ACTA CHEMICA SCANDINAVICA 15 (1961) 1913–1922

Paper Electrophoresis of Carbohydrates in Sulphonated Phenylboronic Acid Buffer

PER J. GAREGG and BENGT LINDBERG

Svenska Träforskningsinstitutet, Träkemiska avdelningen, Stockholm Ö, Sweden

The paper electrophoresis of some carbohydrates in sulphonated phenylboronic acid buffers is described. These allow electrophoresis to be run at neutral pH values and are therefore useful for the separation and characterisation of alkali-labile carbohydrate derivatives. Specific configurations are required for the hydroxyls for complex formation and information of structural significance can be obtained for unknown carbohydrates. The new buffers are a useful complement to those previously described, especially for the separation of glycitols.

In the course of investigations on acetylated carbohydrates it was found desirable to separate various acetylated xylose derivatives by paper electrophoresis. The electrolytes commonly used, borate buffer [1], arsenite [2], basic lead acetate [2], sodium hydroxide [2], and recently germanate buffer [3] are inapplicable to acetylated carbohydrates; due to the alkalinity of these buffer solutions deacetylation or acyl migration may occur during the electrophoresis. Decreasing the pH leads to too low mobility of the carbohydrate derivatives. Paper electrophoresis in molybdate [4] at pH 5 was inapplicable, as xylose and its derivatives show insignificant mobilities in this buffer system. A new concept was therefore tried, namely, the use of phenylboronic acid instead of boric acid in borate electrophoresis, and further by substitution in the benzene ring of a group that dissociates in aqueous solution, the production of increased mobility of the complexes formed between carbohydrates and the hydroxylated boron atom at neutral pH values. At pH 7 no significant increase in the rate of mobility of the sugars tried was found by using phenylboronic acid instead of boric acid. Nitration in the *ortho* [5] or the *meta* [5] position of the benzene ring gave significantly higher mobilities, highest for the *m*-derivative; the poor water solubility of these compounds, however, rendered them unsuitable. Treatment of phenylboronic acid in 25 % oleum [6] at 0° yielded sulphonated phenylboronic acid; the sodium salt of the acid was very soluble in water and gave satisfactory mobilities for a number of carbohydrates at pH values about 7. The present paper deals with an investigation of the rates of migra-

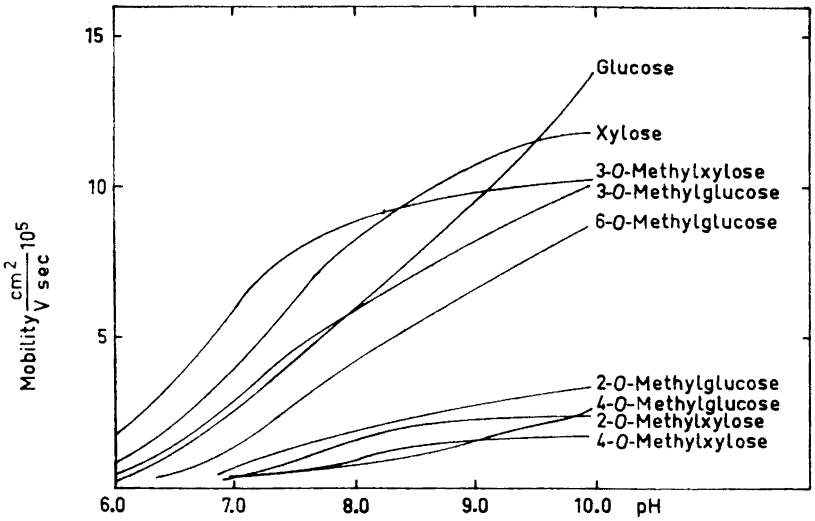

Fig. 1. The mobilities of xylose, glucose and monomethyl ethers in sulphonated phenylboronic acid at various pH values.

tion of various carbohydrates in buffers containing sulphonated phenylboronic acid.

