831. *Paper Ionophoresis of Sugars and Other Cyclic Polyhydroxy-compounds in Molybdate Solution.*

By E. J. Bourne, D. H. Hutson, and H. Weigel.

Paper ionophoresis in molybdate solution at pH 5 has proved a useful analytical method for carbohydrates, complementary to the technique in other electrolytes. Examination of aldoses, derivatives of aldoses, and cyclitols has revealed that compounds with a six-membered ring system form complexes with molybdate if they possess three hydroxyl groups in a *cis-cis*-1,2,3-triol arrangement.

The fact, long known,[1] that molybdate forms complexes with polyhydroxy-compounds found little practical use until Richtmyer and Hudson [2] attempted to use quantitatively the greatly increased specific rotations of the hexitols in acidified ammonium molybdate solutions. Barker *et al.*,[3] comparing the rates of elution of the oligosaccharides of the maltose series from charcoal columns impregnated with borate and molybdate severally, suggested that the two ions formed complexes in different manners and might be used for separations of different pairs of sugars. Complex-formation from polyhydroxy-compounds and borate has been of great value as the complexes, possessing a negative charge, migrate in an electric field,[4] and borate ionophoresis has been studied extensively and brought into general use by Foster.[5]

Use of molybdate complexes in paper ionophoresis was studied for a few sugars by Frahn and Mills.[6] We have examined the paper ionophoresis of a number of cyclic carbohydrates in molybdate solution at pH 5·0. This pH was chosen after trials had confirmed that molybdate forms complexes more strongly in acidic than in alkaline solution.[1,2] The investigation has given some insight into the structure of the molybdate–carbohydrate complex, a hitherto unexplored field, and interpretation was aided by dividing the compounds into groups according to their structure. Sorbitol was used as a standard for the comparison of rates of migration, and glycerol as a non-migrating marker for correction of electro-osmosis. Hence, the migration rates are expressed as M_S values.

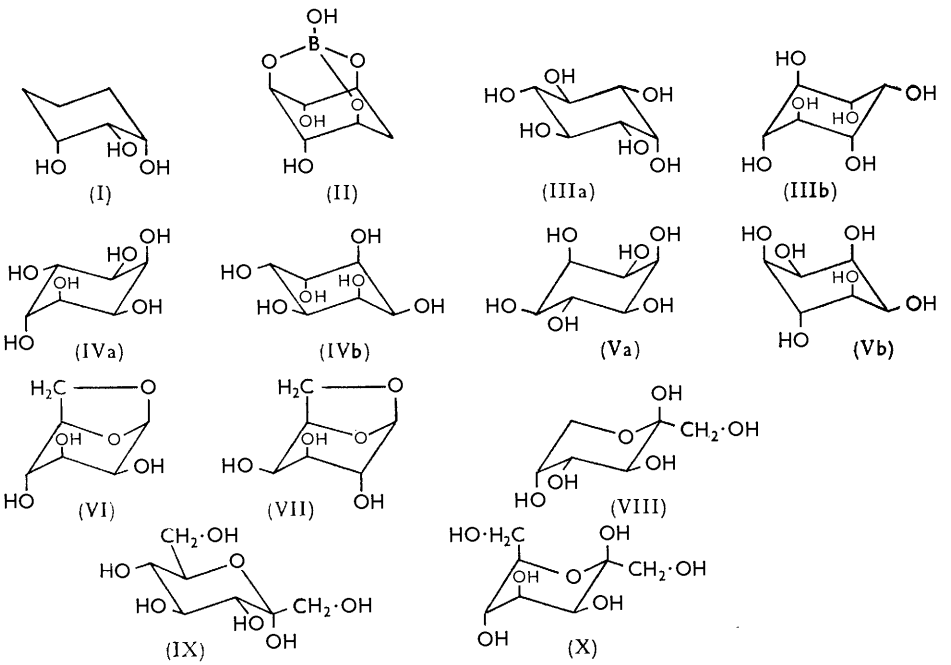
Aldoses and Derivatives.—M_S values of aldoses and their derivatives are given in the Table. Glyceraldehyde and the tetroses were included for completeness. The separation

[1] Honnelâitre, *Ann. Chim.* (*France*), 1925, **3**, 5.
[2] Richtmyer and Hudson, *J. Amer. Chem. Soc.*, 1951, **73**, 2249.
[3] Barker, Bourne, Foster, and Ward, *Nature*, 1957, **179**, 262.
[4] Consden and Stanier, *Nature*, 1952, **169**, 783.
[5] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.
[6] Frahn and Mills, *Austral. J. Chem.*, 1959, **12**, 65.

of the two tetroses, which takes *ca.* 20 min. by this technique, is difficult by any other simple method.

