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CHROMATOGRAPHY OF POLYHYDROXY COMPOUNDS ON CELLULOSE IMPREGNATED WITH TUNGSTATE: DETERMINATION OF PSEUDO-STABILITY CONSTANTS OF COMPLEXES*

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ABSTRACT

Impregnation of cellulose with tungstate affects the chromatographic migration rates of a number of polyhydroxy compounds, offering a means, on small and large scales, of resolution of mixtures not easily achieved by other methods. The effects have been correlated with the structure of the polyhydroxy compounds and their tungstate complexes. Pseudo-stability constants of the complexes have been determined.

INTRODUCTION

Anionic complexes are formed by reaction between a variety of inorganic oxy-acids and polyhydroxy compounds possessing appropriate structural features[1], whereas such compounds as copper(II) acetate afford cationic complexes[2]. Such complexes form the basis of eletrophoresis[1] and ion-exchange chromatography[2,3] of neutral carbohydrates and related compounds. On the other hand, it is known that the incorporation of such materials into chromatographic solvents affects migration rates. For example[4,5], incorporation of boric acid and borates increases and decreases, respectively, the R_F values of a number of carbohydrates. Benzeneboronic acid has been used similarly[6], and assignment of structure[7] to synthetic carbohydrates has been made by chromatography with solvents containing this acid. The products of the reaction of phenyl isothiocyanate with 2-amino-2-deoxy-D-galactose and 2-amino-2-deoxy-D-glucose have been separated by chromatography using paper soaked in sodium molybdate solution[8].

We have investigated the chromatography of polyhydroxy compounds on supports that are impregnated with a variety of inorganic complexing agents[9]. These

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methods are simple, economical, and can easily be applied to small and large quantities of material. This report describes chromatography on paper and cellulose powder impregnated with tungstate.

RESULTS AND DISCUSSION

Table I shows the migration rates of compounds on strips of chromatography paper impregnated with tungstate solution adjusted to pH 6 [$R_{GLC}(W_6)$] or pH 8 [$R_{GLC}(W_8)$]; migration rates are expressed relative to the migration rate of D-glucose. These pH values were chosen because they are close to the values where (*a*) many complexes exhibit maximal stability (pH 5–6) and (*b*) complexes are not readily formed (pH ~9)[10]. The migration rates on ordinary paper (R_{GLC}), as well as electrophoretic mobilities in tungstate solution of pH 5 [$M_S(W)$][10,11], are included for comparison.

The results show that identification of compounds and resolution of mixtures feasible by electrophoresis in tungstate solution can also be achieved by chromatography on paper impregnated with tungstate [*cf.* $M_S(W)$ and $R_{GLC}(W_6)$ of D-glucose and D-gulose]. In addition, compounds that are difficult to separate by electrophoresis or conventional chromatography (*e.g.*, pentitols, hexitols, or 1-deoxyhexitols) have sufficiently different $R_{GLC}(W_8)$ values to allow their separation.

It is impracticable to discuss in detail all possible applications of this method of chromatography, as they will vary with the individual problems encountered. However, Table I shows examples of the usefulness of adjusting to different pH values the tungstate used for impregnation. For example, the hexitols are more readily separated when the tungstate is adjusted to pH 8, whereas the pairs 1-deoxy-D-arabinitol and 1-deoxy-D-lyxitol, and D-gulose and D-mannose, are more efficiently separated at pH 6.

It was noted that tungstate (adjusted to pH 6 or 8) remained immobile during the development of the paper chromatograms. Furthermore, when only an area around the base line of the chromatography paper strip was impregnated with tungstate, tungstate could be detected, after development, only within this area, although several compounds had passed into the unimpregnated area. This shows that the migrating material is the free polyol of the equilibrium mixture. As, in general,

$$1/R_F = (1/k\zeta) + 1, \qquad (1)$$

where the partition coefficient

$$k = [\text{polyol in mobile phase}]/[\text{polyol in stationary phase}], \qquad (2)$$

and the phase ratio, ζ, accounts for the ratio of the volume of the mobile phase (V_m) to that of the stationary phase (V_s), *i.e.*, $\zeta = V_m/V_s$, the stability constant,

