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Anaerobic Alkaline Degradation of D-Glucose, Cellobiose, and Derivatives

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ANAEROBIC ALKALINE DEGRADATION OF D-GLUCOSE, CELLOBIOSE, AND DERIVATIVES

A thesis submitted by

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SUMMARY

Anaerobic degradations of the following reducing sugars were conducted in 0.099N sodium hydroxide at 25°C: D-glucose, 3,6-anhydro-D-glucose, 3-O-methyl-D-glucose, cellobiose, 3,6-anhydro-4-O-methyl-D-glucose, and 3,6-anhydro-cellobiose. In each case, an initial ratio of 4.95 equivalents of NaOH per mole of sugar was employed. Reactions were allowed to go to completion, and final product mixtures were analyzed as trimethylsilyl derivatives by gas-liquid chromatography and mass spectrometry.

3,6-Anhydro-D-glucose and 3-O-methyl-D-glucose degraded primarily to D-glucometasaccharinic acid, in accord with the Nef-Isbell β-alkoxy elimination theory. Trace "fragmentation" products (i.e., having fewer than six carbon atoms) were found in both cases.

Major products from cellobiose degradation were D-glucoisosaccharinic acid and D-glucose, produced via "peeling" (i.e., β-elimination of only the C-4 glucosyloxy substituent). No disaccharide product was detected. Glucose, in turn, degraded to the series of 3- to 6-carbon 3-deoxy-aldonic (metasaccharinic) acids plus glyceric acid, as was shown for the degradation of D-glucose alone. Glucose degradation apparently proceeds primarily via dealdolization to fragmentation products (aldoses) which then undergo "stabilization" (i.e., β-elimination of the C-3 hydroxyl group, keto-enol tautomerization, and benzil-benzilic acid type rearrangement) to 3-deoxy-aldonic acids. Formation of two minor products, tentatively identified as 3,4-dideoxy-aldonic acids, indicates that a second reaction mechanism (in addition to peeling) is operative in cellobiose degradation. A double elimination (of C-3 and C-4 substituents) is proposed to account for one of the dideoxy acids.

The dominant product from degradation of 3,6-anhydro-4-O-methyl-D-glucose was 3-deoxy-D-hexonic acid, suggesting a C-3, C-4 double elimination plus

introduction of a hydroxyl group at C-4. A reaction mechanism is postulated in which a key step is nucleophilic addition of hydroxide ion to a 3,4-dideoxy-hex-3-enosulose intermediate formed by double elimination. Degradation also produced the stabilization product, 4-O-methyl-D-glucometasaccharinic acid, in low yield. In addition, minor fragmentation products were found, indicating that dealdolization occurred.

Degradation of 3,6-anhydro-cellobiose produced D-glucoisosaccharinic acid and the 3- to 6-carbon 3-deoxy-aldonic acids; also, two minor 3,4-dideoxy-aldonic acids and one disaccharide acid were tentatively identified. D-Gluco-isosaccharinic acid, the most abundant product, is postulated to form via elimination of the C-4 glucosyloxy substituent and nucleophilic attack of hydroxide ion at C-3 of the resulting vinyl ether. Two sources are proposed for 3-deoxy-D-hexonic acid: glucose (via stabilization), and 3,6-anhydro-cello-biose via either of two double elimination-nucleophilic addition routes. Glucose, a product and reactive intermediate in the reaction, is a probable source of the 3- to 5-carbon 3-deoxy-aldonic acids. The suspected disaccharide acid may have been formed via the stabilization route.

Anaerobic degradation of D-glucose in 0.041N calcium hydroxide at 25°C [initially, 2.0 equivalents of $Ca(OH)_2$ per mole of sugar] produced two series of 3- to 6-carbon compounds: the 3-deoxy-aldonic acids (as in NaOH), and the 2-C-methyl-aldonic acids. Presence of divalent cations apparently enhanced formation of rearrangement (as opposed to fragmentation) products. A reaction mechanism is proposed to account for formation of the 2-C-methyl-aldonic acids from 4- to 6-carbon aldoses produced via dealdolization.

INTRODUCTION

ALKALINE DEGRADATION OF POLYSACCHARIDES

Why study alkaline degradation reactions of simple reducing sugars? One reason is to gain knowledge of how these sugars, and by inference, analogous polysaccharides, behave in alkaline pulping reactions. Despite a multitude of investigations over a period dating back almost 140 years, much remains to be learned. During the last 15 years, use of sophisticated analytical instruments has shown that reactions of sugars in alkali are far more complex than previously envisioned.

Alkaline pulping is the mainstay of the pulp and paper industry. Almost 80% of all chemical pulp produced in North America and Scandinavia is kraft pulp. While the intent of wood pulping is to solubilize lignin and free the cellulose fibers, some chemical degradation of cellulose and hemicelluloses occurs concurrently. This becomes increasingly important as the ratio of carbohydrates to residual lignin increases. Greater degradation of polysaccharides reduces the yield of useable fibers.

At least three major types of reactions occur between polysaccharides and alkali (1,2). One is "peeling," the stepwise elimination of monomeric units from the reducing end of the polymer chain. Second, the polymer may react to produce an alkali-stable end unit (i.e., a nonreducing moiety), rendering the chain stable to further attack by peeling. This is the so-called "stopping" or stabilization reaction. Third, cleavage of intermonomer linkages within the polymer chain can occur; this reaction is considered to be important only at elevated temperatures (>150°C) and produces a greater number of smaller polymer molecules which can undergo peeling. In terms of reaction rates,

"peeling" is orders of magnitude faster than "hydrolytic" cleavage of glycosidic linkages.

For comparison purposes, Sarkanen and coworkers found that in 5% sodium hydroxide solution, cotton hydrocellulose had an activation energy of 24 kcal/mole for peeling and 32 kcal/mole for stabilization (3); cotton cellulose required 36 kcal/mole for chain cleavage (4).

From a practical standpoint, the pulp and paper industry has recognized the advantages of chemically "stabilizing" polysaccharides to retard their degradation in alkaline pulping. Such modifications as sodium borohydride treatment (5), gaseous hydrogen sulfide pretreatment (6) and polysulfide pulping (7) all enhance yields of polysaccharides from wood. On a more fundamental level, greater insight as to how stabilization occurs chemically in simple reducing sugars may eventually lead to better and less expensive means of accomplishing the same result in reactions between polysaccharides and alkali. Also, solubilized sugars from wood pulping contribute significantly to biochemical oxygen demand (BOD) loads in waste water treatment facilities; greater conservation of polysaccharides during pulping should help to lessen this burden.

PEELING *VERSUS* STABILIZATION REACTIONS FOR CELLOBIOSE

The peeling and stabilization reactions generally proposed for cellobiose, used as a model compound for cellulose, would occur as illustrated in Fig. 1.

To simplify the study of alkaline degradation reactions of mono- and disaccharides, dilute alkali ($\sim$0.1*M*) and mild temperature (e.g., 25°C) are commonly used. Often, the reactions are conducted in nitrogen atmospheres, thereby excluding oxygen which can make a system more complicated by promoting

a competing set of reactions (9-13). All work described herein concerns reactions run exclusively in oxygen-free alkaline media under nitrogen atmospheres.

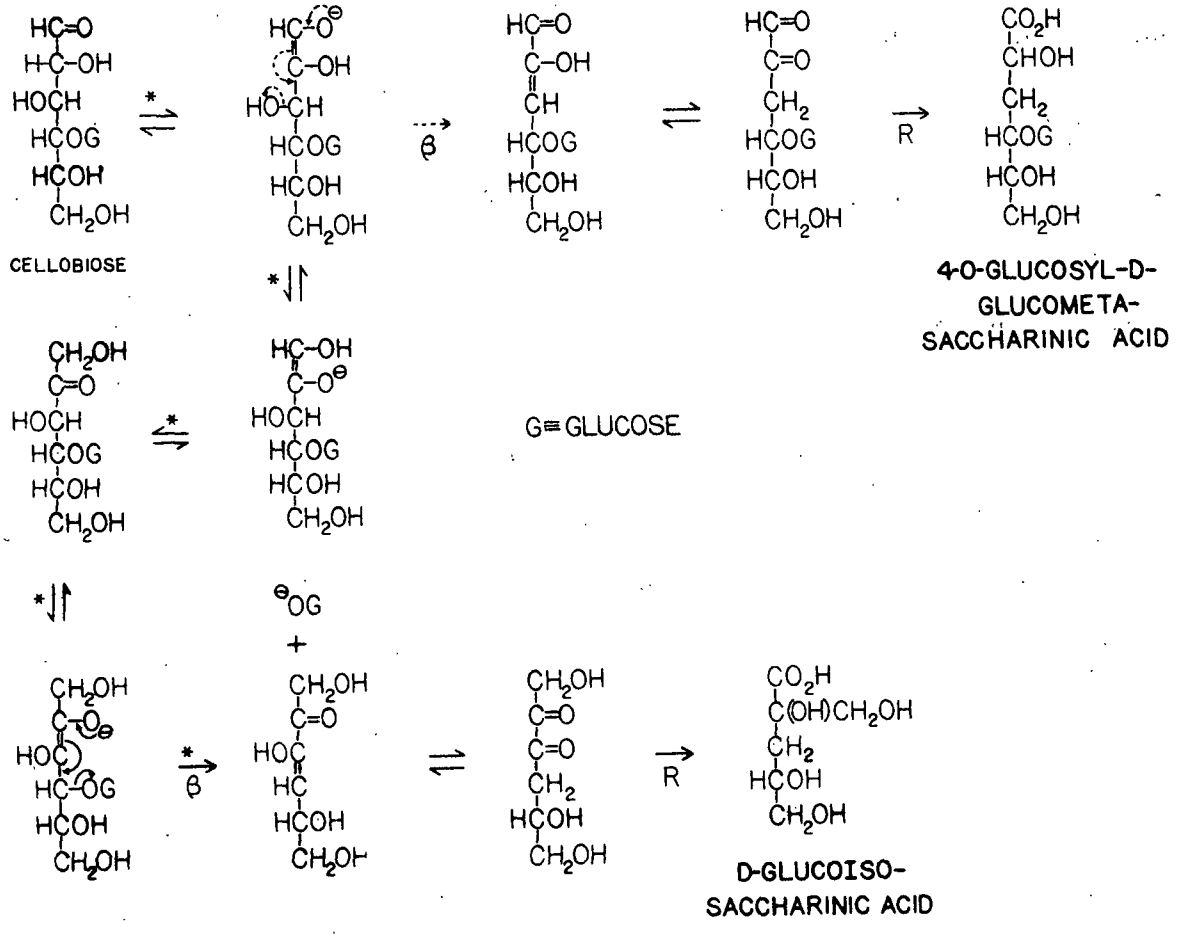


Figure 1. Possible Reaction Mechanisms for Oxygen-Free Alkaline Degradation of Cellobiose (2,8,9); β − β-Elimination; R − Benzil-Benzilic Acid Type Rearrangement; * − Peeling Sequence

As shown in Fig. 1, cellobiose "peels" to generate D-glucoisosaccharinic acid and D-glucose. Theoretically, complete peeling of one mole of cellobiose should yield one mole of the acid plus one mole of glucose. Glucose, also a reducing sugar, undergoes further degradation to acid products. Glucoisosaccharinic acid is a stable product (14).

If, on the other hand, cellobiose follows only the "stabilization" route, the product is one mole of 4-O-glucosyl-D-glucometasaccharinic acid. Such a product would be regarded as stable to further alkaline attack, thus terminating the reaction.

By analogy to the alkaline degradation of cellulose (1), it would be reasonable to expect that the degradation of cellobiose in dilute, oxygen-free alkali would proceed partly via peeling and partly via stabilization. This, however, appears not to be the case. A number of investigators (13,15) have found no evidence for the formation of 4-O-glucosyl-D-glucometasaccharinic acid. The one exception is a report by Lindberg, et al. (16) of the isolation, from cellobiose degradation, of a disaccharide acid, which upon acid hydrolysis yielded glucose and glucometasaccharinic acid. Apparently, this work has never been duplicated.

ALKALINE DEGRADATION OF D-GLUCOSE

Not surprisingly, D-glucose, the monomeric unit of cellulose, has also been the subject of many alkaline degradation studies. The most concise early work was done by Nef (10), who degraded D-glucose (as well as D-mannose and D-fructose) in sodium hydroxide in the presence and absence of oxygen. From D-glucose (100 g) in oxygen-free 8M NaOH at 100°C for 7-8 hours, Nef obtained D,L-lactic acid (40-45 g), D,L-dihydroxybutyrolactone (10-15 g), α,β-glucometasaccharinolactone (20 g) and α,β-glucoisosaccharinolactone (2 g). He also observed that in the presence of air, no "saccharinic" (i.e., six-carbon deoxy-aldonic) acids were formed.

In later work by Kenner and Richards (17), it was determined that degradation of D-glucose in oxygen-free 0.04N calcium hydroxide at 25°C gave D-glucosaccharinic (i.e., 2-C-methyl-D-pentonic) acid as the only six-carbon product.

Again, lactic acid was the dominant product of the reaction; 2,4-dihydroxybutyric acid was described as a very minor product.

More recently, Malinen and Sjostrom (15) degraded D-glucose in dilute oxygen-free sodium hydroxide at 120°C and found the products to be primarily lactic acid (∿57%) and glucometasaccharinic acid (∿28%).

EFFECTS OF SUBSTITUTION

In alkaline degradation of simple sugars substituted at C-3 or C-4 (or both), the substituent has a directing effect upon the course of the reaction (2). For example, 3-O-methyl-D-glucose gives D-glucometasaccharinic acid as the sole product, presumably because the substituent at C-3 is a much better leaving group in the alkaline medium than is the hydroxyl group at C-4 [(18); see also (56)]. Conversely, substitution at C-4, as in 4-O-methyl-D-glucose, directs the reaction toward formation of D-glucoisosaccharinic acid (19). The latter result is also observed for 1,4-linked disaccharides such as cellobiose, lactose and maltose (2).

It is not clear, however, that a substituent at C-3 or C-4 causes the degradation to go only to the meta or iso acid, respectively. Studying the degradation reaction of 4-O-methyl-D-glucose in oxygen-free 2.5N sodium hydroxide at 100°C, Sten and Mustola (20) found, as predicted from the generally accepted theory and earlier results (19), that α- and β-glucoisosaccharinic acids were the main products. However, α- and β-glucometasaccharinic acids were also observed among the products. According to the Isbell theory (9), stabilization reaction products from 4-O-methyl-D-glucose, if any, would be the isomeric pair of 4-O-methyl-D-glucometasaccharinic acids. No such acids were found, even though Sten and Mustola deliberately planned their investigation to search specifically for stabilization products. In this case,

production of the unsubstituted metasaccharinic acids indicates that at least a small proportion of elimination occurred at both C-3 and C-4.

In the alkaline degradation of 3-O-methyl-xylobiose (21), one might expect that the C-3 methoxyl substituent would direct the reaction toward formation of 4-O-xylosyl-D-xylometasaccharinic acid as one of the products. Yet after 6 hours in 1N NaOH at 20°C, no disaccharide product was formed, even though the O-methyl group at C-3 was eliminated as methanol.

EFFECTS OF REACTION VARIABLES

Oxygen-free alkaline degradation reactions are affected by a wide range of variables, among which the more important are: temperature; monovalent versus divalent cations [e.g., NaOH vs. $Ca(OH)_2$]; and initial concentrations of alkali and reducing sugar.

Lindberg, et al. (16) degraded cellobiose, mannobiose and xylobiose in dilute, oxygen-free NaOH and $Ca(OH)_2$. Analyzing for disaccharides in reaction samples, they found significant differences according to reaction temperature (60, 75 and 90°C were employed).

In the same work, differences were observed between reactions conducted in the presence of monovalent (Na^+) and divalent (Ca^{2+}) cations. Similarly, reactions of glucose with $Ca(OH)_2$, $Ba(OH)_2$ and NaOH were affected by the cation in solution, though the differences were relatively small (22). It is generally thought (23,24) that divalent cations catalyze the benzil-benzilic acid type of rearrangement of reactive intermediates, whereas monovalent cations promote fragmentation of the carbon backbone.

It is important to realize that calcium hydroxide has inverse solubility in water. At room temperature, a saturated $Ca(OH)_2$ solution has a normality

not exceeding 0.05 (25). This limits its usefulness with respect to initial sugar concentration when one wants to allow the alkaline degradation reaction to go to completion. The other commonly employed bases, NaOH and $Ba(OH)_2$, do not pose this solubility problem.

Alkali strength at initiation also affects the outcome of degradation reactions. For example, Corbett and Liddle (22) found that glucose produced more equivalents of acid per mole of starting material as the initial concentration of $Ca(OH)_2$ was decreased. The very extensive literature in this field contains references to degradation reactions conducted over the alkali concentration range of 0.01 to 8.0M. Thus, direct comparisons between alkaline degradations of the same reducing sugar may be complicated by differences in the initial alkali strength. A further complication is that initial reducing sugar concentration varies considerably among studies reported in the literature. In general, the effects of initial alkali and sugar concentrations on the progress and outcome of alkaline degradations are not well known.

SYSTEMS SELECTED FOR STUDY

Two examples cited above (4-O-methyl-D-glucose and 3-O-methyl-xylobiose) suggest that use of the classical theory of peeling and stabilization reactions cannot fully explain the results of all alkaline degradation reactions. Sten and Mustola (20) found that degradation of 4-O-methyl-D-glucose gave the "peeling" product (glucoisosaccharinic acid); however, although the stabilization reaction was expected to yield 4-O-methyl-glucometasaccharinic acid, none was observed. Surprisingly, a small amount of glucometasaccharinic acid was formed, for which the normal mechanistic concept of alkaline degradations (2,26) does not account. Aspinall and Ross (21) observed that 3-O-methyl-xylobiose suffered a "double elimination" upon alkaline degradation — the

3-O-methyl group was eliminated as methanol, and no disaccharide acid was produced. It appears, therefore, that alkaline degradation can lead to results not in accord with the generally-accepted theory of how these reactions proceed.

In many alkaline degradation studies of simple sugars under mild conditions, the reactions were not allowed to go to completion. Nor, in most cases, were the reactions monitored as a function of time. Yet titration for alkali consumption (i.e., acids generated) is a well-known procedure (14,17-19, 22) which helps to determine the progress of such reactions. A plateau in the plot of acids generated versus time (for example, see page 17) signals completion of the reaction. Thus, by allowing a reaction to go to completion, one obtains a product mixture that represents the alkali-stable final result and bears directly upon the reaction pathways followed.

Another problem is the common use of calcium hydroxide as the reaction medium. Its very limited solubility in water at room temperature prevents completion of some reactions when the initial sugar concentration exceeds a rather low, yet critical, level. To reach completion in such cases would require running the reaction in a suspension rather than a solution of calcium hydroxide.

In this investigation, aqueous sodium hydroxide was chosen as the reaction medium, and a low initial ratio of alkali to starting material was used. Reactions were allowed to go to completion while mild reaction conditions were maintained.

It was proposed, therefore, to undertake a systematic study of final products from oxygen-free sodium hydroxide degradation of a series of simple reducing sugars under uniform mild conditions. By varying the degree and

position of substitution of the starting material, the investigator could anticipate a wide range of responses. Starting materials chosen included D-glucose (no substitution); 3,6-anhydro-D-glucose and 3-O-methyl-D-glucose (monosubstitution at C-3); cellobiose (equivalent to glucose monosubstituted at C-4); and 3,6-anhydro-4-O-methyl-D-glucose and 3,6-anhydro-cellobiose (disubstitution at C-3 and C-4). Glucose and 3,6-anhydro-D-glucose were particularly important in relation to the behavior in dilute alkali of their disaccharide analogs (cellobiose and 3,6-anhydro-cellobiose, respectively).

Special importance was attached to the determination of final products from cellobiose. As the simplest model compound for cellulose, its response in dilute alkali would be expected to conform to the β-alkoxy elimination theory of peeling and stabilization as commonly postulated for polysaccharides. Yet the literature contains the opposing results that cellobiose does (16) and does not (13,15) yield a classical stabilization product (i.e., 4-O-glucosyl-D-glucometasaccharinic acid). Which result is correct? And is there any evidence that reactions other than stabilization and/or peeling occur?

Ancillary objectives included the following: Can 4-O-glucosyl-D-glucometasaccharinic acid be obtained by alkaline degradation of 3,6-anhydro-cellobiose (analogous to the production of glucometasaccharinic acid from 3,6-anhydro-D-glucose)? Also, what happens when a 3,4-disubstituted reducing sugar (e.g., 3,6-anhydro-4-O-methyl-D-glucose) is subjected to alkaline degradation? This avenue of investigation has received very little study.

An additional note should be made regarding combined gas chromatography-mass spectrometry (GC-MS) techniques. In tandem, these instruments permit the investigator to separate and analyze very complex mixtures of products. The mass spectral data are then used to determine the chemical structures of the

products, for which reference data may be available. The GC-MS method is especially powerful when reference compounds or data do not exist, as, for example, in the identification of previously unknown compounds. For this reason, the GC-MS approach was used extensively in this study and is rapidly becoming the method of choice for many investigators.

RESULTS AND DISCUSSION

SYNTHESIS OF MODEL COMPOUNDS

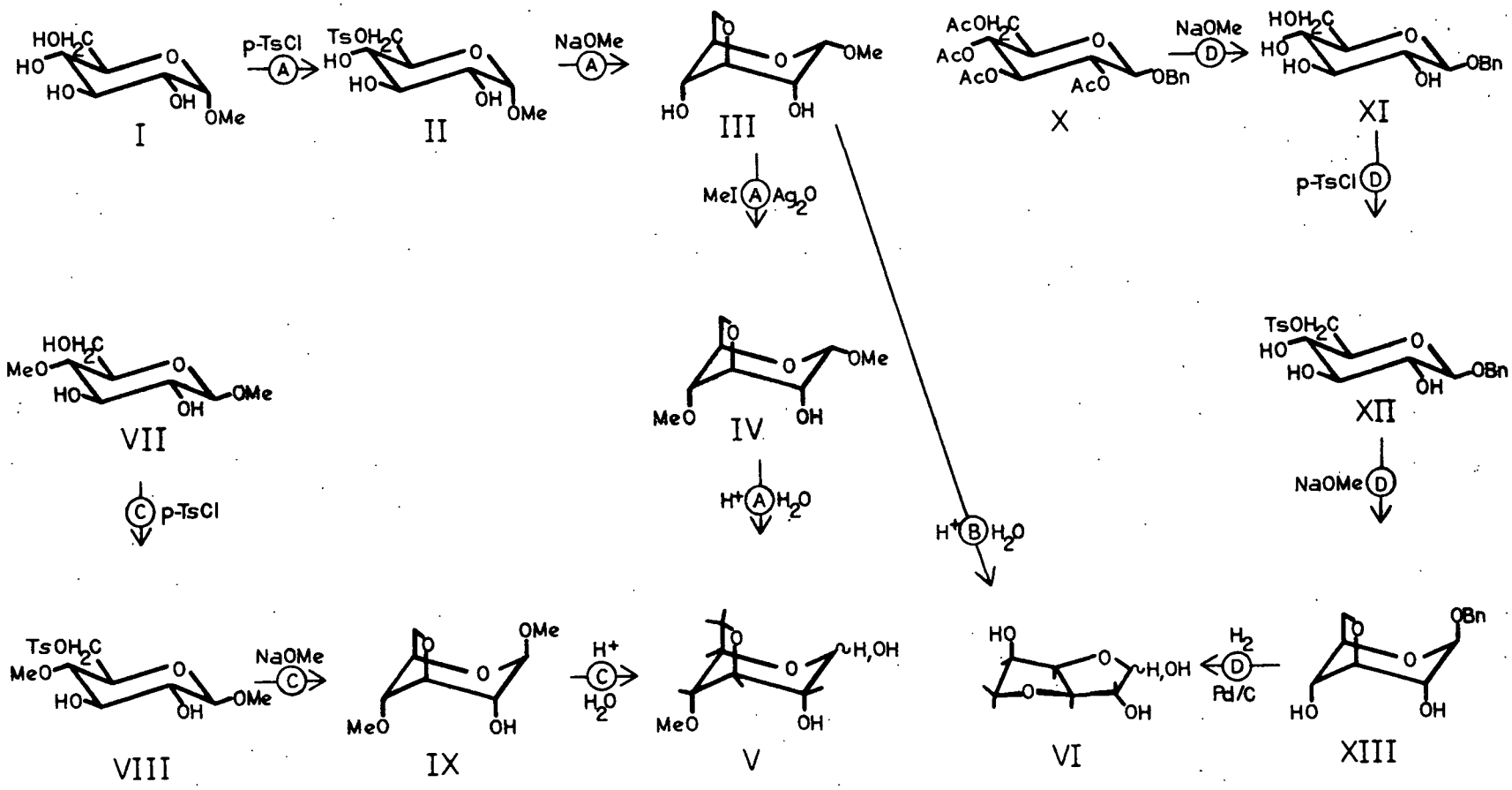
3,6-Anhydro-4-O-methyl-D-glucose (V) and 3,6-anhydro-D-glucose (VI) were each synthesized via two independent routes, as shown in Scheme I. Final products were identical. Routes Ⓐ and Ⓑ of Scheme I provide the simplest routes to these model compounds and are a considerable improvement over reaction sequences previously reported (27).

3,6-Anhydro-cellobiose (XXII), a new compound, was synthesized as illustrated in Scheme II. Compounds XIX through XXI are also reported here for the first time. 3,6-Anhydro-cellobiose can be thought of as 3,6-anhydro-D-glucose bearing a glucosyloxy substituent at C-4. 3,6-Anhydro-glucose and glucose were obtained upon hydrolysis of 3,6-anhydro-cellobiose. Acetylation of XXII provided a hexaacetate (confirmed by proton magnetic resonance spectroscopy) as expected.

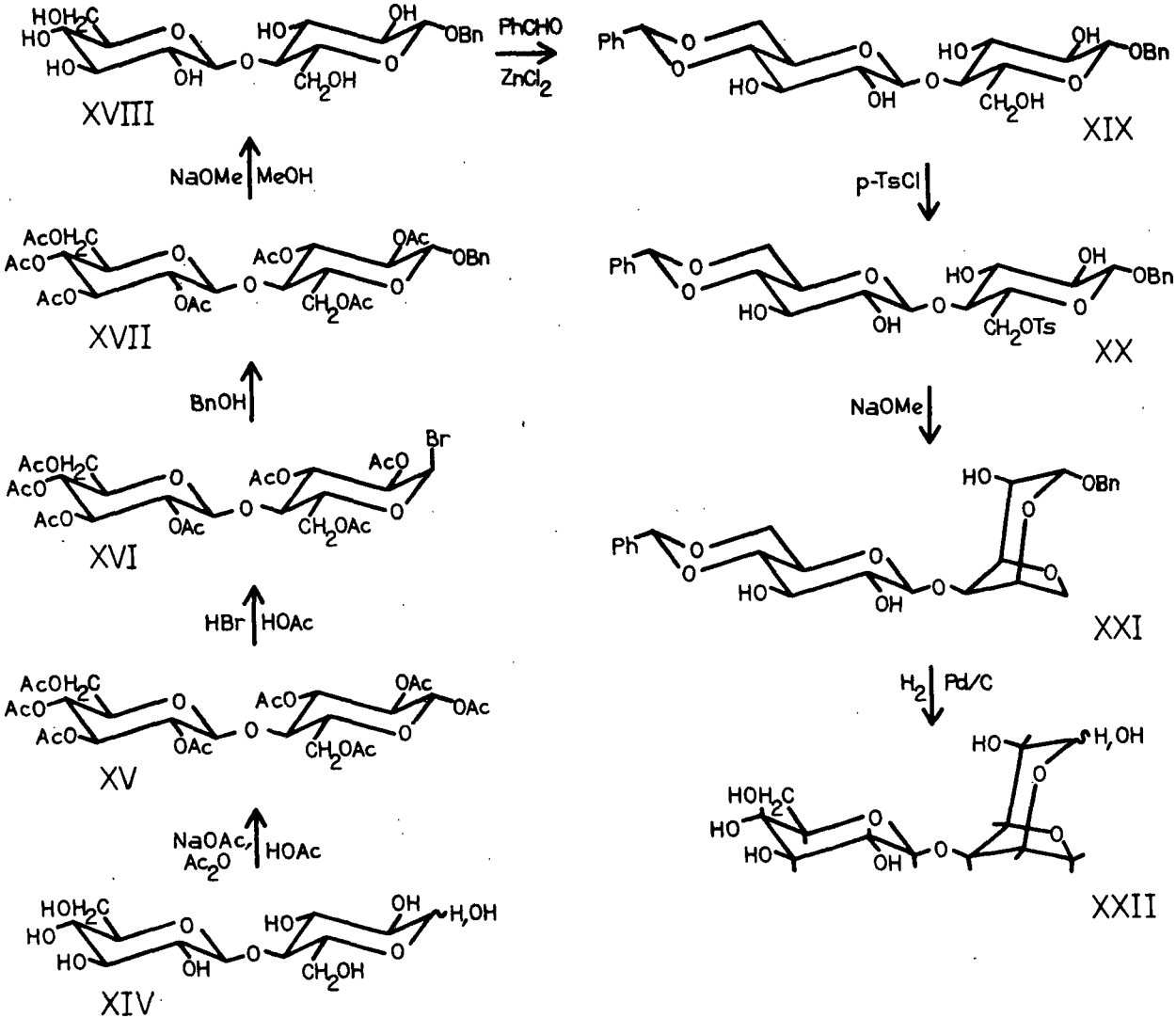
The 3,6-anhydro model compounds all gave positive responses to Fehling solution, characteristic of reducing sugars. In addition, they all restored color to Schiff reagent; this is indicative of 3,6-anhydro sugars, which apparently exist to some extent in the aldehydo form (28).

D-Glucose (XXIII) and cellobiose (XXIV) were obtained commercially and purified by recrystallization. 3-O-Methyl-D-glucose (XXV) was donated by Dr. L. R. Schroeder.

Characterizations of all six model compounds are given in the Experimental section.



Scheme I. Synthesis Routes for Preparation of 3,6-Anhydro-4-O-methyl-D-glucose (V) and 3,6-Anhydro-D-glucose (VI)



Scheme II. Synthesis Route for Preparation of
3,6-Anhydro-Cellobiose (XXII)

REACTIONS IN OXYGEN-FREE SODIUM HYDROXIDE

Standard conditions were used in a systematic study of alkaline degradation reactions of the selected model compounds. All reactions were conducted at 25°C in oxygen-free 0.099N sodium hydroxide under nitrogen atmospheres. An initial ratio of 4.95 equivalents NaOH per mole of reducing sugar was used for each reaction. Reactions were sampled with a nitrogen-purged syringe. Further details are provided in the Experimental section.

All gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS) was conducted with per-O-trimethylsilyl (TMS) derivatives of starting materials, reaction intermediates, products and reference compounds. When GLC and MS results are discussed, the chemical names used denote the actual compounds and their trimethylsilyl derivatives.

All GLC results were obtained on a 5% SE-30 column, with a single exception in the case of glucose products, where a 3% OV-17 column was also used. Conditions A (see Experimental) were used unless otherwise noted.

3,6-ANHYDRO-D-GLUCOSE

3,6-Anhydro-D-glucose (VI) was allowed to react with oxygen-free 0.099N sodium hydroxide in a nitrogen atmosphere for 10 days at 25°C. Samples were withdrawn periodically and titrated to determine alkali consumption. The graph of acids generated versus time is shown in Fig. 2. In terms of acids generated per mole of starting material, the reaction had a half-life of 14.4 hours and reached a plateau at 1.0 equivalent per mole after 6 days.

In this case, and that of 3-O-methyl-D-glucose presented in the following section, only one major product (D-glucometasaccharinic acid) was found, providing reasonable correlation with the observed generation of 1.0 equivalent

of acid per mole of starting material. For the other reducing sugars studied, however, greater numbers of products were formed, and the relationships between acidic products and titration data were more complex and not readily discernible.

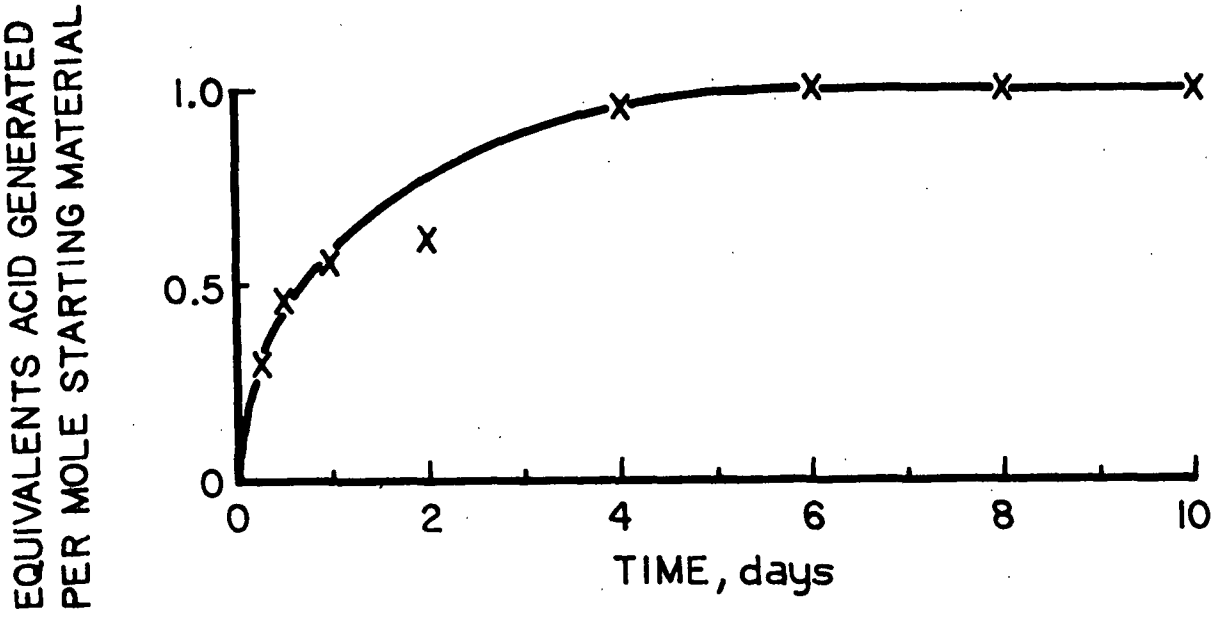


Figure 2. Acids Generated in the Reaction of 3,6-Anhydro-D-glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

Titrated reaction samples were analyzed by paper chromatography, which showed the gradual disappearance of 3,6-anhydro-D-glucose (R_G[1] 1.96) and formation of a single product having mobility identical to that of D-glucometasaccharinic acid (R_G 1.44).

The 10-day sample, analyzed by GLC, gave the results illustrated in Fig. 3. D-Glucometasaccharinic acid was the only major product, accounting for 87%[2] of

[1] R_G denotes the mobility ratio of the named compound to D-glucose.

[2] Product distributions are reported as percentages of total products observed by GLC, calculated on peak areas.

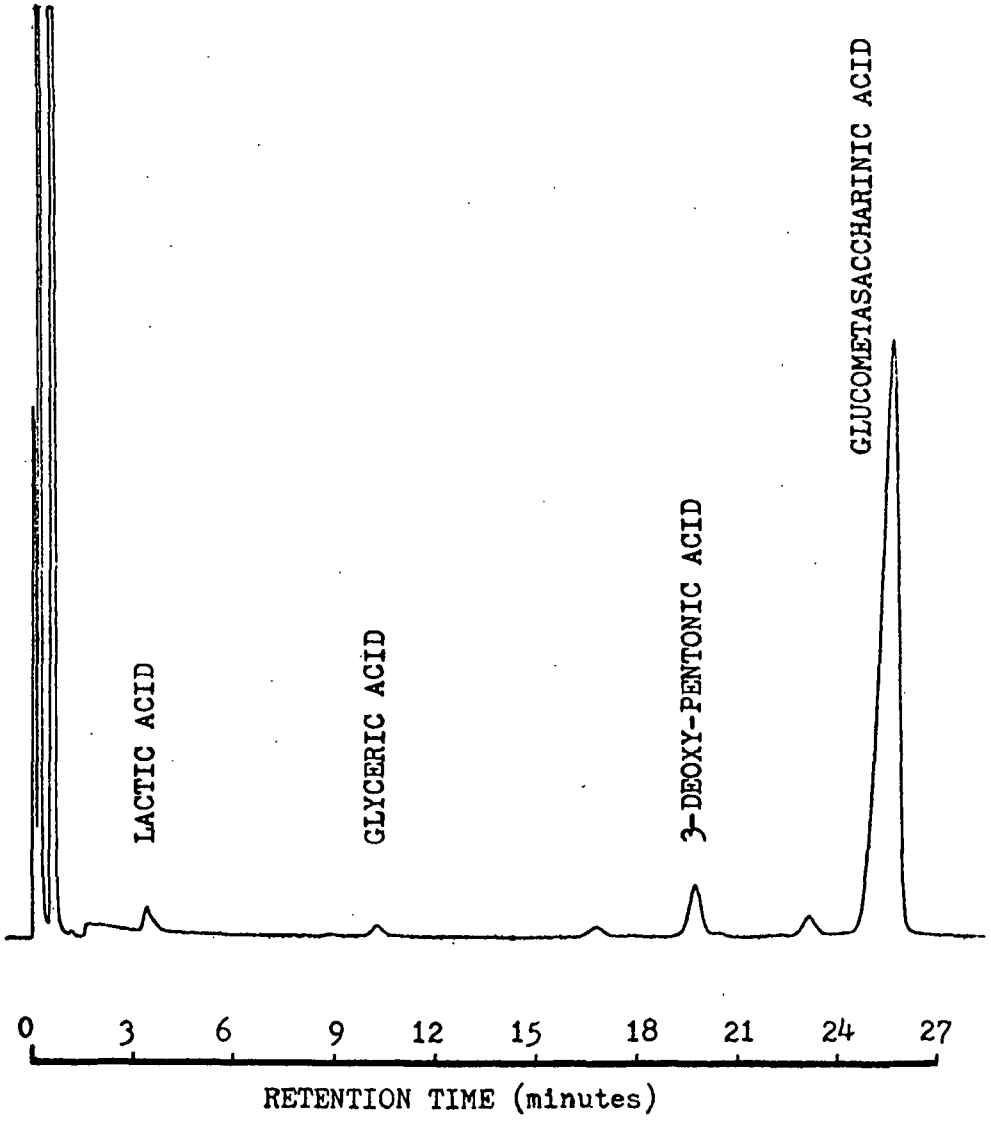


Figure 3. Gas Chromatograms of Products from Reaction of 3,6-Anhydro-D-glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

the products observed. In addition, trace amounts of lactic, glyceric and 3-deoxy-pentonic acids were found, assigned by correlation (according to GLC retention times) with products from cellobiose degradation identified by mass spectrometry.

Following lactonization, the sole important product (GLC, T_r[1] 22.0 min) corresponded to D-glucometasaccharino-1,4-lactone.

Formation of D-glucometasaccharinic acid as the dominant product in this reaction is consistent with the Nef-Isbell β-alkoxy elimination theory[2], and with the results of Corbett and Kenner (29). They reported quantitative yields of the glucometasaccharinic acid isomers from reaction of 3,6-anhydro-D-glucose with oxygen-free 0.04N calcium hydroxide at 25°C. A potential reaction pathway is shown in Fig. 4.

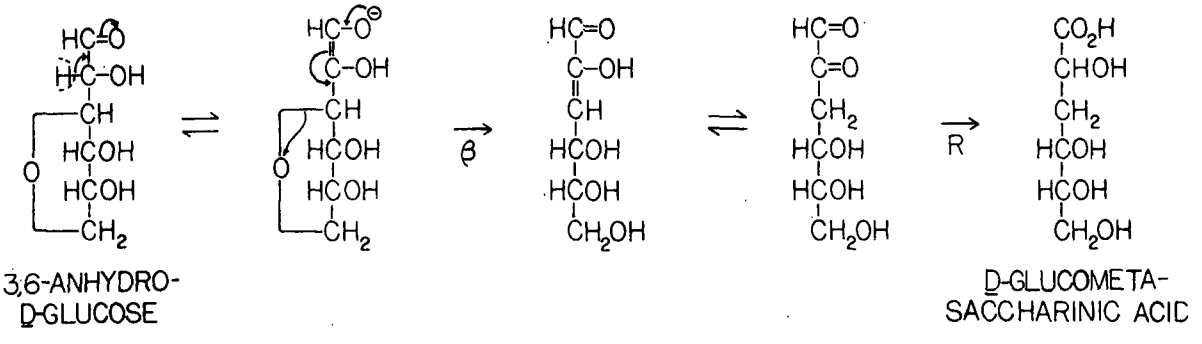


Figure 4. Possible Reaction Mechanism for Formation of D-Glucometasaccharinic Acid from 3,6-Anhydro-D-glucose; β — β-Alkoxy Elimination Step; R — Benzil-Benzilic Acid Type Rearrangement

[1] T_r denotes GLC retention time.

[2] The Nef-Isbell β-alkoxy elimination theory refers to eliminations (beta to carbonyl groups) and subsequent steps as shown in Fig. 1 and 4 and analogous schemes; see References (1,2,24,26).

Clearly, the reaction is not quantitative in 0.099N NaOH, as shown by the formation of small amounts of fragmentation products. These were not evident by paper chromatography, and therefore may also have gone unobserved by Corbett and Kenner. Alternatively, fragmentation products may be more characteristic of NaOH reactions than of the $Ca(OH)_2$ reactions studied by Corbett and Kenner (24); this concept will be dealt with in the discussion of glucose reactions.

3-O-METHYL-D-GLUCOSE

3-O-Methyl-D-glucose (XV) was allowed to react with oxygen-free 0.099N NaOH in a nitrogen atmosphere for 7 days at 25°C. The graph of acids generated versus time is shown in Fig. 5. In terms of acids generated, the reaction had a half-life of 24 hours; completion was reached in 7 days, with production of 1.0 equivalent of acid per mole of starting material.

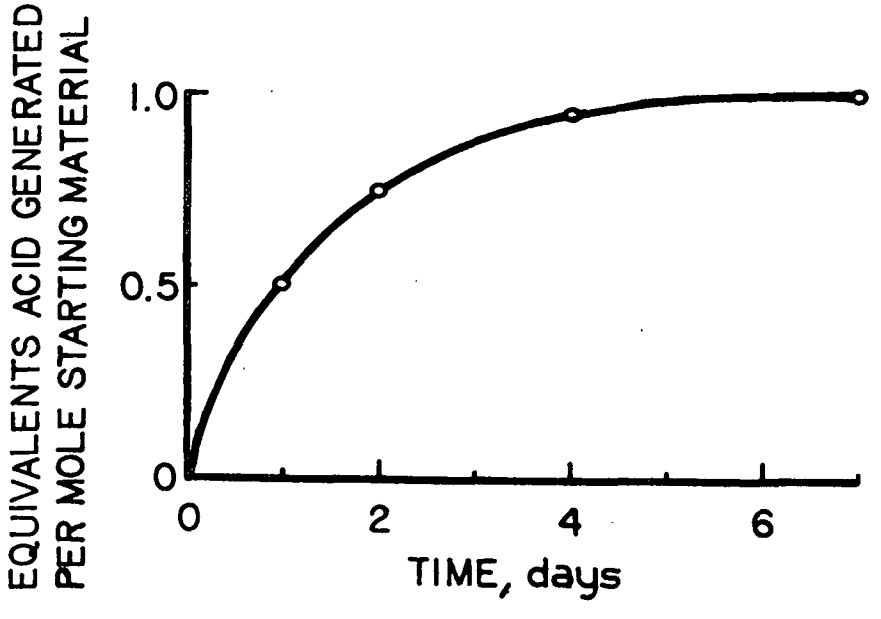


Figure 5. Acids Generated in the Reaction of 3-O-Methyl-D-glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

Gas-liquid chromatographic analysis of the 7-day sample showed a single major product, D-glucometasaccharinic acid (Fig. 6), accounting for 89% of the observed products, the remainder being trace components. Following lactonization, D-glucometasaccharino-1,4-lactone (T_r 22.0 min) was observed as the only major peak.

Again, these results in dilute NaOH are consistent with the prediction of the β-alkoxy elimination theory for a reducing sugar substituted at C-3. The dominant product, D-glucometasaccharinic acid, is probably formed via a route analogous to that illustrated in Fig. 4.

Kenner and Richards (18) reported only D-glucometasaccharinic acid from reaction of 3-O-methyl-D-glucose with oxygen-free 0.04N $Ca(OH)_2$ at 25°C. The trace products found by GLC (Fig. 6), if they were formed in dilute $Ca(OH)_2$, probably would not have been observed by Kenner and Richard's paper chromatographic techniques. In addition, they conducted an analogous reaction of 3-O-methyl-D-glucose in oxygen-free 0.05N KOH and found the initial rate to be slower than that in $Ca(OH)_2$. The reaction in NaOH (this work) also exhibited a slower initial reaction rate than in $Ca(OH)_2$ (18). However, the reactions in NaOH, $Ca(OH)_2$ and KOH all reached completion at 1.0 equivalent of acid in 7-8 days.

