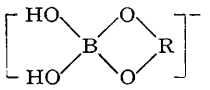
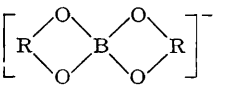
205. *Ionophoresis of Some Disaccharides.*

By A. B. Foster.

Ionophoresis of certain disaccharides on paper, in borate buffer at pH 10, is much more expedient than chromatography in certain cases, and may be of value in the determination of polysaccharide structure. From the migration of numerous methyl sugars in ionophoresis, evidence is obtained which suggests that *aldehydo*-forms, in addition to ring structures of the derivatives, interact with borate ions. An analogy is drawn with the interaction of aldehydes and polyhydric alcohols (Barker, Bourne, and Whiffen, *J.*, 1952, 3865).

Attention has recently been focused on the use of filter-paper ionophoresis as a technique complementary to that of chromatography in structural studies of carbohydrates (Jaenicke, *Naturwiss.*, 1952, **39**, 86; Consden and Stanier, *Nature*, 1952, **169**, 783; Foster, *Chem. and Ind.*, 1952, 828; Woodin, *Biochem. J.*, 1952, **51**, 319; Foster and Stacey, *J. Appl. Chem.*, 1953, **3**, 19). We have been investigating extension of the method and, secondly, the mode of interaction of borate ions with carbohydrates in the alkaline media used.

Migration of certain neutral sugar derivatives, in ionophoresis, occurs at an alkaline pH in the presence of borate ions and results from the formation of the weak negatively charged complexes (I) and (II). These are represented as derived from compounds of the type

(I)   (II)

$R(OH)_2$ which have suitably orientated hydroxyl groups. In acid media the equilibria which involve boric acid [cf. (I) and (II)] lie to the side of the boric acid, but at increasing pH's the concentration of the complexes (I) and (II) extensively increases. The effect of this type of complex formation on the conductivity of 0·5M-boric acid at 25° has been reviewed by Böeseken (*Adv. Carbohydrate Chem.*, 1949, **4**, 189) and the general interaction of boric acid with polyalcohols has been described by Isbell, Brewster, Holt, and Frush (*J. Res. Nat. Bur. Stand.*, 1948, **40**, 129) and by Zittle (*Adv. Enzymology*, 1951, **12**, 493).

As an index of migration of the borate ion–carbohydrate complexes in filter-paper ionophoresis the term M_G has been suggested (Foster, *loc. cit.*) where for any substance

$$M_G = \frac{\text{True distance of migration of the substance}}{\text{True distance of migration of D-glucose}}$$

The true distances of migration are those corrected for movement due to electroendosmotic flow by reference to the movement of 2 : 3 : 4 : 6-tetramethyl D-glucose, which does not form a complex with borate ions. The M_G values recorded in this paper are comparative and for a given system they did not vary by more than $\pm 0{\cdot}02$ about a mean value. The relative positions of the derivatives on the ionophoretograms were invariable.

For extension of the application of ionophoresis, the migration of certain disaccharides has been studied. From the M_G values which are recorded in Table 1 it may be seen that, in the group of six D-glucodisaccharides, maltose, cellobiose, and gentiobiose may be differentiated readily from laminaribiose, *iso*maltose, and the 1 : 3α-linked D-glucodisaccharide. Separation of disaccharides by normal chromatographic procedures is

extremely time-consuming (cf. Partridge, *Biochem. J.*, 1948, **42**, 238; Hough, Jones, and Wadman, *J.*, 1950, 1702; Jeanes, Wise, and Dimler, *Analyt. Chem.*, 1951, **23**, 415) and not

TABLE 1.

Disaccharide	Linkage	M_G	R_{Bz}*	Disaccharide	Linkage	M_G	R_{Bz}
—	1 : 3α	0·69	—	Gentiobiose	1 : 6β	0·75	—
Laminaribiose	1 : 3β	0·69	0·71	Lactose	1 : 4β	0·38	—
Maltose	1 : 4α	0·32	0·62	Melibiose	1 : 6α	0·80	—
Cellobiose	1 : 4β	0·28	—				
*iso*Maltose	1 : 6α	0·69	0·52				

* The R_{Bz} values are calculated from the data given by Bayly and Bourne (*loc. cit.*) for the benzylamine method with reference to the movement of *N*-benzylglucosylamine.