$$K = [\text{complex}]/[\text{polyol}]\,[\text{tungstate}], \qquad (3)$$

TABLE I

CHROMATOGRAPHIC MIGRATION RATES AND PSEUDO-STABILITY CONSTANTS

Compound	*Solvent* 1			*Solvent* 2			$M_S(W)$
	$R_{GLC}(W_6)$	R_{GLC}	Q_6[a]	$R_{GLC}(W_8)$	R_{GLC}	Q_8[b]	
Group A							
Erythritol	0.84	1.68	3.6	1.03	1.51	3.4	0.81–0.94
L-Threitol	1.80	1.63	1.6	0.44	1.48	7.7	0.24
D-Arabinitol	0.30	1.36	8.2	0.49	1.25	5.9	1.04
1-deoxy-	0.61	2.45	7.2	0.99	2.02	4.7	1.09
D-Lyxitol, 1-deoxy-	0.30	2.45	14.7	1.17	1.95	3.8	0.58–0.65
Ribitol	0.48	1.37	5.1	0.63	1.27	4.6	1.03
Xylitol	0.41	1.26	5.5	0.13	1.20	21.2	1.04
1-deoxy-D-	0.30	2.12	12.7	0.51	1.90	8.6	0.82
Allitol	0.26	1.08	7.5	0.39	1.12	6.6	0.86–0.97
D-Altritol	0.25	1.06	7.6	0.24	1.07	10.3	0.83–0.97
1-deoxy-	0.46	1.72	6.7	1.30	1.64	2.9	0.88–1.00
1,6-dideoxy-				1.30	2.07	3.7	1.05
Galactitol	0.25	1.02	7.3	0.33	0.97	6.8	1.00
1-deoxy-L-	0.40	1.88	8.5	0.74	1.56	4.8	1.03
1,6-dideoxy-				1.12	2.05	4.2	1.09
D-Glucitol	0.24	0.98	7.3	0.13	1.00	17.7	1.00
1-deoxy-	0.44	1.68	6.9	0.64	1.59	5.7	0.98
2-*O*-α-D-glucopyranosyl-	0.30	0.59	3.5	0.14	0.46	7.6	0.76
2-*O*-β-D-glucopyranosyl-	0.13	0.53	7.3	0.18	0.50	6.4	0.73
L-Gulitol, 1-deoxy-				0.31	1.42	10.5	0.98
1-*O*-α-D-glucopyranosyl-	0.12	0.42	6.3	0.05	0.35	16.1	0.76
L-Iditol	0.27	0.97	6.5	0.13	1.00	17.7	1.00
D-Mannitol	0.24	1.06	7.9	0.33	1.06	7.4	1.00
L-Mannitol, 1-deoxy-	0.46	1.90	7.4				1.00
1,6-dideoxy-	1.52	2.93	3.5	1.75	2.12	2.8	0.87–0.95
D-Talitol, 1-deoxy-	0.53	1.89	6.4	0.72	1.63	5.2	0.83–1.04
Average			6.9			7.3	
Group B							
D-Lyxose	0.5–0.7	1.45	4.3	1.04	1.40	3.1	0–1.0
D-Ribose	1.39	1.57	2.0	0.80	1.55	4.5	0.2
D-Gulose	0.43	1.10	4.6	0.80	1.15	3.3	1.1
D-Mannose	0.70	1.19	3.1	0.94	1.16	2.8	0–1.1

(Table continued on p. 28)

TABLE I (*continued*)

