7. *Complexes between Molybdate and Acyclic Polyhydroxy-compounds.*

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The compositions of complexes between molybdate and some acyclic polyhydroxy-compounds have been determined. Ionophoresis of reduced oligosaccharides in molybdate solution has been shown to be a useful method for determining the position of the glycosidic linkage to the reducing group of the original oligosaccharide.

It is known [1] that complexes of molybdate with polyhydroxy-compounds in aqueous solution have a maximum stability at acidic pH values and are decomposed by alkali. In a previous paper [2] we discussed the behaviour of sugars and other cyclic polyhydroxy-compounds, with six atoms in the ring, during ionophoresis in molybdate solution. We now report studies on the complexes between molybdate and acyclic polyhydroxy-compounds.

The effect of pH on the specific rotations of some acyclic polyhydroxy-compounds in molybdate solution was examined in order to find suitable conditions for ionophoresis. It can be seen from the results (Fig. 1) that maximal changes in specific rotation, and hence maximal complex-formation, occurred at *ca.* pH 2, but that there was some complex-formation over the whole range of pH 1—8. Ionophoresis was therefore carried out at pH 5, a value which is low enough to assure presence of sufficient complex and high enough to allow reasonable ionisation. The M_S values of acyclic polyhydroxy-compounds are shown in Table 1.

Table 1. M_S *Values of acyclic polyhydroxy-compounds.*

$$M_S = \frac{\text{true distance of migration of compound}}{\text{true distance of migration of sorbitol}}$$

Compound	M_S	Compound	M_S
Ethane-1,2-diol	<0·1	2,3-Di-*O*-methylsorbitol	<0·1
Propane-1,2-diol	<0·1	4-*O*-α-D-Glucopyranosylsorbitol	0·4
Propane-1,3-diol	<0·1	4-*O*-β-D-Glucopyranosylsorbitol	0·4
Butane-2,3-diol	<0·1	4-*O*-β-D-Galactopyranosylsorbitol	0·4
Butane-1,3-diol	<0·1	4-*O*-α-Isomaltosylsorbitol	0·4
Butane-1,4-diol	<0·1	4-*O*-α-Nigerosylsorbitol	0·4
Pentane-1,5-diol	<0·1	5-*O*-α-D-Glucopyranosylsorbitol	0·8
Hexane-1,6-diol	<0·1	6-*O*-α-D-Glucopyranosylsorbitol	0·8
2-Methylpentane-2,4-diol	<0·1	6-*O*-β-D-Glucopyranosylsorbitol	0·8
2-Methylhexane-1,3-diol	<0·1	6-*O*-α-D-Galactopyranosylsorbitol	0·8
Pentaerythritol	<0·1	6-*O*-α-Isomaltosylsorbitol	0·7
Glycerol	<0·1	6-*O*-α-Isomaltotriosylsorbitol	0·6
Erythritol	1·0	6-*O*-α-Isomaltotetraosylsorbitol	0·5
D-Threitol	0·5	6-*O*-α-Isomaltopentaosylsorbitol	0·4
Ribitol	1·1	6-*O*-α-Isomaltohexaosylsorbitol	0·3
D-Arabitol	1·1	6-*O*-α-Isomaltoheptaosylsorbitol	0·25
3-*O*-α-D-Galactopyranosyl-D-arabitol	<0·1	D-Mannitol	1·0
Xylitol	1·1	1-Deoxy-D-mannitol	1·0
Sorbitol	1·0	2-*O*-Methyl-D-mannitol	1·0
2-Deoxysorbitol	1·0	2-*O*-α-D-Glucopyranosyl-D-mannitol	0·8
2-*O*-β-D-Glucopyranosylsorbitol	0·9	2-*O*-α-D-Mannopyranosyl-D-mannitol	0·8
3-*O*-Methylsorbitol	<0·1	3-*O*-α-D-Mannopyranosyl-D-mannitol	<0·1
3-*O*-α-D-Glucopyranosylsorbitol	<0·1	1,2-Di-*O*-methyl-D-mannitol	1·0
3-*O*-β-D-Glucopyranosylsorbitol	<0·1	Galactitol	1·0
3-*O*-α-Maltosylsorbitol	<0·1	6-Deoxy-D-galactitol	1·0

