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Mass Spectra of Partially Methylated Alditol Acetates

Part III. Labelling Experiments in the Mass Spectrometry of Partially Methylated Deoxyalditol Acetates

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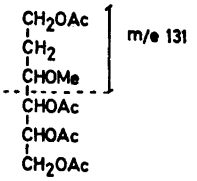
The mass spectra of some partially methylated 2- and 3-deoxyalditol acetates have been studied using deuterium labelling techniques. As a result of these studies the origins of primary fragments have been established; further detailed mechanisms for the formation of secondary fragments are presented.

In previous studies, we have examined the mass spectra of partially methylated alditol acetates and demonstrated that their methoxyl substitution pattern can be determined by analysis of the typical primary fragments, obtained by fission of the alditol chain.[1] The structures of the primary fragments and of secondary fragments formed from these by elimination reactions, were studied using deuterium labelling techniques.[2]

When a deoxy group is introduced into the carbon chain in an alditol acetate, cleavage between the methylene carbon and an adjacent, acetoxylated carbon becomes insignificant.[3] In the mass spectra of the partially methylated 3,6-dideoxyhexitol acetates, derived from *Salmonella* lipopolysaccharides,[4] however, primary fragments obtained by cleavage between a methylene carbon atom and an adjacent methoxylated carbon atom were observed. Some of the secondary fragments observed in these studies were obviously formed by elimination reactions, different from those previously encountered.

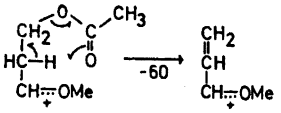
In the present paper, studies (using deuterium labelling techniques) on the origins of the primary and secondary fragments in the mass spectra of some partially methylated deoxyalditol acetates are reported.

One 2-deoxyalditol acetate, 2-deoxy-3-*O*-methyl-D-*ribo*-hexitol acetate (I), and five deuterated analogues (II – VI) were investigated. The mass spectra of these and other substances, discussed below, are given in Table 1. The main primary fragment, N, *m/e* 131, obtained by fission between C-3 and C-4, showed the expected shifts to higher mass numbers when a deuterium atom was introduced at C-1 or C-2 or when the *O*-methyl group was replaced by an *O*-trideuteromethyl group.

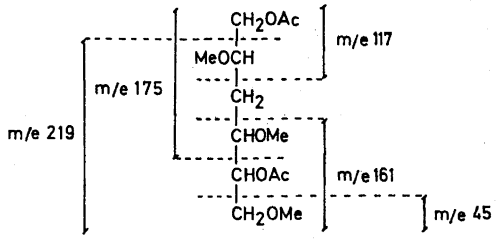


I

A secondary fragment, N_1, *m/e* 71, is formed from N by elimination of acetic acid, and also shows the expected shifts for the deuterated analogues. A deuterium atom at C-2 is only partially retained, as a consequence of a McLafferty rearrangement.



3-Deoxy-2,4,6-tri-*O*-methyl-D-*arabino*-hexitol acetate (XIII), gives primary fragments of *m/e* 45, 117, 161, 175, and 219. The nature of these fragments is confirmed by the results of the deuterium labellings (XIV – XVI). The formation of *m/e* 117 and *m/e* 161 demonstrates that cleavage between a methylene carbon atom and a methoxylated carbon atom is significant. The origin and further fragmentations of these and other primary fragments, devoid of deoxy groups, has been investigated,[2] and will not be elaborated here.



XIII

The primary fragment O, *m/e* 175, is obtained by fission between C-4 and C-5, as deuterium atoms at C-1, C-2, or the *O*-trideuteromethyl group at C-2 are retained in the fragment. The secondary fragment O_1, *m/e* 143, is derived from O by elimination of methanol. A deuterium atom at C-1 is retained, an *O*-trideuteromethyl group at C-2 is lost and a deuterium atom at C-3 is only partially retained, in agreement with the postulated structure.

