4. *Complexes between Polyhydroxy-compounds and Inorganic Oxy-acids. Part V.*[1] *Tungstate Complexes of Acyclic Polyhydroxy-compounds.*

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The paper-electrophoretic behaviour of acyclic polyhydroxy-compounds in tungstate solution is described. The method can be used to determine the position of the glycosidic linkage in oligosaccharides. Structures have been suggested for the complexes formed between the ditungstate ion (and dimolybdate ion) and acyclic vicinal tetritols.

In Part IV [1] we have shown that ionic complexes are formed by reaction between one or two molecules of a polyhydroxy-compound, depending on its structure, and one dimolybdate or ditungstate ion. We now report studies on the complexes formed between tungstate and simple polyhydroxy-compounds.

Fig. 1 shows the effect of pH on the specific rotation of D-mannitol in tungstate solutions. Curve A–B was obtained when (*a*) solutions containing D-mannitol and sodium tungstate were acidified to pH 2—10 by addition of sulphuric acid, and (*b*) D-mannitol was added to sodium tungstate solutions previously adjusted to pH 6—9, the optical rotation being measured immediately. On addition of D-mannitol in case (*b*) the pH increased when initially $>ca.$ 7 and decreased when initially $<ca.$ 7. On storage of the mixture, the rotation, which, after addition of sulphuric acid or D-mannitol, had pH $>ca.$ 6, increased and the final rotations corresponded to curve A′—B. pH values in Fig. 1 were recorded at the time of the polarimetric measurements: the exact reproducibility of curve A depends, of course, on the time required for measurements of optical rotations. Similar results have been obtained with D-glucitol. Although paper electrophoresis in tungstate solution has shown that 1-*O*-α-D-galactopyranosyl- and 3-*O*-β-D-glucopyranosyl-L-gulitol form complexes with tungstate (see below), their specific rotations were not markedly affected by the presence of tungstate.

The composition of the tungstate complexes of D-mannitol and D-glucitol formed at pH 5·5, *i.e.*, where immediate formation of stable complexes occurs, was determined as for molybdate complexes.[2] The rotations became constant (Fig. 2) when 2 mol. of tungstate had been added. Since the ditungstate ion, $W_2O_7^{2-}$, is the complex-forming agent the complexes are derived from one molecule of D-mannitol or D-glucitol and one ditungstate ion. The same composition, *i.e.*, W/D-mannitol = 2, has been found for the D-mannitol complex, which shows a negative rotation and is formed by the addition of the hexitol to a sodium tungstate solution of pH 7·15. The conversion of the (−)-complex into the (+)-complex is a first-order reaction with a rate constant $k = 4{\cdot}31 \times 10^{-3}$ min.$^{-1}$; possibly an intramolecular reaction.

Paper electrophoresis in tungstate solution was carried out at *ca.* pH 5, which allows the immediate formation of stable complexes and moderate ionisation. The mobilities of polyhydroxy-compounds relative to D-glucitol in tungstate [$M_s(W)$] and molybdate solutions [$M_s(Mo)$] [2] are shown in Tables 1—3. Since methyl D-gluco-, D-manno-, and D-galactopyranoside do not form complexes with tungstate [3] (or molybdate [4]), studies on the effect of substitution in hexitols on complex formation were also possible by the use of glycopyranosyl-hexitols.

Acyclic diols do not migrate during electrophoresis in molybdate solution.[2] The same compounds have now been shown not to form complexes with tungstate. Also, 1-deoxy-2,5-di-*O*-methyl-L-mannitol did not migrate in either electrolyte. Of the compounds

[1] Part IV, Angus and Weigel, *J.*, 1964, 3994.
[2] Bourne, Hutson, and Weigel, *J.*, 1961, 35.
[3] Angus, Bourne, Searle, and Weigel, *Tetrahedron Letters*, 1964, 55.
[4] Bourne, Hutson, and Weigel, *J.*, 1960, 4252.

TABLE 1.

Mobilities and structures of tungstate and molybdate complexes of acyclic polyols possessing a vicinal tetritol system.

