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Note

Mass spectrometry of monosaccharide sulphate derivatives

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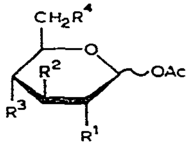
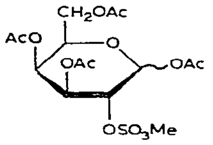
Sulphated polysaccharides are widespread in Nature[1], and investigations of this class of biopolymer require methods for identifying sulphated sugars We have investigated mass spectrometry for this purpose

Monosaccharide sulphates are not sufficiently volatile for mass spectrometry, and we have therefore studied the acetylated sugar methyl sulphates[2] and chlorosulphates[3], which are more volatile Direct introduction into the ion source afforded reproducible mass spectra of the acetylated D-glucose and D-galactose methyl sulphates **1–5** and **8**, and the chlorosulphates **6** and **7** The data are given in Table I The spectrum of the 4-methyl sulphate **5** was unsatisfactory, as the compound could not be isolated pure The data in Table I indicate that fragmentation of the acetylated sugar methyl sulphates is similar to that of the hexose penta-acetates[4]



1 $R^1 = R^3 = R^4 = AcO, R^2 = OSO_3Me$
2 $R^1 = R^2 = R^3 = AcO\ \ R^4 = OSO_3Me$
3 $R^2 = R^3 = R^4 = AcO, R^1 = OSO_3Me$
4 $R^2 = R^3 = R^4 = CD_3COO\ \ R^1 = OSO_3Me$
5 $R^1 = R^2 = R^4 = AcO, R^3 = OSO_3Me$
6 $R^2 = R^3 = R^4 = AcO, R^1 = OSO_2Cl$
7 $R^1 = R^2 = R^3 = AcO\ \ R^4 = OSO_2Cl$



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Series A (Scheme 1) starts with loss of AcO-1 to give the A_2 ion followed by loss of the 3-substituent to give A_2 The formation of subsequent fragments (*m/e* 281, 263, 169, and 109 for **2**, *m/e* 281, 263, 221, 211, 169, and 109 for **3**) depends on the position of the methyl sulphate group Thus, for **1**, the ion formed by elimination of $MeOSO_3H$ and AcOH is not observed, but the ion *m/e* 263 ($A_1 - 2AcOH$) is present Hence,

