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Kinetics of Hot Alkaline Cleavage of the Glycosidic Bonds of Methyl β-D-Glucoside and Methyl β-Cellobioside

E. Vance Best

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KINETICS OF HOT ALKALINE CLEAVAGE OF THE GLYCOSIDIC BONDS OF METHYL β-D-GLUCOSIDE AND METHYL β-CELLOBIOSIDE

A thesis submitted by

E. Vance Best

B.S., 1963, North Carolina State College
M.S., 1965, Lawrence University

in partial fulfillment of the requirements
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Appleton, Wisconsin

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SUMMARY

The alkaline degradation of polysaccharides is generally accepted as occurring in two primary ways. These two degradation reactions are the progressive, endwise degradation from the reducing end of the polymer and the random cleavage of the glycosidic bonds joining the monosaccharide units of the polymeric material. The random alkaline cleavage of β-1,4 glycosidic bonds such as those in cellulose has been investigated. Two cellulose model compounds, methyl β-D-glucoside and methyl β-cellobioside, were used in this investigation.

Methyl β-D-glucoside was shown by an isotope dilution analysis of the alkaline degradation reaction products from methyl β-cellobioside to be the major glycosidic product from the cleavage of the interior glycosidic bond of the cellobioside. Actual glycosidic bond cleavage was indicated by a good mass balance between methyl β-cellobioside, methyl β-D-glucoside, and methanol during the course of the methyl β-cellobioside degradation.

Methyl β-cellobioside was reacted in oxygen-18 enriched aqueous alkali at 170°C. The methanol and the methyl β-D-glucoside produced (and the diethyl dithioacetal derivative of the glucoside) were analyzed on a mass spectrometer. The results showed that the model glycosidic bond was cleaved between the anomeric carbon and the glycosidic oxygen. Interior bond cleavage occurred completely between the anomeric carbon and the glycosidic oxygen.

The pseudo-first-order kinetics of the alkaline degradation of methyl β-cellobioside and methyl β-D-glucoside in molecular-oxygen-free 2.497N sodium hydroxide were investigated over the temperature range of 140 to 170°C. A combination of quantitative paper chromatography and radiochemical analysis was used to measure changes in the concentrations of the two glycosides; a colorimetric technique was used to measure methanol. The cleavage rates were found to have a significant temperature dependence. Linear Arrhenius plots were obtained for the alkaline

cleavage of the interior glycosidic bond of methyl β-cellobioside and for the methyl glycosidic bonds of both glycosides. The Arrhenius activation energies for the cleavage of these three bonds were relatively large and quite similar, varying from 36.3 to 37.8 kcal./g.-mole. These results suggest that the reaction mechanism is the same for cleavage of the three different bonds and that the rate-controlling mechanism is the same over the temperature range investigated. The actual rate of cleavage for the interior bond of methyl β-cellobioside is about five times that for its methyl glycosidic bond; this is believed to be due to leaving group effects and possibly to differences in the equilibrium constants for the C-2, C-2' hydroxyl ionizations.

The effect of hydroxyl ion concentration on the rate of alkaline degradation at 170°C. of methyl β-cellobioside and methyl β-D-glucoside was investigated by measuring the degradation rates in 0.10, 0.36, 1.00, and 2.50N NaOH. The rates have a significant dependence on hydroxyl ion concentration. The experimentally observed reaction order with respect to hydroxyl ion concentration for cleavage of the methyl glycosidic bonds of both compounds was 0.7. The observed reaction order for cleavage of the interior glycosidic bond of methyl β-cellobioside was found to vary in a regular manner from about 0.9 in 0.10N NaOH to about 0.4 in strong alkali. The actual reaction order for both reactions is believed to be 1.0. Consideration of possible activity coefficient effects and salt effects indicates that the experimentally observed reaction order will be less than the true order. Additional complications which could cause the varying reaction order are noted and discussed.

The results described, plus a limited investigation of the reaction products, are consistent with and support a reaction mechanism already postulated in the literature for the random cleavage of glycosidic bonds in oxygen-free aqueous alkali.

INTRODUCTION

In the commercial pulping of wood and other fibrous materials, probably the most basic objective of the process is to obtain the highest possible yield of acceptable quality pulp. The predominant percentage of wood, which is the source of most of the pulp produced today, is composed of only three materials. These are cellulose, hemicelluloses, and lignin. An individual wood fiber may be thought of, at least in a coarse sense, as consisting primarily of cellulose and hemicelluloses. The fibers are, again in a coarse sense, held together by lignin materials. Thus, an ideal pulping process would remove all the lignin, leaving the individual wood fibers, with their cellulosic components in the same form as before pulping. No commercial pulping process approaches this specificity for removing the lignin material, and some degradation or modification of the cellulosic material always occurs. This degradation of the cellulosic components causes the individual pulp fibers to be obtained in lower yields and to have lower physical strength, thereby increasing the cost and lowering the quality of any fibrous products made from the pulp. Even if the pulp is intended as a source of cellulose for further chemical treatment and the fiber characteristics of the pulp are of no importance, the degradation which occurs during pulping causes the cellulose to be of lower quality for many applications due to the decreased degree of polymerization, or decreased intrinsic strength of the polymeric material.

This thesis research is in a broad sense concerned with the degradation of cellulosic materials under conditions similar to those employed in commercial alkaline pulping processes and other alkaline treatments of pulp.

ALKALINE DEGRADATION OF CELLULOSE AND CELLULOSE MODEL COMPOUNDS

CELLULOSE

The degradation of cellulose and other β-1,4-linked polysaccharides in aqueous alkali is generally accepted as occurring predominantly by two distinct reactions: (a) a "peeling" reaction, and (b) a random cleavage of the glycosidic bonds which join the monomer units together to form the polymeric material.

The mechanism for the "peeling" reaction was first suggested by Isbell (1), and it has received considerable study, particularly by Kenner and his associates (2). This mechanism has been applied with success to the alkaline degradation of various β-1,4-linked carbohydrate polymers (3). This endwise type of degradation of the polysaccharide chain begins by the rearrangement of the terminal aldose unit to a ketose at C-2 (4). The acidic hydrogen at C-3 is then easily removed in alkali to form a carbanion. The "peeling" degradation occurs by elimination of the rest of the polysaccharide chain at C-4 as a glycoxy anion, leaving the sugar residue, which rearranges or decomposes to acidic products. The predominant acidic product from the "peeling" degradation of cellulose has been found to be D-glucoisosaccharinic acid (5,6). The terminal reducing group of the glycoxy anion which was eliminated can subsequently undergo the required rearrangement and another monomer unit may be "peeled."

This endwise degradation of a polysaccharide chain could in theory proceed until the complete chain was degraded; however, this does not always occur. Cellulose in aqueous alkali at 100°C. becomes stable to the alkali and suffers no further weight loss after several hours (7). Formation of a terminal D-glucometasaccharinic acid from the reducing end group has been shown to be the basis of this stabilizing or "stopping" reaction (6,8). This reaction is similar to "peeling" except that a

hydrogen is abstracted from the C-2 position, causing the hydroxyl at C-3 to become labile and be eliminated, instead of the polymer chain at C-4 as in "peeling" (4). A benzilic acid-type rearrangement of the resulting diketone end group leads to a D-glucometasaccharinic acid end group, which is alkali-stable. In the reaction of cellobiose in 0.02N sodium hydroxide at 90°C., approximately three percent of the molecules undergo the "stopping" reaction, yielding 4-O-β-D-glucopyranosyl-D-glucometasaccharinic acid (9). This substantiates the general observation that the "stopping" reaction is considerably slower than the "peeling" reaction (3). Some experimental results indicate that, for cellulose in aqueous sodium hydroxide, 50 to 65 glucose residues on the average are split off before a particular chain becomes stable to the alkali (10).

The alkaline random cleavage of glycosidic bonds in polysaccharides, without end-group activation, has not been studied as extensively as has the "peeling" and "stopping" reactions; however, the existence and importance of this degradation may be demonstrated by considering various experimental results. The reaction of cotton hydrocellulose (5) and cellulose (7) in aqueous alkali at 100°C. in the absence of molecular oxygen ultimately resulted in an alkali-stable cellulose. The degradation which did occur was attributed to the "peeling" reaction. Corbett and Richards (11) studied the reaction of cellulose in 0.5N sodium hydroxide at temperatures up to 220°C. and found that at the high temperatures severe degradation occurred. No end group stabilization appeared to be taking place. This indicated that glycosidic linkages were being cleaved, giving rise to new reducing end groups, which could then "peel." The "stopping" reaction was still operating at the high temperatures, but the formation of new reducing end groups more than offset its stabilizing effect. Corbett and Richards also reacted cellulose at high temperature in water buffered to pH of 7.2-7.4 and observed no significant degradation. In addition, they obtained results which indicate that ferric ions in the reaction solution do not catalyze the degradation reaction.

Meier (12) has investigated the alkaline degradation of 4-O-methyl-D-glucuronoxylan at 170°C. The hemicellulose was reacted in a Na_2SO_3-Na_2CO_3 buffered cooking liquor at pH 11.4, and the degree of polymerization of the hemicellulose remained fairly constant for 100 minutes of reaction time. The recoverable polymer had decreased to less than 50% of the original amount at the end of the 100 minutes, however. These results indicated that the "peeling" reaction was the major cause of the degradation. Meier also reacted this hemicellulose in regular sulfate cooking liquor at pH 13.4 and found that the degree of polymerization began decreasing immediately and continued to do so until the reaction was stopped. These results indicate that random alkaline cleavage of the glycosidic bonds is occurring in addition to "peeling" at the higher pH.

Thompson, et al. (13) also investigated the effect of alkali on 4-O-methylglucuronoxylan at 170°C. They found that at pH 14 both the percent recoverable polymer and degree of polymerization decreased quite sharply for the first ten minutes or so of reaction and then continued to decrease, but at a slower rate. At pH 8 the degree of polymerization decreased only slightly during 60 minutes of reaction time; however, the percent recoverable polymer had dropped to about 75% after 30 minutes, but appeared to be stabilizing at longer reaction times. These results also indicate that while "peeling" occurs at both highly alkaline and slightly alkaline pH's, random cleavage of the glycosidic bonds does not occur to a significant amount except at the higher pH levels.

Thompson, Peckham, and Thode (14) have measured the decrease in degree of polymerization of cotton linters as a function of cooking liquor pH. They cooked cotton linters for 4-1/2 hours at 176°C. in liquors whose pH's were varied from 7 to about 12.4. The D.P. of the linters decreased from about 1500 for cooking at pH 7 to about 250 for cooking at pH 12.4. These results also indicate that random

cleavage of glycosidic bonds contributes significantly to polysaccharide degradation at higher hydroxyl ion concentrations.

CELLULOSE MODEL COMPOUNDS

Cellulose model compounds have been used considerably in research work to determine the factors which contribute to the alkaline degradation of cellulose and related polymers. These studies with model compounds have yielded valuable observations and results pertinent to the alkaline degradation of cellulose. The simple reducing sugars have been used with success to study the "peeling" degradation and the "stopping" reaction. Good reviews on the behavior of simple sugars in aqueous alkali are available (15,16). The preceding review on the alkaline degradation of cellulose has indicated, however, that under the reaction conditions of most commercial alkaline pulping processes, the random cleavage of glycosidic bonds is the predominant factor contributing to the serious degradation of the cellulosic materials. The simple sugars are extremely labile under these conditions, and cellulose model compounds which are more alkali-stable have been used in studying the glycosidic bond cleavage occurring under the more severe conditions.

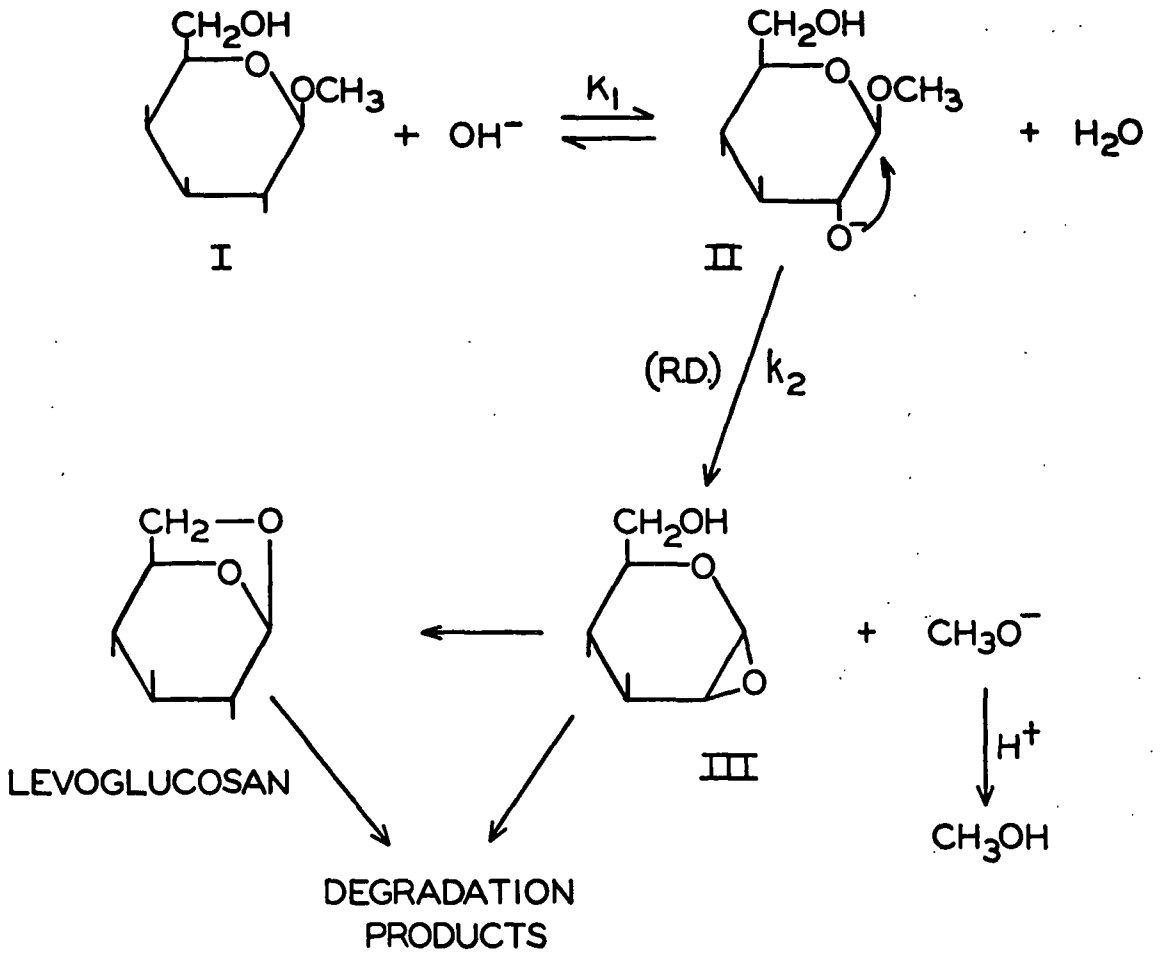
Based upon their work with phenyl β-D-glucoside and other phenyl β-D-glucoside derivatives, McCloskey and Coleman (17) have suggested a mechanism for alkaline degradation of these model compounds. They postulated that the phenoxy aglycone was removed by the formation, with inversion at C-1 of a 1,2-anhydro-D-glucose intermediate, which further reacts, with inversion at C-1, with the proximate C-6 hydroxyl to form 1,6-anhydro-D-glucose (levoglucosan). The C-2 hydroxyl was shown to be necessary for the reaction to take place (17). Phenyl tetra-O-methyl-β-D-glucoside and phenyl 2,3-di-O-methyl-β-D-glucoside were not degraded by NaOH at 120°C., while phenyl 3-methyl-β-D-glucoside was degraded readily. A trans configuration between the C-2 hydroxyl and the aglycone has also been shown to be

necessary for the reaction since phenyl β-D-glucoside is easily degraded under conditions as above (17), while phenyl α-D-glucoside is stable (18). The postulated intermediate is definitely possible since the reaction of Brigl's anhydride (3,4,6-tri-O-acetyl-1,2-anhydro-α-D-glucopyranose), an obviously appropriate model, in hot alkali yields levoglucosan (19). Brigl's anhydride reacts very rapidly with alcohols to form β-glucosides (20), indicating inversion at C-1 upon opening of the ethylene oxide ring. Levoglucosan is the product of the reaction of phenyl β-D-glucoside in hot alcoholic alkali, which shows that the primary hydroxyl rather than the solvent adds to the ethylene oxide ring (19).

McCloskey and Coleman's postulated mechanism has received considerable support and has been applied to the alkaline degradation of methyl glycosides (21). The importance of the C-2 hydroxyl to the reaction mechanism has been shown by the fact that the rate of degradation of methyl 2-O-methyl-β-D-glucoside is less than half the rate of methyl β-D-glucoside under the same conditions (22). This difference is not as significant as is generally expected for a neighboring group participation mechanism, and it is possible that some of the degradation is occurring via an S_N2 mechanism. Lindberg and coworkers (23) have shown that a trans relationship between the aglycone and the C-2 hydroxyl contributes significantly to the rate of alkaline degradation of methyl glycosides. The cleavage rates of both the methyl β- and the methyl α-glycoside derivatives of glucose, mannose, galactose, xylose, and arabinose were measured in 10% NaOH at 170°C. The isomer with the aglycone group trans to the C-2 hydroxyl reacted faster in all cases than did the cis isomer. The authors also note that the rates increase with the conformational instability of the most stable configuration of the glycosides. Robins (24) has recently substantiated these findings for the case of methyl α-D-glucoside during a detailed investigation of the effects of reaction temperature and hydroxyl ion concentration on the alkaline degradation of this model compound.

Brooks (25,26) has studied the kinetics of the alkaline cleavage of the glycosidic bond of methyl β-D-glucoside and confirmed that the mechanism proposed by McCloskey and Coleman (17) would satisfactorily explain his experimental results for cleavage in a molecular-oxygen-free reaction system. McCloskey and Coleman--and other workers since--implicitly assume in their discussion of the formation of the 1,2-anhydrosugar intermediate that the C-2 hydroxyl ionizes to form a secondary carbinolate anion which then by intramolecular nucleophilic attack displaces the aglycone group and thereby forms the 1,2-anhydrosugar. Brooks (25) has stated this more directly and has given the following schematic representation, Fig. 1, of the mechanism. Methyl β-D-glucoside is shown as the reactant. In his work Brooks studied the effect of temperature on the rate of cleavage and found that in going from 140 to 170°C. the cleavage rate increased 22 times for the case of a nitrogen-purged reaction system. In somewhat limited work on the effect of pH on the cleavage rate, Brooks found that about a twentyfold difference in rate was obtained for reaction at pH = 10.2 and at pH = 14 (10% NaOH). Also, in this work, Brooks presents conclusive evidence that the presence of molecular oxygen in the reaction system greatly increased the rate of alkaline degradation.

Lindberg and coworkers (27) have suggested a variation of the McCloskey and Coleman mechanism. They reacted 4-O-β-D-glucopyranosyl-D-glucitol (cellobiitol) in 10% NaOH at 170°C. until the material was almost completely degraded (48 hours). The neutral reaction products were found to consist primarily of glucitol and 1,4-anhydro-D-glucitol plus a small amount of levoglucosan. The stability of levoglucosan under the same reaction conditions was investigated, and the compound was found to be labile--being 75% degraded in 5 hours. The glucitol and the 1,4-anhydro-D-glucitol were found to be very stable, however. The results of this work indicate that a cleavage mechanism in addition to the 1,2-anhydro mechanism was in operation. The authors state that a reasonable assumption for this mechanism is that the 1,4-anhydro-D-glucitol is formed by nucleophilic attack on C-4 by the primary hydroxyl at C-1



I = METHYL β-D-GLUCOSIDE

II = SECONDARY CARBINOLATE ANION, C-2 HYDROXYL IONIZED

III = 1,2-ANHYDRO-D-GLUCOSE

(R.D.) = INDICATES THE RATE-DETERMINING STEP IN THE OVERALL REACTION.

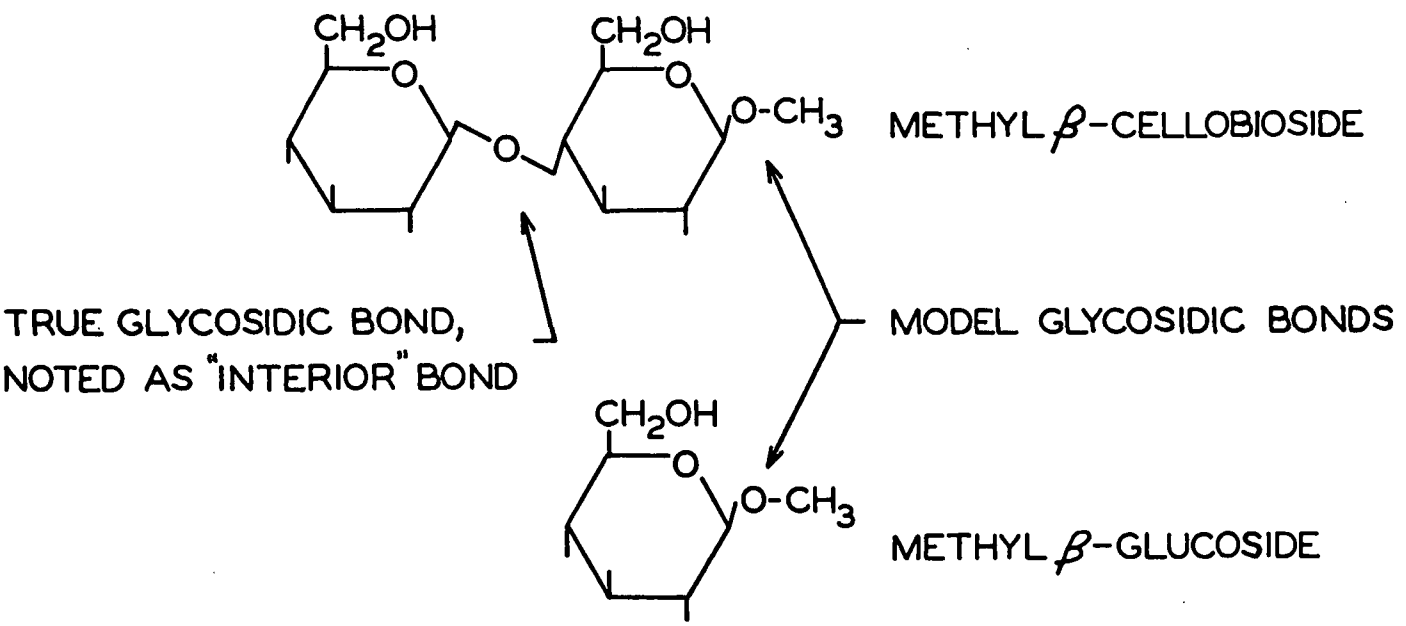
Figure 1. Probable Reaction Pathway for Glycosidic Bond Cleavage in an Oxygen-Free Alkaline Medium. Adapted from Brooks (25)

in the glucitol unit (27). This nucleophilic attack on C-4 could also be from the other glucitol hydroxyls. The strained anhydro rings which would be formed between these other hydroxyls and C-4 could subsequently be opened by the attack of the C-1 primary hydroxyl to form the 1,4-anhydro-D-glucitol. Thus, this additional mechanism is essentially the same as that of McCloskey and Coleman except that it is operating in the aglycone. Janson and Lindberg (22) have obtained some results to support the additional cleavage mechanism by reacting the α- and β-glucosides of ethylene glycol and the monomethyl ether of ethylene glycol in 10% NaOH at 170°C. in the absence of molecular oxygen. Both the glycol glucosides reacted much faster than did the glycol monomethyl ether glucosides. This indicated that in the alkaline hydrolysis of the glycol glucosides, the ability to form an intermediate ethylene oxide ring in the aglycone group apparently increased the cleavage rate. It should be noted that ethylene glycol is relatively acidic and should be a good leaving group as well as being able to form the anion in the aglycone.

DIRECTION OF THIS RESEARCH

Consideration of the information in the literature has indicated that the β-1,4 glycosidic bonds of cellulose are stable in molecular-oxygen-free aqueous alkali at temperatures up to at least 100°C. However, as the reaction temperature is increased to 170°C., this is no longer true, and the overall controlling mechanism for the degradation of cellulose in alkaline solutions changes from the "peeling" reaction to the random cleavage of the glycosidic bonds. Since the breaking of the glycosidic bond gives a new reducing end group which can undergo the stepwise degradation, the rate of the random cleavage should be the significant factor contributing to the overall rate of cellulose degradation, particularly under reaction conditions similar to those employed in the commercial alkaline pulping processes.

No quantitative experimental results have been found in the literature which describes the effect of reaction temperature or alkali strength on the rate of cleavage of the true* glycosidic bond. Limited quantitative results are available for these effects on the rate of cleavage of a model glycosidic bond. The purpose of the current research was to determine quantitatively the effects of reaction temperature and hydroxyl ion concentration on the cleavage rate of both glycosidic bonds and to seek information on how and where the cleavage of these bonds occurs. A cellulose model compound, methyl β-cellobioside, which contains a true and a model glycosidic bond, was chosen for use in this investigation. These two bonds are pointed out in the following sketch.



*In this dissertation, the terms "true glycosidic bond" and "model glycosidic bond" are used relative to the β-1,4 glycosidic bonds of cellulose. The interior glycosidic bond of methyl β-cellobioside should be the same as the glycosidic bonds of cellulose and is regarded as a "true glycosidic bond." The term "model" is used to denote the glycosidic bond between the methyl aglycone and the sugar unit of the particular model compound. The glycosidic bond of methyl β-D-glucoside is, therefore, described as a "model glycosidic bond." Methyl β-cellobioside has a similar "model glycosidic bond" between its methyl aglycone and the cellobiose unit.

Methyl β-D-glucoside, a simple cellulose model compound containing only a model glycosidic bond, was also used in this work and is shown in the sketch. Both these compounds have a nonreducing end group and thus are not subject to the "peeling" degradation. Rates of cleavage in the absence of molecular oxygen over the temperature range of 140 to 170°C. and the hydroxyl ion concentration range of 0.1 to 2.5N were studied.

RESULTS AND DISCUSSION

KINETIC DESCRIPTION OF REACTION SYSTEMS

METHYL β-D-GLUCOSIDE

A major objective of this research was to quantitatively describe the cleavage rates of the glycosidic bonds of both methyl β-D-glucoside and methyl β-cellobioside in a molecular-oxygen-free alkaline medium at high temperatures. Methyl β-D-glucoside, which contains a glycosidic bond similar to those in cellulose, has been used considerably as a cellulose model. Published results of work with methyl β-D-glucoside indicate that the characteristics of its alkaline degradation under conditions similar to those of the current work have been reasonably well established. Brooks (25) concluded from his recent work that a mole of product methanol and a mole of anhydroglucose, which was subsequently degraded, were generated for each mole of glucoside degraded. The current work substantiates his finding that the amount of methanol produced in the reaction system is directly proportional to the amount of glucoside degraded. These results allow the reaction of methyl β-D-glucoside in an alkaline medium free of molecular oxygen to be written schematically as

$$G \xrightarrow{k_{GM}} M \qquad (1)$$

where

G = methyl β-D-glucoside concentration

M = product methanol concentration

k_{GM} = pseudo-first-order rate constant.

Consideration of this kinetic description of the reaction in conjunction with the reaction pathway shown in Fig. 1 will reveal that the pseudo-first-order rate constant, k_{GM}, is composed of at least three factors. From Fig. 1 the rate of reaction, or rate

of disappearance of methyl β-D-glucoside, may be seen to be

$$-dG/dt = k_2[II] \qquad (2)$$

where

$\underline{k_2}$ = specific rate constant for reaction of the secondary carbinolate anion, II, to form the anhydrosugar, III

$\underline{t}$ = reaction time

[II] = secondary carbinolate anion, C-2 hydroxyl ionized.

The concentration of the secondary carbinolate anion may be given as

$$[II] = K_1 G [OH^-] \qquad (3)$$

where

$\underline{K_1}$ = equilibrium constant for the formation of the secondary carbinolate anion.

Thus, the rate of reaction may be written as

$$-dG/dt = K_1 k_2 [OH^-] G \qquad (4),$$

or using the notation from the kinetic equation, this may be written as

$$-dG/dt = dM/dt = k_{GM} G \qquad (5)$$

where

$\underline{k_{GM}} = \underline{K_1}\underline{k_2}[OH^-]$.

The reaction as shown in Fig. 1 has a theoretical reaction order of one with respect to the hydroxyl ion concentration; the order determined experimentally for the reaction is, however, somewhat less than one. This finding and reasons for it will be discussed in detail in a later section. Brooks (25) has demonstrated that the reaction is first order with respect to the methyl β-D-glucoside reactant. This was found to be true in the current work also.

Upon integration, Equation (5) yields the following equations,

$$\ln(G/G_o) = -k_{GM}t \tag{6}$$

$$\ln\left[\frac{G_o - M}{G_o}\right] = -k_{GM}t \tag{7}$$

where

G_o = initial methyl β-D-glucoside concentration.

Equation (6) describes the unused reaction concentration as a function of the initial reactant concentration, the pseudo-first-order rate constant, and the reaction time. Equation (7) describes the product methanol concentration as a function of the initial methyl β-D-glucoside concentration, the pseudo-first-order rate constant, and the reaction time. This equation was used more extensively than was Equation (6) during this work as, in general, methanol was measured instead of unused reactant.

METHYL β-CELLOBIOSIDE

Methyl β-cellobioside should be a more realistic model for cellulose than is methyl β-D-glucoside since its interior glycosidic bond should be identical to the glycosidic bonds of cellulose. The exterior glycosidic bond of methyl β-cellobioside should be essentially the same as the model glycosidic bond of methyl β-D-glucoside. An objective of the current work was to compare the behavior of the two bonds of methyl β-cellobioside.

While the reaction pathway for the alkaline cleavage of methyl β-D-glucoside appeared to be reasonably well established, the same was not true for methyl β-cellobioside, and all possible reaction pathways were considered in its investigation. The various pathways were examined and the ones which were thought to be most probable are shown in Fig. 2. The experimental results of this work are in agreement with the proposed pathways. Reaction pathway A of Fig. 2 shows the cleavage of the interior

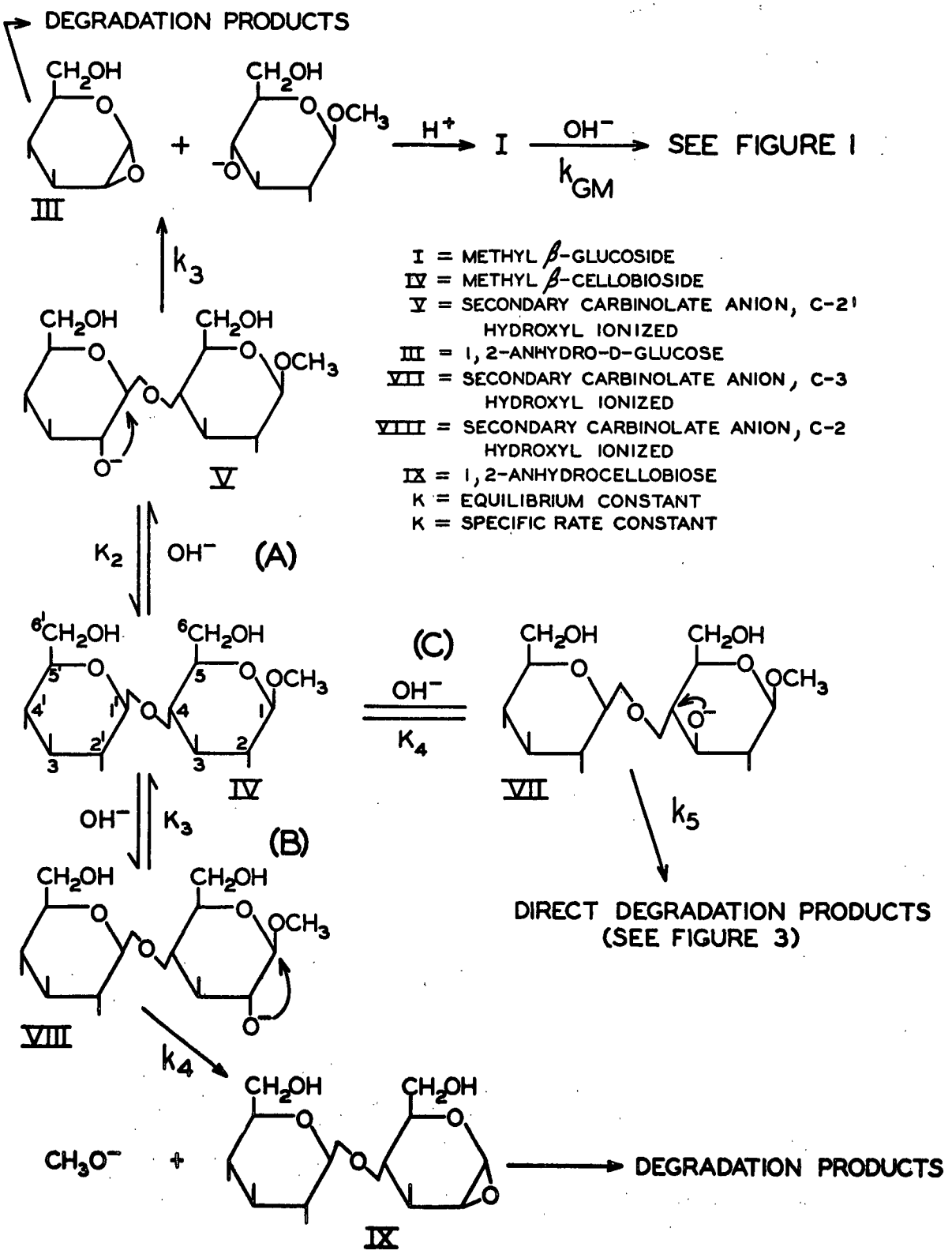
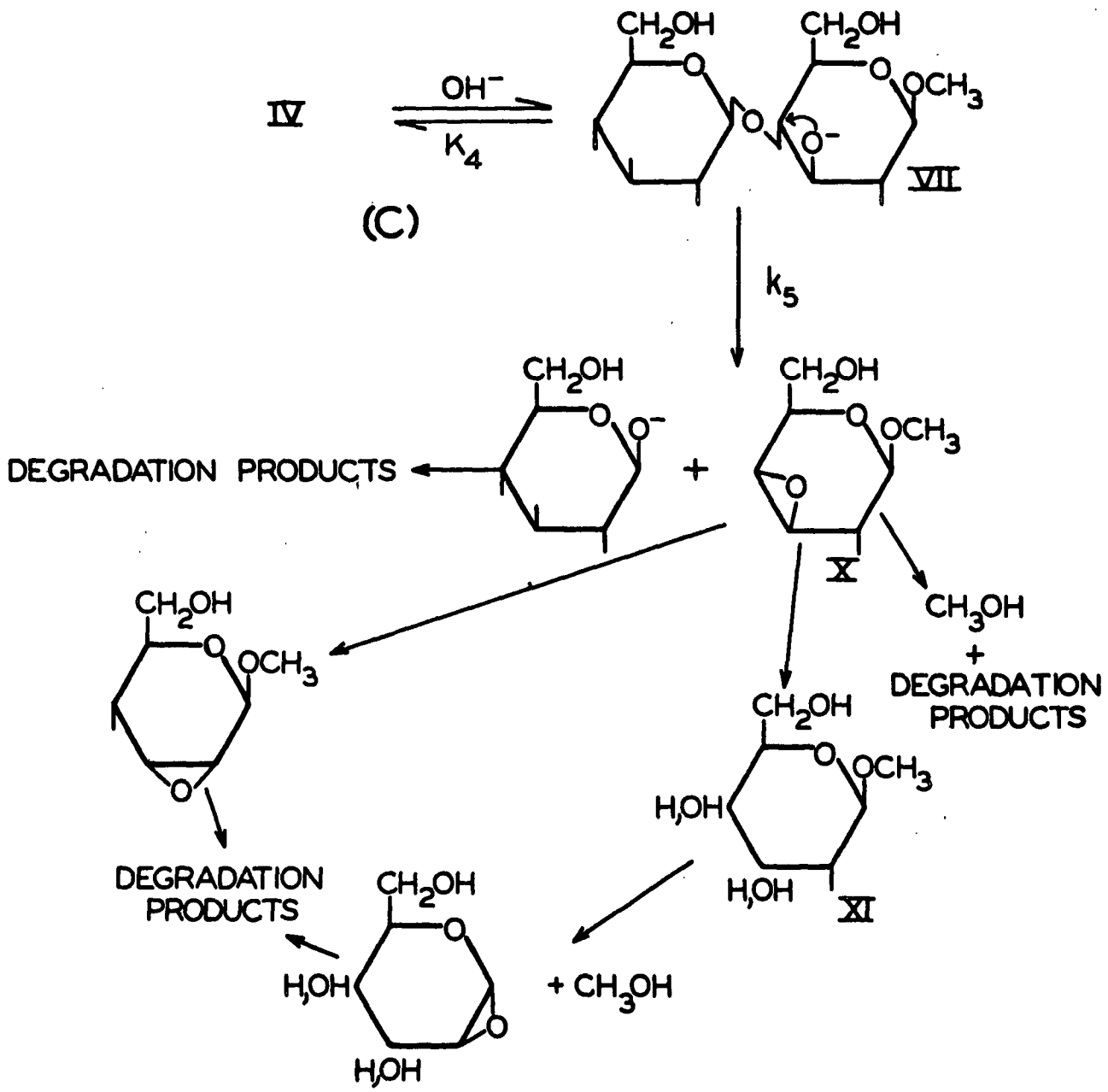


Figure 2. Possible Reaction Pathways for the Alkaline Cleavage of Methyl β-Cellobioside in a Nitrogen-Purged Reaction System

glycosidic bond of methyl β-cellobioside, IV, to yield a molecule each of methyl β-D-glucoside, I, and a postulated 1,2-anhydroglucose, VI. The 1,2-anhydroglucose was subsequently degraded rapidly to unidentified products and levoglucosan under the reaction conditions used. The methyl β-D-glucoside produced is then subject to alkaline cleavage to yield VI and methanol as described earlier. Reaction pathway A is very similar to the reaction pathway for the degradation of methyl β-D-glucoside, and the rate-determining step here is postulated to be the intramolecular, nucleophilic displacement of the methyl β-D-glucoxyl aglycone by the neighboring secondary carbinolate anion at C-2' to produce 1,2-anhydroglucose. The methyl β-D-glucoside 4-anion picks up a proton from the reaction medium to yield methyl β-D-glucoside. The very reactive 1,2-anhydroglucose has never been isolated and is thought to either degrade or react further to form 1,6-anhydroglucose, levoglucosan. The reactive secondary carbinolate anion is assumed to always be in equilibrium with the neutral methyl β-cellobioside.

Reaction pathway B is exactly the same as the pathway for degradation of methyl β-D-glucoside except that 1,2-anhydrocellobiose, instead of 1,2-anhydroglucose, is the postulated intermediate product. The displaced methoxyl anion picks up a proton from the medium and forms methanol. The 1,2-anhydrocellobiose probably degrades rapidly under the severe reaction conditions.