without experimental difficulties (Bayly and Bourne, *Nature*, in the press) but the recent introduction of a chromatographic procedure by Bayly and Bourne (*loc. cit.*), which involved prior conversion of the disaccharides into the *N*-benzylglycosylamine derivatives, has enabled considerable economies of time to be made. Application of filter-paper ionophoresis to the separation of disaccharides permits further large economies of time to be effected in certain instances and moreover facilitates separations which are not possible by chromatography and provides information concerning the structure of the sugars. Thus maltose and *iso*maltose may be separated in an hour by ionophoresis (see Experimental Section) whereas 18 hours were required for the benzylamine method. A mixture containing either maltose or cellobiose and one of the 1 : 3α-, 1 : 3β- or 1 : 6α-linked disaccharides may be separated similarly. Maltose and cellobiose, which cannot be separated by chromatography, may be resolved in 4 hours, and gentiobiose and *iso*maltose in 5 hours. The latter disaccharides were separated only after 28 hours by Bayly and Bourne's method. The benzylamine method, however, permitted separation in 18 hours of laminaribiose and *iso*maltose, which have similar ionophoretic mobilities. Hence by the use of both methods in conjunction, four of the group of six D-glucodisaccharides listed in Table 1 may be recognized quite easily, and the structures of the remaining disaccharides limited to two alternatives.

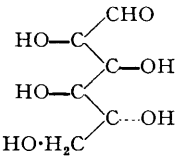
Böeseken (*loc. cit.*) has shown that, in 0·5M-boric acid solution, the interaction of the α-ring forms of D-glucose is restricted to the *cis*-$C_{(1)}$: $C_{(2)}$-hydroxyl groups and that on protection of the $C_{(1)}$-hydroxyl group, as in the methyl-D-glucosides, there is no complex formation. At pH 10 in ionophoresis, the M_G value of α-methyl-D-glucopyranoside shows only a slight interaction with borate ions. The ring structures in the glycosides are fixed and consequently *aldehydo*-forms are absent. The non-reducing moieties of the disaccharides studied may be regarded as similarly fixed and would not be expected therefore to participate in complex formation, unless *cis*-disposed hydroxyl groups were present. The latter condition is fulfilled in lactose and melibiose, which have higher rates of migration than the analogous D-glucodisaccharides, cellobiose and *iso*maltose (see Table 1). No doubt this is due to the extra negative charge afforded to the lactose– and melibiose–borate ion complexes by interaction at the *cis*-$C_{(3)}$: $C_{(4)}$-hydroxyl groups in the D-galactose moiety.

A $C_{(1)}$: $C_{(2)}$-complex cannot be formed by 2-methyl D-glucose but its M_G value of 0·23 implies considerable interaction with borate ions. Polarographic studies on solutions of sugars under equilibrium conditions have revealed the presence of reducible forms which are most probably *aldehydo*-structures. The amount of reducible forms in the equilibrium increases rapidly with increase in pH (Delahay and Strassner, *J. Amer. Chem. Soc.*, 1952, **74**, 893; cf. Ingles, *Nature*, 1949, **163**, 484, and Lippich, *Biochem. Z.*, 1932, **248**, 280). Thus if the interaction of the *aldehydo*-form of 2-methyl D-glucose with borate ions under the conditions of ionophoresis (pH 10) be postulated, its migration may be explained since open-chain carbohydrates, such as the polyalcohols, are known to interact strongly with boric acid even in acid media (Böeseken, *loc. cit.*). In ionophoresis the magnitude of the net charge, and hence of the rate of migration, will therefore depend on the number of contributors to the equilibrium of ring and open-chain forms of the sugar derivatives which can interact with borate ions. Further, it will also depend on the strength of the interactions at the different loci. It is probable that the *aldehydo*-forms of the sugar derivatives

adopt the favoured zig-zag shape of a chain of carbon atoms (cf. McCoubrey and Ubbelohde, *Quart. Reviews*, 1951, **5**, 364). Several possibilities for the interaction of borate ions with various pairs of hydroxyl groups along the open carbon chain therefore exist, and the limiting factor must be the distance the borate ion can span in forming a complex. In this respect there is an analogy with the interaction of aldehydes and polyhydric alcohols discussed by Barker, Bourne, and Whiffen (*J.*, 1952, 3865) who showed that the preferred order of condensation and the stability of the acetal rings thereby formed is strictly governed by the distances separating the various pairs of hydroxyl groups involved in ring formation. Some of the terms and data recorded by these authors will be used in this paper. Thus the Greek letters α′ and β′ refer to the relative position of the hydroxyl groups along the carbon chain in the *aldehydo*-forms, and C and T denote whether these hydroxyl groups are *cis* or *trans* in the usual Fischer projection formulæ. C and T are necessary only when both hydroxyl groups are secondary. The distances separating the pairs of hydroxyl groups relevant to the argument are shown in Table 2. For comparison, *aldehydo*-D-glucose is shown in the Fischer projection formula (III) and in a modification of zig-zag representation (IV) suggested by Barker, Bourne, and Whiffen (*loc. cit.*) for the

