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Mechanisms of Alkaline Glycosidic Bond Cleavage in 1,5-Anhydro-4-O-β-Mannopyranosyl-D-Mannitol

Margaret Esther Henderson

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MECHANISMS OF ALKALINE GLYCOSIDIC BOND CLEAVAGE IN 1,5-ANHYDRO-4-O-β-MANNOPYRANOSYL-D-MANNITOL

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

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To further investigate reaction mechanisms which could be responsible for glycosidic bond cleavage in cellulose model compounds and cellulose under alkaline pulping conditions, the degradation of 1,5-anhydro-4-0-β-mannopyranosyl-D-mannitol (1,5-anhydromannobiitol) was studied.

Degradation of 1,5-anhydromannobiitol in liquors enriched with $^{18}O$ showed that, depending on the reaction conditions, 70-85% of the cleavage occurred at the glycosyl-oxygen bond while the remaining 15-30% took place at the oxygen-aglycon bond. Two products, 1,5-anhydro-D-mannitol (70-82%) and 1,5-anhydro-D-iditol (0-8%), were found. A series of $^{18}O$-incorporation studies showed that 3-5% of the 1,5-anhydro-D-mannitol and 100% of the 1,5-anhydro-D-iditol came from the cleavage of the oxygen-aglycon bond. Depending on the reaction conditions, 0-30% (molar basis) of the aglycon failed to form detectable products.

Kinetic studies of 1,5-anhydromannobiitol were conducted at a variety of temperatures, hydroxide concentrations, and ionic strengths. Apparent thermodynamic functions of activation were calculated from the temperature series. The glycosyl-oxygen bond cleavage had enthalpy and entropy values of 35.3 kcal/mole and $-4.3$ cal/mole-°K, respectively. The enthalpy value for the oxygen-aglycon bond cleavage was $39.7 \pm 8$ kcal/mole, and the entropy was $+3.28 \pm 2$ cal/mole-°K.

Neither glycosyl-oxygen nor oxygen-aglycon cleavage was accelerated by the addition of hydrosulfide ion (SH$^-$), which has been shown to be a stronger nucleophile than hydroxide (OH$^-$) at 170°C. A fivefold increase in the ionic strength of the medium caused an 11% increase in the rate of glycosyl-oxygen bond cleavage and an 82% increase in the rate of oxygen-aglycon bond cleavage. Varying the hydroxide concentration while holding the ionic strength constant
showed that both bond cleavages exhibit a strong positive dependence on hydroxide concentration. Methylating the hydroxyl groups in 1,5-anhydro-4-0-β-manno-pyranosyl-D-mannitol totally stopped cleavage at the glycosyl-oxygen bond and reduced the rate of oxygen-aglycon bond cleavage by a factor of 17.

The above results suggest that the glycosyl-oxygen bond is cleaved primarily by a neighboring group-type mechanism in which the conjugate base of OH-2 attacks at C-1 and displaces the ring oxygen \([SN^1cB(2')-ro]\). Some \(SN^1\)-type reaction may be occurring simultaneously. The oxygen-aglycon bond appears to cleave via a mixed \(SN^1cB(3)/SN^1\) mechanism.
INTRODUCTION

ORIGIN OF THE PROBLEM

High temperature alkaline pulping processes, widely used in the papermaking industry, degrade cellulose and other wood polysaccharides at the same time they remove lignin. Up to 10% of the cellulose and 40-70% of the hemicelluloses are lost.\textsuperscript{1,2} The degree of polymerization of the cellulose can be decreased by a factor of 3 or more,\textsuperscript{1,3,4} resulting in lower pulp viscosity. Because carbohydrate degradation decreases pulp yield and viscosity and hence has negative economic consequences for the pulp and paper industry, it has been studied extensively.

Two major mechanisms of carbohydrate degradation have been identified; glycosidic bond cleavage and peeling. Peeling is the stepwise removal of sugar units from the reducing end of a polysaccharide chain. It occurs at low temperatures and is thought to be responsible for a significant proportion of the yield losses, particularly those which occur during the heat-up portion of the cook. Peeling can be terminated by either chemical or physical stopping reactions. In chemical stopping, the reducing end group rearranges to form an alkali stable group.\textsuperscript{5} In physical stopping, the polymer chain peels until the reducing end group reaches the edge of a crystallite and becomes physically inaccessible to the hydroxide ions.\textsuperscript{6} Thus peeling does not continue unchecked throughout the whole pulping time. Peeling has been reviewed by several authors.\textsuperscript{7-10}

In contrast, glycosidic bond cleavage results in scission of the cellulose or hemicellulose chain at any glycosidic linkage along its length. This type of degradation is generally thought to require temperatures above 140°C and is
primarily responsible for the losses in pulp viscosity.\textsuperscript{4,11} Each chain cleavage also creates a new reducing end from which peeling can begin. It has been estimated that another 40-65 glucose units peel from each new site before a stopping reaction occurs.\textsuperscript{12} Therefore chain cleavage can also contribute to yield loss.

In order to minimize or eliminate chain cleavage in existing pulping systems or to design better new pulping systems, a basic mechanistic understanding of the reactions of chain cleavage is necessary. Many studies have been directed toward this goal. Comprehensive reviews of these studies have recently been published.\textsuperscript{9,11,13,14} Therefore, only the most pertinent ones will be briefly discussed here.

BACKGROUND

Investigating the mechanisms of alkaline glycosidic bond cleavage in cellulose itself is complicated by the simultaneous occurrence of peeling, the presence of physical effects, and the multitude of possible reaction sites and products. Most studies have therefore used model compounds to investigate the mechanisms of glycosidic bond cleavage.

Alkaline Degradation of Aryl Glycosides

Studies of aryl glycosides have shown that, in general, those having a free hydroxyl group on C-2 which is trans to the glycosidic bond react much faster than those whose C-2 hydroxyl group is either cis to the glycosidic bond or blocked by a derivative.\textsuperscript{15-17} In addition, glycosides having this trans-1,2 configuration and a hydroxymethyl group at C-5 cis to the glycosidic bond produce the corresponding 1,6-anhydride in most cases.\textsuperscript{15} These observations led McCloskey and Coleman\textsuperscript{18} to propose that aryl glycosides degrade via an $S_N^{1}cB(2)\star$

\*Unimolecular nucleophilic substitution with neighboring group assistance from the conjugate base of OH-2.
mechanism, as shown in Fig. 1. In this mechanism, the compound must assume a conformation such that the C-1 and C-2 substituents are antiperiplanar. The hydroxyl group at C-2 then ionizes and displaces the oxygen-aglycon group from C-1, forming a 1,2-epoxide. If the C-6 hydroxyl group is axial, it can open this 1,2-epoxide ring to give a 1,6-anhydride.

Further support for this mechanism comes from some recent work by Kyosaka, Murata, and Tanaka.\textsuperscript{19} They showed that phenyl $\alpha$-mannopyranosides (trans-1,2) hydrolyzed 100 to 1000 times faster than their corresponding phenyl $\beta$-mannopyranosides (cis-1,2). In addition, phenyl $\alpha$-D-mannopyranoside degraded much faster than phenyl $\beta$-D-glucopyranoside even though both have a trans-1,2 configuration. The authors suggest that this difference in reactivity is due to the fact that phenyl $\alpha$-D-mannopyranoside does not have to change to its least stable conformation to achieve the necessary antiperiplanar relationship between OH-2 and the glycosidic bond.

The $S_N^1\text{CB}(2)$ pathway is not, however, the only route via which aryl glycosides can degrade. Some phenyl glycosides which have the cis-1,2 configuration still degrade in alkali at 100°C. Both phenyl $\alpha$-D-galactopyranoside and phenyl $\beta$-D-mannopyranoside yielded significant amounts of 1,6-anhydro-D-galactosan and 1,6-anhydro-$\beta$-D-mannopyranose, respectively,\textsuperscript{15,20} although somewhat more slowly than their trans-1,2 analogs. Phenyl $\alpha$-D-glucopyranoside is quite stable in alkali at 100°C, but does degrade at 170°C. Investigators have suggested that this compound degrades by an $S_N^1\text{CB}(6)^*$ mechanism.\textsuperscript{21}

\textsuperscript{*Unimolecular nucleophilic substitution with neighboring group assistance from the conjugate base of OH-6.}
Figure 1. Degradation of phenyl β-D-glucopyranoside via the $S_N^1cB(2)$ mechanism.18,25,26
Thus it appears that the $S_N^1\text{cB}(2)$ mechanism is an important pathway for aryl glycoside degradation, but that there are other, competing mechanisms with just slightly higher free energies of activation.

**Alkaline Degradation of Alkyl Glycosides**

Early studies of methyl glycosides showed that, like the aryl glycosides, compounds having a trans-1,2 relationship between the OH-2 and glycosidic bond reacted significantly faster than their cis-1,2 analogs. This led to the suggestion that the $S_N^1\text{cB}(2)$ mechanism might also be important in the alkaline degradation of these compounds. Lindberg et al. noted, however, that methyl glycosides with a cis-1,2 configuration or with a trans-1,2 configuration, but with OH-2 etherified, still hydrolyzed at significant rates. This indicated that reaction routes other than the $S_N^1\text{cB}(2)$ mechanism also exist.

The alkaline degradations of methyl α- and β-D-glucopyranoside have been studied in more detail. The overall degradation rates and postulated mechanisms for these compounds are shown in Table 1. The rate constants for the degradation of these three compounds are of the same order of magnitude. Methyl α- and methyl 2-O-methyl-β-D-glucopyranosides do react more slowly than methyl β-D-glucopyranoside, but the difference is only a factor of four. Due to the fact that only 18-30% of the methyl β-D-glucopyranoside degraded to 1,6-anhydro-β-D-glucopyranose (levoglucosan), Nault concluded that the glucoside degraded by a mixed mechanism rather than a pure $S_N^1\text{cB}(2)$ one. This work clearly illustrates that there are mechanisms other than $S_N^1\text{cB}(2)$ which play an important role in the degradation of alkyl glycosides.
Table 1. Degradation of methyl glucopyranosides in 2.5M NaOH at 170°C.

<table>
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<th>Mechanism</th>
<th>Reference</th>
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<tr>
<td>Methyl-α-D-glucopyranoside</td>
<td>0.69</td>
<td>$S_{N}^{2a}$</td>
<td>25, 26</td>
</tr>
<tr>
<td>Methyl-β-D-glucopyranoside</td>
<td>2.62</td>
<td>$S_{N}^{1cb}(2) &amp; S_{N}^{1b}$ or $S_{N}^{2}$</td>
<td>24</td>
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<tr>
<td>Methyl 2-O-methyl-β-D-glucopyranoside</td>
<td>0.71</td>
<td>$S_{N}^{2}$</td>
<td>24</td>
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$^{a}$Bimolecular nucleophilic substitution.$^{27}$

$^{b}$Unimolecular nucleophilic substitution.

Alkaline Degradation of Disaccharides

It is likely that there are significant differences between the glycosidic bonds in the simple aryl and alkyl glycoside models and those in polysaccharides. Simkovic et al.$^{28}$ studied the alkaline degradation of methyl β-D-glucopyranoside, methyl β-D-cellobiose, and methyl β-D-cellotrioside and found that the alkali sensitivity of the glycosidic bonds increased throughout this series. Earlier, Best and Green$^{29}$ reported that the interior glucosyl-glucosyl bond in methyl β-D-cellobiose reacted five times faster than the glucosyl-methyl bond. Investigators have therefore studied glycosidic bond cleavage in disaccharides in order to better approximate the reactions occurring in cellulose and other polysaccharides.

One study was made of disaccharide alditols; cellobiitol, lactitol, and maltitol.$^{30}$ All three compounds hydrolyzed at comparable rates. Based on the products, Lindberg postulated that cleavage was occurring at the glucosyl-oxygen bond by a mixed $S_{N}^{1cb}(2')$ and $S_{N}^{2}$ mechanism, and at the oxygen-aglycon bond via both $S_{N}^{2}$ and $S_{N}^{1cb}(1)$ pathways. The $S_{N}^{1cb}(1)$ mechanism proposed by Lindberg is shown in Fig. 2.
Since that time, disaccharides in which the aglycon is a 1,5-anhydro-alditol have been studied. An extensive study of 1,5-anhydro-4-O-β-D-glucopyranosyl-D-glucitol (1,5-anhydrocellobiitol)\textsuperscript{13,14} showed that 10% of the glycosidic chain
cleavage occurs at the oxygen-aglycon bond.* Cleavage of the oxygen-aglycon bond had the characteristics of an $S_N^1$ mechanism. This conclusion was supported by a study of 1,5-anhydro-2,3,6-tri-O-methyl-cellobiitol.$^{31}$ Since methylating the hydroxyl groups in the aglycon did not affect the rate of oxygen-aglycon bond cleavage, it was concluded that the oxygen-aglycon bonds in 1,5-anhydro-cellobiitol and its methylated analog must react via an $S_N^1$ mechanism.

The remaining 90% of the 1,5-anhydrocellobiitol degradation occurred at the glycosyl-oxygen bond.* The results from kinetic studies of glycosyl-oxygen bond cleavage were all consistent with a predominantly neighboring group-type mechanism. However, only 35% of the 1,5-anhydrocellobiitol which cleaved at the glycosyl-oxygen bond formed levoglucosan.$^{13,14}$ In contrast, phenyl $\beta$-D-glucopyranoside$^{15,18}$ and 1,5-anhydro-2,3,6-tri-O-methyl-cellobiitol,$^{31}$ which degrade via $S_N^1cB(2)$ pathways, yield 70-80% levoglucosan. The smaller amount of levoglucosan formed from 1,5-anhydro-cellobiitol led Brandon to conclude that

*The dotted line between G and O represents cleavage at the glycosyl-oxygen bond; the dashed line between O and A indicates cleavage of the oxygen-aglycon bond.
less than 50% of the glycosyl-oxygen bond cleavage was due to an $S_{N1}cB(2)$-type reaction.\textsuperscript{13,14} Degradation of 1,5-anhydrocellobiitol in the presence of $SH^-$ (kraft pulping conditions)\textsuperscript{32,33} was not consistent with an $S_N^2$ mechanism. The mechanism for the remaining portion of the glycosyl-oxygen bond cleavage was therefore tentatively identified as $S_N^1$ in nature.

This review of studies of glycosidic bond cleavage in disaccharides shows that the $S_N^1cB(2)$ mechanism is an important but by no means exclusive pathway for their degradation.

**SCOPE AND OBJECTIVES OF THE THESIS**

The preceding discussion of the mechanisms of alkaline glycosidic bond cleavage in various model compounds clearly shows that these reactions are not fully understood. Although the $S_N^1cB(2)$ mechanism appears to be important, much evidence suggests that other mechanisms play significant roles. In particular, less than 55% of the glycosyl-oxygen bond cleavage in 1,5-anhydrocellobiitol, the model compound most like cellulose, appears to occur via an $S_N^1cB(2)$ mechanism. Since the $S_N^1cB(2)$ mechanism requires that a glucosyl unit convert to its least stable conformation, the $S_N^1cB(2)$ mechanism may not occur at all in the cellulose polymer where the necessary conformational change would be extremely hindered.\textsuperscript{14} Other mechanisms may therefore be the most important ones in cellulose, and warrant further investigation.

The best way to look at these other mechanisms is to eliminate the $S_N^1cB(2)$ route. There are two possible ways to do this: OH-2 can be etherified to prevent ionization or the relationship between OH-2 and the glycosidic bond can be changed from trans-1,2 to cis-1,2. Derivatizing OH-2 so that it cannot ionize
can cause stereochemical and electronic changes in a molecule and thus may alter the competing mechanisms.13,14,31

The relationship between OH-2 and the glycosidic bond can be changed from trans to cis either by switching from a β to α glycosidic linkage or by inverting the OH-2 group. The α-linked analog of 1,5-anhydrocellobiitol, 1,5-anhydro-4-O-α-glucopyranosyl-D-glucitol (1,5-anhydromaltitol), has a cis-1,2 configuration. However, switching the type of bond changes the steric factors and anomeric effects of the bond being broken.13,14 A cis-1,2 configuration is also present in the C-2 epimer of 1,5-anhydrocellobiitol, 1,5-anhydro-4-O-β-mannopyranosyl-D-mannitol (1,5-anhydromannobiitol). Because this compound still has the β-1,4 bond found in cellobiitol, mechanisms other than SN1cB(2) should be less affected by the change. Therefore 1,5-anhydromannobiitol should be a good model compound for investigating the alternate mechanisms of glycosyl-oxygen bond cleavage in cellulose model compounds.

In conclusion, the objective of this thesis was to study the mechanisms of glycosidic bond cleavage in 1,5-anhydromannobiitol under high temperature alkaline conditions. This information should provide additional insight into the mechanisms which may degrade cellulose and other wood polysaccharides.

POTENTIAL MECHANISMS FOR THE ALKALINE DEGRADATION OF 1,5-ANHYDRO-4-O-β-MANNOPYRANOSYL-D-MANNITOL

Glycosidic linkages can be broken in two places; between the glycon and oxygen (glycosyl-oxygen bond), or between the oxygen and aglycon group (oxygen-aglycon bond), as shown in Fig. 3. The potential mechanisms for cleaving each of these bonds in 1,5-anhydromannobiitol are discussed in the following sections.
Figure 3. Potential points of bond cleavage in the glycosidic linkage of 1,5-anhydromannobiitol.

Glycosyl-Oxygen Bond

Unimolecular Nucleophilic Substitution ($S_N^1$)

In a unimolecular nucleophilic substitution ($S_N^1$) mechanism, bond cleavage occurs heterolytically, producing carbocation and anion intermediates. The carbocation is quickly attacked by a nucleophile. The glycosyl-oxygen bond in 1,5-anhydromannobiitol is shown undergoing $S_N^1$-type cleavage in Fig. 4. In an $S_N^1$-type reaction, the reaction rate is strongly affected by the stability of both the carbonium ion formed in the substrate and the anion created in the leaving group. Hence altering either the substrate or leaving group may affect the reaction rate. Since the nucleophile does not participate in the rate-determining step, the rate is independent of the strength of the nucleophile. Compounds which react by an $S_N^1$ mechanism follow first-order kinetics as shown in Eq. (1).

$$\text{rate} = k[\text{reactant}]$$

(1)

where $k =$ rate constant.
Figure 4. Cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobitol via an $S_N^1$ mechanism.
For a neutral substrate undergoing unimolecular substitution, increasing the ionic strength of the medium by the addition of a noncommon ion salt increases the reaction rate. This is due to the fact that the intermediates are more charged than the reactants, and hence stabilized to a greater degree by the increase in ionic strength. Because the rate-determining step in an $S_{N1}$-type reaction requires enough energy to break a bond, and no energy is being generated by the simultaneous formation of another bond, these reactions have relatively high enthalpies of activation. A single reactant molecule forms two ions in the transition state, hence the entropies of activation are relatively positive.\textsuperscript{34,35}

The cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol via an $S_{N1}$ mechanism should produce 1,5-anhydro-D-mannitol. A mixture of $\alpha$- and $\beta$-D-mannose should also be formed, but these compounds will degrade too quickly to be detected.\textsuperscript{36} Another possible product is 1,6-anhydro-\textbeta-D-mannopyranose. Compounds analogous to 1,6-anhydro-\textbeta-D-mannopyranose are unstable under high temperature alkaline conditions, but if the rate constant for formation is larger than the rate constant for degradation, these compounds will still be detected in the reaction mixture.

Bimolecular Nucleophilic Substitution ($S_{N2}$)

The bimolecular nucleophilic substitution ($S_{N2}$) mechanism involves simultaneous attack by the nucleophile and departure of the leaving group. Figure 5 shows the glycosyl-oxygen bond in 1,5-anhydromannobiitol cleaving by this mechanism. Because the nucleophile participates in the rate-determining step, its nucleophilicity has a strong effect on the reaction rate. The leaving ability of the leaving group also affects the rate, so $S_{N2}$-type reactions have second-order rate expressions. In the present alkaline degradation studies, however,
the nucleophile (OH⁻) is present in such excess that the rate expression reduces to a pseudo-first-order form, as given in Eq. (2).

\[
\text{rate} = k' [\text{reactant}]
\]  

(2)

where \(k' = k f[\text{OH}^-] = \text{pseudo-first-order rate constant}\)

\(k = \text{true rate constant}\)

Figure 5. Cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol via an \(S_N^2\) mechanism.
The transition state of this postulated \( S_N^2 \) mechanism has a more delocalized charge than the reactants. Therefore, increasing the ionic strength of the medium stabilizes the reactants more than the transition state, which decreases the reaction rate. Hence \( S_N^2 \)-type reactions generally exhibit negative salt effects.\(^{34,35}\)

In an \( S_N^2 \) mechanistic scheme, the rate determining step involves the simultaneous cleavage and formation of bonds. Some of the energy required for breaking the old bond is recovered by the formation of the new bond. Therefore \( S_N^2 \)-type reactions have relatively low enthalpies of activation. The transition state requires that two molecules form one, decreasing the translational degrees of freedom in the system and giving rise to relatively negative entropies of activation.\(^{34,35}\)

Cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol via an \( S_N^2 \) pathway should produce \( \alpha \)-D-mannose and 1,5-anhydro-D-mannitol. Only the 1,5-anhydro-D-mannitol would be observed because mannose degrades very rapidly under the reaction conditions.\(^{36}\)

Ring Opening with Neighboring Group Assistance [\( S_N^{1cB(2)-ro} \)]

Lindberg \textit{et al.}\(^{37}\) reported that the alkaline degradation of methyl \( \alpha \)-L-arabinofuranoside yielded about 2.6\% methyl \( \alpha \)-L-arabinopyranoside. This observation led them to propose a mechanism in which OH-2 ionizes, attacks C-1, and displaces the ring oxygen. They suggested that ring opening by the nucleophilic attack of a neighboring group [\( S_N^{1cB(2)-ro} \) mechanism] could be responsible for the alkaline degradation of other cis-1,2 glycosides. Figure 6 shows 1,5-anhydromannobiitol reacting according to this mechanism. Once the 1,2-epoxide forms, it can be opened by base to give a hemiacetal. The hemiacetal would
Figure 6. Cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol via the $S_N^{1}cB(2)-ro$ mechanism.
probably rapidly rearrange to an aldose, cleaving the glycosidic bond. An
$S_{N}^{1}cB(2')$-ro mechanism should have characteristics similar to other neighboring
group mechanisms, including a negative salt effect, a relatively low enthalpy of
activation, and an intermediate entropy of activation.$^{34,35}$

Unimolecular Nucleophilic Substitution with Neighboring Group Assistance
from OH-4 [$S_{N}^{1}cB(4')$]

Capon$^{9}$ proposed that phenyl $\beta$-D-mannopyranoside might react via an $S_{N}^{1}cB(4')$ route. This mechanism can also be proposed for 1,5-anhydromannobiitol, as
illustrated in Fig. 7. According to this mechanism, OH-4' ionizes and displaces
the aglycon via a nucleophilic attack at C-1. This requires that the glycon
assume a boat conformation, which is considerably strained, particularly when
the aglycon group is large. DeBruyne$^{16}$ reports that blocking the OH-2 group in
p-nitrophenyl $\beta$-D-xylopyranoside caused the compound to react 1000X more slowly
and switch from an $S_{N}^{1}cB(2)$ to an $S_{N}^{2}Ar$ mechanism rather than follow the $S_{N}^{1}cB(4)$
route. Thus the $S_{N}^{1}cB(4)$ mechanism appears to be a very high energy pathway and
is considered an unlikely mechanism.

*Oxygen-Aglycon Bond*

Unimolecular Nucleophilic Substitution ($S_{N}^{1}$)

Unimolecular nucleophilic substitution ($S_{N}^{1}$) is a possible mode of cleavage
for the oxygen-aglycon bond in 1,5-anhydromannobiitol (Fig. 8). Reaction via
this mechanism could produce 1,5-anhydro-D-mannitol, 1,5-anhydro-D-iditol,
1,5-anhydro-D-talitol, and fragments, as shown. The other characteristics of
the reaction should be the same as those described for the $S_{N}^{1}$ hydrolysis of the
glycosyl-oxygen bond.
Figure 7. Potential cleavage of the glycosyl-oxygen bond in 1,5-anhydro-mannobiitol via an $S_N^{1,4}$ mechanism.
Figure 8. Cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol via an $S_N^1$ mechanism.
Bimolecular Nucleophilic Substitution ($S_N^2$)

A bimolecular nucleophilic substitution ($S_N^2$) mechanism is another way in which the oxygen-aglycon bond could be cleaved. This is illustrated in Fig. 9. The major stable product from $S_N^2$-type cleavage should be 1,5-anhydro-D-talitol. The reaction should have the standard characteristics of an $S_N^2$ mechanism as described previously for glycosyl-oxygen bond cleavage.

Figure 9. Cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol via an $S_N^2$ mechanism.
Unimolecular Nucleophilic Substitution with Neighboring Group Assistance from OH-3 [S_NlcB(3)]

Another possible mechanism for oxygen-aglycon bond cleavage is neighboring group attack by the conjugate base of OH-3 [S_NlcB(3)]. The S_NlcB(3) reaction would result in formation of a 3,4-epoxide intermediate which could be opened by base to give 1,5-anhydro-D-iditol and 1,5-anhydro-D-mannitol, as shown in Fig. 10. This reaction should exhibit the traits common to S_NlcB(i) mechanisms, namely negative salt effects, relatively low enthalpies of activation and intermediate entropies of activation. The rate constants should also have a strong, positive dependence on the hydroxide ion concentration.

