DEVELOPMENT AND APPLICATION OF METHODS FOR THE SEPARATION OF POLYHYDROXY COMPOUNDS ~~AND PHENYLBORONATES OF SOME ACYCLIC PENTITOLS~~

A thesis submitted by

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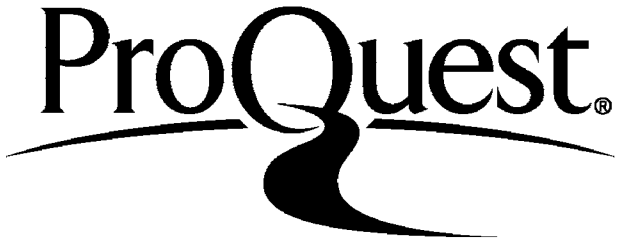

ABSTRACT

Part I. The complexing of molybdate or tungstate ion with polyhydroxy compounds has been studied with a view to utilizing the complexes for the separation of polyhydroxy compounds. The separation of a large number of polyhydroxy compounds by methods such as anion exchange chromatography, paper chromatography, cellulose column chromatography and thin-layer chromatography, has been described. It has been shown that complex forming polyols possessing four adjacent hydroxyl groups can be separated on molybdate or tungstate forms of anion-exchange resin from the polyols which either do not form a complex or complex due to the presence of 1,2,3-cis,cis triol system. The reason for the non-sorption of the polyols of the latter type on the molybdate or tungstate form of resin has beendicussed.

The chromatography on paper partially impregnated with tungstate has divided the polyols into non-complexing polyols, complex forming polyols possessing three adjacent hydroxyl groups and those possessing four adjacent hydroxyl group. The technique of chromatography on paper wholly impregnated with tungstate has, in addition to the separation of a wide variety of substances, further afforded a means of studying the structure of the compounds.

Structures for the polyol-tungstate complexes have been suggested and their correlation with the migration rates (on wholly impregnated paper) has been discussed.

The mode of complex formation of D-galactose has been studied and the conformation suitable for the formation of D-galactose-tungstate comlex has been proposed.

Part II. The preparation of phenylboronates of some acyclic pentitols has been described and the position of hydroxyl group not involved in the ester formation located. Detailed structures of several phenylboronates have been investigated. It has been shown that ribitol and xylitol form 1,3:2,4-bisphenylboronates and 1-deoxy-L-galactitol forms 3,5:4,6-bisphenylboronate. The formation of phenylboronates has been compared with the reaction of aldehydes and ketones with polyols.

To My Father

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C O N T E N T S

A. INTRODUCTION

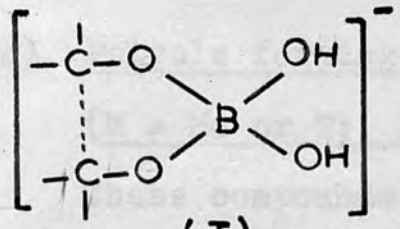
Polyhydroxy-compounds are known to form complexes with ions of several inorganic oxy-acids, e.g. borate,[1] $[B(OH)_4]^-$, germanate,[2,5] $[Ge(OH)_6]^{2-}$, stannate,[3] $[Sn(OH)_6]^{2-}$, antimonate,[4] $[Sb(OH)_6]^-$, tellurate,[5] $[TeO_6]$,[6]- periodate,[6] $[IO_6]^{5-}$, dimolybdate,[7] $[Mo_2O_7]^{2-}$, and ditungstate,[7] $[W_2O_7]^{2-}$. These ions, except the dimolybdate and ditungstate, are themselves the result of co-ordination, e.g.

$$B(OH)_3 + OH^{\ominus} \longrightarrow [B(OH)_4]^-$$

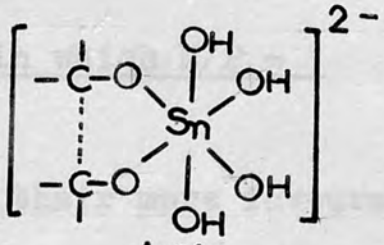
Although the normal molybdate, MoO_4^{2-}, and tungstate ions, WO_4^{2-}, have in their anhydrous salts, e.g. Ag_2MoO_4 and $CaWO_4$, respectively, a tetrahedral symmetry,[8,9] in their known polyacids molybdenum and tungsten are 6-co-ordinated[10]. It is likely that the dimolybdate and ditungstate ions form aquo complexes in which two water molecules around each central atom complete its 6-co-ordination.

$[Mo_2O_7.4H_2O]^{2-}$ $[W_2O_7.4H_2O]^{2-}$

Thus, the formation of complexes between polyhydroxy-compounds can be formulated as the replacement of at least two ligands of the anions by oxygen atoms of the polyhydroxy compound, e.g. (I),(II) and (III).



(I)



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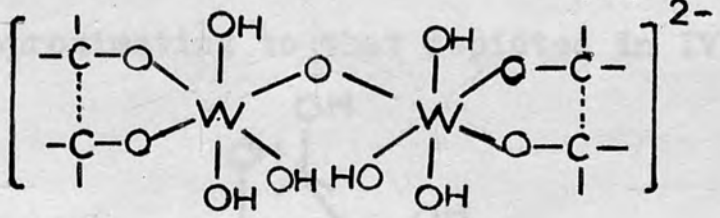


(III)

With the exception of the complexes formed from periodate, which are intermediates in the glycol cleavage reaction,[6] such complexes form the basis of electrophoresis of formally neutral polyhydroxy compounds.[11]

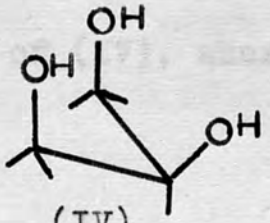
The complex formation with molybdate and tungstate takes place at pH values below those of solutions containing the normal ions (MoO_4^{2-} or WO_4^{2-}) only, i.e. pH <u>ca</u>. 9.[10] Consequently, electrophoresis in molybdate or tungstate solutions has been carried out at pH <u>ca</u>. 5 where immediate formation of stable complexes occurs.[13-16]

Measurement of electrophoretic mobilities and optical rotatory power, and the results of periodate oxidation of a large number of polyols, all carried out in the presence of molybdate or tungstate, has revealed that complex forming polyols fall into two classes:[13-16]

(a) Polyols forming complexes in which M/P = 1

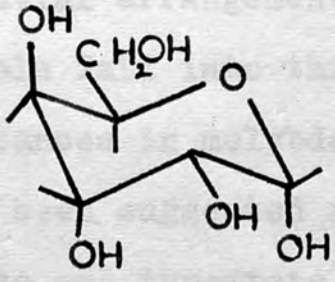
(M = Mo or W; P = polyol)

These compounds possess in their more favourable conformation three hydroxyl groups in a spatial disposition approximating to that depicted in IV.



(IV)

They include pyranoid and furanoid sugars, and inositols which possess a cis-cis-1,2,3-triol system, e.g. α-D-gulopyranose (V), α-D-erythrofuranose (VI) and epi-inositol (VII).



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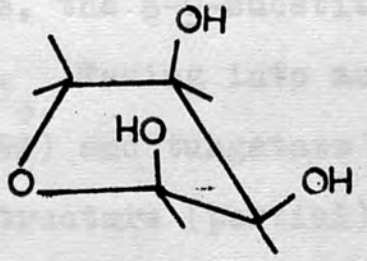

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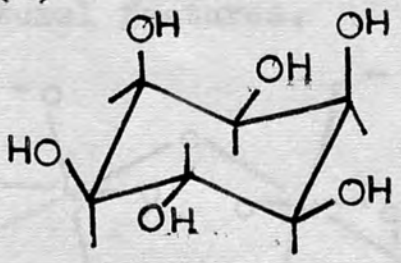
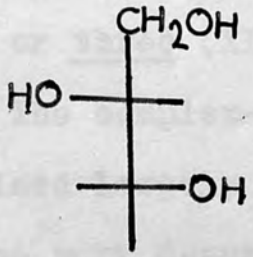


(VII)

Similarly, acyclic polyols which possess a 1,2,3(a,aT) -(VIII) or 1,2,3 (aT,aT)-triol system (IX) (Barker and Bourne nomenclature)[17] will form this type of complex. The hydroxyl groups of (IX), when the carbon chain is in the planar zig-zag conformation, are in the same relative disposition as those of (IV), where as those of (VIII) can



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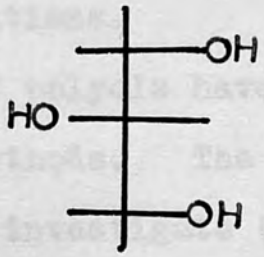
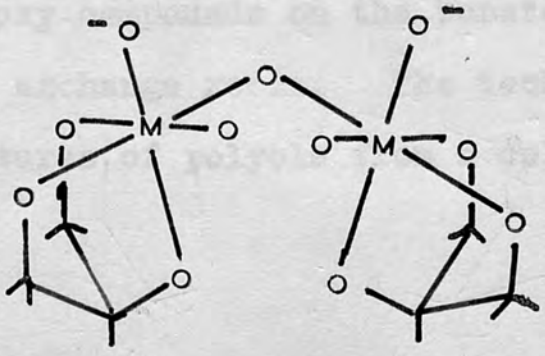


(IX)

be brought, without distortion of the carbon chain, into this spatial arrangement. Thus, the 3-O-substituted L-gulitols fall into this class. Taking into account the O-O distances in molybdate (2.59Å) and tungstate (2.56Å),[7] (X) has been suggested as the structure (partial) of molybdate and tungstate complexes of polyols possessing the above structural features.



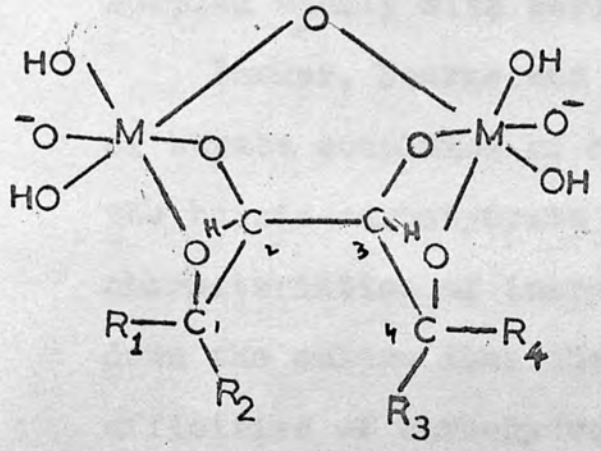
(X) M = Mo or W

(b) Polyols forming complexes in which M/P = 2.

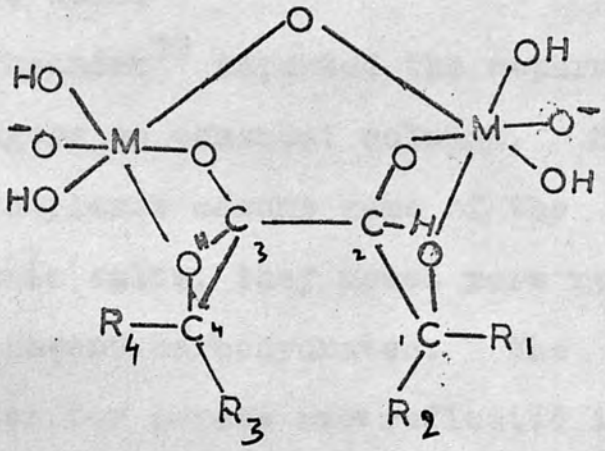
These are the acyclic polyols possessing at least four adjacent hydroxyl groups, irrespective of the configuration at asymmetric carbon atoms. Structures XI-XIV have been postulated for their molybdate and tungstate complexes. These are thus produced from 1,2,3,4-tetritols, the 2,3-diol groups of which have either erythro (XI and XII) or threo (XIII and XIV) configurations.

The complex-forming properties of polyols have been utilised largely in electrophoretic methods. The object of the work described in Part I is to investigate (a) the use of molybdate or tungstate forms of anion exchange resins for the separation of polyols and (b) the chromatographic behaviour of polyols using paper (or cellulose powder) impregnated with molybdate or tungstate. It is thus appropriate to review briefly methods for the separation of polyols, other than electrophoresis, in which complexes of the types mentioned above are employed.

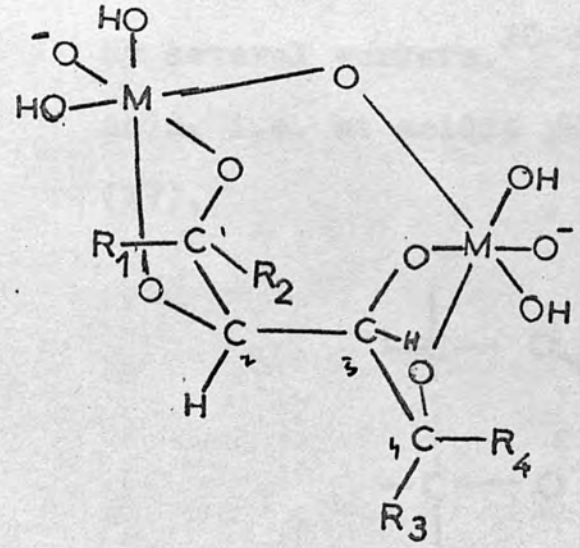
Khym and Zill[18] developed a method for the separation of polyhydroxy compounds on the borate form of a strongly basic anion exchange resin. The technique consists in eluting mixtures of polyols from a column of resin



(XI)



(XII)



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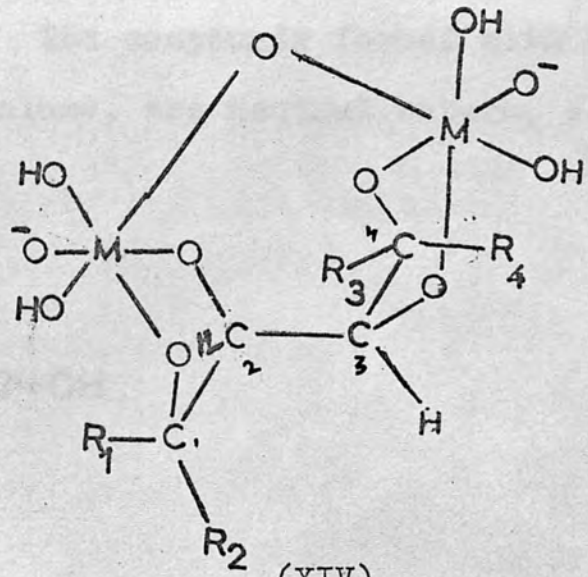
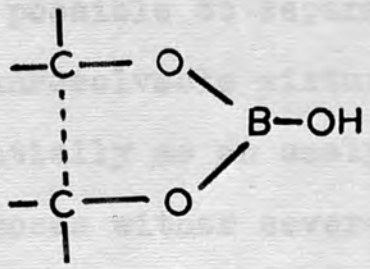


(XIV)

(Dowex-1) by means of aqueous solutions of boric acid or sodium borate. Those polyols which react strongly with borate ions, thereby acquiring high negative charges are more strongly sorped by the resin than those polyols which complex weakly with borate ions.

Barker, Bourne and Theander[19] reported the separation of borate complexes of sugars on charcoal columns. Since the borate-carbohydrate complexes assume some of the characteristics of inorganic salts, they moved more readily down the column than the parent carbohydrates. The affinities of carbohydrates for borate were reflected in their ease of elution from the columns.

Paper chromatography of polyols using solvents containing either boric acid or borates has been reported by several workers.[20-23] The compounds formed with boric acid, i.e. at acidic pH values, are neutral esters, e.g. (XV),



(XV)

whereas those formed under alkaline conditions are anionic complexes (I). Thus, compounds with structure (XV) had higher R_F values in solvents with a stationary aqueous phase than those with structure (I). E.g., D-glucitol had R_G 2.2 (movement with respect to D-glucose) using a solvent containing boric acid and acetic acid, whereas using a solvent containing boric acid and pyridine, i.e. borate ions, it had R_G 0.3.[23]

Use of this phenomenon was made by replacing boric acid in acidic solvents by benzeneboronic acid. The replacement of the hydroxyl group of (XV) by a phenyl group increased the affinity of the ester for the organic solvent and has resulted in an increase in R_G values.[24]

In the field of electrophoresis of carbohydrates, the most commonly used support for the electrolyte, i.e. solutions containing ions or inorganic oxy-acids, is filter paper. Although this technique has made it increasingly possible to separate and identify components of hitherto unresolvable mixtures, the scale restricts its use essentially as an analytical method. For preparative purposes either several runs have to be performed or apparatuses with "filter paper curtains" or columns have to be employed. However, such equipment is costly.

Thus, the aim of the present investigation was to extend the use of molybdate and tungstate complexes of polyols to a preparative scale by methods other than electrophoresis. At the same time it was hoped that such methods would provide information essential for a deeper understanding of the properties of these complexes.

B. RESULTS AND DISCUSSION

Separation of Polyols on Molybdate or Tungstate Forms of Anion-Exchange Resins.

In the light of the data available for the complex formation of carbohydrates and related compounds with molybdate or tungstate,[13-16] it was decided to see whether the complexes could be used for the separation of certain polyols on ion-exchange resins.

Since the polyol-molybdate or polyol-tungstate complexes are anionic, it was likely that they would be adsorbed on the anion-exchange resins. Ward[25] observed the adsorbtion of D-mannitol on the molybdate form of a resin (De-acidite FF), but made no further attempt to utilize this effect.

A mixture of D-glucose and D-glucitol was selected, these compounds being examples of non-complexing and relatively strong complexing polyols. The optimum conditions for the separation had first to be ascertained. Consden and Stanier[26] studied the effect of pH of borate solution on the electrophoretic mobility of a few polyols and obtained the results shown in Table 1. Such measurements permit the selection of a pH range in which certain sugars can be separated effectively. The most effective separation of polyols by electrophoresis in molybdate or

Table 1*

Sugar	Mobilities ($cm.^2/v.\ sec. \times 10^5$) at 20° in borate at various pH values.				
	pH 7.0	pH 8.0	pH 8.6	pH 9.2	pH 9.7
Fructose	8.2	9.7	11.4	12.5	13.1
Sorbose	8.7	10.4	12.2	14.1	14.3
Glucose	2.4	6.5	11.4	14.5	14.6
Galactose	2.8	5.8	9.6	13.0	13.1
Mannose	2.6	4.9	7.8	9.8	10.1
Ribose	7.0	9.1	10.2	10.9	11.0
Arabinose	3.2	6.5	10.3	13.3	13.9
Rhamnose	1.3	2.4	4.4	7.1	7.8
Cellobiose	0.5	0.5	1.5	3.3	4.5

* Data due to Consden and Stanier, ref. 26.

tungstate solutions has been found to occur at pH ca. 5.[13,15]

In Expt. 2(a) the chloride form of Amberlite resin IRA-400(i) was converted into the molybdate form by eluting with sodium molybdate solution of pH 5. After placing the D-glucose, D-glucitol mixture on the column, the column was eluted with water. From the analysis of

(i) Amberlite IRA-400 was used, unless otherwise stated.

the effluent, it was observed that there was some effect of complexing due to which D-glucitol was eluted off the the column comparatively slower than D-glucose. The molybdate or borate ions cannot effectively replace chloride ions from resins.[27] Therefore, the non retention of D-glucitol on the column was presumably due to the incomplete conversion of the resin into the molybdate form.

When the molybdate form of resin was prepared from the free base form (method 2), a part of the D-glucitol was adsorbed on the column and desorbed on decomposing the complex with alkali (Expt. 2(b). The incomplete separation of D-glucose from D-glucitol made it necessary to find out the pH at which maximum adsorbtion will occur and also to find out the D-glucitol holding capacity of the molybdate form of resin. The molybdate form of the resin was acidified to different pH values (Expt. 3), D-glucitol added and the supernatants analysed electrophoretically. Table 14 shows that maximum adsorbtion had occurred at pH 5. Expts. 4(a) and 4(b) show that 5 ml. of this resin can adsorb about 6 mg. of D-glucitol.

An attempt to adsorb the preformed D-glucitol-molybdate complex on the molybdate form of resin [Expts. 5(a) and 5(b)]

showed that the molybdate form of the resin cannot further react with the anionic complex. This indicates that ionizeable sites of the complex do not exchange with the molybdate attached to the resin.

It was observed from the Expt. 4(b) (Table 16) that re-adjustment of the pH, after addition of D-glucitol has improved the adsorbtion of D-glucitol, i.e. if the conditions used are those when pH of the column is adjusted after adding the polyol, a better separation may result. In Expt. 2(c), Table 12, where the pH of the effluent was lowered by washing the column with an excess of water, only a partial separation was achieved. Since the addition of polyol alters the pH of the molybdate solution,[13,14] a similar effect on the molybdate form of resin should occur. This could result in the decomposition of the complex. In order to avoid the above mentioned shortcomings, an aqueous solution containing D-glucose and D-glucitol was added to molybdate form of resin (Expt. 2(d), Table 13), previously maintained at pH 5. The column was eluted with dilute sodium molybdate solution of pH 5.0, to keep the pH constant. The analysis of the fractions revealed that traces of D-glucitol have been eluted off the column, only in the first fraction. This was overcome when D-glucose and D-glucitol were dissolved in dilute sodium molybdate.

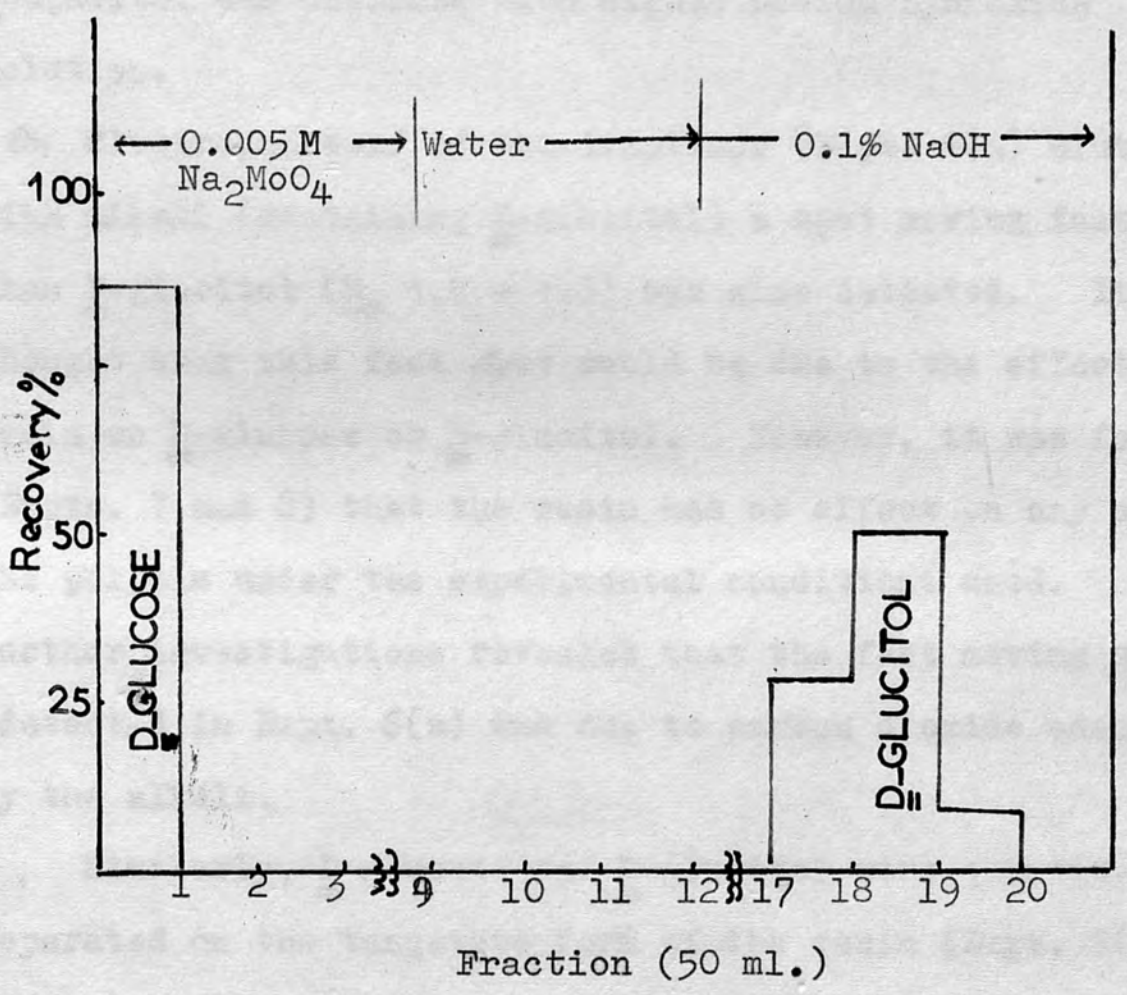
When this solution was added to the column (Expt. 6(a), Table 17) a clear separation of D-glucose from D-glucitol was achieved. By analogy with the borate form of resins columns[18] D-glucitol should have been desorbed on further elution of the column with the same eluant (molybdate) but it was observed that D-glucitol had been adsorbed strongly and further elution with dilute molybdate could not desorb it.

The strong adsorbtion of D-glucitol was further evident from its non-desorbtion even on dilution, i.e. on eluting the column with water.

There could have been a possibility of displacing the molybdate from the resin by introducing other anions, e.g. phosphate, arsenite, tartrate or sulphate which have a stronger affinity for the resin than molybdate,[27] but taking into consideration the difficulties involved in their subsequent removal this was not attempted. Strong molybdate solutions were also not used.

On eluting the column with dilute sodium hydroxide (Expts. 2(d), and 6(a)), the D-glucitol-molybdate was decomposed when the pH of the column reached 9.2 $\pm$ 0.1 (the fractions in which D-glucitol first appeared had pH 9.2 $\pm$ 0.1).

Fig. 1 Separation of D-glucose from D-glucitol on molybdate form of resim Expt. 6(b)



This is the pH at which molybdate exists as its non-complexing, normal ion MoO_4^{2-}. [16]

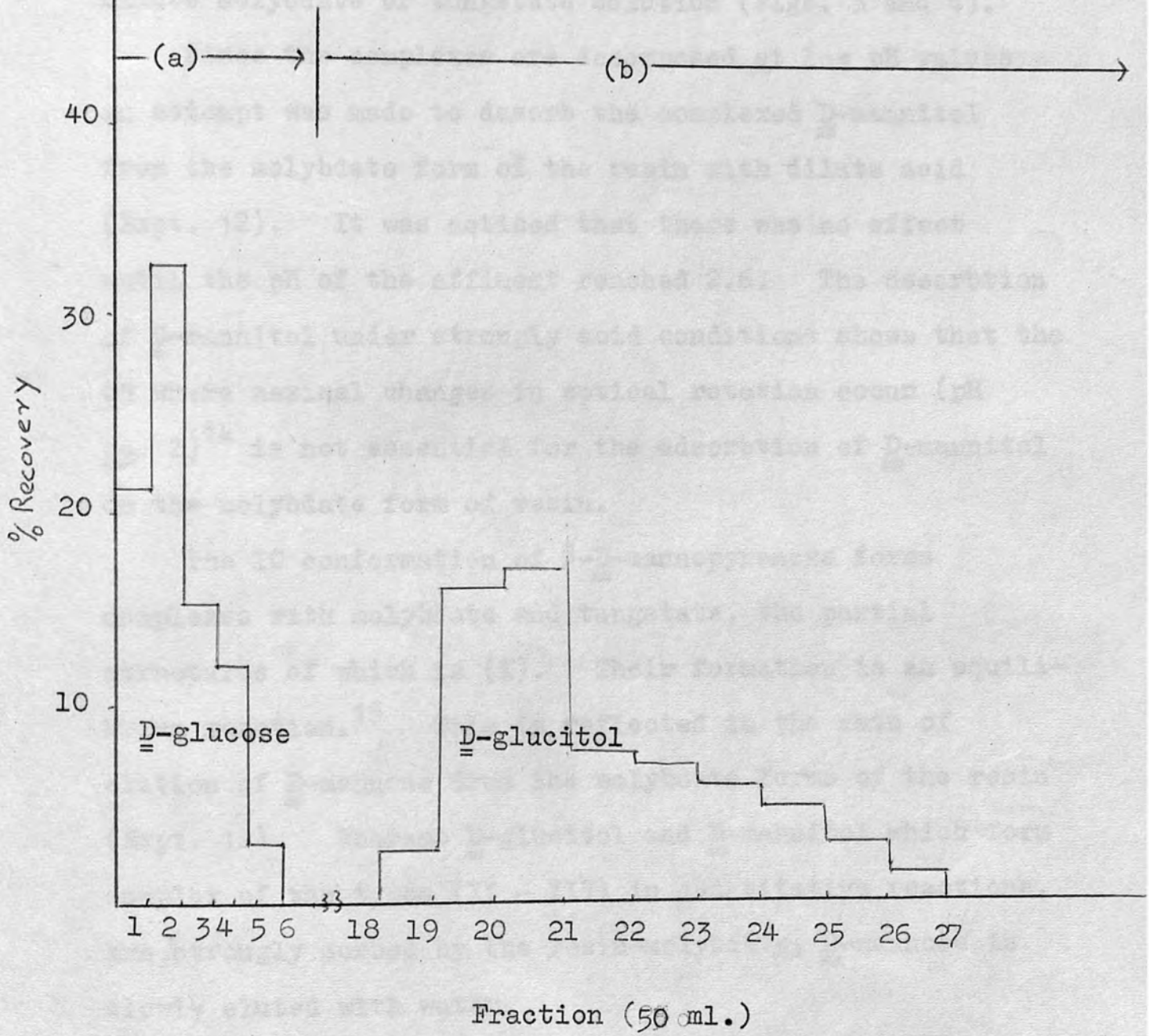
Fig. 1 shows the complete resolution of D-glucose from D-glucitol. Almost all (94%) D-glucose was eluted from the column with a small volume (50 ml.) of the eluate. D-Glucitol was desorbed with dilute sodium hydroxide solution.

On electrophoresis of the fractions (Expt. 6(a) eluted with alkali (containing D-glucitol) a spot moving faster than D-glucitol (M_s 1.2 - 1.3) was also detected. It was thought that this fast spot could be due to the effect of resin on D-glucose or D-glucitol. However, it was found (Expts. 7 and 8) that the resin has no effect on any of the polyols under the experimental conditions used. Further investigations revealed that the fast moving spot (detected in Expt. 6(a) was due to carbon dioxide adsorbed by the alkali.

Similarly, D-glucose and D-glucitol were quantitatively separated on the tungstate form of the resin (Expt. 9(a), Fig. 2).

During the course of studies on the separation of polyols on molybdate and tungstate form of resins, it was observed that if the polyol mixture is allowed to

Fig. 2. Separation of D-glucose from D-glucitol on tungstate form of resin (Expt. 9(a)).
i) Polarimetric (ii) Periodate chromotropic acid method (a) 0.005 M Na_2WO_4 (b) 0.1 N-NaOH



equilibrate on the resin, the complex-forming polyols become more strongly adsorbed. The non complexed polyol can then be eluted from the column with water instead of dilute molybdate or tungstate solution (Figs. 3 and 4).

Since the complexes are decomposed at low pH values an attempt was made to desorb the complexed D-mannitol from the molybdate form of the resin with dilute acid (Expt. 12). It was noticed that there was no effect until the pH of the effluent reached 2.6. The desorbtion of D-mannitol under strongly acid conditions shows that the pH where maximal changes in optical rotation occur (pH ca. 2)[14] is not essential for the adsorbtion of D-mannitol on the molybdate form of resin.

The IC conformation of β-D-mannopyranose forms complexes with molybdate and tungstate, the partial structures of which is (X). Their formation is an equilibrium reaction.[15] This is reflected in the rate of elution of D-mannose from the molybdate forms of the resin (Expt. 12). Whereas D-glucitol and D-mannitol which form complex of the types (XI - XIV) in quantitative reactions, are strongly sorbed by the resin-molybdate, D-mannose is slowly eluted with water.

Fig. 3 Separation of D-glucose from D-glucitol on molybdate form of resin [Expt. 6(c)].
i) Polarimetric.
ii) Periodate/chromotropic acid method.

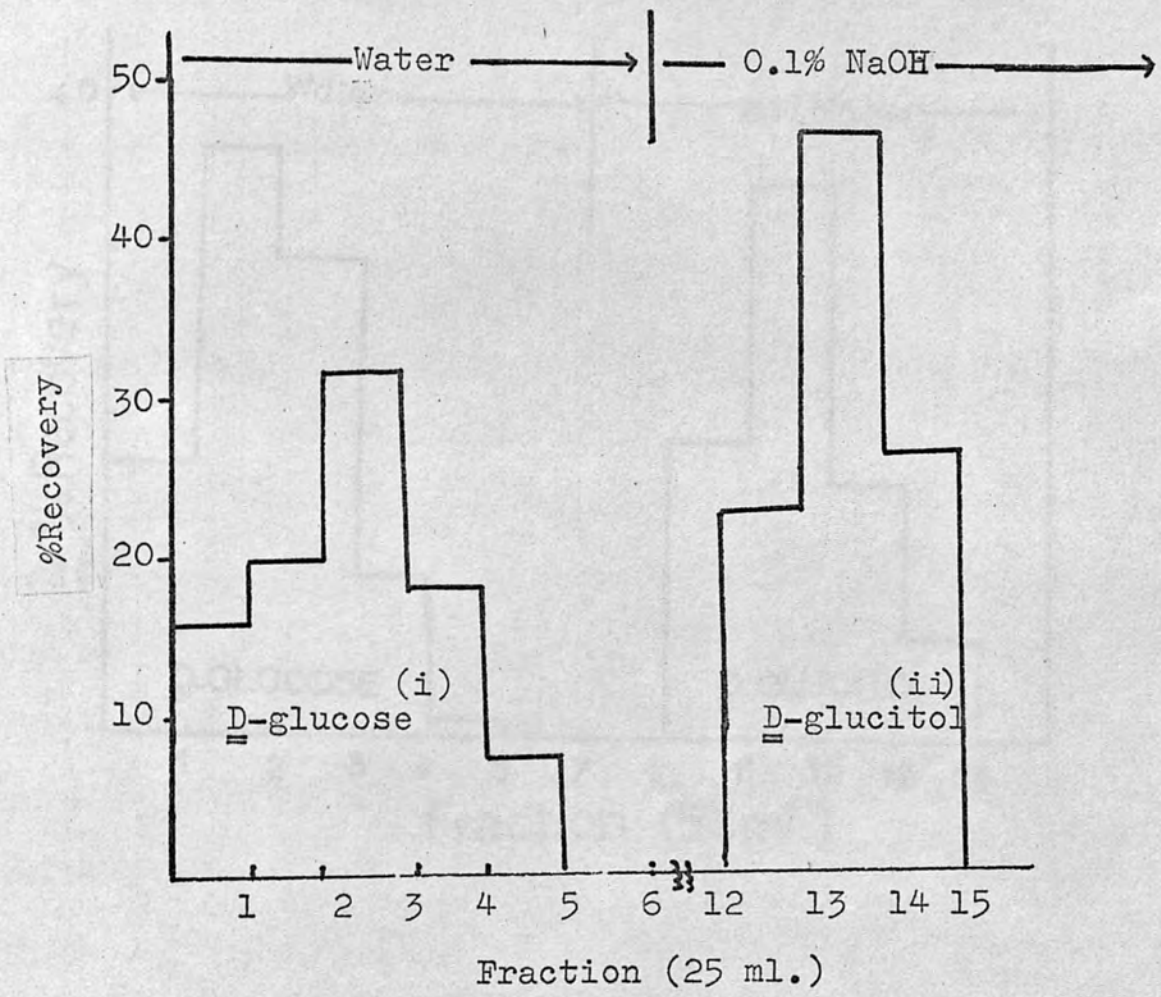
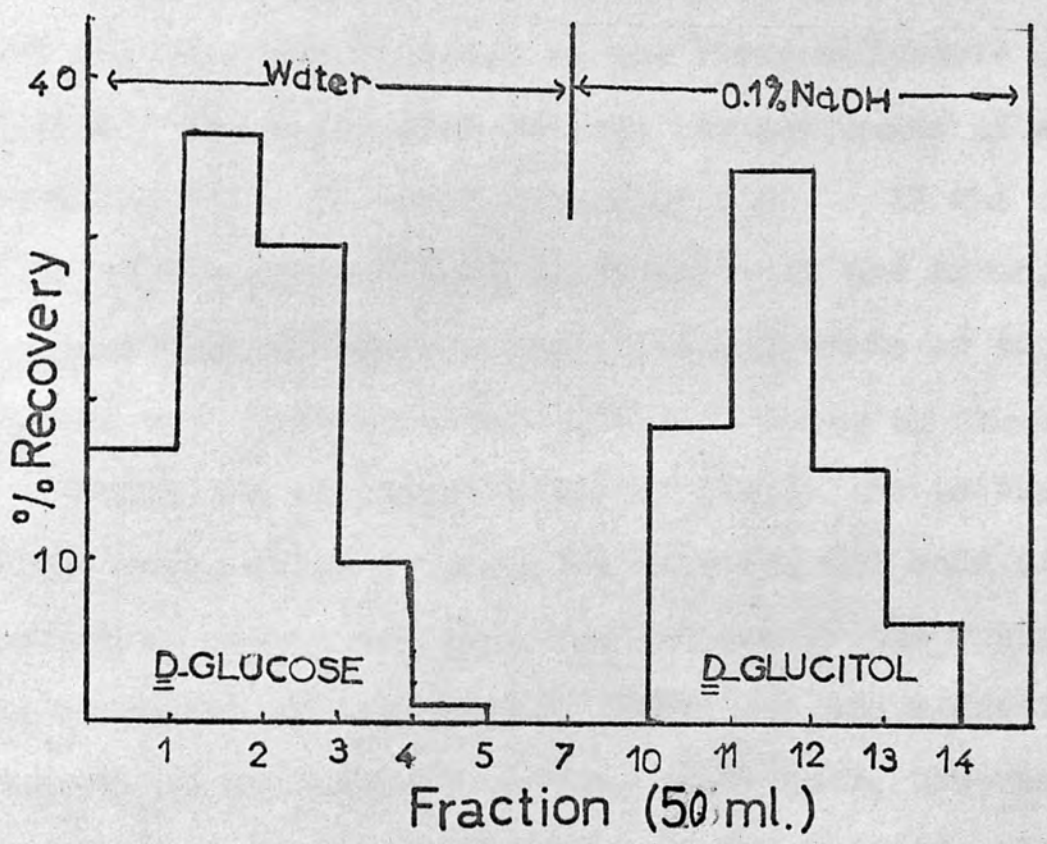


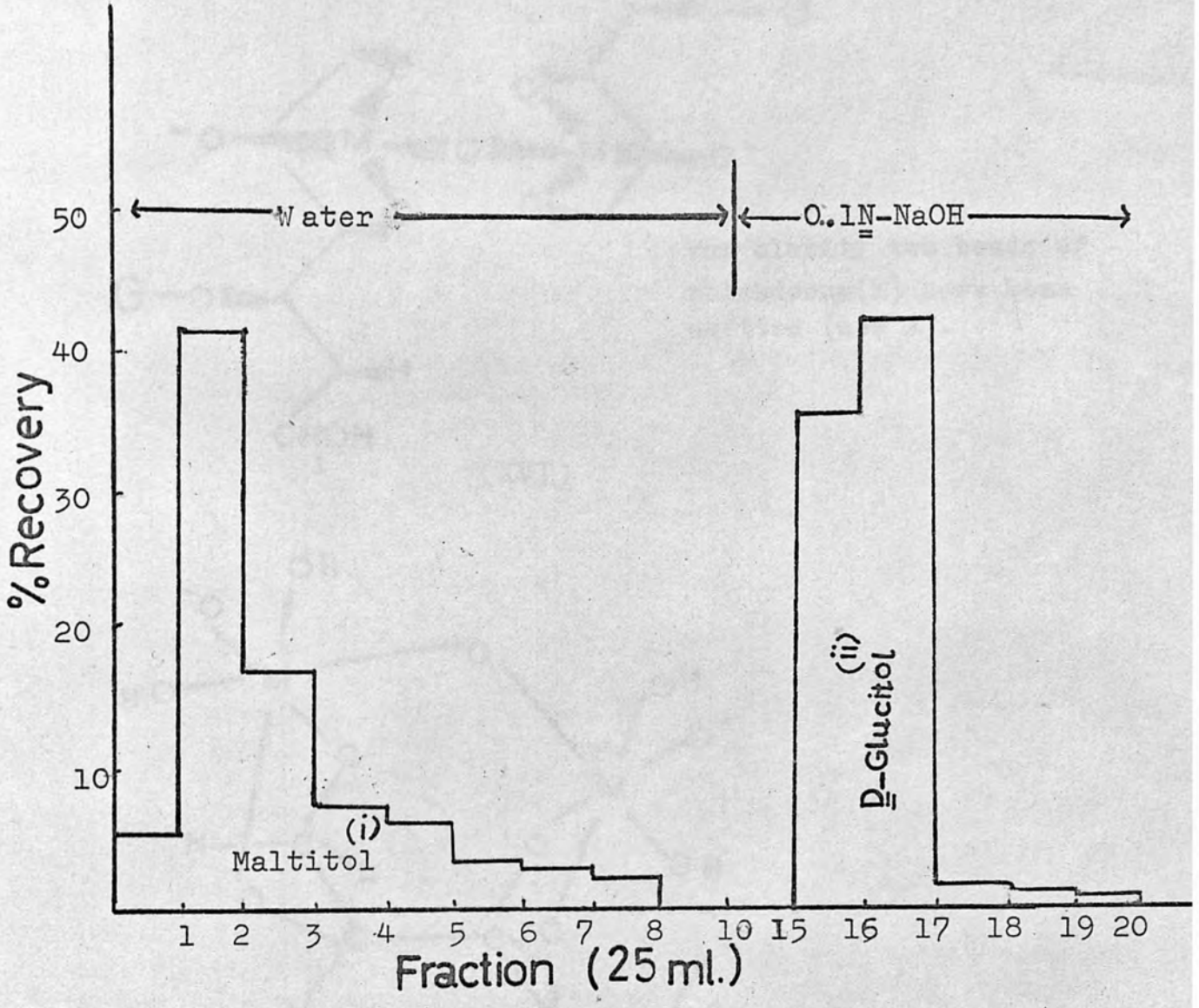
Fig. 4 Separation of D-glucose from D-glucitol on tungstate form of resin [Expt. 9(b)].

