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Electron impact mass spectra of permethylated disaccharide alditols

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ABSTRACT

The electron impact mass spectra (EIMS) of 19 permethylated hexopyranosyl-hexitols, monodeuterated at C′-1, four partially trideuteriomethylated analogues, and some other permethylated hexopyranosides have been investigated. In the formation of the A_1 ion from the hexopyranosyl-hexitols, only a minor part is formed by direct cleavage between the glycosyl group and the "aglycon". The major part is formed by elimination of neutral molecules from different primary ions resulting from cleavage between adjacent carbon atoms in the alditol residue. As a consequence of this facile elimination, these primary ions are not observed or are very weak. From a critical study of the mass spectra, diagnostic ions, from which the position of linkage to the alditol residue in these and related substances can be assigned, have been identified. From the relative intensities of the A_1, A_2, and A_3 ions, glucopyranosyl groups can be distinguished from manno- and galacto-pyranosyl groups.

INTRODUCTION

In structural studies of polysaccharides, they are often degraded to oligosaccharides by specific or less specific methods[1,2]. GLC–MS is a valuable method for the characterization of these products, preferably as their permethylated alditol-*1-d* derivatives[3,4]. Chemical ionizations[5] or fast atom bombardment[6] MS gives information on the molecular weight and the sequence of sugars of different classes. Information on the different linkages involved is obtained by periodate oxidation, borohydride reduction, and methylation, followed by FABMS of the product[7]. EIMS of permethylated oligosaccharide alditols[3,4], monodeuterated on C′-1, gives information on sequences and also on the position of linkage to the alditol residue, and this method is still extensively used. We have, however, observed that the fragmentation is not as simple as previously assumed, and now report more systematic studies of such disaccharide derivatives.

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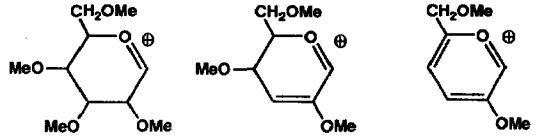
RESULTS AND DISCUSSION

The disaccharides listed in Table I were transformed into their permethylated alditols, monodeuterated at C′-1. A complete list of the ions formed on EIMS of the α-(1 → 2)-, β-(1 → 3)-, α-(1 → 4)-, and α-(1 → 6)-linked D-glucopyranosyl-D-glucitol derivatives is given in Table V in the Experimental section. The naming of the ions follows the nomenclature used by Kochetkov and Chizhov[8], and the intensities are given relative to *m/z* 88, the base ion in all the spectra. The two ions formed by cleavage between two carbon atoms in the alditol chain are designated as, e.g., C′-1–C′-3 and M – (C′-1–C′-3), respectively. The ion formed from aJ_1 by loss of 60 mu, which is sometimes called bA_1 or aJ_2, is called J_2.

The ions of the A series.—On EIMS of a fully methylated methyl hexopyranoside, the A_1 ion is formed by fission between the glycosyl group and the aglycon, and consecutive eliminations of methanol give the A_2 and A_3 ions. Of the three different A_2 ions[8], A_2^2 constitutes ~ 75%, and almost all A_3 is formed from A_2^2. The intensities of the ions in the A series for some permethylated hexopyranosides, relative to the base ion, *m/z* 88, are given in Table II. As seen from the Table, there are drastic differences between the substances, indicating that the major part of A_1 is not a primary ion but is formed by a different route, as will be discussed in more detail below.



