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Positional identification of fluorine in methyl per-*O*-acetyl-x-deoxy-x-fluoro-α-D-hexopyranosides by electron impact and chemical ionisation mass spectrometry

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ABSTRACT

Fully acetylated methyl x-deoxy-x-fluoro-α-D-glucopyranosides have been studied using electron impact and ammonia chemical ionisation mass spectrometry. Mass analysed metastable ion kinetic energy spectroscopy (MIKE), collisional activation (CID), and accelerated voltage scanning have been used to evaluate complete fragmentation schemes. Characteristic differences in the fragmentation of positional isomers were noted on analysis of the spectra, and these make it possible to determine the location of fluorine in the molecules studied. Collisionally activated fragmentation of $[M - OCH_3]^+$ ions, produced by electron impact, provides an alternative method for localisation of the fluorine atoms. To the contrary, MIKE and CID spectra of $[M + NH_4]^+$ cluster ions produced by chemical ionisation did not afford such structural information.

INTRODUCTION

Deoxyfluoro glycosides are important structures for studies of the interaction of carbohydrates with biologically active proteins. They have been employed, for example, to confirm the putative role of hydrogen bonds in the binding of carbohydrate antigens to their homologous antibodies. Using synthetic, specifically fluorinated ligands a great deal of information on binding has been obtained, and it was possible to map the subsites in the combining areas of a series of (1→6)-β-D-galactan- and (1→6)-α-D-glucan (dextran)-specific imunoglobulins[1,2]. In fact, from the approach based on fluoro sugars it has been possible to develop a rational process for elucidating the mode of binding at the molecular level[3].

Mass spectral analysis of acetyl derivatives of saccharides[4] by electron impact (e.i.) or chemical ionisation (c.i.) techniques serve as valuable tools for structural characterisation. The applicable fragmentation schemes can be elucidated by various techniques for measuring metastable transitions, such as mass analysed ion kinetic energy spectroscopy (MIKE), and accelerating voltage scanning (HV). The study of collision induced dissociation (CID) is also a valuable tool for the evaluation of e.i. or

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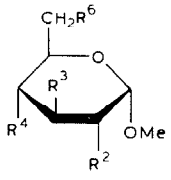
c.i. processes[5]. Instruments with reversed geometry or tandem m.s./m.s. analysers are used for these purposes.

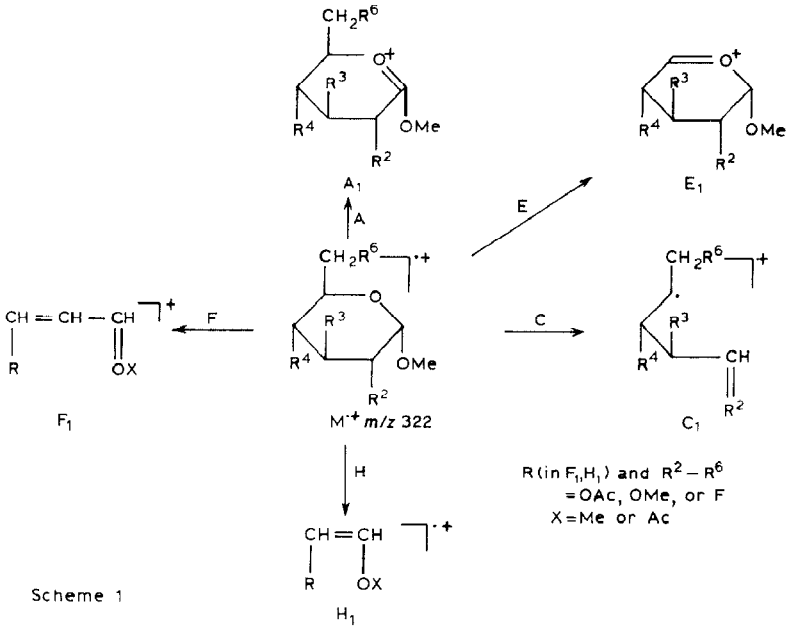
In the present work the e.i. and c.i. mass spectral behaviour of methyl per-*O*-acetyl-x-deoxy-x-fluoro-α-D-glucopyranosides has been studied by the MIKE and CID techniques. The aim was to find a method, alternative to ^{13}C-n.m.r. spectroscopy, for determining the position of fluorine in fluorinated methyl hexopyranosides.