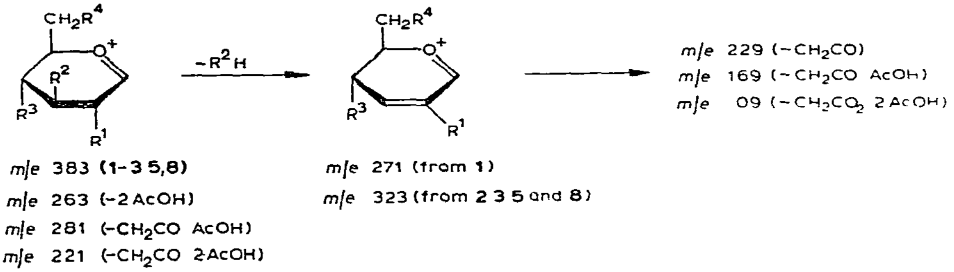
TABLE I

MASS-SPECTRAL DATA FOR COMPOUNDS 1–3 AND 5–7

m/e	*Relative intensity*					
	1	2	3	5	6	7
60	13	27	7		100	100
64				11	90	38
69	8	20	15	78	10	14
70		17	10	53		11
71			15	32		
73	12	25	10	27	16	16
74				45		
80	10	13		20		
81	17	38	27	92	18	41
84		11	10			
85	9	19	15		17	12
86				14	15	
87				36		
97		33	52	58	72	18
98	>100	>100	23	94		78
99	15	20		58	36	
102	5	20		67	9	11
103	15	35	33	81	26	28
108				24		
109	17	35	28	24	13	22
112	5	22	3	62		5
113		20	5	31	8	7
114	12		5		8	5
115	70	80	30	74	45	80
116	11	17				9
125			15			
126	15	17	14	27	29	10
127	12	13	15	53	13	8
131	5		15			
139	7 5	19	75	24	43	8
140	26	80		20		28
141	10	15		71		4
143		15			8	6
144				40	8	
145	18	20	30	40	20	11
149		12	35		5	
151				50		
155	15	33	7	31		11
156					10	
157	100	100	37	50	28	39
158	20	17				8
164		11				
167			85	31		
168					11	
169	27	13	28	58	12	6
173						2
178			5			
182	7		6	11		
183			4 5		10	
186	5	2			5	6
187			5			1
191	6					
192		17	17	28		
196						6 5
197	6 5	67	10	27		
198						3
199	11	9	8	100	2	
200	31			18	3	2 5
203		3			2	
207	6 5					
209	2		100	36		
210	1 3	12		7		
211			17	6	6	2
215				6	4	7
218						2 5
220	6	37		6 5		
221	3	3	17	9		
223			3 5			
225					4	3 5
228	6	2		4	2	
229	3	2	3 5	3	3	3 5
234			3	8		
235				5		
237	6 5					
238	6		3			
239			6 5			
241						0 6
242	35		7	27	2	2 1
243	7			17		1 6
245	2 5	5	70		34	1 6
249	12					0 8
251			40	6		
252		28	40	12		
255	1 5					
256						5 8
258						2 2
259			9		5	
260	2 5					
262	2					
263	7	10	6			
265			3 5			
267	5		4	5	3	0 6
269						0 35
271	2				2	
280	2	9	8			
281	2 5	8 5	5	3		

TABLE I (*continued*)

m/e	*Relative intensity*						m/e	*Relative intensity*					
	1	2	3	5	6	7		1	2	3	5	6	7
284					87	1 6	331	1	1	2			0 65
285						1 4	333						0 25
286						0 75	340	1	6				
287						0 75	341	2 5		1 7	10		
294	1	47	12	10	3		344						0 7
295		14		28			345					0 5	
297	1 2		3				346						0 3
298						5 7	347					1	
300						2 8	351			1			
301					1		355	0 5			4		
303				2 5			368				3		
307						0 35	369	2 5		1 5		0 5	0 4
309	1		2		2	0 55	383	17	19	33	3 5		
311	0 8						384	4	3	8			
317	0 4	1 5				0 4	387					10	0 6
322	2						389					5	0 3
323		6	1 5				403						0 06
326					1		405						0 025
327	0 5				1	0 35							



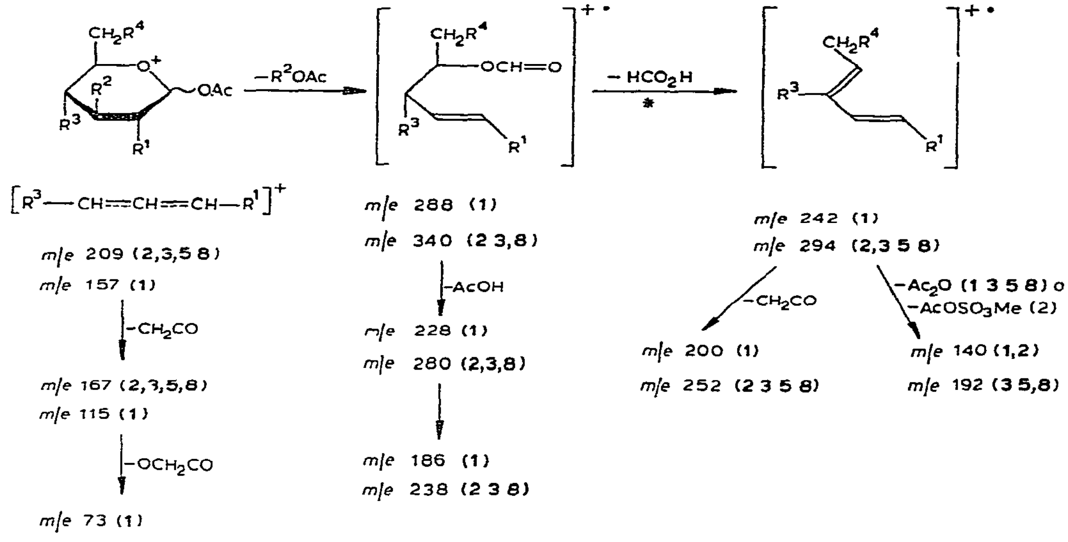
Scheme 1

m/e 263 for **3** should appear by loss of AcOH from C-4 and C-6, and should not be formed from **2**

