Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

MASS SPECTRA OF PARTIALLY METHYLATED ALDITOL ACETATES
PART II. DEUTERIUM LABELLING EXPERIMENTS*

HÅKAN BJÖRNDAL, BENGT LINDBERG, ÅKE PILOTTI, AND SIGFRID SVENSSON
Institutionen för organisk kemi, Stockholms Universitet, Stockholm (Sweden)
(Received May 8th, 1970; accepted for publication, May 29th, 1970)

ABSTRACT

The fragmentation pattern of some partially methylated alditol acetates on electron impact has been studied by using the technique of deuterium labelling. Detailed fragmentation mechanisms are postulated.

INTRODUCTION

In a previous publication[1], mass spectra of a number of partially methylated alditol acetates from hexoses, 6-deoxyhexoses, and pentoses were reported. This information has subsequently been used for the g.l.c.–m.s. identification[2] of methylated sugars obtained on methylation analysis of different polysaccharides. During the course of these studies, m.s. for other partially methylated alditol acetates, *e.g.*, those from methyl ethers of 3,6-dideoxyhexoses, have been determined. The application of g.l.c.–m.s. in methylation analysis has been summarised[3]. The methoxyl substitution pattern of a partially methylated alditol acetate may be determined either by comparison of its m.s. with that of an authentic sample, or by examination of the typical primary fragments obtained by fission between two carbon atoms in the alditol chain. No significant differences in the m.s. of stereoisomers was observed.

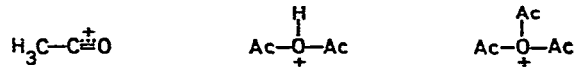
The present paper reports studies of the origin of the primary fragments and also of the secondary fragments formed from these by single or consecutive elimination of acetic acid (60), ketene (42), methanol (32), and formaldehyde (30), using the technique of deuterium labelling.

RESULTS

The most-intense signal in m.s. of partially methylated alditol acetates is generally, but not always, *m/e* 43, the acetylium ion derived from the acetate groups by α-cleavage. For alditol acetates and peracetylated monosaccharides[4], this ion is accompanied by diacetyl and triacetyl oxonium ions, *m/e* 103 and *m/e* 145, respectively. In partially methylated alditol acetates, the acetylium ion is less prominent and the

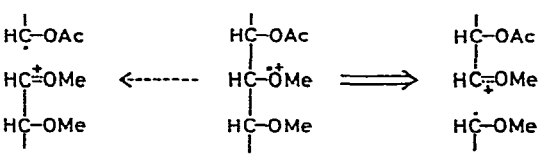
*Dedicated to Professor F. Micheel in celebration of his 70th birthday.

diacetyl and triacetyl oxonium ions are insignificant, except when several adjacent acetyl groups are present as in 1,2,3,4,5-penta-*O*-acetyl-6-*O*-methyl-D-glucitol.



It is reasonable to assume that the charge of the molecular ion obtained from a partially methylated alditol acetate is essentially located on an ether oxygen atom (as in structures **1** and **2**) and not on an ester oxygen atom (as in structures **3** and **4**).

In agreement with this assumption, the fissions indicated for **1** and **2** are significant but not those indicated for **3** and **4**. Further, the fission of **1** is preferred over fission of **2** because the methoxyl radical formed seems to be better stabilized than the acetoxyl radical. Therefore, in structure **5**, the cleavage between the methoxylated carbon atoms predominates. One exception to this rule will be discussed below.



The smallest primary fragment *A*, *m/e* 45, is given by substances having a primary methoxyl group, *e.g.*, a hexitol methylated in the 6-position. Replacement of this methoxyl by a trideuteriomethoxyl group results in the expected shift to *m/e* 48 (for this and other examples, see Table I). The shift is not complete, since a residual signal at *m/e* 45 is still observed. The nature of this fragment is not known but it is also observed in the m.s. of partially methylated alditol acetates lacking a primary methoxyl group. The rather low abundance of the primary fragment *A* ($\simeq$ 15% of the base peak, *m/e* 43) relative to the corresponding fragment *B*, *m/e* 59 (80–90%), obtained from a 6-deoxyhexitol methylated in the 5-position, reflects the low stability of a primary carbonium ion.

