THE RATES OF ACID HYDROLYSIS OF THE BETA-D-GLUCOPYRANOSIDURONIC ACIDS AND BETA-D-GLUCOPYRANOSIDES OF PHENOL, PARA-CRESOL, AND PARA-CHLOROPHENOL

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SUMMARY

Studies of several glycopyranosides have established that replacement of a C_5 hydroxymethyl group with a carboxyl group stabilizes the glycosidic linkage to dilute acid hydrolysis. From the limited amount of data available, the stabilizing effect of the carboxyl group has been generally explained as being due to the inductive effect of the carboxyl group. However, recent and more extensive work on methyl uronosides suggests that uronosides may not hydrolyze via the same mechanism as other glycosides.

To further our understanding of the nature of the carboxyl stabilizing effect, the acid hydrolysis rates of phenyl β-D-glucopyranosiduronic acids (phenyl β-D-glucuronides) and phenyl β-D-glucopyranosides (phenyl β-D-glucosides) were studied as a function of the para-substituent of the aglycon group, acid concentration, and temperature. The aglycon groups studied were p-cresyl, phenyl, and p-chlorophenyl. Since the phenyl substituents were held rigidly at large distances from the glycosyl group, the effects of these substituents were primarily polar in nature. The acid hydrolysis rates were determined in 2.00-20.0 wt. % sulfuric acid at 50-60 ± 0.05°C.

The hydrolysis rates were nearly proportional to the hydronium ion concentration up to about 0.75M but increased faster at higher concentrations. The effects of temperature on the rates obeyed the Arrhenius equation with the activation energies of the phenyl β-D-glucuronides being greater than those of the corresponding phenyl β-D-glucosides (about 33.0 ± 0.2 kcal. per mole compared to 30.8 ± 0.3 kcal. per mole). The rates for the phenyl β-D-glucosides were found to be decreased by phenyl substituents which lowered the electron density in the glycosyl group (Hammett reaction series constants of about -0.49 ± 0.04), while those for the phenyl β-D-glucuronides were affected little by phenyl substituents (Hammett reaction series constants of about -0.11 ± 0.05). Comparison

of the rates of the corresponding phenyl β-D-glucuronides and phenyl β-D-glucosides indicated a substantial carboxyl stabilizing effect, i.e., 13-19 fold higher rates for the phenyl β-D-glucosides at 95°C. in 4.5 wt. % sulfuric acid.

Plots of the logarithms of the rate constants versus the Hammett acidity function were linear with slopes near unity for both glycoside series indicating that the transition states behaved like the conjugate acids. Furthermore, large positive entropies of activation (+9 to +11 cal. per °K per mole) were found which are characteristic of the acid-catalyzed cleavage of carbon-oxygen bonds where the slow heterolysis of the conjugate acid is unimolecular. These results suggested two possible reaction mechanisms: (1) protonation of the glycosidic oxygen with slow unimolecular glycosyl-oxygen heterolysis, A-1(A) mechanism; and (2) protonation of the ring oxygen followed by slow unimolecular ring-opening heterolysis, A-1(B) mechanism. In contrast to work on methyl uronosides, no evidence was found that replacing the C_5 hydroxymethyl group with a carboxyl group caused the mechanism to differ from that of most glycopyranosides.

Recent work on the acid hydrolysis of methyl α-D-glucoside and the acid-catalyzed methanolysis of phenyl α- and β-D-glucosides indicates that these glycosides react via the A-1(A) mechanism. Assuming the A-1(A) mechanism for the glycosides in this study, conformational, intramolecular hydrogen bonding, ponderal, and inductive effects of replacing the C_5 hydroxymethyl group with a carboxyl group were considered. The fact that the lower reactivity of the phenyl β-D-glucuronides was mainly due to higher activation energies suggests that the stabilization is the result of an inductive effect. Furthermore, the lower susceptibility of the phenyl β-D-glucuronide series to phenyl substituent effects suggests a decrease in the polarizability of the glycosyl group due to an inductive effect.

INTRODUCTION

CARBOXYL STABILIZING EFFECT

In aqueous acid media, glycosides* undergo hydrolysis to form their respective sugars and aglycons (1). The rates of the acid hydrolysis are a function not only of the acid concentration and temperature but also the structure of the glycoside. Structural factors such as the ring form of the glycosyl group, the nature of the aglycon, and the nature and position of the substituents are known to influence the acid hydrolysis rates (2). Of recent interest (3-7) has been the stabilizing effect produced when a C_5 hydroxymethyl group of a glycopyranoside is replaced by a carboxyl group (carboxyl stabilizing effect), but its nature is still not well understood. Before the literature on the carboxyl stabilizing effect is discussed the mechanism of the acid hydrolysis of glycosides will be reviewed.

MECHANISM OF ACID-CATALYZED HYDROLYSIS OF GLYCOSIDES

The acid-catalyzed breakage of carbon-oxygen bonds (8), such as in the acid hydrolysis of glycosides, is generally considered to proceed by the formation of the conjugate acid of the molecule through protonation of the oxygen. The proton causes the electrons forming the carbon-oxygen bond to drift toward the oxygen, eventually resulting in complete loss of bonding between the carbon and oxygen. In the following paragraphs, evidence supporting present views of the mechanism of the acid-catalyzed hydrolysis of glycosides will be reviewed briefly. This evidence pertains primarily to glycopyranosides since few mechanistic investigations of glycofuranosides have been made (9).

*Glycosides will be defined as derivatives of the cyclic forms of the sugars and substituted sugars in which the hydrogens of the hemiacetal hydroxyls have been replaced by alkyl or aryl groups.

BOND FISSION

In the acid hydrolysis of glycosides there are two possible types of fission of the glycosidic bond. The first type is glycosyl-oxygen fission whereby the oxygen of the aglycon comes from the glycoside. The second type is aryl or alkyl-oxygen fission with the aglycon oxygen originating from the water. Acid hydrolysis in water enriched with oxygen-18 (O^{18}) has established that for methyl α- and β-D-glucopyranosides (10), phenyl α- and β-D-glucopyranosides (10), methyl α-2-deoxy-D-glucopyranoside (11), and maltose (11) the aglycon is isotopically normal. This result would be obtained only for glycosyl-oxygen fission. The only known exception to this conclusion is for the strongly electron releasing t-butyl group of t-butyl β-D-glucopyranoside which causes alkyl-oxygen fission to predominate (11).

RATE OF PROTONATION

Evidence for the rapid reversible protonation of the glycoside prior to hydrolysis has been obtained by comparing the hydrolysis rate in deuterium oxide with that in water. The rate for methyl α-D-glucopyranoside was found to increase 1.8-1.9 times in deuterium oxide (9) while that of methyl α-2-deoxy-D-glucopyranoside increased 2.5 times (11). Also, the inversion of sucrose was found to proceed 2.05 times faster in deuterium oxide than when water was used as the solvent (12). The deuterium effect (13) was attributed to a higher conjugate-acid concentration, since the lower basicity of deuterium oxide results in deuteron acids having smaller dissociation constants than the corresponding proton acids. If the formation of the conjugate acid were rate controlling, a lower hydrolysis rate would be expected due to the lower zero-point energy of deuterium bonds.

MOLECULARITY OF HETEROLYSIS

In principal, any nucleophilic substitution has two mechanisms of reaction available (14). The first mechanism is the unimolecular mechanism in which a slow heterolysis is followed by the rapid attack of the substituting agent. The second mechanism is the bimolecular mechanism which involves simultaneous attack by the substituting agent and departure of the leaving group in the rate-controlling step.

It follows that the conjugate acid of a glycoside should have two mechanisms available: (1) unimolecular mechanism, A-1, controlled by a heterolysis rate; and (2) bimolecular mechanism, A-2, where a water molecule attacks in the rate-controlling step.

$$\text{A-1:} \quad \begin{cases} GOAH^+ \xrightarrow{\text{slow}} G^+ + AOH \\ G^+ + H_2O \xrightarrow{\text{fast}} \text{products} \end{cases}$$

$$\text{A-2:} \quad GOAH^+ + H_2O \xrightarrow{\text{slow}} \text{products}$$

where $GOAH^+$ = conjugate acid of glycoside

G^+ = carbonium ion

AOH = aglycon

H_2O = water

Several types of evidence have been used to support the conclusion that glycosides usually hydrolyze via an A-1 mechanism. This evidence will be considered in the following paragraphs.

Effect of Acid Concentration

According to the theory of absolute reaction rates (15) an equilibrium may be considered to exist between the conjugate acid of the glycoside and the

transition state. The effect of increasing the acid concentration is to increase the hydrolysis rate primarily by shifting the position of this equilibrium through an increase in the concentration of the conjugate acid. Secondarily, however, changes in acid concentration affect the rate of hydrolysis by affecting the activity coefficients of the species in equilibrium.

Since the A-1 transition state differs from the conjugate acid by a small extension of a C-O bond and by small conformational changes, the activity coefficient of the transition state should be approximately the same as the conjugate acid. The secondary effect of changes in acid concentration can then be shown to be changes in the ratio of the activity coefficients of the glycoside and the hydronium ion to the activity coefficient of the conjugate acid and the activity of water [Equation (9), p. 45]. It has been established for a number of weak uncharged bases (16) that such a ratio is essentially independent of the particular base so long as the medium is of high dielectric constant. This ratio is expressed quantitatively by the Hammett acidity function. It can be shown that a unit proportionality would be expected between the logarithm of the rate constant of an A-1 mechanism and the Hammett acidity function [Equation (11), p. 46]. Many reactions which are regarded as A-1 have been shown to have this correlation (17).

The appropriate activity coefficient ratio for A-2 mechanisms [Equation (12), p. 51] consists of the ratio of the activity coefficients of the glycoside and the hydronium ion to the activity coefficient of the transition state. It would be unlikely that this ratio would be the same as the comparable ratio for the A-1 mechanisms. Even if the acid concentration were low enough so that the activity of water could be considered constant, the A-2 transition state would probably not behave like the conjugate acid since it contains a partially bonded

water molecule in addition to a proton. Indeed, it has been found that those reactions known to be A-2 from other evidence usually show a direct proportionality between the logarithm of the rate constant and the logarithm of the acid concentration rather than the Hammett acidity function (17).

Linear relationships between the logarithm of the rate constant and the Hammett acidity function have been established for methyl α- and β-D-glucopyranosides (10), methyl α- and β-2-deoxy-D-glucopyranosides (11), phenyl α- and β-D-glucopyranosides (10), maltose (11), lactose (11), salicin (11), and cellobiose (18). In addition, the heterogenous acid hydrolysis of laminarin, cellulose, and xylan have been found to be controlled by the rate of the hydrolytic reaction and to correlate directly with the Hammett acidity function (18). Sulfuric, perchloric, hydrochloric, and hydrobromic acids have all been used with essentially the same results (10, 11, 18).

Entropy of Activation

The entropy of activation consists of the entropy change associated with the proton-transfer step plus the entropy change from either the unimolecular decomposition of the conjugate acid, or the bimolecular attack of a water molecule on the conjugate acid. Whalley (19) has shown that the standard entropy change for proton transfer from the ammonium ion to amines was 0 to +10 cal. per °K. per mole and in the case of trimethylamine was +14.6 cal. per °K. per mole. Also, the standard entropy change was similar for the proton transfer from the hydronium ion to amines. By analogy, it is reasonable to expect the entropy change for glycoside protonation to be positive.

For A-1 mechanisms one would expect the transition state to be a looser structure than the conjugate acid (15). In addition, the solvating molecules

should be more loosely bound in the transition state because of greater dispersal of the charge (14). Consequently, the activation process should result in a positive entropy change. It follows that the entropy of activation of A-1 mechanisms should be positive because both the protonation and activation steps should be positive.

When a water molecule attacks the conjugate acid in A-2 mechanisms, a van der Waals bond is replaced in the transition state by a partial bond (14). Such an event should result in a large decrease in entropy which probably more than offsets any increases due to loosening of the structure or to dispersal of charge (19). Depending on the magnitude of increase in entropy from proton transfer, an A-2 mechanism will have a negative or positive entropy of activation.

Estimates of the entropy of activation have been made for a large number of glycosides (7, 9, 11). For the most part these estimates were from +10 to +20 cal. per °K. per mole. Gould (8) states that the entropies of activation for the acid-catalyzed breakage of C-O bonds tend to fall into two ranges: (a) -20 to -25 cal. per °K. per mole, and (b) 0 to +10 cal. per °K. per mole. Those which are believed to be A-2 from other evidence are in the first group while A-1 mechanisms are in the second group. The large positive entropies of activation for glycosides most likely reflect an A-1 mechanism of acid hydrolysis.

Analogy with Simple Acetals

Extensive investigations of the acid hydrolysis of simple acetals has led to the conclusion that they hydrolyze by the unimolecular heterolysis of their conjugate acids (14, 20-23).

NATURE OF UNIMOLECULAR HETEROLYSIS

As noted by several workers (2, 7, 9-11) there are two plausible A-1 mechanisms for glycosides. One involves protonation of the glycosidic oxygen and heterolysis to a cyclic resonating carbonium ion, the A-1(A) mechanism. The other mechanism leads to the formation of an acyclic resonating carbonium ion after protonation of the ring oxygen, the A-1(B) mechanism. These mechanisms are shown schematically in Fig. 1. Since the rates of carbonium ion reactions*, proton transfers, and hemiacetal hydrolyses are generally high, steps subsequent to the formation of the carbonium ion are assumed to be rapid. The effects of structural changes have been generally found to be readily explicable on the basis of the A-1(A) mechanism (24-26). However, direct evidence supporting the A-1(A) mechanism has been obtained recently, and this evidence will be considered in the following paragraphs.

Oxygen Isotope Effect

In the A-1(A) mechanism, glycosyl-oxygen fission is involved in the rate-controlling step. Since one would expect the $C\text{-}O^{18}$ bond to rupture at a slightly slower rate than the $C\text{-}O^{16}$ bond due to the higher zero-point energy of the latter (13), the $O^{18}:O^{16}$ ratio of the aglycon will be lower than normal for partial hydrolysis. In the A-1(B) mechanism, on the other hand, the rate-controlling step includes ring oxygen-C_1 fission, and the isotopic identity of the glycosidic oxygen should have only a minor effect on the heterolysis rate. Therefore, the normal $O^{18}:O^{16}$ ratio would be found in the aglycon for partial hydrolysis.

*Few cases are known where the rate of reaction of carbonium ions is not rapid. However, the reaction of triphenylmethyl chloride with ethanol, water, and phenol in nitromethane has been shown to be controlled by the attack of the nucleophile on a preformed cation (27). This mechanism is due to the powerful conjugative stabilization effects of the three alpha-phenyl groups. Such pronounced stabilization effects are very unlikely in the acid hydrolysis of glycosides.

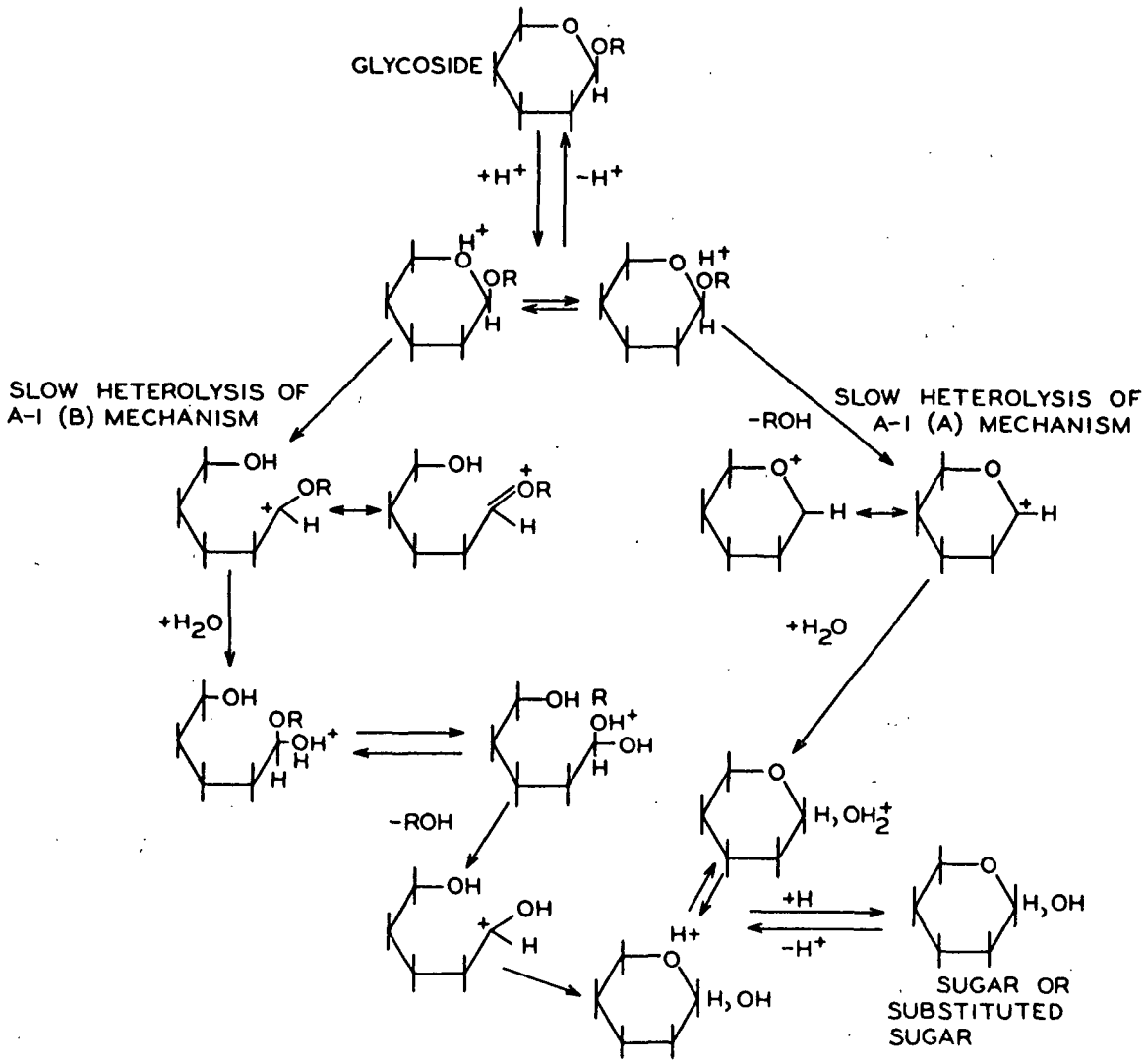

Figure 1. Proposed A-1 Mechanisms of Acid-Catalyzed Glycoside Hydrolysis (2,10,29)

Banks, et al. (28) have determined the $O^{18}:O^{16}$ ratio in the methanol from the partial and complete acid hydrolysis of methyl α-D-glucopyranoside. The isotope ratio was 2.9% lower than normal at 7.5% reaction but it was normal for complete hydrolysis. The magnitude of the effect was close to that predicted for the A-1(A) mechanism but too large to be explained by the A-1(B) mechanism.

Acid-Catalyzed Methanolysis of Glycosides

If the resonating carbonium ion is very unstable and tends to react rapidly with a water molecule*, the receding aglycon tends to protect its side of the carbonium ion thus making attack from the opposite side more probable (14). If the carbonium ion is quite stable, the aglycon will recede to such a distance that attack is equally probable from either side. Hence, the mechanism A-1(A) would be expected to result in the anomeric configuration of the sugar being anywhere from an equal mixture of the alpha and beta forms to the opposite of that of the glycoside.

Hydrolysis by the A-1(B) mechanism leads to the formation of a protonated hemiacetal subsequent to the ring-opening heterolysis (28). The longevity of the hemiacetal might be expected to be sufficient to allow some rotation of the C_1-C_2 bond. Once this rotation is accomplished, the original configuration of the glycoside should have little bearing on the configuration of the later formed sugar. Hydrolysis via mechanism A-1(B) would, therefore, be expected to result in an approximately equal mixture of alpha and beta anomers.

Unfortunately, the anomerization rates of sugars in aqueous acids are so rapid in comparison to the rates of hydrolysis that the relative amounts of alpha

*Ingold (14) has considered the details of the carbonium ion reaction in the unimolecular solvolytic substitution of alkyl halides. It was concluded that the life of the carbonium ion is ended by collapse of its solvation shell rather than penetration of the shell by a single high-energy solvent molecule.

and beta anomers have no diagnostic value. In acidic methanol, however, anomerization is sufficiently slow to allow conclusions to be drawn from the anomeric configuration of the methyl glycoside products. Banks, et al. (28) have studied the acid-catalyzed methanolysis of phenyl α- and β-D-glucopyranosides and 2,3,4,6-tetra-O-methyl-β-D-glycopyranoside. Methanolysis was found to proceed with at least 72-90% inversion. It was concluded that these results are most consistent with the A-1(A) mechanism.

RESISTANCE OF URONOSIDES[1] TO ACID HYDROLYSIS

The first quantitative measure of the carboxyl stabilizing effect was due to Morell and Link (30). They found that the acid hydrolysis rate of methyl α-D-galactopyranoside was only about twice the rate of methyl α-D-galactopyranosiduronic acid in N hydrochloric acid at 60-80°C. No attempt was made to explain this small difference in rates.

The acid hydrolysis rates of 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose (aldobiouronic acid) and the disaccharide alditol, 2-O-(4-O-methyl-α-D-glucopyranosyl)-D-xylitol, were compared by Whistler and Richards (3). In 1.07N sulfuric acid at 95°C., the disaccharide alditol was found to undergo acid hydrolysis at about the same rate as maltose and 18 times the rate of the aldobiouronic acid. To account for the large carboxyl stabilizing effect, it was suggested that stabilization was due mainly to the inductive effect of the carboxyl.[2]

Marchessault and Rånby (4) investigated the acid hydrolysis of cellulosic materials and offered a more detailed explanation of the inductive effect suggested

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[1] Uronosides will be defined as glycosides of uronic acids.

[2] It is well known that groups such as the carboxyl or hydroxymethyl group will tend to induce the electrons away from the rest of the molecule toward these groups. It should be recognized, however, that this inductive effect may be transmitted not only through the chain of atoms attached to these groups but to some extent through the surrounding solvent molecules.

by Whistler and Richards (3). Since the rate-controlling step in mechanism A-1(A) involves a shift of electrons toward the aglycon group, the more electrophilic the glycosyl group the greater the energy barrier for this electron shift, and the slower the hydrolysis rate. The carboxyl group being more electrophilic than the hydroxymethyl group would cause the glycosyl group to be more electrophilic which would result in stabilization of the glycosidic linkage.

Hamilton and Thompson (5) discussed another aspect of the inductive effect in connection with the hydrolytic action of the sulfite cooks on hemicelluloses. They suggested that electrophilic substituents in the glycosyl moiety tended to induce a more positive charge on the glycosidic oxygen thus tending to inhibit the formation of the conjugate acid. Since acid hydrolysis rates are proportional to the concentration of conjugate acid, substituents which retard the formation of the conjugate acid would reduce rates of acid hydrolysis.