Figure 10. Cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol via an SNlcB(3) mechanism.
Unimolecular Nucleophilic Substitution with Neighboring Group Assistance from OH(6) [SN$^1$CB(6)]

Another possible neighboring group mechanism could involve displacement of the oxygen-aglycon bond by the conjugate base of OH-6. This would result in formation of a four-membered ring intermediate. These have been calculated to be very strained$^{38}$ and are seldom if ever found. Reaction via this mechanism would probably yield 1,5-anhydro-D-talitol and 1,5-anhydro-D-mannitol, as shown in Fig. 11. The kinetic behavior of the SN$^1$CB(6) mechanism would be the same as that of the SN$^1$CB(3) mechanism.
Figure 11. Potential cleavage of the oxygen-aglycon bond in 1,5-anhydro-mannobiitol via an $S_{N1cB}(6)$ mechanism.

\[ \text{1,5-Anhydro-D-mannitol} + \text{1,5-Anhydro-D-talitol} \]
RESULTS AND DISCUSSION

SYNTHESIS AND CHARACTERIZATION OF 1,5-ANHYDRO-4-O-β-D-MANNOPYRANOSYL-D-MANNITOL

The model compound used in this study, 1,5-anhydro-4-O-β-D-mannopyranosyl-D-mannitol (1,5-anhydromannobiitol), is a novel compound not previously reported in the literature. It was synthesized from mannobiose, which was isolated from ivory nuts using the procedure developed by Thiem, Sievers, and Karl,

except for a modified acetolysis step. Ground, extracted, and bleached ivory nuts were digested in sodium hydroxide to give mannan A which contained 98.8% mannose. Acetolysis of the mannan A yielded a mixture of acetylated mannosaccharides. Deacetylation and fractionation on Sephadex G-15 produced mannobiose. This compound had a gas chromatographic retention time and NMR spectra identical to those of known mannobiose.

Mannobiose was converted to 1,5-anhydromannobiitol as shown in Fig. 12. Acetylation gave mannobiose octaacetate, which was converted to the hepta-O-acetyl-mannopyranosyl bromide, then reduced with Raney nickel to yield 1,5-anhydromannobiitol heptaacetate. Deacetylation, fractionation, and crystallization yielded pure 1,5-anhydromannobiitol.

The crystalline 1,5-anhydromannobiitol was characterized in several ways. Elemental analysis showed that the carbon, hydrogen, and oxygen contents were almost identical to the expected values. Fehling's test demonstrated that the product was a nonreducing sugar. HPLC analysis showed that acid hydrolysis gave equimolar amounts of 1,5-anhydro-D-mannitol and D-mannose. The 1H- and 13C-NMR spectra were consistent with 1,5-anhydromannobiitol. Gas chromatography showed that this product and 1,5-anhydrocellobiitol had almost identical retention
times, indicating that the two compounds are similar in size and structure. These results demonstrated that the desired product, 1,5-anhydromannobiitol, had been synthesized.

![Chemical structures and reactions for the synthesis of 1,5-anhydromannobiitol from mannobiose.](image)

**Figure 12.** Synthesis of 1,5-anhydromannobiitol from mannobiose.
KINETIC ANALYSES

The concentrations of the reactant and products were measured as a function of time throughout each degradation. The following sections describe how apparent rate constants were calculated from these measurements.

Disappearance of Reactant

The general kinetic expression used for the disappearance of reactant during alkaline degradation was:

$$\frac{dR'}{dt} = -k f[R'] f[OH^-]$$

where 

- $t =$ time, seconds
- $k =$ specific rate constant
- $f[R'] =$ unknown function of reactant activity
- $f[OH^-] =$ unknown function of hydroxide ion concentration

Because the ionic strength is constant throughout a run, it may be assumed that the activity coefficient of $R'$ is constant. Reactant concentration may therefore be used as an estimate of activity. Previous work with similar compounds demonstrated that the reaction is first order with respect to the reactant. Thus

$$f[R'] = R$$

where $R =$ reactant concentration, mole/liter.

The activity of the hydroxide ion will be constant if its activity coefficient and concentration are constant. The activity coefficients depend on temperature, pressure, and ionic strength, which were all constant during a run. The ratio between sodium hydroxide and reactant concentrations was very large (50–250), and thus the hydroxide concentration remained essentially constant throughout a given degradation. For these reasons, Eq. (3) simplifies to:

$$\frac{dR}{dt} = -k_r R$$
where $R =$ reactant concentration, moles/L

$k_r =$ pseudo-first-order rate constant for the disappearance of reactant, sec$^{-1}$.

Integration of (5) and rearrangement yields:

$$\ln R = -k_r t + \ln R_0$$  \hspace{1cm} (6)

where $R_0 =$ initial reactant concentration, moles/L.

Values of $k_r$ were determined by fitting the concentration-time data to Eq. (6) using least-squares linear regression (Program 1, Appendix I). A representative plot of $\ln R$ versus time is shown in Fig. 13. The validity of the use of pseudo-first-order kinetics was checked by verifying that a plot of $\ln R$ versus time was linear over two half-lives.

**Formation of Stable Products**

A reactant can simultaneously undergo two or more independent reactions to give one or more products. This case is known as parallel reactions and has been described mathematically by Frost and Pearson.$^{43}$ The general rate expression for the formation of a product from a parallel reaction is:

$$\frac{dP_i'}{dt} = k_i' f[R] f[OH^-]$$  \hspace{1cm} (7)

where $P_i' =$ activity of product $i$, moles/L

$k_i' =$ specific rate constant

The pseudo-first-order assumptions, discussed previously, reduce this to:

$$\frac{dP_i}{dt} = k_i R$$  \hspace{1cm} (8)

where $P_i =$ concentration of product $i$, moles/L

$k_i =$ pseudo-first-order rate constant for the appearance of product $i$, sec$^{-1}$
Figure 13. Disappearance of 1,5-anhydromannobiitol in 2.5M NaOH at 170°C.
The exponential form of Eq. (6) is:

\[ R = R_0 \exp(-k_r t) \]  

(9)

Substituting this expression for \( R \) into Eq. (8) yields:

\[ \frac{dP_i}{dt} = k_i R_0 \exp(-k_r t) \]  

(10)

Integrating Eq. (10) and evaluating the integration constant leads to the parallel pseudo-first-order rate expression:

\[ (P_i - P_{10}) = k_i (R_0/k_r) [1 - \exp(-k_r t)] \]  

(11)

where \( P_{10} = \) initial product concentration, moles/L

Values for \( k_i \) were determined by fitting each data set to Eq. (11) using least-squares linear regression (Program 2, Appendix I). Figure 14 shows a representative data set plotted according to Eq. (11).

Alternate forms of the equations describing parallel, pseudo-first-order reactions are useful for presenting the data in a consolidated form. The rate equation for the disappearance of reactant can be written as:

\[ \ln X_{r,t} = -k_r t \]  

(12)

where \( X_{r,t} = \) mole fraction of reactant at time \( t \)

\[ k_r = \sum k_i = \) pseudo-first-order rate constant for reactant disappearance, sec\(^{-1}\)

\[ k_i = \) pseudo-first-order specific rate constant for the formation of product \( i, \) sec\(^{-1}\)

Equation (13) describes the appearance of product:

\[ \ln(X_{i,oo} - X_{i,t}) = -k_r t + \ln X_{i,oo} \]  

(13)
\[(P_i - P_0) = k_i (R_0/k_r) \left[1 - \exp(-k_r t)\right]\]

\[k_i = 4.81 \times 10^{-6} \text{sec}^{-1}\]

Figure 14. Appearance of 1,5-anhydro-D-mannitol during the degradation of 1,5-anhydromannobitol in 2.5M NaOH at 170°C.
where $X_{i,t}$ = mole fraction of product i at time t

$X_{i,oo} = X_{i,t}$ at completion (the relative proportion of product i formed)

In these equations:

$$X_{i,t} = \frac{C_i}{Cr,o} \quad (14)$$

and

$$X_{i,oo} = \frac{C_i}{Cr,o-Cr,t} \quad (15)$$

where $C_{i,t}$ = concentration of product i at time t, mole/L

$Cr,o$ = concentration of reactant at $t = 0$, mole/L

$Cr,t$ = concentration of reactant at time t, mole/L

By plotting either $\ln X_{rt}$ or $\ln(X_{i,oo}-X_{it})$ as a function of time, reactant disappearance and product appearance can be plotted on one graph (Program 3, Appendix I). In Fig. 15, one data set is shown plotted in this manner.

**Formation of Unstable Products**

Some products formed may be unstable under the reaction conditions. In this case, the observed concentration of product will be less than what has actually formed and plotting the observed concentration against time using Eq. (11) will not give the correct rate of formation. A mathematical method for determining the pseudo-first-order rate constant for the formation of an unstable product was described by Brandon. The reaction scheme can be pictured as:

Reactant $\xrightarrow{k_r}$ Total Products

$\xrightarrow{k_f}$ Unstable Product $\xrightarrow{}$ Products

The change in the unstable product concentration with respect to time can be expressed as:
Figure 15. Parallel pseudo-first-order kinetic analysis of 1,5-anhydromannobiitol in 2.5M NaOH at 170°C.
\[
\frac{dL}{dt} = k_f R - k_d L
\]  

(16)

where \( L = \) concentration of unstable product, moles/L  
\( R = \) concentration of reactant, moles/L  
\( k_f = \) pseudo-first-order rate constant for unstable product formation, sec\(^{-1}\)  
\( k_d = \) pseudo-first-order rate constant for unstable product degradation, sec\(^{-1}\)

Substituting Eq. (9) into Eq. (12) gives:

\[
\frac{dL}{dt} = k_f R_0 \exp(-k_{f}t) - k_d L
\]  

(17)

Integration and rearrangement yields:

\[
L - L_0 \exp(-k_{d}t) = k_f \left[ \frac{R_0}{(k_d - k_f)} \right] \left[ \exp(-k_{f}t) - \exp(-k_{d}t) \right]
\]  

(18)

The pseudo-first-order rate constant for the formation of an unstable product can be calculated from Eq. (18) if the pseudo-first-order rate constant for the degradation of the unstable product is also known.

**APPARENT THERMODYNAMIC FUNCTIONS OF ACTIVATION**

The Universal Rate Law [Eq. (19)] can be derived from the transition state theory of chemical kinetics.\(^{43,44}\)

\[
\ln(k_{T}/T) = [\ln(k/h) + \Delta S^\ddagger/\mathcal{R}] - \Delta H^\ddagger/\mathcal{R}T
\]  

(19)

where \( k = \) Boltzmann's constant, \( 1.380 \times 10^{-6} \) erg K\(^{-1}\)  
\( T = \) absolute temperature, °K  
\( h = \) Planck's constant, \( 6.625 \times 10^{-27} \) erg sec  
\( \Delta S^\ddagger = \) apparent entropy of activation, cal/mole °K  
\( \mathcal{R} = \) gas constant, \( 1.987 \) cal/mole °K  
\( \Delta H^\ddagger = \) apparent enthalpy of activation, cal/mole
Fitting the rate constants obtained at various temperatures to Eq. (19) using least-squares linear regression (Program 4, Appendix I) allowed calculation of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ from the slope and intercept, respectively. A representative plot is shown in Fig. 16.

$$\ln \left( \frac{k_T}{T} \right) = \left[ \ln \left( \frac{k_B}{n} \right) + \Delta S^\ddagger / R \right] - \Delta H^\ddagger / RT$$

Figure 16. Rate constants for the degradation of 1,5-anhydromannobiitol plotted as a function of temperature according to the Universal Rate Law.

In order to compare rate constants obtained from reactions run at slightly different temperatures, all rate constants were adjusted to a common temperature. This was done using the following form of the Arrhenius equation:

$$k_{T2} = \exp[\ln k_{T1} - (E_a / R) (1/T2 - 1/T1)]$$  \hspace{1cm} (20)
where $k_{T2}$ = pseudo-first-order rate constant at the common temperature, sec$^{-1}$

$k_{T1}$ = pseudo-first-order rate constant at the reaction temperature, sec$^{-1}$

$E_a$ = apparent activation energy, cal/mole

$T_1$ = absolute reaction temperature, °K

$T_2$ = absolute common temperature, °K

A computer program which adjusts the rate constants according to Eq. (20) is available (Program 6, Appendix I). Temperatures were adjusted less than 2°C.

ALKALINE DEGRADATION OF 1,5-ANHYDRO-4-O-β-D-MANNOPYRANOSYL-D-MANNITOL

Analysis of Products

Alkaline degradation of 1,5-anhydromannobiitol produced two stable products, 1,5-anhydro-D-mannitol (70-85%) and 1,5-anhydro-D-iditol (0-8%). These compounds were identified by comparing their gas chromatographic retention times and gas chromatography/mass spectra to those of known compounds. A separate reaction showed that 1,5-anhydro-D-mannitol was stable under typical reaction conditions for over 28 half-lives of 1,5-anhydromannobiitol. Other 1,5-anhydro-D-alditols have been shown to be stable under similar conditions.$^{13,14}$ Since 1,5-anhydro-D-iditol is also an anhydroalditol, it is almost certainly stable under the reaction conditions as well. A 0-30% difference between the moles of 1,5-anhydromannobiitol degraded and moles of products formed from the aglycon was observed. This mole deficit is probably due to fragmentation of the aglycon into products undetectable with the gas chromatography procedures used, and will be referred to as the unidentified fragments.

Because large amounts (ca. 60%) of 1,6-anhydro-β-D-mannopyranose (levomannnosan) reportedly formed from the alkaline degradation of phenyl β-D-manno-pyranoside,$^{45}$ this product was searched for in the 1,5-anhydromannobiitol
system. None was found. Compounds analogous to levomannosan degrade under high
temperature alkaline conditions. If the rate constant for degradation of levo-
mannosan is larger than its rate constant for formation, no levomannosan would
accumulate in the system. Thus none would be detected even if it were forming.

The fraction of levomannosan formed during the degradation of 1,5-anhydro-
mannobiitol can be defined by Eq. (21).

\[ F = \frac{k_f}{k_r} \]  

where \( F \) = the fraction of 1,5-anhydromannobiitol which degrades to
levomannosan

\( k_f \) = rate constant for levomannosan formation

\( k_r \) = rate constant for 1,5-anhydromannobiitol degradation

Substituting Eq. (21) into (18) and assuming that the initial concentration of
levomannosan (\( L_o \)) equals zero gives:

\[ L = F * k_r * \frac{R_o}{(k_d - k_r)} \left[ \exp(-k_r t) - \exp(-k_d t) \right] \]  

Equation (22) can be used to calculate the concentration of levomannosan at any
time if the rate constants for both 1,5-anhydromannobiitol and levomannosan
degradation are known.

The rate constant for levomannosan degradation was therefore measured by
degradating levomannosan under one set of reaction conditions (160°C, 2.5M NaOH).
The measured rate constant was 5.6 \( \times 10^{-6} \) sec\(^{-1}\). Once the rate constant for the
degradation of levomannosan had been determined, the fraction of levomannosan
which could form without being detected in the gas chromatographic analysis
could be calculated using Eq. (22). Figure 17 shows the concentration of levo-
mannosan plotted as a function of time for different fractions of formation.
The minimum concentration of levomannosan detectable by gas chromatography was approximately 0.00002M. Therefore, if more than 1% of the 1,5-anhydromannobiitol had degraded to give levomannosan, levomannosan should have been found in the GC chromatograms.

Figure 17. Predicted concentrations of 1,6-anhydro-β-D-mannopyranose as a function of time at various fractions of formation from the degradation of 1,5-anhydromannobiitol in 2.5M NaOH at 160°C.
Points of Bond Cleavage

The glycosidic linkage in 1,5-anhydromannobiitol can cleave in two places; at either the glycosyl-oxygen or oxygen-aglycon bond. To correctly analyze the kinetic results, it is important to know what fraction of the cleavage occurs at each bond. The major stable product, 1,5-anhydro-D-mannitol, always forms when the glycosyl-oxygen bond cleaves. This same product sometimes forms from oxygen-aglycon bond cleavage as well. To calculate the fractions of glycosyl-oxygen and oxygen-aglycon cleavage, the 1,5-anhydro-D-mannitol produced from each cleavage must be identified. This was done by degrading the 1,5-anhydromannobiitol in liquors enriched with $^{18}O$. 1,5-Anhydro-D-mannitol formed by glycosyl-oxygen bond cleavage does not add any oxygen from the liquor; thus it will not become enriched in $^{18}O$. 1,5-Anhydro-D-mannitol formed by oxygen-aglycon bond cleavage, however, must pick up an oxygen atom from the liquor and therefore will become enriched with $^{18}O$. A mass spectrometer was used to measure the amount of enriched and unenriched 1,5-anhydro-D-mannitol produced from the degradation of 1,5-anhydromannobiitol. From these measurements, it is possible to calculate the fractions of oxygen-aglycon and glycosyl-oxygen bond cleavage. A method for doing this is outlined in Appendix V.

The percent of the total 1,5-anhydro-D-mannitol which formed from oxygen-aglycon bond cleavage is shown in Table 2. The $^{18}O$ incorporation studies also showed that 100% of the 1,5-anhydro-D-iditol results from cleavage of the oxygen-aglycon bond.

Rates of Bond Cleavage

Formation of 1,5-anhydro-D-mannitol from 1,5-anhydromannobiitol is analogous to the formation of 1,5-anhydro-D-glucitol from 1,5-anhydrocellobiitol. In the 1,5-anhydrocellobiitol system, it was assumed that all of the 1,5-anhydro-D-
glucitol resulted from glycosyl-oxygen bond cleavage.\textsuperscript{13,14} Therefore, the rate constant for formation of 1,5-anhydro-D-glucitol was used as the rate constant for glycosyl-oxygen cleavage. Degradation of 1,5-anhydrocellobiitol in \textsuperscript{18}O-enriched liquor showed that less than 1\% of the glucitol formed has been produced by oxygen-aglycon bond cleavage,\textsuperscript{46} hence this was a good assumption. During the degradation of 1,5-anhydromannobiitol, however, significant amounts of 1,5-anhydro-D-mannitol formed from oxygen-aglycon bond cleavage as well as glycosyl-oxygen bond cleavage. Therefore, the overall rate of 1,5-anhydro-D-mannitol formation (\(k_{\text{MNT}}\)) cannot be equated with the rate of glycosyl-oxygen bond cleavage (\(k_{\text{Go}}\)).

Table 2. Percentage of 1,5-anhydro-D-mannitol resulting from the cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol.

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>% MNT-OA\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>161.8</td>
<td>2.51</td>
<td>0.0</td>
<td>3.89</td>
</tr>
<tr>
<td>170.5</td>
<td>2.50</td>
<td>0.0</td>
<td>5.05</td>
</tr>
<tr>
<td>180.7</td>
<td>2.50</td>
<td>0.0</td>
<td>5.37</td>
</tr>
<tr>
<td>190.6</td>
<td>2.51</td>
<td>0.0</td>
<td>5.51</td>
</tr>
<tr>
<td>170.5</td>
<td>2.50</td>
<td>0.0</td>
<td>5.05</td>
</tr>
<tr>
<td>170.5</td>
<td>1.50</td>
<td>1.0</td>
<td>4.14</td>
</tr>
<tr>
<td>170.5</td>
<td>1.00</td>
<td>1.5</td>
<td>3.95</td>
</tr>
<tr>
<td>170.5</td>
<td>0.50</td>
<td>2.0</td>
<td>3.54</td>
</tr>
<tr>
<td>170.5</td>
<td>0.50</td>
<td>0.0</td>
<td>3.81</td>
</tr>
<tr>
<td>171.1</td>
<td>1.51</td>
<td>1.0 (NaSH)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Percentage of the total 1,5-anhydro-D-mannitol formed from cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol.

The rate constants for oxygen-aglycon and glycosyl-oxygen bond cleavage can be calculated, however, once both the overall rate constant for mannitol formation and the percentage of 1,5-anhydro-D-mannitol resulting from cleavage of the oxygen-aglycon bond are known. The mannitol formed by oxygen-aglycon bond
Cleavage and that formed by glycosyl-oxygen bond cleavage must equal the total mannitol formed, so:

\[ k_{MNTGO} = k_{MNT} - k_{MNTOA} \]  

(23)

where \( k_{MNTGO} \) = the rate constant for formation of 1,5-anhydro-D-mannitol via glycosyl-oxygen bond cleavage.

\( k_{MNTOA} \) = the rate constant for formation of 1,5-anhydro-D-mannitol via the oxygen-aglycon bond cleavage.

\( k_{MNT} \) = overall rate constant for 1,5-anhydro-D-mannitol formation.

Cleavage of the glycosyl-oxygen bond always produces mannitol. Therefore the rate constant for the formation of mannitol via glycosyl-oxygen cleavage must equal the rate constant for glycosyl-oxygen bond cleavage:

\[ k_{GO} = k_{MNTGO} \]  

(24)

Cleavage must occur at either the glycosyl-oxygen or oxygen-aglycon bonds. Therefore the overall rate constant for degradation must equal the sum of the rate constants for each bond:

\[ k_r = k_{GO} + k_{OA} \]  

(25a)

or

\[ k_{OA} = k_r - k_{GO} \]  

(25b)

Thus the rate constants for the cleavage of both the glycosyl-oxygen and oxygen-aglycon bonds can be calculated.

**Glycosyl-Oxygen Bond Cleavage**

**Products**

Cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol always produces 1,5-anhydro-D-mannitol, regardless of the mechanism of cleavage. Therefore, production of this product indicates nothing about the mechanisms of cleavage.
Effect of Temperature

Table 3 shows how the rate constant for glycosyl-oxygen bond cleavage varies with temperature. As expected, the rate of glycosyl-oxygen cleavage increased markedly with temperature.

Table 3. Effect of temperature on the glycosyl-oxygen bond cleavage of 1,5-anhydromannobitol in 2.5M NaOH.

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>10^6 k_GO, sec^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>162.2</td>
<td>1.91 ± 0.11</td>
</tr>
<tr>
<td>171.1</td>
<td>4.57 ± 0.13</td>
</tr>
<tr>
<td>181.3</td>
<td>10.9 ± 0.34</td>
</tr>
<tr>
<td>191.9</td>
<td>28.1 ± 0.21</td>
</tr>
</tbody>
</table>

Apparent thermodynamic functions of activation were calculated from this data as described previously. Table 4 shows the enthalpy and entropy values obtained.

Table 4. Apparent thermodynamic functions of activation for cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobitol.

<table>
<thead>
<tr>
<th></th>
<th>ΔH^‡, kcal/mole</th>
<th>ΔS^‡, cal/mole °K</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO bond</td>
<td>35.3 ± 2</td>
<td>-4.3 ± 0.9</td>
</tr>
<tr>
<td>Overall</td>
<td>36.4 ± 2</td>
<td>-1.4 ± 0.3</td>
</tr>
</tbody>
</table>

Apparent thermodynamic functions are relative, and therefore must be compared to the values obtained from compounds degrading via previously identified mechanisms to be meaningful. Table 5 lists the enthalpy and entropy values for some reference compounds.
Table 5. A comparison of the apparent thermodynamic functions of various reactions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mechanism</th>
<th>$\Delta H^\dagger$, kcal/mole</th>
<th>$\Delta S^\ddagger$, cal/mole °K</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO bond in mannobiitol$^a$</td>
<td>?</td>
<td>$35.3 \pm 2$</td>
<td>$-4.3 \pm 0.9$</td>
<td>--$^b$</td>
</tr>
<tr>
<td>Na methyl-(\alpha)-D-glucopyranosiduronate</td>
<td>$S_N^1$</td>
<td>40.0</td>
<td>+15.0</td>
<td>47</td>
</tr>
<tr>
<td>OA bond in 1,5-anhydrocellobitol$^c$</td>
<td>$S_N^1$</td>
<td>41.7</td>
<td>+6.9</td>
<td>13, 14</td>
</tr>
<tr>
<td>Levoglucosan</td>
<td>$S_N^1cB(2)$</td>
<td>32.8</td>
<td>-3.8</td>
<td>25, 26</td>
</tr>
<tr>
<td>GO$^d$ bond in 1,5-anhydro-2,3,6-tri-O-methylcellobiitol</td>
<td>$S_N^1cB(2)$</td>
<td>35.5</td>
<td>-3.2</td>
<td>31</td>
</tr>
<tr>
<td>Methyl (\alpha)-D-glucopyranoside</td>
<td>$S_N^2$</td>
<td>32.4</td>
<td>-13.6</td>
<td>25, 26</td>
</tr>
<tr>
<td>GO bond in 1,5-anhydrocellobiitol$^e$</td>
<td>Mixed $S_N^1$ &amp; $S_N^1cB(2')$</td>
<td>37.1</td>
<td>+1.0</td>
<td>13, 14</td>
</tr>
</tbody>
</table>

$^a$Glycosyl-oxygen bond in 1,5-anhydromannobiitol.
$^b$This work.
$^c$Oxygen-aglycon bond in 1,5-anhydrocellobiitol.
$^d$Glycosyl-oxygen bond.
$^e$Glycosyl-oxygen bond in 1,5-anhydrocellobiitol.

The enthalpy value for the glycosyl-oxygen bond in 1,5-anhydromannobiitol falls between that of the compounds thought to degrade via $S_N^1$ mechanisms and those thought to cleave by $S_N^2$ mechanisms. It is quite similar to the enthalpy value for the glycosyl-oxygen bond in 1,5-anhydro-2,3,6-tri-O-methyl-cellobiitol, which appears to degrade by an $S_N^1cB(2)$ mechanism.

The entropy for the glycosyl-oxygen bond in 1,5-anhydromannobiitol is similar to the values found for levoglucosan and 1,5-anhydro-2,3,6-tri-O-methylcellobiitol, which are both thought to degrade via pure $S_N^1cB(2)$ mechanisms.
The apparent thermodynamic functions of activation for the glycosyl-oxygen bond in 1,5-anhydromannobiitol therefore suggest that this bond is cleaved primarily by an $S_N^{1cB(1)}$-type mechanism.

**Effect of a Stronger Nucleophile**

In an $S_N^2$-type reaction, the nucleophile participates in the rate determining step. Therefore, the addition of a stronger nucleophile should accelerate an $S_N^2$ reaction. In this work, the nonnucleophilic salt sodium tosylate was replaced with strongly nucleophilic sodium hydrosulfide. Earlier studies have shown that the hydrosulfide ion is a stronger nucleophile than hydroxide ion even at $170^\circ C$.\textsuperscript{32,33} Table 6 shows the effect which addition of SH\textsuperscript{-} had on the cleavage of the glycosyl-oxygen bond.

