Carbohydrate Research, 105 (1982) 251–257
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

A contribution to the sequential analysis of oligosaccharides by mass spectrometry

VLADIMÍR KOVÁČIK AND PAVOL KOVÁČ
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava (Czechoslovakia)
(Received July 6th, 1981; accepted for publication, November 6th, 1981)

The molecular mass, the masses of the monomeric units, and the linkage position of disaccharides can be unequivocally determined from the electron-impact (e.i.) mass spectra of their methylated derivatives[1–6]. Methylated trisaccharides give a more complicated fragmentation pattern and, at present, certain[7–9] combinations of units and linkages cannot be unambiguously determined by e.i. mass spectrometry alone. The probability of correct linkage-analysis decreases with the chain-length of the oligosaccharide, since structurally significant data in the literature are scarce:

TABLE I

COMPOUNDS INVESTIGATED

No.	*Compound*	*Symbols*
1	β-D-Xyl*p*-[(1→4)-β-D-Xyl*p*]$_n$ $n = 1$	*a*→4*b*
2	β-D-Xyl*p*-[(1→4)-β-D-Xyl*p*]$_n$ $n = 2$	*a*→4*b*→4*c*
3	β-D-Xyl*p*-[(1→4)-β-D-Xyl*p*]$_n$ $n = 3$	*a*→4*b*→4*c*→4*d*
4	β-D-Xyl*p*-[(1→4)-β-D-Xyl*p*]$_n$ $n = 4$	*a*→4*b*→4*c*→4*d*→4*e*
5	β-D-Xyl*p*-[(1→4)-β-D-Xyl*p*]$_n$ $n = 5$	*a*→4*b*→4*c*→4*d*→4*e*→4*f*
6	β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(2←1)-β-D-Xyl*p*	*a*→4*b*2←*d*
7	β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(3←1)-β-D-Xyl*p*	*a*→4*b*3←*d*
8	β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(2←1)-β-D-GlcA	*a*→4*b*2←*d*
9	β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p* 3 ↑ 1 β-D-Xyl*p*	*a*→4*b*→4*c* 3 ↑ *d*
10	β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p* 3 2 ↑ ↑ 1 1 β-D-Xyl*p* α-D-GlcA	*a*→4*b*→4*c* 3 2 ↑ ↑ *d* *e*

TABLE II

MASS SPECTRA (12 eV) OF PERMETHYLATED XYLO-OLIGOSACCHARIDES **1-5**

m/z	% Σ_{45} × *100*				
	Di (1)	*Tri (2)*	*Tetra (3)*	*Penta (4)*	*Hexa (5)*
975					1
875					2
815				9	2
715				20	9
655				5	4
555			45	44	20
495			8	11	7
494				4	5
469			6		
463			8	11	12
431			8	7	7
421		7	10	11	
409				15	18
395		136	216	90	50
355				37	
349					18
336	2				
335		51	194	253	313
304	2				
303	2	94	216	198	237
281				55	
275	8				
274	4	14			
273			61		
271		32	76	59	85
261	120	180	205	79	52
249		54	80	70	73
235	600	504	583	297	162
229	212	266	313	123	105
219		6			
217				51	55
215		12			
205		12			52
197	12		19		
189		45	78	99	135
175	1200	1671	2442	2314	2825
174	30	21			
161	30	33	50	75	88
159	38	45	43	48	52
157	10	15	7	26	37
145	200	237	84	121	110
143	1180	1700	2122	1916	2175
142		27			
131	32	39	21	26	28
129		57	54	97	110
127				29	23
115	400	473	335	374	350

TABLE II (*continued*)

m/z	% Σ_{45} × *100*				
	Di (1)	*Tri (2)*	*Tetra (3)*	*Penta (4)*	*Hexa (5)*
114	200	166	147	165	105
111	108	281	497	529	625
103		89			
101	1870	916	702	793	600
99	200	266	50	110	90
88	1688	1168	529	804	725
87	184	129			
85	64	51	19	59	35
84	92	123	151	128	108
83	50	36			
75	960	591	421	429	312
71	360	355	173	308	140
69		21			
59	38	24	6	10	
58	36	9	13	34	10
45	66	45	10	64	28

