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Analysis of Acetylated Methyl Glycosides as Their Trimethylsilyl Derivatives by Gas-Liquid Chromatography and Mass Spectrometry

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The separation, by gas liquid chromatography, of the trimethylsilyl ether derivatives of thirty-one methyl D-hexopyranoside acetates with the *O*-acetyl groups in the 2-, 3-, 4-, 6-, 2,3-, 3,6-, and 4,6-positions is described. The number and position(s) of the *O*-acetyl groups are unequivocally determined by mass spectrometry.

Partially *O*-acetylated glycosides are of interest in carbohydrate chemistry from several points of view. They are useful intermediates in synthesis, in particular for the protection of specific hydroxyl groups.[1] Advantage can be taken of their nucleophilic properties in operations involving neighbouring group participation.[2] Their tendency to undergo acyl migration[3] can be troublesome, although this very property has on occasion been put to advantage.[4] Many naturally-occurring polysaccharides are partially *O*-acetylated, Some of these *O*-acetylated polysaccharides are of biological importance and the *O*-acetyl group itself can contribute to the biological activity. Partially *O*-acetylated glycosides are therefore useful in model studies on biological interactions, such as the determination of enzyme specificities and investigations of biological systems. For these reasons, the assignment of the number and positions of *O*-acetyl groups in a glycoside is a problem of general interest. A prerequisite for any method used is that conditions which promote acyl migration must be avoided. Migration of *O*-acetyl groups is catalyzed by acid and base[1,3] and is also promoted by elevated temperature.[5] The number and position(s) of the *O*-acetyl groups can be determined by NMR since the *O*-acetyl function exerts a deshielding effect and the assignments may be facilitated by using lanthanide shift reagents.[6,7] Recently, a method was introduced by de Belder and Norrman in which the free hydroxyl groups are converted into acetal functions. The acetalized compound is then treated with base and methylated and, after hydrolysis, the resulting partially methylated sugars can be analyzed as their alditol acetates, by gas-liquid chromatography (GLC) and mass spectrometry (MS).[8]

In the present paper we describe the separation and analysis of several *O*-acetylated glycosides, as their trimethylsilyl ethers, by GLC. The number and position(s) of the *O*-acetyl groups were determined by MS.

The various acetates used in this study were all available from previous work [9,10] and their synthesis will therefore not be repeated here. The relative retention times of the methyl hexopyranosides acetates on GLC are given in Table 1. The column used (OV 225) generally gives adequate separations for analytical purposes.

Table 1. Relative GLC retention times of acetylated methyl glycosides as their trimethylsilyl derivatives.

	GLC retention time[a]						
Position of *O*-acetylation	2	3	4	6	2,3	3,6	4,6
Methyl glycopyranoside of:							
α-D-Galactose	2.09	2.77	–	2.42	5.23	8.35	3.59
β-D-Galactose	4.25	2.42	1.60	2.43	6.84	–	4.31
α-D-Glucose	2.07	1.64	2.75	4.10	5.25	–	7.77
β-D-Glucose	3.48	2.32	2.73	3.45	–	–	7.46
α-D-Mannose	1.11	1.67	1.54	1.89	–	–	4.45
β-D-Mannose	2.23	1.32	–	–	4.86	–	–

[a] on an OV-225 (S.C.O.T.) column at 165° relative to methyl tetra-*O*-(trimethylsilyl)-α-D-glucopyranoside.

The ions obtained, by electron impact mass spectrometry, from methyl D-hexopyranosides acetylated in the 2-, 3-, 4-, 6-, 2,3-, 3,6-, and 4,6-positions are given in Table 2. Since variations in the configurations of the molecules only gave rise to minor variations in the relative abundance of the ions obtained, only the MS for the methyl β-galactopyranosides (α-anomer of the 3,6-diacetate) are given in Table 2. The fragmentation patterns observed are analogous to those observed by DeJongh and co-workers for methyl glycoside trimethylsilyl ethers.[11] Mass differences are observed, however, owing to the replacement of *O*-trimethylsilyl groups by *O*-acetyl groups and also by the elimination of acetic acid and ketene during the fragmentation of the *O*-acetylated glycosides. In the following discussion, the nomenclature proposed by Kochetkov and Chizhov [12] is used.

