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STRUCTURAL ANALYSIS BY MASS SPECTROMETRY OF OLIGOSACCHARIDES RELATED TO XYLANS

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ABSTRACT

Fully methylated, xylan-type oligosaccharides, namely, positionally and interglycosidically isomeric *O*-D-xylopyranosyl-β-D-xylopyranoses (xylobioses, **2–7**), a complete series of *O*-β-D-xylopyranosyl(1→4)-*O*-β-D-xylopyranosyl-β-D-xylopyranoses (xylotrioses, **8**, **9**, **11**, **12**, and **14**), a branched β-D-xylotetraose (**15**), and α-D-xylopyranosyl β-D-xylopyranoside (**1**), have been studied by 70- and 12-eV mass spectrometry. By using high-resolution techniques metastable-transition measurements, ion species formed through hitherto unknown fragmentation-processes have been found for the (1→1)- and (1→3)-linked disaccharides **1**, **4**, and **5**. Based on the qualitative and quantitative differences in the fragmentations, criteria for unambiguous determination of the positional mode of linkage in xylobioses are proposed. Similarly, by studying the fragmentation of xylotriose (**10**) labelled in the side-chain with trideuteriomethyl groups, the fragmentation-pathways for xylotrioses have been clarified. A new fragmentation series has been discovered in the fission of trioses **13** and **14** having a D-xylopyranosyl group attached to O-3 of the nonreducing end of the basic (1→4)-linked skeleton. Within the series of di- and tri-saccharides studied, the criteria proposed permit reliable determination of any of the theoretically possible branching points. The basic possibilities for structural analysis by mass spectrometry of related pentose tetraoses are also shown.

INTRODUCTION

Sequential analysis of polysaccharides often involves identification of products of partial hydrolysis or methanolysis. From the structures of oligosaccharide segments, important information about the constitution of the parent polysaccharide may then be deduced. Mass spectrometry, alone or in combination with gas chromatography, is a powerful tool for structure elucidation of permethylated oligosaccharides, interpretation of the spectra being usually based on features of the fragmentation of oligosaccharides whose structures have been determined by independent methods[1–7].

Although xylans are components of industrially important woods and other plants[8,9], permethylated oligosaccharides related to them have been studied only incidentally, following electron impact (e.i.)[3,5]. The present work describes results

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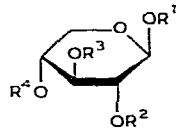
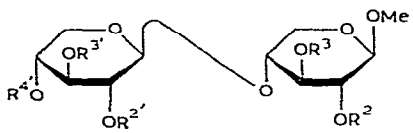
TABLE I

COMPOUNDS INVESTIGATED

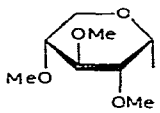


1–7

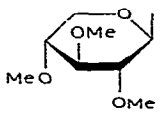


8 – 15

R :



A



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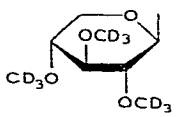


C

No	R^1	R^2	R^3	R^4	$R^{2'}$	$R^{3'}$	$R^{4'}$	*Symbol*
1	A	Me	Me	Me				*a*→*b*
2	Me	A	Me	Me				*a*→2*b*
3	Me	B	Me	Me				
4	Me	Me	A	Me				*a*→3*b*
5	Me	Me	B	Me				
6	Me	Me	Me	A				*a*→4*b*
7	Me	Me	Me	B				
8	Me	Me	Me		Me	Me	B	*a*→4*b*→4*c*
9	Me	B	Me		Me	Me	Me	*a*→4*b* 2 ↑ *d*
10	Me	C	Me		Me	Me	Me	
11	Me	Me	B		Me	Me	Me	*a*→4*b* 3 ↑ *d*
12	Me	Me	Me		B	Me	Me	*a*→4*b* 2 ↑ *d*
13	Me	Me	Me		Me	A	Me	*a*→4*b* 3 ↑ *d*
14	Me	Me	Me		Me	B	Me	
15	Me	Me	Me		Me	B	B	*a*→4*b*→4*c* 3 ↑ *d*

of a systematic study by e.i. mass spectrometry of a series of synthetic, fully methylated D-xylobioses (**1–7**), D-xylotrioses (**8–14**), and a D-xylotetraose (Table I).

