I. REACTIONS OF α-LINKED DISACCHARIDES

II. SYNTHESIS OF THE 2,4-DI-O-METHYL TETROSES

by

KEITH NORMAN SLESSOR

B.Sc., The University of British Columbia, 1960

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

CHEMISTRY

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

August, 1964

In presenting this thesis in partial fulfilment of

the requirements for an advanced degree at the University of

British Columbia, I agree that the Library shall make it freely

available for reference and study. I further agree that per-

mission for extensive copying of this thesis for scholarly

purposes may be granted by the Head of my Department or by

his representatives. It is understood that copying or publi-

cation of this thesis for financial gain shall not be allowed

without my written permission.

Department of ___Chemistry_____

The University of British Columbia,
Vancouver 8, Canada

Date ___Sept 16 / 1964___

The University of British Columbia

FACULTY OF GRADUATE STUDIES

PROGRAMME OF THE

FINAL ORAL EXAMINATION

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

KEITH NORMAN SLESSOR

B.Sc., The University of British Columbia, 1960

TUESDAY, SEPTEMBER 15th, 1964, AT 10:30 A.M.
IN ROOM 261, CHEMISTRY BUILDING

COMMITTEE IN CHARGE

Chairman: I. McT. Cowan

G.G.S. Dutton   T. Money
L.D. Hall   A. Rosenthal
J.P. Kutney   G.M. Tener
C.A. McDowell   J. Trotter

External Examiner: Professor J.K.N. Jones F.R.S.

Queens University, Kingston

I. Reactions of ∝-Linked Disaccharides

II. Synthesis of the 2,4-Di-O-Methyl Tetroses

ABSTRACT

I. Reactions of ∝-Linked Disaccharides

Through reaction of specifically substituted maltoses, ∝-glucosidic disaccharide derivatives have been prepared. Catalytic oxidation of benzyl β-maltoside yielded maltobiouronic acid. Tritylation of 1,6-anhydro-β-maltose made possible the preparation of the 6'-O-tosyl ester. Replacement of the tosylate with azide ion followed by reduction and hydrolysis yielded a small amount of 6'-amino-6'deoxy-maltose. Replacement of the tosylate with thiolacetate allowed the preparation of 6'-deoxy-6'-mercapto-maltose. Iodide replacement of the sulphonyl ester followed by reduction gave the 6'-deoxy-1,6-anhydro derivative which was converted to 6'-deoxy-maltose by acetolysis and deacetylation.

A route for the preparation of 4-O-(∝-D-glucopyranosyluronic acid)-D-xylose by selective decarboxylation of maltosyldiuronic acid was attempted and found unfeasible. Attempts to prepare 6-substituted maltoses by reaction of benzyl 4',6'-O-benzylidene-β-maltoside with various reagents were unsuccessful.

II. Synthesis of the 2,4-Di-O-Methyl Tetroses

The four isomeric 2,4-di-O-methyl tetroses were prepared by periodate oxidation of known methylated sugars. 2,4-Di-O-methyl-D-and L-erythroses were prepared from 4,6-di-O-methyl-D-glucose and 3,5-di-O-methyl-L-arabinose respectively. 2,4-Di-O-methyl-D-and L-threoses were prepared from 3,5-di-O-methyl-D-xylose and 1,4,6-tri-O-methyl-L-sorbose.

The tetroses were characterized as their crystalline 2,4-dinitrophenylhydrazones. The R_f and R_G values of the free sugars were recorded in a variety of solvents including a silica gel thin-layer chromatography system.

GRADUATE STUDIES

<u>Field of Study</u>: Organic Chemistry

Topics in Organic Chemistry	J.P. Kutney
	D.E. McGreer
	R.E. Pincock
Carbohydrate Chemistry	G.G.S. Dutton
	L.D. Hayward
	A. Rosenthal
Physical Organic Chemistry	R. Stewart
Reaction Mechanisms	R.E. Pincock
Natural Products	A.I. Scott
Newer Synthetic Methods	G.G.S. Dutton
	A. Rosenthal
Polysaccharide Chemistry	G.G.S. Dutton

<u>Related Studies</u>:

Topics in Physical Chemistry	A. Bree
	J.R. Coope
	R. Snider
Topics in Inorganic Chemistry	N. Bartlett
	H.C. Clark
	W.R. Cullen
Crystal Structure	N. Bartlett
	S. Melzak
	J. Trotter

PUBLICATIONS

S.A. Black, G.G.S. Dutton and K.N. Slessor,

"Synthetic Disaccharides", Advances in Carbohydrat(
Chemistry, accepted for publication.

G.G.S. Dutton and K.N. Slessor,

"Synthesis of the 2,4,Di-O-Methyl Tetroses",
Canadian Journal of Chemistry, XLII (1964),
pp. 614-619.

G.G.S. Dutton and K.N. Slessor,

"Synthesis of Maltobiouronic Acid (4-O(α-D-
Glucopyranosyl-uronic Acid)-D-glucose)",
Canadian Journal of Chemistry, XLII (1964),
pp.1110-1112.

ABSTRACT

Chairman: Professor G. G. S. Dutton

I.   REACTIONS OF α-LINKED DISACCHARIDES

Through reaction of specifically substituted maltoses, α-glucosidic disaccharide derivatives have been prepared. Catalytic oxidation of benzyl β-maltoside yielded maltobiouronic acid. Tritylation of 1,6-anhydro-β-maltose made possible the preparation of the 6'-O-tosyl ester. Replacement of the tosylate with azide ion followed by reduction and hydrolysis yielded a small amount of 6'-amino-6'-deoxy-maltose. Replacement of the tosylate with thiolacetate allowed the preparation of 6'-deoxy-6'-mercapto-maltose. Iodide replacement of the sulphonyl ester followed by reduction gave the 6'-deoxy-1,6-anhydro derivative which was converted to 6'-deoxy-maltose by acetolysis and deacetylation.

A route for the preparation of 4-0-(α-D-glucopyranosyl-uronic acid)-D-xylose by selective decarboxylation of maltosyldiuronic acid was attempted and found infeasible. Attempts to prepare 6-substituted maltoses by reaction of benzyl 4',6'-O-benzylidene-β-maltoside with various reagents were unsuccessful.

II. SYNTHESIS OF THE 2,4-DI-O-METHYL TETROSES

The four isomeric 2,4-di-O-methyl tetroses were prepared by periodate oxidation of known methylated sugars. 2,4-Di-O-methyl-D-and L-erythroses were prepared from 4,6-di-O-methyl-D-glucose and 3,5-di-O-methyl-L-arabinose respectively. 2,4-Di-O-methyl-D- and L-threoses were prepared from 3,5-di-O-methyl-D-xylose and 1,4,6-tri-O-methyl-L-sorbose.

The tetroses were characterized as their crystalline 2,4-dinitrophenylhydrazones. The R_f and R_G values of the free sugars were recorded in a variety of solvents including a silica gel thin-layer chromatography system.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

I. REACTIONS OF α-LINKED DISACCHARIDES

II. SYNTHESIS OF THE 2,4-DI-O-METHYL TETROSES

ACKNOWLEDGEMENTS

I.   REACTIONS OF α-LINKED DISACCHARIDES

INTRODUCTION *

Purpose

In spite of the importance of starch and similar food polysaccharides, no satisfactory method has been developed for synthesizing the α-glucosidic linkage. Many approaches have been tried, but in nearly all cases the synthesis of α-glucosides has been hindered by very low yields of the desired product.

An obvious route remains for the synthesis of disaccharides containing α-linkages. This is the modification of naturally occurring disaccharides having the α-glucosidic configuration.

The reactions commonly used in monosaccharide chemistry have seldom been effectively applied to disaccharides. Due to the ease of hydrolysis of the glycosidic bond, no reaction in a synthetic sequence may employ strongly acidic conditions. As disaccharides contain twice as many hydroxyl groups, reactions normally exhibiting selectivity in simpler systems often fail when applied to disaccharides. The reactivity normally associated with a specific hydroxyl group may be drastically altered by steric factors originating from the proximity of the two sugar units. It is obvious that

---

* The following conventional abbreviations have been used throughout the text: tosyl = Ts (p-toluenesulphonyl), mesyl = Ms (methanesulphonyl), and trityl = Tr (triphenyl-methyl). All ring-hydrogen atoms are omitted in the Haworth structures.

indiscriminate use of general procedures employed in monosaccharide chemistry will not be successful.

Reactions employed in modifying disaccharides may rely on preferential etherification of primary hydroxyl groups, or blocking of specifically oriented hydroxyls by acetals or ketals. A rather unique functional group, the 1,6-anhydro-linkage, enables simultaneous blocking of the primary hydroxyl of the reducing sugar as well as the reducing function itself. The use of such reactions may permit the modification of disaccharides at specific positions.

In an effort to elucidate some of the reactions that are applicable to disaccharide chemistry, attempts to prepare specifically substituted maltose derivatives were undertaken. Maltose, 4-O-(α-D-glucopyranosyl)-D-glucose, was chosen for three reasons. Maltose is the most common α-linked disaccharide containing glucose. Secondly, the action of various hydrolytic enzymes upon modified maltoses should provide some information on the specificity of these enzymes. This in turn may provide information on the fine structure of starch. Finally, the introduction of reactive substituents into amylitol (1, 2) has provided derivatives with radically different properties. Synthetically altered maltoses would provide not only model compounds for such studies, but also reference compounds for comparison of products obtained by partial hydrolysis.

Chemical Synthesis of α-Glucosidic Disaccharides

Koenigs-Knorr Synthesis

Although several methods exist for the preparation of disaccharides, the method generally used is the elimination of hydrogen halide between a glycosyl halide and a hydroxyl group. The reaction is carried out in the presence of an acid acceptor which may take part directly in a concerted mechanism. The reaction was introduced by Koenigs and Knorr (3), who condensed acetobromglucose with methanol in the presence of various acid acceptors. If the methanol is replaced with a monosaccharide possessing one free hydroxyl group, a disaccharide results. This reaction and the other less common methods of synthesis have been the subject of three reviews (4, 5, 6). Recently a tabular list of synthetic disaccharides has been prepared (7).

The replacement of the bromide in the Koenigs-Knorr reaction generally proceeds with an inversion of carbon atom one. The product of the reaction possesses therefore, the opposite configuration to the reactant glycosyl halide. The configuration of the halide in acetobromglucose has been found to be α. The reasons for this have been summarized by Lemieux (8), "in the absence of large axial substituents at the 3- or 5-positions, the thermodynamically stable anomer for glycopyranosyl halides is the anomer which

has the halogen in axial orientation". Thus, the Koenigs-Knorr reaction proceeds with acetobromo glucose to give mostly the β-glucoside.

A β-glucosyl halide might be expected to give the desired α-anomer upon reaction, but studies have shown that the 1,2-trans halide-acetoxy system reacts by way of an orthoacetate intermediate, preserving the initial configuration (9). The use of a nitro ester as a non-participating group at position two gave isomaltose (6-0-(α-D-glucopyranosyl)-D-glucose) in high yield (10). This yield was later attributed to a very active silver perchlorate catalyst, the synthesis of which could not be repeated (11). The trichloroacetyl ester has been used as a non-participating group, since the electron withdrawing power of the three chlorine atoms of the trichloroacetyl function should deactivate the carbonyl to such an extent that it would be unable to assist in the displacement of the halide (11, 12).

The effect of catalysts, condensing agents, and ratio of reactants has been extensively studied in the case of the non-participating β-glucosyl chlorides, in an attempt to obtain reproducible high yields. Condensations to the primary 6 position were reasonably successful. However, condensations at secondary positions, for example kojibiose (2-0-(α-D-glucopyranosyl)-D-glucose), gave very low yields (11).

Future investigations of more reliable catalysts may
provide higher yielding condensations. At present, the
preparation of α-linked glucoside disaccharides by the
Koenigs-Knorr condensation must be considered impractical.

Inversion at Carbon Atom 2

An interesting approach to α-glucopyranosides was
reported early this year (13). An attempt was made to
replace iodide in alkyl 2-deoxy-2-iodo-α-D-mannopyranoside
triacetates with acetate. The iodo compound is reported
to be readily prepared. Thus a S_{N2} replacement of iodo
by acetate would result in an α-glucopyranosyl configuration.
Unfortunately, the iodide was found highly resistant to
nucleophilic attack on carbon.

Condensation Via 1,2-Anhydro Rings

Alcoholysis of tri-O-acetyl-1,2-anhydro-α-D-glucopyranose
(Brigl's anhydride) at room temperature has been shown to
produce β-glucosides in good yield (14, 15). However, the
steric outcome of the reaction is changed if the reaction is
carried out at elevated temperatures. The reaction of phenol
with Brigl's anhydride at 100° yielded only phenyl 3,4,6-
tri-O-acetyl-α-D-glucopyranoside (14). Lemieux (16)
visualizes the reaction as proceeding through a 1,6-β-D- cyclic
ion which is capable of reacting at carbon atom one to form

the α-glucoside.

Haworth and Hickinbottom (17) were the first to utilize
Brigl's anhydride in the synthesis of disaccharides.
Condensation of this anhydride with 2,3,4,6-tetra-O-acetyl-
β-D-glucose in benzene at 90-100° gave an eight percent
yield of α,β-trehalose.

In a similar manner, Lemieux (18, 19) has succeeded in
synthesizing maltose octaacetate and sucrose octaacetate by
condensation of Brigl's anhydride with 1,2,3,6-tetra-O-acetyl-
β-D-glucopyranose and 1,3,4,6-tetra-O-acetyl-β-D-fructose
respectively. The synthesis of sucrose, although erroneously
reported by Pictet and Vogel (20, 21), had not been previously
accomplished.

Kojibiose was isolated from a mixture obtained by heating
Brigl's anhydride at 116° for several days (22). Two other
disaccharide products were present, one of which was
chromatographically identical to α,α-trehalose.

Condensations with Brigl's anhydride at elevated
temperatures give products in which the α-isomer predominates.
Unfortunately, side reactions such as self-condensation of
the anhydride are known to occur under these conditions. As
a result, in all cases studied, the yields of disaccharide
were low.

Anomerization

The anomerization of alkyl glycosides was first reported
by Pacsu (23) in 1928. Refluxing solutions of stannic
chloride or titanium tetrachloride converted methyl tetra-
O-acetyl-β-D-glucopyranoside into the corresponding α-anomer.

Although varied opinions exist as to the mechanism of
this reaction, Lindberg (24) and Lemieux (25) have shown that
the glycosidic linkage is not completely broken during the
anomerization. Lemieux (16) has discussed the mechanism
postulated by Lindberg (24), and has suggested an alternate
mechanism which he feels meets fewer objections.

A refluxing solution of titanium tetrachloride in
chloroform anomerized gentiobiose octaacetate to the α-
anomer, isomaltose (26). A similar reaction using antimony
pentachloride as the catalyst gave yields of one half that
of the titanium tetrachloride catalysis (27).

A unique combination of reactions, including anomerization,
led to the synthesis of 6-0-(α-D-glucopyranosyl)-D-galactose
(28). A Koenigs-Knorr condensation of tetra-0-acetyl-α-D-
glucopyranosyl bromide and 1,2:3,4-diisopropylidene galactose
gave only the β-1,6-linked disaccharide. Anomerization
of the disaccharide octaacetate with titanium tetrachloride
yielded an equilibrium mixture of the α- and β- glycosidic

forms. De-O-acetylation and digestion of the mixture with β-glucosidase left only the α-1,6-disaccharide.

Anomerization has not been widely used in the synthesis of α-disaccharides for two reasons. The first of these is that recent extension of anomerization to disaccharides has not allowed time for the full utilization of this procedure. Secondly, the reaction seems generally applicable only to disaccharides in which the glycosidic bond is to a primary hydroxyl. It is possible that new catalysts for the anomerization of disaccharides linked in secondary positions will be developed. Such an advancement would enable preparation of all types of α-disaccharides.

Modification Reactions

Due to difficulties involved in the synthesis of α-disaccharides, the possibility of modifying naturally occurring α-glucosyl-disaccharides was investigated. Modifications which allow the alteration of a position on the sugar unit require the reaction to be specific. The means of achieving this specificity usually requires the use of blocking groups on all positions where reaction is undesired. The judicious choice of blocking groups may allow their preferential removal, thus permitting a series of consecutive reactions to be carried out on different positions. The following discussion outlines the different types of blocking

groups and describes their synthetic applications in disaccharide chemistry.

Protection of the Reducing Function

The reducing function of a disaccharide is an aldehyde usually existing in a hemiacetal form. The extreme ease of oxidation and reduction of this group necessitates its protection during most reactions. Chemically, blocking groups for the reducing function can be described as either aldehyde or hemiacetal derivatives. The latter retains the ring structure of the sugar, whereas the former opens the ring to give an acyclic derivative. Emphasis will not be placed on this distinction however, since the chemical properties of the members within these two groups vary so widely.

The benzyl group has been most frequently used in synthetic alterations of disaccharides because of its stability and ease of removal. Benzyl glycosides are base stable and resistant to mildly acidic conditions. Synthesis of the glycoside is generally achieved by Koenigs-Knorr condensation and removal is easily accomplished by hydrogenation under very mild conditions. In his studies on the partially methylated derivatives of maltose and cellobiose (29), Hess used the benzyl group to block the reducing function. Jayme and Demmig (30) obtained cellobiouronic acid, 4-O-(β-D-glucopyranosyl uronic

acid)-D-glucose, by catalytic oxidation of benzyl β-cellobioside and subsequent cleavage of the glycoside. Kremer and Gundlach (31) used benzyl β-maltoside as starting material in the synthesis of a branched tetrasaccharide.

Although a great variety of other glycosides of disaccharides have been prepared (see for example 32), few have been used as synthetic intermediates. Workers have generally regarded glycosides such as methyl biosides as poor intermediates, due to the similarity in ease of hydrolysis of the glycosidic and disaccharide linkages. Compton (33) has shown, however, that acetolysis of the glycoside is considerably faster than the cleavage of the disaccharide. The synthesis of cellobiomethylose (4-O-(6-deoxy-β-D-glucopyranosyl)-6-deoxy-glucose) was achieved in good yield from the acetolysis of the methyl β-glycoside (33). Other than this synthesis and that of sophorose (2-O-(β-D-glucopyranosyl)-D-glucose) (34), acetolysis has been disregarded in synthetic procedures. Caution should be employed when the reaction is to be applied to disaccharides linked in the primary position, i.e. 1—6, as Matsuda and co-workers (35) have shown that disaccharides with 1—2, 1—3, and 1—4 linkages are considerably more stable to acetolysis than are 1—6 linkages.

Alkaline degradation of aryl biosides has been shown to yield 1,6-anhydro rings (36), which may be then hydrolyzed

under weakly acidic conditions to give the parent sugars.
At present, no synthetic route has utilized the aryl glycoside
as a blocking group with removal via the anhydro sugar.

Two important syntheses have proceeded from a disaccharide
blocked with a 1,6-anhydro ring. 1,6-Anhydro-β-cellobiose
has been the starting material for the synthesis of both
cellobiouronic acid (37) and pseudo-cellobiouronic acid,
4-O-(β-D-glucopyranosyl)-D-glucuronic acid (38).

The 1,6-anhydride of maltose was first reported by Pictet
and Marfort (39) as a product of the thermal decomposition of
maltose. Karrer and Kamienski (40) later reported its synthesis
from the base elimination of the methiodide of N,N-dimethyl-
amino hepta-O-acetyl-β-maltoside. Asp and Lindberg (41)
synthesized the hexaacetate by a base elimination of phenyl
hepta-O-acetyl-β-maltoside. Acetolysis of 1,6-anhydro-β-
maltose was first reported by Freudenberg and Soff (42). These
authors showed that the anhydro linkage was cleaved much
faster than the disaccharide linkage.

It should be noted that although anilides (43) and
dithioacetals (44, 45) of disaccharides have been prepared,
these derivatives have not been used as synthetic intermediates.

There exists an ample selection of glycosidic blocking
groups that can be removed under a variety of experimental

conditions. Judicious choice of these blocking agents permits the reaction of other portions of the molecule without destruction of the reducing group.

Use of Anhydro Rings

Anhydro derivatives may be useful as blocking groups as well as reactive centers. The opening of acetylated 1,6-anhydrides with titanium tetrachloride chlorinates on the C-1 position, giving an α-chloride with a free C-6 hydroxyl (46). Opening of ethylene oxide type anhydro rings can be accomplished with a variety of reagents. The chemistry of the anhydro sugars was reviewed in 1946 by Peat (47) and more recently by Newth (48).

The synthesis of 1,6-anhydro-β-maltose (41) was described earlier in the discussion on blocking groups for the reducing function. Formation of 1,2,2',3,3',4',6'-hepta-O-acetyl-β-maltose through opening of the anhydride with titanium tetrachloride, and subsequent reaction of the chloride with mercuric acetate has led to the synthesis of branched tri-saccharides (41, 49).

Methyl 3,6:3',6'-di-anhydro-β-maltoside and β-cellobioside have been prepared by the action of base on their di-mesylates (50). In spite of the interesting reactions undergone by 3,6-anhydrides, their use in the synthesis of modified

disaccharides is severely restricted. The opening of the anhydride requires conditions so severe that cleavage of the disaccharide would surely result (51, 52).

The synthesis of ethylene oxide type rings in the disaccharide series may provide a route for configurational change in the constituent monosaccharides, especially those on the non-reducing portion of the molecule. Such syntheses must await the freeing of a specific hydroxyl within the molecule, as the anhydride is usually prepared by treatment of a sulphonyl ester with alkali.

Use of Esters

There exist many examples of the use of acetates as blocking groups in disaccharide investigations. Three such examples have already been described. Two of these involved the opening of fully acetylated 1,6-anhydro-disaccharides with titanium tetrachloride. Condensation of the halide at C-1 with mercuric acetate leaves only one free hydroxyl in the molecule. This hydroxyl at C-6 has been oxidized in cellobiose (38). In maltose, it has been condensed with glucose to give the anomeric trisaccharides (41, 49).

The isolation of primary hydroxyls in disaccharides has been achieved through tritylation, acetylation, and detritylation. This synthetic route was used in two syntheses already

mentioned: the synthesis of cellobiouronic acid (37), and a tetrasaccharide formed from maltose (31). The synthesis of cyclohexyl 4-O-(α-D-glucopyranosyluronic acid)-β-D-glucopyranosiduronic acid via the ditrityl ether was reported (53). To prepare the 6,6'-di-O-tosyl ester of α,α-trehalose, Bredereck (54) first prepared the ditrityl ether.

In 1923, Brigl and Mistele (55) reported that the action of phosphorus pentachloride on octaacetyl-β-maltose produced hexa-O-acetyl-2-trichloroacetyl-β-maltosyl chloride. No further work seems to have been reported on this compound which would be expected to undergo reactions similar to its glucose analog. From these reactions, the synthesis of the 1,2-anhydro derivative as well as 1,2',3,3',4',6,6'-hepta-O-acetyl-α-maltose could be expected. By the reaction of aqueous sodium acetate with acetobrom sugars, acetates of the α-series with only the 2 hydroxyl unsubstituted have been prepared (56, 57).

In partially acetylated sugars, the migration of acetate groups occurs readily in dilute base (58). Soft glass is reported to be sufficiently alkaline to catalyze this migration (59). This rearrangement could probably be utilized to provide disaccharides with only the 4' position free as is reported for monosaccharide derivatives (60).

Examples in the discussion above have illustrated the use of acetates as a blocking group only. No attempt has been made to include a review of acetate or benzoate derivatives. Articles on the properties and reactions of esters have been published (61, 62). General methods for the preparation and removal of carboxylic acid esters have recently been described (63). The chemistry of trifluoroacetate esters as applied to carbohydrate chemistry has been recently reviewed (64).

Sulphonyl esters are unique because of their manner of reaction with nucleophiles. Whereas carboxylic acid esters undergo acyl-oxygen fission upon reaction, sulphonyl esters undergo alkyl-oxygen cleavage. Nucleophilic attack occurs at the alkyl carbon causing inversion of configuration at that center.

Reactions of sulphonate esters of secondary alcohols proceed much slower than those of primary. Under classical reaction conditions (refluxing acetone) secondary esters did not generally undergo replacement, but with the use of high dielectric aprotic solvents such reactions have been used more frequently. A short discussion of S_{N_2} sulphonyl replacements has been published recently (65).

Replacement of sulphonates with a variety of reagents has led to the synthesis of substituted deoxy sugars. For example, replacement with azide ion followed by reduction

yields an amine. Replacement with iodide and subsequent reduction gives the unsubstituted deoxy sugar. Coupled with their ability to invert configurations, replacement reactions undergone by sulphonates hold unlimited possibilities for modifications in disaccharide molecules.

