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SEPARATION AND IDENTIFICATION OF *O*-ACETYL-*O*-METHYL-GALACTONONITRILES BY GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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ABSTRACT

The gas–liquid-chromatographic retention-times and the mass-spectral features of partially methylated D-galactononitrile acetates are reported. Distinctive fragmentation of each of the mono-*O*-methyl derivatives allows their identification, and the results are applicable to the same substituted derivatives of the other aldohexoses. A new fragmentation-pathway, typical of the acetylated and the *O*-acetyl-*O*-methyl-aldononitriles, is proposed in order to justify previously unexplained fragments. This fragmentation competes with the known ones in derivatives that do not carry vicinal methoxyl groups.

INTRODUCTION

The partially methylated alditol acetates have been used successfully in the application of g.l.c.–m.s. to the investigation of polysaccharide structures[1–3]. The derivatives are easy to prepare, and the method affords simplified gas–liquid chromatograms, but it is limited by the symmetry of some of the alditol molecules, which necessitates the use of sodium borodeuteride for reduction of the aldoses.

The *O*-acetyl-*O*-methylaldononitriles are easier to prepare and afford simple chromatograms, retaining[4] the dissymmetry of C-1. The behavior in g.l.c., and the identification by m.s., of the acetylated aldononitriles derived from the di-, tri-, and tetra-methyl ethers of D-glucose[5,6], D-mannose[7,8], D-xylose[4,9], L-arabinose[9], and 6-deoxy sugars[10] have been reported, but complete information has not been educed[5] for the monomethyl ethers of any aldose, including the monomethyl ethers of D-galactose, despite the fact that the latter are obtained in the hydrolyzates of derivatives of several, naturally occurring polysaccharides.

We now report the gas-chromatographic retention-times and the mass-spectral features of partially methylated D-galactononitrile acetates. Distinctive fragmentation of each of the mono-*O*-methyl derivatives allows their identification, and these results

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are applicable to the similarly substituted derivatives of the other aldohexoses.

A new fragmentation pathway, typical of the acetylated and the *O*-acetyl-*O*-methylaldononitriles, is proposed, in order to justify previously unexplained fragments. This fragmentation competes with the known ones in derivatives that do not carry vicinal methoxyl groups.

RESULTS AND DISCUSSION

Table I shows the relative, g.l.c. retention-times of different *O*-methylated *O*-acetyl-D-galactononitriles in the four systems used. The retention times obtained in columns *A* and *B* are almost identical. Comparing the separations obtained with the OV-225 column, the second program is better, but, even in this case, no differentiation of the 3-*O*-methyl- and 4-*O*-methyl-galactose derivatives was obtained. The best separations and clearer chromatograms were obtained by using the 3% ECNSS-M column (*A*); this is in agreement with the results obtained with the *O*-methylated *O*-acetylmannononitriles[8].

Fig. 1 shows a chromatogram of the separation of a mixture of *O*-acetyl-*O*-methylgalactononitriles obtained in the hydrolysis of a methylated carrageenan[11,12].

As in the case of the alditol acetates, a stepwise decrease in the retention times

TABLE I

RETENTION TIMES OF THE *O*-ACETYL-*O*-METHYL-D-GALACTONONITRILES

O-Methyl	*O-Acetyl*	*Retention times*[a,b]			
		Column			
		A	B	C-1	C-2
2,3,4,6	5	0.11	0.11	0.31	0.27
2,4,6	3,5	0.14	0.13	0.44	0.37
2,3,6	4,5	0.16	0.15	0.45	0.40
2,3,4	5,6	0.19	—	—	—
3,4,6	2,5	0.20	0.20	0.47	0.44
2,6	3,4,5	0.25	0.25[c]	0.51	0.48
4,6	2,3,5	0.27	0.28[c]	0.51	0.50
2,4	3,5,6	0.38	—	0.58	0.52
3,4	2,5,6	0.38	—	—	—
6[d]	2,3,4,5	0.38	0.38	0.57	0.57
2	3,4,5,6	0.56	0.55	0.61	0.63
3	2,4,5,6	0.70	0.68	0.64	0.67
4	2,3,5,6	0.77	0.76	0.64	0.67
—	2,3,4,5,6	0.90	0.90	0.69	0.73

[a]Relative to ($t_{tetra} + t_{Gal}$); see text. [b]Average of at least five determinations. [c]Sometimes only one peak with a shoulder is obtained. [d]The derivatives of 2,3- and 3,6-di-*O*-methylgalactose in a mixture of aldononitriles of partially methylated galactoses from the hydrolysis of a methylated carrageenan are included in this peak.