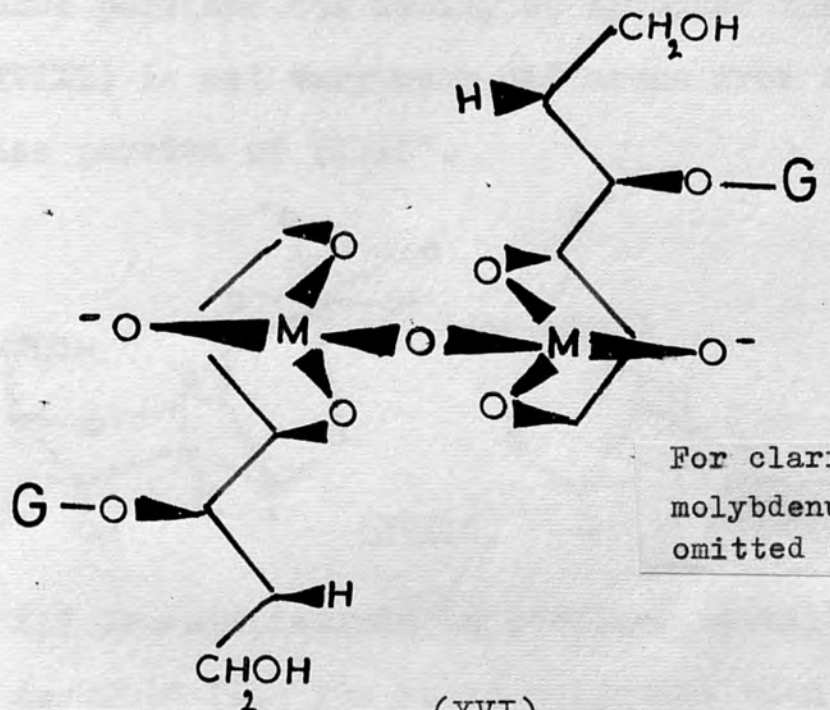


Maltitol (3-O-α-D-glucopyranosyl-L-gulitol) forms the same type of complexes (X) as does D-mannose, but the reaction is, at least with molybdate, quantitative. However, conditions could not be found to adsorb maltitol on either molybdate or tungstate forms of resins. On the other hand isomaltitol (1-O-α-D-glucopyranosyl-L-gulitol) and indeed the reduction products of isomaltose homologues were all strongly adsorbed on the resin molybdate [Expts. 18-19]. One difference between the complexes of maltitol and isomaltitol is their molecular size. If the carbon atoms of the hexitol portion of maltitol are arranged in a planar zig-zag conformation, its molybdate or tungstate complex will have structure (XVI). Those of isomaltitol have structure XVII.

Since the ionisable sites of (XVI) are in the centre of the molecule it is possible that the two ends of (XVI) prevent an easy entry into the lattice of the resin. The complexes of isomaltitol (XVII) and the reduction products of the isomaltose homologues have, however, the shape of a rod with ionisable sites on one end, which would allow their entry into the resin lattice. Molecular models show that (a) rotation around the Mo-O-Mo bonds or (b) union of the two Mo atoms through other oxygen atoms will not reduce the volume of the complex of maltitol to a range approximating the volume of the isomaltitol complex.

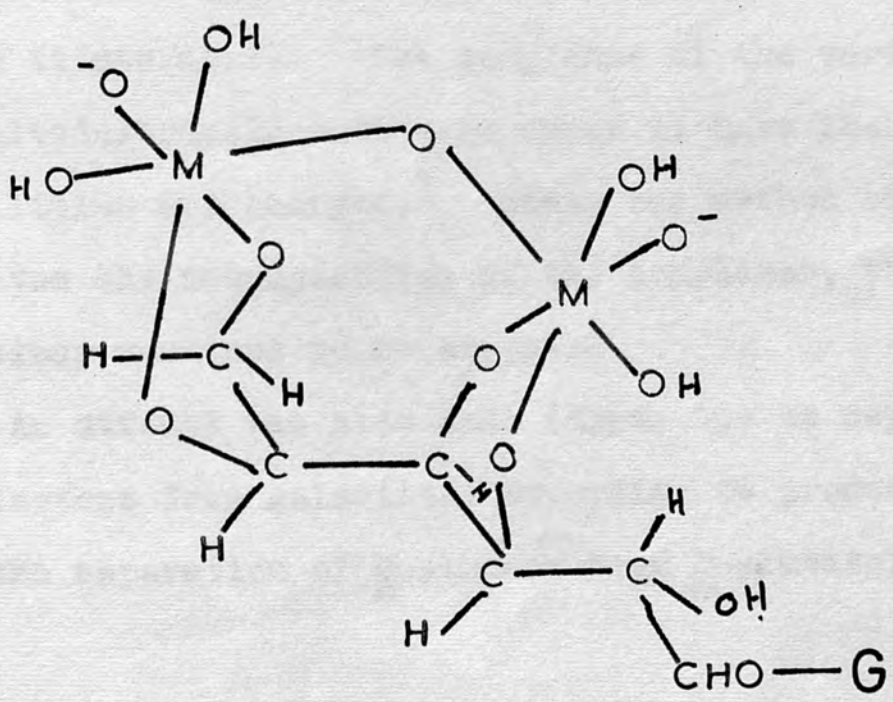
Fig. 5 Separation of maltitol from D-glucitol on molybdate form of resin [Expt. 15].
i) Phenol/sulpuric acid method.
ii) Periodate/chromotropic acid method.





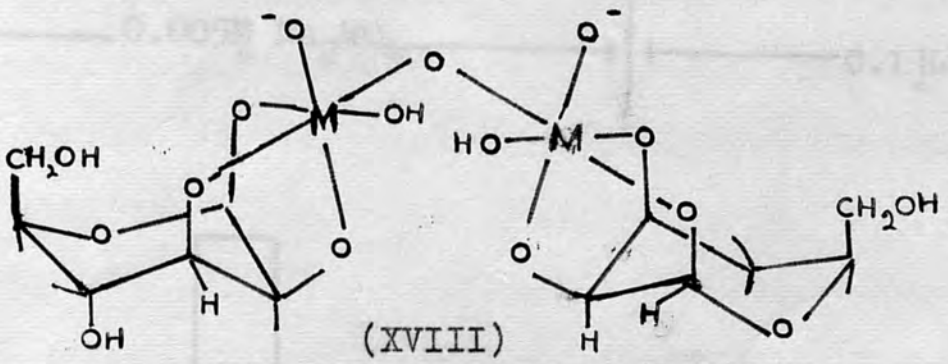
For clarity two bonds of molybdenum(M) have been omitted (see X).

(XVI)



(XVII)

β-D-Mannopyranose forms the same type of complex (XVIII) as does maltitol, i.e. (X). However, its pyranose portions can easily be arranged that the volume of (XVIII) is not very much different from that of the complex portion of (XVII).

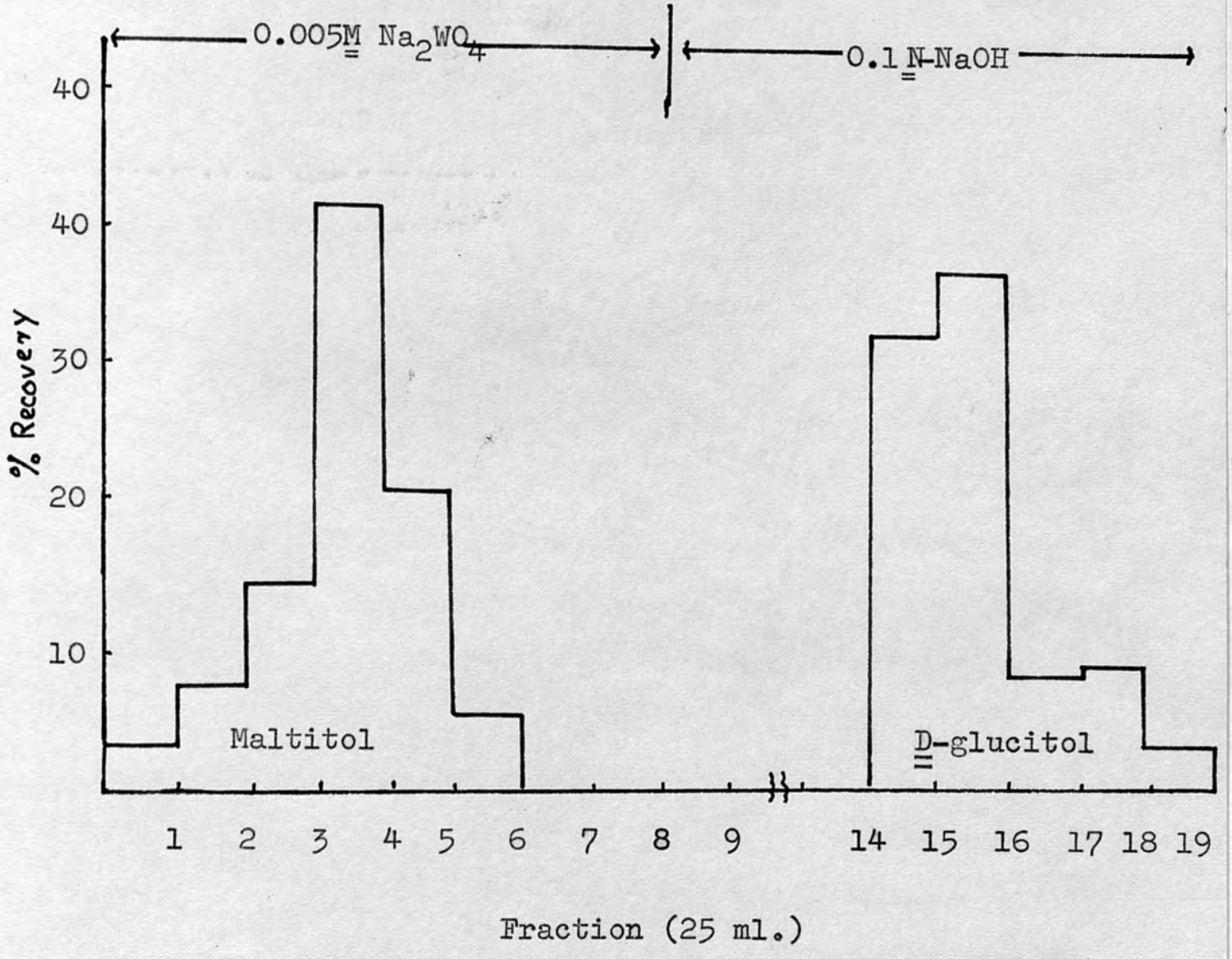


(XVIII)

All isomaltodextrinols (reduced isomaltose homologues) were desorbed from the resin molybdate when the pH of the effluent had reached either 9 (with dilute alkali) or ca.3 (with dilute acid). The complexes of the various isomaltodextrinols have been shown to have identical stabilities and charges.[7] Since the method of desorbtion involves the decomposition of the complexes, the above behaviour was thus to be expected.

An attempt was also made (Expt. 10) to separate D-galactose from galactitol according to procedure described for the separation of D-glucose from D-glucitol (Expt. 6(b).

Fig. 6 Separation of D-glucitol from maltitol on tungstate form of resin (Expt. 14).



D-Galactose has been reported not to complex with molybdate[13,38] or tungstate.[15] Although most of the D-galactose was eluted from the column with the first 500 ml. (Expt. 10), the remaining quantity required a large amount of the eluent for desorbtion. This behaviour of D-galactose indicated some complexing due to which the elution rate of the D-galactose decreased (see p. 90) Polarimetric estimation of D-galactose in the concentrated eluate gave too high results. This also indicated that complex formation does occur, since the optical rotation of complexing polyols is affected by the presence of molybdate.[14]

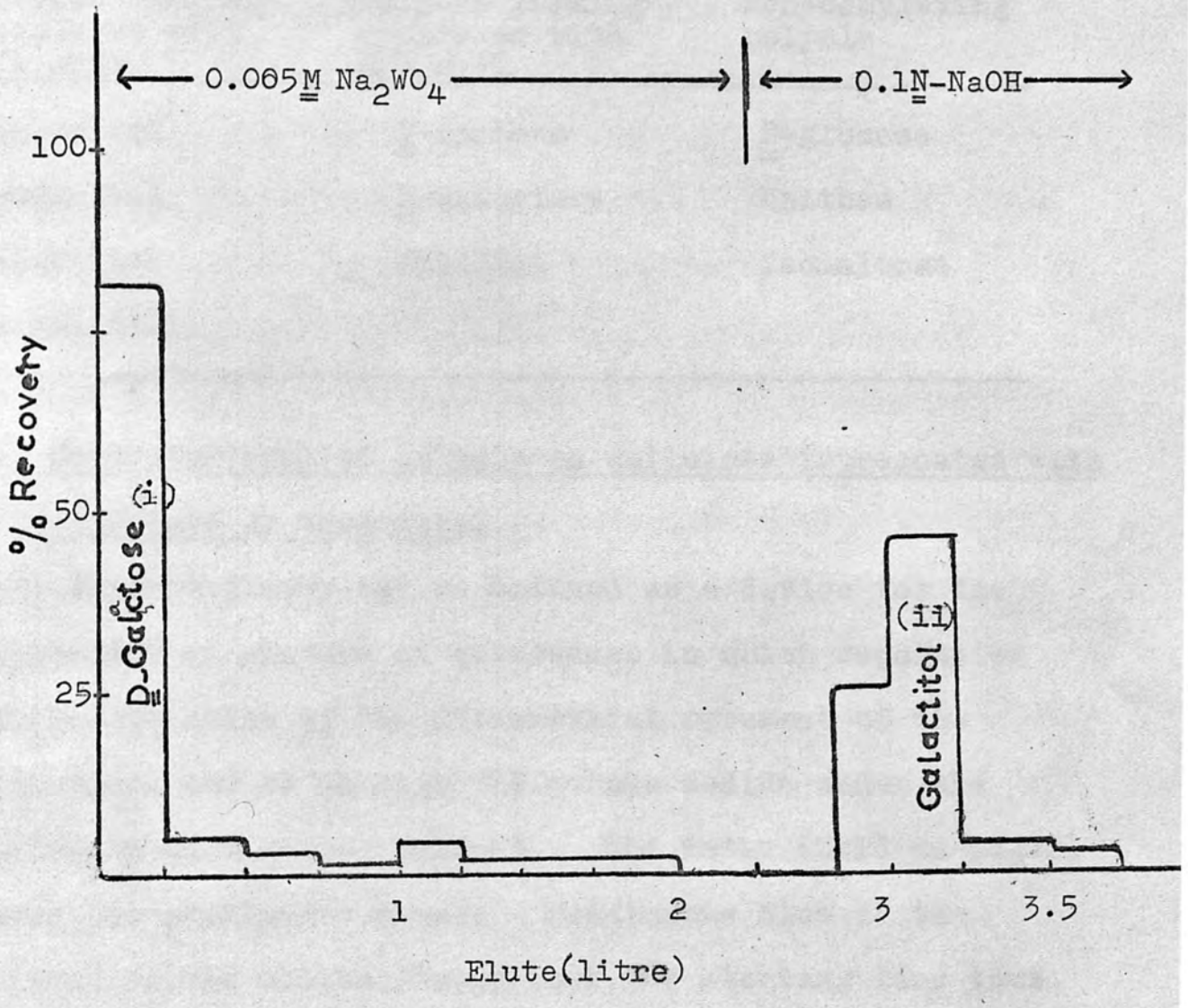
In a further attempt to separate D-galactose from galactitol on a tungstate form of resin column (Expt. 11), a similar effect was observed. However, separation was quantitative (Fig. 7).

Further investigations on the complex formation of D-galactose with molybdate and tungstate are reported on page 90 .

The conclusions drawn from the above experiments may be summarised as follows: The methods of chromatography on molybdate or tungstate forms of anion-exchange resins afford the separation of polyols forming complexes in which M/P = 2 [i.e. (XI-XIV)] from polyols which either do not complex or form complexes in which M/P = 1 [i.e. (X from

Fig. 7 Separation of D-galactose from galactitol on tungstate form of resin [Expt.1] .

i) Phenol/sulphuric acid method.
ii) Periodate/chromotropic acid method.



introduction)]. Separation of members of each group is however not possible. Examples of compounds of each group are shown in Table 2.

Table 2

Polyols forming complexes with M/P = 2.	Polyols forming complexes with M/P = 1.	Non-complexing polyols
D-glucitol	D-mannose	D-glucose
D-mannitol	D-galactose	Maltose
Galactitol	Maltitol	Isomaltose
Isomaltitol		

2. Chromatography of polyols on cellulose impregnated with molybdate or tungstate.

Chromatography can be defined as a device for the separation of mixture of substances in which separation is brought about by the differential movement of the individual solute through the porous medium under the influence of a moving solvent. The water (held on paper) forms the stationary phase. Continuous flow of the solvent on the solute placed near the starting line thus subjects it to innumerable partition between the two phases. The components of the solute, having different solubilities in the two phases, therefore migrate at different rates.

Thus, the process is a continuous liquid - liquid extraction. The rates of migration are measured as R_F values where,

$$R_F = \frac{\text{distance moved by the compound}}{\text{distance moved by the liquid front}}$$

Hanes and Isherwood[28] consider the stationary phase as a water "cellulose complex". A substance in solution will be held more or less strongly in this complex depending on its hydrophillic properties.

Isherwood and Jermyn[29] have made a detailed study of the relation between the sugar structures and their R_F values. They have shown that for a large number of aqueous solvents a straight line graph is obtained from a plot of log $[(1/R_F) - 1]$ against -log N, where N is the mole fraction of water. They have also shown that (a) aldopyranoses having a cis configuration of hydroxyl groups at $C_{(2)}$ and $C_{(3)}$ result in higher R_F values than those having trans hydroxyls at the same position, (b) furanoses have higher R_F values than those with a pyranose ring and (c) the transfer of a hydroxyl group from above to below the sugar ring (in Howarth perspective formula) changes the R_F value in the same direction, if the hydroxyl group is situated at $C_{(2)}$ or $C_{(4)}$, and in the opposite direction if it is at $C_{(3)}$. Similar studies have also been made by Jager and Ramel[30] who showed that the R_F values of the

sugars increase with the increase in number of axial hydroxyl groups.

Paper chromatography of organic substances in the presence of complexing agents has been described by several workers[20-24]. It is often possible to correlate the affinity of certain polyhydroxy compounds for complexing agents with the structure of the polyol. The interaction of boric acid or the borate ion with polyhydroxy compounds results in the formation of a variety of products (i.e. neutral esters or anionic complexes). This has been briefly referred to on p. **7** .

Since the polyol-molybdate or polyol-tungstate complexes are anionic, it was expected that their R_F values would be markedly lower than those of the parent polyols.

In addition the factors which govern the migration rates of a compound during chromatography on paper (or cellulose powder) and electrophoresis are totally different. The electrophoretic mobility of a compound is a measure of frictional resistance to motion under the influence of an electric field and must be related to its effective surface area. Hence electrophoresis in molybdate or tungstate solutions did not separate several compounds, e.g. D-glucitol, D-mannitol and galactitol, as their complexes have identical

charges and stabilities and approximately the same surface area. Migration rates during chromatography on cellulose are on the other hand, related to partition coefficients and these should reflect the general conformation of the compound. It has been reported, e.g. that in the series of aldopyranoses a change of a hydroxyl group from an axial to an equatorial position causes a reduction in R_F value (cf. R_F (in ethyl acetate, pyridin, water, 2:2:1) of D-glucose is 0.195 and that of D-mannose is 0.24).[30] This suggested to the authors that equatorial hydroxyl groups are hydrated more easily than those axially disposed and are thus more readily accessible for the stationary aqueous phase of the chromatographic solvent system.

It was thus expected that further resolution might be achieved by chromatography on cellulose in the presence of molybdate or tungstate. As these reagents are, unlike boric acid, insoluble in the organic phase of suitable solvent systems it was necessary to impregnate the cellulose with molybdate or tungstate solutions adjusted to desirable pH values.

a. Chromatography on cellulose partially impregnated with molybdate on tungstate.

Angus[31] performed experiments with a few polyols when an area around the base line of chromatographic paper was impregnated with tungstate solution (pH 5). He related the R_F values determined under these conditions with the electrophoretic mobilities of polyols during electrophoresis in tungstate solution.

It was thus decided to impregnate the area around the base line of chromatographic paper with molybdate solution of pH 5, i.e. a value suitable for electrophoresis. Table 26 (Expt. 20) indicated that the separation of reducing sugars from their reduction products might be feasible, but the formation of elongated spots was clearly undesirable. In view of the reported effect of the presence of boric acid or borate ions on the R_F values of polyols,[22,23] it was reasonable to find out a suitable pH (if any) for the formation of stable and chromatographically separable polyol complexes of molybdate.

Chromatography of a few polyols was performed on paper partially impregnated with sodium molybdate solutions of different pH values. It was found (Expt. 21, Table 27) that at almost all the pH values the impregnated area either expanded considerably or the polyols gave two spots. The slower spot was inside the expanded molybdate area and the

faster spot was slightly slower than the free polyol. Such effects are clearly undesirable if separations on a large scale should be attempted.

When sodium molybdate was replaced by sodium tungstate, it was observed (Expt. 22(a) Table 28) that no second spot was formed at any of the pH values. Moreover, there was also no effect on the tungstate impregnated area. The best results were obtained when the tungstate solution used for impregnation was adjusted to pH 6.

Consequently, the chromatographic migration rates of a large number of polyols were determined using a tungstate solution adjusted to pH 6 for impregnation of the area around the base line of the paper. Solvent (a) was used for irrigation. Migration rates were expressed relative to the movement of D-glucose spotted outside the impregnated area, thus

$$R_G(W_p) = \frac{\text{Distance travelled by compound spotted within the impregnated area (a)}}{\text{Distance travelled by D-glucose spotted outside the impregnated area}}$$

W_p indicates that the paper is partially (p) impregnated with tungstate (W).

As control the R_G values were determined.

$$R_G = \frac{\text{Distance travelled by compound (b)}}{\text{Distance travelled by D-glucose}}$$

using the same solvent but non-impregnated paper. The results are shown in Tables 3-5. These show that the migration rates of several compounds are affected by the presence of tungstate. The relative effects on the migration rates are the ratios of a/b of the above equations, when measured under standard conditions, thus

$$Q(\underline{W}_p) = \frac{a}{b} = \frac{\underline{R}_G(\underline{W}_p)}{\underline{R}_G}$$

Tables 3-5 show that $Q(\underline{W}_p)$ values range from 0.1-1.0. The migration rates during electrophoresis in tungstate solution [$\underline{M}_s(\underline{W})$] are given for comparison.

As mentioned above, polyols can be divided into three groups:

(a) acyclic compounds possessing four adjacent hydroxyl groups and forming complexes in which W/P = 2, i.e. (XI-XIV).

(b) compounds which possess three hydroxyl groups in a spatial disposition approximating that of (IV) and forming complexes in which W/P = 1, i.e. (X).

(c) non complexing compounds, which do not possess the above structural features.

The compounds examined are accordingly grouped in Tables 3-5.

It can be seen that $Q(W_p)$ of the three groups of compounds are of the following ranges:

group a: $Q(W_p) < 0.27$

group b: $Q(W_p)$ = ca. 0.3-0.9

group c: $Q(W_p) > 0.9$

Table 3

$Q(W_p)$ values of Polyol of group a

$Q(W_p) < 0.27$

Compound	$R_G(W_p)$	R_G	$Q(W_p)$	$M_s(W)$ [16]
Ribitol	0.38	1.37	0.21	1.03
L-Arabinitol	0.23	1.35	0.17	1.04
Xylitol	0.30	1.15	0.26	1.04
Allitol	0.21	1.19	0.17	0.97[e]
D-Altritol	0.20	1.13	0.17	0.97[e]
1-Deoxy-D-altritol	0.34	1.85	0.18	1.00[e]
D-Glucitol	0.13	1.03	0.13	1.00
1-Deoxy-D-glucitol	0.36	1.74	0.20	1.03
Galaditol	0.14	1.08	0.11	1.00
1-Deoxy-L-galactitol	0.33	1.96	0.17	1.03
1-O-methyl-L-gulitol	0.35	1.66	0.21	0.89
2-Deoxy-D-arabino-hexitol	0.36	1.80	0.20	1.00
2-Deoxy-D-lyxo-hexitol	0.41	2.03	0.21	0.61[s]
2-Deoxy-D-ribo-hexitol	0.33	2.10	0.16	0.57[s]
L-Iditol	0.21	1.00	0.21	1.00

D-Mannitol	0.13	1.10	0.12	1.00
1-Deoxy-L-mannitol	0.21	2.10	0.12	1.0
1,6-Dideoxy-L-mannitol	0.39	1.5	0.26	0.95[e]
1-Deoxy-D-talitol	0.36	1.76	0.20	0.94[e]
Exceptions:				
Erythritol	0.88	1.72	0.76	0.90
L-threitol	1.56	1.72	0.90	0.24

This shows that the migration rates of the non complexing compounds (group c) are not significantly affected by the presence of tungstate. Thus this behaviour is complementary to that during electrophoresis when their $M_s(W) = 0$.

It has been suggested that the complexes formed by compounds of group a are more stable than those formed by compounds of group b. The latter are almost always formed by an equilibrium reaction.[15] It is thus not surprising that the $Q(W_p)$ of these compounds fall between those of group a and c. There are some exceptions to these rules:

Exceptions in group a: erythritol
L-threitol
gentiobiitol [Q(W) = 0.57]
melibiitol [Q(W) = 0.42]

It is possible that the relatively free rotation around the terminal C-C bonds in erythritol and L-threitol causes the

complex formation to be an equilibrium reaction. Thus the $Q(\underline{W})_p$) values of these compounds are those of group b.

Table 4

$Q(\underline{W}_p)$ Values of Polyols of group b

$Q(\underline{W}_p) = 0.3 - 0.9$

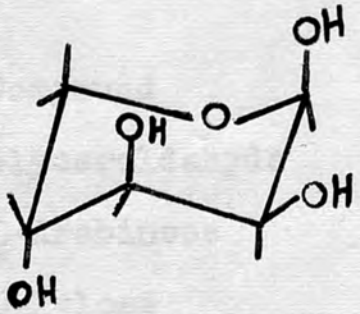
Compound	$\underline{R}_s(\underline{W}_p)$	$\underline{R}_G$	$Q(\underline{W}_p)$	$\underline{M}_s(\underline{W})$[15,11]
D-Erythrose	1.76 - 2.5	2.3	0.76-1.08	0.2-1.1
D-Ribose	1.4 - 1.7	1.66	0.87	0.2
D-Galactose	0.75	0.90	0.83	0-1.1(pH 8)
D-Gulose	0.98	1.3	0.75	1.1
D-Mannose	0.69 - 1.2	1.28	0.54-1.0	1.1
D-Glycero-D-allo-heptose	0.28	0.83	0.34	0.48
D-Glycero-D-gulo-heptose	0.23	0.80	0.29	0.98
D-Glycero-D-manno-heptose	0.59[e]	1.1	0.53	1.0
D-Glycero-L-gluco-heptose	0.60	0.74	0.83	-
D-Arabino-hexulose	1.1	1.27	0.87	0.25
D-Lyxo-hexulose	0.34	1.23	0.90	1.1
L-xylo-hexulose	0.96	1.19	0.88	0.2
D-Gluco-heptulose	1.06	1.1	0.96	

L-Galacto-heptulose	0-0.5	0.91	0-0.55	
D-Manno-heptulose	0.54	1.0	0.54	
Maltitol (α-1 $\longrightarrow$ 4)	0.25	0.43	0.58	0.17
Cellobiitol (β-1 $\longrightarrow$ 4)	0.24	0.42	0.57	0.10
Lactitol (β-1 $\longrightarrow$ 4)	0.18	0.41	0.44	0.10
Exceptions:				
D-Lyxose	0.95-1.46	1.46	0.96-1.0	1.04[s]
6-Deoxy-L-mannose	2.4[e]	2.26	1.06	1.1[s]

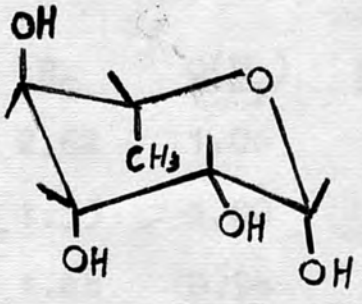
The observed behaviour of gentiobiitol and melibiitol is not understood.

Exceptions in group b: D-lyxose
6-Deoxy-L-mannose

The conformations of D-lyxose and 6-deoxy-L-mannose which could complex with tungstate are (XIX) and (XX), respectively. It is likely that these are not easily adapted and hence the



(XIX)



(XX)

D-lyxose and 6-deoxy-L-mannose behave as non-complexing compounds.

Whereas electrophoresis in tungstate solution can distinguish between complexing and non-complexing compounds, a further division of the former into groups a and b is not always possible by that method (cf. $M_s(W)$ of D-gulose and D-glucitol). However, the method just described, i.e. determination of $Q(W_p)$ values, detects the structural features described above. It is anticipated that it will find application in the structural analysis of carbohydrates and related compounds.

Tables 4 and 5 also show from a practical point of view, that it is now possible to separate components of hitherto unresolvable mixtures. It is thought that large scale separation can be performed on columns of cellulose powder, an upper portion of which is impregnated with tungstate.

Table 5

$Q(W_p)$ values of polyols of group c

$Q(W_p) > 0.90$

Compound	$R(W_p)$	R_G	$Q(W_p)$	$M_s(W)$
Glyceraldehyde	2.70	2.62	1.06	0
L-Arabinose	1.33	1.35	1.00	0
D-Xylose	1.39	1.48	0.94	0
2-Deoxy-D-arabino-hexose	1.80	1.88	1.00	0
2-Deoxy-D-lyxo-hexose	2.03	1.94	1.05	0

2-Deoxy-D-ribo-hexose	2.24	2.34	0.96	0
2-O-methyl-D-galactose	1.88	1.86	1.00	0
6-Deoxy-L-galactose	1.90	1.85	1.06	0
1,6-Anhydro-D-galactose	2.40	2.41	1.00	0
D-Glucose	0.98	1.00	0.98	0
2-O-methyl-D-glucose	1.84	1.86	1.00	0
3-O-methyl-D-glucose	1.78	1.75	1.00	0
4-O-methyl-D-glucose	1.90	1.86	1.00	0
6-Deoxy-D-glucose	1.84	1.94	1.00	0
Methyl-α-D-glucoside	2.08	1.94	1.07	0
Methyl-β-D-glucoside	1.90	1.86	1.04	0
2,3,4,6-tetra-O-methyl-D-glucose	2.30	2.20	1.04	0
Methyl-α-D-mannoside	2.10	2.07	1.00	0
Nigeritol (α-1 → 3)	0.50	0.54	0.91	0
Laminaribiitol (β-1 → 3)	0.57	0.63	0.89	0

b. Wholly impregnated cellulose.

i) Chromatography of polyols on filter paper wholly impregnated with sodium tungstate.

Chromatography of polyols on partially impregnated paper has divided the polyols into three distinct groups (see p. 35). The compounds of group a and b have shown considerable variation in their $Q(W_p)$ values. The variation in the $Q(W_p)$ values of the compounds of group b could be due either to the compounds having (i) identical rate of migration while still within the impregnated area, but different rates of migration when passed out into unimpregnated area; or (ii) different migration rates within and outside the impregnated area. On the other hand the compounds of group a which form complexes of the type (XI - XIV) have also shown some variation in $Q(W_p)$ values while still inside the impregnated area. This behaviour suggests that the majority of complex-forming compounds exhibit different rates of migration on coming in contact with tungstate. But the variation in their $Q(W_p) < 0.3$ (within group) is not sufficient for the study of the structures of the complexes and differentiation could not be made between the stable and comparatively less stable complexes. In order to examine the true effect of the presence of tungstate on the migration rates, i.e.

when the compound are always in contact with tungstate, it was found necessary to impregnate the paper wholly. Moreover since, if the same solvent and pH is used for the irrigation of the wholly impregnated paper, the range of migration values would still be the same, it was also essential to vary the pH and the solvent.

It has been reported that complexes are formed at all the values between pH 1-8[13] and that at values above pH 6, unstable intermediate complexes are formed which are slowly converted into stable complexes.[16] Whereas electrophoresis of polyols in molybdate or tungstate solutions of pH 5 is based on the fact that at ca. pH 5 stable complexes are formed immediately. Immediate formation of complexes is desirable for the fast process like electrophoresis, where compounds are spotted on dry paper and subjected to an electric field soon after soaking the paper in the electrolyte.

Formation of polyol-tungstate complexes at pH 8.0 was confirmed by electrophoresis. None of the polyols showed any signs of migration in solution (pH 8) of molybdate or tungstate. However, when polyols were mixed with tungstate solution of pH 8 and examined by electrophoresis after standing overnight, it was observed that complex-forming polyols migrated towards the anode.

This showed that complex formation takes place also at pH 8, but on standing only. This allows the variation in pH, for the paper chromatography, which in contrast to electrophoresis is a longer process, where immediately formed unstable intermediates will get sufficient time for conversion into stable complexes. Thus paper chromatography of a few polyols was performed on paper wholly impregnated with sodium tungstate solution of varying pH. Varying solvent systems (Expt. 23, Table 29) were also used.

It was found that paper impregnated with 5% (W/V) sodium tungstate dihydrate, acidified to pH 8.0 $\pm$ 0.1 and dried at room temperature overnight or at 70-80°C for 30 min. and the solvent (e) are the most suitable conditions for the study of tungstate-polyol complexes. Thus the available polyols were examined under these conditions. Since the $Q(\underline{W}_p)$ value of D-glucose in common with all non-complexing polyols (group c, table 5) is ca. 1, it was reasonable to assume that presence of tungstate has no effect on the migration rates of these compounds. This was confirmed by chromatography of a few polyols on paper impregnated with salt solutions, such as sodium carbonate or sodium phosphate, when their mobilities were not affected by the presence of these salts. Thus, the migration rates of the polyols on paper wholly impregnated

with tungstate [$\underline{R}_G(\underline{W})$], and on un-impregnated paper [$\underline{R}_G$], were expressed relative to $\underline{D}$-glucose, where -

$$\underline{R}_G(\underline{W}) = \frac{\text{Distance travelled by the compound on paper impregnated with tungstate}}{\text{Distance travelled by } \underline{D}\text{-glucose on the same paper}}$$

($\underline{W}$) indicate that the paper is wholly impregnated with tungstate and

$$\underline{R}_G = \frac{\text{Distance travelled by the compound on unimpregnated paper}}{\text{Distance travelled by } \underline{D}\text{-glucose on the same paper}}$$

The relative effects on the migration rates, i.e. the ratio of the rates of migration of polyols on impregnated paper and on the control paper (both relative to $\underline{D}$-glucose) is

$$Q(\underline{W}) = \frac{\underline{R}_G(\underline{W})}{\underline{R}_G}$$

Polyols of group c, i.e. Non-complexing polyols (see p. 39)

The results (Table 6) show that all the non-complexing polyols have $Q(\underline{W})$ values = 1 or > 1. The increase in the migration rates i.e. $Q(\underline{W}) > 1$ of these compounds seems to be due to an effect similar to salting out, which resulted in the decrease of their solubility in the aqueous phase and increase of their solubility in the mobile organic phase.

Thus $Q(\underline{W}) = 1 \gtrsim 1$ is complementary to $Q(\underline{W}_p) > 0.9$ or $\underline{M}_s(\underline{W}) = 0$.

Polyols of group b (see p. 37).

It can be seen from Table 7 that $Q(\underline{W})$ values of the compounds forming the complexes of type (X) i.e. group b (p. 37) are between 0.5 - 0.9. The variation in $Q(\underline{W})$ values could be due to the spatial disposition of the hydroxyl groups which are not involved in complex formation.

Table 6

Compounds of group C

Compound	$\underline{R}_G(\underline{W})$	$\underline{R}_G$	$Q(\underline{W})$	$\underline{M}_s(\underline{W})$ [15]
Glyceraldehyde	1.13 - 1.9	1.72	0.67-1.1	0
L-Arabinose	1.30	1.19	1.09	0
D-Xylose	1.60	1.43	1.12	0
D-Glucose	1.00	1.00	1.00 (Marker)	0
2-Deoxy-D-arabino-hexose	1.77	1.70	1.04	0
2-Deoxy-D-lyxo-hexose	1.70	1.44	1.18	0
2-Deoxy-D-ribo-hexose	1.94	1.95	1.00	0
6-Deoxy-D-glucose	2.40	1.61	1.49	0
2-O-methyl-D-glucose	2.46	1.61	1.52	0
3-O-methyl-D-glucose	2.70	1.66	1.62	0
Methyl-α-D-glucoside	2.42	1.57	1.54	0
Methyl-β-D-glucoside	2.52	1.58	1.61	0
2-O-methyl-D-galactose	2.06	1.51	1.40	0

6-Deoxy-L-galactose	1.42	1.38	1.06	0
1,6-anhydro-D-galactose	1.95	1.79	1.09	0
Methyl-α-L-mannoside	2.55	1.79	1.42	0
Kojibiose (α 1 → 2)	0.46	0.44	1.04	0
Sophorose (β 1 → 2)	0.63	0.59	1.07	0
Laminaribiose (β 1 → 3)	0.70	0.68	1.03	0
Maltose (α 1 → 4)	0.55	0.54	1.01	0
Glycerol	2.38	1.95	1.22	0
3-O-methyl-D-glucitol	2.68	1.65	1.62	0
Laminaribiitol	0.65	0.65	1.00	0
Scyllo-inositol	0.29	0.29	1.00	0
(+)-Inositol	0.57	0.47	1.20	0
Muco-inositol	0.72	0.73	0.98	0

Table 7

Compounds of group b

11, 15, 16

Compounds	R_G(W)	R_G	Q(W)	M_s(W)
D-Erythrose	1.12	2.08	0.55	1.1
D-Lyxose	1.30[e]	1.49	0.87	1.04
D-Ribose	1.44[e]	1.56	0.92[e]	0.20
D-Galactose	0.66	0.92	0.73	0-1.1 (at pH 8)
D-Mannose	0.91	1.19	0.76	1.10
D-Gulose	0.66	1.06	0.62	1.10

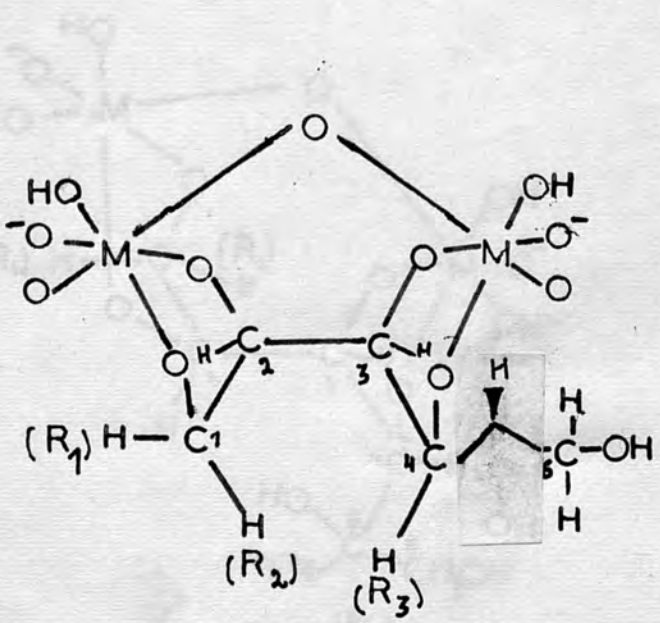
D-Glycero-D-allo-heptose	0.44	0.85	0.52	0.43[e]
D-Glycero-D-gulo-heptose	0.56	0.88	0.64	0.98[e]
D-Glycero-L-gluco-heptose	0.53	0.72	0.74	0
D-Glycero-L-manno-heptose	0.44	0.95	0.46	1.00
D-Glycero-L-galacto-heptose	0-0.51	0.68	0.75[e]	0.94[e]
D-Arabino-hexulose	0.19	1.08	0.17[e]	0.25
D-lyxo-hexulose	0.06-1.0[e]	1.34	0.04-0.74	1.10[s]
L-xylo-hexulose	0.06-1.0	1.34	0.04-0.74	0.20
L-Galacto-heptulose	0-0.50[e]	0.91	0-0.55[e]	-
D-Gluco-heptulose	0-0.54[e]	0.96	0-0.56[e]	-
D-Manno-heptulose	0.62	0.95	0.61	-
D-Allo-heptulose	0.37	1.17	0.31	-
Leucrose	0-0.33[e]	0.54	0.60	0.4
Isomaltalose	0.19	0.63	0.30	0.70[e]
Allo-inositol	0.31	0.62	0.50	0.08
Myo-inositol	0.19	0.60	0.31	0
Epi-inositol	0.23	0.38	0.60	1.00
Maltitol (α-1 $\longrightarrow$ 4)	0.13	0.61	0.21	0.17
Cellobiitol (β-1 $\longrightarrow$ 4)	0.21	0.50	0.42	0.10
Lactitol (β-1 $\longrightarrow$ 4)	0.14	0.42	0.33	0.68

Polyols of group a (see p. 35).

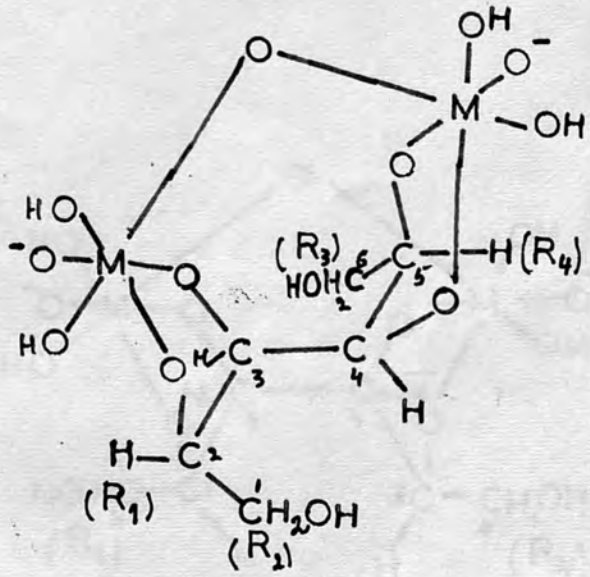
Chromatography of polyols forming complexes of the type (XI-XIV) has also shown (Tables 8 and 9) improvement in their

migration rates on paper wholly impregnated with sodium tungstate solution. It can be seen that $\underline{R}_G(\underline{W})$ values of all the polyols possessing at least four adjacent hydroxyl groups are a fraction of their $\underline{R}_G$ values.

Angus, Bourne and Weigel[16] have related the electrophoretic mobilities and thus the stabilities of the complexes of acyclic polyols possessing at least four adjacent hydroxyl groups, to the sizes of the substituents at R_2 and R_3 (in postulated structures, XI-XIV). They have shown that the compounds of identical molecular size having hydrogen atoms at R_2 and for) R_3 (in the complex) have higher $\underline{M}_s$ values than those possessing carbon atoms. $\underline{D}$-mannitol can form two complexes i.e. (XXI), involving hydroxyl groups at $C_{(1)}$ - $C_{(4)}$ or (XXII) involving hydroxyl groups at $C_{(2)}$ - $C_{(5)}$.

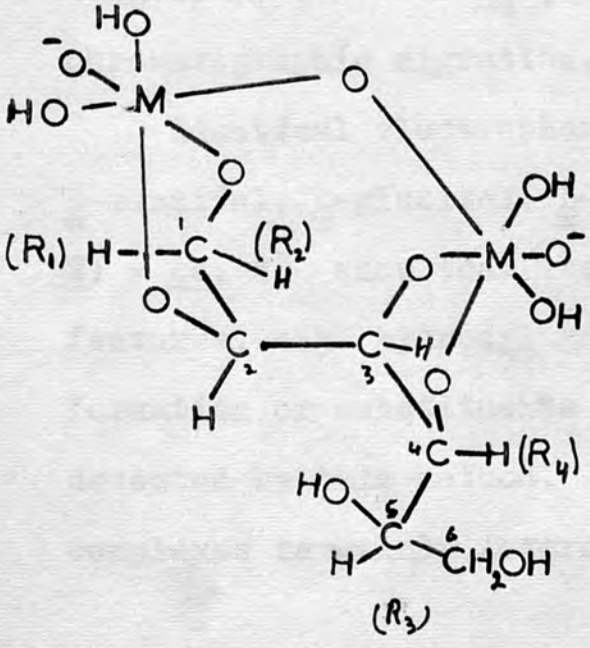


(XXI)

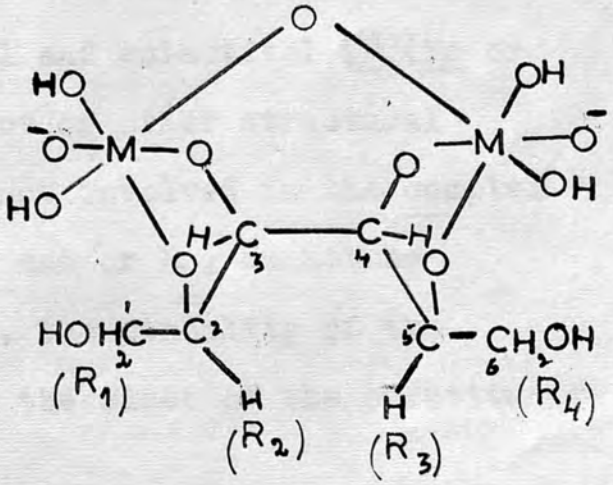


(XXII)

The complex (XXI) has two adjacent hydroxyl groups (at C_5 and C_6), while there is no such glycol in complex (XXII). Thus, partial periodate oxidation of (XXI) will yield D-arabinose, whereas (XXII) will remain unaffected. The formation of D-arabinose from the D-mannitol complex and thus the preferential formation or comparatively greater stability of (XXI) has been confirmed by Angus, Bourne and Weigel.[16] They also studied the partial periodate oxidation of a few other polyol-tungstate complexes and confirmed the type of complex formed preferentially or comparatively stable complex, e.g. the galactitol complex was not affected by the oxidant showing the formation of (XXIV), rather than (XXIII).