The aim of the work was to complete the knowledge available thus far[1-7] on the e.i. mass-spectral fragmentation of scarce, methylated oligosaccharides, particularly branched ones. The theoretical information obtained was employed to find differences significant for linkage analysis, especially the establishment of branch points in oligosaccharides.

RESULTS AND DISCUSSION

Decreasing the electron energy from the conventional 70 to 12 eV resulted in less-pronounced formation of ions formed by deep decomposition and, thus, in simplification of the spectra. The low-energy spectra of the fully methylated xylobioses **1–7** are given in Table II. Table III shows data extracted from the spectra of fully methylated xylotrioses **8**, **9**, and **11–14**, and of the D-xylotetraose **15**.

Capital letters A–K are used to denote the fragment ions formed through processes analogous to the fragmentation of fully methylated monosaccharides[10] and, for the disaccharides, the individual monosaccharide residues are noted by lowercase letters *a* and *b* (Table I). For the rings of the higher oligosaccharides **8–15**, the symbols *a*, *b*, and *c* denote the (1→4)-linked residues and the symbol *d* is reserved for the "branching" moiety (Table I). New fragmentation-series are characterized by the elemental composition of the ions. When there is a possibility for the formation of more, isomeric, ion-species, only one ion species, that formed presumably with the highest probability, is noted.

Fragmentation of fully methylated D-*xylobioses*. — Mass spectrometry of compounds **1–7**, exhibiting different types (1→1, 1→2, 1→3, and 1→4) and stereochemistry of the interglycosidic linkage, showed that the positional isomers give characteristically different spectra. For the (1→1)-linked compound (**1**), a pronounced cleavage according to Series A (*m/z* 175, 143, 111) and F (*m/z* 101) was observed, whereas in the Series H (*m/z* 88) and J (*m/z* 75), this cleavage was much less pronounced. The elimination of methanol from the molecules–ions gives rise to $[M - MeOH]^{+\cdot}$ and $[M - 2MeOH]^{+\cdot}$ fragments appearing in the spectra at *m/z* 334 and 302, respectively. Previously not recognized, characteristic ions at *m/z* 263, 231, 219, and 187 have elemental compositions $C_{12}H_{23}O_6$, $C_{11}H_{19}O_5$, $C_{10}H_{19}O_5$, and $C_9H_{15}O_4$, respectively. Precursors to ions of *m/z* 263 and 219 and, thus, a plausible means for their formation, could not be found by metastable-transition measurements, but a relation between the ions of *m/z* 263 and 231, involving elimination of methanol from the precursors, was established. Also clarified was the mode of disintegration of ions at *m/z* 219 (Scheme 1). The formulation of ions having *m/z* 219 is based, *inter alia*, on the spectra[11] of (1→1)-linked methyl 4-*O*-methyl-2,3-di-*O*-trideuteriomethyl-α,β-D-glucopyranuronates showing, that these ions contain C-2–C-5 substituents of one of the rings, together with a C-2 substituent of the other. Of considerable importance from the viewpoint of analytical application is the forma-

TABLE II

MASS SPECTRA (12 eV) OF PERMETHYLATED XYLOBIOSES **1–7**

m/z	% $\Sigma_{45} \times 100$						
	1	**2**	**3**	**4**	**5**	**6**	**7**
336		9	5	3	3	5	2
335			3				
334	11				5		2
303		46	48	6	5	1	2
302	11						
275	14			55	7	4	8
274					11	5	4
263	37						
261		225	147			165	120
249				28	37	7	
235		1076	1200	1754	2125	306	600
231	108						
229		261	178			212	212
219	147						
212					11		
205	45					6	
197		20	10			11	12
187	204						
175	1276	2141	2376	677	781	896	1200
174	198	148	113	92	104	10	30
173				15	66		
161		13	16	18	7	7	30
159	153	9	12	28	18	20	38
158		12					
157	40	13	12	21	37	11	10
145	62	10	41	148	138	236	200
143	1418	1332	1080	554	519	1320	1180
131	74	164	171	49	40	47	32
129	17					24	
115	453	384	411	1385	1438	472	400
114	340	205	171	200	297	143	200
111	164	118	96	108	60	132	108
103	567						
101	2779	1229	1406	1585	1631	1414	1870
99	822	384	343	215	129	377	200
89	91						
88	96	1332	1382	1323	1043	2122	1688
87	57	41		40	15	165	184
85	62	51	48	86	54	118	64
84		20	21	25	35	80	92
83	51	33	27	43	15	104	50
75	425	410	421	1139	1102	802	960
71	79	92	72	234	163	424	360
59	45	31	24	28	20	123	38
58	51	92	86	68	49	56	36
45	96	97	75	71	25	183	66

