Ionophoresis of Carbohydrates. Part II. Some Pyranose and Furanose Derivatives of* D-*Glucose.*

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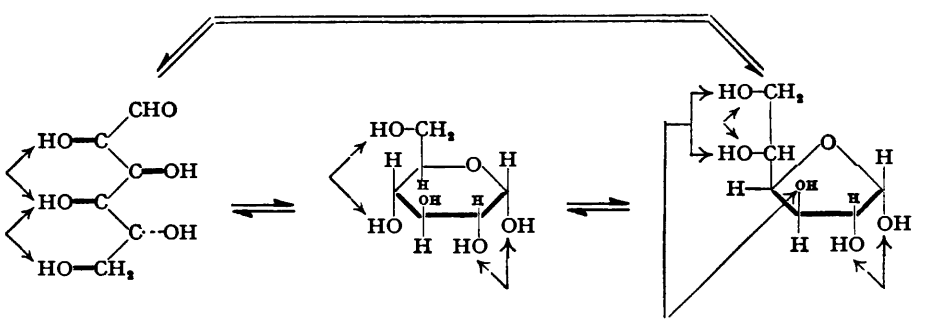
Ionophoresis in alkaline borate buffer of a series of D-glucopyranoside and D-glucofuranoside derivatives has been studied. Hitherto unrecognized reactions between these derivatives and borate ions have been observed. The results obtained give some information about the equilibrium of reducing forms of D-glucose in aqueous alkaline borate solution.

In Part I * evidence was presented which indicated that the reaction of reducing sugar derivatives with borate ions in the aqueous alkaline media used in filter-paper ionophoresis was more complex than that reported to occur in aqueous solutions of boric acid (Böeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189). Thus it appeared that of the cyclic and open-chain forms of the reducing sugar derivatives in equilibrium in aqueous solution, the latter forms (see formulæ) reacted most strongly with borate ions. Other, hitherto unrecognized interactions have now been observed and are described herein.



Representation of part of the equilibrium of the reducing forms of D-*glucose in solution. The β-pyranose and β-furanose forms are not included.*

In order to limit the possibilities for reaction of reducing sugar derivatives with borate ions, a series of D-glucose derivatives has been studied in which the ring type was fixed (for

* Part I, *J.*, 1953, 982.

a preliminary report see Foster and Stacey, Abs. Papers, Amer. Chem. Soc. Meeting, New York, September, 1954). As in Part I, M_G values (rates of migration compared with that of D-glucose) have been taken as measures of borate-ion interaction.

Pyranose Derivatives.—The M_G values of a series of derivatives of D-glucopyranose are listed in Table 1. This shows that methyl α- and β-D-glucopyranoside have a definite, though weak, interaction, approximately in the ratio 1 : 2. The M_G values of the derivatives (V—VIII) indicate that the 2- and 3-hydroxyl groups are not involved in complex formation whereas the zero M_G values of the derivatives (IX—XIII) indicate the complex to be formed across the 4 : 6-hydroxyl groups in the glucopyranosides (I) and (II). There is here a further analogy between the reaction of borate ions and of benzaldehyde with carbohydrates (cf. Part I) [condensation of benzaldehyde with (I) or (II) to give a 4 : 6-*O*-benzylidene derivative is well known]. A further analogy is the condensation of phenyl phosphorodichloridate with methyl α-D-glucopyranoside to give a 4 : 6-(hydrogen phosphate) (Baddiley, Buchanan, and Szabó, *J.*, 1954, 3826).

The wide difference (0·08) in the M_G values of methyl α- (I) and β-D-glucopyranoside (II) was unexpected. In the preferred conformation of the β-anomer all the non-protonic substituents are in equatorial positions round the pyran ring, but the α-anomer has the glycosidic methoxyl group in an axial position where it may impede the movement of borate ions across one face of the molecule. This is reflected in the lower M_G value of the α-anomer (I). That the glycosidic methoxyl group in the β-anomer (II) does not impede the approach of borate ions is indicated by the M_G values of 1 : 5-anhydro-D-glucitol (IV) and 1 : 5-anhydro-2-deoxy-D-glucitol (V) which are close to that of the β-glucoside (II). Non-protonic substituents are absent from $C_{(1)}$ in the anhydro-compounds (IV) and (V). The remoteness of the glycosidic methoxyl group and the groups on $C_{(4)}$ and $C_{(5)}$ in the α-anomer (I) precludes hydrogen bonding which reduces their availability for complex formation. Thus the difference in M_G values of the α- and β-anomers (I) and (II) appears to be a simple steric effect. It is of interest that the M_G value of the 2-deoxyglycoside (VII) is unexpectedly high.

