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Note

Gas–liquid chromatography of partially methylated alditol acetates on capillary columns of OV-17 and OV-225

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Accurate methylation analyses became possible in 1967 with the availability of the g.l.c.–m.s. method to characterize and estimate *O*-methylalditol acetates[1]. The liquid phase ECNSS-M was the one most frequently used in the next 6 to 7 years[2]. Other liquid phases useful for separations of sugars[3] include silicone OV-225 (ref. 4), polyphenyl ether[5], 1,4-butanediol succinate[6], OV-275-XF1150 (ref. 7), and Apiezon greases[8,9]. The latter were considered to be superior to ECNSS-M and OV-225 for separation of *O*-methyl acetates of galactitol, rhamnitol, arabinitol, and xylitol, but not all possible isomers were examined.

The present investigation concerns the characterization, by g.l.c., of acetates of *O*-methyl derivatives of galactitol, glucitol, and mannitol that could arise from microbial polysaccharides. A system of two capillary columns, one coated with OV-17 and the other with OV-225, was used in parallel and separate analyses. The utility of this combination resulted from a series of methylation analyses that employed initially a conventional ECNSS-M column[10], then capillary chromatography[11] using OV-17, and finally, parallel experiments with capillary columns[12] of OV-17 and OV-225. Since the last-cited method appears to offer advantages over those previously used, the comparative retention times of most of the *O*-methylalditol acetates arising from mannopyranosyl, glucopyranosyl, galactofuranosyl, and galactopyranosyl residues have now been measured (Table I).

The comparative retention times of *O*-methylalditol acetates on capillary columns of OV-225 resemble those obtained with a conventional column[13]; however, better peak separations were frequently obtained with the capillary system. For example, a complete separation of peaks of 2,3,4,6-tetra-*O*-methyl-glucitol and -mannitol acetate was obtained, whereas they partially coalesced in the conventional system[13]. The advantage of the OV-17 capillary column lies in the order in which compounds emerge (which differs from that of OV-225), and in its superior properties

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TABLE I

RELATIVE RETENTION TIMES OF *O*-METHYLHEXITOL ACETATES IN CAPILLARY COLUMNS OF OV-17 AND OV-225

Position of methyl groups	T_{M} *values*					
	Mannitol derivatives		*Glucitol derivatives*		*Galactitol derivatives*	
	OV-17	*OV-225*	*OV-17*	*OV-225*	*OV-17*	*OV-225*
2,3,4,6	1.00	1.00	1.00	1.02	1.11	1.13
2,3,5,6					1.04	1.07
2,3,6	1.55	1.79	1.61	2.02	1.58	1.88
3,4,6	1.44	1.64	1.46	1.65	1.68	1.91
2,4,6	1.63	1.71	1.53	1.64	1.72	1.78
2,3,4	1.61	1.89	1.61	1.87	1.93	2.38
2,3,5					1.93	2.28
3,5,6					1.65	1.67
2,5,6					1.57	1.70
2,6	2.21	2.61	2.27	2.87	2.32	2.67
4,6	2.21	2.52	2.29	2.90	2.37	2.72
3,6	2.36	2.97	2.38	3.03	2.50	3.07
2,3	2.48	3.21	2.60	3.63	2.70	3.78
3,4	2.48	3.52	2.52	3.51	3.02	4.36
2,4	2.81	3.60	2.63	3.41	3.16	4.05

[a]Relative to that of 2,3,4,6-tetra-*O*-methylmannitol diacetate (T_{M} 1.00).

for quantitative determination. With OV-225, the peak response tended to be smaller for the di-*O*-methyl as compared with those of the tetra-*O*-methyl derivatives.

With the present system, the four tri-*O*-methylglucitol acetates that may arise from glucopyranosyl units can be completely determined. On OV-17, the 2,3,6- and 2,3,4-isomers cochromatograph, but are distinguishable from the 3,4,6- and 2,4,6-isomers, which are resolved[11]. A different pattern is obtained with OV-225 in the capillary (and conventional[13]) system, the 3,4,6- and 2,4,6-tri-*O*-methyl derivatives being unresolved, but distinct from the 2,3,6- and, in turn, the 2,3,4-isomer. Thus, it appears that resolution of all four isomers is feasible by use of a correct blend of OV-17 and OV-225.

