PERIODATE OXIDATION PRODUCTS OF SOME PARTIALLY METHYLATED HEXOSES AND HEXITOLS

by

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ABSTRACT

Chairman: Professor G.G.S. Dutton

Periodate Oxidation Products of Some Partially Methylated Hexoses and Hexitols

Complete periodate oxidation of 2,3-di-O-methyl-D-glucitol and 2,3-di-O-methyl-D-mannitol produced 2,3-di-O-methyl-L-threose and 2,3-di-O-methyl-D-erythrose respectively. Reduction with sodium borohydride produced the corresponding tetritols, 2,3-di-O-methyl-L-threitol and 2,3-di-O-methyl-erythritol. Incomplete periodate oxidation of the two hexitols lead to the formation of large amounts of the respective pentoses, 3,4-di-O-methyl-L-xylose and 3,4-di-O-methyl-D-arabinose.

Attempts to prepare 4-O-methyl-D-threose from methyl 6-O-methyl-αβ-D-galactofuranoside and 2-O-methyl-D-erythrose from methyl 4-O-methyl-α-D-mannopyranoside by periodate oxidation were only partially successful. Although the oxidation proceeded smoothly, the cleavage of the oxidized product could not be readily effected with mineral acid, methanolysis or mercaptolysis without extensive degradation taking place. Reduction of the aldehydes generated by periodate oxidation allowed the facile preparation of the corresponding tetritols. A scheme to correlate the original aldehyde structure to the reduced tetritol is proposed.

The structure of an unknown component in the hydrolysis of methyl 2,3-di-O-methyl-α-D-glucopyranoside in the presence of 2,3-di-O-methyl-D-glucitol was shown to be 1,4-anhydro-2,3-di-O-methyl-D-glucitol.

The paper chromatographic characteristics of the prepared tetroses, tetritols and pentoses in the two most commonly used solvent systems have been recorded.

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The author also wishes to thank his wife for her patience and support during the course of this work.

INTRODUCTION

One of the most important methods for the determination of the structure of polysaccharides involves the preparation of the polysaccharide methyl ether and subsequent identification of the methylated monosaccharides resulting from its hydrolysis. These methylated sugars are often present in extremely complex mixtures and partition chromatography is the preferred method for the separation of the components. Although many advances have been made in chromatography (1), it is not possible in every case to separate completely all the components nor to identify readily some of the sugars which are resolved (2). Many techniques have been employed to identify these sugars or sugar mixtures: paper electrophoresis (3), determination of hexose or pentose structure (4), demethylation (5), complete methylation (6), optical rotation (7), formation of an osazone to test for substitution at C-2 (8), furanoside formation (9), oxidation to the lactone and subsequent examination of hydrolysis rate, or IR spectra indicating lactone ring size (10), formation of the trityl ether at C-6 (11), and periodate oxidation of the sugar or sugar alcohol (12,13,14).

Periodate oxidation has been employed in both the structural determinations of synthetic methylated sugars and in the identification of partially methylated sugars obtained from hydrolysed methylated polysaccharides. Bell, for example, employed periodate oxidation in the determination of the structure of such sugars as 2,6-di-O-methyl-D-galactose (15) and 3,4-di-O-methyl-D-galactose (16).

Lemieux and Bauer (17) used periodate oxidation as a means of identification for mono-O-methylglucoses. Alkaline hydrolysis of the periodate

oxidation product of any mono-O-methyl glucopyranose yields a substance which can be positively distinguished by paper chromatography from the products formed under the same conditions from the other mono-O-methylglucopyranoses. Extension of the method to di and tri-O-methyl glucoses was discussed. The value of the procedure was illustrated by characterization of the O-methyl glucoses derived from a water-soluble O-methylcellulose. The R_f value of the product obtained from the oxidation of 4-O-methyl-D-glucopyranose was of the order expected for 2-O-methyl-D-erythrose. The reducing substance formed from 2,3,6-tri-O-methyl-D-glucitol was assumed to be 2,3-di-O-methyl-L-threose.

Stephen (18) in the structural investigation of Virgilia oroboides gum obtained a chromatographically fast running spot on periodate oxidation of 2,3,6-tri-O-methyl-D-galactitol, 2,3-di-O-methyl-D-galactitol, 2,3-di-O-methyl-L-arabinitol and 2,3-di-O-methyl-D-glucitol assumed to correspond in each case to 2,3-di-O-methyl-L-threose.

Dutton and Unrau in structural studies of mesquite gum (19) and a number of synthetic polysaccharides: glucan (20), xylan (21), arabinan (22), rhamman (23), galactan (24) and mannan (25) have employed periodate oxidation extensively as a tool in the identification of the components of complex mixtures of methylated sugars and the alcohols derived from them. In the glucan study, these periodate oxidations produced reducing fragments believed to be partially methylated tetroses. In subsequent papers, reduction of the reducing fragments lead to the formation of a considerable number of methylated tetritols which were subsequently analysed by gas liquid chromatography (G.L.C.).

A common factor in the work of Lemieux and Bauer (17), Stephen (18) and Dutton and Unrau was the production of methylated tetrose or tetritol fragments resulting from the periodate oxidation of mixtures of methylated sugars or their derived alcohols. Identification of the unreduced periodate

oxidation products is desirable since the product provides more information than does the derived polyol. For example, reduction of the reducing fragments below yield the same tetritol:

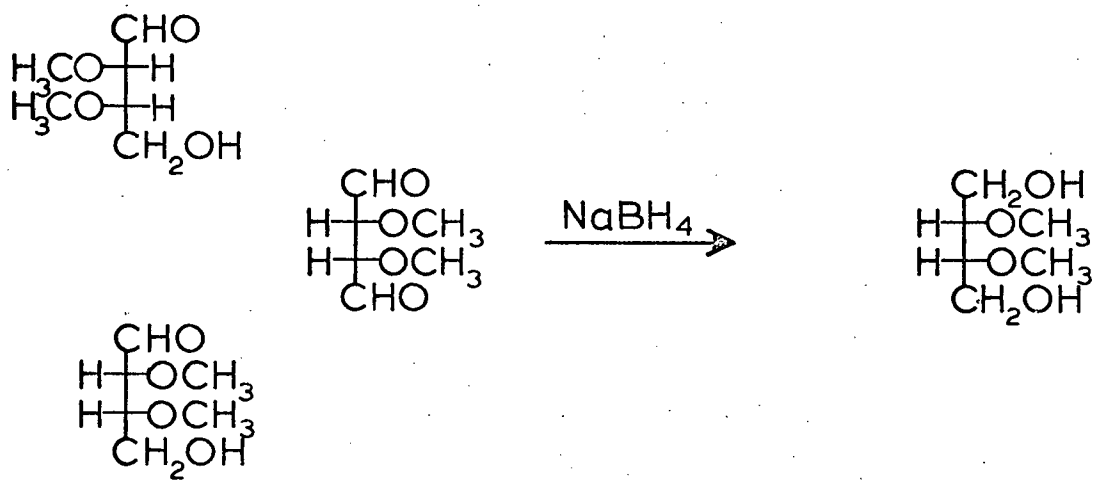


Figure 1. Reduction of three periodate oxidation products to one tetritol.

The studies mentioned above would have been facilitated by the existence of a well characterized series of reference compounds. This laboratory has been engaged in a programme to synthesize these reference compounds. The four isomeric 2,4-di-O-methyl tetroses were synthesized by Slessor and Dutton (26) and in a continuation of this programme this thesis reports the synthesis of 2,3-di-O-methyl-L-threose, 2,3-di-O-methyl-D-erythrose, 2,3-di-O-methyl-L-threitol, 2,3-di-O-methylerythritol, 4-O-methyl-D-threose, 4-O-methyl-D-threitol, 2-O-methyl-D-erythritol and the two pentoses 3,4-di-O-methyl-L-xylose and 3,4-di-O-methyl-D-arabinose.

HISTORICAL

The generic name tetrose refers to the four carbon straight chain aldoses. There are three possible approaches to the synthesis of tetrose derivatives:

1. Lengthening the carbon chain of a lower sugar.
2. Fission of the carbon chain of a higher sugar.
3. Reaction of the tetroses themselves.

The Kiliani and Fischer (27) cyanohydrin synthesis is based on the formation of cyanohydrins (28) from aldehydes and ketones with hydrogen cyanide. Fischer (29) showed that the α-hydroxy acids formed on hydrolysis of the cyanohydrins could be utilized for the preparation of higher aldoses by reduction of their derived aldonic acid lactones. More recently, the nitromethane synthesis has been used to lengthen sugar chains. The addition of nitromethane to aldehydes yields nitroalcohols which can be decomposed in acid solution to yield hydroxyaldehydes. This method, first applied to sugars by Pictet (30), was thoroughly investiaged by Sowden and Fischer (31) as a general method for lengthening the carbon chain of aldoses. The reaction of diazomethane with aldonic acid chlorides results (on decomposition) in the formation of ketoses with one more carbon atom (32). Enzymatic action has also been utilized to lengthen carbon chains of sugars (33,34).

There are three inherent problems in applying chain lengthening by nitromethane and Kiliani-Fischer syntheses to the formation of tetrose derivatives. Firstly, a new asymmetric centre is introduced alpha to the aldehyde, resulting in an epimeric mixture which must be separated. Secondly, the nature of the synthesis makes impossible the formation of a derivative substituted at C-2 unless further reactions are carried out. The third problem is the difficulty of working with glyceraldehyde.

The use of diazomethane is not applicable since aldoses are desired. The use of enzymes would be involved since specific enzymes would be difficult to obtain and they might be expected to reject the compounds if derivatives

were prepared before chain lengthening.

There are several methods for shortening the chain length of monosaccharides. The classical Wohl (35), Ruff (36) and Weerman (37) degradations which eliminate hydrogen cyanide, carbon dioxide and sodium isocyanate respectively, result in the reduction of the chain length by one carbon atom. More recently, it was reported that aldose dithioacetals may be suitable starting materials for the preparation of lower aldoses (38,39). Oxidation of the dithioacetals with monoperphthalic acid yields the disulphone which, in an alkaline medium, loses bis-(ethanesulphonyl)methane yielding an aldose with one less carbon atom. Probably the most useful method is degradation through glycol cleavage. Cleavage between two adjacent hydroxyls by periodate (40), or the less frequently used lead tetracetate (41), results in the formation of two aldehydes. The use of these reagents has been recently reviewed (42,43).

The inherent problem with these degradative methods as a synthetic tool is the necessity of preparing a suitable derivative. The classical cleavages that eliminate the anomeric carbon require that there be no substituent at C-2. The glycol cleaving agents require blockage of all vicinal hydroxyls not to be cleaved in the reaction.

Although utilization of the tetroses as the starting material for preparation of partially methylated derivatives is a feasible approach, little is known about tetrose derivatives and the tetroses needed would normally have to be prepared by the conventional means of oxidative cleavage of higher sugars.

There have been few reports of the synthesis of methylated tetroses and, except for one report, all procedures have employed periodate oxidation. In the first recorded synthesis of a methylated tetrose, Gatzi and Reichstein (44) methylated 1,2-O-isopropylidene-L-threose. Mild hydrolysis yielded

3-O-methyl-L-threose. However, the physical constants of the compound were not reported. Smith et al. (45, 46) in periodate oxidation studies of some partially methylated sugars, had occasion to synthesize two partially methylated tetroses. Periodate oxidation of 3-O-methyl-D-xylose and 2,3-di-O-methyl-D-arabinitol yielded 2-O-methyl-D-threose and 2,3-di-O-methyl-D-threose respectively. Richards (47) oxidized methyl 6-O-methyl-D-galactofuranosides with sodium metaperiodate. The dialdehyde formed was hydrolysed to yield 4-O-methyl-D-threose. The derivative prepared to characterize the compound was the phenylosazone. This derivative destroys the optically active centre at C-2 and hence is not a characteristic derivative of 4-O-methyl-D-threose. This compound has been resynthesised in order to prepare a characteristic derivative. Dutton and Slessor (26) in their synthesis of the four isomeric 2,4-di-O-methyl tetroses employed periodate oxidation of methylated sugar alcohols. 2,4-Di-O-methyl-D- and L-erythroses were prepared from 4,6-di-O-methyl-D-glucitol and 3,5-di-O-methyl-L-arabinitol respectively. The 2,4-di-O-methyl-D- and L-threoses were prepared from 3,5-di-O-methyl-D-xylitol and the mixed alcohols produced on reduction of 1,4,6-tri-O-methyl-L-sorbose.

The method chosen to synthesize the partially methylated tetroses reported in this study was the periodate oxidation of suitably substituted hexoses and hexitols. The reasons for this choice were:-

(a) the quantitative yields resulting from periodate oxidation (48)

(b) the substrates required are known carbohydrates or readily obtained from known carbohydrates

(c) periodate oxidation has been extensively studied in relation to its use in carbohydrate chemistry and the conditions and side reactions are well characterized.

METHODS OF SYNTHESIS

2,3-Di-O-methyl-L-threose.

The synthesis of 2,3-di-O-methyl-L-threose was achieved by periodate oxidation of 2,3-di-O-methyl-D-glucitol. The glucitol was prepared by the sodium borohydride reduction of 2,3-di-O-methyl-D-glucose.

2,3-Di-O-methyl-D-glucose was synthesized by the method of Irvine and Scott (49). The excess benzaldehyde present after the formation of methyl 4,6-O-benzylidene-α-D-glucoside was removed conveniently by steam distillation. Addition of an excess of potassium carbonate to the reaction mixture to neutralize the zinc chloride and any benzoic acid formed by oxidation of benzaldehyde prevents hydrolysis of the acetal during steam distillation. The hydrolysis of methyl 2,3-di-O-methyl-α-D-glucopyranoside to 2,3-di-O-methyl-D-glucose was extremely difficult and required prolonged heating with 2N. sulfuric acid. The reaction required thirty-six hours to reach completion. Thin layer chromatography (T.L.C.) of the hydrolysate, Figure 2, revealed a component running chromatographically faster than the starting material and components running slower than the hydrolysis product.

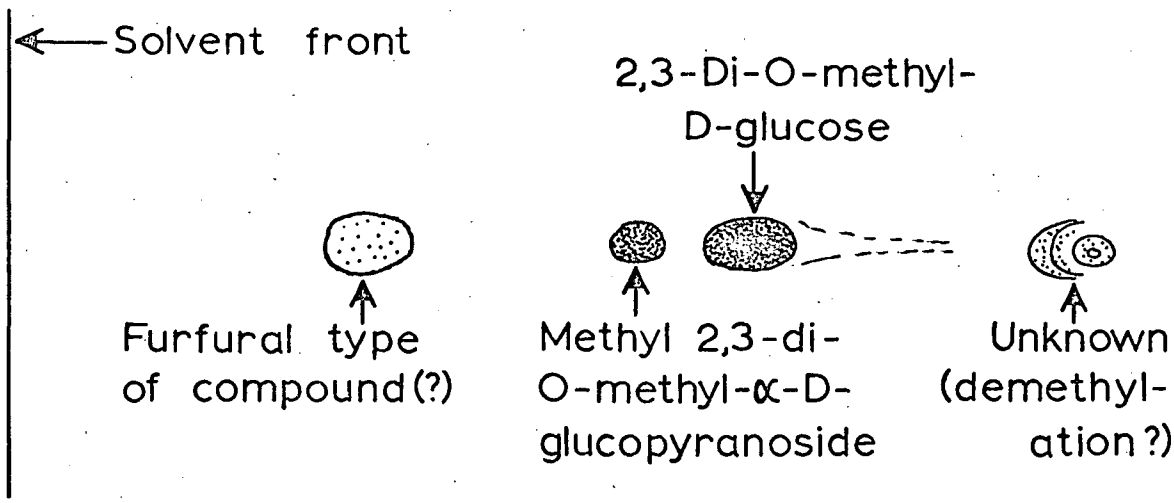


Figure 2. T.L.C. on silica gel, developed with butanone-water azeotrope, of methyl 2,3-di-O-methyl-α-D-glucopyranoside hydrolysate.

Due to the complexity of the hydrolysis problem it was only investigated in part. It is important to note, however, that further study of this problem should be undertaken. Hydrolysis of methylated polysaccharides and sugars has been extensively studied. One of the assumptions that must be made in polysaccharide investigations is that little or no demethylation occurs when methylated polysaccharides are hydrolysed. Demethylation and degradation have been observed, however, and various hydrolysis schemes have been presented in order to minimize these occurrences. Hydrochloric acid, both aqueous (50) and methanolic, causes considerably more demethylation than sulfuric acid. Freudenberg and Boppel (51) found that when 2,3,6-tri-O-methyl-D-glucose was treated with concentrated hydrochloric acid at +5° considerable demethylation to a mixture of di-O-methyl-D-glucoses occurred. Smith et al (52) reported the demethylation of 1,4-di-O-methyl-erythritol to the extent of 1.3 % when refluxed for 18 hours with 3 % methanolic hydrogen chloride. Treatment with aqueous formic acid (53) is reported to result in less degradation than a mixture of acetic and hydrochloric acids. Croon and Lindberg (54) report the use of 72 % sulfuric acid is superior to both methanolysis and formolysis, causing only about 5 % degradation and demethylation of less than 0.5 %. Methyl 2,3-di-O-methyl-α-D-glucopyranoside would appear to provide a good model compound for a systematic investigation of degradation and demethylation since the hydrolysis product is found in the hydrolysis of methylated glucomannans and amylopectin. The starting material and demethylation products are readily synthesized facilitating the detailed study of the demethylation and degradation products.

The reduction of 2,3-di-O-methyl-D-glucose required prolonged treatment with sodium borohydride (24 h.). This finding is in agreement with the resistance reported in the reduction of 3-substituted aldoses (55) with potassium borohydride. The effect is attributed to steric hinderance of the borohydride ion as it approaches the 1,3 system.

