The Institute of Paper Chemistry

Appleton, Wisconsin

Doctor's Dissertation

Alkaline Hydrolysis of
Sodium Methyl α-D-Glucopyranosiduronate and
Methyl α-D-Glucopyranoside

J. Hamilton Robins

June, 1968

ALKALINE HYDROLYSIS OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE AND METHYL α-D-GLUCOPYRANOSIDE

A thesis submitted by

J. Hamilton Robins

B.S. 1963, Miami University
M.S. 1965, Lawrence University

in partial fulfillment of the requirements
of The Institute of Paper Chemistry
for the degree of Doctor of Philosophy
from Lawrence University,
Appleton, Wisconsin

June, 1968

TABLE OF CONTENTS

SUMMARY

From the results of previous kraft studies of 4-O-methylglucuronoxylan, it was hypothesized by others that the replacement of the C5 hydroxymethyl group with a carboxyl group will result in an "activating" effect which significantly increases the rate of cleavage of the glycosidic bond in hot alkali. A review of the literature indicates that the increased reactivity is probably due to a change in reaction mechanism. The magnitude and nature of this "activating" effect were investigated in the present research.

Sodium methyl α-D-glucopyranosiduronate (SMAG) was used as a model compound for 4-O-methylglucuronoxylan and methyl α-D-glucopyranoside (MAG) was used as a control. Both were allowed to react separately in molecular-oxygen-free 2.5N sodium hydroxide at 140-170°C. Pseudo-first-order rate constants were calculated from initial glycoside concentrations, methanol concentrations, and reaction time data. At 170°C., SMAG underwent glycosidic bond cleavage 280 times faster than MAG. The relationship between reaction rate and temperature satisfied the Arrhenius equation with the activation energy of SMAG (40.9 kcal. per mole) being greater than MAG (33.3 kcal. per mole).

The rate dependency on hydroxide ion concentration was ascertained by determining pseudo-first-order rate constants in 0.2-2.50N sodium hydroxide at 170°C. SMAG had a kinetic order of 0.9, and MAG had a value of 0.7. A kinetic order of 1.0 should have been obtained, but in nonideal solutions, salt and solvent effects, manifested in changes in activity coefficients, quite often result in nonunity kinetic orders, especially in solvolysis reactions. These theoretical considerations are noted and discussed.

The kinetic effect of varying nucleophile was investigated by allowing SMAG and MAG to react separately with alkaline sodium iodide and sodium chloride solutions at 158°C. Methanol analysis of the product solutions showed that the rate

of alkaline hydrolysis of both SMAG and MAG was not changed by the presence of a stronger nucleophile. The calculated relative rate constants between nucleophiles had near-unity values. These results indicated that the mechanism(s) of alkaline hydrolysis for SMAG and MAG did not involve nucleophilic bimolecular substitution.

The point of bond cleavage was determined by reacting SMAG and MAG in oxygen-18 (O^{18}) enriched 2.5N sodium hydroxide at 170°C. The methanol product was separated, and analyzed with a mass spectrometer. These results proved that the predominant point of cleavage for both SMAG and MAG was between the anomeric carbon atom and the glycosidic oxygen.

NMR analysis of SMAG and MAG in 0-2.5N sodium deuteroxide at 36°C. indicated the existence of a significant electron-releasing inductive effect that was realized at the anomeric carbon atom of both reactants. The upfield change in the chemical shift of the anomeric proton was used as an empirical measure of the inductive effect. This change was proportional to the hydroxide ion concentration. Hydroxyl groups, especially the C2 hydroxyl, of both reactants ionize at pH values of 13-14 to give alkoxyl anion functions which also exert electron-releasing inductive effects. In 2.5N sodium deuteroxide the accumulative inductive effects caused by the C5 carboxylate anion and the C2 alkoxyl anion in the SMAG molecule is approximately 20% greater than that for the MAG molecule under similar conditions. Since it is the ionized carboxyl and hydroxyl groups that are capable of strong electron-releasing ability, the proportionality between change in chemical shift and hydroxide ion concentration was taken as evidence of an initial base-catalyzed ionization equilibrium step. The association between electron density, screening constant, and chemical shift is discussed in relation to electron-releasing inductive effects.

Based on empirical results and theoretical considerations, it was concluded that SMAG and MAG undergo high-temperature alkaline hydrolysis by two different

reaction mechanisms. The replacement of the C5 hydroxymethyl group with a carboxyl group causes a large increase in reactivity due to a change in reaction mechanism. This change is associated with the electron-releasing ability of the carboxylate anion function. It is proposed that SMAG proceeds by a base-catalyzed unimolecular mechanism and that MAG proceeds by a base-catalyzed intramolecular displacement mechanism. Other possible reaction mechanisms are discussed.

INTRODUCTION

Hemicelluloses constitute the second largest fraction of carbohydrates in wood. The 4-O-methylglucuronoxylans are by far the most abundant polysaccharides in the hemicellulose group occurring in amounts ranging from 20-25% in hardwoods and 7-12% in softwoods (1). The presence of hemicelluloses in papermaking pulps significantly increases the strength and filtration properties of the pulp (2-5), while hemicelluloses present in pulps to be used for chemical conversion display certain detrimental effects (2, 6). Since hemicelluloses are accessible to pulping liquors, they are subject to changes during pulping. Therefore, the chemical behavior of hemicelluloses during alkaline pulping of wood and alkaline refining of wood pulp is of great importance to the pulp, paper, and chemical industries. In the future, knowledge of this kind could provide assistance in relating the physical properties of pulp to its chemical composition.

The most common hemicellulose found in wood is 4-O-methylglucuronoxylan, shown in Fig. 1. Xylose and 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-D-xylose, shown in Fig. 2, are the main products ordinarily obtained upon acid hydrolysis of this polymer (7, 8). During alkaline hydrolysis the 2-O-substituent is readily cleaved from the xylan backbone (9-11). It is the large difference in the reactivity of the glycosidic bond of the aldobiuronic acid that has led to considerable speculation as to the effect of the C5 carboxyl group. Alkaline pulping studies of this acidic hemicellulose have given qualitative determination of its reactivity and degradation products. However, the complicated system does not facilitate accurate, quantitative kinetic measurements. In order to achieve these goals a simple model compound for the aldobiuronic acid of 4-O-methylglucuronoxylan has been used in the present research. This is methyl α-D-glucopyranosiduronic acid, shown in Fig. 3, containing the alpha-linked glycosidic bond, the C5 carboxyl group, and the

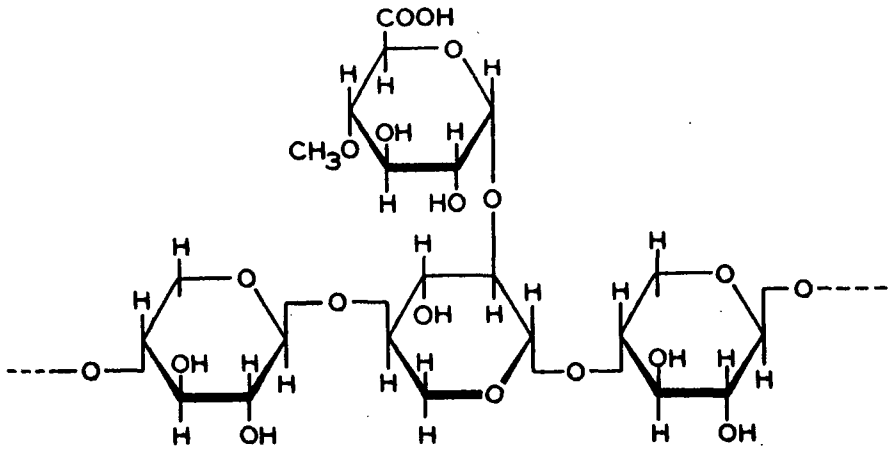

Figure 1. 4-O-Methylglucuronoxylan

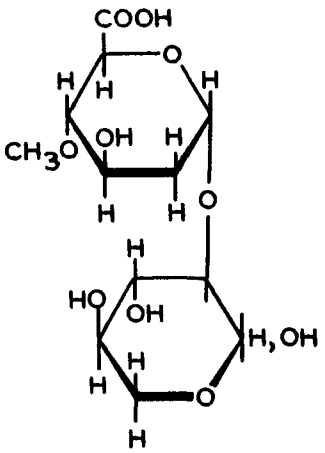

Figure 2. 2-O-(4-O-Methyl α-D-Glucopyranosyluronic Acid)-D-Xylose

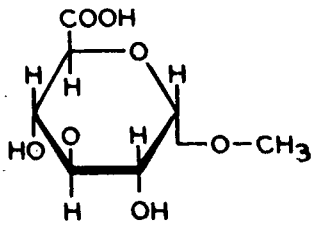

Figure 3. Methyl α-D-Glucopyranosiduronic Acid

conformation of the natural aldobiuronic acid. The methoxyl aglycon represents the xylose group. The absence of the 4-O-methyl substituent should have little or no effect on the reactivity of the glycosidic bond which is the point of concern. This model compound was used in this research to determine the rate of cleavage of the glycosidic bond and to investigate the effect of the C5 carboxyl substituent under alkaline pulping conditions.

DEGRADATION OF POLYSACCHARIDES IN ALKALI

The degradation of sugars and polysaccharides in dilute alkali at room temperature has been studied extensively. Speck (12) and Sowden and Schaffer (13) have reviewed the action of alkali on sugars while Whistler and Be Miller (14) have presented a summary of the degradation reactions of polysaccharides in dilute alkali at room temperature. In dilute, oxygen-free lime water, polysaccharides degrade to form saccharinic acids. The generally accepted mechanism of degradation is the β-alkoxy elimination reaction, commonly called the "peeling" reaction, which was first proposed by Isbell (15). The polysaccharide chains are degraded in a stepwise manner from the reducing end of each chain. The sugar units peeled from the chain rearrange to form saccharinic acids.

The behavior of 1 → 4 linked polysaccharides in strong alkali above room temperature has been studied less thoroughly, because these conditions are not suitable for structural analysis of polysaccharides, and the products of alkaline degradation undergo extensive side reactions. Limited research in this area has shown that the chain peeling mechanism is mainly responsible for the degradation of cellulose during treatment in alkali at the temperatures employed in alkaline refining, e.g., 2.5% sodium hydroxide at 100°C., and sulfate and soda pulping, e.g., 10% sodium hydroxide at 170°C. The presence of isosaccharinic acids and metasaccharinic acids in the alkaline degradation products of hydrocelluloses at

100 and 170°C. (16-18) as well as in sulfate pulping liquors (19) is supporting evidence for the "peeling" reaction. Collier (20) determined the kinetics of alkaline degradation of slash pine hemicellulose in 7.0 and 10% sodium hydroxide at 160-180°C. His results were in agreement with a stepwise end degradation mechanism.

In addition to the chain peeling reaction, Lindberg (21) and Richards (22) report that high-temperature alkaline degradation of cellulose and hemicelluloses may be caused by alkaline hydrolysis or random cleavage of the glycosidic bonds. Each random cleavage will form a new reducing end group which can initiate a new peeling process and further degradation. At room temperature the kinetic rate of the "peeling" reaction is many times that of random cleavage of glycosidic bonds. Brooks (23) and Best (24) have reviewed the relative importance of the random cleavage reaction as a function of temperature and pH. Corbett and Richards (25) have shown that thermal degradation is not a significant factor. Several investigators (9-11) have proposed that the increased rate of overall polymer degradation encountered with increase of pH above 11.0 is caused by an increase in the relative importance of the random cleavage of the glycosidic bonds. The results of Brooks (23), Best (24), and this research prove that random cleavage of the glycosidic bond does occur at a significant rate. Therefore, at higher temperatures, e.g., 140 to 170°C., and pH values of 11 to 14, the random cleavage reaction does become significant and possibly the rate-controlling mechanism of alkaline degradation of polysaccharides.

ALKALINE HYDROLYSIS OF GLUCOSIDES

The sensitivity of the phenyl glucosides to alkali has been long known; in 1894 Tanret (26) showed that the degradation of phenyl β-D-glucopyranoside yields 1,6-anhydro-β-D-glucopyranose. McCloskey and Coleman (27) found that

substitution on the glycosyl portion of phenyl β-D-glucopyranoside with alkali-stable groups could affect the alkaline degradation of this compound. Phenyl 2,3-di-O-methyl-β-D-glucopyranoside is stable to alkali, whereas phenyl 3-O-methyl-β-D-glucopyranoside is degraded readily to 1,6-anhydro-3-O-methyl-β-D-glucopyranose. From these results an intramolecular displacement of the phenoxyl aglycon by the C2 alkoxyl anion function was proposed to yield a 1,2-anhydro sugar intermediate. This unstable intermediate is attacked intramolecularly by the C6 hydroxyl anion or intermolecularly by the solvent or solvent conjugate base. The principal sources of experimental support for this mechanism are studies of products formed (27-29), effect of changes in the structure of the reactant (27-30) effect of hydroxide ion concentration (24, 29, 31), and behavior of a derivative of the proposed intermediate (28).

This double-inversion mechanism requires that the C1-C2 substituent configuration be trans; while the aglycon at C1 and alkyl substituent at C5 be cis. These requirements are satisfied in the β-anomers of phenyl glucosides which are always more reactive than the corresponding α-anomers (30, 32). However, inconsistencies arise because several phenyl glucosides which do not possess the requisite configuration for reaction by the double-inversion mechanism are unusually sensitive to alkali, and some are converted to 1,6-anhydro-D-hexopyranoses (32). To explain these results, McCloskey and Coleman (27) have suggested that the reaction may proceed by a slow intramolecular nucleophilic attack on C1 by the primary hydroxyl anion of C6 with the simultaneous removal of the phenoxyl radical. Other experimental data suggest the possibility of a unimolecular ionic dissociation mechanism (33).

The double-inversion mechanism has been used to explain alkaline hydrolysis of alkyl and aryl glucosides, in general. Brooks (23) and Best (24) have proposed an expanded version of the McCloskey-Coleman mechanism for the alkaline degradation

of methyl β-D-glucopyranoside and methyl β-cellobioside in 10% sodium hydroxide at 140-170°C. The reaction is base catalyzed, and the slow, rate-dependent intramolecular displacement step is preceded by a fast equilibrium step which yields the C2 hydroxyl anion function necessary for neighboring group participation. This proposed mechanism is shown schematically in Fig. 4.

Lindberg, et al. (21, 30) have done an extensive study on the alkaline hydrolysis of a number of glycosides under alkaline pulping conditions, and their results show in each case that the trans-glycosides are more reactive to alkali than the cis-glycosides. They add, however, that since the cis-glycosides show considerable reactivity, there must be other routes of importance other than by the proposed 1,2-anhydride intermediate. Janson and Lindberg (30) report that methyl β-D-glucopyranoside reacts at a rate that is only two times faster than the corresponding α-anomer. The results of Best (24) and this research confirm these results. Janson and Lindberg (30) report that methyl β-D-glucopyranoside is only two times more reactive than its 2-O-methyl analog in 10% sodium hydroxide at 170°C. Under similar conditions methyl α-D-glucopyranoside is only 1.25 times more reactive than its 2-O-methyl analog. Johnson (35) states that this small difference in rates is hardly enough to support the proposed neighboring group effect (C1-C2 intramolecular interaction to give a 1,2-anhydride intermediate). According to Gould (34) a neighboring group effect should give a 10 to 500-fold difference in reaction rates. The similar reactivity of the methyl 2-O-methyl glucosides is strong evidence of other major reaction mechanisms.

The latest work of Lindberg (36) reports the action of alkali on several methyl furanosides. All the furanosides displayed higher reaction rate constants than their corresponding glucopyranosides. These results suggest that the higher reactivity of the furanosides as a group is due to their lower conformational stability compared

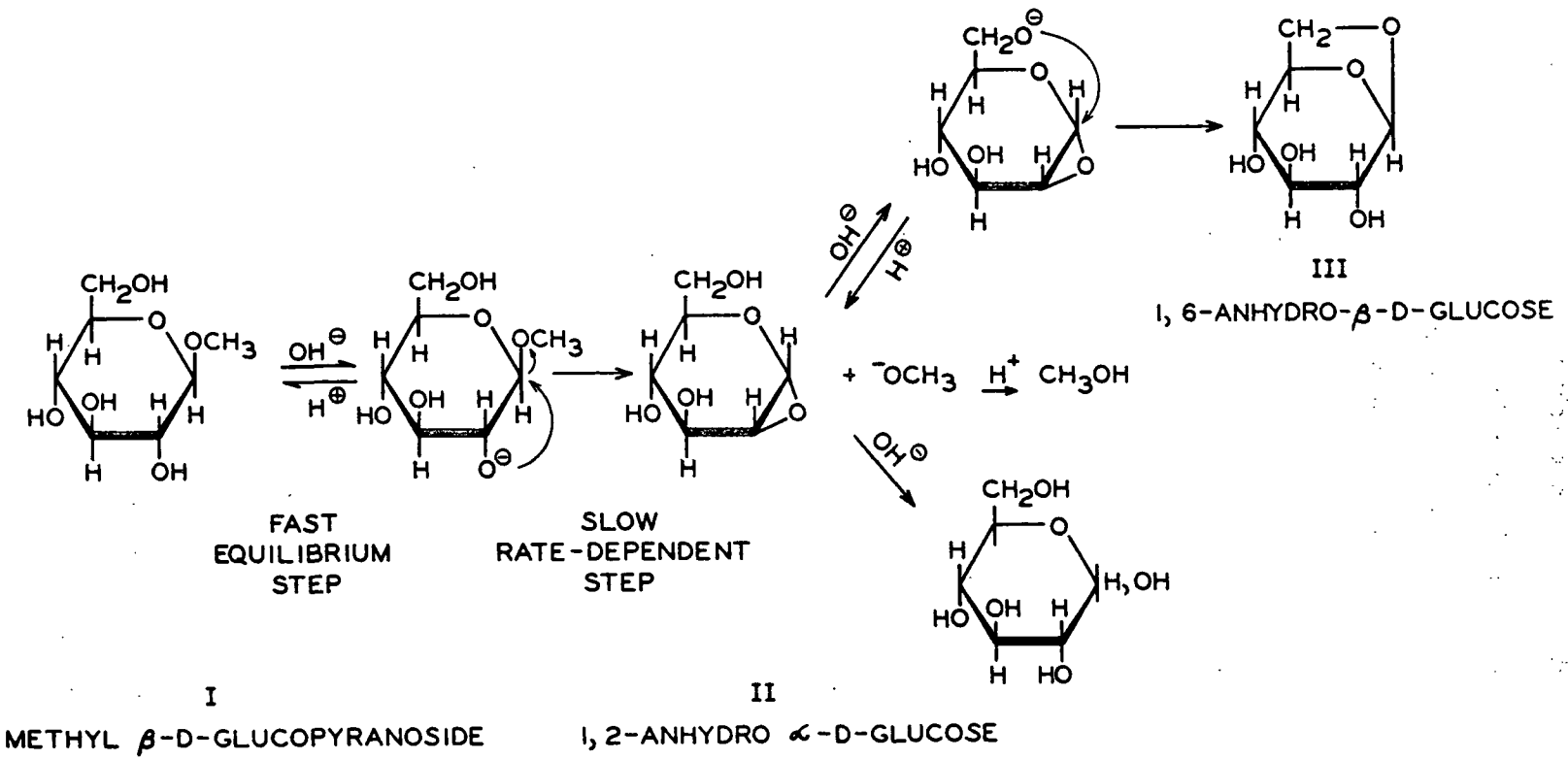

Figure 4. Proposed Base-Catalyzed Intramolecular Displacement Mechanism for the Alkaline Degradation of Methyl β-D-Glucopyranoside (23, 24)

to the glucopyranosides which have the most stable conformation. For the glucopyranosides the reactivity increases with the conformational instability of the most stable chain form (30).

Brooks (23) recently studied the effects of temperature, hydroxyl ion concentration, and molecular oxygen on the rate of cleavage of methyl β-D-glucopyranoside in 10% sodium hydroxide at 140-170°C. The presence of molecular oxygen was shown to increase the rate as much as forty-five times, whereas previous workers (25, 37, 38) reported that oxygen had no significant effect. This contribution by Brooks adds another parameter to the complicated problem of high-temperature alkaline degradation.

Gasman (29) investigated the C2 alkoxyl anion participation in the alkaline degradation of p-nitrophenyl β-D-galactoside and p-nitrophenyl α-D-mannoside. It was demonstrated that the rate was first-order with respect to glycoside and hydroxide ion concentrations. The reaction was base catalyzed with the base functioning to ionize the C2 hydroxyl group. After a fast equilibrium step, the slow rate-dependent step involved intramolecular attack at C1 by the C2 alkoxyl anion function with the simultaneous displacement of the phenoxyl radical. The 2-O-methyl analogs of these p-nitrophenyl glycosides underwent alkaline degradation at a much slower rate by a proposed aromatic nucleophilic substitution mechanism.

CARBOXYL ACTIVATING EFFECT

Several workers (29, 30, 33) have investigated the effects of various aglycons on the rate of alkaline hydrolysis of glycosides. However, very little work has been done on the effect of substitution in the glycon portion of the glycoside. Until this research, no one had compared the alkali sensitivity of the glucuronides to the glucosides or demonstrated the effect of replacing the hydroxymethyl group at C5 with a carboxyl group.

Hamilton and Thompson (9) cooked 4-O-methylglucuronoxylan by the kraft process in a study of the behavior of hemicelluloses during alkaline pulping of wood. The results of this work showed that the 4-O-methyl-D-glucuronic acid substituents were rapidly removed from the xylan polymer. Meier (10) did a similar study, and his results also showed that the 4-O-methyl-D-glucuronic acid substituents are cleaved by alkali, but at a slower rate than that reported by Hamilton and Thompson (9). More recently, Ross (39) determined from a comparative kraft pulping study of 4-O-methylglucuronoxylan and 4-O-methylglucoxylan at 170°C. that the 4-O-methyl-D-glucuronic acid substituents were split off 100 times faster than the 4-O-methyl glucose substituents.

These results support the hypothesis of Hamilton and Thompson (9) that the C5 carboxyl group displays an "activating" effect causing the glycosidic bond to be more sensitive to alkali.

Under acid conditions, Semke (40) has shown that phenyl glucuronides hydrolyze approximately 20 times slower than phenyl glucosides. It is postulated that both the glucuronides and glucosides undergo acid hydrolysis by the same A-1(A) (acid-catalyzed, unimolecular, cyclic) mechanism, but the carboxyl group of the glucuronides stabilizes the glycosidic bond by an electron-withdrawing inductive effect. It is assumed that the electronic effect is transmitted through the pyranose ring-oxygen to the glycosidic bond. This decreases the electron density of the glycosidic oxygen which thereby causes a decrease in the conjugate acid concentration and the rate of hydrolysis.

In alkali, the carboxyl group will be present as a carboxylate anion which normally has an electron-releasing ability. This effect may be transmitted through space or solvent molecules as a field effect or transmitted through chains of atoms as a positive (electron-releasing) inductive effect. The result of either would be

an increase in the electron density of the pyranose ring. If the electron-releasing effect of the carboxyl group is realized at the glycosidic bond, the covalent bond between the anomeric carbon and the glycosidic oxygen will be polarized with the shift in electron density toward the glycosidic oxygen. This electronic effect is shown diagrammatically in Fig. 5. Heterolysis of this covalent bond will be aided by a positive inductive effect. Ingold (41) states that unimolecular nucleophilic substitution (S_N1) reactions are always accelerated by positive inductive effects realized at the reactive center. He adds that a positive inductive effect may be strong enough to cause a change in mechanism from S_N2 or intramolecular nucleophilic substitution to unimolecular heterolysis.

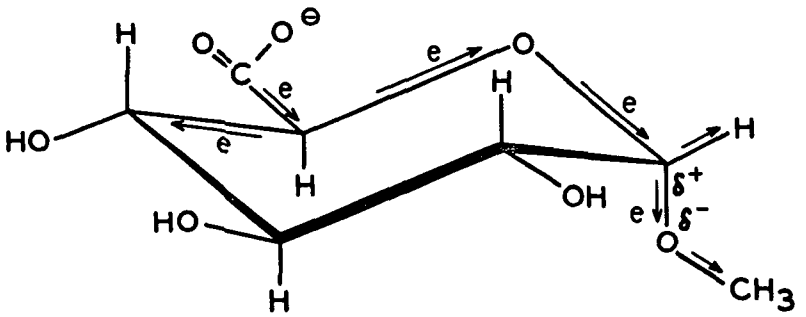

Figure 5. Diagram Showing Bond Polarization Due to the Electron-Releasing Ability of the C5 Carboxylate Anion

NUCLEOPHILIC SUBSTITUTION REACTIONS

A brilliant series of investigations by Hughes, Ingold, and coworkers (43-45) have provided excellent evidence for the existence of a mechanistic spectrum for nucleophilic substitution reactions at a saturated carbon atom.

The terms S_N1 (substitution, nucleophilic, unimolecular) and S_N2 (substitution, nucleophilic, bimolecular) refer to mechanisms at the extremes of the spectrum. Detailed discussions of the nucleophilic substitution mechanisms, including the spectrum intermediate of S_N1 and S_N2, are available in several sources (34, 41, 46, 47).

The physical and theoretical characteristics of nucleophilic substitution reactions are based on experimental studies at near room temperatures (e.g., 15 to 50°C.). It is difficult to say whether these characteristics apply to similar reactions at high temperatures (e.g., 100 to 200°C.). Very little is known about the exact physical, chemical, and thermodynamic properties of nonideal solutions at these elevated temperatures. The values of activity coefficients, and dissociation and equilibrium constants may change significantly from 20 to 170°C. No one has reported a rigorous investigation of a carbohydrate reaction system at 170°C. The effect of high temperature and pressure may cause a significant change in reaction mechanism (34). Conformational and stereochemical factors of the reactive species as well as their potential and kinetic energies may be markedly changed. Also, energy requirements for ionization, solvation, and nucleophilic substitution may be considerably different than those at room temperature. The unknown results of these temperature effects may permit the same or similar reaction mechanism to proceed or cause the incursion or complete change to a distinctly different mechanism.

BIMOLECULAR NUCLEOPHILIC SUBSTITUTION (S_N2)

The S_N2 mechanism is described as a one-step reaction in which one nucleophile (Lewis base) displaces another from a carbon atom. Formation of a covalent bond between the attacking nucleophile and the carbon atom is simultaneous with the heterolysis and cleavage of the initial bond between the carbon atom and the leaving

group. In the transition state, the attacking group and the leaving group are both partially bonded to the carbon atom. Formation of the transition state is the rate-controlling as well as the product-controlling step for the reaction [Equation (1)].

$$\underset{\text{Reactants}}{Y: + R:X} \rightleftarrows \underset{\substack{\text{Transition} \\ \text{state}}}{Y...R...X} \rightarrow \underset{\text{Products}}{Y:R + :X} \qquad (1).$$

The reaction results in a sterically inverted product and is frequently referred to as Walden inversion. The resultant steric inversion is readily discernible when substitution occurs at an asymmetric carbon atom of a reactant which has only one enantiomorphic form.

The kinetic rate exhibits first-order dependence on concentrations of attacking nucleophile and the compound experiencing substitution. The reaction rate is directly dependent on the nucleophilicity of the attacking reagent, the rate increasing with increasing nucleophilicity.

UNIMOLECULAR NUCLEOPHILIC SUBSTITUTION (S_N1)

The S_N1 mechanism consists of a preliminary heterolysis of the carbon-leaving group bond which yields an electron-deficient carbon atom (carbonium ion), which then reacts with the nucleophilic reagent [Equations (2) and (3)].

$$\underset{\text{Reactant}}{R:Y} \rightleftarrows \underset{\text{Transition state}}{R...Y} \rightleftarrows \underset{\substack{\text{Carbonium} \\ \text{ion}}}{R^+} + Y: \qquad (2)$$

$$R^+ + X \rightarrow R : X \qquad (3).$$

The preliminary heterolysis is normally, but not always (48), the slow rate-controlling step, while the combination of the carbonium ion with nucleophile is usually very rapid relative to heterolysis.

The reaction will normally exhibit first-order kinetics with the rate of reaction being dependent only on the concentration of the compound forming the carbonium ion. The rate will not depend on the nucleophilicity of the attacking reagent.

The products in an S_N1 reaction are not necessarily steric inverts as is the case by an S_N2 mechanism. For a carbonium ion formed from an asymmetric carbon atom the products can be inverted or racemized. The extent of inversion or racemization normally depends on the degree of association between the carbonium ion and the liberated anion. Steric factors can also influence the steric nature of the products.

Both S_N1 and S_N2 reaction mechanisms may display distinct salt and solvent effects depending upon the charge type of the reactants. A detailed discussion of these effects as they apply to the specific cases involved in this research is given later in the text.

SOLVOLYSIS REACTIONS

In solvolysis reactions, in which the solvent is the nucleophilic reagent, the mechanistic classification of the reaction is complicated by the fact that kinetic order is no longer diagnostic. Under these conditions, nucleophilic substitution reactions occurring by either mechanism will exhibit first-order kinetics. Only first-order rate dependence on electrophile concentration will be evident.

The rate equations for the S_N1 and S_N2 mechanisms are given in Equations (4) and (5).

$$S_N1: \quad \text{Rate} = k_1 [RY] \tag{4}$$

$$S_N2: \quad \text{Rate} = k_2 [RY][X] \tag{5}$$

where:

$[\underline{RY}]$ = electrophile concentration,

$[\underline{X}]$ = nucleophile concentration,

$\underline{k_1}$ = first-order rate constant, and

$\underline{k_2}$ = second-order rate constant.

