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Note

Gas–liquid chromatographic analysis of partially methylated alditol acetates on a glass capillary column

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Methylation is commonly used in structural studies of polysaccharides and complex carbohydrates. Partially methylated monosaccharides obtained by the acid hydrolysis of the methylated polymers are usually analysed by gas–liquid chromatography (GLC) in the form of their alditol acetates[1]. Many types of packed column systems, including ECNSS-M[2], OV-225[3], OV-275–XF-1150[4], PEGA–PEGS–XF-1150[5], Apiezon greases[6] and Silar-10C[7], have been used for this purpose. However, the efficiency of these columns is inadequate and they often give incomplete separations of closely related compounds and make it difficult to identify each component, even with the use of mass spectrometry (MS)[1].

For a highly efficient capillary column system, OV-225 has mainly been recommended as the stationary phase because of its excellent thermal stability[1]. However, the separation of some closely related compounds is still impossible even with the use of this column. Recently, we tested the application of several liquid stationary phases in glass capillary columns and found that Silar-10C, first introduced by Hirase *et al.*[7], is the most efficient for the present purpose, with high thermal stability and excellent resolution. This paper describes some characteristics of this column and its application to structural studies of some polysaccharides.

EXPERIMENTAL AND RESULTS

GLC was carried out with a Shimadzu GC-6AM gas chromatograph equipped with a flame-ionization detector. A surface-coated open-tubular glass capillary column coated with Silar-10C (G-SCOT, 30 m × 0.28 mm) was purchased from Gasukuro-kogyo Co. (Tokyo, Japan). The column was connected to the gas chromatograph via a Shimadzu CLH-6 all-glass splitter (splitting ratio = 1:59).

Partially methylated alditol acetates were prepared from methylated polysaccharides of known structures or synthesized by the known methods.

Table I shows the relative retention times of various partially methylated alditol acetates. These values are the average of at least three repeated analyses and the reproducibility was excellent (within ± 3%, if the operating conditions were constant). Peaks that have retention times differing by 2% of Table I can be separated from each other. Hence, most of the compounds listed could be separated by this column, with a few exceptions.

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TABLE I

RELATIVE RETENTION TIMES OF PARTIALLY METHYLATED ALDITOL ACETATES ON THE SILAR-10C SCOT COLUMN

Parent sugar	*Position of O-CH_3*	*Relative retention time**	*Parent sugar*	*Position of O-CH_3*	*Relative retention time**
Rhamnose	2,3,4	0.54	Galactose	2,3,4,6	1.19
				2,3,4,6	1.19
Fucose	2,3,4	0.69		2,4,6	1.97
				2,4	5.28
Arabinose	2,3,5	0.59		2,6	3.19
	2,3	1.32			
	3,5	0.94	Mannose	2,3,4,6	0.98
	2,5	1.09		2,3,4	2.16
	2	2.23		2,3,6	2.01
				3,4,6	1.81
Xylose	2,3,4	0.74		3,4	4.56
	2,3 or 3,4	1.48		2,4	4.41
	2	2.70			
	3	2.65			
Glucose	2,3,4,6	1.00			
	2,3,4	2.19			
	2,3,6	2.38			
	2,4,6	1.73			
	2,3	4.75			
	2,4	4.25			
	2,6	3.39			
	3,6	3.82			
	6	4.99			

* 1,5-Di-O-acetyl-2,3,4,6-tetra-O-methylglucitol = 1.00.

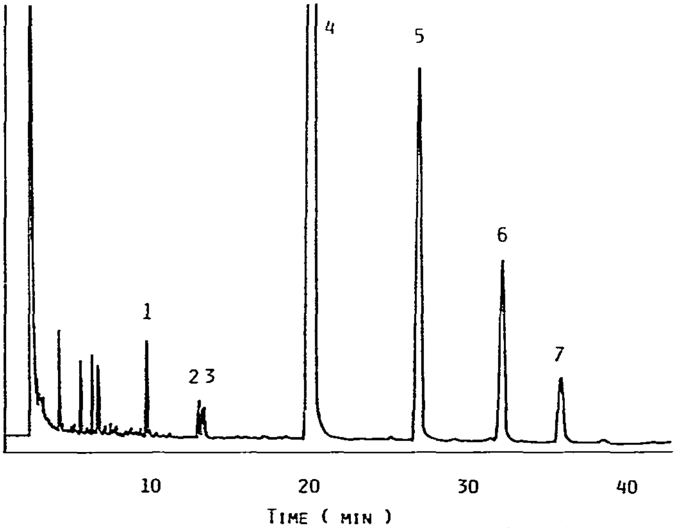

Fig. 1. Separation of 2,3,4,6-tetra-O-methylglucitol diacetate and 2,3,4,6-tetra-O-methylmannitol diacetate. Sample: hydrolysate of the methylated hemicellulose of birch wood. Peaks: 1 = 2,3,4,6-tetra-O-methylxylitol diacetate; 2 = 2,3,4,6-tetra-O-methylmannitol diacetate; 3 = 2,3,4,6-tetra-O-methylglucitol diacetate; 4 = 2,3-di-O-methylxylitol triacetate; 5 = 2,3,6-tri-O-methylmannitol triacetate; 6 = 2,3,6-tri-O-methylglucitol triacetate; 7 = 3-O-methylxylitol tetraacetate.