Glycoside formation reduced the tendency to migrate to a negligible or very low level (cf. D-mannose, D-ribose, and D-lyxose, and the corresponding methyl glycosides), and this suggested that complex formation may have been due to small amounts of *aldehydo*-forms, as in the case of borate-complex formation.[5] However, the amounts of these forms in aqueous solution (*e.g.*, D-ribose 8·5% and D-lyxose 0·4% [7]) would not explain satisfactorily the relative rates of migration. The possibility of the formation of a " tridentate " complex was then examined and it was found that only those aldohexoses and aldopentoses which possess a *cis-cis*-1,2,3-triol system and thus, in at least one of their conformations,[8,9] one equatorial hydroxyl group neighboured by two axial hydroxyl groups (cf. I), migrated during ionophoresis. This type of " tridentate " complex is believed to occur between periodate and D-ribose and other compounds possessing the *cis-cis*-1,2,3-triol system, *e.g.*, 1,6-anhydro-β-D-allopyranose.[10] Similar structures have been postulated for scyllo-quercitol borate [11] (II) and pentaerythritol arsenite.[12]

In all cases, except D-ribopyranose and D-talopyranose, the *cis-cis*-1,2,3-triol system includes the hydroxyl group on the anomeric carbon atom. Substitution in, or replacement of, at least one of the hydroxyl groups of this system destroys the ability to form a " tridentate " complex. This is exemplified by 3,4-di-*O*-methyl-D-mannose, methyl



β-D-mannopyranoside, methyl α- and β-D-lyxopyranoside, and 2-deoxy-D-ribopyranose. D-Ribopyranose possesses the 1(*ax*),2(*eq*),3(*ax*)-triol system (I) in both the C1 and the 1C conformation (Reeves' nomenclature). Methyl α-D-ribopyranoside, however, has this system only in the 1C conformation.

Of the D-*glycero*-aldoheptoses examined, the D-*gulo*-, D-*allo*-, and L-*manno*-compounds

[7] Pigman and Goepp, " Chemistry of Carbohydrates," Academic Press Inc., New York, 1948, p. 68.
[8] Reeves, *Adv. Carbohydrate Chem.*, 1951, **6**, 107.
[9] Ferrier and Overend, *Quart. Rev.*, 1959, **13**, 265.
[10] Barker and Shaw, *J.*, 1959, 584.
[11] Angyal and McHugh, *Chem. and Ind.*, 1956, 1147.
[12] Stevens, *J. Org. Chem.*, 1959, **24**, 1715.

possess the 1(*ax*),2(*eq*),3(*ax*)-triol system (I) in one of their conformations of the pyranose ring. The 4-, 6-, and 7-hydroxyl groups of all heptoses can be brought, without distortion of bond angles, into the same relative positions as those of the 1(*ax*),2(*eq*),3(*ax*)-triol system (I).

Cyclitols.—Less ambiguous results might be obtained from an examination of the behaviour of the cyclitols where the possibility of open-chain and five-membered ring structures does not arise. Of the eleven cyclitols examined, only myoinositol (III), alloinositol (IV), and epi-inositol (V) (Angyal and Anderson's nomenclature [13]) possess the *cis-cis*-1,2,3-triol system and hence migrated. Their rates of migration can be related to the instability factors [8] of their conformations which have the 1(*ax*),2(*eq*),3(*ax*)-triol system (I). Of the two chair conformations of myoinositol (IIIa and b) only the less favoured conformation (IIIb), which has five axial hydroxyl groups, has the 1(*ax*),2(*eq*),3(*ax*)-triol system. The two chair conformations of alloinositol (IVa and b) are mirror images and both have three axial hydroxyl groups. Both chair conformations of epi-inositol (Va and Vb) possess the 1(*ax*),2(*eq*),3(*ax*)-triol system, but (Va) is very much more favoured than (Vb) and it has only two axial hydroxyl groups.

1,6-*Anhydro*-β-D-*aldopyranoses.*—Of those examined (Table) only 1,6-anhydro-β-D-mannopyranose (VI) migrated. The steric arrangement of the three hydroxyl groups

M_S Values of polyhydroxy-compounds.