If the substituting nucleophile, $\underline{X}$, is in large excess, its concentration will remain essentially constant as the reaction proceeds. The rate equation for the S_N2 mechanism [Equation (5)] then becomes

$$S_N2 \text{ (solvolysis): rate} = k'[RY] \tag{6}$$

where:

$$k' = k_2[X] = \text{constant} = \text{pseudo-first-order rate constant}, \tag{7}$$

and for a single reaction the mechanism is kinetically indistinguishable from the S_N1 mechanism [compare Equations (4) and (6)].

As predicted by Equation (7), the rate dependence on nucleophile concentration often may be ascertained by determining whether $\underline{k}'$ is a linear function of $[\underline{X}]$ for a series of reactions in which $[\underline{X}]$ is varied. Another method is to plot the same data according to Equation (8) [derived from Equation (7)] and verify that the slope is approximately equal to one, indicating a first-order dependence on the nucleophile concentration.

$$\log k' = \log k_2 + \log [X] \tag{8}.$$

The results must be interpreted judiciously as a variation of $[\underline{X}]$ can cause a dependence which is due to a change in reaction medium rather than nucleophile concentration.

EXPERIMENTAL RESULTS

ALKALINE DEGRADATION OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE AND METHYL α-D-GLUCOPYRANOSIDE

PSEUDO-FIRST-ORDER RATE CONSTANTS

Sodium methyl α-D-glucopyranosiduronate (0.025M SMAG[1]) and methyl α-D-glucopyranoside (0.025M MAG[2]) were allowed to react in oxygen-free 2.5N sodium hydroxide in stainless steel reaction tubes for 2-240 hours at 140-170°C. Pseudo-first-order rate constants were calculated from initial glycoside concentrations, methanol concentrations, and reaction times. Figures 6 and 7 show typical plots of the natural logarithm of the fraction of unreacted glycoside versus reaction time. The straight-line relationships indicate the degradations are first-order with respect to glycoside concentrations. The slope of each line is the pseudo-first-order rate constant. Tables I and II list the pseudo-first-order rate constants and their calculated standard deviations for the SMAG and MAG systems, respectively.

DETERMINATION OF REACTION STOICHIOMETRY

Critical experiments in this present research have proven that the stoichiometric ratio is 1:1 between both SMAG and MAG reactants and methanol product in 2.5N sodium hydroxide at 140-170°C. Radiochemical analysis of C-14 labeled SMAG gave a pseudo-first-order rate constant of 1.01×10^{-1} hr.$^{-1}$ which differed by 5% from the rate constant of 0.965×10^{-1} hr.$^{-1}$ determined by colorimetric analysis of the methanol product. Similar determinations of the MAG system gave agreement

---

[1] SMAG = sodium methyl α-D-glucopyranosiduronate.

[2] MAG = methyl α-D-glucopyranoside.

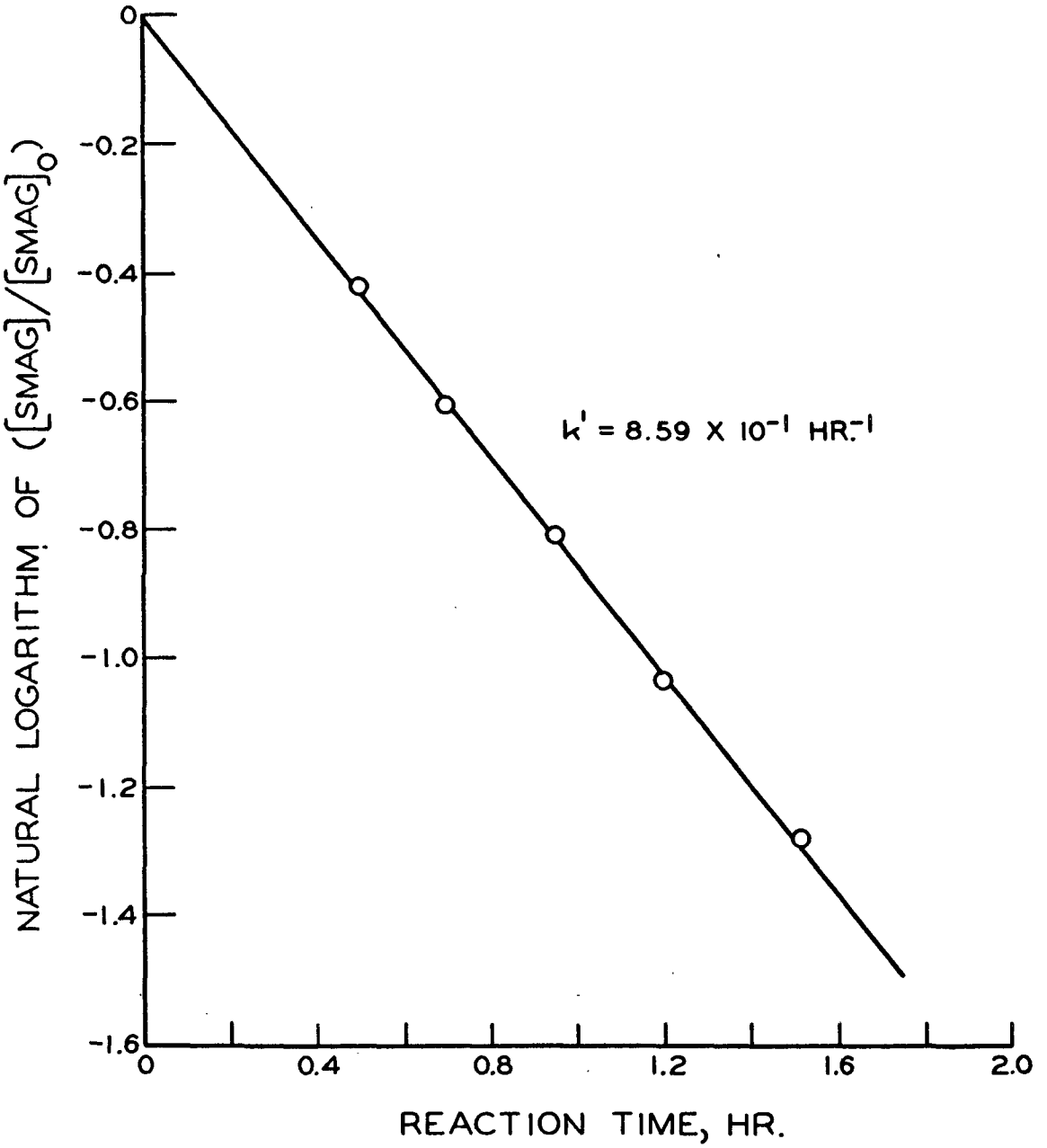

Figure 6. Alkaline Degradation of Sodium Methyl α-D-Glucopyranosiduronate (0.0245M) in Oxygen-Free 2.53N Sodium Hydroxide at 170.2°C.

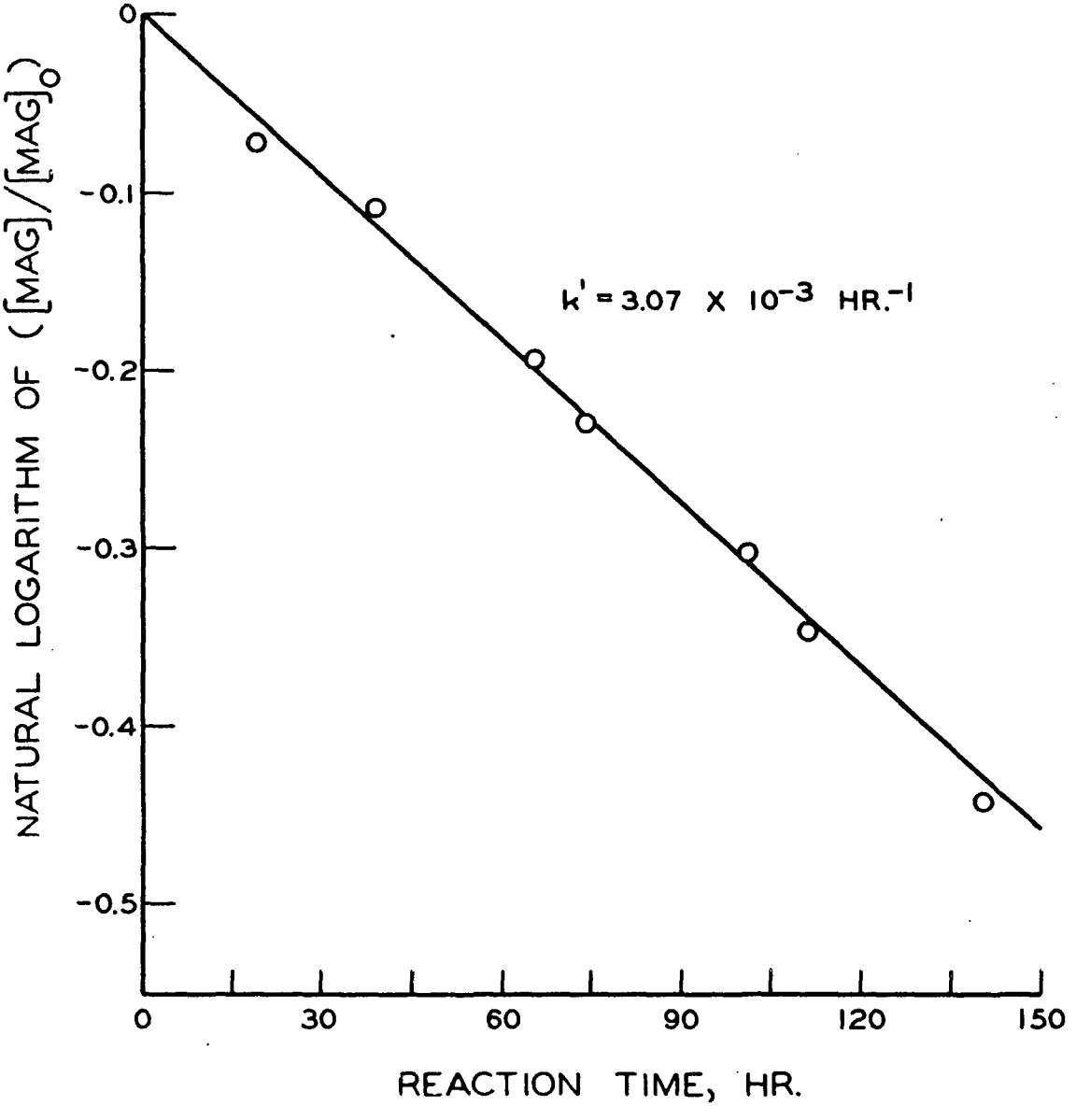

Figure 7. Alkaline Degradation of Methyl α-D-Glucopyranoside (0.0251M) in Oxygen-Free 2.57N Sodium Hydroxide at 170.1°C.

TABLE I

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE ALKALINE DEGRADATION OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE

Temperature, ± 0.5°C.	Initial Reactant Concn., M	Hydroxyl Ion Concn., N[a]	k' x 10, hr.$^{-1}$
139.3	0.0250	2.500	0.265 ± 0.010
149.6	0.0250	2.501	0.872 ± 0.026
150.8	0.0244	2.489	0.965 ± 0.032
158.1	0.0250	2.507	2.36 ± 0.04
170.2	0.0245	2.532	8.59 ± 0.11
170.0	0.1004	2.510	8.79 ± 0.12

[a]Actual hydroxyl ion concentration at the specific reaction temperature.

TABLE II

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE ALKALINE DEGRADATION OF METHYL α-D-GLUCOPYRANOSIDE

Temperature, ± 0.5°C.	Initial Reactant Concn., M	Hydroxyl Ion Concn., N[a]	k' x 10^3 hr.$^{-1}$
139.3	0.0251	2.500	0.181 ± 0.010
150.4	0.0251	2.490	0.526 ± 0.015
158.4	0.0251	2.510	1.07 ± 0.02
160.0	0.0250	2.501	1.33 ± 0.07
170.1	0.0251	2.574	3.07 ± 0.11

[a]Actual hydroxyl ion concentration at the specific reaction temperature.

within 4% and a calculated rate constant of 3.18×10^{-3} hr.$^{-1}$ from radiochemical analysis and 3.07×10^{-3} hr.$^{-1}$ from methanol analysis. The experimental data and calculated rate constants for both SMAG and MAG systems are given in Appendix I.

EFFECT OF HYDROXIDE ION CONCENTRATION

The rate dependency on hydroxide ion concentration was ascertained by determining pseudo-first-order rate constants at various hydroxide ion concentrations. The SMAG system exhibited a linear relationship between the pseudo-first-order rate constant and hydroxide ion concentration and had a rate dependency of 0.90 on hydroxide ion concentration at 158°C. and 0.88 at 170°C. The MAG system exhibited a curvilinear relationship between the same variables and a rate dependency of 0.69 on hydroxide ion concentration at 170°C.

Sodium methyl α-D-glucopyranosiduronate (0.025M SMAG) and methyl α-D-glucopyranoside (0.025M MAG) were allowed to react in oxygen-free 0.2-2.50N sodium hydroxide at 158 and 170°C. Tables III and IV list the pseudo-first-order rate constants and their calculated standard deviations for the SMAG and MAG systems, respectively. Figures 8 and 9 show plots of pseudo-first-order rate constant versus hydroxide ion concentration, and Fig. 10 and 11 show the natural logarithm plots of these data. As discussed in the Introduction, first-order dependence on hydroxide ion concentration is evidenced by a linear relationship between the pseudo-first-order rate constant and hydroxide ion concentration. A near-unit slope for the log-log plot of these variables verifies a first-order rate dependency on hydroxide ion concentration.

EFFECT OF TEMPERATURE

Rate dependency on reaction temperature can usually be represented by an empirical equation proposed by Arrhenius (49).

TABLE III

PSEUDO-FIRST-ORDER RATE CONSTANTS AS A FUNCTION OF HYDROXYL ION CONCENTRATION FOR THE ALKALINE DEGRADATION OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE

Temperature, ± 0.5°C.	Initial Reactant Concn., M	Hydroxyl Ion Concn., N	$k' \times 10^{1}$, $hr.^{-1}$
158.1	0.0250	2.507	2.36 ± 0.04
158.1	0.0249	1.229	1.24 ± 0.06
158.3	0.0251	0.5884	0.671 ± 0.047
158.1	0.0246	0.2270	0.278 ± 0.013
170.2	0.0245	2.532	8.59 ± 0.11
170.1	0.0252	0.2251	1.01 ± 0.02

TABLE IV

PSEUDO-FIRST-ORDER RATE CONSTANTS AS A FUNCTION OF HYDROXYL ION CONCENTRATION FOR THE ALKALINE DEGRADATION OF METHYL α-D-GLUCOPYRANOSIDE

Temperature, ± 0.5°C.	Initial Reactant Concn., M	Hydroxyl Ion Concn., N	$k' \times 10^{3}$, $hr.^{-1}$
170.1	0.0251	2.574	3.07 ± 0.11
170.1	0.0251	1.269	1.91 ± 0.06
170.6	0.0253	0.6398	1.24 ± 0.04
170.9	0.0257	0.2296	0.601 ± 0.028
169.9	0.0251	0.188	0.476 ± 0.019

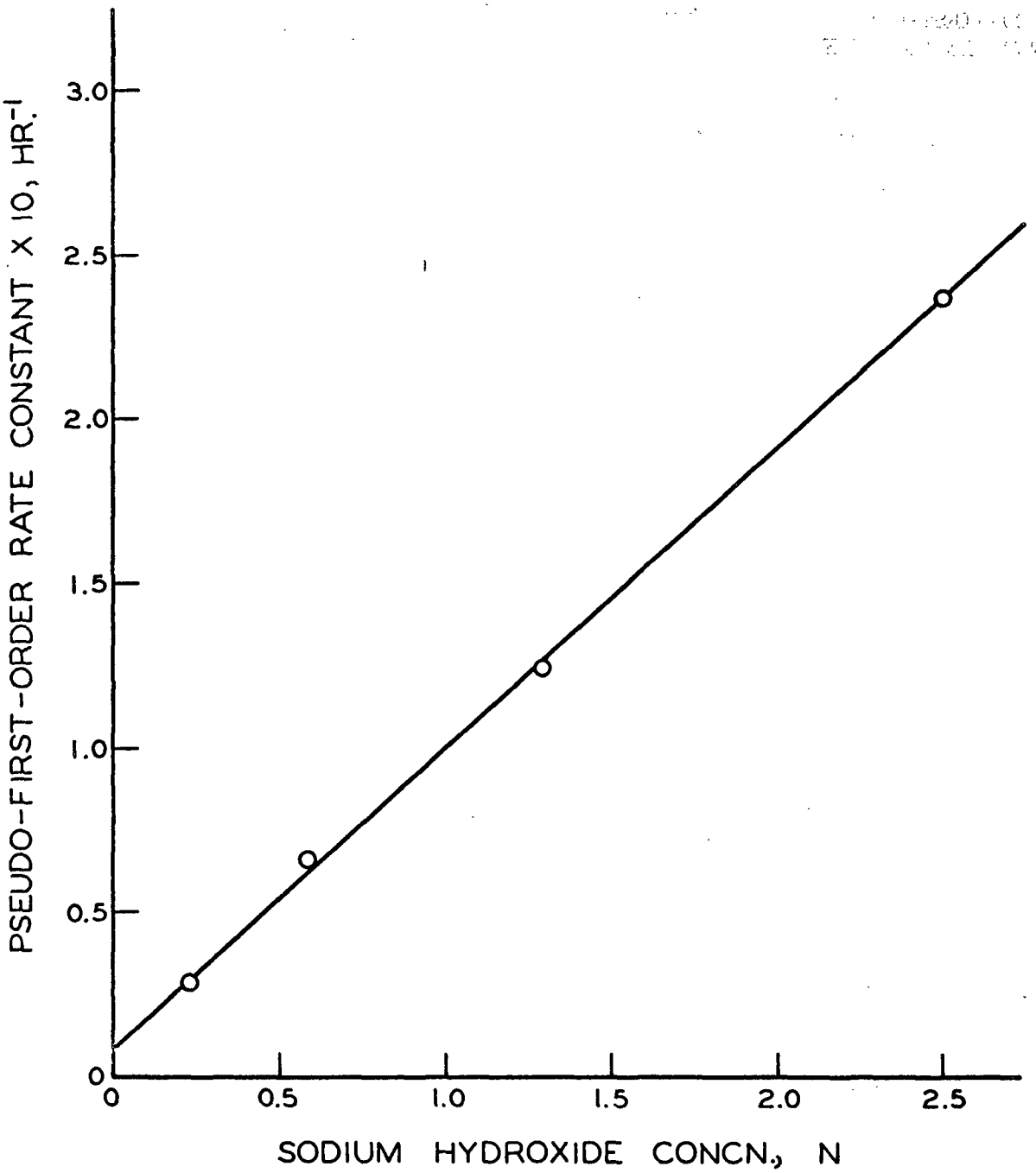

Figure 8. Effect of Hydroxide Ion Concentration on the Rate of Alkaline Degradation of Sodium Methyl α-D-Glucopyranosiduronate (0.025M) at 158.1°C.

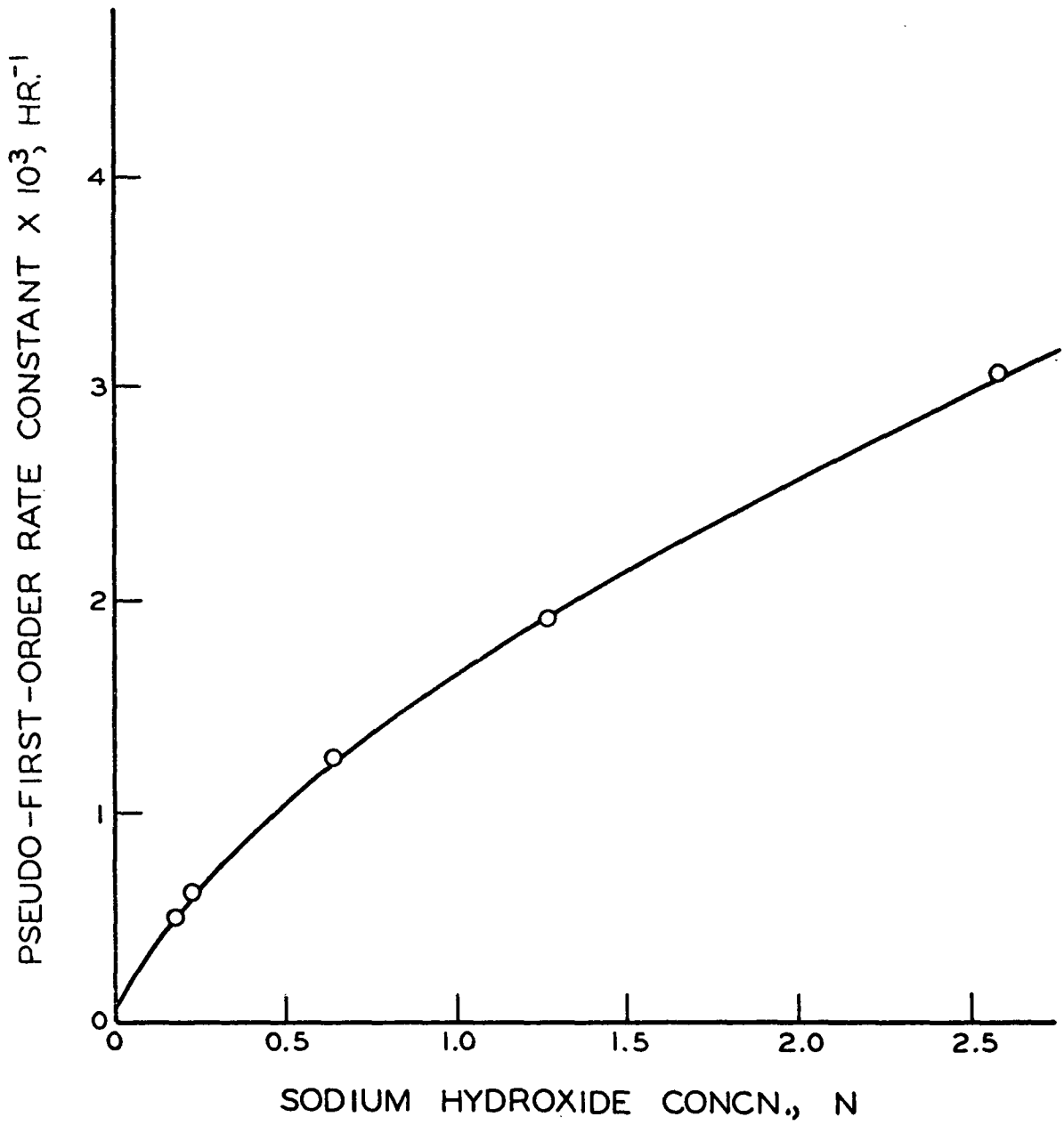

Figure 9. Effect of Hydroxide Ion Concentration on the Rate of Alkaline Degradation of Methyl α-D-Glucopyranoside (0.025M) at 170.6°C.

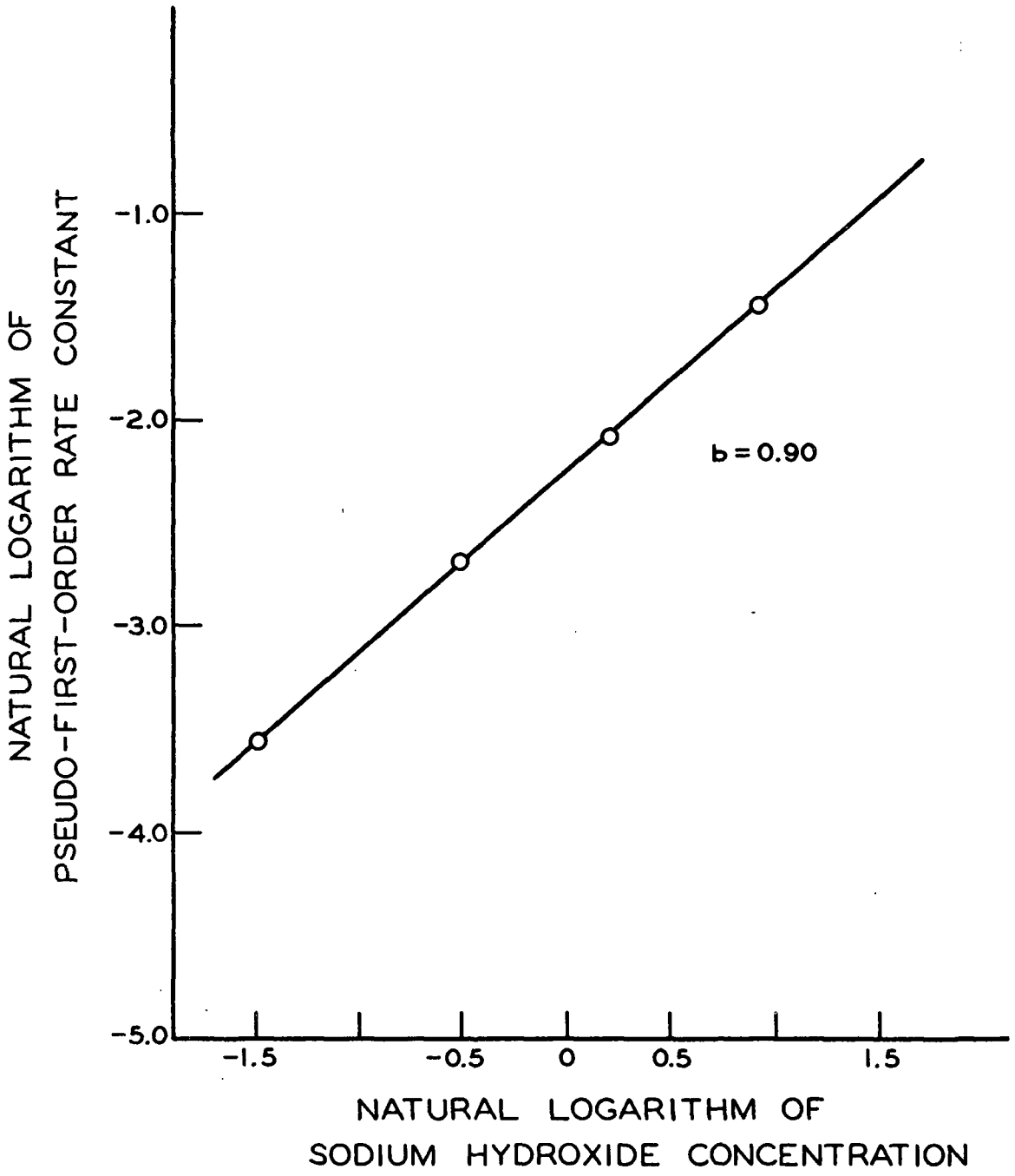

Figure 10. Kinetic Order with Respect to Hydroxide Ion Concentration for the Alkaline Degradation of Sodium Methyl α-D-Glucopyranosiduronate (0.025M) at 158.1°C.

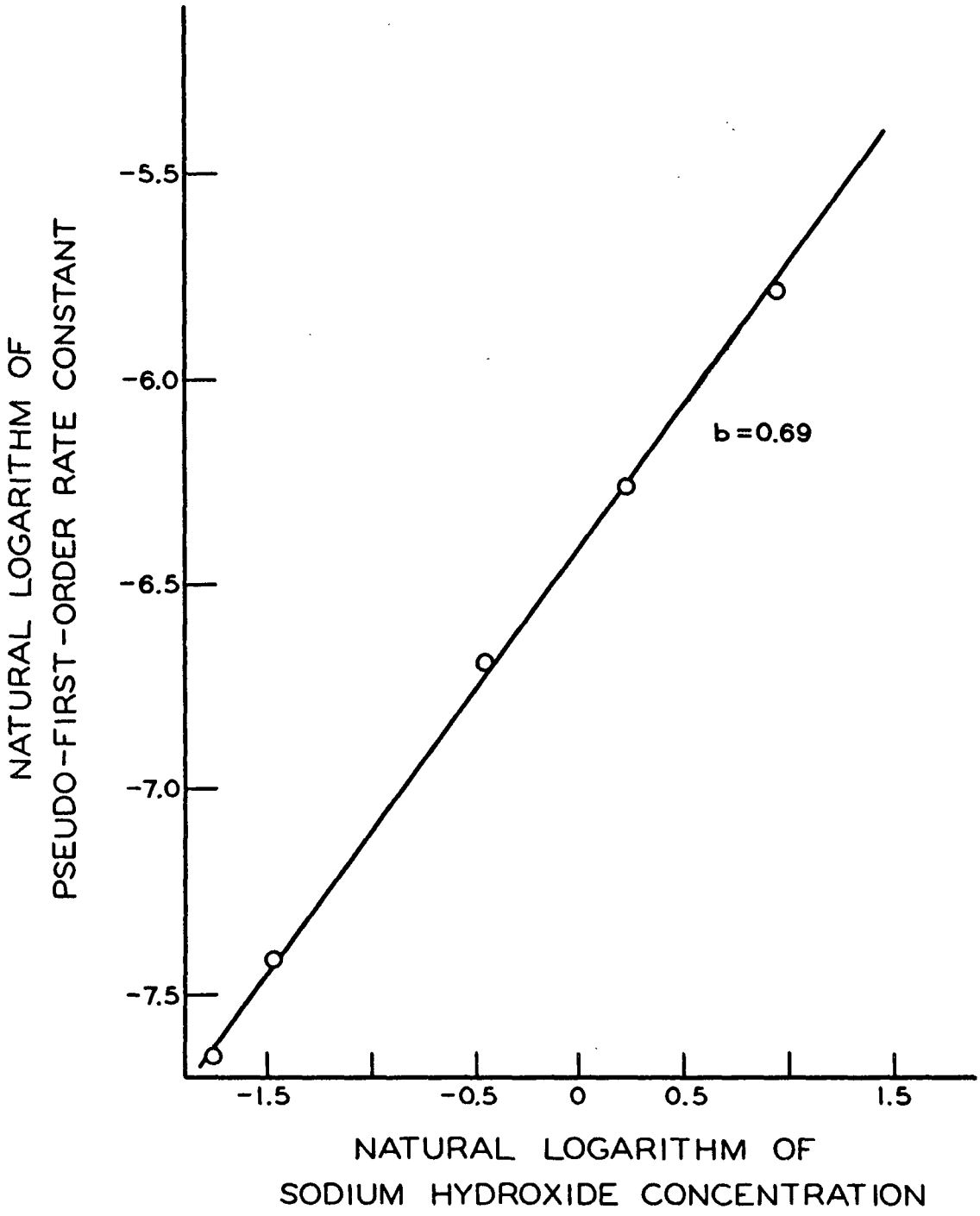

Figure 11. Kinetic Order with Respect to Hydroxide Ion Concentration for the Alkaline Degradation of Methyl α-D-Glucopyranoside (0.025M) at 170.6°C.

$$k = Ae^{-E_a/RT} \qquad (9)$$

where:

$\underline{k}$ = rate constant, $sec.^{-1}$,

$\underline{A}$ = frequency factor, $sec.^{-1}$,

$\underline{E_a}$ = Arrhenius activation energy, $cal.\text{-}mole^{-1}$,

$\underline{R}$ = gas constant, 1.987 $cal.deg.^{-1}\ mole^{-1}$, and

$\underline{T}$ = absolute reaction temperature, °K.