Compound	*Solvent* 1			*Solvent* 2			$M_S(W)$
	$R_{GLC}(W_6)$	R_{GLC}	Q_6[a]	$R_{GLC}(W_8)$	R_{GLC}	Q_8[b]	
Group B							
L-Gulitol, 3-*O*-α-D-glucopyranosyl-	0.36	0.53	2.6	0.07	0.52	17.1	0.17
3-*O*-β-D-glucopyranosyl-	0.37	0.45	2.2	0.15	0.50	7.7	0.10
epi-Inositol	0.16	0.48	5.4	0.28	0.46	3.8	1.0
Average			3.5			6.0	
Group C-a							
D-Arabinose	1.18	1.20	1.8	1.27	1.17	2.1	<0.05, for all
D-Xylose	1.50	1.41	1.7	1.36	1.31	2.2	compounds
D-Galactose	0.63	0.87	2.5	0.67	0.92	3.2	
D-Glucose	1.00[e]	1.00[d]	1.8	1.00[e]	1.00[f]	2.3	
D-Glucitol, 3-*O*-β-D-glucopyranosyl-	0.58	0.59	1.8	0.59	0.63	2.5	
3-*O*-methyl-				1.48	1.52	2.4	
Kojibiose	0.51	0.59	2.1	0.42	0.48	2.6	
Sophorose	0.55	0.54	1.8	0.51	0.53	2.4	
Laminaribiose	0.63	0.61	1.7	0.66	0.62	2.2	
Maltose	0.43	0.46	1.9	0.47	0.44	2.2	
Cellobiose	0.39	0.42	1.9	0.45	0.42	2.1	
Isomaltose	0.36	0.45	2.3	0.27	0.42	3.6	
Gentiobiose	0.34	0.37	2.0	0.26	0.39	3.5	
(+)-Inositol	0.46	0.54	2.1	0.48	0.54	2.6	
muco-Inositol	0.72	0.83	2.1	0.61	0.75	2.8	
myo-Inositol	0.28	0.38	2.4	0.29	0.36	2.9	0.05
scyllo-Inositol	0.30	0.42	2.5	0.26	0.33	2.9	
Average			2.1			2.8	
Group C-b							
Glycerol	3.28	2.16	1.2	3.12	1.92	1.4	<0.05, for all
L-Galactose, 6-deoxy-	1.87	1.53	1.5	1.87	1.40	1.7	compounds
α-D-Glucopyranoside, methyl	2.33	1.59	1.2	2.52	1.50	1.4	
D-Glucose, 3-*O*-methyl-	2.34	1.68	1.3	2.87	1.68	1.3	
Hexose, 2-deoxy-D-*arabino*-	2.27	1.72	1.4	3.13	1.69	1.2	
2-deoxy-D-*lyxo*-	2.00	1.62	1.5	2.70	1.54	1.3	
2-deoxy-D-*ribo*-	2.29	1.82	1.4	3.25	1.77	1.3	
α-D-Mannopyranoside, methyl	3.25	2.09	1.2	3.08	1.75	1.3	
Average			1.3			1.4	

[a] $Q_6 = 1.8\{R_{GLC}/R_{GLC}(W_6)\}$. [b] $Q_8 = 2.3\{R_{GLC}/R_{GLC}(W_8)\}$. [c] $R_F(W_6) = 0.10$. [d] $R_F = 0.18$. [e] $R_F(W_8) = 0.10$. [f] $R_F = 0.23$.

of the complex might then be estimated from the ratio K' of the partition coefficients,

$$K' = k/k(W), \tag{4}$$

where k and $k(W)$ are, respectively, the partition coefficients in the absence and presence of tungstate. However, this would be realistic only if R_F and $R_F(W)$ could be determined under conditions where the stationary phases are truly comparable, *i.e.*, have identical pH and identical ion concentration (non-complexing ions in the case of R_F). On the other hand, the magnitude of the effect of the addition of tungstate to the stationary phase on migration rates of compounds, a pseudo-stability constant, can be expressed by the quotient

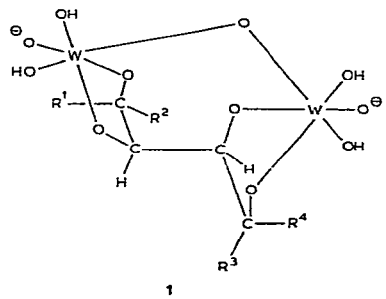
$$Q = R_F/R_F(W). \tag{5}$$

With the solvent systems used, R_{GLC} values are easier to determine than R_F values. The Q values were thus obtained from the experimentally determined R_{GLC} values of compounds and the R_F values of D-glucose, *i.e.*,

$$Q = \{R_F \text{ of D-glucose}/R_F(W) \text{ of D-glucose}\}\ \{R_{GLC}/R_{GLC}(W)\}. \tag{6}$$

The Q_6 and Q_8 values, determined using tungstate adjusted to pH 6 and 8, respectively, are shown in Table I.

In previous reports[10–12], we have shown that, at acidic pH values, anionic complexes are formed as follows. Acyclic compounds possessing a vicinal tetritol system (group A) will form complexes with structures **1**, **2**, or **3**, depending on their stereochemistry. Compounds possessing three hydroxyl groups in a spatial disposition approximating to that of **4** (group B) will form complexes with structure **5** (partial).