(XXIII)



(XXIV)

The confirmed structures of the polyol complexes, e.g. D-mannitol-complex (XXI) and galactitol-complex (XXIV) have only hydrogen atoms at R_2 and R_3 positions while the unstable complexes, e.g. (XXII) and (XXIII) have larger groups ([redacted]) at one or both of these positions. Clearly the presence of substituents other than hydrogen atom at R_2 and/or R_3 introduces a factor of instability.

Paper chromatography of polyols which possess four hydroxyl groups and can form only one type of complex, e.g. 1-deoxy-pentitols and 1,6-dideoxy-hexitols, show that where M_s(W) values of the compounds decrease due to presence of larger groups at R_2 and R_3, the Q(W) values increase (see Table 9). Thus, the electrophoretic behaviour, i.e. low M_s (values), is the reverse of the chromatographic migration, i.e. high Q(W) values.

Identical electrophoretic mobilities of pentitols, D-mannitol, D-glucitol, L-iditol and galactitol [M_s(Mo or W) = ca. 1] show that the effect of other structural features, e.g. hydroxyl groups not involved in the complex formation or substituents at R_1 and/or R_4, cannot be detected by this method. Thus, the stability of the complexes is mainly governed by the sizes of the substituents

at R_2 and R_3. Since the electrophoretic mobilities of the ionic species are mainly based on the ionic charge, molecular size and stability, the complexes of identical molecular size, ionic charge and stability cannot be expected to migrate differently on electrophoresis.

On the other hand migration on paper is governed by the partition (or adsorbtion) co-efficient of the compounds. Suppose two complexes (X and Y) of identical molecular size and identical substituents at R_2 and R_3 have different Q(W) values. The difference in paper chromatographic

Complex X : R_2 = H	Complex Y: R_2 = H
R_3 = CH_2OH	R_3 = CH_2OH

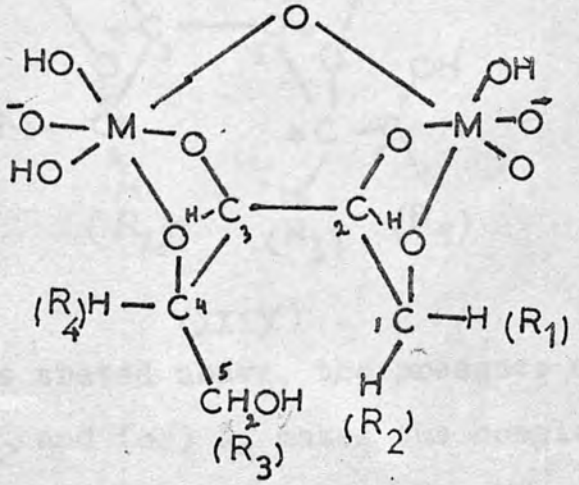
mobilities of X and Y could only be due to (i) different partition coefficient or (ii) some other stability or instability factors, which cannot be revealed by electrophoresis (see below).

The proved structures of the complexes of D-mannitol (XXI) and galactitol (XXIV) are of the same type, i.e. (XI). These two complexes have identical substituents at R_2 and R_3, i.e. a hydrogen atom; the substituents at other positions, e.g.

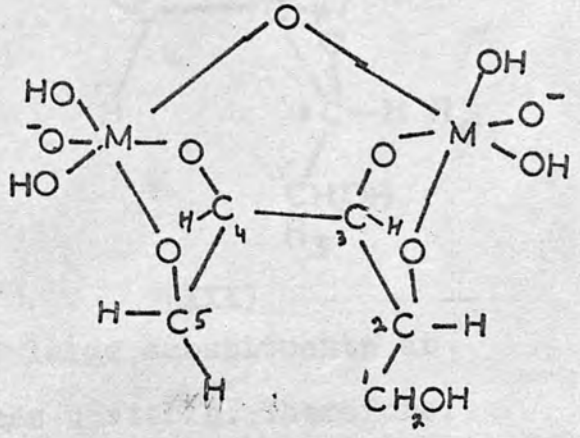
D-mannitol complex: R_1 = H; R_4 = $C_{(5)}HOH$, $C_{(6)}H_2OH$

Galactitol complex: R_1 = $C_{(1)}H_2OH$, R_4 = $C_{(6)}H_2OH$

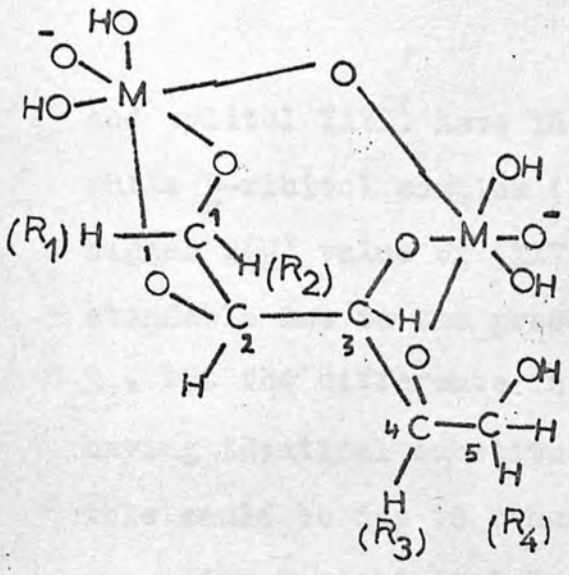
are also more or less the same, and hence have the same $\underline{M}_s$ values, but they have exhibited different Q($\underline{W}$) values, which clearly indicate that some factors (stability or instability) other than those discussed above are also involved for the exhibited paper chromatographic behaviour of the polyols. In the case of pentitols two structures are possible depending on the vicinal tetritols, i.e. $C_{(1)}$- $C_{(4)}$ or $C_{(2)}$- $C_{(5)}$ involved in the complex formation. The two complexes of ribitol are XXV and XXVI and those formed with xylitol are XXVII and XXVIII. Since these complexes differ only in that they are mirror images, only XXV and XXVII will be considered.



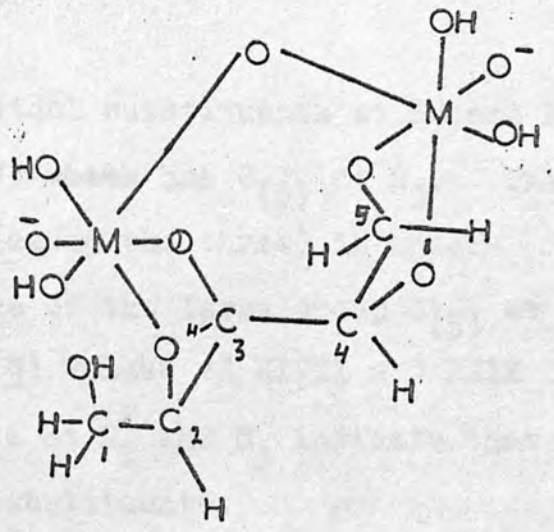
(XXV)



(XXVI)

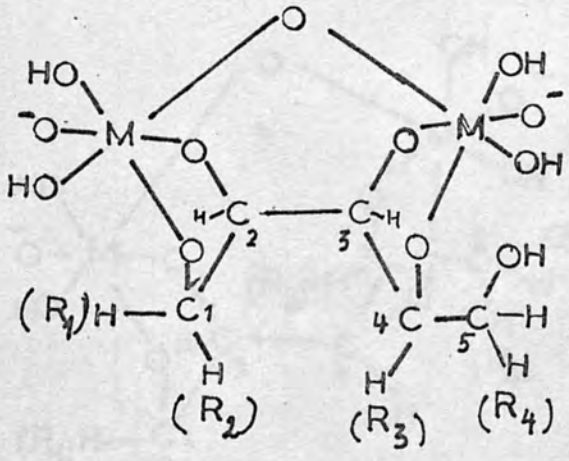


(XXVII)

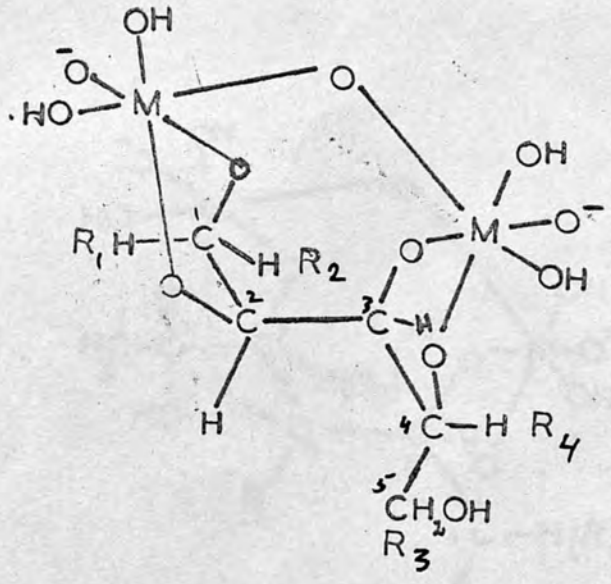


(XXVIII)

The two possible complexes of D-arabinitol are XXIX and XXX.



(XXIX)

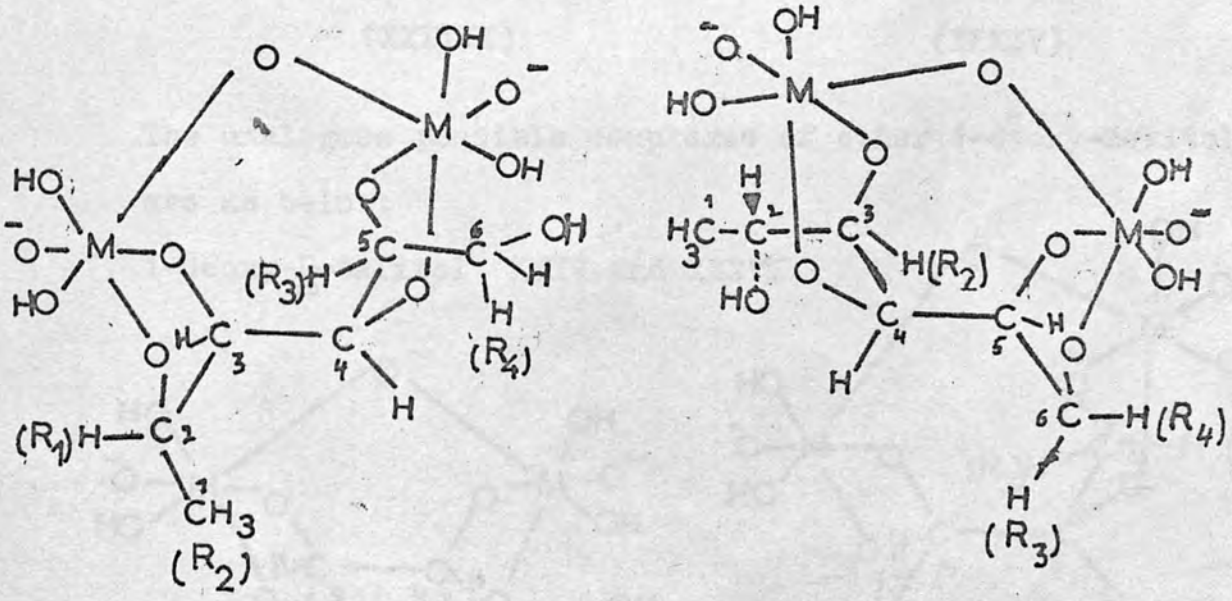


(XXX)

As stated above, the presence of large substituents at R_2 and (or) R_3 makes the complexes unstable. Therefore, the comparatively more stable complex of D-arabinitol will be XXIX. Thus, the complexes of D-arabinitol (XXIX)

and xylitol XXVII have identical substituents at R_2 and R_3, while D-ribitol complex (XXV) ~~which~~ has $C_{(5)}$ at R_3. The higher Q(W) value of (XXV) (among the three) is understandable due to the presence of the large group $C_{(5)}$ at R_3, but the difference in Q(W) values of XXVII and XXIX having identical substituents at R_2 and R_3 indicate that this could be due to other substituents.

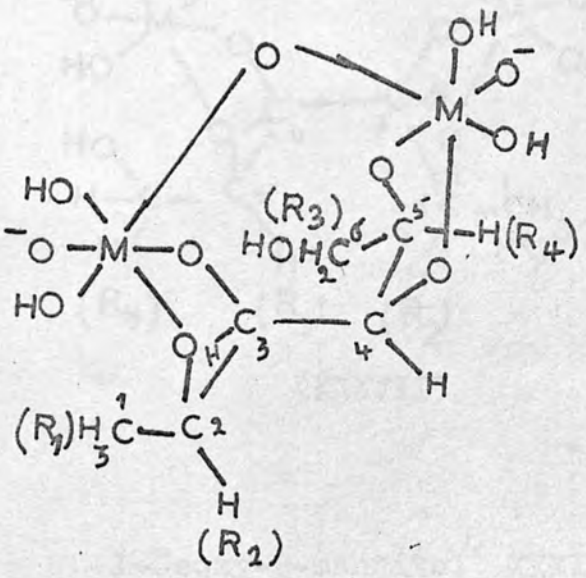
Like D-arabinitol, each 1-deoxy-hexitol can form two complexes; thus, the two possible complexes of 1-deoxy-L-gulitol are XXXI and XXXII.



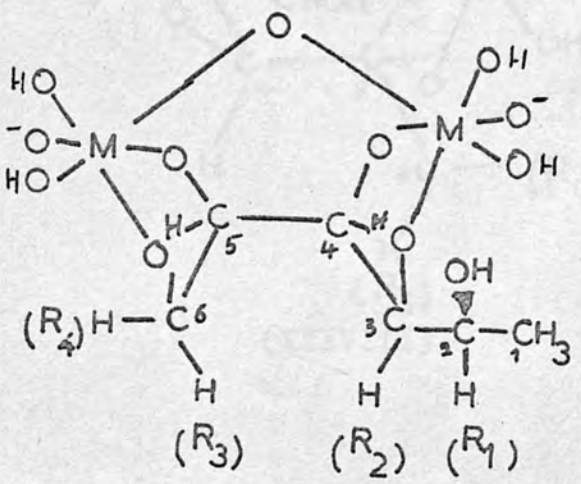
(XXXI) (XXXII)

The comparatively more stable complex will be that having smaller groups at R_2 and R_3, i.e. XXXII. Similarly the

comparatively more stable complex of 1-deoxy-D-glucitol is XXXIV, out of the two possibilities, i.e. XXXIII and XXXIV.



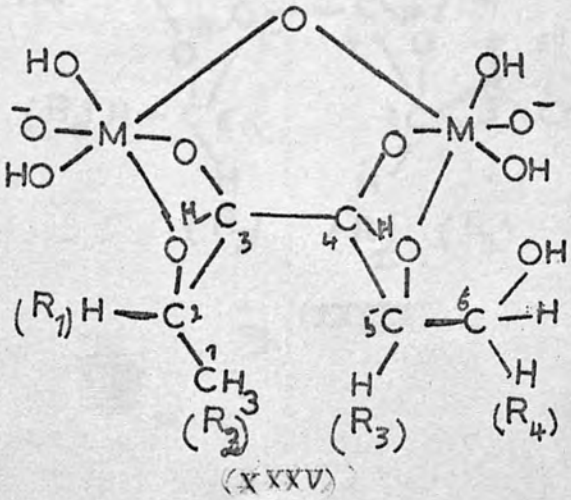
(XXXIII)



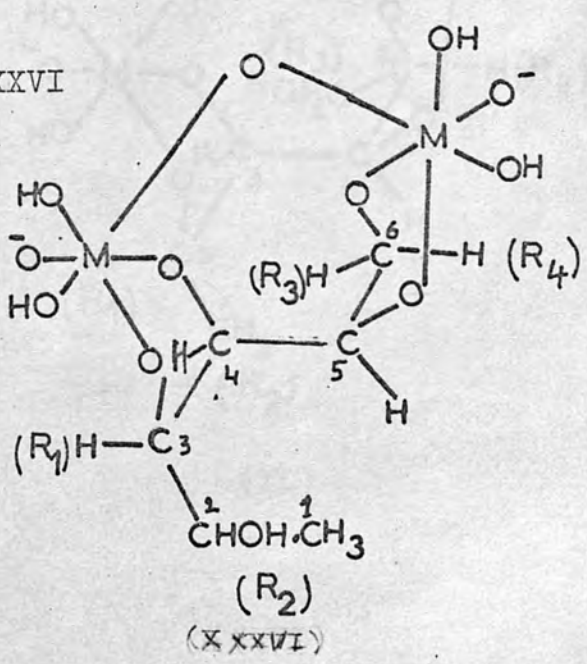
(XXXIV)

The analogous possible complexes of other 1-deoxy-hexitols are as below:

1-deoxy-D-talitol XXXV and XXXVI

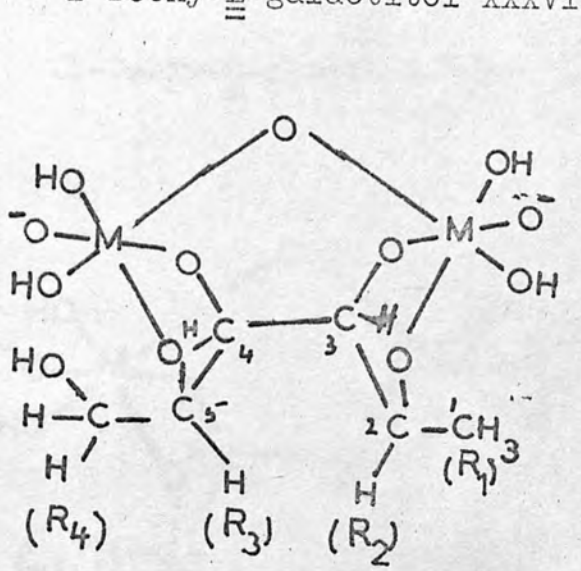


(XXXV)

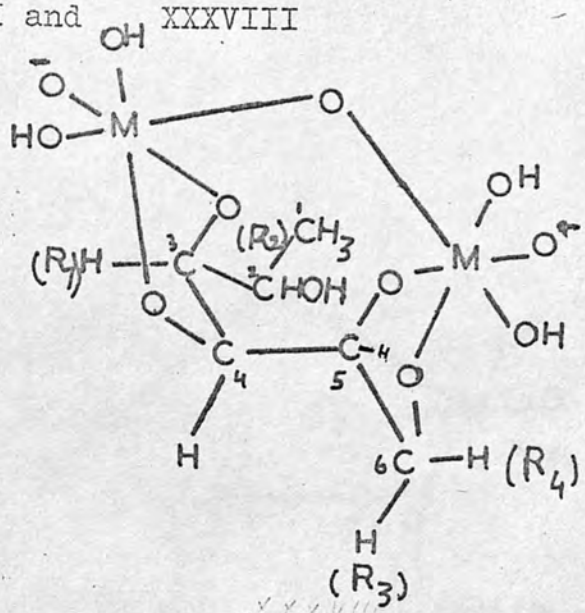


(XXXVI)

1-Deoxy-L-galactitol XXXVII and XXXVIII

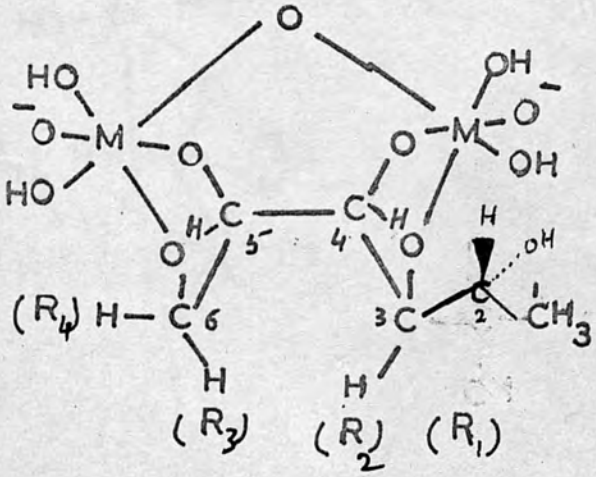


(XXXVII)

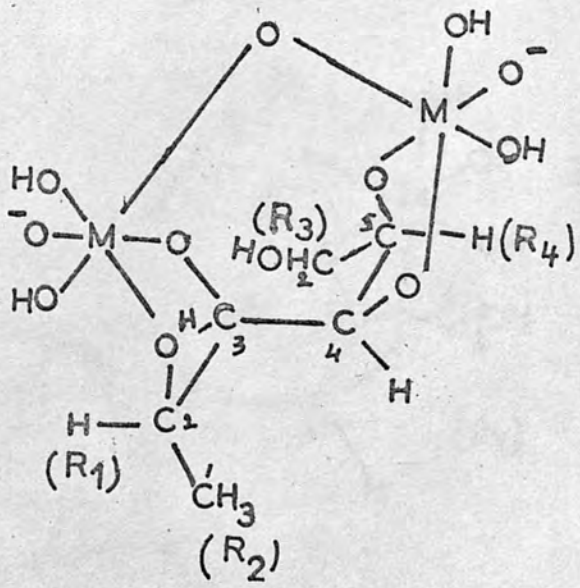


(XXXVIII)

1-Deoxy-D-mannitol XXXIX and XL

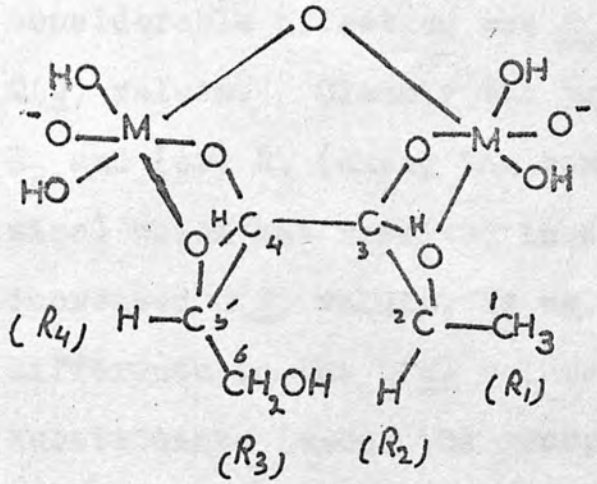


(XXXIX)

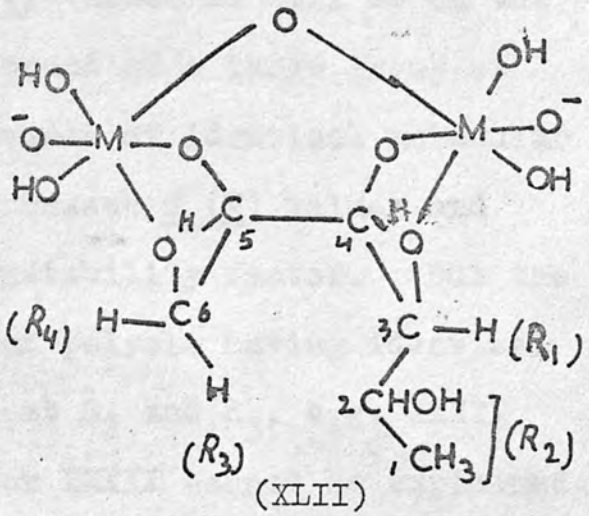


(XL)

1-Deoxy-D-altritol XLI and XLII

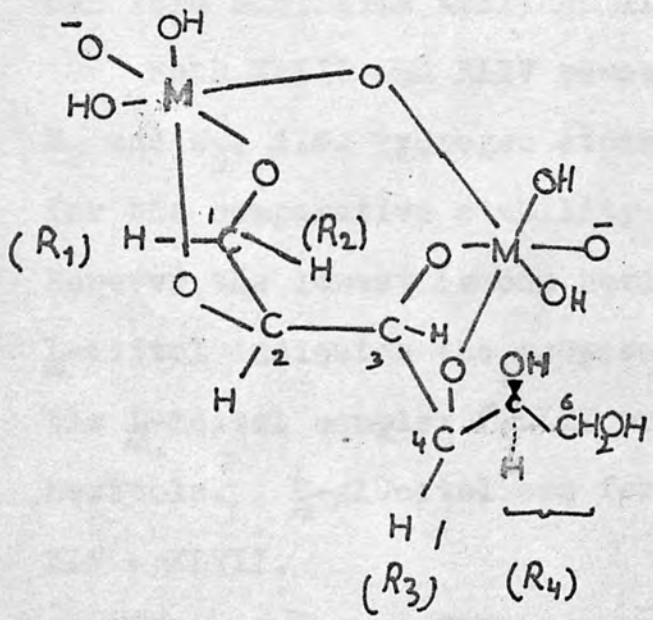


(XLI)

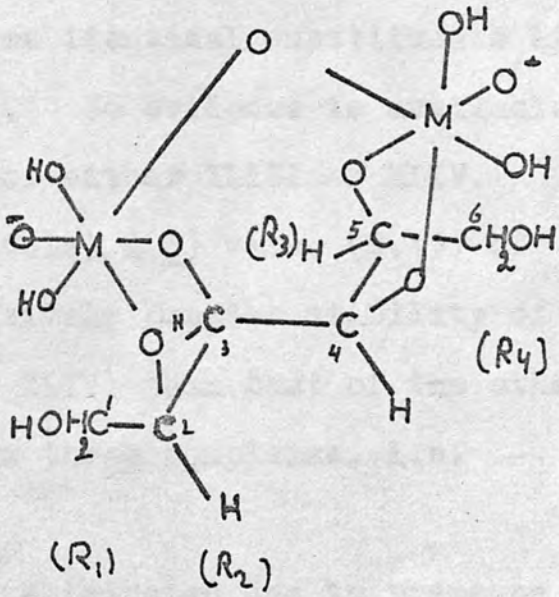


(XLII)

L-Iditol



(XLIII)

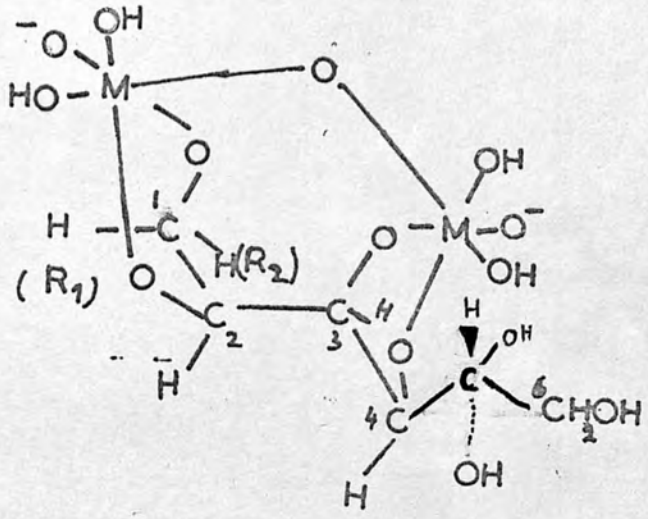


(XLIV)

Out of these complexes the XXXVI, XXVIII, XL and XLII can be eliminated owing to the presence of comparatively larger groups at R_2 and (or) R_3. It can be seen that the presence of a large group at R_2 and (or) R_3 has a considerable effect on the $\underline{M}_s(\underline{W})$ values as well as on the $Q(\underline{W})$ values. Clearly the presence of a large group at R_2 and (or) R_3 (among the compounds of identical molecular size) which has resulted in decreased $\underline{M}_s(\underline{W})$ values and increased $Q(\underline{W})$ values, is an instability factor. But the difference in the $Q(\underline{W})$ values of polyols having identical substituents (among the group) at R_2 and R_3, e.g. XXXII and XXXIV or XXXIV and XXXVII or XXXIX cannot be explained on the basis of substituents at R_2 and R_3 only. L-Iditol can form complexes XLIII and XLIV.

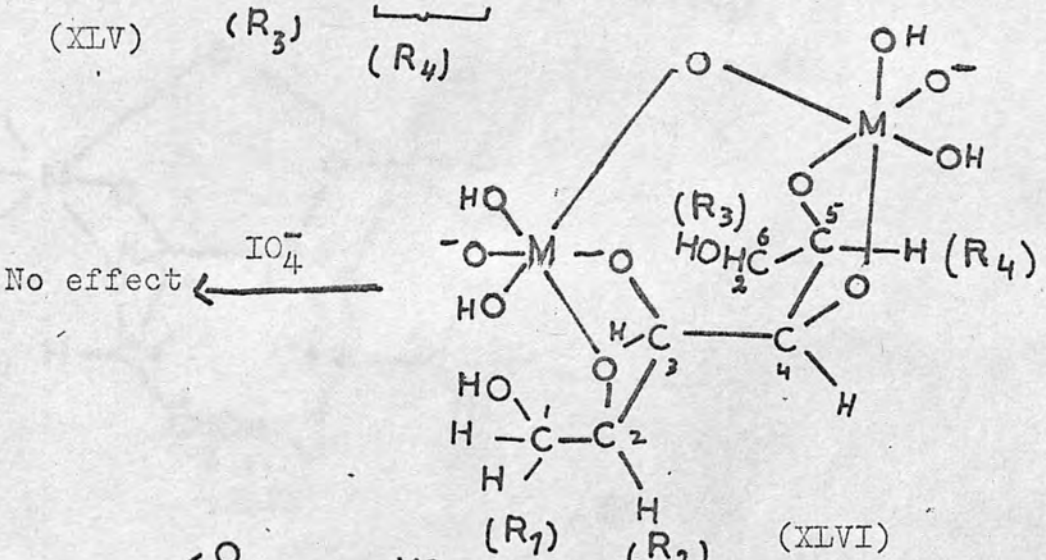
Both XLIII and XLIV possess identical substituents at R_2 and R_3, i.e. hydrogen atoms. No evidence is available for the comparative stability of either XLIII or XLIV. However the lowest (among hexitols) $Q(\underline{W})$ value (0.13) of L-iditol indicates the comparatively greater stability of the L-iditol complex (XLIII or XLIV) than that of the other hexitols. D-glucitol can form three complexes, i.e. XLV - XLVII.

The structure XLVI can be eliminated due to presence of $C_{(6)}$ at R_3. Partial periodate oxidation of the



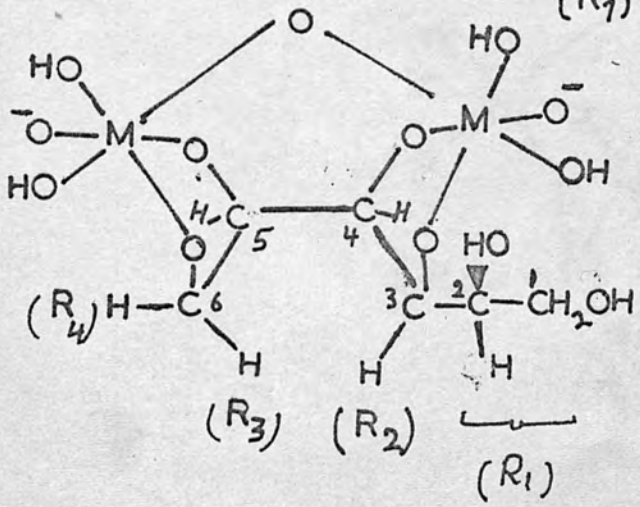
(XLV)

IO_4^- → L-Lyxose

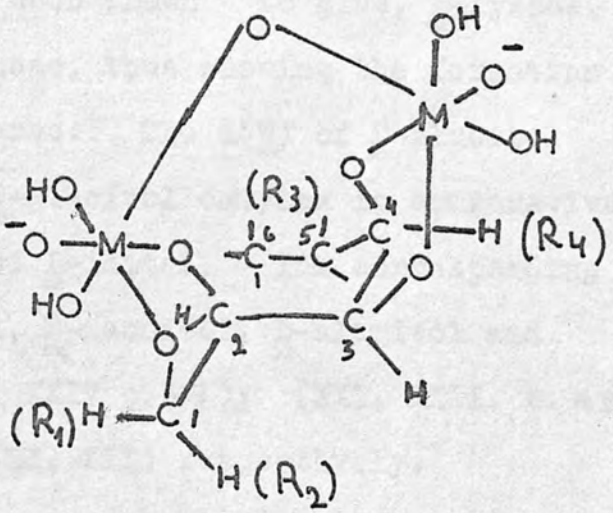


(XLVI)

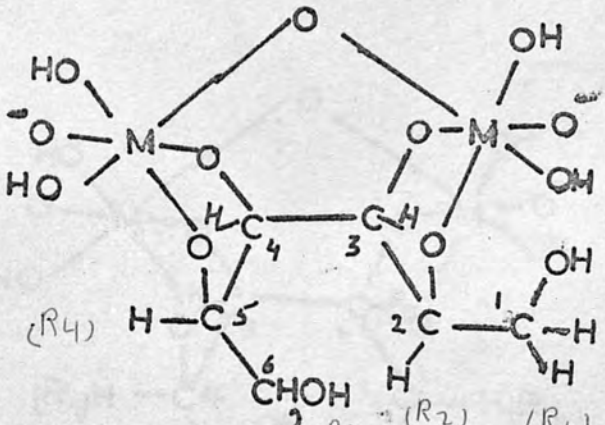
No effect ← IO_4^-



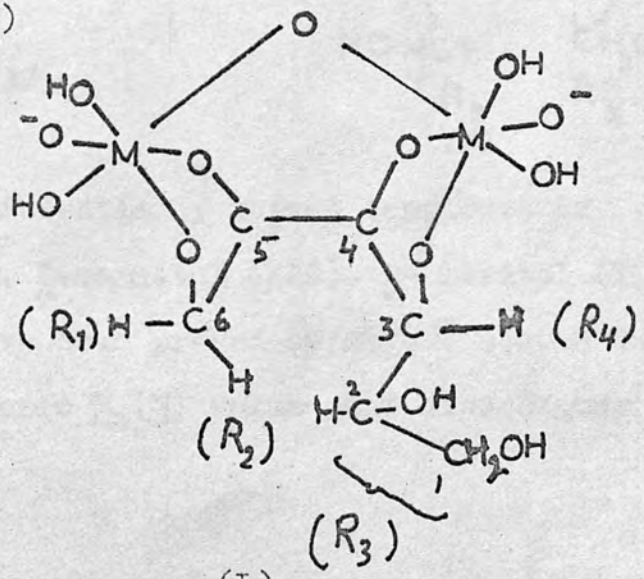
IO_4^- → D-Arabinose



(XLVIII)

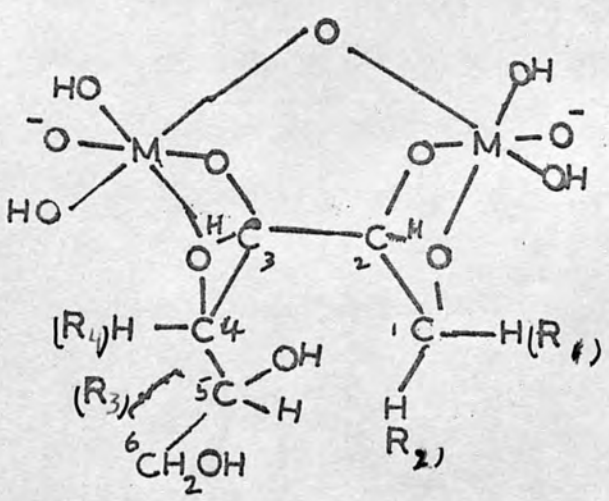


(XLIX)

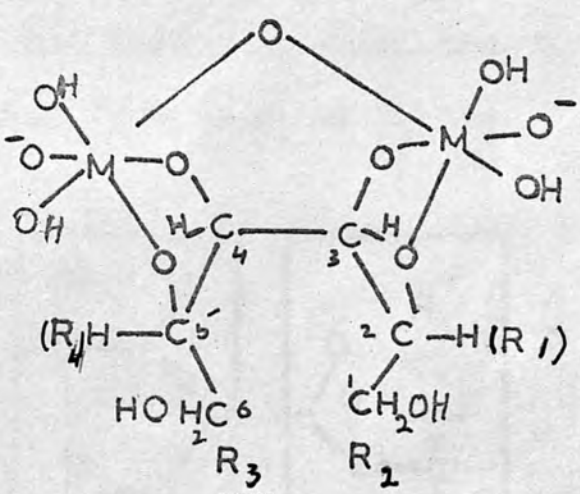


(I.)

D-glucitol-complex has been shown[16] to give, L-lyxose, D-glucitol and D-arabinose, thus showing the formation of all the three complexes. The Q(W) of D-glucitol (0.15) indicates that D-glucitol complex is comparatively less stable than that of L-iditol. The corresponding complexes of galactitol, D-mannitol, D-altritol and D-allitol are; (XXIII, XXIV p. 49); (XXI, XXII, p. 48); (XLVIII, XLIX, L) and (LI, LII) respectively.



LI

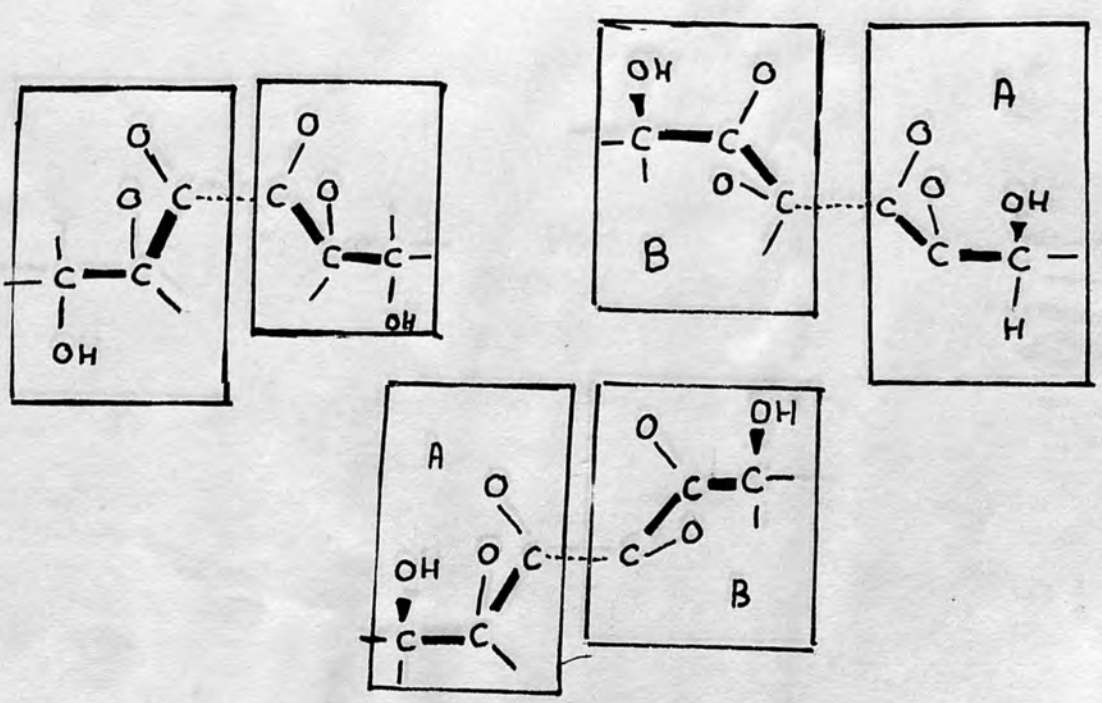


LII

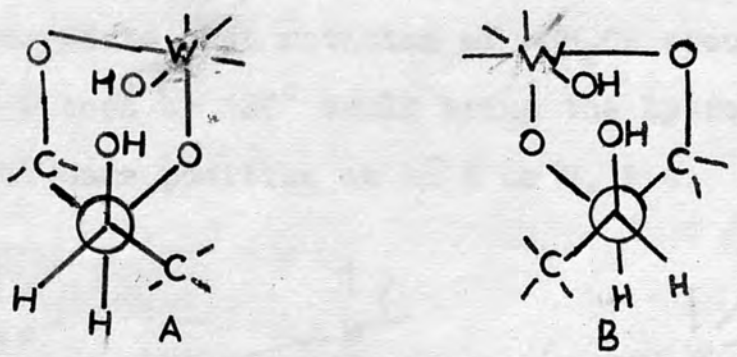
The stable or preferentially formed complexes of galactitol (XXIV), D-mannitol (XXI), D-altritol (XLIX) and D-allitol (LI) have been proved by Angus, Bourne and Weigel.[16] The lower $M_s(W)$ values and also higher Q(W)

values of XLIX and LI show the instability introduced by the large substituents present at R_2 and R_3 of the two complexes respectively. But the other four hexitol complexes possessing identical substituents at R_2 and R_3 have exhibited different Q(W) values as in the case of pentitols and 1-deoxy-hexitols, which indicate that there are definitely some other factors responsible for the chromatographic behaviour of the polyols possessing more than four hydroxyl groups. Thus, it is appropriate to consider the position of substituents at R_1 and R_4.

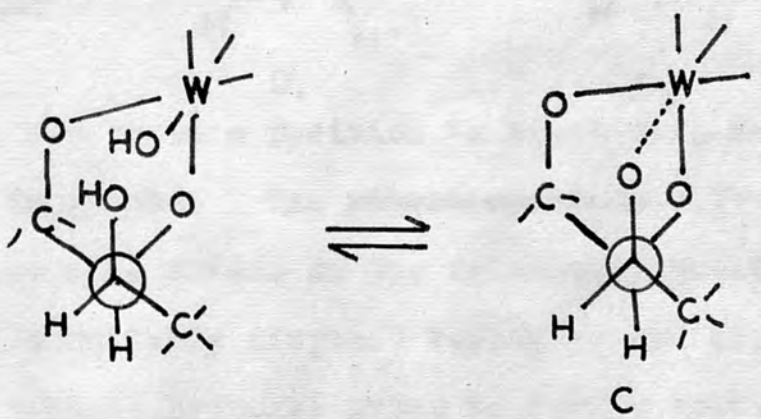
The diagrams discussed above reveal that for each group of compounds having the same molecular size, the Q(W) values are low when a carbon atom at R_1 or R_4 has the following configuration:



When carbon atoms linked by bonds marked with thick lines are arranged in the planar zig-zag conformation, the oxygen atoms of the segments marked □ are in a spatial disposition identical with IV, i.e.

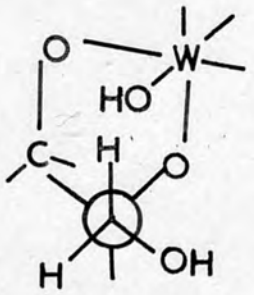


In such a case the hydroxyl groups in A and B are already in such a position that they could react with 6-co-ordinated tungstate, by elimination of water, e.g.