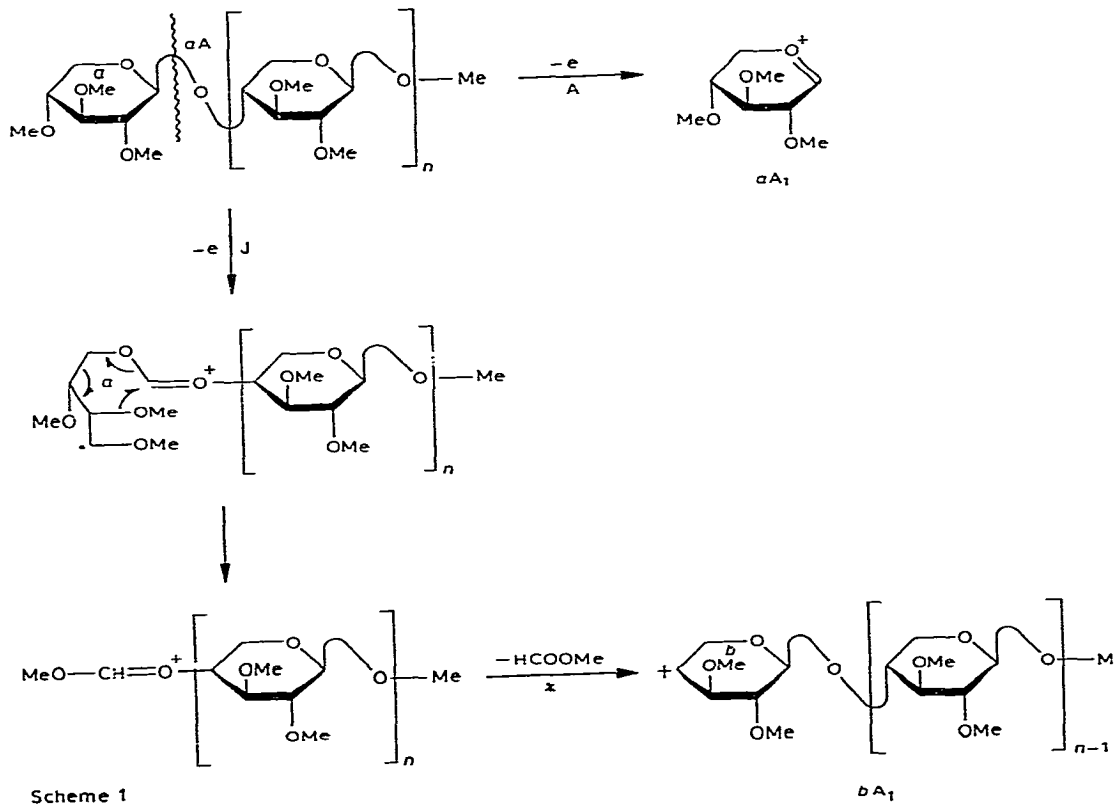
TABLE III

MASS SPECTRUM (12 eV) OF ALDOPENTAOURONIC ACID **10**

m/z	% Σ_{45} × *100*	m/z	% Σ_{45} × *100*	m/z	% Σ_{45} × *100*
841	5	303	78	131	23
773	39	233	624	129	182
713	16	201	1312	115	177
681	8	189	57	114	104
649	8	175	1662	111	520
623	31	174	200	103	73
567	21	169	88	101	831
521	44	167	44	99	171
507	55	161	15	88	650
479	26	159	42	85	47
475	49	157	26	84	112
453	23	149	73	75	260
407	23	145	127	71	49
393	23	143	1558	69	31
361	70	142	130	59	23
329	47	141	169	58	26
				45	26

very few spectra of fully methylated tetrasaccharides[6,10] and only one of a pentasaccharide[10] have been published.

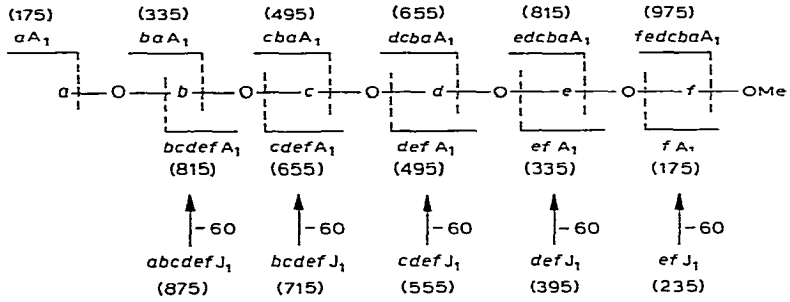
We now report on the e.i. mass spectra of fully methylated, synthetic, (1→4)-linked xylo-oligosaccharides, namely, β-xylotetraose (**3**), β-xylopentaose (**4**), and β-xylohexaose (**5**) (Table I), and the branched aldopentaouronic acid **10**. The features characteristic of the fragmentation of **10** were compared with the fragmentation patterns[6-11] of xylo-oligosaccharides **1**, **2**, and **6–9**.