TABLE III

MASS SPECTRA (12 eV) OF FULLY METHYLATED XYLOTRIOSES **8–9**, **11–14**, AND XYLOTETRAOSE **15**

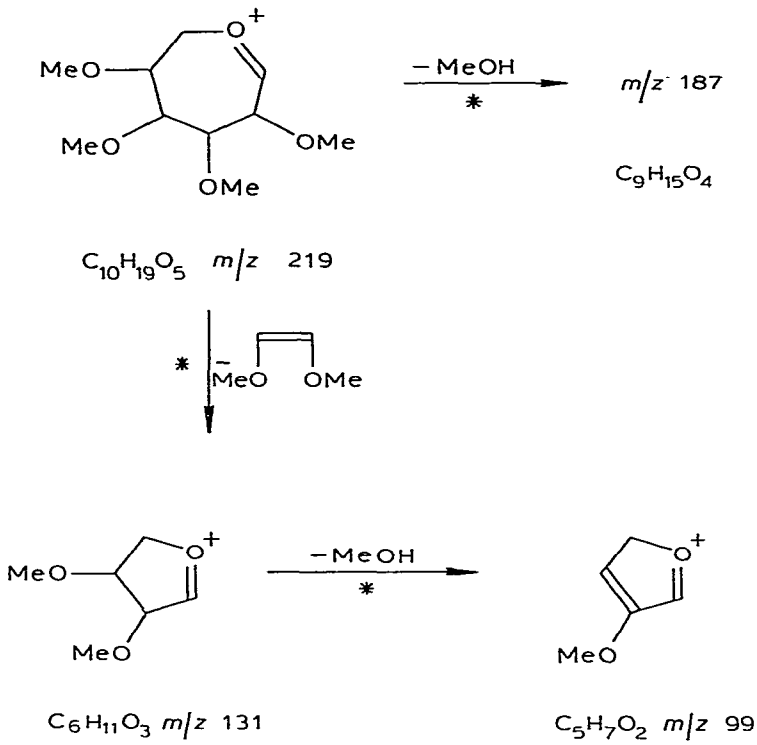
m/z	% $\Sigma_{45} \times 100$						
	8	**9**	**11**	**12**	**13**	**14**	**15**
623							13
595							5
555							142
496		1	1	1			
495		1	3				67
463		4	6	6	27	34	17
435		1	12				
431							18
421	7	33		7	103	92	
405							19
395	136	176	667	326	823	733	
357		8					77
335	51	128	182	274	229	171	26
333							13
321							47
317		38					
304					189	324	
303	94	86	126	613	175	263	196
289							196
285		8					
281					11	165	
275		4			38	43	
274	14						
273					59	85	67
272					113	165	
271	32	13	15	151	30	24	71
261	180		45	104	10	15	105
257							44
249	54		13				
247		33					
245		19	108				
243					103	110	
241							37
235	504		28	274	297	342	115
229	266		66	179	132	128	132
221			10				
219	6	13	13				
215	12	57					
211			20		19	20	
205	12	67					
197				11			12
191		43			30	37	
189	45	100	98	85	60	55	88
183		76					
175	1671	2046	2798	2454	1484	1435	2497
174	21	209	178	179	121	147	236
161	33	10	35	12	22	49	30

