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The Autoxidation of Methyl Glycopyranosides

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THE AUTOXIDATION OF METHYL GLYCOPYRANOSIDES

A thesis submitted by

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SUMMARY

Finely divided, highly purified crystals of methyl \(\beta\)-D-glucopyranoside hemihydrate (hereinafter referred to as MBG), several other glycopyranosides, and \(\alpha\)-D-glucose were heated in the dark at various temperatures and time intervals under compressed oxygen. It was found that these compounds undergo autoxidation (relatively slow chemical reaction with molecular oxygen), this being the first detailed study of that reaction with this class of compounds. Control experiments under nitrogen showed that thermal degradation (as opposed to autoxidation) was very slight under the conditions employed.

Two types of autoxidation can occur with the substances studied. At temperatures below the melting or softening point, a heterogeneous reaction between the crystalline compound and oxygen may take place. For instance, methyl \(\alpha\)-D-glucopyranoside (MAG) was almost quantitatively converted to reaction products after 15.4 hours at 150°C. in a bomb charged with 17.2 atmospheres of oxygen at room temperature (negligible reaction occurred under nitrogen). The melting point of this compound was 168-169°C.; therefore, such a phenomenon could only have been the result of direct reaction between oxygen and the initially crystalline compound. The rate of such a heterogeneous reaction may have a very strong temperature dependence; an approximate value of 50 kcal./mole was deduced for the energy of activation of the heterogeneous autoxidation of MAG. A homogeneous reaction between a molten compound of this type and oxygen dissolved in the melt can occur when the compound is exposed to oxygen at temperatures equal to or above that at which the compound softens or melts, under conditions which insure uniform oxygen concentration in the melt. The heterogeneous reaction, by producing compounds which lower the melting point of the mixture, can gradually lead into the homogeneous reaction by causing the mixture to become molten at the temperature of its surroundings.
The homogeneous autoxidation of MBG under 17.2 atmospheres of oxygen was studied in some detail in the temperature region of 108-130°C. It was found that after the MBG had become molten (which required appreciable time at 108-110°C with the apparatus used), the reaction was well described as being of order 1.5 with respect to MBG concentration over the entire range of MBG concentration. No autocatalysis was observed. The activation energy in this region was 26.4 kcal./mole, which is comparable with the activation energies of other autoxidations. The observed reaction order is consistent with the oxygen-initiation theory of autoxidation; i.e., the reaction is initiated by direct interaction of oxygen with the MBG, rather than by decomposition of reaction products or interaction with trace metals.

The products of the autoxidation of MBG included glucose, gluconic acid and/or its lactones, gentiobiose, traces of other disaccharides, water vapor, small amounts of carbon dioxide, and large amounts of a material presumably similar to the polymeric materials found in autoxidized furfural. Other glycosides appeared to give entirely analogous products upon autoxidation. These products indicated that initial attack probably occurred at the anomeric carbon atom.

A mechanism for the first stages of the homogeneous autoxidation of a glycoside is proposed. This involves attack by oxygen at the hydrogen atom bonded to the anomeric carbon atom, forming a hydroperoxide in a manner similar to the autoxidations of other compounds (such as unsaturated oils). This hydroperoxide could then undergo further reaction to yield the glyconic lactone. It is shown that this mechanism is consistent with the observed kinetics and with the other phenomena which were observed.
INTRODUCTION

The term autoxidation is usually defined as the slow spontaneous reaction of a compound with atmospheric oxygen at room temperature. In this thesis, the reactions of certain methyl glycopyranosides with oxygen were studied at temperatures higher than room temperature but lower than those at which combustion would have occurred; for the sake of convenience, these reactions will be described as autoxidations.

An extremely wide variety of compounds react with oxygen under more or less severe conditions. In the last twenty years, this reaction has come in for a great deal of scrutiny (1). Because of the relative ease of the reaction and its important economic effects (both good and bad), the compounds which have been most thoroughly investigated include the olefins, the drying oils, dietary fats and oils, rubber, and petroleum products. The autoxidations of general organic compounds, such as the ethers, the aldehydes, etc., have received less attention.

In the field of carbohydrate* chemistry, autoxidation is technologically important in at least two areas. The autoxidation ("aging") of alkali cellulose is a vital step in the viscose process of rayon manufacture; the same reaction may also occur during the mercerization of cellulose. The accepted mechanism of alkali cellulose autoxidation was recently challenged by Mattor (2). Another area of importance is in the natural and accelerated aging of paper. It is felt by this author, as a result of this study and other work conducted a few years ago

*The term carbohydrates as used in this thesis includes not only those compounds with empirical formula C(H₂O), but also their simple derivatives.
(2), that autoxidation as a causative factor in the deterioration of paper has not received the attention which it merits. The subject of the natural and accelerated aging of paper is treated at some length in (3).

As far as could be determined from a search of the literature, the autoxidation of simple crystalline carbohydrates has never been investigated. This thesis represents what the author believes to be the first contribution in this field. It is possible that studies such as this one may ultimately prove useful in controlling the technological phenomena described above, apart from their purely scientific interest.

The compound studied in greatest detail here was methyl β-D-glucopyranoside hemihydrate (MBG). This crystalline substance may be regarded as a model for cellulose, since it has the glucopyranose ring and a β-glucosidic linkage. Other compounds studied to a lesser extent include methyl α-D-glucopyranoside (MAG), the anomeric methyl D-galactopyranosides, and methyl α-D-xylopyranoside, as well as α-D-glucose itself. Preparation techniques and physical constants for these compounds are given in Appendix I; the Haworth formulas are shown below.

![Haworth Formulas](image-url)
It should be noted that the autoxidation of these compounds in the \textit{crystalline state} was studied. That is, the pure finely divided crystals were subjected to the action of compressed oxygen. Some of the compounds, when heated, were attacked directly by oxygen without prior melting; others had to be heated to their melting or softening points before observable reaction occurred. In all cases, the effects of a foreign solvent were eliminated. Most previous investigations of autoxidation have dealt with substances which are either liquid at room temperature or in solution. Studies on the autoxidation of pure compounds which are crystalline at room temperature are rare.
THE AUTOXIDATION OF ORGANIC COMPOUNDS IN NONIONIC MEDIA

A summary of many aspects of autoxidation can be found in the two-volume work edited by Lundberg (1). Other reviews are given by Walling (4), Criegee (5), Russell (6), and Waters (7). Some of the more general features of the reaction will be discussed here, along with specific details of the autoxidations of several classes of compounds. This discussion is limited to those autoxidations which occur in nonionic media, as opposed to such reactions as the alkaline autoxidation of cellulose.

GENERAL FEATURES OF AUTOXIDATION

Although the exact electronic structure of molecular oxygen, $O_2$, is somewhat controversial, it is generally agreed (from paramagnetic data) that the molecule in the normal state possesses two unpaired electrons. Many authors draw the structure which first suggests itself, $\cdot O \rightarrow O^\cdot$. Coulson (8) presents the structure of this molecule from the molecular-orbital viewpoint, which gives a different picture but explains the presence of the unpaired electrons on theoretical grounds.

Because of the diradical nature of oxygen and other phenomena observed in autoxidation, this reaction is usually thought of as being a free-radical (as opposed to ionic) process. That is, one-electron transfer reactions are the rule. In support of this theory is the fact that autoxidation usually is initiated at a site in the molecule where homolytic fission of a covalent bond would be expected to occur easily on the basis of other considerations. Carbon-hydrogen bonds are the usual initiation sites; this can be explained on the basis of bond energies and transition-state theory (discussed later).
The course of a typical autoxidation is outlined below. The similarity to other free radical processes, such as addition polymerization, is apparent. As generally conceived, there are three distinct phases in the reaction: initiation, propagation, and termination (all of which are occurring simultaneously in a reacting mass of any appreciable magnitude).

1. Initiation.

Assuming that the substance undergoing autoxidation is represented by the symbol R-H, where H is a labile hydrogen atom at the point of attack, then the creation of the radical R' is required. In the case of photocatalyzed autoxidations, this hydrogen atom may be removed by photolysis of the R-H bond. More often, interaction with an initiating radical, In', is postulated:

\[ R-H + In' \rightarrow R' + In:H \]  

(1)

In cases where direct attack by oxygen occurs, without any other intermediate, then In' is oxygen itself (with two unpaired electrons, rather than one); the perhydroxyl radical, 'O-O-H, is formed as well as R'. When rapid autoxidation of a substance is desired, it is common practice to add compounds such as benzoyl peroxide which readily dissociate into free radicals. The thermodynamics of the initiation phase, as well as the other phases, is discussed later (p. 14).

2. Propagation.

Once it has been created, the radical R' can enter into a cyclic chain-propagation reaction:

\[ R' + O_2 \rightarrow R-O-O' \]  

(2)

\[ R-O-O' + R-H \rightarrow R-O-O-H + R' \]  

(3)
Reactions (2) and (3) are considered to be by far the most frequently occurring reactions in the typical system. Often the hydroperoxide R-O-O-H immediately undergoes decomposition or further reaction; in some cases it may be isolated in good yield from the reaction mixture.

3. Termination.

In the ideal case, only one initiating radical would be required to cause complete autoxidation of all available R-H molecules, since the chain propagation process is repetitive. In practice, however, radicals are removed by termination processes such as (4).

\[ 2 \text{R-O-} \rightarrow \text{Nonradical products} \quad (4) \]

Other termination reactions, such as the combination of two R· radicals, are important at low oxygen concentrations only, since the speed of reaction (2) is usually so great that the species R-O-O· is present in much higher concentrations than R·.

There are other possible autoxidation schemes, but the one just presented appears to be by far the most common. The products of the reaction (before any subsequent reactions occur) are seen to be the hydroperoxide R-O-O-H plus small amounts of the end results of the termination reactions.

THE AUTOXIDATION OF HYDROCARBONS; FACTORS DETERMINING POINT OF INITIAL ATTACK

Autoxidation is usually initiated and propagated at a point in the molecule where an electron can be relatively easily acquired by the strongly electronegative oxygen molecule (or other initiating radical). As already mentioned, this most often occurs as a result of homolytic fission of a carbon-hydrogen
bond. For instance, 2,7-dimethyloctane is preferentially attacked at both tertiary carbon atoms (5). Attack here may be attributed to the relatively high electron density caused by the electropositive methyl substituents; that is, an electron is readily available for removal with the proton as a hydrogen atom and combination with oxygen to form the hydroperoxyl radical.

Perhaps more research has been carried out on the autoxidation of olefinic hydrocarbons and their derivatives than on any other class of compounds. A review of this work, along with many pertinent references, is given in (1). A brief survey of this type of autoxidation is helpful because it illustrates the role of resonance in determining the position at which autoxidation occurs.

In the case of an unconjugated olefin, initial attack usually occurs at a carbon atom in the α-position to a double bond. This is so because the resulting free radical is stabilized by resonance (electron delocalization); only if an electron from this location is lost can this resonance occur, with a consequent decrease in the total energy of the olefin radical.

\[
R-\text{CH}=\text{CH}-\text{CH}_2-R' + \text{In.} \rightarrow \left\{ R-\text{CH}=\text{CH-CH}=R' \leftrightarrow R-\text{CH-CH}=\text{CH-R} \right\} + \text{In.H} \quad (5)
\]

Chemical evidence for this was given by Ross, et al. (2) who showed the presence of isomeric hydroperoxides in the autoxidation products of methyl oleate:

\[
\begin{array}{c}
R-\text{CH}=\text{CH-CH}=R' \\
\text{O-O-H}
\end{array}
\quad
\begin{array}{c}
R-\text{CH}-\text{CH}=\text{CH-R}' \\
\text{O-O-H}
\end{array}
\]

Thus, it is seen that two factors are generally responsible for determining the point of autoxidative attack. First of all, there must be a sufficient electron density at the attacked point to enable a hydrogen atom, with its one electron, to be removed. In the second place, other factors being equal, attack will occur
at the point which results in the lowest energy for the resulting radical. The possible formation of a resonance-stabilized system is often the deciding factor in determining the point of attack in this case.

The first effect, electron density, was noted by Russell (6) in the autoxidation of para-substituted cumenes:

\[ \text{R C-H} \rightarrow \text{C-R H}_3 \]

Attack occurs at the tertiary carbon of the side chain; moreover, the rate of reaction is highest when the substituent \( R \) is an electron releaser, such as the tertiary butyl group, and lowest when it is an electron withdrawer, such as the nitro group.

In cases where attack at a certain carbon atom would be expected to be somewhat inhibited due to an electron-withdrawing inductive effect of a nearby atom or group, yet where the intermediate transition state and the radical (if attack did occur) would be resonance-stabilized, the resonance effect usually appears to be the stronger. Such a situation, discussed below, occurs in the case of the ethers at the carbon atoms adjacent to the oxygen. A somewhat similar situation is encountered when considering the directive influence of groups such as the chlorine atom toward cationic substitution in aromatic molecules (10). The chlorine atom is highly electronegative and might be expected to withdraw electrons from the aromatic nucleus, with consequent meta-directing of substitution; however, due to the existence of unshared electron
pairs on the chlorine atom, the transition state formed when a cationic species attacks at the ortho or para position is more highly resonance-stabilized than when it attacks at the meta position. Consequently, chlorine directs ortho-para, although with deactivation because of the inductive effect.

THE AUTOXIDATION OF ETHERS

That ethers autoxidize readily at room temperature has been known for decades; in many cases, the products are highly explosive. Rieche and Koch (11) give the following scheme for the autoxidation of diethyl ether:

\[
\begin{align*}
\text{CH}_3\text{-CH}_2\text{-O-C}_2\text{H}_5 + O_2 & \rightarrow \text{CH}_3\text{-CH-O-C}_2\text{H}_5 \quad \text{(several steps)} \\
\text{CH}_3\text{-CH-O-C}_2\text{H}_5 + H_2O & \rightarrow \text{CH}_3\text{-CH-OH} + C_2H_5OH \quad \text{(8)} \\
\text{CH}_3\text{-CH-OH} & \rightarrow \text{CH}_3\text{CHO} + H_2O_2 \quad \text{(9)} \\
\text{CH}_3\text{-CH-OH} & \rightarrow [\text{CH}_3\text{-CH-O-O-}]_N + H_2O \quad \text{(10)}
\end{align*}
\]

Reaction (7) presumably proceeds via the usual autoxidation mechanism:

\[
\begin{align*}
\text{CH}_3\text{-CH}_2\text{-O-C}_2\text{H}_5 + \text{In;} & \rightarrow \text{CH}_3\text{-CH-O-C}_2\text{H}_5 + \text{In;H} \quad \text{(11)} \\
\text{CH}_3\text{-CH-O-C}_2\text{H}_5 + O_2 & \rightarrow \text{CH}_3\text{-CH-O-C}_2\text{H}_5 \quad \text{(12)} \\
\text{CH}_3\text{-CH-O-C}_2\text{H}_5 + \text{CH}_3\text{-CH}_2\text{-O-C}_2\text{H}_5 & \rightarrow \text{CH}_3\text{-CH-O-C}_2\text{H}_5 + \text{CH}_3\text{-CH-O-C}_2\text{H}_5 \quad \text{(13)}
\end{align*}
\]

The existence of the following resonance-stabilized transition state* may be responsible for the ease with which diethyl ether undergoes autoxidation:

*This represents an adaptation of Russell’s discussion for alkyl benzyl ethers (6).
The presence of the oxygen atom with its unshared electron pairs facilitates the existence of more extensive resonance than if it were absent. Apparently, this resonance effect is stronger than the inductive effect of the electronegative oxygen, which would tend to discourage removal of the hydrogen atom by causing a somewhat lower electron density at the carbon-hydrogen bond.