Reaction pathway C, shown in Fig. 2, which results in the production of the "Direct Degradation Products" can perhaps be described in more detail, and Fig. 3 gives this further description. No experimental results were available at the outset of this work which could be used to evaluate the accuracy or validity of the pathways shown in Fig. 3. Examination of the possible pathways, however, did show that it was possible for methanol to be produced directly from the "Direct Degradation Products," and also that production of a methyl β-glycoside, XI, was possible. This methyl β-glycoside could then be cleaved to yield additional methanol. The



X = METHYL 3,4-ANHYDRO-β-D-GLUCOSIDE

XI = A METHYL β-GLYCOSIDE

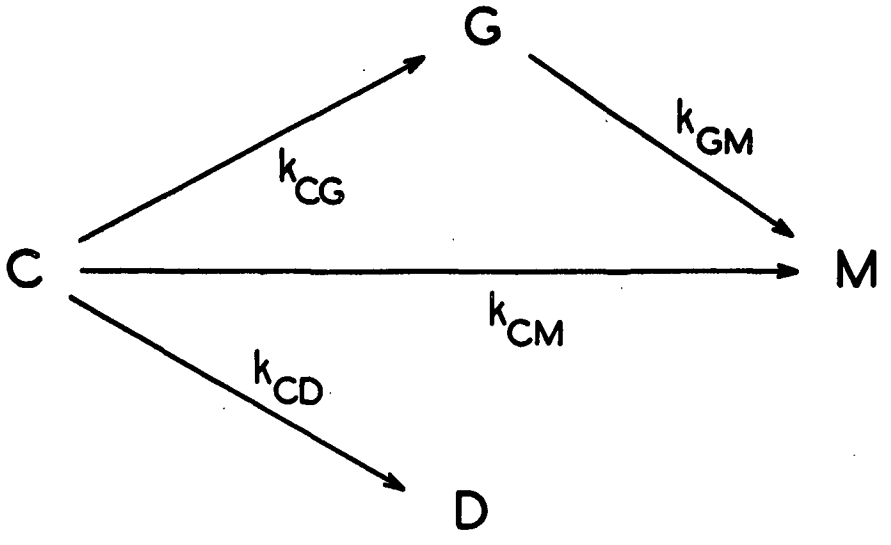
Figure 3. Possible Pathways in the Production of "Direct Degradation Products" by Alkaline Cleavage of Methyl β-Cellobioside. (See Fig. 2)

production of this additional glycoside and methanol would have to proceed through the 3,4-anhydrosugar intermediate, X. The possibility of the additional methyl β-glycoside and methanol produced in this way required that this degradation pathway be considered in the kinetic analysis of the reaction system.

The production of methanol as shown directly from the anhydrosugar, X, (without going through the methyl glycoside) would require that the inherently unstable anhydrosugar be at least somewhat stabilized in the reaction medium in order that the methoxyl group could be cleaved. No basis for such stabilization was apparent, and production of methanol from this source was considered unlikely.

The possibility of producing a methyl β-glycoside, XI, through pathway C was considered to be very possible. The glycoside could be formed from the anhydrosugar by adding the elements of water and breaking the epoxide ring. Methyl β-D-glucoside was considered to be the most probable glycoside to be produced since it has the most stable conformation of the possible product glycosides. The possibility of forming methyl β-D-glucoside by this route was investigated and no significant amount was found to be produced in this way. This investigation will be discussed in detail in a later section. Pathway C was considered in the overall kinetic analysis, however, since methyl β-cellobioside may degrade without yielding products which will be measured by the procedures used. The experimental results of the current work do indicate, however, that only a small and perhaps insignificant amount of methyl β-cellobioside degrades via pathway C.

Examination of the various reaction pathways and reaction products in Fig. 2 showed that it should be possible to kinetically describe the reactions of interest by the equations given in Fig. 4. This schematic representation of the kinetic equations shows that there are four pseudo-first-order reaction rate constants to be determined. The rate constant, k_{GM}, was determined independently from the experimental



C = METHYL β-CELLOBIOSIDE
G = METHYL β-D-GLUCOSIDE
M = METHANOL
D = DIRECT DEGRADATION PRODUCTS
k_{GM} = PSEUDO FIRST-ORDER RATE CONSTANT FOR DEGRADATION OF G TO PRODUCE M.
k_{CG} = PSEUDO FIRST-ORDER RATE CONSTANT FOR DEGRADATION OF C TO PRODUCE G.
k_{CM} = PSEUDO FIRST-ORDER RATE CONSTANT FOR DEGRADATION OF C TO PRODUCE M.
k_{CD} = PSEUDO FIRST-ORDER RATE CONSTANT FOR DEGRADATION REACTION OF C WHICH DO NOT YIELD EITHER G OR M AS PRODUCTS.

Figure 4. Kinetic Pathways Describing the Alkaline Degradation of Methyl β-Cellobioside in a Nitrogen-Purged Reaction System

work which used methyl β-D-glucoside as the reactant. Thus, only three rate constants, k_{CG}, k_{CM}, and k_{CD}, were unknown in the reaction system. The subscripts of the rate constant k's are assigned in such manner as to be a memory aid in discussion of the rate constants, as k_{CM} indicates that this is the pseudo-first-order rate constant for the reaction of methyl β-cellobioside to yield methanol--the "C" being derived from cellobioside and "M" being derived from methanol. The methanol produced in the cleavage reactions is relatively stable in the reaction system, and the two glycosides were found to be stable in the quenched reaction system. These three materials could be measured independently. Therefore, it was possible to determine the three unknown rate constants with the three independent measurements.

Each of the pseudo-first-order rate constants for the reaction mechanism as described in Fig. 2 may be observed to be composed of at least three factors. This is quite similar to the case for the degradation of methyl β-D-glucoside. Each pseudo-first-order rate constant is a product of an equilibrium constant, a specific rate constant, and the hydroxyl ion concentration. The order of the reaction with respect to the hydroxyl ion concentration was thought to be 1.0, but the experimental results indicate that this is not the case, empirically anyway. This finding will be discussed in detail later. The reactions were found to be first-order with respect to methyl β-cellobioside concentration. Equations for the three pseudo-first-order rate constants may be written as

$$k_{CG} = K_2 k_3 [OH^-] \qquad (8),$$

$$k_{CM} = K_3 k_4 [OH^-] \qquad (9),$$

$$k_{CD} = K_4 k_5 [OH^-] \qquad (10)$$

where

K_2 = equilibrium constant for the formation of the secondary carbinolate anion, C-2 hydroxyl ionized, V

$\underline{k}_3$ = specific rate constant for the reaction of V

$\underline{K}_3$ = equilibrium constant for the formation of the secondary carbinolate anion, C-2 hydroxyl ionized, VIII

$\underline{k}_4$ = specific rate constant for the reaction of VIII

$\underline{K}_4$ = equilibrium constant for the formation of the secondary carbinolate anion, C-3 hydroxyl ionized, VII

$\underline{k}_5$ = specific rate constant for the reaction of VII

$\underline{k}_{CG}$, $\underline{k}_{CM}$, and $\underline{k}_{CD}$ are described in Fig. 4.

From the kinetic pathways shown in Fig. 4, equations may be written which will kinetically describe the degradation reactions which occur. The time rate of decomposition of methyl β-cellobioside may be written as

$$-dC/dt = (k_{CG} + k_{CM} + k_{CD})C \qquad (11)$$

or as

$$-dC/dt = k_1 C \qquad (12)$$

where

$$\underline{k}_1 = \underline{k}_{CG} + \underline{k}_{CM} + \underline{k}_{CD}.$$

Equation (12) may be integrated to the forms shown in Equations (13) and (14), which show how methyl β-cellobioside concentration is a function of its initial concentration, $\underline{C}_o$, the reaction time, $\underline{t}$, and the rate constant combination, $\underline{k}_1$.

$$\ln(C/C_o) = -k_1 t \qquad (13)$$

$$C = C_o \exp(-k_1 t) \qquad (14)$$

The time rate of change of the methyl β-D-glucoside concentration may be written as

$$dG/dt = k_{CG}C - k_{GM}G. \qquad (15)$$

Substitution of Equation (14) for $\underline{C}$ into Equation (15) and subsequent integration yield the following form for methyl β-D-glucoside concentration as a function of initial

methyl β-cellobioside concentration; initial methyl β-D-glucoside concentration, G_o; reaction time; and the rate constants, k_{CG}, k_{GM}, and k_1,

$$G = \frac{k_{CG}C_o}{k_1 - k_{GM}} \left[\exp(-k_{GM}t) - \exp(-k_1 t) \right] + G_o \exp(-k_{GM}t). \qquad (16)$$

The time rate of generation of methanol may be written as

$$dM/dt = k_{GM}G + k_{CM}C. \qquad (17)$$

Substitution of Equation (16) for G and Equation (14) for C and subsequent integration yield the following equation for methanol concentration as a function of the various previously defined quantities,

$$M = \frac{k_{CM}C_o}{k_1} \left[1 - \exp(-k_1 t) \right] + \frac{k_{CG}C_o}{k_1} \left\{ 1 - \left[\frac{k_1 \exp(-k_{GM}t) - k_{GM}\exp(-k_1 t)}{k_1 - k_{GM}} \right] \right\} + G_o[1 - \exp(-k_{GM}t)]. \qquad (18)$$

The pseudo-first-order rate constant, k_{CD}, may be obtained by difference upon the subtraction of k_{CG} and k_{CM} from k_1 after these three quantities have been determined. The experimental data obtained in this study of the methyl β-cellobioside reaction system were found to obey quite well the kinetic equations described here.

DEMONSTRATION THAT METHYL β-D-GLUCOSIDE IS A PRIMARY REACTION PRODUCT

Before getting into the discussion and interpretation of the results of the kinetic work with the methyl β-cellobioside reaction system, it will be advantageous to discuss the results of two other related experiments. These will provide additional information and background for the consideration of the kinetic results. The first experiment demonstrated that methyl β-D-glucoside is the primary reaction product

for the alkaline cleavage of the interior glycosidic bond of methyl β-cellobioside. The results of the second experiment indicated the points of cleavage of both the interior and the model glycosidic bonds of methyl β-cellobioside.

As noted previously, only a limited amount of work has been done on the alkaline degradation of methyl β-cellobioside. Nowhere in the literature has proof been found that methyl β-D-glucoside is a product of this degradation. The production of methyl β-D-glucoside from the degradation appears obvious, and it has been ascertained by an isotope dilution analysis of the neutral products of the reaction. It should be noted for this discussion that the methyl β-cellobioside used in this work had a radioactively labelled (carbon-14) atom in the methyl aglycone, and, therefore, any reaction products, such as methyl β-D-glucoside, which contain the methyl group will be radioactively labelled.

Isotope dilution analysis is usually employed to determine the amount of a radioactive compound of known specific activity present in a mixture of compounds. A known amount of the inactive form of the compound of interest is added in great excess to the mixture. Some of the compound is then isolated and purified by convenient methods. The specific activity of the purified material is measured, and the amount of radioactive material present initially may then be calculated. This technique is described more completely along with the details of this particular isotope dilution experiment in the Experimental Procedures section.

The neutral materials from a portion of a particular reaction product solution, which was available from the kinetic work, were diluted with a considerable excess of known, unlabelled methyl β-D-glucoside. Some of the equilibrated methyl β-D-glucoside was then obtained from the mixture and purified. This purified glucoside was found to be radioactive, and its specific activity was determined radiochemically. The amount of radioactive methyl β-D-glucoside present initially in the sample was

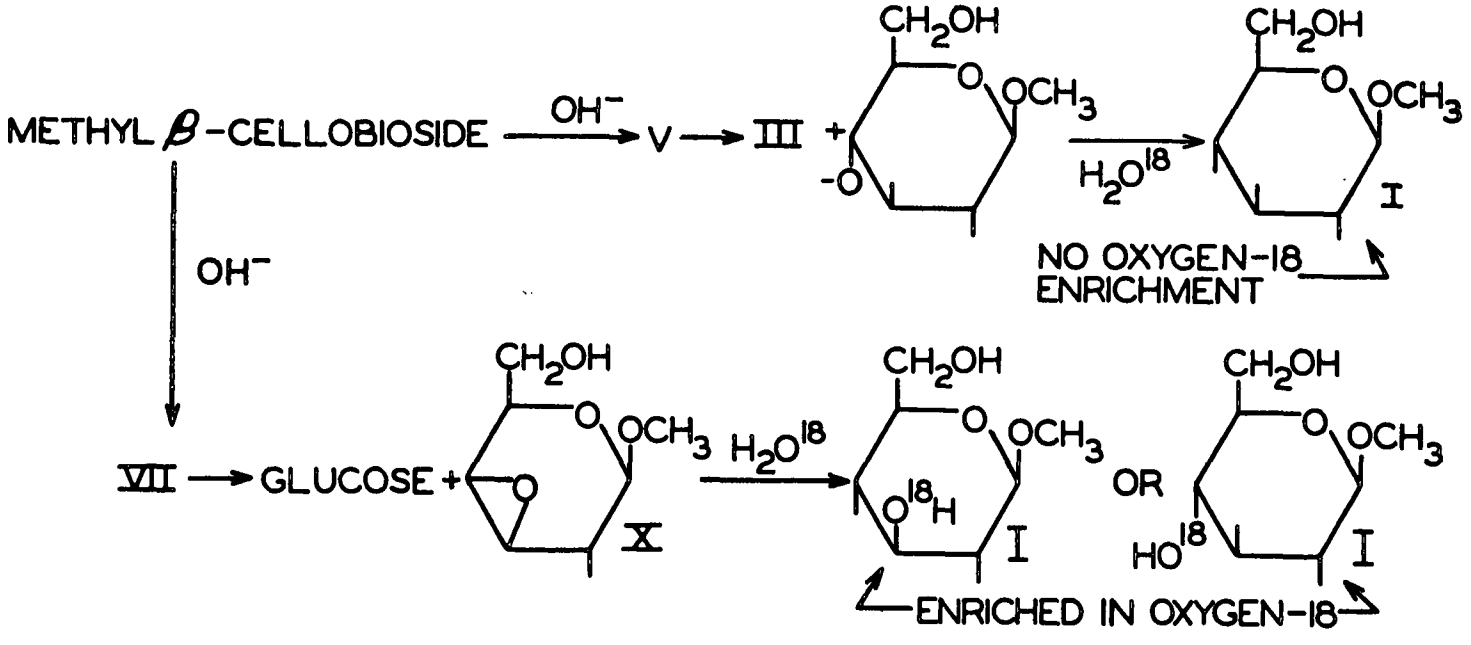
calculated to be 23.4 mg. Analysis of some of the same particular reaction solution during the kinetic work indicated that the portion of the solution used in the isotope dilution analysis should contain 20.4 mg. of methyl β-D-glucoside. This corresponds to a difference of 13.1%, based on the average of the two measurements. This is considered to be reasonable agreement since the measurements are independent and were made several months apart. Thus, the results show positively that methyl β-D-glucoside is produced during the alkaline degradation of methyl β-cellobioside under the conditions of this work. This production of methyl β-D-glucoside apparently occurs upon cleavage of the interior glycosidic bond. The results also show that the technique used to measure methyl β-D-glucoside in the kinetic work is reasonably accurate and that the material measured kinetically as methyl β-D-glucoside actually is methyl β-D-glucoside.

DETERMINATION OF THE POINTS OF CLEAVAGE OF THE GLYCOSIDIC BONDS OF METHYL β-CELLOBIOSIDE

The glycosidic bonds of methyl β-cellobioside were found to be cleaved predominantly on the glycosyl side of the glycosidic oxygen under the conditions of this work. Determination of the point of cleavage for the interior bond was of particular importance for the interpretation of the rate constant, $\underline{k_{CG}}$, for the interior cleavage. In the description of the methyl β-cellobioside reaction system, it was noted that a methyl β-glycoside could be produced via a methyl 3,4-anhydro-β-D-glucoside intermediate. It was also reasoned that methyl β-D-glucoside would be the most probable of the possible glycosides. Thus, methyl β-D-glucoside could apparently be produced from the cleavage of the interior glycosidic bond of methyl β-cellobioside by cleavage occurring either between C-1 of glucose and the glycosidic oxygen or between C-4 of the methyl β-D-glucoxyl aglycone and the glycosidic oxygen. By reacting methyl β-cellobioside in oxygen-18 enriched aqueous alkali and analyzing the methyl β-D-glucoside produced with a mass spectrometer, the amount or percentage of methyl

β-D-glucoside being generated through the 3,4-anhydro intermediate may be determined. This is possible since oxygen-18 enriched water would be added to the product glucoside upon breaking the epoxide ring, thus incorporating the isotope label into either the C-3 or C-4 hydroxyl. The following sketch shows the probable pathways for the interior cleavage and the results for each when done in an O-18 enriched system.

The methyl β-D-glucoside from the reaction of methyl β-cellobioside in oxygen-18 enriched molecular-oxygen-free aqueous sodium hydroxide was separated from the reaction solution, purified, and subjected to mass spectrometric analysis. No significant oxygen-18 enrichment above the normal isotopic amount was detected. The mass spectrum obtained was virtually identical to the spectrum published for known methyl β-D-glucoside (28).



Methyl β-D-glucoside presents some difficulties for mass spectrometric analysis since it is thermally unstable and practically nonvolatile (29). The mass spectra of many carbohydrates do not characteristically exhibit a very significant molecular

ion peak, and this was true for methyl β-D-glucoside. The diethyl dithioacetal derivatives of monosaccharides have been found, however, to show clear, easily detectable molecular ions (30). D-Glucose diethyl dithioacetal was prepared from glucose obtained by hydrolysis of some of the glycosidic product above. This purified derivative was analyzed and found to have only the normal isotopic amount of oxygen-18, thus confirming the results of the first analysis. This spectrum was again virtually identical to a published spectrum for D-glucose diethyl dithioacetal (30).

These results clearly indicate that the cleavage of the interior bond of methyl β-cellobioside occurs predominantly on the glycosyl side of the glycosidic oxygen, or between the C-1 and the glycosidic oxygen. This could have been expected to some degree as the C-2 hydroxyl function is generally found under alkaline conditions to be considerably more acidic or reactive than is the C-3 function (3,31,32). The results indicate that no interior bond cleavage occurs by direct hydroxide ion attack through an S_N2 mechanism.

The methanol from this same experiment was obtained as an aqueous solution and also analyzed for oxygen-18 enrichment. The methanol analyzed here had been generated by the cleavage of methoxyl from both methyl β-cellobioside and the methyl β-D-glucoside produced in the system. The methanol was found to have an oxygen-18 enrichment of 10.9% of that of the enriched water present in the reaction system, indicating that up to 10.9% of the cleavage of the model glycosidic bond was occurring between the glycosidic oxygen and the methyl aglycone. This figure is considered to be a maximum and could in actuality be somewhat lower. Some isotopic oxygen exchange could have occurred between the methanol and the enriched water under the very severe conditions encountered in the mass spectrometer inlet system (33), giving rise to a higher oxygen-18 enrichment in the methanol. No significant amount of such exchange is believed to have occurred, however (33). Also, the reaction conditions of the actual

experiment were rather severe (170°C., 86 hr.), and some isotopic exchange between the methanol and the water could have occurred (exchange with the glucosidic oxygens did not occur, however). The results do indicate, in any case, that the cleavage of the model glycosidic bond occurs predominantly (at least 89% or more) between C-1 and the glycosidic oxygen. This is analogous to cleavage in an acid medium. Bunton, et al. (34) found for methyl β-D-glucoside that 98-99% of the cleavage in acid occurred on the glucosyl side of the glycosidic oxygen. The methanol analysis data permit some speculation that a part of the methyl glycosidic bond cleavage occurs through an S_N2 mechanism by direct hydroxide ion attack. Additional data are necessary before a definite conclusion may be reached.

TEMPERATURE DEPENDENCE OF THE DEGRADATION REACTIONS

METHYL β-D-GLUCOSIDE REACTION

The reaction for the alkaline cleavage of the model glycosidic bond of methyl β-D-glucoside has a significant dependence on temperature. The dependence found in this work is almost exactly that which has already been reported in the literature (25). The work here had to be performed, however, in order to have available exact values of k_{GM} under certain known reaction conditions for substitution into the kinetic analysis of the methyl β-cellobioside reaction system. The methanol and unreacted methyl β-D-glucoside in reaction product solutions were measured as described in the Experimental Procedures section. A typical tabulation of reaction time, methanol concentration, and residual methyl β-D-glucoside concentration is given in Table I. Other similar preliminary results are tabulated in Appendix VIII. The data in Table I show that a mass balance between the unreacted methyl β-D-glucoside and the methanol produced holds reasonably well, substantiating Brooks' (25) finding and indicating that the methyl β-D-glucoside degradation may be satisfactorily followed by measuring methanol formation only. Methanol measurements were usually more precise

than the methyl β-D-glucoside measurements and, in general, were used throughout this work in characterizing methyl β-D-glucoside degradation. The data shown in Table I were used to determine the value of k_{GM} for the particular reaction conditions noted, and the plot of the data is given in Fig. 5. The linear plot indicates that the reaction is first order with respect to the methyl β-D-glucoside. The reaction was pseudo-first-order as no measurable amount of alkali was consumed during any of the 2.497N sodium hydroxide reactions.

TABLE I

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 139.9°C.

Reaction Time, hr.	Methyl β-D-Glucoside, μM/ml.	Methanol, μM/ml.	Sum, μM/ml.
0.0	10.35	0.0	10.35
36.0	10.26	0.17	10.43
72.0	10.19	0.27	10.46
108.0	10.24	0.41	10.65
216.0	9.66	0.80	10.46
252.0	9.67	0.90	10.57
276.0	9.61	0.98	10.59

The pseudo-first-order rate constants for the cleavage of the model glycosidic bond of methyl β-D-glucoside were determined in 2.497N sodium hydroxide over the temperature range of 140 to 170°C. and are tabulated in Table II. The sodium hydroxide concentrations reported in this work are measured at 25°C., and no correction is made for concentration changes due to temperature effects. The rate constant increases by a factor of 20 to 25 times as the temperature is increased from 140 to 170°C. This shows a significant temperature dependence. The Arrhenius activation energy was determined from the usual plot of the natural logarithm of the rate constant versus

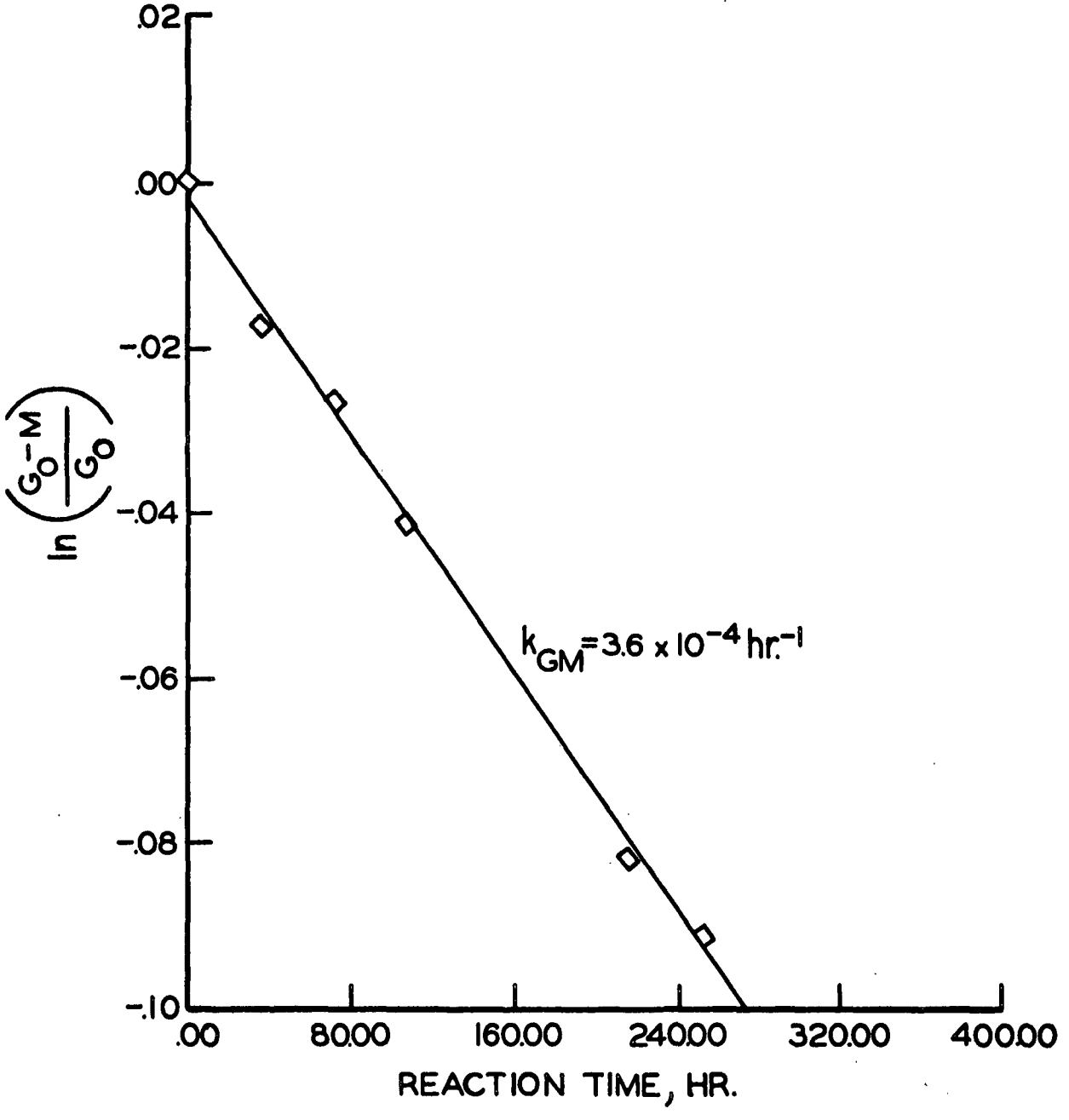


Figure 5. Reaction of Methyl β-D-Glucoside in N_2-Purged 2.497N NaOH at 139.9°C. Methanol Data

the reciprocal absolute temperature and found to be 37.5 kcal./g.-mole. This plot was linear. The energy found is in good agreement with the value of 36.0 kcal./g.-mole reported by Brooks (25).

TABLE II

SUMMARY OF RATE CONSTANTS[a] FOR REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH

Reaction Temperature, °C.	$10^4 k_{GM}$
170.1 (A)	97.5
170.0 (B)	85.8
170.0 (C)	63.9
159.2	24.5
150.3	10.9
139.9	3.6

[a] Pseudo-first-order rate constants, units are $hr.^{-1}$. Determined from methanol measurement.

If the reaction mechanism as described earlier for the degradation of methyl β-D-glucoside is correct, the pseudo-first-order rate constant, k_{GM}, is a product of an equilibrium constant, K_1; a specific rate constant, k_2; and hydroxide ion concentration [see Equations (4) and (5)]. The Arrhenius activation energy is usually thought of as describing the temperature dependence of the specific rate constant for a reaction, but since the energy determined is from the psuedo-first-order rate constant data, its magnitude may be influenced by the other two factors. A given hydroxide ion concentration (or more accurately, activity) should have at most only a slight temperature dependence, but the same is not necessarily true for an equilibrium constant.

The temperature dependence of an equilibrium constant is given by (35)

$$d(\ln K_{eq})/d(1/T) = -\Delta H/R \qquad (19)$$

where

K_{eq} = equilibrium constant

T = absolute temperature

ΔH = standard enthalpy change for the reaction, or heat of ionization for the reaction in point

R = ideal gas law constant.

If the first step of a complex reaction is a rapid equilibrium step followed by a slow rate-determining step, the activation energy for the overall reaction is the sum of the energy for the equilibrium step and the energy for the slow rate-determining step (36). Thus, the measured activation energy for the methyl β-D-glucoside reaction apparently is the sum of the energy for the ionization of the C-2 hydroxyl and the activation energy for the reaction of the carbinolate anion.

The heat of ionization of a weak acid of the approximate strength of water is expected to be fairly small (37). The acid dissociation constant for methyl β-D-glucoside at room temperature is 2.64×10^{-14} (38), very close to that of water, but its actual heat of ionization is not available. The heat of ionization of methanol in water is 10.8 kcal./g.-mole at room temperature (39), and the heat of ionization for phenol in water is 5.65 kcal./g.-mole (40). The dissociation constants for methanol and phenol are 1.87×10^{-17} and 1.05×10^{-10}, respectively. Thus, it appears that between 5 and 10 kcal./g.-mole of the measured activation energy could be due to the equilibrium step prior to the reaction of the carbinolate anion. In the general consideration of the cleavage of the glycosidic bond, it is more important to note, however, that the measured activation energy is the energy barrier for the overall cleavage reaction, and it is of lesser importance to know that this energy is a sum of two contributing factors.

METHYL β-CELLOBIOSIDE REACTION

Temperature Dependence

The reactions for cleavage of both the interior and the model glycosidic bonds of methyl β-cellobioside have a significant and similar temperature dependence. This dependence is the same as that for the alkaline cleavage of methyl β-D-glucoside, and the Arrhenius activation energies are very similar. The interior bond of methyl β-cellobioside was found to be cleaved at a considerably higher rate than was the model bond, however.

A reasonably good mass balance between the methyl β-cellobioside, methyl β-D-glucoside, and methanol was found for the methyl β-cellobioside degradation. This mass balance between the three components may be noted from a typical concentration-reaction time tabulation as given in Table III. All other such tables are included in Appendix IX. The particular reaction data given here show the poorest mass balance, by a factor of almost two, of any data taken during the current work, and even these data show that a mass balance holds reasonably well. This behavior indicates that very little of the methyl β-cellobioside degrades to "direct degradation products" via the previously described Pathway C. The concentration-reaction time data were used as described in the Experimental Procedures section to obtain the values of the pseudo-first-order rate constants which describe the methyl β-cellobioside degradation. A typical example of each of the three plots which show how the rate constants are obtained from the data is included here. All other such plots are included in Appendix IX. Figure 6 shows how $\underline{k}_1$, the rate constant for the overall degradation of methyl β-cellobioside, is obtained. The rate constant, $\underline{k}_{CG}$, for cleavage of the interior glycosidic bond is obtained from the slope of the data plot shown in Fig. 7. The rate constant, $\underline{k}_{CM}$, for the cleavage of the model glycosidic bond may be obtained from the slope of the data plot shown in Fig. 8. The various plotting quantities may be readily identified upon reviewing Equations (13), (16), and (18).

TABLE III

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 139.9°C.

Reaction Time, hr.	Methanol, μM/ml.	Methyl β-D-Glucoside, μM/ml.	Methyl β-Cellobioside, μM/ml.	Sum, μM/ml.
0.0	0.00	0.05	11.55	11.60
24.0	0.07	0.37	11.29	11.73
72.0	0.23	1.00	10.36	11.59
108.0	0.38	1.58	9.99	11.95
144.0	0.47	2.05	9.46	11.98
192.0	0.62	2.49	8.89	12.00
216.0	0.73	2.96	8.44	12.13
276.0	0.88	3.42	7.77	12.07

The various rate constants were determined over the temperature range of 140 to 170°C. and are tabulated in Table IV. The rate constants for cleavage of the interior bond, the model bond, and for overall methyl β-cellobioside degradation increased by a factor of 20-25 times as the reaction temperature was increased from 140 to 170°C. This behavior is the same as that for the rate constant for methyl β-D-glucoside degradation. The values tabulated for k_{CD} are obtained by subtracting k_{CG} and k_{CM} from k_1 for a particular set of reaction conditions. The value of k_{CD} was usually small and positive, indicating that a small amount of methyl β-cellobioside could be degrading through Pathway C. These values obtained by difference for k_{CD} are small enough that they could actually be within the magnitude of the experimental error associated with the other rate constants, k_1 in particular. An exact measure of the amount of experimental error in the rate constants is difficult to obtain due to the somewhat complex relationships between the experimentally determined data and the rate constants. The most reasonable conclusion to be drawn from the k_{CD} results and

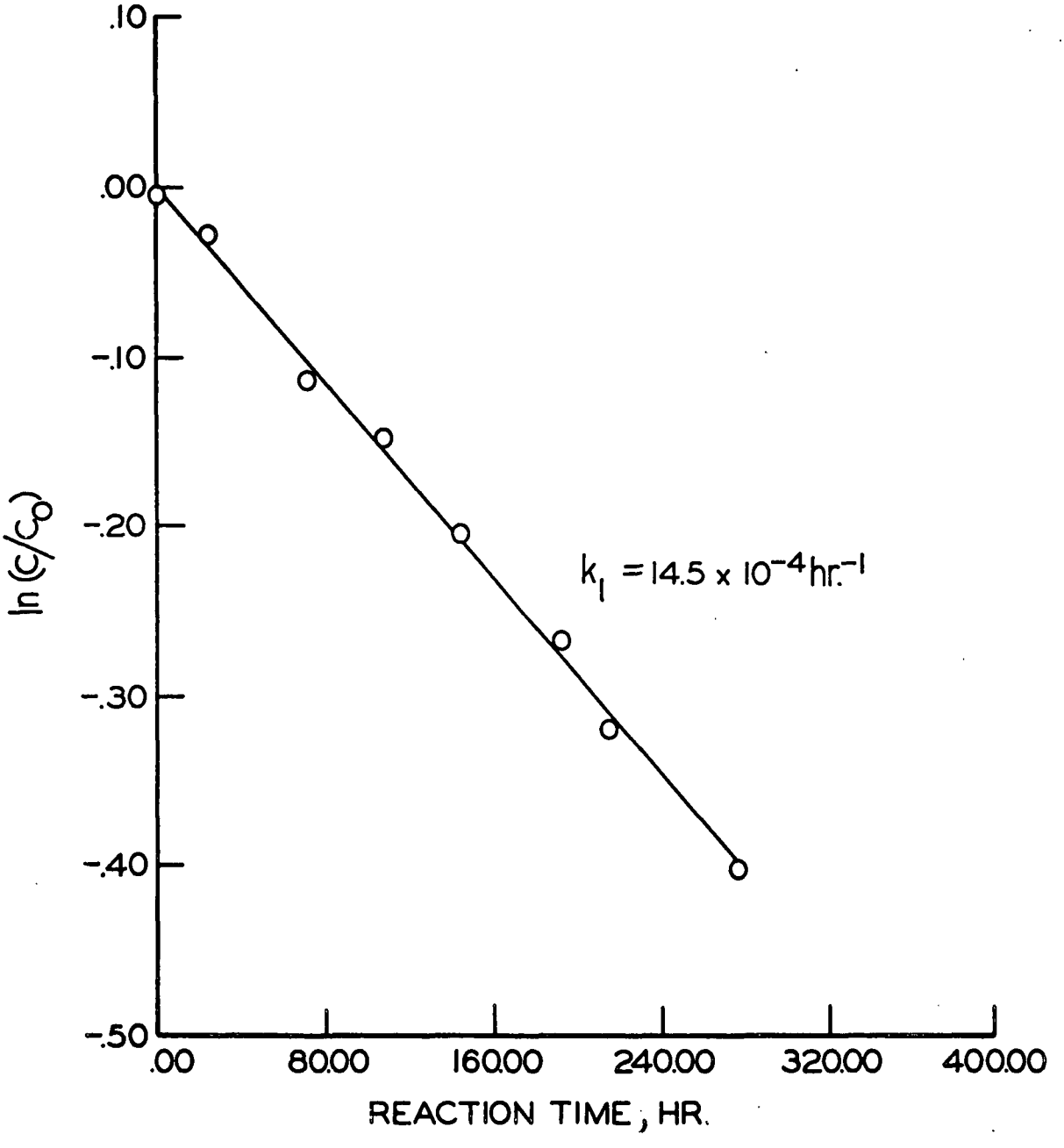


Figure 6. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 139.9°C. Overall Degradation of Methyl β-Cellobioside

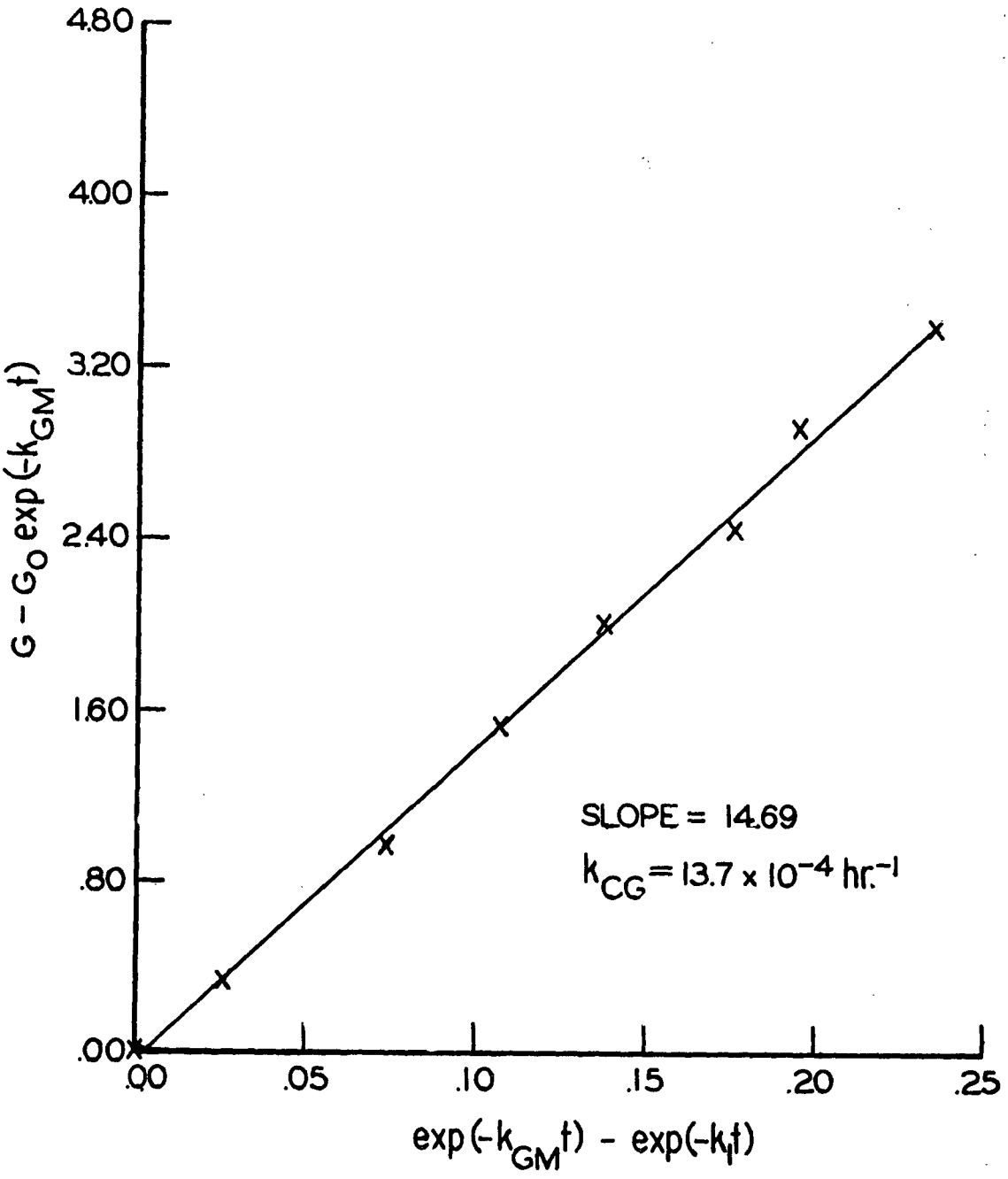


Figure 7. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 139.9°C. Generation of Methyl β-D-Glucoside

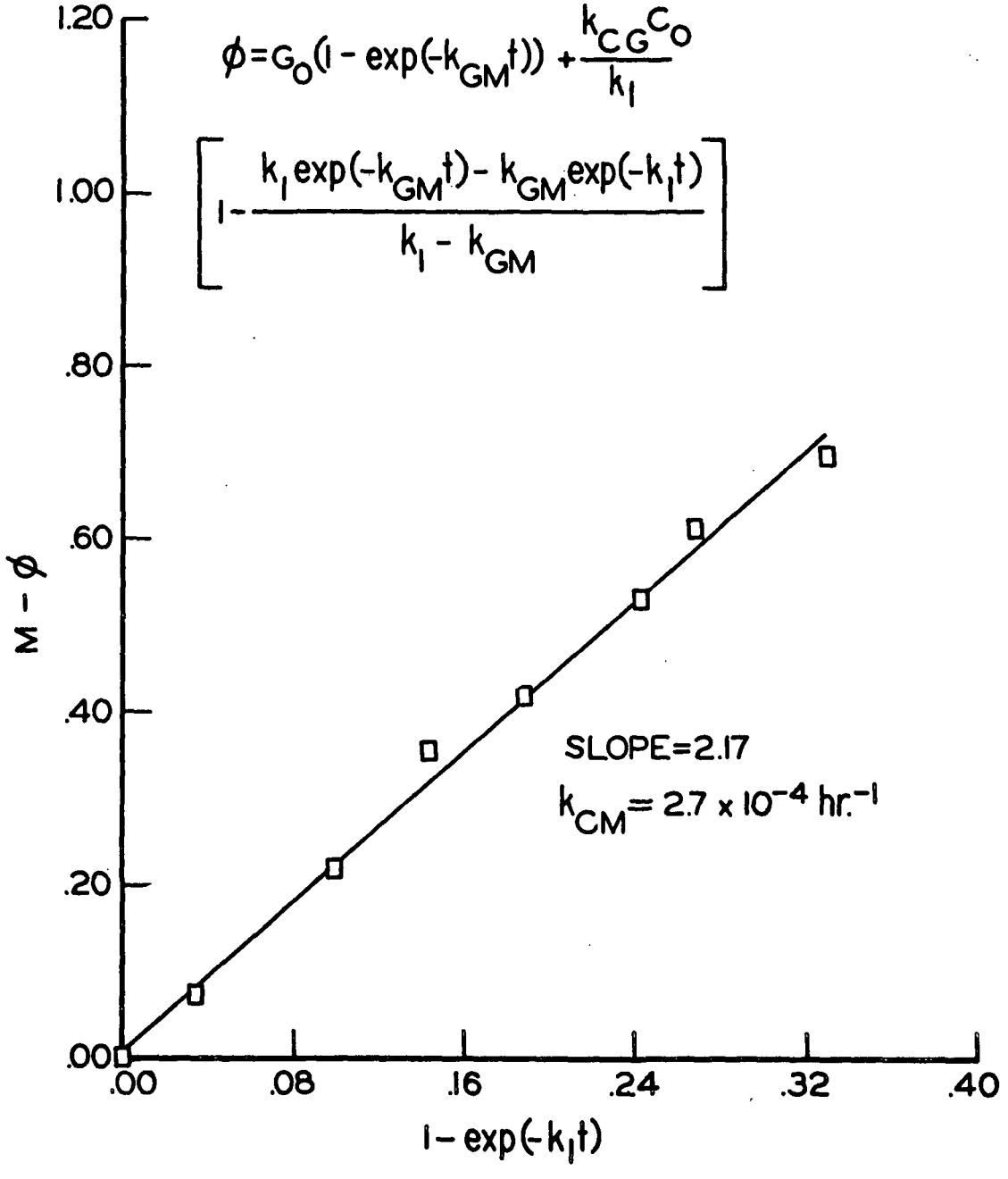


Figure 8. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 139.9°C. Generation of Methanol

the possible experimental errors is that the amount of methyl β-cellobioside degrading via Pathway C (see Fig. 3) is very small and perhaps insignificant.

