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Note

Gas chromatography–mass spectrometry of partially methylated glycoses as their aldononitrile peracetates

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Methylation techniques have been used in the structural analysis of carbohydrate-containing macromolecules for many years. The success of the method depends on complete methylation of the free hydroxyl groups in the polymer, total depolymerisation to monomer residues without loss of the methoxy groups and the ability to analyse the partially methylated products obtained on depolymerisation. The introduction of gas–liquid chromatography (GLC) has led to considerable improvements in the analysis of these products, some of the early work using the methyl glycosides of the methylated sugars[1,2]. Chromatograms of these derivatives are still quite complicated to interpret since the different glycosidic forms of each sugar produced a separate peak. Furthermore, sugar derivatives which still contained a number of hydroxyl groups, *e.g.* mono- and di-O-methylhexoses were not sufficiently volatile to be estimated with accuracy.

Reduction of the partially methylated sugars to their corresponding alditols has considerably simplified the procedure since acetylation of the partially methylated alditol produces a single derivative from each sugar[3] and the characterisation of these peracetylated partially methylated alditols has been made easier by the use of combined gas-liquid chromatography–mass spectrometry (GLC–MS)[4]. The method does suffer from the fact that the hydroxyl group introduced on C-1 of the alditol produces internal symmetry in some molecules. Thus, for example, both 2,3- and 3,4-di-O-methyl-D-xylose will give 1,4,5-tri-O-acetyl-2,3-di-O-methyl-D-xylitol on reduction and acetylation. Reduction of the parent sugar with sodium borodeuteride gives an unambiguous assignment if GLC–MS is used but does not help if GLC alone is available.

Another derivative which is easily prepared and avoids the complication of multiple peaks on GLC is the peracetylated aldononitrile. These derivatives have been used for the compositional analysis of polysaccharides[5] and, in conjunction with the peracetylated alditols, for the determination of the degree of polymerisation of oligo- and polysaccharides[6]. The separation and characterisation of the peracetylated derivatives of partially methylated aldononitriles by GLC and combined GLC–MS have not received the same attention as the corresponding alditol derivatives but some reports have been presented[7,8].

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Since the aldononitrile derivative method does not suffer from the fact that both ends of the molecule have the same functional groups, it has been investigated more thoroughly to show that it can be used routinely as an alternative to the alditol method. Aldononitrile derivatives have another advantage in being more volatile than the corresponding alditol derivatives. The GLC separation is therefore quicker or can be carried out at a lower column temperature. The molar response factors are, however, slightly lower than those of the corresponding alditols.

EXPERIMENTAL

Materials

The 1-O-methyl β-D-glycosides of D-arabinopyranose, D-galactopyranose, D-glucopyranose, and D-xylopyranose were from Sigma (Poole, U.K.). Iodomethane and silver oxide were purchased from B.D.H. (Poole, U.K.).

Apparatus

GLC was performed using a Pye 104 gas chromatograph fitted with dual flame ionisation detectors coupled with a Hewlett-Packard 3373B integrator. Glass columns (4 mm I.D.) were used throughout and were packed with the following materials: (A) 5% OV-225 on 100–120 mesh Chromosorb W-AW-DMCS (2 m); (B) 3% SP-2340 on 100–120 mesh Supelcoport (2 m) and (C) 3% ECNSS-M on 100–120 mesh Gas-Chrom Q (1.5 m). The column temperatures used were: (A) 160 to 210°C at 1° min^{-1}, (B) 150 to 225°C at 2° min^{-1} and (C) 180°C isothermal. Combined GLC–MS was performed on a similar instrument as above, using column A, and coupled, using a single stage glass separator (at 200°C), to an MS3076 mass spectrometer (Kratos) with a source temperature of 200°C and an ionising energy of 70 eV.

Procedure

The methyl glycosides (100 mg), previously dried over phosphorus pentoxide, were dissolved in dry methanol (2 ml) in a round bottom flask (10 ml) fitted with a condenser and drying tube. Iodomethane (0.38 ml) and silver oxide (0.71 g) were added in ten equal portions over a 5 h period. The mixture was stirred at 45°C throughout. After a further 1–3 h, the flask was cooled and the insoluble material removed by filtration. This procedure is a slight modification of the procedure of Purdie and Irvine[9]. The length of reaction was varied between different glycosides to obtain the complete range from the permethylated derivative to the series of monomethyl derivatives. This range was confirmed by analysis of the methyl glycosides obtained above by GLC. To the solution of the partially methylated methyl glycosides in methanol was added an equal volume of myoinositol (2.0 mg ml^{-1}) solution and two volumes of 4 *M* trifluoroacetic acid. The glycosides were hydrolysed by heating at 100°C for 1 h and the solutions were evaporated to dryness in a stream of nitrogen. The aldononitrile acetates were prepared as previously described[6].