In the trehaloses, which comprise two D-glucopyranose residues mutually substituted at the glycosidic centres, the operation of a simple steric effect would suggest a series of increasing M_G values $\alpha\alpha < \alpha\beta < \beta\beta$. From the results in Table 1, movement of ββ-

TABLE 1. *M_G values of derivatives of D-glucopyranose.*

Derivative	M_G	Derivative	M_G
Methyl α-D-glucopyranoside (I)	0·11	1 : 6-Anhydro-β-D-glucopyranose (β-glucosan) (X)	0·00
Methyl β-D-glucopyranoside (II)	0·19	Methyl α-D-xylopyranoside (XI)	0·00
Ethyl α-D-glucopyranoside (III)	0·17	Methyl β-D-xylopyranoside (XII)	0·00
1 : 5-Anhydro-D-glucitol (IV)	0·20	1 : 5-Anhydro-D-xylitol (XIII)	0·00
1 : 5-Anhydro-2-deoxy-D-glucitol (V)	0·20	αα-Trehalose (XIV)	0·19
Methyl 2-deoxy-α-D-glucopyranoside (VI)	0·12	αβ-Trehalose (XV)	0·23
Ethyl 2-deoxy-α-D-glucopyranoside (VII)	0·17	ββ-Trehalose (XVI)	0·19—0·20
Ethyl 2 : 3-dideoxy-α-D-glucopyranoside (VIII)	0·10	Sucrose (XVII)	0·17
Methyl 4-*O*-methyl-β-*D*-glucopyranoside (IX)	0·00		

trehalose appears to be anomalously slow : effects other than simple steric ones appear to be operating here. Other apparently anomalous results have been observed in the reducing disaccharides (cf. Part I), thus maltose (1–4α) and cellobiose (1–4β) migrate at different rates in ionophoresis (M_G 0·32 and 0·28 respectively), as do *iso*maltose (1–6α, M_G 0·69) and gentiobiose (1–6β, M_G 0·75). These effects are being further investigated.

Furanose Derivatives.—Precise formulation of the interaction of derivatives of D-glucofuranose with borate ions is hindered by lack of suitable compounds. The M_G values recorded in Table 2 indicate the occurrence of interactions much stronger than in the

TABLE 2. *M_G values of derivatives of D-glucofuranose and D-xylofuranose.*

Derivative	M_G	Derivative	M_G
Methyl α-D-glucofuranoside (XVIII)	0·73	1 : 2-*O*-*iso*Propylidene-α-D-glucofuranose (XXI)	0·73
Ethyl α-D-glucofuranoside (XIX)	0·70	Methyl α-D-xylofuranoside	0·30
Ethyl β-D-glucofuranoside (XX)	0·65	Methyl β-D-xylofuranoside	0·30

corresponding pyranose derivatives (I) and (II). The M_G value of 1 : 2-*O*-*iso*propylidene-D-glucofuranose (XXI) reveals that the 2-hydroxyl group is not appreciably involved in complex formation. A weak interaction across the 5 : 6-hydroxyl groups would be expected on analogy with propane-1 : 2-diol (M_G 0·2), so that a further strong interaction must be operating which appears to involve the 3-hydroxyl group with either or both of the 5- and 6-hydroxyl groups. The M_G values (both 0·30) of methyl α- and β-D-xylofuranoside indicate that complex formation does occur across the 3 : 5-hydroxyl groups. The considerable difference in M_G values between these xylofuranosides and the glycofuranose derivatives (XVIII—XXI) suggests that complex formation across the 3 : 6-hydroxyl groups may occur in the latter group.

