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Mass Spectra of Partially Methylated Alditol Acetates

Part IV. Deuterium Labelling Experiments on Some Higher Fragments

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The origin and further fragmentation of three isomeric primary fragments of *m/e* 233, obtained on mass spectrometry of certain partially methylated alditol acetates, has been studied using the technique of deuterium labelling. Detailed fragmentation mechanisms are postulated.

In previous studies on mass spectrometry of partially methylated alditol acetates,[1–3] the origin of primary fragments, formed by fission of the alditol chain, and their further fragmentation into secondary fragments have been investigated. These studies, in which deuterium labelling techniques were applied, have now been extended and in the present paper, similar studies on three isomeric primary fragments of *m/e* 233 are reported.

RESULTS

Three isomeric fragments of *m/e* 233, L_1, L_2, and L_3, have been obtained on mass spectrometry of partially methylated alditol acetates. L_1 is obtained

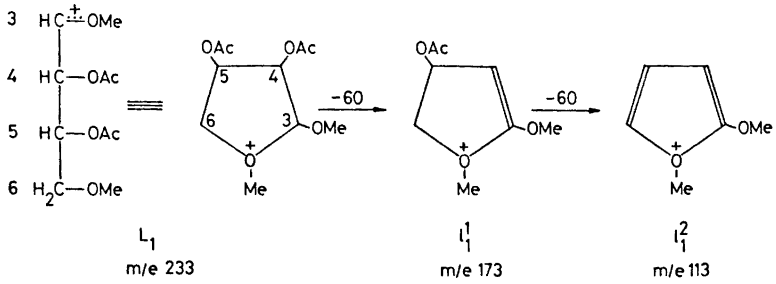


Table 1. Fragments obtained on MS of partially methylated hexitol acetates and some of their deuterated analogues. [a]

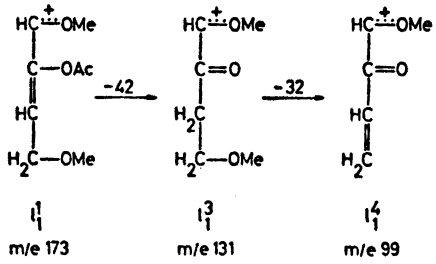
Hexitol acetates methylated in positions	2,3,6					1,2,3,5				2,3,4			
Substance [b]	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Deuteration [c]	0	0 *	6	6 *	0 **	0	0 *	1,5	1,5 *	0	0 *	4	4 *
m/e													
45	20	18	7	7	11	44	26	20	21	11	10	6	5
46					14		28		9				
48			13	14				29	11				
49									27				
59	9	7	5	5		17	9		9				
60							15	9					
61								6	13				
62								11	5				
63									11				
85	6	5	4	4	4	11	11	11	13				
87	17	14	16	16	20	8	9	7	6	21	21	9	
88		4	7	11	7	9	7	7	6	9	7	7	6
89						15	6						
90							13	4	4			16	13
91								7	5				
92								15	4				
93									15				
99	16	15	14	12	8	14	16	12	15	26	30	9	6
100					20								
101	20		31	9	29	58	51	7	9	45	15	45	
102		19	13	38		5	9	4	10	5	46	27	50
103						4	8	4	10				
104							5	60	62				
113	28	26			8								
114					30								
116			29	49									
117	50		60	9	61	61	63	8	7	32		39	
118		49	6	60	6	4	5				34		29
120								63	69				
127						14	17	16	17				
129	9	8	11	11	11					24	28		
131	9	7											
132					8							29	21
133			9	12		7							
134							7						
136								9					
137									7				
161	5		7		6					11		16	
162		6		9							16		14
173	7	6								4	5		
174					8								
176			5	7								5	4
189										8	10		
192												10	8
201						11	10	11	14				
233	16	15				19	16			4	4		
234					26								
236			22	25				22	22			4	6

[a] Only fragments pertinent to the present investigation are included. [b] Substances I–XIII are D-glucitol derivatives. [c] 0 indicates no trideuteriomethoxyl group; 2 indicates a trideuteriomethoxyl group at C-2, *etc.*; 0 *, 4 *, *etc.* indicate that one of the hydrogen atoms are C-1 is replaced by a deuterium atom; and 0 ** that one hydrogen atom at C-6 is replaced by a deuterium atom.

from hexitol acetates, methylated in the 2-, 3-, and 6-positions, such as I (Table 1). When the 6-position is trideuteriomethylated (III) the fragment is shifted to *m/e* 236. Introduction of a deuterium atom at C-1 (II, IV) does not affect the fragment.