Polyol	$M_s(W)$	$M_s(Mo)$	Carbon atoms in polyol corresponding with positions 1—4 in (II—V): 1,2,3,4			2,3,4,5			3,4,5,6		
				R^2	R^3 *		R^2 *	R^3 *		R^2 *	R^5
Tetritols											
Erythritol	0·81—0·94	1·0[2]	II	H	H						
L-Theitol	0·24	0·5[2]	IV	H	H						
Pentitols											
D-Arabinitol	1·04	1·1[2]	V	H	5	III	H	H			
(D-)Ribitol †	1·03	1·1[2]	III	H	5						
(D-)Xylitol †	1·04	1·1[2]	IV	H	H						
Deoxy-pentitols											
1-Deoxy-D-arabinitol	1·09	1·03				III	H	H			
1-Deoxy-D-lyxitol	0·58—0·65	0·95				V	1	H			
1-Deoxy-D-xylitol	0·82	0·96				V	H	H			
Hexitols											
(D-)Allitol †	0·86—0·97	0·94	III	H	5,6	III	1	6			
D-Altritol	0·83—0·97	0·99	V	H	5,6	III	H	6	III	2,1	H
(D-)Galactitol †	1·00	1·0[2]	IV	H	5,6	II	H	H			
D-Glucitol	1·00	1·0[2]	IV	H	H	V	H	6	III	H	H
D-Mannitol	1·00	1·0[2]	II	H	H	V	1	6			
L-Iditol	1·00		IV	H	H	V	H	H			
Monodeoxy-hexitols											
1-Deoxy-D-altritol	0·88—1·00	0·98				III	H	6	III	2,1	H
1-Deoxy-L-galactitol	1·03	1·0[2]				III	H	H	IV	2,1	H
1-Deoxy-D-glucitol	0·98	0·98				V	H	6	III	H	H
1-Deoxy-L-gulitol	0·98	0·94				V	1	H	IV	H	H
1-Deoxy-D-mannitol	1·00	1·0[2]				V	1	6	III	H	H
1-Deoxy-D-talitol	0·83—1·04	1·04				II	1	H	V	2,1	H
2-Deoxy-D-*arabino*-hexitol	1·00	1·0[2]							III	H	H
2-Deoxy-D-*lyxo*-hexitol	0·42—0·61	0·80							V	2,1	H
2-Deoxy-D-*ribo*-hexitol	0·17—0·57	0·13—0·57							III	2,1	H
2-Deoxy-L-*xylo*-hexitol	1·09	1·07							IV	H	H
Dideoxy-hexitols											
1,6-Dideoxy-D-altritol	1·05	0·90				III	H	6			
1,6-Dideoxy-galactitol	1·09	0·98				II	H	H			
1,6-Dideoxy-L-mannitol	0·87—0·95	1·00				IV	1	6			
O-*Methylhexitols*											
1-*O*-Methyl-L-gulitol	0·89	0·93				V	1	H	IV	H	H
2-*O*-Methyl-D-mannitol	0·98	1·0[2]							III	H	H
1,2-Di-*O*-methyl-D-mannitol	0·95	1·0[2]							III	H	H

* The numbers indicate the carbon atoms of the polyol present in the substituents R^2 or R^3.
† The prefix D indicates that the D-isomer only of the 1,2,3,4-complex is illustrated.

TABLE 2.

Electrophoresis in tungstate and molybdate solutions of acyclic polyols possessing a vicinal triol system.

Polyol	$M_s(W)$	$M_s(Mo)$
3-*O*-Methyl-L-gulitol	0·09	0·47
3-Deoxy-L-*xylo*-hexitol	0	0·21

Not complex-forming [$M_s(W$ or $Mo) < 0·05$]
Glycerol
2-Deoxy-D-*erythro*-pentitol
2,3-Di-*O*-methyl-D-glucitol
3-*O*-Methyl-D-glucitol
3-Deoxy-D-*arabino*-hexitol
3-Deoxy-D-*ribo*-hexitol