<table>
<thead>
<tr>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>[NaSH]</th>
<th>$10^6k_{GO}$, sec\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.50</td>
<td>1.00</td>
<td>0.00</td>
<td>2.98 ± 0.06</td>
</tr>
<tr>
<td>1.50</td>
<td>0.00</td>
<td>1.00</td>
<td>3.07 ± 0.10</td>
</tr>
</tbody>
</table>

There is statistically no difference between the rate constants for glycosyl-oxygen cleavage when either sodium tosylate or sodium hydrosulfide is present. This indicates that an $S_N^2$-type mechanism does not govern the glycosyl-oxygen bond cleavage. The reaction site is probably too hindered for the nucleophile to attack and cause $S_N^2$-type cleavage. The possibility that hydroxide ion causes $S_N^2$-type cleavage of the glycosyl-oxygen bond but that hydrosulfide ion is unable to do so because it is larger appears unlikely, since the size difference between these two ions is slight (1.5 vs. 1.85 Å, respectively).\textsuperscript{48,49} However, solvation of the anions plays an unknown role.
Effect of Ionic Strength

The ionic strength of the reaction medium was varied by adding differing amounts of the nonnucleophilic salt, sodium tosylate, to liquors having the same hydroxide concentration. Table 7 illustrates the effect which this had on the rate constant for glycosyl-oxygen bond cleavage.

Table 7. The effect of varying the ionic strength at constant hydroxide concentration on the cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol at 170°C.

<table>
<thead>
<tr>
<th>I (μ)</th>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>(10^6k_{Go}), sec(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.50</td>
<td>0.00</td>
<td>1.61 ± 0.05</td>
</tr>
<tr>
<td>2.50</td>
<td>0.50</td>
<td>2.00</td>
<td>1.79 ± 0.02</td>
</tr>
</tbody>
</table>

A fivefold increase in the ionic strength of the reaction medium caused an 11% increase in the rate of glycosyl-oxygen bond cleavage. The oxygen-agnostic bond in 1,5-anhydrocellobiitol, which is thought to cleave via an \(S_N^1\) mechanism, exhibited a 64% rate increase when the ionic strength was increased five times.\(^{13,14}\) Levoglucosan, which degrades via an \(S_N^{1\text{cB}(2)}\) mechanism, had a negative 19% salt effect.\(^{25,26}\) Therefore, the 11% rate increase observed for the glycosyl-oxygen bond cleavage in 1,5-anhydromannobiitol may indicate a mixed \(S_N^{1\text{cB}(i)}\) and \(S_N^1\) mechanism. Because \(S_N^1\) reactions have strong positive salt effects while \(S_N^{1\text{cB}(i)}\) mechanisms exhibit weak negative salt effects, it is possible that a very minor \(S_N^1\) component to the reaction could produce the small positive salt effect observed here.

Effect of Hydroxide Ion Concentration

A series of degradations at different hydroxide concentrations, in which the ionic strength was maintained at a constant level by adding appropriate amounts of sodium tosylate, were performed. Table 8 shows how these changes in
hydroxide concentration affected the rate constants for glycosyl-oxygen bond cleavage. Increasing the hydroxide ion concentration increases the rate of glycosyl-oxygen bond cleavage.

Table 8. Effect of varying hydroxide concentration at constant ionic strength on glycosyl-oxygen bond cleavage in 1,5-anhydromannobitol at 170°C.

<table>
<thead>
<tr>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>$10^6k_{GO}$, sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>0.00</td>
<td>4.10 ± 0.13</td>
</tr>
<tr>
<td>1.50</td>
<td>1.00</td>
<td>2.98 ± 0.06</td>
</tr>
<tr>
<td>1.00</td>
<td>1.50</td>
<td>2.52 ± 0.10</td>
</tr>
<tr>
<td>0.50</td>
<td>2.00</td>
<td>1.79 ± 0.02</td>
</tr>
</tbody>
</table>

Lai showed that a compound which degrades via an $S_N^1cB(2)$-type mechanism should show a linear reciprocal dependence on hydroxide concentration.$^{50}$ However, $S_N^1$ and $S_N^2$ mechanisms also exhibit linear reciprocal relationships when certain assumptions are made.$^{24-26}$ Figure 18 shows the reciprocal plot for the rate constants of glycosyl-oxygen cleavage vs. hydroxide concentration. The graph is linear.

The rate constants for the glycosyl-oxygen bond in 1,5-anhydrocellobiitol, which is thought to degrade via a mixed $S_N^1cB(2)$ and $S_N^1$ mechanism, are also linear when plotted in this manner, as shown in Fig. 18. This suggests that mixed mechanisms may also exhibit linear reciprocal behavior. Appendix VI gives the derivation of the hydroxide dependence equation for a mixed $S_N^1cB(1)$ and $S_N^1$ cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobitol. The assumed reaction scheme is shown at the top of page 48.
Figure 18. Relationship between reciprocal hydroxide concentration and rate constants for the cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobitol at 170°C.
The resulting equation is:

$$k_{GO} = \frac{k_1 K_e [OH^-] + k_2 K_{e1} [OH^-] + k_3}{1 + K_{e1} [OH^-] + K_{e2} [OH^-]}$$

(26)

where $k_{GO}$ = pseudo-first-order rate constant for cleavage of the glycosyl-oxygen bond.

$k_1$ = rate constant for cleavage of the glycosyl-oxygen bond in ionized MBT via an $S_N^1$ mechanism.

$k_2$ = rate constant for cleavage of the glycosyl-oxygen bond in ionized MBT via an $S_N^{1cB(i)}$ mechanism.

$k_3$ = rate constant for cleavage of the glycosyl-oxygen bond in neutral MBT via an $S_N^1$ mechanism.

$K_{e1}$ = equilibrium constant for ionization of the glycon.

$K_{e2}$ = equilibrium constant for ionization of the aglycon.

$[OH^-]$ = hydroxide ion concentration.

If it is assumed that ionization of the glycon is important in stabilizing the carbocation formed during $S_N^1$ cleavage, then $k_3$ should be much smaller than $k_1$ and $k_2$, and Eq. (26) reduces to:

$$k_{GO} = \frac{(k_1 = k_2) K_{e1} [OH^-]}{1 + (K_{e1} + K_{e2}) [OH^-]}$$

(27)

The inverse of Eq. (27) is:

$$\frac{1}{k_{GO}} = \frac{1}{K_{e1} (k_1 + k_2)} \cdot \frac{1}{[OH^-]} + \frac{(K_{e1} + K_{e2})}{(k_1 + k_2)}$$

(28)

Equation (28) is linear with respect to hydroxide concentration. Therefore, the observed linear plot of $1/k_{GO}$ as a function of $1/[OH^-]$ could be consistent with either a mixed $S_N^1/S_N^{1cB(i)}$ mechanism or a pure $S_N^{1cB(i)}$ mechanism. The fact that a linear relationship was found does not prove that either of these are the "correct" mechanism, since many different mechanisms may yield a linear reciprocal plot.
Effect of Methylating the Hydroxyl Groups

Neighboring group type mechanisms require that the hydroxyl group which acts as the conjugate base be able to ionize. Methylating a hydroxyl group prevents ionization and therefore stops neighboring group cleavage. To further test the importance of hydroxyl group ionization to glycosyl-oxygen bond cleavage in 1,5-anhydromannobiitol, the hydroxyl groups were fully methylated. The model compound was completely instead of selectively methylated for experimental reasons. Selective methylation is a multistep, low-yield process and only a small amount of 1,5-anhydromannobiitol was available for methylation.

Cleavage of the glycosyl-oxygen bond in methylated 1,5-anhydromannobiitol should yield 1,5-anhydro-2,3,6-tri-O-methyl-D-mannitol. This product was not found, suggesting that cleavage of the glycosyl-oxygen bond had stopped entirely. This result is indicative of a neighboring group mechanism. If ionization of the hydroxyl groups is important in stabilizing the cation formed during $S_N^1$ cleavage of the glycosyl-oxygen bond, methylation could also slow or halt this type of reaction as well. However, the oxygen-aglycon bond in 1,5-anhydrocellobiitol is thought to degrade via an $S_N^1$ mechanism and its rate of cleavage was unaffected when the aglycon was methylated.$^{31}$

Summary

Six different mechanistic probes were used to elucidate the mechanism of glycosyl-oxygen bond cleavage in 1,5-anhydromannobiitol. The products found were consistent with all of the proposed mechanisms. The apparent thermodynamic functions of activation were typical for $S_N^1cB(1)$ mechanisms. The lack of a rate increase upon the addition of a stronger nucleophile is not consistent with an $S_N^2$ mechanism. The small positive salt effect was intermediate between that expected for either a pure $S_N^1$ or $S_N^1cB(1)$ mechanism. The reaction rate
constants had a strong positive dependence on hydroxide concentration, which indicates a major $S_N^{1}cB(i)$ component to the mechanism. The plot of reciprocal rate vs. reciprocal hydroxide concentration was linear. This may be consistent with either a pure $S_N^{1}cB(i)$ or mixed $S_N^{1}cB(i)/S_N^{1}$ mechanism, among others. Finally, the complete stoppage of the reaction when the hydroxyl groups were methylated is indicative of an $S_N^{1}cB(i)$ mechanism. Thus it appears that the mechanism of cleavage for the glycosyl-oxygen bond in 1,5-anhydromannobiitol is primarily $S_N^{1}cB(i)$ in nature. The small positive salt effect may indicate that the mechanism also has a minor $S_N^{1}$ component.

The structure of 1,5-anhydromannobiitol prohibits backside neighboring group displacement of the aglycon by the 2, 3, and 6 hydroxyl groups. The hydroxyl at C-4 could conceivably displace the aglycon [an $S_N^{1}cB(4)$ mechanism], but, as discussed previously, this is a very unlikely mechanism. The only other probable neighboring group mechanism is the $S_N^{1}cB(2)$-ro one described earlier. This therefore is probably the major mechanism governing cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol.

**Comparison to the Glycosyl-Oxygen Bond Cleavage in 1,5-Anhydro-4-O-β-Glucopyranosyl-D-Glucitol**

If the glycosyl-oxygen bond in 1,5-anhydromannobiitol cleaves via the $S_N^{1}cB(2')$-ro mechanism, it is interesting to consider whether this same mechanism can occur in 1,5-anhydrocellobiitol. Figure 19 shows that it is indeed possible. Whereas the traditional $S_N^{1}cB(2)$ mechanism requires that 1,5-anhydrocellobiitol flip into its least stable chair conformation, the $S_N^{1}cB(2)$-ro mechanism could take place when the molecule is in its most stable conformation. The $S_N^{1}cB(2)$-ro mechanism should therefore be even more energetically favored than the $S_N^{1}cB(2)$ mechanism for 1,5-anhydrocellobiitol.
Figure 19. Possibility of the $S_{N1}^{1}cB(2)$-ro mechanism in 1,5-anhydrocellobiitol.

Some of the discrepancies observed in Brandon's study of 1,5-anhydrocellobiitol would be resolved if the $S_{N1}^{1}cB(2)$-ro mechanism operates for that compound. As discussed earlier, cleavage of the glycosyl-oxygen bond in 1,5-anhydrocellobiitol had the characteristics of a neighboring group mechanism, but the expected product of $S_{N1}^{1}cB(2)$ cleavage, levoglucosan, was formed only in low yield. Since the $S_{N1}^{1}cB(2)$-ro mechanism would not produce levoglucosan but would otherwise behave like any neighboring group mechanism, it is quite
possible that some cleavage of the glycosyl-oxygen bond via the $S_N^{1}cB(2)$-ro
mechanism accounts for the lack of levoglucosan formation in 1,5-anhydrocello-
biitol degradation.

**OXYGEN-AGLYCON BOND CLEAVAGE**

**Products**

Cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol produced 1,5-
anhydro-D-mannitol (0-22%), 1,5-anhydro-D-iditol (0-40%), and unidentified
fragments (40-100%), as shown in Table 9. If $S_N^{2}$ or $S_N^{1}cB(6)$ mechanisms
governed the reaction, 1,5-anhydro-D-talitol should have been produced, as
discussed earlier. This compound was not found. In general, a 1:2 ratio of
1,5-anhydro-D-mannitol and 1,5-anhydro-D-iditol formed. The opening of a
3,4-anhydro-talopyranosyl intermediate under alkaline conditions gives mannose
and idose derivatives in a 1:2 ratio. A 1,5:3,4-dianhydro-D-talitol inter-
mediate could form from either $S_N^{1}$ or $S_N^{1}cB(3)$ cleavage of the oxygen-aglycon
bond, as shown in Fig. 8 and 10. Therefore, either of these two mechanisms is
consistent with the observed products.

**Effect of Temperature**

Table 10 shows how the rate of oxygen-aglycon bond cleavage varied with
temperature. The rates rise as temperature is increased.

The temperature-rate data were used to calculate apparent thermodynamic
functions of activation. These values are listed in Table 11.

The 95% confidence intervals for $\Delta H^\ddagger$ and $\Delta S^\ddagger$ are large because the values
of $k_{OA}$ are small, and because so many mathematical manipulations of the data are
necessary to compute these rate constants. Comparison of the $\Delta H^\ddagger$ and $\Delta S^\ddagger$ values
for the oxygen-aglycon bond in 1,5-anhydromannobiitol to those values for some compounds with previously identified degradation mechanisms (Table 5) shows that the enthalpy value is most similar to that for an $S_N^1$ mechanism while the entropy is part way between typical $S_N^1$ and $S_N^1cB(1)$ mechanisms. Because of the large errors associated with the thermodynamic functions, however, they are not definitive.

Table 9. Rates of glycosyl-oxygen and oxygen-aglycon bond cleavage in 1,5-anhydromannobiitol.

<table>
<thead>
<tr>
<th>Temp., °C</th>
<th>NaOH, M</th>
<th>NaOTs, a</th>
<th>NaSH, b</th>
<th>$10^6k_{OA}$, sec$^{-1}$</th>
<th>$X_{1,oo}$ c</th>
<th>MNTOA d</th>
<th>X4 e</th>
<th>U r f</th>
</tr>
</thead>
<tbody>
<tr>
<td>162.2</td>
<td>2.5</td>
<td>--</td>
<td>--</td>
<td>0.51</td>
<td>0.15</td>
<td>0.29</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>171.1</td>
<td>2.5</td>
<td>--</td>
<td>--</td>
<td>1.48</td>
<td>0.16</td>
<td>0.27</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>181.3</td>
<td>2.5</td>
<td>--</td>
<td>--</td>
<td>4.30</td>
<td>0.14</td>
<td>0.21</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>191.9</td>
<td>2.5</td>
<td>--</td>
<td>--</td>
<td>10.12</td>
<td>0.16</td>
<td>0.30</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>170.0 g</td>
<td>2.5</td>
<td>1.0</td>
<td>--</td>
<td>1.32</td>
<td>0.16</td>
<td>0.27</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>170.0 g</td>
<td>1.0</td>
<td>1.5</td>
<td>--</td>
<td>1.03</td>
<td>0.12</td>
<td>0.21</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>170.0 g</td>
<td>0.5</td>
<td>2.0</td>
<td>--</td>
<td>0.85</td>
<td>0.13</td>
<td>0.19</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>170.0 g</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
<td>0.51</td>
<td>0.13</td>
<td>0.22</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>170.0 g</td>
<td>1.5</td>
<td>--</td>
<td>1.0</td>
<td>0.28</td>
<td>0.22</td>
<td>0.38</td>
<td>0.40</td>
<td></td>
</tr>
</tbody>
</table>

a Sodium toluenesulfonate.
b Sodium hydrosulfide, added as Na$_2$S.
c Product fractions at infinity, $k_i/k_{OA}$.
d 1,5-Anhydro-D-mannitol resulting from oxygen-aglycon bond cleavage.
e 1,5-Anhydro-D-iditol.
f Unidentified fragments from the aglycon.
g Rate constants adjusted to 170.0°C.

Table 10. Effect of temperature on the rate of oxygen-aglycon bond cleavage for 1,5-anhydromannobiitol in 2.5M NaOH.

<table>
<thead>
<tr>
<th>Temperature, °C</th>
<th>$10^6k_{OA}$, sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>162.2</td>
<td>0.51 ± 0.22</td>
</tr>
<tr>
<td>171.1</td>
<td>1.48 ± 0.16</td>
</tr>
<tr>
<td>181.3</td>
<td>4.30 ± 0.55</td>
</tr>
<tr>
<td>191.9</td>
<td>10.1 ± 0.34</td>
</tr>
</tbody>
</table>
Table 11. Thermodynamic functions of activation for the oxygen-aglycon bond in 1,5-anhydromannobiitol.

<table>
<thead>
<tr>
<th>k_{OA}</th>
<th>ΔH^#, kcal/mole</th>
<th>ΔS^#, cal/mole °K</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{OA}</td>
<td>39.7 ± 8</td>
<td>+ 3.28 ± 2</td>
</tr>
<tr>
<td>overall</td>
<td>36.4 ± 2</td>
<td>- 1.37 ± 0.3</td>
</tr>
</tbody>
</table>

**Effect of a Stronger Nucleophile**

The effect of replacing the nonnucleophilic salt, sodium tosylate (NaOTs) with an equimolar amount of hydrosulfide ion (SH⁻) is shown in Table 12. Although the addition of sodium hydrosulfide appeared to cause a 17% rate increase, the difference between the two rates is not statistically significant.

Table 12. The effect of addition of a stronger nucleophile on the cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol at 170°C.

<table>
<thead>
<tr>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>[NaSH]</th>
<th>10^6k_{OA}, sec⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>1.00</td>
<td>0.00</td>
<td>1.03 ± 0.11</td>
</tr>
<tr>
<td>2.50</td>
<td>0.00</td>
<td>1.00</td>
<td>1.21 ± 0.18</td>
</tr>
</tbody>
</table>

When smaller amounts of SH⁻ (0.2-0.6M) were added to a methyl α-D-gluco-pyranoside reaction, which is known to proceed via an S_N^2 pathway, rate increases of 60-992% were observed. Therefore, even if the 17% rate increase is real, it probably does not indicate any significant S_N^2 pathway in this reaction. A small rate increase might be due to the fact that sodium hydrosulfide is a stronger base than sodium tosylate and therefore would be better at ionizing the hydroxyl groups. It will be shown later that ionization of the hydroxyl groups promotes oxygen-aglycon bond cleavage.

The product distribution also shifts when SH⁻ is added to the system (refer to Table 9). Cleavage of the oxygen-aglycon bond no longer results in formation
of either 1,5-anhydro-D-mannitol or 1,5-anhydro-D-iditol. This suggests that \( \text{SH}^- \) interferes with the product-forming steps. In both the \( S_N^1 \) and \( S_N^1 cB(3) \) mechanisms, 1,5:3,4-dianhydro-D-talitol is the postulated intermediate (see Fig. 8 and 10). The \( \text{SH}^- \) nucleophile may compete very effectively with \( \text{OH}^- \) in opening this 3,4 epoxide ring. Attack by \( \text{SH}^- \) would yield thio-sugars and disulfides instead of anhydro-alditols.

**Effect of Ionic Strength**

The effect of changing the ionic strength of the medium while maintaining the hydroxide concentration is shown in Table 13. A fivefold increase in ionic strength caused an 82% increase in the rate of oxygen-aglycon bond cleavage. Because of the confidence intervals associated with these rates, the effect of the change in ionic strength could actually be anywhere between +25 and +144%. Since \( S_N^1 \)-type reactions typically have positive salt effects of +60-70%, this reaction appears to have significant \( S_N^1 \) character.

<table>
<thead>
<tr>
<th>( I (\mu) )</th>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>( 10^6 k_{OA}, \text{ sec}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.50</td>
<td>0.00</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td>2.50</td>
<td>0.50</td>
<td>2.00</td>
<td>0.51 ± 0.06</td>
</tr>
</tbody>
</table>

**Effect of Hydroxide Ion Concentration**

Table 14 shows that as the hydroxide concentration was increased while holding the ionic strength steady, the rate of oxygen-aglycon bond cleavage also increased sharply.
Table 14. Effect of varying the hydroxide concentration at constant ionic strength on the oxygen-aglycon bond cleavage in 1,5-anhydromannobiitol at 170°C.

<table>
<thead>
<tr>
<th>[NaOH]</th>
<th>[NaOTs]</th>
<th>$10^6 k_{OA}$, sec$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>2.00</td>
<td>0.51 ± 0.06</td>
</tr>
<tr>
<td>1.00</td>
<td>1.50</td>
<td>0.85 ± 0.12</td>
</tr>
<tr>
<td>1.50</td>
<td>1.00</td>
<td>1.03 ± 0.11</td>
</tr>
<tr>
<td>2.50</td>
<td>0.00</td>
<td>1.32 ± 0.16</td>
</tr>
</tbody>
</table>

Figure 20 is a plot of reciprocal rate vs. reciprocal hydroxide concentration. The rates for the cleavage of the oxygen-aglycon bond in 1,5-anhydromannobiitol are linear when plotted in this manner. For comparison, the rates of oxygen-aglycon cleavage in 1,5-anhydrocellobiitol are also plotted in Fig. 18. They follow a very different trend, decreasing somewhat in a non-linear manner as the hydroxide concentration increases.

The strong linear relationship observed for the oxygen-aglycon cleavage rates in 1,5-anhydromannobiitol is indicative of a neighboring group mechanism. As discussed for glycosyl-oxygen bond cleavage, either a pure $S_N^1cB(i)$ or mixed $S_N^1/S_N^1cB(i)$ mechanism could give the observed linear relationship. The fact that oxygen-aglycon bond cleavage depends on the hydroxide concentration more strongly than the glycosyl-oxygen cleavage rates do may reflect the difficulty of ionizing the appropriate hydroxyl group rather than the extent to which an $S_N^1cB(i)$ mechanism is occurring.
Effect of Methylating the Hydroxyl Groups

When 1,5-anhydromannobiitol was fully methylated, the rate of oxygen-aglycon bond cleavage was reduced by a factor of 17, as illustrated in Table 15. This suggests that cleavage of the oxygen-aglycon bond occurs partially via a neighboring group mechanism. The fact that the cleavage does not stop entirely indicates that some other mechanism, probably an $S_N^1$ type, is also operative.
Table 15. The effect of fully methylating 1,5-anhydromannobiitol on the rate of oxygen-aglycon bond cleavage in 2.5M NaOH at 170°C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(10^6k_{OA}, \sec^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,5-anhydromannobiitol</td>
<td>1.48</td>
</tr>
<tr>
<td>1,5-anhydro-2,3,6,2-,3-,4-,6-septa-O-methyl mannobiitol</td>
<td>0.086</td>
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</table>

**Summary**

The six probes used to investigate the mechanism of oxygen-aglycon bond cleavage yielded a variety of information. Lack of 1,5-anhydro-D-talitol formation ruled out the \(\text{SN}_2\) and \(\text{SN}^1\text{cB}(6)\) mechanisms. Based on the products found, either an \(\text{SN}_1\) or \(\text{SN}^1\text{cB}(3)\) mechanism is possible. The enthalpy of activation is characteristic of an \(\text{SN}_1\) mechanism while the entropy indicates a mixed \(\text{SN}_1/\text{SN}^1\text{cB}(i)\) mechanism. These thermodynamic functions are not conclusive, however, due to the large confidence intervals associated with them. The small rate increase observed when a stronger nucleophile than hydroxide, SH\(^-\), was added is inconsistent with a major \(\text{SN}_2\) component. A fivefold increase in ionic strength caused an 80% rise in the rate of oxygen-aglycon bond cleavage, indicating that the mechanism has significant \(\text{SN}_1\) character. The rate of oxygen-aglycon bond cleavage showed a strong positive dependence on hydroxide concentration, which is typical of an \(\text{SN}^1\text{cB}(i)\) mechanism. A plot of reciprocal rate vs. reciprocal hydroxide concentration was linear. Finally, the methylated analog of 1,5-anhydromannobiitol degraded 17 times more slowly than the free sugar. This indicates that a significant fraction of the oxygen-aglycon bond is cleaved via a neighboring group mechanism. Thus there is evidence that both \(\text{SN}_1\) and \(\text{SN}^1\text{cB}(i)\) mechanisms operate in the cleavage of the oxygen-aglycon bond.

As discussed earlier, theoretical considerations and the products found make the \(\text{SN}^1\text{cB}(3)\) mechanism the most likely neighboring group mechanism for this
reaction. Therefore, the oxygen-aglycon bond in 1,5-anhydromannobitol probably cleaves via a mixed $S_N^{1\text{CB}(3)}/S_N^1$ mechanism.

**Comparison to the Oxygen-Aglycon Bond Cleavage in 1,5-Anhydro-4-O-\beta-Glucopyranosyl-D-Glucitol**

The oxygen-aglycon bond in 1,5-anhydromannobitol cleaved approximately twice as fast as the same bond in 1,5-anhydrocellobiitol. The cleavage of the oxygen-aglycon bond in 1,5-anhydrocellobiitol had all the characteristics of an $S_N^1$-type mechanism, while the same bond in 1,5-anhydromannobitol appears to cleave via a mixed $S_N^1/S_N^{1\text{CB}(3)}$ mechanism. This difference in mechanisms was unexpected, since the immediate environment around the oxygen-aglycon bond is quite similar in both compounds.

The $S_N^{1\text{CB}(3)}$ mechanism requires that the OH(3) group and oxygen-aglycon bond at C-4 be trans-diaxial. To achieve this configuration, the aglycon must assume either the alternate chair ($1C_4$) or a quasi-boat conformation. Figure 21 shows both 1,5-anhydrocellobiitol and 1,5-anhydromannobitol in these two conformations. In the $1C_4$ chair conformation, 1,5-anhydrocellobiitol has all its groups in axial positions. 1,5-Anhydromannobitol, on the other hand, has the OH(2) group equatorial. Free-energy calculations show that,\textsuperscript{56} because of this difference, 1,5-anhydromannobitol is about twice as stable as 1,5-anhydrocellobiitol in the $1C_4$ conformation at 25°C. In the quasi-boat conformation, 1,5-anhydrocellobiitol has axial OH(3) and OH(2) groups, whereas manobitol has only an axial OH(3); the OH(2) group is equatorial. Thus the manobitol should also be more stable than cellobiitol in the quasi-boat conformation. Greater stability of the conformation with the trans-diaxial-3,4 configuration should promote an $S_N^{1\text{CB}(3)}$ reaction. This conformational difference is therefore a possible explanation as to why the $S_N^{1\text{CB}(3)}$ mechanism occurs for manobitol but not cellobiitol.
Figure 21. Comparison of 1,5-anhydromannobiitol and 1,5-anhydrocellobiitol in conformations in which OH-3 and the glycosidic bond are trans-diaxial.
CONCLUSIONS

It is proposed that the glycosyl-oxygen bond in 1,5-anhydromannobiitol is cleaved primarily by an $S_N^{1}cB(2')$-ro mechanism. An $S_N^{1}$-type reaction may also be occurring to a small extent. The oxygen-aglycon bond appears to degrade by a mixed $S_N^{1}cB(3)/S_N^{1}$ mechanism.