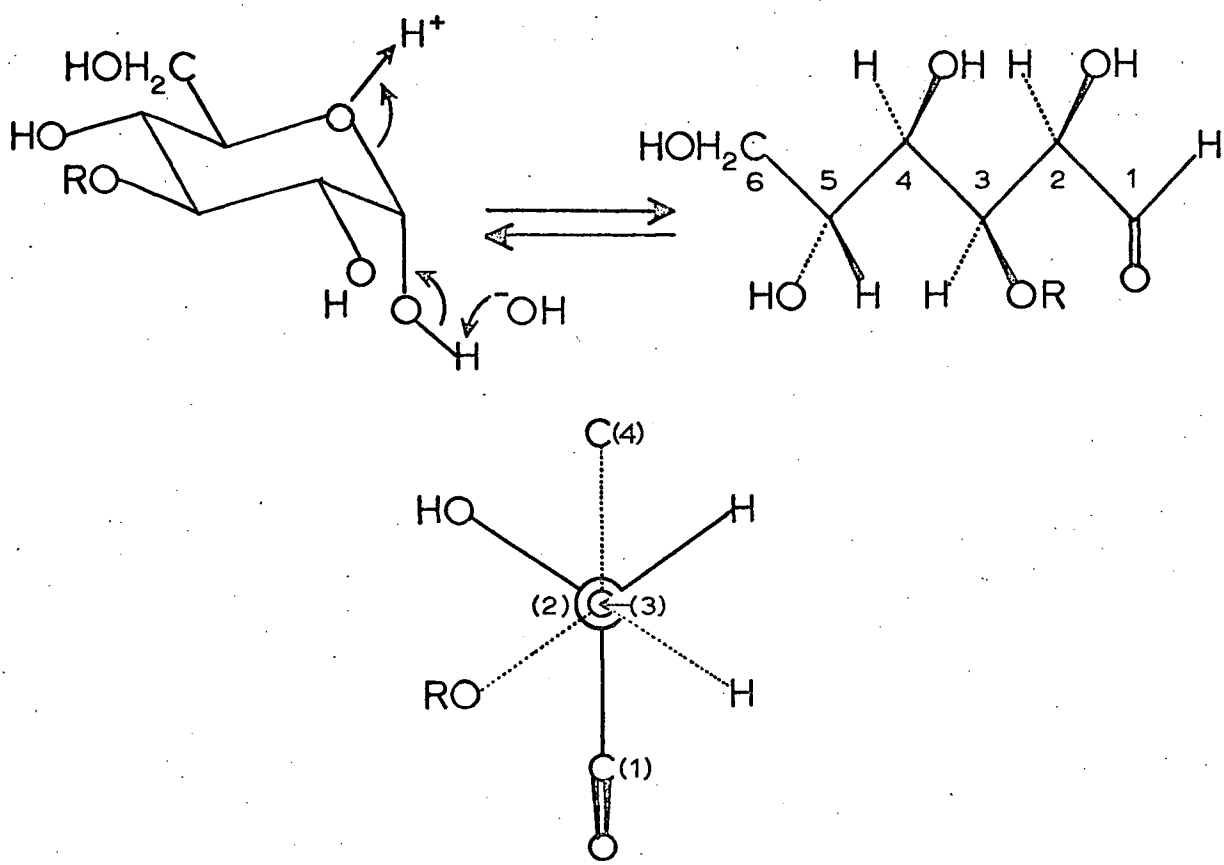


Figure 3. Steric hinderance of substituent at C-3 to borohydride reduction.

Two periodate oxidations were carried out with 2,3-di-O-methyl-D-glucitol, an oxidation with one mole of periodate and an oxidation with excess periodate. The products from the one mole oxidation are 3,4-di-O-methyl-L-xylose and 2,3-di-O-methyl-L-threose. The oxidation with excess periodate yields 2,3-di-O-methyl-L-threose. The products from these oxidations are discussed more fully in section D-3.

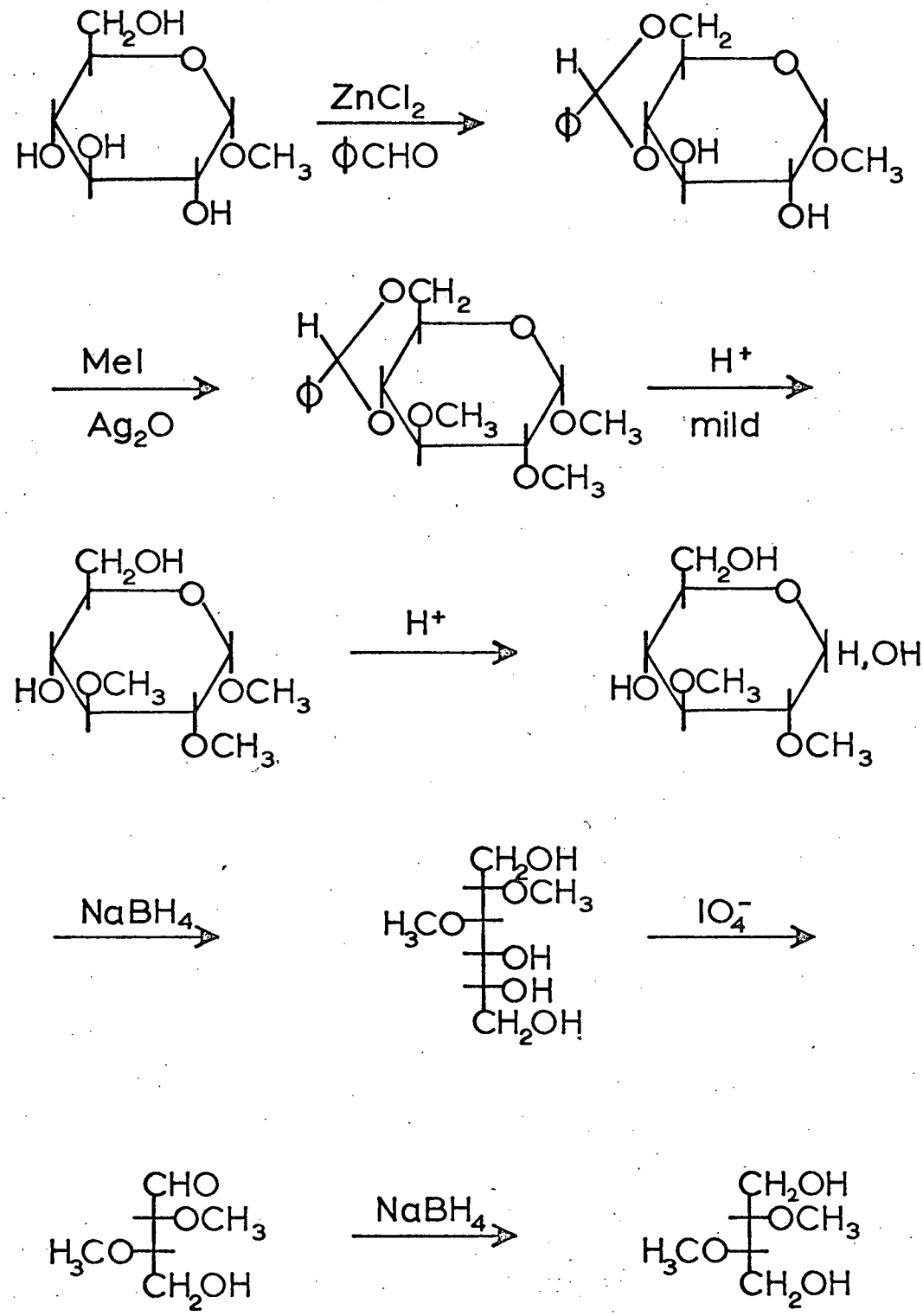


Figure 4. Synthesis of 2,3-di-O-methyl-L-threose.

2,3-Di-O-methyl-D-erythrose

The synthesis of 2,3-di-O-methyl-D-erythrose was achieved by periodate oxidation of 2,3-di-O-methyl-D-mannitol. The mannitol was prepared by borohydride reduction of 2,3-di-O-methyl-D-mannose, see Figure 5.

Methyl α-D-mannopyranoside was prepared by the method of Smith and Van Cleve (56) which is a modification of the Fischer method (57). The benzylidene condensation using benzaldehyde and fused zinc chloride has been used extensively for the preparation of methyl 4,6-O-benzylidene-α-D-glucoside (58,59). The use of these reagents to prepare the corresponding methyl α-D-mannoside derivative is complicated by the cis configuration of the hydroxyls at C-2 and C-3 and the major product formed is methyl 2,3;4,6-di-O-benzylidene-α-D-mannoside (60). This difficulty was overcome by using a procedure provided by Schwarz (61). The methyl α-D-mannopyranoside was ground to a fine powder and dissolved as rapidly as possible in 98-100 % formic acid. Benzaldehyde was added and after five minutes the reaction mixture was poured into a solution of potassium carbonate. Steam distillation was again used to remove the excess benzaldehyde and the methyl 4,6-O-benzylidene-α-D-mannoside was extracted from the resulting aqueous phase with chloroform. The methylation product, methyl 2,3-di-O-methyl-4,6-O-benzylidene-α-D-mannoside on hydrolysis yields 2,3-di-O-methyl-D-mannose. Reduction of the 2,3-di-O-methyl-D-mannose with sodium borohydride yielded crystalline 2,3,di-O-methyl-D-mannitol.

Two periodate oxidations were carried out with the 2,3-di-O-methyl-D-mannitol in a similar manner to the 2,3-di-O-methyl-D-glucitol. Oxidation with one mole of peridate yields 3,4-di-O-methyl-D-arabinose and 2,3-di-O-methyl-D-erythrose. Oxidation with excess periodate yields 2,3-di-O-methyl-D-erythrose. The oxidations and their products will be discussed in section D-3.

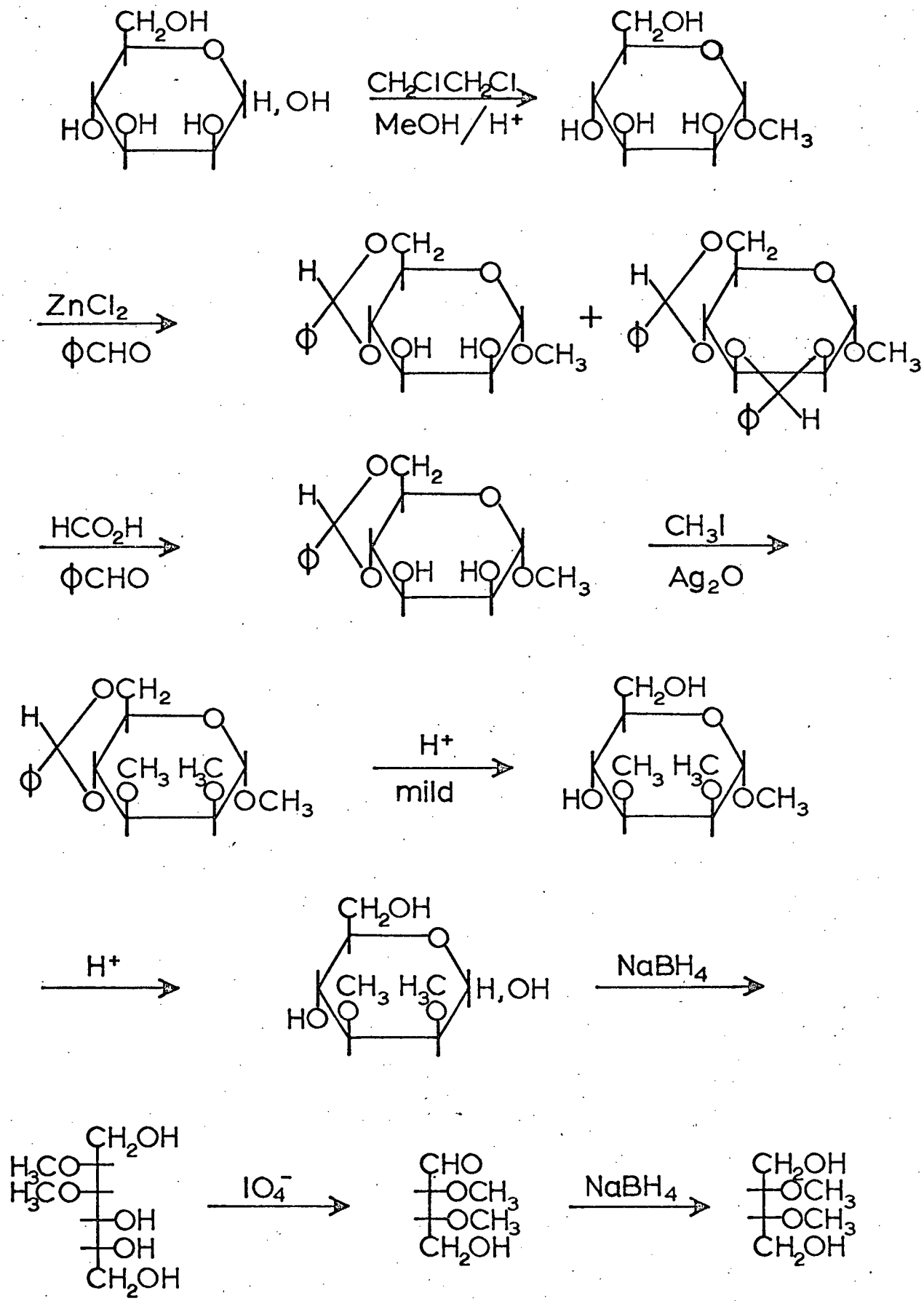


Figure 5. Synthesis of 2,3-di-O-methyl-D-erythrose.

4-O-Methyl-D-threose.

The synthesis of 4-O-methyl-D-threose was accomplished by the method of Richards (47) employing minor modifications.

6-O-Methyl-D-glactose was prepared by methylating 1,2:3,4-di-O-isopropylidene-D-galactose. Mild acid hydrolysis yielded 6-O-methyl-D-galactose (62).

Methyl 6-O-methyl-D-galactofuranosides were prepared by reaction of 6-O-methyl-D-galactose with 0.0125 % methanolic hydrogen chloride (63). The methyl glycosides were separated on a cellulose-hydrocellulose column to yield pure methyl 6-O-methyl-α-D-galactofuranoside, methyl 6-O-methyl-β-D-galactofuranoside and methyl 6-O-methyl-α-D-galactopyranoside. The glycosides were characterized by their proton magnetic resonance spectra (64) and optical rotations. The p-nitrobenzoate derivatives were prepared from the furanosides. The methyl 6-O-methyl-α-D-galactopyranoside crystallized and a mixed melting point with an authentic sample was undepressed.

The periodate oxidations to prepare 4-O-methyl-D-threose were performed on mixed galactofuranosides rather than on pure anomers. As a result, large samples of methyl glycosides could be applied to the column for chromatography. The periodate (0.0076M) consumption for the mixed furanosides was 0.91 moles after 53 hours. Although this is a much longer time than indicated by Richards (47) it is in agreement with the findings of Hudson (65). Richards' shorter reaction time may reflect a certain amount of methyl 6-O-methyl-D-galactopyranoside.

In an attempt to reduce the extensive decomposition reported (47) on hydrolysis of the dialdehyde formed from the periodate oxidation of methyl 6-O-methyl-α,β-D-galactofuranosides, a methanolysis was employed. Decomposition was still encountered, however, and the product was isolated chromatographically as the dimethyl acetal of 4-O-methyl-D-threose. Acid

hydrolysis yielded 4-O-methyl-D-threose. However attempts to prepare the 2,4-dinitrophenylhydrazone were unsuccessful and the compound was characterized as the p-nitrobenzoate of the derived polyol.

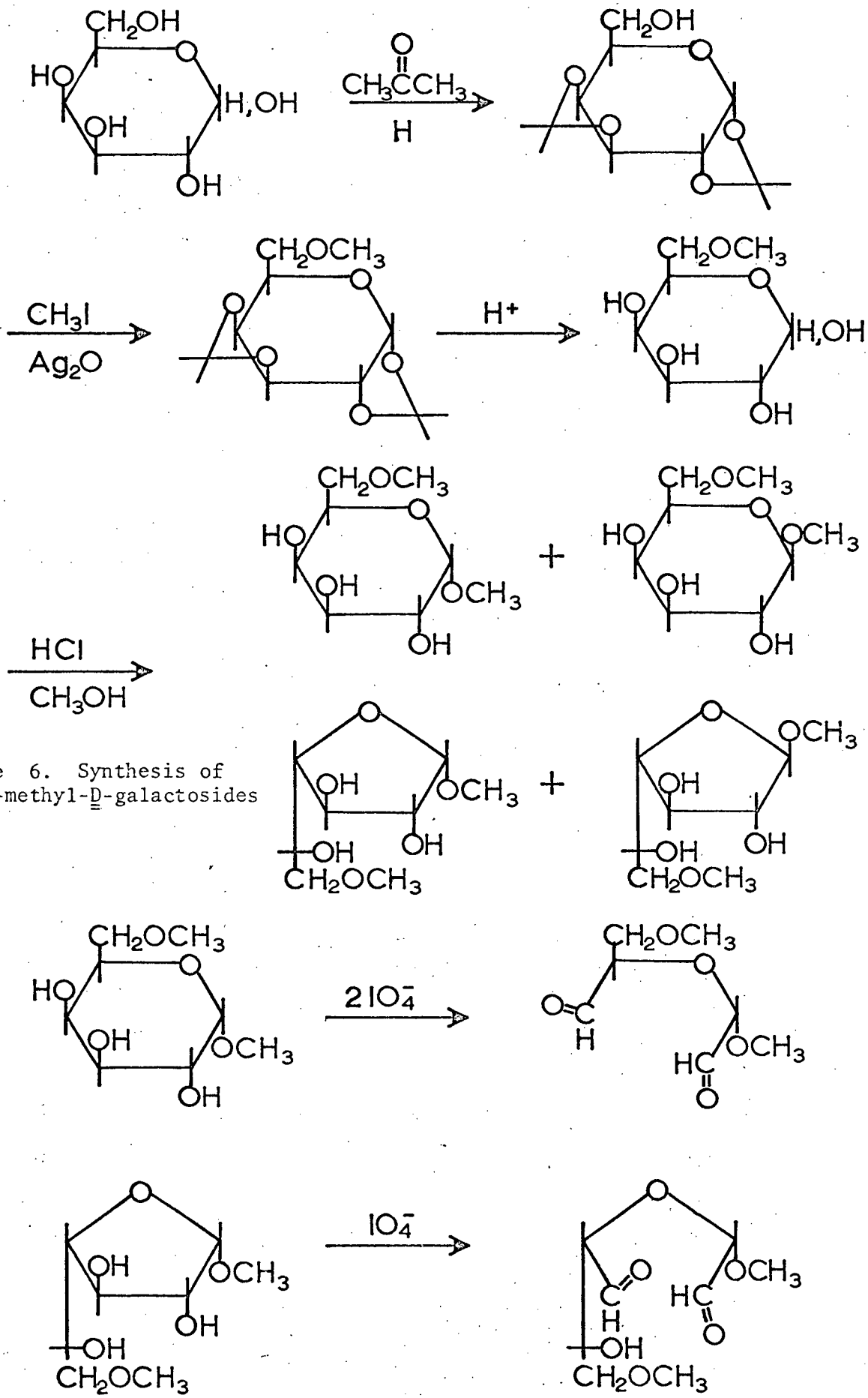


Figure 6. Synthesis of 6-O-methyl-D-galactosides

Figure 7. Modes of periodate oxidation of 6-O-methyl-D-galactosides.