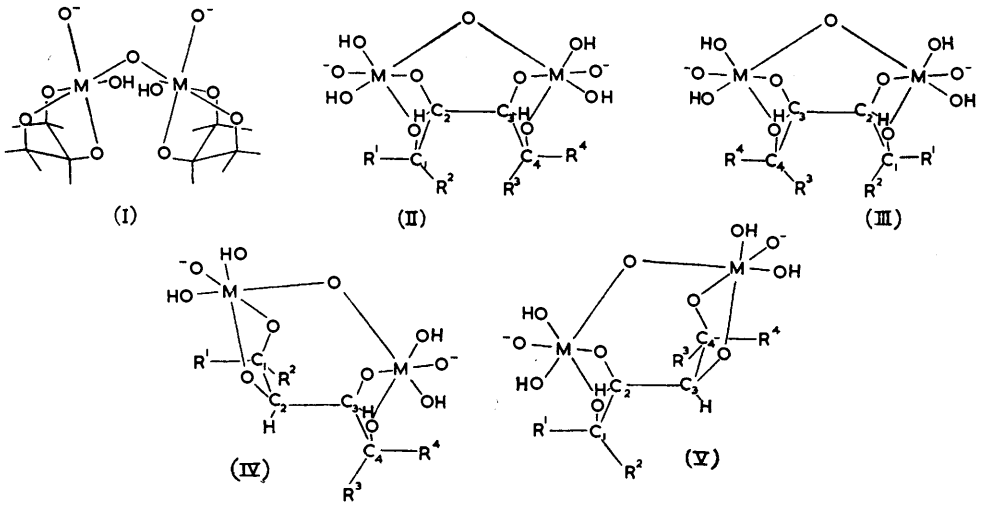
examined that possess only three adjacent hydroxyl groups (in the acyclic polyol portion), only 3-substituted L-gulitols formed complexes, *i.e.*, those with a 1,2,3(α,αT)-triol system (Barker and Bourne's nomenclature [5]). We have already shown [1] that the complexes of compounds containing such structural features are formed from two molecules of the

TABLE 3.

Mobilities of glycopyranosyl-hexitols.

Compound	$M_s(Mo)$	$M_s(W)$	Compound	$M_s(Mo)$	$M_s(W)$
Derivatives of D-*glucitol*			*Derivatives of* L-*gulitol*		
2-*O*-α-D-Glucopyranosyl-	0·69	0·76	1-*O*-α-D-Galactopyranosyl- ...	0·80	0·68
2-*O*-β-D-Glucopyranosyl-	0·70	0·73	1-*O*-α-D-Glucopyranosyl-	0·78	0·76
3-*O*-α-D-Glucopyranosyl-	0	0	1-*O*-β-D-Glucopyranosyl-	0·69	0·66
3-*O*-β-D-Glucopyranosyl-	0	0	2-*O*-α-D-Glucopyranosyl-	0·72	0·76
Derivatives of D-*mannitol*			3-*O*-β-D-Galactopyranosyl- ...	0·40	0·10
			3-*O*-α-D-Glucopyranosyl-	0·46	0·17
2-*O*-α-D-Glucopyranosyl-	0·72	0·70	3-*O*-β-D-Glucopyranosyl-	0·37	0·10
2-*O*-α-D-Mannopyranosyl- ...	0·80				
3-*O*-α-D-Mannopyranosyl-	0	0			

polyol and one ditungstate (or dimolybdate) ion (I, partial structure; M = W or Mo). It would be expected that glycerol and 3-deoxy-L-*xylo*-hexitol also form complexes with tungstate, though this is less likely with glycerol owing to the greater freedom of rotation about C–C bonds. The Tables show that the smaller the 3-substituent of L-gulitol the smaller the mobility (cf. 3-*O*-glycopyranosyl-L-gulitols, 3-*O*-methyl-L-gulitol, 3-deoxy-L-*xylo*-hexitol). On the other hand, 3-deoxy-L-*xylo*-hexitol does form a complex with molybdate, although its $M_s(Mo)$ value is lower than would be expected. These results



agree with our earlier findings that complex formation is less affected by conformational factors with molybdate than with tungstate.[3]

All compounds with at least four adjacent hydroxyl groups (in the acyclic polyol portion) form complexes with tungstate (or molybdate), presumably with the same composition as those produced from D-mannitol and D-glucitol (cf. molybdate complex of 2-deoxy-D-*arabino*-hexitol [2]). There are four possible structures (II—V) for such complexes, the 2,3-diol groups having *erythro* (II and III) and *threo* (IV and V) configurations. Table 1 shows that the electrophoretic mobilities, and thus the stabilities of the complexes, are influenced by the sizes of the substituents R^2 and R^3 in (II—V). The mobilities of the polyols with only four adjacent hydroxyl groups (1-deoxypentitols, and

[5] Barker and Bourne, *J.*, 1952, 905.

