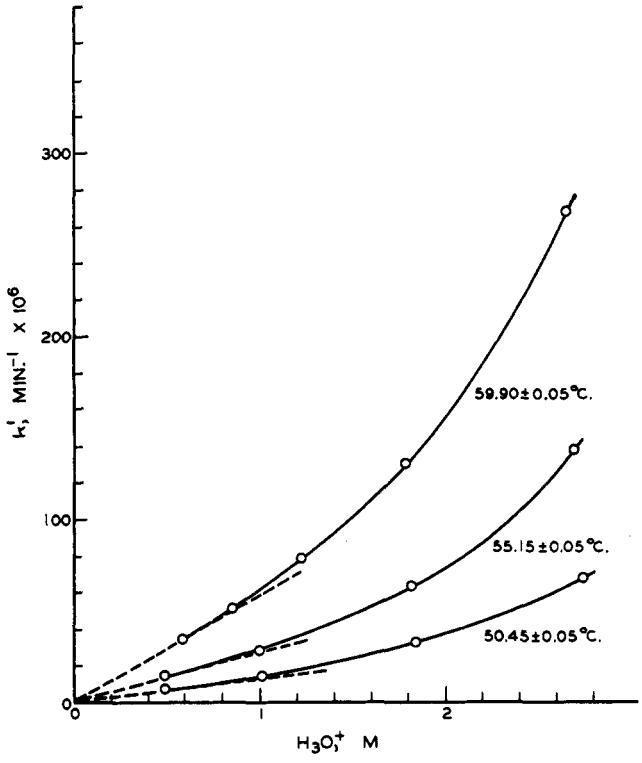

Fig. 2.—Effect of hydronium ion concentration on the hydrolysis rate of phenyl β-D-glucuronide.

acid[13] (1.20, 1.18, and 1.17 times the molarity of sulfuric acid at the 50, 55, and 60° levels, respectively). The rate constants are nearly proportional to the hydronium ion concentration at low concentrations but increase faster above approximately 0.75 *M*. The dotted lines are straight lines through the origin and the first data point. The other phenyl β-D-glucuronides as well as the phenyl β-D-glucosides gave similar rate-constant isotherms. Each isotherm can be represented by the equation $k' = B[H_3O^+] + D[H_3O^+]^3 + F[H_3O^+]^5$. The coefficients B, D, and F in Table I can then be used to calculate the rate constant at any hydronium ion concentration, and were so used in the application of Arrhenius and Hammett equations to the data.

The activation energies and their estimated standard deviations were calculated from the least-squares, straight-line fits of Arrhenius plots. There were no significant differences in activation energies between various levels of hydronium ion concentration nor did the various phenyl substituents produce a measurable change. The activation energies of the phenyl β-D-glucuronides and phenyl β-D-glucosides are 33.0 ± 0.2 and 30.8 ± 0.3 kcal. per mole, respectively. The activation energy of phenyl β-D-glucoside is 30.6 ± 0.2 kcal. per mole as compared to literature values of 32.30 ± 0.43[14] and 31.0 ± 1.2 kcal. per mole.[15]

Shown in Fig. 3 and 4 are plots of the logarithms of the rate constants at various hydronium ion concentrations of the phenyl β-D-glucuronides and the phenyl β-D-glucosides near 60° *vs.* the Hammett substituent constant, σ.[16] Since the activation energies in each series were found to be essentially independent of the substituent, plots of the logarithms of the rate constants in the 50–60° range *vs.* σ determined at 25° will have the same slopes (Hammett reaction series constant,[16] ρ) as if the logarithms at 25° were plotted. The values of ρ were calculated by least-squares, straight-line fits and found to be essentially independent of the hydronium ion concentration. The phenyl β-D-glucuronides showed little sensitivity to polar aglycon group effects ($\rho = -0.09 \pm 0.05$), while the hydrolysis rates of the phenyl β-D-glucosides were reduced by lowering the glycosyl group electron density ($\rho = -0.48 \pm 0.04$). Similar results were obtained at the other temperature levels. For a more extensive series of phenyl β-D-glucosides, Nath and Rydon[17] obtained a ρ of −0.66.

(13) T. F. Young, L. F. Maranville, and H. M. Smith, "The Structure of Electrolytic Solutions," W. J. Hamer, Ed., John Wiley and Sons, Inc., New York, N. Y., 1959, p. 48.

(14) L. J. Heidt and C. B. Purves, *J. Am. Chem. Soc.*, **66**, 1385 (1944).

(15) W. G. Overend, C. W. Rees, and J. S. Sequeira, *J. Chem. Soc.*, 3429 (1962).

(16) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940.

(17) R. L. Nath and H. N. Rydon, *Biochem. J.*, **57**, 1 (1954).

