SYNTHESIS OF A SERIES OF DISACCHARIDES
OF POTENTIAL IMMUNOLOGICAL SIGNIFICANCE

By

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Date May 2, 1974
ABSTRACT

Disaccharides are useful for immunological studies, the identification of isolated polymer fragments, and studies on model compounds but few have been synthesized. The syntheses of four new disaccharides of the 4-O-glycopyranosyl-L-rhamnopyranose series namely 4-O-β-D-glucopyranosyl-L-rhamnopyranose (15), 4-O-α-L-rhamnopyranosyl-L-rhamnopyranose (26), 4-O-α-D-mannopyranosyl-L-rhamnopyranose (38), and 4-O-β-D-mannopyranosyl-L-rhamnopyranose (56) are reported.

The selectively substituted aglycon used for all the members of this series was methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (3) prepared by acetalation of methyl α-L-rhamnopyranoside and purified through crystalline methyl 4-O-acetyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (2).

Compound 2 was deacetalated to give methyl 4-O-acetyl-α-L-rhamnopyranoside which served as a key intermediate for the preparation of the standards 2,3-di-O-methyl-L-rhamnose and 4-deoxy-L-erythritol.

Disaccharide 15 was prepared in an overall yield of 55% based on the aglycon by first condensing 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide with 3 in the presence of mercuric cyanide in acetonitrile to give crystalline methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (10) in 80% yield based on 3. Since the isopropylidene group of 10 could not be acetolyzed it was removed with trifluoroacetic acid to give methyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (11). Acetolysis of 11 gave crystalline 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranose (14). Deacetylation of 11 gave the methyl glycoside of the
disaccharide which, as for the subsequent disaccharides, was subjected to methylation and periodate oxidation studies to confirm the 1,4 linkage. An improved method for the quantitation of periodate oxidation degradation products is described. Deacetylation of 14 gave 15 which was reduced to give 4-O-β-D-glucopyranosyl-L-rhamnitol which upon acetylation gave crystalline 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-rhamnitol.

In an analogous manner disaccharides 26 and 38 were prepared in overall yields of 60 and 55% based on the aglycon by condensing 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide and 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl bromide respectively with 3. The deblocking procedures paralleled those of the D-gluco analog. Disaccharide 26 was characterized as crystalline methyl 2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside, 1,2,3-tri-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranose, and 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-L-rhamnitol. Disaccharide 38 was crystalline and was also characterized as crystalline 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranose and 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-L-rhamnitol. A combined reduction acetylation procedure for the 2,3-di-O-methyl-L-rhamnose obtained from methylated 26 produced a boron-containing derivative of 2,3-di-O-methyl-L-rhamnitol. Proton magnetic resonance spectroscopy was used to substantiate the configuration of the linkage in these disaccharides.

A general method for the synthesis of β-D-mannopyranosides was developed to synthesize disaccharide 56. This was done by preparing 4,6-
-di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl bromide (44) through sequential formation of the carbonate, acetolysis, and bromination of methyl 4,6-O-benzylidene-α-D-mannopyranoside. Bromide 44 was condensed with methanol to give crystalline methyl 4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranoside (45) in 87% yield. Deacylation of 45 gave methyl β-D-mannopyranoside which was characterized as its crystalline isopropylate and peracetate. Disaccharide 56 was prepared in an overall yield of 50% based on the aglycon by condensing 44 with 3 in the presence of silver oxide in chloroform. The deblocking procedures were similar to those for the previous disaccharides. Acetolysis of the disaccharide intermediates in the sequence was profoundly influenced by the presence of the cyclic carbonate group. Disaccharide 56 was characterized as crystalline methyl 4-O-β-D-mannopyranosyl-α-L-rhamnopyranoside isopropylate and 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose.
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1. General

Sugars along with amino acids, nucleotides, and lipids comprise the basic building blocks of nature. As do the other building blocks, sugars occur in nature largely as polymers. These polymers are named according to the number of sugar units, i.e. monosaccharides, that they contain. For example polymers containing two, three, four, two to ten, and more than ten sugar units are called di-, tri-, tetra-, oligo-, and polysaccharides respectively. Many naturally occurring polymers are composed of more than one kind of building block such as glycoproteins, glycolipids, and lipopolysaccharides. Polysaccharides and oligosaccharides are widely distributed in all living organisms and serve four main functions: as food reserves, illustrated by starch, glycogen, and galactomannans; as structural components, exemplified by cellulose, hemicelluloses, and chitin; as protective coatings, illustrated by plant gums and bacterial capsules, and as determinants of specificity, demonstrated by blood group substances, microbial antigens and endotoxins, enzyme regulators, and gangliosides.

Most sugars can be described as polysubstituted tetrahydropyran rings where the substituents are hydroxyl and hydroxymethyl groups and the numbering is as shown below.
Obviously many diastereoisomers are possible and this leads to the specific sugars such as glucose, galactose, and mannose. Optical isomerism also gives rise to the enantiomeric D and L series where C-6 is above the plane of the ring in the D-series and below it in the L-series in most cases. The configuration at C-1 (the anomeric carbon) is designated α when the substituent is on the opposite side of the ring to C-6 and β when it is on the same side of the ring as C-6. These multifunctional compounds contain hemi-acetal and secondary and primary hydroxyl groups. Other functional variations such as 6-deoxy, 2-amino, and 6-carboxyl are common and lead to sugars such as rhamnose, glucosamine, and glucuronic acid.

In polysaccharides all linkages between sugar units are formed from the loss of a molecule of water between C-1 of one sugar and any other hydroxyl on the other sugar, giving rise to a series of acetals. Although there are many combinations possible, natural polysaccharides tend to be repetitive rather than random in both their constituent sugars and linkages. Thus cellulose is composed of β-D-glucose residues linked through C-4. Branching is possible when more than one unit is joined through C-1 to the same sugar residue. Also several kinds of sugar residues can combine to form heteropolymers. In both these cases there is still a high degree of uniformity in that the majority of the branches are attached to a specific position of a certain sugar and the sugars in a heteropolymer are often arranged in a definite order to form repeating units. Again each sugar has a specific configuration and point of attachment. Fragmentation of such polymers, therefore, gives distinct oligosaccharides. This holds true even though polysaccharides do not exist as discrete macromolecules all of the same molecular weight but rather as a homologous series of polymers with a distribution of molecular weights.
about a mean value i.e. they are polydispersed.

2. Rationale for Disaccharide Synthesis

Since their discovery man has been interested in determining the structure of these carbohydrate polymers. Synthetic oligosaccharides of unambiguous structure are invaluable in the structural elucidation of isolated polymer fragments. An early example of this was the acetolysis of cellulose to give cellobiose octaacetate which established the configuration and the linkage between the glucose units in cellulose to be \( \beta\-D-1,4 \). Because synthetic oligosaccharides are often available in reasonable quantities several derivatives, each with distinctive physical properties, may be prepared. This provides a variety of authentic standards from which the one or ones most applicable to a small amount of a particular isolated fragment can be chosen. Synthetic oligosaccharides not only provide important physical constants but also the defined model systems required for the development of new and/or improved methods of structural analysis. Products or results from degradative techniques, such as methylation and periodate oxidation, that cannot be predicted or explained by known mechanisms or reactions can only be discovered and investigated by subjecting compounds of known structure to the same degradative procedures. The ever-increasing use of instrumental techniques, utilizing physical measurements, such as nuclear magnetic resonance, mass spectrometry, and circular dichroism to elucidate structures requires a reservoir of data on compounds of known structure in order to establish the distinguishing characteristics of each structural feature.
The other area of interest to man is the relationship between the chemical structure of these polymers and their biological function. Again, synthetic oligosaccharides of unambiguous structure play an important role. Antigenic polysaccharides contain specific oligosaccharide units which combine with the antibody in the immunological reaction and thus are called immunodominant sites, antigenic determinants, determinants of immunological specificity, or haptens. The structural composition of the determinant of immunological specificity can be determined by hapten inhibition\textsuperscript{13,14,15} or complement fixation inhibition\textsuperscript{16} studies using oligosaccharides of known structure. The binding site and the site of action of enzymes on polysaccharides which are substrates can be determined by inhibition studies or by varying the substrate using oligosaccharides of defined composition\textsuperscript{17}. Specifically labelled radioactive oligosaccharides made available by synthesis\textsuperscript{18,19} can be useful in the elucidation of biosynthetic pathways when used in feeding experiments. Since synthetic antibiotics with minor structural variations can be used to study the structural requirements for activity and since some antibiotics contain sugars it is necessary to have available the methods for coupling the sugar moieties in all their structural variations\textsuperscript{20-22}. Toxins present an analogous case\textsuperscript{23}. In many cases the bare attempt to synthesize a natural product sheds light on its function in nature by revealing the properties, reactivity, stability, and/or lability of certain of its functionalities.

Synthesis provides an excellent testing ground for the development of the fundamental principles of organic chemistry. Each new synthetic endeavour either supports the existing principles or provides another exception which eventually leads to a new principle being formulated.
Synthetic studies of organic compounds enrich organic chemistry in the areas of new reactions, reaction mechanisms, and stereochemistry.

For the foregoing reasons the synthesis of disaccharides was undertaken. As can be seen by Figure 1 the total number of synthetic disaccharides is very limited indicating that disaccharide synthesis is an area in which much development is needed. Although L-rhamnose is widely distributed in nature and is commonly found as a constituent of plant gums, plant glycosides, and bacterial polysaccharides, no disaccharide having L-rhamnose as the aglycon has been synthesized with the exception of 4-O-(3-O-methyl-β-D-galactopyranosyl)-L-rhamnose.

Therefore four new disaccharides having L-rhamnose as the reducing end have been synthesized, namely 4-O-β-D-glucopyranosyl-L-rhamnopyranose (scillabiose), 4-O-α-L-rhamnopyranosyl-L-rhamnopyranose, 4-O-α-D-mannopyranosyl-L-rhamnopyranose, and 4-O-β-D-mannopyranosyl-L-rhamnopyranose. Their structures are shown in Figure 2.

4-O-D-Mannopyranosyl-L-rhamnopyranose occurs with the glycosidic linkage in both the α-D and β-D anomeric configurations in the antigenic cell-wall lipopolysaccharides of different Salmonella species. Serogroups A, B, and D₁ contain an α-D linkage while D₂ and E contain a β-D linkage. The two configurationally different disaccharides isolated from natural sources were originally confused owing to the lack of authentic standards. The synthetic disaccharides would also be useful in immunological studies. 4-O-α-L-Rhamnopyranosyl-L-rhamnopyranose was thought to be a constituent of Shigella flexneri O-antigens by complement fixation inhibition studies but it was later shown that in these antigens the linkage was not 1,4. A rhamnobiose has been isolated from Ulva conglobata and preliminary studies have indicated that it is 4-O-α-L-rhamnopyranosyl-
Figure 1. Chemically Synthesized Disaccharides of the Common Neutral Sugars

<table>
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<tr>
<th>REDUCING</th>
<th>ARABINOSE</th>
<th>RIBOSE</th>
<th>XYLOSE</th>
<th>GALACTOSE</th>
<th>GLUCOSE</th>
<th>MANNOSE</th>
<th>FUCOSE</th>
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</tr>
<tr>
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<td>1</td>
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<tr>
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<tr>
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<tr>
<td>RHAMNOSE</td>
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Figure 2. Disaccharides Described in this Thesis

4-0-β-D-Glucopyranosyl-L-rhamnopyranose (15)

4-0-α-L-Rhamnopyranosyl-L-rhamnopyranose (26)

4-0-α-D-Mannopyranosyl-L-rhamnopyranose (38)

4-0-β-D-Mannopyranosyl-L-rhamnopyranose (56)
-L-rhamnose but comparison of the physical constants with those of the synthetic disaccharide do not confirm this. These structural studies would have been greatly simplified had the authentic standard been available. 4-O-α-L-Rhamnopyranosyl-L-rhamnose is likely to occur in those *Klebsiella* capsular polysaccharides which contain a high percentage of L-rhamnose residues. Scillabiose was first isolated from the partial acid hydrolysates of the glycosides scillarin A and glucoscilliphaeoside obtained from *Scilla maritima* and its identification as 4-O-β-D-glucopyranosyl-L-rhamnose was subsequently established. Since aldobiouronic acids may be easily characterized as the derived neutral disaccharides, 4-O-β-D-glucuronosyl-L-rhamnose from the partial acid hydrolysates of the acidic polysaccharides of the green seaweeds *Acrosiphonia centralis* and *Ulva lactuca* has been characterized as 4-O-β-D-glucopyranosyl-L-rhamnose. Recently 4-O-β-D-glucuronosyl-L-rhamnose has been identified as a structural component of the capsular polysaccharide from *Klebsiella* type 9. These examples give some indication of where these four disaccharides could have been used had they been available, so their real potential lies in facilitating the elucidation of the structures and related functions of oligosaccharides yet to be discovered.

3. Historical Background

Disaccharides can be synthesized either enzymatically or chemically. Only chemical synthesis will be discussed here. There are many chemical methods for disaccharide synthesis. Unsubstituted monosaccharides or partially blocked monosaccharides can be condensed under dehy-
drating conditions such as sulfuric acid, phosphorus pentoxide, or zinc chloride. New disaccharides can also be prepared by the chemical alteration of either the sugar residues or the linkage of available disaccharides. However the least ambiguous method of synthesizing disaccharides is to couple two sugars, one of which has been activated at C-1 and blocked elsewhere and the other which has been selectively blocked to leave a single hydroxyl group reactive. The addition reactions of monosaccharides having one free hydroxyl group to 1,2 anhydrides are examples of this as indicated by Lemieux and Huber's synthesis of sucrose heptaacetate from Brigl's anhydride (3,4,6-tri-O-acetyl-1,2-anhydro-α-D-glucose) and 1,3,4,6-tetra-O-acetyl-D-fructofuranose. Also in this class are the reactions of acetylated monosaccharide 1,2-(alkyl orthoacetates) with other suitably blocked monosaccharides having a free hydroxyl group in the presence of catalytic amounts of mercuric bromide to give good yields of the 1,2-trans glycosides. This reaction has been perfected by Kochetkov and used successfully by others. Another type involves the elimination of an alkali salt such as sodium bromide from the sodium salt of a monosaccharide having a free hydroxyl group and an acylated glycosyl bromide as exemplified by the reaction of the sodium salt of 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide to form gentiobiose octaacetate. However the most important and versatile method is the Koenigs-Knorr reaction which involves the elimination of a hydrogen halide between a substituted glycosyl halide and an unsubstituted hydroxyl group of a second monosaccharide in the presence of an acid acceptor such as a heavy metal salt or organic base. The halide most commonly used is bromide since it is at the same time sufficiently stable to be easily prepared and suffic-
ently reactive towards free hydroxyl groups. The chlorides are less reactive but are more easily prepared in the less common β-D configuration which is sometimes useful. The fluorides do not normally react. The iodides are too reactive to be prepared but may be prepared in situ. The most commonly used condensing agents are silver carbonate, silver oxide, quinoline, and collidine. Silver perchlorate is used with trityl derivatives (Bredereck reaction). Cadmium carbonate has been used with steroidal aglycons. Since the reaction of the hydrogen halide with silver oxide or silver carbonate liberates water which competes with the glycoside formation an internal desiccant such as anhydrous calcium sulfate, sodium sulfate, copper sulfate, or calcium chloride is added. Iodine is also commonly added. The Koenigs-Knorr reaction is usually carried out in an inert solvent such as chloroform or benzene. The other necessary component is a suitably substituted monosaccharide which will react at a single position. The development of the chemistry of hydroxyl blocking groups was necessary in order to provide these. The Koenigs-Knorr reaction has been extensively reviewed and a typical example is the reaction of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide with 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose to give gentiobiose octaacetate.

\[
\begin{align*}
\text{CH}_2\text{OAc}_2\text{O} & \quad \text{Br} \\
\text{Ag}_2\text{O/}{\text{I}_2/\text{CaSO}}_4/\text{CHCl}_3
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{OH}_2\text{O} & \quad \text{OAc} \\
\text{AcO} & \quad \text{OAc}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{OAc}_2\text{O} & \quad \text{O} \\
\text{AcO} & \quad \text{OAc}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{OAc}_2\text{O} & \quad \text{O} \\
\text{AcO} & \quad \text{OAc}
\end{align*}
\]
The anomeric effect generally directs the bromine at C-1 to the axial position. Bromides having an acyloxy group at C-2 cis to the bromine at C-1 normally react with inversion whereas those with an acyloxy group at C-2 trans to the bromine at C-1 react with predominant retention of configuration. In both cases the 1,2 trans glycoside is formed. The first case can be explained either by a $S_{N}^{2}$ type (ion-pair) mechanism or a $S_{N}^{1}$ mechanism followed by participation of the acyloxy group on C-2 to form a cyclic acetonium ion which forces the incoming nucleophile to attack from a trans position at C-1.

Case 1:

The second case can be explained by a $S_{N}^{2}$ assisted displacement of the halogen by the participating acyloxy group on C-2 to form the cyclic acetonium ion followed by replacement by the aglycon from the opposite side.

Case 2:
Anomeric and steric effects are probably also operative but are not predominant when a participating group is present on C-2. Either may become dominant in other circumstances. Thus the Koenigs-Knorr reaction provides a high degree of stereoselectivity which results in the formation of 1,2 trans glycosides almost exclusively.

Later the Koenigs-Knorr reaction was modified by using mercury salts such as mercuric acetate in benzene (Zemplen reaction) or mercuric cyanide and mercuric bromide which were soluble in the newer polar solvents such as acetonitrile and nitromethane (Helferich reaction). Some cases have been reported where the mercury salts give some 1,2 cis glycosides.

The yields of Koenigs-Knorr reactions are limited by two factors, the reactivity of the alcoholic functionality and the side reactions to which the glycosyl bromide is prone. The monosaccharides being large and cyclic are not as reactive as the small aliphatic alcohols such as methanol, ethanol, etc. Usually acyclic sugars are more reactive than cyclic ones, primary hydroxyls are more reactive than secondary ones, and equatorial hydroxyls are more reactive than axial ones. Sterically hindered hydroxyls are also less reactive. Several side reactions as well as the desired glycoside formation may arise from the intermediate cyclic acetoxonium ion as shown in Figure 3. Attack of the incoming nucleophile at B rather than A leads to the formation of two isomeric orthoesters. Attack of cyanide ion at A gives the 1-cyano compound whereas attack at B gives two isomeric products. Water can hydrolyze the bromide to give the 1-hydroxy sugar or attack the cyclic acetoxonium ion at A or B to give the 1- or 2-hydroxy sugars. These are then available to condense and form unwanted disaccharides. Goldschmidt and Perlin have shown that
Figure 3. Side Reactions during Disaccharide Condensations

+ isomer

ROH

Hg(CN)_2

+ isomer

H_2O

and/or
silver oxide itself decomposes the glycosyl bromide leading to products such as 1-hydroxy sugars, dimeric orthoesters, and even a trisaccharide orthoester. They suggest that iodine acts as an inhibitor of these reactions. These side reactions explain why condensation reactions give multicomponent mixtures.

The previously mentioned developments paved the way for the preparation, described in this thesis, of 4-O-β-D-glucopyranosyl-L-rhamnopyranose from 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide and the suitably blocked aglycon methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (3) in the presence of mercuric cyanide in acetonitrile. This provides a typical example of Case 1 steric relationships. In an analogous manner 4-O-α-L-rhamnopyranosyl-L-rhamnopyranose and 4-O-α-D-mannopyranosyl-L-rhamnopyranose were prepared by reacting 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide and 2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl bromide respectively with the aglycon 3 in the presence of mercuric cyanide in acetonitrile. These are typical examples of Case 2 steric relationships.

The synthesis of 4-O-β-D-mannopyranosyl-L-rhamnopyranose required the formation of a 1,2 cis glycoside. The 1,2 cis glycosides have presented much more of a challenge to chemists of organic synthesis, but are interesting because they are often the naturally occurring forms. α-D-Glucosides occur in glycogen, amylose, amylopectin, sucrose, antibiotics, and fungal and bacterial polysaccharides. β-D-Mannosides occur as plant mannans, as glucomannans in hemicelluloses, as galactomannans in seeds, and in Klebsiella capsular polysaccharides. Most of the synthetic work to date on 1,2 cis glycosides has been done on glucose. Once the directing effect of the participating group on C-2 in the formation of 1,2 trans glycosides
was understood the obvious choice for the synthesis of 1,2 cis glycosides was to put a nonparticipating group on C-2. Several nonparticipating groups were tried such as free hydroxyl, methyl, nitro, tosyl, trichloroacetyl and benzyl. In most cases the configuration of the original halide was now inverted by an incoming nucleophile. This was satisfactory for the synthesis of β-D-arabinofuranosides but required a β-halide in order to get α-D-glucosides. In the cases where β-D-glucopyranosyl chlorides were employed α-D-glucosides were produced. However β-chlorides are not readily prepared in high yields. Noting that halides could be stabilized by electron withdrawing groups Ishikawa and Fletcher now synthesized 2-O-benzyl-3,4,6-tri-O-p-nitrobenzoyl-β-D-glucopyranosyl bromide, which combines a nonparticipating group at C-2 with a stabilized β-bromide and demonstrated its stereoselectivity for the production of α-D-glucosides. This halide has proven quite useful in the preparation of α-D-glucosides.

Frechet and Schuerch have extended this idea by using 2,3,4-tri-O-benzyl-D-glucopyranosyl bromides with various electron donating and electron withdrawing groups on C-6 in order to get stereoselective reactions. Zorbach and coworkers (and references therein) and Flowers and coworkers have each studied the effects of substituents at the various positions in the ring on reactions at C-1.

Lemieux and coworkers have developed an alternative method using nitrosyl chlorides for the synthesis of α-D-glucosides and α-D-galactosides. It is particularly useful for disaccharides containing keto and amino sugars. Another method developed by Lemieux and Hendriks involves
substitution with nonparticipating benzyl groups and the rapid equilibra-
tion of the α- and β-bromides in the presence of a quaternary ammonium bro-
mide while doing the condensation with a sufficiently sluggish condensing agent such as diisopropylamine or collidine so that essentially only the more reactive β-bromide reacts. This has been used successfully to make α-L-fucosides.

Some attempts have now been made to replace the halogen at C-1 with a good leaving group that prefers the equatorial position and therefore inversion would lead to α-D-glucosides. On this theme Ferrier has tried phenyl 1-thio-β-D-glucopyranoside and James and Angyal have used the nitrobenzene-p-sulphonyloxy group in another context. Ferrier et al. have also used acylated 2-hydroxyglycals with boron trifluoride for the production of 1,2 cis glycosides. This is particularly useful for the synthesis of disaccharides having 3-deoxy sugars as their non-reducing moieties. West and Schuerch have reviewed the attempts at making 1,2 cis glycosides and have introduced electropositive equatorial substituents at C-1 with nonparticipating groups elsewhere in order to get stereoselective inversion.

The β-D-mannopyranosides first reportedly synthesized were later shown to be incorrectly assigned and were actually α-D-mannopyranosides. Considering the foregoing developments the synthesis of β-D-mannosides should have been straightforward since the commonly available α-bromide should on inversion produce the 1,2 cis glycoside. The only requirement should have been a nonparticipating group on C-2. Nonparticipating groups such as methyl, benzyl, and hydroxyl did not lead to β-D-mannosides stereoselectively. It should be noted that often in the attempts to produce 1,2 cis glycosides
stereoselective inversion was achieved with small relatively strong nucleophiles such as methanol but not with selectively substituted monosaccharides. However, when Gorin and Perlin\textsuperscript{95} used a 2,3 cyclic carbonate as the nonparticipating group stereoselective inversion was achieved. Cyclic carbonates had previously been used\textsuperscript{96,97} to prepare 1,2 \textit{cis} glycosides in the furanose series. Zorbach \textit{et al.}\textsuperscript{98} have condensed a cyclic carbonate of rhamnopyranose with steroidal aglycons. It has been suggested\textsuperscript{72} that the \textit{trans} axial substituent at C-2 of mannose impedes an incoming nucleophile from approaching from that side and hence prevents $S_N^2$ type substitutions of $\alpha$-D-mannoses. Perhaps the fused 5-membered carbonate ring modifies the conformation of the pyranose ring so as to allow this to occur. It has also been proposed\textsuperscript{99} that the modified conformation resists the formation of a planar carbonium ion about C-1.

