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Note

Structural analysis by mass spectrometry of oligosaccharides composed of 6-deoxyhexopyranoses

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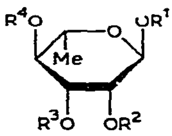
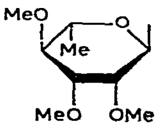
Information about the structure of complex carbohydrate-containing molecules can be obtained when the substance is partially hydrolysed and the resulting oligosaccharides are identified. Mass spectrometry can distinguish between several classes of permethylated oligosaccharides, interpretation of the spectra usually being based on features of the fragmentation of oligosaccharides whose structures have been established by independent methods[1–9]. The mass-spectral behaviour of oligosaccharides containing 6-deoxyhexopyranoses, which has not been studied thus far, is important because 6-deoxyhexopyranoses are repeating-units of some bacterial polysaccharides[10], and components of many plant glycosides and polysaccharides having immunological properties[11,12].

TABLE I

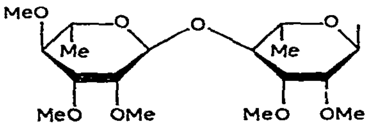
COMPOUNDS INVESTIGATED



1–7



A



B

No.	*R*¹	*R*²	*R*³	*R*⁴	*Symbol*
1	A	Me	Me	Me	*a* → *b*
2	Me	A	Me	Me	*a* → *2b*
3	Me	Me	A	Me	*a* → *3b*
4	Me	Me	Me	A	*a* → *4b*
5	Me	B	Me	Me	*a* → *4b* → *2c*
6	Me	Me	B	Me	*a* → *4b* → *3c*
7	Me	Me	Me	B	*a* → *4b* → *4c*

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TABLE II

MASS SPECTRA (12 eV) OF PERMETHYLATED DI- AND TRI-SACCHARIDES (1–7) OF L-RHAMNOSE

m/z	% Σ_{45} × *100*						
	1	**2**	**3**	**4**	**5**	**6**	**7**
538							8
537					9		12
436					35	25	32
423					121	99	570
377					26		
363		15			125	262	719
362	44						
349		25					
345					13		
331		35			30	43	69
321	11						
319					186	80	452
299					22		
291	82						
287					43		
277	110						
275		146		67	26	22	32
271					22		
262		156	105	128	30	31	74
259		30		28	35	34	86
249	33	1692	784	727	285	176	1468
245	88						
233	121						
219	60					25	
205			79				37
203		40	37	39	87	52	41
201	82						
189	1324	1237	680	783	1903	1510	1396
188	77		42				65
176	38						
175					17	49	
174	49		37				
173	27						
170	33						
161	99						
157	264	156	196	262	221	400	328
145	198	101	1473	615	125	1540	203
143	27						
131	71	45	52	61			
129	115	136	307	157	134	188	57
128	242						
125	88	30	42	78	99	92	154
124					26	15	143
118		30			30		
117	137	25	58	73	22	37	32
115	60				22	18	28
113	82	45	90	78			
101	5104	480	503	615	411	339	148
99	386	141	259	145	151	277	45
97	49	40	84	61			
88	144	4345	4001	4751	4866	3820	3348
85	44	60	28	78	39	37	20
75	375	404	344	447	541	585	349
72	121	505	582	559	234	169	53
59	171	70	137	162	65	74	20

We now report on the mass spectra of some synthetic, fully methylated L-rhamno-oligosaccharides (Table I), namely, α-L-rhamnopyranosyl α-L-rhamnopyranoside (**1**), positionally isomeric *O*-α-L-rhamnopyranosyl-α-L-rhamnopyranoses (**2–4**), and a series of *O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-α-L-rhamnopyranoses (**5–7**). Based on the information obtained, diagnostically important conclusions have been arrived at, according to which the structures of positionally isomeric oligosaccharides, composed of 6-deoxyhexopyranose residues, can be determined.

The advantage, in structural analysis, of 12-eV electron-impact mass spectra of methylated oligosaccharides has been discussed[9]. Low-energy mass spectra of **1–7** are summarised in Table II. The letters A–K are used to denote fragment ions formed through processes analogous to the fragmentation of methylated 6-deoxyhexopyranoses[13,14]. The individual monosaccharide residues in the oligomers are denoted by *a*, *b*, and *c* (Table I). When there is the possibility of formation of isomeric ion-species, only that presumed to be formed with the highest probability is noted.

The spectra of the fully methylated, positionally isomeric L-rhamnose disaccharides, including the non-reducing, trehalose-type substance, show characteristic differences (Table II). A characteristic feature of the fragmentation of the (1→1)-linked compound **1** is the formation of ions of the A-series (*m/z* 189, 157, 125) and the F-series (*m/z* 101). The fission observed for the first time in the fragmentation of permethylated α-D-xylopyranosyl β-D-xylopyranoside[9], and reflected by lines at *m/z* 263, 235, 219, and 187, occurs also with **1** to produce ions of *m/z* 277, 245, 233, and 201, having the elemental composition of $C_{13}H_{25}O_6$, $C_{12}H_{21}O_5$, $C_{11}H_{21}O_5$, and $C_{10}H_{17}O_4$, respectively. This confirms the previous conclusion that the formation of ions of this type is characteristic of the fragmentation of fully methylated (1→1)-linked disaccharides.