Table II lists the carboxyl stabilizing effect, as determined by the ratio of the rate constants of the C-5 hydroxymethyl and C-5 carboxyl glycopyranosides, for several glycosides at 95° in similar acid media. The carboxyl stabilizing effect of the phenyl glycosides was dependent on the phenyl substituent, since the susceptibility of the phenyl β-D-glucosides to polar aglycon effects was greater than the susceptibility of the phenyl β-D-glucuronides. This stabilizing effect was large and about the same magnitude as that for the reduced and unreduced aldobiouronic acid. These results are in sharp contrast to relatively small carboxyl stabilizing effects of the methyl glycosides.

TABLE II

CARBOXYL STABILIZING EFFECT FOR VARIOUS GLYCOSIDES AT 95°

Glycoside pair	Acid catalyst	Carboxyl stabilizing effect[a]	Ref.
Methyl α-D-glucopyranoside/methyl α-D-glucopyranosiduronic acid	1 *N* sulfuric	2.3[b]	8
Methyl β-D-glucopyranoside/potassium (methyl β-D-glucopyranosid)uronate	0.94 *N* sulfuric	2.6[b]	7
Methyl α-D-galactopyranoside/methyl α-D-galactopyranosiduronic acid	1*N* hydrochloric	2.3[b]	3
2-*O*-(4-*O*-Methyl-α-D-glucopyranosyl)-D-xylitol/2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose	1.07 *N* sulfuric	18	4
4-*O*-(α-D-Glucopyranosyl)-D-glucose/2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose	1.07 *N* sulfuric	19	4
p-Cresyl β-D-glucoside/*p*-cresyl β-D-glucuronide	0.855 *N* sulfuric	19[b]	This work
Phenyl β-D-glucoside/phenyl β-D-glucuronide	0.855 *N* sulfuric	16[b]	This work
p-Chlorophenyl β-D-glucoside/*p*-chlorophenyl β-D-glucuronide	0.855 *N* sulfuric	13[b]	This work

[a] Defined as the ratio of the rate constants of the C-5 hydroxymethyl and C-5 carboxy glucopyranosides at 95°. [b] Estimated from data at other temperatures by means of the Arrhenius equation.

According to Hammett,[16] an acid-catalyzed reaction whose transition state behaves like the conjugate acid of the uncharged reactant will show a linear correlation of the unit slope between the logarithm of the rate constant, log k', and the negative of the Hammett acidity function,[18] $-H_0$. Since the activation energies were found to be independent of the hydronium ion concentration, unit slope should still be found when log k' in the 50–60° range is plotted against the Hammett acidity function at the same hydronium ion concentration at 25°. Figure 5 shows such plots for phenyl β-D-glucuronide. The slopes determined by the least-squares, straight-line fits were 0.82–0.85 ± 0.01, which are slightly lower than the predicted value of unity. Similar linear relationships were found for the other phenyl β-D-glucuronides. McIntyre and Long[19] considered deviations of this magnitude in detail and concluded that they were probably due to small deviations

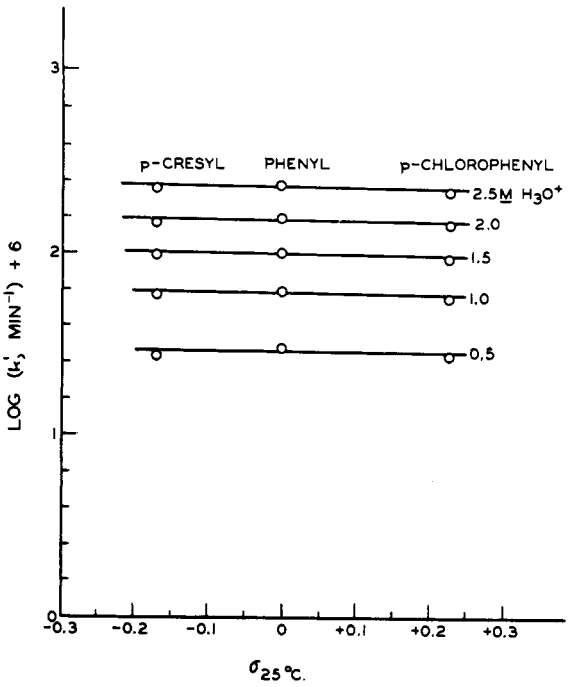

Fig. 3.—Effect of the electron-attracting tendency of the aglycon group (as measured by the Hammett substituent constant of the *para* substituent[15]) on the rates of acid hydrolysis of phenyl β-D-glucuronides at 59.90 ± 0.05°.

