THE METHYLATION OF SUGAR MERCAPTALS

by

YUKIO TANAKA

B. Pharm., Osaka University, 1951

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(Yukio Tanaka)

Department of Chemistry

The University of British Columbia,
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THE METHYLATION OF SUGAR MERCAPTALS

ABSTRACT

Sugar mercaptals were partially methylated with Purdie's reagents in tetrahydrofuran as a solvent, and the relative reactivity of hydroxyl groups was determined by estimation of the ratio of mono-*O*-methyl ethers. The highest reactivity of the 2-hydroxyl group, followed by that of the 3-hydroxyl group, was accounted for by the inductive effect of the mercaptal group and the variation of the ratios among the sugar mercaptals was further explained by the intramolecular hydrogen bonding in the zig-zag conformation of sugar mercaptals. The exceptionally high reactivity of the 3-hydroxyl group of D-galactose diethyl mercaptal was also shown to be explicable in terms of the hydrogen bonding. An appreciable reactivity of the primary hydroxyl groups was noted and attributed to the least steric hindrance at this position. A mechanism for the Purdie methylation was proposed.

Mg values of various mono-*O*-methyl sugars were recorded and some modifications in the separation of mono-*O*-methyl sugars were also described.

5-*O*-Methyl-L-arabinose and two crystalline derivatives were synthesized for the first time, and 2-*O*-methyl-L-arabonolactone, hitherto known as a sirup, was crystallized.

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ABSTRACT

Sugar mercaptals were partially methylated with Purdie's reagents in tetrahydrofuran as a solvent, and the relative reactivity of hydroxyl groups was determined by estimation of the ratio of mono-O-methyl ethers. The highest reactivity of the 2-hydroxyl group, followed by that of the 3-hydroxyl group, was accounted for by the inductive effect of the mercaptal group and the variation of the ratios among the sugar mercaptals was further explained by the intramolecular hydrogen bonding in the zig-zag conformation of sugar mercaptals. The exceptionally high reactivity of the 3-hydroxyl group of D-galactose diethyl mercaptal was also shown to be explicable in terms of the hydrogen bonding. An appreciable reactivity of the primary hydroxyl groups was noted and attributed to the least steric hindrance at this position. A mechanism for the Purdie methylation was proposed.

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INTRODUCTION*

The first sugar mercaptal or dithioacetal was prepared by Fischer (1) in 1894. Since then this class of compounds has attracted considerable attention from carbohydrate chemists in view of the ready formation of their acyclic structures from the parent aldose. Unlike the acid-catalyzed reaction of alcohols with sugars which lead to the formation of cyclic glycosides (e.g. II), thiols, sulphur analogues of alcohols, yield acyclic dithioacetals (e.g. III). The formation of such acyclic structures frees an additional hydroxyl group which is involved in the ring structure of the original sugar (e.g. I) or of the glycoside (Fig. 1).

Sugar mercaptals are of importance in providing the best route to the preparation of acyclic sugar derivatives and have sometimes been used for the characterization of monosaccharides because of the sparing solubility and crystallinity of the majority of mercaptals (2,3,4,5,6). The preparation, reactions, and uses of sugar mercaptals have been reviewed (7,8).

The reactivity of hydroxyl groups in carbohydrate compounds is of great interest and has been a subject of review (9). Some reactions and reactivities of hydroxyl groups in cyclic sugar derivatives are explainable and have been discussed (10, 11) from the view-point of stereochemistry developed in the realm of alicyclic chemistry, and an interesting application of stereospecific reaction has been demonstrated by Aspinall and Zweifel (12) in the

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* The following conventional abbreviations are used in formulations: R (alkyl), Me (methyl), Et (ethyl), Ph (phenyl), Tr (triphenylmethyl), Ac (acetyl), Bz (benzoyl), and Ts (p-toluenesulphonyl). All ring-hydrogen atoms are omitted in the Haworth structures.

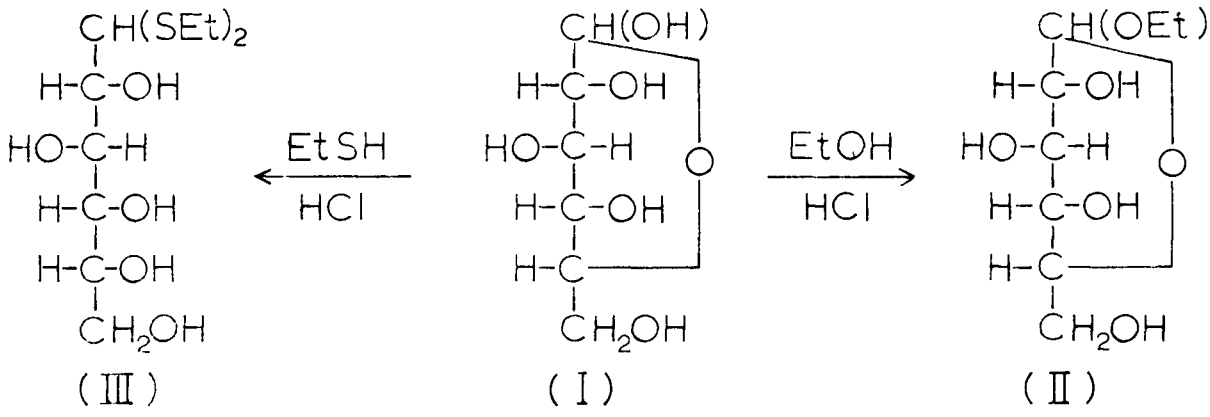


Fig. 1. Formation of glycosides and mercaptals.

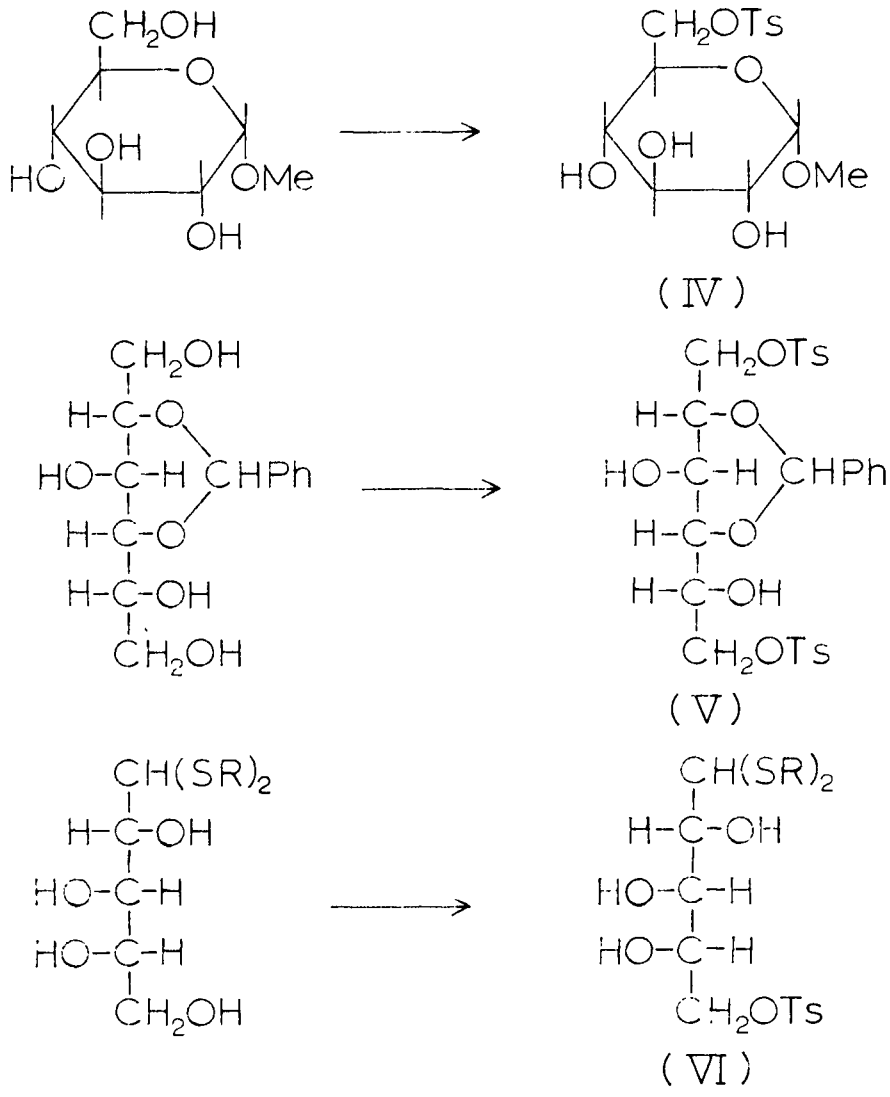


Fig. 2. Selective tosylation of primary hydroxyl group.

syntheses of D-mannose derivatives.

Selective reactions of hydroxyl groups in carbohydrate compounds have been known for a long time. The preferential tosylation of primary hydroxyl groups, leaving secondary hydroxyl groups intact, is one of these selective reactions frequently observed in carbohydrate chemistry (Fig. 2). Compton (13) for example, obtained the 6-O-tosyl derivatives (IV, for the α-form) of methyl α- and β-D-glucopyranosides with tosyl chloride and pyridine, and in a similar manner, von Vargha (14) obtained the 1,6-di-O-tosyl derivative (V) from 2,4-D-benzylidene-D-glucitol. Zinner, Wessely, and Kristen (15) have reported successful tosylation of L-arabinose mercaptals to their 5-O-tosyl derivatives (IV) with tosyl chloride and pyridine at low temperature.

Selective acylation of primary hydroxyl groups has also been observed frequently (Fig. 3). Thus, Richtmyer and Yeakel (16) obtained phenyl 6-O-benzoyl-β-D-glucopyranoside (VII) from the corresponding glucoside, and Müller (17) obtained 1,6-di-O-benzoyl-D-glucitol (VIII) by direct benzoylation of D-glucitol. The reaction of aldose mercaptals with benzoyl chloride at low temperature gave their ω-mono-O-benzoyl derivatives (e.g. IX) (18).

The most widely recognized method of selective etherification of sugars involves the reaction of primary hydroxyl groups with triphenylmethyl (trityl) chloride (Fig. 4). Helferich (19) has described the selective character of this reagent, whose rate of reaction with primary hydroxyl groups is many fold that with secondary hydroxyl groups. Ethyl α-L-arabinofuranoside, for example, gave its 5-O-trityl derivative (X) by the reaction of trityl chloride in pyridine (20). Although Sowden (21) has stated that 'since a considerable portion of the difference in reactivities may arise from steric fac-

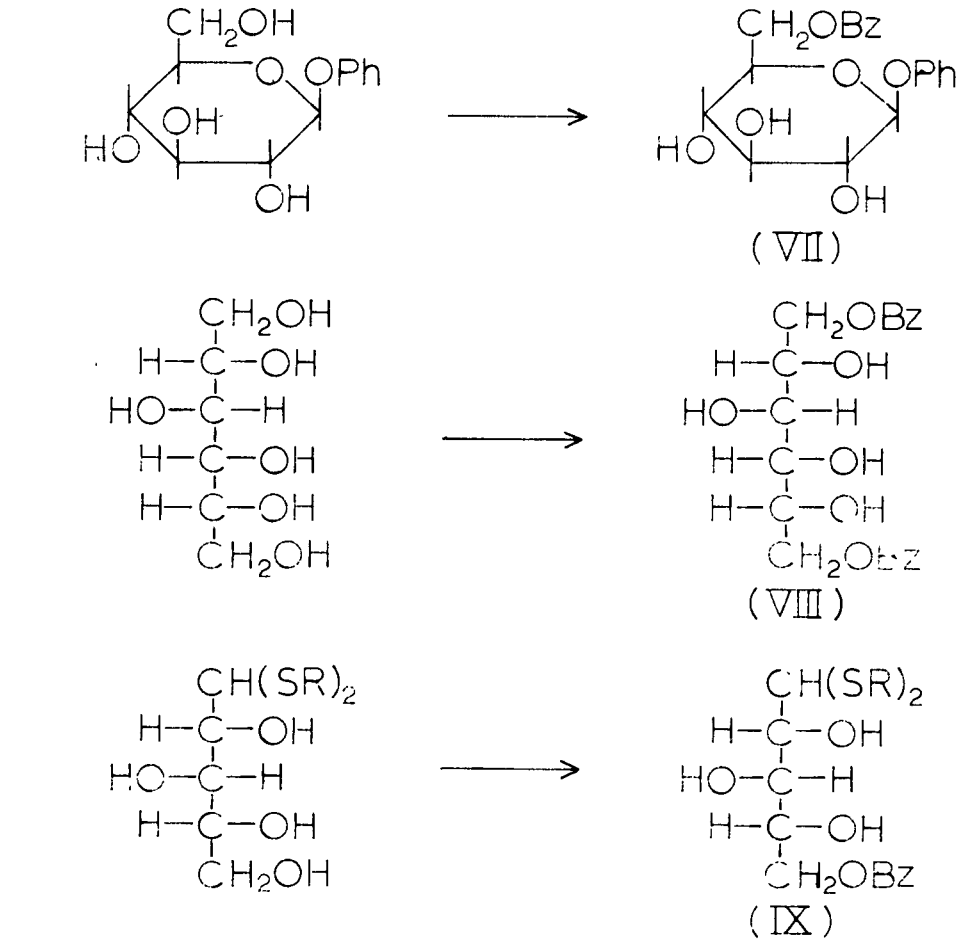


Fig. 3. Selective benzoylation of primary hydroxyl groups.

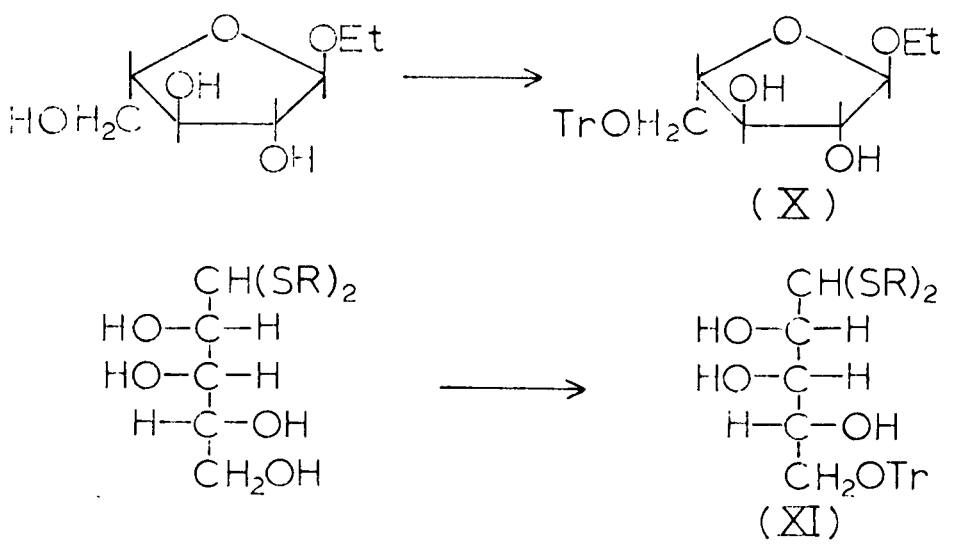


Fig. 4. Selective tritylation of primary hydroxyl groups.

tors of different ring conformations, the difference between acyclic primary and secondary hydroxyl groups may not be so great', Zinner, Braudner, and Rembarz (22) have shown that sugar mercaptals are successfully tritylated on their primary hydroxyl groups (e.g. XI).

Other etherifications, of which methylation has almost exclusively been studied, have been found to be less specific toward primary and secondary hydroxyl groups, and thus selective reaction is more dependent upon the experimental conditions involved. Percival and collaborators (23, 24, 25) prepared complexes of sugars with alkalies and then formed methyl ethers by reaction with dimethyl sulphate. Methyl α- and β-D-glucopyranosides yielded methyl mono-O-methyl-D-glucopyranosides, from which 6-O-methyl-D-glucose, identified as its phenylosazone, was obtained. Application of the same procedure to maltose (4-O-α-D-glucopyranosyl-D-glucose), followed by acid hydrolysis, produced 2-O-methyl- and 2,6-di-O-methyl-D-glucose. In like fashion, sucrose (β-D-fructofuranosyl α-D-glucopyranoside) gave 6-O-methyl-D-glucose, and lactose (4-O-β-D-galactopyranosyl-D-glucose) formed 2-O-methyl- and 2,4-di-O-methyl-D-galactoses. The position of the alkali in the complex was assumed to be at that position where methylation occurred. Similar results were obtained when methyl α-D-glucopyranoside was methylated with methyl iodide in the presence of thallous hydroxide. A mixture of tri-O-methyl-D-glucoses was formed in which substitution in the 2- and 6-positions predominated (26).

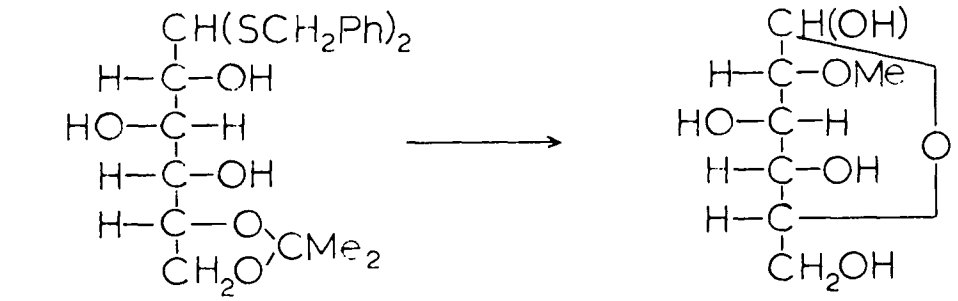
Apart from the stereochemical view on the reactivity of hydroxyl groups in cyclic sugar derivatives and selective reactions of some reagents such as tosyl chloride and trityl chloride, the greater reactivity of 2-hydroxyl groups over other hydroxyl groups in both cyclic and acyclic structures toward methyl-

ation has been noted by many workers. Pacsu (27) has reported that methylation of the 5,6-O-isopropylidene-D-glucose dibenzyl mercaptal, followed by acidic hydrolysis, yielded a mono-O-methyl-D-glucose, to which he erroneously assigned the structure of 4-O-methyl-D-glucose, and the reaction product was re-examined by Schinle (28) to confirm that the methoxyl group was situated on carbon-2, i.e. the product was 2-O-methyl-D-glucose (XII) (Fig. 5).

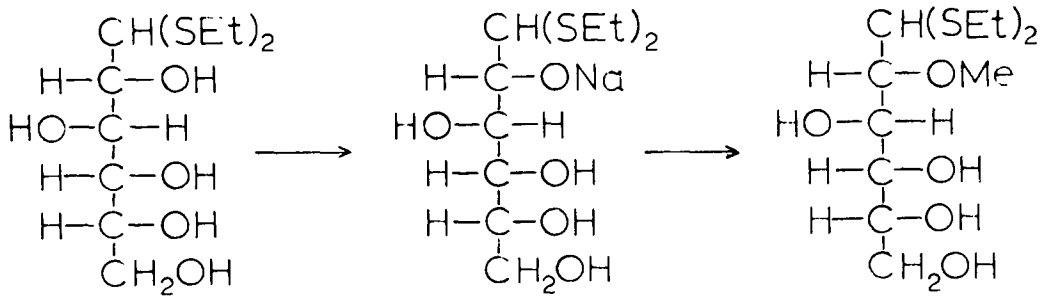
Papadakis (29) has reported the synthesis of 2-O-methyl-D-glucose diethyl mercaptal (XIII) by the action of methyl iodide on mono-O-sodio-D-glucose diethyl mercaptal prepared by Fischer (30) from the mercaptal and sodium ethoxide, obviously indicating an acidic character of the 2-hydroxyl group (Fig. 5).

With starch and cellulose in which cyclic D-glucopyranose units are linked, it has been reported by Lieser (31), by Gaver (32), and by Sugihara and Wolfrom (33) that alkali and methyl iodide lead preponderantly to the 2-O-methyl derivative, although recent work by Hess, Heumann, and Leipold (34) has demonstrated that the methylation of cellulose with the same reagents is random in nature.

The acidity of the 2-hydroxyl group of free sugars has been attributed to the intermediary enediol in the de Bruyn-van Eckenstein transformation (35, 36), for which the mechanism shown in Fig. 6 has been proposed. Sowden and Schaffer (37) have studied the de Bruyn-van Eckenstein transformation, using D-glucose-1-C^{14}, D-fructose-1-C^{14}, and D-glucose in heavy water, and have shown that the radioisotopic dilution analysis for D-glucose and D-fructose could be interpreted in terms of the classical mechanism which includes the enediol intermediate. These results have been supported by conductometric measurements of reducing sugars by Hirsch and Schlags (38), who have shown that



(XII)



(XIII)

Fig. 5. Selective methylation of 2-hydroxyl groups.

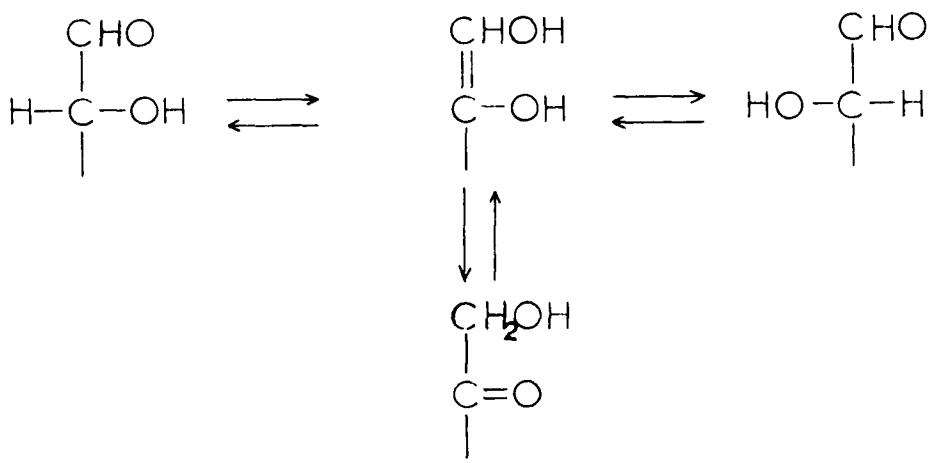


Fig. 6. de Bruyn-van Eckenstein transformation.

reducing sugars behave as weak dibasic acids.

However, Strocchi and Gliozzi (39) have reported that both conductometric and polarographic measurements have indicated acidity and complex formation in such non-reducing sugars as sucrose. Thus, the acidic character of any reducing sugars is explainable on the basis of the acidic enol intermediate of the de Bruyn-van Eckenstein transformation; however, since the analogous enol would not be possible in glycosides, the observed acidity might be the result of a permanent polarization. Sugihara (9) has pointed out that the inductive effect of two oxygen atoms on carbon-1 in non-reducing glycosides can activate the hydroxyl group on carbon-2, resulting in acidity and high reactivity of the 2-hydroxyl group in an alkaline medium. By analogy the high reactivity of 2-hydroxyl groups in sugar mercaptals should then be due to the inductive effect of two sulphur atoms on carbon-1.

In addition to the above observations on the methylation of sugar mercaptals, Lieser and Leckzyck (40) have reported the Purdie methylation of unsubstituted sugar mercaptals. They were able to methylate dimethyl and dibenzyl mercaptals of D-glucose to their 2-O-methyl derivatives, from which 2-O-methyl-D-glucose was obtained by acid hydrolysis. This formation of the 2-O-methyl derivative affords an easy route to 2-O-methyl-D-glucose, which is otherwise prepared more tediously, and has been used by other workers (41). Surprisingly, this procedure failed to methylate diethyl and dibenzyl mercaptals of D-galactose, L-arabinose, and L-rhamnose as well as the dibenzyl mercaptal of D-xylose.

Dutton and Yates (42) have re-investigated these results and confirmed that only the diethyl mercaptal of D-glucose was reactive toward Purdie's

reagents and that the mercaptals of other sugars did not yield methylated products. It seemed to them that there was no a priori reason why other mercaptals should not react like D-glucose mercaptal. Sugar mercaptals are, in general, insoluble in methyl iodide, but D-glucose mercaptal is appreciably soluble. Hence, the lack of reactivity of sugar mercaptals, other than D-glucose mercaptal, towards Purdie's reagents, might be attributed to their insolubilities in methyl iodide, and they assumed that, if methylation was carried out in a homogeneous system, all mercaptals should be methylated to some extent. They have re-examined the procedure of Lieser and Leckzyck on D-glucose mercaptal and have obtained 2-O-methyl-D-glucose mercaptal. They have also demonstrated that not only D-glucose mercaptal, but also other mercaptals were methylated when the methylation was carried out in tetrahydrofuran, in which sugar mercaptals are soluble, and the qualitative observations have been reported. The present study is to demonstrate some selectivity of Purdie's methylation on sugar mercaptals by analysing the ratio of mono-O-methyl ethers in the hydrolysate of partially methylated mercaptals.

Methylation techniques have long been employed in carbohydrate chemistry, particularly in the structural elucidation of polysaccharides; hence the syntheses of methylated monosaccharides, which result from the hydrolysis of fully methylated polysaccharides, occupy an important part of carbohydrate chemistry. Syntheses of partially methylated monosaccharides require preliminary protection of hydroxyl groups which are to be left free in the final product, methylation of unprotected hydroxyl groups, and subsequent removal of protecting groups, thus demanding, in many cases, several steps to synthesize partially methylated sugars. If, as has been demonstrated by Lieser and Leck-

'życk (40), and also by Wolfrom, Lemieux, Olin, and Weisbalt (41), in the methylation of D-glucose mercaptals, selective methylation of any hydroxyl group in sugar mercaptals be observed, partial methylation of sugar mercaptals can supply a convenient route to the synthesis of mono-O-methyl sugars.

The present work has thus two main objectives. The more important has been the study of the relative reactivities towards methylation of the hydroxyl groups in sugar mercaptals. Complementary to this has been the possibility that if any mercaptal showed strongly selective methylation this might provide a convenient route to certain mono-O-methyl sugars. For these reasons this study has been restricted to the more commonly occurring sugars: D-glucose, D-mannose, D-galactose, L-arabinose, and D-xylose. In the preliminary study on the methylation of D-glucose diethyl mercaptal (42), 2-O-methyl-D-glucose diethyl mercaptal crystallized from the methylation product, and no investigation was made on the mother liquor, which might have contained other isomers. The methylation of D-glucose diethyl mercaptal was therefore re-examined in this study.

PROCEDURE

(I) Preparation of Mercaptals.

Only diethyl mercaptals were employed in this study, because they were used in the preliminary investigations (42) and, of all the mercaptans, ethyl mercaptan has been the one most commonly employed in preparing sugar mercaptals. All these diethyl mercaptals were prepared by Fischer's method (1) which comprises the action of ethyl mercaptan on aldoses in the presence of concentrated hydrochloric acid.

It was possible to prepare D-mannose diethyl mercaptal by this method in the first experiment, but it was not possible to repeat the preparation. The reaction mixture of D-mannose with ethyl mercaptan and concentrated hydrochloric acid gave, on addition of ice, a colorless crystalline mass, which was immediately filtered, and the crystals decomposed on washing with ice-water. The reaction mixture was therefore partially neutralized with barium carbonate without addition of ice and, after further dilution with methanol, it was completely neutralized with excess barium carbonate. The mercaptal was then isolated from the methanol solution.

Wolfrom, Newlin and Stahly (43) have prepared crystalline D-xylose diethyl mercaptal by deacetylation of crystalline D-xylose diethyl mercaptal tetraacetate, which was obtained by acetylating the sirupy product of mercaptalation. Zinner, Rembarz, Linke, and Ulbricht (44) have recently reported the direct synthesis of crystalline D-xylose mercaptals from D-xylose by Fischer's method (1) by neutralization of the reaction mixture with ion-exchange resin.

D-Xylose diethyl mercaptal used in this study was first prepared by the

reaction of D-xylose and ethyl mercaptan in tetrahydrofuran containing 65% dry hydrogen chloride. The starting material gradually dissolved in the tetrahydrofuran on shaking. After all the starting material dissolved, the solution was neutralized with barium carbonate, and a sirupy product was obtained by evaporation of the neutral solution. The product failed to crystallize, but paper chromatographic examination showed only one spot (Rf 0.79 in butanone-water azeotrope), and the sirup was assumed to be pure enough to carry on the methylation study. After the methylation study was almost completed, the sirup suddenly crystallized from a concentrated solution in isopropyl alcohol by diluting with a small amount of petroleum ether. It was also prepared by Zinner's method (44), and the sirupy product crystallized on dilution with small amount of isopropyl alcohol and seeding with the crystals obtained by the former method.

(II) Methylation of Mercaptals.

Optimum conditions to give a reasonable amount of mono-O-methyl ethers were chosen for each mercaptal, and analyses of polymethylated and unchanged sugars were not performed. No attempt was made to compare the relative reactivity of the different mercaptals towards Purdie's reagents under identical conditions.

Methylation was performed in dry tetrahydrofuran at room temperature by shaking the solution of mercaptal with suitable amounts of silver oxide, methyl iodide, and anhydrous calcium sulphate (drierite). Due to the photosensitivity of methyl iodide the reaction vessel was covered with aluminum foil. Since D-xylose diethyl mercaptal is freely soluble in methyl iodide, methylation was first attempted in the absence of tetrahydrofuran, but only poly-

methylated D-xylose were detected by paper chromatography, and no appreciable reaction was observed when the methylation in methyl iodide (no tetrahydrofuran) was performed at 0ºC.

(III) Hydrolysis of the Methylated Mercaptals and Isolation of Mono-O-methyl Ethers.

Methylated mercaptals were hydrolyzed in a boiling mixture of alcohol and acid, and the deionized hydrolysates were examined by paper chromatography in butanone-water azeotrope.

It was concluded from examination of reported Rf values of methylated sugars in this solvent (45) that, due to the great similarity in structure, all pyranose-type methyl ethers of a sugar with the same number of O-methyl groups are hardly distinguishable in their Rf values, and that the furanose-type methyl ethers of the sugar travel much faster than the pyranose-type isomers, and as fast as the pyranose-type ethers with one more O-methyl group. It is therefore necessary to examine the components having a Rf value corresponding to both mono-O-methyl-pyranoses and di-O-methyl-pyranoses since the latter may contain mono-O-methyl-furanoses, i.e. 5-O-methyl derivatives.

Although recovery from paper chromatograms by extraction often gives a poor yield, paper chromatography was used to isolate the desired products, whenever the deionized hydrolysates were shown to contain non-migrating components (probably sulphur-containing impurities) which would contaminate the cellulose column. The deionized hydrolysates were chromatographed on Whatman No. 3MM sheets for several hours in butanone-water azeotrope, and the areas containing the mono-O-methyl sugars and the di-O-methyl sugars were located by cutting guide strips from both edges of the sheet and spraying with p-anisi-

dine-trichloroacetic acid reagent. Longer periods of chromatography in the same solvent tended to result in less well defined boundaries making it more difficult to detect minor components. The mono-O-methyl sugars thus located were extracted with methanol (mono-O-methyl-L-arabinoses) or cold water (mono-O-methyl derivatives of other sugars). After three extractions, the extract gave only a faint Molisch test. Evaporation of the extracts gave a sirupy mixture of mono-O-methyl sugars.

Column chromatography on a cellulose-hydrocellulose column in butanone-water azeotrope was also used occasionally, when the non-migrating spots were not detected on paper chromatograms. The desired components were found by examining the fractions by paper chromatography.

(IV) Examination of the Mixture of Mono-O-methyl Sugars.

The mixtures thus isolated were examined by electrophoresis in 0.05 M sodium borate buffer, or by paper chromatography using different solvent systems. In all cases authentic samples were run concurrently and major components were further identified by the preparation of crystalline derivatives (see Section (VII)). The qualitative results are described below and the quantitative results are given under Section (VI).

(1) Mono-O-methyl-D-glucoses.

Electrophoretical examination of the mixture showed two spots, Mg 0.29 (strong) and Mg 0.86 (medium), which corresponded to 2-O-methyl- and 3- or 6-O-methyl-D-glucoses, respectively. Since both 3- and 6-O-methyl-D-glucoses are known to have the same Mg value, 0.80 (46), the medium spot of Mg 0.86 may contain these two isomers. Lindberg and Swan (47) have recently reported their electrophoretical study of some carbohydrates in 0.05 M germ-

anate buffer (pH 10.7), in which all mono-O-methyl-D-glucoses have been separated. The germanate buffer was prepared according to their procedure and the electrophoresis was carried out on our apparatus, but no clear separation was observed probably due to the evolution of heat. Lenz and Holmberg (48) have successfully separated 2-, 3-, and 6-O-methyl-D-glucoses by paper chromatography in 2,4,6-collidine-ethyl acetate-water (2:5:5 by volume, upper layer). This solvent system was therefore tried on the mixture of mono-O-methyl-D-glucoses, and it was shown that the mixture contained 2-, 3-, and 6-O-methyl-D-glucoses, of which the 2-O-methyl-D-glucose was the major component.

(2) Mono-O-methyl-D-mannoses.

Paper chromatography in butanone-water azeotrope, both with and without borax (*vide infra*), and in collidine-ethyl acetate-water did not seem to be fruitful.

Electrophoresis showed that the mixture contained three isomers, one of which was predominant judging from the size and intensity of the spots. These three components had Mg value of 0.38, 0.54, and 0.63, and were shown to be 2-, 3-, and 6-O-methyl-D-mannoses, respectively, by concurrent electrophoretical examination with synthetic samples of these isomers. 2-O-methyl-D-mannose gave the strongest and largest spot, and the others weak and small.

(3) Mono-O-methyl-D-galactoses.

When paper chromatography of the mixture in butanone-water azeotrope was carried out for a long period the original compact spot of Rf 0.08 was found to become longer and longer, indicating considerable differences in the mobilities of mono-O-methyl-D-galactoses in comparison with mono-O-methyl ethers of other sugars. It was hoped that it might be possible to separate the mixture taking

advantage of this fact. It is known that many polyhydroxyl compounds form ionizable complexes with alkali borates or boric acid (49, 50), and that the reaction is governed by stereochemical factors (49). A number of mixtures have been separated by paper electrophoresis in borate buffer (51). Furthermore, it has been shown that in the presence of borate or boric acid, various compounds which may be presumed to undergo complex formation, travel more slowly on paper chromatograms if boric acid is added to the solvent system (52, 53, 54), and that chromatography on paper impregnated with boric acid facilitates the separation of certain mixtures of sugars which are otherwise not resolved (55).

If complex formation is achieved in butanone-water azeotrope in the presence of sodium borate, separation of the mixture of mono-O-methyl-D-galactoses is likely possible. Paper chromatography of standard samples of all pyranose-type mono-O-methyl-D-galactoses in butanone-water azeotrope saturated with sodium borate decahydrate (borax) was found to be suitable to distinguish these isomers. $R_{galactose}$ values of these isomers were: 3.60 (2-O-methyl-), 2.67 (3-O-methyl-), 2.28 (4-O-methyl-), and 3.02 (6-O-methyl-D-galactose) (60 hours). The mixture of mono-O-methyl-D-galactoses was then proved to consist of 2-, 3-, and 6-O-methyl-D-galactoses by the same procedure. These isomers of mono-O-methyl-D-galactoses were also isolated directly from the hydrolysate of the methylated mercaptals by this method. The solvent system, 2,4,6-collidine-ethyl acetate-water, was found to be unsuccessful, due to streaking.