D-GLUCOSE

D-Glucose (XXIII) was allowed to react with oxygen-free 0.099N sodium hydroxide in a nitrogen atmosphere for 76 days at 25°C. The graph of acids generated versus time is presented in Fig. 7 (lower curve). The reaction was complete after 60 days, at a plateau of 1.95 equivalents of acid per mole of starting material. The reaction had a half-life of 9 days in terms of acid

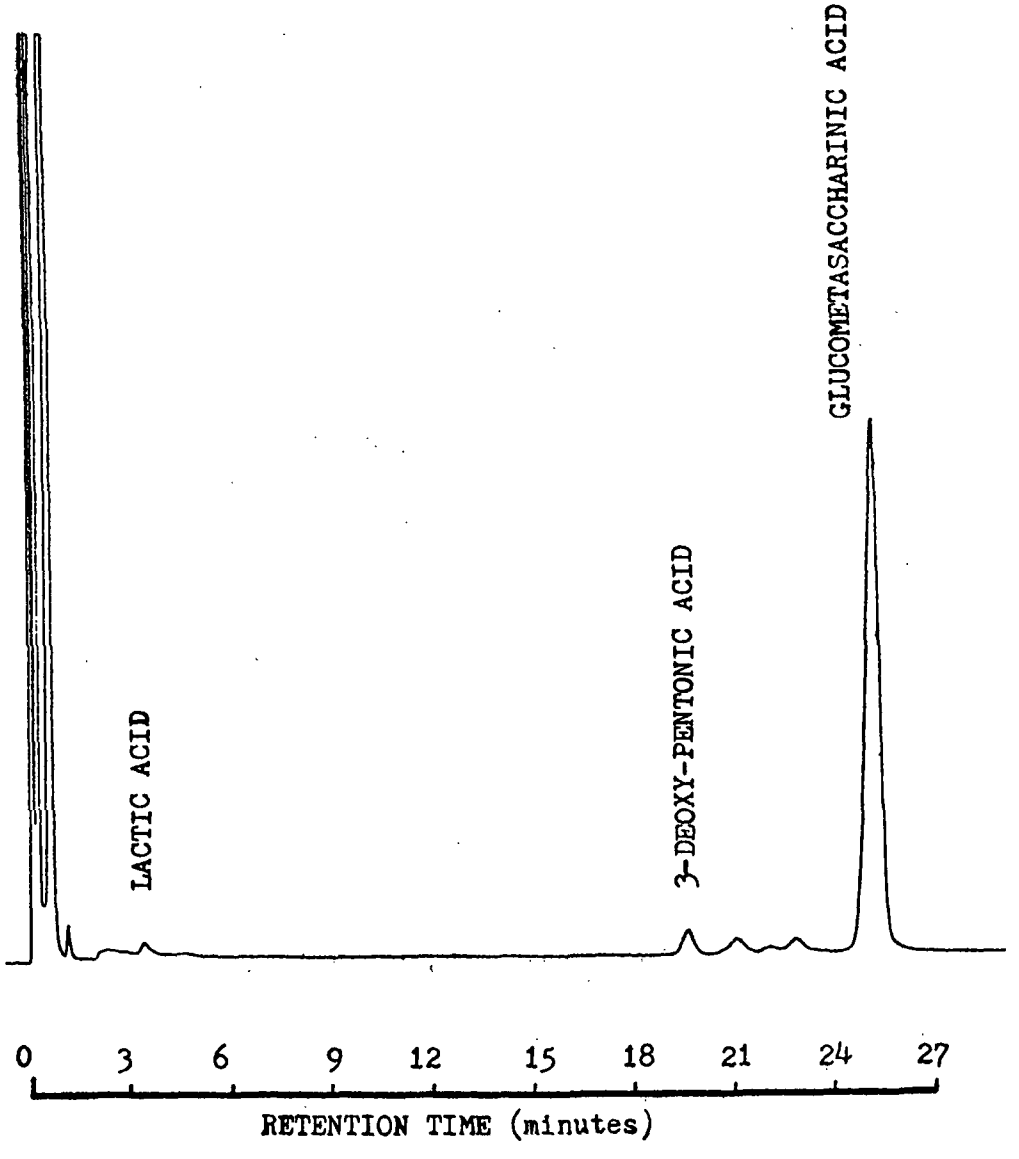


Figure 6. Gas Chromatogram of Products from Reaction of 3-O-Methyl-D-glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

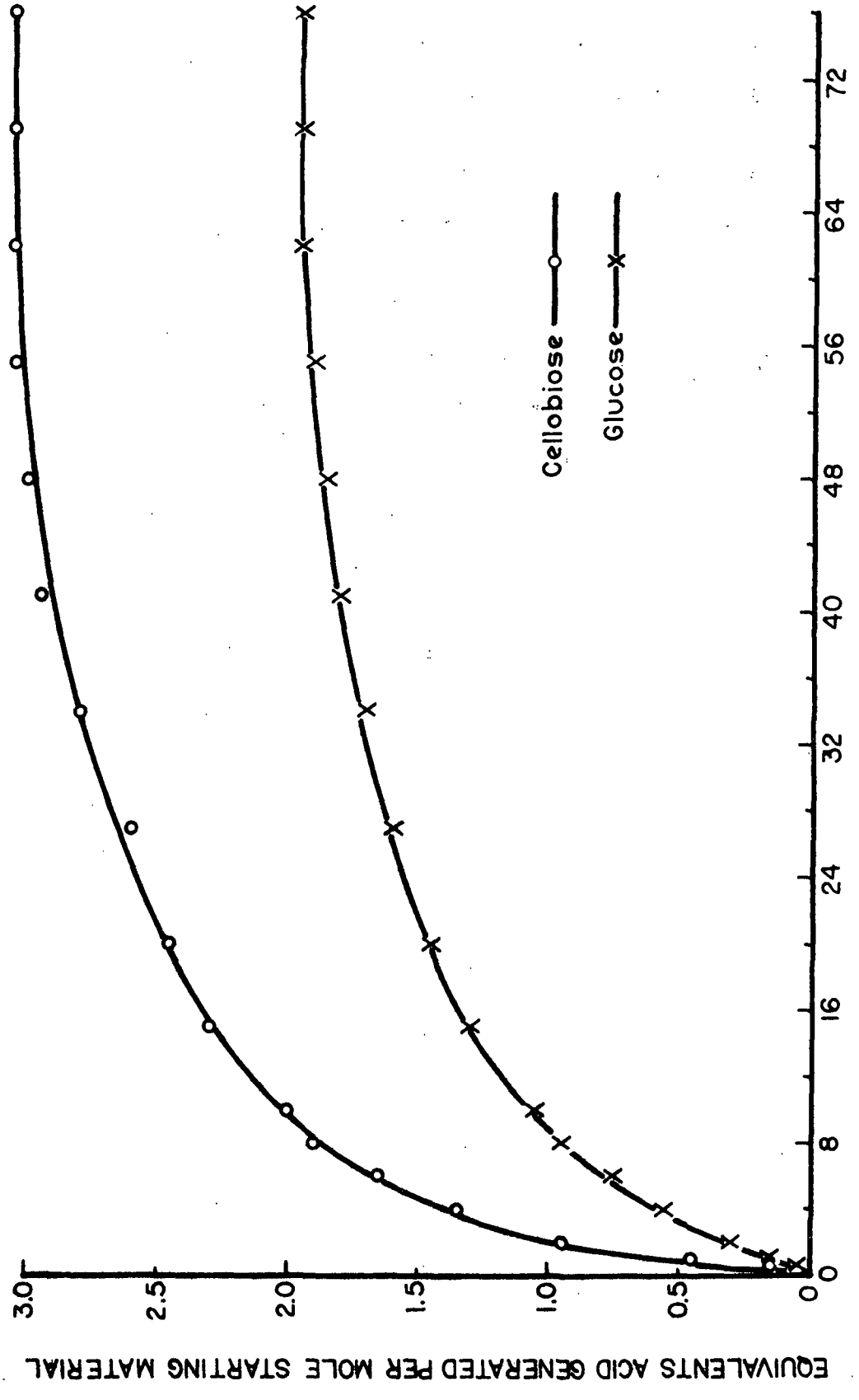


Figure 7. Acids Generated in the Reactions of D-Glucose and Cellobiose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

equivalents generated. Thus, D-glucose was found to be considerably less reactive than 3,6-anhydro-D-glucose or 3-O-methyl-D-glucose; reasons for this difference in reactivity are discussed later in this section.

Paper chromatography showed a very gradual disappearance of glucose accompanied by the formation of several products having R_G values greater than 1.0. One product, R_G 1.44, corresponded to D-glucometasaccharinic acid.

Analysis of the 76-day sample by GLC provided the results shown in Fig. 8. Lactic acid accounted for 56% of the products observed. Its retention time (3.4 min) was identical to that of authentic lactic acid. Glyceric acid, a minor product, comprised 5% of the product mixture; it had a retention time (10.2 min) identical to that of the authentic compound.

2,4-Dihydroxybutyric acid represented 14% of the products, and initially was identified as having the same retention time (18.0 min) on a 3% OV-17 column that Ericsson, et al. (30) reported for this compound. Subsequently, the product mixture from glucose degradation was separated by GLC on a 5% SE-30 column, and mass spectrometry was used to identify Products 3 and 5. Product 3 gave the mass spectrum illustrated in the upper portion of Fig. 9. Below it is reproduced the mass spectrum of 2,4-dihydroxybutyric acid reported by Petersson (31).

Alpha-carbon chain cleavage provides the most abundant ions in the mass spectra of aldonic and deoxy-aldonic acids (31). Also, the 3-deoxy-aldonic acids (or metasaccharinic acids) favor successive α-carbon cleavages from the alcohol end of the molecule. In the case of 2,4-dihydroxybutyric acid (XXVI), cleavage at the C-3–C-4 bond is particularly important, producing an ion of m/e 103 which forms the base peak in the spectrum. 2,4-Dihydroxybutyric acid is the only 3-deoxy-aldonic acid exhibiting a base peak at m/e 103.

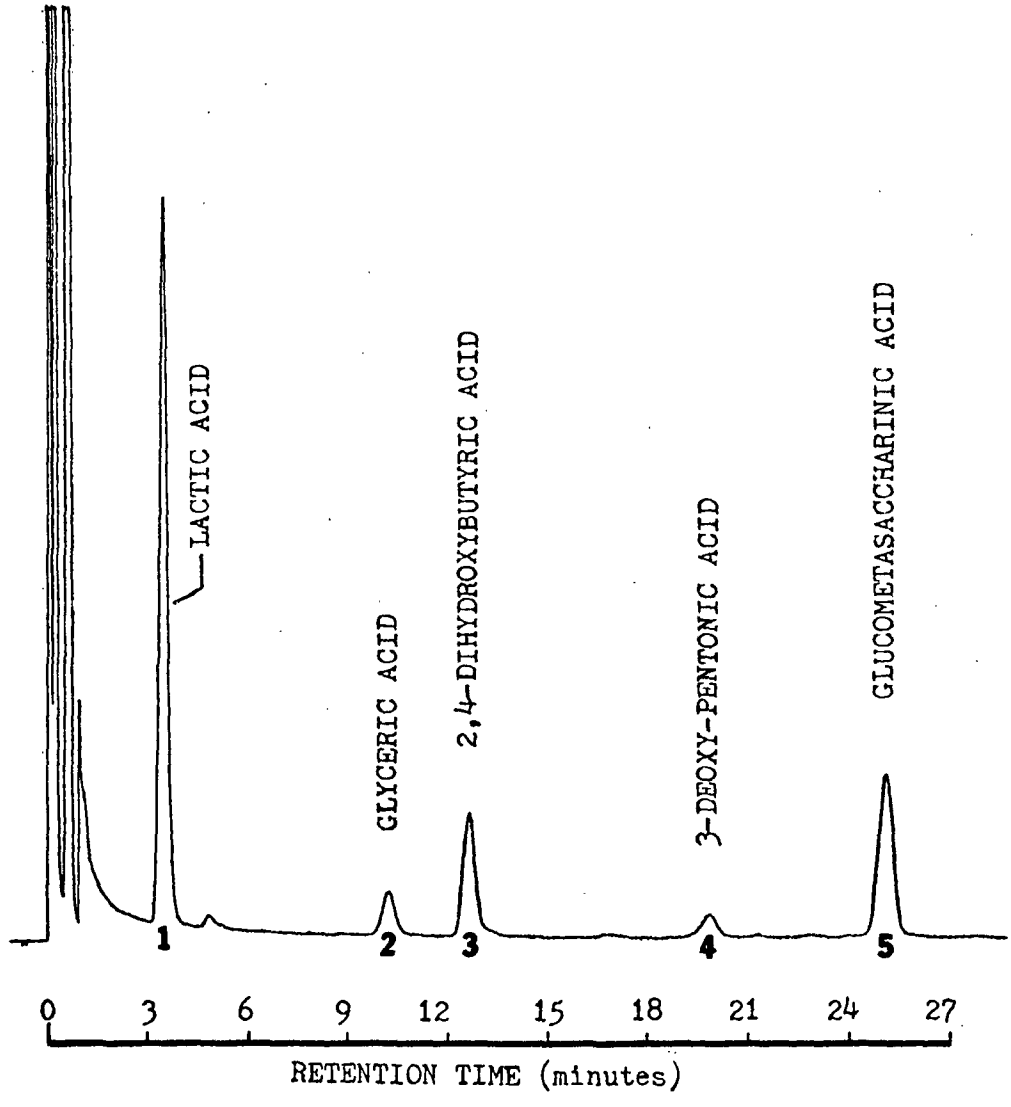


Figure 8. Gas Chromatogram of Products from Reaction of D-Glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

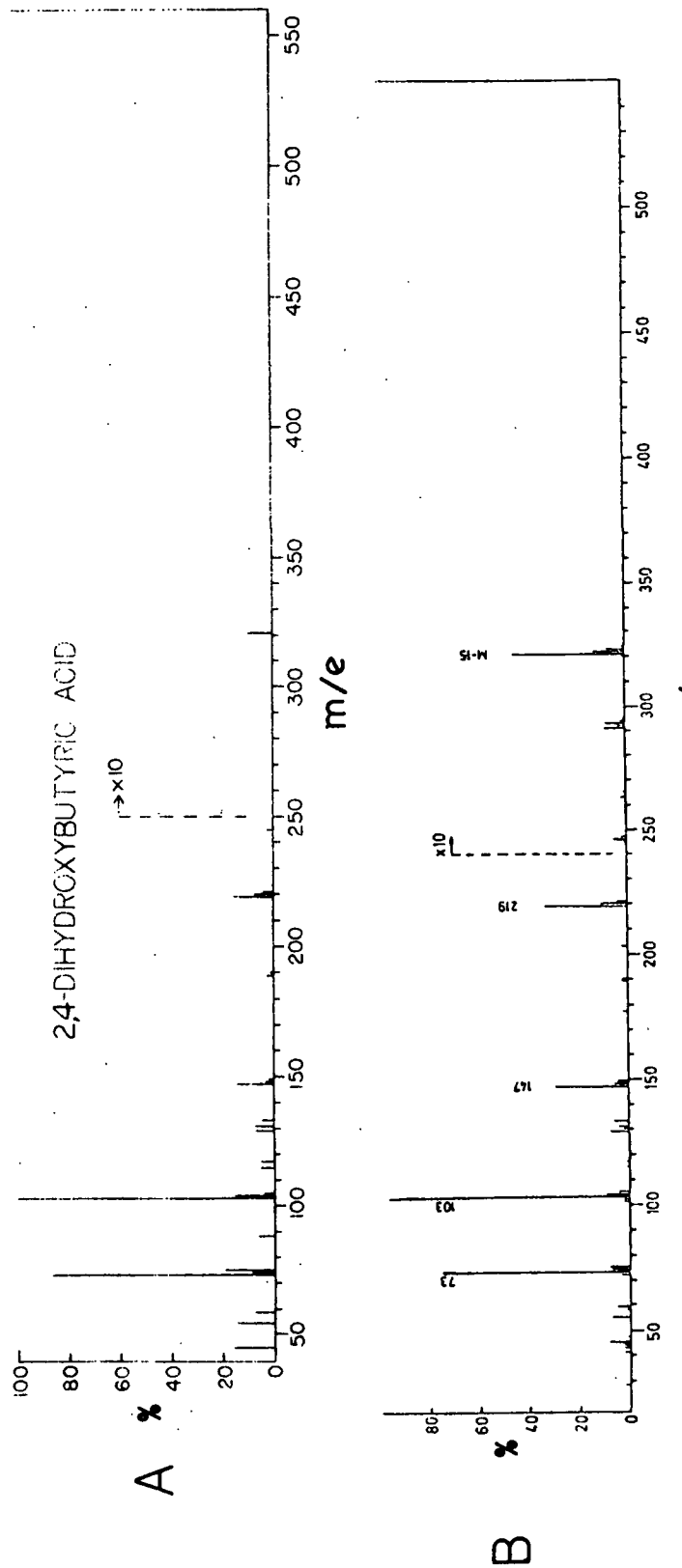
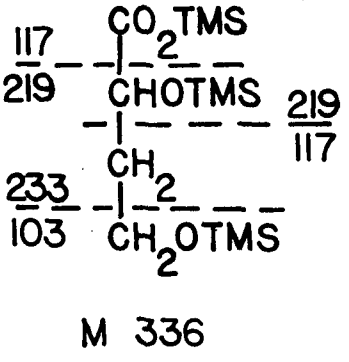


Figure 9. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of 2,4-Dihydroxybutyric Acid; A — This Work; B — Petersson (31)

In addition, deoxy-aldonic acids often provide high abundances of ions resulting from cleavage between carbon atoms alpha and beta to the methylene carbon (32). Thus, in the case of 2,4-dihydroxybutyric acid, the intense peak at m/e



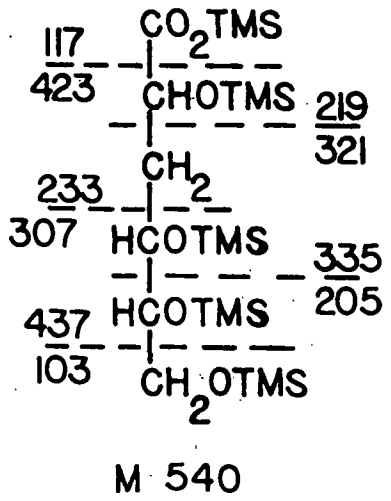
XXVI

219 arises from C-1–C-2 cleavage (i.e., between carbon atoms alpha and beta to the deoxy site); also, C-2–C-3 cleavage yields an ion of m/e 219. The peak at m/e 321 corresponds to the M-15 ion, formed by loss of a methyl group from the parent ion (M^+). Peaks at m/e 73 and 147 are typical of the mass spectra of all trimethylsilyl compounds and have no structural significance (31).

3-Deoxy-D-pentonic acid constituted only 3% of the observed products. It was identified by GLC retention time (19.5 min) identical to a cellobiose reaction product (see following section) confirmed by mass spectrometry as 3-deoxy-D-pentonic acid.

D-Glucometasaccharinic acid accounted for 22% of the product mixture and was the only six-carbon product observed. Its GLC retention time (25.2 min) was identical to that of the authentic material; lactonization of the product mixture gave a peak (T_r 22.0 min) corresponding to D-glucometasaccharino-1,4-lactone.

Analysis by GC-MS provided the mass spectrum of D-glucometasaccharinic acid shown in Spectrum A of Fig. 10; below it is Petersson's (31) spectrum of the same compound.



XXVII

Particularly characteristic of fragmentation of D-glucometasaccharinic acid (XXVII) is cleavage between C-4 and C-5, producing a prominent peak at m/e 335. Subsequently, this ion loses trimethylsilanol (TMSiOH), providing a very intense peak at m/e 245. In the mass spectra of D-glucometasaccharinic acid from glucose, cellobiose and 3,6-anhydro-4-O-methyl-D-glucose, the m/e 245 ion frequently formed the base peak and was considered diagnostic of the compound.

Other important peaks include the series m/e 423 (not seen)→333→243 corresponding to M-117-$(90)_n$, representing C-1–C-2 cleavage followed by loss of TMSiOH groups; m/e 525, the M-15 peak; m/e 437→347, originating from C-5–C-6 cleavage, then loss of TMSiOH; m/e 217, formed from the ion m/e 245 by loss of CO; and chain cleavage ions at m/e 103, 117, 205 and 219. Note that in this case, as in all other mass spectra described herein, a recurring theme is loss of trimethylsilanol, giving pairs of ions differing by 90 mass units.

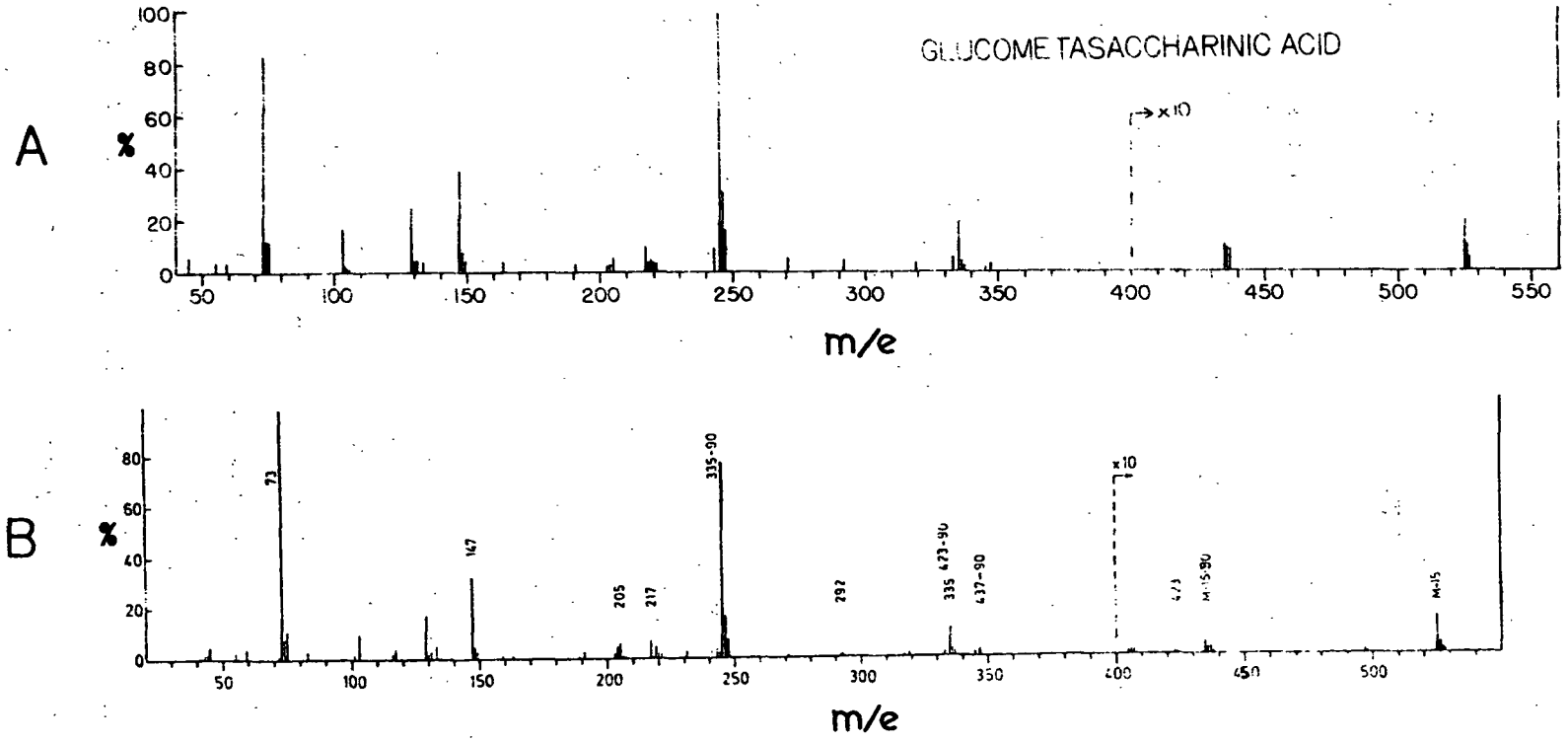


Figure 10. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of D-Glucometasaccharinic Acid; A — This Work; B — Petersson (31)

Several samples taken during the reaction of glucose with oxygen-free NaOH were also analyzed by GLC, providing the chromatograms shown in Fig. 11. Titration data corresponding to these samples are presented in Table I.

TABLE I

SELECTED TITRATION DATA FOR REACTION OF GLUCOSE WITH OXYGEN-FREE 0.099N NaOH AT 25°C

Sample Time, days	Equivalents Acid Generated per Mole Starting Material
0	0.00
2	0.30
6	0.75
30	1.50
55	1.95

Analysis of the GLC results confirmed the slow disappearance of D-glucose, formation (by Lobry de Bruyn-Alberda van Ekenstein isomerization) (33) and disappearance of fructose, and formation of the five final products also shown in Fig. 8. Because the peaks corresponding to α-glucose, fructose and D-glucometasaccharinic acid overlapped, it was not possible to obtain quantitative data for the disappearance of glucose (and isomeric species) as a function of time.

The reaction mechanisms probably operative in the formation of D,L-lactic and D-glucometasaccharinic acids from D-glucose are illustrated in Fig. 12. Lactic acid, the predominant product, stems from dealdolization at C-3–C-4 (24). The result is formation of two three-carbon products, both of which can undergo base-catalyzed isomerization, β-elimination of a hydroxyl group, and subsequent benzil-benzilic acid type rearrangement to produce lactic acid. Only a small amount of glyceric acid was formed. Glyceraldehyde, an

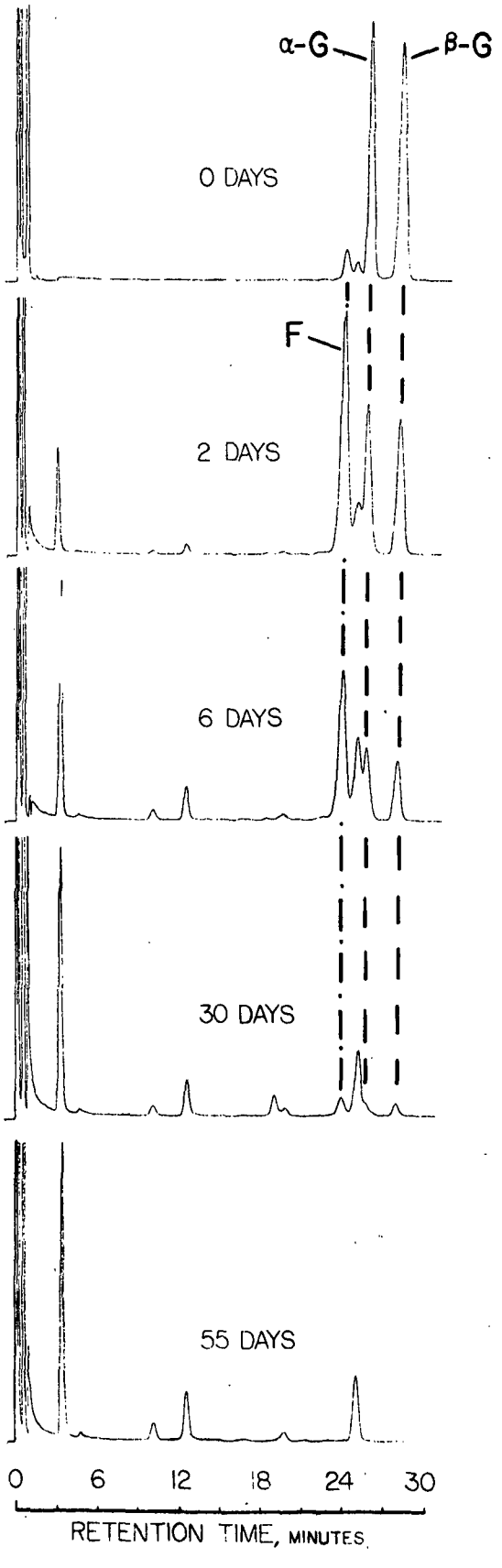


Figure 11. Gas Chromatograms of Intermediates and Products from Reaction of D-Glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C; G = Glucose; F = Fructose

intermediate in Fig. 12, is known to form lactic acid in oxygen-free NaOH (34) and appears not to be the source of glyceric acid. Glyceric acid was also observed as a minor product in degradations of D-glucose (15) and cellobiose (13,15), but a suitable mechanism for its formation remains unclear.

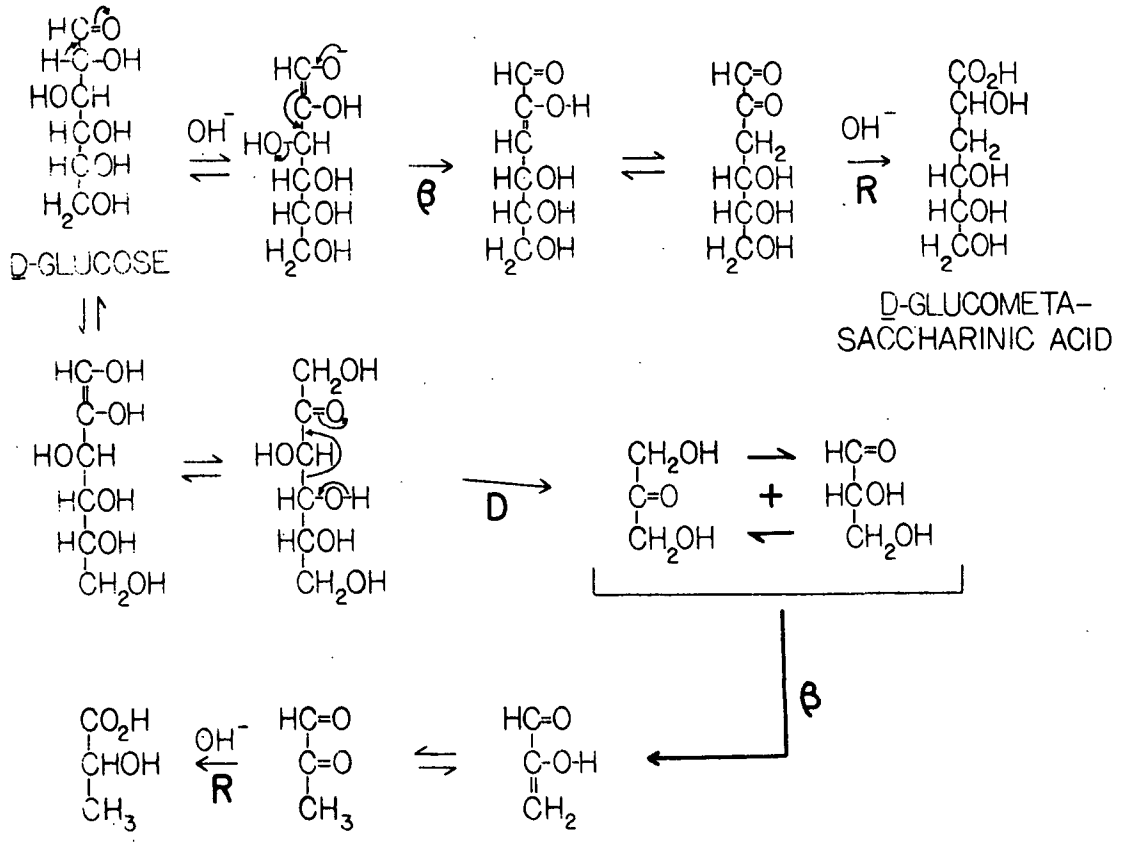
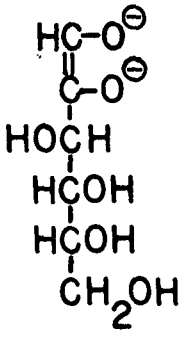


Figure 12. Possible Reaction Mechanisms for Formation of D-Glucometasaccharinic and D,L-Lactic Acids from D-Glucose; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement; D — Dealdolization

D-Glucometasaccharinic acid, the second most abundant product from glucose, is generally agreed (24) to emanate from β-elimination of the hydroxyl group at C-3, keto-enol tautomerization to a 1,2-dicarbonyl intermediate, and rearrangement to the 3-deoxy-aldonic acid.

In the alkaline reaction medium, ionization of hydroxyl groups must be taken into account. In particular, the degree to which the C-3 hydroxyl group of glucose exists as its conjugate base decreases the concentration of substrate able to undergo β-elimination at C-3. This, in turn, is a possible reason for the slower reaction of D-glucose relative to 3,6-anhydro-D-glucose and 3-O-methyl-D-glucose; in the latter cases, the C-3 ether substituents cannot ionize. Also, as ionization of the C-3 hydroxyl group of glucose hinders the β-elimination step in the stabilization sequence, comparatively less D-glucometasaccharinic acid is produced than from the C-3-substituted glucose derivatives. Instead, the reaction is directed primarily toward formation of lactic acid via dealdolization.

In Fig. 12, a monoanionic intermediate was shown preceding the β-elimination of OH-3 leading to D-glucometasaccharinic acid. Such monoanionic species are commonly postulated in the Isbell theory (9). It has also been proposed (17,35) that dianionic species (e.g., XXVIII) are intermediates in alkaline degradation reactions, and that their importance could be equal to or greater than that of monoanionic species.



XXVIII

In particular, Young, et al. (35) proposed that β-elimination of OH-3 from D-glucose under Nef's (10) conditions (8N NaOH, 100°C) was possible because of "coulombic assistance from a second anionic group in the molecule." That is,

the highly alkaline solution converted D-glucose to its dianionic form. They stated that at low alkali concentrations (<0.1M), the monoanion would be the predominant anion species. Yet the product distribution from degradation of D-glucose in oxygen-free 0.099N NaOH at 25°C was remarkedly similar to that reported by Nef under much stronger conditions, as discussed below. It appears, therefore, that a monoanionic intermediate adquately explains β-elimination of OH-3 from D-glucose, and that a dianionic species need not be invoked [see also (24)].

Figure 13 illustrates a possible route for formation of 2,4-dihydroxybutyric acid (36). Similar to the mechanism proposed for lactic acid formation, it involves dealdolization (in this case, at C-2–C-3), β-elimination of OH-3 in the resulting tetrose, then benzil-benzilic acid type rearrangement to form 2,4-dihydroxybutyric acid. Glycolaldehyde is the two-carbon fragment. Typically, aldehydes are not detected among the products of these reactions. However, recombination via aldol condensation is known to occur in alkaline solution (24), and it may be that glycolaldehyde undergoes a self-condensation to generate a tetrose, which in turn degrades to generate more 2,4-dihydroxybutyric acid (10).

The rearrangement of a 1,2-dicarbonyl intermediate to a 3-deoxy-aldonic acid diagrammed in detail in Fig. 13 is representative of the process apparently involved in formation of all of the 3-deoxy-aldonic acids described herein.

A number of workers have reported 3,4-dihydroxybutyric acid as the only four-carbon deoxy-aldonic acid from oxygen-free alkaline degradation of glucose (15) and cellobiose (13,15). In these instances, mass spectrometry was not employed. For 3,4-dihydroxybutyric acid (XXIX), the mass spectrum is quite different from that of 2,4-dihydroxybutyric acid. The spectrum of

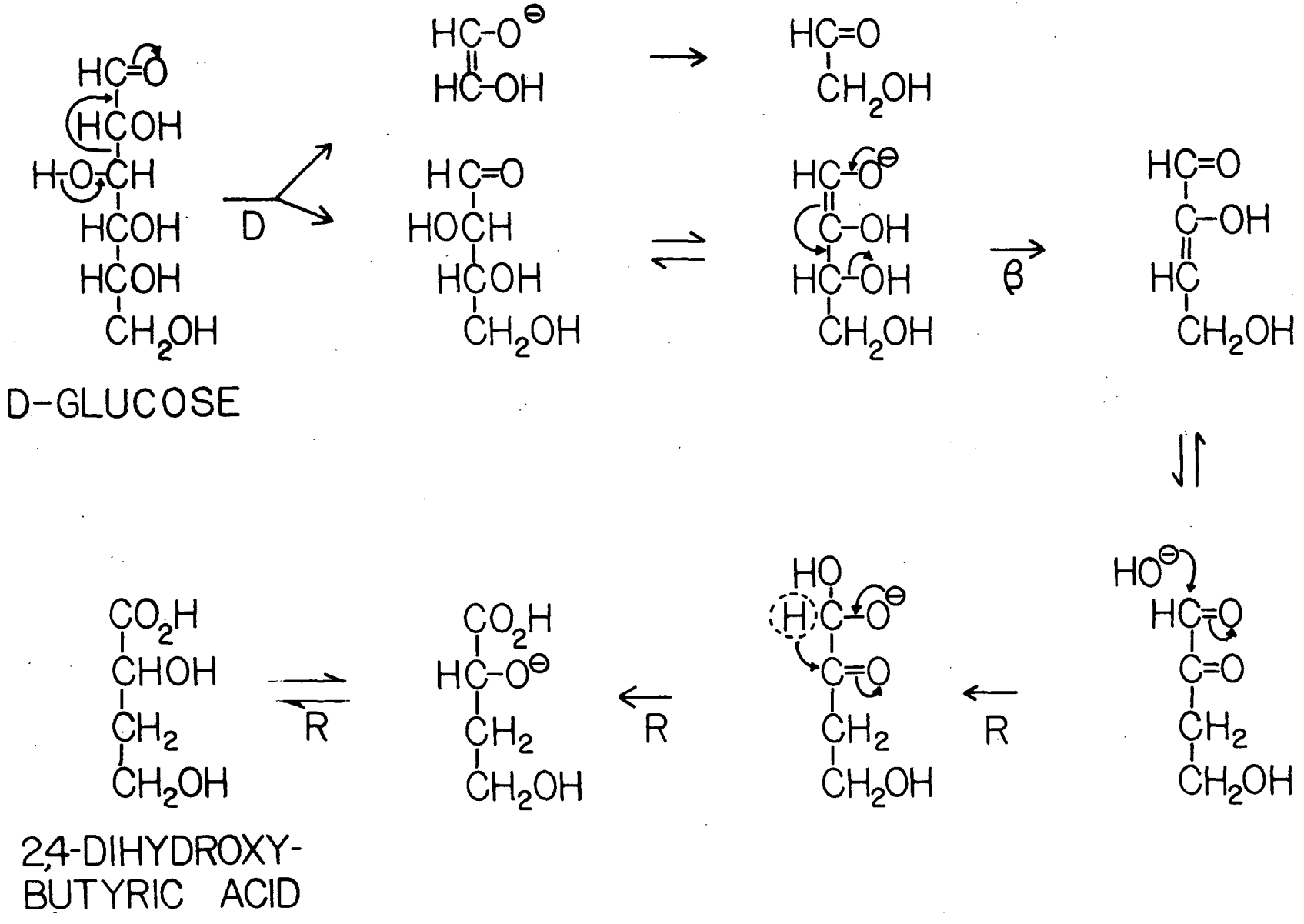
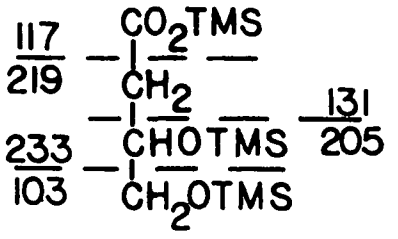


Figure 13. Possible Reaction Mechanism for Formation of D,L-2,4-Dihydroxybutyric Acid from D-Glucose; D — Dealdolization; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement



XXIX

3,4-dihydroxybutyric acid (31) has substantial peaks at m/e 233 (cleavage at C-3–C-4) and 189 (loss of CO_2 from the 233 ion); also, only a trace peak is observed at m/e 103. No 3,4-dihydroxybutyric acid was found among the glucose degradation products reported here. The reason for this apparent difference may be that reactions in the presence of oxygen were also conducted in the work cited, and formation of the 3,4-acid in oxygen systems led the authors to assume that the same occurred in their nitrogen systems. Clearly, 2,4-dihydroxybutyric acid was the only tetronic acid produced from reaction of glucose with dilute oxygen-free NaOH at 25°C, and it is the logical four-carbon member of the 3-deoxy-aldonic acid series. Again, nonasymmetric intermediates provide only the racemic (D,L) product.

3-Deoxy-pentonic acid (presumably the D-erythro and -threo isomers), a minor product from D-glucose, was also reported as a degradation product of glucose in oxygen-free NaOH by Green (37) and Malinen and Sjostrom (15). In neither case was a mechanism proposed for its formation. One possibility is illustrated in Fig. 14, where formation of a C-3 carbonyl intermediate via ene-diol tautomerization (38) permits C-1–C-2 dealdolization, resulting in a pentose sugar and formaldehyde. The pentose then follows the familiar β-elimination and acid rearrangement sequence to produce 3-deoxy-D-pentonic acid. Ishizu, et al. (39) previously showed that 3-deoxy-D-pentonic acid was a product of oxygen-free 8N NaOH degradation of D-fructose.

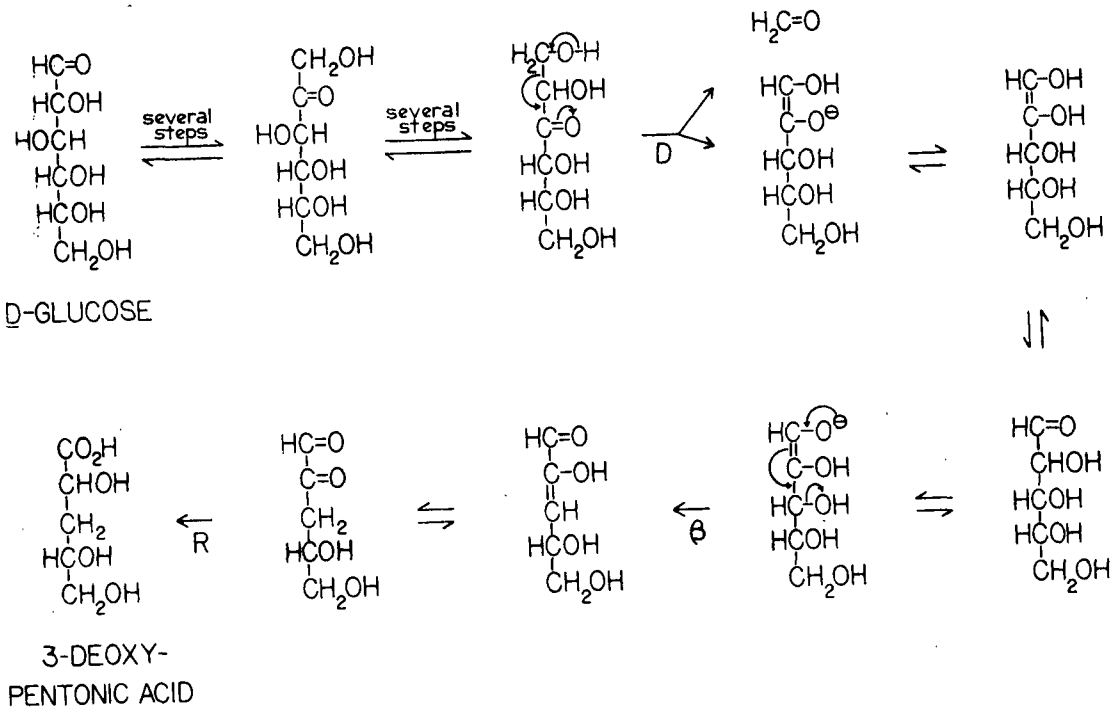


Figure 14. Possible Reaction Mechanism for Formation of 3-Deoxy-D-pentonic Acid from D-Glucose; D — Dealdolization; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement

The fate of formaldehyde in this reaction is unknown; it was neither sought nor detected in the degradations reported here.

Earlier work on oxygen-free alkaline degradation of D-glucose produced various results, some of them conflicting (see Table II). The earliest comprehensive study, by Nef (10), gave the results previously described in the Introduction (p. 6). Nef determined that three major products were formed in oxygen-free 8N NaOH degradation of D-glucose: D,L-lactic acid, D,L-2,4-dihydroxybutyric acid, and D-glucometasaccharinic acid (α- and β-isomers). He also reported a small yield of D-glucoisosaccharinic acid. Despite considerably different reaction conditions (e.g., alkali strength, temperature, duration of reaction), Nef's results and those reported here correlate rather well. Glyceric and 3-deoxy-pentonic acids, observed as minor products by GLC, probably would not

TABLE II

ACIDIC PRODUCTS FROM OXYGEN-FREE ALKALINE DEGRADATION REACTIONS OF D-GLUCOSE[a,b]

	Nef (10), NaOH	Shaffer, et al. (40), KOH	Kenner, et al. (17), $Ca(OH)_2$	Green (37), NaOH	Corbett, et al. (22), $Ca(OH)_2$	Corbett, et al. (22), NaOH	Malinen, et al. (15), NaOH	This work, NaOH	This work, $Ca(OH)_2$
Lactic	++	++	++	++	++	++	++	++	++
Glycolic							+		
Glyceric							+	+	
2-C-Methyl-glyceric									+
2,4-Dihydroxybutyric	++	++	+	++	++	+		++	++
3-Deoxy-pentonic				t			+	+	+
Glucometasaccharinic	++	++		++	++	++	++	++	++
Glucoisosaccharinic	+				+	+			
Glucosaccharinic			++		++	+			++

[a] ++ Denotes major product; + denotes minor product; t denotes trace product.

[b] The results included in this table were obtained at initial alkali concentrations ranging from 0.0375N to 8N and temperatures from 25 to 120°C; see text.

have been isolated by Nef's techniques had they been formed. The reaction in dilute NaOH at 25°C showed no evidence of formation of D-glucoisosaccharinic acid, either in GLC analysis of lactonized final products or in mass spectral data for D-glucometasaccharinic acid. (Though the two six-carbon acids overlapped by GLC, MS revealed the presence of more than one acid in the peak — this point is discussed in the following section on cellobiose.)

Shaffer and Friedmann (40) conducted oxygen-free KOH degradations of D-glucose under various conditions. For example, reaction at 37-40°C in 0.1N KOH produced approximately equal amounts of D,L-lactic acid and all other acids combined (described as 4- and 6-carbon acids).

Kenner and Richards (17) degraded D-glucose in oxygen-free 0.0375N $Ca(OH)_2$ at 25°C, and obtained lactic acid as the dominant product. Also, they reported the sole 6-carbon product to be D-glucosaccharinic acid, not D-glucometasaccharinic acid. 2,4-Dihydroxybutyric acid was variously described as an important and a very minor product. It is known that alkaline degradation in the presence of divalent cations (as opposed to monovalent ones) can produce different compounds or affect the relative proportions of products (8-16). However, Kenner and Richards' results are not in accord with the generally consistent picture of glucose degradation provided by other workers, especially with respect to D-glucometasaccharinic acid.