Although sulphonates show some specificity in reacting preferentially with primary hydroxyls, the presence of so many hydroxyl groups in disaccharides does not allow this selectivity to be operative. In the direct esterification of a disaccharide, a complex mixture of sulphonates is obtained. For this reason many workers prefer to isolate the free primary hydroxyl by formation of the trityl ether. The 6,6'-di-O-tosyl ester of hexa-O-acetyl- α, α'-trehalose was prepared in this manner (54). Replacement of the tosylates with iodide gave the 6,6'-diiodo compound which upon treatment with silver fluoride in pyridine yielded the di-5,6-α-D-glucopyranoseen.

Benzyl hexa-O-acetyl-6'-O-tosyl-β-maltoside was reacted with sodium iodide in acetone to give the 6'-iodo compound (66). Reduction with Raney nickel reduced and deacetylated the iodo acetate to yield benzyl 6'-deoxy-β-maltoside. The latter was not readily cleaved by a brewers yeast preparation which rapidly split benzyl β-maltoside, indicating the importance of the 6'-OH in the enzyme hydrolysis.

By treatment of the 6,6'-di-O-mesylates with alkali, the 3,6:3',6'-di-anhydro derivatives of methyl β-cellobioside and β-maltoside were prepared (50). Although mesylation gave a product which could be recrystallized, the tosylation product was amorphous.

An excellent review on sulphonyl esters was prepared by Tipson in 1953 (67). Due to the many advances in the field, the review is unfortunately out of date. In spite of the extensive use of sulphonates in recent monosaccharide chemistry, there are few examples of their use in the disaccharide field. The development of specifically blocked disaccharides will undoubtedly awaken interest in such esters and their reactions.

Use of Ethers

The general use of ethers as blocking groups is limited by the severe conditions generally necessary for their removal. Two types of ethers, benzyl and trityl, are useful because of characteristic chemical properties which allow their removal under mild conditions. Benzyl ethers are removed by hydrogenation in the presence of a palladium catalyst. Trityl ethers are removed under weakly acidic conditions. These two ethers may be used advantageously in modifications of disaccharides.

The chemistry of the benzyl ethers has been reviewed by McCloskey (68). This article describes the general methods

of synthesis and the stability of the ethers to certain
reagents. The synthesis of 3-O-benzyl-D-glucose has been
reported by benzylation of 1,2:5,6-di-O-isopropylidene-D-
glucose with benzyl bromide and silver oxide. More recently
(69), benzylation has been achieved with benzyl chloride and
sodium hydride at 130° (70). This latter paper describes a
thin layer chromatographic system for these derivatives, as
well as a spectrophotometric method of determining the number
of benzyl residues per molecule. One unfortunate property
of carbohydrate benzyl ethers seems to be their reluctance to
crystallize.

McCloskey has stated that benzylation appears to be
somewhat selective when benzyl chloride and potassium
hydroxide are employed. Benzylation of 1,6-anhydro-β-D-
glucopyranose under these conditions gave varying yields of
the 2,4-di-O-benzyl ether. This 'selectivity' is more
likely due to steric hindrance of the attacking species by
the 1,6-anhydro bridge. Jeanloz (71) has shown that
sulphonation of 1,6-anhydro-β-D-glucopyranose also yields
the 2,4-di-O substituted ester. Selectivity has been shown
in the preparation of 2-O-benzyl-4,5-O-isopropylidene-D-
fucose dimethyl acetal (72). The synthesis was achieved
through the reaction of one mole of sodium and subsequent
treatment with benzyl chloride to give a 42% yield. This
reaction must be considered a preferential alkoxide formation

rather than selective benzylation, since the sodium reacts
preferentially with the more acidic hydroxyl group at carbon
two.

Benzyl ethers have not been used to block positions
other than the reducing function in disaccharides. Their use
in this capacity was outlined earlier.

Helferich has reviewed the chemistry and applications
of trityl ethers (60). Although this review was published
in 1948, the basic applications of trityl ethers have
remained unchanged. Two new developments, however, are
worthy of mention. Synthesis of disaccharides by Koenigs-
Knorr condensations has been achieved by <u>in situ</u> replacement
of primary trityl ethers using silver perchlorate as a
catalyst. Thus the reaction of tetra-0-acetyl-α-D-gluco-
pyranosyl bromide with 1,2,3,4-tetra-0-acetyl-6-0-trityl-
β-D-glucose in nitromethane containing silver perchlorate
gave acetylated gentiobiose in 55-60% yield (73).

An attempt to preferentially remove a primary trityl
ether in the presence of a secondary tetrahydropyranyl ether
in a substituted ribofuranoside was unsuccessful (74). To
circumvent this problem, Khorana and co-workers employed
para-methoxy substituted trityl ethers. They report that
for each methoxyl group substituted on the trityl residue the
rate of hydrolysis increases by a factor of about 10. Thus

the trisubstituted derivative, tri-p-anisylmethyl ether,
hydrolyses about 1000 times as fast as the parent triphenyl-
methyl derivative. Introduction of p-nitro groups into
the trityl residue should increase its resistance to hydrolysis
in comparison with the parent compound. With such substitution,
a trityl ether of any acid stability should be available.

Examples of the few uses of trityl ethers in reactions
of disaccharides have been considered previously under the
discussion of esters. Trityl ethers' major contribution is
their ability to react selectively with primary hydroxyls
even in the presence of a large number of secondary hydroxyls.

The reaction of maltose with excess trityl chloride gave
6,6'-di-O-trityl maltose (75). Recent workers have shown,
however, that insufficient trityl chloride yields the 6'-
O-trityl derivative (76). Tritylation may then lead to
differentiation between not only primary and secondary
hydroxyls, but also primary hydroxyls of different environments.

Use of Acetals and Ketals

Acetals and ketals differ from other blocking groups in
that they block two hydroxyl groups simultaneously, and the
formation of the cyclic structure is highly dependent on the
stereochemistry of the hydroxyl groups involved. The ring
may contain 5 or 6 members depending on the configuration of
the hydroxyls and the reagent employed. Ketones will not

easily form six membered rings due to the 1,3-diaxial inter-
action between the alkyl of the ketone and the two axial
hydrogens of the sugar. Benzaldehyde prefers a six membered
ring where the large aromatic portion can remain equatorial.
In such a compound, the conformation of the fused rings can
be considered fixed since flipping would necessitate the
bulky aromatic ring to assume an axial orientation.

Few examples exist of the use of acetals and ketals in
disaccharide chemistry. Sutra condensed acetaldehyde with
maltose to form a sirupy compound which he regarded as a di-
O-acetal (77). Under the conditions reported for this
condensation, thin layer chromatography indicated the formation
of four major products (78). A crystalline mono-O-benzylidene
compound has been reported to form by condensation of
benzaldehyde with lactose dibenzyl dithioacetal (44). No
structure was assigned to this compound. The removal of
most acetals and ketals can be achieved with weakly acidic
conditions. The replacement of 1,2-O-isopropylidene groups
by acetolysis has been reported (79, 80, 81). Such mild
conditions should enable the removal of these blocking groups
without damage to the disaccharide structure.

Epimerizations and Inversions

In their review on the synthesis of oligosaccharides,
Evans, Reynolds, and Talley (4) give a description of the various

methods of changing configurations within the monosaccharide
units. All of these methods involve change of configuration
of the C-2 or C-3 hydroxyl of the reducing sugar. In a recent
paper describing the inversion of carbohydrates upon acetolysis
of isopropylidene derivatives (82), the author indicates the
similarity of the acid induced inversions to the aluminum
chloride and hydrogen fluoride epimerizations. Although
acid catalyzed rearrangements of this type are now more
fully understood (83), no major developments applicable to
disaccharides have been reported since the review was written.

The judicious application of the reactions described above
will undoubtedly lead to the isolation of many important
disaccharides. Because of the difficulties involved in the
synthesis of glucose disaccharides of α-configuration,
modification offers a practical and chemically interesting
way of obtaining these compounds.

METHODS OF SYNTHESIS

Maltobiouronic Acid   4-O-(α-D-Glucopyranosyluronic Acid)-D-glucose.

Jayme and Demmig (30) synthesized cellobiouronic acid by catalytic aerial oxidation of benzyl β-cellobioside. The catalytic oxidation of carbohydrates has recently been reviewed (84). The oxidation has been shown to be stereospecific, oxidizing primary hydroxyls faster than secondary, and axial hydroxyls more rapidly than equatorial. A system of conformational analysis has been developed from the application of these principles (85). Since catalytic oxidation is highly dependent on stereochemistry, it was interesting to compare the oxidation product of benzyl β-maltoside with that of benzyl β-cellobioside (Fig. 1).

Benzyl hepta-O-acetyl-β-maltoside was synthesized from acetobromomaltose (86) and benzyl alcohol by the condensation method described by Helferich and Berger (87). Deacetylation with methanolic ammonia gave the crystalline benzyl β-maltoside (88). Catalytic oxidation gave a mixture of acidic sugars, which upon esterification with diazomethane and acetylation gave crystalline methyl (benzyl hexa-O-acetyl-β-maltosid)uronate. The methyl ester hexaacetate was obtained in 40% yield, based on the benzyl β-maltoside consumed. Saponification was achieved by warming with aqueous barium hydroxide to yield the barium (benzyl β-maltosid)uronate.

Fig. 1
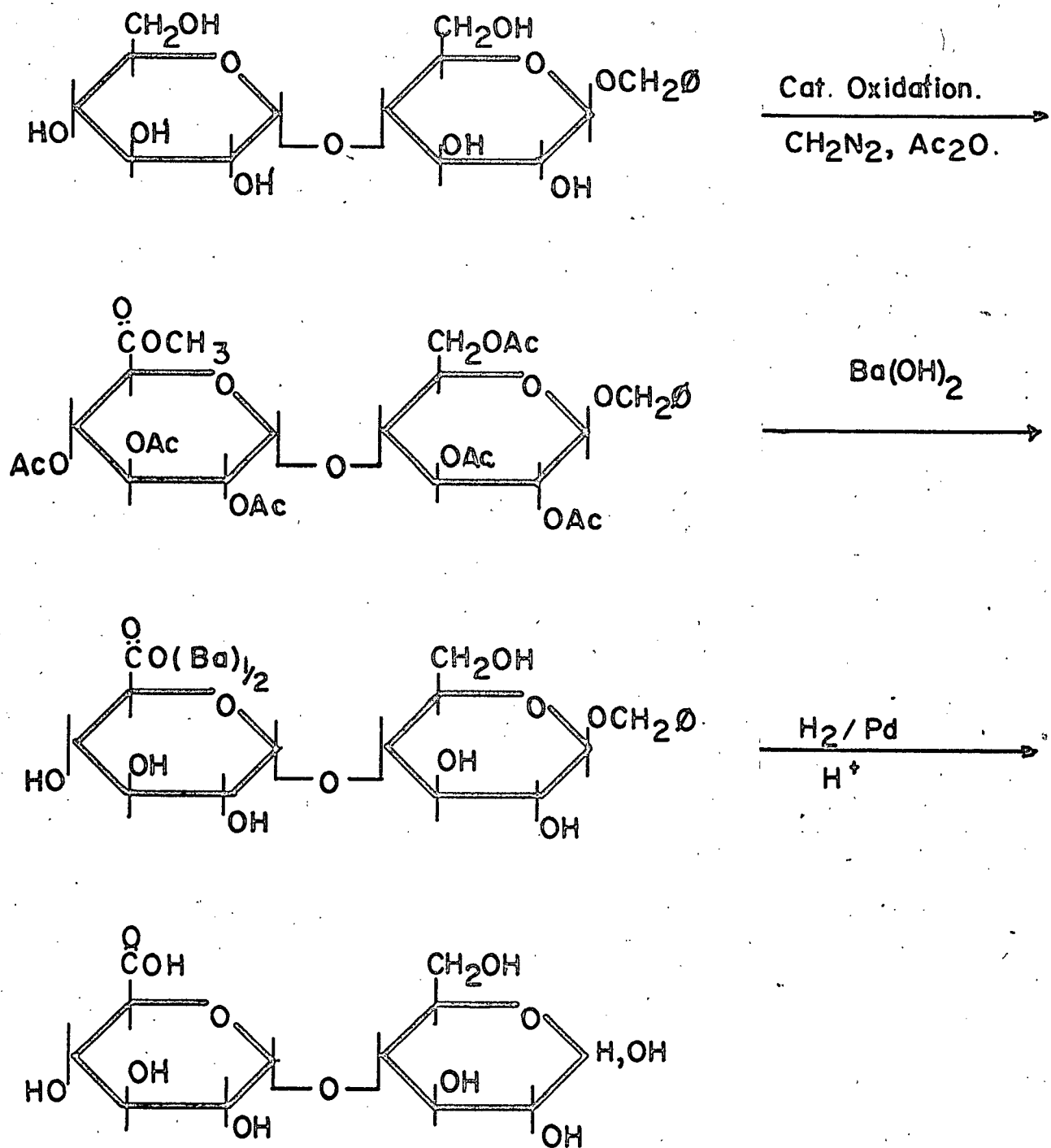
Hydrogenolysis using palladium on barium sulphate (89), followed by acidification with Amberlite IR 120 (H^+) yielded maltobiouronic acid. Esterification of maltobiouronic acid with diazomethane, followed by acetylation with acetic anhydride in pyridine, yielded the crystalline methyl ester heptaacetate.

Borohydride reduction of a sample of the acid, followed by acid hydrolysis indicated only glucuronic acid and sorbitol upon paper chromatography. The structure of the synthetic uronic acid is therefore 4-0-(α-D-glucopyranosyluronic acid)-D-glucose.

The isolation of maltobiouronic acid from the catalytic oxidation of benzyl β-maltoside indicates that the difference between the α-glucosidic derivative (maltose) and the β-glucosidic derivative (cellobiose) is not sufficient to change the site of oxidation. The 6'-position is preferentially oxidized in both compounds, yielding the respective aldo-biouronic acids.

4-0(α-D-Glucopyranosyluronic Acid)-D-xylose.

Due to the importance of aldobiouronic acids, especially those containing D-glucuronic acid and D-xylose, in structural investigations of natural products, the following scheme for the synthesis of 4-0-(α-D-glucopyranosyluronic acid)-D-xylose was proposed (Fig. 2).
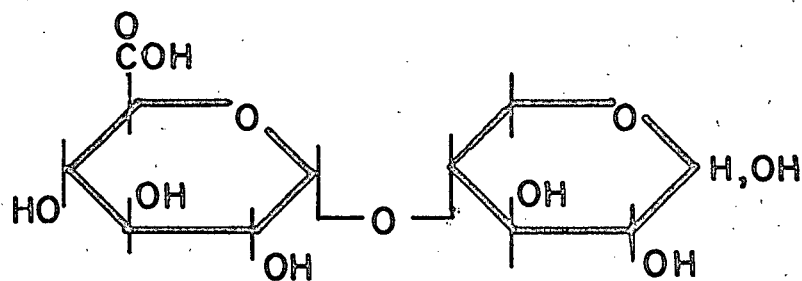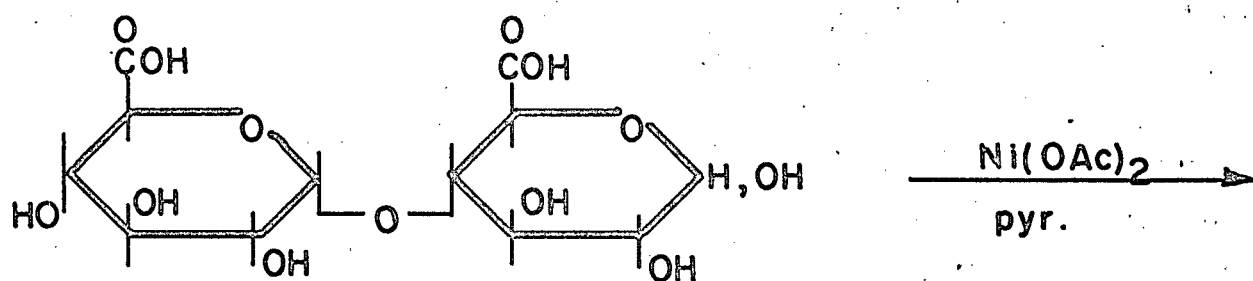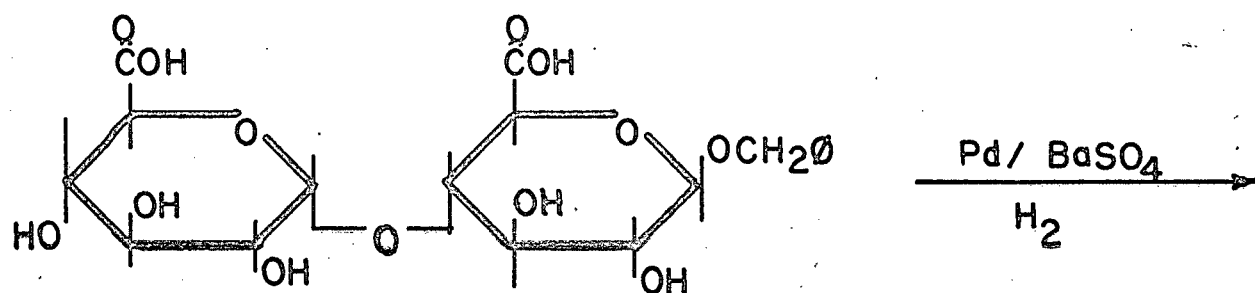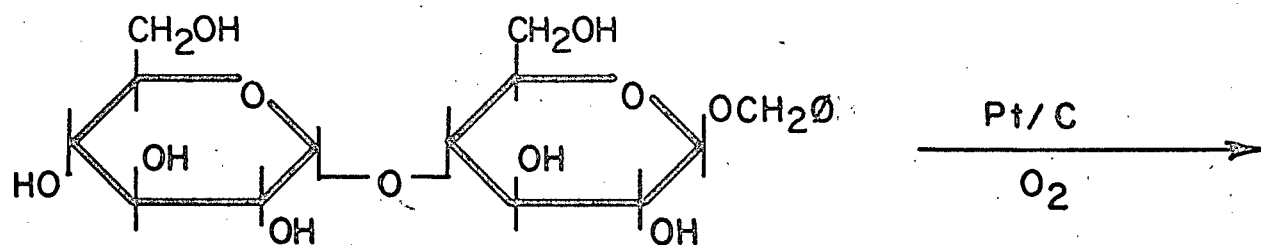
Fig. 2

Prolonged catalytic oxidation of benzyl β-maltoside or permanganate oxidation of benzyl 2,2',3,3',4'-penta-O-acetyl-β maltoside should give the di-uronic acid. Removal of the benzyl glycoside by catalytic hydrogenation would result in 4-O-(α-D-glucopyranosyluronic acid)-D-glucuronic acid. Treatment of this di-acid with nickel acetate in pyridine at 80° as outlined by Zweifel and Deuel (90) should cause decarboxylation of the reducing sugar residue, yielding the desired compound.

The crucial step of this synthetic route is the decarboxylation reaction which, although first reported in 1956, has not been utilized since. Although hot pyridine has been used to effect rearrangements in sugars to their epimers and corresponding ketoses (91), these authors employed pyridine solutions of nickel salts at 80° for decarboxylation. Substitution of the uronate at C-1 as a glycoside prevents the decarboxylation, but esterification of the acid is reported not to hinder the reaction.

The mechanism of the reaction is suggested to be a complexing of the C-1 oxygen lone pair by the metal ion employed as a catalyst (90). It is then postulated that the hydrogen of the C-1 hydroxyl can participate in an electron transfer in which the carboxyl function is eliminated.
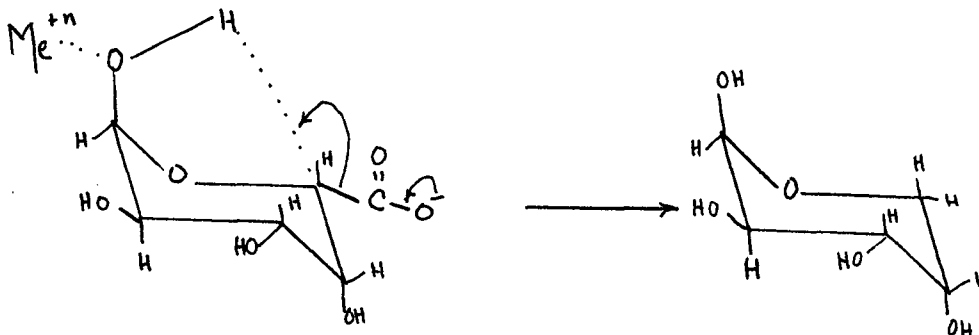
Although Zweifel and Deuel were able to isolate crystalline L-arabinose from the decarboxylation of D-galacturonic acid, no arabinose was detectable by paper chromatography upon repetition of this experiment under the reported conditions. Several more attempts to repeat this work were unsuccessful, and for this reason the proposed synthesis of 4-O-(α-D-glucopyranosyluronic acid)-D-xylose was discontinued.

4-O-(6-Amino-6-deoxy-α-D-glucopyranosyl)-D-glucose.

A possible synthetic route to 6'-amino-6'-deoxy-maltose (4-O-(6-amino-6-deoxy-α-D-glucopyranosyl)-D-glucose) is outlined below (Fig. 3). The methyl ester hexaacetate benzyl glycoside, an intermediate in the synthesis of maltobiouronic acid, upon treatment with methanolic ammonia would deacetylate and form the amide. Reduction of the amide with lithium
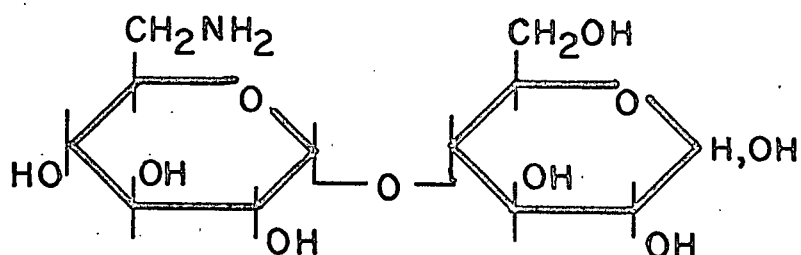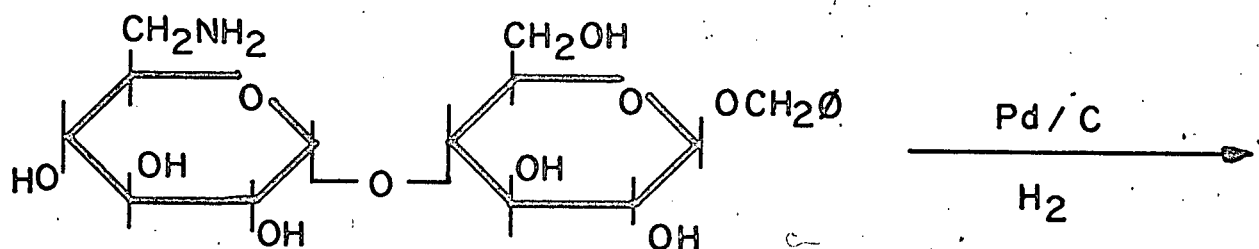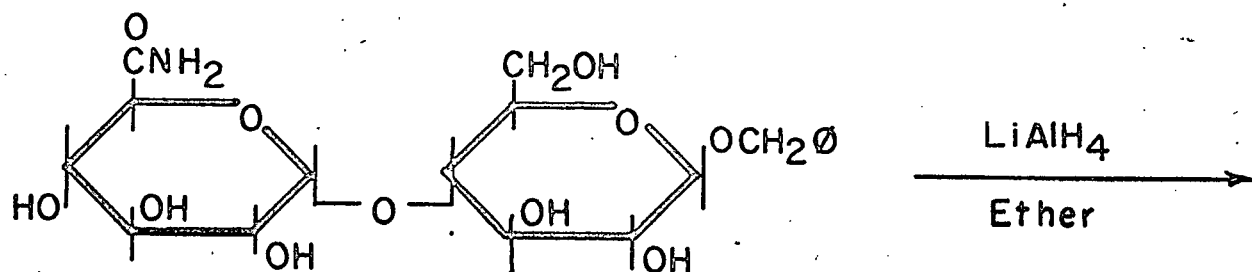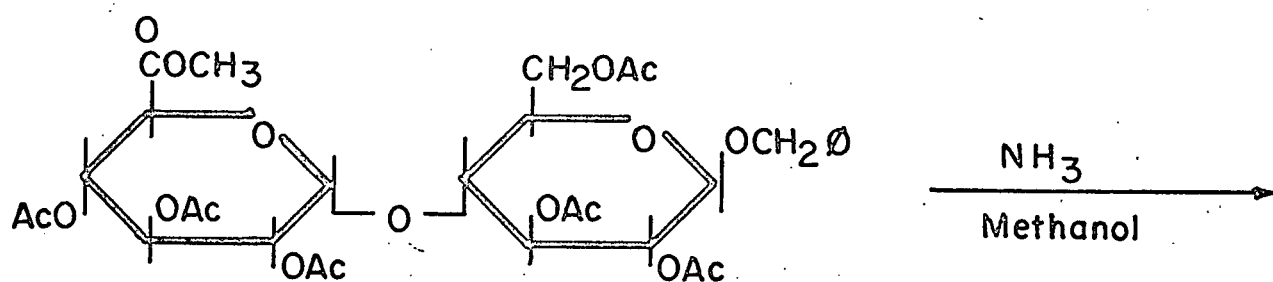
Fig. 3

aluminum hydride would give the primary amine (92), from which the amino sugar could be isolated by catalytic debenzylation.