This mechanism for oxygen-aglycon bond cleavage is quite different from that found for the oxygen-aglycon bond of 1,5-anhydrocellobiitol. Since 1,5-anhydromannobiitol and 1,5-anhydrocellobiitol differ only in the configuration of their OH(2) groups, it is clear that a small change in the conformation of a molecule can drastically affect its reaction mechanisms.

The $S_N^{1}cB(2)$-ro mechanism proposed for the glycosyl-oxygen bond of 1,5-anhydromannobiitol is also a viable route for cleavage of the glycosyl-oxygen bond in 1,5-anhydrocellobiitol. In cellobiitol it can occur without a change in conformation. It is conceivable that the $S_N^{1}cB(2)$-ro mechanism could also occur in the alkaline degradation of cellulose.
EXPERIMENTAL

GENERAL ANALYTICAL METHODS

Melting points were measured on a calibrated Thomas-Hoover capillary apparatus. Optical rotations were determined on a Perkin-Elmer 141-MC polarimeter. Thin layer chromatography (tlc) was done on silica gel D-5 (Camag) coated microscope slides using a sulfuric acid-methanol (1:4, v:v) spray followed by charring to detect components. Elemental analyses were performed by Huffman Laboratories (Wheat Ridge, Colorado).

The 1H- and 13C-NMR spectra were recorded on a Jeol FX-100 spectrometer. Samples were dissolved in deuterated solvents with tetramethylsilane added as an internal standard.

Gas chromatography was done on a Perkin-Elmer Sigma 2 chromatograph equipped with a flame-ionization detector and linked to a Perkin-Elmer Sigma 10 data station. Analyses were performed on a column (stainless steel, 6 ft x 0.125 inch) packed with OV-101 (3%) on Supelcoport (80-100 mesh), rigged for on-column injection. Nitrogen (30 mL/min) was used as the carrier gas. The following operating conditions were used: (A) Injector, 275°C; detector, 300°C; and column, 130°C for 1 min, 5°/min to 275°C, and held at 275°C. (B) Injector, 275°C; detector, 300°C; and column, 130°C for 1 min, 5°/min to 260°C, and held at 260°C. (C) Injector, 275°C; detector, 300°C; and column, 100°C for 1 min, 5°/min to 250°C, and held at 250°C for 1 min.

Fehling's tests were run by placing a few grains or drops of the material to be tested in a test tube, adding 2 drops of solution A (0.277M cupric sulfate pentahydrate) and 2 drops of solution B (2.5M NaOH and 1.23M potassium sodium...
tartrate) or enough to give a dark blue solution. The mixture was then heated with steam for a few minutes. The formation of a red precipitate indicated the presence of a reducing sugar.57

The ferric hydroxamate test for esters was performed by placing several milligrams of sample in a test tube, and adding 1 drop of hydroxylamine in ethanol (0.5M) and 2 drops of methanolic KOH (2M). The mixture was heated to boiling, cooled under tap water, then mixed with several drops of dilute HCl (2N) and one drop of aqueous ferric chloride solution (10%). The formation of a dark red or magenta color indicated the presence of an ester or lactone.57

High Pressure Liquid Chromatography (HPLC) was performed with a Varian 8500 pump using degassed, distilled water at 0.6 mL/min. A Bio-Rad HPX-87 carbohydrate column (300 x 7.8 mm ID) was maintained at 82°C and protected with a Bio-Rad carbohydrate Micro-Guard column. A Valco six-way valve fitted with a 50 μL loop was used to inject samples. Distilled water was used as the trapped reference in a Waters R401 differential refractometer maintained at 30°C. Chromatograms were recorded on a Varian A-25 recorder equipped with a disk integrator.

Gas chromatography/mass spectrometry was performed on a Hewlett-Packard 5985 system. The 18O-enriched liquors were analyzed using a Porapak-Q column, 100°C isothermal conditions, and electron impact mass spectrometry. Analysis of the 18O-content of 1,5-anhydro-D-alditols utilized an OV-225 column and negative chemical ionization mass spectrometry. The operating conditions were: 1 min at 130°C, 4°/min to 200°C. Selective ion monitoring was used to collect data in both cases.
SOLVENTS, REAGENTS, AND CATALYSTS

**Anhydrous Methanol**

Magnesium turnings (20 g) were covered with reagent grade methanol (300 mL). Iodine (0.05 g) was added slowly until a vigorous reaction occurred. After this subsided, additional methanol (1.5 L) was added. The mixture was refluxed for 3 hours, then fractionally distilled. The first 100 mL of distillate were discarded, and the next 1600 mL were collected and stored in tightly-sealed bottles.

**Anhydrous Ethanol**

Absolute ethanol was reacted with magnesium and iodine as described for methanol, but the mixture was heated to initiate the reaction. The dry ethanol was fractionally distilled and stored in dark, tightly-sealed bottles.

**Anhydrous Pyridine**

Pyridine (1500 mL) was refluxed over KOH pellets (250 g) for 3 hours, and then fractionally distilled.

**Dimethylformamide**

Dimethylformamide was dried by refluxing with benzene. The benzene was then distilled off and the dimethylformamide was purified of decomposition products by distilling under vacuum.

**Dimethyl Sulfoxide**

Anhydrous dimethyl sulfoxide was prepared by stirring dimethyl sulfoxide (1500 mL) over powdered calcium hydroxide (30 g) for 2 hours, followed by fractional distillation at ca. 10 mm Hg and 80-90°C.
Acetic Anhydride

Acetic anhydride (reagent grade, 1500 L) was fractionally distilled. The first 200 mL were discarded and the next 1000 mL collected.

Methyl Iodide

Methyl iodide was purified by fractional distillation. It was stored over clean copper wire in a dark bottle.

Sodium Methoxide (1N)

Sodium methoxide (1N) was prepared by dissolving sodium metal (5.8 g) in anhydrous methanol (250 mL). Pea-sized sodium chunks were weighed in mineral oil, washed once with toluene, and twice with methanol, then carefully dropped into the anhydrous methanol.

Triphenylmethyl Chloride

Triphenylmethyl chloride (98%, Aldrich) was crystallized from benzene/acetyl chloride, then recrystallized from benzene/petroleum ether.

p-Toluenesulfonyl Chloride

Crude p-toluenesulfonyl chloride (100 g) was dissolved in a minimum amount of CHCl₃ (200 mL), diluted with low boiling petroleum ether (1250 mL), and decolorized with carbon. The mixture was placed in loosely covered beakers and warmed gently while stirring. When the volume had been reduced 95%, the solution was cooled and crystals formed. These were filtered, washed with petroleum ether, and dried in vacuo. The total yield was 84.62 g (84.6%): m.p. 65.7-70.0°C. Literature: m.p. 67.5-68.5°C.

Silver Nitrate Solution (3%)

Silver nitrate (3 g) was dissolved in water (5 mL), then diluted with acetone (95 mL).
Hydrogen Bromide in Glacial Acetic Acid

Solutions of hydrogen bromide in acetic acid were made by bubbling hydrogen bromide gas through a known weight of glacial acetic acid in tared 1 L bottles. After the first 30 minutes the bottles were cooled in ice. The amount of hydrogen bromide absorbed was monitored gravimetrically, and addition of gas was stopped when the concentration reached 32-40%.\textsuperscript{32,33} The bottles were sealed, wrapped with aluminum foil, and refrigerated.

Raney Nickel (Type W-5)

Raney nickel alloy (400 g) was added slowly to a stirred, cooled sodium hydroxide solution (6.4M, 2080 mL) such that the temperature remained between 45 and 55°C. The slurry was digested at 50°C for 30-60 minutes, decanted, and extracted continuously until the effluent water was neutral. It was then solvent-exchanged into tetrahydrofuran:ethanol (1:1, v:v) by decantation (3 x 200 mL) and centrifugation and decantation (3 x 200 mL).\textsuperscript{63}

SYNTHESIS OF COMPOUNDS

Mannan A

Coarsely ground ivory nuts (\textit{Phytelephas macrocarpa}), obtained from Pfanstiehl, were ground in a Wiley mill using a No. 5 screen. The final consistency was similar to that of beach sand. Ground ivory nuts (165 g) were extracted with chloroform:methanol (1:1, 2000 mL) for twelve hours in a Soxhlet extraction apparatus, then 100 g of the extracted nuts were added to a warm (30°C) solution of sodium chlorite (250 g) in water (2500 mL). After heating to 35°C, glacial acetic acid (250 mL) was added and the mixture was placed in a 30°C water bath for 24 hours. The bleached nuts were filtered, washed with water and methanol, and dried \textit{in vacuo} at 40°C to yield 90 g (90%) of a white solid.
Bleached ivory nuts (75 g) were placed in an NaOH solution (6%, 2.82 L) and left at room temperature for 3 days, with occasional shaking. The mixture was filtered and the collected solids (Mannan B) were washed with water and methanol, then dried. The filtrate was neutralized with glacial acetic acid (300 mL) and Mannan A was precipitated by adding methanol (3 L). The precipitate was collected by filtration, washed with hydrochloric acid (1N, 100 mL), water (5 x 500 mL), and methanol (2 x 500 mL), and dried under vacuum at 40°C. This yielded 15-25 g Mannan A and 35-50 g Mannan B.

Mannobiose

Mannan A (55 g) was mixed with acetic anhydride (330 mL) and cooled to 0°C. First glacial acetic acid (330 mL), then concentrated sulfuric acid (33 mL) were added dropwise. The mixture was stirred at room temperature and the amount of acetalolysis monitored via tlc (chloroform:ethyl acetate, 10:1). After approximately 3 days, the mixture was filtered, poured into water (2 L), and stirred for 1 hour. This mixture was extracted with chloroform (1 x 200 mL, 4 x 50 mL) and the combined extracts were washed with aqueous sodium bicarbonate (saturated, 3 x 200 mL) and water (2 x 250 mL), dried over anhydrous potassium carbonate, and reduced in vacuo to a thick oil (130 g).

Sodium methoxide (1M, 50 mL) was added to a solution of the mannosaccharide acetate mixture (130 g) dissolved in anhydrous methanol (250 mL). The mixture was shaken for an hour and stored overnight in the freezer. A small sample was dissolved in water, deionized, and reduced to an oil. A ferric hydroxamate test was run on this sample to check for the presence of acetate groups. If the ferric hydroxamate test was positive, additional sodium methoxide was added. Otherwise, the mixture was dissolved in water (200 mL), deionized with resin
Dowex 5W-X8H, 5 g), decolorized with carbon (J. T. Baker), then reduced in vacuo to a thick syrup (62 g).

A 5 x 100 cm column was slurry-packed with 500 g of Sephadex G-15 swollen in 10 L of water for 12 hours. The deacetylated syrup was pipetted onto the column, the column eluted with water, and 10 mL fractions were collected. Sugars appeared in fractions 50-115. Analysis by tlc suggested the following identities for the fractions; 93-118, mannose (5.83 g); 83-92, mannobiose (3.44 g); 76-82, mannotriose (1.25 g); 66-74, mannotetrose (0.57 g); 60-65, manno-pentose (0.14 g). Attempts to crystallize the crude mannobiose from isopropanol and n-butanol were unsuccessful. The $^1$H-NMR spectrum (D$_2$O) was consistent with literature spectra.$^{41}$ A $^{13}$C-NMR spectrum (D$_2$O) (Fig. 27, Appendix III) showed: 61.6 (C$_6$), 62.1 (C$_6$'), 67.7 (C$_4$'), 70.1 (a-C$_3$), 71.3 (a-C$_2$), 71.6 (b-C$_2$,2'), 72.0 (a-C$_5$), 72.8 (b-C$_3$), 73.9 (C$_3$'), 75.9 (b-C$_5$), 77.5 (b-C$_4$,5'), 77.9 (a-C$_4$), 94.9 (a-C$_1$, b-C$_1$), 101.3 (C$_1$'). The $^{13}$C spectrum was also consistent with published spectra for mannobiose.$^{41,42}$

**Mannobiose Octaacetate**

Crude mannobiose (19.8 g) was dissolved in hot pyridine, and the solution was cooled to 0°C in an ice bath. Acetic anhydride (300 mL) was added dropwise while cooling and stirring. The solution was stirred for twelve hours, quenched with water (300 mL), and extracted with chloroform (1 x 300 mL, 2 x 50 mL). The combined chloroform extracts were washed with hydrochloric acid (1.0N) until the spent aqueous layer was acidic (10 x 100 mL) and then with water until neutral (5 x 100 mL), dried over potassium carbonate, filtered, and reduced in vacuo to a thick syrup. The $^{13}$C-NMR spectrum is shown in Fig. 28, Appendix III.
1,5-Anhydro-4-O-8-Mannopyranosyl-D-Mannitol

Mannobiose octaacetate syrup (27.4 g) in 1,2-dichloroethane (40 mL) was mixed with hydrogen bromide in glacial acetic acid (36%, 30 mL). The solution was stirred for two hours, then water (200 mL) was added. The mixture was extracted with chloroform (2 x 50 mL). The combined chloroform extracts were washed with water (4 x 100 mL), sodium bicarbonate solution (saturated, 5 mL), and water (2 x 100 mL); dried over potassium carbonate; and reduced in vacuo. The oil was thinned with THF and concentrated twice.

The crude mannobiosyl bromide peracetate was dissolved in tetrahydrofuran (ca. 25 mL) and mixed with Raney nickel (ca. 164 g) and triethylamine (40 mL). The mixture was refluxed for 5 hours. The reaction was monitored with tlc (chloroform:ethyl acetate, 1:1). The mixture was filtered and the Raney nickel solids were washed with tetrahydrofuran, acetone, and tetrahydrofuran. The combined organic filtrates were concentrated to an oil (14.5 g). This oil was purified by silica gel column chromatography (Kieselgel 70-230 mesh, CHCl₃:EtOAc, 4:1).

Purified 1,5-anhydromannobiitol octaacetate (2.0 g) was dissolved in anhydrous methanol (15 mL) and sodium methoxide (1N, 5 mL) was added. When tlc (CHCl₃:EtOAc, 10:1) showed that deacetylation was complete (ca. 30 minutes), the solution was deionized with resin (Dowex H⁺, 5 g), filtered, and reduced in vacuo to an oil. Successive additions of anhydrous ethanol and concentration in vacuo yielded a white solid, which was crystallized (ethanol:water, 10:1); m.p. 231.0-231.5°C; [α]D-39.46 (c 1,506, H₂O); Anal. Calc. C₁₂H₂₂O₁₀: C, 44.17; H, 6.80; O, 49.03. Found: C, 44.09; H, 6.86; O, 49.05; ¹³C-NMR (CDCl₃, δ): 61.9 (t, C₆); 62.2 (t, C₆'); 70.8 (t, C₁); 68.0, 69.6, 71.7, 73.3, 74.2, 77.7, 78.2, and 80.1 (all d, C₂, C₃, C₄, C₅, C₂', C₃', C₄', C₅'); 101.3 (D, C₁').
Fehling's test demonstrated that the product was a nonreducing sugar. The product (0.13 g) was hydrolyzed by refluxing in trifluoroacetic acid solution (2N, 30 mL) for 1 hour. The solution was reduced in vacuo to a syrup, 10 mL of water added, and the mixture evaporated in vacuo to dryness until the pH was neutral (12 times). The residue was taken up in 5 mL water, passed through deionizing resin (MB-3, 5 mL), and evaporated to dryness (0.0448 g). Cyclohexyl-β-D-glucosyl (0.0362 g) was added as an HPLC internal standard, and the mixture was taken up in water (1 mL). Analysis via HPLC demonstrated that the hydrolysate contained equimolar amounts of mannose and 1,5-anhydro-D-mannitol.

1,5-Anhydro-2,3,6-tri-O-methyl-4-O-β-(2,3,4,6-tetra-O-methylmannopyranosyl)-D-mannitol

Pure 1,5-anhydromannobiitol crystals (0.36 g) were dissolved in anhydrous dimethyl sulfoxide (8.5 mL), and powdered sodium hydroxide (1.1 g) was added. Distilled methyl iodide (1.5 mL) was added dropwise over a 10 minute period to the vigorously stirred mixture. After 30 minutes, the reaction was quenched with water (15 mL), and extracted with benzene (1 x 25 mL, 3 x 10 mL). The benzene extracts were washed with saturated sodium chloride solution (3 x 15 mL) and water (1 x 10 mL), dried over sodium sulfate, filtered, and reduced in vacuo to a thick syrup (0.23 g, 49% yield). The aqueous layers were reextracted with benzene (3 x 10 mL). The secondary extracts were treated in a manner similar to the first extracts and yielded additional syrup (0.6 g), which was combined with the first syrup. Gas chromatographic analysis (conditions C) indicated that the product was pure. The $^{13}$C-NMR spectrum (CDCl$_3$, δ) (Fig. 32, Appendix III) had: 29.5 (unassigned), 61.2-56.8 (0-CH$_3$ groups), 64.8 (C$_1$), 71.7 (C$_6$ or C$_{6'}$), 71.9 (C$_{6'}$ or C$_6$), 74.1, 74.6, 75.0, 75.4 (C$_4$, C$_4'$, C$_2$, C$_2'$), 76.1 (CDCl$_3$), 76.7 (C$_5$ or C$_{5'}$), 77.1 (CDCl$_3$), 77.7 (CDCl$_3$), 78.0 (C$_5$ or C$_{5'}$), 81.9 (C$_3$), 84.2 (C$_{3'}$), 100.9 (C$_{1'}$).
1,5-Anhydro-2,3,4,6-Tetra-0-Acetyl-D-Mannitol

D-Mannose (15 g) was mixed with cooled pyridine (105 mL) and acetic anhydride (75 mL). After 12 hours, the solution was poured into chloroform (300 mL) and ice water (300 mL), and stirred vigorously. The water and chloroform layers were separated and the aqueous layer was extracted with chloroform (2 x 100 mL). The combined chloroform extracts were washed with hydrochloric acid (0.1N, 8 x 250 mL) and water (2 x 250 mL), dried over anhydrous potassium carbonate, filtered, and reduced in vacuo to a syrup.

The penta-0-acetyl-D-mannose syrup was reacted with a hydrogen bromide-glacial acetic acid solution (34%, 39 mL) for 3 hours at room temperature. Ethyl ether (300 mL) and ice water (300 mL) were added and the mixture was stirred vigorously for 20 minutes. The ether and aqueous layers were separated, and the aqueous fraction was washed with ether (2 x 150 mL). The combined ether fractions were washed with sodium bicarbonate solution (saturated, 4 x 450 mL) and water (3 x 500 mL), dried over potassium carbonate, filtered, and reduced in vacuo to a syrup.

The 2,3,4,6-tetra-0-acetyl-mannopyranosyl bromide syrup (35.92 g) was mixed with Raney nickel (ca. 100 g) in tetrahydrofuran:ethanol (1:1, 400 mL) at room temperature and the reaction was monitored via tlc. After 15 hours, the temperature was raised to 40°C. Two hours later, triethylamine (15 mL) was added. After an additional seven hours, the reaction mixture was filtered and the filtrate reduced in vacuo to a solid. This solid was crystallized from ethanol. The rhomboidal 1,5-anhydro-D-mannitol tetraacetate crystals were contaminated with a by-product which formed needlelike crystals. These were washed away with water. The total yield of 1,5-anhydro-D-mannitol tetraacetate was 12.32 g (44.28% based on D-mannose); m.p. 64.7-65.7°C, [α]D-38.46 (c 1.498, CHCl₃).
Literature: mp. 66-67°C, [α]D-42 (CHCl₃). The ¹³C-NMR is shown in Fig. 25, Appendix III.

1,5-Anhydro-D-Mannitol

Crystals of 1,5-anhydro-D-mannitol tetraacetate (7.38 g) were dissolved in anhydrous methanol (50 mL). Sodium methoxide (10 mL, 1M) was added and the mixture was shaken for ca. 30 minutes, until tlc (CHCl₃:EtOAc, 10:1) showed that deacetylation was complete. The solution was then deionized with Dowex H⁺ resin (10 g), filtered, and reduced in vacuo to a thick syrup. Crystallization from ethanol yielded needlelike crystals (2.15 g, 60%): m.p. 155.8-156.7°C, [α]D-49.81 (H₂O), c 1.495). Literature: m.p. 156-157°C, [α]D-50° (H₂O, c 1.1). The ¹³C-NMR spectrum (CDCl₃, δ) is shown in Fig. 26, Appendix III.

2,3,4,6-Tetra-O-Acetyl-α-D-Glucopyranosyl Bromide

Glucose pentaacetate (50 g) was mixed with hydrogen bromide/glacial acetic acid solution (33 mL, 34%) and allowed to sit for 2 hours. The solution was then mixed with chloroform (200 mL), poured into ice water (0.75 L) and stirred vigorously for 15 minutes. The chloroform and aqueous layers were separated and the aqueous layer was washed with chloroform (1 x 50 mL). The combined chloroform extracts were washed with sodium bicarbonate solution (saturated, 2 x 200 mL), dried over potassium carbonate, filtered, and reduced in vacuo to a syrup. The syrup was crystallized from diethyl ether (10 mL), yielding 2,3,4,6-O-tetraacetate glucosyl bromide (40.07 g, 76.08%): m.p. 88-89.5°C, [α] = +196.0. Literature: m.p. 88-89°C, [α]D+197.8 (CHCl₃); m.p. 89-90°C, [α]D+197.3°C (c 1.5, CHCl₃).
2-Acetoxyethyl 2,3,4,6-Tetra-O-Acetyl-1-Thio-β-D-Glucopyranoside

2,3,4,6-Tetraacetate-α-D-glucosyl bromide (20 g) was dissolved in chloroform (50 mL) and added dropwise to a solution of methanol (100 mL), KOH (5.5 g), and mercaptoethanol (10 mL). After 4 hours, sodium methoxide (1N, 5 mL) was added. TLC analysis (CHCl₃:EtOAc, 10:1) showed that complete deacetylation had occurred. The mixture was then filtered. The filtrate was neutralized with acetic acid (1N, 50 mL) and concentrated in vacuo to a thick syrup. The residue was extracted with hot pyridine (2 x 50 mL, 1 x 125 mL) and the pyridine was decanted and filtered. The pyridine solution was cooled in ice and mixed with acetic anhydride (60 mL). After 18 hours, TLC analysis showed that the compound was completely acetylated. The reaction mixture was then poured into water (250 mL), stirred vigorously, and extracted with chloroform (2 x 75 mL). The combined chloroform extracts were washed with acetic acid (1N, 4 x 250 mL); then reduced in vacuo to a thick syrup. The yield was 5.54 g (25.29%). The product, crystallized from ethanol, had: m.p. 106.7-108.3°C, [α]D⁻30.6 (c 1.501, CHCl₃). Literature: m.p. 106-106.5°C, [α]D⁻32.0 (c 1.5, CHCl₃);32 m.p. 108.0-108.5°C, [α]D⁻31.5 (CHCl₃).68 The ¹³C-NMR spectrum (CDCl₃, δ) is shown in Fig. 23, Appendix III.

2-Hydroxyethyl 1-Thio-β-D-Glucopyranoside

Sodium methoxide (1N, 5 mL) was added to a solution of 2-acetoxyethyl tetra-O-acetyl-1-thio-β-D-glucopyranoside (5.0 g) in anhydrous methanol (50 mL). The reaction was monitored by TLC (CHCl₃:EtOAc, 1:1). When deacetylation was complete, the solution was deionized with resin (Dowex 5W-X8H, 5 g), filtered, and reduced in vacuo to a thick syrup. The product, crystallized from ethanol, had: m.p. 115.9-117.9°C and [α]D⁻53.4 (c 1.5, H₂O). Literature: m.p. 115.5-116.5°C and [α]D⁻54.9 (c 1.5, H₂O);32,33 m.p. 114-116°C and [α]D⁻61.8 (H₂O).68 The ¹³C-NMR spectrum (CDCl₃, δ) is shown in Fig. 24, Appendix III.
**2-Methoxyethyl 2,3,4,6-Tetra-O-Methyl-1-Thio-β-D-Glucopyranoside**

2-Hydroxyethyl 1-thio-β-D-glucopyranoside (1.59 g) was dissolved in anhydrous dimethyl sulfoxide (31.2 mL) and powdered sodium hydroxide (3.79 g) was added. Distilled methyl iodide (6 mL) was added dropwise over a 40-minute period to the stirred mixture. The mixture was stirred for another 20 minutes, then the reaction was quenched with water (60 mL). After 5 minutes, the resulting yellow solution was extracted with benzene (1 x 50 mL, 3 x 25 mL). The colorless benzene extracts were washed with saturated sodium chloride solution (3 x 50 mL) and water (1 x 25 mL). The aqueous washes were back-extracted with benzene (2 x 45 mL). These second benzene extracts were washed with saturated sodium chloride solution (2 x 30 mL) and water (1 x 15 mL), then added to the earlier extracts. The combined benzene extracts were dried over sodium sulfate, filtered, and reduced in vacuo to a thin syrup (3.12 g). The product was purified by distillation at 0.5 mm Hg and 140°C. Gas chromatographic analysis showed that the middle fraction was pure. The $^{13}$C-NMR spectrum (CDCl$_3$, $\delta$) (Fig. 33, Appendix III) had: 30.1 (C$_b$), 58.5, 59.1, 60.3, 60.5, 60.7 (CH$_3$), 71.4, 72.3 (C$_6$ and C$_a$), 75.7, 77.0, 78.3 (CDCl$_3$), 78.7 (C$_4$), 79.4 (C$_2$), 83.4 (C$_5$), 85.0 (C$_3$), 88.3 (C$_1$).

**KINETIC STUDIES**

**Reactor System**

Kinetic studies were run in the reactor system described previously (Fig. 22). This system consisted of a stainless steel reactor (140 mL capacity) fitted with a magnetic stir bar, thermocouple (Omega Iron-Constantan Type J) and sample line (Type 304 stainless super high pressure tubing, 1/8-inch o.d. x 0.049-inch wall x 18 inches long). Two gyrolak fittings connected the thermocouple and sample line to the removable reactor lid. A high
Figure 22. Schematic diagram of the kinetic reactor system.
pressure seal between the lid and reactor was obtained by compressing a Teflon ring around the lid with heat-treated retaining bolts. The sample line was connected to a Cheminert slider injection valve fitted with a 1 mL sample loop and gas syringe.