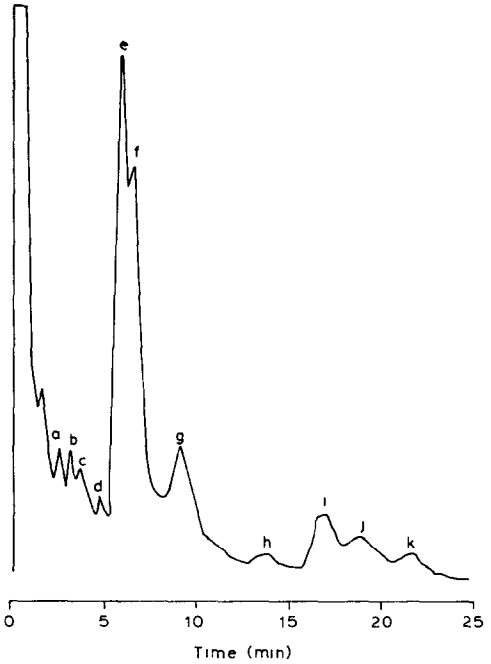


Fig. 1. G.l.c. analysis of a mixture of acetylated *O*-methyl-D-galactononitrile derivatives of partially methylated D-galactoses from the hydrolysis of a methylated carrageenan from *Iridaea undulosa*[11,12] (column *A*). [Key: (a) 2,3,4,6-tetra-*O*-methyl; (b) 2,4,6-tri-*O*-methyl; (c) 2,3,6-tri-*O*-methyl; (d) 3,4,6-tri-*O*-methyl; (e) 2,6-di-*O*-methyl; (f) 4,6-di-*O*-methyl; (g) 6-*O*-methyl that also includes other di-*O*-methylgalactoses; (h) 2-*O*-methyl; (i) 3-*O*-methyl; (j) 4-*O*-methyl; and (k) nonmethylated galactose.]

occurs for the replacement of *O*-acetyl by *O*-methyl groups. An exception is the 6-*O*-methylgalactononitrile derivative, which shows a shorter retention time than predicted, in the range of those of the di-*O*-methylgalactononitrile derivatives.

If derivatives having the same number of methyl groups are considered, those in which O-3 and O-4 are methylated have, in most cases, longer retention-times than those having O-3 and O-4 acetylated, and those that are 6-*O*-methylated have shorter retention-times than the 6-*O*-acetylated.

The more readily exposed, and the more polar, is the group, the greater is the interaction of the product with the stationary phase[13]. Penta-*O*-acetylgalactononitrile has, in pyridine, an extended, planar, zigzag conformation for the carbon chain[14,15], and it is logical to postulate this lower-energy conformation for the vapor phase. Molecular models show that the groups are exposed to outer interactions in the sequence O-6 > O-2 > O-3 $\simeq$ O-4 and, in this order, they will decrease the retention time when a polar group (*O*-acetyl) is replaced by a less-polar one (*O*-methyl).

An advantage of the use of the nitrile over the alditol derivatives is that such compounds as 2,6- and 4,6-di-*O*-methyl galactose, 3-*O*-methylgalactose and galactose, or the triplet composed of 2,3,6-, 2,4,6-, and 3,4,6-tri-*O*-methylgalactose were readily differentiated. The main drawback of the nitrile derivatives is that the 2,3-, 2,4-, 3,4-,