The logarithmic form of Equation (9) is as follows:

$$\ln k = (-E_a/R)\frac{1}{T} + \ln A \qquad (10).$$

According to Equation (10), a straight line should be obtained when the logarithm of the rate constant is plotted against the reciprocal of the absolute reaction temperature, and the Arrhenius activation energy is calculated from the slope of this line.

Listed in Table V are the calculated Arrhenius activation energies and their standard deviations obtained by least squares fit of ln $\underline{k}$ versus $1/\underline{T}$. Sodium methyl α-D-glucopyranosiduronate in oxygen-free 2.50$\underline{N}$ sodium hydroxide at 140-170°C. had an Arrhenius activation energy of 40.9 kcal. per mole. Under similar conditions, methyl α-D-glucopyranoside had an $\underline{E_a}$ value of 33.3 kcal./mole. Both the SMAG and MAG systems had constant slope Arrhenius plots which is indicative of a single reaction mechanism occurring over the investigated temperature range (34). Figures 12 and 13 show these Arrhenius plots. Figure 14 shows the Arrhenius plot for the SMAG system at varying hydroxide ion concentrations. The similar slopes indicate that the Arrhenius activation energy is independent of hydroxide ion concentration.

TABLE V

ARRHENIUS ACTIVATION ENERGIES FOR THE ALKALINE DEGRADATION OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE AND METHYL α-D-GLUCOPYRANOSIDE

System	Hydroxide Ion Concn., N	Temperature Range, ± 0.5°C.	E_a, kcal. mole^{-1}
SMAG	2.50	139.3-170.2	40.9 ± 0.4
SMAG	1.230	158.1-170.1	40.5
SMAG	0.590	158.3-170.1	41.1
SMAG	0.230	158.1-170.1	40.6
MAG	2.50	139.3-170.1	33.3 ± 0.3

EFFECT OF VARYING NUCLEOPHILE

The rate of alkaline degradation of SMAG and MAG was experimentally shown to be essentially independent of the nucleophilicity of the attacking reagent. Sodium methyl α-D-glucopyranosiduronate (0.3M) and methyl α-D-glucopyranoside (0.5M) were allowed to react separately in 1.25N sodium chloride-1.25N sodium deuteroxide and 1.25N sodium iodide-1.25N sodium deuteroxide in sealed glass NMR tubes at 158°C. Samples were analyzed with an NMR spectrometer and each spectrum contained distinct peaks for the protons of methanol and the methoxyl aglycon of the specific reactant. The integral of the spectra was used to measure the heights of the methanol and methoxyl peaks. Calibration studies proved that the ratio of methanol peak height to methoxyl peak height was not equal to but proportional to the true concentration ratio of methanol product to methyl α-glycoside. A relative rate constant (k_{NaI}/k_{NaCl}) was calculated by dividing the peak height ratio (h_{MeOH}/h_{MeO}-), determined in alkaline sodium iodide, by the corresponding ratio determined in alkaline sodium chloride. Table VI lists these results. Both SMAG and MAG had relative rate constants of approximately one. If the alkaline

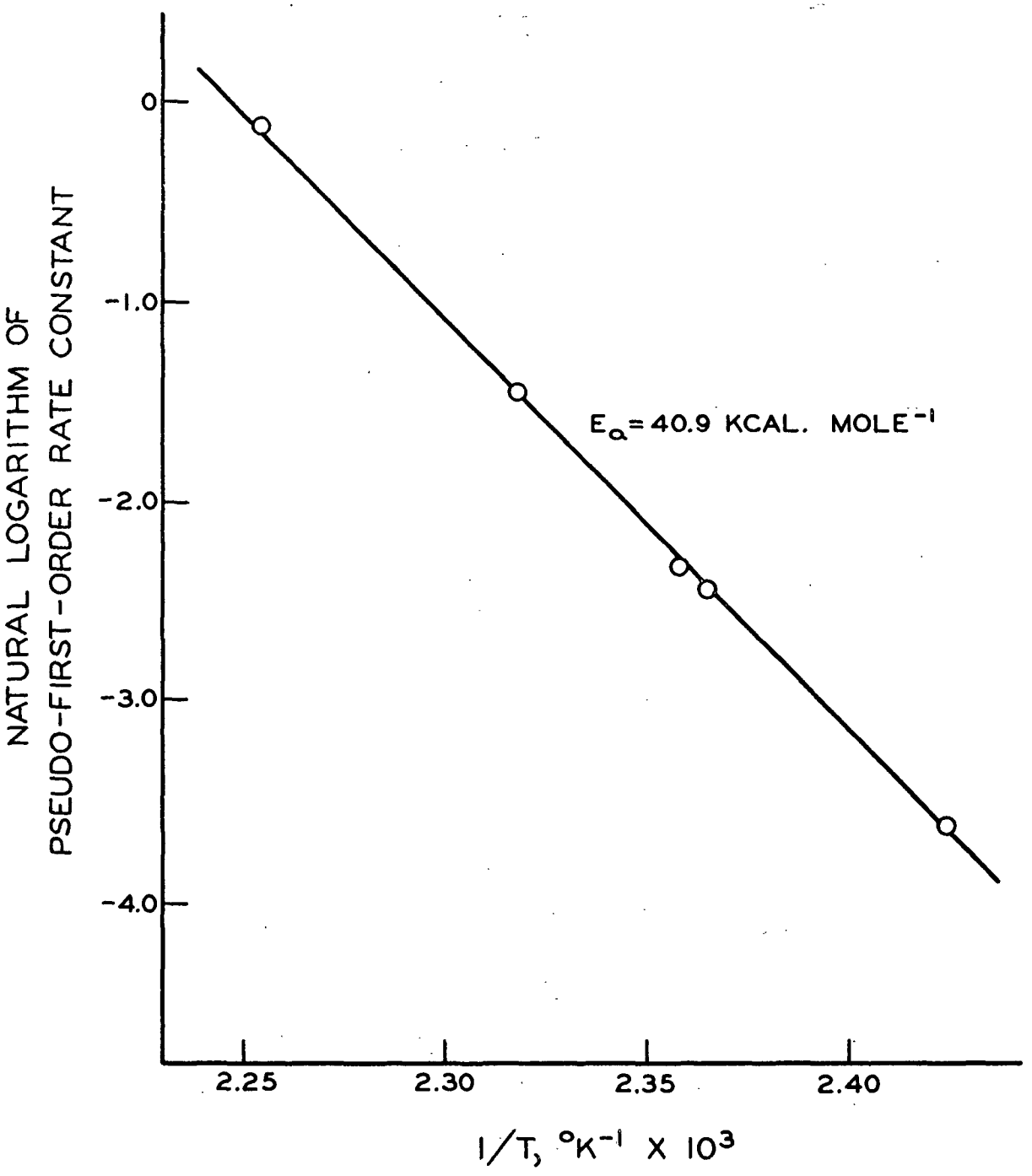

Figure 12. Arrhenius Plot for the Alkaline Degradation of Sodium Methyl α-D-Glucopyranosiduronate (0.025M) in Oxygen-Free 2.5N Sodium Hydroxide at 140-170°C.

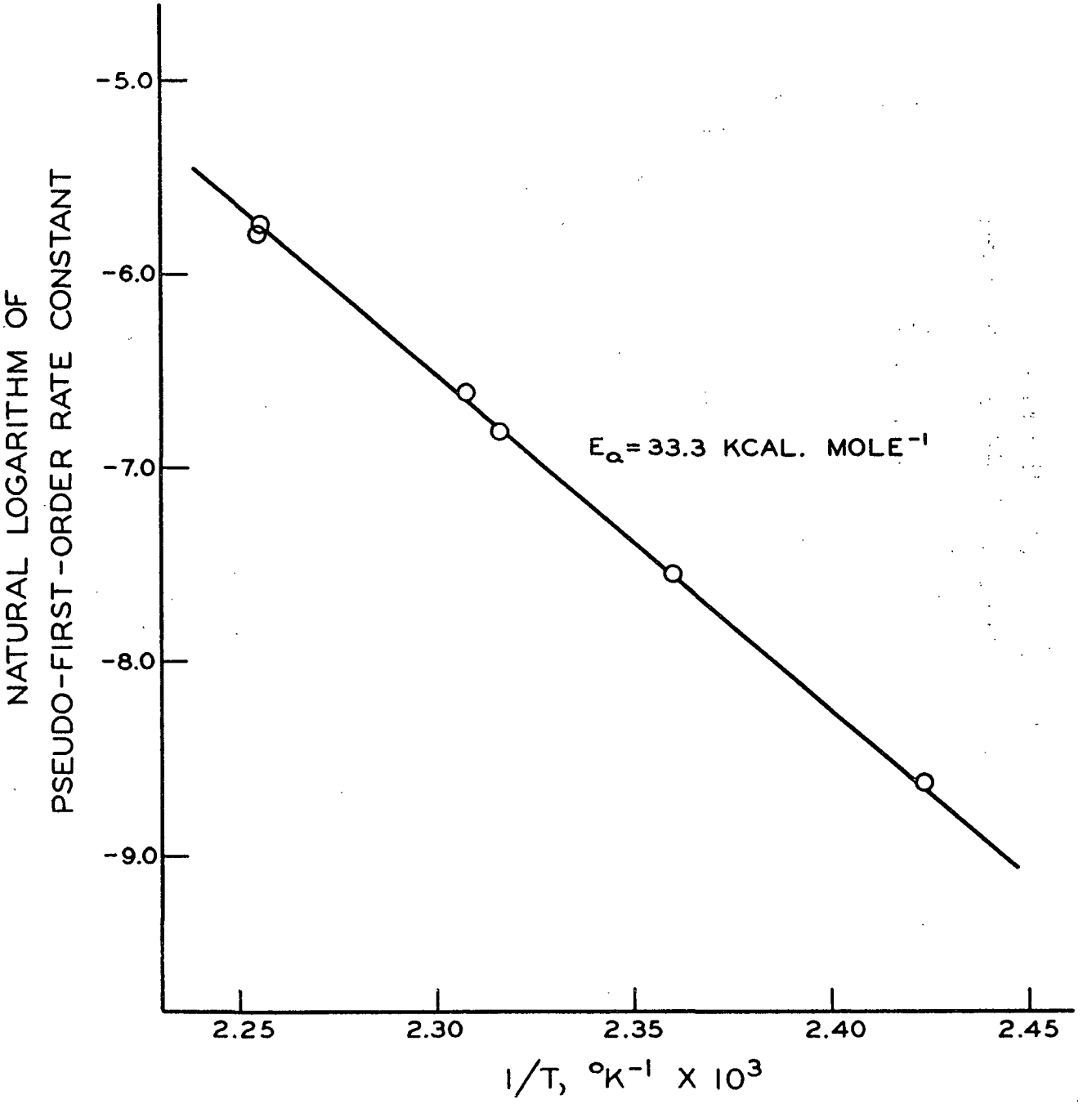

Figure 13. Arrhenius Plot for the Alkaline Degradation of Methyl α-D-Glucopyranoside (0.025M) in Oxygen-Free 2.5N Sodium Hydroxide at 140-170°C.

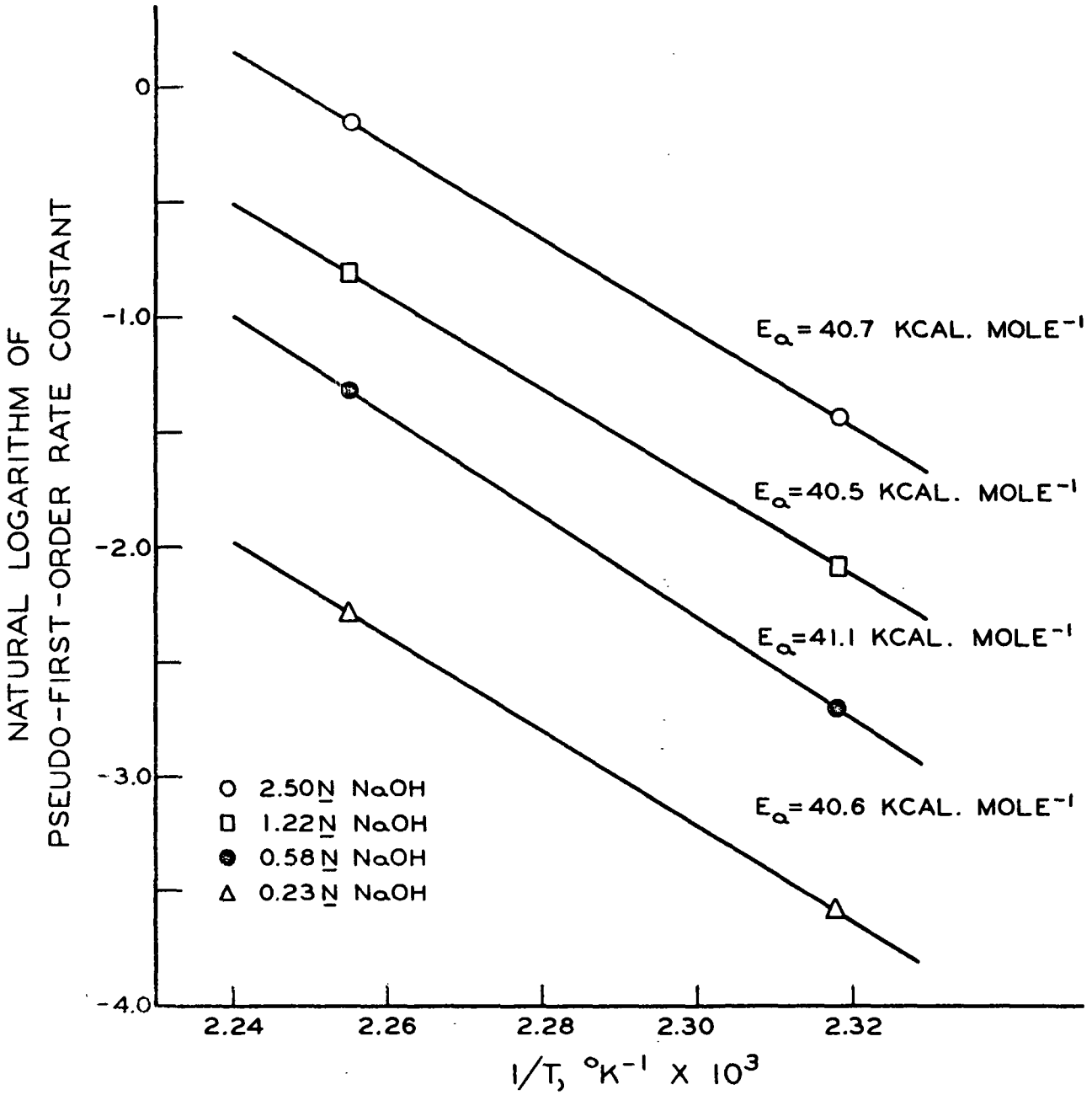

Figure 14. Arrhenius Plot for the Alkaline Degradation of Sodium Methyl α-D-Glucopyranosiduronate (0.025M) as a Function of Hydroxide Ion Concentration

TABLE VI

INVESTIGATION OF THE KINETIC EFFECT OF VARYING NUCLEOPHILE

Sample No.	Initial Reactant Concn., M	Nucleophile Concn., N[a]	Reaction Time, hr.[b]	h_{MeOH}/h_{MeO^-}[c]	k_{NaI}/k_{NaCl}
1-SMAG	0.393	1.25N NaI	17.0	0.0593	1.10
2-SMAG	0.390	1.25N NaCl	17.0	0.0536	
3-SMAG	0.388	1.25N NaI	21.5	0.0830	0.92
4-SMAG	0.384	1.25N NaCl	21.5	0.0908	
1-MAG	0.555	1.25N NaI	250	0.0250	0.88
2-MAG	0.500	1.25N NaCl	250	0.0284	
3-MAG	0.544	1.25N NaI	380	0.046	1.15
4-MAG	0.523	1.25N NaCl	380	0.040	

[a] Each sample contained 1.25N sodium deuteroxide.
[b] Reaction temperature for all samples was 158°C. ± 1°C.
[c] Peak height ratio between methanol and methoxyl proton peaks.

degradation reactions proceeded by pure S_N2 mechanisms, the relative rate constants should have values of 9 to 11 based on Swain nucleophilicity values (46).

Although this investigation of the kinetic effect of varying nucleophile is not thorough, the experimental procedure appeared to be basically sound, yielding reproducible relative results. These results convey definite mechanistic implications.

DETERMINATION OF THE POINT OF BOND CLEAVAGE

Cleavage of a glycosidic bond can occur at two points as represented in Fig. 15.

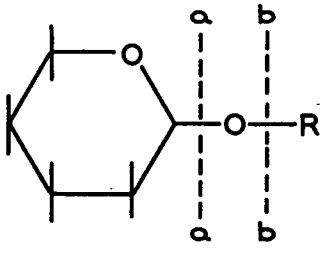

Figure 15. Diagram Showing Possible Points of Cleavage of the Glycosidic Bond

Dotted line aa represents glycosyl bond cleavage between the anomeric carbon atom and the glycosidic oxygen. Glycosidic bond cleavage would occur along dotted line bb between the glycosidic oxygen and the aglycon (R group). Acid hydrolysis of several glycosides (50, 51) in water enriched with oxygen-18 (O^{18}) has established that the aglycon is isotopically normal, which would be obtained

only by glycosyl bond cleavage. The point of cleavage of the glycosidic bond during alkaline degradation of SMAG and MAG has been experimentally determined here for the first time and found to be predominantly of the aa type (glycosyl bond cleavage).

Sodium methyl α-D-glucopyranosiduronate (1.0M) and methyl α-D-glucopyranoside (1.0M) were allowed to react at 158°C. in 2.5N sodium hydroxide in which the water had a 5.0 atom percent enrichment of oxygen-18 (O^{18}). The methanol was separated from each reacted sample by a microdistillation procedure and analyzed with a mass spectrometer by Morgan and Schaffer (52). The results of these analyses are given in Table VII.

If only glycosyl bond cleavage occurred, the methanol product should have zero percent increase of oxygen-18 (O^{18}) content, while glycosidic bond cleavage should result in a 100% increase to a net percent oxygen-18 (O^{18}) content of 5.21 atom percent. The results of the SMAG system indicate that only glycosyl bond cleavage occurs during alkaline degradation. The MAG system undergoes predominantly glycosyl bond cleavage, but some glycosidic bond cleavage may be occurring. Best (24) recently reported an 11% increase of oxygen-18 (O^{18}) content in the methanol product from the alkaline degradation of methyl β-D-glucopyranoside in 2.5N sodium hydroxide at 170°C. The accuracy of the enrichment values is approximately ± 0.2%.

INVESTIGATION OF RELATIVE INDUCTIVE EFFECTS

From NMR analysis of SMAG and MAG at pH values of 8-14, chemical shift data indicate that the carboxylate anion of SMAG exerts a significant electron-releasing inductive effect that is realized at the anomeric carbon atom. At high pH values, secondary hydroxyl groups, especially the C2 hydroxyl, ionize

TABLE VII

SUMMARY DATA FOR THE ALKALINE DEGRADATION OF METHYL α-D-GLUCOPYRANOSIDE AND SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE IN O^{18} ENRICHED WATER AND THE MASS SPECTROMETRIC ANALYSIS OF METHANOL REACTION PRODUCT

Compound	Reactant Concn., M	Reaction Temp., °C.	Reaction Time, hr.	Total Atom % O^{18} Content of Water	Total Atom % O^{18} of Methanol Product[a]	Atom % O^{18} of Natural Methanol[a]	Net % Enrichment of O^{18} in Methanol[b]
MAG	1.0	158	300	5.21	0.473	0.208	5.09
SMAG	1.0	158	12	5.21	0.214	0.208	0.01

[a]Analysis and calculation of results by Morgan and Schaffer Corporation, 5110 Courtrai Av., Montreal 26, Quebec, Canada.

[b]Values were calculated by subtracting the atom percent oxygen-18 content of natural methanol from the experimentally determined total atom percent oxygen-18 content of the methanol product. This difference was divided by the total atom percent oxygen-18 content of the water.

to give alkoxy anion functions which also exert electron-releasing inductive effects that are realized at the anomeric carbon atom. In 2.5N sodium deuteroxide the accumulative inductive effects caused by the C5 carboxylate anion and C2 alkoxy anion functions in the SMAG molecule are approximately 20% greater than that for the MAG molecule under similar conditions.

Inductive effects involve polarization of bonding electrons. These effects arise from ionic charges or from the action of dipoles within the reacting molecule. By convention, electronegative or electron-withdrawing groups exhibit negative inductive (-I) effects; conversely, nucleophilic or electron-donating groups exhibit positive inductive (+I) effects. The magnitude and sign of the inductive effect is a function of the substituent and its relative position to the molecule's reactive center. Hammett (54) has shown a quantitative relationship between structure and equilibrium and rate constants for the reactions of substituted benzene derivatives. A similar relationship demonstrating the polar effects in aliphatic compounds has been determined by Taft (55, 56). Both treatments define a parameter, σ, whose sign and magnitude are characteristics of the electron-withdrawing or electron-donating ability of the substituent. From dipole-moment studies (57), ionization constant data for substituted aliphatic acids (34, 58), and other kinetic studies (12, 59, 60), it has been proven that the carboxyl (-COOH) and hydroxyl (-OH) functions have relatively strong electron-withdrawing abilities. In the ionized forms, as the carboxylate ($-COO^{\ominus}$) and alkoxy ($-O^{\ominus}$) anions, they exhibit an electron-donating ability (34). The methoxyl group ($-OCH_3$) is a weak nucleophile exhibiting a small electron-donating ability and having a small negative Taft σ^* value [$\sigma^* = -0.39$ (56)].

The magnitude of the electron-withdrawing inductive effect of the C5 carboxyl group during the acid hydrolysis of methyl and phenyl glucuronides has not been

agreed upon in the literature (59-63). No explicit measurement of this effect is possible, yet the results of Semke (40) imply that the effect is large and realized at the anomeric carbon atom.

In this research under alkaline conditions, the magnitude of the positive inductive effect will be dependent upon the extent of ionization of the C5 carboxyl group and secondary hydroxyl groups since it is the anion functions that exhibit the electron-releasing abilities. From pK_a data of aryl glucuronides (64) and the ion product of water, it was calculated that at pH 7.0 and 20°C. the carboxylate anion concentration would be 10,000 times the concentration of undissociated carboxyl function. Similar calculations, using the pK_a data of methyl α-D- and methyl β-D-glucopyranoside (65), showed that at pH 7.0 and 20°C. only one in 10^6 C2 hydroxyl groups would be dissociated[1]. In 2.5N sodium hydroxide, nine of ten C2 hydroxyl groups would be dissociated. The details of these calculations are given in Appendix II. It was concluded from these results that the reactive species in the SMAG system was the carboxylate anion in the range of 0-2.5N sodium hydroxide. For the methyl glucoside systems, the reactive species at pH 7.0 was the neutral, undissociated molecule. At pH 14, the anion concentration becomes equal to the undissociated species concentration, and in 2.5N sodium hydroxide the anion species is in a tenfold excess of the undissociated species. The SMAG molecule will also have this increasing alkoxyl anion concentration as a function of pH. Therefore, the principal difference in the inductive abilities of SMAG and MAG molecules is the existence of the carboxylate anion.

Ten-percent solutions of SMAG and MAG were prepared in 99.8% deuterium oxide and analyzed at 36°C. with a Varian NMR spectrometer. The chemical shifts of the anomeric proton and methoxyl protons for each sample were determined. Additional

[1] The C2 hydroxyl is specifically referred to since it is the most acidic hydroxyl.

solutions were prepared in 2.5N sodium deuteroxide and similarly analyzed. The results are listed in Table VIII. The changes in the chemical shift from 0 to 2.5N sodium deuteroxide for the anomeric proton (-0.076) and methoxyl protons (-0.024) were essentially the same for both systems. Since the carboxylate function exists in both the slightly alkaline and highly alkaline SMAG samples, the inductive effect caused by this function cannot be separated. However, the chemical shifts for the anomeric protons and methoxyl protons at pH 7-8 in deuterium oxide are different because under these conditions only the carboxylate anion will be present to exhibit its positive inductive effect. All hydroxyl groups in both compounds will be undissociated. Indeed, this appears to be true since the chemical shift of the SMAG sample for the anomeric proton is 0.021 p.p.m. upfield from the anomeric proton of the MAG molecule. Similarly, the chemical shift for the methoxyl protons of the SMAG molecule are 0.012 p.p.m. upfield from that of MAG. The change in the chemical shifts for the methoxyl protons are less than that for the anomeric protons. This may be explained by the fact that the methoxyl group is a weak nucleophile exhibiting a mild electron-donating ability. This fact indicates that the effective shielding of its protons will be less than that of a single hydrogen atom, resulting in a lower screening constant and a smaller change in the chemical shift.

TABLE VIII

NMR SPECTROMETRIC RESULTS OF METHYL α-D-GLUCOPYRANOSIDE AND SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE IN DEUTERIUM OXIDE AND 2.5N SODIUM DEUTEROXIDE

	Sample	Av. δ for Anomeric Proton[a]	δ for Methoxyl Protons
I.	10% SMAG in D_2O	4.791	3.403
II.	10% SMAG in 2.5N NaDO	4.712	3.380
	Change in chemical shift, $\Delta\delta$[b]	-0.079	-0.023
III.	10% MAG in D_2O	4.812	3.415
VI.	10% MAG in 2.5N NaDO	4.738	3.390
	Change in chemical shift, $\Delta\delta$[b]	-0.074	-0.025
	Change in chemical shift, $\Delta\delta$ between I and III	-0.021	-0.012

[a] Average chemical shift for the anomeric proton doublet. Chemical shift, δ, in p.p.m. corrected for DSS internal standard adjusted to 0.0 p.p.m., accuracy of δ values = ± 0.005 p.p.m.

[b] Change in chemical shift, $\Delta\delta = \delta_{2.5N\ NaDO} - \delta_{D_2O}$.

DISCUSSION OF RESULTS

EFFECT OF HYDROXIDE ION CONCENTRATION

The rate dependency with respect to hydroxide ion concentration was experimentally determined to be 0.88 for SMAG and 0.69 for MAG in oxygen-free 0.25N sodium hydroxide at 158°C. Under similar conditions, Best (24) reported values of 0.66 and 0.74 for methyl β-D-glucopyranoside and methyl β-cellobioside, respectively. These results indicate that the hydroxide ion produces base catalysis or nucleophilic substitution of the glycoside reactant. The nonunity kinetic order values deserve discussion.

If a bimolecular mechanism (S_N2) occurs with the hydroxide ion functioning as a substituting nucleophile, the kinetic order with respect to hydroxide ion concentration should be approximately one. Similarly, if the reaction is unimolecular but base catalyzed, the kinetic rate should be first-order with respect to hydroxide ion concentration. According to the theory of absolute reaction rates (66) the hydroxide ion concentration is related to the pseudo-first-order rate constant by the following expression:

$$k' = kK^* \left(\frac{\gamma_{GM}\ \gamma_{OH^\theta}}{\gamma_{GM^*}} \right) [OH^\theta] \qquad (11)$$

where

k' = pseudo-first-order rate constant,

k = true rate constant,

K^* = overall equilibrium constant,

γ_{GM}, γ_{OH^θ}, γ_{GM^*} = activity coefficients of glycoside, hydroxide ion, and activated complex, respectively,

$[OH^\theta]$ = hydroxide ion concentration.

The natural logarithmic form of Equation (11) is given by Equation (12).

$$\ln k' = \ln kK^* + \ln \left(\frac{\gamma_{GM} \gamma_{OH^\theta}}{\gamma_{GM^*}} \right) + \ln [OH^\theta] \qquad (12).$$

For an ideal solution, the activity coefficient terms would have unit values, the logarithm of the activity coefficient ratio would be zero, and the plot of Equation (12) would be a straight line with unit slope. However, in real or nonideal solutions, especially those of high ionic strength, solvent-solute interactions occur which are manifested in nonunity activity coefficients. Activity coefficients are a function of temperature, pressure, and concentration (67). At constant temperature and pressure, a significant change in the ratio of the activity coefficients with change in hydroxide ion concentration will cause deviation from a linear plot of Equations (11) and (12). Since the concentration of the initial glycoside and its activated complex is always small (less than 0.026M), their activity coefficient values should be near unity and nearly constant as a function of hydroxide ion concentration. Harned and Owen (68) have shown empirically that the mean ionic activity coefficient of sodium hydroxide at 70°C. decreases from 0.76 at 0.1M sodium hydroxide to 0.63 at 1.0M and then increases to 0.71 at 3.0M. Similar studies investigating the effect of temperature showed that the mean ionic activity coefficient of sodium hydroxide had a small negative temperature coefficient in the range of 50 to 100°C. These data indicate that the activity coefficient of the hydroxide ion is probably less than one and passes through a minimum from 0 to 2.5N sodium hydroxide at 140-170°C. Applying this correction qualitatively to Equations (11) and (12) causes an increase in the linearity of the plot of k' vs. $[OH^\theta]$ and raises the slope closer to unity in the plot of ln k vs. ln $[OH^\theta]$. However, the magnitude of this correction is small and the same for both the SMAG and MAG systems. This does not account for the difference in kinetic order values between these two systems.