RESULTS AND DISCUSSION

Preliminary experiments were carried out using methyl β-D-arabinopyranoside,

methyl β-D-xylopyranoside, lactose and panose which had been permethylated by the procedure of Hakomori[10]. The peracetylated partially methylated alditols and aldononitriles were both prepared and their GLC patterns compared. The chromatograms of the alditols were compared with those of published data[3,4] to confirm the sugar derivative and these values were used to characterize the aldononitrile derivatives. Table I shows the MS data for the five peaks obtained on GLC of a mixture of the sugars used. The derivatives of D-glucose and D-galactose in peak III are not separated on the column used. The most intense fragment from both peaks I and II had an *m/e* value of 43. The various fragments from these two compounds were very similar which is not surprising since they were both derived from a 2,3,4-tri-O-methyl pentose. Some differences in the relative intensities such as fragments 101 and 117 may be useful in designating the configuration of the parent sugar. Peaks III and IV had the same most intense ion at *m/e* 129 but there were major differences between them both in intensities (*e.g. m/e* 49) and the fact that ions 101, 145 and 161 were present in III but not in IV while ions 99, 173 and 189 were present in IV but not in III. Similarly the fragment ions from peak V were very distinct to those from peaks IV and III.

TABLE I

THE MAJOR FRAGMENT IONS IN THE MASS SPECTRA OF SOME PARTIALLY METHYLATED ALDONONITRILE PERACETATES

The intensities of the ions are given relative to the most intense ion (100%). The parent sugars are (I) 2,3,4-tri-O-methyl-D-arabinose; (II) 2,3,4-tri-O-methyl-D-xylose; (III) 2,3,4,6-tetra-O-methyl-D-glucose and -D-galactose; (IV) 2,3,4-tri-O-methyl-D-glucose; and (V) 2,3,6-tri-O-methyl-D-glucose.

m/e	*Peak number*				
	I	*II*	*III*	*IV*	*V*
43	100	100	89	91	100
45	20	20	73	15	30
71	4	4	16	11	
73	7	8			
87	7	7	32	48	37
88	26	28	39	29	14
99				39	28
101	68	57	39		
113			11	6	35
114					11
117	70	86			
129	3	2	100	100	26
131					13
145			51		
147					7
158	2	2	7	6	
161	7	5	54		
173				6	7
189				35	4
205			10		
233				2	15

TABLE II

RELATIVE RETENTION TIMES OF PARTIALLY METHYLATED ALDONONITRILE PERACETATES ON COLUMNS A AND B (MYOINOSITOL HEXAACETATE = 1.00; ABSOLUTE RETENTION TIME = 48.0 min)

Parent sugar	*Relative retention times*	
	Column A	*Column B*
2,3,4,6-Me_4-D-Glc	0.291	0.385
2,3,4-Me_3-D-Glc	0.445	0.517
2,3,6-Me_3-D-Glc	0.462	0.530
2,4,6-Me_3-D-Glc	0.369	0.472
3,4,6-Me_3-D-Glc	0.415	0.477
2,3-Me_2-D-Glc	0.624	0.676
2,4-Me_2-D-Glc	0.558	0.625
2,6-Me_2-D-Glc	0.505	0.582
3,4-Me_2-D-Glc	0.505	0.568
3,6-Me_2-D-Glc	0.597	0.661
4,6-Me_2-D-Glc	0.558	0.625
2-Me-D-Glc	0.659	0.729
3-Me-D-Glc	0.784	0.914
4-Me-D-Glc	0.710	–
6-Me-D-Glc	0.687	–
D-Glc	0.830	–

An aliquot of the partially methylated derivatives obtained on methylation of the pure β-methyl glycosides by the Purdie method was hydrolysed, reduced and acetylated. The derivatives were identified by GLC and the relative proportion of each compound was used for the preliminary characterization of the peracetyled partially methylated aldononitrile acetates obtained from another aliquot of the partially methylated glycosides. The relative retention times of all possible peracetylated partially methylated aldononitriles from D-glucopyranose are shown in Table II as typical results. Similar data for the derivatives from D-xylopyranose, D-arabinopyranose and D-galactopyranose has been obtained and is deposited as supplementary information with the British Library, Boston spa.

