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Separation and identification of partially ethylated galactoses as their acetylated aldononitriles and alditols by capillary gas chromatography and mass spectrometry

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ABSTRACT

As an aid to the structural determination of naturally methylated galactans, the separation and identification by capillary gas chromatography and the mass spectral features of ethylated galactoses as their aldononitrile and alditol acetate derivatives are presented and compared with those of their methylated counterparts. Data for some ethylated mono-O-methylgalactose derivatives are also included.

INTRODUCTION

The determination of the position of glycosidic linkages in oligo- and polysaccharides is usually achieved by methylation analysis [1]. The mixtures of partially methylated sugars released by hydrolysis of the methylated polysaccharides are generally derivatized to the alditol acetates [2] or, less frequently, to the aldononitrile acetates [3,4] to produce volatile derivatives for gas chromatographic analysis of the mixtures.

For polysaccharides containing naturally methylated sugars in addition to their non-methylated counterparts, methylation analysis leaves uncertainties in the structural determination. This has been overcome by the use of trideuteriomethyl iodide as alkylating agent [5], but as deuterium labelling does not change retention times, the use of coupled gas chromatography–mass spectrometry (GC–MS) is necessary for unequivocal interpretation of the chromatograms [5].

Another approach is ethylation (or propylation) [6], which produces a significant change in chromatographic behaviour, and therefore can be used without the need for MS. Albersheim and co-workers [7,8] have used ethylation as an alternative to methylation to achieve better chromatographic separations of alditol acetates in packed columns and a laboratory-made, wide-bore capillary column. An advantage of ethylation is that ethyl iodide is far cheaper than trideuteromethyl iodide, and can be synthesized and purified easily.

Seaweed polysaccharides usually contain variable amounts of galactoses naturally methylated at one of the four available positions [9,10]. We now report capillary column GC retention times and the main mass spectral features of partially ethylated D-galactononitrile acetates and galactitol acetates. The data are compared with those for their methylated counterparts. Data for the products of ethylation of some mono-O-methylgalactoses (generated by ethylation of

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naturally methylated seaweed galactans) are also included.

EXPERIMENTAL

Gas chromatography–mass spectrometry

Separations were carried out on a Hewlett-Packard HP 5890 apparatus. Volumes of 1 μl of chloroform solutions of derivatives were introduced via the injector in the split mode (*ca.* 1:100) on a Supelco (Bellefonte, PA, USA) SP-2330 vitreous-silica capillary column (30 m × 0.25 mm I.D., 0.20 μm film thickness), using nitrogen as the gas carrier (100 kPa, flow-rate *ca.* 1 ml/min). Temperature program A was isothermal at 210°C and in program B the temperature was programmed from 160 to 210°C at 2°C/min and from 210 to 240°C at 5°C/min [11]. A flame ionization detector was used, at 230 and 245°C, respectively. For MS, the same apparatus and column were used but using helium as gas carrier (64 kPa), interfaced to a Trio-2 VG Masslab (Manchester, UK) mass spectrometer. Mass spectra were recorded at 70 eV with scan times of 0.90 s (0.10 s reset).

TABLE I

RELATIVE RETENTION TIMES OF ETHYLATED GALACTOSES AS THEIR ACETYLATED ALDONONITRILES ON AN SP-2330 CAPILLARY COLUMN

Retention times relative to 3,4,5-tri-O-acetyl-2,6-di-O-ethylgalactononitrile = 1000.

Acronym	Position of O-ethyl	Programme A	Programme B
1N	2,3,4,6	358	460
2N	3,4,6	568	714
3N	2,4,6	587	729
4N	2,3,4	640	777
5N	2,3,6	662	796
6N	2,6	1000	1000
7N	4,6	1024	1008
8N	3,6	1046	1016
9N	2,4	1283	1098
10N	2,3	1352	1115
11N	6	1537	1155
12N	2	2085	1260
13N	3	2329	1299
14N	4	2554	1333
	–	*ca.* 3400	1488
Xylitol[a]		1505 (19.38 min)	1152 (30.54 min)

[a] Absolute and relative retention times of xylitol pentaacetate are given for comparison.

