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GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY OF METHYLATED AND DEUTERIOMETHYLATED PER-*O*-ACETYL-ALDONONITRILES FROM D-MANNOSE

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ABSTRACT

Peracetylated aldononitriles of the tetra-, tri-, and di-methyl ethers of D-mannopyranose were separated by gas–liquid chromatography, and analyzed by mass spectrometry. Through introduction of deuteriomethyl ether groups, various fragment-ions constituting the mass spectra were identified and related to the parent methylated sugar structures. Also identified were several characteristic series of fragment ions that are common to two or more methylated D-mannopyranosides. As expected, mass spectra of the D-mannose derivatives were identical to those previously observed for D-glucose methylated in the same positions. Distinctive mass spectra were also recorded for all additional di-*O*-methyl-D-mannose derivatives. This information permits use of peracetylated aldononitrile derivatives in methylation–fragmentation analysis of aldohexans.

INTRODUCTION

Aldoses are rapidly and easily converted into peracetylated aldononitriles[1,2] (PAAN), and these derivatives can be separated by gas–liquid chromatography (g.l.c.) to provide a rapid survey method for aldoses[3]. PAAN derivatives of selectively methylated D-xylose have been separated[2] by g.l.c., and the mass spectra of various, selectively methylated PAAN derivatives of D-glucose have also been reported[3].

We now describe the separation by g.l.c. of PAAN derivatives of tetra-, tri-, and di-*O*-methyl-D-mannose, and their analysis by mass spectrometry (m.s.). In addition, CD_3 groups were introduced into certain dimethyl ethers to afford tetra- and tri-*O*-methyl-D-mannose PAAN derivatives containing either one or two OCD_3 groups. The major fragment-ions identified by g.l.c.–m.s. of these deuteriomethylated compounds allow PAAN derivatives to be used in the rapid, methylation analysis of mannans on a microscale. Furthermore, as fragmentation patterns of PAAN

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derivatives depend on the location of the methoxyl groups and are independent of the sugar configuration[3], mass spectra of mannose reference compounds are applicable to the methylation analysis of aldohexans in general.

RESULTS AND DISCUSSION

After selectively methylated methyl α-D-mannopyranosides[4–7] had been hydrolyzed, the reducing sugars resulting were converted into their PAAN derivatives by successive reaction with hydroxylamine and acetic anhydride. PAAN derivatives were formed from all of the possible tetra-, tri-, and di-methyl ethers of α-D-mannopyranose. These derivatives were readily separated by g.l.c. (see Table I), and their relative retention-times were determined on 1,4-butanediol succinate[8]. Neopentyl glycol succinate[3] gave shorter retention-times, but poorer resolution. Both columns could separate all of the PAAN derivatives; however, two pairs of the tri-*O*-methyl derivatives (2,4,6- and 2,3,6-tri-*O*-methyl, and 3,4,6- and 2,3,4-tri-*O*-methyl) were difficult to resolve, and only 1,4-butanediol succinate gave complete, base-line separation for them.

TABLE I

RELATIVE G.L.C. RETENTION-TIMES, ON 5% OF BUTANEDIOL SUCCINATE, OF PERACETYLATED NITRILES OF METHYL ETHERS OF D-MANNOSE

Compound	*Methyl ether*	*Relative retention-time*
2	2,3,4,6-Tetra-	**1.00**[a]
6	2,4,6-Tri-	1.59
8	2,3,6-Tri-	1.65
4	3,4,6-Tri-	1.89
10	2,3,4-Tri-	2.03
20	2,6-Di-	2.28
22	4,6-Di-	2.45
12	2,3-Di-	2.55
18	3,6-Di-	2.85
14	2,4-Di-	3.16
16	3,4-Di-	3.68

[a]6.4 min.

Although it is possible to inject the reaction mixture directly into a g.l.c. column, the solvent front completely obscures the tetra-*O*-methyl PAAN derivative and also makes quantitative determination of the tri-*O*-methyl PAAN derivative difficult. This condition can be avoided by adding the reaction mixture to chloroform, washing twice with water, and injecting the chloroform solution. Back extraction showed that some PAAN derivative remains in the water phase. The PAAN derivatives are stable in chloroform for a month, whereas, if left in the original reaction-mixture, they degrade within a few days. Known mixtures gave a good correlation between the

originally weighed amounts of the methyl α-D-mannopyranosides and the g.l.c. detector-response to PAAN derivatives of these sugars. Quantitative errors arising from sampling such a mixture of sugars are estimated to be less than 5%.

PAAN derivatives of each sugar were injected separately, and the mass spectrum of each g.l.c. peak was recorded. The important *m/e* peaks are shown in Tables II, III, and IV. Mass spectra of partially methylated alditol acetates[9], obtained from the same free sugars by reducing the aldehyde, have been studied extensively[10,11]. Kochetkov *et al.*[3,12] suggested that mass-spectral fragmentation-paths of partially methylated PAAN sugars are similar to those of peracetylated alditols. In contrast either to partially methylated reducing-sugars or their methyl glycosides, the peracetylated alditols give mass spectra that are strikingly different from one another, but can be interpreted on the basis of a few simple rules.

TABLE II

RELATIVE INTENSITIES[a] OF FRAGMENT IONS FROM M.S. OF THE 2,3,4,6-TETRA-*O*-METHYL PERACETYLATED ALDONONITRILE (PAAN) OF D-MANNOSE AND SOME DEUTERIOMETHYL ANALOGS

m/e	*Methyl 2,3,4,6-tetra-O-methyl-D-mannononitrile*								
	2	**23**	**24**	**25**	**26**	**32**	**50**	**44**	**38**
	Deuteriomethyl position								
	None	*2*	*3*	*4*	*6*	*4,6*	*2,4*	*2,6*	*3,6*
43	83	100	100	100	100	82	92	89	100
45	71	72	61	56	23	13	61	26	74
48		5	10	10	42	63	18	59	21
59	5	5	4		3			5	4
71	12	13	9	6	8	3	6	8	8
73	6				4			8	7
74		7	5	7	7	9	11	8	10
83			9	10	4			11	
85	5	5	7	5	3			6	4
87	35	35	25	9	28	6	9	36	19
88	35	25	5	12	28	10	5	30	13
89	12	11			5				6
90				21	3	31	29		25
91		21	24	18		25	35	8	31
92			5	9	4	6	9	6	9
96	6	4	4	3	4	1		4	
99		4		5		5	8		8
101	38	37	34	5	7			5	12
104		17	4	28	24	6	37	36	31
107						32	4		5
113	10	7	3	3	4		4		6
114	6	3		2	3				
116			7	3	4	8	5	4	6
117	7	6	8	5	5	6	8	6	9
119	7	7	6						

Table continued on p. 184.

