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Note

Improved resolution of *O*-methylalditol acetates by gas–liquid chromatography on capillary columns with blended coatings of OV-17 and OV-225*

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Analysis of polysaccharides using the methylation technique has been aided by characterization and quantitative determination of derived *O*-methylalditol acetates by gas–liquid chromatography–mass spectrometry. Recent refinements in the technique include the use of capillary columns, which often results in better resolution of peaks[1].

Recently, with the objective of analyzing microbial polysaccharides, the chromatographic behavior of *O*-methylalditol acetates arising from units of galactose, glucose, and mannose was examined on capillary columns of OV-17 and OV-225. In parallel and separate experiments with each column, it was found that several derivatives emerged in a different order depending on the column used[2]. For example, the four tri-*O*-methylglucitol acetates that can be formed from glucopyranosyl units can be partly resolved on packed[3] and capillary[2] columns of OV-17, the 2,3,4 and 2,3,6

TABLE I

RETENTION TIMES[a] OF *O*-METHYLHEXITOL ACETATES IN CAPILLARY COLUMNS OF OV-17 AND OV-225, AND VARIOUS BLENDED COATINGS

O-Methylalditol acetate derivative	*Column coating, ratio of OV-17 to OV-225*				
	1:0[b]	*37:13*	*12:13*	*1:3*	*0:1*[b]
2,3,4,6-Me_4-Glc	1.00	1.00	1.00	1.00	1.00
2,3,4,6-Me_4-Man	1.00	1.00	1.00	0.99	0.98
3,4,6-Me_3-Glc	1.46	1.58	1.61	1.68	1.62
2,4,6-Me_3-Glc	1.53	1.63	1.64	1.68	1.61
2,3,4-Me_3-Glc	1.61	1.77	1.84	1.90	1.83
2,3,6-Me_3-Glc	1.61	1.81	1.89	2.00	1.98
2,3,6-Me_3-Gal	1.58	1.71	1.84	1.86	1.84
2,3,4-Me_3-Man	1.61	1.77	1.89	1.90	1.85

[a]Relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]See ref. 2.

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isomers emerging together but with a retention time different from those of the 3,4,6 and 2,4,6 isomers, which are resolved from each other. The emergence pattern is different with a capillary column of OV-225, the 3,4,6 and 2,4,6 isomers having the same retention time, which is different from those of the separable 2,3,4 and 2,3,6 isomers[1,2]. The values of the retention times on OV-17 and OV-225 columns (Table I) suggested that a correct blend of the coatings would resolve all four isomers. The present study concerns such a resolution and the chromatographic separation of other commonly encountered mixtures of *O*-methylalditol acetates on various blended-phase, capillary columns.

Three glass-capillary columns were prepared, coated with mixtures of OV-17 and OV-225 in the weight ratios of 1:3, 12:13, and 37:13, respectively. They were used to fractionate mixtures of the aforementioned tri-*O*-methylglucitol acetates, in addition to columns containing OV-17 and OV-225 only. The results presented in Table I show that the coatings with OV-17 and OV-225 in the ratio of 37:13 gives the best resolution and a somewhat poorer resolution takes place by use of the coating with components in the ratio of 12:13.

Methylation analysis of fungal polysaccharides frequently gives rise to a mixture of acetates of 2,3,4-tri-*O*-methylmannitol, 2,3,6-tri-*O*-methylgalactitol, and 2,3,6-tri-*O*-methylglucitol. Previously, it was known[1] that the 2,3,4-tri-*O*-methylmannitol and 2,3,6-tri-*O*-methylgalactitol derivatives were not separable on the commonly used, conventional columns of ECNSS-M, but required use of a packing of neopentylglycol sebacate in a separate experiment[4]. In capillary g.l.c., these compounds can be separated with OV-17, but these conditions do not resolve the acetates of 2,3,6-tri-*O*-methyl derivatives of galactitol and glucitol (Table I). By contrast, OV-225 resolves the 2,3,6-tri-*O*-methyl derivatives of glucitol and galactitol, but cannot separate the acetates of 2,3,6-tri-*O*-methylgalactitol and 2,3,4-tri-*O*-methylmannitol[5]. In the present study, the three compounds were well resolved by a column coated with OV-17 and OV-225 in the ratio of 37:13 (Table I). The characteristics of this column were good for quantitative determination, the size of a 2,3-di-*O*-methylglucitol acetate peak being almost as great as that of an equimolar amount of 2,3,4,6-tetra-*O*-methylglucitol acetate.

Although this column gives excellent resolutions of the aforementioned mixtures, this and the other blended columns did not resolve acetates of 2,3,4,6-tri-*O*-methyl derivatives of glucitol and mannitol. For this purpose, the OV-225 capillary column[2] should be used.

EXPERIMENTAL

G.l.c.–m.s. — G.l.c. was carried out in the capillary columns described below, contained in a Model 400 Finnigan g.l.c.–m.s. unit interfaced with an Inco 2300 Data System. Electron-impact spectra[1] were obtained repetitively every 2 s, scanning from mass 40 to 420. Injections were carried out[6] in the splitless mode at 50°, and then

programmed (40°/min) to 182° (hold). The carrier gas was helium (linear velocity, 22 cm/s).