Nakano and Rånby (6) have presented data on the dilute acid hydrolysis of methyl α- and β-D-glucopyranosiduronic acids (0.061M potassium salts in 0.94N sulfuric acid) and methyl α- and β-D-glucopyranosides (glycosides in 0.94N sulfuric acid). At 65-85°C. the former were two to three times more resistant than the corresponding anomers of the latter. Although the carboxyl stabilizing effect was small, it was suggested that an inductive stabilizing effect was responsible.

Easty (7) found that the acid hydrolysis rate of methyl α-D-glucopyranosiduronic acid was one-half to one-third that of methyl α-D-glucopyranoside at 70-90°C. in N sulfuric acid. Since inductive effects usually increase the activation energy (31), he felt that the lower activation energy of the uronoside (31.6 kcal. per mole compared to 35.6 kcal. per mole) indicated there was no

inductive effect operating. Furthermore, estimates of the entropies of activation at 80°C. indicated this function to be significantly lower for the uronoside (+6.42 cal. per °K. per mole compared to +18.9 cal. per °K. per mole). Easty's analysis of the data of Morell and Link (30) and Nakano and Rånby (6) revealed similar effects on the activation functions for other methyl uronosides. It was suggested that methyl uronosides in particular and, perhaps, uronosides in general may not hydrolyze via the same mechanism as other glycosides.

Dyer, et al. (32) have studied the acid hydrolysis of tetrahydro-2-methoxy-pyran derivatives in 0.001N hydrochloric acid at 30°C. Replacing the hydroxymethyl group at C_6* with the carboxyl group caused the hydrolysis rate to decrease by a factor of 2.8. It was suggested that the more electrophilic carboxyl group caused stabilization through an inductive effect.

*By convention, the positions of substituents in compounds named as derivatives of pyran are denoted by designating the ring oxygen position 1 and the five carbon atoms consecutively 2,3,4,5, and 6. When designating the positions of substituents in the pyranose forms of sugars, the anomeric carbon is designated as position 1 and the four other carbon atoms numbered consecutively 2,3,4, and 5. The pyranose oxygen is not given a numerical designation. Thus, positions 1 and 5 of a pyranose derivative correspond to positions 2 and 6 of a pyran derivative.

STATEMENT OF PROBLEM

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 (6, 7, 30) at a given acid concentration. In some cases, these rates were determined only at a single temperature and acid concentration (3, 32). While the carboxyl stabilizing effect seems well established, the nature of the effect is not well understood. Two explanations have been offered: (1) the inductive effect hypothesis proposes that hydrolysis takes place via the A-1(A) mechanism and that stabilization is due to the inductive effect of the carboxyl; and (2) the mechanistic change hypothesis suggests that replacement of the C_5 hydroxymethyl group with a carboxyl group causes a change in reaction mechanism.

A better understanding of the carboxyl stabilizing effect would be gained through an investigation of the effects of temperature, acid concentration, and the electron affinity of the aglycon on the rates of acid hydrolysis of additional uronosides and their corresponding C_5 hydroxymethyl glycosides. 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. The glycosyl groups are similar to those previously studied in connection with the carboxyl stabilizing effect, and the size of the aglycon groups is of the same order as those of the reduced and unreduced aldobiouronic acid investigated by Whistler and Richards (3). The phenyl substituents are held rigidly at such large distances from the glycosyl group that no steric interactions would occur between the reaction center and the substituent. The effects of these substituents on acid hydrolysis rates would thus be primarily polar in nature. In addition, these compounds are known in pure crystalline form.

EXPERIMENTAL RESULTS

PREPARATION OF PHENYL GLYCOSIDES

PHENYL β-D-GLUCURONIDES

Phenyl, p-chlorophenyl, and p-cresyl β-D-glucuronides were prepared according to the method of Bollenback, et al. (33). First, 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. The acetylated methyl esters of the phenyl β-D-glucuronides were then prepared by fusing methyl tetra-O-acetyl-β-D-glucopyranuronate with the appropriate phenol. The fusion was carried out under vacuum at 110°C. with p-toluenesulfonic acid as a catalyst. The recrystallized acetylated methyl esters were deacetylated with methanolic sodium methoxide, and then the methyl esters were deesterified with dilute aqueous sodium hydroxide. Finally, the sodium (phenyl β-D-glucopyranosid)uronates were converted to their free acids with Amberlite 120-H ion-exchange resin. The phenyl β-D-glucuronides were characterized and the results are listed in Table I. Previously, p-cresyl β-D-glucuronide and p-chlorophenyl β-D-glucuronide had been synthesized only by biological means.

PHENYL β-D-GLUCOSIDES

A sample of phenyl β-D-glucoside was available and this was recrystallized from water. The purified sample was characterized and the results are listed in Table II.

The β-D-glucosides of p-cresol and p-chlorophenol were synthesized by the fusion method of Helferich and Schmitz-Hillebrecht (34). This was done by fusing β-D-glucopyranose pentaacetate with the appropriate phenol under vacuum at 110°C.

TABLE I

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

	β-D-Glucuronide		
	Phenyl	p-Cresyl	p-Chlorophenyl
Melting point, °C.			
Found	162.5-63.5 (corrected)	147.5-48.5 (corrected)	154-55 (corrected)
Literature	161-62 (35) 163-64 (33)	147 (dec.)(36)	151 (37)
Equivalent weight[a]			
Found	272	284	307
Theoretical	270	284	305
Specific rotation, $[\alpha]_D$			
Found	-90.7° (c 1.02, water, 27°C.)	-87.9° (c 1.38, water, 28°C.)	-87.2° (c 1.32, water, 27°C.)
Literature	-90.0° (c 1, water, 25°C.)(33) -87.5° (c 2.10, water, 29°C.)(38)	-76.4° (c 0.4, water, 22°C.)(36)	-87° (c 0.5, water, 19°C.)(37)

[a]Titrated with 0.1N sodium hydroxide.

TABLE II

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

	β-D-Glucoside		
	Phenyl	p-Cresyl	p-Chlorophenyl
Melting point, °C.			
Found	174-76.5 (corrected)	178-79 (corrected)	174.5-76.5 (corrected)
Literature	171-72 (39) 175-76 (40) 174-75 (41)	175-77 (42) 178-79.5 (corrected) (43)	173-75 (39) 173-74 (44)
Specific rotation, $[\alpha]_D$			
Found	-72.0° (c 1.52, water, 29°C.)	-68.1° (c 1.36, water, 25°C.)	-71.6° (c 1.49, water, 28°C.)
Literature	-71.9° (c 2.0, water, 20°C.)(40)	-67.7° (c 2, water, 20°C.)(43)	-82.0° (c 1.13, water, 19°C.)(39)
	-71.0° (water, 20°C.)(41)		-69.5° (c 1.0, water, 20°C.)(44)

with p-toluenesulfonic acid as a catalyst. After recrystallization of their tetraacetates, the phenyl β-D-glucosides were obtained by deacetylation with methanolic sodium methoxide. These compounds were recrystallized and characterized. The results of the characterizations are listed in Table II.

ACID HYDROLYSES

DETERMINATION OF RATE CONSTANTS

Solutions of the β-D-glucuronides and β-D-glucosides of phenol, p-chlorophenol, and p-cresol were prepared which were 0.0200-0.0400M in glycoside and 2.00-20.0 wt. % in sulfuric acid. Four to six ampoules containing a known volume of each solution were plunged into an ethylene glycol bath at 50-60 ± 0.05°C. After predetermined

times, each ampoule was quenched in an ice-water bath and the contents neutralized with standard sodium hydroxide solution. A maximum of 2-3% hydrolysis was allowed.

It was found that essentially all of the reducing power of the hydrolyzates, as determined by the ferricyanide ion (Appendix II), was associated with the hydrolysis products. Hence, the reducing power of the hydrolyzates was calibrated to measure the concentrations of products from which the hydrolysis rates could be determined. Each hydrolyzate was analyzed by reacting it with an excess of mildly alkaline potassium ferricyanide and determining the unreduced ferricyanide colorimetrically. This measurement was made automatically with Technicon's Auto-Analyzer*. The concentrations of products were of the order of 5×10^{-5} M and analyses in this range could be reproduced within $\pm$ 0.75%.

From a knowledge of the concentration of the products and the initial glycoside concentration, the fraction of the glycoside unreacted can be calculated (Appendix III). Figure 2 shows a plot of the natural logarithm of the fraction of glycoside unreacted vs. time for phenyl β-D-glucuronide at 59.90 $\pm$ 0.05°C. and 20.0 wt. % sulfuric acid. The straight-line fit indicates that the hydrolysis was first-order with respect to the glycoside concentration (46). The slope of this line is the rate constant. The small positive intercept in Fig. 2 was due to a small amount of hydrolysis during the period before the ampoules were placed in the ethylene glycol bath, the transient temperature period when the ampoule contents were rising to the bath temperature, and the quenching of the reaction in the ice-water bath. These effects do not affect the slope, however, since the temperature paths before and after entering the bath were identical for each ampoule (Appendix III). Rate constants could be duplicated within $\pm$ 2.0%.

*The AutoAnalyzer is manufactured by the Technicon Instruments Corporation of Chauncey, New York. The reducing power determination was a modification of Technicon's micro-glucose procedure, method 8. The main modification was to eliminate the dialysis.

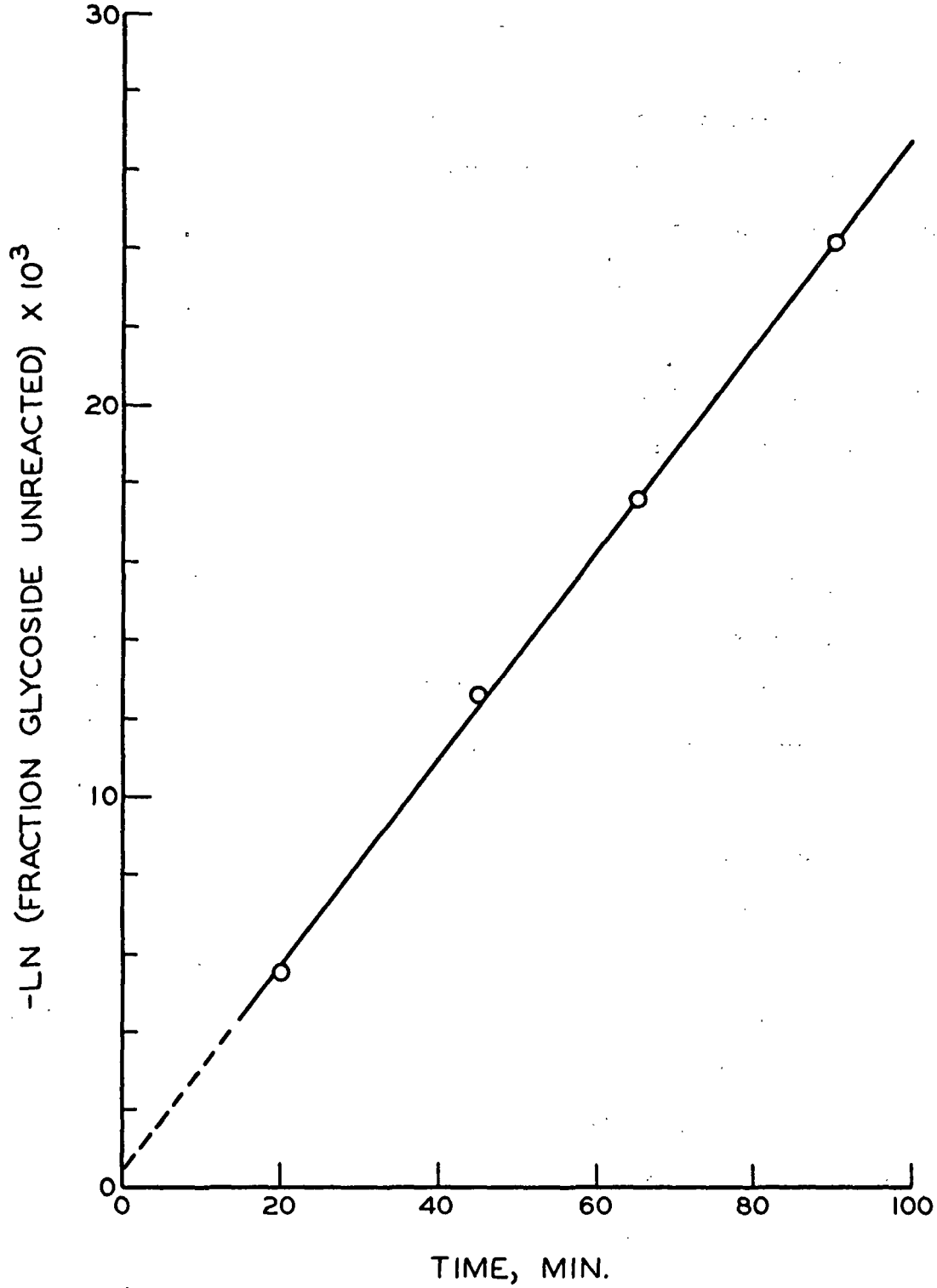


Figure 2. Hydrolysis of Phenyl β-D-Glucuronide at 59.90±0.05°C. in 20.0 wt. % Sulfuric Acid

Listed in Table III-VIII are the first-order rate constants, $\underline{k}'$ and their estimated standard deviations for the three β-D-glucuronides and three β-D-glucosides investigated. In all cases, the rate constants were determined by least-squares straight-line fits.

TABLE III

RATE CONSTANTS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDE

Temperature, ± 0.05°C.	Sulfuric Acid, wt. %	$\underline{k}'$, min.$^{-1}$ x 10^6
50.45	4.00	6.94 ± 0.05
	8.00	14.1 ± 0.2
	14.0	32.3 ± 0.3
	20.0	66.4 ± 1.5
55.15	4.00	14.6 ± 0.6
	8.00	28.0 ± 0.1
	14.0	63.6 ± 1.1
	20.0	137.0 ± 6.0
59.90	4.90	34.8 ± 0.6
	6.96	50.6 ± 1.1
	9.89	78.6 ± 0.9
	14.0	130.0 ± 1.0
	20.0	267.0 ± 4.0

TABLE IV

RATE CONSTANTS FOR THE ACID
HYDROLYSIS OF p-CRESYL β-D-GLUCURONIDE

Temperature, ± 0.05°C.	Sulfuric Acid, wt. %	k' $min.^{-1}$ x 10^6
50.45	4.00	6.40 ± 0.27
	8.00	13.7 ± 0.3
	14.0	30.8 ± 1.1
	20.0	61.2 ± 2.6
55.15	4.00	13.4 ± 0.1
	8.00	26.6 ± 0.2
	14.0	62.1 ± 1.0
	20.0	127.0 ± 2.0
59.90	4.00	27.2 ± 0.5
	8.00	55.3 ± 0.4
	14.0	127.0 ± 2.0
	20.0	251.0 ± 3.0

TABLE V

RATE CONSTANTS FOR THE ACID HYDROLYSIS OF *p*-CHLOROPHENYL β-D-GLUCURONIDE

Temperature, ± 0.05°C.	Sulfuric Acid, wt. %	k', $min.^{-1} \times 10^6$
50.45	4.00	6.26 ± 0.05
	8.00	12.7 ± 0.1
	14.0	28.2 ± 0.4
	20.3	59.2 ± 0.1
55.15	4.00	12.7 ± 0.2
	8.00	25.0 ± 0.8
	14.0	53.7 ± 1.0
	20.0	113.0 ± 2.0
59.90	4.00	26.4 ± 0.3
	8.00	51.4 ± 0.5
	14.0	117.0 ± 1.0
	20.0	238.0 ± 1.0

TABLE VI

RATE CONSTANTS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDE

Temperature, ± 0.05°C.	Sulfuric Acid, wt. %	k', $min.^{-1} \times 10^5$
50.10	2.00	6.91 ± 0.13
	4.00	14.8 ± 0.0
	6.00	24.1 ± 0.3
	6.60	28.0 ± 0.4
	9.14	44.6 ± 0.2
	10.0	52.5 ± 0.9
	12.0	69.7 ± 0.6
	14.0	93.7 ± 1.9
55.00	2.00	13.7 ± 0.1
	4.00	29.1 ± 0.4
	6.49	54.5 ± 0.3
	8.98	86.8 ± 0.6
	11.9	132.0 ± 1.0
	14.0	183.0 ± 2.0
59.95	2.00	27.3 ± 0.2
	4.00	59.1 ± 1.1
	6.38	106.0 ± 2.0
	8.83	167.0 ± 2.0
	11.8	254.0 ± 4.0
	14.0	369.0 ± 11.0

TABLE VII

RATE CONSTANTS FOR THE ACID
HYDROLYSIS OF p-CRESYL β-D-GLUCOSIDE

Temperature, ± 0.05°C.	Sulfuric Acid, wt. %	k', $min.^{-1} \times 10^5$
50.10	2.00	7.45 ± 0.08
	4.00	16.3 ± 0.2
	6.00	27.2 ± 0.7
	10.0	58.5 ± 1.4
	14.0	103.0 ± 1.0
55.00	2.00	15.0 ± 0.3
	4.00	34.8 ± 0.6
	6.00	58.3 ± 0.2
	10.0	116.0 ± 1.0
	14.0	206.0 ± 6.0
59.95	2.00	31.2 ± 0.5
	4.00	65.2 ± 1.7
	6.00	111.0 ± 2.0
	10.0	236.0 ± 7.0
	14.0	400.0 ± 13.0

TABLE VIII

RATE CONSTANTS FOR THE ACID
HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCOSIDE

Temperature, ± 0.05°C.	Sulfuric Acid, wt. %	k', $min.^{-1} \times 10^5$
50.10	4.00	11.0 ± 0.2
	6.00	17.5 ± 0.4
	10.0	38.5 ± 1.7
	14.0	69.7 ± 0.7
55.00	2.00	9.69 ± 0.25
	4.00	22.1 ± 0.5
	6.00	34.3 ± 0.8
	10.0	73.2 ± 0.9
	14.0	132.0 ± 2.0
59.95	2.00	19.3 ± 0.5
	4.00	43.4 ± 0.6
	6.00	69.4 ± 0.8
	10.0	155.0 ± 6.0
	14.0	269.0 ± 6.0

EFFECT OF ACID CONCENTRATION

For purposes of analysis, it is often convenient to express the rate constants at a given temperature as a continuous function of catalyst concentration. It was found that the isothermal rate-constant data could be adequately represented by a least-squares fit to the following equation:

$$k' = B\,[H_3O^+] + D\,[H_3O^+]^3 + F\,[H_3O^+]^5 \qquad (1)$$

where k' = first-order rate constant

$[H_3O^+]$ = hydronium ion concentration

$\underline{B},\underline{D},\underline{F}$ = constants

The hydronium ion concentrations were calculated from the degree of ionization of aqueous sulfuric acid (45). The hydronium ion concentrations were taken as 1.20, 1.18, and 1.17 times the molarity of sulfuric acid at the 50, 55, and 60°C. levels, respectively.

Figures 3 and 4 show plots of the rate constants of phenyl β-D-glucuronide and phenyl β-D-glucoside vs. the hydronium ion concentration. The curves in Fig. 3 and 4 were calculated from Equation (1). The dotted lines are straight lines through the origin and first data point. It will be noted that the rate constants are nearly proportional to the hydronium ion concentration at low concentrations but increase faster above approximately 0.75M.

All of the glycosides gave similar rate-constant isotherms. The constants for each of the isotherm equations are listed in Tables IX and X.

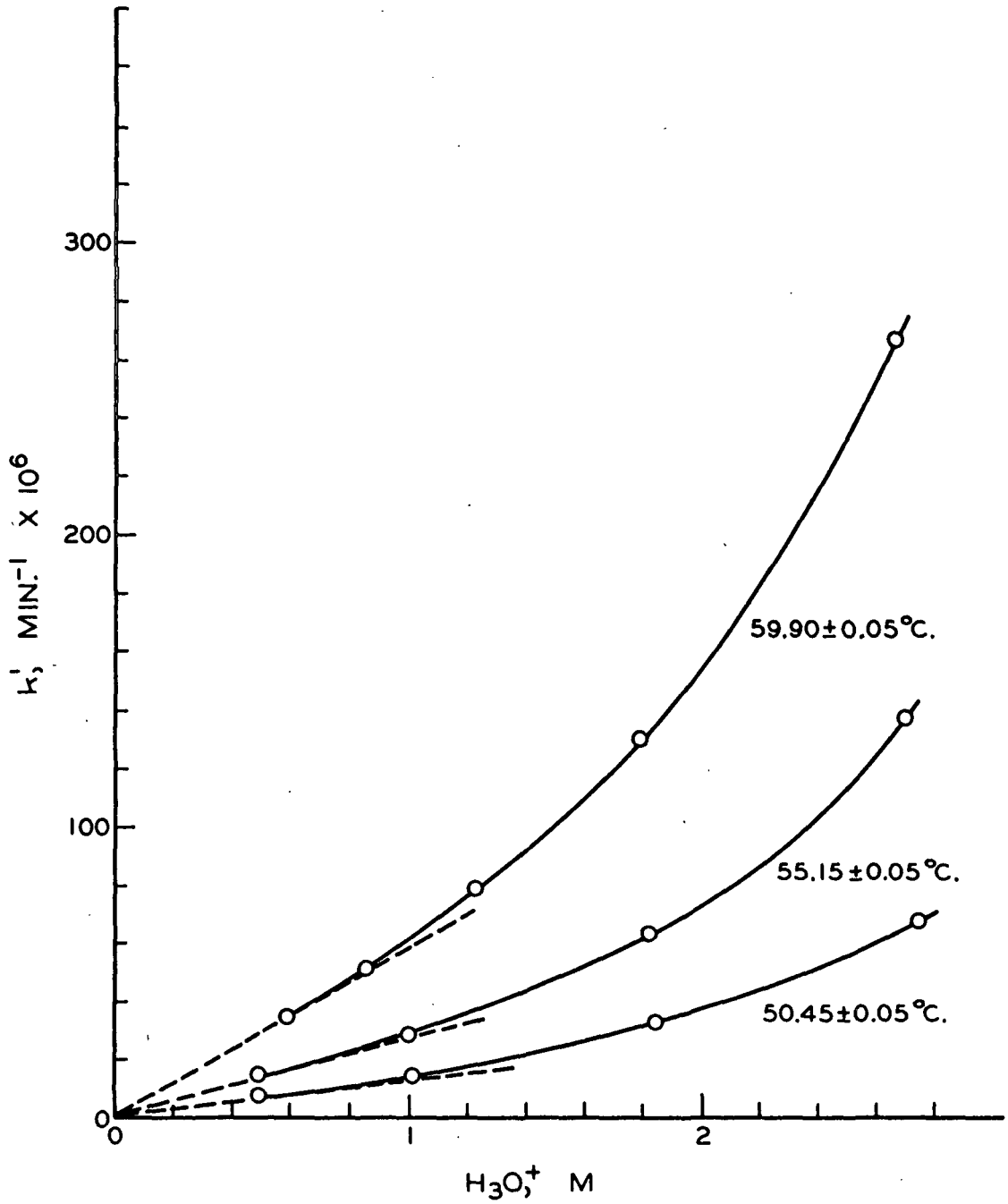


Figure 3. Effect of Hydronium Ion Concentration on the Hydrolysis Rate of Phenyl β-D-Glucuronide