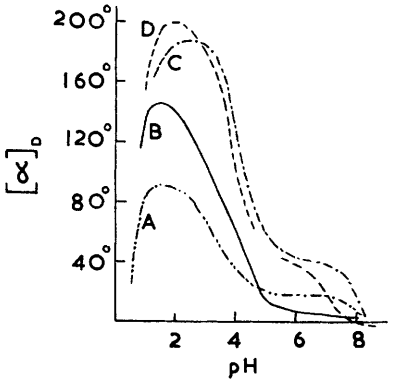
[1] Gernez, *Compt. rend.*, 1891, **112**, 1360; Honnolaître, *Ann. Chim.* (*France*), 1925, **3**, 5; Tanret, *Bull. Soc. chim. France*, 1921, **29**, 670; Richtmyer and Hudson, *J. Amer. Chem. Soc.*, 1951, **73**, 2249; Barker, Bourne, Foster, and Ward, *Nature*, 1957, **179**, 262; Rimbach and Ley, *Z. phys. Chem.*, 1922, **100**, 393; Frèrejacque, *Compt. rend.*, 1935, **200**, 1410; Sonbarew-Chatelain, *Compt. rend.*, 1939, **208**, 1652; Kaputinski, *Zhur. obshchei Khim.*, 1949, **19**, 219; Plško, *Chem. Zvesti*, 1958, **12**, 312.

[2] Bourne, Hutson, and Weigel, *J.*, 1960, 4252.

A further examination was made of the effect of the relative concentrations of the polyhydroxy-compounds and molybdate on the specific rotations of the former at pH values between 2 (Fig. 2) and 5. The rotation became constant when either one or two mol. of molybdate had been added. This revealed the compositions of the complexes which were found to be unchanged over this pH range, although the specific rotations were lower at the higher pH value. The specific rotations, when based on the polyhydroxy-compounds, were also found to be independent of the absolute concentrations of the solutions.

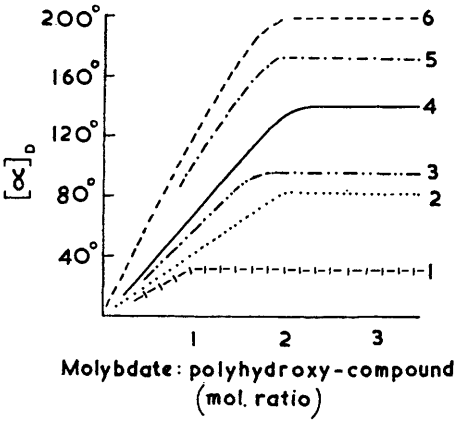
The behaviour of the complexes between polyhydroxy-compounds and molybdate during paper chromatography was examined as a possible analytical method. Impregnation of the paper with acidic molybdate solution [3] before chromatography did not yield reproducible results. When the complexes were formed by dissolving sodium molybdate and the polyhydroxy-compounds in the molar ratio of 2 : 1, and the sodium ions removed

FIG. 1. *Effect of* pH *on* $[\alpha]_D$ *of polyhydroxy-compounds in molybdate solutions (molar ratio molybdate/polyhydroxy-compound = 3).*



A, *Sorbito*. C, 2-*Deoxysorbitol*.
B, *Mannitol*. D, D-*Arabitol*.

FIG. 2. *Effect of relative concentrations of polyhydroxy-compounds and molybdate on* $[\alpha]_D$ *of polyhydroxy-compounds at* pH2.



1, 4-O-β-D-*Glucopyranosylsorbitol*. 2, 6-O-α-D-*Galactopyranosylsorbitol*. 3, *Sorbitol*. 4, D-*Mannitol*. 5, 2-*Deoxysorbitol*. 6, D-*Arabitol*.

with an ion-exchange resin, the resulting solutions could be chromatographed. Paper chromatography in acidic or neutral solvents revealed (Table 2) a trace of molybdic acid remaining on the origin line and traces of the polyhydroxy-compounds which migrated at their normal rate. In each case, between these two spots one or two components could be detected. We believe that these are mono- and di-molybdate complexes of the polyhydroxy-compounds.

