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Note

Separation of isomeric pentitols and hexitols by paper and thin-layer chromatography

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The metabolism of the polyhydric alcohols is a particularly important aspect of carbohydrate biochemistry. Numerous organisms, including many plant species from a variety of taxa, contain one or several polyols[1,2]. Qualitative and quantitative analysis of such compounds, as well as their metabolism in any organism, has hitherto been difficult, as successful chromatographic methods for the reliable and rapid separation or identification of isomeric pentitols and hexitols were scarce. Some procedures[3–7] that have been described in the past few years for the chromatographic analysis of native or synthetic polyols seem to be impractical or to achieve relatively incomplete and unsatisfactory separation of the isomers.

In this paper, some solvent systems are proposed for the identification and characterization of the naturally occurring pentitols (xylitol, arabitol and adonitol) and hexitols (mannitol, dulcitol and sorbitol) by paper (PC) or thin-layer chromatography (TLC). These systems have similar properties and can be applied for analytical as well as preparative purposes.

EXPERIMENTAL

Materials

Paper. Schleicher & Schüll 2043 b Mgl.

Adsorbent. Cellulose MN 300.

Solvents. (A) Ethyl methyl ketone–acetic acid–0.75 *M* boric acid (40:10:9),
(B) 1-Butanol–0.75 *M* boric acid (85:15),
(C) 2-Propanol–acetic acid–0.75 *M* boric acid (70:10:20),
(D) Ethyl methyl ketone–acetic acid–0.7 *M* boric acid (100:10:5),
(E) 1-Butanol–0.75 *M* boric acid (85:10), and
(F) 1-Butanol–distilled water (90:10).

Detection reagents. Ammonium cerium(IV) nitrate–N,N-dimethyl-*p*-phenylenediamine dihydrochloride[7] or sodium periodate–benzidine[8].

Method. Paper chromatograms (solvents D–F) were prepared by the descending techniques using 60-cm × 15-cm sheets; thin-layer chromatography was carried out on 20-cm × 20-cm plates at room temperature (solvents A–C).

RESULTS AND DISCUSSION

On the basis of Boeseken's findings that polyols form borate complexes in solutions of boric acid[9], Rees and Reynolds devised a solvent system[10] that provides a good separation of polyols from their corresponding sugars; our solvents A and D are modifications of this system. These solvents, and mixtures of 1-butanol with aqueous boric acid (solvents B and E), achieve a clear distinction of the naturally occurring polyols and polyol isomers either by TLC (see Table I) or by PC (see Table II). However, the solvent composition used in TLC may not also be in PC if the same efficiency is desired.

TABLE I

MOBILITIES OF SEVERAL POLYOLS ON THIN-LAYER CHROMATOGRAMS

Polyol	$R_{mannitol} \times 100$ *values*		
	Solvent A	*Solvent B*	*Solvent C*
Volemitol*	75	81	78
Mannitol	100	100	100
Dulcitol	90	84	90
Sorbitol	110	100	97
Xylitol	140	123	110
Arabitol	135	134	115
Adonitol	156	180	128
Erythritol	170	200	135
Glycerol	207	280	145

* Prepared as [^{14}C]volemitol from *Pelvetia canaliculata*[12].

As can be seen from Tables I and II, sorbitol and adonitol (ribitol) are clearly separated from the other hexitol and pentitol isomers, whereas mannitol and dulcitol (galactitol) or xylitol and arabitol, respectively, show closer mobilities. These latter polyols can be better separated in solvents B and C. The other C_3, C_4 and C_7 polyols are clearly separable without any problems.

Although a one-dimensional development of the chromatograms is sufficient

TABLE II

MOBILITIES OF SEVERAL POLYOLS ON PAPER CHROMATOGRAMS

Polyol	$R_{mannitol} \times 100$ *values*		
	Solvent D	*Solvent E*	*Solvent F*
Volemitol	65	—	—
Mannitol	100	100	100
Dulcitol	104	83	81
Sorbitol	131	108	81
Xylitol	172	142	139
Arabitol	160	153	157
Adonitol	180	196	157
Erythritol	207	189	236
Glycerol	290	295	380

for a reliable identification of and distinction between the polyols, solvents A and D, and B and E, respectively, can be employed for two-dimensional chromatography. Of course, PC requires longer development times, *e.g.*, 24 h in the first (solvent D) and 48 h in the second (solvent E) direction. Two-dimensional TLC of the polyols in solvents A and B gives satisfactory results with development for 3–4 h in each direction.

For a very sharp separation, especially of mannitol from dulcitol or of xylitol from arabitol, and in particular for preparative purposes, the use of solvent C (TLC) or F (PC) is recommended.

The new solvent systems presented here may be very useful in investigations on the biochemistry and physiology of fungi, algae and lichens. One or more polyols are involved in the metabolism of these organisms, and are probably inter-converted to each other[11]. Rapid chromatography and reliable identification of products and substrates will help in elucidating such metabolic pathways.

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