TABLE II (*Continued*)

m/e	*Methyl 2,3,4,6-tetra-O-methyl-D-mannononitrile*								
	2	**23**	**24**	**25**	**26**	**32**	**50**	**44**	**38**
	Deuteriomethyl position								
	None	*2*	*3*	*4*	*6*	*4,6*	*2,4*	*2,6*	*3,6*
120					3	4			
122				4	6		5	4	5
125						5			
129	100	91	84	14	85	6	10	100	36
130	8	10	7	4	6			10	5
131	5	6		5	3	5		4	4
132				69		100	100		86
133				5		7	8		9
134						3	5		5
145	33	29							8
148			30	23	27		31	27	27
151						33	4		4
155	6				3				
161	67	70	67	7	2	7	4	3	22
162	6	6	5						2
164				42	45		70	69	57
165				3	3		6	5	5
166							2		
167						66			4
168						5			
169						1			
173	0.5								
184	0.5								
186	1								
189			1		1			1	1
192						1			
205	5	5							1
207							5[b]	5[b]	
208			5	1	3				4
211						5			1

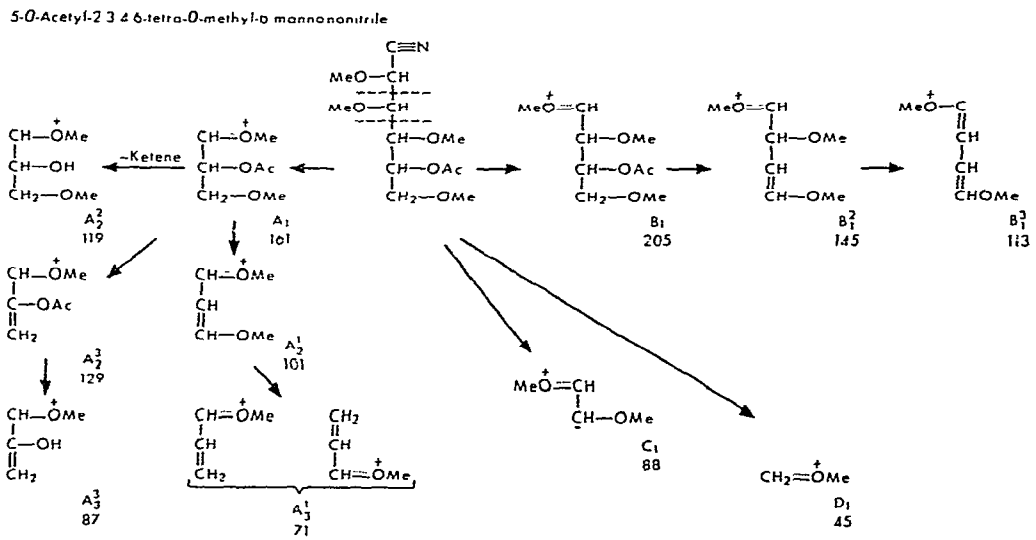
[a]Most-intense peak equals 100. [b]Probable mass marking error.

Fragmentation of alditols proceeds first by a carbon-chain cleavage to yield a radical and a charged, primary fragment. Neutral fragments are then eliminated from the primary ions to yield smaller ions. The fragmentation patterns of the nitriles are similar to those of the alditols, because the charged primary fragments arising from the non-nitrile end of the molecule are identical to those of the corresponding alditol. However, the patterns given by the nitriles are more definitive, because only one primary alcohol group is present in the molecule.

The origin of individual mass-fragments in each spectrum was investigated by selective labeling with $-OCD_3$. A known methyl di-*O*-methyl-α-D-mannopyranoside was subjected to partial Purdie methylation by CD_3I; the methylation reaction was

monitored by thin-layer chromatography (t.l.c.), which could separate the reaction mixture into tetra-, tri-, and di-*O*-methyl components. When the starting di-*O*-methyl compound disappeared, the reaction was stopped, and the products were hydrolyzed and the sugars converted into their PAAN derivatives. This mixture of PAAN derivatives was analyzed by g.l.c.–m.s. Each emerging peak could be identified as the tetra-*O*-methyl- or a given tri-*O*-methyl-PAAN derivative, based on its g.l.c. retention-time compared to those of the known, nondeuterated compounds. Tables II and III also list the mass spectra of deuteriomethylated PAAN derivatives. The spectra of many deuteriomethylated compounds were weak. Rigorous comparisons of relative, fragment-ion intensities were further precluded in these instances by the contributions of background ions. For these reasons, only the more-intense ions representing the major fragmentation-pathways were analyzed. Even so, these spectra made it possible to trace the origins of the various fragment-ions, as the positions of the original and deuterated methyl groups were known.

The mass spectra of eight deuteriomethyl analogs of the 2,3,4,6-tetra-*O*-methyl-D-mannopyranoside PAAN derivative are given in Table II. Because some major fragment-ions in the spectra are displaced by either +3 or +6 mass units, these fragments must contain either one or two deuteriomethoxyl groups. Presumably, these methoxyl groups are still attached at their original positions on the carbon chain of the parent compound. The fragmentation pathway depicted in Scheme 1 is based on this assumption. The fragment ions are labeled according to the rules proposed by



Scheme 1

Kochetkov and Chizhov[12]. These rules are summarized as: "The series of ions related by similar structure or common origin will be denoted as capital letters. The subscript numeral will denote the number of steps needed for the formation of the

fragment from the molecular ion, and the superscript numeral, the ordinal number of the isomeric ion. The lowest ordinal number is given to the isomer having the substituent at the carbon atom of lowest ordinal number or, respectively, containing the lowest ordinal carbon atoms". Because fragment-ions of higher *m/e* value completely shift on deuteriomethylation of certain hydroxyl groups, they probably arise from a single mode of fragmentation. Unlabeled fragment-ions of lower *m/e* value are often present, in addition to a second ion that is 3 mass units greater; either these ions come from more than one part of the molecule, or an intermediate fragment loses a methoxyl group in more than one way.

Fragment ions of *m/e* 205 and 145 contain the 3-, 4-, and 6-methoxyl groups, and therefore represent the C-3–C-6 portion of the chain. As indicated in Scheme 1, the ions probably arise from an initial cleavage between C-2 and C-3. The structure Ac_3O^+ has been proposed[12] for the peak at *m/e* 145 in the mass spectra of acetylated compounds, but the isotopic-substitution data obtained here clearly eliminate this possibility for these compounds. Fragment *m/e* 113 is also affected by isotopic substitution in the 3-, 4-, and 6-methoxyl groups. As the peak is not completely shifted, some *m/e* 113 material is probably formed by an alternative pathway. Almost all of this fragment appears to arise from *m/e* 145, and the amount of isotope incorporated suggests an equal probability that any one of the three methoxyl groups will be lost. Almost all the remaining fragment-ions appear to come from the C-4–C-6 part of the chain. Intense fragments having *m/e* 129 and 87 show that the methoxyl group holding the charge (*i.e.*, C-4) tends to be retained. Incomplete peak-shifting of *m/e* 101 and 71 indicates that these fragments can include C-2, and arise by an alternative pathway. Fragment ions *m/e* 88 and 45 are of special interest. Fragment *m/e* 88 apparently has the odd-electron, ion structure of C_1. Only 6-deuteriomethoxyl substitution results in no mass shift in C_1, and the amount of mass shift resulting from 2-, 3-, or 4-deuteriomethoxyl substitution indicates an equal probability that the fragment arises from C-2,3 or C-3,4. Deuteriomethylation showed that fragment *m/e* 45 can come from any (chain) carbon atom bearing a methoxyl group. The contribution of the 6-methoxyl group is greatest (65%), and it presumably results from simple cleavage. Apparently, other methoxyl groups can contribute as a result of proton transfer during cleavage.