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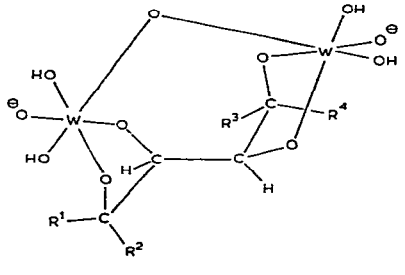

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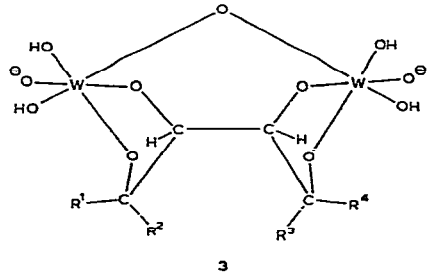

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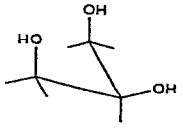

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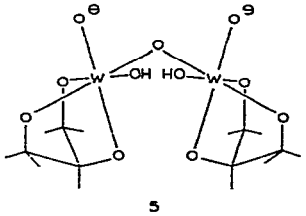

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Compounds lacking the above features in their more stable conformer or tautomer (group C) do not form ionic complexes in quantities detectable by paper electrophoresis. In Table I, the compounds examined are thus grouped. Although the compounds within each group exhibit a range of Q values at both pH values, the average Q values seem to indicate that, in general, the stability constants of the tungstate complexes are of the following order: group A > group B > group C.

As the Q values of the compounds of group C-b are almost unity, it is likely that these compounds do not form complexes at all with tungstate. These Q values probably reflect such factors as the difference between the phase ratios ζ and $\zeta(W)$.

The method described can easily be adapted to larger quantities of material. This is illustrated by the resolution of a mixture containing 1 g each of D-glucose, D-glucitol, and D-mannitol on a column of cellulose powder impregnated with tungstate adjusted to pH 8. The compounds were completely separated and recovered in almost quantitative yields. This method has also been applied[13] to the separation of D-glucose and D-galactose.

The results shown in Table II indicate that the method can also be applied to t.l.c. with tungstate-impregnated cellulose powder. It is anticipated that materials other than cellulose can be coated with tungstate, particularly those which have found application in high-pressure liquid chromatography.

TABLE II

T.L.C. ON CELLULOSE IMPREGNATED WITH TUNGSTATE

Compound	$R_F(W_8)$	R_F	Q_8
Galactitol	0.13	0.31	2.4
D-Glucitol	0.06	0.32	5.3
D-Glucose	0.28	0.32	1.1
D-Mannitol	0.15	0.33	2.2

EXFERIMENTAL

Solvents. — The chromatography solvents used were: *1*, 1-butanol–ethanol–water (40:11:19); *2*, acetone–1-butanol–water (5:3:2); and *3*, acetone–1-butanol–water (5:3:7).

Chromatography on paper impregnated with tungstate. — Strips (12.5 × 57 cm) of Whatman No. 1 paper were dipped in aqueous 5% sodium tungstate dihydrate adjusted with dilute sulphuric acid to either pH 6 (W_6) or 8 (W_8), blotted in folds of filter paper, and dried in air at room temperature. Compounds were spotted on the impregnated paper. Migration rates, $R_{GLC}(W_6)$ and $R_{GLC}(W_8)$, during descending chromatography were expressed relative to the movement of D-glucose. The migration rates, R_{GLC}, of compounds on untreated paper, using the appropriate solvent, were obtained in a similar manner. The R_F, $R_F(W_6)$, and $R_F(W_8)$ values of D-glucose, using the appropriate solvent, were determined separately on a large strip of paper

(12.5 × 150 cm). Compounds were detected with silver nitrate in acetone–ethanolic sodium hydroxide. The results are shown in Table I.

Separation of D-*glucose,* D-*glucitol, and* D-*mannitol by chromatography on cellulose powder impregnated with tungstate.* — Cellulose powder (Whatman, Standard Grade, 400 g) was suspended in aqueous 10% sodium tungstate dihydrate (2 dm^3; adjusted to pH 8 with dilute sulphuric acid) for ~16 h. The cellulose powder was then filtered off, dried in air of room temperature (~2 days), slurried with solvent *2*, and poured into a column (5 × 70 cm).