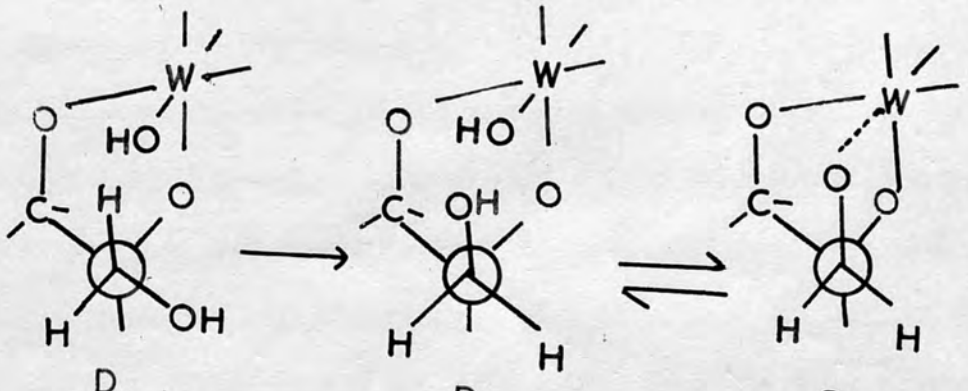
bond formed by elimination of water

which is comparable to the elimination of water as in the formation of X.[7] When R_1 and R_4 are $-CH_2OH$ groups, the hydroxyl group of $-CH_2OH$ would normally extend the planar zig-zag conformation of the carbon chain, i.e.



D

In this case the hydroxyl group of the $-CH_2OH$ is away from the tungstate, but rotation of $-CH_2OH$ around the terminal C-C bond by 120° would bring the hydroxyl group in the same position as in A or B, i.e.



which will now be in a position to react with 6-co-ordinated tungstate. The [redacted] Q(W) are dependent to some extent on the relatively greater ability of a suitably disposed secondary hydroxyl group than of a primary hydroxyl group to form a tridentate complex. To allow for the rotation of the primary hydroxyl group, an entropy term RT ln 1/f has to be added to the free energy change involved in the formation of tridentate D. This could make D less sensitive to a

---

f = Fraction of the molecules may have the the oxygen atoms of the primary hydroxyl groups in a position suitable for the tridentate formation.

tridentate formation than B. Clearly the ability to form a tridentate is a stability factor. The comparison of the Q(W) values of D-glucitol.and galactitol show that the effect of one -CHOH (1a) on the stability, i.e. decrease in Q(W) value is greater than that due to two $-CH_2OH$(2p). Thus, the most stable structure of the polyols (within group) containing more than four hydroxyl groups can be selected (i) by eliminating the structure having large R_2 and (or) R_3 (ii) by selecting the structure capable of forming a tridentate due to a secondary hydroxyl group and (iii) the structure which can form a tridentate due to one primary hydroxyl (p) or 2 primary hydroxyl groups (2p). Table 8 shows the skeleton structures of the complexes formed with pentitols 1-deoxy-hexitols and hexitols (already discussed above). Numbers of the structures correspond to that on p. 48-60 and those in Table 9.

Table 8. Skeleton structures of tungstate complexes of polyols with at least four adjacent hydroxyl groups.

(i) For clarity several atoms have been omitted.

(ii) Skeleton structures of possible complexes, i.e. 2,3 diol grouping is cis (XI or XII) or trans (XIII or XIV). Shown are (a) number of C atoms of polyol (b) substituents

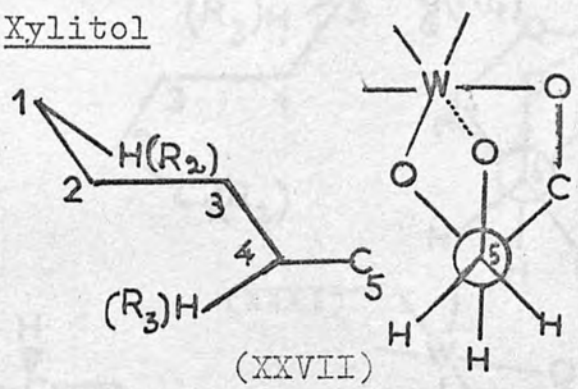
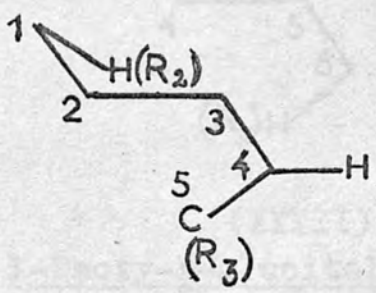
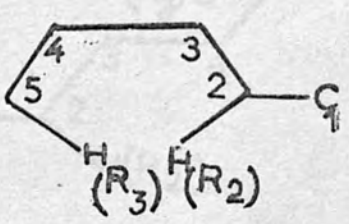
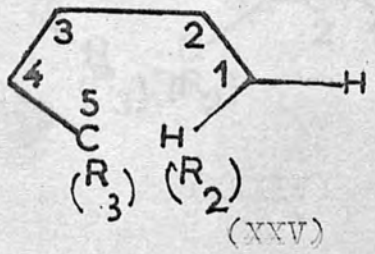
R_2 and R_3 of XI-XIV, i.e. H, $-CH_2OH$ or $CH(OH).CH_2OH$.
(c) substituent R_1 and/or R_4 when they contain C atom(s)
(d) stereochemistry of C atom or R_1 and R_4 (if present)
(iii) Newman projection along $R_1 - C_1$ and $R_4 - C_4$ of (XI-XIV), when R_1 or R_4 contain a hydroxyl group.
The disposition of atoms (or group of atoms) is shown when: (a) R_1 or R_4 contain two carbon atoms. The view is that when $C_{(2)}$, $C_{(1)}$ and those of R_1, or $C_{(3)}$, $C_{(4)}$ and those of R_4 are in a planar zig-zag conformation.
(b) when R_1 or R_4 is a primary hydroxyl group the oxygen of which is placed in a disposition suitable for tridentate attachment (i.e. rotation by 120° from its otherwise usual preferred conformation (c) bond formed by elimination of water is shown by broken lines (------). (see p. 66)

Table 9 shows the most stable structures of polyols selected on the basis of the above mentioned choices, with their $\underline{R_G}(\underline{W})$ and $\underline{M_s}(\underline{W})$ values. The structures of tetritols, 1-deoxy-pentitols and 1,6-dideoxy-hexitols are also included for comparison.

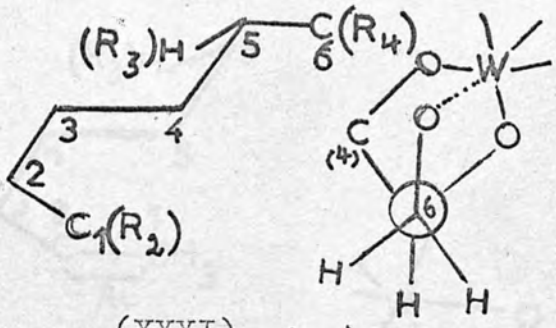
Tetritols.

The complexes of L-threitol (LIII) and erythritol (LIV) are of the trans and cis type i.e. XIII and XI respectively. Both (LIII) and (LIV) possess identical substituents at R_2 and R_3, i.e. hydrogen atoms.

Table 8

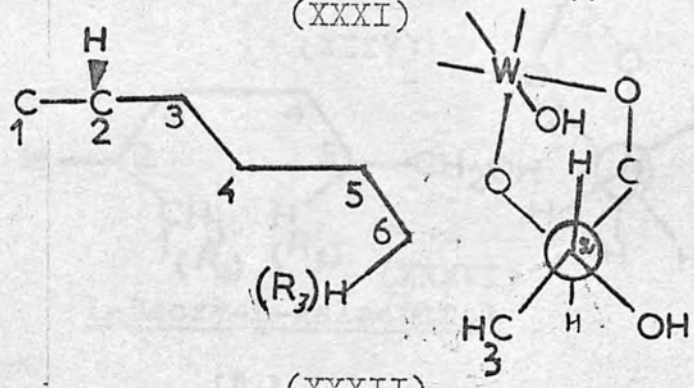
Compound	Instability factors, i.e. carbon atoms at R_2 and/or R_3	Stability factors a= -CHOH p= $-CH_2OH$	Q(W)
Xylitol  (XXVII)	-	1_p	0.14
D-Arabinitol  (XXX)	$C_{(5)}$	-	
 (XXIX)	-	1_p	0.48
Ribitol  (XXV)	$C_{(5)}$	-	0.58

1-Deoxy-L-gulitol



(XXXI)

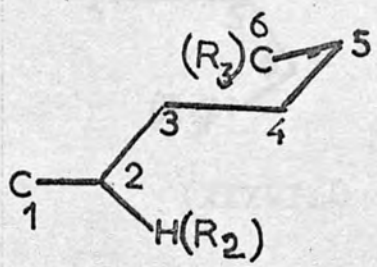
C(1) l_p



(XXXII)

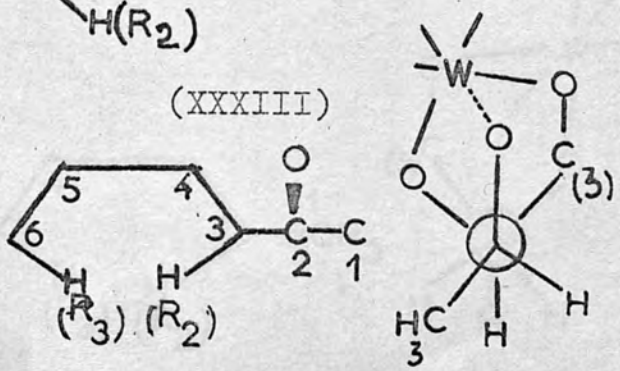
\- - 0.19

1-Deoxy-D-glucitol



(XXXIII)

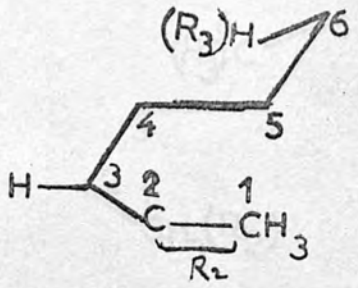
C(6) -



(XXXIV)

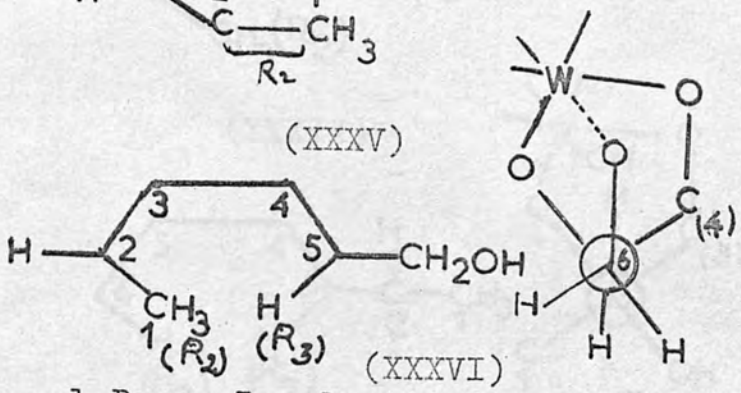
\- la 0.31

1-Deoxy-D-talitol



(XXXV)

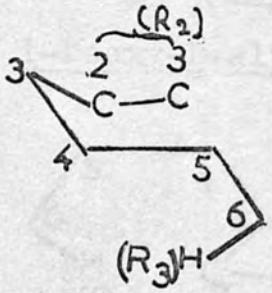
C_2-C_1 -



(XXXVI)

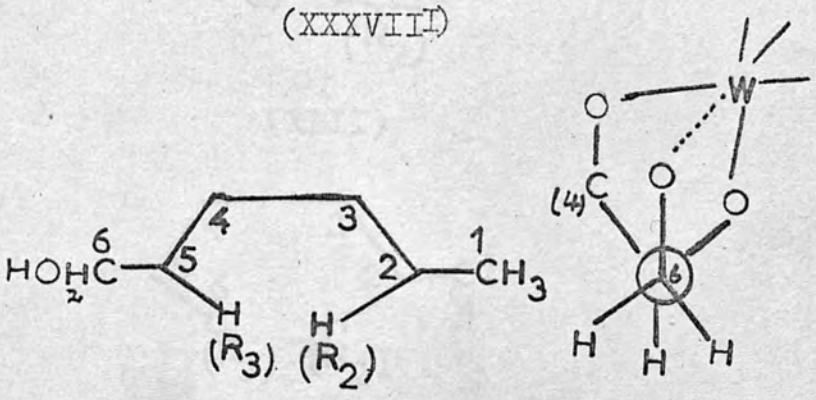
C_1 lp 0.45

1-Deoxy-L-galactitol



(XXXVIII)

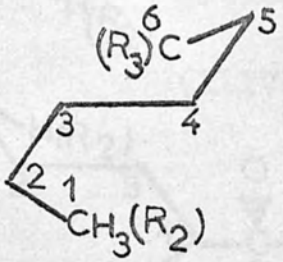
C_2-C_1 -



(XXXVII)

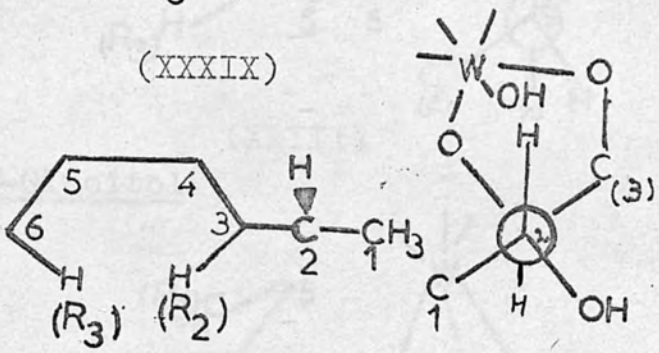
- lp 0.54

1-Deoxy-D-mannitol



(XXXIX)

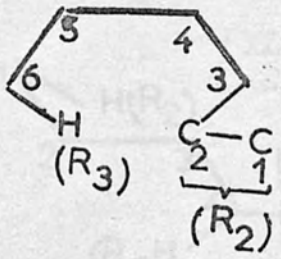
C_1, C_6 - -



(XL)

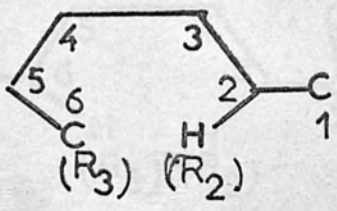
0.55

1-Deoxy-D-altritol



(XLII)

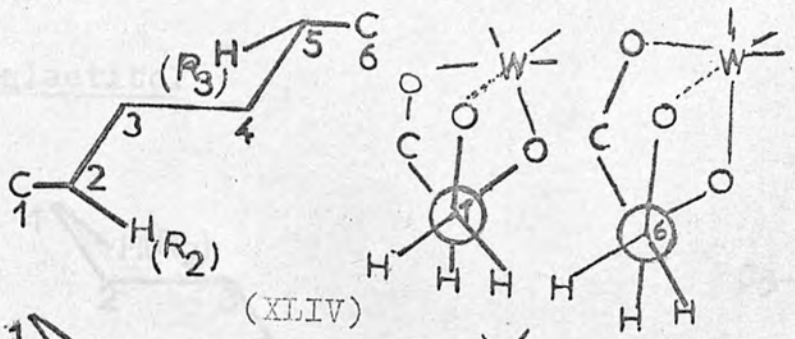
C_2-C_1 - -



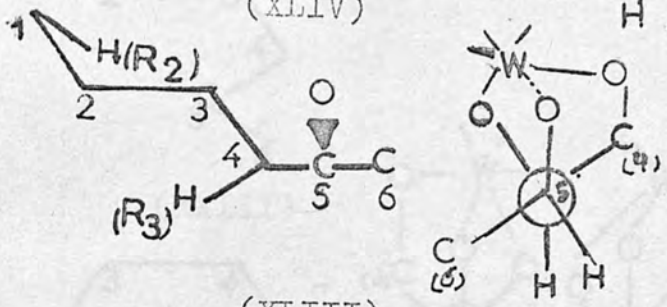
(XLI)

C_6 - 0.79

L-Iditol

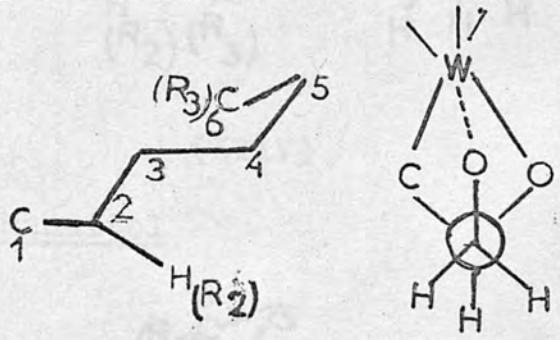


(XLIV) - 2p

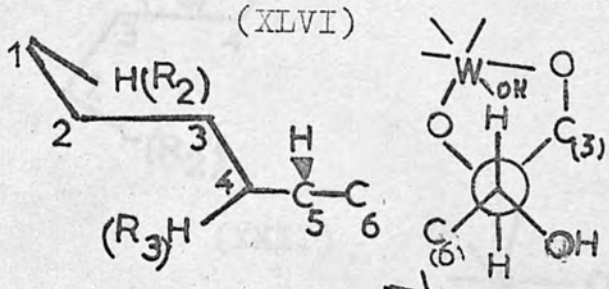


(XLIII) - l_a 0.13

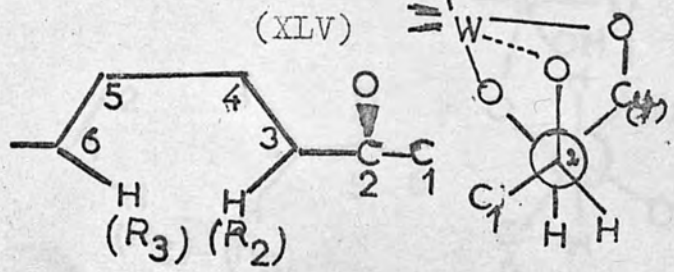
D-Glucitol



(XLVI) C_6 l_p

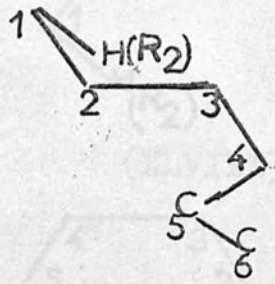


(XLV) - -



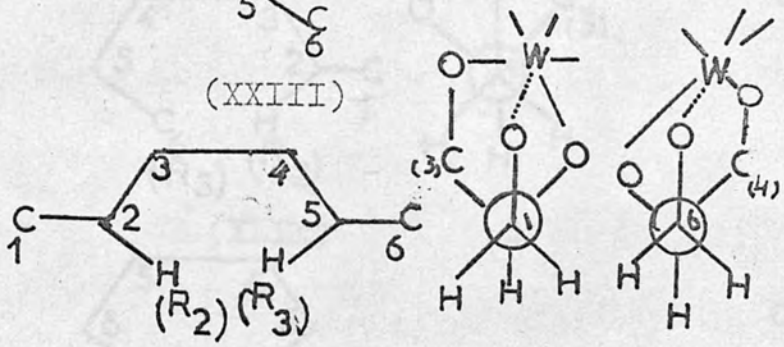
(XLVII) - l_a 0.15

Galactitol



(XXIII)

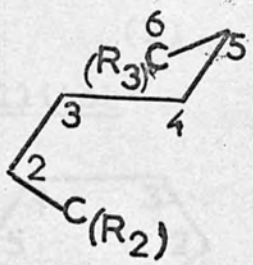
C_5-C_6 - -



(XXIV)

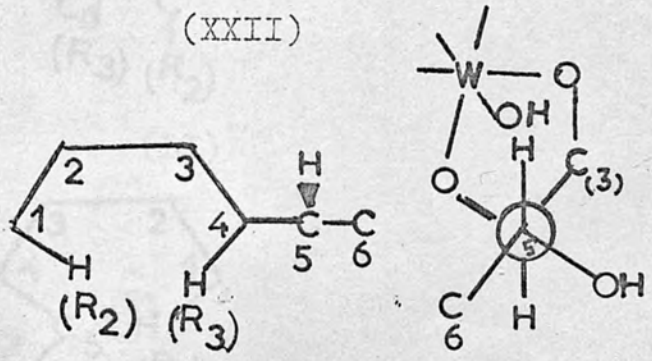
- 2_p 0.39

D-Mannitol



(XXII)

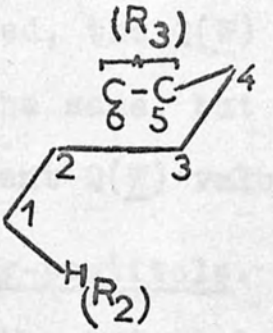
C_1, C_6 - -



(XXI)

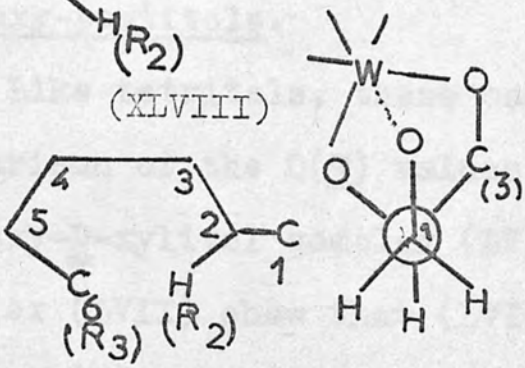
- - 0.44

D-Altritol



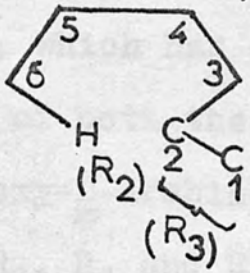
(XLVIII)

C_5-C_6 -



(XLIX)

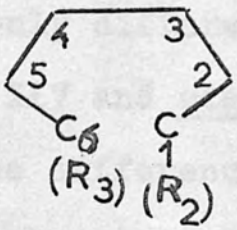
C_6 l_p 0.45



(L)

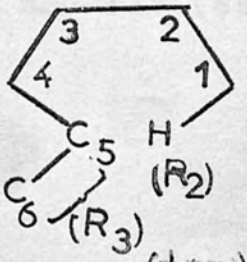
C_2-C_1 -

D-Allitol



(LI)

C_1, C_6 -



(LII)

C_5-C_6 - 0.61

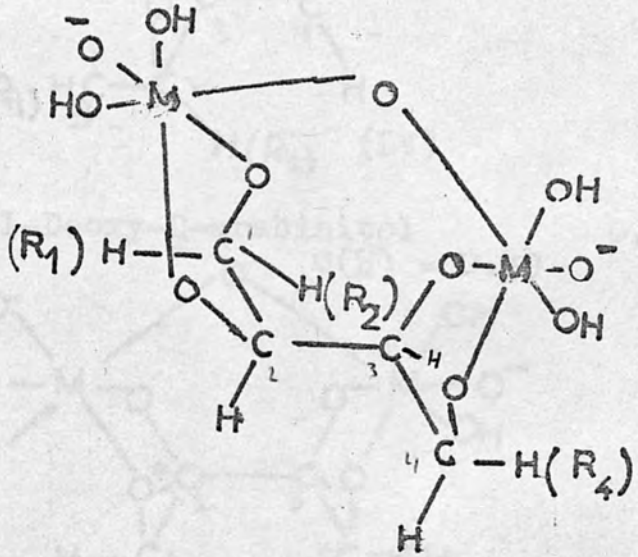
Since no other stability or instability factors are involved, the Q(W) values were expected to be more or less the same, but in fact the compounds have exhibited different Q(W) values.

1-Deoxy-pentitols.

Like tetritols, these can also form only one complex. Comparison of the Q(W) values and $M_s(W)$ values of the 1-deoxy-D-xylitol complex (LV) and the 1-deoxy-D-lyxitol complex (LVII) show that (LVII), having $C_{(1)}$ at R_2 has lower $M_s(W)$ value and higher Q(W) value than those of (LV), which has only hydrogen atoms at R_2 and R_3. In the case of both the 1-deoxy-D-xylitol complex (LV) and the 1-deoxy-D-arabinitol complex (LVI), the substituents at R_1, R_2, R_3 and R_4 are identical. Therefore, the expected Q(W) values would be more or less the same. However, they have exhibited different Q(W) values. The only difference in LV and LVI is that, these are trans type XIV and cis type XII respectively. This is similar to the difference both in Q(W) and the types of structures encountered in the case of the tetritol complexes, i.e. LIII and LIV (see above).

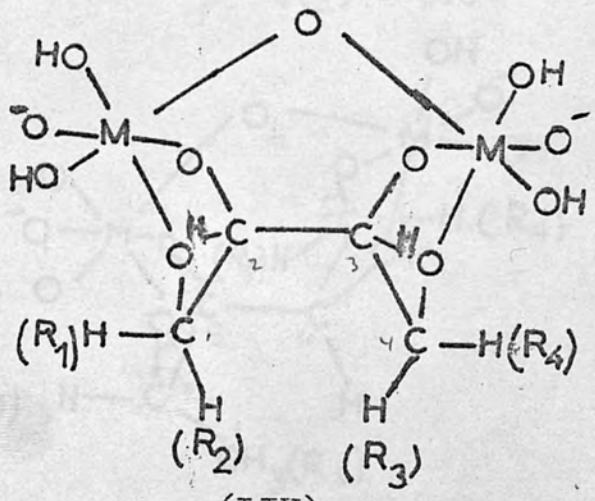
Table 9

Compound		$R_G(W)$	R_G	$M_s(W)$[15]
L-Threitol	Q(W)=0.32	0.47	1.46	0.24



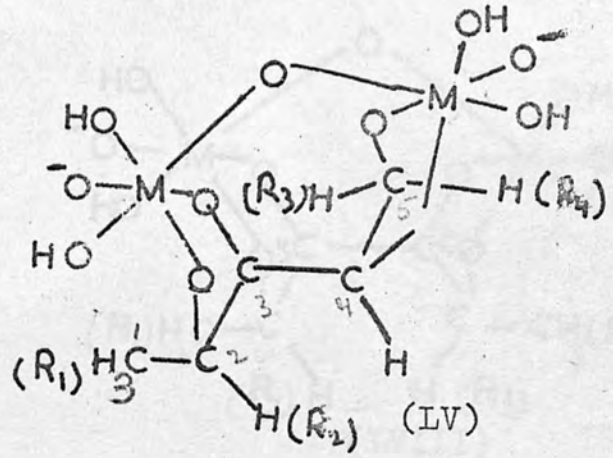
(LIII) (R_3)

Erythritol	Q(W)= 0.93	1.43	1.57	0.90



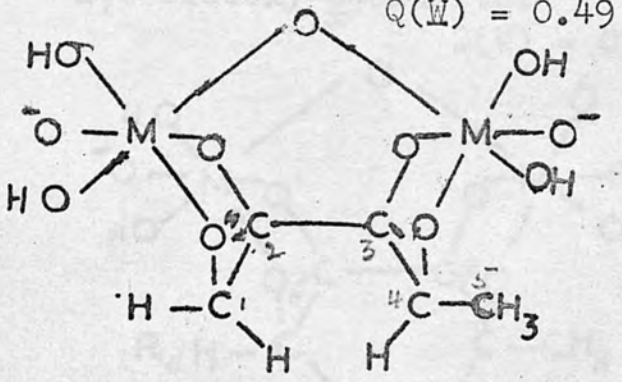
(LIV)

1-Deoxy-D-xylitol Q(W) = 0.27	0.51	1.90	0.82



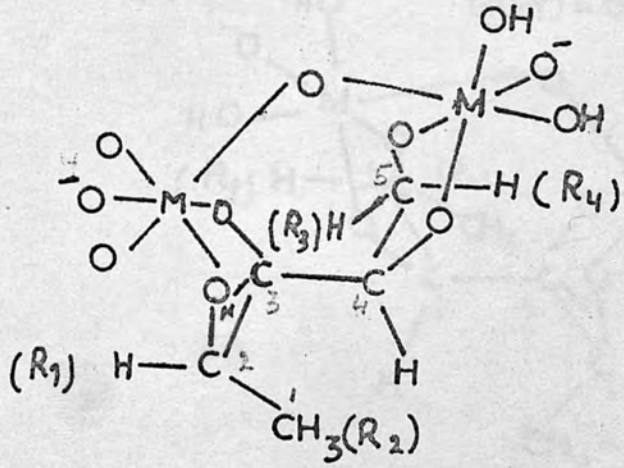
(LV)

1-Deoxy-D-arabinitol Q(W) = 0.49	0.96	1.96	1.09



(LVI)

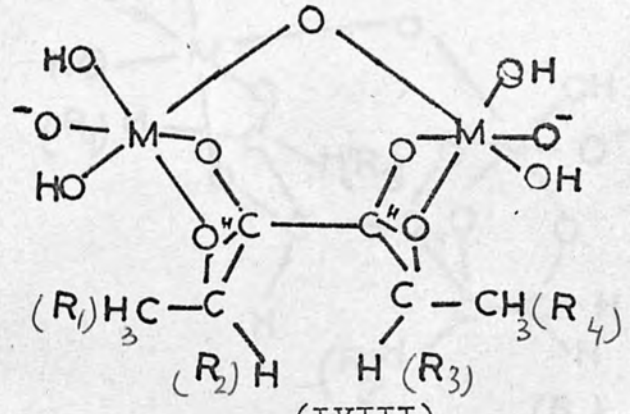
1-Deoxy-D-lyxitol Q(W) = 0.64	1.25	1.95	0.65[e]



(LVII)

1,6-Dideoxy-galactitol 1.33 2.2 1.09

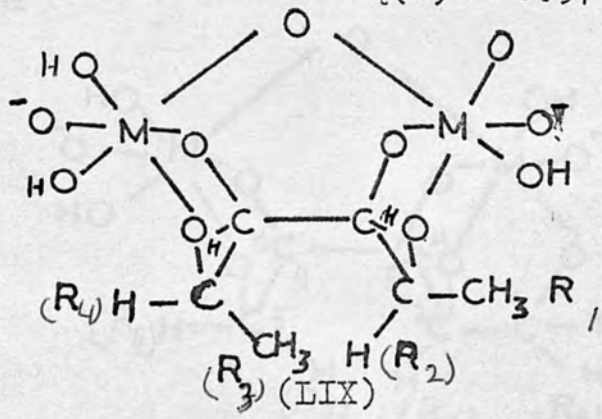
Q(W) = 0.61



(LVIII)

1,6-Dideoxy-D-altritol 1.97 2.02 1.05

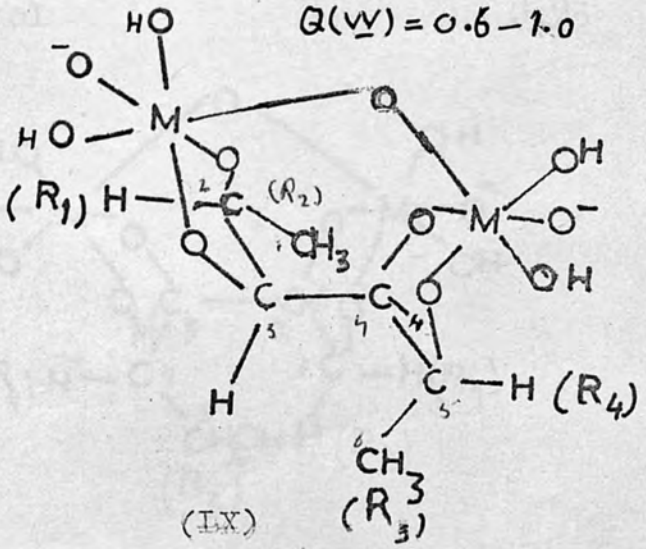
Q(W) = 0.97



(LIX)

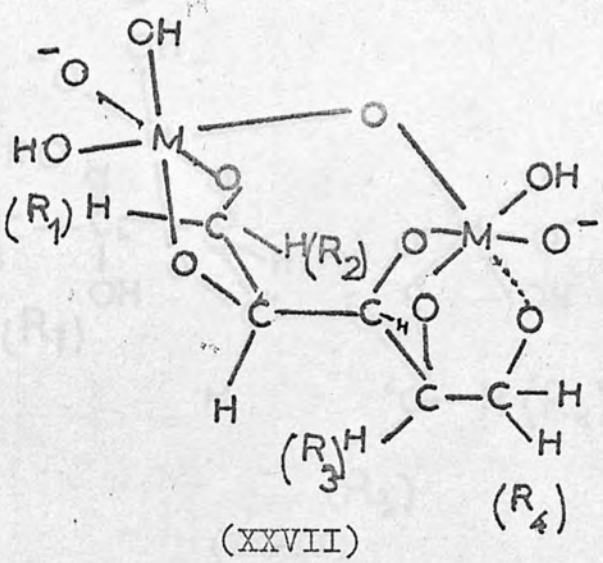
1,6-Dideoxy-L-mannitol 1.3-2.2 2.08 0.95[e]

Q(W) = 0.6-1.0



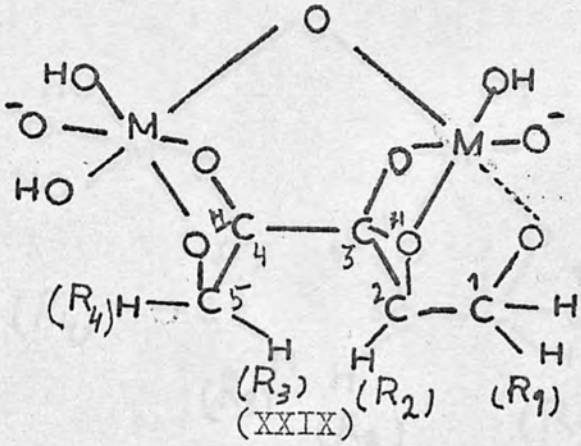
(LX)

Xylitol 0.16 1.17 1.04



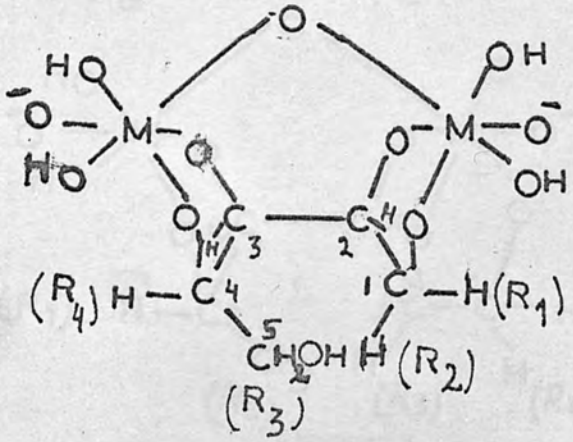
(XXVII)

D-arabinitol 0.60 1.24 1.03

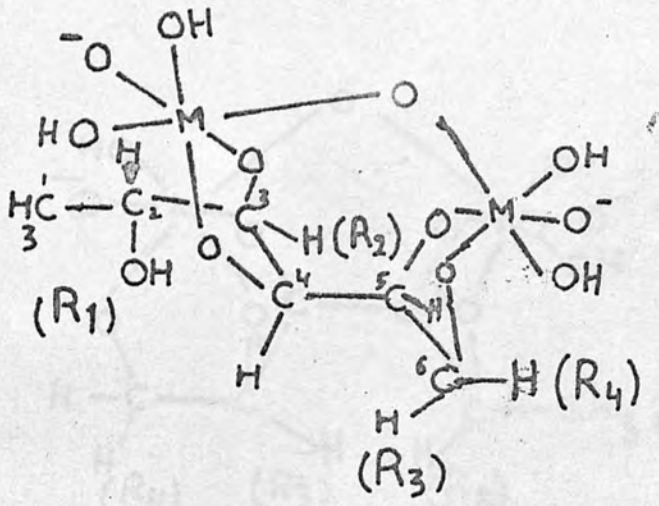


(XXIX)

Ribitol 0.73 1.26 1.04

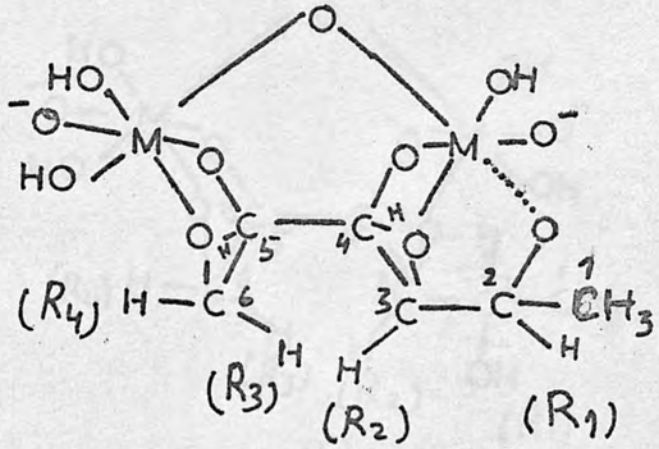


1-Deoxy-L-gulitol	0.32	1.66	0.98



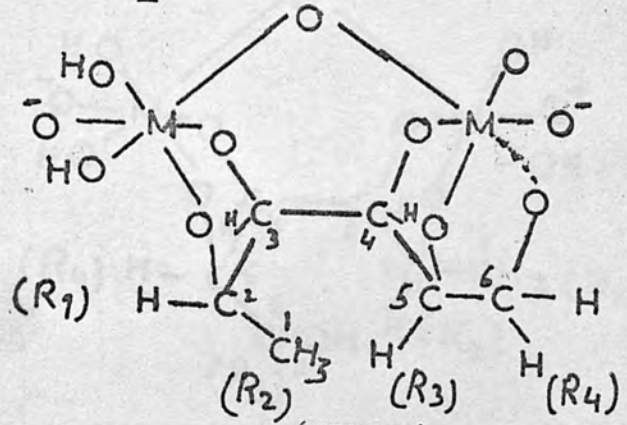
(XXXII)

1-Deoxy-D-glucitol	0.47	1.5	0.98



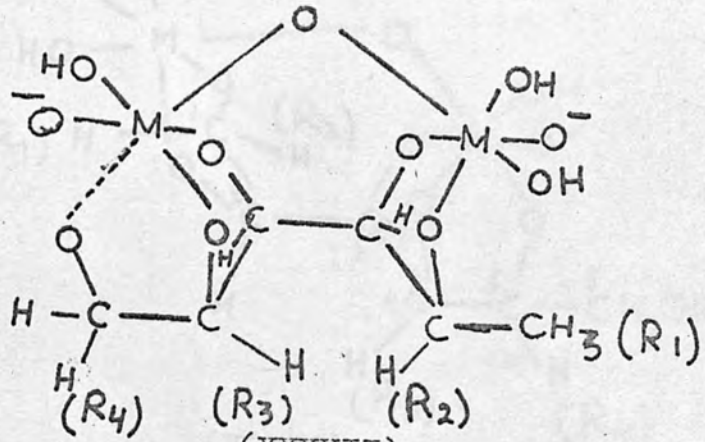
(XXXIV)

1-Deoxy-D-talitol	0.74	1.64	0.94[e]



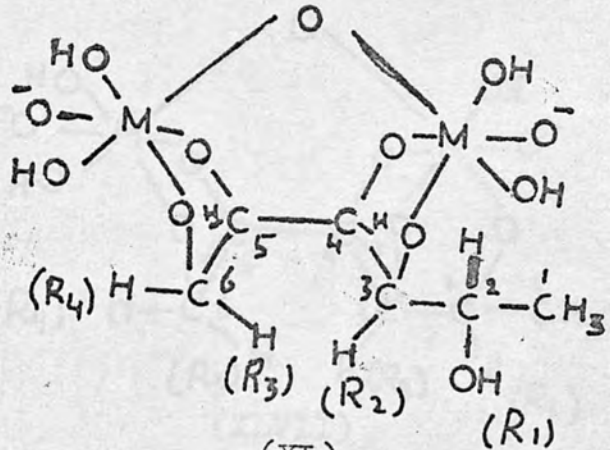
(XXXVI)

1-Deoxy-L-galactitol 0.89 1.64 1.03



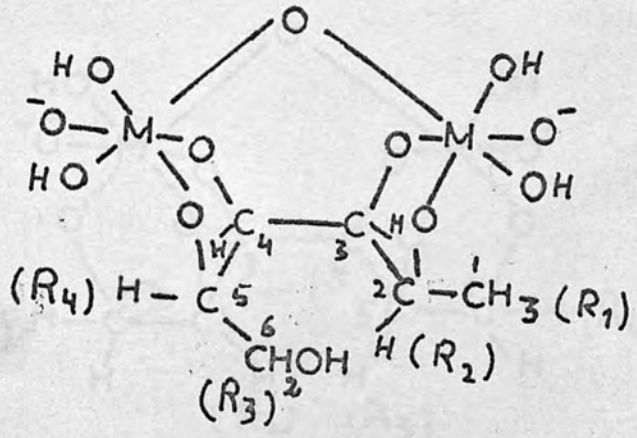
(XXXVII)

1-Deoxy-D-mannitol 0.94 1.72 1.00



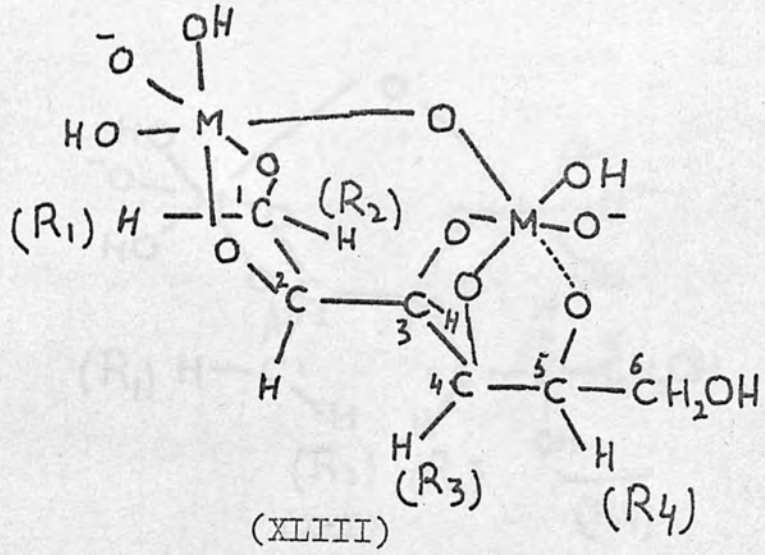
(XL)

1-Deoxy-D-altritol 1.3 1.64 1.00



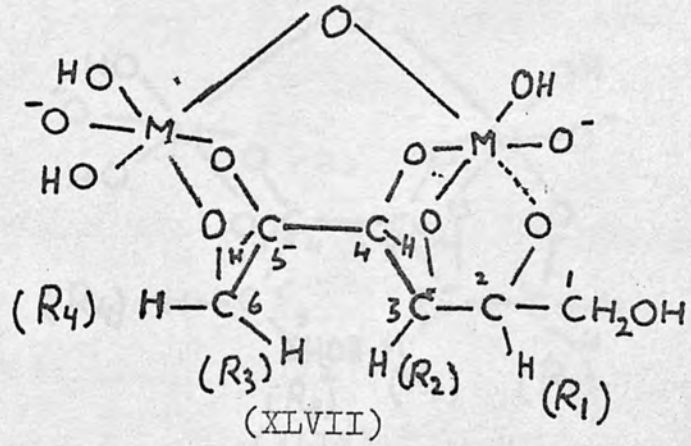
(XLI)

L-Iditol 0.13 1.00 1.00



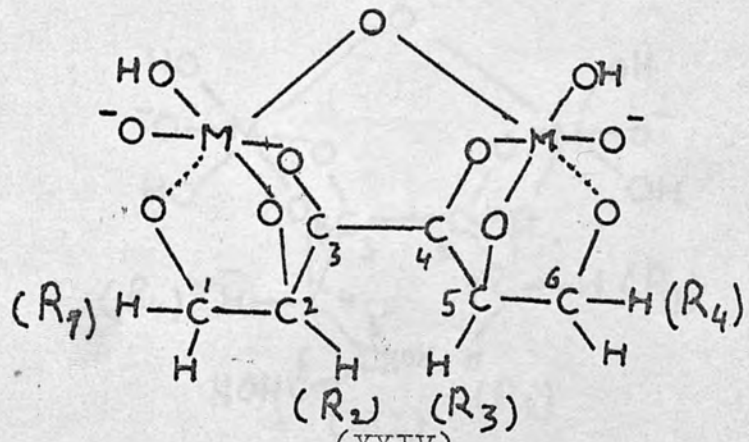
(XLIII)

D-Glucitol 0.15 1.01 1.00



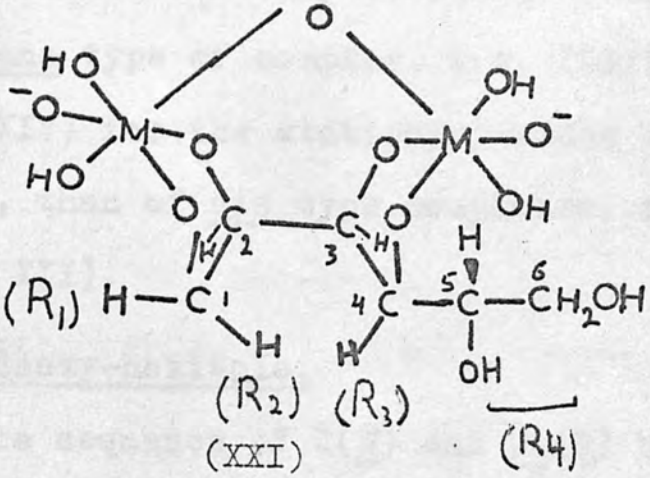
(XLVII)

Galactitol 0.41 1.04 1.00



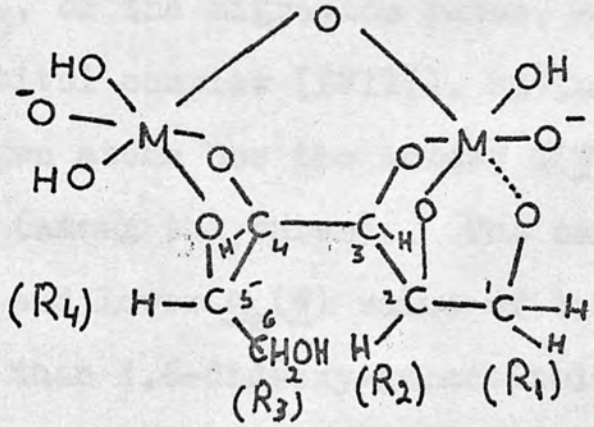
(XXIV)

D-Mannitol 0.49 1.12 1.00



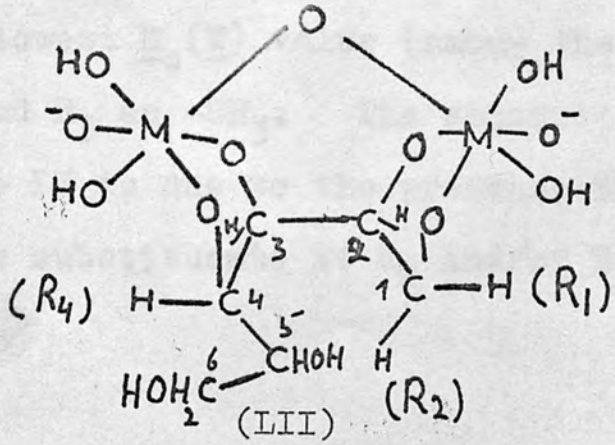
(XXI)

D-Altritol 0.46 1.03 0.97[e]



(XLIX)

D-Alitol 0.46 1.03 0.97



(LII)

It is therefore, reasonable to assume that the comparatively lower Q(W) values of LIII than LIV and LV than LVI could only be due to greater affinity of the trans type of complex, i.e. (LIII = XIII) and (LV = XIV) for the stationary water phase of the solvent system, than of cis type complexes, i.e. (LIV = XI) and (LVI = XII).

