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Paper Electrophoresis of Carbohydrates in Germanate Buffer

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The paper electrophoretic mobility of some carbohydrates in germanate buffer has been studied. The method seems to be a useful complement to other methods for the separation of these substances. Information of structural significance can be obtained from the mobilities of unknown carbohydrates.

Paper electrophoresis of neutral substances requires the presence of a complexing agent that will give charged complexes with the substances under investigation. Defined spots are obtained either when there is a rapid interconversion between complex and substance or when the equilibrium is displaced strongly towards the complex. The former is preferable as it permits greater differences in mobility between individual substances. Paper electrophoresis of neutral carbohydrates is usually done in borate buffer [1] but hydrogen sulphite [2], arsenite [3], basic lead acetate [3], molybdate [4] and sodium hydroxide [3] have also been used.

Germanate is known to give complexes with polyalcohols, and the reaction has recently been studied by Antikainen [5]. In the present investigation a study has been made of the electrophoretic mobility of some carbohydrates in germanate buffer. After some preliminary experiments the electrophoreses were run in 0.05 M sodium germanate buffer of pH 10.7 at 40° and 1 500 V (25—30 V/cm). The mobilities are given as M_G-values, that is relative to the mobility of glucose. Under these conditions glucose moves about 11 cm in 90 min. The mobilities of the various carbohydrates investigated are given in Tables 1—7. For some substances the M_G-values in borate buffer have also been given. These values are taken from Foster [1] or Frahn and Mills [3] or were determined in separate experiments. The conditions used by Foster [1] and by Frahn and Mills [3] are slighty different, but their M_G-values are very similar and could be compared directly.

As can be seen from Table 1 there are large differences between the mobilities of the monosaccharides in borate and in germanate. In borate, glucose is the fastest sugar and pairs of pentose and hexose sugars with the same configuration, *e. g.* xylose and glucose, have almost the same mobility. In germanate, glucose is the slowest sugar and pentoses move considerably faster than the corresponding hexoses. As discussed below, furanosidic 1,2-*cis*-diols seem

to contribute more to the mobility in germanate than the corresponding pyranosidic groupings, and an M_G-value in this buffer may reflect the conformational stability of the most stable pyranosidic chair form of the sugar. The individual differences between the ketoses is rather small in both buffers, but their mobilities relative to glucose are considerably higher in the germanate buffer.

A study of the mobilities of various partially methylated sugars has enabled some conclusions to be drawn about the structures of the borate complexes [1]. The mobilities in germanate and borate of some sugar derivatives, chiefly methyl ethers, are given in Table 2. Some special effects in germanate buffer can be seen. Firstly, the glucose and xylose derivatives, in which the hydroxyl group at $C_{(2)}$ is replaced by a methoxyl or amino group or by a hydrogen atom, show no mobility at all. This result strongly indicates that germanate complexes with reducing sugars are formed exclusively by 1,2-*cis*-diols in a cyclic form of the sugar. Since sugar alcohols, *e. g.* 2-*O*-methyl-D-glucitol (Table 6), have rather high mobilities, the contributions from complexes with the open chain form of the sugar must be negligible. In the light of these results is seems probable that the same is also true for borate complexes with the sugars. 2-*O*-Methyl-D-galactose, which has a 3,4-*cis*-diol grouping in its pyranose forms, gives a charged complex with germanate.

The high mobilities in germanate of glucose and xylose derivatives in which the $C_{(3)}$-hydroxyl group is etherified or replaced by hydrogen is difficult to understand. A possible explanation may be that, in the parent sugar, this hydroxyl takes part in the formation of an uncharged complex, competing with the charged complex of the α-pyranose or α-furanose form. When the 3-hydroxyl is absent, the uncharged complex cannot be formed and the mobility is therefore higher. The same effect also occurs in the galactose series, where the contribution from the 3,4-*cis*-diol is restricted to the pyranose forms and is probably not very important. In 3-*O*-methyl-D-mannose however the mobility relative to mannose is considerably reduced. In mannose both furanose and pyranose forms can give 2,3-*cis*-diol complexes. The 1,2-complexes are given by the β-forms only and may be less significant than the 2,3-complexes.

Table 1. Monosaccharides.

Substance	M_G in germanate	M_G in borate
D-Glucose	1	1
D-Mannose	1.4	0.72
D-Allose	1.8	0.83
D-Galactose	1.3	0.93
D-Xylose	1.4	1.00
D-Lyxose	1.9	0.71
D-Ribose	2.1	0.77
L-Arabinose	1.5	0.96
L-Rhamnose	1.3	0.52
D-Fructose	2.1	0.90
L-Sorbose	2.0	0.95
D-Tagatose	2.4	0.95

Table 2. Sugar derivatives.