Table 1. Fragments obtained on MS of partially methylated deoxyalditol acetates and some of their deuterated analogues.
(1) 0 indicates no deuterium labelling at deoxy or methoxyl groups. 2 indicates monodeuteration at 2-deoxy groups or a trideuteromethoxyl group at C-2, *etc.* 0*, 2*, *etc.* indicate that one of the hydrogen atoms at C-1 is replaced by a deuterium atom.
(2) Substances I–VI are 2-deoxy-D-*ribo*-derivatives and substances VII–XVI are 3-deoxy-D-*arabino*-derivatives disregarding the orientation of an eventual deuterium atom at C-2 or C-3.

Hexitol acetate	3-deoxy 2-OMe				3-deoxy 2,4,6-OMe			
Deuterated at[1]	3	3*	2,3	2,3*	0	0*	2,3	2,3*
Substance No.[2]	IX	X	XI	XII	XIII	XIV	XV	XVI
m/e								
43	100	100	100	100	100	100	100	100
44	4	4	4	5	4	4	3	3
45	4	4	4	4	31	29	33	30
59								
60	4	4						
69			2		30	1	9	
70	4	4	3	2		26	15	10
71	1			3	8	10	4	17
72	4	4						
73								
74								
75	5	5						
76								
77								
78			4	3				
81	19	19	18	19				
82	21	21	20	21				
83					7	8	5	5
85					8	6	4	4
87					8	7	8	7
88								
99								
101					17	8	9	8
102	14					9		
103	5	18	5	6				
104								
105			10				12	3
106				10				15
113	14	14			7	6		2
114	39	38						
115	3	5						
116			12	12			2	2
117	10		35	31	35		4	4
118		10				32		
120			9				33	4
121				9				33
126	2							
127	5	2						
128	2	5						
129		2	2	2	13	12	12	12
130			4	2				

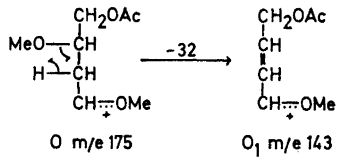
Table 1. Continued.

131			2	4				
132				1				
133								
135								
136								
142	2	2						
143					31		13	
144	1					33	24	12
145	2	4	4	5			2	23
146								4
155	3	3			3	3		
156	9	9						
158			3	4			1	1
159			8	9			2	2
161					3	3	2	2
174	2	1						
175		1			3			
179						4	2	
180								1
187					5	5		
190							2	1
191							3	3
202	2	2	2	1				
203				1				
205		1						
207			1					
208				1				
215	1	1						
216	7	7						
217	1	1						
219			6	7	2	2		
223							2	2
275								
276	3	3						
279			3	4				

Hexitol acetate	2-deoxy 3-OMe						3-deoxy 2-OMe	
Deuterated at[1]	0	0*	2	2*	2,3	2,3*	0	0*
Substance No.[2]	I	II	III	IV	V	VI	VII	VIII
m/e								
43	100	100	100	100	100	100	100	100
44	5	3	14	13	13	10	3	3
45							2	2
59							2	2
60							1	1
69								
70			4					3
71	155	12	60	4				
72	7	150	100	53				3
73		12	10	95				
74				8	46			
75					98	63		
76					7	100		
77						7		
78								
81							30	27

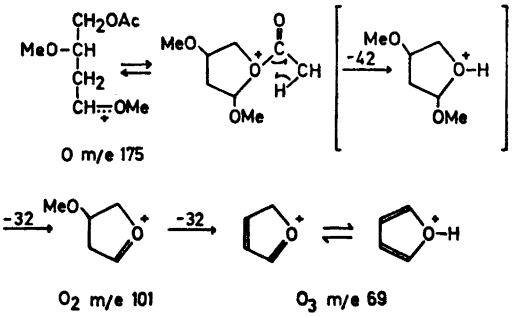
Table 1. Continued.