TABLE II

RELATIVE RETENTION TIMES OF ETHYLATED GALACTOSES AS THEIR ACETYLATED ALDITOLS ON AN SP-2330 CAPILLARY COLUMN

Retention times relative to 1,3,4,5-tri-O-acetyl-2,6-di-O-ethylgalactitol = 1000.

Acronym	Position of O-ethyl	Programme A	Programme B	Ref. 7[a]
1A	2,3,4,6	418	518	588
3A	2,4,6	652	771	804
2A	3,4,6	659	781	810
5A	2,3,6	676	796	810
4A	2,3,4	738	847	889
6A	2,6	1000	1000	1000
7A	4,6	1016	1005	967
8A	3,6	1128	1061	1018
10A	2,3	1287	1120	1065
9A	2,4	1375	1145	1092
11A	6	1501	1172	1105
12A	2	1952	1264	1209
13A	3 (=4)	2421	1337	1294
	–	3095	*ca.* 1440	1490
Xylitol[b]		1778	1233	1176

[a] Reported data on a 1% PEGS, 1% PEGA, 4% XF-1150 capillary column [7].
[b] Relative retention times of xylitol pentaacetate are given for comparison; absolute values are given in Table I.

General methods

Xylogalactans from *Corallina officinalis* were isolated, fractionated, purified and characterized as described previously [10]. They were composed of xylose, galactose and four mono-O-methylgalactoses [10]. Cystocarpic carrageenans from *Iridaea undulosa* were isolated and fractionated by means of potassium chloride; they are devoid of methylated galactoses [12]. These seaweed polysaccharides were ethylated as their triethylammonium salts, as described previously for methylation [12,13], but a second addition of methylsulphinyl carbanion and iodoethane was made. Carbanion was allowed to react for 150 min and iodoethane for 1 h each time. Iodoethane was synthesized from iodine, ethanol and red phosphorus [14], purified by distillation and found by ^{1}H NMR to be pure.

Ethylated polysaccharides were hydrolysed with 45% formic acid at 100°C for 16 h and divided into two roughly equal portions: one was derivatized as the alditol acetates [7] and the other as the aldononitrile acetates [3]. Samples of α-methyl galactopyranoside (Sigma, St. Louis, MO, USA) were ethylated using variable but limiting amounts of iodoethane [7], and hydrolysed as described.

TABLE III

RELATIVE RETENTION TIMES OF METHYLATED GALACTOSES AS THEIR ACETYLATED ALDONONITRILES ON SP-2330 CAPILLARY COLUMNS

Retention times relative to 3,4,5-tri-O-acetyl-2,6-di-O-methylgalactononitrile = 1000. Data for an older and a newer column are presented (see text).

Position of O-methyl	Programme A		Programme B	
	ca. 3 years	<1 year	*ca.* 3 years	<1 year
2,3,4,6	411	393	576	536
2,4,6	624	612	785	755
3,4,6	678	664	832	803
2,3,6	748	737	878	852
2,3,4	794	782	911	883
2,6	1000	1000	1000	1000
4,6	1082	1082	1028	1030
3,6	1132	1129	1042	1046
2,4	1356	1361	1099	1116
2,3	1458	1461	1125	1139
6	1423	1433	1123	1132
2	1898	1916	1209	1223
3	2357	2372	1292	1298
4	2571	2590	1325	1328
–	2858	2912	1377	1380
Xylitol[a]	1254		1077	

[a] Relative retention times of xylitol pentaacetate are given for comparison; absolute values are given in Table I.