RESULTS AND DISCUSSION

The conventional mass spectra of positional isomers **1–4** are presented in Table I. In analogy to the per-*O*-acetylated methyl glycosides[4,5], the molecular ions of deoxyfluoro derivatives fragment by five pathways, depicted in Scheme 1. Fragments are denoted by the capital letters A–K, in accordance with commonly used nomenclature[5,6]. Pathway E, characteristic of nonfluorinated hexopyranosides, is followed to a very limited



	R^2	R^3	R^4	R^6
1	F	OAc	OAc	OAc
2	OAc	F	OAc	OAc
3	OAc	OAc	F	OAc
4	OAc	OAc	OAc	F



Scheme 1

TABLE I

EI mass spectra of methyl per-*O*-acetyl-x-deoxy-x-fluoro-α-D-glucopyranosides

m/z	*Relative intensities (%)*				*Ion type*
	2-F	*3-F*	*4-F*	*6-F*	
291	7.7	4.0	3.2	4.3	A_1
262	4.7	1.5			C_1
249	3.7	5.9	2.5		A_2
248	3.7				
231	1.7		17.0	3.1	A_2
220	3.6	8.0			C_2
219		6.0			
203	56.9	1.3	60.6	32.9	C_2
202	8.0	1.5	12.8	40.1	C_2
200		5.2			
189	17.7		12.0	12.3	A_3
188	2.0				
182			4.0		
177	6.5	5.4	3.2		
175		5.4			
171	6.2			8.8	A_3
169	3.7	11.9	17.6	12.9	A_3
168	4.1				
165		10.2			
161	13.7		19.8	13.6	C_3
160	69.4		80.9	72.6	C_3
158		11.3			
157	2.8	17.0	2.2	64.5	F_1
147			3.2		
145	37.2	4.7	28.7	23.0	
144	21.4		33.2	23.8	H_1
143	5.9	3.0	8.5		
142	9.6	4.5	19.8		
140		11.3	8.5		
131	6.9	15.6	12.0	18.0	
130	18.0	2.8			
129	49.1	15.6	19.1	39.2	F_1, A_4
128	7.0	4.1			
127	12.2	14.3	10.6		
119				12.9	
118	26.5	6.1	26.1		
117	32.2	9.4	18.4	13.8	F_1
116	28.7	64.4	5.0	12.2	H_1
115	49.0	44.1	8.5	61.6	F_2
112	15.0	17.8	18.4	25.3	
103	86.0	17.4	76.1	64.0	
102	45.7		100.0	100.0	H_1
101	33.4	3.7	57.6	82.6	C_4
100	30.6	8.2	97.6	37.6	C_4
99	16.1	17.7	44.1	19.2	
98	14.1	33.1	22.2	4.5	
89	38.1	44.7	4.5	7.6	F_1
87	100.0	5.2	17.1	37.8	F_2
86	10.7	3.6	8.8		

(continued)

TABLE I (continued)

m/z	*Relative intensities (%)*				*Ion type*
	2-F	*3-F*	*4-F*	*6-F*	
85				18.4	
76	41.9				H_1
75	6.9		12.7	8.9	F_2
74		74.9	57.4	33.5	H_2
73	21.9	15.8	17.9	21.3	
72	21.4	100.0	20.0	5.0	F_2
71				12.6	
70	7.1	2.0	45.0	42.6	
69	5.7	5.4	25.4	6.6	
61	23.7	13.4	40.6	26.7	
60	8.2		23.5	22.7	H_3

extent. The assignment of ions to particular fragmentation pathways was based on MIKE, HV, and CID analysis.