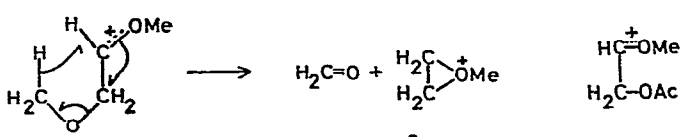
A m/e 45 B m/e 59

The primary fragment *C*, *m/e* 89, is obtained when a primary and a secondary methoxyl group are adjacent (hexitols methylated in the 5- and 6-position; *e.g.*, substance VII, Table I), by fission between the methoxylated and the acetoxylated carbon atoms (C-5 and C-4). This fission is favoured over that between the methoxylated carbon atoms (C-5 and C-6) and occurs significantly only in the present situation, reflecting the decreased stability of the primary ion formed in the latter fission. Accordingly, when the 6-position (substance VIII) was trideuteriomethylated, *m/e* 89 shifted to *m/e* 92, and when both the 5- and the 6-position were trideuteriomethylated (substance XII), it shifted to *m/e* 95.

A secondary fragment c_1, *m/e* 59, is formed from *C* by loss of formaldehyde. As expected, this fragment shifted from *m/e* 59 to *m/e* 63 when both the 5- and 6-position (substance XII) were trideuteriomethylated. However, when only the 6-position was trideuteriomethylated (substance VIII), *m/e* 59 disappeared, and instead two fragments of equal intensities, at *m/e* 60 and 62, were observed. This could be explained by a tautomeric equilibrium between two identical forms of *C*, which become distinguishable when one of the methoxyl groups is deuterated.

C m/e 89

This formaldehyde elimination is probably favoured by the formation of an oxironium ion as indicated below.



C m/e 89 c_1 m/e 59 D m/e 117

The primary fragment *D*, *m/e* 117, is obtained from alditol derivatives having an acetoxyl group at C-1 and a methoxyl group at C-2. By introducing a deuterium atom at C-1 (substance II), fragment *D* became *m/e* 118; on further replacement of the C-2 methoxyl with a trideuteriomethoxyl group (substance III), the signal shifted to *m/e* 121. Deuterium labelling caused no significant shift in the lower mass fragments that could be derived from *D*; it is therefore concluded that *D* is a fairly stable ion.

Two primary fragments F_1 and F_2, *m/e* 161, would be expected from 1,5-di-*O*-

TABLE I

FRAGMENTS OBTAINED ON M.S. OF PARTIALLY METHYLATED HEXITOL ACETATES AND SOME OF THEIR DEUTERATED ANALOGUES[a]