RESULTS AND DISCUSSION

Table I gives the GC relative retention times of different O-acetyl-O-ethyl-D-galactononitriles on an SP-2330 capillary column using two different conditions. Table II gives the same data for the corresponding alditols. Both tables were constructed using ethylation data for several carrageenans and other galactans (see Experimental) for which methylation data are available [12,15], and complemented with intentionally underethylated samples of methyl α-D-galactopyranoside [7]. In the former case, the general pattern after ethylation was similar to that of methylation. Sweet *et al.* [7] reported the retention times of ethylated galactitol acetates on three different packed columns and a laboratory-made capillary ("wide-bore") column. Their results are included in Table II.

The retention times of the same polysaccharide components, as their partially methylated galactononitrile and galactitol acetates, are presented in Tables III and IV, respectively. A previous publication reported the data for the former derivatives in packed columns [3], and for the latter derivative several reports have been made [11,16], one of which (using an SP-2330 column) is included in Table IV. To aid comparison between Tables I–IV, the retention time of xylitol pentaacetate with respect to each standard is included in each table.

SP-2330 is regarded as an excellent support for separating methylated alditol acetates [11]. Good separation is achieved for all ethylated galactononitrile acetates (Table I), being comparable to the corresponding methylated derivatives (Table III). In spite of the molecular mass increment, ethylated aldononitrile derivatives appear earlier than corresponding methylated derivatives, owing to their lower polarity. The order of elution for the derivatives is, however, not the same: there are reversals for 3,4,6- and 2,4,6-tri-O-alkyl, the 2,3,4- and 2,3,6-tri-O-alkyl and the 6-mono- and 2,3-di-O-alkyl derivatives.

TABLE IV

RELATIVE RETENTION TIMES OF METHYLATED GALACTOSES AS THEIR ACETYLATED ALDITOLS ON SP-2330 CAPILLARY COLUMNS

Retention times relative to 1,3,4,5-tri-O-acetyl-2,6-di-O-methylgalactitol = 1000. Data for an older and a newer column are presented (see text).

Position of O-methyl	Programme A		Programme B		
	ca. 3 years	<1 year	*ca.* 3 years	<1 year	Ref. 11[a]
2,3,4,6	487	466	663	626	606
2,4,6	699	687	840	817	803
3,4,6	752	740	880	859	847
2,3,6	773	760	892	871	859
2,3,4	942	928	982	970	964
2,6	1000[b]	1000[b]	1000	1000	1000
4,6	1005[b]	1005[b]	1000	1000	1000
3,6	1153	1159	1051	1059	1073
2,3	1433	1436	1119	1136	1175
2,4	1474	1486	1127	1148	1188
6	1270	1283	1079	1094	1150
2	1795	1822	1187	1208	1262
3 (=4)	2305	2342	1273	1287	1342
–	2352	2406	1282	1298	1353
Xylitol[c]	1361		1101		1160

[a] Literature data [11] using programme B are included.
[b] Unresolved peaks.
[c] Relative retention times of xylitol pentaacetate are given for comparison; absolute values are given in Table I.

TABLE V

RELATIVE RETENTION TIMES OF ETHYLATED MONO-O-METHYLGALACTOSES AS THEIR ACETYLATED ALDONONITRILES AND ALDITOLS ON AN SP-2330 CAPILLARY COLUMN

Retention times relative to the derivative of the corresponding 2,6-di-O-methylgalactose = 1000.

Position of O-ethyl	Position of O-methyl	Aldononitriles			Alditols		
		Acronym	Programme A	Programme B	Acronym	Programme A	Programme B
3,4,6	2	**15N**		478	**15A**	461	570
2,3,6	4	**16N**	398	514	**16A**	470	583
2,4,6	3	**17N**		520	**17A**		595
2,4	6	**18N**	633	762			
3,6	4	**19N**	651	780			
3,6	2	**20N**	705	823	**20A**	774	864
2.4	3	**21N**		868			
2,6	3	**22N**	776	876	**22A**	800	884
4	2				**23A**	1588	1198
	2,6[a]		1000 (15.45 min)	1000 (28.36 min)		1000 (14.24 min)	1000 (27.74 min)

[a] Absolute retention times of the derivatives of the corresponding 2,6-di-O-methylgalactose are given for comparison.