Cyclic ethers undergo an analogous reaction, as shown by Robertson (12) with tetrahydrofuran:

\[
CH_2\bigg\vert\bigg\vert CH_2 + O_2 \rightarrow CH_2\bigg\vert\bigg\vert CH_2 C=O-O-H
\]

(15)

THE AUTOXIDATION OF ACETAIS

Acetals, having a carbon atom adjacent to two oxygen atoms, are readily autoxidized, presumably via mechanisms very similar to those already outlined. It is apparent that an even greater number of resonance forms of the transition state are possible. According to Criegee (5), 2-methyl-1,3-dioxolane and paraldehyde autoxidize as follows, although this has not been definitely proved:

\[
\begin{align*}
\text{In.} & + H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \quad \rightleftharpoons \quad \left\{ \begin{array}{c} 
\text{In:}^- \quad H^- \quad H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \end{array} \right. \\
\text{In:}^- \quad H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \quad \rightleftharpoons \quad \left\{ \begin{array}{c} 
\text{In:H}^- \quad H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \\
\text{In:H}^- \quad H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \end{array} \right. \\
\left\{ \begin{array}{c} 
\text{In:H}^- \quad H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \\
\text{In:H}^- \quad H\cdot\overset{\text{H}}{\cdot}C\cdot\overset{\text{H}}{\cdot}C_2H_5 \end{array} \right. \right. \\
\end{align*}
\]

(14)
These observations are highly significant with respect to this thesis, since methyl glycopyranosides are acetals.

THE AUTOXIDATION OF CELLULOSE

Tryon and Wall (13) present a brief review of the meager knowledge concerning the autoxidation of cellulose. They point out the lack of fundamental research in this field; most studies of the interaction of cellulose and oxygen have been concerned with the loss of strength properties. A review of the phenomenon of alkali cellulose autoxidation, which may be an ionic rather than a radical process, has already been cited (2). Other, rather empirical studies are reviewed by McBurney (14). The autoxidation of cellulose derivatives, such as ethyl cellulose, has apparently been more widely studied than the autoxidation of cellulose itself.

A study of the degradation of cellulose under oxygen was conducted in recent years by Major (15). A temperature of 170°C. was employed. Under oxygen the reaction was an autoxidation as defined in this thesis (as shown by control experiments under nitrogen), although Major did not use this term. He concluded that oxygen was responsible for the reaction which occurred when dry cellulose
fibers (from cotton) were heated at 170°C. in an atmosphere of dry oxygen at atmospheric pressure. The oxidation, termed nonspecific, was concentrated in the amorphous regions of the cellulose, where extensive depolymerization occurred; a very wide variety of products resulted. Almost no reaction occurred under nitrogen. A study of the mechanism of the reaction was not a part of the thesis; however, Major suggested that studies be made of the oxygen oxidation of model compounds, which would help in the elucidation of the details of the reaction. This suggestion provided part of the impetus for the present thesis.

THERMODYNAMICS OF AUTOXIDATION

Autoxidation is generally an exothermic reaction. For example, Bolland and Gee (16) found that the heat of reaction for the formation of the monohydroperoxide of ethyl linoleate was -53.3 kcal./mole. An estimate of the heats of reaction of the individual reactions in autoxidation may be made if the pertinent bond dissociation energy values are known.

The bond dissociation energy of the peroxidic O-O bond has been a matter of controversy for a number of years. Pauling (17) gives a value of 34.9 kcal./mole; however, Bolland and Gee (16) determined it as 66 kcal./mole from the heat of autoxidation of ethyl linoleate (this was possible because all other bond dissociation energy values pertaining to this reaction were known).

In the following discussion, the energy values employed by Bolland and Gee will be used. The values they used were corrections of Pauling's values, using a new value for the heat of sublimation of carbon. These values are given in Table I; it should be mentioned that these are average figures and that the true figures are dependent on the environment of the bond. Bolland and Gee also used
15 kcal./mole as the resonance energy of the hydroperoxyl radical, \( \cdot O-O-H \), and various values for the resonance energies of the unsaturated free radicals with which they were concerned. The value for the hydroperoxyl radical was admittedly somewhat arbitrary.

### TABLE I

**BOND DISSOCIATION ENERGIES (16)**

<table>
<thead>
<tr>
<th>Bond</th>
<th>Bond Dissociation Energy, kcal./mole</th>
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<tbody>
<tr>
<td>O-O (peroxide)</td>
<td>66</td>
</tr>
<tr>
<td>C-C</td>
<td>81</td>
</tr>
<tr>
<td>C-O</td>
<td>87</td>
</tr>
<tr>
<td>C-H</td>
<td>99</td>
</tr>
<tr>
<td>O-H</td>
<td>110</td>
</tr>
<tr>
<td>( O_2 ) (molecule)</td>
<td>118</td>
</tr>
</tbody>
</table>

If oxygen itself initiated the autoxidation, the reaction

\[
R-H + O_2 \rightarrow R^+ + \cdot O-O-H
\]  

(18)

would have a heat of reaction of \( 99 + 118 - 66 - 110 - 15 - A \) kcal./mole, where \( A \) is the resonance energy of \( R^+ \). Thus, \( \Delta H = 26 - A \) kcal./mole, and unless \( A \) were greater than 26 kcal./mole, such an initiation reaction would be endothermic. This type of argument is used by Uri (18) to support his hypothesis that such initiation reactions do not occur, and that trace amounts of metal ions are needed to catalyze autoxidations. However, although \( A \) may not be as great as 26 kcal./mole, it nevertheless can have a value not far from it in some cases, making the reaction only slightly endothermic. To initiate an autoxidation, it is not necessary that a very large number of initiating reactions occur (since
it is a chain process), and the author believes that enough oxygen-initiated reactions can occur out of pure chance (even with unfavorable energetics) to enable autoxidation to occur at a reasonable rate. This is the belief held by Bolland and Gee (16) and other investigators (discussed later) and supported by the results of this thesis.

The following propagation reactions are definitely exothermic.

\[
R^\cdot + O_2 \rightarrow R-O-O^\cdot \quad (19)
\]

\[
R-O-O^\cdot + R-H \rightarrow R-O-O-H + R^\cdot \quad (20)
\]

For (19), \( \Delta H = A + 118 - 66 - 87 - B \)

\( = A - 35 - B \)

where \( B \) = resonance energy of \( R-O-O^\cdot \) radical.

For (20), \( \Delta H = B + 99 - 110 - A \)

\( = B - 11 - A \)

The total \( \Delta H \) for these reactions is -46 kcal./mole, which represents the net reaction \( R-H + O_2 \rightarrow R-O-O-H \). The heat of autoxidation of ethyl linoleate was greater than this (16) because the hydroperoxide was stabilized by more extensive resonance than was the original ester. The resonance energies of \( R-H \) and \( R-O-O-H \) were not included in the previous discussion. In the case of saturated compounds, these energies would be negligible, if not zero.

KINETICS OF AUTOXIDATION

THEORETICAL BACKGROUND

There are at present several theories of the kinetics of organic autoxidations in the liquid phase. All are based on the following general postulates:
1. A free-radical chain mechanism accounts for the reaction.

2. The concentration of free radicals is small and tends to remain constant (concept of the steady state).

3. The reaction chains are long, and hence the number of initiation and termination reactions is small compared with the number of propagation reactions.

The various theories differ principally with regard to the nature of the chain initiation reactions. Some investigators believe that oxygen itself initiates reaction chains (19-21); others hold that this is impossible on thermodynamic grounds and postulate that metals of variable valence are required (18); still others (16) are of the opinion that, while some direct initiation by oxygen is possible, decomposition of hydroperoxides (autocatalysis) is chiefly responsible for the initiation of new chains. Evidence presented later points to the initiation of carbohydrate autoxidation by oxygen itself; little or no autocatalysis has been observed in these reactions.

The oxygen-initiation theory will be developed here for future reference when the results of the autoxidation of MBG are presented. The rate equation which should be obeyed if autocatalysis occurred in this autoxidation will also be developed; it will be shown that the autoxidation of MBG is not well described by this theory.

**Oxygen-Initiated Kinetics**

A good summary of this theory [through Equation (25)] is given by Cooper and Melville (19). The concept of the steady state is discussed by Frost and Pearson (22).
Let the individual reactions of the liquid-phase, homogeneous, oxygen-initiated, nonphotocatalytic, and nonautocatalytic autoxidation of an organic compound \( R-H \) and their respective rates be given by the following scheme*. 

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. ( R-H + O_2 \rightarrow R^+ + O-O-H )</td>
<td>( k_i [R-H][O_2] )</td>
</tr>
<tr>
<td>B. ( R-H + O-O-H \rightarrow R^+ + H_2O_2 )</td>
<td>( k_i [R-H][O-O-H] )</td>
</tr>
<tr>
<td>C. ( R^+ + O_2 \rightarrow R-O-O^+ )</td>
<td>Fast</td>
</tr>
<tr>
<td>D. ( R-O-O^+ + R-H \rightarrow R-O-O-H + R^- )</td>
<td>( k_p [R-O-O^-][R-H] )</td>
</tr>
<tr>
<td>E. ( 2 R-O-O^- \rightarrow ) Inactive products</td>
<td>( k_t [R-O-O^-]^2 )</td>
</tr>
</tbody>
</table>

Reaction (C) is generally assumed to be fast with respect to the other reactions. Consequently, the concentration of \( R-O-O^- \) is high compared with that of \( R^+ \), and it is not necessary to consider termination reactions such as the combination of \( R-O-O^- \) and \( R^+ \) (except at very low oxygen concentrations).

As mentioned earlier, it is assumed that the concentration of free radicals reaches a low but constant value. Therefore, the rate of free radical production must be equal to the rate of free radical removal. It is seen from the above scheme that the only reactions which involve the net production or removal of free radicals are (A) and (E); moreover, each involves two free radicals. Hence, their rates must be equal:

\[
k_i [R-H][O_2] = k_t [R-O-O^-]^2
\]  

(21)

From (21) it follows that

\[
[R-O-O^-] = (k_i/k_t)^{0.5} [R-H]^{0.5} [O_2]^{0.5}
\]  

(22)

*For symbols, see Nomenclature (p. 76).
We want an expression for the rate of consumption of R-H. Now R-H is ostensibly consumed in Reactions (A), (B), and (E). However, it is assumed that the reaction chain, comprising Reactions (C) and (D), is long. Therefore, nearly all of the R-H will be consumed in Reaction (D); or

\[ \frac{-d[R-H]}{dt} = k_p [R-O-O^\cdot][R-H] \]  

(23)

Substituting the expression for \([R-O-O^\cdot]\) from Equation (22), it is found that

\[ \frac{-d[R-H]}{dt} = k_p (k_i/k_t)^{0.5} [R-H]^{1.5} [O_2]^{0.5} \]  

(24)

This basic equation shows that the kinetics of autoxidation of R-H should be of order 1.5 with respect to the concentration of R-H and of order 0.5 with respect to the concentration of oxygen in the liquid phase, if all necessary conditions are met. Combination of the three rate constants into one, \(k_a\), gives

\[ \frac{-d[R-H]}{dt} = k_a [R-H]^{1.5} [O_2]^{0.5} \]  

(25)

The concentration of oxygen in the liquid phase, \([O_2]\), is assumed to follow Henry's Law and to be proportional to the partial pressure of oxygen in the gas phase:

\[ [O_2] = k_h P_{O_2} \]  

(26)

where \(k_h = \text{Henry's Law constant.}\)

If it is further assumed that \(k_h\) is not changed appreciably upon reaction of R-H with oxygen, then at constant temperature in the presence of a large excess of oxygen in the gas phase, \([O_2]\) will remain approximately constant. In any event, the fact that the concentration of oxygen is raised to the 0.5 power diminishes the effect of small changes in its value upon the over-all rate of
reaction. For convenience, the symbol \( k_\circ \) will represent \([O_2]^{0.5}\). The rate equation is now

\[
-d[R-H]/dt = k_a k_\circ [R-H]^{1.5}
\]

(27)

The reason for not combining \( k_a \) and \( k_\circ \) will become evident later.

Equation (27) may be integrated directly to yield the important integrated rate expression:

\[
2\left(1/[R-H]^{0.5} - 1/[R-H]_0^{0.5}\right) = k_a k_\circ t
\]

(28)

where \([R-H]_0 = [R-H] \text{ at } t = 0\).

A plot of the expression on the left side of Equation (28) versus time (at constant temperature) should yield a straight line with slope \( k_a k_\circ \) if the particular compound under investigation is undergoing an autoxidation reaction of order 1.5.

Consider now the dependence of the rate of such a reaction upon temperature. If, as is usually assumed, each of the individual initiation, propagation, and termination reaction rates has a simple Arrhenius temperature dependence, it can be shown (22) that the over-all reaction rate will also have a simple Arrhenius temperature dependence. That is,

\[
d \ln k_a/dT = E_a/RT^2
\]

(29)

where \( T = \text{absolute temperature}; \)

\( E_a = \text{energy of activation}; \text{ and} \)

\( R = \text{gas constant}. \)

\( E_a \) is in fact a function of the energies of activation of the individual reactions:
\[ E_a = E_p + \left( \frac{1}{w} \right) (E_i - E_t) \]  

(30)

where \( E_p \) = energy of activation of propagation reaction; 
\( E_i \) = energy of activation of initiation reaction; 
\( E_t \) = energy of activation of termination reaction; and 
\( w \) = order of chain-breaking step (generally 2).

Integration of (29) between the limits \( k_a = k_{a1} \) at \( T = T_1 \) and \( k_a = k_{a2} \) at \( T = T_2 \) gives

\[ \log_{10} \left( \frac{k_{a2}}{k_{a1}} \right) = \left( \frac{E_a}{2.303R} \right) \left( \frac{T_2 - T_1}{T_2T_1} \right) \]  

(31)

Determination of \( k_a \) at two temperatures will allow the determination of the energy of activation by substitution in Equation (31). In practice, at least three values of \( k_a \) are determined, and \( \log_{10} k_a \) is plotted against \( 1/T \). All of the points should fall near the same straight line; this serves as a check on the value of the experimental data. The basis for this procedure is found in the integrated form of Equation (29), using indefinite integrals:

\[ \log_{10} k_a = \left( \frac{E_a}{2.303RT} \right) + C \]  

(32)

where \( C = \) constant of integration.

**Autocatalyzed Kinetics**

The phenomenon of autocatalysis is often encountered in autoxidation. This occurs when hydroperoxides (the usual products) decompose into free radicals which then initiate new propagation chains \( (16) \). At appreciable hydroperoxide concentrations this decomposition is thought to be a bimolecular reaction; the rate equation which should be obeyed if this occurs will now be developed. A brief treatment of a similar situation (not involving autoxidation) is given by Frost and Pearson \( (23) \).

