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Determination of the Number and Position of Methoxyl Groups in Methylated Aldohexoses by Mass Spectrometry of their Trimethylsilyl Derivatives

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Trimetylsilylering av metylerade aldohexopyranoser och masspektrometri av de blandade derivaten möjliggör en enkel bestämning av metoxylgruppernas antal och position. Metoden grundas på den nära analogin mellan fragmenteringen för blandade derivat och fullständigt metylerade derivat. Referensprov behövs inte för bestämningen. Fragmenteringen diskuteras på grundval av spektra för tjugo föreningar.

Spektra av derivat av hexofuranoser och etylerade hexopyranoser tyder på att metoden kan tillämpas också för dessa föreningar.

Dimetylsilylderivat uppvisar en fragmentering liknande den för trimetylsilylderivaten.

Trimethylsilylation of methylated aldohexopyranoses and mass spectrometry of the mixed derivatives permits a simple determination of the number and location of the methoxyl groups. Advantage is taken of the close analogy between the fragmentation of the mixed derivatives and of fully methylated species. Reference substances are not necessary for identification. The fragmentation is discussed on the basis of the spectra of twenty species.

Spectra of derivatives of hexofuranoses and ethylated hexopyranoses indicate that the method can be applied to these species as well.

Dimethylsilyl derivatives are shown to exhibit a fragmentation behavior similar to that of the trimethylsilyl derivatives.

Herstellung von Trimethylsilylderivaten methylierter Aldohexopyranosen und Massenspektrometrie der resultierenden gemischten Derivate ermöglicht eine einfache Bestimmung der Anzahl und Stellung der Methoxylgruppen. Die Methode gründet sich auf eine weitreichende Ähnlichkeit der Fragmentation gemischter Derivate und Derivate, die vollständig methyliert worden sind. Eine Referenzprobe ist nicht erforderlich. Die Fragmentation wird an Hand von Spektren von 20 Substanzen diskutiert.

Die Spektren von Hexofuranosederivaten und äthylierten Hexopyranosen zeigen, dass die Methode auch für diese Substanzen angewandt werden kann.

Dimethylsilylderivate weisen eine Fragmentation auf, die jener von Trimethylsilylderivaten ähnlich ist.

In an earlier paper (1) it was shown that a close analogy exists between the fragmentation of trimethylsilyl (TMS) derivatives of partially methylated aldopentopyranoses and that of fully methylated aldopentopyranoses previously studied (2, 3). The mass spectra of the TMS-derivatives were shown to permit a simple determination of the number and position of the methoxyl groups in partially methylated aldopentopyranoses.

The purpose of the present paper is to describe a similar method for the analysis of methylated hexoses, the study of which is of great importance in structure determinations of hemicellulose and other polysaccharides.

EXPERIMENTAL

About 1 mg of each sugar was used as starting material. The trimethylsilyl derivatives were prepared according to the method of Sweeley *et al.* (4). Dimethylsilyl (DMS) derivatives were prepared in an analogous manner (5) using tetramethyldisilazane and dimethylmonochlorosilane. The pyridine and excess reagents were removed in a rotating vacuum evaporator at 40°C and the silyl derivatives were dissolved in diethyl ether.

The mass spectral measurements were carried out on a LKB 9000 gas chromatograph—mass spectrometer. The silyl derivatives (less than 5 μg) were introduced through columns with 1 % SE-30 on Chromosorb P or 3 % QF-1 on Gas Chrom Q as stationary phases. Column temperatures below 170°C were used throughout. For the molecule separators a temperature in the interval 200—220°C was used for the TMS-derivatives and 190°C for the DMS-derivatives. The temperature of the ion source was 270°C. All spectra were measured at the ionizing voltage 70 eV.

A correction for column bleeding was made by subtracting bleeding spectra. The peak intensities

Table 1. Peak intensities of characteristic fragment ions of fully substituted TMS-derivatives of aldohexopyranoses and partially methylated aldohexopyranoses.