Hot 8N NaOH treatment of D-glucose by Green (37) gave a mixture of products which were converted to anilide derivatives and separated on a cellulose column. The anilides of 2,4-dihydroxybutyric acid and β-D-glucometasaccharinic acid were obtained in crude yields of 11 and 20%, respectively. Green also reported low yields of two five-carbon anilides, presumably 3-deoxy-pentonic acids.

Investigating glucose degradation in oxygen-free saturated $Ca(OH)_2$ and 0.05N NaOH at 25°C, Corbett and Liddle (22) found lactic acid to be the major product in both cases. Formation of 2,4-dihydroxybutyric acid was 1.6 times greater in $Ca(OH)_2$; formation of D-glucometasaccharinic acid was 1.7 times greater in NaOH. D-Glucosaccharinic acid was 2.7 times more abundant in $Ca(OH)_2$. There was evidence for D-glucoisosaccharinic acid in both reactions, and unidentified products were present in both cases. These results give credibility to the concept of formation of glucometasaccharinic and glucosaccharinic acids from $Ca(OH)_2$ degradation of glucose. A reinvestigation of this reaction was performed, using GLC and GC-MS techniques. The results, presented below in the discussion of $Ca(OH)_2$ reactions, confirmed that both six-carbon acids were indeed produced, though not in the proportions found by Corbett and Liddle. Their observation of six-carbon acids other than glucometasaccharinic acid in the NaOH reaction disagrees with the results reported here as well as those of Green (37) and Malinen and Sjostrom (15).

In oxygen-free 0.25N NaOH at 120°C, Malinen and Sjostrom (15) found the following acid products from D-glucose: lactic — 56.6%; glycolic — 3.2%; glyceric — 2.2%; 3-deoxy-pentonic — ∿1.3%; D-glucometasaccharinic — 27.7%. These results are very similar to those reported in this work with regard to formation of lactic, 3-deoxy-pentonic and glucometasaccharinic acids. As in this work, Malinen and Sjostrom used GLC to analyze their reaction products; however, they lactonized product mixtures prior to derivatization to TMS ethers. For the product 2,4-dihydroxybutyric acid, our results differed considerably: they reported none, whereas degradation at 25°C (this work) gave a yield of 14%. Also, they obtained a 3.2% yield of glycolic acid; however, in the present work at 25°C, no product was observed which correlated with the retention time (4.0 min) of authentic glycolic acid (see Fig. 8). In both cases, glyceric acid was a minor product.

Although similar results were reported earlier for reactions in 8N NaOH at 100°C (10,37,39), this work demonstrated that the complete series of 3- to 6-carbon 3-deoxy-aldonic (i.e., metasaccharinic) acids are also produced from D-glucose under considerably milder conditions (0.099N NaOH at 25°C). Glyceric acid was also formed. No products having fewer than three carbon atoms were found; glycolic acid was not a product, and formic acid, if formed, would not have been detected by the GLC analysis employed.

CELLOBIOSE

Cellobiose (XXIV) was allowed to react with oxygen-free 0.099N sodium hydroxide in a nitrogen atmosphere for 76 days at 25°C. The graph of acids generated versus time is shown in the upper curve of Fig. 7 (p. 23). Completion was attained in ∿55 days, at a plateau of 3.05 equivalents of acid per mole of starting material. In terms of acids generated, the reaction's half-life was 5.5 days.

Analysis of intermediate reaction samples by paper chromatography showed disappearance of cellobiose (R_G 0.53) after ∿15 days, plus formation of glucose and several other products having R_G values greater than 1.0. Spots observed at R_G 1.45 and 1.40 apparently correlated with the known values for D-glucometasaccharinic (R_G 1.44) and D-glucoisosaccharinic (R_G 1.38) acids.

Analysis of the 76-day sample by gas-liquid chromatography gave the chromatogram shown in Fig. 15. Once again, lactic acid was a major product, corresponding to 33% of the products observed. Glyceric acid (3%), 2,4-dihydroxybutyric acid (9%) and 3-deoxy-D-pentonic acid (4%) were also found, as in the oxygen-free NaOH degradation of D-glucose. Two unknowns (GLC T_r 15.1 and 18.2 min, Products 4 and 5) were observed, corresponding to 4 and 5%, respectively, of total products observed.

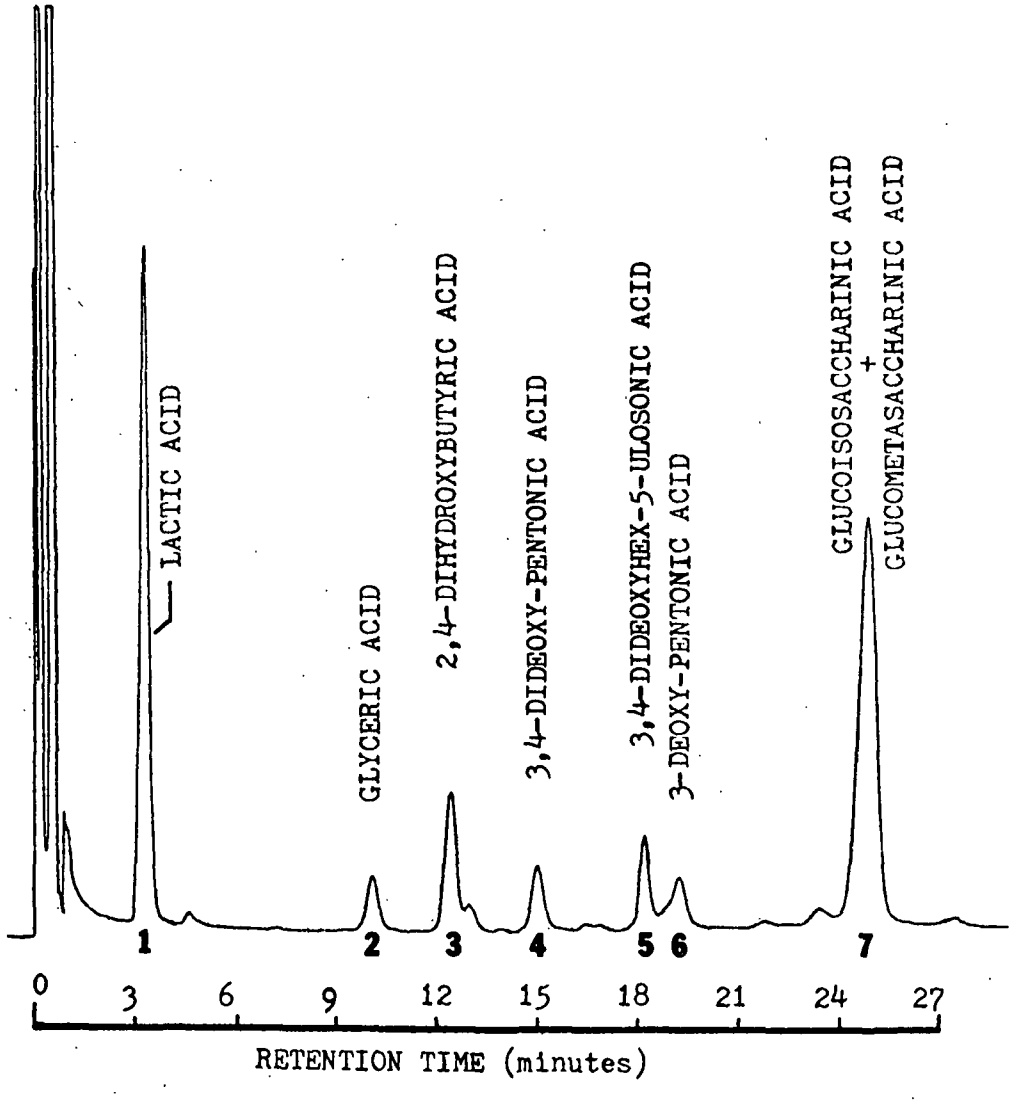


Figure 15. Gas Chromatogram of Products from Reaction of Cellobiose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

The largest peak (T_r 25.0 min) represented 41% of the products observed. Mass spectral data (Fig. 16, Spectrum B) for this peak provided evidence for both D-glucoisosaccharinic and D-glucometasaccharinic acids. For example, intense peaks were seen at m/e 335 and 245, indicative of glucometasaccharinic acid but not of glucoisosaccharinic acid. On the other hand, the relative abundance of peaks at m/e 205, 243, 257, 347, 435, and 437 correlated well with the mass spectrum of the iso but not the meta acid. It was concluded that Peak 7 represented both D-glucoisosaccharinic (see Fig. 1, p. 5) and D-glucometasaccharinic acids.

As cellobiose is postulated to degrade in oxygen-free alkali to glucoisosaccharinic acid and glucose (2), and because glucose yields glucometasaccharinic acid under the same conditions, both of these 3-deoxy-aldonic acids are expected products from cellobiose degradation.

Following lactonization of the product mixture, GLC analysis gave the results shown in Fig. 17. D-Glucoisosaccharino-1,4-lactone (T_r 20.0 min) and D-glucometasaccharino-1,4-lactone (T_r 22.0 min) were found in a ratio of 2.6:1. Assuming that the acids lactonized equally well (41), the iso/meta ratio in the mixture of the two acid products was also 2.6:1, corresponding to relative yields of 30% (glucoisosaccharinic acid) and 11% (glucometasaccharinic acid).

All products generated from cellobiose were subjected to mass spectrometric analysis. The mass spectrum of lactic acid is shown in Fig. 18, along with that obtained by Petersson (31) for the same compound. The very intense peak at m/e 117 stems from C-1—C-2 cleavage (see XXX): both fragments so produced can give ions of mass 117. The M-15 peak (m/e 219) is clearly seen; in this molecule, it can arise from loss of the methyl group attached to C-2 as well as from loss of a methyl group from either trimethylsilyl substituent.

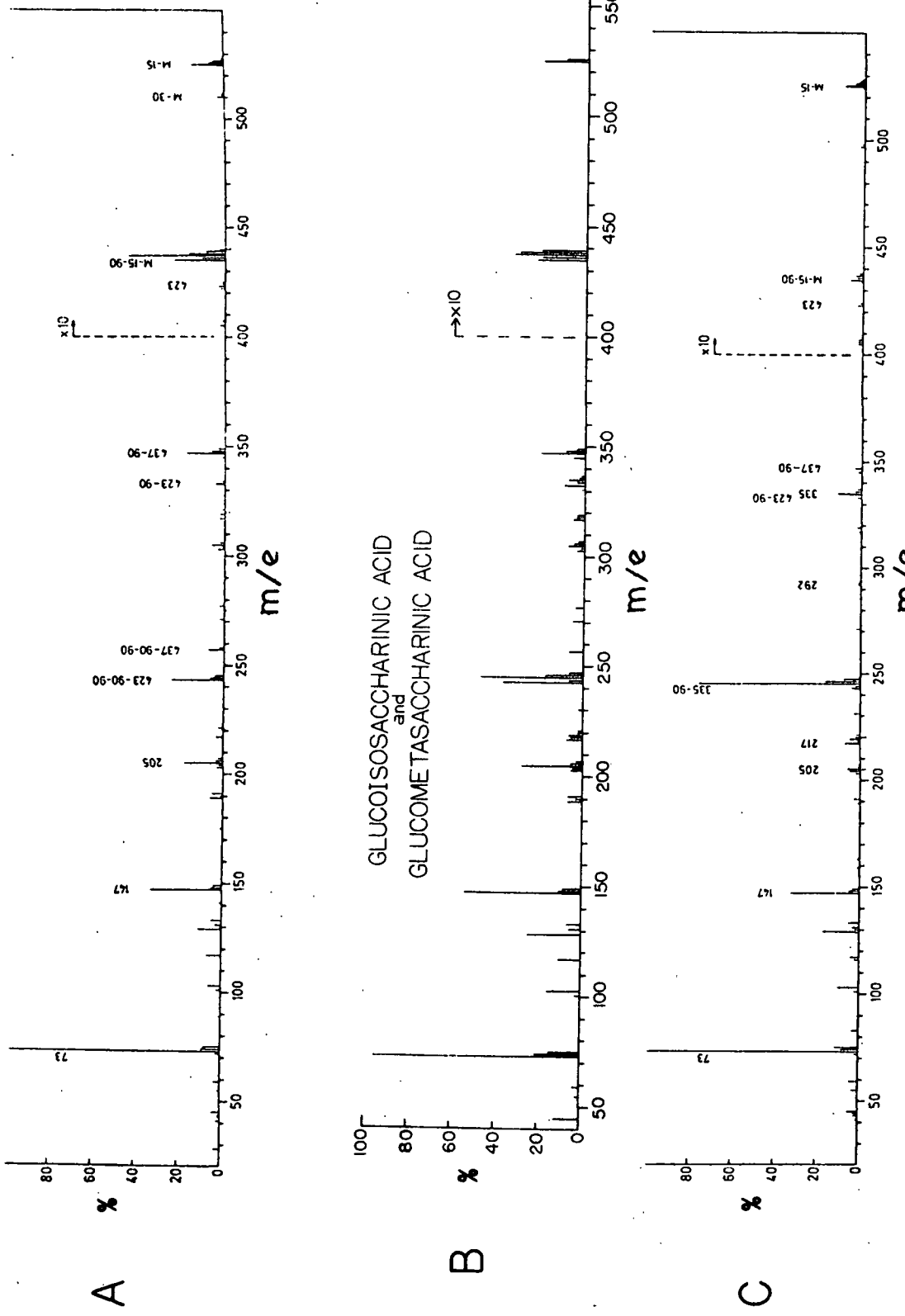


Figure 16. Mass Spectra at 70 ev of Trimethylsilyl Derivatives of a Mixture of D-Glucoisosaccharinic and D-Glucometasaccharinic Acids; A — D-Glucoisosaccharinic Acid, Petersson (31); B — Acid Mixture, This Work; C — D-Glucometasaccharinic Acid, Petersson (31)

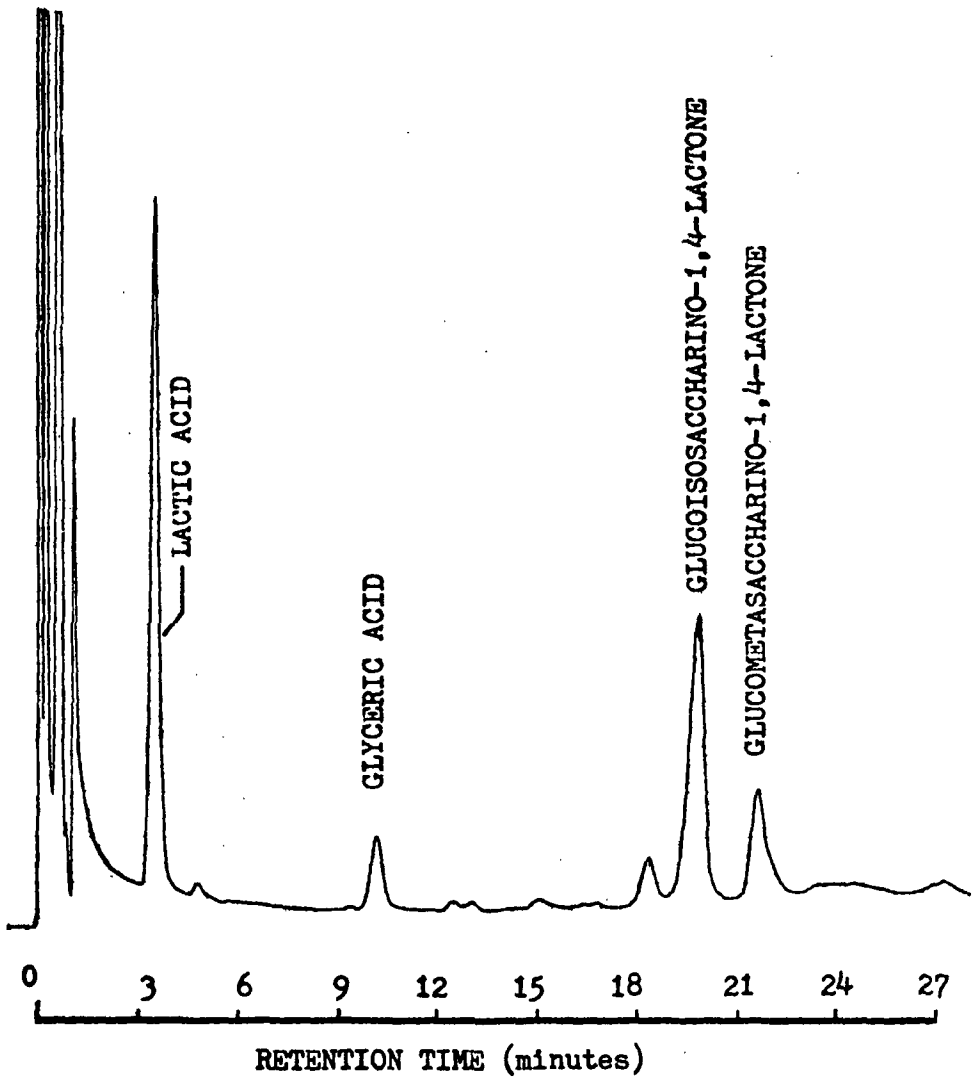


Figure 17. Gas Chromatogram of Products from Reaction of Cellobiose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C Following Lactonization

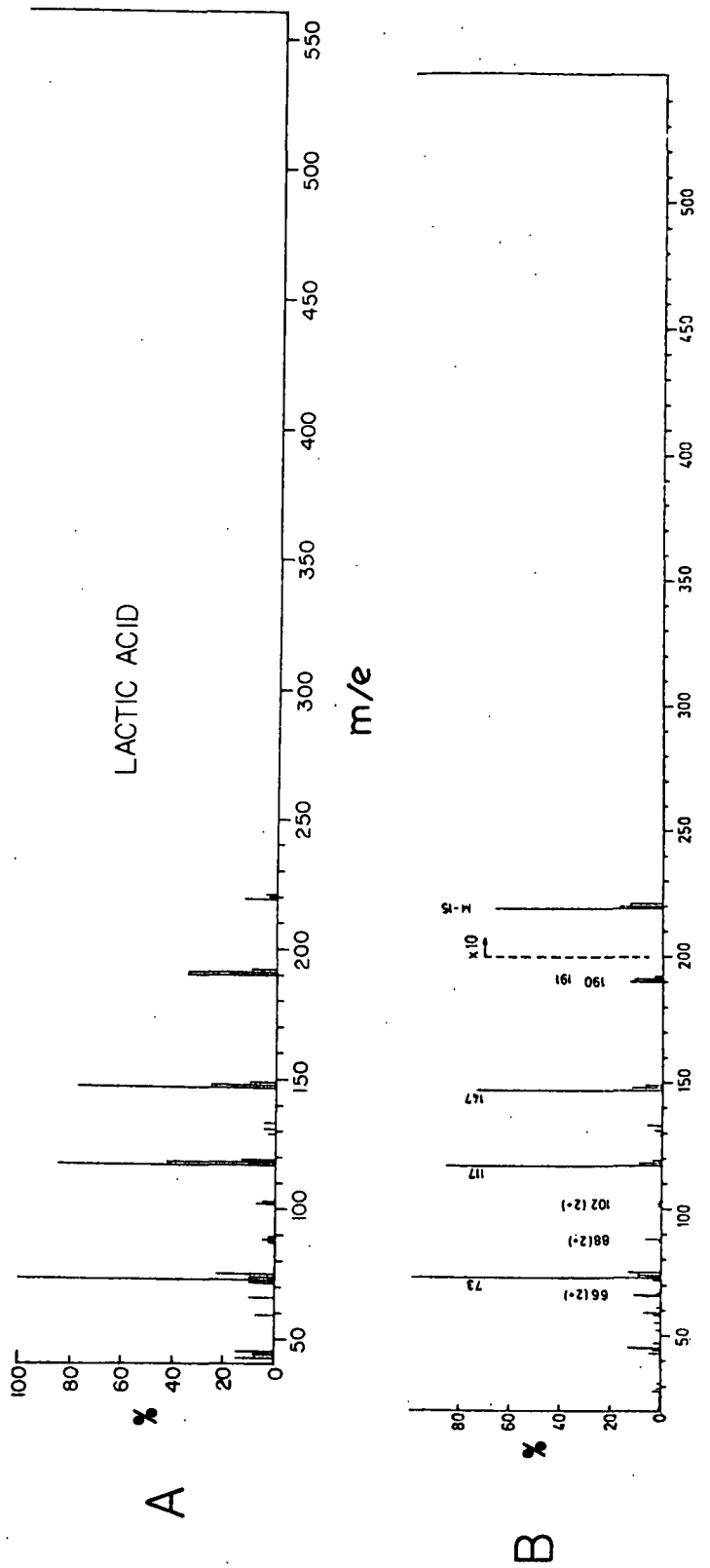
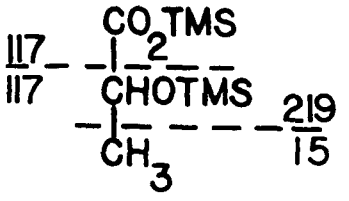


Figure 18. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of Lactic Acid; A — This Work; B — Petersson (31)

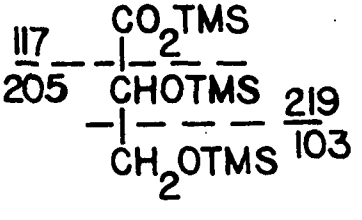


XXX

M 234

Significant even-numbered ions, relatively uncommon for other 3-deoxy-aldonic acids, are found at m/e 102, 88, and 66; Petersson attributed these peaks to doubly-charged ions (31). The odd-electron ion at m/e 190 was suggested to arise by loss of acetaldehyde from the parent ion.

Mass spectral analysis of glyceric acid provided the spectrum shown in Fig. 19; Petersson's mass spectrum of glyceric acid is also reproduced. For this compound (see XXXI), the parent ion (M^+, m/e 322) shows up clearly, as does the M-15 ion (m/e 307). Consecutive loss of carbon monoxide and trimethylsilanol from the M-15 ion results in the prominent peak at m/e 189. Peaks at m/e 205 and 103 stem from cleavages at C-1—C-2 and C-2—C-3, respectively. In addition, the intense peak at m/e 292 is indicative of a McLafferty-type rearrangement possible for aldonic acids having hydroxyl groups at C-2 and C-3 (31).



XXXI

M 322

Mass spectral data for 2,4-dihydroxybutyric acid are given in Appendix II (p. 134). Correlation with Petersson's data for this compound was excellent, as was correlation with data for 2,4-dihydroxybutyric acid obtained as a product from glucose degradation (see previous section).

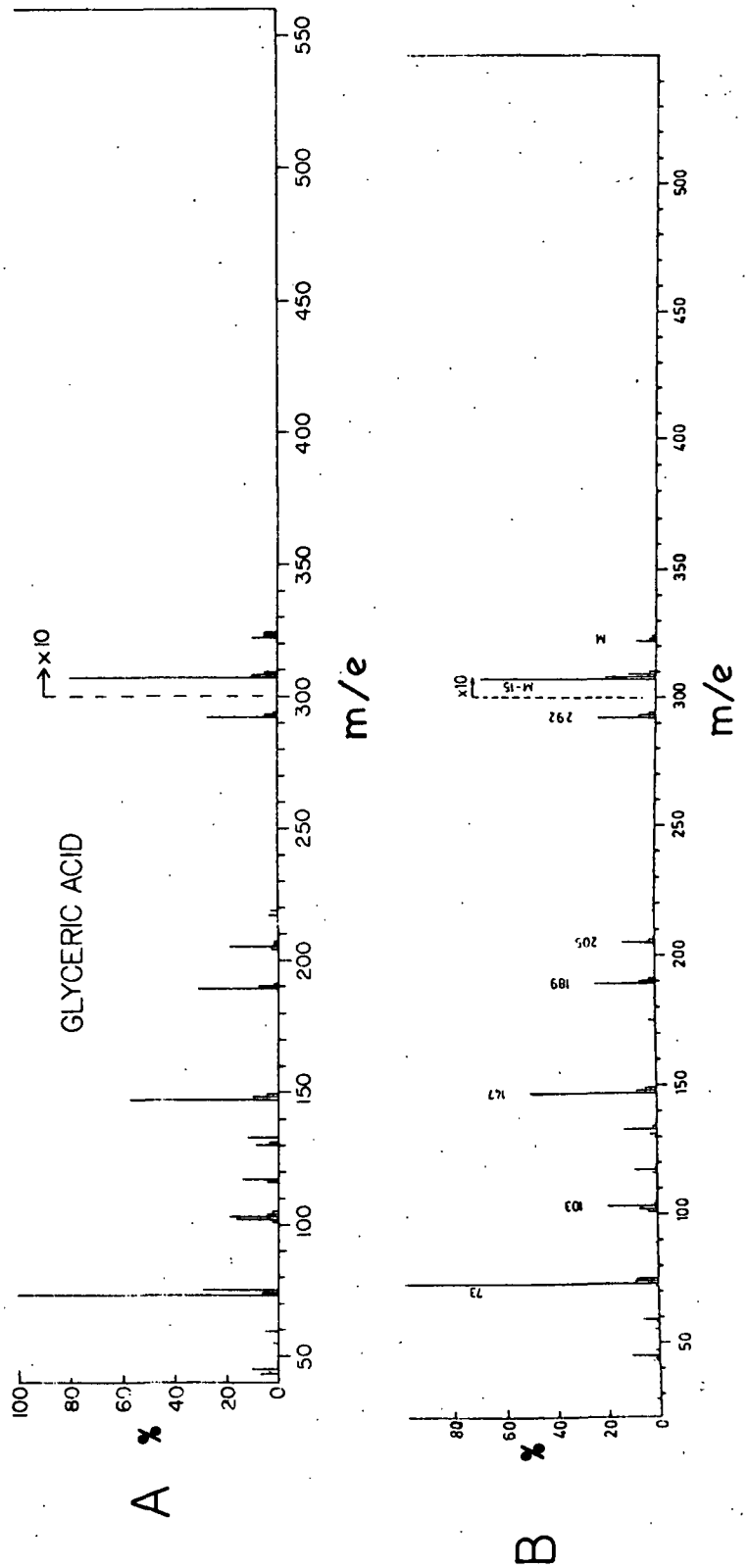
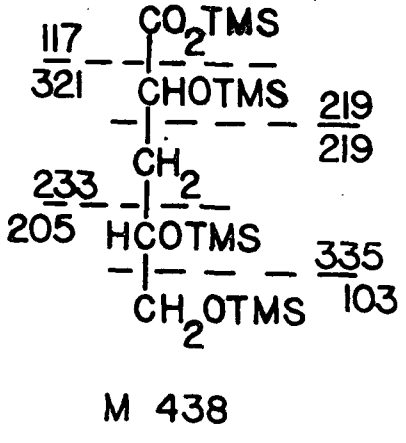


Figure 19. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of Glyceric Acid; A — This Work; B — Petersson (31)

Figure 20 presents the mass spectrum of 3-deoxy-D-pentonic acid, accompanied by Petersson's spectrum of the same compound. The intense peak at m/e 335 comes from C-4—C-5 cleavage (see XXXII); subsequent loss of TMSiOH provides a very intense peak at m/e 245 (this process is identical to the main fragmentation route for D-glucometasaccharinic acid). The M-15 ion (m/e 423) stands out distinctly, being the only ion observed in the upper portion (m/e >350) of the spectrum. Loss of TMSiOH from the M-15 ion results in a significant peak at m/e 333. Cleavage at C-1—C-2 provides an ion of mass 321 (not seen in Spectrum A) which loses TMSiOH to give an intense peak at m/e 231. The ion at m/e 205 corresponds to C-3—C-4 cleavage with charge retention at C-4.



XXXII

Two minor products from cellobiose degradation did not correspond to any products from glucose degradation under identical conditions. These compounds (see Fig. 15, Products 4 and 5, T_r 15.1 and 18.2 min, respectively) were analyzed by mass spectrometry, providing the spectra shown in Fig. 21.

Product 4 (Spectrum A) exhibited an unusual mass spectrum dominated by a base peak at m/e 143. Also noteworthy were peaks at m/e 71, 233, and 335. Petersson (31) reported the main features of the mass spectrum of 3,4-dideoxypentonic acid (see XXXIII) to be a base peak at m/e 143, second and third most

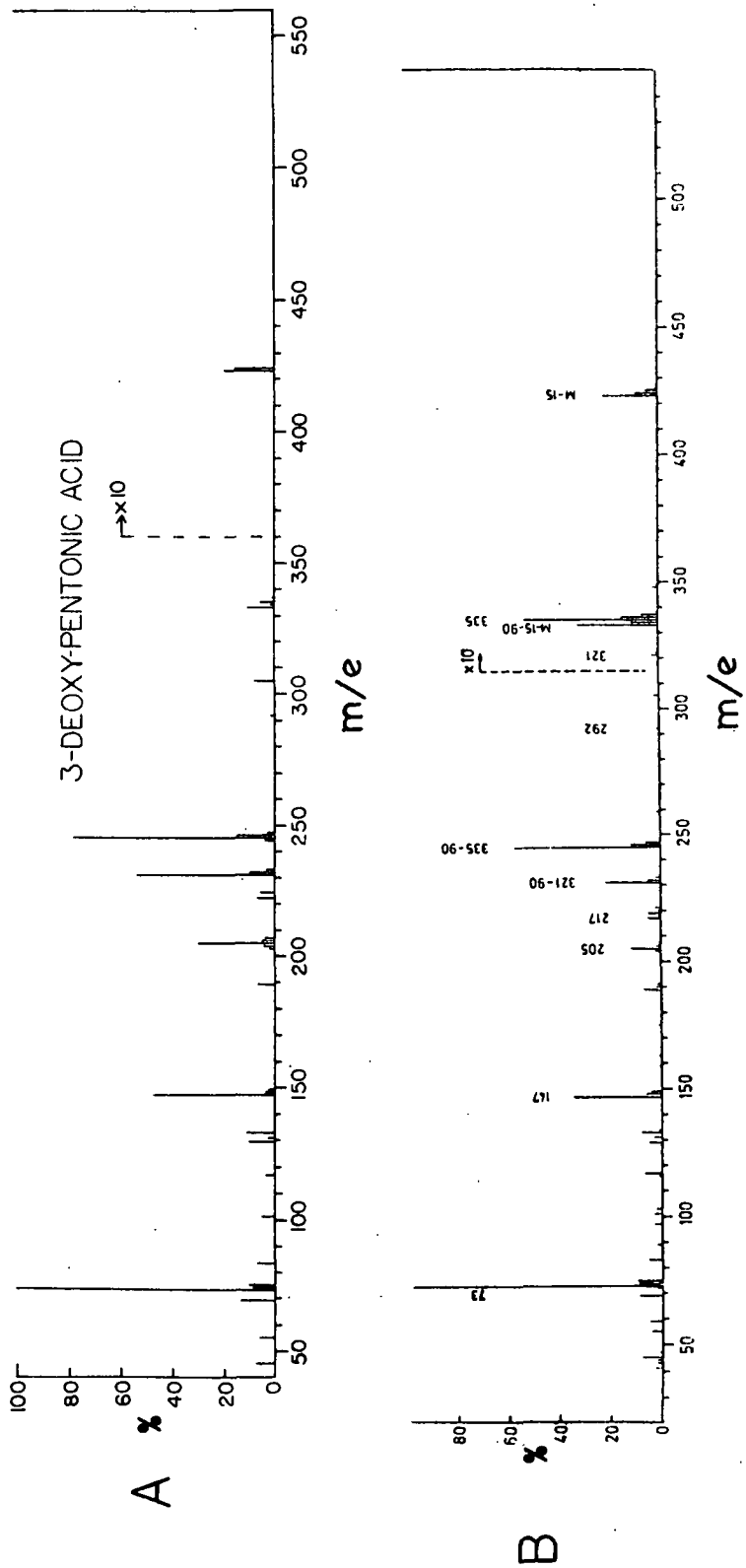


Figure 20. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of 3-Deoxy-D-pentonic Acid; A — This Work; B — Petersson (31)

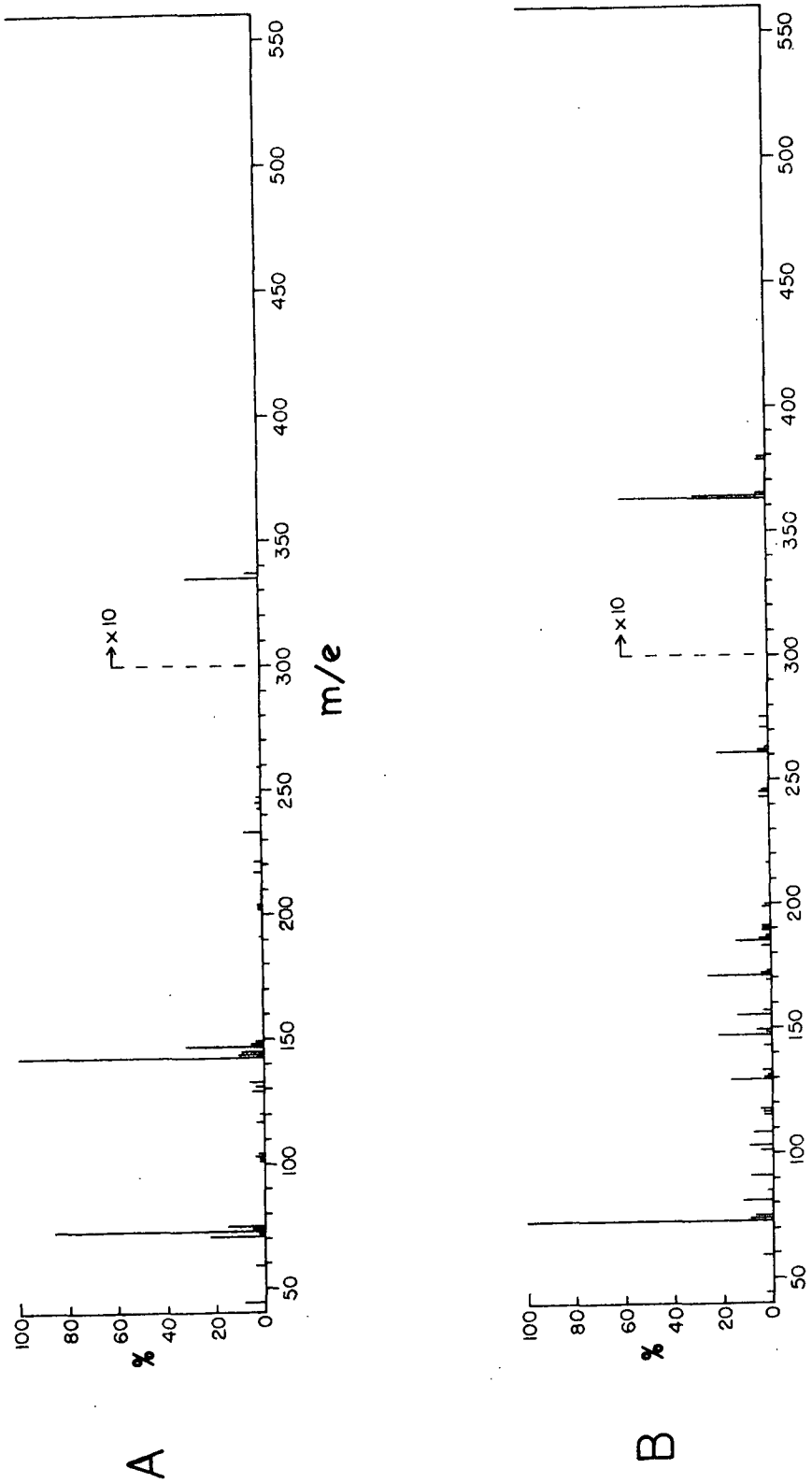
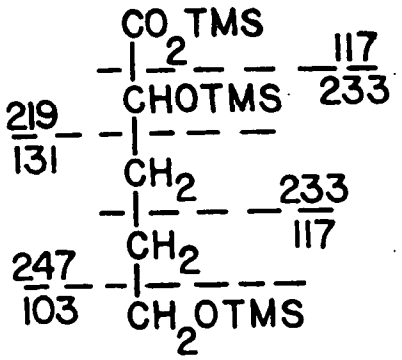


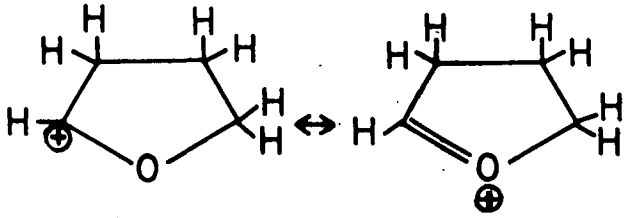
Figure 21. Mass Spectra at 70 ev of Trimethylsilyl Derivatives of Products from Cellobiose Degradation; A — Product 4 (Tentatively 3,4-Dideoxy-pentonic Acid); B — Product 5 (Tentatively 3,4-Dideoxyhex-5-ulosonic Acid)



XXXIII

M 350

intense peaks at m/e 73 and 147, respectively, plus prominent peaks at m/e 233 and 71. Petersson's findings correlate very well with Spectrum A, Fig. 21. The significant peak at m/e 335 thus represents the M-15 ion. Favored cleavage at C-1—C-2 provides the ion of mass 233, which in turn fragments to m/e 143 by loss of TMSiOH. According to Petersson, the significant peak at m/e 71 could be due to a tetrahydrofuryl ion formed by cyclization. A proposed structure for such an ion is illustrated below (see XXXIV).

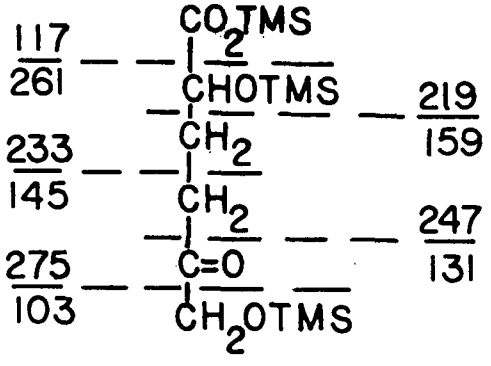


XXXIV

m/e 71

Spectrum B, Fig. 21, was recorded for cellobiose Product 5. The only strong peak in the upper portion of the spectrum (m/e >350) was observed at m/e 363; it was attributed to the M-15 ion, as a very weak ion at m/e 378 (M^+) was also found. Relative to the base peak (m/e 73), none of the other ions exceeded 30% intensity.

A possible structure for Product 5 could be 3,4-dideoxyhex-5-ulosonic acid (XXXV), formed by elimination of the C-3 (hydroxyl) and C-4 (glucosyloxy) substituents from cellobiose via a pathway similar to that proposed by Aspinall and Tam (42). They found evidence for the same compound from oxygen-free NaOH degradation of 3,4-di-O-methyl-D-glucose; they reduced the 5-keto acid to 3,4-dideoxy-hexonic acid prior to mass spectral analysis.



XXXV

M 378

3,4-Dideoxyhex-5-ulosonic acid would have M and M-15 ions of m/e 378 and 363, respectively. The M-15-28-$(90)_n$ series produces ions at m/e 335 (not seen), 245 and 155. Cleavage at C-1–C-2 leads to an ion at m/e 261; by sequential loss of TMSiOH groups, ions at m/e 171 and 81 are formed. Cleavage at C-5–C-6 results in fragments at m/e 103 and 275; the latter, losing TMSiOH, provides an ion at m/e 185. Supporting evidence for this structural assignment is the observation that Product 5 was unchanged by lactonization conditions, as seen by comparing the gas chromatograms of the acid products (Fig. 15) and lactones (Fig. 17).

Samples taken during the first ten days of reaction of cellobiose in oxygen-free 0.099N NaOH were analyzed by GLC, providing the chromatograms shown in Fig. 22. Under the conditions chosen (GLC Conditions B; see Experimental), the

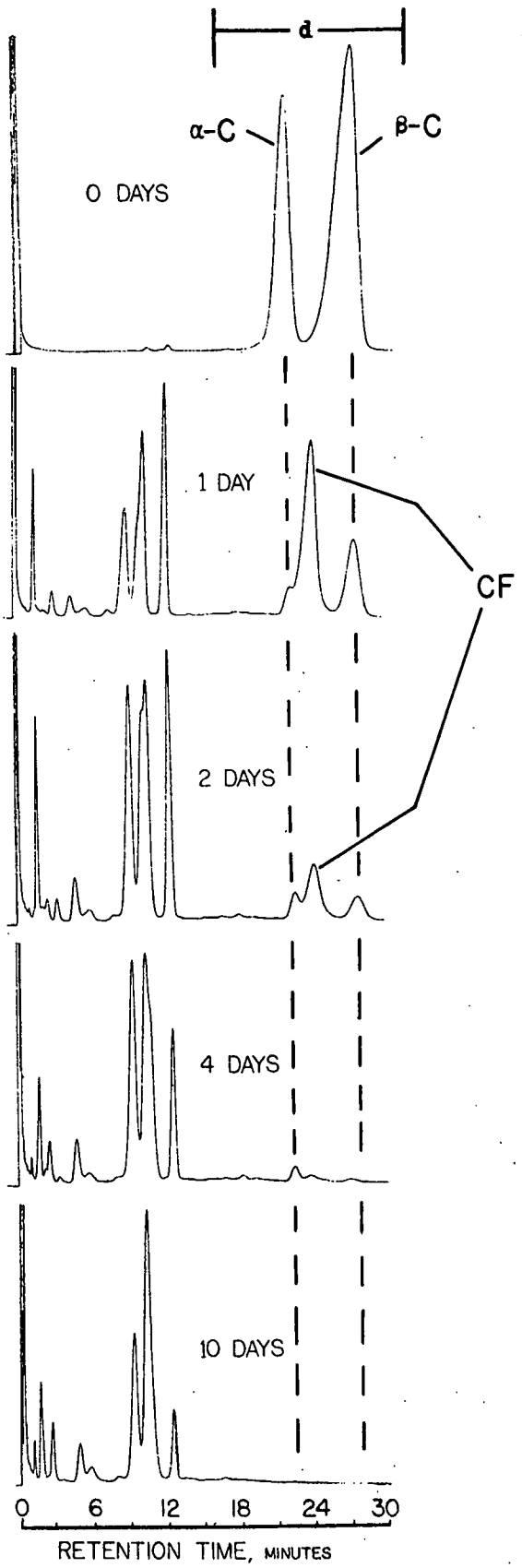


Figure 22. Gas Chromatograms of Intermediates and Products from Reaction of Cellobiose with Oxygen-Free 0.099N NaOH at 25°C; C — Cellobiose; CF — Cellobiulose; d — Disaccharide Region

disaccharide trimethylsilyl ethers were well separated from hexose and smaller derivatives. Cellobiose (α- and β-anomers) was observed to degrade quite rapidly despite mild reaction conditions; the starting material completely disappeared within 10 days. A third disaccharide, located between the cellobiose anomers, was especially prominent in the chromatograms of the 1- and 2-day samples; it is thought to be cellobiulose, formed by Lobry de Bruyn-Alberda van Ekenstein isomerization of cellobiose and subsequently degraded (14,43). Cellobiulose, a 2-ketose, makes β-elimination of the glucosyloxy anion from C-4 possible (see Fig. 1, p. 5).

Titration data for the samples shown in Fig. 22 are given in Table III. Note that after ten days, the reaction was not complete in terms of generation of final acid products (see Fig. 7, p. 23). Though cellobiose was totally consumed, glucose which was produced continued to react in the alkaline medium.

TABLE III

SELECTED TITRATION DATA FOR REACTION OF CELLOBIOSE WITH OXYGEN-FREE 0.099N NaOH AT 25°C

Sample Time, days	Equivalents Acid Generated per Mole Starting Material
0	0.00
1	0.45
2	0.95
4	1.65
10	2.00

The results shown in Fig. 15 and 22 demonstrated conclusively that degradation of cellobiose in oxygen-free 0.099N NaOH yielded no detectable alkali-stable disaccharide product. This further confirms the results of Rowell (8,13) and Malinen and Sjostrom (15) and discounts the findings of Lindberg, et al. (16) for cellobiose degradation in oxygen-free alkali.

Figure 1 (see Introduction, p. 5) illustrated the generally-accepted mechanism for formation of D-glucoisosaccharinic acid from cellobiose, proceeding through cellobiulose and involving the usual β-elimination and benzil-benzilic acid type rearrangement. As glucose is also a product of cellobiose degradation, the mechanisms previously used to account for formation of lactic, 2,4-dihydroxybutyric, 3-deoxy-D-pentonic and D-glucometasaccharinic acids from D-glucose apply equally well in the cellobiose reaction under identical conditions. Glyceric acid could also come from glucose, though the mechanism is unknown.

By analogy to dealdolization of D-glucose into 1- and 5-carbon fragments (see Fig. 14), a similar process in the cellobiose case provides an additional route for the formation of 3-deoxy-D-pentonic acid. As shown in Fig. 23, the 3-keto intermediate can undergo dealdolization at C-1—C-2, producing 1- and 11-carbon fragments. The latter, by rearrangement, provides an aldopentose bearing a 3-O-glucosyl substituent. The C-3 substituted pentose is an ideal candidate for the stabilization sequence of reactions, as illustrated, resulting in formation of 3-deoxy-D-pentonic acid.

Dealdolization at C-3—C-4, similar to that shown for D-glucose (Fig. 12), is precluded in cellobiose because C-4 bears a glucosyloxy substituent, not a hydroxyl group. A dealdolization of cellobiose at C-2—C-3 could be postulated, analogous to the process shown for D-glucose (Fig. 13), the larger fragment being a tetrose bearing the glucosyloxy group at C-2. But reducing sugars substituted at C-2 resist alkaline degradation (2); as no disaccharide product was detected from cellobiose degradation, dealdolization at C-2—C-3 apparently does not occur.