Although benzyl ethers are stable to lithium aluminum hydride under moderate conditions (93), the reaction of benzyl glycosides under similar conditions has not been investigated. For this reason, the action of lithium aluminum hydride in refluxing ether on benzyl hepta-O-acetyl-β-maltoside was investigated. Acetylation of the reaction product after one hour yielded material which indicated two major components on silica gel thin layer chromatography (94). The faster spot corresponded to the starting material, benzyl hepta-O-acetyl-β-maltoside, and the slower spot was presumably nona-O-acetyl-maltitol. The presence of compounds other than benzyl hepta-O-acetyl-β-maltoside indicated that the benzyl glycoside was unstable to conditions necessary for the reduction of the amide. For this reason, this synthetic route was not considered practical and was abandoned.

An alternate route for the synthesis of 6'-amino-6'-deoxy-maltose is the azide replacement of a 6'-O-sulphonate ester. Reduction of a primary azide yields a primary amine. 1,6-Anhydro-β-maltose was selected as the starting material. It was hoped that tosylation of this material would give preferentially the 6'-O-tosylate. Unfortunately tosyl chloride
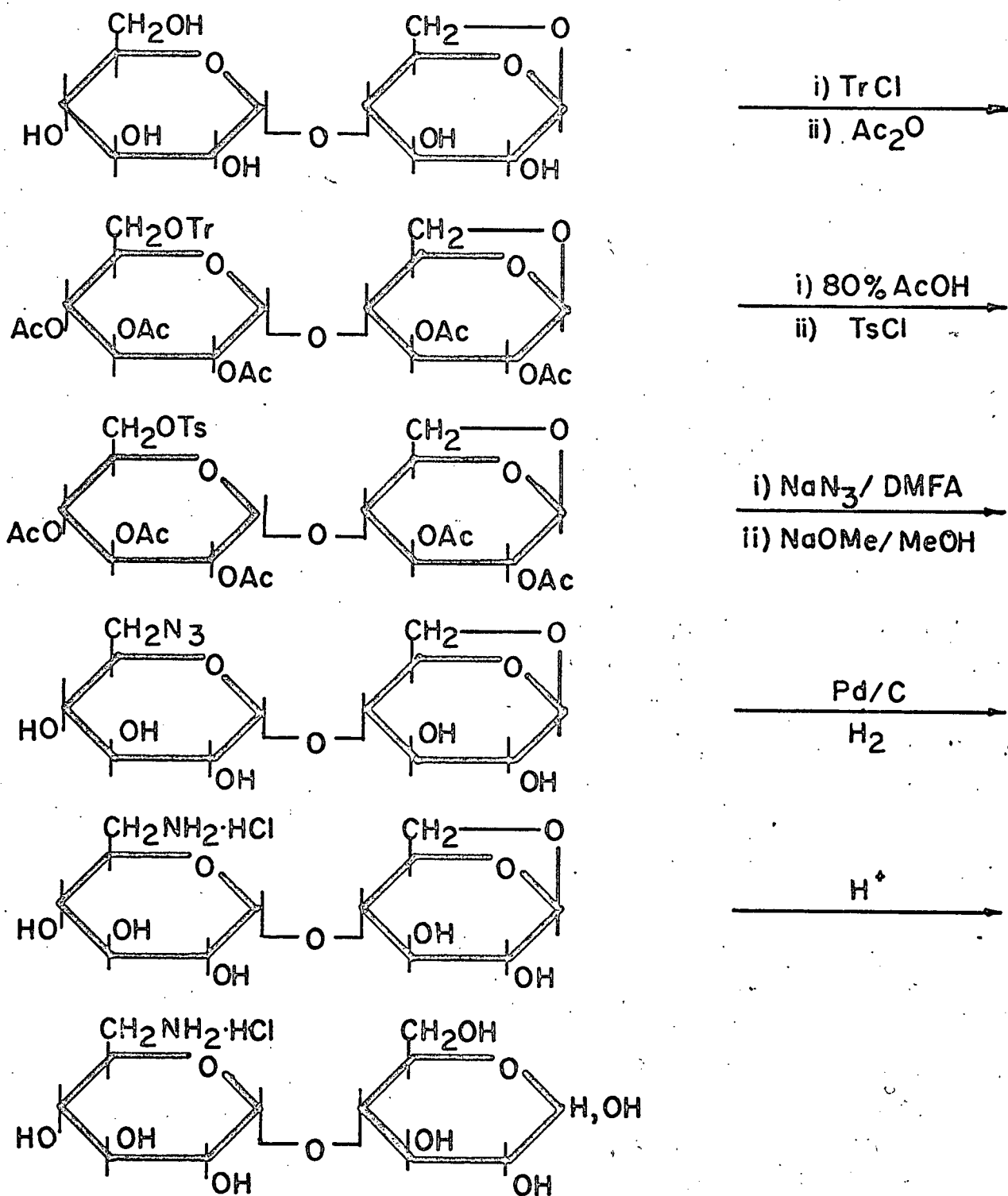
Fig. 4

was not sufficiently specific and thin layer chromatography
indicated two monotosylates and several ditosylates in addition
to starting material. Tritylation followed by acetylation
and detritylation with 80% acetic acid gave 2,2',3,3',4'-
penta-0-acetyl-1,6-anhydro-β-maltose. Tosylation of this
substrate was then carried out quickly and in good yield by
the use of excess tosyl chloride. Replacement of the
tosyl ester was accomplished by heating the ester with sodium
azide in N,N-dimethylformamide. Deacetylation followed by
catalytic reduction yielded chromatographically pure 6'-
amino-1,6-anhydro-6'-deoxy-β-maltose. The ability of the
6-amino group to hinder acid hydrolysis (95) would be expected
to stabilize the glycosidic linkage of a 6-amino glucoside.
This has, in fact, been reported by Cramer and co-workers (96).
Thus mild acid hydrolysis should open the 1,6-anhydro ring
without cleavage of the disaccharide, yielding 6'-amino-6'-
deoxy-maltose.

4-0-(6-Deoxy-6-mercapto-α-D-glucopyranosyl)-D-glucose

The replacement of sulphonyl esters by potassium thiolacetate
was investigated by Chapman and Owen (97) and found to be a
convenient method of introducing a sulfhydryl group into a
molecule. Reaction of 2,2',3,3',4'-penta-0-acetyl-1,6-anhydro-
6'-0-tosyl-β-maltose with potassium thiolacetate in N,N-
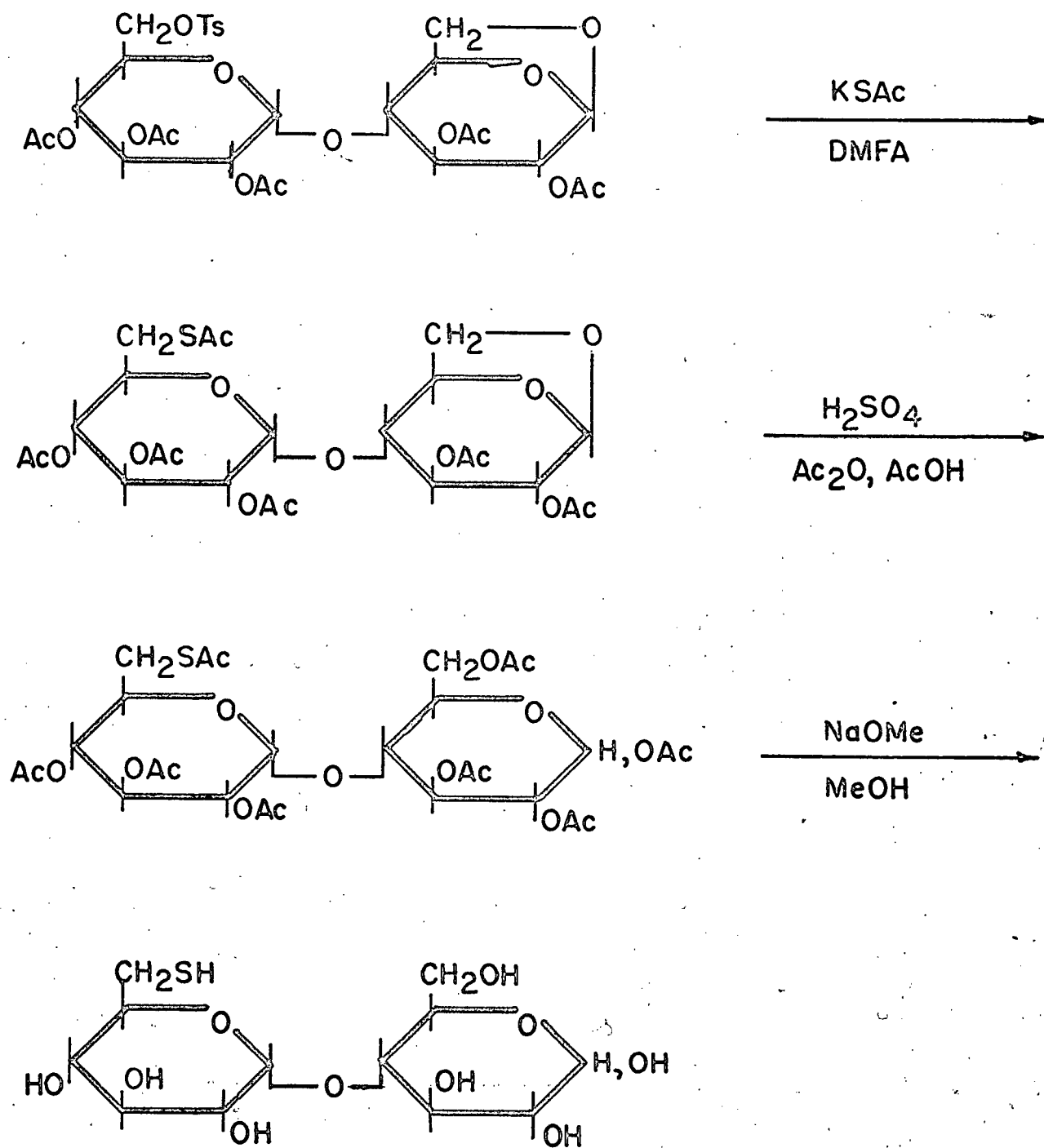
Fig. 5

dimethylformamide gave 2,2',3,3',4'-penta-0-acetyl-6'-S-
acetyl-1,6-anhydro-β-maltose in good yield. Acetolysis
was used to open the 1,6-anhydro ring without cleavage of
the disaccharide linkage. Deacetylation of the octaacetate
yielded the free thio sugar, which oxidized very rapidly
with atmospheric oxygen to give the disulphide. Addition of
excess 2-mercaptoethanol regenerated the 6'-deoxy-6'-mercapto-
maltose.

4-0-(6-Deoxy-α-D-glucopyranosyl)-D-glucose

Although benzyl 6'-deoxy-β-maltoside had been synthesized
(66) for enzyme studies, the removal of the benzyl glycoside
was not attempted. An attempt to prepare 6-deoxy-, 6'-
deoxy-, and 6,6'-dideoxy-maltose via tosylation of methyl
β-maltoside failed to yield characterizable products (98).

The synthesis of 6'-deoxy-maltose (4-0-(6-deoxy-α-D-
glucopyranosyl)-D-glucose) was attempted by the replacement
of the 6'-0-tosylate by iodide. The catalytic reduction of
the iodide yielded 2,2',3,3',4'-penta-0-acetyl-1,6-anhydro-
6'-deoxy-β-maltose. Acetolysis and deacetylation yielded
6'-deoxy-maltose.
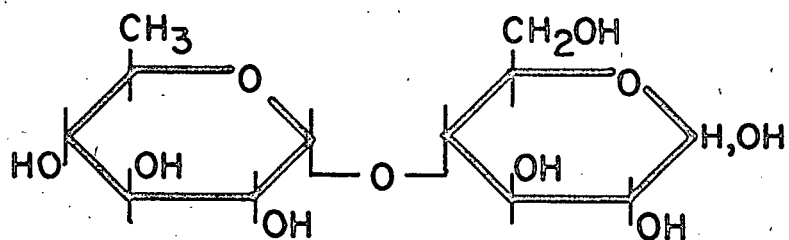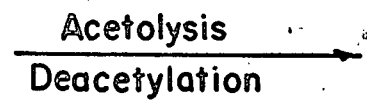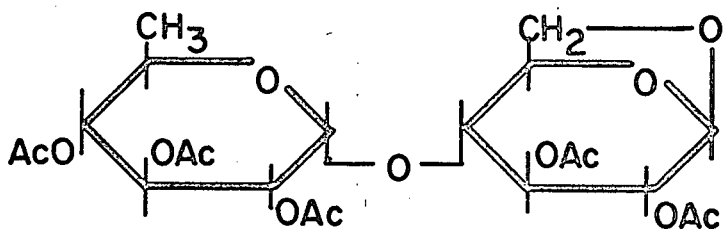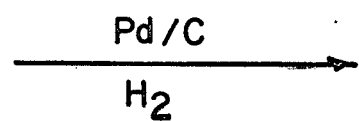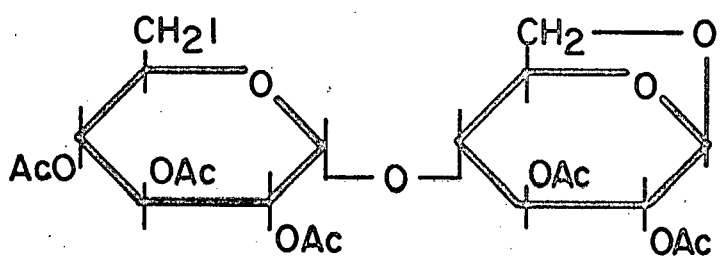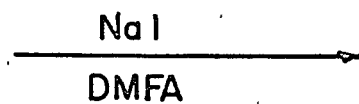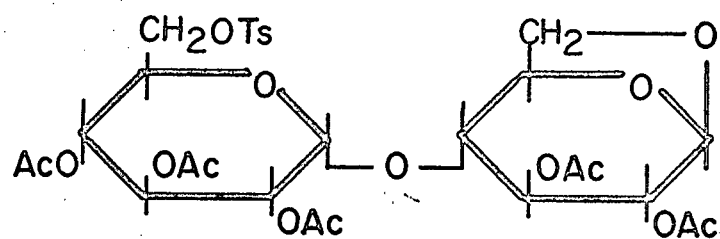
Fig. 6

4-O-(3-Amino-3-deoxy-α-D-glycopyranosyl)-D-glucose

A general synthesis of 3-amino-3-deoxy-sugars was first
introduced to carbohydrate chemistry by Baer and Fischer (99)
and has been investigated in a very thorough manner by
Baer in subsequent publications (100). The subject has been
recently reviewed by Lichtenthaler (101). The synthesis is
a modification of the Sowden-Fischer synthesis in which two
aldehyde groups within the same molecule react with nitro-
methane in a typical aldol condensation. The dialdehyde
is produced from glycol oxidation with periodate or lead
tetraacetate (Fig. 7). Condensation of the dialdehyde with
nitromethane under the influence of base leads to a C-nitro
alcohol. Upon acidification, the nitro group invariably
takes up an equatorial orientation. The configurations of
carbons 2 and 4 can give rise to four possible isomers but
in practice, manipulation of the condensation conditions allows
the preferential formation of certain of the configurations.
Subsequent reduction of the C-nitro-alcohol yields the 3-amino-
3-deoxy-glycoside.

Recent nuclear magnetic resonance work (102) has shown that
2,3,4-tri-0-acetyl-1,6-anhydro-β-D-glucose exists primarily
in a 1C rather than a 3B conformation.
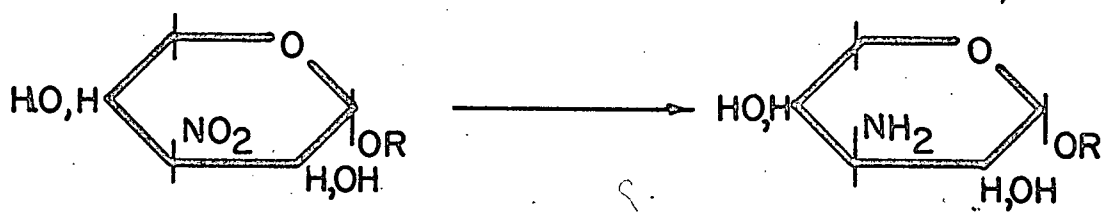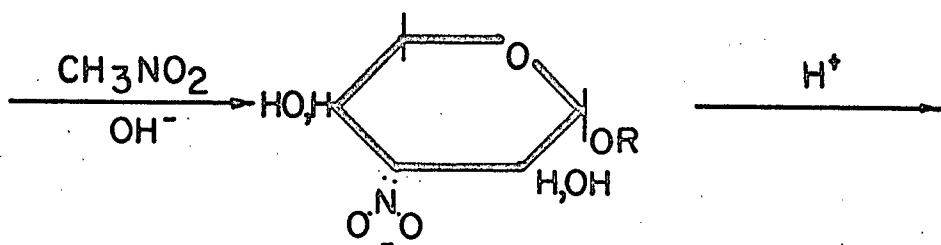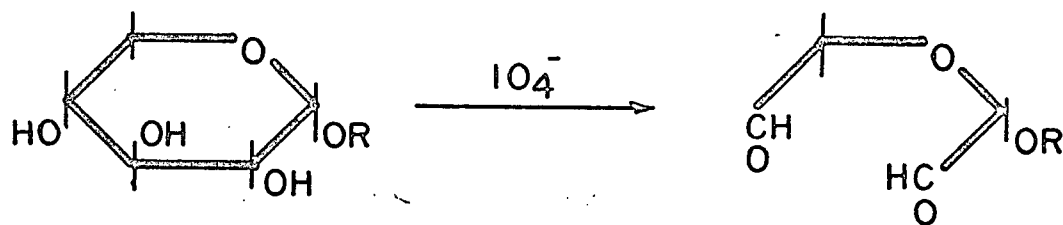
Fig. 7
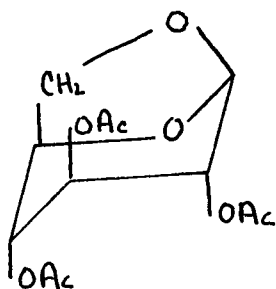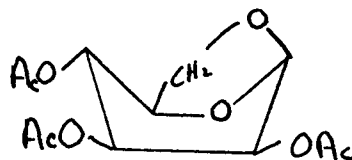
IC   3B

Replacement of the relatively bulky acetyl groups with
hydrogen, would stabilize the 1C conformation as there would
be less crowding of the axial C3 substituent against the 1,6-
anhydro bridge and less C2, C4 1,3-diaxial interaction.
1,6-Anhydro-β-D-glucose would be expected then, to possess
the 1C conformation.

Reeves (103) has deduced from the optical rotation of
cuprammonium solutions of 1,6-anhydro-β-D-glucose that the
sugar does indeed exist in a 1C conformation. There is
little difference in the optical rotation of the complex
formed from either 1,6-anhydro-3-0-methyl-β-D-glucose or
its parent 1,6-anhydro-β-D-glucose. This indicates that
hydroxyl groups 2 and 4 are involved in the complex formation
which they could only do if they are in a diaxial (i.e. 1C)
conformation.

1,2-Diols are oxidatively cleaved by lead tetraacetate at a rate which is dependent upon the geometry of the diol system (104). The closer the two hydroxyl groups approach each other the faster the rate of oxidation. For example, cis-cyclohexandiol is oxidized 23 times more quickly than trans-cyclohexandiol (105) and 1,6-anhydro-β-D-glucofuranose is not attacked at all (106). In the latter the 1,2-diol system is locked and the projected bond angle between the hydroxyl groups is very near 120°.

From the information discussed above, a synthesis of 4-O-(3-amino-3-deoxy-α-D-glycopyranosyl)-D-glucose was proposed (Fig. 8). Lead tetraacetate oxidation at 5° of 1,6-anhydro-β-maltose prepared by deacetylation of the known hexaacetate (41), should give the dialdehyde, as the hydroxyl groups on the non-reducing residue being trans diequatorial should oxidize more quickly than the trans diaxial orientation of hydroxyls on the anhydro glucose portion of the molecule (Fig. 9).

Condensation of the dialdehyde with nitromethane would then give a C-3' nitro-disaccharide which after reduction could be hydrogenolyzed to the free amino sugar.

Before attempting the tetraacetate oxidation of 1,6-anhydro-β-maltose, the oxidation of model compounds was attempted. The two compounds selected as models of both
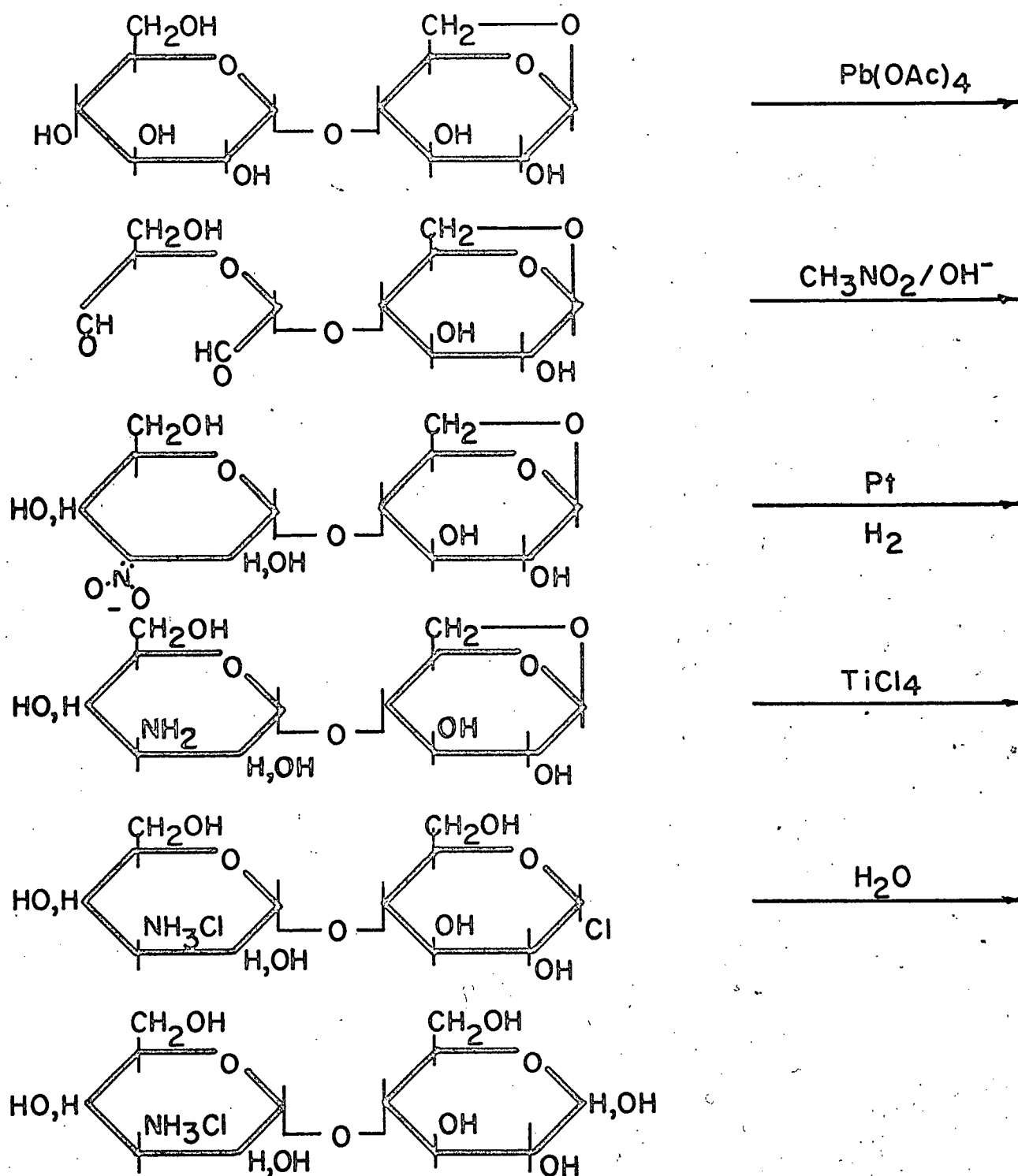
Fig. 8

portions of the 1,6-anhydro-β-maltose molecule were methyl α-D-glucoside and 1,6-anhydro-β-D-glucose (Fig. 9).

It was rather surprising, therefore, to find that 1,6-anhydro-β-D-glucose oxidized at a faster rate than methyl α-D-glucoside (Fig. 10). From consideration of the existing data outlined above, 1,6-anhydro-β-D-glucose would be expected to oxidize at $^1/_{10}$ or less the rate of methyl α-D-glucoside due to the orientations of the respective hydroxyl groups. Such is not the case, in fact, the two molecules oxidize at essentially the same rate.