TABLE IV

SUMMARY OF RATE CONSTANTS[a] for REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH

Reaction Temp., °C.	$10^4 k_{GM}$ Used	$10^4 k_1$	$10^4 k_{CG}$	$10^4 k_{CM}$	$10^4 k_{CD}$ (by difference)	k_{CG}/k_{CM}	k_{CD}/k_1, %
170.1 (A)	97.5	343.0	255.0	63.5	24.7	4.0	7.2
170.0 (B)	85.8	348.5[b]	289.0	57.7	2.2	5.0	0.6
170.0 (C)	63.9	354.0	293.0	59.2	1.6	5.0	0.5
159.2	24.5	131.0	101.0	21.0	9.3	4.8	7.1
150.3	10.9	50.6	39.4	7.3	3.9	5.4	7.7
139.9	3.6	14.5	13.7	2.7	-2.0	5.1	-13.8

[a] Pseudo-first-order rate constants; units are $hr.^{-1}$.

[b] Average value of k_1 for the (A) and (C) reactions (see Table XXIII in Appendix IX).

The Arrhenius activation energies for cleavage of the two bonds of methyl β-cellobioside were determined from the appropriate plot of the kinetic results in Table IV. A composite Arrhenius plot using all the rate constant data in Table IV is given as Fig. 9. The activation energies which were determined from the plot are tabulated in Table V. The earlier discussion of the effect of the equilibrium constant on the measured activation energy is applicable here, but need not be repeated.

The Arrhenius activation energy for the cleavage of the interior bond of methyl β-cellobioside appears to be only slightly lower than the energy for cleavage of the model bond. The energies for cleavage of the model bond in both methyl β-D-glucoside and methyl β-cellobioside are virtually the same. The close agreement between the

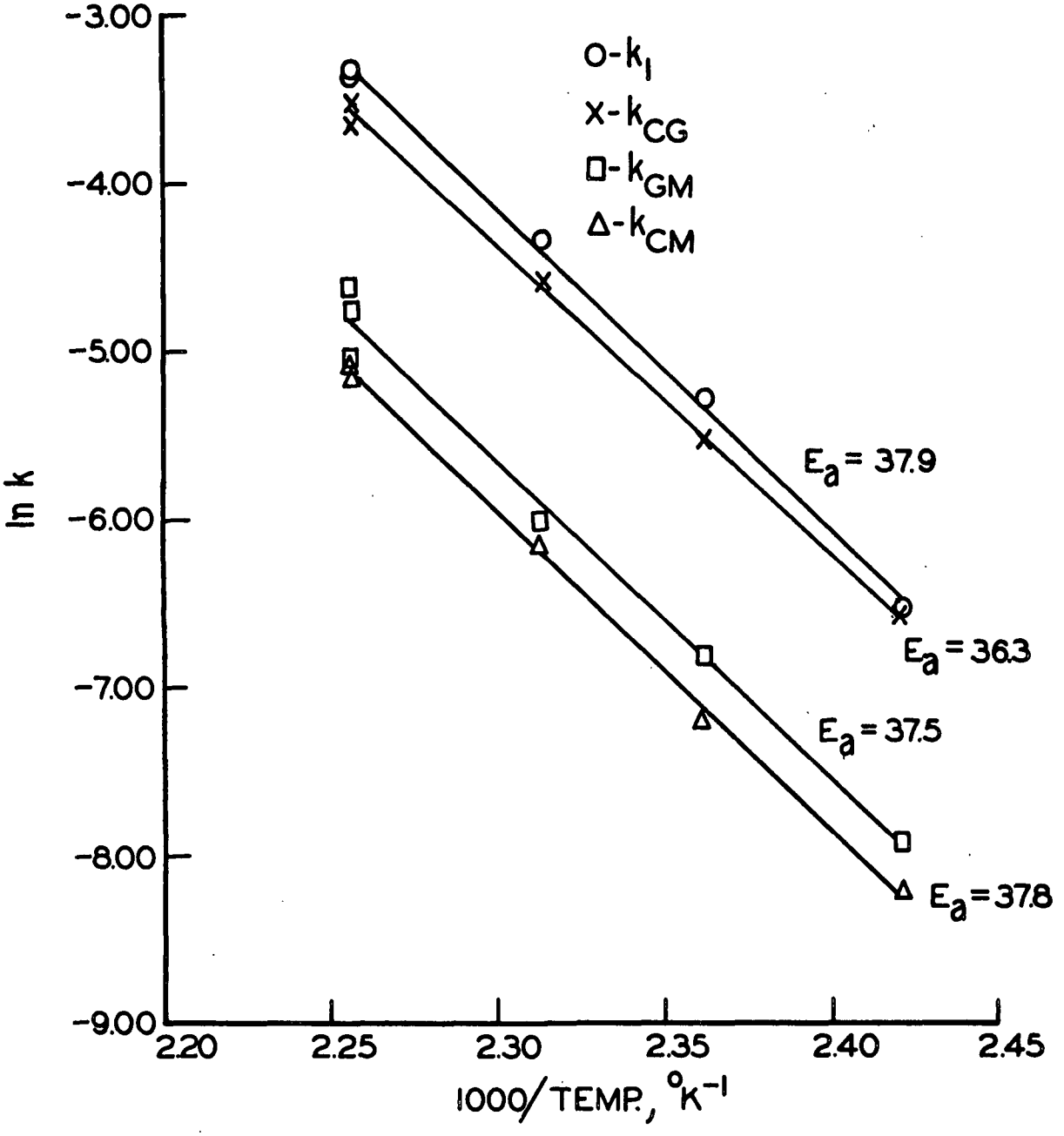


Figure 9. Arrhenius Correlations for the Reaction of Methyl β-D-Glucoside and Methyl β-Cellobioside in N_2-Purged 2.497N Sodium Hydroxide

activation energies suggests that the three bonds may be reacting by the same mechanism. The linear Arrhenius plots suggest that the rate-controlling reaction mechanism is the same over the temperature range investigated.

TABLE V

ARRHENIUS ACTIVATION ENERGIES FOR THE CLEAVAGE OF THE GLYCOSIDIC BONDS OF METHYL β-D-GLUCOSIDE AND METHYL β-CELLOBIOSIDE IN N_2-PURGED AQUEOUS ALKALI

Glycosidic Bond Cleaved	Arrhenius Activation Energy, kcal./g.-mole
Model bond of methyl β-D-glucoside	37.5
Model bond of methyl β-cellobioside	37.8
Interior bond of methyl β-cellobioside	36.3

The Arrhenius activation energy for cleavage of a glycosidic bond is considerably greater than that for the overall degradation of a hemicellulose under similar reaction conditions. The energy for the alkaline degradation of a hemicellulose is reported to be about 17 kcal./g.-mole and to have a rate constant of about 1.2 $hr.^{-1}$ in 10% (2.5N) NaOH at 170°C. (41). This compares to an energy of about 36 kcal./g.-mole and a rate constant of 0.029 $hr.^{-1}$ for the random cleavage of glycosidic bonds under the same conditions. Comparison of the rates shows that the hemicellulose degrades much faster than the glycosidic bonds are cleaved. The major portion of the degradation is probably due to "peeling" as noted earlier; however, if glycosidic bonds are being cleaved, this can significantly affect the overall rate since random cleavage of a glycosidic bond produces a new "peelable", reducing end group.

A comparison of the Arrhenius activation energies points out the much greater sensitivity of the glycosidic bond cleavage to temperature rises. The rate constants for the glycosidic bond cleavage shows that only a small amount of random

cleavage occurs at reaction temperatures less than 130-140°C., but at higher temperatures this is no longer true. The rate of random cleavage increases very rapidly as the temperature is increased over 140°C. or so. The reaction rate for "peeling" also increases as the temperature increases, but the relative rate increase is not nearly as great as for the random cleavage. At 170°C. and higher temperatures, this random cleavage of the glycosidic bonds undoubtedly would cause a significant increase in the overall rate of degradation of a cellulosic material. This increase in degradation would be due both to more "peeling" from the polymer chain ends and to the generation of shorter chain polymeric material which could dissolve in the reaction solution and be degraded even faster.

The ratio of k_{CG} to k_{CM} remains relatively constant at about five over the temperature range studied for the random cleavage in 2.497N NaOH and indicates that the interior glycosidic bond is somewhat more labile or easily cleaved than is the model bond. This could be due to leaving group effects or to different amounts of C-2 and C-2' hydroxyl ionization. The reaction involves the breaking of the C-1- and the C-1'-glycosidic oxygen bonds. The more electronegative or electron withdrawing the aglycone group, the weaker the glycosidic bond, and the easier it is for the aglycone to be displaced by an attacking nucleophile (42). On this basis, the interior bond should be easier to cleave since the methyl β-D-glucoxyl aglycone has two quite electronegative oxygen atoms within the inductive range to pull electrons away from the glycosidic bond, facilitating the nucleophilic attack of the C-2' carbinolate anion. There are no highly electronegative atoms in the methyl aglycone, and the cleavage rate for the bond should be relatively less than the rate for the interior glycosidic bond cleavage.

The dissociation of the C-2 hydroxyls of cellobiose has been reported to be greater than that for the C-2 hydroxyl of glucose (32). If this result could

possibly be extended to methyl β-D-glucoside and methyl β-cellobioside, then the cellobioside would be expected to react faster than the glucoside due to the increased concentration of the reactive ionized species. This helps explain the higher rate of cleavage for the interior bond as compared to the model bond of methyl β-D-glucoside, but does little to explain why the model bond of cellobioside reacts at a considerably slower rate than does the interior bond. Degradation of methyl β-cellobioside by two competing mechanisms could explain the ratio of interior to model cleavage rates, but the Arrhenius plot suggests a single mechanism.

The k_{CG}/k_{CM} ratio of about five could also be due to entropy effect differences which are not apparent between the two cleavage reactions. The Gibbs free energy change for a reaction is related to the equilibrium constant for the particular reaction in the following way

$$\Delta F = -RT \ln K \tag{20}$$

and for an isobaric, isothermal change,

$$\Delta F = \Delta H - T\Delta S \tag{20a}$$

so the equilibrium constant for the reaction may be written as

$$K = \exp(-\Delta H/RT)\exp(\Delta S/R) \tag{21}$$

where

ΔF = standard change in Gibbs free energy for the reaction

T = absolute temperature

R = ideal gas law constant

K = equilibrium constant

ΔH = standard change in enthalpy for the reaction

ΔS = standard change in entropy for the reaction (37).

According to the transition-state theory, a rate constant, k_r, for a particular reaction may be written as

$$k_r = (kT/h)K^{\ddagger} \qquad (22)$$

where

k = Boltzmann constant

h = Planck's constant

$K^{\ddagger}$ = an equilibrium constant for the overall equilibrium between the reactants and the transition-state intermediate.

The (kT/h) term represents a universal rate constant for the reaction to products of the transition-state intermediate. This expression for k_r may be rewritten in terms of the thermodynamic activation energies

$$k_r = (kT/h)\exp(-\Delta H^{\ddagger}/RT)\exp(\Delta S^{\ddagger}/R) \qquad (23)$$

The Arrhenius activation energy, E_a, is related to the Gibbs free energy of activation by the following relation for reaction in solution (37)

$$\Delta H^{\ddagger} = E_a - RT \qquad (24)$$

and substitution of this yields

$$k_r = (ekT/h)\exp(-E_a/RT)\exp(\Delta S^{\ddagger}/R). \qquad (25)$$

This equation shows that two reactions which have the same Arrhenius activation energy may have a different rate due to entropy effects which are different for the two reactions.

Investigation of Reaction Products

During the course of the temperature dependence work, limited investigation of the reaction products produced additional evidence supporting the postulated reaction mechanism, the results of the isotope dilution work, and the conclusion that little methyl β-cellobioside degrades through Pathway C. 1,6-Anhydro-D-glucose (levoglucosan), a reaction product expected from the postulated reaction mechanism, was identified as a reaction product by qualitative paper chromatography in three different solvent systems. Some of the material thought to be levoglucosan was isolated by preparative paper chromatography and determined by radiochemical analysis to be nonradioactive. Only very small amounts were found in the reaction product solutions from the methyl β-D-glucoside reaction, but easily observable amounts were present in the methyl β-cellobioside reaction product solutions. Only a small concentration of levoglucosan can be expected to accumulate under the reaction conditions as its rate of degradation is about fifty times that of methyl β-D-glucoside (27).

Qualitative paper chromatography, using three different solvent systems, also showed that no detectable amounts of methyl β-D-glucoside or methyl β-D-galactoside were present in the methyl β-cellobioside reaction product solutions. These two glycosides could be produced from the postulated methyl 3,4-anhydro-β-D-glucoside intermediate.

EFFECT OF HYDROXYL ION CONCENTRATION ON THE DEGRADATION REACTIONS

METHYL β-GLUCOSIDE REACTION

The alkaline cleavage of the glycosidic bond of methyl β-D-glucoside is dependent upon hydroxyl ion concentration, but the empirical dependence is not the same as the dependence predicted by the postulated reaction mechanism. The

mechanism predicts the reaction to be first order with respect to hydroxyl ion concentration, but the experimentally determined order is about 0.7 order. The actual order, however, is believed to be 1.0 since there are obvious factors operative in the reaction system which would tend to decrease the experimentally determined reaction order.

The effect of hydroxyl ion concentration [OH^-], on the random cleavage of glycosidic bonds in N_2-purged aqueous alkali was investigated by measuring the rate of degradation of methyl β-D-glucoside at 170°C. in such reaction systems whose [OH^-] were varied from 0.10 to 2.5 moles/liter. The [OH^-] was measured at 25°C., and no correction was made for temperature effects. The pseudo-first-order rate constants, which were determined from measurement of methanol, are tabulated in Table VI. At 170°C., the rate of glycosidic bond cleavage increases by a factor of 7-9 times as the [OH^-] is increased from 0.10N (0.4%) to 2.5N (10%) NaOH.

Changes in the rate of cleavage of the glycosidic bond were expected to be directly proportional to changes in [OH^-], but this was not found. Increases in [OH^-] did not effect directly proportional increases in the reaction rate, as may be noted from Table VI; an eightfold increase in the pseudo-first-order rate constant for a 25-fold increase in alkali concentration. The behavior observed is demonstrated in Fig. 10. For first-order dependence on [OH^-], a linear plot should have been obtained in Fig. 10. The empirical order was determined by a log-log plot (Fig. 11) of the kinetic data and found to be about 0.7.

Some of the factors which can affect the experimentally determined reaction order are activity coefficients of the various species in the reaction system, salt effects, and dielectric constant of the reaction medium; activity coefficient

TABLE VI

SUMMARY OF RATE CONSTANTS[a] FOR REACTION OF METHYL -D-GLUCOSIDE AND METHYL β-CELLOBIOSIDE IN N_2-PURGED AQUEOUS ALKALI AT 170°C.

NaOH Concentration, moles/liter	$10^4 k_{GM}$	$10^4 k_1$	$10^4 k_{CG}$	$10^4 k_{CM}$	$10^4 k_{CD}$ by difference	k_{CG}/k_{CM}	k_{CD}/k_1 %
2.497 (A)	97.5	343.0	255.0	63.5	24.7	4.0	7.2
2.497 (B)	85.8	348.5[b]	289.0	57.7	2.2	5.0	0.6
2.497 (C)	63.9	354.0	293.0	59.2	1.6	5.0	0.5
1.000	41.9	246.0	206.0	28.2	1.9	7.3	4.8
0.359	23.8	148.0	130.0	13.9	3.5	9.4	2.4
0.101	9.9	56.1	49.8	5.5	0.8	9.0	1.4

[a] Pseudo-first-order rate constants; units are $hr.^{-1}$

[b] Average value of k_1 for the (A) and (C) reactions.

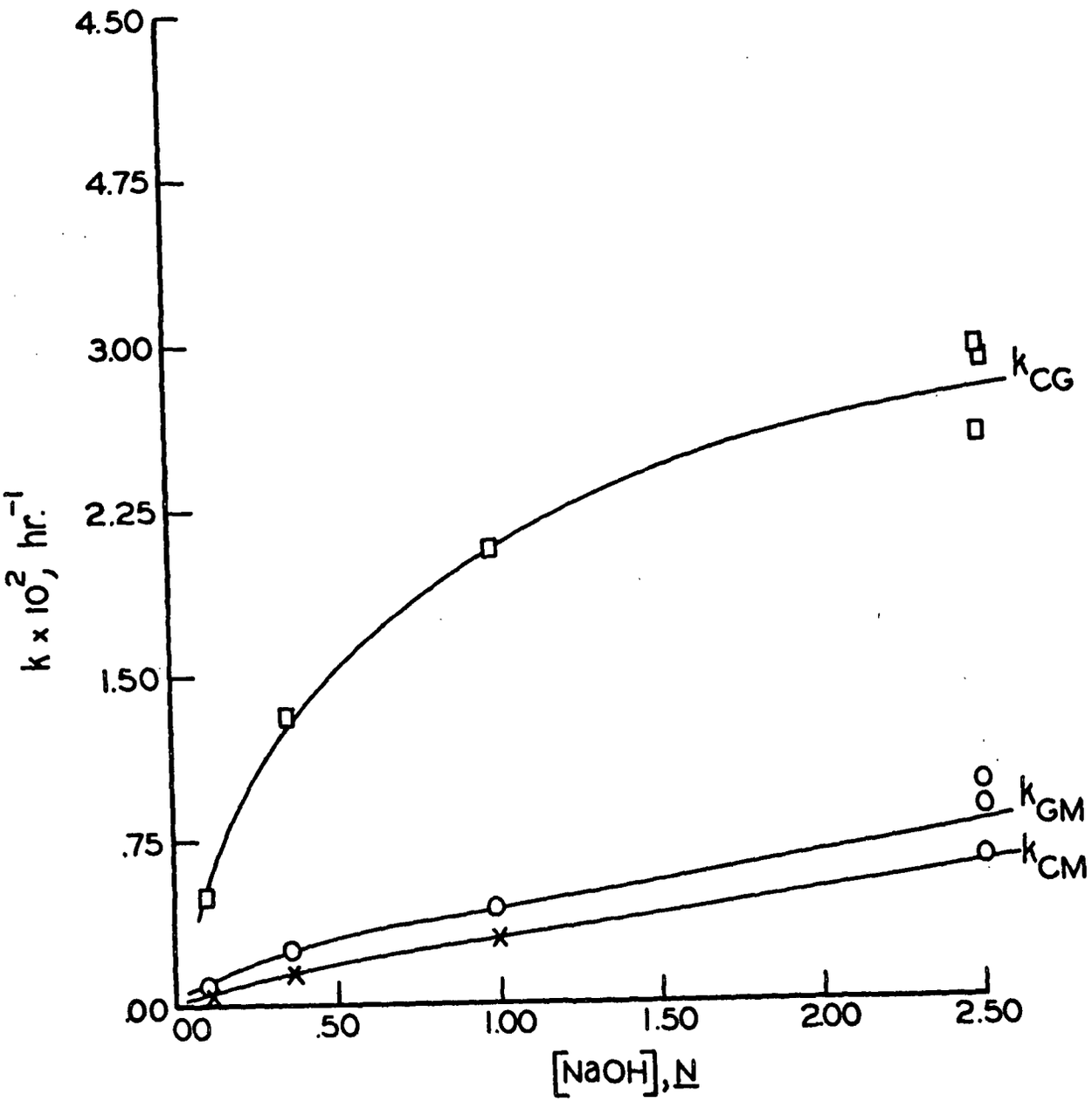


Figure 10. Dependence of Pseudo-First-Order Rate Constants on NaOH Concentration for Reaction of Methyl β-D-Glucoside and Methyl β-Cellobioside in Aqueous Alkali

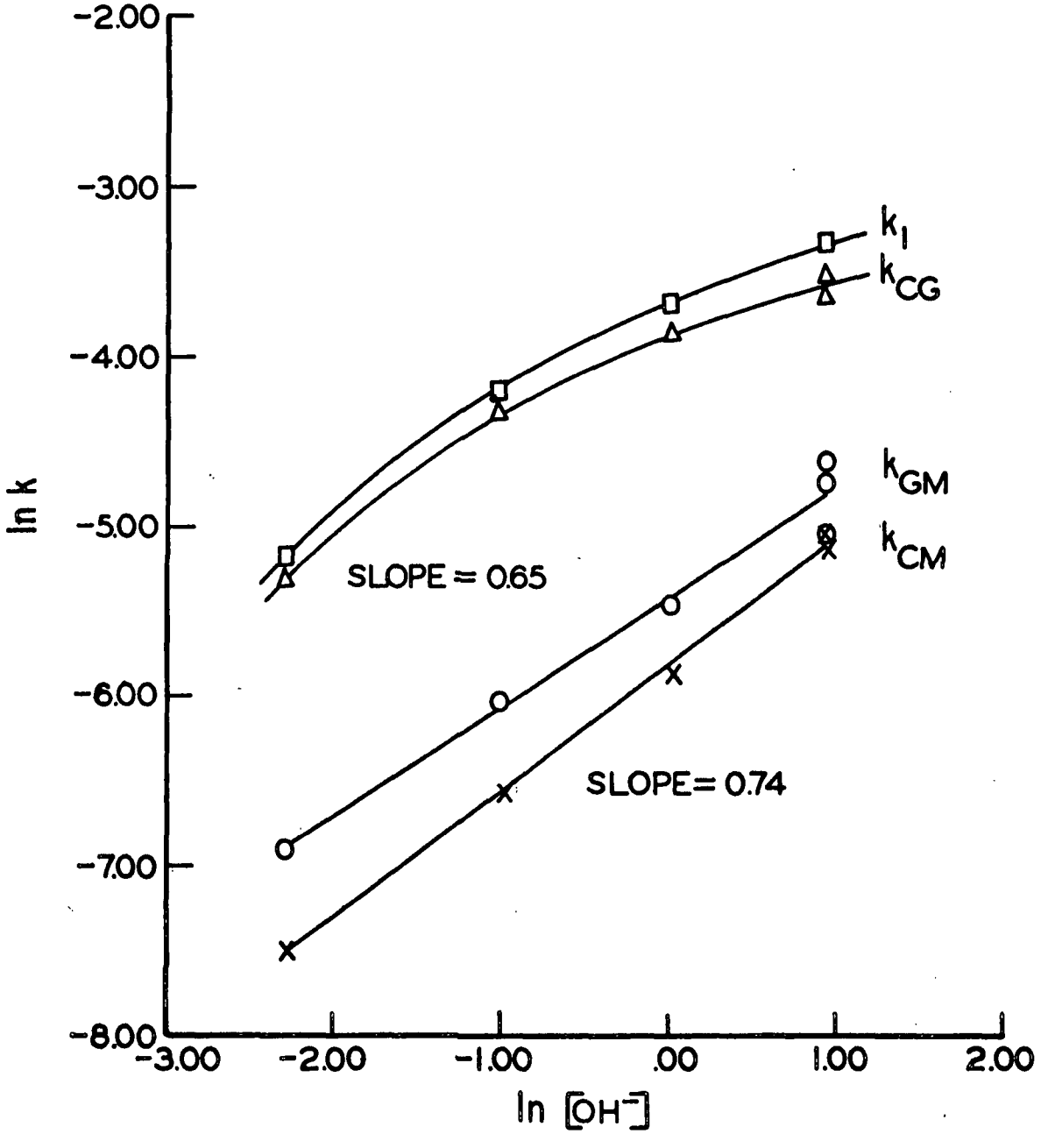


Figure 11. Determination of Empirical Reaction Order with Respect to Hydroxyl Ion Concentration for the Reaction of Methyl β-D-Glucoside and Methyl β-Cellobioside in N_2-Purged Aqueous Alkali at 170°C.

effects are probably the most important. The activity coefficient of the hydroxide ion obviously will have a major effect on the observed reaction order as demonstrated in the following discussion.

Equations (4) and (5) show theoretically how k_{GM} is a function of $[OH^-]$. This relation of k_{GM} may be written more accurately as

$$k_{GM} = k_1 k_2 (\alpha_{OH^-}) \tag{26}$$

where

α_{OH^-} = activity of the hydroxide ion.

The activity, α_{OH^-}, may be represented as the product of the $[OH^-]$ and the activity coefficient of the hydroxide ion, γ_{OH^-},

$$\alpha_{OH^-} = \gamma_{OH^-}[OH^-] \tag{27}$$

and

$$k_{GM} = K_1 k_2 (\gamma_{OH^-}[OH^-]). \tag{28}$$

If the activity coefficient, γ_{OH^-}, is not a constant, but is some function of the $[OH^-]$, it may be written as

$$\gamma_{OH^-} = f([OH^-]) \tag{29}$$

and substituted into Equation (22) to yield

$$k_{GM} = K_1 k_2 \left\{ f([OH^-]) \right\} [OH^-]. \tag{30}$$

Taking logarithms of this equation gives

$$\ln k_{GM} = \ln(K_1 k_2) + \ln\left\{ f([OH^-]) \right\} + \ln[OH^-]. \tag{31}$$

Examination of Equation (31) shows that the slope of the usual ln k_{GM} versus ln[OH^-] plot will not necessarily be 1.0, the theoretical reaction order with respect to the hydroxide ion. This discussion is applicable here since the γ_{OH^-} in water has been shown to be a complex function of [OH^-] and temperature (44,45). At 70°C., the highest temperature for which data are available, γ_{OH^-} decreases from near 1.0 for [OH^-] approaching zero to about 0.63 in one molal NaOH, and then γ_{OH^-} begins to increase as [OH^-] increases (45). The data reported indicate that the minimum in the activity coefficient-hydroxide ion concentration plot gets lower and moves to higher [OH^-] as the temperature is increased. These changes are in the proper direction to cause the experimentally observed reaction order to be decreased from the true value with respect to the [OH^-].

In addition to the changes in γ_{OH^-} as noted above, changes in the ionic strength of the reaction system, or salt effects, can cause changes in the activity coefficients of the other species participating in the overall reaction. The expression for the ionization equilibrium constant involves a ratio of activities of the various species. Activity as noted before is a product of the activity coefficient and the concentration. Thus, any changes in activity coefficient of any species can cause changes in this ratio, and thereby change the effective value of the equilibrium constant and the rate of reaction. This activity or activity coefficient ratio is very important to a reaction order plot; changes in the ratio with changes in the ionic strength can be expected usually, as the salt effects vary considerably with molecular size and chemical nature (46). The Debye-Huckel theory relates changes in activity coefficient to changes in ionic strength of a reaction medium, but this theory may be applied only to very dilute solutions (usually less than 0.01 molar). At higher ionic strengths where the Debye-Huckel theory no longer applies, the activity coefficient of a species depends on things

other than its charge, and there is no simple method of predicting the magnitude or direction of the salt effect (47).

The dielectric constant of the reaction system, which increases with increasing sodium hydroxide concentration, can influence the reaction rate. The rate of reaction of an ion and a neutral molecule, in which the charge is neutralized or dispersed over a larger area in the formation of the (rate-determining) reaction intermediate will be decreased in a medium of high dielectric constant and high ionic strength (48). This indicates that the reaction rate for formation of the postulated 1,2-anhydroglucose intermediate will be decreased relative to what would be expected as [OH^-] is increased.

METHYL β-CELLOBIOSIDE REACTION

The rates of cleavage of both glycosidic bonds of methyl β-cellobioside have a significant dependence on the hydroxide ion concentration of the reaction system. The dependence for the model bond is very similar to that for the model bond of methyl β-D-glucoside. The dependence observed for the interior bond is different in that the reaction order with respect to the hydroxyl ion concentration varies as a function of this concentration.

The cleavage rates for the two bonds of methyl β-cellobioside were determined under the same conditions as described for methyl β-D-glucoside, and the kinetic analysis results are in Table VI, which was presented earlier. The alkali consumption was measured for the 0.10N NaOH reaction, and at termination of the reaction about 20% of the alkali had been consumed. The data for the reaction were examined, but no significant effect attributable to the alkali consumption was noted, so no correction was necessary.

The results in Table VI show that k_{GM} appears to be more sensitive to alkali concentration changes than is k_{CG}. The value of k_{CM} increases about tenfold for a 25-fold alkali concentration increase while k_{CG} increases by only 5-6 times for the same alkali change. The same nonlinear behavior between rate constant and alkali concentration as noted earlier for k_{GM} is also true for both k_{CM} and k_{CG}. This is shown in Fig. 10. A log-log plot of the kinetic results shows that the reaction for cleavage of the model glycosidic bond of methyl β-cellobioside has an order of about 0.7, just as found for the model bond cleavage in methyl β-D-glucoside. This log-log plot, shown in Fig. 11, also shows that the reaction for cleavage of the interior bond of methyl β-cellobioside does not have a constant reaction order with respect to $[OH^-]$. The order appears to decrease in a regular manner from an order of about 0.9 at low $[OH^-]$ to about 0.4 at the higher concentrations.

The discussion given earlier for activity coefficient effects, salt effects, and dielectric constant effects on the reaction order for methyl β-D-glucoside is applicable here to the cleavage reactions for both bonds in methyl β-cellobioside. In addition, there are two possible additional factors which could be contributing to the varying reaction order for the interior bond cleavage. These have been discussed somewhat in the temperature dependence section and may be expanded somewhat here since in addition to being temperature dependent, they may also be dependent on the hydroxyl ion concentration.

The equilibrium constants for the C-2 and the C-2' hydroxyl ionizations may have different sensitivities to hydroxyl ion concentration changes--this appears obvious if the previous discussion on activity coefficient effects is kept in mind. This will produce a different relative concentration of the two reactive species as a function of $[OH^-]$, and this causes a change in the k_{CG}/k_{CM} ratio as $[OH^-]$ is changed.

The character of the methyl β-D-glucoxyl leaving group may also be changed as $[OH^-]$ changes, whereas similar changes for the methoxyl leaving group are not apparent. The methyl β-D-glucoxyl leaving group is considerably more complex, and as the $[OH^-]$ is increased, this leaving group could become more negatively charged due to increased ionization of the various hydroxyls on the group. This increased negativity could tend to repress the nucleophilic attack of the C-2' oxygen anion. The degree of repression would appear to be a function of the $[OH^-]$ and would contribute to a change in the relative amounts of model and interior glycosidic bond cleavage as the $[OH^-]$ was changed.

CONCLUSIONS

The interior glycosidic bond as well as the model glycosidic bond of methyl β-cellobioside is cleaved in oxygen-free aqueous sodium hydroxide solution at reaction temperatures of 140 to 170°C. This glycosidic bond cleavage is important to the overall alkaline degradation of cellulosic materials at high temperatures since random cleavage of a glycosidic bond in the polymer produces a new reducing end group which can undergo the "peeling" reaction. The shorter chain material produced by the random bond cleavage is more soluble in the reaction system and in general is lost. The cleavage of the interior and the model glycosidic bond occurs predominantly between the glycosidic oxygen atom and the anomeric carbon atom (C-1) of the glucose monomer.

Arrhenius activation energies for cleavage of both the interior and the model glycosidic bonds are relatively high and quite similar. This suggests that both bonds are probably reacting by the same mechanism. Linear Arrhenius plots for cleavage of both bonds suggests that the reaction mechanism remains the same over the temperature range investigated. The actual reaction rates are small, but quite temperature-dependent; glycosidic bond cleavage should not become significant in even strongly alkaline solutions until temperatures of 120°C. or so are reached. The rate of cleavage of the interior glycosidic bond is about five times that for the model glycosidic bond in strongly alkaline solutions. This is believed due to leaving group effects and possible differences in amount of C-2 hydroxyl ionization, and not to differences in reaction mechanism.

Rates of cleavage of both the interior and model glycosidic bonds under conditions of the current work were significantly dependent on the hydroxyl ion concentration of the reaction system. The reaction for cleavage of model glycosidic bonds has an empirical reaction order of 0.7 with respect to hydroxyl ion concentration.

The reaction for the interior glycosidic bonds has an empirical order which varies in a regular manner from about 0.9 in very dilute alkali to about 0.4 in strong alkali. The actual order of both cleavage reactions with respect to hydroxyl ion concentration is believed to be 1.0 as there are several factors operative in the reaction system which will tend to cause the experimentally observed reaction order to be lower than its true value. The observed order for the cleavage of the interior glycosidic bond is further complicated by leaving group effects and possible C-2 hydroxyl ionization equilibrium effects.

EXPERIMENTAL PROCEDURES

PREPARATION OF LABELLED METHYL(C-14) β-D-GLUCOSIDE

2,3,4,6-Tetra-O-acetyl-α-D-glycopyranosyl bromide was prepared from commercial glucose pentaacetate by the method of Fischer and Fischer (49,50), in which a solution of 30% hydrogen bromide in glacial acetic acid is the reaction medium. The product (acetobromoglucose) was obtained in 73% yield by crystallization from anhydrous diethyl ether by the addition of petroleum ether.

This acetobromoglucose was used to prepare radioactively labelled methyl(C-14) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside by the procedure of Schroeder and Green (51). This procedure was modified in that only 1.6 times the stoichiometric amount of the methanol, which was radioactively labelled (Nuclear Chicago Corp.), was added. The methyl(C-14) β-D-glucoside tetraacetate was obtained in 67% yield, and was deacetylated in dry methanol containing catalytic amounts of sodium methoxide (52). The resulting methyl(C-14) β-D-glucopyranoside was obtained in 93% yield; the overall yield, starting from the pentaacetate, was 45.5%.

The methyl(C-14) β-D-glucoside was recrystallized from methanol, dried, and stored *in vacuo* over sodium hydroxide pellets. Its melting point was 109-110°C. and its specific optical rotation was $[\alpha]_D^{26} = -33.6°$ (water). Literature values are 108-110°C. and $[\alpha]_D = -34.2°$ (water) (50).

The specific activity of the product was measured and found to be 6070 disintegrations per minute per milligram (dis./min./mg.). The purity of the glucoside was investigated with paper chromatography and no impurities were detected. Even a tenth of a percent of glucose, the most probable impurity, should have been detected. A detailed procedure for the preparation of the methyl(C-14) β-D-glucoside is given in Appendix I.

PREPARATION OF LABELLED METHYL(C-14) β-CELLOBIOSIDE

4-O-β-D-Glucopyranosyl-β-methyl(C-14)-D-glucopyranoside [methyl(C-14) β-cellobioside] was prepared from cellulose in four preparation steps. The method of Braun (53) was used to prepare α-cellobiose octaacetate by acetolysis of powdered cellulose. The octaacetate was obtained in 36% yield.

α-Acetobromocellobiose was prepared from the α-cellobiose octaacetate by the method of Fischer and Zemplen (54). The reaction mixture was mechanically shaken for the entire reaction period instead of allowing it to set after an initial period of shaking. An average yield of 61.5% was obtained for the preparation of nine separate batches.

The method of Schroeder and Green (51) was slightly modified and used to prepare the methyl(C-14) hepta-O-acetyl-β-cellobioside from the α-acetobromocellobiose. The amount of radioactively labelled methanol added to the reaction mixture was only five times the stoichiometric amount required for complete reaction. The chloroform-methanol(C-14) portion of the reaction mixture was recovered, refortified with labelled methanol, and reused several times. The average yield of methyl(C-14) β-cellobioside heptaacetate was 71%.

Methyl(C-14) β-cellobioside was prepared by the deacetylation of the methyl heptaacetate with catalytic amounts of sodium methoxide in dry methanol (52). An average yield of 89% was obtained for the deacetylation step. The overall yield of recrystallized methyl(C-14) β-cellobioside from the α-cellobiose octaacetate was 33%. Several batches of the cellobioside were combined and recrystallized from methanol. The recrystallized material was kept over sodium hydroxide pellets in a vacuum desiccator until needed. The specific optical rotation of the cellobioside was $[\alpha]_D^{24} = -18.3°$ (water). Literature values are $[\alpha]_D = -18.4°$ (water) (55) and $[\alpha]_D^{17} = -19.1°$ (water) (50).

Some difficulty was experienced in obtaining a dependable value for the melting point for the methyl(C-14) β-cellobioside. The crystals appeared to lose some solvent at about 140°C. and after that the actual melting point was difficult to judge, but they appeared to melt at 170.5-174°C. The literature value is 193°C. (55). The crystals from a second crop of methyl(C-14) β-cellobioside had a better melting point--185.5-189°C.--than the first crop.

The purity of the synthesized material was investigated by paper chromatography, and a small amount of cellobiose was present as the only impurity. The amount of cellobiose present was estimated to be less than 0.5%. The cellobioside was not purified further since the small amount of cellobiose would not complicate or affect the kinetic measurements.

The specific activity of the methyl(C-14) β-cellobioside was found to be 2790 disintegrations/minute/milligram (dis./min./mg.). The specific activity of methyl-(C-14) β-D-glucoside generated from this cellobioside by cleavage of the cellobioside's interior bond would be 5119 dis./min./mg. A more detailed description of the preparation procedures for obtaining the radioactive cellobioside is given in Appendix II.

PREPARATION OF REACTION SOLUTIONS

A volume of 2.497N NaOH sufficient for use throughout the kinetic work was prepared in a single container. Freshly boiled and cooled triply distilled water (56) was used to dilute concentrated aqueous sodium hydroxide which was taken from the supernatant portion of a 78% sodium hydroxide solution. The diluted sodium hydroxide solution (2.497N) was kept under a nitrogen atmosphere in a rubber-stoppered glass bottle. For the hydroxyl ion concentration effect work, appropriate volumes of this 2.497N stock solution were diluted with freshly boiled and cooled triply distilled water. All the alkali normalities were measured at room temperature

(usually about 25°C.) by titration with dilute hydrochloric acid to the phenolphthalein end point.