For the monoacetates, ions are produced from the parent ion ($M^{\cdot+}$) corresponding to ($M^{\cdot+}-CH_3^{\cdot}$), ($M^{\cdot+}-CH_3^{\cdot}-CH_3OH$), ($M^{\cdot+}-CH_3^{\cdot}-CH_3COOH$), ($M^{\cdot+}-CH_3^{\cdot}-CH_3OH-CH_3COOH$) and [$M^{\cdot+}-CH_3^{\cdot}-CH_3COOH-(CH)_3SiOH$]. The parent ions [$M^{\cdot+}$] were not observed.

The A-series type of fragmentation produces a fragment *m/e* 331 (A_2) for the 2-, 3-, and 6-acetates.

$$M^{\cdot+} \xrightarrow{-{}^{\cdot}OCH_3} A_1 \xrightarrow{-TMSOH} A_2{}^1 \ m/e\ 331$$

Table 2. Fragments obtained on MS of acetylated methyl glycosides as their trimethylsilyl derivatives.

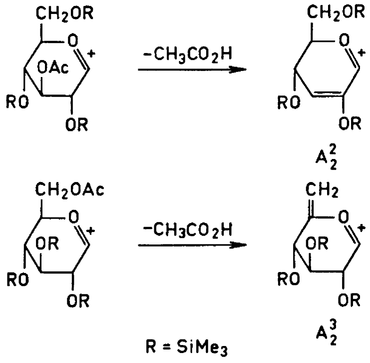
	Relative peak intensities[a]					Relative peak intensities[a]			
m/e	2-acetate	3-acetate	4-acetate	6-acetate	*m/e*	2-acetate	3-acetate	4-acetate	6-acetate
437	0.2	0.2	0.2	0.1	203	4	2	–	–
405	0.2	0.1	0.3	0.2	199	–	1	–	–
378	0.4	–	–	0.2	193	–	2	–	–
377	0.5	1.5	0.3	0.4	192	–	4	–	–
363	0.2	0.8	–	–	191	10	21	4	3
362	–	–	–	0.2	189	5	3	3	3
361	–	0.3	–	0.4	187	3	–	–	–
349	0.6	–	–	–	185	–	2	–	2
347	1.1	–	0.2	0.3	175	5	5	–	4
346	–	–	0.2	–	174	12	28	–	–
345	0.7	0.9	0.8	0.4	173	–	2	–	–
335	0.8	0.9	–	0.4	171	3	2	–	–
334	1.5	2.9	–	–	169	–	3	2	–
333	5.0	7.9	0.5	–	163	–	2	–	–
332	0.6	3.2	0.3	0.3	161	3	2	3	–
331	0.6	1.3	–	0.6	159	6	4	2	3
319	–	–	–	1.2	157	–	3	2	2
318	0.6	–	0.2	1.6	155	–	3	3	1
317	1.4	0.8	0.9	5.3	149	4	4	3	3
306	0.8	–	–	0.7	148	5	3	2	3
305	3.1	0.6	0.2	2.6	147	32	27	16	23
304	1.4	–	–	–	146	–	4	2	4
303	5.3	–	0.5	–	145	9	4	5	5
291	0.5	–	0.7	0.6	143	4	5	4	3
290	–	–	–	0.5	142	–	13	9	–
289	1.3	2.0	0.6	0.3	135	–	–	–	1
288	–	0.9	0.7	0.3	134	3	4	2	2
287	1.2	2.7	2.3	1.1	133	19	18	22	23
276	–	0.7	–	–	132	43	69	6	–
275	1.0	2.4	–	–	131	5	6	5	4
274	0.5	1.8	–	–	130	–	4	–	1
273	1.8	2.4	1.0	0.5	129	11	34	8	8
272	–	–	–	0.4	119	2	4	6	–
271	–	1.5	–	1.2	118	2	2	2	–
265	0.7	–	–	–	117	16	26	28	5
261	1.3	1.4	2	–	116	4	7	3	2
260	3.7	1.2	8	1.2	115	–	–	–	1
259	1.1	1.0	–	0.5	113	–	2	–	2
257	–	0.8	–	–	103	14	11	8	3
248	–	–	–	–	101	3	3	3	2
247	4	2	–	1	95	2	2	3	11
246	1	–	–	–	93	5	6	8	5
245	5	3	2	–	89	6	11	9	5
244	2	5	3	–	85	–	–	–	2
243	4	11	5	2	81	3	2	3	3
233	1	–	1	–	75	18	24	28	15
232	–	2	2	–	74	9	6	7	5
231	4	3	8	–	73	100	100	100	73
230	2	3	–	–	72	–	2	2	–
229	–	–	–	–	71	–	1	–	–
220	–	2	–	–	69	–	2	3	–
219	9	8	–	3	61	–	–	–	1
218	31	24	–	8	59	5	6	5	4
217	39	61	3	43	55	–	2	–	2
207	–	–	–	3	45	8	7	8	6
206	–	–	8	8	44	7	6	5	4
205	6	3	26	22	43	16	15	21	16
204	7	2	99	100					