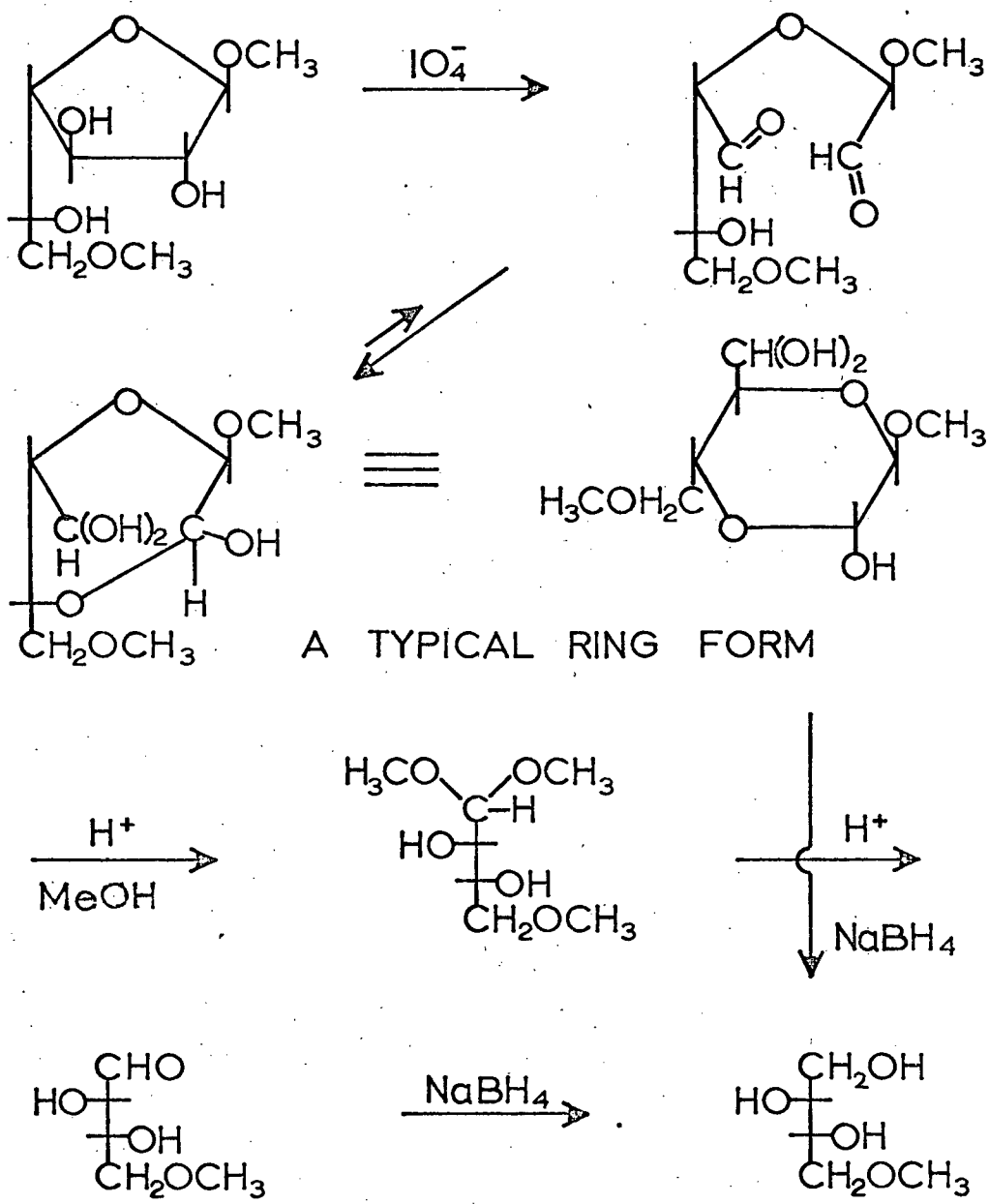


Figure 8. Synthesis of 4-O-methyl-D-threose.

2-O-Methyl-D-erythrose.

The synthesis of 2-O-methyl-D-erythrose was accomplished by periodate oxidation of methyl 4-O-methyl-α-D-mannoside. Methyl 4-O-methyl-α-D-mannoside was synthesized by the procedure of Smith (66).

Attempts in this laboratory and another (67) to synthesize methyl 2,3-O-isopropylidene-α-D-mannopyranoside by the method of Ault, Haworth and Hirst (68) were unsuccessful. A modification of the benzylidene condensation provided by Schwarz (61) was devised in a successful attempt to synthesize this compound. Methyl α-D-mannoside was dissolved in 98-100 % formic acid and acetone was added to the reaction mixture. The formic acid was neutralized after several days of reaction by pouring into a saturated solution of potassium carbonate. A mixture of methyl 2,3-O-isopropylidene-α-D-mannoside and methyl 2,3: 4,6-di-O-isopropylidene-α-D-mannoside was extracted from the aqueous phase with chloroform. Extraction of the mixture with light petroleum (B.p. 30-60°) removed the diisopropylidene derivative from the monoisopropylidene derivative.

During the Kuhn (69) methylation of methyl 6-O-trityl-2,3-O-isopropylidene-α-D-mannoside, T.L.C. monitoring revealed detritylation in the prolonged treatment. Column chromatography was employed, therefore, in order to obtain a pure sample of methyl 4-O-methyl-α-D-mannoside. Periodate oxidation of 4-O-methyl-α-D-mannoside produced a dialdehyde which was extensively degraded in all hydrolysis attempts. The best results were obtained when formation of the dithioacetal was attempted. However, insufficient material was obtained and characterization was not possible. The dialdehyde was reduced and hydrolysed to give 2-O-methyl-D-erythritol.

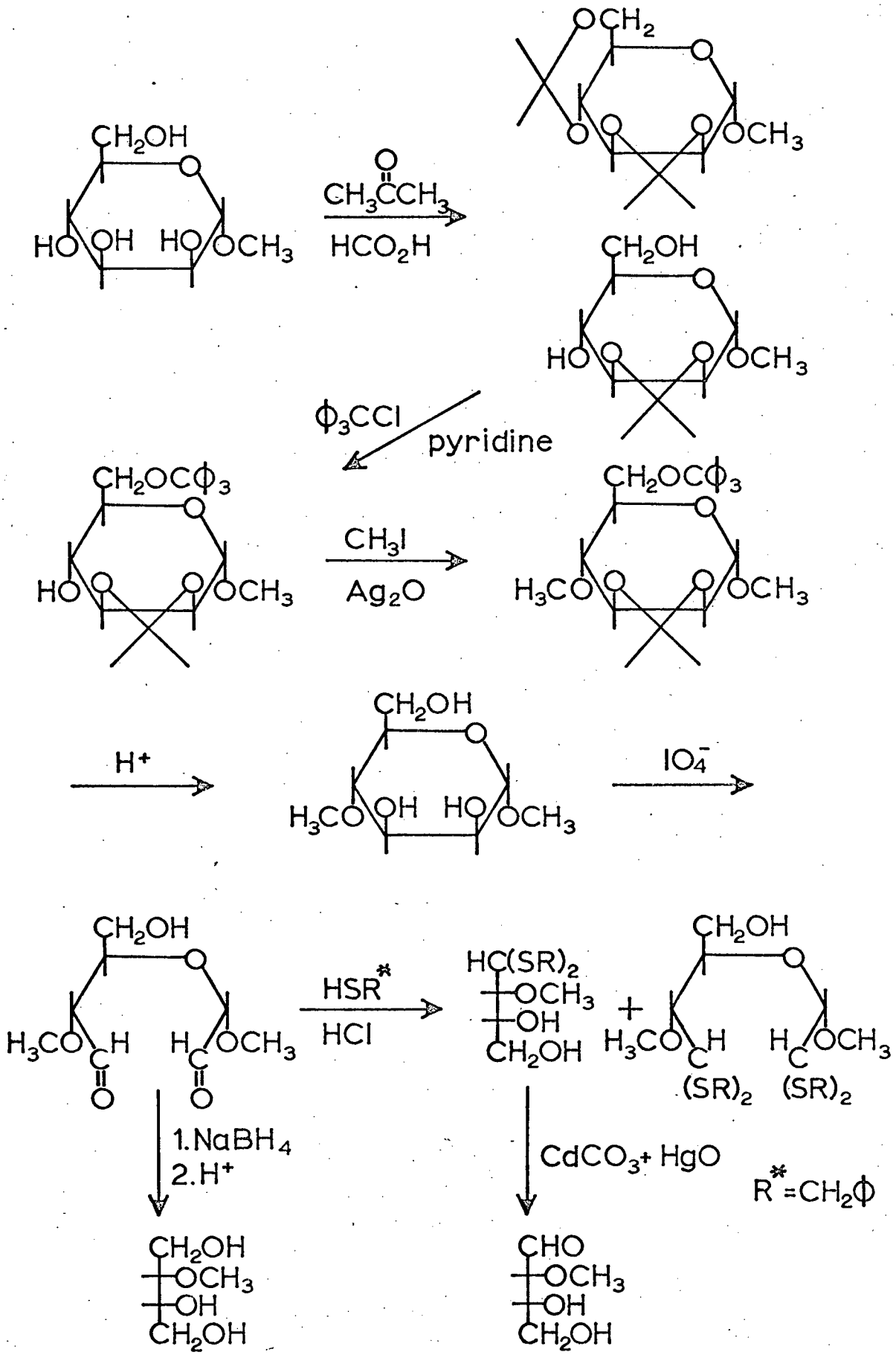


Figure 9. Synthesis of 2-O-methyl-D-erythritol.

D-1. Unknown in the hydrolysis of methyl 2,3-di-O-methyl-α-D-glucopyranoside.

In the preparation of 2,3-di-O-methyl-D-erythrose, the presence of small amounts of methyl 2,3-di-O-methyl-α-D-glucopyranoside in the 2,3-di-O-methyl-D-glucitol did not interfere with the periodate oxidation because of its inability to be oxidized. The larger R_f value of the tetrose allowed its easy removal from the trace of glycoside. However, during the oxidation of 2,3-di-O-methyl-D-glucitol with one mole of periodate to form 3,4-di-O-methyl-L-xylose it was necessary that no trace of methyl 2,3-di-O-methyl α-D-glucopyranoside remained, as the R_f's of the 3,4-di-O-methyl-L-xylose and the methyl 2,3-di-O-methyl-α-D-glucopyranoside are very similar in solvent A. The 2,3-di-O-methyl-D-glucose however, moves with nearly the same R_f as its methyl glycoside and in order to clearly see that the hydrolysis had taken place the sample was reduced after hydrolysis and the slower R_f of the polyol allowed a clear look at any remaining glycoside.

Resubjecting a sample which had been treated in the above manner to further hydrolysis, resulted in a sample which contained a unique, chromatographically fast-moving component which was not the furfural type of compound seen in previous hydrolyses of methyl 2,3-di-O-methyl-α-D-glucopyranoside. Reduction of the 2,3-di-O-methyl-D-glucose with sodium borohydride produced no change in the unknown components R_f indicating that there was no free hemiacetal function in the molecule. Due to the large amount of this substance (20 %), it was decided to determine its structure. Isolation was achieved by subjecting the aqueous sample of 2,3-di-O-methyl-D-glucitol to continuous chloroform extraction. The chloroform solution on evaporation yielded a relatively pure fraction of the unknown compound.

Proton magnetic resonance (60 mc.p.s.) studies on this product as initially isolated, provided little information because of the environmental similarity of the protons. It was not possible however, to see a proton resonance at low field which is characteristic of the anomeric hydrogen (70). This would appear to indicate that no acetal functionality existed in the unknown component. Addition of trifluoroacetic acid shifted the hydroxyl resonances to lower field but the spectrum was not appreciably improved.

Further purification of the unknown compound was achieved by acetylation and subsequent gas liquid chromatography. Acetylation was chosen for several reasons:-

(a) the influence an acetate has on the position of resonance of of the hydrogen joined to the carbon whose hydroxyl has been acetylated (71);

(b) the acetylation mixture of acetic anhydride and pyridine can be injected directly onto the column of the gas chromatograph without work-up of the reaction (72);

(c) the acetate blocking group can be readily removed to yield the starting material (73) and

(d) a large body of information regarding the mass spectra of sugar acetates is available (74).

Proton Magnetic Resonance (p.m.r.)

The proton magnetic resonance spectra of the acetylated unknown at 60 and 100 mc.p.s. were considerably different to those of the unacetylated unknown. Certain of the protons, those which were situated on carbons whose free hydroxyl group had been acetylated, were shifted to lower field. The integral indicated eight hydrogen atoms in addition to two methoxyls and two acetates. This would be consistent with a loss of one mole of water from 2,3-di-O-methyl-D-glucitol to form an anhydro ring. There are several

possibilities as to the structure of such a dehydration product, an epoxide, a five membered ring or a six membered ring. Of these three possibilities only the five membered ring is consistent with the p.m.r. spectra and the lack of chemical reaction (75).

The 100 mc.p.s. proton magnetic resonance spectrum of the acetylated unknown in chloroform, disclosed a septet at τ 4.76 consistent with a proton on C-5 geminal to an acetate and coupled with three hydrogens (H-6_1, H-6_2 and H-4). The two C-6 hydrogens (H-6_1 and H-6_2) were also shifted downfield to τ 5.42 and 5.87 indicating that the C-6 hydroxyl was also acetylated. The H-4 resonance at τ 5.91 could be seen clearly, coupled to H-5. Irradiation of H-5 and H-6 separately confirmed the coupling between these four hydrogens. Since neither C-6 nor C-5 were involved in the formation of a ring, there must be a furan type ring between C-1 and C-4. Analysis of the ring structure can be carried no further since the environmental similarity of the remaining hydrogens introduces second order coupling and isotopic substitution would be necessary to further assign the remaining four hydrogens. The assignment of H-4, however, allows one to evaluate the configuration at C-4. The coupling constant "J" between H-4 and H-3 has a value of 3.7 c.p.s. This is consistent with an envelope conformation for a five membered ring in which C-4 is below the plane of the ring and the molecule has the gluco configuration.

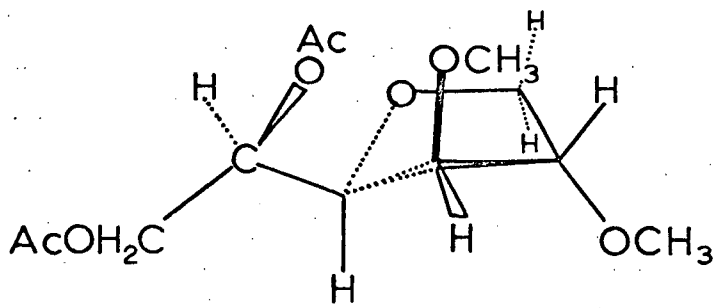


Figure 10. Envelope conformation of gluco configuration.

