THE METHYLATION OF SUGAR MERCAPTALS

by

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B.A., University of British Columbia, 1956

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Chemistry

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1957

ABSTRACT

It was reported in 1934 by Lieser and Leckzyck that D-glucose diethyl mercaptal could be selectively methylated by use of methyl iodide and silver oxide at 0°C. to give exclusively 2-O-methyl-D-glucose diethyl mercaptal in 52% yield. They stated that the mercaptals of D-galactose, L-arabinose, D-xylose and L-rhamnose failed to react in a similar way and isolated no products. The present investigation was undertaken to determine to what extent these statements are correct, and if methylation does occur with the mercaptals of other sugars, to determine the nature of the methylated products. It was also of interest to determine if reaction predominates at any one position as in D-glucose mercaptal.

The original observations of Lieser and Leckzyck were substantially confirmed by a repetition of their experiments. The present work, however, has shown that some methylation does occur with mercaptals other than that of D-glucose but to a much smaller extent, and that this is partly due to the insolubility of these mercaptals in methyl iodide.

Tetrahydrofuran was found to be a suitable methylation solvent in which the diethyl mercaptals of D-glucose, D-galactose, L-arabinose and D-mannose have comparable solubility. These mercaptals were methylated in this solvent by a modification of Lieser's procedure. Investigation of the

products by paper chromatography indicated that all four gave similar results. Further there did not appear to be any significant difference in the reactions when they were carried out at 3°, 22°, and 50°C. These experiments were all on an exploratory scale. Suitable conditions were established for larger scale methylations and separations of products.

One such large scale methylation and separation was carried out on D-glucose diethyl mercaptal and the products hydrolysed and separated as the free sugars by means of column chromatography. Six distinct methylated glucoses were separated showing that the reaction is by no means as selective when tetrahydrofuran is used as reaction solvent. The largest product, but no longer dominantly so, was positively identified as the 2-O-methyl derivative, again showing that reactivity is greatest at the 2-position. This was found to be the only monomethyl ether produced.

A second large scale methylation and separation was carried out in an exactly similar manner on D-mannose diethyl mercaptal. Eight distinct methylated mannoses were separated with the extent of methylation being comparable with that above. Two major monomethyl products were isolated and identified by periodate oxidation studies and their osazones as the 6-O-methyl and 5-O-methyl derivatives. The results indicate that the 6-position is most reactive in D-mannose diethyl mercaptal.

A third similar methylation of L-arabinose diethyl mercaptal, followed by separation of products by a combination

of column and paper chromatography, yielded seven distinct methylated arabinoses. There were no major monomethyl products, reaction appearing to have gone largely to the dimethyl stage. Three monomethyl arabinoses were separated in negligible amounts and could not be identified. Reaction apparently does not predominate at any one position in L-arabinose diethyl mercaptal.

Preliminary experiments on D-galactose diethyl mercaptal indicated methylation to be of a highly random nature. An exploratory separation of products showed that no one monomethyl ether predominated. The large scale methylation and separation was not repeated on this mercaptal.

Of the compounds investigated, unusual reactivity of the 2-hydroxyl group is only clearly manifested by D-glucose mercaptal. No marked reactivity of this position is shown by the other mercaptals, although there is evidence that the 6- and 5-hydroxyls of D-mannose mercaptal are unusually reactive.

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Date September 10th., 1957.

ACKNOWLEDGEMENT

Thanks are extended to Dr. G.G.S. Dutton for his patient and invaluable direction of this research, and also to the National Research Council for financial assistance.

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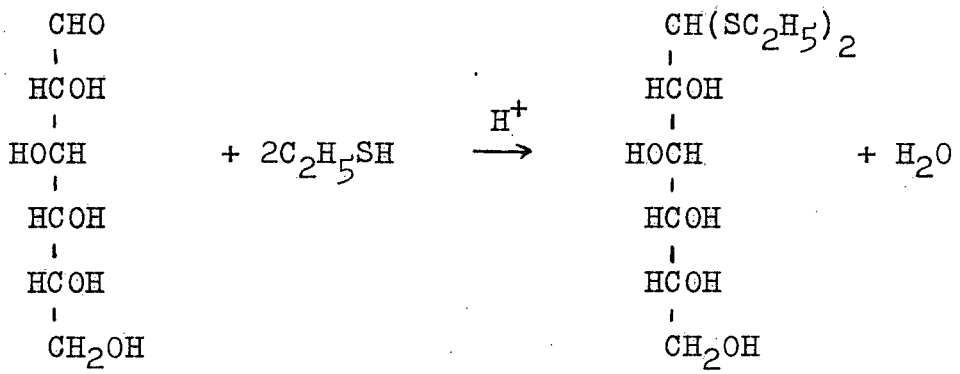
HISTORICAL INTRODUCTION

In 1894, Fischer[4] prepared a new series of carbohydrate derivatives, the thioacetals, or as they are more usually called, the mercaptals. These compounds were formed by the addition of mercaptans to carbonyl compounds in the presence of acid catalysts. An example of this reaction is shown below.



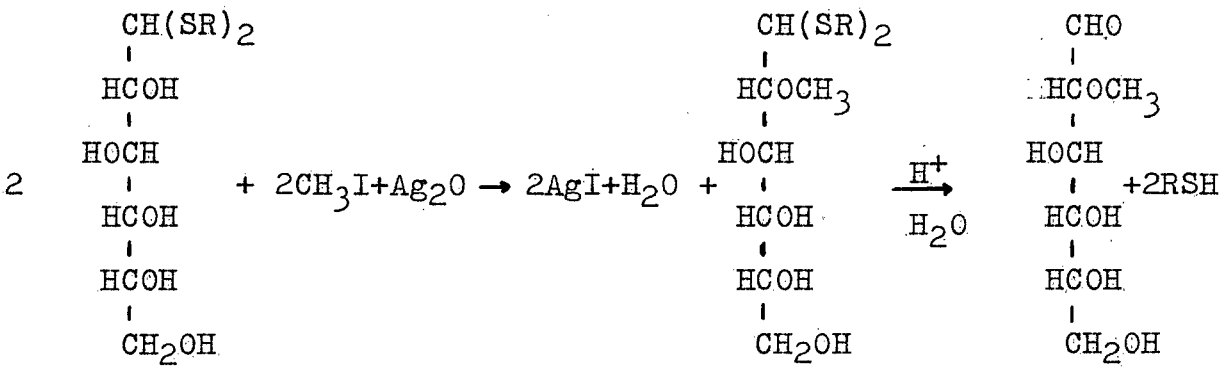
D-glucose (aldehydo form) — ethyl mercaptan — D-glucose diethyl mercaptal

Though the reaction is by no means peculiar to carbohydrate aldehydes and ketones, it is in this field that it has found its widest application.

The mercaptals have interesting properties in that they are necessarily acyclic since they no longer possess a carbonyl function. For the same reason they are quite stable to bases and mild oxidants and can therefore be used in such reactions as the Purdie[16] methylation with methyl iodide and silver oxide, unlike the simple sugars. Since none of their

hydroxyl groups are involved in ring formation, they lend themselves to the preparation of partially substituted sugars whose syntheses would be unnecessarily complicated starting from the various cyclic forms.

It was reported in 1934 by Lieser and Leckzyck[12] that D-glucose diethyl mercaptal (I) could be selectively methylated by the use of methyl iodide and silver oxide at 0°C. to give exclusively 2-O-methyl-D-glucose diethyl mercaptal (II) in 52% yield. Similar results were also obtained with D-glucose dibenzyl mercaptal (III). The corresponding 2-O-methyl-D-glucose (IV) could easily be obtained by acid hydrolysis of the mercaptal groups. For this reason it is the most easily accessible glucose methyl ether.



I R = $-C_2H_5$ II

III R = $-CH_2C_6H_5$

Since the method showed promise of an easy route to specifically substituted sugars, they attempted the same reaction with the mercaptals of D-galactose, L-arabinose, D-xylose and L-rhamnose. They stated that these failed to react in a similar way, since they were able to isolate no products, and implied that the

reaction was peculiar to D-glucose mercaptals, although there appears to be no a priori reason why this should be so. They did not investigate further, since at that time the problem of separating the complex mixtures of products which may have resulted was almost insuperable. This was unfortunate for if the reaction, albeit an incomplete one, could have been extended to other sugars in general, the usual lengthy and difficult introduction and removal of blocking groups could be avoided in the preparation of partially methylated sugar derivatives. The latter have assumed great importance in the determination of polysaccharide structure.

However, since the introduction of partition chromatography in 1941 by Martin and Synge[13] a valuable technique is available for the separation of complex mixtures of carbohydrates such as might result from incomplete methylations.

Apart from the above practical significance, Lieser's work has considerable theoretical interest with respect to the study of relative reactivities of hydroxyl groups in sugars. This subject has been extensively reviewed by Sugihara.[18] The problem is by no means clarified however, and this applies in particular to the reactivities of the hydroxyl groups in sugar mercaptals, upon which relatively little work has been done.

In this latter respect, Lieser[12] has shown that in the methylation of D-glucose diethyl and dibenzyl mercaptals,

the reactivity of the 2-hydroxyl so far exceeds any other that its methyl ether results exclusively. This is in contrast to the more random nature of methylation in the free sugars or glycosides, but his work has not indicated that this unusual reactivity is generally exhibited by mercaptals. Further, Zinner[20] has recently shown that in partial esterifications of sugar mercaptals, the terminal or primary hydroxyl group is considerably more reactive than the secondary hydroxyls, a phenomenon which is not as markedly exhibited by other types of carbohydrates. In other reactions, such as tritylation,[7] the mercaptals appear to follow the general trends established for carbohydrates.

A factor which results in the mercaptal hydroxyls exhibiting different relative reactivities compared to the free sugars or glycosides is their acyclic nature. Barker and co-workers[1] have shown that for the polyols at least, there is a marked tendency for the carbon chain to adopt the planar zig-zag form, resulting in selectivity in certain reactions. It is reasonable to suppose that the mercaptals are similar in nature and that the resultant differences in conformation of their hydroxyl groups compared to those already established for cyclic sugars will affect their reactivities. Further, the proximity of the bulky, easily polarisable sulphur atoms is bound to exert an effect on the reactivity of the remainder of the mercaptal molecule. Fischer[4] has shown that D-glucose diethyl mercaptal is weakly acidic, giving a 2-sodium salt in aqueous alkaline solution.

In view of the above considerations, the aims of the present work are as follows:

(1) A reinvestigation of Lieser's original work to determine firstly if methylation is exclusive at the 2-position and secondly, if any reaction does occur with mercaptals other than those of D-glucose, and if so, to what extent.