Electrophoretical examination of the mixture confirmed that 2-, 3-, and 6-O-methyl-D-galactoses were included in the mixture, Mg values of these isomers being 0.45, 0.81, and 0.90, respectively.

(4) Mono-O-methyl-L-arabinoses.

The extract of the mono-O-methyl ethers showed only one spot (Mg 0.33) by electrophoresis. Of three possible mono-O-methyl-L-arabinoses, the 2- and 3-isomers are known, and the spot of Mg 0.33 was assumed to be the 2-O-methyl derivative, since examination of reported Mg values of D-glucose derivatives (51) showed that absence of a free hydroxyl group on carbon-2 tends to lower the Mg value. Electrophoresis of the extract with synthetic 2- and 3-O-methyl-L-arabinoses showed that the spot of Mg 0.33 corresponded to that of 2-O-methyl-L-arabinose.

(5) Mono-O-methyl-D-xyloses.

The mixture of mono-O-methyl-D-xyloses was first examined electrophoretically with a mixture of 2- and 3-O-methyl-D-xyloses isolated from a methylated hemicellulose, and was shown to be a mixture of these two compounds. The mixture was also examined chromatographically in butanone-water azeotrope, which clearly distinguished 2- and 3-O-methyl isomers in the mixture, particularly when one drop of concentrated ammonia was added to the solvent, and development continued for at least 20 hours. The 2-O-methyl isomer showed a stronger spot than that of 3-O-methyl isomer.

(V) Examination of Fast-Running Spots.

As stated under Section (III), the spots whose Rf values correspond to those of pyranose-type di-O-methyl ethers may contain the furanose-type mono-O-methyl ether, i. e. 5-O-methyl derivative, and therefore a group of fast-running components (those running next faster than the mono-O-methyl ethers) was also isolated from the paper chromatograms or by column chromatography and was examined by electrophoresis. With all these components of the hexo-

ses, strong spots of non-migrating or very slowly (slower than the 2-O-methyl ether) migrating components were detected. It is true, some mono-O-methyl ethers may migrate slower than the 2-O-methyl isomer, as in the case of 4-O-methyl-D-galactose, Mg 0.30 (cf. Mg 0.43 of 2-O-methyl-D-galactose), but it was apparent that those spots did not contain any mono-O-methyl-pyranoses, nor mono-O-methyl-furanoses, because the former should not be contained in the fast-running components of chromatography and the latter is expected to have a high Mg value (see the Discussion).

In the case of L-arabinose, a weak spot of Mg 0.80 and a strong spot of Mg 0.00 were detected. The spot of Mg 0.80 in the mixture of di-O-methylated L-arabinoses was shown electrophoretically to be 5-O-methyl-L-arabinose by comparison with synthetic 5-O-methyl-L-arabinose.

Examination of the fast-running components isolated from the mixture of O-methylated D-xyloses showed three spots, Mg 1.00 (weak), Mg 0.37 (very weak), and Mg 0.00 (medium), and the fast-migrating spot (Mg 1.00) was proved to be 5-O-methyl-D-xylose by comparison with a synthetic sample of this compound.

Some poly-O-methylated sugars have high Mg values; for example, 3,4-di-O-methyl-D-glucose (Mg 0.31) migrates faster than 2-O-methyl-D-glucose (Mg 0.23), and 3,5,6-tri-O-methyl-D-glucose has an Mg value of 0.71 (51). It is therefore natural to expect some spots with high Mg values in the mixture of di-O-methyl sugars. The fact that only non-migrating or very slowly migrating spots were detected is, however, understandable because the 2-hydroxyl group is reactive in every case, and therefore, in all di-O-methyl derivatives, this position is presumably substituted, preventing the complex form-

ation between the 1,2-hydroxyl grouping and the borate, which would have an important role in giving a high Mg value. Consequently, the possibility of the fast-migrating spots detected in the mixtures of di-O-methylated L-arabinoses and of di-O-methylated D-xyloses being a di-O-methyl ether migrating as fast as the 5-O-methyl ether is very small.

(VI) Estimation of the Ratio of Mono-O-methyl Isomers.

The phenol-sulphuric acid method, established by Dubois, Gilles, Hamilton, Rebers, and Smith (56), was used to estimate the ratio of mono-O-methyl ethers. It was assumed that all mono-O-methyl ethers of any particular sugar give almost superimposable standard curves, and therefore only readily available isomers were used to make the standard curves.

By paper chromatography, mono-O-methyl-D-glucoses were separated in 2,4,-6-collidine-ethyl acetate-water, mono-O-methyl-D-galactoses in butanone-water azeotrope saturated with borax, and mono-O-methyl-D-xyloses in butanone-water azeotrope plus one drop of concentrated ammonia.

By electrophoresis, mono-O-methyl-D-mannoses, mono-O-methyl-D-xyloses, a mixture of 5-O-methyl-and di-O-methyl-L-arabinoses, and a mixture of 5-O-methyl- and di-O-methyl-D-xyloses were separated; in the last two cases the di-O-methyl ethers were assumed to give almost superimposable standard curves with that of a mono-O-methyl ether of each sugar and the ratio of the 5-O-methyl ether to the other mono-O-methyl ethers was calculated by the combination of the colorimetric results and the weights of mono- and di-O-methyl ethers isolated**. It is known that the borate ion interferes color development in the

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**Colorimetric analysis was performed on the mixtures of mono-O-methyl-D-xyloses isolated from the methylation products of both sirupy and crystalline D-xylose mercaptals.

phenol-sulphuric acid method (57); and the extracts of each components and their blanks were evaporated to dryness and the residues were refluxed for 30 minutes in 0.1N methanolic hydrogen chloride in order to destroy the borate complexes.

The compounds used as standard were: 3-O-methyl-D-glucose, 2-O-methyl-D-mannose, 6-O-methyl-D-galactose, 5-O-methyl-L-arabinose, and 2-O-methyl-D-xylose. The approximate ratios are shown in Table I.

Using gas-liquid partition chromatography, Gunner, Jones and Perry (58) have recently established an elegant method of quantitative analysis of glycose mixtures, which were converted to the mixture of their acetylated glycitols. They have stated that the results are at least as accurate as those obtained by the analysis of glycose mixtures using paper chromatographic or colorimetric procedures and this new method has been shown to require less time and material than the older methods and in addition it provides a very sensitive method for the detection and estimation of minor components in glycose mixtures. They have not described the analysis of mixture of partially methylated glycoses, but this method apparently seems to be applicable equally to the mixtures of partially methylated glycoses. This method was, however, not used in the present study, simply because some mono-O-methyl-glycoses, e.g. 2- and 5-O-methyl-D-mannoses, give an identical product by reduction to their glycitols.

(VII) Identification of Major Components as their Crystalline Derivatives.

Only major components of mono-O-methyl derivatives were identified as crystalline derivatives, and only in a few cases was identification possible as the free sugar. No attempt was made here to identify 2-O-methyl-D-glucose,

since it has been identified in the preliminary work (42).

(1) 2-O-Methyl-D-mannose.

The mixture of mono-O-methyl-D-mannoses was separated by electrophoresis, and the appropriate area extracted with water. The extract was evaporated to a sirup, which was then treated with methanol many times (59). The residue was condensed with phenylhydrazine in warm ethanol to give 2-O-methyl-D-mannose phenylhydrazone, m.p 161 - 163°C (60).

(2) 2-O-Methyl-D-galactose.

This was isolated from the mixture by paper chromatography in butanone-water azeotrope saturated with borax. Although the extract gave negative test on tumeric test paper, indicating the absence of borate ion, the extract was evaporated to dryness and then treated with methanol to destroy possible traces of borate complex. The treated residue was crystallized from ethanol-ether, m.p. 152 - 154°C (61).

(3) 3-O-Methyl-D-galactose.

Extraction from the same chromatograms provided an electrophoretically pure sirup which failed to crystallize. It was converted into 3-O-methyl-D-galactose phenylosazone, m.p. 177 - 179°C, and identified by comparison with the same compound derived from 2,3-di-O-methyl-D-galactose (62).

(4) 6-O-Methyl-D-galactose.

The electrophoretically pure sirup isolated from the same chromatograms did not crystallize. It was identified as its phenylhydrazone, m.p. 171 - 173°C (63).

(5) 2-O-Methyl-L-arabinose

The sirup was oxidized by bromine in water and the resulting 2-O-methyl-

L-arabonolactone was converted into 2-O-methyl-L-arabonomide, m.p. 131 - 132°C (64), which gave a negative Weerman test.

(6) 2-O-Methyl-D-xylose.

The mixture of mono-O-methyl-D-xylose was dissolved in absolute ethanol and addition of petroleum ether gave a cloudy solution, from which 2-O-methyl-D-xylose, m.p. 133°C (65), crystallized on seeding.

(7) 3-O-Methyl-D-xylose.

The mixture from which 2-O-methyl-D-xylose was crystallized still showed a strong spot of 2-O-methyl-D-xylose by paper chromatography. The mixture was chromatographed in butanone-water azeotrope with one drop of concentrated ammonia added and the 3-O-methyl-D-xylose was extracted with water. The resulting sirup was converted into 3-O-methyl-D-xylose phenylosazone, m.p. 171 - 172°C (66).

(VIII) Synthesis of Standard Samples.

2-, 3-, and 6-O-methyl-D-glucoses, 6-O-methyl-D-galactose, and 2-O-methyl-D-xylose were available in our laboratory. 2-, 3-, and 4-O-methyl-D-galactoses were obtained from Dr. B. Lindberg, Stockholm, Sweden. Syntheses of other standard compounds needed in this study are described briefly here.

(1) 2-O-Methyl-D-mannose.

This compound was synthesized by the method of Curtis and Jones, except that they used the dimethyl mercaptal instead of diethyl, (67), following the reaction scheme shown in Fig. 7.

It was also prepared by the method of Aspinall and Zweifel (12). This method is the first application, in carbohydrate chemistry, of the well-established fact that in a cyclohexane system equatorial hydroxyl groups are more

readily esterified than are axial hydroxyl groups. In methyl 4,6-O-ethylidene-α-D-mannopyranoside (XIV) (Fig. 8) there is little doubt that the pyranoside ring is held in the C1 conformation, disposing the 2-hydroxyl group axial, and 3-hydroxyl group equatorial. The equatorial hydroxyl group is selectively tosylated to give XV, from which 2-O-methyl-D-mannose is obtained by a series of reactions.

The synthesis according to their procedure showed, however, that the product was accompanied with small amount of the 3-O-methyl ether.

(2) 3-O-Methyl-D-mannose.

Starting with XIV, Aspinall and Zweifel (12) have also been able to synthesize 3-O-methyl-D-mannose, according to the scheme shown in Fig. 9.

Following their procedure, XIV was partially O-nitrated in acetic anhydride, and the infrared spectrum of the product showed bands of O-acetate at 5.76 μ (C=O stretching) and at 8.13 μ (C-O stretching), indicating partial acetylation during the nitration. It was assumed, however, that the attack by nitrate is much faster than that by acetate, and therefore the O-nitration had taken place almost exclusively on the 3-hydroxyl group. Bell and Synge (68) have successfully deacetylated XVI (Fig. 10) in a mixture of methanol and chloroform with a trace of sodium. Application of this method on the partially acetylated product gave a sirup which showed no band of O-acetate in its infrared spectrum. Completion of Aspinall and Zweifel's process with this deacetylated product showed, however, that the final product was accompanied with a small amount of 2-O-methyl-D-mannose.

(3) 6-O-Methyl-D-mannose.

This has been synthesized by Waters, Hockett, and Hudson (69) by a series

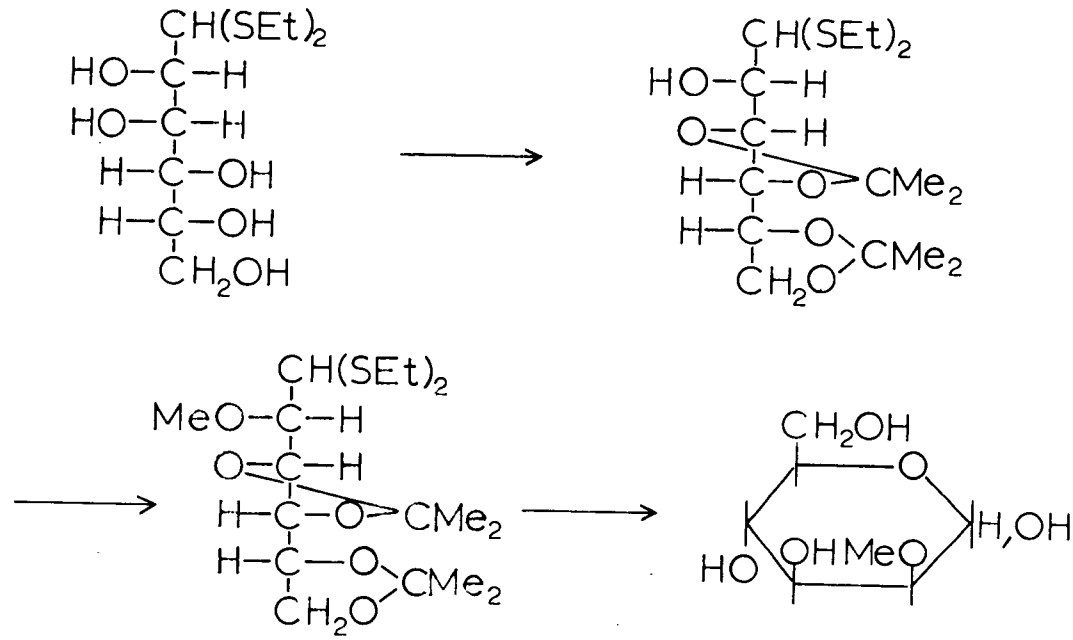

Fig. 7. Synthesis of 2-O-methyl-D-mannose.

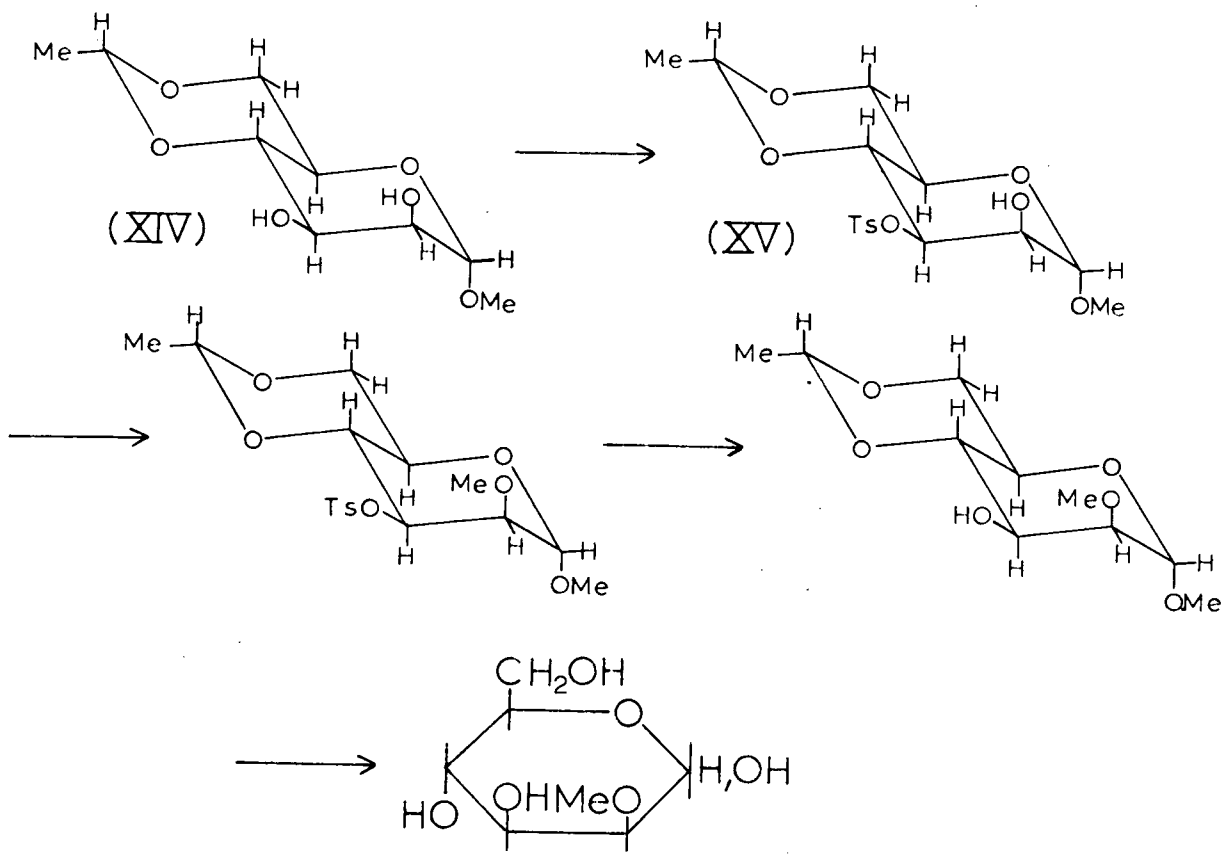

Fig. 8. Synthesis of 2-O-methyl-D-mannose.

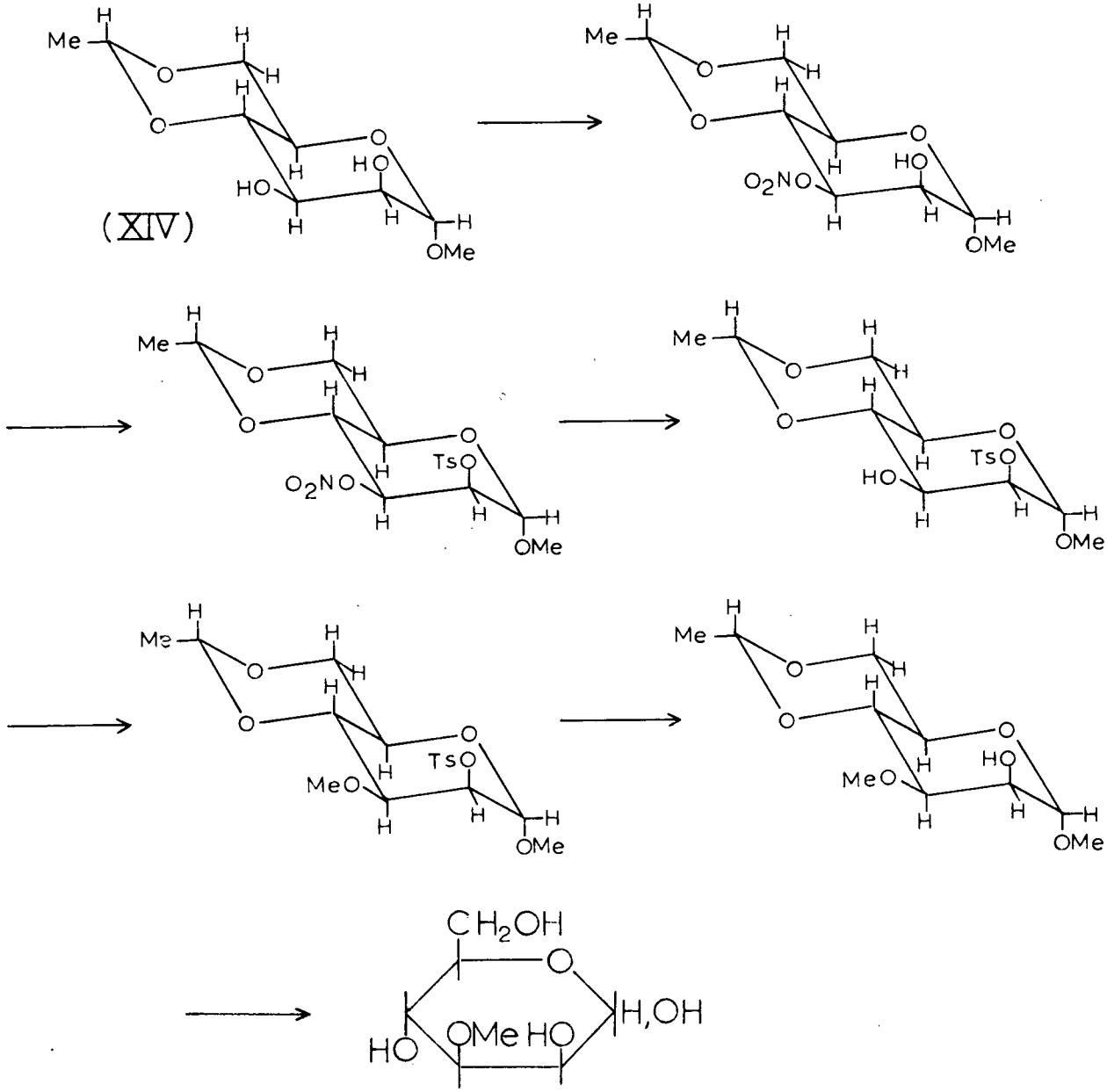

Fig. 9. Synthesis of 3-O-methyl-D-mannose.

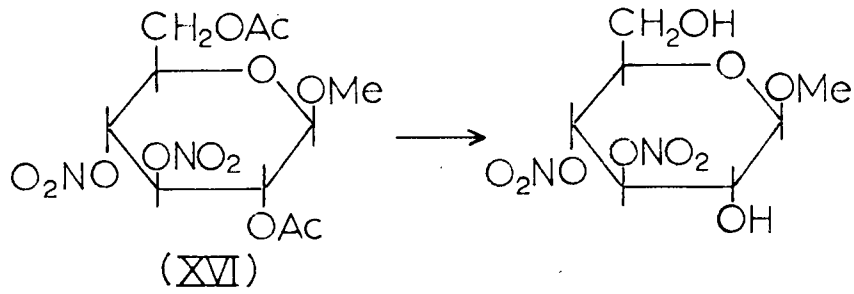

Fig. 10. Deacetylation of O-nitrated sugar.

of reactions shown in Fig. 11. Their procedure was followed, but the product was shown to be a mixture of mono-O-methyl-D-mannoses by electrophoresis, presumably due to acetyl migration during methylation. A very low yield of electrophoretically pure 6-O-methyl-D-mannose was obtained by column chromatography in butanone-water azeotrope, and the pure sirup was converted to its phenylosazone, m.p. 171°C.

(4) 2,3-Di-O-methyl-D-galactose.

This compound was synthesized by the method of Robertson and Lamb (62) (Fig. 12) and was converted into 3-O-methyl-D-galactose phenylosazone, m.p. 177 - 179°C.

(5) 2-O-Methyl-L-arabinose.

The method of Jones, Kent, and Stacey (64) (Fig. 13) was used to prepare this compound. The 2-O-methyl-L-arabonolactone, hitherto known as a sirup, was crystallized for the first time, m.p. 88°C.

(6) 5-O-Methyl-L-arabinose.

At the beginning of this study, it was presumed that the primary hydroxyl group of sugar mercaptals should be methylated to some extent. This compound was unknown at this time, although its possible presence in the hydrolysis products of methylated wheat bran hemicellulose had been reported by Adams (70). It was therefore synthesized (20) according to the scheme shown in Fig. 14, and the sirupy product was characterized as its phenylosazone, m.p. 154.5°C, and the lactone, m.p. 135°C. The structure of this compound was confirmed by periodate oxidation, in which 3 moles of periodate were consumed yielding 3 moles of formic acid.

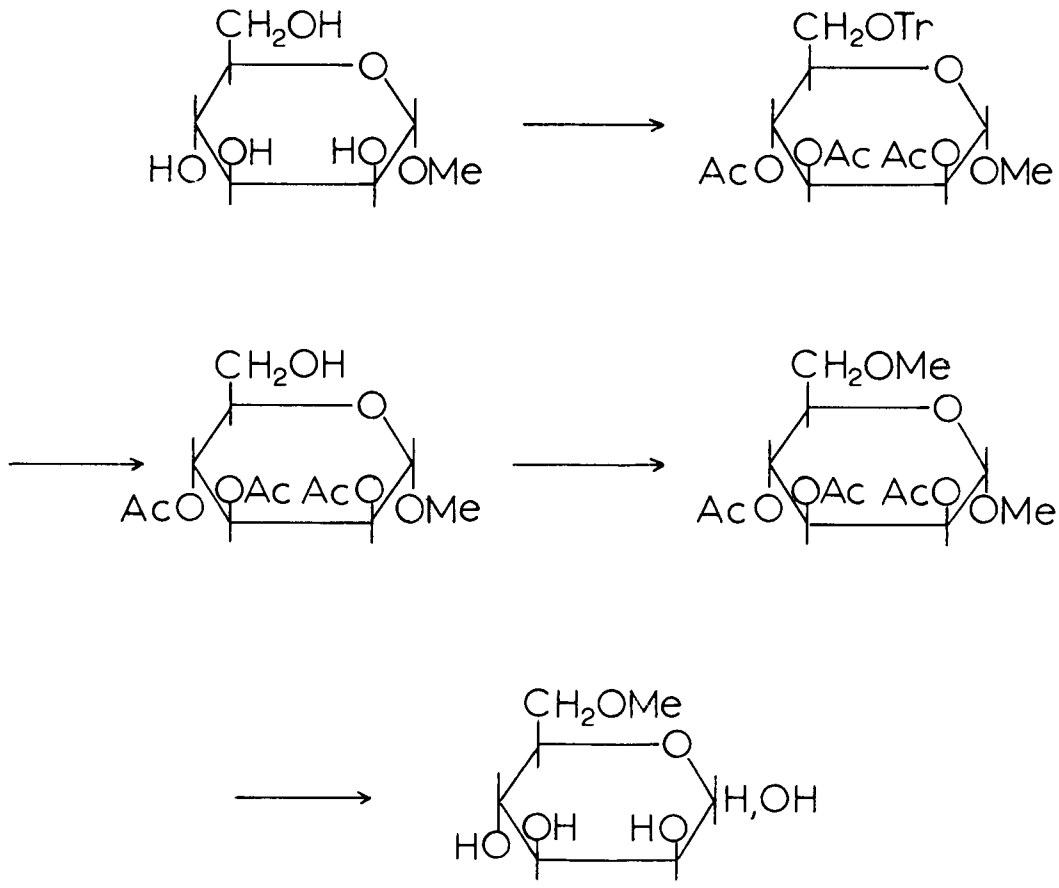

Fig. 11. Synthesis of 6-O-methyl-D-mannose.

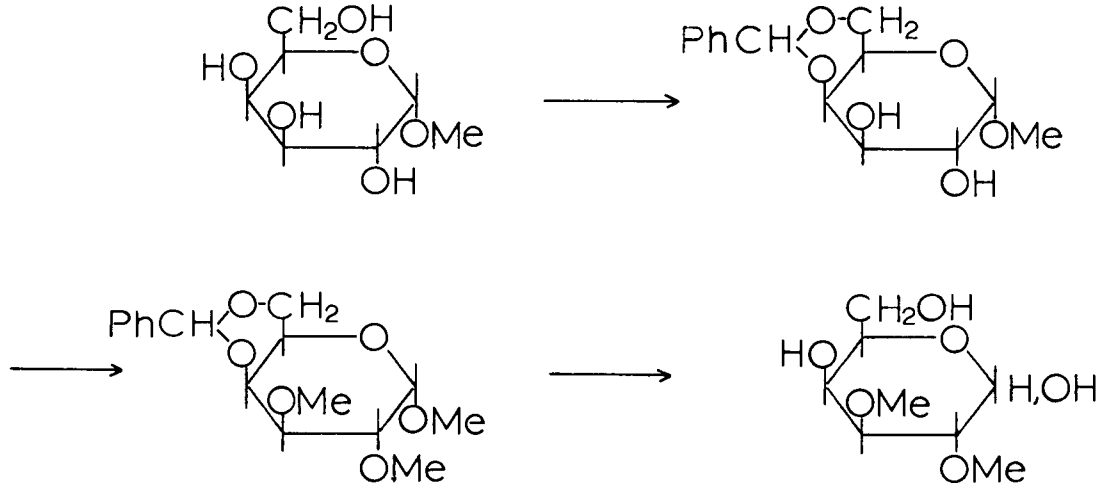

Fig. 12. Synthesis of 2,3-di-O-methyl-D-galactose.

Siddiqui, Adams and Bishop (71) have recently reported the synthesis of this compound by a similar process, in which benzyl, instead of acetyl, was used as the blocking group for the 2- and 3-hydroxyl groups. They claim that the possible acetyl migration which has not been observed in our synthesis may lead to an impure product. However, examination of molecular models shows that acetyl migration is very unlikely because of the highly twisted ortho-ester intermediate.

(7) 3-O-Methyl-D-xylose.

This compound was synthesized by the method of Levene and Raymond (72) (Fig. 15). The trityl group was used to block the 5-hydroxyl group, because, when crystalline 1,2-O-isopropylidene-5-O-benzoyl-D-xylose was methylated by the Purdie reagents according to the procedure of Levene and Raymond, a strong odour of methyl benzoate was experienced from the extracts of the methylation mixture and paper chromatography of the hydrolysis product gave several strong spots. The tritylated product of crude 1,2-O-isopropylidene-D-xylose gave, after methylation and hydrolysis, a sirup which showed only weak spots of poly-O-methylated D-xyloses by paper chromatography.

(8) 5-O-Methyl-D-xylose.

The method of Levene and Raymond (72) (Fig. 16) provided this compound.

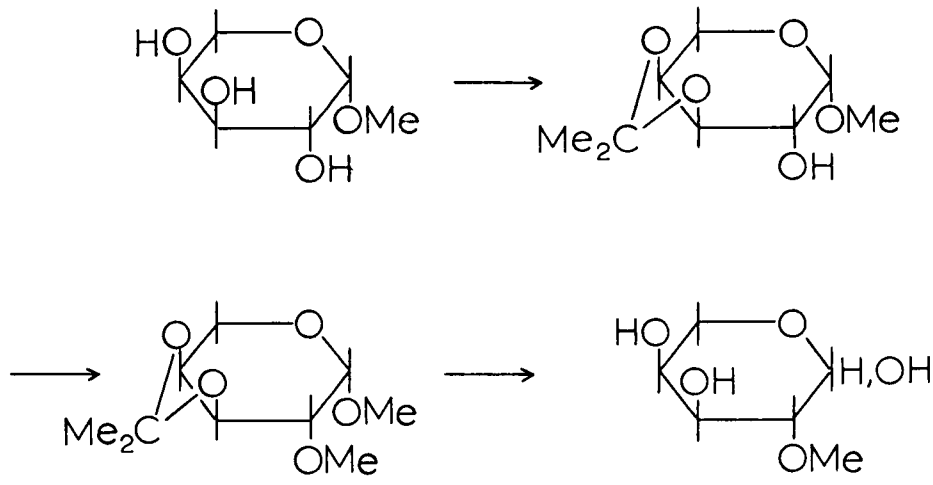

Fig. 13. Synthesis of 2-O-methyl-L-arabinose.

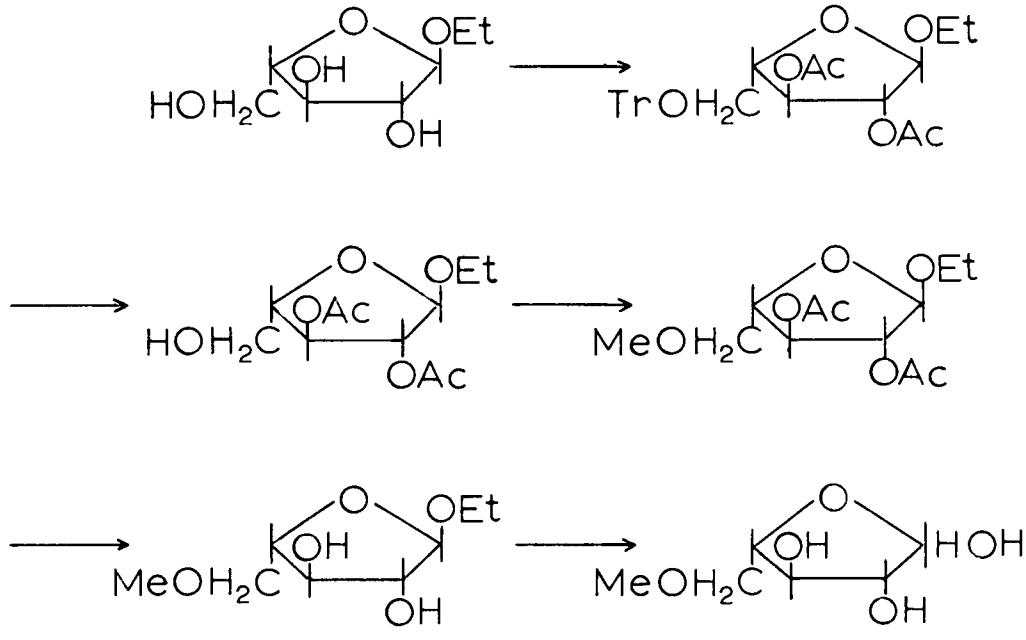

Fig. 14. Synthesis of 5-O-methyl-L-arabinose.

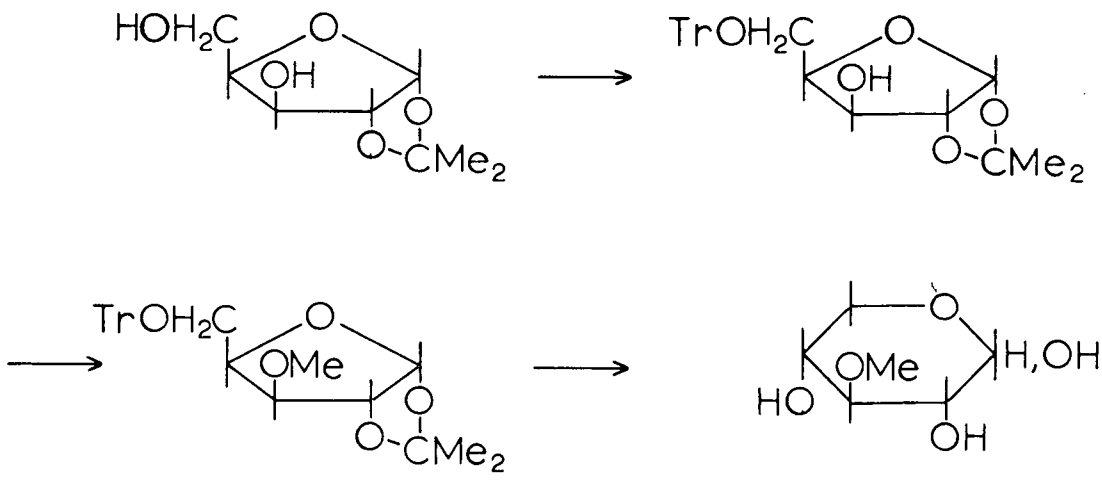

Fig. 15. Synthesis of 3-O-methyl-D-xylose.