Substance	M_G in germanate	M_G in borate
2-*O*-Methyl-D-glucose	0.0	0.23
2-Deoxy-D-glucose	0.0	0.29
2-Amino-2-deoxy-D-glucose	0.1	—
3-*O*-Methyl-D-glucose	1.4	0.80
3-*O*-Benzyl-D-glucose	1.2	—
3-Deoxy-D-glucose	1.6	0.85
4-*O*-Methyl-D-glucose	0.3	0.24
4-*O*-Benzyl-D-glucose	0.2	0.17
5-*O*-Methyl-D-glucose	1.6	0.65
6-*O*-Methyl-D-glucose	0.96	0.80
2,3-Di-*O*-methyl-D-glucose	0.0	0.12
3-*O*-Methyl-D-mannose	0.8	—
2-*O*-Methyl-D-galactose	0.6	0.43
3-*O*-Methyl-D-galactose	1.4	0.63
4-*O*-Methyl-D-galactose	0.4	0.30
6-*O*-Methyl-D-galactose	1.2	0.86
2,3-Di-*O*-methyl-D-galactose	0.1	—
2,4-Di-*O*-methyl-D-galactose	0.0	—
2,6-Di-*O*-methyl-D-galactose	0.5	—
2-*O*-Methyl-D-xylose	0.0	0.39
3-*O*-Methyl-D-xylose	1.7	0.66
4-*O*-Methyl-D-xylose	0.3	0.21

The low mobilities of 4-*O*-methyl-sugars in borate has been attributed to their inability to give a furanose structure [1]. The mobilities of these sugars are also low in germanate, and this explanation is supported by the very high mobility of 5-*O*-methyl-D-glucose, which can only assume this form. The results also show that the greater complex-forming ability of 1,2-*cis*-diols in a furanose ring relative to that of 1,2-*cis*-diols in a pyranose ring is more pronounced in germanate than in borate.

Table 3. Pyranosides and related substances.

Substance	M_G in germanate	M_G in borate
Methyl-β-D-glucopyranoside	0.0	0.19
Methyl-α-D-glucopyranoside	0.0	0.11
1,5-Anhydro-D-glucitol	0.05	0.20
Methyl-β-D-mannopyranoside	0.4	0.31
Methyl-α-D-mannopyranoside	0.5	0.42
1,5-Anhydro-D-mannitol	0.6	0.40
Methyl-β-D-galactopyranoside	0.5	0.38
Methyl-α-D-galactopyranoside	0.6	0.38
Methyl-β-D-xylopyranoside	0.0	0.0
Methyl-α-D-xylopyranoside	0.0	0.0
Methyl-β-L-arabinopyranoside	0.6	0.38
Methyl-α-L-arabinopyranoside	0.7	0.38
1,6-Anhydro-β-D-glucopyranose	0.0	—

Table 4. Furanosides and related substances.

Substance	M_G in germanate	M_G in borate
1,4-Anhydro-D-glucitol	0.4	—
Methyl-α-D-mannofuranoside	1.4	—
Ethyl-β-D-galactofuranoside	0.2	0.31
Methyl-α-L-arabinofuranoside	0.0	0.035
Methyl-β-D-xylofuranoside	0.05	0.33
1,6-Anhydro-α-D-galactofuranose	0.0	—

Methylation of glucose or galactose in the 6-position gives a slight reduction in the M_G-value in germanate. This is probably due to the elimination of the interaction with the 5,6-diol in the furanose form. With borate, complexes may also be formed at 4,6-positions in the glucopyranoses.

Of the glycopyranosides and the related 1,5-anhydrides of the glycitols (Table 3), only those with a 1,2-*cis*-diol grouping show significant mobility in germanate. There are rather small differences between the individual substances containing one such grouping. For an anomeric pair of glycosides, the anomer with the aglyconic group in an axial position in the most stable chair form has a somewhat higher mobility. Interactions with the 4,6-position, which contribute to the mobility in borate, *e. g.* in the glucosides, seem to be of no importance in germanate. One of the furanosides, which has a 1,2-*cis*-diol grouping, shows a high mobility in germanate but the others (Table 4), without such a grouping, show low mobilities. The xylofuranoside gives a complex with borate, probably due to interaction with the 3,5-hydroxyls [1], but no complex of this type seems to be formed with germanate. Methyl-α-D-glucofuranoside has a high mobility in borate ($M_G = 0.73$), which can be attributed to a tridentate complex involving the 3,5,6-hydroxyls [1]; 1,4-anhydro-D-glucitol would be expected to have a similar mobility in borate. It is questionable whether a tridentate complex is also formed with germanate and the relatively low mobility can also be due, at least to some extent, to complex formation with the 5,6-hydroxyls.

The M_G-values for a number of oligosaccharides that are given in Table 5 can easily be rationalised in terms of contributions from the reducing and non-reducing monomer residues, based on M_G-values for the analogous sugar ethers and glycosides. The effect of a 3-substituent in the glucose and galactose series is also clearly marked in the disaccharides.