(2) An investigation into the generality of the reaction.

(3) The identification of the principal monomethyl products of the reaction to establish which are the most highly reactive positions, if any such clearly exist as in D-glucose mercaptals.

(4) The establishment of whether with an effective separation technique, an incomplete reaction such as the above can be profitably extended to the preparation of specific methylated sugars.

The success of the investigation in achieving these aims will depend on the complexity of the mixtures of reaction products obtained and the adaptability of the separation techniques of partition chromatography.

DESCRIPTION AND RESULTS OF PRESENT RESEARCH

I. Preliminary Investigation of Methylation Reactions

The diethyl mercaptals of D-glucose, D-mannose, D-galactose and L-arabinose were methylated on an exploratory scale by the method of Lieser and Leckzyck.[12] Upon separation of the products from excess methyl iodide and silver oxide, syrups were obtained. The syrup from the reaction of D-glucose diethyl mercaptal crystallised 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. Thus in a general way Lieser's results were confirmed. The mother liquor from the above 2-O-methyl-D-glucose mercaptal and the remaining three reaction mixtures were investigated by means of paper partition chromatography. The developed and detected chromatograms gave a strikingly similar pattern of spots, as shown in Appendix A, indicating qualitatively that the reaction is more general than had been supposed. The presence of a further amount of 2-O-methyl-D-glucose diethyl mercaptal in the mother liquor is also indicated, suggesting that if desired, the yield of this product could be improved to approach Lieser's 52%.

It can be seen from the chromatograms and Table II that the reaction in each case gave products of two types. Those products with considerably lower rates of travel than the unsubstituted mercaptals are presumably strongly polar

compounds and are of unknown constitution. However, it is clear that they result from a modification of the mercaptal groups in some way, since reaction at the hydroxyl groups would not be expected to give rise to an increased polarity. The mercaptal groups have not been removed completely, since these compounds gave no spot for a reducing sugar with p-anisidine hydrochloride. It is also apparent that these compounds are probably carbohydrate in nature since their R_F values vary from sugar to sugar, suggesting that the molecule contains part of the original sugar moiety. On hydrolysis with acid, however, they did not regenerate the parent sugars. The most intense of these "slow" spots can be produced by reacting the mercaptal with methyl iodide alone, which indicates that this major "slow" component may possibly be a type of methyl sulphonium iodide. This would account for the increase in polarity. However, any attempt at postulating structures for any of these compounds is highly speculative and is in effect a separate problem. It can be said definitely from their rates of travel, their different colour reaction with iodine vapour[5] and their failure to yield sugars on acid hydrolysis,[4] that these compounds are not mercaptals and were not investigated further at this time.

From comparison with known standards, the "fast" spots which gave a yellow colour reaction with iodine-vapour are obviously mercaptals and methylated mercaptals. From the distribution and intensity of these it appeared that reaction had been most extensive in the case of D-glucose since no spot

for unchanged mercaptal was detected. Somewhat less reaction appeared to have occurred in the case of D-galactose and only a small amount in the cases of the other two mercaptals.

Due to the high R_F values of the mercaptals and consequent low degree of chromatographic separation, the products were hydrolysed and reinvestigated by paper chromatography as the free sugars thus produced. The results of this are shown in Appendix B. From a comparison with the characteristic R_G values of known compounds[8] it appears that the first row of spots correspond to unsubstituted sugars, the second to their monomethyl derivatives and the remainder to more highly substituted sugars. (Table III.) From this it can be seen that some monomethylation has occurred in each case, which is in apparent disagreement with Lieser's statement that the reaction fails for sugar mercaptals other than those of D-glucose. In the latter case the reaction gives exclusively monomethylation and since no unsubstituted D-glucose was detected, all the original mercaptal must have reacted. This agrees with the findings above and also explains the absence of a spot of $R_X = 0.95$ in the glucose column in Table II. In the reaction of D-mannose and L-arabinose mercaptals methylation appears to have gone to the monomethyl stage also, but to a much lesser extent since large amounts of the unchanged sugars were detected. However, in the reaction of D-galactose mercaptal some polymethylation has also taken place. These results suggest the reason for Lieser's failure to isolate any crystalline products except in the case of D-glucose;

in two instances the small amounts of products seem to be responsible and in the other, the complexity of the reaction mixture.

From the above evidence it again appeared that the selectivity of the reaction, if not the extent, was more general than had been supposed, at least as far as D-glucose, D-mannose and L-arabinose were concerned. The evidence from the D-galactose hydrolysate was somewhat in disagreement.

During this preliminary work it was observed that there were considerable differences in the solubilities of the various mercaptals in methyl iodide. Under the conditions used, it was found that the extent of methylation corresponded approximately to the degree of solubility of the particular mercaptal being reacted. In this respect D-glucose diethyl mercaptal was found to be most soluble and its unusual reactivity appeared in part due to the fortuitous choice of methyl iodide as reaction solvent. Large amounts of D-mannose and L-arabinose mercaptals were found undissolved at the end of the reaction and these mercaptals appeared to have reacted to the least extent.

In order to eliminate this solubility effect it was decided to incorporate an inert reaction solvent in which all four mercaptals had comparable solubilities and compare the reaction of each again on a more equal basis. The most suitable of the common organic solvents which are inert to methylation was found to be tetrahydrofuran. Since the

presence of a large amount of inert solvent was found to quench the reaction almost entirely, increased amounts of methyl iodide and silver oxide were used. In order to determine if changing the original reaction temperature of 0°C. had any critical effect on the nature of the products, three parallel reactions were carried out on each mercaptal at 3°, 22°, and 50°C.

The syrupy products were separated from the reaction mixtures as before, however in this case none of them crystallised spontaneously. They were again investigated by paper chromatography, the results being shown in Appendix C and Table IV. The generality of the reaction was again indicated from the similarity of the pattern of spots obtained. The unusual reactivity of D-glucose diethyl mercaptal was not now so markedly apparent since a spot was obtained for unchanged mercaptal at all three temperatures. The products were hydrolysed and rechromatographed as before, the results of this being shown in Appendix D and Table V. Some monomethylation has again occurred in each case and also polymethylation which was previously absent in three of the reactions when carried out in methyl iodide. The extent of methylation appeared more comparable in each case. The following conclusions were drawn from these results. Firstly, the peculiar selectivity of the reaction of D-glucose diethyl mercaptal was no longer apparent when carried out in tetrahydrofuran. Secondly, the reaction appeared more general in this solvent. Thirdly, the effect of varying the reaction

temperature did not seem to be of critical importance.

II. Large Scale Methylations and Separations

In the light of the above evidence it was decided to repeat the methylations on a larger scale to obtain workable quantities of the products and attempt a separation and isolation of the products as quantitatively as possible. Although complete recovery of the products is not feasible due to chromatographic losses, the relative yields which can be obtained are of value in establishing which are the major products. It was further decided to investigate the monomethyl fractions most thoroughly to determine if preferential methylation had occurred at the 2-position.

A large scale methylation of D-glucose diethyl mercaptal was carried out in tetrahydrofuran at 22°C. It was decided to remove the undesired "slow" components which might later contaminate the other products. The syrupy reaction products were therefore subjected to a crude preliminary separation by column chromatography[19] into a mercaptal and non-mercaptal fraction. The former was hydrolysed as before and the mixture of free sugars separated by a more refined application of column chromatography. The fractions collected were investigated systematically by paper chromatography and polarimetry to locate individual sugars. The results given in Table VI show that there are six different methyl glucoses in addition to some unchanged sugar. The R_F values and methoxyl contents were obtained to determine the degree of methylation

in each of the products.

The monomethyl fraction was shown to be one component only by its chromatographic purity and constancy of optical rotation throughout the fraction. It is thus the largest single product of the reaction. This fraction crystallised spontaneously and failed to depress the melting point of an authentic sample of 2-O-methyl-D-glucose on admixture with it. It was further characterised as 2-O-methyl-D-glucose through its p-toluidide derivative.

The polymethyl fractions were not further investigated. The yields of the original products of methylation were estimated and are given below.

Unreacted D-glucose diethyl mercaptal				2.02%
Monomethyl	"	"	"	8.36%
Dimethyl	"	"	"	10.55%
Trimethyl	"	"	"	5.19%

It should again be emphasized that these do not take into account mechanical losses and are only of value in showing the general extent of methylation.

The important result is that although the only monomethyl derivative resulting from the methylation is the 2-O-methyl, the reaction in this solvent is no longer so selective as to yield this exclusively. The addition of a solvent seems to favour polymethylation, possibly due to the increased solubility of the reactants. Hence the singularity of glucose mercaptals in Lieser's work appears to be a

consequence of the reaction solvent used.

The above methylation and separation of products was repeated on D-mannose diethyl mercaptal. The results are given in Table VII, showing that there are eight distinct methyl mannoses in addition to some unchanged D-mannose. The degree of methylation in each product was determined as before.

Of the four monomethyl mannoses, two were present in negligible amounts and were considered to be relatively unimportant. The two predominant monomethyl sugars were subjected to periodate oxidation studies. The results of this, given in Table VIII, indicated that the larger was 6-O-methyl- and the lesser 5-O-methyl-D-mannose. These were further characterised through their phenylosazones.

The yields of the original products were estimated as before and are given below.

Unreacted	D-mannose	diethyl	mercaptal	0.53%
Monomethyl	"	"	"	12.07%
Dimethyl	"	"	"	3.88%
Trimethyl	"	"	"	21.45%

The results in this case show that reaction does not predominate at the 2-position nor at any one position. Nevertheless the primary hydroxyl is apparently most reactive, with the 5-position also being highly reactive. Further, the total yield of methylated products is now more comparable with that obtained from D-glucose diethyl mercaptal than when methyl Iodide was used as reaction solvent. However, the monomethyl derivatives are not the largest products of the reaction,

since methylation has apparently proceeded largely to the trimethyl stage.

A large scale methylation and preliminary crude fractionation was carried out on L-arabinose diethyl mercaptal exactly as before. The final refined separation, however, was effected by paper chromatography, followed by recovery of the individual sugars from the developed chromatograms by elution of the appropriate zones. The results are given in Table IX, showing that there are seven distinct methyl arabinoses present but no unchanged L-arabinose. The yields of the original products were estimated as before and are given below.