Three possibilities allow explanation of the oxidation results:

a) Lead tetraacetate oxidation is not specific and oxidizes glycol groups without regard to their geometry. While this possibility must be kept in mind it is extremely unlikely. The basis for the use of lead tetraacetate has depended on the specificity of its oxidative cleavage and it is difficult to see why 1,6-anhydro-β-D-glucose should pose an exception.

b) The 1,6-anhydro-linkage is hydrolyzed very quickly in glacial acetic acid, which is the solvent used for lead tetraacetate oxidations, giving D-glucose. D-Glucose would then be attacked very quickly by the oxidant with a total

Fig. 9

Catalyzed Lead Tetraacetate Oxidation

1,6-Anhydro-β-D-glucose

Methyl α-D-Glucoside

molar uptake

(min.)

Fig. 10

-43-
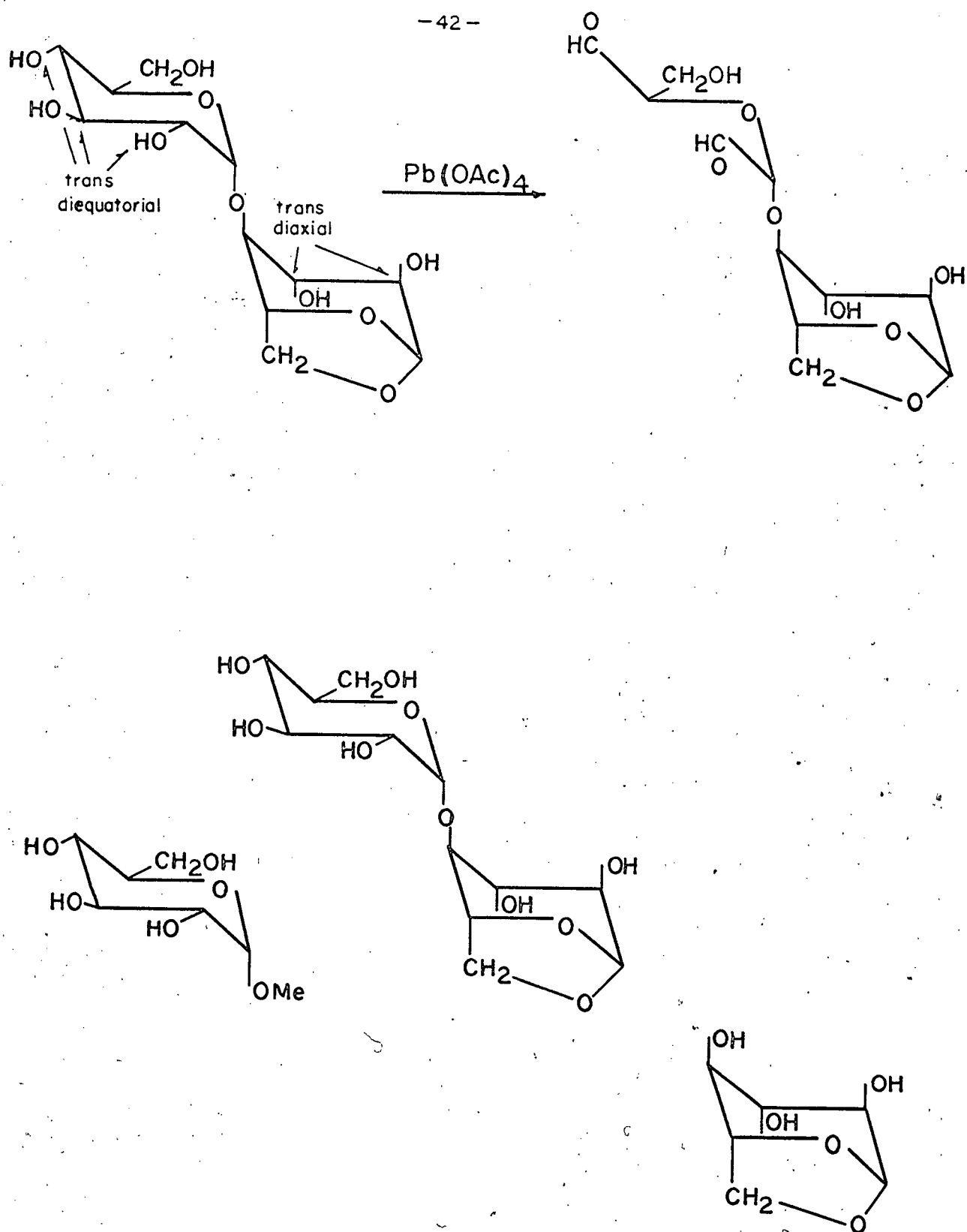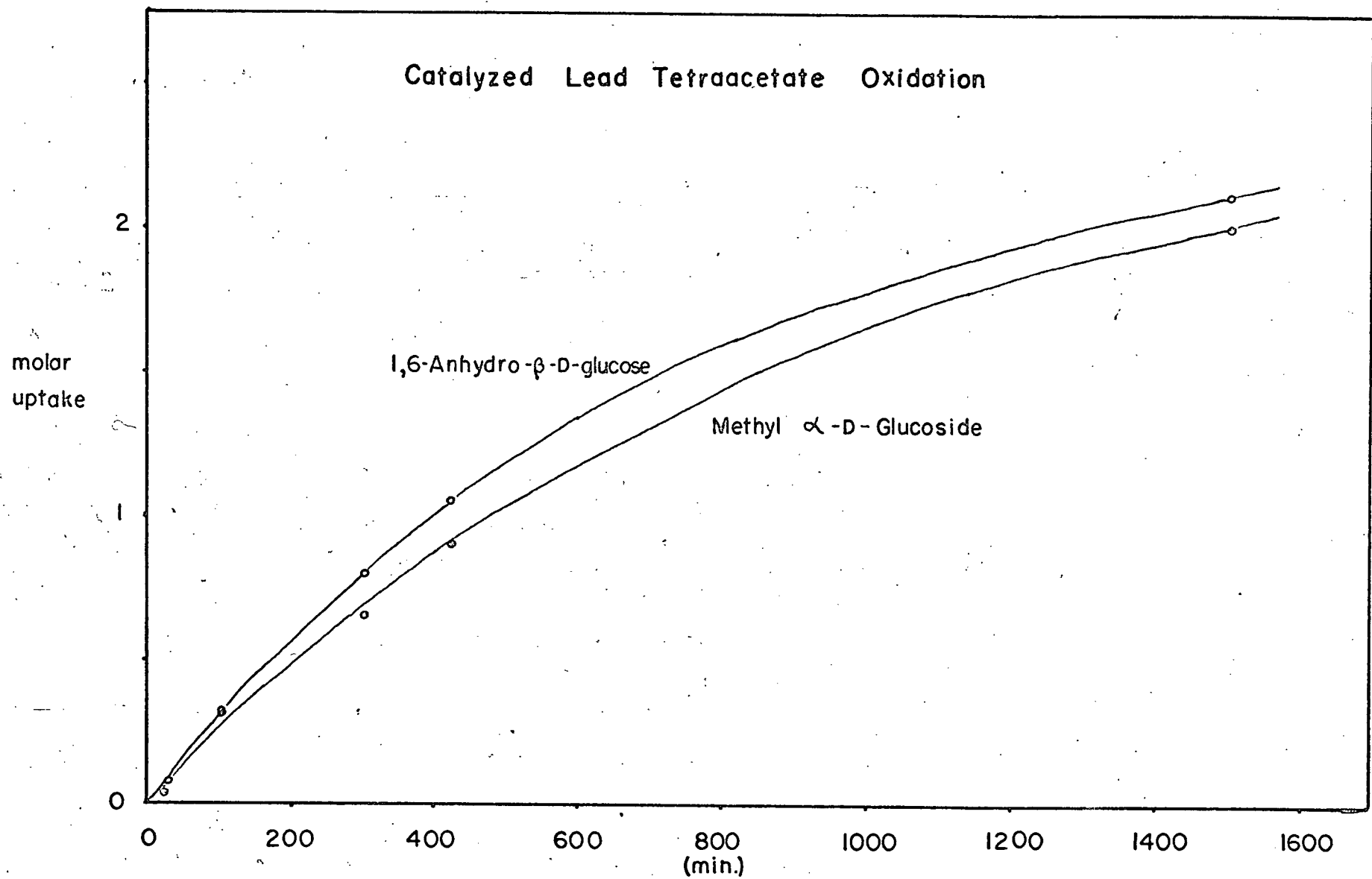
rate for the two reactions slightly greater than the rate for the glucoside. This attractive possibility was eliminated by observing the optical rotation of a solution of 1,6-anhydro-β-D-glucose in glacial acetic acid. If any hydrolysis occurred the rotation would change from a strongly negative value (ca. -70°) to a positive value (ca. +53°). In 24 hours no change in rotation of the levorotatory solution was observed.

c) 1,6-Anhydro-β-D-glucose in glacial acetic acid actually exists in a 3B conformation. Such a conformation would allow tetraacetate oxidation to proceed at a rate equal to that of methyl α-D-glucopyranoside. The slightly slower rate of oxidation of the glucoside may possibly be explained by the formation of a 6-O-formyl ester which slows the rate of oxidation of formic acid to carbon dioxide. This esterification cannot occur in 1,6-anhydro-β-D-glucose because the anhydro linkage blocks the 6-position. Change in conformation with solvent is not a common occurrence but has been observed in methyl 2-deoxy-α-D-ribopyranoside (107). However, in these compounds, the ring remains in a chair with only the disposition of the substituents varying.

The proposed synthesis of the amino disaccharide was discontinued at this point due to the lack of information

on the oxidation step. Further work is to be carried out in
an effort to determine the cause of the anomalous oxidative
behaviour of 1,6-anhydro-β-D-glucose.

Attempts to Synthesize 4-O-(α-D-Glucopyranosyl)-6-deoxy-6-
substituted-D-glucose Derivatives

In an attempt to synthesize 6-substituted derivatives of
maltose, benzyl 4',6'-O-benzylidene-β-maltoside was employed.
In this molecule only one primary hydroxyl remains free and
attempts to selectively tosylate this hydroxyl group were
unsuccessful. The reaction of a more specific reagent,
trityl chloride, was investigated. Although removal of
trityl in the presence of benzylidene would not have been
possible, the use of a trimethoxy trityl derivative (74) would
have permitted such a manipulation. Had tritylation proceeded
in a normal fashion, the corresponding methoxy derivative
would have been prepared. Specific tritylation of the 6-
position was not achieved. The lack of reaction of the
trityl chloride may have been due to steric hindrance and for
this reason mesylation was attempted. Mesylation was the
most effective of the three methods tried. However, the
reaction was abandoned as a preparative method, because
sulphonation occurred in only about 30% yield as indicated
by thin layer chromatography.

DISCUSSION

Maltobiouronic Acid

The synthesis of maltobiouronic acid by the catalytic oxidation of benzyl β -maltoside was accomplished slightly in advance of this work by Hirasaka (108). Knowledge of his results was obtained shortly after publication of our work early this year (109). The physical constants of all compounds common to both publications were in agreement.

Hirasaka (110) reported, at the same time, the synthesis of maltobiouronic acid by the permanganate oxidation of 1,2, 2',3,3',4',6-hepta-0-acetyl-β -maltose. This acetate was also treated in acetic acid with chromium trioxide followed by permanganate. Acid chromate oxidation of the alcohol is presumably faster than permanganate, whereas the reverse is true of the oxidation of the aldehyde. By using both reagents a faster overall oxidation was accomplished.

Maltobiouronic acid would be expected to be one of the products obtained from the partial acid hydrolysis of oxidized amylose. Partially acetylated amylose was prepared from amylose by tritylation, acetylation and detritylation. Chromate-permanganate oxidation of this substrate yielded material oxidized at 50% of the primary alcoholic positions (111). Although partial hydrolysis was not reported, the authors

stated that the oxidized amylose had physical constants quite different from nitric acid oxidized amylose.

The non-reducing residue in maltose has been assigned both skew (112) and chair (113) conformations by various authors. Had catalytic oxidation of benzyl β-maltoside given a major product other than maltobiouronic acid, a conformation might have been assigned to the starting material. With the isolation of maltobiouronic acid as the major product nothing can be said regarding the conformation of benzyl β-maltoside.

Acid Hydrolysis of 1,6-Anhydrides

Acid hydrolysis of 1,6-anhydro rings has been reported in a number of communications (114, 115, 116). 0.2$\underline{N}$ Hydrochloric acid for 4 hours on a steam bath (115) indicates the severity of conditions necessary to effect this hydrolysis. Under such conditions the rates of hydrolysis of the 1,4-β linkage and the anhydro ring of 1,6-anhydro-β-cellobiose were found to be nearly equal (116). In studies of 6-amino-6-deoxy-D-glucose (96), methyl 6-amino-6-deoxy-α-D-glucoside was not hydrolyzed by 0.2$\underline{N}$ hydrochloric acid at 100° for 8 hours. Extending these conditions to 6'-amino-1,6-anhydro-6'-deoxy-β-maltose, the anhydro ring should hydrolyze while the glycosidic linkage should not, because of the stabilization by the 6-amino function.

Hydrolysis of 6'-amino-1,6-anhydro-6'-deoxy-β-maltose
with 0.15$\underline{N}$ hydrochloric acid for 13 hours at 100° yielded,
as indicated by paper chromatography, glucose, the expected
amino disaccharide, some degradation products, and a small
amount of starting material. The presence of glucose was
somewhat mysterious as there did not appear to be an equal
amount of 6-amino-6-deoxy-glucose present in the hydrolysate.
The reluctance of the anhydro ring to open may be a further
example of the difficulty with which amino sugars are
hydrolyzed (95). This is probably due to the necessity of
double protonation for hydrolysis, as the amine readily
accepts the initial proton and further protonation is
hindered by the molecule's positive charge. The isolation
of a fraction recognizable as the amino-disaccharide indicates
a definite stabilization of the glycosidic linkage by the 6-
amino function.

Acetolysis of 1,6-Anhydrides

Acetolysis of 1,6-anhydro rings was first reported by
Freudenberg and Soff (42). These authors showed that the
reaction gave an equilibrium mixture of the α and β forms
with the α predominating (88% for D-glucose). Haskins, Hann
and Hudson showed that the 1,6-anhydro linkage was acetolyzed
much faster than the 1,4-β disaccharide linkage. These
procedures were incorporated in the chemical syntheses of
cellobiose (117) and lactose (118).

Thin layer chromatography of carbohydrate acetates has
been described by a number of authors (94, 119). In an
attempt to follow the acetolysis of 1,6-anhydro derivatives
by thin layer chromatography, samples were removed during
the course of the reaction and spotted directly onto thin
layer plates. The addition of two drops of pyridine to the
plate was sufficient to neutralize the sulphuric acid present.
The spot was then dried at room temperature. The plates
were developed with ethyl ether-toluene (2:1, v/v., solvent
D), a solvent described by Gee (120) for thin layer
chromatography of fully methylated sugars. The acetates
were detected by spraying with sulphuric acid and heating.
A typical pattern observed in the acetolysis of penta-O-
acetyl-6'-S-acetyl-1,6-anhydro-6'-deoxy-β-maltose is shown
in Figure 11.

Obviously, two consecutive reactions are taking place.
The first is the acetolysis of the anhydro ring to an approximately
equal mixture of the α and β acetates. The second reaction
is the anomerization of the two acetates to give an equilibrium
mixture in which the α predominates. The identity of the
second reaction was confirmed by the formation of the
equilibrium mixture upon dissolving octa-O-acetyl-β-maltose
in the acetolysis solution. It is quite likely the opening
of the anhydro ring is initiated by the formation of a

Solvent Front

0   30   60   90   120

Reaction Time
(min.)
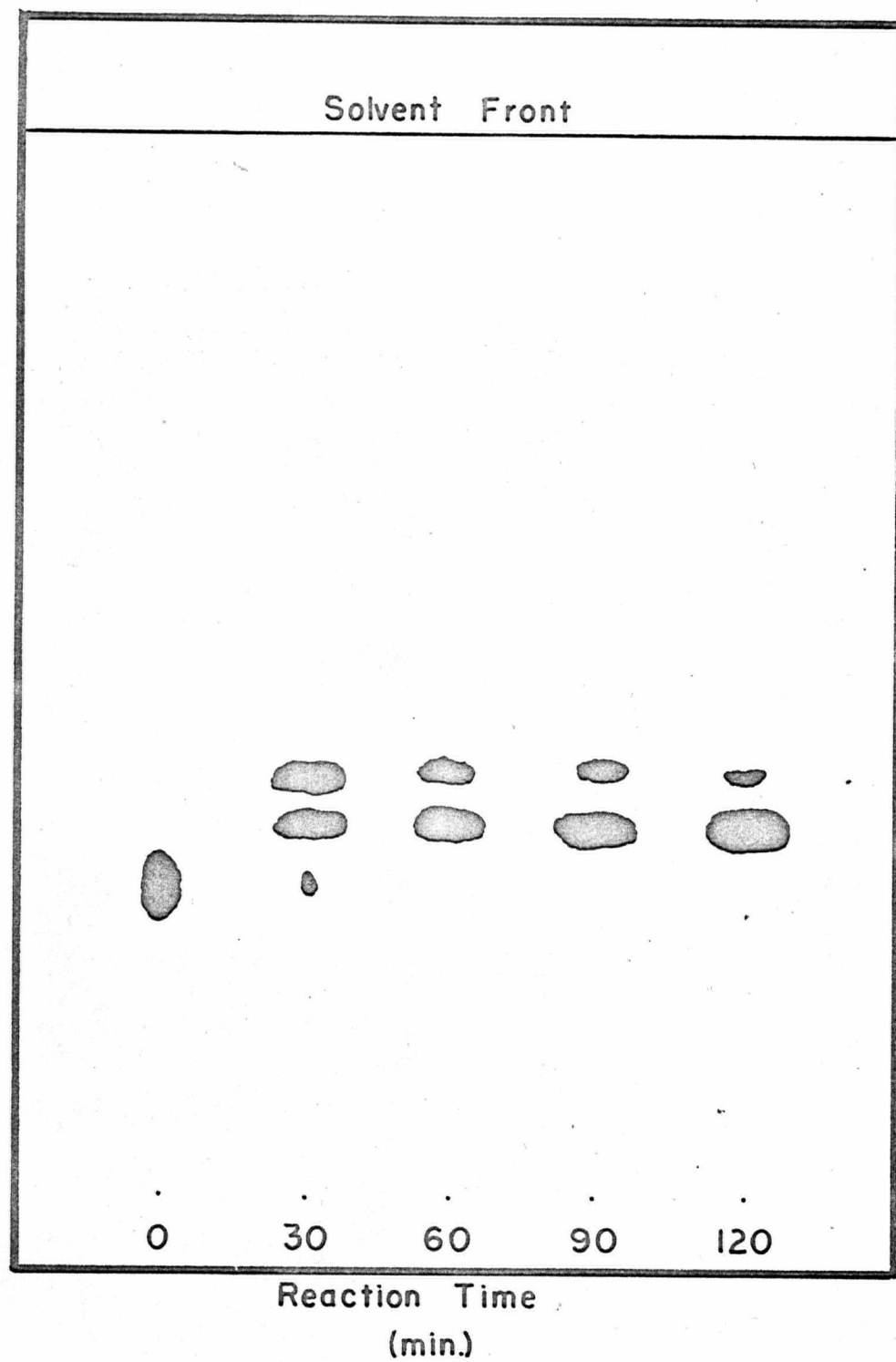
Fig. II

1,6-β-D-cyclic ion which rearranges to a more stable
carbonium ion. This ion reacts with a molecule of acetic
acid to form an equal mixture of the α and β anomers.
Anomerization, catalyzed by the sulphuric acid, then shifts
the ratio of isomers to the equilibrium value (Fig. 12).

Mercapto Sugars
_____

Although the replacement of sulphonates by thiolacetate
is a convenient method of introducing sulphur into a sugar,
complications arise upon deacetylation. Thiols are known
to undergo atmospheric oxidation and this reaction is
accelerated in the presence of alkali. Acid catalyzed
deacetylation has been suggested (121) but these conditions
do not seem advisable for disaccharide derivatives.
Regeneration of the thiol from the disulphide can be
accomplished by addition of excess 2-mercapto-ethanol (122).

Several alternate methods exist for the introduction of
sulphur into sugar molecules and these have been reviewed by
Hutson and Horton (65). Possibly the most promising method
is the replacement of sulphonates by thiosulphates forming
the 'Bunte salt'. As this molecule now possesses a negative
charge, ion exchange chromatography can be conveniently
carried out. Generation of the free mercapto sugar is
accomplished by addition of excess low molecular weight
thiols such as 2-mercapto-ethanol. The use of thiosulphate
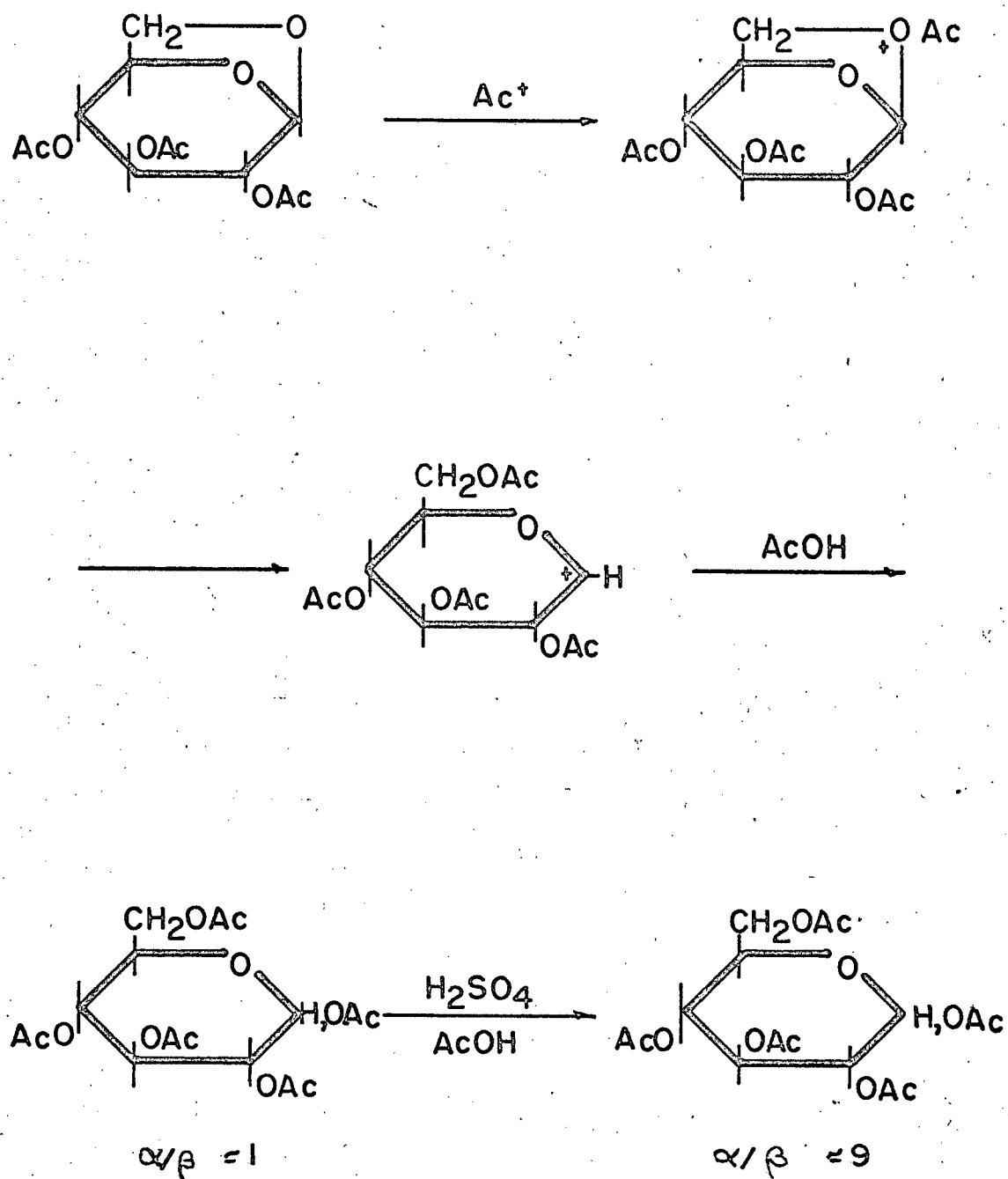
Fig. 12

derivatives was first introduced by Swan (122) for the study of sulphur containing proteins.

Comparative Reactivity of Primary Hydroxyl Groups

As investigations on disaccharides continue, one fact is becoming apparent. The primary hydroxyl group on the reducing residue of 1,4 linked glucose disaccharides is less reactive than the other primary hydroxyl.