A satisfactory explanation cannot be offered for the origin of fragments *m/e* 186 and 96. Although of even *m/e*, the fragment of *m/e* 186 evidently lacks the nitrile group, as isotopic labeling with $-CD_3$ showed that it apparently contains the 3-, 4-, and 6-methoxyl groups.

Fragments from the nitrile end of the tetra-*O*-methyl PAAN derivative have not been identified. Major cleavages of the backbone occur between adjacent carbon atoms bearing methoxyl groups. Apparently, if alternative routes are available, fragments involving either the nitrile end or the acetoxyl, charge-stabilized radical are not intense.

Because fragments contributed from the nitrile end are either absent or weak, all primary fragment-ions and their resulting, secondary fragments are the same as

those observed for the corresponding 2,3,4,6-tetra-*O*-methylalditol acetate. In contrast to the alditol, however, the fragmentation pattern of the nitrile is simpler, as only one end of the molecule contributes intense, primary fragments.

Table III records the mass spectra of tri-*O*-methyl PAAN derivatives of D-mannose, as well as their deuteriomethylated analogs derived from known dimethyl ethers. Mass shifts in major fragment-ions due to deuteriomethyl substitution were used to construct the fragmentation pathways illustrated in Schemes 2–5.

TABLE III

RELATIVE INTENSITIES[a] OF FRAGMENT IONS OBTAINED FROM M.S. OF TRI-*O*-METHYL PAAN DERIVATIVES OF D-MANNOSE AND SOME DEUTERIOMETHYL ANALOGS

m/e	*Methyl tri-O-methyl-D-mannononitrile*											
	4	**49**	**43**	**6**	**37**	**8**	**48**	**31**	**10**	**42**	**36**	**30**
	Methylated in positions											
	3,4,6			*2,4,6*		*2,3,6*			*2,3,4*			
	Deuteriomethyl position											
	None	*4*[b]	*6*[b]	*None*	*6*[b]	*None*	*2*	*6*	*None*	*2*[b]	*3*[b]	*4*[b]
43	98	100	95	100	100	100	100	100	98	[c]	100	[c]
45	54	48	8	32	8	32	26	5	18	14	19	12
48		6	43		17		3	25		4		9
71	10	3	5						9	8		4
73									4	7		7
74	6	9	7									7
83										10		10
87	34	13	30	5		41	35	18	55	52	40	
90		23						25	26	19		6
91										4	12	21
99				8		26	27	14	43	36	44	14
101	40			32					12	6		6
102								15			10	37
104		26	27		32							3
112				16	18							
113						39	36		7	3		
116								41				6
119	5											
122		3										
126	18		5	3					4			
127				3								
129	100	20	100	21	26	32	27		100	100	37	7
131						14	13		3	7		3
132		84						21				100
134								16				
142	6	5	4									
147		9				5	6					
148			8									

Table continued on p. 188.

TABLE III (*Continued*)

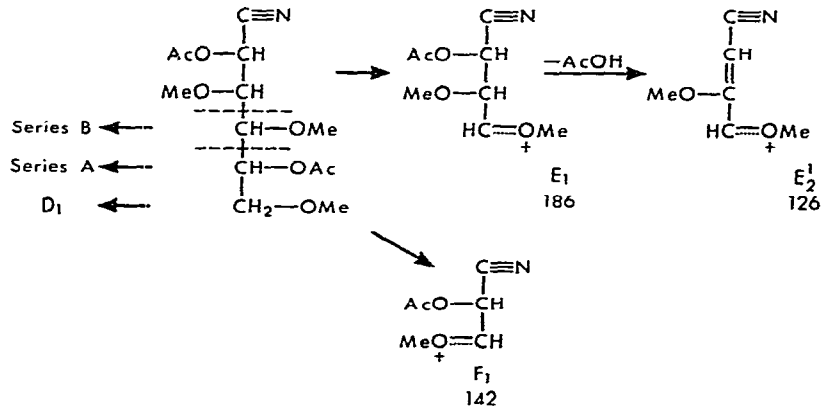
m/e	*Methyl tri-O-methyl-D-mannononitrile*											
	4	49	43	6	37	8	48	31	10	42	36	30
	Methylated in positions											
	3,4,6			*2,4,6*		*2,3,6*			*2,3,4*			
	Deuteriomethyl position											
	None	*4*[b]	*6*[b]	*None*	*6*[b]	*None*	*2*	*6*	*None*	*2*[b]	*3*[b]	*4*[b]
150								7				
154				8	8							
158								9				
159				3			3		3	3		
161	64			11						6		9
164		60	58		17							
173						5	5	1	7	6		
175								6[d]				
176												6
186	2		3	15	16							
189		2		1		4	3	2	34	28	11	1
192								3				42
205	1											
216				1								
233						15	13		3	3		
235								13[d]				
247									1			

[a]Most-intense peak equals 100. [b]Weak spectra not permitting rigorous comparison of relative intensities. [c]Intensity at *m/e* 43 not recorded. [d]Probable mass marking error.

The mass spectrum of the 3,4,6-tri-*O*-methyl-D-mannose PAAN derivative (see Scheme 2) is similar in many respects to that of the tetra-*O*-methyl compound. This similarity was expected, because only the group at O-2 has been changed, and because fragment ions from the tetra-*O*-methyl compound come from the non-nitrile end. Accordingly, primary fragments A and B, with their accompanying secondary fragments, are observed; and deuteriomethyl substitution confirms that they arise from the pathways shown in Scheme 1. Three additional fragment-ions of even *m/e* (126, 142, and 186) appear in these spectra, and deuteriomethyl substitution confirms that they arise from the nitrile end.