Monomethyl L-arabinose diethyl mercaptal 2.1%

Dimethyl " " " 26.8%

The results do not show that methylation predominates at any one position. Apart from the less specific nature of methylation, reaction appears to have gone largely to the dimethyl stage, with three of the four possible monomethyl arabinoses being present only in negligible amounts. The total yield of methylated products is now more comparable with that from D-glucose mercaptal than when methyl iodide was used as reaction solvent.

From the diversity of spots obtained in the preliminary investigation of D-galactose diethyl mercaptal it did not appear that any particular selectivity of reaction was exhibited. Indeed, an exploratory separation of the products showed that the monomethyl fraction was not

homogeneous, indicating that reaction did not predominate at any one position. It was felt that the value of the results to be obtained did not justify a repetition of the above large scale work on D-galactose diethyl mercaptal at this time.

III. Conclusions and Theoretical Implications

Of the mercaptals investigated both by Lieser and in the present work, only the D-glucose derivatives exhibit unusual reactivity of the 2-hydroxyl group. Under Lieser's conditions methylation has been shown to occur exclusively at this position and although reaction is less selective when an inert solvent is used, the 2-O-methyl is the sole monomethyl ether formed. The reasons for this peculiar reactivity are not clear. Lieser[12] stated that the reactivity of the 2-hydroxyl is particularly increased by the neighbouring mercaptal groups, presumably by an electronic effect. If this is true, it is difficult to see why this effect is not equally present in other mercaptals which are identical with those of D-glucose except for the configuration of their hydroxyl groups. That there is such an effect appears to be established by the acidity of the 2-hydroxyl as demonstrated by Fischer[4], and also by the fact that non-mercaptalated glucoses do not show this peculiar reactivity of the 2-position in methylation. It is doubtful, however, that this effect is solely responsible for producing the unusual reactivity of the 2-hydroxyl.

The possibility of a purely steric effect operating in conjunction with the above electronic effect was considered. It is reasonable to assume that the proximity of the mercaptal groups confers potential reactivity on the 2-position in all the sugar mercaptals. However, if the availability of this position to attack were reduced due to blocking by either the bulky mercaptal groups or the rest of the molecule, this potential reactivity would be diminished and perhaps no longer apparent. A study of models of the four mercaptal molecules was made. Various spatial arrangements of the groups were considered in an attempt to minimize non-bonded interactions, but in no case did these reveal any pronounced differences between the various mercaptals in the availability of the 2-position. Hence it appears that this steric effect is not responsible for the marked difference between glucose and the other mercaptals.

A consideration of the conformation of the hydroxyl groups in the planar zig-zag form, established by Barker[1] for the polyols, suggested the possibility of differences in hydrogen-bonding being responsible for the singularity of glucose mercaptals. The greater the extent of hydrogen-bonding, the less reactivity would be expected from an alcohol in a nucleophilic substitution reaction such as methylation. The infra-red spectra of the four mercaptals (Appendix E) show marked differences in the position of the hydroxyl peak. The peak for glucose mercaptal at 3395 cm^{-1}

is shifted the furthest towards the high frequency region of the spectrum indicating least hydrogen-bonding, with progressively more for galactose, mannose and arabinose mercaptals as indicated by the positions of their peaks at 3320, 3280 and 3275 cm^{-1} respectively. Thus the greater freedom of the hydroxyl groups in cooperation with the effect of the neighbouring mercaptal groups suggests an explanation for the unusual behaviour of glucose mercaptal compared to the other mercaptals.

A complication is introduced when tetrahydrofuran is the reaction solvent. Since it is capable of solvent-solute hydrogen bonding whereas methyl iodide is not, it might be expected that differences in the inter- and intramolecular hydrogen-bonding of the various mercaptals would tend to be smoothed out in the presence of a large number of bonding solvent molecules. This would result in more similar reactivity of the 2-hydroxyls. The presence of tetrahydrofuran does indeed reduce the marked reactivity of the 2-hydroxyl over the other hydroxyls in glucose mercaptal as evidenced by the occurrence of polymethylation, but apparently does not significantly increase the reactivity of this position in other mercaptals.

The effect of the dielectric constant of the solvent used has been considered, but since all the reactions involve methyl iodide they probably take place by the same mechanism. Hence changes in the dielectric constant of the medium would

not be expected to affect particular hydroxyl groups.

The complexity of the various electronic, electrostatic and conformational effects simultaneously operative makes any complete explanation of the peculiarity of glucose mercaptals impossible without recourse to further investigation. A study of the relative importance of these factors seems desirable.

A knowledge of these factors would also be of value in extending the reaction to the preparation of specifically substituted derivatives of sugars other than D-glucose. There is promise of this in the results from the methylation of D-mannose mercaptal. Adjusting the reaction conditions should lead to more practical yields of the 5-O-methyl and 6-O-methyl ethers. Although the results from the methylations of D-galactose and L-arabinose mercaptals do not show any promise under the conditions used, partial methylations of mercaptals of these and other sugars not yet investigated may be of preparative and theoretical value if suitable conditions can be determined.

EXPERIMENTAL

All melting points were taken by means of a Leitz electrically heated melting point block and are corrected. All evaporations were done *in vacuo* at 40°C. Methoxyl analyses were carried out by the method of Viebock and Schwappach as described by Clark.(2)

I. Preparation of Diethyl Mercaptals

The following mercaptals were prepared by the method of Fischer(4), and their physical constants are given in Table I..

Table I

Physical Constants of Diethyl Mercaptals

Mercaptal	M.P.	Lit.M.P.	$[\alpha]_D^{20}$	$[\alpha]_D^{20}$(4)	Yield
D-glucose	128-129°C.	127-128°C.(4)	-27°(H_2O)*	-29.8°(H_2O)*	50%
D-mannose	133-134°C.	134°C.(10)	0°(Pyridine)	-	53%
D-galactose	140-142°C.	140-142°C.(4)	-9°(H_2O)	-10°(H_2O)	64%
L-arabinose	124-125°C.	124-126°C.(4)	-5°(Pyridine)	-	60%

* Rotation of this compound was measured at 50°C.

II. Methylation of Mercaptals in Methyl Iodide

D-glucose diethyl mercaptal was methylated by the method of Lieser and Leckzyck(12). The mercaptal (0.94 gm.) was shaken with silver oxide (1.5 gm.) and methyl iodide

(10 ml.) for 21 hours at 0°C. under anhydrous conditions. Finally, the mixture was allowed to attain room temperature (one hour) and filtered. The residue was extracted successively with boiling chloroform, acetone and methanol. The filtrate and extracts were combined and evaporated to a syrup which was taken up in a small amount of methanol. On standing this solution deposited crystals (0.46 gm.) of 2-O-methyl-D-glucose diethyl mercaptal, m.p. 154-155°C. Lit.m.p. 155-156°C. (12) Anal: Calc. for $C_{11}H_{24}O_5S_2$: $-OCH_3$= 10.37%. Found: $-OCH_3$ = 10.99. Yield 46%.

The diethyl mercaptals of D-mannose, D-galactose and L-arabinose were also methylated by the above procedure, but the syrups obtained failed to yield any crystalline product.

III. Chromatographic Investigation of the Reaction Products

Chromatograms of the four reaction products above were run on Whatman No. 1 paper using n-butanol-ethanol-water (40:10:50) as developer. Detection of the chromatograms with iodine vapour (5) showed the pattern of spots represented in Appendix A. Table II gives the R_x values and descriptions of the spots produced.

Further duplicate chromatograms were detected with p-anisidine hydrochloride but failed to yield any spots for reducing sugars.

Table II

Results of Chromatograms of the Products of Methylations in Methyl Iodide

D-Glucose mercaptal		D-Mannose mercaptal		D-Galactose mercaptal		L-Arabinose mercaptal	
R_X*	Description	R_X	Description	R_X	Description	R_X	Description
0.13	OF	0.10	OF	0.13	OF	0.13	OF
0.21	OF	0.18	OF	0.21	OF	0.21	OF
0.36	OI	0.36	OI	0.40	OI	0.40	OI
-		-		0.47	OF	0.46	OF
-		0.94**	YI	0.95**	YF	0.95**	YI
1.00	YI	1.07	YF	1.03	YI	1.05	YF

*R_X is based on the arbitrary standard 2-O-methyl-D-glucose diethyl mercaptal whose rate is set equal to 1.00.

**These values correspond to standards of the unsubstituted mercaptals.

Key: O = Orange Y = Yellow I = Intense F = Faint.

IV. Investigation of the Effect of Individual Reagents

Two samples of D-glucose diethyl mercaptal (10 mgm.) were dissolved in carbon tetrachloride (5 ml.) and shaken for 21 hours at 0°C. with methyl iodide and silver oxide respectively. After filtering, the solutions were chromatographed with standards and detected as before. The results are given below.

A. Methyl iodide treated mercaptal: Three spots of R_X = 0.21, 0.35 (Intense) and 0.94 (unchanged mercaptal).

B. Silver oxide treated mercaptal: One spot of R_x = 0.95 (unchanged mercaptal).

V. Hydrolysis of the Reaction Products

The reaction products from II were dissolved in 20% aqueous ethanol containing 5% hydrogen chloride. The solution was heated to reflux, a stream of nitrogen being bubbled through continuously. When chromatography of the solutions failed to detect any unchanged mercaptals (5-6 hours), they were passed through a column of Duolite A-4 anion exchange resin and concentrated by evaporation.

VI. Chromatographic Investigation of the Hydrolysates

The above hydrolysates were chromatographed as in III and detected with p-anisidine hydrochloride, the results being shown in Table III.

Table III

Results of Chromatograms of Hydrolysed Products of Methylations in Methyl Iodide

Glucose Hydrolysate	Mannose Hydrolysate	Galactose Hydrolysate	Arabinose Hydrolysate
-	0.11 I	0.08 F	0.12 I
0.23 I	0.28 F	0.22 F	0.34 F
-	-	0.39 F	-
-	-	0.72 F	-
-	-	0.86 F	-

(The values given are R_G values based on 2,3,4,6-tetra-*O*-methyl-D-glucose = 1.00)

VII. Methylation of Mercaptals in Tetrahydrofuran

The mercaptal (0.3 gm.) was dissolved in dry peroxide free tetrahydrofuran (20 ml.). After silver oxide (1 gm.) and methyl iodide (10 ml.) were added, the whole was shaken vigorously for 22 hours. Three parallel experiments were carried out on each mercaptal at 3°, 22° and 50°C. The products were then extracted as before, no crystalline materials being obtained.

VIII. Chromatographic Investigation of the Methylations in Tetrahydrofuran

Chromatograms of the syrupy products from the above were run as before and detected with iodine vapour. The mixtures were then hydrolysed as in V, rechromatographed in n-butanol-ethanol-water (40:10:50) and detected with p-anisidine hydrochloride. The results are presented in Tables IV and V respectively.