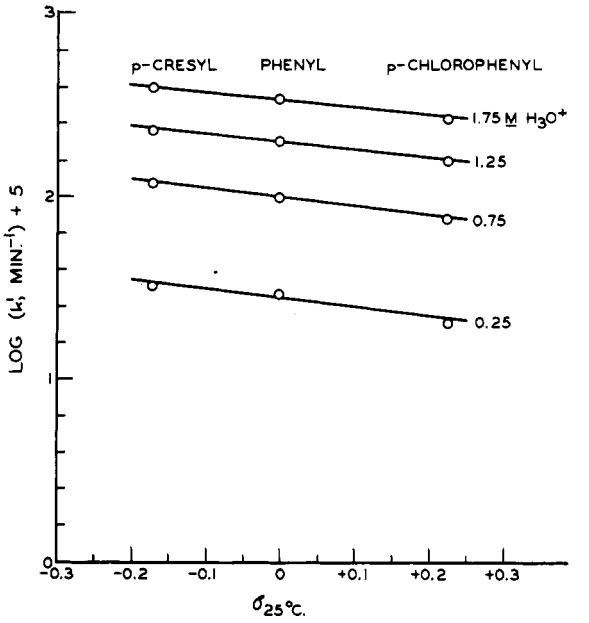

Fig. 4.—Effect of the electron-attracting tendency of the aglycon group (as measured by the Hammett substituent constant of the *para* substituent[15]) on the rates of acid hydrolysis of phenyl β-D-glucosides at 59.95 ± 0.05°.

of the uncharged base activity coefficient from those of the bases used to establish the Hammett acidity scale. All of the slopes for the phenyl β-D-glucosides were the predicted value of unity (1.00–1.03 ± 0.01). Bunton and co-workers[20] found a slope of 0.94 for phenyl β-D-glucoside at 72.9° in perchloric acid.

Calculations of the thermodynamic activation functions of the two glycoside series were made based on

(18) M. A. Paul and F. A. Long, *Chem. Rev.*, **57**, 1 (1957).
(19) D. McIntyre and F. A. Long, *J. Am. Chem. Soc.*, **76**, 3240 (1954).
(20) C. Armour, C. A. Bunton, S. Patai, L. H. Selman, and C. A. Vernon, *J. Chem. Soc.*, 412 (1961).

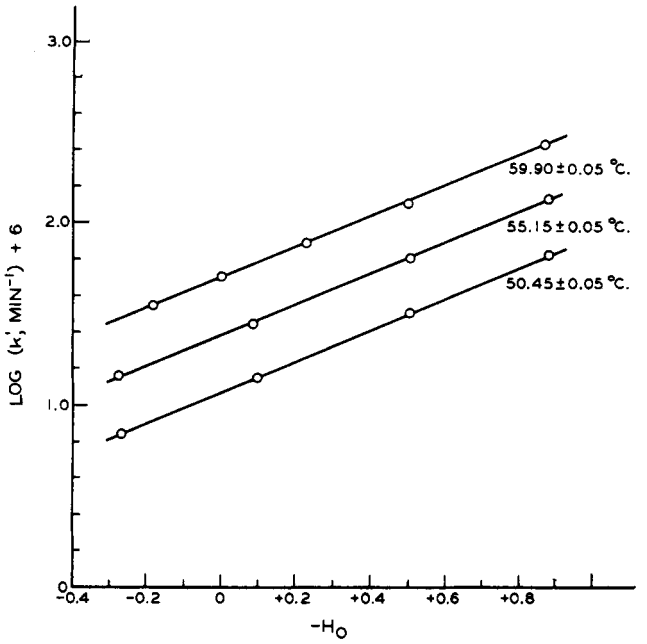

Fig. 5.—Relationship between the Hammett acidity function and the acid hydrolysis rate of phenyl β-D-glucuronide.

the theory of absolute reaction rates.[21] The specific rates employed in determining the free energies of activation were calculated from the slopes of the rate-constant isotherms as the acid concentration approached zero. The activation energies used to calculate the enthalpies of activation were average values taken over the range of hydronium ion concentration investigated. The calculated activation functions are listed in Table III. The activation functions within each series were not significantly different. Since the entropies of activation for the two series were essentially the same, the differences in free energy of activation of the two series were reflected primarily in the enthalpy function. This difference was about 2.0 kcal. per mole.