The mass spectra data for the peracetylated partially methylated glucononitriles are summarised in Table III. The data for the other sugars have been obtained and are also supplied as supplementary information. The fragmentation patterns have many similarities to those already reported for the corresponding alditol acetate, but some of the ions are not found in the alditol acetates since they are derived from the nitrile end of the molecule. It was not easy to detect if fragmentation occurred between C-1 and C-2 since ions of *m/e* 26 are too small to be confidently confirmed in the above system but fragmentation of aldononitriles between C-2 and C-3 did not appear to occur since ions at *m/e* 70 or 98 were not found. These should arise from a methyl or acetyl group, respectively, being present on C-2. Fragmentation between C-3 and C-4 did seem to occur since ions of *m/e* 114 and 142 were both found, corresponding to fragments containing two methyl, and one methyl and one acetyl substituent, respectively. No fragments from the nitrile end of the molecules which had acetyl substituents on both C-2 and C-3 were found. Fragmentation appeared to be easier the greater the distance from the nitrile group. Ions from the nitrile end

TABLE III

INTENSE PEAKS IN THE MASS SPECTRA OF THE ALDONONITRILE ACETATES OF PARTIALLY METHYLATED DERIVATIVES OF D-GLUCOPYRANOSE

Parent sugar	*m/e*																			
	45	*71*	*85*	*87*	*88*	*99*	*100*	*101*	*103*	*112*	*113*	*114*	*115*	*127*	*129*	*131*	*142*	*145*	*147*	*154*
2,3,4,6-Me_4-D-Glc	+	+		+	+			+			+				+			+		
2,4,6-Me_3-D-Glc	+	+		+				+		+					+					
2,3,6-Me_3-D-Glc	+					+					+	+			+					
2,3,4-Me_3-D-Glc				+		+					+				+					
3,4,6-Me_3-D-Glc	+	+			+			+			+				+		+			
2,6-Me_2-D-Glc	+														+					
4,6-Me_2-D-Glc	+		+					+		+					+					+
3,6-Me_2-D-Glc	+			+		+					+				+	+			+	
2,4-Me_2-D-Glc			+							+					+					
2,3-Me_2-D-Glc			+	+		+						+	+	+						
3,4-Me_2-D-Glc				+						+			+		+					
2-Me-D-Glc			+							+			+	+				+		
3-Me-D-Glc			+			+	+						+	+			+			
4-Me-D-Glc				+									+		+			+		+
6-Me-D-Glc																				
D-Glc									+				+					+		

TABLE III (*continued*)

m/e	*157*	*158*	*159*	*161*	*173*	*184*	*186*	*187*	*189*	*197*	*201*	*212*	*214*	*217*	*229*	*233*	*242*	*261*	*286*	*289*	*314*
2,3,4,6-Me_4-D-Glc		+		+																	
2,4,6-Me_3-D-Glc				+			+														
2,3,6-Me_3-D-Glc																					
2,3,4-Me_3-D-Glc							+														
3,4,6-Me_3-D-Glc																			+		
2,6-Me_2-D-Glc	+		+			+															
4,6-Me_2-D-Glc					+				+							+					
3,6-Me_2-D-Glc																+					
2,4-Me_2-D-Glc			+				+		+					+				+			
2,3-Me_2-D-Glc	+		+																		
3,4-Me_2-D-Glc								+											+	+	
2-Me-D-Glc	+							+						+				+			
3-Me-D-Glc	+		+										+								
4-Me-D-Glc									+												
6-Me-D-Glc												+		+			+			+	+
D-Glc	+																				

TABLE IV
THE ANALYSIS OF THREE PERMETHYLATED XYLANS BY THE PERACETYLATED PARTIALLY METHYLATED ALDONONITRILE METHOD (USING COLUMN B)

Parent sugar	*Relative peak area from methylated xylan*		
	Ryegrass	*Alfalfa*	*Hay*
2,3,5-Me_3-Ara	4	0.3	8
2,3,4-Me_3-Xyl	2	1.2	4
2,3,4,6-Me_4-Gal	0.5	0.7	2
2,3,4,6-Me_4-Glc	0.7	0.3	2
2,4-Me_2-Xyl	0.9	0.5	6
2,3-Me_2-Ara	3	1.0	5
3,4-Me_2-Xyl	0.7	0.5	3
2,3-Me_2-Xyl	100	100	100
2-Me-Xyl	26	5	31
3-Me-Xyl	3.5	6	11
2,6-Me_2-Glc	–	–	9

of molecules split between C-4 and C-5 were plentiful (*m/e* 158, 186, 214 and 242). Similar fragmentation pathways were observed for the D-galactose derivatives and derivatives of D-xylose and D-arabinose could be confirmed by analogous comparisons.

The method has been applied to the analysis of xylans from ryegrass, alfalfa and hay and the results are shown in Table IV. The results for the ryegrass and alfalfa xylan are very similar to the values obtained by other methods[11,12] and so the peracetylated partially methylated aldononitriles can be confidently used for the analysis by GLC or GLC–MS of partially methylated products obtained by hydrolysis of methylated polysaccharides.

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