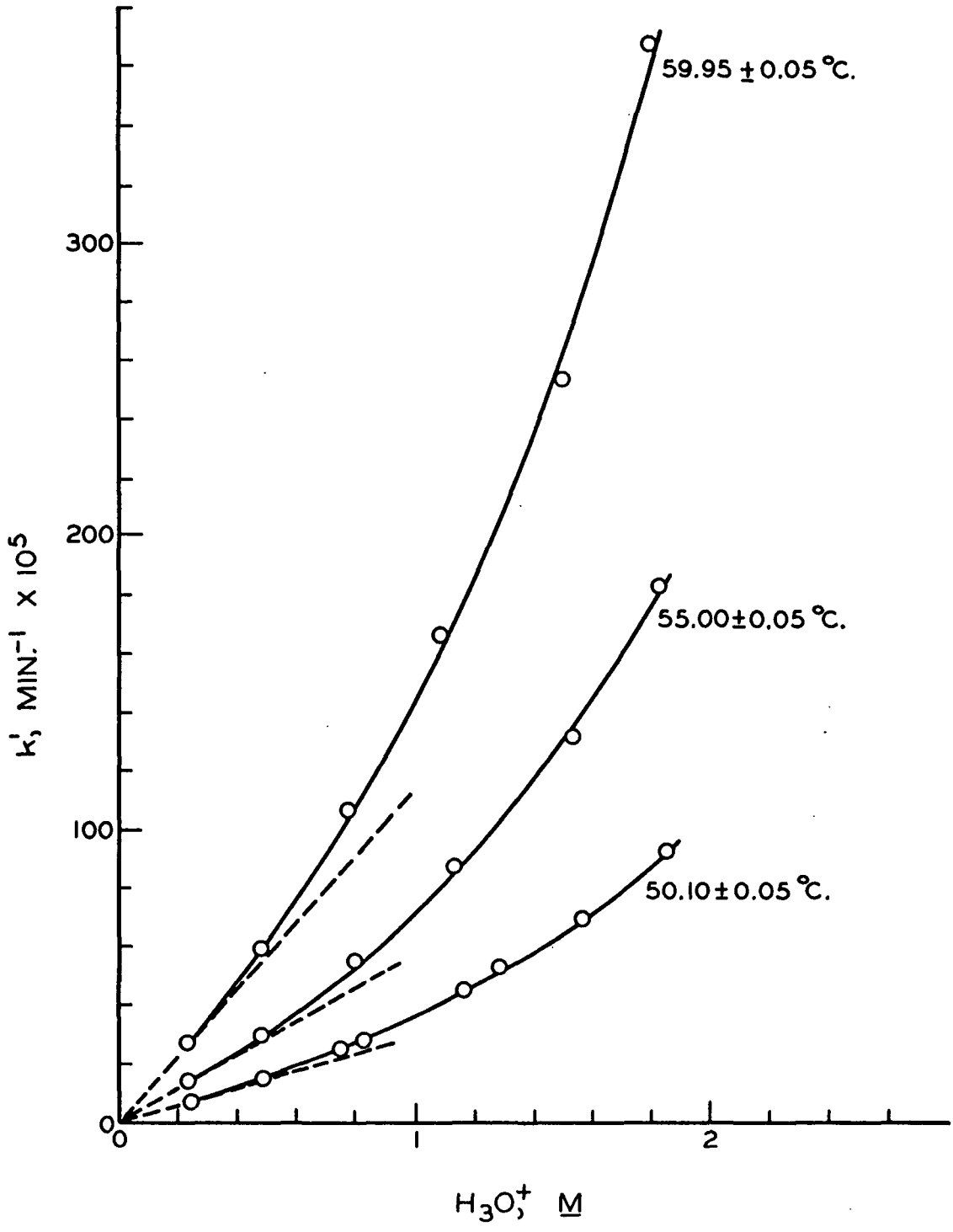


Figure 4. Effect of Hydronium Ion Concentration on the Hydrolysis Rate of Phenyl β-D-Glucoside

TABLE IX

RATE-CONSTANT ISOTHERMS OF p-CRESYL, PHENYL, AND p-CHLOROPHENYL β-D-GLUCURONIDES

Temperature, ± 0.05°C.	Substituent of Aglycon Group	Isotherm Constants[a]		
		B	D	F
50.45	p-CH_3	12.49	1.136	0.02422
	none	13.34	0.9341	0.06984
	p-Cl	12.14	0.6564	0.06738
55.15	p-CH_3	25.82	1.924	0.1393
	none	28.36	0.7967	0.3168
	p-Cl	25.21	0.3303	0.2712
59.90	p-CH_3	53.50	4.617	0.1653
	none	56.97	3.805	0.3275
	p-Cl	51.78	2.663	0.3792

[a] $k' = B\,[H_3O^+] + D\,[H_3O^+]^3 + F\,[H_3O^+]^5$

where k' = rate constant, $min.^{-1} \times 10^6$

$[H_3O^+]$ = hydronium ion concentration, moles per liter.

TABLE X

RATE-CONSTANT ISOTHERMS OF p-CRESYL, PHENYL, AND p-CHLOROPHENYL β-D-GLUCOSIDES

Temperature, ± 0.05°C.	Substituent of Aglycon Group	Isotherm Constants[a]		
		B	D	F
50.10	p-CH_3	30.14	11.16	-1.058
	none	27.94	8.237	-0.4670
	p-Cl	20.31	6.333	-0.3466
55.00	p-CH_3	65.54	20.28	-1.783
	none	57.13	15.13	-0.6746
	p-Cl	41.01	11.37	-0.5358
59.95	p-CH_3	126.6	49.49	-6.040
	none	118.2	25.02	0.4544
	p-Cl	80.30	33.47	-3.643

[a] $k' = B\,[H_3O^+] + D\,[H_3O^+]^3 + F\,[H_3O^+]^5$

where k' = rate constant, $min.^{-1} \times 10^5$

$[H_3O^+]$ = hydronium ion concentration, moles per liter.

EFFECT OF TEMPERATURE

The variation of reaction rates with temperature is most conveniently expressed by means of the Arrhenius equation (46).

$$\log k' = -\frac{E}{2.3\,RT} + \text{constant} \qquad (2)$$

where E = activation energy

R = gas constant

T = absolute temperature

The effect of temperature is characterized by the activation energy which may be evaluated from the slope of the straight line obtained by plotting log $\underline{k}'$ versus $1/\underline{T}$.

Figures 5 and 6 show plots of log $\underline{k}'$ versus $1/\underline{T}$ for phenyl β-D-glucuronide and phenyl β-D-glucoside, respectively. The linear relationships indicate that the Arrhenius equation is applicable over the temperature range studied. Similar results were obtained for the other glycosides studied. Since at a given level of weight per cent sulfuric acid the hydronium ion concentration is a function of temperature, the activation energies at constant hydronium concentration could not be evaluated directly from the rate-constant data. Hence, the first-order rate constants, such as those plotted in Fig. 5 and 6, were calculated from the appropriate rate-constant isotherms.

Listed in Tables XI and XII are the activation energies and their estimated standard deviations obtained by least-squares fit of log $\underline{k}'$ versus $1/\underline{T}$. There appears to be no significant difference in activation energies between various levels of hydronium ion concentration nor do the various phenyl substituents produce a measurable change. The activation energies of the phenyl β-D-glucuronides are 2.0-2.3 kcal. per mole greater than those of corresponding phenyl β-D-glucosides. The activation energy of phenyl β-D-glucoside was 30.6 ± 0.2 kcal. per mole as compared to literature values of 31.0 ± 1.2 (9) and 32.30 ± 0.43 (47) kcal. per mole.

EFFECTS OF PHENYL SUBSTITUENTS

Within reaction series of meta and para-substituted side-chain derivatives of benzene, the substituents are held rigidly at such large distances from the reaction center that no steric interaction occurs between the substituents and the reaction

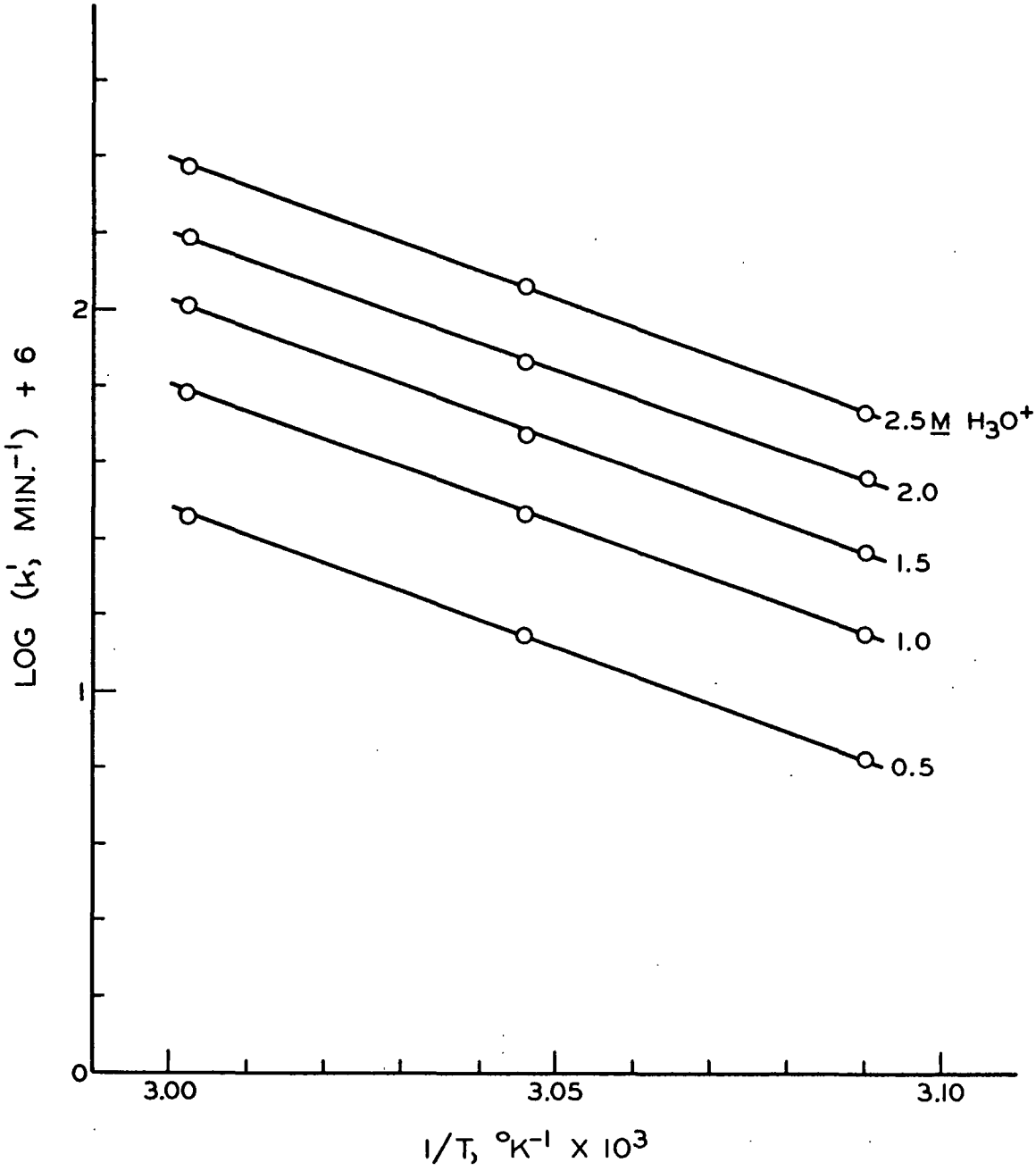


Figure 5. Effect of Temperature on the Hydrolysis Rate of Phenyl β-D-Glucuronide

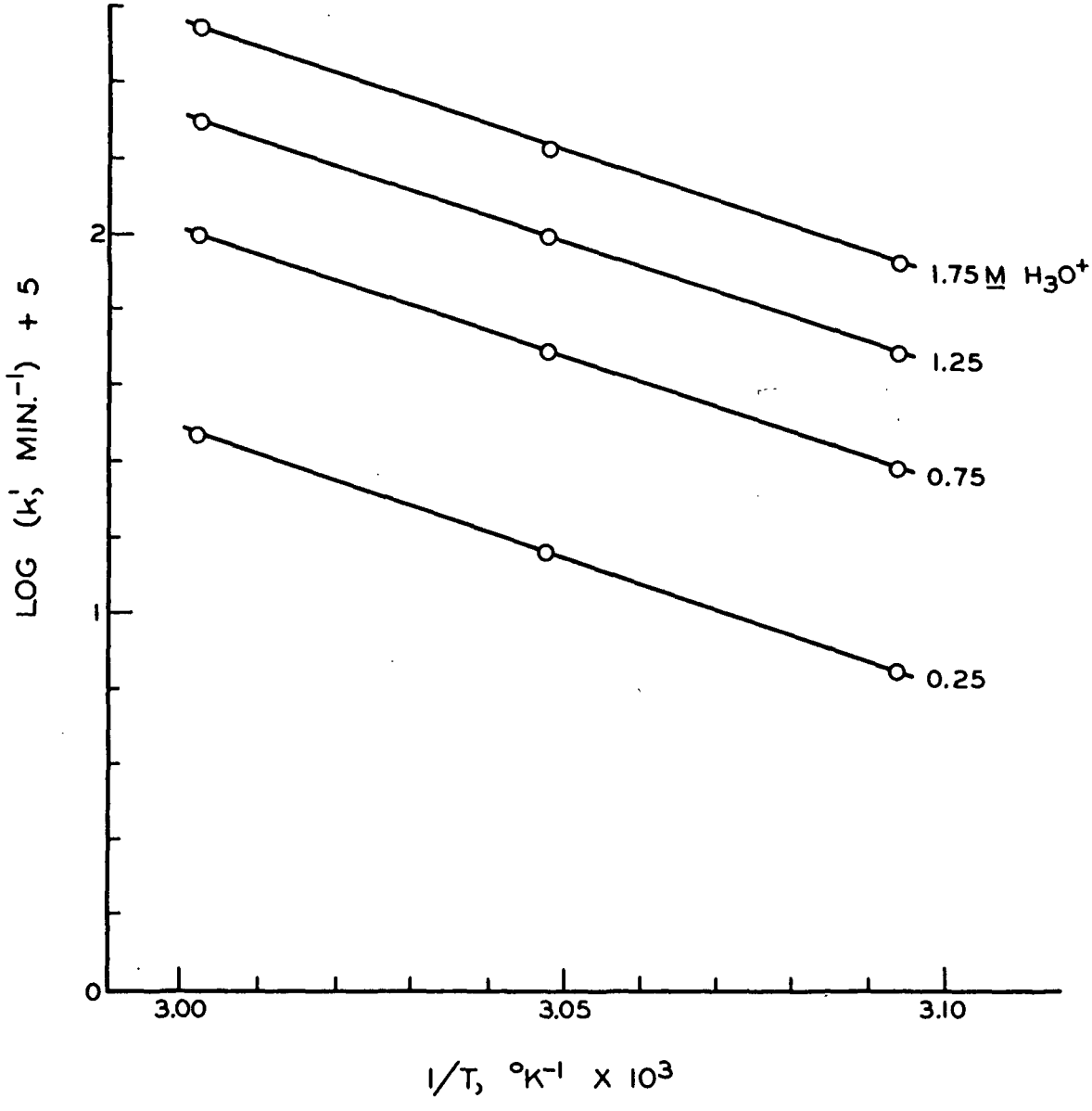


Figure 6. Effect of Temperature on the Hydrolysis Rate of Phenyl β-D-Glucoside

TABLE XI

ACTIVATION ENERGIES FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDES

Hydronium Ion, M	E, kcal. per mole		
	p-Cresyl	Phenyl	p-Chlorophenyl
0.5	33.0 ± 0.3	32.9 ± 0.3	32.9 ± 0.3
1.0	32.9 ± 0.5	32.9 ± 0.3	32.9 ± 0.9
1.5	32.9 ± 0.6	32.8 ± 0.8	33.0 ± 1.6
2.0	33.0 ± 0.4	32.9 ± 0.8	33.2 ± 1.9
2.5	33.2 ± 0.2	32.9 ± 0.1	33.7 ± 1.4
Average	33.0 ± 0.2	32.9 ± 0.2	33.1 ± 0.6

TABLE XII

ACTIVATION ENERGIES FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDES

Hydronium Ion, M	E, kcal. per mole		
	p-Cresyl	Phenyl	p-Chlorophenyl
0.25	31.2 ± 1.2	31.2 ± 0.3	--
0.75	31.3 ± 0.6	30.6 ± 0.2	30.8 ± 0.7
1.25	31.2 ± 0.0	30.1 ± 0.2	31.2 ± 1.4
1.75	30.5 ± 0.1	30.5 ± 0.5	30.5 ± 1.1
Average	31.0 ± 0.3	30.6 ± 0.2	30.8 ± 0.6

center in either the initial state or the transition state. The effect of these substituents on reaction rate is nearly always determined by a single factor, the polar effect of the substituent. The result of this simplification is the most general relationship known for correlating structure with reactivity, the Hammett equation (48).

$$\log (k'/k'_o) = \rho \cdot \sigma \qquad (3)$$

where σ = substituent constant independent of the nature of the reaction

ρ = proportionality constant which is a function of the reaction series and the conditions

k', k'_o = rate constants of substituted and unsubstituted derivatives, respectively

The substituent constant, σ, is a measure of the relative tendency of a substituent to change the electron density within the side chain.

$$\sigma = \log \frac{K}{K_o} \qquad (4)$$

where K = ionization constant of substituted benzoic acid

K_o = ionization constant of benzoic acid

The constant, ρ, is a measure of the susceptibility of a given reaction series to polar substituent effects. If it is positive, the reaction is facilitated by lower side-chain electron densities, whereas a negative value denotes inhibition of the reaction by lower side-chain electron densities.

Shown in Fig. 7 and 8 are plots of the rate constants of phenyl β-D-glucuronides and phenyl β-D-glucosides versus σ. The straight lines are the least-squares fits. The good linear relationships indicate that the Hammett equation holds for the substituents studied. The small deviations from linearity were due partly to

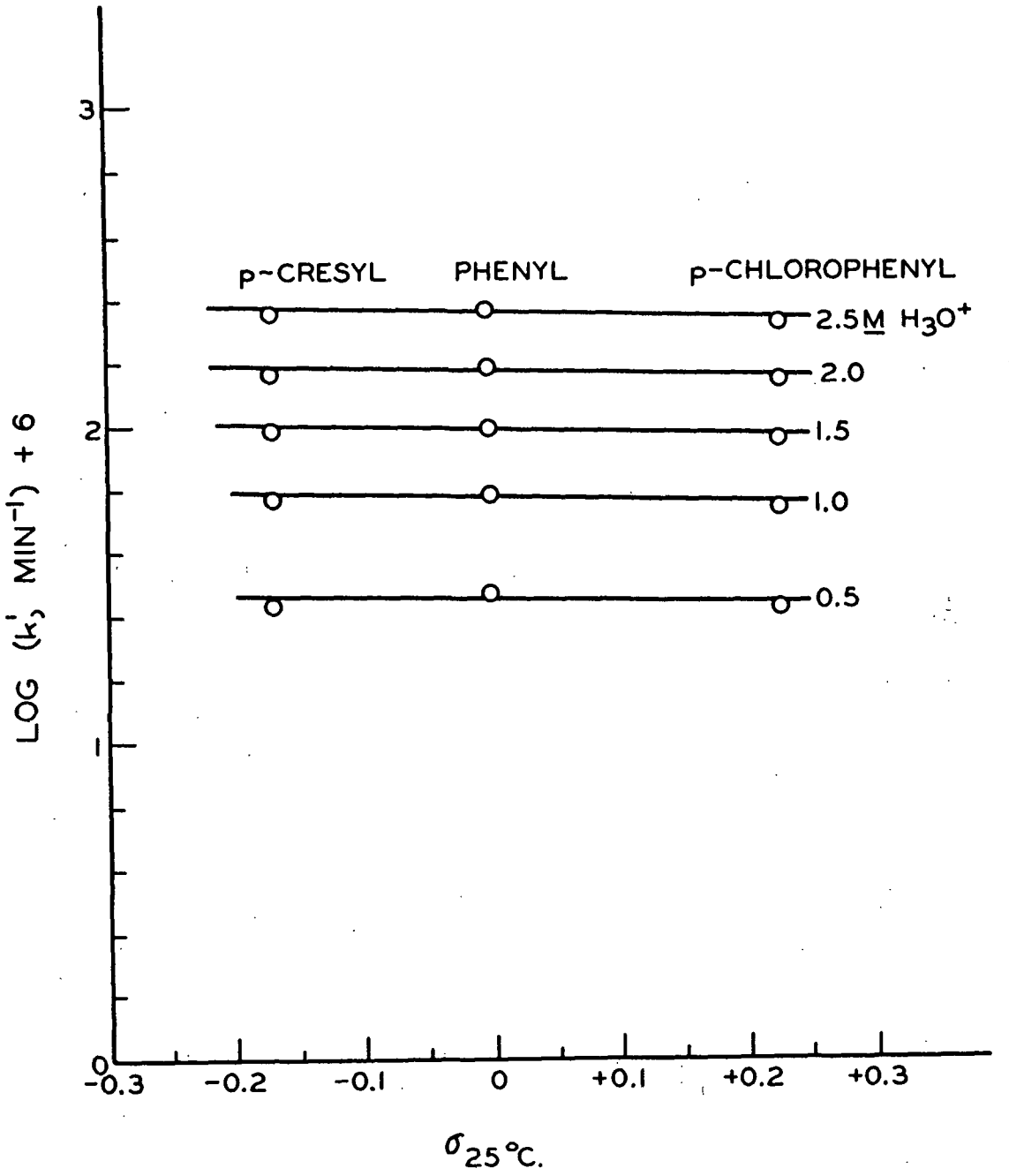


Figure 7. Effect of the Electron-Attracting Tendency of the Aglycon Group (as Measured by the Hammett Substituent Constant of the para-Substituent) on the Rates of Acid Hydrolysis of Phenyl β-D-Glucuronides at 59.90±0.05°C.

