Preprinted from the Journal of the Chemical Society, March, 1957, (269), pages 1432—1433.

269. *The Paper Chromatography of Cyclitols.*

By S. J. Angyal, D. J. McHugh, and P. T. Gilham.

Cyclitols are particularly suitable for paper chromatography: they are in most cases easily separated and readily detected, and the separations can be translated to a preparative scale by the use of cellulose-powder chromatography. R_F values for several inositols in aqueous acetone were reported [1] and recently Posternak [2] gave values for many cyclitols and related compounds in several solvent systems. In the Table we now list R_F values for all the inositols and for the known quercitols and inositol methyl ethers. Aqueous acetone is the solvent of choice in most cases because by its use chromatograms can be run, dried, and developed within 3—4 hr. In the authors' laboratory chromatograms are now run as a routine matter in aqueous acetone before the working-up of reaction mixtures.

The best method for detecting cyclitols on paper is the silver nitrate–sodium hydroxide reagent described by Trevelyan *et al.*,[3] as modified by Anet and Reynolds;[4] fixation by thiosulphate gives black spots which can be preserved as a permanent record. The older ammoniacal silver nitrate reagent [1] is less sensitive and usually causes darkening of the whole paper. The sensitivity of the reagent decreases with decreasing number of hydroxyl groups; while 10 μg. of an inositol is readily detected, about 50 μg. of a monomethyl inositol and 500 μg. of dambonitol are required to cause a similar spot: heating the paper for a short time increases the sensitivity to methyl ethers. For dambonitol, Lemieux and Bauer's permanganate–periodate reagent [5] is more suitable; it is a good reagent for all cyclitols and its sensitivity does not vary much with the number of hydroxyl groups, but the spots are not permanent. The Scherer reagent [6] is specific for inositols only but lacks sensitivity. In some cases Hockenhull's [7] borate–phenol-red reagent is useful because it is more sensitive to those cyclitols which form

R_F values of cyclitols.

Solvents: *A*, acetone–water (4 : 1, v/v); *B*, phenol–water (4 : 1, w/w); *C*, butanol–acetic acid–water (4 : 1 : 1); *D*, ethanol–water–conc. ammonia solution (20 : 4 : 1).

Solvent	*A*	*B*	*C*	*D*
D-Glucose	0·43	0·35	0·175	0·64
Inositols				
scyllo- (1 : 3 : 5/2 : 4 : 6)	0·17	0·155	0·07	0·40
(+)- (1 : 2 : 4/3 : 5 : 6)	0·27	0·195	0·10	0·50
neo- (1 : 2 : 3/4 : 5 : 6)	0·19	0·24	0·085	0·42
myo- (1 : 2 : 3 : 5/4 : 6)	0·185	0·205	0·08	0·41
muco- (1 : 2 : 4 : 5/3 : 6)	0·35	0·265	0·145	—
allo- (1 : 2 : 3 : 4/5 : 6)	0·30	0·29	0·135	—
epi- (1 : 2 : 3 : 4 : 5/6)	0·22	0·33	0·105	0·46
cis- (all-*cis*)	0·22	0·35	0·125	—
Quercitols				
scyllo- (1 : 3 : 5/4 : 6)	0·31	0·37	0·145	0·63
proto- (1 : 4/2 : 3 : 5)	0·405	0·38	0·20	0·67
vibo- (1 : 2 : 4/3 : 5)	0·33	0·42	0·155	0·63
epi- (1 : 2 : 3 : 5/4)	0·34	0·41	0·16	—
cis- (all-*cis*)	0·39	0·54	0·205	—
Inositol methyl ethers				
1-Me-*myo*- (bornesitol)	0·30	0·50	0·15	0·58
2-Me-*myo*-	0·35	0·50	0·16	0·61
4-Me-*myo*- (ononitol)	0·33	0·50	0·16	—
5-Me-*myo*- (sequoyitol)	0·33	0·47	0·165	0·58
1-Me-(−)	0·43	0·49	0·19	0·64
2-Me-(−) (quebrachitol)	0·39	0·46	0·185	0·64
3-Me-(+) (pinitol)	0·44	0·45	0·20	0·64
1 : 3-diMe-*myo*- (dambonitol)	0·50	0·69	0·27	0·69

complexes readily with borate,[8] *e.g.*, small amounts of *cis*inositol can be detected in the presence of *myo*inositol. It is also useful when the cyclitols are to be preserved, for example, for elution; all the other reagents referred to destroy the compounds.

For the separation of *cis*- and *epi*-inositol ethyl acetate–acetic acid–water (3 : 1 : 1; v/v) is useful, the R_F values being: *myo*- 0·15, (±)- 0·20, *epi*- 0·20, and *cis*-inositol 0·25.

The R_F values were determined on descending chromatograms (although the ascending technique is used for routine testing). With butanol–acetic acid–water, the chromatograms were run for 24 hr., the solvent dripping off the serrated end of the paper to give relative R_F values; absolute values were calculated by comparison with a chromatogram of a few substances (of higher R_F values) for which the solvent was run only to the end of the paper. Whatman No. 1 paper was used throughout. Papers wetted with phenol or butanol were dried overnight but those with acetone require only a few minutes' drying. The R_F values vary considerably, unless stringent precautions are taken to assure constant conditions, but their ratio to the R_F value of glucose (the R_G value) is easily reproducible; glucose was therefore incorporated in all chromatograms and its R_F values are given in the Table.

N.S.W. University of Technology,
School of Applied Chemistry, Sydney.

[*Received, September 3rd*, 1956.]

[1] Ballou and Anderson, *J. Amer. Chem. Soc.*, 1953, **75**, 648.
[2] Posternak, Reymond, and Haerdi, *Helv. Chim. Acta*, 1955, **38**, 1911.
[3] Trevelyan, Proctor, and Harrison, *Nature*, 1950, **166**, 444.
[4] Anet and Reynolds, *ibid.*, 1954, **174**, 930.
[5] Lemieux and Bauer, *Analyt. Chem.*, 1954, **26**, 920.
[6] Fleury, Courtois, and Malangeau, *Bull. Soc. Chim. biol.*, 1953, **35**, 537.
[7] Hockenhull, *Nature*, 1953, **171**, 982.
[8] Angyal and McHugh, preceding paper.

Printed in Great Britain by Richard Clay and Company, Ltd.,
Bungay, Suffolk.