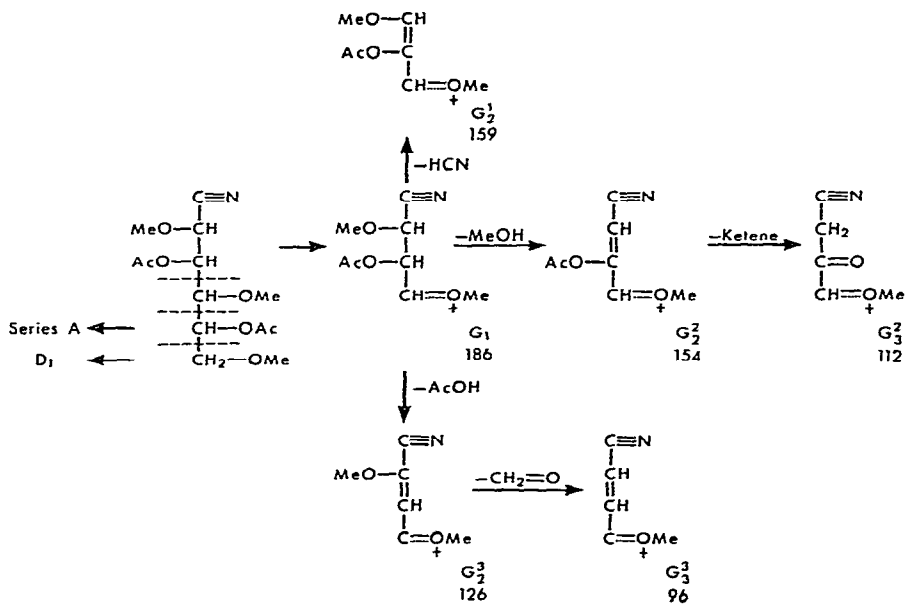
An acetoxyl group near C-1 apparently stabilizes fragments containing the nitrile group. Stabilization of the nitrile end by acetoxyl groups is further seen in the mass spectrum of the 2,4,6-tri-*O*-methyl PAAN derivative (see Scheme 3). The last three carbon-attached groups are identical to those of the tetra-*O*-methyl derivative, and give rise to the primary fragment-ion A_1, *m/e* 161, and its expected secondary fragments (*m/e* 129, 119, 101, 87, and 71). In addition, five extra fragments of even *m/e* (96, 112, 126, 154, and 186) are present.

2,5-Di-*O*-acetyl-3,4,6-tri-*O*-methyl-D-mannononitrile



Scheme 2

3,5-Di-*O*-acetyl-2,4,6 tri-*O* methyl-D-mannononitrile

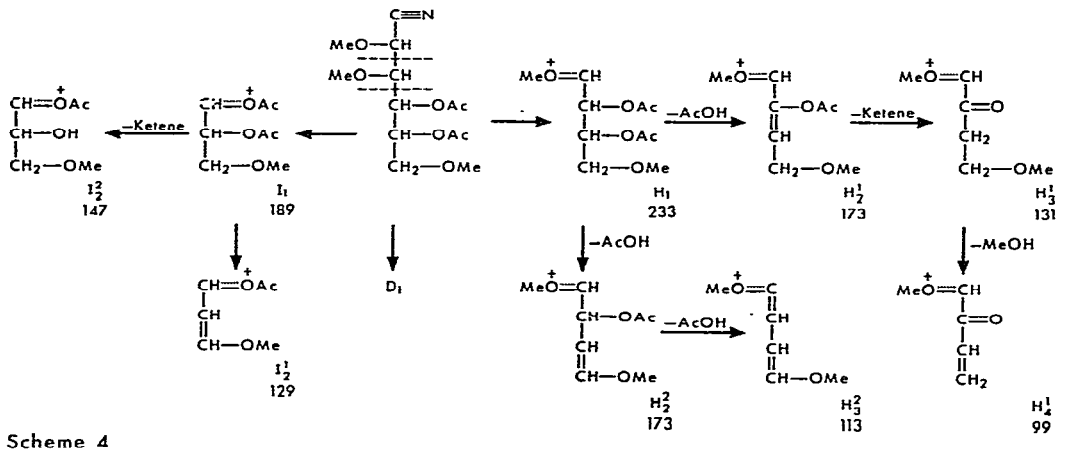


Scheme 3

Both 2,3,6- and 2,3,4-tri-*O*-methyl-D-mannose PAAN derivatives yield mass spectra considerably different from that of the 2,3,4,6-tetra-*O*-methyl PAAN derivative. This difference is due to new acetoxyl groups in the non-nitrile ends of the compounds.

Scheme 4 explains the mass spectrum of the 2,3,6-tri-*O*-methyl-D-mannose PAAN derivative. Two primary fragment-ions are postulated. The first is H_1,

4,5-Di-O-acetyl-2,3,6-tri O-methyl D mannononitrile

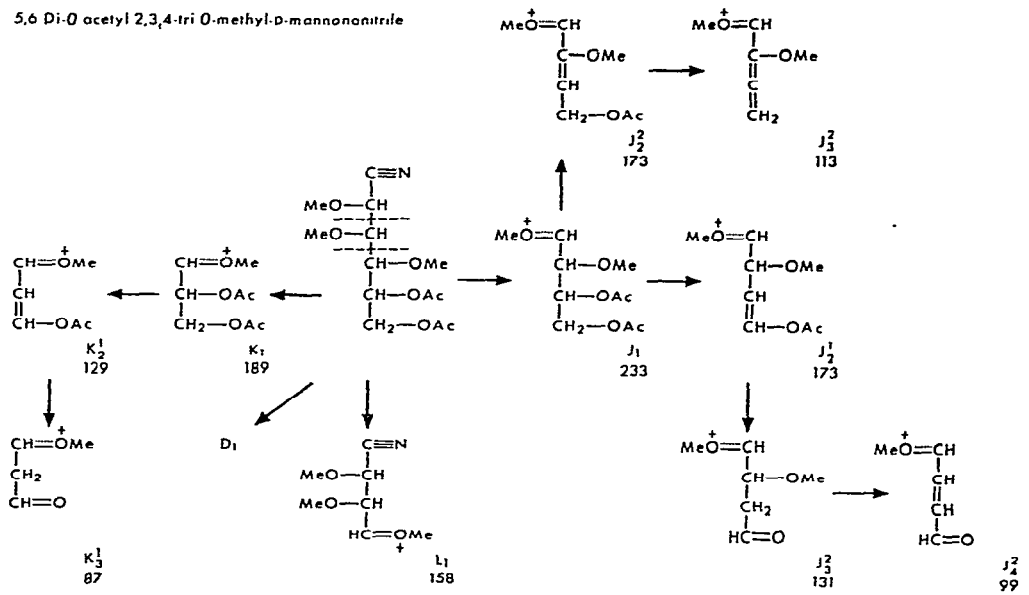


Scheme 4

m/e 233, which gives rise to a secondary-fragmentation series of *m/e* 173, 131, 113, and 99. Fragments of *m/e* 173, H_2^1 and H_2^2, can be formed from H_1 by loss of acetic acid in several ways. Ions H^1 and H^2 can lose ketene and acetic acid, respectively, to yield H_3^1 (*m/e* 131) and H_3^2 (*m/e* 113). Loss of methanol from H_3^1 leads to formation of H_4^1 (*m/e* 99). In the second, primary fragment-ion I_1 (*m/e* 189) and its secondary fragments (*m/e* 147 and 129), the charge is stabilized by an acetoxyl group. Deuteriomethyl substitution shows that both *m/e* 147 and 129 contain the 6-methoxyl group. The frequently occurring, *m/e* 189 fragment does not show a complete shift upon 6-*O*-CD_3 substitution, and it is also affected by substitution on C-2. Possibly, two fragments of the same *m/e* value arise from different sources. It is not clear whether the shift of +2 mass units for fragment *m/e* 233 upon 6-*O*-CD_3 substitution arises from a rearrangement or is the result of a mass marking error. As a similar shift is noted in Table II for fragments *m/e* 207 in mass spectra taken during the same experiment, the latter possibility is the more likely. Unfortunately, no material was available to permit re-examination of the sample.