TABLE III *(continued)*

m/z	% Σ_{45} × 100						
	8	9	11	12	13	14	15
159	45	33	42	13	35	30	47
157	15	15	17	20	30	43	
145	237	286	168	292	191	183	108
143	1700	1903	1049	1180	917	733	1653
142	27						
131	39	48		66	38	30	27
129	57	43	420	26	29	24	371
115	473	333	280	330	1309	1161	277
114	166	176	245	198	377	428	273
111	281	176	203	151	648	367	641
103	89				30	37	
101	916	1404	888	613	985	1100	641
99	266	381	203	330	76	49	162
88	1168	1237	1294	991	432	490	742
87	129			75	30	31	30
85	51	43	28	38	21	24	30
84	123	48	301	75	59	67	250
83	36			19			
75	591	428	336	472	540	611	337
71	355	91	31	274	121	134	88
69	21	41	17				11
59	24	12	14	28	19	18	7
58	9	18	24	38	16	12	16
45	45	66	14	94	21	24	18

tion of ions at *m/z* 219 and 187 ($C_{10}H_{19}O_5$ and $C_9H_{15}O_4$, respectively), which may lead to a misleading conclusion that the sample under investigation might be contaminated with a hexose-containing impurity[10]. However, the commercial D-xylose used as the starting material for making **1** was chromatographically pure, and the peak intensities at *m/z* 219 and 187 in the spectrum of **1** did not change after one, two, and three recrystallizations. Although the origin of ions at *m/z* 103 ($C_4H_7O_3$) could not be determined, they constitute an additional characteristic feature of the fission of fully methylated, nonreducing, (1→1)-linked xylobiose.

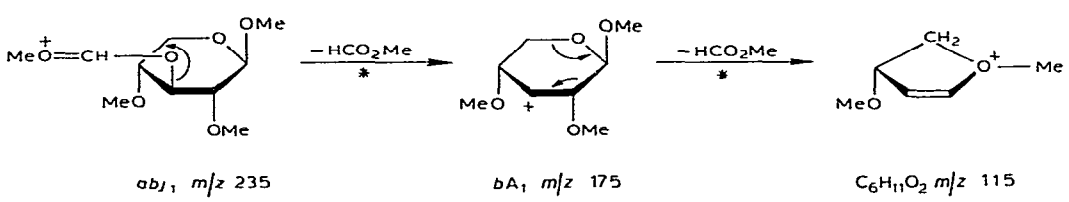
The ions produced in the fragmentation of (1→2)- and (1→4)-linked per-*O*-methyl-D-xylobioses are formed in the same manner (symbol, *m/z*): *ba*B_1, 336; *ba*A_2, 303; *ba*C_2, 275; *ba*F_1, 261; *ab*J_1, 235; [*ba*F_1 — MeOH]$^{+\cdot}$, 229; [*ba*F_1 — 2MeOH]$^{+\cdot}$, 197; *a*A_1, 175; *a*A_2, 143; *a*C_2, 115; *a*A_3, 111; *a*F_1, 101; *a*H_1, 88 and *b*J_1, 75. Peaks at *m/z* 99 represent ions having elemental composition $C_5H_7O_2$. Ions $C_7H_{13}O_4$ (ref. 7), appearing at *m/z* 161, suggested[1,6] as suitable for distinguishing between (1→2)- and (1→4)-linked disaccharides, have intensities too low to be of reliable diagnostic value. On the other hand, fragmentation of the (1→2)-linked compounds **2** and **3** gives rise to intense ions having *m/z* 131 ($C_6H_{11}O_3$), and these



Scheme 1

are represented in the spectra of differently linked disaccharides **1**, and **4–7**, only as very weak peaks. It is worth mentioning that, in the spectra of (1→2)-linked disaccharides **2** and **3**, the peak intensity at *m/z* 131 is comparable to that of F-type ions at *m/z* 261 and 229. For the (1→4)-linked disaccharides **6** and **7**, the peak height at *m/z* 131 is ~20% of those at 261 and 229. Another difference in the fragmentation of (1→2)- and (1→4)-linked disaccharides studied herein is the much readier cleavage of the interglycosidic bond, resulting in the formation of $^{a}A_1$ ions (*m/z* 175) from the (1→2)-linked substances **2** and **3**.