The infrared spectrum, using the potassium bromide pellet technique, of the mono sodium salt of the sulphonated phenylboronic acid gave some indication of the position of substitution. The absorption at 6.25 μ and the weak one at 6.7 μ are typical of mono-, *ortho-* and *meta*-substituted benzene [7,8] the bands

Table 1. Monosaccharides.

$$M_G = \frac{\text{true distance of migration of the substance}}{\text{true distance of migration of glucose}}$$

Substance	M_G in sulphonated phenylboronic acid pH 6.5	M_G in germanate pH 10.7	M_G in borate pH 10.0
D-Xylose	1.8	1.4	1.00
D-Lyxose	2.3	1.9	0.71
L-Arabinose	2.4	1.5	0.96
D-Ribose	4.7	2.1	0.77
L-Rhamnose	0.50	1.3	0.52
D-Glucose	1	1	1
D-Mannose	1.1	1.4	0.72
D-Galactose	1.8	1.3	0.93
D-Altrose	5.8		0.97
L-Sorbose	8.5	2.0	0.95
D-Tagatose	8.6	2.4	0.95
D-Fructose	9.3	2.1	0.90

Table 2. Glycosides.

Substance	M_G in sulphonated phenylboronic acid pH 6.5	M_G in germanate pH 10.7	M_G in borate pH 10.0
Methyl α-D-xylofuranoside	2.3	0.05	0.33
Methyl β-D-xylopyranoside	0.0	0.0	0.0
Methyl β-D-glucofuranoside	2.0	—	—
Methyl α-D-glucopyranoside	0.0	0.0	0.11
Methyl α-D-mannofuranoside	16.0	1.4	—
Methyl β-D-mannopyranoside	0.0	0.4	0.31
Methyl β-D-galactofuranoside	0.4	0.2	0.31
Methyl β-D-galactopyranoside	0.0	0.5	0.38

at 14.15 μ and possibly at 14.45 μ are typical of mono- and *meta*-substituted benzene, the strong absorption band at 13.55 μ is characteristic for *ortho*-substitution only, a weaker diffuse band at 12.3—12.6 μ is typical of *m*-substitution only, while the absence of absorption at 6.10 μ and 6.60 μ rules out *para*-substitution. It therefore seems probable that the sulphonic acid is largely the *ortho*-sulphonic acid while a smaller part is the *meta*-isomer; it seems improbable that the *para*-isomer is present.

The buffers were 0.05 M aqueous solutions of sulphonated phenylboronic acid containing the requisite amount of sodium hydroxide for the desired pH. Additional pH stability of the buffers was obtained by the addition of monopotassium phosphate and sodium hydroxide. Electrophoreses were run at 40° and 500 V (about 10 V/cm). Under these conditions the mobility of glucose ranged from 0.08 cm/h at pH 6.0 to 4.5 cm/h at pH 10 as shown in Fig. 1. At pH 6.5, which was generally used throughout this work, the average mobi-

Table 3. Methyl ethers of glucose and xylose.

Substance	M_G in sulphonated phenylboronic acid pH 6.5	M_G in germanate pH 10.7	M_G in borate pH 10.0
D-Xylose	1.8	1.4	1.00
2-*O*-Methyl-D-xylose	0.0	0.0	0.39
3-*O*-Methyl-D-xylose	2.9	1.7	0.66
4-*O*-Methyl-D-xylose	0.0	0.3	0.21
5-*O*-Methyl-D-xylose	13.0	—	—
3,5-Di-*O*-methyl-D-xylose	9.3	—	—
D-Glucose	1	1	1
2-*O*-Methyl-D-glucose	0.0	0.0	0.23
3-*O*-Methyl-D-glucose	1.3	1.4	0.80
4-*O*-Methyl-D-glucose	0.0	0.3	0.24
6-*O*-Methyl-D-glucose	0.5	0.96	0.80

Table 4. Glycitols.

$$M_M = \frac{\text{true distance of migration of the substance}}{\text{true distance of migration of mannitol}}$$