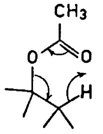
Table 2. Continued

	Relative peak intensities [a]				Relative peak intensities [a]		
m/e	2,3-acetate	3,6-acetate	4,6-acetate	*m/e*	2,3-acetate	3,6-acetate	4,6-acetate
407	0.2	–	0.4	187	14	–	2
375	0.1	1.0	0.1	185	3	4	–
348	0.2	–	–	183	3	5	2
347	0.2	0.5	0.4	175	4	8	5
333	0.3	1.0	–	174	–	25	–
322	–	2.1	–	173	4	–	–
319	0.6	–	–	172	3	–	–
317	0.6	–	–	171	16	–	–
314	–	–	0.7	170	7	–	–
306	0.8	–	–	169	3	–	–
305	3.5	–	–	161	4	–	3
304	1.4	–	–	159	4	5	–
303	3.9	2.1	–	158	6	–	–
302	0.6	2.1	–	157	7	–	–
301	0.6	–	–	155	5	7	6
291	1.3	–	–	149	5	–	–
289	0.8	2.1	–	148	3	–	–
288	0.5	–	0.5	147	23	7	8
287	2.5	5	2.8	146	3	13	3
275	0.8	–	–	145	27	22	5
274	1.4	–	–	143	6	7	4
273	5.2	4	0.4	142	–	13	11
272	–	–	0.4	134	–	6	3
271	–	3	0.4	133	13	25	21
262	1.3	–	0.9	132	9	89	4
261	5.0	–	3.1	131	4	8	3
260	–	–	9.5	130	3	–	–
259	2.6	–	–	129	23	23	3
257	1.3	–	1.1	119	9	6	6
246	1	–	–	118	3	7	–
245	4	5	1	117	41	48	17
244	1	7	–	116	7	11	4
243	5	33	2	115	3	–	2
242	2	–	–	113	3	–	–
241	–	3	–	112	4	–	–
233	–	–	1	109	–	–	2
231	3	–	–	103	19	4	3
230	2	–	–	101	5	5	2
221	–	–	1	95	4	5	2
220	3	–	–	93	8	14	5
219	8	6	–	89	8	13	5
218	29	11	–	87	8	–	–
217	29	51	2	85	4	4	2
215	4	–	–	81	6	8	4
213	4	–	–	75	29	43	19
206	–	–	7	74	10	9	7
205	6	6	16	73	100	100	67
204	–	6	100	69	–	–	3
203	4	–	–	61	3	4	–
201	–	–	3	59	7	9	4
199	3	–	–	55	3	4	–
191	8	7	–	45	13	16	7
189	5	–	3	44	14	36	10
188	3	5	–	43	84	73	44

[a] Peaks of relative intensity <1 were omitted below m/e 250.