A_1 A_2^2 A_3

The relative intensities of the A_1, A_2, and A_3 ions depend upon the configuration of the glycopyranosyl group[8]. For the ten glucopyranosyl-hexitol derivatives listed in Table I, the proportions were on an average 1 : 7(5–9) : 1(1–2); for the six galactopyranosides, 1 : 3(2.5–4.3) : 0.5(0.5–1); and for the three mannopyranosides, 1 : 2(1.4–2.3) : 0.3(0.2–0.5), respectively. The numbers in brackets give the extreme values. From these results, it should be possible to distinguish between a glucopy-

TABLE I

Disaccharides investigated

Linkage	1 → 2	1 → 3	1 → 4	1 → 6
Sugars	α-Glc-Glc	α-Glc-Glc	α-Glc-Glc	α-Glc-Glc
	α-Glc-Gal	β-Glc-Glc	β-Glc-Glc	β-Glc-Glc
	α-Man-Man	α-Glc-Man	β-Gal-Glc	α-Glc-Man
			β-Gal-Man	α-Gal-Glc
			β-Man-Man	β-Gal-Gal
				α-Gal-Man
				β-Gal-Man
				β-Man-Gal

TABLE II

The A series of ions on EIMS of some permethylated hexopyranosides

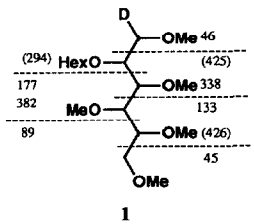
Glycosyl group	Aglycon	Linkage	Relative intensity (%)		
			A_1	A_2	A_3
α-Glc	Methanol	1→1	– [a]	2.6	–
β-Gal	Glycerol	1→2	–	2.7	–
β-Gal	D-Erythritol	1→2	–	3.5	1.3
β-Gal	D-Xylitol	1→4	3.0	13	3.5
α-Glc	D-Glucitol	1→2	9.2	59	12
β-Glc	D-Glucitol	1→3	15	68	17
α-Glc	D-Glucitol	1→4	12	75	13
α-Glc	D-Glucitol	1→6	7.3	57	10

[a] –, Less than 1%.

ranosyl group and a galactopyranosyl or mannopyranosyl group. The differences are even more pronounced between fully methylated glycuronides of the *gluco* and *galacto* configurations[9].

Fragmentation of permethylated hexopyranosyl-hexitols.—The possible ions formed on cleavage between two adjacent carbon atoms in the alditol chain of a (1 → 2)-linked derivative (**1**) are indicated in the formula. Of these ions, however, those in brackets, *m/z* 426, 425, and 294, are not observed or are very weak. It seems probable that the disappearance of these ions and the presence of a strong A_1 ion are interrelated. For the ions formed by cleavage on either side of the glycosyloxylated carbon, e.g., **2**, the facile elimination of an aldehyde should give A_1. For the ion of *m/z* 426 (**3**), the elimination of a five-membered cyclic fragment (a pentofuranoside) should also give A_1. Similar eliminations of 4- and 3-membered cyclic molecules from *m/z* 382 and 338 should be less favourable. The proposed formation of the A_1 ion from the three former ions was supported by studies of metastable transitions, using both DADI[10] and defocusing techniques.

The (1 → 3)- and (1 → 4)-linked derivatives (**4** and **5**) should give identical ions were it not for the deuterium labelling. According to the principles discussed above, all ions except those formed on cleavage at either side of the glycosyloxylated carbon should be present, which was also observed.



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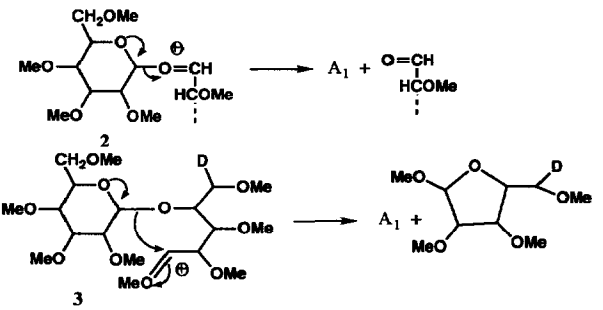

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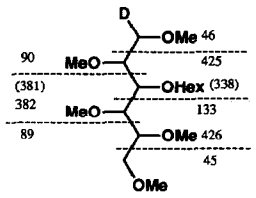

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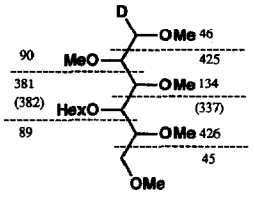