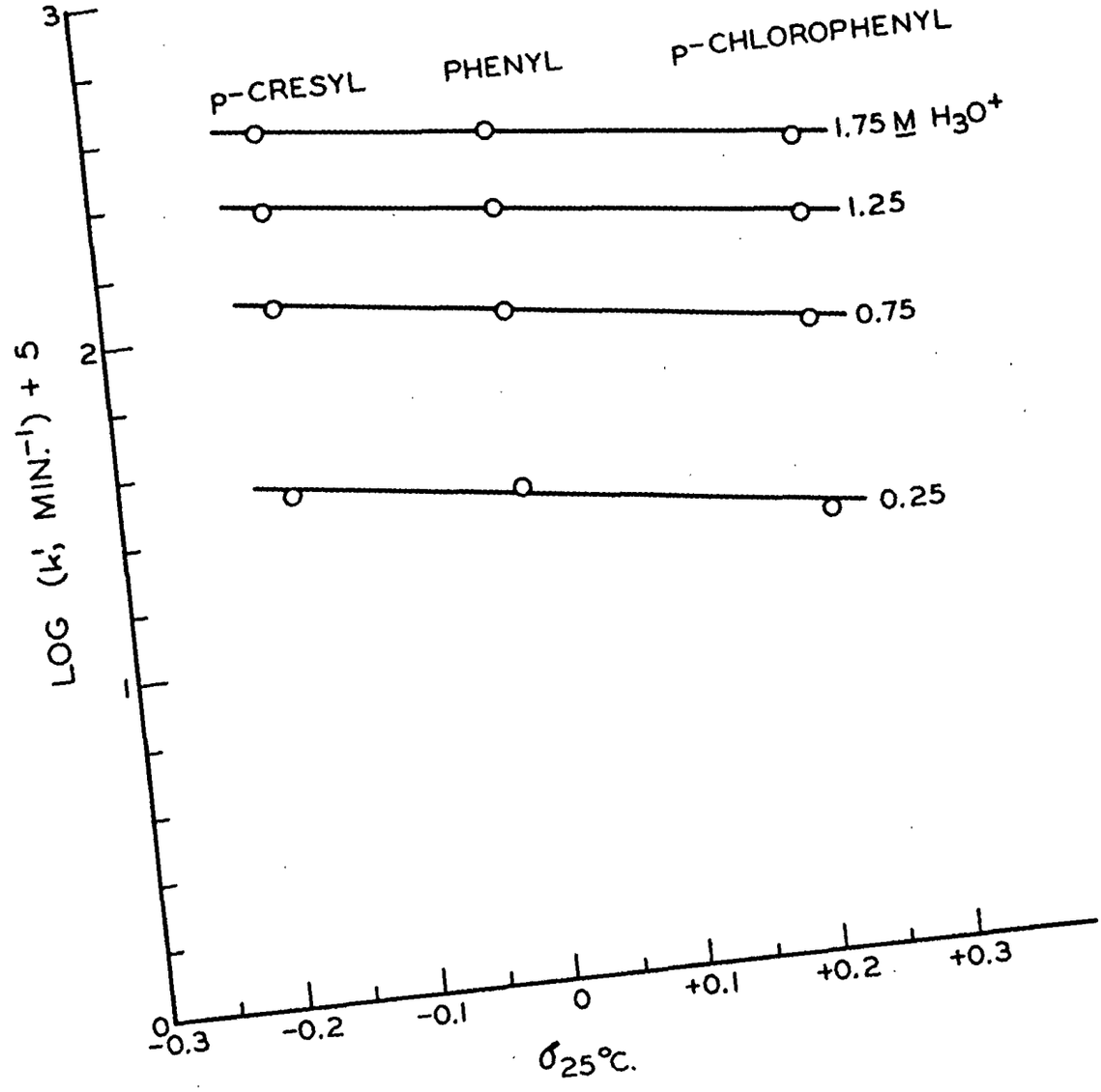


Figure 8. Effect of the Electron-Attracting Tendency of the Aglycon Group (as Measured by the Hammett Substituent Constant of the Para-Substituent) on the Rates of Acid Hydrolysis of Phenyl β-D-Glucosides at 59.95±0.05°C.

the fact that the Hammett equation is not an exact relationship. Rate constants were calculated from the rate-constant isotherms so that the rates could be compared at the same hydronium ion concentration. Since the activation energies in each series were found to be essentially independent of the substituent, the logarithms of the rate constants at one temperature differed from those at another temperature by a constant amount*. Hence, plots of the logarithms of the rate constants in the 50-60°C. range *vs.* those determined at 25°C. had the same slope, ρ, as if the logarithms at 25°C. were plotted. Similar log *k'* versus σ plots were obtained at all temperature levels.

The values of ρ were calculated by least-squares straight-line fits and these are listed in Tables XIII and XIV. The values of ρ were essentially independent of hydronium ion concentration and temperature. The latter is the result of the activation energies being independent of the phenyl substituent. The phenyl β-D-glucuronides showed little sensitivity to polar aglycon group effects while the hydrolysis rates of the phenyl β-D-glucosides were reduced by lowering the glycosyl group electron density. For a more extensive series of phenyl β-D-glucosides, Nath and Rydon (39) obtained a ρ of -0.66 as compared to about -0.5 found in this study.

*Combining the logarithmic forms of the Arrhenius equation at temperatures T_1 and T_2, yields,

$$\log k'_2 = \log k'_1 + \frac{E}{2.3R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

where k'_1, k'_2 = first-order rate constants at T_1 and T_2.
The second term on the right-hand side of this equation is the same for each member of either the phenyl β-D-glucoside or phenyl β-D-glucuronide series since E is the same. Hence, the logarithm of the first-order rate constant at T_1 will differ from the logarithm at T_2 by the same amount for each member of a series.

TABLE XIII

REACTION SERIES CONSTANTS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDES

Hydronium Ion, M	ρ 50.45±0.05°C.	55.10±0.05°C.	59.90±0.05°C.
1.0	-0.07 ± 0.09	-0.09 ± 0.11	-0.07 ± 0.09
1.5	-0.10 ± 0.10	-0.14 ± 0.11	-0.10 ± 0.09
2.0	-0.11 ± 0.10	-0.17 ± 0.11	-0.10 ± 0.09
2.5	-0.10 ± 0.13	-0.16 ± 0.14	-0.08 ± 0.10
Average	-0.10 ± 0.05	-0.14 ± 0.06	-0.09 ± 0.05

TABLE XIV

REACTION SERIES CONSTANTS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDES

Hydronium Ion, M	ρ 50.10±0.05°C.	55.00±0.05°C.	59.95±0.05°C.
0.25	--	-0.52 ± 0.08	-0.51 ± 0.14
0.75	-0.46 ± 0.09	-0.53 ± 0.06	-0.49 ± 0.06
1.25	-0.47 ± 0.07	-0.53 ± 0.06	-0.47 ± 0.05
1.75	-0.44 ± 0.07	-0.50 ± 0.07	-0.44 ± 0.08
Average	-0.46 ± 0.04	-0.52 ± 0.03	-0.48 ± 0.04

MAGNITUDE OF CARBOXYL STABILIZING EFFECTS

At a given concentration of acid and temperature, the carboxyl stabilizing effect may be defined quantitatively as follows:

$$S = \frac{k'_{CH_2OH}}{k'_{COOH}} \qquad (5)$$

where $\underline{S}$ = stabilizing effect of carboxyl group

$\underline{k}'_{CH_2OH}$ = rate constant of C_5 hydroxymethyl glycoside

$\underline{k}'_{COOH}$ = rate constant of C_5 carboxyl glycoside

When $\underline{S}$ is greater than one, the C_5 hydroxymethyl glycoside is more reactive than the corresponding C_5 carboxyl glycoside. Unity $\underline{S}$ indicates both compounds are equally reactive and there is no carboxyl stabilizing effect.

In Table XV are listed the carboxyl stabilizing effects for several glycosides at 95°C. in similar acid media. The carboxyl stabilizing effect of the phenyl glycosides is dependent on the phenyl substituent since the susceptibility of the phenyl β-D-glucosides to polar aglycon effects is greater than the phenyl β-D-glucuronides. This stabilizing effect is 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. As noted by Easty (7), a large carboxyl stabilizing effect should not be regarded as completely general.

TABLE XV

CARBOXYL STABILIZING EFFECT FOR VARIOUS GLYCOSIDES AT 95°C.

Glycoside Pair	Acid Catalyst	Carboxyl Stabilizing Effect	Ref.
Methyl α-D-glucopyranoside/methyl α-D-glucopyranosiduronic acid	N sulfuric	2.3[a]	(7)
Methyl β-D-glucopyranoside/potassium (methyl β-D-glucopyranosid)uronate	0.94N sulfuric	2.6[a]	(6)
Methyl α-D-galactopyranoside/methyl α-D-galactopyranosiduronic acid	N hydrochloric	2.3[a]	(30)
2-O-(4-O-methyl-α-D-glucopyranosyl)-D-xylitol/2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose	1.07N sulfuric	18	(3)
4-O-(α-D-glucopyranosyl)-D-glucose/2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose	1.07N sulfuric	19	(3)
p-Cresyl β-D-glucoside/p-cresyl β-D-glucuronide	0.855N sulfuric	19[a]	this work
Phenyl β-D-glucoside/phenyl β-D-glucuronide	0.855N sulfuric	16[a]	this work
p-Chlorophenyl β-D-glucoside/p-chlorophenyl β-D-glucuronide	0.855N sulfuric	13[a]	this work

[a]Estimated from data at other temperatures by means of the Arrhenius equation.

DISCUSSION OF RESULTS AND CONCLUSIONS

In order to understand how the C_5 carboxyl group of the phenyl β-D-glucuronides stabilizes the glycosidic linkage to acid hydrolysis, one must ascertain whether the reaction mechanism is the same as that of the phenyl β-D-glucosides. If the reaction mechanisms are the same, one may then proceed to consider ways in which the carboxyl group causes an increase in stability. If the reaction mechanisms are different, the stabilizing effect is due to a change of mechanism and one may consider reasons for this change.

MECHANISMS OF ACID HYDROLYSIS

The acid hydrolysis of glycosides may involve either glycosyl-oxygen or aglycon-oxygen fission. There is now a considerable amount of data indicating that when hydrolyses are carried out in O^{18} enriched water the liberated aglycon has the normal oxygen isotope composition (10, 11). This result would be expected for glycosyl-oxygen fission only. Bunton, et al. (10) have established that phenyl β-D-glucoside fissions at the glycosyl-oxygen bond. Therefore, the hydrolysis of p-chlorophenyl and p-cresyl β-D-glucoside as well as the phenyl β-D-glucuronides would be expected to follow the glycosyl-oxygen mode of bond fission.

Several studies (9, 11, 12) have shown that the rates of acid hydrolysis of glycosides in deuterium oxide are about twice as great as when water is the solvent. This effect is interpreted to mean that the rate of formation of the conjugate acid is very rapid and the less basic deuterium oxide shifts the equilibrium toward the conjugate acid (13). Although the proton-transfer rates for the acid hydrolysis of phenyl glycosides have not yet been studied, the rapid rates for similar glycosides and the generally high protonation rates of oxygen, nitrogen, and sulfur bases (17) suggest that the phenyl β-D-glucuronides and phenyl β-D-glucosides will be in equilibrium with their conjugate acids.

Assuming glycosyl-oxygen fission and rapid protonation, the mechanistic implications of the experimental results of the acid hydrolysis of phenyl β-D-glucuronides and phenyl β-D-glucosides will be considered in the following paragraphs.

MOLECULARITY OF HETEROLYSIS

Effect of Acid Concentration

From the viewpoint of the theory of absolute reaction rates (15), A-1 mechanisms may be represented as follows:

$$GA + H_3O^+ \underset{}{\overset{K_1}{\rightleftharpoons}} GAH^+ + H_2O$$

$$GAH^+ \overset{K_2^*}{\rightleftharpoons} M^* \xrightarrow{k} \text{products (G, AH)}$$

where GA = glycoside

H_3O^+ = hydronium ion

GAH^+ = conjugate acid

H_2O = water

M* = transition state

k = rate constant

K_1, K_2^* = equilibrium constants

G = sugar or substituted sugar

A, AH = aglycon group and aglycon, respectively

The appropriate rate expression would be,

$$-\frac{d[M^*]}{dt} = \frac{d[G]}{dt} = k\,[M^*] \qquad (6)$$

where t = time

$[M^*]$, $[G]$ = concentrations of transition state and sugar, respectively.

The equilibrium between the initial states and the transition state may be written as follows:

$$K_3^* = K_1 \cdot K_2^* = \frac{[M^*]}{[GA]} \cdot \frac{\gamma_{M^*}}{\gamma_{GA}} \cdot \frac{a_{H_2O}}{a_{H_3O^+}} \qquad (7)$$

where K_3^* = over-all equilibrium constant

γ_{M^*}, γ_{GA} = activity coefficients of transition state and glycoside, respectively

a_{H_2O}, $a_{H_3O^+}$ = activity of water and hydronium ion, respectively.

Combining Equation (6) and Equation (7) yields,

$$\frac{d[G]}{dt} = k K_3^* \cdot \frac{\gamma_{GA}}{\gamma_{M^*}} \cdot \frac{a_{H_3O}}{a_{H_2O}} \cdot [GA] \qquad (8)$$

For low glycoside concentrations and small amounts of hydrolysis, only [GA] will vary with time. Assuming only a small fraction of the total glycoside present exists as the conjugate acid, Equation (8) is the differential form of the first-order rate equation (46). This leads to the following expression for the first-order rate constant, k'.

$$k' = k K_3^* \cdot \frac{\gamma_{GA}\, \gamma_{H_3O^+}}{\gamma_{M^*}} \cdot \frac{1}{a_{H_2O}} \cdot [H_3O^+] \qquad (9)$$

where $[H_3O^+]$ = hydronium ion concentration

$\gamma_{H_3O^+}$ = activity coefficient of hydronium ion.

Since the A-1 transition state differs from the conjugate acid by an extension of a carbon-oxygen bond and small conformational changes, it seems likely that the activity coefficient of the transition state will be approximately equal to that of the conjugate acid. Examination of Equation (9) reveals that the general shape of the first-order rate-constant isotherms of A-1 mechanisms will be independent of the glycoside if the ratio of the activity coefficient of the glycoside to its conjugate acid is the same for each glycoside considered. This is reasonable since Hammett and Deyrup (16) and Bascombe and Bell (49) have shown that when uncharged bases in media of high dielectric constant undergo a single protonation, the ratio of the activity coefficients of the base to its conjugate acid is largely independent of the base involved. This generality is expressed quantitatively by the Hammett acidity function.

The Hammett acidity function may be expressed as follows:

$$10^{-H_o} = \frac{\gamma_B}{\gamma_{BH^+}} \cdot \frac{\gamma_{H_3O^+}}{a_{H_2O}} \cdot [H_3O^+] \qquad (10)$$

where H_o = Hammett acidity function

γ_B = uncharged base activity coefficient

γ_{BH^+} = conjugate acid activity coefficient

Combining Equation (9) and Equation (10), and taking the logarithm,

$$\log k' = -H_o + \log \frac{\gamma_{GA}}{\gamma_B} \cdot \frac{\gamma_{BH^+}}{\gamma_{M^*}} + \log k K_3^* \qquad (11)$$

The activity coefficient term in Equation (11) should be close to unity and its logarithm zero. Hence, a plot of the logarithm of k' versus the negative of H_o should have unit slope.

Figure 9 shows plots of log k' versus $-H_o$ at 25°C. for phenyl β-D-glucoside and the least-squares straight-line fits. The values of H_o were those given by Paul and Long (50) which are based on the generally accepted ionization constants of the indicators used to establish the Hammett acidity scale. Similar linear relationships were found for the other phenyl β-D-glucosides. Since the activation energies of all of the phenyl glycosides were found to be independent of the hydronium ion concentration, unit slope should still be found when log k' in the 50-60°C. range is plotted against the Hammett acidity function at the same hydronium ion concentration at 25°C. (Results, p. 39). The slopes of the least-squares straight-line fits and their estimated standard deviations were calculated and these are listed in Table XVI. Within experimental error, all of the slopes could be considered to be unity. Bunton, et al. (10) found a slope of 0.94 for phenyl β-D-glucoside at 72.9°C. in perchloric acid. Therefore, the effects of acid concentration on the hydrolysis rates of the phenyl β-D-glucosides are consistent with the A-1 mechanism.

TABLE XVI

SLOPES OF LOG k' VERSUS $-H_o$ FOR ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDES

Temperature, ± 0.05°C.	Slopes		
	p-Cresyl	Phenyl	p-Chlorophenyl
50.10	1.01 ± 0.01	1.00 ± 0.01	1.02 ± 0.01
55.00	1.01 ± 0.02	1.00 ± 0.02	1.00 ± 0.01
59.95	1.00 ± 0.02	1.01 ± 0.01	1.03 ± 0.01

Figure 10 shows plots of log k' versus $-H_o$ at 25°C. for phenyl β-D-glucuronide and the least squares straight-line fits. Similar linear relationships were found for the other phenyl β-D-glucuronides. The slopes of the least-squares straight-line

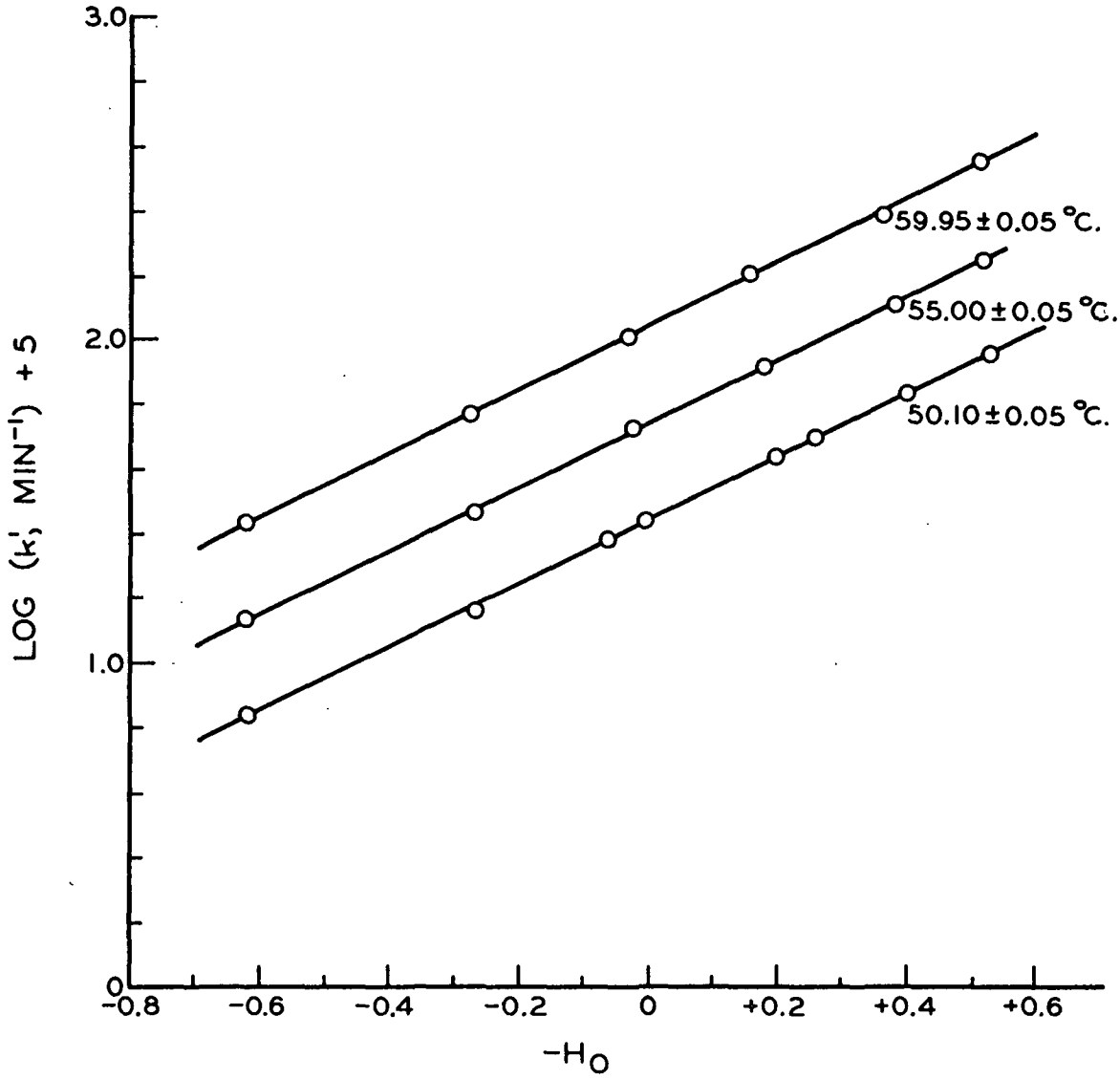


Figure 9. Relationship Between the Hammett Acidity Function and the Acid Hydrolysis Rate of Phenyl β-D-Glucoside

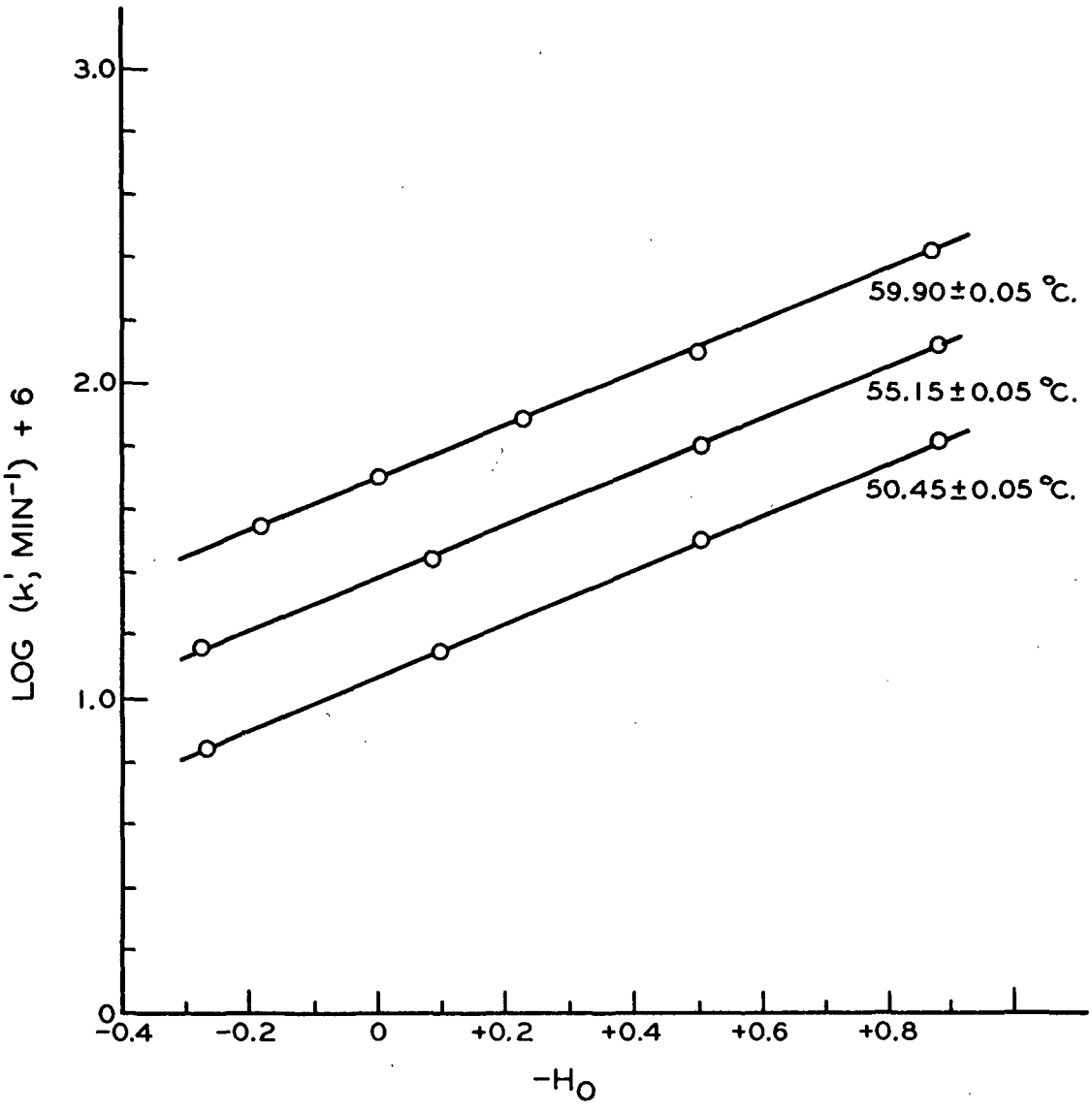


Figure 10. Relationship Between the Hammett Acidity Function and the Acid Hydrolysis Rate of Phenyl β-D-Glucuronide

fits and their estimated standard deviations were calculated and these are listed in Table XVII. The slopes were consistently 15-18% lower than the predicted value of unity. McIntyre and Long (20) considered deviations of this magnitude in detail and concluded that they were probably due to small deviations of the uncharged base activity coefficient from those of the bases used to establish the Hammett acidity functions. Studies of the acid hydrolysis of methylal (51), for example, revealed that the predicted slope of unity could be obtained by correcting for the difference between the activity coefficients of the acetal and the appropriate Hammett base. Therefore, the effects of acid concentration on the hydrolysis rates of the phenyl β-D-glucuronides are reasonably consistent with the A-1 mechanism*.