The splitting off of a methoxyl radical from molecular ions produces the A_1 ions. The major subsequent processes are eliminations of AcOH and CH_2CO molecules, giving rise to ions at *m/z* 249, 231, 189, 171, and 111. Surprisingly, in the case of the A_1 ion formed from the 3-deoxy-3-fluoro-glycoside **2**, an additional pathway can be observed producing the ions *m/z* 271, 211, 169, and 109. A molecule of HF is eliminated under the conditions of MIKE and CID measurements (Fig. 1), but this elimination does not take place in the A series ions of the other positional isomers.

The MIKE and CID spectra of the 2-fluoro derivative **1** exhibit a large signal at *m/z* 249, indicating loss of a CH_2CO molecule from the A ion at *m/z* 291, while the MIKE spectrum of the 4-fluoro derivative **3** shows a large peak at *m/z* 231, indicating

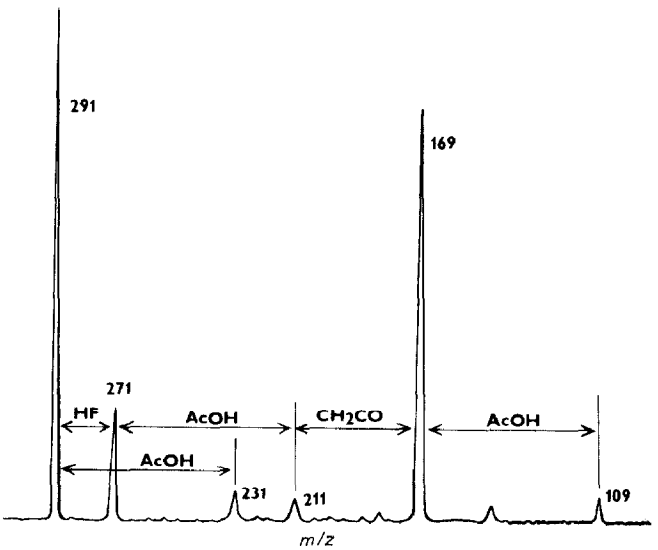


Fig. 1. MIKE spectrum of A_1 ions of 3-fluoro derivative **2**.

the elimination of AcOH. These ions are also formed from the 6-substituted isomer **4**, which also gives an additional ion at *m/z* 171, due to the loss of two AcOH molecules. The same differences are found in the CID spectra of the isomeric deoxyfluoro glycosyl cations A_1. Thus, both the MIKE and CID spectra[7] of $[M - OCH_3]^+$, which represent the A series of e.i. fragmentations, can be used for the unambiguous determination of the position of fluorine in per-*O*-acetylated methyl deoxyfluoro glycosides.

The C_1 ions, at *m/z* 262, originate from the molecular ions via the elimination of an HCO_2Me molecule (Scheme 1). The decay of C_1 ions then follows two pathways. The first begins with the splitting off of an OAc radical. The product ions of *m/z* 203 fragment, not only by elimination of AcOH and CH_2CO molecules, but also by elimination of a molecule of HF. Fragmentation of the C_2 ions provides the species having *m/z* 161, 143, 141, 129, 101, and 81 (Table I). The second mode of the decay of C_1 ions (*m/z* 262) begins with the elimination of CH_2CO or AcOH. The ions appearing at *m/z* 220 (or 202) then fragment by stepwise elimination of CH_2CO or AcOH, giving rise to the ions *m/z* 160, 118, 100, and 98 (Table I). The extent of HF elimination is low. Fig. 2 illustrates the CID mass spectrum of the C_2 ion (*m/z* 203) derived from the 6-fluoro glycoside **4**. The production of ions with *m/z* 160 is demonstrated in Fig. 3 by an HV analysis of the 4-fluoro derivative **3**. This spectrum is the only one showing the molecular ions (*m/z* 322) produced under e.i. conditions.

The conjugated transfer of electrons along the pyranoid ring results in the production of H-type fragments (Scheme 1). The H_1 ions possess *m/z* 144, 116, or 76, according to the substituents present (144 for Ac,Ac; 116 for Ac,Me; 76 for Me,F). The Ac,F combination has not been observed. The H_1 ions containing an OAc group eliminate a molecule of CH_2CO giving rise to H_2 ions having *m/z* 102 or 74.