IX. Large Scale Methylation of D-Glucose Diethyl Mercaptal

Silver oxide (6.7 gm.) and methyl iodide (67 ml.) were added to a solution of D-glucose diethyl mercaptal (2.0 gm.) in purified tetrahydrofuran (100 ml.). The vessel was flushed with nitrogen, sealed and shaken at room temperature for 22 hours. The products were extracted as in II, an amber syrup (2.07 gm.) being obtained.

Table IV

Results of Chromatograms of the Products of Methylations in Tetrahydrofuran*

D-Glucose			D-Mannose			D-Galactose			L-Arabinose		
3°	22°	50°	3°	22°	50°	3°	22°	50°	3°	22°	50°
0.11	0.10	0.12	0.10	0.10	0.10	0.12	0.13	0.12	0.12	0.13	0.14
0.20	0.20	0.20	0.17	0.18	0.20	0.21	0.21	0.22	0.21	0.21	0.22
0.36	0.36	0.36	0.36	0.36	0.36	0.39	0.39	0.40	0.38	0.40	0.41
0.95	0.95	0.94	0.95	0.96	0.95	0.96	0.96	0.96	-**	-**	0.97
1.00	1.00	1.00	1.01	1.00	1.01	1.01	1.02	1.01	1.02	1.03	1.03

* The values given are R_x values based on 2-O-methyl-D-glucose diethyl mercaptal = 1.00

** The absence of a spot for unreacted mercaptal corresponding to the spots for L-arabinose produced on hydrolysis may be due to the greater sensitivity of the p-anisidine.

Paper chromatography of the above syrup using methyl ethyl ketone-water azeotrope as developer gave essentially the same results as shown in the first column of Table IV. An aliquot (1.96 gm.) was placed on a cellulose column (2.8 cm. diam. x 40 cm. length) and developed with the same solvent. Fractions were collected at one hour intervals for eight hours. (Two column front times) Two fractions were then collected at two hour intervals and the remaining material was removed from the column by developing for a further 80 hours, (20 front times) without any attempt at fractionation. Paper chromatograms of the first eight fractions developed with methyl ethyl

Table V

Results of Chromatograms of the Hydrolysed Products of Methylations in Tetrahydrofuran

D-Glucose Hydrolysate			D-Mannose Hydroylsate			D-Galactose Hydrolysate			L-Arabinose Hydrolysate		
3°	22°	50°	3°	22°	50°	3°	22°	50°	3°	22°	50°
0.09	0.09	0.11	0.12	0.13	0.13	0.07	0.08	0.08	0.13	0.12	0.12
0.23	0.23	0.26	0.25	0.26	0.28	0.18	0.20	0.23	0.32	0.32	0.34
0.40	0.42	0.43	0.42	0.45	0.45	0.36	0.38	0.40	0.44	0.44	0.43
0.46	0.47	0.52							0.50	0.50	0.49
									0.62	0.64	0.65

The values given are R_G values based on 2,3,4,6-tetra-O-methyl-D-glucose = 1.00

ketone-water and detected with iodine vapour indicated fairly good separation had been achieved. Fractions 2-5 were found to contain all the mercaptalated products with slight traces of undesired impurity. These were combined (0.879 gm.) and designated as mercaptal fraction. The higher fractions were discarded.

The mercaptal fraction was hydrolysed as before, passed through a Duolite A-4 column and the eluate concentrated to a syrup (0.425 gm.) by evaporation. Two chromatograms of the latter were run in methyl ethyl ketone-water and detected with iodine vapour and p-anisidine hydrochloride respectively. The first chromatogram showed no spots corresponding to glucose mercaptals indicating hydrolysis was complete. The second

showed four spots of R_F's 0.014, 0.06, 0.21 and 0.47 corresponding to the characteristic R_F ranges[3] of glucose, mono-, di- and trimethyl glucoses respectively.

An aliquot (0.406 gm.) of the above hydrolysate in the minimum amount of methyl ethyl ketone was placed on a column (2.8 x 42 cm.) of cellulose-hydrocellulose (1:1) and developed with methyl ethyl ketone-water azeotrope. Fractions were collected at 30 minute intervals at a flow rate of 40 ml. per hour. After 300 fractions had been collected no further carbohydrate material could be detected in the eluate by application of the Molisch test and fractionation was terminated. The fractions were investigated systematically by paper chromatography, measurement of optical rotation and determination of methoxyl value to obtain the following classification.

Table VI.

Glucose Fractions Separated on Cellulose-Hydrocellulose (1:1) Column

Classification	Tube No.	Wt.(mgm.)	$[\alpha]_D^{20}$	R_F*	% OCH_3**
Hexose	238-270	23	+48°(H_2O)	0.017	-
Monomethyl	169-235	104	+59°(H_2O)	0.05	15.7
Dimethyl A	57-74	50	+ 82°(Acetone)	0.28	26.5
Dimethyl B	86-108	47	+32°(Acetone)	0.18	24.8
Dimethyl C	30-40	42	+61°(MeOH)	0.47	27.3
Trimethyl A	28-29	11	+91°(MeOH)	0.50	36.6
Trimethyl B	12-19	63	+20°(H_2O)	0.80	36.7

*R_F values determined in methyl ethyl ketone-water azeotrope.

**Calculated methoxyl values for mono-, di- and trimethyl hexoses respectively are 16.0, 29.8 and 41.9%.

All the above fractions were found to be chromatographically pure except for some overlap between Dimethyls A and B. The monomethyl fraction also showed constancy of optical rotation throughout the fraction and yielded a crystalline product melting at 153-157°C. Admixture with an authentic sample of 2-O-methyl-D-glucose gave a melting point of 154-158°C. The p-Toluidide was prepared by the method of Smith[17], m.p. 144-146°C. Lit. m.p. 150-151°C. (14).

X. Large Scale Methylation of D-Mannose Diethyl Mercaptal

D-Mannose diethyl mercaptal (2.1 gm.) was methylated in an exactly similar manner to D-glucose mercaptal, yielding a syrup (2.21 gm.) which was fractionated into a mercaptal fraction and a non-mercaptal fraction as before. Hydrolysis of the mercaptal fraction yielded a syrup (0.79 gm.). Two chromatograms were run on this syrup as before. One detected with iodine vapour gave negative results, whereas the other detected with p-anisidine hydrochloride gave three discrete spots of R_F's 0.023, 0.08 and 0.20 and a trailing spot of R_F range 0.50-0.80.

The mannose mercaptal fraction hydrolysate was separated and the fractions investigated exactly as for D-glucose. The results are given in Table VII.

Table VII

Mannose Fractions Separated on Cellulose-Hydrocellulose (1:1) Column

Classification	Tube No.	Wt.(mgm.)	$[\alpha]_D^{20}$	R_F(MEK)	%OCH_3
Hexose	200-225	7	-	0.023	-
Monomethyl A	142-175	107	+30°(H_2O)	0.08	15.4
Monomethyl B	102-139	53	+17.3°(H_2O)	0.09	15.3
Monomethyl C*	89-99	9	+11.6°(MeOH)	0.13	-
Monomethyl D*	83-87	3	-	0.15	-
Dimethyl A	45-55	27	+26°(MeOH)	0.23	28.1
Dimethyl B	35-41	32	+59°(MeOH)	0.28	27.2
Trimethyl A	13-22	141	+38.3°(H_2O)	0.53	34.1
Trimethyl B	7-11	208	+36.0°(MeOH)	0.75	34.1

* Classification is based on R_F values only and is subject to confirmation.

All the above fractions were found to be chromatographically pure except for some overlap between Trimethyls A and B.

XI. Periodate Oxidation of Mannose Monomethyl A Fraction

Solutions containing monomethyl A (21.76 mgm., 0.112 mmole.) and sodium metaperiodate (103.2 mgm., 0.482 mmole.) were mixed and immediately made up to 50 ml. with distilled water and maintained at 20°C. Aliquots (5 ml.) were withdrawn at intervals and excess sodium bicarbonate and potassium iodide added, the solutions being left to stand for 15 minutes to allow complete iodine formation. Consumption of periodate was then determined by adding a measured excess of 0.1N sodium arsenite

solution and back-titrating with 0.1N iodine solution[9]. When periodate consumption had reached a constant value, formic acid production was determined by neutralising an aliquot to methyl red with standard alkali. A further aliquot was treated with dimedone reagent[9] to determine formaldehyde, but failed to yield any precipitate even after prolonged standing. The results are tabulated below.

A. Periodate Consumption

Time (minutes)	5	10	15	30	60	120
Consumption (mmoles.)	0.404	0.410	0.413	0.414	0.417	0.417
Consumption (moles per mole)	3.60	3.66	3.69	3.70	3.73	3.73

B. Formic Acid 0.403 mmole. (3.6 moles per mole sugar.)

C. Formaldehyde None.

XII. Periodate Oxidation of Mannose Monomethyl B. Fraction

Monomethyl B (16.2 mgm., 0.084 mmole.) was treated with sodium metaperiodate (62.8 mgm., 0.294 mmole.) exactly as before, the following results being obtained.

A. Periodate Consumption

Time (minutes)	5	10	15	30	60	90	120
Consumption (mmoles.)	0.03	0.13	0.15	0.19	0.23	0.26	0.26
Consumption (moles per mole)	0.36	1.55	1.79	2.26	2.74	3.09	3.09

B. Formic Acid 0.242 mmole. (2.88 moles per mole sugar.)

C. Formaldehyde None.

The values obtained in XI and XII are compared with the theoretical values for the monomethyl mannoses in Table VIII.

Table VIII

Theoretical and Obtained Results of Periodate Oxidation of Monomethyl Mannoses

Monomethyl Mannose	Moles Periodate Consumed	Moles Formic Acid Produced	Moles Formaldehyde Produced
2-O-methyl	3.0	2.0	1.0
3-O-methyl	3.0	2.0	1.0
4-O-methyl	3.0	2.0	1.0
5-O-methyl	3.0	3.0	0.0
6-O-methyl	4.0	4.0	0.0
Monomethyl A	3.7	3.6	0.0
Monomethyl B	3.1	2.9	0.0

XIII. Preparation of Derivatives of Monomethyls A and B

Treatment of the above sugars with phenylhydrazine by the method of Hamilton[6] yielded crystalline phenylosazones. Their melting points are compared below with those of the corresponding methyl glucose phenylosazones which are identical in structure with the 6-O-methyl- and 5-O-methyl-D-mannose phenylosazones.