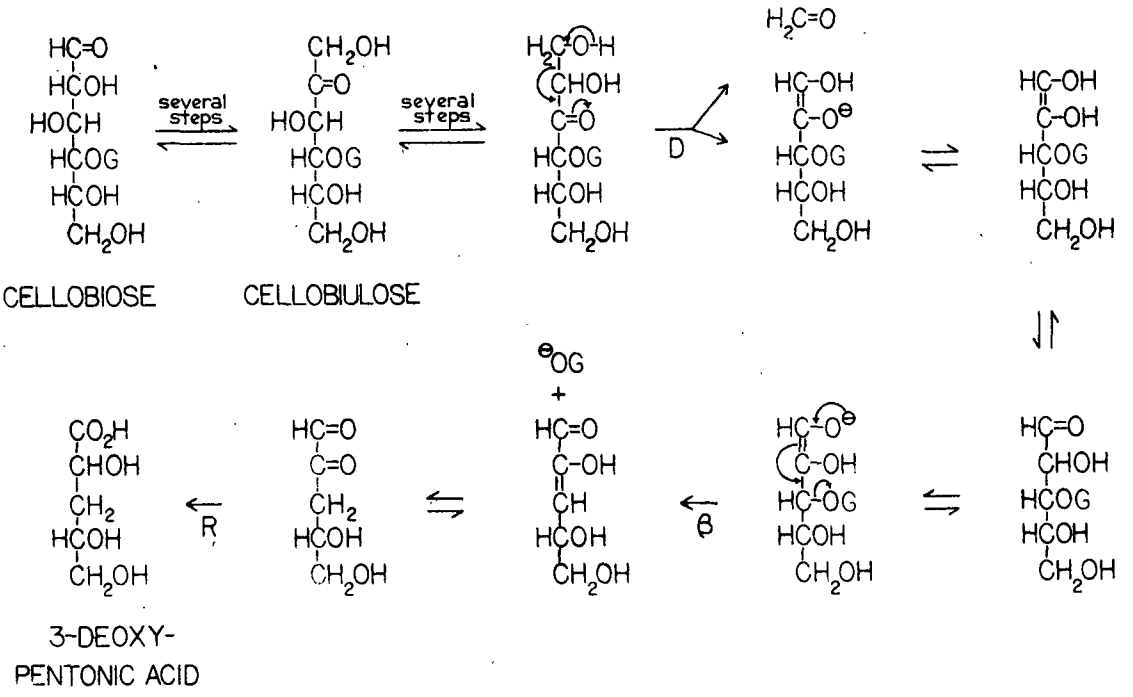


Figure 23. Possible Reaction Mechanism for Formation of 3-Deoxy-D-pentonic Acid from Cellobiose; D — Dealdolization; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement

No evidence of 3,4-dideoxy-pentonic or 3,4-dideoxyhex-5-ulosonic acids was found in the glucose degradation reaction. Consequently, formation of these products must be related to the 4-O-β-D-glucosyl-D-glucose structure.

A possible reaction pathway to 3,4-dideoxyhex-5-ulosonic acid is illustrated in Fig. 24. Sequential β-eliminations of the C-3 (hydroxyl) and C-4 (glucosyloxy) substituent from cellobiose would result in a vinyl ketone intermediate. In the alkaline medium, abstraction of the acidic proton at C-5 leads to a resonance-stabilized enolate intermediate; protonation and keto-enol tautomerization provide 3,4-dideoxyhexos-2,5-diulose. The final step is rearrangement to 3,4-dideoxy-hex-5-ulosonic acid.

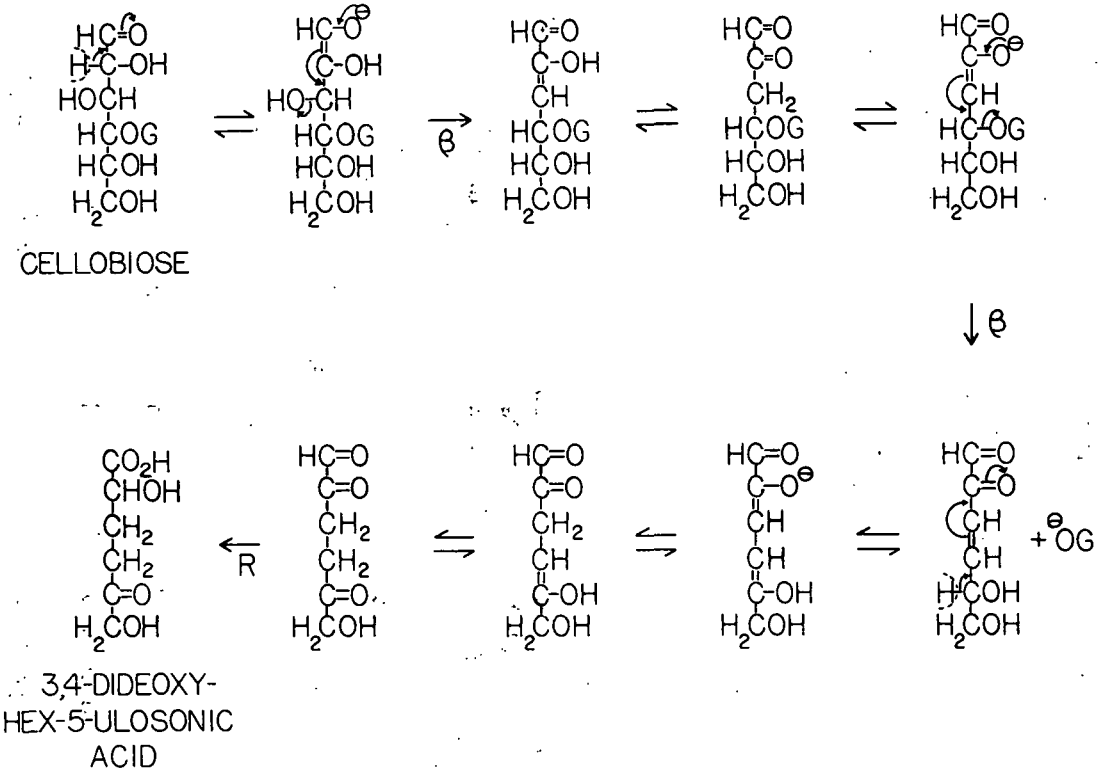


Figure 24. Possible Reaction Mechanism for Formation of 3,4-Dideoxyhex-5-ulosonic Acid from Cellobiose; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement

Aspinall and Tam (42) proposed a route from 3,4-di-O-methyl-D-glucose to 3,4-dideoxyhex-5-ulosonic acid via the same vinyl ketone intermediate. However, their literature citation for a similar conversion of santonin to santonic acid (44) involved very drastic alkaline conditions which far exceeded the temperature and alkali concentration used in their own work and that reported here, and direct comparison may not be valid.

The reaction mechanism proposed in Fig. 24 does not account for formation of 3,4-dideoxy-pentonic acid from cellobiose. Presumably, double elimination takes place, but a suitable explanation for subsequent steps to the dideoxy-aldonic acid is unavailable at the present time.

As in the case of D-glucose degradation, earlier work on oxygen-free alkaline degradation of cellobiose produced some conflicting results (see Table IV). Corbett and Kenner (14) allowed cellobiose to react with oxygen-free 0.0425N $Ca(OH)_2$ at 25°C; they found that cellobiose was 90% decomposed after 6 days, accompanied by production of D-glucoisosaccharinic acid and glucose. By paper chromatography, they also observed the formation and disappearance of cellobiulose. Follow-up work by Corbett and Liddle (22) revised the decomposition rate of cellobiose to 90% disappearance after 10 days under the same reaction conditions.

Green (37) degraded cellobiose in hot oxygen-free 8N NaOH. After converting the products to anilides, he separated the derivatives by column chromatography on cellulose and obtained α-D-glucoisosaccharinic, β-D-gluco-metasaccharinic and D,L-2,4-dihydroxybutyric anilides in crude yields of 15, 7 and 8%, respectively.

TABLE IV

ACIDIC PRODUCTS FROM OXYGEN-FREE ALKALINE DEGRADATION REACTIONS OF CELLOBIOSE[a,b]

	Corbett, et al. (14) $Ca(OH)_2$	Green (37) NaOH	Lindberg, et al. (16) NaOH	Rowell (13) $Ba(OH)_2$	Malinen, et al. (15) NaOH	This Work, NaOH
4-O-Glucosyl-D-gluco-metasaccharinic			+			
Lactic		++	++		++	++
Glycolic			++		+	t
Glyceric				+	+	+
2,4-Dihydroxybutyric		++				+
3,4-Dihydroxybutyric[c]				+	t	
3-Deoxy-pentonic						+
3,4-Dideoxy-pentonic (tentative)						+
3,4-Dideoxyhex-5-ulosonic (tentative)						+
Arabinonic				+	t	
Glucometasaccharinic		++	++		++	++
Glucoisosaccharinic	++	++	++	++	++	++
Glucosaccharinic			+			

[a] ++ Denotes major product; + denotes minor product; t denotes trace product.

[b] The results included in this table were obtained at initial alkali concentrations ranging from 0.04 to 8N and temperatures from 25 to 120°C.

[c] Product described as 3,4-dihydroxybutyric acid is probably 2,4-dihydroxybutyric acid in nitrogen systems (see p. 34,36).

Lindberg, et al. (16) reported formation of a small amount of 4-O-glucosyl-D-glucometasaccharinic acid from the reaction of cellobiose with oxygen-free 0.15N NaOH at 50°C. Apparently, this is the only reported instance in which a classical disaccharide stabilization product was obtained from cellobiose degradation. Other reaction products included lactic, glycolic, D-glucosaccharinic, D-glucoisosaccharinic, and D-glucometasaccharinic acids. The reported formation of D-glucosaccharinic acid in a dilute, oxygen-free NaOH system also seems questionable — none was found under milder conditions (this work), nor under more drastic conditions (15).

Rowell (8,13) found no disaccharide metasaccharinic acid from oxygen-free 0.04N $Ba(OH)_2$ degradation of cellobiose at 25 and 50°C, but did observe significant yields of D-glucoisosaccharinic acid and D-glucose, along with smaller quantities of glyceric, 3,4-dihydroxybutyric and D-arabinonic acids.

Malinen and Sjostrom (15) reported D-glucoisosaccharinic acid (38.1% of products observed), D-glucometasaccharinic acid (18.0%) and lactic acid (34.4%) to be the major products from the degradation of cellobiose in oxygen-free 0.25N NaOH at 120°C. Glycolic acid (4.3%) and glyceric acid (1.9%) were minor products. Malinen and Sjostrom's results are in general agreement with those reported here, with several exceptions: in this work, smaller quantities of 6-carbon acids were found, glycolic acid was at most a trace product, and 2,4-dihydroxybutyric acid was an important product.

The hitherto satisfactory explanation for the degradation products from reaction of cellobiose with oxygen-free alkali (see Fig. 1) clearly does not account for all the results reported here. As no 4-O-glucosyl-D-glucometasaccharinic acid was produced, β-elimination of the glucosyloxy group was apparently complete, implying production of equal proportions of D-glucose and D-glucoisosaccharinic acid. Yet D-glucoisosaccharinic acid, representing 30% of the

products observed, was well below the predicted level. Furthermore, new products were identified which did not come from the nonreducing portion of cellobiose. A reaction mechanism has been proposed to account for one of two tentatively identified 3,4-dideoxy acids from cellobiose degradation. It is intended to supplement the β-alkoxy elimination theory which, along with dealdolization, successfully explains most of the results of this reaction.

3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE

3,6-Anhydro-4-O-methyl-D-glucose (V) was allowed to react with oxygen-free 0.099N sodium hydroxide in a nitrogen atmosphere for 15 days at 25°C. The graph of acids generated versus time is shown in Fig. 25.

The reaction was complete in 10 days and had generated 1.0 equivalent of acid per mole of starting material; the half-life was 9.6 hours (in terms of acids generated).

Analysis of the 15-day sample by GLC gave the results shown in Fig. 26. The dominant product (T_r 25.0 min) corresponded to D-glucometasaccharinic acid; it accounted for 41% of the products observed. Following lactonization, the major peak (T_r 22.0 min) had a retention time identical to that of D-glucometasaccharino-1,4-lactone.

Other acid products readily identified by their GLC retention times included lactic, glyceric and 3-deoxy-D-pentonic acids. Many unidentified products were also observed, none of which apparently correlated with acids produced in the reactions discussed previously. Products 2-6 were present in quantities too small to be analyzed by mass spectrometry.

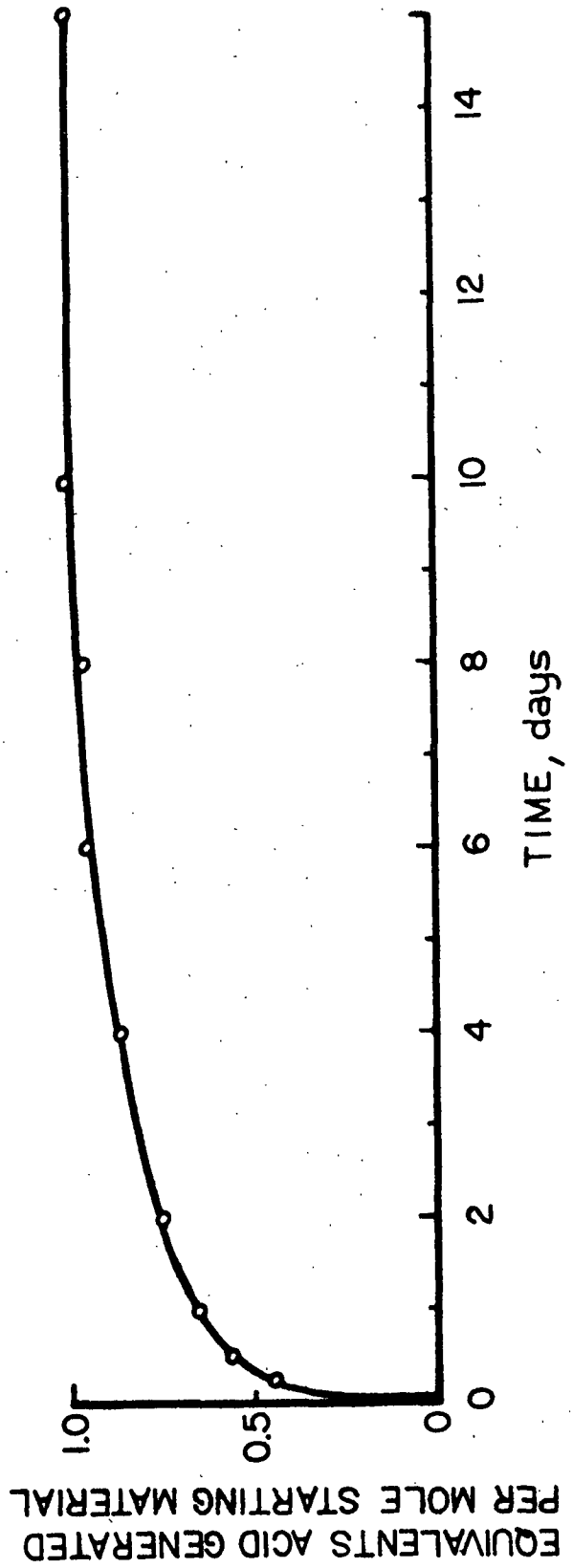


Figure 25. Acids Generated in the Reaction of 3,6-Anhydro-4-O-methyl-D-glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

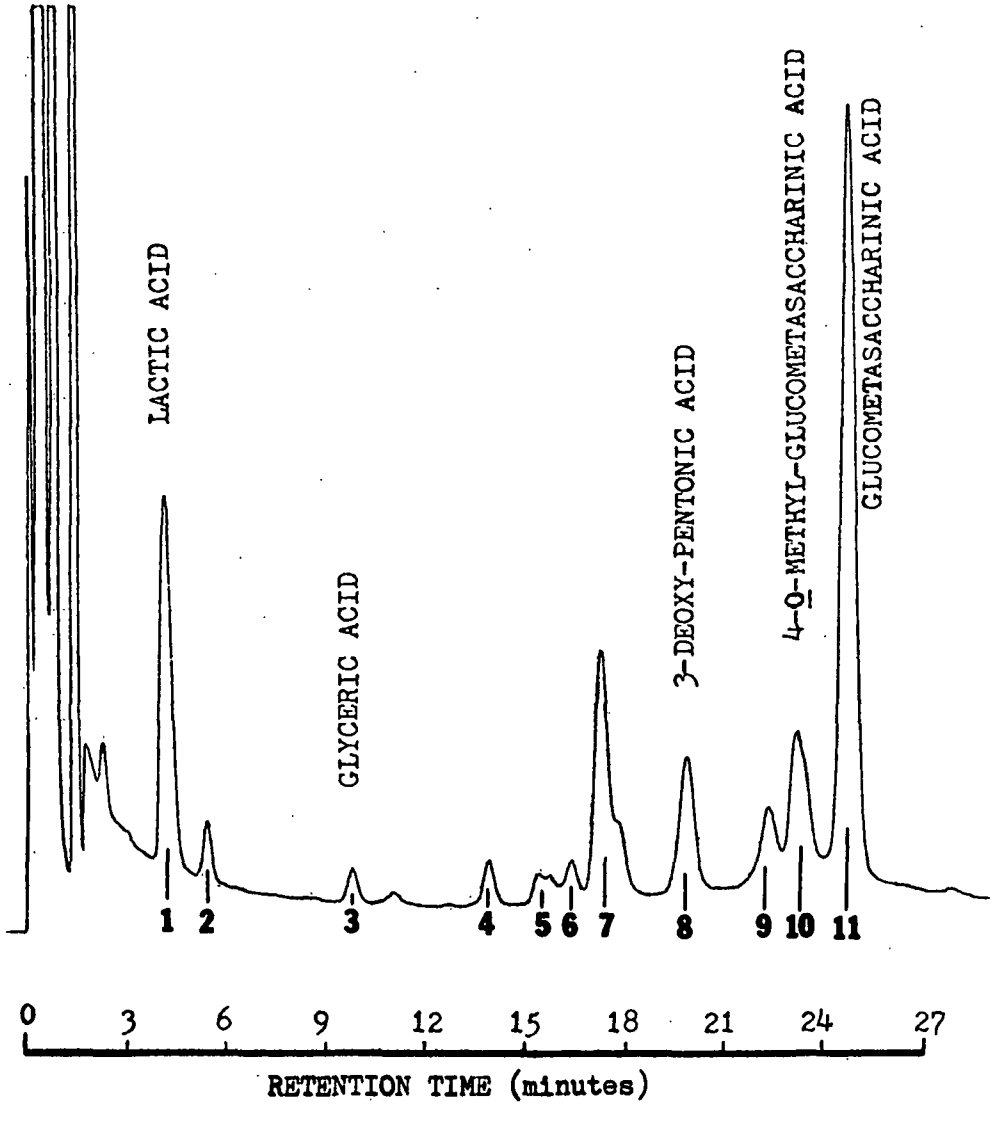


Figure 26. Gas Chromatogram of Products from Reaction of 3,6-Anhydro-4-O-methyl-D-glucose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

Mass spectral analysis of Products 7-11 was conducted. Product 11, the dominant final product, provided mass spectral data (Appendix II, p. 139) virtually identical to that for D-glucometasaccharinic acid previously found in the glucose and cellobiose degradations and as published by Petersson (31). For Product 8, the mass spectrum (Appendix II, p. 137) correlated well with that of 3-deoxy-D-pentonic acid reported by Petersson and also identified among the products from cellobiose degradation.

The mass spectrum of Product 7 is shown in Spectrum A of Fig. 27. Though the compound was a lactonizable acid (i.e., GLC analysis of the lactonized product mixture showed no peak at T_r 17.2 min), a suitable structure could not be determined from the mass spectral data, and Product 7 remains unidentified.

An adequate mass spectrum of Product 9 was difficult to obtain, due to its low yield and proximity (by GLC) to Product 10. The mass spectrum of Product 9 (Appendix II, p. 138) could not be resolved.

The mass spectrum recorded for Product 10 is presented in Spectrum B of Fig. 27. Applying the reaction mechanism given for degradation of 3,6-anhydro-D-glucose (see Fig. 4, p. 19) to this case, the expected product would be 4-O-methyl-D-glucometasaccharinic acid (XXXVI). The mass spectrum of Product 10 fits this compound well. Thus, the peak at m/e 467 is the M-15 ion. By analogy to the previously discussed fragmentation of D-glucometasaccharinic acid, C-4—C-5 cleavage of XXXVI provides a prominent ion at m/e 277 which loses TMSiOH to give a very intense peak at m/e 187; the ion at m/e 97 is probably the third in this series. Cleavage at C-2—C-3 results in ions of me/ 219 and, by loss of TMSiOH, 129. Loss of carbon monoxide from the M-15 ion (m/e 467), followed by sequential losses of TMSiOH units, could account for peaks at m/e

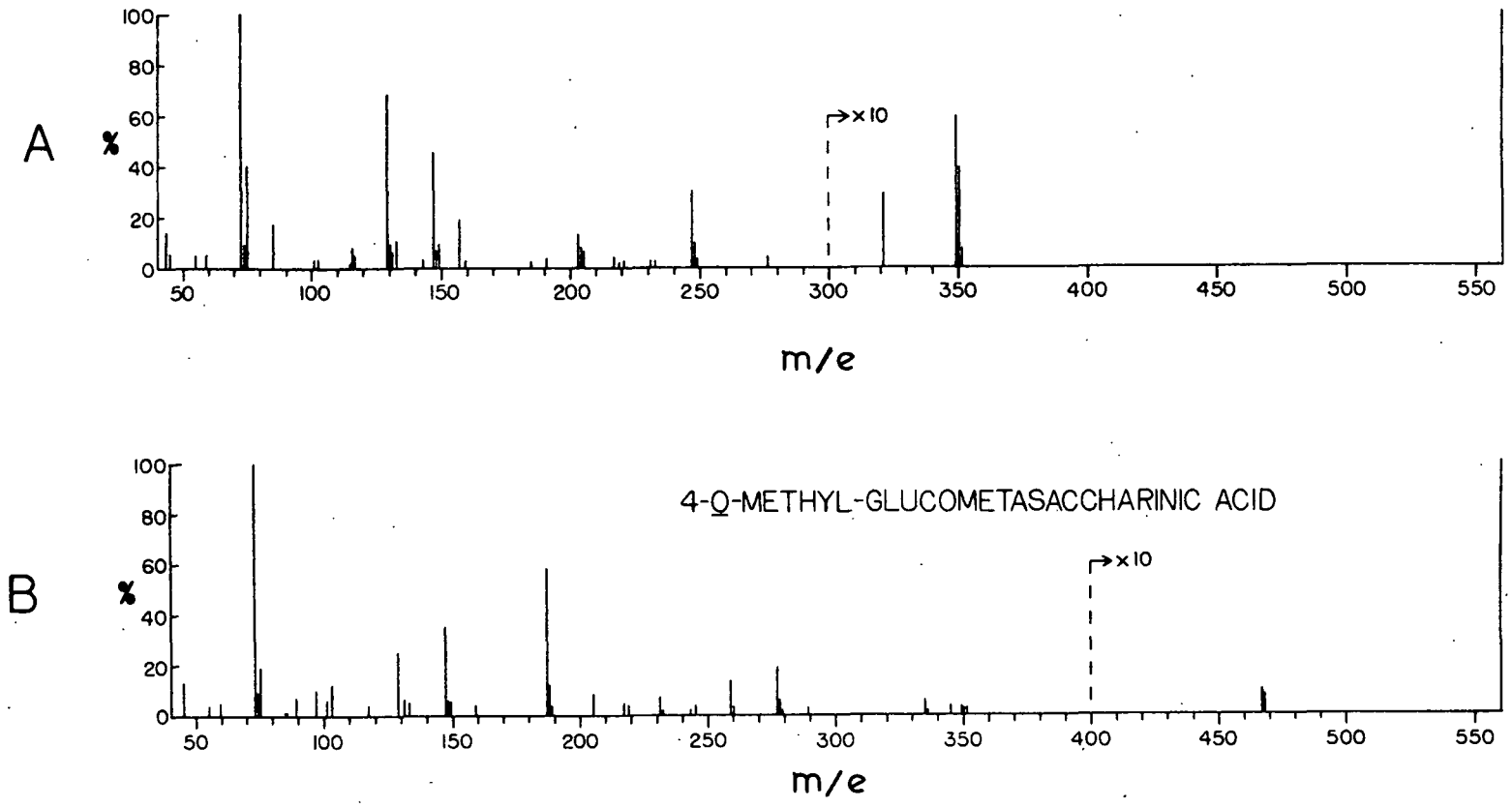
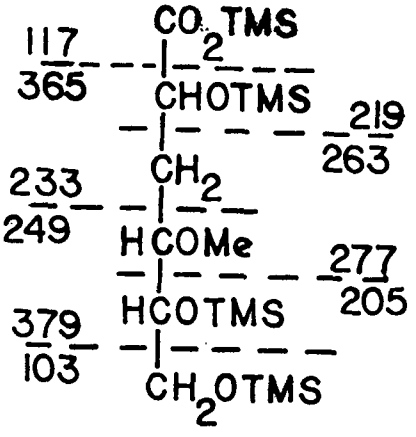


Figure 27. Mass Spectra at 70 ev of Trimethylsilyl Derivatives of Products from 3,6-Anhydro-4-O-methyl-D-glucose Degradation; A — Product 7 (Unidentified Acid); B — Product 10 (4-O-Methyl-D-glucometasaccharinic Acid)

439 (not observed), 349 and 259. Other important peaks at m/e 103, 117 and 205 stem from carbon-carbon cleavage.



XXXVI

M 482

4-O-Methyl-D-glucometasaccharinic acid, identified as described above, accounted for 13% of the products observed from degradation of 3,6-anhydro-4-O-methyl-D-glucose. Its low yield, relative to that of D-glucometasaccharinic acid, indicated preference for a reaction route different from stabilization but also involving only 6-carbon compounds.

Possible reaction mechanisms resulting in formation of 4-O-methyl-D-glucometasaccharinic and D-glucometasaccharinic acids are illustrated in Fig. 28. Following the conventional stabilization route, β-elimination of the C-3 substituent from 3,6-anhydro-4-O-methyl-D-glucose would lead to formation of 4-O-methyl-D-glucometasaccharinic acid.

However, the C-4 substituted 3-deoxy-hexosulose intermediate shown in Fig. 28 may also undergo β-elimination, as the 4-O-methyl group is now located beta to a carbon site (C-2). The result of the second β-elimination is formation of a 3,4-dideoxyhex-3-enosulose. Structurally, this intermediate is a vinyl ketone (see XXXVII) which, under nucleophilic attack, directs the nucleophile to the alkene carbon atom (β) away from the carbonyl group. The resulting

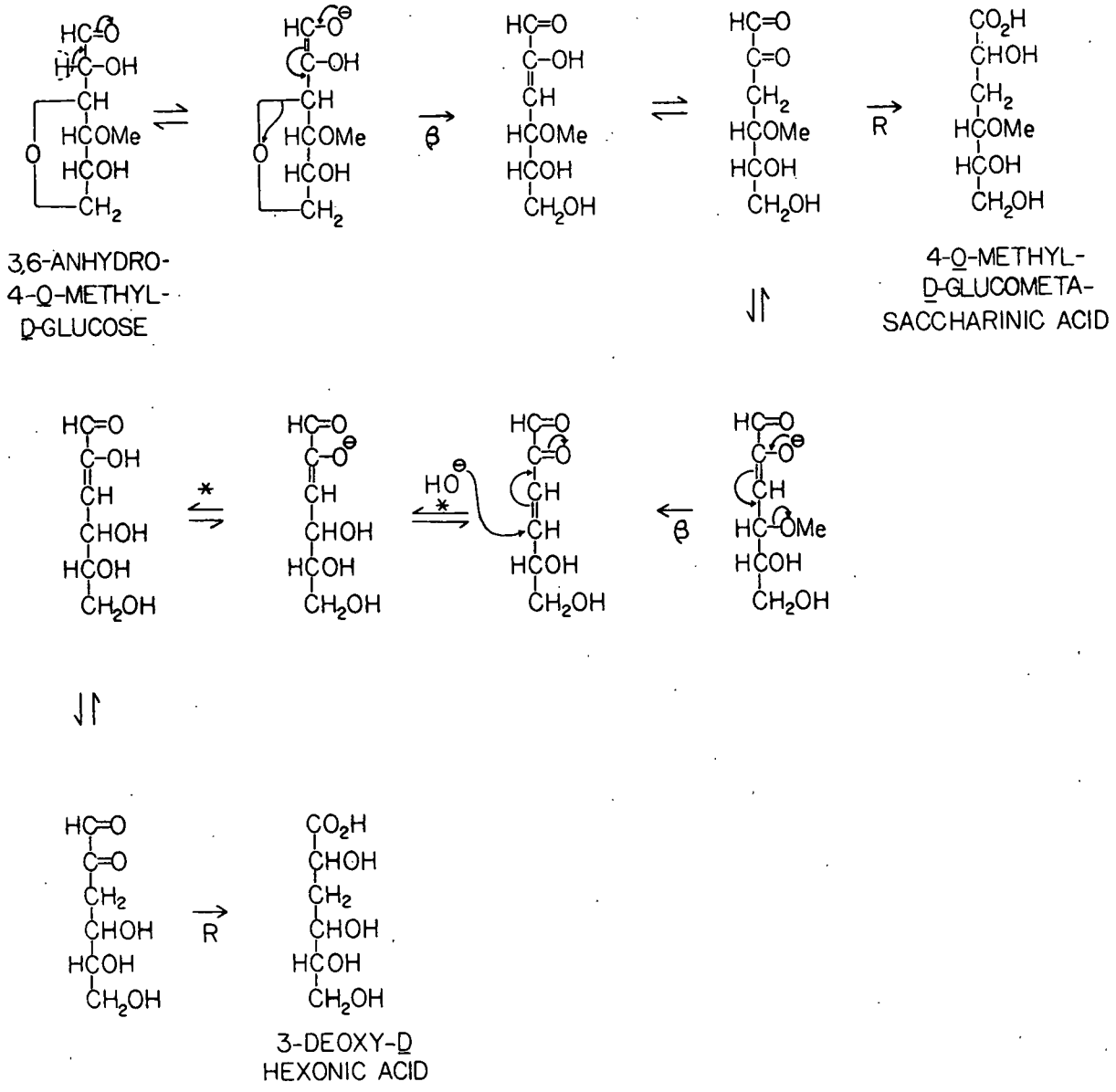
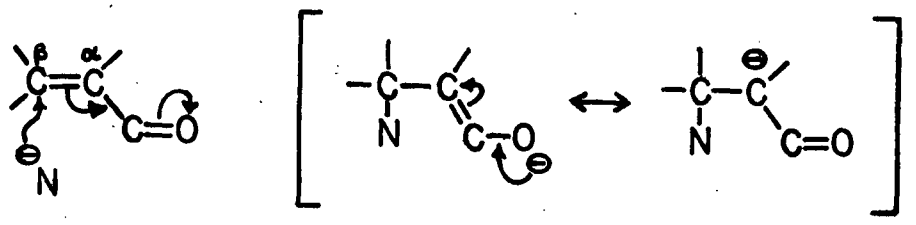


Figure 28. Possible Reaction Mechanisms for Formation of 4-O-Methyl-D-glucometasaccharinic Acid and 3-Deoxy-D-hexonic Acid from 3,6-Anhydro-4-O-methyl-D-glucose; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement

carbanion is effectively resonance-stabilized by the conjugated carbonyl group. Nucleophilic attack at the alkene carbon atom (α) adjacent to the



XXXVII

carbonyl group is unfavorable, because it would lead to a carbanion having no resonance stabilization (45,46). However, the presence of a second substituent at the α-carbon may affect the direction of nucleophilic attack, as discussed in the following section on 3,6-anhydro-cellobiose.

Thus, nucleophilic attack of hydroxide ion at C-4 of the 3,4-dideoxyhex-3-enosulose intermediate leads to a resonance-stabilized sugar anion which, by protonation, forms an enol. Keto-enol tautomerization to the hexosulose and subsequent rearrangement provide the 3-deoxy-D-hexonic acid.

Because addition of the hydroxide ion occurs at C-4, it is likely that both D-gluco- and D-galacto-metasaccharinic acids are produced. The GLC and GC-MS methods employed in this work would not distinguish between these two compounds.

Addition to the vinyl ketone, as described above, is termed a 1,4-nucleophilic addition by March (45), in that protonation takes place at the (carbonyl) oxygen atom four positions away from the site of nucleophilic attack. Because keto-enol tautomerization occurs, the net result in this case is addition of water to the double bond. Nucleophilic addition of this type is also thought

to be the mechanism operating in the reaction of cellulose with acrylonitrile (47,48), but in this case cellulose acts as the nucleophile.

The low yield of lactic acid (17% of the products observed) appears to be due to the 3,6-anhydro bridge, which effectively forestalls dealdolization of the starting material to two 3-carbon fragments. In fact, it is only the 3-deoxy-hexosulose precursor to 3-deoxy-D-hexonic acid which has the requisite configuration for dealdolization. The result, as shown in Fig. 29, is a C-3–C-4 cleavage in which the two fragments react further to produce lactic acid. The identical process could account for the trace amounts of lactic acid observed in the degradation of 3,6-anhydro-D-glucose and 3-O-methyl-D-glucose.

No 2,4-dihydroxybutyric acid was found among the products from degradation of 3,6-anhydro-4-O-methyl-D-glucose. The probable reason is that a hydroxyl group is never available at C-3 to initiate dealdolization to 2- and 4-carbon fragments (see Fig. 13, p. 35).

A plausible dealdolization of 3,6-anhydro-4-O-methyl-D-glucose to 1- and 5-carbon fragments could not be deduced, and the mechanistic pathway to 3-deoxy-D-pentonic acid in this reaction remains unknown.

Following oxygen-free NaOH degradation of 3,4-di-O-methyl-D-glucose, Aspinall and Tam (42) found approximately equal amounts of 4-O-methyl-D-gluco-metasaccharinic acid, 3-deoxy-D-hexonic acid and 3,4-dideoxyhex-5-ulosonic acid. It appears that nucleophilic addition of hydroxide ion to the 3,4-dideoxyhex-3-enosulose intermediate, identical to the one discussed above, provides a logical explanation for production of 3-deoxy-D-hexonic acid in their system.

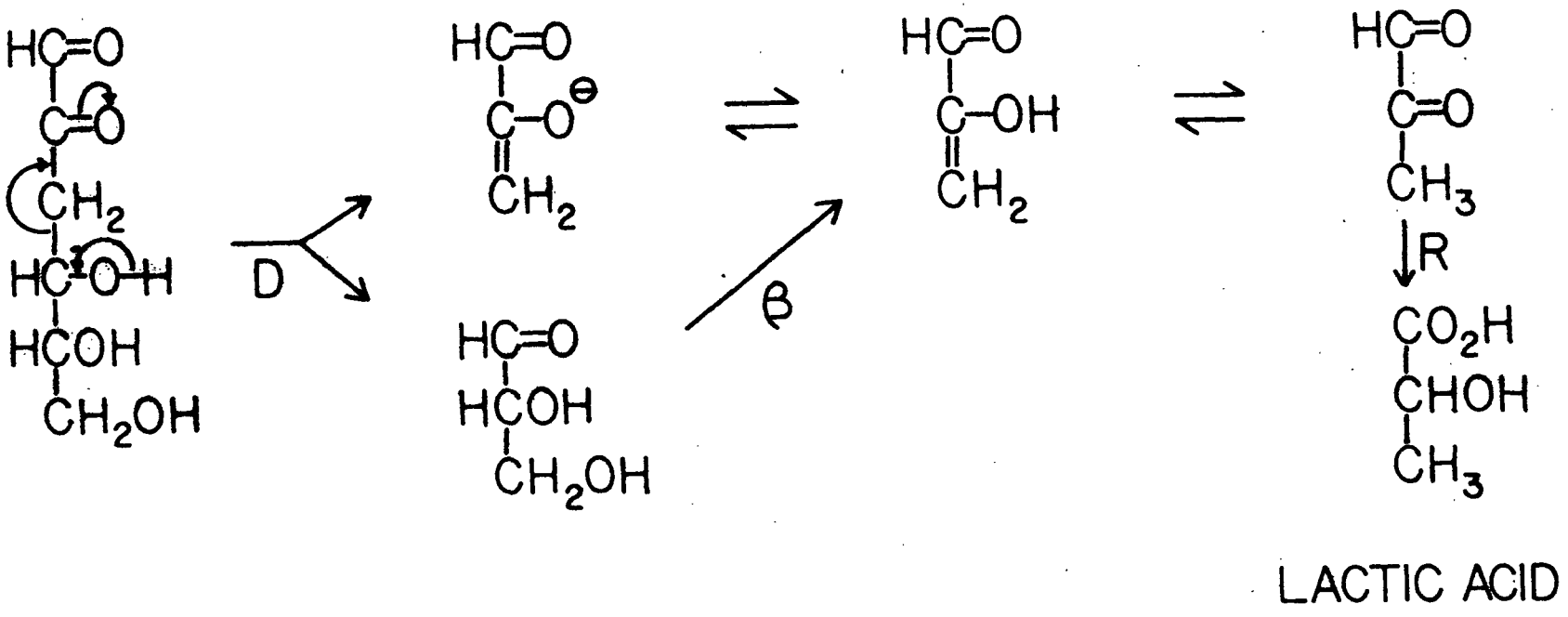


Figure 29. Possible Reaction Mechanism for Formation of Lactic Acid from a 3-Deoxy-hexosulose Intermediate in the Degradation of 3,6-Anhydro-4-O-methyl-D-glucose; D — Dealdolization; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement

In this work, no 3,4-dideoxyhex-5-ulosonic acid was found from degradation of 3,6-anhydro-4-O-methyl-D-glucose. However, it was tentatively identified among the products from cellobiose degradation, and a possible reaction mechanism for its formation was presented in the previous section.

In earlier work, Aspinall and Ross (21) reported that degradation of 3-O-methyl-xylobiose in oxygen-free NaOH resulted in "almost complete" destruction of the disaccharide. Methanol and D-xylose were identified as products, meaning that elimination of the C-3 and C-4 substituents occurred. In a companion article, Aspinall and coworkers (49) proposed sequential β-eliminations of C-3 and C-4 substituents from 3-O-methyl-xylobiose to generate a 3,4-dideoxypent-3-enosulose intermediate. The nucleophilic addition mechanism proposed above provides a plausible route from the 5-carbon vinyl ketone intermediate to final acid products.

Apparent addition of hydroxide ion to the 3,4-dideoxyhex-3-enosulose intermediate via the nucleophilic addition mechanism also explains the occurrence reported by Sten and Mustola (20) of a small amount of D-glucometasaccharinic acid among the products from oxygen-free NaOH degradation of 4-O-methyl-D-glucose. In this case, the yield of the 3-deoxy-D-hexonic acid was very low, indicating a preference for "peeling" (i.e., β-elimination of the C-4 substituent only) to generate D-glucoisosaccharinic acid, the dominant product.

3,6-ANHYDRO-CELLOBIOSE

3,6-Anhydro-cellobiose (XXII) was allowed to react with oxygen-free 0.099N sodium hydroxide in a nitrogen atmosphere for 36 days at 25°C. Figure 30 shows the graph of acids generated versus time for the reaction.

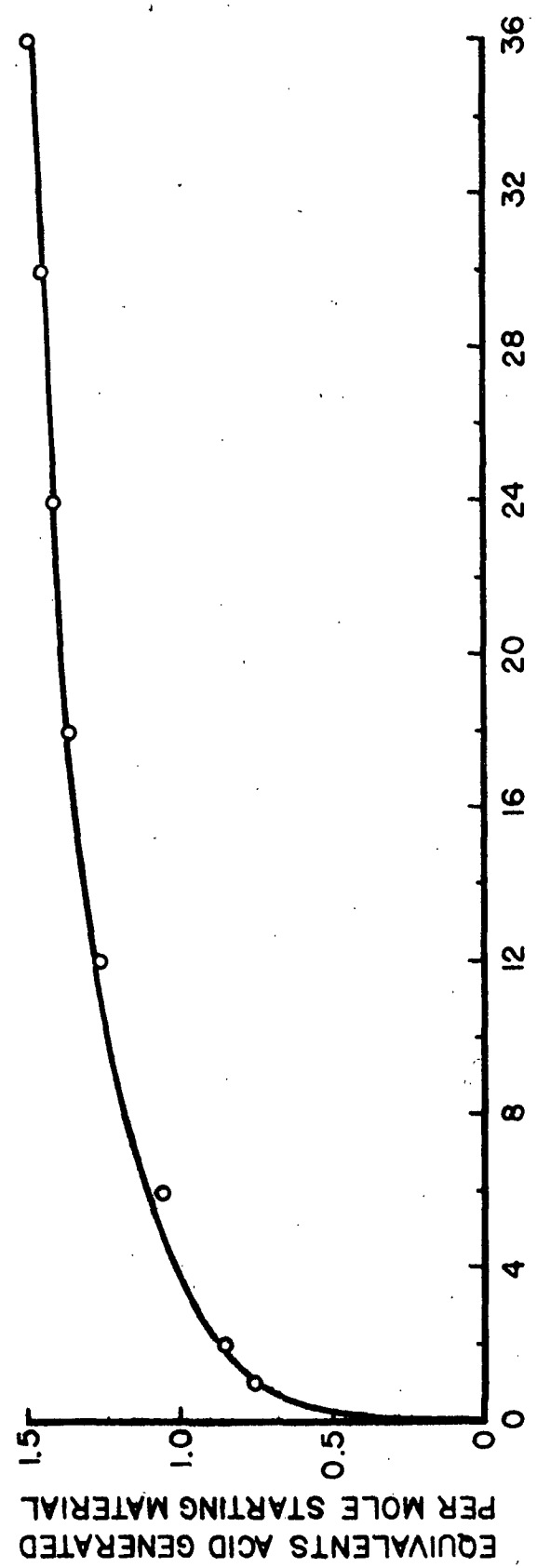


Figure 30. Acids Generated in the Reaction of 3,6-Anhydro-Cellobiose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

In 36 days the reaction generated 1.52 equivalents of acid per mole of starting material. Apparently, completion was not attained; beyond 24 days the reaction proceeded at an almost negligible rate. In terms of acids generated, the reaction had a half-life of approximately 24 hours. Similar to the degradations of 3,6-anhydro-D-glucose and 3,6-anhydro-4-O-methyl-D-glucose, the initial reaction rate was very fast.

By paper chromatography, the starting material (R_G 0.55) was observed to disappear, accompanied by the formation of glucose and several products having R_G values greater than unity.

Analysis of the 36-day sample by GLC gave the results shown in Fig. 31. Products typical of the degradation of D-glucose were readily identified: lactic acid (28% of the products observed), glyceric acid (3%), 2,4-dihydroxy-butyric acid (6%) and 3-deoxy-D-pentonic acid (2%). Product 4 (1%) correlated well, by retention time, with 3,4-dideoxy-pentonic acid previously identified among the products of cellobiose degradation.

In addition, small peaks were observed at T_r 21.9 and 42.8 minutes, plus a major peak (Product 7) at T_r 24.9 minutes. Upon lactonization, Product 7 was replaced by two peaks at T_r 19.9 and 22.0 minutes. The latter compounds were identified as D-glucoisosaccharino-1,4-lactone and D-glucometasaccharino-1,4-lactone, respectively, at a ratio of 3.5:1. Product 7 (T_r 24.9 min) represented 50% of the products observed; therefore, D-glucoisosaccharinic and D-glucometasaccharinic acids corresponded to 39 and 11%, respectively, of the total products found by GLC.