Lindberg\textsuperscript{100} has recently developed an alternative method for the synthesis of $\beta$-D-mannosides which involves the synthesis of $\beta$-D-glucosides and subsequent selective epimerization.

Following up the idea of Gorin and Perlin\textsuperscript{95} 4,6-di-O-acetyl-2,3-$\alpha$-carbonyl-D-mannopyranosyl bromide (44) was used to synthesize 4-O-$\beta$-D-mannopyranosyl-L-rhamnopyranose. An improved synthetic route for the preparation of this valuable bicyclic bromide and condensation conditions modified to make this a practical method for the preparation of $\beta$-D-mannopyranosides are described in this thesis.
II RESULTS AND DISCUSSION

The discussion will fall into five main areas: the aglycon which also served as a key intermediate for the preparation of necessary standards; 4-\(\alpha\)-\(\beta\)-D-glucopyranosyl-L-rhamnopyranose which functioned as a model for the proof of structure, de-blocking, and characterization procedures of the series; 4-\(\alpha\)-L-rhamnopyranosyl-L-rhamnopyranose which served as a testing ground for an alternative reduction acetylation procedure which led to an anomalous boron-containing product; 4-\(\alpha\)-D-mannopyranosyl-L-rhamnopyranose which demonstrates the utility of proton magnetic resonance spectroscopy in assigning configurations; and 4-\(\beta\)-D-mannopyranosyl-L-rhamnopyranose for which it was necessary to develop a general method for the synthesis of \(\beta\)-D-mannopyranosides. Many of the reactions employed in this work are standard methods which will not be discussed in detail but the results will be illustrated by structures. Only the intriguing and potentially important facets of this work will be discussed.

1. Aglycon

As previously mentioned a requirement for discaccharide synthesis is a monosaccharide selectively substituted so that a single hydroxyl group is reactive. Methyl 2,3-\(\alpha\)-isopropylidene-\(\alpha\)-L-rhamnopyranoside (3) fills these requirements.
It also happens that the hydroxyl group on C-4 of \( \beta \) is relatively reactive and hence leads to good yields of disaccharides. L-Rhamnose has other advantages for the synthesis of disaccharides such as the fact that under the visualization procedures used for thin-layer chromatography 6-deoxy sugars such as L-rhamnose appear yellow whereas all the ordinary hexoses appear black and hence compounds containing only L-rhamnose (yellow), half L-rhamnose (olive green), and no L-rhamnose (black) can be distinguished. The proton magnetic resonance spectrum of compounds containing 6-deoxy sugars such as L-rhamnose is particularly informative because the quantitation of the high field doublet arising from the C-6 protons provides an internal standard for determining the amount of any other signal relative to L-rhamnose. This provides an easy method for determining the number of the various substituents per L-rhamnose moiety in any molecule. The disaccharides synthesized in this thesis all have a common aglycon, namely \( \beta \), so therefore form a series of disaccharides all linked through position 4 of L-rhamnose, that is a series of 4-O-glycopyranosyl-L-rhamnopyranoses.

The synthetic scheme for the preparation of \( \beta \) is shown in Figure 4. Methyl \( \alpha \)-L-rhamnopyranoside (1) was prepared essentially as described by Levene and Muskat\(^{101} \). Compound 1 crystallizes readily when seeded, but only when seeded, and thus provides a starting material for the aglycon which is pure in anomeric configuration and locked in the pyranose ring structure. This pure methyl \( \alpha \)-L-anomer assures a permanent configuration around C-1 for all subsequent derivatives including derived disaccharides. This greatly facilitates their crystallization. This stabilized linkage is also beneficial for immunochemical studies where the methyl glycoside of a disaccharide can be used to study the specificity of two linkages in
Figure 4. Synthetic Sequence for the Preparation of the Aglycon
sequence. The methyl α-L-glycosides of 38 and 56 are particularly relevant as it has been shown that L-rhamnose is α-linked in the *Salmonella* lipopolysaccharides.

Insoluble acids in the form of cation exchange resins can be used to catalyze acetal formation and were found to be the most convenient method for preparing 3 from 1. The condition of the resin is the most influential factor. It performs best when it is freshly regenerated and dehydrated as much as possible with anhydrous methanol. As recommended, it was found advantageous to use 2,2-dimethoxypropane as the acetalating reagent but since the starting material did not dissolve in 2,2-dimethoxypropane, acetone was also employed. The fact that internal desiccants did not improve the reaction may be explained by the greater reactivity of 2,2-dimethoxypropane and hence the production of methanol rather than water. The formation of acetone polymers can be controlled by carrying out the reaction at 0°. Under these conditions resins are sufficient catalysts for the acetal formation yet weak enough acids that they do not disturb the methyl glycoside appreciably during the time required for the reaction. It was found necessary to filter the product through calcium oxide to remove the traces of acid resulting from bleeding of the resin. Compound 3 was reported crystalline once in the literature and also crystallized neat once in this work but could not be persuaded to crystallize from solvent. Hence compound 3 was purified through crystalline methyl 4-O-acetyl-2,3-O-isopropylidene-α-L-rhamnopyranoside (2). Because acetalation never goes 100% to completion, some acetone polymers are always formed, and since deacetylation is a clean quantitative procedure this crystalline intermediate 2 provides an easy method for obtaining the pure 3 required for disaccharide synthesis. Care must be taken during the deacetylation when
deionizing the sodium methoxide with cation exchange resin to keep the
temperature of the solution around $5^\circ$ and the time of contact with the
resin minimal in order to prevent hydrolysis of the isopropylidene group.
Subsequent passage of the solution through an anion exchange resin removes
the traces of acid resulting from bleeding of the cationic resin and pre­
vents deacetalation.

Shapiro et al.\textsuperscript{108} have reported a very mild method for the removal
of isopropylidene groups in the presence of acetates. It involves the use
of trifluoroacetic acid in wet chloroform which is conveniently removed by
evaporation. This procedure selectively removes isopropylidene groups
while leaving methyl glycosides and acetates intact. Subjecting compound
2 to this treatment results in the preparation of crystalline methyl
4-O-acetyl-\(\alpha\)-L-rhamnopyranoside (4).

Since compound 4 has a substituent on C-4 it serves as an analog of the
methyl glycosides of the disaccharides and thus can be used to prepare the
necessary standards for the methylation and periodate oxidation products
resulting from the L-rhamnose moiety as shown in Figure 5. Compound 4
should also be a useful intermediate for the preparation of L-rhamnose
residues selectively substituted to leave position 3 reactive as it has
been demonstrated that vicinal hydroxyls react with ethyl orthoacetate to
Figure 5. Preparation of Methylation and Periodate Oxidation Standards from Methyl 4-O-acetyl-α-L-rhamnopyranoside
give monoacetylation of the axial hydroxyl. This would then lead to the interesting series of disaccharides linked through position 3 on L-rhamnose but will not be discussed further here.

Methylation of compound 4 leads to the standards required for comparison with the product obtained from the methylation of the L-rhamnose moiety of the disaccharides. Since until recently all methylation procedures involved the use of strong bases, compounds containing base labile groups such as acetates could not be methylated. However Gros et al. have developed a procedure for methylating compounds containing base labile groups by using boron trifluoride and diazomethane. Using this procedure compound 4 was methylated to give methyl 4-O-acetyl-2,3-di-O-methyl-α-L-rhamnopyranoside. It was not found advantageous to cool the reaction mixture to -78° as has been suggested but the suggestion to repeat the procedure if it is not complete the first time has proven practical. Care should be taken to obtain pure starting material and to maintain anhydrous conditions. Subsequent deacetylation gave methyl 2,3-di-O-methyl-α-L-rhamnopyranoside (5). Compound 5 was characterized as crystalline methyl 2,3-di-O-methyl-4-O-toluene-p-sulphonyl-α-L-rhamnopyranoside. Now the standard methyl 2,3-di-O-methyl-α-L-rhamnopyranoside (5) can be hydrolyzed readily to give standard 2,3-di-O-methyl-L-rhamnose (6) which can be reduced to give standard 2,3-di-O-methyl-L-rhamnitol which in turn can be acetylated to give standard 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (7).

Periodate oxidation of 4 leads to the standards required for comparison with the product obtained from the periodate oxidation of the L-rhamnose moiety of the disaccharides. Therefore periodate oxidation of 4 followed by deacetylation, reduction and methanolysis gave 4-deoxy-L-
-erythritol (1-deoxy-D-erythritol) (8). Methanolysis rather than hydrolysis was employed in order to convert the reducing portion of the molecule to volatile glycolaldehyde dimethyl acetal which could be conveniently removed by evaporation. Methanolysis also prevents the unwanted formation of acetals between glycolaldehyde and 8. This aspect will be discussed more fully in the periodate oxidation of the disaccharides. Compound 8 was characterized as its crystalline tri-p-nitrobenzoate. Standard 4-deoxy-L-erythritol (8) was acetylated with sodium acetate and acetic anhydride to give standard 4-deoxy-L-erythritol triacetate. Sodium acetate was used as a catalyst instead of the usual pyridine because the evaporation conditions used for the removal of pyridine may lead to the loss of some of this fairly volatile product.

2. 4-O-β-D-Glucopyranosyl-L-rhamnopyranose

Since 4-O-β-D-glucopyranosyl-L-rhamnopyranose (15) results from the condensation of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) (9) with the aglycon 3 and since acetobromoglucose is the cheapest, most commonly used, best understood, and most easily prepared glycosyl halide available this condensation was the one used to determine the optimal condensation conditions for this series of disaccharides. Many variations of the standard Koenigs-Knorr conditions were compared with the Helferich conditions since Kamiya et al. have recommended the Helferich reaction for the preparation of L-rhamnopyranosides. However it was noted that the isopropylidene group of 3 was not stable to the Helferich reaction conditions. By doing a series of control experiments where only 3 was subjected to condensation conditions each varying in one component it was deter-
mined that the mercuric bromide was causing hydrolysis of the isopropylidene group. Neither the acetonitrile alone nor in combination with mercuric cyanide caused cleavage but when mercuric bromide was added considerable deacetalation was observed. Kamiya et al.\textsuperscript{110} did not report any deacetalation of their aglycons in the presence of mercuric bromide which perhaps indicates the lability of the isopropylidene group of 3. Consequently the Helferich reaction conditions were modified in that the mercuric bromide was omitted. Parallel microscale condensations were performed with silver oxide and anhydrous calcium sulfate in chloroform; silver oxide, iodine, and anhydrous calcium sulfate in chloroform; silver oxide, silver perchlorate, and anhydrous calcium sulfate in chloroform; and mercuric cyanide in acetonitrile. Consistently the modified Helferich reaction conditions gave rise to a more complete reaction; that is, gave a higher yield of product and less of fewer degradation products. The major degradation product observed in all the condensation reactions described in this thesis was the so called "hydrolysis product" of the bromide, that is the product with a free hydroxyl at C-1 resulting from hydrolysis of the glycosyl bromide. This by-product was identified by thin-layer chromatographic comparison with the product obtained by deliberate hydrolysis of the bromide with aqueous silver nitrate. The major difference between the silver oxide condensations and the mercuric cyanide condensation is that the latter is a homogeneous system whereas the others are heterogeneous. Therefore acetobromoglucose 9 was condensed with 3 in the presence of mercuric cyanide in acetonitrile, as shown in Figure 6, to give crystalline methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (10) in 80% yield.
Figure 6. 4-β-D-Glucopyranosyl-L-rhamnopyranose

R₁ R₂ R₃ R₄ R₅ R₆ R₇

10 Me CH₃ CH₃ Ac Ac Ac Ac
11 Me H H Ac Ac Ac Ac
12 Me H H H H H H H
13 Me Me Me Me Me Me Me Me
14 Ac Ac Ac Ac Ac Ac Ac Ac
15 H H H H H H H H
Since 4-O-β-D-glucopyranosyl-L-rhamnopyranose in the form of 10 was the most easily obtained member of the 4-O-glycopyranosyl-L-rhamnopyranose series it was the most appropriate one to use to work out the proof of structure procedures and the deblocking and derivatization reactions for the series. Although the mode of synthesis gives a good indication of the structure of a synthetic disaccharide it cannot be used to assign the structure unambiguously without confirmation from the usual proof of structure procedures such as methylation and periodate oxidation. By practicing these procedures on a system of predicted structure the procedures can be improved to give optimum results in general and/or for the specific structural feature under investigation and then these improved procedures can be extended to systems of unknown or suspected structure.

The methyl glycoside of the disaccharide is the most convenient derivative to use for methylation and periodate oxidation studies because it has the ring size and configuration of the reducing sugar locked, thus only one degradation product results from the reducing moiety of the disaccharide. The methyl glycoside also prevents base degradation from the reducing end during methylation and prevents the formation and slow hydrolysis of a formate ester during periodate oxidation. Consequently 10 was deacetalated by the previously discussed method of Shapiro et al.\textsuperscript{108} to give methyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (11) which was deacetylated to give methyl 4-O-β-D-glucopyranosyl-α-L-rhamnopyranoside (12).

The methyl glycoside 12 was methylated by the Hakomori method\textsuperscript{111} as detailed by Sandford and Conrad\textsuperscript{112}. Since the disaccharide could not be dialyzed the methyl sulphoxide extract was diluted with a small amount of
water and extracted with large amounts of petroleum ether (b.p. 65-70°). This procedure is satisfactory for separating the permethylated disaccharide 13 from the methyl sulfoxide and provides a clean permethylated product as nothing else is extracted by the petroleum ether. The permethylated disaccharide 13 was then cleaved into monomeric units since the determination of the methyl substitution pattern of the derived monomeric units indicates the position of linkage. The methyl substitution pattern is routinely established by gas-liquid and paper chromatographic comparison with authentic standards and by mass spectrometric fragmentation analysis. A variety of derivatives of the partially methylated monomeric units can be used to determine the substitution pattern as illustrated by the various ones used for the monomeric fragments of 13 shown in Figure 7. Methanolysis of 13 gave the monomeric methyl glycosides but they were not separable by gas-liquid chromatography. However since the L-rhamnose derivatives had a free hydroxyl group but the D-glucose derivatives did not, it was possible to make the trimethylsilyl derivative thus decreasing substantially the retention time of the L-rhamnoses while leaving the D-glucoses unchanged. This method has been used with particular success in the D-mannose series 113,114. Now that the fragments were separated they were shown to cochromatograph with authentic standards of the trimethylsilyl derivatives of the methyl 2,3-di-O-methyl-L-rhamnopyranosides and with the authentic standards of the methyl 2,3,4,6-tetra-O-methyl-D-glucopyranosides. Methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside was collected and characterized as a crystalline derivative. Hydrolysis of 13 gave 2,3-di-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-glucose as indicated by paper chromatographic comparison with authentic standards. They could also be separated
Figure 7. Hydrolysis Products of Methylated Disaccharide
and identified by gas-liquid chromatographic analysis of their acetate derivatives\textsuperscript{115}. Hydrolysis of \textsuperscript{13} followed by reduction and subsequent acetylation gave the alditol acetates. These were not separable by gas-liquid chromatography using butanediol succinate\textsuperscript{116} as a liquid phase but when OS138 was used as suggested by Lindberg and coworkers\textsuperscript{117} separation was achieved on an ordinary packed column. The two equimolar peaks then obtained were identified as 1,4,5-tri-0-acetyl-2,3-di-0-methyl-L-rhamnitol and 1,5-di-0-acetyl-2,3,4,6-tetra-0-methyl-D-glucitol by gas-liquid chromatographic and mass spectrometric comparison with authentic standards. Thus 2,3-di-0-methyl-L-rhamnose and 2,3,4,6-tetra-0-methyl-D-glucose can be separated and identified by paper chromatography and by gas-liquid chromatography as either their methyl glycosides, their acetates, or their alditol acetates. In all cases authentic standards are required which emphasizes the importance of compounds such as \textsuperscript{5}, \textsuperscript{6} and \textsuperscript{7}.

The methyl glycoside \textsuperscript{12} was subjected to periodate oxidation degradation as shown in Figure 8. Structural information is obtained from both the consumption of periodate and the products obtained after degradation. A consumption of three moles per mole of \textsuperscript{12} indicated that the linkage was either 1,2 or 1,4 but could not be 1,3. The products obtained from the subsequent degradation procedures distinguished 1,2 and 1,4 linkages. In both cases the non-reducing portion of the molecule would produce glycerol and if the linkage was 1,2 the reducing portion would produce 3-deoxy-L-glycerol but if the linkage was 1,4 the reducing portion would produce 4-deoxy-L-erythritol. The 1,4 linkage was confirmed by the production of glycerol and 4-deoxy-L-erythritol as identified by paper chromatography as well as the gas-liquid chromatography and mass spectrometry of their
Figure 8. Periodate Oxidation of Disaccharide
peracetylated derivatives with the use of authentic standards such as 8. Glycerol and 4-deoxy-L-erythritol should be produced quantitatively and in an equimolar ratio. Although this is not critical for this series of disaccharides it should hold true if the method is to be generally useful for undefined systems where more components are present and their ratios are informative. As mentioned previously (page 25) hydrolysis of the polyalcohol intermediate led to the formation of acetals between glycolaldehyde and glycerol and 4-deoxy-L-erythritol. Gas-liquid chromatographic analysis of the acetylated mixture indicated one large peak and several smaller ones in addition to the peaks expected for glycerol and 4-deoxy-L-erythritol. Therefore hydrolysis conditions are not satisfactory if quantitative procedures are required. This and related problems have been reviewed. Methanolysis produced a much more quantitative reaction. The dimethyl acetal of glycolaldehyde is formed during methanolysis thus rendering it inert toward the formation of acetals with the higher alcohols. Glycolaldehyde is also conveniently removed by evaporation as its dimethyl acetal. Gas-liquid chromatographic analysis of the acetylated product obtained from methanolysis showed two major equimolar peaks corresponding to glycerol and 4-deoxy-L-erythritol with a few other smaller components. Methanolysis gave satisfactory results for all the disaccharides in this thesis, however on the suggestion of P.E. Reid the polyalcohol was also hydrolyzed with cation exchange resin in the presence of an anion exchange resin. The anion exchange resin irreversibly adsorbs the glycolaldehyde (or any aldehyde) as soon as it is released thus effectively removing it from the reaction mixture. Gas-liquid chromatographic analysis of the acetylated product obtained from mixed resin hydrolysis showed only the two peaks expected corresponding
to glycerol and 4-deoxy-L-erythritol plus one other very small peak. This method
certainly looks promising for the quantitation of more complicated mixtures.

To be of use in the identification of isolated polymer fragments
synthetic disaccharides of unambiguous structure have to be characterized
as derivatives, preferably crystalline, that can be readily prepared from
the isolated fragments. The peracetate of the disaccharide was an obvious
candidate. Since acetolysis has been used successfully to replace methyl
glycosides \(^{81,120,121}\), benzyldene acetals \(^{121,122}\), and isopropylidene
ketal \(^{120,123}\) with acetates it was assumed that this would provide a
convenient one-step procedure for converting \(\text{10}\) into 1,2,3-tri-O-acetyl-
-4-O-(2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranosyl)-\(\alpha\)-L-rhamnopyranose
(scillabiose heptaacetate) \((14)\). However, all of the several sets of
acetolysis conditions tried only gave the product in a very low yield (up
to 15%). Once crystalline 1-O-acetyl-2,3-O-isopyropylidene-4-O-(2,3,4,6-
tetra-O-acetyl-\(\beta\)-D-glucopyranosyl)-\(\alpha\)-L-rhamnopyranose as identified by
its proton magnetic resonance spectrum was isolated. A closer investiga-
tion of the literature revealed that acetolysis had only been used success-
fully for isopropylidene groups spanning a primary and a secondary hydroxyl
i.e. \(1,2\)-; \(5,6\)-; \(6,7\)-O-isopropylidene groups and not for isopropylidene
groups spanning two secondary hydroxyl groups such as the 2,3-O-isopropylid-
ene group in \(\text{10}\). It has also been noted \(^{120}\) that secondary-secondary
methylene acetals are considerably less susceptible to acetolysis than are
primary-secondary methylene acetals. Acetolysis of isopropylidene ketal
formed between two secondary hydroxyl groups has been studied by Sowa\(^{124}\)
in extending an earlier observation made by Jerkeman\(^{125}\). They give examples
of \(cis\) 2,3-O-isopropyldene ketal of furanoses that are epimerized at C-2.
when acetolyzed in high concentrations of acetic acid. It seemed probable that the isopropylidene group of 10 could not be acetolyzed to any extent but may have been hydrolyzed during the work-up thus making the product water soluble and therefore lost during the washing of the organic solvent layer. This would account for the observed 15% yield of uncontaminated predicted product. Once it was realized that the isopropylidene group could not be acetolyzed it was easily removed by the mild method of Shapiro et al.\textsuperscript{108} to give methyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (11). Now the acetolysis of 11 essentially as described by Aspinall and coworkers\textsuperscript{121} gave 14 in good yield (70%). Since acetolysis preferentially cleaves small aglycons\textsuperscript{126} it is a convenient method for removing the methyl glycoside while leaving the disaccharide linkage intact. Since 1,4 linkages are least susceptible to cleavage by acetolysis it is most appropriate for this series of disaccharides. Scillabiose heptaacetate 14 thus obtained had m.p. 139-140° and the proton magnetic resonance spectrum showed clearly the presence of seven acetate groups per L-rhamnose moiety. Neither the melting point nor the optical rotation agree with that given in the literature\textsuperscript{35,36} for "scillabiose hexaacetate". Although satisfactory analytical data are given for a hexaacetate there is no indication why such an acetate should be formed and Bailey\textsuperscript{28} has recognized this ambiguity by listing only scillabiose acetate.

Straightforward deacetylation of 14 gave the free disaccharide, 4-O-β-D-glucopyranosyl-L-rhamnopyranose (15). Two other derivatives, the free alditol, 4-O-β-D-glucopyranosyl-L-rhamnitol (16) and its crystalline peracetate, 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-L-rhamnitol (17) were prepared by standard procedures.
In order to extend the data of Percival\textsuperscript{127} the gas-liquid chromatographic retention times of the per-O-(trimethylsilyl) derivatives of 15 and 16 relative to per-O-(trimethylsilyl) sucrose were recorded.

The configuration of the disaccharide linkage can be determined by Hudson's rules or by enzymatic hydrolysis studies but most conveniently by proton magnetic resonance spectroscopy as demonstrated by van der Veen\textsuperscript{128}, pursuing the earlier observations of Lemieux et al.\textsuperscript{129}. Because C-1 is bonded to two oxygen atoms the resonance of the proton on C-1 (the anomeric proton) is to low field of the other ring protons and hence can be distinguished. \(\alpha\)-Linked disaccharides have an equatorial H-1 which resonates at lower field (4.5 - 5.0 \(\tau\)) than the axial H-1 of \(\beta\)-linked disaccharides which resonates at 5.0 - 5.5 \(\tau\). Coupling constants are also informative for distinguishing \(\alpha\)-D- and \(\beta\)-D-linked glucosides since an \(\alpha\)-D-linkage leads to a small (\(\sim 3\) Hz) axial-equatorial coupling constant whereas a \(\beta\)-D-linkage leads to a large (\(\sim 7\) Hz) axial-axial coupling constant. As has been reported\textsuperscript{130} the proton magnetic resonance spectra of permethylated disaccharides also distinguish \(\alpha\)- and \(\beta\)-anomers. Whyte\textsuperscript{131} has pointed out the advantage of using disaccharide alditols for determining the glycosidic configuration.
as they only exhibit the one anomic proton of interest and eliminate complications arising from the anomic protons of the reducing moiety. Proton magnetic resonance spectroscopy was used to confirm the β-D-linkage of 4-O-β-D-glucopyranosyl-L-rhamnopyranose. The signals from the anomic protons of the free disaccharide, the permethylated disaccharide, and the free alditol provide particularly clear evidence of a β-D-linked glucoside as shown in Figure 9 which compares the α-D-\(^{83}\) and β-D-linked glucosides.