The 2,3,4-tri-*O*-methyl-D-mannose PAAN derivative yields fragments similar to those observed in spectra previously discussed, but apparently arising by different pathways (see Scheme 5). Cleavage between C-2 and C-3 gives the J series, which has fragments of *m/e* identical to those of the H series (see Scheme 4). Cleavage between C-3 and C-4 produces primary fragment K_1, *m/e* 189, and its secondary fragments. Ion L_1, *m/e* 158, is formed by cleavage between C-4 and C-5. Assignments of the J, K, and L fragment-ion pathways are in agreement with those for spectra of isotopically substituted products.

Although no isotopically substituted di-*O*-methyl compounds were prepared, our studies provide sufficient information to propose fragmentation-pathways based on data in Table IV. The 3,4-di-*O*-methyl-D-mannose PAAN derivative would be expected to give a mass spectrum similar to that of the corresponding 2,3,4-tri-*O*-methyl compound, as they differ only at C-2. In fact, the spectra are similar, both



Scheme 5

TABLE IV

RELATIVE INTENSITIES[a] OF FRAGMENT IONS FROM M.S. OF DI-*O*-METHYL PAAN DERIVATIVES OF D-MANNOSE

m/e	*Methyl di-O-methyl-D-mannononitrile*					
	12	**14**	**16**	**18**	**20**[b]	**22**
	Methylated in positions					
	2,3	*2,4*	*3,4*	*3,6*	*2,6*	*4,6*
43	100	100	96	[c]	100	100
45	5	5	5	83	20	27
71	1	4			3	25
74	2			7	3	
75	2	11		10	4	
78		4				
79				8		
83				22		
84				20		
85	15			16		
87	4	26	50	100	21	9
88	5					
96		14				
98				10		
99	12	10	15	32	9	4
101						*55*
103	2	5				

Table continued on p. 192.

TABLE IV (*Continued*)

m/e	*Methyl di-O-methyl-D-mannononitrile*					
	12	**14**	**16**	**18**	**20**[b]	**22**
	Methylated in positions					
	2,3	*2,4*	*3,4*	*3,6*	*2,6*	*4,6*
112		25				9
113				46		
115	7			11	12	
117	2			16	9	
126		4	19			
127	13	11			4	
129	1	57	100	78	12	21
131				37		
142			8	30		2
147				13	2	
154		19				7
157					4	
159	3	20			15	
161						8
169				5	13	
173			2	5		5
184				13	9	4
186		29	4			
187	2					
189		20	34	7	1	
201	2				2	
211					4	1
212				4		
214						10
233				16		
244					1	
261	2					
286					2	

[a]Most-intense peak equals 100. [b]Kindly provided by Dr. M. B. Perry, National Research Council, Ottawa, Canada, as 2,6-di-*O*-methyl-D-mannopyranose (ref. 7). [c]Intensity at *m/e* 43 not recorded.

being composed primarily of fragments described in J and K pathways. The spectrum of the 3,4-dimethyl ether also contains the E series and fragment F_1, *m/e* 142, which are also present in the spectrum of the 3,4,6-trimethyl ether (see Scheme 2).

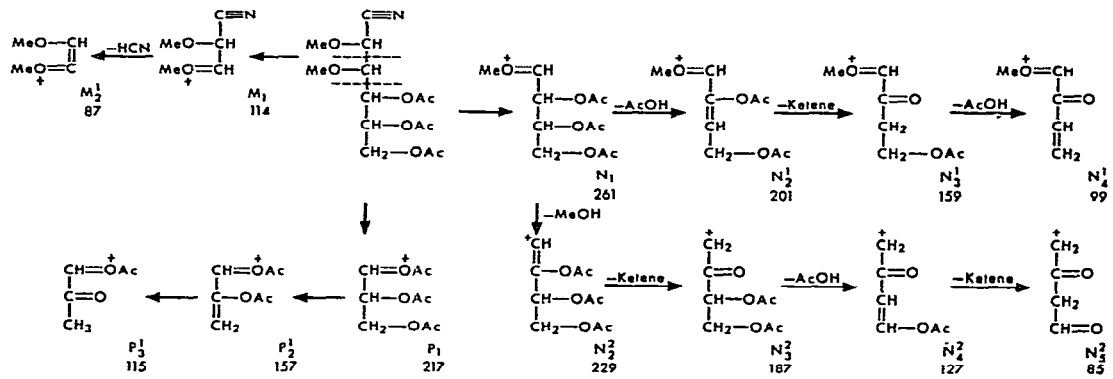
The spectrum of the 3,6-di-*O*-methyl-D-mannose PAAN derivative is likewise a composite of previous pathways. The compound may cleave toward the nitrile end, between C-3 and C-4, to give *m/e* 142 (F_1). Fragments *m/e* 189 (series I), *m/e* 233 (series H), and *m/e* 45 (D_1) are formed by cleavage toward the 6-methoxyl end. With the exception of a few weak ions (*m/e* 131 and 101), all the secondary fragmentation-ions belonging to these series are observed.

Fragments produced from the acetoxyl end of the 2,4-di-*O*-methyl-D-mannose PAAN derivative correspond to those of the 2,3,4-tri-*O*-methyl compound (*i.e.*,

series K). This di-*O*-methyl derivative also yields fragments similar to those from the nitrile end of the 2,4,6-tri-*O*-methyl compound (*i.e.*, series G).

The 2,3-di-*O*-methyl-D-mannose PAAN derivative yields a complicated spectrum unlike any of the ones previously discussed. This complexity is, presumably, due to the large number of acetoxyl groups at the non-nitrile end. A probable explanation of these peaks is given in Scheme 6.

