Carbohydrate Research, 62 (1978) 363–367

Note

G.l.c. of partially methylated alditol acetates

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(Received April 13th, 1977; accepted for publication May 11th, 1977)

It was only after the introduction[1] of the liquid phase ECNSS-M in 1965 that adequate separations of alditol acetates[2] were achieved in g.l.c. and that their application in studies of polysaccharide structure was stimulated. Methylated alditol acetates[3] can be separated on ECNSS-M and, coupled with the technique[4,5] for the rapid methylation of micro quantities of poly- and oligo-saccharides, this further stimulated their use in the study of the structure of oligo- and poly-saccharides.

However, the thermal stability of ECNSS-M is rather low and its useful operating life is rather short in comparison with those of other phases. For this reason, the silicone phase OV-225, which has greater thermal stability (up to 250°) and characteristics similar to those of ECNSS-M, was recommended[6]. Other phases which have been used include polyphenyl ether[7], butanediol succinate[8], OV-275–XF1150[9], and Apiezon greases[10–12]. Hitherto, the Apiezon greases have been used to separate simple mixtures of methylated alditol acetates and no systematic study has been reported. We now report that the Apiezon greases are extremely useful, and in many instances superior to ECNSS-M and OV-225, in the separation and analysis of mixtures of partially methylated alditol acetates.

Table I lists the retention times (T) of thirteen galactitol acetates on columns of Gas Chrom Q coated with six Apiezon greases. Identical T values were obtained using 15% Apiezon grease K, N, or T. The T values of the galactitol acetates decrease with increasing concentration of Apiezon grease, but peak-broadening is evident at 7.5% which decreases the value of these columns. However, such columns, when operated at 180°, are extremely useful for the rapid separation and analysis of simple mixtures of methylated galactitol acetates (galactitol hexa-acetate elutes in ~25 min). Excellent separations are achieved with 15% of an Apiezon grease; an increase above 15% does not improve the separation. Apiezon L, which has the lowest vapour pressure and highest thermal stability (350°) of the Apiezon greases, is the phase of choice, especially for g.l.c.–m.s.

Table II shows the T values of partially methylated galactitol, arabinitol, xylitol, and rhamnitol acetates on columns of Gas Chrom Q coated with ECNSS-M, OV-225, and Apiezon T. The best separation of 2,4,6- and 2,3,6-tri-*O*-methylgalactitol

TABLE I

RETENTION TIMES (*T*) OF PARTIALLY METHYLATED GALACTITOLS ON APIEZON GREASES

Precursor of alditol acetate	*Stationary phase*[a]	*Apiezon M (20%)*	*Apiezon L (20%)*	*Apiezon T (15%)*	*Apiezon K (15%)*	*Apiezon N (15%)*	*Apiezon N (7½%)*
		190°	*190°*	*180°*	*180°*	*180°*	*165°*
2,3,4,6-Me_4Gal[b]		1.00 (15.40)[c]	1.00 (15.40)	1.00 (14.80)	1.00 (15.0)	1.00 (15.70)	1.00 (12.85)
2,4,6-Me_3Gal		1.33	1.31	1.35	1.34	1.34	1.39
2,3,6-Me_3Gal		1.16	1.13	1.17	1.16	1.17	1.14
2,3,4-Me_3Gal		1.56	1.52	1.61	1.60	1.61	1.63
2,6-Me_2Gal		1.40	1.36	1.44	1.43	1.43	1.48
4,6-Me_2Gal		1.50	1.46	1.54	1.53	1.53	1.60
3,6-Me_2Gal		1.51	1.47	1.57	1.55	1.55	1.60
2,4-Me_2Gal		2.08	2.01	2.19	2.18	2.17	2.32
2,3-Me_2Gal		1.77	1.72	1.83	1.83	1.82	1.89
2-MeGal		2.10	2.05	2.22	2.23	2.20	2.36
(3)4-MeGal		2.36	2.29	2.52	2.53	2.51	2.68
6-MeGal		1.68	1.65	1.75	1.75	1.75	1.85
Gal		2.54	2.46	2.72	2.72	2.70	2.94

[a]Solid support, Gas Chrom Q (100–120 mesh). [b]2,3,4,6-Me_4Gal connotes 2,3,4,6-tetra-*O*-methylgalactose, *etc.* [c]Values in parentheses are *T* values (min) of standards.