TABLE III

ESTIMATED THERMODYNAMIC ACTIVATION FUNCTIONS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDES AND PHENYL β-D-GLUCOSIDES

Glycoside	ΔF^*, kcal. per mole[c]	ΔH^*, kcal. per mole	ΔS^*, cal. per °K. per mole
p-Cresyl β-D-glucuronide[a]	28.8 ± 0.1	32.3 ± 0.2	+10 ± 1
Phenyl β-D-glucuronide[a]	28.7 ± 0.1	32.2 ± 0.2	+10 ± 1
p-Chlorophenyl β-D-glucuronide[a]	28.8 ± 0.1	32.4 ± 0.6	+11 ± 2
p-Cresyl β-D-glucoside[b]	26.7 ± 0.1	30.3 ± 0.3	+11 ± 1
Phenyl β-D-glucoside[b]	26.7 ± 0.1	29.9 ± 0.2	+10 ± 1
p-Chlorophenyl β-D-glucoside[b]	27.0 ± 0.1	30.1 ± 0.6	+ 9 ± 2

[a] Calculated at 59.90 ± 0.05°. [b] Calculated at 59.95 ± 0.05°. [c] The standard deviation of ΔF^* was estimated from the standard deviation of the first-order rate constants at the lowest acid concentrations rather than from the standard deviations of the slopes of the rate-constant isotherms as the acid concentration approached zero.

(21) E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt, New York, N. Y., 1960.

Discussion

There is now a considerable amount of data indicating that the hydrolysis of glycosides usually proceeds with glycosyl-oxygen fission[10,20] and that protonation of glycosides is rapid.[15,20] Furthermore, studies[10,15,20] have consistently indicated that the slow heterolysis step is unimolecular. Two possible unimolecular mechanisms have been proposed and these are shown schematically in Fig. 1. The fact that the effect of acid concentration on the hydrolysis rates of phenyl β-D-glucuronides and phenyl β-D-glucosides indicates that the transition states behave like the conjugate acids, agrees with either of the proposed unimolecular mechanisms. Furthermore, the large positive entropies of activation, +9 to +11 cal. per °K. per mole, are characteristic of the acid-catalyzed cleavage of carbon–oxygen bonds where the slow heterolysis step is unimolecular.[21] Hence, the results of this investigation are consistent with either the A-1 (A) or A-1 (B) mechanism.

The work of Banks and co-workers[22] on the acid hydrolysis of methyl α-D-glucoside indicates that the A-1 (A) mechanism predominates. Also, their studies of the acid-catalyzed methanolysis of phenyl α- and β-D-glucoside suggest the A-1 (A) mechanism. Some indication of the generality of this to glycosides is gained from the fact that the entropies of activation of the glycosides studied by Banks and co-workers[22] are about the same as most other glycosides (+10 to +20 cal. per °K. per mole[15]) including the phenyl β-D-glucosides and phenyl β-D-glucuronides. Therefore, a reasonable assumption would be that the phenyl β-D-glucosides and phenyl β-D-glucuronides hydrolyzed *via* the A-1 (A) mechanism.

As to the origin of the C-5 carboxyl group stabilization of the glycosidic linkage, Whistler and Richards[3] considered the increased conformational resistance to the formation of the A-1 (A) carbonium ion and concluded that this effect was too small to account for large carboxyl stabilizing effects. The molecular models show that when the phenyl β-D-glucuronides are in the Cl conformation the oxygens of the carboxyl group are in good position to hydrogen bond with the C-4 hydroxyl. Since this tendency would be even greater in the A-1 (A) transition state, one would expect less conformational resistance[23] and an activating rather than a stabilizing effect. A large ponderal effect[24] would be surprising since the maximum difference in the mass between any of the glycoside pairs studied was only about 5%. Thus, none of these effects appear to be an adequate explanation for the carboxyl stabilization.

According to the inductive effect hypothesis,[3] the greater inductive effect of the carboxyl group compared to the hydroxymethyl group decreases the rate of the heterolysis step by opposing the migration of the electron pair of the glycosyl-oxygen bond to the glycosidic oxygen.[4] In addition, the induction of a smaller partial negative charge on the glycosidic oxygen would diminish the ease of protonation and decrease the con-

(22) B. E. Banks, Y. Meinwald, A. J. Rhind-Tutt, I. Sheft, and C. A. Vernon, *J. Chem. Soc.*, 3240 (1961).

(23) J. T. Edward, *Chem. Ind.* (London), 1102 (1955).

(24) P. B. D. De la Mare, L. Fowden, E. D. Hughes, C. K. Ingold, and D. H. Mackie, *J. Chem. Soc.*, 3200 (1955).

centration of conjugate acid.[5] Finally, since the ring oxygen stabilizes the carbonium ion by a drift of electron density toward C-1 giving the ion some oxonium ion character, the induction of the electron cloud of the ring oxygen toward C-5 would hinder this drift. Stabilization of the carbonium ion would thus be more difficult, making the hydrolysis rate lower. Considering the fact that the ring oxygen is α to the carboxyl group, this latter factor may be the dominant aspect of the inductive effect. Thus, even if the inductive effect of a C-5 substituent extended only to the ring oxygen, a stabilizing effect would result.