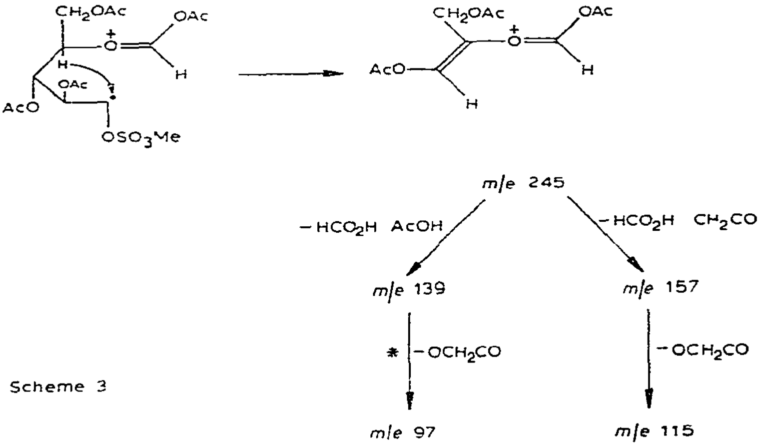
Series B and C are chiefly distinguished by the inability of the methyl sulphate group to eliminate as a ketene-like fragment (Scheme 2)

The mass spectrum of **3** also contains peaks of considerable intensity at *m/e* 245, 139, and 97, and fragments with *m/e* 157 and 115 The formation of these ions is represented in Scheme 3, this being in agreement with the shifts of the corresponding peaks from the deuterated compound **4**

The mass spectra of **3** and **8** are almost identical Thus, the mass spectra of the acetates of sugar methyl sulphates are characteristic and reflect the location of the sulphate groups



Scheme 2



Scheme 3

The mass spectrum of the chloro derivative **6** does not contain ions of the B and C series, probably because of the increased electronegativity of the substituent at C-2 The peaks at *m/e* 126, 168, and 186 are probably due to fragments of unsaturated products of the thermal decomposition of **6**

The fragmentation of **7** is similar to that of the tetra-acetate of 6-chloro-6-deoxy-β-D-glucopyranose Our spectrum of the latter compound differs somewhat from that published[5], in that it contains peaks at *m/e* 317 ($M-CH_2Cl$), 323 and 325 ($M-Ac$), and 331 ($M-Cl$)

EXPERIMENTAL

The mass spectra were recorded on CH6 (**1** and **2**) and LKB 9000S instruments (**3**–**8**)

T l c was performed on silica gel with chloroform–acetone (98 2) and detection by charring with sulphuric acid

Acetylated sugar methyl sulphates (**1**–**5**, **8**) — The barium salt (0 1 g) of each acetylated sugar sulphate dissolved in methanol (3 ml) was eluted with methanol from a column of Dowex-50W (H^+) resin The eluate was neutralized with ethereal diazomethane, then kept overnight in a refrigerator, and subsequently chromatographed on a column (13 × 0 7 cm) of silica gel with chloroform–acetone (97 3) Yield ~60 mg

Chlorosulphates — The acetylated sugar chlorosulphates were obtained by a slightly modified procedure[3] (*a*) A solution of 1,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose (1 g) in chloroform (1 ml) and pyridine (4 ml) at −75–80° was treated dropwise with sulphuryl chloride (2 6 ml) After 15 min, the mixture was poured into aqueous 10% magnesium sulphate (100 ml) at 0° The precipitate was removed and the filtrate was extracted with chloroform The extracts were washed with 5% sulphuric acid, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated to give 1,2,4,6-tetra-*O*-acetyl-*β*-D-glucopyranose 2-chlorosulphate (0 8 g), m p 102–103°, $[\alpha]_D^{20}$ +98° (*c* 0 8, chloroform)

Anal Calc for $C_{14}H_{19}ClO_{12}S$ C, 37 6 H, 4 2, S, 7 1, Cl, 7 9 Found C, 37 7, H, 4 4, S, 7 1, Cl, 7 9

(*b*) Using a procedure similar to that in (*a*), 1,2,3,4-tetra-*O*-acetyl-*α*-D-glucopyranose (1 g) was converted into 1,2,3,4-tetra-*O*-acetyl-*α*-D-glucopyranose 6-chlorosulphate (0 7 g), m p 91–92° (dec), $[\alpha]_D^{20}$ +16° (*c* 0 64, chloroform)

Anal Calc for $C_{14}H_{19}ClO_{12}S$ C, 37 6, H, 4 2, S, 7 1, Cl, 7 9 Found C, 36 95, H, 4 3, S, 7 1, Cl, 7 8

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