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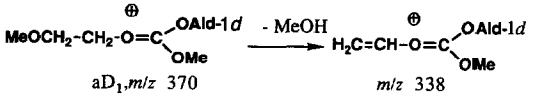


aD_1, *m/z* 370 *m/z* 338

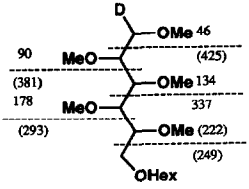
A weak ion of *m/z* 338, present in the spectra of all the (1 → 4)-linked derivatives, is, however, not accounted for by this type of fragmentation, but has to be formed by a different route. In order to investigate this problem, some disaccharide derivatives with trideuteriomethyl groups in defined positions were investigated for the presence of aJ_1 and this fragment or a deuterated analogue (Table III). As seen from Table III, trideuteriomethyl groups in the alditol residue are incorporated in these ions, independent of their positions. It is, however, only the trideuteriomethyl group in the 3-position of the glycosyl group that is incorporated. A plausible explanation of these results is that the *m/z* 338 ion is formed by elimination of methanol from a tautomer of the aD_1 ion. In aD_1, which is the precursor of aJ_1, the methoxyl originally on C-3 has migrated to C-1[8].

TABLE III

The formation of ions aJ_1 and *m/z* 338 or a deuterated analogue on EIMS of some fully methylated D-glucopyranosyl-D-glucitols with trideuteriomethyl groups in defined positions [a]

Linkage	OCD_3 in positions	aJ_1	*m/z* 338 or analogue
β-(1 → 2)		296 (2.7)	338 (1.2)
β-(1 → 3)		296 (23)	338 (2.3)
β-(1 → 3)	2, 2′	299 (5)	341 (2.0)
β-(1 → 3)	3, 1′, 5′	305 (19)	347 (2.4)
β-(1 → 4)		296 (7)	338 (0.2)
β-(1 → 4)	4, 1′, 5′	302 (5)	344 (0.1)
β-(1 → 6)		296 (13)	338 (0.4)
β-(1 → 6)	6, 1′, 5′	302 (12)	344 and 347 [b]

[a] Relative percentage in brackets. [b] Traces only.



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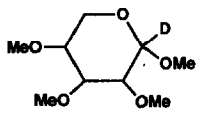

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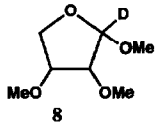

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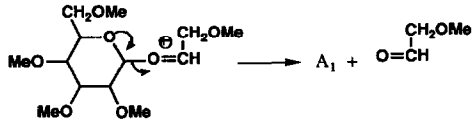

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On cleavage between adjacent carbon atoms in the alditol chain, the (1 → 6)-linked derivative (**6**) could, in principle, give the ions indicated in the formula. Of these, *m/z* 425 and 381, which are not observed, should readily eliminate a pyranoside (**7**) and a furanoside (**8**) derivative, respectively, and give the A_1 ion.

TABLE IV

Diagnostic fragments, formed by cleavage between adjacent carbon atoms in the alditol part of a permethylated hexopyranosyl-hexitol-*1-d*

m/z	Relative intensity (%)			
	1 → 2	1 → 3	1 → 4	1 → 6
133	6–8	10	1–2	1–4
134	4–5	1	6–8	7–9
177	5–7			1
178	1			5–7
337				1–2
338	1–2	0.1–0.3	0.1–0.3	0.3–0.4
381	0.1–0.2	0.1–0.2	0.6–2	
382	2–4	2–3	0.3–0.5	
425		0.1–0.5	0.1–0.5	
426		0.1–0.5	0.1–0.5	

Also in agreement with results discussed above, m/z 249 should give the A_1 ion by elimination of formaldehyde. The absence of m/z 293 may be explained by elimination of methoxyacetaldehyde from its tautomeric form (**9**). If this explanation is correct, this ion should be much more labile than other ions formed by cleavage between two carbon atoms adjacent to the glycosyloxylated carbon, e.g., m/z 338 from the (1 → 2)-linked derivative, which could eliminate a ketone by a similar mechanism.