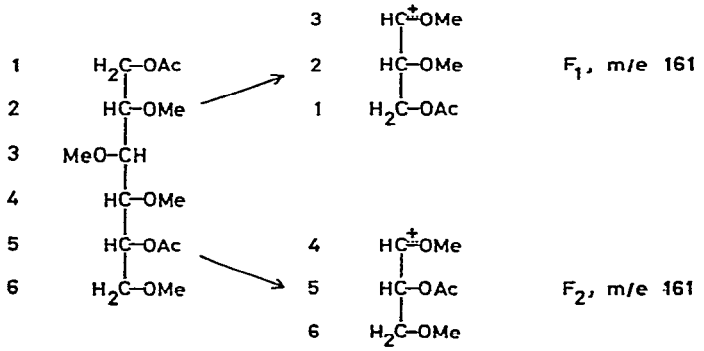
Hexitol acetates methylated in positions	*2,3,4,6*						*2,3,5,6*			*3,5,6*			*2,3*			*3*		
Substance[b]	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*	*XIV*	*XV*	*XVI*	*XVII*	*XVIII*
Deuteration[c]	*0*	*0**	*2**	*3**	*4**	*6**	*0*	*6**	*0***	*0*	*0**	*5,6**	*0*	*0**	*2,3**	*0*	*0**	*3**
m/e																		
45	36	40	30	29	33	10	29	10	10	24	24	4	5	8		5	3	3
47									12									
48			3	3	4	27		15				12						
59	4	7					24			29	26			3				
60								12	8		3			3	3			
61									29			7						
62								13				4			4			
63								3				22						
87	15	18	13	10	7	17	16	9	16	34	8	4	11	9		29	8	
88	6	12	6	6	6	9	8	9	6	11	31	28		6			16	
89	6	9					57		5	49	52			3				
90				4	11			5		4					7			9
91								7	61						2			16
92								45										
95								4				51						
99	4	5					8	5	3	11	6	4	15	21		17	14	
101	60	21	12	13		9	80	25	69	18	22		18	4				
102	7	64	4	6	46	37	6	33	4		7			18	13			13
103		7	5	4		4		3				6		4	7	5	5	9
104					17	9		10				19						
105			36	45	5			12										
108															17			
117	34	3	4	6	4	4	53	7	45	6		4	60	3		4		
118		37		29	33	36	3	32						55				
121			26	3				7							54			
127													19	15	15	22	15	16
129	32	40	23	26		34	8	4	7	65	7	6	6	4		52	3	
130	3	6	3	3		6		4		9	55	48				5	38	
131	4						6			4	7	4					3	
132					30	5		3				4			3			4
133									8			4						39
140											12	11						
143							7											
145	28	37	22	3			3			4	5			6	4			3
147									4	4								
148				25	28	31		4										
151												5						
161	31	21	12	13	6		9		8				6					
162		21		5	15	17		6						3	4			5
164				5	13	17												
165			12	13	7			3										
173							4			5	7							
175									12									
176								5				4						
189										23						20		

TABLE I (*continued*)

Hexitol acetates methylated in positions	*2,3,4,6*						*2,3,5,6*			*3,5,6*			*2,3*			*3*		
Substance[b]	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*	*X*	*XI*	*XII*	*XIII*	*XIV*	*XV*	*XVI*	*XVII*	*XVIII*
Deuteration[c]	*0*	*0**	*2**	*3**	*4**	*6**	*0*	*6**	*0***	*0*	*0**	*5,6**	*0*	*0**	*2,3**	*0*	*0**	*3**
m/e																		
190											17	17					12	
201																		13
205		9	15	7				18			19	12						
207										25								
208					9	9	10		9									
211												13						

[a]Only fragments affected by the deuterium labellings performed are included. [b]Substances I–VI and IX–XVIII are D-glucitol derivatives; substances VII–VIII are D-galactitol derivatives. [c]0 indicates no trideuteriomethoxyl group; 2 indicates a trideuteriomethoxyl group at C-2, etc.; 0*, 2*, etc. indicate that one of the hydrogen atoms at C-1 is replaced by a deuterium atom; and 0** that both hydrogen atoms at C-6 are replaced by deuterium atoms.

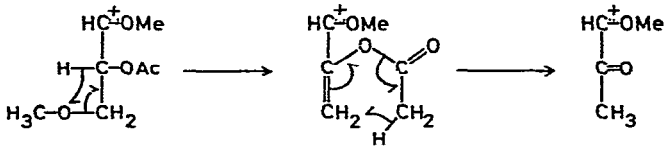
acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol by fission between C-3 and C-4. These fragments and their further reactions were investigated with deuterated analogues (substances I–VI, Table I), all of which contained one deuterium atom at C-1. Introduction of a trideuteriomethoxyl group at C-2 or C-3 did not affect F_2 (*m/e* 161) but



shifted F_1 from *m/e* 162 to 165. Analogously, introduction of a trideuteriomethoxyl group at C-4 or C-6 did not affect F_1 (*m/e* 162) but shifted F_2 to *m/e* 164. The two fragments have about the same intensities.