The methyl β-D-glucoside reaction solutions were prepared at a glucoside concentration of 0.0103 molar (10.3 μmoles/ml.), and the methyl β-cellobioside reaction solutions were prepared at a concentration of 0.0112 molar (11.2 μmoles/ml.); thus alkali was always in considerable excess. The amount of crystalline material necessary was weighed on an analytical balance. Care was taken to protect the reaction solution from atmospheric oxygen as much as possible during its preparation. Usually 500 ml. of a particular reaction solution was prepared. The solutions were prepared within one day of the beginning of the particular kinetic run. Just prior to the filling of the reaction tubes with solution, the solutions were purged for an hour or so with a slow stream of nitrogen.

The stability of methyl β-D-glucoside and methyl β-cellobioside in N_2-purged 2.5N NaOH at room temperature was investigated, and both were found to be stable for over thirty days. The results of this investigation are given in Appendix V.

DESCRIPTION OF THE PHYSICAL REACTION SYSTEM

The reaction tubes for this work were constructed from 50-inch lengths of standard 3/8-inch Type 316 stainless steel tubing. The tubes would contain a 55-ml. aliquot of reaction solution with sufficient extra space to accommodate the thermal expansion of the solutions at the high reaction temperatures. Some tubes for 25-ml. aliquots were only 26 inches long. One end of a tube was plugged and the other end fitted with threaded caps.

The procedure used to fill a reaction tube is as follows: Nitrogen pressure was used to force fill a buret with reaction solution from a reservoir (in which the N_2 purging of the reaction solution was done). The buret, initially filled

with nitrogen, was fitted with a rubber balloon reservoir at the top to accommodate the nitrogen displaced from the buret by the reaction solution. The tip of the filled buret was inserted into the mouth of a reaction tube, which was filled with nitrogen, and the proper volume of solution delivered into the tube. The mouth of the tube was held in a stream of nitrogen as the cap was put in place and the tube sealed. Teflon tape was used on the pipe threads to insure good seals.

An oil bath of about 75-gallon capacity, equipped with a rack for holding the reaction tubes, was used to provide and maintain the high reaction temperatures. Texaco Regal K heat transfer oil was used as the heating medium. The oil temperature was regulated to $\pm$ 1°C. by an automatic controller; a recorder provided a continuous record of bath temperature for the longer reaction times.

Measurement of reaction time was begun when the reaction tubes were placed in the heated bath, and stopped when the tube was removed for quenching. A reaction tube was cooled quickly to room temperature by immersing it in a standpipe of kerosene-based degreaser with a temperature of about 15°C. The possibility of an error in measuring reaction time due to the heat-up or quench time for a reaction tube is not serious since, over the course of the work, only two reaction tubes were removed from the bath with less than three hours reaction time.

METHANOL ANALYSIS PROCEDURE AND CALIBRATION

The methanol from a particular reaction solution was obtained in highly dilute aqueous solution, usually with a maximum methanol concentration of 50-60 micrograms per milliliter of solution. An analysis procedure described by Boos (57) was used to determine the amount of methanol in a particular solution.

This procedure involves the oxidation of the methanol present to formaldehyde by potassium permanganate under dilute acid conditions. The excess permanganate is

reduced by addition of sodium bisulfite. The test solution is made strongly acid with concentrated sulfuric acid, and chromotropic acid is added to form a colored complex with formaldehyde. The absorbance of the solution is measured at 580 nm. on a spectrophotometer. The amount of methanol present in the initial sample may be calculated from the calibration results and the measured absorbance.

The details of the analysis technique and its calibration are given in Appendix III. The equation, obtained from this calibration, relating methanol concentration in a product solution and absorbance of the test solution is

$$M = (162.15)A - 1.234 \qquad (32)$$

where

M = methanol concentration in the product solution, μg./ml.

A = absorbance value.

PAPER CHROMOTOGRAPHY

QUALITATIVE

In the qualitative investigation of the reaction product solutions and the neutral reaction products, three chromatography solvent systems were used. They were

A. ethyl acetate:pyridine:water (8:2:1)

B. ethyl acetate:acetic acid:water (9:2:2)

C. ethyl acetate:acetic acid:formic acid:water (18:3:1:4).

Paper chromatograms were detected by either of two techniques which are

1. silver nitrate:sodium hydroxide:sodium thiosulfate (dip) (58)

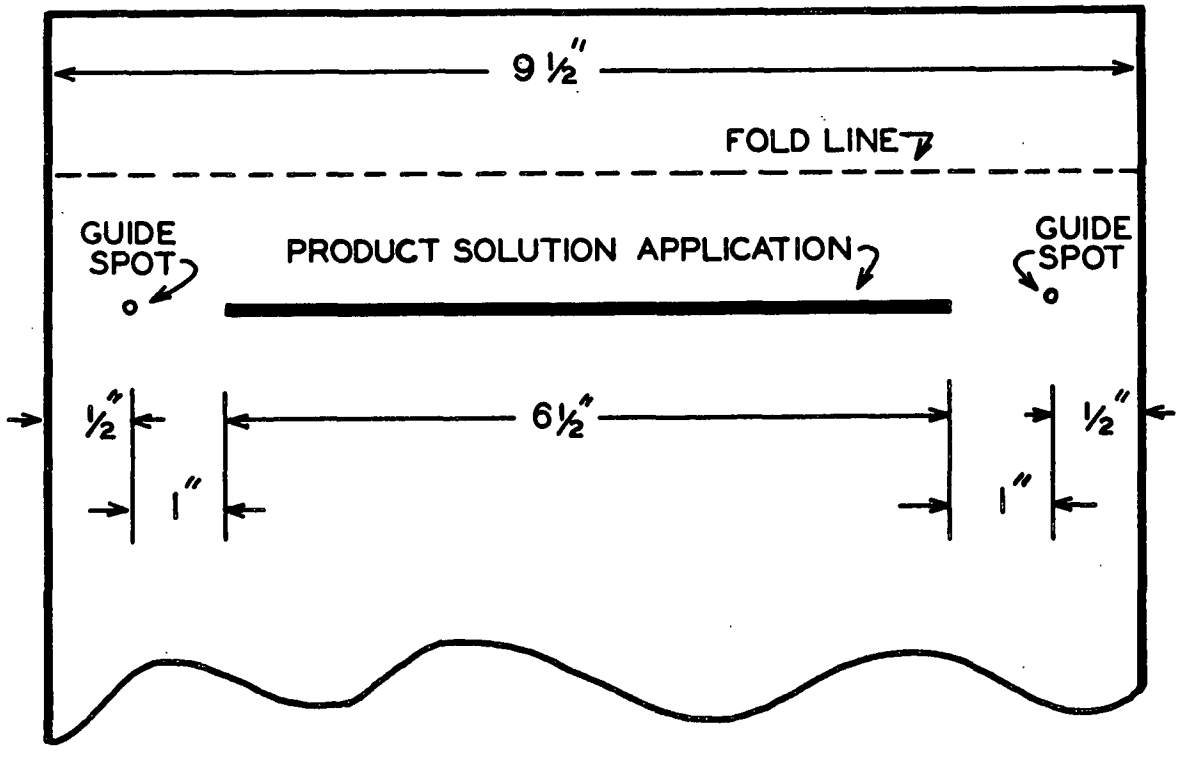
2. p-anisidine hydrochloride (spray) (59).

In general, solvent System A and detection Technique 1 were used for most of the qualitative work.

Whatman No. 1 paper was used in almost all the qualitative work, but Whatman 3MM was used for some preparative work. The descending technique for developing the chromatograms was used. Known compounds were used to compare to the materials present in reaction product solutions.

QUANTITATIVE

Quantitative paper chromatography was used in the separation and measurement of carbon-14-labelled methyl- -D-glucoside and methyl -cellobioside which were present in the same reaction product solution. The descending technique using solvent System A and 9-1/2 by 22-1/2 inch sheets of Whatman No. 1 chromatography paper were used. The top of a chromatographic sheet was marked off as shown in the following sketch.



Small amounts of nonradioactive glucoside and cellobioside were spotted on the points a half-inch in from each side of the sheet. Two hundred fifty microliters of the five-milliliter aqueous solution of the neutral materials from a particular reaction product solution were spotted with the aid of an ultramicroburet (Manostat Corp., New York) along the interior 6-1/2 inch line. The 250 µl. were applied in 40-50 µl. portions with the sheet being dried by a stream of air between applications. A quantitative paper chromatogram was developed for 8-10 hours in Solvent A to separate the radioactive methyl β-cellobioside and methyl β-D-glucoside into two separate and distinct bands on the sheet.

A "guide" strip of about 3/4-inch width was trimmed from each side of the developed chromatogram and detected by treatment with silver nitrate:sodium hydroxide. These strips were used to locate the bands of radioactive glucoside and cellobioside on the undetected portion of the chromatogram. Each band was excised, a wick of No. 1 paper was stapled on, and the water-soluble material was eluted off the paper band and into a combustion tube (for subsequent use in the Van Slyke analysis equipment). The bottom of the excised band was slit, folded, and stapled to form a drip-tip to insure delivery of the eluate into the combustion tube. The excised bands were usually about four inches wide, and about ten milliliters of eluate were collected from each band.

The combustion tubes containing aqueous eluates were placed over calcium chloride in a vacuum desiccator, and a partial vacuum pulled (about 28 inches Hg). The nonvolatile materials, including the desired radioactive glycosides, remained in the tubes after all the water had evaporated. The contents of a tube were then ready for radiochemical analysis by the Van Slyke technique.

RADIOCHEMICAL ANALYSIS

The wet combustion technique of Van Slyke, et al. (60,61) was used to oxidize to carbon dioxide the carbon in the organic materials obtained by the quantitative paper chromatography. Upon completion of combustion, the carbon dioxide produced was absorbed in an alkaline hydrazine solution, and the other gases in the system were expelled. Lactic acid was used to neutralize the alkali and release the carbon dioxide, which was then measured manometrically. The carbon dioxide was transferred quantitatively to a Bernstein-Ballentine proportional counting tube with the aid of a methane flushing step (62). The Bernstein-Ballentine tube was adjusted to atmospheric pressure with methane and attached to a Nuclear Chicago Model 182 scaling unit for measuring the amount of the radioactivity of the tube contents. The tube used in this work was calibrated and operated at the proper voltage as described in the literature (63).

This procedure was also used to analyze accurately weighed samples of the synthesized methyl(C-14) β-D-glucoside and methyl(C-14) β-cellobioside to determine their specific activities. A description of the Van Slyke apparatus, its operation, and a sample calculation using data from an analysis, as performed in the current work, has been reported recently (64). Current use of the equipment deviated from this account only in that a vacuum pump was used to evacuate the system prior to beginning a combustion instead of using the mercury pumping procedure.

ANALYSIS OF A REACTION PRODUCT SOLUTION

METHANOL

A reaction tube was kept at room temperature from the time it was quenched from the high temperature bath until it was opened and the contents analyzed for methanol, glucoside, and unreacted cellobioside. For analysis, a particular tube

was opened and the contents quickly and carefully poured into a small beaker. A 25-ml. aliquot was pipetted into a 100-ml. 24/40 round-bottom flask. Twenty-five milliliters of triply distilled, freshly boiled and cooled water were added; the flask's void space purged with nitrogen, and the flask attached to a N_2-purged distillation apparatus.

This apparatus consisted of a 40-cm. Vigreux column connected through a glass elbow and tee to a 300-mm. straight tube, water-cooled condenser. All joints of the apparatus were 24/40 ground glass. Heat was supplied through a 100-ml. electric heating mantle. A short length (4-5 inches) of small rubber tubing was attached to the condenser outlet and conducted the condensate beneath the surface of the condensate already collected in a 25-ml. volumetric flask. Two milliliters of triply distilled water were placed in the receiving flask before the distillation apparatus was purged and the distillation of a sample begun. The receiving flask was cooled in an ice bath during the distillation.

The distillation was stopped when the volumetric flask was within a milliliter or so of being to the mark. After coming to room temperature, the collected condensate, containing the methanol, was adjusted exactly to the mark and was ready for analysis of its methanol concentration by the technique already described.

The distillation procedure was shown in preliminary work to be satisfactory. No methanol is generated by the procedure, and all the methanol present in a reaction solution is quantitatively distilled.

METHYL β-D-GLUCOSIDE AND METHYL β-CELLOBIOSIDE

Upon completion of the distillation of the methanol from a sample, the residual alkaline solution in the distillation pot was quantitatively washed into a small beaker, diluted to about 100 ml., and placed on a prewashed ion exchange column.

Columns approximately with 1/2-inch i.d. and packed with 140-150 milliliters of Amberlite MB-3 mixed bed ion exchange resin (Rohm and Haas Co.) were used. The sample solution was followed by 500-600 ml. of distilled water. The eluate, containing the neutral materials from the reaction product solution, was concentrated under reduced pressure at 50°C. to about two milliliters. This concentrated solution was diluted to exactly five milliliters. This 5-ml. concentrate was then ready for analysis of the methyl(C-14) β-cellobioside and methyl(C-14) β-D-glucoside present by the combination of the quantitative paper chromatography and subsequent radiochemical analysis. The amount of cellobioside which would have been present in an initial 25-ml. aliquot if no degradation had occurred was 100 mg.

CALCULATION OF PSEUDO-FIRST-ORDER RATE CONSTANTS

METHYL β-D-GLUCOSIDE SYSTEM

The pseudo-first-order rate constant, k_{GM}, was obtained from the slope of a plot such as the one shown earlier in Fig. 5. Equation (7) describes the relationship between the variables of interest. The value used for G_o was 10.3 μM./ml., which was the methyl β-D-glucoside concentration of the reaction solutions as prepared.

The concentrations of methanol in the reaction product solution were calculated from the absorbance measurements and the calibration equation relating these two quantities. The calculated concentrations were converted to molar basis and the ordinate plotting quantities were calculated. A least squares linear regression analysis was used to obtain the best value for k_{GM}.

METHYL β-CELLOBIOSIDE SYSTEM

The rate constant determined first in the methyl β-cellobioside system was k_1, the constant for overall degradation of the cellobioside. The slope of the

plot shown as Fig. 6 is k_1, and its relationship to the reaction time and the cellobioside concentration is described in Equation (13). Proper consideration of calibration and correction factors, size of aliquots spotted on the chromatograms, etc., permitted the molar concentration of the methyl β-cellobioside in a particular reaction solution to be calculated. The concentration values obtained by this overall analysis procedure were investigated for accuracy by running some samples of known cellobioside concentration through the procedure. The values calculated were not the true concentrations; however, the overall analysis technique was found to yield dependable results, and it was satisfactorily calibrated. This calibration is

$$C_{actual} = (1.1428)C_{measured} - 0.3023 \qquad (33)$$

where C = methyl β-cellobioside concentration, μmole/ml. Appendix IV gives the details of this accuracy investigation.

The value used for C_o in a particular set of calculations was obtained by extrapolation of a ln C versus reaction time plot to zero reaction time instead of using the measured C_o value. This was done since the extrapolated value should be more accurate than a single measured value; the measured C_o was always within 2% of the extrapolated C_o, however.

The ordinate plotting quantities were calculated, and the data subjected to a least squares regression analysis, which yielded the best value for k_1. This k_1, as well as the value of k_{GM} under identical reaction conditions, was then available for use in determining k_{CG}.

The value of k_{CG} was obtained from the slope of a plot as shown in Fig. 7. This plot is described by Equation (16). The product glucoside concentrations for the various reaction times were obtained from the radiochemical analysis results. As in the case for the cellobioside, the accuracy for the overall analysis procedure

was investigated. The calibration equation for the glucoside concentration analysis is

$$G_{actual} = (1.0072)G_{measured} + 0.0162 \qquad (34)$$

where $\underline{G}$ = methyl β-D-glucoside concentration, μmole/ml.

The ordinate plotting quantities were calculated for the particular reaction times. The only variable in the abscissa plotting quantity at this point was the reaction time, and these quantities were calculated. Regression analysis of these data provided the best value for the slope of the plot. The value for $\underline{k}_{CG}$ was calculated from the plot slope since the value of the slope was the product of $\underline{k}_{CG}$ and $\underline{C}_o/(\underline{k}_1 - \underline{k}_{GM})$.

The methanol concentration for the various reaction times was calculated as described earlier. The ordinate plotting quantities described in Fig. 8 were then calculated from the methanol concentrations, the reaction times, and the other known constants. Reaction time is the only variable in the abscissa plotting quantities, and these quantities were calculated. The relationship between these quantities is described in Equation (18). Regression analysis of the data yielded the best value for the slope of the plot. The slope was a product of $\underline{k}_{CM}$ and $(\underline{C}_o/\underline{k}_1)$, and the value of $\underline{k}_{CM}$ was easily obtained.

The value of $\underline{k}_{CD}$ was obtained by subtraction of $\underline{k}_{CM}$ and $\underline{k}_{CG}$ from the value of $\underline{k}_1$. An IBM 1620 Model II digital computer was used in performing the calculations required for determining the various rate constants.

ISOTOPE DILUTION ANALYSIS OF PRODUCT METHYL β-D-GLUCOSIDE

The technique of isotope dilution may be used to measure the amount of a particular radioactive species of known specific activity which is in a mixture of compounds. This is done by adding in considerable excess a known amount of the same, but nonradioactive chemical species. Some of the radioactively diluted material is then separated by some convenient method and purified. The radioactivity of the purified material is measured, and by consideration of a total activity balance, the amount of the radioactive species present initially, x, may be calculated. This total activity balance may be written as

$$xA + yB = (x + y)C \qquad (35)$$

where

x = initial amount of labelled species

A = specific activity of the initial labelled species

y = amount of the unlabelled species added

B = specific activity of the unlabelled species

C = specific activity of the diluted species

Every factor except x is known or may be measured directly, thus x may be calculated.

The following isotope dilution analysis was performed in order to ascertain that the predominant reaction product formed by the cleavage of methyl(C-14) β-cellobioside's interior glycosidic bond is methyl(C-14) β-D-glucoside. Eight hundred eleven milligrams of unlabelled glucoside were added to three milliliters of the concentrate of a deionized reaction mixture, or to the equivalent of 15 ml. of a 25-ml. reaction product solution aliquot. The particular reaction mixture concentrate chosen for the isotope dilution was the 54-hour sample from the reaction of cellobioside in 1.00N NaOH at 170.0°C. The reason for choosing this sample was the fact that it had a relatively high and known amount of glucoside present. From the kinetic data

taken earlier, the amount of glucoside present in the 3-ml. sample was calculated to be 20.4 mg.

The solution of the unlabelled glucoside and the deionized reaction solution was concentrated to dryness, and the glucoside crystallized from methanol. The crystalline material was washed with methanol and recrystallized a second time from methanol. The second crop was washed and recrystallized from absolute ethanol. The third set of crystals was washed, dried, and subjected to radiochemical analysis. The crystals, which appeared to be pure methyl β-D-glucoside by qualitative paper chromatography, had a specific activity of 173 dis./min./mg. The unlabelled glucoside was also analyzed and found to have an activity of 30 dis./min./mg. The net increase in radioactivity was 143 dis./min./mg. By taking a total activity balance, the amount of radioactive methyl(C-14) β-D-glucoside present in the initial sample was calculated to be 23.4 mg.; the glycosidic product was assumed to have an activity of 5119 dis./min./mg. since the parent cellobioside had an activity of 2790 dis./min./mg.

REACTION OF METHYL β-CELLOBIOSIDE IN OXYGEN-18 ENRICHED AQUEOUS ALKALI AT 170°C.

Methyl β-cellobioside was reacted in oxygen-18 enriched alkali to determine if a measurable percentage of the interior bond cleavage occurred between the glycosidic oxygen and C-4 of the aglycone. Such cleavage was thought to generate a methyl 3,4-anhydro-β-D-glucoside intermediate, which, upon opening of the ethylene oxide ring, would incorporate O-18 into the C-3 and/or C-4 hydroxyls. The experimental details of this investigation are as follows.

Four hundred milligrams of methyl β-cellobioside and 254 mg. of sodium hydroxide pellets were dissolved in two grams of 30-atom-% O-18-enriched water (Bio-Rad Corp.). This reaction solution was placed in a small, Type 316 stainless steel bomb, which was subsequently purged with nitrogen and sealed. The bomb was

kept in a 170°C. oven for 86 hours. The cooled bomb was carefully opened and the contents transferred to a small improvised distillation apparatus and slowly distilled. The first two drops of condensate were caught in a curved glass tube condenser which was cooled in a dry ice-methyl cellosolve trap. The only outlet to the atmosphere from the distillation-condenser apparatus was protected by a Drierite-filled drying tube. The glass tube condenser containing the two frozen drops was sealed on both ends with a torch, and submitted to Morgan-Schaffer Corporation, Montreal, Canada, for mass spectrometric analysis.

The reaction solution remaining in the distillation pot was deionized on an Amberlite MB-3 mixed bed ion exchange resin column. The deionized solution was concentrated to about one milliliter and spotted on several sheets of Whatman No. 3MM chromatography paper. The preparative chromatograms were developed, and the methyl β-D-glucoside bands located with the aid of guide strips. The glucoside was eluted from the chromatograms. The eluate was filtered through Celite and concentrated to about ten milliliters. One milliliter was transferred to a small vial and allowed to air dry--the glucoside present later crystallized from the sirup and was submitted for analysis.

It was desired to prepare the mercaptal derivative from the glucoside, so the remaining material was hydrolyzed by refluxing with an aqueous suspension of Amberlite IR 120 (H^+) ion exchange resin for 24 hours. The hydrolysis mixture was filtered through Celite, concentrated to about three-fourths milliliter, and transferred to a small vial. The vial was placed over calcium chloride in a vacuum desiccator to allow the solution to concentrate to dryness. The resulting sirup had some yellow-brown color which was probably derived from the ion exchange resin.

The D-glucose diethyl dithioacetal derivative was obtained by the reaction of hydrochloric acid and ethyl mercaptan with the above sirup. The mercaptal did not

crystallize from the reaction mixture as it should have, however. The reaction mixture was neutralized and then deionized. The desired mercaptal was separated from the sirupy mixture and obtained in a relatively clean form by preparative paper chromatography on Whatman No. 3MM paper. About 25 mg. of this material was obtained in crystalline form. Paper chromatography showed that it was predominantly D-glucose diethyl dithioacetal. This material was thought to be sufficiently pure for mass spectrometric analysis and was submitted.

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APPENDIX I

PROCEDURES FOR THE PREPARATION OF METHYL(C-14) β-GLUCOSIDE

PREPARATION OF ACETOBROMOGLUCOSE

The following procedure, essentially that of Fischer and Fischer (49), was used to prepare acetobromoglucose (ABG). One hundred grams of commercial glucose pentaacetate was added to 100 ml. of a 30 to 32% hydrogen bromide in glacial acetic acid solution. The mixture was shaken at room temperature on a mechanical shaker for one and a half hours. The yellow solution was poured into a 2-liter separatory funnel containing 200 ml. of chloroform and slightly over a liter of ice and cold water. The mixture was shaken and the chloroform phase separated. The aqueous phase was back-extracted once with 75 ml. of chloroform, which was added to the first chloroform phase. The combined chloroform solutions were washed with one liter of ice cold water and separated. The wash water was back-extracted with 50 ml. of chloroform, which was added to the chloroform phase. The chloroform phase was then washed with one liter of a cold, saturated sodium bicarbonate solution and separated. The aqueous phase was back-extracted as above. The combined chloroform solutions were dried with about 150 ml. of anhydrous calcium chloride. Two to three grams of activated carbon were also added to the yellow-orange solution, which was allowed to stand at room temperature for a half hour. The mixture was filtered through a Celite pad and concentrated under reduced pressure at 35°C. to a thick sirup. The sirup was taken up in warm anhydrous diethyl ether. This solution was treated with small amounts of petroleum ether (30 to 60°C. b.p.) until a persistent turbidity was observed. The crystallization solution was allowed to cool to room temperature and then refrigerated for several hours. The crystalline ABG was collected on a Buchner funnel, washed with petroleum ether, and dried at 35°C. *in vacuo* for 4 hours. The dry ABG was stored in a desiccator until used.

A second crop of ABG (of lower quality) was usually obtained by concentration of the mother liquor and retreatment with petroleum ether. The ABG was obtained in 73% yield.

PREPARATION OF METHYL(C-14) β-GLUCOSIDE TETRAACETATE

The methyl(C-14) β-glucoside tetraacetate (M*BGA) was prepared by modification of the procedure of Schroeder and Green (51). The ABG (200 g., 0.49 mole) was dissolved in 250 ml. of purified chloroform and placed in a dropping funnel. To a 2-liter, 3-necked round-bottomed flask equipped with a mercury-sealed mechanical stirrer and an air outlet through a calcium chloride drying tube, was added the following materials: 300 g. of 10-20 mesh, nonindicating, and freshly dried Drierite; 90 g. of yellow mercuric oxide; 5 g. of mercuric bromide; 350 ml. of purified chloroform; and 31.2 ml. (0.78 mole) of C-14-labelled methanol (diluted to a calculated specific activity of 64×10^{-5} millicuries/millimole of methanol). The dropping funnel was put in place, and the contents of the flask were stirred for an hour. Then the ABG solution in the dropping funnel was added dropwise to the flask over a one and a half hour period. The stopcock of the dropping funnel was closed and the stirring was continued overnight. The mixture was then filtered through a Celite pad and the filtrate concentrated under reduced pressure at 50°C. to a thick sirup. The sirup was taken up in 200 ml. of hot chloroform and refiltered through Celite (to remove any insoluble mercuric bromide). The filtrate was concentrated to a thick sirup and taken up in 500 ml. of boiling hot absolute ethanol. The crystallization solution was allowed to cool slowly to room temperature. Crystals of M*BGA had formed, and after an hour or so, the solution was placed in the refrigerator overnight. The crystalline M*BGA was collected, washed with cold absolute ethanol, and dried overnight *in vacuo* at 40°C. The M*BGA was stored in a desiccator until used. No appreciable second crop of M*BGA was obtained. A M*BGA yield of 67% was obtained.

This same procedure was used for synthesis of unlabelled MBGA except that an eightfold excess of purified (unlabelled) methanol was used.

PREPARATION OF METHYL(C-14) β-GLUCOSIDE

Methyl(C-14) β-glucoside (M*BG) was prepared by the deacetylation of M*BGA by the following procedure (50,52). M*BGA (120 g.) was dissolved in 900 ml. of warm anhydrous methanol, and then 65 ml. of 0.22N sodium methoxide in dry methanol was added (this is 1/95 the theoretical amount). The solution was warmed to about 40°C., allowed to cool slowly, and left overnight at room temperature. The solution was concentrated under reduced pressure at 35°C. to 250 ml., and 36 ml. of Amberlite MB-3 mixed bed ion exchange resin added. About a half gram of active carbon was also added. After stirring the warm solution for a few minutes, it was filtered through Celite and refrigerated for crystallization of the M*BG. After refrigeration for at least 48 hours, the crystalline M*BG was collected, washed with cold methanol, and dried *in vacuo* at 50°C. overnight. The M*BG was stored in a desiccator until needed. A second crop of M*BG was obtained by concentration and refrigeration of the mother liquor. The yield of M*BG from the deacetylation step was 93%.

APPENDIX II

PROCEDURES FOR THE PREPARATION OF METHYL(C-14) β-CELLOBIOSIDE

PREPARATION OF CELLOBIOSE OCTAACETATE FROM CELLULOSE

The general procedure of Braun (53) was used, with some modifications, to prepare α-cellobiose octaacetate from cellulose. Five hundred-twenty grams of air-dried cellulose powder were slowly and continuously added over a one and a half hour period to a 3-liter, 3-necked reaction flask which contained 2000 ml. acetic anhydride and 180 ml. concentrated sulfuric acid. The flask was equipped with a thermometer, powder funnel, and a mechanical stirrer (glass stirring element). The reaction temperature was controlled to between 45 and 55°C. with a water bath and by the rate of cellulose powder addition. After the cellulose addition was completed, the temperature was held at 50 to 55°C. for an hour, and then allowed to cool to about 30°C. The reaction mixture was poured into two large beakers, which were covered with plastic film and placed in a 30°C. oven for 10 days. Then the thick, deep red, highly crystalline acetolysis mixture was poured slowly into about 3 gallons of ice and water, stirred vigorously with a mechanical stirrer for 10 to 15 minutes, and then allowed to stand. The crystalline material settled, and the supernatent liquor was siphoned off and discarded. This washing and siphoning step was repeated twice, and the crystalline material was collected on a large Buchner funnel. The crystalline material was masticated with a liter of warm methanol, cooled, and again collected on a Buchner, where it was washed with three 100-ml. portions of cold methanol. The crystals were dried overnight *in vacuo* at 50°C. The crude cellobiose octaacetate was purified by dissolution in 3 liters of chloroform, filtration through a Celite pad, concentration under reduced pressure to about 2 liters, and then re-crystallization by pouring into 4 liters of hot methanol. The crystallization of the octaacetate began almost immediately. The crystallization solution was covered and allowed to cool to room temperature overnight. The solution was refrigerated for 24

hours and the cellobiose octaacetate was collected. The crystals were washed 3 times with 100-ml. portions of cold methanol, and dried overnight in vacuo at 40°C. The dry cellobiose octaacetate was kept in a closed glass jar until used. The cellobiose octaacetate was obtained in 36% yield from the cellulose powder.

PREPARATION OF ACETOBROMOCELLOBIOSE

Acetobromocellobiose (ABC) was prepared from cellobiose octaacetate by the procedure of Fischer and Zemplen (54). Cellobiose octaacetate (30 g.) was added to 150 g. (110 ml.) of a cold 30 to 32% hydrogen bromide in glacial acetic acid solution. The mixture was shaken at room temperature on a mechanical shaker for 90 minutes. The honey-colored sirup was poured into a 2-liter separatory funnel containing about one liter of ice and cold water. Chloroform (200 ml.) was added and the mixture shaken. The chloroform phase was separated, and the aqueous phase back-extracted with 75 ml. of chloroform. The combined chloroform solutions were washed with one liter of cold water, which was then back-extracted with about 50 ml. of chloroform. The combined chloroform solutions were also washed with one liter of cold, saturated sodium bicarbonate solution. The aqueous phase was again back-extracted with a small amount of chloroform. About 150 ml. of calcium chloride, a gram of sodium bicarbonate, and about one-half gram of activated carbon were added to the combined chloroform phase, which was allowed to stand with occasional stirring for 30 minutes. The mixture was filtered through Celite, and the nearly colorless filtrate was concentrated to about 200 ml. under reduced pressure at 50°C. The concentrated solution was treated while warm with petroleum ether (30 to 60°C. b.p.) until a persistent turbidity was obtained. The turbid solution was allowed to cool slowly to room temperature and refrigerated overnight. The crystalline ABC was collected, washed with petroleum ether, and dried in vacuo at 40°C. for about 6 hours. The dried ABC was kept in a vacuum desiccator until used. A second crop of ABC was usually

obtained by concentration of the mother liquor and retreatment with petroleum ether. Average yield for ABC preparation was 61.5%.

PREPARATION OF METHYL(C-14) β-CELLOBIOSIDE HEPTAACETATE

The basic procedure of Schroeder and Green (51) for the synthesis of alkyl β-glucoside tetraacetates was modified for the synthesis of methyl(C-14) β-cellobioside heptaacetate (M*BCA) from ABC. Acetobromocellobiose (100 g., 0.143 mole) was dissolved in 200 ml. of purified chloroform and placed in a dropping funnel (the solution was protected from atmospheric moisture with a calcium chloride drying tube). To a 1000-ml., 3-necked, round-bottomed flask, equipped with a mercury-sealed mechanical stirrer, a drying tube-protected air outlet, and a one-holed rubber stopper for admission of the dropping funnel delivery tip, was added the following materials: 200 g. of 10-20-mesh, freshly dried, nonindicating Drierite; 35.0 g. of yellow mercuric oxide; 2.0 g. of mercuric bromide; 300 ml. of purified chloroform, and 28.8 ml. (0.72 mole) of C-14-labelled methanol (diluted to a calculated specific activity of 72 x 10^{-5} mc./mM). The dropping funnel was put in place, and the contents of the flask were stirred with the exclusion of atmospheric moisture for one hour. The ABC solution in the dropping funnel was admitted dropwise to the flask over a one and a half hour period. Stirring with the exclusion of moisture was continued overnight. The reaction mixture was filtered through Celite, and the filtrate concentrated under reduced pressure to a thick sirup or solid [a large portion of the chloroform-methanol(C-14) mixture was recovered for reuse]. The concentrate was dissolved in the minimal amount of hot chloroform and filtered through Celite to remove any insoluble mercuric bromide. The filtrate was reconcentrated to a thick sirup or solid and taken up in somewhat more than the minimal amount of boiling hot methanol (usually about 1300 ml.). The crystallization solution was allowed to cool slowly to room temperature and

refrigerated for at least 36 hours. The crystalline M*BCA was collected, washed with cold methanol, and dried overnight in vacuo at 50°C. The M*BCA was stored in a desiccator until used. A second crop of crystals was obtained by concentration and refrigeration of the mother liquor. An average yield of 71% was obtained.

PREPARATION OF METHYL(C-14) β-CELLOBIOSIDE

Methyl(C-14) β-cellobioside (M*BC) was prepared by the deacetylation of M*BCA with catalytic amounts of sodium methoxide in dry methanol (50,52). Two hundred forty grams of M*BCA were placed in 1750 ml. of dry methanol, and 155 ml. of 0.22N sodium methoxide were added (1/80 of the theoretical amount). The mixture was placed in a 40°C. water bath for one and a half hours and then allowed to cool slowly to room temperature. The M*BC crystallized upon overnight refrigeration. The crystalline material was collected, washed with cold, dry methanol, and dried overnight in vacuo at 50°C. A second crop of M*BC was obtained upon concentration and refrigeration of the mother liquor. The M*BC was further purified by recrystallization from methanol. The dry, recrystallized M*BC was kept in a vacuum desiccator until needed. The yield of M*BC from the deacetylation was 89%.

A purification procedure which was used with impure M*BCA and impure M*BC is the following. For each gram of impure material, 2 to 3 ml. of 1N sodium hydroxide was added, and the mixture heated on a steam bath for 30 minutes. The mixture was then deionized by passing through an Amberlite MB-3 ion exchange resin column. The deionized solution was decolorized with activated carbon, filtered through Celite, and concentrated to a sirup under reduced pressure. The sirup was taken up in an appropriate amount of hot methanol and refrigerated for crystallization of the M*BC.

The crystalline M*BC (and the M*BG) used in the kinetic work was exposed to a high humidity atmosphere for a week or so and redried in order to eliminate any methanol (the solvent used in crystallization) which might be associated with the crystals.

APPENDIX III

METHANOL ANALYSIS PROCEDURE

The analysis procedure as described by Boos (57) has been modified only slightly, as may be noted below. All chemicals used were reagent grade, and triply-distilled water was used in preparing the aqueous solutions. The sodium salt form of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) was obtained from K & K Laboratories.

The actual analysis for methanol concentration is performed as follows. One milliliter of the aqueous methanol solution is pipetted into a 10-ml. GGS volumetric flask. Four drops of 5% phosphoric acid are added to the flask and then 5 drops of 5% potassium permanganate. The flask is lightly stoppered and then gently swirled occasionally. Exactly 10 minutes after the permanganate is added, 20% sodium bisulfite is added dropwise until the solution is colorless. The flask is cooled in an ice bath as 4 ml. of concentrated sulfuric acid are added. Four drops of 2% chromotropic acid are added and the lightly stoppered flask is placed in a boiling water bath for 15 minutes. The flask is then cooled in an ice bath as water is added almost to the mark. After allowing the flask to come to room temperature, water is added exactly to the mark. The absorbance of the solution is measured at 580 nm. on a Beckman DU Spectrophotometer, and the methanol concentration obtained from either the calibration plot or calculated from the slope-intercept equation.

Aqueous methanol solutions of known methanol concentration were prepared and used to determine the calibration plot shown as Fig. 12. The data are tabulated in Table VII. The data were subjected to regression analysis, and the linear correlation coefficient for the plot was 0.99932. The equation for the correlation is

$$M = (162.15)\ A - 1.234 \qquad (32)$$

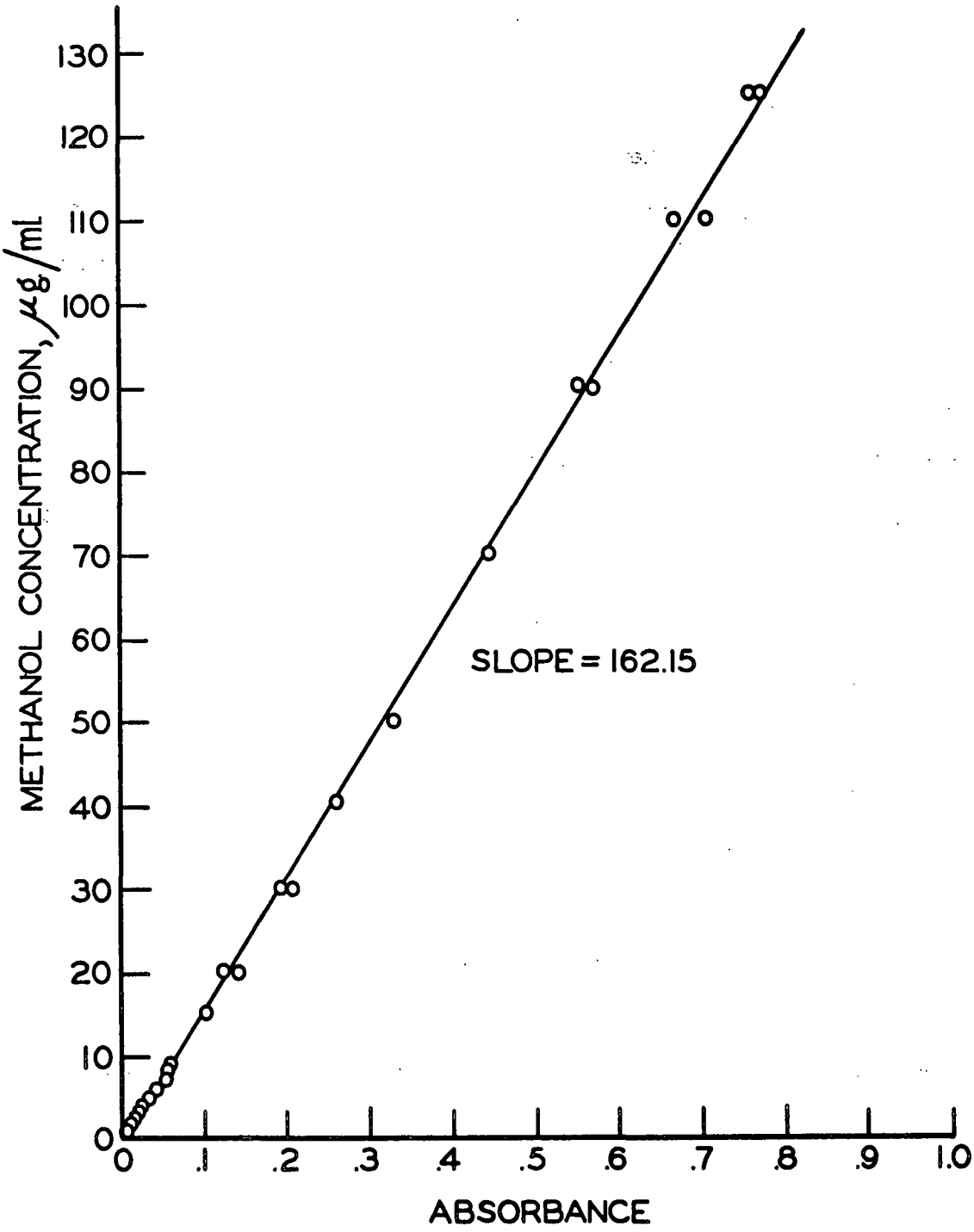

Figure 12. Methanol Concentration versus Absorbance Calibration

where

$\underline{M}$ = methanol concentration, µg./ml.

$\underline{A}$ = absorbance.