The synthesis of 4-O-β-D-glucopyranosyl-L-rhamnopyranose described here has now been published\(^{132}\).

3. 4-O-α-L-Rhamnopyranosyl-L-rhamnopyranose

4-O-α-L-Rhamnopyranosyl-L-rhamnopyranose (26) was prepared in a manner analogous to \(^{15}\) by condensing 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide (19) with \(^3\) in the presence of mercuric cyanide in acetonitrile as suggested by Kamiya et al.\(^{110}\) to give methyl 2,3-O-isopropylidene-4-O-\(-(2,3,4\text{-tri-O-acetyl-α-L-rhamnopyranosyl})\)-α-L-rhamnopyranoside (20) as shown in Figure 10. In trial condensations of 19 Kamiya et al.\(^{110}\) found that whereas the Koenigs-Knorr conditions gave mainly orthoesters the Helferich conditions produced the desired disaccharides.

Since 4-O-β-D-glucopyranosyl-L-rhamnopyranose was the only disaccharide of this series where the direct product of the condensation crystallized and since it was desired to prepare the methyl glycoside, the peracetate, the free disaccharide, the free alditol, and the alditol acetate for each disaccharide of the series, it was necessary to use crystalline intermediates for purifying the condensation mixture. As mentioned earlier
Figure 9. 4-O-D-Glucopyranosyl-L-rhamnopyranose; Proton Magnetic Resonance Spectra of the Anomeric Region
Figure 10. 4-O-α-L-Rhamnopyranosyl-L-rhamnopyranose
Condensations are never very clean reactions and thus produce multicomponent mixtures. In almost all cases there is a small amount of the other anomer formed which is similar to, and therefore difficult to separate from, the major product. Chromatography can be used for purification but it is tiresome for large quantities and not as absolute as crystalline intermediates. Thus crystalline intermediates are useful stepping stones between the direct condensation product and the desired derivatives. The crystalline intermediates are obtained on a trial and error basis by preparing small quantities of the various intermediates possible, purifying them chromatographically, and utilizing the ones that crystallize. Since each disaccharide has different crystalline intermediates the exact sequence of deblocking and derivatization procedures will vary from disaccharide to disaccharide although the same general operations are employed in each case.

For example, in the 4-O-α-L-rhamnopyranosyl-L-rhamnopyranose case removal of the isopropylidene group and subsequent acetylation gave crystalline methyl 2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamno-pyranosyl)-α-L-rhamnopyranoside (22) which served as a crystalline intermediate for the preparation of methyl 4-O-α-L-rhamnopyranosyl-α-L-rhamnopyranoside (23). In fact 22 is also a crystalline derivative since it could be prepared from the isolated disaccharide although it is not a commonly used derivative. The first crystalline intermediate is also used to calculate the yield of the condensation as no meaningful numbers can be arrived at until a pure product is obtained. In this case 22 was obtained in 65% yield based on 3. This yield could probably be increased by using benzobromorhamnose rather than acetobromorhamnose (19) as Kamiya et al. obtained higher yields when the benzoyl analog was employed. Benzobromo-
mannose has also been reported\textsuperscript{95} to give better yields and higher stereo-selectivity than acetobromomannose so was chosen for the synthesis of 4-0-\alpha-D-mannopyranosyl-L-rhamnopyranose (38). Although a higher yield (76%) was obtained, benzoates introduce their own difficulties as will be mentioned later. So although acetates may not produce as high a yield as benzoates they offer a more attractive synthetic sequence.

The methyl glycoside 23 obtained by deacetylation of crystalline 22 was subjected to methylation and periodate oxidation studies to substantiate the 1,4 linkage. As for subsequent disaccharides it was decided to characterize the fragments of the permethylated disaccharide 24 only as their alditol acetates. The alditol acetates were chosen so that each sugar would result in only one compound and hence give only one peak when subjected to gas-liquid chromatographic analysis. They also lend themselves to mass spectrometric analysis and the authentic standards i.e. 7 were available.

Albersheim et al.\textsuperscript{133} have devised a procedure for the combined reduction and acetylation of aldoses which circumvents the isolation of the free alditol. It involves destroying the excess borohydride with acetic acid and removing the borate as methyl borate thus leaving the sodium acetate to catalyze the subsequent acetylation with acetic anhydride. It was assumed that this would provide a convenient method for the preparation of the methylated alditol acetates. However, when this method was employed three peaks designated Peak 1, Peak 2, and Peak 3 in a 4:1:3 ratio respectively rather than the expected two peaks were observed on gas-liquid chromatographic analysis. Peak 1 and Peak 3 were identified as 1,5-di-0-acetyl-2,3,4-tri-0-methyl-L-rhamnitol and 1,4,5-tri-0-acetyl-2,3-di-0-methyl-L-rhamnitol (7) respectively by gas-liquid chromatographic and mass spectro-
metric comparison with authentic standards.

Peak 2 has been assigned the structure shown below on the basis of the mass spectrum, the proton magnetic resonance spectrum, and the product obtained after methanolysis and subsequent acetylation.

The mass spectrum obtained showed peaks at m/e 161, 131, 117, 101, and 71 indicating Fragment 1. It also contained m/e 87, possibly indicating Fragment 2. The peak at m/e 143 which arises from Fragment 3 in \(7\) could also arise from the proposed structure.

The proton magnetic resonance spectrum showed the presence of two acetate substituents and four methyl substituents per rhamnose moiety. The fact that Peak 2 can be transformed into \(7\) by methanolysis and subsequent acetylation also supports this structure. Consequently it was concluded that 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol and 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (7) were obtained in an equimolar ratio thus sub-
stantiating the 1,4 linkage, but that this combined reduction acetylation method when applied to the preparation of methylated alditol acetates does not produce quantitative results. The hydrolyzates of the permethylated derivatives of the other disaccharides in this series were also subjected to this procedure and in each case a portion of the 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol appeared as this (1,5-di-O-acetyl-2,3-di-O-methyl-L-rhamnityl)-4-dimethyl borate, although the ratios varied slightly from disaccharide to disaccharide. Therefore, the phenomenon is not associated with the other compounds present but only with 2,3-di-O-methyl-L-rhamnose itself. Albersheim et al.\textsuperscript{133} noted that the borate present formed a complex with the alditols and suggested the addition and evaporation of five portions of methanol to remove it as methyl borate. However, this borate compound is not decomposed by successive additions and evaporation of methanol but requires methanolysis at reflux overnight for complete removal. This type of compound has since been observed in this laboratory with other methylated aldoses when subjected to this procedure. Whenever the free alditols were isolated (as was done in the scillabiose case), that is the sodium ions were removed with cation exchange resin prior to removal of the borate as methyl borate, the borate was removed completely by successive additions and evaporation of methanol and this anomalous compound did not arise. This was the procedure adopted for the subsequent disaccharides since it appears that this combined reduction acetylation procedure is not satisfactory for methylated aldoses. This is a prime example of the utility of defined systems for checking degradative procedures before they are applied to undefined systems as should this extraneous peak appear in a gas-liquid chromatographic analysis of a permethylated
polysaccharide hydrolyzate it would severely hamper meaningful interpretation.

The derivatization procedures were carried out in a manner analogous to those for 4-O-β-D-glucopyranosyl-L-rhamnopyranose and proceeded without incident. 4-O-α-L-Rhamnopyranosyl-L-rhamnopyranose (26) was characterized as crystalline 1,2,3-tri-O-acetyl-4-0-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranose (25) and as crystalline 1,2,3,5-tetra-O-acetyl-4-0-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-L-rhamnitol (28). The α-L-linkage was confirmed by the chemical shift of the non-reducing anomeric proton in the proton magnetic resonance spectra of all the derivatives prepared but particularly in the free disaccharide where the anomeric proton of the non-reducing sugar is clearly to low field and therefore axial. Although it was not possible to assign the signals from the C-6 protons to the reducing and non-reducing sugars, comparison with the C-6 protons from the reducing sugar of the other members of the series suggests that the high field signal comes from the reducing sugar in most cases.

A report of the synthesis and properties of 4-O-α-L-rhamnopyranosyl-L-rhamnopyranose described here is in press.

4. 4-O-α-D-Mannopyranosyl-L-rhamnopyranose

4-O-α-D-Mannopyranosyl-L-rhamnopyranose (38) was prepared in a manner parallel to 15 and 26 by first condensing 2,3,4,5-tetra-O-benzoyl-α-D-mannopyranosyl bromide (30) with 3 in the presence of mercuric cyanide in acetonitrile to give methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (31) as shown in Figure.
11. Benzobromomannose \textsuperscript{30} was used rather than the more common acetate analog since Gorin and Perlin\textsuperscript{95} have reported that it gives higher yields and greater stereoselectivity than acetobromomannose. However, benzoates also present some complications since deacylation with sodium methoxide produces methyl benzoate which has to be removed by steam distillation. They also tend to retard the separation by thin-layer chromatography of closely related compounds. When several benzoates are present on one molecule the product forms a hard glass and becomes too insoluble to crystallize readily as was the case with \textsuperscript{31} which was debenzoylated and acetylated to give crystalline methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-\alpha-D-mannopyranosyl)-\alpha-L-rhamnopyranoside (32) in 76\% yield based on 3.

The deblocking and derivatization sequence was essentially the same as for \textsuperscript{15}. In this case the free disaccharide \textsuperscript{38} crystallized and was also characterized as crystalline 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-\alpha-D-mannopyranosyl)-\alpha-L-rhamnopyranose (37) and crystalline 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-\alpha-D-mannopyranosyl)-L-rhamnitol (40). The alditol acetates of disaccharides frequently crystallize since they only exist in one anomeric form. Again the 1,4 linkage was substantiated by methylation and periodate oxidation studies.

Proton magnetic resonance spectroscopy has been used extensively in this work for confirming the number and type of substituents present in a molecule. It has also been used\textsuperscript{135} to determine the number of free hydroxyl groups in a molecule. The ring size can often be confirmed by proton magnetic resonance spectroscopy\textsuperscript{136,137} as will be illustrated later. As previously mentioned the other area where proton magnetic resonance spectros-
Figure 11. 4-\(\alpha\)-D-Mannopyranosyl-L-rhamnopyranose

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copy provides valuable information is in assigning the configuration of the disaccharide linkage. Proton magnetic resonance spectroscopy does not differentiate α-D- and β-D-mannopyranosides as well as it does α-D- and β-D-gluco or galactopyranosides since the coupling constant for equatorial-equatorial hydrogens does not differ substantially from that of equatorial-axial hydrogens. Also the chemical shift difference between the α-D- and β-D-anomers of mannopyranosides is not as great as for glucopyranosides or galactopyranosides. However, the pair of anomic mannopyranosides synthesized in this thesis demonstrates that there is consistently a significant small difference in the chemical shift of the anomic proton of α-D- and β-D-mannopyranosides as well as in their coupling constants as shown in Figure 12. Lindberg et al. 138-143 when determining the structure of the Salmonella cell-wall lipopolysaccharides containing these disaccharides 38 and 56 used the proton magnetic resonance spectra of the per-O-(trimethylsilyl) oligosaccharide alditols to aid the assignment of the configuration of the glucose and galactose residues but could not assign the configuration of the mannose residues by this technique, although their data are consistent with the fact that the β-D-linkages, assigned polarimetrically, resonate at least 0.2 parts per million higher than the corresponding α-D-linkages. Ballou et al. 144 assigned the configuration of mannose and 3-O-methylmannose units in a cyclic polysaccharide on the basis of the chemical shift of their anomic protons. Gorin et al. 145 used the difference between the chemical shifts of the anomic protons to distinguish α-D- and β-D-mannose residues in yeast mannans. Gahan, Sandford, and Conrad 146 assigned the configuration of D-mannose in an oligosaccharide isolated from the capsular polysaccharide of Klebsiella type 2 on the basis of the chemical shift of
Figure 12. 4-O-D-Mannopyranosyl-L-rhamnopyranose; Proton Magnetic Resonance Spectra of the Anomeric Region
the anomeric proton. Dutton et al. 69,147 have used chemical shifts to determine the configuration of D-mannose residues in the intact capsular polysaccharides of Klebsiella types 5 and 24. It seems apparent that α-D- and β-D-mannopyranosides can be distinguished by their proton magnetic resonance chemical shifts particularly when both anomers are available. In most cases the β-D-anomer resonates about 0.2 to 0.3 parts per million higher than the α-D-anomer. The actual position of the resonance depends on the derivative being examined i.e. per-O-(trimethylsilyl) derivative, permethylated derivative, or free oligosaccharide and on the position of the linkage i.e. 1,2; 1,3; 1,4; or 1,6. Therefore, in order to assign configuration from a single resonance, appropriate standards are required such as the disaccharides and their derivatives described in this thesis. As noted by Gorin et al. 145 the β-D-glycosides of mannose consistently exhibit a smaller coupling constant than the α-D-glycosides. Although the difference is not large, for small oligosaccharides it can easily be observed with the present technology. Thus proton magnetic resonance spectroscopy complements polarimetry and enzyme studies in the assignment of the configuration of oligosaccharide linkages as illustrated by 4-O-α-D-mannopyranosyl-L-rhamnopyranose and the proton magnetic resonance data presented here will extend the usefulness of proton magnetic resonance spectroscopy for the assignment of 1,4-linked α-D- and β-D-mannopyranosides.

The synthesis of 4-O-α-D-mannopyranosyl-L-rhamnopyranose described here has now been published 148.
5. 4-O-β-D-Mannopyranosyl-L-rhamnopyranose

In order to synthesize the complementary anomer of 38, namely 4-O-β-D-mannopyranosyl-L-rhamnopyranose it was necessary to develop a general method for the synthesis of β-D-mannopyranosides. It was decided to pursue the 2,3 cyclic carbonate procedure of Gorin and Perlin95. Considering the low yields obtained in many of their steps an alternative synthetic route for the preparation of 4,6-di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl bromide (44) was devised as shown in Figure 13. In view of the previous success in acetolyzing methyl glycosides it was decided to use methyl 4,6-O-benzylidene-α-D-mannopyranoside (41) rather than benzyl 6-O-triphenylmethyl-α-D-mannopyranoside for preparing the 2,3 carbonate. Compound 41 was easily prepared although not in high yield by the method described by Gibney149. Methyl 4,6-O-benzylidene-2,3-O-carbonyl-α-D-mannopyranoside (42) was prepared by modifying the procedure of Doane et al.150 for the D-gluco analog which readily produced cyclic carbonates in high yields by employing the convenient reagent ethyl chloroformate in the presence of triethylamine. The molar quantities recommended by Doane et al.150 for the D-gluco analog produced in addition to the desired product substantial quantities of the acyclic O-ethyloxy carbonyl derivatives in the D-manno series. However, if instead of adding the amount of triethylamine suggested by Doane et al. just enough triethylamine to make the reaction mixture basic was added the desired compound was essentially the only product. The addition of any more triethylamine only removes any cyclic carbonate already formed as cyclic carbonates are very base labile. This procedure worked well for small reactions (under one gram) but was less satisfactory for larger quantities.
Figure 13. Synthetic Route for the Preparation of 4,6-Di-O-acetyl-2,3-O-carbonyl-α-D-mannopyranosyl bromide

Methyl α-D-mannopyranoside → \text{PhCHO/HCOOH} → \text{ClCOOEt/Et}_3N → \text{41} → \text{Ac}_2O/H^+ → \text{43} → \text{HBr/ACOH} → \text{44}
Since it was noted (by thin-layer chromatography) that very little reaction took place until the reaction mixture became basic and since it was believed that the triethylamine should aid the removal of the hydroxylic proton, the triethylamine was combined with the starting material and the ethyl chloroformate was added dropwise instead of the reverse procedure employed by Doane et al. The molar ratios of the previous reaction were not changed, that is the amount of triethylamine to just make a certain quantity of ethyl chloroformate basic was added to the reaction mixture and that certain quantity of ethyl chloroformate was added dropwise. Doing the reaction at 0° prevents decomposition of the cyclic carbonate once it is formed. It is imperative for the reaction to proceed that the mixture remain basic. Furthermore, if it is neutral the excess ethyl chloroformate will slowly hydrolyze during the various stages of the work-up thus producing ethanol and hydrogen chloride which will hydrolyze the benzylidene group and basic neutralization procedures will remove the cyclic carbonate. Using this modified procedure a very good yield of a substantial quantity of cyclic carbonate 42 was easily obtained. The large difference between the optical rotations of 41 (+64.3°) and 42 (-19.3°) is attributed to a modification of the conformation of the pyranose chair towards a boat when a five-membered carbonate ring is superimposed.

Acetolysis of 42 provides 1,4,6-tri-O-acetyl-2,3-O-carbonyl-α-D-mannopyranose (43). The mild acetolysis conditions used (0.75% (v/v) concentrated sulfuric acid in acetic anhydride for two hours) replace the methyl glycoside and the benzylidene group with acetates while leaving the cyclic carbonate intact. Acetolysis furnishes a method by which 42 can be converted to 43 without a change in ring form. It has been shown that
2,3-O-carbonyl-D-mannose exists in the furanose form since a five-membered ring fused to a five-membered ring is more stable than a five-membered ring fused to a six-membered ring. Therefore, if at any time C-1 and C-4 were simultaneously free the mannoapyranose ring with the fused five-membered carbonate ring would convert to a mannofuranose system. The mechanism of acetolysis is such that a position is blocked with an acetate as it is relieved of its previous substituent so that no position ever had a free hydroxyl substituent during acetolysis. This permits replacement of the benzylidene group and the methyl glycoside with acetates while preserving the pyranose ring form. The pyranose ring structure is substantiated by the proton magnetic resonance spectrum \( \text{H-5} \) will be geminal to an acetoxy group and hence downfield of H-4 but in the pyranose form H-4 will be geminal to an acetoxy group and hence downfield relative to H-5 as is observed for 43. The discrepancy between the optical rotation presented here and that of Gorin and Perlin\(^95\) is probably due to the fact that they did not have a pure anomer.

4,6-Di-O-acetyl-2,3-O-carbonyl-\( \alpha \)-D-mannopyranosyl bromide (44) was prepared from 43 essentially as described by Gorin and Perlin\(^95\). The reaction time was extended somewhat since reactions worked up after two hours often contained a small amount of starting material which was difficult to remove as it cocrystallized with the product. It was found advantageous to filter the chloroform extract of 44 through calcium oxide and silica gel to remove remaining traces of acid which lead to decomposition. The synthetic sequence presented here permits the preparation of the valuable bicyclic bromide 44 in four easy steps.

Condensation conditions which engender a \( S_N^2 \) type mechanism for the
replacement of the bromine of 44 by an incoming nucleophile thus producing net inversion are required for the formation of β-D-mannopyranosides from this α-D-bromide with a nonparticipating group on C-2 and C-3. If a free carbonium ion is produced at C-1 the thermodynamically more stable α-D-mannopyranosides will be formed since both the anomeric effect and the steric relationships favor α-D-mannopyranosides. The use of tremendous excesses of nucleophile facilitates the operation of the $S_N^2$ type mechanism. Condensation of the bromide 44 in the presence of mercuric cyanide in acetonitrile produces β-D-mannopyranosides stereoselectively only when a relatively strong nucleophile such as methanol is used in excess as was done for the preparation of crystalline methyl 4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranoside (45) in 87% yield. Again the discrepancy between the optical rotation obtained here and that of Gorin and Perlin95 is probably due to the fact that they did not obtain a crystalline product and therefore a pure anomer. Compound 45 was deacylated to give methyl β-D-mannopyranoside (46) which was characterized as its crystalline isopropylate and as its crystalline peracetate 47.

When a monosaccharide nucleophile such as 3, even in ten-fold excess, was condensed with the bromide 44 in the presence of mercuric cyanide in acetonitrile equal amounts of α-D- and β-D-mannopyranosides were produced and when equimolar ratios were condensed the α-D-anomer was produced almost exclusively. The ratios of anomers presented here were those observed by thin-layer chromatography of the reaction product. The β-D-anomer has a significantly smaller $R_f$ value than the α-D-anomer. Thin-layer chromatography was also used for monitoring the progress of the reaction153. Also the fact that the condensation of 44 with methanol in equimolar quantities
in the presence of mercuric cyanide in acetonitrile gave largely the α-D-anomer indicates the inappropriateness of these condensation conditions for the synthesis of β-D-mannopyranosides. The use of other solvents such as nitromethane and benzene instead of acetonitrile increases the percentage of the β-D-anomer (up to 70% when equimolar ratios were employed) but does not produce a stereoselective reaction. Consistently the addition of ethyl acetate to any condensation conditions tried increased the percentage of the α-D-anomer.

Gorin and Perlin\textsuperscript{95} used silver oxide and iodine in chloroform and a ten-fold excess of aglycon to obtain a stereoselective reaction with 44. These conditions also produce the desired product when 3 is used as the aglycon but the use of a ten-fold excess of aglycon is most impractical for the synthesis of disaccharides. The use of large excesses of aglycon is acceptable only when the aglycon is easily obtained and when the excess is readily separated from the product such as is the case with methanol. When the aglycon is a specifically substituted monosaccharide this is not the case so the necessity of using large excesses of aglycon makes the method unmanageable except for the preparation of very small quantities of product. When the aglycon is not present in excess these condensation conditions (silver oxide and iodine in chloroform) produce \( \sim 75\% \) β-D-mannopyranosides; however, if the iodine is omitted the condensation proceeds with stereoselective inversion. The use of mercuric acetate in benzene (Zemplen reaction) also provides condensation conditions which generate stereoselective inversion and hence β-D-mannopyranosides from 44 when the aglycon is present in an equimolar ratio, but more bromide degradation products are formed than when silver oxide in chloroform is employed.
Preliminary condensation experiments of 44 with other aglycons such as 1,2,3,4-tetra-O-acetyl-β-D-glucopyranose and 1,2;3,4-di-O-isopropylidene-α-D-galactopyranoside in the presence of silver oxide in chloroform indicated that again the product was the β-D-anomer thus confirming the generality of the method for the preparation of β-D-mannopyranosides.

Consequently 44 was condensed with 3 in the presence of silver oxide in chloroform with anhydrous calcium sulfate as an internal desiccant to give the desired methyl 4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-manno-pyranosyl)-2,3-O-isopropylidene-α-L-rhamnopyranoside (48) as shown in Figure 14. Alcohol-free chloroform is required for these condensations since any primary alcohol of low molecular weight such as ethanol would condense preferentially with the bromide thus producing unwanted glycosides. The α-D-anomer was formed in less than 5% yield as it was just detectable on thin-layer chromatography but not by proton magnetic resonance spectroscopy.

As before, the synthetic sequence was determined by the crystalline intermediates and derivatives. In this case removal of the isopropylidene group to give methyl 4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (49) and subsequent acetylation gave the crystalline intermediate methyl 2,3-di-O-acetyl-4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (50) in 73% yield based on 3. Deacylation of 50 gave methyl 4-O-β-D-mannopyranosyl-α-L-rhamnopyranoside (51) which crystallized as an isopropylate containing two moles of 51 per mole of 2-propanol. Methylation and periodate oxidation studies of 51 confirmed the 1,4 linkage. Acetylation of 51 gave sirupy methyl 2,3-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (53).
Figure 14. 4-O-β-D-Mannopyranosyl-L-rhamnopyranose

\[ \text{Diagram of molecular structures} \]

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<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
<th>( R_4 )</th>
<th>( R_5 )</th>
<th>( R_6 )</th>
<th>( R_7 )</th>
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<td>( 48 )</td>
<td>( \text{Me} )</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
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<tr>
<td>( 50 )</td>
<td>( \text{Me} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
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<tr>
<td>( 51 )</td>
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<td>( \text{Me} )</td>
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<tr>
<td>( 53 )</td>
<td>( \text{Me} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
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<tr>
<td>( 54 )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
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<tr>
<td>( 55 )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
<td>( \text{Ac} )</td>
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<tr>
<td>( 56 )</td>
<td>( \text{H} )</td>
<td>( \text{H} )</td>
<td>( \text{H} )</td>
<td>( \text{H} )</td>
<td>( \text{H} )</td>
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</tbody>
</table>
For this disaccharide the most direct route to the peracetate 55 (1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose) appeared to be the acetolysis of the methyl glycoside 51. On the basis of the previous success of the acetolysis of methyl 4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (33) to give the corresponding peracetate 37 it was assumed that the acetolysis of 51 would conveniently provide 55. However, the acetolysis of either 51 or its peracetate 53 gave two compounds in approximately equal amounts as indicated by thin-layer chromatography. Proton magnetic resonance spectroscopy identified the slower moving compound as the desired peracetate 55. The ratio of the two components could be varied by varying the concentration of the sulfuric acid and the amount of acid relative to the amount of starting material but at no time could the desired product be obtained in more than 65% yield.