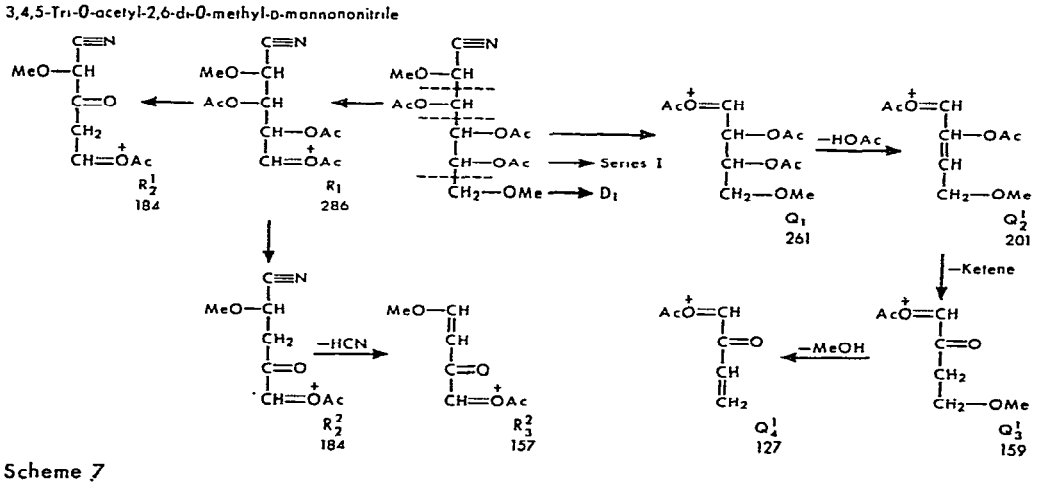
4,5,6-Tri-O-acetyl-2,3-di-O-methyl-D-mannononitrile



Scheme 6

The 2,6-di-*O*-methyl-D-mannose PAAN derivative (see Scheme 7) gives the Q and R series, in which charged acetoxyl fragments preponderate, as alternative pathways leading to fragments stabilized by methoxyl groups are not available.

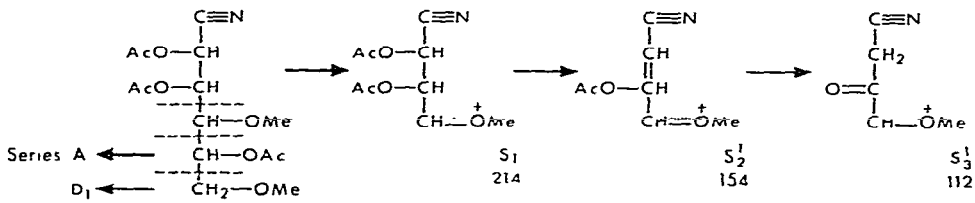
3,4,5-Tri-O-acetyl-2,6-di-O-methyl-D-mannononitrile



Scheme 7

Cleavage of the 4,6-di-*O*-methyl-D-mannose PAAN (see Scheme 8) occurs at the 4-methoxyl position, to give series A fragments from the non-nitrile end and a new series, S, from the nitrile end.

2,3,5-Tri-*O*-acetyl-4,6-di-*O*-methyl-D-mannononitrile



Scheme 8

In addition to the analogies drawn between tri- and di-*O*-methyl derivatives, the relative intensities of peaks composing the various series may be considered. It could be expected that a primary fragment would undergo the same fragmentation-pathway regardless of its origin, assuming equal original internal-energy. Therefore, each of the various series would contain fragments in similar ratios of intensity. For most series in the mass spectra of two or more compounds, there is good agreement between the relative intensities of the fragment ions.

EXPERIMENTAL

Preparation of PAAN derivatives. — Syntheses of the various methyl ethers of methyl α-D-mannopyranoside have been described previously[4–6]. These D-mannosides were hydrolyzed to their corresponding D-mannose ethers and then these were converted into peracetylated aldononitriles by the method of Lance and Jones[2], which was modified by a final extraction with chloroform. The methylated methyl α-D-mannopyranoside (3 mg) together with 2M hydrochloric acid (8 drops) in a glass vial (Teflon screw-cap) was heated for 1 h in boiling water. The solution was transferred to a cylindrical reaction-flask (3 × 7 cm), and evaporated to dryness at 40°. Ethanol (1 ml) was added to and evaporated from the residue. The flask was air-dried (15 min), and then hydroxylamine hydrochloride (15 mg) and pyridine (12 drops) were added, and the stirred mixture was heated for 20 min at 60°. Acetic anhydride (12 drops) was added, and heating and stirring were continued for 20 min. The solution was cooled, transferred to 1:2 chloroform–water, and vigorously agitated. The chloroform layer was re-extracted with water (2 ml), and dried with a few pellets of 4 Å molecular sieve, and was immediately ready for g.l.c. injection. (The water/chloroform extraction significantly lessened tailing of the g.l.c. solvent peak, which obscured that of the tetra-*O*-methyl derivative and seriously interfered with those of the tri-*O*-methyl derivatives).

Gas–liquid chromatography. — A gas chromatograph equipped with a hydrogen-flame ionization detector, helium carrier, and stainless-steel column (1.83 m × 3.18 mm) was used. Column A consisted of 3% of neopentyl glycol succinate on Supelcoport (an acid-washed, silane-treated Chromosorb W), and was temperature-programmed from 120 to 150° at 1°/min, at a carrier flow-rate of 30 ml/min. Column B consisted of

5% of butanediol succinate on Supelcoport, temperature-programmed from 185 to 210° at 1°/min, and then held at 210°, with a carrier flow-rate of 30 ml/min.

Mass spectrometry. — Mass spectra were recorded at 70 eV on a duPont 21-492-1 mass spectrometer. A Packard 7401 gas chromatograph coupled to the mass spectrometer by means of a jet separator was used to introduce all samples. Glass columns (1.83 m × 3.18 mm), packed with 5% of butanediol succinate on Supelcoport, were operated at 180–220°. Transfer lines from the gas chromatograph to the mass spectrometer were held at 220°.

Partial Purdie deuteriomethylations. — A micro-reaction flask, consisting of a 1-dram vial fitted with a small, magnetic stirring-bar and a cork stopper pierced with a hypodermic needle attached to a short length of rubber tubing packed with 4 Å molecular sieve was used. A serum cap having a hole cut in the bottom was pushed over the vial, and the resulting, upper flange was packed with Dry Ice. A methyl ether of methyl α-D-mannopyranoside (10 mg), deuteriomethyl iodide (0.3 ml), and silver oxide (50 mg) were added, and the bottom of the vial was held at the surface of an oil bath maintained at 60°, atop a combined magnetic stirrer–hot plate. Additional portions of silver oxide were added as the reaction was monitored by t.l.c. Plates (silica gel) were developed with 1:100 methanol–chloroform, and spots were made visible by spraying with 1:10 methanol–sulfuric acid and heating. The tetra-, tri-, and di-*O*-methyl derivatives separated clearly. At the end of the reaction, the clear solution was pipetted away from the silver oxide, and evaporated to dryness. The sample was then hydrolyzed, and the product converted into the peracetylated aldononitrile.