TABLE XVII

SLOPES OF LOG k' VERSUS $-H_o$ FOR ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDES

Temperature, ± 0.05°C.	p-Cresyl	Phenyl	p-Chlorophenyl
50.45	0.85 ± 0.01	0.85 ± 0.01	0.82 ± 0.00
55.15	0.85 ± 0.01	0.85 ± 0.02	0.82 ± 0.01
59.90	0.85 ± 0.01	0.84 ± 0.01	0.83 ± 0.01

The A-2 mechanisms may be represented as follows:

$$GA + H_3O^+ \overset{K_4}{\rightleftharpoons} GAH^+ + H_2O$$

$$GAH^+ + H_2O \overset{K_5^*}{\rightleftharpoons} M^* \xrightarrow{k} \text{products (G, AH)}$$

where K_4, K_5^* = equilibrium constants.

*Since the transition state for the protonation of a glycoside would involve a glycoside-hydronium ion complex, the observation that the transition state behaves like the conjugate acid would suggest that the protonation of the glycoside is rapid. Hence, the linear relationships between the logarithms of the rate constants and the Hammett acidity supports the assumption that the protonation of the phenyl β-D-glucuronide and phenyl β-D-glucosides is rapid.

An analysis similar to that for the A-1 case yields the following expression for the first-order rate constant, k'.

$$k' = k K_6^* \cdot \frac{\gamma_{GA}\, \gamma_{H_3O^+}}{\gamma_{M^*}} \cdot [H_3O^+] \qquad (12)$$

where K_6^* = over-all equilibrium constant.

If by coincidence the activity coefficient term in Equation (12) for the A-2 mechanisms varied with acid concentration in the same manner as the comparable term in Equation (9) for the A-1 mechanisms, the effect of acid concentration could not distinguish between the two types of mechanisms. Usually, however, it is found that a plot of the logarithm of the rate constant versus the logarithm of the acid concentration is nearly linear (17) for A-2 mechanisms. This indicates that the activity coefficient terms for A-2 mechanisms are generally less affected by acid concentration than those for A-1 mechanisms. Figure 11 shows that such plots are not linear for the acid hydrolysis of phenyl β-D-glucuronide. Similar results were obtained for the other glycosides studied. Therefore, the A-2 type mechanism is not supported by the effect of acid concentration on the hydrolysis rates of phenyl β-D-glucuronides and phenyl β-D-glucosides.

Entropy of Activation

The changes in the entropy function when the reacting system passes from the initial state to the transition state often provide information as to the molecularity of the acid-catalyzed cleavage of carbon-oxygen bonds (8). These changes in the entropy function may be calculated from the changes in the free energy and enthalpy functions by the following thermodynamic equation (15):

$$\Delta S^* = \frac{\Delta H^* - \Delta F^*}{T} \qquad (13)$$

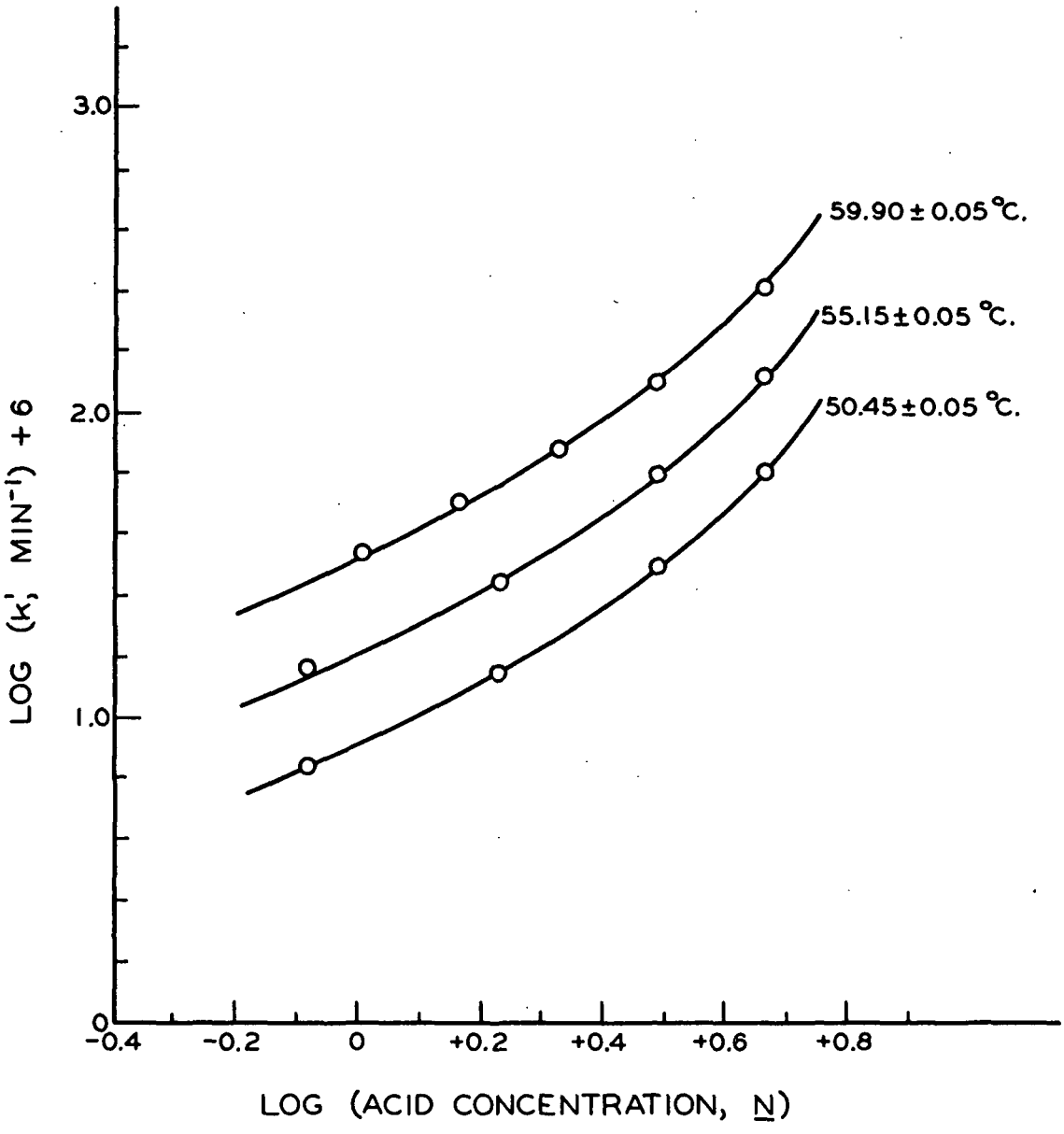


Figure 11. Effect of Acid Concentration on the Acid Hydrolysis Rate of Phenyl β-D-Glucuronide

where ΔS^* = entropy of activation

ΔH^* = enthalpy of activation

ΔF^* = free energy of activation

T = absolute temperature

The enthalpy of activation and free energy of activation may be estimated with the aid of the theory of absolute reaction rates (15).

Equation (9) for the A-1 mechanisms and Equation (12) for the A-2 mechanisms have the same general form.

$$k' = k\,K^* \cdot Q \cdot [H_3O^+] \qquad (14)$$

where K^* = K^*_3 or K^*_0

Q = ratio of activity coefficients, or activity coefficients and activity of water.

By convention, the activity of pure water at a given temperature is unity (52) so that at low ionic strengths the activity of water in aqueous solutions approaches unity (53). At low ionic strengths, the activity coefficients of neutral molecules approach unity (53) and those of ions are functions solely of their ionic charges (54). At low acid concentrations, therefore, Equation (14) will assume the following form:

$$k'_o = k\,K^*\,[H_3O^+] \qquad (15)$$

where k'_o = first-order rate constant at low acid concentrations.

Differentiating Equation (15) with respect to $[H_3O^+]$ yields

$$\frac{dk'_o}{d\,[H_3O^+]} = k\,K^* \qquad (16)$$

Since $\Delta F^* = -2.3\ R\ T \log K^*$ (15),

$$\Delta F^* = 2.3\ R\ T \left[\log k - \log \frac{d\ k'_o}{d[H_3O^+]} \right] \tag{17}$$

In order to calculate ΔF^*, k must be known but k cannot be readily determined by experiment. Assuming the time average collision frequency of reactants in solution is the same as in the gas phase, the value of k becomes (15),

$$k = \frac{k_B}{h} \cdot T \cdot \varkappa \tag{18}$$

where k_B = Boltzmann's constant

h = Planck's constant

T = absolute temperature

$\varkappa$ = transmission factor

The transmission factor is usually near unity (15).

The quantity $d\ k'_o / d\ [H_3O^+]$ may be estimated by differentiating Equation (1) with respect to $[H_3O^+]$ and letting $[H_3O^+]$ approach zero.

$$\frac{d\ k'}{d\ [H_3O^+]} = B + 3D\ [H_3O^+]^2 + 5F\ [H_3O^+]^4 \tag{19}$$

$$\underset{[H_3O^+] \longrightarrow 0}{\text{limit}} \frac{d\ k'}{d\ [H_3O^+]} = \frac{d\ k'_o}{d\ [H_3O^+]} = B \tag{20}$$

Combining Equations (17), (18), and (20) results in the following expression for estimating ΔF^*.

$$\Delta F^* = 2.3\ R\ T \left[\log \frac{k_B}{h} T - \log B \right] \tag{21}$$

Examination of Equation (14) shows that k' will vary with temperature according to how k, K^*, and Q vary. The quantity Q would be expected to change considerably less than the other two terms since it is composed of terms which do not usually show large temperature coefficients (53). Making this assumption, differentiation of the logarithmic form of Equation (14) yields

$$\frac{d \log k'}{d (1/T)} = \frac{d \log k}{d (1/T)} + \frac{d \log K^*}{d (1/T)} \qquad (22)$$

From Equation (2) and Equation (18) it can be shown that

$$\frac{d \log k'}{d (1/T)} = - \frac{E}{2.3R} \qquad (23)$$

$$\frac{d \log k}{d (1/T)} = - \frac{T}{2.3} \qquad (24)$$

Since $d \log K^*/d(1/T) = -\Delta H^*/2.3R$ (15), combining Equations (22), (23), and (24) yields,

$$\Delta H^* = E - R T \qquad (25)$$

Estimates of the free energies, enthalpies, and entropies of activation were calculated from the appropriate equations and the results are listed in Tables XVIII and XIX. The average values of E listed in Tables XI and XII were used to calculate the enthalpy of activation. There were no large differences in the entropies of activation within the two glycoside series. The difference in reactivity between the phenyl β-D-glucuronide and phenyl β-D-glucoside series (carboxyl stabilizing effect) was reflected almost entirely in the enthalpy function.

Due to the similar entropies of activation, it seems likely that all of the glycosides hydrolyzed via the same or similar reaction mechanisms. In addition, the magnitudes of the entropies of activation were similar to those estimated for

most other glycosides (+10 to +20 cal. per °K. per mole) suggesting that most glycosides hydrolyze via the same or similar mechanisms. As discussed previously (Introduction, p. 8) large positive entropies of activation suggest A-1 mechanisms rather than A-2 mechanisms.

TABLE XVIII

ESTIMATED THERMODYNAMIC ACTIVATION FUNCTIONS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDES AT 59.90 ± 0.05°C.

	Substituent of Aglycon Group		
	p-CH_3	none	p-Cl
ΔF^*, kcal. per mole	28.8	28.7	28.8
ΔH^*, kcal. per mole	32.3	32.2	32.4
ΔS^*, cal. per °K. per mole	+10	+10	+11

TABLE XIX

ESTIMATED THERMODYNAMIC ACTIVATION FUNCTIONS FOR THE ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDES AT 59.95 ± 0.05°C.

	Substituent of Aglycon Group		
	p-CH_3	none	p-Cl
ΔF^*, kcal. per mole	26.7	26.7	27.0
ΔH^*, kcal. per mole	30.3	29.9	30.1
ΔS^*, cal. per °K. per mole	+11	+10	+9

The low entropies of activation of the methyl uronosides (7) has led to the suggestion that uronosides hydrolyze via a different mechanism than other glycosides. Since no comparable entropy effects were observed for the phenyl β-D-glucuronides, low entropies of activation cannot be completely general for uronosides.

Furthermore, it seems doubtful that the mechanistic change hypothesis can be applied to the phenyl β-D-glucuronides.

NATURE OF UNIMOLECULAR HETEROLYSIS

As noted previously, there are two possible A-1 mechanisms (Fig. 1). Both mechanisms are consistent with the observed effects of acid concentration on the hydrolysis rates, and the large entropies of activation could be accounted for by either transition state.

Phenyl substituents at the para position will affect hydrolysis rates by their effects on the distribution of the electron clouds in the glycosyl side-chain (9). There are two opposing ways by which phenyl substituents that make the aglycon more electrophilic will affect the hydrolysis rate by the A-1(A) mechanism. First, protonation will be inhibited by the lower electron density at the glycosidic oxygen when the electron cloud is shifted toward the benzene ring. This in turn will lower the conjugate-acid concentration and reduce the hydrolysis rate. Second, the pair of electrons of the C_1-glycosidic oxygen bond will be shifted toward the glycosidic oxygen. Since the A-1(A) heterolysis involves migration of this electron pair from C_1 to the glycosidic oxygen, the heterolysis rate, and consequently the hydrolysis rate, will be increased. The observed decrease in the hydrolysis rates of the phenyl β-D-glucosides as the aglycon group was made more electrophilic may be explained as a predominance of the protonation effect in the A-1(A) mechanism. The small effect of phenyl substituents on the hydrolysis rates of the phenyl β-D-glucuronides suggests a near cancellation of the protonation and heterolysis effects.

There are three ways by which phenyl substituents which make the aglycon more electrophilic will affect the rate by the A-1(B) mechanism. First, protonation

will be inhibited by the lower electron density of the ring oxygen when the electron cloud is shifted toward C_1. This will lower the conjugate-acid concentration and reduce the hydrolysis rate. Second, the pair of electrons of the C_1-ring oxygen bond will be shifted toward C_1. Since the A-1(B) heterolysis involves migration of this electron pair from C_1 to the ring oxygen, the heterolysis rate, and consequently the hydrolysis rate, will be decreased. Third, the electron cloud about the glycosidic oxygen will be shifted toward the benzene ring and away from C_1. Since the A-1(B) carbonium ion is stabilized by a drift of electron density from the glycosidic oxygen to C_1, the carbonium ion will be stabilized to a lesser extent with the result that the hydrolysis rate will be lower. The observed decrease in hydrolysis rates of the phenyl β-D-glucosides as the aglycon was made more electrophilic may be the result of the combination of effects on protonation, heterolysis, and carbonium ion stabilization. The small effect of phenyl substituents on the hydrolysis rates of phenyl β-D-glucuronides may be due to the resultant of these effects being small.

Therefore, from the available data, it is not possible to choose clearly between the A-1(A) or A-1(B) mechanism for either the phenyl β-D-glucuronides or the phenyl β-D-glucosides.

NATURE OF CARBOXYL STABILIZING EFFECT

The work of Banks, et al. (28) on 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-glucosides suggest the A-1(A) mechanism. The question arises as to whether these conclusions hold for other glycosides. Some indication of this is gained from the fact that the entropies of activation of the glycosides studied by Banks, et al. (28) are about the same as

most other glycosides including the phenyl β-D-glucosides and phenyl β-D-glucuronides. Overend, et al. (9) calculated the following entropies of activation at 60°C.: methyl α-D-glucoside, +14.8 cal. per °K. per mole; phenyl β-D-glucoside, +10.8 cal. per °K. per mole; and phenyl α-D-glucoside +13.3 cal. per °K. per mole. The entropies of activation for the phenyl β-D-glucuronides and phenyl β-D-glucosides of this study ranged from +9 to +11 cal. per °K. per mole at approximately 60°C. Therefore, a reasonable assumption at this point would be that the phenyl β-D-glucosides and phenyl β-D-glucuronides hydrolyze via the A-1(A) mechanism.

If the A-1(A) mechanism predominates in the acid hydrolysis of phenyl β-D-glucuronides and phenyl β-D-glucosides, the question then arises as to how the carboxyl group stabilizes the glycosidic linkage. In the following paragraphs several effects will be considered as possible explanations of the carboxyl stabilizing effect.

CONFORMATIONAL RESISTANCE

The conformational aspects of the carboxyl stabilizing effect may be considered from the viewpoint of Foster and Overend (26), and Edward (25). As the aglycon recedes, the carbonium ion of the A-1(A) mechanism is stabilized by resonance between a carbonium ion and an oxonium ion. As illustrated in Fig. 12, this requires the C_2-C_1-O-C_5 chain to be planar. The formation of this planar half-chair conformation from the initial chair conformation involves rotation about the C_2-C_3 and C_4-C_5 bonds which alters the nonbonded interaction between substituents. Since the conformation of the transition state would be expected to approach that of the carbonium ion, changes in nonbonded interaction would be expected. The transition state would be formed with greater or less difficulty depending on whether the nonbonded interaction was increased or decreased. For D-glucopyranose-type configurations,

these conformational changes result in closer proximity of the C_4 and C_5 substituents with increased repulsive interaction. As the size of these substituents increases, the interaction would be expected to increase, causing a decrease in hydrolysis rate (25, 26).

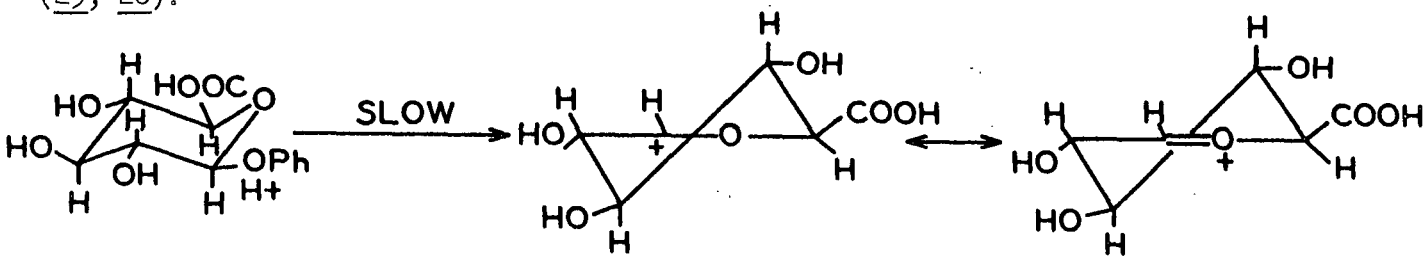


Figure 12. Conformational Changes in Formation of A-1(A) Carbonium Ion for Acid Hydrolysis of Phenyl β-D-Glucuronide (25)

As pointed out by Whistler and Richards (3), the difference in conformational effects between the C_5 carboxyl and hydroxymethyl groups would be about the same as between the C_5 1,2-dihydroxyethyl and hydroxymethyl groups. However, the C_5 1,2-dihydroxyethyl methyl glycosides are only about twofold more resistant than the corresponding C_5 hydroxymethyl glycosides (1, 55). Therefore, it seems that conformational effects while they may contribute to the carboxyl stabilizing effect are too small to account for large stabilizing effects.

If an increase in conformational resistance were responsible for the carboxyl stabilizing effect, the increase in the steric restraints of the C_4 and C_5 substituents in the transition state should be greater for the phenyl β-D-glucuronides than for the phenyl β-D-glucosides. Since the entropy of activation is a measure of the difference in freedom from restraint in the initial and transition states, one would expect the entropies of activation of the phenyl β-D-glucuronides to be less than those of the phenyl β-D-glucosides. The fact that the estimated entropies

of activation of the two series were essentially the same suggests that differences in conformational resistance are not responsible for the carboxyl stabilizing effect.

INTRAMOLECULAR HYDROGEN BONDING

It is known that the carboxyl group forms hydrogen bonds more readily than the hydroxyl group (56) so that intramolecular hydrogen bonding might be responsible for the carboxyl stabilizing effect. The Cenco-Petersen* molecular models indicate that when phenyl β-D-glucuronides are in their expected C1 conformation, the hydroxyl of the C_5 carboxyl and the C_4 hydroxyl can achieve good position for hydrogen bonding. The spatial arrangements are illustrated in Fig. 13. Estimates of the oxygen-oxygen separations indicate these may approach as little as about 2.6 A. Syrkin and Dyatkina (57) present data on a number of hydrogen bonds indicating oxygen-oxygen distances range from 2.55 to 2.78 A. Pauling (56) states that for most hydrogen bonded oxygen atoms the oxygens are separated by 2.50 to 2.80 A. According to the conformational analyses of the A-1(A) carbonium ion (25, 26), the C_5 carboxyl and the C_4 hydroxyl would be somewhat closer (about 2.5 A. as compared to 2.6 A.) in the

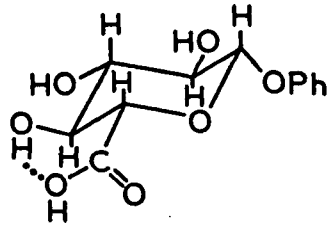


Figure 13. Possible Intramolecular Hydrogen Bond Formation in Expected C1 Conformation of Phenyl β-D-Glucuronide

*The Cenco-Petersen molecular models were purchased from the Central Scientific Company, Chicago, Illinois.

initial state leading to a more favorable spatial arrangement for the hydrogen bonding in the former*. This increased tendency for hydrogen bonding in the transition state should result in a somewhat increased attraction between the C_4 and C_5 substituents in the transition state. One would expect, therefore, that such hydrogen bonding would tend to decrease the resistance to the conformational changes necessary for the formation of the A-1(A) transition state.