Ion de-notation	m/e - value	α-D-glucose	β-D-glucose	α-D-mannose	α-D-galactose	Me α-D-glucoside	2-O-Me-glucose	3-O-Me-glucose	6-O-Me-glucose	Me 4-O-Me-β-D-glucoside	2.3-di-O-Me-glucose	2.4-di-O-Me-glucose	2.4-di-O-Me-galactose	3.4-di-O-Me-galactose	2.6-di-O-Me-galactose	3.6-di-O-Me-glucose	4.6-di-O-Me-glucose	Me 2.3-di-O-Me-β-D-glucoside	2.3.4-tri-O-Me-glucose	2.3.6-tri-O-Me-glucose	2.3.4.6-tetra-O-Me-glucose
H_1	88	0	0	0	0	0	0	0	0	0	83	0	0	5	0	0	0	100	100	100	100
	146	0	0	0	0	7	100	91	0	17	9	100	100	80	100	100	12	10	4	10	3
	204	100	100	100	100	100	8	4	100	100	0.8	0.2	0.3	2.8	5.0	3	100	0.3	0.4	0.5	-
J_1	75	7	6	6	6	7	8	9	7	9	9	10	11	9	8	9	8	49	16	11	15
	133	5	4	4	4	37	9	100	6	42	100	4	4	100	9	64	9	12	95	77	68
	191	40	38	39	44	7	40	6	29	2	4.3	34	35	2.5	46	4	35	2.6	0.9	1.7	-
F_1G_1	101	1	1	1	2	2	2	2	0	3	6	5	10	4	2	2	5	14	33	10	36
C_2	159	0	0	0	0	2	9	8	3	21	40	9	11	60	30	12	12	58	9	47	3
	217	17	17	16	25	27	8	73	25	6	6.7	1.2	1.1	3.5	2.4	80	4	5.2	0.8	1.9	-
S_1	45	4	3	3	4	5	5	6	7	7	6	5	5	6	14	16	12	12	11	19	24
	103	6	6	6	8	8	8	10	3	6	9	5	7	9	4	4	3	7	9	3	2
-	117	6	4	6	4	9	7	12	2	12	11	8	6	9	3	3	1	14	16	2	0
A_2	187	0	0	0	0	0	0	0	0	0	1.2	0	0	0.6	0	0	0	1.1	0.8	0.5	0.6
	245	0.4	0.3	0.3	0.6	0.5	0.6	0.9	0.5	0.9	0.5	0.3	0.3	1.6	0.8	0.5	2.1	0.5	0.2	0.3	-
	303	0.2	0.3	0.3	0.4	1.0	0.2	0.3	1.5	0.6	0.1	1.6	1.3	0.2	3.1	0.5	0.2	0.6	-	-	-
	361	1.0	0.8	0.4	0.6	1.7	2.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
A_1B_{T1}	219	3	2	2	3	4	2	8	2	1	1.8	0.6	0.5	0.8	0.5	7	0	2.4	0.2	0.2	0.2
	277	0.1	0.1	0.1	-	-	0.1	-	0.1	0.5	0.3	0.1	-	1.1	0.3	-	0.2	0.1	-	0.2	-
	335	-	-	-	-	0.6	0.4	2.1	0.1	-	0.1	-	-	0.3	0.1	1.8	2.1	-	-	-	-
	393	1.1	0.4	1.1	2.0	0.1	-	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-
B_{T2}	175	1	0	0	0	0	0	0	0	2	3.5	0	0	1.4	0	0	0	2.3	2.7	3.8	2.9
	233	0.7	0.7	0.5	0.4	1.7	2.3	3.3	0.7	2.2	1.2	2.1	1.0	2.7	2.4	3.7	1.2	0.7	0.3	1.1	-
	291	2.0	1.7	1.2	1.6	1.4	0.4	0.7	1.9	-	0.3	0.1	-	0.3	0.3	0.8	0.8	0.1	-	-	-