2-deoxy- and 1,6-dideoxy-hexitols) that form a complex in which R^2 and/or R^3 contain carbon atoms are smaller than those of compounds of the same molecular size that form the same type of complex (*i.e.*, type II and III, or IV and V), the substituents R^2 and/or R^3 of which, however, are hydrogen atoms (*i.e.*, 1-deoxy-D-lyxitol, 1-deoxy-D-xylitol; 2-deoxy-D-*ribo*-hexitol, 2-deoxy-D-*arabino*-hexitol; 2-deoxy-D-*lyxo*-hexitol, 2-deoxy-L-*xylo*-hexitol; 1,6-dideoxy-D-altritol, 1,6-dideoxy-galactitol).

On the other hand, pentitols, hexitols, and 1-deoxy-hexitols might form two or three complexes involving their various vicinal tetritol systems. The facts that galactitol, D-glucitol, D-mannitol, and L-iditol have identical mobilities and that those of 1-deoxy-L-galactitol, -D-glucitol, -L-gulitol, and -D-mannitol are comparable indicate preferential formation of complexes in which R^2 and R^3 are hydrogen. The substituents R^2 and/or R^3 of all possible complexes of allitol, D-altritol, 1-deoxy-D-altritol, and 1-deoxy-D-talitol contain carbon atoms. Consequently, these compounds streak during electrophoresis in tungstate solution (less so for molybdate; see above).

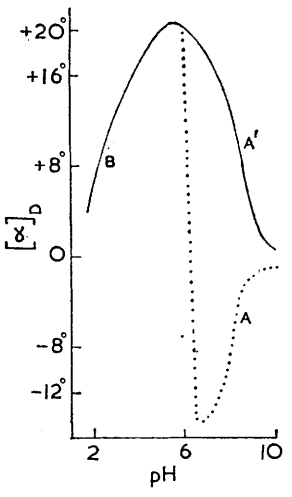


FIG. 1. Effect of pH on $[\alpha]_D$ of D-mannitol in tungstate solution.

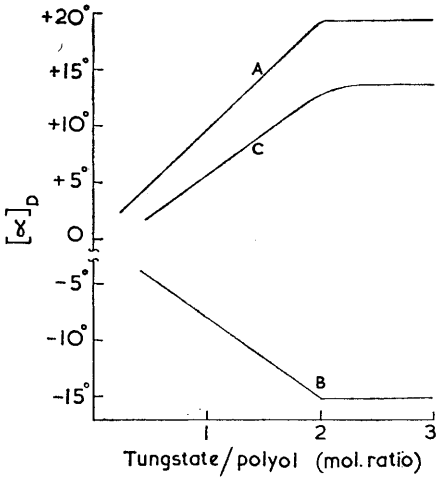


FIG. 2. Effect of relative concentrations of polyols and tungstate on $[\alpha]_D$ of polyol. A, D-mannitol, method (i); B, D-mannitol, method (ii); C, D-glucitol, method (i).

The mobilities of erythritol and L-threitol suggest that complexes of types (II) and (III) are generally more stable than those of types (IV) and (V).

Table 3 shows that during electrophoresis in tungstate solution, glycopyranosyl-hexitols fall into the same three classes, as in molybdate:[2] (*a*) 3-*O*-glycopyranosyl-D-glucitols; (*b*) 2-*O*-glycopyranosyl-D-glucitols, 1- and 2-glycopyranosyl-L-gulitols; and (*c*) 3-*O*-glycopyranosyl-L-gulitols. In addition, the α-isomers of D-glucopyranosyl-D-glucitols and -L-gulitols have slightly higher mobilities than their corresponding β-isomers. Thus, electrophoresis of D-glucopyranosyl-D-glucoses[6] and their reduction products in borate and tungstate or molybdate[2] solutions, respectively, can indicate the position of the glycosidic linkage to the reducing moiety in the original disaccharide.