The oil bath, which held ca. 4.5 gallons of HTF-100 UCON high temperature oil, was fitted with a motor-driven magnetic stirrer and movable rack to raise and lower the reactor. Two continuous heaters (250 and 500 V) and a variable heater (500 V) controlled by a fixed-point thermostat and electronic relay maintained the bath temperature at ± 0.1°C of the desired temperature. The reactor temperature was measured by connecting the thermocouple to a precision potentiometer (Leeds and Northrup 8691-2) through an electronic cold junction compensator (Omega CJ-J).

**Stock Sodium Hydroxide**

The stock NaOH solution was prepared by slowly adding sodium hydroxide pellets (500 g) to cooled, stirred, triply-distilled water (500 mL). The resulting cloudy solution was filtered and stored in a paraffin-lined glass bottle. The concentration was measured by diluting an aliquot of stock solution to approximately 2N and titrating against a known amount of potassium acid phthalate using a phenolphthalein indicator.

**Stock Sodium Sulfide**

Crystals of sodium sulfide nonahydrate were placed in a Buchner funnel and washed with water until the crystals and filtrate were colorless. Under nitrogen, the purified sodium sulfide crystals (400 g) were dissolved in oxygen-free water (600 g) by stirring for three hours. The solution was stored in a paraffin-lined bottle. This bottle was opened only under nitrogen. The
concentration was measured by diluting 10 mL (ca. 11.2 g) of stock sodium sulfide solution to 100 mL. Aliquots of this solution (10 mL) were titrated with 0.1M HgCl₂ solution in the presence of sodium thiosulfate (5 g) and sodium hydroxide (1M, 100 mL). The titration was followed potentiometrically using an Orion Ag/AgS electrode in conjunction with a double junction reference electrode.

Oxygen-Free Water

Oxygen-free water was made by boiling 1400 mL of triply-distilled water until the volume was reduced to 1200 mL, then purging it with nitrogen while cooling in ice. The container was sealed tightly and the water was used within 24 hours.

Standard Sodium Hydroxide

For each run, a standard sodium hydroxide solution having twice the sodium hydroxide concentration desired in the final liquor was prepared. Under nitrogen, the appropriate amount of stock sodium hydroxide solution, calculated according to Eq. (29), was weighed into a tared volumetric flask and diluted with oxygen-free water.

\[ X = \frac{M_S V_S \delta}{M_{STK}} \]  

(29)

where \( X \) = weight of stock solution

\( M_S \) = desired molarity of standard solution

\( V_S \) = desired volume of standard solution, room temperature

\( \delta \) = density of stock solution

\( M_{STK} \) = molarity of stock solution

The density of the standard sodium hydroxide solution was determined and the concentration was measured by titrating a known amount of potassium acid phthalate.
Reaction Liquor

The reaction liquors were prepared from standard solutions and crystalline salts under nitrogen. The thermal expansion of the solution must be accounted for when the amount of standard sodium hydroxide solution needed to give the desired liquor concentration is calculated. This is done using the volume expansivity factor, $F_V$, which is defined according to Eq. (30):

$$F_V = \frac{V_T}{V_{RT}} \tag{30}$$

where $V_T =$ volume of water at reaction temperature, mL

$V_{RT} =$ volume of water at room temperature, mL

Volume expansivity factors are listed in Table 16.

<table>
<thead>
<tr>
<th>Reaction Temperature</th>
<th>Volume Expansivity Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>160°C</td>
<td>1.101</td>
</tr>
<tr>
<td>170°C</td>
<td>1.113</td>
</tr>
<tr>
<td>180°C</td>
<td>1.126</td>
</tr>
<tr>
<td>190°C</td>
<td>1.140</td>
</tr>
</tbody>
</table>

Equating $V_S$ with $V_{RT}$ and substituting Eq. (30) into (29) gives (31):

$$X_S = \frac{M_RF_VV_{RT}\delta}{M_S} \tag{31}$$

where $X_S =$ weight of standard solution

$M_R =$ desired molarity of reaction liquor

$\delta =$ density of standard solution

$M_S =$ molarity of standard solution
The analogous equation for solids is:

\[ Y = M_C V_{RT} F_V W_{MW} \]  

(32)

where \( Y \) = weight of sodium tosylate needed, g

\[ M_C \] = desired molarity of solids in liquor, moles/L

\[ W_{MW} \] = molecular weight of solid

\[ F_V \] = volume expansivity factor

\[ V_{RT} \] = volume of liquor at room temperature, mL

In liquors which contained only sodium hydroxide, the necessary volume of standard NaOH was diluted to the appropriate volume with oxygen-free water. The density of the reaction liquor was measured and the concentration determined via titration of potassium acid phthalate.

Some liquors contained sodium tosylate in addition to sodium hydroxide. In these cases, the sodium tosylate needed to give the desired concentration was calculated from Eq. (32). The necessary amount of sodium tosylate was weighed into a tared 100 mL volumetric flask. Under nitrogen, the appropriate amount of standard sodium hydroxide solution, calculated according to Eq. (31), was added, and the mixture was diluted to the mark with oxygen-free water. After measuring the density of the mixture, the flask was tightly stoppered, removed from the glove bag, and heated over steam until all the sodium tosylate dissolved. Because sodium tosylate interferes with the potassium acid phthalate titration, the sodium hydroxide concentration in the liquor could not be measured.

Liquors which contained sodium hydroxide and sodium sulfide were prepared by weighing appropriate amounts of standard sodium hydroxide solution and stock sodium sulfide solution, both determined using Eq. (31), into a tared volumetric
flask under nitrogen. The liquor was diluted with oxygen-free water and weighed to determine the density.

The concentration of sodium sulfide in the reaction liquor was measured by titrating 2-mL aliquots of liquor with 0.1000 M HgCl₂ in the presence of sodium sulfite (5 g), sodium hydroxide (4 M, 25 mL), and water (75 mL).³²,³³,⁶⁹ Active alkali was determined by titrating liquor aliquots (ca. 10 mL, determined gravimetrically) and formaldehyde (5 mL) with 1.0 M hydrochloric acid to the phenolphthalein endpoint.³²,³³,⁷⁰ The sodium hydroxide concentration was found by subtracting the sodium sulfide concentration from the active alkali.

**Loading the Reactor**

The amount of carbohydrate needed for each degradation was calculated according to Eq. (32). If the carbohydrate was a solid, the appropriate amount was weighed into a weighing bottle, then added to the reaction under nitrogen. Reaction liquor (100 mL) was then added to the reactor. If the carbohydrate was an oil, it was dried under vacuum in a flask. A portion of the weighed liquor (100 mL) was added to the flask, swirled until the carbohydrate dissolved, and poured into the reactor. The flask was rinsed with additional portions of the reaction liquor. The reactor was sealed, connected to the temperature measurement and sampling lines, and placed in a hot oil bath.

When a liquor contained sodium tosylate, one reactor port was sealed with a cap instead of the sample line. The reactor was partially immersed in the bath, and when the temperature reached 65-70°C, the cap was replaced with the sample line. The reactor was then fully immersed in the bath, and the heat-up continued as usual.
Sampling

Sampling was started when the reactor reached temperature. The sample line was purged twice, then the timer was started and the first sample taken. Duplicate samples were withdrawn into tared, 4-mL vials at logarithmically-spaced intervals throughout the reaction. The sample line was purged once prior to withdrawing each set of samples. Internal standard solution (ca. 0.005M, 1 mL) was added to one of the samples in each set. Both the sample size and amount of internal standard solution added were determined gravimetrically.

Sample Analysis

Samples containing only sodium hydroxide and carbohydrates were passed through a fresh column of ion-exchange resin (MB-3, 10 mL), eluted with water (10 mL), and reduced in vacuo to dryness. Samples which contained 1.0M sodium tosylate were passed through a 10 mL column of the MB-3 resin, eluted with water (10 mL), passed through the same resin column a second time and again eluted with water (10 mL). They were reduced in vacuo to dryness. Samples which contained 1.5 or 2.0M sodium tosylate were passed through 10 mL of MB-3 resin, eluted with 5 mL water, passed through the same column again, eluted with 10 mL water, then passed through 5 mL of fresh MB-3 resin and eluted with 10 mL of water. These samples were also reduced to dryness in vacuo.

Samples were acetylated by adding pyridine (0.66 mL) and acetic anhydride (0.33 mL) and agitating overnight. The reaction was quenched with water (10 mL) and extracted with chloroform (2 x 5 mL). The combined chloroform extracts were washed with HCl (1N, 10 mL) and water (2 x 10 mL), reduced to dryness, taken up in chloroform (ca. 2 mL), transferred to a 4-mL vial, and reduced to dryness. The residue was taken up in chloroform (15 drops) and injected on the gas chromatograph using conditions B. The peak areas for each compound were converted
to concentrations using a calculator program (Program 5, Appendix I) based on Eq. (33):

\[ M_X = \frac{A_X}{A_{IS}} \cdot M_{IS} \cdot \frac{W_{IS}}{W_X} \cdot \frac{\delta_X}{\delta_{IS}} \cdot F_X \]  

(33)

where 

- \( M_X \) = concentration of unknown (M) at room temperature
- \( M_{IS} \) = concentration of internal standard solution (M)
- \( A_X \) = area of unknown peak
- \( A_{IS} \) = area of internal standard peak
- \( W_X \) = weight of unknown (sample) solution
- \( W_{IS} \) = weight of internal standard solution added
- \( \delta_X \) = density of unknown (sample) solution
- \( \delta_{IS} \) = density of internal standard solution
- \( F_X \) = response factor

Response factor samples were prepared by pipetting appropriate amounts of internal standard and carbohydrate solutions into 4-mL vials. The solutions were treated and derivatized in the same manner as the reaction samples. Response factors were calculated according to Eq. (34):

\[ F_X = \frac{A_{IS}}{A_X} \left( \frac{M_X}{M_{IS}} \right) \]  

(34)

Samples of fully-methylated sugars were neutralized with aqueous hydrochloric acid (0.5 mL, 5M), then extracted with chloroform (3 x 3 mL), reduced in vacuo just to dryness, transferred to a 4-mL vial using ca. 2 mL of chloroform, and reduced to dryness again. The residue was then taken up in chloroform (15 drops) and injected on the GC using conditions C. Concentrations were calculated according to Eq. (33). Response factor samples were treated in the same manner, and response factors were calculated using Eq. (34).
OXYGEN-18 INCORPORATION STUDIES

Reactor System

The $^{18}O$ incorporation studies were carried out in 4-mL capacity stainless steel pressure vessels. These were placed in a rotating, motor-driven plate and immersed in an oil bath. The oil bath was filled with ca. 3.5 gallons of HTP-100 UCON high temperature oil (Blue M). The temperature was maintained with 2 continuous heaters (250, 500 V) and a variable heater (500 V) controlled by a fixed point thermostat and electronic relay, and monitored by a thermocouple inserted in the oil and connected to a digital thermometer (Omega Model 199) and strip chart recorder.

Oxygen-18 Water (50%)

In a nitrogen-filled glovebag, 95% $^{18}O$-water and oxygen-free water were mixed in a 1.07:0.82 (w:w) ratio. One drop of this mixture was set aside for mass spectral analysis.

Oxygen-18 Enriched Liquors (20%)

Liquors were prepared in 5-mL volumetric flasks. Appropriate amounts of sodium tosylate were weighed into these flasks. Under nitrogen, the proper weights of stock sodium hydroxide solution and 50% $^{18}O$-water were added, then each solution was diluted to 5 mL with oxygen-free water.

Loading the Reactors

In a nitrogen-filled glovebag, 1,5-anhydromannobiitol was weighed into bombs which had been heated at 120°C for two hours and cooled under vacuum. Liquors which contained sodium tosylate were heated on the steam cone until all the tosylate dissolved. Under nitrogen, 4-mL of liquor was pipetted into each
bomb. Bombs were covered with the inner cap, wrapped with Teflon tape, and sealed with the outer cap. A few drops of each liquor were set aside for mass spectral analysis.

The bombs were placed in the rack, the rack was put into the bath, and the timer was started when the bath temperature stabilized.

**Sampling**

To remove a bomb, the rotating plate was stopped in an upright position, the bomb grasped with pliers, and pulled out of the rack, being careful to not loosen the cap. It was immediately plunged into a metal beaker containing ice water to cool it rapidly.

**Sample Analysis**

The contents of each bomb were weighed into three 4-mL vials. Samples which contained no sodium tosylate were passed through an MB-3 resin column (10 mL), and eluted with water (20 mL). Samples which did contain sodium tosylate were passed through an MB-3 resin column (10 mL), eluted with water (10 mL), then passed through the same column a second time and eluted with more water (20 mL). All samples were reduced to dryness under vacuum in 25-mL Erlenmeyer flasks. Small quantities of water were used to transfer them to 6-mL hypovials, in which they were again reduced to dryness. They were then dried under vacuum at 35°C in the presence of phosphorus pentoxide.

Under nitrogen, anhydrous pyridine (freshly distilled, 0.5 mL) and N-methyl-bis(trifluoroacetamide) reagent (1 mL, Pierce) were added to each sample, the samples were tightly sealed with a Teflon disk and crimp-cap, and heated at 55°C for one hour. They were then injected on the gas chromatograph-mass spectrometer the same day.
ACKNOWLEDGMENTS

The author gratefully acknowledges the contributions of her thesis advisory committee: Dr. L. R. Schroeder, Dr. D. R. Dimmel, and Dr. N. S. Thompson. Special thanks are due to Dr. Schroeder for his guidance and support throughout the course of this work.

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Finally, the author wishes to thank her family, particularly her husband, Jim, for support and encouragement throughout the course of this thesis.
LITERATURE CITED


70. TAPPI Standard Method T 625 ts-64.


APPENDIX I

COMPUTER AND CALCULATOR PROGRAMS

Program 1: MH/DISAPPEAR

100   SRESET
200   C   LINEAR REGRESSION PROGRAM
         '00   C   FOR THE ANALYSIS OF PSEUDO-FIRST ORDER REACTIONS
400   C   WRITTEN BY D. SLYTHE
450   C   MODIFIED BY H. HENDERSON
500   C   THIS PROGRAM IS DESIGNED TO GENERATE PSEUDO-FIRST ORDER RATE
600   C   CONSTANTS AND THEIR 95% CONFIDENCE LIMITS DIRECTLY FROM RAW
700   C   CONCENTRATION MEASUREMENTS.
800   C
1100 C   THE OUTPUT CONSISTS OF A TABLE SHOWING EACH SAMPLE TIME
1200 C   (IN SECONDS), THE MEAN CONCENTRATION (AT TEMPERATURE),
1300 C   THE CALCUATED (REGRESSION) CONCENTRATIONS, AND THE
1400 C   DIFFERENCE BETWEEN THE TWO CONCENTRATIONS. THE OUTPUT ALSO
1500 C   INCLUDES THE SLOPE (AND ITS 95% CONFIDENCE INTERVAL), THE
1600 C   INTERCEPT, AND THE CORRELATION COEFFICIENT FOR THE LEAST-SQUARES
1700 C   REGRESSION LINE.
1710 C   IF THE OUTPUT IS TO BE DIRECTED TO THE PRINTER,
1720 C   THEN REMOVE THE COMMENT CHARACTER '*ROM:
1730 FILE 6(KIND=PRINTER,HYUSE=OUT,MAXRECSIZE=22)
1740 C   AND COMPILE WITH FORTRAN.
1741
1742 C   A FILE CONTAINING THE SAMPLE TIME (IN SECONDS) AND
1744 C   THE LN OF THE AVERAGE MBT CONCENTRATION AT EACH TIME IS
1746 C   ALSO GENERATED. THIS FILE CAN BE USED WITH ‘ST*/PLOT-
1748 C   TO PLOT THE DATA. IT IS DEFINED BY:
1750 FILE 1(KIND=DISK,TITLE="PLOT13/MBT",PROTECTION=SAVE,
1751   *AREAS=1,AREASIZE=200)
1800 C   THE INPUT DATA FILE IS DEFINED BY:
1850 FILE 5(KIND=DISK,TITLE="DATA13/MBT")
1900 C   A FILE WITH THE GIVEN TITLE MUST BE CREATED BEFORE RUNNING
2000 C   THIS PROGRAM AND MUST CONTAIN A LED RECORD GIVING THE
2050 C   REACTION NUMBER, THE REACTION TEMPERATURE, THE NAOH
2100 C   MOLARITY, AND THE VOLUME EXPANSIVITY FACTOR. THEN UP TO TWENTY DATA RECORDS (TIME IN MINUTES,
2205 C   NUMBER OF CONCENTRATION MEASUREMENTS).
2210 C   FOLLOWED BY UP TO SIX INDIVIDUAL CONCENTRATION MEASUREMENTS)
2220 C   AND AN END RECORD OF 0.000.
2700 C
2800 INTEGER NO,X,Y,I,NUM
2900 REAL THIN,CONC(6),TSEC(20),AVG,CHIT20),POINT(20),TEMP,TFACT
3000 REAL SUMX,SUMX2,SUMY,SYMXY,4*8,DENOM,CC,HOL,XPIC(20)
3100 REAL SSSY,SSX,SSXY,THETAR,ERROR,T(20),CALC(20),DIFF(20)
3200 READ(5,/)NUM,TEMP,TFACT
3210 WRITE(6,9000)NUM
3300 9000 FORMAT(1H/,* THIS IS REACTION NUMBER 'I5,'/
3400 WRITE(6,9001)TEMP
3500 9001 FORMAT(1H/,* THE TEMPERATURE OF THE REACTION WAS 'F8.2,'/
3520 WRITE(6,8000)TFACT
3540 8000 FORMAT(1H/,* THE MOLARITY OF THE REACTION (AT TEMP) WAS 'F5.2,'/
3541 +F5.2,'/
3600 Y=1
3700 X=1
3900 C   THIS LOOP FINDS THE MEAN OBSERVED CONCENTRATIONS
3900 C   NO IS THE NUMBER OF RAW CONCENTRATION MEASUREMENTS
4000 1 READ(5,/)THIN:NO,(CONC(I),I=1,NO)
4100 9002 FORMAT(F10.1*14*6F10.9)
4200 C   THIS TESTS FOR THE LAST DATA CARD
4300 IF (NO) 99 P=2
4400 2 TSEC(X) = THIN*60.
4500 X = X + 1
4600 AVG = 0.
4700 DO 3 I = 1, NO
  4900 3 AVG = CONC(I) * AVG
      C CHI IS THE MEAN OBSERVED CONCENTRATION
5000 CHI(Y) = (AVG/NO)/FACT
5100 POINT(Y) = ALOG(CHI(Y))
5200 Y = Y + 1
5300 GO TO 1
5400 C N IS THE NUMBER OF SAMPLING TIMES (DATA CARDS)
5500 9 N = Y - 1
5600 WRITE (6, 9003) N
5700 9003 FORMAT (1H/" THE NUMBER OF DATA POINTS IS "I15")
5800 C THIS SECTION FINDS THE REGRESSION PARAMETERS
5900 SUMX = 0.
6000 SUMX2 = 0.
6100 SUMY = 0.
6200 SUMY2 = 0.
6300 SUMXY = 0.
6400 DO 10 I = 1, N
6500 SUMX = TSEC(I) + SUMX
6600 SUMX2 = TSEC(I) * TSEC(I) + SUMX2
6700 SUMY = POINT(I) + SUMY
6800 SUMY2 = POINT(I) * POINT(I) + SUMY2
6900 10 SUMXY = TSEC(I) * POINT(I) + SUMXY
7000 C CC IS THE CORRELATION COEFFICIENT
7100 CC = (SUMXY - SUMX * SUMY)/N)/DENOM
7200 00 CC = (SUMXY - SUMX * SUMY)/N)/DENOM
7300 C B IS THE INTERCEPT
7400 B = (SUMX2 * SUMY - SUMX * SUMXY)/(N * SUMX2 - SUMX * SUMX)
7500 C M IS THE SLOPE
7600 M = (N * SUMXY - SUMX * SUMY)/(N * SUMX2 - SUMX * SUMX)
7700 C THIS SECTION FINDS THE CONFIDENCE INTERVAL OF THE SLOPE
7800 SSY = SUMY2 - (SUMY * SUMY)/N
7900 SSX = SUMX2 - (SUMX * SUMX)/N
8000 SSXY = SUMXY - (SUMX * SUMY)/N
8100 THETA = (CSSY - HSSXY)/N)**.5
8200 C THIS IS A TABLE OF STUDENT'S T VALUES AT THE 0.95 LEVEL
8300 TC(1) = 12.706
8400 TC(2) = 4.303
8500 TC(3) = 3.182
8600 TC(4) = 2.776
8700 TC(5) = 2.571
8800 TC(6) = 2.447
8900 TC(7) = 2.365
9000 TC(8) = 2.306
9100 TC(9) = 2.262
9200 TC(10) = 2.228
9300 TC(11) = 2.201
9400 TC(12) = 2.179
9500 TC(13) = 2.160
9600 TC(14) = 2.145
9700 TC(15) = 2.131
9800 TC(16) = 2.120
9900 TC(17) = 2.110
1000 TC(18) = 2.101
1010 TC(19) = 2.093
1020 TC(20) = 2.086
1030 ERROR = TC(N-2) * THETA *((N/((N-2) * SSX))**.5)
10400 C THIS CALCULATES THE REGRESSION CONCENTRATIONS
DO 30 I=1,N
CALC(I)=EXP(M*TSEC(I)+B)
30 DIFF(I)=CHI(I)-CALC(I)
C THIS SECTION WRITES THE OUTPUT DATA
WRITE(6,9004)
9004 FORMAT( SECONDS CONCENTRATION CALC. CONC. DIFFERENCE )
9005 FORMAT(F10.1,F15.6)
WRITE(C6,9005)(TSEC(I),CHI(I),CALC(I),DIFF(I),I=1,N)
WRITE(6,9006)
9006 FORMAT( THE SLOPE OF THE REGRESSION LINE IS *E12.4 / )
9007 FORMAT( THE CONFIDENCE INTERVAL ON THE SLOPE IS *E12.4 / )
WRITE(6,9008)
9008 FORMAT( THE INTERCEPT OF THE LINE IS *E12.4 / )
WRITE(C6,9009)
9009 FORMAT( THE CORRELATION COEFFICIENT IS *F6.4 / )
C THIS WRITES TO FILE USED FOR PLOTTING
DO 40 I=1,N
XPT(I)=TSEC(I)/1000
WRITE(1,9010)XPT(I),POINT(I),I=1,N
9010 FORMAT(2F10.4)
STOP
END
Program 2: MH/APPEAR

LINEAR REGRESSION PROGRAM
FOR ANALYSIS OF THE FORMATION OF REACTION PRODUCTS

WRITTEN BY D. BLYTHE
MODIFIED BY M. HENDERSON

THIS PROGRAM IS DESIGNED TO GENERATE PSEUDO-FIRST ORDER
RATE CONSTANTS AND THEIR 95% CONFIDENCE LIMITS DIRECTLY
FROM RAW CONCENTRATION MEASUREMENTS.

THE OUTPUT CONSISTS OF A TABLE SHOWING EACH SAMPLE
TIME (IN SECONDS), THE MEAN CONCENTRATION (AT TEMPERATURE),
THE CALCULATED (REGRESSION) CONCENTRATION, AND THE
DIFFERENCE BETWEEN THE TWO CONCENTRATIONS.

THE OUTPUT ALSO INCLUDES THE SLOPE (AND IT'S 95% CONFIDENCE INTERVAL), THE INTERCEPT, AND THE CORRELATION
COEFFICIENT FOR THE LEAST-SQUARES REGRESSION LINE.

IF THE OUTPUT IS TO BE DIRECTED TO THE PRINTER,
THEN REMOVE THE COMMENT CHARACTER FROM:

FILE 6(KIND=PRINTER,MYUSE=OUT,MAXRECSIZE=22)

AND COMPILE WITH FORTRAN.

A FILE CONTAINING THE X AND Y PARAMETERS WHICH MUST
BE PLOTTED TO OBTAIN K1 IS ALSO GENERATED. THIS FILE CAN
BE USED WITH 'ST/PLOT'. IT IS DEFINED BY:

FILE I(KIND=DISK,TITLE="PLOT15/X4",PROTECTION=SAVE,AREAS=1,*AREA_SIZE=200)

THE INPUT DATA FILE IS DEFINED BY:

FILE 5(KIND=DISK,FILETYPE=7,TITLE="DATA15/X4")

A FILE WITH THE GIVEN TITLE MUST BE CREATED BEFORE
RUNNING THIS PROGRAM. IT MUST CONTAIN A RECORD GIVING
THE REACTION NUMBER.

THE TEMPERATURE AND VOLUME EXPANSIVITY OF THE REACTION,
THE INITIAL CONCENTRATION OF REACTANT (AT TEMPERATURE),
THE REACTANTS RATE OF DISAPPEARANCE (IN MOLES/LITER),
AND THE FINAL CONCENTRATION OF REACTANT (AT TEMPERATURE);
THEN UP TO 20 DATA CARDS (SAMPLE TIME IN MINUTES, NUMBER OF
CONCENTRATION MEASUREMENTS AT THAT TIME, AND UP TO SIX
INDIVIDUAL CONCENTRATION MEASUREMENTS);
AND AN END RECORD OF 0.0000.

NOTE - THE FIRST DATA CARD MUST BE THE TIME ZERO
CARD, EVEN IF THE CONCENTRATION OF THE PRODUCT IS ZERO.