---

**Note:** The mathematical expressions and derivations are presented as they appear in the text, with the assumption that all necessary mathematical symbols and notation are standard. The formatting and notation have been adjusted for clarity and readability in the digital context.
As before, let the various reactions and their respective rates be given by the scheme below.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. 2 R-O-O-H → R-O· + R-O-O- + H₂O</td>
<td>(k_1 [R-O-O-H]^2)</td>
</tr>
<tr>
<td>G. R-O· + R-H → R-O-H + R·</td>
<td>(k_F [R-O·][R-H])</td>
</tr>
<tr>
<td>H. R· + O₂ → R-O-O·</td>
<td>Fast</td>
</tr>
<tr>
<td>J. R-O-O· + R-H → R-O-O-H + R·</td>
<td>(k_J [R-O-O·][R-H])</td>
</tr>
<tr>
<td>K. 2 R-O-O· → Inactive products</td>
<td>(k_k [R-O-O·]^2)</td>
</tr>
</tbody>
</table>

In this scheme, it is assumed that a very small concentration of the hydroperoxide R-O-O-H has been formed by direct oxygen initiation; thereafter, autocatalysis is responsible for chain initiation. The steady state is assumed, and reaction (G) is assumed to account for a negligible amount of R-H consumption as compared with the main chain reactions (H) and (J). Setting the rate of (F) equal to that of (K) (required by the steady state hypothesis) gives

\[
\frac{k_1}{k_t} [R-O-O-H]^2 = \frac{k_k}{k_t} [R-O-O·]^2
\]

From this it is found that

\[
[R-O-O·] = \left(\frac{k_1}{k_t}\right)^{0.5} [R-O-O-H]
\]

The rate of autoxidation of R-H is given by

\[
-d[R-H]/dt = k_p [R-O-O·][R-H]
\]

Substitution of the previous expression for [R-O-O·] gives

\[
-d[R-H]/dt = k_p \left(\frac{k_1}{k_t}\right)^{0.5} [R-O-O-H][R-H]
\]
Now if R-O-O-H is the only product of autoxidation (which is true in the case of some olefin autoxidations), \([R-O-O-H] = [R-O-O-H]_o + [R-H]_o - [R-H]\), where \([R-O-O-H]_o\) and \([R-H]_o\) are the initial concentrations of R-O-O-H and R-H. Substitution gives the complete rate equation:

\[
-d[R-H]/dt = k_a[R-H]([R-O-O-H]_o + [R-H]_o - [R-H]) \tag{37}
\]

where \(k_a\) = combined rate constant.

Integration of (37) gives the integrated rate expression:

\[
1/G \ln ([R-O-O-H]/[R-H]) = k_a t + 1/G \ln ([R-O-O-H]_o/[R-H]_o) \tag{38}
\]

where \(G = [R-H]_o + [R-O-O-H]_o\).

Now if \([R-O-O-H]_o\) is small, then \([R-O-O-H]\) is approximately the amount of R-H that has reacted. A method of data treatment will be introduced when the autoxidation of MBG is discussed in which the percentage MBG reacted or unreacted will be the reaction variable, rather than its concentration. If this method of data treatment is applied in the present case, it is seen that

\[
1/G \ln (\% R-H reacted/\% R-H unreacted) = k_a t + 1/G \ln ([R-O-O-H]_o/[R-H]_o) \tag{39}
\]

It is seen from Equation (39) that if R-H undergoes an autocatalytic autoxidation of the type described, then a plot of \(\ln (\% R-H reacted/\% R-H unreacted)\) versus reaction time, \(t\), should give a straight line with slope \(Gk_a\) and y-intercept \(\ln ([R-O-O-H]_o/[R-H]_o)\). With this method of data treatment, \([R-H]_o = 100\).

The actual behavior of the concentration of R-H (or some other easily comprehended reaction variable, such as the percentage R-H reacted) as a function of
reaction time is somewhat difficult to understand from the above equations. For purposes of illustration, let it be assumed that \([R-H]_o = 100\) and that \([R-O-O-H]_o = 0.01\). The percentage \(R-H\) reacted is given by

\[
\text{Percentage } R-H \text{ reacted } = \frac{[R-H]_o - [R-H]}{100 - [R-H]} \quad (40)
\]

Lengthy algebraic manipulation of Equation (38) then gives

\[
\% \text{ } R-H \text{ reacted } = 0.01 \left[ \frac{100.01}{0.01 + 100 \exp(-100.01 k_a t)} \right] - 1 \quad (41) *
\]

In Fig. 1 is shown a plot of percentage \(R-H\) reacted versus \(k_a t\). The sigmoid shape is characteristic of autocatalytic reactions and is interpreted as follows. In the early stage of the reaction, the rate is very low. However, \(R-O-O-H\) begins to build up and hence the reaction rate becomes increasingly greater. Eventually, however, the substrate \(R-H\) undergoes depletion and the rate necessarily slows down again. In a nonautocatalytic reaction, whatever the order, the reaction rate decreases from the very start due to depletion of substrate.

RELATED STUDIES

Several kinetic studies have been carried out which are relevant to the present investigation. Some of these show that the oxygen-initiated kinetic scheme does in fact describe organic autoxidations under certain conditions. Others give an idea of the order of magnitude of the energy of activation in organic autoxidations. The work of McBurney (24), Cooper and Melville (19), Miller and Mayo (20), and Russell (21) will be reviewed in chronological order.

McBurney (24) studied the autoxidation of ground ethyl cellulose film (degree of substitution approximately 2.5) under 1 atmosphere of oxygen in the

*The notation "exp \(y\)" is used in place of the more awkward \(e^y\).
Figure 1. Autocatalyzed Autoxidation
temperature range of 69 to 108°C. The reaction was followed by measuring the velocity of oxygen uptake and by measurements of peroxide and carboxyl groups in the oxidized polymer. He quite correctly concluded that no simple rate expression could describe the kinetics, since in a semisolid polymer there is restriction of movement of active centers (i.e., the reaction is inhomogeneous). However, at zero degree of reaction the reaction is approximately homogeneous. Therefore, he measured initial reaction rates by a graphical method and determined an energy of activation by treating the measured rates as rate constants. If the reaction order does not vary with temperature, this is valid, since the rate constants will be proportional to the rates at a given degree of reaction (zero degree of reaction in this case). The activation energy determined by this indirect method was 25 kcal./mole in the temperature range of 69 to 90°C. The point for 108°C. did not fall near the Arrhenius straight line; the discrepancy was attributed to diffusion effects of oxygen in the ethyl cellulose film. Autocatalysis appeared to occur, although its extent could not be quantitatively estimated.

McBurney found that the reaction could be accelerated by benzoyl peroxide and inhibited by phenols and diphenylamine. Such behavior is typical of an autoxidation because of the free-radical character of the mechanism. He also postulated attack by oxygen not only on the secondary carbon of the ethoxyl groups (which is responsible for loss of ethoxyl and formation of acetaldehyde) but also on the hydrogen attached to the glycosidic carbon atom, in a manner similar to that proposed later in this thesis with simple glycosides. McBurney proposed that this attack and formation of peroxidic linkages would lead to chain scission by a mechanism which he did not specify; he did not measure the extent of chain scission in his ethyl cellulose.
Cooper and Melville (19) studied the thermal and photochemical autoxidation of highly purified liquid decanal at 5°C. A very efficient stirring apparatus was necessary because of the rapidity of oxygen uptake, even at this relatively low temperature. Although reproducibility of only ±10% was attained in the case of the thermal reaction, the initial rate was apparently proportional to the 1.5 power of the aldehyde concentration and to the 0.5 power of the oxygen pressure, indicating that direct initiation by oxygen at the aldehyde hydrogen atom was occurring. The reaction was only carried to 6% conversion because at this point solid products began to precipitate. Autocatalysis was not observed, so that the experimental rate expression had the same form as Equation (25) of the earlier theoretical discussion.

Miller and Mayo (20) investigated the autoxidation of styrene in the presence and absence of azodiisobutyronitrile, a frequently used initiator. In the absence of this material the observed initial rate of oxygen uptake was proportional to the 0.4 power of the oxygen pressure and the 1.4 power of the concentration of styrene, in good agreement with theory. In the temperature range of 35 to 85°C., the over-all energy of activation was 23.0 kcal./mole.

Russell (21) studied the autoxidation of the hydrocarbon indene. At 50°C., in the absence of any initiator, the typical empirical rate equation was obtained, involving the concentration of the substrate to the 1.5 power and the concentration of oxygen to the 0.5 power. Again, no autocatalysis was observed, so the rate expression did not have to include terms representing the concentration of decomposable products.
EXPERIMENTAL METHODS AND GENERAL PROCEDURES

The experimental methods used in the investigation of the autoxidation of the compounds chosen for study were straightforward. The compounds were prepared and purified to a high degree; the details of these preparations are given in Appendix I. The apparatus employed and the actual autoxidation procedures, having been developed especially for this work, are described below. Chromatographic, titrimetric, microscopic, and precipitation methods used in analyzing the reaction products are described in Appendices II-V.

The general procedure was to subject a small sample (0.1-0.2 g.) of the pure chosen compound to the action of compressed oxygen or nitrogen under isothermal conditions in the absence of visible and ultraviolet light for a specific time interval. Since no data were available concerning the autoxidation of these compounds in the pure state, a considerable amount of trial and error was required to locate the temperature levels at which they underwent well-defined reaction. The reaction products were then analyzed by means of the various methods described. Control experiments were conducted under nitrogen to demonstrate that the observed reaction was an autoxidation.

AUTOXIDATION APPARATUS

PARR BOMB AND OVEN

Autoxidations were conducted in the apparatus illustrated in Fig. 2.

The bomb used was a Parr No. 1101 double valve oxygen bomb (Parr Instrument Company, 211 53rd St., Moline, Illinois). This bomb is supplied with Neoprene gaskets and seals, but performance is much improved at high temperatures when
Figure 2. Autoxidation Apparatus
all gaskets and seals are Teflon. A main sealing ring of Teflon can be supplied by the company, but the other Teflon seals must be custom-made. It is necessary to hold the bomb in a vise and tighten the screw cap with a large pipe wrench to make an adequate seal when a main sealing ring of Teflon is used. The gas outlet valve is of the needle type and allows relief of oxidation gases at any desired rate. The mass of the bomb is 3.03 kg. and its internal volume is 360 ml.

There are advantages and disadvantages in using a bomb as the container for autoxidation. Advantages are the large heat capacity (which helps to keep the temperature constant once the desired value has been reached), the total absence of visible and ultraviolet light from its interior (which eliminates photocatalytic reactions), the ease and rapidity with which all undesired gases can be removed from its interior, and its ability to withstand high pressures. Disadvantages include the relatively long time required to bring the bomb to the desired temperature, and the difficulty in measuring its internal temperature and oxygen consumption during autoxidation.

During autoxidation runs the bomb rested inside an enamelled steel can which was supported by a perforated steel shelf inside the oven. Dow Corning 550 Silicone Fluid (heat stable and nonvolatile) was used as the heat transfer medium. The oil level was kept slightly below the top of the bomb to avoid contaminating the valves. A glass rod bent as shown stirred the oil; it was rotated by an electric motor mounted outside of the oven. Two thermometers were used, one for temperatures below 110°C. and the other for temperatures greater than this. The thermometer used at the lower temperatures was calibrated in this laboratory; the other thermometer had a National Bureau of Standards certificate.
The oven itself was a Thelco laboratory model. A circulating fan was made by attaching a cooling fan from an old electric motor to an aluminum shaft. A hole was drilled in the top of the oven to allow the fan shaft to be attached to an external electric motor. This motor rotated the shaft at about 2,000 revolutions per minute, causing a strong circulating draft in the oven.

The Thermocap relay (Niagara-Electron Laboratories, Andover, New York) is operated by the very small change in capacitance which occurs when the mercury in the controlling thermometer approaches or recedes from the clip soldered to the lead from the instrument. The fineness of control is a function of the thickness of the thermometer stem and the distance between subdivisions on the thermometer scale. The best control is obtained with the use of a thin stem thermometer which contains a large mass of mercury and which has relatively widely spaced graduations. In the present case, the average air temperature in the oven remained constant, with variations of a few tenths of a degree C. about the average temperature; this variation was cyclic with a period of about one minute, so there was no appreciable temperature variation of the bomb and its contents. Long-term drift generally did not exceed 0.5°C.

The time required for the dish containing the compound inside of the bomb to come to the temperature of the bomb (as indicated by the reading of the thermometer in the stirred oil bath) was estimated from the laws of radiative heat transfer. The heat transferred by conduction cannot be estimated simply, but it will further shorten the required time. Assuming that at zero time the temperature of the bomb is 110°C. and remains constant, then radiative heat transfer will raise the dish temperature from 25 to 109°C. in about 43 minutes. An intuitive estimate by a member of the Institute Physics Group* was that,

*Dr. R. W. Nelson.
including conduction, not more than 15 minutes would be required for reasonable equilibrium to be attained.

A Dickson Minicorder circular chart temperature recorder (The Dickson Company, 7420 Woodlawn Avenue, Chicago 19, Illinois) was used to record oven air temperature continuously. The recorder was not especially sensitive (± 2°C.) but served to show any gross temperature variations and to prove that the oven did not go off at night due to electric service interruptions.

The connection and gage used to introduce oxygen or nitrogen into the bomb was calibrated by charging the bomb to a certain indicated pressure and measuring the volume of gas within the bomb by relieving it into a calibrated inverted bottle filled with water (displacing a volume of water equal to the gas volume). Since the volume of the bomb was known, the pressure within it could be calculated. It was found that the true pressure was a nearly linear function of the indicated pressure, and that an indicated pressure of 20 atmospheres corresponded to a true pressure of 17.2 atmospheres.

OXYGEN AND NITROGEN GAS

The oxygen used was Matheson Extra Dry Grade (The Matheson Company, Inc., P. O. Box 966, Joliet, Illinois). Its purity is quoted in the catalog as 99.6% minimum and its dew point as less than -70°F. The gas was found by actual analysis to contain 0.04% by weight of water vapor and an equal amount of carbon dioxide.

The nitrogen used was Matheson Prepurified Grade. This is quoted as having a minimum purity of 99.996%, a typical dew point of -85°F., and a typical oxygen content of 8 parts per million (0.0008%). It was found by actual analysis to contain 0.02% water vapor and 0.01% carbon dioxide.
GAS ABSORPTION APPARATUS

A train of gas absorption tubes was used to estimate the amounts of water vapor and carbon dioxide (plus any other acidic gases, such as formic acid) in the relief gas from the bomb. The first two tubes contained Anhydron (anhydrous magnesium perchlorate) to pick up water vapor, and the third tube contained Ascarite (sodium hydroxide-asbestos carbon dioxide absorbent) backed up by Anhydron. At the end of the train was a tall form gas washing bottle containing 100 ml. of sulfuric acid; this served as a flowmeter and to prevent atmospheric gases from entering the train from this end. Before bomb gas was relieved through the train, the latter was thoroughly flushed with prepurified nitrogen and the tubes were carefully weighed. The weight gains of the tubes after gas passage represented the amounts of water vapor and carbon dioxide (plus other acidic gases) in the relief gas. Blank runs were made to determine the amounts of these gases in the oxygen and nitrogen used; these corrections were subtracted from the gross weight gains after bomb gas passage to yield the net amounts of evolved gas.