A number of glycitols and glycitol methyl ethers were studied; their M_G-values are listed in Table 6. As these substances have less fixed conformations and a variety of competing complexes may be formed, it is rather difficult to interpret the results. An analysis of the M_G-values obtained show , however, that charged complexes are preferentially formed with 1,2-*trans*-diols (*threo*-configuration) and 1,2-diols in which one of the hydroxyls is primary. 1,2-*cis*-Diols (*erythro*-configuration) and 1,3-diols may also contribute, but to a much lesser extent. The same difference between 1,2-*cis*- and 1,2 *trans*-diols can be observed in borate buffer [1]. It seems to be difficult to draw any conclusions of

Table 5. Oligosaccharides.

Substance	Component * sugars	Linkage **	M_G in germanate	M_G in borate
Nigerose	G→G	α(1→3)	1.3	0.69
Laminarobiose	»	β(1→3)	1.1	0.69
Maltose	»	α(1→4)	0.4	0.32
Cellobiose	»	β(1→4)	0.3	0.23
Isomaltose	»	α(1→6)	0.9	0.69
Gentiobiose	»	β(1→6)	1.0	0.75
Lactose	Ga→G	β(1→4)	0.7	0.38
Melibiose	»	α(1→6)	1.4	—
Galactosido-galactose	Ga→Ga	β(1→3)	1.4	0.69
» »	»	β(1→4)	0.7	0.50
» »	»	β(1→6)	1.4	0.83
Mannosido-mannose	M→M	β(1→4)	1.1	0.66
Mannosido-glucose	M→G	β(1→4)	0.7	0.43
Glucosido-mannose	G→M	β(1→4)	0.8	0.58
Sucrose	G↔F	—	0.1	—
Raffinose	Ga→G↔F	—	0.4	—

* G = Glucose, Ga = Galactose, M = Mannose and F = Fructose.
** All linkages pyranosidic, except in the last two oligosaccharides.

Table 6. Glycitols.

Substance	M_G in germanate	M_G in borate
Glycol	0.0	—
Glyceritol	0.4	0.44
Erythritol	1.0	0.75
D-Ribitol	1.2	—
L-Arabinitol	1.8	0.90
D-Xylitol	1.7	—
D-Glucitol	1.9	0.89
D-Mannitol	1.9	0.90
Galactitol	2.1	0.98
Lactitol	1.5	—
Cellobiitol	1.2	—
Melibiitol	1.5	—
2-*O*-Methyl-D-xylitol	1.2	—
3-*O*-Methyl-D-xylitol	0.5	—
2-*O*-Methyl-D-glucitol	1.5	—
3-*O*-Methyl-D-glucitol	0.8	—
4-*O*-Methyl-D-glucitol	1.4	—
2-*O*-Methyl-D-galactitol	1.7	—
3-*O*-Methyl-D-galactitol	1.4	—
6-*O*-Methyl-D-galactitol	1.6	—

Table 7. Cyclitols.

Substance	M_G in germanate	M_G in borate
scyllo-Inositol	0.2	0.05
myo-Inositol	0.7	0.53
D-Inositol	1.0	0.63
epi-Inositol	1.8	0.73
Sequoitol	0.5	0.18
Pinitol	1.0	0.66
Mytilitol	0.2	0.20

structural significance from the electrophoretic mobilities of open chain polyhydroxy compounds in germanate buffer. It does however appear to be a useful method for the separation of for instance the pairs ribitol and arabitol or 2- and 3-*O*-methyl-D-glucitol. Electrophoresis in molybdate [4] seems to be more suitable for the separation of substituted glycitols.

The mobilities of some cyclitols in germanate (Table 7) are rather similar to the corresponding values in borate. It has been demonstrated that tridentate complexes are formed in borate [6] and it is possible that similar complexes are formed with germanate, especially as *scyllo*-inositol shows a definite mobility in this buffer.

DISCUSSION

In a germanate ion with the coordination number six, the distance between two oxygen atoms is 2.64 Å. This is rather close to the distance between the oxygen atoms in tetrahedrally coordinated borate, 2.72 Å (Values for the atomic distances are taken from Pauling [7]). The results described above indicate that germanate gives complexes with 1,2-*cis*-diols in furanosides (O—O = 2.51) and pyranosides (O—O = 2.86) and with open chain 1,2-diols with a *threo*-configuration (the O—O distances are taken from Reeves [8]). The same type of complex is formed with borate but the complexes between germanate and furanosidic 1,2 *cis*-diols seem to be especially strong. The same seems to be true for the complexes with arsenite [3]. Borate also gives complexes with 1,3-diols but this possibility seems to be very limited with germanate. Thus methyl-β-D-glucopyranoside and methyl-β-D-xylofuranoside, although they have low mobilities in germanate (almost nil) show significant mobilities in borate, almost certainly due to the formation of complexes at the 4,6- and 3,5 positions, respectively.