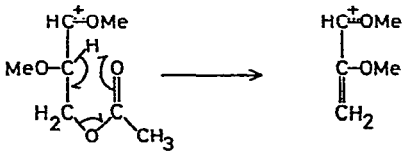
Two secondary fragments, *m/e* 129 (f_2) and *m/e* 101 (f_2''), were assumed to be derived from *m/e* 161 by elimination of methanol and acetic acid, respectively. Inspection of the values in Table I reveals that elimination of methanol could occur only from F_2; furthermore, it was the methoxyl group at C-6 that was eliminated. The secondary fragment f_2, *m/e* 129, can eliminate ketene yielding f_2', *m/e* 87. This signal shifted to *m/e* 90 when position 4 was trideuteriomethoxylated. Accordingly, no peak

of *m/e* 129 (f_2) is obtained in the m.s. of 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol, which gives F_1 but not F_2 as a primary fragment.

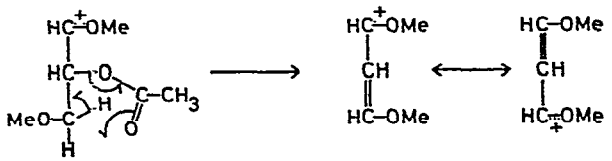


F_2 m/e 161 f_2 m/e 129 f_2' m/e 87

It is evident from Table I that elimination of acetic acid occurs both from F_1 and F_2, although the fragment obtained from F_1 is about three times more abundant than that from F_2. Acetic acid elimination from F_1, as well as methanol elimination from F_2, are facilitated by the acidity of the hydrogen atom eliminated. The elimina-



F_1 m/e 161 f_1 m/e 101



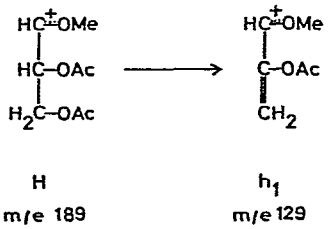
F_2 m/e 161 f_2'' m/e 101

tion of acetic acid from F_2 is probably facilitated by the formation of the symmetrical, mesomeric ion f_2''.

A secondary fragment of *m/e* 71 was asssumed[1] to be derived from f_1 and/or f_2 by loss of formaldehyde. The labelling experiments have, however, not given support for this assumption, and its origin is still uncertain.

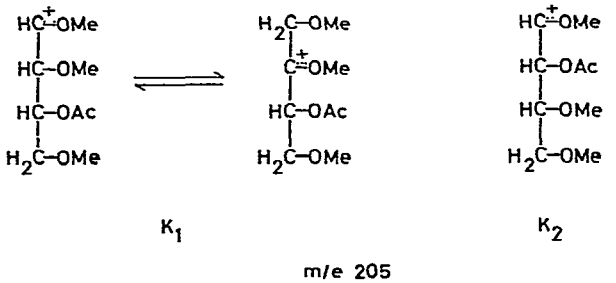
The primary fragment *H*, *m/e* 189, is derived from alditols acetylated in the 1- and 2-positions and methylated in the 3-position, *e.g.*, from 1,2,4,5,6-penta-*O*-acetyl-3-*O*-methyl-D-glucitol (substance XVI). On introduction of a deuterium atom at C-1 (substance XVII), it shifted to *m/e* 190. On replacement of the methoxyl with a trideuteriomethoxyl group in the compound deuterated at C-1 (substance XVIII), the

signal shifted to 193. The secondary fragment h_1, *m/e* 129, is formed from *H* by elimination of acetic acid. When *H* was monodeuterated at C-1 in the original alditol derivative, h_1 shifted completely to *m/e* 130. As acetic acid is certainly eliminated by



a McLafferty rearrangement[5], this result also demonstrated that it was the acetoxyl group at C-1 that was eliminated. The fragment h_1 is therefore identical with the f_2 fragment discussed above, and consequently it can further eliminate a molecule of ketene, yielding *m/e* 87.