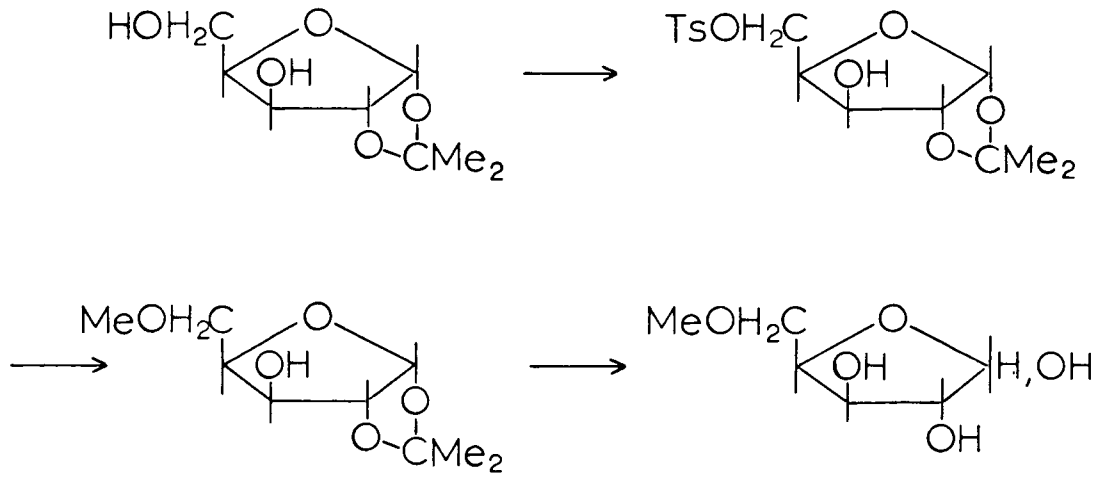

Fig. 16. Synthesis of 5-O-methyl-D-xylose.

RESULTS AND DISCUSSION

The mono-O-methyl ethers detected in the methylation products are listed in Table I, with their approximate ratios in parentheses.

TABLE I.

Mono-O-methyl ethers detected in the methylated mercaptals and their ratios.

Diethyl mercaptal of-	Mono-O-methyl ethers detected-		
D-glucose	2 (6)	3 (1)	6 (1)
D-mannose	2 (12)	3 (1)	6 (1)
D-galactose	2 (1)	3 (1)	6 (1)
L-arabinose	2 (30)		5 (1)
D-xylose	2 (16)	3 (10)	5 (1)

From Table I, it is seen that there is a marked tendency to yield 2-O-methyl ethers by methylation of mercaptals, and that 3- and primary hydroxyl groups are also reactive, but to lesser extent, with the exception of D-galactose diethyl mercaptal, where approximately the same amount of 2-, 3-, and 6-O-methyl ethers were found.

The structures of sugar mercaptals are somewhat similar to those of polyols, the reduction products of free sugars, in the sense that both mercaptals and ployols have acyclic structures. In their study of the configuration of carbohydrate compounds, Macpherson and Percival (73) have observed that D-glucose diethyl mercaptal, as well as straight-chain polyhydroxyl com-

pounds such as dulcitol and sorbitol, showed a strongly positive effect on the conductivity of boric acid solution.

As one of the typical and important reactions of polyhydroxyl compounds the formation of acetals and ketals from carbonyl compounds and sugar alcohols has been extensively explored, and an accumulation of data has led to empirical rules concerning these reactions. In order to simplify the discussion, a code system used by Barker and Bourne (74) will be employed, whereby the position and size of an acetal ring can be indicated precisely. The Greek letters, α, β, and γ, signify the relative positions, along the carbon chain of the polyhydric alcohols, of the two hydroxyl groups engaged in the cyclisation, and C and T indicate whether these two hydroxyl groups are disposed cis or trans in the usual Fischer projection formula; C and T are required only when both hydroxyl groups are secondary. Thus a system in which an alkylidene residue spans two secondary hydroxyl groups, located on adjacent carbon atoms and having a trans-orientation, contains an αT-ring, whereas β-ring is present when one of the hydroxyl groups concerned is primary and the other is situated on the β-carbon atom.

A study of the methylene acetals of proven structure derived from dulcitol, mannitol, sorbitol, and ribitol enabled Hann and Hudson (75) to reach certain conclusions, which, when expressed in terms of the Barker-Bourne code, were as follows: (a) a βC-ring is favoured, but a βT-ring is not; (b) a β-ring is favoured; (c) a βC-ring is preferred to a β-ring; (d) a γT-ring is favoured, but a γC-ring is not; (e) an α-ring may be formed when a β-ring is not permissible. Later, Ness, Hann and Hudson (76) added a rider to the eff-

ect that a γT-ring (previously believed, from considerations of the behaviour of methylene acetals during acetolysis, to be highly favoured) may not be formed when β- and βC-rings are permissible. They observed, too, that these rules were compatible with the data then available for benzylidene acetals of the polyhydric alcohols. The Hann-Hudson rules have long been recognized as being an outstanding contribution in this field, and have been of great value in the interpretation of the problems of acetal formation and in the prediction of the structures of acetals of the polyhydric alcohols.

In the light of all the relevant information concerning the acetals of the tetritols and their higher homologues which has been garnered since the introduction of the Hann-Hudson rules, Barker and Bourne (74) have extended them to cover virtually all known cases of the formation of benzylidene, ethylidene, and methylene derivatives of the polyhydric alcohols. The extended rules are: (1) the first preference is for a βC-ring; (2) the second for a β-ring; (3) the third for an α-, αT-, βT-, or γT-ring; (4) in methylenation, a βT-ring takes precedence over an αT- or a γT-ring; (5) in benzylidenation and ethylidenation, an αT-ring takes precedence over a βT- or a γT-ring; (6) rules (4) and (5) may not apply when one (or both) of the carbon atoms carrying the hydroxyl groups concerned is already part of a ring system. Rule (5) is to be regarded as tentative because of the small number of examples upon which it can be based. It is well known that the condensation of an aldehyde with a polyhydric alcohol does not afford a γ-, αC-, or γC-ring. It should be noted that the participation of two hydroxyl groups, possessing a trans-orientation, in acetal formation is made possible by free rotation within the

carbon skeleton of the polyhydric alcohol, a situation which is not encountered when the carbon atoms carrying these hydroxyl groups are already part of a ring system; it is for this reason that it is necessary to include rule (6).

These rules – at first sight rather arbitrary – might be expected to have an underlying, unifying principle, and Barker, Bourne, and Whiffen (77) have shown that the marked tendency for a carbon chain to adopt the planar zig-zag form (78), which enables the largest groups attached to adjacent carbon atoms to become trans to one another, is sufficient to explain the main features of the rules, since it appears that the most favoured rings involve the least energy for distortion of the planar chain.

If it is accepted that the most stable arrangement in a polyhydric alcohol is that in which the carbon chain assumes a planar zig-zag form, then D-glucitol and D-mannitol are represented by the structures (XVII) and (XVIII), respectively (Fig. 17), viewed perpendicularly to the plane through the carbon chain with bold and dotted lines to indicate the substituents on the carbon atoms in conventional manner; for comparison, the Fischer projection formulae for these hexitols are also given in Fig. 17. From the comparison of these two types of structures, it is seen that the adjacent cis-hydroxyl groups in the Fischer projection formulae (e.g., 4- and 5-hydroxyl groups of D-glucitol, and 2- and 3-hydroxyl groups of D-mannitol) are threo to each other.*

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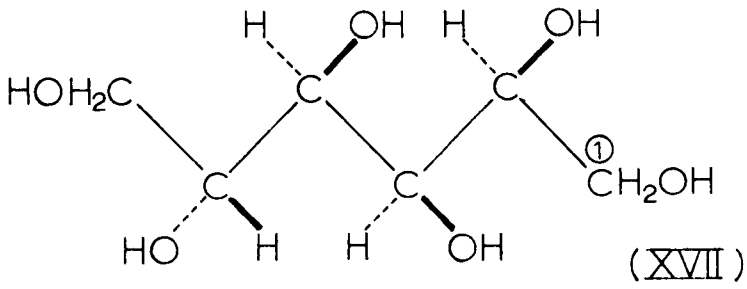
* In the present discussion cis- and trans-configurations refer to the Fischer projection formulae or the Haworth structures and erythro- and threo- to the zig-zag conformations.

The zig-zag form of the carbon chain as the most favoured conformation for polyhydric alcohols has been supported by the results reported by Schwarz (79), who, working with hexitols, has shown that preferential attack by periodate occurs at the 2,3- and 3,4-positions of D-glucitol (XVII), 3,4-position of D-mannitol (XVIII), and 2,3- and 4,5-positions of D-galactitol (XIX) (Fig. 17), all these pairs of hydroxyl groups being trans-oriented in the Fischer projection formulae, but erythro- in the zig-zag structures. These structures are further supported by the results reported by Courtois and Guernet (80). By assuming the same zig-zag carbon chain, Bragg and Hough (81) have explained the slower reduction of 3-O-substituted aldoses and 4-O-substituted hexuloses with potassium borohydride, in terms of steric hindrance of these substituents toward approach of the reagent to carbon-1 of aldoses and to carbon-2 of hexuloses. Foster (46), too, emphasizing the importance of the open-chain aldehydo-form as a contributor to the equilibria of borate complex formation in electrophoresis, has explained the mobilities of various free sugars, for which he assumed the zig-zag structure as the principal form in alkaline medium.

'These results,' Ferrier and Overend (11) have stated, 'do not prove that the zig-zag conformations are adopted in solution. The phenomenon may be due to differences in stability of the cyclic intermediates derived from the threo- and erythro-systems.' But it is known (77) that the formation of a cyclic acetal is a two-stage process, involving first the formation of a semiacetal and then cyclisation, with accompanying dehydration, and therefore no cyclic intermediate is involved in the formation of an acetal.

The conformation of sugar mercaptals is not known, but it seems to be reasonable to assume the zig-zag carbon chain as the most favourable conformation. In connection with this assumption it has already been noted that Macpherson and Percival (73) have observed parallel results between mercaptals and polyhydric alcohols in the conductivity of boric acid solutions, and further Littleton (82) has shown by X-ray analysis a nearly planar six-membered zig-zag carbon chain for the gluconate ion (Fig. 18) in a crystalline state. If this assumption is accepted, then the rules proposed for the formation of cyclic acetals of polyhydric alcohols should also be able to explain the same reaction of sugar mercaptals.

Cyclic acetals of sugar mercaptals are known only in a limited number of cases, because of the instability of the mercaptal group towards acidic catalysts which are required in acetal formation. Huebner, Pankratz, and Link (83) have obtained a di-O-benzylidene-L-arabinose diethyl mercaptal by the action of dry hydrogen chloride on the mercaptal in benzaldehyde. Since a β-ring is permissible in L-arabinose diethyl mercaptal (XX), the structure (XXI) (Fig. 19) is predicted from the Barker-Bourne rules, accomodating the first benzylidene group on the 3,5-hydroxyl groups and the second on the 2,4-hydroxyl groups to form a less favourable βT-ring. However, they proposed the structure (XXII) (Fig. 19) for their di-O-benzylidene derivative on the basis of lead tetraacetate oxidation, in glacial acetic acid, of the partially hydrolyzed mono-O-benzylidene-L-arabinose diethyl mercaptal which consumed two moles of the oxidant very rapidly and an additional two moles very slowly. They interpreted these observations to mean



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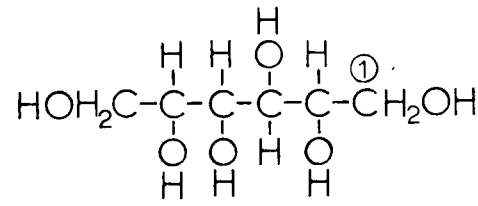

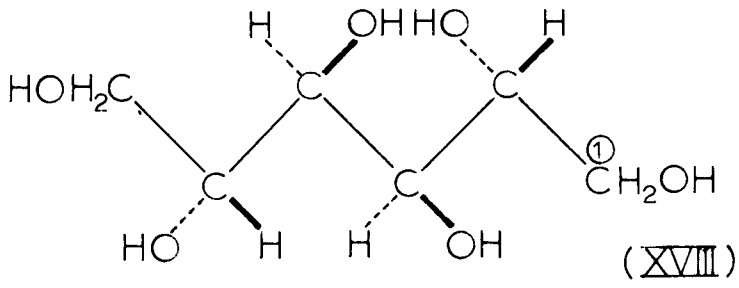

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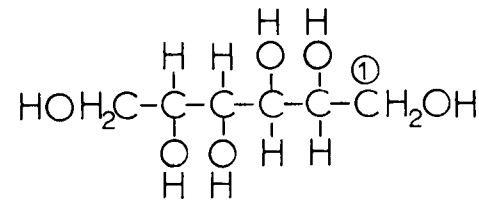

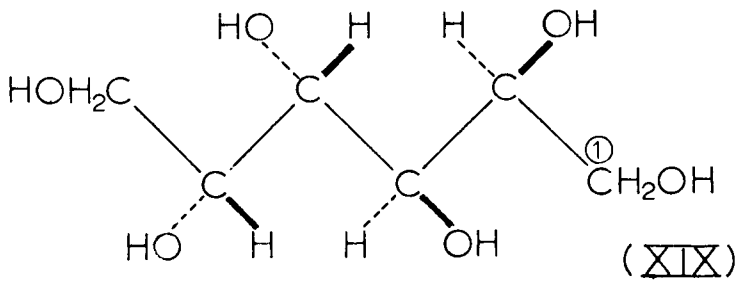

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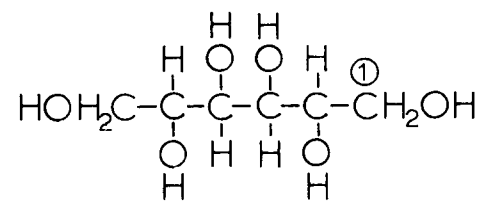


Fig. 17. Zig-zag structure of some glycitols.

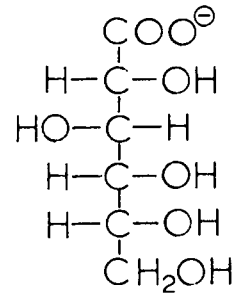


Fig. 18. Gluconate ion.

that the vicinal hydroxyl groups on carbons-4 and -5 consumed one mole of the oxidant rapidly, one of the sulphur atoms one mole rapidly, the formaldehyde formed from the carbon-5 one mole slowly, and the second sulphur atom one mole slowly. Since their di-O-benzylidene derivative and D-galactose diethyl mercaptal tetraacetate consumed one mole of the oxidant very rapidly and an additional one mole very slowly, they suggested a fast oxidation of a sulphur atom, followed by slow oxidation of the second sulphur atom. They have also isolated formaldehyde, as the dimedon condensation product, using a smaller amount of oxidant to prevent over-oxidation of formaldehyde. They have assigned the structure (XXIII) (Fig. 19) to the mono-O-benzylidene derivative from these observations. Zinner and Wittenburg (84) have observed a partial hydrolysis of the O-benzylidene group during lead tetraacetate oxidation in benzene. Since partial hydrolysis of O-benzylidene groups is more likely in acidic medium, the observations by Huebner, Pankratz, and Link might be the results of complex reactions involving partial hydrolysis of O-benzylidene group. The definite formulation of their mono- and di-O-benzylidene-L-arabinose diethyl mercaptals must therefore await more conclusive observations concerning their structures.

Zinner, Rembarz, Linke, and Ulbricht (44) were the first to prepare mono- and di-O-benzylidene-D-xylose mercaptals. They obtained the di-O-benzylidene derivatives by the partial hydrolysis of the former compounds. They have assigned the structures of 2,3-mono- and 2,3;4,5-di-O-benzylidene-D-xylose mercaptals to these derivatives (XXIV) and (XXV), respectively (Fig. 20), on the basis of their observations that the mono-O-benzylidene derivatives con-

sumed one mole of lead tetraacetate and formed formaldehyde (yield not stated). The Barker-Bourne rules predict, however, the 2,4; 3,5-di-O-benzylidene derivative (XXVII) for D-xylose mercaptals. Furthermore, it is known that lead tetraacetate oxidation of sugar mercaptals is an unreliable guide to the presence of hydroxyl groups on adjacent carbon atoms, due to the oxidation of the mercaptal group (83, 85), and Zinner, Bock, and Klöcking (86), too, have observed the oxidation of mercaptal group by lead tetraacetate in benzene. Curtis and Jones (87), in connection with the synthesis of a disaccharide, have proved that the structure (XXIV) proposed by Zinner, Rembarz, Linke and Ulbricht (44) is to be corrected to XXVI, and consequently XXV to XXVII. Zinner and Wittenburg (84) have also recognized that these compounds are to be formulated as XXVI and XXVII, but without mentioning the correction by Curtis and Jones (87). These results suggest that the Barker-Bourne rules can also be applied to sugar mercaptals.

Zinner and Wittenburg (84) have also prepared di-O-benzylidene-D-ribose mercaptals, to which they have assigned the structure (Fig. 21) after careful proof by a series of reactions, as well as lead tetraacetate oxidation on the mono-O-benzylidene derivative and the consideration of the Barker-Bourne rules. The structure was also supported by further chemical evidence presented by Zinner and Schmandke (88). Here again applicability of the Barker-Bourne rules to sugar mercaptals was proved.

Wolfrom and Tanghe (89) have reported that 6-O-benzoyl-D-glucose diethyl mercaptal obtained by selective esterification of the mercaptal afforded the di-O-benzylidene derivative which on saponification with alkali yielded di-O-benzylidene-D-glucose diethyl mercaptal. The position of these two ben-

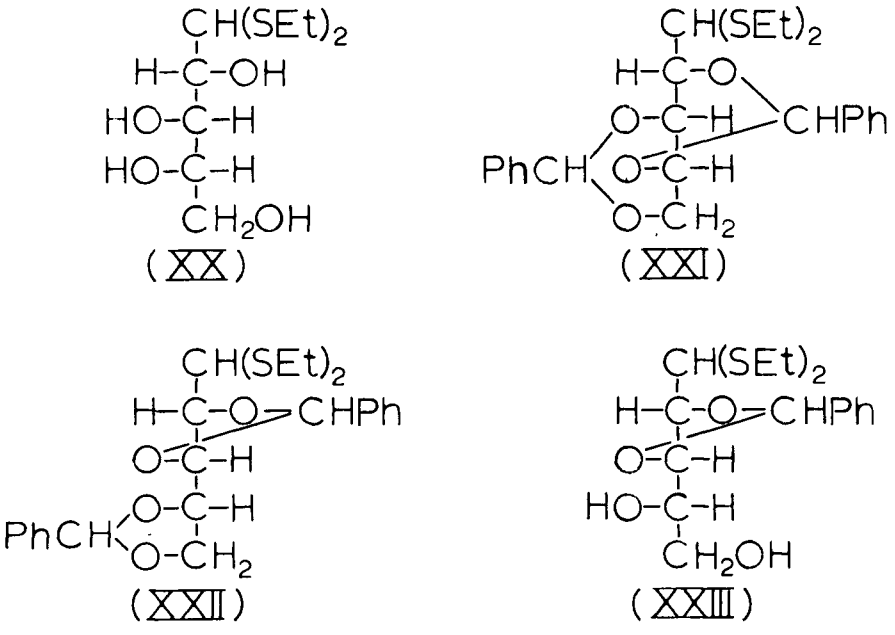


Fig. 19. Benzylidene derivatives of L-arabinose diethyl mercaptal.

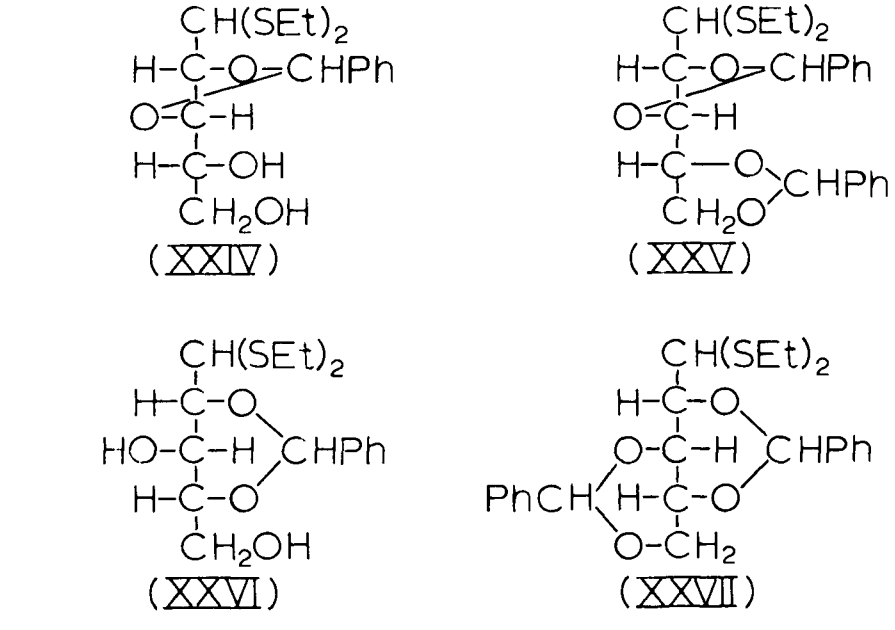


Fig. 20. Benzylidene derivatives of D-xylose diethyl mercaptal.

zylidene groups was not assigned; thus the compound was described as (2,3,4,-5)-di-O-benzylidene-D-glucose diethyl mercaptal. Since the favourable βC-ring is permissible, the first benzylidene group should occupy the 2- and 4-positions, and since the 6-position is blocked with a benzoyl group, the second benzylidene group is forced to occupy the 3- and 5-positions forming a βT-ring. Hence, the di-O-benzylidene-D-glucose diethyl mercaptal is very likely to be 2,4;3,5-di-O-benzylidene-D-glucose diethyl mercaptal (Fig. 22).

Although it has been stated (74) that the Barker-Bourne rules do not apply to the isopropylidene derivatives of polyhydric alcohols, and that there is a marked tendency to form a five-membered ring in these derivatives, the zig-zag structure for sugar mercaptals can also explain why the 2,3-hydroxyl groups of D-mannose methyl mercaptal do not form an isopropylidene derivative. In the zig-zag structure of D-mannitol (XVIII) (Fig. 17), the 2,3-hydroxyl groups which are cis-oriented in the Fischer projection formula are threo- in the zig-zag structure, whereas the 3,4-hydroxyl groups are in erythro-relationship in the zig-zag structure, but trans- in the Fischer projection formula; thus from D-mannose dimethyl mercaptal the 3,4;5,6-di-O-isopropylidene derivative is obtained (67) (Fig. 7).

The mechanism of the Purdie methylation is not known, but, due to the inevitable presence of a trace of barium hydroxide in silver oxide preparations, and also because of the basicity of silver oxide in the presence of moisture, the methylation is considered to take place in a basic medium. This is the reason why Purdie's reagents cause the migration of acetyl groups during methylation; an ortho acid ester is formed as an intermediate by the

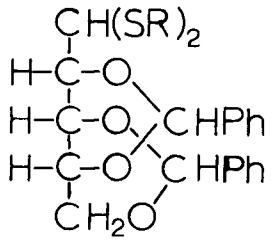


Fig. 21. Di-O-benzylidene-D-ribose mercaptal.

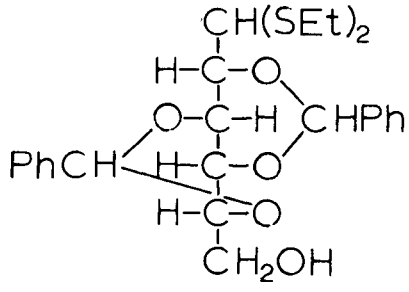


Fig. 22. Di-O-benzylidene-D-glucose diethyl mercaptal.

basic reagents (Fig. 23). The first step of the methylation should therefore be the ionization of an hydroxyl group ((1) in Fig. 24), and the methylation is then completed by substitution with a methyl group according to the scheme (2) (Fig. 24). It will be seen that the equilibrium of the step (1) is shifted to the right, if any electron-withdrawing group is present near the hydroxyl group concerned. Since in glycosides only carbon-1 is linked to two such groups, i.e. oxygen atoms, the acidity of the hydroxyl groups of glycosides would be the greatest at position 2 and much less at position 3. As Sugihara (9) has pointed out, this can explain the fact that non-reducing sugars exhibit a measurable acidity in conductometry and polarography, and that methylation tends to yield 2-O-methyl derivatives from glycosides. This is also true in the case of sugar mercaptals in which carbon-1 carries two electron-withdrawing atoms, i.e. sulphur atoms, and the greater reactivity of the 2-hydroxyl group of these compounds towards methylation can be explained in terms of this inductive effect; some examples have been quoted in the Introduction.

The present study has again demonstrated that in the Purdie methylation of sugar mercaptals the 2-hydroxyl group has the greatest reactivity. In general, a much lower reactivity of the 3-hydroxyl group of sugar mercaptals compared to that of 2-hydroxyl group was demonstrated clearly for the first time. Since the inductive effect rapidly decreases progressively down the carbon chain, this effect on the β-carbon is very much weaker than on the α-carbon. The much lower reactivity of the 3-hydroxyl group can thus be explained in terms of the inductive effect of the two sulphur atoms on carbon-1. The

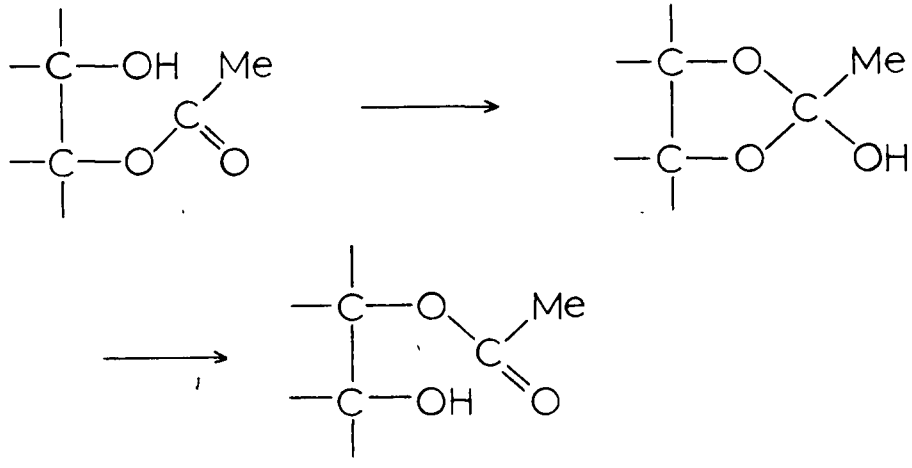


Fig. 23. Migration of acetyl group.

$$R\text{-}OH + B \rightleftharpoons R\text{-}O^{\ominus} + BH^{\oplus} \quad (B{:}base) \qquad (1)$$

$$R\text{-}O^{\ominus} + Me\text{-}I + Ag\text{-}O\text{-}Ag$$

$$\longrightarrow [\, R\text{-}\overset{\ominus}{O}\!\curvearrowright Me\text{-}\overset{\curvearrowright}{I}\, Ag\text{-}O\text{-}Ag \,]$$

$$\longrightarrow R\text{-}O\text{-}Me + AgI + AgO^{\ominus} \qquad (2)$$

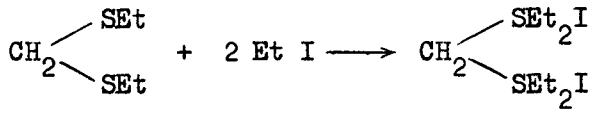
Fig. 24. Mechanism of Purdie methylation.

appreciable reactivity of the primary hydroxyl group of sugar mercaptals is explicable by the fact that the primary hydroxyl group is sterically the least hindered.

It should be noted here that the preliminary work (42) has described the following results. Sugar mercaptals were methylated by the method of Lieser and Leckzyck (40) and upon separation of the products from excess methyl iodide and silver oxide, sirups were obtained. The sirup from the reaction of D-glucose diethyl mercaptal crystallized spontaneously to give a 46% yield of 2-O-methyl-D-glucose diethyl mercaptal, the others failing to yield any crystalline material except unchanged mercaptal. The mother liquor from the 2-O-methyl-D-glucose mercaptal and the remaining three sirups were investigated by paper chromatography. On development of the chromatogram with iodine vapour, the presence of several compounds was noted, qualitatively indicating that the reaction is more general than had been supposed. The reaction in each case gave products of two types; those having a high Rf values corresponded to methylated and unchanged mercaptals, while those having lower Rf values were presumably more polar compounds whose constitution is at present unknown. It can, however, be stated that in these slow-running compounds the mercaptal group has not been removed completely since they gave no spot for reducing sugar with p-anisidine hydrochloride. It is also apparent that these compounds are probably carbohydrate in nature since their Rf values varied from sugar to sugar, but on hydrolysis with acid they did not regenerate the parent sugars. It was observed that the most intense of these slow spots can be produced by reacting the mercaptal with methyl iodide in the ab-

sence of silver oxide.

It is well known that alkyl sulphides form stable sulphonium compounds with alkyl halides, and that as a disulphide, mercaptals of ordinary aldehydes also form bis-sulphonium derivatives with halides- e.g.



Kuhn and Trischmann (90) have recently observed that dimethylsulphoxide, the basicity of the sulphur atom in which would be much lower than that of thioethers, forms the addition compound, trimethylsulphoxonium iodide, with methyl iodide. A similar type of reaction likely occurs in the action of methyl iodide on sugar mercaptals. Some of the compounds having lower Rf values which were observed in the preliminary work (42) would possibly be these sulphonium compounds because polar compounds usually travel slowly in paper chromatography. The low yield of O-methyl sugars from the methylation of sugar mercaptals can be accounted for by the formation of these polar compounds during methylation since the sulphonium compounds would not be hydrolyzed by acid to their parent free sugars. If these sulphonium compounds are formed, the acidity of the 2-hydroxyl group is much more enhanced than it would be by the mercaptal group alone and the predominant methylation at the 2-hydroxyl group is equally well explained in terms of the inductive effect of the sulphonium group.

The formation of sulphonium compounds appears to provide another mechanism, in addition to the direct methylation of an activated hydroxyl group, for the interpretation of the high reactivity of the 2-hydroxyl group towards

Purdie's reagents. The ionized oxygen on carbon-2 is suitably located to attack the rear-side of the methyl group on the sulphonium grouping and the methylation on the 2-hydroxyl group may be effected through the intermediate (XXVIII)* (Fig. 25).

This 'transmethylation' from the sulphur to the oxygen is also possible with the 3-hydroxyl group forming a six-membered intermediate (XXIX) (Fig. 25) which incidentally would be more stable than XXVIII. However, the much weaker acidity of the 3-hydroxyl than the 2-hydroxyl group would account for the preponderant formation of 2-O-methyl derivatives.

A criticism may arise: why has the 2-O-ethyl derivative not been isolated in the methylation of diethyl mercaptals? In these mixed sulphonium compounds, transfer of the ethyl group, rather than the methyl group, from the sulphur to the oxygen atom is very unlikely for two reasons; (1) the terminal methyl of the ethyl group would sterically prevent the approach of the oxygen towards the methylene of the ethyl group, and (2) the methylene carbon atom would be less electropositive compared with the carbon of the methyl group on the sulphur atom because the terminal methyl of the ethyl group tends to repel the bond-electrons toward the methylene of the ethyl group, and the oxygen would exclusively attack the methyl group on the sulphur atom. In this connection a kinetic study (91) of the substitutive Hofmann degradations of dilute sulphonium hydroxides in water must be cited, wherein a much slower

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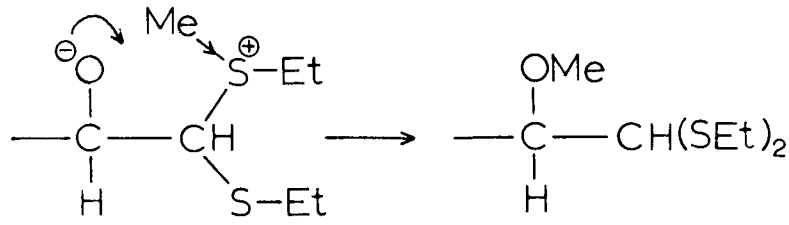
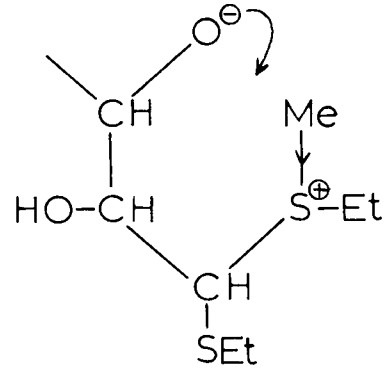
* Only the mono-sulphonium compound is shown here. The bis-sulphonium compound is less probable under the mild conditions of the Purdie methylation since the positive charge on the sulphur atom would lower the basicity of the second sulphur atom.



(XXVIII)



(XXIX)

Fig. 25. Mechanism of "transmethylation."

reaction of the ethyl derivative compared to that of the methyl derivative has been demonstrated. The fact that the 2-O-methylated mercaptals (not the sulphonium compounds) have often been isolated from the Purdie methylation products, does not eliminate the mechanism of this transmethylation. In the presence of a neighbouring hydroxyl group (2- or 3-position) as an acceptor of the methyl group, the sulphonium compounds would immediately be transformed into the O-methylated mercaptals.

Hydrogen bonding in sugar molecules has been observed for some time and a chapter has been devoted to the subject in a recently published review (92). In polyhydroxyl compounds like sugar mercaptals, the presence of intramolecular hydrogen bonding seems to be quite possible. In fact, in acyclic diols, where free rotation around the carbon-carbon bonds is possible, the five-, six-, and seven-membered intramolecular hydrogen bondings are known (92). Foster (46) has given the distances between various oxygen atoms of polyhydric alcohols in the zig-zag conformation (Table II). It is known that hydrogen bonding between two oxygen atoms is possible when these oxygen atoms are separated by 2.5 - 2.9Å and that in pentaerythritol the hydrogen bond distance was observed to be 2.69 ± 0.03Å (93). From the table it is seen that

TABLE II.

Distances between the oxygen atoms of polyhydric alcohols.

Relation of hydroxyl groups	βC,β	αT,α	βT	αC
Separating distance, Å	2.51	2.83	3.43	3.68

intramolecular hydrogen bonding will be possible between two hydroxyl groups in βC=, β-, αT-, or α-relationships, but it would be definitely weak because rectilinear hydrogen bonding, where the hydrogen bond is strong, is not possible without large distortion of the zig-zag conformation.

Several examples in which the effect of hydrogen bonding is the determining factor of a reaction have been demonstrated (92). The hydrogen bonding of the type O-H····X (X : negative atom) tends to increase the basicity of the oxygen atom, and hence its reactivity toward electrophilic reagents is increased. Therefore, the reactivity of a hydroxyl toward methylation would be lowered if the hydroxyl group is engaged in hydrogen bonding, i.e. the equilibrium (1) (Fig. 24) (p. 44) would be shifted to the left. In hydrogen bonding between two hydroxyl groups, the electrons to form the hydrogen bond will be donated to the hydrogen from the more negative oxygen atom, and in the case of sugar mercaptals, the electrons would be donated from the oxygen atom which is located far from the mercaptal group since the electronegativity of the oxygen atom closer to the mercaptal group would be less than the other due to the electron-attracting character of the two sulphur atoms. Consequently, if the hydroxyl group is not hydrogen-bonded, the reactivity toward methylation would be higher than that of the hydrogen-bonded hydroxyl group, the hydrogen atom of which is involved in the hydrogen bonding.