In studying the relationships between the electronic theories of reactivity, the influence of substituents, and the activation energies, Hinshelwood, Laidler, and Timm[25] concluded that changes in reactivity which result from inductive effects are reflected primarily in the activation energies. The difference in reactivity of the phenyl β-D-glucosides and phenyl β-D-glucuronides is almost entirely associated with changes in the enthalpies of activation. Since the enthalpy of activation nearly equals the energy of activation,[21] these results are consistent with the view that the carboxyl group stabilizes the glycosidic linkage by an inductive effect.

A greater electron affinity of the aglycon group decreases the ease of protonation of the glycosidic oxygen but increases the rate of glycosyl-oxygen bond heterolysis[15]; thus, the negative ρ-values of the two series indicate that the effect on protonation predominates. If the hypothesis of the carboxyl inductive effect is valid, the carboxyl group would tend to lower the effective electron density on the atoms of the reaction center (O–C-1–O) reducing its polarizability. According to Smith and Eyring,[26] the inductive effect of a substituent is dependent on the electron density at the reaction center. Therefore, the polar aglycon group effects should be less for the phenyl β-D-glucuronide series than for the phenyl β-D-glucoside series. Thus, the low Hammett constants, ρ, of the former as compared to the latter support the inductive effect hypothesis.

The inductive effect of *n*-alkyl carboxylic acids are well-known,[27] and it is recognized that this inductive effect may be transmitted not only through the atoms attached to these groups but also to some extent through the surrounding solvent molecules. However, the ionization constants of *meta*- and *para*-substituted phenyl carboxylic acids are measurably affected by phenyl substituents even when a methyleneoxy[28] or two methylene[16] groups have been interposed between the carboxyl and phenyl groups. Hence, the polar effects of phenyl substituents could extend to the ring oxygen. Therefore, the inductive effects of the phenyl substituents and the C-5 substituent could interact. Such an interaction could cause the phenyl β-D-glucuronides to be less susceptible to polar aglycon effects since the C-5 carboxyl would be expected to reduce the polarizability of the reaction center.

It might be argued that the large negative ρ-values of the phenyl β-D-glucosides compared to the phenyl β-D-glucuronides indicate a change from the A-1 (A) mechanism for the former to the A-1 (B) mechanism for the latter. However, although the A-1 (A) and A-1 (B) mechanisms are similar, it would be surprising to find nearly identical entropies of activation as was observed.

In contrast to this, the activation entropies of methyl α-D-glucuronide[7] and of methyl α-D-galacturonide[2] were significantly different from their corresponding glucosides, suggesting that these glycopyranosiduronic acids hydrolyzed *via* a different mechanism than most glycopyranosides. Indeed, it might be expected that replacing the hydroxymethyl group of methyl α-D-glucoside or methyl α-D-galactoside with a carboxyl group would cause a change in mechanism, since they are among the least susceptible of the glycosides to acid hydrolysis, and further stabilization by a C-5 carboxyl group may place them in a region of reactivity where some other mechanism predominates. Easty[7] calculated the free energy of activation for the acid hydrolysis of methyl α-D-glucoside to be 28.2 kcal. per mole at 80°. Assuming that replacement of the C-5 hydroxymethyl group with a carboxyl group increases the enthalpy of activation by the observed 2.0 kcal. per mole, the free energy of activation of methyl α-D-glucuronide would be 30.2 kcal. per mole for the A-1 (A) transition state. Easty calculated 28.6 kcal. per mole for the free energy of activation of methyl α-D-glucuronide at 80°. An alternate mechanism is thus feasible for these methyl glycopyranosiduronic acids.

It appears, therefore, that the A-1 (A) mechanism is applicable to the acid hydrolysis of most glycopyranosides, and that the carboxyl stabilizing effect is due to the greater inductive effect of the C-5 carboxyl group compared to the hydroxymethyl group. In cases of relatively acid-resistant C-5 hydroxymethyl methyl glycopyranosides, the introduction of the C-5 carboxyl group may cause some other mechanism to predominate.

Experimental

Preparation of Phenyl β-D-Glucuronides.—Methyl tetra-*O*-acetyl-β-D-glucopyranuronate was prepared by the methanolysis of D-glucuronolactone in alkaline methanol followed by the pyridine-catalyzed acetylation of the methyl ester with acetic anhydride as described by Bollenbeck and co-workers.[29] The product was isolated in 29% yield, m.p. 176–177.5°, $[\alpha]^{29}D$ +6.7° (*c* 2.2, chloroform) (lit. m.p. 176.5–178°,[29] 178°[30]), and $[\alpha]^{23}D$ +7.4° (*c* 2, chloroform),[31] +8.7° (*c* 1, chloroform).[32]

The methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)uronates were prepared by fusing methyl tetra-*O*-acetyl-β-D-glucopyranuronate with *p*-cresol, phenol, or *p*-chlorophenol under vacuum at 110° with *p*-toluenesulfonic acid as catalyst, as described by Bollenbeck and co-workers.[26] The yields were about 30% in all instances. Methyl (*p*-chlorophenyl tri-*O*-acetyl-β-D-glucopyranosid)uronate had m.p. 151–152° (lit. m.p. 152–153°[29] and 151–152°[32]); methyl (*p*-cresyl tri-*O*-acetyl-β-D-glucopyranosid)uronate, m.p. 137.5–139° (lit. m.p. 137–138°[29] and 140°[32]); methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)uronate, m.p. 118–119° (lit. m.p. 126–27.5°[29] and 116°[33]).