Of the ions discussed here, the (1→3)-linked D-xylobioses **4** and **5** do not produce $^{ba}F_1$ ions (*m/z* 261) and, therefore, nor are the peaks representing ions



Scheme 2

TABLE IV

CRITERIA FOR LINKAGE ANALYSIS IN POSITIONALLY ISOMERIC, FULLY METHYLATED XYLOBIOSES[a]

m/z	% Σ_{45}			
	a→b	a→*2*b	a→*3*b	a→*4*b
261	0	...	0	...
115	...	...	xx	...
101/88	30	1	1	1
261/131 (or 229/131)	0	1	0	5

[a]Peak intensities (Tables IV and VI): ., 0.5%; .., 0.5–1.0%; ..., 1–5%: x, 5–10%; xx, 10–20%; xxx, 20%.

resulting from the elimination of methanol from them (*m/z* 229 and 197) present in their spectra. Characteristic of the fragmentation of **4** and **5** are intense ions of *m/z* 115 ($C_6H_{11}O_2$). Metastable-transition measurements showed that these ions are formed from bA_1 ions, originating from the abJ_1 ions (*m/z* 235, Scheme 2).

The foregoing data show that the spectra of positionally isomeric per-*O*-methyl-*β*-D-xylobioses show characteristic differences. Criteria for simple differentiation among the variously linked substances, extractable from both 12- and 70-eV spectra, are summarized in Table IV. As may be seen, the type of linkage can be deduced from the peak intensities at *m/z* 261 and 115, and from the peak intensity-ratios 101/88 and 261/131.

The differences in the peak intensities in the spectra (Table II) of *α*,*β* pairs of positionally isomeric, fully methylated xylobioses were found insufficient to allow conclusions to be made about the configuration of the interglycosidic linkage.

Fragmentation of fully methylated D-*xylotrioses.* — Electron impact upon the title class of compounds results in the formation of ions (Table III) common to all compounds in this series. The notation of ions is shown for compound **9** (*d*→2*a*→4*b* type). In the upper part of the spectra, the following ions containing carbon atoms from all three rings are present (symbol, *m/z*); $abcB_1$, 496; $badA_2$, 463; $badC_2$, 435; $badF_1$, 421; and $dabJ_1$, 395. Fragments containing carbon atoms from only two rings are as follows: bdA_1, 335; bdA_2, 303; bdA_3. 271; baF_1, 261; and $[baF_1 - MeOH]^+$, 229. In the lower part of the spectra, peaks are present representing ions containing carbon atoms from only one ring: aA_1, 175; $[C_7H_{13}O_3]^+$, 145; $[C_6H_9O_3]^+$, 129; $[C_5H_7O_2]^+$, 99; dH_1, 88 and bJ_1, 75.

To find the contribution of the individual, glycosidically linked rings to the abundance of fragments formed from xylotrioses located at points of branching in xylans, compound **10**, an analogue of **9**, trideuteriomethylated in the side-unit *d*, was synthesized and studied. Table V shows the observed contributions of the isomeric ions to the abundance of characteristic ions.

TABLE V

CONTRIBUTIONS OF ISOMERS TO THE ABUNDANCE OF CHARACTERISTIC IONS FORMED FROM **10**[a]

Symbol	m/z	*Contribution*, %	
		70 eV	*12 eV*
$badF_1$	421(430)[a]	100	100
$dabJ_1$	395(398)	59	54
$abdJ_1$	395(404)	41	46
baA_1	335(335)	10	8
bdA_1	335(344)	90	92
baA_2	303(303)	50	50
bdA_2	303(312)	50	50
aA_1	175(175)	65	65
dA_1	175(184)	35	35
aA_2	143(143)	61	72
dA_2	143(149)	39	28
aF_1	101(101)	52	59
dF_1	101(107)	48	41
aH_1	88(88)	42	40
dH_1	88(94)	58	60
bJ_1	75(75)	62	68
cJ_1	75(81)	38	32

[a]When referring to the spectrum of **10**, *m/z* values are given in parentheses.