Galactomannans from *Aspergillus* spp. contain a (1→6)-linked α-D-manno-pyranosyl main-chain, partially substituted by chains of (1→5)-linked β-D-galacto-furanosyl residues. The resulting methylation mixture contained acetates of 2,3,4-tri-*O*-methylmannitol and 2,3,6-tri-*O*-methylgalactitol, which were not resolvable[13] on ECNSS-M and required neopentylglycol sebacate in a separate experiment[10]. A separation is now possible with a capillary column of OV-17 (but not OV-225; see Table I). However, when such a galactomannan contains glycogen as a contaminant, it is necessary to use OV-225 in order to separate[12] 2,3,6-tri-*O*-methylglucitol acetate from the peak consisting of acetates of 2,3,6-tri-*O*-methylgalactitol and 2,3,4-tri-*O*-methylmannitol.

TABLE II

T_M VALUES OF COMPONENTS OF STANDARD MIXTURES OF *O*-METHYLHEXITOL ACETATES

Position of methyl groups and parent hexose	T_M *value*		*Position of methyl groups and parent hexose*	T_M *value*	
	OV-17	*OV-225*		*OV-17*	*OV-225*
Group 1			*Group 4*		
2,3-Glc	2.60	3.63	3,4,6-Man	1.44	*1.64*
3,4-Glc	2.52	3.51	3,4,6-Glc	1.46	*1.66*
2,4-Glc	2.63	3.41	2,4,6-Glc	1.53	*1.64*
2,4-Man	2.81	3.60	2,3,6-Man	1.55	1.79
2,3-Man	*2.48*	3.21			
3,4-Man	*2.48*	3.52			
Group 2			*Group 5*		
2,3,6-Gal	1.58	*1.88*	4,6-Gal	*2.37*	2.72
2,4,6-Man	1.63	1.71	3,6-Glc	*2.38*	3.03
2,3,6-Glc	*1.61*	2.02	3,6-Man	*2.36*	2.97
2,4,6-Gal	1.72	1.78	4,6-Glc	2.29	2.90
2,3,4-Man	*1.61*	*1.89*			
2,3,4-Glc	*1.61*	1.87	*Group 6*		
3,4,6-Gal	1.68	1.91	2,6-Gal	2.32	2.67
Group 3			2,6-Glc	2.27	2.87
3,5,6-Gal	1.65	1.67	2,6-Man	*2.21*	2.61
2,3,6-Gal	1.60	1.88	4,6-Man	*2.21*	2.52
2,5,6-Gal	1.57	1.70	*Group 7*		
5,6-Gal	2.16	2.30	2,4-Gal	3.16	4.05
			3,4-Gal	3.02	4.36

The retention times of *O*-methylhexitol acetates, summarized in Table I, are expressed relative to that of 2,3,4,6-tetra-*O*-methylmannitol acetate (T_M 1.00). A difference of T_M 0.01–0.02 between materials with T_M 1.0–1.5 signifies that they are separable. Compounds having longer retention times (T_M 1.5–4.5) may be resolved where their retention times differ by T_M 0.02–0.03. The resolution of the method was established by standards whose components had T_M values close to each other; different orders of peak emergence occurring with capillary columns of OV-17 and OV-225 were observed (see Table II). Unresolved acetates in seven standard mixtures for each column are italicized. The components of the mixtures were chosen because they had closely related retention-times.

EXPERIMENTAL

Analyses were performed with a Model 4000 Finnigan g.l.c.–m.s. unit, interfaced with an Incos 2300 Data System. Electron-impact spectra[13] were obtained repetitively every 2 s, scanning from *m/z* 40 to 420. G.l.c. was performed in columns (0.25 mm i.d. × 30 m), one coated with OV-17 (J and W Scientific, 11505 Douglas Road, Rancho Cordova, CA 95670), and the other with OV-225 (Chrompack Canada,

R.R. 2, Blenheim, Ontario NOP 1AO). Injections were made in the splitless mode at 50° in order to obtain the "Grob solvent effect"[14], and the elution was quickly programmed (40°/min) to 182° (hold). The carrier gas was helium with a linear velocity of 22 cm/s.

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