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(27) N. A. Lange and G. M. Forkner, "Handbook of Chemistry," Handbook Publishers, Inc., Sandusky, Ohio, p. 1198.

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(31) W. F. Goebel and F. H. Babers, *J. Biol. Chem.*, **106**, 63 (1934).

(32) I. A. Kamil, J. N. Smith, and R. T. Williams, *Biochem. J.*, **50**, 235 (1952).

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TABLE IV

PROPERTIES OF PHENYL, *p*-CHLOROPHENYL, AND *p*-CRESYL β-D-GLUCURONIDES

	Aglycon group of β-D-glucuronide		
	Phenyl	*p*-Cresyl	*p*-Chlorophenyl
Melting point, °C.			
Found	162.5–163.5	147.5–148.5	154–155
Literature	161–162[a] 163–164[d]	147 dec.[b]	151[c]
Equivalent weight[e]			
Found	272	284	307
Theoretical	270	284	305
Specific rotation, [α]D			
Found	−90.7° (*c* 1.02, water, 27°)	−87.9° (*c* 1.38, water, 28°)	−87.2° (*c* 1.32, water, 27°)
Literature	−90.0° (*c* 1, water, 25°)[d] −87.5° (*c* 2.10, water, 29°)[f]	−76.4° (*c* 0.4, water, 22°)[b]	−87° (*c* 0.5, water, 19°)[c]

[a] See ref. 33. [b] H. G. Bray, W. V. Thorpe, and K. White, *Biochem. J.*, **46**, 275 (1950). [c] See ref. 29. [c] B. Spencer and R. T. Williams, *Biochem. J.*, **47**, 279 (1950). [d] See ref. 29. [e] Titrated with 0.1 *N* sodium hydroxide. [f] K. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **75**, 1042 (1953).

TABLE V

PROPERTIES OF PHENYL, *p*-CHLOROPHENYL, AND *p*-CRESYL β-D-GLUCOSIDES

	Aglycon group of β-D-glucoside		
	Phenyl	*p*-Cresyl	*p*-Chlorophenyl
Melting point, °C.			
Found	174–176.5	178–179	174.5–176.5
Literature	171–172[a] 175–176[c] 174–175[f]	175–177[b] 178–179.5[d]	173–175[a] 173–174[e]
Specific rotation, [α]D			
Found	−72.0° (*c* 1.52, water, 29°)	−68.1° (*c* 1.36, water, 25°)	−71.6° (*c* 1.49, water, 28°)
Literature	−71.9° (*c* 2.0, water, 20°)[c] −71.0° (*c* 3.91, water, 20°)[f]	−67.7° (*c* 2, water, 20°)[d]	−82.0° (*c* 1.13, water, 19°)[a] −69.5° (*c* 1.0, water, 20°)[e]

[a] See ref. 17. [b] See ref. 34. [c] E. Fischer and L. Mechel, *Ber.*, **49**, 2813 (1916). [d] See ref. 30. [e] A. Dyfverman and B. Lindberg, *Acta Chem. Scand.*, **4**, 878 (1950). [f] E. Fischer and E. F. Armstrong, *Ber.*, **34**, 2885 (1901).

Deacetylation was accomplished by allowing 6.0 mequiv. of methanolic sodium methoxide to react with 0.025 mole of the methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)uronates in 200 ml. of dry methanol for 30 min. The solutions were evaporated to dryness at 35° in a rotary evaporator. Sufficient 1 *N* sodium hydroxide was added to the residues to saponify the remaining methyl ester groups. After deionization with Amberlite IR 120H resin and decolorizing with charcoal, the filtrates were evaporated to 60 ml. and stored at 4.5°. The crystalline products which formed overnight were dried over phosphorus pentoxide. The yields in all instances were about 50% and the properties of the three phenyl β-D-glucuronides are given in Table IV.

Preparation of Phenyl β-D-Glucosides.—A sample of phenyl β-D-glucoside was crystallized from water at 4.5° in 64% yield. The melting point and specific optical rotation are listed in Table V.