TABLE VI

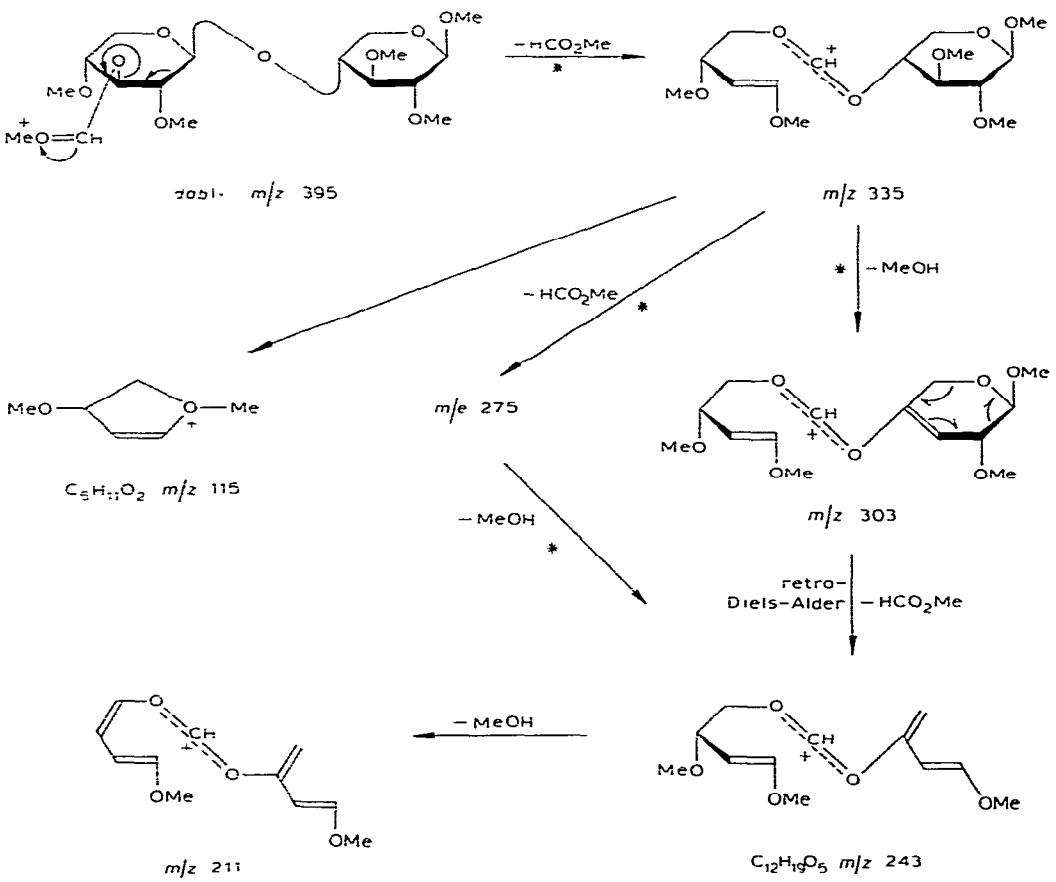
CRITERIA FOR LINKAGE ANALYSIS IN POSITIONALY ISOMERIC, FULLY METHYLATED D-XYLOTRIOSES

m/z	% Σ_{45}				
	a→*4*b→*4*c	a→*4*b*2*←d	a→*4*b*3*←d	d→*2*a→*4*b	d→*3*a→*4*b
261	...	0	.	...	.
243	0	0	0	0	...
235	x	0	.	...	...
229	...	0	..	...	...
129	..	.	...	.	.
115	...	...	...	...	xx
115/129	10	10	0.5	10	50
261/271	5			0.5	

By comparing mass spectra of **8–14**, characteristic differences in the fragmentation of positionally isomeric D-xylotriose derivatives could be found (Table VI) that make it possible, in the range of applied electron energy (12–70 eV), to identify compounds of this class, that is, they allow location of the point of branching in xylan-type trisaccharides.