TABLE II

RETENTION TIMES OF PARTIALLY METHYLATED ALDITOL ACETATES ON APIEZON T, OV-225, AND ECNSS-M

Precursor of alditol acetate	*Stationary phase*	*Apiezon T (15%)*	*OV-225 (3%)*	*ECNSS-M (3%)*
		180°	*195°*	*172°*
2,3,4,6-Me_4Gal		1.00 (14.80)[a]	1.00 (6.95)	1.00 (4.3)
2,4,6-Me_3Gal		1.35	1.55	1.72
2,3,6-Me_3Gal		1.17	1.66	1.80
2,3,4-Me_3Gal		1.61	2.10	2.47
2,6-Me_2Gal		1.44	2.32	2.67
4,6-Me_2Gal		1.54	2.35	2.68
3,6-Me_2Gal		1.57	2.60	3.10
2,4-Me_2Gal		2.19	3.43	4.47
2,3-Me_2Gal		1.83	3.19	4.08
2-MeGal		2.22	4.26	5.60
(3)4-MeGal		2.52	5.35	7.45
6-MeGal		1.75	3.17	3.70
Gal		2.72	5.64	7.90
2,3,5-Me_3Ara		0.46	0.46	0.47
2,3,4-Me_3Ara		0.57	0.55	0.59
2,3-Me_2Ara		0.74	0.93	1.15
2,5-Me_2Ara		0.61	0.85	0.93
3,4-Me_2Ara		0.77	0.96	1.20
3,5-Me_2Ara		0.63	0.73	0.87
Ara		1.09	2.00	2.60
2,3,4-Me_3Rha		0.55	0.46	0.49
2,4-Me_2Rha		0.78	0.84	0.80
3,4-Me_2Rha		0.72	0.79	0.76
2-MeRha		0.84	1.19	1.23
3-MeRha		0.96	1.37	1.35
Rha		1.02	1.52	1.61
2,3,4-Me_3Xyl		0.56	0.58	0.61
2,3-Me_2Xyl		0.74	1.03	1.28
Xyl		1.09	2.55	2.91

[a]Values in parentheses give *T* values (min) of standards.

acetates is achieved on Apiezon T; the order of elution is reversed compared with that for the OV-225 and ECNSS-M columns. The 2,6- and 4,6-di-*O*-methylgalactitol acetates are also separated on Apiezon T in contrast to OV-225 or ECNSS-M, although they are separated on an OV-225 (SCOT) column.

The separations achieved on Apiezon T are extremely useful in the analysis of the alditol acetates derived from the methylation products of certain galactan sulphates isolated from red seaweeds. Many of these galactans contain (1 → 3)- and (1 → 4)-linked galactose and galactose sulphate residues, and 2,6- and 4,6-di- and