Complex-forming	M_S value	Not complex-forming (M_S <0·1)	
Aldoses and their derivatives.			
		Glyceraldehyde	
D-Erythrose	0·9	2-Deoxy-D-ribose	Methyl α-D-glucopyranoside
L-Threose	0·6	D-Arabinose	Phenyl β-D-glucopyranoside
D-Ribose	0·4	Methyl α-D-arabopyranoside	Catechol β-D-glucopyranoside
Methyl α-D-ribopyranoside	0·1	Methyl β-D-arabopyranoside	3,4-Di-*O*-methyl-D-mannose
		1,2-Dideoxy-D-arabinose	Methyl α-D-mannopyranoside
D-Lyxose	1·1	D-Xylose	Methyl β-D-mannopyranoside
D-Mannose	0 → 0·9	Methyl α-D-xylofuranoside	D-Galactose
D-Gulose	1·1	Methyl α-D-lyxopyranoside	Sophorose
D-Talose	0·7	Methyl β-D-lyxopyranoside	Nigerose
L-Rhamnose	0 → 0·6	D-Altrose	Laminaribiose
D-*glycero*-D-*allo*Heptose	0·9	D-Glucose	Maltose
D-*glycero*-L-*gluco*Heptose	0·2	3-*O*-Methyl-D-glucose	Cellobiose
D-*glycero*-L-*manno*Heptose	0·8	2,3,4-Tri-*O*-methyl-D-glucose	Lactose
D-*glycero*-D-*gulo*Heptose	1·1	2,3,6-Tri-*O*-methyl-D-glucose	Isomaltose
D-*glycero*-D-*ido*Heptose	1·0	2,3,4,6-Tetra-*O*-methyl-D-glucose	Gentiobiose
D-*glycero*-D-*gala*Heptose	0·4	2-Deoxy-D-glucose	Melibiose
D-*glycero*-L-*gala*Heptose	0·4		
Cyclitols.			
Myoinositol (III)	0·2	Mucoinositol	Quebrachitol
Alloinositol (IV)	0·4	Scylloinositol	(−)-Viboquercitol
Epi-inositol (V)	1·1	Mytilitol	Scylloquercitol
		Pinitol	(+)-Protoquercitol
1,6-*Anhydro*-β-D-*aldopyranoses.*			
1,6-Anhydro-β-D-mannopyranose (VI)	0·5	1,6-Anhydro-β-D-glucopyranose	
		1,6-Anhydro-β-D-gulopyranose	
		1,6-Anhydro-β-D-galactopyranose (VII)	
Ketoses.			
D-Fructose (VIII)	0·5	Sucrose	
L-Sorbose	0·3		
D-Glucosone	0·9		
D-*gluco*Heptulose (IX)	1·0		
D-*manno*Heptulose (X)	0·4		
Leucrose	0·4		
Turanose	0·1		

(*eq*, *ax*, *ax*) occurs also in 1,6-anhydro-β-D-galactopyranose (VII). However, a unique feature of 1,6-anhydro-β-D-mannopyranose is the *ax*, *eq*, *ax* relation of the 2- and 3-hydroxyl

[13] Angyal and Anderson, *Adv. Carbohydrate Chem.*, 1959, **14**, 135.

groups and the anhydro-ring oxygen atom. A possible explanation for complex formation in this compound is that a cyclic complex across the 2- and 3-hydroxyl groups is stabilised by hydrogen bonding between a molybdenum-hydroxyl group and the oxygen atom of the anhydro-ring. There was no evidence from the behaviour of other compounds to refute this suggestion.

Ketoses.—The general tendency of ketoses in this section to migrate (see Table) suggests that an examination of the behaviour of more ketoses is warranted. Comparison with the aldoses shows that several ketose–aldose separations are possible. These may be of particular use in the oligosaccharide field. The nature of the molybdate–ketose complex is uncertain but three possibilities could arise: (*a*) complex-formation by an open-chain form; (*b*) slight enolisation of the keto-group and complex-formation by the resultant ene-diol with molybdate; [14] and (*c*) " tridentate " complex formation with either the furanose or the pyranose ring form. For example, in the furanose and pyranose form of D-fructose (VIII) and L-sorbose, the 1-, 2-, and 3-hydroxyl groups are able to form a structure of the same relative spacings as those of the 1(*ax*),2(*eq*),3(*ax*)-triol system (I). It is probable, by virtue of the α–β-equilibrium and the free rotation of the 2-hydroxymethyl group, that unsubstituted 1-, 2-, and equatorial 3-hydroxyl groups of the pyranose form of any 2-ketose can form a structure approximating to that of (I). The same applies for unsubstituted 1-, 2-, and 3-hydroxyl groups of the furanose form of any 2-ketose. The large difference in M_S values of D-*gluco*heptulose (IX) and D-*manno*heptulose (X) confirms this, as the latter, when in the pyranose form, has an equatorial 3-hydroxyl group only in its unfavoured conformation (X). The relatively high migration rate of leucrose (5-*O*-substituted fructopyranose), the low migration rate of turanose (3-*O*-substituted fructopyranose), and the immobility of sucrose (2-*O*-substituted fructofuranose) support this.