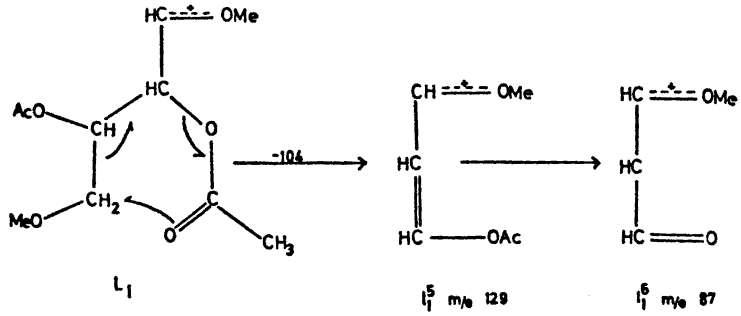
Secondary fragments l_1^1, *m/e* 173 and l_1^2, *m/e* 113, are formed from L_1 by two consecutive eliminations of acetic acid, and it is assumed that these fragments are cyclic.

Another route, starting from the non-cyclic form of l_1,[1] gives l_1^3 131 and l_1^4 99, by consecutive elimination of ketene and methanol.



The suggested route to l_1^2 is corroborated by the partial shift of l_1^2 from *m/e* 113 to *m/e* 114 when a deuterium atom is introduced at C-6. Both the suggested routes are in accordance with the labelling experiments. On introduction of a trideuteriomethoxyl group at C-6 (III – IV), l_1^1, l_1^2, and l_1^3 are shifted to the higher mass numbers but l_1^4 is unaffected.

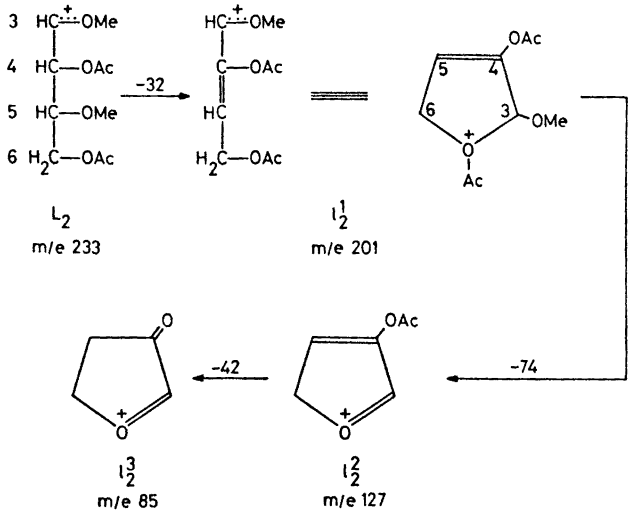
The fragments l_1^5, *m/e* 129, and l_1^6, *m/e* 87, may be formed from L_1 by consecutive eliminations of methoxymethyl acetate (104) and ketene. This mechanism is supported by the fact that neither a trideuteriomethoxyl at C-6 (III, IV) nor a deuterium atom at C-1 or C-6 are retained in l_1^5 or l_1^6. The McLafferty type elimination of methoxymethylacetate has been observed for another fragment, K_2, in which a similar situation exists.