AUTOXIDATION PROCEDURE

A general routine was followed in essentially all of the autoxidation runs; a description of this routine is given in this section.

The desired quantity (usually 0.2000 g.) of the compound under study was weighed out into a Pyrex "gum test" dish of mass 12.7060 g. The dish, the bottom of which was evenly covered with a thin layer of the compound, was carefully lowered into the bomb with the aid of the loop electrode (unscrewed from the head). The electrode remained in the bomb during the run so that the dish
could be easily lifted out at the end. The bomb cap was screwed down very tightly with the aid of an 18-inch pipe wrench, the bomb being held in a vise. The special charging fitting (loaned by the Analytical Group of the Institute) was attached to the gas supply cylinder and to the bomb. The bomb was filled twice to an indicated pressure of 30 atmospheres and relieved twice, these operations being conducted slowly so as not to disturb the sample in the dish; this operation effectively removed extraneous gases from the bomb. Three chargings and relievings were the rule when nitrogen was used. The bomb was then filled to the desired pressure (generally 20 atmospheres indicated, 17.2 atmospheres actual total pressure) and the valves tightly sealed.

After the filling and charging operations, the bomb was placed in the enamelled can containing the silicone oil, and the assembly was installed in the oven. The oven, can, and oil were preheated to reduce the heating-up time required. Zero time was taken when the indicated oil temperature reached the desired level; this usually required 0.5 to 1.0 hour from the time the can assembly was placed in the oven. Temperature was controlled by moving the Thermocap relay clip along the stem of the control thermometer to the desired temperature. At the end of the predetermined time interval the bomb was removed from the oven, wiped dry of oil, and quickly cooled with a stream of cold water. The gas was relieved through the tared gas absorption tubes at a rate of about 3-5 ml./second. At the end of this operation the tubes were immediately weighed to detect evolved water and carbon dioxide. The dish containing the reaction products was removed from the bomb and weighed to determine the actual weight loss of the material. The appearance of the products was noted, and generally the products were dissolved in distilled water and made up to 10 ml. in a volumetric flask. In nearly all cases, the products were completely soluble in water at room temperature.
RESULTS AND DISCUSSION

THE AUTOXIDATION OF CARBOHYDRATES

EVIDENCE FOR AUTOXIDATION

To the author's knowledge, it has never been shown previously that the compounds studied in this work are attacked by molecular oxygen in the absence of visible and ultraviolet light, in the pure state, at temperatures less than those which would cause pyrolysis or combustion. That such an attack does occur is shown in Table II. The only significant difference between Runs 44G* and 45G-N, 63 and 62-N, and 57A and 87A-N was the substitution of nitrogen for oxygen in the second run of each of these pairs (Run 45G-N was heated for a slightly longer period than 44G, but this is inconsequential).

TABLE II

COMPARISON OF EFFECTS OF OXYGEN AND NITROGEN

<table>
<thead>
<tr>
<th>Run</th>
<th>Compound</th>
<th>Gas</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>Reaction of Compound, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>44G</td>
<td>α-D-glucose</td>
<td>O₂</td>
<td>130</td>
<td>46.6</td>
<td>70-80ᵇ</td>
</tr>
<tr>
<td>45G-N</td>
<td>α-D-glucose</td>
<td>N₂</td>
<td>130</td>
<td>48.9</td>
<td>5-10ᵇ</td>
</tr>
<tr>
<td>63</td>
<td>MBG</td>
<td>O₂</td>
<td>130</td>
<td>24.0</td>
<td>98ᶜ</td>
</tr>
<tr>
<td>62-N</td>
<td>MBG</td>
<td>N₂</td>
<td>130</td>
<td>24.0</td>
<td>5ᵈ</td>
</tr>
<tr>
<td>57A</td>
<td>MAG</td>
<td>O₂</td>
<td>150</td>
<td>23.6</td>
<td>95-100ᵇ</td>
</tr>
<tr>
<td>87A-N</td>
<td>MAG</td>
<td>N₂</td>
<td>150</td>
<td>23.6</td>
<td>5ᵈ</td>
</tr>
</tbody>
</table>

ᵃPressure in all runs was 17.2 atmospheres at room temperature.
ᵇEstimated from appearance and chromatograms.
ᶜDetermined from quantitative chromatogram.
ᵈEstimated from appearance and specific rotation.

*For meaning of letter suffixes, see Nomenclature.
Excluding the possibility that oxygen catalyzed the reaction, which seems remote, the only reasonable interpretation of these results is that the compounds were autoxidized. The small amounts of reaction noted under nitrogen could have been at least partially due to traces of oxygen in the prepurified nitrogen.

One characteristic of autoxidations is that they are inhibited by small amounts of materials such as hydroquinone which are able to terminate the reaction chains without starting new ones. This phenomenon, known as antioxidation, is discussed at length in Lundberg's two-volume work (1). That hydroquinone effectively inhibited the reaction of MBG with oxygen is shown in Table III. This inhibition is taken as further evidence that a free-radical autoxidation was involved.

**TABLE III**

**EFFECT OF HYDROQUINONE ON MBG-OXYGEN REACTION**

<table>
<thead>
<tr>
<th>Run</th>
<th>MBG, mg.</th>
<th>Hydroquinone, mg.</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>Reaction of MBG, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>200.0</td>
<td>0.0</td>
<td>110</td>
<td>22.0</td>
<td>65.0</td>
</tr>
<tr>
<td>76HQ</td>
<td>200.0</td>
<td>10.3d</td>
<td>110</td>
<td>24.0</td>
<td>8.9</td>
</tr>
</tbody>
</table>

\(^a^\) Pressure, 17.2 atmospheres at room temperature.

\(^b^\) As hemihydrate.

\(^c^\) Determined by quantitative paper chromatography.

\(^d^\) Ground with MBG in mortar.

**HETEROGENEOUS AND HOMOGENEOUS AUTOXIDATION**

In Table IV are given the results of runs in which pure α-D-glucose, MAG, and methyl β-D-galactopyranoside were heated in oxygen at temperatures well below their melting points.* A control run was made with MAG under nitrogen.

*The effect of pressure upon the melting point of one of the compounds (MAG) was found to be small; indeed, the melting point was raised slightly. The calculations are given in Appendix VI.
TABLE IV
HETEROGENEOUS AUTOXIDATION

<table>
<thead>
<tr>
<th>Run</th>
<th>Compound</th>
<th>M.P., °C.</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>Reaction of Compound, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>44G</td>
<td>α-D-glucose</td>
<td>148-150</td>
<td>130</td>
<td>46.6</td>
<td>70-80</td>
</tr>
<tr>
<td>102A</td>
<td>MAG</td>
<td>168-169</td>
<td>147</td>
<td>21.2</td>
<td>95-100</td>
</tr>
<tr>
<td>101A</td>
<td>MAG</td>
<td>168-169</td>
<td>150</td>
<td>15.4</td>
<td>95-100</td>
</tr>
<tr>
<td>87A-N</td>
<td>MAG</td>
<td>168-169</td>
<td>150</td>
<td>23.6</td>
<td>5</td>
</tr>
<tr>
<td>58X</td>
<td>Me β-D-galactoside</td>
<td>178-179</td>
<td>150</td>
<td>23.7</td>
<td>95-100</td>
</tr>
</tbody>
</table>

a Pressure, 17.2 atmospheres of oxygen at room temperature, except in Run 87A-N.
b Estimated from appearance, specific rotation, and chromatograms.
c Pressure, 17.2 atmospheres of nitrogen at room temperature.
d Products crystalline at end of run.

It was determined microscopically that none of these pure compounds exhibited any detectable sign of fusion when heated in air for 5 to 10 minutes at the temperatures listed in Table IV, although some sublimation did occur (for microscopic techniques, see Appendix IV). Yet in each case where very extensive reaction occurred, it was evident from examination of the reaction products that fusion had occurred at some point during the reaction; the crystal structure had disappeared and the products were in the form of a transparent yellow tar or glass. Therefore, oxygen presumably reacted directly with the crystals. Such a reaction, involving more than one phase, is termed a heterogeneous reaction (as opposed to a homogeneous reaction, which takes place in only one phase). Heterogeneous autoxidations of crystalline organic compounds have been only rarely observed and apparently never investigated to any great extent. An example is the slow autoxidation of crystalline abietic acid, reported in 1928 (25).
The heterogeneous autoxidation of carbohydrates was investigated further, using MAG at 147 and 150°C. A series of autoxidations was carried out; the specific rotation of the reaction products in water was used as a measure of the degree of reaction (it will be shown later that in the case of MBG, at least, there is a well-defined relationship between the specific rotation of the products and the degree of reaction of the glucoside). The results of these experiments are summarized in Table V and Fig. 3.

From the observations of the physical state of the products, their melting points, and their specific rotations, it seems likely that these autoxidations followed a specific pattern. A relatively slow heterogeneous reaction between molecular oxygen and the crystalline glucoside occurred first. This reaction produced compounds such as glucose and gluconic acid which lowered the melting point of the mixture (originally 168-169°C.). As the melting point became lower, it eventually decreased below the temperature of the surroundings, and melting occurred. In the liquid phase the molecules attained a much greater degree of mobility than they previously possessed; consequently, free radical chain transfer reactions were facilitated. Also, oxygen dissolved in the melt and reaction was no longer limited to regions near the surface of the phase; the reaction became essentially homogeneous. These factors caused the reaction velocity to increase greatly, with the result being a precipitous drop in the specific rotation of the mixture and a dramatic change in its general physical properties. A similar lowering of the melting point and change in specific rotation occurred during the autoxidation of crystalline abietic acid at room temperature (25).

Figure 3 provides an idea of the temperature dependence of the heterogeneous autoxidation. A difference of 3°C. made a difference of several hours in the
TABLE V
AUTOXIDATION OF METHYL \( \alpha \)-D-GLUCOPYRANOSIDE

<table>
<thead>
<tr>
<th>Run(^a)</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>State of(^b) Products</th>
<th>Specific Rotation(^c) of Products, degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>94A</td>
<td>147</td>
<td>5.0</td>
<td>Crystalline</td>
<td>+158.5</td>
</tr>
<tr>
<td>95A</td>
<td>147</td>
<td>10.0</td>
<td>Crystalline; M.P. 168-169°C.</td>
<td>+158.2</td>
</tr>
<tr>
<td>100A</td>
<td>147</td>
<td>18.0</td>
<td>Crystalline; M.P. 158-168°C.</td>
<td>+153.1</td>
</tr>
<tr>
<td>102A</td>
<td>147</td>
<td>21.2</td>
<td>Yellow glass</td>
<td>+91.6</td>
</tr>
<tr>
<td>96A</td>
<td>150</td>
<td>8.2</td>
<td>Sintered crystals; M.P. 154-168°C.</td>
<td>+152.6</td>
</tr>
<tr>
<td>98A</td>
<td>150</td>
<td>13.4</td>
<td>95% yellow tar(^d), 5% crystalline</td>
<td>+124.5</td>
</tr>
<tr>
<td>101A</td>
<td>150</td>
<td>15.4</td>
<td>Yellow glass</td>
<td>+93.1</td>
</tr>
<tr>
<td>97A-N(^e)</td>
<td>150</td>
<td>8.2</td>
<td>Crystalline; M.P. 168-169°C.</td>
<td>+158.4</td>
</tr>
<tr>
<td>87A-N(^e)</td>
<td>150</td>
<td>23.6</td>
<td>Crystalline</td>
<td>+152.6</td>
</tr>
</tbody>
</table>

\(^a\)Pressure, 17.2 atmospheres of oxygen at room temperature except as noted.
\(^b\)Melting points determined microscopically. M.p. of pure MAG = 168-169°C.
\(^c\)In water with sodium D line. Specific rotation of pure MAG = +159.0°.
\(^d\)Relative amounts of tar and crystals estimated.
\(^e\)Pressure, 17.2 atmospheres of prepurified nitrogen at room temperature.
Figure 3. Autoxidation of MAG
time required for the reaction to be transformed from heterogeneous to essentially homogeneous. Thus, the rate of the heterogeneous autoxidation is rather strongly temperature-dependent. If the rate of the heterogeneous reaction is arbitrarily defined as being inversely proportional to the time required for the specific rotation to decrease to 155°, then with the aid of Fig. 3 it may be estimated from Equation (31) (p. 21) that the energy of activation for the heterogeneous reaction is approximately 50 kcal./mole. This calculation was made using an estimate of 6.4 hours for the time required to reach the stated rotation at 150°C. and 15.0 hours for the time required at 147°C. Since such a small temperature range was used, this estimate of the energy of activation is probably not very accurate, but it does convey an idea of the order of magnitude of the temperature dependence of this reaction.

As with any heterogeneous reaction, the rate of the heterogeneous autoxidation of MAG is no doubt partially dependent on the surface area available for reaction. A very finely ground specimen would be expected to be attacked more rapidly than a less finely ground specimen. All autoxidation runs with MAG were made with samples from the same batch; these crystals had been ground to a state of medium fineness with an ordinary porcelain mortar and pestle. Quite an extensive size distribution was evident under magnification, the particles ranging in size from a few microns up to 100-150 microns in greatest dimension.

Since the rate of heterogeneous autoxidation of carbohydrates may have a very strong temperature dependence, it is not surprising that with some compounds it may be difficult to observe, simply because the compound has a low melting point. At temperatures above the melting point, the reaction tends toward homogeneity, especially under conditions which favor a relatively high concentration of oxygen in the melt. In the case of MBG, the kinetics of the homogeneous
autoxidation of which will be discussed later, very little heterogeneous autoxida-
tion has been noted. MBG begins to soften at about 106°C., although a well-defined
liquid phase does not appear until 109°C. is reached. Table VI summarizes the
results of some runs in the vicinity of the softening point.

**TABLE VI**

AUTOXIDATION OF MBG IN VICINITY OF SOFTENING POINT

<table>
<thead>
<tr>
<th>Run</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>State of Products</th>
<th>Specific Rotation&lt;sup&gt;b&lt;/sup&gt; of Products, degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>103</td>
<td>47.4</td>
<td>Slightly discolored crystals</td>
<td>-31.9</td>
</tr>
<tr>
<td>33</td>
<td>106</td>
<td>48.0</td>
<td>Pale yellow tar</td>
<td>-24.6</td>
</tr>
<tr>
<td>34</td>
<td>106</td>
<td>71.5</td>
<td>Yellow glass</td>
<td>-10.7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pressure, 17.2 atmospheres of oxygen at room temperature.
<sup>b</sup>In water with sodium D line. Specific rotation of pure MBG (anhydrous) = -34.1°.

It is likely that melting would intervene, in the case of MBG, before the
temperature could be raised to a point at which heterogeneous autoxidation would
occur at anywhere near the rate with which it takes place, for example, with MAG
at 150°C., given the same specific surface area of the crystals.