The results obtained in this study will be discussed individually, but only the trend of reactivity can be considered because the estimation of the ratio of mono-O-methyl ethers involves an assumption that all mono-O-

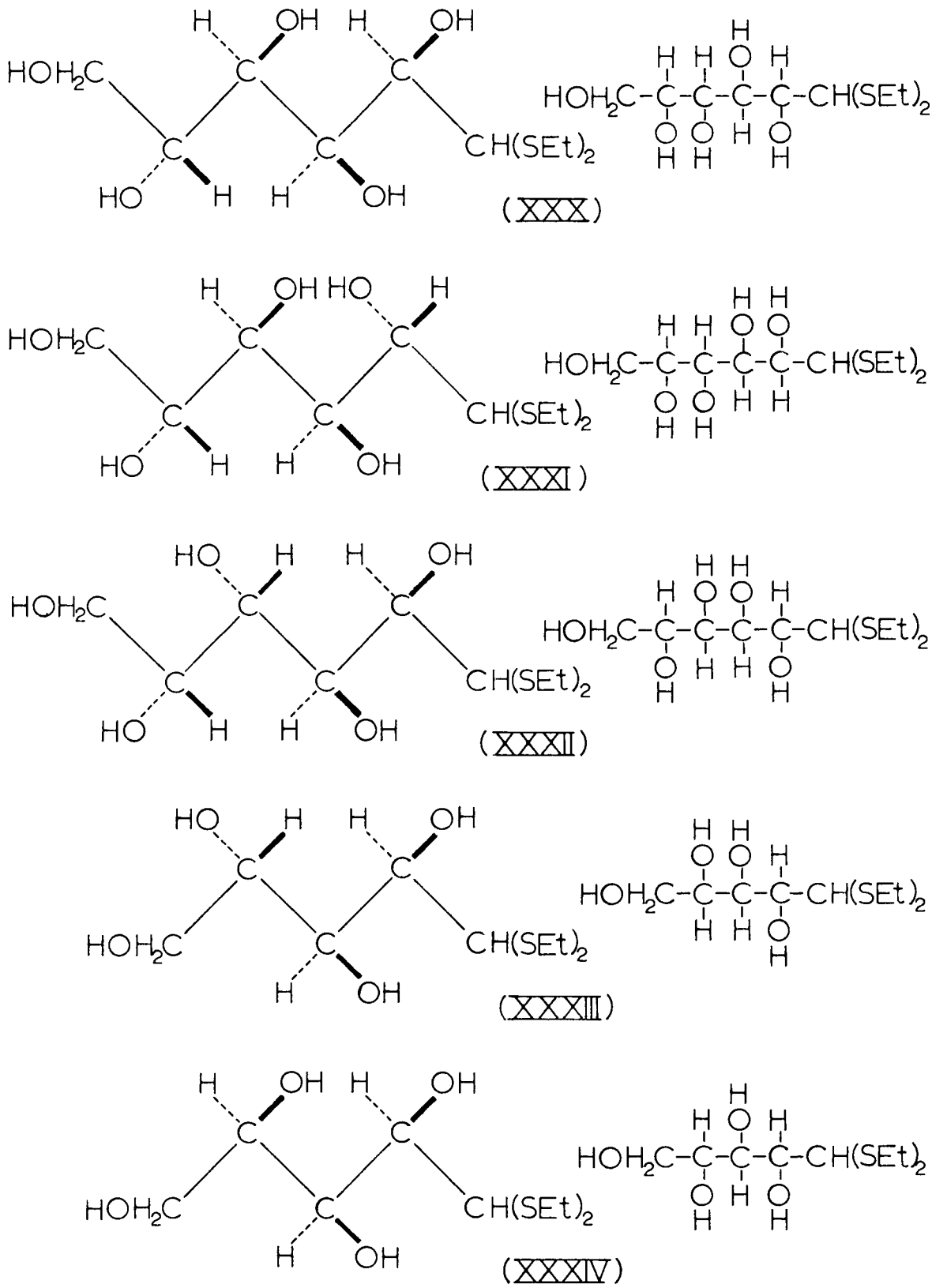


Fig. 26. Zig-zag conformation of sugar mercaptals.

methyl ethers of particular sugars give almost superimposable standard curves in the colorimetric analysis. The zig-zag conformation, together with the Fischer projection formula, of the sugar mercaptals are shown in Fig. 26.

(1) D-Glucose Diethyl Mercaptal (XXX).

From the methylation product of D-glucose diethyl mercaptal 2-, 3-, and 6-O-methyl-D-glucoses were detected in a ratio of approximately 6 : 1 : 1.

The highest reactivity of the 2-hydroxyl group, followed by that of the 3-hydroxyl can be understood by the inductive effect of the mercaptal group or of the sulphonium group. The appreciable reactivity of the 6-hydroxyl group is due to the least steric hindrance, as discussed before. Methylation on the 6-hydroxyl group apparently proceeds by direct attack of the reagents.

(2) D-Mannose Diethyl Mercaptal (XXXI).

The 2-, 3-, and 6-O-methyl ethers were found in the methylation mixture in the approximate ratio of 12 : 1 : 1. This ratio, compared with that of mono-O-methyl-D-glucoses (6 : 1 : 1), is more favourable to the 2-O-methyl ether. The only possible hydrogen bonding between the secondary hydroxyl groups of D-mannose diethyl mercaptal is that between the 3- and 4-hydroxyl groups. Since the 2- and 4-hydroxyl groups are threo-oriented, separating them as far as 3.43Å, no interaction between these two hydroxyl groups is permissible, but the 3- and 4-hydroxyl groups are close enough (2.83Å) to form a weak hydrogen bond, thus decreasing the reactivity of the

3-hydroxyl group toward methylation, and furthermore, the αC-relationship (3.68Å) between the 2- and 3-hydroxyl groups makes the former entirely free from hydrogen bonding. In D-glucose diethyl mercaptal, the reactivity of the 2-hydroxyl group is lowered by the hydrogen bonding with the 3- or 4-hydroxyl group, and the greater degree of methylation at the 2-hydroxyl group of D-mannose diethyl mercaptal would be accounted for by the difference between these situations.

(3) D-Galactose Diethyl Mercaptal (XXXII)

Approximately equal amounts of 2-, 3-, and 6-O-methyl-D-galactoses were found in the methylation products. If the results are compared with those for D-glucose or D-mannose diethyl mercaptals, the much higher reactivity of the 3-hydroxyl group of the former is unexpected. The results of the colorimetric analysis are supported by the fact that almost identical ratios were obtained from the relative weights of these isomers isolated from paper chromatograms. If the inductive effect is the most important factor controlling the rate of methylation, somewhat similar results to that of D-glucose diethyl mercaptal should be obtained. In order to explain the high reactivity of the 3-hydroxyl group (as high as the 2-hydroxyl group), another factor is apparently important in the methylation of D-galactose diethyl mercaptal.

In D-glucose diethyl mercaptal, hydrogen bonding is expected between 2,3-, 2,4-, or 3,4-hydroxyl grouping, and as soon as the most reactive 2-hydroxyl group is methylated, the first two possibilities are removed. (Hydrogen bonding between 2-methoxyl and 3- or 4-hydroxyl groups would not be possible owing to the low basicity of the oxygen atom in the methoxyl

group.) However, the reactivity of the 3-hydroxyl group is still suppressed by the hydrogen bonding from the 4-hydroxyl group. In the case of D-galactose diethyl mercaptal the only possible hydrogen bonding is that between the 2- and 3-hydroxyl groups, and the latter is threo-oriented to both the 4- and 5-hydroxyl groups so that no hydrogen bonding is permissible among these hydroxyl groups. Thus, as discussed before, the reactivity of the 3-hydroxyl group of D-galactose diethyl mercaptal is expected to be higher than that of other mercaptals. This provides support for the mechanism of "transmethylation" since the 3-hydroxyl group, free from hydrogen bonding, could approach the sulphonium group easily. Croon has observed (94) that in the Haworth methylation of cyclic glucose derivatives the 3-hydroxyl group is "doubled" when the 2-hydroxyl group is methylated. He has attributed this to an increase in the acidity of the 3-hydroxyl group when the 2-hydroxyl becomes methylated. This finds a parallel in the present instance where the 3-hydroxyl group of D-galactose diethyl mercaptal can not be hydrogen bonded. If the di-O-methyl galactose ethers were examined it is likely that 2,3-di-O-methyl-D-galactose would be found as the principal component.

(4) L-Arabinose Diethyl Mercaptal (XXXIII).

Only 2- and 5-O-methyl-L-arabinoses were obtained by the methylation and their ratio was estimated to be 30 : 1 by the combination of the weights of the fast- and slow-moving components and the colorimetric analysis of the fast-moving components. From the comparison with a synthetic sample of 3-O-methyl-L-arabinose on electrophoresis, the presence of this compound in the methylation mixture was denied.

The stereochemical relationship of the 2-, 3-, and 4-hydroxyl groups

of L-arabinose diethyl mercaptal is the same as that of D-galactose diethyl mercaptal; but the 5-hydroxyl group of the former is primary, whereas in the latter compound it is secondary, and on account of the zig-zag conformation hydrogen bonding between 3- and 5-hydroxyl groups is not possible in the latter. In L-arabinose diethyl mercaptal, a rather strong hydrogen bonding between 3- and 5-hydroxyl groups (2.51Å) is possible and particularly when the 2-hydroxyl group is methylated the reactivity of the 3-hydroxyl group is largely reduced by the hydrogen bonding with the 5-hydroxyl group. This hydrogen bonding would be the reason for the absence of 3-O-methyl-L-arabinose in the methylation product.

(5) D-Xylose Diethyl Mercaptal (XXXIV).

From the methylation product 2-, 3-, and 5-O-methyl-D-xyloses were detected in the approximate ratio of 16 : 10 : 1. The fact that 5-O-methyl-D-xylose was not detected in the electrophoresis of the methylation product of the sirupy D-xylose diethyl mercaptal is explicable by the weakness of the spot detected in the methylation product of the crystalline D-xylose diethyl mercaptal; 5-O-methyl-D-xylose might be missed.

Due to the high solubility of D-xylose diethyl mercaptal in methyl iodide, the methylation was first carried out in the absence of the diluent, tetrahydrofuran, yielding only poly-O-methyl-D-xyloses. The higher ratio of 3-O-methyl-D-xylose compared with that of 3-O-methyl-D-glucose, the stereochemical relationships of the 2-, 3-, and 4-hydroxyl groups of D-glucose and D-xylose diethyl mercaptals being equal, may be accounted for by the higher concentration of methyl iodide in tetrahydrofuran in the methylation of D-

xylose diethyl mercaptal (see Experimental) compared with that of D-glucose diethyl mercaptal, regardless of the hydrogen bonding between the 3- and 5-hydroxyl groups in D-xylose diethyl mercaptal.

In the preceding discussion the 4-O-methyl ethers of pentoses and 4- and 5-O-methyl ethers of hexoses were not mentioned. Those spots corresponding to 2-, 3-, or 6-O-methyl ethers may contain the 4-O-methyl derivative, but when the predominant effect of the mercaptal group on the methylation of the neighbouring hydroxyl groups is considered, it is natural to assume that the 4-O-methyl derivatives are not formed in any appreciable amount. Furthermore, the reactivity of the 4-hydroxyl group of the hexose diethyl mercaptals would be largely depressed by the hydrogen bonding with the 6-hydroxyl group and a similar situation exists in the case of the 3-hydroxyl group of L-arabinose diethyl mercaptal. The 4-O-methyl ethers exclude the furanose form and, therefore, the contribution of the 1,2-hydroxyl grouping of this form to the equilibria in borate complex formation need not be considered; hence, a low Mg value is expected in the 4-O-methyl ethers (e.g., 4-O-methyl-D-glucose: Mg 0.24 (46) and 4-O-methyl-D-galactose: Mg 0.30 (61)). 4-O-Methyl-D-mannose possesses the 2,3-cis-hydroxyl grouping in the pyranose form and may migrate relatively faster than the 4-O-methyl ethers of other sugars in which no cis-hydroxyl grouping exists in the pyranose form, but due to the powerful effect of the mercaptal group and the hydrogen bonding between the 4- and 6-hydroxyl groups the presence of this isomer in the mixture is very unlikely. Similarly, the 4-O-methyl ethers of L-arabinose and D-xylose are expected to have a low Mg value, al-

though there is a possible contribution of the β-hydroxyl grouping, the effect of which is less significant.

The 4-hydroxyl group is invariably hydrogen-bonded as noted above and since it is well known that inductive effects fall off rapidly along a chain one may assume that the 4-hydroxyl groups will not be activated. If it is true that the methylation of mercaptals proceeds through the 'transmethylation' mechanism, the 4-hydroxyl group can not approach sufficiently close to the sulphonium group as shown by the examination of molecular models of the zig-zag conformation, thus further demonstrating the lack of reactivity at this position. In the case of D-galactose it was possible to substantiate this hypothesis as 4-O-methyl-D-galactose is readily differentiated from its isomers and was found to be absent.

Absence of the 5-O-methyl ethers of hexoses in the mixtures is very clear. In all cases the β-hydroxyl grouping in addition to the 1,2-cis-hydroxyl and 3,6-hydroxyl groupings in the furanose form are possible (95), and moreover the βC- in the aldehyde-form of 5-O-methyl-D-glucose, and the 2,3-cis-hydroxyl grouping in the pyranose form of 5-O-methyl-D-mannose are able to form borate complexes. Therefore, a high Mg value is expected from each 5-O-methyl ether.

As discussed above, the reactivity of the 2- and 3-hydroxyl groups of all sugar mercaptals is the result of the electron-attracting groups, mercaptal or sulphonium group, on the carbon-1, and it is further affected by the intramolecular hydrogen bonding in the zig-zag conformation. This does not imply that the 4- and 5-hydroxyl groups of the hexose mercaptals and the

4-hydroxyl group of the pentose mercaptals are not reactive towards the Purdie methylation. In fact poly-O-methylated sugars were detected when methylation was carried out for a longer period, and by the Haworth methylation, which comprises the treatment with dimethyl sulphate and alkali, D-glucose and D-mannose diethyl mercaptals are known to be methylated up to the penta-O-methyl derivatives (96). Being located far from the activating mercaptal group, the complete methylation of the secondary hydroxyl groups other than the 2- and 3-hydroxyl groups would be extremely difficult and in an attempt to prepare 2,3,4,5-tetra-O-methyl-L-arabinose by the Purdie methylation of the mercaptal the methylation products, after acidic hydrolysis, have always been accompanied by several components with a lower degree of methylation (97).

EXPERIMENTAL

Tetrahydrofuran used here was pre-dried over potassium hydroxide pellets, refluxed for several hours with lithium aluminum hydride, and then distilled to collect the fraction with b.p. 64-66°C. Ethanol (95%), unless otherwise specified, and petroleum ether (b.p. 30-60°C) were used throughout.

Methylation was performed by shaking the mixture in a stoppered flask covered with aluminum foil on a mechanical shaker. Evaporation was carried out *in vacuo* at 40-50°C. All melting points were taken on a Fisher-John's apparatus or E. Leitz microscopic heating block, and only uncorrected melting points are given.

Paper chromatography was performed, unless otherwise stated, in butanone-water azeotrope using Whatman No. 1 filter paper, and components were located by spraying with p-anisidine-trichloroacetic acid reagent and drying in an oven at 100-110°C. Intensities of spots are described using the following abbreviations: m (medium), s (strong), w (weak), vs (very strong) etc.

Electrophoresis was done in 0.05M sodium borate solution under the conditions specified in each case, and in calculating Mg values, D-glucose (Mg 1.00) and 2,3,4,6-tetra-O-methyl-D-glucose (Mg 0.00) were used as standards. Intensities of spots are given using the same abbreviations used for chromatography.

Infrared spectra were taken by a Perkin-Elmer Infracord. Colorimetric

analyses were performed following the procedure of Dubois et al (56), and using a Bausch and Lomb colorimeter. When electrophoresis was used to separate components, the extracts were evaporated to dryness, and the residues were refluxed for 30 minutes in 0.1N methanolic hydrogen chloride in order to destroy the borate complexes.

A. D-Glucose Diethyl Mercaptal.

Methylation of D-Glucose Diethyl Mercaptal (I).

D-Glucose diethyl mercaptal (1.00g) was dissolved in tetrahydrofuran (50ml), and silver oxide (4.00g), drierite (anhydrous calcium sulphate) (5g), and methyl iodide (30.0ml) were added to the solution. The mixture was shaken for 12 hours at room temperature and then filtered in order to remove inorganic substances, which were washed with tetrahydrofuran (20ml). The filtrate and washing were evaporated to a sirup which was redissolved in chloroform (20ml) and a small amount of inorganic impurities was removed by filtration. The filtrate was evaporated giving a light yellow sirup (796mg).

The sirup (796mg) was refluxed for 5 hours in a mixture of ethanol (20ml) and 18% hydrochloric acid (3ml). During the hydrolysis ethyl mercaptan was evolved. The hydrolysis solution was neutralized with Duolite A-4 resin and the neutral solution was evaporated to a sirup which was treated with charcoal in hot water. A light brown sirup (561mg) was obtained by evaporation of the decolorized solution.

The hydrolysate was shown by paper chromatography to be a mixture of mainly poly-O-methylated D-glucoses; Rf values being as follows:

Rf 0.95 (s), 0.87 (s), 0.68 (m), 0.31 (w), 0.25 (w), 0.10 (vw), and 0.02 (vw).

The product was not investigated further.

Methylation of D-Glucose Diethyl Mercaptal (II).

The same quantities of reagents used in methylation (I) were used, and the mixture was shaken for 3 hours at room temperature. Similar treatment of the methylation mixture gave a yellow sirup (820mg), which was hydrolysed under the same conditions, and treated in a similar fashion to yield a light brown sirup (529mg). Paper chromatography of the sirup revealed a strong spot of mono-O-methyl-D-glucoses (Rf 0.10), a strong spot of unchanged D-glucose (Rf 0.02), and a few very weak spots of poly-O-methyl-D-glucoses.

The sirup (529mg) was chromatographed on eight 15cm-wide Whatman No.3MM sheets for 10 hours. Guide strips were cut off and sprayed, and the areas containing the mono-O-methyl-D-glucoses were cut and extracted with cold water (100ml each time). After three extractions the extract gave a faint Molisch test. The extracts were combined and evaporated, and the residue was dissolved in methanol (2ml) and filtered through glass wool in order to remove cellulosic impurities. Evaporation of the methanol solution yielded a sirupy mixture (40.3mg) of mono-O-methyl-D-glucoses (mixture (II)).

Methylation of D-Glucose Diethyl Mercaptal (III).

D-Glucose diethyl mercaptal (1.00g) was dissolved in tetrahydrofuran (100ml), and silver oxide (4.00g), drierite (5g), and methyl iodide (30.0ml) were added to the solution. The mixture was shaken for 3 hours at room temperature. The reaction mixture was treated as described under methylation

(I), and a yellow sirup (807mg) was obtained. Hydrolysis of the sirup was performed similarly and a light brown sirup (545mg) thus obtained was shown to consist mainly of mono-O-methyl-D-glucoses (Rf 0.10 (s)) and unchanged D-glucose (Rf 0.02 (s)), plus a small amount of poly-O-methylated D-glucoses (all very weak spots). The mono-O-methyl ethers were isolated by paper chromatography on six 15cm-wide Whatman No. 3MM sheets for 10 hours, as described under methylation (II), and the yield was 32.7mg (mixture (III)).

Electrophoresis of the Mixture of Mono-O-methyl-D-glucoses.

The mixtures of mono-O-methyl-D-glucoses (II) and (III) were examined electrophoretically (950-1050 V, 25mA, 45 minutes). The results are shown in Table III.

Chromatographic Separation of the Mixtures of Mono-O-methyl-D-glucoses.

The mixtures (II) and (III) were chromatographed for 48 hours in 2,4,6-collidine-ethyl acetate-water (2 : 5 : 5 by volume, upper layer). The results are shown in Table IV.

Examination of Fast-Running Spots by Electrophoresis.

Since no clear spot of any fast-running components was observed, the remainder of the paper chromatograms used for separation of the mono-O-methyl-D-glucoses in the mixture (III) was extracted with cold water (ca. 200ml), and the evaporation residue was examined by electrophoresis (950-1050 V, 25mA, 45 minutes). Only a weak, but large spot (several components?) of Mg 0.00 was detected.

Colorimetric Analysis of the Mixtures (II) and (III).

(1) Preparation of standard curve.

From the original solution containing 832γ/ml of 3-O-methyl-D-gluc-

TABLE III.

Electrophoresis of mono-O-methyl-D-glucoses

Mixture (II)	Mg 0.29 (s), 0.86 (m)
Mixture (III)	Mg 0.29 (s), 0.86 (m)
2-O-Methyl-D-glucose	Mg 0.29
3-O-Methyl-D-glucose	Mg 0.86
6-O-Methyl-D-glucose	Mg 0.86

TABLE IV.

Chromatographic separation of mono-O-methyl-D-glucoses

	Travelling distance (mm) of spots
Mixture (II)	338 (w), 303 (s), 247 (w)
Mixture (III)	338 (w), 303 (s), 247 (w)
2-O-Methyl-D-glucose	303 ($R_{glucose}$ 3.79)
3-O-Methyl-D-glucose	338 ($R_{glucose}$ 4.23)
6-O-Methyl-D-glucose	247 ($R_{glucose}$ 3.09)
D-Glucose	80

ose, five solutions given in Table V were prepared. The results are shown in Table V, and the curve is given in Figure 27.

(2) Analysis of the mixtures.

The mixtures of mono-O-methyl-D-glucoses (II) and (III) were chromatographed for 72 hours in 2,4,6-collidine-ethyl acetate-water (2 :5 :5 by volume, upper layer) on a 15cm-wide Whatman No. 1 sheet. The results are shown in Tables VI and VII.

B. D-Mannose Diethyl Mercaptal.

D-Mannose Diethyl Mercaptal.

D-Mannose (15g) was mixed with concentrated hydrochloric acid (15g) in an ice-water bath. Ethyl mercaptan (10g) was added portionwise with vigorous shaking. After all the ethyl mercaptan was added, the mixture was shaken mechanically for 20 minutes. The clear solution thus obtained was partially neutralized with barium carbonate, and then completely neutralized with excess barium carbonate after addition of methanol (100ml) to the mixture. Barium chloride and excess barium carbonate were filtered and evaporation of the filtrate gave a sirupy residue, from which inorganic impurities were removed by extraction with cold absolute ethanol (ca. 150ml). The solvent was evaporated and the residue was recrystallized from ethanol-ether. Yield 14.2g (60%). M.p. and mixed m.p. with a sample prepared by Fischer's method were 132-134°C.

Methylation of D-Mannose Diethyl Mercaptal (I).

D-Mannose diethyl mercaptal (1.00g) was dissolved in tetrahydrofuran (70ml), and silver oxide (3.35g), drierite (5g), and methyl iodide (35.0ml)

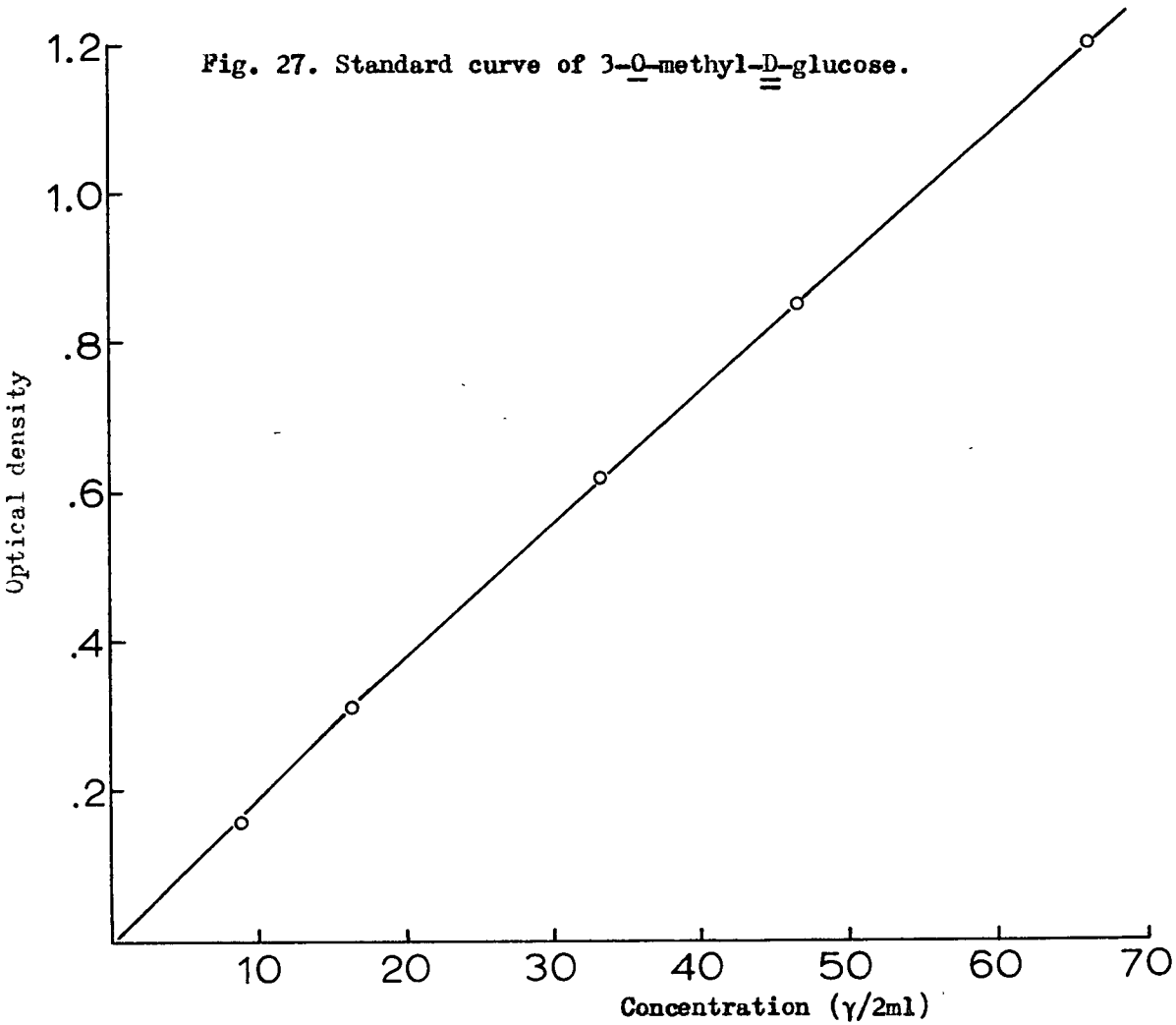


Fig. 27. Standard curve of 3-O-methyl-D-glucose.

TABLE V.

Preparation of standard curve of 3-O-methyl-D-glucose

Solution	Concentration (γ/2ml)	Readings at 490mμ			Average
1	66.6	1.2	1.2	1.2	1.2
2	46.6	0.85	0.85	0.85	0.85
3	33.3	0.62	0.62	0.62	0.62
4	16.7	0.312	0.320	0.315	0.313
5	8.3	0.160	0.160	0.152	0.157

Table VI. Colorimetric analysis of mono-O-methyl-D-glucoses (II)

Component		Readings at 490 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-glucose	(sugar	1.5	1.5	1.4	1.47	1.217	67.5
	(blank	0.245	0.245	0.260	0.250		
3-O-Methyl-D-glucose	(sugar	0.420	0.390	0.410	0.404	0.299	16.0
	(blank	0.115	0.100	0.100	0.105		
6-O-Methyl-D-glucose	(sugar	0.370	0.370	0.350	0.363	0.222	12.0
	(blank	0.130	0.145	0.148	0.141		

Table VII. Colorimetric analysis of mono-O-methyl-D-glucoses (III)

Component		Readings at 490 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-glucose	(sugar	1.2	1.2	1.2	1.2	0.95	52.5
	(blank	0.250	0.250	0.260	0.25		
2-O-Methyl-D-glucose (x2 dilution)	(sugar	0.62	0.63	0.62	0.62	0.49	26.5
	(blank	0.130	0.130	0.130	0.130		
3-O-Methyl-D-glucose	(sugar	0.310	0.300	0.310	0.307	0.214	11.5
	(blank	0.090	0.100	0.090	0.093		
6-O-Methyl-D-glucose	(sugar	0.300	0.290	0.320	0.303	0.151	8.0
	(blank	0.155	0.155	0.145	0.152		

were added to the solution. The mixture was shaken for 15 hours at room temperature and then filtered in order to remove inorganic substances which were washed with tetrahydrofuran (20ml). Evaporation of the filtrate and washings gave a sirup which was redissolved in chloroform (20ml). The filtered chloroform solution yielded, on evaporation, an amber sirup (950mg).

The sirup (950mg) was hydrolysed by refluxing in a mixture of ethanol (20ml) and concentrated hydrochloric acid (2ml) for 5 hours; ethyl mercaptan was evolved during hydrolysis. The hydrolysate was deionized with Duolite A-4 resin, and the neutralized hydrolysate was concentrated to yield a brown sirup, which after treatment with charcoal in hot water gave a light brown sirup (686mg).

Paper chromatography of the sirup showed the following spots, which indicated polymethylation of the mercaptal. Rf 0.93 (vs), 0.89 (s), 0.84 (s), and several faint slower-moving spots. The sirup was not investigated further.

Methylation of D-Mannose Diethyl Mercaptal (II).

D-Mannose diethyl mercaptal (1.00g) was dissolved in tetrahydrofuran (100ml), and silver oxide (1.50g), drierite (3g), and methyl iodide (10.0 ml) were added to the solution, which was then shaken at room temperature for 21 hours. During this period additional methyl iodide (20.0ml) was added to the reaction mixture in aliquots of 10ml at intervals of 7 hours. The inorganic substances were filtered and then washed with tetrahydrofuran (20ml). The filtrate and washings were concentrated to a sirup, which

was redissolved in chloroform (20ml) in order to remove inorganic impurities in the sirup by filtration. A light brown sirup (998mg) was obtained by evaporation of the chloroform solution.

The sirup (998mg) was refluxed for 5 hours in a mixture of ethanol (10ml) and 10% hydrochloric acid (10ml). The hydrolysate was neutralized with Duolite A-4 resin and evaporation of the deionized solution gave, after treatment with charcoal, a brown sirup (574mg).

The product showed the following spots on paper chromatography: Rf 0.25 (m), 0.08 (s) (two components?), 0.01 (s), and almost undetectably faint spots of higher Rf values.

The sirup (574mg) was chromatographed on six 15cm-wide Whatman No. 3MM sheets for 10 hours, and the fast (Rf 0.25)-and slow (Rf 0.08)-running components were extracted with cold water (100ml x 3). The yield of the fast- and slow-running components was 8.3 and 42.7mg, respectively.

Methylation of D-Mannose Diethyl Mercaptal (III).

Using the same quantities of the reactants and conditions, a sirupy mixture (502mg) was obtained.

The sirup (502mg) was chromatographed similarly and the fast-running (7.9mg) and slow-running (31.3mg) (mixture (III)) components were isolated.

Electrophoresis of the Slow- and Fast-Running Components.

The slow-running component (Rf 0.08) (66.7mg) isolated from the methylations (II) and (III) was examined by electrophoresis (900-1050V, 25-30mA, 40 minutes). The results are shown in Table VIII.

The fast-running component (Rf 0.25) was also examined electrophor-

TABLE VIII.

Electrophoresis of mono-O-methyl-D-mannoses (mixture (III))

Compound	Mg value
Sirup of Rf 0.08	0.38 (s), 0.54 (m), 0.63 (w)
2-O-Methyl-D-mannose	0.38
3-O-Methyl-D-mannose	0.54
6-O-Methyl-D-mannose	0.63

etically (950-1100V, 25-30mA, 40 minutes), but only a non-migrating spot (Mg 0.00) was detected.

Colorimetric Analysis of the Slow-Running Component (Mixture (III)).

(1) Preparation of the standard curve.

From the original solution containing 875γ/ml of 2-O-methyl-D-mannose (electrophoretically and chromatographically pure sirup), five solutions of various concentrations were prepared. The results are shown in Table IX, and the curve is given in Fig. 28.

(2) Analysis of the mixture.

The sirup (mixture (III)) was separated by electrophoresis (1100-1200V, 25-35mA, 60 minutes). The results are shown in Table X.

Methylation of D-Mannose Diethyl Mercaptal (IV).

To the solution of D-mannose diethyl mercaptal (1.00g) in tetrahydrofuran (100ml), silver oxide (1.50g), drierite (3g) and methyl iodide (10.0ml) were added, and the mixture was shaken at room temperature for 12 hours. After another 10.0ml of methyl iodide was added, the mixture was shaken at room temperature for a further 12 hours. The mixture was treated as before to give a sirup (1157mg), which was hydrolyzed for 5 hours in a boiling mixture of ethanol (10ml) and 2N sulphuric acid (5ml). Evaporation of the deionized solution with Duolite A-4 resin gave a sirup (528mg). Paper chromatography showed the following spots:

Rf 0.34 (vw), 0.22 (m), 0.07 (s), and a few spots (vvw) of high Rf values.

From the same quantities of the reactants, another sirup (508mg) of O-methylated D-mannoses was obtained and paper chromatography showed the foll-

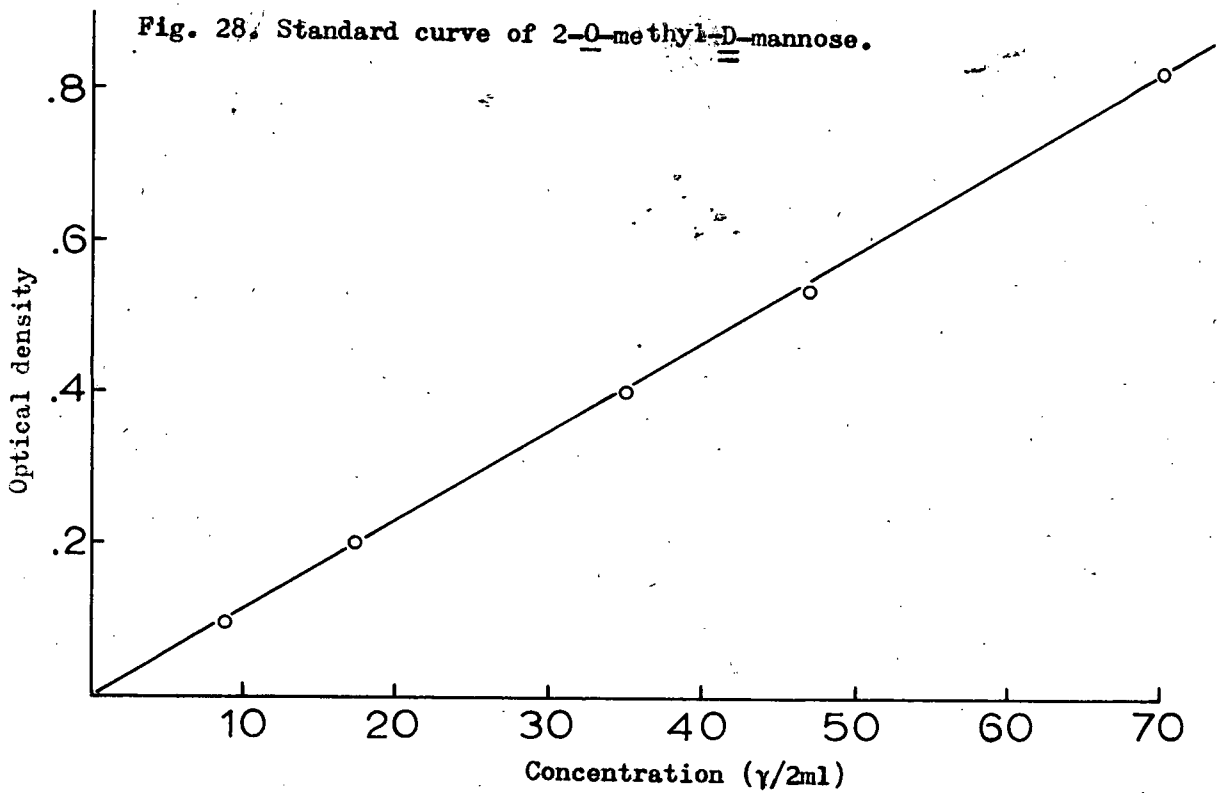


Fig. 28. Standard curve of 2-O-methyl-D-mannose.