82								
83								
85								
87								
88								
99							3	3
101							12	
102								11
103							2	5
104								
105								
106								
113							38	41
114							4	5
115							1	2
116								
117							9	
118								8
120								
121								
126							7	
127							1	7
128								2
129								
130								
131	20							
132		21	21					
133				21				
135					24			
136						26		
142								
143								
144								
145							2	2
146								
155							10	10
156								
158								
159								
161								
174								
175								
179								
180								
187								
190								
191								
202								
203								
205								
207								
208								
215							8	8
216							1	1
217								
219								
223								
275							3	3
276								
279								



O m/e 175 O_1 m/e 143

A secondary fragment, O_2, *m/e* 101, could conceivably be derived from O_1 by the elimination of ketene. However, since ketene usually only is eliminated from *O*-acetyl linked to unsaturated carbon atoms, this possibility is slight. Another secondary fragment (f_2''),[2] with the same mass number, *m/e* 101, is obtained from the primary fragment *m/e* 161, by the loss of acetic acid. Inspection of Table 1 reveals that this fragment is obtained from XIII as well as from its deuterated analogues and is, as expected, unaffected by the deuterium labelling at C-1, C-3, or at the C-2 methoxyl. It seems reasonable to assume that for the deuterated analogues, the entire peak at *m/e* 101 is due to f_2'', since O_2 is shifted to higher values on account of the labelling. If this is correct, it is evident that deuterium at C-1, C-3, and at the methoxyl at C-2 is retained in O_2, which consequently cannot be derived from O_1. An alternative fragmentation sequence, starting from a cyclic form of O, may also explain these results, as indicated below.

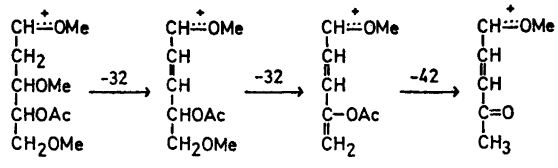


O m/e 175

O_2 m/e 101 O_3 m/e 69

The abundant fragment O_3, *m/e* 69, is derived from O_2 by the elimination of methanol. As a deuterium atom at C-1 is retained, while a deuterium atom at C-3 is only partially retained, the methanol arises essentially as a result of elimination between C-2 and C-3.

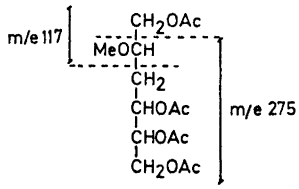
The primary fragment P, *m/e* 219, is obtained from 3-deoxy-2,4,6-tri-*O*-methyl-D-*arabino*-hexitol acetate (XIII) by fission between C-1 and C-2. This is confirmed by the retention of deuterium labelling in positions other than C-1.



P m/e 219 P_1 m/e 187 P_2 m/e 155 P_3 m/e 113

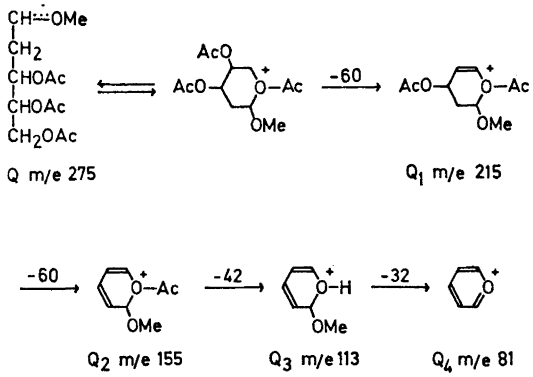
The peaks P_1, *m/e* 187, P_2, *m/e* 155, P_3, *m/e* 113 represent secondary fragments arising from P and are formed by consecutive eliminations of methanol and ketene. The methoxyl group at C-2 is retained in these fragments which are assumed to be acyclic.

The primary fragment Q, *m/e* 275, is obtained from 3-deoxy-2-*O*-methyl-D-*arabino*-hexitol acetate (VII) by fission between C-1 and C-2.



