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Note

Thin-layer chromatography on cellulose impregnated with tungstate: a rapid method of resolving mixtures of some commonly occurring carbohydrates

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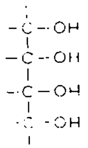
Neutral, polyhydroxy compounds can often be separated as the ionic complexes which they form with inorganic oxy-acids or metal ions. The methods may involve electrophoresis[1] in the appropriate electrolyte, or the use of ion-exchange resins[2]. Recently[3], we reported that impregnation of paper strips with tungstate affects the chromatographic migration-rates of a number of polyhydroxy compounds. It was shown that the migrating material is the free polyol of the equilibrium mixture, and that the magnitude of the effect of the addition of tungstate to the stationary phase on migration rates of compounds can serve to construct a sequence of pseudo-stability constants.

One of the disadvantages of paper chromatography is the relatively long time of development (*e.g.*, 48 and 12 h with solvents *1* and *2*, respectively), whereas speedy development is one of the features of t.l.c.[4]. We have already briefly indicated[3] that tungstate-impregnated cellulose powder, obtained by slurrying the powder in tungstate solution, can be used for t.l.c. We have now found that it is more expedient to prepare thin layers of tungstate-impregnated cellulose by simply dipping commercially available cellulose powder–polythene sheets in aqueous tungstate solution. In our experience, this treatment does not impair the quality of the sheets.

Table I shows the R_F values of compounds on cellulose t.l.c.-sheets when the tungstate solutions used for impregnation had been adjusted to pH 6 [R_F (W_6)] and pH 8 [R_F (W_8)]. The R_F values on untreated, cellulose t.l.c.-sheets are included for comparison. The choice of pH values was explained in our previous report[3]. As before[3], the grouping of compounds was made on the following grounds: group *A* comprises acyclic compounds possessing a vicinal tetraol system (**1**); compounds of



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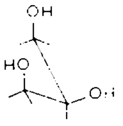

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TABLE I

T.L.C. MIGRATION-RATES ON CELLULOSE IMPREGNATED WITH TUNGSTATE

Compound	*Solvent* 1			*Solvent* 2		
	$R_F(W_6)$	R_F	Q_6	$R_F(W_8)$	R_F	Q_8
Group A						
Erythritol	0.19	0.56	2.9	0.19	0.58	3.1
L-Threitol	0.26	0.54	2.1	0.03	0.56	18.7
D-Arabinitol	0.11	0.48	4.4	0.05	0.47	9.4
1-deoxy-	0.16	0.65	4.1	0.19	0.69	3.6
Ribitol	0.16	0.48	3.0	0.09	0.48	5.3
Xylitol	0.12	0.46	3.8	0.03	0.46	15.3
Allitol	0.11	0.42	3.8	0.09	0.39	4.3
D-Altritol	0.11	0.42	3.8	0.03	0.39	13.0
1-deoxy-				0.11	0.62	5.6
Galactitol	0.10	0.41	4.1	0.03	0.36	12.0
1-deoxy-L-	0.13	0.60	4.6	0.12	0.60	5.0
D-Glucitol	0.10	0.41	4.1	0.02	0.37	18.5
D-Mannitol	0.11	0.42	3.8	0.07	0.38	5.4
L-Mannitol, 1-deoxy-	0.13	0.61	4.7	0.14	0.63	4.5
D-Talitol, 1-deoxy-	0.19	0.59	3.1	0.10	0.60	6.0
Group B						
D-Lyxose	0.17	0.50	2.9	0.18	0.52	2.9
D-Ribose	0.24	0.50	2.1	0.07	0.56	8.0
D-Mannose				0.10	0.46	4.6
L-Gulitol, 3-*O*-α-D-glucopyranosyl-	0.09	0.27	3.0	0.02	0.16	8.0
3-*O*-β-D-glucopyranosyl-	0.10	0.26	2.6	0.06	0.16	2.7
L-Gulose	0.16	0.43	2.7	0.12	0.41	3.4
Group C-a						
D-Arabinose	0.20	0.44	2.2	0.17	0.41	2.4
D-Xylose	0.24	0.49	2.0	0.16	0.49	3.1
D-Galactose	0.15	0.37	2.5	0.06	0.30	5.0
D-Glucose	0.18	0.41	2.3	0.12	0.37	3.1
(+)-Inositol	0.11	0.27	2.5	0.05	0.18	3.6
Group C-b						
Glycerol	0.46	0.62	1.3	0.45	0.66	1.5
L-Galactose, 6-deoxy-	0.28	0.51	1.8	0.31	0.50	1.6
D-Glucose, 3-*O*-methyl-	0.44	0.58	1.3	0.50	0.60	1.2
Hexose, 2-deoxy-D-*arabino*-	0.39	0.58	1.5	0.47	0.59	1.3
2-deoxy-D-*lyxo*-	0.33	0.53	1.6	0.44	0.54	1.2

group *B* possess three hydroxyl groups in a spatial disposition approximating to that of **2**; compounds of group *C* lack the above features in their more-stable conformer or tautomer, but those of group *C-b* do not seem to form complexes with tungstate. Table I gives representative examples of each group.

As before, we have expressed the magnitude of the effect of the addition of tungstate to the stationary phase on migration rates by the quotient $Q = R_F/R_F(W)$.

Table I shows that, in general, the results obtained with paper and t.l.c. sheets are comparable, *i.e.*, the Q values of the tungstate complexes are in the order: group A > group B > group C. However, within each group, the sequences are not strictly identical. The differences that occur may be due, *inter alia*, to small differences between the pH values of the tungstate in the stationary phases on the paper and the t.l.c. sheets. As changes in pH also affect the equilibrium constant, and as the magnitude of the effect varies with the polyol, such differences in pH could give rise to differences in Q values.

Nevertheless, the results show the method to be a simple, inexpensive, and speedy means of resolution of mixtures of polyhydroxy compounds not easily achieved by other methods. For example, the R_F values, using untreated, cellulose t.l.c.-sheets, of the pentoses and hexoses do not differ greatly from those of their reduction products (in most cases, the difference is $<10\%$). However, the $R_F(W_6)$ and/or $R_F(W_8)$ values of the monosaccharides examined differ from those of their reduction products by factors of 1.4 (for D-mannose–D-mannitol) to 6 (for D-glucose–D-glucitol, using tungstate adjusted to pH 8). The resolution of corresponding mixtures can therefore be achieved on tungstate–cellulose t.l.c.-sheets in less than 2 h. As no derivatisation is necessary (unlike in g.l.c.), the method is particularly suitable in investigations that require such compounds to be rapidly monitored.

EXPERIMENTAL

T.l.c. sheets (20 × 20 cm; Eastman Chromatogram, 6064 Cellulose, without fluorescent indicator) were dipped in aqueous 5% sodium tungstate dihydrate adjusted with M sulphuric acid to either pH 6 (W_6) or 8 (W_8), dried in air at room temperature, and then at 60° for 30 min. Compounds were spotted on the impregnated, as well as the untreated, t.l.c.-sheets, and detected with silver nitrate in acetone–ethanolic sodium hydroxide. The chromatographic solvents used were: *1*, 1-butanol–ethanol–water (40:11:19); and *2*, acetone–1-butanol–water (5:3:2); with development times of ~2.5 and 1.5 h, respectively.

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