Osazone	M.p.	
Monomethyl A	181-185°C.	
6-O-methyl-D-glucose	184-187°C.	(11)
Monomethyl B	126-130°C.	
5-O-methyl-D-glucose	128°C.	(15)

XIV. Large Scale Methylation of L-Arabinose Diethyl Mercaptal

L-Arabinose diethyl mercaptal (3.0 gm.) was methylated by the same procedure as for the D-glucose and D-mannose mercaptals, yielding a syrup (2.757 gm.) which was crudely fractionated as before. Hydrolysis of the mercaptal fraction yielded a syrup (0.863 gm.) which when chromatographed in n-butanol-ethanol-water-ammonia (40:10:49:1) gave no spots with iodine vapour and seven spots with p-anisidine hydrochloride of R_G's 0.18, 0.22, 0.25, 0.33, 0.37, 0.39 and 0.45.

The arabinose mercaptal fraction hydrolysate was streaked on the starting lines of sheets of Whatman No. 3 MM paper which had been pre-run in n-butanol-ethanol-water-ammonia. The load of material was approximately 3 mgm. per cm. of paper width. The strips were developed for 15 hours using the same solvent. Marker strips 1.5 cm. wide were then cut from the edges of the dried developed chromatograms. Detection of these with p-anisidine hydrochloride located the zones containing products. The products were recovered by Soxhlet extraction of the separated zones using 5% aqueous acetone. Rotations, methoxyls and R_F values were determined as before and are given in Table IX.

Table IX

Arabinose Fractions Separated on Whatman No. 3MM Paper

Classification	Wt. (mgm.)	$[\alpha]_D^{20}$	R_F (MEK)	% OCH_3 *
Monomethyl A**	5.6	+106° (H_2O)	0.09	-
Monomethyl B	13.4	+52.5° (H_2O)	0.16	18.5
Monomethyl C	18.5	+37° (H_2O)	0.23	16.6
Dimethyl A	91.4	+58° (H_2O)	ca0.41 (Trails)	33.9
Dimethyl B	147.9	+77° (H_2O)	ca0.50 (Trails)	36.1
Dimethyl C	243.2	± 0° (MeOH)	0.80	34.4

* Calculated methoxyl values for mono- and dimethyl pentoses respectively are 18.9 and 34.8%.

** Classification of this product is based on R_F only.

All the above fractions were found to be chromatographically pure except Dimethyl B which was an overlap fraction of two components which failed to separate. The monomethyl fractions were not investigated further since present in only negligible amounts.

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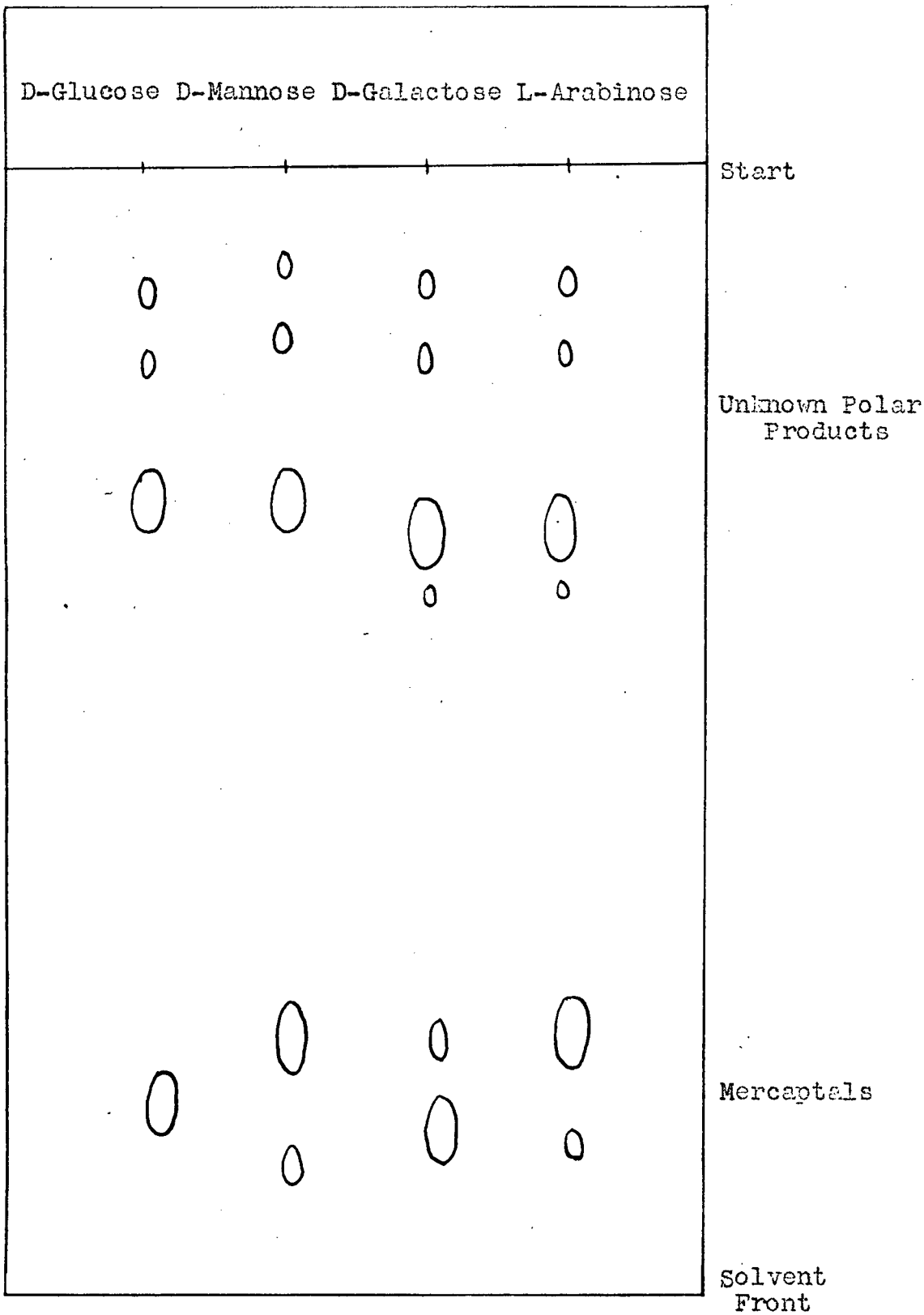
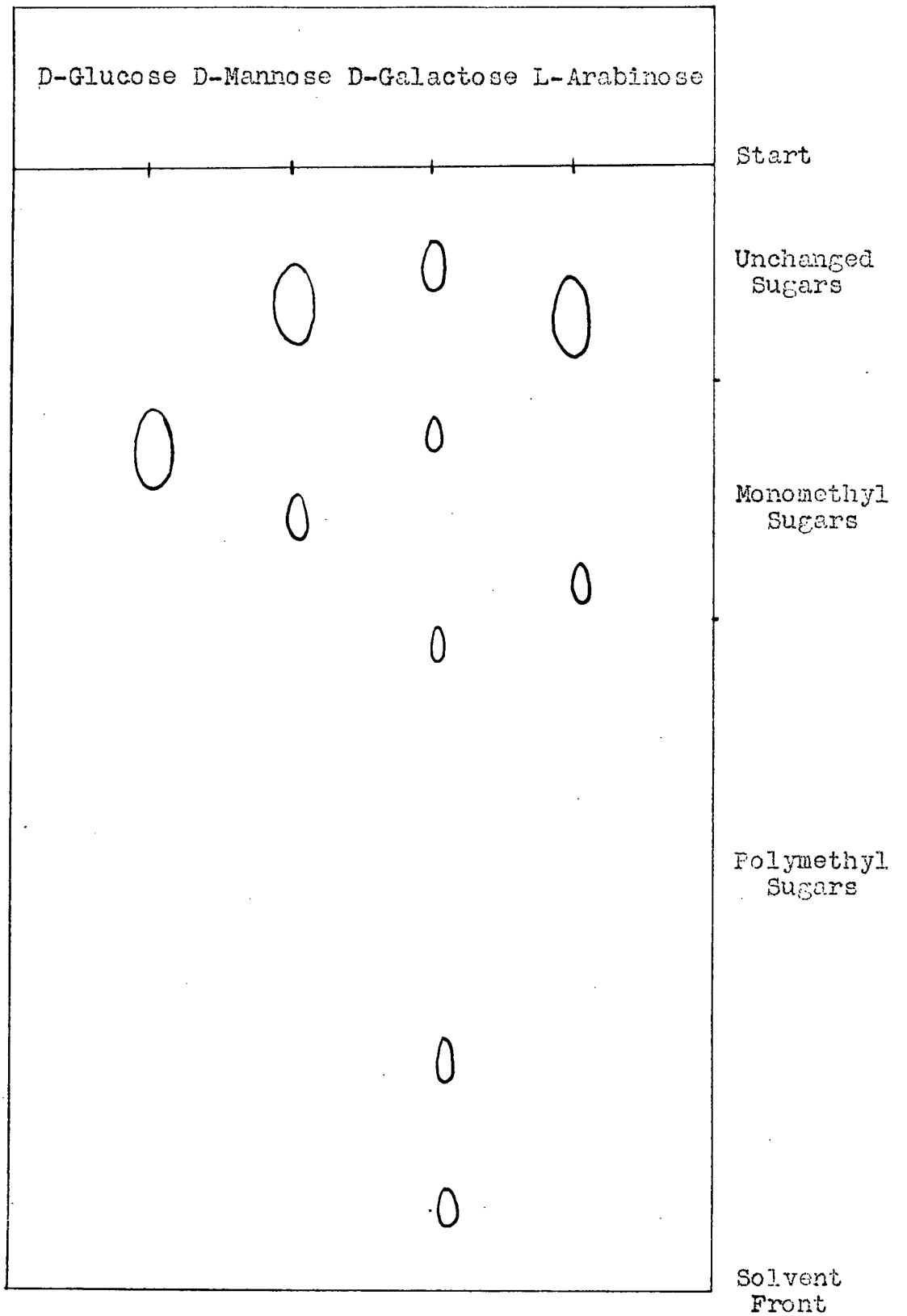
APPENDIX A