While discussing the kinetic order with respect to hydroxide ion concentration, the effect of ionic strength should be considered. Unimolecular S_N1 mechanisms involving charge separation will be aided by a more ionic medium, while S_N2 mechanisms involving charge dispersion will be retarded and the reaction rate decreased slightly as ionic strength increases (41). These primary salt effects are often manifested empirically in nonunity kinetic order values. However, the existence and magnitude of salt effects are not readily discernible since changes in activity coefficients may cause the same results. Ingold (41) states that mechanistic implications from kinetic order values are quite often vague and interpretation of results must be exercised with caution and preferably in conjunction with other criteria.

EFFECT OF ELECTRON-DONATING SUBSTITUENTS

The realization of a significant electron-donating inductive effect at the anomeric carbon atom of both glycosides has been demonstrated empirically by NMR analysis. This inductive effect has been shown to depend upon hydroxide ion concentration. The significant change in chemical shift of the methoxyl protons indicates that this effect is even carried out past the glycosidic oxygen. For MAG, the C2 alkoxyl anion function is primarily responsible for this effect, while SMAG has both the C2 alkoxyl and C5 carboxylate anion functions operating to cause this effect[1]. The additional influence of the carboxylate anion causes the glucuronide molecule to have approximately 20% greater screening of its anomeric proton than occurs in methyl α-D-glucopyranoside. These results are reasonable since the C2 alkoxy anion is adjacent to the anomeric carbon, while the C5

[1] It must be recognized that other hydroxyls may be ionized and contributing a positive inductive effect. However, in comparison, their effect will be smaller since they ionize to a lesser extent and are farther removed from the anomeric carbon atom.

carboxylate anion is three atoms removed. Inductive effects diminish rapidly after three or four carbon atoms (12,46). However, the second atom from the carboxylate anion is oxygen, which should be more efficient in transferring its inductive effect to the anomeric carbon since it is more electronegative than carbon and has a greater ability for bond polarization.

Constitutional influences in glycosyl radicals may affect nucleophilic substitution. In the rate-determining stage of unimolecular nucleophilic substitution (S_N1), there is an electron transfer from the carbon atom to the leaving group, without any compensating gain of electrons by the carbon atom. Hence, a large kinetic polar effect is expected, and its direction is unambiguous. Electron-releasing substituents must accelerate such substitutions (41). Bimolecular nucleophilic substitutions (S_N2) involve simultaneous electron transfer from the substituting nucleophile to the carbon atom and from the carbon atom to the leaving group. In general, these transfers will not be balanced exactly in the transition state of the reaction, so that a small polar effect on the rate is to be expected. However, it cannot be said whether the carbon at the reaction site is more positive or more negative in the activated complex than in the initial reactant; consequently, an S_N2 reaction might be accelerated or retarded by electron-releasing substituents (41,46).

Ingold (41) discusses the influence of electron-releasing substituents on reaction mechanisms and describes several reaction series that proceed from S_N2 to S_N1 mechanisms as the electron-releasing ability of the alkyl radical increases. He concludes that if a substituent capable of electron release produces a large increase in substitution rate, then it is very probable that the reaction of the substituted compound is a unimolecular substitution. Evidence of a possible change from bimolecular to unimolecular mechanism is given by the fact that SMAG undergoes alkaline degradation 280 times faster than MAG in 2.5N

sodium hydroxide at 170°C. The only constitutional difference is the replacement of the C5 hydroxymethyl group with the carboxylate anion which has a strong electron-releasing ability.

EFFECT OF VARYING NUCLEOPHILE

The constitutional effects of the substituting nucleophile may be used to distinguish the bimolecular and unimolecular mechanisms of substitution. The rate of S_N2 substitution increases with increasing nucleophilicity of the attacking group (41,46). In unimolecular substitution, the slow, rate-dependent process does not involve the attacking nucleophile, and the specific rate is independent of the nucleophilicity of the attacking group.

It has been shown empirically that the rate of alkaline degradation of both SMAG and MAG is not changed by the presence of a stronger nucleophile. These results, although semiquantitative and rather limited, generally disprove the existence of an S_N2 mechanism. However, the fact that both systems demonstrate a definite rate dependency on hydroxide ion concentration indicates that the reaction is base catalyzed. If the reactive species in both systems are the dissociated anions, then base catalysis would be involved in the ionization of the C5 carboxyl, C2 and C6 hydroxyl groups of the reactant molecules. The anion species may then undergo unimolecular heterolysis or intramolecular displacement to cleave the C1-glycosidic oxygen bond and yield methanol.

If base catalysis occurs first to produce anion species which then react further to cleave the glycosidic bond, it must be ascertained whether the first or second step is rate controlling. Gasman (29) determined a hydrogen isotope effect, $k_i D_2O/k_i H_2O$, of 1.35 for p-nitrophenyl-β-D-galactoside and 1.41 for p-nitrophenyl α-D-mannoside in 0.48N sodium hydroxide at 45°C. These results clearly indicated that the proposed base-catalysis step was not rate controlling. A similar conclusion regarding the reaction systems of this research may be drawn from what is known about the rates of proton transfer reactions. Proton transfer rates between atoms with unshared electron pairs are of the order 10^{11} to 10^{-1} liters/mole sec. at 25°C. (46). Since the measured

rate constants for the glycoside reactions are of the order 10^{-5} to 10^{-7} $sec.^{-1}$, it appears quite probable that the base-catalysis step is not rate controlling.

Supporting evidence for base-catalyzed unimolecular or intramolecular substitution reactions is found in the literature. Kraft pulping studies of cellulose and hemicelluloses (9, 10, 39) have shown that alkaline degradation is related directly to effective alkali content of the cooking liquor and not sulfidity. Legg and Hart (69) report that beyond a certain effective alkali concentration, no increase occurs in the rates of pulping and alkaline degradation. Investigation of saccharinic acids formed during kraft cooking of hardwoods (19) has not shown the presence of sulfur atoms. A sulfur atom should be present if the reaction proceeded by an S_N2 mechanism which involved the hydrosulfide anion ($SH^{\ominus}$) because its nucleophilic activity is ten times greater than that of the hydroxide anion (70, 71).

CONFORMATIONAL ANALYSIS

Consideration of conformational effects indicates that SMAG and MAG undergo alkaline degradation by two different reaction mechanisms. The configuration of SMAG and MAG will not permit a Walden inversion mechanism involving C2 neighboring group participation. This is the generally supported mechanism for the alkaline degradation of methyl β-D-glucopyranoside (20, 21, 29). It appears that MAG undergoes alkaline degradation by an intramolecular process involving the substitution of the methoxyl aglycon at C1 by the C6 hydroxyl anion function which yields 1,6-anhydro β-D-glucopyranose (levoglucosan) as the proposed initial product.

According to an S_N1 mechanism, as the methoxyl aglycon recedes, the resulting carbonium ion is stabilized by resonance between a carbonium ion and oxonium ion, as shown in Fig. 16. This requires that the C2-C1-O-C5 chain be planar. The formation of this planar half-chair conformation from initial chair conformation involves rotation about the C2-C3 and C4-C5 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 activated complex would be formed with greater or less difficulty, depending on whether the nonbonded interaction was increased or decreased. According to Eliel, et al., (74) for D-glucopyranose-type configurations, these conformational changes result in closer proximity of the C4 and C5 substituents with increased repulsive interaction. However, this change is not clearly demonstrated with Cenco-Peterson molecular models, and it may well be that the C4 and C5 substituents actually move to a greater separation with this conformational change. Therefore, no accurate prediction can be made whether the rate would increase or decrease as the size of the substituent increased.

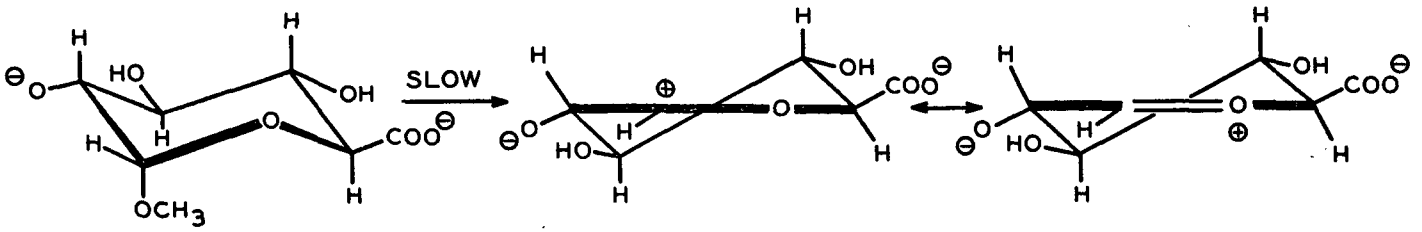

Figure 16. Resonance Stabilization Between Carbonium and Oxonium Ions

Whistler and Richards (8) point out that during acid hydrolysis the difference in conformational effects between methyl glycosides with C5 carboxyl versus hydroxymethyl groups would be about the same as that between the same methyl glycosides with C5 1,2-dihydroxyethyl versus hydroxymethyl groups. Methyl α-glucoheptoside with the bulkier C5 1,2-dihydroxyethyl group ($-CH(OH)CH_2OH$) is

only 0.36 times as reactive as the configurationally related hexoside (MAG) which possesses a C5 hydroxymethyl group (72, 73). This small difference in reactivity does not compare to the large difference in reactivity between the SMAG and MAG systems. It appears that differences in nonbonded interactions between these systems cannot account for the carboxyl activating effect.

The preferred conformations of the α-glycosides have the aglycon in an axial position, which results in it being shielded by substituents in the pyranose ring in adjacent relationship and by the ring itself. This shielding may retard nucleophilic attack. Methyl pyranosides of D-glucose have the preferred C1 conformation, permitting the hydroxyls to exist in the equatorial position. It is likely that in the pyranose ring, axial alkoxyl groups at C1 are in general more stable than an equatorial aglycon due to an anomeric effect (74, 75). Electrostatic repulsive forces between the unshared orbitals of the pyranose ring oxygen and the glycosidic oxygen favor the alkoxyl aglycon in the α- rather than the β-position. If a common reaction mechanism is occurring, the configurations of the transition state of both the α- and β-methyl glucosides should be energetically rather similar. The lower stability of the β-form of the initial reactant will be reflected in a faster rate of reaction. This is borne out in the fact that methyl β-D-glucopyranoside is 2.5 times as reactive as its α-anomer in 2.5N sodium hydroxide at 170°C. (30). Dryselius, et al. (76) report that the rate of alkaline hydrolysis of glycosides increases with conformational instability of the most stable chair form of the glycoside. Similar studies of various methyl glucopyranosides (30, 31) have shown the trans-glucosides were always more reactive than were the cis-anomers.

Since neighboring group participation requires a coplanar arrangement of the atomic centers involved, a conformational change from C1 to 1C occurs in the β-glycoside systems that proceed through a proposed 1,2-anhydro intermediate (see Fig. 17). The development of a mutual interaction between the C1, C3, and C5

substituents accompanies this change. The interaction for methyl β-D-glucopyranoside will involve the methoxyl aglycon at C1 while its α-anomer in the same 1C conformation will have its methoxyl aglycon in an equatorial position. The result should be less nonbonded interaction for MAG during this conformational change. If MAG and MBG proceeded by the same reaction mechanism, MAG would be expected to be more reactive which is not the actual case.

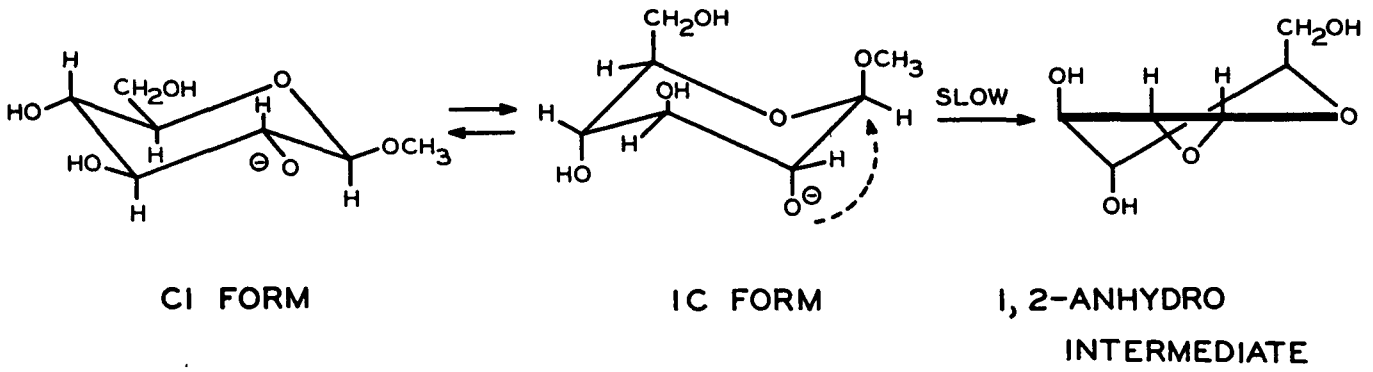

Figure 17. Diagram Showing Conformational Change from C1 to 1C for β-Glucosides Reacting to 1,2-Anhydro Intermediates

Intramolecular displacements, as do their intermolecular analogs, require that the attacking nucleophile must approach from the backside, while the leaving group departs from the frontside. This is called Walden inversion. Both SMAG and MAG have a cis-configuration between their C1-C2 substituents. For Walden inversion to occur there must be a trans-configuration between these substituents. Therefore, neither SMAG or MAG can undergo C2 neighboring group participation to form the 1,2-anhydro intermediate without subjecting these molecules to considerable strain. It has been shown that intramolecular displacements leading to epoxides are, as a group, at least 100 times faster than their intermolecular counterparts (44). Since MBG is only 2.5 times more reactive than MAG, a different intramolecular process or unimolecular process is suggested, while an S_N2 process

involving a free hydroxyl ion as the attacking nucleophile appears to be quite improbable. Also, since methyl α- and methyl β-D-glucopyranosides undergo alkaline degradation only twice as fast as their 2-O-methyl analogs which are not eligible for C2 neighboring group participation, additional evidence of other significant reaction pathways is given.

An intramolecular nucleophilic attack at C1 by the hydroxyl anion at C6 is quite feasible. This mechanism has been proposed by McCloskey and Coleman (27) to explain the reactivity of methyl α-D-glucopyranoside. The requisite trans-configuration for Walden inversion exists between the C1 methoxyl and C5 hydroxymethyl groups. According to this mechanism, the initial reaction product would be levoglucosan. Several workers (27, 30, 33) report the presence of small amounts of levoglucosan in product solutions from the alkaline digestion of glycosides with cis C1-C2 substituents and trans C1-C5 substituents. No attempt was made in the present research to identify levoglucosan in the reaction product solution. Since this product cannot be obtained by C2 neighboring group participation, further evidence of additional reaction mechanisms is given. The small amount of levoglucosan is well explained by the fact that Dryselius, et al. (78) have shown that levoglucosan is degraded at a rate one hundred times faster than MAG in 2.5N sodium hydroxide at 170°C.

Although the proposed 1,6 intramolecular displacement reaction for the alkaline degradation of MAG appears quite probable, a unimolecular heterolysis mechanism is also possible. The electron-releasing inductive effect of the C2 alkoxyl anion will aid heterolysis of the C1-glycosidic oxygen bond. The unstable carbonium ion intermediate could be attacked intramolecularly by the C6 alkoxyl anion to give levoglucosan. Although the large difference in reactivity between MAG and SMAG strongly indicates a significant difference in reaction mechanism, the possibility of a unimolecular mechanism for MAG cannot be eliminated.

ENTHALPY AND ENTROPY OF ACTIVATION

The estimated thermodynamic activation functions of SMAG and MAG in 2.5N sodium hydroxide at 170°C. are given in Table IX. The details of these calculations based on the theory of absolute reaction rates (66) are given in Appendix IV. The values for the free energy and entropy functions are not absolute but relative since they contain empirical errors based on the fact that nonunity kinetic order values were obtained with respect to hydroxide ion concentration.

TABLE IX.

THERMODYNAMIC ACTIVATION FUNCTIONS FOR SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE AND METHYL α-D-GLUCOPYRANOSIDE IN 2.5N SODIUM HYDROXIDE AT 170°C.

Function	SMAG	MAG
E_a, kcal. $mole^{-1}$	40.9	33.3
ΔH^*, kcal. $mole^{-1}$	40.0	32.4
ΔF^*, kcal. $mole^{-1}$	33.5	38.4
ΔS^*, cal. $°K.^{-1}$ $mole^{-1}$	+14.7	-13.6

Pseudo-first-order rate data, used in the calculation of these functions, contain values for an equilibrium constant and hydroxide ion concentration. Any errors in the calculated functions due to temperature dependence of these variables should be small. The hydroxide ion concentration in both systems was maintained constant as a function of temperature. The temperature coefficient for the equilibrium constant should be small as is the general case for ionic equilibria (79). The heat of ionization should be approximately 5-10 kcal. per mole and nearly constant as a function of temperature based on the ionization data of methanol (80) and phenol (81) whose dissociation constants are similar to that of methyl α-D-glucopyranoside (65).

If the enthalpies and entropies of activation for SMAG and MAG are to be compared on a common basis, the values of these equilibrium constants must be nearly equal. This should be the case since the equilibrium step predominantly involves the ionization of the C2 hydroxyl group in both molecules. It is impossible to state what the exact reactive species are in both systems, but it is reasonable to assume that the equilibrium concentrations of hydroxyl anion functions should be nearly equal for both SMAG and MAG at constant temperature and hydroxide ion concentration. Since methyl β-D-glucopyranoside apparently undergoes a similar equilibrium step, a comparison of the calculated thermodynamic functions of these three systems under equal conditions should be valid.

The large Arrhenius energy of activation for SMAG (ca. 40.9 kcal./mole) is consistent with an S_N1 mechanism. Disregarding solvation and steric effects, the S_N1 mechanism will inherently have a higher energy of activation than the S_N2 mechanism because of a greater extension of the polarized carbon-methoxyl bond in the transition state. In the S_N2 mechanism, the partially formed covalent bond aids the heterolysis and electron transfer to the leaving group, resulting in a lower activation energy than S_N1 which does not enjoy such a helping effect. Brown and Hudson (82) report, in mechanism studies of the hydrolysis of substituted benzoyl chlorides, that the activation energies for S_N1 reactions were larger than for the S_N2 reactions.

The difference in the activation energies for MAG (ca. 33.3 kcal./mole) and its β-anomer [ca. 37.5 kcal./mole (24)]may be due in part to solvation effects. During the activation process, the solvent cage of the attacking nucleophile must be disrupted and a new cage built up around the activated complex. Any difficulty in breaking up the solvent cage of the attacking species must be reflected in an increase in the activation energy (41). It is possible that the C2 hydroxyl anion

will have a stronger, closer packed, more symmetrical solvent cage than the C6 hydroxyl anion. More energy will be required to disrupt the stronger solvent cage before intramolecular displacement can occur.

The formation of the 1,6-anhydro sugar by intramolecular displacement produces a five-membered ring which is considerably less strained than the 1,2-anhydro sugar. This lower ring strain will be reflected in a lower activation energy.

The large entropy differential between SMAG (ca. +14.7 e.u.) and MAG (ca. -13.6 e.u.) indicates the occurrence of two separate reaction mechanisms. Schaleger and Long (83) state that bimolecular and intramolecular substitution processes should reflect a loss of translational and rotational freedom of the bound nucleophile by a decrease in the entropy of activation when compared to the unimolecular process. This prediction is borne out by entropies of activation for A-1 and A-2 ester hydrolyses with typical entropy values of 0 to +10 e.u. for A-1 and -15 to -30 e.u. for A-2. Recent work by Long, et al. (84) indicates that the entropy differences are not usually so large as implied by the ester data.

Since neighboring group participation requires a coplanar arrangement of the atomic centers involved, a significant restriction of bond motion occurs on going from a flexible monocyclic to a relatively strained bicyclic system. Although six flexible forms are possible for the monocyclic pyranose ring, the bicyclic 1,2-anhydro and 1,6-anhydro sugars are limited to two formations (74). The loss of rotational and vibrational degrees of freedom in the formation of the 1,6-anhydro sugar should be greater than that in the 1,2-anhydro formation. This effect may account for part of the entropy differential between MAG and its β-anomer.

Steric effects encountered during the formation of the activated complex are reflected in the entropy of activation as well as the energy of activation (41).

If SMAG and MAG proceeded by a common unimolecular mechanism, a definite entropy relationship would be expected. The rotation about the C2-C3 and C4-C5 bonds to form the planar half-chair conformation of the carbonium ion would involve a greater nonbonded interaction for SMAG due to the bulkier C6 carboxyl group. Since entropy of activation is a measure of the difference in freedom from restraint in the initial and transition states, one would expect the entropy function for SMAG to be less than that of MAG. This is not the case since the calculated entropy value for SMAG is 21.1 e.u. larger than that of MAG. The large entropy differential indicates two different reaction mechanisms and suggests that conformational and entropy effects are not responsible for the great difference in reactivity between SMAG and MAG.

CONCLUSIONS

Based on empirical results and theoretical considerations, it is concluded that sodium methyl α-D-glucopyranosiduronate (SMAG) and methyl α-D-glucopyranoside (MAG) undergo high-temperature alkaline degradation by two different reaction mechanisms. The replacement of the C5 hydroxymethyl group with a carboxyl group causes a large increase in reactivity due to a change in reaction mechanism. This change is associated with the strong electron-releasing ability of the carboxylate anion function.

It is proposed that SMAG proceeds by a base-catalyzed unimolecular heterolysis mechanism (B-1) which is diagrammatically shown in Fig. 18. The base functions to ionize the C5 carboxyl and C2 hydroxyl groups. A fast equilibrium step occurs between the ionized and nonionized species which is followed by a slow, rate-dependent heterolysis of the covalent bond between C1 and the glycosidic oxygen. The resultant carbonium ion is an unstable intermediate that is immediately attacked intermolecularly by hydroxide ions or intramolecularly by the C2 hydroxyl anion function. Either product will be rapidly degraded to saccharinic acids by the β-alkoxy elimination reaction. The slow heterolysis step is accelerated by a positive inductive effect realized at the anomeric carbon atom which is due primarily to the electron-releasing ability of the C5 carboxylate anion function.

It is proposed that the alkaline degradation of MAG proceeds by a base-catalyzed intramolecular displacement (B-i) mechanism which is diagrammatically shown in Fig. 19. The base functions to ionize primary and secondary hydroxyl groups and to establish a rapid equilibrium between ionized and nonionized species. The slow, rate-dependent step involves the intramolecular attack at C1 by the C5 hydroxymethyl anion function with the simultaneous displacement of the

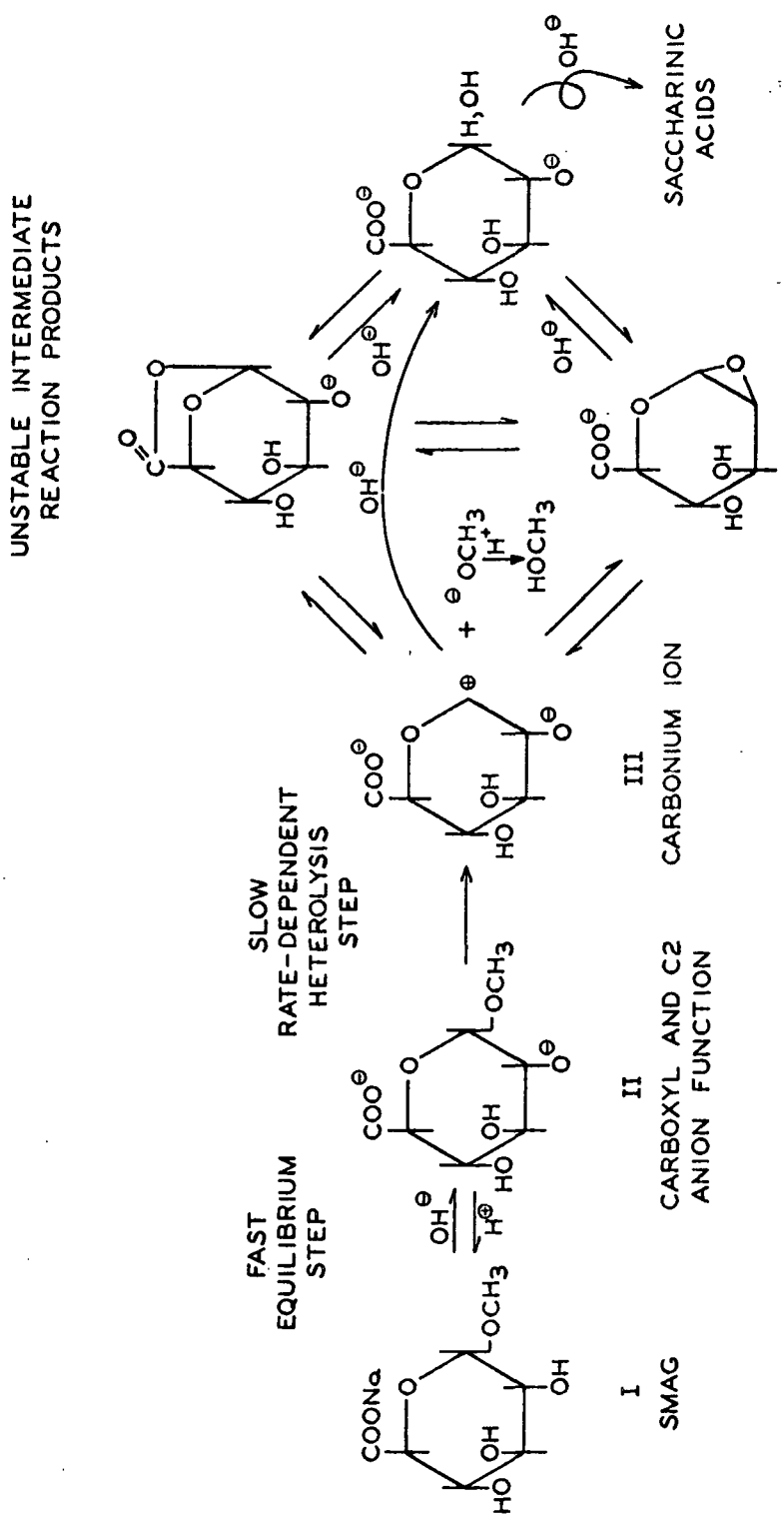

Figure 18. Proposed Base-Catalyzed Unimolecular Heterolysis (B-1) Mechanism for the Alkaline Degradation of Sodium Methyl α-D-Glucopyranosiduronate in Oxygen-Free 2.5N Sodium Hydroxide at 140-170°C.

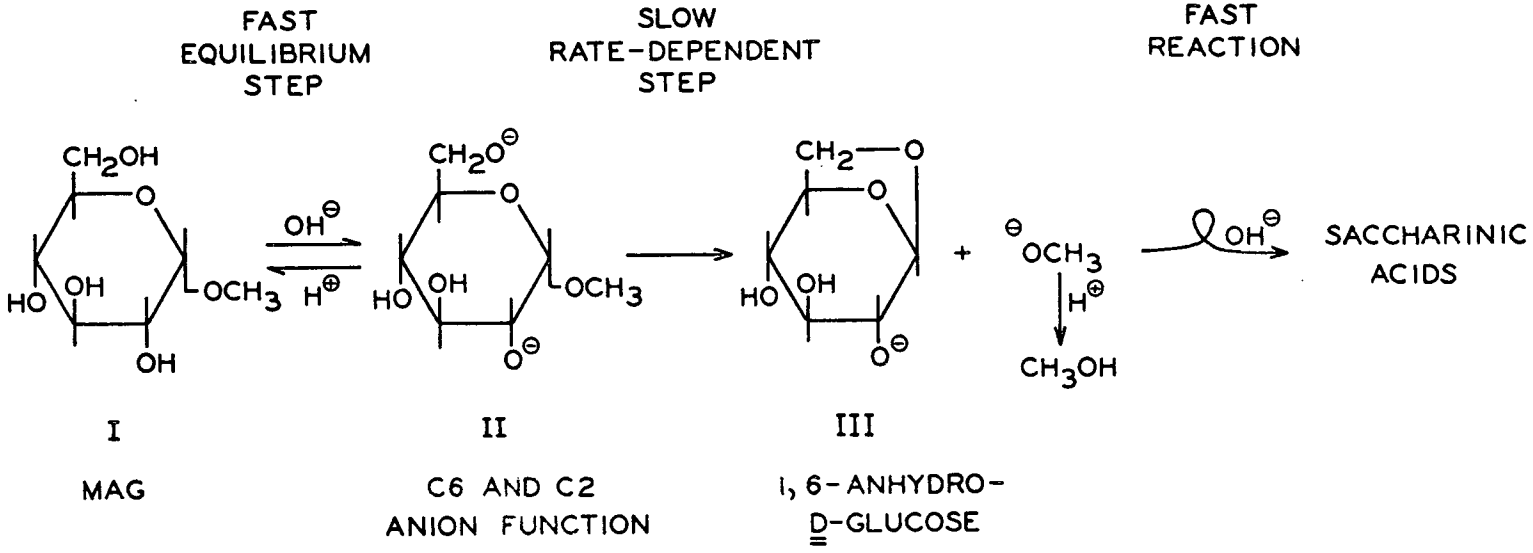

Figure 19. Proposed Base-Catalyzed Intramolecular Displacement (B-i) Mechanism for the Alkaline Degradation of Methyl α-D-Glucopyranoside in Oxygen-Free 2.5N Sodium Hydroxide at 140-170°C.

methoxyl aglycon. The proposed initial product is levoglucosan which would undergo further alkaline degradation by the β-alkoxy elimination reaction.