Scheme 1

The mass-spectral data for the methylated xylo-oligosaccharides **1–5** and **10**, shown in Tables II and III, reveal that fragmentation of permethylated oligosaccharides occurs independently in each sugar ring. This information served as a starting point for evaluation of the spectra of oligomers and, eventually, the polymer from which the oligomer originated. In order to obtain information about the masses of the individual units and, thus, about the sequences of units differring in mass, it is important to consider fragmentations of the A- and J-Series. This is demonstrated in Scheme 1 for cleavage of ring *a* in the fragmentation of xylo-oligosaccharides **1–5**.

The cleavage of glycosidic linkages gives A_1 ions. The formation of ions J_1 is followed by elimination of methyl formate to give ions bA_1, whose structure is



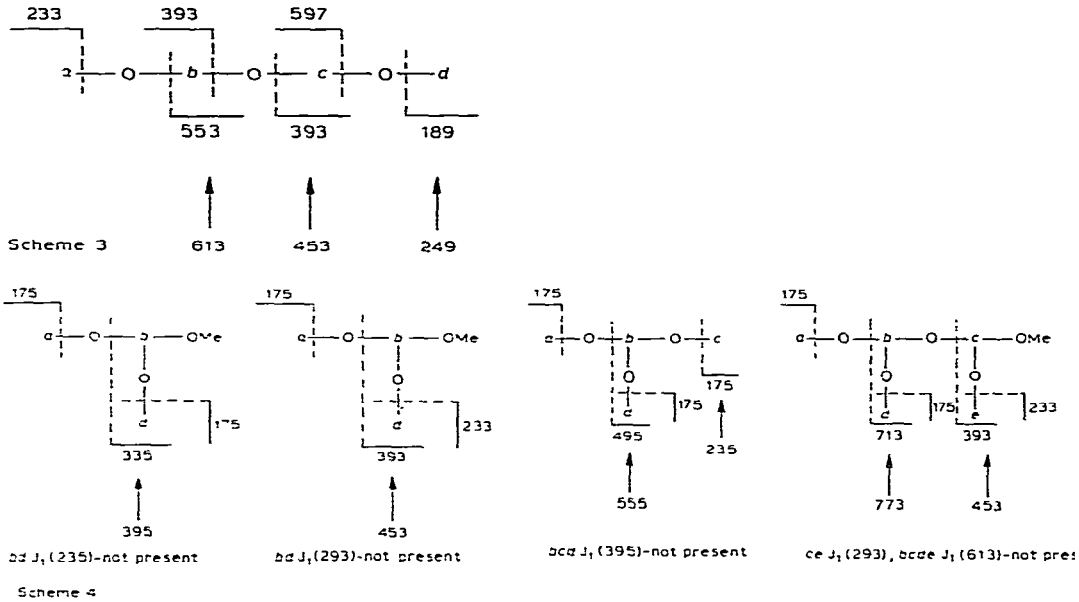
Scheme 2

different from that of aA_1 ions. Peaks reflecting the formation of bA_1 ions, together with peaks of their precursor abJ_1 ions (heavier by 60 mass units), characterise the mass of the fragment originating from the portion of the molecule to the right of the glycosidic oxygen at which the fission had occurred. The characteristic fissions occurring for the fully methylated xylohexaose **5** are depicted in Scheme 2, where the A and J ions are denoted by the generally accepted symbols[2,6] and *m/z* values.

In the spectrum of the methylated xylopentaose **4**, the number of ion species of the same origin is one less than for **5**: the peaks of ions of *m/z* 975 and 875 are missing. Similarly, in addition to the latter peaks, the spectrum of the methylated xylotetraose **3** does not contain peaks at *m/z* 815 and 715. On the other hand, a spectrum of a xyloheptaose, or any pentoheptaose, would contain peaks at *m/z* 1135 and 1035, and that of any pento-octaose peaks at *m/z* 1295 and 1195. The *m/z* values of the "right side" and the "left side" A-ions can be used to determine the molecular mass of an oligosaccharide (*e.g.*, M = 815 + 175 + 16, M = 335 + 655 + 16, or M = 495 + 495 + 16 for xylohexaose **5**).

Replacement of a pentose by a hexose in an oligosaccharide is reflected in the spectrum, and in a scheme analogous to Scheme 2, by an increase of the mass of a single unit by 44. When a 6-deoxyhexose or a hexuronic acid is present, these increases are by 14 and 58 mass units, respectively. Thus, as a result of the presence of units other than a pentose, the respective *m/z* values in the spectrum will be different than those shown in Scheme 2. For example, if the spectrum of an unknown oligomer contained peaks at *m/z* 189, 233, 249, 393, 453, 553, 597, and 613, only the structure shown in Scheme 3 would be possible. In this structure, unit *a* is a hexuronic acid, unit *b* is a pentose, unit *c* is a hexose, and unit *d* is a 6-deoxyhexose. The validity of this approach has been verified also by using published spectra of various oligosaccharides[2,3,5,8–10]. Thus, except for the stereochemistry, the *m/z* values of the A and J ions determine the sequence of monomeric units (according to their masses) in oligosaccharides.