TABLE II

MOST-INTENSE PEAKS IN THE SPECTRA OF THE *O*-ACETYL-*O*-METHYL-D-GALACTONONITRILES

m/z	*Tetra*-O-*methyl*	*Tri*-O-*methyl*				*Di*-O-*methyl*				*Mono*-O-*methyl*				*Non-methylated*
	2,3,4,6	*2,4,6*	*2,3,6*	*2,3,4*	*3,4,6*	*2,6*	*4,6*	*2,4*	*3,4*	*2*	*3*	*4*	*6*	—
43	100	100	100	100	100	100	100	100	100	100	100	100	100	100
45	54	36	24	24	72	44	30		5				31[a]	
70					7			3						
71	10	8	6	8	13			4						
73			4		12									
74	7		3		11			5				3		
75	7		4		7	5								
83											10[a]			
85	7		6							7	16			
87	20	13	26	26	34	32	8	18	27			12	18	
88	22		7	17	18									
89	7				9									
95			4				4					5		
96		12						11[a]						
99		14	14	26	14	10		10	12		10[a]			
100					12									
101	10	30	5	6	45		20[a]							
103					5			3		9	4	6		13
112		27[a]					28	26				30[a]		
113	7	5	20	6	8		9					11		
114			5		5									
115		4	7			21				10	12		9	11
117	10	4	6	7		14							10[a]	
119					8[a]									
126		6	3		4			3	28					
127		8[a]				5		7		9	16			
129	36	30	25	38	71	16	22	32	56			23	8	
131			8											
142					13						13[a]			
145	27				25			4		9		5		15
147			4[a]											
154		18[a]					16	18				19[a]		
155							6					7	4	
157			3			7				5			5	5
159				5		18		8			4		9	
161	25	13	3		38		11[a]							
169						14								
184						14				9[a]				
186		19						20	6					
187										14[a]				4
189				7[a]				9	17			6[a]		
197													10[a]	
201						1					5[a]			
205					4[a]									
211						4								
212													5[a]	6

TABLE II *(contined)*

m/z	*Tetra*-O-*methyl*	*Tri*-O-*methyl*				*Di*-O-*methyl*				*Mono*-O-*methyl*				*Non-methylated*
	2,3,4,6	*2,4,6*	*2,3,6*	*2,3,4*	*3,4,6*	*2,6*	*4,6*	*2,4*	*3,4*	*2*	*3*	*4*	*6*	—
214							16[a]					12[a]		
215							2					2		
217										3	4			2
225														2[a]
261						1					3	2		
273										2		2		
286						1[a]				2				
289										2[a]				
314														2

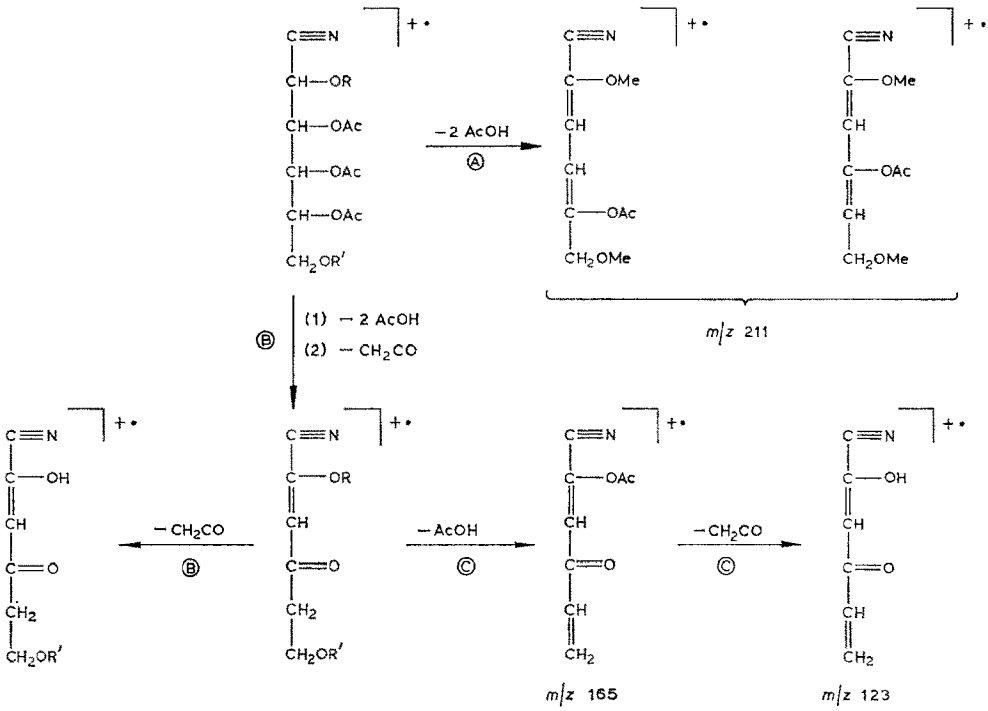
[a]Specific peaks for each class of methylated compounds. In the case of the di-*O*-methyl derivatives, the specificity was evaluated by taking into account the spectral data given by Seymour *et al.*[7] for the 2,3- and 3,6-di-*O*-methyl-D-mannononitrile derivatives.