INTEGER NO_X,Y,I,N,NUM
REAL TEMP,CONC(6),TSEC(20),AVG,CHT(20),POINT(20),TEMP,FACT
REAL SUMX,SUMX2,SUMY,SUMY2,SUMXY,M,B,DENOH,CC,R,KR,XVAL(20)
REAL SXY,SSX,SSXY,THETAP,ERROR(20),CALC(20),DIFF(20)
REAL XPT(20),YPT(20),CRF

READ(5,/)NUM,TEMP,FACT,R,KR

PRINT(69010,9)
WRITE(6,'(F10.9)')TEMP

9000 FORMAT(6F10.9)
1700 WRITE(6,'(G9.2)')TEMP
4800 FORMAT(1H /, 'THE TEMPERATURE OF THE REACTION WAS ',E8.2/)}
4900 WRITE(6,'(G9.2)')R
5000 FORMAT(* THE INITIAL REACTANT CONCENTRATION WAS ',E8.4/)}
5100 WRITE(6,'(G9.2)')XVAL(20)
5200 FORMAT(* THE RATE OF DISAPPEARANCE OF REACTANT WAS ',E12.4/)
C THIS LOOP FINDS THE MEAN OBSERVED CONCENTRATIONS
9500 READ (5,/) TMIN,NO,(CONC(I),I=1,NO)
9504 FORMAT(10,1X,I4,GF10.9)
950 C THIS TESTS FOR THE LAST DATA CARD
9600 IF(NO)9,9,2
9610 TSEC(X)=TMIN*60.
9620 XVAL(X)={(R/KR)*(.--EXP(-KR*TSEC(X)))}
9630 X=X+1
9640 AVG=0.
9650 DO 3 I=1,NO
9660 AVG=CONC(I)+AVG
9670 C CHI IS THE MEAN OBSERVED CONCENTRATION
9680 CHI(Y)=(AVG/NO)/TFACT
9690 POINT(Y)=CHI(Y)-CHI(I)
9700 Y=Y+1
9710 GO TO 1
9720 C N IS THE NUMBER OF SAMPLING TIMES (DATA CARDS)
9730 N=Y-1
9740 WRITE(6,9005)N
9750 9005 FORMAT(1H THE NUMBER OF DATA POINTS IS *,I5S/)n
9760 C THIS SECTION FINDS THE REGRESSION PARAMETERS
9770 SUMX=0.
9780 SUMX2=0.
9790 SUMY=0.
9800 SUMY2=0.
9810 SUMXY=0.
9820 DO 10 I=1,N
9830 SUMX=XVAL(I)*SUMX
9840 SUMX2=XVAL(I)*XVAL(I)+SUMX2
9850 SUMY=POINT(I)*SUMY
9860 SUMY2=POINT(I)+SUMY2
9870 10 SUMXY=XVAL(I)*POINT(I)+SUMXY
9880 C CC IS THE CORRELATION COEFFICIENT
9890 DENOM=((SUMX2-SUMX*SUMX)/N)*((SUMY-SUMY)/N)**2.5
9900 CC=(SUMXY*(SUMX*SUMY)/N)/DENOM
9910 C B IS THE INTERCEPT
9920 B=(SUMX2*SUMY-SUMX*SUMXY)/(N*SUMX2-SUMX*SUMX)
9930 C M IS THE SLOPE
9940 M=(SUMXY-SUMX*SUMY)/(N*SUMX2-SUMX*SUMX)
9950 C THIS SECTION FINDS THE CONFIDENCE INTERVAL ON THE SLOPE
9960 SSY=(SUMY2-SUMY/SUMX/N)
9970 SSX=(SUMX2-SUMX/SUMX/N)
9980 SSXY=(SUMXY-SUMX*SUMY/N)
9990 THETA=((SSY-SSXY)/N)**2.5
10000 C THIS IS A TABLE OF STUDENT'S T VALUES AT THE 0.95 LEVEL
10100 TI(1)=12.706
10200 TI(2)=4.303
10300 TI(3)=3.182
10400 TI(4)=2.766
10500 TI(5)=2.571
10600 TI(6)=2.447
10700 TI(7)=2.365
10800 TI(8)=2.306
10900 TI(9)=2.262
11000 TI(10)=2.228
11100 TI(11)=2.201
11200 TI(12)=2.179
11300 TI(13)=2.160
11400 TI(14)=2.145
11500     T(15)=2.131
11600     T(16)=2.120
11700     T(17)=2.110
11800     T(18)=2.101
11900     T(19)=2.093
    200     T(20)=2.086
     .100     ERROR=T(N-2)*THETA*(N/(N-2)*SSX)*.5
12200     C THIS CALCULATES THE REGRESSION CONCENTRATIONS
12300     DO 30 I=1,N
12400       CALC(I)=A+XVAL(I)+B+CHI(I)
12500     30       DIFF(I)=CHI(I)-CALC(I)
12600     C THIS SECTION WRITES THE OUTPUT DAta
12700     WRITE(6,9006)
12800     9006 FORMAT(9 SECONDS CONCENTRATION CALC. CONC. DIFFERENCE*)
12900     WRITE(6,9007)(TSEC(I),CHI(I),CALC(I),DIFF(I),I=1,N)
13000     9007 FORMAT(F10.1,3F15.6)
13100     WRITE(6,9008)
13200     9008 FORMAT(H /* THE SLOPE OF THE REGRESSION LINE IS*E12.4*)
13300     WRITE(6,9009)ERROR
13400     9009 FORMAT(' THE CONFIDENCE INTERVAL ON THE SLOPE IS*E12.4*)
13500     WRITE(6,9010)B
13600     9010 FORMAT(' THE INTERCEPT OF THE LINE IS*E12.4*)
13700     WRITE(6,9011)CC
13800     9011 FORMAT(' THE CORRELATION COEFFICIENT IS*F6.4*)
13802     DO 7 I=1,N
13804       XPT(I)=XVAL(I)/10
13806     7       YPT(I)=POINT(I)*100
13808     WRITE(1,9012)(XPT(I),YPT(I),I=1,N)
13810     9012 FORMAT(F10.4,F10.4)
13900     STOP
14000     END
Program 3: PARALLEL

100 SRESET FREE
200 C CALCULATION OF PRODUCT DISAPPEARANCE USING PARALLEL FIRST-ORDER KINETICS
300 C
400 C WRITTEN BY M. HENDERSON
500 C
600 C This program uses parallel, first-order kinetics to calculate rates of product formation and reactant disappearance from raw product concentrations and generates a file for use in plotting the appearance of product according to parallel first-order kinetics.
700 C
800 C Data is read in from the file Data(No) and defined by:
900 C FILE 5(KIND=DISK, FILETYPE=7, TITLE="DATA13/X4")
1000 C This file must be created before running the program, and contains a lead record giving the reaction number, temperature-volume expansivity factor, initial reactant concentration (at temperature), the reactants rate of disappearance (in moles/liter), and the final reactant concentration (at temperature).
1100 C This record is followed by up to 20 records each containing the time (in minutes), the number of individual concentration measurements at that time, and up to six concentration product measurements. The file must terminate with an end record of 0.0.0.
1200 C
1300 C The output consists of a table listing the mean concentrations of product (at temperature), the calculated concentrations, and the differences as a function of time. The regression parameters of the product disappearance described by parallel, first-order kinetics are also printed.
1400 C If this output is to be directed to the printer, remove the comment character from:
1500 C FILE 6(KIND=PRINTER, NYUSE=OUT, MAXRECSIZE?=22)
1600 C A file containing the x and y values of the regression line is generated on disk. It can be used for plotting and is defined by:
1700 C FILE 1(KIND=DISK, TITLE="PARALLEL/X4", PROTECTION=SAVE, AREAS=1, AREASIZE=200)
1800 C INTEGER NUMX, YNOJN
1900 C REAL TEMPT, FACT, CRO, KR, CRF, TMIN, CORCI(20), TSEC(20), TOT
2000 C REAL MEAN(20), MIN(20), Mi(T20), YPOINT(20), SUMX, SUMX2, SUMY, SUMY2
2100 C REAL SUMXY, CC, DENOM, 9, MESSY, SSX, SSY, THEETA(20), CALC(20)
2200 C REAL DIFF(20), ERROR, XPT(T20), KI
2300 C READ IN PRODUCT CONCENTRATIONS AND CALCULATE MEAN CONCS
2400 C READ(5,/) NUMX, TEMPT, FACT, CRO, KR, CRF
2500 C WRITE(6,9001) NUMX
2600 C 9001 FORMAT(1H/, 'THIS IS REACTION NUMBER', I5,/) / WRITE(6,9002) TEMPT
2700 C 9002 FORMAT(1H/, 'THE REACTION TEMPERATURE WAS ', F6.2,/) / WRITE(6,9003) CRO
2800 C 9003 FORMAT(1H/, 'THE INITIAL REACTANT CONCENTRATION WAS ', F8.5,/) / WRITE(6,9004) CRF
2900 C 9004 FORMAT(1H/, 'THE FINAL REACTANT CONCENTRATION WAS ', F8.5,/) / WRITE(6,9005) X, Y
3000 1 READ(5,/) TMIN, NOJN(CORCI(J), J=1, NO)
3100 C TEST FOR LAST CARD
IF (NO)>9,9=2
2 TSECC(X)=TMIN=60.
500 TOT=0.
550 DO 3 I=1,NO
560 3 TOT= CONC(I)*TOT
570 MEANIC(X)=(TOT/NO)/FACT
580 X=X+1
590 Y=Y+1
600 GO TO 1
610 9 N=Y-1
700 C This section calculates the parameters to be regressed. MIOO
710 C is the mole fraction of product at infinity. MIT is the mole
720 C fraction of product at some time.
730 C 100=MEANIC(N)/CRO-CRF)
740 DO 6 X=1,N
750 6 MIT(X)=MEANIC(X)/CRO
760 YPOINT(X)=ALOGC(MIOO-MIT(X))
770 C This section calculates a regression line for YPOINT as
780 C a function of time. This provides a value for KR.
790 SUMX=0.
800 SUMX2=0.
810 SUMY=0.
820 SUMY2=0.
830 SUMXY=0.
840 DO 10 I=1,N
850 SUMX=TSEC(I)*SUMX
860 SUMX2=TSEC(I)*TSEC2*SUMX2
870 SUMY=YPOINT(I)*SUMY
880 SUMY2=YPOINT2(I)*YPOINT(I)*SUMY2
890 10 SUMXY=TSEC(I)*YPOINT(I)*SUMXY
900 C CC IS THE CORRELATION COEFFICIENT
910 DENOM=(SUMX2-(SUMX*SUMX)/N)*(SUMY2-(SUMY*SUMY)/N)**.5
920 CC=(SUMXY-(SUMX*SUMY)/N)/DENOM
930 C B IS THE INTERCEPT
940 B=(SUMX2*SUMY-SUMXY*SUMX)/(N*SUMX2-SUMY*SUMX)
950 C M IS THE SLOPE
960 N=(N*SUMXY-SUMX*SUMY)/(N*SUMX2-SUMY*SUMX)
970 C THIS SECTION FINDS THE CONFIDENCE INTERVAL OF THE SLOPE
980 SSY=SUMY2-(SUMY*SUMY)/N
990 SSS=SUMX2-(SUMX*SUMX)/N
1000 SSXY=SUMXY-(SUMX*SUMY)/N
1010 THEETA=(SSXY-M*SSXY)/N)**.5
1020 C THIS IS A TABLE OF T VALUES AT THE 95% LEVEL
1030 TTC=12.706
1040 TTC=4.303
1050 TTC=3.182
1060 TTC=2.776
1070 TTC=2.571
1080 TTC=2.447
1090 TTC=2.365
1100 TTC=2.306
1110 TTC=2.262
1120 TTC=2.228
1130 TTC=2.201
1140 TTC=2.179
1150 TTC=2.160
1160 TTC=2.145
1170 TTC=2.131
1180 TTC=2.120
1190 TTC=2.110
1200 TTC=2.101
1210 TTC=2.093
THE REGRESSION CONCENTRATIONS
DO 30 I=1,N
CALC(I)=CRO*(MEANI(N)/(CRO-CRF))-EXP(N*TSEC(I)+B))
30  DIFF(I)=MEANI(I)-CALC(I)
C This section calculates the rate of product appearance
  KI=-(N*MEANI(N))
C This section calculates the rate of product appearance
  KI=-N*HEANI(N)
THIS SECTION WRITES A TABLE OF TIMES AND CONCENTRATIONS
WRITE(6,9006)
9006 FORMAT(' SECONDS CONCENTRATION CALC. CONC. DIFFERENCE ')
WRITE(6,9007)(TSEC(I),MEANI(I),CALC(I),DIFF(I),I=1,N)
9007 FORMAT(F10.1,3F15.6)
WRITE(6,9008)N
9008 FORMAT(' THE SLOPE OF THE REGRESSION LINE IS*E12.4*/')
WRITE(6,9009)ERROR
9009 FORMAT(' THE 95%CONFIDENCE INTERVAL ON THE SLOPE IS*E12.4*/')
WRITE(6,9010)B
9010 FORMAT(' THE INTERCEPT OF THE LINE IS*E12.4*/')
WRITE(6,9011)CC
9011 FORMAT(' THE CORRELATION COEFFICIENT IS*F6.4*/')
WRITE(6,9015)KI
9015 FORMAT(' THE RATE OF PRODUCT APPEARANCE IS*E12.4*/')
C THIS SECTION GENERATES A FILE ON DISK CONTAINING YPOINT
DO 12 I=1,N
12  XP(T(I)=TSEC(I)/1000
WRITE(1*,9012)(XP(T(I),YPOINT(I),I=1,N)
9012 FORMAT(2F10.4)
STOP
END
Program 4: MH/UNIVERSAL

LINEAR REGRESSION PROGRAM
FOR ANALYSIS OF THE LAW OF UNIVERSAL
REACTION RATES

BY M. HENDERSON

THIS PROGRAM USES THE UNIVERSAL RATE EQUATION TO FIND THE
ACTIVATION ENERGY OF A REACTION. THE INPUT CONSISTS OF
RATE CONSTANT, TEMPERATURE (C) PAIRS, FOLLOWED BY
A RECORD OF O. THE END RECORD THEN SPECIFIES THE
TEMPERATURE FOR THE ENTROPY, ENTHALPY, AND FREE ENERGY.
CALCULATIONS. THE DATA FILE IS DEFINED BY:

FILE 5(KIND=DISK, FILETYPE=7, TITLE="DATA/KOAW")

THE OUTPUT CONSISTS OF A TABLE SHOWING EACH TEMPERATURE,
THE OBSERVED RATE, THE CALCULATED (REGRESSION) RATE, AND
THE DIFFERENCE BETWEEN THE RATES. THE SLOPE, INTERCEPT,
AND CORRELATION COEFFICIENT OF THE REGRESSION LINE ARE GIVEN.
THE ENTHALPY, ENTROPY, AND FREE ENERGY OF ACTIVATION ARE CALCULATED, WITH
THEIR CONFIDENCE INTERVALS.

IF THE OUTPUT IS TO BE DIRECTED TO THE PRINTER,
THEN REMOVE THE COMMENT CHARACTER FROM:

FILE 6(KIND=PRINTER, TITLE="OUT/UNIVERSAL")

AND COMPILE WITH FORTRAN.

A DATA FILE CONTAINING THE DATA TO BE PLOTTED
IS CREATED ON DISK. IT IS DEFINED BY:

FILE 'PLOT/200'

INTEGER N
REAL XAVE, YAVE, CI, S, SS, SSR, SE, ERROR, TERM
REAL YCALC, DIFF, PC, C20, Y0, X0, XTEMP
REAL SD, SDEV, PDEV, SH, CI, LS

THIS SECTION READS AND MANIPULATES THE INPUT DATA

READ (5,/) K1, TEMP

THIS TESTS FOR THE LAST DATA CARD

IF(TEMP)9,9,9
X(N)=1/(TEMP*273.)
Y(N)=ALOG(K1*X(N))
CC(N)=TEMP
RC(N)=KI
N=N+1
GO TO 1

WRITE(6,9003) N

9003 FORMAT(1H9, THE NUMBER OF DATA POINTS IS 9,151)

THIS SECTION FINDS THE REGRESSION PARAMETERS

SUMX=0.
SUMY=0.
SUMXY=0.
SUMXX=0.
SUNY=

DO 20 I=1,N
5700 20 SUMY=Y(I)+SUMY
5800 XAVE=SUMX/N
5900 YAVE=SUMY/N

DO 50 I=1,N
6000 SXY=SXY + (X(I)-XAVE)*(Y(I)-YAVE)
6100 SXX=SXX + (X(I)-XAVE)**2
6200 SYY=SYY + (Y(I)-YAVE)**2
6210 C CALCULATE RESIDUALS
6220 S2XY=SXY*SXY
6230 SSREG=S2XY/SXX
6240 SSRESI=SYY-SSREG
6250 MEANSQ=(SSRESI/(N-2))**.5
6300 C N IS THE SLOPE
6400 M=SXY/SXX
6500 C B IS THE INTERCEPT
6600 B=YAVE-M*XAVE
6700 C R2 IS THE CORRELATION COEFFICIENT
6800 R2=SSREG/SYY

C THIS IS A TABLE OF STUDENT'S T VALUES AT THE .095 LEVEL
6802 T(1)=12.706
6803 T(2)=4.303
6804 T(3)=3.182
6805 T(4)=2.776
6806 T(5)=2.571
6807 T(6)=2.447
6808 T(7)=2.365
6809 T(8)=2.306
6810 T(9)=2.262
6811 T(10)=2.228
6812 T(11)=2.201
6813 T(12)=2.179
6814 T(13)=2.160
6815 T(14)=2.145
6816 T(15)=2.131
6817 T(16)=2.120
6818 T(17)=2.110
6819 T(18)=2.101
6820 T(19)=2.093
6821 T(20)=2.086

C THIS SECTION FINDS THE CONFIDENCE INTERVAL FOR THE SLOPE
6900 C SS= SYY-(S2XY/SXX)
6910 LS = (SS/(N-2))**.5
7000 CISLSP= (TC(N-2)+LS)/(CSS*.5)
7100 C THIS FINDS THE CONFIDENCE INTERVAL FOR THE INTERCEPT
7110 CIINT=TC(N-2)*(((SUM**2)/(N*SXX))**.5)*LS
7200 C THIS WRITES THE SUMS AND MEANSQ
7300 WRITE(6,800) MEANSQ,SXX,SUMX
7400 800 FORMAT(1H*/'MEANSQ='E12.4' SXX='E14.4' SUMX='E12.4'/)
7500 WRITE(6,8005) SYY
7600 8005 FORMAT(1H*/'SYY='E12.4'/)

C THIS CALCULATES THE REGRESSION RATES
9400 DO 30 I=1,N
9500 YCALC(I)=(M*X(I)+B)
9600 KCALC(I)=EXP(YCALC(I))*(C(I)+273)
9700 30 DIFF(I)=R(I)-KCALC(I)

C THIS SECTION WRITES THE OUTPUT DATA
9900 WRITE(6,9004)
10000 9004 FORMAT(* TEMPERATURE RATE CALC RATE DIFFERENCE
10100 WRITE(6,9005)(C(I)+R(I)-KCALC(I),DIFF(I),I=1,N)
Program 5: TEMP/ADJ

100 $RESET FREE
200 C RATE CONSTANT TEMPERATURE ADJUSTMENT
300 C WRITTEN BY D. BLYTHE
400 C THIS PROGRAM USES THE ARRENHIUS EQUATION TO ADJUST
500 C A RATE CONSTANT FROM ONE TEMPERATURE TO ANOTHER.
600 C THE INPUT CONSISTS OF RECORDS SPECIFYING THE OBSERVED
700 C RATE OF REACTION AND TEMPERATURE (T), THE ACTIVATION
800 C ENERGY OF THE REACTION (CAL), AND THE DESIRED TEMPERATURE.
900 C THE END CARD IS 00,0,0,0.
1000 C THE OUTPUT SHOWS THE INPUT DATA AND THE CALCULATED
1100 C REACTION RATE AT THE DESIRED TEMPERATURE.
1200 C IF THE OUTPUT IS TO BE DIRECTED TO THE PRINTER,
1300 C THEN REMOVE THE COMMENT CARD FROM:
1400 C FILE 6 (KIND=PRINTER,MYUSE=OUT,MAXRECS=22)
1500 C AND COMPILe WITH FORTRAN
1600 C REAL TSET,K1,T1,KSET,ACTE
1700 C 8887 FORMAT(1H \*
1800 C INPUT \*
1900 C INPUT \*
2000 C INPUT \*
2100 C KSET)
2200 C READ(5,/)T1,T1,ACTE,TSET
2300 C 9999 FORMAT(2F10.5)
2400 C IF(K1)4,4,3
2500 C KSET=EXP(ALOG(K1)-(ACTE/1.9872)*C1(TSET273.)-1/(T1+273.)))
2600 C WRITE(6,8888)T1,K1,ACTE,TSET,KSET
2700 C 8888 FORMAT(F10.2,E15.5,F14.0,E15.2,E15.5)
2800 C GO TO 1
2900 C 4 STOP
3000 C END
Program 6: ALL/AN

This HP-41 program converts peak areas obtained from the gc to concentrations according to the following equation:

$$M_x = \left[ \frac{\frac{M_{IS}}{F_v}}{\rho x} \right] \left[ \frac{W_{IS}}{\rho IS} \right] \left[ A_{IS} \cdot F_{xi} \right]$$

where $M_x$ = molarity of component i

$M_{IS}$ = molarity of internal standard solution

$F_v$ = expansivity factor

$\rho IS$ = density of internal standard solution

$\rho x$ = density of sample

$W_{IS}$ = weight of internal standard added

$x$ = weight of sample

$A_{IS}$ = area of internal standard

$A_{xi}$ = area of component i

$F_{xi}$ = response factor for component i

Registers used: 05 $M_{IS}$ 06 $\rho x$ 07 $\rho IS$ 08 $F_v$ 09 $\frac{M_{IS} \rho x}{F_v \rho IS}$

10 $\frac{W_{IS}}{W x A_{IS}}$ 11 $M_{bitl}$ 12 $M_{nitr}$ 13 $F_{LM}$ 14 $F_x$

Program Steps:

1. GTO ALLAN
2. RCL 05
3. Enter
4. RCL 06
5. $	imes$
6. RCL 07
7. 
8. RCL 08
9. $rac{1}{x}$
10. STO 09
11. WIS? Enter
12. XEQ
13. Prompt
14. Alpha
15. Enter
16. X=0?
17. GTO 02
18. RCL 11
19. $	imes$
20. XEQ A
21. LBL 02
22. Prompt
23. Alpha
24. AMNITL?
25. Enter
26. X=0?
27. GTO 03
28. RCL 12
29. X
30. XEQ A
31. LBL 03
32. Prompt
33. Alpha
34. ALM?
35. Enter
36. X=0?
37. GTO 04
38. RCL 13
39. $	imes$
XEQ A
LBL 04
[Alpha] AX? Alpha
XEQ
[Alpha] Prompt Alpha
Enter
X=0?
GTO 05
[Alpha] FX? Alpha
XEQ
[Alpha] Prompt Alpha
x
XEQ A
GTO 05
LBL A
RCL 10
x
RCL 09
x
R/S
RTN
LBL 05
END
Program 7: 018AN.BAS

10 REM This program calculates the fraction of the mannitol which forms from OA cleavage based on the amount of 018 incorporated into it.
20 REM
30 DIM A(25), T(25), U(25), V(25)
40 INPUT "Label for this degradation run ", LABEL$
50 'PRINT LABEL'
60 LPRINT "This is degradation "; LABEL$
70 REM This section calculates the fractions of the various isotopes present in natural mannitol.
80 'Print Prompt'
90 PRINT "Enter no. counts for natural mannitol"
100 INPUT "No. counts at M=548 "; M548
110 INPUT "No. counts at M=549 "; M549
120 INPUT "No. counts at M=550 "; M550
130 INPUT "No. counts at M=551 "; M551
140 'Print Header and Label'
150 LPRINT "This is the natural MNT for degradation "; LABEL$
160 LPRINT "Pass Alpha M M017 M018"
170 A(1)= .15
180 I=0
190 'I=I+1'
200 'Calculate M017 and M018'
210 M=M548
220 M017=M549-(A(I)* M)
230 M018=M550-(A(I)* M017)
240 'Write values from this pass'
250 LPRINT USING "## "; I;
260 LPRINT USING "#.#### "; A(I);
270 'Calculate new A'
280 A(I+1)= M551/M018
290 'Compare last two A values'
300 IF I<25, GOTO 330
310 PRINT "25 passes, no convergence"
320 GOTO 1400
330 DIFA=ABS(A(I)-A(I+1))
340 IF DIFA > .0001, GOTO 190
345 ALPHA = A(I+1)
350 'Calculate Isotope Fractions'
360 MC13= ALPHA * M
370 MC13017 = ALPHA * M017
380 MC13018 = ALPHA * M018
390 MTOT = M+M017+M018+MC13+MC13017+MC13018
400 BETA = M017/MTOT
410 GAMMA = M018/MTOT
420 DELTA = M/MTOT
430 REM End of this section
440 REM Calculation of the numbers of various MBT species
450 RTOT = 100
460 R017 = BETA * RTOT
470 R018 = GAMMA * RTOT
480 R = DELTA * RTOT
490 RC13 = ALPHA * R
500 RC13017 = ALPHA * R017
510 RC13018 = ALPHA = RO18
520 REM End of this Section
530 REM This section inputs the counts observed for the
540 REM experimentally observed mixture of natural and
550 REM enriched mannitol and normalizes them.
560 PRINT "Enter ma counts for product mannitol"
570 INPUT "No. counts at M=548"; M548P
580 INPUT "No. counts at M=549"; M549P
590 INPUT "No. counts at M=550"; M550P
600 INPUT "No. counts at M=551"; M551P
610 ETOT = M548P + M549P + M550P + M551P
620 NORMF = ETOTP/ETOT
630 E548 = M548P/NORMF
640 E549 = M549P/NORMF
650 E550 = M550P/NORMF
660 E551 = M551P/NORMF
670 REM End of this section
680 REM This section calculates the fractions of the various
690 REM isotopic species present in the liquor.
700 'Print Prompt'
710 PRINT "Enter me counts for liquor"
720 INPUT "No. counts at M=16"; M16
730 INPUT "No. counts at M=17"; M17
740 INPUT "No. counts at M=18"; M18
750 INPUT "No. counts at M=19"; M19
760 HTOT = M16 + M17 + M18 + M19 + M20
770 'Enter initial values for T, U, and V'
780 T(1) = .75
790 U(1) = .2
800 V(1) = .05
810 'Print header and label'
820 LPRINT
830 LPRINT "This is the liquor for deg. " LABELS
840 LPRINT "Pass T U V 016 017 018"
850 I = 0
860 'Compare last two T, U, and V values'
870 IF I<25, GOTO 1070
880 DIFT = ABS(T(I)-T(I+1))
890 IF DIFT > .0002, GOTO 900
900 DIFU = ABS(U(I)-U(I+1))
910 IF DIFU > .0002, GOTO 900
920 'Calculate new T, U, and V'
930 V(I+1) = M16/T(I)
940 U(I+1) = (M17-(U(I)*V(I)))/T(I)
950 'Calculate 016, 017, and 018'
960 O16 = (M18-(V(I)*O17)-(V(I)*V(I)))/T(I)
970 O17 = (M19-(U(I)*O17))/T(I)
980 O18 = (M20-(U(I)*V(I)))/T(I)
990 'Compare last two T, U, and V values'
1000 IF I<25, GOTO 1070
1010 DIFT = ABS(T(I)-T(I+1))
1020 DIFU = ABS(U(I)-U(I+1))
1030 IF DIFT > .0002, GOTO 900
1040 IF DIFU > .0002, GOTO 900
1050 LPRINT "25 PASSES, NO CONVERGENCE"
1110 DIFV = ABS(V(I) - V(I+1))
1120 IF DIFV > .0002, GOTO 900
1130 'Now calculate fractions of various species'
1140 C = 016/HTOT
1150 D = 017/HTOT
1160 E = 018/HTOT
1170 REM End of liquor calculation section
1180 REM This section calculates OA, the fraction of cleavage
1190 REM at the oxygen-aglycon bond.
1195 LPRINT
1200 LPRINT "These are OA fractions for Deg. "; LABEL$
1210 'Calculate OA from M548 measurement'
1220 OA548 = (E548-R)/(R*C-R)
1230 LPRINT "From M548, OA = "
1240 LPRINT USING "###.####"; OA548
1250 'Calculate OA from M549 measurement'
1260 OA549 = (E549- (RC13 + R017))/(RC13*C-RC13*R+D*R017*C-R017)
1270 LPRINT "From M549, OA = "
1280 LPRINT USING "###.####"; OA549
1290 'Calculate OA from M550 measurement'
1300 NUM = E550 - R018-RC13017
1310 DEN = R*E + R018*C + RC13*D + RC13017*C + R017*D - R018-RC13017
1320 OA550 = NUM/DEN
1330 LPRINT "From M550, OA = ";
1340 LPRINT USING "###.####"; OA550
1350 END
APPENDIX II

MASS SPECTRA

Table 17. CI mass spectrum of TFA derivative of 1,5-anhydro-D-mannitol, OV-1 column.