The result of such hydrogen bonding would be to activate rather than to stabilize the glycosidic linkage. Hence, it must be concluded that if significant hydrogen bonding of this type does occur it is not the dominant effect in the lower reactivity of the phenyl β-D-glucuronides. It must be remembered that when a favorable spatial arrangement for hydrogen bonding exists it does not necessarily follow that significant hydrogen bonding will occur. In this case, intramolecular hydrogen bonding must

*If in the transition state, the C_5 orbitals changed from sp^3 hybrid orbitals to sp^2 hybrid orbitals plus one p_z orbital, the C_2-C_1-O-C_5-C_4 chain would become coplanar with the carboxyl group. The p_z orbitals of C_1, the ring oxygen, C_5, and the carboxyl group could then form a π-electron system. Such a carbonium ion would delocalize the positive charge to a greater extent than the carbonium ion depicted in

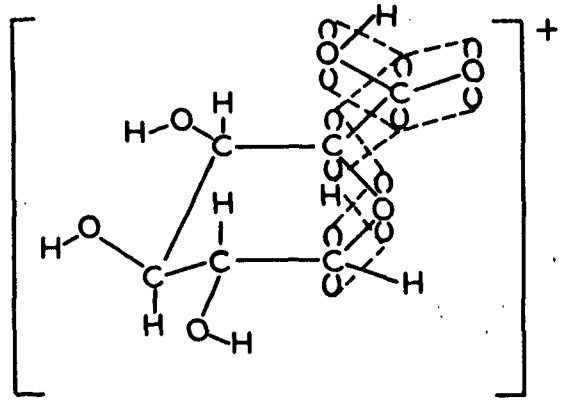


Fig. 12 (p. 60). To achieve the required conformation, however, would necessitate the expenditure of considerable energy. First, energy would be required for rehybridization of the C_5 orbitals. Second, energy would be needed to overcome the repulsion between the oxygen of the C_4 hydroxyl group and one of the oxygens of the C_5 carboxyl group since these atoms would be required to lie within about 2.3 A. of each other [2.4 A. is the shortest known hydrogen bond (the proton lies midway between the oxygens) (56) so that it is likely that a strong repulsion will exist between oxygens closer than 2.4 A.].

compete with the hydrogen bonding between the substituents and water molecules. Hydrogen bonds which lead to dimers of carboxylic acids in solvents like benzene or chloroform, for example, do not persist in hydroxylic solvents like water (56).

PONDERAL EFFECT

De la Mare, et al. (58) theoretically treated the effects of alkyl branching on the exchange of bromide ion with alkyl bromides. The transition state was considered to be bimolecular with the incoming and outgoing bromine atoms equidistant from the alpha carbon atom, and the bond angles and bond lengths chosen to minimize the free energy. A particularly interesting result was that the quantum-mechanical relationships derived for the entropy included the masses of the species involved. Hence, any alteration of structure which increases the mass of the reactant or alters the distribution of mass may change the free energy of activation through changes in the entropy of activation. Replacing a hydrogen by a heavy alkyl group invariably made the entropy of activation more negative. These effects were called ponderal effects.

Since no similar treatment has been attempted for a unimolecular transition state such as the A-1(A) transition state for glycosides, the importance of ponderal effects in the formation of the A-1(A) transition state cannot be readily calculated. A large ponderal effect would be surprising, however, since the maximum difference in mass between any of the glycoside pairs studied was only about five per cent. If ponderal effects enter the entropy function, it can be seen from the estimated entropies of activation presented in Tables XVIII and XIX that they must not be very significant for the glycosides studied. Therefore, ponderal effects appear to be a rather dubious explanation for the carboxyl stabilizing effect.

INDUCTIVE EFFECT*

Whistler and Richards (3) suggested that the carboxyl stabilizing effect was due to the inductive effect of the carboxyl group. Development of this hypothesis led to the idea that the inductive effect would stabilize the glycosidic linkage in at least two ways. First, the inductive pull of the carboxyl group would decrease the rate of the heterolysis step by opposing the migration of electrons from C_1 to the glycosidic oxygen (4). Secondly, the induction of a smaller partial negative charge on the glycosidic oxygen would diminish the ease of protonation and decrease the concentration of conjugate acid (5).

Another effect of the induction of electrons by the carboxyl group may also be suggested. 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 of electron density. Stabilization of the carbonium ion would thus be more difficult, making the hydrolysis rate lower. Ingold (14) estimated that the extra ethoxyl group which diethyl acetal contains increases its acid hydrolysis rate over that of diethyl ether by a factor of about 10^{11}. The change in the ability of the ring oxygen to stabilize the carbonium ion, therefore, might be expected to be a rather important aspect of the carboxyl inductive effect. Considering the fact that the ring oxygen is alpha to the carboxyl group this may be the dominant aspect of the carboxyl inductive effect (8).

Hinshelwood, Laidler, and Timm (31) have studied the relationships between the electronic theories of reactivity, the influence of substituents, and the

*An alternate inductive effect hypothesis may be suggested for the carboxyl stabilizing effect based on the phenyl β-D-glucosides and phenyl β-D-glucuronides hydrolyzing via the A-1(A) and A-1(B) mechanisms, respectively. This hypothesis is stated in Appendix V.

activation energies. It was pointed out that an inductive effect will influence the repulsive and attractive forces in the formation and decomposition of the transition state as well as the strengths of the bonds cleaved. They concluded that changes in reactivity which result from inductive effects are reflected primarily in the activation energies.

The difference in reactivity between the phenyl β-D-glucoside and phenyl β-D-glucuronide series was almost entirely associated with changes in the enthalpies of activation. Since the enthalpy of activation nearly equals the energy of activation [Equation (25), p. 55], these results are consistent with the view that the carboxyl group stabilizes the glycosidic linkage by an inductive effect. Overend, et al. (9) suggested that the greater reactivity of the 2-deoxy glycosides compared to the fully hydroxylated glycosides is due mainly to the inductive effect of the C_2 hydroxyl. Of the 5.2 kcal. per mole increase in the free energy of activation at 60°C. in going from methyl β-2-deoxy-D-glucoside to methyl β-D-glucoside, 4.4 kcal. per mole is due to an increase in the enthalpy function.

The difference in the enthalpy of activation between the phenyl β-D-glucuronide and phenyl β-D-glucoside series also suggests a reason why Easty (7) found evidence of a change in mechanism when comparing the acid hydrolysis of methyl α-D-glucoside and methyl α-D-glucuronide. Easty estimated the free energy of activation for methyl α-D-glucoside to be 28.2 kcal. per mole at 80°C. Assuming that the effect of replacing the C_5 hydroxymethyl group with a carboxyl group is to increase the enthalpy of activation by about 2.0 kcal. per mole, the free energy of activation of methyl α-D-glucuronide for the A-1(A) transition state would be 30.2 kcal. per mole. This free energy of activation is large compared to that of most other glycosides at similar temperatures (9), so that a region may have been entered where some other transition state has a lower free energy than the A-1(A) transition

state. If this is the case, the acid hydrolysis of methyl α-D-glucuronide would occur via this other transition state, since a reaction will proceed through the pathway whose transition state has the lowest free energy. Easty calculated 28.6 kcal. per mole for the free energy of activation of methyl α-D-glucuronide at 80°C. which is 1.6 kcal. per mole less than that estimated above for hydrolysis via the A-1(A) mechanism.

If the hypothesis of the carboxyl inductive effect is valid, the carboxyl group would tend to induce a more positive partial charge on the other atoms of the glycoside than the hydroxymethyl group. This tendency would be greatest for atoms closest to the carboxyl group such as the other atoms of the glycosyl group. Other things being equal, the more positive the partial charge of an atom the greater is the control of the nuclear charge over the electron cloud, and the greater will be the energy required to induce a given additional partial charge. As stated by Smith and Eyring (59), the inductive effect of a substituent is dependent on the electron density at the reaction center. The carboxyl group, therefore, would be expected to reduce the polarizability of the glycosyl group. It follows that phenyl substituent effects on the hydrolysis rates should be less for the phenyl β-D-glucuronide series than for the phenyl β-D-glucoside series since they depend on changing electron density of the glycosyl group. Therefore, the low Hammett reaction series constants of the phenyl β-D-glucuronide series as compared to those of the phenyl β-D-glucoside series support the inductive effect hypothesis for the carboxyl stabilizing effect.

Easty (7) has argued that the three atoms between the C_5 substituent and the glycosidic oxygen make a significant inductive effect improbable. In addition, the low polarizability of carbon-oxygen single bonds was cited as evidence against the inductive effect.

It will be observed, however, that the A-1(A) mechanism involves not only the glycosidic oxygen but also the ring oxygen and C_1 (Fig. 1). One would expect, therefore, that any structural change which affects the electron density at one or more of these atoms could alter the hydrolysis rate. As noted previously, the inductive effect of the carboxyl group will lower the conjugate-acid concentration, slow the heterolysis rate, and diminish carbonium ion stabilization by the ring oxygen. Since these effects act in the same direction, any inductive effect which extends at least to the ring oxygen would cause a stabilizing effect.

The relative dissociation constants of several carboxylic acids are listed in Table XX. Comparison of the constants for γ-hydroxybutyric acid and n-propionic acid indicates that the hydroxymethyl group shifts the electrons away from the carboxyl group thereby easing the departure of the proton. This has been accomplished at a distance of two methylene groups. Since inductive effects in aliphatic systems diminish rapidly with distance and carbon-oxygen single bonds are known to be less polarizable than carbon-carbon bonds (60), it seems doubtful that the inductive effect of a hydroxymethyl group at the C_5 position of a glycoside would extend much beyond the ring oxygen*.

The dissociation constants listed in Table XX for the dicarboxylic acids correspond to the first proton removed. In the absence of any inductive interaction between the carboxyl groups, the dissociation constant of a dicarboxylic

*It is not necessarily a straightforward matter to determine whether a substituent has an inductive effect on some atom of a glycoside simply from the effects of the substituent on the ionization constants of carboxylic acids. In either the carboxylic acids or the glycoside the mode of transmission of the inductive effect sometimes may be mainly through the solvent rather than through the atoms attached to the substituent. Since the spatial arrangement of the substituent and the carboxyl group of the acid may differ from that of the substituent and the atom of the glycoside under consideration, deciding whether an inductive effect is operative from the number of atoms interposed between the substituent and the atom of interest may in some instances be misleading.

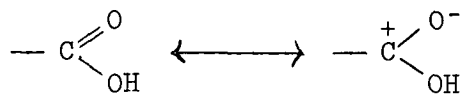
TABLE XX

RELATIVE DISSOCIATION CONSTANTS OF CERTAIN CARBOXYLIC ACIDS AT 25°C. IN WATER (61, 62)

Acid	Formula	Relative Dissociation Constants
n-Propionic	$H\text{-}CH_2CH_2\text{-}COOH$	1.00
γ-Hydroxybutyric	$HOCH_2\text{-}CH_2CH_2\text{-}COOH$	1.4
Succinic	$HOOC\text{-}CH_2CH_2\text{-}COOH$	4.8
n-Butyric	$H\text{-}CH_2CH_2CH_2\text{-}COOH$	1.12
Glutaric	$HOOC\text{-}CH_2CH_2CH_2\text{-}COOH$	3.38
n-Valeric	$H\text{-}CH_2CH_2CH_2CH_2\text{-}COOH$	1.16
Adipic	$HOOC\text{-}CH_2CH_2CH_2CH_2\text{-}COOH$	2.77

acid should be twice as large as a monobasic acid (8). The dissociation constant of succinic acid, however, is considerably more than twice as great as either n-propionic or even γ-hydroxybutyric acid. Evidently, the carboxyl group is more electrophilic than the hydroxymethyl group causing a greater shift of the electrons with a greater increase in the dissociation constant. This would be expected from a consideration of resonance structures of the carboxyl group*. Since the carboxyl group is a stronger electron attractor than the hydroxymethyl group one would expect the inductive effect of the carboxyl group to extend over greater distances. Referring to Table XX, the effect of the number of interposed methylene groups on the inductive effect of the carboxyl group can be seen. The carboxyl group is such a strong electron attractor that a significant effect carries through even four

*Since the electrons of the carbon-oxygen double bond may be polarized by the carbonyl oxygen with relative ease, the following resonance structures are possible (8):



Thus, the positive character of the carbon atom causes it to attract electrons from its neighboring atoms more strongly than it would in the absence of such resonance structures.

methylene groups. It seems likely, therefore, that the carboxyl group at the C_5 position of a glycoside could produce an inductive effect at C_1 which is at a distance of two atoms.

Such considerations also support the suggestion that the difference in the susceptibility of the phenyl β-D-glucuronide and phenyl β-D-glucoside series to polar aglycon effects was due to changes in polarizability of the reaction center. Table XXI lists the Hammett constant, ρ, for meta- and para-substituted phenyl carboxylic acids. Interposing groups between the phenyl group and the carboxyl group reduces polar effects, but they remain significant even after a methyleneoxy or two methylene groups have been added. It seems likely, therefore, that the polar effects of the phenyl substituents of phenyl glycosides will extend to the ring oxygen. If the inductive effect of the carboxyl group can extend to C_1, there will be an interaction of the inductive effect of the carboxyl group and the polar effects of the aglycon. Such an interaction could lead to differences in susceptibility to polar aglycon effects between the phenyl β-D-glucuronide and phenyl β-D-glucoside series.

TABLE XXI

EFFECTS OF THE INTERPOSITION OF GROUPS BETWEEN THE PHENYL AND CARBOXYL GROUPS ON THE IONIZATION OF PARA AND META SUBSTITUTED PHENYL CARBOXYLIC ACIDS IN WATER AT 25°C.

Acid Series	Formula[a]	Hammett Constant of Series, ρ	Ref.
Benzoic	X-Ph-COOH	+1.000	(48)
Phenylacetic	X-Ph-CH_2-COOH	+0.471	(48)
Phenylpropionic	X-Ph-CH_2CH_2-COOH	+0.212	(48)
Phenoxyacetic	X-Ph-OCH_2-COOH	+0.29	(63)

[a] X = para or meta substituent of phenyl group.

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LITERATURE CITED

1. Wolfrom, M. L., and Thompson, A. Glycosides, simple acetals, and thioacetals. In Pigman's The carbohydrates. p. 209. New York, Academic Press, 1957.

2. Shafizadeh, F., Adv. Carbohydrate Chem. 13:24-43(1958).

3. Whistler, R. L., and Richards, G. N., J. Am. Chem. Soc. 80:4888-91(1958).

4. Marchessault, R. H., and Rånby, B. G., Svensk Papperstid. 62, no. 7:230-40 (1959).

5. Hamilton, J. K., and Thompson, N. S., Pulp Paper Mag. Can. 61, no. 4:263-72 (April, 1960).

6. Nakano, J., and Rånby, B. G., Svensk Papperstid. 65, no. 2:29-33(1962).

7. Easty, D. B. The synthesis and acid hydrolysis of methyl alpha-D-gluco-pyranosiduronic acid. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1961.

8. Gould, E. S. Mechanism and structure in organic chemistry. Chap. 7 and 8. New York, Henry Holt, 1960.

9. Overend, W. G., Rees, C. W., and Sequeira, J. S., J. Chem. Soc. 1962:3429-40.

10. Bunton, C. A., Lewis, T. A., Llewellyn, D. R., and Vernon, C. A., J. Chem. Soc. 1955:4419-23.

11. Armour, C., Bunton, C. A., Patai, S., Selman, L. H., and Vernon, C. A., J. Chem. Soc. 1961:412-16.

12. Gross, P., Steiner, H., and Suess, H., Trans. Faraday Soc. 32:883-9(1936).

13. Wiberg, K. B., Chem. Revs. 55:713-43(1955).

14. Ingold, C. K. Structure and mechanism in organic chemistry. Chap. VII. Ithaca, New York, Cornell University Press, 1953.

15. Glasstone, S., Laidler, K. J., and Eyring, H. The theory of rate processes. 1st ed. New York, McGraw-Hill, 1941. 599 p.

16. Hammett, L. P., and Deyrup, A. J., J. Am. Chem. Soc. 54:2721-39(1932).

17. Long, F. A., and Paul, M. A., Chem. Revs. 57:935-1010(1957).

18. Novikova, L. I., and Konkin, A. A., Zh. Prikl. Khim. 32, no. 5:1081-5(1959); C.A. 53:15726(1959).

19. Whalley, E., Trans. Faraday Soc. 55:798-808(1959).

20. McIntyre, D., and Long, F. A., J. Am. Chem. Soc. 76:3240-2(1954).

21. Skrabal, A., and Zlatewa, M., Z. Phys. Chem. 122:349-56(1926).

22. O'Gorman, J. M., and Lucas, H. J., J. Am. Chem. Soc. 72:5489-90(1950).

23. Whalley, E., and Koskikallio, J., Trans. Faraday Soc. 55:809-14(1959).

24. McKee, S. An investigation of the hydrolysis of a reduced 4-O-methyl-glucuronoxylan. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1961.

25. Edward, J. T., Chem. & Ind. 1955:1102-4.

26. Foster, A. B., and Overend, G. W., Chem. & Ind. 1955:566-7.

27. Gelles, E., Hughes, E. D., and Ingold, C. K., J. Chem. Soc. 1954:2918-29.

28. Banks, B. E., Meinwald, Y., Rhind-Tutt, A. J., Sheft, I., and Vernon, C. A., J. Chem. Soc. 1961:3240-6.

29. Hall, A. N., Hollingshead, S., and Rydon, H. N., J. Chem. Soc. 1961:4290-5.

30. Morell, S., and Link, K. P., J. Biol. Chem. 104:183-8(1934).

31. Hinshelwood, C. N., Laidler, K. J., and Timm, E. W., J. Chem. Soc. 1938: 848-58.

32. Dyer, E., Glaudemans, C. P. J., Koch, M. J., and Marchessault, R. H., J. Chem. Soc. 1962:3361-4.

33. Bollenback, G. N., Long, J. W., Benjamin, D. G., and Lindquist, J. A., J. Am. Chem. Soc. 77:3310-15(1955).

34. Helferich, B., and Schmitz-Hillebrecht, E., Ber. Deut. Chem. Ges. 66:378-83 (1933).

35. Porteous, J. W., and Williams, R. T., Biochem. J. 44:46-55(1949).

36. Bray, H. G., Thorpe, W. V., and White, K., Biochem. J. 46:275-8(1950).

37. Spencer, B., and Williams, R. T., Biochem. J. 47:279-84(1950).

38. Tsou, K., and Seligman, A. M., J. Am. Chem. Soc. 75:1042-4(1953).

39. Nath, R. L., and Rydon, H. N., Biochem. J. 57:1-10(1954).

40. Fischer, E., and Mechel, L., Ber. Deut. Chem. Ges. 49:2813-20(1916).

41. Fischer, E., and Armstrong, E. F., Ber. Deut. Chem. Ges. 34:2885-2900(1901).

42. Ryan, J., J. Chem. Soc. 75:1054-7(1899).

43. Helferich, B., and Scheiber, H., Z. Physiol. Chem. 226:272-80(1934).

44. Dyfverman, A., and Lindberg, B., Acta. Chem. Scand. 4:878-84(1950).

45. Young, T. F., Maranville, L. F., and Smith, H. M. Raman spectral investigations of ionic equilibria in solutions of strong electrolytes. In Hamer's The structure of electrolytic solutions. p. 48-59. New York, Wiley & Sons, 1959.

46. Daniels, F., and Alberty, R. A. Physical chemistry. 1st ed. Chap. 13. New York, Wiley & Sons, 1955.

47. Heidt, L. J., and Purves, C. B., J. Am. Chem. Soc. 66:1385-9(1944).

48. Hammett, L. P. Physical organic chemistry. 1st ed. Chapter VII. New York, McGraw-Hill, 1940.

49. Bascombe, K. N., and Bell, R. P., J. Chem. Soc. 1959:1096-1104.

50. Paul, M. A., and Long, F. A., Chem. Revs. 57:1-45(1957).

51. McIntyre, D., and Long, F. A., J. Am. Chem. Soc. 76:3243-7(1954).

52. Lewis, G. N., and Randall, M. Thermodynamics. 2nd ed. Chap. 20. New York, McGraw-Hill, 1961.

53. Harned, H. S., and Owen, B. B. The physical chemistry of electrolytic solutions. Chap. 12 and 13. New York, Reinhold, 1958.

54. Glasstone, S. Textbook of physical chemistry. 2nd ed. p. 956-60. New York, D. Van Nostrand Co., 1946.

55. Isbell, H. S., and Frush, H. L., J. Res. Natl. Bur. Std. 24:125-51(1940).

56. Pauling, L. The nature of the chemical bond. 3rd ed. p. 477-85. Ithaca, New York, Cornell University Press, 1960.

57. Syrkin, Y. K., and Dyatkina, M. E. Structure of molecules and the chemical bond. p. 273. New York, Interscience, 1950.

58. De la Mare, P. B. D., Fowden, L., Hughes, E. D., Ingold, C. K., and Mackie, J. D. H., J. Chem. Soc. 1955:3200-6.

59. Smith, R. P., and Eyring, H., J. Am. Chem. Soc. 75:5183-6(1953).

60. Alexander, E. R. Ionic organic reactions. p. 5-12, New York, John Wiley, 1950.

61. Hodgman, C. D., editor in chief. Handbook of chemistry and physics. 44th ed. p. 1753-9. Cleveland, Ohio, The Chemical Rubber Publishing Co., 1962.

62. Lange, N. A., and Forkner, G. M. Handbook of chemistry. 10th ed. p. 1198-1202. New York, McGraw-Hill, 1961.

63. Hayes, N. V., and Branch, G. E. K., J. Am. Chem. Soc. 65:1555-64(1943).

64. Goebel, W. F., and Babers, F. H., J. Biol. Chem. 106:63-9(1934).

65. Kamil, I. A., Smith, J. N., and Williams, R. T., Biochem. J. 50:235-40(1952).

66. Hach, W., and Benjamin, D. G., J. Am. Chem. Soc. 76:917-18(1954).

67. Ehrlich, F., and Rehorst, K., Ber. Deut. Chem. Ges. 62:628-34(1929).

APPENDIX I

PREPARATION OF PHENYL GLYCOSIDES

PREPARATION OF PHENYL β-D-GLUCURONIDES

Phenyl, p-chlorophenyl, and p-cresyl β-D-glucuronides were prepared by the method of Bollenback, et al. (33). After dissolving 0.0637 mole of sodium hydroxide in 750 ml. methanol, 0.568 mole of D-glucuronolactone was added and the resulting solution stirred for one hour. The methanol was removed over a period of five hours using a rotary vacuum evaporator with a 60°C. bath and a water aspirator followed by another five hours at room temperature under 0.1 mm. Hg. The orange gummy residue was acetylated by adding a mixture of 3.10 moles of pyridine and 4.00 moles of acetic anhydride over a one-hour period while keeping the temperature at 15-30°C. After standing overnight at 4.5°C., the acetate was filtered from the reaction mixture, washed with 95% ethanol, and then recrystallized overnight from 1000 ml. 95% ethanol at 4.5°C. The crystals were filtered, washed and dried. The following were determined: yield, 61.6 g. (29%); melting point, 176-77.5°C. (corrected), and $[\alpha]_D^{29.2}$ + 6.7° (c 2.2, chloroform). The literature gives the following properties for methyl tetra-O-acetyl-β-D-glucopyranuronate: melting point, 176.5-78°C. (33) and 178°C. (64); and $[\alpha]_D^{23}$ + 7.4° (c 2, chloroform) (33) and $[\alpha]_D^{24}$ + 8.7° (c 1, chloroform) (64).