Lindberg (38) has remarked on the difficulty of oxidizing the 6-position with respect to the 6'-position. The partial tritylation of maltose gives only the 6'-derivative, and no 6-substituted derivative (110). Catalytic oxidation of maltose or cellobiose derivatives lead to 6'-oxidized products (30, 108, 109). The unsuccessful attempts to prepare 6-substituted derivatives as described in this work serve as additional examples of what may be a general effect in 1,4 linked disaccharides.

EXPERIMENTAL

Evaporations were carried out under reduced pressure at a bath temperature of 40-45°. Optical rotations are equilibrium values measured on a Bendix-Ericson ETL-NPL Automatic Polarimeter (Type 143A) at 21 $\pm$ 2°. Melting points quoted are uncorrected. Chromatograms were run by the descending technique in the following solvents: (A) ethyl acetate - acetic acid - formic acid - water (18:3:1:4); (B) 1-butanol - ethanol - water (4:1:5); (C) ethyl acetate - acetic acid - water (18:7:8). Silica gel thin layer plates were run by the ascending technique in the following solvents: (D) ethyl ether - toluene (2:1); (E) benzene - methanol (19:1); (F) butanone - water azeotrope; (G) 1-butanol - acetic acid - ethyl ether - water (9:6:3:1). Sugars were detected on paper chromatograms by the p-anisidine-trichloroacetate spray (123) for reducing sugars and periodate-permanganate spray (124) for non-reducing sugars. Detection of sugars on thin layer plates was accomplished by spraying with concentrated sulphuric acid and heating the plate at 150°.

Benzyl Hepta-O-acetyl-β-maltoside

To acetobromomaltose (16.6 g) prepared from maltose (10 g) by the method of Barczai-Martos and Korosy (86), was added freshly distilled benzyl alcohol (32 ml) and mercuric cyanide (6 g). The mixture was heated to 85° on a water bath and

stirred vigorously for 45 minutes. The resulting solution
was poured with stirring into ethanol (90 ml) and cooled to
5° for 3 hours. The crude product (10.5 g, 61% based on the
acetobrom sugar) was collected by suction filtration.
Recrystallization from ethanol yielded material melting at
124-125° Lit. (88), m.p. 125°.

Benzyl β -Maltoside

Deacetylation of benzyl hepta-0-acetyl-β-maltoside was
accomplished with methanol saturated with ammonia as described
by Fischer and Kogl (88). Recrystallization from methanol-
ethyl acetate gave the crystalline glycoside, m.p. 148-149°.
Lit. (87), m.p. 147-148°.

Platinum Oxidation Catalyst

Modification of a method of Brown (125) facilitated the
preparation of an active platinum catalyst. To carbon (10 g,
Darco G-60), digested overnight in 6N hydrochloric acid,
washed free of chloride with distilled water and dried at 120°
for four hours, a solution of chloroplatinic acid (5 g) in
50% aqueous ethanol (50 ml) was added. To this mixture, a
solution of sodium borohydride (2.5 g) in ethanol (100 ml) was
added slowly with stirring. After 5 minutes 6N hydrochloric
acid (10 ml) was added and the suspension suction filtered.
The precipitate was washed free of chloride with distilled

water and dried in vacuum over phosphorus pentoxide. The
product (12 g) was pyrophoric and in later experiments
drying was not carried out and the product was kept as a
moist paste.

Catalytic Oxidation of Benzyl β-Maltoside

To a solution of benzyl β-maltoside (5 g) and sodium
bicarbonate (1 g) in distilled water (100 ml) was added
platinum catalyst (5 g). The mixture was maintained at 65°
and magnetically stirred for 6 hours during which time a
stream of oxygen was passed into the solution through a
gas dispersion tube. After cooling the mixture was filtered
through Celite, acidified with Amberlite IR 120 (H^{+}) and passed
slowly through a column of Duolite A-4 (OH^{-}). The Duolite
resin was washed with water (500 ml) and the combined eluates
evaporated to recover unreacted benzyl β-maltoside (3-4 g).
The Duolite resin was eluted with $\underline{N}$ NaOH (15 ml), washed
with water (100 ml) and the combined eluate acidified by
passage through Amberlite IR-120 (H^{+}). The acid mixture
obtained was evaporated to a brown sirup (1 g).

Methyl(Benzyl Hepta-O-acetyl-β-maltosid)uronate

To the acid mixture (1.5 g) dissolved in methanol (15 ml)
excess diazomethane in ether was added. After ten minutes
the excess diazomethane was decomposed with glacial acetic acid,

and the solution evaporated to a sirup. This material was acetylated in pyridine (20 ml) with acetic anhydride (50 ml) overnight. Recovery in the normal way yielded a sirup which crystallized. Recrystallization from ethanol or methanol yielded the methyl ester hexaacetate (0.8 g) m.p. 164-165°, $[\propto]_D$ 33.8° (c, 2.04 in $CHCl_3$). Calculated for $C_{32}H_{40}O_{18}$: C, 53.95; H, 5.62%. Found: C, 53.80; H, 5.71%.

Barium (Benzyl β-Maltosid)uronate

To a solution of the methyl ester hexaacetate (415 mg) in methanol (20 ml) a saturated aqueous barium hydroxide solution (40 ml) was added and the solution refluxed on a steam bath for 90 minutes. The solution was neutralized with carbon dioxide, filtered, and the precipitate washed with water (15 ml). The combined filtrates were acidified by passage through Amberlite IR-120 (H^+) and evaporated to a glassy solid. The solid was dissolved in water (2 ml) and neutralized with saturated aqueous barium hydroxide. The precipitate obtained upon the addition of ethanol (30 ml) was centrifuged, washed with ethanol (10 ml) and was dried by solvent exchange with ether to give a white powder (300 mg). $[\propto]_D$ 30° (c, 1.53 in H_2O).

Maltobiouronic Acid

A small scale experiment (8.25 mg of barium salt) was conducted in a Warburg type microhydrogenator. Hydrogenolysis was complete after two hours with the hydrogen uptake constant at 1.04 moles.

To a solution of the barium salt (202 mg) in water (10 ml) was added palladium oxide on barium sulphate catalyst (270 mg). The mixture was hydrogenolyzed at room temperature in a Paar apparatus at a pressure of one atmosphere. After two hours the mixture was filtered, the precipitate washed with water (10 ml) and the combined filtrates acidified by passage through Amberlite IR-120 (H^+) and evaporated to a sirup (138 mg). Freeze-drying yielded a product which analyzed as a monohydrate. After drying overnight _in vacuo_ (0.02 mm) at 80° the product had lost a weight equivalent to 1.2 moles of water. Upon drying at 100° for 36 hours a further 0.5 moles of water was lost. Drying was discontinued at this stage as the acid began to darken indicating decomposition. Neutralization equivalent: calculated for $C_{12}H_{22}O_{13}$ (monohydrate), 374; found 367, $[\alpha]_D$ 116° (_c_, 2.52 in H_2O). Calculated for $C_{12}H_{22}O_{13}$ (monohydrate): C, 38.50; H, 5.88%. Found: C, 38.26; H, 5.76%. R_f 0.051, $R_{glucose}$ 0.33, $R_{maltose}$ 0.90, solvent system A.

Methyl (Hepta-O-acetyl-β-maltosid)uronate

To maltobiouronic acid (55 mg) in methanol (5 ml) was
added excess diazomethane in ether, and after 10 minutes the
solution was evaporated to dryness. Acetic anhydride (10 ml)
and anhydrous sodium acetate (0.5 g) were added and the
mixture heated on a steam bath for two hours. The mixture
was poured into ice water and extracted into chloroform in
the usual manner. Crystals (30 mg) were obtained which
upon recrystallization from methanol melted at 197-198°.
$[\alpha]_D$ 77° (c, 0.54 in $CHCl_3$). Calculated for $C_{27}H_{36}O_{19}$:
C, 48.80; H, 5.42%. Found: C, 49.29; H, 5.53%.

Hydrogenolysis of methyl (benzyl-hexa-O-acetyl-β-maltosid)-
uronate (145 mg) in glacial acetic acid (5 ml) with Pd/C
catalyst (75 mg) for 10 hours at atmospheric pressure gave
a material which upon acetylation as described above gave
methyl (hepta-O-acetyl-β-maltosid)uronate (43 mg). Melting
point and mixed melting point 197-198°.

Constitution of Maltobiouronic Acid

To maltobiouronic acid (6 mg) in water (5 ml) sodium
borohydride (30 mg) was added. After seventeen hours at
room temperature the solution was neutralized with acetic
acid and evaporated to dryness. The sirup was evaporated
with 3% HCl in methanol (3 x 5 ml) and the residue dissolved

in water (5 ml), passed through Amberlite IR-120 (H^+) and
evaporated to dryness. The resulting material was dissolved
in $\underline{N}$ H_2SO_4 (3 ml) and hydrolyzed at 100° in a sealed tube
overnight. Neutralization of the hydrolyzate with washed
barium carbonate gave a filtrate which upon acidification by
passing through Amberlite IR-120 (H^+) indicated only glucuronic
acid and sorbitol upon paper chromatography.

Reaction of Benzyl Hepta-O-acetyl-β-maltoside with $LiAlH_4$

To a suspension of $LiAlH_4$ (0.2 g) in dry tetrahydrofuran
(5 ml) was added a solution of benzyl hepta-O-acetyl-β-
maltoside (54 mg) in tetrahydrofuran (5 ml). The mixture was
stirred under reflux for 1 hour, after which time excess
$LiAlH_4$ was destroyed with ethyl acetate. The mixture was
then neutralized with glacial acetic acid and evaporated to
dryness. To the residue anhydrous sodium acetate (0.2 g)
and acetic anhydride (5 ml) were added and the mixture left
at room temperature overnight. The acetylation mixture was
poured into ice water (50 ml), extracted with chloroform
(3 x 20 ml), extracts washed in the usual way and the dried
extract evaporated to a sirup. The sirup upon silica gel
thin layer chromatography (solvent E) indicated two major
components. The faster of the two corresponded to the starting
material, benzyl hepta-O-acetyl-β-maltoside. The second spot
was more intense and was presumably nona-O-acetyl maltitol.

1,6-Anhydro-β-maltose

To a suspension of hexa-0-acetyl-1,6-anhydro-β-maltose
(41) (7 g) in methanol (95 ml) was added a solution of 0.1$\underline{M}$
sodium methoxide in methanol (5 ml). After 4 hours the
acetate had dissolved, and after a further 14 hours the
solution was neutralized with IR 120 (H$^+$). The solution was
filtered and evaporated to give a sirup which was dissolved
in refluxing methanol (50 ml). The hot solution was thinned
with ethyl acetate (100 ml) and allowed to cool. The crystals
(3.2 g) were filtered and recrystallized in the same manner.
M.p. 156-158°. $[\alpha]_D$ 108° ($\underline{c}$, 7.3 in H_2O). Lit. (40),
m.p. 150°. $[\alpha]_D$ 79°. Calculated for $C_{12}H_{20}O_{10}$: C, 44.44;
H, 6.17%. Found: C, 44.02; H, 5.93%.

Direct Tosylation of 1,6-Anhydro-β-maltose

To 1,6-anhydro-β-maltose (100 mg) dissolved in pyridine
(1 ml) was added tosyl chloride (53 mg) in pyridine (1 ml).
The solution was left 17 hours at 5°, after which time thin
layer chromatography (solvent F) indicated three monotosylates
and two ditosylates in addition to starting material. Varying
the temperature of the reaction and concentration of the
reagents did not seem to change the relative amounts of the
products as indicated by thin layer chromatography. Direct
acetylation of the tosylation mixture indicated only two
tosylated products on thin layer chromatography in solvent D,

presumably due to the inability of the chromatographic system to separate individual isomers. Tosyl esters were identified by spraying with diphenylamine in ethanol and viewing under ultraviolet radiation (126).

1,6-Anhydro-6'-O-trityl-β-maltose

To 1,6-anhydro-β-maltose (2.2 g) powdered and dried over P_2O_5 in vacuo freshly distilled pyridine (25 ml) was added. The mixture was warmed to effect solution and cooled to room temperature. Recrystallized trityl chloride (2.85 g., 1.5 mole equiv.) was added and the solution left at room temperature for 5 hours. The pyridine solution was added dropwise to ice water (500 ml) with continuous stirring. The mixture was then extracted with chloroform (2 x 100 ml), and the chloroform extracts were amalgamated and filtered through Celite. The clear solution was thinned with an equal volume of petroleum ether (30-60°) and the precipitate collected (1.9 g). The trityl ether was recrystallized three times from ethanol-water (1:2, 75 ml/g) to yield material melting at 141-142°. $\left[\alpha\right]_D$ 40.8° (c, 2.53 in MeOH). Calculated for monohydrate $C_{31}H_{34}O_{10} \cdot H_2O$: C, 63.70; H, 6.16%. Found: C, 63.78; H, 6.26%.

Penta-O-acetyl-1,6-anhydro-6'-O-trityl-β-maltose

To 1,6-anhydro-6'-O-trityl-β-maltose (1.5 g) dissolved

in pyridine (15 ml) acetic anhydride (15 ml) was added and the solution left overnight at room temperature. Dropwise addition of the acetylation solution into ice water (1 L) gave the acetylated ether (2.2 g). Recrystallization from ethanol - water (3:1, 30 ml/g) gave material melting at 101-102°. $[\alpha]_D$ 55.0° ($\underline{c}$, 1.55 in $CHCl_3$). Calculated for $C_{41}H_{44}O_{15}$: C, 63.40; H, 5.67%. Found: C, 62.98; H, 5.84%.

2,2',3,3',4'-Penta-O-acetyl-1,6-anhydro-β-maltose

Penta-O-acetyl-1,6-anhydro-6'-O-trityl-β-maltose (1 g) was dissolved in 80% aqueous acetic acid (40 ml) and the solution was kept 3 hours at 50°. To the warm solution, water (90 ml) was added and the mixture kept at 5° for 18 hours. The mixture was filtered, the precipitate washed with 25% aqueous acetic acid (10 ml), and the combined filtrates evaporated to a sirup. Crystals (665 mg) were obtained by dissolving the sirup in hot 2-propanol (25 ml). Recrystallization from the same solvent (40 ml/g) gave crystals melting at 82-83°. $[\alpha]_D$ 43.4° ($\underline{c}$, 2.43 in $CHCl_3$). Calculated for $C_{22}H_{30}O_{15}$: C, 49.44; H, 5.62%. Found: C, 49.87; H, 5.70%.

Penta-O-acetyl-1,6-anhydro-6'-O-tosyl-β-maltose

To the pentaacetate (4 g) dissolved in dry pyridine (18 ml) tosyl chloride (7.5 g) was added. After 5 hours at room

temperature, 3 drops of water were added to hydrolyze excess

tosyl chloride and the solution was poured into ice water

(80 ml). This mixture was extracted with chloroform (3 x 100 ml),

and the chloroform extracts were combined and washed with

water (7 x 100 ml). The solution was dried with calcium

chloride, filtered and evaporated to give a sirup which,

when dissolved in hot 2-propanol (155 ml), gave the

crystalline tosylate (4.25 g). Recrystallization from the

same solvent (36 ml/g) gave material melting at 170-171°.

$[\alpha]_D$ 55.7° ($\underline{c}$, 1.00 in $CHCl_3$). Calculated for $C_{29}H_{36}O_{17}S$:

C, 50.58; H, 5.23; S, 4.65%. Found: C, 50.48; H, 5.11;

S, 4.36%.

Penta-O-acetyl-1,6-anhydro-6'-azido-6'-deoxy-β-maltose

Penta-O-acetyl-1,6-anhydro-6'-O-tosyl-β-maltose (1.5 g)

dissolved in $\underline{N}$, $\underline{N}$-dimethylformamide (75 ml) was heated on

a steam bath with sodium azide (1.5 g) for one hour and was

stirred frequently. After cooling, the solution was poured

into an equal volume of water and the mixture extracted

with chloroform (4 x 100 ml). The combined chloroform

extract was thoroughly washed with water (8 x 200 ml), dried

over calcium chloride, filtered, and evaporated to a sirup.

The sirup was dissolved in hot 2-propanol and the solution

cooled to give crystals (1.06 g) of the 6'-azide. Recrystal-

lization from the same solvent (50 ml/g) yielded crystals

melting at 151-152°. $[\alpha]_D$ 47.9° (c, 2.53 in $CHCl_3$).
Calculated for $C_{22}H_{29}O_{14}N_3$: C, 47.23; H, 5.19; N, 7.51%.
Found: C, 47.68; H, 5.49; N, 7.38%.

6'-Amino-6'-deoxy-maltose

To a solution of penta-O-acetyl-1,6-anhydro-6'-azido-6'-
deoxy-β-maltose (560 mg) in methanol (20 ml) was added
0.1 M sodium methoxide in methanol (4 ml). After 18 hours
at room temperature, the solution was neutralized with
IR 120 (H^+) and evaporated to a sirup which plainly showed
an infrared absorption band near 2100 cm^{-1} characteristic
of the azide group (127). The sirup was dissolved in ethanol
(15 ml) and 10% palladium on charcoal (300 mg) was added.
The mixture was maintained at 65° by means of a water bath
while a fine stream of hydrogen was bubbled through the
solution. After 45 minutes the solution was made slightly
acidic with 3% hydrogen chloride in methanol and the
hydrogenation continued for a further 2 hours. The catalyst
was removed by filtration and the solution evaporated to give
a chromatographically pure sirup R_f 0.054, solvent B, detected
with ninhydrin. This material was dissolved in 0.15N hydrochloric
acid and heated in a sealed tube for 13 hours at 100°. The
hydrolysate was neutralized with silver carbonate, filtered
and adjusted to pH 5 with 0.3N HCl. Chromatography in
solvent B indicated only two ninhydrin positive materials

R_f 0.041 and 0.054. However p-anisidine-trichloroacetate

spray indicated glucose, the material showing R_f 0.041 and

slower running degradation products. The presence of glucose

was indicated by comparison with standards in solvent systems

A, B, C, and also by thin layer chromatography (128) in

solvent G. Preparative paper chromatography on Whatman 3MM

paper in solvent B gave a small amount (10 mg) of the

R_f 0.041 material. Hydrolysis of a sample of this material

indicated glucose and 6-amino-6-deoxy-glucose, identical

to material prepared by the method of Cramer and co-workers

(96). This indicates that the R_f 0.041 material is

6'-amino-6'-deoxy-maltose. The very low yield prevented any

attempts to prepare suitable derivatives. $[\alpha]_D$ 88°

(c, 0.3 in H_2O). Calculated for $C_{12}H_{23}O_{10}N \cdot HCl$: N, 3.70%.

Found: N, 3.97%.

Potassium Thiolacetate

To a solution of potassium carbonate (36 g) in water (500

ml) was added redistilled thiolacetic acid (22 ml). The

solution was evaporated to dryness and the residue was

extracted with ethanol (2 x 250 ml). The combined extract

was filtered and evaporated to dryness. The residue was

dissolved in hot absolute ethanol (150 ml), decolourizing

carbon (1 g) was added, the solution filtered and allowed to

cool. After 4 hours at 5°, the salt was filtered off/and dried

over calcium chloride. Yield 14.6 g (129).

Penta-O-acetyl-6'-S-acetyl-1,6-anhydro-6'-deoxy-β-maltose

To a solution of penta-O-acetyl-1,6-anhydro-6'-O-tosyl-

β-maltose (500 mg) in N,N-dimethylformamide (5 ml) was

added potassium thiolacetate (220 mg). The mixture was

heated on a steam bath for half an hour. After cooling,

water (50 ml) was added and the mixture extracted with

chloroform (4 x 50 ml). The combined chloroform solution

was thoroughly washed with water (7 x 100 ml), dried over

calcium chloride, filtered and evaporated to a sirup. The

sirup was dissolved in hot 2-propanol (45 ml) which upon

cooling gave crystals (300 mg) of the S-acetate. Recrystal-

lization of this material from 2-propanol (80 ml/g) yielded

crystals melting at 219-220°. $[\alpha]_D$ 34.6° (c, 0.72 in

$CHCl_3$). Calculated for $C_{24}H_{32}O_{15}S$: C, 48.66; H, 5.41; S, 5.41%.

Found: C, 48.74; H, 5.57; S, 5.24%.

Hepta-O-acetyl-6'-S-acetyl-6'-deoxy-α-maltose

Penta-O-acetyl-6'-S-acetyl-1,6-anhydro-6'-deoxy-β-maltose

(150 mg) was dissolved in the acetolysis mixture (3 ml)

(H_2SO_4:Ac_2O:AcOH = 1:70:30). After 3 hours at room temperature

the solution was added dropwise to ice water (25 ml). The

white powder so obtained was filtered and a portion recrystallized

from ethanol-water (1:3, 100 ml/g). Further recrystallization did not remove a small amount of the β-anomer which was apparent on thin layer chromatography in solvent D. M.p. 73-75°. $[\alpha]_D$ 113° ($\underline{c}$, 0.79 in $CHCl_3$). Calculated for $C_{28}H_{38}O_{18}S$: C, 48.42; H, 5.49%. Found: C, 48.67; H, 5.79%.

6'-Deoxy-6'-mercapto-maltose

To hepta-O-acetyl-6'-S-acetyl-6'-deoxy-α-maltose (120 mg) in methanol (4 ml) was added a solution of 0.1$\underline{M}$ sodium methoxide in methanol (1 ml). After the solution had been kept at room temperature overnight, it was neutralized with IR 120 (H^+), filtered and evaporated to a sirup. Chromatography in solvent A showed two products $\underline{R}_f$ 0.018 and 0.23 which were the disulphide and the free thiol respectively. When excess 2-mercapto-ethanol and 1 drop of concentrated ammonia were added, only the more mobile compound was present. Chromatography of the acid hydrolysate of 6'-deoxy-6'-mercapto-maltose indicated glucose and 6-deoxy-6-mercapto-glucose. The latter was prepared by a route similar to that employed by Akagi, Tejima and Haga (130). Borohydride reduction followed by hydrolysis indicated sorbitol and 6-deoxy-6-mercapto-glucose. Attempts to crystallize the chromatographically pure disaccharide were unsuccessful. Insufficient material was recovered to obtain suitable analyses. $[\alpha]_D$ 137.6° ($\underline{c}$, 2.02 in H_2O).

Penta-O-acetyl-1,6-anhydro-6'-deoxy-6'-iodo-β-maltose

To a solution of the 6'-O-tosylate (1.2 g) in $\underline{N},\underline{N}$-dimethylformamide (50 ml) was added finely powdered sodium iodide (4 g). The solution was heated on a steam bath for $1\frac{1}{2}$ hours. After cooling, the solution was diluted with water (100 ml), and extracted with chloroform (4 x 50 ml). The combined chloroform extract was washed with water (6 x 100 ml), dried over calcium chloride, filtered and evaporated. The sirup obtained was dissolved in hot 2-propanol (100 ml) from which crystals (1.04 g) were obtained on cooling. Recrystallization from the same solvent (100 ml/g) gave material melting at 194-195°. $\left[\alpha\right]_D$ 42.0° ($\underline{c}$, 1.03 in CHCl$_3$). Calculated for $C_{22}H_{29}O_{14}I$: C, 40.99; H, 4.50%. Found: C, 41.09; H, 4.58%.

Penta-O-acetyl-1,6-anhydro-6'-deoxy-β-maltose

To a solution containing penta-O-acetyl-1,6-anhydro-6'-deoxy-6'-iodo-β-maltose (0.5 g) and pyridine (0.1 ml) in ethanol (10 ml) was added 10% palladium on charcoal (0.5 g). The mixture was kept at 60° by means of a water bath and a stream of hydrogen was introduced through a capillary. After four hours the catalyst was filtered and the solution evaporated. The resulting sirup was dissolved in chloroform (30 ml) washed with 1% sodium thiosulphate (15 ml) and finally washed with water (4 x 30 ml). The chloroform solution was

dried over calcium chloride and evaporated to give a faintly
yellow sirup. The sirup was dissolved in hot 2-propanol
(25 ml) which upon cooling gave the crystalline deoxy sugar
(252 mg). Recrystallization from 2-propanol (40 ml/g) gave
material melting at 141-142°. $[\alpha]_D$ 44.5° ($\underline{c}$, 0.88 in
$CHCl_3$). Calculated for $C_{22}H_{30}O_{14}$: C, 50.96; H, 5.77%.
Found: C, 50.87; H, 5.89%.