Substance	M_M in sulphonated phenylboronic acid pH 6.5	M_M in germanate pH 10.7	M_M in borate pH 10.0
Glycol	0.0	0.0	—
Glyceritol	0.0	0.2	0.54
Erythritol	0.1	0.5	0.83
L-Threitol	0.3	—	0.83
erythro Butane 2,3-diol	0.0	—	0.14
threo Butane 2,3-diol	0.3	—	0.56
D-Ribitol	0.3	0.6	0.93
L-Arabinitol	0.6	0.9	0.96
Xylitol	0.9	0.9	0.87
D-Glucitol	1.3	1.0	0.91
D-Mannitol	1	1	1
L-Iditol	1.4	—	0.89
Galactitol	1.0	1.1	1.07
2-*O*-Methyl-L-arabinitol	0.1	0.1	
4-*O*-Methyl-L-arabinitol	0.5	0.5	
2-*O*-Methyl-D-xylitol	0.4	0.6	
3-*O*-Methyl-xylitol	0.1	0.3	
5-*O*-Methyl-D-xylitol	0.6	—	
1-Deoxy-D-glucitol	0.9		
2-*O*-Methyl-D-glucitol	1.2	0.8	
3-*O*-Methyl-D-glucitol	0.1	0.4	
4-*O*-Methyl-D-glucitol	1.3	0.7	
6-*O*-methyl-D-glucitol	0.6		
6-Deoxy-L-mannitol (Rhamnitol)	0.8	—	
1,6-Di-*O*-acetyl-D-mannitol	0.8	—	
2-*O*-Methyl-D-galactitol	0.5	0.9	
3-*O*-Methyl-D-galactitol	1.0	0.7	
6-*O*-Methyl-D-galactitol	0.8	0.8	

lity of glucose was 0.38 cm/h and that of mannitol, used as standard for measuring the relative mobility of sugar alcohols was 3.4 cm/h. The mobilities for the carbohydrates and related compounds are shown in Tables 1 to 6 and in Figs. 1, 2 and 3. The M_G and $M_{mannitol}$ (M_M) values in germanate are taken from Lindberg and Swan [3] and those in borate from Foster [1] and Frahn and Mills [2].

As will be seen from Table 1 the spread in mobilities of monosaccharides in sulphonated phenylboronic acid is much greater than in either germanate or borate. In borate pairs of aldopentoses and aldohexoses with similar configura-

tion, *e.g.* arabinose and galactose, xylose and glucose, lyxose and mannose have very similar mobilities. In both sulphonated phenylboronic acid and in germanate the pentoses are the faster in each pair, and, except for L-rhamnose, glucose is the slowest sugar in both buffers while being the fastest in borate. The high mobility of the three ketohexoses in sulphonated phenylboronic acid probably, as discussed below in connection with Table 2, reflects the high proportion of the furanose forms of these sugars present in aqueous solution since most of the mobility is due to furanosidic 1,2-*cis*-diols, as is the case in germanate [3]. Similarly the differences in the mobilities of the aldopentoses and the aldohexoses possibly reflect the stability of the most stable pyranosidic chair form of these sugars. The differences in mobility among the three ketohexoses is small in all three buffers.

In Table 2 are given the mobilities of a few glycosides. The very high mobility of the mannofuranoside indicates that furanosidic *cis*-1,2-diols are by far the most important in complex formation. In mannofuranoside the hydroxyls on $C_{(2)}$, $C_{(3)}$ and $C_{(5)}$ also are situated for a possible tridental complex formation with boron [1]. The much lower mobility of the xylofuranose is probably due to complex formation on $C_{(3)}$ and $C_{(5)}$.

The possibility of contribution to the mobility of the furanosides by the $C_{(5)}$ and $C_{(6)}$ hydroxyls [1] is unlikely as glyceritol does not migrate (see Table 4).

No contribution to the mobility of the sugars comes from pyranosidic *cis*-1,2-diols or from pyranosidic *cis*-1,3-diols as shown by the lack of mobility of the mannopyranoside, galactopyranoside, and D-inositol, likewise no contribution is given by the $C_{(4)}$ and $C_{(6)}$ hydroxyls in the glucoside configuration.

These results are different to those obtained with germanate and borate where, apart from the interactions mentioned above, contributions are given by pyranosidic *cis*-1,2-diols and possibly also by 1,3-diols of suitable configuration, *e.g.* $C_{(4)}$ and $C_{(6)}$ in glucopyranose.