The possibility of the formation of tridentate complexes especially with the cyclitols, can not be excluded, but the mobilities of this substances may also be due to complexes with 1,2-*cis*-diols. *Scyllo*-Inositol, however, has no such groupings but may give an 1,3-*cis*-complex in a boat conformation. With open chain sugars, complexes with 1,3-diols seem to be of lesser importance. As in the case of borate complexes and cyclic acetals, there is little sign of the formation of germanate complexes with 1,2-*trans*-diols in a pyran ring or with open chain 1,2-diols with an *erythro*-configuration.

Germanium has also the coordination number four. For tetrahedrally coordinated germanate the distance between two oxygen atoms is 3.32 Å and any complex formed with this should be uncharged. In furanose rings, the distance between the oxygen atoms in a 1,2-*trans*-diol is 3.45 Å, and such a diol might therefore give uncharged complexe with germanate. There are also other diols, in which the distance between the oxygen atoms can assume values close to 3.32 Å. As mentioned above, derivatives of glucose and xylose, in which the 3-hydroxyl group is replaced by another group, rather unexpectedly have higher mobilities than the parent sugars; this can be explained by the formation of uncharged complexes which will compete with the charged complexes *. It must be admitted, however, that this effect is not perfectly understood.

As can be seen from the Tables, paper electrophoresis in germanate buffer is a useful complement to electrophoresis in borate [1] and other buffers [2-4]. Many separation problems can be dealt with better by electrophoresis in germanate instead of borate and other buffers. For instance, this is probably the only method by which all five mono-*O*-methyl-D-glucoses can be separated in one operation. It should also be possible to obtain information on the structures of unknown carbohydrates from their electrophoretic mobilities in germanate. This is true especially for partially etherified sugars and for reducing oligosaccharides, in which rather few groupings contribute to the mobility and the effect of substitution is often considerable.

Germanate is non-toxic and requires no special precautions. Non-reducing sugars are sometimes difficult to detect on germanate impregnated paper. The same difficulty is observed in borate electrophoresis and may be overcome by increasing the amount of the component or by modifying the method used for localisation. The same spray reagents can be used with both buffer systems.

EXPERIMENTAL

Buffer. An 0.05 M solution of germanate was prepared by adding 50 % aqueous sodium hydroxide to a suspension of germanium dioxide in water until a clear solution of pH 10.7 was obtained. This solution turns milky on keeping but is still quite suitable for use. The milkiness depends on the formation of polygermanates and is accelerated if the solution is heated. After each run, the solutions at the cathode and the anode should be mixed, as the pH increases in the former and decreases in the latter. The pH of the buffer should be checked regularly.

Procedure. The apparatus, of the Kunkel and Tiselius type [9], and the technique were essentially the same as described by Foster [1]. Whatman No. 1 filter paper was used. The electroendosmotic flow is rather high, and the starting line was placed 20 cm from the cathode end of the cooling plate. 2,3,4,6-Tetra-*O*-methyl-D-glucose and D-glucose were run on all papers, the first in order to make a correction for the electroendosmosis, the latter as an internal standard. The electrophoreses were run for 90 min. at 1 500 V, equivalent to 25—30 V/cm. Water at 40° was circulated through the cooling-plate. Under these conditions the migration of D-glucose was about 11 cm.

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* *Added in proof.* This explanation is probably not valid as we have recently observed that 3-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose have higher mobilities than their parent sugars also on electrophoresis in sulphonated phenylboronic acid buffers of low pH-values (6—7). In these buffers all complexes must be charged. (P. Garegg and B. Lindberg. *Unpublished results.*)

Localisation of spots. The reducing sugars were localised by spraying with anisidine hydrochloride [10]. Non-reducing sugars were localised either with the silver nitrate — sodium ethoxide reagent [11] or with periodate-benzidine [12], after neutralisation of the paper by spraying with acetic acid. Some spots could not be detected with the former reagent, but the latter, although somewhat more complicated, was more reliable. Distinct, circular spots were usually obtained. The migration distance was measured from the centre of the spots.

Substances. Most of the substances studied were available in this laboratory or at the wood chemistry department of the Swedish Forest Products Research Laboratory. 5-*O*-Methyl-D-glucose and 3-*O*-methyl-D-mannose were kindly supplied by Professor O. Th. Schmidt, Heidelberg, and Dr. G. O. Aspinall, Edinburgh, respectively. The partially methylated glycitols were prepared, in small quantities, by reduction of the corresponding methylated sugars with Raney nickel according to Karabinos and Ballun [13]. These substances, most of which are not described in the literature, were not characterised.

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