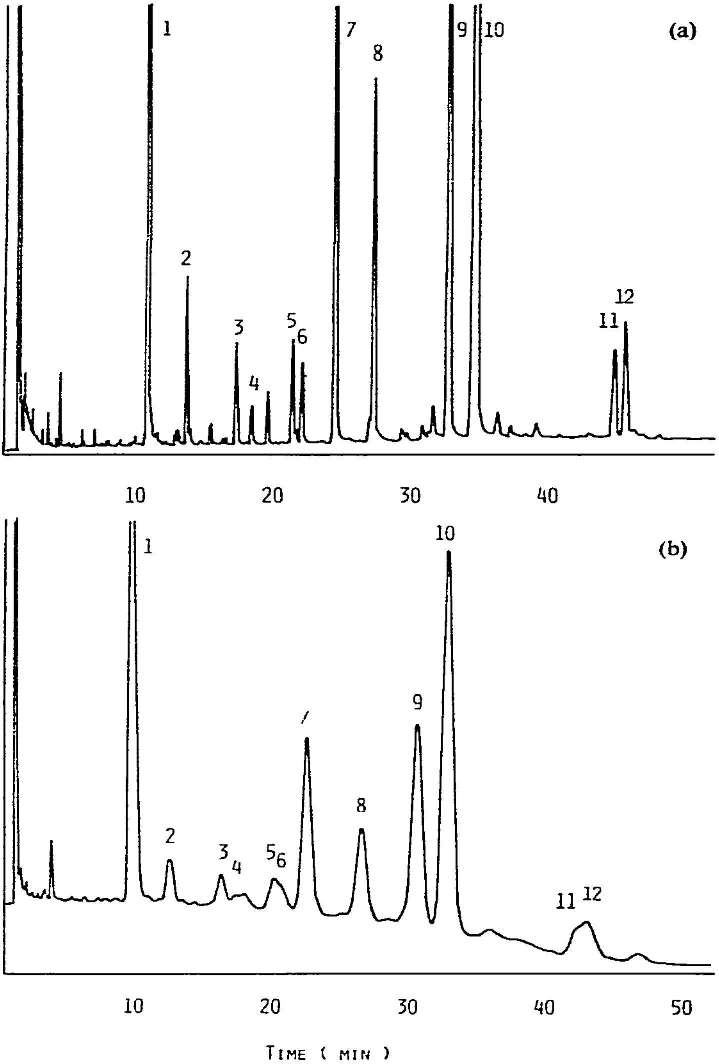

Fig. 2. Comparison of the separation abilities of a Silar-10C SCOT column and an ECNSS-M packed column. (a) Gas chromatogram obtained with the Silar-10C SCOT column. (b) Gas chromatogram obtained with the ECNSS-M packed column. Sample: hydrolysate of the methylated hemicellulose of rice endosperm cell wall[11]. Peaks: 1 = 2,3,5-tri-O-methylarabinitol diacetate; 2 = 2,3,4-tri-O-methylxylitol diacetate; 3 = 3,5-di-O-methylarabinitol triacetate; 4 = 2,3,4,6-tetra-O-methylglucitol diacetate; 5 = 2,3,4,6-tetra-O-methylgalactitol diacetate; 6 = 2,3-di-O-methylarabinitol triacetate; 7 = 2,3-di-O-methylxylitol triacetate; 8 = 2,4,6-tri-O-methylglucitol triacetate; 9 = 2,3,6-tri-O-methylglucitol triacetate; 10 = 2-O-methylxylitol tetraacetate; 11 = xylitol pentaacetate; 12 = 2,3-di-O-methylglucitol tetraacetate. Column temperature programmed from 150 to 190°C at 1°C/min in both experiments.

For example, 2,3,4,6-tetra-O-methylglucitol acetate ($T = 1.00$) and 2,3,4,6-tetra-O-methylmannitol acetate ($T = 0.98$) derived from the hydrolysate of methylated glucomannan were clearly separated (Fig. 1). It has previously been difficult to identify these compounds by GLC or GLC–MS because they could not be separated from each other by the column systems so far reported and, moreover, they showed the same fragmentation patterns when the commonly used electron impact mass spectrometry was applied[8]. They can only be distinguished by the chemical ionization technique[9], but this is laborous and expensive and the determination of each component in a mixture is still difficult.

Some closely related derivatives, the analysis of which is often difficult, can also be separated with the proposed column; thus, 2,3,4-tri-O-methylhexitol acetates (glucitol, mannitol and galactitol), 2-O- and 3-O-methylxylitol acetates, and 2,3,4-tri-O-methylxylitol acetate and the corresponding derivative of fucose were separated from each other. On the other hand, 2,4,6- and 3,4,6-tri-O-methylmannitol acetates overlapped completely, suggesting that the column is not suitable for the analysis of some polymers such as yeast mannan, which contains 2-linked and 3-linked mannose residues in the same molecule[10] (although they can be distinguished by GLC–MS).

Fig. 2 shows the application of the Silar-10C capillary column to the analysis of the complicated mixture of methylated alditol acetates derived from the methylated crude hemicellulose of rice endosperm cell wall[11]. The gas chromatogram obtained with a packed column (ECNSS-M) is also shown for the comparison. Many peaks were separated inadequately by the packed column, whereas they could be separated very clearly by the capillary column.

One of the disadvantage of glass capillary columns that has limited their wide use is their high cost and relatively short life. Because of the excellent thermal stability of Silar-10C liquid stationary phase[7], the column has been improved significantly in the latter respect. After 100 analyses at 180–190°C, only a small decrease in the number of theoretical plates (less than 5%) was observed.

The variability of the relative peak area of each component was also examined by comparing the results of six repeated analyses of a sample with different operating conditions (column temperature, flow-rate). The relative peak area varied within ±5%, indicating a good reproducibility of the quantitative analysis.

The characteristics of the proposed column suggest that it will be useful for structural studies of polysaccharides and related compounds with complicated structures.

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