In the fragmentation of (1→2)-, (1→3)- and (1→4)-linked dimers, as in that of fully methylated 6-deoxyhexopyranoses[13,15], intense ions of the H-series (*m/z* 88) are formed, while the F and J ions (*m/z* 101 and 75) are produced to a lesser extent.

TABLE III

CRITERIA FOR LINKAGE-ANALYSIS IN POSITIONALLY ISOMERIC, FULLY METHYLATED DISACCHARIDES OF L-RHAMNOSE[a]

m/z	% Σ_{45}			
	a → b	a → 2b	a → 3b	a → 4b
275	0	...	0	..
145	...	...	xx	x
101/88	.35	0.1	0.1	0.1
249/145		15	15	1

[a]Peak intensities: ., 0.5%; .., 0.5–1.0%; ..., 1–5%; x, 5–10%; xx, 10–20%; xxx, 20%.

The ions produced in the fragmentation of the permethylated, (1→2)- and (1→4)-linked disaccharides **2** and **4** are formed in the same manner (symbol, *m/z*): *ab*F_1, 275; *ab*H_1, 262; *ab*J_1 249; *a*A_1 189; *a*A_2, 157; *a*C_2, 129; *a*A_3, 125; *a*F_1, 101; $[C_5H_7O_2]^+$, 99; *a*H_1, 88; *b*J_1, 75; *a*K_1, 72. The two types of linkages can be distinguished according to the intensity ratio of the peaks at *m/z* 249 and 145 (Table III). In the fragmentation of the (1→2)-linked substance **2**, these ions are produced in a ratio of ~15 (for 70-eV spectra, this ratio is ~10), whereas the ratio of these peaks in the spectra of the (1→4)-linked disaccharide **4** is ~1, regardless of the electron energy applied.

Characteristic of the fragmentation of the (1→3)-linked disaccharide **3** is the formation of intense ions at *m/z* 145, having the elemental composition $C_7H_{13}O_3$. The previously discussed ions, except for the species *ba*F_1 appearing at *m/z* 275, are also formed.

The trisaccharides **5**–**7** contain the *O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl group (Table I) linked to various positions (*b*→2*c*, *b*→3*c*, *b*→4*c*) of the "reducing" α-L-rhamnopyranose residue. In the higher-mass region of the spectra, the following ions, containing carbon atoms of all three rings, were identified (symbol, *m/z*): *cab*A_1, 537; *abc*H_1, 436; and *abc*J_1, 423. Fragments containing carbon atoms from only two rings are as follows: *ba*A_1, 363; *ba*A_2, 331; *ba*B_2, 319; *bc*F_1, 275; *bc*H_1, 262; and *bc*J_1, 249. In the lower-mass region of the spectra, peaks representing ions containing carbon atoms from only one ring are present: *a*A_1, 189; *a*A_2, 157; $[C_7H_{13}O_3]^+$, 145; *a*C_2, 129; *a*A_3, 125; *a*F_1, 101; $[C_5H_7O_2]^+$, 99; *a*H_1, 88; *c*J_1, 75; and K_1, 72. The type of linkage on ring *b* can be deduced from the abundance of ions of *m/z* 249 (*bc*J_1) and 145 ($[C_7H_{13}O_3]^+$), and from their intensity ratio (Table IV). Diagnostic of the (1→3) linkage in this type of trimer is a high intensity of ions of *m/z* 145. A high intensity of ions of *m/z* 249, together with the characteristic ratio of intensities (~7) of the peaks at *m/z* 249 and 145, is diagnostic of a (1→4) linkage. The intensity ratio of the peaks at *m/z* 249 and 145 in a 70-eV spectrum is ~1 for (1→2)-, and ~6 for (1→4)-linked compounds.

TABLE IV

CRITERIA FOR LINKAGE-ANALYSIS IN POSITIONALLY ISOMERIC, FULLY METHYLATED *O*-α-L-RHAMNOPYRANOSYL-(1→4)-*O*-α-L-RHAMNOPYRANOSYL-*O*-α-L-RHAMNOPYRANOSIDES[a]

m/z	% Σ_{45}		
	a → *4*b → *2*c	a → *4*b → *3*c	a → *4*b → *4*c
249	...	...	xx
145	...	xx	...
249/145	2	0.1	7

[a]Peak intensities, see footnote to Table III.

EXPERIMENTAL

Compounds 1–7 were obtained by methylation (with methyl iodide and sodium hydride in *N,N*-dimethylformamide) of the corresponding methyl α-L-glycosides of compounds[16–19] containing free hydroxyl groups.

Mass spectra (70 and 12 eV) were obtained at an emission of 300 μA with a JMS D 100 instrument by the direct sample-introduction technique. The temperature at the site of evaporation was, depending on the volatility of the substances, 70–160°, and that of the ionising chamber was 180°. The peak intensities (Tables II–IV) are expressed as percentages of the total ionisation $\%\sum_{45}$. Exact mass measurements were performed at a resolution of 10,000 with perfluorokerosene as the reference. The elemental compositions obtained are given in the text.

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