By mass spectrometry, Product 6 (Fig. 32, Spectrum A) corresponded to 3,4-dideoxy-D-hexonic acid (XXXVIII). The peak at m/e 437 is the M-15 ion,

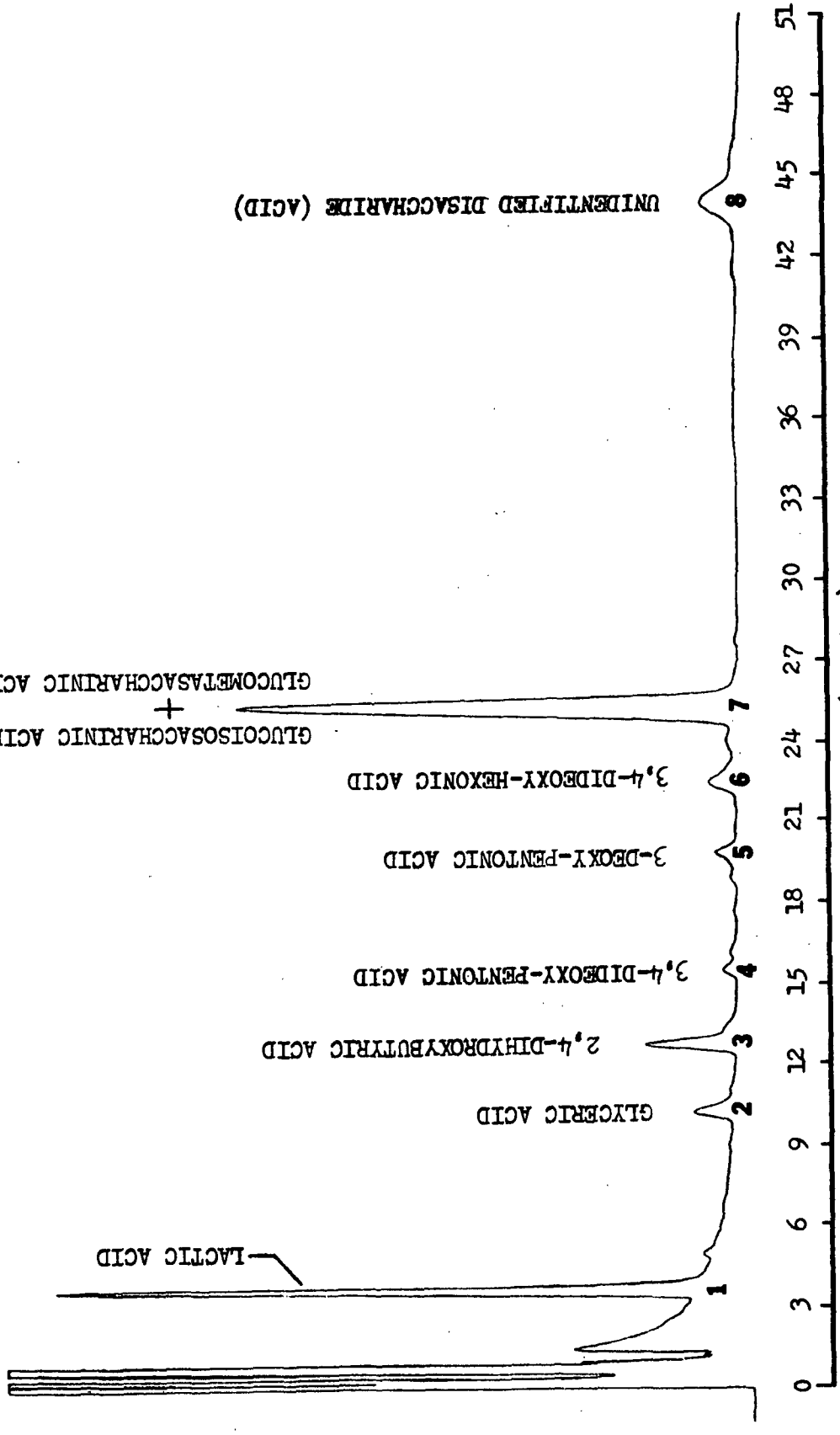


Figure 31. Gas Chromatogram of Products from Reaction of 3,6-Anhydro-Cellobiose with Oxygen-Free 0.099N Sodium Hydroxide at 25°C

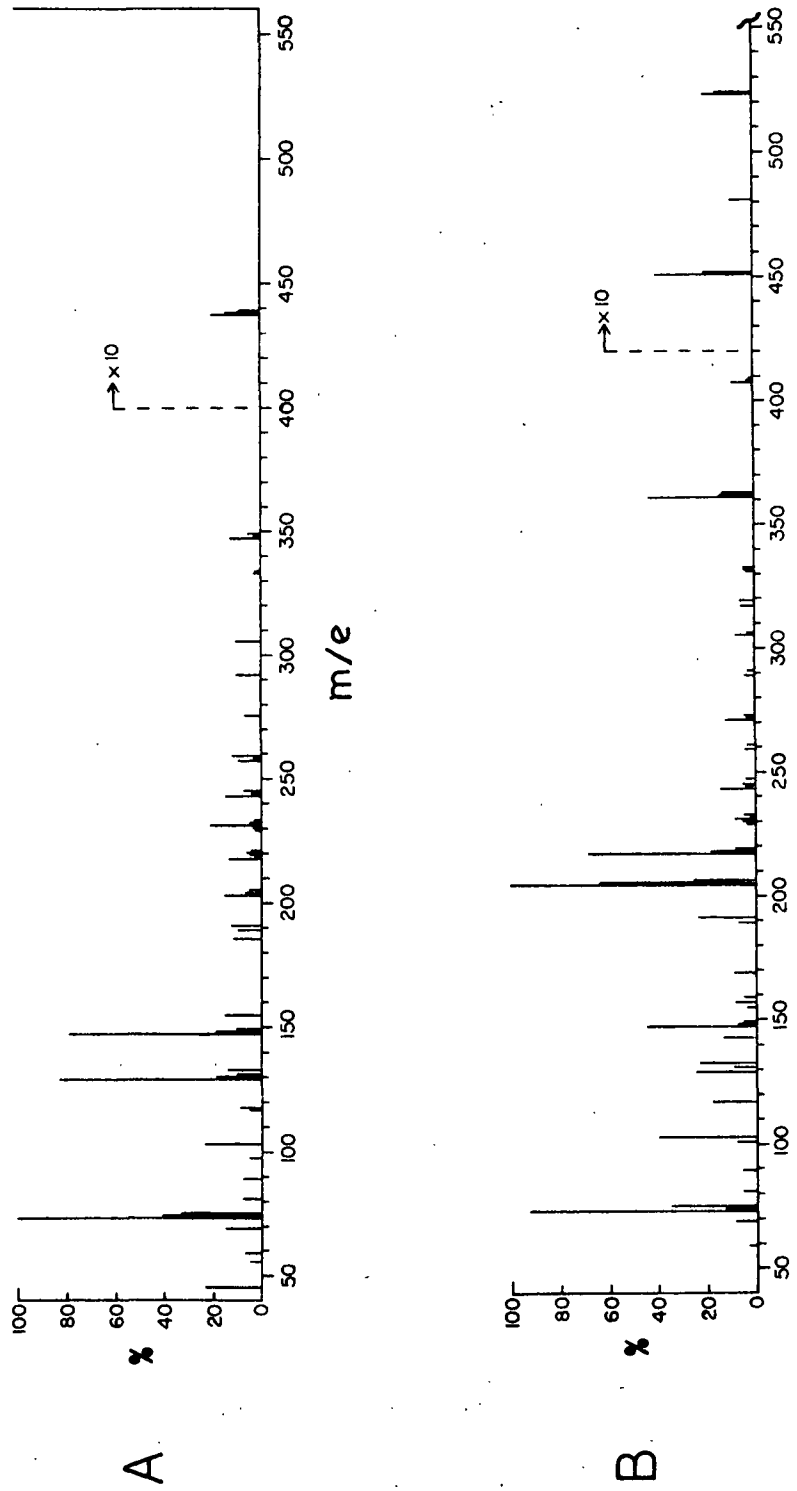
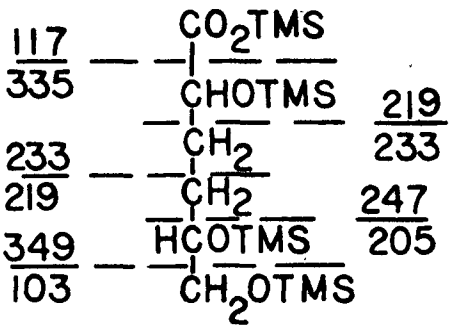


Figure 32. Mass Spectra at 70 ev of Trimethylsilyl Derivatives of Products from 3,6-Anhydro-Cellobiose Degradation; A — Product 6 (Tentatively 3,4-Dideoxy-D-hexonic Acid); B — Product 8 (Unknown Disaccharide Acid) (Partial Spectrum)

which by loss of TMSiOH groups produces ions at m/e 347 and 257. Cleavage at C-5—C-6 results in ions at m/e 103 and 349; the latter loses TMSiOH to form the m/e 259 ion. Cleavage at C-2—C-3 produces ions at m/e 219 and 129 (219-90).



XXXVIII

M 452

The very intense ion at m/e 129 appears to indicate that C-2—C-3 cleavage is highly favored in this molecule. Note, however, that C-3—C-4 cleavage may also contribute ions at m/e 219 and 129. 3,4-Dideoxy-D-hexonic acid accounted for 3% of the products observed from 3,6-anhydro-cellobiose.

The mass spectrum of Product 7 (Appendix II, p. 136) exhibited ions typical of D-glucoisosaccharinic acid (e.g., m/e 243, 257, 347) and D-glucometasaccharinic acid (e.g., m/e 245, 335), thereby confirming that both acids were indeed present. The same approach was discussed earlier regarding identification of the same two acids from cellobiose degradation.

According to its long GLC retention time (T_r 42.8 min), Product 8 appeared to be a disaccharide. Analyzed by mass spectrometry, its mass spectrum (Fig. 32, Spectrum B) exhibited many peaks above the range normally investigated (i.e., m/e 40-560). Unfortunately, measurement of peak heights and assignment of mass numbers became unreliable above m/e 560, due to very small peak sizes and the lack of adequate spacing between consecutive peaks. Nevertheless, ions at

least as high as m/e 700 were observed, indicating that Product 8 was a disaccharide.

In addition, the portion of the spectrum below m/e 460 showed that a glucopyranosyl unit was part of the structure of Product 8. Thus, the base peak was found at m/e 204, accompanied by intense peaks at m/e 191, 217, 231, 243, 271, 361, and 451. The significant peak at m/e 451 corresponds to the G^+ ion typically observed upon fragmentation of TMS derivatives of disaccharides incorporating glucopyranose units (50-52). Loss of TMSiOH groups from the G^+ ion provides the peaks at m/e 361 and 271.

The monosaccharide moieties of the disaccharide should fragment independently and produce two "families" of ions (32). Whereas the ions from the glucopyranose unit were readily distinguished, the remaining peaks were weak and did not permit assignment of a satisfactory structure for what was presumably the acidic portion of the molecule. Note, however, that a significant peak was found at m/e 117, apparently corresponding to C-1–C-2 cleavage of the TMS derivative of an aldonic acid with charge retention at C-1.

Thus, Product 8 may be 4-O-glucosyl-D-glucometasaccharinic acid (formed via the stabilization reaction), but proof of this could not be established. Sufficient material was not available to allow isolation of Product 8 by paper- or ion-exchange chromatography and reanalysis, after hydrolysis, by GLC and GC-MS.

In the degradation of 3,6-anhydro-cellobiose, glucose was observed to be a product (and a reactive intermediate) by paper chromatography and GLC. Thus, glucose is a probable source of lactic, glyceric, 2,4-dihydroxybutyric and 3-deoxy-D-pentonic acids in this reaction. Reaction mechanisms accounting

for formation of these acids were discussed previously for degradation of D-glucose and cellobiose under identical reaction conditions.

D-Glucometasaccharinic acid may be produced from 3,6-anhydro-cellobiose via a number of mechanistic routes. It could come directly from glucose formed in the reaction, the result of β-elimination of OH-3 followed by tautomerization and acid rearrangement (see Fig. 12, p. 32). Alternatively, 3,6-anhydro-cellobiose could undergo a double elimination of C-3 and C-4 substituents, like that postulated for 3,6-anhydro-4-O-methyl-D-glucose (see Fig. 28, p. 68), followed by nucleophilic addition of hydroxide ion to the 3,4-dideoxyhex-3-enosulose and subsequent steps leading to 3-deoxy-D-hexonic acid. Again, as discussed earlier, the isomeric 3-deoxy-D-hexonic acids so formed may exist in the gluco- and galacto-configurations.

The glucosyloxy substituent at C-4 should be an excellent leaving group, just as in the case of cellobiose degradation. Thus, isomerization to 3,6-anhydro-cellobiulose could lead to β-elimination of the glucosyloxy anion, as shown in Fig. 33. The intermediate product, however, is a vinyl ether, because the 3,6-anhydro ring remains intact. Nucleophilic addition of hydroxide ion to the vinyl ether might be expected to occur at either C-3 (Route A, Fig. 32) or C-4 (Route B). An analogous nucleophilic addition of hydroxide ion to C-4 of a 3,4-dideoxyhex-3-enosulose intermediate was proposed previously in the degradation of 3,6-anhydro-4-O-methyl-D-glucose (see Fig. 28, p. 68). In the 3,6-anhydro-cellobiose case, the electron-donating ability of the ether oxygen atom can polarize the C-3–C-4 bond (see XXXIX), thereby directing nucleophilic attack to C-3 (53).

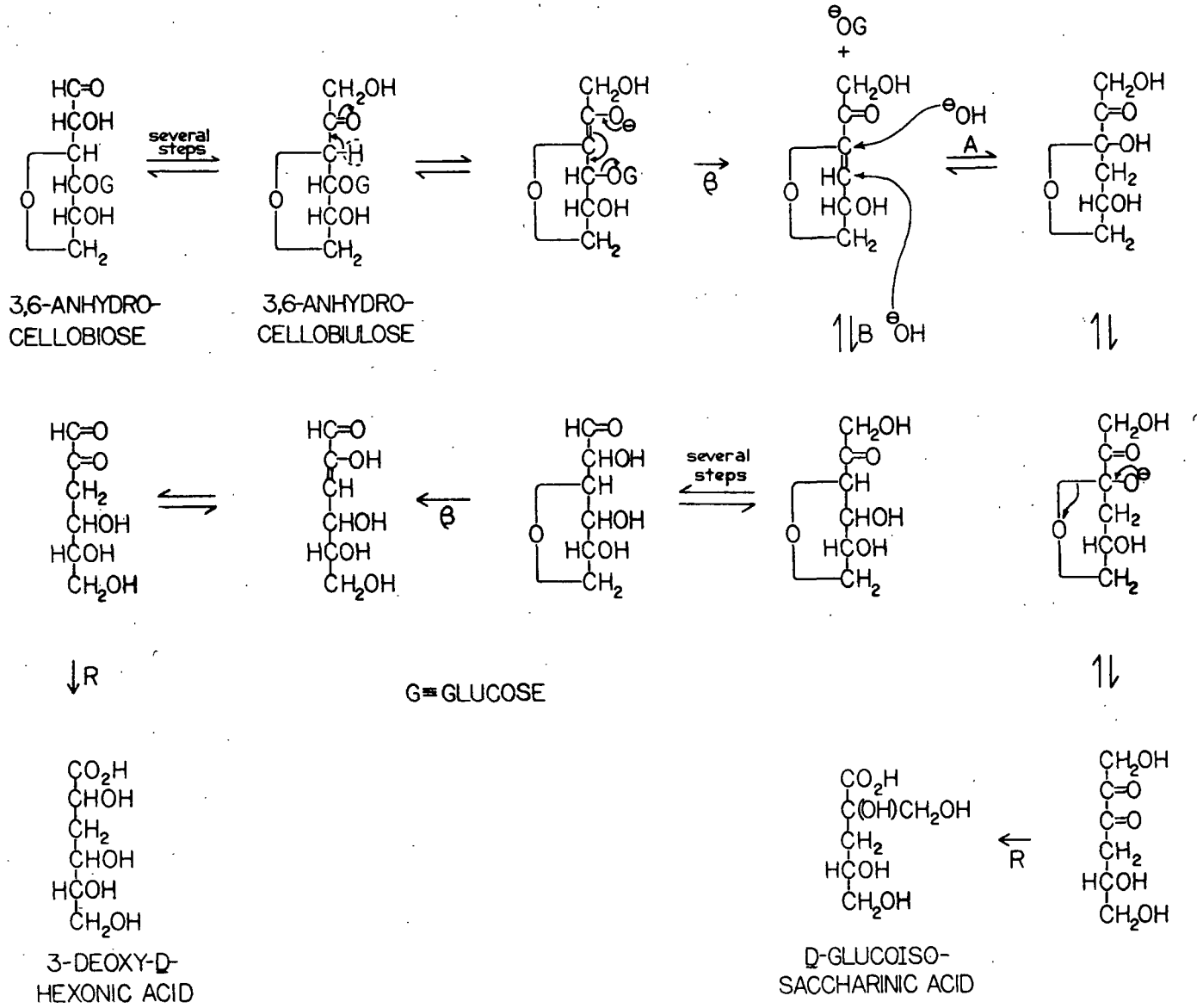
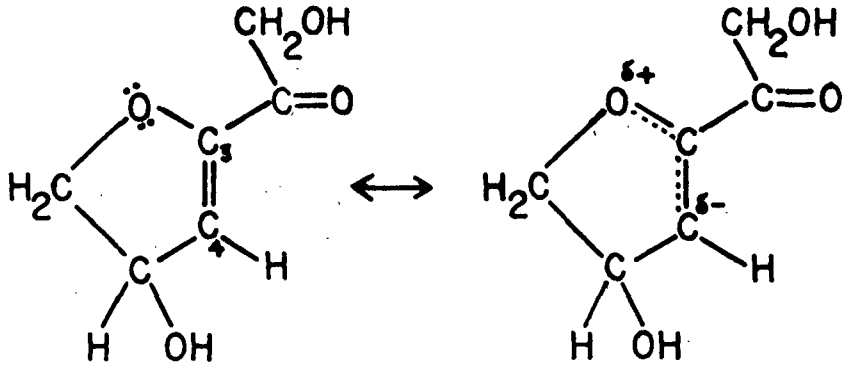


Figure 33. Possible Reaction Mechanisms for Formation of D-Glucoisosaccharinic and 3-Deoxy-D-hexonic Acids from 3,6-Anhydro-Cellobiose; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement



XXXIX

The hemiacetal thus formed at C-3 would ionize in the alkaline solution and then eliminate the ether substituent to give the 2,3-hexodiulose. The final step is rearrangement to D-glucoisosaccharinic acid, the most abundant product from the degradation of 3,6-anhydro-cellobiose at 25°C.

If nucleophilic attack of hydroxyl ion occurred instead at C-4 of the vinyl ether (Route B, Fig. 32), good resonance stabilization of the carbanion could be expected because the conjugated carbonyl group (C-2) is available to delocalize the charge. Following protonation, isomerization of the 2-keto compound back to the aldose form provides a 3,6-anhydro-hexose. The latter compound would react in the same manner as 3,6-anhydro-D-glucose, resulting in formation of 3-deoxy-D-hexonic acid.

As discussed previously, formation of 3,4-dideoxy acids (in this case, pentonic and hexonic) apparently proceeds via β-elimination of both the C-3 and C-4 substituents from the starting material. However, a plausible reaction mechanism accounting for conversion of vinylic intermediates to 3,4-dideoxy acids remains unclear, and therefore the identifications of Products 4 and 6 are tentative. 3,4-Dideoxy-pentonic acid and 3,4-dideoxy-hexonic acid comprised 1 and 3%, respectively, of the final products observed following degradation of 3,6-anhydro-cellobiose.

If 3,6-anhydro-cellobiose degraded to some extent via the stabilization route (i.e., β-elimination only at C-3, followed by tautomerization and acid rearrangement), the expected product would be 4-O-glucosyl-D-glucometasaccharinic acid. Product 8mm may be this disaccharide metasaccharinic acid, but a positive identification could not be made. Product 8av accounted for 7% of the products from 3,6-anhydro-cellobiose.

REACTIONS IN OXYGEN-FREE CALCIUM HYDROXIDE

All of the model compounds degraded in oxygen-free 0.099N NaOH at 25°C were also allowed to react with oxygen-free saturated calcium hydroxide [~2.0 equivalents of $Ca(OH)_2$ per mole of sugar] at the same temperature. The $Ca(OH)_2$ reactions were monitored by titration, except in the case of 3-O-methyl-D-glucose [which was degraded as the preparative method for D-glucometasaccharinic acid subsequently used as a reference compound (54)]. Titrated samples were analyzed by paper chromatography. In the reaction of D-glucose with oxygen-free $Ca(OH)_2$, the product mixture obtained after 28 days was also analyzed by GLC and GC-MS.

The calcium hydroxide degradations were conducted as a check of previously reported data for $Ca(OH)_2$ reactions, particularly with regard to titration data.

D-GLUCOSE AND CELLOBIOSE

D-Glucose (XXIII) and cellobiose (XXIV) were allowed to react with oxygen-free 0.041N $Ca(OH)_2$ in nitrogen atmospheres at 25°C. Graphs of acids generated *versus* time for both reactions are shown in Fig. 34, accompanied by titration data reported in the literature for oxygen-free $Ca(OH)_2$ degradations of D-glucose (17) and cellobiose (14) performed under virtually identical conditions. It is readily apparent from Fig. 34 that the reactions reported here were quite similar to those reported earlier.

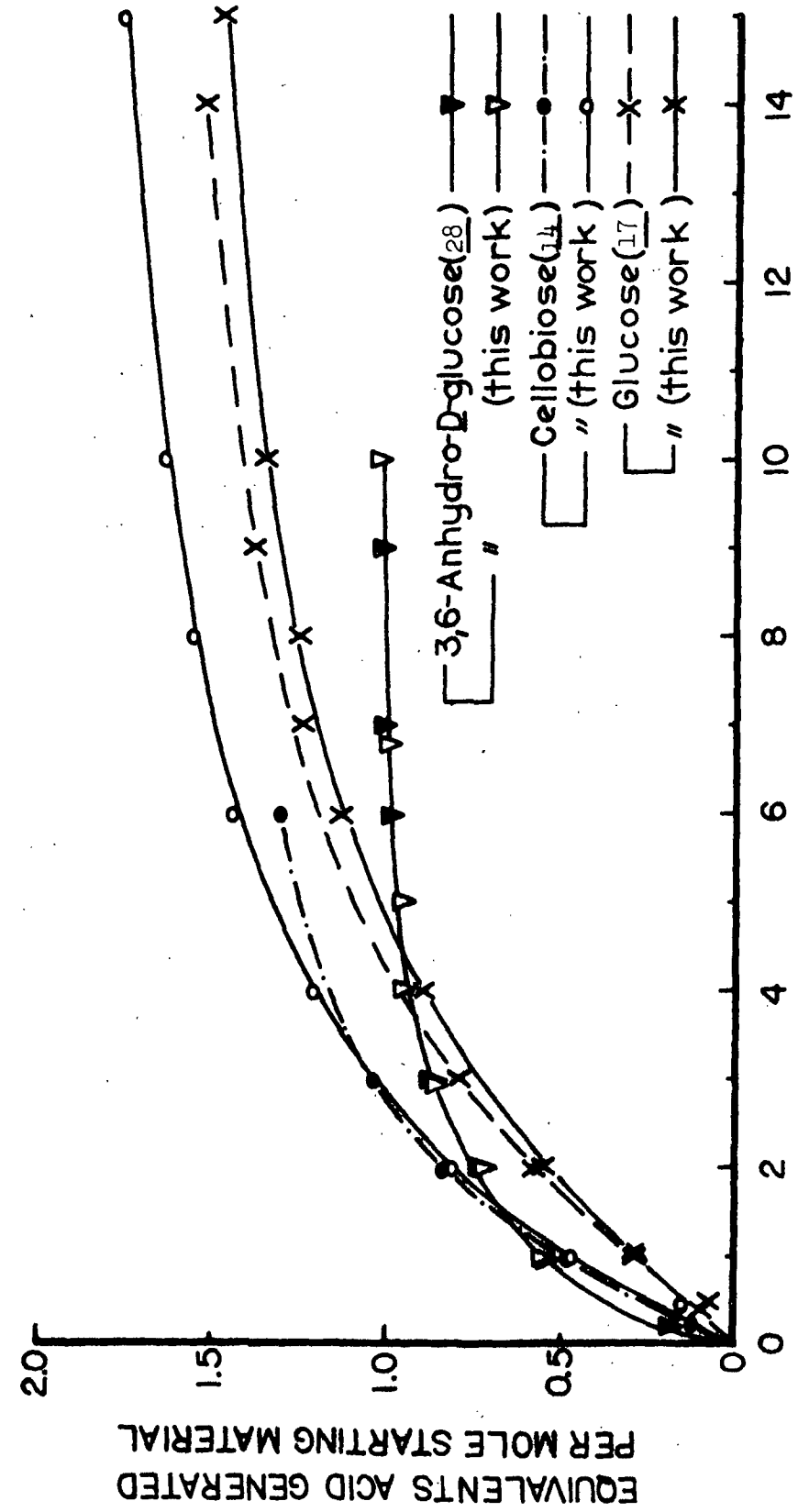


Figure 34. Acids Generated in Reactions of D-Glucose, Cellobiose and 3,6-Anhydro-D-glucose with Oxygen-Free Saturated Calcium Hydroxide at 25°C

After 20 days, D-glucose degraded to 1.64 equivalents of acid per mole of starting material, and cellobiose generated 1.93 equivalents of acid. Neither reaction had reached completion. The reactions proceeded at very slow rates beyond approximately 8 days, due to substantial depletion of the base. After 20 days, 80 and 94% of the initial alkali was consumed in the glucose and cellobiose reactions, respectively. Thus, in the glucose case, insufficient alkali remained to allow the reaction to go to completion in a reasonable amount of time; for cellobiose, there was simply not enough alkali available to generate the full complement of final products.

By paper chromatography, D-glucose was observed to degrade to a number of products with R_G values greater than 1.0. A trace amount of starting material remained visible in the 20-day sample. One product, R_G 1.45, corresponded to D-glucometasaccharinic acid, in agreement with the results of Corbett and Liddle (22).

Paper chromatographic analysis of samples from the cellobiose reaction showed the gradual disappearance of starting material, accompanied by formation of glucose and several more mobile products. The faster products (R_G values >1) correlated well with those found from reaction of D-glucose itself, except for one component (R_G 1.40) which corresponded to D-glucoisosaccharinic acid.

A second reaction of D-glucose with oxygen-free 0.041N $Ca(OH)_2$ was run in a nitrogen atmosphere at 25°C for 28 days, at which time ~1.70 equivalents of acid per mole of starting material had been generated. The 28-day sample was analyzed by GLC, providing the distribution of products shown in Fig. 35.

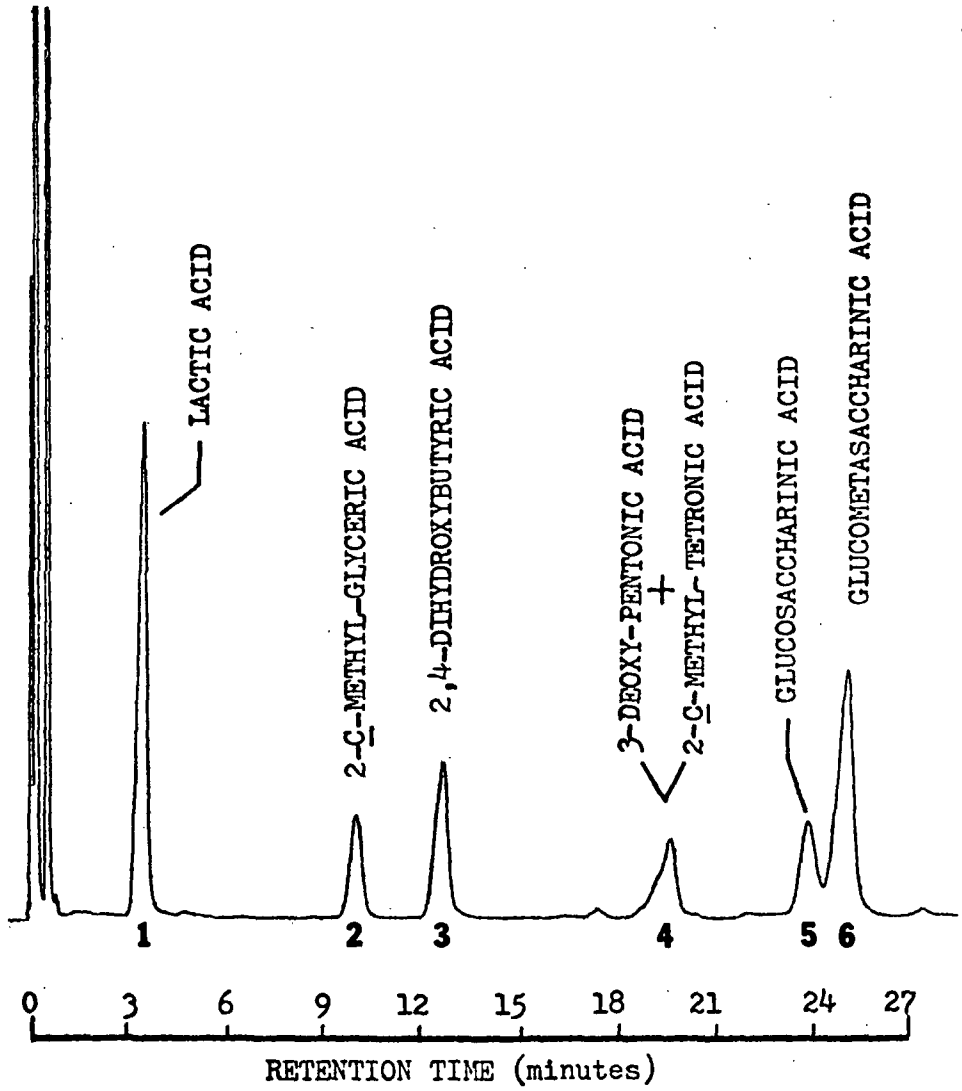


Figure 35. Gas Chromatogram of Products from Reaction of D-Glucose with Oxygen-Free 0.041N Calcium Hydroxide at 25°C

According to GLC retention times, several of the products appeared to be similar to those from oxygen-free NaOH degradation of D-glucose (compare with Fig. 8, p. 25). A sixth component was observed at T_r 23.7 minutes. The relative proportions of products differed greatly from those found in the glucose-NaOH system. Upon lactonization, the compounds having T_r values of 23.7 and 24.9 minutes disappeared, and peaks at T_r 18.8 and 22.1 minutes were observed, correlating well with the known retention times of authentic D-glucosaccharino-1,4-lactone and D-glucometasaccharino-1,4-lactone, respectively.

In order to confirm the identities of products from oxygen-free $Ca(OH)_2$ degradation of D-glucose, all six components were analyzed by mass spectrometry. Mass spectral data for lactic acid (Product 1) are given in Appendix III (p. 141). Good correlation was found with Petersson's data (31) for this compound, and with data for lactic acid produced in the NaOH degradation of cellobiose.

The mass spectrum of Product 2 (T_r 10.0 min) is presented in Fig. 36, accompanied by Petersson's spectrum (31) of 2-C-methyl-glyceric acid. Despite some differences in relative intensities, the two spectra are in accord for all major ions, thereby identifying Product 2 as 2-C-methyl-glyceric acid (XL), not glyceric acid as found in the NaOH degradation reaction. The parent ion (M^+, m/e 336) is observed, and the strong peak at m/e 321 is the M-15 ion. As in the case of glyceric acid, a McLafferty-type rearrangement ion can occur, but now it is seen at m/e 306 rather than 292, indicative of the presence of the 2-C-methyl substituent. No peak is seen at m/e 292, confirming the absence of glyceric acid. The m/e 293 peak is attributed to loss of carbon monoxide from the M-15 ion. Fragmentation at C-1—C-2 and C-2—C-3 with charge

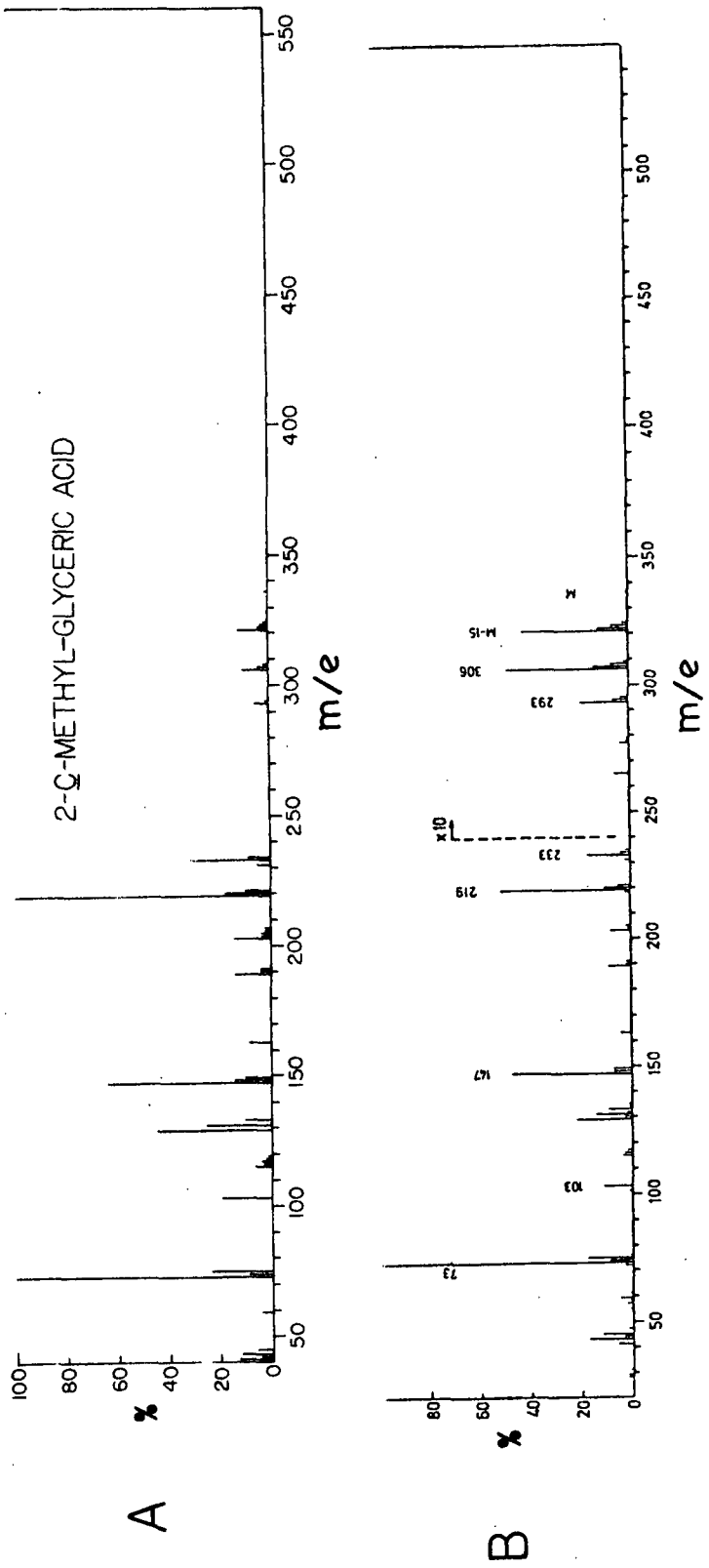
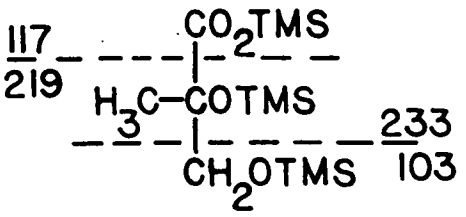


Figure 36. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of 2-C-Methyl-Glyceric Acid; A — This Work; B — Petersson (31)



XL

M 336

retention at C-2 produces very intense peaks at m/e 233 and 219; the 2-C-methyl substituent enhances charge stabilization of these ions (31). The peak at m/e 203 corresponds to loss of TMSiOH from the m/e 293 ion, analogous to what occurs in glyceric acid; the peak is shifted 14 mass units higher than that found with glyceric acid because of the methyl group attached to C-2.

Product 3 was identified as 2,4-dihydroxybutyric acid; the mass spectrum for this product (Appendix III, p. 142) correlated well with those obtained for the same compound from NaOH degradations of D-glucose and cellobiose and that reported by Petersson (31).

Product 4 gave mass spectral data illustrated in Spectrum A of Fig. 37, which agreed in part with the mass spectra of 3-deoxy-pentonic acid from NaOH degradations of cellobiose and 3,6-anhydro-4-O-methyl-D-glucose and with Petersson's spectrum (31) of 3-deoxy-pentonic acid. Several significant differences (e.g., unusually intense peaks at m/e 103, 205, 217, 231, and 306) plus the nonsymmetrical shape of the GLC response (see Product 4, Fig. 35) suggested, however, that a second component was also present.

One possibility is formation of 2-C-methyl-D-tetronic acid (XLI). This C-2 substituted acid is isomeric with 3-deoxy-D-pentonic acid, thus sharing many characteristic ions: m/e 423 (M-15), 335, 333, 245, 231, 217, and 205.

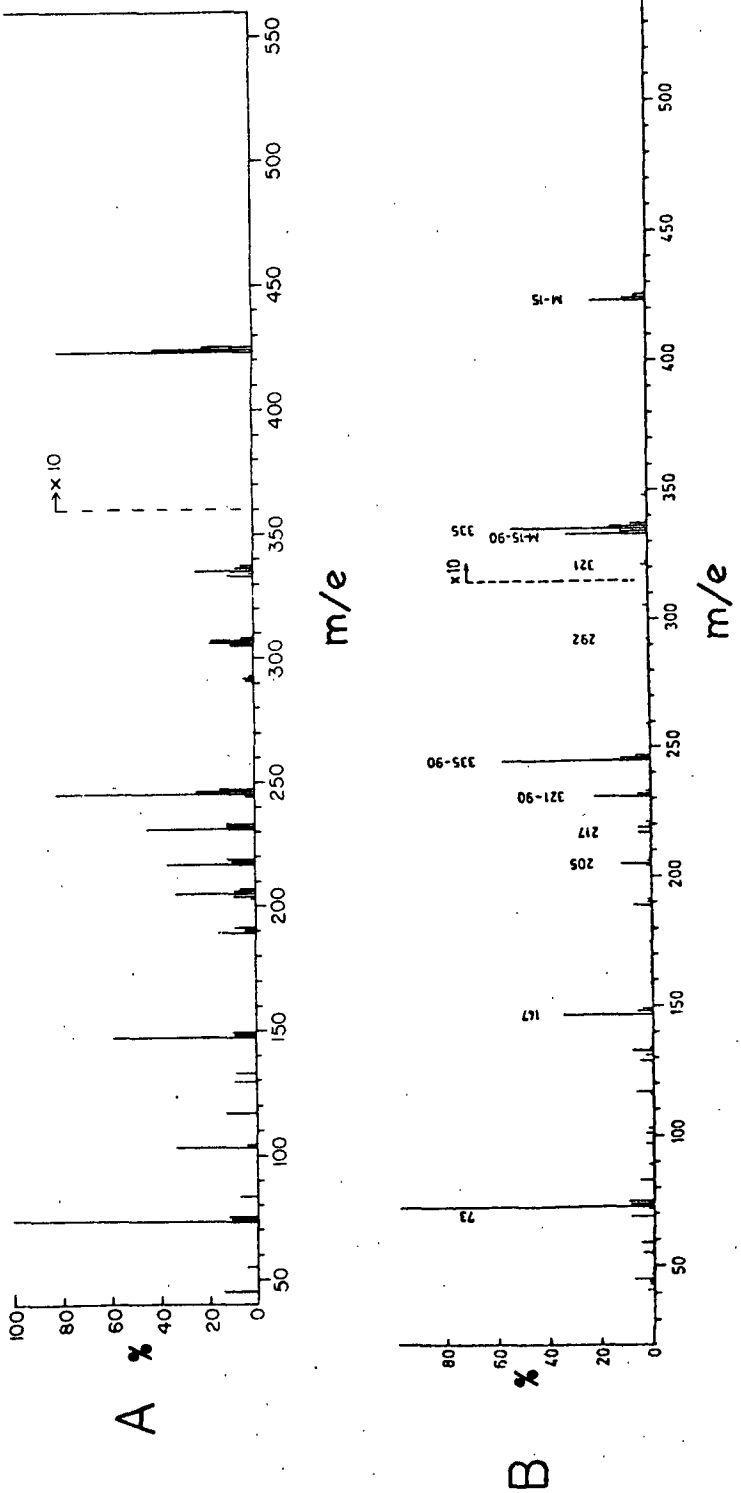
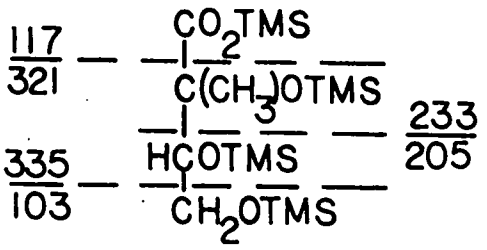


Figure 37. Mass Spectrum at 70 ev of Trimethylsilyl Derivatives of a Mixture of Acids from Glucose Degradation in $Ca(OH)_2$; A — Mixture of 3-Deoxy-D-pentonic Acid and 2-C-Methyl-D-tetronic Acid; B — 3-Deoxy-D-pentonic Acid, Petersson (31)



XLI

Particularly indicative of 2-C-methyl-D-tetronic acid, however, is a strong peak at m/e 306, corresponding to a McLafferty-type rearrangement ion in a 2-C-methyl aldonic acid, as discussed above for 2-C-methyl-glyceric acid. In addition, an intense peak at m/e 103 is seen in Spectrum A; this suggests a second component in Product 4, because the m/e 103 peak for 3-deoxy-D-pentonic acid alone is negligible.

Thus, it appears from the mass spectral data for Product 4 that both 3-deoxy-pentonic and 2-C-methyl-tetronic acids are present. These two acids, both having four hydroxyl groups and identical molecular weights, could be expected to behave similarly (as their TMS derivatives) under the GLC conditions employed, which explains why the compounds were not separated.

The mass spectrum of Product 5 is shown in Fig. 38, along with Petersson's spectrum (31) of D-glucosaccharinic acid. Good correlation was observed for all major peaks, confirming that D-glucosaccharinic acid (XLII) is indeed a product of the oxygen-free $Ca(OH)_2$ degradation of D-glucose. Diagnostic ions are found at m/e 525 (M-15), 437 and 103 (C-4–C-5 cleavage), 435 (M-15-90), 306 (McLafferty-type rearrangement ion), 307 and 233 (C-2–C-3 cleavage), 335 and 205 (C-3–C-4 cleavage) and 217 (307-90).

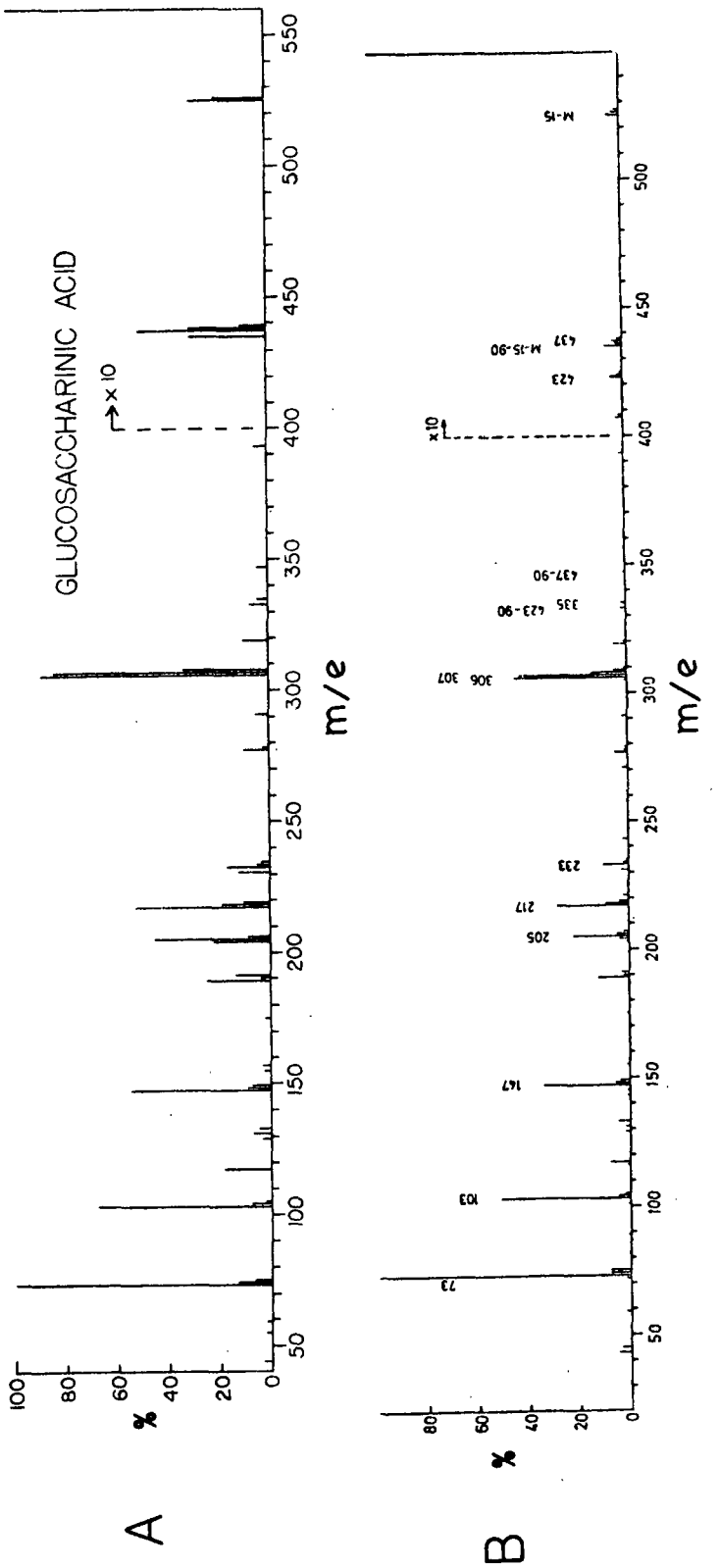
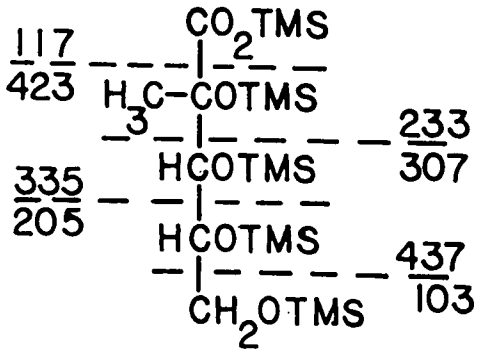


Figure 38. Mass Spectra at 70 ev of the Trimethylsilyl Derivative of D-Glucosaccharinic Acid; A — This Work; B — Petersson (31)



XLII

M 540

Product 6 exhibited a strong mass spectrum (Appendix III, p. 144) fully in agreement with those of D-glucometasaccharinic acid previously found from oxygen-free NaOH degradations of D-glucose, cellobiose and 3,6-anhydro-4-O-methyl-D-glucose.

Possible reaction mechanisms accounting for the series of 3-deoxy-aldonic acids from oxygen-free NaOH degradation of D-glucose were discussed earlier. The same 3-deoxy acids were produced in the $Ca(OH)_2$ reaction. It seems likely, therefore, that the same mechanisms could also be applicable in the $Ca(OH)_2$ case.

However, D-glucose also produces a corresponding series of 2-C-methyl-aldonic acids upon reaction with oxygen-free $Ca(OH)_2$, the individual members of this series containing three to six-carbon atoms. Lactic acid can be considered both a 3-deoxy and a 2-C-methyl-aldonic acid.