1,6-Dideoxy-hexitols.

The sequence of Q(W) and M_s(W) values of 1,6-dideoxy-hexitols clearly show the effect of substituents at R_2 and R_3, on the migration rates, e.g. the 1,6-dideoxy-galactitol complex (LVIII), having both R_2 and R_3 as hydrogen atoms has the lowest Q(W) and the highest M_s(W) value (among the three). The comparatively higher Q(W) value and lower M_s(W) value of 1,6-dideoxy-D-altritol (LIX) than 1,6-dideoxy-galactitol (LVIII) is clearly due to the $-CH_3$ group at R_3. Similarly the 1,6-dideoxy-L-mannitol complex (LX) which has the highest Q(W) value and lowest M_s(W) value (among the group), possesses both R_2 and R_3 as $-CH_3$. The sequence of Q(W) values of LVIII > LIX > LX is due to the presence of none, one and two large substituents at R_2 and/or R_3 respectively.

Pentitols.

The sequence of Q(W) values of the three pentitols is D-ribitol > D-arabinitol > xylitol. The $-C_{(5)}H_2OH$ of the xylitol-complex (XXVII) and the D-arabinitol complex (XXIX) form tridentates. Whereas ribitol-complex (XXV) forms no such tridentate, it also possesses a large group, i.e. $C_{(5)}$ at R_3 and hence is the most unstable among the pentitol complexes. The difference in the Q(W) values of xylitol and D-arabinitol which possesses identical stability factors in their complexes XXVII and XXIX, here again shows the different affinity of trans-type complex XXVII (=XIII) for the stationary phase of the solvent system than that of cis type complex XXIX (=XII).

1-Deoxy-hexitols.

The selected complexes of 1-deoxy-D-altritol (XLI), 1-deoxy-D-mannitol (XL) and 1-deoxy-L-galactitol (XXXVII) are of the cis type (i.e. XII). The ~~higher~~ Q(W) values of XXXVII and XL show the comparative stability (i.e. low Q(W) of XXXVII, which clearly indicate that a tridentate has formed. The 1-deoxy-D-talitol complex (XXXVI) can give a tridentate like 1-deoxy-L-galactitol complex (XXXVII). It has a $-CH_3$ group at R_2, therefore the expected Q(W) value would be higher than that of XXXVII but its Q(W) value was in fact less than the Q(W) value

of XXXVII, showing the exceptional behaviour of 1-deoxy-D-talitol complex (XXXVI). The difference in the Q(W) values of the complexes of 1-deoxy-D-glucitol (XXXIV) and 1-deoxy-L-galactitol (XXXVI) shows that the stability effect due to one -CHOH (as in XXXIV) is greater than that due to $-CH_2OH$ (as in XXXVII).

Hexitols.

In common with all the polyols discussed above, the sequence of Q(W) values of the six hexitols clearly shows the effect of the following: (i) sizes of R_2 and/or R_3; (ii) tridentate formed due to $-CH_2OH$ or -CHOH and also (iii) the type of structure (i.e. XI, XII or XIII, XIV), on the migration rate and hence the stability of the complex, e.g.

The D-allitol complex (LII) which has exhibited the highest Q(W) value (among the six hexitols), has $C_{(5)}$-$C_{(6)}$ at R_3 and possesses no other stability factors. The comparatively higher stability of the D-altritol complex (XLIX) than LII is in agreement with the fact that it has only one carbon atom at R_3 and that a tridentate is formed with $-C_{(1)}H_2OH$. The galactitol complex (XXIV) and D-mannitol complex (XXI) are of the same type and stability (see p. 48). The comparative low Q(W) of (XXIV) clearly shows that two

tridentates have formed (2p), while there is no possibility of a tridentate formation in (XXI). Out of the two remaining possibilities of the comparative stable complexes of D-glucitol, i.e. XLV and XLVII (see p. 58), both have identical substituents at R_2 and R_3, i.e. hydrogen atoms except with the difference that, these are of the trans type (XLV = XIII) and cis type (XLVII = XII) and that XLVII has formed a tridentate with a -CHOH(1a), thus the most stable structure of D-glucitol complex is XLVII. In XXIV two tridentates are formed due to 2p, the low Q(W) value of XLVII where one tridentate is formed due to one -CHOH(1a) show that, one -CHOH(1a) has greater effect towards the stability than two tridentates formed due to two $-CH_2OH$(2p), thus the most stable complex of L-iditol, out of XLIV and XLIII, which which can give tridentates with two $-CH_2OH$(2p) and one -CHOH respectively, will be XLIII. Comparison of the L-iditol complex (XLIII) with that of D-glucitol (XLVII) shows that both have identical stability factors, i.e. $R_1 = R_2 = R_3 = H$, $R_4 = CH(OH).CH_2OH$ and a tridentate formed due to a -CHOH, except with the difference that XLIII is a trans type complex (XLII) and XLVII is a cis type complex (XII), which explains the lower Q(W) of XLIII

than XLVII. It is therefore predicted that partial periodate oxidation of the L-iditol complex will yield D-xylose as a major component. An objection could be raised at this point that since a tridentate is formed in XLIII and also in XLVII, there should be no effect of periodate. The tridentate formation takes place like ester formation, by elimination of water and is an equilibrium reaction. The reaction with periodate will shift the equilibrium, i.e. breakage of the tridentate formed and hence the cleavage of -CHOH - CH_2OH bond.

Conclusion.

From the above results it is concluded that, the wide range of Q(W) values (ca. 0.1-1.0) exhibited by the polyols forming complexes of the types XI-XIV, has indeed resolved all the polyols of group a. Thus, this method i.e. the determination of Q(W) values, is an important analytic tool. This can also be used for the separation of polyols on a preparative scale (see below).

ii) Chromatography of Polyols on Wholly Impregnated Cellulose Powder.

Separation of monosaccharides on a cellulose column was first described by Hough et.al.[32] in 1948. Cellulose

columns have essentially the same general behaviour as paper strips. Therefore it was considered that if a column is prepared like that of tungstate impregnated paper (Expt. 23(b)), it may be useful for the macro separation of polyols.

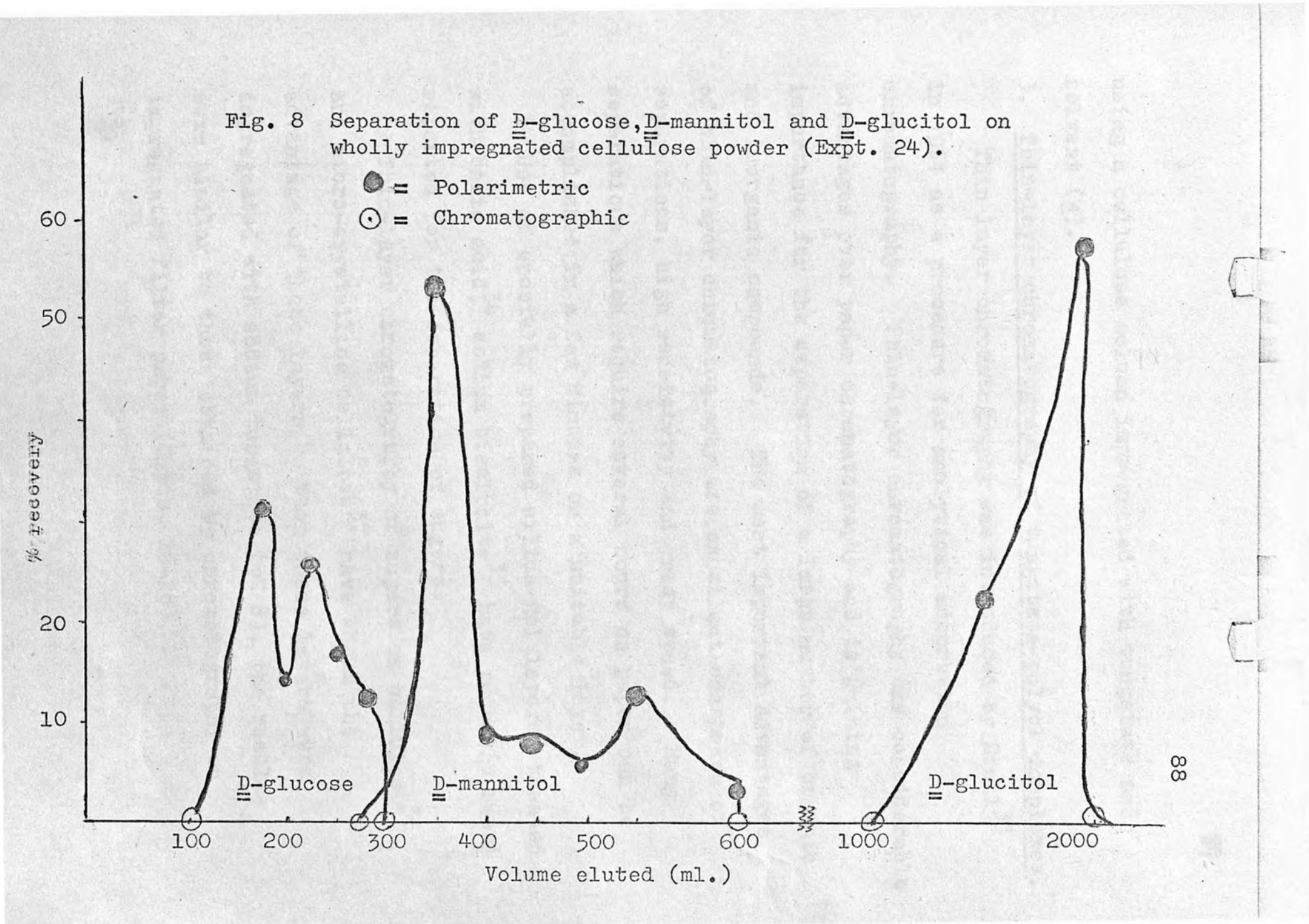
Separation of D-mannitol, D-glucitol and D-glucose.

A mixture of D-glucose, D-mannitol and D-glucitol has been separated on cellulose powder impregnated with sodium tungstate (Expts. 24 and 25). Fig.8 shows their rate of elution. It was observed that all the three polyols separated on the above mentioned column contained some eluted tungstate. The polyols were isolated in pure state by passing through ultrasorb/120/240 (Expt. 25).

Preparative semi micro separation of D-glucose from D-galactose.

It can be seen from Tables 6 and 7 that the difference in migration rates of D-glucose and D-galactose, i.e. R_G(W) 1 and 0.66 respectively, is reasonable enough to give complete resolution of the two isomers. It has already been shown (Expts. 24 and 25) that a cellulose column impregnated with tungstate has all the properties of the tungstate impregnated paper. Therefore Worth and Pridham have recently performed the successful resolution of D-glucose and D-galactose, obtained from a tobacco pectin hydrolysate,

Fig. 8 Separation of D-glucose, D-mannitol and D-glucitol on wholly impregnated cellulose powder (Expt. 24).



using a cellulose column impregnated with tungstate and solvent (e).

3. Thin-layer chromatography of tungstate-polyol complexes.

Thin-layer chromatography was introduced by Stahl[33] in 1961 as a procedure for analytical adsorbtion chromatography. Thin-layer chromatography has considerable advantages over paper chromatography and is gaining importance for the separation of a large number of organic and inorganic compounds. The most important advantages of thin-layer chromatography are, excellent sharpness of separations, high sensitivity and great speed. Many separations which require several hours on paper can be accomplished in a few minutes on a suitable layer.

Use of specially prepared silica-gel layers, treated with boric acid,[34] sodium bisulphite[35] have recently been reported for the separation of sugars.

Thin-layer chromatography of sugars on cellulose[36] and micro-crystalline cellulose[37] have shown the advantage of these layers. When these layers were impregnated with sodium tungstate (pH 8), the results were similar to those obtained by chromatography on impregnated filter paper (Expts. 26-28).

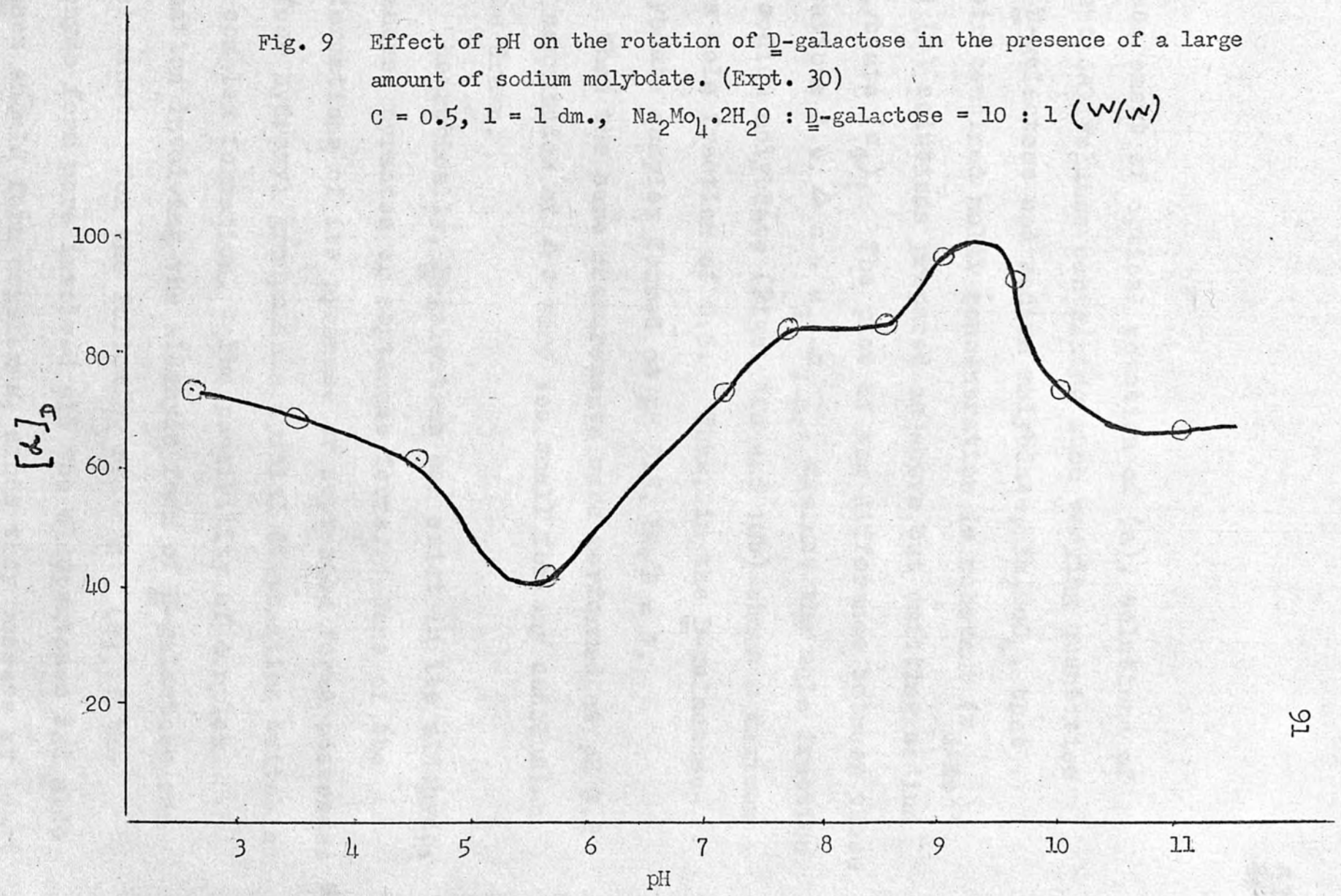
4. Complex formation of D-galactose with molybdate and tungstate.

D-Galactose has been reported by two laboratories[13,38] not to complex with molybdate. Since the above results (p.26) are in apparent conflict with these reports, it was necessary to investigate further the effect of molybdate on D-galactose.

In the presence of an equimolar quantity of molybdate there was no appreciable change in the rotation of D-galactose at pH 5, but on addition of excess (~~mol.~~ ratio 10:1) of molybdate (Expt. 29, Table 31), a considerable decrease in its specific rotation was observed. This indicated that complexing has occurred. Study of the effect of pH on the rotation of D-galactose in the presence of excess (10:1 mol.) sodium molybdate (Expt. 30, Fig.9) revealed that there is a steady fall and rise in the rotation of D-galactose on the addition of acid or alkali respectively. A minimum specific rotation (+ 39.5°) was recorded at pH 5.5 and a maximum (+ 99.0°) at pH 9.2, while the specific rotation of D-galactose (only) was unaffected by acid or alkali.

The composition of the D-galactose-molybdate complex formed at pH 5.5 was determined by Job's[39] "continuous variation" method.39 (Expt. 31). This consists of

Fig. 9 Effect of pH on the rotation of D-galactose in the presence of a large amount of sodium molybdate. (Expt. 30)
C = 0.5, l = 1 dm., $Na_2Mo_4.2H_2O$: D-galactose = 10 : 1 (w/w)



measurement of optical rotation of (a), solutions of identical volumes containing such varying quantities of D-galactose and sodium molybdate, Na_2MoO_4, that their combined molar concentration is constant (a_{G-Mo}), and (b) solutions prepared as above but omitting sodium molybdate (a_G). The plot of the difference between these rotations i.e. $\Delta a = a_G - a_{G-Mo}$, against the mole fraction of sodium molybdate (Figs. 10a and 10b) shows a maximum at a mole fraction of 0.5. Thus, in the D-galactose-molybdate complex formed at pH 5.5, Mo/P = 1.

When the same measurements were performed at pH 9.2 the magnitudes of Δa were too small for any conclusion to be drawn.

Theoretically, D-galactose can exist in its aldehydo, furanose, pyranose or septanose forms. None of the conformations of its pyranose or septanose forms possesses three or four hydroxyl groups in a spatial disposition suitable for complex formation. The possibility of complex formation involving the aldehydo form of D-galactose can be eliminated for the following reasons: (a) if the aldehydo form were involved all the aldopentoses and aldohexoses should form complexes, since they possess at least

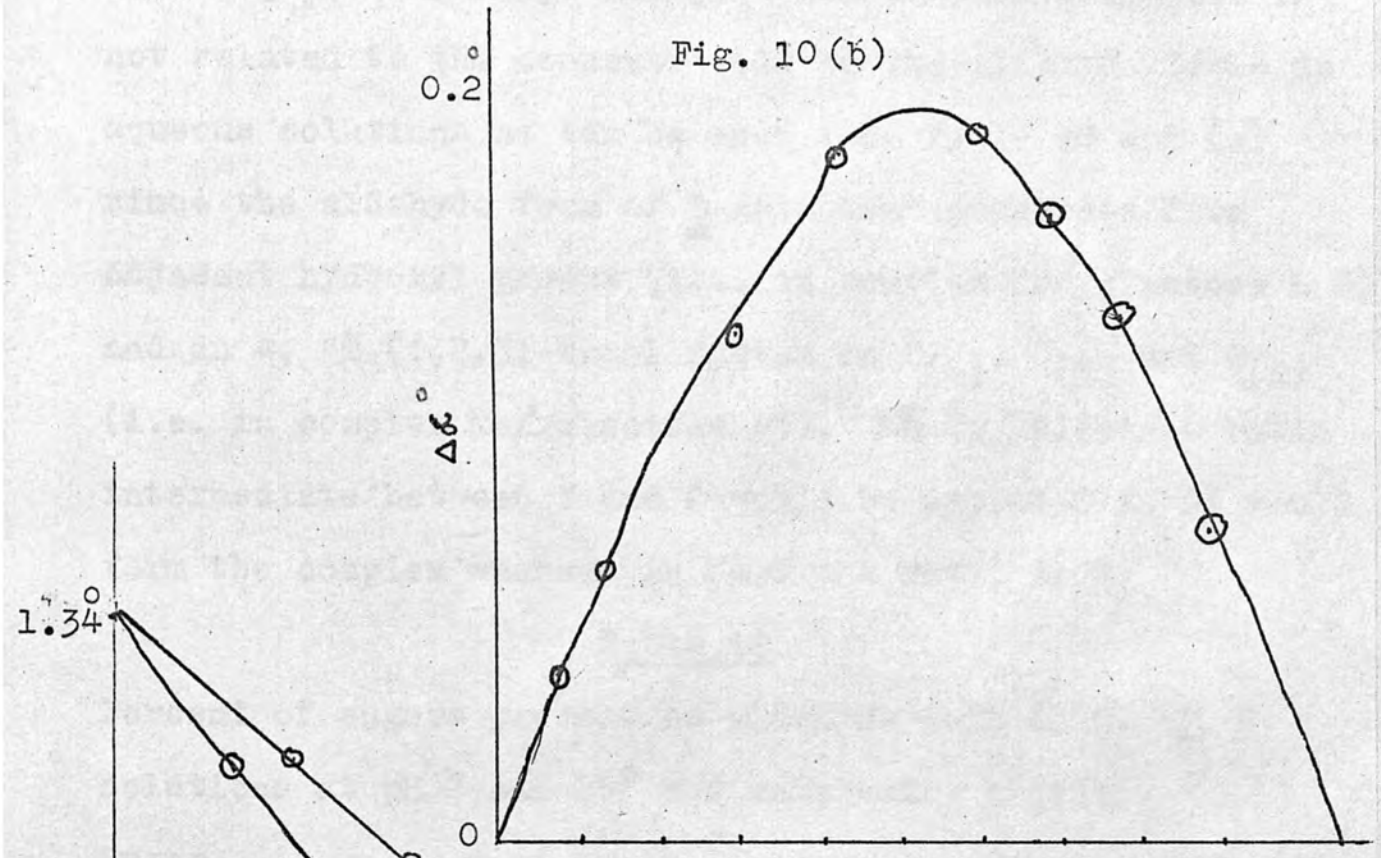


Fig. 10 (b)

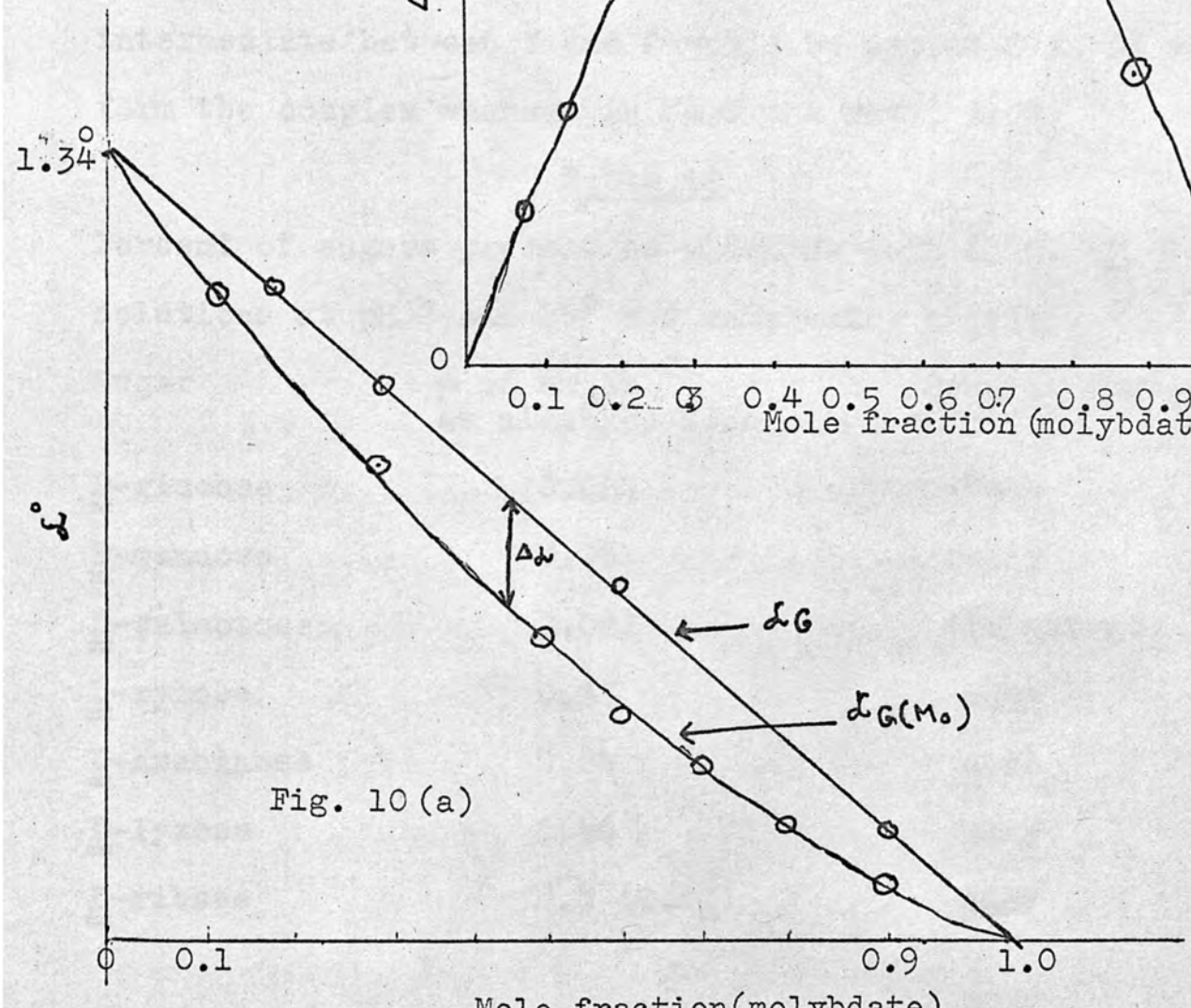


Fig. 10 (a)

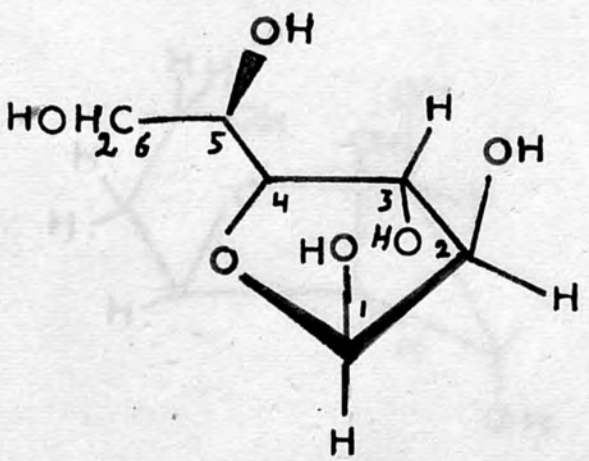
four adjacent hydroxyl groups; (b) complex formation is not related to the concentration of the aldehydo forms in aqueous solutions as can be seen from Table 10 and (c) since the aldehydo form of D-galactose possesses five adjacent hydroxyl groups (i.e. in complex Mo/galactose = 2) and an α, αT (1,2,3)-triol system on $C_{(4)}$, $C_{(5)}$ and $C_{(6)}$ (i.e. in complex Mo/galactose =1). The Mo/galactose ratio intermediate between 1 and 2 would be expected if it would form the complex whereas in fact the ratio is 1.

Table 10

Percent of sugars present as aldehydo form in 0.25M solutions at pH 7 and 25° and complexing ability.

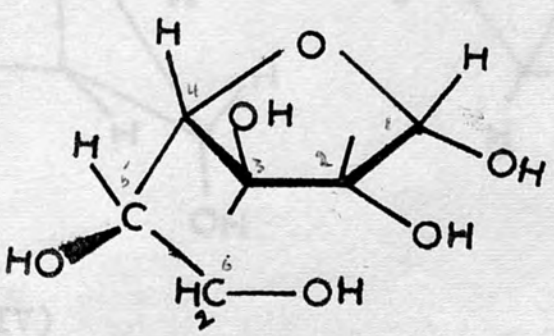
Sugar	% of sugar[40] as aldehydo form	Complex formation
D-glucose	0.024	None
D-mannose	0.064	easy
D-galactose	0.082	reluctant
D-xylose	0.17	none
D-arabinose	0.24	none
D-lyxose	0.40	easy
D-ribose	8.5 (0.1M)	easy

On the other hand, the hydroxyl groups on $C_{(1)}$, $C_{(2)}$ and $C_{(5)}$ of α-D-galactofuranose (LXI), are in a



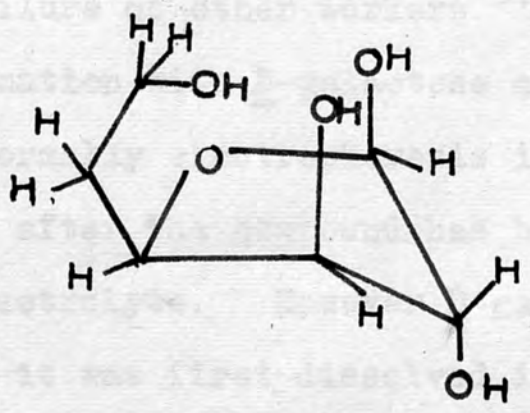
(LXI)

spatial disposition closely resembling that of (IV) when $C_{(3)}$, $C_{(4)}$, $C_{(5)}$ and $C_{(6)}$ are in a planar zig-zag conformation, alternatively rotation around the bond between $C_{(4)}$ and $C_{(5)}$ would bring the hydroxyl group on $C_{(1)}$, $C_{(2)}$ and $C_{(6)}$ into a spatial disposition as shown in LXII.



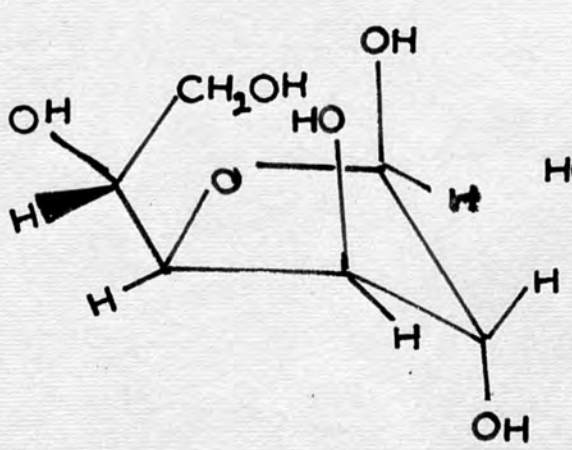
(LXII)

5-Deoxy-β-D-xylohexofuranose (5-Deoxy-β-D-gluco-furanose) which cannot exist in a pyranose form complexes with molybdate and tungstate,[15] and a conformation such as (LXIII) has been suggested as that involved in complex

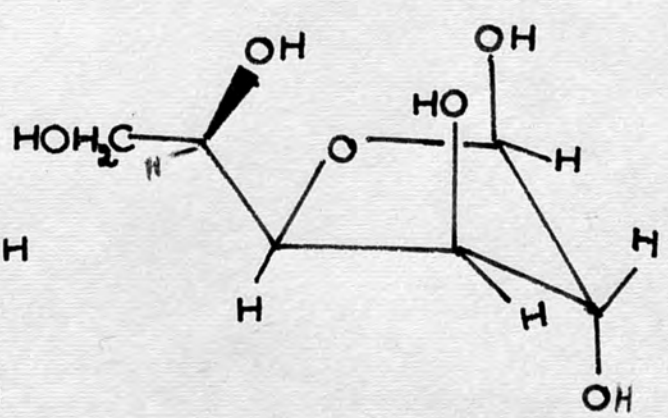


(LXIII)

formation. Thus, the β-isomer of D-glucofuranose (LXIV or LXV) might have been expected to complex with molybdate or tungstate, but no evidence for this has been obtained.



(LXIV)



(LXV)

It is possible that D-glucose adopts the furanose form

even more reluctantly than does D-galactose because $C_{(5)}$ and the hydroxyl group on $C_{(3)}$ are cis related and are thus in close proximity, whereas the same groups of D-galactofuranose are trans related.

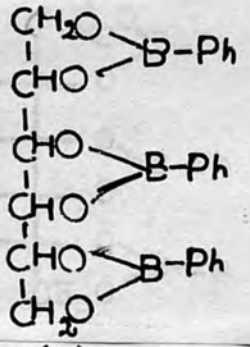
The failure of other workers[13,15,38] to detect complex formation with D-galactose may be due to a time factor. Normally electrophoresis is carried out immediately after the compound has become in contact with the electrolyte. However D-galactose had M_S(Mo) = 0-1.1, when it was first dissolved in molybdate solution (pH 8) and the solution allowed to stand for 18 hrs. before electrophoresis was carried out.

Part II

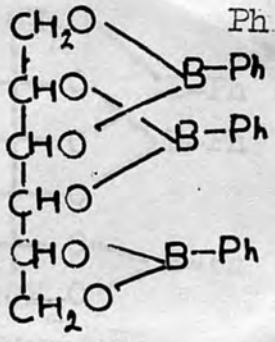
Phenylboronates of some Pentitols

A. INTRODUCTION

Phenylboronic acid $C_6H_5B(OH)_2$ forms cyclic esters with polyhydroxy compounds. It can be removed from the polyhydroxy compounds under very mild conditions and hence is an attractive protecting agent. In 1954 Kuivila[44] and collaborators prepared phenylboronic esters of some diols and polyols. The phenylboronates of D-mannitol and D-glucitol prepared by them were found to be trisphenylboronates. They suggested structures (I or II) for the D-mannitol trisphenylboronate, without taking the spatial configuration of D-mannitol into consideration.



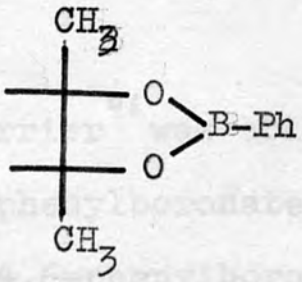
(I)



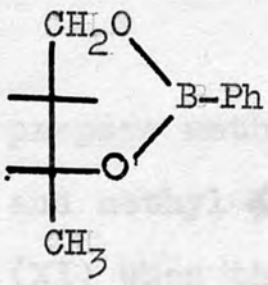
(II)

Ph = C_6H_5-

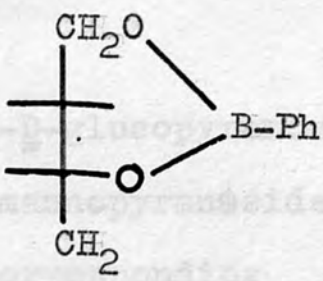
Sugihara and Bowman[45] reported the preparation of cyclic phenylboronates of galactitol, D-glucitol and D-mannitol $C_6H_8O_4(C_6H_5B)_3$ but made no attempt to elucidate the structure of these compounds. Among the other phenylboronates prepared by them were those of cis butane 2,3-diol(III), butane 1,3-diol(IV) and butane 1,4-diol(V).



(III)

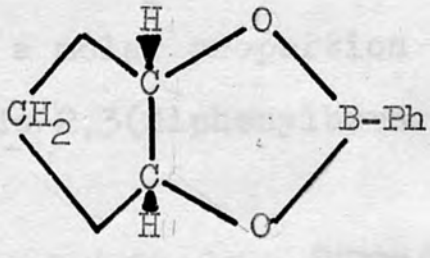


(IV)

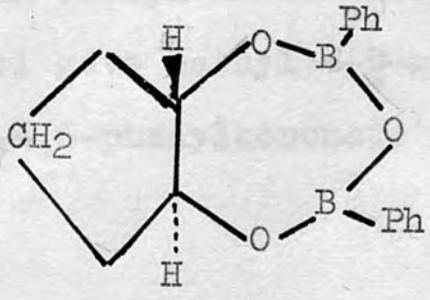


(V)

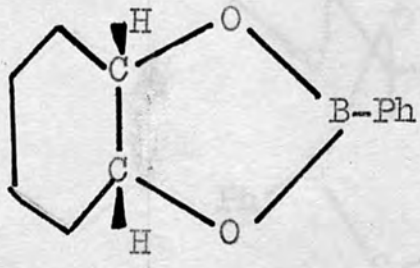
Cyclic phenylboronates are formed with cis and trans cyclopentane 1,2-diol. It has been found that the phenylboronate formed with cyclopentane cis-1,2-diol is a five-membered ring (VI), whereas that formed with the corresponding trans diol is a seven-membered ring (VII). Similarly cyclic phenylboronates of cis and trans-cyclohexane-1,2-diol are found to be composed of five- (VIII) and seven membered rings (IX) respectively.



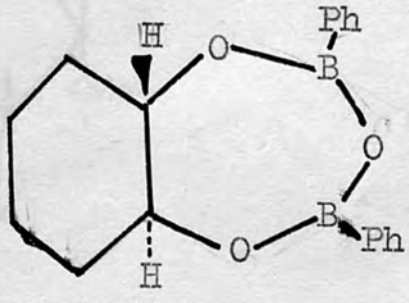
(VI)



(VII)

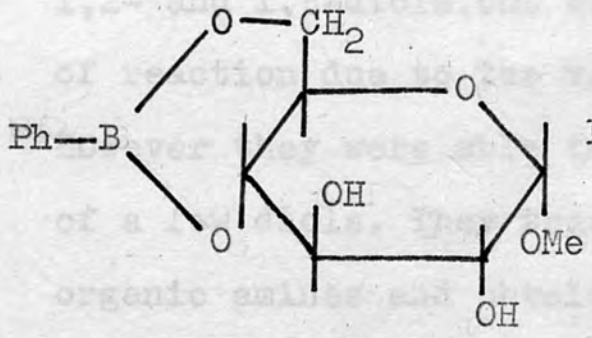


(VIII)

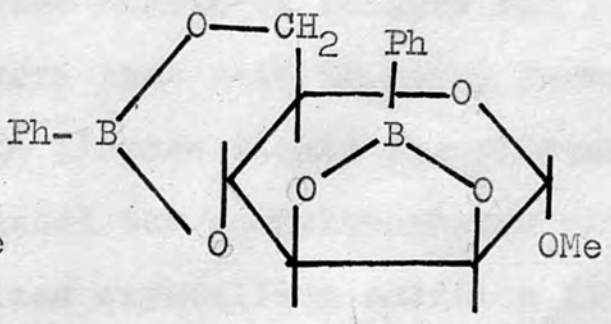


(IX)

Ferrier[46] was able to prepare methy α-D-glucopyranoside -4,6-phenylboronate (X) and methyl α-D-mannopyranoside -2,3:4,6-phenylboronate (XI) when the corresponding glucosides were mixed in the molar proportion of 1 and 2, respectively.

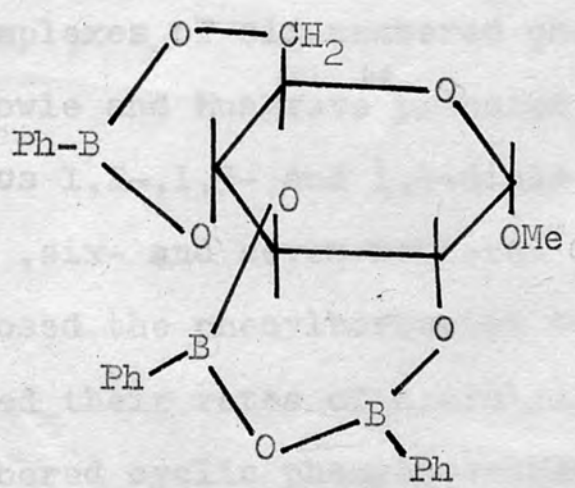


(X)



(XI)

The addition of 2 mol. of phenylboronic acid to methyl α-D-glucoside gave an amorphous mixture. Mixing in a molar proportion of 3:1 gave methyl α-D-glucopyranoside-2,3(diphenylboronate),4,6-phenylboronate (XII).