Application of Scheme 2 to branched oligosaccharides **6–10** produces Scheme 4. As shown there, spectra of substances containing side-units linked glycosidically to branch-points do not contain peaks of all of the J-type ions. In the spectra of trimers **6–8** having unit *d* linked to unit *b* of the "main chain", peaks of ions bdJ_1 (*m/z* 235 in the spectra of **6** and **7**, and at *m/z* 293 in the spectrum of **8**) are absent. In the



spectrum of **9**, peaks of *bcd*J ions of *m/z* 395 are missing; in the spectrum of **10**, ions of ceJ_1 and $bcdJ_1$ (*m/z* 293 and 613) are missing. Thus, by using the generally accepted[2] terminology of ions, the first letter in the symbol denotes the missing ion and determines unambiguously the branch unit in an oligomer.

Determination of sequences of units and the branching units in oligosaccharides should be the first step in methylation analysis of a polysaccharide. Information about the linkage positions, obtainable from the same spectrum (prefentially obtained with low-energy electrons), should provide further evidence for solution of a particular structural problem. When necessary, partial hydrolysis of a higher oligosaccharide to less-complex molecules and methylation analysis thereof provide additional data for determination of the overall structural features of polysaccharides and other carbohydrate-containing substances.

EXPERIMENTAL

Mass spectra (70 and 12 eV) were obtained at an emission of 300 μA, with a JMS D-100 spectrometer, applying the direct-insertion technique. Depending upon the volatility of substances, the temperature at the site of evaporation was 200–250°, and that in the ionising chamber was 180°. The peak intensities (Tables II and III) are expressed as percentages of total ionisation. Syntheses of **1–10** are described elsewhere[11–18].

REFERENCES

1 O. S. Chizhov, L. A. Poljakova, and N. K. Kochetkov, *Dokl. Akad. Nauk SSSR*, 158 (1964) 291–294.

2 V. KOVÁČIK, V. MIHÁLOV, J. HIRSCH, AND P. KOVÁČ, *Biomed. Mass Spectrom.*, 5 (1978) 136–145.
3 J. MOOR AND E. S. WAIGHT, *Biomed. Mass Spectrom.*, 2 (1975) 26–45.
4 K. G. DAS AND B. THAMAYUNAVAN, *Org. Mass Spectrom.*, 6 (1972) 1063–1069.
5 E. G. DE JONGH, W. HEERMA, J. HAVERKAMP, AND J. P. KAMERLING, *Biomed. Mass Spectrom.*, 5 (1979) 72–77.
6 V. KOVÁČIK, V. MIHÁLOV, AND P. KOVÁČ, *Carbohydr. Res.*, 88 (1981) 189–201.
7 V. KOVÁČIK, P. KOVÁČ, AND A. LIPTÁK, *Carbohydr. Res.*, 98 (1981) 242–246.
8 J. KÄRKKÄINEN, *Carbohydr. Res.*, 17 (1971) 1–10.
9 V. KOVÁČIK, Š. BAUER, AND J. ROSÍK, *Carbohydr. Res.*, 8 (1968) 291–294.
10 J. COMTAT, J.-P. JOSELEAU, C. BOSSO, AND F. BARNOUD, *Carbohydr. Res.*, 38 (1974) 217–224.
11 J. HIRSCH, P. KOVÁČ, J. ALFÖLDI, AND V. MIHÁLOV, *Carbohydr. Res.*, 88 (1981) 146–152.
12 P. KOVÁČ, *Collect. Czech. Chem. Commun.*, 45 (1980) 892–900.
13 J. HIRSCH AND P. KOVÁČ, *Carbohydr. Res.*, 77 (1979) 241–244.
14 P. KOVÁČ, *Collect. Czech. Chem. Commun.*, 44 (1979) 928–932.
15 P. KOVÁČ, *Chem. Zvesti*, 34 (1980) 234–240.
16 P. KOVÁČ AND J. HIRSCH, *Carbohydr. Res.*, 100 (1982) 177–193.
17 P. KOVÁČ, J. HIRSCH, AND V. KOVÁČIK, *Carbohydr. Res.*, 75 (1979) 109–116.
18 P. KOVÁČ, J. HIRSCH, V. KOVÁČIK, AND P. KOČIŠ, *Carbohydr. Res.*, 85 (1980) 41–49.