The primary fragments K_1 and K_2, *m/e* 205, are formed, for example, from a 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol and a 1,4-di-*O*-acetyl-2,3,5,6-tetra-*O*-methylhexitol respectively. On replacement of the methoxyl group at either C-3, C-4, or C-6 with a trideuteriomethoxyl group, K_1 is shifted to *m/e* 208, and K_2 to *m/e* 211 when trideuteriomethoxyl groups are introduced at C-5 and C-6, respectively. K_1 probably exists in two tautomeric forms and produces a secondary fragment, k_1,

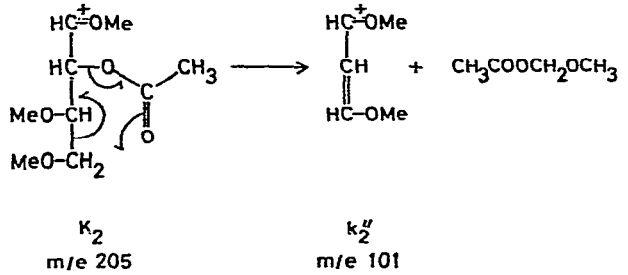


m/e 145, by elimination of acetic acid. On deuterium labelling of the methoxyl groups at either C-3, C-4, or C-6, this fragment became *m/e* 148. No other fragments derived from K_1 could be detected.

Two secondary fragments, k_2', *m/e* 173 and k_2, *m/e* 145, are formed from K_2 by elimination of methanol and acetic acid, respectively. On introduction of trideuteriomethoxyl groups at C-6, or at both C-5 and C-6, k_2' was shifted to *m/e* 176, demonstrating that the C-5 methoxyl was eliminated. On similar labelling, k_2 shifted to *m/e* 148 and 151, respectively. The fragmentation patterns of K_1 and K_2 therefore seem to be analogous to those of F_1 and F_2 discussed above.

A secondary fragment k_2'', *m/e* 101, was observed in the m.s. of 1,2,4-tri-*O*-acetyl-3,5,6-tri-*O*-methyl-D-glucitol deuterated at C-1 (substance XI). Further, by

introduction of trideuteriomethoxyl groups at both C-5 and C-6, this fragment changed to *m/e* 104. This demonstrates that only one of these methoxyl groups was



lost and the other remained in the fragment. Replacing the two hydrogen atoms at C-6 by deuterium (substance IX) did not affect fragment k_2'', and it is therefore concluded that the bond between carbons C-5 and C-6 was broken. Direct elimination of methoxymethyl acetate from K_2 by a reaction analogous to a McLafferty elimination of acetic acid would account for the observed results.

DISCUSSION

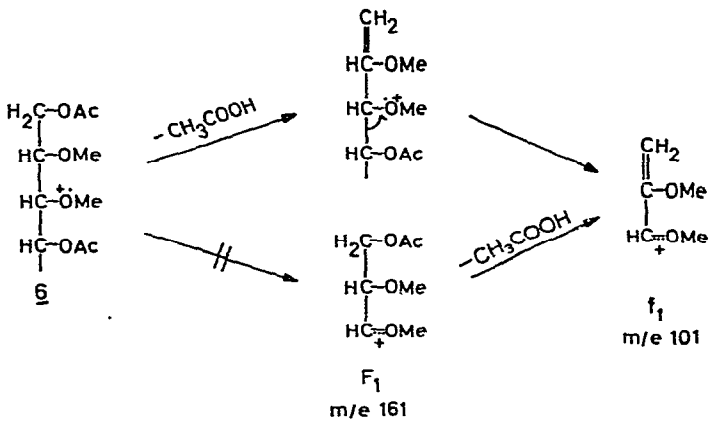
The nature of the primary fragments *A*, *m/e* 45, *C*, *m/e* 89, *D*, *m/e* 117, F_1 and F_2, *m/e* 161, *H*, *m/e* 189, and K_1 and K_2, *m/e* 205, has been confirmed by deuterium labelling experiments. The investigations have also given some information on different elimination mechanisms by which secondary fragments are formed from the primary ones. Some of these mechanisms have been studied previously[5].