The spectra of the linear D-xylotriose **8** ($a\rightarrow4b\rightarrow4c$ type), representing the main or a longer side-chain of the polysaccharide, are characterized by intense peaks of fragments baF_1 (261), bcJ_1 (235), and $[baF_1 - MeOH]^+$ (229), while the ones at m/z 129 and 115 of the elemental composition $C_6H_9O_3$ and $C_5H_7O_2$, respectively, are weak. The same characteristic features are present also in the spectra of **12** ($d\rightarrow2a\rightarrow4b$ type). As with the (1→2)- and (1→4)-linked D-xylobioses (Table IV), the differences in the fragmentation of **8** and **12** are in the intensity of series baA (adA in the case of **12**), namely, in the intensity of peaks at m/z 335, 303, and 271 which, in the spectrum of **12** ($d\rightarrow2a\rightarrow4b$ type), is about 5 times higher than that in the spectrum of **8** ($a\rightarrow4b\rightarrow4c$ type). This significant difference is expressed in the formulation of one of the criteria (Table VI) for identification of this class of compound. It may be seen that the ratio of intensities of the peaks at m/z 261 and 271 is 5 for **8** and 0.5 for **12**.

The spectra of **9** and **11** branched in ring b ($a\rightarrow4b2\rightarrow d$ and $a\leftarrow4b3\leftarrow d$ type) differ from those of the other trioses by the absence of ion peaks at m/z 261 and 235



Scheme 3

(very weak peaks at these *m/z* values in the spectra of **11** are neglected). The two compounds may be readily distinguished from each other by the intensity of the peak present in their spectra at *m/z* 129, more intense by a factor of 10 with **11** than with **9**. This forms the criterion (Table VI) expressed by the intensity-ratio of the peaks at *m/z* 115 and 129, which is 10 and 0.5 for the *a*→4*b*2→*d* and *a*→4*b*3→*d* arrangements, respectively.

Compounds **12–14** "branched" at the *a* ring constitute *d*→2*a*→4*b* and *d*→3*a*→4*b* types (compounds **13** and **14** differ merely in the stereochemistry of the interglycosidic linkage, and their mass spectra did not show significant differences). The fragmentation of **13** and **14** differs significantly from that of **12** by a new fragmentation series occurring with the former compounds, giving rise to ions appearing in their spectra at *m/z* 275, 243, and 211 (of the respective elemental composition $C_{13}H_{23}O_6$, $C_{12}H_{19}O_5$, and $C_{11}H_{15}O_4$). Another characteristic feature of the fragmentation of **13** and **14** is the formation of highly abundant ions of *m/z* 115 of the elemental composition $C_6H_{11}O_2$. The process in the new series clarified, *inter alia*, by metastable-transition measurements, involve combined eliminations of methyl formate (Scheme 3, retro-Diels–Alder fragmentation), and methanol from *dab*J_1 ions.

Thus, D-xylotrioses containing the *a*→4*b* arrangement of the two xylose moieties may be clearly distinguished from the other type of compound, and the attachment of the third residue may be unambiguously determined solely by e.i. mass spectrometry (at any electron energy) of their fully methylated derivatives.

Fragmentation of the fully methylated, branched D-*xylotetraose.* — The mass spectrum of **15** (*a*→4*b*→4*c* type) is characterized by high-intensity fragments con-

$$\begin{matrix} 3 \\ \uparrow \\ d \end{matrix}$$

taining carbon atoms belonging to only one ring (the region below *m/z* 175). In the upper part of the spectrum, ions (symbol *m/z*) *badc*A_2, 623; *abcd*C_2, 595; *abcd*J_1, 555; *bcd*A_1, 495; *bcd*A_2, 463; and *bcd*A_3, *m/z* 431 may be identified. The molecular weight of similar tetraoses may be calculated by using data extracted from their e.i. mass spectra according to the equation: $M = aA_1 + bcdA_1 + 16$. As with **11**, the mass spectrum of **15** contains a highly pronounced peak at *m/z* 129 representing ions of elemental composition $C_6H_9O_3$. The peak of the same ions was identified[12] in the spectrum of a branched, fully methylated aldopentaouronic acid of the *a*→4*b*→4*c* type,

$$\begin{matrix} 3 & 2 \\ \uparrow & \uparrow \\ d & e \end{matrix}$$

where *abcd* = β-D-xylopyranosyl, and *e* = methyl 4-*O*-methyl-α-D-glucopyranosyluronate). As a characteristic, structural feature common to these compounds is the *a*→4*b*3→*d* arrangement, an intense peak at *m/z* 129 in the spectra of oligosaccharides related to xylans may reasonably be considered diagnostic of the arrangement of three pentose residues discussed. Detailed determination of the structure of position-

ally isomeric xylotetraoses would, however, require further studies on related model compounds.