<table>
<thead>
<tr>
<th>M/E</th>
<th>Intensity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>577</td>
<td>2.1</td>
<td>M+29</td>
</tr>
<tr>
<td>549</td>
<td>49.7</td>
<td>M+1</td>
</tr>
<tr>
<td>435</td>
<td>100.0</td>
<td>M-113</td>
</tr>
<tr>
<td>321</td>
<td>8.8</td>
<td>M-114-113</td>
</tr>
<tr>
<td>115</td>
<td>16.2</td>
<td>Protonated TFA</td>
</tr>
</tbody>
</table>

Table 18. CI mass spectrum of TFA derivative of product 1,5-anhydro-D-iditol, OV-1 column.

<table>
<thead>
<tr>
<th>M/E</th>
<th>Intensity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>549</td>
<td>11.6</td>
<td>M+1</td>
</tr>
<tr>
<td>435</td>
<td>53.8</td>
<td>*,a</td>
</tr>
<tr>
<td>321</td>
<td>8.1</td>
<td>*,b</td>
</tr>
<tr>
<td>207</td>
<td>6.1</td>
<td>*,c</td>
</tr>
<tr>
<td>115</td>
<td>100.0</td>
<td>Protonated TFA</td>
</tr>
</tbody>
</table>

*aCorresponds to the protonated molecular ion which has lost (a) one, (b), two, or (c) three TFA groups.

Table 19. NCI mass spectrum of TFA derivative of authentic 1,5-anhydro-D-iditol.

<table>
<thead>
<tr>
<th>M/E</th>
<th>Intensity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>548</td>
<td>3.6</td>
<td>M</td>
</tr>
<tr>
<td>435</td>
<td>1.1</td>
<td>M-TFA anion</td>
</tr>
<tr>
<td>275</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>227</td>
<td>100.0</td>
<td>TFA+TFA anion</td>
</tr>
<tr>
<td>113</td>
<td>37.0</td>
<td>TFA anion</td>
</tr>
</tbody>
</table>
Table 20. NCI mass spectrum of TFA derivative of product 1,5-anhydro-D-iditol.

<table>
<thead>
<tr>
<th>M/E</th>
<th>Intensity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>548</td>
<td>3.1</td>
<td>M-1</td>
</tr>
<tr>
<td>275</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>227</td>
<td>100.0</td>
<td>TFA+TFA anion</td>
</tr>
<tr>
<td>113</td>
<td>42.0</td>
<td>TFA anion</td>
</tr>
</tbody>
</table>

Table 21. EI mass spectrum of the TFA derivative of authentic 1,5-anhydro-D-iditol.

<table>
<thead>
<tr>
<th>M/E</th>
<th>Intensity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>320</td>
<td>11.5</td>
<td>M-2TFAH</td>
</tr>
<tr>
<td>207</td>
<td>15.9</td>
<td>M-2TFAH-TFA</td>
</tr>
<tr>
<td>193</td>
<td>33.8</td>
<td>M-CH$_2$OC(CF$_3$)O</td>
</tr>
<tr>
<td>97</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>100.0</td>
<td>CF$_3^+$</td>
</tr>
</tbody>
</table>

Table 22. EI mass spectrum of the TFA derivative of product 1,5-anhydro-D-iditol.

<table>
<thead>
<tr>
<th>M/E</th>
<th>Intensity</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>320</td>
<td>7.6</td>
<td>M-2TFAH</td>
</tr>
<tr>
<td>207</td>
<td>10.4</td>
<td>M-2TFAH-TFA</td>
</tr>
<tr>
<td>193</td>
<td>23.8</td>
<td>M-CH$_2$OC(CF$_3$)O</td>
</tr>
<tr>
<td>97</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>100.0</td>
<td>CF$_3^+$</td>
</tr>
</tbody>
</table>
APPENDIX III
NUCLEAR MAGNETIC RESONANCE SPECTRA

Figure 22. 13C-NMR spectrum of 2-acetoxethyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside tetraacetate in CDCl₃.
Figure 24. $^{13}$C-NMR spectrum of 2-hydroxyethyl 1-thio-β-D-glucopyranoside in D$_2$O.
Figure 25. 13C-NMR spectrum of 1,5-anhydro-2,3,4,6-tetra-O-acetyl-D-mannitol in CDCl₃.
Figure 26. $^{13}$C-NMR spectrum of 1,5-anhydro-D-mannitol in D$_2$O.
Figure 27. $^{13}$C-NMR spectrum of mannosiose in D$_2$O.
<table>
<thead>
<tr>
<th>Position (ppm)</th>
<th>Assignment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>170.21</td>
<td></td>
</tr>
<tr>
<td>170.09</td>
<td></td>
</tr>
<tr>
<td>169.69</td>
<td></td>
</tr>
<tr>
<td>169.27</td>
<td></td>
</tr>
<tr>
<td>167.98</td>
<td></td>
</tr>
<tr>
<td>97.97</td>
<td>$\alpha-1$, $\beta-1$</td>
</tr>
<tr>
<td>90.59</td>
<td>$\beta-1'$</td>
</tr>
<tr>
<td>90.35</td>
<td>$\alpha-1'$</td>
</tr>
<tr>
<td>78.40</td>
<td>CDC13</td>
</tr>
<tr>
<td>77.11</td>
<td>CDC13</td>
</tr>
<tr>
<td>75.08</td>
<td>CDC13, $\alpha-4'$, $\beta-4'$</td>
</tr>
<tr>
<td>72.54</td>
<td>$\alpha-2$, $\beta-2$, $\beta-3'$ or $\beta-5'$</td>
</tr>
<tr>
<td>71.07</td>
<td>$\beta-3$, $\alpha-3$, $\beta-3'$ or $\beta-5'$</td>
</tr>
<tr>
<td>70.72</td>
<td>$\beta-5$, $\alpha-5$, $\beta-5'$</td>
</tr>
<tr>
<td>68.73</td>
<td>$\alpha-2'$, $\alpha-3'$, $\beta-2'$</td>
</tr>
<tr>
<td>65.98</td>
<td>$\alpha-4$, $\beta-4$</td>
</tr>
<tr>
<td>62.46</td>
<td>$\alpha-6$, $\beta-6$, $6'$</td>
</tr>
<tr>
<td>20.86</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>20.68</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>0.29</td>
<td>TMS</td>
</tr>
<tr>
<td>0.00</td>
<td>TMS</td>
</tr>
</tbody>
</table>

Figure 28. $^{13}$C-NMR spectrum of mannobiose octaacetate in CDC$_3$. 
Figure 29. $^{13}$C-NMR spectrum of 1,5-anhydro-2,3,6-tri-O-acetyl-4-O-$\beta$-\((2,3,4,6$-\text{tetra-O-acetyl$-\beta$-mannopyranosyl})$-\text{D-mannitol.}$
Figure 30. $^{13}$C-NMR spectrum of 1,5-anhydro-4-O-β-mannopyranosyl-D-mannitol in D$_2$O.
<table>
<thead>
<tr>
<th>Resonance (ppm)</th>
<th>Assignment $^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>101.9</td>
<td>C-1</td>
</tr>
<tr>
<td>76.6</td>
<td>C-5</td>
</tr>
<tr>
<td>72.4</td>
<td>C-4</td>
</tr>
<tr>
<td>71.1</td>
<td>C-3</td>
</tr>
<tr>
<td>66.8</td>
<td>C-2</td>
</tr>
<tr>
<td>65.8</td>
<td>C-6</td>
</tr>
</tbody>
</table>

Figure 31. $^{13}$C-NMR spectrum of 1,6-anhydro-β-D-mannopyranose in D$_2$O.
Figure 32. $^{13}$C-NMR spectrum of 1,5-anhydro-2,3,6-tri-O-methyl-4-O-$\beta$-(2,3,4,6-tetra-O-methyl-mannopyranosyl)-D-mannitol in CDC$_3$. 
Figure 32. 13C-NMR spectrum of 2-methoxyethyl 2,3,4,6-tetra-O-methyl-1-thio-β-D-glucopyranoside in CDCl₃.
Table 23. Stability of 1,5-anhydro-D-mannitol (0.01M) in 2.553M sodium hydroxide at 180.36 ± 0.09°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MNT]$^a$, M (\times 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0105</td>
</tr>
<tr>
<td>1440.0</td>
<td>0.0100</td>
</tr>
<tr>
<td>2880.0</td>
<td>0.0100</td>
</tr>
<tr>
<td>4320.0</td>
<td>0.0102</td>
</tr>
<tr>
<td>5760.0</td>
<td>0.0101</td>
</tr>
<tr>
<td>7200.0</td>
<td>0.0097</td>
</tr>
<tr>
<td>8640.0</td>
<td>0.0103</td>
</tr>
<tr>
<td>10080.0</td>
<td>0.0101</td>
</tr>
<tr>
<td>11520.0</td>
<td>0.0099</td>
</tr>
<tr>
<td>12960.0</td>
<td>0.0094</td>
</tr>
<tr>
<td>14400.0</td>
<td>0.0097</td>
</tr>
<tr>
<td>15840.0</td>
<td>0.0100</td>
</tr>
<tr>
<td>17280.0</td>
<td>0.0096</td>
</tr>
<tr>
<td>18720.0</td>
<td>0.0096</td>
</tr>
<tr>
<td>21600.0</td>
<td>0.0095</td>
</tr>
</tbody>
</table>

$^a$[MNT] = concentration of 1,5-anhydro-D-mannitol at reaction temperature.
Table 24. Degradation of 1,5-anhydromannobiitol (0.01M) in 2.500M NaOH at 171.13 ± 0.1°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>[MBT]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>[X4]&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>11.069</td>
<td>0.130</td>
<td>0.000</td>
</tr>
<tr>
<td>120.0</td>
<td>10.912</td>
<td>0.476</td>
<td>0.058</td>
</tr>
<tr>
<td>240.0</td>
<td>10.117</td>
<td>0.833</td>
<td>0.063</td>
</tr>
<tr>
<td>360.0</td>
<td>9.780</td>
<td>1.159</td>
<td>0.094</td>
</tr>
<tr>
<td>480.0</td>
<td>9.167</td>
<td>1.515</td>
<td>0.108</td>
</tr>
<tr>
<td>720.0</td>
<td>8.601</td>
<td>2.129</td>
<td>0.168</td>
</tr>
<tr>
<td>960.0</td>
<td>7.839</td>
<td>2.745</td>
<td>0.216</td>
</tr>
<tr>
<td>1440.0</td>
<td>6.631</td>
<td>3.585</td>
<td>0.256</td>
</tr>
<tr>
<td>1800.0</td>
<td>5.834</td>
<td>4.325</td>
<td>0.329</td>
</tr>
<tr>
<td>2160.0</td>
<td>5.076</td>
<td>4.834</td>
<td>0.382</td>
</tr>
<tr>
<td>2880.0</td>
<td>3.914</td>
<td>6.020</td>
<td>0.527</td>
</tr>
<tr>
<td>3600.0</td>
<td>3.010</td>
<td>6.418</td>
<td>0.524</td>
</tr>
</tbody>
</table>

-\[
  k_r = 6.05 \pm 0.10 \times 10^{-6}, \text{sec}^{-1}.
\]

-\[
  k_{\text{MNT}} = 4.81 \pm 0.13 \times 10^{-6}, \text{sec}^{-1}.
\]

-\[
  k_{\text{X4}} = 0.267 \pm 0.025 \times 10^{-6}, \text{sec}^{-1}.
\]

MNT from OA cleavage = 5.05%.

-\[
  k_{\text{GO}} = 4.57 \pm 0.13 \times 10^{-6}, \text{sec}^{-1}.
\]

-\[
  k_{\text{OA}} = 1.48 \pm 0.16 \times 10^{-6}, \text{sec}^{-1}.
\]

---

<sup>a</sup>MBT = concentration of 1,5-anhydromannobiitol at reaction temperature, M x 10<sup>3</sup>.

<sup>b</sup>MBT = concentration of 1,5-anhydro-D-mannitol at reaction temperature, M x 10<sup>3</sup>.

<sup>c</sup>X4 = concentration of 1,5-anhydro-D-iditol at reaction temperature, M x 10<sup>3</sup>, calculated using MNT response factor.
Table 25. Degradation of 1,5-anhydromannobitol (0.01M) in 2.508M NaOH at 162.18 ± 0.1°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]a</th>
<th>[MBT]b</th>
<th>[X4]c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>9.078</td>
<td>0.073</td>
<td>0.000</td>
</tr>
<tr>
<td>360.0</td>
<td>8.992</td>
<td>0.391</td>
<td>0.032</td>
</tr>
<tr>
<td>720.0</td>
<td>8.029</td>
<td>0.840</td>
<td>0.073</td>
</tr>
<tr>
<td>1080.3</td>
<td>7.702</td>
<td>1.172</td>
<td>0.100</td>
</tr>
<tr>
<td>1440.0</td>
<td>7.115</td>
<td>1.332</td>
<td>0.091</td>
</tr>
<tr>
<td>1800.3</td>
<td>7.053</td>
<td>1.698</td>
<td>0.136</td>
</tr>
<tr>
<td>2160.0</td>
<td>6.676</td>
<td>1.939</td>
<td>0.159</td>
</tr>
<tr>
<td>2880.0</td>
<td>5.985</td>
<td>2.734</td>
<td>0.206</td>
</tr>
<tr>
<td>3600.3</td>
<td>5.849</td>
<td>3.256</td>
<td>0.213</td>
</tr>
<tr>
<td>4320.0</td>
<td>4.864</td>
<td>3.615</td>
<td>0.286</td>
</tr>
<tr>
<td>5040.0</td>
<td>4.378</td>
<td>3.919</td>
<td>0.282</td>
</tr>
<tr>
<td>5760.3</td>
<td>3.828</td>
<td>4.105</td>
<td>0.332</td>
</tr>
</tbody>
</table>

\[ k_T = 2.42 \pm 0.19 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{MNT}} = 1.99 \pm 0.11 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{X4}} = 0.15 \pm 0.01 \times 10^{-6}, \text{sec}^{-1}. \]

MNT from OA cleavage = 3.89%.

\[ k_{\text{GO}} = 1.91 \pm 0.11 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{OA}} = 0.51 \pm 0.22 \times 10^{-6}, \text{sec}^{-1}. \]

\( a \) [MBT] = concentration of 1,5-anhydromannobitol at reaction temperature, M x 10^3.

\( b \) [MBT] = concentration of 1,5-anhydro-D-mannitol at reaction temperature, M x 10^3.

\( c \) [X4] = concentration of 1,5-anhydro-D-iditol at reaction temperature, M x 10^3, calculated using MNT response factor.
<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>[MBT]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>[X4]&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10.886</td>
<td>0.173</td>
<td>0.000</td>
</tr>
<tr>
<td>60.4</td>
<td>10.364</td>
<td>0.601</td>
<td>0.036</td>
</tr>
<tr>
<td>120.4</td>
<td>9.636</td>
<td>1.026</td>
<td>0.075</td>
</tr>
<tr>
<td>180.0</td>
<td>9.201</td>
<td>1.382</td>
<td>0.095</td>
</tr>
<tr>
<td>240.0</td>
<td>8.877</td>
<td>1.825</td>
<td>0.138</td>
</tr>
<tr>
<td>360.4</td>
<td>7.860</td>
<td>2.549</td>
<td>0.195</td>
</tr>
<tr>
<td>480.0</td>
<td>6.898</td>
<td>3.022</td>
<td>0.234</td>
</tr>
<tr>
<td>600.4</td>
<td>6.234</td>
<td>3.783</td>
<td>0.298</td>
</tr>
<tr>
<td>720.0</td>
<td>5.537</td>
<td>4.232</td>
<td>0.298</td>
</tr>
<tr>
<td>960.0</td>
<td>4.729</td>
<td>4.871</td>
<td>0.377</td>
</tr>
<tr>
<td>1200.0</td>
<td>3.659</td>
<td>5.764</td>
<td>0.450</td>
</tr>
<tr>
<td>1440.0</td>
<td>2.882</td>
<td>6.079</td>
<td>0.475</td>
</tr>
</tbody>
</table>

\[
k_r = 15.19 \pm 0.43 \times 10^{-6}, \text{sec}^{-1}.
\]
\[
k_{\text{MNT}} = 11.48 \pm 0.34 \times 10^{-6}, \text{sec}^{-1}.
\]
\[
k_{X4} = 0.92 \pm 0.04 \times 10^{-6}, \text{sec}^{-1}.
\]

MNT from OA cleavage = 5.37%.

\[
k_{GO} = 10.89 \pm 0.34 \times 10^{-6}, \text{sec}^{-1}.
\]
\[
k_{OA} = 4.30 \pm 0.55 \times 10^{-6}, \text{sec}^{-1}.
\]

<br>

<sup>a</sup>[MBT] = concentration of 1,5-anhydromannobiitol at reaction temperature, M x 10<sup>3</sup>.

<sup>b</sup>[MBT] = concentration of 1,5-anhydro-D-mannitol at reaction temperature, M x 10<sup>3</sup>.

<sup>c</sup>[X4] = concentration of 1,5-anhydro-D-iditol at reaction temperature, M x 10<sup>3</sup>, calculated using MNT response factor.
Table 27. Degradation of 1,5-anhydromannobitol (0.01M) in 2.507M NaOH at 191.86 ± 0.1°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]&lt;sup&gt;a&lt;/sup&gt;</th>
<th>[MBT]&lt;sup&gt;b&lt;/sup&gt;</th>
<th>[X4]&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>11.203</td>
<td>0.339</td>
<td>0.000</td>
</tr>
<tr>
<td>30.0</td>
<td>10.258</td>
<td>0.926</td>
<td>0.070</td>
</tr>
<tr>
<td>60.6</td>
<td>8.945</td>
<td>1.471</td>
<td>0.114</td>
</tr>
<tr>
<td>120.0</td>
<td>8.292</td>
<td>2.624</td>
<td>0.211</td>
</tr>
<tr>
<td>180.0</td>
<td>6.866</td>
<td>3.277</td>
<td>0.272</td>
</tr>
<tr>
<td>240.0</td>
<td>6.717</td>
<td>3.861</td>
<td>0.338</td>
</tr>
<tr>
<td>300.0</td>
<td>5.383</td>
<td>4.352</td>
<td>0.404</td>
</tr>
<tr>
<td>360.0</td>
<td>4.431</td>
<td>5.199</td>
<td>0.491</td>
</tr>
<tr>
<td>420.0</td>
<td>4.058</td>
<td>5.370</td>
<td>0.505</td>
</tr>
<tr>
<td>480.0</td>
<td>3.615</td>
<td>6.458</td>
<td>0.565</td>
</tr>
<tr>
<td>600.0</td>
<td>3.023</td>
<td>7.134</td>
<td>0.706</td>
</tr>
<tr>
<td>720.0</td>
<td>1.966</td>
<td>7.323</td>
<td>0.728</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{k}_r &= 38.25 \pm 0.3 \times 10^{-6}, \text{sec}^{-1}. \\
\text{k}_{\text{MNT}} &= 29.78 \pm 0.2 \times 10^{-6}, \text{sec}^{-1}. \\
\text{k}_{\text{X4}} &= 3.00 \pm 0.20 \times 10^{-6}, \text{sec}^{-1}. \\
\text{MNT from OA cleavage} &= 5.51\%. \\
\text{k}_{\text{GO}} &= 28.13 \pm 0.21 \times 10^{-6}, \text{sec}^{-1}. \\
\text{k}_{\text{OA}} &= 10.12 \pm 0.34 \times 10^{-6}, \text{sec}^{-1}.
\end{align*}
\]

\[\text{a}[\text{MBT}] = \text{concentration of 1,5-anhydromannobitol at reaction temperature, } M \times 10^3.\]
\[\text{b}[\text{MBT}] = \text{concentration of 1,5-anhydro-D-mannitol at reaction temperature, } M \times 10^3.\]
\[\text{c}[\text{X4}] = \text{concentration of 1,5-anhydro-D-iditol at reaction temperature, } M \times 10^3, \text{calculated using MNT response factor.}\]
Table 28. Degradation of 1,5-anhydromannobiitol (0.01M) in 1.5M NaOH and 1.0M NaOTs at 170.43°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]a</th>
<th>[MBT]b</th>
<th>[X4]c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10.710</td>
<td>0.108</td>
<td>0.000</td>
</tr>
<tr>
<td>120.0</td>
<td>10.566</td>
<td>0.328</td>
<td>0.000</td>
</tr>
<tr>
<td>240.0</td>
<td>10.166</td>
<td>0.566</td>
<td>0.027</td>
</tr>
<tr>
<td>480.0</td>
<td>9.542</td>
<td>1.033</td>
<td>0.063</td>
</tr>
<tr>
<td>720.0</td>
<td>8.917</td>
<td>1.460</td>
<td>0.081</td>
</tr>
<tr>
<td>960.0</td>
<td>8.558</td>
<td>1.873</td>
<td>0.117</td>
</tr>
<tr>
<td>1440.0</td>
<td>7.552</td>
<td>2.547</td>
<td>0.166</td>
</tr>
<tr>
<td>2160.0</td>
<td>6.190</td>
<td>3.553</td>
<td>0.229</td>
</tr>
<tr>
<td>2880.0</td>
<td>5.234</td>
<td>4.452</td>
<td>0.291</td>
</tr>
<tr>
<td>3600.0</td>
<td>4.403</td>
<td>4.973</td>
<td>0.332</td>
</tr>
</tbody>
</table>

\[ k_r = 4.18 \pm 0.09 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{MNT}} = 3.24 \pm 0.06 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{X4}} = 0.226 \pm 0.008 \times 10^{-6}, \text{sec}^{-1}. \]

MNT from OA cleavage = 4.14%.

\[ k_{\text{GO}} = 3.10 \pm 0.06 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{OA}} = 1.08 \pm 0.11 \times 10^{-6}, \text{sec}^{-1}. \]

---

a[MBT] = concentration of 1,5-anhydromannobiitol at reaction temperature, \( M \times 10^3 \).
b[MBT] = concentration of 1,5-anhydro-D-mannitol at reaction temperature, \( M \times 10^3 \).
c[X4] = concentration of 1,5-anhydro-D-iditol at reaction temperature, \( M \times 10^3 \), calculated using MNT response factor.
Table 29. Degradation of 1,5-anhydromannobitol (0.01M) in 1.0M NaOH and 1.5M NaOTs at 171.20°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT](^a)</th>
<th>[MBT](^b)</th>
<th>[X4](^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10.506</td>
<td>0.084</td>
<td>0.000</td>
</tr>
<tr>
<td>120.0</td>
<td>10.067</td>
<td>0.288</td>
<td>0.031</td>
</tr>
<tr>
<td>240.0</td>
<td>9.892</td>
<td>0.500</td>
<td>--</td>
</tr>
<tr>
<td>480.0</td>
<td>9.212</td>
<td>0.904</td>
<td>0.057</td>
</tr>
<tr>
<td>720.0</td>
<td>8.850</td>
<td>1.366</td>
<td>0.081</td>
</tr>
<tr>
<td>1200.0</td>
<td>7.898</td>
<td>2.107</td>
<td>0.117</td>
</tr>
<tr>
<td>1680.0</td>
<td>6.923</td>
<td>2.606</td>
<td>0.175</td>
</tr>
<tr>
<td>2160.0</td>
<td>6.226</td>
<td>3.330</td>
<td>0.234</td>
</tr>
<tr>
<td>2880.0</td>
<td>5.404</td>
<td>4.223</td>
<td>0.283</td>
</tr>
<tr>
<td>3600.0</td>
<td>4.623</td>
<td>4.793</td>
<td>0.294</td>
</tr>
<tr>
<td>4320.0</td>
<td>3.890</td>
<td>5.373</td>
<td>0.317</td>
</tr>
</tbody>
</table>

\[ k_r = 3.79 \pm 0.1 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{MNT}} = 2.95 \pm 0.07 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{X4}} = 0.19 \pm 0.02 \times 10^{-6}, \text{sec}^{-1}. \]

MNT from OA cleavage = 3.95%.

\[ k_{\text{GO}} = 2.83 \pm 0.07 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{OA}} = 0.96 \pm 0.12 \times 10^{-6}, \text{sec}^{-1}. \]

\(^a\)[MBT] = concentration of 1,5-anhydromannobitol at reaction temperature, \(M \times 10^3\).