TABLE VII

METHANOL CONCENTRATION VERSUS ABSORBANCE CORRELATION DATA

Known Methanol Concentration, µg./ml.	Measured Absorbance	Known Methanol Concentration, µg./ml.	Measured Absorbance
1.001	0.007	30.026	0.203
2.002	0.011	40.035	0.256
3.003	0.018	40.035	0.260
4.003	0.028	50.044	0.332
5.004	0.034	50.044	0.328
6.005	0.042	70.061	0.443
7.006	0.052	70.061	0.443
8.007	0.053	90.078	0.567
9.008	0.059	90.078	0.558
15.013	0.103	110.096	0.707
15.013	0.108	110.096	0.669
20.017	0.125	125.109	0.775
20.017	0.140	125.109	0.759
30.026	0.193		

APPENDIX IV

INVESTIGATION OF THE ACCURACY OF THE REACTION MIXTURE ANALYSIS PROCEDURE

The procedure used to measure the amounts of M*BG and M*BC in a reaction mixture was investigated to determine its accuracy. Overall, this analysis procedure includes the deionization of the reaction mixture, its concentration to a known volume, the quantitative paper chromatography and elution, and the radiochemical analysis. In order to test the analysis procedure, several samples containing known amounts of M*BG and M*BC were prepared and analyzed. Each sample was prepared to simulate a normal 25-ml. reaction mixture aliquot. For example, Sample 2 (see Table VIII) contained 90.0 mg. of M*BC and 5.0 mg. of M*BG. A 25-ml. aliquot from a reaction tube which had been reacted at a high temperature for a relatively short time might actually contain almost these exact amounts of the two glycosides. Volumetric solutions of M*BG and of M*BC were prepared, and each sample for analysis was prepared by combining the appropriate amounts of each glycoside solution. Before starting a sample through the analysis procedure, 10 ml. of 6.25N NaOH--the equivalent of 25 ml. of 2.5N NaOH--was added. From this point each sample was treated as if it were an actual reaction mixture aliquot. The results are tabulated in Table VIII.

A plot of measured amount of M*BG *versus* known amount of M*BG was made and is included as Fig. 13. The slope of the plot was found to be 0.99287 and to have an intercept of -0.078034 mg. M*BG/25 ml., yielding the following equation:

$$G_{measured} = (0.99287)\, G_{actual} - 0.078034 \qquad (36)$$

where

G = methyl β-glucoside, mg./25 ml.

This equation should be valid over the range of 2-30 mg. MBG/25 ml. By solving Equation (36) for G_{actual} as a function of $G_{measured}$ and changing the units to

TABLE VIII

RESULTS OF THE ACCURACY EVALUATION OF THE ANALYSIS PROCEDURE FOR MEASURING METHYL β-GLUCOSIDE AND METHYL β-CELLOBIOSIDE[a]

	Methyl β-Glucoside			Methyl β-Cellobioside		
Sample No.	Known Amount, mg.	Measured Amount, mg.	Recovery, %	Known Amount, mg.	Measured Amount, mg.	Recovery, %
1	2.00	1.96	98.0	100.0	89.7	89.7
2	5.00	4.80	96.0	90.0	80.6	89.6
3	10.00	9.83	98.3	80.0	73.3	91.6
4	15.00	14.8	98.7	64.0	58.1	90.8
5	22.50	22.4	99.7	45.0	42.0	93.3
6	30.00	29.6	98.7	30.0	28.4	94.6

[a] Each sample is analogous to a 25-ml. reaction mixture aliquot.

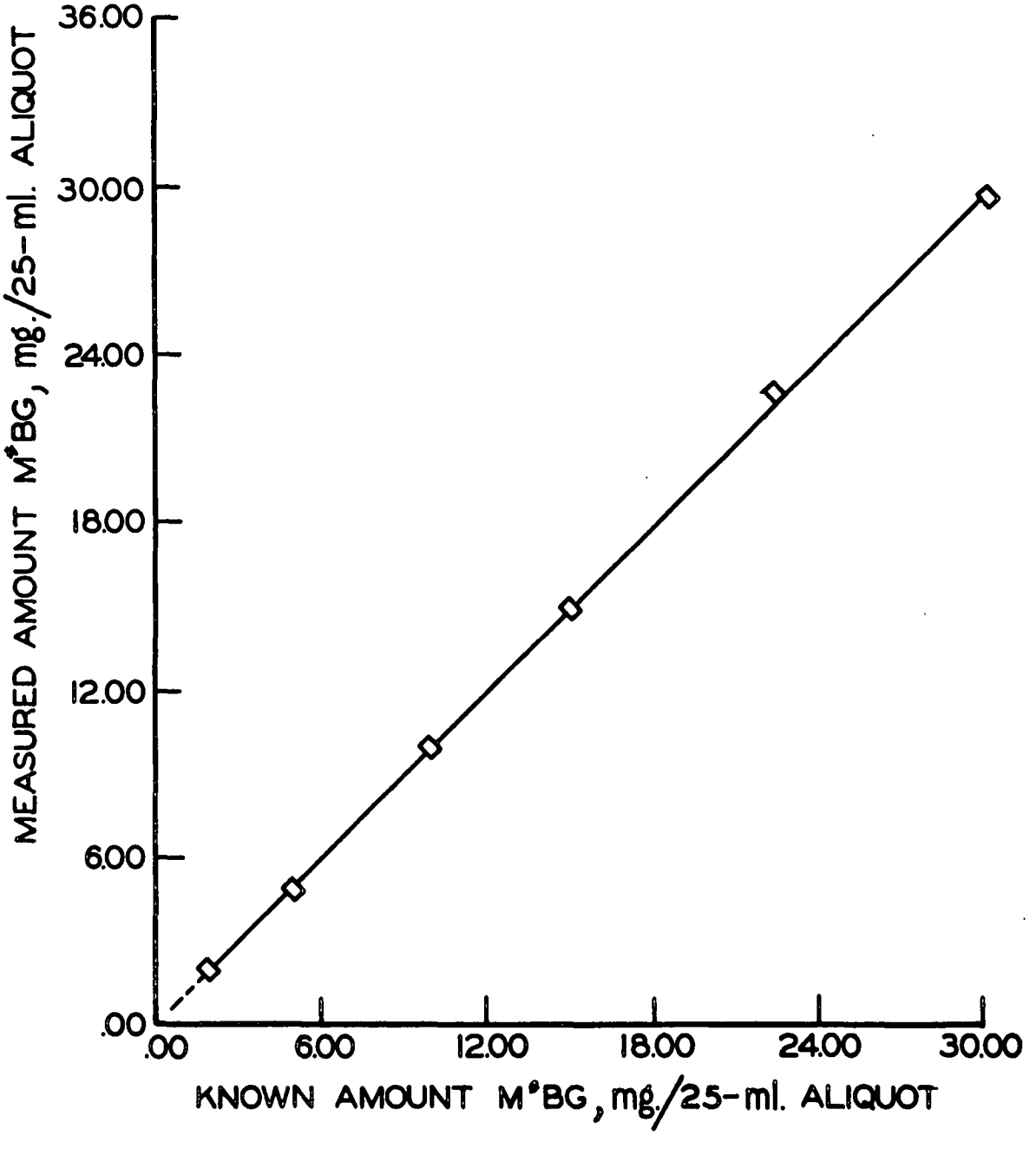

Figure 13. Accuracy of Measuring Methyl β-Glucoside with the Reaction Mixture Analysis Procedure

molar basis, the following equation is obtained:

$$G_{actual} = (1.0072)\ G_{measured} + 0.0162 \qquad (34)$$

where

$\underline{G}$ = methyl β-glucoside, μmole/ml.

A plot was also made for the results of the M*BC measurements. The plot is included as Fig. 14. The following equation, which should be valid over the range of 30-100 mg. MBC/25 ml., was obtained by regression analysis of the results;

$$C_{measured} = (0.87506)\ C_{actual} + 2.3564 \qquad (37)$$

where

$\underline{C}$ = methyl β-cellobioside, mg./25 ml.

Upon rearranging Equation (37) and changing the units, the following equation is obtained:

$$C_{actual} = (1.1428)\ C_{measured} - 0.3023 \qquad (33)$$

where

$\underline{C}$ = methyl β-cellobioside, μmole/ml.

These results show that the analysis procedure is quite accurate in measuring M*BG with over 98% of the actual amount being measured over the entire concentration range. The measurement of M*BC is somewhat inaccurate, but apparently quite precise; therefore, the procedure may be calibrated satisfactorily. Thus, for the case of both M*BG and M*BC, the actual amount of glycoside present in an aliquot may be calculated from the measured amount by the use of Equation (33) or Equation (34). The correction for the M*BG measurement will be quite small, but the correction for the M*BC measurement will be more significant--up to about 10% at the high M*BC concentrations.

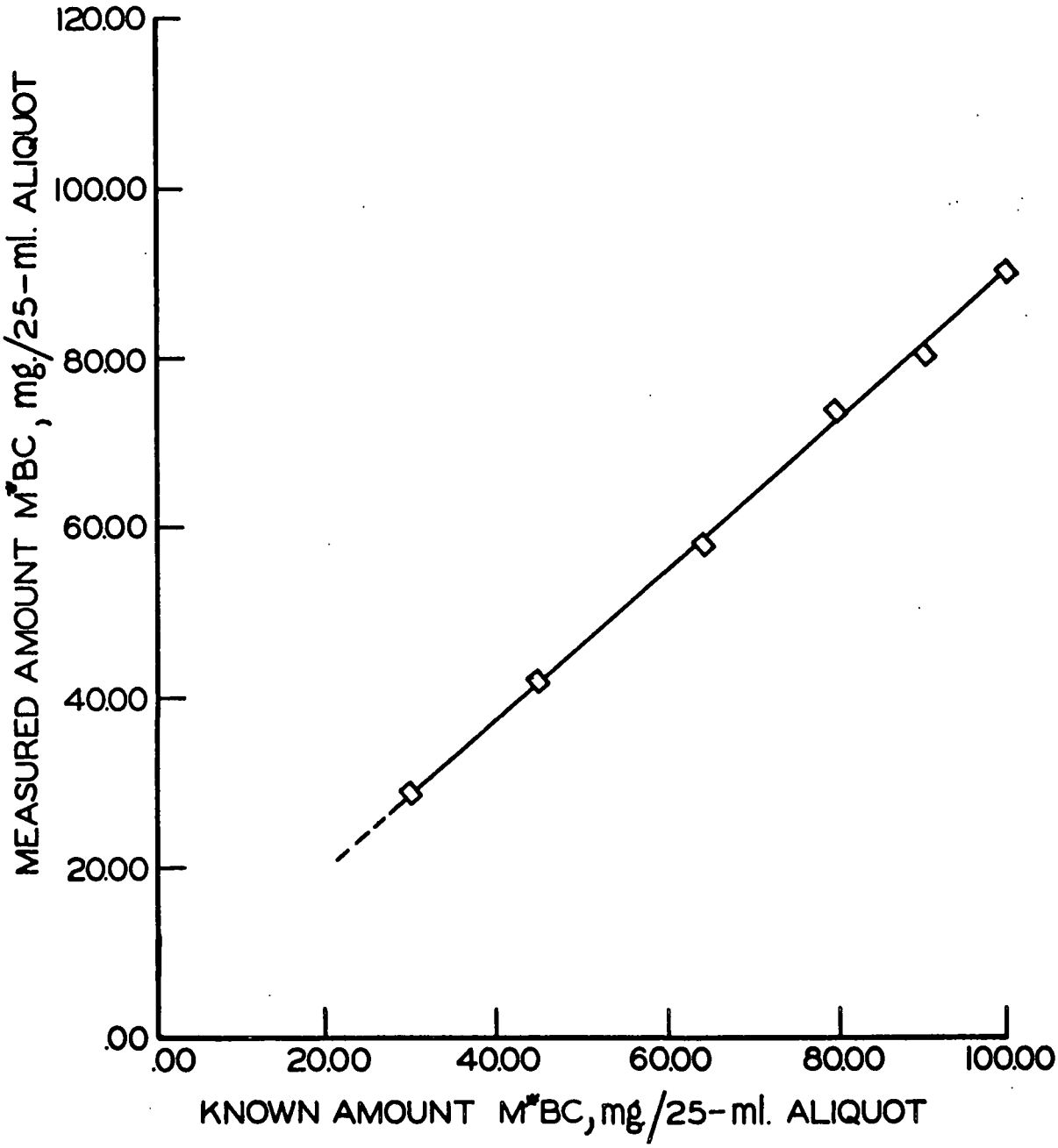

Figure 14. Accuracy of Measuring Methyl β-Cellobioside with the Reaction Mixture Analysis Procedure

APPENDIX V

STABILITY OF METHYL β-GLUCOSIDE AND METHYL β-CELLOBIOSIDE IN 2.5N NaOH AT ROOM TEMPERATURE

The stability of M*BG and M*BC in aqueous alkali at room temperature was determined by preparing such solutions and observing their optical rotation for a period of time. The M*BG solution was prepared by dissolving 0.789 g. M*BG in 25 ml. of 2.55N NaOH. A similar M*BC solution was prepared with 0.997 g. M*BC. Care was taken to protect both solutions from atmospheric oxygen. A two-decimeter polarimeter tube was filled with each solution, and the optical rotation of each was measured every few days for a period of 36 days. The solutions remained in the polarimeter tubes and at the prevailing laboratory temperature for the entire period. The temperature over this period fluctuated from about 16 to 26°C. The results are shown in Table IX. Regression analysis of the data shows virtually no dependence of observed rotation on reaction time for either M*BG or M*BC, indicating that both are quite stable to 2.5N NaOH under N_2-purged conditions at normal room temperatures.

TABLE IX

STABILITY OF METHYL β-GLUCOSIDE AND METHYL β-CELLOBIOSIDE IN 2.5N NaOH AT ROOM TEMPERATURE

Reaction Time, days	Me β-Glucoside Obs. Opt. Rotation, °	Me β-Cellobioside Obs. Opt. Rotation, °
2	-2.08	-1.66
5	-2.00	--
6	-2.00	-1.72
9	-2.05	-1.75
12	-1.99	-1.71
15	-2.04	-1.69
17	-2.03	-1.68
20	-2.06	-1.63
24	-2.06	-1.73
28	-2.03	-1.70
34	-2.01	-1.70
35	-2.06	-1.72
36	-2.03	-1.70

APPENDIX VI

HIGH TEMPERATURE STABILITY OF METHANOL IN N_2-PURGED 2N NaOH

The stability of aqueous methanol in highly alkaline solution at 167°C. was reasonably well ascertained by actual reaction of such solutions for up to 120 hours. An aqueous methanol solution in 2N NaOH was prepared and 55-ml. aliquots placed in the stainless steel reaction tubes. The tubes were purged with nitrogen, sealed, and placed in an oil bath at 167°C. Tubes were removed after varying reaction times and 50-ml. aliquots were analyzed for methanol concentration by the distillation procedure described in a previous section of this report. The data shown in Table X were obtained and subjected to linear regression analysis. No significant correlation was found between amount of methanol recovered and the reaction time. A plot of the data with a zero slope is given in Fig. 15.

TABLE X

STABILITY OF METHANOL IN N_2-PURGED 2N NaOH AT 167°C.

Reaction Time, hr.	Amount of Charged Methanol Recovered, %
0.0	97.5
2.0	97.3
4.5	105.5
8.0	98.5
14.5	100.3
24.0	100.1
35.5	102.1
45.0	97.1
48.0	100.4
61.0	98.5
72.0	95.5
82.0	100.8
94.0	100.8
106.0	100.6
120.0	104.5

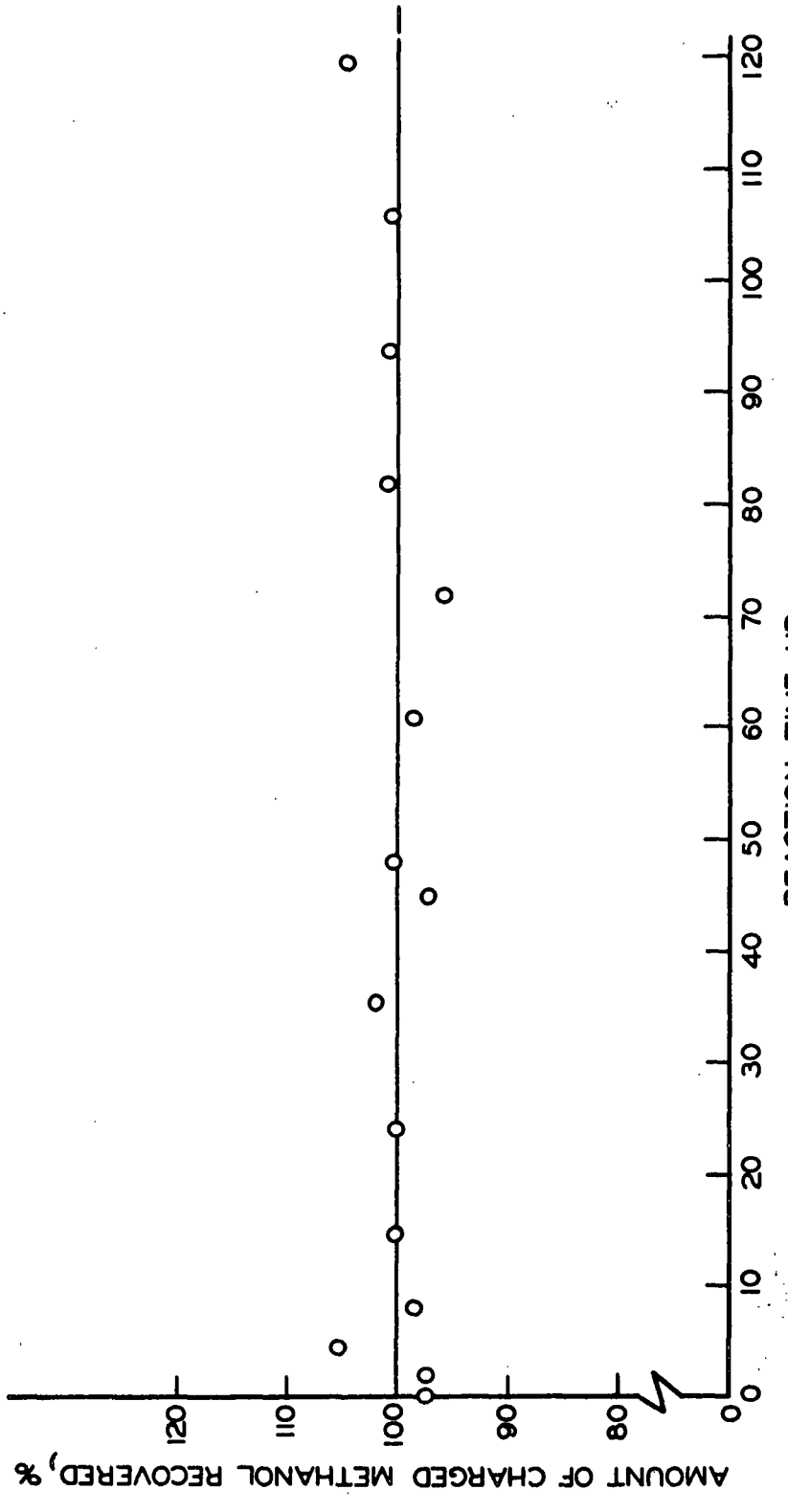

Figure 15. Stability of Methanol in N_2-Purged 2N NaOH at 167°C.

APPENDIX VII

RESULTS OF MASS SPECTROMETRIC ANALYSES

The mass spectrometric analysis work on the products from the reaction of methyl β-cellobioside in O-18 enriched aqueous alkali was done by Morgan-Schaffer Corporation, Montreal, Quebec, Canada. The results of the analysis of the O-18 enriched aqueous solution of the methanol were the following:

Compound	O-18 Content	O-16 Content
methanol	2.25%	97.75%
water	20.5%	79.5%

The results of the analysis of the glucoside are tabulated in Table XI, and results of the analysis of the D-glucose diethyl dithioacetal derivative are shown in Table XII.

TABLE XI

MASS SPECTRUM OF METHYL β-D-GLUCOSIDE FROM THE REACTION OF METHYL β-CELLOBIOSIDE IN H_2O^{18}-ENRICHED AQUEOUS ALKALI

m/e	Peak Height, cm.	Relative Intensity	m/e	Peak Height, cm.	Relative Intensity
15	5.22	9.2	72	1.420	2.50
17	1.32	2.3	73	30.500	54.00
18	5.85	10.3	74	30.500	54.00
19	4.66	8.2	75	6.410	11.30
27	3.51	6.2	76	0.350	0.60
28	4.70	8.3	77	0.050	0.00
29	7.24	12.8	79	1.400	2.40
30	1.00	1.7	85	2.660	4.70
31	10.57	18.7	86	1.100	1.90
32	0.63	1.1	87	2.780	4.90
33	4.56	8.0	88	0.340	0.60
39	1.70	3.0	89	0.620	1.00
41	3.52	6.2	97	2.490	4.40
42	4.45	7.8	98	2.430	4.30
43	9.57	16.9	99	0.810	1.40
44	4.17	7.3	103	1.420	2.50
45	8.98	15.9	104	0.910	1.60
46	0.46	0.8	116	1.430	2.50
47	0.83	1.4	117	0.250	0.40
52	1.15	2.0	121	0.530	0.90
53	0.51	0.9	131	3.850	6.80
54	0.68	1.2	132	0.210	0.30
55	4.23	7.5	133	0.250	0.40
56	6.94	12.3	144	0.772	1.36
57	22.30	39.5	145	0.810	1.43
58	1.50	2.6	161	0.011	0.01
59	4.15	7.3	162	0.010	0.01
60	56.40	100.0	163	0.556	0.98
61	30.80	54.6	164	0.038	0.06
62	0.82	1.4	165	0.012	0.02
69	3.71	6.5	193	0.026	0.04
70	3.14	5.5	194	0.002	0.00
71	12.30	21.8	195	0.005	0.01

TABLE XII

MASS SPECTRUM OF D-GLUCOSE DIETHYL DITHIOACETAL DERIVATIVE OF PRODUCT MBG FROM THE REACTION OF MBC IN H_2O^{18}-ENRICHED AQUEOUS ALKALI

m/e	Peak Height, cm.	Relative Intensity	m/e	Peak Height, cm.	Relative Intensity
15	2.40	7.6	87	2.10	6.6
17	1.54	4.8	88	0.35	1.1
18	4.95	15.6	89	2.10	6.6
19	1.82	5.7	91	1.23	3.8
26	1.50	4.7	97	1.03	3.2
27	7.35	23.3	101	0.71	2.2
28	3.68	11.6	102	0.40	1.2
29	12.03	38.1	103	4.16	13.1
30	1.22	3.8	104	6.64	21.0
31	6.76	21.4	105	14.37	45.5
32	1.73	5.4	106	2.42	7.6
34	1.10	3.4	107	9.58	30.3
35	1.53	4.8	108	0.70	2.2
39	1.04	3.2	109	1.10	3.4
41	2.93	9.2	115	1.13	3.5
42	1.89	5.9	117	1.90	6.0
43	8.11	25.7	127	1.22	3.8
44	2.84	9.0	129	0.71	2.2
45	7.25	22.0	133	1.39	4.4
46	2.09	6.6	134	0.21	0.6
47	8.77	27.8	135	31.54	100.0
48	1.17	3.7	136	9.01	28.5
55	2.39	7.5	137	3.43	10.8
56	1.13	3.5	138	0.86	2.7
57	3.36	10.6	145	1.33	4.2
58	1.46	4.6	146	0.22	0.6
59	4.78	15.1	147	0.35	1.1
60	3.63	11.5	163	1.86	5.8
61	9.50	30.1	164	0.26	0.8
62	4.92	15.5	165	0.24	0.7
63	2.68	8.4	177	1.02	3.2
69	1.51	4.7	178	0.15	0.4
71	1.95	6.1	179	0.14	0.4
72	0.80	2.5	193	0.36	1.1
73	5.12	16.2	207	1.27	4.0
74	1.40	4.4	208	0.17	0.5
75	9.31	29.5	209	0.12	0.3
76	2.65	8.4	225	0.300	0.95
77	4.81	15.2	226	0.050	0.15
78	0.70	2.2	227	0.047	0.14
79	1.02	3.2	228	0.020	0.06
83	0.83	2.6	286	0.246	0.77
85	3.58	11.3	287	0.034	0.10
86	0.53	1.6	288	0.028	0.08

APPENDIX VIII

KINETIC RESULTS OF METHYL β-D-GLUCOSIDE REACTION IN N_2-PURGED AQUEOUS ALKALI

The experimental data, intermediate results, and figures (Tables XIII-XXI and Fig. 16-23) which are supplementary to those included in the text of this dissertation are given in this appendix for the kinetic work involving the reaction of methyl β-D-glucoside. A sample calculation for obtaining the methanol concentration in a reaction product solution is also given here.

$$[\text{MeOH}] = \frac{(162.15)\ (\text{Abs}_{\text{meas}} - \text{Abs}_{o})\ (\text{Vol. dist. condensate})}{(\text{Molecular weight})(\text{Vol. of product sol'n distilled})}$$

The calculation for the 15-hr. mixture for Reaction (A) of methyl β-D-glucoside in 2.497N NaOH at 170.1°C. is

$$[\text{MeOH}] = \frac{(162.15)(0.224 - 0.00)(25.0\ \text{ml.})}{(32.04)(25.0\ \text{ml.})} = 1.13\ \mu\text{mole MeOH/ml.}$$

TABLE XIII

REACTION (A) OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 170.1°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.000	0.0	0.00
3.0	0.016	25.0	0.08
6.0	0.063	25.0	0.31
9.0	0.085	25.0	0.43
12.0	0.196	25.0	0.99
15.0	0.224	25.0	1.13
18.0	0.159	12.5	1.60
21.0	0.179	12.5	1.81
24.0	0.207	12.5	2.09
27.0	0.210	12.5	2.12

TABLE XIV

REACTION (B) OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.000	0.0	0.00
1.0	0.050	50.0	0.12
2.0	0.044	25.0	0.22
4.0	0.114	50.0	0.28
6.0	0.186	50.0	0.47
9.0	0.291	50.0	0.73
12.0	0.204	25.0	1.03
15.0	--	--	--
18.0	0.294	25.0	1.48

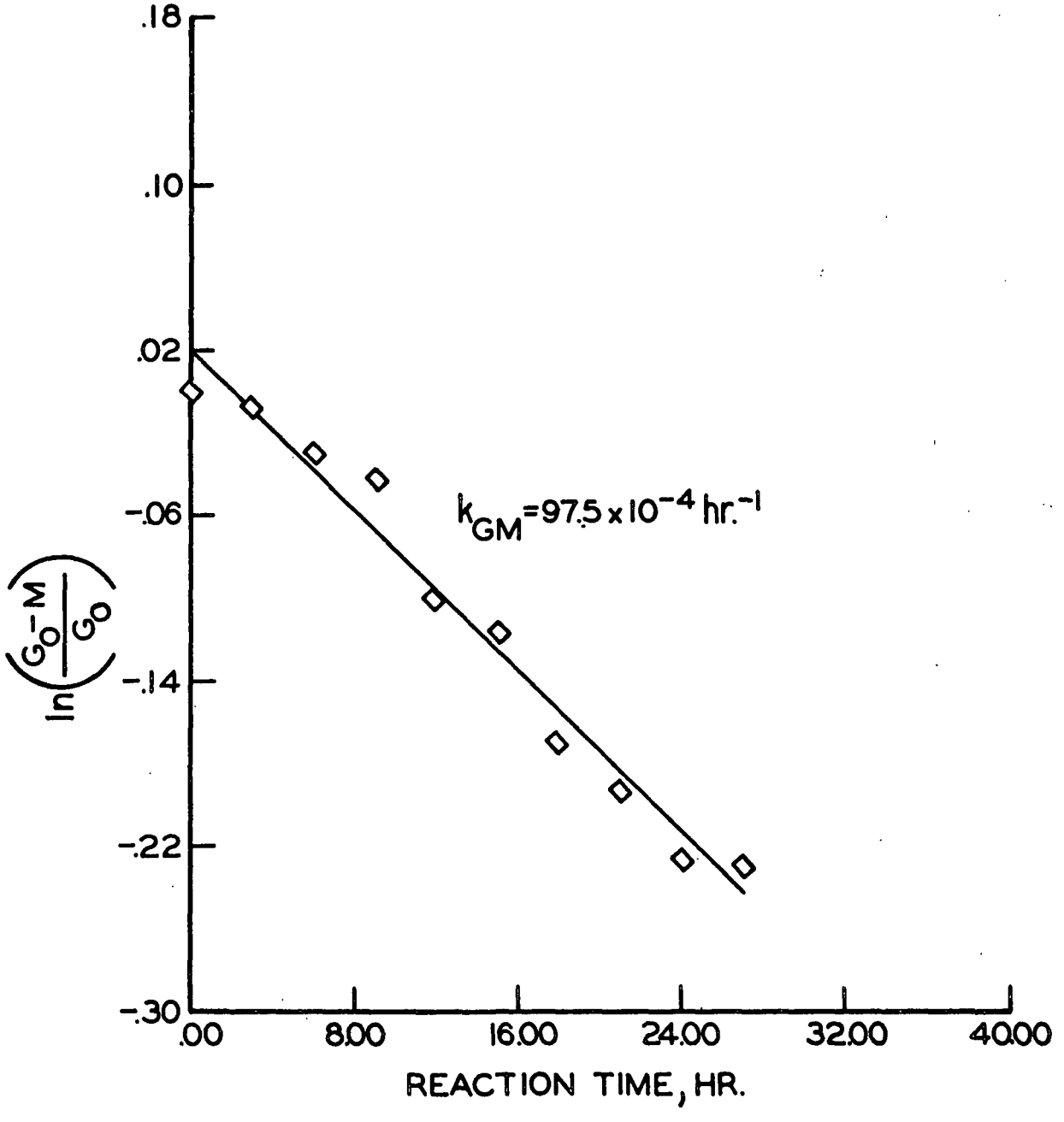

Figure 16. Reaction (A) of Methyl β-D-Glucoside in N_2-Purged 2.497N NaOH at 170.1°C. Methanol Data

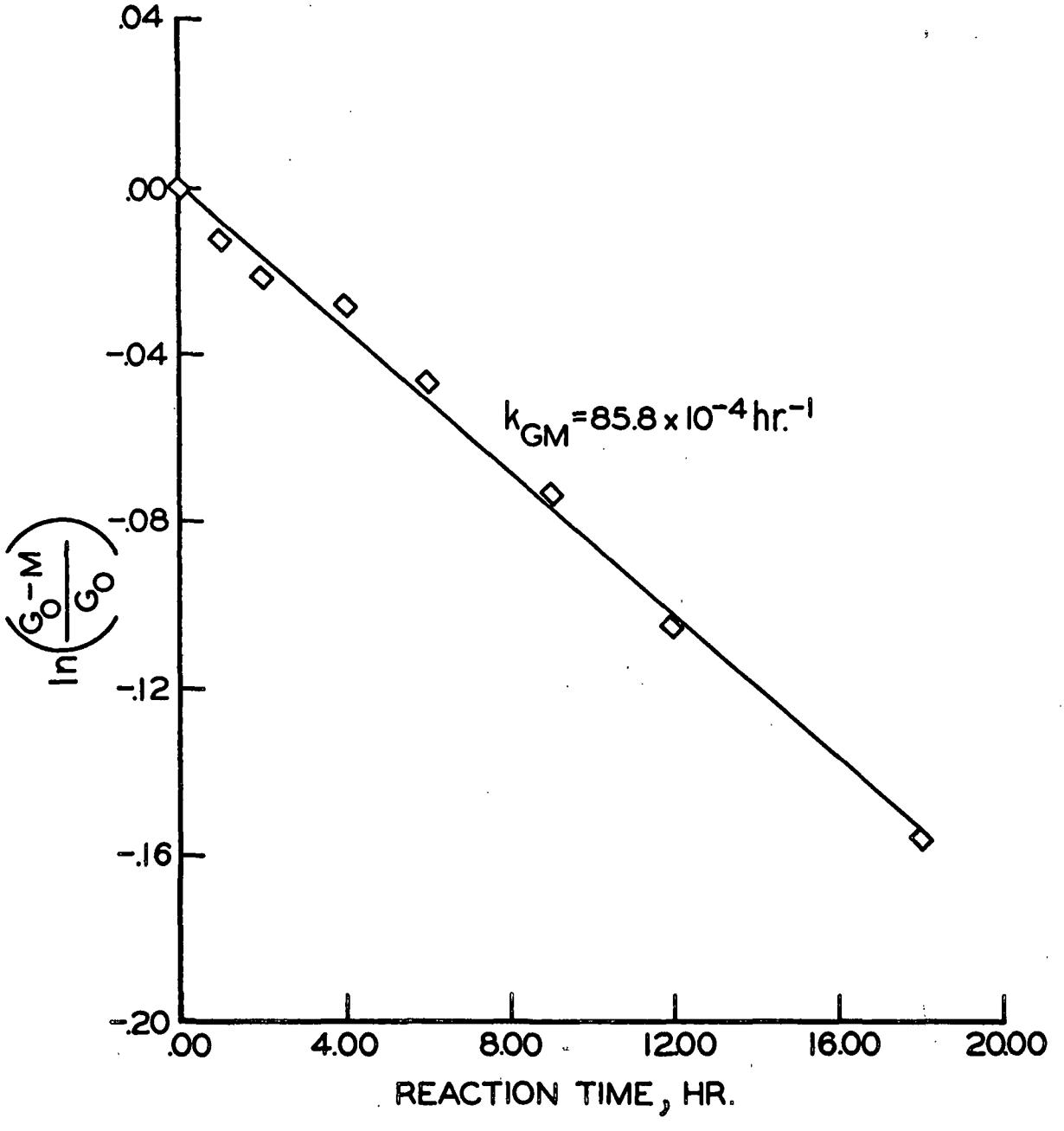

Figure 17. Reaction (B) of Methyl β-D-Glucoside in N_2-Purged 2.497N NaOH at 170.0°C. Methanol Data

TABLE XV

REACTION (C) OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	-0.003	25.0	0.00
6.0	0.094	25.0	0.49
12.0	0.184	25.0	0.94
18.0	0.259	25.0	1.32
24.0	0.322	25.0	1.64
36.0	0.444	25.0	2.26
48.0	0.536	25.0	2.72

TABLE XVI

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 159.2°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.000	25.0	0.00
2.0	0.015	25.0	0.07
5.0	0.030	25.0	0.15
8.0	0.039	25.0	0.19
12.0	0.065	25.0	0.32
21.0	0.119	25.0	0.60
27.0	0.165	25.0	0.83
34.2	0.174	25.0	0.88
42.0	0.201	25.0	1.01
50.5	0.241	25.0	1.21
60.0	0.276	25.0	1.39

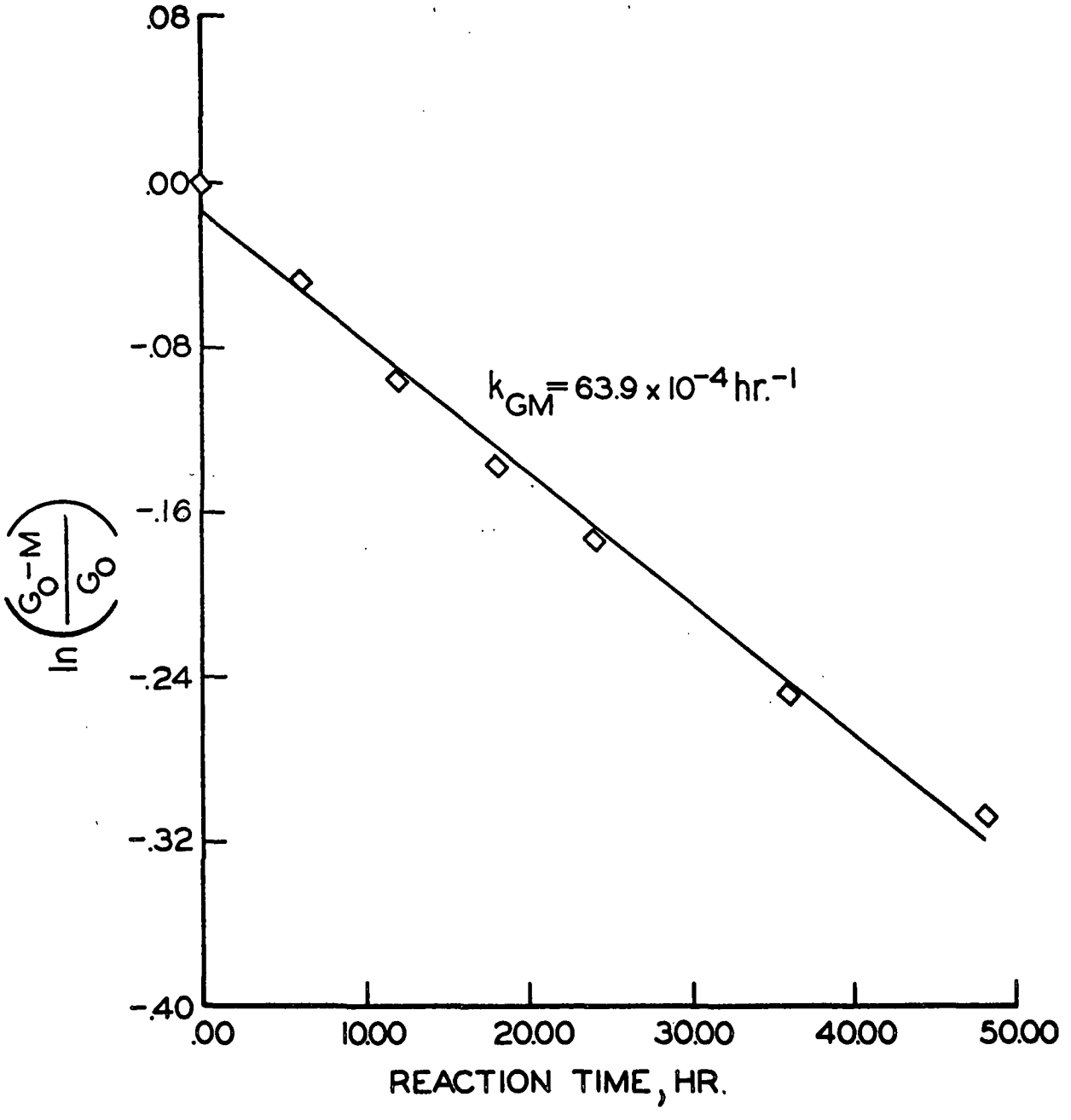

Figure 18. Reaction (C) of Methyl β-D-Glucoside in N_2-Purged 2.497N NaOH at 170.0°C. Methanol Data