The interaction of reducing sugar derivatives with borate ions in aqueous alkali is complicated by equilibration of furanose, pyranose, and open-chain forms, any or all of which may be involved in complex formation. In the reducing derivatives of D-glucose (cf. Part I) this equilibrium may be affected, first, by the intensity of interaction of each form with borate ions and, secondly, by substitution of the hydroxyl groups; *e.g.*, protection of the 4- or 5-hydroxyl group precludes the formation of furanose and pyranose forms respectively. The M_G values recorded so far and the interactions known to occur in solutions of boric acid (Böeseken, *loc. cit.*), which are presumed to be intensified in the presence of alkali, lead to the complexes shown in the formulæ (p. 1778). For the α-furanose form it is not possible in alkaline borate solutions to obtain a measure of the contribution of the 1 : 2-hydroxyl groups alone since the 4-hydroxyl group must be simultaneously unprotected, and hence complex formation across the 2 : 4-hydroxyl groups in the open-chain form is also possible. The M_G values for 3 : 4-di-*O*-methyl-D-glucose (0·31) must largely originate from the 1 : 2-hydroxyl groups in the α-pyranose form (see formulæ). Although, from the results in Table 2, the furanose forms of D-glucose are seen to form firm complexes elsewhere than at the 1 : 2-hydroxyl groups, these forms appear to be minor contributors to the equilibrium. This is indicated by (*a*) the low M_G value (0·23) of 2-*O*-methyl-D-glucose for which a firm complex across the 2 : 4-hydroxyl groups in the open-chain form is prelcuded but complexes across the 3 : 5 : 6-hydroxyl groups of the furanose forms are possible, and (*b*) the high M_G value (0·82) of 3-*O*-methyl-D-glucose where the reverse is the case. That the 3 : 5 : 6-hydroxyl groups are involved to some extent in complex formation is indicated by the M_G value (0·12) of 2 : 3-di-*O*-methyl-D-glucose, where this type of complex is precluded, compared with that of 2-*O*-methyl-D-glucose (0·23), where it can occur.

The M_G values reported here and in Part I also indicate that the type of complex formation between borate ions and the reducing forms of the D-glucose derivatives is apparently too weak to disturb appreciably the equilibrium shown in the formulæ. If this were not so, a much higher M_G value for 2-*O*-methyl-D-glucose would be expected, where only the furanose forms can interact strongly with borate ions. Böeseken (*loc. cit.*) observed that the rate of mutarotation of the α- or β-form of D-glucopyranose is not appreciably affected in aqueous boric acid, although as mutarotation proceeds complex formation decreases and increases respectively.

From the ionophoretic studies on derivatives of D-glucose the significant fact emerges that in alkaline borate solution *all* the hydroxyl groups appear to be involved in complex formation to a greater or smaller extent, whereas in aqueous boric acid (Böeseken, *loc. cit.*) only the 1 : 2-hydroxyl groups in the α-pyranose and α-furanose forms are involved.

Ionophoresis of the series of 1–6α-linked D-gluco-oligosaccharides in alkaline borate buffer, where fast migration has been observed, reveals that the lower members may be separated. After ionophoresis at 900 v for 3 hr. (see Experimental section), separation was complete and the M_G values, D-glucose 1·00, *iso*maltose 0·69, *iso*maltotriose 0·57, *iso*maltotetraose 0·50, were observed. Similar results would be expected in the series of 1–6β- and 1–3-linked D-gluco-oligosaccharides. In the series of 1–4α-linked D-gluco-oligosaccharides, where slow migration occurs, M_G values decreased from 0·32 to 0·28 as the molecular size increased from maltose to maltohexaose, and no separation could be obtained. Similar results would be expected in the series of 1–4β- and 1–2-linked D-gluco-oligosaccharides [sophorose, a 1–2β-linked D-glucodisaccharide, has a low M_G value (0·23)].

Northcote (*Biochem. J.*, 1954, **58**, 353) has reported the mobilities of a series of amylose and amylopectin preparations in borate buffer at pH 9·2. Amylopectin was observed to migrate faster than amylose, which itself had an appreciable rate of migration. It is possible that the mobility of these polysaccharides is due, in part at least (amylopectin is known in certain cases to contain a small number of phosphate groups), to charges conferred on the molecules by complex formation of the type shown to occur in methyl α- and β-D-glucopyranoside. This would be expected to occur at the non-reducing ends of the chains and in this respect amylopectin, because of its highly branched nature and consequently larger number of non-reducing end-groups, would be expected to form firmer complexes than amylose. In view of the mobility of amylose, other types of complex formation at present unrecognized may also operate.

Experimental

The apparatus and technique for ionophoresis have been described by Foster (*Chem. and Ind.*, 1952, 1050). Ionophoreses were carried out on Whatman No. 3 paper in 0·2M-sodium borate at pH 10 under potentials of 900—1500 v, which gave final currents of 25—40 milliamps. Non-reducing sugar derivatives were located on the ionophoretograms by the use of ammoniacal silver nitrate. The durations of ionophoreses mentioned in the discussion refer to these conditions.

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