\(^b\)[MBT] = concentration of 1,5-anhydro-D-mannitol at reaction temperature, \(M \times 10^3\).

\(^c\)[X4] = concentration of 1,5-anhydro-D-iditol at reaction temperature, \(M \times 10^3\), calculated using MNT response factor.
Table 30. Degradation of 1,5-anhydromannobiitol (0.01M) in 0.5M NaOH and 2.0M NaOTs at 170.85°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]a</th>
<th>[MBT]b</th>
<th>[X4]c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>9.407</td>
<td>0.081</td>
<td>0.000</td>
</tr>
<tr>
<td>180.0</td>
<td>9.178</td>
<td>0.261</td>
<td>0.000</td>
</tr>
<tr>
<td>360.0</td>
<td>8.899</td>
<td>0.454</td>
<td>0.000</td>
</tr>
<tr>
<td>720.0</td>
<td>8.585</td>
<td>0.818</td>
<td>0.031</td>
</tr>
<tr>
<td>1080.0</td>
<td>8.046</td>
<td>1.168</td>
<td>0.045</td>
</tr>
<tr>
<td>1440.0</td>
<td>7.650</td>
<td>1.541</td>
<td>0.094</td>
</tr>
<tr>
<td>2160.0</td>
<td>6.923</td>
<td>2.116</td>
<td>0.108</td>
</tr>
<tr>
<td>2880.0</td>
<td>6.190</td>
<td>2.749</td>
<td>0.148</td>
</tr>
<tr>
<td>3600.0</td>
<td>5.517</td>
<td>3.187</td>
<td>0.175</td>
</tr>
<tr>
<td>4320.0</td>
<td>5.013</td>
<td>3.670</td>
<td>0.198</td>
</tr>
<tr>
<td>5760.0</td>
<td>4.097</td>
<td>4.438</td>
<td>0.243</td>
</tr>
<tr>
<td>7200.0</td>
<td>3.148</td>
<td>5.079</td>
<td>0.296</td>
</tr>
</tbody>
</table>

\[ k_T = 2.49 \pm 0.06 \times 10^{-6}, \sec^{-1}. \]
\[ k_{\text{MNT}} = 2.01 \pm 0.02 \times 10^{-6}, \sec^{-1}. \]
\[ k_{\text{X4}} = 0.16 \pm 0.01 \times 10^{-6}, \sec^{-1}. \]
\[ \text{MNT from OA cleavage} = 3.54\%. \]
\[ k_{\text{GO}} = 1.94 \pm 0.02 \times 10^{-6}, \sec^{-1}. \]
\[ k_{\text{OA}} = 0.55 \pm 0.06 \times 10^{-6}, \sec^{-1}. \]

\[ a[\text{MBT}] = \text{concentration of 1,5-anhydromannobiitol at reaction temperature, } \ M \times 10^3. \]
\[ b[\text{MBT}] = \text{concentration of 1,5-anhydro-D-mannitol at reaction temperature, } \ M \times 10^3. \]
\[ c[X4] = \text{concentration of 1,5-anhydro-D-iditol at reaction temperature, } \ M \times 10^3, \text{ calculated using MNT response factor.} \]
Table 31. Degradation of 1,5-anhydromannobiitol (0.01M) in 0.5070M NaOH at 171.35 ± 0.1°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]a</th>
<th>[MBT]b</th>
<th>[X4]c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10.809</td>
<td>0.099</td>
<td>0.000</td>
</tr>
<tr>
<td>360.0</td>
<td>10.422</td>
<td>0.431</td>
<td>0.000</td>
</tr>
<tr>
<td>720.0</td>
<td>9.883</td>
<td>0.845</td>
<td>0.040</td>
</tr>
<tr>
<td>1080.0</td>
<td>9.434</td>
<td>1.303</td>
<td>0.054</td>
</tr>
<tr>
<td>1440.0</td>
<td>9.012</td>
<td>1.712</td>
<td>0.076</td>
</tr>
<tr>
<td>2160.0</td>
<td>8.329</td>
<td>2.372</td>
<td>0.117</td>
</tr>
<tr>
<td>2890.0</td>
<td>7.412</td>
<td>3.028</td>
<td>0.148</td>
</tr>
<tr>
<td>3600.0</td>
<td>6.891</td>
<td>3.706</td>
<td>0.220</td>
</tr>
<tr>
<td>4320.0</td>
<td>6.155</td>
<td>4.187</td>
<td>0.252</td>
</tr>
<tr>
<td>5041.5</td>
<td>5.588</td>
<td>4.429</td>
<td>0.256</td>
</tr>
<tr>
<td>5760.0</td>
<td>5.310</td>
<td>5.171</td>
<td>0.270</td>
</tr>
<tr>
<td>6480.0</td>
<td>4.672</td>
<td>5.602</td>
<td>0.332</td>
</tr>
<tr>
<td>7200.0</td>
<td>4.205</td>
<td>5.894</td>
<td>0.364</td>
</tr>
<tr>
<td>7920.0</td>
<td>3.962</td>
<td>6.047</td>
<td>0.355</td>
</tr>
</tbody>
</table>

\[ k_T = 2.16 \pm 0.05 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{MNT}} = 1.91 \pm 0.05 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{X4}} = 0.122 \pm 0.009 \times 10^{-6}, \text{sec}^{-1}. \]
\[ \text{MNT from OA cleavage} = 3.81\%. \]
\[ k_{\text{GO}} = 1.84 \pm 0.05 \times 10^{-6}, \text{sec}^{-1}. \]
\[ k_{\text{OA}} = 0.32 \pm 0.07 \times 10^{-6}, \text{sec}^{-1}. \]

\[ a \] [MBT] = concentration of 1,5-anhydromannobiitol at reaction temperature, M x 10^{3}.
\[ b \] [MBT] = concentration of 1,5-anhydro-D-mannitol at reaction temperature, M x 10^{3}.
\[ c \] [X4] = concentration of 1,5-anhydro-D-iditol at reaction temperature, M x 10^{3}, calculated using MNT response factor.
Table 32. Degradation of 1,5-anhydromannobitol (0.01M) in 1.506M NaOH and 1.10M SH\textsuperscript{-} at 171.65°C.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>[MBT]\textsuperscript{a}, (M \times 10^3)</th>
<th>[MNT]\textsuperscript{b}, (M \times 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>10.069</td>
<td>0.102</td>
</tr>
<tr>
<td>120.0</td>
<td>9.721</td>
<td>0.377</td>
</tr>
<tr>
<td>240.0</td>
<td>9.200</td>
<td>0.668</td>
</tr>
<tr>
<td>480.0</td>
<td>8.598</td>
<td>1.155</td>
</tr>
<tr>
<td>720.0</td>
<td>7.844</td>
<td>1.617</td>
</tr>
<tr>
<td>960.0</td>
<td>7.245</td>
<td>2.046</td>
</tr>
<tr>
<td>1200.4</td>
<td>6.586</td>
<td>2.471</td>
</tr>
<tr>
<td>1440.4</td>
<td>6.222</td>
<td>2.808</td>
</tr>
<tr>
<td>2160.0</td>
<td>5.153</td>
<td>3.535</td>
</tr>
<tr>
<td>2880.0</td>
<td>4.111</td>
<td>4.465</td>
</tr>
<tr>
<td>3600.0</td>
<td>3.311</td>
<td>4.861</td>
</tr>
<tr>
<td>5040.0</td>
<td>2.192</td>
<td>5.753</td>
</tr>
</tbody>
</table>

\(k_r = 5.03 \pm 0.1 \times 10^{-6}, \text{sec}^{-1}\).  
\(k_{\text{MNT}} = 3.59 \pm 0.1 \times 10^{-6}, \text{sec}^{-1}\).  
MNT from OA cleavage = 0.07%.  
\(k_{\text{GO}} = 3.59 \pm 0.10 \times 10^{-6}, \text{sec}^{-1}\).  
\(k_{\text{OA}} = 1.44 \pm 0.18 \times 10^{-6}, \text{sec}^{-1}\).  

\(\text{a}[\text{MBT}] = \text{concentration of 1,5-anhydromannobitol at reaction temperature, } M \times 10^3\).  
\(\text{b}[\text{MBT}] = \text{concentration of 1,5-anhydro-D-mannitol at reaction temperature, } M \times 10^3\).  

---
APPENDIX V

CALCULATION OF THE FRACTIONS OF GLYCOSYL-OXYGEN AND OXYGEN-AGLYCON CLEAVAGE
FROM THE AMOUNT OF OXYGEN-18 INCORPORATION IN 1,5-ANHYDRO-D-MANNUITOL

The product mixture from the degradation of 1,5-anhydromannobiitol in
18O-enriched liquors was derivatized with TFA and analyzed via NCI gc/ms.
Molecular ion peaks were found for each isotopic species of 1,5-anhydro-D-
mannitol and iditol. To calculate the fractions of GO and OA bond cleavage from
these peak areas, it was necessary to take into account the natural isotopes
present, the isotopic distribution of oxygen atoms in the liquor, and the isoto-
opes present in the products. To do this, the problem was set up as shown in
Scheme 1.

\[
\begin{array}{cccccc}
\text{M} & \text{R} & \text{N} & \text{N}_{17} & \text{N}_{18} \\
\text{MC}_{13} & \text{RC}_{13} & \text{NC}_{13} & \text{NC}_{13017} & \text{NC}_{13018} \\
\text{MO}_{17} & \text{RO}_{17} & \text{NO}_{17} & \text{NO}_{17017} & \text{NO}_{17018} \\
\text{MO}_{18} & \text{RO}_{18} & \text{NO}_{18} & \text{NO}_{18017} & \text{NO}_{18018} \\
\text{MC}_{13017} & \text{RC}_{13017} & \text{NC}_{13017} & \text{NC}_{13017017} & \text{NC}_{13017018} \\
\text{MC}_{13018} & \text{RC}_{13018} & \text{NC}_{13018} & \text{NC}_{13018017} & \text{NC}_{13018018} \\
\text{MTOT} & \text{RTOT} & \text{NTOT} \\
\end{array}
\]

where R = number of mannonbiitol molecules which degrade to mannitol.
M = number of mannobiitol molecules resulting from GO bond cleavage.
N = number of mannitol molecules resulting from OA bond cleavage.
C13, O17, and O18 represent the presence of a carbon-13, oxygen-17, or
oxygen-18 atom, respectively.
a = fraction of OA bond cleavage.
b = fraction of GO bond cleavage.
TOT = total number of molecules.

Some reactant molecules naturally contain 13C, 17O, and 18O atoms.
Cleavage of the GO bond will produce 1,5-anhydro-D-mannitol with the same
isotopic distribution present in 1,5-anhydromannobiitol. This can be expressed by Eq. (35a-f).

\[ b \* R = M \] (35a)

\[ b \* RC_{13} = MC_{13} \] (35b)

\[ b \* R_{017} = M_{017} \] (35c)

\[ b \* R_{018} = M_{018} \] (35d)

\[ b \* RC_{13017} = MC_{13017} \] (35e)

\[ b \* RC_{13018} = MC_{13018} \] (35f)

Those 1,5-anhydromannobiitol molecules which cleave at the OA bond must react with a hydroxide ion from solution to form mannitol. Depending upon whether the mannobiitol intermediate happens to react with a hydroxide ion which contains \( ^{16}O \), \( ^{17}O \), or \( ^{18}O \), the mannitol formed may be enriched in \( ^{17}O \) or \( ^{18}O \), as shown in Scheme 1. The fractions of \( ^{16}OH^- \), \( ^{17}OH^- \), and \( ^{18}OH^- \) present (c, d, and e, respectively), determine the chance that an intermediate will react with a given species. For example, an unlabelled mannobiitol molecule, \( R \), could react with \( ^{16}OH^- \) to give unlabelled mannitol (N), with \( ^{17}OH^- \) to give mannitol containing one \( ^{17}O \) atom (N017), or with \( ^{18}OH^- \) to give mannitol containing one \( ^{18}O \) atom (N018). The chance of each of these reactions occurring is expressed by Eq. (36a-c).

\[ a \* R \* c = N \] (36a)

\[ a \* R \* d = NO_{17} \] (36b)

\[ a \* R \* e = NO_{18} \] (36c)

Similar equations can be written for each isotopic mannobiitol species.

In the mass spectra, peaks were found at M/E values of 548, 549, 550, and 551. These are due to a mixture of M and N species as shown in Eq. (37a-d).
M548' = M + N \quad (37a)
M549' = MC13 + M017 + NC13 + N017 \quad (37b)
M550' = M018 + MC13O17 + N018 + NC13O17 + N017O17 \quad (37c)
M551' = MC13O18 + NC13O18 + N018O17 \quad (37d)

Values for the M and N quantities cannot be measured separately. When Eq. (35) and (36) are substituted into Eq. (37), the following set of equations results.

M548' = (b*R) + (a*R*c) \quad (38a)
M549' = (a*RC13*c) + (a*R*d) + (a*R017*c) + (b*RC13) + (b*R017) \quad (38b)
M550' = (a*R*e) + (a*RO18*c) + (a*RC13*d) + (a*RC13017*c) + (a*R017*d) + (b*R018) + (b*RC13017*c) \quad (38c)
M551' = (a*RC13*e) + (a*RC13O18*c) + (A*R017*d) + (b*RC13O18) \quad (38d)

To solve these equations for a and b, it is necessary to determine the values of c, d, and e, the fractions of each isotopic oxygen species present in the liquor, and the isotopic distribution in the reactant, 1,5-anhydromannobitol.

**Liquor**

To determine the fractions of the various isotopic hydroxide ions present in the liquor, the liquors were analyzed on the mass spectrometer. Ions were found at masses of 16 through 20. These must be due to the species shown:

M16 = 0 \quad (39a)
M17 = OH + 017 \quad (39b)
M18 = H2O + 017H + 018 \quad (39c)
M19 = H2O17 + 018H \quad (39d)
M20 = H2O18 \quad (39e)

where M16 through M20 = number of counts observed at that mass.
0, OH, H2O, etc. = number of molecules of that type.
To solve for the fractions of the various species, let:

\[ X = \text{number of all 016 containing species} \]
\[ Y = \text{number of all 017 containing species} \]
\[ Z = \text{number of all 018 containing species} \]
\[ \alpha = \text{fraction present as H}_2\text{O} \]
\[ \beta = \text{fraction present as OH} \]
\[ \delta = \text{fraction present as O} \]

Substituting these values into (39a-e) gives:

\[
\begin{align*}
M_{16} &= \delta \cdot X \\
M_{17} &= \beta \cdot X + \delta \cdot Y \\
M_{18} &= \alpha \cdot X + \beta \cdot Y + \delta \cdot Z \\
M_{19} &= \alpha \cdot Y + \beta \cdot Z \\
M_{20} &= \alpha \cdot Z
\end{align*}
\]

By definition:

\[ \alpha + \beta + \delta = 1 \] (41)

This is a system of 6 equations and 6 unknowns and can therefore be solved by successive approximation.

Now the fraction of each isotopic species of oxygen present can be calculated:

\[
\begin{align*}
\text{HTOT} &= X + Y + Z \\
\frac{X}{\text{HTOT}} &= c = \text{fraction of unlabelled molecules} \\
\frac{Y}{\text{HTOT}} &= d = \text{fraction of 017 labelled molecules}
\end{align*}
\]
\[
\frac{Z}{HTOT} = e = \text{fraction of } \text{O}18 \text{ labelled molecules} \quad (45)
\]

**Unenriched (Natural) Mannitol**

The natural isotopic distribution in 1,5-anhydromannobiitol could not be measured because no molecular ions were found in its mass spectrum. Instead, the isotopic distribution of natural mannitol was measured in a separate experiment and assumed to be the same as that of mannobiitol.

The mass spectrum of natural mannitol contained four molecular ion peaks. These come from the species shown in Eq. (46a-d).

\[
\begin{align*}
M_{548} &= M \\ 
M_{549} &= MC13 + M017 \\ 
M_{550} &= M018 + MC13017 \\ 
M_{551} &= MC13018 
\end{align*} 
\quad (46a-d)
\]

where \( M_{548} \) through \( M_{551} \) = number of counts observed at that mass

- \( M \) = number of MNT molecules containing no isotopes
- \( M017 \) = number of MNT molecules containing one \text{O}17 atom
- \( M018 \) = number of MNT molecules containing one \text{O}18 atom
- \( MC13 \) = number of MNT molecules containing one \text{C}13 atom
- \( MC13017 \) = number of MNT molecules which contain a \text{C}13 and \text{O}17 atom
- \( MC13018 \) = number of MNT molecules which contain a \text{C}13 and \text{O}18 atom

To solve this set of equations, let

\[
\alpha = \text{the fraction of molecules containing a carbon-13 atom},
\]

\[
\alpha = \frac{MC13}{M} = \frac{MC13017}{M017} = \frac{MC13018}{M018} \quad (47)
\]
Substituting (47) into Eq. (46) gives:

\[ M_{548} = M \]  
\[ M_{549} = \alpha \ast M + M_{017} \]  
\[ M_{550} = M_{018} + \alpha \ast M_{017} \]  
\[ M_{551} = \alpha \ast M_{018} \]

This is a system of 4 equations and 4 unknowns so it can be solved by successive approximation.

Once values for variables in Eq. (48) have been determined, the distribution of oxygen isotopes can be calculated.

\[ MTOT = M + MC_{13} + M_{017} + M_{018} + MC_{13017} + MC_{13018} \]

= total number of mannitol molecules

and

\[ \frac{M_{017}}{MTOT} = \beta = \text{fraction of MNT molecules which naturally contain an } 0_{17} \text{ atom} \]

\[ \frac{M_{018}}{MTOT} = \phi = \text{fraction of MNT molecules which naturally contain an } 0_{18} \text{ atom} \]

\[ \frac{M}{MTOT} = \delta = \text{fraction of MNT molecules which naturally contain no isotopes} \]

**Isotopic Distribution in 1,5-Anhydromannobiitol**

This same isotopic distribution can be applied to 1,5-anhydromannobiitol. To do this, choose an arbitrary total number of 1,5-anhydromannobiitol molecules (i.e., 100) to use as a basis.

\[ RTOT = 100 = \text{total number of mannobiitol molecules} \]

\[ = R + RC_{13} + R_{017} + R_{018} + RC_{13017} + RC_{13018} \]
The isotope fractions determined for natural mannitol can now be used to calculate the relative number of each species of mannobiitol present.

\[
\frac{R_{017}}{R_{TOT}} = \beta = \text{fraction of mannobiitol molecules which contain an O17 atom} \tag{54}
\]

or

\[
R_{017} = \beta \times R_{TOT} \tag{54b}
\]

Likewise

\[
R_{018} = \phi \times R_{TOT} \tag{55}
\]

\[
R = \delta \times R_{TOT} \tag{56}
\]

\[
R_{C13} = \alpha \times R \tag{57}
\]

\[
R_{C13017} = \alpha \times R_{017} \tag{58}
\]

\[
R_{C13018} = \alpha \times R_{018} \tag{59}
\]

**Overall**

Except for \(a\) and \(b\), the fractions of cleavage at the OA and GO bond, the variables in Eq. (38a-d) have all been calculated. Since \(a + b\) equals 1, it is possible to solve any of these equations for \(a\) or \(b\). This yields the fraction of cleavage occurring at each bond. Program 7 (Appendix I) performs these calculations.
APPENDIX VI

DERIVATION OF RECIPROCAL HYDROXIDE-RATE RELATIONSHIP FOR GLYCOSYL-OXYGEN BOND CLEAVAGE IN 1,5-ANHYDROMANNOBIIOTOL VIA A MIXED $S_{N^1}cB(2')$-RO/$S_{N^1}$ MECHANISM

The reaction scheme for cleavage of the glycosyl-oxygen bond in 1,5-anhydromannobiitol via a mixed $S_{N^1}cB(2')$-RO/$S_{N^1}$ mechanism is shown in Eq. (60).

\[
\begin{align*}
\text{G-A} & \quad \xrightleftharpoons[k_1]{K_{el}} \quad \text{G-A}^- \\
\text{G-A}^- & \xrightleftharpoons[k_2]{K_{e2}} \quad \text{G-A} \\
\text{P} & \quad \xrightarrow{k_1} \quad \text{G-A} \\
\text{P} & \quad \xrightarrow{k_2} \quad \text{G-A}^- \\
\end{align*}
\]

(60)

where

- $\text{G-A}$ = unionized mannobiitol which undergoes glycosyl-oxygen bond cleavage
- $\text{G-A}^-$ = mannobiitol with an ionized hydroxyl on the glycon which undergoes glycosyl-oxygen bond cleavage
- $\text{G-A}^-$ = mannobiitol with an ionized hydroxyl on the aglycon which undergoes glycosyl-oxygen bond cleavage
- $k_1$ = rate constant for cleavage of $\text{G-A}$ via an $S_{N^1}$ mechanism
- $k_2$ = rate constant for cleavage of $\text{G-A}$ via an $S_{N^1}cB(2')$-RO mechanism
- $k_3$ = rate constant for cleavage of $\text{G-A}$ via an $S_{N^1}$ mechanism
- $\text{P}$ = products from glycosyl-oxygen bond cleavage
- $K_{el}$ = equilibrium constant between $\text{G-A}$ and $\text{G-A}^-$
- $K_{e2}$ = equilibrium constant between $\text{G-A}$ and $\text{G-A}^-$

The equilibrium constants can be expressed as:

\[
K_{el} = \frac{[\text{G-A}^-]}{[\text{G-A}][OH^-]} \quad (61a)
\]

\[
[G-A] = K_{el}[G-A][OH^-] \quad (61b)
\]

\[
K_{e2} = \frac{[G-A^-]}{[G-A][OH^-]} \quad (62a)
\]

\[
[G-A^-] = K_{e2}[G-A][OH^-] \quad (62b)
\]

where $[OH^-]$ = concentration of hydroxide ion.
The appearance of products is described by Eq. (63):

$$\frac{d[P]}{dt} = k_1[G^-A] + k_2[G^-A] + k_3[G-A]$$  \hspace{1cm} (63)

where $[P]$ = concentration of products at time, $t$.

The concentration of glycoside at any time can be expressed as:

$$[G-A]_t = [G-A]_o - [G^-A]_t - [G-A^-]_t - [P]_t$$  \hspace{1cm} (64)

where

- $[G-A]_t$ = concentration of neutral glycoside which degrades via glycosyl-oxygen bond cleavage at any time, $t$
- $[G-A]_o$ = initial concentration of glycoside which undergoes glycosyl-oxygen bond cleavage
- $[G^-A]_t$, $[G-A^-]_t$ = concentrations of conjugate bases of the glycoside at any time, $t$
- $[P]_t$ = concentrations of products of glycosyl-oxygen bond cleavage at any time, $t$

Substituting Eq. (61) and (62) into (64) gives:

$$[G-A]_t = [G-A]_o - (Ke_1[G-A][OH^-]) - (Ke_2[G-A][OH^-]) - [P]_t$$  \hspace{1cm} (65)

Solving Eq. (65) for $[G-A]$ yields:

$$[G-A] = \frac{[G-A]_o - [P]_t}{(1 + Ke_1[OH^-] + Ke_2[OH^-])}$$  \hspace{1cm} (65b)

When (61) is substituted into (63), Eq. (66) results.

$$\frac{d[P]}{dt} = k_1Ke_1[G-A][OH^-] + k_2Ke_1[G-A][OH^-] + k_3[G-A]$$  \hspace{1cm} (66)

Rearranging gives:

$$\frac{d[P]}{dt} = [G-A] \{k_1Ke_1[OH^-] + k_2Ke_1[OH^-] + k_3\}$$  \hspace{1cm} (66b)
An expression for \([G-A]\) is given by Eq. (65b). Use this in Eq. (66b) to obtain an integratable expression for product formation.

\[
\frac{d[P]}{dt} = \frac{([G-A]_o - [P]_t)}{\left(1 + K_{e1}[OH^-] + K_{e2}[OH^-]\right)} \times \left(k_1K_{e1}[OH^-] + k_2K_{e1}[OH^-] + k_3\right) \quad (67)
\]

To simplify Eq. (67), let:

\[
N = k_1K_{e1}[OH^-] + k_2K_{e1}[OH^-] + k_3 \quad (68)
\]

and

\[
D = 1 + K_{e1}[OH^-] + K_{e2}[OH^-] \quad (69)
\]

Substituting (68) and (69) into (67) simplifies it to:

\[
\frac{d[P]}{dt} = \frac{N}{D} \times ([G-A]_o - [P]) \quad (70)
\]

Equation (70) can be rearranged to:

\[
\frac{d[P]}{([G-A]_o - [P])} = \frac{N}{D} \frac{dt}{dt} \quad (70b)
\]

Integrating yields:

\[
-log([G-A]_o - [P]) = \frac{N}{D} \times t + C \quad (71)
\]

When \(t = 0\), \([P] = 0\), so:

\[
C = -log([G-A]_o) \quad (72)
\]

Plugging (72) into (71) and rearranging gives:

\[
log \frac{[G-A]_o - [P]}{[G-A]_o} = -\frac{N}{D} \times t \quad (73)
\]
Letting \([R]' = [G-A]_0 - [P]\) and substituting into Eq. (73) gives:

\[
\log \frac{[R]'}{[G-A]_0} = -\frac{N}{D} * t
\]  

(74)

and

\[
\log \frac{[R]'}{[G-A]_0} = -k_\tau t
\]  

(75)

Comparing Eq. (74) and (75) and expanding \(N\) and \(D\) according to Eq. (68) and (69) shows that:

\[
k_\tau = \frac{\{k_1K_{el}[OH^-] + k_2K_{el}[OH^-] + k_3\}}{\{1 + K_{el}[OH^-] + K_{e2}[OH^-]\}}
\]  

(76)

Assuming that \(k_3 << k_1\) and \(k_2\) reduces Eq. (76) to:

\[
k_\tau = \frac{(k_1 + k_2) K_{el}[OH^-]}{1 + (K_{el} + K_{e2})[OH^-]}
\]  

(77)

Which when inverted gives:

\[
\frac{1}{k_\tau} = \frac{1}{K_{el} (k_1 + k_2)} * \frac{1}{[OH^-]} + \frac{(K_{el} + K_{e2})}{(k_1 + k_2)K_{el}}
\]  

(78)

Equation (78) predicts a linear relationship between \(1/k_\tau\) and \(1/[OH^-]\).