Further evidence for preferential formation of complexes with the smallest interactions between R^2 and R^3 (II—V) was provided by periodate oxidation of hexitol–tungstate complexes. Solutions of complexes were prepared by adjusting solutions containing sodium tungstate and the polyols in the molar ratio of 2·1—3·0 : 1 to *ca.* pH 5·2 at which

[6] Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.

immediate formation of stable complexes occurs, and then to pH 7·8, in order to carry out the periodate oxidations in more neutral solutions.

The tungstate complexes of D-glucitol, D-mannitol, galactitol, allitol, D-altritol, and 2-deoxy-D-*arabino*-hexitol were treated with 2 mol. (per polyol) of sodium periodate. The components of the reaction mixtures, disclosed by paper-chromatographic analysis, both before and after adjustment of the solution to pH 2, are shown in Table 4. (We had already found that the R_F values of complex-forming polyols are affected by the presence of tungstate at pH 7—8, but are unaffected when the solutions are adjusted to pH 2.) The materials obtained from the complexes of D-glucitol, D-mannitol, and galactitol were fractionated on charcoal columns, further purified by paper chromatography, and converted into derivatives.

TABLE 4.

Periodate oxidation of polyol–tungstate complexes.

	Component of reaction mixture			Component of reaction mixture
Polyol	Paper-chromatographic identity	Derivative (m. p.)	Polyol	Paper-chromatographic identity
D-Glucitol	L-Xylose	155—156° *	Allitol	(DL-)Ribose
	D-Arabinose	182—183 †	D-Altritol	D-Altritol
	D-Glucitol	128—129 ‡	2-Deoxy-D-*arabino*-hexitol	2-Deoxy-D-*arabino*-hexitol
D-Mannitol	D-Arabinose	182—183 †		
	D-Mannitol (trace)			
Galactitol	Galactitol	169—170 §		

* L-Xylose *p*-nitrophenylhydrazone. † D-Arabinose *p*-nitrophenylhydrazone. ‡ Hexa-*O*-benzoyl-D-glucitol. § Hexa-*O*-acetylgalactitol.

The results shown in Table 4 are not those expected from the partial periodate oxidation of the polyols themselves [7,8] and should thus indicate the vicinal tetritol system of the polyols involved in complex formation. It is evident that tungstate forms complexes with the tetritol systems on C-1, C-2, C-3, and C-4 of allitol and D-mannitol, and those on C-2, C-3, C-4, and C-5 of D-altritol and galactitol. Table 1 shows that the interaction between R^2 and R^3 of the complexes formed with these tetritol systems is the smallest. The materials obtained from D-glucitol arise from its three possible complexes of comparable stability. As expected, the tungstate complex of 2-deoxy-D-*arabino*-hexitol was unaffected by periodate.

The results of the periodate oxidation of the polyol–tungstate complexes are thus in agreement with the above correlation between their structures and mobilities during paper electrophoresis.

EXPERIMENTAL

Effect of pH *on Optical Rotations of Acyclic Polyhydroxy-compounds in Tungstate Solution.*—(i) Several solutions containing sodium tungstate and D-mannitol or D-glucitol (*ca.* 0·6—1%) in the molar ratio of 3 : 1 were adjusted with sulphuric acid to pH values between 2 and 10. The optical rotations, expressed as $[\alpha]_D$ and based on the polyhydroxy-compound, were measured immediately. The results obtained with D-mannitol are shown graphically (Fig. 1, curve A—B). The rotation of solutions adjusted to pH $>$ *ca.* 6 increased on storage, the final rotations after 2 days corresponding to curve A′—B. D-Glucitol exhibited max. $[\alpha]_D$ 13·5°. Addition of alkali to solutions at pH 4 resulted in changes in optical rotation represented essentially by curve A′—B (Fig. 1, for D-mannitol).