VII

The results with the deuterated analogues of VII are in agreement with the postulated structure for Q. A number of secondary fragments, Q_1, *m/e* 215, Q_2, *m/e* 155, and Q_3, *m/e* 113, are clearly derived from Q, as they are not affected by deuteration at C-1, but are shifted to higher mass number when the methoxyl at C-2 is replaced by a trideuteromethoxyl group. A deuterium atom at C-3 is also partially retained in these fragments. These fragments are formed by consecutive eliminations of acetic acid and thus could be formed analogously to P_1, P_2 and P_3. The abundant fragment Q_4 (*m/e* 81) is most probably derived from Q_3 by elimination of methanol. It is not formed from P_3, hence P_3 and Q_3 must have different structures. An alternative fragmentation sequence, starting from a cyclic isomer of Q, is therefore indicated, as shown below. The fragment P, which does not carry an acetoxyl group at C-6, will not undergo these reactions.



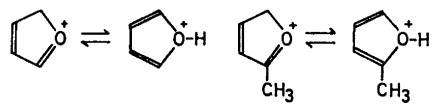
DISCUSSION

The results reported above confirm the observation that, on mass spectrometry, fission between the methylene carbon and an adjacent acetoxylated carbon in deoxyalditol acetates is insignificant. Fission between the methylene

carbon and a methoxylated carbon atom is preferred, but is less important than fission between a methoxylated and an acetoxylated carbon atom.

Several sizable fragments appear in the mass spectra of partially methylated deoxyalditol acetates, which are not observed for the corresponding non-deoxy derivatives, the most important being *m/e* 69 and *m/e* 81. These are best formulated as the cyclic ions, O_3 and Q_4. It is assumed that the corresponding primary fragments O and Q, which carry an *O*-acetyl at an appropriate distance from the formal carbonium ion, are in equilibrium with cyclic ions, which by elimination of methanol, acetic acid, and ketene give rise to the secondary fragments. Whether O_2 is derived by consecutive eliminations of ketene and methanol from O or by a single elimination of methyl acetate is difficult to ascertain. Although the proposed reaction schemes are in agreement with the results of the deuterium labelling experiments, they must nevertheless be regarded as hypothetical and only representing reasonable alternatives.

3,6-Dideoxyhexitol acetates, *e.g.* abequitol acetate [4] on mass spectrometry, give fragments at *m/e* 69 and *m/e* 83, which next to the base peak, *m/e* 43, are the strongest ions in the spectrum. In the light of the present results, they should be formulated as O_3 and its methylated analogue.



O_3 m/e 69 m/e 83

Similarly, the ions O_3 and Q_4 give peaks of high intensity in the mass spectrum of a 3-deoxy-D-*ribo*-hexitol acetate. It seems reasonable that also other, four carbon or longer fragments from alditol derivatives should form cyclic ions. The equilibrium between the cyclic and acyclic forms should be influenced by stereochemical factors. Mass spectra of stereoisomeric alditol derivatives are not very different, but it is possible that careful examination of the spectra of stereoisomers may reveal differences, due to these effects.

EXPERIMENTAL

General methods. Concentrations were performed at reduced pressure. Melting points are corrected. Optical rotations were determined at room temperature using a Perkin-Elmer 141 polarimeter. Gas-liquid chromatography was carried out on a Perkin-Elmer model 900 instrument fitted with a 3 % nitrile silicone-polyester copolymer (ECNSS-M) column. The separations were run at 170°. For gas liquid chromatography-mass spectrometry, the various alditol acetates, in acetone solution, were injected on an ECNSS-M column fitted in a Perkin-Elmer 270 gas chromatograph-mass spectrometer. The spectra were recorded at a manifold temperature of 200°, an ionisation potential of 60 eV, an ionisation current of 80 μA, and an ion source temperature of 80°.

Methylations were performed by treating the appropriate glycoside with methylsulphinyl sodium and methyl iodide or trideuteromethyl iodide in methyl sulphoxide as devised by Hakomori.[5]

Hydrolyses of 2-deoxyhexosides were performed by treatment with 0.3 M aqueous sulphuric acid for 15 min at 100°. 3-Deoxyhexosides were hydrolysed as above for 12 h.

Table 2. Physical constants of deoxy sugar derivatives. (literature values in brackets).