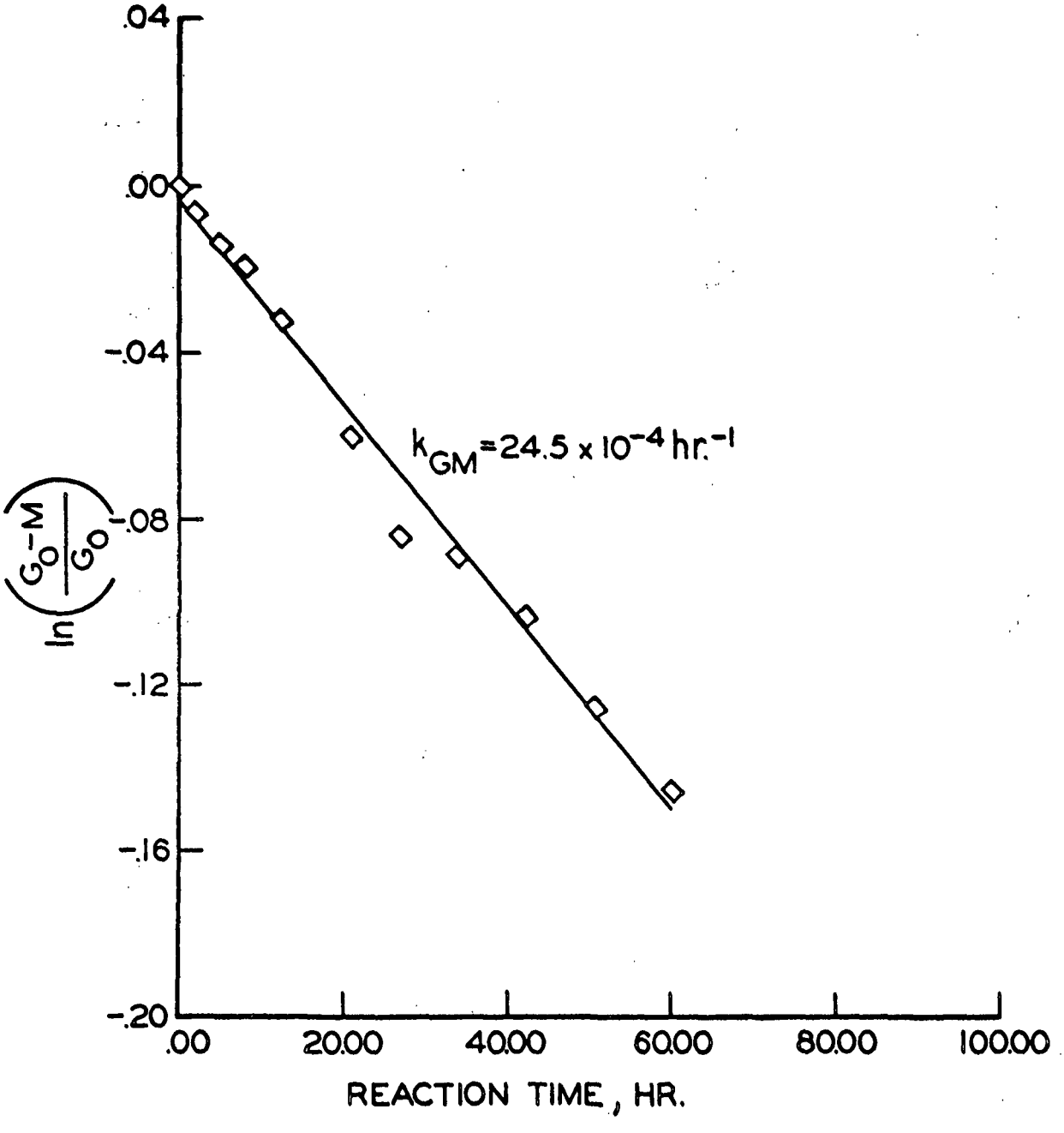

Figure 19. Reaction of Methyl β-D-Glucoside in N_2-Purged 2.497N NaOH at 159.2°C. Methanol Data

TABLE XVII

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 150.3°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.018	25.0	0.00
20.0	0.062	25.0	0.22
40.0	0.103	25.0	0.43
62.0	0.144	25.0	0.63
120.0	0.259	25.0	1.21
144.0	0.320	25.0	1.52

TABLE XVIII

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 1.00N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.007	25.0	0.00
11.0	0.108	25.0	0.51
22.0	0.207	25.0	1.01
33.0	0.284	25.0	1.40
44.0	0.376	25.0	1.86
55.0	0.445	25.0	2.21
77.0	0.583	25.0	2.91
88.0	0.643	25.0	3.21
99.0	0.698	25.0	3.49

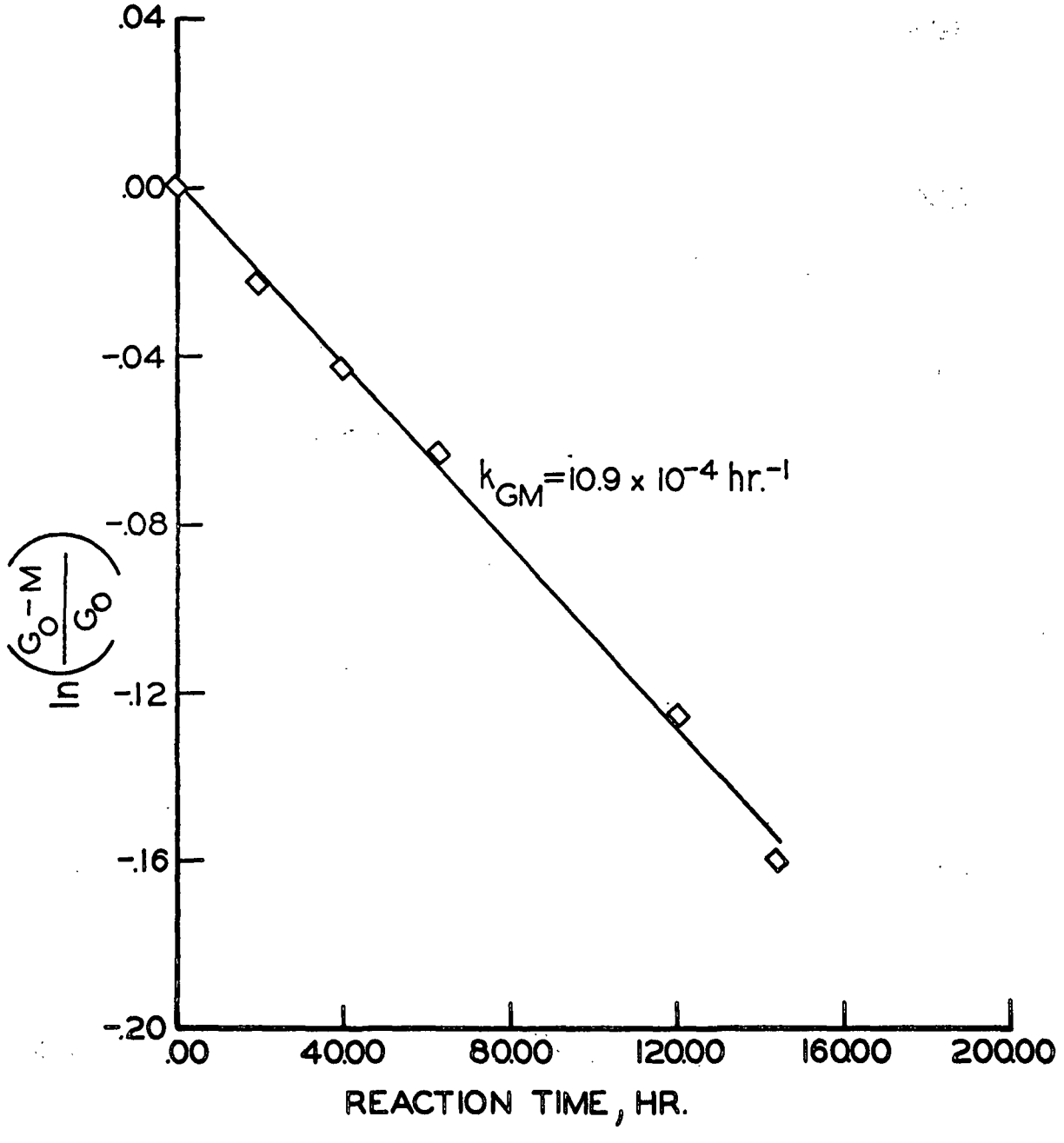

Figure 20. Reaction of Methyl β-D-Glucoside in N_2-Purged 2.497N NaOH at 150.3°C. Methanol Data[2]

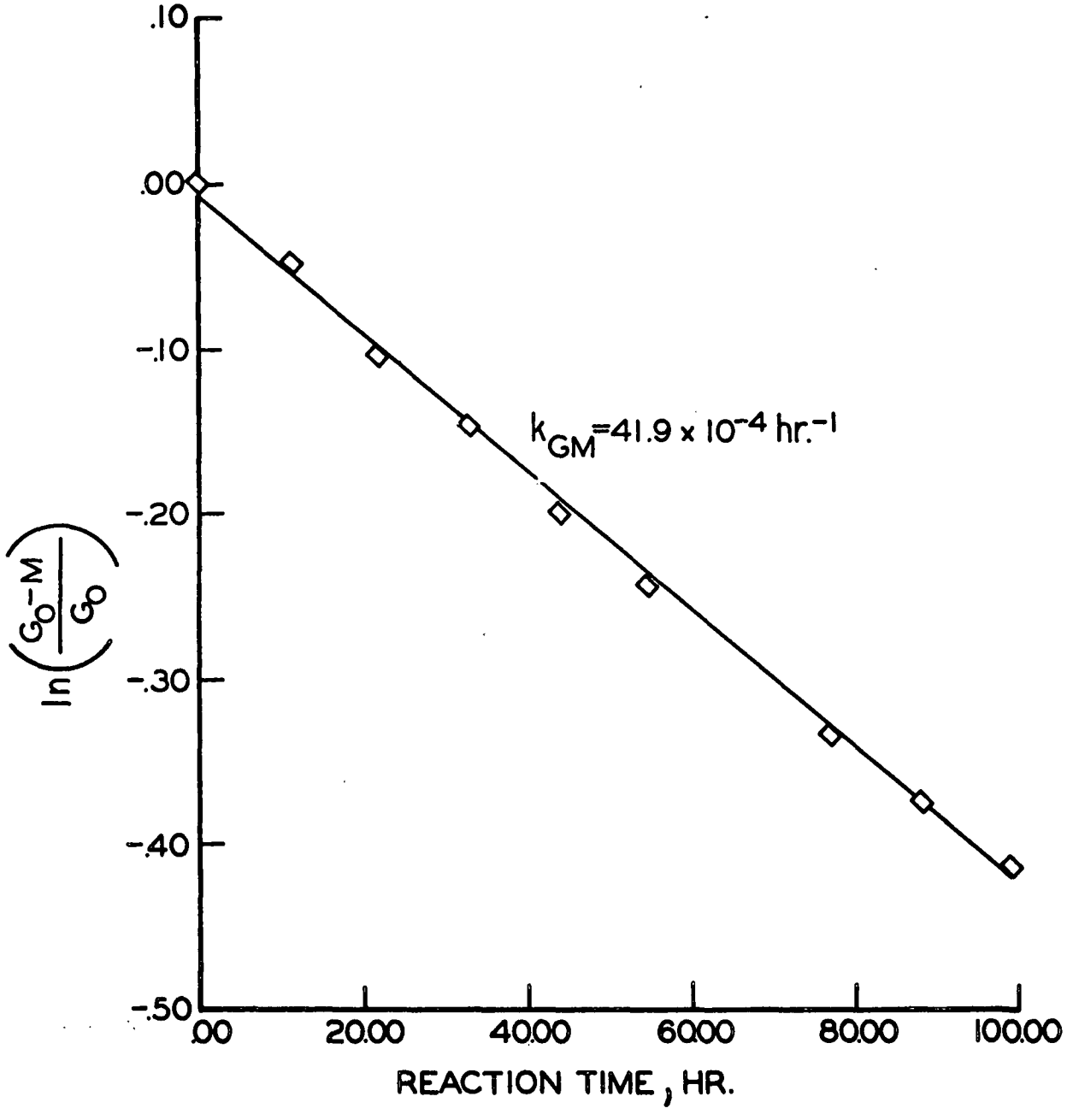

Figure 21. Reaction of Methyl β-D-Glucoside in N_2-Purged 1.00N NaOH at 170.0°C. Methanol Data

TABLE XIX

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 2.497N NaOH AT 139.9°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Radiochemical Data		
			Scales	Partial Scale	Count Time, min.
0.0	0.008	25.0	489	87	10.00
36.0	0.043	25.0	799	238	16.50
72.0	0.062	25.0	337	111	7.00
108.0	0.090	25.0	823	75	17.00
216.0	0.168	25.0	456	147	10.00
252.0	0.186	25.0	457	175	10.00
276.0	0.203	25.0	1135	241	25.00

Reaction Time, hr.	Unreacted Glucoside, μmole/ml.	Methanol, μmole/ml.	Sum, μmole/ml.
0.0	10.35	0.00	10.35
36.0	10.25	0.17	10.43
72.0	10.19	0.27	10.46
108.0	10.24	0.41	10.65
216.0	9.65	0.80	10.46
252.0	9.67	0.90	10.57
276.0	9.60	0.98	10.59

TABLE XX

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 0.359N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.002	25.0	0.00
4.0	0.024	25.0	0.11
9.0	0.058	25.0	0.28
22.0	0.117	25.0	0.58
30.0	0.154	25.0	0.76
39.0	0.188	25.0	0.94
49.0	0.230	25.0	1.15
57.0	0.263	25.0	1.32
67.0	0.308	25.0	1.54

TABLE XXI

REACTION OF METHYL β-D-GLUCOSIDE IN N_2-PURGED 0.101N NaOH AT 170.3°C.

Reaction Time, hr.	Absorbance	Aliquot Distilled, ml.	Methanol, μmole/ml.
0.0	0.003	25.0	0.00
24.5	0.053	25.0	0.25
48.0	0.106	25.0	0.52
72.0	0.145	25.0	0.71
85.0	0.165	25.0	0.81
97.0	0.197	25.0	0.98
132.0	0.252	25.0	1.26
145.0	0.279	25.0	1.39
168.0	0.318	25.0	1.59

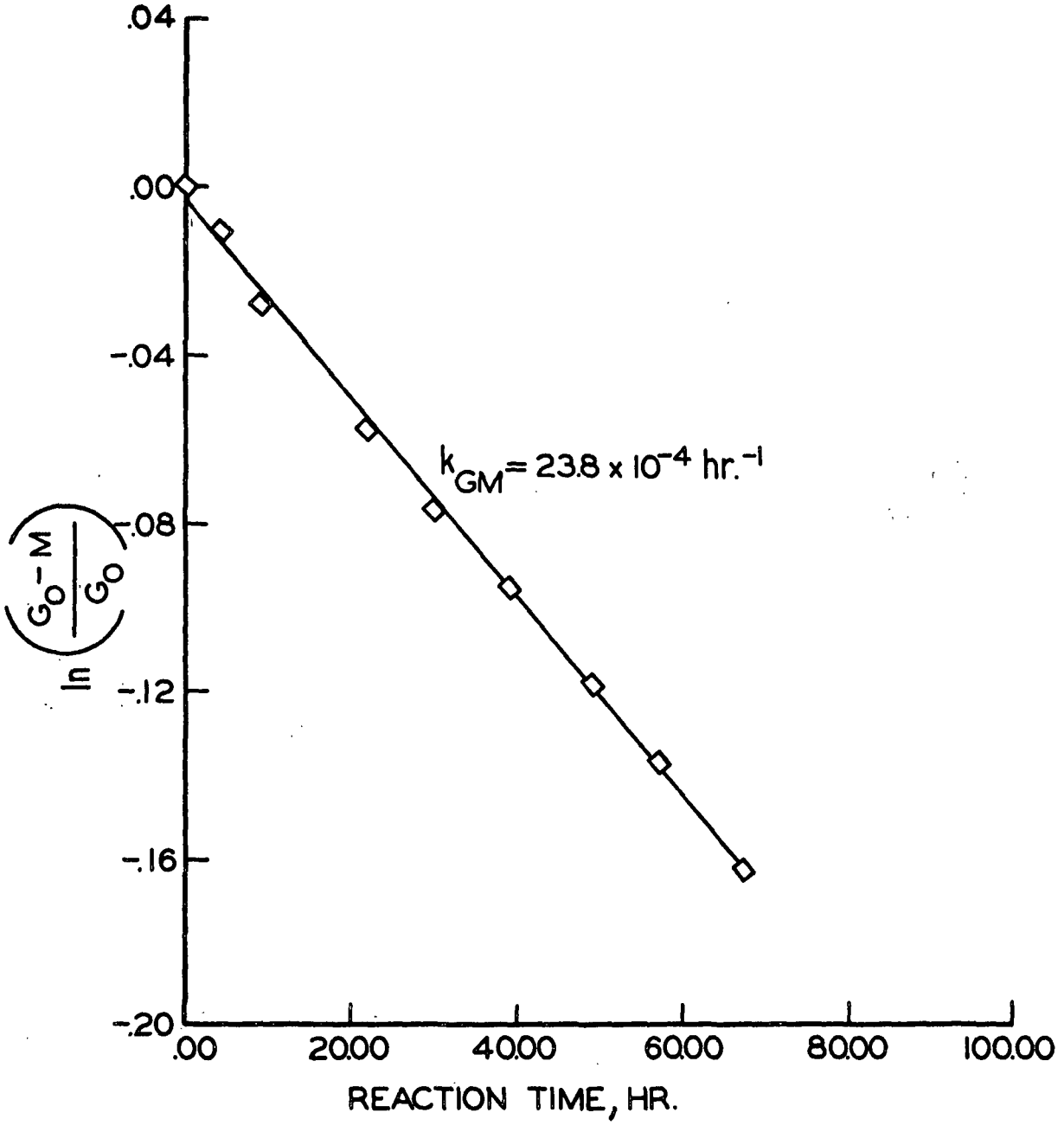

Figure 22. Reaction of Methyl β-D-Glucoside in N_2-Purged 0.359N NaOH at 170.0°C. Methanol Data[2]

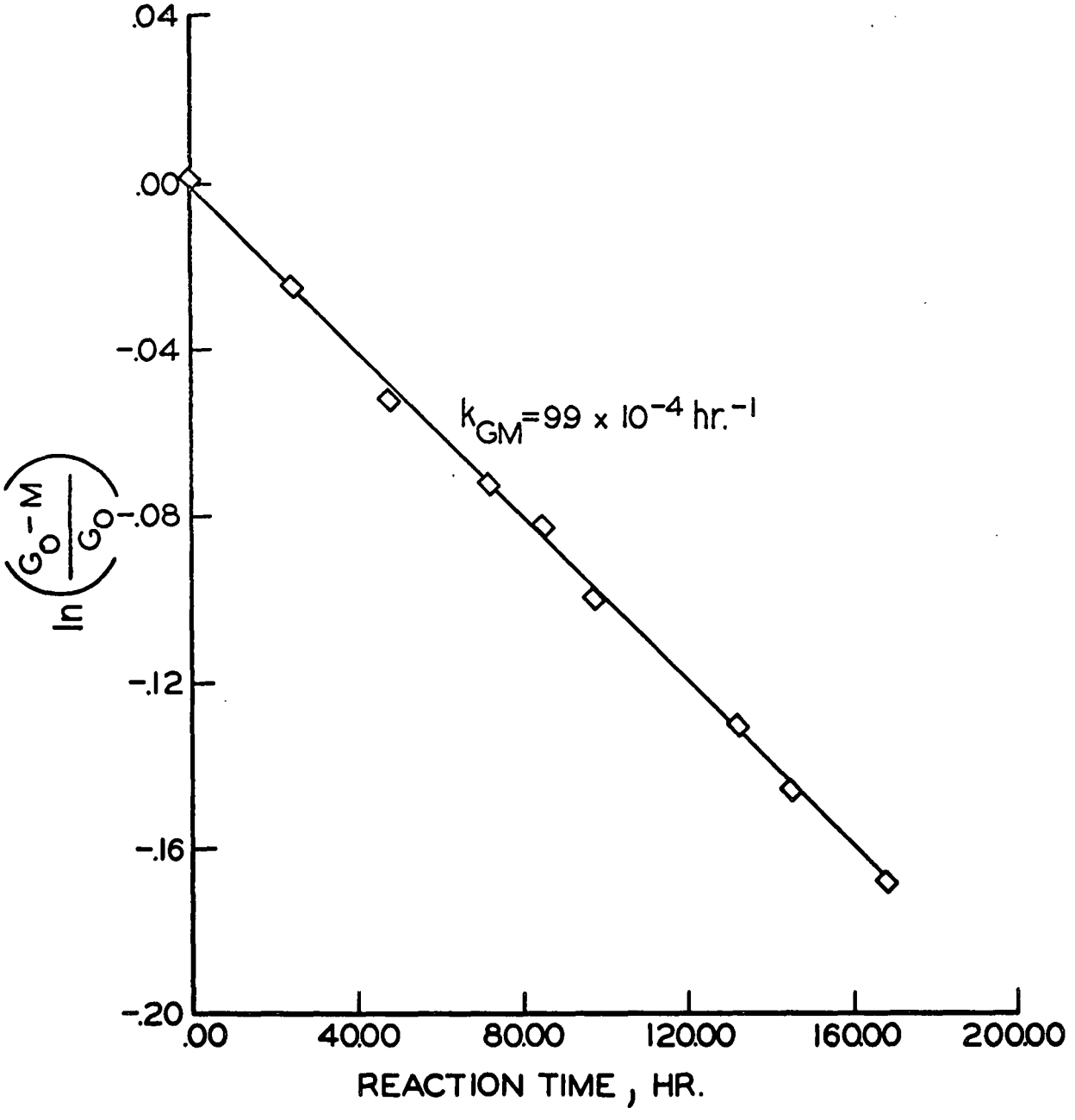

Figure 23. Reaction of Methyl β-D-Glucoside in N_2-Purged 0.101N NaOH at 170.3°C. Methanol Data

APPENDIX IX

KINETIC RESULTS OF METHYL β-CELLOBIOSIDE REACTION IN N_2-PURGED AQUEOUS ALKALI

The experimental data, calculated results, and figures (Tables XXII-XXX and Fig. 24-46) which are supplementary to those included in the text of this dissertation are given in this appendix for the kinetic work involving the reaction of methyl β-cellobioside. Sample calculations are described below.

The methanol concentration in a reaction solution is calculated as described in Appendix VIII. A sample calculation for obtaining the unreacted cellobioside concentration in a reaction solution is as follows:

$$C_{meas} = \frac{\left(\frac{\text{Total Radioactive Counts}}{\text{counting time}}\right) - \left(\text{Background Count}\right)}{(V_r)\left(\text{Efficiency Factor}\right)\left(\text{Specific Activity}\right)\left(\text{Molecular Weight}\right)\left(\text{Fraction of Total Aliquot Spotted}\right)\left(\text{Vol. of Reaction Solution Aliquot}\right)}$$

For the zero-reaction-time sample of the Reaction (A) of methyl β-cellobioside in 2.497N NaOH at 170.1°C., this calculation is

$$C_{meas} = \frac{\left[\frac{(2872)(256) + 103}{29.25}\right] - 160}{(.828)(.98)(2790)(356.3)(250/5000)(50)}$$

$$C_{meas} = 12.35 \text{ μmole/ml.}$$

and

$$C_{actual} = (1.1428)(12.35) - 0.3023 = 13.85 \text{ μmole/ml.}$$

Methyl β-D-glucoside concentrations are calculated from the radiochemical data in a similar manner. It may be noted from the above calculation that a "scale" corresponds to 256 counts.

TABLE XXII

REACTION (A) OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 170.1°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	0.414	12.00	13	125	29.25	2872	103
3.0	0.456	21.25	230	159	--	--	--
6.0	0.540	--	--	--	24.25	1914	43
9.0	0.550	21.50	404	79	16.25	1171	171
12.0	0.612	20.00	484	200	13.00	865	236
15.0	0.674	--	--	--	13.50	829	207
18.0	--	15.00	524	190	--	--	--
21.0	0.798	16.50	599	127	21.00	1009	91
27.0	0.888	13.50	610	111	--	--	--

Background count = 160 cts./min.
Distillation aliquot = 50 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.08	13.85	13.93
3.0	0.20	1.32	--	--
6.0	0.63	--	11.05	--
9.0	0.68	2.34	10.06	13.09
12.0	0.99	3.03	9.26	13.30
15.0	1.31	--	8.52	--
18.0	--	4.40	--	--
21.0	1.93	4.58	6.57	13.10
27.0	2.39	5.71	--	--

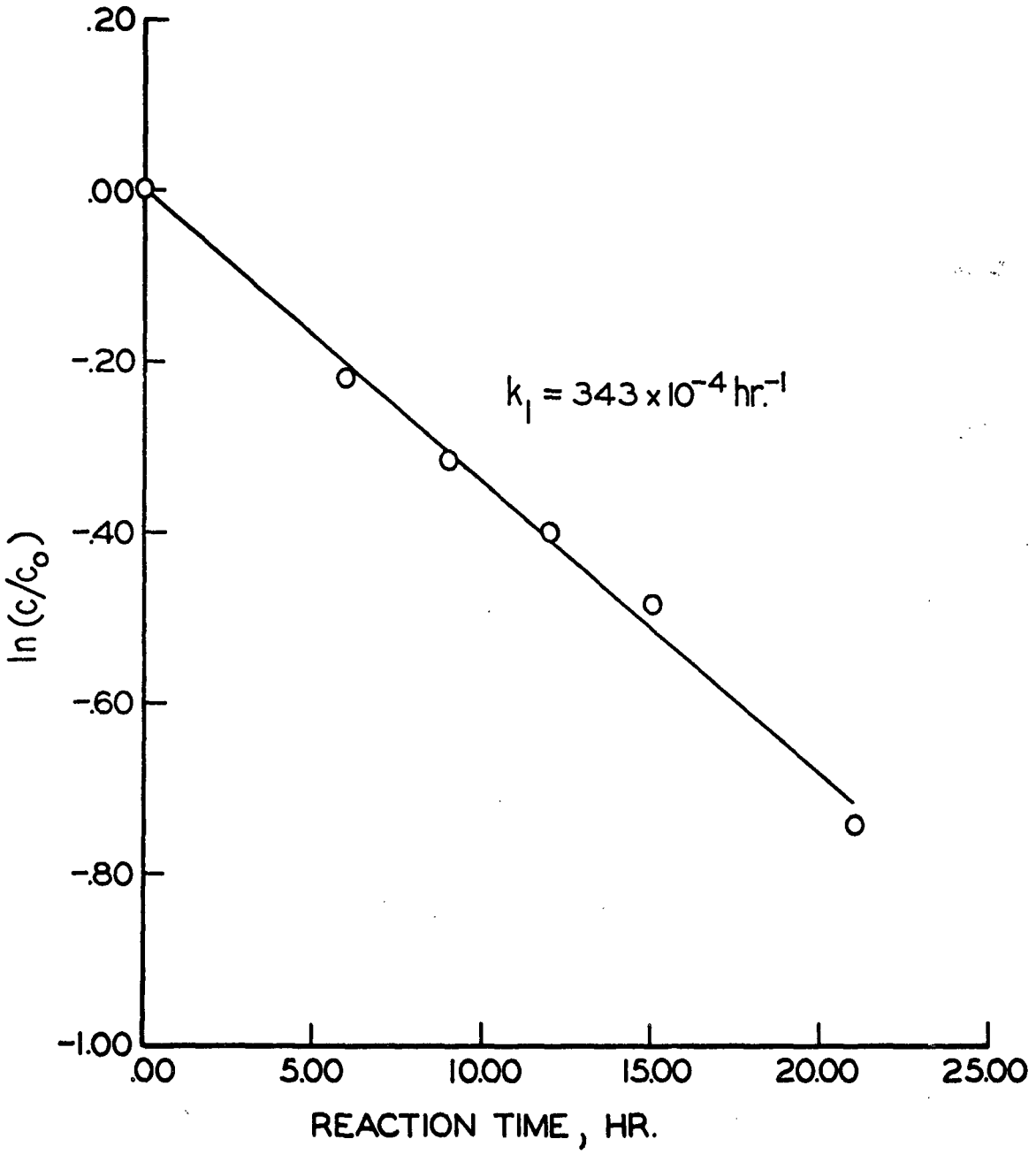

Figure 24. Reaction (A) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.1°C. Overall Degradation of Methyl β-Cellobioside

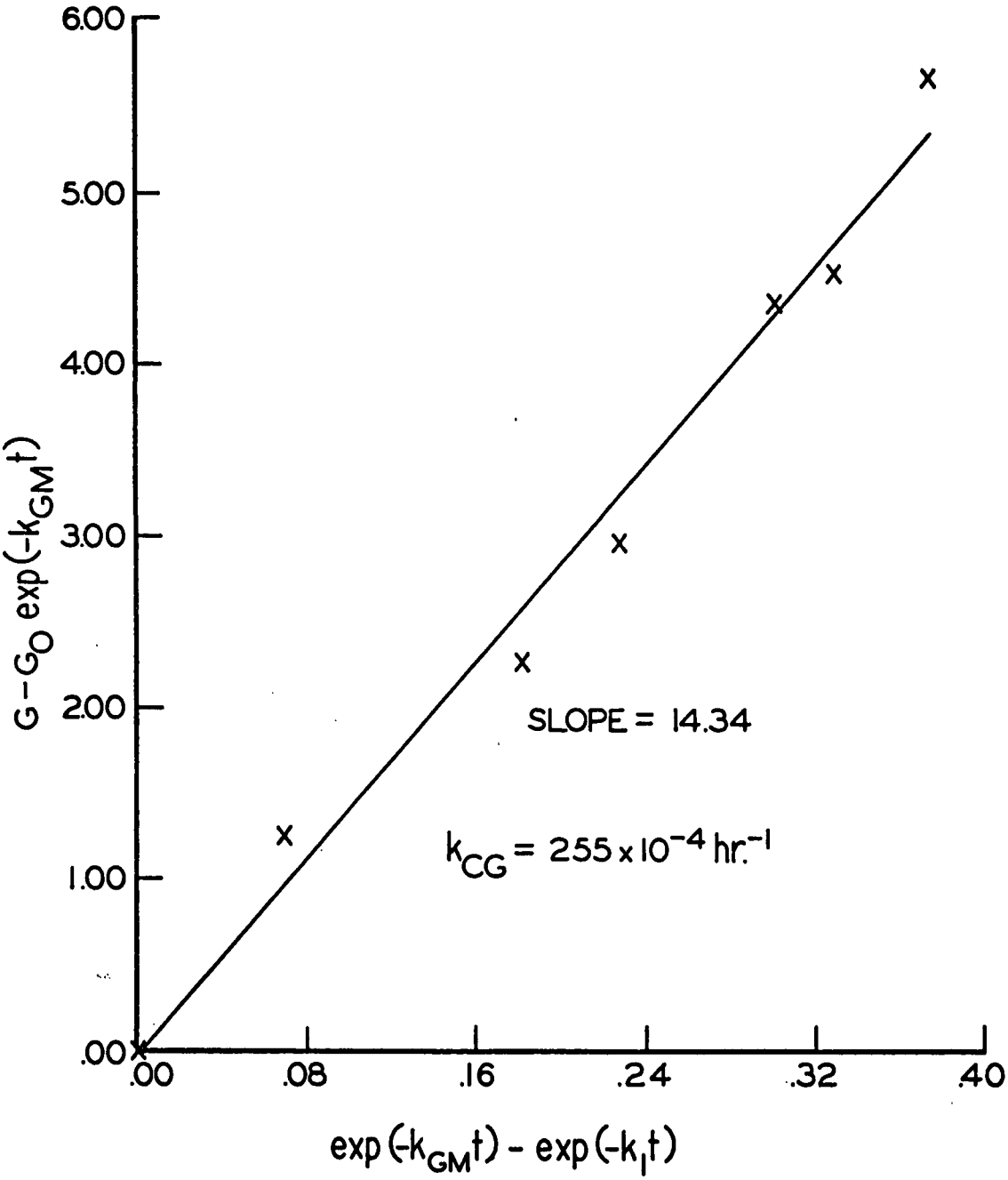

Figure 25. Reaction (A) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.1°C. Generation of Methyl β-D-Glucoside

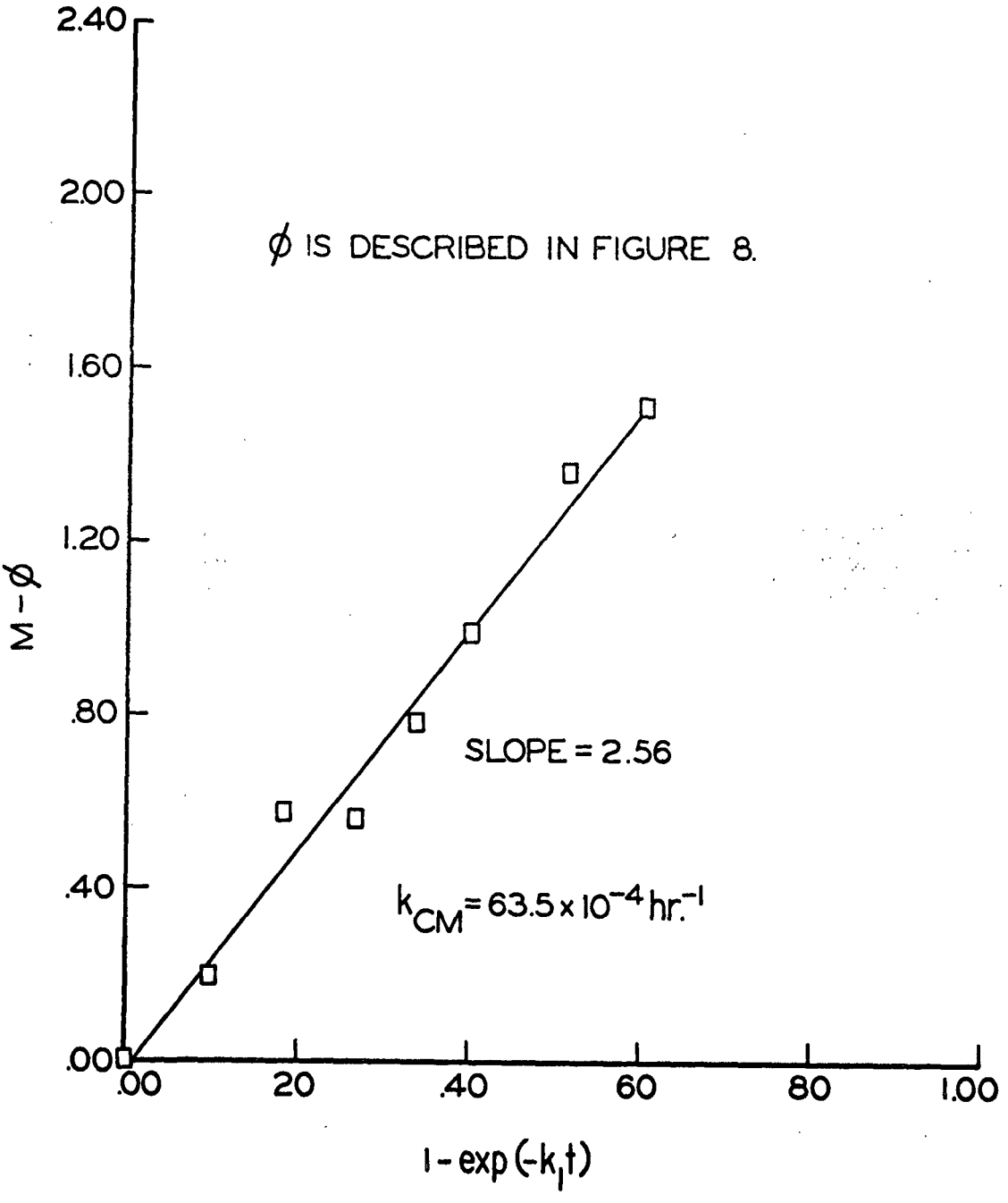

Figure 26. Reaction (A) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.1°C. Generation of Methanol

TABLE XXIII

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside		
		Time, min.	Scales	Part Scale
0.0	0.000	27.50	18	310
1.0	0.014	9.50	29	0
2.0	0.021	18.50	96	135
3.0	0.045	17.00	122	40
4.0	0.048	--	--	--
6.0	0.077	19.50	261	100
8.0	0.113	22.00	370	155
10.0	0.120	16.50	343	175
13.0	0.193	16.00	419	169
16.0	0.213	20.50	625	15

Background count = 157 cts./min.
Difficulty was experienced in obtaining good cellobioside data during this particular series, and the average k_1 value for the reaction series (A) and (C) was fed into the calculations for this (B) series. All three series were performed under the same conditions. The resulting values for k_{CG} and k_{CM} were as expected and they are believed to be valid results.
Distillation aliquot = 50 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.
0.0	0.00	0.02
1.0	0.07	0.32
2.0	0.10	0.60
3.0	0.22	0.85
4.0	0.24	--
6.0	0.38	1.65
8.0	0.57	2.09
10.0	0.60	2.60
13.0	0.97	3.29
16.0	1.07	3.83

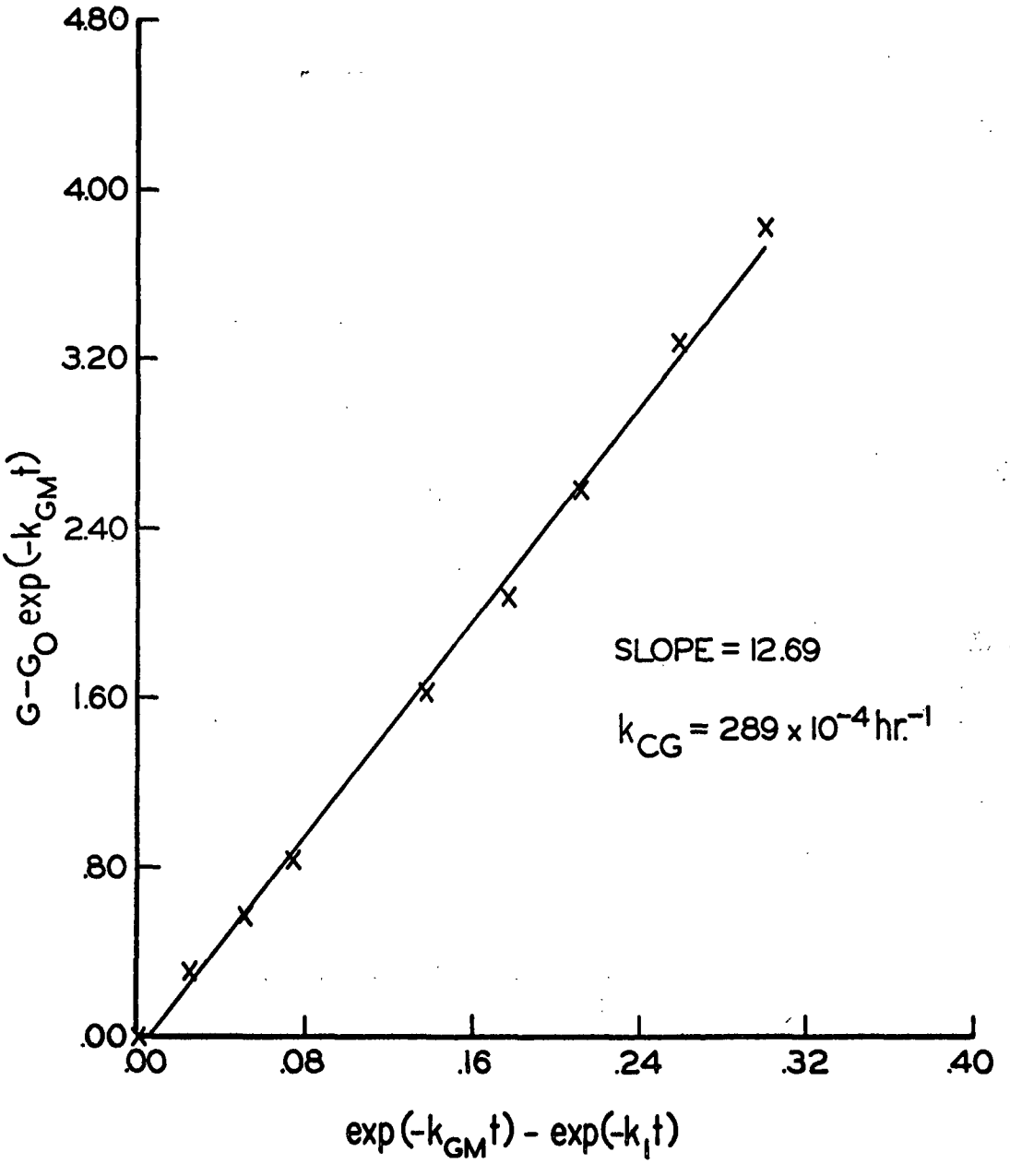

Figure 27. Reaction (B) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.0°C. Generation of Methyl β-D-Glucoside