Capillary-drawing procedure. — Pyrex tubing (i.d., 2 mm; o.d., 7 mm; length, 1.2 m) was subjected to the following pretreatment. It was cleaned by storage in 10% aqueous potassium hydroxide, saturated with potassium permanganate, for 3 weeks, and rinsed several times with distilled water. Following treatment with concentrated hydrochloric acid for 1 h, it was rinsed with water, and pure methanol and acetone passed successively through the tube. Drying was carried out by suction for 16 h, the inlet air being passed through a Teflon tube containing a layer of activated charcoal followed by Drierite. A capillary tube (i.d., 0.25 mm; length 33 m) was drawn with the aid of the apparatus of Hupe and Busch, model 1045A.

The column was coated with sodium chloride[7], dried at room temperature for 12 h with a rapid current of nitrogen, and then heated for 1 h at 280° in a g.l.c. instrument (flow rate of helium, 20 cm/s).

Deactivation of column surface. — This was carried out by the procedure of Grob and Grob[8]. The column coils were put into a vertical plane and attached to a buffer column (10 m) with a 1/16″ Swagelok union-system. Approximately 10% of the column coils was filled by suction with 1% polyethylene glycol (molecular size 20M) in dichloromethane, and the solution propelled through the column at 1 cm/s by nitrogen under pressure. The column was dried, heated at 280° in the g.l.c. instrument with no helium flow for 20 min, and then helium was passed through (linear velocity, 20 cm/s) before cooling. The coating procedure was then repeated.

Coating of column surface and column conditioning. — Coating solutions consisted of 0.3% of solute in distilled, ethanol-free, chloroform. One end of the column was dipped into the coating solution which was contained in a vessel of 10-mL capacity and subjected to a positive pressure of nitrogen, thus forcing the solution through the tubing (Fig. 1). The column outlet was attached to a tube of Tygon (i.d., 0.2 mm; length, 3 cm) and when this tube was filled with the coating solution, it was immersed in a bath containing ethanol. A glass plug was inserted into the end of the Tygon tube to prevent bubbles of gas from entering during the following procedure. The column was removed from the ethanol bath and immersed for 12 h in a water-bath controlled at 40°, and a static coating was carried out by application of a vacuum (3–4 mm of Hg) at the other end, which evaporated the solvent. When

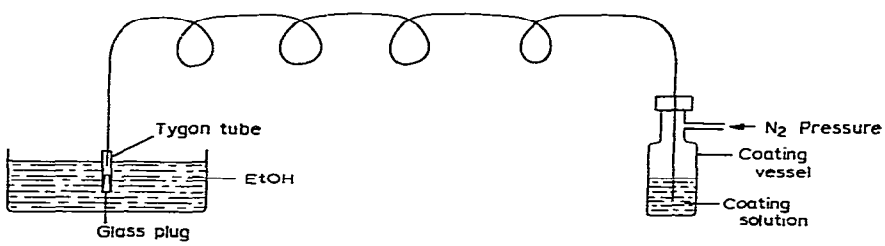

Fig. 1. Column attachments used in the coating procedure.

the column was empty, nitrogen was passed through for 1 h to evaporate remaining solvent. Four coils of ~ 1.5 m were removed from the end of the column and discarded. The remaining column ends were straightened by flame, and 1% polyethylene glycol (20 M) in dichloromethane was applied to the ends in order to replace the deactivator.

The column was conditioned in a Dani 3900 GC apparatus with a Grob split-splitless injector. Helium was passed (linear velocity, 20 cm/sec) with the following temperature program: 30° for 1 h; increase of 0.5°/min to 80°; hold for 1 h; increase of 0.5°/min to 200°; hold for 1 h; increase of 0.5°/min to 230°; and hold for 16 h.

Column-testing results. — Three columns containing three different blends of OV-17 and OV-225 were tested to estimate their efficiencies with a Dani 3900 G.C. apparatus equipped with a FID detector (helium carrier velocity, 20 cm/sec; nitrogen flow, 30 mL/min; hydrogen flow, 30 mL/min; air flow, 260 mL/min; column temperature, 130°) as follows:

(*a*) *Column 1322/185* (*length 31 m*). This was coated with OV-17 (16.5 mg) and OV-225 (17.6 mg) dissolved in chloroform (10 mL). It had an efficiency of 2700 effective theoretical plates/m for a C_{17} hydrocarbon at k 5.0. (The Grob test-mixture[9] consists of 12 compounds: dicyclohexylamine, 2-ethylhexanoic acid, decane, undecane, nonanal, 1-octanol, 2,3-butanediol, 2,6-dimethylaniline, 2,6-dimethylphenol and methyl esters of C_{10}-, C_{11}-, and C_{12}-linear carboxylic acids). The size of the 1-octanol peak was 40% of that of each hydrocarbon peak. Those of 2,6-dimethylaniline and 2,6-dimethylphenol were 80% of that of the C_{12}-methyl ester.

(*b*) *Column 1322/200* (*length 28 m*). This was coated with OV-17 (26.2 mg) and OV-225 (9.2 mg) in chloroform (10 mL). Its efficiency was 2587 theoretical plates/m for a C_{16} hydrocarbon at k 6.7. The size of the 1-octanol peak was 90% of that of each hydrocarbon peak. The sizes of the 2,6-dimethylaniline and 2,6-dimethylphenol peaks were 90% of that of the C_{12}-methyl ester.

(*c*) *Column 1403/4A* (*length 29 m*). This was coated with OV-17 (8.7 mg) and OV-225 (25.5 mg) in chloroform (10 mL). Its efficiency was 2500 theoretical plates/m for a C_{17} hydrocarbon at k 5.3. The 1-octanol peak was very small compared with the hydrocarbon peak. The 2,6-dimethylaniline and 2,6-dimethylphenol peaks were 50 and 70%, respectively, of the size of the C_{12}-methyl ester peak.

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