The faster moving component on thin-layer chromatography was identified as 1,2,3-tri-O-acetyl-4-O-(1,2,3,4,5,6-hexa-O-acetyl-aldehydo-D-mannosyl)-α-L-rhamnopyranose on the basis of the proton magnetic resonance spectrum, the mass spectrum, and the products obtained on deacylation. The structure is shown below.
The proton magnetic resonance spectrum indicated the presence of nine acetate groups per rhamnose moiety and also that both anomeric protons were very low field (τ 4.05) and therefore probably both geminal to acetoxy groups. The mass spectrum contained peaks at m/e 43, 85, 115, 145, 157, 229, 289, and 331 indicative of the peracetylated acyclic mannose portion of the molecule and a peak at m/e 273 corresponding to the rhamnose portion of the molecule. The fact that this compound exhibits one spot on thin-layer chromatography (appearing olive green indicating a hybrid of rhamnose and mannose) but when deacetylated gives monomeric rhamnose and mannose supports this hemiacetal structure since hemiacetals hydrolyze in base whereas full acetals (glycosides) do not.

This compound presumably arises from attack of the acetylium ion on the ring oxygen rather than on the C-1 oxygen as shown in Figure 15. Attack at the C-1 oxygen would lead to cleavage of the disaccharide linkage and concurrent acetylation which is the phenomenon normally observed in the acetolysis of polysaccharides. Acetolysis at the reducing anomeric carbon proceeded as in the previous disaccharides by replacing the methyl glycoside with an acetate group.

The three cis substituents on C-1, C-2, and C-3 of β-D-mannopyranosides create a sterically strained system which is relieved when the ring opens to form the acyclic compound. Another example of ring opening to relieve steric strain during acetolysis, observed in this laboratory, was the acetolysis of methyl 4,6-O-benzylidene-2,3-O-carbonyl-α-D-glucopyranoside to give 1-O-methyl-1,4,6-tri-O-acetyl-2,3-O-carbonyl-aldehyde-D-glucose. Acyclic products have also been observed in boron trifluoride catalyzed acetolyses.
Figure 15. Competing Pathways in Acetolysis
Conversely, when 50 was subjected to acetolysis only one product resulted and it was identified as the expected 1,2,3-tri-O-acetyl-4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-L-rhamnopyranose (54). The 2,3 cyclic carbonate appears to distort the conformation of the ring so as to relieve the steric crowding resulting from the three cis substituents and therefore prevents ring opening during acetolysis. This is another example of the 2,3 cyclic carbonate altering the steric relationships in its environment and consequently the course of reactions therein.

To obtain the peracetate 55 it was now necessary to deaclylate 54 and acetylate it. The latter step was achieved with pyridine and acetic anhydride but the resulting peracetate contained a substantial quantity of the sirupy β-L-anomer so was anomerized with zinc chloride\textsuperscript{159,160} to give crystalline 55. The acetylation could have been done directly with zinc chloride in acetic anhydride but the free disaccharide did not dissolve sufficiently in zinc chloride and acetic anhydride at room temperature and when heated considerable degradation was observed. The anomerization proceeded smoothly at room temperature. No doubt this anomerization procedure could have been used for other compounds in this thesis to improve the yield of crystalline product such as 14 or 43. The free disaccharide 4-O-β-D-mannopyranosyl-L-rhamnopyranose (56) could be obtained directly from 54 but was preferably obtained from crystalline 55 by deacetylation. The derived alditol 4-O-β-D-mannopyranosyl-L-rhamnitol (57) and its peracetate 58 were prepared by the usual procedures.

Thus the method of Gorin and Perlin\textsuperscript{95} for the synthesis of β-D-mannopyranosides has been refined and is being extended to other aglycons.
6. Conclusions

The uniqueness of this work lies not only in the syntheses of four new disaccharides of potential immunological significance but also in the thoroughness with which these syntheses have been investigated. Many disaccharides are synthesized with the sole objective of acquiring a small quantity of a certain disaccharide for a specified purpose with no exploration into determining the best general method for the synthetic sequence employed. In this work an attempt was made to improve the methods used for the synthesis and characterization of these disaccharides as well as to obtain new products. This involved comparing various reaction conditions in order to determine the ones which produced the maximum yield of the desired product most conveniently. These refined procedures can then be extended to other systems. Another requirement for an optimal yield is an adequate method of isolation which is fulfilled by chromatography or more attractively by crystallization. Thus much time and effort has been spent in obtaining the many crystalline intermediates and derivatives presented here. The improved reaction conditions combined with the ease and efficiency of isolation provided by crystalline intermediates allows the synthesis of these disaccharides in sufficient quantity to furnish defined systems for examining the reliability of, and selecting the optimal procedures for, the methods of structural elucidation both instrumental and chemical as well as to prepare and characterize several derivatives to serve as standards for the identification of isolated polymer fragments as shown in Figure 16.

In the course of this detailed synthetic study many noteworthy points have been brought to light. For example, the homogeneous system,
Figure 16. Physical Properties of Disaccharides and Derivatives

<table>
<thead>
<tr>
<th>Glc $\beta$</th>
<th>Rha</th>
<th>Glc $\alpha$</th>
<th>Rha</th>
<th>Man $\beta$</th>
<th>Rha</th>
<th>Man $\alpha$</th>
<th>Rha</th>
<th>m.p.</th>
<th>[a]$_D$ (H$_2$O)</th>
<th>[a]$_D$ (CHCl$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Methyl glycoside</td>
<td>-58°</td>
<td>-109°</td>
<td>13°</td>
<td>-74.8°</td>
<td>[a]$_D$</td>
<td>23 (H$_2$O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.30, 7.7</td>
<td>4.81,1.8</td>
<td>5.01,1.8</td>
<td>5.16,0.9</td>
<td>τ H-1', J$_1$,2', (D$_2$O)</td>
<td>2.9</td>
<td>4.0</td>
<td>2.9</td>
<td>1.44</td>
<td>R$_{glucose}$ (solvent C)</td>
<td></td>
</tr>
<tr>
<td>139-140°</td>
<td>162-163°</td>
<td>149.5-150.5°</td>
<td>164-165°</td>
<td>m.p.</td>
<td>[a]$_D$</td>
<td>23 (CHCl$_3$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peracetate</td>
<td>-62.3°</td>
<td>-63.6°</td>
<td>-5.5°</td>
<td>-67.8°</td>
<td>[a]$_D$</td>
<td>23 (CHCl$_3$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>132-133°</td>
<td>138.5-139.5°</td>
<td>84-85°</td>
<td>m.p.</td>
<td>[a]$_D$</td>
<td>23 (CHCl$_3$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free disaccharide</td>
<td>-24°</td>
<td>-68°</td>
<td>60.3°</td>
<td>-46°</td>
<td>[a]$_D$</td>
<td>23 (H$_2$O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.26,7.5</td>
<td>4.82,1.7</td>
<td>5.03,1.8</td>
<td>5.20,0.9</td>
<td>τ H-1', J$_1$,2', (D$_2$O)</td>
<td>1.2</td>
<td>2.2</td>
<td>1.0</td>
<td>0.6</td>
<td>R$_{glucose}$ (solvent C)</td>
<td></td>
</tr>
<tr>
<td>Free alditol</td>
<td>-50°</td>
<td>57°</td>
<td>-36°</td>
<td>[a]$_D$</td>
<td>23 (H$_2$O)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.37,7.2</td>
<td>5.02,1.7</td>
<td>4.89,1.9</td>
<td>5.26,0.9</td>
<td>τ H-1', J$_1$,2', (D$_2$O)</td>
<td>0.8</td>
<td>1.3</td>
<td>0.5</td>
<td>0.5</td>
<td>R$_{glucose}$ (solvent C)</td>
<td></td>
</tr>
<tr>
<td>Alditol peracetate</td>
<td>-78.6°</td>
<td>-67.2°</td>
<td>-4.4°</td>
<td>-67°</td>
<td>[a]$_D$</td>
<td>23 (CHCl$_3$)</td>
<td></td>
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</table>
mercuric cyanide in acetonitrile, is a superior condensing agent for the synthesis of 1,2 \textit{trans} glycosides aided by a participating group on C-2. Some isopropylidene groups are not stable in the presence of mercuric bromide in acetonitrile. The fact that secondary-secondary isopropylidene groups cannot be acetolyzed and that sterically strained systems can ring open during acetolysis to relieve that strain contributes to the pool of information about acetolysis which will eventually lead to an encompassing mechanism. An excellent method for the quantitation of periodate oxidation degradation products has been elaborated and boron containing compounds were discovered when the borate was not adequately removed after reduction with sodium borohydride.*

This work demonstrates the exceptional ability of the cyclic carbonate group to act not only as a nonparticipating blocking group but more importantly as an implement for altering the steric relationships around it so as to allow otherwise inaccessible reactions to take place. The evidence for this is provided by the preparation of $\beta$-D-mannopyranosides from 44 whereas they could not be prepared from 3,4,6-tri-\textit{O}-acetyl-2-\textit{O}-benzyl-\textit{a}-D-mannopyranosyl bromide and by the acetolysis of 50 to give the desired product whereas the acetolysis of 51 or 53 led to a different product. An amended procedure for the preparation of cyclic carbonates has also been devised. The most significant development in this work has been the facile entry into the $\beta$-D-mannopyranoside series presented here. The method of Gorin and Perlin* has been promoted to a practicable procedure.

*Since writing this thesis it has been noted that carbohydrate borate esters have been prepared and examined [Ya. Ya. Makarov-Zembyanskii and V.V. Gertsev, \textit{Zh. Obshch. Khim.}, 35 (1965) 272; \textit{Chem. Abstr.}, 62 (1965) 13216 g].
III EXPERIMENTAL

General Methods

Melting points were obtained for samples between glass slides on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter at 23 ± 1°. Proton magnetic resonance (p.m.r.) spectra were recorded on a Varian XL-100 instrument, with tetramethylsilane as the internal standard, except as noted. The integrated area, multiplicity, and type of proton are indicated in parentheses. Gas-liquid partition chromatography (g.l.c.) was conducted with an F and M 720 instrument equipped with dual, thermal-conductivity detectors using helium as a carrier gas at a flow-rate of 60 ml/min, with the following columns: (a) 2 ft x 0.25 in. of 20% of SE-30 (F and M Division, Hewlett Packard, Avondale, Pennsylvania), (b) 4 ft x 0.25 in. of 5% of butanediol succinate on Diatoport S (80 - 100 mesh), and (c) 6 ft x 0.25 in. of 15% of OS-138 on Gas Chrom Q (100 - 120 mesh). Peak areas were determined with an Infotronics CRS-100 electronic integrator. Mass spectra were recorded either with a Micromass 12 gas-liquid chromatography-mass spectrometer, or on an AEI MS 9 instrument. Thin-layer chromatography (t.l.c.) was performed with solvent systems A and B on silica gel G (from EM Reagents, type 60); solvent A, ethyl ether-toluene (2:1); solvent B, butanone-water azeotrope. The plates were dried and components were detected by spraying with 35% ethanolic sulfuric acid and heating for 3 - 5 min at ~ 150°. Paper-chromatographic separations were conducted on Whatman No. 1 paper with the upper layer of solvent systems C and D; solvent C, ethyl acetate-pyridine-
-water (4:1:1); solvent D, 1-butanol-ethanol-water (4:1:5). Zones were made visible by using silver nitrate in acetone for reducing and non-reducing underivatized compounds and p-anisidine in trichloroacetic acid for methylated reducing sugars. \( R_G \) indicates with respect to 2,3,4,6-tetra-O-methyl-D-glucose. Microanalyses were performed by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia, Vancouver. Solutions were evaporated below 50° under diminished pressure.

Dry solvents where indicated were prepared as follows:

- methanol - dried over sodium metal and then distilled,
- acetone - dried with anhydrous calcium sulfate and then distilled,
- pyridine - dried by refluxing with solid potassium hydroxide, distilled, and stored over potassium hydroxide pellets,
- acetic anhydride - fractionally distilled or used as received from Mallinckrodt (analytical reagent),
- acetonitrile - stirred with calcium hydride and then distilled,
- methyl sulfoxide - stirred with calcium hydride overnight, distilled under diminished pressure, and stored over Linde type 4A molecular sieves,
- ethyl ether (anhydrous) - used as received from Mallinckrodt,
- petroleum ether (b.p. 65-70°) - dried over sodium metal and distilled,
- benzene - refluxed over sodium metal and then distilled,
- chloroform (alcohol-free) - prepared according to Reynolds and Evans. 
Descriptions of typical recurring operations are described in detail on the pages indicated below and thereafter are merely cited. The actual amounts of reagents were adjusted to the quantity of substrate to preserve the same molar ratios.

- acetylation with pyridine, compound 2, page 68
- deacetylation (deacetylation), compound 3, page 69
- deacetalation, compound 4, page 69
- reduction, compound 6, page 72
- acetylation with sodium acetate, compound 8, page 73
- Helferich condensation (work-up), compound 10, page 74
- methylation, compound 12, page 75
- periodate oxidation, page 77
- acetolysis (work-up), compound 14, page 78
- trimethylsilylation, compound 15, page 79

**Methyl α-L-rhamnopyranoside (1)**

Compound (1) was prepared essentially as described by Levene and Muskat. L-Rhamnose monohydrate (10.0 g, commercial preparation, from Eastman Kodak) was dissolved in hydrogen chloride in methanol (0.4 M, 1.6%, 100 ml, prepared by adding 2.6 ml of acetyl chloride to 100 ml of methanol) and refluxed for 2 h. The cooled solution was rendered neutral to pH indicator paper with lead carbonate filtered, and evaporated to a thick sirup. The remaining lead chloride was removed by dissolving the sirup in ethyl acetate (13 ml) and filtering. The product crystallized from the ethyl acetate immediately upon being seeded; yield 7.80 g (80%). Recrystallization from ethyl acetate gave pure 1, m.p. 108.5 - 109°; \([\alpha]_D^0 - 60.4°\) (c 9.6, water); lit. \[164\] m.p. 109 - 110°, \([\alpha]_D^{20} - 62.48°\) (c 10.2, water),
lit. 165 m.p. 108 - 109°, $[\alpha]_D^{20} - 62.5^\circ$ (c 9.1, water); $R_f$ 0.39 (solvent B); p.m.r. (D$_2$O, external tetramethylsilane): $\tau$ 5.36 (1 H doublet, $J_{1,2}$ 1.5 Hz, H-1), 6.64 (3 H singlet, OMe), 8.75 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

**Methyl 4-0-acetyl-2,3-0-isopropylidene-α-L-rhamnopyranoside (2)**

A solution of 1 (5.00 g, finely powdered) in dry acetone (150 ml) was magnetically stirred for 5 h in an ice-water bath with 2,2-dimethoxy-propane (50 ml) and Amberlite IR-120 (H$^+$) resin (10 ml, freshly regenerated, soaked in dry methanol overnight). The resin was removed by filtration through a layer of calcium oxide to remove remaining traces of acid and the filtrate was evaporated to a sirup. T.l.c. (solvent B) showed a major component with $R_f$ 0.76 and a small amount of starting material. The sirupy product was acetylated with dry pyridine (30 ml) and acetic anhydride (30 ml) overnight at room temperature. The excess acetic anhydride was removed as ethyl acetate by successive additions and evaporations of ethanol. The excess pyridine was removed as an azeotrope by successive additions and evaporations of water. Insoluble impurities resulting from pyridine decomposition were removed by dissolving the dried sirup in ethyl acetate and filtering. The resulting acetate 2 crystallized from ethanol (25 ml); yield 4.90 g (67%). Recrystallization from ethanol gave pure 2, m.p. 66 - 67°; $[\alpha]_D - 16.5^\circ$ (c 2.4, chloroform); lit. 166 m.p. 61 - 63°, $[\alpha]_D - 14.5^\circ$ (c 2.8, chloroform); $R_f$ 0.69 (solvent A); p.m.r. (CDCl$_3$): $\tau$ 5.10 (1 H singlet, H-1), 6.62 (3 H singlet, OMe), 7.92 (3 H singlet, OAc), 8.44, 8.66 (3 H singlets, CMe$_2$), 8.83 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).
Methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (3)

Compound 2 (5.90 g) was deacetylated with sodium methoxide in dry methanol (0.2 M, 30 ml, prepared by reacting sodium metal (0.5 g) with methanol (100 ml) for 1 h at room temperature. Sodium ions were quickly removed from the chilled solution with Amberlite IR-120 (H\(^+\)) resin (60 ml), rinsed with methanol and the remaining traces of acid were removed with Duolite A-4 (OH\(^-\)) resin (rinsed with methanol). The sirup obtained on filtration and evaporation showed one spot on t.l.c., \(R_f\) 0.54 (solvent A); yield 4.90 g (99%); [\(\alpha\)]\(_D\) - 29.5° (c 5.0, chloroform), [\(\alpha\)]\(_D\) - 16.4° (c 3.1, acetone); lit.\(^{101}\) [\(\alpha\)]\(_D\)\(^{24}\) - 14.1° (c 1.5, water), lit.\(^{107}\) m.p. 36°, [\(\alpha\)]\(_D\)\(^{20}\) - 15.9° (c 1.6, acetone); p.m.r. (CDCl\(_3\)): \(\tau\) 5.14 (1 H singlet, H-1), 6.62 (3 H singlet, OMe), 8.48, 8.65 (3 H singlets, endo-, exo- OMe\(^2\))\(^{167}\), 8.70 (3 H doublet, \(J_{5,6}\) 6 Hz, CH\(_3\)).

Methyl 4-O-acetyl-α-L-rhamnopyranoside (4)

A solution of compound 2 (3.00 g) in chloroform (135 ml) was deacetylated with trifluoroacetic acid containing 1% (v/v) water (15 ml) for 1 h at room temperature. The reaction mixture was then concentrated, and remaining trifluoroacetic acid was removed by addition and evaporation of toluene. The resulting sirup crystallized neat; yield 2.50 g (98%). It was further purified by recrystallization from ethyl acetate (50 ml) or by
sublimation, m.p. 117°; $[\alpha]_D$ - 104.1° ($c$ 2.2, chloroform), $[\alpha]_D$ - 56.0° ($c$ 2.8, water); lit. 166 m.p. 112 - 116°, $[\alpha]_D$ - 55° ($c$ 1.8, water); $R_f$ 0.66 (solvent B); p.m.r. (CDCl$_3$): $\tau$ 5.30 (1 H doublet, $J_{1,2}$ 1.5 Hz, H-1), 6.64 (3 H singlet, OMe), 7.90 (3 H singlet, OAc), 8.80 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).


Methyl 2,3-di-O-methyl-α-L-rhamnopyranoside (5)

Compound 4 (0.50 g) in dichloromethane (5 ml) was cooled in an ice-salt water bath (-5°). Boron trifluoride etherate solution (1 ml, prepared by adding 0.4 ml of boron trifluoride etherate to 10 ml of dichloromethane) was added and then diazomethane in dichloromethane (at -78°, prepared according to Vogel 168 except that dichloromethane was substituted for ether) was added dropwise while swirling the flask until a faint yellow color persisted. The reaction mixture was kept at -5° for 30 min and then allowed to warm up. The solid polymethylene was removed by filtration and all volatile components were removed from the filtrate by evaporation. T.l.c. (solvent A) showed a major spot at $R_f$ 0.47 with a minor monomethyl component at $R_f$ 0.29. The product was purified by a preparative t.l.c. separation 169 (solvent A); yield 0.45 g (80%); p.m.r. (CDCl$_3$): $\tau$ 5.25 (1 H doublet, $J_{1,2}$ 1.5 Hz, H-1), 6.49, 6.58, 6.61 (3 H singlets, 3 OMe), 7.93 (3 H singlet, OAc), 8.80 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$). The methyl 4-O-acetyl-2,3-di-O-methyl-α-L-rhamnopyranoside (0.45 g) was deacetylated in the usual way (see page 69) to give 5 as a sirup; yield 0.36 g (96%), $[\alpha]_D$ - 6° ($c$ 2.0, water), $[\alpha]_D$ - 28° ($c$ 2.0, chloroform); lit. 170 $[\alpha]_D$ - 6° ($c$ 2.0, water), lit. 171 $[\alpha]_D$ - 14° ($c$ 1.97), lit. 172 $[\alpha]_D$ - 26.2° ($c$ 0.3, chloro-
form); \( R_f \) 0.24 (solvent A); p.m.r. (CDCl\(_3\)): \( \tau \) 5.19 (1 H doublet, \( J_{1,2} \) 1.5 Hz, H-1), 6.46, 6.48, 6.57 (3 H singlets, 3 OMe), 8.65 (3 H doublet, \( J_{5,6} \) 6 Hz, CH\(_3\)).

A portion of 5 (0.20 g) was dissolved in dry pyridine (0.2 ml) and treated with p-toluenesulfonyl chloride (0.3 g, recrystallized from benzene-petroleum ether) at 55° for 24 h. T.l.c. (solvent A) showed the reaction to be complete with one spot of \( R_f \) 0.57. A few drops of water were added to the reaction mixture which was left standing for 1 h and then diluted with chloroform (100 ml). The chloroform extract was successively washed with water (2 x 75 ml), saturated sodium hydrogen carbonate solution (2 x 75 ml), hydrochloric acid (0.5 \( M \), 2 x 75 ml) and water (3 x 75 ml). The syrup obtained on evaporation crystallized from 2-propanol (3 ml); yield 0.30 g (86%). Recrystallization from 2-propanol gave pure methyl 2,3-di-O-methyl-4-O-toluene-p-sulphonyl-\( \alpha \)-L-rhamnopyranoside, m.p. 114°; \([\alpha]_D\) \(-36.7°\) (c 2.6, chloroform); lit. \(^{170}\) m.p. 111°, \([\alpha]_D\) \(-33°\) (c 2.0, chloroform); p.m.r. (CDCl\(_3\)): \( \tau \) 2.12 - 2.74 (4 H, \( \delta \)H); 5.30 (1 H doublet, \( J_{1,2} \) 1.5 Hz, H-1), 6.56, 6.68, 7.06 (3 H singlets, 3 OMe), 7.58 (3 H singlet, \( \delta \)Me), 8.67 (3 H doublet, \( J_{5,6} \) 6 Hz, CH\(_3\)).


2,3-Di-O-methyl-L-rhamnose (6)

The methyl glycoside 5 (0.30 g) was hydrolyzed with trifluoroacetic acid (2 \( M \), 30 ml) by refluxing for 5 h. The syrup (0.26 g, 93%) obtained on evaporation showed one spot on t.l.c. \( R_f \) 0.41 (solvent B) and on paper
chromatography gave a reddish-brown spot with $R_G$ 0.85 (solvent D) when
sprayed with aniline trichloroacetate spray; $[\alpha]_D^1$ 42° ($c$ 1.5, water); 
$[\alpha]_D^1$ lit. 40° ($c$ 0.7, water), lit. $R_G$ 0.83.