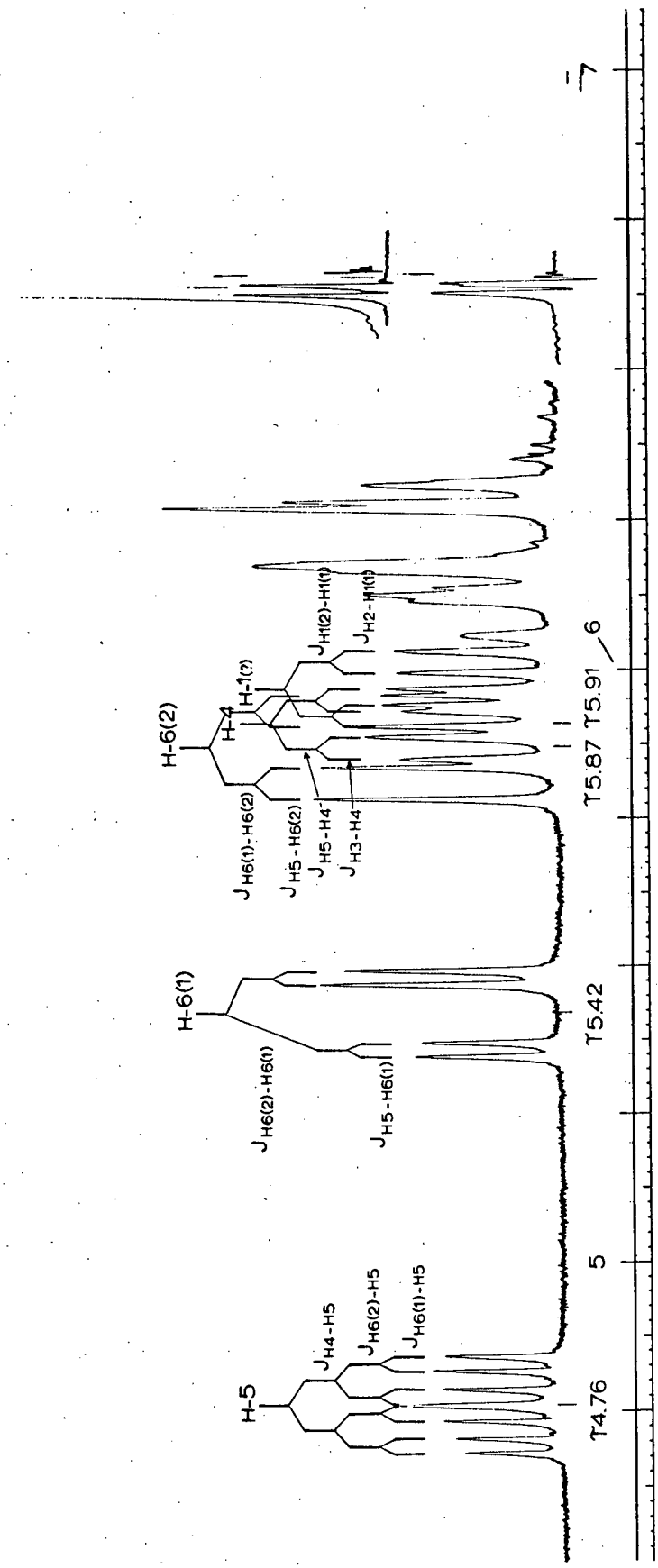


Figure 11. P.M.R. spectrum of 5,6-di-O-acetyl-1,4-anhydro-2,3-di-O-methyl-D-glucitol.

(This splitting may be seen to occur in the 5,6 epoxide of isopropylidene-α-D-glucofuranose (76)). This is, in addition, chemically sound since formation of the 1,4-anhydride of sorbitol and mannitol proceeds with retention of configuration (77). One may also be certain that because of the relative stability of the methoxyl blocking group, no migration of these functions will have taken place. Hence it is possible to assign the structure of the unknown compound as 1,4-anhydro-2,3-di-O-methyl-D-glucitol.

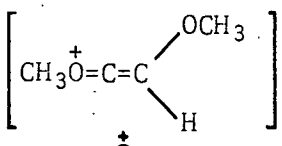
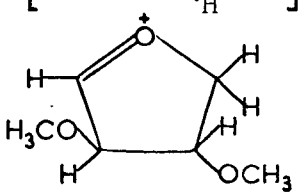
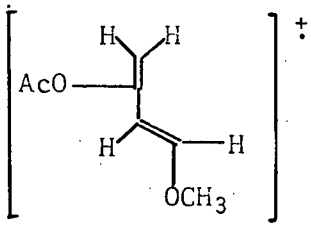
Mass Spectra

The mass spectrum of the acetylated unknown compound was determined and the fragmentation patterns are consistent with an assigned structure of 5,6-di-O-acetyl-1,4-anhydro-2,3-di-O-methyl-D-glucitol. Although it is not possible to provide absolute identification of peaks since no labelling experiments were carried out, the volume of published spectra (74,78) allows assignments and interpretation, all of which are consistent with the proposed structure. In addition, it is not possible to assign the mass spectrum to any other conceivable structure.

The mass spectrum of 1,4;3,6-dianhydro-2,5-di-O-methyl-D-glucitol was determined and the assignment of peaks as a result of previous work, allows one to indicate the correlation of known spectra with those of compounds not previously subjected to mass spectral analysis. The major peaks at m/e 58 and 69 can be seen to arise in other similar types of compounds in which the structure of these peaks has been assigned. The peak m/e 58 assigned as characteristic of methyl 3,6-anhydro-2,5-di-O-methyl-furanoside (78) sugars is seen to occur in the methylated dianhydrohexitol and presumably arises from the same functionality in the molecule.

TABLE 1

Molecular Ion Peaks for 5,6-di-O-acetyl-1,4-anhydro-2,5-di-O-methyl-D-glucitol

m/e	Species	Source	Ref.
43	$CH_3\overset{+}{C}O$	acetylium ion. -base peak in all published spectra for acetates.	(79)
45	$CH_2=\overset{+}{O}-CH_3$	C-3, C-2 contribute to this peak.	(80)
58	$CH_3O\,\overset{H}{\underset{+}{C}}-\dot{C}H_2$	C-2, C-1	(78)
71	$CH_3\overset{+}{O}=CH-CH=\dot{C}H_2$	C-3-C-2-C-1	(78)
87		C-3 - C-2	(81)
131		$C_1 \rightarrow C_4$ loss of C-5 - C-6	(82)
142		C-6 → C-3 also M-CH_3COOH, CH_2=CO, MeOH	
153	M-CH_3COOH, CH_3OH, CH_3O		
184	M-CH_3COOH, CH_3OH		
203	M-$CH_2O\overset{O}{\overset{\|}{C}}CH_3$		
216	M-CH_3COOH		
217	M-$CH_3COO^{\bullet}$		
233	M-$\overset{+}{C}H_3CO$		
244	M-CH_3OH		

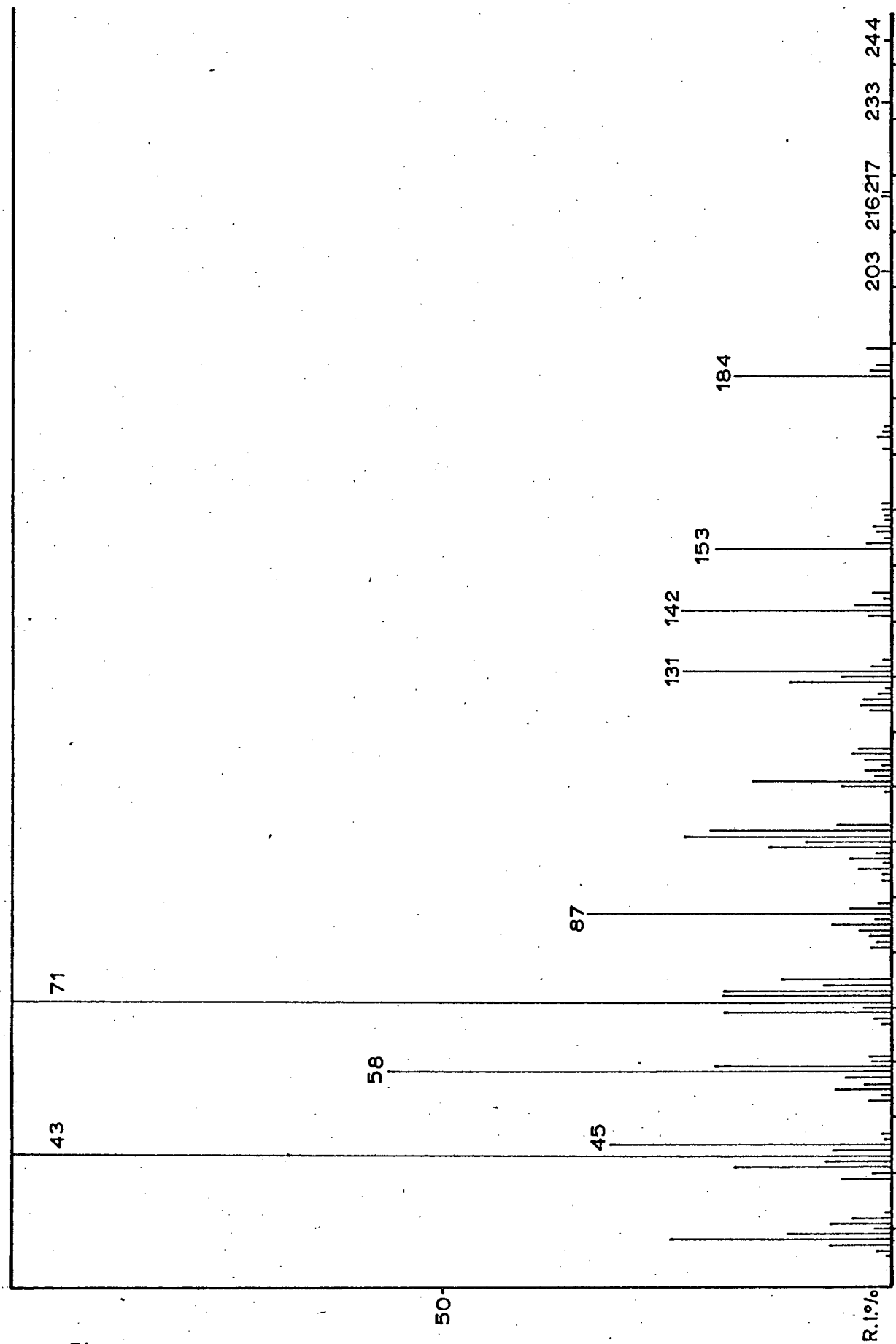


Figure 12. Mass spectrum of 5,6-di-O-acetyl-1,4-anhydro-2,3-di-O-methyl-D-glucitol.

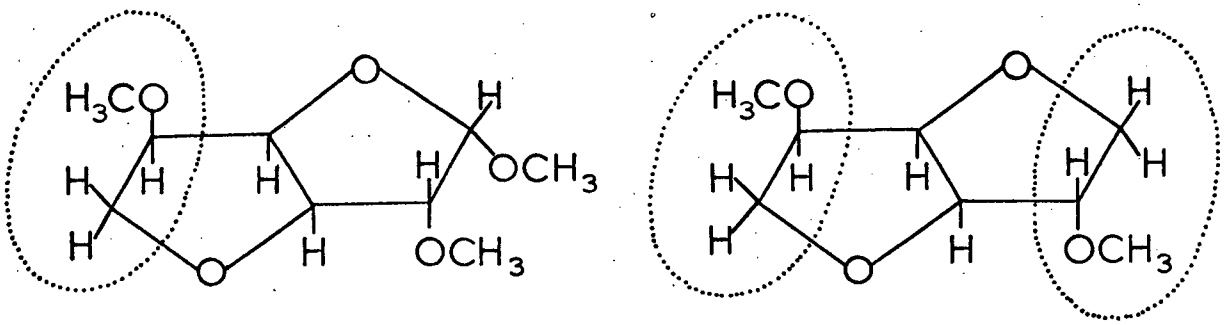


Figure 13. Source of m/e 58 peak.

The peak m/e 69 assigned as characteristic of 1,4:3,6-dianhydro-2-O-methyl-β-D-glucose (78) can be seen as well in the 3,6-anhydrofuranoside and the 1,4:3,6-dianhydro-2,5-di-O-methyl-D-glucitol and this arises from the five membered anhydro rings. As mentioned, the absolute assignment of these peaks requires isotopic labelling-this has not been carried out.

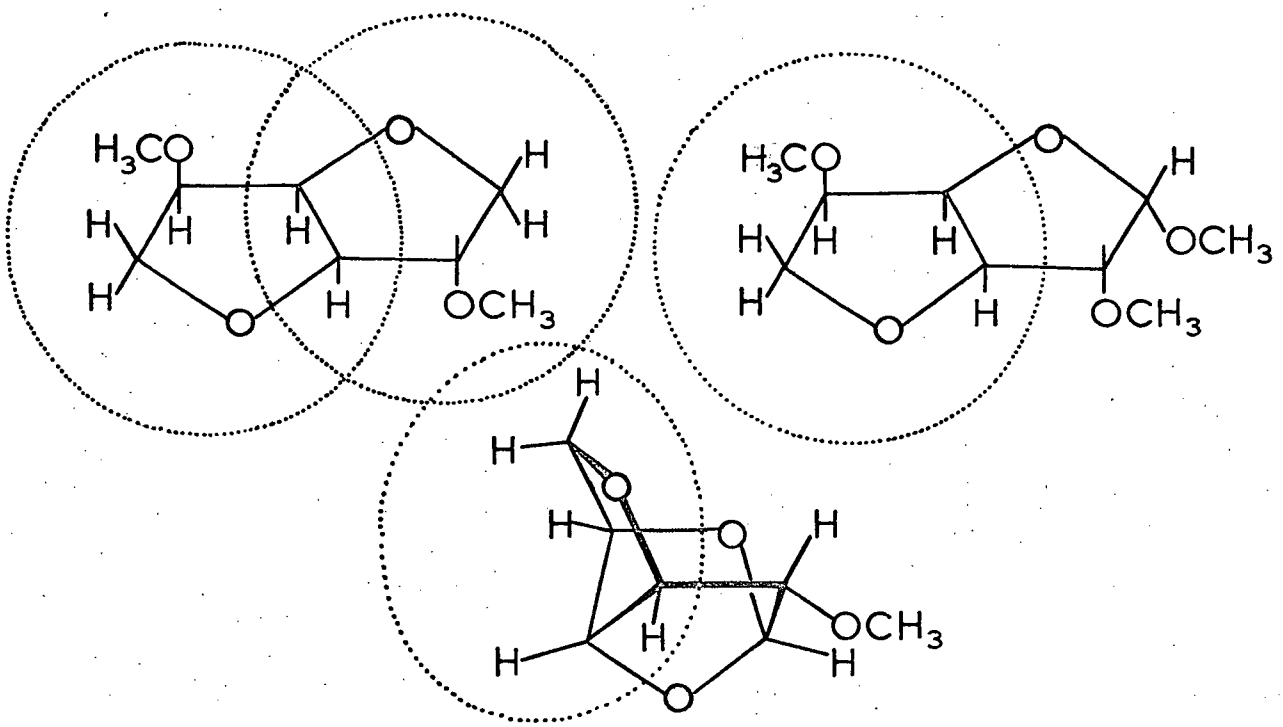
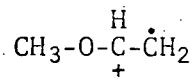
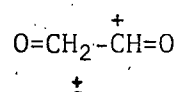
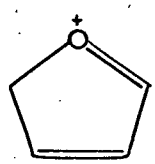
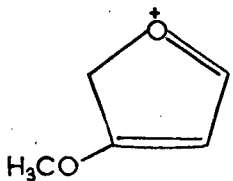
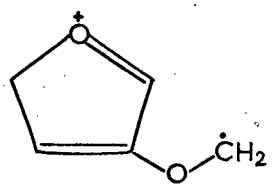
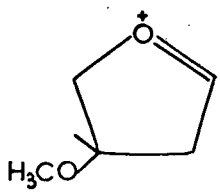


Figure 14. Source of m/e 69 peak.

TABLE 2

Molecular Ion Peaks for 1,4:3,6-dianhydro-2,5-di-O-methyl-D-glucitol

m/e	Species	Source
58		C 1 and C 2 C 5 and C 6
59		C 3 and C 4
69		C 1-C 4 and C 3 -C 6
99	 	
100	M-OCH_2-CHOMe	
101		
142	M-CH_3OH	
143	M-CH_3O•	
174	M^+	

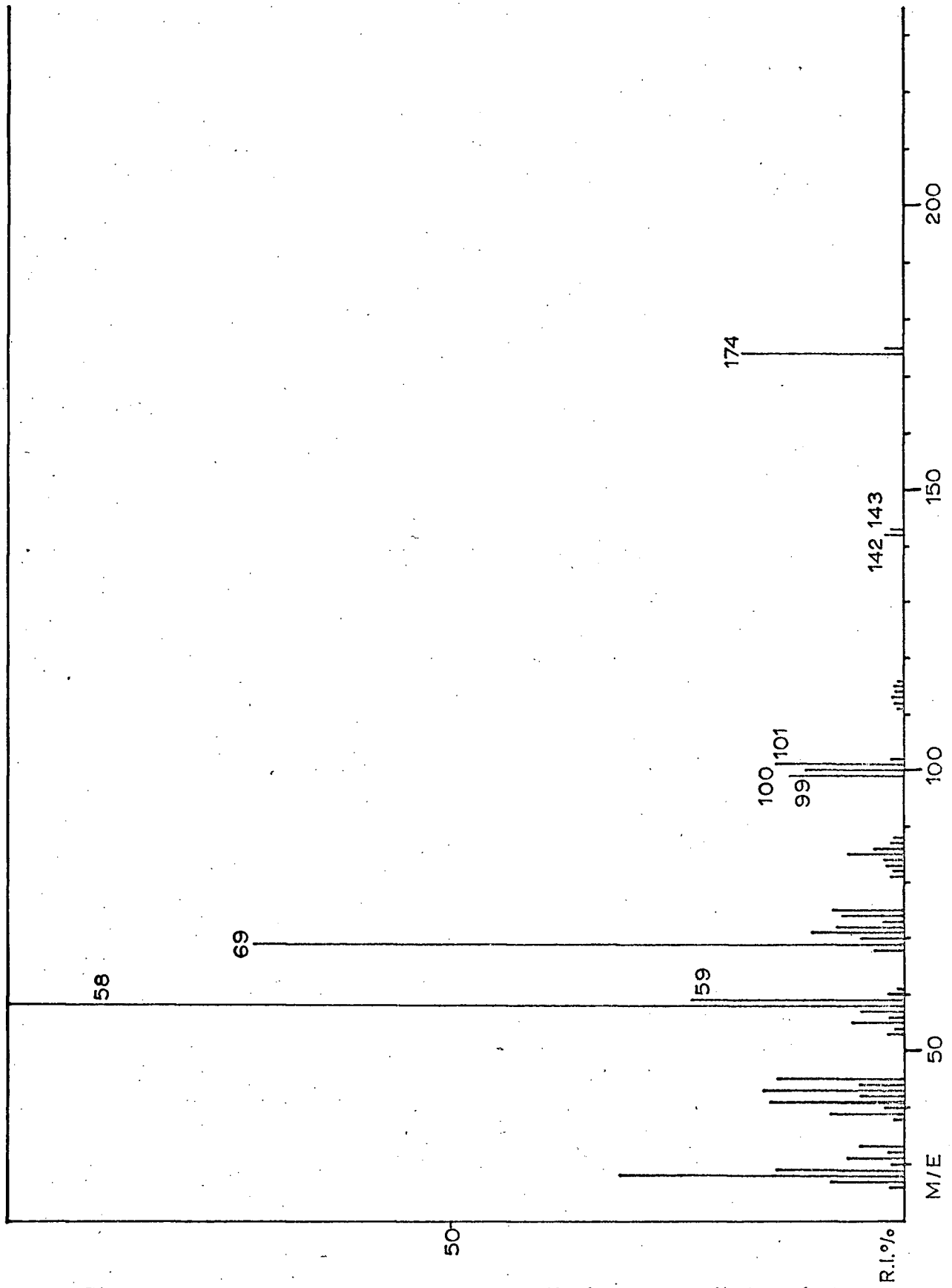


Figure 15. Mass spectrum of 1,4:3,6-dianhydro-2,5-di-O-methyl-D-glucitol.