(XII)

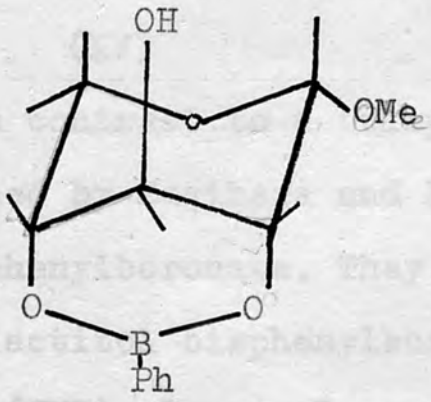
Wolfrom and Solms[47] have prepared crystalline bis-phenylboronates from the pentoses and 6-deoxy-hexoses but gave no indication of the detailed structure of the derived compounds.

Finch and Lockhart[48] attempted to study the rate of formation of five- and six-membered phenylboronates of some 1,2- and 1,3-diols, but were unable to measure the rate of reaction due to the very fast rate of ester formation. However they were able to prepare liquid phenylboronates of a few diols. They treated the phenylboronates with organic amines and obtained crystalline addition (1:1) complexes. They found that the amino complexes of five-membered cyclic phenylboronates are formed at room temperature while six-membered cyclic phenylboronates gave the complex on cooling to -80°. They have also shown that the amino complexes of five-membered phenylboronates are comparatively stable and less hygroscopic than the amino complexes of six-membered phenylboronates.

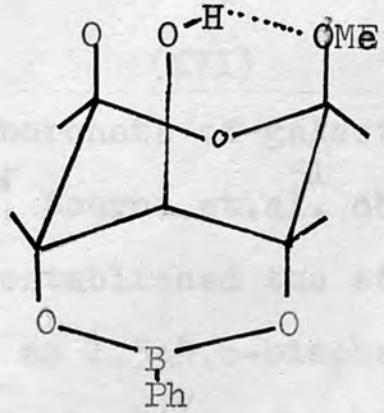
Bowie and Musgrave[49] prepared cyclic phenylboronates of various 1,2-, 1,3- and 1,4-diols which were composed of five-, six- and seven-membered rings respectively. They exposed the phenylboronates to moist air and determined their rates of hydrolysis. They observed that five-membered cyclic phenyboronates possess considerable

ring strain and are hydrolysed rapidly, six-membered cyclic phenylboronates are free from ring strain and therefore, take up water slowly. In the case of seven -membered cyclic phenylboronates, the ring is much more flexible than the corresponding six-membered rings. The bond interaction is weaker and there is little obstruction to hydrolysis.

Recently Ferrier et. al.[50] prepared phenylboronates of α-D-xylopyranoside (XIII) and β-D-xylopyranoside (XIV)



(XIII)

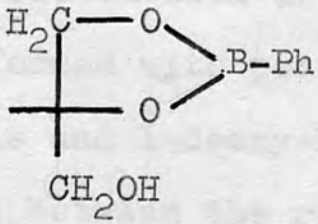


(XIV)

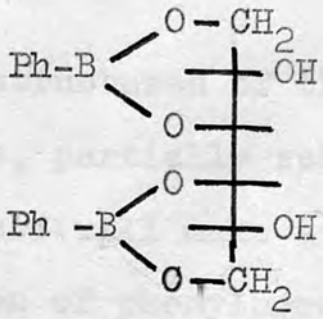
Infrared studies of (XIII and XIV) have shown that XIII was free from hydrogen bonding, while XIV gave an absorption peak at 3623 $cm.^{-1}$ showing that the axial oxygen atom at $C_{(1)}$ was intramolecularly hydrogen bonded to the axial oxygen at $C_{(3)}$.

Investigation of the detailed structure of phenyl-boronates formed with acyclic polyols was not attempted

until recently. Bourne,Lees and Weigel[51] prepared phenylboronates of glycerol,erythritol,D-arabinitol and a few hexitols by Kuivila's[44] method. They also prepared phenylboronates of some partially substituted hexitols by refluxing the reactants in acetone. The product obtained from glycerol was found to be the 1,2-phenylboronic ester (XV).



(XV)



(XVI)

In contrast to a trisphenylboronate of galactitol obtained by Sugihara and Bowman[45], Bourne,et.al.[51] obtained a bisphenylboronate. They have established the structure of galactitol bisphenylboronate as 1,3:4,6-bisphenylboronate (XVI). Bourne,Lees and Weigel also prepared D-mannitol trisphenylboronate and D-glucose bisphenylboronate,but have given no suggestion about the structure of these compounds.

It has been mentioned above that phenylboronic acid reacts with polyhydroxy compounds to form five- ,six- and in some cases seven-membered cyclic esters. Therefore investigations must be carried out to ascertain the type

of diol grouping prefferred for the formation of cyclic phenylboronic esters. Since monophenylboronates could not be obtained from hexitols, the polyols possessing five hydroxyl groups, e.g. pentitols and 1-deoxy-hexitols are suitable for this purpose. At least one hydroxyl group of such polyols will remain unsubstituted in the phenylboronates.

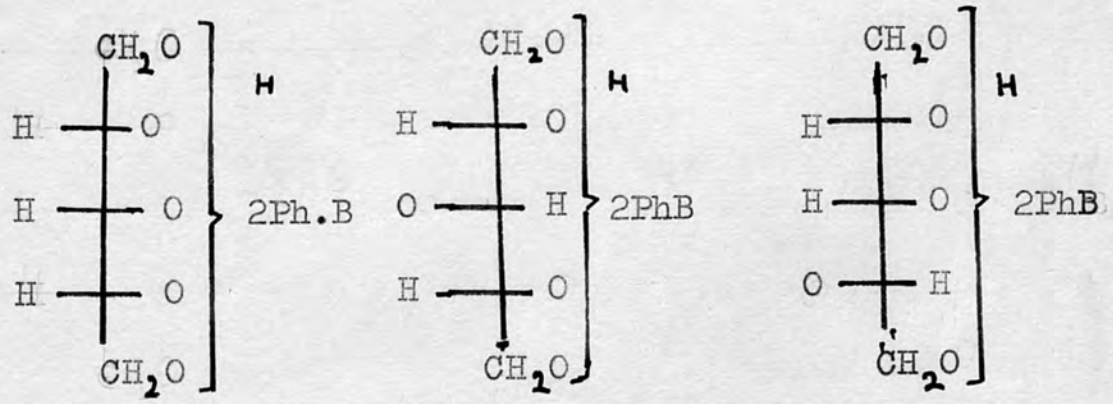
The elucidation of the structures of the phenylboronates formed with pentitols, partially substituted pentitols and 1-deoxy-hexitols will make a comparison possible between the reaction of phenylboronic acid with polyols and the condensation products of aldehydes and ketones formed with polyols.

B. DISCUSSION

1. Preparation of Bisphenylboronates of some Acyclic Pentitols

i) From ribitol, xylitol and L-lyxitol

When aqueous solution of ribitol, xylitol or L-lyxitol were mixed with methanolic solutions of phenylboronic acid, white precipitates were obtained (Epts. 33-35). The precipitates gave crystalline products (A, B and C) on recrystallization. In each case analysis figures were correct for $C_{17}H_{18}B_2O_5$, i.e. a bisphenylboronate.

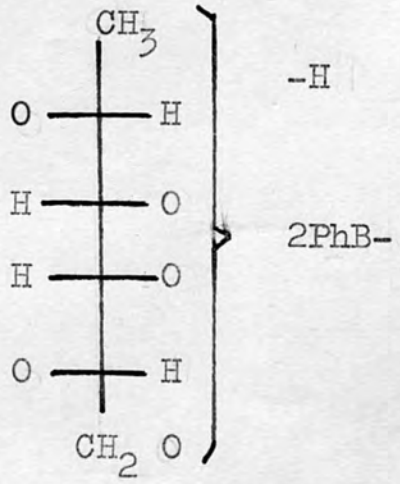


A B C

The infrared spectrum of A, B and C when examined as a mull with nujol exhibited an absorption peak at 3400 $cm.^{-1}$ indicating the presence of hydroxyl group, and a peak at 1600 $cm.^{-1}$ for phenyl group.

ii) From 1-deoxy-L-galactitol

When an aqueous solution of 1-deoxy-L-galactitol was mixed with a methanolic solution of phenylboronic acid (in 1:2 molar ratio), a white precipitate was obtained, which gave a crystalline solid on recrystallization (Expt. 43β). It had m.p. 113-114° with analysis figures correct for $C_{18}H_{20}B_2O_5$,i.e. 1-deoxy -L-galactitol bisphenylboronate D.



D

The infrared spectrum of the compound D, when examined in the form of mull with nujol exhibited absorption at 3400 cm^{-1} (hydroxyl group) and at 1600 cm^{-1} (phenyl group).

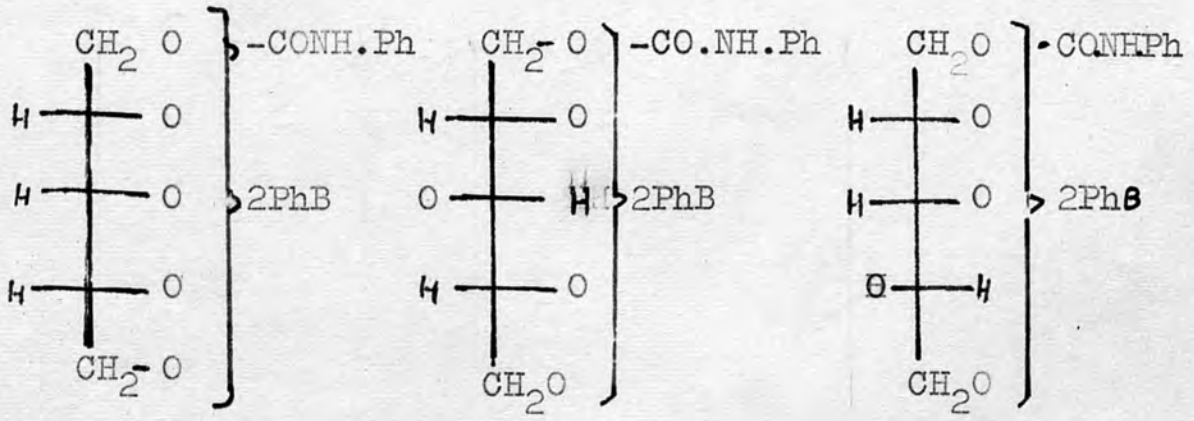
2. Location of Unsubstituted Hydroxyl group in Bisphenylboronates of some pentitols

a) Periodate oxidation of phenylcarbamates derived from bisphenylboronates of (i) ribitol, xylitol and L-lyxitol

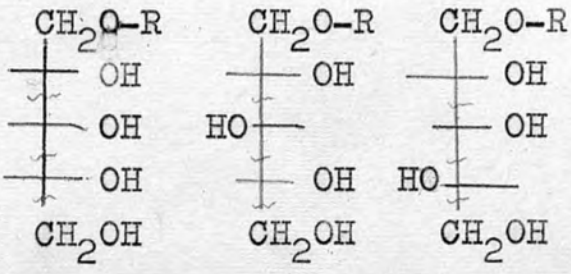
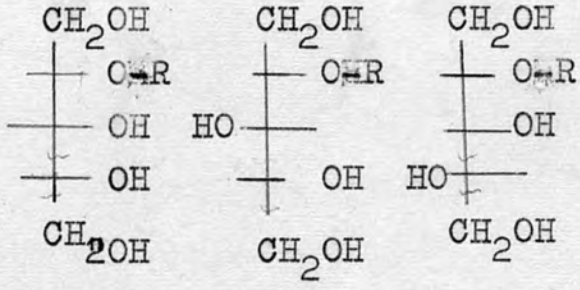
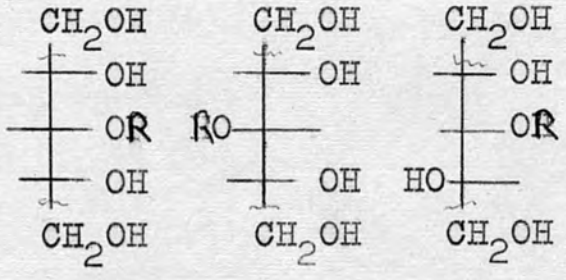
It was conceived that the free hydroxyl group in the phenylboronates of pentitols could be located by substituting its hydrogen by a phenylcarbamoyl group, followed by the removal of the phenylboronate group and subsequent periodate oxidation of the mono-O-phenylcarbamates thus obtained. Accordingly a portion of each of the three phenylcarbamates A, B and C was refluxed in benzene with phenyl isocyanate (Expts. 37, 38 and 36). The derived crystalline solids F, G and H gave analysis figures correct for $C_{24}H_{23}B_2NO_6$, i.e. mono-O-phenylcarbamoyl pentitol bisphenylboronate. The mono-O-phenylcarbamoyl ribitol bisphenylboronate F, mono-O-phenylcarbamoyl xylitol bisphenylboronate G, and mono-O-phenylcarbamoyl-L-lyxitol bisphenylboronate H, showed no absorption in the hydroxyl region of their infrared spectrum showing the absence of a free hydroxyl group.

It is known that when phenylboronates are dissolved in aqueous organic solvents, hydrolysis of

the cyclic ester occurs, leaving in solution the polyol and phenylboronic acid.[51] It was decided to oxidize the compounds F, G and H without removal of phenylboronic acid. The oxidations were carried out in 25% aqueous



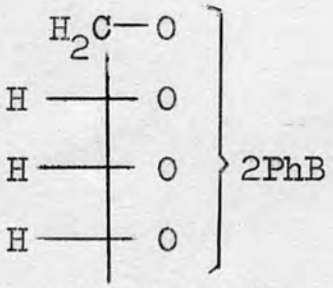
dioxane (Expt. 49(a)). The periodate uptake was estimated by the arsenite method[76] and the formaldehyde produced by chromotropic acid method.[77] Page 110 show the expected amount of periodate consumed by pentitol phenylcarbamates and the formaldehyde liberated. The mono-O-phenylcarbamoyl ribitol bisphenylboronate F consumed 4.47 mol. (Expt. 49(a)), of periodate over a period of 24 hours. This higher consumption of periodate by the compound F made it necessary to find out the reason for this over-consumption. Consequently phenylboronic anhydride and ribitol bisphenylboronate A, were subjected to periodate oxidation under the

Structure	Mol. IO_4^- consumed	Mol. HCO_2H	Mol. HCHO
F or G or H			
	3	2	1
	2	1	1
	2	0	2

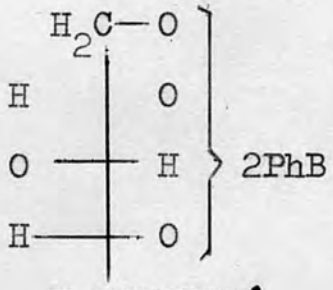
R = -CONH.Ph

identical conditions. Phenylboronic anhydride consumed 0.22 mole of periodate per mole of phenylboronic acid and ribitol bisphenylboronate consumed 4.8 mol. of periodate. However, when periodate oxidation was ~~periodate oxidation was~~ carried out in the presence of sodium bicarbonate (Expt. 49 (b), the mono-O-phenylcarbamoyl-xylitol-bisphenylboronate G and mono-O-phenylcarbamoyl-L-lyxitol bisphenylboronate H consumed 3.03 and 3.25 mol. periodate respectively. It was thought that the over-consumption of periodate in the case of mono-O-phenylcarbamoyl-ribitol-bisphenylboronate F and that encountered by Bourne, Lees and Weigel on the periodate oxidation of glycerol 1,2-phenylboronate[51] could be due the hydrolysis of the phenylcarbamoyl group, but a practically negligible amount of periodate was consumed by ethylcarbamate ($C_2H_5O\overset{CO}{}NH.C_6H_5$), which indicated that the phenylcarbamoyl group was not hydrolysed under these conditions. When periodate oxidation was carried out using aqueous dimethylformamide as a solvent (Expt. 49(c) instead of dioxane, the amount of periodate consumed by the mono-O-phenylcarbamoyl-xylitol-bisphenylboronate G and mono-O-phenylcabamoyl-L-lyxitol bisphenylboronate H, was 3.01 and 3.14 mol. respectively (Table 38). Clearly dioxane was responsible for the erroneous results.

The cosumption of ca. 3 mol. of periodate by the three pentitol derivatives, i.e. F, G, and H and the formation of ca. 1 mol. of formaldehyde (Table 38) revealed that these compounds, A, B and C possess four adjacent hydroxyl groups and that the phenylcarbamoyl group is at a terminal position. Thus, the phenylcarbamates F, G and H obtained via the pentitol-bisphenylboronates can be represented as 5-O-phenylcarbamoyl -DL-ribitol bisphenylboronate (XVIII), 5-O-phenylcarbamoyl -DL-xylitol bisphenylboronate (XIX) and 5-O-phenylcarbamoyl -L-lyxitol bisphenylboronate (XX a) or 1-O-phenylcarbamoyl -L-lyxitol bisphenylboronate (XXI a). For (XX a) and (XXIa) see p. 118.



(XVIII)

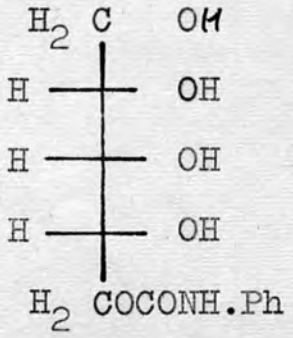


(XIX)

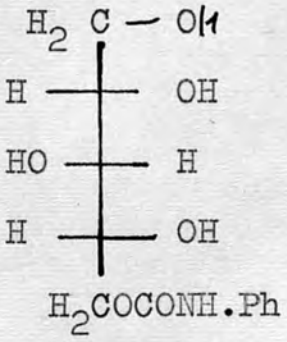
As mentioned above the phenylboronates of many polyols are easily hydrolysed even during chromatography with a water containing solvent, e.g. ethyl acetate, acetic acid, water (9:2:2). In this solvent the phenylboronic acid which can be detected under U.V. light, moves almost with

the solvent front and can be easily separated from the polyhydroxy compounds. However, this was found unsuitable for the isolation of mono-O-phenylcarbamoyl pentitols since their R_F values in several chromatographic solvent systems were close to the R_F values of phenylboronic acid. Ferrier [50] has developed a method for the removal of phenylboronate group involving a transesterification reaction. Accordingly the solutions of mono-O-phenylcarbamoyl bisphenylboronated in acetone were treated with propane-1,3-diol. The propane-1,3-diolphenylboronate produced was extracted with petroleum ether. Each pentitol gave a crystalline solid, $C_{12}H_{17}NO_6$, i.e. a mono-O-phenylcarbamate (Expts. 39,40 and 41).

Based on the evidence of periodate oxidation (Table 38) they are thus, 5-O-phenylcarbamoyl-DL-ribitol (XXII), 5-O-phenylcarbamoyl-DL-xylitol (XXIII) and 5-O-phenylcarbamoyl-L-lyxitol (XXIV) or 1-O-phenylcarbamoyl-L-lyxitol (XXV).



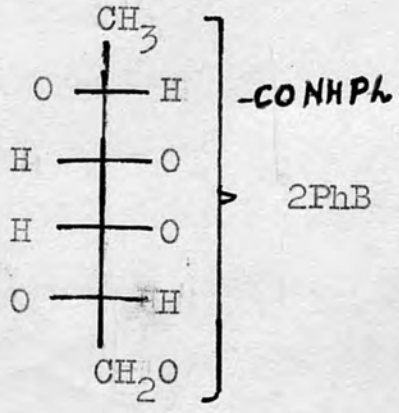
(XXII)



(XXIII)

ii) 1-deoxy-L-galactitol

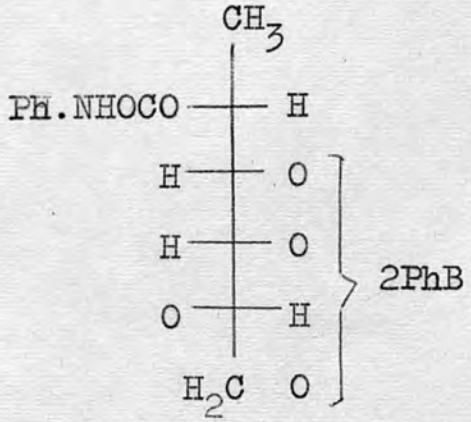
A portion of the 1-deoxy-L-galactitol bisphenylboronate D, was refluxed with phenyl isocyanate in benzene (Expt. 44) and a crystalline solid I, m.p. 135-136° was obtained. Elemental analysis gave figures ($C_{25}H_{25}B_2NO_6$), correct for mono-O-phenylcarbamoyl-1-deoxy-L-galactitol bisphenylboronate. The absence of absorption for hydroxyl group in its IR spectrum indicated the complete substitution of the free hydroxyl group.



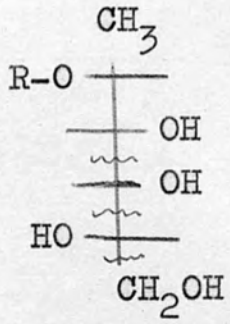
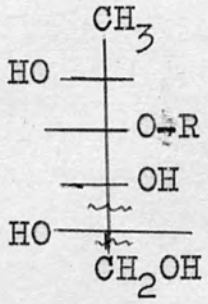
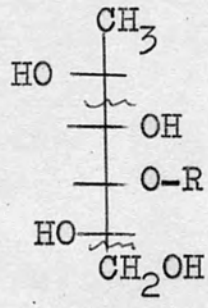
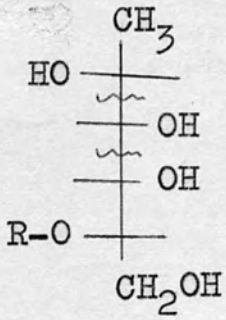
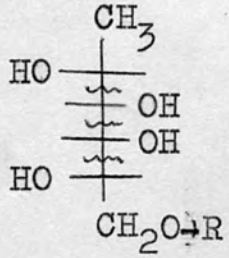
I

Paper chromatography of I, in solvent (e) gave a compound (R_G 4.7) and a compound corresponding to phenylboronic acid (R_F 0.98)(both visible under U.V. light).
The appearance of a separate spot for phenylboronic acid show the hydrolysis of I. Chromatography of the compound I, on paper, wholly impregnated with tungstate 72

gave a compound at Q(W) = 0.76. The Q(W) < 1 and M_s(W) = 0.2-0.67 shows that the hydrolysis product of the compound I forms a complex with tungstate and that it possesses four or at least three adjacent hydroxyl groups. The possible structures for the 1-deoxy-L-galactitol monocarbamate produced on the hydrolysis of the compound I are shown on p. 116. Distinction between these structures was made by the periodate oxidation of 1-deoxy mono-O-phenylcarbamoyl-L-galactitol bisphenylboronate, I. The periodate uptake was estimated by the arsenite method[76] and the formation of formaldehyde was confirmed by making the dimedone[78] derivative. The consumption of 3.08 mol. (Table 38) periodate and liberation of formaldehyde shows that the compound I is infact 1-deoxy,2-O-phenylcarbamoyl-L-galactitol bisphenylboronate (XXVI).



(XXVI)

Structure	Mol. IO_4^- consumed	Mol. HCO_2H	Mol. HCHO
	3	2	1
	2	1	1
	2	0	1
	2	1	0
	3	2	0

R = -CONH·Ph

b. Location of unsubstituted primary hydroxyl group in L-lyxitol bisphenylboronate

The evidence presented above show that one of the primary hydroxyl group of the L-lyxitol bisphenylboronate C, is not involved in the formation of the phenylboronate. Due to the asymmetry of L-lyxitol the bisphenylboronate could be either (XX) or(XXI). A distinction between (XX) and (XXI) could be made by converting their primary hydroxyl group into a carboxyl group. However,since the phenylboronate group is easily removed by hydrolysis (cf. addition of water to dioxane solution of pentitol phenylboronates), it was decided to oxidize the phenylcarbamate (XXIV) or (XXV) obtained via the bisphenylboronate,in situ.

The phenylcarbamate (XXIV) would give, on oxidation and subsequent hydrolysis L-lyxonic acid (XXVII), whereas (XXV) would give L-arabonic acid (XXVII a).

The hydrolysis of mono-O-phenylcarbamoyl-L-lyxitol bisphenylboronate (XX a) or (XXI a) to give the phenylcarbamate was effected by dissolution in aqueous acetone. The phenylcarbamate was then oxidized in situ with oxygen in the presence of a platinum catalyst, according to a method developed by Barker,Bourne and Fleetwood.[71] (Experiment 47).

CH2O
O
O
2PhB
O
CH2CH
(XX)
CH2O
O
O
2PhB
O
CH2 OCONHPh
(XX a)
CH2OH
OH
OH
HO
CH2 OCONHPh
(XXIV)
CO2H
OH
OH
HO
CH2 OCONHPh
(XXVI)
CO2H
OH
OH
HO
CH2 OH
(XXVII)
CH2OH
O
O
2PhB
O
CH2O
(XXI)
CH2OCONHPh
O
O
2PhB
O
CH2O
(XXI a)
CH2OCONHPh
OH
OH
HO
CH2OH
(XXV)
CH2OCONHPh
OH
OH
HO
CO2H
(XXV a)
CO2H
OH
OH
HO
CH2OH
(XXVII a)

Paper chromatography and electropheretic analysis of the reaction mixture revealed, in addition to the phenylboronic acid and O-phenylcarbamoyl-L-lyxitol, the presence of an acidic component. The latter was visible under U.V.light (as are all phenylcarbamates) and was presumably (XXVI) or (XXVa). It was removed from the reaction mixture by preparative chromatography. The acidic component was treated with sodium methoxide in methanol to remove the phenylcarbamoyl group, and the product was converted into a crystalline salt. The melting point of the potassium salt(162°) showed no depression on admixture with authentic potassium-D-lyxonate, whereas it showed a depression in m.p. on admixture with potassium-L-arabonate. It was thus potassium-L-lyxonate. The phenylboronate obtained from L-lyxitol was thus (XX). The yield of potassium-L-lyxonate represents a ca. 19% conversion of (XXa) into (XXVII).

In the group of cyclic acetals of the polyols, assignment of ring structure has been made inter alia. from a study of (a) the intermediate products formed during reactions to give fully substituted polyols and (b) the products of partial hydrolysis of the polyacetals. All attempts to obtain similarly the monophenylboronates were unsuccessful. It was thus decided to investigate the detailed ring structure of the phenylboronates by a physical method, i.e. infrared spectroscopy.

3. Assignment of Ring Structures of Phenylboronates.

Infrared spectroscopy has been a valuable tool for many years in helping to recognise the presence of structural units in compounds and in many cases it has proved possible to correlate, "group" vibration frequencies with the position of the group in the molecule. These correlations have been based to a great extent on experimental observations, though theoretical calculations of the frequencies at which the band occur are possible for very simple molecules. A great deal of work along these lines has been published and a comprehensive survey has been given by Ballamy.[52]

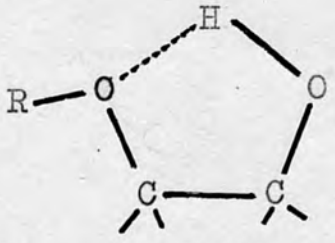
Ballamy, et.al[53] have studied the infrared spectra of boron compound and have made certain frequency assignments for, e.g. B-O, B-N and B-C bonds. They have also studied the IR spectra of B-aryl compounds and have assigned frequencies for B-Ph at 1280-1250 cm.$^{-1}$. No particular frequencies have so far been described for the five- or six-membered phenylboronate rings. On the other hand much information is available about the inter- and intra-molecular hydrogen bonding occurring in

carbocyclic and acyclic diols. The intermolecular hydrogen bonding is critically dependent on the concentration of the diol and can be detected in concentrated solutions (or as nujol mull) at ca. 3400 cm.$^{-1}$. It becomes negligible in solutions in carbon tetrachloride which are less than 0.005M with respect to the diol. Infrared spectra of certain diols examined under these conditions, i.e. dilute solutions has shown two absorption bands. The high frequency band at ca. 3630 cm.$^{-1}$ has been assigned to the free hydroxyl group and the low frequency band at ca. 3580 cm.$^{-1}$ to the intramolecularly hydrogen bonded hydroxyl group.[54-58]

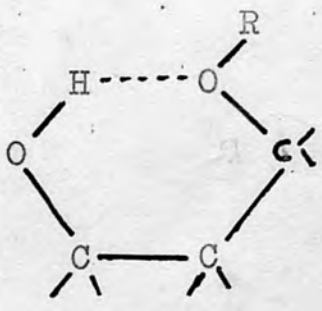
(continued on p. 122)

A parallel increase in arithmetical difference between these two frequency bands (ν free OH – ν bonded OH) occurs, as the ring formed by the intramolecular hydrogen bond increases from five to seven membered rings.

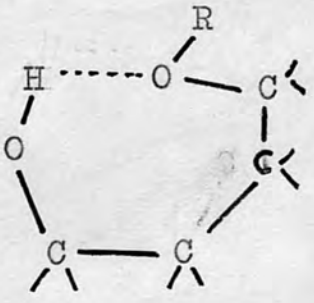
Frequencies (in parenthesis) below have been assigned[54-58] to those intramolecular hydrogen bonds which may occur in diols and lead to the formation of five-(3606 ± 10 cm.$^{-1}$), six-(3560 ± 5 cm.$^{-1}$) and seven-(ca. 3484cm.$^{-1}$) membered rings as depicted in XXVIII, XXIX and XXX respectively (bond formed by hydrogen bonding is shown as broken line).



(XXVIII)



(XXIX)

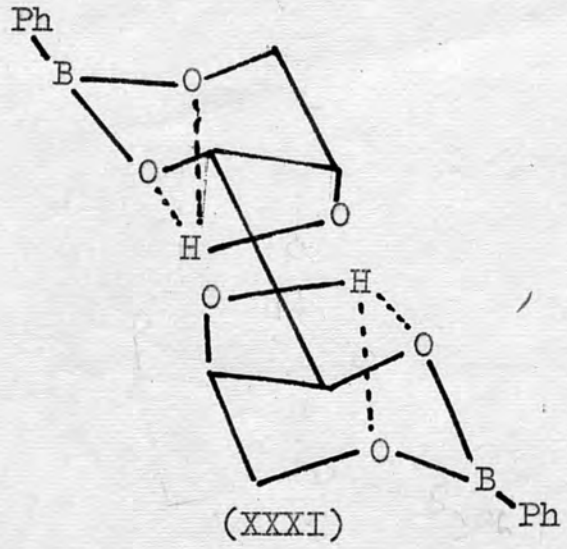


(XXX)

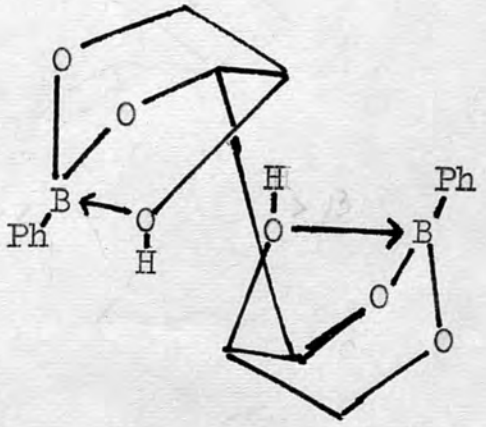
It was conceived that an investigation of the type of intramolecular hydrogen bonding occurring in the bisphenylboronates of pentitols (possessing one unsubstituted hydroxyl group) might similarly make a detailed assignment of their ring structures possible.

Bourne, Lees and Weigel[51] studied the IR spectra of

glycerol 1,2-phenylboronate (XV) and galactitol 1,3:4,6-bisphenylboronate (XVI) p.164). From the complete absence of intramolecular hydrogen bonding in galactitol 1,3:4,6-bisphenylboronate which would be expected in structure (XXXI) they concluded that the oxygen atoms at $C_{(2)}$ and $C_{(5)}$ are instead co-ordinated with the corresponding boron atom as shown in XXXII.



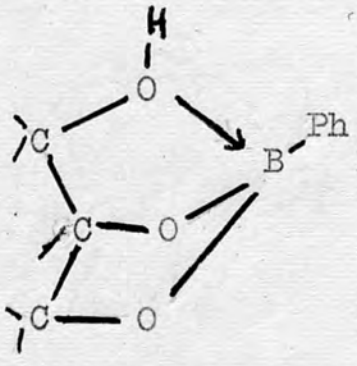
(XXXI)



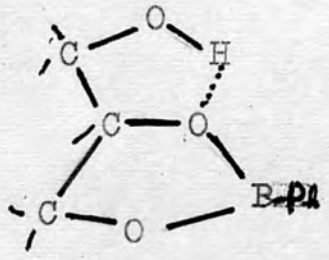
(XXXII)

Glycerol-1,2-phenylboronate exhibited absorption at 3630 $cm.^{-1}$ corresponding to a free hydroxyl group and at 3597 $cm.^{-1}$ corresponding to a bonded hydroxyl group (XXXIV) forming a five membered ring. The ratio of the intensities (1:2) of these two bands suggested to the authors that the small amount of absorption for the free hydroxyl group, may

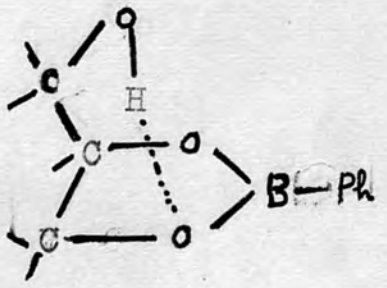
be due to (a) the ring strain inherent in forming the hydrogen bond from the extramnular primary hydroxyl group or (b) co-ordination of the oxygen atom of the primary hydroxyl group with the boron atom. Since in compound formulated as XXXIII, boron could not adapt a regular tetrahedral symmetry, it is more likely that the smaller amount of free hydroxyl abosrption is due to reason (a) above rather than reason (b).



(XXXIII)



(XXXIV)



(XXXV)

A further possibility of ring formation would be a hydrogen bond leading to six membered ring as shown in (XXXV). The absence of absorption at ca. 3561 cm.$^{-1}$ corresponding to hydrogen bond forming a six membered ring indicates that this ring is not formed. Presumably the lone pair of

electrons on the corresponding oxygen atom point away from the ring and are thus not available to the hydrogen atom of the hydroxyl group.

The infrared spectra of the pentitol bisphenylboronates in Table 37 were determined for dilute solutions ($< 0.005\underline{\underline{M}}$) in dry carbon tetrachloride. [Expt. 55(a)].

Table 37

Compound	Absorption $cm.^{-1}$		
	bonded hydroxyl group		
	Free O-H 3625 ± 10	5-membered ring 3606 ± 14	6-membered ring 3560 ± 5
Glycerol 1,2-phenyl boronate	3630(1)	3597(2)	-
Galactitol 1,3:4,6-bisphenylboronate	3630	-	-
DL-Ribitol-bis-phenylboronate	-	3608(1.5)	3562(1)
DL-Xylitol-bis-phenylboronate	3635(1.3)	3609(1)	-
L-Lyxitol-bis-phenylboronate	3626(1)	3611(1.1)	-
1-Deoxy-L-galactitol bisphenylboronate	-	3608(4)	3560(1)

Figures in parenthesis refer to the ratio of the intensities observed.

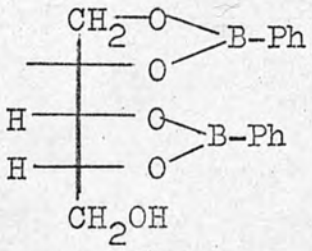
It is known that phenylboronic acid reacts with polyols to

form five-membered cyclic esters (cf. glycerol) as well as six-membered cyclic esters (cf. galactitol). Therefore, the possible five- and six-membered ring modifications based on the evidence described above are those shown on p. 127. If phenylboronates of pentitols were five-membered esters, i.e. (XXXVI), (XXXVIII), (XL) and (XLII), their pattern of absorption between 3500-3600 cm.$^{-1}$ should be that of glycerol 1,2-phenylboronate. In addition intramolecular hydrogen bonding resulting a seven-membered ring (ca. 3484 cm.$^{-1}$) might be expected. However, only the L-lyxitol derivative conformed to this expectation. The five-membered phenylboronates of other pentitols, i.e. ribitol, xylitol and 1-deoxy-L-galactitol can be eliminated. Thus, the phenylboronates of ribitol, xylitol and 1-deoxy-L-galactitol are six-membered cyclic esters (XXXVII), (XXXIX) and (XLIII) respectively.

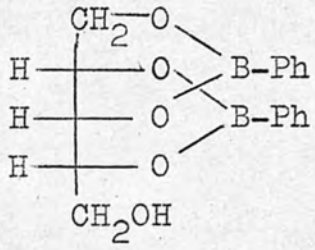
DL-Ribitol 1,3:2,4-bisphenylboronate (XXXVII).

When ring junction in two six-membered fused ring is trans, only one conformation in which both rings are in a chair form, is possible.[61] Thus, the ribitol 1,3:2,4-bis-phenylboronate which possesses two trans fused rings is (XLIV).

DL-Ribitol bisphenylboronate

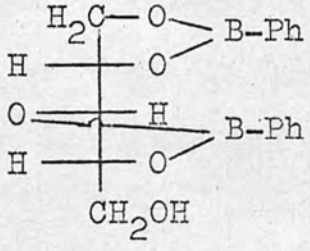


(XXXVI)

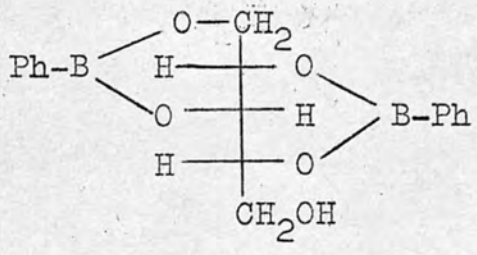


(XXXVII)

DL-Xylitol bisphenylboronate

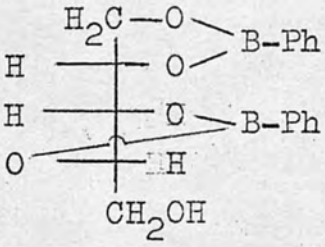


(XXXVIII)

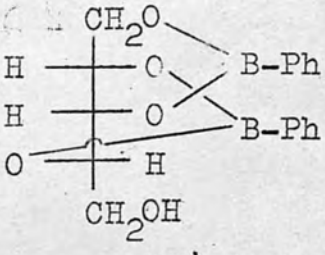


(XXXIX)

L-Lyxitol bisphenylboronate

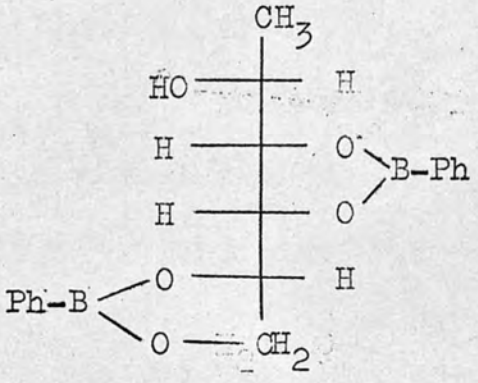


(XL)

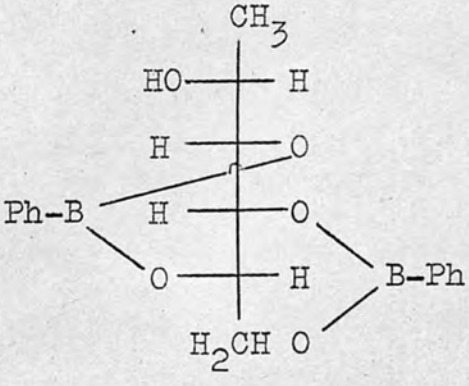


(XLI)

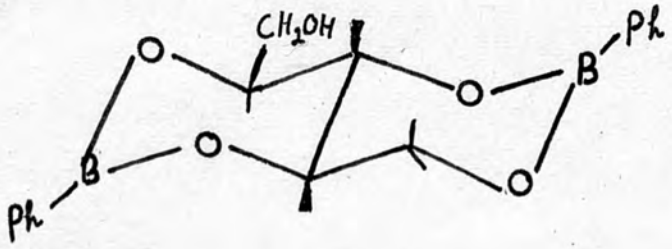
1-Deoxy-L-galactitol bisphenylboronate



(XLII)

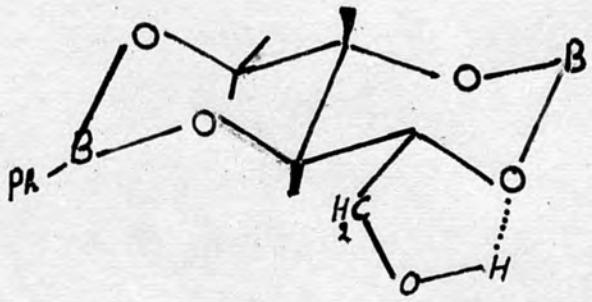


(XLIII)

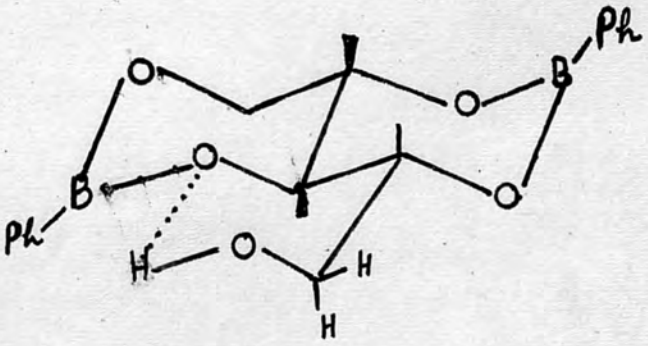


XLIVa

The type of intramolecular bonding which can occur in XLIVa is shown in XLIVb and XLIVC, i.e. involving five- and six-membered rings.



(XLIVb)



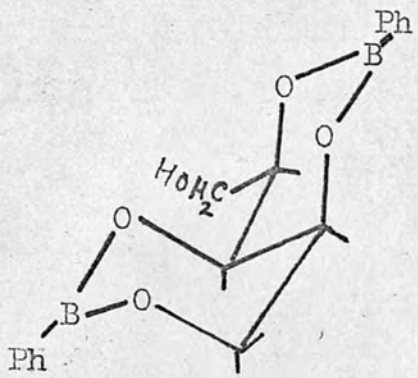
(XLIVc)

The pattern of IR absorption encountered (Table 37), is in agreement with this structure. Conversion of the two rings of XLIVa into boat form would not alter the relative disposition of the atoms involved in intramolecular hydrogen bonding. However, the chair conformations are more likely than the boat conformations.

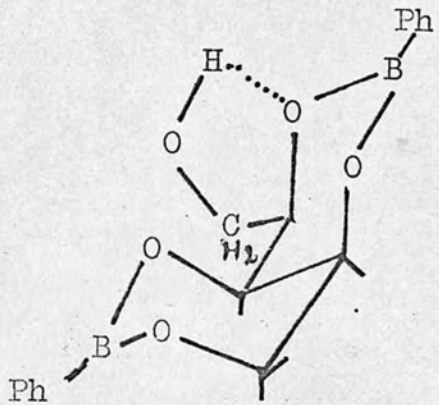
DL-Xylitol 1,3:2,4-bisphenylboronate (XXXIX).

This compound contains two cis fused rings. With cis ring fusion two conformations with both rings in a chair form are possible. These have been denoted as "O-inside" and "H-inside" forms.[61] Formulae XLVa and XLVIa represent the "O-inside" and "H-inside" conformations of DL-xylitol 1,3:2,4-bisphenylboronate. Following Mill's[61] argument for the analogous cyclic acetals, it would be expected that structure XLVa (O-inside) would be more stable than XLVIa (H-inside). The intramolecular hydrogen bonds which can occur in XLVa is shown in structure XLVb and XLVc, involving a five- and six-membered ring respectively. However, the observed pattern of absorption in which there is no absorption at the frequency corresponding to a six-membered ring, is not in agreement with these structures.