and 3,6-di-*O*-methylgalactoses cannot be separated, and often appear together with the 6-*O*-methylgalactose derivative.

It was noted that the relative retention-times of the derivatives diminished with the age of the columns. The sequence is not altered, and the effect is not important for sugars whose retention times are near the standard, but becomes significant in the other cases. The use of two standards, as suggested by Lindberg and coworkers[1], lessens this aging effect.

The fragmentation of the molecules of partially methylated galactononitrile acetates is analogous to that described for methylated alditol acetates[3], *i.e.*, the fission takes place mainly between carbon atoms carrying vicinal methoxyl groups. The primary fragments formed eliminate molecules of hydrocyanic acid, formaldehyde, methanol, ketene, or acetic acid, to yield more-intense, secondary fragments. The *m/z* values of the more intense peaks in the mass spectra of partially methylated galactononitrile acetates are listed in Table II. Those of the di-, tri-, and tetra-*O*-methyl-D-galactoses agree with those published for similar derivatives of other aldohexoses[5,7]. An inspection of the data indicated that unambiguous characterization of methylated monosaccharides is possible.

There is also another fragmentation pathway, shown in Scheme 1, which presents losses of neutral fragments (acetic acid and ketene) from the molecular ion, giving radical ions that are important in the less-methylated compounds. This pathway is specific to the aldononitrile derivatives, and the radical-ions produced are stabilized by conjugation along the carbon chain; this was suggested by Szafranek *et al.*[16], and justifies the fragments *m/z* 225, 183, 165, and 123 in the spectra of per-*O*-acetylated gluconononitrile. The corresponding shift on deuteroacetylation, in that work[16], also supports this postulation.



Ⓐ R = R′ = Me m/z 211

Ⓑ R = R′ = Me m/z 169
R = Ac, R′ = Me m/z 197 ⟶ m/z 155
R = R′ = Ac m/z 225 ⟶ m/z 183