The results obtained for methyl ethers of xylose and glucose, Table 3, can with the exception of the 3-*O*-substituted sugars be explained as for the furanosides and pyranosides. Blocking of the $C_{(2)}$ and $C_{(4)}$ hydroxyls yields derivatives which are immobile in sulphonated phenylboronic acid. The lack of mobility of the 2-*O*-methyl ethers indicates, as has been discussed by Foster [1], that these derivatives are not to any appreciable degree present in the furanose form; substitution in the 4-position entails pyranose forms with which no interaction occurs.

As in germanate buffer, 3-substitution yields enhanced mobility of the two sugars. This is difficult to interpret on the basis of present knowledge and will be the subject of a subsequent investigation. Substitution on the $C_{(6)}$ hydroxyl in glucose leads to decreased mobility in sulphonated phenylboronic acid, more so than in borate, while in germanate this has little effect. While the reduction in mobility in borate may be attributed to blocking the interactions with the 4,6- or 5,6-hydroxyls this should not be the case in sulphonated phenylboronic acid, since α-D-glucopyranoside and glycerol are immobile on electrophoresis; the explanation possibly lies in the furanose-pyranose and/or in the α-β-equilibria of glucose and 6-*O*-methylglucose. A similar example is the slower mobility of rhamnose compared to mannose. (Table 1). As would be expected, the mobilities of 5-*O*- and 3,5-di-*O*-methylxylose are high in

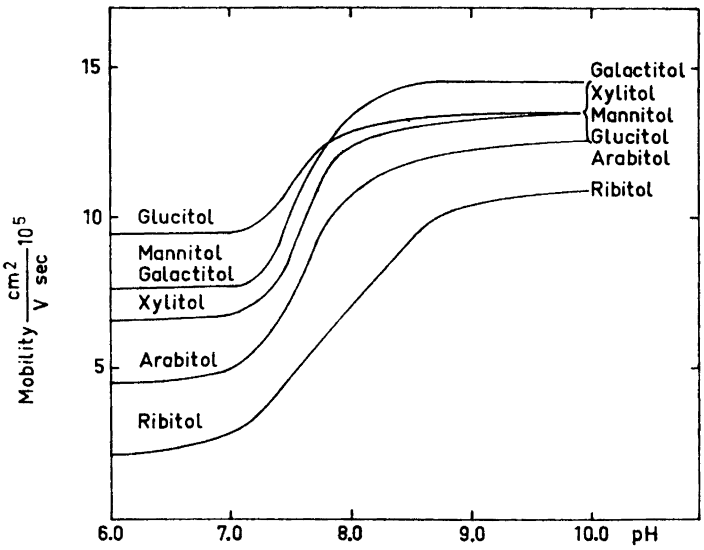

Fig. 2. The mobilities of some glycitols in sulphonated phenylboronic acid at various pH values.

sulphonated phenylboronic acid, this again reflects the importance of furanosidic *cis*-1,2-diols in complex formation. The differences between the two methyl ethers may reflect different α-β-equilibria under the conditions used.

In Fig. 1 are given the results of runs in sulphonated phenylboronic acid at various pH values for xylose, 2-, 3- and 4-*O*-methyl-xylose and the similar series for glucose including the 6-*O*-methyl ether. At pH 9 to 10 the relative mobilities strongly resemble those obtained with borate. As the pH decreases, fewer possibilities for complex formation exist, and important differences in relative mobilities arise, *e.g.* for the sugars glucose, xylose, 3-*O*-methyl-glucose and 3-*O*-methylxylose. Superimposed on this is the general decrease in mobility of all sugars with decreasing pH due to decreasing ionisation of the boronic acid.

A series of runs similar to the one described in connection with Fig. 1, but where each sugar and methyl sugar was dissolved in buffer solution at the appropriate pH and kept for a minimum period of 24 h did not give any difference to the mobilities depicted in Fig. 1, outside that due to experimental error, indicating that the equilibria between the various forms of the sugars and the complex were rapidly established.