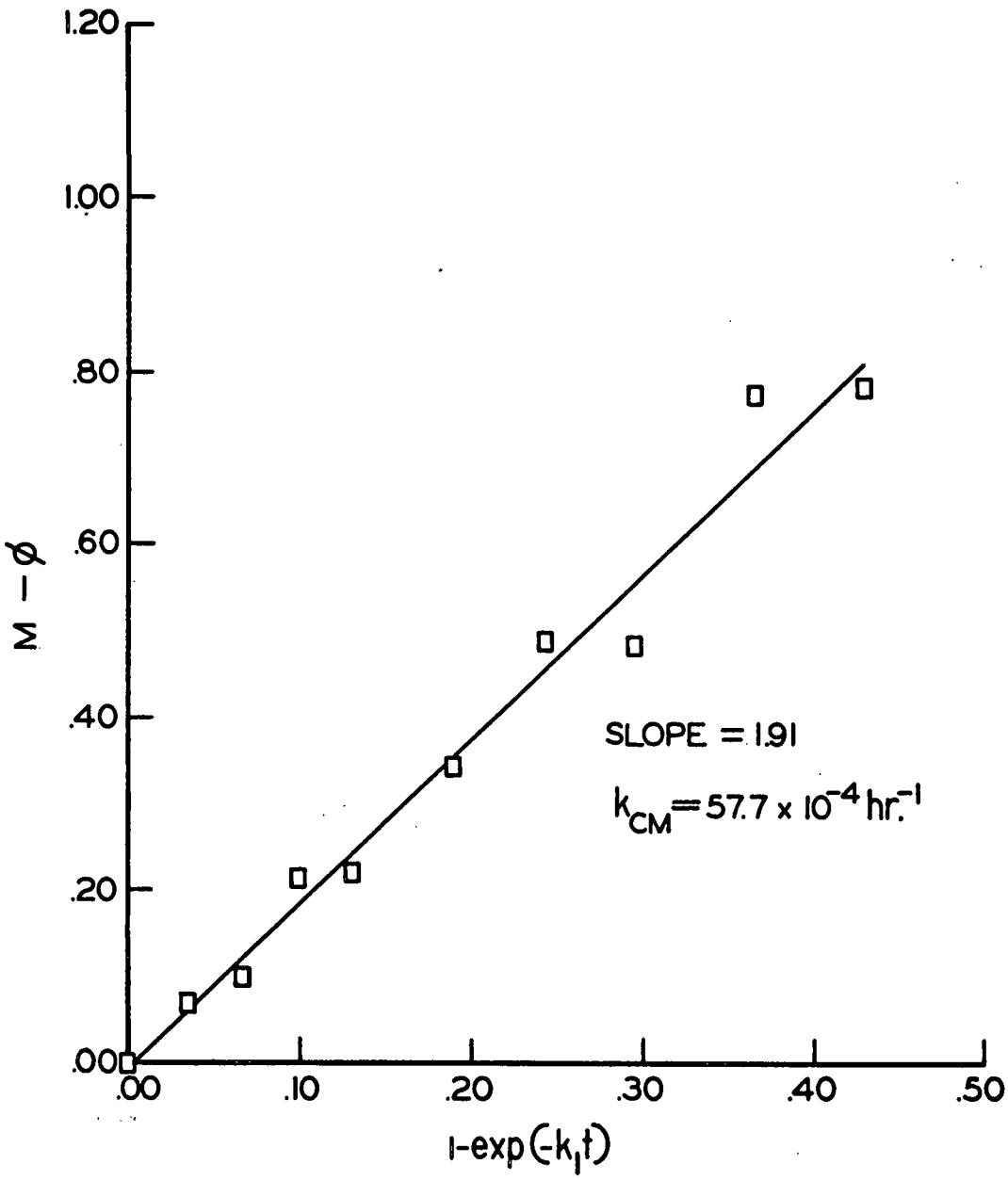

Figure 28. Reaction (B) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.0°C. Generation of Methanol

TABLE XXIV

REACTION (C) OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	-0.009	9.00	6	112	9.02	408	192
3.0	0.034	9.00	44	221	7.00	275	79
6.0	0.078	9.00	74	233	9.00	347	58
12.0	0.163	16.00	228	55	14.00	433	127
15.0	0.204	9.00	150	15	8.00	230	59
21.0	0.298	10.00	206	235	10.00	214	199
24.0	0.328	13.00	294	123	10.00	205	51

Background count = 153 cts./min.
Distillation aliquot = 25 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.04	12.67	12.71
3.0	0.21	1.13	10.93	12.29
6.0	0.44	1.99	10.71	13.15
12.0	0.87	3.51	8.50	12.88
15.0	1.07	4.12	7.87	13.07
21.0	1.55	5.15	5.75	12.46
24.0	1.70	5.65	5.47	12.83

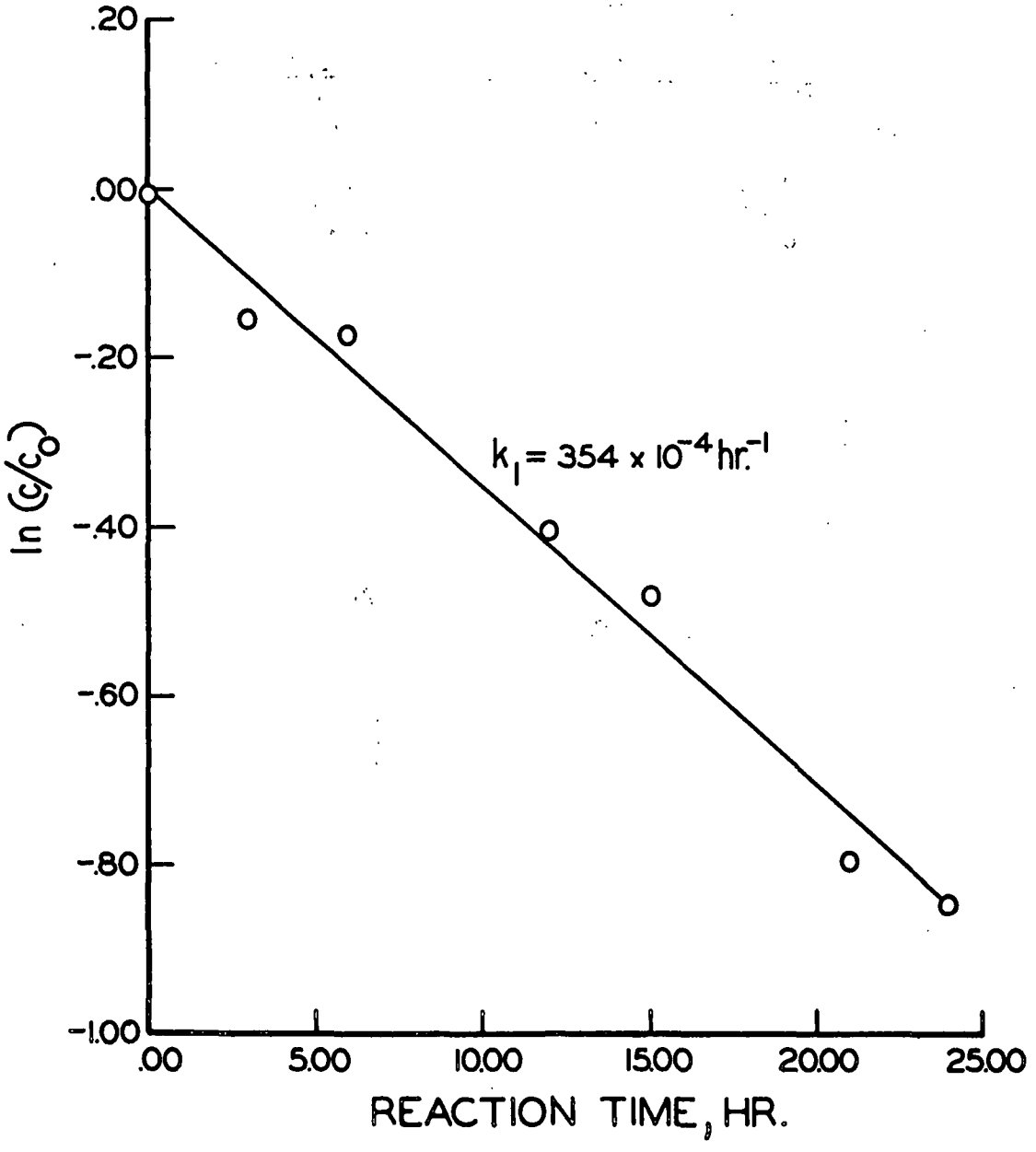

Figure 29. Reaction (C) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.0°C. Overall Degradation of Methyl β-Cellobioside

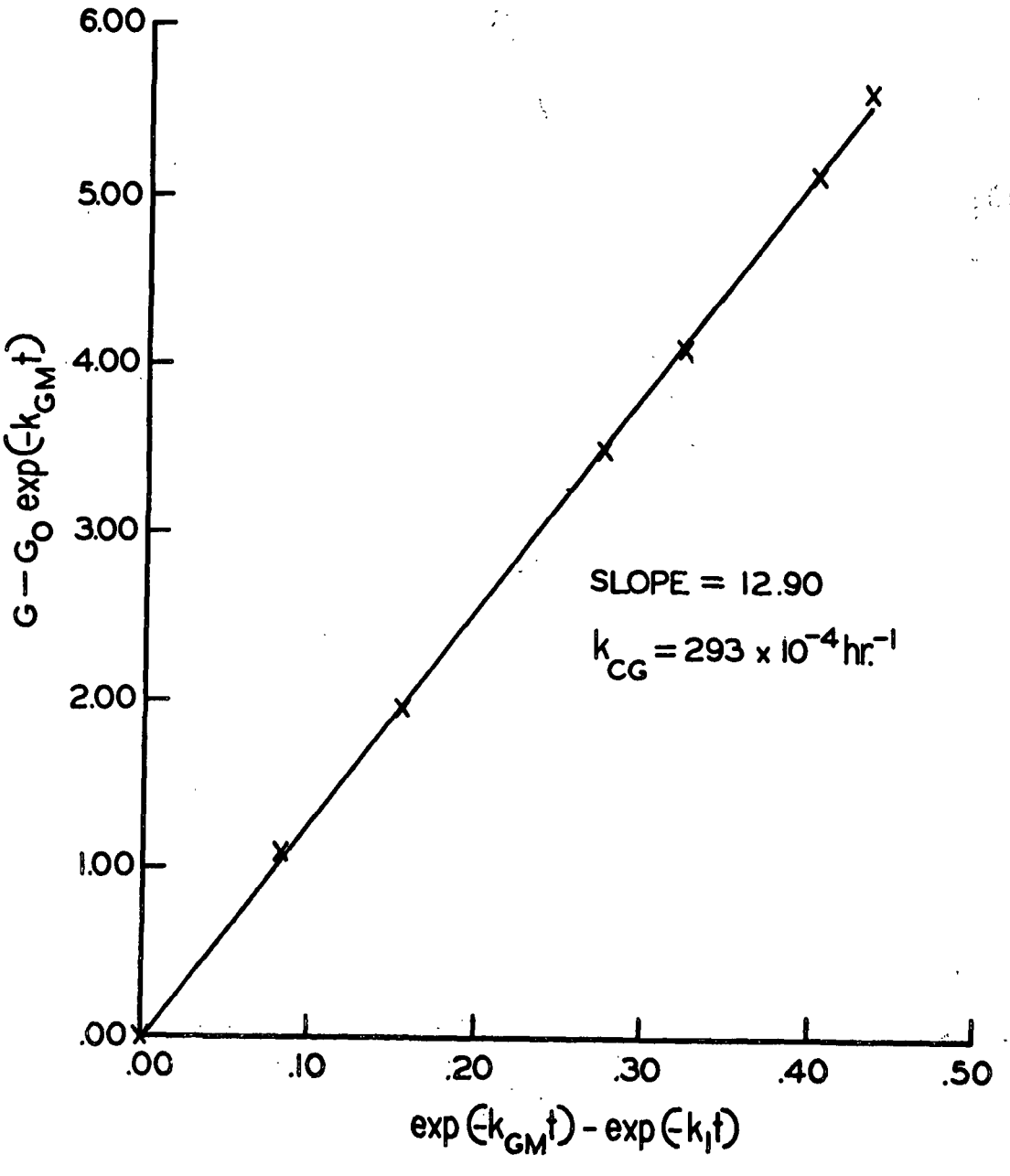

Figure 30. Reaction (C) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.0°C. Generation of Methyl β-D-Glucoside

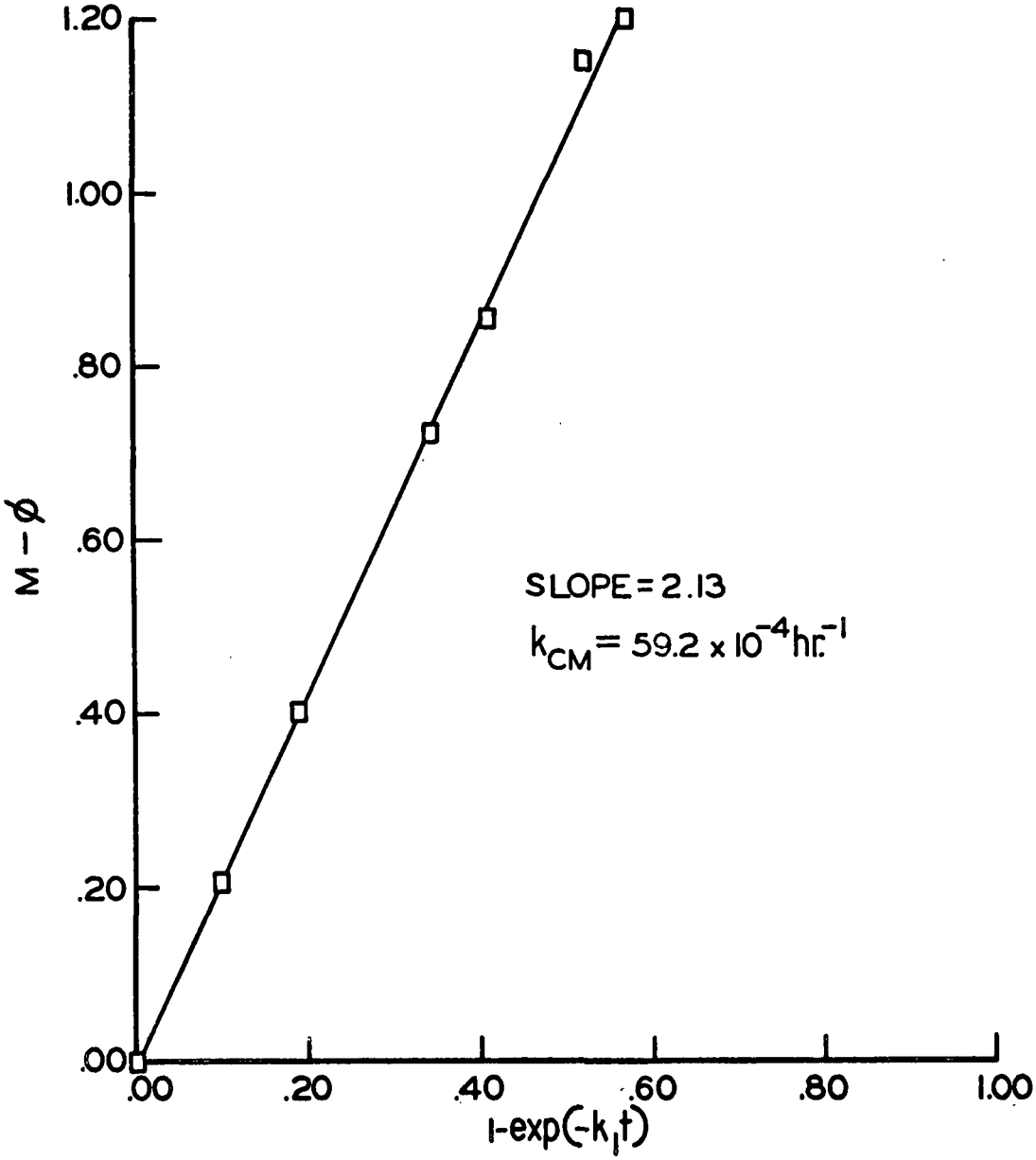

Figure 31. Reaction (C) of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 170.0 °C. Generation of Methanol

TABLE XXV

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 159.2°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	0.205	26.50	24	59	9.00	402	115
2.0	0.215	8.50	13	165	11.00	483	59
5.0	0.226	9.00	27	213	7.00	297	105
8.0	0.247	14.20	59	151	9.00	375	100
12.0	0.258	13.40	69	227	8.00	322	83
16.0	0.276	9.00	68	220	--	--	--
21.0	0.301	10.00	94	140	7.10	236	195
27.0	0.344	21.00	260	47	8.00	270	11
34.2	0.382	7.00	100	63	10.00	285	173
42.0	0.428	10.30	152	227	10.00	278	107
50.5	0.453	9.00	162	15	12.00	285	183

Background count = 150 cts./min.
Distillation aliquot = 25 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.10	12.50	12.60
2.0	0.05	0.27	12.27	12.60
5.0	0.10	0.65	11.85	12.61
8.0	0.21	0.93	11.62	12.78
12.0	0.26	1.20	11.21	12.68
16.0	0.35	1.82	--	--
21.0	0.48	2.28	9.20	11.97
27.0	0.70	3.03	9.32	13.05
34.2	0.89	3.52	7.81	12.24
42.0	1.12	3.66	7.60	12.39
50.5	1.25	4.47	6.43	12.16

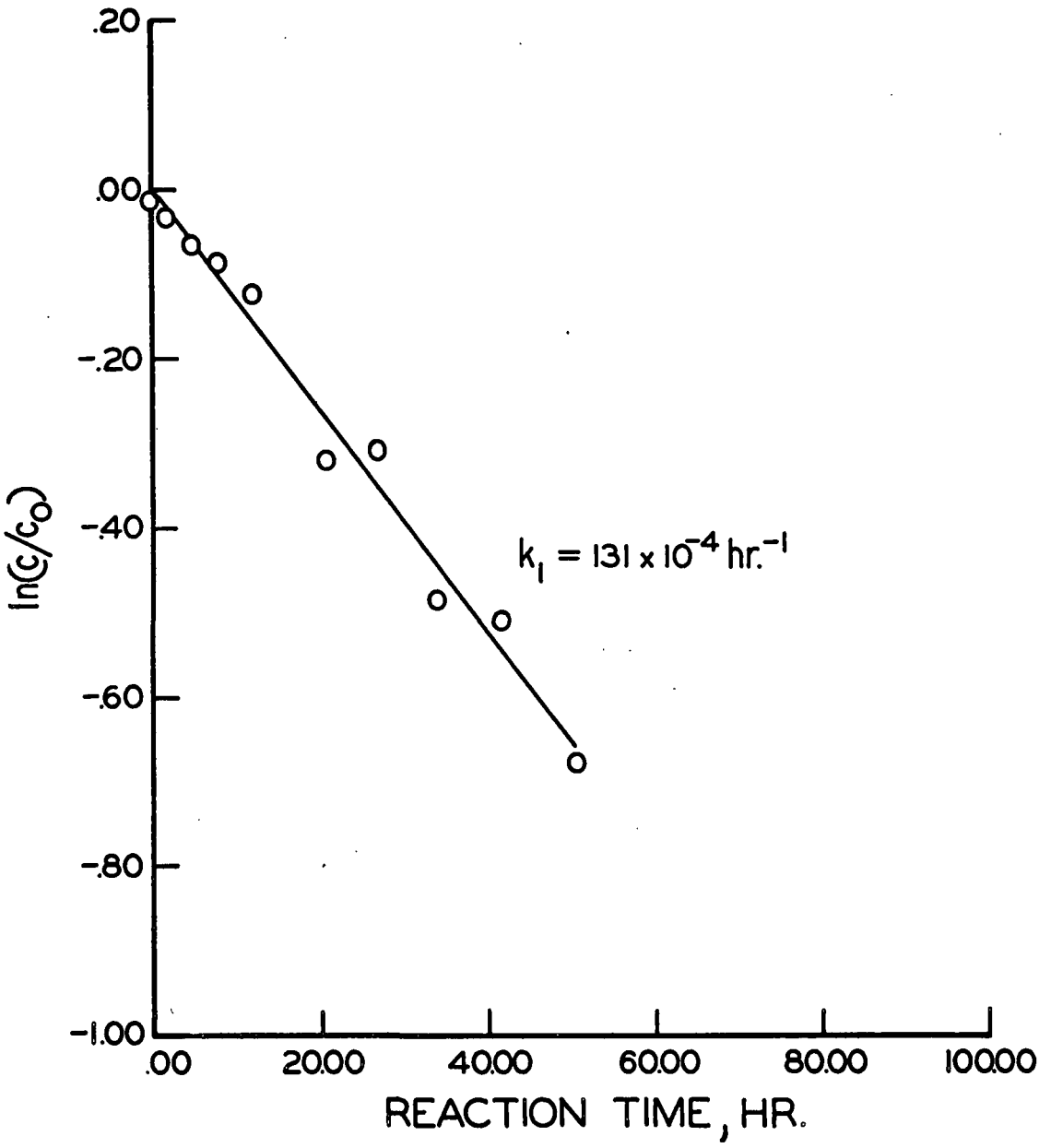

Figure 32. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 159.2°C. Overall Degradation of Methyl β-Cellobioside

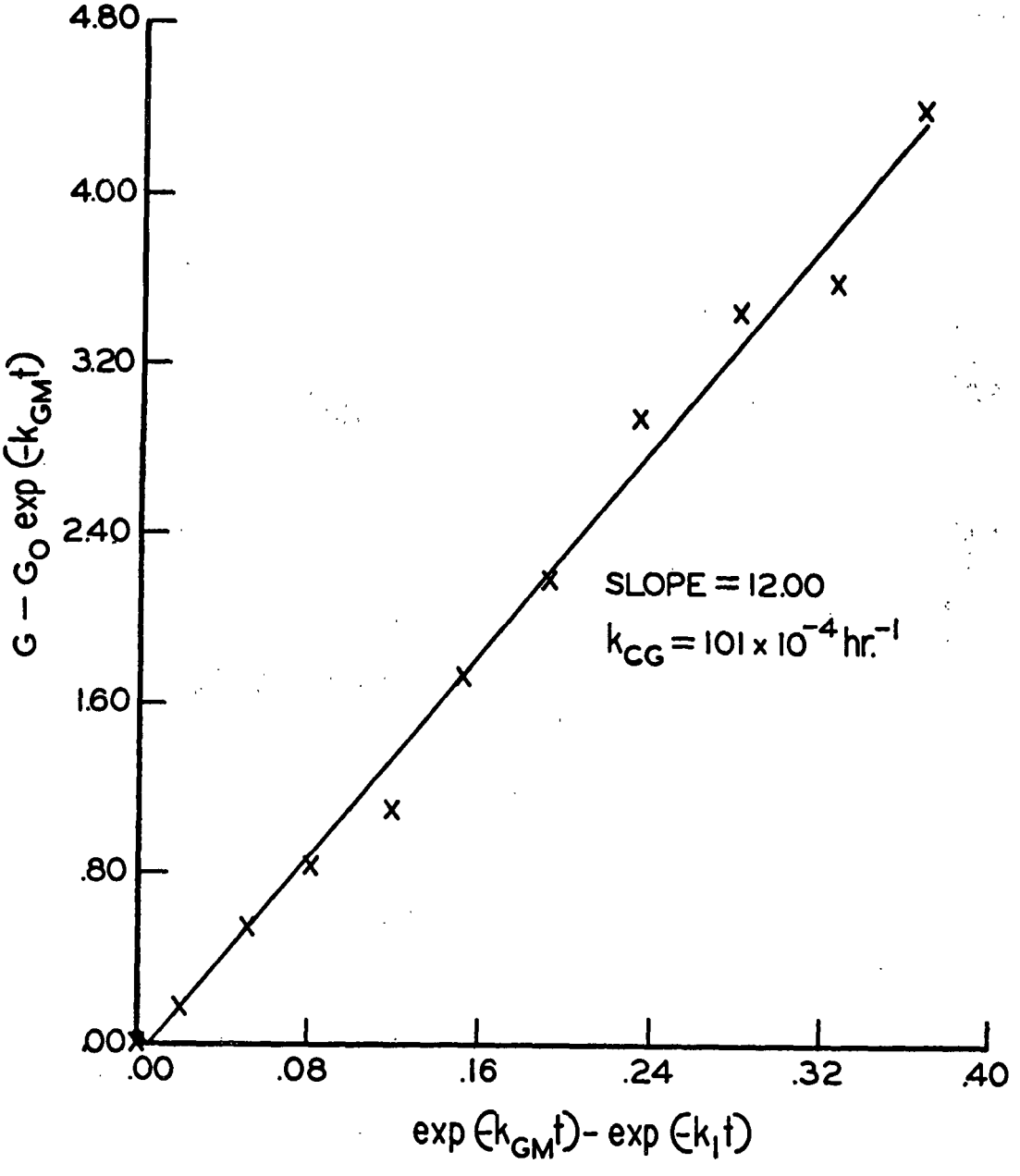

Figure 33. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 159.2°C. Generation of Methyl β-D-Glucoside

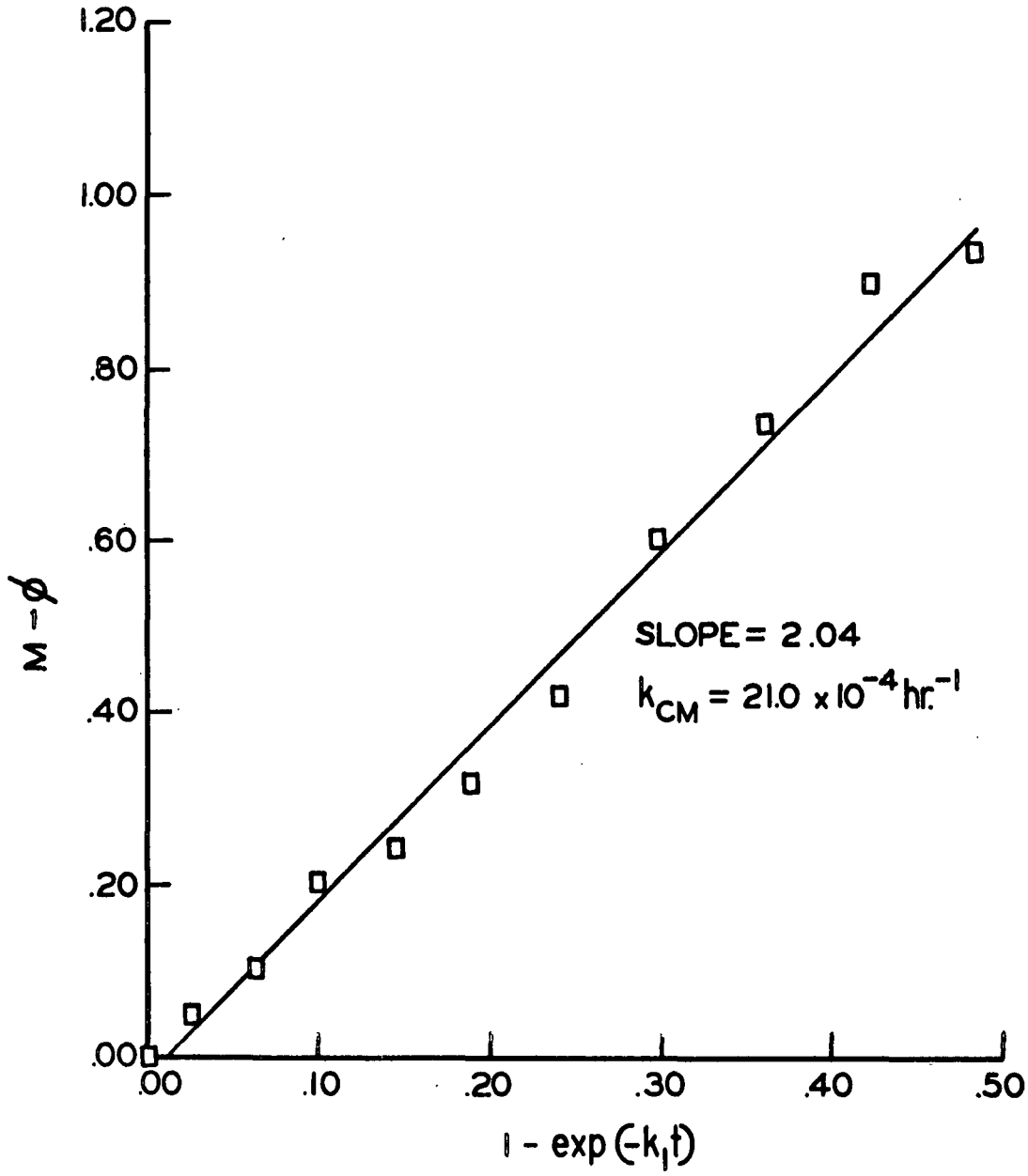

Figure 34. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 159.2°C. Generation of Methanol

TABLE XXVI

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 150.3°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	0.005	17.00	13	4	--	--	--
6.0	--	--	--	--	9.00	399	167
20.0	0.042	9.00	39	208	--	--	--
30.0	0.063	16.00	95	11	8.00	310	203
40.0	--	--	--	--	11.00	421	55
50.0	0.092	10.00	90	255	--	--	--
62.0	0.122	8.00	83	240	9.00	304	9
70.0	--	--	--	--	8.00	265	83
91.5	0.183	11.00	160	111	--	--	--
100.0	0.202	10.00	153	183	9.00	249	223
110.0	--	--	--	--	10.05	279	219
120.0	0.227	10.00	170	75	15.10	379	43

Background count = 152 cts./min.
Distillation aliquot = 25 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.06	--	--
6.0	--	--	12.40	--
20.0	0.18	0.99	--	--
30.0	0.29	1.38	10.79	12.47
40.0	--	--	10.63	--
50.0	0.44	2.19	--	--
62.0	0.59	2.54	9.32	12.46
70.0	--	--	9.14	--
91.5	0.90	3.59	--	--
100.0	0.99	3.79	7.58	12.37
110.0	--	--	7.60	--
120.0	1.12	4.21	6.81	12.15

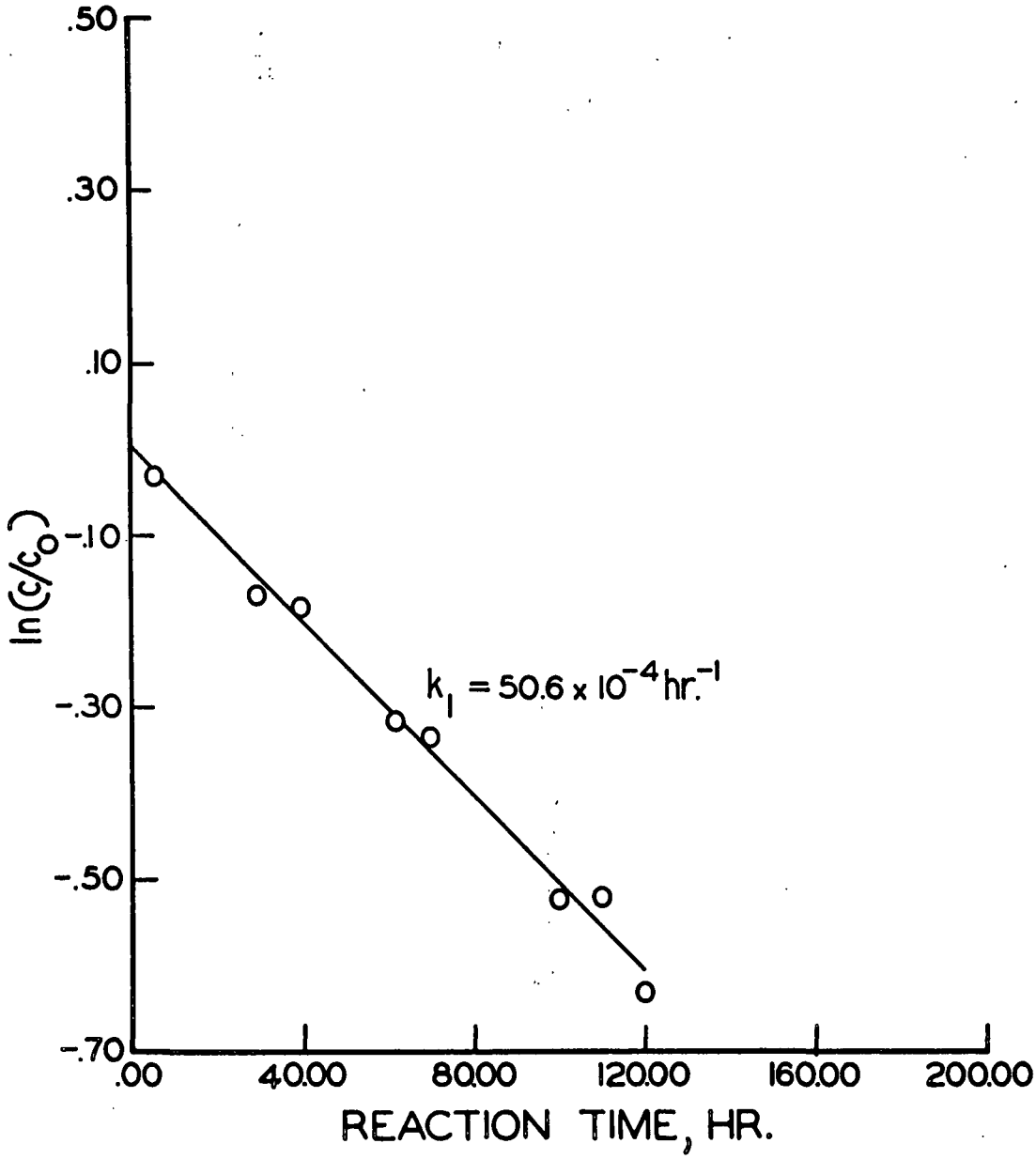

Figure 35. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 150.3°C. Overall Degradation of Methyl β-Cellobioside

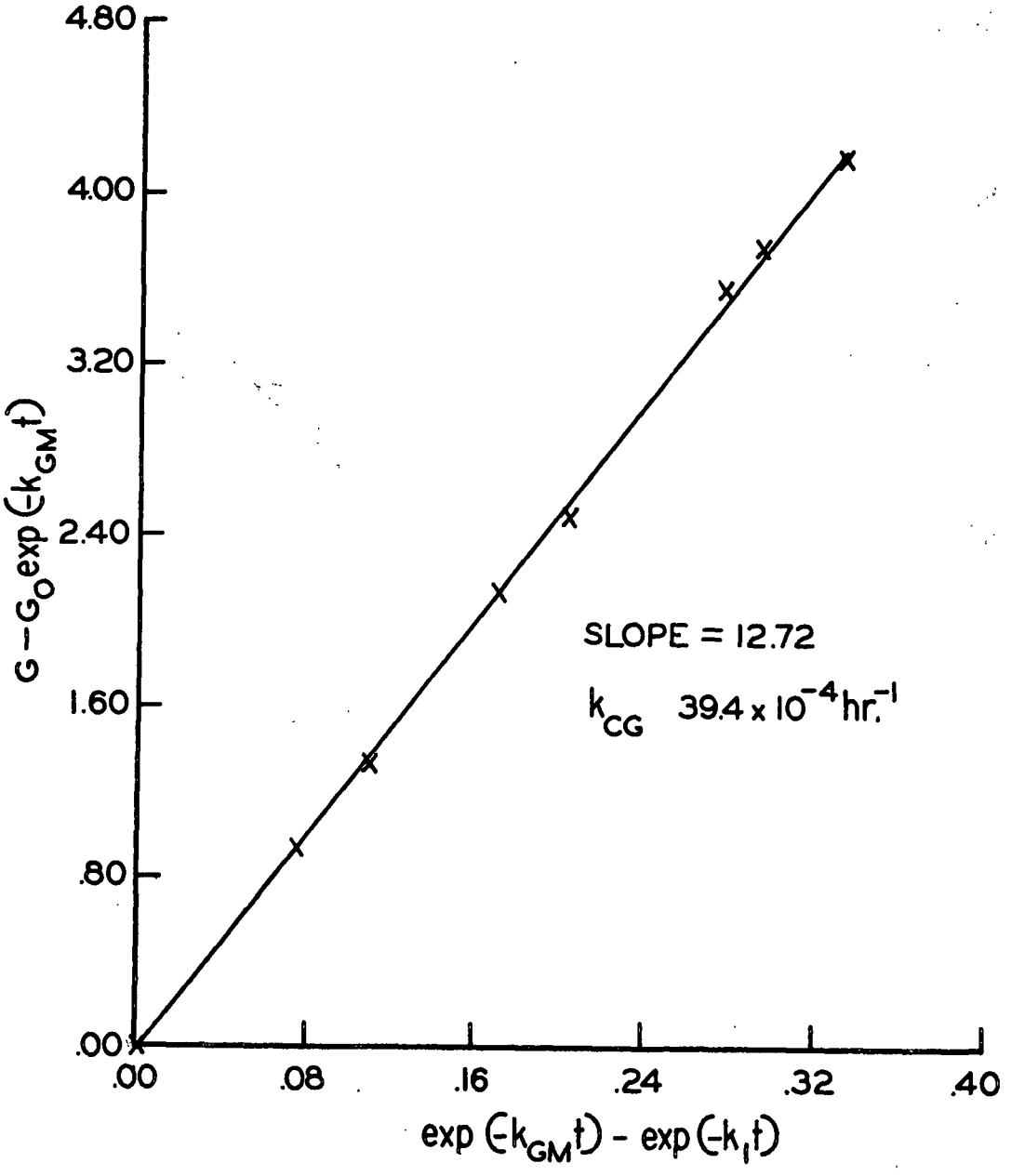

Figure 36. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 150.3°C. Generation of Methyl β-D-Glucoside

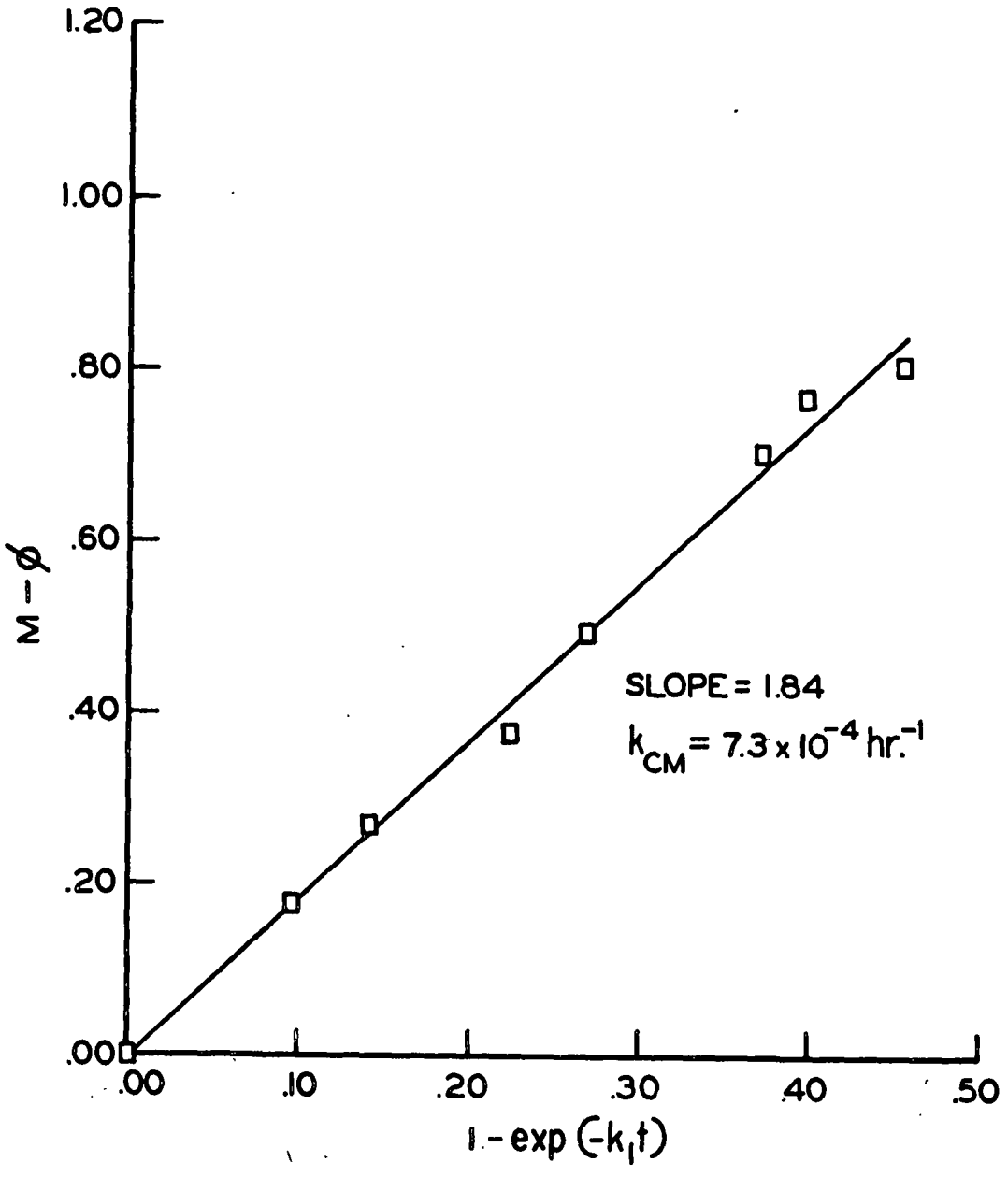

Figure 37. Reaction of Methyl β-Cellobioside in N_2-Purged 2.497N NaOH at 150.3°C. Generation of Methanol

TABLE XXVII

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 2.497N NaOH AT 139.9°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	-0.003	9.00	6	97	8.00	331	171
24.0	0.012	9.00	18	16	8.00	324	43
72.0	0.043	10.00	44	120	8.00	298	175
108.0	0.073	13.00	86	159	12.00	433	32
144.0	0.091	9.00	76	227	8.00	273	210
192.0	0.121	11.00	112	245	9.00	290	67
216.0	0.142	9.00	108	240	8.00	245	151
276.0	0.172	12.00	166	175	22.00	624	235

Background count = 150 cts./min.
Distillation aliquot = 25 ml.