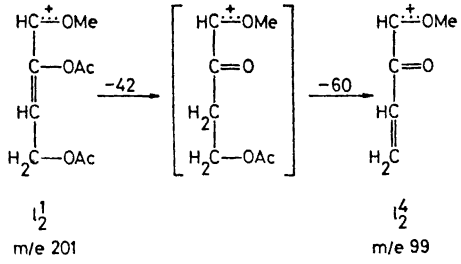
The fragment L_2, *m/e* 233, is formed from 4,6-di-*O*-acetyl-1,2,3,5-tetra-*O*-methyl-D-glucitols (VI). Of the different primary fragments obtained from VI all but *m/e* 277, *m/e* 233, and *m/e* 117 should retain a deuterium atom introduced

at C-1 (VII), which was useful for the identification of secondary fragments derived from L_2. On introduction of a trideuteriomethoxyl group at either C-3 or C-5, L_2 shifted to *m/e* 236.

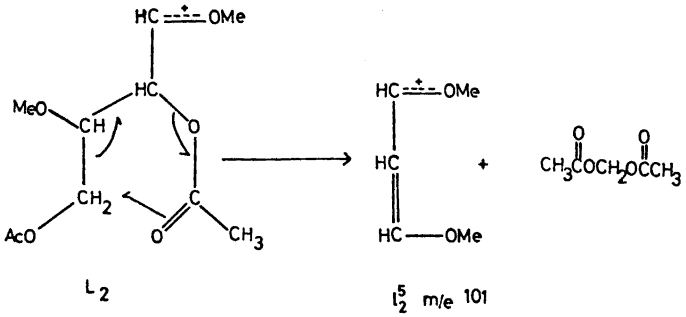
Elimination of methanol from L_2 yields l_2^1, *m/e* 201. As expected, introduction of a trideuteriomethoxyl group at C-5 does not affect l_2^1. Further eliminations of methyl acetate and ketene, assumed to proceed from cyclic ions, yield l_2^2, *m/e* 127 and l_2^3, *m/e* 85.



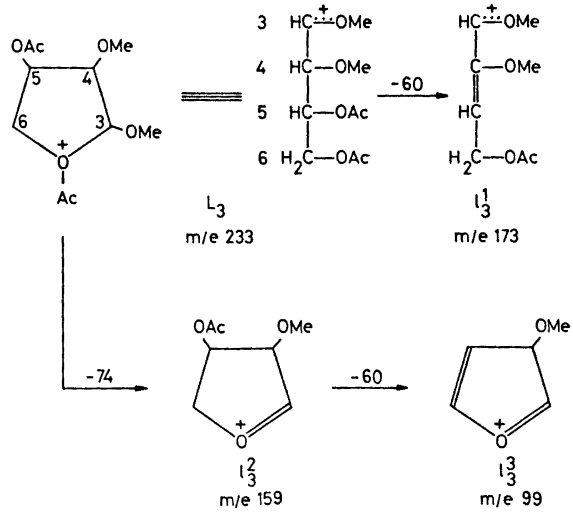
The formation of l_2^4, *m/e* 99, by consecutive eliminations of ketene and acetic acid *via* a non-cyclic route, is analogous to the sequence $l_1^1 - l_1^3 - l_1^4$, discussed above.



The secondary fragment l_2^5 (*m/e* 101) must be derived from either L_2 or the larger fragment, *m/e* 277, since it is not affected by deuterium labelling at C-1 (substance VII). The most plausible mechanism is elimination of acetoxymethyl acetate analogous to elimination of methoxymethyl acetate from L_1, as discussed above. Inspection of mass spectra of 2,4-di-*O*-methyl-pentitol acetates and 2,4-di-*O*-methyl-hexitol acetates, which do not give *m/e* 277, showed that these substances also give the fragment *m/e* 101.



The L_3 fragment is obtained from 1,5,6-tri-*O*-acetyl 2,3,4-tri-*O*-methyl-D-glucitol (X). The trideuteriomethoxyl group at C-4 (XII, XIII) is retained in the fragment but not the deuterium atom at C-1 (XI, XIII). The secondary fragment l_3^1 is obtained from L_3 by elimination of acetic acid.



Consecutive elimination of methyl acetate and acetic acid from L_3 yields l_3^2, *m/e* 159 and l_3^3, *m/e* 99. In all these fragments, the methoxyl group at C-4 in the alditol derivative is retained, as revealed by the deuterium labelling.

