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Note

Improved method for the thin-layer chromatographic identification of alditols

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Many methods for thin-layer chromatographic (TLC) separation and identification of low-molecular-weight carbohydrates have recently been described, since the use of Kieselguhr[1] or cellulose layers[2] has been recommended for the chromatographic characterization of mono- and disaccharides. Similar methods have also been applied to the separation of aliphatic polyhydroxy alcohols (alditols). For an analysis of such compounds, particularly in biological materials, rapid and reliable procedures are required, especially with respect to the occurrence of isomeric alditols which often dominate the specific low-molecular-weight carbohydrate pattern of a wide variety of algal and lichen species[3,4]. On the other hand the methods which have hitherto been proposed[5–8] imply the disadvantage that not all the naturally occurring, frequently encountered alditols (hexitols and pentitols) can be easily differentiated during a single chromatographic run.

The TLC method described below is a modification of the procedure which has previously been described by Hansen[9] for the separation of mono- and oligosaccharides. This method is very suitable for a direct identification of alditols from C_3 to C_7, *e.g.*, from crude plant extracts.

EXPERIMENTAL

TLC

Glass plates (20 × 20 cm) pre-coated with silica gel 60 (*e.g.* article No. 5715 from Merck, Darmstadt, G.F.R.) were impregnated by spraying with *ca.* 10 ml 0.5 *M* NaH_2PO_4 in 50% ethanol, were then allowed to stand for 15 min and subsequently activated by drying for 60 min at 110°. No remarkable decrease in the efficiency of separation was observed when plates prepared according to this procedure had been stored in a desiccator for longer periods.

Solvent

The solvent system used is isopropanol–acetone–0.2 *M* lactic acid (60:30:10). A fresh mixture should be prepared daily. At room temperature the solvent front will migrate about 15 cm within 4 h.

Detection

For the detection of the alditols in the crude extracts analyzed or of the co-chromatographed authentic references (1 μl of a 0.1% alditol solution) the reagents specified earlier[7,8] have been employed.

RESULTS AND DISCUSSION

It can be seen from Table I that the compounds mostly encountered as polyhydroxy constituents of plant extracts are clearly separated by the TLC method described above. Even mannitol, sorbitol, and dulcitol are reliably distinguished with simultaneous separation from the heptitols included. In the same system, the pentitols can also be easily differentiated. As Hansen[9] has demonstrated for the diverse sugars investigated in his study, the use of lactic acid in the solvent prevents unfavourable "tailing" of the samples and references and achieves the migration of clearly defined spots on the chromatograms. This observation also proved to be true for the separation of the alditols.

TABLE I

R_F AND R_M VALUES OF SEVERAL ALDITOLS AND SOME CORRESPONDING MONOSACCHARIDES ON LAYERS OF IMPREGNATED SILICA GEL 60

Compound	*R_F value*	*R_M value*
C_3 Glycerol	0.73	2.43
C_4 Erythritol	0.64	2.13
Threitol	0.61	2.03
C_5 Ribitol (Adonitol)	0.53	1.76
Arabitol	0.47	1.56
Xylitol	0.38	1.26
C_6 Mannitol	0.30	1.00
Dulcitol (Galactitol)	0.25	0.84
Sorbitol (Glucitol)	0.21	0.70
C_7 Volemitol	0.18	0.61
Perseitol	0.13	0.42
Fructose	0.34	1.10
Glucose	0.28	0.93
Galactose	0.16	0.53
Xylose	0.55	1.85
Ribose	0.47	1.54
Arabinose	0.37	1.23

This TLC method is now routinely applied to the separation and identification of alditols mostly from crude extracts of lower plants. However, it has additionally been found that the solvent system proposed here is also suitable for a reliable differentiation of the single alditols from their corresponding monosaccharides. Table I includes the mobilities of, *e.g.*, fructose, glucose, and galactose corresponding to mannitol, sorbitol, and dulcitol, respectively. Even the various pentitols show mobilities different from those of the equivalent pentoses. This observation may be of significance for studies of the biochemical interconversion of these compounds. Moreover, the solvent proposed here provides a clear separation of certain amino

acids and carbohydrates: the difficulty of a chromatographic differentiation between, *e.g.*, aspartate and sucrose or between glutamate and mannitol (*cf.* ref. 2) are well-known and almost classic problems in biochemical plant physiology.

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