The *p*-chlorophenyl and *p*-cresyl tetra-*O*-acetyl-β-D-glucosides were prepared by fusing β-D-glucopyranose pentaacetate with *p*-cresol or *p*-chlorophenol under vacuum at 110° with *p*-toluenesulfonic acid as a catalyst as described by Bollenbeck and coworkers[29] for the preparation of the methyl (phenyl tri-*O*-acetyl-β-D-glucopyranosid)uronates. The yields were about 35%. *p*-Chlorophenyl tetra-*O*-acetyl-β-D-glucoside had m.p. 123.5–124.5° (lit. m.p. 123–124°[31]); *p*-cresyltetra-*O*-acetyl-β-D-glucoside, m.p. 119–120° (lit. m.p. 119–120°[34] and 116–118°[30]).

Deacetylation was accomplished by treating 2.0 mequiv. of methanolic sodium methoxide with 0.025 mole of the phenyl β-D-glucosidtetraacetates in 200 ml. of anhydrous methanol for 1 hr. The solutions of the glycosides were evaporated to dryness at 35° in a rotary evaporator. The residues were slurried in 50 ml. of water, neutralized with acetic acid, heated to effect complete solution, and allowed to crystallize overnight at 4.5°. A commercial sample of phenyl β-D-glucoside was crystallized from water under similar circumstances. The yields of all three glycosides were about 63%, and their properties are given in Table V.

Hydrolysis Procedure.—Solutions were prepared containing 0.0200–0.0400 *M* glycoside and 2.00–20.0 wt. % sulfuric acid. Four to six aliquots (*ca.* 1.9 ml.) were placed in glass ampoules with a syringe whose delivered volume was reproducible within ±0.1%. The ampoules were sealed and then simultaneously plunged into an ethylene glycol bath maintained at constant temperatures ±0.05° in the region 50–60°. Each ampoule was removed at a predetermined time and plunged into an ice–water bath. After 3 min., the ampoule contents were transferred to a 10-ml. volumetric flask and neutralized with sodium hydroxide solution. A maximum of 2–3% hydrolysis was allowed.

Analysis of Hydrolysates.—The reducing power of the samples was determined by the method of Hoffman.[35] An AutoAnalyzer (Technicon Controls, Inc., Chauncey, N. Y.) was employed to measure automatically the change in intensity at 420 mμ due to unreduced potassium ferricyanide. A flow cuvette with a 6-mm. light path was employed. It was found that essentially all of the reducing power of the hydrolysates was associated with the hydrolysis products. Hence, the reducing power of the hydrolysates was calibrated to measure the concentrations of products from which the hydrolysis rates could be calculated. Each hydrolysate was analyzed four times and the results averaged. The concentrations of products were of the order of 5×10^{-5} *M* and analyses in this range could be reproduced within ±0.75%.

(34) J. Ryan, *J. Chem. Soc.*, **75**, 1054 (1899).

(35) W. S. Hoffman, *J. Biol. Chem.*, **120**, 51 (1937).

The AutoAnalyzer was calibrated with equal molar solutions of D-glucose or sodium D-glucuronate monohydrate and the appropriate phenol. The calibration for the instrument was established with three to four analyses at four to six concentration levels.

Calculation of Rate Constants.—From a knowledge of the concentration of the products and the initial glycoside concentration, the fraction of the glycoside unreacted was calculated. It was assumed that each mole of glycoside hydrolyzed produced 1 mole of D-glucuronic acid or D-glucose. Plots of the natural logarithm of the fraction of glycoside unreacted *vs.* time were made. The first-order rate constants were calculated by least-squares, straight-line fits with estimated standard deviations about ±1–2%. Duplicate rate-constant determinations agreed within ±2.0%.

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Synthetic Nucleosides. LVIII.[1,2] Studies on the Synthesis of *cis*-2,3-Diamino Sugars. I. The Nitroguanidine Neighboring Group

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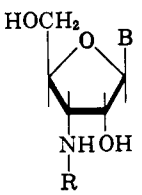
1-(2-Mesyloxyethyl)-3-nitroguanidine (XIV) rapidly cyclized in pyridine at 40° to the imidazoline (XV), a precursor to the ethylenediamine system formed by a neighboring group reaction. In contrast, the sugar pyranoside, methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-mesyl-3-(3-nitroguanidino)-α,D-altropyranoside (XXII), failed to cyclize even in boiling pyridine. Anionic cyclization of XXII with a strong base led to the thermodynamically unstable tricyclic imino sugar derivative (XXIV) rather than the expected and more stable tricyclic imidazoline (XXI). Even methyl 3-acetamido-4,6-*O*-benzylidene-3-deoxy-2-*O*-mesyl-α,D-altropyranoside (XXVI), when treated with a strong base, was converted to the imino sugar (XXV) *via* an anion; this result contrasts to the cyclization of XXVI to an oxazoline (VI) in the presence of sodium acetate.