The dihydroxy-compounds examined did not migrate during ionophoresis (Table 1) and hence do not form complexes with molybdate. This suggests that at least three hydroxyl groups are required for complex-formation with molybdate, as is the case for sugars and other cyclic polyhydroxy-compounds with six ring-atoms.[2] In agreement with Richardson's results,[4] glycerol did not form a complex.

It can be seen from Fig. 2 and Table 2 that sorbitol and 2-deoxysorbitol will each form a complex containing two molybdenum atoms per molecule. A 3-*O*-substituted sorbitol does not migrate during ionophoresis in molybdate solution (Table 1) and hence does not give a complex. Since the α- and β-D-glucopyranosides and D-galactose do not give a

[3] Swain, *Biochem. J.*, 1953, **53**, 200.
[4] Richardson, *J. Inorg. Nuclear Chem.*, 1959, **9**, 267.

complex,[2] it is evident that each molecule of 4-*O*-substituted sorbitol will form a complex containing one molybdenum atom, and each molecule of 6-*O*-substituted sorbitol a complex containing two molybdenum atoms (Fig. 2). As a mixture of 5-*O*-α-D-glucopyranosyl-sorbitol and 2-*O*-α-D-glucopyranosyl-D-mannitol, obtained by reduction of leucrose, migrated during ionophoresis at the same rate as 6-*O*-α-D-galactopyranosylsorbitol it is reasonable to assume that a 5-*O*-substituted sorbitol also will form a complex containing two molybdenum atoms.

D-Mannitol and D-arabitol each form a complex containing two molybdenum atoms (Fig. 2 and Table 2). 3-*O*-Substituted D-arabitol does not form a complex (Table 1). Richardson [4] has found by conductometric measurements that erythritol forms a complex containing one molybdenum atom: our chromatographic method (Table 2) has confirmed this.

It is clear that more studies are necessary before the detailed structures of the complexes between molybdate and acyclic polyhydroxy-compounds can be assigned. The problem is more difficult than in the case of cyclic polyhydroxy-compounds: *e.g.*, the number of conformations which can be adopted by an acyclic polyhydroxy-compound is very much greater. It was thought that the application of ionophoresis of polyhydroxy-compounds in molybdate solution was of greater immediate value than the assignment of the structure of the complexes.

In a previous paper [2] we reported the M_S values (defined in Table 1) of sugars and other cyclic polyhydroxy-compounds. It can now be seen that the common sugars may easily be separated from their reduction products. The method has found routine use in checking the complete reduction of sugars during treatment with sodium borohydride.

The compositions of the complexes between molybdate and substituted sorbitols suggested that the M_S values of *O*-glycosylsorbitols obtained by reduction of oligosaccharides with D-glucose as the reducing end-group would fall into three well-defined groups, *i.e.*, of those with 1,3-, 1,4-, and 1,2- or 1,5- or 1,6-glycosidic linkages.[5] This has been shown to be so (Table 1). The M_S values of the three groups of *O*-glycosylsorbitols derived from di- and tri-saccharides are $<0{\cdot}1$, $0{\cdot}4$, and $0{\cdot}7$—$0{\cdot}9$, respectively, thus allowing an efficient separation. Foster [6] has shown that disaccharides of D-glucose with 1,2- or 1,4-glucosidic linkages can be differentiated from those with 1,3- or 1,6-glucosidic linkages by ionophoresis in borate solution. Ionophoresis in molybdate solution is hence complementary to that in borate solution.

Ionophoresis in molybdate solution can be applied also to the reduction products of oligosaccharides containing up to 8 glucose units, as the M_S values are reasonably high, especially if the reducing glucose unit of the original oligosaccharide was linked by a 1,6-linkage. Table 1 shows the M_S values of the reduced oligosaccharides of the isomaltose series.