Ⓒ R = R′ = Ac m/z 165 ⟶ m/z 123

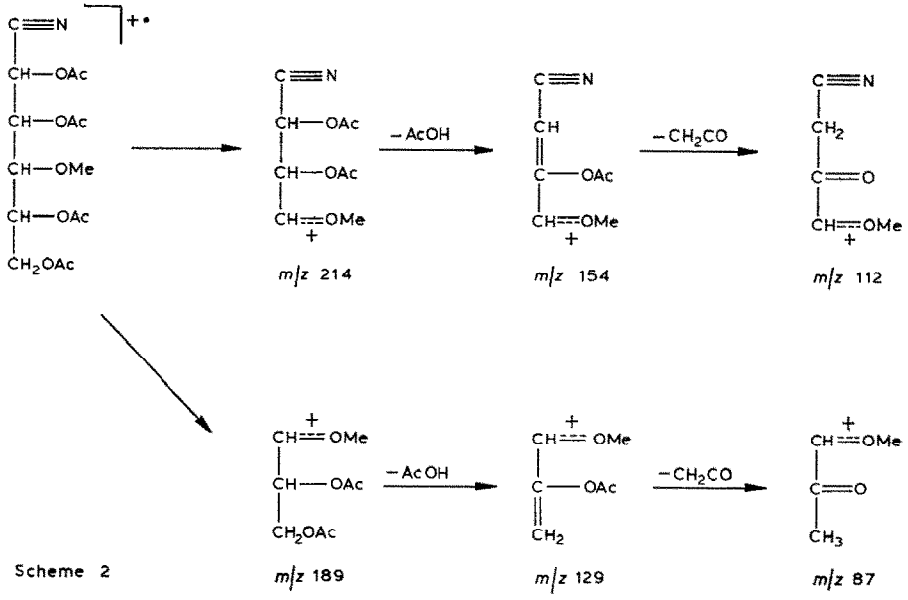
Scheme 1

This rationalization also explains the fragment *m/z* 197 (see Scheme 1) produced by the 6-*O*-methylgalactose derivative, and the previously unexplained peaks at *m/z* 169 and 211 (see Scheme 1) produced by some di-*O*-methylgalactose derivatives (mainly 2,6-di-*O*-methyl). This pathway is not observed for tri- or tetra-*O*-methyl derivatives, where the cleavages between carbon atoms carrying vicinal methoxyl groups, or a methoxyl and an acetoxyl group, predominate.

The fragmentation patterns of the tetra-*O*-acetyl-2-*O*-methyl-, -3-*O*-methyl-, -4-*O*-methyl-, and -6-*O*-methyl-D-galactononitriles, which up to now have not been analyzed, follow the same rules as those for the other partially methylated aldononitrile acetates, and, in addition, present distinctive, primary fragmentation for the mono-*O*-methyl derivatives[8].

The 6-*O*-methyl derivative shows, specifically, the ion *m/z* 45, formed by a fragmentation arising from C-5–C-6 bond cleavage, and the ion *m/z* 117, by cleavage of the C-4–C-5 bond. These fragments also appear for other 5-*O*-acetyl-6-*O*-methyl derivatives. Also specific to the monomethyl ethers is the ion *m/z* 212, formed by

secondary fissions of the C-1–C-5 part of the molecule, producing a five-membered ring[16]. The most-specific peak, that does not appear in the mass spectra of any of the tetra-, tri-, di-, or mono-*O*-methyl, or non-methylated derivatives is the ion *m/z* 197 (see Scheme 1).