TABLE IX.

Standard curve of 2-O-methyl-D-mannose

Solution	Concentration (γ/2ml)	Readings at 490mμ			Average
1	70.0	0.81	0.81	0.80	0.81
2	46.7	0.53	0.53	0.52	0.53
3	35.0	0.410	0.402	0.400	0.404
4	17.5	0.202	0.200	0.200	0.201
5	8.8	0.100	0.097	0.099	0.099

Table X. Colorimetric analysis of mono-O-methyl-D-mannoses (mixture (III))

Component		Readings at 490 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-mannose	(sugar	0.77	0.76	0.77	0.77	0.70	61.5
	(blank	0.080	0.065	0.070	0.072		
2-O-Methyl-D-mannose (x2 dilution)	(sugar	0.415	0.420	0.410	0.415	0.374	32.8
	(blank	0.040	0.042	0.042	0.041		
3-O-Methyl-D-mannose	(sugar	0.095	0.087	0.092	0.091	0.051	4.5
	(blank	0.043	0.039	0.038	0.040		
6-O-Methyl-D-mannose	(sugar	0.117	0.109	0.119	0.115	0.055	5.0
	(blank	0.062	0.060	0.059	0.060		

owing spots:

Rf 0.43 (vw), 0.29 (m), 0.11 (s), 0.03 (s), and a few spots (vvw) of high Rf values.

These combined sirups (528mg + 508mg) were separated on a cellulose-hydrocellulose column (rate of flow: 10ml/20 minutes), and the component of Rf 0.07 was detected in tubes No. 80-200, whereas the components of Rf 0.22 and 0.35 were found in tubes No. 40-70, but without clear separation. The yield of these fractions was 115.7mg (mixture (IV)) and 21.3mg (mixture (IVa)), respectively.

Methylation using the same quantities of the reactants was performed twice more, and two sirups (484mg and 419mg) of O-methylated D-mannoses were obtained. These sirups showed the following spots by paper chromatography:

Rf 0.27 (vw), 0.22 (m), 0.11 (w), 0.07 (m), 0.02 (m), and a few spots (vvw) of high Rf values.

These sirups were combined and chromatographed on a cellulose-hydrocellulose column (rate of flow: 10ml/20 minutes). The eluant was not collected for the first 10 hours. The components corresponding to the mono-O-methyl ethers were detected in tubes No. 60-175, and those corresponding to di-O-methyl ethers were found in tubes No. 15-45. No clear separation of these two fractions was obtained. The yield of these fractions was 70.5mg (mixture (V)) and 11.7mg (mixture (Va)), respectively.

Electrophoresis of the Mixtures (IV), (V), (IVa) and (Va).

Since the mixtures (IV) and (V) did not show any clear separation of

the spots by electrophoresis; (probably due to impurities), these sirups were treated together in hot water with charcoal. A clear sirup (170mg) (mixture (VI)), was recovered, and the electrophoretic examination (950-1050V, 25-30mA, 50 minutes) showed three spots Mg 0.32 (s), 0.48 (w) and 0.60 (w), which corresponded to 2-, 3-, and 6-O-methyl-D-mannoses, respectively.

The electrophoresis of the mixtures (IVa) and (Va) showed one large spot (two or three (?) components) of Mg 0.14 and 0.15, respectively.

Colorimetric Analysis of the Mixture (VI).

The sirup (mixture (VI)) was separated by electrophoresis (1050-1200V, 25-35mA, 60 minutes). The results are shown in Table XI.

Isolation of 2-O-Methyl-D-mannose and the Preparation of 2-O-Methyl-D-mannose Phenylhydrazone.

The mixture (VI) was separated by electrophoresis (950-1050V, 50 minutes) on three 15cm-wide Whatman No. 3MM sheets, and 2-O-methyl-D-mannose was located on the guide strips. The appropriate areas of the sheets were extracted with cold water (30ml x 3). The residue obtained by evaporation of the extracts was treated with cold methanol (about 5ml) and the solvent was evaporated. After this procedure was repeated about twenty times, a sirupy residue (28.7mg) was obtained.

Absolute ethanol (2ml) and phenylhydrazine (16.0mg) in absolute ethanol (0.09ml) were added to the residue (28.7mg), and the mixture was warmed for 20 minutes. After cooling, petroleum ether was added to precipitate the phenylhydrazone. The precipitates were separated by centrifugation,

Table XI. Colorimetric analysis of mono-O-methyl-D-mannoses (mixture (VI))

Component		Readings at 490 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-mannose	(sugar	0.78	0.76	0.78	0.774	0.688	60.3
	(blank	0.083	0.087	0.087	0.086		
3-O-Methyl-D-mannose	(sugar	0.157	0.152	0.149	0.153	0.114	10.0
	(blank	0.038	0.043	0.035	0.039		
6-O-Methyl-D-mannose	(sugar	0.091	0.089	0.085	0.088	0.047	4.5
	(blank	0.045	0.038	0.040	0.041		

and then recrystallized from ethanol-petroleum ether, m.p. 161-163°C. Admixture with synthetic 2-O-methyl-D-mannose phenylhydrazone showed no depression of the melting point.

Preparation of 2-O-Methyl-D-mannose.

D-Mannose diethylmercaptal (5.0g), and anhydrous copper sulphate (3.0g) were added to dry acetone (100ml) to which concentrated sulphuric acid (0.50ml) had been added previously. The mixture was shaken mechanically for 48 hours at room temperature. The filtrate from the reaction mixture was neutralized with Duolite A-4 resin after addition of water (5ml). The neutralized solution was evaporated and the residue was redissolved in chloroform.(20ml). The solution was dried over anhydrous magnesium sulphate and evaporation gave a colorless sirup (5.51g).

The sirup (5.40g) was dissolved in methyl iodide (100ml) and the solution was refluxed with vigorous stirring for 24 hours, during which period silver oxide (20g) was added portionwise. The reaction mixture was filtered and evaporation of the filtrate yielded a sirup in which a small amount of inorganic impurities was observed. The sirup was extracted with ether (about 50ml) and a brown sirup was obtained on evaporation of the extract. No hydroxyl band was observed in the infrared spectrum of the sirup. Yield 3.01g.

The sirup (3.01g) was hydrolyzed by refluxing for 6 hours in a mixture of ethanol (20ml) and 2N sulphuric acid (10ml). Neutralization of the hydrolyzate with Duolite A-4 resin and evaporation gave a brown sirup (968mg), paper chromatography of which showed a strong spot (R_f 0.07) of a monomethyl-D-mannose as well as a medium spot of unchanged mannose and a series of weak spots

of poly-O-methylated mannoses. A paper chromatography for a prolonged period (48 hours) showed the following spots-

$R_{mannose}$ 2.50 (s), $R_{mannose}$ 1.40 (w), and $R_{mannose}$ 1.00 (m).

Chromatographically and electrophoretically pure 2-O-methyl-D-mannose (103mg) was obtained by chromatography on a cellulose-hydrocellulose column with butanone-water azeotrope.

2-O-Methyl-D-mannose Phenylhydrazone.

2-O-Methyl-D-mannose (19.5mg) was dissolved in absolute ethanol (2ml) and phenylhydrazine (10.8mg) in absolute ethanol (0.06ml) was added. The mixture was warmed at 80°C for 20 minutes. A part of the solvent was evaporated and the resulting solution gave on cooling a yellow precipitate (21.3mg) which was recrystallized from ethanol-light petroleum ether. M.p. 161-163°C.

Preparation of 2-O-Methyl-D-mannose (II).

A solution of methyl 4,6-O-ethylidene-α-D-mannopyranoside (3.00g) in dry pyridine (20ml) was cooled to -7°C. An ice-cold solution of tosyl chloride (2.60g) in dry pyridine (20ml) was added to the solution, and the mixture was kept at -7°C for 60 hours and then at 0°C for 24 hours. The reaction mixture was poured into ice-water and extracted with chloroform (20ml x 5). The extract was washed with ice-cold 1 N sulphuric acid (50ml x 5), and then with a saturated solution of potassium carbonate. Evaporation of the extract gave a sirup(199g), λmax 2.90μ (OH), 6.32μ (aromatic C=C), 7.37μ (O-tosylate), and 8.52μ (O-tosylate).

The tosylated sirup (886mg) was dissolved in methyl iodide (40ml). Drierite

(5g) and silver oxide (5g) were added to the solution and the mixture was refluxed vigorously for 24 hours. The solvent was evaporated and the residue was exhaustively extracted with boiling chloroform (20ml x 3). Evaporation of the extract gave a sirup (350mg), λ max 2.85μ (OH).

The detosylated sirup (350mg) was heated in 0.5N hydrochloric acid (20ml) for 6 hours in boiling water (evolution of acetaldehyde). The hydrolysate was neutralized with silver oxide, filtered, treated with hydrogen sulphide, and centrifuged. The clear supernatant solution was concentrated to a sirup, which was extracted with absolute ethanol. The extract gave a sirup (190mg) which showed three spots ($R_{mannose}$ 3.32, 2.54, and 1.00 (48 hours)) on paper chromatography. The sirup (190mg) was chromatographed on a cellulose hydrocellulose column (20ml/30 minutes) and in tubes No. 101-140 the component of $R_{mannose}$ 2.54 was detected. The component (78mg) was shown by paper chromatography (96 hours) using butanone-water azeotrope saturated with borax to be contaminated with a small amount of 3-O-methyl-D-mannose ($R_{mannose}$ 2.40 (s) and 2.22 (w)). This was further examined electrophoretically and shown to be identical with 2-O-methyl-D-mannose made from D-mannose diethyl mercaptal (vide supra) which appeared to be a better method.

Preparation of 3-O-Methyl-D-mannose.

To a suspension of methyl 4,6-O-ethylidene-α-D-mannopyranoside (3.50g) in ice-cold acetic anhydride (8ml) was added ice-cold fuming nitric acid (3.50ml) in acetic anhydride (8ml) and the mixture was shaken in an ice-water bath for 15 minutes. The acetic anhydride solution was decanted into ice-water (100ml). Some starting material did not react and the treatment was repeated three more times. The acetic anhydride solutions were poured into the same ice-water. The aqueous layer was decanted from the sirupy

product (A), neutralized with solid sodium carbonate, and extracted with chloroform (50ml x 5). Evaporation of the solvent gave a sirup (B) 210mg), λ max 2.90μ (OH), 5.76μ (O-acetate), 6.10μ (O-nitrate), 8.13μ (O-acetate).

The sirupy product (A) was dissolved in chloroform and washed with aqueous sodium carbonate solution. Evaporation of the chloroform solution gave a sirup (3.38g), λ max 2.90μ (OH), 5.76μ (O-acetate), 6.10μ (O-nitrate), 7.85μ (O-nitrate), 8.13μ (O-acetate). The OH stretching band at 2.90μ was weaker compared with that of the above sirup.

The combined sirup ((A) + (B)) (3.59g) was dissolved in a mixture of methanol (150ml) and chloroform (150ml) and sodium (3.0g) was added to the solution, and the reaction mixture was kept in a cold water bath for 1 hour. The solution was neutralized with glacial acetic acid and then evaporated. The residue was dissolved in aqueous sodium carbonate solution and extracted with chloroform (50ml x 5). Evaporation of the chloroform solution gave a sirup (2.92g), λmax 2.90μ (OH), 8.10μ (O-nitrate), 7.85μ (O-nitrate).

The deacetylated sirup (1.47g) was dissolved in chloroform (10ml) and a solution of tosyl chloride (3.2g) in pyridine (5ml) was added to the solution. The mixture was kept at 50-60°C for 48 hours, poured into ice-water, and extracted with chloroform (30ml x 4). The extracts were successively washed twice with 2N sulphuric acid, with aqueous sodium carbonate, and with water. Evaporation of the chloroform solution gave a sirup (1.64g), λ max 6.10μ (O-nitrate), 6.30μ (aromatic C=C), 7.30μ (O-tosylate), 7.85μ (O-nit-

rate), 8.52μ (O-tosylate).

The tosylated sirup (1.64g) was dissolved in glacial acetic acid (35ml) and a mixture of iron (2.5g) and zinc (2.5g) was added portionwise. The mixture was stirred at room temperature for 15 minutes, and then filtered in order to remove the inorganic substances which were washed with chloroform (30ml x 3). The filtrate and washings were washed with aqueous sodium carbonate solution. Evaporation of the chloroform solution gave a sirup (1.42g), λ max 2.90μ (OH), 6.30μ (aromatic C=C), 7.35μ (O-tosylate), 8.50μ (O-tosylate).

The denitrated sirup (1.42g) was methylated three times using methyl iodide (50ml), silver oxide (10g), and drierite (10g) (48 hours each time at the boiling point of methyl iodide). A sirup (1.32g) was obtained and the infrared spectrum showed an almost negligible free hydroxyl band.

The methylated sirup (1.32g) was dissolved in a mixture of water (3ml) and methanol (27ml). After 4% sodium amalgam (12.0g) was added, the mixture was stirred for 48 hours at room temperature. The liberated mercury was filtered off and the filtrate was neutralized with dry ice. The residue obtained by evaporation of the neutralized filtrate was extracted with chloroform (20ml x 3). A sirup (470mg), λ max 2.86μ (OH), was obtained by evaporation of the extracts.

The detosylated sirup (470mg) was refluxed for 10 hours in 0.5N hydrochloric acid (20ml). The hydrolysate was neutralized with silver carbonate, treated with hydrogen sulphide, evaporated to a sirup (204mg), which showed two spots (Mg 0.87 (w) (mannose?) and 0.50 (s) by electrophoresis (1000-

1100V, 25-35mA, 45 minutes), and three spots ($R_{mannose}$ 3.34 (w), 2.50 (s), 1.00 (m), (8 hours)) and two spots ($R_{mannose}$ 2.26 (s) and 1.00 (w) (54 hours)) by paper chromatography.

The sirup (204mg) was chromatographed on a 15cm-wide Whatman No. 3MM sheet for 72 hours and from the area of $R_{mannose}$ 2.26 a sirup (51.3mg) was recovered. The sirup was examined by paper chromatography for 96 hours in butanone-water azeotrope saturated with sodium borate and gave a strong spot ($R_{mannose}$ 2.22) and a weak spot ($R_{mannose}$ 2.40=2-O-methyl-D-mannose) without any clear separation.

The sirup (14.3mg) was dissolved in 20% acetic acid (2ml) to which phenylhydrazine (26.5mg) in absolute ethanol (0.15ml) was added. The mixture was heated in boiling water for several hours. On cooling of the reaction mixture, yellow crystals precipated. The crystals melted at 165°C after recrystallization from ethanol-water. No depression of the melting point was observed when mixed with synthetic 3-O-methyl-D-glucose phenylosazone.

Preparation of 6-O-Methyl-D-mannose.

Methyl α-D-mannopyranoside (2.60g) was dissolved in pyridine (25ml) and trityl chloride (5.20g) was added to the solution. The mixture was left to stand overnight at room temperature (precipitates of pyridine hydrochloride), and after dilution of the reaction mixture with pyridine (20ml) acetic anhydride (40ml) was added and the mixture was kept at room temperature for 48 hours. The reaction mixture was poured into ice-water and extracted with chloroform (100ml x 3). The extracts, after washing

with ice-cold 1 N sulphuric acid (150ml x 2) and with saturated sodium bicarbonate solution, gave a sirup (5.51g).

The sirup (2.57g) was dissolved in glacial acetic acid (20ml) and cooled in an ice bath, and treated with glacial acetic acid saturated with hydrogen bromide. After a few minutes white crystals (triphenylmethyl bromide) precipitated. The reaction mixture was filtered and washed with glacial acetic acid, and the filtrate and washing were poured into ice-water. The sirupy product was extracted with chloroform (50ml x 3) and washed with saturated sodium bicarbonate solution. The chloroform solution gave, on evaporation, a sirup (1.40g) in which white crystals (triphenyl carbinol) were also observed.

The detritylated product (1.40g) was methylated three times using methyl iodide (50ml), silver oxide (10g), and drierite (10g) (24 hours each time under reflux), and the methylated product (1.30g) was hydrolysed by refluxing for 5 hours in a mixture of ethanol (20ml) and 15% hydrochloric acid (10ml). The deionized (Duolite A-4 resin) hydrolysate gave a sirup (759mg) which was chromatographed on a cellulose-hydrocellulose column (45 x 3cm) (rate of flow 10ml/20 minutes). Tubes No. 79-93 gave a sirup (17.7mg) which showed only one spot of Mg 0.68 and tubes No. 94-145 a sirupy mixture (41.6mg) (Mg 0.81, 0.69, 0.50, and 0.41).

The sirup (10.0mg) of Mg 0.68 was dissolved in 20% acetic acid (2ml) and was heated for 5 hours in boiling water with phenylhydrazine (20.0mg) in absolute ethanol (0.1ml) and a little sodium bisulphite. The phenylosazone which precipitated on cooling melted at 171°c after recrystalliza-

tion from ethanol-water.

C. D-Galactose Diethyl Mercaptal.

Methylation of D-Galactose Diethyl Mercaptal (I).

D-Galactose diethyl mercaptal (0.50g) was dissolved in tetrahydrofuran (150ml), and silver oxide (2.00g), drierite (3g), and methyl iodide (20ml) were added to the solution. The mixture was shaken at room temperature for 5 hours. After methyl iodide (20.0ml) was added, the mixture was shaken for another 5 hours. The inorganic substances were filtered and then washed with tetrahydrofuran (20ml). The filtrate and washing were evaporated to a sirup which was redissolved in chloroform (20ml) and the undissolved impurities were removed by filtration. Evaporation of the chloroform solution gave a sirup (470mg).

The sirup (470mg) was hydrolyzed by refluxing in a mixture of ethanol (10ml) and 18% hydrochloric acid (2ml) for 5 hours (evolution of ethyl mercaptan). The hydrolysate was neutralized with Duolite A-4 resin, and the evaporation gave a brown sirup (275mg). The sirup was decolorized by treatment with charcoal in hot water, and a yellow sirup (259mg) was obtained.

Chromatographic examination showed that the sirup consisted of poly-O-methylated D-galactoses; Rf values were as follows:
Rf 0.89 (s), 0.77 (m), 0.69 (s), 0.50 (s), 0.39 (m), 0.17 (s), and 0.05 (vw). The sirup was not investigated further.

Methylation of D-Galactose Diethyl Mercaptal (II).

D-Galactose diethyl mercaptal (0.50g) was dissolved in tetrahydrofur-

an (150ml), and silver oxide (2.00g), drierite (3g), and methyl iodide (20.0ml) were added to the solution. The mixture was shaken for 7 hours at room temperature. The inorganic substances were filtered and then washed with tetrahydrofuran (20ml). The filtrate and washing were evaporated into a sirup, which was redissolved in chloroform (20ml) in order to remove a small amount of inorganic impurities by filtration. The chloroform solution gave on evaporation a yellow sirup (470mg).

The methylation product (470mg) was dissolved in ethanol (10ml), and 18% hydrochloric acid (2ml) was added to the solution. Hydrolysis was effected by refluxing the solution for 5 hours (evolution of ethyl mercaptan). A yellow sirup (255mg) (mixture(II)) was obtained by evaporation of the neutralized hydrolysate (with Duolite A-4 resin) and treatment with charcoal.

Paper chromatography showed that the sirup was a mixture of mono-O-methyl-D-galactoses (Rf 0.05 (s)) and unchanged D-galactose (Rf 0.01 (s)), plus several fast-running components whose Rf values were difficult to calculate due to the weakness of their spots.

Methylation of D-Galactose Diethyl Mercaptal (III).

Another methylation under the same conditions as for methylation (II), using double quantities of the mercaptal and the reagents gave a sirup (529mg) (mixture (III)) of the O-methylated D-galactoses. Paper chromatography showed a similar pattern.

Methylation of D-Galactose Diethyl Mercaptal (IV).

Double quantities of the mercaptal and the reagents were shaken for 6 hours at room temperature, and the methylation product (1.09g) gave on

hydrolysis a sirup (580mg) (mixture (IV)), paper chromatography of which showed a similar pattern.

Chromatographic Separation of the Mixture of Methylated D-Galactoses.

To butanone-water azeotrope (100ml) sodium borate decahydrate (1g) was added and the mixture was left to stand for several hours after shaking. The mixture was decanted and the supernatant solution was used as a developing solvent.

Authentic samples of all mono-O-methyl-D-galactopyranoses were pre-examined by paper chromatography using this solvent for 60 hours. The results are shown in Table XII.

The mixture (II) of O-methylated D-galactoses was chromatographed in this solvent for 40 hours, and was shown to contain three mono-O-methyl-D-galactoses, namely 2-,3-, and 6-O-methyl-D-galactoses. The results are shown in Table XIII.

Neither authentic samples nor methylation product (II) gave clear separation of these mono-O-methyl-D-galactoses when they were chromatographed for 60 hours in butanone-water azeotrope (no sodium borate).

Isolation of the Mono-O-methyl-D-galactoses in the Mixture (II) and Electrophoresis of the Components.

The mixture (II) (255.3mg) was chromatographed on four 15cm-wide Whatman No. 3MM sheets for 60 hours in butanone-water azeotrope saturated with sodium borate. The spots were located on both edges of each sheet, and the area of each component was extracted with cold water (50ml x 3). Evaporation of the extracts gave a sirup from each component. These sirups were

examined by electrophoresis (950-1050V, 25-30mA, 45 minutes). The results are shown in Table XIV.

Chromatographic Isolation of Mono-O-methyl-D-galactoses in the Mixture (III) and (IV) and Electrophoresis of the Components.

The mixture (III) (529mg) was chromatographed on eight 15cm-wide Whatman No. 3MM sheets for 60 hours in butanone-water azeotrope saturated with sodium borate decahydrate, and the components were located on both edges of each sheet. Each component was extracted with cold water (50ml x 3), and the extracts were evaporated to a sirup which was then examined electrophoretically (950-1050V, 25-30mA, 40 minutes). The results are shown in Table XV.

The mixture (IV) (580mg) was separated similarly, and the results are shown in Table XVI. The electrophoresis was performed under similar conditions (800-1000V, 25mA, 60 minutes).

Methylation of D-Galactose Diethyl Mercaptal (V).

D-Galactose diethyl mercaptal (2.00g) was dissolved in tetrahydrofuran (600ml), and silver oxide (8.00g), drierite (12g), and methyl iodide (80ml) were added to the solution. The mixture was shaken for 7 hours at room temperature, and then treated as described before. The sirupy methylation product (1.73g) was hydrolyzed by refluxing for 5 hours in a mixture of ethanol (20ml) and 2N sulphuric acid (20ml). The hydrolysate was treated as before to give a yellow sirup (1.00g). Paper chromatography showed the following Rf values:

Rf 0.34 (vw), 0.17 (vw), 0.12 (w), 0.05 (m) and 0.01 (m).

TABLE XII.

Chromatographic separation of standard mono-O-methyl-D-galactoses in butanone-water azeotrope saturated with sodium borate decahydrate.

Compound	Travelling distance (mm) after 60 hours	$R_{galactose}$
2-O-Methyl-D-galactose	335	3.60
3-O-Methyl-D-galactose	239	2.67
4-O-Methyl-D-galactose	212	2.28
6-O-Methyl-D-galactose	281	3.02
D-Galactose	93	1.00

TABLE XIII.

Chromatographic separation of the mixture (II) of O-methylated D-galactoses in butanone-water azeotrope saturated with sodium borate decahydrate.

Compound	Travelling distance (mm) of spots after 40 hours
Mixture (II)	205 (m), 176 (m), 143 (m)
2-O-Methyl-D-galactose	205 ($R_{galactose}$ 3.46)
3-O-Methyl-D-galactose	143 ($R_{galactose}$ 2.34)
4-O-Methyl-D-galactose	127 ($R_{galactose}$ 2.16)
6-O-Methyl-D-galactose	176 ($R_{galactose}$ 2.89)
D-Galactose	59 ($R_{galactose}$ 1.00)

TABLE XIV.

Electrophoresis of the isolated components of mono-O-methyl-D-galactoses in the mixture (II).

Component	Mg value
Sirup of $R_{galactose}$ 3.46	0.43
Sirup of $R_{galactose}$ 2.89	0.90
Sirup of $R_{galactose}$ 2.43	0.76
2-O-Methyl-D-galactose	0.43
3-O-Methyl-D-galactose	0.76
6-O-Methyl-D-galactose	0.90

TABLE XV.

Electrophoresis of the isolated components of mono-O-methyl-D-galactoses in the mixture (III)

Component	Yield (mg)	Mg value
Sirup of $R_{galactose}$ 3.37	13.2	0.45
Sirup of $R_{galactose}$ 2.88	8.1	0.90
Sirup of $R_{galactose}$ 2.38	14.7	0.81
2-O-Methyl-D-galactose	—	0.45
3-O-Methyl-D-galactose	—	0.81
6-O-Methyl-D-galactose	—	0.90

TABLE XVI.

Electrophoresis of the isolated components of mono-O-methyl-D-galactoses in the mixture (IV).

Component	Yield (mg)	Mg value
Sirup of $R_{galactose}$ 3.34	10.2	0.43
Sirup of $R_{galactose}$ 2.82	5.8	0.87
Sirup of $R_{galactose}$ 2.37	16.3	0.76
2-O-Methyl-D-galactose	—	0.43
3-O-Methyl-D-galactose	—	0.76
6-O-Methyl-D-galactose	—	0.87

The sirup (1.00g) was chromatographed on a cellulose-hydrocellulose column (45 x 3cm) in butanone-water azeotrope (rate of flow: 10ml/10 minutes). In tubes No. 80-200, the component of Rf 0.05 was found, but paper chromatography for 24 hours showed that these tubes contained several components without any clear separation. These fractions (tubes No. 80-200) were evaporated and a sirup (48.2mg) (mixture (V)) was obtained.

In tubes No. 40-70, the components of Rf 0.17 and 0.12 were detected but no clear separation was observed of these sugars. Evaporation of these fractions (tubes No. 40-70) gave a sirup (21.2mg), which was electrophoretically examined (vide infra).

Electrophoresis of the Fast-running Component.

The fast-running sirup separated on the column from methylation V was examined electrophoretically (950-1050V, 25-30mA, 50 minutes) and two strong spots (Mg 0.23 and 0.28) were detected.

Colorimetric Analysis of the Mixture (V).

(1) Preparation of standard curve.

From the original solution containing 914γ/ml of 6-O-methyl-D-galactose, five solutions given in Table XVII were prepared. The results are shown in the Table, and the curve is given in Fig. 29.

(2) Analysis of mixture (V) of mono-O-methyl-D-galactoses.

The mixture (V) was chromatographed for 60 hours in butanone-water azeotrope saturated with sodium borate decahydrate on a 15cm-wide Whatman No. 1 sheet. The results are shown in Table XVIII.

TABLE XVII.

Preparation of standard curve of 6-O-methyl-D-galactose.

Solution	Concentration (γ/2ml)	Readings at 490mμ	Average
1	73.1	0.74 0.75 0.74	0.74
2	51.2	0.56 0.53 0.52	0.54
3	36.6	0.390 0.390 (0.352)	0.390
4	18.3	0.200 (0.255) 0.202	0.201
5	9.1	0.093 0.100 0.088	0.937

Fig. 29. Standard curve of 6-O-methyl-D-galactose.

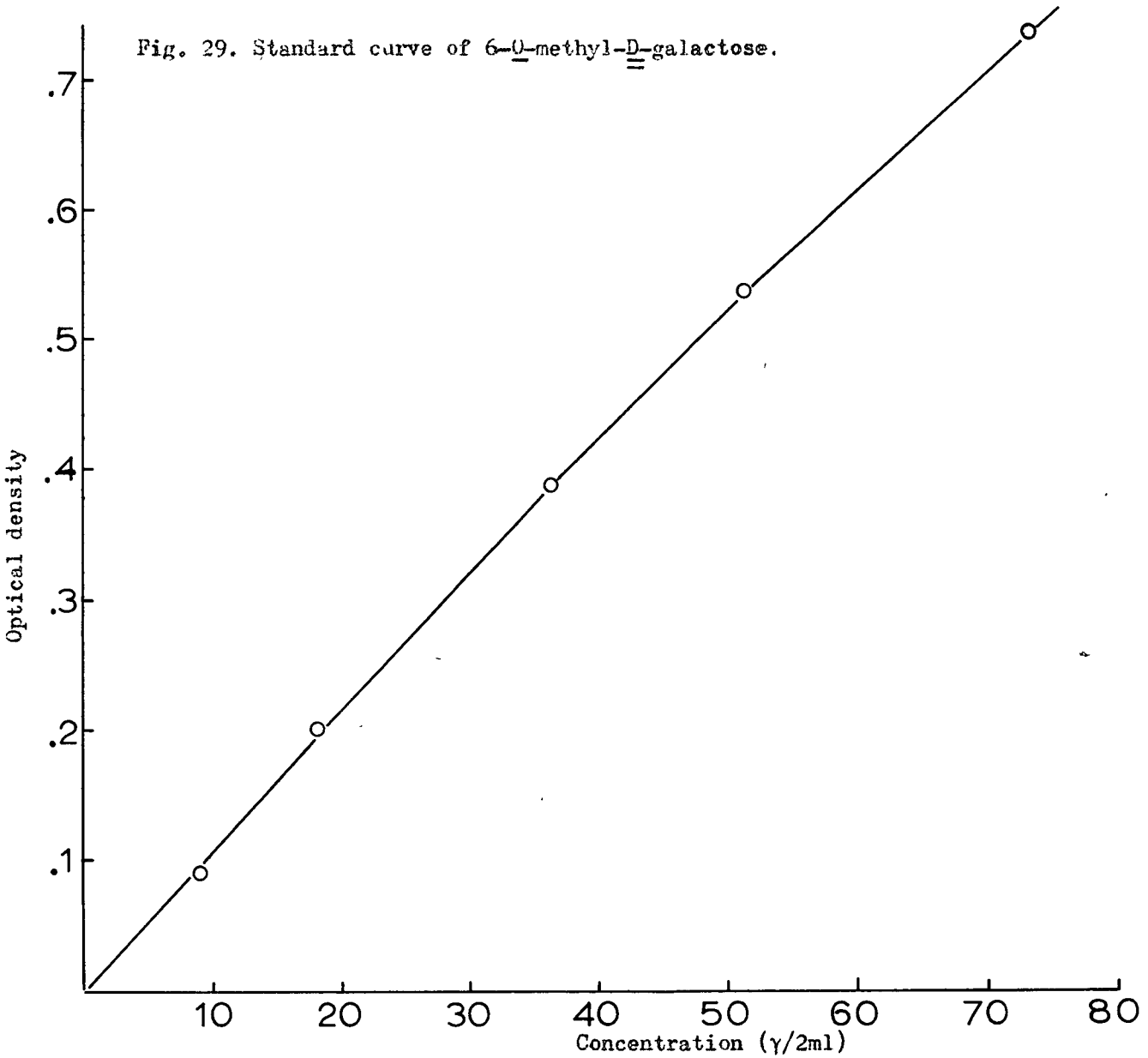


Table XVIII. Colorimetric analysis of mono-O-methyl-D-galactoses

Component		Readings at 490 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-galactose	(sugar	0.280	0.290	0.300	0.290	0.269	25.0
	(blank	0.115	0.125	0.123	0.121		
3-O-Methyl-D-galactose	(sugar	0.440	0.460	0.440	0.447	0.305	28.0
	(blank	0.135	0.140	0.150	0.142		
6-O-Methyl-D-galactose	(sugar	0.290	0.290	0.275	0.285	0.185	18.0
	(blank	0.100	0.100	0.100	0.100		

Identification of the Component $R_{galactose}$ 3.60 as 2-O-Methyl-D-galactose.

The components which corresponded to 2-O-methyl-D-galactose ($R_{galactose}$ 3.60) from the mixtures (III) and (IV) were combined. Although the concentrated solution of the sirup in water gave a negative test on tumeric test paper, the following procedure was applied in order to remove possible traces of borate complexes.

To the sirup (18.2mg) dried *in vacuo* at the boiling point of acetone for several hours, was added methanol (ca. 5ml) and the solution was evaporated *in vacuo* without heating. After the procedure was repeated about twenty times, the residue was dissolved in a small amount of warm ethanol (absolute) and ether was added to turbidity. After seeding with an authentic sample of 2-O-methyl-D-galactose, colorless crystals were precipitated on the wall, and flocculent impurities (cellulose?) were also observed in the solution. The solvent was decanted and the crystals on the wall were washed with ether. The crystals melted at 152-154ºC. Admixture with authentic 2-O-methyl-D-galactose (m.p. 153-156ºC) gave a melting point of 152-154ºC.

Identification of the Component $R_{galactose}$ 2.67 as 3-O-Methyl-D-galactose Phenylosazone.

The sirup of $R_{galactose}$ 2.67 isolated from the mixtures (III) and (IV) failed to crystallize after treatment with methanol.

The sirup (23.4mg) was dissolved in 20% acetic acid (3ml). Phenylhydra-

zine (39.1mg) in absolute ethanol (0.22ml) and a trace of sodium metabisulphite were added to the solution and the mixture was heated for 5 hours in boiling water. On cooling, yellow crystals precipitated and a brown tar was also found in the solution. The crystals were filtered and recrystallized from water after treatment with charcoal. The yellow crystals m.p. 177-179ºC, showed no depression of the melting point when mixed with 3-O-methyl-D-galactose phenylosazone derived from 2,3-di-O-methyl-D-galactose.

Identification of the Component $R_{galactose}$ 3.02 as 6-O-Methyl-D-galactose Phenylhydrazone.

The sirup of $R_{galactose}$ 3.02 isolated from the mixtures (III) and (IV) failed to crystallize after treatment with methanol.

The sirup (16.5mg) was dissolved in absolute ethanol (1ml) and phenylhydrazine (9.2mg) in absolute ethanol (0.06ml) was added to the solution. The mixture was warmed for about 20 minutes and on addition of petroleum ether, white crystals precipitated. The crystals (14.4mg) was recrystallized from ethanol-petroleum ether and the purified product melted at 161-162ºC. When the product was recrystallized from methanol-water the melting point was raised to 171-173ºC. Mixed m.p. with authentic 6-O-methyl-D-galactose phenylhydrazon, m.p. 172-173ºC, was 171-172ºC.

Synthesis of 2,3-Di-O-methyl-D-galactose.

(1) Methyl 4,6-O-benzylidene-α-D-galactopyranoside.

Anhydrous methyl α-D-galactopyranoside (0.82g) was shaken for 24 hours in freshly distilled benzaldehyde (20ml) with fused zinc chloride (3g). Water (20ml) and methanol (2ml) were added to the mixture and the excess ben-

zaldehyde was extracted with petroleum ether. A slight excess of saturated sodium carbonate solution was added to the aqueous layer. The precipitated inorganic substances were filtered and then extracted with boiling methanol (30ml x 2). The filtrate and the extracts were evaporated to dryness and the residue was recrystallized from ethanol-petroleum ether, m.p. 177-178°C. Yield 1.14g.

(2) Methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-galactopyranoside.