6'-Deoxy-maltose

Penta-O-acetyl-1,6-anhydro-6'-deoxy-β-maltose (485 mg)
was dissolved in the acetolysis mixture (10 ml) (H_2SO_4:Ac_2O:
AcOH = 1:70:30). The optical rotation varied from $[\alpha]_D$
89.2° (5 min) to 138.8° (2 h) after which time it was constant.
The flask was cooled in ice after 3 hours and ice (5 g) was
added. The sulphuric acid was neutralized by the addition of
$\underline{M}$ barium acetate and the precipitate removed by centrifugation.
The solution was evaporated to give a sirup which was evaporated
to dryness with ethanol (3 x 10 ml). The hepta-O-acetate
could be obtained as an amorphous powder on precipitation from
alcohol-water. The sirupy acetate was dissolved in methanol
(10 ml) and a solution of 0.1 $\underline{M}$ sodium methoxide in methanol
(2 ml) was added. After 17 hours at room temperature the
solution was neutralized with IR 120 (H^+), filtered and
evaporated to a sirup. The sirup, which was chromatographically
pure, could not be induced to crystallize even after chromatography

on Dowex 50 W x 2 (Li$^+$ salt) (131). Hydrolysis of a sample

of the sugar with 2 $\underline{N}$ sulphuric acid indicated glucose and

material identical to 6-deoxy-glucose prepared by the procedure

of Fischer and Zach (132). A second sample, which had been

reduced with sodium borohydride before hydrolysis, indicated

sorbitol and 6-deoxy-glucose. $[\alpha]_D$ 112.7° ($\underline{c}$, 2.77 in

H_2O). Calculated for $C_{12}H_{22}O_{10} \cdot H_2O$: C, 41.86; H, 6.93%.

Found: C, 41.39; H, 6.96%.

Hepta-O-acetyl-6'-deoxy-β-maltose

A portion of the 6'-deoxy-maltose (23.3 mg) was dissolved

in pyridine (1 ml) and acetic anhydride (4 ml) was added.

The solution was left 30 hours at room temperature, poured

into ice water (40 ml), and extracted with chloroform (3 x

30 ml). The combined extract was washed thoroughly with

water (6 x 50 ml), dried over calcium chloride, filtered and

evaporated. The resulting sirup was dissolved in hot ethanol

(2 ml) and the solution, upon cooling, yielded crystalline

hepta-O-acetyl-6'-deoxy-β-maltose. M.p. 183-185°. $[\alpha]_D$

64° ($\underline{c}$, 0.68 in $CHCl_3$). Calculated for $C_{26}H_{36}O_{17}$: C, 50.32;

H, 5.80%. Found: C, 49.57; H, 5.55%.

Catalyzed Lead Tetraacetate Oxidations

Methyl α-D-Glucopyranoside

To a solution of methyl α-D-glucopyranoside (26.4 mg.,

1.36 x 10^{-4} moles) in glacial acetic acid (5 ml) was added

50% aqueous potassium acetate (0.2 ml) and 0.0622 $\underline{M}$ lead

tetraacetate in glacial acetic acid. Lead tetraacetate

consumption was estimated by iodimetry as outlined by Perlin (104).

1,6-Anhydro-β-D-glucose

1,6-Anhydro-β-D-glucose m.p. 174°, lit. (133), m.p.

172°, (19.0 mg., 1.17 x 10^{-4} moles) was oxidized using the

same quantities as for methyl α-D-glucopyranoside.

Table 1

Time (min)	Methyl α-D-glucopyranoside	1,6-Anhydro-β-D-glucose
	moles of tetraacetate consumed per mole of sugar	
30	0.07	0.03
120	0.34	0.33
300	0.66	0.75
420	0.91	1.04
1500	2.02	2.12

Benzyl 4',6'-O-Benzylidene-6-O-tosyl-β-maltoside

To a solution of benzyl 4',6'-O-benzylidene-β-maltoside

(650 mg) (134) in dry pyridine (5 ml) was added recrystallized

tosyl chloride (286 mg) in dry pyridine (5 ml). The reaction

proceeded 40 hours, during which time samples were removed for

thin layer chromatography (solvent F). Chromatography
indicated that three major products were formed. Two of
the products travelled at a rate corresponding to mono-
tosylates, the other corresponding to a ditosylate. It was
obvious that the selectivity of tosylation was not sufficiently
specific to obtain the 6-tosyl derivative.

Benzyl 4',6'-O-Benzylidene-6-O-trityl-β-maltoside

To a solution of benzyl 4',6'-O-benzylidene-β-maltoside
(35 mg) in dry pyridine (0.35 ml) was added trityl chloride
(25 mg). The reaction was followed by thin layer chromatography
in solvent D, which indicated no reaction had occurred even
after 3 days at room temperature.

Benzyl 4',6'-O-Benzylidene-6-O-mesyl-β-maltoside

To a solution of benzyl 4',6'-O-benzylidene-β-maltoside
(300 mg) in dry chloroform (25 ml) was added dry pyridine (5 ml)
and the solution cooled to 5°. Mesyl chloride (0.06 ml) was
then added. The solution remained at 5° for 5 hours, and a
drop of water was then added to hydrolyze any excess reagent.
The solution was extracted with water (6 x 30 ml), dried over
calcium chloride, and evaporated. Crystals which melted at
86-89° were obtained from ethyl acetate - petroleum ether
(30-60°). The crystalline product was shown by thin layer

chromatography to be composed of about 70% starting material. The minor component appeared chromatographically homogeneous and was presumably the 6-O-mesyl derivative.

BIBLIOGRAPHY

1. R. L. Whistler and B. Shasha. J. Org. Chem. 29, 880 (1964).

2. M. L. Wolfrom, M. I. Taha and D. Horton. J. Org. Chem. 28, 3553 (1963).

3. W. Koenigs and E. Knorr. Ber. 34, 957 (1901).

4. W. L. Evans, D. D. Reynolds and E. A. Talley. Advan. Carbohydrate Chem. 6, 27 (1951).

5. M. G. Blair and W. Pigman. Angew. Chem. 69, 422 (1957).

6. R. W. Bailey and J. B. Pridham. Advan. Carbohydrate Chem. 17, 121 (1962).

7. S. Black, G. G. S. Dutton and K. N. Slessor. A Tabular List of Synthetic Disaccharides. Department of Chemistry, University of British Columbia, Vancouver, B. C.

8. R. U. Lemieux and B. Fraser-Reid. Can. J. Chem. 42, 532 (1964).

9. P. A. J. Gorin and A. S. Perlin. Can. J. Chem. 39, 2474 (1961).

10. M. L. Wolfrom, A. O. Pittet and I. C. Gillam. Proc. Natl. Acad. Sci. 47, 700 (1961).

11. D. R. Lineback. Ph.D. Thesis. The Ohio State University, (1962).

12. H. Bredereck, A. Wagner, D. Geissel and H. Ott. Chem. Ber. 95, 3064 (1962).

13. R. U. Lemieux and B. Fraser-Reid. Can. J. Chem. 42, 539 (1964).

14. W. J. Hickinbottom. J. Chem. Soc. 3140 (1928).

15. P. Brigl. Z. physiol. Chem. 122, 245 (1922).

16. R. U. Lemieux. Advan. Carbohydrate Chem. 9, 1 (1954).

17. W. N. Haworth and W. J. Hickinbottom. J. Chem. Soc. 2847 (1931).

18. R. U. Lemieux. Can. J. Chem. 31, 949 (1953).

19. R. U. Lemieux and G. Huber. J. Am. Chem. Soc. 75, 4118 (1953).

20. A. Pictet and H. Vogel. Ber. 62, 1418 (1929).

21. A. Pictet. Helv. Chim. Acta. 16, 144 (1933).

22. S. Haq and W. J. Whelan. Nature 178, 1222 (1956).

23. E. Pacsu. Ber. 61, 137, 1508 (1928).

24. B. Lindberg. Acta. Chem. Scand. 3, 1153 (1949).

25. R. U. Lemieux, G. Huber and W. P. Shyluk. Can. J. Chem. 33, 148 (1955).

26. B. Lindberg. Acta. Chem. Scand. 3, 1355 (1949).

27. I. Karasawa and R. Onishi. J. Agr. Chem. Soc. (Japan) 35, 813 (1961).

28. I. J. Goldstein and W. J. Whelan. J. Chem. Soc. 4264 (1963).

29. K. Hess, H. Hammerstein and W. Gramberg. Ber. 70, 1134 (1937).

30. G. Jayme and W. Demmig. Chem. Ber. 93, 356 (1960).

31. A. Klemer and F. Gundlach. Chem. Ber. 96, 1765 (1963).

32. S. Matsubara. Bull. Soc. Chem. (Japan) 34, 718 (1961).

33. J. Compton. J. Am. Chem. Soc. 60, 1203 (1938).

34. O. T. Schmidt. Methods in Carbohydrate Chem. 1, 349 (1962).

35. K. Matsuda, H. Watanabe, K. Fujimoto and K. Aso. Nature 191, 278 (1961).

36. E. M. Montgomery, N. K. Richtmeyer and C. S. Hudson. J. Am. Chem. Soc. 65, 1848 (1943).

37. B. Lindberg and L. Selleby. Acta. Chem. Scand. 14, 1051 (1960).

38. I. Johanson, B. Lindberg and O. Theander. Acta. Chem. Scand. 17, 2019 (1963).

39. A. Pictet and A. Marfort. Helv. Chim. Acta. 6, 129 (1923).

40. P. Karrer and C. Kamienski. Helv. Chim. Acta. 15, 739 (1932).

41. L. Asp and B. Lindberg. Acta. Chem. Scand. 6, 941 (1952).

42. K. Freudenberg and K. Soff. Ber. 69, 1245 (1936).

43. S. Tsuiki. Tohuku. J. Exptl. Med. 61, 267 (1955). C.A. 50, 4043 (1956).

44. J. Stanek and J. Sada. Coll. Czech. Chem. Coll. 14, 540 (1949). C.A. 44, 5820 (1950).

45. Z et. Heweihi. Chem. Ber. 86, 862 (1953).

46. G. Zemplen and Z. Csuros. Ber. 62, 993 (1929).

47. S. Peat. Advan. Carbohydrate Chem. 2, 37 (1946).

48. F. H. Newth. Quart. Rev. 13, 30 (1959).

49. R. de Souza and I. J. Goldstein. Tetrahedron Letters 1215 (1964).

50. F. H. Newth, S. D. Nicholas, F. Smith and L. F. Wiggins. J. Chem. Soc. 2550 (1949).

51. T. L. Cottrell and E. G. V. Percival. J. Chem. Soc. 749 (1942).

52. T. J. Painter. J. Chem. Soc. 1396 (1964).

53. B. Lythgoe and S. Trippett. J. Chem. Soc. 1983 (1950).

54. H. Bredereck. Ber. 63, 959 (1930).

55. P. Brigl and P. Mistele. Z. physiol Chem. 126, 120 (1923).

56. B. Helferich and W. Ost. Chem. Ber. 95, 2616 (1962).

57. B. Helferich and J. Zirner. Chem. Ber. 96, 385 (1963).

58. E. Fischer, M. Bergmann and A. Rabe. Ber. 53, 2362 (1920).

59. B. Helferich and W. Klein. Ann. 450, 219 (1926).

60. B. Helferich. Advan. Carbohydrate Chem. 3, 79 (1948).

61. A. Thompson and M. L. Wolfrom. "Esters" in "The Carbohydrates" (edited by W. Pigman), Academic Press Inc., New York, N. Y., p. 138 (1957).

62. F. Micheel. Chemie Der Zucker und Polysaccharide Akademische Verlagsgesellschaft. Leipzig p. 102 (1956).

63. M. L. Wolfrom and A. Thompson. Methods of Carbohydrate Chemistry 2, 211 (1963).

64. T. G. Bonner. Advan. Carbohydrate Chem. 16, 59 (1961).

65. D. Horton and D. H. Hutson. Advan. Carbohydrate Chem. 18, 162 (1963).

66. B. Helferich and W. Speicher. Ann. 579, 106 (1953).

67. R. S. Tipson. Advan. Carbohydrate Chem. 8, 107 (1953).

68. C. M. McCloskey. Advan. Carbohydrate Chem. 12, 137 (1957).

69. I. Croon and B. Lindberg. Acta. Chem. Scand. 13, 593 (1959).

70. M. E. Tate and C. T. Bishop. Can. J. Chem. 41, 1801 (1963).

71. R. W. Jeanloz, A. M. C. Rapin, and S. Hakomori. J. Org. Chem. 26, 3939 (1961).

72. O. T. Schmidt and E. Wernicke. Ann. 558, 70 (1947).

73. H. Bredereck, A. Wagner, G. Faber, H. Ott and J. Rauther. Chem. Ber. 92, 1135 (1959).

74. M. Smith, D. H. Rammler, I. H. Goldberg and H. G. Khorana. J. Am. Chem. Soc. 84, 430 (1962).

75. K. Josephson. Ann. 472, 230 (1929).

76. Y. Hirasaka, I. Matsunaga, K. Umemoto and M. Sukegawa. Yakugaku Zasshi. 83, 966 (1963).

77. R. Sutra. Bull. Soc. Chim. 9, 794 (1942).

78. G. G. S. Dutton and K. N. Slessor. Unpublished Results.

79. H. Borjeson, P. Jerkeman and B. Lindberg. Acta. Chem. Scand. 17, 1705 (1963).

80. P. Jerkeman and B. Lindberg. Acta. Chem. Scand. 17, 1709 (1963).

81. H. H. Schlubach, W. Rauchenberger and A. Schultze. Ber. 66, 1248 (1933).

82. P. Jerkeman. Acta. Chem. Scand. 17, 2769 (1963).

83. E. J. Hedgley and H. G. Fletcher, Jr. J. Am. Chem. Soc. 84, 3726 (1962).

84. K. Heyns and H. Paulsen. Advan. Carbohydrate Chem. 17, 169 (1962).

85. K. Heyns. Staerke 15, 425 (1964).

86. M. Barczai-Martos and F. Korosy. Nature 165, 369 (1950).

87. B. Helferich and A. Berger. Chem. Ber. 90, 2492 (1957).

88. H. Fischer and F. Kogl. Ann. 436, 219 (1924).

89. R. Kuhn and H. J. Haas. Angew. Chem. 67, 785 (1955).

90. G. Zweifel and H. Deuel. Helv. Chim. Acta. 39, 662 (1956).

91. H. O. L. Fischer, C. Taube and E. Baer. Ber. 60, 479 (1927).

92. N. G. Gaylord. Experientia 10, 423 (1954).

93. R. Allerton and H. G. Fletcher, Jr. J. Am. Chem. Soc. 76, 1757 (1954).

94. M. E. Tate and C. T. Bishop. Can. J. Chem. 40, 1043 (1962).

95. R. W. Jeanloz and A. M. C. Rapin. J. Org. Chem. 28, 2978 (1962).

96. F. Cramer, H. Otterbach and H. Springmann. Chem. Ber. 92, 384 (1959).

97. J. H. Chapman and L. N. Owen. J. Chem. Soc. 579 (1950).

98. M. Abdullah. Ph.D. Thesis. University of London (1960).

99. H. H. Baer and H. O. L. Fischer. Proc. Nat. Acad. Sci. 44, 991 (1958).

100. H. H. Baer and A. Ahammad. Can. J. Chem. 41, 2931 (1963).

101. F. W. Lichtenthaler. Angew. Chem. 76, 84 (1964).

102. L. D. Hall and L. Hough. Proc. Chem. Soc. 382 (1962).

103. R. E. Reeves. J. Am. Chem. Soc. 71, 2116 (1949).

104. A. S. Perlin. Advan. Carbohydrate Chem. 14, 9 (1959).

105. Ibid. p. 12.

106. R. J. Dimler. Advan. Carbohydrate Chem. 7, 37 (1952).

107. R. U. Lemieux and S. Levine. Can. J. Chem. 42, 1473 (1964).

108. Y. Hirasaka. Yakugaku Zasshi. 83, 960 (1963).

109. G. G. S. Dutton and K. N. Slessor. Can. J. Chem. 42, 1110 (1964).

110. Y. Hirasaka, I. Matsunaga, K. Umemoto and M. Sukegawa. Yakugaku Zasshi 83, 971 (1963).

111. Ibid. pp. 1073, 1078.

112. R. Bentley, J. Am. Chem. Soc. 81, 1952 (1959).

113. V. S. R. Rao and J. F. Foster. J. Phys. Chem. 67, 951 (1963).

114. A. E. Knauf, R. M. Hann and C. S. Hudson. J. Am. Chem. Soc. 63, 1447 (1941).

115. R. M. Hann and C. S. Hudson. J. Am. Chem. Soc. 63, 2241 (1941).

116. K. Freudenberg and W. Nagai. Ber. 66, 27 (1933).

117. W. T. Haskins, R. M. Hann and C. S. Hudson. J. Am. Chem. Soc. 64, 1289 (1942).

118. W. T. Haskins, R. M. Hann, and C. S. Hudson. J. Am. Chem. Soc. 64, 1852 (1942).

119. M. L. Wolfrom, R. M. de Lederkremer and L. E. Anderson. Anal. Chem. 35, 1357 (1963).

120. M. Gee. Anal. Chem. 35, 350 (1963).

121. Y. U. Zhdanov, G. A. Korol'chenko and G. N. Dorofeenko. Dokl. Akad. Nauk. SSSR. 143, 852 (1962). C.A. 57, 4747 (1962).

122. J. M. Swan. Nature 180, 643 (1957).

123. L. Hough, J. K. N. Jones and W. L. Wadman. J. Chem. Soc. 1702 (1950).

124. R. U. Lemieux and H. F. Bauer. Anal. Chem. 26, 920 (1954).

125. H. C. Brown and C. A. Brown. J. Am. Chem. Soc. 84, 2827 (1962).

126. M. Jackson and L. D. Hayward. J. Chromatog. 5, 166 (1961).

127. R. D. Guthrie and D. Murphy. J. Chem. Soc. 5288 (1963).

128. G. W. Hay, B. A. Lewis and F. Smith. J. Chromatog. 11, 479 (1963).

129. B. Bannister and F. Kagan. J. Am. Chem. Soc. 82, 3363 (1960).

130. M. Akagi, S. Tejima and M. Haga. Chem. Pharm. Bull. (Tokyo) 10, 562 (1962).

131. J. K. N. Jones, R. A. Wall and A. O. Pittet. Can. J. Chem. 38, 2285 (1960).

132. E. Fischer and K. Zach. Ber. 45, 3761 (1912).

133. R. B. Ward. Methods in Carbohydrate Chem. 2, 394 (1963).

134. A. Klemer. Chem. Ber. 92, 218 (1959).

II.   SYNTHESIS OF THE 2,4-DI-O-METHYL TETROSES

INTRODUCTION

Purpose

The aim of preparing partially methylated tetroses was
to facilitate the identification of small quantities of
partially methylated sugars by characterization of their
periodate oxidation products. Periodate oxidation may supply
not only information on the number of free vicinal hydroxyls
present, but also, analysis of the products for formic acid
and formaldehyde gives information as to their location.
Formaldehyde then, indicates the presence of a primary alcohol
in a 1,2-glycol system, whereas formic acid indicates a
secondary alcohol flanked by two other alcohol groups.
Identification of the fragments might allow full characterization
of the sugar.

Two recent communications have emphasized the need for
reference compounds in the tetrose series. Had identification
of the tetroses produced by periodate oxidation been possible,
the intensive investigation necessary to determine the parent
sugar would not have been necessary.

Stephen (1), in his structural investigation of Virgillia
oroboides gum, used periodate oxidation to establish the
positions of substitution on partially methylated sugars.

Several chromatographically fast running products were obtained which, from consideration of the assigned structure of the parent sugar, were believed to be of the tetrose series. Stephen assigned probable structures for these products, only after extensive investigation to establish the structure of the parent sugar. Partially methylated glucoses, obtained from hydrolysis of a methylated glucan (2), gave, upon periodate oxidation, methylated reducing fragments, believed to be tetroses.

To provide reference compounds for structural determinations of this type, the synthesis of the 2,4-di-O-methyl tetroses was undertaken.

Background

Tetrose is the generic name describing four carbon sugars. The class of compounds belonging to this family, which contain a straight carbon chain terminated by an aldehydic functional group, possess two asymmetric carbon atoms and therefore consists of four stereoisomeric compounds.

The synthesis of tetroses can be approached from two directions. Starting with D or L-glyceraldehyde, an ascent of the series can be carried out through addition of a further carbon atom by various chemical means, increasing the length of the three carbon sugar by one. The other major approach

is through degradation of higher carbon sugars by cleavage of
a carbon-carbon bond.

Ascent of the series can be accomplished by the classical
Kiliani synthesis (3), in which the aldose is treated with
hydrogen cyanide forming a nitrile, which, through hydrolysis
and reduction, regenerates an aldehyde containing one more
hydroxy-methylene group than its precursor. A newer method,
developed by Sowden and Fischer (4, 5), involves the
condensation of the aldose with nitromethane. Subsequent
hydrolysis of the nitro compound gives the new aldehyde in
good yield. An inherent drawback in any method for ascent
of the series is the introduction of a further asymmetric
carbon atom into the molecule, giving rise to the possibility
of two products. Generally, both possible products are
formed, one usually in higher yield. The separation of these
epimers constitutes the major drawback to methods which
involve ascent of the series.

Several methods are available for the degradation of the
carbon chain of a sugar. A classical method such as the
Wohl degradation (6) requires the elimination of hydrogen
cyanide from the α-hydroxyl nitrile. A newer method (7)
involves the oxidation of diethyl dithio acetals with per-
acids. Subsequent treatment with base eliminates the disulfone,

yielding an aldehyde of one carbon less. Probably one of
the most useful and highest yielding methods is degradation
through glycol cleavage. Cleavage between suitably disposed
1,2-diol systems by periodate (8), or less frequently lead
tetraacetate, results in two aldehydes. The use of both
of these reagents has been recently reviewed (9, 10). The
yields from periodate cleavage are quantitative, in fact
Malaprade (8) originally introduced this reaction as a method
of quantitatively estimating periodate. Limitations in
degradative methods are realized when it is seen that a
suitably orientated and substituted pentose must be used in
the synthesis of a tetrose by the C-1 elimination method,
and that contiguous hydroxyl groups should not be present
in a product expected to be produced by glycol cleavage.

The chemistry of the twenty-four partially O-methylated
tetroses has only been briefly investigated. Prior to this
work, only three reports of synthetic partially methylated
tetroses had been published. Periodate oxidation of 3-O-
methyl-D-xylose gave 2-O-methyl-D-threose (11), and similarly
2,3-di-O-methyl-D-arabinitol gave 2,3-di-O-methyl-D-threose (12).
Although the preparation of 3-O-methyl-L-threose has been
described (13), no physical properties of the product were
described at that time. Previous workers in this group have
attempted synthesis of 2,3-di-O-methyl-L-threose (14),

2,3-di-O-methyl-D-erythrose (14), 2-O-methyl-D-erythrose (15), and 2,4-di-O-methyl-L-erythrose (16).

METHODS OF SYNTHESIS

2,4-Di-0-methyl-D-erythrose

The synthesis of 2,4-di-0-methyl-D-erythrose was achieved by periodate oxidation of 4,6-di-0-methyl-D-glucitol. The glucitol was prepared by borohydride reduction of 4,6-di-0-methyl-D-glucose.

4,6-Di-0-methyl-D-glucose was synthesized by the method of Bell and Lorber (17) which depends on the formation of a 4,6-benzylidene group on methyl α-D-glucoside allowing blockage of hydroxyls 2 and 3 as benzyl ethers. The lability of the benzaldehyde acetal to weak acid allows preferential removal of this group. Subsequent methylation gives the di-0-methyl derivative. Reductive cleavage of the benzyl ethers and acid hydrolysis of the glycoside yield the free sugar (Fig. 1).