D-2. A solution to hydrolysis problems.

The extreme difficulty encountered in the hydrolysis of the periodate oxidized methyl 6-O-methyl-D-galactofuranosides and methyl 4-O-methyl-α-D-mannopyranoside indicates that this procedure at present cannot be used as a valuable tool in the identification of unknown partially methylated sugar moieties. The degradation during acid hydrolysis is extreme and it is questionable if one could evaluate the structure of an unknown component or mixture of components using this method. It is, however, well known that facile hydrolysis can be accomplished after reduction of the aldehydic functions formed by periodate oxidation. A problem still exists however, since one must evaluate the amount of a compound that may be present in an unknown mixture as well as its structure.

The techniques needed for the evaluation of structure and quantity are presently available and need only be applied to a series of compounds to show their general applicability. As noted, reduction of three different isomers leads to formation of the same tetritol. However, if reduction were carried out employing sodium borodeuteride, the reduced end of the molecule would be substituted with one deuterium. Mass spectral analysis of this compound, as its acetate or silyl ether, would clearly indicate the nature of the substitution of the deuterium in relation to the methyl ether function. Using the previous example one could find the existence of characteristic fragments.

The remaining problem is the evaluation of the quantity of an unknown or known compound. The proposed procedure is determination of the molar response factor (M.R.F.) for a compound when it is passed through the gas chromatograph. This allows one to give a quantitative estimate of the amount of a compound in a mixture.

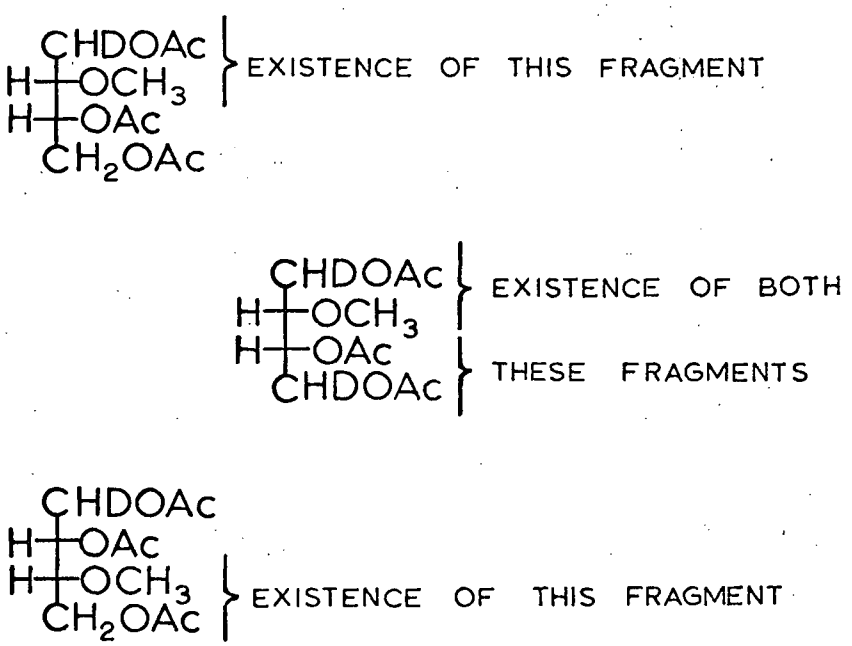


Figure 16. Characteristic fragments from labelled isomeric tetritols.

The standard procedure for determining the M.R.F. for a compound is to weigh a sample and add an internal standard to which all samples will be related. A problem arises however, when new or unknown compounds are to be used. If the amount of material available is very small, is a syrup or a mixture, then large errors can occur as a result of inaccurate weights. For example, with a syrup it is very difficult to remove all the solvent in which the compound was dissolved.

To get around the previous problem, it was decided to measure M.R.F.'s by weighing the sample eluted. The component was collected in a capillary tube after injection and resolution on the gas chromatograph. Hence the sample weight would not only give a molar response factor with regard to some internal standard, but would also give an absolute response factor.

There are two possible sources of error in this procedure. It is possible that degradation occurs on the column so that a specific amount is lost on each injection. This does not appear to be a significant factor with the amounts that could be measured. A twenty fold change in sample size did not show a significant fluctuation attributable to thermal degradation. The second possible source of error was the possibility of incomplete collection of the sample. Only in the case of the disilyl ether of ethylene glycol did this factor appear to influence the amount of the sample collected. As previously mentioned, sample size variation did not affect the response factor found and this is also an indication of total collection.

The response factor is calculated using the molecular weight of the sample collected and, in the case of an unknown, this would be obtained from the mass spectrum. A large percentage of the weight of sample is due to the

TABLE 3

Molar Response Factors for some Sample Compounds

Compound as Silyl ether	Av.R.F. (at ATT 4) peak area/wt in µg	Mol.Wt. gm/mole	M.R.F.(abs.) area/mole $x10^6$
Ethylene glycol	1.109 ± .115	204	5.33×10^{-3}*
Butane 1,4-diol	.395 ± .003	234	1.69×10^{-3}
Erythritol	.367 ± .015	410	0.895×10^{-3}
2-O-Methyl erythritol	.331 ± .003	352	0.940×10^{-3}
4-O-Methyl threitol	.303 ± 011	352	0.861×10^{-3}

*may be some loss of silyl ether during collection.

silyl ethers, hence only small amounts of unknown compounds are required in order to determine the molar response factor. For example, 2-O-methyl-erythritol has a molecular weight of 136, while its silyl ether derivative has a

molecular weight of 352. Assuming that three approximately 1 mg samples are required for the accurate determination of the M.R.F., then only 1.2 mg. of 2-O-methyl erythritol would be required. In addition, the mass spectrum could easily be obtained from one of the samples collected for the M.R.F. The presence of small or large amounts of impurity would not interfere with the determination of the M.R.F. since their weight would not be considered.

The obvious extension of this technique to fields other than carbohydrates should prove invaluable to workers dealing with small quantities of compounds, where accuracy is hampered by difficulties in handling techniques, lack of pure standards or complicated reaction mixtures.

D-3. Review and Discussion of Periodate Oxidation

Fleury and Lange (83) pointed out that the reduction of periodic acid studied by Malaprande (84) could be considered selective for hydroxyl groups attached to adjacent carbon atoms. The carbon chain is broken and two aldehyde groups are produced.

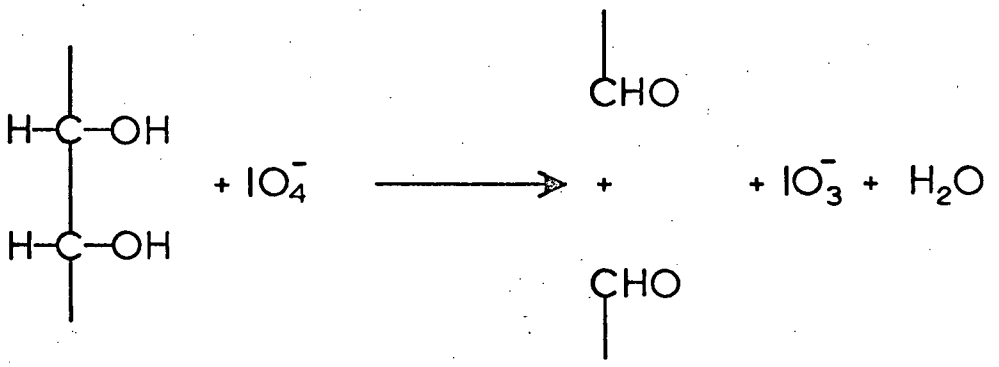


Figure 17. Periodate oxidation of adjacent hydroxyl groups.

The scope of the reaction was extended to the oxidation of 1,2-di-ketones and α-hydroxy ketones by the work of Clutterbuck and Reuter (85). Nicolet and Shinn (86) extended the reaction to the oxidation of a series of α-amino acids and found that those containing an α-hydroxy-amine structure were oxidized rapidly.

Compounds containing three or more hydroxyl groups on adjacent carbon atoms are oxidized by n-1 moles of periodate, where n is the number of adjacent hydroxyls, and are cleaved to yield two aldehyde groups and n-2 moles of formic acid. The formic acid may be estimated by titration and can provide information about the arrangement of hydroxyl groups.

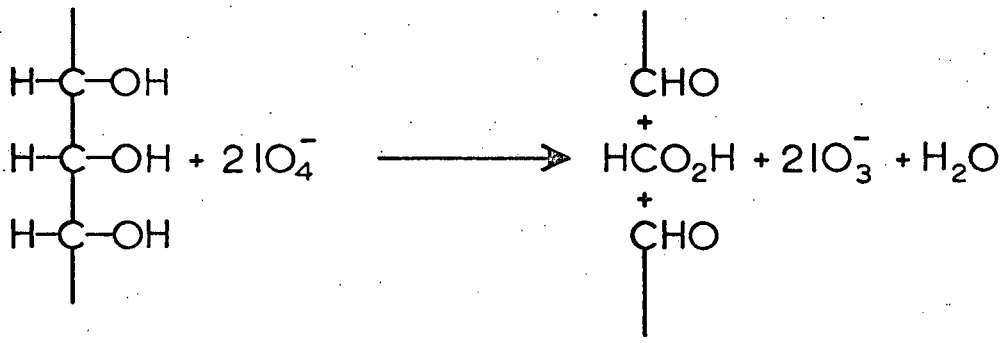


Figure 18. Formation of formic acid.

If a primary alcohol group is involved in the oxidation, then formaldehyde is produced. The amount may be estimated and used to determine the number of periodate oxidizable primary hydroxyls.

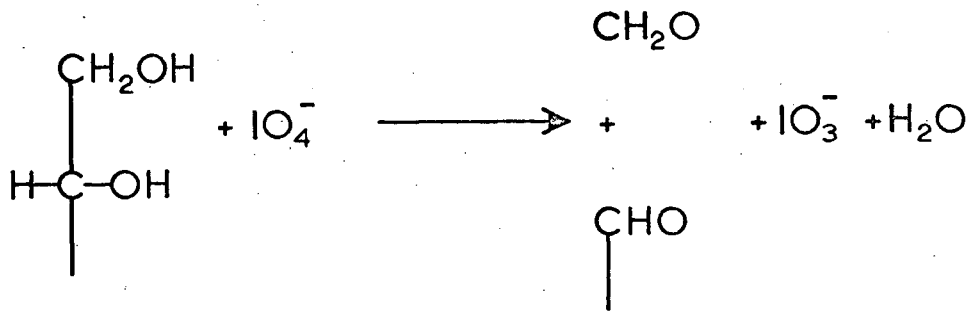


Figure 19. Periodate oxidation of a primary hydroxyl

Although the exact course of periodate oxidation is unknown, the most widely accepted interpretation is that suggested by Criegee (87,88). It is assumed that a cyclic ester is formed and the formation of a cyclic intermediate has been confirmed by kinetic (89) and polarimetric measurements (90, 91).

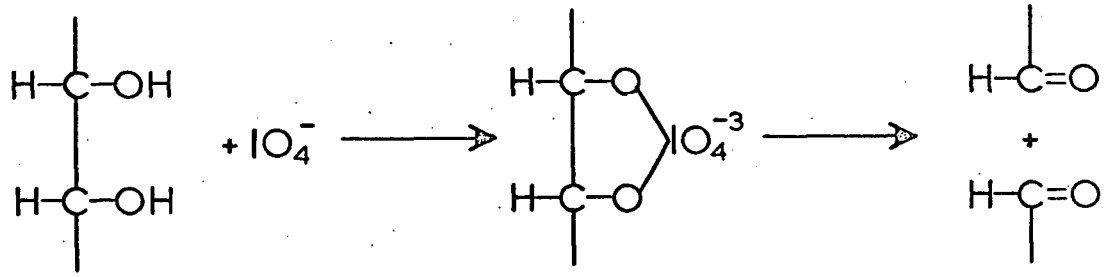


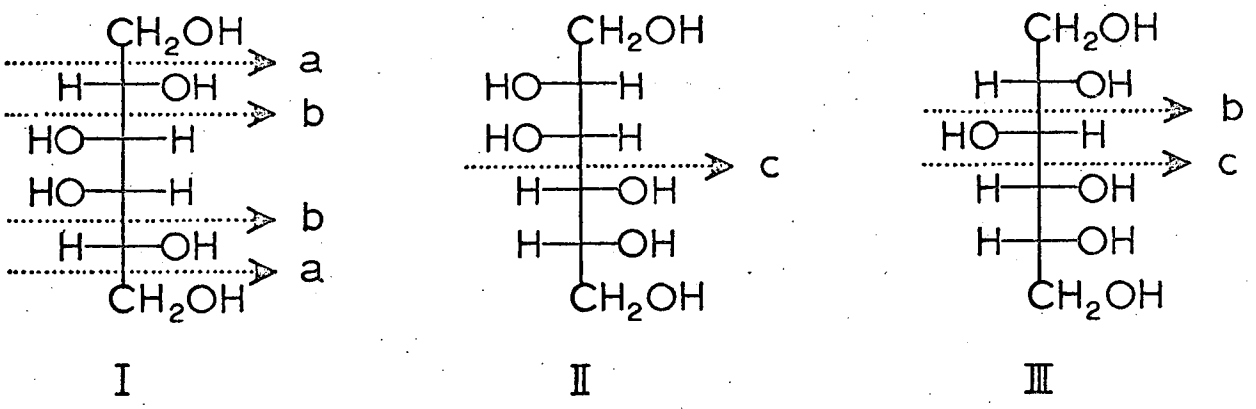
Figure 20. Course of periodate oxidation.

In agreement with the concept of the above oxidation mechanism, is the fact that the vicinal cis-hydroxyl groups in cyclohexanediol are oxidized more rapidly than the trans hydroxyl groups (92). Similarly, methyl aldohexopyranosides with cis-hydroxyl groups such as methyl α-D-galactopyranoside and methyl α-D-mannopyranoside are oxidized faster than glycosides with trans-

hydroxyl groups, as occurs in methyl α-D-glucopyranoside (93). In cases where the trans α-glycol groups are held rigidly apart by a fixed stereochemistry, as in 1,6-anhydro-β-D-glucofuranose (80) or methyl 4,6-O-benzylidene-α-D-altroside (89), no oxidation takes place because of the difficulty in forming the cyclic ester. In this work, the periodate oxidation of methyl 6-O-methyl-D-galactofuranosides proceeded at a much slower rate than normal for a periodate oxidation probably because of the steric requirements of the trans vicinal hydroxyls oxidized.

Steric and perhaps electronic factors influence the rate of attack of periodate on vicinal hydroxyls. Smith and co-workers (95) have shown that the position of cleavage of phenyl β-D-glucoside and methyl 6-O-trityl-α-D-glucoside when oxidized with one mole of periodate is subject to steric effects causing preferential cleavage between C-3, C-4 and C-2, C-3 respectively. However, interference by bulky substituents does not appear to fully account for the retarded oxidative rate of the 1,6-di-O-trityl-D-fructofuranosides, as the corresponding 1,6-ditosyl derivative reacts readily and quantitatively (96).

Schwarz (97), in his examination of the steric effects in the oxidation of hexitols with periodate, has shown that there is preferential attack at threo-glycol groups. This was accomplished by investigation of the intermediate compounds formed when galactitol (I), mannitol (II) and glucitol (III) were oxidized with 0.1 moles of periodate. For example, chromatographic examination of the oxidation products resulting from galactitol indicated formation of a tetrose (DL-threose) (oxidation at "b") while oxidation of mannitol gave mainly glyceraldehyde (oxidation at "c") and only a trace of tetrose. In addition, a trace of pentose was found, indicating some oxidation at "a". A similar oxidation of glucitol gave results suggesting comparable quantities of glyceraldehyde and erythrose, as well as traces of xylose and arabinose. These results were confirmed by studies preformed by Courtois and Guernet (98).



I II III

Figure 21. Positions of periodate oxidation in various hexitols.