Methyl 4,6-O-benzylidene-α-D-galactopyranoside (1.14g) was methylated for 48 hours in methyl iodide (50ml) with silver oxide (10g, added in five portions) and drierite (10g). The methylation was repeated again and the sirupy product (1.12g) showed no hydroxyl band in its infrared spectrum. The sirup crystallized on standing.

(3) 2,3-Di-O-methyl-D-galactose.

Methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-galacto-pyranoside (1.08g) was hydrolyzed for 3 hours in boiling 1N sulphuric acid (20ml). The liberated benzaldehyde was extracted with petroleum ether. After neutralization with Duolite A-4 resin, the aqueous solution was evaporated to a sirup (601mg) having Rf 0.29 and $R_{galactose}$ 5.00 (20 hours) in ethyl acetate-pyridine-water), $[\alpha]_D^{25}$ +51.8° (c, 2.15 in water).

(4) 3-O-Methyl-D-galactose phenylosazone.

2,3-Di-O-methyl-D-galactose (229mg) was dissolved in 20% acetic acid (10ml), and phenylhydrazine (430mg) in absolute ethanol (2.4ml) and a little sodium metabisulphite were added. The mixture was heated for 5 hours in boiling water. The reaction mixture was treated with charcoal to remove a brown

tar, and from the filtrate yellow crystals (42.1mg) precipitated on cooling. The crystals were recrystallized from water, m.p. 178°C.

Preparation of 6-O-Methyl-D-galactose Phenylhydrazone.

6-O-Methyl-D-galactose (50.0mg) was dissolved in water (1ml), and phenylhydrazine hydrochloride (100mg) and crystalline sodium acetate (150mg) were added. The phenylhydrazine base was liberated as a brown oil and on scratching white crystals precipitated after several minutes. After standing for an hour, the crystals (72.3mg) were filtered and recrystallized from methanol-water, m.p. 172-173°C.

D. L-Arabinose Diethyl Mercaptal.

Methylation of L-Arabinose Diethyl Mercaptal.

L-Arabinose diethyl mercaptal (0.50g) was dissolved in tetrahydrofuran (50ml), and silver oxide (0.50g) and drierite (3g) were added to the solution. Methyl iodide (10.0ml) was added to the mixture which was then shaken at room temperature for 20 hours, during which time methyl iodide (30.0ml) was added in aliquots of 10ml at 5 hours' intervals. The reaction mixture was filtered in order to remove inorganic substances which were washed with tetrahydrofuran (20ml). The filtrate and washing were evaporated to a sirup in which a small amount of inorganic impurities was observed. The sirup was redissolved in chloroform (20ml) and the inorganic impurities were removed by filtration. The chloroform solution was evaporated into a light yellow sirup (564mg).

The sirup (564mg) was dissolved in a mixture of ethanol (10ml) and con-

centrated hydrochloric acid (2.5ml) and refluxed for 5 hours. After neutralization with Duolite A-4 resin, the solution was evaporated into a light brown sirup (291mg). The sirup showed the following spots by paper chromatography:

Rf 0.91-0.75 (two(?) components) (w), 0.59 (w), 0.47 (w), 0.36 (w), 0.30 (w), 0.15 (vs), and 0.05 (s).

Chromatographic Separation of the Methylated L-Arabinoses.

The sirup was chromatographed for 13 hours on four 15cm-wide Whatman No. 3MM papers. The components which corresponded to Rf 0.30 and 0.15 were detected on both edges of the chromatograms, and the parts containing these components were extracted with boiling methanol (30ml). Yields of the fast- and slow-running components were 7.0 and 32.6mg, respectively.

These components were examined by electrophoresis (750-1000v, 25mA, 1.5 hours, and the results are shown in Table XIX.

TABLE XIX.

Electrophoresis of the fast- and slow-running components of O-methylated L-arabinoses

Compound	Mg value
Slow-running component (Rf 0.15)	0.33
Fast-running component (Rf 0.30)	0.80 (w), 0.00 (s)
2-O-Methyl-L-arabinose	0.33
5-O-Methyl-L-arabinose	0.80

Identification of the Slow-Running Component as 2-O-Methyl-L-arabinose.

The sirup (20.7mg) was dissolved in water (2ml) and excess bromine (about 1ml) was added to the solution. The solution was kept in the dark at room temperature. After 24 hours the solution showed no p-anisidine-positive spot on paper chromatography. Water (5ml) was added to the reaction mixture and excess bromine was removed by aeration. The clear solution was then neutralized with silver carbonate, and silver bromide and excess silver carbonate were filtered off. The filtrate was treated with hydrogen sulphide and then centrifuged. Evaporation of the supernatant solution followed by drying *in vacuo* in boiling water for several hours gave a colorless sirup (11.0mg), which was dissolved in absolute ethanol (10ml), saturated with dry ammonia at 0°C and kept overnight at room temperature. Evaporation of the solvent gave a sirup (9.0mg) which was crystallised from ethanol-petroleum ether. Yield was 45% based on the monomethylarabinose. Melting point and mixed m.p. with synthetic 2-O-methyl-L-arabonamide were 131-132°C. Mixed m.p. with 3-O-methyl-L-arabonamide was 80-105°C.

Weerman Test of 2-O-Methyl-L-arabonamide.

2-O-Methyl-L-arabonamide (10.1mg) was dissolved in water (1ml) and, after addition of 5% sodium hypochlorite solution (0.2ml), the solution was stored at 0°C for 30 minutes. Excess hypochlorite was destroyed with 0.1N sodium thiosulphate solution (3 drops). Crystalline sodium acetate (excess) and the saturated solution of semicarbazide hydrochloride (3 drops)

were added to the solution. No precipitation occurred after 24 hours at room temperature.

Colorimetric Analysis of the Fast-Running Components.

(1) Preparation of standard curve.

From the original solution containing 1940γ/ml of 5-O-methyl-L-arabinose (electrophoretically and chromatographically pure sirup), six solutions of various concentrations were prepared. The results are shown in Table XX, and the curve is given in Fig. 30.

(2) Analysis of the fast-running component.

A part of the fast-running component was separated electrophoretically (950-1100V, 25mA, 60 minutes). The results are shown in Table XXI. The analysis was performed twice, and in the second run (Table XXII) two sheets of the same size were used in view of the low concentration of the spot of Mg 0.80 in the first run.

Synthesis of 2-O-Methyl-L-arabinose.

This compound was prepared by the published method from methyl-β-L-arabinopyranoside (m.p. 168°C) (4.00g), anhydrous copper sulphate (50.0g) and dry acetone (200ml). The sirupy 3,4-O-iso-propylidene derivative (3.29g) was methylated three times by Purdie's method and the methylated product (2.45g), which showed no appreciable band of hydroxyl group in its infrared spectrum, was hydrolyzed with 1N sulphuric acid (20ml) for 4 hours on steam bath. Neutralisation with Duolite A-4 resin and evaporation gave a sirup (1.67g) which on chromatographic examination showed two strong spots ($R_{arabinose}$ 3.76 and 10.7) and a weak spot ($R_{arabinose}$ 1.00). Separation of the sirup on a cellulose-hydrocellulose column yielded chromatographically ($R_{arabinose}$ 3.76) and electrophoretically (Mg 0.33) pure 2-O-methyl-

TABLE XX.

Preparation of standard curve of 5-O-methyl-L-arabinose

Solution	Concentration (γ/2ml)	Readings at 480mμ			Average
1	77.6	0.67	0.68	0.66	0.67
2	51.7	0.460	0.455	0.455	0.457
3	38.8	0.358	0.350	0.360	0.356
4	19.4	0.185	0.195	0.195	0.192
5	9.7	0.102	0.100	0.102	0.101
6	4.9	0.052	0.050	0.055	0.052

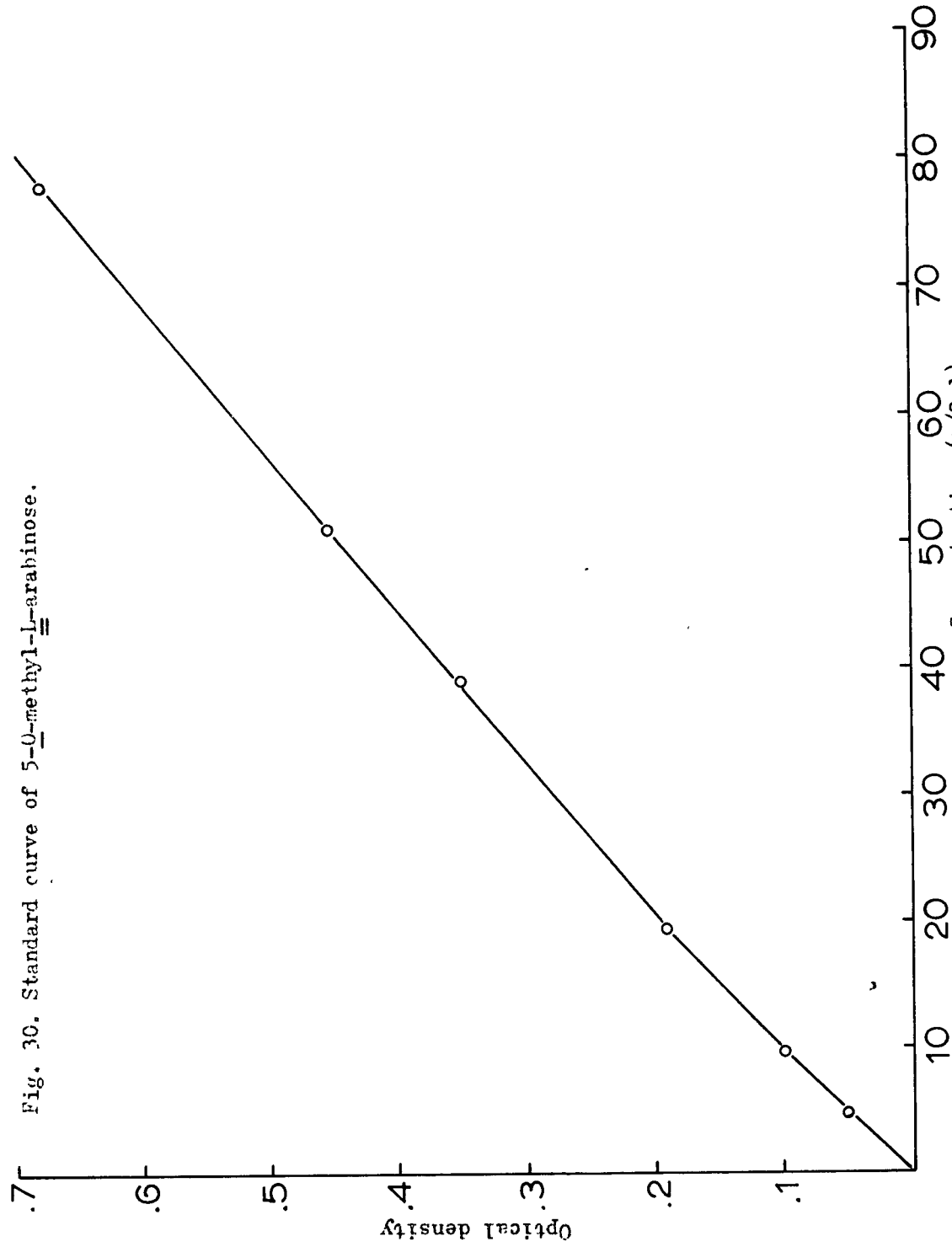


Fig. 30. Standard curve of 5-O-methyl-L-arabinose.

Table XXI. Colorimetric analysis of the fast-running components of O-methylated L-arabinoses

Component		Readings at 480 mμ			Average	Difference	Concentration (γ/2 ml)
5-O-Methyl-L-arabinose	(sugar	0.057	0.055	0.060	0.057	0.018	1.7
	(blank	0.040	0.040	0.038	0.039		
Poly-O-methyl-L-arabinoses							
	(sugar	0.160	0.150	0.175	0.162	0.095	9.0
	(blank	0.070	0.065	0.065	0.067		

Table XXII. Colorimetric analysis of the fast-running components of O-methylated L-arabinoses

Component		Readings at 480 mμ			Average	Difference	Concentration (γ/2 ml)
5-O-Methyl-L-arabinose	(sugar	0.153	0.130	0.130	0.138	0.053	5.0
	(blank	0.085	0.080	0.084	0.083		
Poly-O-methyl-L-arabinoses							
	(sugar	0.390	0.388	0.398	0.392	0.240	25.0
	(blank	0.151	0.155	0.150	0.152		

L-arabinose (sirup, 691mg). $[\alpha]_D^{25}$ + 94.0º (c, 0.26 in water).

When the preparation of this compound was attempted by refluxing for 24 hours a mixture of the sirupy glycosides (10g) with anhydrous copper sulphate (50g) and dry acetone (250ml), the yield of chromatographically pure 2-O-methyl-L-arabinose was only 438mg.

2-O-Methyl-L-arabono-γ-lactone and -arabonamide.

Synthetic 2-O-methyl-L-arabinose (173mg) was dissolved in water (5ml) and excess bromine (ca. 2ml) was added to the solution. The mixture was kept in the dark at room temperature for 24 hours, completion of oxidation being demonstrated by the absence of the original p-anisidine-positive spot on paper chromatography. Liberated hydrogen bromide was neutralized with silver carbonate and the filtrate, on evaporation after treatment with hydrogen sulphide and centrifugation, gave the lactone (153mg, 89.5%) as a sirup which was crystallized from acetone or ethyl acetate as fine needles, m.p. 88ºC, $[\alpha]_D^{25}$ -49.1º → -46.3º (after 24 hours) (c, 0.128 in water), λ_{max}^{nujol} 5.73μ (c=O). Anal. Calc. for $C_6H_{10}O_5$: OCH_3, 19.2%. Found: OCH_3, 19.2, 19.5%.

The lactone (111mg) was dissolved in dry methanol (5ml) and the solution was saturated with dry ammonia in ice-water bath. The solution was kept overnight at room temperature, and evaporation of the solvent gave the solid amide (92mg, 76%) which was recrystallized from ethyl acetate-acetone or ethyl acetate-methanol as fine needles. M.p. 131ºC. The infrared spectra of this amide and that obtained from the slow-running component, i.e. 2-O-methyl-L-arabinose, were identical.

Synthesis and Characterisation of 5-O-Methyl-L-arabinose.

(1) Synthesis.

To ethyl 2,3-di-O-acetyl-5-O-trityl-α-L-arabinoside (8.30g) were added water (62ml) and glacial acetic acid (250ml), and the mixture was heated for an hour in boiling water. The solution was concentrated to give a mixture of tritanol and yellow sirup. The mixture was dissolved in chloroform (10ml) and adsorbed on an alumina column (3.2 x 20cm) which was washed previously with benzene. Tritanol was eluted first with benzene (800ml) and ethyl 2,3-di-O-acetyl-α-L-arabinofuranoside was eluted with chloroform (1500ml). Evaporation of the eluate gave a yellow sirup (3.59g, 83%). $[\alpha]_D^{25}$ -58.8º (c, 0.218 in chloroform). Infrared spectrum of the sirup showed complete lack of aromatic character.

The detritylated sirup (3.59g) and drierite (5g) were stirred under reflux in methyl iodide, (50ml) for 60 hours. Silver oxide (10.0g) was added in ten portions at about 30 minutes' intervals. Excess methyl iodide was removed by distillation and the residue was extracted with boiling chloroform (50ml x 5). Evaporation of the solvent gave a yellow sirup (2.99g).

The procedure was repeated again under the same conditions and the yellow sirup (2.48g, 65.5%, $[\alpha]_D^{25}$ -49.0º (c, 0.306 in chloroform)), showed no hydroxyl band on the infrared spectrum.

The methylated sirup (2.48g) was heated in a mixture of 1N sodium hydroxide (40ml) and methanol (40ml) for an hour in boiling water, and then neutralized with IR-120 resin. The neutral solution was concentrated to a volume of 65ml and 1N hydrochloric acid (65ml) was added. The mixture was heated in

boiling water and the hydrolysis was followed polarimetrically. The initial rotation (-1.440º in 1dm tube) changed after 4 hours to a constant value (0.155º in 0.5dm tube). The acidic solution was neutralized with Duolite A-4 resin and then concentrated into a sirup (1.10g, 74%). Paper chromatography of the sirup showed a strong spot of Rf 0.30 and weak spots of Rf's 0.64, 0.53, 0.22, and 0.12.

The hydrolysis product (1.10g) was dissolved in methanol (2ml) and was chromatographed on a cellulose-hydrocellulose column (3 x 40cm) using butanone-water azeotrope (rate of flow: 10ml/30 minutes). Each fraction (10ml) was checked by paper chromatography. In tubes No. 13-23 a compound with Rf 0.30 was found with others of Rf 0.64 and 0.53, but in tubes No. 24-40 only the compound of Rf 0.30 was detected. On evaporation of the fractions No. 24-40 a colorless sirup (387mg) was obtained. Re-chromatography of fractions No. 13-23 under the same conditions gave a further quantity (120mg) of the sirup of Rf 0.30. Both were chromatographically and electrophoretically (Mg 0.80, 750-1000V, 25mA, 1.5 hours) pure and the total yield of the sirup was 507mg (46%). Anal. Calc. for $C_6H_{12}O_5$: OCH_3, 18.9%. Found: OCH_3 18.8, 19.0%. $[\alpha]_D^{25}$ -32.0º (c, 0.484 in water).

(2) Periodate Oxidation of 5-O-Methyl-L-arabinose.

Oxidation was carried out in the dark and at 5ºC.

(i) Determination of periodate consumption.

5-O-Methyl-L-arabinose (7.5mg) was dissolved in water (10ml), and 0.23M sodium metaperiodate solution (1ml) was added to the solution. A blank was

run concurrently. Aliquot portions (1ml) were withdrawn at intervals and the consumption of periodate was determined by means of the arsenite method. The results are shown below.

(ii) Estimation of formic acid produced.

After reacting overnight, an aliquot was titrated with 0.01N sodium hydroxide solution using methyl red as an indicator. The results are shown in Table XXIII.

TABLE XXIII.

Periodate oxidation of 5-O-methyl-L-arabinose

	Periodate consumed					Formic acid produced
Time, hours	0	1	2	3	overnight	overnight
Moles	1.97	2.68	2.70	2.86	2.86	3.09

(3) 5-O-Methyl-L-arabinose Phenylosazone.

5-O-Methyl-L-arabinose (111mg) was dissolved in 20% acetic acid (5ml), and phenylhydrazine (1ml) and sodium bisulphite (110mg) were added. The solution was kept for an hour at 80°C and then at room temperature overnight. The precipitated yellow crystals (50mg) were filtered and recrystallized from aqueous acetone, m.p. 154.5°C, $[\alpha]_D^{25}$ -16.6° (c, 0.4 in methanol). Dilution of the filtrate with water gave a further 70mg of the crystals which showed m.p. 154.5°C after recrystallization from aqueous acetone. Total yield of the phe-

nylosazone was 120mg (52%). Anal. Calc. for $C_{18}H_{22}O_3N_4$: C, 63.1; H, 6.5; N, 16.4; OCH_3, 9.1%. Found: C, 62.9; H, 6.4; N, 16.3, 16.6; OCH_3, 9.0, 9.2%.

(4) 5-O-Methyl-L-arabonolactone.

5-O-Methyl-L-arabinose (120mg) was dissolved in water (2ml) and kept at room temperature in the dark after addition of bromine (10 drops). The oxidation mixture showed no original spot (Rf 0.30) in paper chromatography after 30 hours. Water (10ml) was added to the mixture and excess bromine was removed by aeration, and the solution was treated with silver carbonate in order to neutralize hydrogen bromide. The inorganic substances were separated from the solution by centrifugation. Hydrogen sulphide was passed through the solution and the metal sulphide was removed by centrifugation. The solution was concentrated to dryness and heated in vacuo for several hours in boiling water to give a solid (77mg) which, after recrystallization from acetone-light petroleum ether, gave colorless needles, m.p. 135°C; $[\alpha]_D^{25}$ -76.2° (c, 0.22 in water) (no change after 20 hours); λ_{max}^{nujol} 5.62 μ (c=O). Anal. Calc. for $C_6H_{10}O_5$: OCH_3, 19.2%. Found- OCH_3, 19.1, 19.3%.

E. D-Xylose Diethyl Mercaptal.

Preparation of D-Xylose Diethyl Mercaptal (I).

D-Xylose (15.0g) was suspended in dry tetrahydrofuran (100ml) which contained 65% hydrogen chloride. (Hydrogen chloride was introduced into dry tetrahydrofuran at 0°C.) The suspension was cooled in an ice-water bath.

Ethyl mercaptan (10g) was added portionwise with vigorous shaking and with occasional cooling in ice-water. After all the ethyl mercaptan was added (about 10 minutes), the mixture was shaken for another 20 minutes, during which time all the D-xylose dissolved to give a homogeneous solution. Dry tetrahydrofuran (100ml) was added to the solution and the diluted solution was neutralized with excess barium carbonate. Anhydrous calcium chloride was added in order to remove the water formed during the condensation reaction. After the mixture was left for several hours, the inorganic substances were filtered and evaporation of the filtrate gave a mixture of sirup and a small amount of inorganic impurities. Extraction with dry tetrahydrofuran (ca. 50ml) and evaporation of the filtered extract gave a light yellow sirup (20.3g, 79%), which crystallized on dilution with a small amount of isopropyl alcohol. Recrystallization from isopropyl alcohol-petroleum ether gave colorless crystals (15.8g), which melted at 63-64°C. No depression of melting point was observed on admixture with D-xylose diethyl mercaptal prepared by method (II).

Preparation of D-Xylose Diethyl Mercaptal (II).

D-Xylose (4.5g), ethyl mercaptan (4.5g), and concentrated hydrochloric acid (4.5g) were shaken for 30 minutes with occasional cooling in ice-water. To the clear reaction mixture were added ice-water (50ml) and then sufficient Duolite A-4 resin to neutralize the acid. An additional amount (50ml) of water was needed to facilitate stirring. The neutral solution was filtered and the filtrate was evaporated to a light yellow sirup which was diluted

with isopropyl alcohol (10ml). Petroleum ether (3-4ml) was added to the solution, and after several hours' storage of the solution at 5°C a crystalline mass was obtained. The crude crystals were recrystallized from isopropyl alcohol-petroleum ether. Yield 3.2g (42%) M.p. 62-63°C.

Methylation of D-Xylose Diethyl Mercaptal (I).

D-Xylose diethyl mercaptal (1.50g, sirup) was dissolved in methyl iodide (25.0ml). Silver oxide (10.0g) and drierite (10g) were added to the solution and the mixture was shaken for 10 hours at room temperature. Initially the reaction was mildly exothermic and then gradually subsided. The reaction mixture was filtered and the inorganic substances were washed with dry tetrahydrofuran (10ml). The filtrate and washing were evaporated to a sirup, which was redissolved in chloroform (10ml) in order to remove by filtration inorganic impurities. Evaporation of the chloroform solution gave a sirup (1.32g).

The sirup (1.32g) was dissolved in ethanol (10ml), and water (1.5ml) and concentrated hydrochloric acid (1.5ml) were added. Hydrolysis was effected by refluxing the solution for 5 hours. The hydrolysate was neutralized with Duolite A-4 resin and evaporation of the neutral solution gave a light brown sirup (801mg) after treatment with charcoal.

Paper chromatography showed the following results which suggested polymethylation of the mercaptal.

Rf 0.94 (vs), 0.87 (s), 0.81 (vs), 0.65 (vw), 0.55 (s), 0.47 (w), 0.19 (w), and 0.04 (vvw).

Methylation of D-Xylose Diethyl Mercaptal (II).

D-Xylose diethyl mercaptal (600mg, sirup) was dissolved in a mixture of

methyl iodide (10.0ml) and dry tetrahydrofuran (10.0ml). After silver oxide (3.0g) and drierite (3g) were added, the mixture was shaken for 12 hours at room temperature. No evolution of heat was observed. Similar treatment of the reaction mixture gave a brown sirup (551mg) which was hydrolyzed by 5 hours' refluxing in a mixture of ethanol (10ml), water (0.75ml), and concentrated hydrochloric acid (0.75ml). A light yellow sirup (308mg) was obtained by similar treatment of the hydrolyzate. The sirup showed the following Rf values, indicating polymethylation of the mercaptal.
Rf 0.97 (s), 0.90 (s), 0.84 (vs), 0.67 (w), 0.58 (w) and 0.19 (w).

A methylation using the same quantities of reactants gave similar results after 5 hours- shaking, and unchanged D-xylose was detected on paper chromatograms when the methylation was carried out at 0ºC.

Methylation of D-Xylose Diethyl Mercaptal (III).

D-Xylose diethyl mercaptal (3.00g, sirup), was dissolved in a mixture of methyl iodide (35.0ml) and dry tetrahydrofuran (35.0ml). Silver oxide (7.0g) and drierite (7g) were added to the solution, and the mixture was shaken at room temperature for 3 hours. The reaction mixture was treated as described before, and a light brown sirup (2.49g) was obtained. Hydrolysis was performed by refluxing for 5 hours in a mixture of ethanol (20ml), water (1.5ml), and concentrated hydrochloric acid (1.5ml). A light yellow sirup (1.42g) was obtained by similar treatment of the hydrolysate. The sirup showed the following Rf values:
Rf 0.94 (w), 0.87 (vw), 0.79 (vw), 0.62 (w), 0.54 (vw), 0.46 (vvw), 0.18 (s) and 0.05 (m).

Methylation of D-Xylose Diethyl Mercaptal (IV).

Using the same quantities of reactants under the same conditions a sirup (3.12g) of the methylated mercaptal was obtained. Hydrolysis of this sirup provided a sirup (1.97g) of the methylated D-xyloses which showed two strong spots (Rf 0.20 and 0.06) and a few very weak spots with higher Rf values.

Isolation and Chromatographic and Electrophoretic Examinations of Mono-O-Methyl-D-xyloses.

The sirup (1.42g) of the methylation (III) was chromatographed on twelve 15cm-wide Whatman No. 3MM sheets for 7 hours, and the mono-O-methyl-D-xyloses located by means of the guide strips were extracted with cold water (200ml x 3). Evaporation of the extracts gave a sirup, which was redissolved in methanol (ca. 5ml) and the mixture (96.7mg) of mono-O-methyl-D-xyloses was obtained by the evaporation of the filtered methanolic solution.

In a similar manner a sirup (118.6mg) of mono-O-methyl-D-xyloses was isolated from the hydrolysate of methylation (IV).

The mixtures were chromatographed for 20 hours and two spots (R_{xylose} 3.16 (s) and 3.65 (w)) were shown to be 2- and 3-O-methyl-D-xyloses, respectively. Separation of these two spots was clearer when one drop of concentrated ammonia was added to the solvent.

Electrophoretic examination (900-1000V, 20-90mA, 90 minutes) of the mixtures also showed that they consisted of 2-O-methyl-D-xylose (Mg 0.49 (s)) and 3-O-methyl-D-xylose (Mg 0.92 (w)).

Isolation and Electrophoretical Examination of the Fast-moving Spots.

From the same chromatograms used for the isolation of mono-O-methyl-D-xylopyranoses, the area which corresponded to the di-O-methyl ethers (Rf 0.54 and 0.46) was extracted with cold water (200ml x 3). A sirup (21.1mg) was obtained by evaporation of the extracts, followed by the removal of methanol-insoluble impurities. The sirup gave only one spot (Mg 0.00).

Colorimetric Analysis of the Mixture of Mono-O-methyl-D-xyloses.

(1) Preparation of standard curve.

From the original solution containing 424γ/ml of 2-O-methyl-D-xylose, five solutions given in Table XXIV were prepared. The results are shown in Table XXIV and the curve is given in Fig. 31.

(2) Analysis of the mixture.

The mixture of mono-O-methyl-D-xyloses isolated from the methylation (III) was separated electrophoretically (1000-1100V, 25mA, 60 minutes). The results are shown in Table XXV.

Identification of the Component R_{xylose} 3.16 as 2-O-Methyl-D-xylose.

The mixture isolated from the methylation (III) was dissolved in warm ethanol (absolute) and after cooling petroleum ether was added to turbidity. Colorless crystals, m.p. 133°C, precipitated from the solution on seeding. Admixture with an authentic sample gave no depression of the melting point.

Identification of the Component R_{xylose} 3.65 as 3-O-Methyl-D-xylose Phenylosazone.

After 2-O-Methyl-D-xylose was precipitated, the mother liquor was decanted, and evaporated to a sirup which still showed a strong spot for 2-O

TABLE XXIV.

Preparation of standard curve of 2-O-methyl-D-xylose

Solution	Concentration (γ./2ml)	Readings at 480 mμ			Average
1	85.0	0.76	0.76	0.76	0.76
2	51.0	0.50	0.499	0.50	0.50
3	42.5	0.429	0.430	0.429	0.429
4	21.3	0.237	0.234	0.230	0.230
5	10.7	0.120	0.119	0.123	0.121

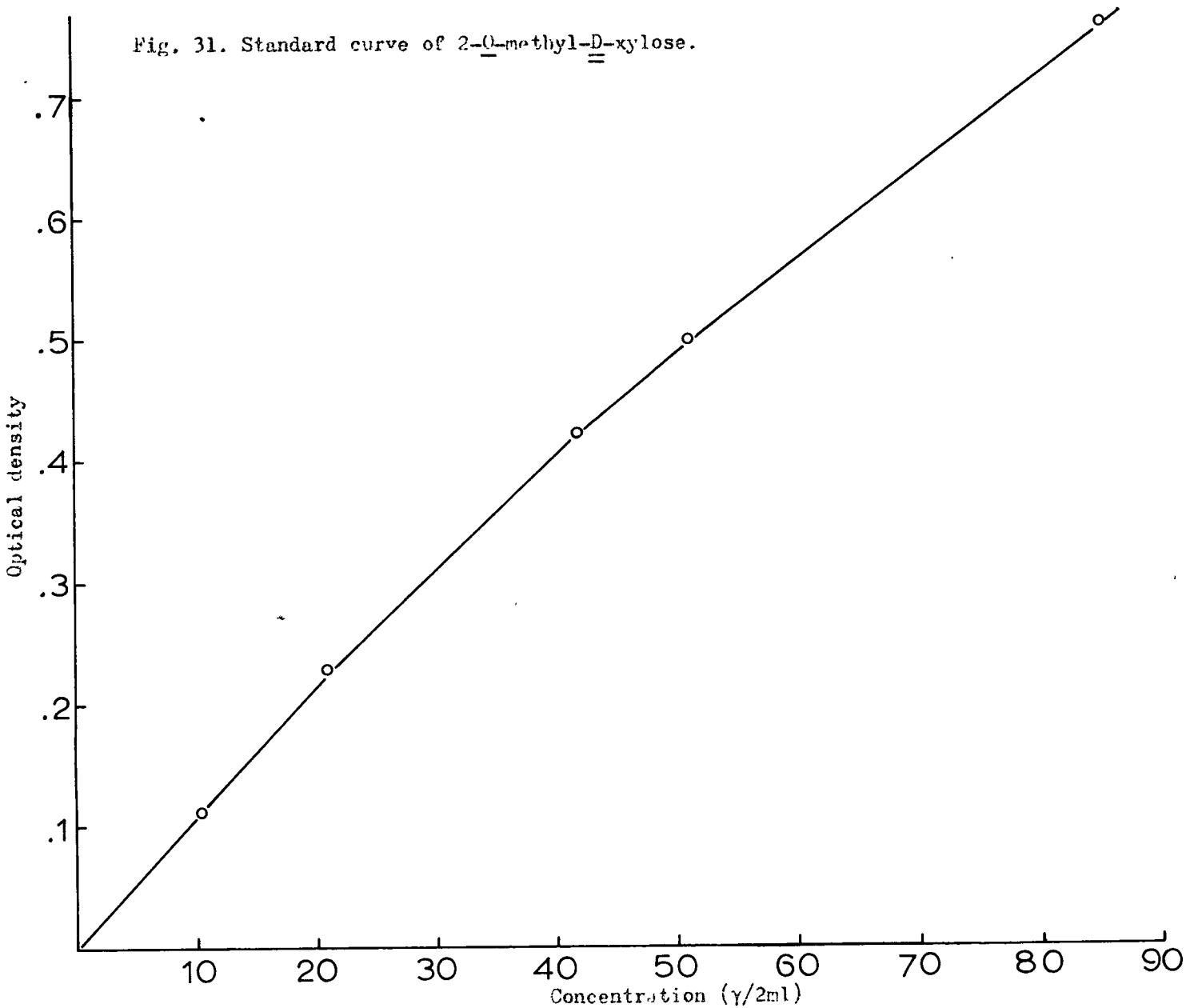


Fig. 31. Standard curve of 2-O-methyl-D-xylose.

Table XXV. Colorimetric analysis of mono-O-methyl-D-xyloses (mixture III)

Component		Readings at 480 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-xylose	(sugar	0.455	0.460	0.470	0.462	0.443	44.0
	(blank	0.020	0.018	0.020	0.019		
3-O-Methyl-D-xylose	(sugar	0.192	0.170	0.187	0.183	0.154	14.0
	(blank	0.022	0.034	0.030	0.029		

methyl-D-xylose.

The sirup (52.1mg) was combined with that (118.6mg) obtained from the methylation (IV) and the combined sirup was chromatographed for 20 hours on three 15cm-wide Whatman No. 3MM sheets using butanone-water azeotrope to which one drop of concentrated ammonia was added. The 3-O-methyl-D-xylose was extracted with cold water (50ml x 3), and evaporation of the extracts gave a sirup (28.5mg), which was electrophoretically pure.

The sirup (28.5mg) was dissolved in water (3ml) and phenylhydrazine (56.2mg) in ethanol (0.31ml) and a small amount of sodium metabisulphite were added. The mixture was heated for 5 hours in boiling water and yellow crystals precipitated on cooling. The crystals (21.6mg) were recrystallized from ethanol-water, m.p. 171-172°C. No depression of the melting point was observed when mixed with synthetic 3-O-methyl-D-xylose phenylosazone.

Methylation of D-Xylose Diethyl Mercaptal (V).

Crystalline D-xylose diethyl mercaptal (1.00g) was dissolved in a mixture of methyl iodide (12.0ml) and tetrahydrofuran (12.0ml). Silver oxide (2.40g) and drierite (5g) were added to the solution and the mixture was shaken for 3 hours at room temperature. The methylation product was treated similarly as described under the methylation (III). The sirup (991mg) thus obtained was hydrolyzed in a similar manner, and a light yellow sirup (583mg) was obtained which showed the following Rf values:
Rf 0.91 (w), 0.85 (vw), 0.73 (vw), 0.60 (w), 0.51 (w), 0.40 (w), 0.20 (s), and 0.07 (m).

Isolation and Electrophoretic Examination of Mono-O-methyl-D-xyloses and Di-O-methyl-D-xyloses.

The sirup (583mg) was chromatographed on six 15cm-wide Whatman No. 3MM sheets for 7 hours, and the areas of Rf 0.20 and of 0.51 plus 0.40 were separately extracted with cold water (100ml x 3). The yield of these two components was 41.9mg and 16.3mg, respectively.

The component of Rf 0.20 showed two spots (Mg 0.45 (s) and 0.91 (w)) by electrophoresis (950-1100V, 25mA, 60 minutes), whereas the components of Rf 0.51 and 0.40 showed three spots (Mg 1.00 (w), 0.37 (vw), and 0.00 (s)) (950-1100V, 25-35mA, 70 minutes). The spot of Mg 1.00 was shown to be 5-O-methyl-D-xylose by a concurrent examination with a synthetic sample (Mg 1.00).

Colorimetric Analysis of the Extracts.