Other types of A_2-fragments, at *m/e* 361, are also given by the 3- and 6-acetates, by the elimination of acetic acid from the A_1-fragment.





In the ensuing fragmentation, $A_2{}^2$ and $A_3{}^2$ can lose a molecule of trimethyl silanol [TMSOH (90)] yielding A_3-fragments at *m/e* 271.

Fragments corresponding to the B-, C-, D- and E-series are weak. Strong fragments derived from the H- and K-series type of fragmentation are observed (Tables 2 and 3).

In order to distinguish between the monoacetates, reference may be made to the spectra in Table 2. Alternatively, the substitution pattern of a fully trimethylsilylated methyl hexopyranoside monoacetate may be unequivocally determined from some of the most abundant fragments (Table 3).

Table 3. Abundant fragments obtained from monoacetylated methyl glycosides as their trimethylsilyl derivatives.

Fragment (*m/e*)	Probable major structure (TMS = $(CH_3)_3Si$-)	Relative peak intensities 2-acetate	3-acetate	4-acetate	6-acetate
219	$[TMSO-{\cdot}CHCH_2CH_2OTMS]^+$	9	8	–	3
218	$[TMSO-CH=CHCH_2OTMS]^+$	31	24	–	8
217	$[TMSO-CH=CH-CH=OTMS]^+$	39	61	3	43
205	$[TMSO-{\cdot}CH-CH_2OTMS]^+$	6	3	26	22
204	$[TMSO-CH=CH-OTMS]^+$	7	2	99	100
174	$[TMSO-CH=CH-OAc]^+$	12	28	–	–
142	Probably C_3-C_4 fragm.	–	13	9	–
132	$[TMSO-CH_2CHO]^+$	43	69	6	–

The molecular ions of the diacetates are not seen, but fragments are obtained corresponding to $(M^{\cdot+}-CH_3{}^{\cdot}-CH_3COOH)$. The A_1-, B-, C-, D-, and E-series of fragments are only found in low abundance. The position of the

O-acetyl groups can readily be determined by inspection of the H- and K-type fragments (Table 4) or from a comparison with the reference spectra.

Table 4. Abundant fragments obtained from diacetylated methyl glycosides as their trimethylsilyl derivatives.

Fragment (*m*/*e*)	Probable major structure (TMS = $(CH_3)_3Si$-)	Relative peak intensities		
		2,3-acetate	3,6-acetate	4,6-acetate
243	possibly $M^{\cdot+} - TMSO^{\cdot} - TMSOH$	5	33	2.5
218	$[TMSO-CH=CH-CH_2OTMS]^+$	29	11	–
217	$[TMSO-CH=CH-CH=OTMS]^+$	29	51	2
204	$[TMSO-CH=CH-OTMS]^+$	–	6	100
174	$[AcO-CH=CH-OTMS]^+$	–	25	–
171	–	16	–	–
147	$[(CH_3)_3SiOSi(CH_3)_2]^+$	23	7	8
145	–	27	22	5
142	Probably C_3-C_4 fragm.	–	13	11
132	$[TMSO-CH_2CHO]^+$	9	89	4
129	$[TMSO-{\cdot}CH-CH=CH_2]^+$	23	23	3

EXPERIMENTAL

The various glycoside acetates were available from previous studies.[9,10] They were converted into the fully trimethylsilylated derivatives as described by Sweeley.[13] GLC was performed on a Perkin-Elmer 900 instrument fitted with an OV-225 S.C.O.T. column at 165°. The mass spectra, using a Perkin-Elmer 270 gas chromatograph mass spectrometer, 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 120°.

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