There is an extensive literature on heterogeneous reactions such as the
catalytic hydrogenation of olefins by metal surfaces, as well as discussions of
the nature of solid surfaces themselves (26, 27). Because of the extreme non-
uniformity of most solid surfaces, it is generally very difficult to describe
them in detail or to discuss their reactions in quantitative terms. The surface
of a fractured crystal of, say, MAG is complex. All possible crystal planes are
exposed, and there are undoubtedly numerous small cracks and fissures into which
oxygen can penetrate.
Physical adsorption of oxygen on MAG, especially at the temperatures used in this work, would be very slight, due to the relatively low critical temperature of this gas (-119°C.). Whether or not true chemisorption, in the sense of valence-bond formation, occurs prior to heterogeneous autoxidation of MAG is highly questionable [in contrast to the behavior of the surface of charcoal, for instance (26)].

The author believes that the heterogeneous autoxidation of a glycoside may occur in the following fashion. The crystalline surface is continually bombarded by oxygen molecules; since there is presumably only a very small amount of physical adsorption, the concentration of oxygen is not much greater near the surface than in the bulk gas phase. There is probably a small but appreciable amount of oxygen inside the crystals, due to penetration into surface cracks as well as some actual diffusion into the crystal structure. Occasionally, an oxygen molecule happens to strike a hydrogen atom which is bonded to an anomeric carbon atom in such a manner that the transition state required for abstraction of this hydrogen atom is formed. After removal of the perhydroxyl radical, oxygen then adds rapidly to the glycoside free radical. Eventually, due to vibrations in the crystal structure, the radical thus created may be able to abstract a hydrogen atom from a neighboring glycoside molecule; alternatively, the perhydroxyl radical created in the initial reaction may reorient and combine with the glycoside radical to form the hydroperoxide (for the structure of these postulated entities, see the discussion of the hypothesized mechanism for glycoside autoxidation beginning on p. 67). In such a manner, the reaction may spread from an initiation point. This hydroperoxide formation and subsequent reactions gradually lower the melting point in the vicinity of the reaction until it drops below the local temperature; melting then occurs, and the reaction rate becomes
greater because of (a) the greatly increased degree of mobility of the molecules, and (b) the availability of dissolved oxygen for reaction throughout the system.

Since MAG was observed to sublime to some extent as it was being heated on the Fisher-Johns apparatus (Appendix IV), there was no doubt a small but appreciable concentration of it in the vapor phase during the autoxidation runs at 147 and 150°C. An attempt was made to calculate the concentration of MAG in the vapor phase, using an estimated value of the heat of sublimation of 130 cal./g., but this could not be done without knowing the vapor pressure at one temperature (which apparently has never been determined). The possibility exists that some autoxidation of MAG might have occurred homogeneously in the vapor phase. However, except in regions very close to the crystal surfaces, the concentration of MAG in the vapor phase would be small, and hence there would be only limited opportunities for the necessary chain transfer reactions between MAG molecules. Therefore, it is believed that such a homogeneous autoxidation in the gas phase was negligible in comparison with the heterogeneous reaction between the gas and the crystals.

In all cases observed during this work, a glycoside was autoxidized whenever it was subjected to compressed oxygen at a temperature higher than its melting point. This was true for MAG, MBG, both anomic methyl D-galactopyranosides, and methyl α-D-xylopyranoside. In Table VII are given data for the autoxidation of methyl α-D-galactopyranoside (Run 52Y) and methyl α-D-xylopyranoside (Run 65K).

These results allow a tentative generalization to be advanced: All crystalline methyl glycosides are subject to autoxidation in the dark when exposed to molecular oxygen at temperatures equal to or above their melting or softening points. In addition, it is probable that these compounds are in general subject
to heterogeneous autoxidation at temperatures below their melting points, but since these reactions may be strongly temperature-dependent it may become increasingly difficult to observe such reactions as compounds with progressively lower melting points are studied.

TABLE VII

AUTOXIDATION OF METHYL α-GALACTOSIDE AND α-XYLOSIDE

<table>
<thead>
<tr>
<th>Run</th>
<th>M.P. of Pure Compound, °C.</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>State of Products</th>
<th>Specific Rotation of Products, degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>52Y</td>
<td>116-118</td>
<td>120</td>
<td>46.0</td>
<td>Yellow tar</td>
<td>+98.6°c</td>
</tr>
<tr>
<td>65K</td>
<td>90-92</td>
<td>100</td>
<td>24.0</td>
<td>Colorless gum</td>
<td>+143.9°d</td>
</tr>
</tbody>
</table>

aPressure, 17.2 atmospheres of oxygen at room temperature.
bIn water with sodium D line.
cSpecific rotation of pure galactoside = +196°.
dSpecific rotation of pure xyloside = +153°.

KINETICS OF THE AUTOXIDATION OF MBG

Procedure

In each of the kinetic runs, the substrate was 0.2000 g. (0.985 mmole) of finely powdered MBG hemihydrate, equivalent to 0.1912 g. (0.985 mmole) of anhydrous MBG. Details of the general autoxidation procedure have already been presented. At the end of each run, the weighed products were dissolved in distilled water and made up to 10 ml. in a volumetric flask. The specific rotation of the solution was determined in sodium light soon after solution and again the following day to check for mutarotation. The percentage of unreacted MBG was determined by quantitative paper chromatography as described in Appendix II.

Runs were made at four temperatures—108, 110, 120, and 130°C. Five runs were
made at each of the two lower temperatures and four runs at each of the two higher temperatures. To demonstrate that the results were reproducible, duplicate runs were made at the first two time intervals at 120°C.

**Method of Data Treatment**

Certain modifications in the previously discussed kinetic scheme are necessary in order to make use of the data obtained in this work. These involve the way in which the concentration of oxygen in the MBG melt is handled, and the method of expressing the concentration of MBG.

It is desired to determine the energy of activation for the autoxidation of MBG. Assume that the data have been found to fit Equation (28) (p. 20) and that values of $k_{-2} k_{-1}$ have been determined at several temperatures. It is seen that if $k_{-1}$ were temperature-independent, then the ratio $\frac{k_{-2} k_{-1}}{k_{-2} k_{-1}}$ would equal $\frac{k_{-1}}{k_{-2}}$ and an unequivocal value for $E_a$ could be calculated from Equation (31) or (32), using the experimentally determined values of $k_{-2} k_{-1}$. From Equation (26),

$$k_{-1} = \left(k_h P_{O_2} \right)^{0.5} \tag{42}$$

In the great majority of cases, the solubility of a gas in a liquid decreases with increasing temperature; that is, $k_h$ decreases as the temperature increases. However, in the system under consideration in this work, a sealed bomb charged to the same pressure at room temperature is used in each oxidation run. Consequently, as the temperature of this bomb rises, the pressure of oxygen within it increases in approximate accordance with the gas law:

$$P_{O_2} = \frac{nRT}{V} \tag{43}$$
where \( n \) = number of moles of oxygen within the bomb (constant for each run at 0.246);

\[ R = \text{gas constant;} \]
\[ T = \text{absolute temperature; and} \]
\[ V = \text{volume of bomb (constant).} \]

The increase in pressure and the assumed decrease in \( k_2 \) with increasing temperature will tend to offset one another, keeping the product \( k_2 P_{O_2} \) fairly constant over the temperature range under consideration (from about 105° to 130°C.). Again, as mentioned earlier, the dependence of \( k_o \) upon the square root of \( k_2 P_{O_2} \) diminishes the effect of small variations in the latter expression upon the reaction rate.

As an example, these quantities have been calculated in the temperature range of from 80 to 100°C., using water instead of MBG as the substrate. It is seen in Table VIII that \( k_o \) varies only 1% over the 20°C. temperature range, in the case of water. It will be assumed that \( k_o \) is constant with the MBG-oxygen system and that the energy of activation can be determined with Equation (32), using the values of \( k_2 k_o \) in place of \( k_2 \).

**TABLE VIII**

**SOLUBILITY OF OXYGEN IN WATER**

<table>
<thead>
<tr>
<th>Temp., °C.</th>
<th>( P_{O_2} )^a</th>
<th>( k_{H_2} )^b</th>
<th>( k_2 P_{O_2} )^b</th>
<th>( k_o )^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>atm.</td>
<td>mole l.(^{-1}) atm.(^{-1})</td>
<td>mole l.(^{-1}) ( x 10^4 )</td>
<td>mole l.(^{-1}) ( x 10^2 )</td>
</tr>
<tr>
<td>80</td>
<td>20.4</td>
<td>7.86</td>
<td>160.3</td>
<td>12.65</td>
</tr>
<tr>
<td>90</td>
<td>20.9</td>
<td>7.68</td>
<td>160.5</td>
<td>12.67</td>
</tr>
<tr>
<td>100</td>
<td>21.5</td>
<td>7.59</td>
<td>163.2</td>
<td>12.78</td>
</tr>
</tbody>
</table>

\(^a\)Initial pressure, 17.2 atmospheres at 25°C.

\(^b\)Calculated from values in Handbook of Chemistry and Physics, 37th ed.
One other approximation which will be made in the treatment of the data should be noted here. Instead of the concentration of MBG, the percentage unreacted MBG will be used as the reaction variable. This is done because the volume of the melt is unknown, and the percentage unreacted MBG can be conveniently determined. The two expressions should be equivalent provided the volume of the melt does not change upon reaction. During the reaction, oxygen is absorbed in solution as well as in actual chemical reaction with MBG; water vapor and other gases are given off. An actual weight loss of up to 10% was observed. The consequent shrinkage in volume that might be expected is probably somewhat counteracted by the presumably lesser extent of hydrogen bonding in the products (since there are probably fewer hydroxyl groups in the products than in the reactants), leading to a smaller degree of intermolecular association and hence a larger specific volume. With the above method of data treatment, \([R-H]_0 = [MBG]_0 = 100\) and the second term of the left-hand side of Equation (28) has the value 0.1. A similar type of approximation was used by Liskowitz (28) in his study of the thermal condensation of glucose in vacuo.

Results

The data obtained are presented in Table IX. The percentage MBG reacted, rather than the percentage unreacted, is given, since it seems to present a clearer picture of the course of the reaction. \(F\) is the symbol for the function defined by the left-hand side of Equation (28). This function is plotted against reaction time for the four temperatures in Fig. 4. It is seen that fairly good straight lines are obtained, which shows agreement with the oxygen-initiation mechanism discussed earlier.
TABLE IX

KINETIC DATA FOR AUTOXIDATION OF MBG

<table>
<thead>
<tr>
<th>Run</th>
<th>Temp., °C.</th>
<th>Time, hr.</th>
<th>Reaction of MBG, %</th>
<th>Specific Rotation of Total Products, degrees</th>
</tr>
</thead>
<tbody>
<tr>
<td>74</td>
<td>108</td>
<td>9.0</td>
<td>0.0\textsuperscript{d}</td>
<td>0.0000</td>
</tr>
<tr>
<td>80</td>
<td>108</td>
<td>11.0</td>
<td>13.0\textsuperscript{d}</td>
<td>0.0142</td>
</tr>
<tr>
<td>71</td>
<td>108</td>
<td>13.5\textsuperscript{a}</td>
<td>21.7</td>
<td>0.0260</td>
</tr>
<tr>
<td>54</td>
<td>108</td>
<td>22.4</td>
<td>47.7</td>
<td>0.0764</td>
</tr>
<tr>
<td>55</td>
<td>108</td>
<td>46.6</td>
<td>80.4</td>
<td>0.2518</td>
</tr>
<tr>
<td>81</td>
<td>110</td>
<td>6.0</td>
<td>3.2</td>
<td>0.0036</td>
</tr>
<tr>
<td>61</td>
<td>110</td>
<td>8.4</td>
<td>27.5</td>
<td>0.0348</td>
</tr>
<tr>
<td>79</td>
<td>110</td>
<td>15.0</td>
<td>40.3</td>
<td>0.0566</td>
</tr>
<tr>
<td>53</td>
<td>110</td>
<td>22.0</td>
<td>65.0</td>
<td>0.1380</td>
</tr>
<tr>
<td>64</td>
<td>110</td>
<td>47.4</td>
<td>84.4</td>
<td>0.3062</td>
</tr>
<tr>
<td>69\textsuperscript{e}</td>
<td>120</td>
<td>2.0</td>
<td>20.7</td>
<td>0.0244</td>
</tr>
<tr>
<td>92\textsuperscript{e}</td>
<td>120</td>
<td>2.0</td>
<td>19.0</td>
<td>0.0222</td>
</tr>
<tr>
<td>68\textsuperscript{f}</td>
<td>120</td>
<td>5.0</td>
<td>44.1</td>
<td>0.0676</td>
</tr>
<tr>
<td>91\textsuperscript{f}</td>
<td>120</td>
<td>5.0</td>
<td>45.3</td>
<td>0.0704</td>
</tr>
<tr>
<td>66\textsuperscript{f}</td>
<td>120</td>
<td>12.0</td>
<td>74.9</td>
<td>0.1992</td>
</tr>
<tr>
<td>67\textsuperscript{f}</td>
<td>120</td>
<td>19.0</td>
<td>84.6</td>
<td>0.3094</td>
</tr>
<tr>
<td>82\textsuperscript{f}</td>
<td>130</td>
<td>1.5</td>
<td>29.6</td>
<td>0.0384</td>
</tr>
<tr>
<td>84\textsuperscript{f}</td>
<td>130</td>
<td>2.3</td>
<td>37.5</td>
<td>0.0526</td>
</tr>
<tr>
<td>83\textsuperscript{f}</td>
<td>130</td>
<td>33.0</td>
<td>49.0</td>
<td>0.0802</td>
</tr>
<tr>
<td>70\textsuperscript{f}</td>
<td>130</td>
<td>5.0</td>
<td>73.2</td>
<td>0.1860</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determined by quantitative chromatography, except as noted.

\textsuperscript{b} Function defined by left side of Equation (28).

\textsuperscript{c} In water with sodium D line; tabulated here for later reference.

\textsuperscript{d} Estimated from specific rotation.

\textsuperscript{e} Duplicate of Run 69.

\textsuperscript{f} Duplicate of Run 68.
Figure 4. Kinetics of MBG Autoxidation
The slopes of the lines were calculated by the method of least squares; these slopes were taken as estimates of the rate constants $k_{1,5}$ for the 1,5-order reaction. These rate constants, their 90% confidence limits, and their logarithms (for calculation of the energy of activation) are given in Table X.

### TABLE X

RATE CONSTANTS FOR 1,5-ORDER AUTOXIDATION OF MBG

<table>
<thead>
<tr>
<th>Temp., °C.</th>
<th>Rate Constant, $k_{1,5}$, hr.$^{-1} \times 10^4$</th>
<th>$\log_{10} k_{1,5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>108</td>
<td>66.9 ± 5.2</td>
<td>1.825 ± 0.033 - 0.035</td>
</tr>
<tr>
<td>110</td>
<td>72.5 ± 9.3</td>
<td>1.860 ± 0.053 - 0.059</td>
</tr>
<tr>
<td>120</td>
<td>168.7 ± 9.2</td>
<td>2.227 ± 0.023 - 0.024</td>
</tr>
<tr>
<td>130</td>
<td>439 ± 160</td>
<td>2.642 ± 0.135 - 0.196</td>
</tr>
</tbody>
</table>

A plot of the logarithms of the rate constants versus the reciprocal of the absolute temperature is given in Fig. 5. This shows that the data obey the Arrhenius equation [Equation (32)] fairly well. The energy of activation, calculated by the method of least squares, is 26.4 kcal./mole, which is comparable with the values obtained in other autoxidations (discussed earlier) and which is typical for a homogeneous chemical reaction in which diffusion is not rate-controlling (29).