(III)

CHO	1
H–C–OH	2
HO–C–H	3
H–C–OH	4
H–C–OH	5
CH_2·OH	6



(IV)

In (IV), — represents valencies projecting above the plane of the carbon atoms, and ···· represents valencies projecting below the plane of the carbon atoms. The hydrogen atoms at positions 2, 3, 4, and 5 in (IV) are not shown.

polyhydric alcohols. The disposition of the free hydroxyl groups of the partially substituted derivatives of D-glucose listed in Table 3 may easily be ascertained by reference to (III) and (IV).

In the case of the D-glucose derivatives, listed in Table 3, interaction of borate ions with the contributors to the equilibrium at pH 10 appears to be limited *in the main* to

TABLE 2.

Relation of hydroxy groups	β′C	β′	α′T	,α′	β′T	α′C
Separating distance, Å	2·51	2·51	2·83	2·83	3·43	3·68

TABLE 3.

Substance	M_G	Complexes formed	M_G	Disaccharide	Linkage
D-Glucose	1·00	X, β′C (β′T)	—	—	—
6-Methyl D-glucose	0·82	X, β′C (β′T)	0·80 *	Melibiose	1 : 6α
	—	X, β′C (β′T)	0·75	Gentiobiose	1 : 6β
	—	X, β′C (β′T)	0·69	*iso*Maltose	1 : 6α
3-Methyl D-glucose	0·82	X, β′C (α′)	0·69	—	1 : 3α
	—	X, β′C (α′)	0·69	Laminaribiose	1 : 3β
3 : 5 : 6-Trimethyl D-glucose	0·71	X, β′C	—	—	—
3 : 4-Dimethyl L-rhamnose	0·36	X	—	—	—
3 : 4-Dimethyl D-glucose	0·31	X (α′)	—	—	—
4-Methyl D-galactose	0·27	X (α′T, α′)	0·38 *	Lactose	1 : 4β
4-Methyl D-glucose	0·24	X (α′T, α′)	0·32	Maltose	1 : 4α
	—	X (α′T, α′)	0·23	Cellobiose	1 : 4β
2-Methyl D-glucose	0·23	β′ (β′T)	—	—	—
2 : 3-Dimethyl D-glucose	0·12	β′	—	—	—
α-Methyl-D-glucopyranoside	<0·09	—	—	—	—
2 : 4-Dimethyl D-glucose	<0·05	(α′)	—	—	—
2 : 3-Dimethyl L-rhamnose	<0·05	(α′C)	—	—	—
2 : 4-Dimethyl L-rhamnose	<0·05	(β′T)	—	—	—
2 : 3 : 4-Trimethyl D-glucose	0·00	(α′)	—	—	—
2 : 3 : 6-Trimethyl D-glucose	0·00	(α′C)	—	—	—

* Includes a contribution from the $C_{(3)}$: $C_{(4)}$-hydroxyl groups in the non-reducing moiety. The central column shows the complexes formed and, in parentheses, the remaining possibilities which do not facilitate extensive complex formation. In the derivatives of D-glucose X represents the $C_{(1)}$: $C_{(2)}$-*cis*-hydroxyl groups in the α-ring forms; in the *aldehydo*-forms the location of the hydroxyl groups mentioned are as follows: β′C $C_{(2)}$: $C_{(4)}$; β′ $C_{(4)}$: $C_{(6)}$; α′T $C_{(2)}$: $C_{(3)}$; α′ $C_{(5)}$: $C_{(6)}$; α′C $C_{(4)}$: $C_{(5)}$.