General Observations.—It is thus established that sugars and other six-membered cyclic polyhydroxy-compounds form complexes significantly with molybdate only if they possess a *cis-cis*-1,2,3-triol system, or can assume an equivalent system. It can be seen from the Table that several useful separations are obtained by ionophoresis in molybdate solution which are very difficult by chromatography or ionophoresis in borate solution. The rapidly migrating sorbitol, used as a standard, is very quickly separated from glucose and the aldose–alditol separation is fairly general. In an investigation of the action of Fenton's reagent on sorbitol,[15] where the main products are D-glucose, L-gulose, D-fructose, and L-sorbose, the technique was most useful. Ionophoresis of such a mixture in borate solution was virtually useless. Also molybdate forms its complexes most strongly in acid, whereas the borate complexes are more stable in alkali. The two methods can thus be regarded as complementary.

It is generally accepted that when a borate ion reacts with a polyhydroxy-compound a cyclic diester is usually formed.[5] Angyal and McHugh [11] postulated the formation of a " tridentate " borate complex (II) with certain cyclitols. Molybdate, however, seems to form complexes with sugars and other six-membered cyclic polyhydroxy-compounds only when three or more hydroxyl groups are available in the correct relative positions. There is one exception to this rule. Compounds containing the " ene-diol " group, *e.g.*, ascorbic acid and *o*-dihydroxybenzene, form stable complexes with molybdate at pH *ca.* 5·0, giving deep orange solutions. This complex has been used by Pridham [16] and by Halmekoski [17] for the ionophoresis and chromatography of phenolic compounds. *m*-Dihydroxybenzene does not form a complex with molybdate, and an " ene-diol " group seems to be essential. The participation of only two hydroxyl groups in the " ene-diol " system need not be at variance with our suggestion. The intense colour of the complex of " ene-diol " compounds

[14] Weinland and Gaisser, *Z. anorg. Chem.*, 1919, **108**, 231.
[15] Bourne, Hutson, and Weigel, unpublished work.
[16] Pridham, *J. Chromatography*, 1959, **2**, 605.
[17] Halmekoski, *Suomen Kem.*, 1959, *B*, **32**, 170.

indicates a conjugated system. Thus the diester structure could be resonance-stabilised. When only two suitable hydroxyl groups were involved in a sugar complex, as in 1,6-anhydro-β-D-mannopyranose (VI), the complex was probably stabilised by hydrogen-bonding.

Ionophoresis of sugars and other six-membered cyclic polyhydroxy-compounds in molybdate solution is not only a tool for the separation of such compounds. Applications might be found in the determination of structures. Affinity for molybdate can be regarded as a diagnosis for the ability of a compound possessing a *cis-cis*-1,2,3-triol system to adopt a conformation with a 1(*ax*),2(*eq*),3(*ax*)-triol system. For example, α-D-ribopyranose possesses this system in the C1 and the 1C conformation. β-D-Ribopyranose has this spatial arrangement of three hydroxyl groups in the 1C conformation. The equilibrium mixture of D-ribose thus migrates during ionophoresis in molybdate solution. However, methyl α-D-ribopyranoside possesses the 1(*ax*),2(*eq*),3(*ax*)-triol system only in the 1C conformation and its low rate of migration suggests that it will not easily adopt this.

In the discussion above only " chair " conformations of six-membered ring compounds have been considered. Reeves [8] concluded that a " chair " conformation is adopted in preference to any " boat " conformation whenever both are structurally possible. However, complex formation with molybdate could also easily occur with " boat " conformations of the compounds possessing the *cis-cis*-1,2,3-triol system, as at least one of their " boat " conformations possesses a 1(*bax*),2(*bs*),3(*bax*)-triol system (Angyal and Mills's nomenclature [18]), which is spatially identical with the 1(*ax*),2(*eq*),3(*ax*)-triol system.

Experimental

Paper Ionophoresis.—The apparatus used was built according to a design kindly provided by Dr. D. Gross of Tate and Lyle, Ltd., and was capable of delivering up to 5000 v at 100 mA. Ionophoresis was carried out on 10 cm. wide sheets of Whatman No. 3MM filter paper. The electrolyte was prepared by dissolving sodium molybdate dihydrate (25 g.) in water (1200 ml.) and adjusting the whole to pH 5·0 with concentrated sulphuric acid. Ionophoretograms were prepared by applying a voltage of 30—60 v/cm. for 1—2 hr. Compounds were detected by spraying with acetone–silver nitrate–ethanolic sodium hydroxide [19] or *p*-anisidine hydrochloride in butan-1-ol.[20] Migration rate was expressed relative to the movement of sorbitol which migrated *ca.* 25 cm. in 2 hr. at 60 v/cm. By comparison with 2,3,4,6-tetra-*O*-methyl-D-glucose, glycerol was shown not to form a complex and was used for the correction of electro-osmosis.

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Royal Holloway College, University of London,
Englefield Green, Surrey. [*Received, April 1st,* 1960.]

[18] Angyal and Mills, *Rev. Pure Appl. Chem.* (*Australia*), 1952, **2**, 185.
[19] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[20] Hough, Jones, and Wadman, *J.*, 1950, 1702.