In Fig. 6 it is shown that Equation (39) does not describe the autoxidation of MBG very well. A very large deviation from linearity is evident. This is taken as evidence that the type of autocatalysis for which this equation was derived does not occur in the case of MBG. Supporting evidence is the fact that no peroxides have been observed in the autoxidation products of MBG; the reaction products would have to be almost exclusively peroxides for this kinetic scheme to be valid.
Figure 5. Arrhenius Plot For MBG Autoxidation
Figure 6. Nonconformity of MBG Autoxidation to Autocatalytic Scheme
PRODUCTS OF GLYCOSIDE AUTOXIDATION

Gaseous Products

The evolution of considerable amounts of water vapor, judged from its absorption by Anhydrone, and small amounts of a gas which is absorbed by Ascarite (probably carbon dioxide) has been observed when glycosides are autoxidized. In the case of MBG, the water vapor has two distinct sources. The first to be evolved is the hemihydrate water of crystallization, amounting to 4.4% of the weight of the hemihydrate (8.8 mg./200 mg. hemihydrate). It is not necessary for autoxidation to occur for this water to be evolved; it was noted when MBG was heated in nitrogen (Run 62-N). After this has been evolved, and if autoxidation occurs, actual water of constitution begins to be given off. One possible source of this is dehydration involving a hydroxyl group on a certain carbon atom and a hydrogen atom on an adjacent carbon atom, thus giving rise to a double bond. Such dehydrations occur when sugars are treated with hot strong acid, giving rise to furfural derivatives, and indeed in the autoxidation of furfural itself (30).

The actual weight loss of MBG, determined by weighing the products immediately after autoxidation, is a fairly well-defined function of the percentage reaction of the MBG, as seen in Fig. 7. While there is some scatter of the points, no significant differences can be noted between temperatures. This supports the argument that the general course of the reaction did not vary with temperature.

In general, not more than 1% of the weight of any of the glycosides studied was evolved as carbon dioxide or other acidic gas, even at high degrees of autoxidation. Thus, carbon dioxide is not an important autoxidation product under these conditions.
Figure 7. Weight Loss of MBG During Autoxidation
An over-all material balance of the total products (residue plus evolved gases) was not obtained in these autoxidations. After consideration of the probable amounts of oxygen absorbed and water vapor and carbon dioxide evolved, some of the glycoside weight loss remained unaccounted for with MBG, this discrepancy increased in an approximately linear fashion with degree of autoxidation, reaching a value of 10% of the original weight of the MBG at nearly 100% reaction of MBG, indicating that perhaps some undetected gas was being evolved. As possibilities may be mentioned methanol, hydrogen peroxide, formaldehyde, and low molecular weight hydroperoxides. On occasions when the evolved gases were not passed through the gas absorption train, the gases were smelled; a distinct but unrecognized odor was noticed.

**Parent Sugars and Disaccharides**

Glucose (presumably in the D configuration) was detected in solutions of autoxidized MBG with the aid of paper chromatography; galactose was detected in autoxidized methyl α-D-galactopyranoside. These sugars are easily detected chromatographically because of their extreme sensitivity to a variety of spray and dip reagents (see Appendix II). The presence of glucose in autoxidized MBG was indicated with five different developing systems (A, B, C, E, and F). In addition, infrared spectral evidence was obtained for its presence. As described in Appendix II, that section of a heavy paper chromatogram which presumably contained glucose was cut out and eluted with water. A potassium bromide pellet made from the evaporated eluate was subjected to infrared examination and the spectrum compared with that of known glucose treated in a similar manner (31). The two spectra are compared in Fig. 8, from which the presence of glucose is inferred. The sample shown was from Run 27, in which MBG had been heavily autoxidized so as to obtain as large an amount as possible of glucose; chromatograms
Figure 8. Infrared Evidence for Glucose in Autoxidized MBG
indicated that the absolute amount of glucose increased with degree of autoxida-
tion up to about 2 weight per cent of the original MBG at nearly 100% reaction
of MBG. It is probable that glucose is continuously formed and autoxidized in
the mixture, but the rate of formation slightly exceeds the rate of autoxidation.

At least three substances with $R_g$ values in the disaccharide range were
observed in autoxidized MBG. Only one of these, gentiobiose, was identified
chromatographically with any degree of certainty (in autoxidized MBG). Genti-
obiose was the slowest moving of the compounds in both systems $C$ and $E$; it appeared
to be present in about the same amounts as glucose. Table XI summarizes the
attempts to identify these substances chromatographically (each of the disacchar-
ides listed is composed solely of glucose units linked in the specified manner).
The disaccharides other than gentiobiose were present in very minor amounts; they
were reducing, as shown by their reaction with the aniline phthalate spray reagent.
Similar materials were observed in the autoxidation products of other methyl gly-
copyranosides, but they were not extensively investigated.

**TABLE XI**

**ATTEMPTED IDENTIFICATION OF DISACCHARIDES IN AUTOXIDIZED MBG**

<table>
<thead>
<tr>
<th>Disaccharide</th>
<th>Type of Linkage</th>
<th>System $C^a$</th>
<th>System $E^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sophorose</td>
<td>$1 \rightarrow 2$ ($\beta$)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Laminaribiose</td>
<td>$1 \rightarrow 3$ ($\beta$)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>$1 \rightarrow 4$ ($\alpha$)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>$1 \rightarrow 4$ ($\beta$)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Isomaltose</td>
<td>$1 \rightarrow 6$ ($\alpha$)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>$1 \rightarrow 6$ ($\beta$)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

$^a$ Presence (existence of spot beside known reference spot) indicated by +; absence
indicated by -. Composition of solvent systems given in Appendix II.
Disaccharides are often found in the reaction mixture when aqueous glucose is heated in the presence of acid or when glycosides are hydrolyzed (32); even the simple melting of crystalline glucose in vacuo yields these substances (33). Overend, et al. (32) proposed that gentiobiose, which they found in hydrolyzed MAG, was formed by a reaction between the intermediate cyclic cation (formed by heterolysis of the conjugate acid of MAG) and glucose. In view of the strongly dehydrating conditions in the present work, it is perhaps more likely that these compounds were synthesized by the direct elimination of water from the appropriate hydroxyl groups. Since autoxidation of glucose alone under the conditions used in this work yielded these substances, it appears that their presence in autoxidized glycosides was the result of dehydration reactions between glycoses (formed during the autoxidation of the glycosides). The possibility of their formation via an acid-catalyzed mechanism cannot be ruled out, however, especially since solutions of autoxidized glycosides were found to be acidic (discussed below).

**Acidic Products**

Solutions of autoxidized MBG have been observed in this work to be invariably acidic, concentrations of 2% exhibiting pH's in the neighborhood of 3. Good chromatographic evidence has been obtained for the presence of gluconic acid and its deltalactone, using systems D and E with a bromcresol green spray; yellow spots were obtained beside yellow reference gluconic acid spots. The acid and lactone were also detected with the silver nitrate dip on chromatograms run in system E. The amount of gluconic acid did not vary over a very wide range at different degrees of autoxidation; the weight of gluconic acid appeared to be about 15% of the original weight of the MBG. Glucuronic acid was not detected in the reaction products, either chromatographically or with the aid of the naphthoresorcinol test (34).
Titrations as described in Appendix III were made with the products of Runs 53, 54, and 55; these solutions were found to contain 0.19, 0.32, and 0.25 milliequivalent of acid, respectively. This is a relatively large amount of acid, considering that only 0.985 millimole of MBG were originally present in each case. It seemed apparent from chromatograms that not all of this acid could be accounted for by gluconic acid. The most reasonable place for the excess carboxyl groups appears to be as substituents on the polymeric materials whose existence is postulated later.

Absence of Peroxides

Peroxides were not found in any of the autoxidized glycosides. Two qualitative tests were used in an attempt to detect these compounds, which are present at one stage or another in practically all autoxidations. The tests were the common iodine-starch test (35) and the titanium sulfate test (36); both are sensitive to quite small concentrations of peroxides, on the order of a few parts per million. Generally, autoxidations are carried out at considerably lower temperatures than those employed in this work, and under these less severe conditions the peroxides are apparently relatively stable. All of the other evidence in this work suggests that a typical autoxidation occurs with glycosides, with the formation of peroxides as initial products. The author believes that this does occur, but immediate decomposition in a nonautocatalytic fashion precludes the detection of these products.

Further Autoxidation Products

Chromatograms have shown the existence, in solutions of autoxidized glycosides, of substances which move slowly or not at all in the various solvent systems. The amounts of these substances increased with the degree of autoxidation of the glycoside. It seems likely that by far the greater portion of the
glycoside which undergoes autoxidation is eventually converted into such materials, which are most likely polymeric in nature. In some cases where reaction was allowed to proceed to nearly complete disappearance of the glycoside, the products were not completely soluble in water at room temperature; specifically, with MAG (Runs 101A and 102A), small pieces of film which rolled up into tight tubular structures were noticed in amounts estimated as 20 to 30% of the entire material (the rest promptly dissolved). Upon warming on a steam bath, these film particles eventually dissolved; hydrolysis may have played a part in their dissolution. These film particles were not further investigated; however, a fraction of this presumed polymer (from MBG) was isolated by solvent precipitation, as described below.

During the autoxidation of glycosides, an amber color is gradually formed. In the case of MBG, this is first evident to the eye at about 40% conversion of the MBG. At 95% conversion the color is a deep orange. Solutions of autoxidized MBG exhibit an absorption maximum in the ultraviolet in the vicinity of 270 to 280 millimicrons, indicating the probable existence of the carbonyl group; the absorption "tail" extends into the blue region of the visible spectrum, causing the amber appearance. It is of interest that chromatograms sprayed with an acidic solution of 2,4-dinitrophenylhydrazine show some degree of orange color at low R_2 values, indicating that carbonyl groups are possibly associated with the slow-moving, presumably polymeric products; this was substantiated by direct addition of the above reagent to a sample of isolated "polymer," as described below.

The specific rotation (in water with sodium light) of the complete reaction mixture from autoxidized MBG, including unreacted MBG, is a well-defined function of the percentage reaction of MBG, as shown in Fig. 9. The correlation enables
Figure 9. Specific Rotation of Solutions of Autoxidized MBG
a reasonable estimate of the degree of reaction to be made from the easily determined specific rotation.

Knowing the weight of the reacted mixture, its specific rotation, the specific rotation of anhydrous MBG, and the percentage reaction of MBG, it was possible to calculate the specific rotation of the non-MBG materials in the reaction mixture. Equation (44) was used for this purpose; it was derived from the basic assumption that the total observed rotation is equal to the sum of the observed rotation due to MBG and the observed rotation due to the other materials in the mixture.

\[
[\alpha]^D_{\text{Non-MBG products}} = \frac{[\alpha]^D_{\text{Total}} - [\alpha]^D_{\text{MBG}}(U)}{(1-U)} \tag{44}
\]

where \( U \) = weight fraction of MBG in reaction products.

A plot of the specific rotation of the non-MBG products as a function of the percentage reaction of MBG is shown in Fig. 10. As the percentage reaction increases, the postulated polymeric products become a progressively larger fraction of the total products (as shown by chromatograms). Hence, it was deduced that this presumed polymer, if it were isolated, would be strongly dextro-rotatory.

A fraction of the presumed polymer was isolated by precipitation from a 10% aqueous solution of rather heavily autoxidized MBG with isopropanol and subsequent centrifugation. The techniques used are given in detail in Appendix V. The material, obtained as a yellow tar, exhibited \([\alpha]^D_{25} = +100^\circ (c 0.3, \text{ water})\). The tar proved to be chromatographically homogeneous, being immobile in solvent systems C and E. It contained carbonyl groups, as shown by precipitation with 2,4-dinitrophenyhydrazine, and was slightly acidic to litmus.
Figure 10. Specific Rotation of Non-MBG Autoxidation Products of MBG
At high degrees of autoxidation, the products of MBG autoxidation were largely this presumably polymeric material; however, some glucose and gluconic acid (with positive specific rotations, but less than +100 degrees) still remained. Thus, the average rotation of the non-MBG products would be expected to be less than +100 degrees, which agrees with what was actually observed.

It will be recalled that the specific rotation of the autoxidation products of MAG decreases from the value for pure MAG as autoxidation proceeds (Fig. 3) and approaches the value for the products from MBG. At high degrees of autoxidation, the specific rotation of the total mixture was less than what might be expected if a polymer identical with that from MBG were being formed; this can be explained as being due to the presence of materials with lower specific rotations. At still higher degrees of autoxidation, a slow increase in specific rotation back to a value in the neighborhood of +100 degrees might be expected, as the materials with lower rotations were converted into the polymer. In any event, the specific rotation data indicate that the ultimate products of the autoxidation of MAG and MBG under the conditions used have similar if not identical properties, which supports the hypothesis that attack occurs principally, if not exclusively, at the anomeric carbon atom.

It has been shown by Wolfrom, et al. (37) and by Singh, et al. (38) that furfural and its derivatives are intimately associated with the degradation and discoloration which take place when glucose is heated in aqueous mineral acids. In addition, Dunlop, et al. (30) found that when furfural underwent autoxidation, presumably polymeric products were formed. The author believes that processes similar in general nature to these are responsible for the formation of the colored and presumably complex polymeric materials observed in autoxidized glycosides.
According to Dunlop, et al. (30), furfural can be autoxidized at room temperature to yield furoic acid, formic acid, and β-formylacrylic acid. The latter was thought to polymerize to a water-soluble "resin acid" containing carbonyl and end carboxyl groups and having the following structure:

\[
\begin{align*}
&\quad H - C = C - H \\
&\quad H - C - C - O - \quad \text{[\text{\(n\)}]} \\
&\quad \text{\(\text{\(n\)}\)} \\
&\quad O \quad O \\
&\quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad
\end{align*}
\]

Such a material would be acidic, react with carbonyl-sensitive reagents, and presumably show absorption in the ultraviolet in the vicinity of 270 millimicrons. Subsequent loss of water is postulated, with a shift in double-bond arrangements and formation of lactone groups. Although it contains asymmetric carbon atoms, it should be optically inactive because a racemic mixture should be formed from the originally inactive furfural (39).

As has been brought out, the presumably polymeric products formed during the autoxidation of glycosides display some of the above characteristics. However, the evidence indicates that they are strongly dextrorotatory. This indicates that dehydration to a furfural-type compound is not completed before polymerization begins; that is, a number of asymmetric centers remain in the individual units. This is supported by the observation that no more than roughly one mole of water vapor was evolved per mole of glycoside even after very extensive autoxidation, whereas dehydration to 5-(hydroxymethyl)furfural would require the loss of three moles of water per mole of glycoside.