That the ion m/z 222 is not observed has some analogies. In the EI-mass spectra of mono-*O*-acetylpenta-*O*-methylhexitols, the expected four-carbon ions are generally strong but the corresponding five-carbon ions are very weak or absent. The primary ions formed on EIMS of permethylated glucitol are m/z 45 (100%), 89 (55%), 133 (12%), 177 (11%), and 221 (1.7%), and also here the five-carbon fragment is weak.

Diagnostic fragments.—In Table IV, the diagnostic fragments, formed by cleavage between two adjacent carbon atoms in the alditol residue of permethylated hexopyranosyl-hexitols, monodeuterated at C′-1, are summarized. As discussed above, a weak ion of m/z 338 may also be formed by a different mechanism. The ions of m/z 134 and 133 may also be formed by other mechanisms, and are therefore of limited diagnostic value. The ion of m/z 89 is strong in the spectra of all isomers and has no diagnostic value. For an unambiguous identification, it is therefore necessary that all diagnostic fragments, in the expected relative proportions, are observed. The principles established for the hexopyranosyl-hexitol derivatives can also be applied for disaccharide alditols containing other sugar components and for higher oligosaccharide alditols.

EXPERIMENTAL

General methods.—Mass spectra were recorded with a Varian 311A instrument, equipped with Spectrosystem 100, at an ion-source temperature of 150°C, an ionization current of 3 mA, and an electron energy of 70 eV, using either direct inlet or inlet via GLC on an SE-30 glass capillary column. The instrument was focused for a maximum intensity at the ion m/z 207. High resolution MS was carried out at a resolution of 15000. Metastable ions were studied either by the DADI[10] technique or by the defocusing method.

Preparation of permethylated hexopyranosyl-hexitols.—The different disaccharides (Table I) were reduced with $NaBD_4$ and permethylated. The mass spectra of four D-glucopyranosyl-D-glucitol derivatives are given in Table V.

Fully methylated laminaran, amylose, or dextran was hydrolyzed in 90% formic acid at 80°C for 1 h, and the product reduced with $NaBD_4$ and permethylated, using CD_3I, to give a mixture containing the partially trideuteriomethylated disaccharide derivative. The mass spectra of the three desired products were determined using GLC–MS.

Laminaran (200 mg) in a mixture of Me_2SO (20 mL), propanal (5 mL), and

TABLE V

Ions formed on EIMS of four linkage isomers of permethylated D-glucopyranosyl-D-glucitol-*1-d*

m/z	Relative intensity (%)				Ion designation [a]
	α-(1→2)	β-(1→3)	α-(1→4)	α-(1→6)	
439		0.1		0.1	aE_1
426		0.8	0.4		M−C′-6
425	0.1	0.5	0.5	0.1	M−C′-1
412		0.4			
411			0.2	0.1	
394	0.1	0.2		0.1	
393		0.1		0.1	
382	3.1	3.0	0.6	0.1	M−(C′-5–C′-6)
381	0.2	0.2	0.6	0.1	M−(C′-1–C′-2)
362	0.1	0.1	0.1	0.1	
361	0.2	0.2		0.3	
350	1.7	3.0	0.2	0.1	M−(C′-5–C′-6)−32
349	0.1	0.2	0.3	0.2	M−(C′-1–C′-2)−32
338	1.2	2.3	0.2	0.4	M−(C′-4–C′-6)
337	0.1	0.1	0.1	1.9	M−(C′-1–C′-3)
321		0.1	0.4	0.1	
320		0.2	0.1	0.1	
309		0.1	0.2	0.1	
307	1.0	0.4	0.4	0.1	
306	1.3	1.0	0.2	0.6	M−(C′-4–C′-6)−32
305	0.4	0.2	0.7	1.9	M−(C′-1–C′-3)−32
296	2.7	23	7.0	13	aJ_1
275	1.8		1.4		
251	1.1	1.3	1.1	1.0	
250		1.9	1.5	2.7	
249		1.2			
237	7.1	5.5	4.7	3.8	
236	59	53	36	30	J_2
219	9.2	15	12	7.3	aA_1
204		3.0	1.8	3.6	J_2-32
190		4.6	1.3	4.1	
188		7.9	7.8	5.5	
187	59	68	75	57	aA_2
178			1.0	12	C′-1–C′-4
177	4.8		1.0	1.7	C′-3–C′-6
175				3.8	
172	10	28	10	12	$J_2-(2\times32)$
171	1.5	2.5	1.1	1.5	
159	1.3	4.4	2.2	1.6	aC_2
158	2.3	9.2	4.0	4.5	
157	2.0	4.5	3.7	3.6	
156	1.4	1.9	2.1	2.3	
155	12	17	13	10	aA_3
147	2.5	1.7	1.0	8.6	
146	19	5.4	1.4	30	(C′-1–C′-4)−32
145	20	8.4	7.3	7.4	(C′-3–C′-6)−32
143	4.1	6.7	4.8	4.9	
140	3.3	5.1	2.1	2.0	
134	3.5	1.4	7.5	9.6	C′-1–C′-3