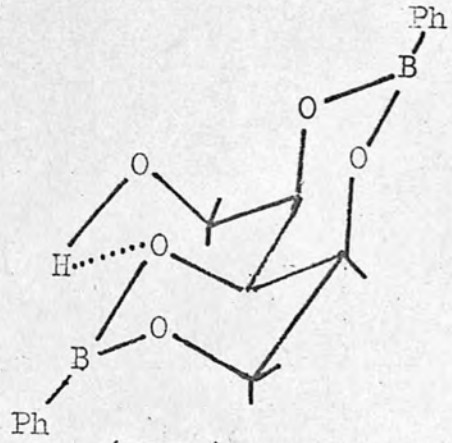
Intramolecular hydrogen bonding involving five- and six-membered rings might also occur in "H-inside" (XLVIa) conformation as shown in XLVIb and XLVIc. However, in XLVIc the oxygen atom of the primary hydroxyl group is in such a close proximity to the strongly electrophilic boron atom that the co-ordination reaction will compete with the formation of intramolecular hydrogen bonding. Since the



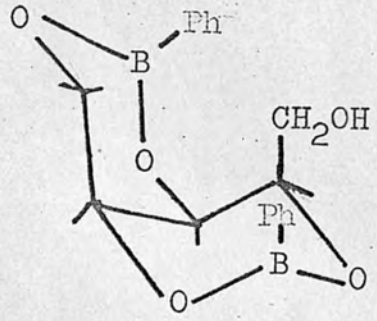
(XLV a)



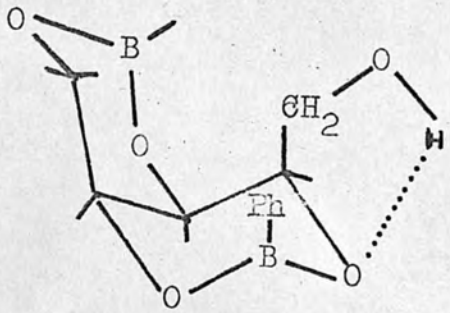
(XLV b)



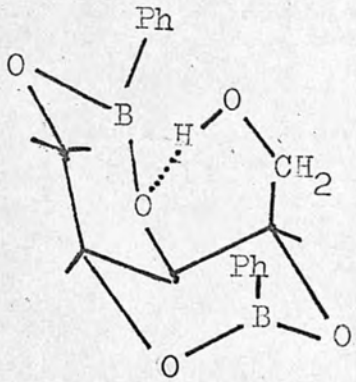
(XLV c)



(XLVI a)

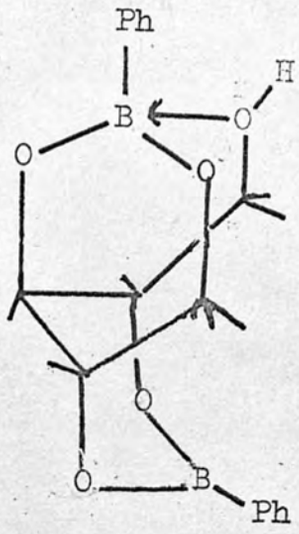


(XLVI b)

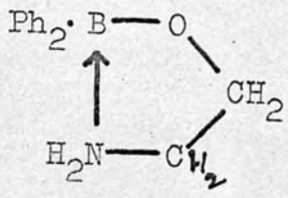


(XLVI c)

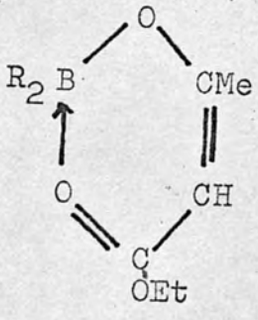
co-ordinating electron pair of an oxygen atom is not directly involved in the formation of an O-H band, the influence of this type of co-ordination on the O-H stretching frequency would be small. Thus, the co-ordination of the oxygen atom of the hydroxymethyl group with boron atom would leave a free hydroxyl group, which was indeed revealed by the infrared spectrum of the DL-xylitol bisphenylboronate. A free hydroxyl absorption may however, be due to the ring strain inherent in forming a hydrogen bond of the type shown in structure XLVIb, which is analogous to that occurring in glycerol 1,2-phenyl-boronate (XXXIV). In such an event the ratio of intensities of absorption at 3635 and 3609 $cm.^{-1}$ would be expected to be ca.1:2 (cf. glycerol 1,2-phenylboronate). The observed ratio was in fact 1.3:1. It is thus concluded that DL-xylitol 1,3:2,4-bisphenylboronate adapts the "H-inside" conformation and that in a large number of molecules the oxygen of the hydroxymethyl group co-ordinates with the boron atom of the 1,3-phenylboronate ring (XLVII). Similar type of internal co-ordination is known to occur in many boron compounds, which affects the properties of such compounds, e.g. exceptional stability of, 2-amino ethyl diphenyl borinate and 2-ethoxy carbonyl-1-methylvinyl di-n-butyl-borinate to hydrolysis and storage have been



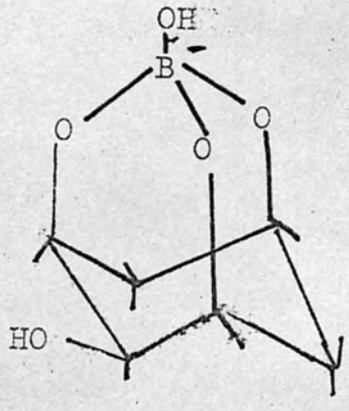
(XLVII)



(XLVIII)



(XLIX)



(L)

R = n-butyl

explained by postulating internal co-ordination between boron and an electron donating atom, i.e. nitrogen atom and oxygen atom in XLVIII and XLIX respectively.[59,60]

It is likely, that the energy difference between the "O-inside" and "H-inside" conformations is largely overcome by the energy of co-ordination. The structure XLVII is comparable to the structures postulated by Angyal and McHugh[62] for the borate complexes of inositols e.g. L.

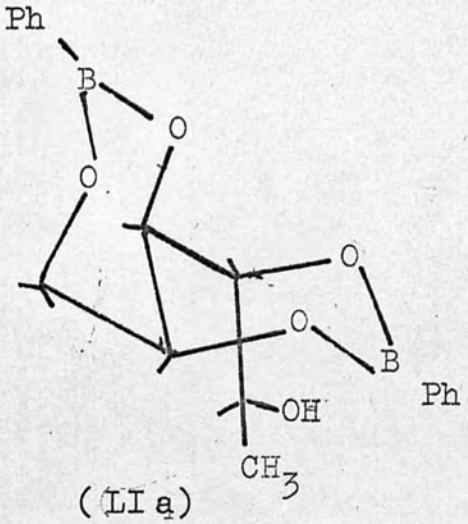
1-Deoxy-L-galactitol 3,5:4,6-bisphenylboronate (XLII).

This contains two cis fused rings and may thus adapt conformations LIa or LIIa. The IR spectrum of this compound has revealed intramolecular hydrogen bonding involving five- and six-membered rings. The "O-inside" conformation LIa can give rise to hydrogen bonding involving only a five-membered ring as shown in LIb, whereas the "H-inside" conformation LIIa can give rise to hydrogen bonding involving both five- and six-membered rings as shown in LIIb and LIIc respectively.

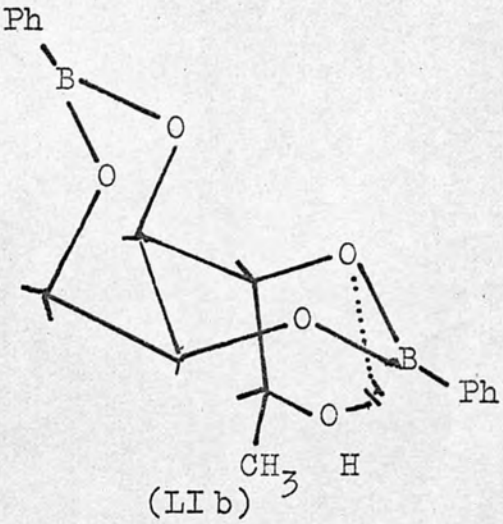
It is thus concluded that the conformation adapted by this compound is LIIa.

L-Lyxitol bisphenylboronate (XL or XLI).

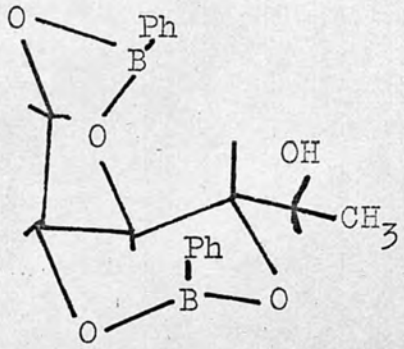
From consideration of the periodate oxidation of mono-O-phenylcarbamoyl-L-lyxitol and its conversion into L-lyxonic acid (see p.118), two possible structures of



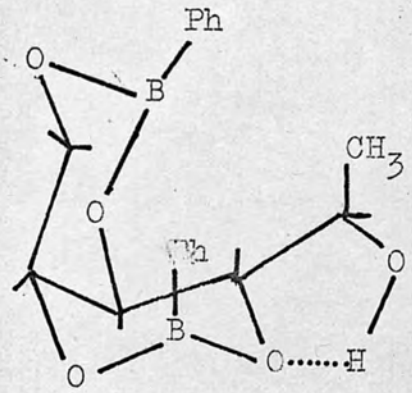
(LI a)



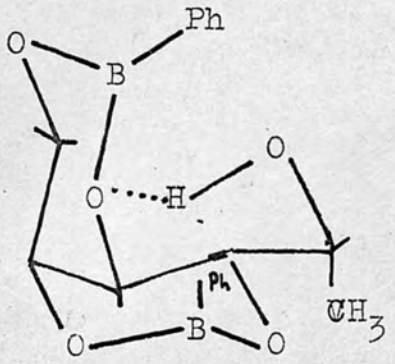
(LI b)



(LII a)

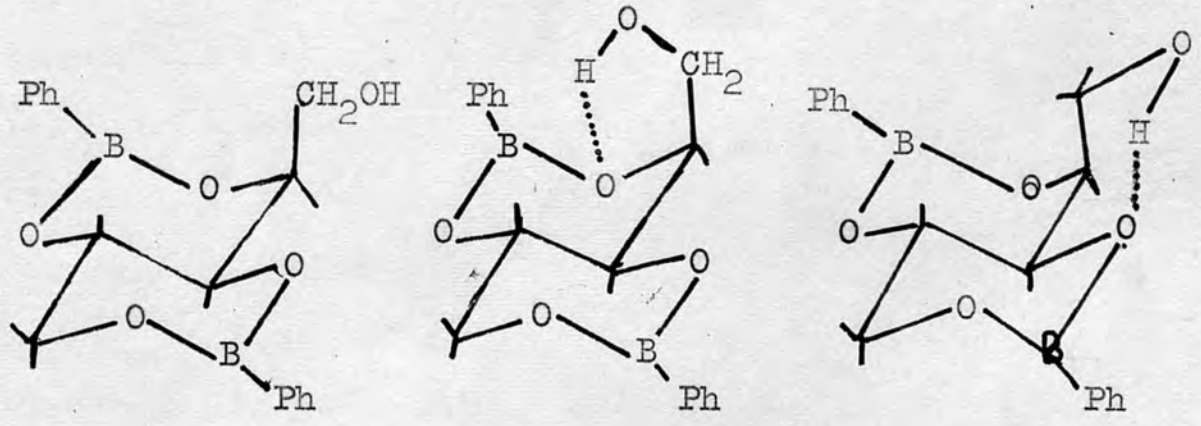


(LII b)



(LII c)

L-lyxitol bisphenylboronate are XL and XLI (shown on p.127). Unlike the phenylboronates discussed above, the pattern of IR absorption exhibited by this compound between 3500 - 3600 cm.$^{-1}$ was similar to that of glycerol 1,2-phenylboronate, i.e. it possesses a free hydroxyl group and an intramolecularly hydrogen bonded hydroxyl group forming a five-membered ring. The intensities of absorption at the corresponding frequencies are ca. 1:1.1. The type of intramolecular hydrogen bonding possible in the two trans fused rings (LIIIa) of 1,3:2,4-bisphenylboronate. are those shown by structures (LIIIb) and (LIIIc).



(LIIIa) (LIIIb) (LIIIc)

The fact that the compound did not absorb at a frequency (ca. 3562 cm.$^{-1}$) corresponding to hydrogen bonding involving a six-membered ring as expected from the structure LIIIc, could suggest that the structure is XL, i.e. L-lyxitol 1,2:3,4-bisphenylboronate. However,

the boron atom of 2,4-phenylboronate ring in (LIIIb) might exert sufficient attraction of the hydroxymethyl group to prevent its rotation to a position suitable for the formation of structure LIIIc. In such an event the expected frequencies of absorption and the ratio of absorption could be those found experimentally (Table 37). However, further investigations are necessary to distinguish between structure XL and XLI for the L-lyxitol bisphenylboronate.

Carbohydrate chemistry is especially rich in examples of five-or six-membered rings fused to another five- or six-membered rings. For example aldehydes react with polyhydric alcohols to give a variety of condensation products. The configuration of the hydroxyl groups in the polyhydric alcohol is a major factor governing the course of acetal formation. Barker and Bourne[63] have examined a large number of acetals and ketals and have compiled certain rules, concerning the preferred hydroxyl group arrangement of the polyols for reaction with aldehydes and ketones. They have stated that when aldehydes react with polyols, the first preference is for a βC (Barker and Bourne nomenclature)[17]. The second preference is for a β arrangement and the third preference is for an α-, βT- or γT- arrangement. In the case of methylation, a βT-ring

takes precedence over an αT or γT ring.

Comparison of the pentitol bisphenylboronates with the analogous known examples of acetals reveal that the bisphenylboronates of ribitol, xylitol and 1-deoxy-galactitol comply with these rules.

The DL-ribitol derivatives (XXXVII) consist of one βC and a β ring. In fact, with this compound no other possibilities for six-membered ring formation exist. It would be expected, by analogy with 1,3:2,4-dimethylene-DL-ribitol[63] that the βC ring is more stable than β ring, and if it were possible to isolate the mono-phenylboronate, this would most likely be the 2:4-phenylboronate.

The same situation exists with the xylitol derivative (XXXIX) where a βC and β ring are also formed, analogously to 2,4:3,5 dimethylene DL-xylitol.

The bisphenylboronate (XLIII) formed with 1-deoxy-L-galactitol (~~XLIII~~) needs further explanation. If the β ring is formed first, between the oxygen atoms at $C_{(4)}$ and $C_{(6)}$, there are two further possibilities for ring formation, i.e. either an αT- ring involving oxygen atom at $C_{(2)}$ and $C_{(3)}$ or βT involving oxygen atoms at $C_{(2)}$ and $C_{(4)}$. By strict analogy with the acetals, an αT-ring (5-membered) would be expected. However the six-membered βT ring is in fact formed. It is worth noting that Barker

and Bourne[63] mentioned that βT-ring formation takes precedence over αT- in methylene ring formation.

The structure of L-lyxitol derivative cannot be derived completely from the infrared spectrum (see p.133). However XLI involves a β- and a βT- ring, whereas XL involves α- and αT- rings. By analogy with the acetals, and also with the phenylboronates discussed above the structure XLI is more likely.

EXPERIMENTAL

General Methods

Electrophoresis.

Electrophoresis was carried out on 10 cm. wide strips of Whatman No.3 paper, using "Shandon's", high voltage electrophoresis apparatus, which was capable of producing a potential difference of up to 10,000 V. Mobilities are expressed as $\underline{M}_S$ values, i.e.

$$\underline{M}_S = \frac{\text{True distance travelled by the compound}}{\text{True distance travelled by } \underline{D}\text{-glucitol}}$$

Electrolytes used.

Molybdate:[13,14] Aqueous sodium molybdate dihydrate (1.5% W/V) acidified to pH 5.0 with dilute sulphuric acid.

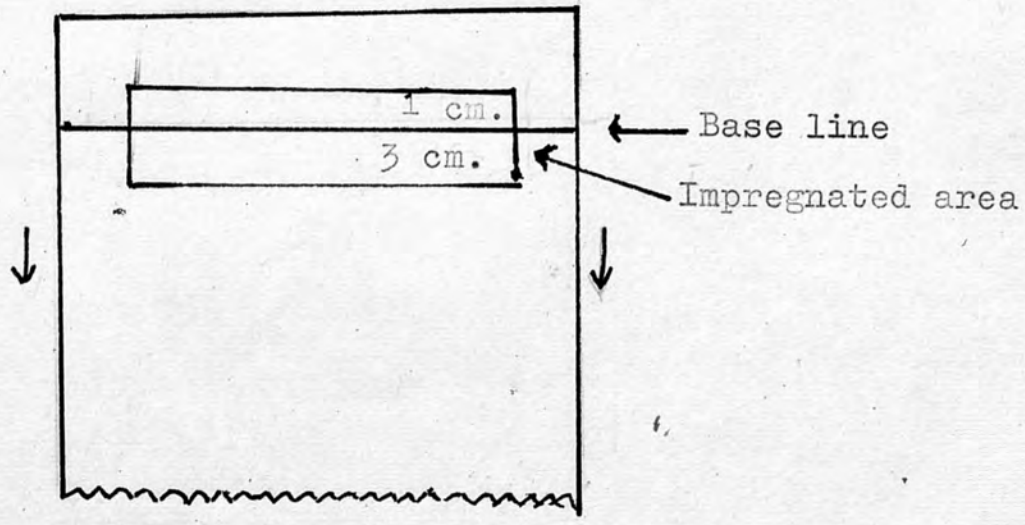
Tungstate:[15] Aqueous sodium tungstate dihydrate (2% W/V) acidified to pH 5.0 as above.

Paper chromatography.

Paper chromatography was carried out on Whatman No.1 or No.3 paper by the descending method.

Paper Chromatography on paper (i) partially and (ii) wholly impregnated with sodium molybdate or sodium tungstate solution.

(i) An area of 1x3 cm. (3 cm. above and 1 cm. below the base line) was impregnated (as shown below) with sodium molybdate or sodium tungstate solution of desired pH and



concentration as mentioned in the text, with the help of a [redacted] jet tube. The paper was dried at room temperature and the impregnated area was outlined in pencil under ultra violet light.

Polyols to be examined were spotted within the impregnated area. D-Glucose (or marker) was applied outside the impregnated area. A control was run in the same tank on unimpregnated paper.

(ii) The paper was dipped in molybdate or tungstate solution of desired concentration and pH (as shown in the text) blotted in folds of filter paper and dried.

Solvents

(a) n-Butanol, ethanol, water (4:1:5), organic phase.

(b) Acetone, n-butanol, water (5:4:3).

(c) Acetone, n-butanol, water (4:4:2).

(d) Acetone, n-butanol, water (5:4:2.5).

(e) Acetone, n-butanol, water (5:3:2).

(f) Pyridine, ethyl acetate, water, acitic acid (5:5:1:3).

(g) n-Butanol, pyridine, water (10:4:3).

Spray reagents for detecting the compounds.

(i) Silver nitrate/sodium hydroxide.[41]

(ii) Aqueous aniline oxalate.[42]

(iii) Bromocresol green.[43]

Polarimetry.

The rotations were measured in 2 or 4 dm. tubes at the wavelength of mercury light or at the wavelength of sodium light.

Ultraviolet absorption measurements.

These were made on one of the following apparatus as mentioned in the text.

(i) Unicam SP 500 spectrophotometer.

(ii) Hilger Spekker absorptiometer.

Thin-layer plates (TLP).

Plates for the thin-layer chromatography were prepared

using "Sandon's" basic outfit No.2805.

Detection of compounds on TLP.

Compound on Kieselgel plates were detected by standing the plates in iodine vapour. Compounds on the cellulose and Avicel[69] layers were detected by the spray reagents mentioned above under paper chromatography.

Experiment 1. Preparation of the molybdate form of anion exchanger.

Method 1: Amberlite IRA-400 (Cl^-) was slurried into a glass column (1.4 cm. dia.) to a height of 40 cm. and allowed to settle under gravity. After thorough washing with water the anion exchanger was converted into the molybdate form by passing sodium molybdate dihydrate (4%, W/V), pH of which was previously adjusted to 5.0 with Amberlite IR-120(H^+), until the amount of molybdate in the effluent was the same as that of influent.[ii] A water wash removed excess of molybdate from the column.

Method 2: Amberlite IRA-400 (Cl^-) was converted into free base form by eluting with aqueous sodium hydroxide (2N) until the washings gave a negative test for chloride ion (ca. 3 bed volumes). The column was then washed with water to remove excess alkali. Sodium molybdate dihydrate (4%, W/V) was passed through the column until the saturation point[i] was reached. Eluent was replaced with

sodium molybdate dihydrate [0.005M, pH 5) adjusted with IR-120(H^+)] until the pH of the effluent reached 5.0±0.1.

Method 3: Free base form of the resin, prepared according to method 2, was packed into a glass column (as above) and converted into the molybdate form by passing 4% sodium molybdate dihydrate (W/V) until saturation point(i) was reached. The column was then washed with large excess of water until the pH of the washings reached to the pH of water (5.5).

(i) Potassium thiocyanate spot test.[64]

Experiment 2(a) Separation (attempted) of D-glucose from D-glucitol.

The molybdate form of resin (50 ml.) prepared as in method 1, was packed into a glass column (1.4 cm. dia.) and washed with water (50 ml.) to remove free molybdate.

A solution containing D-glucose (15 mg.) and D-glucitol (15 mg.) in water (15 ml.) was placed on the top of the column which was then eluted with water. Fractions (10 ml.) were collected on an automatic fraction collector. Each fraction was examined by paper electrophoresis in molybdate solution, which revealed that both D-glucose and D-glucitol were eluted at the same rate. The first two fractions were comparatively rich in D-glucose content.

Experiment 2(b) Attempted separation of D-glucose from D-glucitol on molybdate form of resin.

The molybdate form of resin prepared according to method 2, was packed in a column with an inside diameter of 1.4 cm. to a height of 40 cm. A solution containing D-glucose (12.5 mg.) and D-glucitol (12.5 mg.) in water (12.5 ml.) was added to the column, which was then eluted with water. Fractions (10 ml.) were collected and examined by electrophoresis in molybdate solution. After none of the added polyol could be detected, the eluent was then replaced by 0.1N sodium hydroxide, fractions were collected and examined as above. Electrophoresis revealed an incomplete separation. The results are shown in Table 11.

Table 11

Fraction (10 ml.)	Elute	Polyol* detected	Intensity of spots (visual).
1-10	Water	1,2	dense spot for 1, traces of 2.
11-15	"	"	" "
16-70	"	"	" "
71-78	0.1N NaOH	2 only	dense spot in fraction 71-74, traces in 75-78.

* 1. D-glucose, 2. D-glucitol.

Experiment 2(c). Attempted separation of D-glucose from D-glucitol on molybdate form of resin column.

Molybdate form of resin prepared according to method 3 was packed in a glass column (1.4 cm., dia.) up to a height of 32 cm. washed with water (250 ml.). A solution containing D-glucose (10 mg.) and D-glucitol (10 mg.) in water (5 ml.) was placed on the column. Fractions (50 ml.) were collected by eluting with water at a flow rate of 2-2.5 ml., per minute and were examined by electrophoresis in molybdate solution.

Fraction 1 showed the presence of only D-glucose while there was a trace of D-glucitol in the 2nd. fraction. Rest of D-glucitol came out of the column comparatively slowly, i.e. in fractions 3-10. The results are shown in Table 12.

Table 12

Fraction (50 ml.)	Elute	Volume total (ml.)	Polyol detected	Remarks
1	Water	50	glucose	D-glucose + D-glucitol
2	"	100	D-glucose	traces of D-glucitol
3-10	"	500	D-glucitol	D-glucitol only.

Experiment 2(d) Attempted separation of D-glucose from D-glucitol on molybdate form of resin.

A solution containing D-glucose (10 mg.)and D-glucitol (10 mg.) in water (10 ml.) was placed on the top of a molybdate form of resin column (1.4 x 32.0 cm.) prepared according to method 2. The column was then eluted with sodium molybdate solution (0.005M, pH 5.0). Fractions (50 ml.) were collected on an automatic fraction collector and were examined by electrophoresis in molybdate solution, until no more D-glucose could be detected in the effluent. Eluent was then changed to water and finally to 0.1% sodium hydroxide. Fractions (50 ml.) were collected and examined as above. The results are shown in Table 13.

Table 13

Fraction (50 ml.)	Eluent	Polyol detected	Remarks
1	Na_2MoO_4 (0.005M)	D-glucose	D-glucitol in traces.
2	"	"	"
3-8	"	-	-
9-11	water	-	-
12-17	NaOH (0.1%)	-	-
18-20	"	D-glucitol	Bulk in 19 and 20
21	"	"	traces.

Experiment 3. Effect of pH on adsorbtion of D-glucitol, on molybdate form of resin.

Aliquots (5 ml.) of thoroughly washed Amberlite IRA-400(OH^-) were placed in different beakers. Sodium molybdate dihydrate (4%, 20 ml.) was added to each and the supernatants were acidified to pH values between 2-7. The whole was allowed to stand for 2 hours. The resin was filtered and washed with water. D-glucitol (1 mg.) was added to each after suspending the resin in water (5 ml.). The solutions were shaken and the supernatants examined electrophoretically in molybdate solution. Results are given in Table 14.

Table 14

No.	pH	Spot intensity
1	2.0	dense
2	3.0	"
3	4.0	moderate
4	5.0	very faint
5	6.3	moderate
6	7.0	dense

Experiment 4(a). Capacity (approximate) of the molybdate form of resin for the adsorbtion of D-glucitol.

Aliquots (5 ml.) of the molybdate form of resin (method 2) was suspended in water (5 ml.). D-glucitol

(2-10 mg.) in water (1 ml.) was added to each. The supernatant was examined electrophoretically in molybdate solution. Results are given in table 15.

Table 15

No.	pH (original)	D-glucitol added (mg.)	spot intensity (visual)
1	5	2	faint
2	"	4	"
3	"	6	medium
4	"	8	dense
5	"	10	dense

Experiment 4(b) Approximate capacity of the molybdate form of resin for the adsorbtion of D-glucitol.

Experimental procedure was the same as in Expt. 4(a) except that pH of the supernatants was readjusted with 2N-sulphuric acid after the addition of D-glucitol. Results are shown in Table 16.

Table 16

No.	pH (original)	D-glucitol added (mg.)	pH after adding D-glucitol (readjusted)	spot intensity (visual)
1	5.0	2	5.0	-
2	5.0	4	5.1	-
3	5.0	6	5.0	v. faint
4	5.0	8	5.0	dense
5	5.0	10	5.0	"

Experiment 5(a) Adsorbtion and desorbtion of preformed D-glucitol/molybdate complex on molybdate form of resin.

D-Glucitol/molybdate complex was prepared by dissolving sodium molybdate dihydrate and D-glucitol in the molar ratio of 2:1. The pH was adjusted to 2.0 with 2N-sulphuric acid.

Above solution (10 ml.) containing 40 mg. of D-glucitol was placed on a column (1.4 cm. dia.) packed with molybdate form of resin (40 ml.), prepared according to method 2, which was then eluted with water. Washings were collected and examined by electrophoresis, in molybdate solution which revealed that the D-glucitol-molybdate complex had been eluted with water.

Experiment 5(b). Adsorbtion and desorbtion of preformed D-glucitol/molybdate complex on molybdate form of resin.

All the experimental procedure was the same as in Expt. 5a except that the pH was adjusted to 5.0. The results were the same as in Expt. 5a i.e. the preformed complex could not be retained by the resin.

Experiment 6(a) Separation of D-glucose from D-glucitol using molybdate form of anion exchanger

A solution (5 ml.) containing D-glucose (10 mg.) and D-glucitol (10 mg.) in sodium molybdate dihydrate (0.005M), of pH 5 [adjusted with IR-120(H^+)], was placed on

on a molybdate form of resin column (1.4 x 32 cm., method 2). The column was then eluted with sodium molybdate dihydrate (0.005M) solution of pH 5 [adjusted with Amberlite IR-120(H^+)], followed by water and 0.1% sodium hydroxide. Fractions (50 ml.) were collected and examined by electrophoresis in molybdate solution.

A fast moving spot (M_s = 1.2 - 1.3) was also detected in fractions eluted with alkali. Results are given in Table 17.

Table 17

Fraction (50 ml.)	Elute	Polyol detected	Remarks
1	$Na_2MoO_4 2H_2O$		-
	(0.005M)	D-glucose	-
2	"	"	
3-8	"	nil	-
9-11	Water	"	
12-17	0.1%-NaOH	"	A spot M_s 1.2-1.3
18-20	"	D-glucitol	".

Experiment 6(b) Separation of D-glucose from D-glucitol on molybdate form of resin.

All the experimental procedure was the same as in Expt. 6(a), except that the elution was followed quantitatively. The results are shown below and also in Fig.1.

Fraction	Eluent	Polyol	% recovery	Total recovery %
1	0.005M Na_2MoO_4	D-glucose	94.00(i)	94.0
2-8	"	-	-	-
9-12	Water	-	-	-
13-17	0.1N-NaOH	-	-	-
18	"	D-glucitol	30.0(ii)	91.0
19	"	"	52.0	
20	"	"	9.0	

(i) Estimated polarimetrically based on $[\alpha]_D$ of D-glucose, using wavelength of sodium light.

(ii) Estimated by periodate/chromotropic acid method[65], after deionizing with IR-120(H^+) and IRA-400(OH^-) on Spekker using filter No.606.

Experiment 6(c). Separation of D-glucose from D-glucitol on molybdate form of resin.

The molybdate form of resin prepared as in method 3, was packed in a glass column (1.4 x 32 cm.).

A solution containing D-glucose (40 mg.) and D-glucitol (40 mg.) in water (ca. 5 ml.) was added to the column. The polyol solution was pushed in by flowing the column (ca. 0.5 ml./min), the column was then allowed to stand for six hours. The column was then eluted with water.

Fractions (25 ml.) were collected and were examined by electrophoresis in molybdate solution. After all the D-glucose had been eluted, the eluent was changed to dilute alkali (0.1% NaOH). Fractions were collected and examined as above. D-Glucose was estimated polarimetrically (Na light, l = 2) D-glucitol containing fractions were deionized and estimated as in Expt. 6(b). The results are shown in Table 18 and also in Fig. 3.

Table 18

Fraction (25 ml.)	Elute	Polyol detected	recovery %	
1	water	D-glucose	16.1	
2	"	"	19.5	
3	"	"	31.7	92.6%
4	"	"	18.0	
5	"	"	7.3	
6	"	-	-	
7-12	0.1% NaOH	-	-	
13	"	D-glucitol	22.5	
14	"	"	46.2	90%
15	"	"	21.3	

Experiment 7. Effect of anion exchanger on D-glucose and D-glucitol.

Aliquots (5 ml.) of chloride, molybdate and hydroxyl form of Amberlite IRA-400 were placed in six different beakers. D-Glucose or D-glucitol (ca. 5 mg.)., was added to each. The suspensions were shaken and the supernatants were examined by electrophoresis in molybdate solution. In each case only the added material was detected.

Experiment 8. Optimum pH for the desorbtion of D-glucitol from the molybdate form of resin.

A solution containing D-glucitol (10 mg.) in water was placed on a molybdate form of resin column (1.4 x 32 cm.) prepared according to method 2. The column was eluted with water (5 ml.) and the resin was removed from the column into a beaker. The resin was then divided into five equal portions (10 ml.). Water (5 ml.) was added to each and the pH of the supernatants was adjusted to values between ca. 6 and 11 with 2N-sodium hydroxide. After standing (2 hr.) the supernatants were examined by electrophoresis in molybdate solution. The results are shown in table 19.

Table 19

No.	pH	Intensity of spot (visual)
1	6.8	very faint.
2	7.8	"

(Table 19 continued)

3	8.4	very faint
4	9.3	Dense spot
5	10.2	" "
6	11.0	" "

Experiment 9(a) Separation of D-glucose from D-glucitol using tungstate form of anion exchanger

Tungstate form of resin was prepared according to method 2 using sodium tungstate dihydrate. The resin was packed in a glass column with an inside diameter of 1.8 cm. to a height of 65 cm. After washing with water, the column was then eluted with sodium tungstate dihydrate (0.005M) acidified to pH 6.0 with Amberlite IR-120(H^+).

A solution (ca. 8 ml.) containing D-glucose (80 mg.) and D-glucitol (80 mg.) in sodium tungstate (0.005M, pH 6.0) was placed on the column, which was then eluted with sodium tungstate (0.005M, pH 6.0). Fractions (50 ml.) were collected and examined by electrophoresis in tungstate solution. The results are shown in Table 20 and in Fig.2.

Table 20

Fraction (50 ml.)	Elute	Polyol	% recovery	
1	0.005M	D-glucose[(i)]	21.0	
2	Na_2WO_4	"	35.87	
3	"	"	15.00	86.87%
4	"	"	12.00	
5	"	"	3.00	

(Table 20 continued)

6	Na_2WO_4	D-glucose[1]	traces	
7-18	0.1N NaOH	-	-	
19	"	D-glucitol[i]	2.5	
20	"	"	16.0	
21	"	"	17.0	
22	"	"	7.5	
23	"	"	7.0	86.0%
24	"	"	6.0	
25	"	"	5.0	
26	"	"	3.0	
27	"	"	2.0	

(i) D-glucose and D-glucitol were estimated as in Expt. 6(b)

Experiment 9(b) Separation of D-glucose from D-glucitol on tungstate form of resin.

Experimental procedure was exactly the same as in Expt. 6(c) except that tungstate form of resin and electrophoresis in tungstate solution was employed. The results are shown in Table 21 and in Fig. 4.

Table 21

Fraction	Eluent	Polyol (detected)	Recovery %	
1	water	D-glucose(i)	16.6	
2	"	"	36.66	
3	"	"	30.00	93.3%
4	"	"	10.00	
5	"	"	traces	
6,7	"	-	-	
8-10	0.1% NaOH	-	-	
11	"	D-glucitol(ii)	19.1	
12	"	"	35.0	
13	"	"	16.6	77.3%
14	"	"	6.6	

(i) polarimetric

(ii) periodate/chromotropic acid/Spekker filter No.606.

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Experiment 10. Separation of D-galactose from galactitol using molybdate form of resin.

The molybdate form of resin (300 ml.) prepared according to method 2, was packed in a column (2.7 cm. dia.). After lowering the pH of the effluent (see method 2) to 5.0, a solution containing D-galactose (300 mg.) and galactitol (300 mg.) in sodium molybdate (0.005M, pH 5.0) was added to the column which was then eluted with 0.005M sodium molybdate (pH 5.0). Fractions (300 ml.) were collected and examined by electrophoresis, in molybdate solution. It was noticed that D-galactose came out of the column very slowly (28 fractions). Fractions containing D-galactose were evaporated to a small

volume (250 ml.) and estimated polarimetrically at wavelength of sodium light. The % recovery of D-galactose (151.33%) based on $[\alpha]_D$ of D-galactose was found to be very high.

After all the D-galactose has been washed out of column, the eluent was replaced by sodium hydroxide (0.1N). Fractions (300 ml.) were collected and examined as above. Galactitol desorbed when the pH of the effluent reached 9.0. The fractions containing galactitol were combined together and were deionized by successive treatment with IR-120(H^+) and IRA-400(OH^-)(i) and estimated by periodate chromotropic and method (recovery 85%). The deionized solution containing galactitol was evaporated to a syrup and characterised as galactitol hexacetate, m.p. and mixed m.p. 168°C.

(i) For removal of molybdate or tungstate from the solutions containing complex forming polyols, it is necessary to add sufficient IRA-400(OH^-) to make the solution alkaline.

Experiment 11. Separation of D-galactose from galactitol on tungstate form of resin.

The tungstate form of resin (300 ml.) was prepared according to method 2, using sodium tungstate dihydrate (4%). The resin was packed in a glass column (2.7 cm., dia.).

The column was eluted with sodium tungstate dihydrate (0.005M), acidified to pH 5.5 until the pH of the effluent reached 5.5 ± 0.2. A solution (30 ml.) containing D-galactose (300 mg.) and galactitol (300 mg.) in sodium tungstate dihydrate (0.005M) of pH 5.5, was placed on the column. The column was then eluted with tungstate solution (0.005M, pH 5.0).

Fractions (500 ml.) were collected and examined by electrophoresis in tungstate solution. After no more D-galactose could be detected electrophoretically, eluent was replaced with 0.1N-sodium hydroxide. Fractions (250 ml.) were collected and examined by tungstate electrophoresis. D-galactose and galactitol were deionized and were estimated as in expt.6(b). The results are shown in Table 22 and Fig. 7.

Table 22

Fraction (500 ml.)	Eluent	Polyol (detected)	recovery %	
1	0.005M	D-galactose	33.33	
2	Na_2WO_4	"	3.33	
3	"	"	1.66	
4	"	"	1.00	93.96%
5	"	"	2.66	
6	"	"	0.66	
7	"	"	0.66	
8	"	"	0.66	

Table 22 (continued).

9	0.005M Na_2WO_4	D-galactose	-	
10-12 (250 ml.)	0.1N NaOH	-	-	
13	"	Galactitol	25.0	77.66%
14	"	"	47.33	
15	"	"	3.33	
16	"	"	2.00	

Experiment 12. Separation of D-mannose from D-mannitol on molybdate form of resin.

A solution (2 ml.) containing D-mannose (30 mg.) and D-mannitol (30 mg.) in water was placed on a molybdate form of resin column (1.4 x 32 cm.) prepared as in method 3. The column was allowed to stand for 4-5 hours and was then eluted with water. Fractions (25 ml.) were collected and examined as in Expt. 6. Water washed out only D-mannose from the column. After all the D-mannose had been eluted, dilute hydrochloric acid of pH 2.5 (prepared by dropwise addition of 0.1N-hydrochloric acid to water) was passed through the column. D-mannitol desorbed when the pH of the effluent reached ca. 2.60. The results are shown in Table 23.

Table 23

Fraction	Eluent	Polyol (detected)	recovery %
1-26	water	D-mannose	98.78
27	"	"	-
28-34	dil.HCl	-	-
35	"	D-mannitol	N.E.
36	"	"	Bulk, N.E.
37	"	"	N.E.
38	"	"	N.E.

N.E. not estimated.

Experiment 13. Attempted separation of maltose from maltitol on tungstate form of resin.

A column (1.4 cm.dia.) containing tungstate form of resin was prepared according to Expt. 9(a).

A solution containing maltose (45 mg.) and maltitol (45 mg.) in tungstate solution (10 ml.) of pH 5.0 [adjusted as in Expt. 9(a)] was added to the column. The column was then eluted with sodium tungstate solution (0.005M, pH 5.0).

Fractions (25 ml.) were collected and examined by electrophoresis in tungstate solution. Both maltose and maltitol were present in fractions (1-8). Changing of the eluent to 0.1N NaOH showed that no maltitol was adsorbed on the resin.

Experiment 14. Separation of D-glucitol from maltitol on a tungstate form of resin column.

A column (1.4 x 32 cm.) containing tungstate form of resin was prepared as in Expt. 9(a). A solution containing D-glucitol (31 mg.) and maltitol (31 mg.) in sodium tungstate (0.005M, pH 5, 3 ml.) was added to the column. The column was then eluted with tungstate solution and 0.1N-sodium hydroxide successively as in Expt. 10. Fractions (25 ml.) were collected and examined by electrophoresis in tungstate solution. Fractions containing the separated polyols were deionized as in Expt. 10. The maltitol was estimated by phenol sulphuric acid colorimetric method[66] and D-glucitol was estimated as in Expt. 10. The results are shown in Table 24 and in Fig.6.

Table 24

Fraction	Eluent	Polyol(i)	recovery %	
1	0.005M Na_2WO_4	Maltitol(ii)	3.20	
2	"	"	7.75	Total: 95.60%
3	"	"	15.00	
4	"	"	42.18	
5	"	"	21.87	
6	"	"	5.60	

Table 24 (continued)

7	-	-	-	
8 - 14	0.1N NaOH	-	-	
15	"	D-glucitol(iii)	32.80	Total 88.15%
16	"	"	36.75	
17	"	"	7.60	
18	"	"	8.50	
19	"	"	2.50	
20	"	"	-	

(i) Polyol detected.

(ii) Estimated by phenol/H_2SO_4 method[66] on Unicam SP.500 at 490 mμ

(iii) Periodate/chromotropic acid method[65]/Spekker filter No.606.

Experiment 15. Separation of D-glucitol from maltitol on molybdate form of resin.

A column (1.4 cm.dia.) containing molybdate form of resin (40 ml.) was prepared according to method 3 and washed with large excess of water until pH of the washings reached that of water (5.5 ± 0.1). A solution containing D-glucitol (31 mg.) and maltitol (31 mg.) in water (5 ml.) was added to the column and was allowed to flow at the rate of ca. 0.5 ml./min. in order to push-in the sample solution into the column, after which the column was allowed to stand for 4-5 hours. The column was then eluted with

water. Fractions (25 ml.) were collected and examined by electrophoresis in molybdate solution. After all the maltitol had been eluted, the eluent was replaced by 0.1N-sodium hydroxide. Fractions were collected and examined as above. The separated polyols were deionized and estimated as in Expt. 10. The results are shown in Table 25, and in Fig. 5.

Table 25

Fraction	Eluent	Polyol	recovery %	
1	water	Maltitol	6.10	
2	"	"	41.90	
3	"	"	17.30	Total =
4	"	"	7.25	85.49%
5	"	"	6.29	
6	"	"	3.35	
"	"	"	3.30	
8-10	-	-	-	
11-16	0.1N-NaOH			
16	"	D-glucitol	36.1	
17	"	"	42.74	
18	"	"	1.65	Total =
19	"	"	1.45	83.24%
20	"	"	1.3	

Experiment 16. Attempt to adsorb maltitol complex on various anion-exchange resins.

Anion exchange resins such as Amberlite IRA-400, IR-45, De-acidite FF.SRA-61 and De-acidite FF.SRA-69 were converted into molybdate or tungstate form according to method 3, and using the experimental procedure described for the separation of D-glucose from D-glucitol (Expts. 6(c) and 6(b). Several attempts were made to adsorb maltitol. It was noticed that none of the above mentioned resins in molybdate or tungstate form could retain maltitol.

Experiment 17. Adsorbtion of Isomaltitol on De-acidite FF/SRA-61 molybdate form.

De-acidite FF/SRA-61(Cl^-) was converted into molybdate form according to method 2 and packed into a column (1.4 x 32 cm.). A solution containing isomaltitol (27 mg.) in sodium molybdate (0.005M), of pH 5 (5 ml.) was added to the column. The column was allowed to stand overnight and was then eluted with sodium molybdate (0.005M) acidified to pH 5 with Amberlite IR-120 (H^+). Fractions (100 ml.) were collected and examined by electrophoresis in molybdate solution. No isomaltitol desorbed on elution with molybdate solution. On replacing the eluent to 0.1N-sodium hydroxide, isomaltitol desorbed from the resin, when the pH of the effluent reached 9.0.

Experiment 18. Adsorption of isomaltitol on Amberlite IRA-400 molybdate form.

Isomaltitol (30 mg.) dissolved in aqueous sodium molybdate (0.005M) of pH 5 (ca. 5 ml.) was added to a column containing molybdate form of Amberlite IRA-400 (40 ml.) prepared according to method 2. Rest of the procedure was the same as in Expt. 17. Results were the same as in Expt. 17, i.e. isomaltitol was adsorbed by the resin and desorbed on eluting the column with alkali.

Experiment 19(a). Attempted fractionation of reduced oligosaccharides of isomaltose series on molybdate form of resin column.