Summary of Autoxidation Products of MBG

When the reaction had progressed to the point at which about 85% of the MBG had undergone reaction of some kind (Run 67), the total products had about
the following composition: unreacted MBG, 15%; water vapor, 15%; carbon dioxide, 1%; unidentified gaseous products, 10%; gluconic acid and/or its lactones, 10%; glucose, 2%; disaccharides, 2%; and the postulated polymer, 45%. Although a quantitative summation of the reaction products was not carried out in the strict sense of the term, it is felt that the above estimates are reasonably precise. As the reaction progressed, the postulated polymer became a progressively larger percentage of the nongaseous products.

A HYPOTHESIZED MECHANISM FOR THE AUTOXIDATION OF MBG

On the basis of the evidence presented in this thesis, as well as previous knowledge concerning autoxidation, it is possible to propose a reasonable mechanism for the first stages of the autoxidation of a methyl glycopyranoside. The author believes that this is the first time that the concepts of autoxidation have been considered with reference to simple glycosides.

Although MBG is the reference compound in the following discussion, it should be realized that other glycopyranosides would most probably be autoxidized in a similar fashion. The initiation, propagation, and termination phases of the free-radical reaction will be discussed individually; consideration will then be given to reactions of the postulated hydroperoxide which could account for some of the actual products observed.

INITIATION

Since the specific rotations of the autoxidation products of MAG and MBG converged toward a common value, and since gluconic acid was detected in the products, it appears that attack by oxygen occurred principally if not exclusively at the anomeric carbon-hydrogen bond. The theoretical considerations discussed
earlier also make it likely that direct attack occurs at this point. (For greater
clarity, those hydroxyl groups and hydrogen atoms which do not take part in the
hypothesized reaction have been omitted).

Presumably the transition state which leads to the formation of (II) would
be stabilized in a manner similar to that illustrated in Reaction (I4), except
that now two oxygen atoms with two unshared electron pairs each are adjacent to
the central carbon atom, allowing still more possible resonance forms. As con-
ceived here, this resonance effect would be stronger than the discouraging
inductive effect of the two oxygen atoms. After separation of the hydroperoxyl
radical, species (II) would be stabilized as follows:

\[
\begin{align*}
\text{H} & \text{O} \quad \text{OCH}_3 \\
\text{II} & \quad \text{II} \\
\end{align*}
\]

It is difficult to estimate the actual value of the resonance energy, but it should
definitely be appreciable and cause a decrease in the endothermicity of (45).
Species (II) would tend toward coplanarity at the anomeric carbon atom because
of the trigonal structure. Hence, subsequent addition at this point should
result in the formation of the \(\alpha\) and \(\beta\) anomers.

It would not be impossible for initiation to occur at the other carbon atoms;
each is adjacent to an oxygen atom with two unshared electron pairs. However, the
anomeric carbon atom is the only one adjacent to two oxygen atoms, and it would thus be expected that attack would be confined principally to this point.

**PROPAGATION**

Once species (II) has been formed, a typical chain propagation process is easily envisioned. This reaction sequence would be expected to be exothermic.

\[
\begin{align*}
\text{(II)} & \quad \text{OCH}_3 + \text{O}_2 \quad \text{Fast} \quad \text{(III)} \\
\text{(III)} & \quad \text{OCH}_3 + \text{H} \quad \text{OCH}_3 \quad \text{(IV)} \\
& \quad \text{(II)} \\
\end{align*}
\]

Compounds similar to (IV) have been synthesized and isolated. Milas, et al. (40) prepared 1-hydroperoxycyclohexanol, m.p. 76-78°C., from cyclohexanone and hydrogen peroxide:

\[
\begin{align*}
\text{C}_{12} & \quad \text{O} + \text{H}_2\text{O}_2 \quad \text{Fast} \quad \text{O-H} \\
& \quad \text{O-O-H} \\
\end{align*}
\]

The presence of the same compound was indicated in the autoxidation products of cyclohexanol (41). Criegee, et al. have isolated similar products (42).
TERMINATION

Reaction (49) is probably the most frequent termination reaction because of the high concentration of (III) as compared with other radicals. This is due to the rapidity of Reaction (45). In such a termination reaction various rearrangements are possible, such as those proposed by Russell wherein oxygen is evolved (43). In most free radical reactions, terminations account for only a very minor fraction of the total products, because the propagation chains are usually quite long.

FURTHER PEROXIDE REACTIONS

It is postulated that the hydroperoxide (IV) is unstable and can react as shown below.

\[ 2 \text{(III)} \rightarrow \text{Inactive products} \]  

Reaction (50) is analogous to that discussed by Rust and Youngman (44) in which the final product of the autoxidation of a secondary alcohol is the corresponding ketone:

\[ \text{R-C-R'} + O_2 \rightarrow \text{R-C-R'} + \text{H}_2\text{O}_2 \]
In both cases, the hydroxy- (or alkoxy-)hydroperoxide loses a hydroperoxide with the formation of the keto group. In the case of the glucoside, the "ketone" is the delta-lactone of gluconic acid. A reaction analogous to (51), except in the reverse direction, was carried out by Milas, et al. (46), as discussed on p. 69.

If hydronium ions are available, (IV) may be subject to hydrolysis:

\[
\begin{align*}
\text{H}_2\text{O} + \begin{array}{c}
\text{OCH}_3 \\
\text{O-O-H}
\end{array} & \xrightarrow{\text{H}_2\text{O}^+} \begin{array}{c}
\text{O} \\
\text{O-O-H}
\end{array} + \text{CH}_2\text{OH}
\end{align*}
\]  

(VI) could then lose hydrogen peroxide to give gluconic acid. The necessary hydronium ions could be derived from the gluconic acid formed by hydrolysis of gluconic lactone [from Reaction (50)]. Although water is probably in scarce supply in this system because of the high temperature, it will be recalled that it is continually being evolved during the reaction; it is possible that some of the water participates in these hydrolyses before escaping. It may be noted in passing that if hydrolysis of (IV) proceeded with the formation of a cyclic carbonium ion in a manner similar to the hydrolysis of normal glycosides (45), such a carbonium ion would probably be extensively resonance-stabilized due to the availability of the electron pairs on the adjacent peroxy oxygen atom, as well as the ring oxygen atom.

The formation of glucose during the autoxidation of MBG seems to have been a side reaction not directly associated with the autoxidation proper. That it could have been due to hydrolysis of MBG was shown by the observation that small amounts of glucose were detected chromatographically when a finely ground mixture
of nine parts MBG and one part gluconic delta-lactone was heated for four hours at 120°C. under 17.2 atmospheres of nitrogen, in the presence of water vapor (to simulate the water evolved during autoxidation).

APPLICATIONS TO CELLULOSE AUTOXIDATION

One of the original aims of this work was to clarify further, if possible, the details of the thermal autoxidation of cellulose. Major (15) showed that dry cotton cellulose was subject to autoxidation (as defined in the present thesis) when heated in a dry oxygen atmosphere at 170°C.; the reaction appeared to be concentrated in the amorphous regions of the cellulose. A very wide variety of compounds was produced; water of constitution was evolved.

The results of this thesis show clearly that the glycosidic bond (at least in methyl glycopyranosides) is labile to nonphotochemical autoxidation. It seems probable that autooxidative decreases in the degree of polymerization of cellulose, with attendant changes in strength properties, are traceable to direct attack by oxygen at the glycosidic carbon-hydrogen bonds.

The fact that the autoxidation of cellulose occurs in the amorphous rather than in the crystalline regions can be at least partly explained as being due to the much greater mobility of the polymer chains in the amorphous regions. Mobility is a prerequisite for reasonably rapid autoxidation, as shown in this thesis, presumably because of the necessity for free radical chain transfer and propagation reactions. It should be noted that dry cellulose autoxidation, whether in the amorphous or crystalline regions, is a heterogeneous reaction.

The apparent absence of uronic acid groups in autoxidized glycosides is worthy of notice, because Major reported their detection in autoxidized cellulose.
Although these groups are relatively sensitive to decarboxylation, the possibility exists that they might be formed at 170°C. and not at the lower temperatures used in this work. In any event, the present results make it seem probable that a larger fraction of the acidity noted in autoxidized cellulose (for example, in naturally and artificially aged paper) is due to gluconic acid than to glucuronic.
CONCLUSIONS

Pure methyl glycopyranosides in general appear to be subject to homogeneous autoxidation when exposed to molecular oxygen at temperatures equal to or higher than their melting or softening points, in the absence of visible and ultraviolet light. Autoxidation of these compounds in the dark at temperatures below their melting points (i.e., in the pure crystalline state) is probably also a general reaction, but since this heterogeneous reaction may be strongly temperature-dependent, it may be difficult to observe with relatively low-melting compounds.

In the case of methyl β-D-glucopyranoside (MBG), the homogeneous autoxidation is well described as being a nonautocatalytic reaction of order 1.5 with respect to MBG concentration in the temperature range of from 108 to 130°C. The energy of activation for the disappearance of MBG in this temperature range is 26.4 kcal./mole, which is comparable with the energies of activation of several other autoxidations. The observed reaction order, as well as other evidence, suggest that a typical free radical chain mechanism, involving direct initiation by oxygen, is operative; attack appears to occur principally if not exclusively at the anomeric carbon-hydrogen bond.

The products of the autoxidation include water vapor, small amounts of carbon dioxide, glucose, gluconic acid and/or its lactones, gentiobiose, other disaccharides (or similar substances), and a dextrorotatory, presumably polymeric material. Analogous materials are formed upon the autoxidation of other methyl glycopyranosides.

The specific rotation, in aqueous solution, of MBG together with its autoxidation products is directly related to the degree of autoxidation of MBG. Thus, the course of the reaction may be conveniently followed by specific rotation measurements. This is probably true for other methyl glycopyranosides as well.
SUGGESTIONS FOR FUTURE WORK

During this investigation a number of topics have suggested themselves as possible lines of fruitful future research. Foremost among these would be a more detailed study of the heterogeneous autoxidation of pure organic crystals—not only in the carbohydrate field but also with other types of organic compounds. Studies of this kind of reaction are very rare; the results of this work showed that such reactions may possibly have unusually high energies of activation, a hypothesis which merits further investigation.

Another interesting line of research would be further studies of the homogeneous autoxidation of carbohydrates. It would be of great interest to learn whether or not this reaction is generally of order 1.5, because this reaction order implies direct initiation of the chain reaction by oxygen.

A study of the structure and properties of the postulated polymeric materials noted among the autoxidation products of glycosides would be in order. The formation of small tubular structures composed of tightly wound thin films, upon the addition of water to extensively autoxidized glycosides, was an intriguing phenomenon.
NOMENCLATURE

CHEMICAL COMPOUNDS

In⁺ = generalized initiating free radical
MAG = methyl α-D-glucopyranoside
MBG = methyl β-D-glucopyranoside
R-H = generalized organic compound with labile hydrogen atom

PHYSICAL QUANTITIES AND CONSTANTS

A = resonance energy of a free radical R·
B = resonance energy of a free radical R-O-O·
C = constant of integration
E_pitä = over-all energy of activation for autoxidation
E_i = energy of activation for initiation reaction
E_p = energy of activation for propagation reaction
E_t = energy of activation for termination reaction
F = \(2 \left( \frac{1}{[R-H]^{0.5}} - \frac{1}{[R-H]_o^{0.5}} \right)\)
G = \([R-H]_o + [R-O-O-H]_o\)
\(\Delta H\) = heat of reaction
\(\Delta H_f\) = heat of fusion
k_p = combined rate constant for autoxidation
k_h = Henry's Law constant for oxygen dissolved in R-H
k_i = rate constant for initiation reaction
k_0 = square root of oxygen concentration in R-H
k_p = rate constant for propagation reaction
k_r = rate constant for post-initiation reaction
k_t = rate constant for termination reaction
n = number of moles of oxygen in bomb
\[ P_{O_2} = \text{pressure of oxygen in bomb} \]
\[ R = \text{gas constant} \]
\[ T = \text{absolute temperature} \]
\[ T_m = \text{melting point} \]
\[ t = \text{time} \]
\[ U = \text{weight fraction of MBG in autoxidation products} \]
\[ V = \text{volume of bomb} \]
\[ v_s = \text{specific volume of molten MAG} \]
\[ v_c = \text{specific volume of crystalline MAG} \]
\[ w = \text{order of chain-breaking step in autoxidation} \]

**AUTOXIDATION RUN NUMBER SUFFIXES**

<table>
<thead>
<tr>
<th>Suffix</th>
<th>Denotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Substrate was MBG</td>
</tr>
<tr>
<td>A</td>
<td>Substrate was MAG</td>
</tr>
<tr>
<td>G</td>
<td>Substrate was ( \alpha )-D-glucose</td>
</tr>
<tr>
<td>HQ</td>
<td>Hydroquinone present</td>
</tr>
<tr>
<td>K</td>
<td>Substrate was methyl ( \alpha )-D-xylopyranoside</td>
</tr>
<tr>
<td>-N</td>
<td>Run was made in atmosphere of prepurified ( N_2 )</td>
</tr>
<tr>
<td>X</td>
<td>Substrate was methyl ( \beta )-D-galactopyranoside</td>
</tr>
<tr>
<td>Y</td>
<td>Substrate was methyl ( \alpha )-D-galactopyranoside</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS

The author would like to express his sincere appreciation to the members of his Thesis Advisory Committee, Drs. J. W. Green, K. Ward, Jr., and N. S. Thompson, for their guidance and helpful suggestions during this work.

Thanks are also due the following:

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33. Thompson, N. S. Personal communication, 1963.


METHYL \( \beta \)-D-GLUCOPYRANOSIDE HEMIHYDRATE

Methyl \( \beta \)-D-glucopyranoside hemihydrate (MBG) was prepared by the method of Raymond and Schroeder (46). In this procedure, anhydrous dextrose is treated under reflux with methanol containing 3% by weight of dry hydrogen chloride; a reflux time of 1.5 hr. seemed to give the best yield in the present work. After cooling, methyl \( \alpha \)-D-glucopyranoside (MAG) separates upon seeding. This is filtered off and MBG is separated as the crystalline potassium acetate complex. The complex is decomposed with tartaric acid; after removal of precipitated potassium acid tartrate, MBG crystallizes from the concentrated solution.

MBG prepared by the above method seems invariably to contain glucose and small amounts of a disaccharide. These are not readily removable by recrystallization from ethanol. To remove the glucose, recourse was had to the well-known reaction of reducing sugars with strong alkali to form the saccharinic acids. Impure MBG was treated with an aqueous \( 1\text{N} \) solution of sodium hydroxide on a steam bath. The solution became yellow, indicating reaction of the glucose. After twenty minutes the cooled solution was passed through a column of Amberlite IR-120 resin (H\(^+\)) to remove the sodium and neutralize the solution, which was then passed through a column of Amberlite IR-4B resin (free base). This resin retained the saccharinic acids; the effluent was concentrated and worked up with ethanol to yield crystalline MBG. This procedure was used by Theander (47) to purify MBG.