Diagram of Chromatographed Products of Methylations in Methyl Iodide



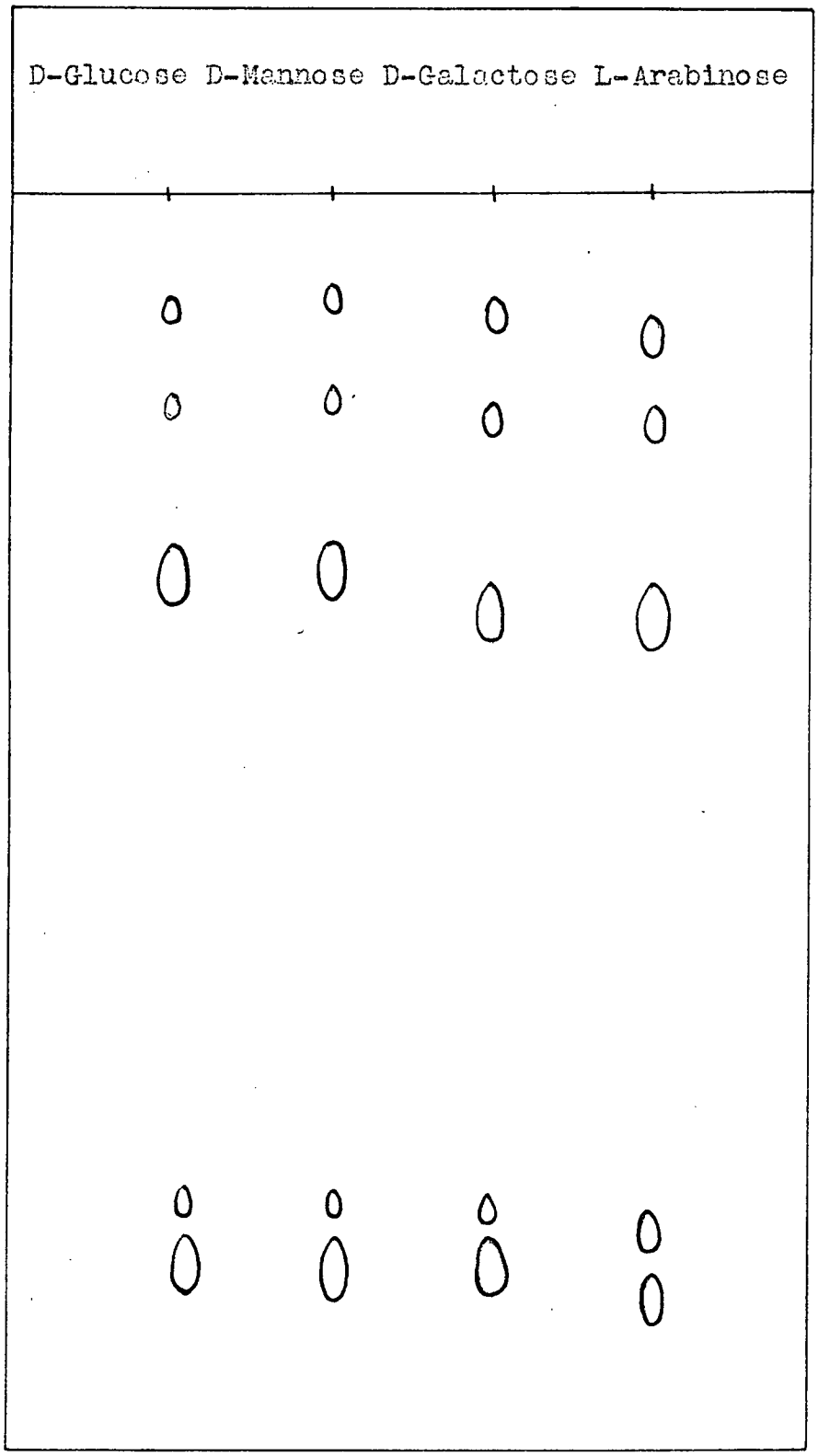
APPENDIX B

Diagram of Chromatographed Hydrolysates from Methylations in Methyl Iodide



APPENDIX C

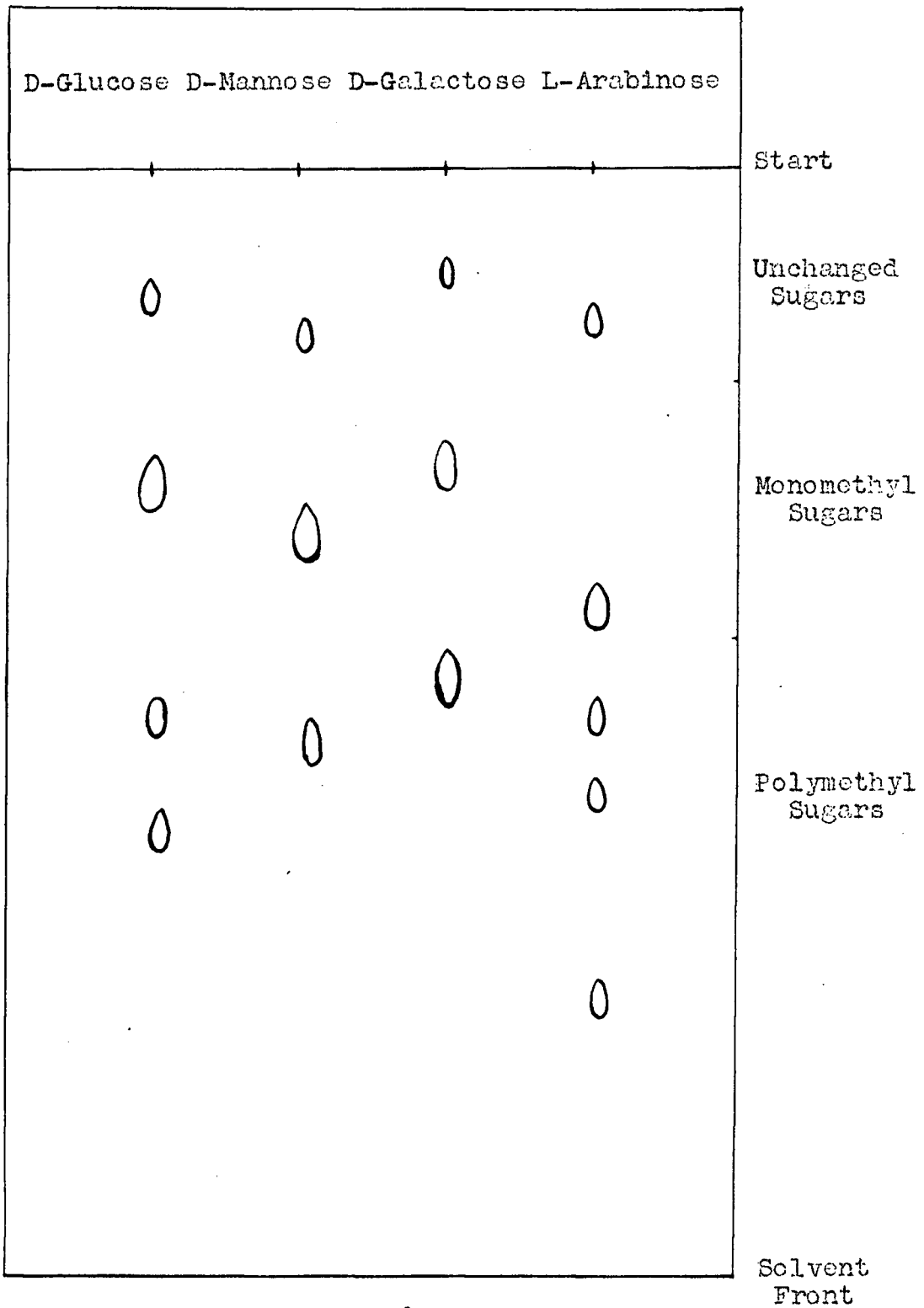
Diagram of Chromatographed Products of Methylations in Tetrahydrofuran*



* This shows products at 50° only.

APPENDIX D

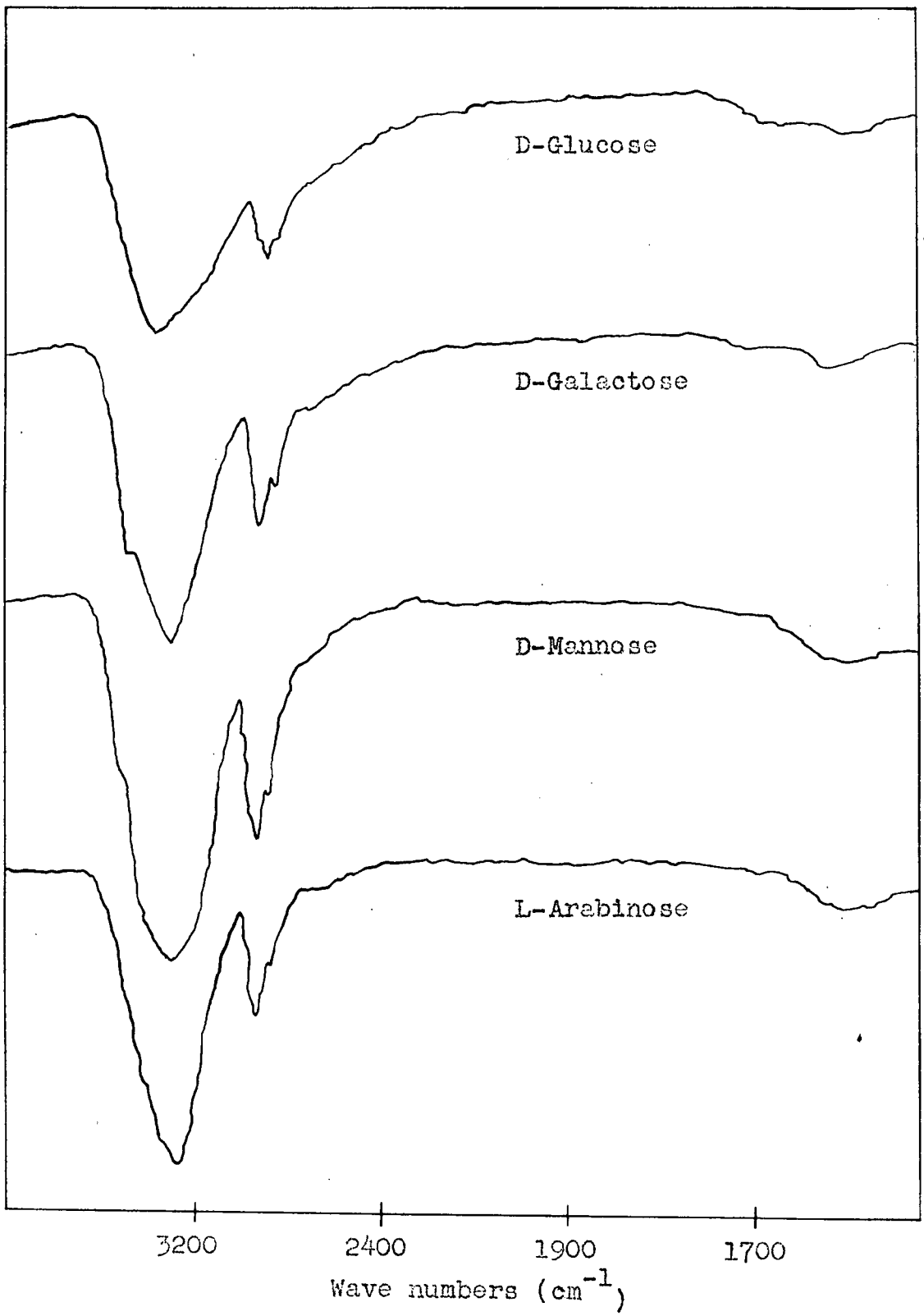
Diagram of Chromatographed Hydrolysates from Methylations in Tetrahydrofuran*



* This shows products at 50° only.