Better separations are obtained for sugar alcohols run in sulphonated phenylboronic acid than those obtained in both borate and germanate (Table 4); the separations at low pH values are better than those obtained at higher pH as will be seen from Fig. 2. At pH 10 the relative mobilities in sulphonated phenylboronic acid resemble those in borate at the same pH. A lowering of the pH of borate does not lead to separations as good as those obtained with

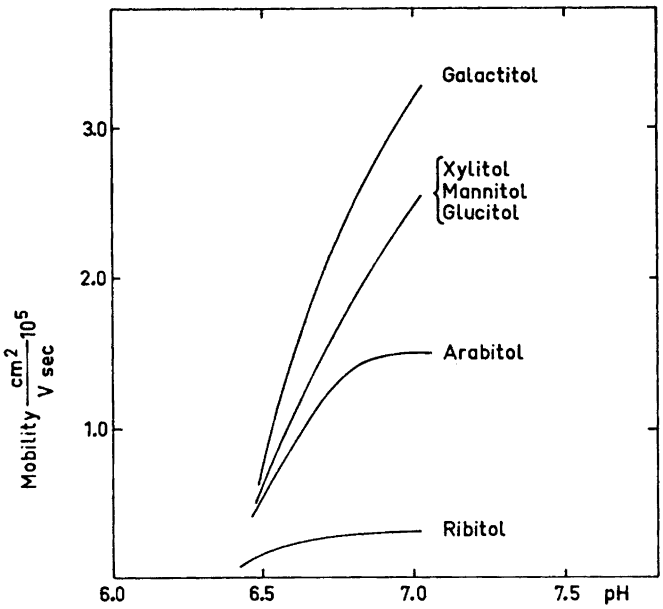

Fig. 3. The mobilities of some glycitols in boric acid below pH 7.

phenylboronic acid as shown in Fig. 3; at low pH values the rate of migration in borate of the glycitols approaches zero and relative differences in mobility between the substances cannot be observed. The lack of restriction on the conformations of the glycitols and their derivatives makes the rationalisation of their relative mobilities in sulphonated phenylboronic acid rather difficult. From a study of the M_M values of the glycitols and their methyl ethers it does however seem probable that 1,2-*trans*-diols are important in complex formation as shown by the mobilities of the glycitols up to and including the pentitols, and also by the high mobility of iditol.

From the lack of mobility of glycol and glycerol it may be inferred that primary 1,2-diols as well as 1,2-diols with one primary hydroxyl do not form complexes. That primary hydroxyl groups play some part in complex formation is, however, indicated by the reduction in mobility by substituting xylitol on $C_{(1)}$ (or $C_{(5)}$) and by 1-deoxy-D-glucitol, 6-*O*-methyl-D-glucitol as well as 1,6-di-*O*-acetyl-D-mannitol and L-rhamnitol which all move slower than their parent hexitols. Substitution on the primary hydroxyls in threitol has no effect on the mobility while in erythritol this leads to a mobility too low to measure, as indicated by the mobilities of *threo*- and *erythro*-butane-2,3-diol. The importance of 1,2-*trans*-diols is reflected by the low mobilities of 2-*O*-methyl-L-arabinitol, 2-*O*-methyl-D-galactitol, 3-*O*-methyl-xylitol and 3-*O*-methyl-D-sorbitol. In the hexitol series however, the M_M values for the glycitols and their derivatives indicate that the situation is too complex to be accounted for by the number of 1,2-*trans*-diol groupings only. It might be possible that tridental

Table 5. Reduced disaccharides.

Substance	Linkage	M_M in sulphonated phenylboronic acid pH 6.5
Sophoritol	β (1→2)	1.2
Laminaribiitol	β (1→3)	0.1
Cellobiitol	β (1→4)	1.1
Gentiobiitol	β (1→6)	0.6

Table 6. Inositols.

Substance	M_M in sulphonated phenylboronic acid pH 6.5	M_M in germanate	M_M in borate
myo-Inositol	0.0	0.1	0.59
D-Inositol	0.0	0.4	0.70
epi-Inositol	1.8	0.9	0.81

complex formation occurs with at least some of the glycitols; that tridental complex formation is possible is also indicated by the high mobility of *epi*-inositol (Table 6).

The relative mobilities of the reduced disaccharides in sulphonated phenylboronic acid in Table 5 closely correspond to those of the corresponding methyl glycitols. This might be useful for the characterisation of disaccharides and glycityl glycosides.