Compound 6 (0.20 g) was reduced with sodium borohydride (0.1 g)
in water (3 ml) overnight. Passage through Amberlite IR-120 (H$^+$) resin
to remove the sodium ions, concentration, and addition and evaporation of
methanol (5 x 50 ml) to remove the borate as methyl borate gave 2,3-di-O-
methyl-L-rhamnitol which was acetylated with pyridine and acetic anhydride
(see page 68) to give 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (7)
(0.30 g, 90%); p.m.r. (CDCl$_3$): $\delta$ 6.55, 6.63 (3 H singlets, 2 OMe), 7.87, 7.90,
7.94 (3 H singlets, 3 OAc), 8.75 (3 H doublet, J$_{5,6}$ 6 Hz, CH$_3$).
Injection of 7 in ethyl acetate onto column b programmed from 150 to 200°
at 2°/min gave a major peak at 24.4 min and on column c at 225° gave a
major peak at 38.6 min. A sample was collected and the mass spectrum obtained
showed fragments at m/e 43, 44, 45, 58, 59, 69, 71, 74, 85, 87, 101,
113, 117, 143, 161, and 203.

4-Deoxy-L-erythritol (1-deoxy-D-erythritol) (8)

Reaction of 4 (2.40 g) with sodium metaperiodate (0.05 M, 400 ml)
at 5° in the dark reached a constant uptake of 1 mole per mole of sugar
(monitored by titration with sodium thiosulfate) in 20 h. The iodate
and excess periodate were precipitated with barium acetate solution and
removed by centrifugation. The residual polyaldehyde was deacetylated by
standard procedures (see page 69) and reduced with sodium borohydride (2 g)
ownight at 5°. The polyalcohol, obtained after the usual work-up (see
Glycolaldehyde dimethyl acetal and solvent were evaporated and the resulting crude sirup (1.10 g, 95%) gave a small positive rotation (\( \epsilon = 1.6 \), methanol); lit.\(^{176} \) \([\alpha]_D^7 = 7.4^\circ\) (\( \epsilon = 2 \), methanol); \( R_f = 0.57 \) (solvent D); lit.\(^{177} \) \( R_f = 0.53 \) (solvent D). A portion (0.05 g) was acetylated with sodium acetate (0.1 g) and acetic anhydride (1 ml) for 30 min on the steam bath. The excess acetic anhydride was removed as ethyl acetate by successive additions and evaporations of ethanol. The addition of ethyl acetate (15 ml) and subsequent filtration removed the sodium acetate. Evaporation of most of the ethyl acetate and injection onto column b at 140° gave a major peak at 12 min. The mass spectrum was obtained and showed fragments at m/e 43, 44, 70, 71, 86, 87, 103, 115, 117, 130, 145, 159, and 189.

Another portion of 8 (1.00 g) in dry pyridine (60 ml) was treated with \( p \)-nitrobenzoyl chloride (5.6 g) for 24 h at room temperature (initially warmed to aid solution). The reaction mixture was diluted with a few drops of water and left stand for 2 h. The pyridine was evaporated under diminished pressure and the resulting sirup triturated with saturated sodium hydrogen carbonate solution. The aqueous phase was decanted and the crystalline residue dissolved in chloroform (100 ml) and washed with saturated sodium hydrogen carbonate solution (2 x 100 ml) and then with water (2 x 200 ml). The amorphous residue obtained on evaporation crystallized from acetone (10 ml); yield 2.70 g (52%). Recrystallization from acetone gave pure 4-deoxy-L-erythritol tri-\( p \)-nitrobenzoate, m.p. 161 - 162°; \([\alpha]_D^{1}\) 81.9° (\( \epsilon = 2.2 \), chloroform); lit.\(^{177} \) m.p. 159 - 160°, \([\alpha]_D^{1}\) 81.07° (\( \epsilon = 2.05 \), chloroform).

Anal. Calcd. for \( C_{25}H_{19}O_{12}N_3 \): C, 54.26; H, 2.36; N, 7.59.
Found: C, 54.08; H, 3.31; N, 7.81.

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (9)

Compound 9 was prepared from anhydrous D-glucose either as reported by Lemieux in 68% yield or more conveniently but in lower yield (55%) by the method of Dale. In both cases recrystallization from ether gave pure 9, m.p. 90.5 - 91°; \([\alpha]_D^{199.9°} (c 2.2, \text{chloroform}); \]
it. m.p. 88 - 89°, \([\alpha]_D^{197.8°} (c 2, \text{chloroform}).\]

Methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-α-L-rhamnopyranoside) (10)

To a solution of 3 (4.90 g, 22.5 mmoles) and dry mercuric cyanide (5.0 g) in dry acetonitrile (50 ml) was added the bromide 9 (15.0 g) with stirring over 2 h and the stirring was continued for 3 h. The acetonitrile was evaporated under diminished pressure and the resulting residue was dissolved in chloroform (150 ml). The chloroform extract was washed successively with potassium bromide (1 M, 2 x 75 ml), water (75 ml), saturated sodium hydrogen carbonate solution (75 ml), and water (2 x 75 ml). The sirup obtained on evaporation was dissolved in warm ethanol (50 ml) and crystallized upon cooling; yield 9.90 g, 18.0 mmoles (80% based on 3). Recrystallization from ethanol gave pure 10, m.p. 158.5 - 159°; \([\alpha]_D^{159°} (-30.6° (c 1.7, \text{chloroform}); \]
Rf 0.56 (solvent A); p.m.r. (CDCl₃): \(6.66 (3 \text{ H singlet, OMe}), 7.96 - 8.03 (12 \text{ H, 4 OAc}), 8.49, 8.67 (3 \text{ H singlets, CMe}_2), 8.75 (3 \text{ H doublet, J}_5,6 6 \text{ Hz, CH}_3).\]

Methyl 4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (11)

Compound 10 (2.00 g) was deacetalated with trifluoroacetic acid in chloroform by a previously described procedure (see page 69). The resulting sirup showed one spot on t.l.c. with $R_f$ 0.09 (solvent A) and 0.66 (solvent B); yield 1.83 g (99%); p.m.r. ($CDCl_3$): $\delta$ 6.65 (3 H singlet, OMe), 7.93 - 8.01 (12 H, 4 OAc), 8.70 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

Methyl 4-O-β-D-glucopyranosyl-α-L-rhamnopyranoside (12)

Compound 11 (1.83 g) was deacetylated in the usual way (see page 69). T.l.c. (solvent B) showed a major component with $R_f$ 0.04 and a small amount of a faster running compound; yield 1.10 g (90%). A portion of the sirupy product was purified by preparative t.l.c. for analytical purposes, $[\alpha]_D$ - 58° ($c$ 1.5, methanol); $R_{glucose}$ 2.9 (solvent C); p.m.r. ($D_2O$, external tetramethylsilane): $\delta$ 5.30 (1 H doublet, $J_{1',2'}$ 7.7 Hz, H-1'), 5.32 (1 H doublet, $J_{1,2'}$ 1.5 Hz, H-1), 6.61 (3 H singlet, OMe), 8.65 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

The methyl glycoside 12 was methylated by the Hakomori method. Sirupy 12 (1.10 g) was dissolved in dry methyl sulfoxide (20 ml) and shaken with methyl sulfinyl anion (1.5 $M$, 20 ml, prepared according to Sandford and Conrad) for 4 h in an atmosphere of nitrogen supplied via a hypodermic needle through a serum cap. A small portion was withdrawn with a syringe and tested for excess anion by reaction with triphenylmethane. To the reaction mixture, maintained in a nitrogen atmosphere and cooled to 0°, was added methyl iodide (6 ml) with shaking and the shaking was continued.
overnight. The excess methyl iodide was evaporated under diminished pressure and the resulting solution was diluted with water (20 ml). The solution was extracted with petroleum ether (b.p. 65 - 70°, 5 x 100 ml). The sirup 13 obtained on evaporation showed one spot on t.l.c. (solvent A) with $R_f$ 0.28; yield 1.00 g (73%); $[\alpha]_D$ -51° (c 2.6, methanol); lit. $^{39}$ $[\alpha]_D$ 7° (c 2.0, methanol); p.m.r. ($CDCl_3$): $\tau$ 5.26 (1 H doublet, $J_{1,2}$ 1.5 Hz, H-1), 5.37 (1 H doublet, $J_{1,2}$ 7.7 Hz, H-1'), 6.36-6.63 (21 H, 7 OMe), 8.68 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$). Methanolation of 13 (0.30 g) with hydrogen chloride in methanol (3%, 30 ml, prepared by adding 5 ml of acetyl chloride to 100 ml of methanol) refluxing for 8 h, neutralization with Duolite A-4 (OH$^-$) resin, and concentration gave the corresponding monomeric methyl glycosides which were examined by g.l.c. as the trimethylsilyl derivatives (prepared as described on page 79). Injection in ethyl acetate onto column b programmed from 110 to 205° at 2°/min gave peaks corresponding to the methyl 2,3-di-O-methyl-L-rhamnopyranosides (6.8, 9.8 min) and the methyl 2,3,4,6-tetra-O-methyl-D-glucopyranosides (15.4, 20.7 min), identified by cochromatography with authentic standards (i.e. compound 5). The peak corresponding to methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside was collected, m.p. 36°; lit. $^{180}$ m.p. 39 - 41°. The mixed melting point with authentic standard was undepressed. Hydrolysis of 13 (0.30 g) in methanol (5 ml) with trifluoroacetic acid (2 M, 25 ml) refluxing for 16 h followed by evaporation gave a mixture of 2,3-di-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-glucose as shown by paper chromatography (solvent D) using authentic standards (i.e. compound 6). The mixture was acetylated in the usual way (see page 68) and then could be separated$^{115}$ by g.l.c. on column b programmed from 110 to 210° at 2°/min. The glucose derivatives gave peaks at 27.9 min (29%) and 30.2 min
(21%) while the rhamnose derivatives gave peaks at 37.0 min (38%) and 40.7 min (4%). Two unidentified peaks at 16.1 min (5%) and 18.5 min (3%) may be rhamnofuranose derivatives. The peaks were collected and subjected to t.l.c. in order to assign the parent sugars, as L-rhamnose and derivatives appear yellow under the visualization conditions. A portion of the hydrolysate was reduced by standard procedure (see page 72) and subsequently acetylated (see page 68) to give the corresponding alditol acetates. Injection in ethyl acetate, onto column b at any temperature gave one peak while on column c at 225° gave an equimolar ratio of two peaks which cochromatographed with authentic standards of 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (7) (38.6 min) and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol (46.3 min). Samples were collected and the mass spectra obtained corresponded to those of the authentic standards.

Reaction of the methyl glycoside of the disaccharide \( 12 \) (0.625 g) with sodium metaperiodate (0.05 \( M \), 40 ml) at 5° in the dark showed a rapid uptake of 1 mole per mole of sugar with the consumption of periodate becoming constant (3.0 moles per mole of \( 12 \), monitored by titration with sodium thiosulfate) in 48 h. The iodate and excess periodate were precipitated with barium acetate solution and removed by centrifugation. The residual polyaldehyde in the supernatant (500 ml) was reduced with sodium borohydride (0.6 g) overnight at 5°. Passage through Amberlite IR-120 (H\(^+\)) resin to remove the cations, concentration, and addition and evaporation of methanol (5 x 50 ml) to remove the borate as methyl borate and the excess acetic acid as methyl acetate gave the polyalcohol which was subsequently subjected to methanolysis with hydrogen chloride in methanol (3%, 10 ml, prepared by adding 5 ml of acetyl chloride to 100 ml of methanol) by refluxing for 6.5 h.
The chloride ions were removed with Duolite A-4 (OH\textsuperscript{−}) resin and the glycolaldehyde dimethyl acetal and solvents were evaporated. The resulting sirup showed two major spots on paper chromatography (solvent D) corresponding to standard 4-deoxy-L-erythritol (8) and glycerol. A portion was acetylated with sodium acetate and acetic anhydride as on page 73. Injection in ethyl acetate, onto column b at 140° gave a 1:1 ratio of peaks identical to authentic standards of the peracetylated derivatives of 4-deoxy-L-erythritol (8) (12 min) and glycerol (18 min). Mass spectra were obtained and corresponded to those of the authentic standards.

Alternative hydrolysis of the polyalcohol

The polyalcohol (0.01 g) in water (10 ml) was refluxed overnight in the presence of Amberlite IR-120 (H\textsuperscript{+}) resin (4 ml) and Duolite A-4 (OH\textsuperscript{−}) resin (4 ml). The resins were removed by filtration and the solvent evaporated. The resulting sirup was acetylated with sodium acetate and acetic anhydride as on page 73. Injection in ethyl acetate, onto column b at 140° gave two major peaks in a 1:1 ratio identical to authentic standards of the peracetylated derivatives of 4-deoxy-L-erythritol (8) and glycerol plus one small unidentified peak.

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\text{Compound 11 (1.00 g) in acetic anhydride (5 ml) was shaken with 2\% (v/v) concentrated sulfuric acid in acetic anhydride (10 ml) for 5 h at room temperature. The reaction mixture was diluted with chloroform (100 ml) and successively washed with ice-cold water (2 x 100 ml), saturated sodium}\]
hydrogen carbonate solution (2 x 100 ml), and water (2 x 100 ml). The chloroform was evaporated under diminished pressure and remaining acetic anhydride, and/or acetic acid was removed as ethyl acetate by addition and evaporation of ethanol. The resulting sirup, containing only a trace of cleavage products as indicated by t.l.c., was dissolved in 2-propanol (20 ml) and crystallized immediately when nucleated with a crystal obtained from a t.l.c. separation of a small portion; yield 0.85 g (70%). Recrystallization from 2-propanol gave pure 14, m.p. 139 - 140°; [α]_D
-62.3° (c 2.6, chloroform); R_f 0.34 (solvent A); p.m.r. (CDCl_3): τ 4.04 (1 H doublet, J_1,2 1.5 Hz, H-1), 7.86 - 8.04 (21 H, 7 OAc), 8.70 (3 H doublet, J_5,6 6 Hz, CH_3).

Anal. Calcd. for C_{26}H_{36}O_{17}: C, 50.32; H, 5.85. Found: C, 50.07; H, 5.84.

4-O-β-D-Glucopyranosyl-L-rhamnopyranose (15)

Scillabiose heptaacetate 14 (0.50 g) was deacetylated by the customary method (see page 69). The resulting sirup (0.25 g, 95%) showed one spot on a paper chromatogram (solvent C) with R_gucose 1.2; [α]_D
-24° (c 1.1, water); lit. 35 [α]_D - 24.8° (c 3.3, water); p.m.r. (D_2O, external tetramethylsilane): τ 4.88 (0.63 H doublet, J_1,2 1.5 Hz, H-1, α-L-form), 5.12 (0.37 H doublet, J_1,2 0.8 Hz, H-1, β-L form), 5.26 (1 H doublet, J_1',2' 7.5 Hz, H-1'), 8.65 (3 H doublet, J_5,6 6 Hz, CH_3).

The per-O-(trimethylsilyl) derivative of 15 was prepared by dissolving 15 (2-6 mg) in pyridine (0.4 ml) and reaction with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) for 2 h at 40°. The
excess reagents and solvent were evaporated in anhydrous conditions. G.l.c. (column a at 250°) of the per-O-(trimethylsilyl) disaccharide gave one peak (74%) at 10.1 min and a second peak which has the same retention time as per-O-(trimethylsilyl) sucrose at 12.6 min.

4-O-β-D-Glucopyranosyl-L-rhamnitol (16)

The free disaccharide 15 (0.20 g) was reduced with sodium borohydride (0.05 g) in water (10 ml) overnight. The usual work-up (see page 72) gave 16 as a sirup; yield 0.20 g (99%); Rf glucose 0.8 (solvent C); p.m.r. (D₂O, external tetramethylsilane): τ 5.37 (1 H doublet, J₁',₂', 7.2 Hz H-1'), 8.70 (3 H doublet, J₅,₆ 6 Hz, CH₃).

G.l.c. (column a at 250°) of the per-O-(trimethylsilyl) alditol (prepared by the procedure described on page 79) gave one peak at 13.3 min [per-O-(trimethylsilyl) sucrose, 16.6 min]¹²⁷.

The alditol 16 (0.20 g) was acetylated in the usual way (see page 68) to give crystalline 4-O-β-D-glucopyranosyl-L-rhamnitol octaacetate (scillabiitol octaacetate) (17) (0.38 g, 94%, crystallized from ethanol (20 ml)). Recrystallization from ethanol gave pure 17, m.p. 132 - 133°; [α]D - 78.6° (c 2.0, chloroform); Rf 0.33 (solvent A); p.m.r. (CDCl₃): τ 7.84 - 7.99 (24 H, 8 OAc), 8.66 (3 H doublet, J₅,₆ 6 Hz, CH₃).

Anal. Calcd. for C₂₈H₄₀O₁₈: C, 50.60; H, 6.07. Found: C, 50.63; H, 6.06.
L-Rhamnose monohydrate (1.00 g, commercial preparation, from Eastman Kodak) was acetylated with pyridine (25 ml) and acetic anhydride (25 ml) for 5 h at room temperature. This is essentially the procedure of Fischer et al.\textsuperscript{181}, except that the excess reagents were removed by successive evaporations with ethanol, and then with water (see page 68), yielding the sirupy product (1.80 g, 98%); \([\alpha]_D\) - 63° (c 2.3, chloroform); lit.\textsuperscript{182} \([\alpha]_D\) - 61.7° (c 2.7, chloroform); R\textsubscript{f} 0.58 (solvent A); p.m.r. (CDCl\textsubscript{3}): \(\tau\) 3.98 (1 H doublet, J\textsubscript{1,2} 1.4 Hz, H-1), 7.85, 7.86, 7.95, 8.01 (3 H singlets, 4 OAc), 8.77 (3 H doublet, J\textsubscript{5,6} 6 Hz, CH\textsubscript{3}).

2,3,4-Tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl bromide (19)

Compound 18 (6.00 g) was stirred with glacial acetic acid (6 ml), chloroform (6 ml), and hydrogen bromide in acetic acid (30 - 32%, 12 ml, commercial preparation, from Eastman Kodak) for 3 h at 0°, essentially as described by Fischer et al.\textsuperscript{181}. The mixture was diluted with chloroform (100 ml), quickly washed with ice-water (2 x 75 ml), saturated sodium hydrogen carbonate solution (2 x 75 ml), and then ice-water (2 x 75 ml), dried over sodium sulfate and calcium sulfate at 5°, filtered through a layer of calcium oxide and silica gel, and evaporated to a sirup that crystallized from ethyl ether (anhydrous, 6 ml) upon the gradual addition of petroleum ether (b.p. 65 - 70°, 6 ml) at -10°; yield 5.10 g (80%). Recrystallization from ethyl ether gave pure 19, m.p. 64.5 - 65.6°, \([\alpha]_D\) - 173.5° (c 2.4, chloroform); lit.\textsuperscript{181} m.p. 71 - 72°, \([\alpha]_D\) - 169.0° (c 12.3, tetrachloroethane); R\textsubscript{f} 0.65 (solvent A); p.m.r. (CDCl\textsubscript{3}): \(\tau\) 3.70 (1 H doublet, J\textsubscript{1,2} 1.6 Hz, H-1), 7.86,
Methyl 2,3-O-isopropylidene-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-β-L-rhamnopyranoside (20)

To a stirred solution of compound 3 (1.00 g; 4.6 mmoles) and dry mercuric cyanide (1.0 g) in dry acetonitrile (2 ml) was added the bromide 19 (3.50 g), in portions during 3 h. At 4 h, the standard work-up (see page 74) gave an impure, amorphous material (3.00 g) which showed a major component (~80%) on t.l.c. with Rf 0.62 (solvent A). For analytical purposes a small amount was purified by preparative t.l.c.169; [α]D - 74° (α 2.0, chloroform); p.m.r. (CDCl3): τ 4.66 (1 H doublet, J1,2' 1.9 Hz, H-1'), 5.12 (1 H singlet, H-1), 6.60 (3 H singlet, OMe), 7.83, 7.94, 8.01 (3 H singlets, 3 OAc), 8.45, 8.65 (3 H singlets, CM€2), 8.66 (3 H doublet, J5,6 6 Hz, CH3), 8.75 (3 H doublet, J5,6 6 Hz, CH3).

Methyl 4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (21)

A solution of impure, amorphous compound 21 (3.00 g) in chloroform (135 ml) was deacetalated by a previously described procedure (see page 69). The resulting syrup (2.70 g) showed mainly one spot on t.l.c., Rf 0.13 (solvent A) and 0.78 (solvent B); p.m.r. (CDCl3): τ 6.63 (3 H singlet, OMe), 7.86, 7.95, 8.01 (3 H singlets, 3 OAc), 8.66 (3 H doublet, J5,6 6 Hz, CH3), 8.77 (3 H doublet, J5,6 6 Hz, CH3).
Methyl 2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (22)

Crude compound 21 (2.70 g) was acetylated with pyridine (25 ml) and acetic anhydride (25 ml), for 5 h at room temperature. The sirup resulting from the usual work-up (page 68) crystallized from ethanol (13 ml) when nucleated with a crystal obtained from a t.l.c. separation; yield 1.60 g, 3.0 mmoles (65% based on 3). Recrystallization from ethanol gave pure 22, m.p. 182 - 183°; [α]D - 51.7° (c 2.3, chloroform); Rf 0.51 (solvent A); p.m.r. (CDCl3): τ 5.02 (1 H doublet, J1,2 1.4 Hz, H-1'), 5.42 (1 H doublet, J1,2 1.2 Hz, H-1), 6.62 (3 H singlet, OMe), 7.88 - 8.03 (15 H, 5 OAc), 8.63 (3 H doublet, J5,6 6 Hz, CH3), 8.78 (3 H doublet, J5,6 6 Hz, CH3).


Methyl 4-O-α-L-rhamnopyranosyl-α-L-rhamnopyranoside (23)

Compound 22 (1.30 g) was deacetylated with sodium methoxide (0.2 M, 35 ml) in the customary way (see page 69). The sirup obtained on evaporation showed one spot on t.l.c., Rf 0.19 (solvent B); yield 0.73 g (97%); [α]D - 109° (c 2.5, water); Rglucose 4.0 (solvent C); p.m.r. (D2O, external tetramethylsilane): 4.81 (1 H doublet, J1,2 1.8 Hz, H-1'), 5.28 (1 H doublet, J1,2 1.0 Hz, H-1), 6.58 (3 H singlet, OMe), 8.64 (3 H doublet, J5,6 6 Hz, CH3), 8.68 (3 H doublet, J5,6 6 Hz, CH3).

The methyl glycoside 23 (0.30 g) was methylated by a method
previously described (see page 75), to give the corresponding hexamethyl ether 24; yield 0.30 g (82%). A small amount was purified by preparative t.l.c. for analytical purposes; \( R_f \) 0.29 (solvent A); \([\alpha]_D \) - 81.5° (c 2.1, chloroform); p.m.r. (CDCl₃): \( \tau \) 4.77 (1 H doublet, \( J_{1',2'} \) 1.7 Hz, H-1'), 5.26 (1 H doublet, \( J_{1,2} \) 1.6 Hz, H-1), 6.45 - 6.63 (18 H, 6 OMe), 8.69 (3 H doublet, \( J_{5,6} \) 6 Hz, CH₃), 8.74 (3 H doublet, \( J_{5,6} \) 6 Hz, CH₃). Hydrolysis (as on page 76) of 24 (0.10 g) gave 2,3-di-O-methyl-L-rhamnose and 2,3,4-tri-O-methyl-L-rhamnose as shown by paper chromatography (solvent D) using authentic standards (i.e. compound 6). The hydrolysate was reduced with sodium borohydride (0.1 g) in water (10 ml) overnight. Acetic acid was added dropwise until the effervescence ceased and the mixture was neutral to litmus paper. The solvent was evaporated and successive portions of methanol (5 x 50 ml) were added and evaporated to remove the borate as methyl borate. The resulting residue (containing sodium acetate) was acetylated with acetic anhydride (3 ml) for 1 h on a steam bath. The excess acetic anhydride was removed as ethyl acetate by successive additions and evaporations of ethanol. The resulting residue was dissolved in ethyl acetate, filtered to remove the sodium acetate, and injected onto column c at 220°. Three peaks in a 4:1:3 ratio were eluted at 14.0, 18.8, and 23.6 min respectively. The peaks at 14.0 and 23.6 min cochromatographed with authentic standards of 1,5-di-O-acetyl-2,3,4-tri-O-methyl-L-rhamnitol and 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (7) respectively. Samples were collected and the mass spectra obtained corresponded to those of the authentic standards.