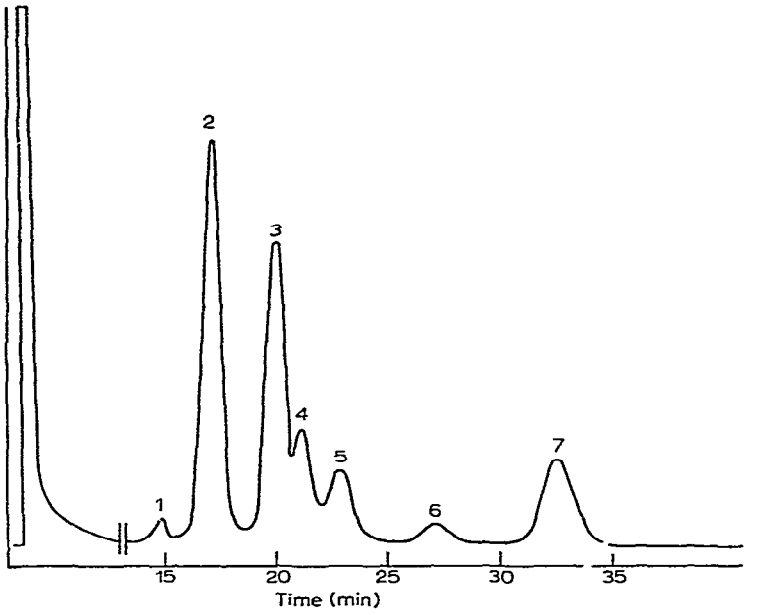

Fig. 1. Separation of *O*-methylgalactitol acetates on Apiezon T; 1, 2,3,4,6-tetra-*O*-methylgalactitol diacetate; 2, 2,3,6-tri-*O*-methylgalactitol triacetate; 3, 2,4,6-tri-*O*-methylgalactitol triacetate; 4, 2,6-di-*O*-methylgalactitol tetra-acetate; 5, 4,6-di-*O*-methylgalactitol tetra-acetate; 6, 2,3-di-*O*-methylgalactitol tetra-acetate; 7, 2,4-di-*O*-methylgalactitol tetra-acetate.

2,4,6- and 2,3,6-tri-*O*-methylgalactoses are frequently encountered as hydrolysis products of the methylated polysaccharides. The mixed OV-275–XF 1150 phase[9], which gives a better separation of partially methylated alditol acetates obtained from cell-wall samples than ECNSS-M and OV-225, does not give as good a separation of the tri-*O*-methylgalactitol acetates as does Apiezon T. Darvill *et al.*[9] do not give a retention time for 4,6-di-*O*-methylgalactitol acetate. Fig. 1 shows the separation on Apiezon T of the alditol acetates derived from a methylated galactan sulphate. All of the methylated galactitol acetates possessing both a 6-*O*-methyl and a 4-acetoxy group are eluted far more rapidly from Apiezon than from either of the other two phases, presumably due to reduced polar interactions.

Surprisingly, the Apiezon T column does not give as good a separation of the partially methylated alditols of arabinose, rhamnose, and xylose as either ECNSS-M or OV-225. However, the methylated galactitol acetates are well separated from the methylated rhamnitol, arabinitol, and xylitol acetates, thus allowing easy identification and quantitation of methylated galactoses in certain plant gums and mucilages.

The suggestion[9] that the superior separation achieved on the OV-275–XF1150 mixed-phase column replaces the need for using two columns applies only when the composition of the mixture is known. With mixtures of unknown composition, it is always prudent to investigate separations by trying different phases, preferably with different characteristics.

EXPERIMENTAL

Materials. — The methylated sugars used had either been isolated from the hydrolysis products of methylated polysaccharides or had been synthesized by known routes.

Preparation of alditol acetates. — The methylated sugars were reduced in water–methanol with sodium borohydride. The excess of borohydride was destroyed by using Amberlite IR-120 (H^+) resin, and the boric acid was removed by distillation with methanol. Acetylation of the alditols was carried out with pyridine–acetic anhydride (1 : 1).

G.l.c. — A Beckman GC-4 chromatograph equipped with dual flame-ionization detectors and glass columns (220 × 0.22 cm) was used, with nitrogen as the carrier gas (70–80 ml/min). The column temperatures are listed in Tables I and II.

All the stationary phases were coated onto Gas Chrom Q (100–120 mesh) by the "slurry" method and, with the exception of Apiezon N and T which were obtained from Shell Chemicals, were supplied by Applied Science Laboratories. Apiezon T was purified by elution from silica gel with light petroleum (b.p. 40–60°).

Retention times (T) are reported relative to that of tetra-*O*-methylgalactitol acetate. A minimum of three injections per methylated alditol acetate was used in establishing T values.

ACKNOWLEDGMENTS

The authors thank Professor G. O. Aspinall and (the late) Professor J. K. N. Jones for samples of methylated arabinoses, and the C.S.I.R. and Rhodes University for financial support.

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