It is interesting to note that while Bell and Lorber's method begins with the readily available methyl α-D-glucoside, the intermediate compounds are either sirups or difficultly crystallizable compounds. Dennison and McGilvray (18) on the other hand, start with the less common methyl β-D-glucoside using the same sequence of reactions to give the β-glycoside. In this anomeric series all the intermediates were found to be crystalline. This further substantiates
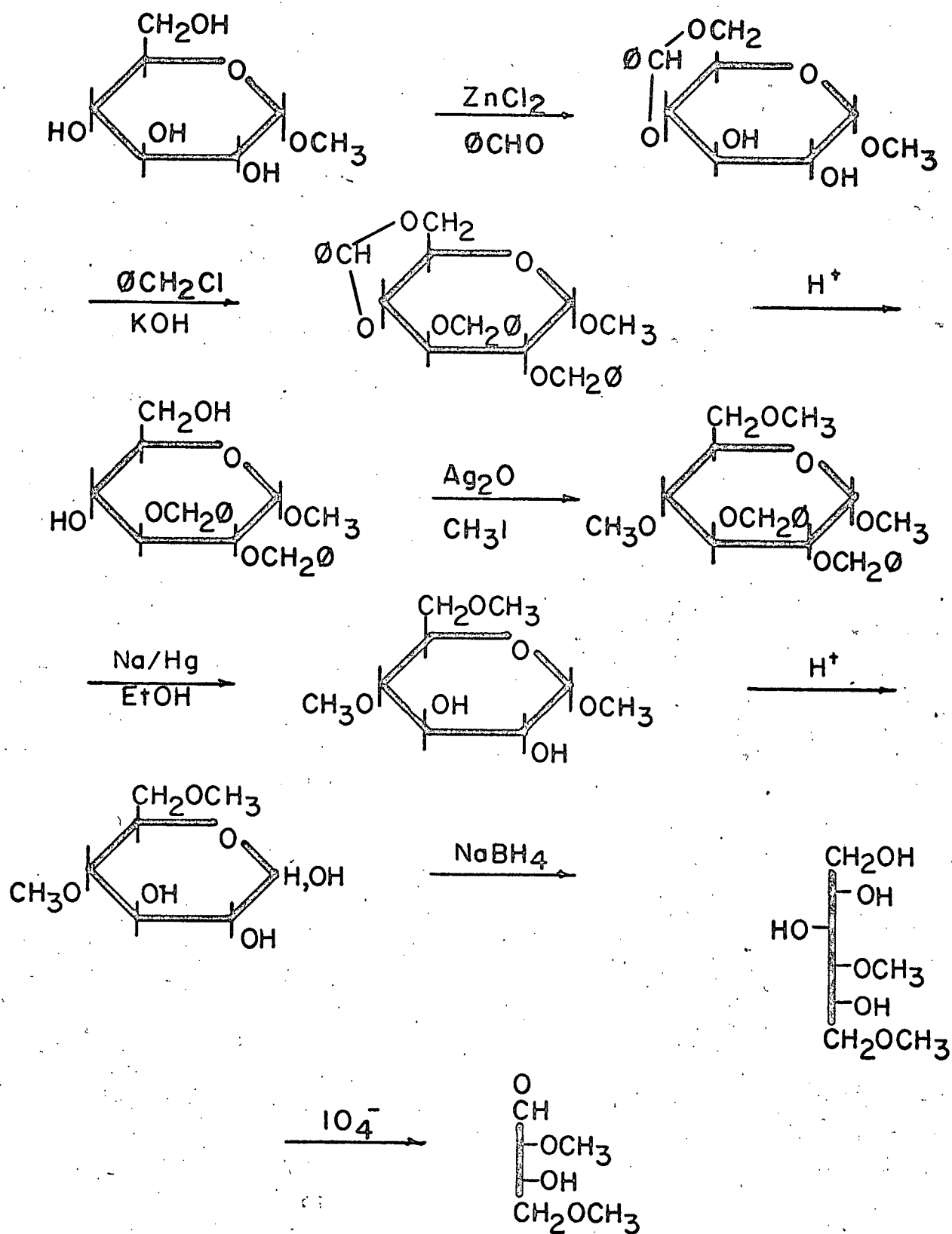
Fig. I

Zemplen's statement (19) that compounds containing α-linkages are more difficult to crystallize than the corresponding compounds with the β-linkage.

Periodate oxidation of the reduced 4,6-di-0-methyl-D-glucose resulted in a 2.03 molar uptake of periodate per mole of sugar. After precipitation of the iodate and excess periodate as barium salts, the solution was evaporated to give a sirup.

Characterization was achieved by formation of the 2,4-dinitrophenylhydrazone and measurement of the optical rotation.

2,4-Di-0-methyl-L-erythrose

2,4-Di-0-methyl-L-erythrose was synthesized by periodate oxidation of 3,5-di-0-methyl-L-arabinitol. This alcohol was obtained through the borohydride reduction of 3,5-di-0-methyl-L-arabinose.

3,5-Di-0-methyl-L-arabinose was synthesized according to the procedure of Hirst, Jones and Williams (20). This route relies on the acid stability of the 5-tosyl ester formed by treating an α, β mixture of methyl arabinofuranosides with tosyl chloride in pyridine. The acid stability allows the replacement of the methyl glycoside with 1,2-isopropylidene without concurrent ring expansion. In this way 1,2-isopropy-

lidene-5-tosyl-arabinose is isolated. Removal of the 5-
tosyl group by reductive cleavage leaves the 3 and 5 hydroxyls
free to be methylated. Acid hydrolysis of the isopropylidene
group of the methylated derivative yields 3,5-di-O-methyl-L-
arabinose (Fig. 2).

A possible source of 3,5-di-O-methyl-L-arabinose is
fully methylated mesquite gum. Several workers (21, 22)
have hydrolyzed this methylated gum with weak acid to cleave
the arabinofuranose linkages. The dimethyl sugar was isolated
from the hydrolyzate mixture by fractional distillation of
the methyl glycosides. Unfortunately, traces of 2,5-di-O-
methyl-L-arabinose are present in such preparations (23), and
cannot easily be removed.

2,4-Di-O-methyl-L-erythrose is identical to its optical
isomer 2,4-di-O-methyl-D-erythrose, in all its properties
except optical rotation. The specific rotation was equal
in magnitude but opposite in sign as would be expected.

2,4-Di-O-methyl-D-threose

Periodate oxidation of 3,5-di-O-methyl-D-xylitol yielded
2,4-di-O-methyl-D-threose which, upon purification, yielded a
crystalline product. The pentitol was obtained from the known
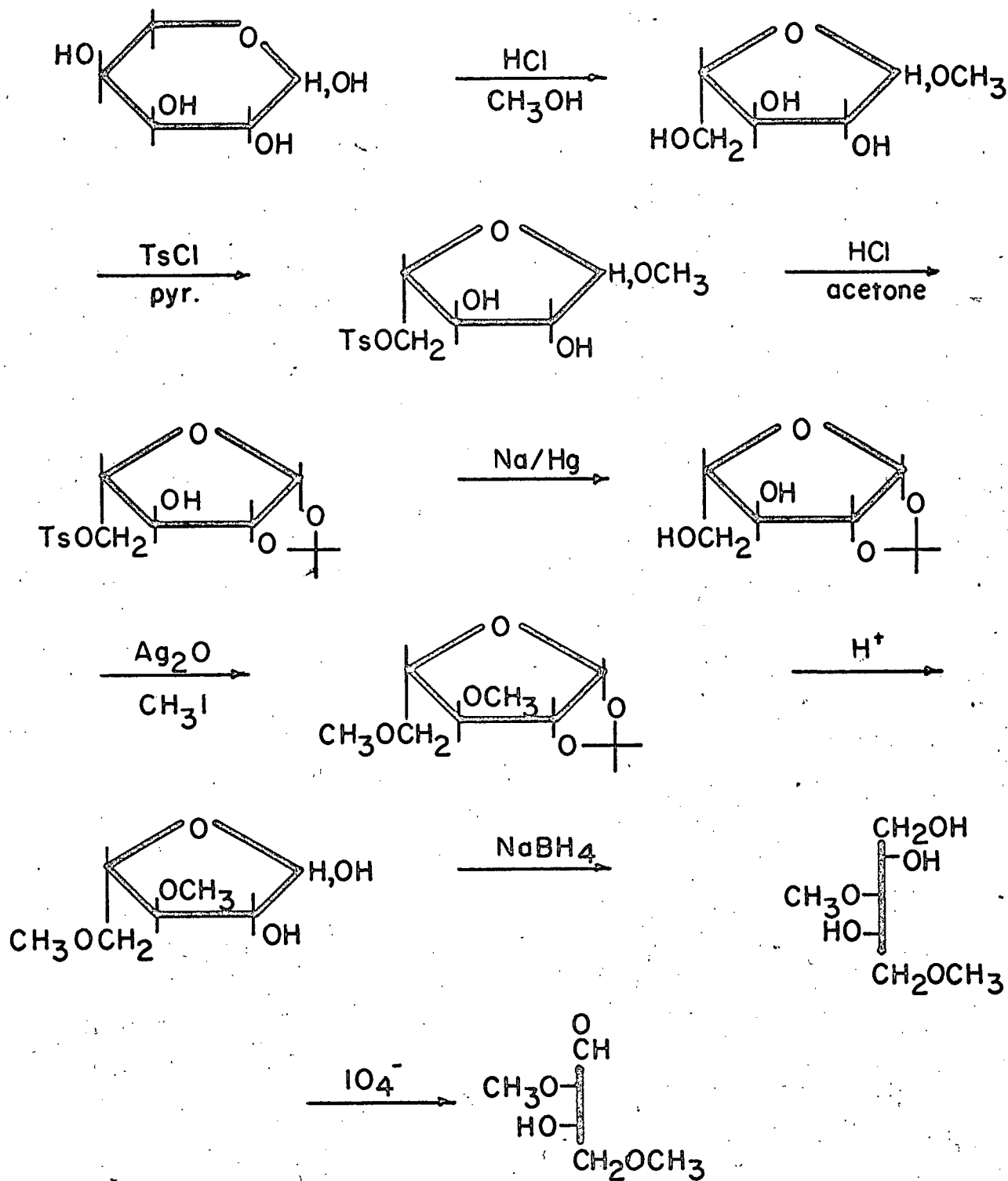3,5-di-O-methyl-D-xylose (24) by borohydride reduction.

Fig. 2

3,5-Di-O-methyl-D-xylose was prepared by the method
of Levene and Raymond (24) (Fig. 3). D-Xylose was condensed
with two molecules of acetone in the presence of concentrated
sulphuric acid to give 1,2:3,5-diisopropylidene-D-xylose.
Partial hydrolysis of the diisopropylidene compound with
dilute aqueous acid yielded 1,2-isopropylidene-D-xylo-
furanose which was recrystallized for the first time to
give the pure compound.

Methylation of 1,2-isopropylidene-D-xylofuranose by the
method of Kuhn et al (25) yielded a sirup which was purified
by vacuum distillation. Hydrolysis of the isopropylidene
group with 25% aqueous acetic acid yielded chromatographically
pure 3,5-di-O-methyl-D-xylose. Borohydride reduction followed
by periodate oxidation yielded 2,4-di-O-methyl-D-threose which
crystallized after purification by silica gel column chromatog-
raphy.

2,4-Di-O-methyl-L-threose

2,4-Di-O-methyl-L-threose was obtained from the periodate
oxidation of the borohydride reduction product of 1,4,6-tri-
O-methyl-L-sorbose. This sugar was synthesized by the method
of Schlubach and Olters (26). Condensation of L-sorbose with
acetone yielded 2,3:4,6-diisopropylidene-L-sorbose. Partial
acid hydrolysis yielded 2,3-isopropylidene-L-sorbofuranose
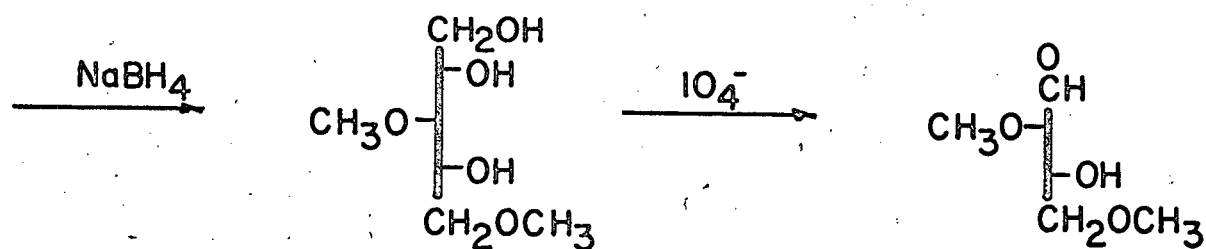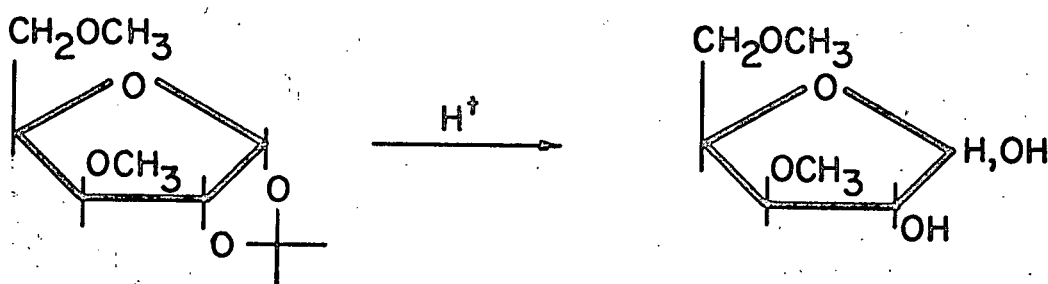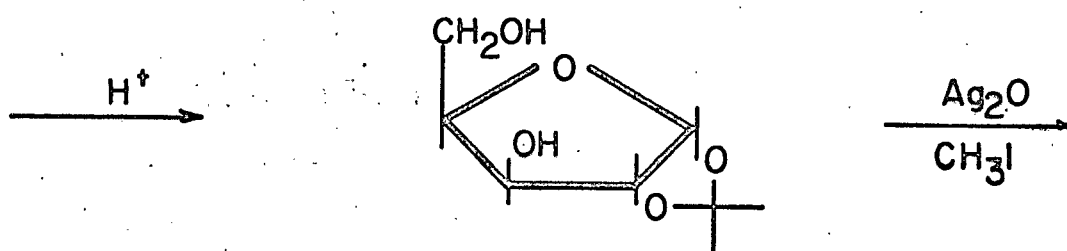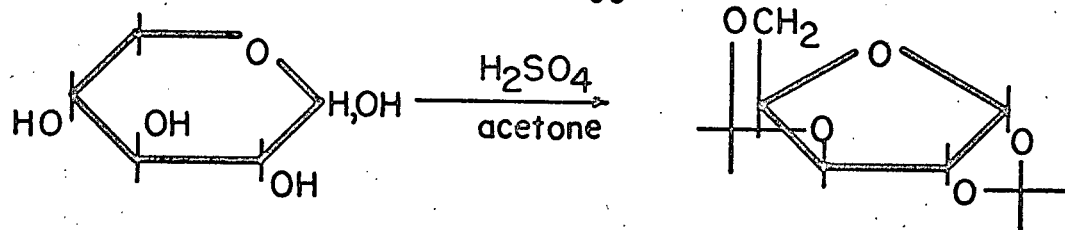which, upon methylation, yielded the 1,4,6-tri-O-methyl

Fig. 3

derivative. The trimethyl ether was obtained as a crystalline compound, but no attempt was made to recrystallize it due to its low melting point (m. p. 15-17°). Acid hydrolysis of 2,3-isopropylidene-1,4,6-tri-O-methyl-L-sorbose liberated the trimethyl ether as the free sugar.

Borohydride reduction of 1,4,6-tri-O-methyl-L-sorbose followed by periodate oxidation yielded 2,4-di-O-methyl-L-threose (Fig. 4). The sirup could not be induced to crystallize even when seeded with its optical isomer.

It is important to note that reduction of a ketose such as sorbose may yield two hexitols. In the reduction of 1,4,6-tri-O-methyl-L-sorbose, 1,4,6-tri-O-methyl-L-gulitol (or 1,3,6-tri-O-methyl-D-glucitol) and 1,4,6-tri-O-methyl-L-iditol (or 1,3,6-tri-O-methyl-L-iditol) may be formed. The configuration of the derived tetrose has no bearing on the configuration of the carbon atom at position 2, as it is eliminated in the oxidative cleavage.

Silica gel thin layer chromatography of the reduction mixture indicated only one major component, the stereochemistry of which can possibly be inferred. Bragg and Hough have shown that borohydride reduction of aldoses and ketoses proceeds through the acyclic staggered zig-zag conformation (27). 1,4,6-Tri-O-methyl-L-sorbose, when represented in this fashion
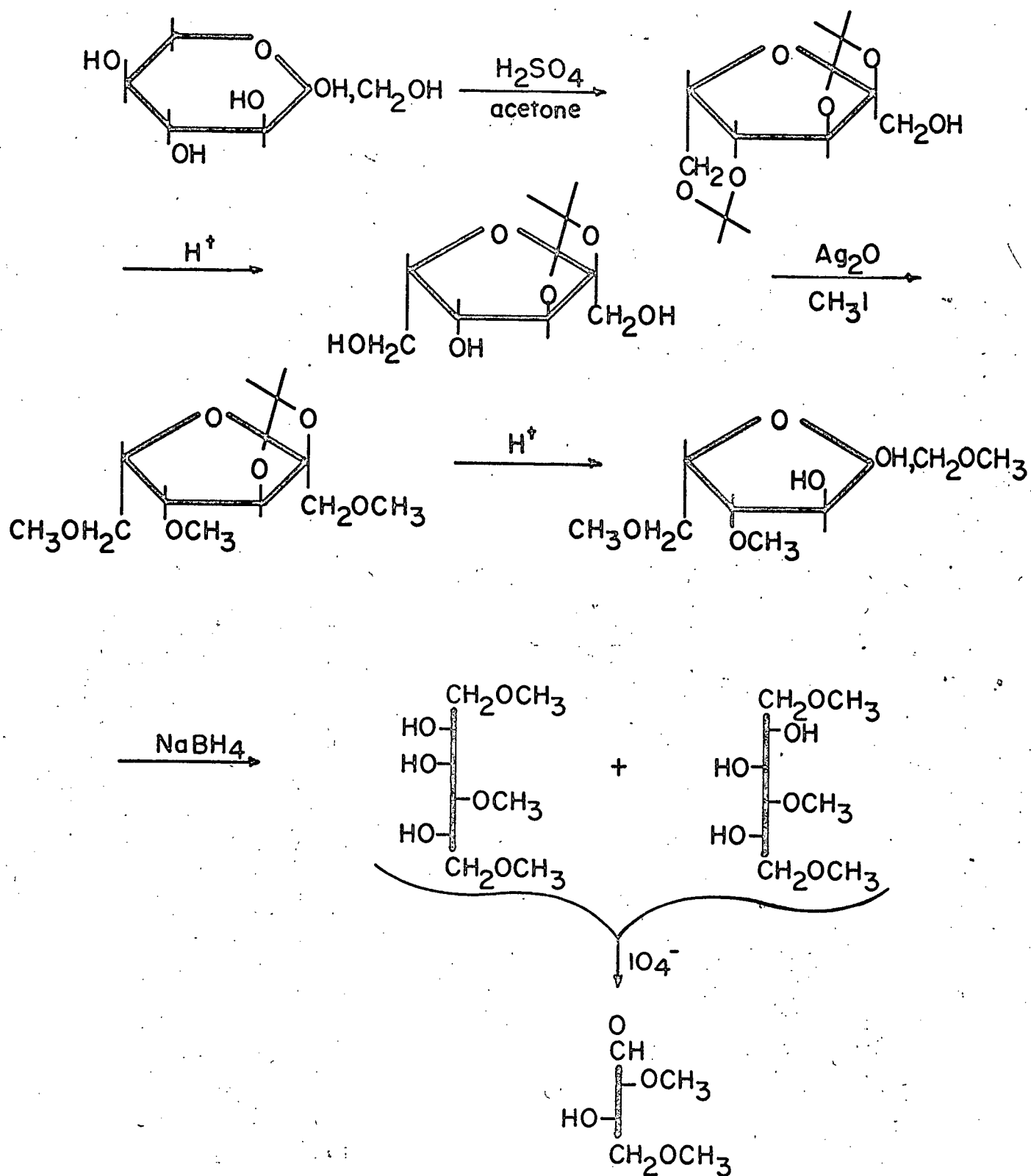
Fig. 4

(Fig. 5), has the bulky C_4 methoxyl above the plane of the paper. Approach of the borohydride will come then, predominantly, from below the plane of the paper. Such attack will leave the newly formed hydroxyl group above the plane of the paper. The derivative so formed is 1,4,6-tri-O-methyl-L-iditol as represented in Fig. 5. Since evidence of the configuration of carbon atom 2 is not easily obtained, the validity of this prediction cannot be evaluated.
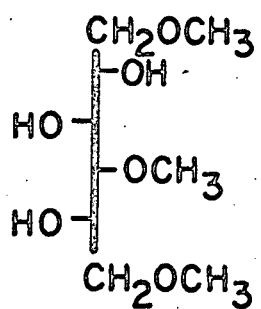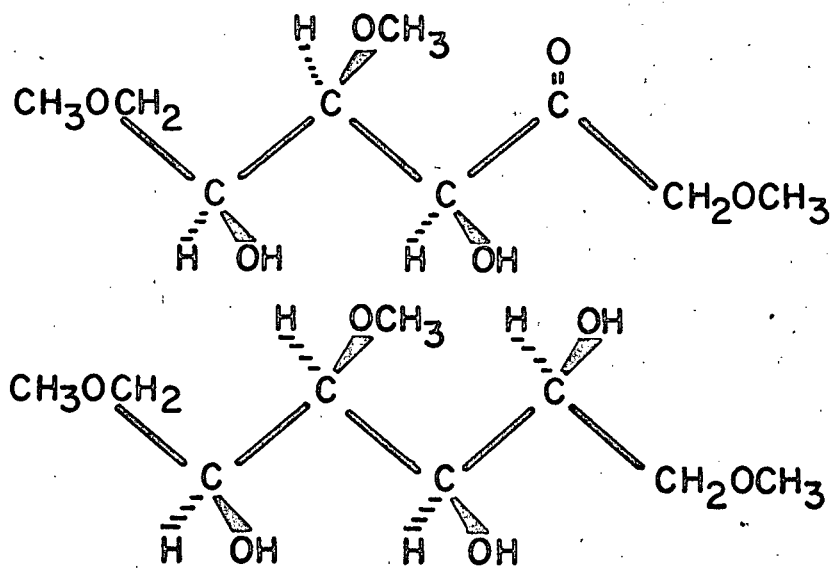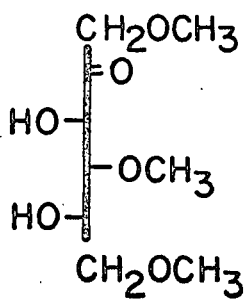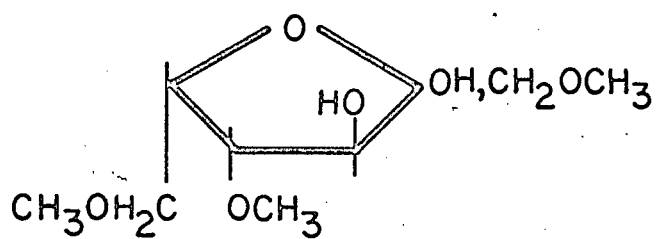
Fig. 5

DISCUSSION

Incomplete Periodate Oxidation of Reducing Sugars

Incomplete periodate oxidation of reducing sugars has been reported (1) and was substantiated in this work. The reason for this resistance to periodate oxidation is likely due to the formation of formyl esters and cyclic inner acetals. Oxidative cleavage of the C-1 carbon of an aldopyranose or aldofuranose sugar yields a formyl ester still attached to the sugar. The removal of this formyl ester must precede any cleavage that involves the esterified hydroxyl. Inner cyclic acetals have been shown to form (28) whenever a free aldehyde group is δ to a hydroxyl group, that is, the cyclic hemiacetal formed contains a six membered ring. In this way a hydroxyl group which would be expected to undergo periodate oxidation can be inactivated by the formation of such an inner acetal.

Stephen (1), circumvented the danger of formyl ester formation by reduction of the free sugar to the corresponding hexitol. In a similar manner, to obtain theoretical periodate uptake values in the synthesis of the 2,4-di-O-methyl tetroses, it was found necessary to reduce the free sugar to the alcohol prior to the glycol cleavage.

One of the most convenient methods of reducing sugars is
treatment with aqueous sodium borohydride. As discussed
earlier (see page 94) Bragg and Hough (27) have shown that
borohydride reduction is preceded by opening of the sugar
into its acyclic zig-zag conformation. In this conformation
any bulky substituent in the position β to the carbonyl
function will retard the rate of reduction. They attribute
this to the steric hindrance of approach of the borohydride
ion to the 1,3-system.

Although 3-O-substituted aldoses constitute the majority
of examples of such rate reduction, 4-O-substituted ketoses
belong to this stereochemical class and show the same effect.
This was discussed earlier in the reduction of 1,4,6-tri-O-
methyl-L-sorbose.

In the preparation of 3,5-di-O-methylpentitols from the
parent pentoses and 1,3,6-tri-O-methylhexitols from 1,4,6-
tri-O-methyl-L-sorbose, borohydride reduction was not complete
after 24 hours. If longer times were used, detectable amounts
of degradation products were formed; these were, presumably,
due to Lobry de Bruyn - van Ekenstein transformations.
Reductions were carried out overnight and subsequent separation
of the product from the unreduced material was effected by
chromatography on a silica gel column using butanone-water
azeotrope as the solvent.

Chromatography

The use of silica gel columns originated with Bell (29), who estimated the chain length of methylated polysaccharides by use of this method. Elution of the fully methylated sugars, formerly non-reducing end groups, allowed estimation of the ratio of non-reducing end group to backbone residues. With the advent of cellulose chromatography, the use of silica gel columns was neglected. In purifications, such as described in this work, silica gel column chromatography offers comparable resolution in approximately one-fourth of the time. It should be noted that thin layer chromatography is a fast, efficient method of analyzing methylated sugar fractions (30) obtained from either cellulose or silica gel columns.

Because the importance of partially methylated tetroses lies in their chromatographic recognition, R_f and R_G values were recorded in a large number of solvent systems (Table 1).