TABLE XXVIII

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 1.00N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	-0.002	24.00	16	52	30.00	1431	185
6.0	0.025	8.00	54	191	25.00	977	167
12.0	0.092	8.00	80	198	17.00	618	178
18.0	0.139	16.00	261	143	18.00	538	122
36.0	0.283	10.00	238	43	13.00	263	187
42.0	0.302	8.00	201	151	9.00	158	0
48.0	0.363	9.00	243	123	15.00	239	219
54.0	0.427	7.00	197	171	13.00	173	115

Background count = 152 cts./min.
Distillation aliquot = 25 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.03	13.37	13.40
6.0	0.13	1.61	10.87	12.62
12.0	0.47	2.44	10.08	13.00
18.0	0.71	4.04	8.20	12.96
36.0	1.44	5.95	5.41	12.80
42.0	1.53	6.30	4.61	12.46
48.0	1.84	6.78	4.16	12.79
54.0	2.17	7.08	3.39	12.65

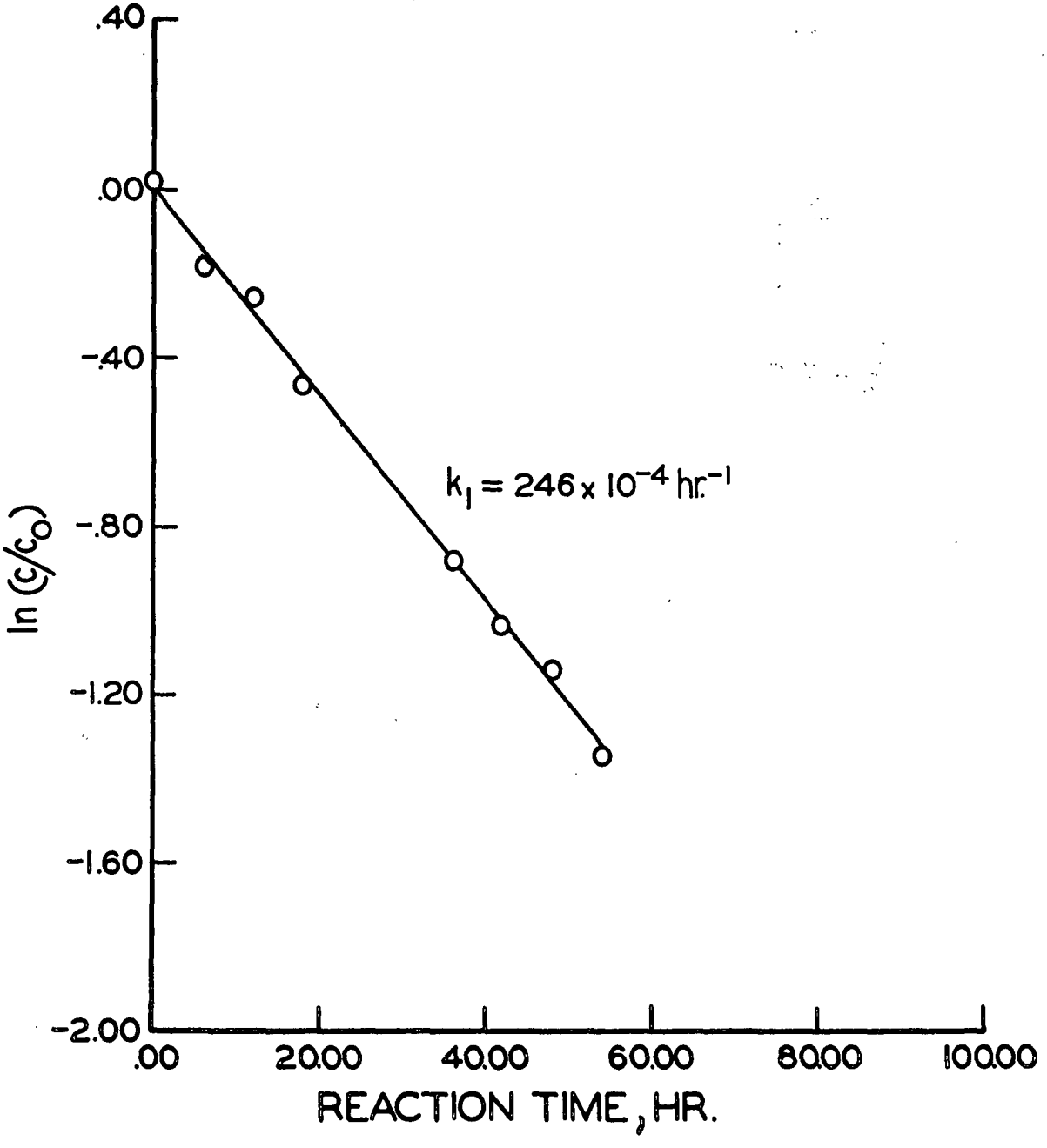

Figure 38. Reaction of Methyl β-Cellobioside in N_2-Purged 1.00N NaOH at 170.0°C. Overall Degradation of Methyl β-Cellobioside

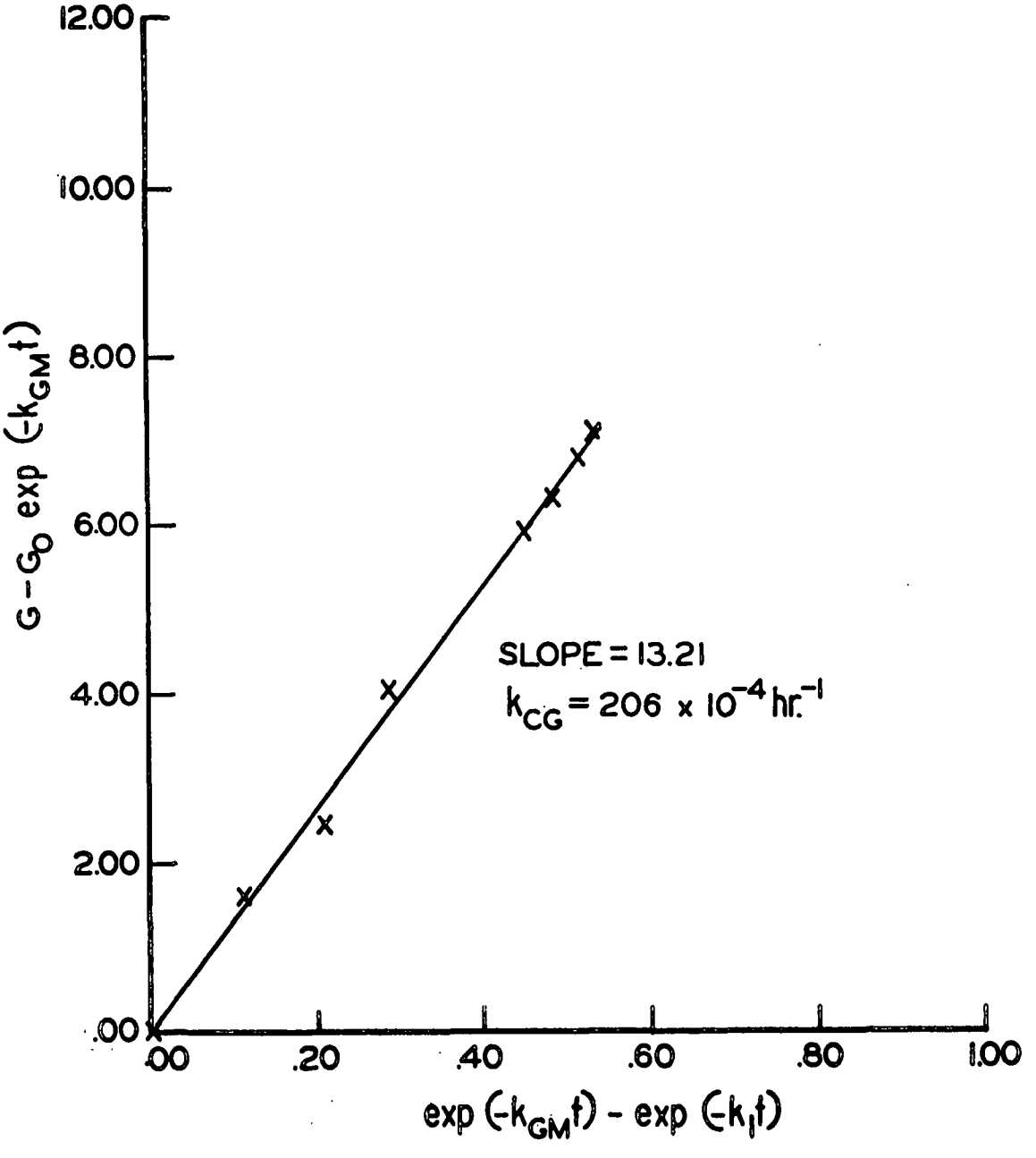

Figure 39. Reaction of Methyl β-Cellobioside in N_2-Purged 1.00N NaOH at 170.0°C. Generation of Methyl 2-β-D-Glucoside

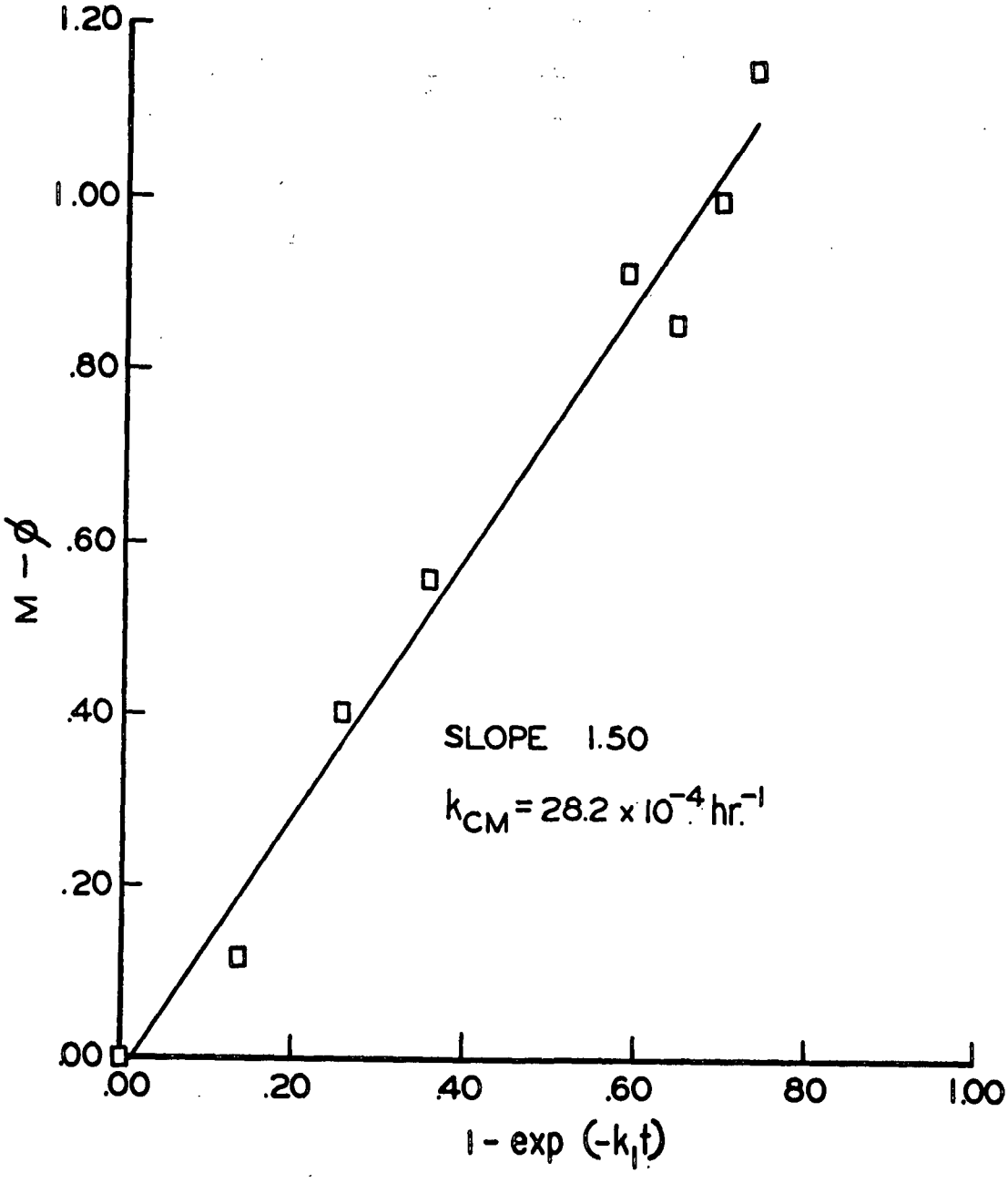

Figure 40. Reaction of Methyl β-Cellobioside in N_2-Purged 1.00N NaOH at 170.0°C. Generation of Methanol

TABLE XXIX

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 0.359N NaOH AT 170.0°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	-0.001	10.00	8	48	10.00	498	223
4.0	0.016	27.00	101	16	11.00	514	79
9.0	0.049	11.00	77	107	15.00	674	139
22.0	0.101	10.00	148	27	8.00	300	227
30.0	0.138	16.00	296	30	8.00	268	225
39.0	0.185	19.01	410	159	10.00	300	207
49.0	0.217	10.00	246	67	18.00	466	0
57.0	0.263	10.00	266	68	10.00	221	59
67.0	0.302	9.01	260	25	6.00	117	195

Background count = 161 cts./min.
Distillation aliquot = 25 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.06	13.98	14.05
4.0	0.08	0.81	13.08	13.97
9.0	0.25	1.65	12.56	14.47
22.0	0.51	3.64	10.42	14.58
30.0	0.70	4.58	9.26	14.55
39.0	0.94	5.37	8.24	14.56
49.0	1.10	6.15	7.02	14.28
57.0	1.33	6.66	5.93	13.93
67.0	1.53	7.23	5.20	13.98

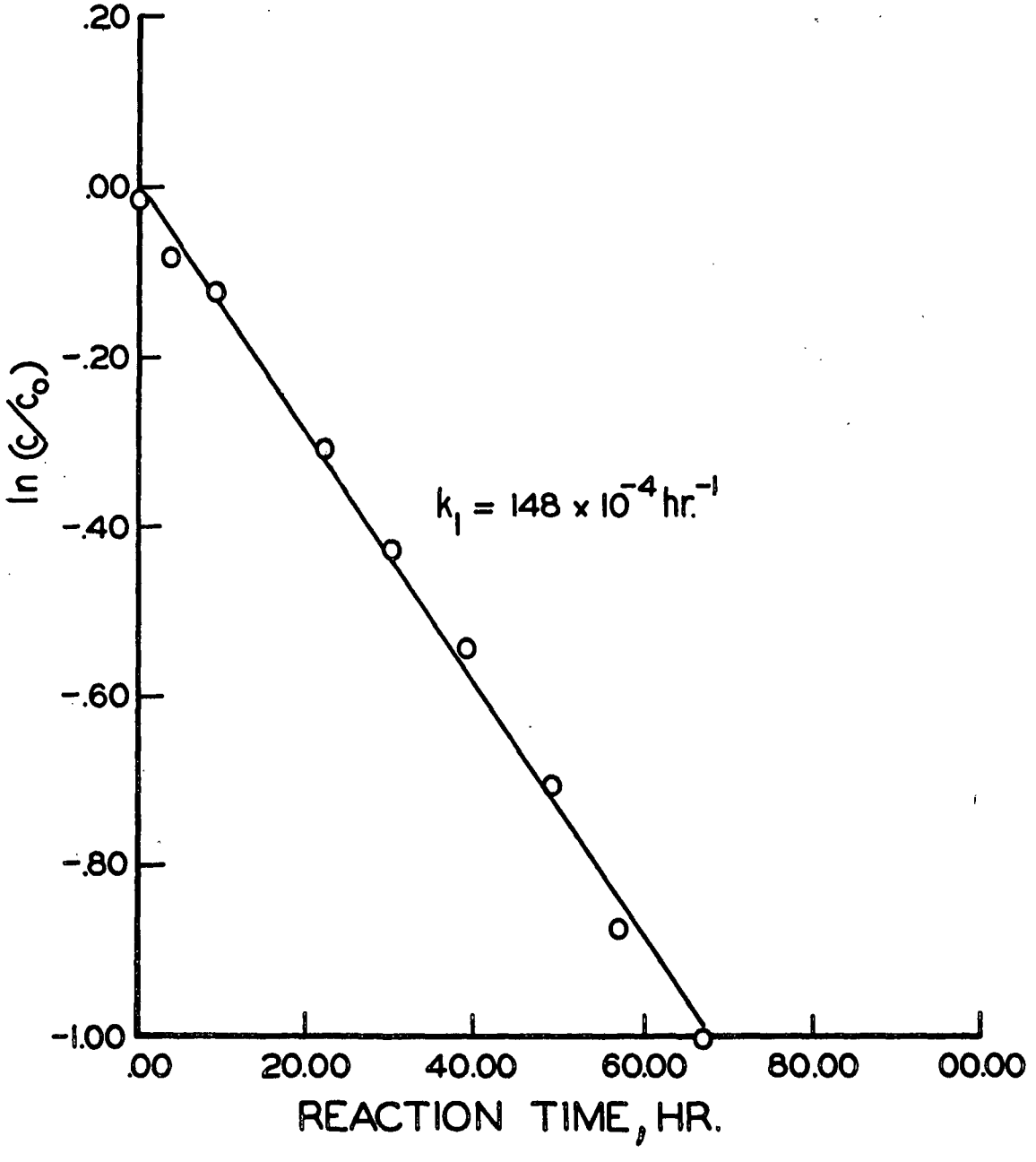

Figure 41. Reaction of Methyl β-Cellobioside in N_2-Purged 0.359N NaOH at 170.0°C. Overall Degradation of Methyl β-Cellobioside

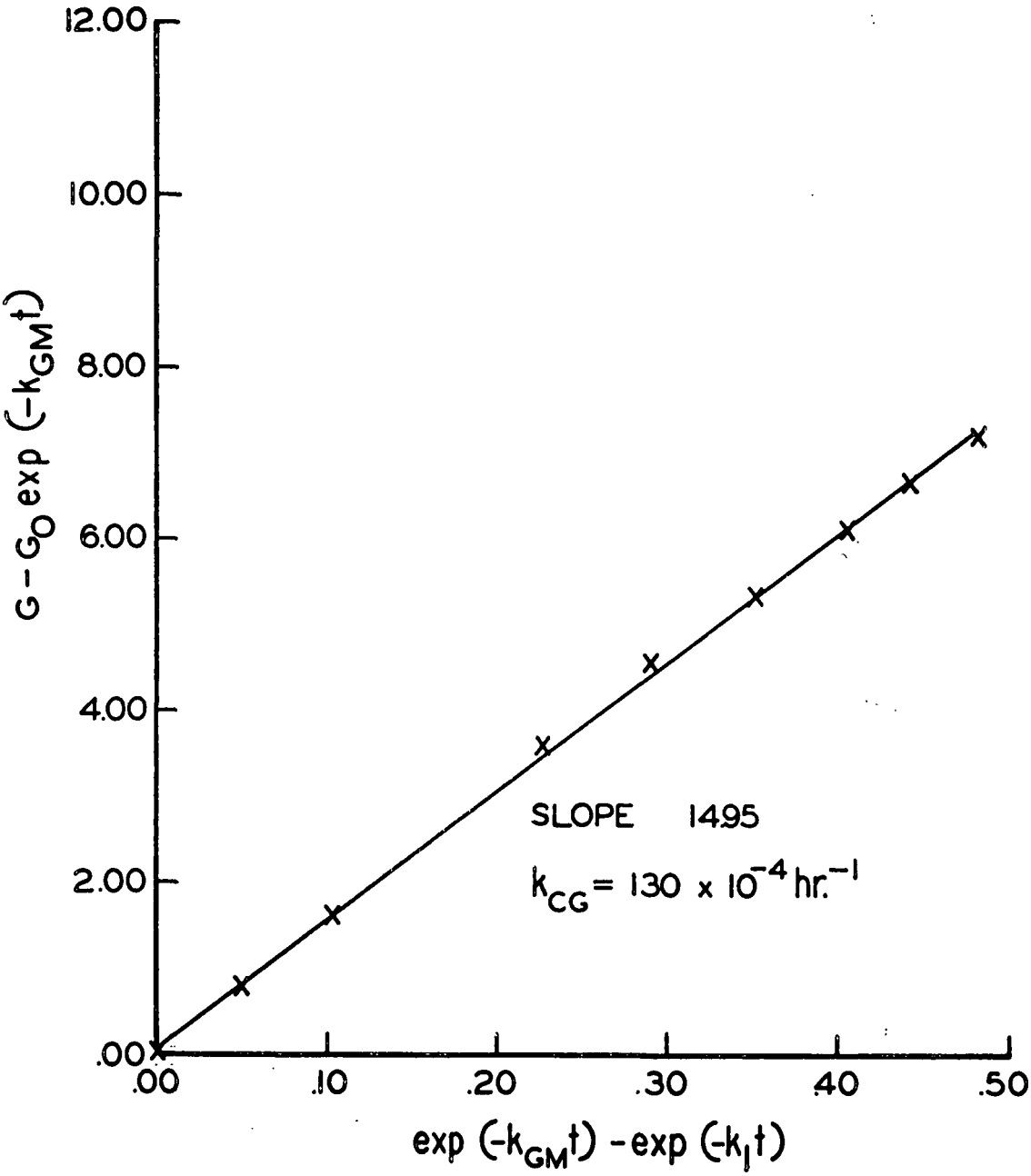

Figure 42. Reaction of Methyl β-Cellobioside in N_2-Purged 0.359N NaOH at 170.0°C. Generation of Methyl β-D-Glucoside

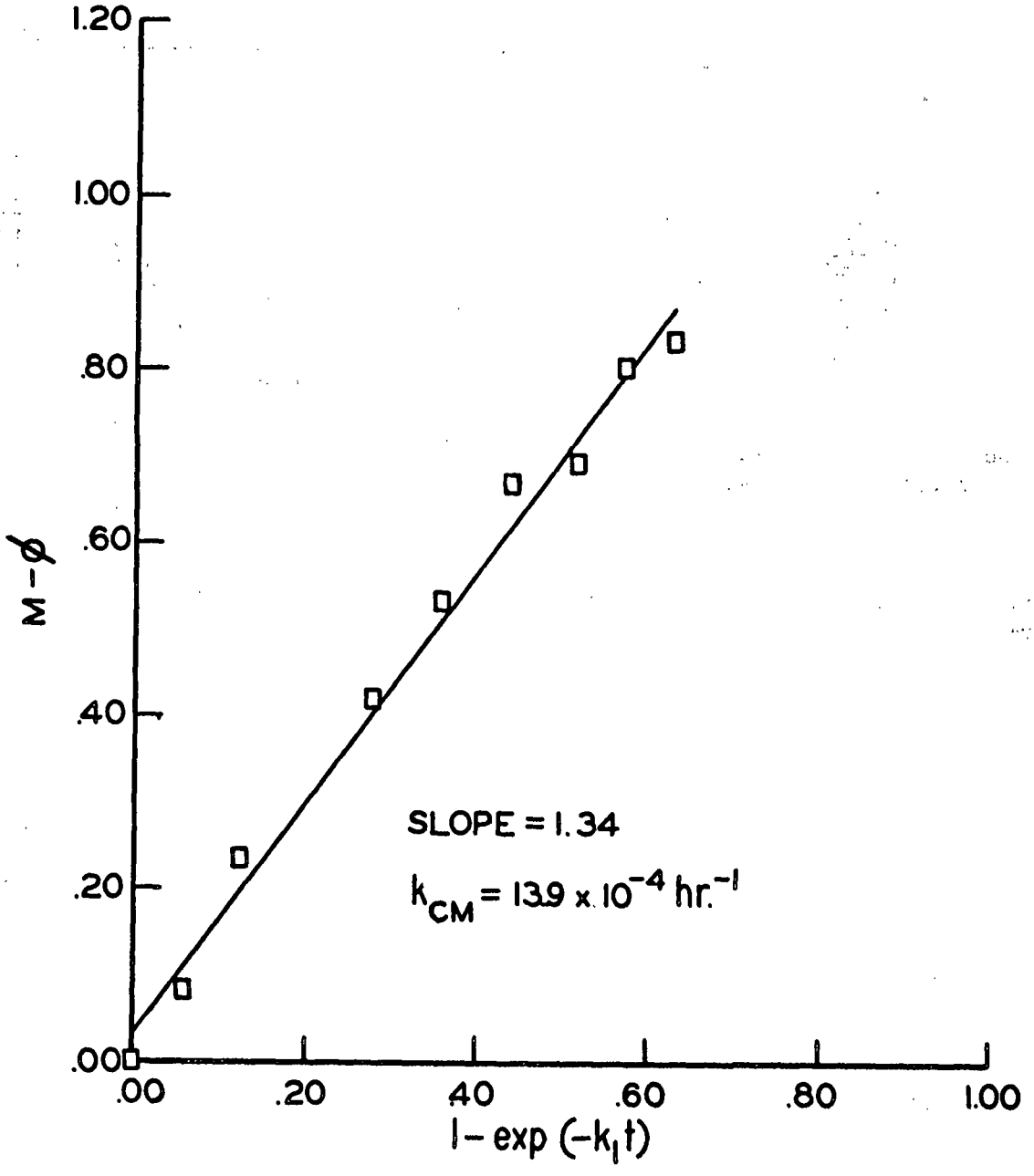

Figure 43. Reaction of Methyl β-Cellobioside in N_2-Purged 0.359N NaOH at 170.0°C. Generation of Methanol

TABLE XXX

REACTION OF METHYL β-CELLOBIOSIDE IN N_2-PURGED 0.101N NaOH AT 170.3°C.

Reaction Time, hr.	Absorbance	Methyl β-D-Glucoside Time, min.	Scales	Part Scale	Methyl β-Cellobioside Time, min.	Scales	Part Scale
0.0	-0.004	29.00	20	236	18.00	846	112
24.5	0.038	9.00	61	251	10.13	405	39
48.0	0.074	9.00	105	251	8.00	290	203
72.0	0.123	9.00	138	159	8.00	248	107
97.0	0.156	9.00	175	139	8.00	223	155
120.0	0.199	8.00	173	15	14.00	352	75
132.0	0.220	9.00	203	111	9.00	203	103
145.0	0.235	7.00	164	55	10.00	217	111
168.0	--	15.00	400	175	7.00	132	171

Background count = 150 cts./min.
Distillation aliquot = 25 ml.

Reaction Time, hr.	Methanol, μmole/ml.	Glucoside, μmole/ml.	Cellobioside, μmole/ml.	Sum, μmole/ml.
0.0	0.00	0.05	13.17	13.22
24.5	0.21	1.62	11.13	12.97
48.0	0.39	2.87	10.07	13.34
72.0	0.64	3.80	8.53	12.98
97.0	0.80	4.85	7.63	13.30
120.0	1.02	5.39	6.82	13.25
132.0	1.13	5.64	6.08	12.86
145.0	1.20	5.86	5.83	12.91
168.0	--	6.69	5.02	--

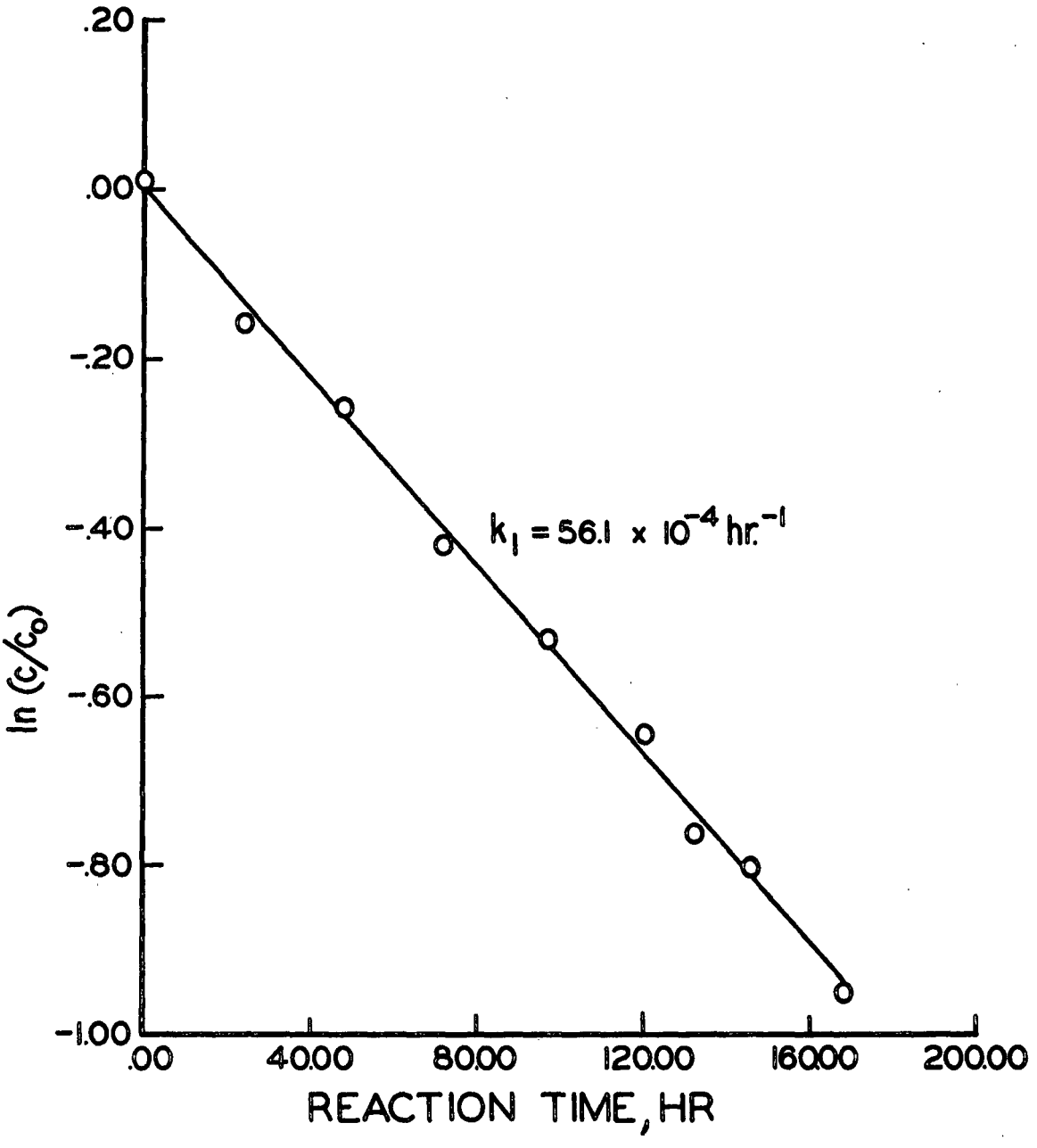

Figure 44. Reaction of Methyl β-Cellobioside in N_2-Purged 0.101N NaOH at 170.3°C. Overall Degradation of Methyl β-Cellobioside

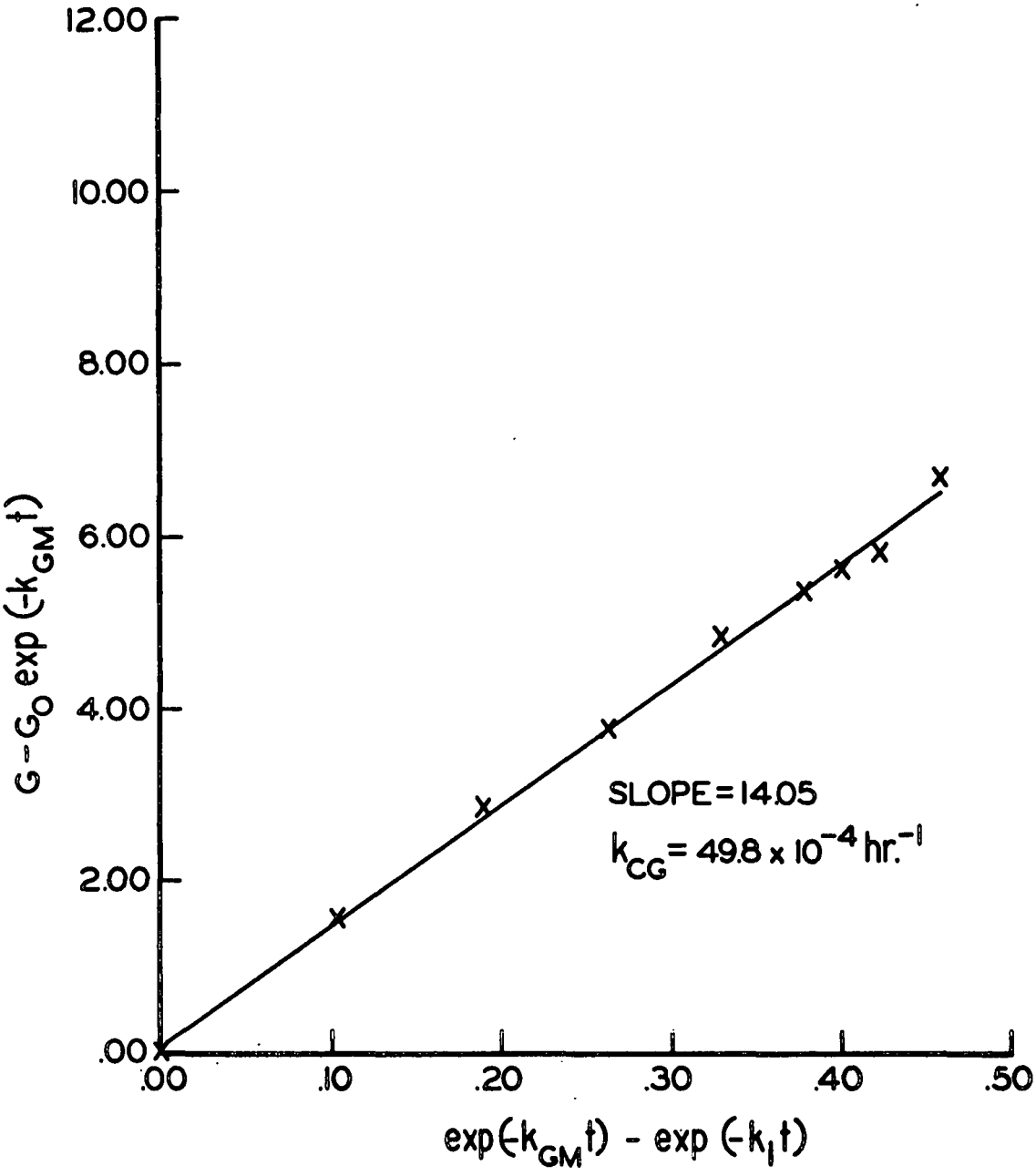

Figure 45. Reaction of Methyl β-Cellobioside in N_2-Purged 0.101N NaOH at 170.3°C. Generation of Methyl β-D-Glucoside

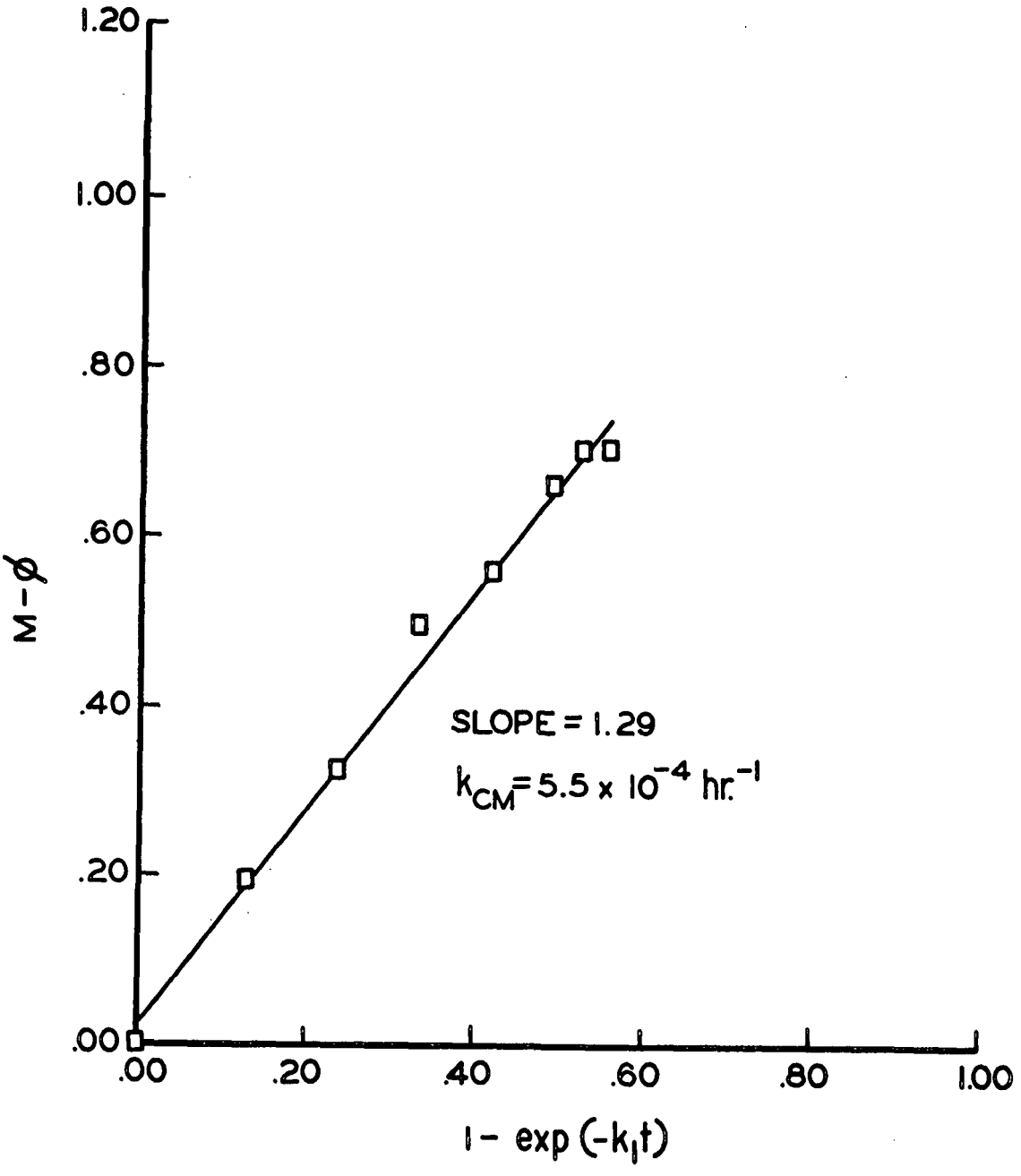

Figure 46. Reaction of Methyl β-Cellobioside in N_2-Purged 0.101N NaOH at 170.3°C. Generation of Methanol