Impregnated cellulose powder (~10 g; prepared as above) was added to a solution of D-glucose (1 g), D-glucitol (1 g), and D-mannitol (1 g) in aqueous 10% sodium tungstate dihydrate (10 cm^3; pH adjusted as above). The whole mixture was freeze-dried, and then dried *in vacuo* over calcium chloride, slurried with solvent *2*, and placed on the aforementioned column. The column was then eluted with solvent *2*. Fractions (50 cm^3) were examined by chromatography on paper impregnated with tungstate (see above). Fractions 66–86 (fraction *A*) and 105–165 (fraction *B*) contained D-glucose and D-mannitol, respectively. After fraction 268, the eluent was changed to solvent *3*. Fractions 272–283 (fraction *C*) contained D-glucitol.

Fraction *A* was evaporated to dryness and the residue dissolved in a small volume of water. D-Glucose was adsorbed on a column of acid-washed Ultrasorb S.C. 120/140 (activated charcoal; British Carbo Norit Union, Ltd.), and the column was washed until free from tungstate. Aqueous ethanol (2.5%) eluted D-glucose, which was isolated by freeze-drying and further dried *in vacuo* over P_2O_5. The weight of the residue was 0.97 g. A portion was converted into penta-*O*-acetyl-β-D-glucopyranose, m.p. and mixture m.p. 130–132°.

Fractions *B* and *C* were treated similarly, except that the aqueous solutions were adjusted to pH 9.3 before being placed on the charcoal. Fraction *B* gave crystalline D-mannitol (0.94 g, from 95% ethanol). The weight of the final residue of fraction *C* was 0.91 g. A portion was converted into hexa-*O*-acetyl-D-glucitol, m.p. and mixture m.p. 101–102°.

T.l.c. on cellulose impregnated with tungstate. — (*a*) Avicel (8 g; American Viscose Co.) was slurried with water (32 cm^3). Layers (0.25 mm) were spread on glass plates, which were then dried at 80° for 30 min and stored in a desiccating box over silica gel.

(*b*) Avicel (7 g) was slurried with aqueous 5% sodium tungstate dihydrate (20 cm^3) adjusted to pH 8 with dilute sulphuric acid. Layers were prepared as described in (*a*).

(*c*) Chromatograms were developed with solvent *2*. Compounds were detected with silver nitrate in acetone–ethanolic sodium hydroxide.

REFERENCES

1 H. Weigel, *Adv. Carbohydr. Chem.*, 18 (1963) 61–97.
2 E. J. Bourne, F. Searle, and H. Weigel, *Carbohydr. Res.*, 16 (1971) 185–187.
3 E. Lederer and M. Lederer, *Chromatography*, Elsevier, Amsterdam, 2nd edition, 1957, p. 243.

4 G. R. BARKER AND D. C. C. SMITH, *Chem. Ind. (London)*, (1954) 19.
5 E. J. BOURNE, J. HARTIGAN, AND H. WEIGEL, *J. Chem. Soc.*, (1959) 2332–2337.
6 E. J. BOURNE, E. M. LEES, AND H. WEIGEL, *J. Chromatogr.*, 11 (1963) 253–257.
7 R. J. FERRIER, W. G. OVEREND, G. A. RAFFERTY, H. M. WALL, AND N. R. WILLIAMS, *Proc. Chem. Soc.*, (1963) 133.
8 J. E. SCOTT, *Biochem. J.*, 82 (1962) 43P–44P.
9 N. A. SUFI, Ph.D. Thesis, University of London, 1966.
10 H. J. F. ANGUS, E. J. BOURNE, AND H. WEIGEL, *J. Chem. Soc.*, (1965) 21–26.
11 H. J. F. ANGUS, E. J. BOURNE, F. SEARLE, AND H. WEIGEL, *Tetrahedron Lett.*, (1964) 55–60.
12 H. J. F. ANGUS AND H. WEIGEL, *J. Chem. Soc.*, (1964) 3994–4000.
13 H. G. J. WORTH, Ph.D. Thesis, University of London, 1965.