The elimination of formaldehyde seems to be unusual and was only demonstrated with certainty for *C*, *m/e* 89. Fragments F_1 and K_1 have structures related to that of *C* but do not eliminate formaldehyde, probably because acetic acid elimination is more favourable.

Significant elimination of methanol is only observed when a methoxyl group is in the β-position relative to the formal carbonium ion, as in F_2 and K_2. This structural feature also exists in one of the tautomeric forms of K_1, but elimination of methanol is insignificant here because of the more facile elimination of acetic acid.

Acetic acid is readily eliminated when an acetoxyl group is in the β-position to the formal carbonium ion, as in F_1, *H*, and K_1. The signals of secondary fragments formed by this reaction always have high intensities. A number of substances having the partial structure **6** give strong signals at *m/e* 101, but only small intensities for F_1, *m/e* 161. Possibly, the main route to *m/e* 101 from these substances does not proceed *via* F_1 but by the route depicted below.

When a deuterium atom was introduced at C-1 (substance XIV), the *m/e* 101 fragment shifted to *m/e* 102; further, when both methoxyl groups at C-2 and C-3 were replaced by trideuteriomethoxyl groups, a shift from *m/e* 101 to 108 was observed (substance XV). These results are in agreement with the structure for f_1.



Acetic acid can also be eliminated when the acetoxyl group is in the α-position to the formal carbonium ion, provided that the resulting ion is stabilized by resonance, as observed for F_2 and K_2. The secondary fragments formed in these reactions have relatively low intensities.

Ketene is never eliminated directly from a primary fragment. Its elimination must be preceded by an elimination of either acetic acid or methanol and must also leave behind an acetoxyl group attached to an unsaturated carbon atom, as in the reactions of F_2 and H. A similar situation exists after elimination of methanol from K_2, and a peak at m/e 131 (205 − 32 − 42) is also observed.

The elimination of methoxymethyl acetate from K_2 (205) suggests that the fragmentation mechanisms of higher primary fragments may be considerably more complicated and will be the subject of future studies.

EXPERIMENTAL

G.l.c. was performed with a Perkin–Elmer model 900 instrument fitted with a 3% nitrilesilicone–polyester copolymer (ECNSS-M) column. Separations were performed at 170°. For g.l.c.–m.s., the alditol acetates, dissolved in acetone, were injected into a Perkin–Elmer 270 gas chromatograph–mass spectrometer. The mass spectra were recorded at a manifold temperature of 200°, ionisation potential of 60 eV, ionisation current of 80 μamp, and an ion-source temperature of 80°. T.l.c. was performed on silica gel G (E. Merck AG) with the solvent systems (*A*) chloroform–methanol (95:5; v/v) and (*B*) toluene–ether (1:2; v/v).

Methylations were performed by treating the appropriate glycoside with methylsulphinyl sodium–methyl iodide or trideuteriomethyl iodide as devised by Hakomori[6]. Hydrolyses were done in 0.3M sulphuric acid for 12 h at 100°. The hydrolysate was neutralised with barium carbonate, filtered, and concentrated to dryness.

Preparation of alditol acetates. — The sugars were reduced with sodium borohydride in water or with sodium borodeuteride in deuterium oxide. After 3 h, the solutions were treated with an excess of Dowex-50 (H^+) resin, filtered, and concen-

trated to dryness. Boric acid was removed by distillation of methanol from the residue. The alditols were acetylated by treatment with acetic anhydride and pyridine for 15 min at 100°.

Syntheses of the alditol acetates I–XVIII (Table I). — The alditol acetates I, II, VII, X, XI, XIII, XIV, and XVII were prepared from the corresponding methylated sugars[7].