These proposed mechanisms are consistent with the experimental results of this research. However, sufficient data have not been obtained to eliminate other possible mechanisms. The SMAG system may undergo alkaline degradation by intramolecular displacement of the C1 methoxyl aglycon by attack of the C5 carboxylate anion function to form an unstable ester. This product would readily degrade to saccharinic acids. As an alternative to the proposed mechanism for the MAG system, unimolecular heterolysis of the C1-glycosidic oxygen bond may be occurring. The presence of the C2 alkoxyl anion capable of electron-releasing ability may provide the driving force for such a displacement mechanism. Complete elucidation of the exact reaction mechanisms is dependent upon future kinetic studies and development of greater knowledge of high-temperature physical organic chemistry.

The results of this research indicate that random cleavage of glycosidic bonds of cellulose and hemicelluloses is a significant mechanism of high-temperature alkaline degradation. Since the presence of a carboxyl group significantly increases the sensitivity of the glycosidic bonds to hot alkali, it should be profitable during alkaline pulping of wood to minimize the concentration of carboxyl groups. This possibly could be done by pulping in a reducing atmosphere or with the aid of reducing agents. The result should be a greater yield pulp with greater strength and hydration properties due to a higher degree of polymerization and hemicellulose content.

EXPERIMENTAL PROCEDURES AND APPARATUS

SYNTHESIS OF METHYL (C-14) α-D-GLUCOPYRANOSIDE

The procedure of Bollenback (85) was used on a reduced scale. Recrystallized anhydrous glucose (6.70 g.) and methanol-washed, ovendried Dowex 50 X-2 cation-exchange resin (1.66 g.) were added to a 50-ml. round-bottom flask containing 20 ml. of anhydrous methanol with a total activity of 1.0 mc. of methanol C-14. The reaction flask was attached to a condenser which contained a glass cold finger. The mixture was stirred by a Teflon-coated stirring bar and magnetic stirring motor while heat was applied through a paraffin oil bath on a hot plate. The reaction solution was refluxed gently and stirred continuously for 26 hours. The hot reaction solution was decolorized by adding activated charcoal (Darco G-60), stirring, and filtering on a Celite bed. The filtrate was cooled slowly to room temperature after adding two seed crystals of methyl α-D-glucopyranoside. After 20 hours, a crystalline solid had formed. Ten milliliters of anhydrous ethanol was mixed with the crsytalline solid and its mother liquor. The mixture was filtered and the crystals thoroughly washed with anhydrous ethanol. The filtrate was kept for recovery of methanol C-14. The crude MAG was recrystallized once in absolute ethanol as a 10% (w/v) solution by refluxing for 3 hours, decolorizing with activated charcoal, filtering on a Celite bed, and cooling slowly to 8°C. in the presence of a few seed crystals. After 10 hours at 8°C., long, white, needle-shaped crystals were formed. The crystals were washed with absolute ethanol, acetone, and diethyl ether, and dried in a vacuum oven at 50°C. for 10 hours. The weight of the dried crystalline product was 1.3 g. or 20% of the theoretical yield. The estimated specific activity was 9000 dis./min.//mg. C. Approximately 9 ml. of unused methanol C-14 was recovered by fractional distillation of the mother liquor. This reclaimed methanol was used in subsequent repeat reactions.

This procedure was repeated until 7.7 g. of methyl (C-14) α-D-glucopyranoside was accumulated. This material was added to 22.1 g. of nonradioactive pure methyl α-D-glucopyranoside and recrystallized twice from absolute ethanol. The final dried product weighed 25.3 g. with an estimated specific activity of 2250 dis./min.//mg. C. The actual specific activities were determined by the wet combustion procedure of Van Slyke and Folch (86) and the determination of total carbon and its radioactivity by the procedure of Van Slyke, Steele, and Plazin (87). These results with other characteristics of methyl (C-14) α-D-glucopyranoside are given in Table X. Quantitative paper chromatography proved that the content of glucose impurity was less than 0.02%, while gas-liquid chromatography of the trimethyl silyl derivative gave no detection of glucose or methyl β-D-glucopyranoside.

TABLE X

PROPERTIES OF METHYL (C-14) α-D-GLUCOPYRANOSIDE

Melting point, °C.	
This work:	166-167°C. (corrected)
Literature:	167-168°C. (corrected) (88) 167°C. (corrected) (89)
Specific rotation, $[\alpha]_D$	
This work:	+159.1° (c 4.0, water, 20°C.)
Literature:	+159.0° (c 2.0, water, 25°C.) (88) +159.0° (c 3.0, water, 20°C.) (89)
Specific activity, dis./min.//mg. C	
Before dilution	9,004[a]
After dilution	2,166[a]

[a]Average of three separate determinations.

SYNTHESIS OF SODIUM METHYL (C-14) α-D-GLUCOPYRANOSIDURONATE

PREPARATION OF PLATINUM-CARBON CATALYST

A modification of the procedure described by Trenner (90) was used to prepare an active platinum-carbon catalyst. Darco G-60 activated charcoal was digested in 10% HCl, washed with water until free of chloride ion, filtered, vacuum dried, and degassed for 24 hours in an evacuated tube heated to 400°C. This procedure was used by Farrar (91) to purify charcoal for polymer adsorption studies.

Purified Darco G-60 (17.2 g.) was mixed with chloroplatinic acid ($H_2PtCl_6 \cdot 6H_2O$) (7.4 g.) in 600 ml. of water. After neutralizing to pH 7.5 with sodium bicarbonate, the mixture was heated to 80°C. with continuous stirring, 55 ml. of 38% formaldehyde was added over 45 minutes, and sodium bicarbonate was simultaneously added at such a rate that the pH remained between 7.5 and 8.5. The mixture was held at 80°C. for 2 hours with constant stirring. The pH was continuously measured with a Beckman pH meter. After filtering, the catalyst was washed with 80°C. solutions of potassium chloride of decreasing concentration and finally with water until the filtrate was free of chloride ion. The catalyst was dried overnight in a vacuum oven at 60°C. An assay on the prepared catalyst was performed by ashing a blank and two catalyst samples at 900°C. for 15 hours in a muffle furnace. The results showed that the prepared catalyst contained 13.85% Pt, and subsequent catalytic oxidation reactions proved that the catalyst had a high activity.

CATALYTIC AIR OXIDATION

The procedures of Mehltretter (92) and Easty (93) were modified to produce large quantities of crude sodium methyl α-D-glucopyranosiduronate (SMAG). Seventy-five grams of methyl α-D-glucopyranoside (MAG) were catalytically air oxidized to

give a 68% yield of crude methyl α-glucuronide which was precipitated in absolute ethanol as the sodium salt. Sodium hydroxide was continuously added to neutralize the acid product while a pH meter and combination electrode were used to monitor the pH of the reaction solution. The details and apparatus of this procedure are given in Appendix IV.

ESTERIFICATION OF THE CRUDE METHYL α-GLUCURONIDE WITH DIAZOMETHANE

The procedure of De Boer and Backer (94) was expanded to produce sufficient ethereal diazomethane to esterify 25 g. of crude methyl α-glucuronide. The details and precautions of this procedure for generating diazomethane are given in Appendix V. The sample to be esterified was cation-exchanged to generate the free acid and concentrated to a thick yellow-orange sirup. This sirup was reconcentrated five times from absolute ethanol to remove water, then diluted to 500 ml. with methanol. The methanolic solution was cooled to -30°C. in a Dewar flask containing acetone and dry ice. The ethereal diazomethane was added to this solution with mild stirring until the yellow color of excess diazomethane persisted. The solution was stirred at -30°C. for three hours, warmed slowly to room temperature, and concentrated on a rotary vacuum evaporator (equipped with a cold trap) to remove excess diazomethane. The methyl ester was reconcentrated several times from absolute ethanol and diluted to 500 ml. with methanol. Titration of the unreacted acids in methanol solution showed that the esterification procedure gave a 95% theoretical yield.

PREPARATION OF METHYL α-GLUCURONIDE HYDRAZIDE

The procedure of Wolfrom, Kowkabany, and Binkley (95) was used to convert the methyl ester to the hydrazide. The hydrazide solution was prepared by mixing 95%+ hydrazine (Eastman Organic Chemicals) with absolute methanol. Five times

the theoretical requirement of hydrazine solution was slowly added with rapid stirring to a methanol solution of the methyl ester. After 5 minutes the glucuronide hydrazide crystallized from solution. After filtering, the hydrazide was washed with absolute ethanol and vacuum dried. The yield of the crude hydrazide from the methyl ester was 59.3%.

RECRYSTALLIZATION OF METHYL α-GLUCURONIDE HYDRAZIDE

Ten grams (10 g.) of crude methyl α-glucuronide hydrazide were dissolved in 250 ml. of triply distilled water. This solution was filtered through a 0.2 nm. Millipore filter into a 1-liter round-bottom flask and concentrated with a rotary vacuum evaporator to 75 ml. After adding a Teflon-coated stirring bar, 600 ml. of distilled ethanol was added to the flask. A condenser was attached, and the mixture was refluxed and stirred for 30 minutes causing complete dissolution of the hydrazide. Two grams of ethanol-washed, oven-dried Darco G-60 activated charcoal were added to the flask, the mixture was refluxed for 5 minutes and filtered on a Celite bed formed in a steam-heated Büchner funnel. The Celite bed was washed with 300 ml. of boiling absolute ethanol. The filtrate and washings were allowed to cool slowly to room temperature. After 10-hr. equilibration time at room temperature, the white, needle-shaped crystals and mother liquor were filtered on no. 40 Whatman filter paper. The crystals were washed with a 50-50 v/v solution of absolute ethanol and diethyl ether, and dried in a vacuum oven at 40°C. for 10 hr. The weight of the dried crystals was 7.0 g. or a 70% theoretical yield. Two recrystallizations by this procedure were required to yield the pure hydrazide. The characteristics of methyl α-D-glucopyranosiduronic acid hydrazide were determined and these results are given in Table XI.

TABLE XI

PROPERTIES OF METHYL α-D-GLUCOPYRANOSIDURONIC ACID HYDRAZIDE

Melting point, °C.			
This work:	232-233°C. (corrected)		
Literature:	231.5-232.5°C. (corrected) (93) 234°C. (corrected) (96)		
Specific rotation, $[\alpha]_D$			
This work:	+150.5°C. (c 0.99, water, 25°C.)		
Literature:	+150°C. (c 0.62, water, 22°C.) (93) +151°C. (c 1.00, water, 25°C.) (96)		
Elemental analysis, %	C, %	H, %	N, %
This work:[a]	37.75	6.49	12.53
Literature: (96)	37.93	6.44	12.57
Theoretical:	37.84	6.35	12.61

[a]Determinations done by Geller Microanalytical Laboratories.

ALKALINE HYDROLYSIS OF THE HYDRAZIDE AND PREPARATION OF SODIUM METHYL (C-14) α-D-GLUCOPYRANOSIDURONATE

A modification of the procedure described by Easty (93) was used. Eleven grams (11.0 g.) of recrystallized methyl α-glucuronide hydrazide were dissolved in 200 ml. of water and added to a refluxing solution of 50 g. of crystalline barium hydroxide [$Ba(OH)_2 \cdot 8H_2O$] in 1000 ml. of water. The solution was refluxed for 10 minutes. Normal sulfuric acid was added until the solution pH was 5.0, causing barium sulfate to precipitate. This precipitate was removed by filtration on a Celite bed and liberated hydrazine and excess barium cations were removed on an IR-120 (R^+) cation exchange column. The decolorized free acid solution was concentrated with a rotary vacuum evaporator to approximately 300 ml. and the pH was raised to 7.5-8.0 by the addition of 2.0N sodium hydroxide. Darco G-60

activated charcoal was added, stirred, and removed by filtration. The filtrate was concentrated with a rotary vacuum evaporator to 50 ml. of clear, water-white solution.

The concentrated SMAG was slowly added to 1500 ml. of Millipore-filtered ethanol which was vigorously stirred by a Teflon-coated stirring bar and magnetic stirring motor. A white, amorphous precipitate was formed which was filtered, washed with absolute ethanol, acetone, and diethyl ether, respectively, and dried overnight in a vacuum oven at 40°C. The dried sodium salt weighed 10.3 g. at a 93% theoretical yield. The SMAG was characterized and the results are given in Table XII. It was experimentally determined from potentiometric titrations and specific rotation measurements that sodium methyl α-D-glucopyranosiduronate exists as a dihydrate.

The average total accumulative yield over all these procedures for preparing pure sodium methyl α-D-glucopyranosiduronate from methyl α-D-glucopyranoside was 17%. These procedures were used on a reduced scale for the synthesis of sodium methyl (C-14) α-D-glucopyranosiduronate using methyl (C-14) α-D-glucopyranoside as the initial reactant.

PREPARATION OF OXYGEN-FREE REACTION SAMPLES

The thermal expansivity data of water (98) and specific gravity data of sodium hydroxide solutions (68) were used to calculate the appropriate sodium hydroxide concentration at 20°C. which would provide a concentration of 10% (2.5N) at specific reaction temperatures of 140-170°C. Carbonate-free sodium hydroxide (16.2N) was diluted with distilled water, boiled for 20 minutes, and diluted again with fresh, triply distilled water. This solution was titrated with potassium hydrogen phthalate, diluted, and retitrated until the desired sodium hydroxide concentration was obtained.

TABLE XII

A. PROPERTIES OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE

Specific rotation, $[\alpha]_D$

This work:	+101° (c 1.05, water, 22°C.)
Literature:	+93° (c 1.0, water, 25°C.)[a] (97) +118° (c 0.53, water, 25°C.)[b] (93)

C, H, Na, MeO analysis	C, %	H, %	Na, %	MeO, %
This work:[c]	36.35	4.81	10.20	14.78
Theoretical:	36.52	4.78	10.00	14.90

B. PROPERTIES OF METHYL α-D-GLUCOPYRANOSIDURONIC ACID

Specific rotation, $[\alpha]_D$

This work:	+130° (c 0.44, water, 22°C.)
Literature:	+110° (c 0.50, water, 20°C.)[d] (97) +167° (c 0.13, water, 25°C.)[e] (93)
Equivalent point	7.01 pH units

[a] Reported value for the potassium salt.

[b] Reported value for the barium salt.

[c] Determinations done by Geller Microanalytical Laboratories.

[d] Calculated value based on quantitative removal of K^+ ions.

[e] Calculated value based on quantitative removal of Ba^{++} ions.

Approximately 5 g. of SMAG was placed in a weighing bottle, dried in a vacuum oven at 40°C. for 2 hours, covered, and cooled to room temperature. Sufficient material was weighed out to give a 0.025M solution in 250 ml. of sodium hydroxide. This solution was transferred to a 300-ml. round-bottom flask and purged with prepurified nitrogen for 3 hours to remove dissolved oxygen. Analysis of a purged reaction solution with a Weston and Stack D.O. Analyzer showed the content of dissolved oxygen to be less than 0.1 p.p.m.

A special apparatus was constructed to charge the reaction tubes with their samples in a nitrogen atmosphere because the presence of molecular oxygen significantly increases the rate of high-temperature alkaline degradation of glycosides (23). A picture of this apparatus is shown in Fig. 20. While the reaction solution was being purged with nitrogen, the reaction tube and delivery system were evacuated, filled with nitrogen, and evacuated again. This was repeated four times. Next, the sample delivery system was charged with nitrogen until the balloon on top of the buret was partly inflated. Nitrogen pressure was used to force reactant solution into the buret. By turning proper valves, the delivery system was connected with the evacuated reaction tube and twenty-five milliliters (25 ml.) of reactant solution was delivered to the reaction tube. The charged tube was evacuated and filled with nitrogen four times and capped as nitrogen swept air away from the top of the tube. Average filling time was 10 minutes per tube, and the precision of the delivered sample volume was $\pm$ 0.5 ml.

REACTION APPARATUS

STAINLESS STEEL REACTION TUBES

Reaction tubes were made of no. 314 stainless steel pipe (1/4 in. i.d. x 2-ft. length). One end was plugged with a threaded bolt and sealed with silver solder. The other end was threaded, wrapped with Teflon tape, and fitted with a no. 314 stainless steel cap. The capacity of each tube was 35 ml. Each tube was charged with 25 ml. of 2.5N NaOH and heated for 150 hr. at 170°C. No leakage or loss of solution was detected during this time. It was experimentally determined that 3.0 minutes were required for the contents of the tube to reach equilibrium with the surrounding oil bath at 170°C. Five minutes (5 min.) were required to cool the contents of the reaction digester from 170 to 30°C. by immersion in a water-cooled kerosene quench tank.

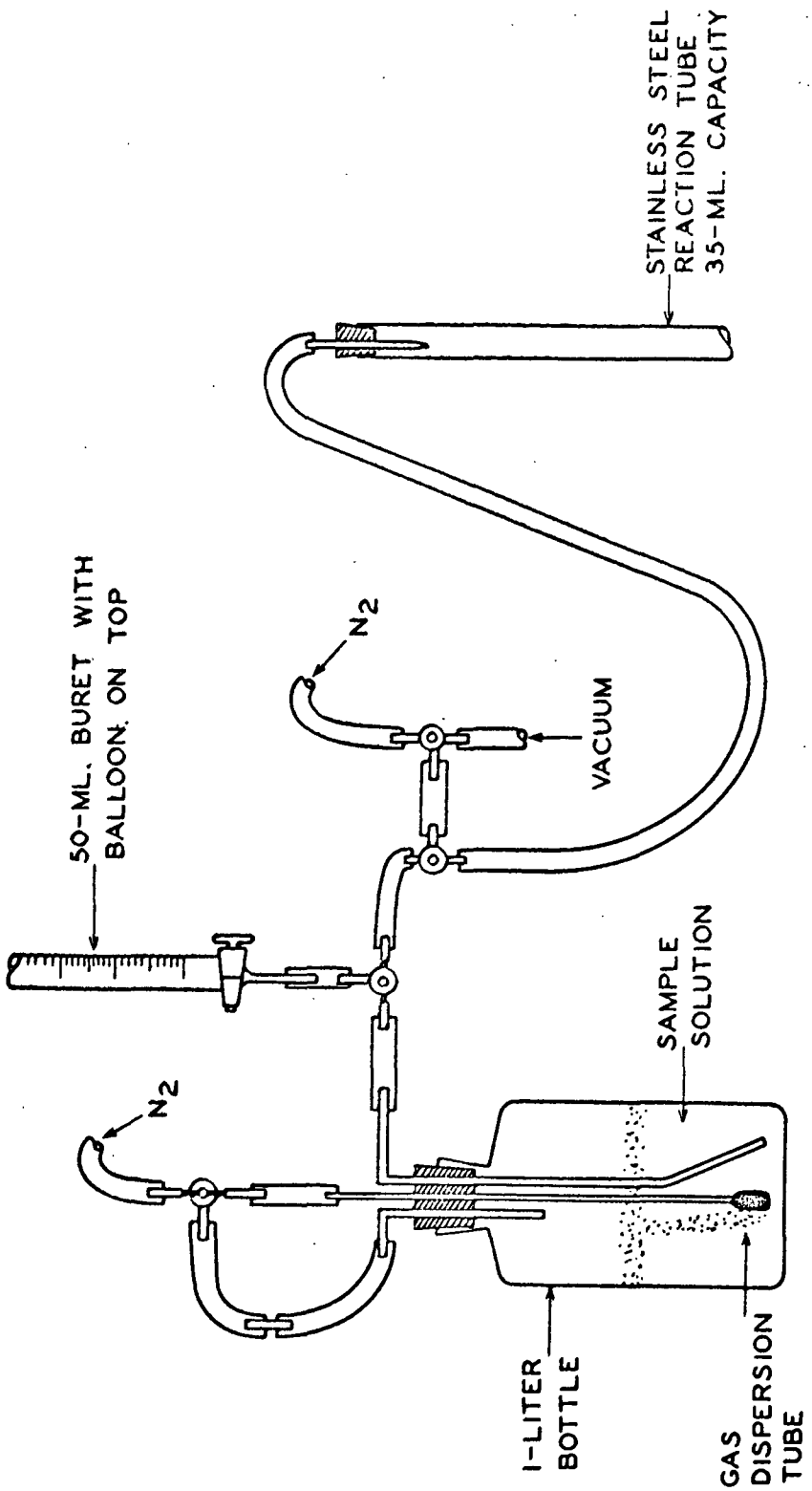

Figure 20. Apparatus for Charging Reaction Tubes in a Nitrogen Atmosphere

THERMOSTATED OIL BATH

All reactions were run in a 70-gallon oil bath filled with Regal "K" heat transfer oil. The bath was equipped with a recirculating pump to provide uniform temperature distribution and a rocker arm to agitate the reaction tubes.

Auxiliary equipment included two iron-constantan thermocouple probes and leads attached to a Py-ro-vane automatic temperature controller and a Bristol's Pyromaster potentiometer for continuous temperature recording. A Brooklyn "high precision" calibrated thermometer (-2 to 200°C., 0.2° div.) was used to accurately measure the bath oil temperature. This system maintained the reaction temperature to within $\pm$ 0.5°C.

REACTION QUENCH TANK

After a specified reaction time, each sample tube was removed from the oil bath and immediately immersed in a quench tank to stop the reaction. This tank consisted of an aluminum conduit (6 in. x 5 ft.) filled with a kerosene "Gunk" degreaser solution which was cooled to 13°C. by a surrounding water jacket. Tubes were removed after 15 min., rinsed with water, and placed in a 10°C. cold room until their contents were analyzed.

DISTILLATION OF METHANOL FROM REACTION PRODUCT SOLUTIONS

After a specific kinetic run, each reaction tube was equilibrated to room temperature for 6 hr. The tube was carefully uncapped and its contents poured into a 50-ml. beaker. Twenty milliliters (20 ml.) of reaction product solution were pipeted into a 100-ml. round-bottom flask containing 40 ml. of fresh, triply distilled water. After adding two small boiling chips and a Teflon sleeve gasket, the flask was attached to the distillation apparatus. One milliliter of fresh, triply distilled water was placed in a 25-ml. volumetric flask, the tip of the

condensate delivery tube was immersed below the level of the water in the flask, and the flask was immersed in a salt ice-water bath. This arrangement caused all noncondensed distillate vapors to be scrubbed with water. The assembled apparatus is shown in Fig. 21. Heat was applied and the distillation allowed to proceed until approximately 24 ml. of condensate had been collected in the receiver flask. The latter was removed, diluted to volume (25 ml.), stoppered, sealed with paraffin film, and placed in a 10°C. refrigerator until the sample was analyzed for methanol.

The characteristics of the distillation procedure were experimentally determined using samples of known methanol concentration. Over a concentration range of 1-300 μg. methanol per milliliter, the distillation of methanol from reaction product solutions was quantitative with a mean error of ± 0.5%. Samples of 2.50N sodium hydroxide containing known amounts of methanol were distilled according to the above procedure, and the distillate samples were quantitatively analyzed for methanol by a modified procedure of Boos (99). Previous calibration of this analysis during this research gave a mean error of ± 1.5% for the methanol determination. Boos (99) reports a mean error of ± 2%, while Larson (100) reports ± 1.8%.

METHANOL ANALYSIS

Methanol concentrations were determined with a mean error of ± 1.5% by a modified procedure of Boos (99). The details of this procedure are given in Appendix VI. Two methanol calibration curves were determined from solutions of known methanol concentration. These curves are shown in Fig. 22 and 23, and their characteristics are given in Table XIII. When the methanol concentration of the distilled sample exceeded 70 μg. MeOH/ml., the sample was diluted ten times with water, the normal procedure was followed, and the methanol concentration determined from Methanol Calibration Curve II.

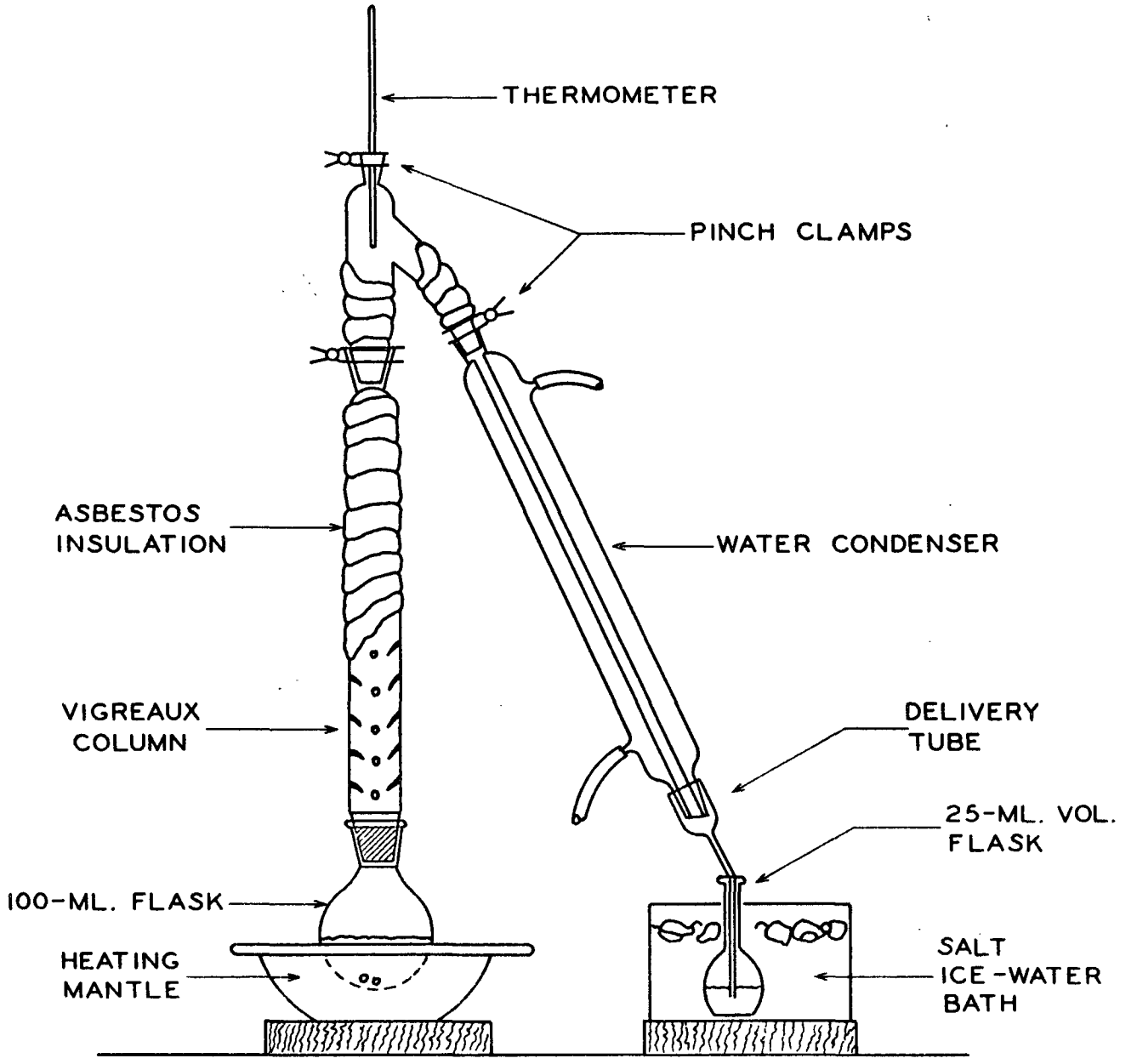

Figure 21. Apparatus for the Distillation of Methanol from Reaction Product Solutions