TABLE I

Physical properties of 2,4-di-O-methyl tetroses

	2,4-Di-O-methyl D-erythrose	2,4-Di-O-methyl L-erythrose	2,4-Di-O-methyl D-threose	2,4-Di-O-methyl L-threose
Melting point	Sirup	Sirup	114–116° (dimer?)	Sirup
$[\alpha]_D$ of free sugar	60.1° (c, 1.4 in MeOH)	−61.4° (c, 4.9 in MeOH)	−14.8° (c, 1.17 in MeOH) −9° (3 min) → + 0.9° (15 min) (c, 0.35 in 1 N H$_2$SO$_4$)	−14.3° (c, 5.7 in MeOH) −17° (5 min) → −3° (15 min) (c, 0.55 in 1 N H$_2$SO$_4$)
2,4-Dinitrophenyl-hydrazone melting point	105–106°	107–108°	148–149°	149–150°
Solvent system[1]				
Butanone Water Azeotrope	R_F 0.70, 0.64 R_G 0.86, 0.78	Silica gel T. L. C. R_F 0.62, 0.50	R_F 0.66, 0.57 R_G 0.89, 0.76	Silica gel T. L. C. R_F 0.65, 0.50
Ethyl acetate Pyridine Water (8:2:1)	R_F 0.77 R_G 0.92	-	R_F 0.81 R_G 0.96	
Butan-1-ol Ethanol Water (4:1:5) upper layer	R_F 0.82 R_G 0.96		R_F 0.84 R_G 0.08	
Ethyl acetate Acetic acid Formic acid Water (18:3:1:4)	R_F 0.80, 0.75 R_G 0.95, 0.88		R_F 0.86, 0.75 R_G 1.02, 0.88	

[1]Descending paper chromatography was carried out using Whatman No. 1 paper and R_G values are relative to 2,3,4,6 tetra-O-methyl-D-glucose. Sugars were detected by use of p-anisidine trichloracetate spray reagent. Optical isomers showed identical chromatographic patterns in all solvents. R_F and R_G values quoted for D-threose refer to the sirup before crystallization. The crystalline modification gave only the slower running component.

Ascending silica gel thin-layer chromatography was adopted for rapid examination of column fractions. Detection was achieved by use of 5% HNO$_3$ in H$_2$SO$_4$. Subsequent heating at 150° was sufficient for development.

Anomalous Behaviour of Optical Isomers

Paper chromatography of the tetroses in some solvents and chromatography on a silica gel thin-layer system indicated the existence of two modifications of the tetroses, which were inseparable by silica gel column chromatography. Upon standing for extended lengths of time (3 months), sirupy tetroses were observed to become more viscous. Infrared spectra of the viscous sirup showed reduction in the carbonyl stretching band at 1750 cm^{-1} and enhancement of the hydroxyl band at 3400 cm^{-1}, with respect to the more mobile sirup. The infrared spectrum of the crystalline 2,4-di-O-methyl-D-threose showed no carbonyl stretching band but contained a hydroxyl band which was split into two equal-intensity absorptions at 3375 and 3480 cm^{-1}. The anomalous optical rotation of the threose isomers (D, -14.8°; L, -14.3°) in methanol is attributed to the existence of two or more modifications present in the solution of the sirupy L-threose compound. Upon solution of the isomers in 1 $\underline{N}$ sulphuric acid, the rotations dropped to near-zero values with opposite signs. These results suggest that 2,4-di-O-methyltetroses easily undergo dimerization to a compound of structure similar to that from 5-aldo-1,2-O-isopropylidene-D-xylopentofuranose (31) (Fig. 6). The structure of the crystalline modification

of 2,4-di-O-methyl-D-threose may therefore be of a cyclic

acetal nature (Fig. 7). Thin layer chromatography of

the tetroses isolated as sirups contained appreciable

quantities of modifications of this type. Because of

the small amounts of product, molecular weight and nuclear

magnetic resonance studies were not undertaken.
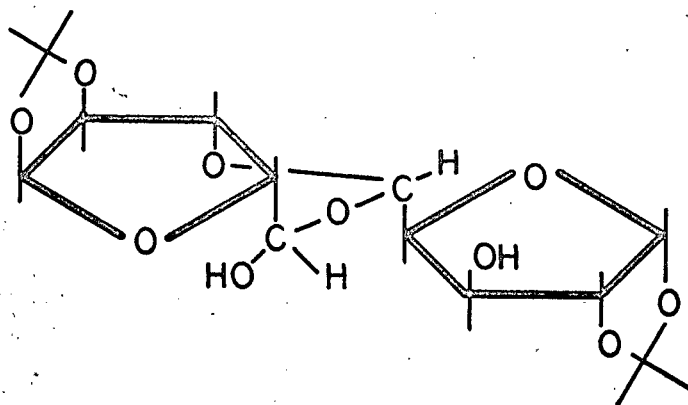
Fig. 6
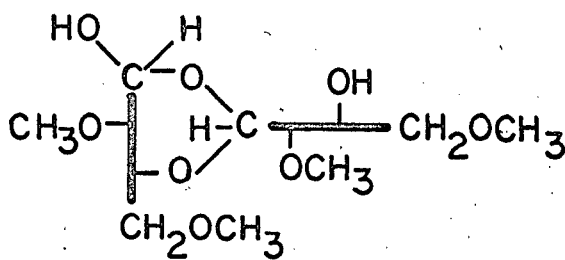
Fig. 7

Derivatives

Attempts to prepare crystalline derivatives of the glycitols were unsuccessful. Although some acetates and p-nitrobenzoates were prepared and shown to be homogeneous by thin-layer chromatography (32), crystallization could not be induced.

Acceptable analytical values were seldom obtained for the sirupy di-O-methyltetroses presumably because of occluded solvent. No attempt was made to distil them because of the small amounts obtained and their volatility. Several different reagents were tried for characterizing the tetroses but only 2,4-dinitrophenylhydrazine gave satisfactory crystalline derivatives in all four cases. This reagent was used in neutral solution to give the phenylhydrazone rather than the osazone. In retrospect, the difficulty of making crystalline derivatives may be due to the equilibrium with a dimeric form.

EXPERIMENTAL

Evaporations were carried out under reduced pressure at a bath temperature of 40-45°. Optical rotations are equilibrium values measured on either a Bendix ETL-NPL Automatic Polarimeter (Type 143 A) or a Rudolph Polarimeter (Model 219) at 21 $\pm$ 2°. Melting points quoted are uncorrected. Periodate oxidation estimations were carried out by quenching oxidation aliquots in buffered arsenite and back-titrating with iodine. Solvent system A: butanone - water azeotrope.

Preparation of the Silica Gel Column

To silica gel (Fisher S-157, 200 g) that had been screened to pass through 60 mesh was added butanone - water azeotrope (500 ml) with stirring. The slurry was allowed to sit for 1 hour with occasional stirring. The mixture was then slurried into a column (3 x 36 cm), fitted with a fritted disk, and the silica was packed tightly by tapping the outside of the column until no further settling was apparent. A 1 cm layer of sand was carefully placed on top of the silica gel.

The column was equilibrated by passing butanone - water azeotrope through the column for two days. At the end of this time the flow rate was constant at 1.8 ml per minute.

The movement of the solvent can be approximated with Sudan IV dye which shows an R_f of about 0.95 on thin layer

plates. Using this as a marker the front time for the column was about 1 hour.

Fractions from the column were analyzed by running samples on silica gel thin layer plates using the same solvent system as that used on the column.

Synthesis of 2,4-Di-O-methyl-D-erythrose

4,6-Di-O-methyl-D-glucose

4,6-Di-O-methyl-D-glucose was prepared by the method of Bell and Lorber (17). After recrystallization from ethyl acetate the constants were: m.p. 154-156°. $[\alpha]_D$ 116° $\longrightarrow$ 70.5° (c, 1.23 in H_2O). Lit., (17), m.p. 156-157; $[\alpha]_D$ 108° $\longrightarrow$ 65.7° (c, 4 in H_2O).

4,6-Di-O-methyl-D-glucitol (1,3-Di-O-methyl-L-gulitol)

4,6-Di-O-methyl-D-glucose (2.7 g) was dissolved in water (50 ml) and sodium borohydride (0.5 g) was added. The solution was neutralized with acetic acid after 18 hours, and evaporated to dryness. The resulting solid was treated with 3% HCl in methanol (3 x 15 ml) and evaporated to dryness. The residue was dissolved in water (25 ml) and the solution de-ionized with Amberlite IR-120 (H^+) and Duolite A-4 (OH^-). Evaporation of the resulting solution gave a non-reducing sirup (2.6 g), $[\alpha]_D$ 7° (c, 1.5 in $CHCl_3$).

4,6-Di-O-methyl-D-glucitol Phenylurethan

To 4,6-di-O-methyl-D-glucitol (100 mg) in pyridine (1 ml)
was added phenylisocyanate (0.3 ml) and the solution was
heated on a steam bath for 3 hours. Anhydrous methanol (1 ml)
was added and heating continued for a further 15 minutes.
The cooled solution was poured dropwise into cold water (25 ml)
and the precipitate filtered. Recyrstallization from ethanol-
acetone gave a product melting at 195-199°. By recrystal-
lization from ethyl acetate - petroleum ether (30-60°)
the melting point could be raised to 203-205°. The mixed
melting point with diphenylurea (m.p. 238°) was depressed but
nitrogen analyses were always high. Calculated for $C_{36}H_{38}O_{10}N_4$:
N, 8.16%. Found: N, 9.57, 9.61%.

2,4,Di-O-methyl-D-erythrose

4,6-Di-O-methyl-D-glucitol (524 mg, 2.5 mmoles) in water
(25 ml) was added to 0.12 $\underline{M}$ sodium periodate (50 ml, 6 mmoles).
The oxidation was complete in 25 minutes with an uptake of
2.03 moles of periodate per mole of hexitol. The solution
was neutralized with washed barium carbonate, and diluted with an
equal volume of methanol, filtered and evaporated to give
a mobile sirup which was purified by chromatography on a
silica gel column to give 2,4-di-O-methyl-D-erythrose
(297 mg) $[\alpha]_D$ 60.1° ($\underline{c}$, 1.4 in MeOH).

2,4-Dinitrophenylhydrazone of 2,4-Di-O-methyl-D-erythrose

2,4-Di-O-methyl-D-erythrose (100 mg) was dissolved in 100% ethanol (3 ml) and recrystallized 2,4-dinitrophenylhydrazine (110 mg) was added. The mixture was refluxed on a steam bath for 5 hours, evaporated to dryness, dissolved in ethyl acetate (10 ml) and petroleum ether (30-60°) (10 ml) was added to precipitate excess reagent. The mixture was filtered after 10 minutes, the filtrate evaporated to a sirup, dissolved in chloroform (2 ml) and applied to an alumina column (3 x 20 cm). Collection of the light yellow band eluted with chloroform afforded yellow-orange crystals melting at 102-103°. Subsequent recrystallization from ethyl acetate - petroleum ether (30-60°) gave crystals melting at 105-106°. Calculated for $C_{12}H_{16}O_7N_4$: N, 17.07; OCH_3, 18.90%. Found: N, 17.04; OCH_3, 19.23%.

Synthesis of 2,4-Di-O-methyl-L-erythrose

3,5-Di-O-methyl-L-arabinose

3,5-Di-O-methyl-L-arabinose was obtained by the procedure of Hirst, Jones and Williams (20). The sirup showed only one component (R_f 0.59) upon paper chromatography in solvent system A and had $[\alpha]_D$ -36° (c, 6.7 in MeOH). Lit. (20), $[\alpha]_D$ -39° (25% aqueous acetic acid).

3,5-Di-O-methyl-L-arabinonolactone

3,5-Di-O-methyl-L-arabinose (75 mg) in water (4 ml) was
treated with bromine (4 drops) and the mixture left at room
temperature overnight. Aeration and treatment with silver
carbonate followed by filtration gave a solution which was
passed through IR-120 (H^+), and evaporated to a sirup.
Sublimation at 65-70° (0.05 mm) gave crystals of 3,5-di-O-
methyl-L-arabinonolactone, m.p. 72-73°. Lit. (20), m.p. 73°.

3,5-Di-O-methyl-L-arabinonamide

3,5-Di-O-methyl-L-arabinonolactone (6 mg) was dissolved
in methanol saturated with ammonia (1 ml) and the solution
was left overnight at room temperature. The solution was
evaporated to give crystals, which after recrystallization
from acetone, melted at 144°. Lit. (20), m.p. 144°.

3,5-Di-O-methyl-L-arabinitol (1,3-Di-O-methyl-L-lyxitol)

3,5-Di-O-methyl-L-arabinose (1.7 g) dissolved in water
(30 ml) was treated with excess sodium borohydride (1 g) and
left at room temperature overnight. The solution was worked
up in the same manner as described for 4,6-di-O-methyl-D-
glucitol. The sirup containing 3,5-di-O-methyl-L-arabinitol
and 3,5-di-O-methyl-L-arabinose (c.a. 9:1) was separated
completely on the silica gel column. The arabinose (R_f 0.56 on

T.L.C. same solvent system) was found in the eluate, 175-300 mls after the dye marking the front. The arabinitol (R_f 0.40) was found in the 675-1000 ml fraction. Evaporation of the fraction containing arabinitol yielded a non-reducing sirup (1.4 g) $[\alpha]_D$ -7° (c, 5.8 in $CHCl_3$).

2,4-Di-O-methyl-L-erythrose

3,5-Di-O-methyl-L-arabinitol (527 mg, 2.93 mmoles) in water (200 ml) was treated with 0.08 M sodium periodate (50 ml, 4.0 mmoles). The oxidation was complete in 10 minutes and was worked up as described for its optical isomer. The final periodate uptake was 1.04 moles per mole of pentitol. Purification on silica gel gave a colourless sirup (365 mg) $[\alpha]_D$ -61.4° (c, 4.85 in MeOH).

2,4-Dinitrophenylhydrazone of 2,4-Di-O-methyl-L-erythrose

The hydrazone was prepared using the same quantities as that for its optical isomer, giving crystals, which upon recrystallization from ethyl acetate - petroleum ether (30-60°), melted at 107-108°. Calculated for $C_{12}H_{16}O_7N_4$: N, 17.07; OCH_3, 18.90%. Found: N, 16.97; OCH_3, 19.08%.

Synthesis of 2,4-Di-O-methyl-D-threose

1,2-Isopropylidene-D-xylofuranose

1,2-Isopropylidene-D-xylofuranose was prepared by the acid hydrolysis of 1,2:3,5-diisopropylidene-D-xylose. The product was recrystallized from ethyl acetate - petroleum ether (30-60°) (1:4) m.p. 67-69°. $[\alpha]_D$ -21.9° ($\underline{c}$, 3.15 in H_2O). Lit. (33), m.p. 41-43° $[\alpha]_D$ -19.0° ($\underline{c}$, 2 in H_2O). Calculated for $C_9H_{14}O_5$: C, 50.52; H, 7.37%. Found: C, 50.68; H, 7.65%.

3,5-Di-O-methyl-1,2-isopropylidene-D-xylose

1,2-Isopropylidene-D-xylofuranose was methylated by the method of Kuhn et al (25). The compound was isolated as a sirup, which was distilled under vacuum, collecting the fraction boiling at 80-82° (0.05 mm). The mobile liquid had a specific rotation $[\alpha]_D$ -57.3° ($\underline{c}$, 5.2 in $CHCl_3$). Lit. (34), $[\alpha]_D$ -60° ($\underline{c}$, 1.09 in $CHCl_3$).

3,5-Di-O-methyl-D-xylose

3,5-Di-O-methyl-1,2-isopropylidene-D-xylose (5 g) was dissolved in 25% aqueous acetic acid and heated on a steam bath for 5 hours after which time the optical rotation was constant. After evaporation a yellow sirup (2.1 g) remained which showed only one component ($\underline{R_f}$ 0.57) upon paper

chromatography in solvent system A, $[\alpha]_D$ 23.5° (c, 3.9 in H_2O). Lit. (34), $[\alpha]_D$ 25° (c, 1.13 in H_2O).

p-Bromophenylosazone of 3,5-Di-O-methyl-D-xylose

The osazone was prepared by the method of Applegarth, Dutton and Tanaka (35). 3,5-Di-O-methyl-D-xylose (75 mg) and p-bromophenylhydrazine (280 mg) were dissolved in glacial acetic acid (4.3 ml). Water (2.2 ml) was added and the solution heated 6 minutes on a steam bath and allowed to cool. After 2 hours at 5° the crystals were filtered and recrystallized from ethyl acetate - petroleum ether (30-60°) (1:7) m.p. 106.5-107.5°. Lit. (24), m.p. 107-108°.

3,5-Di-O-methyl-D-xylitol (1,3-Di-O-methyl-L-xylitol)

3,5-Di-O-methyl-D-xylose (390 mg) dissolved in water (15 ml) was treated with excess sodium borohydride (200 mg). The solution was left at room temperature overnight, and worked up in the manner described for the arabinitol. The sirup (330 mg) obtained showed no reducing properties $[\alpha]_D$ -5.4° (c, 6.6 in $CHCl_3$).

2,4-Di-O-methyl-D-threose

3,5-Di-O-methyl-D-xylitol (212 mg, 1.17 mmoles) in water (40 ml) was treated with 0.203 M periodic acid (10 ml, 2.03 mmoles). The oxidation was complete in 10 minutes and the

final periodate uptake amounted to 0.91 moles per mole of pentitol. Work up was identical to that described earlier for the erythroses to give a mobile sirup (124 mg). Purification of this material on a silica gel column yielded crystalline (dimeric?) 2,4-di-O-methyl-D-threose. Recrystallization from ethyl acetate - petroleum ether (30-60°) (1:5) gave crystals melting at 114-116°. $[\alpha]_D$ -14.8° (c, 1.17 in MeOH) showing no mutarotation after 24 hours. $[\alpha]_D$ -9° (3 min) $\longrightarrow$ 0.9° (15 min) (c, 0.35 in 1 N H$_2$SO$_4$). Calculated for C$_6$H$_{12}$O$_4$: C, 48.64; H, 8.11; OCH$_3$, 41.89%. Found: C, 48.78; H, 8.22; OCH$_3$, 41.51%.

2,4-Dinitrophenylhydrazone of 2,4-Di-O-methyl-D-threose

The hydrazone was prepared in the same manner and using the same quantities as for the 2,4-di-O-methyl erythroses. The derivative after recrystallization from ethyl acetate - petroleum ether (30-60°) melted at 148-149°. Calculated for C$_{12}$H$_{16}$O$_7$N$_4$: N, 17.07; OCH$_3$, 18.90%. Found: N, 17.26; OCH$_3$, 18.43%.

Synthesis of 2,4-Di-O-methyl-L-threose

2,3-Isopropylidene-1,4,6-tri-O-methyl-L-sorbose

2,3-Isopropylidene-1,4,6-tri-O-methyl-L-sorbose was prepared by the method of Schlubach and Olters (26). Vacuum distillation

(0.05 mm) gave a fraction boiling at 105-115°. The sirup
crystallized upon being kept in a freezer (-10°) for six
months, m.p. 15-17°. No attempt was made to recrystallize
this material. $[\alpha]_D$ 34.2° (c, 4.05 in CHCl$_3$). Lit. (26),
$[\alpha]_D$ 29.6° (c, 1.0 in CHCl$_3$).

1,4,6-Tri-O-methyl-L-sorbose

1,4,6-Tri-O-methyl-L-sorbose was prepared by mild acid
hydrolysis of 2,3-isopropylidene-1,4,6-tri-O-methyl-L-sorbose
(26). The sirup showed only one component (R_f 0.72 upon
paper chromatography in solvent system A.) $[\alpha]_D$ -1.85°⟶
3.16° (c, 7.2 in CHCl$_3$). Lit. (26), $[\alpha]_D$ 3.8° (c, 1.5
in CHCl$_3$).

1,4,6-Tri-O-methyl-hexitol (1,3,6-Tri-O-methyl-D-glucitol
     or 1,3,6-Tri-O-methyl-L-iditol)

1,4,6-Tri-O-methyl-L-sorbose (520 mg) in water (15 ml)
was treated with excess sodium borohydride (1 g) and left
overnight at room temperature. The methylated hexitols were
isolated in the same manner as previously described. The
resulting sirup showed only one major component (R_f 0.45) on
silica gel thin layer chromatography using solvent system A.
This component was isolated by chromatography on a silica
gel column to give a sirup (280 mg) having a rotation $[\alpha]_D$
5.6° (c, 1.5 in CHCl$_3$).

2,4-Di-O-methyl-L-threose

To the tri-O-methyl hexitol (650 mg, 2.9 mmoles) dissolved in water (5 ml), 0.2 $\underline{M}$ sodium periodate (20 ml, 4.0 mmoles) was added. The oxidation was complete in 5 minutes with an uptake of 0.98 moles of periodate per mole of hexitol. Recovery of the tetrose in the manner described above and subsequent purification on a silica gel column yielded a colourless sirup (370 mg) $[\alpha]_D$ -14.3° ($\underline{c}$, 5.7 in MeOH). $[\alpha]_D$ -17° (5 min) $\longrightarrow$ -3° (15 min) ($\underline{c}$, 0.55 in 1 $\underline{N}$ H_2SO_4).

2,4-Dinitrophenylhydrazone of 2,4-Di-O-methyl-L-threose

The hydrazone was prepared using the same quantities as that of its optical isomer, giving the derivative, which upon recrystallization from ethyl acetate – petroleum ether (30-60°) melted at 149-150°. Calculated for $C_{12}H_{16}O_7N_4$: N, 17.07; OCH_3, 18.90%. Found: N, 16.87; OCH_3, 19.16%.

BIBLIOGRAPHY

1. A. M. Stephen. J. Chem. Soc. 2030 (1962).

2. G. G. S. Dutton and A. M. Unrau. Can. J. Chem. 41, 2439 (1963).

3. H. Kiliani. Ber. 18, 3066 (1885).

4. J. C. Sowden and H. O. L. Fischer. J. Am. Chem. Soc. 69, 1963 (1947).

5. J. C. Sowden. Advances in Carbohydrate Chem. 6, 291 (1951).

6. A. Wohl. Ber. 26, 730 (1893).

7. D. L. MacDonald and H. O. L. Fischer. J. Am. Chem. Soc. 74, 2087 (1952).

8. L. Malaprade. Bull. Soc. chim. (France) 43, 683 (1928).

9. J. M. Bobbitt. Advances in Carbohydrate Chem. 11, 1 (1956).

10. A. S. Perlin. Advances in Carbohydrate Chem. 14, 9 (1959).

11. G. W. Huffman, B. A. Lewis, F. Smith and D. R. Spriestersbach. J. Am. Chem. Soc. 77, 4346 (1955).

12. I. J. Goldstein, H. Sorger-Domenigg and F. Smith. J. Am. Chem. Soc. 81, 444 (1959).

13. K. Gatzi. Helv. Chim. Acta. 21, 195 (1938).

14. K. E. Pierre. M.Sc. Thesis. University of British Columbia. (1962).

15. S. C. Ho. B.Sc. Thesis. University of British Columbia. (1959).

16. A. E. Barlay. M.Sc. Thesis. University of British Columbia. (1961).

17. D. J. Bell and J. Lorber. J. Chem. Soc. 453 (1940).

18. J. C. Dennison and D. I. McGilvray. J. Chem. Soc. 1616 (1951).

19. G. Zemplen and Z. Bruckner. Ber. 64, 1852 (1931) as quoted in: W. L. Evans, D. D. Reynolds and E. A. Talley. Advances in Carbohydrate Chem. 6, 27 (1951).

20. E. L. Hirst, J. K. N. Jones and E. Williams. J. Chem. Soc. 1062 (1947).

21. J. I. Cunneen and F. Smith. J. Chem. Soc. 1146 (1948).

22. E. V. White. J. Am. Chem. Soc. 68, 272 (1946).

23. S. A. Black and G. G. S. Dutton. Unpublished results.

24. P. A. Levene and A. L. Raymond. J. Biol. Chem. 102, 331 (1933).

25. R. Kuhn, I. Low and N. Trischman. Ber. 90, 203 (1957).

26. H. H. Schlubach and P. Olters. Ann. 550, 140 (1942).

27. P. D. Bragg and L. Hough. J. Chem. Soc. 4347 (1957).

28. M. Guernet, A. Jurado-Soler and P. Malangeau. Bull. Soc. chim. (France) 1183 (1963).

29. D. J. Bell. J. Chem. Soc. 473 (1944).

30. G. W. Hay, B. A. Lewis and F. Smith. J. Chromatog. 11, 479 (1963).

31. R. Schaffer and H. S. Isbell. J. Am. Chem. Soc. 79, 3864 (1957).

32. M. E. Tate and C. T. Bishop. Can. J. Chem. 40, 1043 (1962).

33. O. Svanberg and K. Sjoberg. Ber. 56, 863 (1923).

34. R. A. Laidlaw. J. Chem. Soc. 2941 (1952).

35. D. A. Applegarth, G. G. S. Dutton and Y. Tanaka. Can. J. Chem. 40, 2177 (1962).