The following alditol acetates (in brackets) were prepared from the partially substituted sugar derivatives listed below, by methylation with trideuteriomethyl iodide and subsequent hydrolysis, reduction, and acetylation: methyl 2,4,6-tri-*O*-methyl-α,β-D-glucoside(III), methyl 2,3,6-tri-*O*-methyl-α,β-D-glucopyranoside (IV), methyl 3,4,6-tri-*O*-methyl-α-D-glucoside(V), methyl 2,3,4-tri-*O*-methyl-α,β-D-glucoside(VI), 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose(XII), methyl 4,6-*O*-benzylidine-α-D-glucoside(XV), and 1,2:5,6-di-*O*-isopropylidene-α-D-glucose(XVIII). The anomeric mixtures of glucosides used for the syntheses of three of the substances above were prepared by Fischer syntheses from the corresponding methylated sugars.

Alditol acetate VIII was prepared by the following sequence of reactions: A solution of ethyl β-D-galactofuranoside (1.5 g) and triphenylmethyl chloride (2.4 g) in pyridine (6 ml) was kept for 4 days at room temperature. Pyridine (9 ml) was added and the solution cooled to 0°, after which acetic anhydride (9 ml) was added and the solution was kept at room temperature for 3 days. The solution was then poured into ice–water (300 ml) and extracted with ether (3 × 30 ml). The ether solution was washed with aqueous potassium hydrogen sulphate, aqueous sodium hydrogen carbonate, and water, dried over magnesium sulphate, and concentrated. The product was separated from triphenylmethanol by preparative t.l.c. (solvent system *B*) and crystallised from aqueous ethanol, to yield ethyl 2,3,5-tri-*O*-acetyl-6-*O*-triphenylmethyl-β-D-galactofuranoside, m.p. 133–134°, $[\alpha]_{578}^{20}$ −52° (*c* 1.0, chloroform) (Found: C, 68.9; H, 6.35. $C_{33}H_{36}O_9$ calc.: C, 68.8; H, 6.25%).

Methylation of this product, during which the *O*-acetyl groups were split off, yielded amorphous ethyl 2,3,5-tri-*O*-methyl-6-*O*-triphenylmethyl-β-D-galactofuranoside. Part of this product was hydrolysed to the sugar, and subsequently converted into the alditol acetate, which had the expected structure, as demonstrated by g.l.c.–m.s. Another part was detritylated by treatment with hydrogen bromide[8] in acetic acid, methylated with trideuteriomethyl iodide, and converted into VIII.

Alditol acetate IX was prepared from methyl α-D-glucofuranosidurono-6,3-lactone by reduction[9] with sodium borodeuteride, followed by methylation, hydrolysis, reduction, and acetylation.

All syntheses were followed by t.l.c. and, when appropriate, by g.l.c.–m.s. The structures of the different components are defined from their modes of synthesis and were also consistent with their behaviour on g.l.c.–m.s.

ACKNOWLEDGMENTS

We are indebted to the Swedish Natural Science Research Council, Harald Jeanssons Stiftelse, and Stiftelsen Sigurd och Elsa Goljes Minne for financial support.

REFERENCES

1 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Carbohyd. Res.*, 5 (1967) 433.
2 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Acta Chem. Scand.*, 21 (1967) 1801.
3 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem.*, 82 (1970) 643.
4 K. BIEMANN, D. C. DE JONGH, AND H. K. SCHNOES, *J. Amer. Chem. Soc.*, 85 (1963) 1763.
5 L. S. GOLOVKINA, N. S. WULFSON, AND O. S. CHIZHOV, *Zh. Org. Khim.*, 4 (1968) 738.
6 S. HAKOMORI, *J. Biochem.* (Tokyo), 55 (1964) 205.
7 R. L. WHISTLER, *Methods Carbohyd. Chem.*, 5 (1965) 298.
8 G. A. BARKER, *Methods Carbohyd. Chem.*, 2 (1963) 170.
9 D. D. PHILIPS, *J. Amer. Chem. Soc.*, 76 (1954) 3598.