Courtois and Guernet (99) extended the study made by Schwarz to include an expanded range of concentrations and compounds. The concentrations of periodate were varied from 5 to 40 % of the quantity reducible with an excess of oxidant. The compounds studied were glycerol, erythritol, mannitol, glucitol, galactitol, mannose, glucose and galactose. They found that attack on the hexoses was more regular than attack on the polyols. Attack started preferentially at the reducing end and gave initially the corresponding pentose. The degradation scheme was similar to that of Perlin and Brice (100) for the lead tetraacetate oxidation of hexoses, i.e. hexose $\longrightarrow$ pentose $\longrightarrow$ tetrose. The small amount of formaldehyde formed indicated that there was little attack between C-5 and C-6 at low concentrations of periodate.

Periodate oxidation of erythritol lead to preferential attack at one extremity. The glyceraldehyde produced was then oxidized from the reducing end in preference to the remaining erythritol. This sequence of oxidation was also observed for glycerol. Oxidation yielded glycolic aldehyde which was subsequently attacked at a faster rate than the initial attack on glycerol itself.

The attack of periodate on the hexitols occurred most rapidly at the carbon-carbon bond joining hydroxyls in a threo configuration, as outlined

previously. The extended range of periodate concentrations, however, allowed Courtois and Guernet to show that the composite aldehydes formed from the initial attack were then attacked more rapidly than the polyol from which they were derived.

In the present study, periodate oxidations were carried out, using one mole of periodate to oxidize 2,3-di-O-methyl-D-glucitol and 2,3-di-O-methyl-D-mannitol. The hydroxyls on C-4 and C-5 are in the erythro configuration and hence, might be expected to oxidize more slowly than the terminal C-5, C-6 position. This represents an analogous situation to the oxidation of erythritol performed by Courtois and Guernet (99). The further oxidation of the pentose thus formed is complicated by two factors: the presence of a methyl group on C-3 and the cyclization of the aldehyde and the free hydroxyl at C-5 of the newly formed pentose to form a pyranose ring form. One might expect the oxidation of the pentose formed to occur more slowly than oxidation of the hexitol (101). The methyl ether on C-3 of the pentose might also be expected to interfere with oxidation between C-1 and C-2 of the pentose in a manner similar to the steric hinderance of borohydride reduction (55). In an analogous argument, one might expect the C-3 methyl of the polyol to interfere with oxidation between C-4 and C-5. The formation of a considerable amount of pentopyranose structure prior to oxidation has been shown in this work by the formation of the corresponding formyl esters, 4-O-formyl-2,3-di-O-methyl-L-threose and 4-O-formyl-2,3-di-O-methyl-D-erythrose from the complete oxidation of the respective hexitols. Oxidation with one mole of peridate of 2,3-di-O-methyl-D-glucitol and 2,3-di-O-methyl-D-mannitol gave relatively high yields of the pentose products: 3,4-di-O-methyl-L-xylose and 3,4-di-O-methyl-D-arabinose respectively.

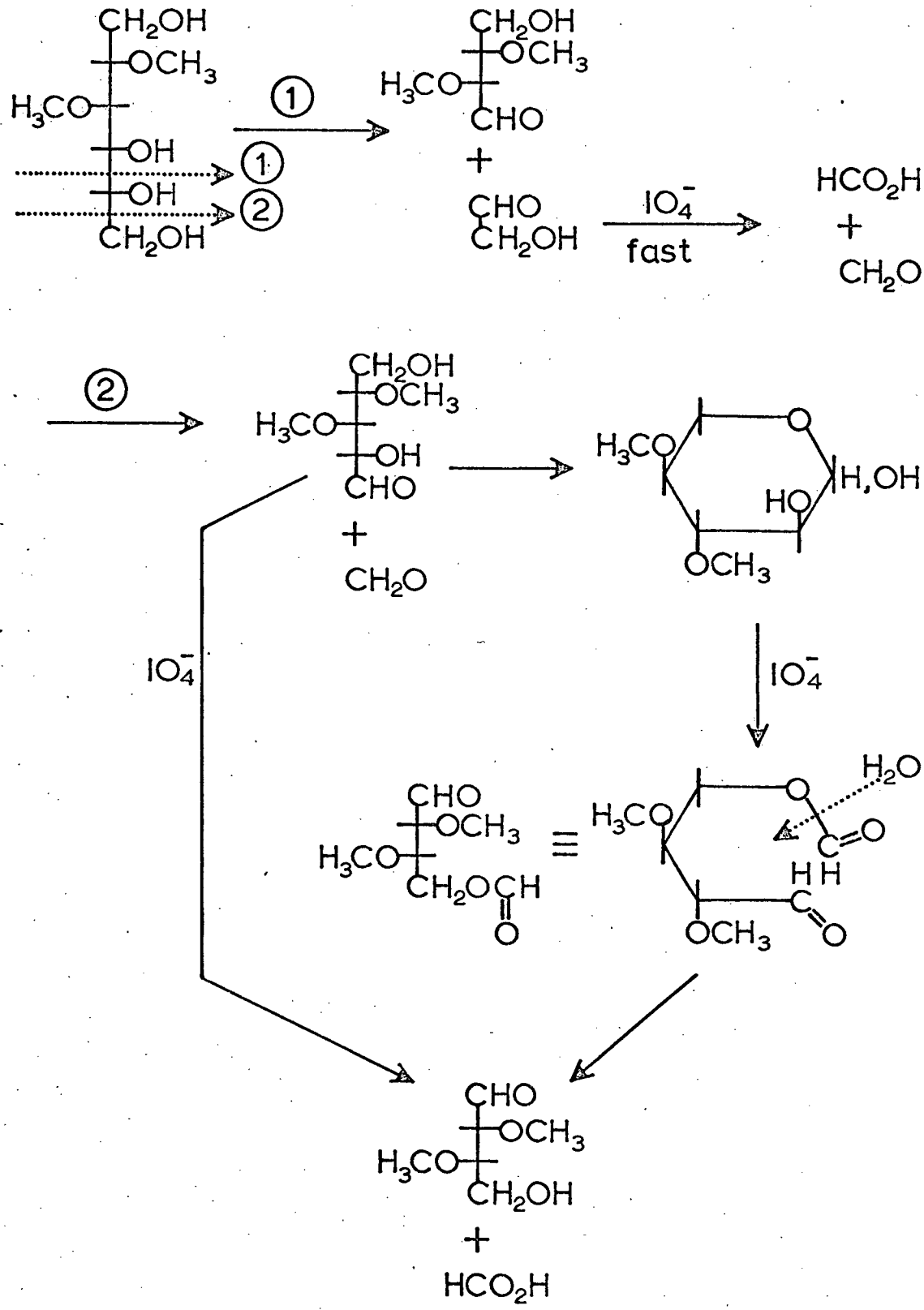


Figure 22. Periodate oxidation of 2,3-di-O-methyl-D-glucitol.

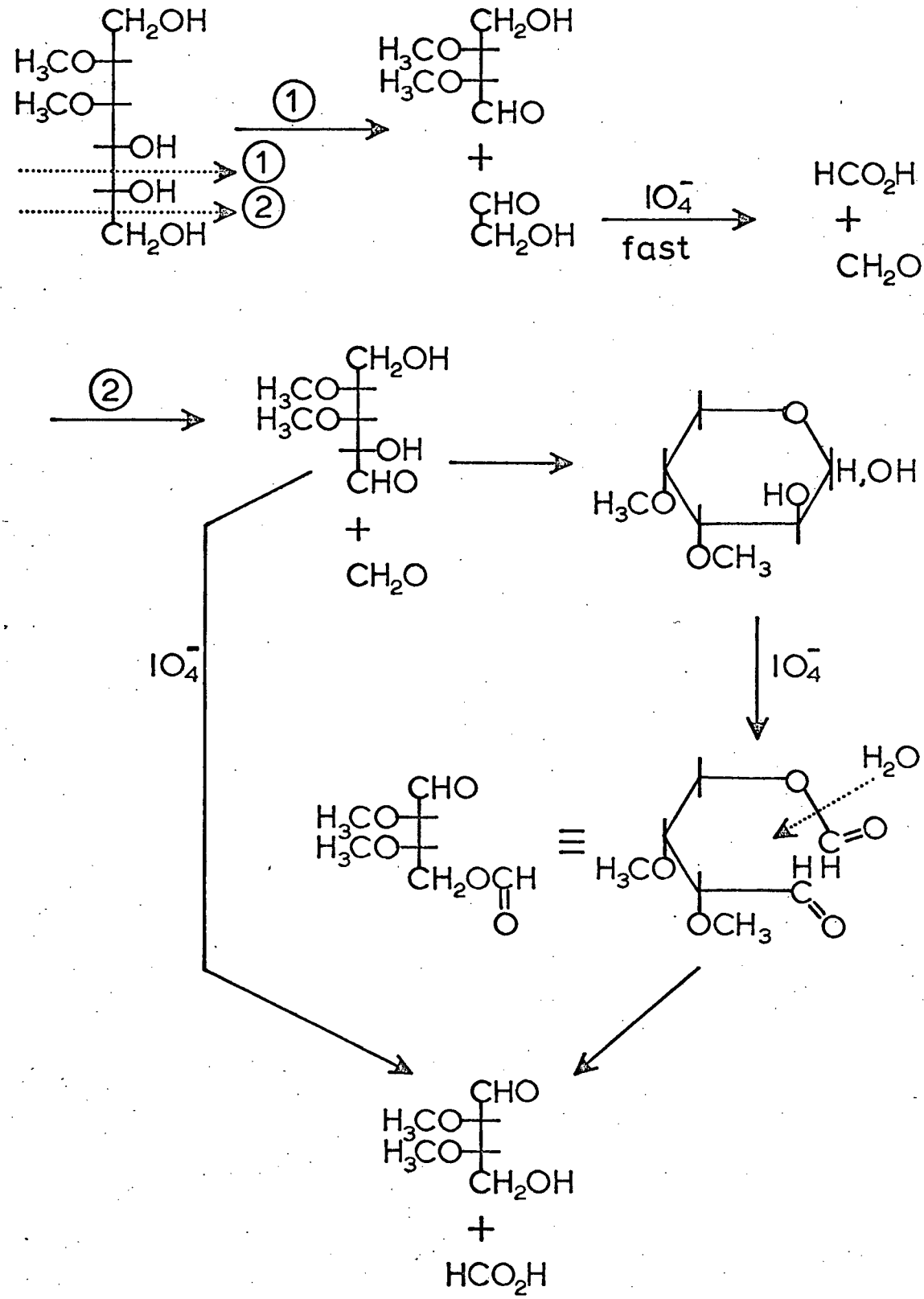


Figure 23. Periodate oxidation of 2,3-di-O-methyl-D-mannitol.

The periodate oxidation of carbohydrates can be complicated by incomplete (102) or over oxidation (103). Incomplete oxidation usually results from formation of formyl esters or blockage of one of two vicinal hydroxyls by the cyclic acetal (104) of the sugar formed.

Figure 24. Inhibition of periodate oxidations.

Over oxidation results from the oxidation of the C-H bond of an active methylene such as occurs in a malondialdehyde (105).

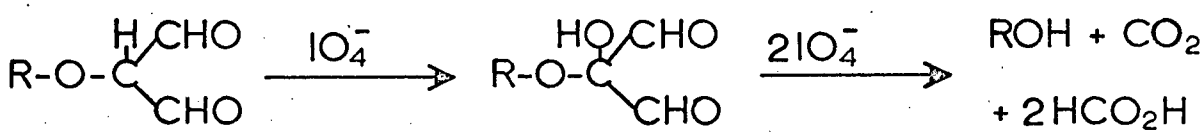


Figure 25. Periodate oxidation of a malondialdehyde.

Over oxidation may be further complicated by the formation of elemental iodine (106,107).

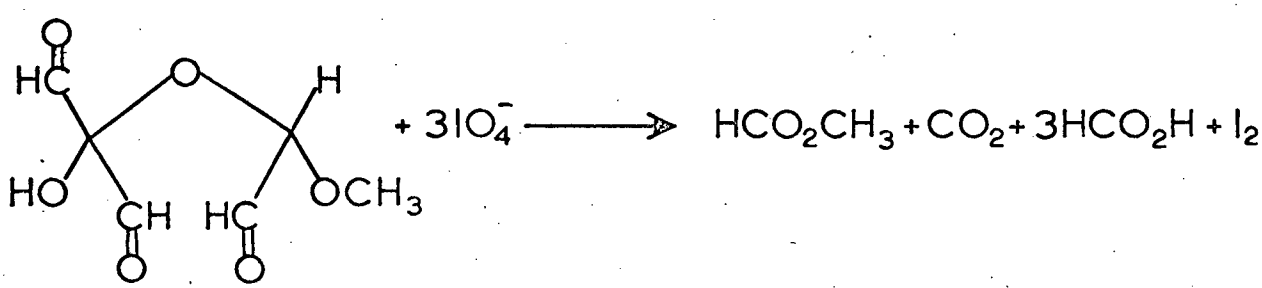


Figure 26. Formation of elemental iodine on periodate oxidation.

The oxidation of ketoses is complicated by two different courses of oxidation (108, 109).

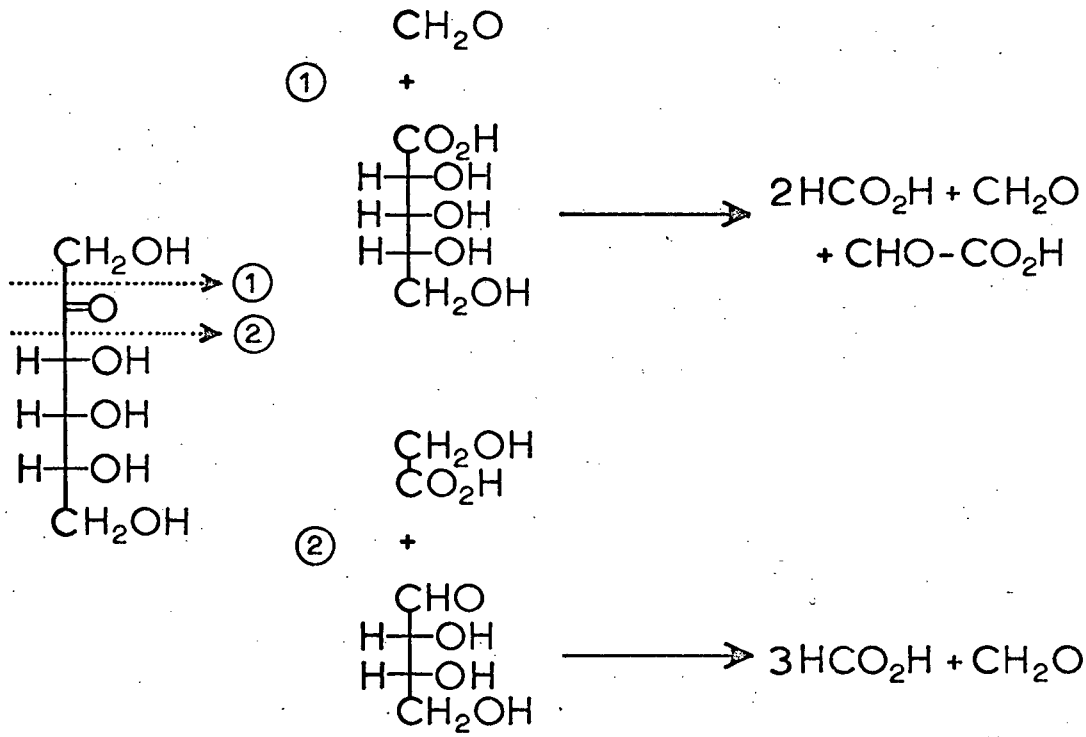


Figure 27. Course of periodate oxidation of ketoses.

As discussed previously, periodate oxidation of partially methylated sugars and sugar alcohols can lead to the formation of fragments which, if identified, aid in the structural elucidation of the parent compound. However, one may look at periodate oxidation also as a preparative tool. While partially methylated derivatives have been prepared in this way, the preparation of D-erythrose from 4,6-O-benzylidene-D-glucitol (110), and L-xylose from 2,4-benzylidene-D-glucitol has been reported (111). Periodate oxidation has also been applied to sugar phosphates (112,113), benzyl ethers of sugars (114) and O-isopropylidene sugars (115,116).

Formyl Ester

Measurement of the acidity produced in the periodate oxidation of carbohydrates is confined mainly to the estimation of formic acid. One of the

more difficult problems encountered in obtaining a valid formic acid assay is the formation of formate esters. The formate is formed from the cyclic, hemiacetal structure which is the equilibrium form of most sugars in solution. The oxidation of D-glucose proceeds with consumption of three moles of periodate to an intermediate product, 2-O-formyl-D-glyceraldehyde. The rate of further oxidation is dependent on the rate of hydrolysis of the formyl ester (117).