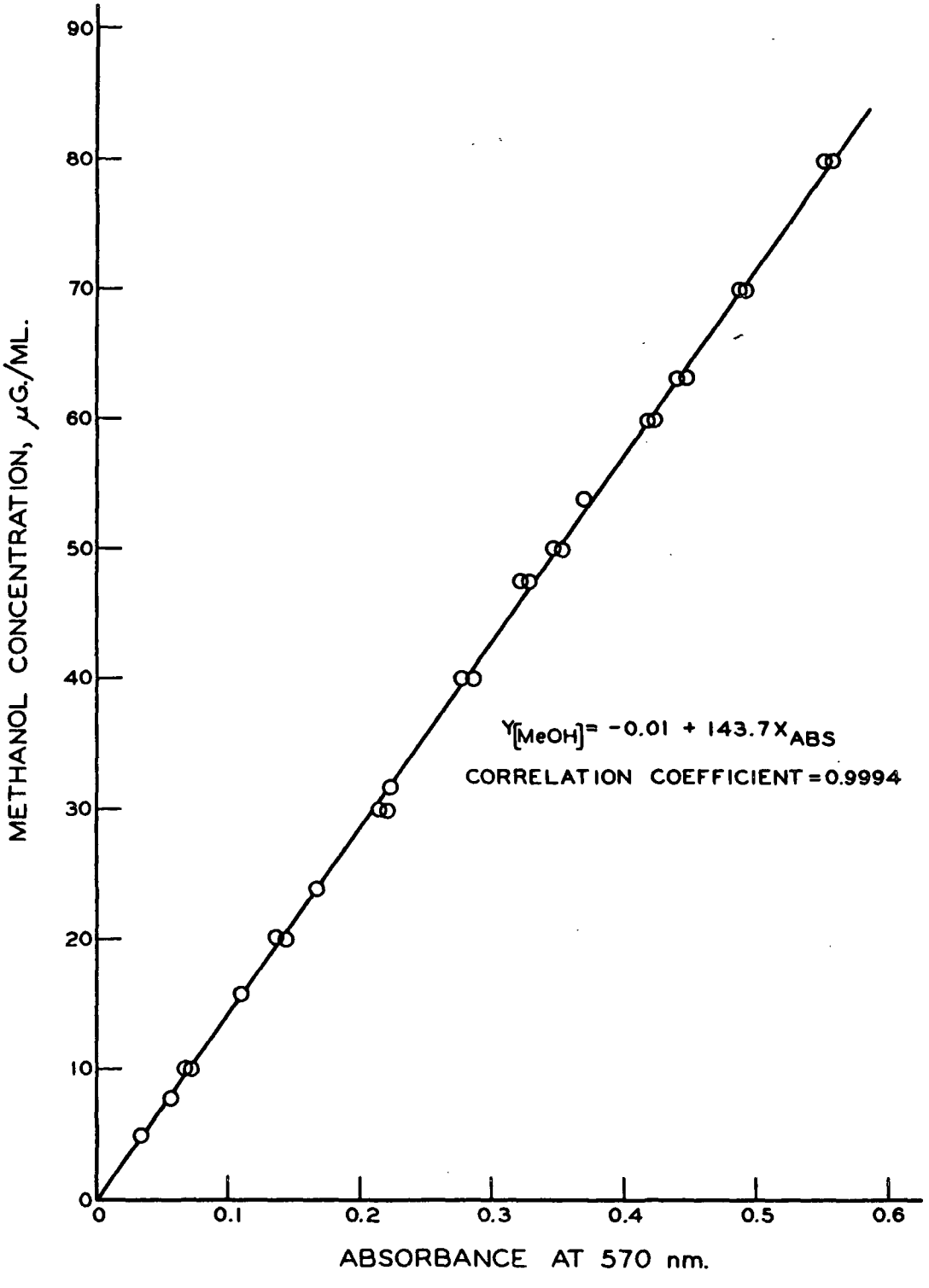

Figure 22. Methanol Calibration Curve I

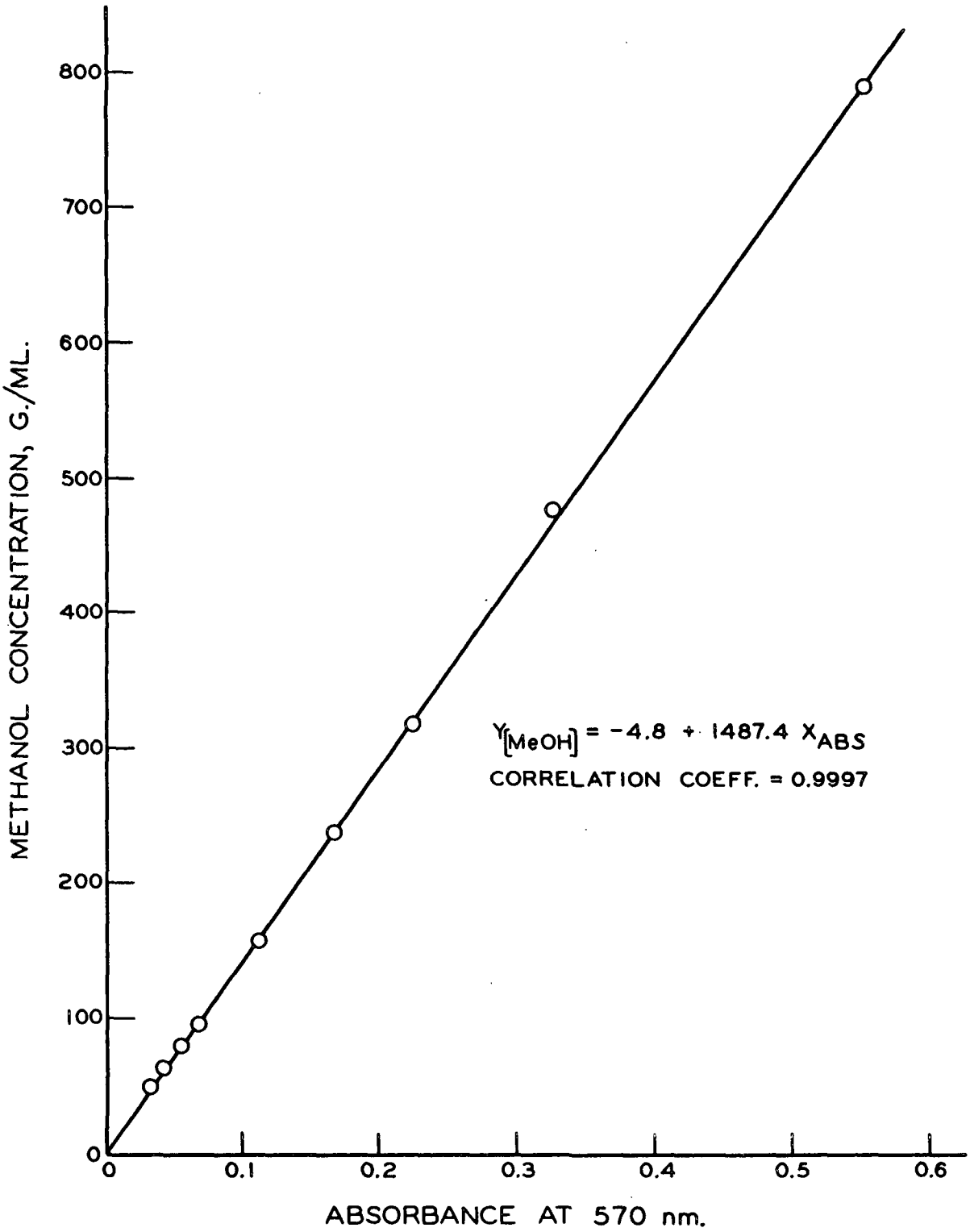

Figure 23. Methanol Calibration Curve II

TABLE XIII

CHARACTERISTICS OF METHANOL CALIBRATION CURVES

	Methanol Calibration Curve I	Methanol Calibration Curve II
Concentration range	0-80 μg. MeOH/ml.	0-800 μg. MeOH/ml.
Line of regression	$Y_{[MeOH]} = -0.01 + 143.7X_{abs}$	$Y_{[MeOH]} = -4.8 + 1487.4X_{abs}$
Correlation coeff.	0.9994	0.9997
Mean error	± 1.4%	± 1.6%

CORRECTION FACTORS FOR KINETIC RATE DETERMINATION

REACTION TIME CORRECTION

Reaction time correction factors were experimentally determined as the time required to raise the temperature of the reactant solution from 32°C. to the oil bath reaction temperature (140-170°C.). Three minutes were required to raise the solution temperature to 170°C. Since the glucuronide system at 170°C. undergoes 1.8% reaction in three minutes, this heat-up time was quite significant. Correction was made by subtracting the heat-up time factor from the gross reaction time (defined as the total time from immersion of the reaction tubes in the oil bath to immersion in the quench tank). This net reaction time or true reaction time at constant reaction temperature was used in the calculation of the reaction rate constant. These reaction time corrections were made for both the glucoside and glucuronide systems.

A standard reaction tube charged with 25 ml. of 2.5N sodium hydroxide was equipped with a calibrated thermocouple probe whose leads were attached to a high-speed recorder. The tube was immersed in the oil bath, and temperature rise versus time profiles were recorded for four reaction temperatures. The results are summarized in Table XIV and temperature rise profiles for the four ranges are

shown in Fig. 24 and 25. Similar experiments determined that 5 minutes were required to cool the contents of the reaction tube from 170 to 32°C. Figure 26 shows a representative cooling curve.

TABLE XIV

REACTION TIME CORRECTION FACTOR

Temp. Range, °C.	Heat-up Time, min.
31.0 to 139.8	2.8
32.0 to 149.8	3.1
35.0 to 160.0	3.0
32.0 to 170.2	3.2

METHANOL BLANK CORRECTION

Since kinetic rate determinations for the glucoside and glucuronide systems are dependent on methanol product concentrations, an accurate methanol blank is essential. In this experimental program there are four sources of methanol: (1) methanol produced at an increasing exponential rate during the heat-up period, (2) methanol produced at a constant rate during constant temperature, (3) methanol produced at a decreasing exponential rate during the quench period, and (4) methanol produced during distillation of reaction product solutions. Since the kinetic rate determination must be based on the methanol produced during constant-temperature reaction time, this methanol concentration is determined by subtracting the sum of sources 1, 3, and 4 from the total methanol concentration (sum of sources 1-4). This is explained mathematically by the following equations where the subscripts indicate the source of methanol:

$$\text{Total } [MeOH] = [MeOH]_1 + [MeOH]_2 + [MeOH]_3 + [MeOH]_4$$

$$[MeOH]_2 = \text{total } [MeOH] - ([MeOH]_1 + [MeOH]_3 + [MeOH]_4)$$

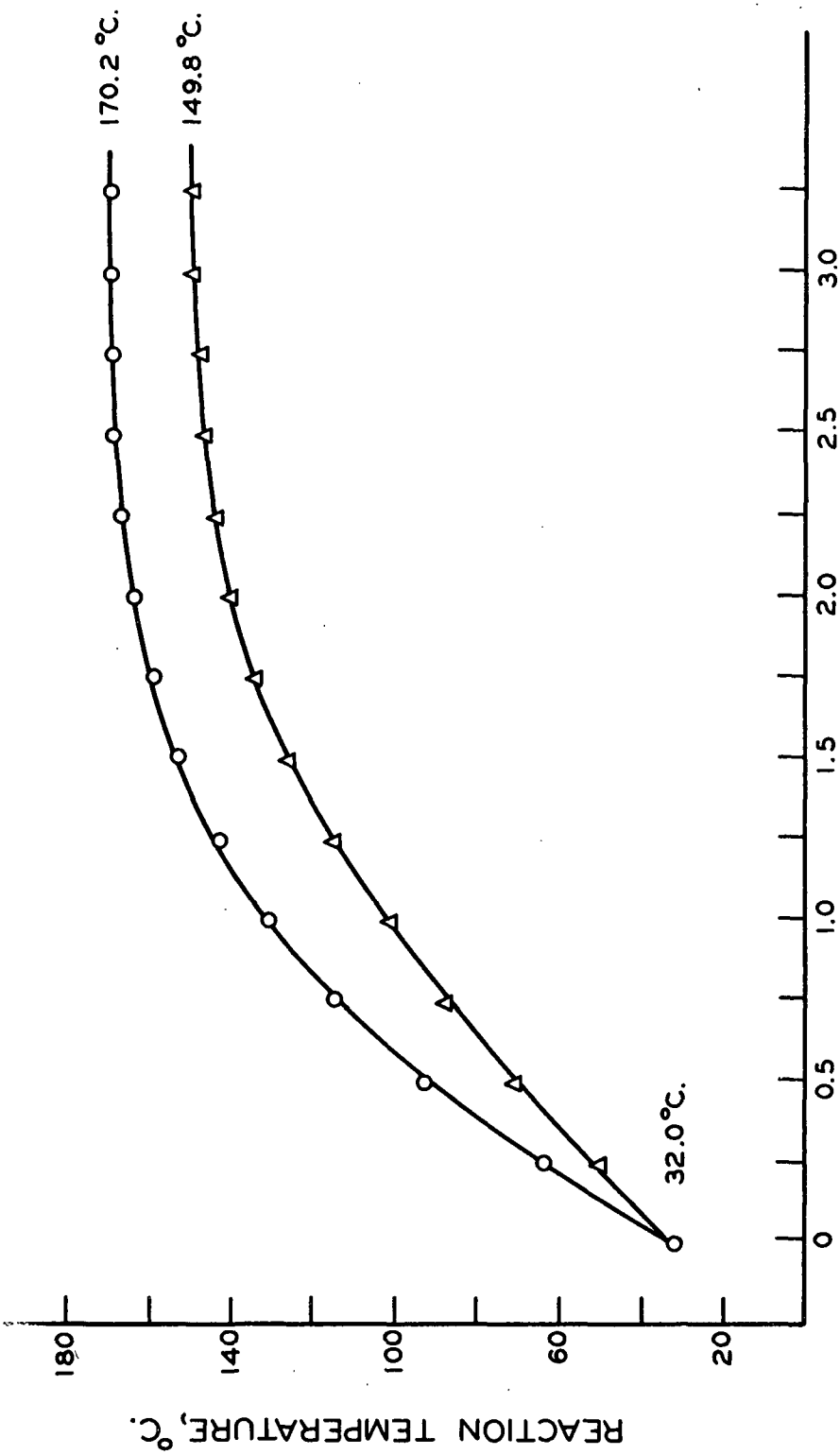

Figure 24. Plot of Reaction Solution Temperature Versus Time Required to Attain Equilibrium with Oil Bath Reaction Temperature

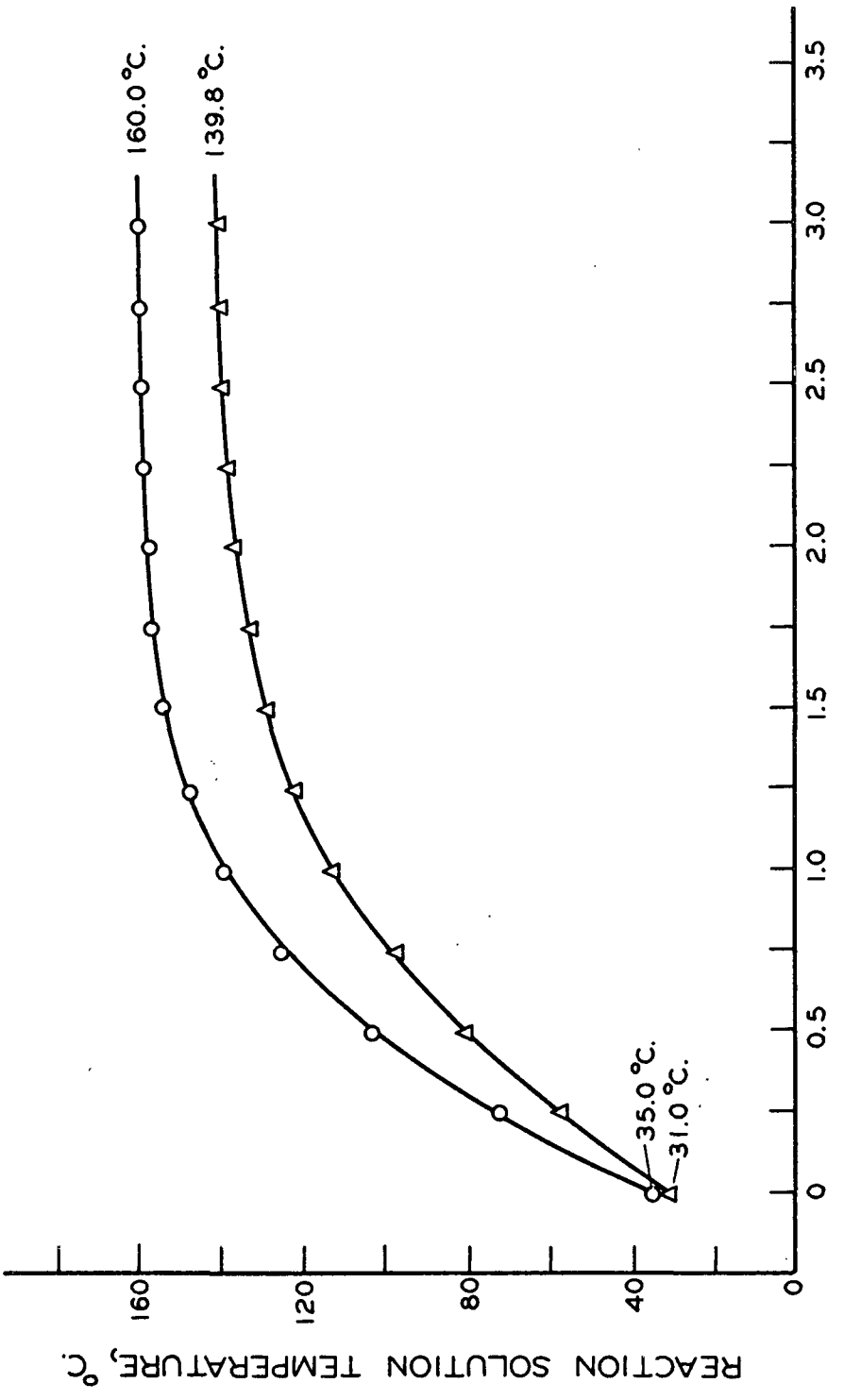

Figure 25. Plot of Reaction Solution Temperature Versus Time Required to Attain Equilibrium with Oil Bath Temperature

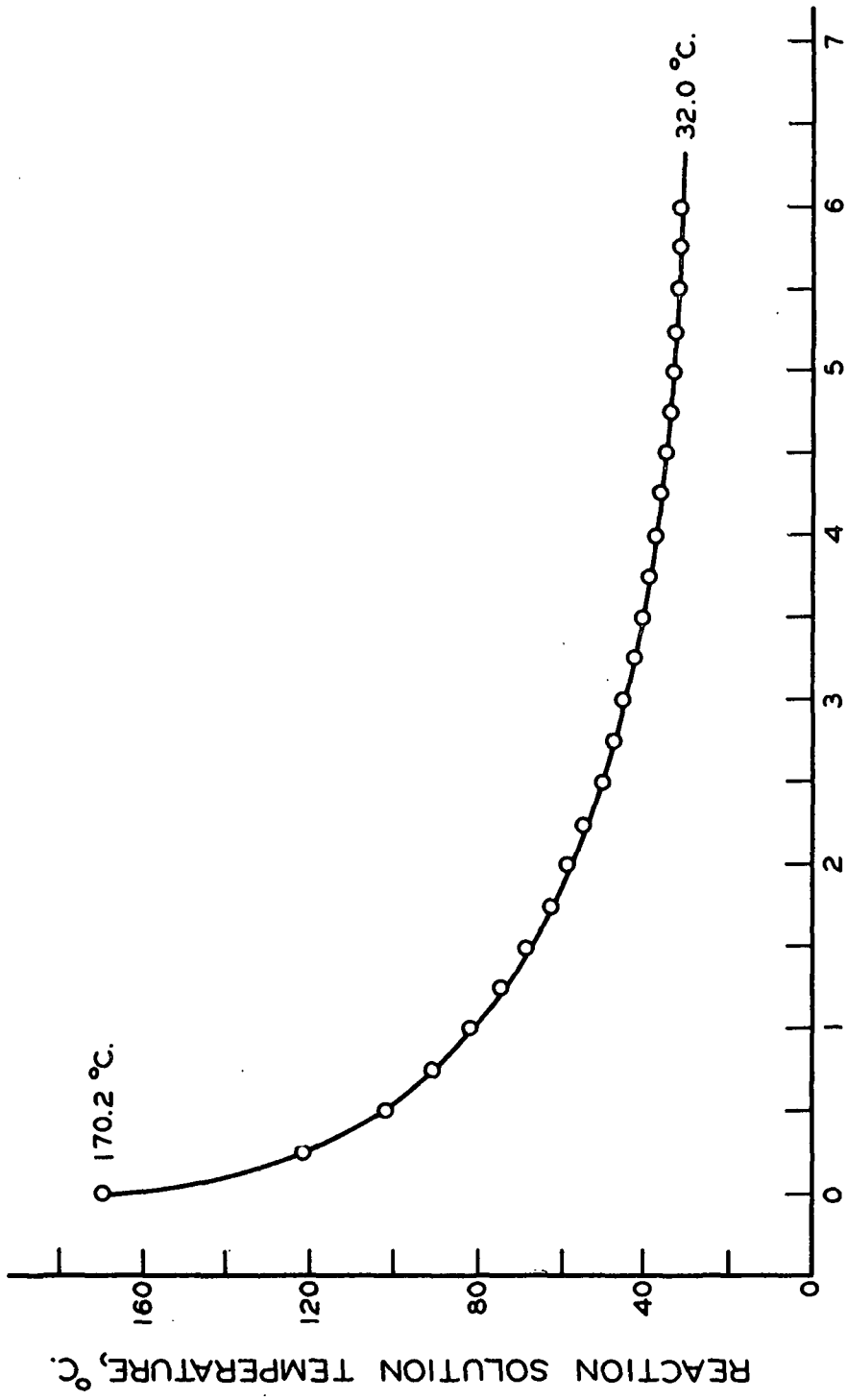

Figure 26. Plot of Reaction Solution Temperature Versus Time Required to Cool from 170.2 to 32.0°C.

$$[MeOH]_2 = \text{total } [MeOH] - B$$

$$\text{Methanol blank} = B = ([MeOH]_1 + [MeOH]_3 + [MeOH]_4) .$$

Reaction tubes containing known SMAG-sodium hydroxide solutions were immersed in the oil bath for the time required to attain equilibrium with the oil bath temperature (see Table XIV). They were immediately removed, quenched, distilled according to normal procedure, and analyzed for methanol. Blanks were run at four reaction temperatures and four hydroxyl ion concentrations. The results are summarized in Table XV. Figures 27 and 28 show the methanol blank correction factor as a function of reaction temperature and hydroxide ion concentration, respectively. Figures 29 and 30 show the semilogarithmic plots of these data. The equations describing these relationships were used to calculate the methanol blank value at a specific reaction temperature and hydroxyl ion concentration. This value was subtracted from the total methanol concentration to give the true methanol concentration produced during constant-temperature reaction time. These data were coupled with the corrected reaction time data to calculate the true reaction rate constant.

TABLE XV

METHANOL BLANK CORRECTION FACTORS

Reaction Temp., °C.	Initial SMAG Concn., M	NaOH, N	MeOH Concn., M x 10^{-5}
170.2	0.0249	2.507	18.8
158.2	0.0249	2.511	11.8
149.8	0.0249	2.518	9.4
139.8	0.0249	2.527	7.0
170.2	0.0249	2.507	18.8
170.2	0.0249	2.000	13.1
170.2	0.0249	1.277	6.8
170.2	0.0249	0.6359	4.6

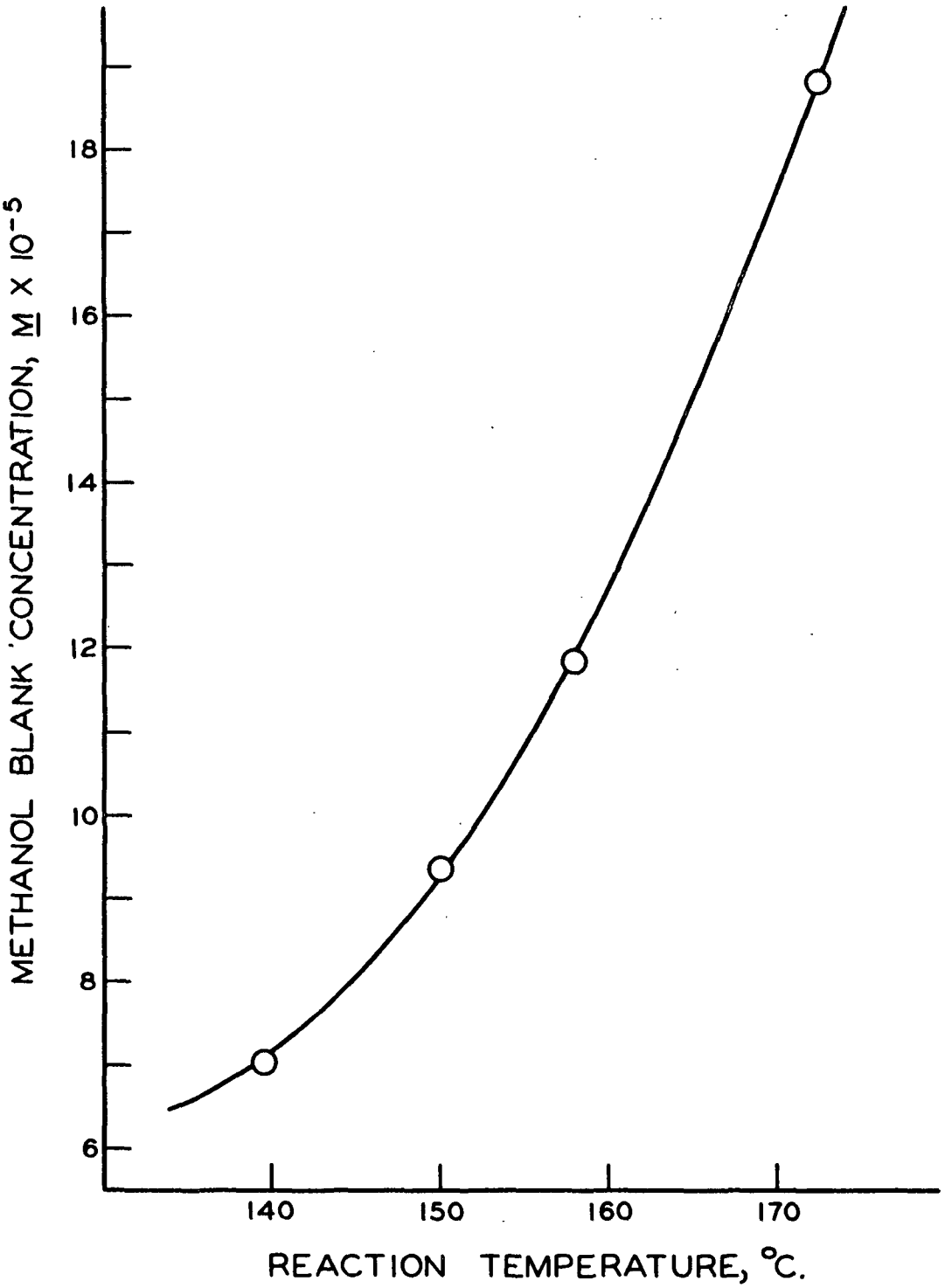

Figure 27. Plot of Methanol Blank Correction Factor Versus Reaction Temperature at Constant Hydroxyl Ion Concentration (2.51N)

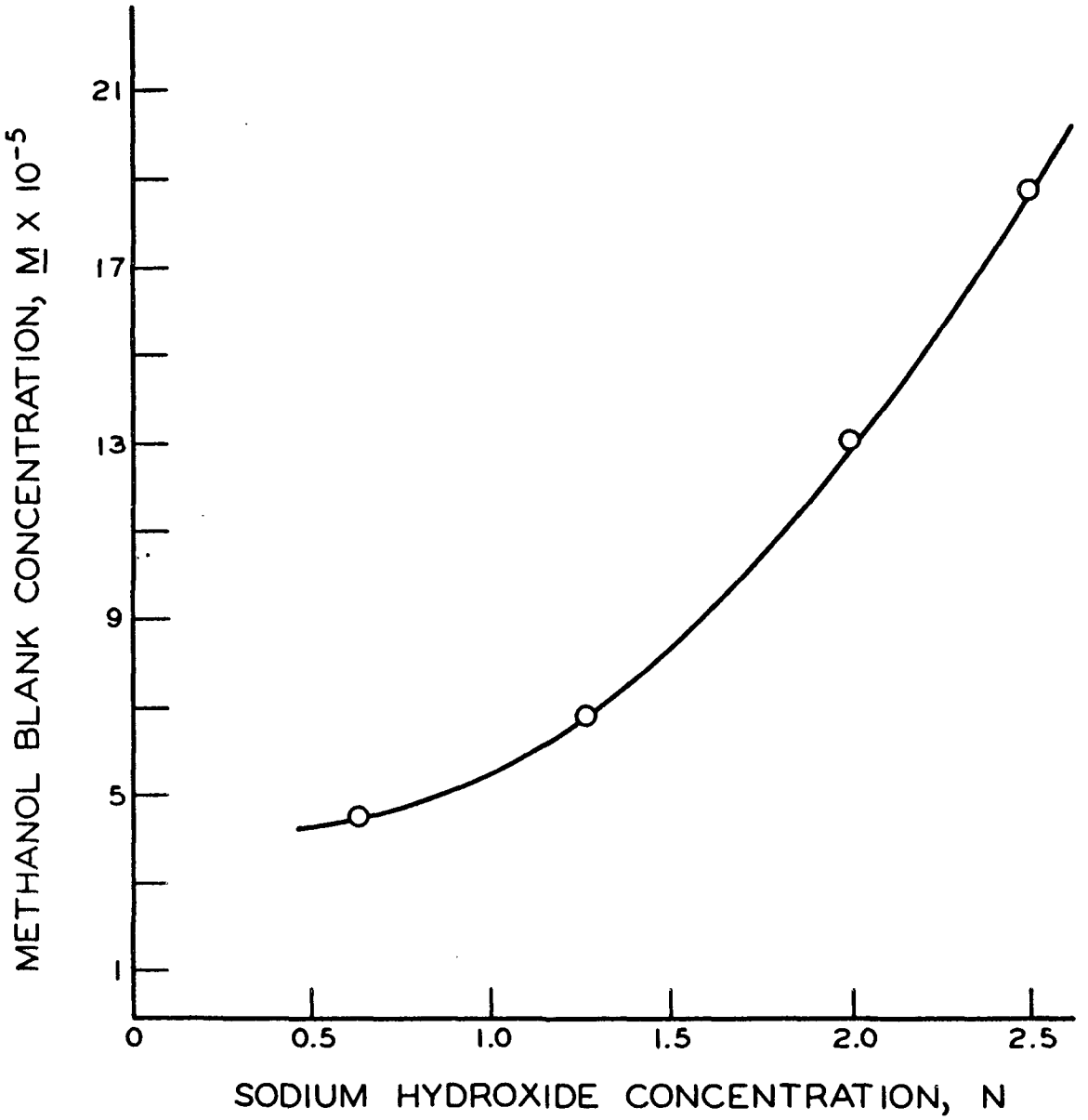

Figure 28. Plot of Methanol Blank Correction Factor Versus Hydroxyl Ion Concentration at 158°C.