Substance	Derived alditol acetate	m.p.	$[\alpha]_D$	Ref.
Methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*hexopyranoside		127–129° (118–120, 127–129°)	$[\alpha]_D$ 153° (*c*, 0.2 $CHCl_3$) ($[\alpha]_D^{19}$ 156° (*c*, 0.65 $CHCl_3$))	6
Methyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-*ribo*-hexopyranoside	I, II	(99–100°)	($[\alpha]_D^{16}$ 126,8° (*c*, 0.707 $CHCl_3$))	9
Methyl 4,6-*O*-benzylidene-2-deoxy-2-deutero-α-D-*altro*-pyranoside		128–129°	$[\alpha]_D$ 148° (*c*, 0.57 $CHCl_3$)	
Methyl 4,6-*O*-benzylidene-2-deoxy-2-deutero-3-*O*-methyl-α-D-*altro*-pyranoside	III, IV	99–100°	$[\alpha]_D$ 131° (*c*, 0.18 $CHCl_3$)	
Methyl 4,6-*O*-benzylidene-2-deoxy-2-deutero-3-*O*-trideuteromethyl-α-D-*altro*-pyranoside	V, VI	98–100°	$[\alpha]_D$ 133° (*c*, 0.43 $CHCl_3$)	
Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*arabino*-hexopyranoside		108–110° (111–112°)	$[\alpha]_D$ 102° (*c*, 0.43 $CHCl_3$) ($[\alpha]_D^{22}$ 107° (*c*, 0.94 $CHCl_3$))	7
Methyl 4,6-*O*-benzylidene-3-deoxy-2-*O*-methyl-α-D-*arabino*-hexopyranoside	VII, VIII	88–89° (86–87°)	$[\alpha]_D$ 86° (*c*, 0.33 $CHCl_3$) ($[\alpha]_D^{12}$ 90° (*c*, 0.91 $CHCl_3$))	8
Methyl 4,6-*O*-benzylidene-3-deoxy-3-deutero-α-D-*altro*-pyranoside		107–110°	$[\alpha]_D$ 99° (*c*, 0.53 $CHCl_3$)	
Methyl 4,6-*O*-benzylidene-3-deoxy-3-deutero-2-*O*-methyl-α-D-*altro*pyranoside	IX, X	87–89°	$[\alpha]_D$ 91° (*c*, 0.25 $CHCl_3$)	
Methyl 4,6-*O*-benzylidene-3-deoxy-3-deutero-2-*O*-trideuteromethyl-α-D-*altro*pyranoside	XI, XII	85–87°	$[\alpha]_D$ 84° (*c*, 0.40 $CHCl_3$)	
Methyl 3-deoxy-2,4,6-tri-*O*-methyl-α-D-*arabino*-hexopyranoside	XIII, XIV	Syrup (Syrup)	$[\alpha]_D$ 107° (*c*, 0.23 MeOH) ($[\alpha]_D^{20}$ 113° (*c*, 2.06 MeOH))	9
Methyl 3-deoxy-3-deutero-2-*O*-trideuteromethyl-4,6-di-*O*-methyl-α-D-*altro*-pyranoside	XV, XVI	Syrup	$[\alpha]_D$ 103° (*c*, 0.20 MeOH)	

Preparation of alditol acetates. The reducing sugars were treated with either sodium borohydride in water or with sodium borodeuteride in deuterium oxide. After 3 h at room temperature, the solutions were treated with excess Dowex 50 (H^+), filtered and concentrated to dryness. Boric acid was removed by codistillation with methanol. The resulting alditols were acetylated by treatment with acetic anhydride and pyridine (1:1) at 100° for 15 min.

Preparation of substances. The various substances, from which the corresponding alditol acetates were prepared are listed in Table 2. Apart from the introduction of deuterium at methylene positions and in methoxyl groups, they are all known and were prepared by standard procedures as described in the various references. Trideuteromethyl groups were introduced by methylating with trideuteromethyl iodide as described above; the deuterium at methylene carbons was introduced by opening of the appropriate 2,3-oxirane rings with lithium aluminium deuteride.

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