Rearrangement processes are involved in the production of the intense F-type ions seen in our spectra. The F_1 ions, containing three carbon atoms of the pyranoid

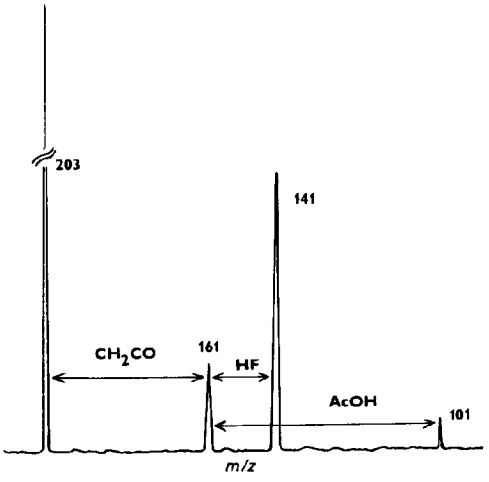


Fig. 2. CID spectrum of C_2 ions of 6-fluoro derivative **4**.

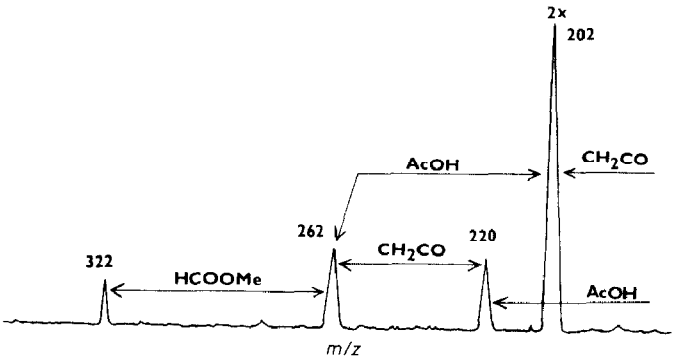


Fig. 3. HV spectrum of 4-fluoro derivative **3**, showing pathways to ions having *m/z* 160.

TABLE II

The abundance of structurally characteristic H- and F-type ions from fluorodeoxy hexopyranosides

Ion type	m/z	*Relative abundance*[a]			
		2-F	*3-F*	*4-F*	*6-F*
F_1	157				XXX
H_1	76	XX			
F_2	87	100		XXX	
	72	X	100	X	
H_2	102	XX		100	100

[a] X < 25%, 25 < XX < 50%, 50 < XXX < 100%, 100 = base peak.

ring, possess *m/z* values of 157, 129, 117, and 89, according to the nature of the substituents present (157 for Ac,Ac; 129 for Ac,Me; 117 for Ac,F; 89 for Me,F). The F_1 ions containing OAc groups eliminate a molecule of CH_2CO, giving rise to F_2 ions having *m/z* 115 or 87. Also, the HV measurements prove that F_1 ions of *m/z* 157 and 129 fragment with the production of ions $CH{=}OAc^+$ at *m/z* 72. This phenomenon is particularly noticeable in the case of the 3-fluoro derivative 2. The relative abundances of ions of the F and H series depend on the position of fluorine in methyl per-*O*-acetyl-x-deoxy-x-fluoro-hexopyranosides, as shown in Table II.

The e.i. spectra of methyl per-*O*-acetyl-x-deoxy-x-fluoro-α-D-glucopyranosides do not exhibit peaks corresponding to the molecular ions. To find new possibilities for structure elucidation, we studied the c.i. mass spectral behaviour of these compounds. The protonated species of hydrocarbon reactants such as methane and isobutane do not form adduct ions with the glycosides. As expected, in contrast to the hydrocarbon ions, the ammonium ions produced from NH_3 under the c.i. conditions[7–9] yield abundant cluster ions, $[M + NH_4]^+$, which appear at *m/z* 340. This phenomenon allows direct determination of the relative molecular masses of per-*O*-acetylated methyl deoxyfluoro glycosides.