DISCUSSION

The unpaired electron and the positive charge on the molecular ion of a partially methylated alditol acetate are separated on fission of the alditol chain, which gives a positive ion (primary fragment) and a radical. For this reason the further reactions of the primary fragment are rather simple. The following types of reactions have been observed.

1. Elimination of methanol or acetic acid when a methoxyl or acetoxyl group is in the β-position to the formal carbonium ion.

2. Elimination of acetic acid, when an acetoxyl group is in α-position to the formal carbonium ion may also occur.[2] An analogous elimination of methoxymethyl acetate has been indicated for two ions, K_2[2] and L_1.

3. Double bonds are formed by the above eliminations, and when an acetoxyl is linked to an unsaturated carbon atom, ketene is eliminated with the formation of a carbonyl group.

4. Methanol and acetic acid are eliminated when a methoxyl or acetoxyl group, respectively, is in the β-position to a carbonyl group.

5. Elimination of formaldehyde, which is unusual, has been discussed previously.[2]

6. Some fragmentations are assumed to proceed *via* cyclic ions. When the methoxyl group, linked to the formal carbonium ion, is eliminated as methyl acetate ($l_2^1 \rightarrow l_2^2$, $l_3^1 \rightarrow l_3^2$) this pathway seems to be the most likely. There are other sequences of reactions which are also best explained by assuming the existence of cyclic ions, *e.g.* $L_1 \rightarrow l_1^1 \rightarrow l_1^2$.

The different fragmentation mechanisms discussed above should be looked upon as reasonable assumptions used to rationalize the observed fragmentations.

EXPERIMENTAL

General methods. GLC was carried out at 170° on a Perkin-Elmer 900 instrument fitted with a 3 % nitrile-polyester copolymer (ECNSS-M) column. For GLC-MS the same column in a Perkin-Elmer 270 gas chromatograph-mass spectrometer was used. The spectra were recorded at a manifold temperature of 200°, an ionization potential of 70 eV, an ionization current of 80 μA and an ion source temperature of 80°.

Methylations were performed by the Hakomori[4] or, for some substances, the Purdie[5] methods. Glycosides were hydrolysed in 0.3 M sulphuric acid for 12 h at 100°, followed by neutralisation with barium carbonate and concentration.

Alditols were prepared from the reducing sugars by treatment of an aqueous solution with an excess of sodium borohydride (borodeuteride) for 3 h, addition of excess of Dowex 50 (H^+), filtration and concentration. Boric acid was removed by codistillations with methanol and the resulting alditols were acetylated by treatment with acetic anhydride-pyridine (1:1) at 100° for 15 min.

Syntheses of alditol acetates I-XIII. Alditol acetates I and II were prepared from 2,3,6-tri-*O*-methyl-D-glucose,[6] alditol acetates X and XI from 2,3,4-tri-*O*-methyl-D-glucose.[7] Alditol acetates XII and XIII were prepared from the corresponding sugar, obtained on trideuteriomethylation of methyl 2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside,[8] followed by detritylation and hydrolysis.

Alditol acetates VI and VIII were prepared from 4,6-di-*O*-benzyl-2,3-di-*O*-methyl-D-glucitol (A, see below) by methylation or trideuteriomethylation, respectively, followed by catalytic hydrogenation and acetylation. VII and IX were prepared analogously, but from a D-glucitol derivative containing a deuterium atom at C-1.

The sugar which on reduction and acetylation yielded III and IV was prepared from methyl 4-*O*-benzyl-2,3-di-*O*-methyl-α-D-glucopyranoside (B, see below) by trideuteriomethylation, catalytic hydrogenation and hydrolysis.

Reduction of the aldehyde C (see below) with sodium borodeuteride yielded an analogue of B, containing a deuterium atom at C-6. The preparation of V from this substance was analogous to the synthesis of III, discussed above.

The identities of all the alditol acetates were confirmed by the primary fragments obtained on mass spectrometry. All the methyl glycosides obtained as intermediates gave NMR spectra in agreement with the postulated structures.