To prepare the methyl (phenyl tri-O-acetyl-β-D-glucopyranosid)-uronates, an intimate mixture of 0.0768 mole of methyl tetra-O-acetyl β-D-glucopyranuronate, 0.266 mole phenol, p-chlorophenol, or p-cresol, and 0.0035 mole of p-toluenesulfonic acid was fused for 1.5 hr. at 95-105°C. and 29.5 in. Hg. vacuum. The cooled melt was dissolved in 400 ml. benzene and extracted with two 150-ml. portions of 2N potassium hydroxide solution. The benzene solution was washed

until the aqueous phase was neutral, and then treated with charcoal and anhydrous sodium sulfate for one hour. After filtering, the pale yellow solution was taken to a sirup which was dissolved in 200 ml. of hot isopropanol. After crystallizing overnight at 4.5°C., the methyl (phenyl tri-O-acetyl-β-D-glucopyranosid)-uronate was filtered, washed, and again crystallized from 200 ml. isopropanol. The yields were about 30%. The melting points were determined and these are listed in Table XXII.

TABLE XXII

MELTING POINTS OF METHYL (PHENYL TRI-O-ACETYL β-D-GLUCOPYRANOSID)-URONATES

Aglycon Group	Melting Point, °C. Found	Literature
p-Chlorophenyl	151-52 (corrected)	152-53 (33) 151-52 (65)
p-Cresyl	137.5-39 (corrected)	137-38 (33) 140 (65)
Phenyl	118-19 (corrected)	126-27.5 (33) 116 (35)

To deacetylate, 6.0 meq. of methanolic sodium methoxide were added to 0.025 mole of the methyl (phenyl tri-O-acetyl-β-D-glucopyranosid)-uronate in 200 ml. dry methanol and the solution was allowed to stand 30 min. at room temperature. This yielded the methyl (phenyl β-D-pyranosid)-uronate. The solution was taken to dryness while keeping the temperature below 35°C. By adding 50 ml. water to the residue, the sodium methoxide was converted to sodium hydroxide which hydrolyzed the methyl ester groups until the liberated carboxyl groups neutralized the solution. Sufficient N sodium hydroxide was then added to hydrolyze the remaining methyl ester groups. The resulting solution of sodium (phenyl β-D-glucopyranosid)-uronate was passed through an Amberlite 120 H resin column to convert the salt to

the phenyl β-D-glucuronide. The glucuronide solution was treated with charcoal, filtered, and concentrated to about 60 ml. The crystals of the phenyl β-D-glucuronide which formed overnight at 4.5°C. were filtered and then dried over phosphorous pentoxide under vacuum. The yields based on the methyl (phenyl tri-O-acetyl-β-D-glucopyranosid)-uronates were about 50%. The melting points, equivalent weights, and specific optical rotations of phenyl, p-chlorophenyl and p-cresyl β-D-glucuronides were determined and these are listed in Table I.

PREPARATION OF PHENYL β-D-GLUCOSIDES

A sample of phenyl β-D-glucoside was crystallized overnight from water at 4.5°C. The crystals were filtered, washed, and dried. The yield was 64%. The melting point and specific optical rotation were determined, and these are listed in Table II.

To synthesize p-chlorophenyl and p-cresyl β-D-glucosides, the method of Helferich and Schmitz-Hillebrecht (34) was employed. An intimate mixture of 0.0768 mole β-D-glucopyranose pentaacetate, 0.266 mole p-cresol or p-chlorophenol, and 0.0035 mole p-toluenesulfonic acid was fused for 1.5 hr. at 95-105°C. and 29.5 in. Hg vacuum. The cooled melt was dissolved in 400 ml. benzene and extracted with three 150-ml. portions 2N potassium hydroxide solution. The benzene solution was washed with water until the aqueous phase was neutral, and then treated with charcoal and anhydrous sodium sulfate for one hour. After filtering, the pale yellow solution was taken to a sirup which was dissolved in 200 ml. hot isopropanol. After crystallizing overnight at 4.5°C., the p-chlorophenyl and p-cresyl tetra-O-acetyl-β-D-glucosides were filtered, washed, and again crystallized from 200 ml. isopropanol. The yields were about 35%. The melting points were determined and these are listed in Table XXIII.

TABLE XXIII

MELTING POINTS OF PHENYL
TETRA-O-ACETYL-β-D-GLUCOSIDES

Aglycon Group	Melting Point, °C. Found	Literature
p-Chlorophenyl	123.5-24.5 (corrected)	123-24 (uncorrected) (44)
p-Cresyl	119-20 (corrected)	119-20 (42) 116-18 (corrected) (43)

To deacetylate, 2.0 meq. of methanolic sodium methoxide was added to a solution of 0.025 mole of the tetraacetate in 200 ml. dry methanol. After one hour at room temperature, the solution was taken to dryness while keeping the temperature below 35°C. The residue was slurried in 50 ml. water and neutralized with a few drops of acetic acid. The slurry was heated and crystals were allowed to form overnight at 4.5°C. The crystals were filtered, washed, and dried. The yields for the deacetylation were about 60%. The melting point and specific rotation of both p-chlorophenyl and p-cresyl β-D-glucoside were determined and these are listed in Table II.

APPENDIX II

MEASUREMENT OF RATE CONSTANTS

HYDROLYSIS PROCEDURE

Solutions were prepared which were 0.0200-0.0400M in glycoside and 2.00-20.0 wt. % in sulfuric acid. Four to six aliquots (ca. 1.9 ml.) were placed in glass ampoules with a syringe. The syringe was modified with a stop which allowed the barrel to be filled with a reproducible volume. The delivered volume was reproducible within ± 0.1%. The ampoules were sealed with a small gas-oxygen torch and then simultaneously plunged into an ethylene glycol bath. The bath temperature ranged from 50-60°C. and it could be held within ± 0.05°C. The bath thermometer was calibrated with a thermometer calibrated by the National Bureau of Standards. Each ampoule was removed at a predetermined time and plunged into an ice-water bath. After three minutes, the ampoule contents were quantitatively transferred to a calibrated 10 ml. volumetric flask and neutralized with standard sodium hydroxide solution. Upon dilution to volume, the neutrality of the hydrolyzate was verified with pH paper. A maximum of 2-3% hydrolysis was allowed.

ANALYSIS OF HYDROLYZATES

The reducing power of the hydrolyzates, as determined by the ferricyanide ion, was calibrated to measure the concentrations of products from which the hydrolysis rates could be calculated. The reducing power was measured automatically with Technicon's AutoAnalyzer. The hydrolyzate was mixed first with 3.6 times its volume of alkaline ferricyanide solution (0.177 g. per l. potassium ferricyanide and 20.0 g. per l. sodium carbonate) and then 0.75 times its volume of cyanide solution (10.0 g. per l. potassium cyanide). The resulting solution was heated to 94°C. for three minutes and then the unreacted ferricyanide determined

colorimetrically at 420 mμ. Each hydrolyzate was analyzed four times and the results averaged. Analyses could be reproduced within ± 0.75%.

Heating D-glucuronic acid, D-glucuronolactone, and D-glucose under the conditions of the hydrolyses resulted in no significant loss of reducing power due to acid degradation. Furthermore, D-glucuronic and D-glucuronolactone gave the same molar reducing power as sodium D-glucuronate monohydrate which indicated a rapid conversion of these forms to sodium D-glucuronate in the alkaline medium. In addition, it was found that all of the glycosides showed virtually no reducing power under the conditions of the analyses. Since it is well known that the acid hydrolysis of a glycoside yields principally the sugar and the aglycon, it was concluded that the reducing power of the hydrolyzates was a measure of the extent of hydrolysis.

The AutoAnalyzer was calibrated with equal molar solutions of D-glucose or sodium D-glucuronate monohydrate and the appropriate phenol. The D-glucose was obtained from the National Bureau of Standards and the sodium D-glucuronate monohydrate, $[\alpha]_D^{29}$ + 21.7° (water, c 2.10), was prepared according to the method of Hach and Benjamin (66). The literature lists $[\alpha]_D^{20}$ + 22.5° (water) (67) for sodium D-glucuronate monohydrate. Gas chromatography showed the phenol, p-chlorophenol, and p-cresol to be chromatographically pure. To prevent biological activity in the standard solutions, about 0.5% benzoic acid was added to the standards and they were kept at 4.5°C. when not in use. The calibration for the instrument was established with three to four analyses at four to six concentration levels.

APPENDIX III

CALCULATION OF RATE CONSTANTS

Although the hydrolysis of a glycoside at a given acid concentration and bath temperature occurred mostly while the aliquots were at the temperature of the constant temperature bath, small amounts of hydrolysis occurred before and after this period. The total hydrolysis period may be divided into the following time, t, periods:

t_o to t_1: The acid solution of the glycoside was prepared (t_o) and aliquots of this solution were placed in ampoules. During this period the temperature and acid concentration were essentially constant.

t_1 to t_2: All of the ampoules were plunged simultaneously into the constant temperature bath (t_1) and the temperature rose to that of the bath (t_2).

t_2 to t_3: Constant rate period with the temperature being that of the bath.

t_3 to t_4: Each ampoule was quenched at a predetermined time (t_3) causing the temperature to fall to that of the ice-water bath (t_4). The aliquot was then neutralized and diluted to a known volume.

The change in glycoside concentration during the period t_2 to t_3 may be expressed by the integrated form of the first-order rate equation (46).

$$- \ln \frac{C_3}{C_2} = k' (t_3 - t_2) \qquad (26)$$

where C_2, C_3 = glycoside concentration at t_2 and t_3, respectively

k' = first-order rate constant at the bath temperature.

The quantity $-\ln (C_3/C_2)$ may be written as follows:

$$-\ln \frac{C_3}{C_2} = -\ln \frac{C_4}{C_o} + \ln \frac{C_1}{C_o} + \ln \frac{C_2}{C_1} + \ln \frac{C_4}{C_3} \tag{27}$$

where C_o, C_1, C_4 = glycoside concentration at t_o, t_2, and t_4, respectively.

The change in glycoside concentration from t_o to t_1, t_1 to t_2, and t_3 to t_4 may be expressed with the first-order rate equation.

$$\ln \frac{C_1}{C_o} = -k_a (t_1 - t_o) \tag{28}$$

$$\ln \frac{C_2}{C_1} = -\int_{t_1}^{t_2} k_b \, dt \tag{29}$$

$$\ln \frac{C_4}{C_3} = -\int_{t_3}^{t_4} k_c \, dt \tag{30}$$

where k_a, k_b, k_c = first-order rate constants for t_1 to t_o, t_1 to t_2, and t_3 to t_4, respectively.

Substituting Equations (25), (28), (29), and (30) into Equation (27), and adding and subtracting $k' (t_2-t_1)$ from the resulting expression yields,

$$-\ln \frac{C_4}{C_o} = k' (t_3-t_1) - k' (t_2-t_1) + k_a (t_1-t_o) + \int_{t_1}^{t_2} k_b \, dt + \int_{t_3}^{t_4} k_c \, dt \tag{31}$$

The temperature paths from t_1 to t_2 and t_3 to t_4 as well as the quantities t_2-t_1 and t_1-t_o are the same for each ampoule. Hence, the last four terms in Equation (27) are constants.

$$-\ln \frac{C_4}{C_o} = k' (t_3 - t_1) + I \tag{32}$$

where I = constant.

Both the initial glycoside concentration, $\underline{C}_o$, and the glycoside concentration at $\underline{t}_4$, $\underline{C}_4$, may be calculated from measurable quantities. Hence, a plot of the logarithm of the fraction of the glycoside unreacted, $-\ln (\underline{C}_4/\underline{C}_o)$, versus the measurable quantity, $\underline{t}_3-\underline{t}_1$, will be a straight line. The slope of this straight line will be the first-order rate constant, $\underline{k}'$, and the intercept will be $\underline{I}$.

The following is an example of how the first-order rate constants were calculated. This example will be for phenyl β-D-glucuronide at 59.90 ± 0.05°C. in 20.0 wt. % sulfuric acid.

The initial glycoside concentration, $\underline{C}_o$, was calculated as follows:

$$C_o = \frac{W}{V_1} \cdot \frac{100-M}{100} = \frac{0.0771\ (100-6.2)}{9.99 \times 100} = 0.00724 \qquad (33)$$

where $\underline{C}_o$ = initial glycoside concentration, g. per ml.

$\underline{W}$ = weight of glycoside dissolved, g.

$\underline{V}_1$ = total volume of acidic glycoside solution, ml.

$\underline{M}$ = per cent moisture in glycoside sample

The final glycoside concentration, $\underline{C}_4$, was calculated from $\underline{C}_o$ and the concentration of sodium glucuronate in the neutralized and diluted ampoule, $\underline{C}_s$. It was assumed that each mole of phenyl β-D-glucuronide hydrolyzed produced one mole of D-glucuronic acid.

$$C_4 = C_o - \frac{M_{GA}}{M_S} \cdot \frac{V_3}{V_2} \cdot \frac{C_s}{10^5} \qquad (34)$$

$$= 7.24 \times 10^{-3} - \frac{270.23}{234.15} \cdot \frac{10.00}{1.9200} \cdot C_s \times 10^{-5}$$

$$= 7.24 \times 10^{-3} - 6.01 \times 10^{-5}\ C_s$$

where C_4 = concentration of glycoside at t_4, g. per ml.

M_{GA}, M_S = molecular weights of phenyl β-D-glucuronide and sodium glucuronate monohydrate, respectively

V_2 = volume of aliquot, ml.

V_3 = volume of diluted aliquot, ml.

C_s = concentration of sodium glucuronate monohydrate, mg. per 100 ml.

To obtain C_s, the AutoAnalyzer was calibrated with standard solutions so that the colorimetric measurements could be related to the sugar concentration. The following calibration data were obtained:

C_s, mg. per 100 ml.	Transmission, %
0.00	33.75
"	33.75
"	33.75
"	33.75
1.02	40.50
"	40.50
"	40.50
"	40.50
2.05	49.00
"	49.25
"	49.25
"	49.25
3.06	58.25
"	58.25
"	58.50
"	58.25

The calibration data were fitted by least squares to the following empirical equation:

$$\ln (\%T) = X + Y \cdot C_s + Z \cdot C_s^2 \qquad (35)$$

$$= 3.5174 + 0.1873 \cdot C_s - 0.025 \cdot C_s^2$$

where $\%\underline{T}$ = per cent transmission

$\underline{X},\underline{Y},\underline{Z}$ = constants

The transmission of each aliquot was measured four times and the results averaged. The values of $\underline{C}_s$ were then calculated from Equation (35), and these values used to calculate $\underline{C}_4$ from Equation (34). The quantity $\ln (\underline{C}_4/\underline{C}_o)$ could then be evaluated at various times, $\underline{t}_3-\underline{t}_1$.

Time, min.	Average Transmission, %	$\underline{C}_s$, mg. per 100 ml.	$\underline{C}_4$, g. per ml. x 10^3	$-\ln (\underline{C}_4/\underline{C}_o)$ x 10^3
20.00	38.1	0.66	7.20	5.54
45.00	44.4	1.51	7.15	12.6
65.00	49.4	2.10	7.11	17.6
90.00	56.7	2.89	7.07	24.3

A plot of $\ln (\underline{C}_4/\underline{C}_o)$ versus time is shown in Fig. 2. The slope of this plot was calculated by least-squares straight-line fit. An estimate of the standard deviation of the slope was also made. The first-order rate constant calculated was $2.67 \pm 0.04 \times 10^{-4}$ min.$^{-1}$.

The first-order rate constants for phenyl β-D-glucuronide at other acid concentrations and temperatures were calculated in the manner described above. Also, the first-order rate constants for the other glycosides at various levels of acid concentration and temperature were calculated by this method. The data relevant to these calculations are listed in Appendix IV, and the first-order rate constants are listed in Tables III to VIII. Duplicate rate constant determinations agreed within $\pm$ 2.0%.

APPENDIX IV

EXPERIMENTAL DATA*

ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDE AT 50.45±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
550.00	36.8	4.17
1,320.00	40.6	9.36
1,965.00	44.1	13.9
2,802.00	49.0	19.8

initial glycoside concentration = 0.00624 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.5268, $\underline{Y}$ = 0.1837, and $\underline{Z}$ = -0.0018

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
287.00	37.4	4.48
537.00	40.0	7.77
735.00	42.6	10.8
1,305.00	50.0	18.8

initial glycoside concentration = 0.00693 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.5268, $\underline{Y}$ = 0.1837, and $\underline{Z}$ = -0.0018

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
84.00	36.4	3.23
231.00	40.0	7.84
380.00	44.1	12.7
525.00	48.5	17.5

initial glycoside concentration = 0.00685 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.5268, $\underline{Y}$ = 0.1837, and $\underline{Z}$ = -0.0018

*The diluted volume of all aliquots was 10.00 ± 0.02 ml.

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
70.00	37.6	5.15
152.00	41.8	10.8
220.00	45.5	15.4
270.00	48.1	18.4

initial glycoside concentration = 0.00634 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.5268, $\underline{Y}$ = 0.1837, and $\underline{Z}$ = -0.0018

ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDE AT 55.15±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
295.00	37.6	4.74
548.00	40.1	7.69
760.00	43.5	11.5
1,325.00	51.2	19.5

initial glycoside concentration = 0.00715 g. per ml.
volume of glycoside solution per aliquot = 1.9196 ml.
calibration equation constants: $\underline{X}$ = 3.5196, $\underline{Y}$ = 0.1915, and $\underline{Z}$ = -0.0044

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
85.00	36.2	3.20
272.00	40.5	8.37
398.00	43.6	11.9
560.00	47.9	16.5

initial glycoside concentration = 0.00702 g. per ml.
volume of glycoside solution per aliquot = 1.9196 ml.
calibration equation constants: $\underline{X}$ = 3.5196, $\underline{Y}$ = 0.1915, and $\underline{Z}$ = -0.0044

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
56.00	38.0	4.44
100.00	40.5	6.87
113.00	41.5	7.81
285.00	54.3	18.9

initial glycoside concentration = 0.00851 g. per ml.
volume of glycoside solution per aliquot = 1.9196 ml.
calibration equation constants: $\underline{X}$ = 3.5196, $\underline{Y}$ = 0.1915, and $\underline{Z}$ = -0.0044

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
42.00	39.1	5.85
70.00	43.1	9.82
126.00	50.2	16.5
182.00	61.1	25.4

initial glycoside concentration = 0.00807 g. per ml.
volume of glycoside solution per aliquot = 1.9196 ml.
calibration equation constants: $\underline{X}$ = 3.5196, $\underline{Y}$ = 0.1915, and $\underline{Z}$ = 0.0044

ACID HYDROLYSIS OF PHENYL β-D-GLUCURONIDE AT 59.90±0.05°C.

Sulfuric Acid: 4.90 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
165.00	36.8	6.12
300.00	40.0	10.5
405.00	43.1	14.3
600.00	49.0	21.2

initial glycoside concentration = 0.00648 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.4809, $\underline{Y}$ = 0.1901, and $\underline{Z}$ = -0.0038

Sulfuric Acid: 6.96 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
90.00	35.9	4.65
195.00	40.3	10.1
275.00	44.0	14.4
390.00	49.0	19.8

initial glycoside concentration = 0.00696 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.4809, $\underline{Y}$ = 0.1901, and $\underline{Z}$ = -0.0038

Sulfuric Acid: 9.89 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
55.00	36.3	4.70
102.00	39.3	8.15
180.00	45.2	14.4
240.00	50.1	19.2

initial glycoside concentration = 0.00757 g. per ml.
volume of glycoside solution per aliquot = 1.9204 ml.
calibration equation constants: $\underline{X}$ = 3.4809, $\underline{Y}$ = 0.1901, and $\underline{Z}$ = -0.0038

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
35.00	37.6	4.87
80.00	42.7	10.7
110.00	46.5	14.7
190.00	57.4	25.0

initial glycoside concentration = 0.00722 g. per ml.
volume of glycoside solution per aliquot = 1.9200 ml.
calibration equation constants: $\underline{X}$ = 3.5174, $\underline{Y}$ = 0.1873, and $\underline{Z}$ = -0.0025

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Trans. mission, %	-ln (Fraction Unreacted) x 10^3
20.00	38.1	5.54
45.00	44.4	12.6
65.00	49.4	17.6
90.00	56.7	24.3

initial glycoside concentration = 0.00724 g. per ml.
volume of glycoside solution per aliquot = 1.9200 ml.
calibration equation constants: $\underline{X}$ = 3.5174, $\underline{Y}$ = 0.1873, and $\underline{Z}$ = -0.0025

ACID HYDROLYSIS OF p-CRESYL β-D-GLUCURONIDE AT 50.45±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
556.00	39.4	4.05
1,210.00	44.4	8.50
1,590.00	47.6	11.1
2,071.00	50.6	13.7

initial glycoside concentration = 0.00730 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5564, $\underline{Y}$ = 0.2566, and $\underline{Z}$ = -0.0133

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
180.00	38.6	3.09
380.00	41.8	5.82
564.00	44.4	7.94
1,200.00	56.5	17.1

initial glycoside concentration = 0.00783 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5564, $\underline{Y}$ = 0.2566, and $\underline{Z}$ = -0.0133

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
133.00	40.4	4.98
195.00	42.1	6.47
255.00	44.7	8.73
429.00	50.9	14.0

initial glycoside concentration = 0.00728 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5564, $\underline{Y}$ = 0.2566, and $\underline{Z}$ = -0.0133

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
60.00	40.1	5.13
122.00	43.4	8.30
180.00	47.9	12.5
240.00	51.9	16.0

initial glycoside concentration = 0.00673 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5564, $\underline{Y}$ = 0.2566, and $\underline{Z}$ = -0.0133

ACID HYDROLYSIS OF p-CRESYL β-D-GLUCURONIDE AT 55.15±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
228.00	38.8	3.66
415.00	41.2	6.09
590.00	43.8	8.51
1,225.00	53.3	17.0

initial glycoside concentration = 0.00653 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5642, $\underline{Y}$ = 0.2544, and $\underline{Z}$ = -0.0099

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
172.00	41.0	5.51
300.00	44.6	8.80
425.00	48.6	12.2
580.00	53.7	16.3

initial glycoside concentration = 0.00693 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5642, $\underline{Y}$ = 0.2544, and $\underline{Z}$ = -0.0099

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
42.00	39.0	3.59
105.00	43.1	7.29
162.00	47.3	10.9
216.00	51.5	14.4

initial glycoside concentration = 0.00702 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5642, $\underline{Y}$ = 0.2544, and $\underline{Z}$ = -0.0099