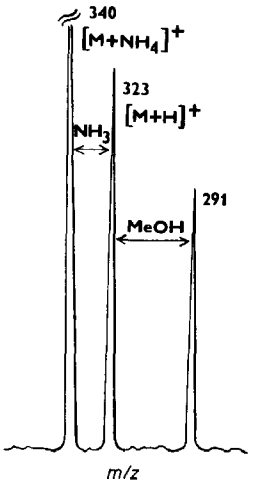


Fig. 4. MIKE spectrum of $[M + NH_4]^+$ ion of 3-fluoro derivative **2**.

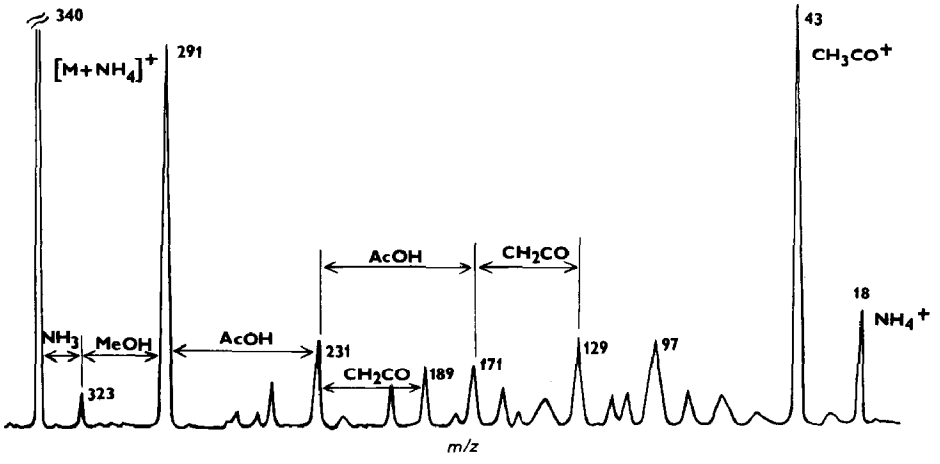


Fig. 5. CID spectrum of $[M + NH_4]^+$ ions of 3-fluoro derivative **2**.

The $[M + NH_4]^+$ cluster ions have been isolated in the magnetic sector of the instrument with reversed geometry and were subjected to MIKE and CID measurements. Figs. 4 and 5 present the MIKE and CID spectra of acetylated methyl 3-deoxy-3-fluoro-α-D-glucopyranoside **2**.

The only reactions observed in the MIKE spectrum are the sequential loss of NH_3 and MeOH molecules, yielding the ions $[M + H]^+$ and A_1 having *m/z* 323 and 291, respectively. The CID spectrum contains also the products of deeper fragmentation, characterised by eliminations of AcOH and CH_2CO, giving rise to ions having *m/z* 231, 189, 171, 129, and 97. The elimination of HF does not take place. The relative intensities of peaks in the MIKE and CID spectra of the $[M + NH_4]^+$ cluster ions do not show

differences that could be used for the localisation of fluorine in the positional isomers of the deoxyfluoro glycoside series.

EXPERIMENTAL

The synthesised[10,11] methyl x-deoxy-x-fluoro-α-D-glucopyranosides were acetylated by conventional treatment with acetic anhydride–pyridine.

The e.i. spectra were obtained with a single-focussing CH-5 mass spectrometer, operated at 70 eV ionisation energy. The oily samples were introduced into the ion source, maintained at ~ 180°, via a direct inlet system and a leak valve. The spectra were normalised using the instrument's on-line computer. Ions were also produced by electron impact in a VG ZAB-2F mass spectrometer, using the same conditions. These ions were focussed magnetically into the second field-free region of the instrument, after the magnet, and MIKE or HV spectra were recorded by scanning the deflection voltage of the electrostatic analyser or the accelerating voltage of the instrument, respectively. The CID spectra were obtained by the same techniques, while He was introduced into the collision chamber of the second field-free region until the intensity of the parent peak was reduced to ~ 50% of full value.

For measuring c.i. mass spectra ammonia was introduced into the c.i. ion source of the VG ZAB-2F instrument until the pressure reading at the ion source housing was 10^{-6}–10^{-5} mbar.

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