(1) Analysis of the component of Rf 0.20.

The mixture was chromatographed for 20 hours in butanone-water azeotrope plus one drop of concentrated ammonia. The results are shown in Table XXVI.

(2) Analysis of the Components of Rf 0.51 and 0.40.

The mixture was separated by electrophoresis (950-1100V, 25-35mA, 60 minutes). The results are shown in Table XXVII.

Preparation of 3-O-Methyl-D-xylose.

Crude 1,2-O-isopropylidene-D-xylose (2.97g) (positive Benedict test) was dissolved in pyridine (30ml). Trityl chloride (4.50g) was added to the solution and the mixture was kept at room temperature for 24 hours.

Table XXVI. Colorimetric analysis of mono-O-methyl-D-xyloses (methylation (V))

Component		Readings at 480 mμ			Average	Difference	Concentration (γ/2 ml)
2-O-Methyl-D-xylose	(sugar	0.51	0.465	0.485	0.487	0.448	44.5
	(blank	0.045	0.035	0.038	0.039		
3-O-Methyl-D-xylose	(sugar	0.325	0.292	0.300	0.306	0.276	26.0
	(blank	0.032	0.028	0.030	0.030		

Table XXVII. Colorimetric analysis of the fast-running components of O-methylated D-xyloses

Component		Readings at 480 mμ			Average	Difference	Concentration (γ/2 ml)
5-O-Methyl-D-xylose	(sugar	0.100	0.101	0.095	0.099	0.035	3.0
	(blank	0.063	0.071	0.059	0.064		
Component (Mg 0.37)	(sugar	(0.130)	0.065	0.068	0.067	0.017	1.5
	(blank	0.053	0.049	0.048	0.050		
Component (Mg 0.00)	(sugar	0.363	0.368	0.370	0.367	0.315	30.0
	(blank	0.048	0.058	0.050	0.052		

The reaction mixture was poured into ice-water and extracted with chloroform (50ml x 3). The extracts were washed twice with ice-cold 1N sulphuric acid (ca. 100ml), with aqueous sodium carbonate, and with water. Evaporation of the dried extract ($MgSO_4$) gave a sirup (6.00g).

The tritylated sirup (6.00g) was methylated with methyl iodide (80ml) and silver oxide (20g). After two methylations, the sirup (5.92g) showed no appreciable free hydroxyl band in its infrared spectrum.

The methylated sirup (5.92g) was hydrolyzed in 2N sulphuric acid (20ml) (in boiling water). The hydrolysate was filtered in order to remove the liberated tritanol and the filtrate was deionized with Duolite A-4 resin and then evaporated to a sirup, which was extracted with cold water. The water extracts gave, on evaporation, a sirup (1.49g) which showed the following Rf values:

Rf 0.87 (vw), 0.79 (vw), 0.60 (m), 0.22 (vs), and 0.06 (w).

The sirup (1.49g) was chromatographed using butanone-water azeotrope on a cellulose-hydrocellulose column (40 x 3cm) (rate of flow: 10ml/10 minutes), and the component of Rf 0.22 was found in tubes No. 30-80. A sirup (758mg) of 3-O-methyl-D-xylose was obtained.

The sirup (238mg) was treated in water (5ml) with phenylhydrazine hydrochloride (650mg) and anhydrous sodium acetate (400mg), and 3-O-methyl-D-xylose phenylosazone (m.p. 179-181°C) was obtained.

Preparation of 5-O-Methyl-D-xylose.

Crude 1,2-O-isopropylidene-D-xylose (5.12g) was dissolved in pyridine (25ml) and the solution was cooled in an ice-water bath. A solution of tos-

yl chloride (5.50g) in chloroform (10ml) was added to the pyridine solution and the mixture was kept in an ice-water bath for an hour and then at room temperature overnight. The reaction mixture was poured into water and extracted with chloroform (50ml x 3). The dried ($MgSO_4$) extracts gave on evaporation a sirup which crystallized on dilution with small amount of ether. The colorless crystals (6.28g) melted at 135-136°C.

1,2-O-Isopropylidene-5-O-tosyl-D-xylose (2.70g) was added to a methanol (10ml) solution of sodium (5.50g). The mixture was sealed in a tube and was heated for 4 hours in boiling water. The content of the tube was washed out with water and then evaporated to dryness. The residue was redissolved in water and extracted with chloroform (30ml x 3). Evaporation of the extracts gave a sirup (1.23g) which spontaneously crystallized (m.p. 85°C).

1,2-O-Isopropylidene-5-O-methyl-D-xylose (1.23g) was dissolved in 1N sulphuric acid (20ml) and the solution was heated for 1 hour in boiling water. The hydrolysate was deionized with Duolite A-4 resin and evaporation of the neutral solution gave a sirup (793mg) which showed the following Rf values:

Rf 0.32 (vs), 0.19 (w), 0.09 (w), and 0.04 (w).

The sirup (793mg) was separated by chromatography on a cellulose-hydrocellulose column using butanone-water azeotrope (rate of flow: 10ml/20 minutes) and the component of Rf 0.32 was found in tubes No. 45-65. Evaporation of these fractions gave a sirup (593mg).

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*All abbreviations are as given in the "Annual Reports on the Progress of Chemistry" with the exception of the "Journal of the Chemical Society" (J. Chem. Soc.).

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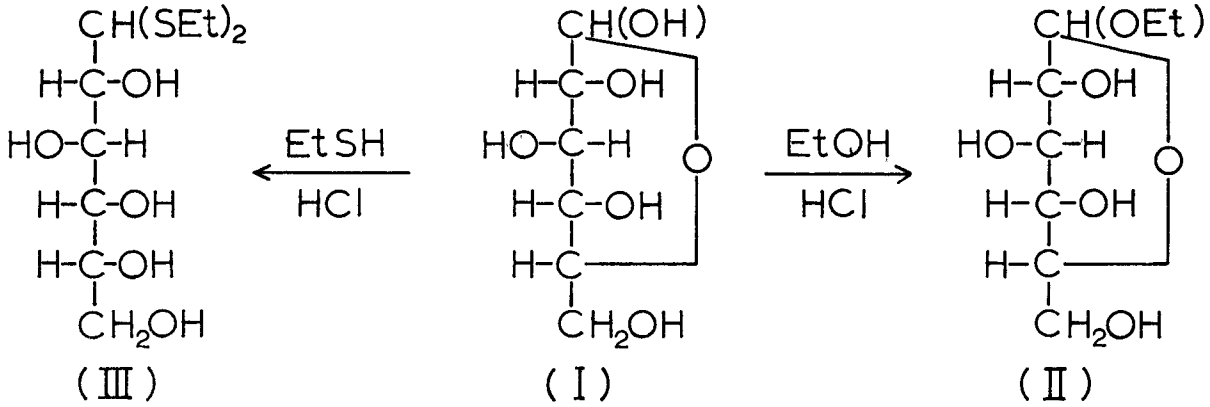


Fig. 1. Formation of glycosides and mercaptals.

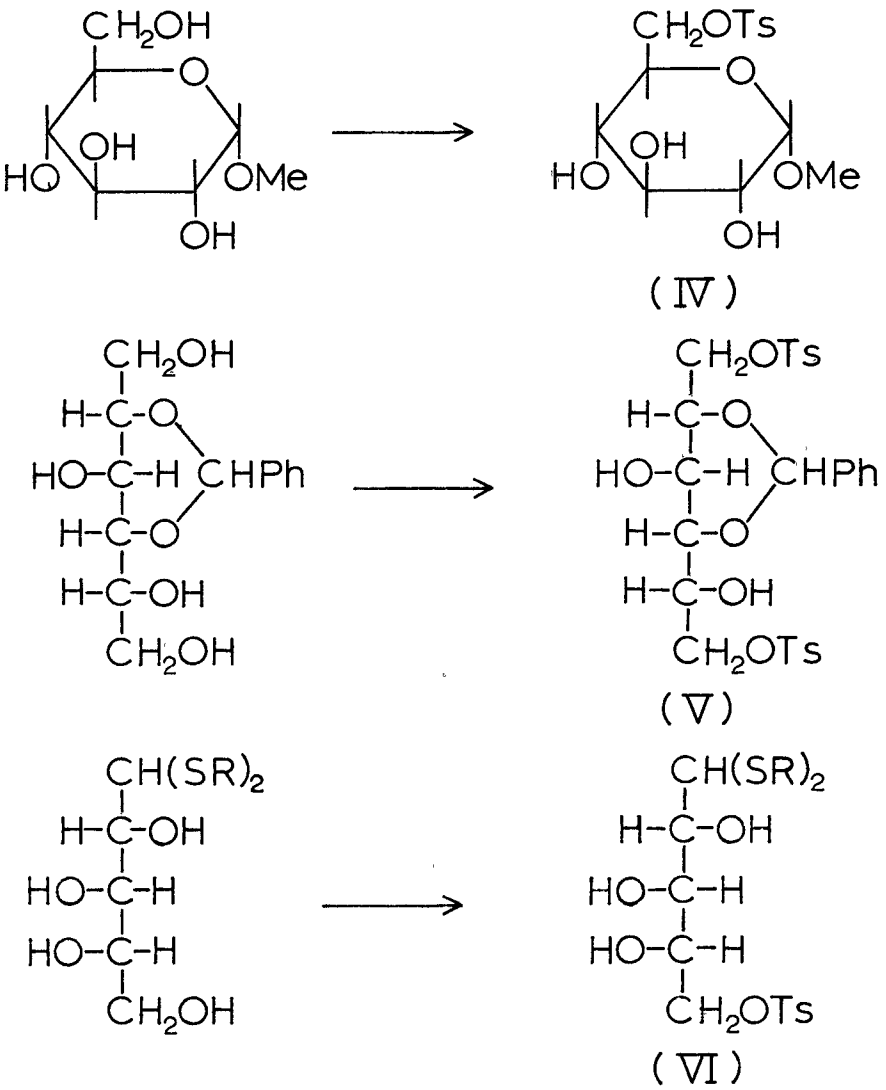


Fig. 2. Selective tosylation of primary hydroxyl group.

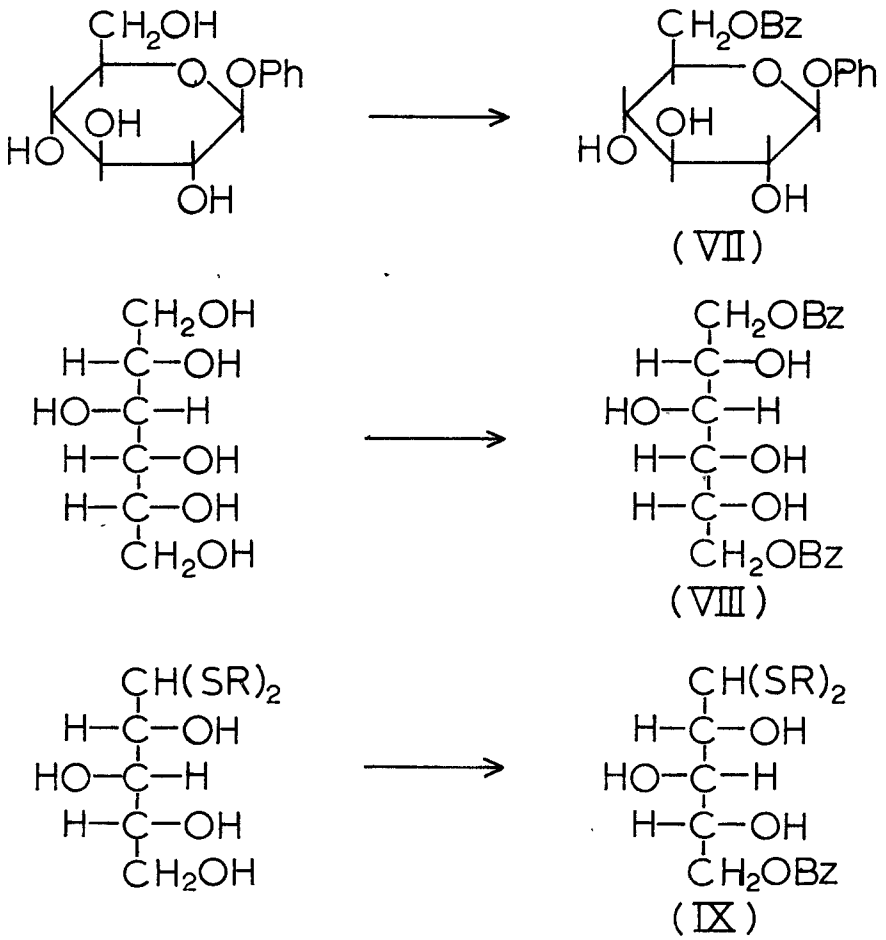


Fig. 3. Selective benzoylation of primary hydroxyl groups.

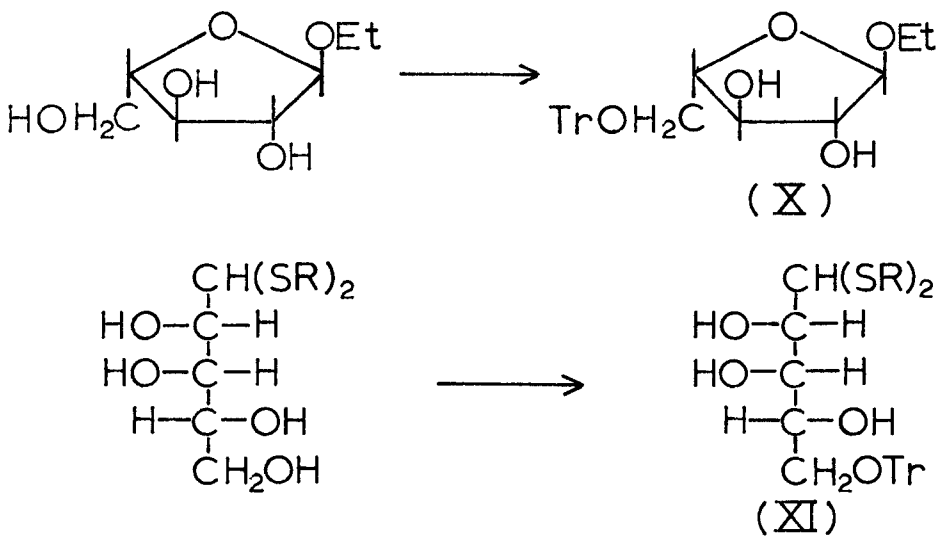
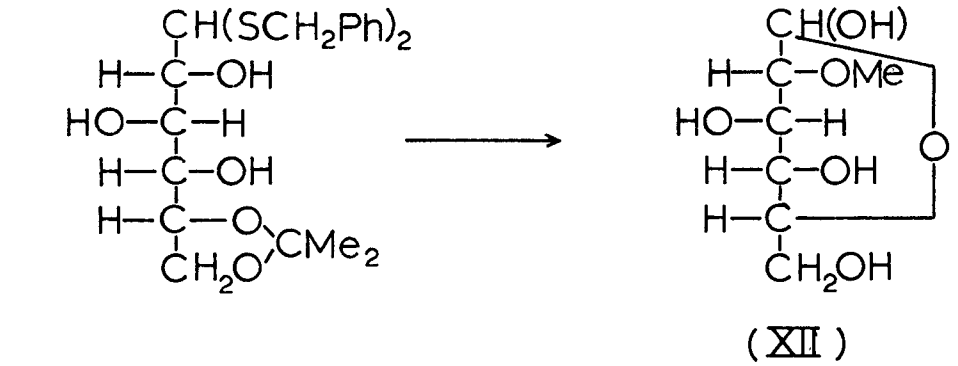
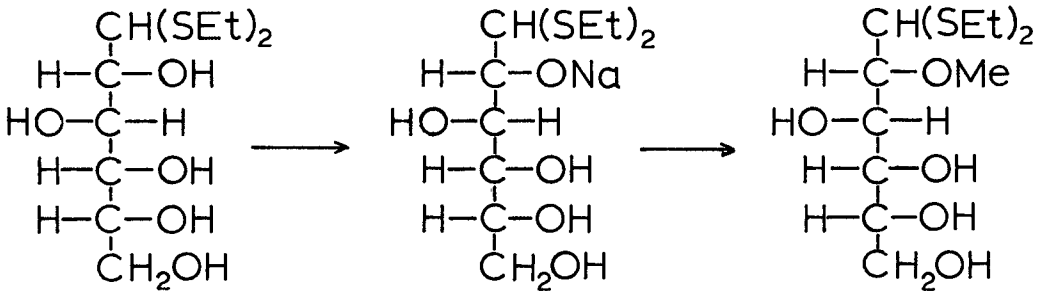


Fig. 4. Selective tritylation of primary hydroxyl groups.



(XII)



(XIII)

Fig. 5. Selective methylation of 2-hydroxyl groups.

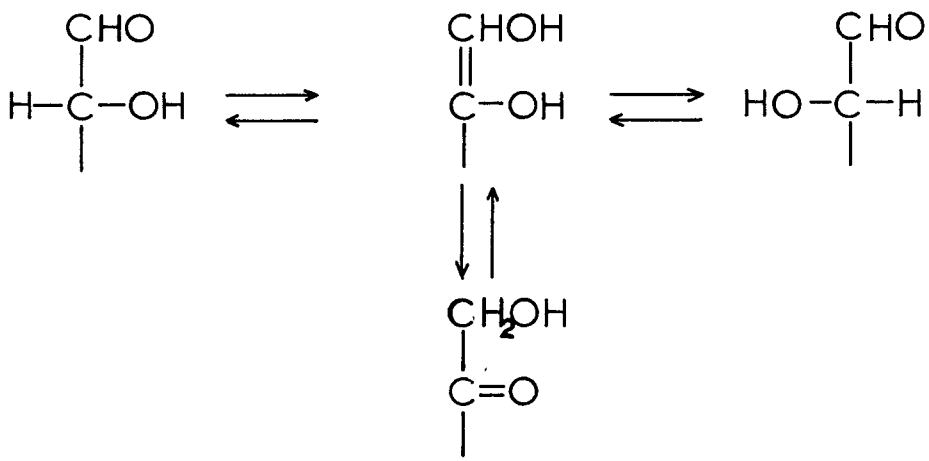


Fig. 6. de Bruyn-van Eckenstein transformation.

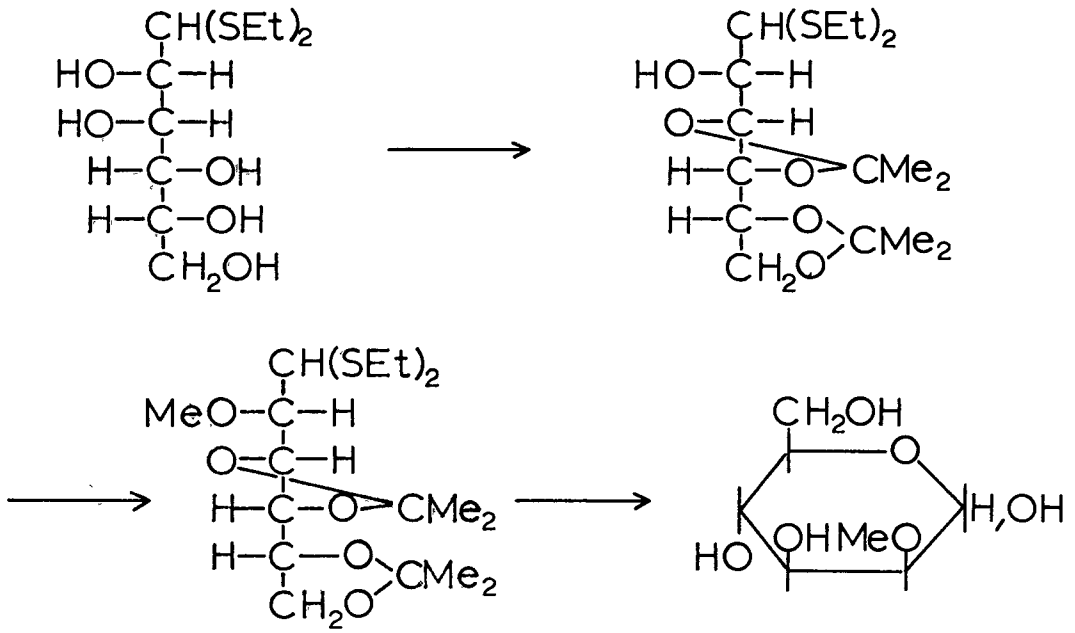


Fig. 7. Synthesis of 2-O-methyl-D-mannose.

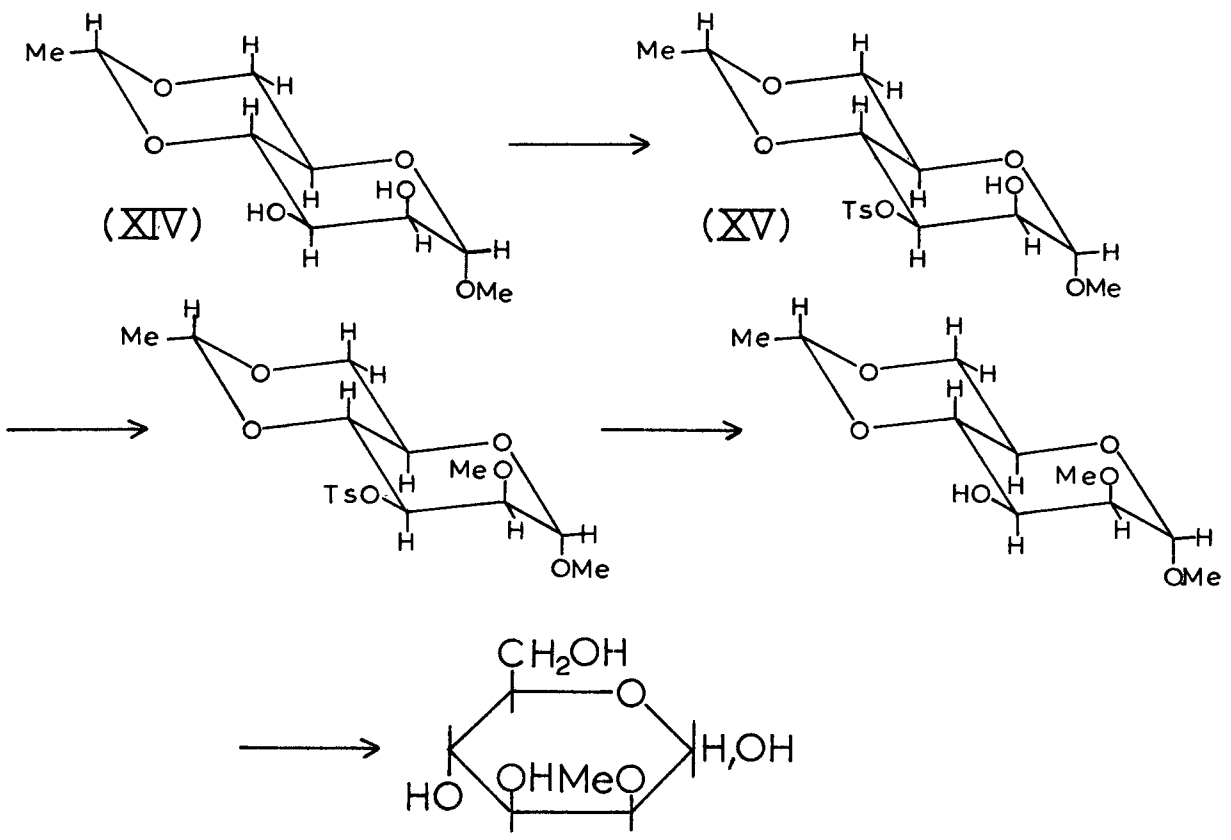


Fig. 8. Synthesis of 2-O-methyl-D-mannose.

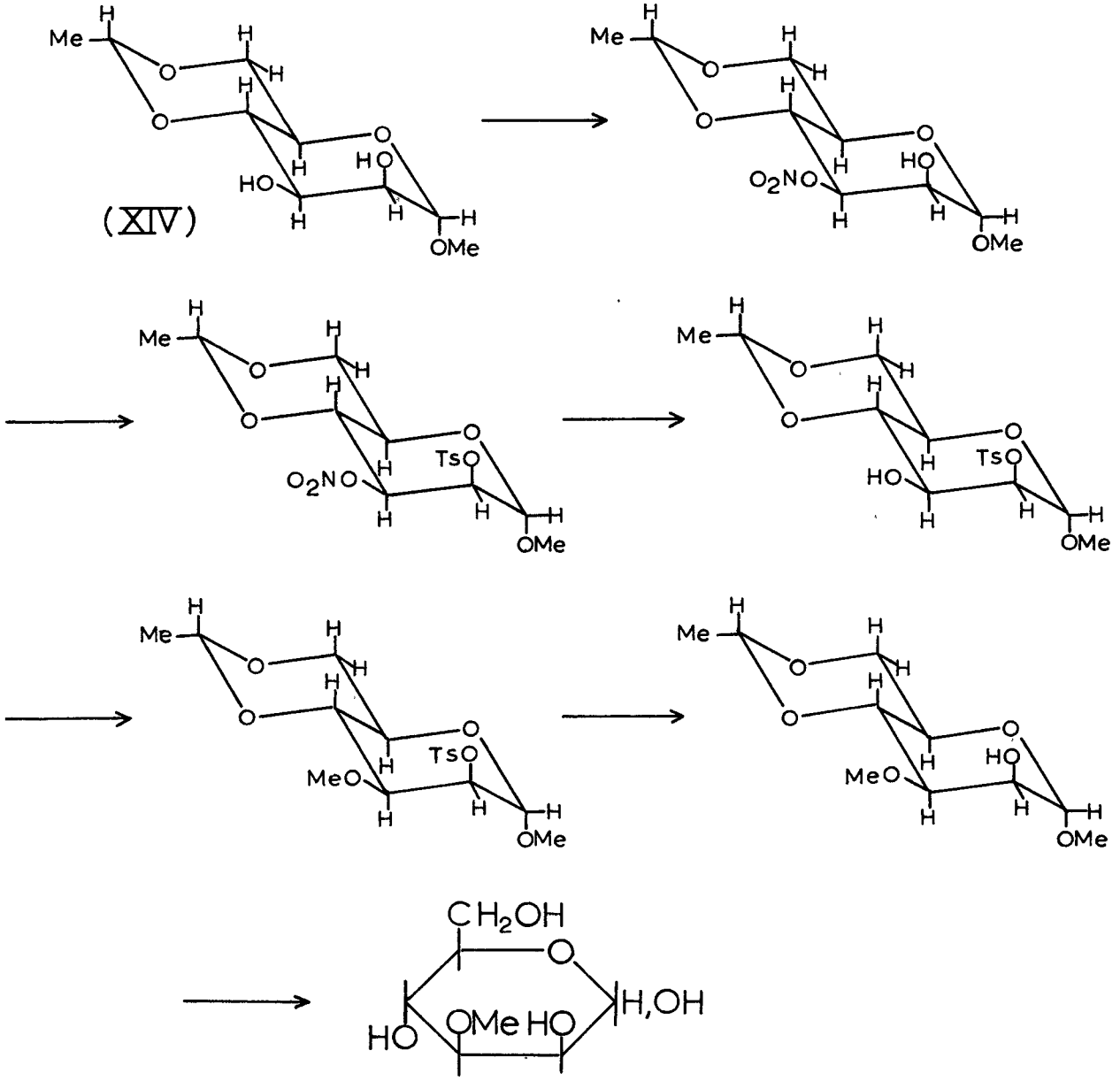


Fig. 9. Synthesis of 3-O-methyl-D-mannose.

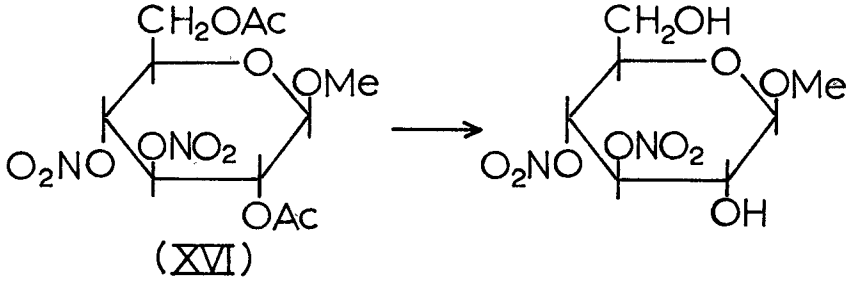


Fig. 10. Deacetylation of O-nitrated sugar.

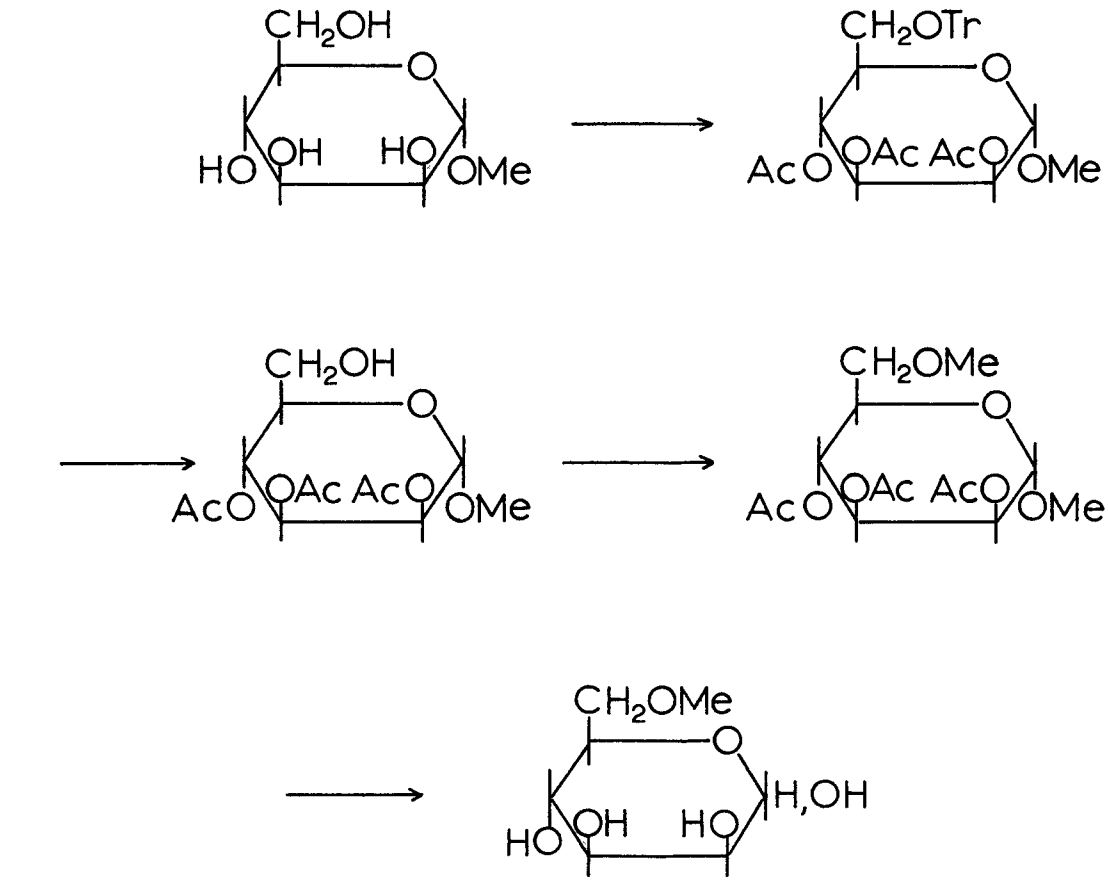


Fig. 11. Synthesis of 6-O-methyl-D-mannose.

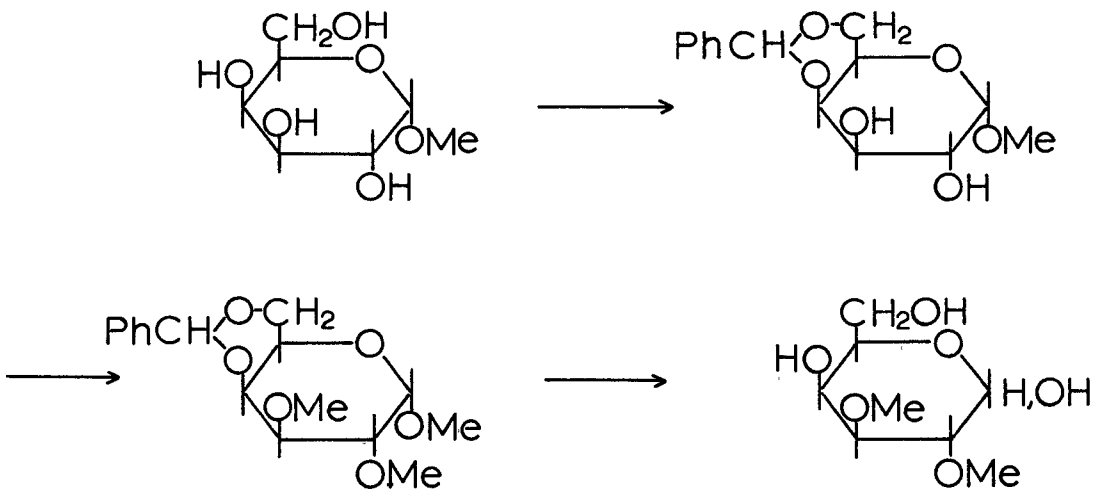


Fig. 12. Synthesis of 2,3-di-O-methyl-D-galactose.

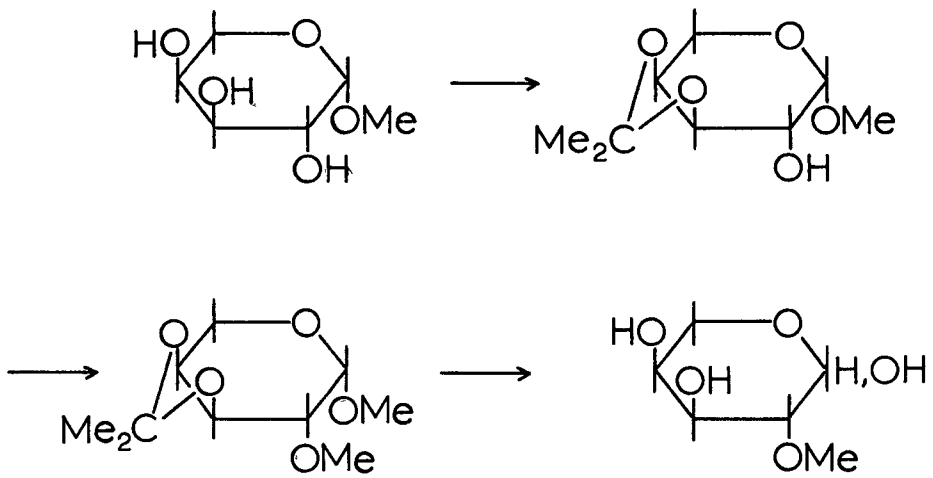


Fig. 13. Synthesis of 2-O-methyl-L-arabinose.