TABLE V (continued)

m/z	Relative intensity (%)				Ion designation [a]
	α-(1 → 2)	β-(1 → 3)	α-(1 → 4)	α-(1 → 6)	
133	6.6	9.8	1.0	4.3	C′-4–C′-6
131	4.2	5.0	2.9	5.5	aB_2
129	3.0	3.7	3.1	2.7	
128	2.4	4.9	2.7	2.6	
127	9.6	18	12	13	aC_3
126		1.7	1.4	1.1	
125	1.8	2.8	1.5	1.9	
117	1.8	4.8	2.5	1.9	
116	12	41	10	10	
115	9.0	15	16	11	
114	2.8	3.1	2.2	2.9	
113	3.3	4.5	2.9	2.6	
112	4.3	5.6	3.6	3.4	
111	51	67	56	45	aE_4
104	2.2		2.3	3.9	
103	6.0	6.3	6.7	7.8	
102	15	16	18	21	aK_1
101	88	82	81	94	aF_1
100	1.7	1.9	1.8	1.5	
99	7.6	11	7.4	9.0	
98	1.7	2.6	1.5	1.5	
95	4.1	6.9	3.6		
90	6.1	28	8.8	28	
89	44	42	32	23	
88	100	100	100	100	aH_1
87	2.0	4.1	1.9	3.4	
86	3.1	4.8	2.5	2.5	
85	4.3	8.8	5.0	4.6	
76	3.0	5.6	3.3	3.6	
75	42	68	53	50	
74	4.6	5.4	3.9	4.8	
73	13	16	8.7	12	aH_2
72	21	13	11	16	
71	35	51	36	41	aK_2
69		4.3		2.3	
68	1.4	1.6	1.0	1.1	
67		2.0			
60	3.9	24	8.5	13	
59	21	24	20	11	
58	2.0	2.0	2.6	3.9	
55	1.1	6.0			
53	1.6	2.8	1.5	1.7	
47		3.5	1.0	2.3	
46	12	14	21	21	C′-1
45	79	74	63	58	C-6 and C′-6

[a] Several ions have in part, or for some weak ions totally, origins other than those indicated.

$BF_3 \cdot Et_2O$ (0.8 mL) was stirred at 50°C for 16 h. The acetalized polysaccharide was recovered by dialysis, the product was methylated using CD_3I, then hydrolyzed as above, and the product was methylated using CH_3I. The mixture containing the

permethylated glucopyranosyl-glucitol derivative, with OCD_3 groups in the C-2 and C′-2 positions, was investigated by GLC–MS. Pertinent ions in the mass spectra of the four partially trideuteriomethylated derivatives are given in Table III.

ACKNOWLEDGMENTS

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