4,6-Di-O-benzyl-2,3-di-O-methyl-D-glucitol (A) was prepared from 4,6-di-*O*-benzyl-2,3-di-*O*-methyl-α-D-glucoside.[8] This substance (230 mg) was hydrolysed in a mixture of acetic acid (4.3 ml) and 0.6 M sulphuric acid (2.4 ml) for 12 h at 100°, neutralised with barium carbonate and concentrated. The purity of the product (178 mg) was checked on TLC (silica gel, petroleum ether/ethyl acetate 1:2). Hydrogenation of A, using palladium on carbon as a catalyst, followed by reduction with sodium borodeuteride and acetylation gave a product that was indistinguishable from 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol on GLC, and gave the expected mass spectrum.

Methyl 4-O-benzyl-2,3-di-O-methyl-α-D-glucoside (B). Methyl 2,3-di-*O*-methyl-6-*O*-trityl-α-D-glucopyranoside [6] (200 mg) was benzylated by treatment with sodium hydroxide (500 mg) and benzyl chloride (5 ml) for 48 h at room temperature.[9] The reaction mixture was poured into water and extracted with chloroform. The chloroform layer was concentrated and volatile products in the residue removed by repeated distillations with water. The crude reaction mixture (290 mg) was detritylated by treatment with excess hydrogen bromide in glacial acetic acid [11] for 5 minutes. The reaction mixture was then neutralised with sodium carbonate – ice, and extracted with chloroform. The product was isolated by chromatography on a silica gel column, irrigated with chloroform/acetone (1:1). The product was recrystallized from hexane, yielding the pure substance (98 mg), m.p. 95 – 96° and $[\alpha]_D = 133.6$ (*c* 0.25 chloroform). (Found: C 61.6; H 7.62; O 30.6. Calc. for $C_{16}H_{24}O_6$: C 61.5; H 7.75; O 30.7.)

Methyl 4-O-benzyl-2,3-di-O-methyl-α-D-gluco-hexodialdo-1,5-pyranoside (C). B (100 mg) was oxidized in a mixture of methyl sulphoxide (0.35 ml), benzene (0.1 ml) pyridine (0.02 ml), orthophosphoric acid (85 %, 0.01 ml) and *N,N'*-dicyclohexylcarbodiimide (250 mg) by stirring for 5 h at room temperature.[10] The reaction mixture was filtered and stirred with oxalic acid (0.1 g) in methanol (0.25 ml) for 1 h, filtered and the filtrate washed with aqueous sodium hydrogen carbonate. The aqueous phases were washed with ethyl acetate, the organic phases were combined, dried, evaporated and dissolved in acetone. After filtration (to remove *N,N'*-dicyclohexylurea) and evaporation, the resulting syrup was fractionated by preparative thin layer chromatography (silica gel, ethyl acetate/toluene, 1:1), yielding C (77 mg) as a syrup. NMR was in agreement with the postulated structure and showed, *inter alia*, a one proton signal at τ 0.16 (singlet [10]), for the aldehyde proton. Reduction of C with sodium borohydride produced B, indistinguishable from the starting material.

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REFERENCES

1. Björndal, H., Lindberg, B. and Svensson, S. *Carbohyd. Res.* **5** (1967) 433.
2. Björndal, H., Lindberg, B., Pilotti, Å. and Svensson, S. *Carbohyd. Res.* **15** (1970) 339.
3. Borén, H., Garegg, P., Lindberg, B. and Svensson, S. *Acta Chem. Scand.* **25** (1971) 3299.
4. Hakomori, S. *J. Biochem. (Tokyo)* **55** (1964) 205.
5. Purdie, T. and Irvine, J. C. *J. Chem. Soc.* **83** (1903) 1021.
6. Robertson, G. J. *J. Chem. Soc.* **1933** 737.
7. Robertson, A. and Waters, R. B. *J. Chem. Soc.* **1931** 1709.
8. Freudenberg, K. and Plankenhorn, E. *Ber.* **73** (1940) 621.
9. Tate, M. E. and Bishop, C. T. *Can. J. Chem.* **41** (1963) 1801.
10. Horton, D., Nakadate, M. and Tronchet, J. M. J. *Carbohyd. Res.* **7** (1968) 56.
11. Barker, G. R. *Methods Carbohyd. Chem.* **2** (1963) 169.

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