The compound with retention time 18.8 min was collected and had the following p.m.r. spectrum (CDCl₃): \( \tau \) 6.51 - 6.56 (12 H, 4 OMe, expansion
indicated four separate methoxy groups), 7.90, 7.94 (3 H singlets, 2 OAc), 8.66 (3 H doublet, J$_{5,6}$ 6 Hz, CH$_3$). The mass spectrum obtained showed m/e 43, 45, 58, 71, 87, 101, 117, 130, 131, 143, 149, 161, 177, 191 and 203. This compound with retention time 18.8 min when subjected to methanalysis (3% hydrogen chloride in methanol, prepared by adding 5 ml of acetyl chloride to 100 ml of methanol) overnight at reflux, neutralization with Duolite A-4 (OH$^-$) resin, evaporation, and subsequent acetylation, cochromatographed (column c, 220°) with standard 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol (7).

Periodate oxidation of 23 by a procedure previously described (see page 77) showed a total consumption of 3.0 moles per mole of 23 in 70 h. The reaction sequence corresponding to that described previously (see page 77) gave 3-deoxy-L-glycerol diacetate and 4-deoxy-L-erythritol triacetate, identified by comparative g.l.c. (column b, 60 - 220°, programmed at 2°/min, retention times 9.2 and 35.6 min, respectively) and mass-spectrometric analysis with authentic standards (i.e. 8).

l,2,3-Tri-O-acetyl-4-O-(2,3,4-tri-O-acetyl-a-L-rhamnopyranosyl)-a-L-rhamnopyranose (25)

Crude compound 21 (2.70 g) in acetic anhydride (15 ml) was shaken with 1% (v/v) concentrated sulfuric acid in acetic anhydride (30 ml) for 2 h at room temperature. The sirup resulting from the conventional work-up (see page 78) crystallized from ethanol (7 ml) when nucleated with a crystal obtained from a t.l.c. separation; yield 1.70 g, 3.0 mmoles (65% based on 3). Recrystallization from ethanol gave pure 25, m.p. 162 - 163°;
$[\alpha]_D - 63.6^\circ \ (c \ 2.1, \ \text{chloroform})$; $R_f \ 0.51 \ (\text{solvent A}); \ \text{p.m.r. (CDCl}_3): \ \tau$

4.00 (1 H doublet, $J_{1,2} \ 1.7 \text{ Hz}, \ H-1$), 4.99 (1 H doublet, $J_{1,1',2'} \ 1.9 \text{ Hz}, \ H-1'$), 7.83 - 8.02 (18 H, 6 OAc), 8.62 (3 H doublet, $J_{5,6} \ 6 \text{ Hz}, \ CH_3$),

8.77 (3 H doublet, $J_{5,6} \ 6 \text{ Hz}, \ CH_3$).

**Anal. Calcd. for C$_{24}$H$_{34}$O$_{15}$**: C, 51.25; H, 6.09. **Found**: C, 51.27; H, 6.10.

4-O-$\alpha$-L-Rhamnopyranosyl-L-rhamnopyranose (26)

The peracetate 25 (0.80 g) was deacetylated with sodium methoxide (0.2 M, 20 ml) for 1 h at room temperature. After the usual processing (see page 69), the resulting sirup (0.40 g, 91%) had $[\alpha]_D - 68^\circ \ (c \ 2.2, \ \text{water})$; $R_{\text{glucose}} \ 2.2 \ (\text{solvent C}); \ \text{p.m.r. (D}_2\text{O, \ external tetramethylsilane)}: \ \tau$

4.82 (1 H doublet, $J_{1',2'} \ 1.7 \text{ Hz}, \ H-1'$), 4.88 (0.64 H doublet, $J_{1,2} \ 1.3 \text{ Hz}, \ H-1, \ \alpha$-L form), 5.14 (0.36 H doublet, $J_{1,2} \ 1.0 \text{ Hz}, \ H-1, \ \beta$-L form), 8.67, 8.68 (3 H doublets, $J_{5,6} \ 6 \text{ Hz}, \ 2 \ CH_3$).

G.l.c. (column a at 230$^\circ$) of the per-O-(trimethylsilyl) disaccharide (prepared as on page 79) gave one peak (77.5%) at 7.2 min and a second peak at 11.4 min [per-O-(trimethylsilyl) sucrose, 19.4 min]$^{127}$.

4-O-$\alpha$-L-Rhamnopyranosyl-L-rhamnitol (27)

The free disaccharide 26 (0.20 g) was reduced with sodium borohydride (0.08 g) in water (5 ml) for 6 h. The usual work-up (see page 72) gave 27 as a sirup; yield 0.19 g (95%); $[\alpha]_D - 50^\circ \ (c \ 2.1, \ \text{water})$; $R_{\text{glucose}} \ 1.3 \ (\text{solvent C}); \ \text{p.m.r. (D}_2\text{O, \ external tetramethylsilane)}: \ \tau$

5.02 (1 H doublet, $J_{1',2'} \ 1.7 \text{ Hz}, \ H-1'$), 8.71 (6 H doublet, $J_{5,6} \ 6 \text{ Hz}, \ 2 \ CH_3$).
G.l.c. (column a at 230°) of the per-O-(trimethylsilyl) alditol (prepared by a previously described procedure, see page 79) gave one peak at 11.6 min [per-O-(trimethylsilyl) sucrose, 19.8 min].

The alditol 27 (0.19 g) was acetylated (see page 68) to give 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-L-rhamnitol (28) which crystallized from ethanol (3 ml); yield 0.35 g (95%). Recrystallization from ethanol gave pure 28, m.p. 138.5 - 139.5°; [α]D - 67.2° (c 2.1, chloroform); Rf 0.42 (solvent A); p.m.r. (CDCl3): τ 5.13 (1 H doublet, J1',2' 1.8 Hz, H-1'), 7.83 - 7.99 (21 H, 7 OAc), 8.59 (3 H doublet, J5,6 6 Hz, CH3), 8.75 (3 H doublet, J5,6 6 Hz, CH3).


G.l.c. (column a at 260°) of the peracetylated alditol 28 gave one peak at 6.4 min (sucrose octaacetate, 16.2 min).

l,2,3,4,6-Penta-O-benzoyl-α-D-mannopyranose (29)

Compound 29 was prepared essentially as described by Fischer and Oetker. α-D-Mannose (25.0 g finely ground, dry) was added in portions to a solution of dry chloroform (375 ml), benzoyl chloride (125 ml) and dry pyridine (125 ml) at 0°. The mixture was stirred at 0° until a clear solution resulted and the stirring was continued for an additional 3 h. The pyridine was removed by washing the reaction mixture with sulfuric acid (3 M, 5 x 200 ml) and then with water (3 x 200 ml). Ethanol (500 ml) was added to the chloroform extract which was then evaporated to a sirup that crystallized from ethanol (1600 ml); yield 90.0 g (93%). Recrystalliza-
tion from ethanol gave pure 29, m.p. 152 - 153°; \([\alpha]_D - 19.9° \text{ (c 2.9, chloroform)}\); \(R_f 0.80 \) (solvent A); p.m.r. (CDCl$_3$): \(\tau 1.74 - 2.75 \) (25 H, 5 OBz), 3.35 (1 H doublet, \(J_{1,2} 1.9 \text{ Hz, H-1}\)).

2,3,4,6-Tetra-O-benzoyl-\(\alpha\)-D-mannopyranosyl bromide (30)

Compound 30 was prepared by the procedure of Ness, Fletcher and Hudson. A solution of 29 (10.0 g) in ethylene dichloride (20 ml) was treated with hydrogen bromide in acetic acid (30 - 32%, 20 ml, commercial preparation, from Eastman Kodak) overnight at room temperature. The reaction mixture was diluted with ethylene dichloride (100 ml) and washed successively with ice-water (2 x 100 ml), saturated sodium hydrogen carbonate solution (2 x 100 ml), and ice-water (2 x 100 ml), dried with sodium sulfate and calcium sulfate, and filtered through a layer of calcium oxide and silica gel. The sirup obtained on evaporation showed one spot on t.l.c. (solvent A) with \(R_f 0.83\) and on one occasion the sirupy product crystallized neat but it was not possible to recrystallize it from solvent; yield 9.10 g (97%); \([\alpha]_D 11° \text{ (c 3.3, chloroform)}\); \(R_f 0.83\) m.p. 152 - 153°, \([\alpha]_D - 18.6° \text{ (c 1.6, chloroform)}\); \(R_f 0.80 \) (solvent A); p.m.r. (CDCl$_3$): \(\tau 1.74 - 2.75 \) (25 H, 5 OBz), 3.35 (1 H doublet, \(J_{1,2} 1.9 \text{ Hz, H-1}\)).

Methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-benzoyl-\(\alpha\)-D-mannopyranosyl)-\(\alpha\)-L-rhamnopyranoside (31)

The bromide 30 (15.0 g) in dry acetonitrile (20 ml) was added to a stirred solution of 3 (4.00 g, 18.3 mmoles) and dry mercuric cyanide...
(4.0 g) in dry acetonitrile (10 ml) during 2 h and the stirring was continued for an additional 4 h. The standard work-up procedure (see page 74) gave a crude amorphous solid (17.4 g) which showed a major component (~80%) on t.l.c. (solvent A) with Rf 0.71. A small amount was purified by preparative t.l.c. for analytical purposes; p.m.r. (CDCl3): τ 1.82 - 2.78 (20 H, 4 OBz), 4.78 (1 H doublet, J1',2' 1.5 Hz, H-1'), 5.14 (1 H singlet, H-1), 6.63 (3 H singlet, OMe), 8.42, 8.66 (3 H singlets, CMe2), 8.63 (3 H doublet, J5,6 6 Hz, CH3).

Methyl 2,3-O-isopropylidene-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (32)

Compound 31 (crude amorphous solid, 17.3 g) was debenzoylated with sodium methoxide (0.2 M, 200 ml) for 1 h at room temperature. Following the usual work-up (see page 69) the remaining methyl benzoate was removed by steam distillation under diminished pressure. The resulting sirup (Rf 0.31 (solvent B)) was acetylated with pyridine (100 ml) and acetic anhydride (100 ml) overnight at room temperature. The acetate 32, obtained on conventional work-up (see page 68), crystallized from ethanol (25 ml); yield 7.60 g, 13.9 mmoles (76% based on 3). Recrystallization from ethanol gave pure 32, m.p. 119 - 120°; [α]D 30.7° (c 2.2, chloroform); Rf 0.53 (solvent A); p.m.r. (CDCl3): τ 5.01 (1 H doublet, J1',2' 1.7 Hz, H-1'), 5.14 (1 H singlet, H-1), 6.63 (3 H singlet, OMe), 7.84, 7.90, 7.97, 8.01 (3 H singlets, 4 OAc), 8.48, 8.66 (3 H singlets, CMe3), 8.72 (3 H doublet, J5,6 6 Hz, CH3).

Methyl 4-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl)-\(\alpha\)-L-rhamnopyranoside (33)

A solution of 32 (2.00 g) in chloroform (90 ml) was deacetalated by a previously described procedure (see page 69). The resulting sirup showed one spot on t.l.c., \(R_f\) 0.17 (solvent A) and 0.59 (solvent B); yield 1.82 g (98%); p.m.r. (CDCl\(_3\)): \(\tau\) 5.04 (1 H doublet, \(J_{1,1}\) 1.7 Hz, H-1'), 5.31 (1 H singlet, H-1), 6.62 (3 H singlet, OMe), 7.85, 7.90, 7.95, 7.99 (3 H singlets, 4 OAc), 8.67 (3 H doublet, \(J_{5,6}\) 6 Hz, CH\(_3\)).

Methyl 4-O-\(\alpha\)-D-mannopyranosyl-\(\alpha\)-L-rhamnopyranoside (34)

Compound 33 (1.83 g) was deacetylated in the usual way (see page 69). The sirup obtained on evaporation showed one spot on t.l.c., \(R_f\) 0.03 (solvent B); yield 1.17 g (96%), \([\alpha]_D\) 13° (c 2.2, water); \(R_{glucose}\) 2.9 (solvent C); p.m.r. (D\(_2\)O, external tetramethylsilane): \(\tau\) 5.01 (1 H doublet, \(J_{1,1}\) 1.8 Hz, H-1'), 5.29 (1 H doublet, \(J_{1,2}\) 1.5 Hz, H-1), 6.60 (3 H singlet, OMe), 8.67 (3 H doublet, \(J_{5,6}\) 6 Hz, CH\(_3\)).

Methylation of 34 by a previously described procedure (see page 75) gave the corresponding heptamethyl ether 35, which showed one spot on t.l.c. (solvent A) with \(R_f\) 0.14; \([\alpha]_D\) 10° (c 1.0, chloroform); p.m.r. (CDCl\(_3\)): \(\tau\) 4.93 (1 H doublet, \(J_{1,1}\) 1.6 Hz, H-1'), 5.27 (1 H doublet, \(J_{1,2}\) 1.6 Hz, H-1), 6.46 - 6.63 (21 H, 7 OMe), 8.72 (3 H doublet, \(J_{5,6}\) 6 Hz, CH\(_3\)).

Hydrolysis (as on page 76) of 35 gave 2,3-di-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-mannose identified by paper chromatography (solvent D) using authentic standards (i.e. 6). Subsequent reduction and acetylation (see page 72) gave equimolar amounts of 1,4,5-tri-O-acetyl-2,3,6-di-O-methyl-
-L-rhamnitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-mannitol, identified by g.l.c. (column c, 220°, retention times 33.0 and 41.0 min, respectively) and mass-spectrometric comparison with authentic standards (i.e. 7).

Periodate oxidation of 34 by a previously described procedure (see page 77) showed a rapid uptake of 2 moles of periodate per mole of 34 with the consumption becoming constant (3.0 moles per mole) in 30 h. Subsequent reduction, methanolysis, and evaporation (see page 77) gave a sirup that showed two major spots on paper chromatography (solvent D) corresponding to standard 4-deoxy-L-erythritol (8) and glycerol. Acetylation gave 4-deoxy-L-erythritol triacetate and glycerol triacetate, identified by g.l.c. (column b, 140°, retention times 7.2 and 10.0 min, respectively) and mass-spectrometric comparison with authentic standards.

Methyl 2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranoside (36)

Compound 33 (0.10 g) was acetylated by standard procedures (see page 68); yield 0.11 g (94%). A portion of the sirupy product was purified by preparative t.l.c. for analytical purposes; [α]_D 4° (c 1.3, chloroform); R_f 0.41 (solvent A); p.m.r. (CDCl_3): τ 5.01 (1 H doublet, J_1',2', 1.6 Hz, H-1'), 5.40 (1 H doublet, J_1,2 1.5 Hz, H-1), 6.61 (3 H singlet, OMe), 7.84 - 8.01 (18 H, 6 OAc), 8.60 (3 H doublet, J_5,6 6 Hz, CH_3).
1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-L-rhamnopyranose (37)

Compound 37 (1.00 g) in acetic anhydride (5 ml) was shaken with 2% (v/v) concentrated sulfuric acid in acetic anhydride (10 ml) for 3 h at room temperature. The sirup resulting from the conventional work-up (see page 78) crystallized from ethanol (10 ml); yield 0.95 g (78%). Recrystallization from ethanol gave pure 37, m.p. 149.5 - 150.5°; [α]D -5.5° (c 4.3, chloroform); Rf 0.39 (solvent A); p.m.r. (CDCl3): δ 4.00 (1 H doublet, J 1.5 Hz, H-l), 5.00 (1 H doublet, J 1',2' 1.8 Hz, H-1'), 7.82 - 8.00 (21 H, 7 OAc), 8.60 (3 H doublet, J 5,6 6 Hz, CH3).


4-O-α-D-Mannopyranosyl-α-L-rhamnopyranose (38)

The peracetate 37 (1.20 g) was deacetylated with sodium methoxide (0.2 M, 30 ml) for 1 h at room temperature followed by the usual work-up (see page 69). The sirup (0.60 g, 95%) obtained on evaporation crystallized neat after long standing. Pure 38 was obtained by recrystallization from a 1:1 mixture of methanol and 2-propanol (20 ml/g), m.p. 143 - 145°; [α]D 53.0° mutarotating to 60.3° in 2 h (c 1.0, water); Rf glucose 1.0 (solvent C); p.m.r. (D2O, external tetramethylsilane): δ 4.90 (0.64 H doublet, J 1,2 1.5 Hz, H-1, α-L form), 5.03 (1 H doublet, J 1',2' 1.8 Hz, H-1'), 5.16 (0.36 H doublet, J 1,2 1.0 Hz, H-1, β-L form), 8.71 (3 H doublet, J 5,6 6 Hz, CH3).

Anal. Calcd. for C12H22O10: C, 44.17; H, 6.80. Found: C, 43.91; H, 6.86.
G.I.c. (column a at 240°) of the per-O-(trimethylsilyl) disaccharide (prepared by the procedure on page 79) gave one peak (80%) at 8.5 min and a second peak at 11.7 min [per-O-(trimethylsilyl) sucrose, 14.5 min].

The disaccharide 38 (0.10 g) was dissolved in sodium acetate buffer (0.3 M, pH 4.5, 2 ml) and incubated at 37° with crude α-mannosidase (2 ml) prepared from jack bean meal essentially by the procedure of Li. The free disaccharide 38 was ~80% cleaved in 10 h and completely cleaved in 22 h as indicated by paper chromatography (solvent C) using authentic standards. Under these conditions methyl β-D-mannopyranoside (46) was not cleaved but methyl α-D-mannopyranoside (commercial preparation, from Calbiochem) was hydrolyzed. The disaccharide reaction mixture was precipitated into ethanol and centrifuged. The supernatant was deionized with Amberlite IR-120 (H+) and Duolite A-4 (OH-) resins. The resulting solution of mannose and rhamnose was reduced and acetylated by the standard procedure (see page 72). Injection of the mixture onto column b programmed from 150 to 220° at 2°/min gave a 1:1 ratio of peaks identical to authentic standards of L-rhamnitol pentaacetate and D-mannitol hexaacetate. Samples were collected and the mass spectra obtained corresponded to those of the authentic standards. Furthermore, the D-mannitol hexaacetate had m.p. 120 - 122°, undepressed by an authentic sample; lit. m.p. 125°.

4-O-α-D-Mannopyranosyl-L-rhamnitol (39)

The free disaccharide 38 (0.50 g) was reduced with sodium borohydride (0.20 g) in water (25 ml) overnight. The routine work-up (see page 72) gave sirupy 39; yield 0.49 g (97%); [α]D 57° (c 3.5, water); Rglucose
0.5 (solvent C); p.m.r. (D$_2$O, external tetramethylsilane): $\tau$ 4.89 (1 H doublet, $J_{1',2'}$ 1.9 Hz, H-1'), 8.73 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

G.l.c. (column a at 240°) of the per-O-(trimethylsilyl) alditol (preparation analogous to that on page 79) gave one peak at 11.5 min [per-O-(trimethylsilyl) sucrose, 12.9 min]$^{127}$.

The alditol 39 (0.30 g.) was acetylated with pyridine (10 ml) and acetic anhydride (10 ml) to give 1,2,3,5-tetra-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranosyl)-L-rhamnitol (40), which was crystallized from ethanol (5 ml); yield 0.56 g (92%). Recrystallization from ethanol gave pure 40, m.p. 84 - 85°; $[\alpha]_D^0$ - 4.4° (c 1.9, chloroform); $R_f$ 0.33 (solvent A); p.m.r. (CDCl$_3$): $\tau$ 4.98 (1 H doublet, $J_{1',2'}$ 1.6 Hz, H-1'), 7.84 - 8.02 (24 H, 8 OAc), 8.63 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

Anal. Calcd. for C$_{38}$H$_{40}$O$_{18}$: C, 50.60; H, 6.07. Found: C, 50.39; H, 6.13.

G.l.c. (column a at 270°) of the peracetylated alditol 40 gave one peak at 7.0 min (sucrose octaacetate, 10.0 min).

*Methyl 4,6-O-benzylidene-\(\alpha\)-D-mannopyranoside (41)*

Compound 41 was prepared essentially as described by Buchanan and Schwarz$^{187}$ with the modification of Gibney$^{149}$. Methyl \(\alpha\)-D-mannopyranoside (10.0 g, finely powdered, commercial preparation, from Calbiochem) was dissolved as rapidly as possible in formic acid (98 - 100%, 50 ml) and benzaldehyde (50 ml, freshly distilled) was immediately added to the solution. After standing 5 min with occasional shaking, the solution was poured with stirring into a mixture of water (200 ml) and anhydrous potassium carbonate (137 g). The excess benzaldehyde was immediately removed by steam
distillation of the resulting solution. The aqueous phase was extracted with chloroform in a continuous extractor. The chloroform extract was evaporated to a solid residue that crystallized from benzene (210 ml); yield 5.50 g (38%). Recrystallization from benzene gave pure 41, m.p. 146-147°; $\alpha_D^\circ 64.3^\circ$ (c 2.1, chloroform); lit. 187 m.p. 140-143°, $\alpha_D^\circ 61^\circ$ (c 1.84, chloroform), lit. 188 m.p. 146-147°, $\alpha_D^\circ 71.7^\circ$ (c 1.185, chloroform); $R_f$ 0.23 (solvent A); p.m.r. (CDCl$_3$): $\tau$ 2.44 - 2.66 (5 H, $\beta$-H), 4.44 (1 H singlet, $\beta$-CH), 6.62 (3 H singlet, OMe).

Methyl 4,6-0-benzylidene-2,3-O-carbonyl-α-D-mannopyranoside (42)

Compound 42 was prepared with some reference to Doane et al. 150. Compound 41 (3.00 g, dry) in p-dioxane (15 ml) was treated with a solution of triethylamine (7.5 ml) in benzene (45 ml) and subsequently cooled in an ice-water bath. While the suspension was being magnetically stirred ethyl chloroformate (25 ml) was added dropwise so as to keep the effervescence under control (during ~ 20 min). The reaction mixture (basic to litmus paper) was stirred in the ice-water bath for an additional 20 min, then diluted with benzene (200 ml) and washed successively with ice-water (2 x 200 ml), hydrochloric acid (1 M, 200 ml), water (200 ml), saturated sodium hydrogen carbonate solution (200 ml), and water (2 x 200 ml). Evaporation of the solvent gave a solid residue which was crystallized from ethanol (9 ml); yield 3.10 g (94%). Recrystallization from ethanol gave pure 42, m.p. 125 - 127°; $\alpha_D^\circ - 21.0^\circ$ (c 2.1, chloroform); lit. 151 m.p. 125 - 126°, $\alpha_D^\circ - 19.3^\circ$ (chloroform); $R_f$ 0.75 (solvent A); p.m.r. (CDCl$_3$): $\tau$ 2.46 - 2.70 (5 H, $\beta$-H), 4.43 (1 H singlet, $\beta$-CH), 4.99 (1 H singlet, H-1),
6.62 (3 H singlet, OMe).

1,4,6-Tri-O-acetyl-2,3-O-carbonyl-\(\alpha\)-D-mannopyranose (43)

Compound 42 (3.00 g) was suspended in acetic anhydride (15 ml) and shaken with 1% (v/v) concentrated sulfuric acid in acetic anhydride (30 ml) for 2 h at room temperature. The sirup resulting from the conventional work-up (see page 78) crystallized from ethanol (10 ml) to yield 2.00 g of crystals. A further 0.60 g of sirup containing some \(\beta\)-anomer was obtained by pressure column chromatography on silica gel H (from EM Reagents) and elution with solvent A; total yield 2.60 g (81%). Recrystallization from ethanol gave pure 43, m.p. 117.5 - 118.5°; \([\alpha]_{D} 15.6° (c 2.5,\) chloroform); lit. \([\alpha]_{D} 7.5° (c 1.0,\) chloroform); \(R_f 0.21\) (solvent A); p.m.r. (CDCl₃): \(\tau 3.60\) (1 H singlet, H-1), 7.86, 7.88, 7.92 (3 H singlets, 3 OAc).