Molybdate form of resin (250 ml.) prepared as in method 3, was packed into a column (2.7 cm., dia.). An aqueous solution containing D-glucitol and isomaltodextrinols, the whole obtained from the acid hydrolysis and subsequent borohydride reduction of dextran, (310 mg.) was added to the column and was allowed to stand overnight. The column was then eluted with dilute sodium hydroxide of pH values 6.5, 7.5, 9 and 10 (prepared by dropwise addition of 0.1N-NaOH to water), and finally with 0.1N sodium hydroxide. Fractions (250 ml.) were collected and examined by electrophoresis in molybdate solution. It was observed that all the polyols had adsorbed on the

resin and were desorbed together, when the pH of the effluent reached 9.0.

Experiment 19(b) Attempted fractionation of isomaltodextrinols on a molybdate form of resin column by lowering the pH.

A molybdate form of resin column (1.4 x 32 cm.) was prepared according to method 3. An aqueous solution (5 ml.) containing D-glucitol and isomaltodextrinols obtained from partial acid hydrolysis and subsequent borohydride reduction[12] of dextran (40 mg.) was added to the column. The column was allowed to stand overnight, and was then eluted with water, dilute hydrochloric acid of pH values between 5.5 - 1.5 (adjusted by adding 0.1N HCl to water). Fractions (50 ml.) were collected and examined as in Expt. 19(a). It was observed that all the polyols desorbed when the pH of the effluent reached 3.

Experiment 20. Chromatography of polyols on paper partially impregnated with sodium molybdate.

Whatman chromatography paper No.1 was impregnated along the base line (as shown on page 140) with sodium molybdate dihydrate (1.5% W/V) acidified (pH 5.0) with 2N-sulphuric acid. Papers were dried in air at room temperature and the molybdate impregnated area was outlined in pencil under ultraviolet light. The polyols were spotted within the

impregnated area using D-glucose as marker outside the impregnated area and were irrigated in solvent (a). Separate chromatograms were run on unimpregnated paper as a control. The chromatograms were dried in air and the molybdate boundary re-marked (as above). The polyols were detected with spray (i). The results are shown in Table 26.

Table 26

Compound	R_G	R_G(Mo)
D-Glucose	1.0	0.9
D-Glucitol	1.0	0.2 (elongated)
D-Galactitol	1.08	0.19 (elongated)
D-Galactose	0.94	0.80 (elongated)

Width of original molybdate impregnated area = 4 cm.

Width of molybdate area after irrigation = 8 cm.

$$R_G = \frac{\text{Distance migrated by compound on unimpregnated paper}}{\text{Distance migrated by D-glucose on the same paper}}$$

$$R_G(Mo) = \frac{\text{Distance travelled by the compound, spotted in the molybdate area}}{\text{Distance travelled by D-glucose spotted outside the impregnated area}}$$

Experiment 21. Effect of pH of the molybdate solution on the paper chromatographic mobilities of polyhydroxy compounds.

Sodium molybdate solutions (5% W/V) adjusted to pH values between 2 - 9.1 were used for impregnating the paper.

Rest of the experimental procedure was the same as in Expt. 20. The results are shown in Table 27

Table 27

pH	Width of impregnated area (cm.) (i)	(ii)	R_G(Mo) D-Mannitol	D-Glucitol	Galactitol
2.0	4.0	15.4	1.29	0.97	1.32
3.0	3.4	4.5	0.84,0.55*	0.49,0.47*	0.57,0.84*
4.0	3.5	4.5	0.44,0.75*	0.31,0.51*	0.24-0.63, 0.75
5.0	4.0	5.5	0.2-0.3	0.2	0.2
6.0	4.0	5.6	0.2,e	0.2,e	,0.2e
7.2	4.0	5.1	0.2,0.75*	0.2,0.94*	0.74 0.2*
8.0	4.0	4.7	0.31,0.74*	0.37,0.6*	0.28,0.74*

Impregnated area (i) orignal (ii) after irrigation
e elongated spot * two spots

Experiment 22(a) Effect of pH on the mobilities of polyols, on paper partially impregnated ~~paper~~ with sodium tungstate.

An area (as shown on p.140) was impregnated on Whatman No. 1 chromatography paper with sodium tungstate solution (5%,W/V),acidified with 2N-sulphuric acid to pH values between 3.9-7.8. Rest of the experimental procedure was the same as in Expt. 20. The results are shown in Table 28.

Table 28

pH	Effect on impregnated area	R_D-glucitol D-Mannitol	D-Glucitol	Galactitol
3.9	-	0.22e	0.22e	0.22e
5.0	-	0.24	0.24	0.24
6.0	-	0.13	0.13	0.13
7.8	expanded	0.27	0.23	0.27

$$R_{D\text{-glucitol}} = \frac{\text{Distance travelled by the compound spotted within impregnated area}}{\text{Distance travelled by D-glucitol spotted outside the impregnated area}}$$

Experiment 22(b) Chromatography of polyols on paper partially impregnated with sodium tungstate solution of pH 6.0.

Experimental procedure was the same as in Expt. 22(a), except that papers were impregnated with 5% sodium tungstate solution of pH 6.0. The results are shown in Tables 3-5.

Experiment 23(a). Chromatography of polyhydroxy compounds on paper wholly impregnated with sodium tungstate solution (5%, pH 8) in different solvents systems.

Wholly impregnated papers were prepared (as shown on p. 140), polyols spotted and the papers were developed for 18-24 hours depending on the speed of the solvent system. The following solvent compositions gave elongated streaks extending from base line or no separation.

Solvent composition	Remarks
Acetone, n-butanol, water (7:2:1)	No migration
Acetone, ethyl acetate, water (5:4:2)	Elongated streaks
Acetone, ethyl acetate, water (5:4:3)	" "
Acetone, ethyl acetate, water (5:4:1)	" "
Acetone, ethyl acetate, water (6:2:2)	" "
Acetone, ethyl acetate, water (4:5:3)	" "
Acetone, ethyl acetate, water (1:1:1)	" "

Note: other solvent systems such as ethyl acetate, acetic acid, water (9:2:2), n-butanol, ethanol, water (40:11:19), ethyl acetate, acetic acid, formic acid, water (18:3:1:4) were also tried but results were neither useful nor reproducible. Results with some other solvents are given in Table 29.

Table 29

Compound	Solvent System (b)		(c)		(d)		(e)	
	R_G	R_G(W)	R_G	R_G(W)	R_G	R_G(W)	R_G	R_G(W)
D-glucose	-	1.0	1.0	1.0	1.0	1.0	1.0	1.0
D-Glucitol	-	0.16	0.9	0.18	1.0	0.15	1.08	0.15
D-mannitol	-	0.5	1.0	0.21	1.0	0.58	1.0	0.49
Galactitol	-	0.43	1.0	0.21	1.0	0.46	1.04	0.41
Xylitol	1.17	0.37	1.18	0.3	-	0.28	-	0.16
L-Arabinitol	1.25	-	1.25	-	1.25	0.82	-	0.6

Experiment 23(b). Chromatography of polyhydroxy compounds, on paper wholly impregnated with tungstate solution.

Twenty five centimeter wide strips of Whatman No.1 paper were dipped in sodium tungstate dihydrate (5% W/V) adjusted to pH 8.0 ± 0.1 (with dil. sulphuric acid), and dried at room temperature (overnight) after blotting in folds of filter paper.

Polyols were spotted in the usual manner and the chromatograms were irrigated in solvent (e) overnight. Polyols were detected with spray (i). Separate chromatograms were also run in the same tank as a control on untreated chromatography paper. The results are shown in Tables 6-9.

Experiment 24. Column chromatography of polyols on tungstate impregnated cellulose.

(i) Preparation of tungstate impregnated cellulose.

B.D.H. cellulose powder (200 g.) was suspended in sodium tungstate dihydrate (10%, W/V, 1.5 l.) acidified to pH 8 (with dil. sulphuric acid). The suspension was mixed well in an electrical blender (10 min.) and was allowed to stand (overnight). The cellulose was then filtered under suction and pressed in folds of thick filter paper to remove maximum possible moisture. The cellulose was then dried at room temperature (2 days).

(ii) Packing of column.

Above prepared dry cellulose was slurried with freshly prepared solvent (e). The slurry was mixed well in an electrical blender and poured into a glass column (1.8 cm. dia.) containing some solvent (e). After the cellulose had settled under gravity, it was pressed with a glass rod flatened at the end and thus a column height of 52 cm. was attained.

(iii) Preparation of polyol sample.

An aqueous solution (ca. 1 ml.) containing D-glucose (100 mg.), D-glucitol (102 mg.) and D-mannitol (98.6 mg.) was added to above prepared dry cellulose (ca. 2 g.) which was then evacuated in a vacuum desiccator over calcium chloride (5-6 hr.) and placed on the column after making a thin slurry with the solvent (e).

(iv) Elution.

The column was then eluted with the same solvent at a speed of ca. 0.5 ml./min. and fractions (5 ml.) were collected on an automatic fraction collector. Each fraction was examined by chromatography on paper wholly impregnated with tungstate [Expt. 23b]. On the basis of the chromatographic observations about the polyol contents of each fraction, the fractions were combined together to give separate lots of the different polyols.

After all the D-mannitol had been eluted off the column a stage was reached when only D-glucitol appeared in the eluate. At this stage solvent composition was changed to acetone: n-butanol: water (5:3:7), in order to increase the elution rate of D-glucitol. Separate lots of D-glucose, D-mannitol and D-glucitol thus obtained were evaporated to a syrup, taken up in water and estimated as described below:

(v) Estimation of D-glucose:

D-glucose was estimated polarimetrically using 2 dm. tube and a mercury light polarimeter.

(vi) Estimation of D-mannitol and D-glucitol.[16,31]

To the concentrates containing D-mannitol or D-glucitol, solid sodium tungstate dihydrate (ca. 100 mg.) was added, were acidified with 2N-sulphuric acid to pH 5.5 and 5.0 respectively, volume made up (25 ml.) and rotation measured as above (v). The results are shown in Table 30 also in Fig. 8.

Table 30

Fraction (5 ml.)	Total volume	Recovery (%)	Solute
1-24	0 - 120 ml.	-	-
25-35	120-180	31.4	D-Glucose
36-40	180-205	14.6	"
41-45	205-230	26.3	"
46-50	230-255	17.1	"
51-56	255-285	12.1	"
57-59	285-300	traces	D-Glucose and D-mannitol
60-71	300-355	54.0	D-mannitol
72-80	355-400	9.1	"
81-90	400-450	8.1	"
91-100	450-500	5.8	"
101-110	500-550	12.9	"
111-125	550-625	3.2	"
126-200	625-1 lit.	nil	No solute
201-300	1 lit.-1.5 lit.	22.0	D-glucitol
301-400	1.5 lit.(i.e. 500 ml. 5:3:7)	57.7	"

Experiment 25. Column chromatography of polyols on tungstate impregnated cellulose and isolation of polyols.

(i) A tungstate impregnated cellulose column (5 x 70 cm.) was prepared according to procedure outlined in Expt. 24. An aqueous solution (5 ml.) containing D-glucose (0.99 g.) D-mannitol (1.0 g.) and D-glucitol (0.995 g.) mixed with impregnated cellulose (ca. 10 g.) and treated as in Expt. 24, was added to column. The column was then eluted with freshly prepared solvent (e). Fractions (50 ml.) were collected and examined as in Expt. 24. Elution data based on chromatographic results is given hereunder.

Fractions (50 ml.)	Solvent	Polyol detected
1-65	(e)	-
66-86	"	D-glucose (A)
87-104	"	-
105-165	"	D-mannitol (B)
166-268	"	-
269-271	5:3:7*	-
272-283	"	D-glucitol (C) bulk in 278.

* Acetone: n-butanol: water, 5:3:7

(ii) Isolation of D-glucose.

A charcoal column (2.6 x 20 cm.) containing acid washed Ultrasorb S.C. 120/240 was prepared.[67] The column was washed with ethanol (250 ml.) and the alcohol was removed by washing with excess of water. Fractions containing D-glucose (see A above) were concentrated to a small volume under reduced pressure at 40° and added to the column. The column was washed with water until washings were free from tungstate ($KSCN/SnCl_2$ spot test).[64] Elute was then replaced by aqueous ethanol (2.5% V/V) until free from D-glucose (phenol/sulphuric acid test,[68] ca. 800 ml.). The eluate was then concentrated to a small volume under reduced pressure and was freeze dried. This was further dried over phosphorous pentoxide, in a vacuum desiccator to constant weight (0.969 g. 97.9%).

(iii) Isolation of D-mannitol.

Acid washed Ultrasorb S.C. 120/240 as prepared[67] above was packed into a sintered glass funnel (7 x 10 cm.). Fraction (B, above) was made alkaline with 2N-sodium hydroxide to pH 9.2 and placed on the charcoal containing funnel after standing overnight which was then washed with water until washings were free from tungstate. D-mannitol was then desorbed by eluting with aqueous ethanol (2.5%, 750 ml.). The washings containing D-mannitol were evaporated to dryness, taken up in water (ca. 5 ml.) made

alkaline to pH 9.2 and re-eluted through the same to obtain pure D-mannitol, which was then concentrated and crystallized from 95% ethanol (wt., 0.944 mg., 94.3%), m.p. 165°.

(iv) Isolation of D-glucitol.

Fractions (c, above) containing D-glucitol were evaporated to small volume and passed through charcoal (twice, as in (iii) above). The tungstate free D-glucitol was freeze dried and the syrup obtained was dried over phosphorus pentoxide under vacuum to constant weight (0.91 g., 81.2%).

Experiment 26. Thin-layer chromatography of polyhydroxy compounds on cellulose (CC-41) layers.

Whatman cellulose powder No. CC-41 (20 g.) was slurried with water (90 ml.). After mixing in an electrical blender (2 min.) layers (250 μm) were spread on glass plates. The plates were dried in an hot air oven (80°C) for 30 min. and stored in a desiccating box containing silica gel. Polyol solutions (5-10 μg.) were spotted as usual. Plates were developed in separate tanks containing solvent (e) and (g) for 30 and 45 min., respectively. Solvents were evaporated at room temperature and polyols were detected with spray (ii). The results are given in Table 31.

Table 31

Compound	R_F solvent (e)	(f)
L-arabinose	0.48	0.27
D-xylose	0.54	0.29
D-glucose	0.43	0.17
D-mannose	0.48	0.19
D-galactose	0.37	0.14

Experiment 27. Thin-layer chromatography of polyols on layers of cellulose, impregnated with sodium tungstate.

Thin-layer plates of Whatman cellulose CC-41 were prepared (Expt. 26) and sprayed evenly with sodium tungstate dihydrate (5% pH 8). The plates were re-dried in a hot air oven (80°C) for 20 min. Polyols were spotted and the plates were developed in solvent (e) and solvent (f). When the solvent front had reached to about 13 cm. the plates were removed from the tanks and the solvent evaporated in air.

Spot were detected with spray (a). After the spots had developed, the plates were dipped carefully in sodium thiosulphate solution (2.5%) to get permanent brown spots on white background. The results are shown in Table 32.

Table 32

Compound	R_F	
	Solvent (e)	Solvent (f)
D-glucose	0.26	0
D-glucitol	0.05	0
Galactitol	0.12	0
D-mannitol	0.16	0
Ribitol	0.36	0
D-arabinitol	0.32	0
Xylitol	0.23	0

Experiment 28. Thin-layer chromatography of polyols on layers of avicel.

(i) Avicel[69] (8 gm.) was made into slurry with water (32 ml.), was blended in an electrical blender (2-3 min.). Layers (0.25 mm.) were spread on glass plates. The plates were dried in an hot air oven (80°C) for 30 min. and stored in a desiccating box over silica gel.

(ii) Avicel[69] (7 g.) was slurried with sodium tungstate dihydrate (5%, 20 ml.) acidified to pH 8. The layers were prepared and dried as above.

Polyols were spotted on the plates as usual and the plates were developed in solvent (e) for ca. 45 min. The solvent was evaporated at room temperature and the polyols were detected with spray (i). The plates were finally dipped in sodium thiosulphate solution (2.5%) to get

permanent brown spots on white background. The results are shown in Table 33.

Table 33

Compound	R_F (controls) (i)	R_F (tungstate) (ii)	$\frac{R_F(W)}{R_F}$
D-glucose	0.32	0.28	-
D-glucitol	0.32	0.066	0.20
D-mannitol	0.33	0.15	0.45
Galactitol	0.31	0.13	0.39
L-arabinitol	-	0.33	-
Xylitol	-	0.17	-

Experiment 29. Effect of the presence of sodium molybdate on the specific rotation of D-galactose.

A solution of D-galactose (3.7232 g.) in water (200 ml.) was prepared and a portion (50 ml.) of this solution was diluted to 100 ml., (solution a). To another portion (50 ml.) sodium molybdate dihydrate (10 g.) was added and the whole acidified (pH 5.0) with 2N-sulphuric acid and the volume made up (100 ml., solution b) with water. Rotations of these solutions measured at the wavelength of sodium light, using 1 dm., polarimetric tube. The results are given in Table 34.

Table 34

Solution	Polarimetric (reading)	Specific rotation based on D-galactose
(a)	0.77°	82.83°
(b)	0.51°	54.79°

Experiment 30. Effect of pH on the rotation of D-galactose in the presence of a large amount of sodium molybdate.

D-Galactose (5.0018 g.) and sodium molybdate dihydrate (66.4 g.) were dissolved in water (500 ml.). The pH of the aliquots (25 ml.) was adjusted to values between 2.6 - 11.2 by addition of sulphuric acid or sodium hydroxide (2N). The volume of each was made to 50 ml. The optical rotation was measured immediately. The results are given in Table 35 and Fig. 9.

Table 35

pH	Specific rotation $[\alpha]_{D,}^{l} l = 1$ based on D-galactose
2.6	72.0°
3.5	68.0°
4.5	60.0°
5.2	42.0°
5.9	58.0°
6.5	60.0°
7.1	74.0°
7.6	84.0°
8.0	84.0°
8.5	85.0°
9.0	96.0°

Table 35 (continued)

9.6	92.0°
10.6	68.0°
11.2	66.0°

Experiment 31. Effect of relative concentration of D-galactose and molybdate on the optical rotation of D-galactose.

(a) Different volumes (2 ml. - 20 ml.) of equilibrated D-galactose solution (0.2 M) were mixed with varying volumes (20 ml. - 2 ml.) of sodium molybdate solution (0.2 M). The resulting solutions adjusted to pH 5.5 ± 0.1 with 2N-sulphuric acid and made up to 25 ml. Optical rotation (α_{G-Mo}) was measured in 0.5 dm. tube on a mercury light polarimeter.

(b) Solutions containing the same amount, (as above) of D-galactose were made up to 25 ml. and optical rotation (α_G) was measured.

The difference in optical rotation i.e. $\Delta\alpha = [(\alpha_G - (\alpha_{G-Mo})]$, has been plotted against mole fraction in Fig. 10(2), the results are also shown in Table 36.

Table 36.

Mole fraction molybdate	α_G	α_{G-Mo}	$\Delta\alpha$
0.0	1.34°	-	-
0.1	-	1.1	-
0.2	1.07°	-	-
0.3	0.94°	0.80°	0.14
0.4	0.80°	0.63°	0.17

(Table 36 continued)

0.5	0.67°	0.49°	0.18
0.6	-	0.35°	-
0.7	0.40°	0.27°	0.13
0.9	0.14°	0.08°	0.06

Experiment 32. Preparation of phenylboronic acid.[70]

Tetraphenyl tin (200 g.) was placed in a three necked flask fitted with a coldfinger mercury seal stirrer and a dropping funnel. The flask was cooled in a dry ice-acetone bath for 15 min. Boron trichloride (240 g.) cooled as above was added dropwise with continuous stirring. After all the boron trichloride had been added the reaction mixture was further stirred for 15 minutes. The bath was then removed and the contents were allowed to reflux at room temperature. After the vigorous reaction was over, the contents were refluxed over moderate heat for 2 hours. The reaction mixture was then cooled and the clear supernatant was decanted into a distillation flask, which was then distilled and the fraction distilling between 168-176°C was collected and was redistilled. (ca. 236 g.).

Phenylboron dichloride prepared above (236 g.) was diluted with carbon tetrachloride (150 ml.) and was cooled (-80°C) as above. This was then added dropwise to ice cold

water (2.0) over a period of 45 minutes. The carbon tetrachloride was then removed and phenylboronic acid was crystallized from hot water. The product was treated with decolourizing carbon and was recrystallized (115.5 g.). It had m.p. 217°C.

Experiment 33. Preparation of ribitol bisphenylboronate.

A solution containing phenylboronic anhydride (4.1 g., 1 mol. = 3 mol. phenylboronic acid) in methanol (20 ml.) was added to an aqueous solution (20 ml.) containing ribitol (2 g.). The resulting solution was shaken well and allowed to stand for 30 min. The white precipitate formed was filtered and washed with cold methanol and was dried in vacuo. Recrystallization from dry hexane afforded ribitol bisphenylboronate (4.25 g. 92%), m.p. 100-101°. (Found: C,63.66%; H,5.93%; B,6.66% required for $C_{17}H_{18}B_2O_5$, C,63.03%; H,5.6%; B,6.67%).

Experiment 34. Preparation of xylitol bisphenylboronate.

A solution containing phenylboronic anhydride (4.1 g., 1 mol = 3 mol. phenylboronic acid) in methanol (20 ml.) was added to xylitol (2.0 g. 1 mol.) in water. The precipitate obtained was filtered and washed with cold methanol. Recrystallization from benzene-hexane gave a crystalline solid (4.06 g. 95.3%). It had m.p. 93-94° (Found: C,63.07%; H,5.57%; B,6.61%. $C_{17}H_{18}B_2O_5$ requires

C,63.03%, H, 5.6%; B,6.67%).

Experiment 35. Preparation of L-lyxitol bisphenylboronate.

Phenylboronic anhydride (4.1 g., 1 mol. = 3 mol. phenylboronic acid) in methanol (20 ml.) was added to a solution containing L-lyxitol (2 g., 1 mol.) in water (20 ml.). The white precipitate produced was filtered and washed with cold methanol. Recrystallisation from a dry benzene-hexane mixture afforded L-lyxitol bisphenylboronate (3.45 g., 77.5%), m.p. 113-115°C. Found: C,63.25% H,5.59%, B,6.72. $C_{17}H_{18}O_5B_2$ requires C,63.03: H,5.6, B,6.67%).

Experiment 36. Preparation of mono-O-phenylcarbamoyl-ribitol bisphenylboronate.

The ribitol bisphenylboronate (1.35 g.) was dissolved in dry benzene (15 ml.). Phenyl isocyanate (0.5 ml. 1 mol.) was added and the solution was refluxed overnight. The reaction mixture deposited white needles on cooling. The product was filtered and washed with dry hexane. Recrystallization from dry benzene (hot) gave mono-O-phenyl-carbamoyl-ribitol-bisphenylboronate (1.3 g. 73%) m.p. 197-198°. (Found: C,64.98%; H,5.45; N,3.48% -Ph, 53.8%. $C_{24}H_{23}B_2NO_6$ requires C,65.05%; H,5.23%, N,3.16%; -Ph, 52.14%).

Experiment 37. Preparation of mono-O-phenylcarbamoyl xylitol bisphenylboronate.

To a solution containing xylitol bisphenylboronate

(2.1 g.) in dry benzene (25 ml.), phenyl isocyanate (0.8 ml.) was added and the resulting solution was refluxed overnight. The needles separated on cooling were recrystallized from dry benzene. The product (1.8 g., 81.1%) had m.p. 199-200°. (Found: C,65.05%; H,5.21%; N,2.94%; -Ph,53.8%. $C_{24}H_{23}B_2NO_6$ requires C,65.05%; H, 5.23%; N,3.16%; -Ph, 52.14%).

Experiment 38. Preparation of mono-O-phenylcarbamoyl-L-xylitol bisphenylboronate.

The L-lyxitol bisphenylboronate (3.5 g.) was dissolved in dry benzene (50 ml.). Phenyl isocyanate (2 ml., 1 mol.) was added and the solution was refluxed for 18 hours. The reaction mixture was concentrated to half the volume under reduced pressure and dry hexane (15 ml.) was added. The solid produced was filtered and washed with dry hexane. Recrystallization from a benzene-hexane mixture afforded mono-O-phenylcarbamoyl-L-arabinitol bisphenylboronate (3.1 g., 69.3%), m.p. 129-130°C. (Found: C,65.08%; H,5.17%; N,3.06%; -Ph, 50.57% $C_{24}H_{23}B_2NO_6$ requires C,65.05%; H,5.23%; N,3.16%; -Ph, 52.14%).

Experiment 39. Preparation of mono-O-phenylcarbamoyl -L-lyxitol.

The mono-O-phenylcarbmoyl-L-lyxitol bisphenylboronate (5 g.) was dissolved in acetone (100 ml.) and propane-1,3-diol (1.6 g., 2 mol.) was added which soon deposited a white

precipitate. The solvent was removed under reduced pressure at 40° and the residue was repeatedly extracted with petroleum ether (b.p. 60-80°) until free from boron (flame test). The residue on recrystallisation from methanol gave mono-O-phenylcarbamoyl-L-lyxitol (2.1 g., 65.4%) m.p. 178-180°C. (Found: C,52.99%, H,6.26%; N,5.19% $C_{12}H_{17}NO_6$ requires C,53.12%; H,6.32%; N,5.16%).

Experiment 40. Preparation of mono-O-phenylcarbamoyl -DL-ribitol.

The mono-O-phenyl carbamoyl-ribitol bisphenylboronate (105 mg.) was dissolved in acetone (3.5 ml.) containing propane-1,3-diol (36 mg. 2 mol.). The acetone was removed under reduced pressure at 40°. The residue was dissolved in acetone (1ml.) and was crystallized from light petroleum (35 mg. 54%) m.p. 119-120°. (Found: C,52.46%; H,6.26%; N,5.38%. $C_{12}H_{17}NO_6$ requires C,53.12%; H,6.32%; N,5.16%.

Experiment 41. Preparation of mono-O-phenylcarbamoyl-xylitol.

To a solution containing mono-O-phenylcarbamoyl-xylitol bisphenylboronate (1 g.) in acetone (5 ml.), propane 1,3-diol (0.35 ml. 2 mol.) was added. The solution was warmed on a water bath (ca. 2 min.). The solvent was removed and the residue was extracted with ether, until free from boron (flame test). The residue was recrystallized from ethanol. (m.p. 122°) (Found: C,53.35%; H,6.44%; N,5.29%. $C_{12}H_{17}NO_6$ requires

C,53.12%; H,6.32%; N,5.16%).

Experiment 42. Preparation of ethyl-N-phenylcarbamate.[80]

Phenyl isocyanate (5 ml.) was added to dry ethanol (5 ml.) and the solution was heated under reflux for 10 min. The solvent was removed, final traces of un-reacted phenyl isocyanate were eliminated at 0.1 mm. and the residue was recrystallized from light petroleum. It had m.p. 50°C; the yield was 6.7 g. (97%).

Experiment 43. Preparation of 1-deoxy-L-galactitol bisphenylboronate.

A solution (5 ml.) containing phenylboronic acid (730 mg. 2 mol.) in methanol was added to a solution containing 1-deoxy-L-galactitol (500 mg. 1 mol.) in water (5 ml.). The precipitate was filtered after standing (1 hr.) and washed with cold methanol (ca. 10 ml.). The precipitate was dried over calcium chloride under vacuum and was recrystallized from benzene-petroleum ether. Shining needles (0.85 g. 84.1%) had m.p. 113-114°C (Found: C,63.94%; H,6.11%; B,6.23%. $C_{18}H_{20}B_2O_5$ requires C,63.96%; H,5.96%; B,6.34%).

Experiment 44. Preparation of mono-O-phenylcarbamoyl--1-deoxy-L-galactitol bisphenylboronate.

To a solution containing 1-deoxy-L-galactitol bisphenylboronate (350 mg.) in dry benzene (15 ml.) phenyl isocyanate (0.2 ml., 1 mol.) was added and the solution

was refluxed overnight. The solvent was removed under reduced pressure, benzene (10 ml.) was added and was re-evaporated as before. The product was stored over sulphuric acid in vacuo to remove final traces of unreacted phenyl isocyanate. The product was then recrystallized from benzene-petroleum ether (100-120°). The product (330 mg., 75.14%) had m.p. 135-136°. (Found: C,65.75%; H,5.56%; N,3.26% -Ph,53.18%). $C_{25}H_{25}B_2NO_6$ requires C,65.69%; H,5.51%; N,3.06%; -Ph, 52.14%.

Experiment 45. Preparation of 3-O-methyl-D-glucitol bisphenylboronate.

A solution containing 3-O-methyl-D-glucitol (520 mg.) in water (5 ml.) was added to a solution containing phenylboronic acid (650 mg.) in methanol (5 ml.). On shaking a white gelatinous precipitate was deposited. The precipitate was washed with cold methanol and dried over calcium chloride in vacuo. Recrystallization from benzene-hexane afforded a crystalline solid (880 mg. 90.7%) m.p. 142° (Found: C,62.0%; H,6.04%; B,5.55%. $C_{19}H_{22}B_2O_6$ requires C,62.0%; H,6.02%; B,5.88%).

Experiment 46. Preparation of mono-O-phenyl carbamoyl -3-O-methyl-D-glucitol bisphenylboronate.

To a solution (10 ml.) containing 3-O-methyl-D-glucitol bisphenylboronate (336 mg.) in dry benzene, phenyl isocyanate (0.5 ml.) was added. The solution was refluxed (overnight), and filtered hot. After standing 1 hr., in the cold, in a well stoppered flask, white crystals separated from the filtrate. The product was recrystallized from dry benzene, had m.p. 221°, yield 290 mg. (66%). (Found: C,64.36%; H,5.45%; N,2.78%. $C_{26}H_{27}B_2NO_6$ requires C,64.1%, H,5.58%; N,2.87%).

Experiment 47. Catalytic oxidation of phenylcarbamate obtained from L-lyxitol bisphenylboronate (XXa or XXIa).

(i) Preparation of catalyst.

Platinum oxide (Adams catalyst), 1g. was suspended in aqueous acetone (50%, 50 ml.). A continuous stream of hydrogen was percolated through the suspension with continuous stirring until a finely divided black suspension was obtained (4-5 hrs.). The flask containing the platinum catalyst was placed in a desiccator and was evacuated repeatedly to remove hydrogen.

(ii) Oxidation.

Platinum catalyst (ca. 100 mg.) was suspended in a solution (40 ml.) containing mono-O-phenylcarbamoyl-L-

lyxitol bisphenylboronate (500 mg.) in 50% aqueous acetone. A continuous stream of oxygen was percolated through the suspension. The solution was heated to 30 ± 0.2° and was maintained at this temperature. Sodium bicarbonate (1%) solution was added dropwise to maintain the pH of the reaction mixture between 8.5 ± 0.1, thus a total of ca. 9 ml. had been added when pH of the solution became constant.

Samples were withdrawn at intervals and examined by paper chromatography in solvent (g) which revealed, in addition to phenylboronic acid and O-phenylcarbamoyl-L-lyxitol, the presence of a compound at R_F 0.23 which was visible under U.V. light. Oxidation was stopped after 15 hr., the catalyst was filtered and washed with water. The filtrate and the washings were deionized with Amberlite IR-120(H^+) and concentrated to a syrup. The slow migrating component (R_F 0.23) was separated from the reaction mixture by preparative chromatography in solvent (g) and concentrated to a syrup (ca. 116 mg.) paper electrophoresis in phosphate (pH 7.2) showed it to be a single component ($M_{gal.Acid}$ = 1.0). The syrup was dissolved in methanol (10 ml.), a small piece of metallic sodium was added and the solution heated on water bath (ca. 30 min). After standing overnight, water (5 ml.) was added and methanol was removed under reduced pressure. The resulting solution was deionized with Amberlite IR120(H^+)

and concentrated under reduced pressure to a syrup (34.5 mg.). Paper chromatography of this syrup in solvent (g) and spray reagent (iii) gave a yellow spot at R_F 0.18, which was invisible under U.V. light. The syrup was dissolved in a mixture of water (ca. 0.1 ml.) and methanol (ca. 0.2 ml.), 4% methanolic potassium hydroxide was added dropwise with shaking until no more precipitate was formed. The precipitate was filtered, dissolved in water, decolourized with charcoal and recrystallized as above (43.1 mg.). It had m.p. 162°. There was no depression in its m.p. on admixture with authentic potassium-D-lyxonate (m.p. 166°)[73] whereas it showed depression in m.p. on admixture with potassium-L-arabonate.

Experiment 48. Estimation of -Ph-B and -Ph in phenyl-boronates and phenyl-carbamoyl phenyl-boronates.

Phenylboronic anhydride (102.5 mg.) was dissolved in 50% (V/V) aqueous methanol (1.25 l.). Aliquots (1-10 ml.) of this solution were diluted to 100 ml. with aqueous methanol (50%, V/V.). The absorption of these standard solutions was measured at 219 mμ on a unicam SP-500 ultraviolet absorptiometer.

The optical density of the solution was as follows:

Volume taken (ml.)	Volume made up (ml.)	Optical density
1	100	0.075
1.5	"	0.115
2.0	"	0.147
3.0	"	0.226
4.5	"	0.333
5.5	"	0.40
8.5	"	0.64
10.0	"	0.73

The optical density was plotted against concentration and used as a standard graph for the estimation of Ph-B and -Ph group.

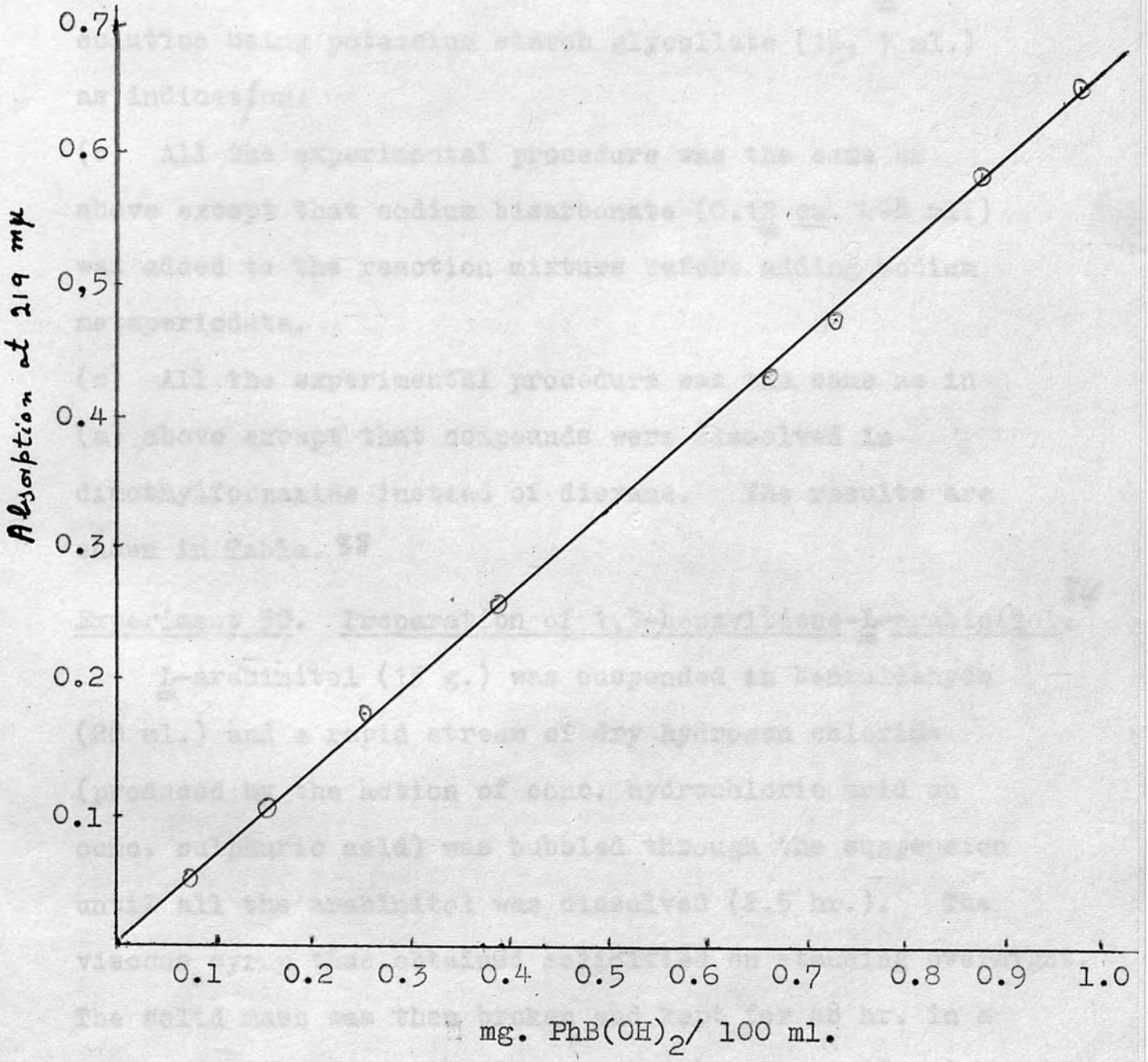
Experiment 49. Periodate oxidation of phenylboronates and mono-O-phenylcarbamoyl-phenylboronates.

(a) The periodate oxidation was carried out according to the procedure described in ref. 79.

Known weights were dissolved in dioxan (25 ml.) and water (25 ml.) and a solution of sodium metaperiodate (10 ml. 0.25N) was added. The volume was made up to 100 ml. with water.

(b) Aliquots (10 ml.) were withdrawn at intervals to which water (ca. 20 ml.), saturated sodium bicarbonate

Fig. 11 Standard graph for the estimation of PhB- and Ph- group (Expt. 48)



solution (ca. 4-6 ml.), potassium iodide solution (20%, ml.) and standard sodium arsenite (0.1N) were added. After 15 minutes the excess of arsenite was estimated by titration with standard iodine (0.1N) solution using potassium starch glycollate (1%, 1 ml.) as indication.

(b) All the experimental procedure was the same as above except that sodium bicarbonate (0.1N ca. 4-8 ml.) was added to the reaction mixture before adding sodium metaperiodate.

(c) All the experimental procedure was the same as in (a) above except that compounds were dissolved in dimethylformamide instead of dioxane. The results are shown in Table. 38

Experiment 50. Preparation of 1,3-benzylidene-L-arabinitol. 74

L-arabinitol (15 g.) was suspended in benzaldehyde (20 ml.) and a rapid stream of dry hydrogen chloride (produced by the action of conc. hydrochloric acid on conc. sulphuric acid) was bubbled through the suspension until all the arabinitol was dissolved (2.5 hr.). The viscous syrup thus obtained solidified on standing overnight. The solid mass was then broken and kept for 48 hr. in a vacuum dessicator containing conc. sulphuric acid and potassium hydroxide (pellets). The product was then

successively triturated with ether, dilute sodium hydrogen carbonate and water. Recrystallization from ethanol afforded, 1,3-benzylidene-L-arabinitol (8.9 g. 37.6%) m.p. 148-150°C).

Experiment 51. Preparation of 1,3-benzylidene-L-arabinitol monophenylboronate.

A solution of phenylboronic anhydride (0.7 g. 0.33 mol.= 1 mol. phenylboronic acid) in methanol (10 ml.) was added to a solution of 1,3-benzylidene-L-arabinitol (1 g.) in hot water. The resulting solution was shaken well and was allowed to stand overnight. The precipitate was filtered and washed with cold water. Recrystallization from benzene-hexane (dry) afforded 1,3-benzylidene-L--arabinitol monophenylboronate (1.4 g., 93.33%), m.p. 131°. (Found: C,66.46%; H,6.02%. $C_{18}H_{19}B_1O_5$ requires C,66.28%; H,5.87%).

Experiment 52. Attempted preparation of ribitol-monophenylboronate.

Phenylboronic anhydride (340 mg. 0.33 mol. = 1 mol. phenylboronic acid), in benzene (12.5 ml.) was added to ribitol (500 mg. 1 mol.). The mixture was shaken vigorously and was heated to 40°C. A thick white precipitate was obtained. The precipitate was dissolved in dry benzene and was filtered (residue ca. 240 mg., ribitol).

The filtrate deposited a crystalline solid on the dropwise addition of hexane. It had m.p. 93-99 and showed no depression in m.p. on admixture with ribitol bisphenylboronate.

Experiment 53. Attempted preparation of tri-O-phenyl carbamoyl-ribitol mono-phenylboronate.

Phenylboronic anhydride (1.039 g. 0.33 mol. = 1 mol. phenylboronic acid) in dry benzene (4.0 ml.) was added to a solution containing ribitol (1.52 g. 1 mol.) in dry dioxane (75 ml.). The resulting solution was refluxed under a Dean and Stark head overnight. The solvents (ca. 80 ml.) were distilled off and phenyl isocyanate (3.8 ml.) was added. The reaction mixture was refluxed overnight and evaporated to dryness under reduced pressure. The resulting solid was triturated with dry hexane to remove unreacted phenyl isocyanate. The amorphous solid thus obtained was insoluble in benzene, petroleum ether and ligroin, had m.p. 210-215°C.

Repeated attempts were made to prepare monophenyl-boronate from pentitols, but all were unsuccessful.

Experiment 54. Estimation of formaldehyde by chromotropic acid.

Erythritol (6.47 mg.) was dissolved in water and excess sodium metaperiodate (0.42 mg.) was added. The solution was adjusted to 100 ml. with water. After standing (in dark) for ca. 2 hours portions of this solution were diluted to make standard solutions of varying concentrations. To 1 ml. of each solution 20% sodium sulphite (0.1 ml.), water (1 ml.) and chromotropic acid reagent (8.4 ml.) was added and were heated on boiling water bath for 15 min. The tubes were cooled to room temperature and half saturated thiourea (0.5 ml.) was added to each. The absorption at 5700Å was measured against a blank containing chromotropic acid reagent. The following values were obtained.

Concentration HCHO/µg. ml.	Absorption at 5700Å
15.9	0.095
31.8	0.178
63.6	0.360
79.5	0.442
95.4	0.545

Portions (1 ml.) of the reaction mixture (Expt. 49) were treated with chromotropic acid and absorptions measured as described above. Results are shown in Table 38.

Table 38

Compound	Method	Periodate consumed (mol.)	Formaldehyde produced (mol.)
Phenylboronic anhydride	a	0.22x3	-
Ribitol bisphenylboronate	a	4.8	-
"	b	4.18	1.9
"	c	4.06	1.83
Mono-O-phenylcarbamoyl-ribitol bisphenylboronate	a	4.47	-
"	b	3.03	0.95
"	c	3.03	1.01
Mono-O-phenylcarbamoyl ribitol	c	2.91	0.90
L-lyxitol bisphenylboronate	b	4.35	2.21
Mono-O-phenylcarbamoyl-L-lyxitol bisphenylboronate	b	3.25	1.05
"	c	3.01	0.97
Mono-O-phenylcarbamoyl-L-lyxitol	b	3.14	0.88
Xylitol bisphenylboronate	b	3.68	1.69
Mono-O-phenylcarbamoyl-xylitol bisphenylboronate	c	3.13	1.01
Mono-O-phenylcarbamoyl, 1-deoxy-L-galactitolbis-phenylboronate	c	3.08	-
Ethylcarbamate ($C_2H_5CO_2NHPh$)	a	0.29	-
"	b	0.12	-

Experiment 55. Infrared spectroscopy.

(a) The infrared spectra in the hydroxyl stretching region of the pentitol bisphenylboronates in Table 37 were determined for 0.005 M solutions in dry carbon tetrachloride by using a Unicam SP-100 Spectrophotometer and 4 cm. cells. Collaboration of Dr. D. Steel during this experiment is greatly acknowledged.

(b) The IR spectra of all the other compounds mentioned in the text were obtained using a Perkin Elmer Infracord spectrophotometer. The samples used were in the form of a mull with nujol.

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