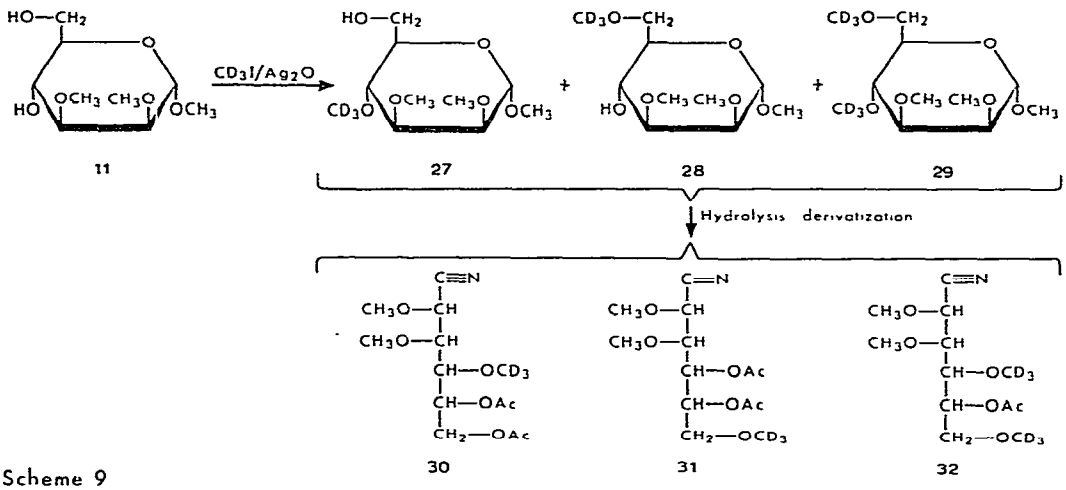
Non-deuteriomethylated methyl α-D-mannopyranosides were hydrolyzed and derivatized to yield the following: methyl 2,3,4,6-tetra-*O*-methyl-α-D-mannopyranoside (**1**) gave 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannononitrile (**2**); methyl 3,4,6-tri-*O*-methyl-α-D-mannopyranoside (**3**) gave 2,5-di-*O*-acetyl-3,4,6-tri-*O*-methyl-D-mannononitrile (**4**); methyl 2,4,6-tri-*O*-methyl-α-D-mannopyranoside (**5**) gave 3,5-di-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannononitrile (**6**); methyl 2,3,6-tri-*O*-methyl-α-D-mannopyranoside (**7**) gave 4,5-di-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannononitrile (**8**); methyl 2,3,4-tri-*O*-methyl-α-D-mannopyranoside (**9**) gave 5,6-di-*O*-acetyl-2,3,4-tri-*O*-methyl-D-mannononitrile (**10**); methyl 2,3-di-*O*-methyl-α-D-mannopyranoside (**11**) gave 4,5,6-tri-*O*-acetyl-2,3-di-*O*-methyl-D-mannononitrile (**12**); methyl 2,4-di-*O*-methyl-α-D-mannopyranoside (**13**) gave 3,5,6-tri-*O*-acetyl-2,4-di-*O*-methyl-D-mannononitrile (**14**); methyl 3,4-di-*O*-methyl-α-D-mannopyranoside (**15**) gave 2,5,6-tri-*O*-acetyl-3,4-di-*O*-methyl-D-mannononitrile (**16**); methyl-3,6-di-*O*-methyl-α-D-mannopyranoside (**17**) gave 2,4,5-tri-*O*-acetyl-3,6-di-*O*-methyl-D-mannononitrile (**18**); 2,6-di-*O*-methyl-α-D-mannopyranose (**19**) gave 3,4,5-tri-*O*-acetyl-2,6-di-*O*-methyl-D-mannononitrile (**20**); and methyl 4,6-di-*O*-methyl-α-D-mannopyranoside (**21**) gave 2,3,5-tri-*O*-acetyl-4,6-di-*O*-methyl-D-mannononitrile (**22**).

Methyl α-D-mannopyranosides completely deuteriomethylated, hydrolyzed, and then derivatized to their corresponding peracetylated aldononitriles were as follows: methyl 3,4,6-tri-*O*-methyl-α-D-mannopyranoside (**3**) gave 5-*O*-acetyl-2-*O*-(deuteriomethyl)-3,4,6-tri-*O*-methyl-D-mannononitrile (**23**); methyl 2,4,6-tri-*O*-methyl-α-D-

mannopyranoside (**5**) gave 5-*O*-acetyl-3-*O*-(deuteriomethyl)-2,4,6-tri-*O*-methyl-D-mannononitrile (**24**); methyl 2,3,6-tri-*O*-methyl-α-D-mannopyranoside (**7**) gave 5-*O*-acetyl-4-*O*-(deuteriomethyl)-2,3,6-tri-*O*-methyl-D-mannononitrile (**25**); and methyl 2,3,4-tri-*O*-methyl-α-D-mannopyranoside (**9**) gave 5-*O*-acetyl-6-*O*-(deuteriomethyl)-2,3,4-tri-*O*-methyl-D-mannononitrile (**26**).

Compounds partially deuteriomethylated were as follows:

Methyl 2,3-di-*O*-methyl-α-D-mannopyranoside (**11**) yielded a mixture of methyl 4-*O*-(deuteriomethyl)-2,3-di-*O*-methyl-α-D-mannopyranoside (**27**), methyl 6-*O*-(deuteriomethyl)-2,3-di-*O*-methyl-α-D-mannopyranoside (**28**), and methyl 4,6-di-*O*-(deuteriomethyl)-2,3-di-*O*-methyl-α-D-mannopyranoside (**29**). This mixture of compounds was hydrolyzed and derivatized to yield 5,6-di-*O*-acetyl-4-*O*-(deuteriomethyl)-2,3-di-*O*-methyl-D-mannononitrile (**30**), 4,5-di-*O*-acetyl-6-*O*-(deuteriomethyl) 2,3-di-*O*-methyl-D-mannononitrile (**31**), and 5-*O*-acetyl-4,6-di-*O*-(deuteriomethyl)-2,3-di-*O*-methyl-D-mannononitrile (**32**) (see Scheme 9).