4,6-Di-O-acetyl-2,3-O-carbonyl-\(\alpha\)-D-mannopyranosyl bromide (44)

Compound 44 was prepared essentially as described by Gorin and Perlin. Compound 43 (2.00 g) in chloroform (200 ml) was stirred with hydrogen bromide in acetic acid (30-32%, 40 ml, commercial preparation, from Eastman Kodak) for 3 h at 0°. The chloroform solution was quickly washed successively with ice-water (2 x 200 ml), saturated sodium hydrogen carbonate solution (2 x 200 ml), and ice-water (2 x 200 ml), dried with anhydrous sodium sulfate and calcium sulfate, filtered through a layer of
calcium oxide and silica gel, and evaporated to a sirup that crystallized from ethyl ether (anhydrous, 25 ml) at -10°. Petroleum ether (b.p. 65-70°, 10 ml) was added in portions to complete the crystallization; yield 1.75 g (82%). Recrystallization from ethyl ether gave pure 44, m.p. 79 - 80°; [α]_D 89.8° (c 3.3, chloroform); lit. 95 [α]_D 55° (c 1.0, chloroform); R_f 0.63 (solvent A); p.m.r. (CDCl_3): τ 3.33 (1 H singlet, H-1), 7.86, 7.92 (3 H singlets, 2 OAc).

Methyl 4,6-di-O-acetyl-2,8-O-carbonyl-β-D-mannopyranoside (45)

Compound 44 (1.60 g) was stirred with dry acetonitrile (10 ml), dry methanol (10 ml), and dry mercuric cyanide (1.60 g) for 4 h. Standard work-up procedures (see page 74) gave a sirup that crystallized from ethanol (12 ml); yield 1.20 g (87%). Recrystallization from ethanol gave pure 45, m.p. 137 - 138°; [α]_D - 85.9° (c 2.7, chloroform); lit. 95 [α]_D - 37° (c 1.0, chloroform); R_f 0.22 (solvent A); p.m.r. (CDCl_3): τ 5.17 (1 H doublet, J_1,2 3 Hz, H-1), 6.43 (3 H singlet, OMe), 7.89, 7.91 (3 H singlets, 2 OAc).

Anal. Calcd. for C_{12}H_{16}O_{9}: C, 47.37; H, 5.30. Found: C, 47.49; H, 5.45.

Methyl β-D-mannopyranoside 2-propyl alcoholate (46)

Compound 45 (0.50 g) was deesterified with sodium methoxide (0.2 M, 15 ml) for 1 h at room temperature, followed by the usual work-up (see page 69). The sirup obtained on evaporation crystallized from 2-propanol (15 ml) after addition and evaporation of 2-propanol (2 x 30 ml); yield 0.36 g (86%). Recrystallization from 2-propanol gave pure 46, m.p.
72 - 73°; \([\alpha]_D^\circ\) = 52.7° (c 2.2, water); lit.\(^{190}\) m.p. 74 - 75°, \([\alpha]_D^\circ\) = 53.3° (c 4, water); \(R_f\) 0.02 (solvent B); \(R_{glucose}\) 1.62 (solvent C); p.m.r. (D\(_2\)O, external tetramethylsilane): \(\tau\) 5.41 (1 H doublet, \(J_{1,2}\) 0.9 Hz, H-1), 6.43 (3 H singlet, OMe).

**Methyl 2,3,4,6-tetra-O-acetyl-\(\beta\)-D-mannopyranoside (47)**

Compound 46 (0.10 g) was acetylated in the usual way (see page 68). The resulting product crystallized neat or from ethanol (3 ml); yield 0.18 g (96%). Recrystallization from ethanol gave pure 47, m.p. 163 - 164°; \([\alpha]_D^\circ\) = 50.1° (c 2.2, chloroform); lit.\(^{95}\) m.p. 161 - 162°, lit.\(^{190}\) m.p. 159 - 160°, \([\alpha]_D^\circ\) = 47.8°; \(R_f\) 0.43 (solvent A); p.m.r. (CDCl\(_3\)): \(\tau\) 5.42 (1 H doublet, \(J_{1,2}\) 1.1 Hz, H-1), 6.47 (3 H singlet, OMe), 7.83, 7.92, 7.97, 8.02 (3 H singlets, 4 OAc).

**Methyl 4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-\(\beta\)-D-mannopyranosyl)-2,3-O-isopropylidene-\(\alpha\)-L-rhamnopyranoside (48)**

Compound 3 (1.00 g, 4.6 mmoles) was stirred magnetically in the dark for 1 h at room temperature with silver oxide (3 g, freshly prepared, dry), calcium sulfate (4 g, anhydrous), and chloroform (10 ml, dry, alcohol-free). Compound 44 (3.00 g) in chloroform (20 ml, dry, alcohol-free) was added dropwise during 3 h and the stirring was continued for an additional 0.5 h. The reaction mixture was filtered and evaporated to a crude sirup (3.75 g) which showed a major component (∼80%) on t.l.c. (solvent A) with \(R_f\) 0.36. For analytical purposes a small amount was purified by preparative t.l.c.\(^{169}\); \([\alpha]_D^\circ\) = 65° (c 3.1, chloroform); p.m.r. (CDCl\(_3\)): \(\tau\) 5.15 (1 H
singlet, H-1), 5.19 (1 H doublet, $J_{1',2'} = 1.4$ Hz, H-1'), 6.65 (3 H singlet, OMe), 7.90, 7.92 (3 H singlets, 2 OAc), 8.49, 8.66 (3 H singlets, CMe$_2$), 8.63 (3 H doublet, $J_{5',6'} = 6$ Hz, CH$_3$).

*Methyl 4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (49)*

A solution of crude compound 4J3 (3.75 g) in chloroform (135 ml) was deacetalated by a previously described procedure (see page 69). The resulting sirup (3.60 g) showed mainly one spot on t.l.c., $R_f$ 0.04 (solvent A) and 0.62 (solvent B); p.m.r. (CDCl$_3$): $\tau$ 6.65 (3 H singlet, OMe), 7.89, 7.92 (3 H singlets, 2 OAc), 8.61 (3 H doublet, $J_{5',6'} = 6$ Hz, CH$_3$).

*Methyl 2,3-di-O-acetyl-4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranoside (50)*

Crude compound 49 (3.60 g) was acetylated with pyridine (20 ml) and acetic anhydride (20 ml) overnight at room temperature. The excess reagents were removed as before (see page 68). The resulting sirup crystallized from ethanol (25 ml); yield 1.80 g, 3.37 mmoles (73% based on 3). Recrystallization from ethanol gave pure 50, m.p. 204.5 - 205.5°; $[\alpha]_D$ - 61.8° (c 2.4, chloroform); $R_f$ 0.08 (solvent A); p.m.r. (CDCl$_3$): $\tau$ 6.65 (3 H singlet, OMe), 7.92, 7.94, 8.03 (3 H singlets, 4 OAc), 8.61 (3 H doublet, $J_{5',6'} = 6$ Hz, CH$_3$).

Anal. Calcd. for C$_{22}$H$_{30}$O$_{15}$: C, 49.44; H, 5.66. Found: C, 49.27; H, 5.69.
Methyl 4-O-β-D-mannopyranosyl-α-L-rhamnopyranoside (51)

Compound 50 (1.40 g) was deesterified with sodium methoxide (0.2 M, 40 ml) for 1 h at room temperature. The catalyst was destroyed as formerly described (see page 69). The sirup (0.85 g, 95%), obtained on evaporation, showed one spot on t.l.c., $R_f$ 0.03 (solvent B), and crystallized from 2-propanol (50 ml) as an alcoholate after addition and evaporation of 2-propanol (2 x 50 ml); yield 0.90 g of crystals (97%). Recrystallization from 2-propanol gave pure 51 alcoholate, m.p. 113-114.5° $[\alpha]_D$ -74.8° (c 2.2, water); $R_f$ glucose 1.44 (solvent C); p.m.r. (D$_2$O, external tetramethylsilane): $\tau$ 5.16 (1 H doublet, $J_{1',2'}$ 0.9 Hz, H-1'), 5.35 (1 H doublet, $J_{1,2}$ 1.3 Hz, H-1), 6.64 (3 H singlet, OMe), 8.69 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$). The 2-propanol of crystallization was lost by exchanging the active hydrogens with deuterium oxide.

Anal. Calcd. for C$_{29}$H$_{56}$O$_{21}$: C, 47.02; H, 7.62. Found: C, 46.82; H, 7.81.

The methyl glycoside 51 was methylated by a method previously described (see page 75), to give the corresponding heptamethyl ether 52. A small amount was purified by preparative t.l.c. 169 by analytical purposes; $R_f$ 0.17 (solvent A); $[\alpha]_D$ - 87.5° (c 1.4, chloroform); p.m.r. (CDCl$_3$): $\tau$ 5.25 (1 H doublet, $J_{1,2}$ 1.6 Hz, H-1), 5.37 (1 H doublet, $J_{1',2'}$ 0.8 Hz, H-1'), 6.40 - 6.63 (21 H, 7 OMe), 8.63 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$). Hydrolysis (as described on page 76) of 52 gave 2,3-di-O-methyl-L-rhamnose and 2,3,4,6-tetra-O-methyl-D-mannose as identified by paper chromatography (solvent D) using authentic standards (i.e. 6). Subsequent reduction and acetylation (see page 72) gave equimolar amounts of 1,4,5-tri-O-acetyl-2,3-di-O-methyl-L-rhamnitol and 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-mannitol, identi-
fied by g.l.c. (column c, 220°, retention times 21.8 and 26.6 min, respectively) and mass-spectrometric comparison with authentic standards (i.e. 7).

Periodate oxidation of 51 by a procedure previously described (see page 77) showed a total consumption of 3.0 moles of periodate per mole of 51 in 50 h. The usual processing (see page 77) gave a mixture of 4-deoxy-L-erythritol and glycerol, identified by paper chromatography (solvent D) using authentic standards (i.e. 8) and as the peracetates by comparative g.l.c. (column b, 130°, retention times 8.4 and 12.8 min, respectively) and mass-spectrometric analysis with authentic standards.

Methyl 2,3-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-\beta-D-mannopyranosyl)-\alpha-L-rhamnopyranoside (53)

Compound 51 (0.20 g) was acetylated with pyridine (10 ml) and acetic anhydride (10 ml) for 5 h at room temperature. The customary work-up (see page 68) produced the sirupy product; yield 0.33 g (95%). For analytical purposes a small amount was purified by preparative t.l.c. 169; 

[\alpha]_D = 57° (c 2.1, chloroform); Rf 0.36 (solvent A); p.m.r. (CDCl₃): 5.25 (1 H doublet, J₁'₂, 0.8 Hz, H-1'), 5.42 (1 H doublet, J₁₂ 1.6 Hz, H-1), 6.65 (3 H singlet, OMe), 7.88 - 8.04 (18 H, 6 OAc), 8.64 (3 H doublet, J₅₆ 6 Hz, CH₃).
Unsuccessful acetolysis attempts

Compound 51 or compound 53 (0.15 g) in acetic anhydride (0.75 ml) was shaken with 1% (v/v) concentrated sulfuric acid in acetic anhydride (1.5 ml) for 2 h at room temperature. The sirup resulting from the usual work-up procedures (see page 78) showed two spots on t.l.c. in approximately equal amounts, one with $R_f$ 0.36 (solvent A) and the other with $R_f$ 0.42 (solvent A). The two compounds were separated by preparative t.l.c. and the p.m.r. spectra obtained. The compound with $R_f$ 0.36 (solvent A) was identified as the desired 1,2,3-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)-α-L-rhamnopyranose (55). The compound with $R_f$ 0.42 (solvent A) had the following p.m.r. spectrum (CDCl₃): $\tau$ 4.05 (2 H, H-1, H-1'), 7.87 - 7.99 (27 H, 9 OAc, on expansion signals for nine separate acetate groups were indicated), 8.80 (3 H doublet, $J = 5$ Hz, CH₃). The mass spectrum obtained showed m/e 43, 83, 85, 115, 127, 145, 157, 169, 229, 242, 259, 273, 289, and 331. Deacetylation of the compound with $R_f$ 0.42 (solvent A) by the usual procedure (see page 69) gave a mixture of rhamnose and mannose as identified by paper chromatography (solvent C) using authentic standards.

1,2,3-Tri-O-acetyl-4-O-(4,6-di-O-acetyl-2,3-O-carbonyl-β-D-mannopyranosyl)-α-L-rhamnopyranose (54)

Compound 50 (1.50 g) in acetic anhydride (7.5 ml) was shaken with 1% (v/v) concentrated sulfuric acid in acetic anhydride (15 ml) for 2 h at room temperature. The sirup resulting from the usual work-up procedures (see page 78) showed essentially one spot on t.l.c., $R_f$ 0.07 (solvent A);
yield 1.50 g (95%). For analytical purposes a small amount was purified by preparative t.l.c. \(^{169}\); \([\alpha]_D\) - 64° (c 1.7, chloroform); p.m.r. (CDCl\(_3\)): \(\tau\) 3.99 (1 H doublet, \(J_{1,2}\) 1.6 Hz, H-1), 7.84, 7.85, 7.88, 7.90, 7.95 (3 H singlets, 5 OAc), 8.58 (3 H doublet, \(J_{5,6}\) 6 Hz, CH\(_3\)).

1,2,3-Tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-\(\beta\)-D-mannopyranosyl)-\(\alpha\)-L-rhamnopyranose (55)

Compound 54 (1.50 g) was deesterified with sodium methoxide (0.2 M, 40 ml) for 1 h at room temperature. The standard work-up procedure (see page 69) was employed. The sirup obtained on evaporation was acetylated with pyridine (25 ml) and acetic anhydride (25 ml) overnight at room temperature. The excess reagents were removed in the usual way (see page 68). The resulting sirup was anomerized \(^{159,160}\) with zinc chloride (0.10 g, freshly fused) in acetic anhydride (10 ml) for 4 h at room temperature. The reaction mixture was diluted with chloroform (200 ml), successively washed with ice-water (2 x 200 ml), saturated sodium hydrogen carbonate solution (2 x 200 ml), and water (2 x 200 ml), and the remaining acetic anhydride and/or acetic acid was removed by addition and evaporation of ethanol. The resulting sirup crystallized from ethanol (9 ml) when nucleated with a crystal obtained from a t.l.c. separation \(^{169}\); yield 1.30 g (79%). Recrystallization from ethanol gave pure 55, m.p. 164 - 165°; \([\alpha]_D\) - 67.8° (c 1.3, chloroform); \(R_f\) 0.36 (solvent A); p.m.r. (CDCl\(_3\)): \(\tau\) 4.06 (1 H doublet, \(J_{1,2}\) 1.7 Hz, H-1), 5.27 (1 H doublet, \(J_{1',2'}\) 1.0 Hz, H-1'), 7.89 - 8.04 (21 H, 7 OAc), 8.64 (3 H doublet, \(J_{5,6}\) 6 Hz, CH\(_3\)).

4-O-β-D-Mannopyranosyl-L-rhamnopyranose (56)

The peracetate 55 (0.40 g) was deacetylated with sodium methoxide (0.2 M, 15 ml) for 1 h at room temperature. After the usual operations (see page 69), the resulting sirup (0.20 g, 95%), had $[\alpha]_D^0 - 46°$ (c 2.5, water); $R_{\text{glucose}}$ 0.63 (solvent C); p.m.r. ($D_2O$, external tetramethylsilane): \(\tau\) 4.97 (0.64 H doublet, J",1,2) 1.4 Hz, H-1, α-L form), 5.20 (1 H doublet, J",1',2', 0.9 Hz, H-1'), 5.23 (0.36 H doublet, J",1,2) 1.0 Hz, H-1, β-L form), 8.77 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

G.l.c. (column a at 240°) of the per-O-((trimethylsilyl) disaccharide (prepared by the procedure on page 79) gave one peak (73%) at 8.2 min and a second peak at 10.8 min. [per-O-((trimethylsilyl) sucrose, 11.2 min]$_{127}$.

4-O-β-D-Mannopyranosyl-L-rhamnitol (57)

The free disaccharide 56 (0.10 g) was reduced with sodium borohydride (0.05 g) in water (5 ml) overnight. The customary reaction sequence (see page 72) gave 57; yield 0.10 g (99%); $[\alpha]_D^0 - 36°$ (c 2.4, water); $R_{\text{glucose}}$ 0.51 (solvent C); p.m.r. ($D_2O$, external tetramethylsilane): \(\tau\) 5.26 (1 H doublet, J",1',2', 0.9 Hz, H-1'), 8.77 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).

G.l.c. (column a at 240°) of the per-O-((trimethylsilyl) alditol (preparation analogous to that on page 79) gave one peak at 14.5 min [per-O-((trimethylsilyl) sucrose, 11.2 min]$_{127}$.

The alditol 57 (0.10 g) was acetylated with pyridine (4 ml) and acetic anhydride (4 ml) overnight at room temperature to give the corresponding octaacetate 58 (0.20 g, 98%) as a sirup; $[\alpha]_D^0 - 67°$ (c 2.6, chloroform); $R_f$ 0.33 (solvent A); p.m.r. (CDCl$_3$): \(\tau\) 5.21 (1 H doublet, J",1',2', 0.9 Hz, H-1'), 7.86 - 8.02 (24 H, 8 OAc), 8.72 (3 H doublet, $J_{5,6}$ 6 Hz, CH$_3$).
G.l.c. (column a at 270°) of the peracetylated alditol 58 gave one peak at 7.0 min (sucrose octaacetate, 9.2 min).
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APPENDIX

In the course of the synthetic studies described in this thesis other peripheral topics became of interest and hence were investigated in collaboration with other members of the laboratory.

Methods to facilitate the separation of compounds by gas-liquid chromatography are continually under investigation. The utility of sugars as acetates was being considered and it was shown that although 2,3,4,6-tetra-O-methyl-D-glucose and 2,3-di-O-methyl-L-rhamnose were difficult to separate as methyl glycosides or alditol acetates they could be conveniently resolved as acetates.

The inadequacy of current analytical methods for readily determining the number of free hydroxyl groups in a molecule was emphasized during investigations of certain of the intermediates isolated during the synthesis of disaccharides. The use of proton magnetic resonance spectroscopy of the trimethylsilyl derivative of these compounds was being examined and it was shown that the proton magnetic resonance spectrum of the trimethylsilyl derivative of a product obtained by partial methylation of methyl 4-O-acetyl-\(\alpha\)-L-rhamnopyranoside indicated one O-acetyl, two O-methyl, and one O-tri-methylsilyl (therefore one hydroxyl) group per rhamnose moiety. Integration of the trimethylsilyl signal at \(\tau\) 9.92 thus furnishes a non-destructive method for the determination of hydroxyl groups on a microscale.

Semimicro methods for determining the configuration (D or L) of sugars from isolated polysaccharides by the circular dichroism of their alditol acetates were being explored. 4-O-Methyl-D-galactose (3-O-methyl-\(\alpha\)-L-galactose) was prepared and the circular dichroism of its alditol acetate
shown to be opposite in sign to that of 3-O-methyl-D-galactose.

Proton magnetic resonance spectroscopy was being extended to polysaccharides with distinct repeating units in order to determine the number of pyruvate ketal, O-acetyl groups, and 6-deoxy sugars per repeating unit as well as the configurations of the linkages of the sugars. It was shown that *Klebsiella* type 18 capsular polysaccharide contained no pyruvate ketal and no O-acetyl groups but four α- and two β-linked sugars including two non-identical rhamnose residues per repeating unit.

The four enclosed reprints are evidence of the scope of these complementary investigations.
Separation by gas-liquid chromatography of tetra-O-methylaldohexoses and other sugars as acetates

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Gas-liquid chromatography was first applied in the carbohydrate field to methyl glycosides of methylated sugars. Since that time, data on the retention times of many such compounds have been published. The use of methyl glycosides means that, in most cases, one compound will produce several peaks; these complicate the chromatogram even though the pattern of the peaks for a particular sugar may be a useful diagnostic feature. The most common way of avoiding this problem is to reduce the methylated sugars and to separate them as methylated alditol acetates.

In situations where neither of these methods give satisfactory resolution of certain components of a mixture it has been possible, in many cases, to achieve separations of methyl glycosides and free sugars as their O-trimethylsilyl derivatives or as acetylated methyl glycosides. Unfortunately none of these combinations gives satisfactory resolution of a mixture containing 2,3,4,6-tetra-O-methyl-D-glucose, -D-galactose, and -D-mannose. The necessity for achieving this separation arises in studies on galactoglucomannans, such as occur in wood, where each of the tetra-O-methylaldohexoses in a mixture must be determined or, alternatively, the absence of one in the presence of the other two needs to be confirmed positively. Table I shows that these three ethers

TABLE I
RETENTION TIMES OF METHYLATED ALDOHEXOSE ACETATES

<table>
<thead>
<tr>
<th>Compound (as peracetate)</th>
<th>Retention time (min)</th>
<th>Approximate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>peak 1</td>
<td>peak 2</td>
</tr>
<tr>
<td>(a) Isothermal at 170°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-methylglucose</td>
<td>9.4</td>
<td>11.5</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-methylgalactose</td>
<td>13.4</td>
<td>20.2</td>
</tr>
<tr>
<td>2,3,5,6-Tetra-O-methylgalactose</td>
<td>15.1</td>
<td>23.2</td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-methylmannose</td>
<td>17.9</td>
<td>23.2</td>
</tr>
<tr>
<td>(b) Isothermal at 185°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-Tetra-O-methylglucose</td>
<td>4.8</td>
<td>5.2</td>
</tr>
<tr>
<td>2,3,6-Tri-O-methylglucose</td>
<td>14.8</td>
<td>17.0</td>
</tr>
<tr>
<td>2,3,6-Tri-O-methylmannose</td>
<td>31.2</td>
<td>39.7</td>
</tr>
<tr>
<td>2,3-Di-O-methylglucose</td>
<td>34.8</td>
<td>39.7</td>
</tr>
</tbody>
</table>

Carbohydr. Res., 23 (1972) 430-432
may be separated readily in the form of their acetates. Although this method causes each compound to give two peaks, the retention times of these peaks serve for characterization.

Table I also shows that, at a slightly higher column-temperature, di-O-methylaldohexoses are sufficiently volatile to be separated as their acetates; particularly noteworthy is the marked difference in retention times between 2,3,6-tri-O-methyl-D-glucose and the corresponding D-mannose analog. This is particularly useful since these two compounds as alditol acetates and as methyl glycosides have similar retention times.

These results demonstrate that, for the examples cited, separation of methylated sugars as their acetates is the preferred method. This system is thus a useful complement to those previously described, and has the additional merit that the free sugar may be readily regenerated for further characterisation. The only other report of the use of acetates of methylated sugars appears to be that of H. G. Jones, J. K. N. Jones, and Perry, who used this method in a study on the separation of mono-, di-, tri-, and tetra-O-methyl-D-glucose derivatives.

EXPERIMENTAL

2,3,4,6-Tetra-O-methyl D-glucose, D-galactose, and D-mannose were prepared from the corresponding sugars by a standard procedure. 2,3,6-Tri-O-methyl-D-glucose was obtained by hydrolysis of a commercial methylated cellulose. 2,3,6-Tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-glucose were available from previous studies. All compounds and/or their derivatives had physical properties in accord with literature values.

Sugars were acetylated by dissolving them in acetic anhydride-pyridine (1:1) and heating the mixtures under anhydrous conditions (conveniently in a sealed tube) for 15 min on a steam bath. The acetates were extracted with chloroform and the solution was washed twice with M hydrochloric acid, followed by aqueous sodium hydrogen carbonate, and water. The dried (CaCl₂) extract was concentrated to small volume for injection. Omission of the washing procedure gave extraneous peaks.