three possibilities. These involve (A) the β'C hydroxyl groups at $C_{(2)} : C_{(4)}$ in the *aldehydo*-forms, (B) the *cis*-hydroxyl groups at $C_{(1)} : C_{(2)}$ in the α-ring (furanose and pyranose) forms, and (C) the β' hydroxyl groups at $C_{(4)} : C_{(6)}$ in the *aldehydo*-forms. Interaction at (C) is possible in certain cases where a β'C complex is precluded but if both possibilities are present then β'C takes preference over β'. The reasons given by Barker, Bourne, and Whiffen (*loc. cit.*) for the preference of β'C rings over β' in acetal formation apply equally to complex formation with borate ions. It should be noted that stereochemical variations in sugars other than D-glucose may involve possibilities for complex formation different from those enumerated for the D-glucose derivatives. Interactions other than at (A), (B), and (C) probably occur in the derivatives of D-glucose, but to a negligible extent.

From Table 3 it may be seen that, with respect to M_G values the derivatives listed fall into four groups where (1) interaction at (A) and (B) is possible (high M_G values), (2) interaction at (B) only is possible (moderate M_G values), (3) interaction at (C) only is possible (moderate M_G values), and (4) interaction at (A), (B), and (C) is precluded (low M_G values). The pronounced fall in the M_G values when complex formation at (A) is precluded, as in 4-methyl D-glucose and the 1 : 4-linked D-glucodisaccharides, suggests that the β'C hydroxyl groups at $C_{(2)} : C_{(4)}$ play a major rôle in complex formation. The M_G values of 2 : 3 : 4- and 2 : 3 : 6-trimethyl D-glucose and 2 : 4-dimethyl L-rhamnose imply that α', α'C, or β'T complexes respectively are not formed to any appreciable extent and, further, the results in group 2 suggest that β'T complexes also are not favoured to any great extent. Table 3 shows the complexes presumed to be formed, on interaction with borate ions, for all the derivatives studied, together with the remaining possibilities which do not apparently facilitate complex formation. The correlation of M_G values with the presence of specific structural features is clearly shown and provides strong support for the theory presented in this paper.

It is of interest that Böeseken, who used conductivity increments as a measure of the interaction of polyalcohols with boric acid, recorded an increment of 327 for D-arabitol and 625 for D-xylitol (*op. cit.*, p. 191). The $C_{(2)} : C_{(4)}$-hydroxyl groups in D-xylitol are β'C whereas only β' relationships are present in the D-arabitol molecule between the $C_{(1)} : C_{(3)}$- and $C_{(3)} : C_{(5)}$-hydroxyl groups. A much stronger interaction is therefore to be expected in the case of D-xylitol.

The rate of migration of the D-glucodisaccharides is controlled by, and hence gives some indication of, the position at which the non-reducing moiety is attached to the reducing moiety, since this will determine the mode of interaction of the reducing moiety with boric acid. Similarly the migration of trisaccharides will be governed largely by the point of attachment of the remainder of the molecule to the reducing moiety. This was demonstrated on ionophoresis of the trisaccharide fraction obtained from a partial hydrolysate of the polyglucosan produced by *Aspergillus niger* (strain 152) described by Barker, Bourne, and Stacey (*Chem. and Ind.*, 1952, 755). The trisaccharide fraction appeared to be homogenous on chromatography by the benzylamine method (Bayly and Bourne, *loc. cit.*) but from the R_G value the presence of both a 1 : 3- and a 1 : 4-link was inferred. Ionophoresis showed the presence of two components with mobilities similar to the 1 : 3α- and 1 : 4α-linked disaccharides. This result is further evidence in support of the structure of alternate 1 : 3- and 1 : 4-links postulated for the polyglucosan by Bayly and Bourne (*loc. cit.*) since on partial hydrolysis such a structure would afford a mixture of trisaccharides linked 1 : 3-1 : 4 and 1 : 4-1 : 3.

In the case of polysaccharides containing sugars other than D-glucose, a consideration of the stereochemistry of the reducing moiety would no doubt facilitate a correlation of structure and ionophoretic migration of the di- and tri-saccharides obtained on partial hydrolysis. For this reason the method may be of wide potential application in polysaccharide structural studies.

Experimental

The apparatus and technique of the ionophoreses have previously been described in detail 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 an applied potential of 900 v, which gave a final current

of 30—35 milliamps. The duration of ionophoreses mentioned in the paper refer to these conditions. Location of the reducing sugars on the ionophoretograms was achieved by the use of aniline hydrogen phthalate (Partridge, *Nature*, 1948, **164**, 443).

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