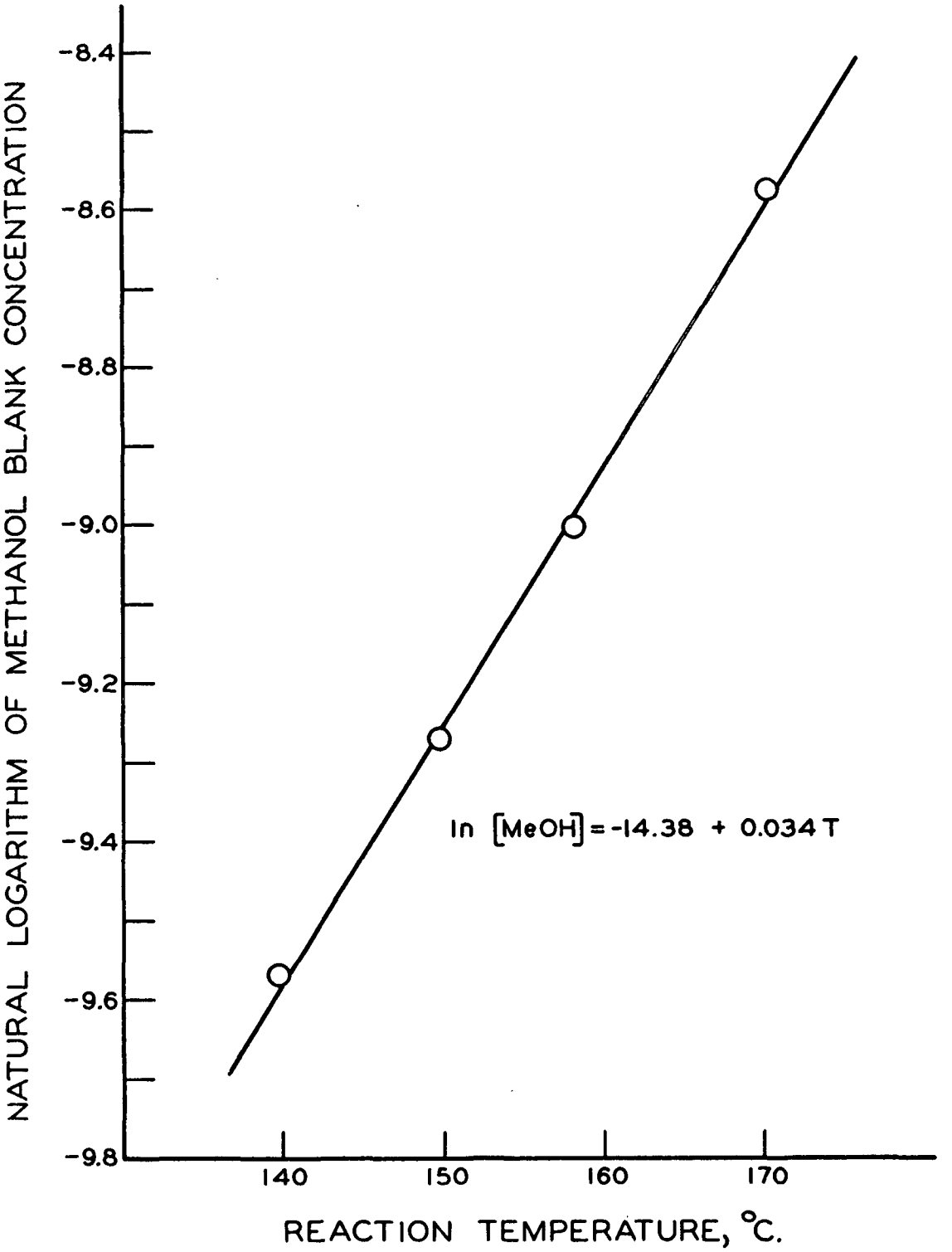

Figure 29. Semilogarithmic Plot of Methanol Blank Correction Factor Versus Reaction Temperature at Constant Hydroxyl Ion Concentration (2.51N)

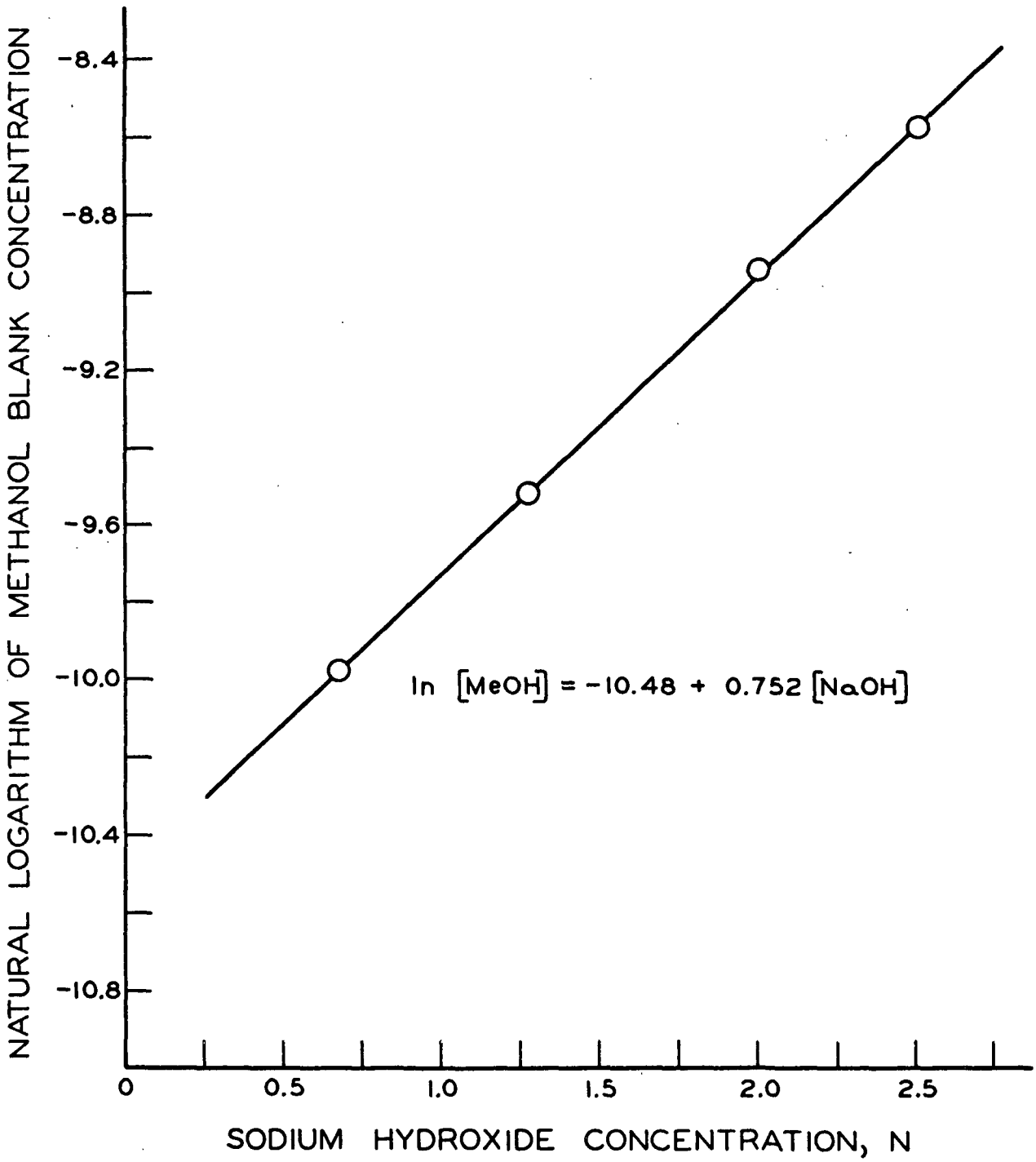

Figure 30. Semilogarithmic Plot of Methanol Blank Correction Factors Versus Hydroxyl Ion Concentration at 158°C.

Blanks were run on the methyl β-glucoside system at 170°C. The three-minute heat-up time was insignificant to the first sample reaction time of 20 hr. Similarly, the methanol produced during heat-up and cooling periods was too small to measure. Therefore, the methanol blank for the glycoside system was only the methanol produced during the distillation process.

CALCULATION OF PSEUDO-FIRST-ORDER RATE CONSTANTS

A 1620 Model II computer and Fortran II program were used to calculate pseudo-first-order rate constants from uncorrected reaction time data, absorbance values, and initial glycoside concentration. The program converted absorbance values to methanol concentrations and subtracted the methanol blank correction factor to give the true methanol concentration of each sample. Based on a proven 1:1 stoichiometric ratio between glucoside or glucuronide and methanol product, the methanol concentration was subtracted from the initial reactant concentration to give the unused reactant concentration. The natural logarithm of the ratio of unused reactant concentration to initial reactant concentration was calculated, and these values were used with corresponding corrected reaction times in a linear regression analysis to yield a correlation coefficient as a measure of the precision of data about the line of regression. A nonlinear regression analysis solved the exponential form of the pseudo-first-order rate equation, and gave the most accurate value of the rate constant as the slope of the nonlinear line of regression.

DETERMINATION OF REACTION STOICHIOMETRY

METHYL α-D-GLUCOPYRANOSIDE SYSTEM

Labeled methyl (C-14) α-D-glucopyranoside (0.025M) in oxygen-free 2.5N sodium hydroxide was reacted for various times (0-142 hr.) at 170°C. Each sample was distilled by the normal procedure and the distillate analyzed for its methanol

concentration. The contents of the distillation flask were diluted ten times with water, placed on a prewashed column of MB-3 anion-cation exchange resin, eluted slowly and washed with 500 ml. of water. The eluate and washings were concentrated, transferred to a 100-ml. flask, reconcentrated to less than 5 ml. with a rotary vacuum evaporator, and transferred to a 5-ml. volumetric flask. The 100-ml. flask was rinsed with 3-4 ml. of water which was concentrated to a few drops and transferred to the 5-ml. flask. This was repeated until the flask was diluted to volume. Using a calibrated Manostat apparatus, 200-400 µl. of solution were applied to Whatman no. 1 chromatography paper and 2-4 µl. were applied on each border as guide strips. The chromatogram was developed for 10 hr. in ethyl acetate:pyridine:water (8:2:1). The guide strips were cut off, detected with silver nitrate:sodium hydroxide:sodium thiosulfate reagent, and used to mark the area of the chromatogram containing methyl α-D-glucopyranoside. This area was cut out, attached to a paper wick, and eluted with water until 5 ml. of eluate collected in a Van Slyke combustion tube. This eluate sample was concentrated to dryness in a vacuum desiccator containing anhydrous calcium chloride. The dry sample was combusted by the procedure of Van Slyke and Folch (86) and analyzed for total carbon and its radioactivity by the procedure of Van Slyke, Steele, and Plazin (87). Using the known volume of solution applied to the chromatogram, the concentration of unreacted methyl α-D-glucopyranoside was calculated.

SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE SYSTEM

The same procedure as above was used except that IR-120 (H^+) cation exresin replaced MB-3 anion-cation exchange resin and the chromatograms were developed for 14 hr. in ethyl acetate:acetic acid:formic acid:water (18:3:1:4).

The Van Slyke radiochemical data and calculated rate constants for SMAG and MAG are given in Appendix I. These results are compared to the rate constants determined from methanol analysis.

DETERMINATION OF THE POINT OF BOND CLEAVAGE

SAMPLE PREPARATION AND ALKALINE DIGESTION

A 2.5N sodium hydroxide solution was prepared by dissolving 0.404 mg. of sodium hydroxide in 4.0 ml. of 5-atom % oxygen-18 (O^{18}) enriched water. A 1.0M solution of methyl α-D-glucopyranoside was prepared by dissolving 390 mg. in 2.0 ml. of 5-atom-percent oxygen-18 (O^{18}) enriched 2.5N sodium hydroxide. The solution was purged with nitrogen, placed in a Type 314 stainless steel bomb (3-ml. capacity), sealed and heated in a hot-air oven at 160°C. for 300 hr.

MICRODISTILLATION OF METHANOL

After allowing the bomb to cool to room temperature, its contents were transferred to a 6-ml. round-bottom flask, two boiling chips were added, and the flask was attached to a specially designed microdistillation apparatus equipped with an air condenser and capillary delivery tube that passed through an ethylene-glycol-dry ice bath. The tip was connected to a drying tube containing Drierite. Heat was applied through a silicon oil bath. Distillation was continued until the first drop of water vapor condensed in the delivery tube. At this time, all the methanol was distilled and condensed in the area of the tube immersed in the cooling bath. The delivery tube was quickly sealed at both ends by a hot flame and submitted to Morgan-Schaffer, Inc., Montreal, Canada, for mass spectrometric analysis.

The same procedures were used for the sodium methyl α-D-glucopyranosiduronate except that the reaction time was only 12 hr.

DETERMINATION OF RELATIVE INDUCTIVE EFFECTS

Ten-percent (10%) solutions of MAG and SMAG were prepared by dissolving 100 mg. in 1.0 ml. of 99.8-atom percent deuterium oxide. Both samples measured[1] approximately pH 7-8. These were labeled "neutral samples." Concentrated sodium deuteroxide (16.3N) was diluted with deuterium oxide to 2.5N. One hundred milligrams (100 mg.) of SMAG and MAG were each dissolved in 1.0 ml. of 2.5N sodium deuteroxide. These samples measured pH 14, and were labeled "alkaline samples."

All four samples were analyzed at 36°C. with a Varian NMR Spectrometer (Model V-6040). The sodium salt of 3-(trimethylsilyl)-propane-sulfonic acid (DSS) was used in each sample as an internal standard. Sweep width and spectrum amplitude were varied to fully investigate the regions of the anomeric proton and methoxyl protons. The chemical shifts of these peaks were recorded for comparison between neutral and alkaline samples.

KINETIC EFFECT OF VARYING NUCLEOPHILE

Five-milliliter (5-ml.) solutions of 1.25N sodium iodide and 1.25N sodium chloride were prepared with standard reagents and 99.8-atom-percent deuterium oxide. Concentrated sodium deuteroxide (16.3N) was diluted with deuterium oxide to 1.25N. One hundred milligrams of SMAG and MAG were added to separate NMR tubes and accurate sample weights were obtained by difference. To each tube was added 0.50 ml. of 1.25N sodium deuteroxide and 0.5 ml. of 1.25N sodium chloride or 0.5 ml. of 1.25N sodium iodide. Duplicate samples of each glycoside

---

[1] The approximate pH of each solution was determined with Hydrion paper.

with each salt combination were prepared. Equal amounts of the sodium salt of 3-(trimethylsilyl)-propane-sulfonic acid (DSS) were added to each tube as an internal standard. A blank sample was prepared consisting of DSS, 0.5 ml. of 1.25N sodium deuteroxide, and 0.5 ml. of 1.25N sodium iodide. All tubes were sealed in a gas flame and agitated thoroughly until dissolution was complete.

All tubes were simultaneously immersed in a silicon oil bath thermostated at 158°C. ± 1°C. The oil was stirred rapidly by means of a Teflon-coated stirring bar and magnetic stirring motor. After specified reaction times, the sample tubes were removed and quenched in 20°C. water. Each tube was cleaned to remove residual oil and then analyzed at 36°C. by a Varian NMR spectrometer.

Samples of SMAG and MAG containing varying amounts of methanol were analyzed with the NMR, and a characteristic peak for the methanol protons was proved to occur at a δ value of 3.33 p.p.m. This peak was distinctly separated from the primary peak of the methoxyl protons which occurred at a δ value of 3.44 p.p.m. Analysis of the reacted DSS blank gave its normal NMR spectrum with no evidence of new peak formation indicating no degradation of the standard.

The NMR spectrum of each reacted sample was integrated and the height of the methanol peak relative to the methoxyl peak was determined. This ratio of peak heights was used with reaction time data to calculate a specific rate constant. Since the peak height ratio was not equal to but proportional to the concentration ratio of methanol to SMAG or MAG, an absolute rate constant could not be calculated. For means of comparison, a relative rate constant between the specific rate constants of the two nucleophilic systems was calculated. This experimental procedure gave reproducible results between duplicate samples.

NOMENCLATURE

$\underline{A}$ = frequency factor, $sec.^{-1}$

$\underline{b}$ = empirical kinetic order with respect to hydroxide ion concentration

$\underline{E_a}$ = Arrhenius activation energy, kcal. $mole^{-1}$

$\underline{e}$ = base for Napierian logarithm, 2.7183

$\Delta\underline{F}^{\ddagger}$ = Gibbs free energy of activation, kcal. $mole^{-1}$

$\Delta\underline{H}^{\ddagger}$ = enthalpy of activation, kcal. $mole^{-1}$

$\underline{K}^*$ = overall equilibrium constant

$\underline{K_A}$ = dissociation constant

$\underline{K_e}$ = equilibrium constant for proton transfer reaction

$\underline{K_w}$ = ion product of water

$\underline{k}$ = rate constant, $sec.^{-1}$

$\underline{k'}$ = pseudo-first-order rate constant, $sec.^{-1}$

$\underline{k_i}$ = initial first-order rate constant

$\underline{R}$ = gas constant, 1.987 cal. $deg.^{-1}$ $mole^{-1}$

$\Delta\underline{S}^{\ddagger}$ = entropy of activation, cal. $°K.^{-1}$ $mole^{-1}$

$\underline{T}$ = absolute temperature, °K.

γ = activity coefficient

δ = chemical shift, p.p.m.

$\Delta\delta$ = change in chemical shift, p.p.m.

ACKNOWLEDGMENTS

The author gratefully acknowledges the assistance and encouragement of his thesis advisory committee: Drs. J. W. Green, chairman, N. S. Thompson, and Mr. E. E. Dickey.

In addition, the author wishes to express his sincere gratitude to Dr. L. R. Schroeder for his consultation on interpretation of the experimental results. Indebtedness is due to L. O. Sell for performing all NMR analyses.

To my parents and personal friends, I express true appreciation for the continuing encouragement, guidance, and confidence given throughout this research.

Finally, I wish to thank Mrs. Betty Cary for the typing of this and other manuscripts throughout my graduate studies at The Institute of Paper Chemistry.

LITERATURE CITED

1. Whistler, R. L., and Corbett, W. M. In The carbohydrates. Pigman, W., Ed. p. 666. New York, Academic Press, Inc., 1957.

2. Wise, L. E. In Wood chemistry. 2d. ed. p. 399. Wise, L. E., and Jahn, E. C., Eds. New York, Reinhold Publishing Corp., 1952.

3. Cottrall, L. G., Tappi 33:471(1950).

4. Sitch, D. A., and Marshall, H. B., Can. J. Res. F28:376(1950).

5. Giertz, H. W., Svensk Papperstid. 55:72(1952).

6. Haas, H., Battenberg, E., and Teves, D., Tappi 35:116(1952).

7. Timell, T. E., Glaudemans, C. P. J., and Gilham, J. K., Tappi 42:623-34(1959).

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

9. Hamilton, J. K., and Thompson, N. S., Pulp Paper Mag. Can. 61:T263-72(1960).

10. Meier, H., Svensk Papperstid. 65:299(1962).

11. Thompson, N. S., Peckham, J. R., and Thode, E. F., Tappi 45:433(1962).

12. Speck, J. C., Jr., Adv. Carbohydrate Chem. 13:63-103(1958).

13. Sowden, J. C., and Schaffer, R., J. Am. Chem. Soc. 74:499(1952).

14. Whistler, R. L., and Be Miller, J. N., Adv. Carbohydrate Chem. 13:289-329 (1958).

15. Isbell, H. S., J. Res. Natl. Bur. Stds. 32:45-59(1944).

16. Machell, G., Richards, G. N., and Sephton, H. H., Chem. Ind. 1957:467-9.

17. Machell, G., and Richards, G. N., J. Chem. Soc. 1957:4500-6.

18. Alfredsson, B., and Samuelson, O., Tappi 46:379-81(1963).

19. Green, J. W., Tappi 39:472-7(1956).

20. Collier, R. W., Tappi 43:15-18(1960).

21. Lindberg, B., Svensk Papperstid. 59:531(1956).

22. Richards, G. N. Alkaline degradation. In Methods in carbohydrate chemistry. Vol. III. p. 154. R. L. Whistler, Ed. New York, Academic Press, 1963.

23. Brooks, R. D. A kinetic study of the rate of cleavage of the glycosidic bond of methyl β-glucopyranoside in an alkaline medium. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1965.

24. Best, E. V. Kinetics of hot alkaline cleavage of the glycosidic bonds of methyl β-D-glucoside and methyl β-D-cellobioside. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1968.

25. Corbett, W. N., and Richards, G. N., Svensk Papperstid. 60:792(1957).

26. Tanret, C., Compt. rend. 119:58(1894).

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

28. Bardolph, M. S., and Coleman, G. H., J. Org. Chem. 15:169(1950).

29. Gasman, R. C. Nucleophilic displacement reactions at the anomeric carbon of p-nitrophenyl β-D-galactoside and p-nitrophenyl α-D-mannoside. Doctor's Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1966.

30. Janson, J., and Lindberg, B., Acta Chem. Scand. 13:138(1959).

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

32. Ballou, C. E., Adv. Carbohydrate Chem. 9:59(1954).

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

34. Gould, E. S. In Mechanism and structure in organic chemistry. p. 567. New York, Holt, Rinehart, and Winston, Inc., 1960.

35. Johnson, D. C. Personal communication, 1965.

36. Lindberg, B., and Janson, J., Acta Chem. Scand. 14:2051(1960).

37. Prey, V., Waldmann, E., and Swoboda, H., Monatsch. Chem. 84:98, 888(1953).

38. Prey, V., Waldmann, E., and Swoboda, H., Holzforsch. 9:10(1955).

39. Ross, J. R. The behavior of 4-O-methylglucuronoxylan and 4-O-methylglucoxylan in hot alkali. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1964.

40. Semke, L. K. The rates of acid hydrolysis of the β-D-glucopyranosides of phenol, para-cresol, and para-chlorophenol. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1963.

41. Ingold, C. K. In Structure and mechanism in organic chemistry. p. 324. New York, Cornell University Press, 1953.

42. Hughes, E. D., Ingold, C. K., and Gleave, J. L., J. Chem. Soc. 1935:236, 244.

43. Hughes, E. D., Ingold, C. K., and Shapiro, V. G., J. Chem. Soc. 1936: 225.

44. Hughes, E. D., Trans. Faraday Soc. 34:185(1938).

45. Hughes, E. D., Trans. Faraday Soc. 37:603(1941).

46. Hine, J. Physical organic chemistry. 2d. ed. New York, McGraw-Hill Book Co., Inc., 1962. 552 p.

47. Streitwieser, A., Jr. Solvolytic displacement reactions. New York, McGraw-Hill Book Co., Inc., 1962. 214 p.

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

49. Arrhenius, S., Z. Phys. Chem. 1:631(1887).

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

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

52. Morgan, J. E., and Schaffer, R. Personal communication, 1967.

53. Cohn, M., and Urey, H. C., J. Am. Chem. Soc. 60:679(1938).

54. Hammett, L. P. Physical organic chemistry. 1st ed. Chap. VII. New York, McGraw-Hill Book Co., Inc., 1940.

55. Taft, R. W., J. Am. Chem. Soc. 74:2729; 3120(1952).

56. Taft, R. W., J. Am. Chem. Soc. 75:4231(1953).

57. Smyth, C. P. Dielectric behavior and structure. p. 202-392. New York, McGraw-Hill Book Co., Inc., 1955.

58. Brown, H. C., McDaniel, D. H., and Haflinger, O. In Braude, E. A., and Nachod, F. C.'s Determination of organic structures by physical methods. p. 567-662. New York, Academic Press, Inc., 1955.

59. Marchessault, R. H., and Ranby, B. G., Svensk Papperstid. 62:230(1959).

60. Nakano, J., and Ranby, B. G., Svensk Papperstid. 65:29(1962).

61. Timell, T. E., Enterman, W., Spencer, F., and Soltes, E. J., Can. J. Chem. 43:2296(1965).

62. McKee, S. C., and Dickey, E. E., J. Org. Chem. 28:1561(1963).

63. Saunders, M. D., and Timell, T. E., Carbohydrate Res. 5:453(1967).

64. Glor, P. Unpublished work, The Institute of Paper Chemistry.

65. Michaelis, L., Ber. 46:3683(1913).

66. Glasstone, S., Laidler, K. J., and Eyring, H. The theory of rate processes. New York, McGraw-Hill Book Co., Inc., 1941. 611 p.

67. Denbigh, K. The principles of chemical equilibrium. Chap. 9. Cambridge University Press, 1963.

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

69. Legg, G. W., and Hart, J. S., Tappi 45:5(1960).

70. Swain, C. G., and Scott, C. E., J. Am. Chem. Soc. 75:4627(1953).

71. Swain, C. G., and Scott, C. B., J. Am. Chem. Soc. 77:3727, 3731, 3737(1955).

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

73. Isbell, H. S., and Frush, H. L., J. Res. NBS 24:125-51(1940).

74. Eliel, E. L., Allinger, N. L., Angyal, S. J., and Morrison, G. A. Conformational analysis. Chap. 6. New York, John Wiley & Sons, Inc., 1965.

75. Jungius, C. L., Z. Phys. Chem. 52:97(1905).

76. Dryselius, E., Lindberg, B., and Theander, O., Acta Chem. Scand. 12:340(1958).

77. Angyal, S. J., and McHugh, D. J., Chem. Ind. (London) 1955:947.

78. Dryselius, E., and Lindberg, B., Acta Chem. Scand. 11:663(1957).

79. Daniels, F., and Alberty, R. A. Physical chemistry. 2d. ed. Chap. 9. New York, John Wiley & Sons, Inc., 1962.

80. Koskikallio, J., Suomen Kem. 30B:155(1957); C.A. 52:3481f(1958).

81. Fernander, L. P., and Hepler, L. G., J. Am. Chem. Soc. 81:1783(1959).

82. Brown, D. A., and Hudson, R. F., J. Chem. Soc. 1953:3352.

83. Schaleger, L. L., and Long, F. A., Adv. Phys. Org. Chem. 1:1(1963).

84. Long, F. A., Pritchard, J. G., and Stafford, F. E., J. Am. Chem. Soc. 79: 2362(1957).

85. Bollenback, G. N. In Methods in carbohydrate chemistry. Vol. III. p. 326. New York, Academic Press, 1963.

86. Van Slyke, D. D., and Folch, J., J. Biol. Chem. 136:509-41(1940).

87. Van Slyke, D. D., Steele, R., and Plazin, J., J. Biol. Chem. 192:769-805 (1951).

88. Bollenback, G. N. Methyl glucoside. p. 19-20. New York, Academic Press, 1958.

89. Macpherson, H. T., and Percival, E. G. V., J. Chem. Soc. 1937:1920.

90. Trenner, N. R., U. S. pat. 2,428,438(1947); 2,483,251(1949).

91. Farrar, N. O. Partitioning and reversibility of polymer adsorption in the polystyrene 1,2-dichloroethane carbon black system. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1967.

92. Mehltretter, C. L., U. S. pat. 2,562,200(1951).

93. Easty, D. B. The synthesis and acid hydrolysis of methyl α-D-glucopyranosiduronic acid. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1963.

94. De Boer, T. J., and Backer, H. J., Rec. Trav. Chim. 73:229-34(1954).

95. Wolfrom, M. L., Kowkabany, G. N., and Binkley, W. W., J. Am. Chem. Soc. 76:4011-12(1954).

96. Hardegger, E., and Spitz, D., Helv. Chim. Acta 33:337-42(1950).

97. Nakano, J., and Ranby, B. G. Paper presented before the Cellulose Division of the American Chemical Society, New York City Meeting, Sept., 1960.

98. Perry, J. H. Chemical engineers' handbook. 3d. ed. p. 277. New York, McGraw-Hill Book Co., Inc., 1950.

99. Boos, R. N., Anal. Chem. 20:964(1948).

100. Larson, K. An investigation of pectic substance in the developing xylem of Populus tremuloides. Doctoral Dissertation. Appleton, Wis., The Institute of Paper Chemistry, 1967.

APPENDIX I

DETERMINATION OF REACTION STOICHIOMETRY

TABLE XVI

VAN SLYKE RADIOCHEMICAL DATA FOR KINETIC RATE DETERMINATION OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE (0.024M) IN 2.5N SODIUM HYDROXIDE AT 150°C.

Reaction Time, hr.[a]	Dis./Min.//Sample	Carbon, mg.[b]	Methyl α-Glucuronide Concn., M
0.95	6239	2.41	0.0216
1.45	5903	2.28	0.0203
1.95	5411	2.09	0.0187
2.95	4893	1.89	0.0168

Result of radiochemical analysis: $\underline{k}' = 1.01 \times 10^{-1}$ hr.$^{-1}$.

Result of methanol analysis[c]: $\underline{k}' = 0.965 \times 10^{-1}$ hr.$^{-1}$.

Error = 4.9%.

---

[a] Includes reaction time correction factor.

[b] Based on a known specific activity of 2589 dis./min.//mg. C.

[c] Includes reaction time and methanol blank correction factors.

TABLE XVII

VAN SLYKE RADIOCHEMICAL DATA FOR KINETIC RATE DETERMINATION OF METHYL α-D-GLUCOPYRANOSIDE (0.024M) IN 2.5N SODIUM HYDROXIDE AT 170°C.

Reaction Time, hr.[a]	Dis./Min.//Sample	Carbon, mg.[b]	Methyl α-Glucoside Concn., M
39.33	7798	3.60	0.0215
74.25	6845	3.16	0.0188
111.50	5957	2.75	0.0164
140.17	5350	2.47	0.0147

Result of radiochemical analysis: $\underline{k}' = 3.18 \times 10^{-3}$ hr.$^{-1}$.