Table 1 also shows that 3-*O*-glycosyl-D-mannitol and 2-*O*-glycosyl-D-mannitol can be readily separated by ionophoresis in molybdate solution.

Experimental

Effect of pH *on Optical Rotation of Acyclic Polyhydroxy-compounds in Molybdate Solutions.*—Several solutions containing hydrated sodium molybdate and the polyhydroxy-compound (*ca.* 1—3%) in the molar ratio of 3 : 1 were adjusted with sulphuric acid to pH values between 1 and 8. The optical rotations measured were expressed as $[\alpha]_D$ and based on the polyhydroxy-compound. The results are shown graphically in Fig. 1.

Paper Ionophoresis.—Paper ionophoretograms were prepared by applying a voltage of 20—80 v per cm. across 10 cm. wide lengths of Whatman No. 3MM filter-paper for 1—2 hr. in

[5] Bourne, Hutson, and Weigel, *Chem. and Ind.*, 1959, 1047.
[6] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.

an apparatus similar to that described by Gross,[7] to whom we are grateful for advice. The electrolyte consisted of hydrated sodium molybdate (25 g.) in water (1200 ml.) adjusted to pH 5 with sulphuric acid. Compounds were detected with acetone–silver nitrate–alcoholic sodium hydroxide [8] or by spraying the paper with 0·1N-sulphuric acid and heating it at 120° for 10 min. The latter treatment caused all compounds containing primary hydroxyl groups to appear as bluish-green spots. Migration rates (Table 1) were expressed as M_S values.[2]

Effect of Relative Concentrations of Acyclic Polyhydroxy-compounds and Molybdate on the Optical Rotation of the Former.—Solutions containing the polyhydroxy-compound (*ca.* 1—14%) and hydrated sodium molybdate in varying molar ratios were adjusted to pH 2 with sulphuric acid. The optical rotations measured were expressed as $[\alpha]_D$ and based on the polyhydroxy-compound. The results are shown graphically (Fig. 2) by plotting $[\alpha]_D$ against the molar ratio of molybdate and polyhydroxy-compound. Repetition of the experiments at pH 5 resulted in lower values of $[\alpha]_D$ but no change in the shapes of the curves.

Paper Chromatography of Pre-formed Complexes between Molybdate and Acyclic Polyhydroxy-compounds.—Complexes were formed by dissolving polyhydroxy-compounds (1 mol.) and hydrated sodium molybdate (2 mol.) in water and adjusting the solutions to pH 2 with Amberlite IR-120 (H^+). The solutions were then spotted on to Whatman No. 1 filter-paper and the paper was irrigated with the organic layer of a mixture of butan-1-ol–acetic acid–water (4 : 1 : 5). Acetone–silver nitrate–alcoholic sodium hydroxide [8] was used to locate polyhydroxy-components, and an aqueous solution of catechol [9] (5%) to locate molybdate-containing components. The results are shown in Table 2.

TABLE 2.

Compound	$R_{Glucose}$	$R_{Glucose}$ of components of pre-formed complexes			
Sorbitol	1·0	0·9 *	0·6 *†	0·5 *†	0 †
2-Deoxysorbitol	1·2	1·2 *	0·7 *†	0·4 *†	0 †
D-Mannitol	1·1	0·9 *	0·7 *†	0·5 *†	0 †
Galactitol	1·1	1·1 *	0·7 *†	0·5 *†	0 †
D-Arabitol	1·1	1·1 *	0·7 *†	0·4 *†	0 †
Erythritol	1·7	1·7 *	1·2 *†		0 †
Glycerol	2·2	2·2 *			0 †
Molybdic acid					0 †

* Detected with acetone–silver nitrate–alcoholic sodium hydroxide.
† Detected with aqueous solution of catechol.

The organic phase of a mixture of butanol–ethanol–water (4 : 1 : 5) gave a wider separation of the components but the spots were less discrete.

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[7] Gross, *Chem. and Ind.*, 1959, 1219.
[8] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.
[9] Pridham, *J. Chromatog.*, 1959, **2**, 605.