APPENDIX E

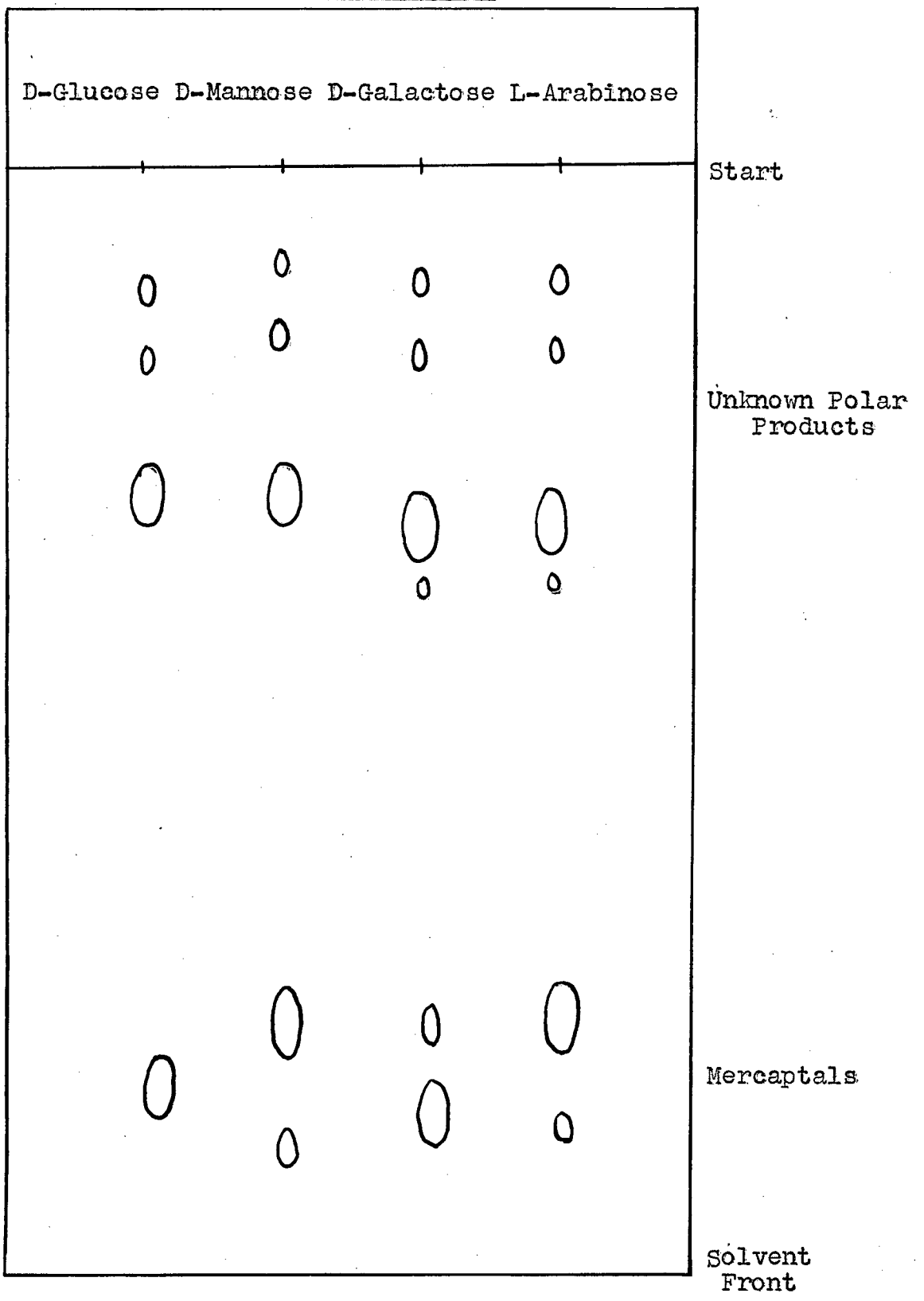
Infra-Red Spectra of Diethyl Mercaptals



The infra-red absorption was measured in a Perkin-Elmer Recording Spectrophotometer using pressed potassium bromide pellets.