After the above treatment, the MBG was found to be free of glucose but to still contain traces of the previously mentioned disaccharide. This was
effectively removed by formation and decomposition of the potassium acetate complex once more. The final product exhibited a corrected melting point of 109-110.5°C. and $\left[\alpha\right]_D^{25} = -32.6^\circ (c\ 10,\ water)$. Because of the difficulty in purifying this compound, a relatively wide range of melting points and specific rotations may be found in the literature (48); it is believed that the above constants are valid, since the product was shown to have a purity of 99.9+% by chromatography.

The quoted melting point of MBG requires some clarification. This is the temperature range in which what is normally termed "melting" occurs. However, at 106-107°C., loss of crystallinity and consolidation of the mass in the capillary tube is observed, without the actual formation of a liquid phase. This phenomenon is termed "softening" in this thesis; it is probably due to evolution of the hemihydrate water of crystallization. The same phenomenon was observed by Crossman (49), using MBG prepared by a completely different method. The specific rotation obtained by Crossman was identical to that reported above.

**METHYL $\alpha$-D-GLUCOPYRANOSIDE**

The MAG used was obtained as a by-product of the synthesis of MBG. It also contained glucose, which was removed by recrystallization from methanol. The final product had a melting point of 168-169°C. and $\left[\alpha\right]_D^{25} = +159.0^\circ (c\ 2,\ water)$; these figures agree with those reported in the literature (48).

**$\alpha$-D-GLUCOSE**

The $\alpha$-D-glucose was Baker Reagent Grade Anhydrous Dextrose. It had a melting point of 148-150°C. and an equilibrium $\left[\alpha\right]_D^{25} = +52.6^\circ$ (specified on label).
METHYL α-D-GALACTOPYRANOSIDE

The methyl α-D-galactopyranoside used was obtained in crude form from Dr. J. W. Green. After removal of galactose with sodium hydroxide, it crystallized as the hydrate. The water of crystallization was removed by heating it at 90°C. in vacuo in the presence of Anhydron for 24 hours. The final product had a melting point of 116-118°C. and [α]D25 = +196° (c 1, water). The literature reports a melting point of 114-116°C. and [α]D = +196.6° (50).

METHYL β-D-GALACTOPYRANOSIDE

A quantity of methyl β-D-galactopyranoside was obtained through the courtesy of E. Malcolm. It contained a small amount of galactose, which was removed by sodium hydroxide treatment. The final product had a melting point of 178-179°C. and [α]D20 = 0.0° (c 1, water). The literature gives a melting point of 178°C. and [α]D = -0.4° (50).

METHYL α-D-XYLOPYRANOSIDE

Some crude methyl α-D-xylopyranoside was obtained from Dr. J. W. Green. After two recrystallizations from ethanol it had a melting point of 90-91°C. and [α]D25 = +153.0° (c 2, water). The literature gives a melting point of 90-92°C. and [α]D = +153.5° (51).
APPENDIX II

CHROMATOGRAPHIC METHODS

QUALITATIVE CHROMATOGRAPHY

Descending paper partition chromatography was extensively used in this work for identification of the reaction products of the various autoxidations. Whatman papers were used exclusively, No. 1 being used routinely for qualitative identification as well as for quantitative analysis of unreacted MBG in the kinetic study.

The following solvent systems were employed.

A. Butanol:acetic acid:water (5:1:4 v/v).


C. Ethyl acetate:pyridine:water (8:2:1 v/v).

D. Isobutyric acid:water (8:1 v/v).

E. Ethyl acetate:acetic acid:water (9:2:2 v/v).


The most generally useful systems were C and E. System C was used for quantitative analysis of MBG, since a very clean separation of MBG from all other products was obtained in 12 hours' running time. Acidic materials were stationary in this system but were well resolved in E. System A separates acids well but moves rather slowly down the sheet. Formic acid in system B is very difficult to remove from the sheet upon drying, even in an oven, and this causes difficulties with various spray reagents. System D is slow-moving and disagreeable to work with; however, it was useful in the identification of gluconic acid. Acidic materials streak extensively in F when the lactones are present.
For spot detection, the following dips and sprays were used.

(1). Silver nitrate-sodium hydroxide-sodium thiosulfate (52).
(2). p-Anisidine hydrochloride (53).
(3). Aniline phthalate (54).
(4). Bromcresol green solution (E. H. Sargent & Co.).
(5). 2,4-Dinitrophenylhydrazine (0.4% in 2N HCl).

Reagent (1), a three-stage dip followed by a water wash, detects a wide range of compounds and was the most generally useful method of spot detection. Sprays (2) and (3) detect reducing sugars, especially with the aid of an ultraviolet lamp. Spray (4) gives a yellow spot with acidic materials and was used to show the presence of gluconic acid. Spray (5) is a well-known reagent for carbonyl groups, giving an orange spot in most cases.

Some important \( R_g \) values are given in Table XII.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_g ) C</th>
<th>( R_g ) E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>MGB</td>
<td>2.47</td>
<td>2.24</td>
</tr>
<tr>
<td>Gluconic acid</td>
<td>0.08</td>
<td>1.22; 2.89(^a)</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>0.00</td>
<td>0.94; 3.30(^a)</td>
</tr>
<tr>
<td>Gentiose</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0.36</td>
<td>0.35</td>
</tr>
</tbody>
</table>

\(^a\) Lactone.
IDENTIFICATION OF GlUCOSE

A method similar to that used by Henderson (31) was used to demonstrate the presence of glucose in autoxidized MBG. A heavy paper chromatogram (Whatman No. 17) was prewashed with water, dried, and then streaked with enough autoxidized MBG solution to yield an estimated 1 mg. of glucose. Guide strips of known glucose were spotted on either side. The chromatogram was developed in system C. After thorough drying, glucose was located on the guide strips and the portion of the main strip which presumably contained glucose was cut out. This section was eluted with water, and some spectral-grade potassium bromide was dissolved in it. The solution was evaporated to dryness on the steam bath with addition of ethanol, and the crystals were dried at 90°C. in a vacuum oven over Anhydrone. A pellet was made from the potassium bromide; its spectrum was determined with a Perkin-Elmer Model 21 infrared spectrophotometer. It was found that a sufficient amount of water was present in the pellet, even after drying, to cause interference; therefore, a fraction of the pellet was made into a new pellet with additional anhydrous potassium bromide. The spectra obtained, No. 1720 and No. 1722 (from Runs 27 and 50), were compared with Henderson's spectra No. 111 and No. 113. The agreement between them (No. 1720 and No. 113 are compared in Fig. 8) is evidence for the presence of glucose in solutions of autoxidized MBG.

QUANTITATIVE CHROMATOGRAPHY

The phenol-sulfuric acid method (55), with slight modifications, was used for the quantitative determination of MBG, after separation of the MBG from the other compounds present in the autoxidation mixtures. In this method, exactly 10 microliters of solution (made by dissolving the MBG and its reaction products
in water and diluting to 10 ml. in a volumetric flask) are spotted on Whatman No. 1 paper. These spots are made in duplicate, and side guide strips are also spotted. The chromatogram is developed in system C for approximately 12 hours. It is then thoroughly dried and the side strips are cut off and given the silver nitrate dip to locate the MBG. The untreated areas containing the MBG are cut out, keeping all areas the same. These, together with a blank from the same chromatogram containing no MBG or oxidation products, are eluted with water into small collection pipets of volume 0.375 ml. These are discharged into 10-ml. volumetric flasks, rinsing each pipet twice into the flask. To each flask is then added 1 ml. of a 5% aqueous phenol solution and 5 ml. of concentrated sulfuric acid. It is difficult to add sulfuric acid quantitatively from a pipet because of its high viscosity, but small variations in the volume of acid delivered have no effect because all solutions are brought to the same final volume (10 ml.). The hot sulfuric acid–water solution hydrolyzes and dehydrates the MBG to 5-(hydroxymethyl)furfural, which condenses with the phenol to give an orange product. After 15 minutes the flasks are cooled and the contents diluted to the mark in two dilutions (cooling the flask between dilutions). The absorbance of each solution is measured relative to the blank at 490 millimicrons with a Beckman Model DU spectrophotometer.

It was necessary to make a calibration curve, using known amounts of MBG and following the above procedure. The curve obtained is shown in Fig. 11. From the least squares regression line, it was calculated that the percentage MBG unreacted in a given run is given by the following formula:

\[
\text{Percentage unreacted MBG} = 163.8 (A - 0.006) \tag{52}
\]

where \( A \) = mean of duplicate absorbance measurements.
Figure 11. Calibration Curve for MBG Determination
Using standard statistical methods (56), it was calculated that the 90% confidence limits for a determination of percentage unreacted MBG in a given autoxidation run, using the mean of duplicate observations of optical density (absorbance), are ± 4%. The precision could be somewhat improved by determining more points on the calibration curve and by making MBG determinations in triplicate, but there are inherent limitations in this method, such as great sensitivity to microscopic dust particles. However, it is probably the best method now available for determining MBG in a complex mixture.
A volumetric method based on that used by Isbell (57) in his studies on gluconic acid was used to estimate the total acidity in solutions of autoxidized MBG. The method involves the addition of an excess of sodium hydroxide (which saponifies lactones and converts them to the sodium salts of the free acids) to an aliquot of the solution and back-titration with sulfuric acid, using a pH meter.

A 1-ml. aliquot of the solution under study (total volume 10 ml.) was diluted with water and to it was added 5.00 ml. of 0.1N sodium hydroxide (0.5 milliequivalent). The solution was then back-titrated with 0.1N sulfuric acid, noting the pH after incremental additions of acid. When the pH was plotted against the volume of acid added, the typical sigmoid neutralization curve appeared. The equivalence point was taken as the point of greatest rate of change of pH. The milliequivalents of titratable acid in the aliquot were equal to the difference between the milliequivalents of acid needed to neutralize 5.00 ml. of alkali and the milliequivalents of acid needed to neutralize 5.00 ml. of alkali in the presence of acid from the aliquot. The total amount of acid in the solution under study was equal to ten times the amount found in the aliquot.

These titrations were found to be reproducible, and they gave accurate results with known amounts of gluconic delta-lactone. In addition, glucose was found not to interfere with the titration; evidently, the formation of saccharinic acids from glucose and sodium hydroxide is slow enough at room temperature to be unimportant.
A polarizing microscope was used in conjunction with a Fisher-Johns melting point apparatus to study the effect of slight autoxidation on the crystal properties of MAG. With this apparatus, the influence of autoxidation on the melting point and other properties of very small crystals could be observed. In addition, the behavior of the pure crystals could be microscopically observed over a range of temperatures up to the melting point. These observations lend credence to the theory of heterogeneous autoxidation stated in this thesis.

The procedure used in these observations was as follows. A small quantity of finely powdered crystals was placed on a circular cover glass, which was placed in the hollow of the Fisher-Johns apparatus. The metal block, from which a thermometer protruded from a deep socket, was electrically heated. By means of a variable resistance, the heating rate was adjusted so that a very slow temperature rise (approximately 1°C. per minute) occurred in the vicinity of the melting point. The crystals were observed with both reflected light and light transmitted through a small hole drilled in the block. A magnification of 100 diameters was generally used.

With the pure compounds studied in this work, melting was observed to occur very sharply and rapidly at the melting point. At a point on the block directly over the thermometer bulb, it was found that the same melting points were obtained as with a capillary melting point apparatus, indicating that the thermometer calibration was satisfactory. Other points on the block were as much as 3°C. lower in temperature than the point directly over the bulb.
APPENDIX V
POLYMER PRECIPITATION AND ANALYSIS

A fraction of the presumably polymeric material noted in autoxidized MBG (and materials similar which were very probably present in other autoxidized glycosides) was isolated by precipitation from aqueous solution by the addition of isopropanol. A sample of MBG (0.6 g.) was autoxidized at 140°C. for six hours, after which time about 90% of the MBG had undergone reaction. The resulting orange glass was entirely soluble in 5 ml. of water. To half of this solution was added five times its volume of isopropanol; an immediate cloudiness was noted. After standing a few minutes, the suspension was centrifuged; a small quantity of yellow tarry material separated. The supernatant was decanted, and the residue was redissolved in 1.5 ml. of warm water and reprecipitated with isopropanol. After centrifugation, the yellow tar was again obtained as a residue. Chromatographic examination in systems C and E, using the silver nitrate dip method, showed that the residue was homogeneous; it contained only material which was immobile on the chromatograms, in contrast to the original aqueous solution (which contained glucose, unreacted MBG, gluconic acid, etc.). Upon addition of more isopropanol to the supernatant from the centrifugation, more precipitation was noted; however, only the first fraction (presumably containing the highest molecular weight material) was further investigated. This fraction was dissolved in a small volume of water, filtered through Celite, and made up to 3 ml. for determination of its specific rotation in a 2-dm. small diameter polarimeter tube. After the observed rotation was obtained in sodium light, a weighed aliquot of the solution was concentrated to dryness and the residue (in the form of a hard yellow film) was weighed. The yield was 10 mg., and the specific rotation was calculated to be +100° (c 0.3, water).
A solution of the residue in water gave a precipitate with an acidic solution of 2,4-dinitrophenylhydrazine when warmed for a short period over a flame. A solution of glucose of equal concentration also gave a precipitate with this reagent when warmed, but only after a considerably longer heating time. It is probable that the isolated, presumably polymeric material contained free carbonyl groups. Its aqueous solution was slightly acidic to litmus, so the presence of carboxyl groups was also inferred. Further analysis was not attempted.
The effect of pressure upon the melting point of a crystalline substance is given by the Clausius-Clapeyron equation (52):

\[
\frac{\Delta T_m}{\Delta P} = \frac{T_m (v^L - v^S)}{\Delta H_f}
\]  

(52)

where \( T_m \) = melting point, °K.;

\( P \) = pressure, atm.;

\( v^L \) = specific volume of melt, cc./g.;

\( v^S \) = specific volume of solid, cc./g.; and

\( \Delta H_f \) = heat of fusion of solid, cc.-atm./g.

In the case of MAG, \( v^S \) is 0.675 cc./g., as calculated from the value of its density given by Bollenback (48). The quantity \( v^L \) was estimated as 0.76 cc./g. by measuring the volume of a known weight of molten MAG in a capillary tube [knowing the radius of the tube (0.070 cm.), the volume of melt was calculated by measuring the length of the column occupied by the melt].

The heat of fusion was estimated from the approximate relation given by Glasstone (52):

\[
\frac{\Delta H_f}{T_m} = 13 \text{ cal./mole °K.}
\]  

(53)

Since for MAG, \( T_m = 442 \) °K., \( \Delta H_f \) was calculated to be 30 cal./g., or 1239 cc.-atm./g. Substitution in Equation (52) resulted in a calculated elevation of the melting point of MAG of 0.029 °K./atm. If the bomb is charged with 17.2 atm. of oxygen at room temperature, then the pressure at 150°C. is 24.4 atm. Hence, the elevation of the melting point is 0.71°C. at this temperature. The melting point is
elevated because the density of the solid phase is greater than that of the liquid phase; hence, increases in pressure favor the solid phase, or make it stable at a higher temperature than was the case at the lower pressure.