Result of methanol analysis[c]: $\underline{k}' = 3.07 \times 10^{-3}$ hr.$^{-1}$.

Error = 3.4%.

---

[a] Includes reaction time correction factor.

[b] Based on a known specific activity of 2166 dis./min.//mg. C.

[c] Includes reaction time and methanol blank correction factors.

APPENDIX II

CALCULATION OF THEORETICAL CARBOXYL AND ALKOXYL ANION FUNCTION CONCENTRATIONS

I. Derivation of a theoretical equilibrium constant for the following reaction:

$$RCOOH + OH^- \overset{K_e}{\rightleftarrows} RCOO^- + H_2O$$

where:

RCOOH = undissociated methyl α-glucuronide,

K_e = equilibrium constant for proton transfer reaction of carboxyl group, and

$RCOO^-$ = dissociated methyl α-glucuronide with C5 carboxylate anion.

$$RCOOH + H_2O \overset{K_i}{\rightleftarrows} RCOO^- + H_3O^+ \tag{13}$$

$$H_2O \overset{K_w}{\rightleftarrows} H^+ + OH^- \tag{14}$$

$$K_i = \frac{[RCOO^-][H_3O^+]}{[RCOOH][H_2O]} \tag{15}$$

$$K_A = K_i[H_2O] = \frac{[RCOO^-][H_3O^+]}{[RCOOH]} \tag{16}$$

$$K_w = [H^+][OH^-] = [H_3O^+][OH^-],\ [H_3O^+] = \frac{K_w}{[OH^-]} \tag{17}$$

$$K_A = \frac{[RCOO^-][H_3O^+]}{[RCOOH]} = \frac{[RCOO^-]}{[RCOOH]}\ \frac{K_w}{[OH^-]} \tag{18}$$

$$pK_A = -\log K_A = -\log \frac{[RCOO^-]}{[RCOOH][OH^-]}\ -\log K_w \tag{19}$$

$$-\log \frac{[RCOO^-]}{[RCOOH][OH^-]} = -\log K_A + \log K_w \tag{20}$$

$$K_e = \frac{K}{[H_2O]} = \frac{[RCOO^-][H_2O]}{[RCOOH][OH^-]} = K_A - K_w \qquad (21)$$

$$K_e = \frac{[RCOO^-][H_2O]}{[RCOOH][OH^-]} = K_A - K_w \qquad (22)$$

$$pK_{e_{COOH}} = pK_{A_{SMAG}} - pK_w \qquad (23).$$

Similar treatment gives the following expression for the proton transfer reaction involving the hydroxyl groups of SMAG and MAG: $pK_{e_{OH}} = pK_{A_{MAG}} \; pK_w$ ..

II. Calculation of Theoretical Equilibrium Constant

$$K_{e_{COOH}} = K_{A_{SMAG}} - K_w, \; K_{e_{OH}} = K_{A_{MAG}} - K_w$$

Phenyl β-glucuronide (64) $pK_A = 3.13$[1]

Methyl α-glucoside (65) $pK_A = 13.71$[1]

Ion product of water (98) $pK_w = 14.17$[1]

$pK_{e_{COOH}} = -11.04$ $\qquad$ $pK_{e_{OH}} = -0.46$

$K_{e_{COOH}} = 1.10 \times 10^{11}$ $\qquad$ $K_{e_{OH}} = 2.88$

III. Calculation of the ratio of dissociated to undissociated glycoside species.

$$K_{e_{COOH}} = \frac{[RCOO^-][H_2O]}{[RCOOH][OH^-]}, \qquad K_{e_{OH}} = \frac{[R_2CHO^-][H_2O]}{[R_2CHOH][OH^-]}$$

$$K_{e_{COOH}}[OH^-] = \frac{[RCOO^-]}{[RCOOH]}, \qquad K_{e_{OH}}[OH^-] = \frac{[R_2CHO^-]}{[R_2CHOH]}$$

[1] Determined by potentiometric titrations at 20°C.

$$(10^{11.04})[OH^-] = \frac{[RCOO^-]}{[RCOOH]}, \qquad (10^{0.46})[OH^-] = \frac{[R_2CHO^-]}{[R_2CHOH]} .$$

At pH 7, $[OH^-] = 10^{-7}$,

$$\frac{[RCOO^-]}{[RCOOH]} = (10^{11.04})(10^{-7}), \qquad \frac{[R_2CHO^-]}{[R_2CHOH]} = (10^{0.46})(10^{-7})$$

$$\frac{[RCOO^-]}{[RCOOH]} = 10^{4.04}, \qquad \frac{[R_2CHO^-]}{[R_2CHOH]} = 10^{-6.54} .$$

Therefore, at pH 7.0 ten thousand carboxylate anions will be present for each undissociated methyl α-glucuronide molecule while only one in 10^6 molecules will have alkoxyl anion functions. Table XVIII lists these calculated ratios as a function of hydroxide ion concentration.

TABLE XVIII

RELATIVE RATIOS OF IONIZED AND UNIONIZED SPECIES AS A FUNCTION OF HYDROXIDE ION CONCENTRATION

pH	Hydroxide Ion Concn., N	$\frac{[RCOO^-]}{[RCOOH]}$	$\frac{[R_2CHO^-]}{[R_2CHOH]}$
7	10^{-7}	$10^{4.0}$	$10^{-6.5}$
11	10^{-3}	10^{8}	$10^{-2.5}$
14	1.0	10^{11}	$10^{0.5}$
--	2.50	--	$10^{0.9}$

APPENDIX III

LARGE-SCALE PREPARATION OF CRUDE SODIUM METHYL α-GLUCURONIDE BY CATALYTIC AIR OXIDATION

Dissolve 78 g. of recrystallized methyl α-glucoside in 1300 ml. of distilled water and clean the solution with activated charcoal. Filter through a Celite mat and transfer the filtrate to a 3-liter Morton stirring flask which is immersed in a hot water bath regulated at 50°C. ± 1°C. As the glucoside solution warms to temperature, adjust the air flow rate to 650 liters/hr., standardize the combination pH electrode in a pH 7.0 buffer solution at 50°C., and start the stirring motor to provide gentle mixing. Once the reactant solution is at 50°C., adjust its pH to 8.5 by adding a few drops of 2.5N NaOH, raise the stirring motor speed to the point just before cavitation. This is approximately 1300 r.p.m. Add 10.3 g. of platinum-carbon catalyst (14% Pt) to the rapidly stirred solution and insert the Beckman combination pH electrode into the reaction flask (see Fig. 31).

The reaction begins spontaneously as the catalyst is added. Begin adding 170 ml. of 2.5N NaOH to the reaction flask and adjust the flow from the buret so that the pH of the reaction solution is maintained between 7.5 and 8.5. The reaction is completed after all the base has been added and the pH of the reaction solution remains constant. The average reaction time is six hours, with a final pH value of 8.0.

The reaction solution is filtered to remove the catalyst and is concentrated to a 300-ml. volume on a rotary vacuum evaporator. This product solution is yellow-green in color. Since sodium hydroxide is used to neutralize the glucuronide, the sodium salt of the free acid is directly precipitated in absolute ethanol. Due to the hygroscopic nature of methyl glucuronide, this precipitation is delicate and should be done batchwise. Slowly add 50 ml. of the concentrated

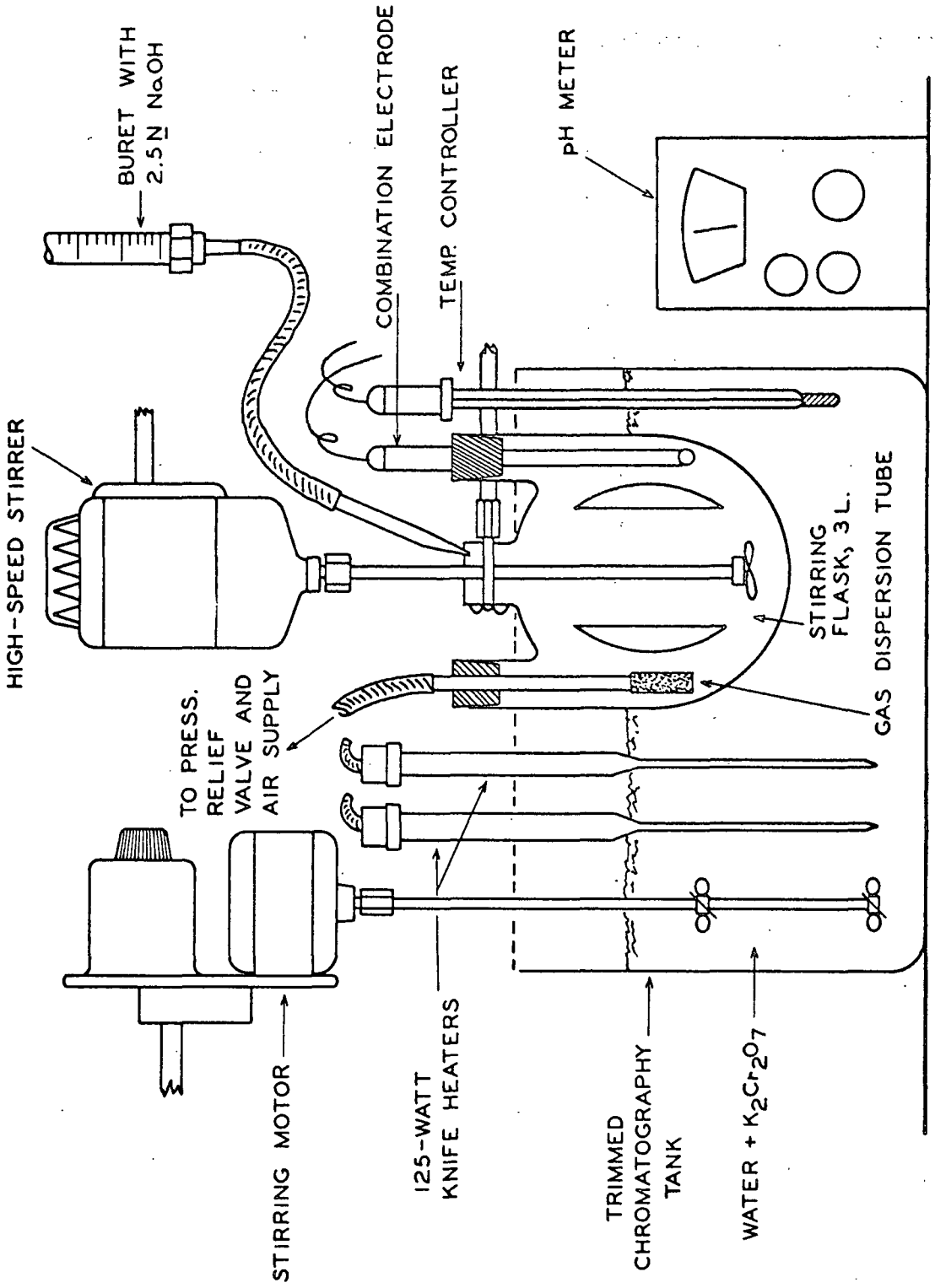

Figure 31. Apparatus for Catalytic Air Oxidations

glucuronate solution to 1500 ml. of rapidly stirred ethanol. The sodium salt precipitates as a fine white solid. After filtering, the precipitate is solvent exchanged with diethyl ether and petroleum ether, in that order. The precipitate is quickly transferred to a vacuum desiccator and dried at 40°C. under 29 in. Hg vacuum. Following this procedure, the average yield of crude sodium methyl α-glucuronate is 68%.

APPENDIX IV

CALCULATION OF THERMODYNAMIC ACTIVATION FUNCTIONS

The Arrhenius activation energies (E_a) were calculated by the method of least squares, according to the logarithmic form of the Arrhenius equation [Equation (24a)]:

$$k' = A \exp(-E_a/RT) \tag{24}$$

$$\ln k' = \ln A - E_a/RT \tag{24a}$$

where:

k' = pseudo-first-order rate constant

A = empirical "frequency factor."

The enthalpy of activation ($\Delta H^{\ddagger}$) was calculated from Equation (25) (68):

$$\Delta H^{\ddagger} = E_a - RT + p\Delta V^{\ddagger} \tag{25}$$

where $p\Delta V^{\ddagger} = 0$, since $\Delta V^{\ddagger}$, the volume change in the reaction, is assumed to be zero,

T = temperature, °K., and

R = gas constant, 1.9865 cal./°K mole .

The entropy of activation ($\Delta S^{\ddagger}$) was calculated from Equation (27), which is derived from the Arrhenius equation [Equation (24)], and the relationship between the rate constant and the entropy of activation [Equation (26)]. Equation (26) was derived from the theory of absolute reaction rates (66) which relates the free energy of activation to the rate constant:

$$k' = (ekT/h) \exp(-E_a/RT) \exp(\Delta S^{\ddagger}/R) \tag{26}$$

where:

$\underline{e}$ = base for Napierian logarithm, 2.7183,

$\underline{k}$ = Boltzmann constant, 1.380×10^{-16} erg/°K

$\underline{h}$ = Planck constant, 6.625×10^{-27} erg sec.

$$\Delta S^{\ddagger} = R \ln (A/T) + R \ln (h/ek) \qquad (27)$$

The free energy of activation ($\Delta \underline{F}^{\ddagger}$) was calculated from Equation (28):

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \quad . \qquad (28)$$

APPENDIX V

PREPARATION OF ETHEREAL DIAZOMETHANE (94)

A 500-ml. distilling flask is fitted with a condenser and a long-stemmed 500-ml. dropping funnel. The condenser is connected by means of an adapter to a 1000-ml. Erlenmeyer flask. Through a second hole in the stopper of the Erlenmeyer flask is placed an outlet tube so as to pass into and nearly to the bottom of a second Erlenmeyer flask which is not stoppered but plugged with cotton. Both receivers are cooled in an ice-salt mixture; in the first is placed 40 ml. of anhydrous diethyl ether, and in the second 120 ml. of ether. The inlet tube passes below the surface of the ether in the second flask. A picture of the assembled apparatus is shown in Fig. 32. All glassware is free from scratches and sharp edges, while all connections are made with rubber stoppers. Checkers of this procedure attribute the explosions frequently reported to the presence in the system of sharp or rough surfaces, especially ground joints.

In the distilling flask are placed 60 ml. of potassium hydroxide solution (60 g. KOH dissolved in 100 ml. of water), 140 ml. of monoethyl ether of diethylene glycol, and the Teflon-coated bar of a magnetic stirrer. The dropping funnel is attached and adjusted so that the stem is just above the surface of the solution in the distilling flask. There is placed in the dropping funnel 40 ml. of anhydrous ether. The distilling flask is heated in a silicone oil bath at 70-75°C., the stirrer is started, and the ether is added at a regular rate during five minutes. When the dropping funnel is nearly empty of ether, it is filled with a solution of 86.0 g. (0.4M) of p-tolysulfonylmethylnitrosamide (Diazald, Aldrich Chemical Co.) in 550 ml. of ether. The nitrosamide solution is added at a regular rate over a 90-minute period. After all this solution has been added, additional ether (50-100 ml.) is placed in the dropping funnel and added at the previous rate until

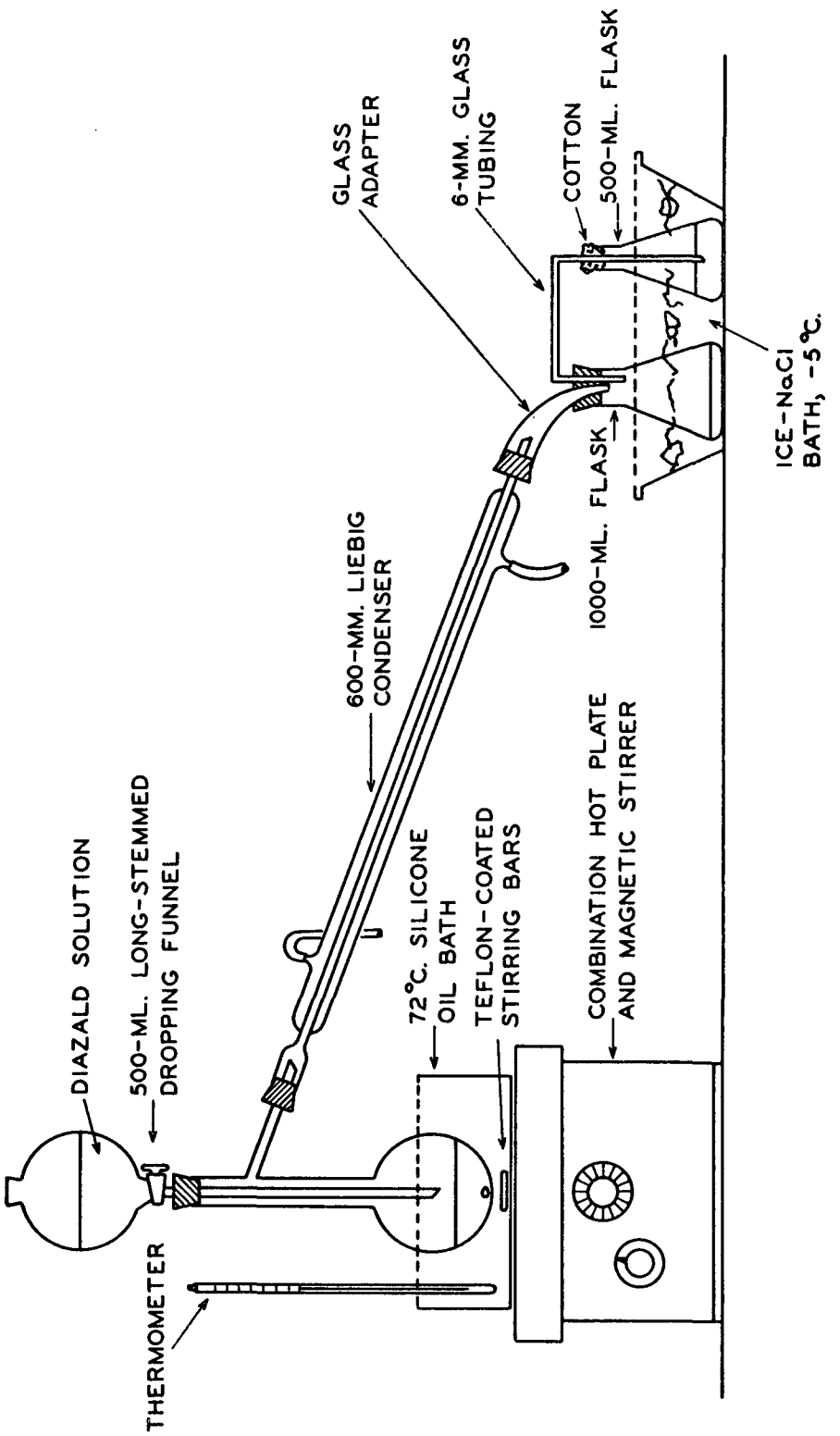

Figure 32. Apparatus for the Generation of Ethereal Diazomethane

the distillate is colorless. The distillate volume totals approximately 800 ml. and contains 10.8-11.6 g. (64-69%) of diazomethane.

Note: Caution! Diazomethane is toxic and prone to cause development of specific sensitivity. A well-ventilated hood with a safety glass door should be used for the entire procedure. Also, safety glasses and heavy gloves are to be worn at all times when working with the diazomethane solution.

APPENDIX VI

PROCEDURE FOR THE COLORIMETRIC ANALYSIS OF METHANOL

The following procedure was developed from the basic method of Boos (99). For the concentration range of 1 to 300 μg. MeOH/ml., the mean error was less than ± 2.0%.

I. REAGENTS

A. 5% Chromotropic Acid

Weigh 1.250 g. of chromotropic acid on an aluminum weighing dish, dissolve in 20 ml. of distilled water, transfer to a 25-ml. volumetric flask, and dilute to volume. Filter the solution on no. 50 Whatman filter paper, collect the filtrate in a dark glass bottle wrapped with aluminum foil. Store in a 10°C. refrigerator except when being used. Since this reagent decomposes with time, a fresh acid solution should be prepared after the original is two weeks old.

B. 5% Potassium Permanganate

Weigh out 1.250 g. of potassium permanganate crystals, dissolve in 20 ml. of distilled water, transfer to a 25-ml. volumetric flask, dilute to volume, and store in the dark.

C. 10% Sodium Bisulfite

Dissolve 5.0 g. of sodium bisulfite in 30 ml. of distilled water, dilute to 50 ml., filter on no. 50 Whatman filter paper, collect the filtrate in a dark glass bottle, and store in the refrigerator.

D. 5% Phosphoric Acid

Dilute 4.54 ml. of 86.4% phosphoric acid to 100 ml. with distilled water, and store in the refrigerator.

II. PROCEDURE

1. Carefully pipet 1.00 ml. of the sample to be analyzed into a 10-ml. volumetric flask. Place the flask in a salt-ice water bath.

2. Carefully add four (4) drops of 5% phosphoric acid to each sample and shake mildly. Ten samples can be most conveniently analyzed in one batch determination.

3. Add five (5) drops of 5% potassium permanganate to the first acidified sample and activate a stopwatch after the first drop is added. Shake mildly and replace in the cold bath.

4. Oxidize each sample for 10.0 minutes, shaking each sample frequently. Stop the oxidation by adding eight (8) drops of 10% sodium bisulfite and shaking.

5. Add 4.0 ml. of 5°C. concentrated sulfuric acid to each sample. Swirl the flask in a salt-ice water bath while the acid is being added. A 25-ml. buret should be used to deliver the acid, and its stopcock should be lubricated only with sulfuric acid.

6. Preheat a water bath to 60°C. Carefully add four (4) drops of 5% chromotropic acid to each sample and place in the hot water bath. Digest each sample for 16 minutes with frequent shaking. Remove each sample and quench in an ice-water bath. Use a stopwatch for a timer.

7. After 10 minutes' cooling time, add freshly distilled water to each sample until the solution is within 1/2 inch of the dilution line. Stopper, shake, and allow an hour for equilibration to room temperature.

8. Tap each sample to drive off any adhering gas bubbles, and dilute to 10.0 ml.

9. Measure the absorbance of each sample against a water blank with a D.U. spectrophotometer at 570 nm. Matched sample cuvettes should be used. Two reagent blanks should be run with each set of determinations. Any blank with an absorbance higher than 0.050 indicates that fresh reagents should be prepared.

APPENDIX VII

EXPERIMENTAL DATA

TABLE XVIII

ALKALINE HYDROLYSIS OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE

Time,[a] hr.	Methanol[b] Concn., $(\underline{M}) \times 10^3$	Unreacted SMAG Concn., $(\underline{M})$, $\underline{G}$	$\ln (\underline{G}/\underline{G}_o)$
	Temp., $\underline{T}$ = 139.3°C.; NaOH concn., [NaOH] = 2.500$\underline{N}$; init. reactant concn., $[\underline{G}_o]$ = 0.025$\underline{M}$		
1.95	0.40	0.02459	-0.05482
2.95	0.91	0.02408	-0.07557
3.95	1.54	0.02345	-0.10226
4.95	2.23	0.02276	-0.13189
5.95	2.85	0.02214	-0.15989
	$\underline{T}$ = 149.6°C.; [NaOH] = 2.501$\underline{N}$; $[\underline{G}_o]$ = 0.0250$\underline{M}$		
0.98	1.22	0.02377	-0.09013
1.95	2.79	0.02220	-0.15860
2.45	3.84	0.02115	-0.20685
2.95	4.83	0.02016	-0.25467
3.45	5.93	0.01906	-0.31096
3.95	6.63	0.01836	-0.34822
	$\underline{T}$ = 150.8°C.; [NaOH] = 2.489$\underline{N}$; $[\underline{G}_o]$ = 0.0244$\underline{M}$		
0.95	1.14	0.02325	-0.09171
1.95	3.28	0.02111	-0.18826
	$\underline{T}$ = 158.1°C.; [NaOH] = 2.507$\underline{N}$; $[\underline{G}_o]$ = 0.0250$\underline{M}$		
0.45	2.01	0.02298	-0.10733
0.95	4.45	0.02054	-0.21956
1.45	6.89	0.01810	-0.34599
1.95	8.81	0.01618	-0.45792
5.95	7.90	0.01719	-0.45982
7.57	10.22	0.01487	-0.60501
	$\underline{T}$ = 170.2°C.; [NaOH] = 2.532$\underline{N}$; $[\underline{G}_o]$ = 0.0245$\underline{M}$		
0.50	8.9	0.01644	-0.39842
0.70	12.0	0.01329	-0.61034
0.95	14.3	0.01098	-0.80358
1.51	18.5	0.00676	-1.28932
1.20	17.0	0.00833	-1.11174

[a] See end of table for footnotes.

TABLE XVIII (Continued)

ALKALINE HYDROLYSIS OF SODIUM METHYL α-D-GLUCOPYRANOSIDURONATE

Time,[a] hr.	Methanol[b] Concn., (M) x 10^3	Unreacted SMAG Concn., (M), G	ln (G/G_o)
	T = 158.1°C.; [NaOH] = 1.229N; [G_o] = 0.0249M		
0.95	1.07	0.02382	-0.12908
1.95	3.46	0.02143	-0.23437
2.95	5.61	0.01928	-0.34003
4.20	8.98	0.01591	-0.53204
5.12	10.72	0.01417	-0.64800
	T = 158.3°C.; [NaOH] = 0.5884N; [G_o] = 0.0251M		
1.45	0.93	0.02416	-0.11978
4.45	5.34	0.01975	-0.32123
5.95	7.90	0.01719	-0.45982
7.57	10.22	0.01487	-0.60501
	T = 158.1°C.; [NaOH] = 0.2270N; [G_o] = 0.0246		
2.95	1.95	0.02265	-0.08152
5.45	3.42	0.02118	-0.14860
9.63	5.77	0.01883	-0.26613
10.98	6.42	0.01818	-0.30127
	T = 170.1°C.; [NaOH] = 0.2251N; [G_o] = 0.0252M		
4.53	9.02	0.01618	-0.44401
6.20	11.81	0.01339	-0.63121
7.83	13.71	0.01149	-0.78502
10.95	16.73	0.00847	-1.09235

[a] Including reaction time correction.

[b] Including methanol blank correction.

TABLE XIX

ALKALINE HYDROLYSIS OF METHYL α-D-GLUCOPYRANOSIDE

Time,[a] hr.	Methanol[b] Concn., (M) x 10^3	Unreacted MAG Concn., (M), G	ln (G/G_o)
	T = 139.3°C.; [NaOH] = 2.500N; [G_o] = 0.0251M		
39.95	0.19	0.02490	-0.00763
79.95	0.38	0.02471	-0.01555
119.95	0.48	0.02461	-0.01964
179.95	0.78	0.02431	-0.03180
239.95	1.08	0.02401	-0.04435
	T = 150.4°C.; [NaOH] = 2.490N; [G_o] = 0.0251M		
44.95	0.61	0.02449	-0.02401
77.50	1.00	0.02410	-0.03978
94.50	1.22	0.02388	-0.04983
124.43	1.65	0.02345	-0.06682
145.65	1.89	0.02321	-0.07707
168.45	2.12	0.02298	-0.08798
	T = 158.4°C.; [NaOH] = 2.510N; [G_o] = 0.0251M		
35.95	0.80	0.02429	-0.03273
50.20	1.22	0.02387	-0.05021
71.95	1.62	0.02347	-0.06705
100.95	2.45	0.02264	-0.10312
120.95	2.86	0.02223	-0.12125
149.95	3.56	0.02153	-0.15311
171.20	4.14	0.02095	-0.18046
	T = 160.0°C.; [NaOH] = 2.501N; [G_o] = 0.0250M		
23.95	0.70	0.02429	-0.02846
48.95	1.35	0.02364	-0.05563
73.45	2.02	0.02297	-0.08428
96.95	3.08	0.02191	-0.13172
150.15	4.36	0.02063	-0.19182
	T = 170.1°C.; [NaOH] = 2.574N; [G_o] = 0.0251M		
19.95	1.52	0.02357	-0.06275
39.28	2.33	0.02276	-0.09767
65.45	4.19	0.02090	-0.18287
74.20	4.95	0.02014	-0.21967
101.20	6.34	0.01875	-0.29139
111.45	7.15	0.01794	-0.33572
140.12	8.78	0.01631	-0.43078

[a]See end of table for footnotes.

TABLE XIX (Continued)

ALKALINE HYDROLYSIS OF METHYL α-D-GLUCOPYRANOSIDE

Time,[a] hr.	Methanol[b] Concn., (M) x 10^3	Unreacted MAG Concn., (M), G	ln (G/G_o)
T = 170.1°C.; [NaOH] = 1.269N; [G_o] = 0.0251M			
10.53	0.48	0.02461	-0.01964
24.03	1.07	0.02402	-0.04365
30.95	1.37	0.02372	-0.05634
38.95	1.72	0.02337	-0.07112
50.95	2.33	0.02276	-0.09764
T = 170.6°C.; [NaOH] = 0.6398N; [G_o] = 0.0253M			
12.95	0.37	0.02492	-0.01475
25.20	0.74	0.02455	-0.02972
35.95	1.06	0.02423	-0.04283
60.45	1.85	0.02344	-0.07603
65.95	1.90	0.02339	-0.07819
T = 170.9°C.; [NaOH] = 0.2296N; [G_o] = 0.0257M			
17.45	0.44	0.02525	-0.01762
33.95	0.74	0.02495	-0.02947
58.20	1.08	0.02461	-0.04306
69.95	1.31	0.02438	-0.05246
T = 169.9°C.; [NaOH] = 0.188N; [G_o] = 0.0251M			
49.45	1.02	0.02407	-0.04178
73.12	1.20	0.02389	-0.04927
98.53	1.53	0.02356	-0.06323
135.95	2.01	0.02308	-0.08369
172.95	2.35	0.02274	-0.09863

[a] Including reaction time correction.

[b] Including methanol blank correction.