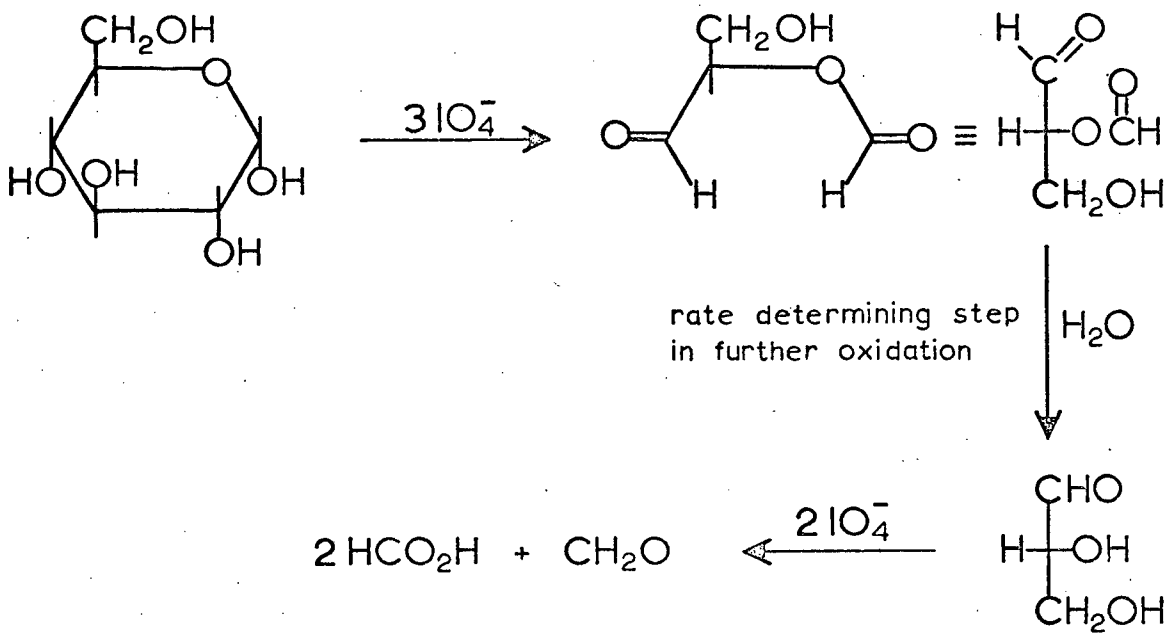


Figure 28. Periodate oxidation showing formate ester formation.

The isolation of the 4-O-formyl esters of 2,3-di-O-methyl-D-erythrose and 2,3-di-O-methyl-L-threose provides further proof that oxidation occurs to a considerable extent between C-6 and C-5 of the respective hexitols. The pentoses thus formed are then oxidized, resulting in the 4-O-formyl derivatives. The nature of the position of the formyl proton produces a very low Tau (τ) value nuclear magnetic resonance signal which allows one to confirm the presence of this grouping. In addition, the signal for the hydrogen on the aldehyde may be observed at an even lower τ value.

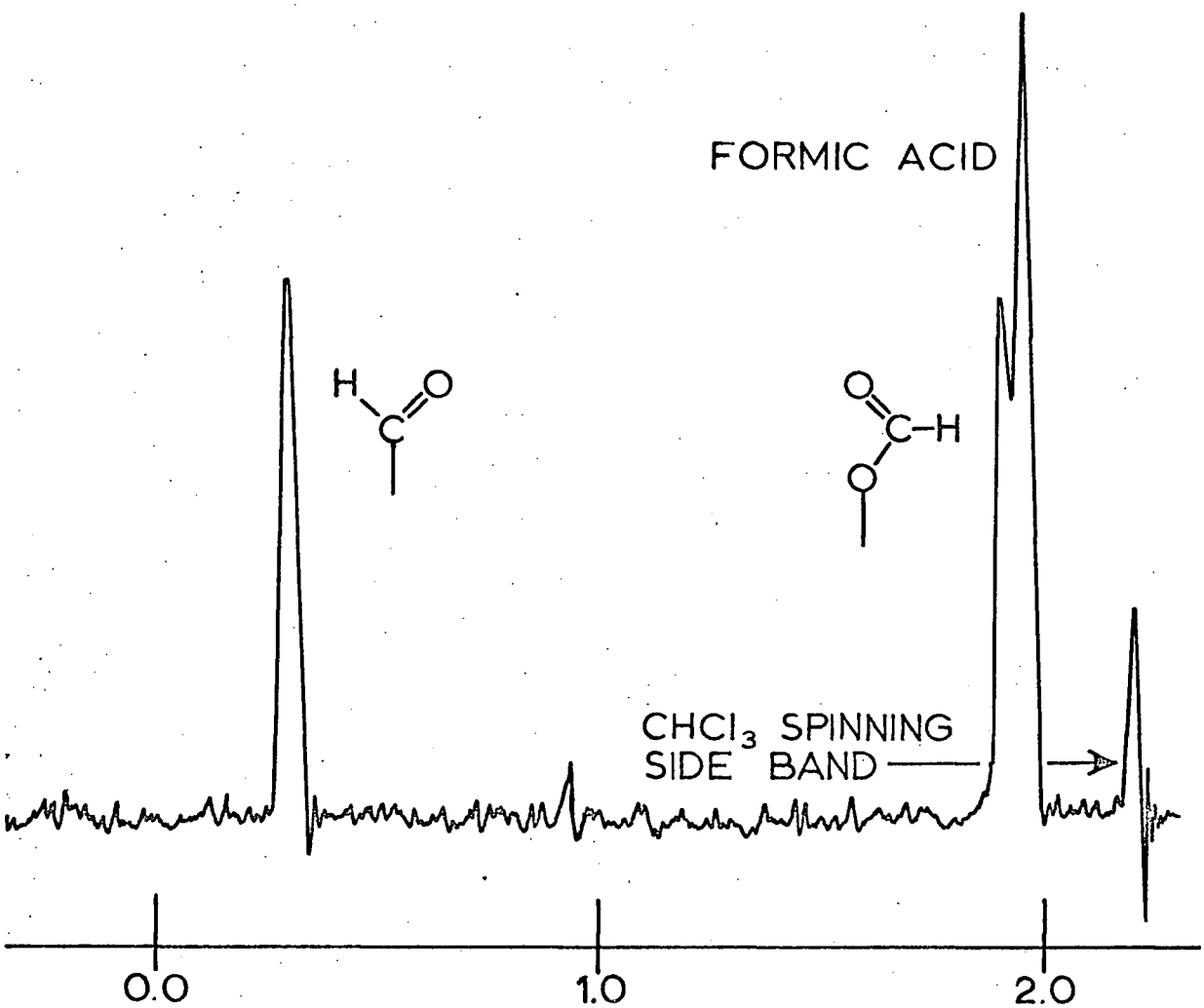


Figure 29. P.M.R. spectrum of formyl and aldehyde hydrogens.

Thin layer chromatography for monitoring carbohydrate reactions.

One of the most useful techniques that has been developed in this laboratory is the monitoring of carbohydrate reactions with thin layer chromatography (TLC) (118). Microscope slides developed in large weighing bottles allow one to rapidly follow the course of a reaction. The reaction mixture may be applied directly to the TLC plate and excess reagent may then be inactivated. For example, hydrolyses may be applied and the excess acid neutralized with a small amount of pyridine, or, when following borohydride reductions, the excess sodium borohydride is destroyed on the plate with acetic acid. One may follow virtually any reaction provided the concentration is such that a small number

of applications of the solution are all that is required to produce a detectable spot. The use of a destructive spray, such as 5 % nitric acid in sulfuric acid, allows one to detect all organic compounds which are relatively involatile. In this laboratory, all thin layer chromatograms were run on silica gel plates which were conditioned at 120°C for one hour and stored in the open room.

Table 4: Paper Chromatographic Characteristics of Known and New Tetroses, Pentoses and Tetritols.

	R_F or R_G/sol.	$[\alpha]_D$	Ref.
2-O-Methyl-D-threose	R_F 0.58/a	$[-28]^{23°}$ (C, 2.0 in H_2O)	31
2-O-Methyl-D-erythritol	R_F 0.165/a	[+12°] (C, 0.8 in H_2O)	
	R_F 0.46/c		
3-O-Methyl-D-threose	(not characterized)		30
4-O-Methyl-D-threose	R_G 0.80/b	$[+3°]^{18°}$ (C, 2 in H_2O)	32
4-O-Methyl-D-threitol	R_F 0.23/a	[+4.7°] (C, 1.2 in H_2O)	
	R_F 0.51/c		
2,3-Di-O-methyl-L-threose	(not characterized		33
2,3-Di-O-methyl-L-threose	R_F 0.83/a	[+12°] (C, 1.6 in MeOH)	
	R_F 0.80/c		
2,3-Di-O-methyl-D-erythrose	R_F 0.69/a	[-55.3] (C, 1.13 in MeOH)	
2,3-Di-O-methyl-L-threitol	R_F unknown*	[+7.6°] (C, 1.4 in MeOH)	
2,3-Di-O-methylerythritol	R_F unknown*	-	
2,4-Di-O-methyl-D-erythrose	R_F 0.70/a	$[+60.1]^{21°}$ (C, 1.4 in MeOH)	34
	R_F 0.64/a		
2,4-Di-O-methyl-L-erythrose	R_F 0.70/a	$[-61.4°]^{21°}$ (C, 4.9 in MeOH)	34
	R_F 0.64/a		
2,4-Di-O-methyl-D-threose	R_F 0.66/a	$[+14.8°]^{21°}$ (C, 1.17 in MeOH	34
	R_F 0.57/a		
2,4-Di-O-methyl-L-threose	R_F 0.66/a	$[-14.3°]^{21°}$ (C, 5.7 in MeOH)	34
	R_F 0.57/a		
3,4-Di-O-methyl-D-arabinose	R_F 0.195/a	$[-117°]_D$ (C, 2.4 in H_2O)	
	R_F 0.49/c		
3,4-Di-O-methyl-L-xylose	R_F .55/a	$[-29°]_D$ (C, 2.1 in H_2O)	
	R_F .65/c		

R_G values are relative to 2,3,4,6-tetra-O-methyl-D-glucose.

Solvent (a) refers to butanone-water azeotrope
(b) refers to butan-1-ol-pyridine-benzene-water (4:2:1:1)
(c) refers to butan-1-ol-ethanol-water (4:1:5)

*R_F unknown due to the lack of a proper detection reagent.

EXPERIMENTAL

Gas Liquid Chromatography

All analytical runs for the determination of M.R.F. were carried out in an identical manner. The gas chromatograph used was an F.and M.720 temperature programmed, dual column gas chromatograph equipped with a disc integrator. The injector port was 270°C, the detector block was 290°C. The bridge power was set at 150 milliamps. The helium flow rate was 6.8 sec/10 mls. and the copper column was 8 feet (') x 1/4, packed with 20% SF-96 silicone fluid on 60-80 mesh "Diataport-S". The column was run isothermally at 130°C for six minutes then programmed at 3° per minute. The programme stopped at 220°C and held at this point. The gas chromatograph was employed routinely to analyse reaction mixtures, prepare samples for the mass spectrometer and determine purity of prepared samples. Samples were examined as acetates, silyl ethers and methyl ethers, generally using SF-96 as the liquid phase. Preparative G.L.C. was performed using an 8' by 1/2 inch column packed with 20% SF-96 on 80-100 mesh "Diataport S".

Silylation of Samples for Gas Chromatography

Samples silylated for separation on the gas chromatograph were prepared by the method of Sweeley et al.(119) with some increase in the concentration of sugar depending on the number of free hydroxyl groups. Removal of the silyl ethers from the separated products was accomplished by refluxing the sample in methanol-water followed by evaporation under reduced pressure to obtain the starting material.

Periodate Oxidations

The course of periodate oxidations was followed by pipetting samples into an excess of pH 6.98 buffer (120) and sufficient 10% potassium iodide to

reduce all periodate to iodate. The liberated iodine was titrated with standardized sodium thiosulfate using starch as the indicator.

Evaporations were carried out under reduced pressure at a bath temperature of 35-45°. Optical rotations were equilibrium values measured on either a Bendix ETL-NPL Automatic Polarimeter (Type 143 A) or a Rudolph Polarimeter(Model 219) at 21 ± 2°. Melting points are uncorrected. Mass spectra were obtained at 70 eV on an Atlas MS-9 Mass Spectrometer.

Synthesis of 2,3-di-O-methyl-L-threose

Methyl 2,3-di-O-methyl-α-D-glucopyranoside was synthesized by the method of Irvine and Scott (49). The preparation of the benzylidene compound has been modified to avoid losses in the work-up of the reaction mixture.

Methyl 4,6-benzylidene-α-D-glucoside

Methyl α-D-glucopyranoside (144 g.) was added to a mixture of finely powdered, fused zinc chloride (116 g.) and freshly distilled benzaldehyde (420 g.) and the mixture was shaken for ninety (90) hours at room temperature. The reaction was monitored by running thin layer chromatograms. The solution was poured in a fine stream into rapidly stirred ice and water (2 l.). The benzylidene compound and the benzaldehyde separated as a slurry. The ice water was decanted and sufficient sodium carbonate was added to neutralize any remaining zinc chloride and render the solution slightly basic. The resulting mixture was transferred to a round bottom flask and the benzaldehyde was removed by steam distillation. Insoluble zinc carbonate was filtered from the hot solution and the methyl 4,6-O-benzylidene-α-D-glucoside crystallized from the cooled filtrate. The product was recrystallized successively from water and 65-110° petroleum, (178.5 g.) yield 85% m.p. 163-164° $[\alpha]_D$ +115.3 (c, 1.2 in $CHCl_3$). Lit.(121), m.p. 161-162°, $[\alpha]_D$ +117.5 (C, 1 in $CHCl_3$).

Methyl 2,3-di-O-methyl-α-D-glucopyranoside

Methyl 2,3-di-O-methyl-α-D-glucopyranoside was recrystallized from ethyl acetate-petroleum ether (65-110°) m.p. 84-85°. $[\alpha]_D$ +142.9 (C, 2.5 in EtOH). Lit. (122) m.p. 83-85°. $[\alpha]_D$ +143.1 (C, 5 in EtOH).

2,3-Di-O-methyl-D-glucitol

The hydrolysis of methyl 2,3-di-O-methyl-α-D-glucopyranoside resulted in some decomposition as discussed in "Methods of Synthesis". As a result it was not possible to crystallize 2,3-di-O-methyl-D-glucose. Recently however, a sample of 2,3-di-O-methyl-D-glucose started to crystallize spontaneously and it has been possible to isolate the β anomer of 2,3-di-O-methyl-D-glucose. Recrystallization from anhydrous acetone gave 2,3-di-O-methyl-β-D-glucose m.p. 112° $[\alpha]_D$ +6.4 → 59.6° (C, 2.1 in H_2O). Lit. (49) m.p. 110° $[\alpha]_D$ +10.6 → +64.4° (C, 5.0 in H_2O).

Non-crystalline 2,3-di-O-methyl-D-glucose (3.0 g.) was dissolved in water (50 ml.) and sodium borohydride (0.6 g.) was added. The solution was neutralized with acetic acid after 36 hours. The solution was passed through a column of Amberlite IR-120 (H^+) resin to remove sodium ions. The eluant was evaporated to dryness under reduced pressure and the solid (boric acid) and syrup were dissolved in methanol and evaporated to dryness three times. The syrupy non-reducing product (3.1 g.) could not be induced to crystallize, $[\alpha]_D$ +15 (C, 3.0 in H_2O). Lit. (123) $[\alpha]_D$ +13 (C, 2.54 in H_2O).

2,3-Di-O-methyl-1,4,5,6-tetra-O-p-nitrobenzoyl-D-glucitol

2,3-Di-O-methyl-D-glucitol (100 mg.) was dissolved in anhydrous pyridine and freshly distilled p-nitrobenzoyl chloride (400 mg.) was added. The reaction mixture was heated on a steam bath for one hour after which a few drops of water were added. The mixture was allowed to cool for fifteen minutes. The cool solution was poured into a stirred saturated solution of

sodium bicarbonate. The reaction product, 2,3-di-O-methyl-1,4,5,6-tetra-O-p-nitrobenzoyl-D-glucitol separated as a yellow gum (453 mg.) which was crystallized from acetone-methanol, m.p. 88-91°. Found: C, 52.3: H, 3.8. $C_{36}H_{30}O_{18}N_4$ requires C, 52.0; H, 3.6 %.

2,3-Di-O-methyl-L-threose

To non-crystalline 2,3-di-O-methyl-D-glucitol (2.02 g.) in water (1000 ml.) was added three molar equivalents of sodium metaperiodate (6.12 g.). The oxidation was allowed to proceed for 4 hours. The excess periodate and the iodate produced were removed by the addition of an excess of 10% barium acetate. The solution was filtered and then de-ionized with Amberlite IR-120 (H^+) and Duolite A-4 (OH^-). The neutral solution was evaporated to give a mobile syrup (1.35 g.) containing two components R_F 0.67 and 0.59 solvent A, T.L.C. Attempts to separate the formyl ester component on a cellulose-hydrocellulose column using solvent A were unsuccessful due to ester hydrolysis. The syrup was dissolved in water and one drop of pyridine was added. The product was evaporated to yield pure 2,3-di-O-methyl-L-threose, $[\alpha]_D$ +12° (c, 1.6 in MeOH). Found: C, 48.4; H, 8.3. $C_6H_{12}O_4$ requires C, 48.6; H, 8.2 %.

2,3-Di-O-methyl-L-threitol

2,3-Di-O-methyl-L-threose (100 mg.) was treated with excess sodium borohydride (200 mg.) After 16 hours, the excess borohydride was decomposed with a slight excess of acetic acid. The solution was deionized by passing through a column of Amberlite IR-120 (H^+). Evaporation of the resulting solution followed by evaporation of methanol (10 ml.) three times removed boric acid, yielding a colourless syrup (65 mg.) of 2,3-di-O-methyl-L-threitol. $[\alpha]_D$ +76° (c, 1.4 in MeOH). Found: C, 48.2; H, 9.5. $C_6H_{14}O_4$ requires C, 48.0; H, 9.3 %.

2,3-Di-O-methyl-1,4-di-O-p-nitrobenzoyl-L-threitol

A portion of the 2,3-di-O-methyl-L-threitol (30 mg.) was dissolved in pyridine (1 ml.) and treated with p-nitrobenzoyl chloride (100 mg.) in the usual manner, to give 2,3-di-O-methyl-1,4-di-O-p-nitrobenzoyl-L-threitol. m.p. 143-144°. Found: C, 53.3; H, 4.6. $C_{20}H_{20}O_{10}N_2$ requires C, 53.6; H, 4.5 %.