TABLE VI

MAIN PEAKS IN THE MASS SPECTRA OF THE O-ACETYL-O-ETHYL-D-GALACTONONITRILES ($m/z > 44$)

m/z	Compound																			
	1N	**15N**	**16N**	**17N**	**2N**	**19N**	**3N**	**18N**	**4N**	**21N**	**5N**	**20N**	**22N**	**6N**	**7N**	**8N**	**9N**	**10N**	**11N**	**12N**	**13N**	**14N**
45	15	23	16	23				78		26			48									
57				17																18		
59	57	43	69	83	25	58	33	21		30	64	58	31	74	51	41			100			
71			41	28		27														16		
73	15	19		18								15						17		33	15	
85	51	42	60	70	18	24	19	24	23	26	30	27	19	23	20		15	100		85	100	
87			37	36		29		16					31									
97																					27	
98								20							24						38	26
99				18						20			33					15		16	16	
101	19	23	14	24	18	15	14	14	18	38	100	100	100	100	23	100	10	25	57	29	29	16
102										14		18	25									
103		24	15	24						14				12				20		84	26	
109															16							
113	10	10				16	25	27	43	43	19	16	15	49			27	94		11	82	
114											10							21			17	
115	14	22	54	34		50	12	65	11	18		12	13	77	10		14	32	28	89	52	14
116	17	13	11									12						26		14		
117	16						11	18									14					
126					16		59	61						18	71		85			10		85
127				10									52	19			11	60		69	55	31
128																					29	
129	46	32	100	100	25	100	100	17					15	12	100					28		
131			33	36		23				13	10			32				13	14		16	
140						24	10	14							18		16					17
141											44	31				12						
142				14						11	13							11				
143	100	100		94	100	22	56	100	100	100	66	70	58	51	63	59	100		20	13		100
145	19	17		22	17								29		10		10			85	12	12
152														13					9			
153														21								
154					18		9	12						6			11					
155											6							8		11		
156	6					6			6							20					84	
157								5			5			19					9	21	15	
159	5									9	26	23			7	23						
161											6	7	7			6						
168							58	73						19	79		61		6	9		54
169							5								13		5		10	11	5	11
173			24	26					5	6				48			20	10	21		13	
175			55	33		72		48													5	
186		5								8										8		
187	15	15								6			12					5		100	9	
189	59	75		50	65		29								27							
196														6								
197														34								
198														24	7	6				56	9	
200	2					8			3													
201						2			10	3	10	7				3						
203						5			32	29	3	5	6	9		3	27		2			14
211																			37	10	4	
212						2													11	3		
214				2	6		48	85						17			73					
215														6				13			12	
217																		4		11	10	
228													3		60							26
229																						3
230												2	4									
233			10	12																		
239														15								
240																3						
244											2											
247	10	6								6			10									
256														3								
261									4		7	4				2						
275														3			2	3			3	

TABLE VII

MAIN PEAKS IN THE MASS SPECTRA OF THE O-ACETYL-O-ETHYL-D-GALACTITOLS ($m/z > 44$)