A possible route for formation of D-glucosaccharinic acid in the $Ca(OH)_2$ degradation of D-glucose is illustrated in Fig. 39. This reaction route (24) requires a 3-hexulose intermediate (identical to the one shown in Fig. 14, p. 37) which has the necessary configuration to promote β-elimination of the hydroxyl group attached to C-1. Subsequent steps are keto-enol tautomerization to the 2,3-hexodiulose and benzil-benzilic acid type rearrangement to D-glucosaccharinic

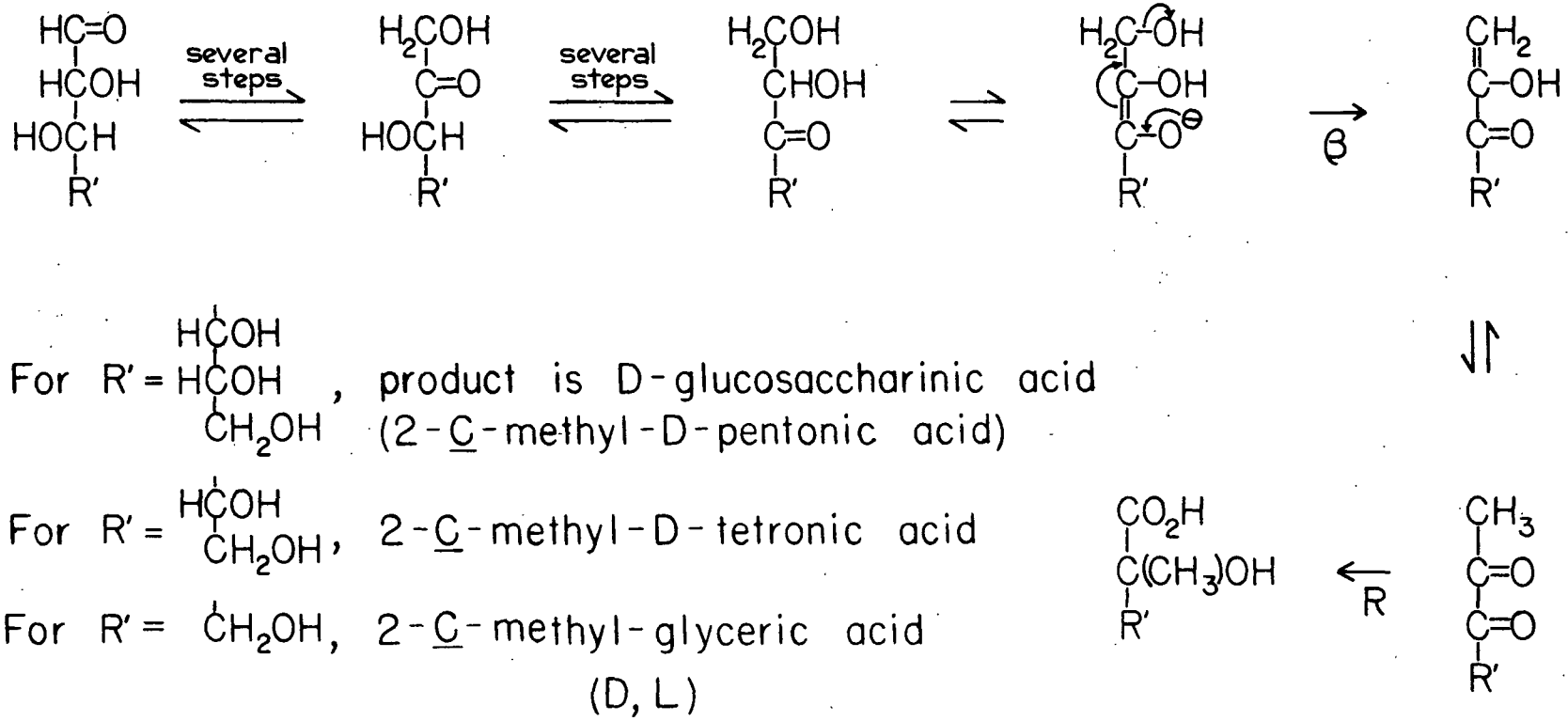


Figure 39. Possible Reaction Mechanism for Formation of 2-C-Methyl-aldonic Acids from D-Glucose; β — β-Elimination; R — Benzil-Benzilic Acid Type Rearrangement

(i.e., 2-C-methyl-D-ribo-pentonic) acid. According to Ishizu, et al. (55), the ribo (or "α") acid is a much more abundant product than the arabino (or "β") acid in this process. The predominance of α-D-glucosaccharinic acid has been ascribed to stereoselective control of the acid rearrangement step, due to an asymmetrically substituted carbon atom adjacent to the 2,3-dicarbonyl position. Such is not the case in the formation of either D-glucometasaccharinic or D-glucoisosaccharinic acids, where the dicarbonyl groups are adjacent to deoxy sites. In the latter two instances, equal proportions of the C-2 isomers are formed (55).

As in the case of NaOH degradation of D-glucose, dealdolization probably is responsible for the formation of products having fewer than 6-carbon atoms. In fact, the C-3 carbonyl intermediate discussed above was postulated earlier to account for dealdolization of D-glucose in NaOH to give a pentose which then degraded to 3-deoxy-D-pentonic acid. Dealdolization could also produce a tetrose which would degrade to 2,4-dihydroxybutyric acid. And by analogy to the formation of D-glucosaccharinic acid from D-glucose (Fig. 38), the pentoses and tetroses could be converted to 2-C-methyl-D-tetronic and 2-C-methyl-glyceric (D,L) acids, respectively. The mechanistic route to lactic acid in NaOH solution also applies to the $Ca(OH)_2$ reaction.

Apart from product identities, another important difference between NaOH and $Ca(OH)_2$ degradations of D-glucose in this work was the production of almost twice as much lactic acid in the NaOH reaction, accompanied by only about half the amount of 6-carbon product(s) (see Table V). This finding agrees with the frequently cited observation that degradation reactions in the presence of divalent cations (e.g., Ca^{2+}) favor production of rearrangement products, whereas solutions containing monovalent cations (e.g., Na^{+}) produce

greater quantities of fragmentation products (8). Whether divalent cations are important in stabilizing ene-diol intermediates by formation of complexes (56-58) or are operative in the benzil-benzilic acid type rearrangement step (59,60) has not been shown conclusively.

TABLE V

DISTRIBUTION OF PRODUCTS FROM OXYGEN-FREE ALKALINE DEGRADATIONS OF D-GLUCOSE AT 25°C

Product	Product Ratio,[a] %	
	0.099N NaOH	0.041N $Ca(OH)_2$
D,L-Lactic acid	56	29
D,L-Glyceric acid	5	
D,L-2-C-Methyl-glyceric acid		8
D,L-2,4-Dihydroxybutyric acid	14	13
3-Deoxy-D-pentonic acid	3	
2-C-Methyl-D-tetronic acid		10[b]
D-Glucosaccharinic acid		11
D-Glucometasaccharinic acid	22	29

[a] Product ratio given as percentage of total products observed by GLC, calculated on peak areas.

[b] Combination of 3-deoxy-D-pentonic and 2-C-methyl-D-tetronic acids.

3,6-ANHYDRO SUGARS

3,6-Anhydro-D-glucose (VI) was degraded in oxygen-free 0.040N calcium hydroxide in a nitrogen atmosphere at 25°C for 10 days. The graph of acids generated versus time is given in Fig. 33 (p. 80). As can be seen readily, the curve was superimposable upon that reported by Corbett and Kenner (28) for $Ca(OH)_2$ degradation of 3,6-anhydro-D-glucose under virtually identical conditions. The reaction reached a plateau of 1.01 equivalents of acid generated per mole of starting material after 7 days; in terms of acids generated, the reaction had a half-life of 21.6 hours.

By paper chromatography, the starting material (R_G 1.95) was seen to disappear, concurrent with formation of a single product (R_G 1.45) corresponding to D-glucometasaccharinic acid. Lactonization of a portion of the final product (10-day sample) and reanalysis showed only one spot (R_G 2.25) which correlated well with the known value (R_G 2.23) for D-glucometasaccharino-1,4-lactone.

3,6-Anhydro-4-O-methyl-D-glucose (V) and 3,6-anhydro-cellobiose (XXII) were allowed to react with oxygen-free 0.041N $Ca(OH)_2$ in nitrogen atmospheres at 25°C for 7 and 15 days, respectively. Graphs of acids generated versus time for the reactions are shown in Fig. 40.

The reactions of both 4-substituted 3,6-anhydro sugars were characterized by very rapid initial rates which began to decline after about one day. Neither reaction was allowed to go to completion.

Paper chromatographic analysis of 7-day samples from both reactions showed formation of many overlapping products, making identifications very difficult. It appeared, however, that a component corresponding to D-glucometasaccharinic acid (R_G 1.44) was present in both reaction product mixtures. Substantial depletion of the base in the 3,6-anhydro-cellobiose case indicated that the reaction probably would not reach completion; therefore, both reactions were discontinued in favor of NaOH degradations.

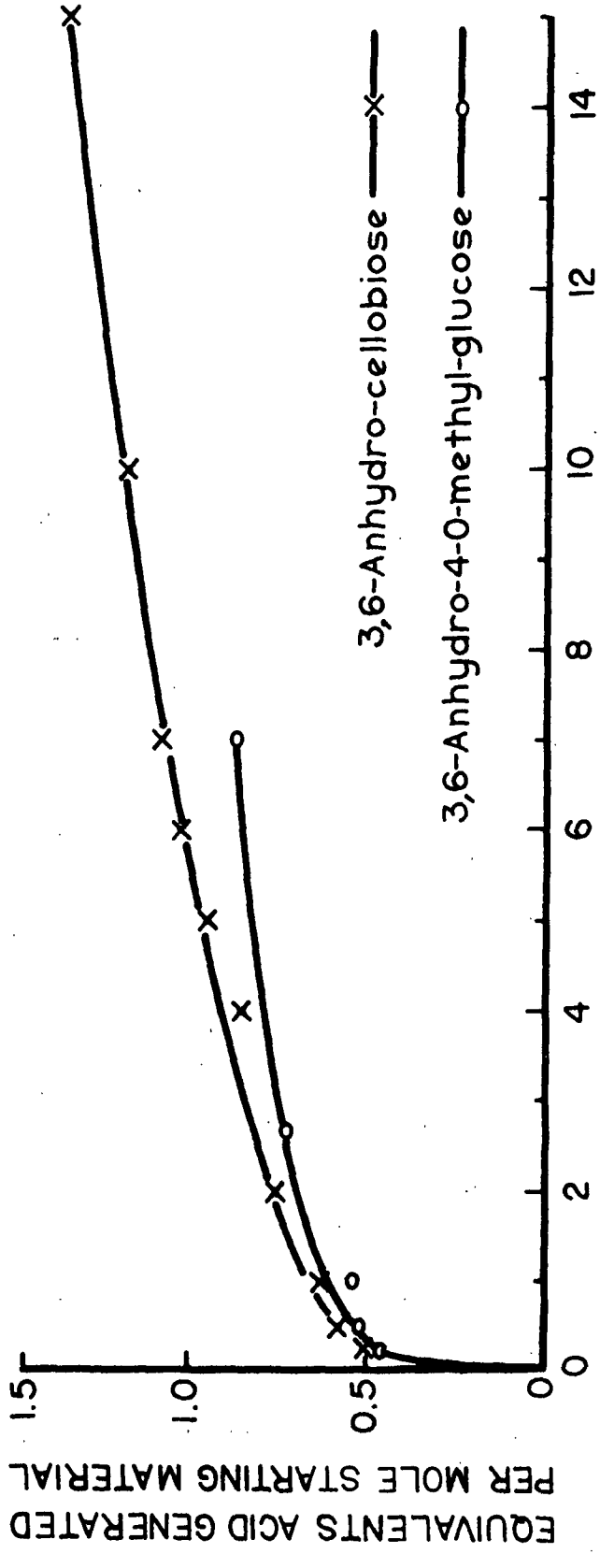


Figure 40. Acids Generated in the Reactions of 3,6-Anhydro-Cellobiose and 3,6-Anhydro-4-O-methyl-D-glucose with Oxygen-Free 0.04$\underline{1}$N Calcium Hydroxide at 25°C

CONCLUSIONS

In dilute, oxygen-free sodium hydroxide and calcium hydroxide degradations of D-glucose, the generally-accepted Nef-Isbell β-alkoxy elimination theory, supplemented by dealdolization, successfully accounts for the products observed. Such is also true for degradations of 3,6-anhydro-D-glucose and 3-O-methyl-D-glucose in oxygen-free sodium hydroxide, which proceed almost exclusively via the "stabilization" route.

For cellobiose, most of the products from sodium hydroxide degradation can be accounted for by the Nef-Isbell theory; no disaccharide product was found. However, previously unreported minor products were tentatively identified which suggest that β-elimination occurred at both C-3 and C-4 of cellobiose.

Formation of 3-deoxy-hexonic acid as the dominant product from sodium hydroxide degradation of 3,6-anhydro-4-O-methyl-D-glucose indicates that a double elimination (of C-3 and C-4 substituents) occurs, following which a key step is nucleophilic addition of hydroxide ion to a 3,4-dideoxyhex-3-enosulose intermediate.

3,4-Dideoxyhex-3-enosuloses appear to be important intermediates in alkaline degradations of 3,6-anhydro-cellobiose and other substituted sugars as well, as proposed in earlier work by Aspinall and coworkers. The nature of the alkene carbon substituents apparently directs the nucleophilic attack of hydroxide ions during addition to the alkene intermediate: a carbonyl group at C-2 leads to hydroxide ion addition at C-4 in the 3,6-anhydro-4-O-methyl-D-glucose case, whereas the additional presence of an ether linkage at C-3 promotes hydroxide ion addition at C-3 in the 3,6-anhydro-cellobiose case.

EXPERIMENTAL

GENERAL ANALYTICAL PROCEDURES AND EQUIPMENT

Melting points were determined on a Thomas Hoover Unimelt apparatus which was calibrated with known compounds.

Thin-layer chromatography was conducted on microscope slides coated with silica gel G (Brinkman Instruments, Des Plaines, IL). To visualize compounds, chromatograms were sprayed with anisaldehyde (5%)-sulfuric acid (5%)-acetic acid (1%) in 95% ethanol and heated to 100°C for 5 minutes (61).

Paper chromatography was conducted on Whatman No. 1 paper using ethyl acetate:acetic acid:water (3:1:1, vol.) as the developer. Chromatograms were developed for 22-24 hours and visualized with alkaline silver nitrate reagents (62). For detection of lactones, hydroxylamine-ferric chloride spray reagents were used (63).

Polarimetric measurements were made on a Perkin-Elmer 141 MC polarimeter.

A Varian A-60A spectrometer was used to determine proton magnetic resonance spectra. Tetramethylsilane was used as the internal standard for deuterochloroform ($CDCl_3$) and dimethyl sulfoxide-d_6 (DMSO-d_6); for deuterium oxide (D_2O), sodium 2,2-dimethyl-2-silapentane-5-sulfonate was the internal standard. Unless otherwise indicated, spectra were determined at normal probe temperature.

Gas-liquid chromatography was conducted on a Varian Aerograph 1200-1 chromatograph equipped with a hydrogen flame ionization detector. Chromatograms were recorded on a Honeywell Electronic 16 recorder equipped with a Disc integrator. The carrier gas was prepurified nitrogen (Matheson Gas

Products). A 5% SE-30 on 60/80 mesh Chromosorb W column (5 ft by 0.125 inch, stainless steel) was used for all analyses except under Conditions D. On-column injection was used throughout. Conditions were:

Conditions A: Nitrogen, 10 ml/min; column, 80 → 200°C at 4°/min; injector, 230°C; detector, 250°C.

Conditions B: Nitrogen, 10 ml/min; column, 80 → 240°C at 4°/min; injector, 230°C; detector, 270°C.

Conditions C: Nitrogen, 18 ml/min; column, 150°C for 5 min, then 150 → 240°C at 6°/min; injector, 260°C; detector, 290°C.

Conditions D: 3% OV-17 on 100/120 mesh Chromosorb W (5 ft by 0.125 inch, stainless steel); nitrogen, 30 ml/min; column, 70 → 170°C at 4°/min; injector, 200°C; detector, 250°C.

Mass spectra were determined on a Du Pont Instruments 21-491 spectrometer interfaced via a jet separator with a Varian Aerograph 1440-1 gas chromatograph equipped with a hydrogen flame ionization detector. Chromatograph response was recorded on a Hewlett-Packard 7128A recorder; mass spectra were recorded on a Century GPO 460 oscillographic recorder.

Chromatographic separations were performed on either of two columns: 10% SE-30 on 60/80 mesh Chromosorb W (5 ft by 0.125 inch, stainless steel) or 5% SE-30 on 60/80 mesh Chromosorb W (5 ft by 0.125 inch, stainless steel). The conditions were as follows: helium (UHP, $\geq$ 99.999%, Matheson Gas Products), 30 ml/min; column, 80 → 200°C at 4°/min; injector, 230°C; detector, 250°C.

Separated compounds were routed from the gas chromatograph to the mass spectrometer via a proportioning valve to the jet separator. The interfacing apparatus was maintained at the following temperatures: valve-interface block, 300°C; connecting tube, 280°C; jet separator, 250°C.

Control settings for the mass spectrometer were: oven temperature, 110°C; source temperature, 225°C; sensitivity, 7.5; ionizing voltage, 70 ev; scan, 10 seconds per decade; chart speed, 4 inches/sec; pressure during sample introduction, $\sim 1 \times 10^{-6}$ Torr.

REAGENTS AND SOLUTIONS

ACETONE

Reagent grade acetone was dried over Drierite, fractionally distilled with exclusion of moisture, and stored in a sealed glass bottle.

CHLOROFORM

Absolute chloroform was prepared as described by Snyder, et al. (64). It was stored under nitrogen in a dry, brown glass bottle in the dark to retard the formation of phosgene.

FEHLING SOLUTION

Fehling solution (Parts 1 and 2) was prepared and used according to Shriner, et al. (65).

HYDROGEN BROMIDE IN ACETIC ACID

Cold glacial acetic acid was saturated with gaseous hydrogen bromide introduced through a gas dispersion tube. The concentration of HBr in acetic acid was determined by weight gain; 35-37% HBr was normal. The reagent was stored in a sealed glass bottle at ~10°C.

METHANOL

Reagent grade methanol was refluxed with magnesium and iodine for 3-4 hours, fractionally distilled with exclusion of moisture (66), and stored in a sealed glass bottle.

PALLADIUM-ON-CARBON CATALYST

Palladium-on-carbon catalyst was prepared according to the method of Mozingo (67).

PYRIDINE

Reagent grade pyridine was dried over potassium hydroxide pellets, fractionally distilled with exclusion of moisture, and stored in a sealed glass bottle.

SCHIFF REAGENT

Schiff reagent was prepared and used according to Shriner, et al. (68).

SODIUM METHOXIDE IN METHANOL

Sodium metal (stored under kerosene) was rinsed clean in anhydrous methanol, then added stepwise to a known volume of anhydrous methanol. Sufficient metallic sodium was dissolved in methanol to prepare an approximately 1N sodium methoxide solution.

SYNTHESIS OF 3,6-ANHYDRO SUGARS

METHYL 6-O-TOLUENESULFONYL-α-D-GLUCOPYRANOSIDE (II)

Methyl-α-D-glucopyranoside (I, 30 g) was dissolved in pyridine (anhydrous, 200 ml) and cooled to 0°C. p-Toluenesulfonyl chloride (33 g, 1.1 molar equivalents) was added, and the reaction solution was allowed to regain room temperature gradually. The reaction was continued for 72 hours, at which time TLC showed only a trace of starting material. The product was diluted with chloroform and cooled, washed with cold 10% hydrochloric acid, cold water, dried (Na_2SO_4, anhydrous) and concentrated *in vacuo* to a syrup. The product crystallized from ethyl acetate at 0°C (35 g, 65%); m.p. 120-121°C. Lit. (69): m.p. 124°C. The PMR[1] spectrum of II in DMSO-d_6 exhibited a broad 4-proton multiplet at τ 2.10-2.60, assigned to the toluenesulfonyl ring protons, and a sharp 3-proton singlet at τ 7.56 which corresponded to the *para*-methyl group on the toluenesulfonyl substituent. A sharp 3-proton singlet at τ 6.75 indicated the presence of one O-methyl group (at C-1).

METHYL 3,6-ANHYDRO-α-D-GLUCOPYRANOSIDE (III)

Methyl 6-O-toluenesulfonyl-α-D-glucopyranoside (II, 8.0 g) was dissolved in absolute methanol (25 ml); 1N sodium methoxide in methanol (40 ml) was added, and the reaction was run for 16 hours at room temperature. TLC showed the reaction to be ~80% complete; more NaOMe (20 ml) was added, and the reaction was continued for 24 hours. Water (40 ml) was then added, and the reaction solution was deionized with IRC-50 (H^+) resin. The eluate was concentrated *in vacuo* to dryness and kept overnight at 50°C in a vacuum oven.

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[1] Proton magnetic resonance spectra are given in Appendix IV, p. 145.

The solid residue was extracted with acetone under reflux for 2 hours, and the acetone solution was concentrated *in vacuo* to a dry syrup (3.3 g, 82%). A portion of the syrup was crystallized from ethyl acetate by addition of petroleum ether; m.p. 112-114°C, $[\alpha]_D^{25}$ + 61.1° (*c* 3.80, MeOH). Lit. (27): m.p. 108°C, $[\alpha]_D^{20}$ + 56° (*c* 1.0, H_2O). The PMR spectrum of III in DMSO-d_6 showed a sharp 3-proton singlet at τ 6.53, corresponding to one *O*-methyl group (at C-1). No signals were observed in the regions τ 2.10-2.60 or τ 7.55, indicating complete removal of the toluenesulfonyl group.

3,6-ANHYDRO-D-GLUCOSE (VI)

Methyl 3,6-anhydro-α-D-glucopyranoside (III, 360 mg) in 0.1*N* sulfuric acid (20 ml) was refluxed for 2 hours, at which time TLC showed one product and no starting material. The solution was cooled, deionized with IR-4B resin (OH^- form), and the eluate was concentrated *in vacuo* to a dry syrup (280 mg, 84%). The product reduced Fehling solution and restored color to Schiff reagent. It was chromatographically pure (TLC). The PMR spectrum of VI in D_2O showed no *O*-methyl resonance signals, confirming the complete removal of the *O*-methyl group from C-1 of III.

Analysis of 3,6-anhydro-D-glucose (TMS ether) by GLC (Conditions A) showed five distinct peaks; these represented the furanose (2), pyranose (2) and open-chain (or aldehydo) configurations possible in this unusual compound.

Benzyl 3,6-anhydro-β-D-glucopyranoside (XIII, 700 mg) was dissolved in absolute methanol (10 ml). The solution was added to palladium-on-carbon catalyst (500 mg) in a teflon-lined Parr bomb. After purging the bomb three times with hydrogen, 30 psig H_2 was applied for 24 hours with magnetic

stirring. The mixture was filtered through Celite, and the filtrate was concentrated *in vacuo* to a dry syrup which was chromatographically pure (TLC) (405 mg, 90%); $[\alpha]_D^{25}$ + 53° (*c* 4.0, H_2O). The compound resisted crystallization. It gave positive responses to Fehling solution and Schiff reagent. A small portion (70 mg) of VI was acetylated with acetic anhydride in anhydrous pyridine. The PMR spectrum of the peracetylated product in $CDCl_3$ showed a multiplet at τ 7.88-7.96 integrating to 9 protons, indicating the presence of three *O*-acetyl substituents as expected.

METHYL 3,6-ANHYDRO-4-*O*-METHYL-α-D-GLUCOPYRANOSIDE (IV)

Methyl 3,6-anhydro-α-D-glucopyranoside (III, 2.8 g) was dissolved in acetone (25 ml, anhydrous) and transferred to a flask fitted with a condenser and a magnetic stirrer. Methyl iodide (9.0 ml, AR) was added, then silver oxide (0.9 g). The mixture was stirred continuously at 40°C with exclusion of moisture. After 12 hours, a second addition of Ag_2O (0.9 g) was made. The reaction was complete (by TLC) after 24 hours. The reaction mixture was filtered through Celite, and the silver salts were washed thoroughly with boiling acetone. The filtrate was concentrated *in vacuo* and the thin syrup crystallized from acetone by addition of ethyl ether (1.8 g, 60%); m.p. 150-152°C; $[\alpha]_D^{25}$ + 29.9 (*c* 1.07, H_2O). Lit. (27): m.p. 152°C; $[\alpha]_D^{17}$ + 24° (*c* 1.1, H_2O). The PMR spectrum of IV in $CDCl_3$ showed two sharp 3-proton singlets at τ 6.37 and 6.45, indicating the presence of two *O*-methyl substituents.

3,6-ANHYDRO-4-*O*-METHYL-D-GLUCOSE (V)

Methyl 3,6-anhydro-4-*O*-methyl-α-D-glucopyranoside (IV, 400 mg) in 0.1*N* sulfuric acid (20 ml) was refluxed for 1 hour, at which time TLC showed one product and no starting material. The reaction solution was cooled, deionized

with Amberlite IR-4B (OH^-) resin, and the eluate was concentrated *in vacuo* to a dry syrup (312 mg, 84%); $[\alpha]_D^{25}$ - 15.2° (*c* 3.12, H_2O). Lit. (27): $[\alpha]_D^{18}$ - 17° (*c* 0.7, H_2O). The product reduced Fehling solution, characteristic of reducing sugars, and restored color to Schiff reagent, indicative of 3,6-anhydro sugars (29). The PMR spectrum of V in DMSO-d_6 exhibited a single *O*-methyl resonance signal (τ 6.57), confirming that the *O*-methyl group had been removed from C-1 of IV.

Methyl 3,6-anhydro-4-*O*-methyl-β-D-glucopyranoside (IX, 300 mg) was dissolved in 0.1*N* sulfuric acid (20 ml) and refluxed for 60 minutes. TLC showed one product and no starting material. The reaction solution was cooled, neutralized with solid barium carbonate and filtered through Celite. The filtrate was concentrated *in vacuo* to a dry syrup (220 mg, 79%); $[\alpha]_D^{25}$ - 11° (*c* 1.09, H_2O). Lit. (27): $[\alpha]_D^{18}$ - 17° (*c* 0.7, H_2O). The product reduced Fehling solution, demonstrating that it was a reducing sugar. Its PMR spectrum in DMSO-d_6 showed only one sharp *O*-methyl signal (τ 6.63), indicating complete removal of the *O*-methyl group from C-1 of IX. By TLC, the compound was identical to 3,6-anhydro-4-*O*-methyl-D-glucose synthesized by the alternate route previously described.

METHYL 4-*O*-METHYL-6-*O*-TOLUENESULFONYL-β-D-GLUCOPYRANOSIDE (VIII)

Methyl 4-*O*-methyl-β-D-glucopyranoside (70) (VII, 1 g) was dissolved in pyridine (15 ml, anhydrous) and cooled to 0°C; *p*-toluenesulfonyl chloride (1.10 g, 1.2 molar equivalents) was added. The solution was allowed to regain room temperature slowly; after 36 hours, TLC showed that the starting material was consumed. The solution was diluted with chloroform and cooled, washed with cold 10% hydrochloric acid, cold water, and concentrated *in vacuo* to a dry syrup (1.43 g, 82%). The PMR spectrum of VIII in $CDCl_3$

showed two distinct 3-proton singlets at τ 6.47 and 6.53, assigned to the O-methyl substituents. A 4-proton multiplet at τ 2.10-2.70 was assigned to the toluenesulfonyl ring protons; a sharp 3-proton singlet at τ 7.53 was attributed to the para-methyl group on the toluenesulfonyl substituent.

METHYL 3,6-ANHYDRO-4-O-METHYL-β-D-GLUCOPYRANOSIDE (IX)

Methyl 4-O-methyl-6-O-toluenesulfonyl-β-D-glucopyranoside (VIII, 1.19 g) in absolute methanol (10 ml) and 1N sodium methoxide in methanol (10 ml) were allowed to react for 24 hours. The solution was warmed to 50°C, and more NaOMe (10 ml) was added. After 12 hours, no starting material was detectable by TLC. Water (10 ml) was added, and the solution was deionized with Amberlite IRC-50 (H^+) resin. The eluate was concentrated to dryness in vacuo and kept overnight in a vacuum oven at 50°C. The solid residue was extracted with acetone under reflux for 2 hours, and the acetone solution was concentrated in vacuo to a dry syrup (420 mg, 67%). The PMR spectrum of IX in $CDCl_3$ exhibited distinct 3-proton singlets at τ 6.47 and 6.55, corresponding to two O-methyl substituents. The absence of signals in the regions τ 2.10-2.70 and 7.55 confirmed the complete removal of the toluenesulfonyl group.

BENZYL 2,3,4,6-TETRA-O-ACETYL-β-D-GLUCOPYRANOSIDE (X)

Powdered Drierite (5 g), yellow mercuric oxide (2 g), mercuric bromide (100 mg), absolute chloroform (25 ml) and benzyl alcohol (15 ml, dried over Drierite) were placed in a sealed flask and stirred magnetically for 1 hour. Tetra-O-acetyl-α-D-glucosyl bromide (71) (5 g) was added, and the reaction was stirred continuously for 48 hours at room temperature. The reaction mixture was filtered through Celite into 10% aqueous potassium iodide (100 ml). The $CHCl_3$ layer was separated, washed with 10% KI solution, washed with cold water, dried (Na_2SO_4, anhydrous) and concentrated in vacuo to remove

$CHCl_3$. The warm, concentrated solution was dissolved in absolute ethanol, and the product crystallized at 0°C (3.6 g, 66%); m.p. 94-95°C. Lit. (72): m.p. 96-101°C. The PMR spectrum of X in $CDCl_3$ exhibited a 5-proton singlet at τ 2.68 corresponding to the benzyl ring protons; a multiplet at τ 7.90-8.05 integrated to 12 protons, indicative of four O-acetyl substituents.

BENZYL β-D-GLUCOPYRANOSIDE (XI)

Benzyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (X, 3.5 g) in absolute methanol (10 ml) and 1N sodium methoxide in methanol (0.5 ml) were allowed to react for 3 hours. TLC showed no starting material. The solution was neutralized with IR-120 (H^+) resin and filtered. The filtrate was concentrated *in vacuo* to a white solid which was dried in a vacuum oven at 50°C for 24 hours (2.0 g, 93%); m.p. 121-122°C. Lit. (72): m.p. 123-125°C. The PMR spectrum of XI in DMSO-d_6 showed a broad 5-proton singlet at τ 2.63 corresponding to the benzyl ring protons. Absence of signals in the range τ 7.90-8.10 indicated complete removal of the O-acetyl substituents.

BENZYL 6-O-TOLUENESULFONYL-β-D-GLUCOPYRANOSIDE (XII)

Benzyl β-D-glucopyranoside (XI, 2.0 g) was dissolved in pyridine (10 ml, anhydrous) and cooled to 5°C. p-Toluenesulfonyl chloride (1.55 g, 1.1 molar equivalents) was added to the solution. After 72 hours at room temperature, the solution was diluted with chloroform and cooled, washed with cold 10% hydrochloric acid, cold water, dried (Na_2SO_4, anhydrous), and concentrated *in vacuo* to a white solid which was dried in a vacuum oven at 50°C for 24 hours (2.0 g, 64%); m.p. 114-117°C. Lit. (69): 116-117°C. A small portion (~100 mg) of XII was acetylated with acetic anhydride in anhydrous pyridine (73). The peracetylated product, m.p. 141-142°C, was pure by TLC. Its PMR spectrum in $CDCl_3$ exhibited a 9-proton multiplet at τ 2.15-2.70

which was attributed to the toluenesulfonyl (4H) and benzyl (5H) ring protons. A 3-proton singlet at τ 7.58 was assigned to the para-methyl substituent on the toluenesulfonyl group. A sharp 9-proton signal was observed at τ 8.02, indicative of three O-acetyl groups as expected for the peracetylated derivative of XII.

BENZYL 3,6-ANHYDRO-β-D-GLUCOPYRANOSIDE (XIII)

Benzyl 6-O-toluenesulfonyl-β-D-glucopyranoside (XII, 1.9 g) in absolute methanol (10 ml) and 1N sodium methoxide in methanol (2 ml) were allowed to react in a sealed flask for 24 hours at room temperature. TLC showed no starting material. The reaction solution was neutralized with IR-120 (H^+) resin, filtered, and the filtrate was concentrated in vacuo to a dry syrup (894 mg, 79%). The syrup was pure by TLC; however, it resisted crystallization. A small portion (~75 mg) of XIII was acetylated with acetic anhydride in anhydrous pyridine. The PMR spectrum of the peracetylated product in $CDCl_3$ showed a sharp 5-proton singlet at τ 2.64 which was assigned to the benzyl ring protons; two sharp 3-proton singlets at τ 7.85 and 7.98 indicated the presence of two O-acetyl substituents as expected. No signals corresponding to a toluenesulfonyl group were observed.

OCTA-O-ACETYL-β-CELLOBIOSE (XV)

Acetic anhydride (1200 ml, AR) was added to a 3-liter beaker equipped with two steam-cold water fingers. Under continuous stirring, sodium acetate (100 g) and then crude cellobiose (185 g) were added, and the temperature was raised slowly to 75°C. Glacial acetic acid (150 ml) was added. The temperature was brought to 90°C and maintained for 3 hours. TLC showed no remaining cellobiose. The reaction mixture was allowed to cool slowly to 50°C, poured into 6 liters of vigorously stirred ice water, and stirred for 2 hours.

The white precipitate was filtered, washed with cold water and dried *in vacuo* at 50°C for 24 hours (249 g, 68%). Pure XV had m.p. 195-197°C. Lit. (73): m.p. 202°C.

HEPTA-*O*-ACETYL-α-CELLOBIOSYL BROMIDE (XVI)

Finely powdered octa-*O*-acetyl-β-cellobiose (XV, 150 g) was added to hydrogen bromide in acetic acid (35%, 300 ml). The mixture, in a sealed flask, was stirred magnetically for 2 hours at room temperature, then poured slowly into 3 liters of vigorously stirred ice water. The white precipitate was filtered, washed with cold water, rinsed with petroleum ether (b.p. 30-60°C) and dried *in vacuo* at 40°C (108 g, 70%); m.p. 176°C (decomp.). Lit. (74): m.p. 180°C (decomp.).

BENZYL HEPTA-*O*-ACETYL-β-CELLOBIOSIDE (XVII)

Drierite (10-20 mesh, 50 g), mercuric oxide (yellow, 20 g), mercuric bromide (1 g), absolute chloroform (250 ml), and benzyl alcohol (150 ml, dried over Drierite) in a 500 ml stoppered flask were stirred magnetically for 2 hours. Hepta-*O*-acetyl-α-cellobiosyl bromide (XVI, 55 g) was added, and stirring was continued for 24 hours. The reaction mixture was filtered through Celite into 10% aqueous potassium iodide (200 ml). The chloroform layer was separated, washed with 10% KI, cold water, dried (Na_2SO_4, anhydrous) and concentrated *in vacuo* to a thin syrup. The syrup, still warm, was poured into absolute ethanol (400 ml) under strong stirring. The white precipitate was filtered and dissolved in a minimum of hot $CHCl_3$, from which crystallization was achieved by addition of absolute EtOH (37 g, 66%); m.p. 189-191°C, $[\alpha]_D^{25}$ - 43.5° (*c* 1.86, $CHCl_3$). Lit. (75): m.p. 188-190°C.

BENZYL β-CELLOBIOSIDE (XVIII)

Benzyl hepta-O-acetyl-β-cellobioside (XVII, 37 g) was dissolved in absolute methanol (200 ml) and absolute chloroform (100 ml), and 1N sodium methoxide in methanol (10 ml) was added to the solution. After 3 hours, TLC showed no starting material. The solution was neutralized with Amberlite IR-120 (H^+) resin and concentrated *in vacuo* to a syrup. The syrup was crystallized from ethanol:methanol (5:1, vol.) (19.5 g, 89%); m.p. 190-191°C; $[\alpha]_D^{25}$ - 34.2° (*c* 1.14, H_2O), $[\alpha]_D^{25}$ - 39.1° (*c* 1.01, MeOH). Lit. (76): m.p. 187°C; $[\alpha]_D^{25}$ - 35.5° (H_2O).

BENZYL 4',6'-O-BENZYLIDENE-β-CELLOBIOSIDE (XIX)

Powdered zinc chloride (35 g, anhydrous), benzaldehyde (70 ml, AR), and benzyl β-cellobioside (XVIII, 17 g, finely powdered) were placed in a stoppered flask and mechanically shaken for 48 hours at room temperature. The syrupy mixture was poured slowly into vigorously stirred cold aqueous 10% sodium bisulfite (1 liter). After 30 minutes, the white precipitate was filtered, washed with cold 10% $NaHSO_3$, cold water, rinsed with petroleum ether (b.p. 30-60°C) and dried for 24 hours *in vacuo*. The solid product was crystallized from absolute ethanol (12.8 g, 62%); m.p. 191-193°C, $[\alpha]_D^{25}$ - 46.6° (*c* 1.08, MeOH). Lit. (77): m.p. 191°C, $[\alpha]_D^{20}$ - 47.0° (MeOH). The PMR spectrum of XIX in DMSO-d_6 showed a 10-proton multiplet at τ 2.45-2.75, indicating the presence of two phenyl moieties (i.e., the benzyl and benzylidene substituents). A 1-proton singlet at τ 4.38 was assigned to the nonaromatic benzylidene proton.

BENZYL 4',6'-O-BENZYLIDENE-6-O-TOLUENESULFONYL-β-CELLOBIOSIDE (XX)

Benzyl 4',6'-O-benzylidene-β-cellobioside (XIX, 12 g) was dissolved in pyridine (60 ml, anhydrous) and cooled to 5°C. p-Toluenesulfonyl chloride (4.83 g, 1.1 molar equivalents) was added, and the mixture was shaken until the p-TsCl dissolved. After 48 hours at room temperature, TLC showed no starting material. The solution was diluted with chloroform and cooled, washed with 10% hydrochloric acid, cold water, dried ($CaCl_2$, anhydrous) and concentrated in vacuo to a white solid. The product was crystallized from ethanol:methanol (4:1, vol.) (10.2 g, 66%); m.p. 159°C (decomp.); $[\alpha]_D^{25}$ - 33.7°C (c 1.01, MeOH). The PMR spectrum of XX DMSO-d_6 showed a 14-proton multiplet at τ 2.05-2.70 which was assigned to the combined ring-proton signals of the benzyl (5H), benzylidene (5H), and toluenesulfonyl (4H) substituents. The nonaromatic benzylidene proton singlet was observed at τ 4.38. A 3-proton singlet at τ 7.60 was assigned to the para-methyl group attached to the toluenesulfonyl substituent.

BENZYL 3,6-ANHYDRO-4',6'-O-BENZYLIDENE-β-CELLOBIOSIDE (XXI)

Benzyl 4',6'-O-benzylidene-6-O-toluenesulfonyl-β-cellobioside (XX, 7.2 g) in absolute MeOH (25 ml) and 1N sodium methoxide in methanol (15 ml) were allowed to react in a sealed flask for 24 hours at room temperature. TLC showed no starting material. The solution was neutralized with IRC-50 (H^+) resin to a pH slightly above 7 and concentrated in vacuo to a thin syrup which crystallized from absolute ethanol (4.1 g, 74%); m.p. 156-157°C; $[\alpha]_D^{25}$ - 132° (c 0.75, $CHCl_3$). The PMR spectrum of XXI in $CDCl_3$ showed a 10-proton multiplet at τ 2.45-2.70 which was assigned to the aromatic protons of the benzyl and benzylidene substituents. The nonaromatic benzylidene proton singlet was observed at τ 4.48. Signals typical of a p-toluenesulfonyl group (i.e.,

4-proton broad multiplet, τ 2.05-2.60; 3-proton singlet, τ 7.60) were absent, indicating the complete removal of the 6-O-toluenesulfonyl group. A small amount of XXI (∿100 mg) was acetylated with acetic anhydride in anhydrous pyridine. The peracetylated product was crystallized from absolute ethanol; m.p. 186-189°C, $[\alpha]_D^{25}$ - 160.7° (c 1.70, $CHCl_3$). Its PMR spectrum in $CDCl_3$ showed a 9-proton multiplet at τ 7.85-8.10, indicating, as expected, the presence of three O-acetyl substituents (at C-2, C-2' and C-3').

3,6-ANHYDRO-CELLOBIOSE (XXII)

Benzyl 3,6-anhydro-4',6'-O-benzylidene-β-cellobioside (XXI, 1.9 g) in absolute methanol:absolute ethanol (50 ml; 1:1, vol.) and palladium-on-carbon catalyst (1.9 g) were placed in a teflon-lined Parr bomb. After purging the bomb three times with hydrogen, 30 psig H_2 was applied for 24 hours with magnetic stirring. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo to a white solid product which was chromatographically pure (TLC) (786 mg, 86%); $[\alpha]_D^{25}$ + 19.4° (c 2.11, H_2O); $[\alpha]_D^{25}$ + 7.2° (c 1.32, MeOH).

The PMR spectrum of XXII in DMSO-d_6 showed no signals in the aromatic region (τ 2.00-3.00) nor in the vicinity of τ 4.38, confirming the complete removal of the benzyl and benzylidene blocking groups from XXI. A small amount (∿150 mg) of XXII was acetylated with acetic anhydride in anhydrous pyridine. Although the peracetylated product was chromatographically pure (TLC) it resisted crystallization. Its PMR spectrum in $CDCl_3$ showed O-acetyl signals (τ 7.85-8.00) integrating to 18 protons, as required for the hexa-acetate of XXII.

3,6-Anhydro-cellobiose gave a positive response to Fehling solution, indicative of a reducing sugar. It restored color to Schiff reagent, characteristic of 3,6-anhydro sugars.

Following complete hydrolysis of a sample of 3,6-anhydro-cellobiose in 2N HCl, GLC analysis showed the products to be identical to authentic D-glucose and 3,6-anhydro-D-glucose.

SYNTHESIS OF REFERENCE COMPOUNDS

D-GLUCOMETASACCHARINIC ACIDS (XLIII) (54) (3-DEOXY-D-ARABINO- AND RIBO-HEXONIC ACIDS)

3-O-Methyl-D-glucose (78) (6.0 g) was treated with saturated, oxygen-free calcium hydroxide solution (0.04N, 1 liter) under a nitrogen atmosphere for 170 hours at room temperature. The reaction solution was neutralized with solid carbon dioxide, boiled to precipitate calcium carbonate and filtered. The filtrate was decolorized with activated carbon and concentrated in vacuo to a dry syrup (5.5 g, 89%).

The syrup was dissolved in 25% aqueous ethanol (20 ml); after 7 days at 5°C, the calcium arabino-hexonate ("β" salt) precipitated. The precipitate was isolated on a cold filter and dissolved in warm water. The solution was deionized with excess IR-120 (H^+) resin (pH 2-3) under stirring for 3 hours at room temperature and concentrated in vacuo to syrupy 3-deoxy-D-arabino-hexono-1,4-lactone. The product was pure by TLC, GLC and paper chromatography; $[\alpha]_D^{25}$ + 7.5° (c 2.20, H_2O). Lit. (54): $[\alpha]_D$ + 8° (H_2O). It gave a positive response to the ferric hydroxamate test for lactones (61).

According to the literature (54), the calcium ribo-hexonate can be isolated by fractional precipitation using progressively greater concentrations

of ethanol in aqueous solution. This approach resulted in little success, and attempts to crystallize the 1,4-lactone from ethyl acetate (54) were also fruitless.

D-GLUCOISOSACCHARINO-1,4-LACTONE (XLIV) (79)
[3-DEOXY-2-C-(HYDROXYMETHYL)-D-PENTONO-1,4-LACTONE]

Saturated oxygen-free calcium hydroxide solution (0.04N, 1 liter) and maltose (Pfanstiehl Laboratories Inc., Waukegan, IL, 5.0 g) were allowed to react under a nitrogen atmosphere for 14 days at room temperature. The solution was neutralized with solid carbon dioxide, heated to boiling, and filtered to remove precipitated calcium carbonate. The filtrate was concentrated to 50 ml. After standing for 4 days at 5°C, calcium 3-deoxy-2-C-(hydroxymethyl)-D-erythro-pentonate (α-salt) crystallized from solution. The salt was isolated by filtration, dissolved in hot water and lactonized by treatment with excess IR-120 (H^+) resin under stirring for 3 hours. The resin was removed by filtration and the filtrate was concentrated in vacuo to a dry syrup (650 mg). The product was pure by TLC, GLC and paper chromatography, and it responded positively to the ferric hydroxamate test for lactones.

D-GLUCOSACCHARINO-1,4-LACTONE (XLV)
(2-C-METHYL-D-RIBO-PENTONIC ACID)

This material was kindly donated by Dr. J. W. Green. It was pure by TLC, GLC and paper chromatography; m.p. 159°C. Lit. (80): m.p. 160-151°C. The material gave a positive response to the ferric hydroxamate test for lactones.

D-GLUCOSE

Dextrose (Mallinckrodt Chemical Works, St. Louis, MO) was recrystallized twice from 85% aqueous ethanol. Melting point 145-146°C; $[\alpha]_D^{25}$ + 52.0°

(c 1.07, H_2O, equil.). Lit. (81): m.p. 146°C; $[\alpha]_D^{20}$ + 52.5° (H_2O). No impurities were detected by paper chromatography or GLC.

CELLOBIOSE

Twice-recrystallized cellobiose was obtained from Dennis J. Czappa (82). Melting point 238°C (decomp.); $[\alpha]_D^{25}$ + 34.7° (c 1.41, H_2O, equil.). Lit. (81): m.p. 239°C (decomp.); $[\alpha]_D^{20}$ + 34° (c 1.0, H_2O). Analysis by paper chromatography and GLC showed no impurities.

3-O-METHYL-D-GLUCOSE

3-O-Methyl-D-glucose was kindly donated by Dr. L. R. Schroeder; m.p. 165-166°C. Lit. (78): m.p. 166-168°C. The material was pure by TLC, GLC and paper chromatography.

PROCEDURE FOR CONDUCTING ALKALINE DEGRADATION REACTIONS

REACTIONS IN OXYGEN-FREE SODIUM HYDROXIDE SOLUTION

A standard technique was employed for the preparation, initiation and monitoring of oxygen-free sodium hydroxide reactions as follows.