1,4-Anhydro-2,3-Di-O-methyl-D-glucitol

A mixture of methyl 2,3-di-O-methyl-α-D-glucopyranoside and 2,3-di-O-methyl-D-glucitol (3 g.) was treated with 2N. sulfuric acid (100 ml.) at 100° for 24 hours. The reaction was neutralized with barium carbonate and the neutral product was filtered and subsequently deionized by passing through successive columns of Amberlite IR-120 (H^+) and Duolite A-4 (OH^-). The neutral solution was reduced with an excess of sodium borohydride for 24 hours. The excess borohydride was destroyed with acetic acid. Sodium ions were removed by passing the solution down a column of Amberlite IR-120 (H^+) and the boric acid was removed by evaporation to dryness of the water solution followed by three evaporations of methanol solutions. The 1,4-anhydro-2,3-di-O-methyl-D-glucitol was removed from the 2,3-di-O-methyl-D-glucitol by continuous chloroform extraction of an aqueous solution of the mixed polyols. The chloroform solution was evaporated under reduced pressure to give a highly enriched fraction of 1,4-anhydro-2,3-di-O-methyl-D-glucitol (0.8 g.).

The enriched fraction was acetylated using acetic anhydride (2 ml.) in pyridine (10 ml.). The reaction after 16 hours was concentrated under reduced pressure to a volume of approximately 3 mls. This solution was subjected to separation on the preparative G.L.C. column to give a pure fraction of 5,6-di-O-acetyl-1,4-anhydro-2,3-di-O-methyl-D-glucitol (0.5 g.)

A portion of the 5,6-di-O-acetyl-1,4-anhydro-2,3-di-O-methyl-D-glucitol (0.2 g.) was deacetylated using a trace of sodium methoxide in methanol. The sodium ions were removed by treatment of the methanol solution with Amberlite IR-120 (H^+) and water. The solution was evaporated under reduced pressure to give syrupy 1,4-anhydro-2,3-di-O-methyl-D-glucitol (0.13). Found: C, 49.9; H, 8.4. $C_8H_{16}O_5$ requires C, 50.0; H, 8.4%.

1,4-Anhydro-2,3-di-O-methyl-5,6-di-O-p-nitrobenzoyl-D-glucitol

A portion of the pure 1,4-anhydro-2,3-di-O-methyl-D-glucitol (0.05 g.) was dissolved in pyridine (1.0 ml.) and treated with p-nitrobenzoyl chloride (150 mg.) in the usual manner, to give 1,4-anhydro-2,3-di-O-methyl-5,6-di-O-p-nitrobenzoyl-D-glucitol. m.p. 114°. Found: C, 53.5; H, 4.7; N, 5.5. $C_{24}H_{22}N_2O_{11}$ requires C, 53.85; H, 4.5; N, 5.7%.

Synthesis of 2,3-di-O-methyl-D-erythrose

Methyl α-D-mannopyranoside

D-Mannose (180 g.) was added to 3 % methanolic hydrogen chloride (150 ml.) and ethylene dichloride (300 ml.). The mixture was refluxed on a steam bath for six hours. During the course of the reaction, a two phase liquid system formed, the lower layer of which formed a crystalline mass. The cooled mixture was filtered and washed with a little ice-cold methanol, followed by ether. The mother liquors and methanol washings were evaporated to dryness under reduced pressure and fresh 3 % methanolic hydrogen chloride (75 ml.) and ethylene dichloride (150 ml.) were added. The mixture was refluxed for a further three hours and a second crop of crystals was isolated. The methyl α-D-mannopyranoside (154 g.) was recrystallized from ethanol water, m.p. 190-191°. $[\alpha]_D$ +77.3 (c., 1.2 in H_2O) Lit. (56) m.p. 191-192° $[\alpha]_D$ +79.0 (c., 1.0 in H_2O).

Methyl 4,6-O-benzylidene-α-D-mannopyranoside

Finely powdered methyl α-D-mannopyranoside (50 g.) was dissolved as rapidly as possible in 98-100 % formic acid (250 ml.) and freshly distilled benzaldehyde (250 ml.) was immediately added to the solution. After being allowed to stand five minutes, the solution was poured with stirring into a mixture of water (1l.) and anhydrous potassium carbonate (685 g.). The excess benzaldehyde was removed by steam distillation of the resulting solution. The aqueous phase was extracted with chloroform in a continuous extractor. The methyl 4,6-O-benzylidene-α-D-mannoside (23.0 g.) was recrystallized from benzene, m.p. 143-145°. Lit. (124), m.p. 147-148°.

Methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-mannopyranoside

Methyl 4,6-benzylidene-α-D-mannopyranoside (5 g.) was dissolved in dimethyl formamide (50 ml.) and methylated by Kuhn's method (69) using methyl iodide (20 ml.) and silver oxide (20 g.) added in portions three times during the reaction, at the start, at 8 hours and at 32 hours. Methyl 4,6-benzylidene-2,3-di-O-methyl-α-D-mannopyranoside (4.3 g.) was isolated as a syrup which could not be induced to crystallize. $[\alpha]_D$ +60.7 (c., 2.4 in $CHCl_3$). Lit. (124), $[\alpha]_D$ +62.7 (c., 1.178 in $CHCl_3$).

2,3-Di-O-methyl-D-mannitol

Acid hydrolysis of methyl 4,6-benzylidene-2,3-di-O-methyl-α-D-mannopyranoside (3.0 g.) followed by reduction with sodium borohydride yielded crystalline 2,3-di-O-methyl-D-mannitol)1.8 g.). The polyol was recrystallized from ethyl acetate, m.p. 101-102°. Lit. (125), m.p. 101-103°.

2,3-Di-O-methyl-D-erythrose

To 2,3-di-O-methyl-D-mannitol (1.00 g.) in water (500 ml.) was

added three molar equivalents of sodium metaperiodate (3.06 g.). The oxidation was allowed to proceed for four hours. The reaction was worked up in the usual manner with barium acetate. The neutral solution was evaporated to give a mobile syrup (885 mg.) containing two components, solvent A. (TLC Silica gel). The faster running 4-O-formyl-2,3-di-O-methyl-D-erythrose could be converted to the slower component, 2,3-di-O-methyl-D-erythrose by treatment with mild base. The pure 2,3-di-O-methyl-D-erythrose was separated as a mobile syrup from trace impurities by column chromatography on a cellulose-hydrocellulose column using solvent A. $[\alpha]_D$ -55.3° (c., in MeOH). Found: C, 48.4; H, 8.3. $C_6H_{12}O_4$ requires C, 48.6; H, 8.2%.

2,3-Di-O-methylerythritol

A sample of 2,3-di-O-methyl-D-erythrose (50 mg.) was reduced with sodium borohydride (100 mg.). The reaction was worked up in the usual manner after 16 hours. Evaporation of the neutral solution gave 2,3-di-O-methyl-erythritol (35 mg.). Found: C, 48.3; H, 9.25. $C_6H_{14}O_4$ requires C, 48.0; H, 9.4 %.

2,3-Di-O-methyl-1,4-di-O-p-nitrobenzoylerythritol

Treatment of a portion of the syrupy 2,3-di-O-methylerythritol (25 mg.) with p-nitrobenzoyl chloride (100 mg.) in the usual manner gave 2,3-di-O-methyl-1,4-di-O-p-nitrobenzoylerythritol. m.p. 178°. Found: 53.5; H, 4.4. $C_{20}H_{20}O_{10}N_2$ requires C, 53.55; H, 4.5 %.

Synthesis of 2-O-Methyl-D-erythritol

Methyl 2,3-isopropylidene-α-D-mannoside.

Methyl α-D-mannopyranoside (25 g.) was dissolved in 98-100 % formic acid (100 ml.) and acetone (750 ml.) was added. The reaction was allowed to proceed for fourteen days. "Drierite" (30 g.) drying agent was added on the

fourth day to aid in maintaining anhydrous conditions. Undissolved methyl α-D-mannopyranoside and "Drierite" were removed by filtration. The solution was neutralized by pouring into a slight excess of 15 % sodium hydroxide. The aqueous mixture formed two layers and the excess acetone was removed by evaporation under reduced pressure. The solution was extracted with chloroform (3 x 500 ml.) and ethyl ether (3 x 500 ml.). The extracts were combined and evaporated to yield a syrup which was dissolved in water. Extraction of the water with 30-60° petroleum (3 x 300 ml.) removed all the methyl 2,3;4,6-di-O-isopropylidene-α-D-mannopyranoside. The aqueous phase was evaporated under reduced pressure to yield a syrup. The syrup was dissolved in hot ethyl acetate and on cooling methyl 2,3-O-isopropylidene-α-D-mannopyranoside (10.92 g.) crystallized out. m.p. 107-108°. Lit. (68), m.p. 105°.

Methyl 4-O-methyl- -D-mannopyranose

Methyl 4-O-methyl-α-D-mannopyranoside was prepared by the method of Smith (66). There was however, detritylation during the methylation and the methanolysis product was purified by column chromatography on a cellulose-hydrocellulose column using solvent A. Methyl 4-O-methyl-α-D-mannopyranoside crystallized from butanone on prolonged standing m.p. 101-102°. Lit. (126), m.p. 101-103°.

2-O-Methyl-D-erythritol

Methyl 4-O-methyl-α-D-mannopyranoside (0.8 g.) in water (200 ml.) was oxidized with 1.5 mole equivalents of sodium metaperiodate (1.2 g.) for twenty-four hours. The reaction was worked up with barium acetate in the usual manner. The neutral solution was reduced with an excess of sodium borohydride for 24 hours and the excess borohydride was destroyed with acetic acid. Sodium ions were removed by passing the solution down a column of Amberlite IR-120 (H^+), the boric acid was removed by repeated distillations

of methanol. Mild acid hydrolysis of the hemiacetal gave a sample of 2-O-methyl-D-erythritol (0.39 g.) $[\alpha]_D$ +12° (c, 0.8 in H_2O). Found: C, 44.2; H, 8.8. $C_5H_{12}O_4$ requires C, 44.1; H, 8.9 %.

2-O-Methyl-1,3,4-tri-O-p-nitrobenzoyl-D-erythritol

Treatment of a portion of 2-O-methyl-D-erythritol (50 mg.) with p-nitrobenzoyl chloride (200 mg.) in pyridine (2 ml.) in the usual manner gave 2-O-methyl-1,3,4-tri-O-p-nitrobenzoyl-D-erythritol m.p. 218-220° mixed m.p. with authentic sample 219-220°.

Synthesis of 4-O-methyl-D-threose

6-O-Methyl-D-galactose

6-O-Methyl-D-galactose was synthesized by the methylation of diacetone galactose followed by mild acid hydrolysis. After recrystallization from ethanol it had the following constants, m.p. 128° $[\alpha]_D$ +115° (c., 2.1 in H_2O). Lit. (62), m.p. 128° $[\alpha]_D$ +114° (c., 2.1 in H_2O).

Methyl 6-O-methyl-α-D-galactofuranoside

Methyl 6-O-methyl-β-D-galactofuranoside

Methyl 6-O-methyl-α-D-galactopyranoside

A two percent methanolic solution of 6-O-methyl-D-galactose (5 g.) containing 0.012 to 0.013 per cent hydrogen chloride was refluxed 18 hours until the free sugar content was very low, as indicated by tests on a sample with Fehling's solution. The hydrogen chloride was removed by the addition of silver oxide. The filtered solution was evaporated to dryness and chromatographed on a cellulose-hydrocellulose column with solvent A to yield three fractions. Fraction one, (2.475 g.) methyl 6-O-methyl-β-D-galactofuranoside $[\alpha]_D$ = 117° (c., 4.7 in MeOH). Fraction two, (0.735 g.) methyl 6-O-methyl-α-D-galactofuranoside. $[\alpha]_D$ +95° (c., 1.96 in MeOH). Fraction three (0.151 g.) methyl 6-O-methyl-α-D-galactopyranoside, m.p. 132-134° mixed m.p. with

authentic sample m.p. 132-134°.

Methyl 6-O-methyl-2,3,5-tri-O-p-nitrobenzoyl-α-D-galactofuranoside

Treatment of methyl 6-O-methyl-α-D-galactofuranoside (50 mg.) with p-nitrobenzoyl chloride in the usual manner gave methyl 6-O-methyl-2,3,5-tri-O-p-nitrobenzoyl-α-D-galactofuranoside. m.p. 107-110°. Found: C, 53.1; H, 3.8. $C_{29}H_{25}O_{15}N_3$ requires C, 53.2; H, 3.85 %.

Methyl 6-O-methyl-2,3,5-tri-O-p-nitrobenzoyl-β-D-galactofuranoside

Treatment of methyl 6-O-methyl-β-D-galactofuranoside (50 mg.) with p-nitrobenzoyl chloride in the usual manner gave methyl 6-O-methyl-2,3,5-tri-O-p-nitrobenzoyl-β-D-galactofuranoside. m.p. 82-85°. Found C, 53.0; H, 3.75 %.

4-O-Methyl-D-threose-dimethylacetal

Methyl 6-O-methyl-αβ-D-galactofuranoside (3.0 g.) was oxidized with 1.5 mole equivalents of sodium metaperiodate (75 mls. ~0.3M) in water (1.2 l.). The reaction was allowed to proceed for three days. Excess sodium metaperiodate and iodate were removed in the usual manner with barium acetate and Amberlite IR-120 (H^+). Evaporation of the deionized solution gave 2.873 g. of the periodate oxidized product. This mixture was subjected to a methanolysis with 3 % methanolic hydrogen chloride for 24 hours and no slow moving component on TLC (solvent A) remained. Chromatography of this mixture (1.67 g.) on the cellulose-hydrocellulose column with solvent A gave a fraction (0.63 g.) with properties consistant with the dimethyl acetal of 4-O-methyl-D-threose, having three methoxyl and two free hydroxyls in its N.M.R. spectra. $[\alpha]_D$ +5 (c., 0.8 in MeOH).

4-O-Methyl-D-threose

Hydrolysis of this fraction with dilute sulphuric acid (.5N) gave 4-O-methyl-D-threose. $[\alpha]_D$ +5° (c., 1.3 in H_2O). Lit.(47), $[\alpha]_D$ +3° (c., 2 in H_2O).

4-O-Methyl-D-threitol

Reduction of a portion of periodate oxidized furanosides (1.0 g.) with sodium borohydride, followed by mild acid hydrolysis gave a sample of 4-O-methyl-D-threitol. $[\alpha]_D$ +4.7° (c., 1.2 in H_2O), Found: C, 44.1; H, 8.7. $C_5H_{12}O_4$ requires C, 44.1; H, 8.9%.

4-O-Methyl-1,2,3-tri-O-p-nitrobenzoyl-D-threitol

Treatment of 4-O-methyl-D-threitol (50 mg.) in pyridine (1 ml.) with p-nitrobenzoyl chloride (150 mg.) in the usual manner gave 4-O-methyl-1,2,3-tri-O-p-nitrobenzoyl-D-threitol m.p. 129°. Found: C, 53.3 H, 3.6. $C_{26}H_{21}O_{13}N_3$ requires C, 53.5; H, 3.6.

Controlled periodate oxidations

Controlled periodate oxidations were performed on 2,3-di-O-methyl-D-glucitol and on 2,3-di-O-methyl-D-mannitol. One mole of sodium metaperiodate was allowed to react completely with each hexitol.

3,4-Di-O-methyl-L-xylose

2,3-Di-O-methyl-D-glucitol (1.11 g.) was added to a solution (175 ml.) containing one mole equivalent of sodium metaperiodate (1.12 g.) and allowed to react for twenty-four hours. Removal of the ions in the usual manner yielded a neutral solution which on T.L.C. in solvent A showed one major and three lesser spots. The major spot was isolated by column chromatography, Solvent A, to give a fraction, 3,4-di-O-methyl-L-xylose (0.74 g.) which could

not be induced to crystallize. $[\alpha]_D$ -14.6° (c., 2.1 in MeOH). Lit. (128) D isomer +13° (c., 1.6 in MeOH).

Methyl 3,4-di-O-methyl-α-L-xyloside

A portion of the 3,4-di-O-methyl-L-xylose (2.0 g.) was refluxed with 3% methanolic hydrogen chloride for twenty-four hours. The solution was neutralized with silver oxide and evaporated to give a syrup of the two possible glyosides. The glycosides were then purified by preparative G.L.C. to give a crystalline sample of methyl 3,4-di-O-methyl-α-L-xylose, m.p. 87-89°. Lit. (127), m.p. 89-90°. Found: OMe, 48.15. $C_8H_{16}O_5$ requires OMe, 48.35 %.

3,4-Di-O-methyl-D-arabinose

2,3-Di-O-methyl-D-mannitol (1.15 g.) was added to a solution containing one mole equivalent of sodium metaperiodate and allowed to react for twenty-four hours. The neutral solution obtained by the usual work-up showed on T.L.C. chromatography in solvent A one major and three lesser spots. The major spot was isolated by column chromatography using solvent A to give a fraction, 3,4-di-O-methyl-D-arabinose (0.83 g.) which could not be induced to crystallize. $[\alpha]_D$ -118° (c., 1.3 in H_2O). Lit. (128) $[\alpha]_D$ +125° (c., 2.4 in H_2O) (L-isomer).

3,4-Di-O-methyl-D-arabonamide

3,4-Di-O-methyl-D-arabinose (50 mg.) was oxidized four days with bromine water. The lactone was recovered in the usual manner. The lactone (40 mg.) was dissolved in methanol (5 ml.) saturated with ammonia. The reaction was allowed to stand overnight at 0°C. Removal of the solvent gave the amide which was recrystallized from acetone. m.p. 132-33°. Lit. (128), m.p. 133° (L-isomer). Found: C, 43.45; H, 7.9. $C_7H_{15}N_1O_5$ requires C, 43.5; H, 7.8 %.

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