(ii) D-Mannitol (0·12 g.) or D-glucitol (0·12 g.) in water (1 ml.) was added to each of several solutions of anhydrous sodium tungstate (0·4 g.) in water (15 ml.) previously adjusted to pH values between 6 and 9 by treatment with Amberlite IR-120(H^+). The pH and the optical rotation, expressed as $[\alpha]_D$ and based on the hexitols, of the solutions were measured immediately (Fig. 1, curve A—B) and again after *ca.* 18 hr. (Fig. 1, curve A′—B). The results

[7] Schwarz, *J.*, 1957, 276.
[8] Hutson and Weigel, *J.*, 1961, 1546.

obtained with D-mannitol and D-glucitol were essentially identical, except for the magnitude of rotational changes (D-glucitol: min. $-9{\cdot}3°$, max. $+14{\cdot}0°$). On addition of hexitols to the tungstate solutions the pH increased when initially >7 and decreased when initially <7.

Kinetic Measurements.—D-Mannitol (0·22 g.) in water (5 ml.) was added to a solution of anhydrous sodium tungstate (0·8 g.) in water (10 ml.) adjusted to pH 7·15 by treatment with Amberlite IR-120(H^+), and the whole was made up to 25 ml. and kept at room temperature. The optical rotatory power of the solution was measured (4 dm. tube, sodium light) initially (α_0), at time intervals (α) and after *ca.* 18 hr. (α_∞). The plot of $\log[(\alpha_\infty - \alpha_0)/(\alpha_\infty - \alpha)]$ against time gave a straight line. From this the rate constant $k = 4{\cdot}31 \times 10^{-3}$ min.$^{-1}$ was calculated.

Effect of Relative Concentrations of Acyclic Polyhydroxy-compounds and Tungstate on Optical Rotation.—(i) The same method was used as for molybdate complexes,[2] but with pH *ca.* 5·5. Optical rotations were expressed as $[\alpha]_D$, based on the polyhydroxy-compound. The results are shown graphically in Fig. 2.

(ii) To each of several solutions of hydrated sodium tungstate (0·8 g.) in water (10 ml.) adjusted to *ca.* pH 7 was added 4·42% D-mannitol solution of such volume to give molar ratio of tungstate to D-mannitol between 0·5 : 1 and 3 : 1. The whole was made up to 25 ml. The optical rotation, expressed as $[\alpha]_D$ and based on D-mannitol, was measured immediately. The results are shown graphically in Fig. 2.

Paper Electrophoresis.—The methods used were those described previously.[3,4]

Paper Chromatography.—The solvent system was butan-1-ol–ethanol–water (4 : 1 : 5) (organic phase).

Periodate Oxidation of Polyol–Tungstate Complexes.—(i) Separate solutions containing sodium tungstate and D-glucitol (1·8%), D-mannitol (1·8%), galactitol (1·8%), allitol (0·2%), D-altritol (0·2%), or 2-deoxy-D-*arabino*-hexitol (0·4%) in the molar ratio of 2·1—3·0 : 1 were first adjusted with 2N-sulphuric acid to pH 5·2 and then, after *ca.* 1 hr., with N-sodium hydroxide to pH 7·8. To each was added standard sodium periodate solution (2 mol., relative to polyol) and the whole set aside overnight. The mixtures were analysed by paper chromatography. Small portions were adjusted with 2N-hydrochloric acid to pH 2 and, next morning, were analysed by paper chromatography. The results are shown in Table 4.

(ii) *Characterisation of products from D-glucitol–, D-mannitol–, and galactitol–tungstate complexes.* The components of the reaction mixtures were fractionated on a column (50 × 3·5 cm.) of Ultrasorb S.C. 120/140 (activated charcoal) (supplied by British Carbo Norit Union, Ltd.). Water (*ca.* 500 ml.) removed inorganic materials. Aqueous ethanol (3 : 1) eluted (*a*) L-xylose, D-arabinose, and D-glucitol (from the D-glucitol complex and in that order), (*b*) D-arabinose (from the D-mannitol complex), and (*c*) galactitol (from the galactitol complex). These materials were further purified by paper chromatography. The pentoses were converted into their *p*-nitrophenyl hydrazones, D-glucitolinto its hexabenzoate, and galactitol into its hexa-acetate (m. p.s unchanged when mixed with authentic specimens).

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