Preparation of Oxygen-Free NaOH Solution

Deionized water was boiled for 30 minutes and then allowed to cool to room temperature in a sealed container. Sodium hydroxide (0.1N) was prepared from a commercial standardized solution and deoxygenated water. The reagent solution was transferred to a sealable bottle fitted with an inlet-outlet tube and a gas dispersion tube. Prepurified nitrogen was bubbled through the NaOH solution for 60 minutes to remove any remaining oxygen, and the solution was stored under nitrogen. Sodium hydroxide solution was removed

by pressurizing the storage bottle with N_2 and dispensing the solution into N_2-purged containers. The normality was established by titration with 0.01N H_2SO_4 to the phenolphthalein end point.

Initiation of Reactions

All reactions had an initial ratio of 4.95 equivalents sodium hydroxide per mole of starting material. The sugar substrate was dried to constant weight in vacuo. All containers, substrates and the reagent solution were placed inside a polyethylene glove bag. The bag was evacuated and then filled with prepurified nitrogen. The evacuation-filling cycle was repeated three times. The substrate was dissolved in NaOH solution and the reaction solution was transferred to a 125-ml glass bottle which was sealed with an air-tight rubber septum and metal retaining ring. When all manipulations in the N_2 atmosphere were completed, the sealed reaction solutions were transferred from the glove bag to a water bath maintained at 25°C. At all times, positive nitrogen pressure was maintained in each reaction bottle as indicated by an upward bulge at the top of the septum.

The reaction solutions, initially colorless, eventually became colored, usually yellow but tending toward brown in the case of 3,6-anhydro-4-O-methyl-D-glucose. Intensity of color increased fastest in reactions of the 3,6-anhydro sugars.

Sampling

Samples were withdrawn periodically from the reaction solutions, using a 5-ml glass syringe thoroughly purged and then filled with prepurified nitrogen. The samples were titrated with a standard solution of 0.05N sulfuric acid to the phenolphthalein end point (pH 8). Samples to be used for product analysis were concentrated in vacuo to dryness, sealed and stored.

For paper chromatography, samples were "puddled" with water, applied to Whatman No. 1 paper, chromatographed by the descending solvent technique with ethyl acetate:acetic acid:water (3:1:1, vol.) and developed using alkaline silver nitrate reagents (62).

For GLC analysis, previously dried samples were dissolved in dimethylsulfoxide (0.3-0.4 ml, silylation grade) and reacted with Tri-Sil Concentrate (0.1 ml) (both reagents from Pierce Chemical Co., Rockford, IL). The mixture was shaken mechanically for 10-16 hours and allowed to settle. A two-layer (liquid-liquid) mixture was obtained. A sample for GLC analysis was drawn from the upper layer and injected directly into the chromatograph.

Lactonization of product mixtures was accomplished by either of two methods: A — the dried sample was redissolved in a minimum of distilled water; one drop of concentrated hydrochloric acid was added, and the sample was reconcentrated to dryness; and B — the dried sample was redissolved in water, stirred with an excess of IR-120 (H^+) resin for 30 minutes and reconcentrated to dryness. Both procedures gave satisfactory results upon derivatization and GLC analysis, including trials with reference saccharinic acids in their Na- and Ca-salt forms. Procedure A was preferred for its simplicity and speed.

Termination of Reactions

Reactions were terminated by titrating the remaining reaction solution with 0.05N H_2SO_4 to the phenolphthalein end point. Titrated solutions were concentrated in vacuo to dryness and stored in sealed flasks.

REACTIONS IN OXYGEN-FREE CALCIUM HYDROXIDE SOLUTION

The standard procedure was also employed for all calcium hydroxide reactions, with slight variations in quantities of materials.

Oxygen-free calcium hydroxide solution was prepared by dissolving excess solid $Ca(OH)_2$ (AR) in previously-boiled deionized water at 5°C; the solution was purged with prepurified nitrogen for 60 minutes, then stored in a sealed bottle at 25°C. The normality of the solution was established by titration with 0.05N H_2SO_4 to the phenolphthalein end point.

The starting material was weighed into a 125-ml glass bottle. In a nitrogen-filled glove bag, oxygen-free calcium hydroxide solution was added to the bottle, which was then sealed with an air-tight rubber septum and metal retaining ring. Reactions were conducted at constant temperature (25 ± 1°C).

Unless otherwise noted in Results and Discussion, two equivalents of saturated $Ca(OH)_2$ (approximately 0.04N, determined exactly at the start of each reaction) were used per mole of starting material. Samples were withdrawn, titrated, concentrated and stored as previously described for NaOH reactions.

As a check on reaction procedures, calcium salts of D-glucometasaccharinic, D-glucoisosaccharinic and cellobionic acids were converted by ion exchange (IR-120, Na^+) to their sodium salts and then subjected to the same conditions as described for oxygen-free NaOH reactions of the reducing sugars. After 7 weeks no reaction was observed in any of the three cases, confirming that these compounds were stable at the given conditions. Johansson and Samuelson (83) reported that D-glucometasaccharinic and D-glucoisosaccharinic acids were stable when subjected to 5% NaOH at 170°C for 7 hours.

Blanks (no reducing sugar added) were run parallel to most of the degradation reactions. In no case was any change in alkali strength observed. Great care was taken to assure no oxygen contamination of all reactions. Even minute amounts of oxygen can affect the outcome of alkaline degradations (23).

NOMENCLATURE

$[\alpha]_D$ = specific optical rotation at 589 nm

τ = chemical shift (ppm) relative to internal standard (τ 10.00)

GLC = gas-liquid chromatography

GC-MS = gas chromatography-mass spectrometry (combined)

m/e = mass-to-charge ratio (mass spectrometry)

m.p. = melting point

M = molarity (moles per liter)

MS = mass spectrometry

N = normality (equivalents per liter)

R_G = mobility relative to D-glucose (paper chromatography)

PMR = proton magnetic resonance

TLC = thin-layer chromatography

TMS = trimethylsilyl

TMSiOH = trimethylsilanol

T_r = retention time (gas-liquid chromatography)

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APPENDIX I

ANALYSIS OF REACTION PRODUCT MIXTURES BY GAS-LIQUID CHROMATOGRAPHY

This appendix contains GLC data for reference compounds and product mixtures from degradation reactions in NaOH and $Ca(OH)_2$. All compounds were analyzed as their trimethylsilyl (TMS) ethers. All data are given for GLC Conditions A (see Experimental), except for products from the NaOH degradation of 3,6-anhydro-cellobiose, where Conditions B were used. Reference compounds had identical retention times under Conditions A and B. (See Tables VI through XIII.)

TABLE VI

GAS CHROMATOGRAPHIC ANALYSIS OF REFERENCE COMPOUNDS

	T_r, min
Lactic acid	3.4
Glycolic acid	4.0
Glyceric acid	10.2
D-Glucosaccharino-1,4-lactone	19.0
D-Glucoisosaccharino-1,4-lactone	20.0
D-Glucometasaccharino-1,4-lactone	22.0
D-Glucoisosaccharinic acid	25.0
D-Glucometasaccharinic acid	25.3

TABLE VII

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF 3,6-ANHYDRO-D-GLUCOSE WITH OXYGEN-FREE 0.099N SODIUM HYDROXIDE AT 25°C

Product	T_r, min	Product Ratio,[a] %
D,L-Lactic acid	3.4	2
D,L-Glyceric acid	10.2	1
3-Deoxy-D-pentonic acid	19.7	5
D-Glucometasaccharinic acid	25.1[b]	87
Unknowns		5

[a]Product ratio given as percentage of total products observed, calculated on peak areas.

[b]Upon lactonization, the major peak shifted to T_r 22.1 minutes.

TABLE VIII

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF 3-O-METHYL-D-GLUCOSE WITH OXYGEN-FREE 0.099N SODIUM HYDROXIDE AT 25°C

Product	T_r, min	Product Ratio,[a] %
D,L-Lactic acid	3.3	1
3-Deoxy-D-pentonic acid	19.7	4
D-Glucometasaccharinic acid	25.1[b]	89
Unknowns		6

[a]Product ratio given as percentage of total products observed, calculated on peak areas.

[b]Upon lactonization, the major peak shifted to T_r 22.0 minutes.

TABLE IX

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF D-GLUCOSE WITH OXYGEN-FREE 0.099N SODIUM HYDROXIDE AT 25°C

Product	T_r, min	Product Ratio,[a] %
D,L-Lactic acid (1)	3.4	56
D,L-Glyceric acid (2)	10.2	5
D,L-2,4-Dihydroxybutyric acid (3)	12.6	14
3-Deoxy-D-pentonic acid (4)	19.6[b]	3
D-Glucometasaccharinic acid (5)	25.1[b]	22

[a] Product ratio given as percentage of total products observed, calculated on peak areas.

[b] Upon lactonization, this peak shifted to T_r 22.0 minutes.

TABLE X

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF CELLOBIOSE WITH OXYGEN-FREE 0.099N SODIUM HYDROXIDE AT 25°C

Product	T_r, min	Product Ratio,[a] %
D,L-Lactic acid (1)	3.4	33
D,L-Glyceric acid (2)	10.1	3
D,L-2,4-Dihydroxybutyric acid (3)	12.5	9
3,4-Dideoxy-pentonic acid (4)	15.1	5
3,4-Dideoxyhex-5-ulosonic acid (5)	18.2	5
3-Deoxy-D-pentonic acid (6)	19.5	4
D-Glucoisosaccharinic acid → (7)	25.0[b]	41 < 30
D-Glucometasaccharinic acid → (7)		41 < 11

[a] Product ratio given as percentage of total products observed, calculated on peak areas.

[b] Upon lactonization, this peak was converted to two peaks at T_r 20.0 and 22.0 minutes, in a ratio of 2.6:1.

TABLE XI

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF 3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE WITH OXYGEN-FREE 0.099N SODIUM HYDROXIDE AT 25°C

Product	T_r, min	Product Ratio,[a] %
D,L-Lactic acid (1)	3.5	17
Unknown acid (2)	4.9	
D,L-Glyceric acid (3)	10.1	
Unknowns – (4)	14.0	[b]
Unknowns – (5)	15.4	
Unknowns – (6)	16.5	
Unknown acid (7)	17.2	14
3-Deoxy-D-pentonic acid (8)	19.7	9
Unknown acid (9)	22.3	6
4-O-Methyl-D-glucometasaccharinic acid (10)	23.3	13
3-Deoxy-D-hexonic acid (11)	25.0[c]	41

[a] Product ratio given as percentage of total products observed, calculated on peak areas.

[b] Trace products, peak areas not measured.

[c] Upon lactonization, this peak shifted to T_r 22.1 minutes.

TABLE XII

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF 3,6-ANHYDRO-CELLOBIOSE WITH OXYGEN-FREE 0.099N SODIUM HYDROXIDE AT 25°C[a]

Product	T_r, min	Product Ratio,[b] %
D,L-Lactic acid (1)	3.5	28
D,L-Glyceric acid (2)	10.0	3
D,L-2,4-Dihydroxybutyric acid (3)	12.4	6
3,4-Dideoxy-pentonic acid (4)	15.0	1
3-Deoxy-D-pentonic acid (5)	19.5	2
3,4-Dideoxy-hexonic acid (6)	21.9	3
D-Glucoisosaccharinic acid → (7)	24.9[c]	50 (39)
3-Deoxy-D-hexonic acid → (7)		50 (11)
Unknown disaccharide acid (8)	42.8	7

[a] GLC Conditions B were used for this analysis only.

[b] Product ratio given as percentage of total products observed, calculated on peak areas.

[c] Upon lactonization, this peak was converted to two peaks at T_r 19.9 and 22.0 minutes, in a ratio of 3.5:1.

TABLE XIII

GAS CHROMATOGRAPHIC ANALYSIS OF PRODUCTS FROM REACTION OF D-GLUCOSE WITH OXYGEN-FREE 0.041*N* CALCIUM HYDROXIDE AT 25°C

Product	T_r, min	Product Ratio,[a] %
D,L-Lactic acid (1)	3.5	29
D,L-2-*C*-Methyl-glyceric acid (2)	10.0	8
D,L-2,4-Dihydroxybutyric acid (3)	12.5	13
3-Deoxy-D-pentonic acid ⟶ (4) 2-*C*-Methyl-D-tetronic acid ⟶ (4)	19.5	10
D-Glucosaccharinic acid (5)	23.7[b]	11
D-Glucometasaccharinic acid (6)	24.9[c]	29

[a] Product ratio given as percentage of total products observed, calculated on peak areas.

[b] Upon lactonization, this peak shifted to T_r 19.0 minutes.

[c] Upon lactonization, this peak shifted to T_r 22.0 minutes.

APPENDIX II

ANALYSIS OF PRODUCTS FROM NaOH REACTIONS BY MASS SPECTROMETRY

This appendix contains the mass spectral data for identification of final products from oxygen-free NaOH degradation reactions. A constant multiplier of 16 was used to convert peak height values from the first to the second scale of the MS chart.

Product designations in parentheses refer to GLC results as follows: D-glucose — Fig. 8, p. 25; cellobiose — Fig. 15, p. 42; 3,6-anhydro-4-O-methyl-D-glucose — Fig. 26, p. 64; 3,6-anhydro-cellobiose — Fig. 30, p. 73. (See Tables XIV through XXX.)

TABLE XIV

MASS SPECTRUM OF D,L-2,4-DIHYDROXYBUTYRIC ACID TMS DERIVATIVE[a] (GLUCOSE PRODUCT 3)

m/e	Peak Height, mm	Relative Abundance, %	m/e	Peak Height, mm	Relative Abundance, %
321 (M-15)	1	1	130	2	2
221	4	4	129	6.5	6
220	7.5	7	105	4	4
219	16	15	104	15	14
190	1	1	103	108	100
189	2	2	75	20.5	19
149	1	1	74	8	7
148	3	3	73	93	86
147	15	14	59	7.5	7
133	4	4	55	22	20
131	4	4	45	17	16

[a] Data represent the average of two spectra.

TABLE XV

MASS SPECTRUM OF D-GLUCOMETASACCHARINIC ACID TMS DERIVATIVE
(GLUCOSE PRODUCT 5)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
527	3.5	0.6	218	18	3
526	5	1	217	56	10
525(M-15)	11	2	205	33	6
437	4	1	204	23	4
436	4	1	203	21	4
435	4	1	191	17	3
347	22	4	163	21	4
345	13	2	149	25	4
337	13	2	148	37	7
336	24	2	147	216	39
335	109	19.5	133	22	4
333	33	6	131	22	4
319	18	3	130	25	4
317	12	2	129	134	24
292	21	4	105	6	1
271	31	5.5	104	8	1.5
247	89	16	103	89	16
246	180	32	75	65	12
245	558	100	74	65	12
243	50	9	73	468	84
221	19	3	59	22	4
220	15	3	55	20	4
219	30	5	45	32	6

TABLE XVI

MASS SPECTRUM OF D,L-LACTIC ACID TMS DERIVATIVE
(CELLOBIOSE PRODUCT 1)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
221	2	5	103	2	5
220	1	2.5	102	3	7.5
219 (M-15)	5	12.5	89	1	2.5
192	4	10	88	2	5
191	14	35	87	1	2.5
190	14	35	75	9	22.5
149	4	10	74	4	10
148	10	25	73	40	100
147	31	77.5	72	4	10
133	2	5	66	4	10
131	2	5	59	3	7.5
129	1	2.5	45	6	15
119	5	12.5	44	3	7.5
118	17	42.5	43	6	15
117	34	85			

TABLE XVII

MASS SPECTRUM OF D,L-GLYCERIC ACID TMS DERIVATIVE (CELLOBIOSE PRODUCT 2)

m/e		Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
324		1	0.5	148	23	10
323		1	0.5	147	128	57
322	(M)	2	1	133	27	12
309		1	0.5	131	6	3
308		3	1.5	130	19	8
307	(M-15)	17	8	117	28	13
294		3	1.5	116	9	4
293		12	5	105	4	2
292		61	27	104	9	4
219		4	2	103	53	24
217		7	3	102	49	22
207		2	1	101	4	2
206		3	1.5	75	64	29
205		41	18	74	14	6
204		4	2	73	224	100
191		3	1.5	59	12	5
190		15	7	55	4	2
189		88	36	45	23	10
149		9	4	43	16	7

TABLE XVIII

MASS SPECTRUM OF D,L-2,4-DIHYDROXYBUTYRIC ACID TMS DERIVATIVE (CELLOBIOSE PRODUCT 3)

m/e		Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
336	(M)	< 1	1	131	1.5	7
321	(M-15)	< 1	2	129	1.5	7
245		< 1	2	117	1	5
220		2	10	115	1	5
219		7	33	105	1.5	7
177		1	5	104	1	5
165		2	10	103	21	100
161		1	5	88	1	5
149		1	5	75	1	5
148		1	5	74	1.5	7
147		6	29	73	18	86
				55	3	14

TABLE XIX

MASS SPECTRUM OF 3,4-DIDEOXY-PENTONIC ACID TMS DERIVATIVE (CELLOBIOSE PRODUCT 4)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
335 (M-15)	8	3	133	15	6
259	3	1	131	6	3
245	2	1	129	12	5
233	17	7	119	4	2
221	6	3	117	6	3
217	8	3	104	5	2
202	3	1	103	9	4
191	3	1	102	3	1
149	6	3	75	35	15
148	11	5	74	12	5
147	73	32	73	198	86
145	21	9	71	52	23
144	22	10	59	9	4
143	231	100	45	18	8

TABLE XX

MASS SPECTRUM OF 3,4-DIDEOXYHEX-5-ULOSONIC ACID TMS DERIVATIVE (CELLOBIOSE PRODUCT 5)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
378(M^+)	1	<1	169	5	2
365	1	<1	157	8	3
364	8	3	155	33	14
363(M-15)	13	6	149	13	6
275	6	3	148	5	2
271	6	3	147	50	22
263	3	1	143	7	3
262	9	4	133	10	4
261	49	21	131	4	2
246	6	3	130	7	3
245	9	4	129	40	17
243	10	4	117	12	5
217	3	1	116	10	4
200	5	2	115	7	3
199	8	3	108	16	7
191	7	3	103	24	10
189	7	3	101	12	5
187	5	2	91	20	9
186	12	5	85	4	2
185	33	14	81	28	12
183	6	3	75	15	7
173	4	2	74	21	9
172	10	4	73	230	100
171	59	26	59	10	4
			46	6	3

TABLE XXI

MASS SPECTRUM OF 3-DEOXY-D-PENTONIC ACID TMS DERIVATIVE
(CELLOBIOSE PRODUCT 6)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
424	1.5	1.5	205	28	29
423(M-15)	2	2	204	4	4
335	6	6	203	2	2
333	12	12	189	6	6
305	8	8	171	4	4
292	3	3	149	2	2
271	2	2	148	3	3
259	4	4	147	46	47
247	3	3	133	11	11
246	14	14	131	2	2
245	76	78	129	10	10
244	4	4	119	4	4
233	3	3	117	3	3
232	10	10	101	5	5
231	52	54	83	6	6
221	3	3	75	10	10
219	6	6	74	8	8
217	7	7	73	97	100
207	4	4	69	13	13
206	5	5	45	5	5

TABLE XXII

MASS SPECTRUM OF A MIXTURE OF D-GLUCOISOSACCHARINIC AND D-GLUCOMETASACCHARINIC ACID TMS DERIVATIVES[a]
(CELLOBIOSE PRODUCTS 7)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
526	5	1	245	241	47
525(M-15)[a]	11	2	244	32	6
439	10	2	243	189	37
438	13	3	231	12	2
437	17	3	221	11	2
436	10	2	220	8	2
435	11	2	219	33	6
349	16	3	218	26	5
348	41	8	217	37	7
347	102	20	207	11	2
345	24	5	206	27	5
337	6	1	205	137	27
336	10	2	204	21	4
335	34	7	203	26	5
334	15	3	191	29	6
333	46	9	189	30	6
319	16	3	149	43	8
318	16	3	148	53	10
317	25	5	147	275	53
307	10	2	133	30	6
306	21	4	131	24	5
305	38	7	129	126	24
304	7	1	117	52	10
303	17	3	103	76	15
277	15	3	101	11	2
271	22	4	75	68	13
257	39	6	74	105	20
247	31	6	73	515	100
246	86	17	45	56	11

[a] Both TMS derivatives have the same molecular weight.

TABLE XXIII

MASS SPECTRUM OF UNKNOWN ACID TMS DERIVATIVE[a]
(3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE PRODUCT 7)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
351	3	1	149	31	10
350	12	4	148	25	8
349(M-15)	19	6	147	145	47
321	9	3	143	12	4
277	15	5	133	34	11
249	13	4	131	22	7
248	34	11	130	31	10
247	92	30	129	213	69
233	9	3	117	19	6
231	9	3	116	25	8
221	9	3	115	6	2
219	6	2	103	13	4
217	15	5	101	12	4
205	22	7	85	55	18
204	25	8	75	126	41
203	43	14	74	40	13
191	12	4	73	308	100
185	8	3	59	19	6
159	9	3	55	18	6
158	6	2	45	19	6
157	59	19	43	46	15

[a]Data represent the average of two spectra.

TABLE XXIV

MASS SPECTRUM OF 3-DEOXY-D-PENTONIC ACID TMS DERIVATIVE[a]
(3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE PRODUCT 8)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
423 (M-15)	2	2	147	33	34
335	13.5	14	133	6	6
333	5	5	131	3	3
305	6	6	129	9	9
245	41.5	43	117	7	7
231	22	23	101	5	5
219	2	2	88	7	7
217	9	9	83	4	4
205	19	20	73	97	100
191	7	7	69	4	4
189	12	12	55	8	8
163	4	4	45	6	6

[a]Data represent the average of two spectra.

TABLE XXV

MASS SPECTRUM OF UNKNOWN ACID TMS DERIVATIVE
(3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE PRODUCT 9)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
363	3	6	201	3	6
349	2	4	155	5	10
348	1.5	3	149	3	6
347	1	2	148	2	4
293	1.5	3	147	7	15
292	3	6	133	5	10
259	5	10	131	6	12
247	3	6	129	23	48
245	4	8	103	2	4
243	1	2	75	4	8
232	1.5	3	74	6	13
231	6	13	73	48	100
204	3	6	69	1	2
203	3	6	55	5	10
202	3	6			

TABLE XXVI

MASS SPECTRUM OF 4-O-METHYL-D-GLUCOMETASACCHARINIC ACID TMS DERIVATIVE[a]
(3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE PRODUCT 10)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
467(M-15)	1	1	189	3	3
351	3	3	188	12	12
350	3	3	187	57.5	57.5
349	4	4	159	3.5	3.5
345	4	4	149	6	6
333	5.5	5.5	148	6	6
289	3.5	3.5	147	35	35
279	2	2	133	4.5	4.5
278	6	6	131	6	6
277	19	19	129	24.5	24.5
260	3	3	117	4	4
259	12.5	12.5	103	11.5	11.5
245	4	4	101	6	6
243	2	2	97	10	10
232	2	2	89	7	7
231	7	7	75	19.5	19.5
221	3	3	74	9	9
219	3.5	3.5	73	101	100
217	5	5	59	5	5
205	7.5	7.5	55	4	4
			45	12.5	12.5

[a] Data represent the average of two spectra.

TABLE XXVII

MASS SPECTRUM OF D-GLUCOMETASACCHARINIC ACID TMS DERIVATIVE
(3,6-ANHYDRO-4-O-METHYL-D-GLUCOSE PRODUCT 11)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
526	4	3	219	13	9
525(M-15)	4	3	218	6	4
437	2	1	217	24	16
436	2	1	207	6	4
435	5	3	206	3	2
347	12	8	205	16	11
345	4	3	204	13	9
337	16	11	203	6	4
336	17	11	163	8	5
335	51	34	149	10	7
333	8	5	148	30	20
319	6	4	147	72	48
317	11	7	133	6	4
292	7	5	131	11	7
271	10	7	129	36	24
257	8	5	103	22	15
247	28	19	75	15	10
246	59	39	74	12	8
245	150	100	73	130	87
243	10	7	59	12	8
221	9	6	55	10	7
220	5	3	45	9	6

TABLE XXVIII

MASS SPECTRUM OF 3,4-DIDEOXY-D-HEXONIC ACID TMS DERIVATIVE
(3,6-ANHYDRO-CELLOBIOSE PRODUCT 6)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
439	5	<1	217	82	13
438	9	1.5	205	31	5
437(M-15)	13	2	204	37	6
349	33	5	203	95	15
348	21	3	191	75	12
347	74	12	189	56	9
334	13	2	185	71	11
333	21	3	155	91	15
307	21	3	149	64	10
306	27	4	148	112	18
305	64	10	147	496	79
292	64	10	133	80	13
275	40	6	131	64	10
259	72	12	130	112	18
257	65	10	129	512	82
245	22	4	117	55	9
243	92	15	116	34	5
233	16	3	103	144	23
232	34	5	97	31	5
231	128	21	89	41	7
230	17	3	81	42	7
229	10	2	75	256	41
221	26	4	74	208	33
220	35	6	73	624	100
219	28	4	69	93	15
218	15	2	59	46	7
			45	144	23

TABLE XXIX

MASS SPECTRUM OF A MIXTURE OF D-GLUCOISOSACCHARINIC AND D-GLUCOMETASACCHARINIC ACID TMS DERIVATIVES (3,6-ANHYDRO-CELLOBIOSE PRODUCTS 7)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
527	5	10	244	27	56
526	7	15	243	39	81
525(M-15)	13	27	231	8	17
439	4	8	221	11	23
438	8	17	219	12	25
437	19	38	217	22	46
436	7	15	207	10	21
435	15	31	206	13	27
349	13	27	205	35	73
348	25	52	204	19	40
347	35	73	203	11	23
345	11	23	191	18	38
337	4	8	189	17	35
336	11	23	149	16	33
335	30	63	148	17	35
319	12	25	147	37	77
317	14	29	133	16	33
305	18	38	131	10	21
303	11	23	129	34	71
293	4	8	117	20	42
292	6	13	103	27	56
277	7	15	83	7	15
271	18	38	75	31	65
257	28	58	74	26	54
247	20	42	73	48	100
246	29	60	59	8	17
245	44	92	55	5	10
			45	19	40

TABLE XXX

MASS SPECTRUM OF AN UNKNOWN DISACCHARIDE ACID TMS DERIVATIVE (3,6-ANHYDRO-CELLOBIOSE PRODUCT 8)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
524[a]	10	1.5	230	33	5
523	15	2	229	17	3
481	5	<1	219	51	8
452	10	2	218	112	18
451(OG^+)	23	4	217	432	68
409	8	1	206	160	25
408	15	2	205	400	63
407	50	8	204	640	100
363	80	13	191	144	23
362	81	13	189	47	7
361	272	43	169	55	9
333	23	4	159	34	5
332	25	4	157	53	8
331	16	3	155	25	4
319	39	6	149	33	5
317	30	5	148	35	5
306	18	3	147	288	45
305	50	8	143	70	11
291	17	3	133	144	23
289	25	4	131	57	9
273	28	4	129	160	25
272	19	3	117	112	18
271	76	12	103	256	40
261	19	3	101	52	8
259	24	4	89	40	6
247	25	4	81	36	6
245	33	5	75	224	35
244	25	4	74	80	13
243	88	14	73	592	93
233	31	5	69	56	9
232	10	2	59	26	4
231	48	8			

[a]Although many peaks were observed above m/e 524, it was impossible to assign reliable mass numbers beyond that point due to scale compression on the mass spectral chart.

APPENDIX III

ANALYSIS OF PRODUCTS FROM A $Ca(OH)_2$ REACTION BY MASS SPECTROMETRY

This appendix contains the mass spectral data for all products from oxygen-free $Ca(OH)_2$ degradation of D-glucose. A constant multiplier of 16 was used to convert peak height values from the first to the second scale of the MS chart.

Product designations in parentheses refer to GLC results shown in Fig. 34, p. 83. (See Tables XXXI through XXXVI.)

TABLE XXXI

MASS SPECTRUM OF D,L-LACTIC ACID TMS DERIVATIVE (GLUCOSE PRODUCT 1)

m/e	Peak Height, mm	Relative Abundance, %	m/e	Peak Height, mm	Relative Abundance, %
221	1	3	117	40	100
220	2	5	103	1.5	4
219 (M-15)	11	28	102	2	5
192	4	11	101	3	8
191	18	45	88	4	10
190	20	50	75	13	33
149	7	18	74	9	23
148	11	28	73	38	95
147	36	90	66	8	20
133	5	13	59	5	13
131	2.5	6	55	3	8
129	1	3	45	17	43
119	4	10	44	3	8
118	14	35	43	5	13

TABLE XXXII

MASS SPECTRUM OF D,L-2-C-METHYL-GLYCERIC ACID TMS DERIVATIVE[a]
(GLUCOSE PRODUCT 2)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
336(M)	2	1	190	11	4
324	2	1	189	34	13
323	5	2	163	21	8
322	8	3	149	26	10
321(M-15)	29	11	148	37	14
308	5	2	147	169	64
307	11	4	133	26	10
306	26	10	131	66	25
294	2	1	129	119	45
293	13	5	119	5	2
234	21	8	118	8	3
233	82	31	117	18	7
231	13	5	116	11	4
221	24	9	115	13	5
220	45	17	103	66	25
219	259	98	75	74	28
207	8	3	74	21	8
206	5	2	73	264	100
205	8	3	59	13	5
204	8	3	45	16	6
203	37	14	43	29	11
191	11	4	42	18	7
			41	34	13

[a]Data represent the average of two spectra.

TABLE XXXIII

MASS SPECTRUM OF D,L-2,4-DIHYDROXYBUTYRIC ACID TMS DERIVATIVE
(GLUCOSE PRODUCT 3)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
323	2	5	133	9	21
322	4	10	131	9	21
321(M-15)	14	33	130	5	12
294	2	5	129	16	38
292	2	5	117	4	10
221	10	24	115	3	7
220	27	64	105	10	24
219	40	95	104	18	43
203	4	10	103	42	100
190	3.5	8	75	19	45
189	3	7	74	12	29
177	7	17	73	39	93
149	6.5	15	59	11	26
148	10	24	55	13	31
147	35	83	45	13	31

TABLE XXXIV

MASS SPECTRUM OF A MIXTURE OF 3-DEOXY-D-PENTONIC ACID AND 2-C-METHYL-D-TETRONIC ACID TMS DERIVATIVES (GLUCOSE PRODUCT 4)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
425	10	2	207	29	6
424	21	4	206	40	8
423(M-15)	39	8	205	160	32
337	24	5	204	40	8
336	34	7	191	38	8
335	112	23	190	18	4
333	43	9	189	73	15
308	24	5	149	42	9
307	85	17	148	39	8
306	84	17	147	288	58
305	42	9	133	42	8
293	4	1	130	19	4
292	18	4	129	47	9
291	13	3	117	58	12
247	64	13	104	14	3
246	112	23	103	160	32
245	400	81	83	32	6
233	55	11	75	53	11
232	56	11	74	48	10
231	216	44	73	496	100
219	56	11	59	9	2
218	42	9	55	19	4
217	176	35	45	64	13

TABLE XXXV

MASS SPECTRUM OF D-GLUCOSACCHARINIC ACID TMS DERIVATIVE (GLUCOSE PRODUCT 5)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
526	8	2	205	187	45
525(M-15)	11	3	204	85	21
439	6	1	191	54	13
438	12	3	190	12	3
437	21	5	189	94	25
435	12	3	175	9	2
393	22	5	157	13	3
347	16	4	155	9	2
335	18	4	149	30	7
333	27	7	148	38	9
319	42	10	147	221	54
308	136	33	133	19	5
307	340	83	131	29	7
306	357	88	129	13	3
291	21	5	117	73	18
278	8	2	105	10	2
277	41	10	104	28	7
235	10	3	103	272	67
234	20	5	75	23	6
233	66	16	74	53	13
231	49	12	73	408	100
219	41	10	59	7	2
218	92	23	55	8	2
217	213	52	45	13	3
206	44	8			

TABLE XXXVI

MASS SPECTRUM OF D-GLUCOMETASACCHARINIC ACID TMS DERIVATIVE (GLUCOSE PRODUCT 6)

m/e	Peak height mm	Relative abundance %	m/e	Peak height mm	Relative abundance %
527	25	4	217	290	41
526	40	6	207	31	5
525(M-15)	51	7	206	49	7
497	11	2	205	161	23
438	27	4	204	112	16
437	46	7	203	80	11
436	32	5	191	81	11
435	50	7	189	34	5
423	161	23	149	80	11
347	175	25	148	112	16
345	79	11	147	495	70
337	79	11	133	96	13
336	111	16	131	56	8
335	449	64	130	35	5
333	97	14	129	320	45
319	64	9	117	128	18
317	61	9	103	240	34
293	44	5	101	25	4
292	67	9	83	48	7
271	145	20	75	161	23
247	190	27	74	192	27
246	466	66	73	704	100
245	672	95	59	25	3
231	50	7	55	42	7
219	77	11	43	80	11
218	81	11			

APPENDIX IV

PROTON MAGNETIC RESONANCE SPECTRA OF INTERMEDIATES IN THE SYNTHESIS OF THE 3,6-ANHYDRO MODEL COMPOUNDS

In Fig. 41-55 may be found the proton magnetic resonance spectra of intermediates in the synthesis of 3,6-anhydro model compounds.

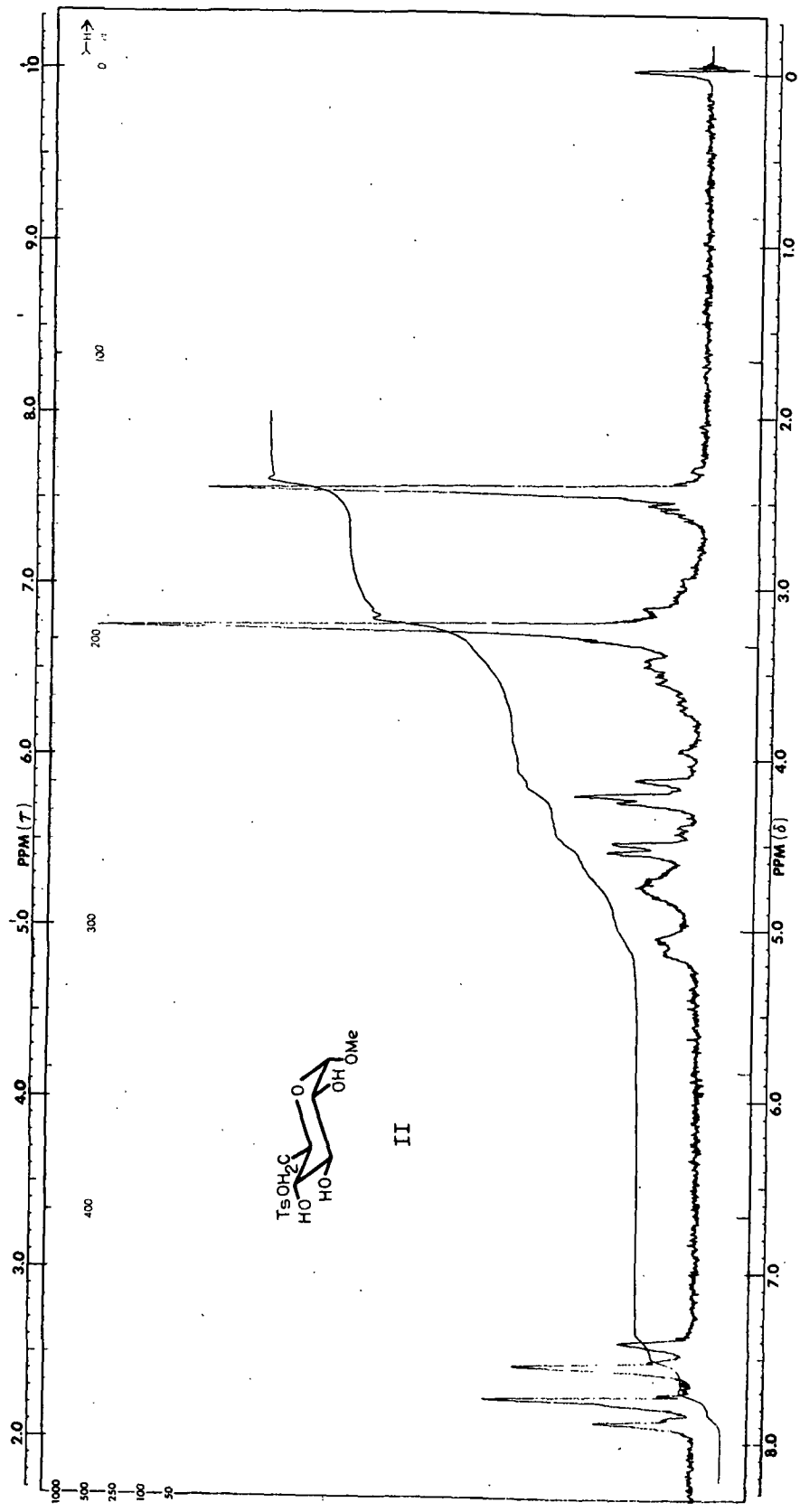

Figure 41. Proton Magnetic Resonance Spectrum (60 MHz) of Methyl 6-O-Toluenesulfonyl-α-D-glucopyranoside in DMSO-d_6

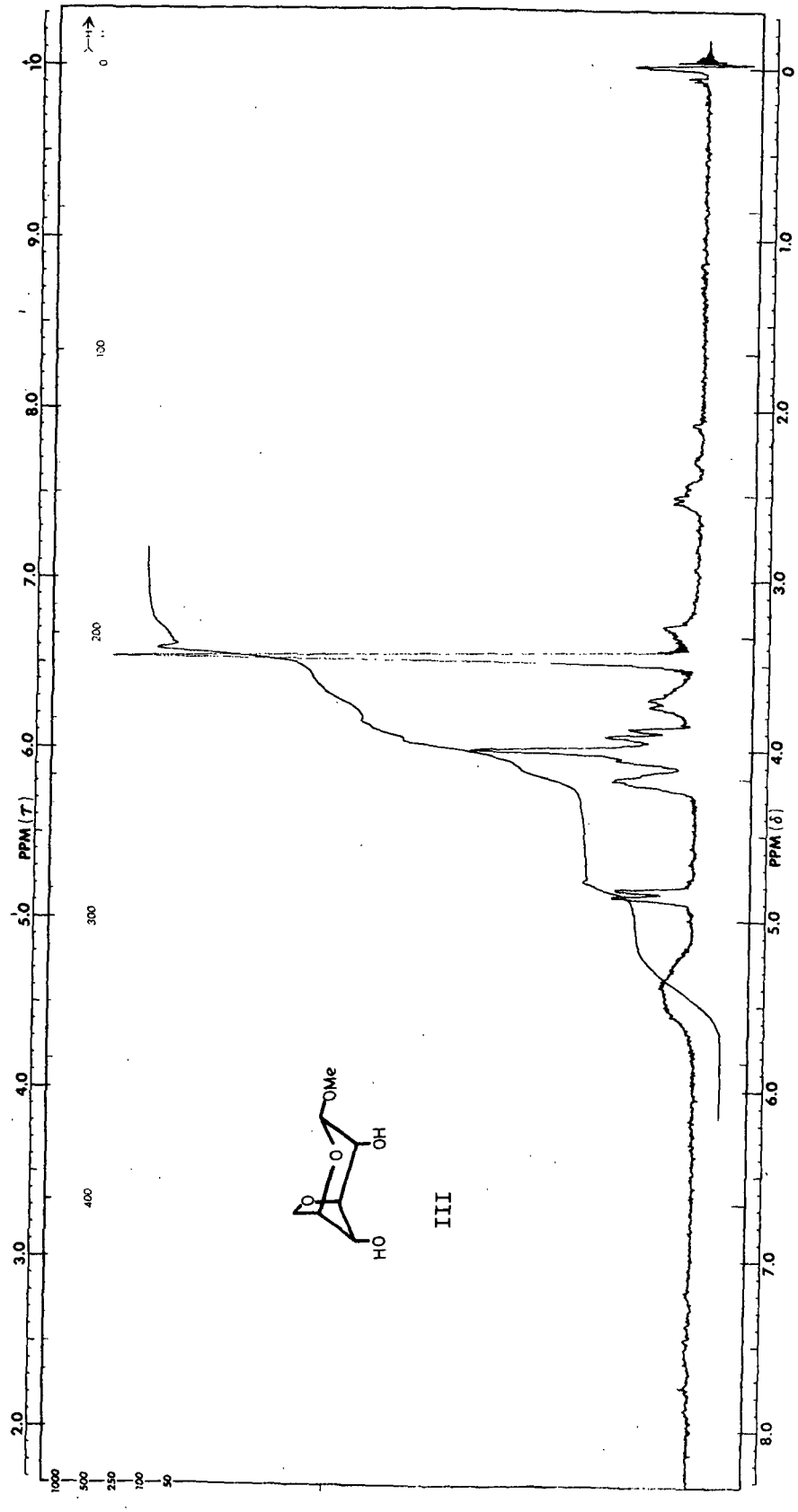

Figure 42. Proton Magnetic Resonance Spectrum (60 MHz) of Methyl 3,6-Anhydro-α-D-glucopyranoside in DMSO-d_6

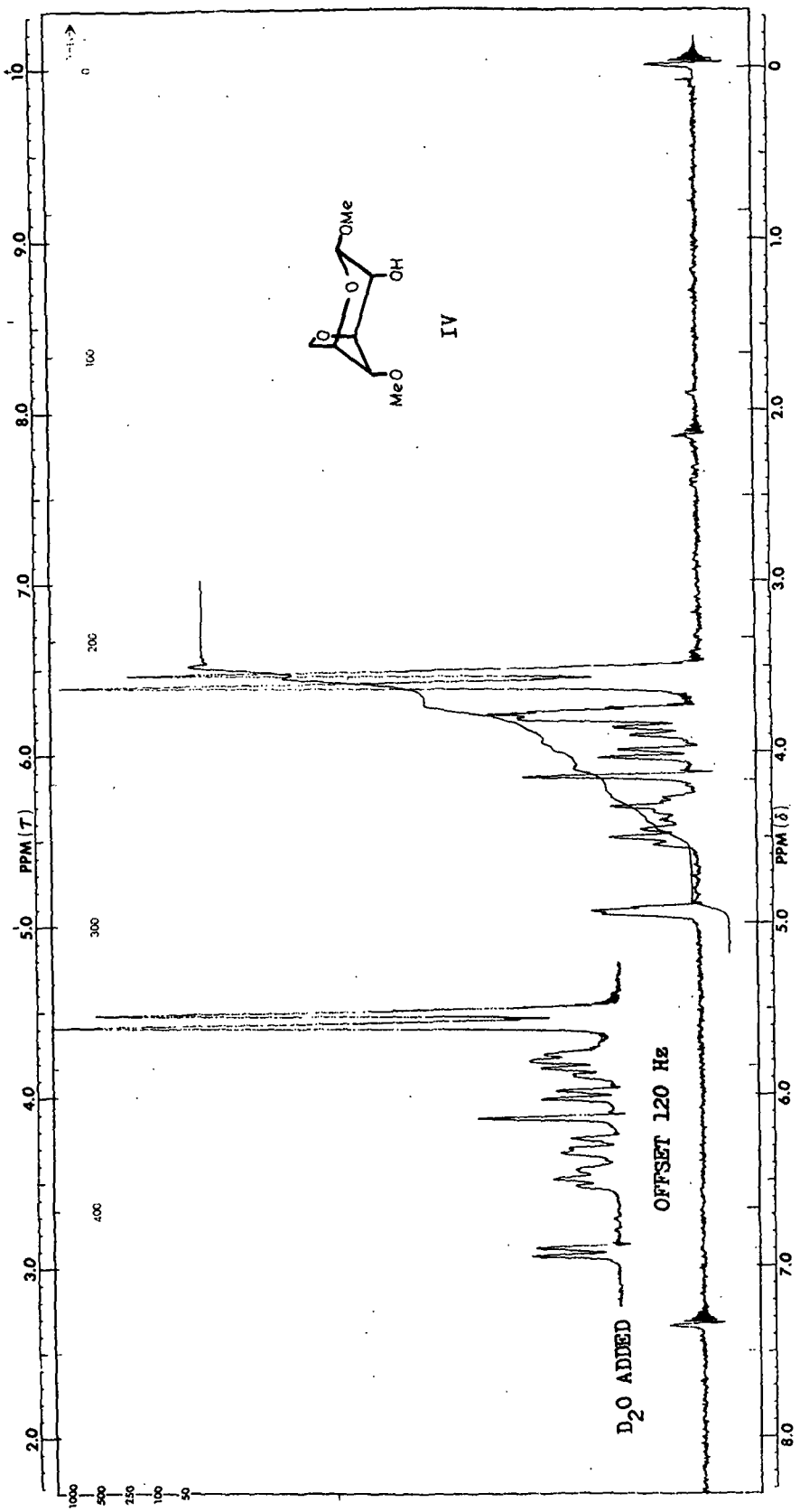

Figure 43. Proton Magnetic Resonance Spectrum (60 MHz) of Methyl 3,6-Anhydro-4-O-methyl-α-D-glucopyranoside in $CDCl_3$