The rate of migration of three inositols is shown in Table 6. Of these, *myo*-inositol and *epi*-inositol in one of the chair conformations have three axial *cis*-hydroxyls in 1,3,5-positions that may form tridental complexes [1]. *Myo*-inositol with 5 axial hydroxyls in this conformation is immobile while *epi*-inositol with 4 axial hydroxyls has a high mobility. The high mobility of *epi*-inositol may however be due to a complex with the two axial hydroxyls in the 1,3-position in its most stable chair form. D-Inositol has in its most stable chair form two axial hydroxyls, these however are 1,2-*trans* and D-inositol does not migrate.

Buffers containing sulphonated phenylboronic acid have proved useful for separating mono-acetylated monosaccharides [9] at sufficiently low pH values to avoid deacetylation, the mobilities closely correspond to those of the corresponding methyl ethers under the same conditions.

DISCUSSION

The substitution of phenylboronic acid with an ionisable group and paper electrophoresis with this substance at neutral pH values, thus decreasing the ionisation of the boric acid residue of the molecule, leads to much more selective complex formation than is the case with borate at pH 10. At the

same time, reasonable rates of migration of the complexes are obtained. With reducing sugars and glycosides by far the largest contribution to the mobilities is given by furanosidic 1,2-*cis*-diols. A smaller contribution is given by 3,5-hydroxyls, *e.g.* in the xylofuranose structure. No contributions to the mobilities are given by pyranosidic *cis*-1,2-diols or by the $C_{(4)}$ and $C_{(6)}$ hydroxyls in the glucose configuration. The very fast rate of movement of *epi*-inositol suggests either tridental complex formation with inositols with a *cis-cis*-1,3,5-triol grouping in one of its chair conformations provided no more than four of the hydroxyls are axial in this conformation, or a complex involving two axial hydroxyls in 1,3-position.

Substitution on $C_{(3)}$ in xylose and glucose gives enhanced mobility; this phenomenon is not perfectly understood and is currently being investigated *.

The number of conformations possible makes the rationalisation of the mobilities of the glycitols difficult. It is however obvious that *trans*-1,2-diol groupings are important in complex formation, and it is possible that tridental complexes are present in the equilibrium mixtures.

Electrophoresis in sulphonated phenylboronic acid at neutral pH values makes possible a number of separations of carbohydrates substituted with alkali-labile groups. Some separations are better in this than in other buffer systems due to the specific configurations required for the hydroxyls for complex formation with sulphonated phenylboronic acid. With reducing sugars and glycosides the separations obtained resemble those in germanate at pH 10.6, although the steric requirements for complex formation are more extreme than for germanate.

With the glycitols, however, the separations are much better than those obtained with borate [1] or germanate [3]. The separations to some extent resemble those reported for sodium arsenite [2] and basic lead acetate [2] buffers but is different from those obtained in molybdate buffer [4] and sulphonated phenylboronic acid can be regarded as a complement to these buffers.

Sulphonated phenylboronic acid buffers should also prove useful for separating compounds that form strong complexes with this buffer from those that form weak complexes on anion exchange resins.[10]

EXPERIMENTAL

Evaporations were performed at 40° in a vacuum.

Sulphonation of phenylboronic acid [6]. Phenylboronic anhydride (100 g) was added in small portions during 1 h to 25 % oleum (600 g) cooled in an ice-salt or dry ice-alcohol bath to −5 to 0° under vigorous mechanical stirring, the temperature was maintained at about 0° to control the exothermic reaction. At the end of the additions the mixture was stirred at 0° for a further 10 min and then poured into crushed ice (3 l) under vigorous stirring. The precipitate (probably biphenyl sulphone) was filtered off and the filtrate neutralised to pH 7 with aqueous sodium hydroxide under external cooling. Some precipi-

* *Added in proof.* Bishop and Cooper [15] have studied the equilibrium mixtures in the Fischer syntheses starting from xylose and from some of its methyl ethers. They found that the percentages of the furanosides were much higher with 3-*O*-methyl-D-xylose than with D-xylose as starting material. If this reflects the equilibrium mixture of the two sugars in aqueous solutions, the high mobility of 3-*O*-methylxylose in sulphonated phenylboronic acid and in germanate solution would seem to be explained.