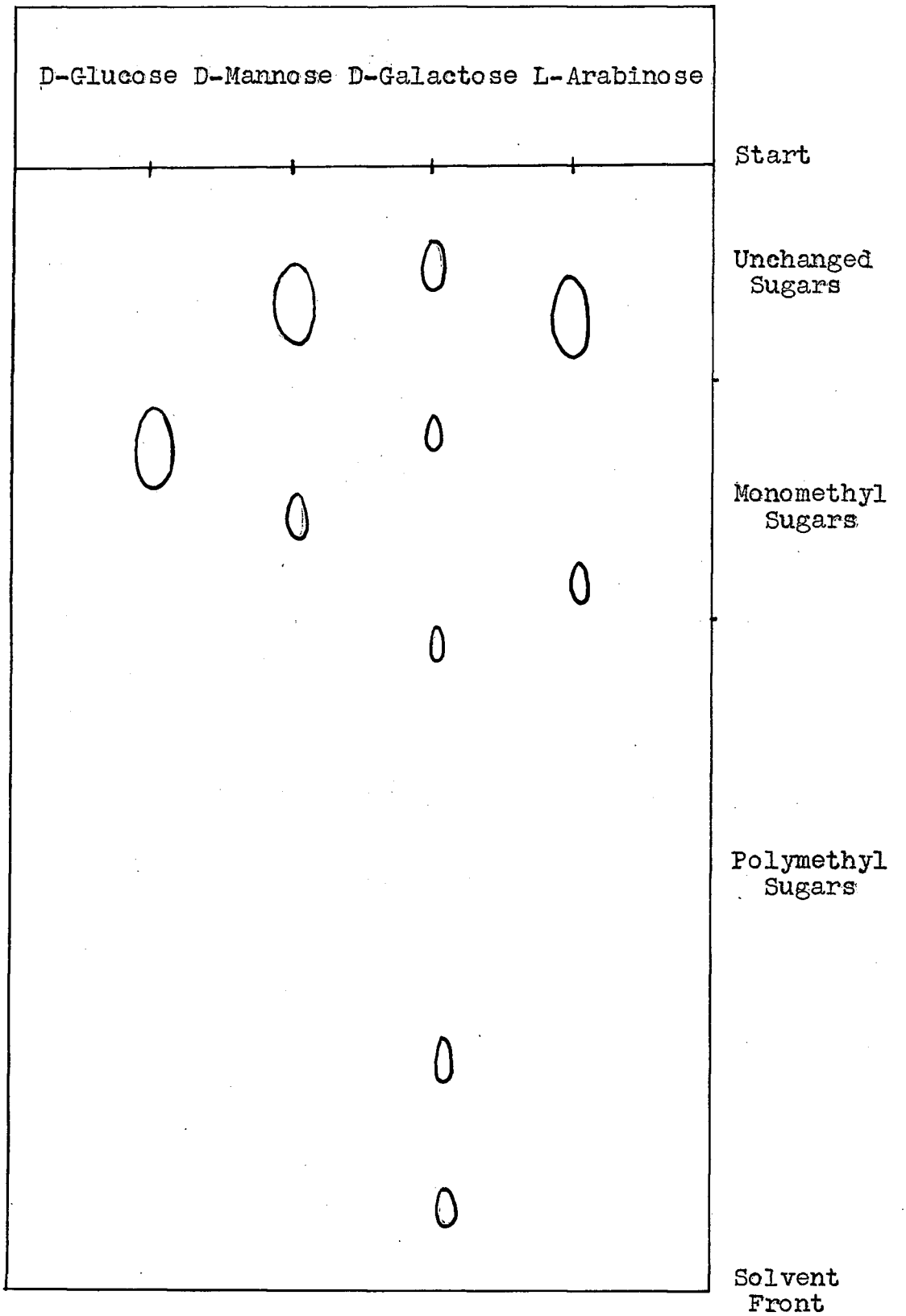
APPENDIX A

Diagram of Chromatographed Products of Methylations in Methyl Iodide



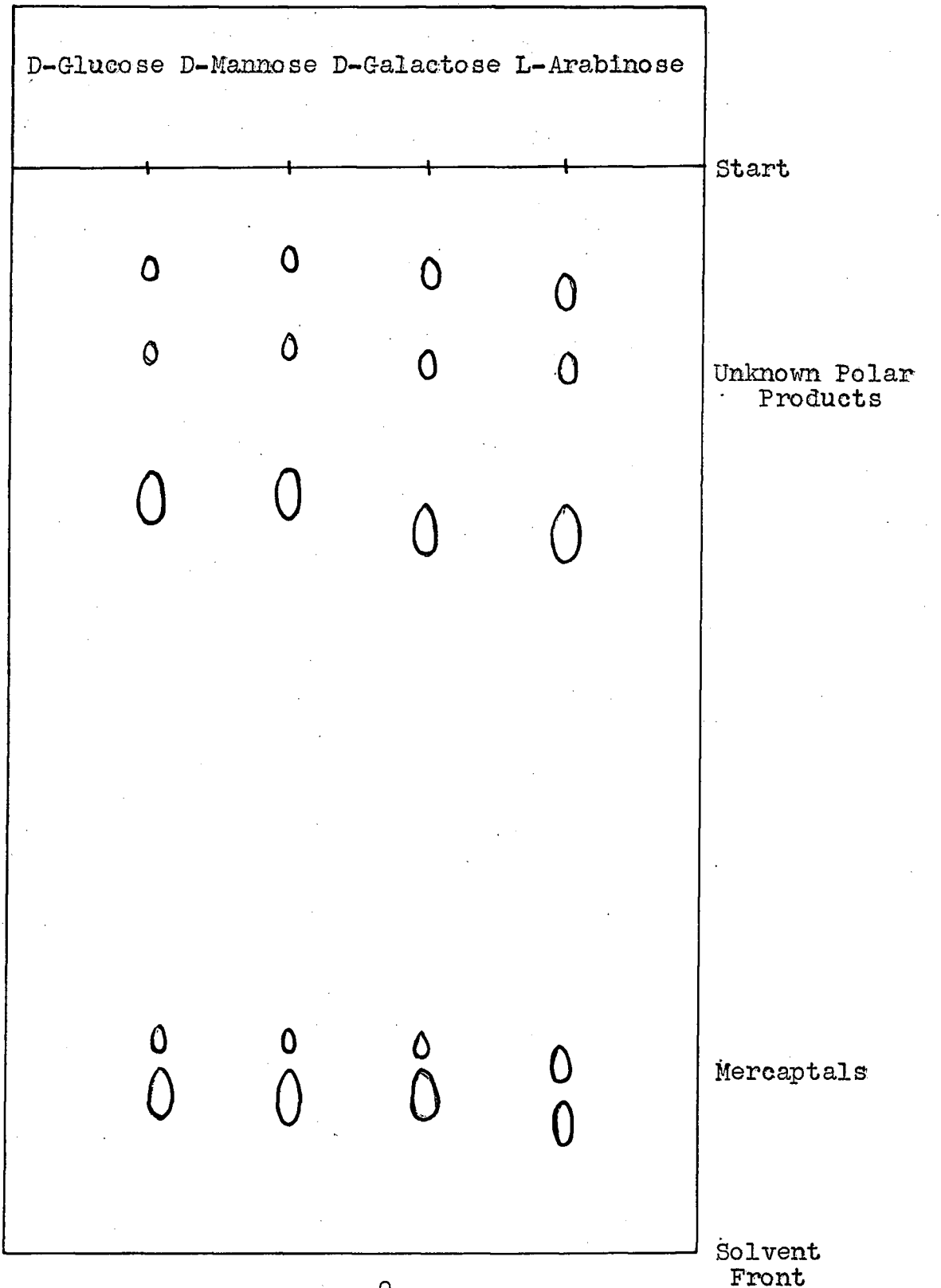
APPENDIX B

Diagram of Chromatographed Hydrolysates from Methylations in Methyl Iodide



APPENDIX C

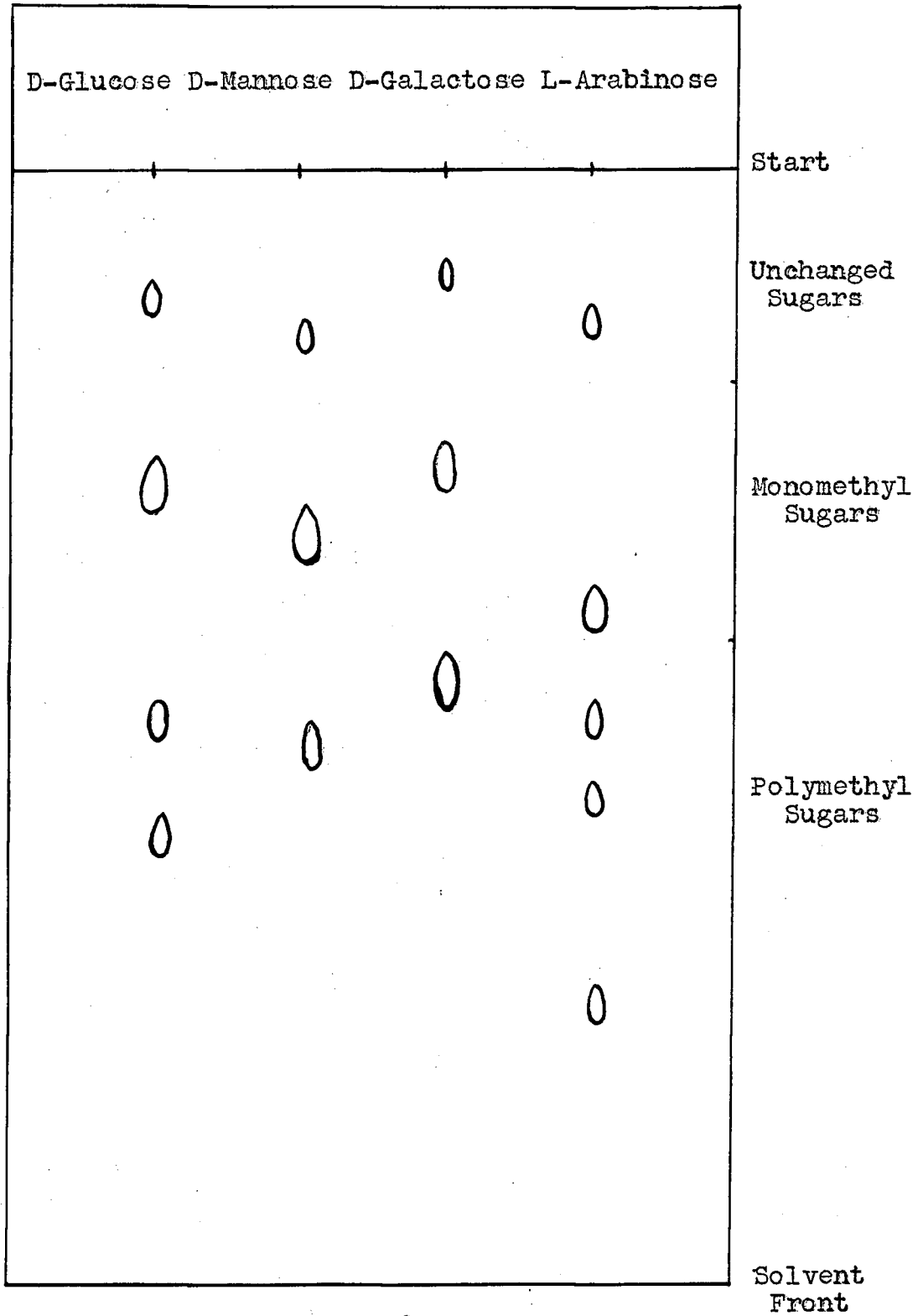
Diagram of Chromatographed Products of Methylations in Tetrahydrofuran*



* This shows products at 50° only.

APPENDIX D

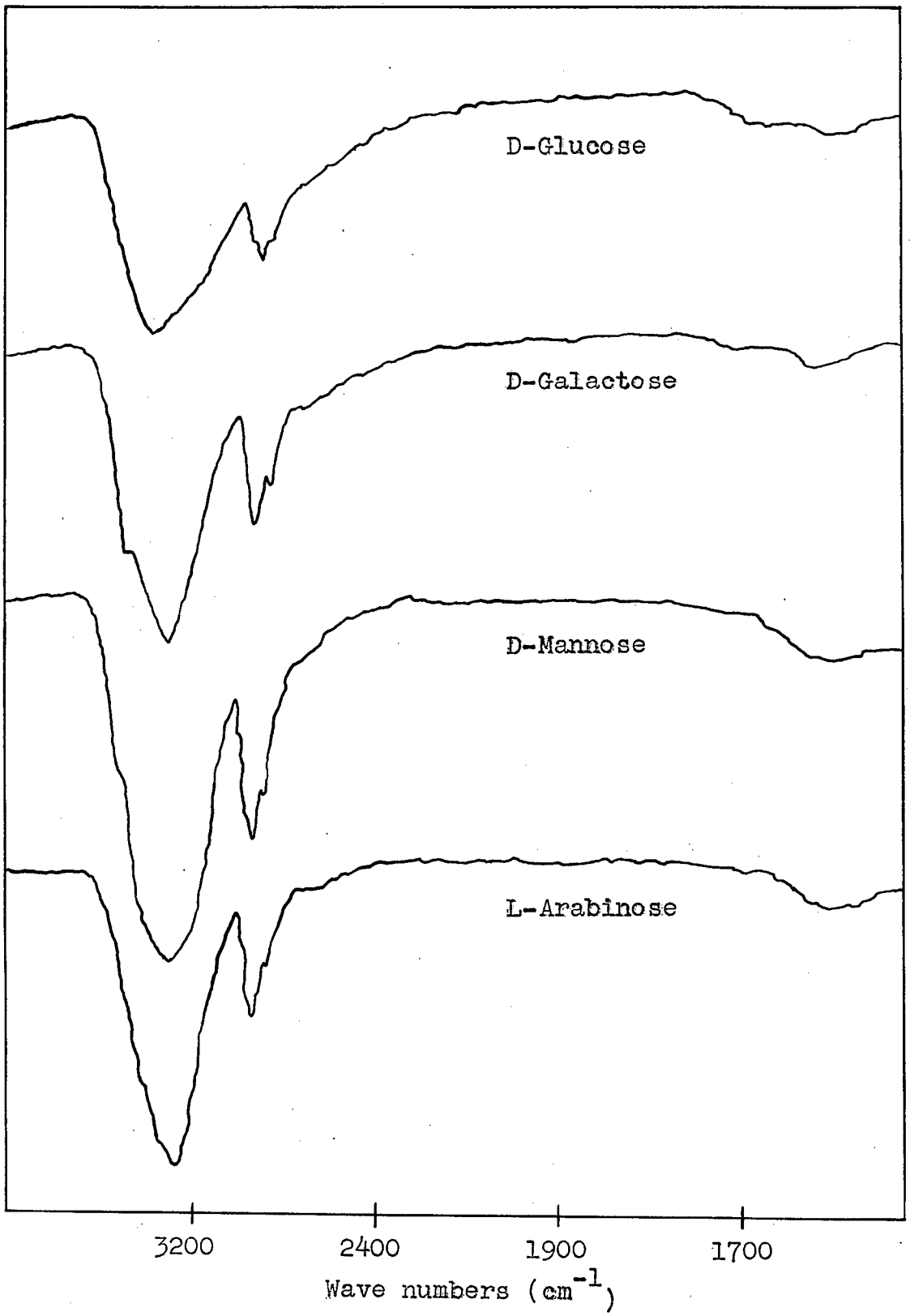
Diagram of Chromatographed Hydrolysates from Methylations in Tetrahydrofuran*



* This shows products at 50° only.

APPENDIX E

Infra-Red Spectra of Diethyl Mercaptals



The infra-red absorption was measured in a Perkin-Elmer Recording Spectrophotometer using pressed potassium bromide pellets.