EXPERIMENTAL

General methods. — The 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, 150–220°, and that of the ionizing chamber was 180°. The peak intensities (Table II–IV, and VI) are expressed in per cent of the total ionization % $\sum_{+5^{e}}$. Exact mass measurements were performed at a resolution of 10,000 with perfluorokerosene as the reference. The elemental compositions obtained are given in Schemes and the text. Metastable-transition measurements were performed by using an MS-MT-01 metastable-ion detector.

Melting points were measured on a Kofler hot-stage. Optical rotations were determined with a Perkin–Elmer automatic polarimeter, Model 141. Thin-layer chromatography (t.l.c.) on Silica Gel G and column chromatography on Silica Gel 60 (Merck, A.G., Darmstadt) were effected with *A*, 8:1 benzene–acetone, *B*, 5:1 chloroform–acetone; *C*, 10:1 benzene–acetone; *D*, 9:1 chloroform–methanol; and *E*, 3:1 benzene–acetone.

Methyl 3-O-benzyl-2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4-O-(2,3,4-tri-O-benzyl-β-D-xylopyranosyl)-β-D-xylopyranoside. — To a mixture of methyl 3-*O*-benzyl-4-*O*-(2,3,4-tri-*O*-benzyl-β-D-xylopyranosyl)-β-D-xylopyranoside[13] (1 g, 1.5 mmol), Drierite (2 g), and mercuric cyanide (0.38 g, 1.5 mmol) in dry acetonitrile (20 mL) was added 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide[14] (1.03 g, 3.05 mmol) and the mixture was stirred for 1 h. The crude product was isolated conventionally, chromatographed, and the main product (1.1 g, R_F 0.5, solvent *A*) was crystallized from ethanol, m.p. 126–128°, $[\alpha]_D^{22}$ −47° (*c* 1.5, chloroform) (Found: C, 65.65; H, 6.33. $C_{50}H_{58}O_{16}$ calc.: C, 65.53; H, 6.39%).

Methyl 3-O-methyl-2-O-(2,3,4-tri-O-trideuteriomethyl-β-D-xylopyranosyl)-4-O-(2,3,4-tri-O-methyl-β-D-xylopyranosyl)-β-D-xylopyranoside (**10**). — The foregoing compound (1 g) was deacetylated (Zemplén) and the product, obtained as a chromatographically pure syrup (R_F 0.1, solvent *B*), was treated with trideuteriomethyl iodide and sodium hydride in *N*,*N*-dimethylformamide. The main product (R_F 0.5, solvent *C*), isolated by column chromatography, was dissolved in ethanol and hydrogenated at room temperature over 5% palladium-on-charcoal to give chromatographically pure product (R_F 0.5, solvent D). Methylation and isolation of the main product in the afore-described manner gave the title compound (0.3 g, 51%) which, after crystallization from isopropyl ether/hexane had the same physical properties as its fully methylated analogue[16]: the dimorphous material (long needles changed at 82–84° to fibrous crystals, m.p. 88–90°) showed $[\alpha]_D^{22}$ −87°, (*c* 1, chloroform), lit.[16] $[\alpha]_D^{22}$ −89° for the fully methylated analogue.

2,3,4-Tri-O-methyl-α-D-xylopyranosyl 2,3,4-tri-O-methyl-β-D-xylopyranoside

(**1**). — α-D-Xylopyranosyl β-D-xylopyranoside[14] (0.15 g) was methylated as just described and some undermethylated material (R_F <0.2, solvent *E*) was removed by elution of the crude product from a column of silica gel. The chromatographically pure, title compound (R_F 0.6, solvent *E*) thus obtained solidified after trituration with cyclohexane. Recrystallization from the same solvent gave material melting at 62–64°, $[\alpha]_D^{22}$ +59° (*c* 0.7, chloroform) (Found: C, 52.61; H, 8.33. $C_{16}H_{30}O_5$ calc.: C, 52.44; H, 8.25%).

Syntheses of **2–9** and **11–15** have been described elsewhere[16–20].

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