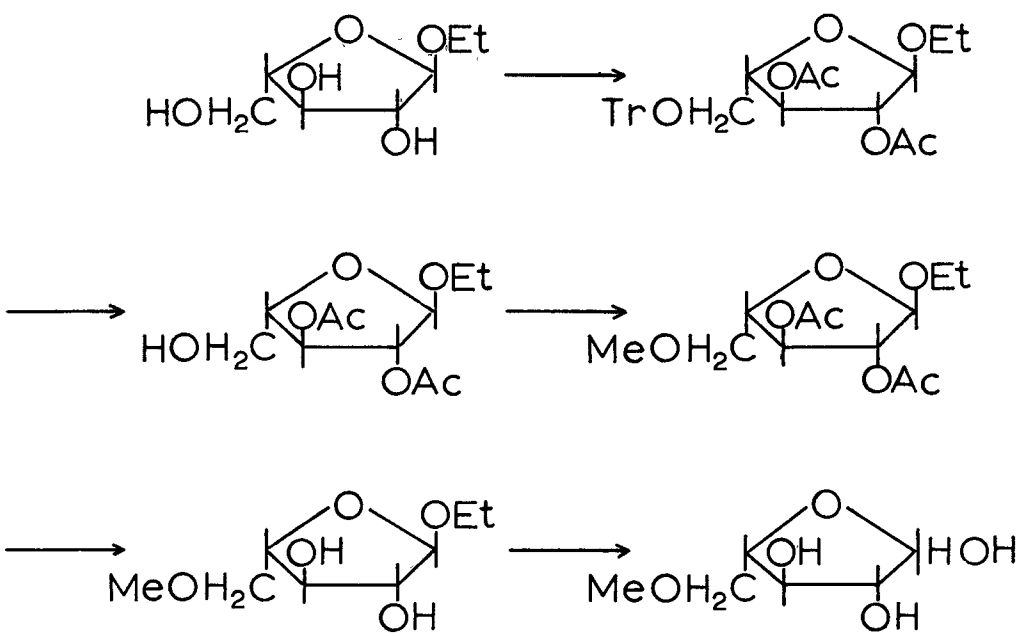


Fig. 14. Synthesis of 5-O-methyl-L-arabinose.

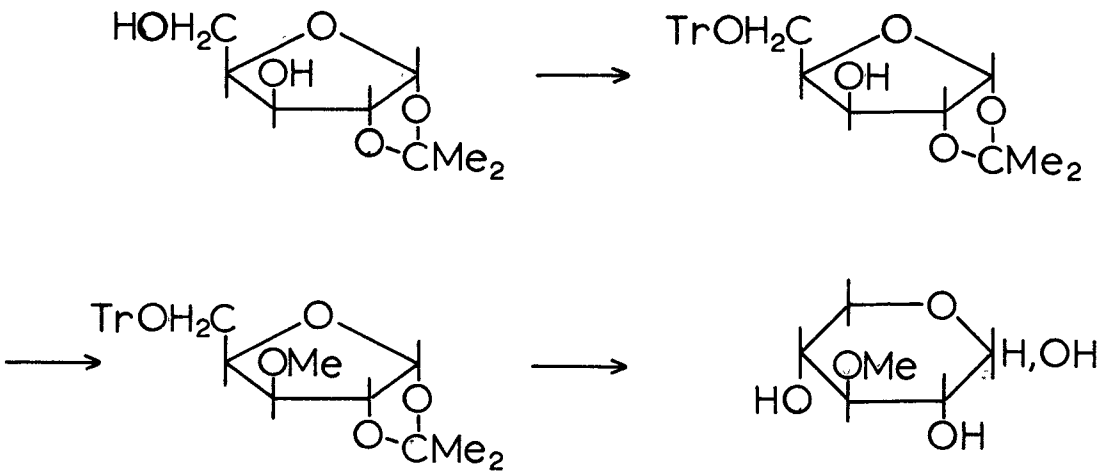


Fig. 15. Synthesis of 3-O-methyl-D-xylose.

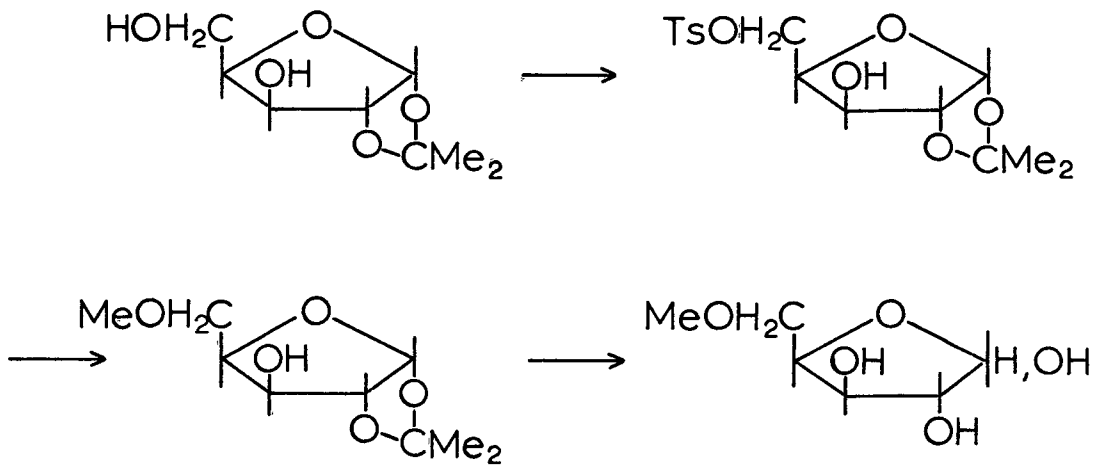


Fig. 16. Synthesis of 5-O-methyl-D-xylose.

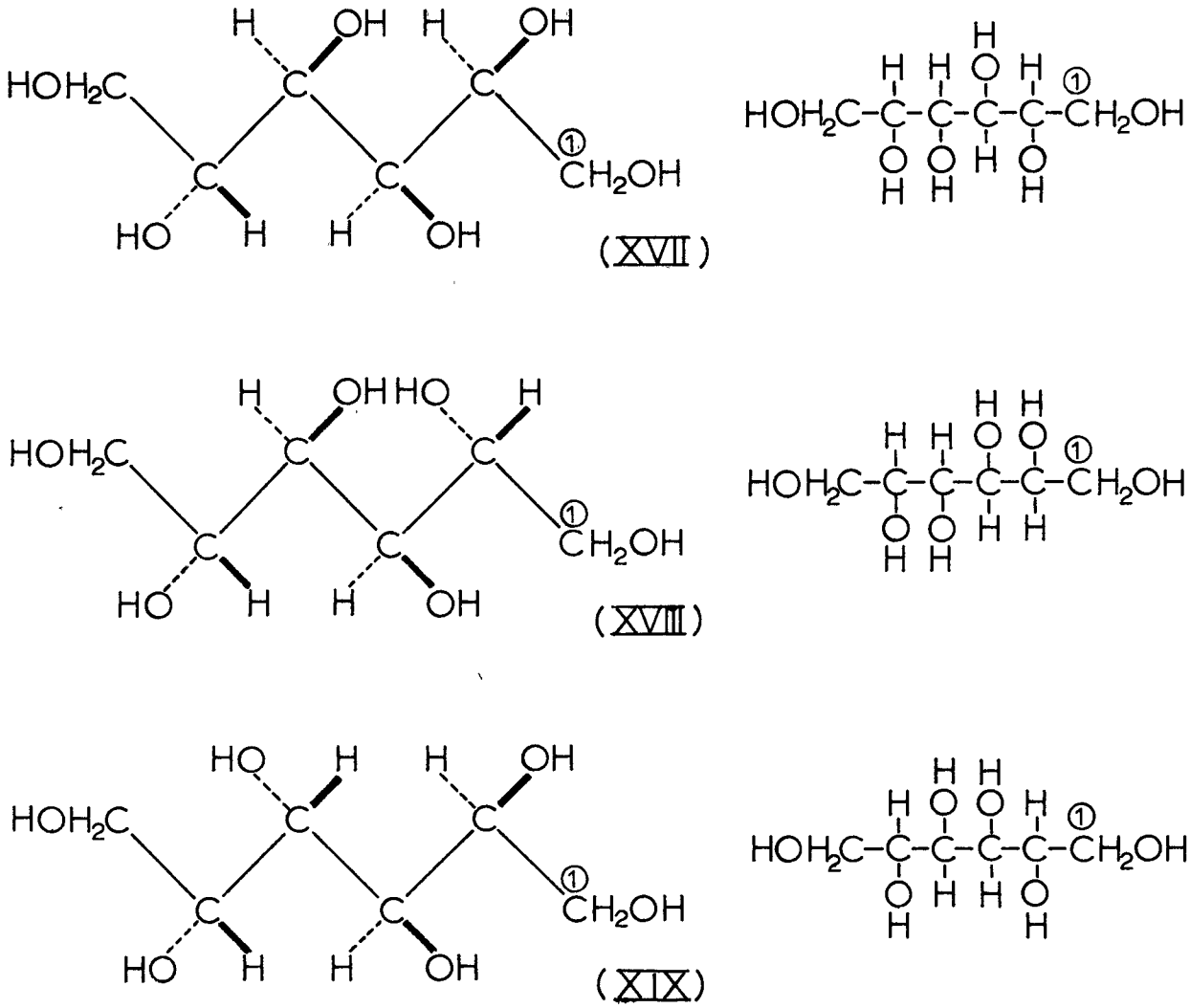


Fig. 17. Zig-zag structure of some glycitols.

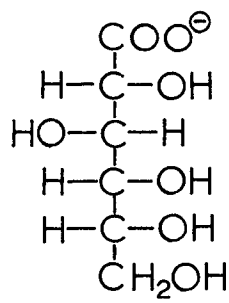


Fig. 18. Gluconate ion.

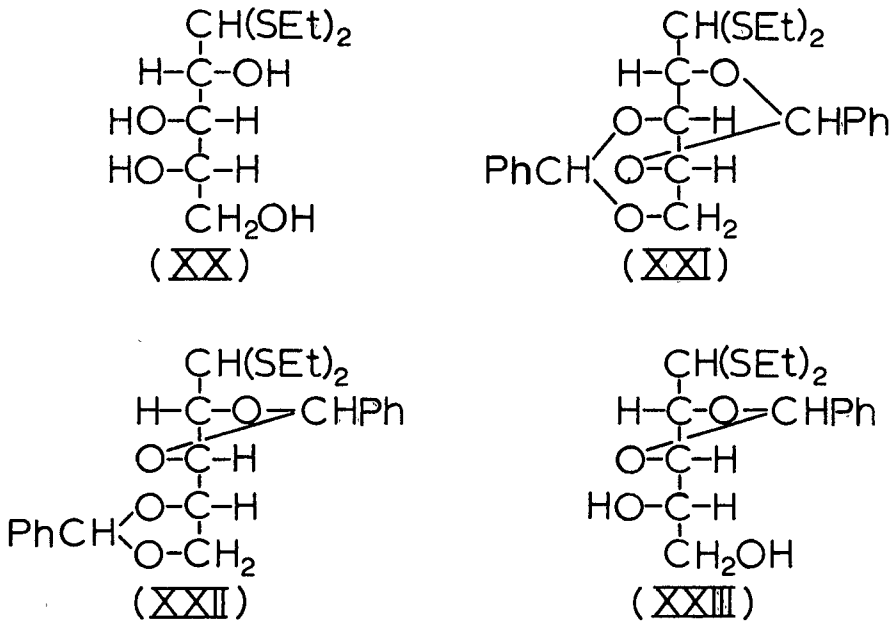


Fig. 19. Benzylidene derivatives of L-arabinose diethyl mercaptal.

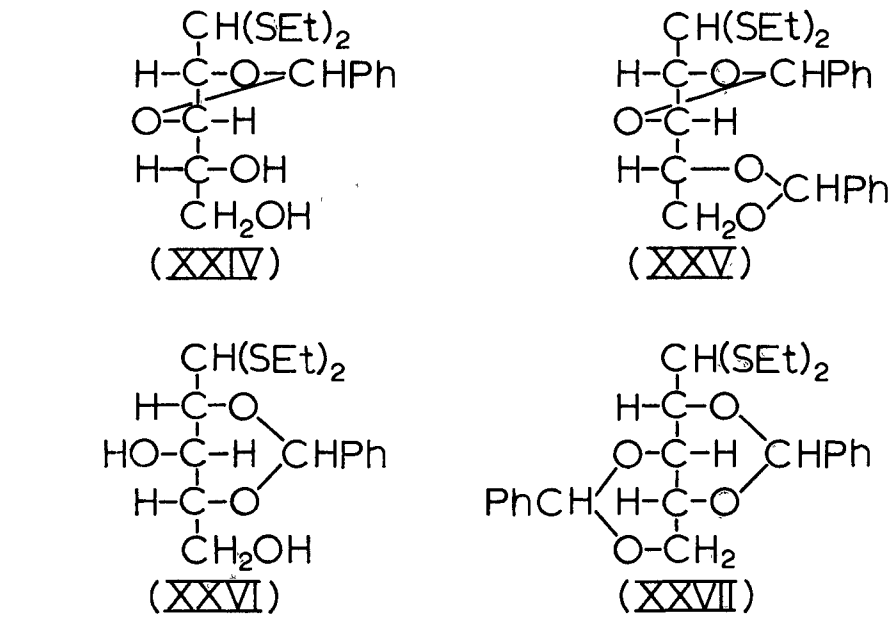


Fig. 20. Benzylidene derivatives of D-xylose diethyl mercaptal.

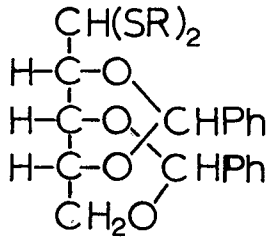


Fig. 21. Di-O-benzylidene-D-ribose mercaptal.

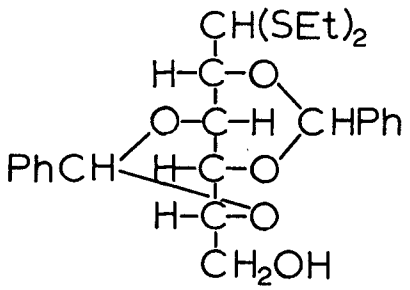


Fig. 22. Di-O-benzylidene-D-glucose diethyl mercaptal.

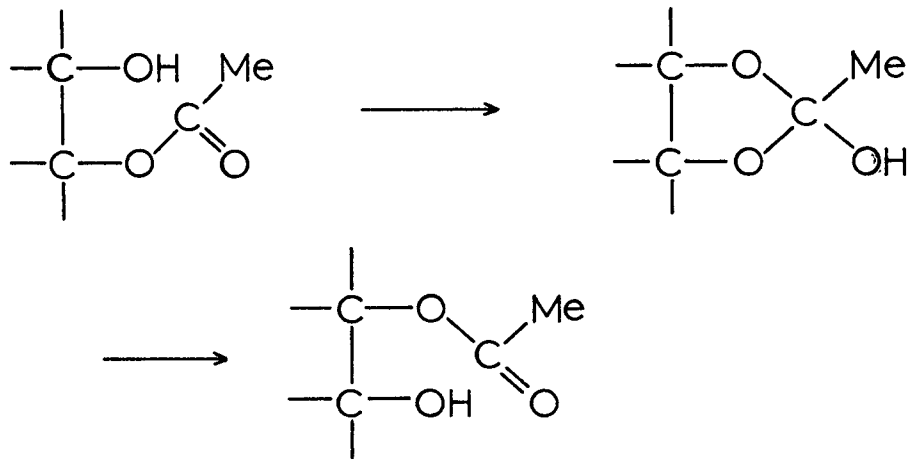


Fig. 23. Migration of acetyl group.

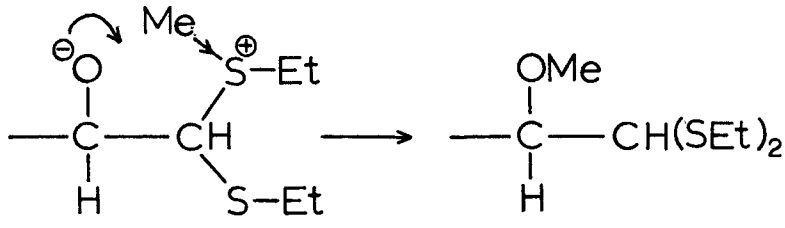
$$R\text{-}OH + B \rightleftharpoons R\text{-}O^{\ominus} + BH^{\oplus} \quad (B{:}base) \qquad (1)$$

$$R\text{-}O^{\ominus} + Me\text{-}I + Ag\text{-}O\text{-}Ag$$

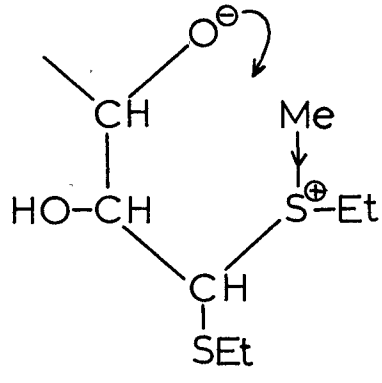
$$\longrightarrow [\, R\text{-}O^{\ominus} \cdots Me \cdots I \cdots Ag\text{-}O\text{-}Ag \,]$$

$$\longrightarrow R\text{-}O\text{-}Me + AgI + AgO^{\ominus} \qquad (2)$$

Fig. 24. Mechanism of Purdie methylation.



(XXVIII)



(XXIX)

Fig. 25. Mechanism of "transmethylation."

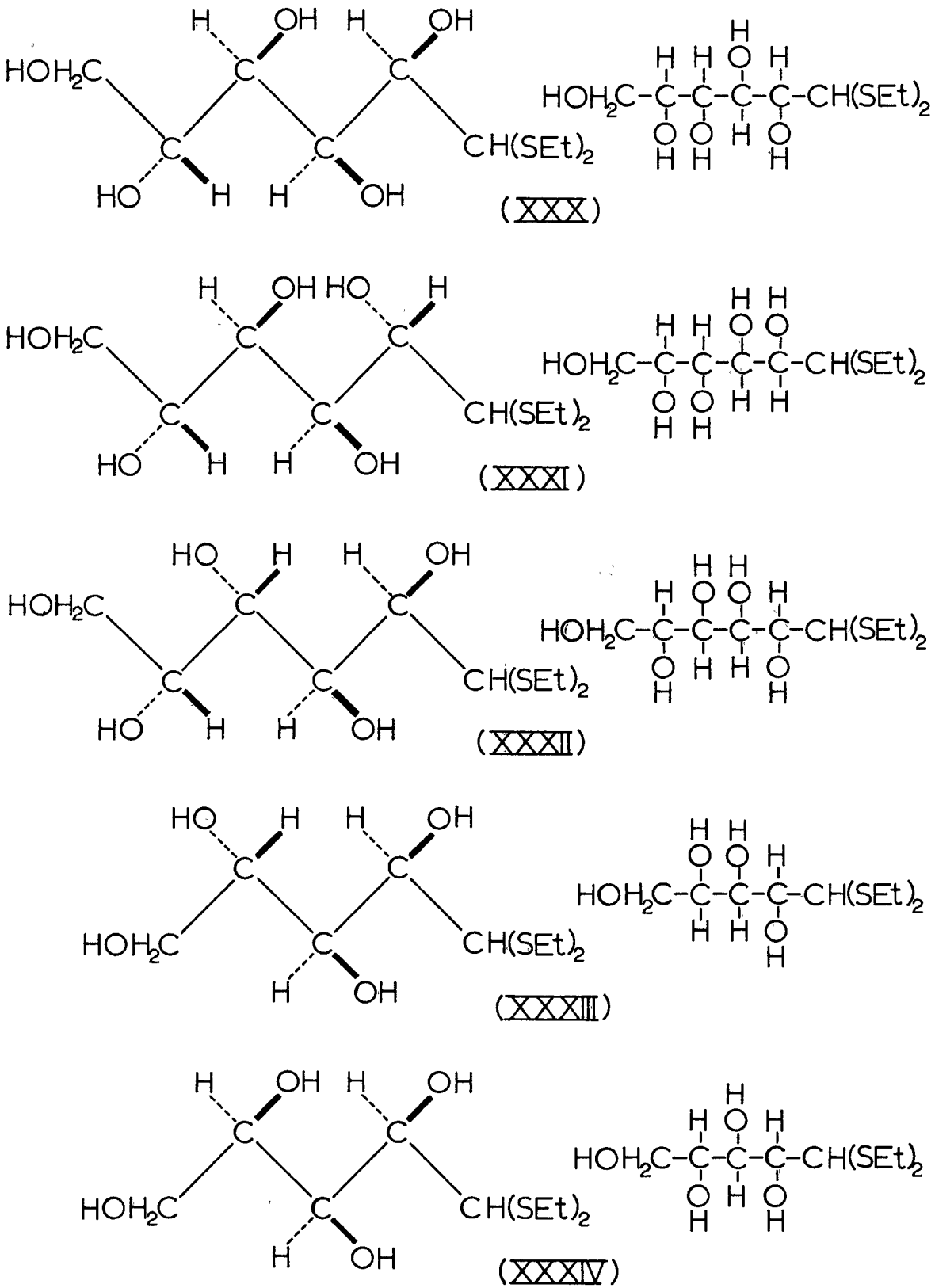


Fig. 26. Zig-zag conformation of sugar mercaptals.

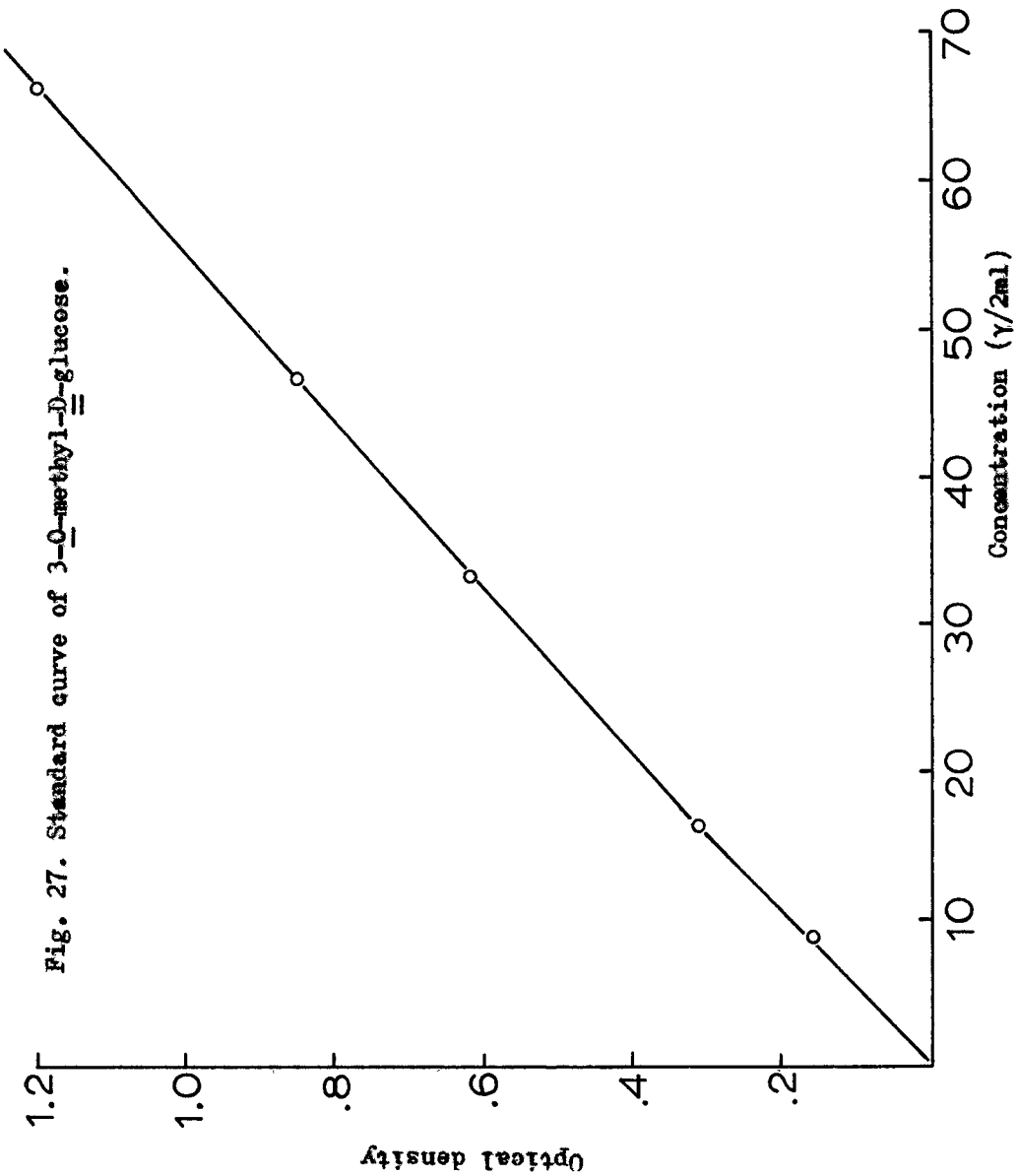


Fig. 27. Standard curve of 3-O-methyl-D-glucose.

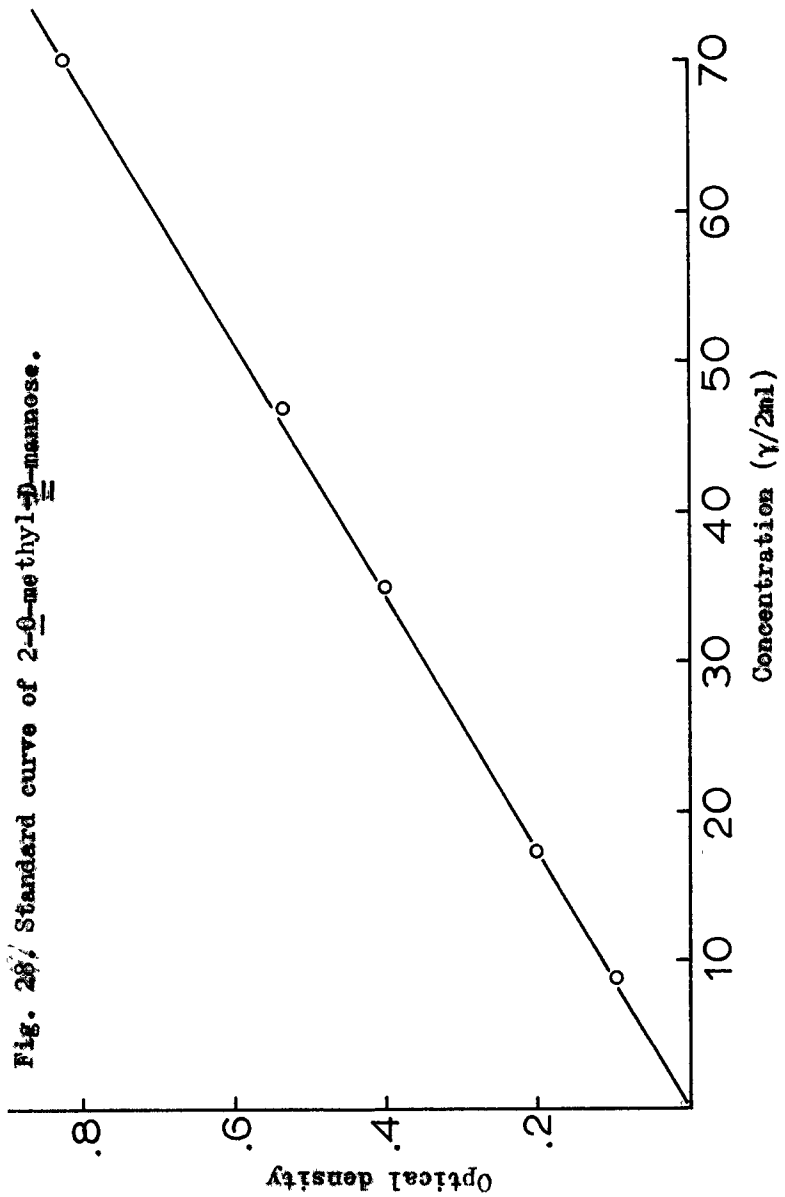


Fig. 20/ Standard curve of 2-O-methyl-D-mannose.

Fig. 29. Standard curve of 6-O-methyl-D-galactose.

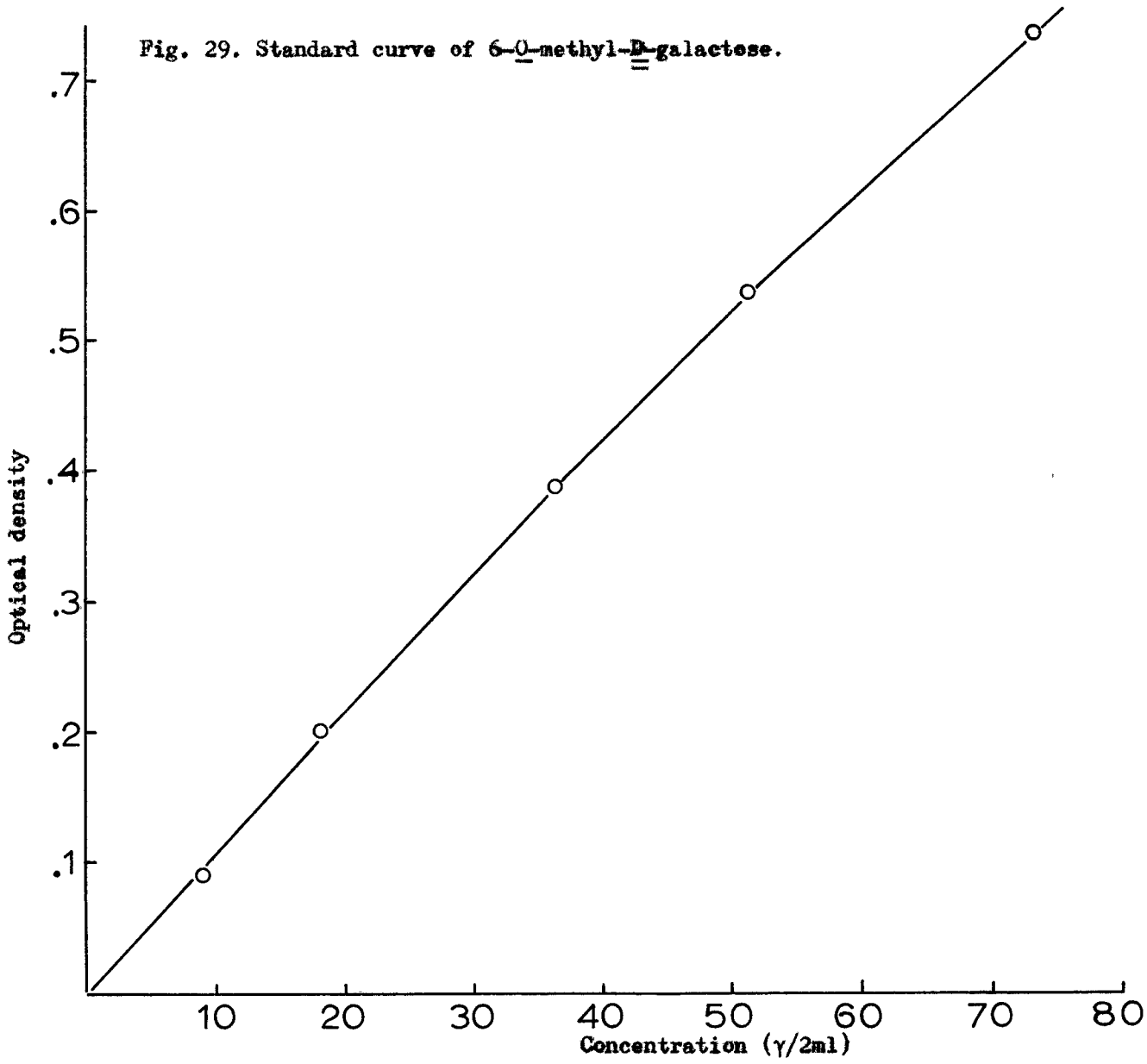


Fig. 30. Standard curve of 5-O-methyl-L-arabinose.

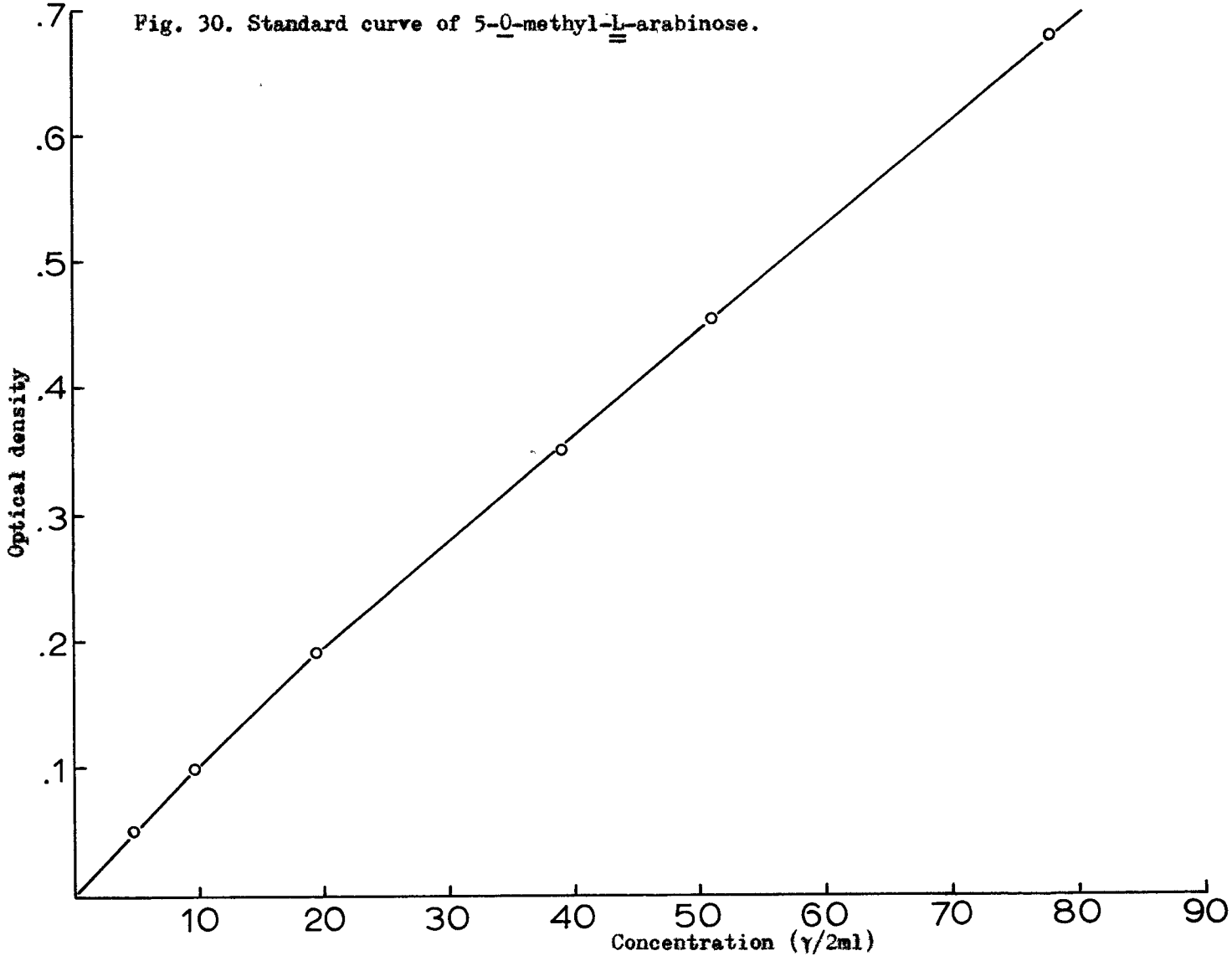


Fig. 31. Standard curve of 2-O-methyl-D-xylose.