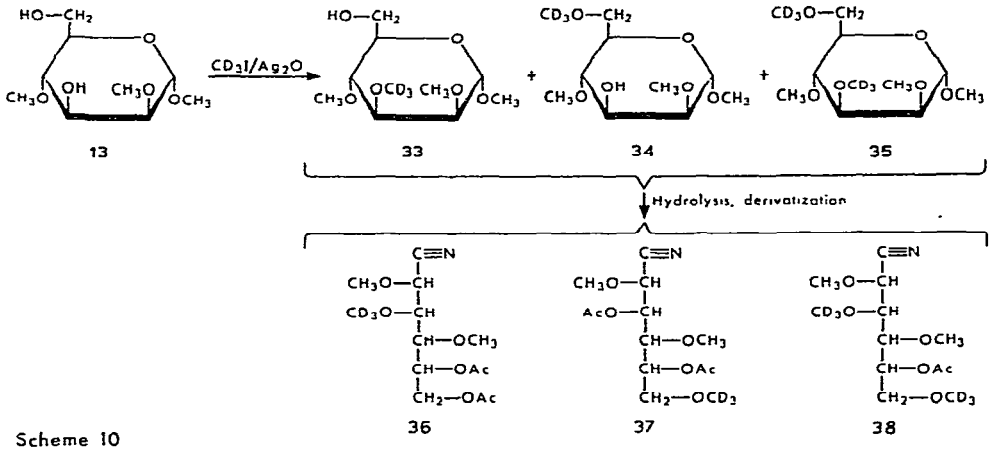
11 27 28 29

30 31 32

Scheme 9

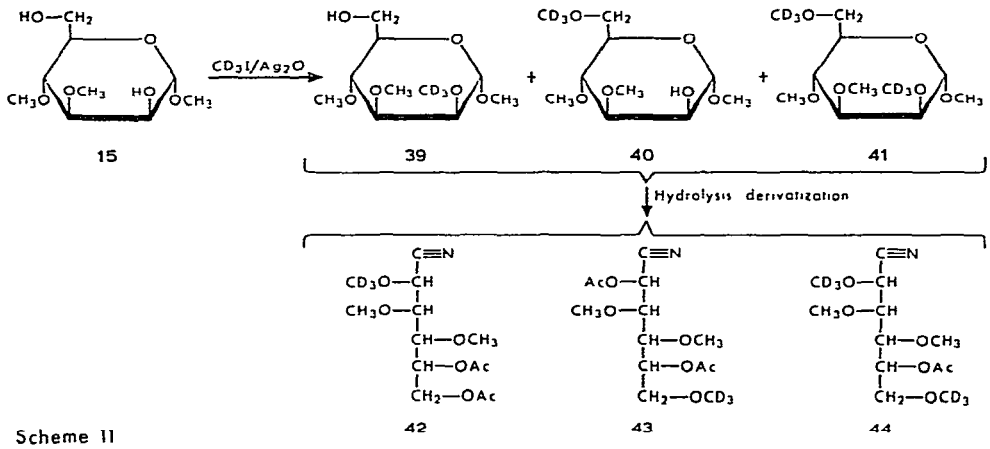
Methyl 2,4-di-*O*-methyl-α-D-mannopyranoside (**13**) yielded a mixture of methyl 3-*O*-(deuteriomethyl)-2,4-di-*O*-methyl-α-D-mannopyranoside (**33**), methyl 6-*O*-(deuteriomethyl)-2,4-di-*O*-methyl-α-D-mannopyranoside (**34**), and methyl 3,6-di-*O*-(deuteriomethyl)-2,4-di-*O*-methyl-α-D-mannopyranoside (**35**). This mixture of compounds was hydrolyzed and derivatized to yield 4,5-di-*O*-acetyl-3-*O*-(deuteriomethyl)-2,4-di-*O*-methyl-D-mannononitrile (**36**), 3,5-di-*O*-acetyl-6-*O*-(deuteriomethyl)-2,4-di-*O*-methyl-D-mannononitrile (**37**), and 5-*O*-acetyl-3,6-di-*O*-(deuteriomethyl)-2,4-di-*O*-methyl-D-mannononitrile (**38**) (see Scheme 10).

Methyl 3,4-di-*O*-methyl-α-D-mannopyranoside (**15**) yielded a mixture of methyl 2-*O*-(deuteriomethyl)-3,4-di-*O*-methyl-α-D-mannopyranoside (**39**), methyl 6-*O*-(deuteriomethyl)-3,4-di-*O*-methyl-α-D-mannopyranoside (**40**), and methyl 2,6-di-*O*-(deuteriomethyl)-3,4-di-*O*-methyl-α-D-mannopyranoside (**41**). This mixture of compounds was hydrolyzed and derivatized to yield 5,6-di-*O*-acetyl-2-*O*-(deuteriomethyl)-



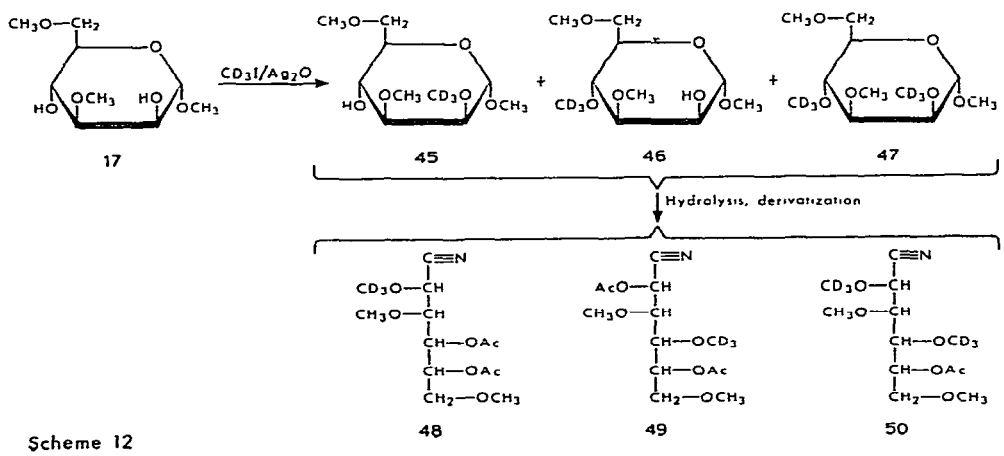
13 33 34 35 36 37 38

Scheme 10



15 39 40 41 42 43 44

Scheme 11



17 45 46 47 48 49 50

Scheme 12

3,4-di-*O*-methyl-D-mannononitrile (**42**), 2,5-di-*O*-acetyl-6-*O*-(deuteriomethyl)-3,4-di-*O*-methyl-D-mannononitrile (**43**), and 5-*O*-acetyl-2,6-di-*O*-(deuteriomethyl)-3,4-di-*O*-methyl-D-mannononitrile (**44**) (see Scheme 11).

Methyl 3,6-di-*O*-methyl-α-D-mannopyranoside (**17**) yielded a mixture of methyl 2-*O*-(deuteriomethyl)-3,6-di-*O*-methyl-α-D-mannopyranoside (**45**), methyl 4-*O*-(deuteriomethyl)-3,6-di-*O*-methyl-α-D-mannopyranoside (**46**), and methyl 2,4-di-*O*-(deuteriomethyl)-3,6-di-*O*-methyl-α-D-mannopyranoside (**47**). This mixture of compounds was hydrolyzed and derivatized to yield 4,5-di-*O*-acetyl-2-*O*-(deuteriomethyl)-3,6-di-*O*-methyl-D-mannononitrile (**48**), 2,5-di-*O*-acetyl-4-*O*-(deuteriomethyl)-3,6-di-*O*-methyl-D-mannononitrile (**49**), and 5-*O*-acetyl-2,4-di-*O*-(deuteriomethyl)-3,6-di-*O*-methyl-D-mannononitrile (**50**) (see Scheme 12).

Deuteriomethylated compounds had relative g.l.c. retention-times identical to those of the corresponding methylated compounds.

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