Gas-liquid chromatography was carried out on a F and M 720 instrument using dual column (8 ft x 3/16 in.) of 3% ECNSS-M on Gas-Chrom Q with a helium flow-rate of 86 ml/min.

ACKNOWLEDGMENT

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Note added in proof (11 April 1972). 2,3,4,6-Tetra-O-methyl-l-glucose and 2,3-di-O-methyl-
l-rhamnose, a pair of compounds difficult to separate as methyl glycosides or alditol acetates, may be resolved conveniently as acetates. Using a column (4 ft × 1/4 in.) containing 5% butanediol succinate on Diatoprot 5 (60–100 mesh) and a temperature program from 110 to 210° at 2°/min, the acetates of 2,3,4,6-tetra-O-methyl-l-glucose were eluted at 27.9 and 30.2 min, and those of 2,3-di-O-
methyl-l-rhamnose at 37.0 and 40.7 min. In addition, two unidentified peaks (ca 7% of total peak area) were eluted at 16.1 and 18.5 min.

DETERMINATION OF HYDROXYL GROUPS BY P.M.R. SPECTROSCOPY OF TRIMETHYLSILYL ETHERS

KEY WORDS: P.m.r. spectroscopy, trimethylsilyl ethers, hydroxyl group determination.

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ABSTRACT

Trimethylsilyl ethers are shown to give sharp p.m.r. signals at 9.8-9.9 and to have distinct chemical shifts. The technique is useful for determining the number of hydroxyl groups in a molecule. Because each trimethylsilyl group consists of nine protons strong signals are obtained from small amounts of sample. The method is thus suitable for analyzing eluates collected by gas-liquid chromatography.

INTRODUCTION

The O-trimethylsilyl ethers (-OSiMe$_3$) are widely used for the analysis of carbohydrates by gas-liquid chromatography following the introduction of this technique by Sweeley and co-workers. These applications have been reviewed by Dutton. More recently O-trimethylsilyl ethers have been used as convenient carbohydrate derivatives for the p.m.r. spectroscopic determination of the geometry of the anomeric proton in mono- and oligo-saccharides. In these studies attention was concentrated on the region of the spectrum $\tau=5$ and the signals due to the O-trimethylsilyl ethers were dismissed as having $\tau \approx 10$.
The hydroxy compound was dissolved in pyridine and reacted with hexamethyldisilazane and chlorotrimethylsilane according to Sweeley and co-workers. Where the hydroxy compound was crystalline the solvent and excess reagents were removed by evaporation in vacuo at 40° and the residue was dissolved in chloroform or benzene for p.m.r. measurements. In the case of starting materials which were syrups the O-trimethylsilylated product was dissolved in hexane and purified by g.l.c. on a column of 20% SF-96 on Diatoport S (60-80 mesh) held at 130° for 6 min and then programmed at 3°/min to 200°. Portions of the g.l.c. eluate were collected and used for p.m.r. spectroscopy. Pure α-D-glucose was mutarotated in water and evaporated to a syrup before silylation (g.l.c. program 190-220° at 3°/min).

P.m.r. spectra were obtained on Varian HA-100 and XL-100 instruments operated in the frequency sweep mode and locking onto the chloroform or benzene signal.

RESULTS AND DISCUSSION

Figures 1-9 show that the protons of the (-OSiMe₃) group give a sharp signal at 9.8-9.9 and, furthermore, that the chemical shifts of non-identical -OSiMe₃ ethers are different. Such p.m.r. measurements thus provide clear evidence of the number of trimethylsilyl ether groups, and hence hydroxyl groups, in a molecule. The majority of the spectra shown were obtained in chloroform solution but it was subsequently found that resolution of the signals was greater in benzene, compare Figs. 3 and 4; 5 and 6; a situation similar to that which exists for O-methyl ethers.

The use of O-trimethylsilyl ethers for the determination of hydroxyl groups by p.m.r. spectroscopy is similar to other methods.
DETERMINATION OF HYDROXYL GROUPS

FIGS. 1-4

P.m.r. spectra of per-O-trimethylsilyl derivatives of:
1. glycerol,
2. erythritol,
3. methyl α-D-galactopyranoside,
4. as 3 in C₆H₆.
For Figs. 1-9 only the spectrum in the region τ 9.9 is shown. Positions of peaks are given in Hz downfield from TMS.

FIGS. 5-8

P.m.r. spectra of per-O-trimethylsilyl derivatives of:
5. 6-O-(6-D-glucopyranosyl)-1,2:3,4-di-isopropylidene-D-galactose,
6. as 5 in C₆H₆,
7. αβ-D-glucofuranose,
8. β-D-glucopyranose.
which have employed O-acetyl\textsuperscript{6} or O-trifluoroacetyl\textsuperscript{7} derivatives but has certain significant advantages.

Every \(-\text{OSiMe}_3\) group introduced increases, in effect, the number of protons on each hydroxyl group by a factor of nine. In this sense a trimethylsilyl group may be considered to act as a "signal multiplier". This is of particular advantage when only small quantities of sample are available, and the signals from other protons may be lost in background noise. This feature is illustrated by the following experiment which also demonstrates the feasibility of examining the nature of \(\text{O-trimethylsilyl}\) derivatives separated by gas-liquid chromatography by p.m.r. spectroscopy.

The gas chromatogram of per-\(\text{O-trimethylsilylated}\) \(\text{D-glucose}\) often shows a small peak, amounting to about 1\% of total peak area, representing an unidentified compound, in addition to major peaks of \(\alpha\)-D- and \(\beta\)-D-glucopyranose. Although the unknown was thought to be a furanose it could not be ruled out that it was due to undersilylation or an anhydro form of \(\text{D-glucose}\). Repeated injection of per-\(\text{O-trimethylsilylated}\) \(\text{D-glucose}\) permitted the collection\textsuperscript{8} of 2 mg of material. The p.m.r. spectrum of this component (Fig. 7) indicates the presence of five \(-\text{OSiMe}_3\) groups. The resolution of the signals is less distinct in this case since, presumably, the component was a mixture of \(\alpha\) and \(\beta\) anomers. With a sample of this size the signals for the other protons in the molecule were obscured by spectrometer noise. For the pure pyranose anomers the resolution is good (e.g. Fig. 8).

In the domain of carbohydrate chemistry it is often necessary to know the number of O-methyl groups in a molecule. It is usually not possible to do this by direct p.m.r. spectroscopy of the \(-\text{OCH}_3\) signal since the methyl protons resonate at frequencies similar to the protons of the carbohydrate ring (Fig. 10). This problem may be resolved by...
DETERMINATION OF HYDROXYL GROUPS

FIGS. 9 and 10

P.m.r. spectra of per-O-trimethylsilyl derivatives of: 9. methyl 2,3-di-O-methyl-α-D-galactopyranoside, 10. Unknown methyl O-acetyl-0-methyl-α-L-rhamnoside (CDCl₃). Fig. 10 shows the region r 6.5-10. All spectra were run in CHCl₃ except as noted.

conversion of the sample to the O-trimethylsilyl ether and determination of the number of unmethylated hydroxyl groups; the number of methyl ethers may then be determined by difference. This is shown in Fig. 9 which represents the spectrum of the derivative of methyl 2,3-di-O-methyl-α-D-glucoside and also by Fig. 10. Since methyl ethers of carbohydrates are often separated by g.l.c. as their O-trimethylsilyl derivatives this illustrates another example of a useful combination of the two techniques.

A further advantage of -OSiMe₃ ethers is that they may be used for determining the number of free hydroxyl groups in a molecule in the presence of O-acetyl groups⁹. Not only are the p.m.r. signals of O-acetyl and O-trimethylsilyl groups widely separated but, more importantly, the latter group may be introduced and removed under conditions which do not affect the former. Thus an unknown material isolated in other research¹⁰ was shown (Fig. 10) to contain one O-acetyl and one free hydroxyl group by conversion to the O-trimethylsilyl ether.

These results show clearly the utility of O-trimethylsilyl ethers as an analytical tool for determining the number of hydroxyl groups in
organic molecules. While the examples shown are mainly carbohydrates the technique is clearly of general application.

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Semimicro Determination of Sugar Configuration by Measurement of Circular Dichroism of Alditol Acetates


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The configuration (D or L) of a sugar may be determined conveniently by circular dichroism measurements at 213 nm on alditol acetates, or their methylated derivatives, where the acetoxy group acts as a chromophore. Only milligram quantities of material are required and the method is well suited to analyzing fractions obtained by gas-liquid chromatography. Structural information which may be derived from the c.d. spectra is briefly discussed.

La configuration (D or L) d'un sucre peut être établie par le dichroisme circulaire à 213 nm soit des alditol acetates soit de leur dérivés méthyles, où le groupement acétate joue le rôle de chromophore. On ne nécessite que de très petites quantités de substance et la méthode s'adapte très bien à l'analyse des fractions obtenues par chromatographie en phase gazeuse. On discute brièvement des renseignements structuraux que fournissent les spectres d.c.

Determinations of the structure of a polysaccharide requires that the nature and proportion of the constituent sugars be known as well as the positions through which the monosaccharide units are linked in the polymer. Such investigations are facilitated by the use of gas-liquid chromatography (g.l.c.) and sugar ratios are customarily determined as trimethylsilyl ethers or as alditol acetates (1). Similarly, partially methylated sugars are conveniently separated by g.l.c. of their derived alditol acetates and the pattern of methylation is determined by mass spectrometry (2). The technique of mass spectrometry does not readily differentiate between diastereoisomers but this problem may be resolved by collection of the methylated alditol acetate from the effluent gas stream (3) followed by demethylation and characterization of the parent alditol as the peracetate (4).

None of these chromatographic methods is capable of determining the configuration (D or L) of the constituent sugars of a polysaccharide, an aspect of structural studies which is sometimes ignored. One great merit of procedures involving gas-liquid chromatography and mass spectrometry is the small quantity of material required and this is of particular value when only limited amounts of sample are available as, for example, with bacterial polysaccharides. The advantage of these techniques is lost if the determination of the configurational series must be made on macroscopic amounts of specimen. In the particular cases of D-glucose and D-galactose specific oxidases are available (e.g. Glucostat and Galactostat reagents, Worthington Biochemical Corporation) but no such method is available for other commonly occurring sugars; even so these are destructive methods.

We have now demonstrated that the sugar configuration (D or L) may be determined unambiguously on milligram quantities of monosaccharide derivatives from the circular dichroism (c.d.) band at ca. 213 nm of the cor responding alditol acetates. Just as a sugar of D-configuration may have either a positive or negative specific rotation so also D-alditol acetates may have c.d. bands of either sign. Thus, this sign must be determined empirically and a selection of such data is presented in Table 1. While the method is obviously inapplicable to meso-alditols, such as galactitol, it is readily extended...
to their chiral methylated acetates obtained in structural studies. Conversely, in order to avoid the necessity of measuring c.d. bands for all possible methylated alditols these compounds may conveniently be demethylated (4) where the parent alditol is chiral.

Circular dichroism measurements were made using a Jasco J-20 automatic recording spectropolarimeter, accurately weighed milligram quantities of the alditol acetate being dissolved in acetonitrile (0.15 ml, spectroscopic grade) and placed in a quartz spectrophotometer cell with path length of 0.1 cm. The spectrometer was calibrated using the value of ε = 1.00 for (+) 10-camphorsulfonic acid in water (5). The c.d. curves obtained at a scan-rate of 5 nm/min were bell shaped and symmetrical as far as could be ascertained (measurements below 205 nm were not reproducible). While there is no doubt about the sign of the c.d. curve in each case examined, the numerical values of Δε are sometimes hard to reproduce particularly for those compounds which have small values of Δε. This is an instrumental problem since the acetate band lies near the extreme range of the instrument used.

The initial experiments described here were carried out during an investigation, to be described later (6), into the structure of the capsular polysaccharide of Klebsiella K-type 7 and have subsequently been employed in other, related structural studies. Measurements have been made on the alditol acetates used for quantitative analysis of polysaccharide hydrolyzates (7), as for Klebsiella K-type 21, on methylated alditol acetates and on alditol acetates obtained by demethylation and acetylation. The first method is particularly convenient for mannose where no simple micromethod exists.

The capsular polysaccharide of Klebsiella K7 contains glucose, galactose, mannose, and glucuronic acid. From the methylated polysaccharide 2-O-methyl- and 2,4,6-tri-O-methylglucose are obtained as well as 2,4-di-O-methylglucose which results from reduction of the glucuronic acid residue. These compounds were separated by g.l.c. of their alditol acetates. Demethylation and acetylation of the individual fractions yielded (g.l.c.) glucitol hexaacetate, each sample of which gave a positive c.d. band with Δε values ranging from +0.28 to +0.33 depending on the amount of contaminating liquid phase caused by bleeding of the column. Thus each glucose residue in Klebsiella K7 is shown to be of D-configuration as is the glucuronic acid. Mannitol hexaacetate samples were obtained similarly from the methylated mannose derivatives, namely the 4,6-di- and 3,4,6-tri-O-methyl ethers, and each sample showed a strong positive c.d. band (Δε +1.4 to +1.7) thus demonstrating that all mannose units in the polysaccharide are of D-configuration.

The galactose in Klebsiella K7 gives only 2,3,4,6-tetra-O-methylgalactose which, on reduction and conversion to the syrupy diacetate, gave a positive c.d. band (Δε +0.54) similar to that obtained from an authentic sample of the methylated sugar of D-configuration and to that obtained by methylation of a wood galactoglucosmannan (8). One sample of galactitol hexaacetate prepared by demethylation and acetylation was shown to be achiral.

In addition, a sample of 2-O-methyl-D-glucitol pentaacetate obtained from K7 polysaccharide was recrystallized from ethyl acetate – petroleum ether (b.p. 30–60°) and had m.p. 56–57°. The c.d. measurement showed a positive band with Δε +0.71, a value significantly greater than for D-glucitol hexaacetate. Measurements of c.d. spectra may also provide alternative means for determining the position of methoxyl substitution, distinguishing between isomers and estimating the composition of mixtures. Thus,
acetates and their methylated derivatives is thus not depress the m.p. of authentic samples respectively. Each sugar was crystalline and did -galactopyranoside (10), -D 2,3,6-tri-O-benzoyl-a -galactofuranose (9) and of methyl -D charides.

The pattern of substitution in such monosaccharides is now under investigation as is the possibility that differences in rotational strength may be useful in distinguishing isomeric methylated alditol acetates. The c.d. spectra of sugars (12, 13) and of monoacetates of methyl glycosides (14) have been discussed recently.

We are grateful to the National Research Council of Canada for financial support, to Professor J. K. N. Jones, F.R.S. for an authentic sample of 3-O-methyl-D-galactose and to Mrs. E. Dumitrescu for skilled assistance in taking spectra. One of us (A.M.) is indebted to the University of Cape Town for study leave and to C.S.I.R. for the award of a bursary.

The symmetry rule relating molecular conformation and the sign of the c.d. band for alditol acetates is now under investigation as is the possibility that differences in rotational strength may be useful in distinguishing isomeric methylated alditol acetates. The c.d. spectra of sugars (12, 13) and of monoacetates of methyl glycosides (14) have been discussed recently.

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The symmetry and utility of this is demonstrated by the following experiments.

In structural studies on xylans both 2- and 3-O-methyl-D-xyloses are commonly encountered and this pair of compounds is not separable as alditol acetates by g.l.c. Collection of the monoacetate fraction from the g.l.c., in a study on western red cedar (8), and measurement of $\Delta \varepsilon$ showed that the 2-O-methyl derivative was present to the extent of 35%, a value in close agreement with that (33%) obtained by integration of the well resolved methoxyl signals in the p.m.r. spectrum of the mixture of alditol acetates. A control experiment verified that 3-O-methyl xylitol is achiral.

The 3- and 4-O-methyl-D-galactoses were synthesized by methylation of 1,2:5,6-di-O-isopropylidene-D-galactofuranose (9) and of methyl 2,3,6-tri-O-benzoyl-D-galactopyranoside (10), respectively. Each sugar was crystalline and did not depress the m.p. of authentic samples (10, 11).

Measurement of c.d. spectra of chiral alditol acetates and their methylated derivatives is thus proposed as a convenient, non-destructive method for determining the configurational series of a sugar. The technique requires very small samples and is admirably suited to examining individual fractions obtained by gas–liquid chromatography. By combining these techniques with mass spectrometry most partially methylated monosaccharides may be identified completely, including the configurational assignment.

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Measurement of c.d. spectra of chiral alditol acetates and their methylated derivatives is thus proposed as a convenient, non-destructive method for determining the configurational series of a sugar. The technique requires very small samples and is admirably suited to examining individual fractions obtained by gas–liquid chromatography. By combining these techniques with mass spectrometry most partially methylated monosaccharides may be identified completely, including the configurational assignment.
Proton Magnetic Resonance Spectroscopy of *Klebsiella* Capsular Polysaccharides

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The presence of acetate and pyruvate groups in *Klebsiella* capsular polysaccharides may be demonstrated and estimated quantitatively by running the proton magnetic resonance spectrum of the polysaccharide (as sodium salt) in deuterium oxide at 95°C. Such spectra also permit an assessment to be made of the number of α- and β-linkages in the repeat unit of the polysaccharide structure.

Pyruvic acid is found covalently linked to a sugar residue in a variety of polysaccharides, especially those which form the capsule of *Klebsiella* bacteria (3). There is a lack, however, of a nondestructive method for the estimation of pyruvate. It has been shown recently (Y. M. Choy et al., Anal. Lett. 5:675, 1972) that certain capsular polysaccharides (molecular weights 5 to 9 × 10⁵) from *Klebsiella*, after exchange with deuterium oxide (D₂O), give proton magnetic resonance (PMR) spectra in which the presence of a pyruvic acid ketal is clearly demonstrated by the signal at δ 8.5, characteristic of CH₃—C. Derivatives of these ketals, prepared during structural studies (Y. M. Choy et al., Anal. Lett. 5:675, 1972), also give signals at δ 8.5 to 8.6.

MATERIALS AND METHODS

In PMR spectra the chemical shift (i.e., position) of peaks is measured downfield from the signal given by tetramethylsilane (TMS) which is assigned a value of δ = 10. The integral gives the area beneath a peak which is proportional to the number of protons resonating at that particular frequency. Labile hydrogen atoms in poly- and oligosaccharides are deuterated by repeated exchange with D₂O. Further details may be found in many reviews (4, 5).

Some difficulty was experienced in making quantitative determination of the pyruvic acid content because of the large peak present due to partially deuterated water (HOD). This peak appeared at approximately δ 5 to 6, partially covering those regions of the spectrum associated with anomeric and ring protons. The magnitude of the HOD peak was largely due to the necessity of working with solutions of less than 2% concentration because of their viscosity. This same viscosity prevented the solutions from being cooled in order to move the HOD peak downfield. The HOD peak may be moved downfield by running the spectrum on a solution of polysaccharide in trifluoroacetic acid which permits integration of the pyruvic acid and ring proton signals (Y. M. Choy et al., Anal. Lett. 5:675, 1972). This is not very precise because of the disparity in size of the integrals being compared. The fact that the acid may degrade the polysaccharide is a further disadvantage. Conversely, the HOD signal is moved upfield when the polysaccharide (in free acid form) is dissolved in methyl sulfoxide-d₆ and the spectrum is run at 95°C. This method also suffers from the disadvantage of heating solutions of acidic polysaccharides (Y. M. Choy et al., Anal. Lett. 5:675, 1972).

We have now found that excellent PMR spectra may be obtained by dissolving the sodium salt of the polysaccharide, after exchange, in D₂O and running the spectra at 95°C. This is currently used as a routine screening process from which the polysaccharide samples may be recovered unchanged. Some representative results are discussed and the figures illustrate spectra obtained from *Klebsiella* capsular polysaccharides containing O-acetyl or pyruvate ketal groups or both. Polysaccharides were isolated as described elsewhere (1; Y. M. Choy and G. G. S. Dutton, Can. J. Chem., in press), converted to their sodium salts (It is convenient to use samples whose equivalent weight has been determined by titration.), and exchanged 2 or 3 times with D₂O. Spectra were run on solutions of approximately 2% concentration in D₂O at 95°C using a Varian XL100 instrument with tetramethylsilane as the external standard.

RESULTS

Figure 1A shows the spectrum of K21 polysaccharide and illustrates the sharp signal at δ 8.5 for the CH₃—C of the pyruvate ketal. The four signals in the range δ 4.5 to 5.5 are due to the anomeric protons and integration shows clearly that there are five anomeric protons to...
The PMR spectra (100 MHz) run in D₂O at 95°C of capsular polysaccharides from Klebsiella: A. K21; B. K24; C K5. The numbers beside the integrals represent the areas under each peak in arbitrary units. Thus, in A the signal at τ 8.5 is due to the methyl group of the pyruvic acid ketal, and therefore represents three protons; hence one proton = 46/3 units ~ 15 units. It is then clear that the peaks around τ 5 correspond to 1 + 2 + 1 + 1, or five protons. The signals around τ 5 are those given by the anomeric protons, and therefore there are five sugar units and one pyruvic acid ketal per repeat unit. In C the signals at τ 7.8 and τ 8.5 each correspond to 3 protons (CH₃ of acetate and pyruvate, respectively); thus, taking the average, 3 protons = 19 units and therefore the integral of the signals at τ 5.5 shows that there are three anomeric protons (i.e., 3 sugar residues) per repeat unit. Case B is less precise but suggests 1-O-acetyl group (τ 7.8, 3H = 50 units) for about 7 sugar residues.

Each pyruvate ketal. Furthermore, an anomeric signal above τ 5.0 is indicative of a hexose unit linked in the β-D-configuration (6). The PMR spectrum therefore suggests that in this K21 polysaccharide, only one of the five sugar units in the repeat unit has a β-D-linkage. This polysaccharide contains β-galactose and β-mannose, in addition to β-glucuronic acid. The splitting of 7 Hz at the signal of τ 5.15 shows that the protons on C-1 and C-2 are trans-diaxial; i.e. they have a dihedral angle of 180°. This signal therefore cannot be due to a β-mannose unit, since in this sugar the protons on C-1 and C-2 are either trans-diequatorial (α-D-) or cis-axial-equatorial (β-D-) and in each case have a splitting (coupling constant) of 2-3 Hz (4).
This splitting is not distinct when the spectrum is run in methyl sulfoxide-\(d_6\). Detailed examination of K21 polysaccharide has subsequently confirmed that the structure has a repeat unit of five sugar residues, of which one D-galactose unit is \(\beta\)-linked (Y. M. Choy and G. G. S. Dutton, Can. J. Chem., in press).

Figure 1B is the spectrum for K24 polysaccharide which shows the presence of an O-acetyl group (\(r\ 7.8\)) and the absence of pyruvate. The anomeric signals integrate for five protons, but in this case, the chemical shifts suggest that three sugar units are \(\alpha\)-\(\beta\)-linked and 2 are \(\beta\)-\(\alpha\)-linked. The acetate content corresponds to one O-acetyl group per seven or eight sugar units. This indicates a certain degree of random character in the acetate substitution or loss of O-acetyl during the isolation of the polysaccharide. The latter is unlikely in view of the mild procedures used.

Figure 1C shows the spectrum for K5 polysaccharide and demonstrates that there are one O-acetyl group and one pyruvate ketal to every repeat unit of three sugars, each of which is linked by a \(\beta\)-\(\alpha\)-bond. This is in accord with the chemical structure as subsequently determined (2).

In a similar manner, the ratio of pyruvate to L-fucose in K6 has been shown to be 1:1; K7 has one pyruvate to 8 to 9 sugar residues; K18 has neither O-acetyl nor pyruvate. In K22, the ratio of pyruvate to L-rhamnose is 1:4, and in K56 the ratio is 1:1, with the L-rhamnose being one member of a five-sugar repeat unit.

The sharpness of the signals due to the acetate and pyruvate groups, as well as those of the anomeric protons, may be taken to indicate that such structural features all have a constant environment in these bacterial polysaccharides. In other words, the nature of these signals is further evidence that Klebsiella capsular polysaccharides are indeed composed of true repeat units.

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LITERATURE CITED

PUBLICATIONS