The antibiotic, puromycin (I), was the first example of an inhibitor derived from a nucleoside by replacement of a hydroxyl group by an amino function.[3,4] The corresponding aminonucleoside (II) also had interesting biological properties.[4,5] Among the analogs of puromycin synthesized for biological evaluation was the adenine analog (III),[6] which was subsequently



IV

I, B = 6-dimethylamino-9-purinyl
R = *p*-methoxy-L-phenylalanyl
II, B = 6-dimethylamino-9-purinyl
R = H
III, B = 6-amino-9-purinyl
R = H

isolated from *Helminthosporium* sp., and *Cordyceps militaris*.[7] The corresponding 2,3-diamino-2,3-dideoxy-D-ribonucleosides (IV) represented a logical extension of structural modification of II that would be worthy of biological evaluation if these could be synthesized; in addition, no synthesis of a *cis*-2,3-diamino sugar had been reported,[8] and the projected synthetic schemes represented an unexplored area of neighboring group reactions.

The use of a neighboring group reaction for inversion of the configuration of an amino sugar from a *trans* system (V) to *cis* (VI) was introduced into the carbohydrate field by Baker and Schaub[9,10] and fruitfully has been extended by Jeanloz, *et al.*,[11] and others (Scheme I). That the same principle[12] could be used for the introduction of an amino function (VIII)[13] or a sulfur function (X)[14,15] into the pyranose ring of a glycoside subsequently was shown. A logical extension for synthesis of the *cis*-diamino system (XII) would be the use of the appropriate nitrogen derivative of amino sugar XI. In this paper is presented our investigations with the nitroguanidine neighboring group; in the accompanying papers are presented studies with the urea, guanidine, and thiourea neighboring groups.

(1) This work was generously supported by Grant CY-5845 of the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series, see B. R. Baker and H. S. Sachdev, *J. Org. Chem.*, **28**, 2135 (1963).

(3) For a review of the studies leading to the synthesis of puromycin and some of its analogs, see B. R. Baker, "The Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Conner, Ed., J. and A. Churchill Ltd., London, 1957, pp. 120–133.

(4) For a review of the biological properties of puromycin and its analogs, see B. H. Hutchings, *ibid.*, pp. 177–191; M. B. Yarmolinsky and G. L. de la Haba, *Proc. Natl. Acad. Sci. U. S.*, **45**, 1721 (1959), have shown that puromycin can inhibit protein synthesis at the s-RNA level.

(5) B. R. Baker, J. P. Joseph, and J. H. Williams, *J. Am. Chem. Soc.*, **76**, 2838 (1954); **77**, 1 (1955).

(6) B. R. Baker, R. E. Schaub, and H. M. Kissman, *ibid.*, **77**, 5911 (1955); E. J. Reist and B. R. Baker, *J. Org. Chem.*, **23**, 1083 (1958).

(7) (a) N. N. Gerber and H. L. Lechevalier, *ibid.*, **27**, 1731 (1962); (b) A. J. Guarino and N. M. Kredich, *Biochem. Biophys. Acta*, **68**, 317 (1963).

(8) R. D. Guthrie and D. Murphy, *Chem. Ind.* (*London*), 1473 (1962), recently have synthesized methyl 2,3-diamino-2,3-dideoxy-α,D-mannopyranoside by treatment of methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-tosyl-α,D-altropyranoside with sodium azide, followed by reduction. The synthesis of the corresponding alloside from a 3-azido-2-tosyl-D-altropyranoside was unsuccessful (private communication from Dr. Guthrie).

(9) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **20**, 646 (1954); *J. Am. Chem. Soc.*, **75**, 3864 (1953).

(10) This reaction was based on a similar study in the cyclohexane area by G. E. MacCasland, R. K. Clark, Jr., and H. E. Carter, *ibid.*, **71**, 637 (1949).

(11) R. W. Jeanloz, *ibid.*, **79**, 2591 (1957), and related papers by Jeanloz and co-workers.

(12) S. Winstein and R. Boschan, *ibid.*, **72**, 4669 (1950), have discussed the probable generality of neighboring group reactions for introduction of other hetero atoms into a chain or ring, but did not carry the study past that reported by MacCasland, *et al.*[10] Later F. L. Scott, R. E. Glick, and S. Winstein, *Experientia*, **13**, 183 (1957), reported results with urea and urethane neighboring groups.

(13) B. R. Baker, K. Hewson, L. Goodman, and A. Benitez, *J. Am. Chem. Soc.*, **80**, 6577 (1958).

(14) L. Goodman and J. E. Christenson, *ibid.*, **83**, 3823 (1961); **82**, 4738 (1960).

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