m/z	Compound																	
	1A	**15A**	**16A**	**17A**	**2A**	**3A**	**4A**	**5A**	**20A**	**22A**	**6A**	**7A**	**8A**	**9A**	**23A**	**10A**	**11A**	**12A**	**13A**
57		27	15	20															
59	56	66	42	66	18	21		22	68			44	28				100		
71			18																
73	35	31	21	38			30					21				17			
85	87	91	60	71	19		21	17	41			64				32	27		41
87		44	25	27					34										
89	24	15		24											19				
97																	21	12	
98																	29		
99				17												28			
101	52	36	39	52	16		50	24	34	11	13	32	33	10	19	26	96		17
103	17	21	16	38		12	12			10		16		10		17	24		
113	11	16		14	17	10	75	11	24			90	10		10	34	13		47
114												12				10			
115	16	88	30	53	11				47	13		22	10		10		85		16
116		10		13															
117	26	80		41					100						100				11
127				11						27		59				20	16		30
129	100	67	100	100	13	20	63	18	10	20		57				13			
130	14	11		10															
131	89	29	97	57		100	100	100	33	100	100	17		100	21	100	25	100	17
139																	58	17	
141	11	11						30	59				21						
143	90	100	19	37	100	44	50	30	32		22	100	100	55	56		90	12	100
145	16	22	12	30	10							18					29		
153																	13		
156									5							9	24		
157				13					5			5					64		
159	8	7		5				16	38				12						
170																	7		
171						6					10								
173			31	39								8				6	16		7
175		31	20	32					15										
185									3								9	6	
187	28	26		5											5		6		
189	70	53	24	13	36	29	15	5				58							
198																	25		
199																	12		
201	3	3		2	2	7	10	7	15				5	5	7				
203	2	2		3	18		14						32	14	19		5		36
213											7								
215						4						17		2		11	2	2	17
217				4													6		
231						2					2								
233			30	28															
245																	2		2
247	31	26	2	2	2				2	9					13				
259																	4		
261				2	2	11	8	14	29				10	6					
275												28				20			20
333											2								
347																	3		

Compared with methylation, ethylation improves the separation between 6- and 2,3-di-O-alkyl galactose derivatives, but worsens that between 2,6-, 4,6- and 3,6-di-O-alkyl derivatives, even though the resolution is not impaired.

The ethylated alditol acetates (Table II) show more overlapping peaks (*e.g.*, 2,4,6- and 3,4,6-tri-O-ethylgalactitol and 2,6- and 4,6-di-O-ethylgalactitol) than the aldononitrile derivatives; the same trend was observed for methylated products [3]. The only reversal found in the elution order is for the 6-O-alkyl derivative, which appears earlier than some dialkylated products in methylation (Table IV), but later when comparing ethylated derivatives, *i.e.*, the number of alkyl groups has a greater effect on the elution order in the ethylated derivative series.

As noted previously for methylated derivatives on packed columns [3], ethylated galactononitrile acetates yield better separations than the alditol derivatives. However, each run takes longer as they are less volatile (*cf.*, their retention times relative to xylitol pentaacetate).

Table V reports the retention times of several ethylated mono-O-methylgalactoses separated as their aldononitrile and alditol acetates encountered in the ethylation of the polysaccharides from *Corallina officinalis* [15]. As shown, aldononitrile derivatives allow better separations.

Tables III and IV show the differences in retention times recorded on a newer (less than 1 year old) and an older (*ca.* 3 years old) column. It is noteworthy that as the SP-2330 column ages, the retention times become closer to the standard, *i.e.*, resolution diminishes. However, this effect is not large (Tables III and IV), and even with the 3-year-old column good resolution of peaks is achieved. For Tables I, II and V only the retention times for an aged column (2–3 years old) are shown.

Tables VI and VII show mass spectral data for ethylated galactoses and mono-O-methylgalactoses as aldononitrile acetates and alditol acetates, respectively. The fragmentation patterns are similar to those already predicted for methylated derivatives [2,3] and ethylated alditols [8], *i.e.*, the fission takes place mainly between carbon atoms carrying vicinal alkoxyl groups, and the primary fragments generated eliminate simple molecules to yield secondary fragments. Another fragmentation pathway which arises by loss of neutral fragments from the molecular ion, yielding radical ions, was postulated earlier [3] for aldononitriles and is supported by the ethylation data. Inspection of all the data shows that unambiguous characterization of the products is possible.

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