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
27.00	40.7	4.20
54.00	45.6	7.72
93.00	52.7	12.5
120.00	58.6	16.1

initial glycoside concentration = 0.00860 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5642, $\underline{Y}$ = 0.2544, and $\underline{Z}$ = -0.0099

ACID HYDROLYSIS OF p-CRESYL β-D-GLUCURONIDE AT 59.90±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
125.00	39.3	4.02
274.00	43.1	7.89
407.00	46.7	11.4
587.00	52.3	16.6

initial glycoside concentration = 0.00625 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5739, $\underline{Y}$ = 0.2495, and $\underline{Z}$ = -0.0082

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
67.00	41.4	4.38
115.00	45.2	7.10
225.00	54.4	13.1
284.00	60.1	16.4

initial glycoside concentration = 0.00882 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5739, $\underline{Y}$ = 0.2495, and $\underline{Z}$ = -0.0082

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
30.00	40.3	4.53
57.00	43.9	7.79
80.00	47.4	10.8
105.00	51.4	14.1

initial glycoside concentration = 0.00703 g. per ml.
volume of glycoside solution per aliquot = 1.9152 ml.
calibration equation constants: $\underline{X}$ = 3.5739, $\underline{Y}$ = 0.2495, and $\underline{Z}$ = -0.0082

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
16.00	40.0	4.34
29.00	43.6	7.66
46.00	48.2	11.8
60.00	52.5	15.5

initial glycoside concentration = 0.00665 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5720, $\underline{Y}$ = 0.2581, and $\underline{Z}$ = -0.0127

ACID HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCURONIDE AT 50.45±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
590.00	36.5	3.96
1,392.00	40.0	9.14
2,068.00	43.0	13.3
2,768.00	46.4	17.6

initial glycoside concentration = 0.00648 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5269, $\underline{Y}$ = 0.1864, and $\underline{Z}$ = -0.0003

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
300.00	36.7	4.17
570.00	39.0	7.57
715.00	40.4	9.49
1,380.00	46.9	17.9

initial glycoside concentration = 0.00661 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5269, $\underline{Y}$ = 0.1864, and $\underline{Z}$ = -0.0003

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
110.00	36.4	3.85
290.00	40.0	9.08
384.00	42.0	11.8
580.00	46.1	17.1

initial glycoside concentration = 0.00653 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5269, $\underline{Y}$ = 0.1864, and $\underline{Z}$ = -0.0003

Sulfuric Acid: 20.3 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
95.00	38.9	6.77
190.00	43.5	12.4
277.00	48.1	17.6
373.00	53.8	23.2

initial glycoside concentration = 0.00728 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5269, $\underline{Y}$ = 0.1864, and $\underline{Z}$ = -0.0003

ACID HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCURONIDE AT 55.15± 0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
300.00	37.3	3.92
527.00	39.5	7.03
711.00	41.0	9.08
1,305.00	47.0	16.7

initial glycoside concentration = 0.00668 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5468, $\underline{Y}$ = 0.1887, and $\underline{Z}$ = -0.0017

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
105.00	36.9	3.29
290.00	40.0	7.56
525.00	44.4	13.2
710.00	48.9	18.5

initial glycoside concentration = 0.00684 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5468, $\underline{Y}$ = 0.1887, and $\underline{Z}$ = -0.0017

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
75.00	39.2	4.97
196.00	45.6	11.3
279.00	51.2	16.2
380.00	57.6	21.2

initial glycoside concentration = 0.00887 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5468, $\underline{Y}$ = 0.1887, and $\underline{Z}$ = -0.0017

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
60.00	39.9	7.69
115.00	45.0	14.4
195.00	52.6	23.4
245.00	57.6	28.6

initial glycoside concentration = 0.00662 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5468, $\underline{Y}$ = 0.1887, and $\underline{Z}$ = -0.0017

ACID HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCURONIDE AT 59.90±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
199.00	38.6	5.55
435.00	43.5	11.7
612.00	47.8	16.6
765.00	51.4	20.4

initial glycoside concentration = 0.00754 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5423, $\underline{Y}$ = 0.1788, and $\underline{Z}$ = -0.0007

Sulfuric Acid: 8.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
130.00	40.0	7.46
216.00	43.4	11.7
309.00	47.7	16.6
425.00	53.4	22.6

initial glycoside concentration = 0.00749 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5423, $\underline{Y}$ = 0.1788, and $\underline{Z}$ = -0.0007

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
60.00	39.4	7.88
120.00	44.2	14.8
198.00	51.7	24.2
252.00	56.9	30.3

initial glycoside concentration = 0.00642 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5423, $\underline{Y}$ = 0.1788, and $\underline{Z}$ = -0.0007

Sulfuric Acid: 20.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
30.00	39.2	7.49
60.00	43.9	14.7
90.00	49.0	21.9
120.00	54.5	28.9

initial glycoside concentration = 0.00608 g. per ml.
volume of glycoside solution per aliquot = 1.9207 ml.
calibration equation constants: $\underline{X}$ = 3.5474, $\underline{Y}$ = 0.1818, and $\underline{Z}$ = -0.0020

ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDE AT 50.10±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
50.00	39.4	3.39
100.00	43.0	6.72
171.00	48.5	11.5
210.00	52.2	14.5

initial glycoside concentration = 0.00652 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5847, $\underline{Y}$ = 0.3052, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
27.00	40.1	3.81
50.50	44.0	7.29
81.00	49.6	11.8
106.00	54.5	15.5

initial glycoside concentration = 0.00683 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5847, $\underline{Y}$ = 0.3052, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
16.00	39.8	3.81
29.00	43.0	6.93
42.00	46.3	9.96
51.00	48.9	12.3

initial glycoside concentration = 0.00631 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5847, $\underline{Y}$ = 0.3052, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 6.60 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
12.00	37.1	3.49
24.00	40.0	7.13
36.00	42.8	10.4
48.00	45.6	13.5
60.00	48.9	17.1

initial glycoside concentration = 0.00648 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.5421, $\underline{Y}$ = 0.2315, and $\underline{Z}$ = -0.0014

Sulfuric Acid: 9.14 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
8.00	37.4	3.68
16.00	40.4	7.33
24.00	43.4	10.8
32.00	46.9	14.5
40.00	50.6	18.1
48.00	54.2	21.5

initial glycoside concentration = 0.00671 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.5421, $\underline{Y}$ = 0.2315, and $\underline{Z}$ = -0.0014

Sulfuric Acid: 10.0 wt %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
7.50	40.4	3.84
15.00	45.6	7.96
20.00	49.0	10.5
25.00	52.5	13.0

initial glycoside concentration = 0.00737 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5847, $\underline{Y}$ = 0.3052, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 12.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
5.00	37.5	4.41
10.00	40.0	7.86
15.00	42.5	11.1
20.00	45.5	14.9
25.00	48.4	18.2
30.00	51.6	21.8

initial glycoside concentration = 0.00587 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.5421, $\underline{Y}$ = 0.2315, and $\underline{Z}$ = -0.0014

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
5.00	41.2	4.52
10.00	47.4	9.48
14.00	52.6	13.2
19.00	59.1	17.6

initial glycoside concentration = 0.00729 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5847, $\underline{Y}$ = 0.3052, and $\underline{Z}$ = -0.0103

ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDE AT 55.00±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
27.00	41.5	3.52
50.00	46.0	6.71
82.00	52.7	11.0
105.00	58.0	14.3

initial glycoside concentration = 0.00792 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.6098, $\underline{Y}$ = 0.3129, and $\underline{Z}$ = -0.0101

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
15.00	39.2	4.24
27.50	44.0	8.02
40.00	49.0	11.7
54.00	54.8	15.6

initial glycoside concentration = 0.00764 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5351, $\underline{Y}$ = 0.3106, and $\underline{Z}$ = -0.0106

Sulfuric Acid: 6.49 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
6.00	34.9	3.31
12.00	37.2	6.43
18.00	39.8	9.71
24.00	42.6	13.0
30.00	45.6	16.4
36.00	48.6	19.6

initial glycoside concentration = 0.00612 g. per ml.
volume of glycoside solution per aliquot = 1.9678 ml.
calibration equation constants: $\underline{X}$ = 3.4848, $\underline{Y}$ = 0.2459, and $\underline{Z}$ = -0.0021

Sulfuric Acid: 8.98 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
4.00	35.0	3.42
8.00	37.5	6.80
12.00	40.1	10.1
16.00	43.1	13.7
20.00	46.2	17.2
24.00	49.6	20.8

initial glycoside concentration = 0.00609 g. per ml.
volume of glycoside solution per aliquot = 1.9678 ml.
calibration equation constants: $\underline{X}$ = 3.4848, $\underline{Y}$ = 0.2459, and $\underline{Z}$ = -0.0021

Sulfuric Acid: 11.9 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	36.9	3.75
6.00	39.8	7.52
9.00	42.9	11.4
12.00	46.4	15.4
15.00	50.2	19.6
18.00	53.9	23.4

initial glycoside concentration = 0.00627 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.5329, $\underline{Y}$ = 0.2363, and $\underline{Z}$ = -0.0050

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	40.0	4.91
6.00	47.0	10.3
8.00	52.4	14.0
10.00	58.2	17.7

initial glycoside concentration = 0.00764 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5351, $\underline{Y}$ = 0.3106, and $\underline{Z}$ = -0.0106

ACID HYDROLYSIS OF PHENYL β-D-GLUCOSIDE AT 59.95±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
14.00	42.1	3.48
23.00	46.1	6.02
41.00	54.6	10.8
53.00	61.1	14.2

initial glycoside concentration = 0.00894 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.6098, $\underline{Y}$ = 0.3129, and $\underline{Z}$ = -0.0101

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
9.00	42.0	4.86
14.25	45.6	7.94
21.25	50.9	12.3
28.00	55.8	16.0

initial glycoside concentration = 0.00650 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.6044, $\underline{Y}$ = 0.3200, and $\underline{Z}$ = -0.0152

Sulfuric Acid: 6.38 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	37.4	6.61
6.00	39.8	9.68
9.00	42.1	12.7
12.00	45.3	16.4
15.00	47.7	19.0
18.00	51.2	22.6

initial glycoside concentration = 0.00619 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.4939, $\underline{Y}$ = 0.2275, and $\underline{Z}$ = 0.0017

Sulfuric Acid: 8.83 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	36.1	4.22
5.00	38.9	7.69
7.00	41.6	10.8
9.00	45.0	14.4
13.00	51.8	20.9

initial glycoside concentration = 0.00687 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.4939, $\underline{Y}$ = 0.2275, and $\underline{Z}$ = 0.0017

Sulfuric Acid: 11.8 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	37.2	6.37
4.00	39.2	9.16
5.00	41.3	11.8
6.00	43.4	14.5
7.25	45.9	17.3
8.25	47.9	19.8

initial glycoside concentration = 0.00612 g. per ml.
volume of glycoside solution per aliquot = 1.9677 ml.
calibration equation constants: $\underline{X}$ = 3.4939, $\underline{Y}$ = 0.2275, and $\underline{Z}$ = 0.0017

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	47.9	8.30
4.00	53.9	12.3
5.00	59.9	16.0
6.00	65.6	19.4

initial glycoside concentration = 0.00772 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.6044, $\underline{Y}$ = 0.3200, and $\underline{Z}$ = -0.0152

ACID HYDROLYSIS OF p-CRESYL β-D-GLUCOSIDE AT 50.10±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
30.00	40.4	2.47
66.00	45.5	5.15
100.00	50.4	7.60
135.00	56.2	10.3

initial glycoside concentration = 0.00931 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5869, $\underline{Y}$ = 0.3923, and $\underline{Z}$ = -0.0251

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
17.00	40.0	2.80
37.00	45.1	6.20
51.00	48.5	8.37
69.00	53.4	11.3

initial glycoside concentration = 0.00739 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5869, $\underline{Y}$ = 0.3923, and $\underline{Z}$ = -0.0251

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
6.00	38.9	1.68
29.00	50.0	7.78
38.00	55.3	10.4

initial glycoside concentration = 0.00885 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5869, $\underline{Y}$ = 0.3923, and $\underline{Z}$ = -0.0251

Sulfuric Acid: 10.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
6.00	41.5	3.51
9.00	44.0	5.05
14.00	49.4	8.19
18.00	53.4	10.4

initial glycoside concentration = 0.00806 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5869, $\underline{Y}$ = 0.3923, and $\underline{Z}$ = -0.0251

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
4.00	42.8	3.86
5.00	44.9	4.98
8.00	50.8	8.03
10.00	55.0	10.1

initial concentration = 0.00903 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5869, $\underline{Y}$ = 0.3923, and $\underline{Z}$ = -0.0251

ACID HYDROLYSIS OF p-CRESYL β-D-GLUCOSIDE AT 55.00±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
20.00	41.5	3.18
40.50	45.9	6.09
60.00	51.0	9.28
75.25	54.6	11.4

initial glycoside concentration = 0.00736 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.6113, $\underline{Y}$ = 0.3899, and $\underline{Z}$ = -0.0242

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
8.00	39.6	2.40
16.00	43.5	5.15
24.00	48.0	8.15
35.00	53.9	11.8

initial glycoside concentration = 0.00763 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5961, $\underline{Y}$ = 0.3591, and $\underline{Z}$ = -0.0151

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
6.00	40.0	2.94
10.00	43.0	5.29
15.00	47.0	8.22
20.00	51.2	11.1

initial glycoside concentration = 0.00695 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5961, $\underline{Y}$ = 0.3591, and $\underline{Z}$ = -0.0151

Sulfuric Acid: 10.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	40.9	3.20
5.25	44.6	5.70
8.00	49.9	9.04
10.00	53.8	11.3

initial glycoside concentration = 0.00791 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5961, $\underline{Y}$ = 0.3591, and $\underline{Z}$ = -0.0151

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	43.0	5.17
4.00	46.1	7.39
5.00	48.6	9.18
6.00	52.0	11.4

initial glycoside concentration = 0.00711 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5961, $\underline{Y}$ = 0.3591, and $\underline{Z}$ = -0.0151

ACID HYDROLYSIS OF p-CRESYL β-D-GLUCOSIDE AT 59.95±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
8.00	40.1	2.28
17.00	44.4	5.25
29.00	49.9	8.77
40.25	55.9	12.4

initial glycoside concentration = 0.00720 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.6113, $\underline{Y}$ = 0.3899, and $\underline{Z}$ = -0.0242

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
5.00	39.1	3.01
9.00	42.8	5.92
14.00	46.8	8.89
18.00	50.6	11.6

initial glycoside concentration = 0.00655 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5708, $\underline{Y}$ = 0.3870, and $\underline{Z}$ = -0.0228

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	38.4	2.61
6.25	42.6	6.13
8.00	45.2	8.28
10.00	47.9	10.3

initial glycoside concentration = 0.00614 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5708, $\underline{Y}$ = 0.3870, and $\underline{Z}$ = -0.0228

Sulfuric Acid: 10.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
4.00	49.1	7.94
5.00	52.9	10.2
6.00	57.2	12.7

initial glycoside concentration = 0.00766 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.6136, $\underline{Y}$ = 0.3782, and $\underline{Z}$ = -0.0215

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	47.4	9.70
4.00	53.5	14.1
5.00	59.3	18.1
6.00	64.9	21.7

initial glycoside concentration = 0.00630 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5708, $\underline{Y}$ = 0.3870, and $\underline{Z}$ = -0.0228

ACID HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCOSIDE AT 50.10±0.05°C.

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
35.00	38.6	3.91
82.00	42.4	8.80
112.00	45.6	12.5
155.00	49.7	17.0

initial glycoside concentration = 0.00687 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5757, $\underline{Y}$ = 0.2406, and $\underline{Z}$ = -0.0003

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
18.00	38.4	3.29
37.00	42.5	6.82
53.00	46.0	9.66
71.00	49.8	12.5

initial glycoside concentration = 0.00829 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5542, $\underline{Y}$ = 0.2943, and $\underline{Z}$ = -0.0054

Sulfuric Acid: 10.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
9.00	37.5	3.15
18.00	40.9	7.13
28.00	44.4	10.9
36.00	47.0	13.5

initial glycoside concentration = 0.00640 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5542, $\underline{Y}$ = 0.2943, and $\underline{Z}$ = -0.0054

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
5.00	38.2	3.18
11.00	42.9	7.42
16.00	46.9	10.7
22.00	52.6	15.1

initial glycoside concentration = 0.00798 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.5542, $\underline{Y}$ = 0.2943, and $\underline{Z}$ = -0.0054

ACID HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCOSIDE AT 55.00±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
29.00	40.4	2.97
60.50	44.1	5.88
89.00	48.4	8.95
120.00	52.5	11.7

initial glycoside concentration = 0.00864 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.6058, $\underline{Y}$ = 0.3043, and $\underline{Z}$ = -0.0062

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
18.00	39.0	3.67
32.00	42.6	7.10
51.00	47.0	11.1
66.00	50.8	14.3

initial glycoside concentration = 0.00725 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5680, $\underline{Y}$ = 0.3060, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
10.00	39.4	3.73
20.00	42.9	6.88
29.00	46.8	10.0
38.00	51.0	13.3

initial glycoside concentration = 0.00786 g. per ml.
volume of glycoside solution per aliquot - 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5680, $\underline{Y}$ = 0.3060, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 10.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
5.50	39.9	4.06
10.00	43.6	7.23
15.00	48.2	10.9
20.00	53.3	14.7

initial glycoside concentration = 0.00809 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5680, $\underline{Y}$ = 0.3060, and $\underline{Z}$ = -0.0103

Sulfuric Acid: 14.0 wt %.

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
4.00	40.6	5.05
7.00	45.0	8.97
9.00	48.0	11.5
12.00	53.2	15.6

initial glycoside concentration = 0.00754 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5680, $\underline{Y}$ = 0.3060, and $\underline{Z}$ = -0.0103

ACID HYDROLYSIS OF p-CHLOROPHENYL β-D-GLUCOSIDE AT 59.95±0.05°C.

Sulfuric Acid: 2.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
16.00	39.5	2.99
30.00	42.4	6.02
42.00	44.5	8.15
59.00	47.9	11.4

initial glycoside concentration = 0.00653 g. per ml.
volume of glycoside solution per aliquot = 1.9214 ml.
calibration equation constants: $\underline{X}$ = 3.6058, $\underline{Y}$ = 0.3043, and $\underline{Z}$ = -0.0062

Sulfuric Acid: 4.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
8.00	39.7	3.19
16.00	43.4	6.89
25.00	47.3	10.6
33.00	51.3	14.1

initial glycoside concentration = 0.00684 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.6034, $\underline{Y}$ = 0.3018, and $\underline{Z}$ = -0.0071

Sulfuric Acid: 6.00 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
6.00	41.0	4.18
11.00	44.8	7.57
15.00	48.2	10.5
20.00	52.5	13.9

initial glycoside concentration = 0.00743 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.6034, $\underline{Y}$ = 0.3018, and $\underline{Z}$ = -0.0071

Sulfuric Acid: 10.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
4.00	40.1	5.23
6.00	43.0	8.14
8.00	46.0	11.1
10.00	49.9	14.6

initial glycoside concentration = 0.00687 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5664, $\underline{Y}$ = 0.2976, and $\underline{Z}$ = -0.0064

Sulfuric Acid: 14.0 wt. %

Time, min.	Average Transmission, %	-ln (Fraction Unreacted) x 10^3
3.00	41.3	6.47
4.25	44.7	9.83
5.00	47.1	12.1
6.25	50.5	15.2

initial glycoside concentration = 0.00685 g. per ml.
volume of glycoside solution per aliquot = 1.9205 ml.
calibration equation constants: $\underline{X}$ = 3.5664, $\underline{Y}$ = 0.2976, and $\underline{Z}$ = -0.0064

APPENDIX V

ALTERNATE INDUCTIVE EFFECT HYPOTHESIS FOR CARBOXYL STABILIZING EFFECT

The carboxyl stabilizing effect observed in this study may be explained by the hypothesis that both glycoside series hydrolyzed via an A-1 mechanism, but that the phenyl β-D-glucosides hydrolyzed via the A-1(A) mechanism while the phenyl β-D-glucuronides hydrolyzed via the A-1(B) mechanism. The mechanisms shown schematically in Fig. 1 suggest a reason, based on differences in inductive effects of the C_5 substituent, why replacing the C_5 hydroxymethyl group with a carboxyl group could cause a change in reaction mechanism. Although the greater inductive effect of the carboxyl group would decrease the tendency for the protonation (lower the basicity of the ring oxygen) required for the A-1(B) mechanism, it would tend to favor a greater rate of heterolysis (decrease the energy required to transfer the electron pair of the C_1-ring oxygen bond to the ring oxygen). Hence, the carboxyl group would either decrease or increase the hydrolysis rate by the A-1(B) mechanism. The introduction of the carboxyl group would tend to inhibit protonation, slow the heterolysis rate, and diminish carbonium ion stabilization in the A-1(A) mechanism (p. 64), and, therefore, tend to decrease the hydrolysis rate. Perhaps the opposing directions of the effects of the carboxyl group on the rate by the A-1(B) mechanism would cause the rate by the A-1(B) mechanism to be less affected by the introduction of the carboxyl group than the rate by the A-1(A) mechanism where all of the effects of the carboxyl group tend to decrease the rate. Thus, the rate by the A-1(A) mechanism may be decreased to such an extent that it becomes less than the rate by the A-1(B) mechanism.

Both the A-1(A) and A-1(B) mechanisms would be expected to have positive entropies of activation since the protonation and heterolysis steps should have

positive entropy changes (p. 7). It is difficult to say whether the formation of the two fragments in the A-1(A) transition state or the ring-opening in the A-1(B) transition state would cause the entropy of activation to be more positive. Hence, it is possible that the phenyl β-D-glucosides and phenyl β-D-glucuronides could hydrolyze via the A-1(A) and A-1(B) mechanisms, respectively, and yet have nearly identical entropies of activation (p. 55).

Polar aglycon effects on the A-1(B) mechanism inhibit the protonation step, decrease the rate of heterolysis, and diminish the stabilization of the carbonium ion (p. 57), while polar aglycon effects in the A-1(A) mechanism depend on inhibiting the protonation step and decreasing the rate of heterolysis (p. 57). If the dominant aspects of polar aglycon effects in the A-1(B) mechanism are either the inhibition of protonation* or the decrease of the heterolysis rate, the electrons important to these effects are those surrounding the ring oxygen and the electron pair constituting the C_1-ring oxygen bond. Since polar aglycon effects in the A-1(A) mechanism involve the electrons of the glycosidic oxygen and the electron pair of the C_1-glycosidic oxygen bond, the electrons important to the polar aglycon effects would be further removed from the aglycon group in the A-1(B) mechanism than in the A-1(A) mechanism, and, therefore, they would be less affected by changes in the electron affinity of the aglycon. Hence, one would expect to find lower reaction series constants for the phenyl β-D-glucuronide series than for the phenyl β-D-glucoside series (p. 13).

*If the phenyl β-D-glucosides are assumed to hydrolyze via the A-1(A) mechanism, inhibition of protonation is the dominate aspect of the polar aglycon effects (p. 57).