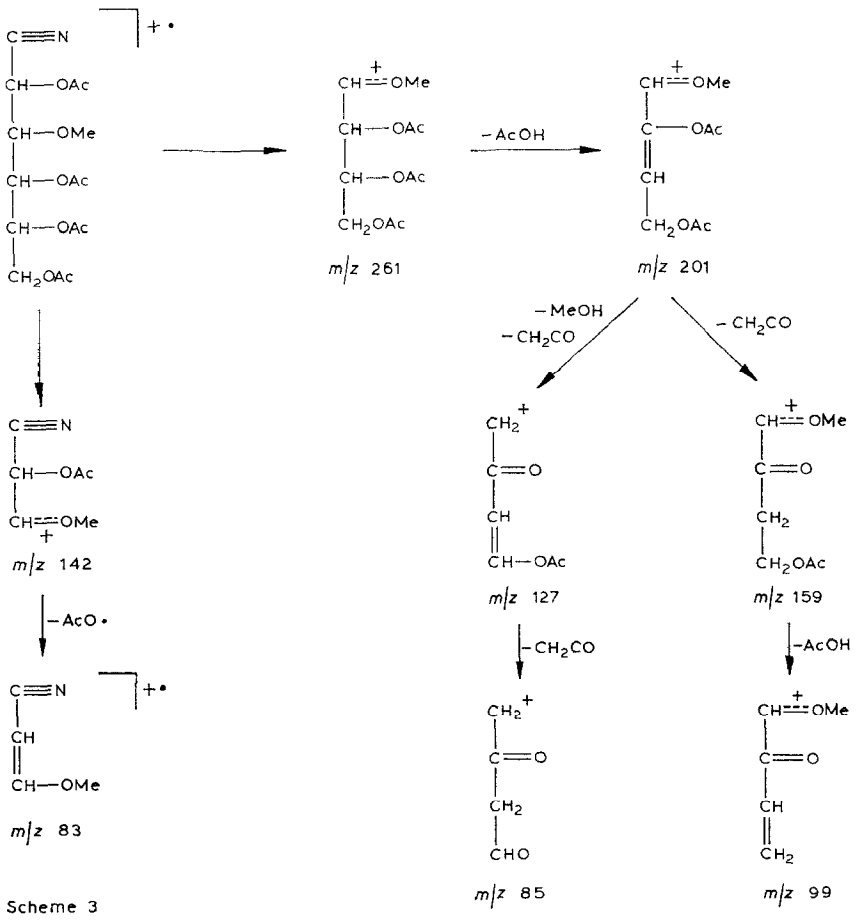


Scheme 2

The spectrum of the 4-*O*-methyl derivative shows two characteristic fragmentation-pathways (see Scheme 2). Both are produced by the primary fissions of carbon–carbon bonds of vicinal atoms carrying acetoxyl and methoxyl groups, and, in both cases, the fragments produced retain the methoxyl group. The three peaks of the first fragmentation-sequence are specific, the one at *m/z* 112 being the most prominent of the spectrum. The second sequence shows only one specific peak, namely, the primary fragment, *m/z* 189.

The spectrum of the 3-*O*-methyl derivative also shows two characteristic fragmentation-sequences (see Scheme 3). In the first, fragments *m/z* 201 and 99 are specific. In the second sequence, peaks *m/z* 142 and 83 are also specific. The loss of acetate radical, with the formation of the *m/z* 83 fragment, is justified by the production of a conjugate, stabilized system.

The spectrum of the 2-*O*-methyl derivative shows two characteristic fragmentation-sequences: (*a*) 286→184→157, and (*b*) 289→187→127→85. The first is an unusual one, produced by the cleavage of the C-5–C-6 bond and giving a fragment containing the nitrile end of the molecule, which, by loss of acetic anhydride and HCN, gives rise to the secondary fragments. Only the peak *m/z* 184 is specific, because the other two have isomers that appear in spectra of other mono-*O*-methyl derivatives. The second sequence, which also appears in the spectrum of galactononitrile penta-



Scheme 3

acetate, is produced by cleavage of the C-2–C-3 bond, maintaining, in this case, the charge on the acetoxyl group. Ion *m/z* 187 is the most important, and can distinguish this derivative specifically. The fragment *m/z* 70, which would be formed by cleavage of the C-2–C-3 bond, is not detected, in agreement with the statement that the fragment from the nitrile end is stable only when carrying an acetoxyl group near C-1.

EXPERIMENTAL

The partially methylated D-galactononitrile acetates were prepared from the corresponding, partially methylated D-galactoses, or from mixtures of them obtained from the hydrolysis of methylated galactans, according to Seymour *et al.*[7]. The final residue was dissolved in chloroform (20 μL) and an aliquot was injected into the g.l.c. column.

G.l.c. was conducted in a Hewlett–Packard Research Gas Chromatograph model 5830-A equipped with a dual-flame detector and a Hewlett–Packard 18850-A

g.l.c. terminal. The glass columns (180 cm × 2 mm i.d.) and the operating conditions were as follows: (*A*) 3% of ECNSS-M on Gas Chrom Q (100–120 mesh), isothermal at 180°; (*B*) 0.2% of poly(ethylene glycol succinate), 0.2% of poly(ethylene glycol adipate) and 0.4% of silicone XF 1150 on Gas Chrom P, isothermal at 170°; and (*C*) 3% of OV-225 on Gas Chrom Q (100–120 mesh): programmed (*1*) from 150 to 245° at 8°.min^{-1}, and afterwards isothermally, and (*2*) from 185 to 245° at 4°.min^{-1}, and afterwards isothermally.

The retention times given are relative to a standard calculated by addition of the retention times of the derivatives having shorter and longer retention-times, namely, 5-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactononitrile and penta-*O*-acetylgalactononitrile, respectively. This standard was used in order to lessen the aging effect.

Mass spectra were recorded with a Varian MAT CH7 mass spectrometer at an ionizing energy of 70 eV, a trap current of 100 μA, and a source pressure of 10^{-9} atm. Computerized g.l.c.–m.s. was conducted, under the same conditions as before, with a column (120 cm × 2 mm i.d.) containing system *A*.

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