tated sodium sulphate was filtered off and the filtrate concentrated to dryness under reduced pressure. The sodium salt of the sulphonated phenylboronic acid was extracted with several portions of boiling 80 % aqueous ethanol, the combined extracts were evadorated to dryness. The crystalline residue was extracted with several portions of boiling 90 % aqueous ethanol, the combined extracts were concentrated and poured into absolute ethanol and kept at −4° overnight. The precipitate was collected by filtration and air dried. The yield of sodium sulphate-free material varied between 80 to 120 g depending upon the temperature control of the sulphonation.

Buffer. The buffers were 0.05 M solutions of sulphonated phenylboronic acid containing sodium hydroxide to give the desired pH and also monopotassium phosphate and sodium hydroxide in the ratio giving the same pH; the molarity of the solution was 0.06 with respect to phosphate.

Method. The apparatus was similar to that described by Kunkel and Tiselius [11] and the procedure as described by Foster [1]. Whatman No. 1 filter paper was used, the starting line was placed 20 cm from the cathode end of the cooling plate. Hydroxymethylfurfural was used to mark the starting line and glucose or mannitol as standard references for the relative rates of migration. Water at 40° was passed through the cooling plate. The voltage applied was 0.5 kV corresponding to about 10 V/cm and the time was 3 or 6 h. The electrolytes in the two electrolyte vessels were mixed again after each run, and after running electrophoresis for each 3 h at 0.5 kV the electrophoresis was interrupted and the solutions in the two vessels were mixed again to avoid too large pH variations. Good reproducibility for the relative rates of migration at various pH values was obtained.

The reducing sugars were located by spraying with anisidine hydrochloride [12] and the non-reducing sugars and inositols with periodate-benzidine [13]. Distinct, although occasionally somewhat elongated spots were obtained, and migration distances were measured from the centres of the spots.

Substances. Most of the substances were available at this laboratory or at the Institution of Wood Chemistry, The Royal Institute of Technology [3]. The partially methylated glycitols not previously available [3] were obtained from the corresponding reducing sugars by reduction with Raney nickel [14].

Acknowledgement. The authors are indebted to Miss Hillevi Svensson for her skilful assistance and to Dr. K. Torssell for a gift of the sodium salt of sulphonated phenylboronic acid, and for directions concerning its preparation.

REFERENCES

1. Foster, A. B. *Advances in Carbohydrate Chem.* **12** (1957) 81.
2. Frahn, J. L. and Mills, J. A. *Australian J. Chem.* **12** (1959) 65.
3. Lindberg, B. and Swan, B. *Acta Chem. Scand.* **14** (1960) 1043.
4. Bourne, E. J., Hutson, D. H. and Weigel, H. *Chem. & Ind., London* **1959** 1047; *J. Chem. Soc.* **1961** 35.
5. Saeman, W. and Johnson, J. R. *J. Am. Chem. Soc.* **53** (1931) 711.
6. Torssell, K. *Private communication.*
7. Colthup, N. B. *J. Opt. Soc. Am.* **40** (1950) 397.
8. McMurry, H. L. and Thornton, V. *Anal. Chem.* **24** (1952) 318.
9. Garegg, P. J. *Unpublished results.*
10. Khym, J. X. and Zill, L. P. *J. Am. Chem. Soc.* **73** (1951) 2399; **74** (1952) 2090.
11. Kunkel, H. G. and Tiselius, A. *J. Gen. Physiol.* **35** (1951) 89.
12. Hough, L., Jones, J. K. N. and Wadman, W. H. *J. Chem. Soc.* **1950** 1702.
13. Cifonelli, J. A. and Smith, F. *Anal. Chem.* **26** (1954) 1132.
14. Karabinos, J. V. and Ballun, A. T. *J. Am. Chem. Soc.* **75** (1953) 4501.
15. Bishop, C. T. and Cooper, F. P. *Abstract Papers Am. Chem. Soc.* **140** (1961) 3 D.

Received May 17, 1961.