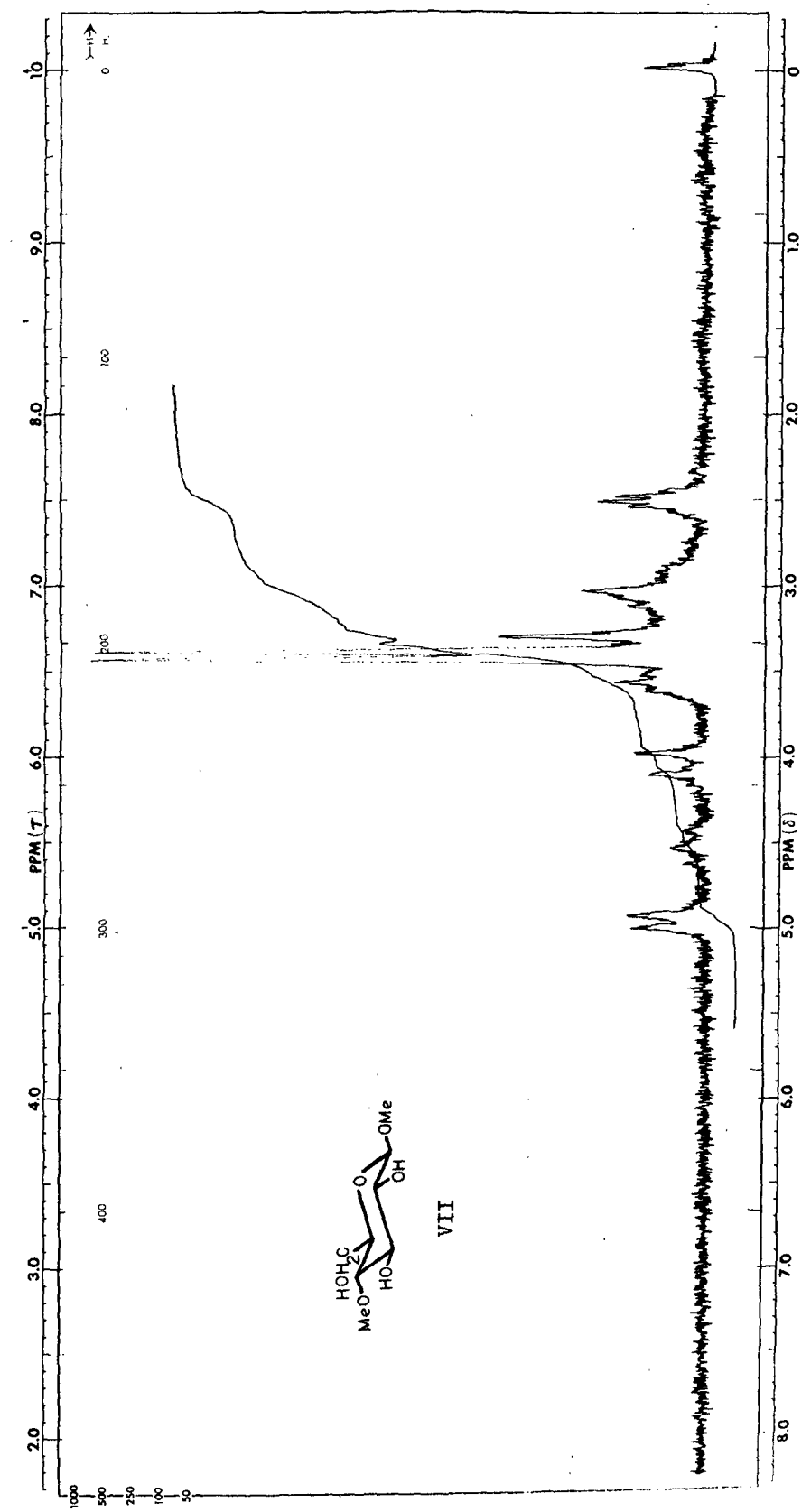

Figure 44. Proton Magnetic Resonance Spectrum (60 MHz) of Methyl 4-O-Methyl-β-D-glucopyranoside in DMSO-d_6

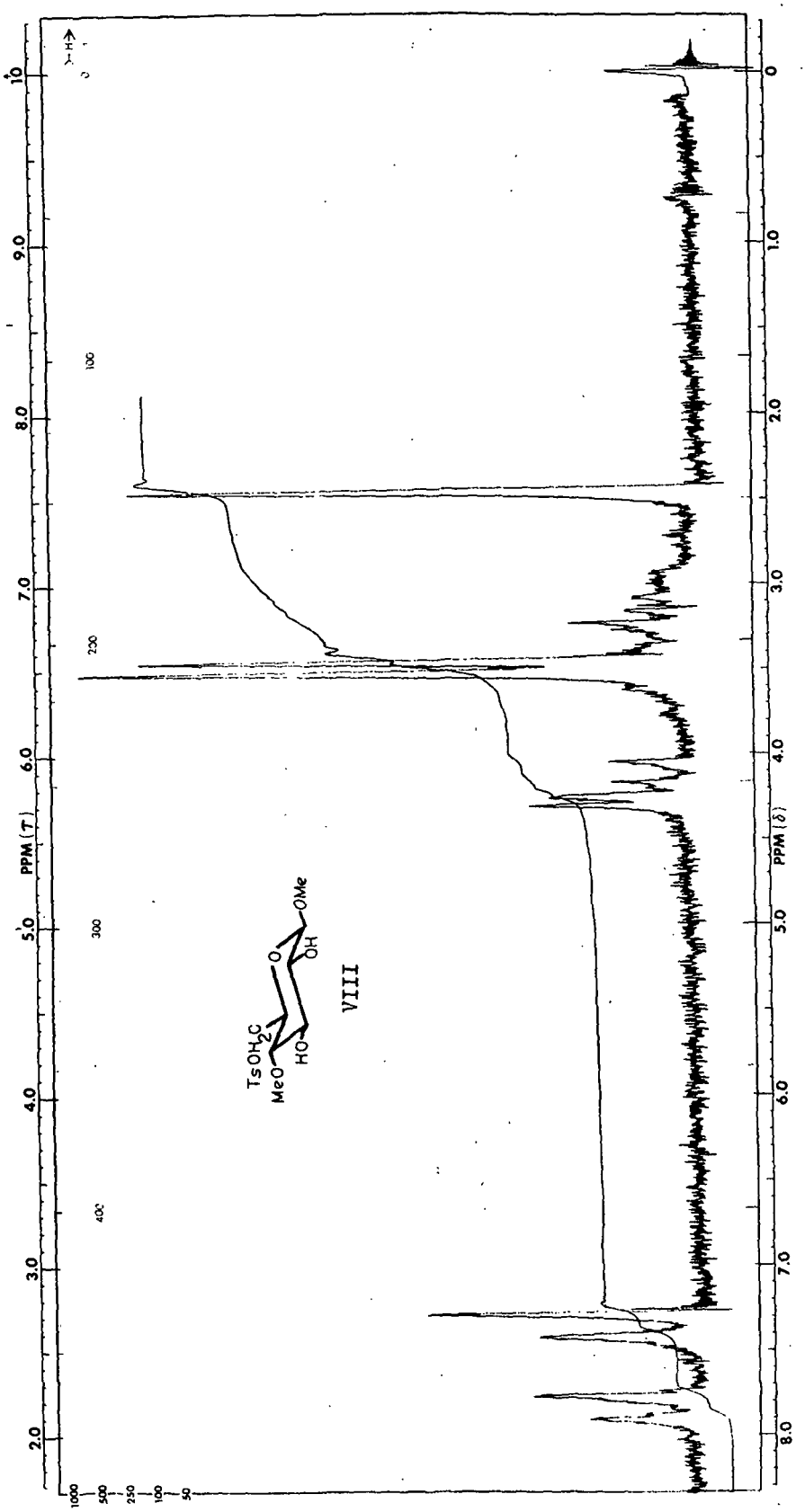

Figure 45. Proton Magnetic Resonance Spectrum (60 MHz) of Methyl 4-O-Methyl-6-O-toluenesulfonyl-β-D-glucopyranoside in $CDCl_3$

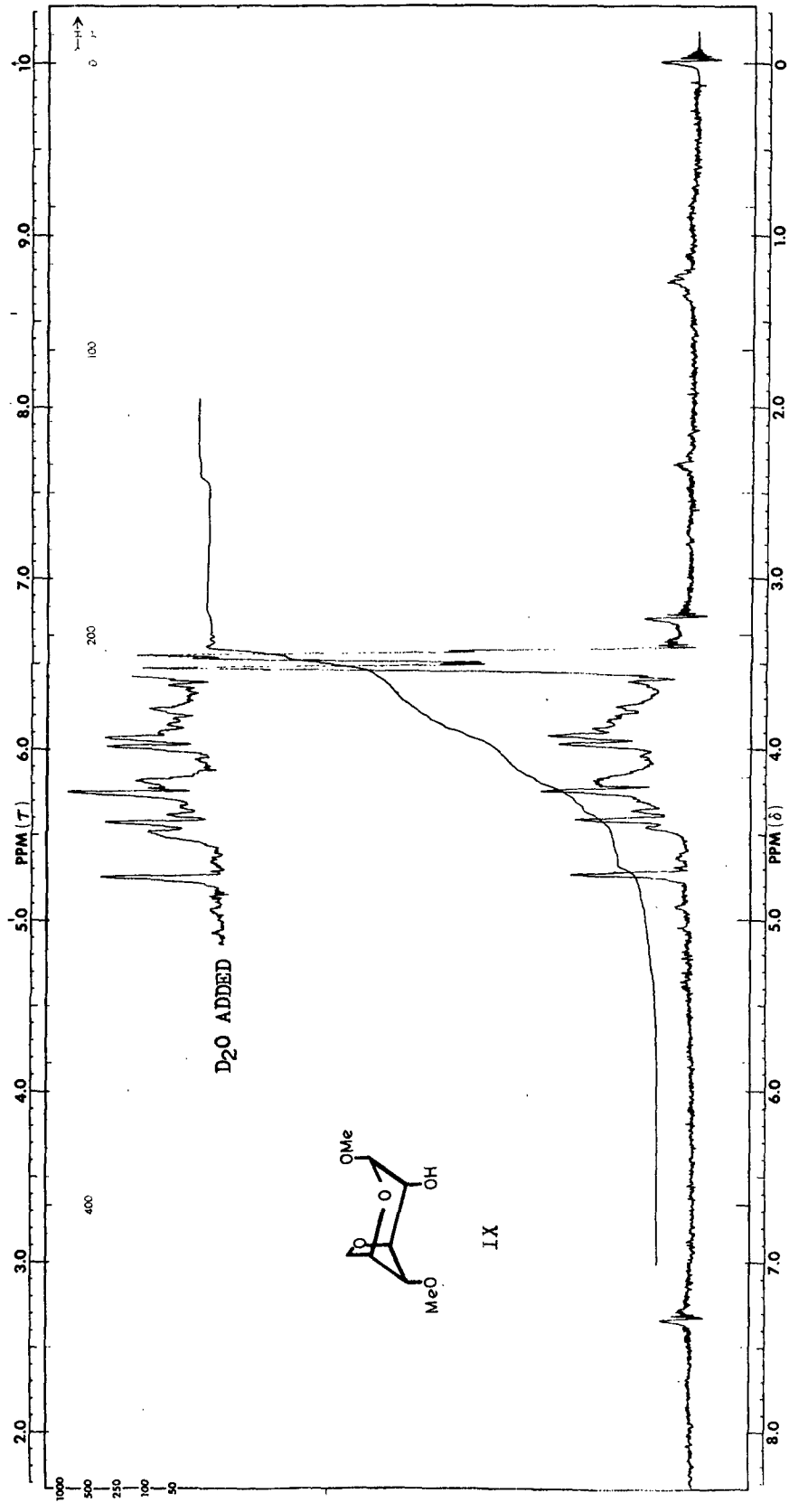

Figure 46. Proton Magnetic Resonance Spectrum (60 MHz) of Methyl 3,6-Anhydro-4-O-methyl-β-D-glucopyranoside in $CDCl_3$

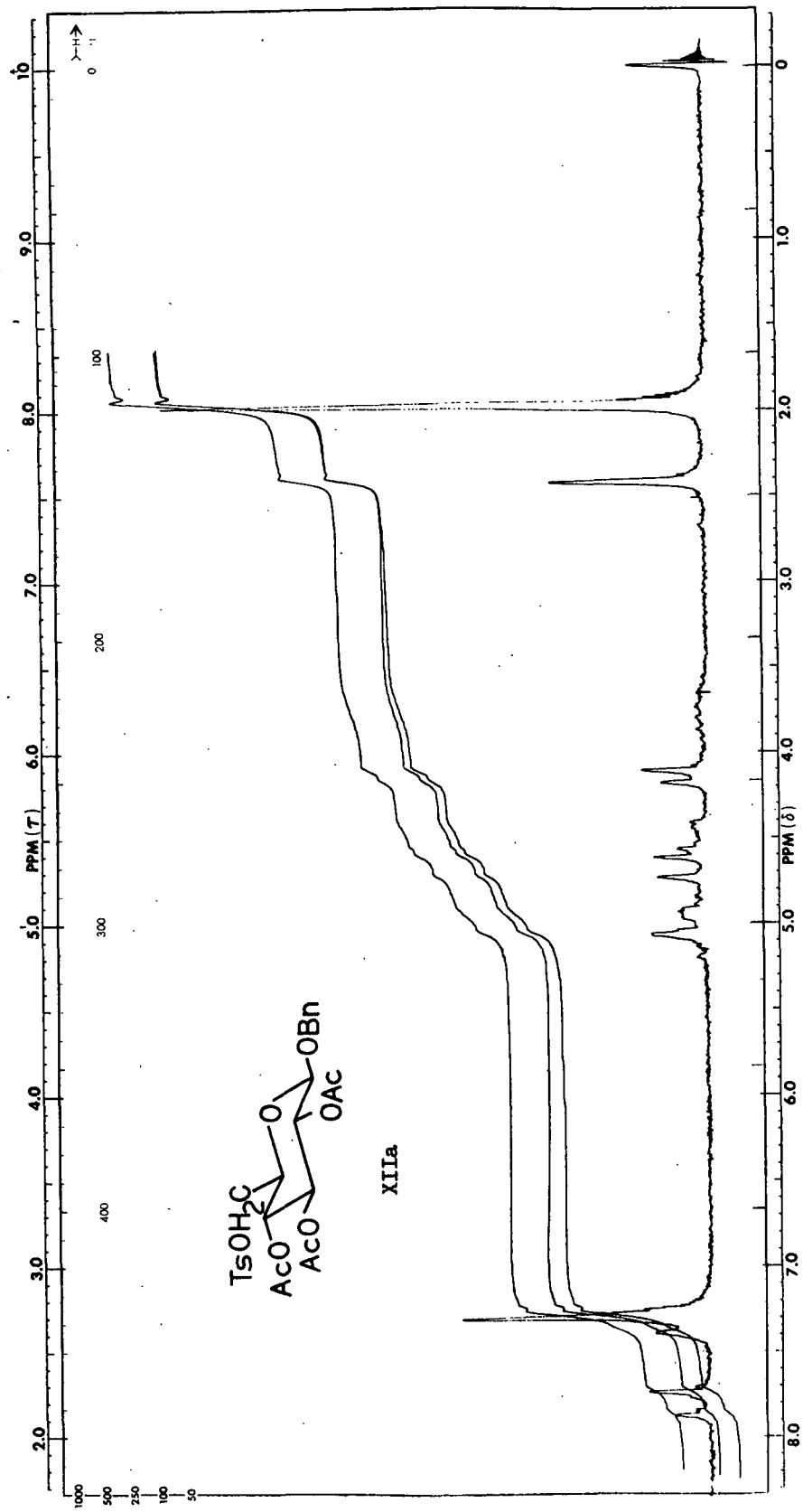

Figure 47. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl 2,3,4-Tri-O-acetyl-6-O-toluenesulfonyl-β-D-glucopyranoside in $CDCl_3$

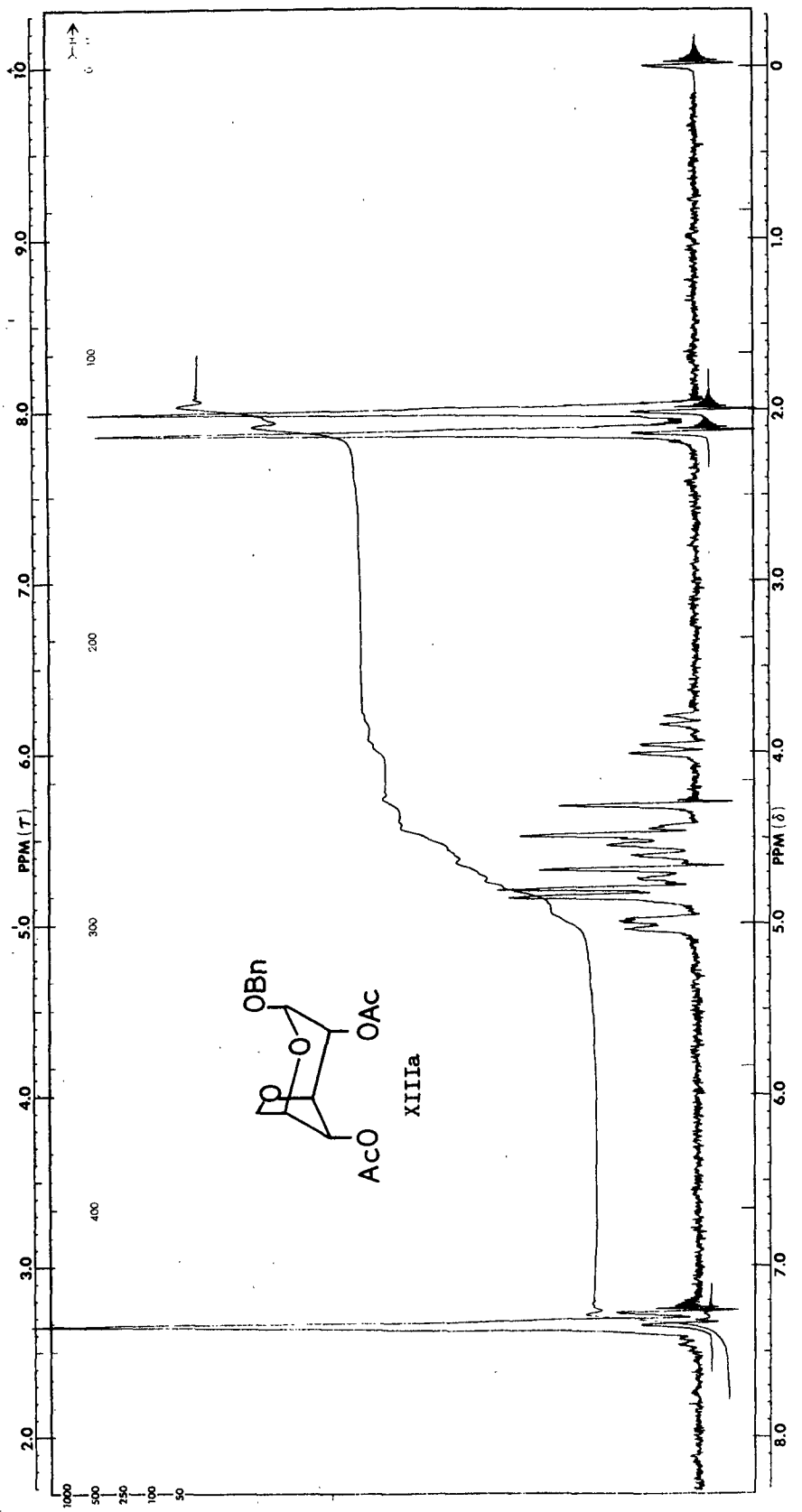

Figure 48. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl 2,4-Di-O-acetyl-3,6-anhydro-β-D-glucopyranoside in $CDCl_3$

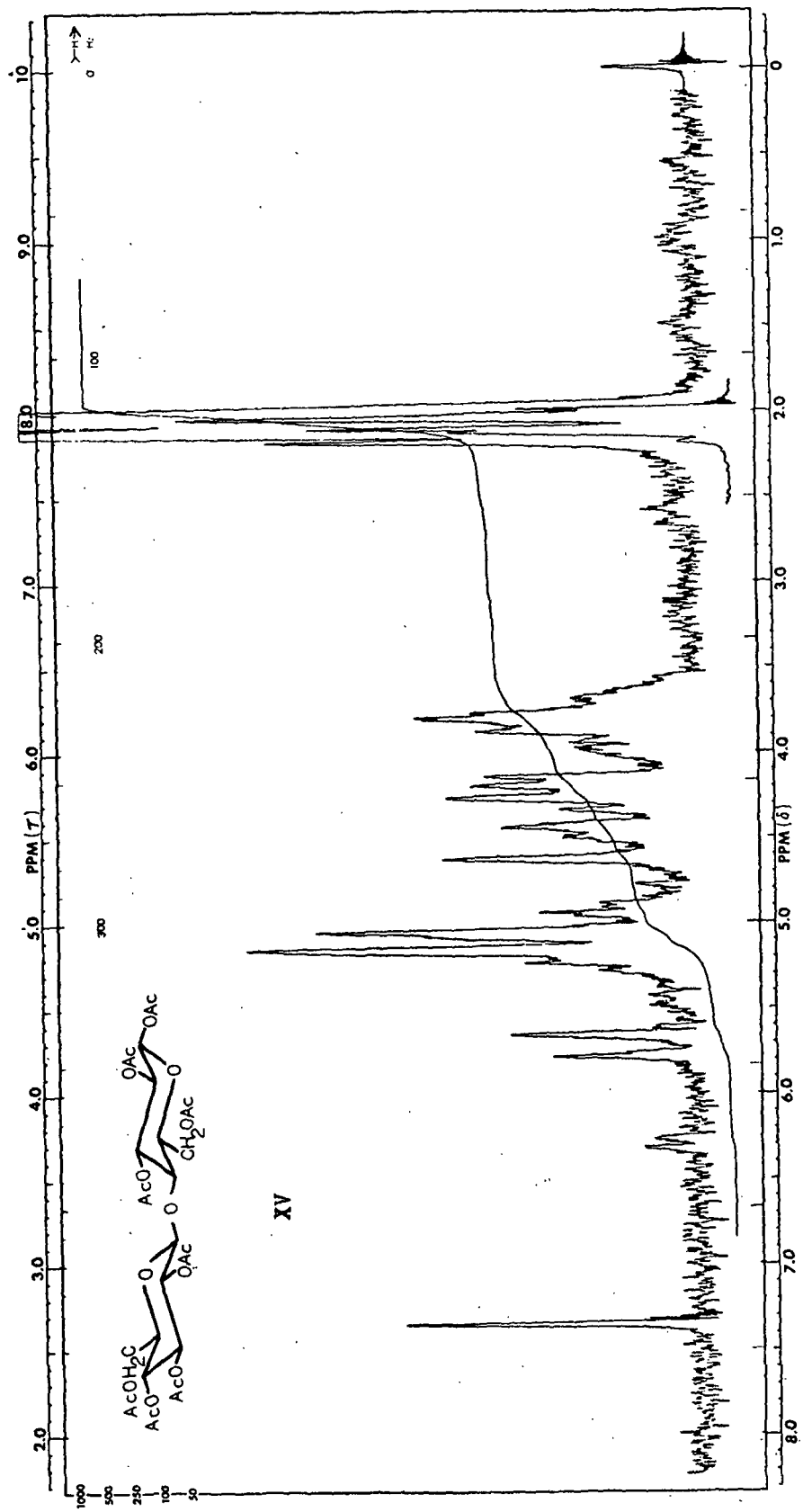

Figure 49. Proton Magnetic Resonance Spectrum (60 MHz) of Octa-O-acetyl-β-cellobiose in $CDCl_3$

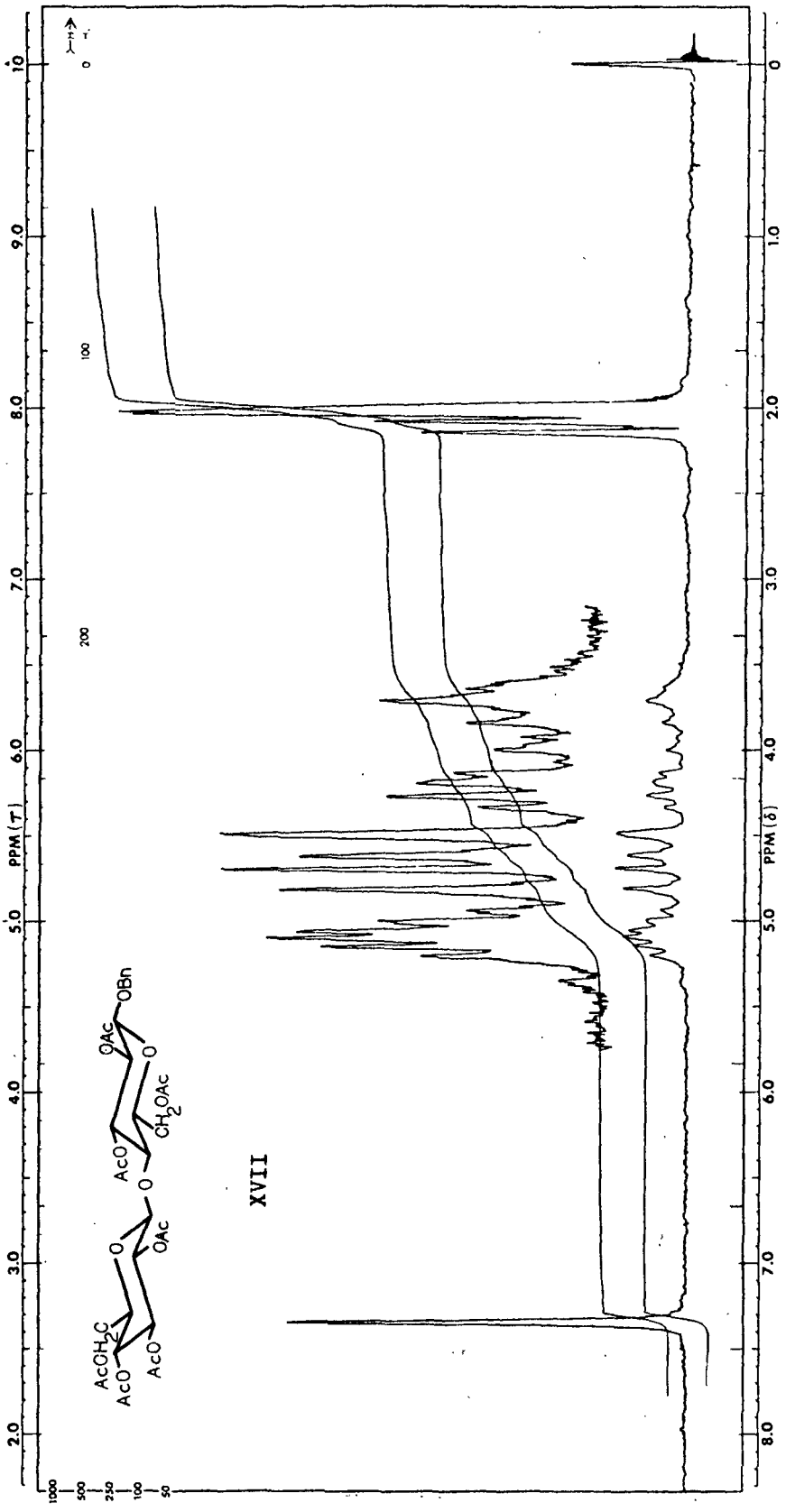

Figure 50. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl Hepta-O-acetyl-β-cellobioside in $CDCl_3$

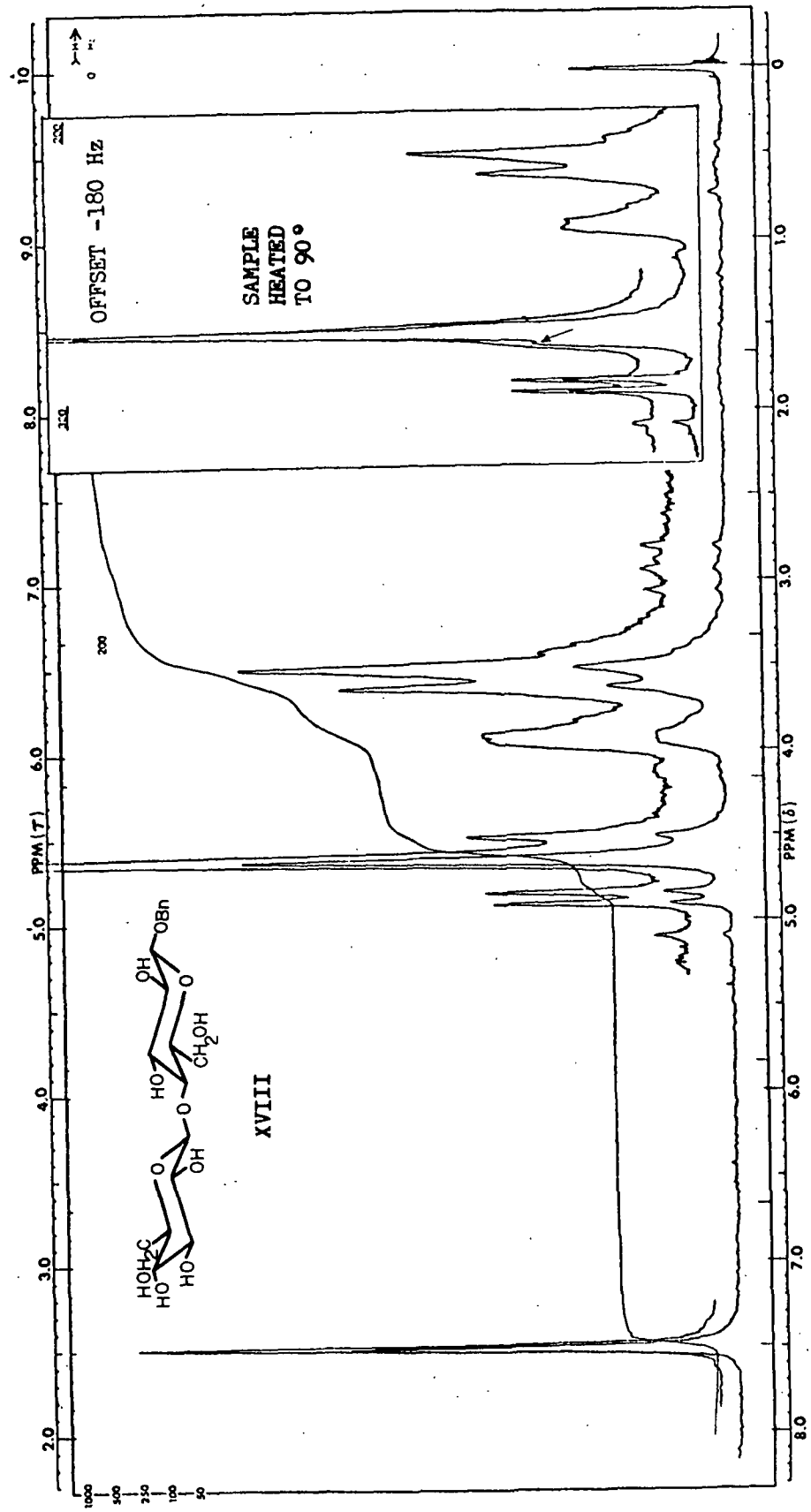

Figure 51. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl β-Cellobioside in D_2O

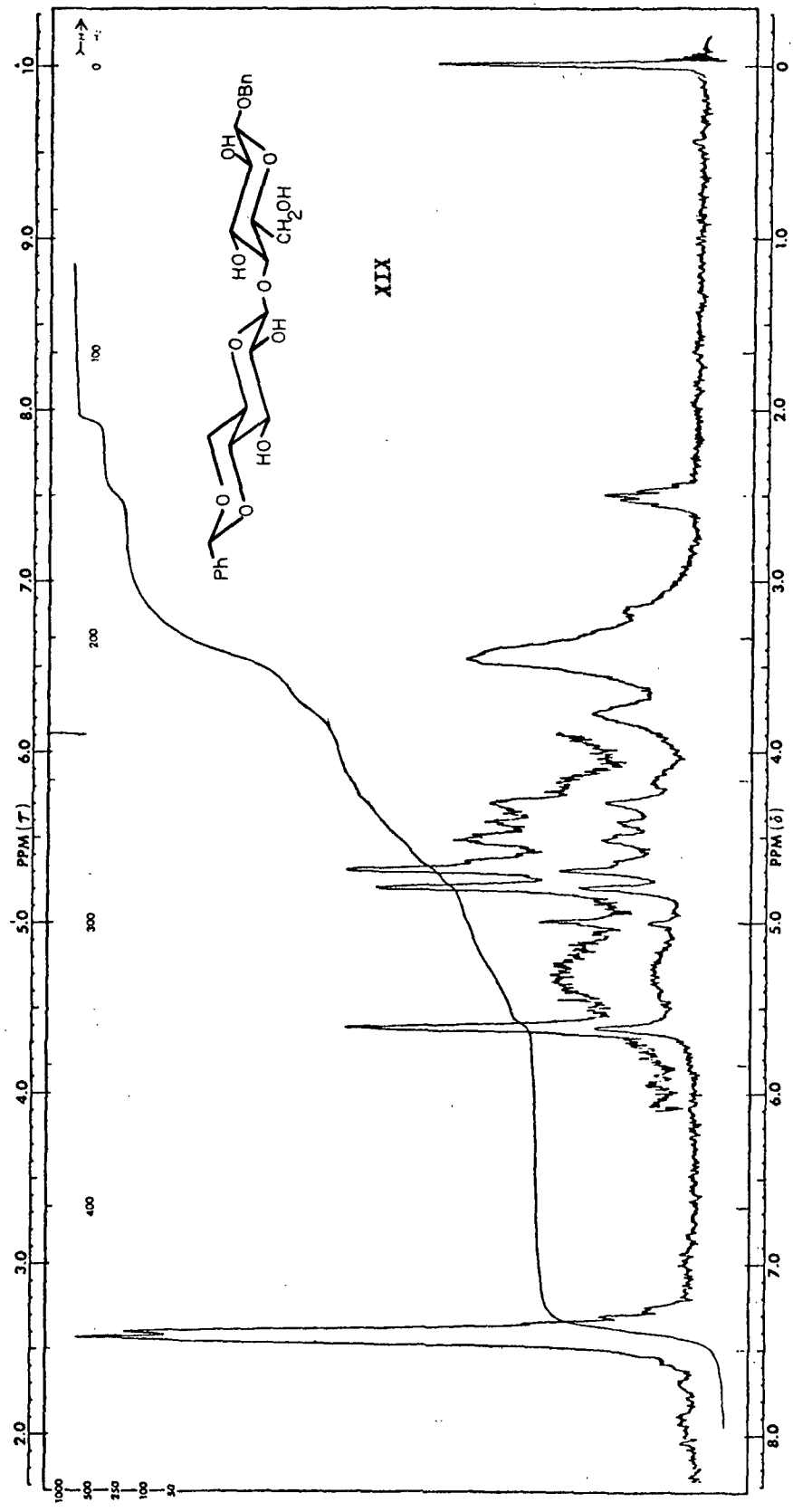

Figure 52. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl 4',6'-O-Benzylidene-β-cellobioside in DMSO-d_6

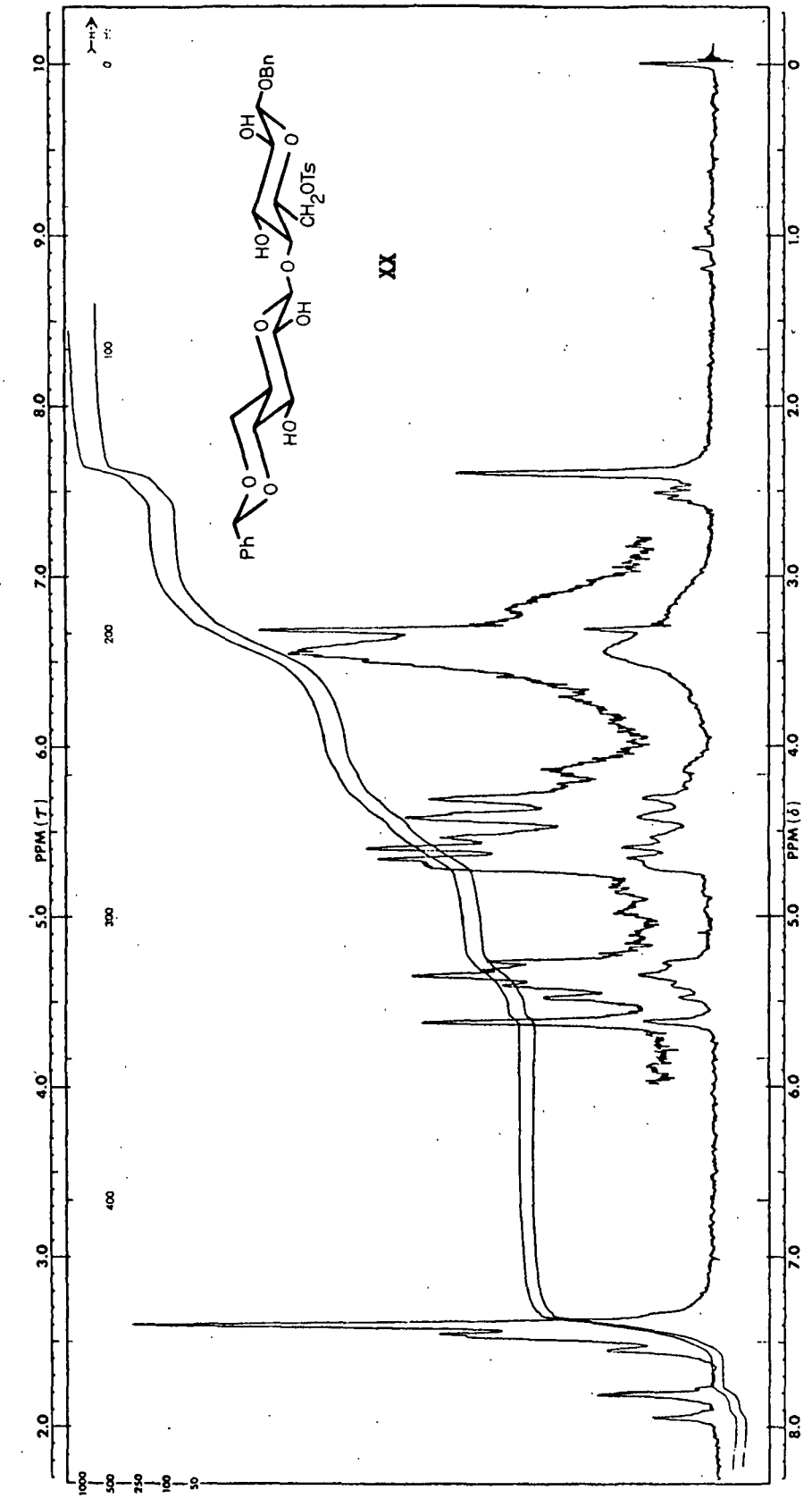

Figure 53. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl 4',6'-O-Benzylidene-6-O-toluenesulfonyl-β-cellobioside in DMSO-d_6

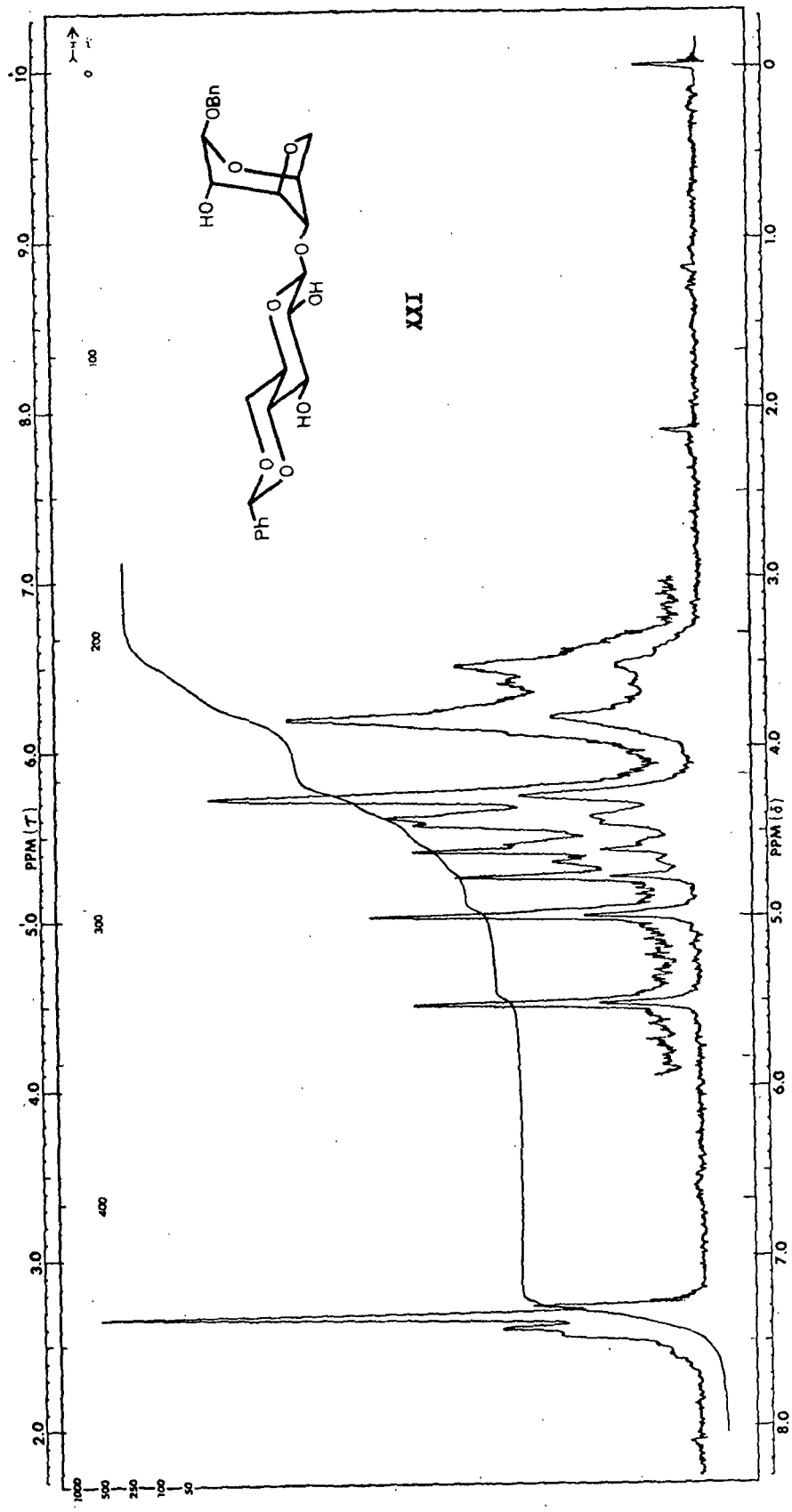

Figure 54. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl 3,6-Anhydro-4',6'-O-benzylidene-β-cellobioside in $CDCl_3$

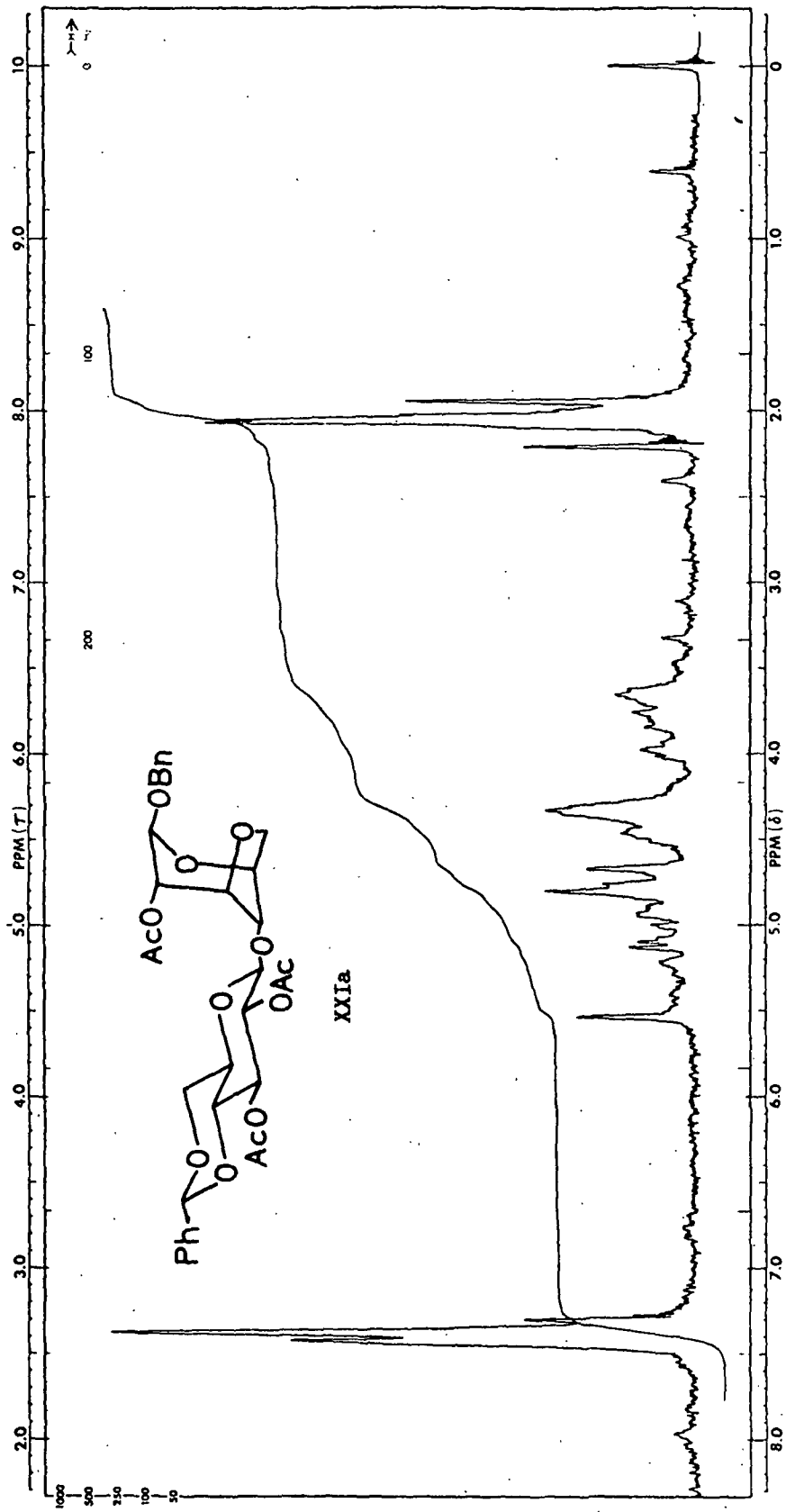

Figure 55. Proton Magnetic Resonance Spectrum (60 MHz) of Benzyl 2,2',3'-Tri-O-acetyl-3,6-anhydro-4',6'-O-benzylidene-β-cellobioside in $CDCl_3$