B_2	131	3	2	2	3	5	8	9	3	7	7	11	9	13	12	12	4	6	6	7	6
	189	3	4	4	3	3	2	3	4	2	2.4	1	1	4.0	3	2	3	1.9	1.2	1.4	0.3
	247	0.2	0.2	0.1	-	1.0	1.3	2.0	0.3	1.6	0.4	0.6	0.4	0.6	2.4	1.2	1.6	0.3	-	0.1	-
	305	2.5	2.1	2.0	2.8	1.7	0.8	0.1	2.2	0.2	0.1	0.2	0.2	0.1	0.7	0.1	0.2	0.1	-	-	-
C_2	275	-	-	-	-	0.1	0.1	0.2	0.4	0.3	1.0	0.3	0.5	0.9	1.9	0.8	0.3	0.9	-	-	-
	333	0.4	0.3	0.3	0.7	0.5	0.5	0.7	0.1	0.2	-	-	-	-	-	-	-	-	-	-	-
C_3	127	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	3	4
	185	0	0	0	0	0	1	0	0	1	3.9	2	3	2.2	2	2	2	4.5	2.7	1.6	0.3
	243	1.4	1.0	0.8	2.1	1.6	1.3	3.5	1.0	1.2	1.1	0.5	0.7	2.0	0.9	1.1	0.4	0.8	-	0.1	-
T_1	293	0.4	0.3	0.2	0.2	0.3	0.2	0.2	0.4	0.2	-	-	-	-	-	-	0.2	-	-	-	0.1
	351	-	-	-	-	-	-	-	-	0.3	-	-	-	-	-	-	-	-	0.2	0.4	-
	409	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	0.6	-	-	-	-	-
	467	-	-	-	-	0.2	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-	-
T_2	261	-	-	-	-	0.3	0.3	0.5	0.4	1.1	0.6	0.7	0.3	0.9	1.3	0.6	0.4	0.2	0.2	0.3	0.5
	319	1.2	1.5	1.2	1.5	1.1	1.3	0.4	1.3	2.1	0.3	0.8	0.4	0.3	0.8	0.2	0.8	2.1	2.1	0.4	-
	377	-	-	-	-	2.1	1.1	0.4	1.1	1.2	1.9	0.5	0.2	2.2	0.3	1.1	0.4	-	-	-	-
	435	1.7	1.8	1.1	0.7	0.5	0.2	2.7	0.2	-	-	-	-	-	-	-	-	-	-	-	-
T_3	229	0.4	0.6	0.4	0.4	0.4	0.3	0.3	0.3	0.9	0.5	0.5	0.4	0.2	0.4	0.2	0.3	1.1	1.0	0.4	0.8
	287	-	-	-	-	0.7	0.6	0.3	0.6	1.4	1.2	0.7	0.4	0.9	0.5	0.4	0.5	0.8	1.1	0.3	-
	345	1.0	1.0	0.6	0.5	0.7	0.6	0.7	0.5	0.3	0.3	0.3	0.2	1.8	-	0.2	-	-	-	-	-
R	174	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0	0.5	3.4	0.2	3.5
	232	0.7	0.5	0.5	0.5	0.8	0.9	0.4	0.2	2.1	3.8	0.3	0.4	3.1	0.2	0.2	0.3	0.5	-	3.2	-
	290	-	-	-	0.3	1.2	-	2.4	-	0.1	0.2	-	-	0.1	-	1.5	-	0.3	-	-	-
-	59	2	2	2	2	4	4	7	3	8	8	6	6	7	6	8	5	8	9	11	7
	73	89	77	78	93	80	68	93	63	68	69	41	46	69	54	71	58	57	45	44	23
	89	2	0	1	1	6	7	14	5	16	17	13	14	18	12	19	8	20	26	28	18
	147	24	21	19	24	24	27	31	17	13	14	17	15	19	24	21	14	12	2	5	2
	145	0	0	0	0	0	1	3	2	2	2	2	2	3	3	4	1	2	4	5	1
	203	3	2	2	3	4	2	1	1	0	1.6	0.7	0.5	0.8	0.7	0	0	1.3	0.7	0.6	0.3
	173	0	0	0	0	0	0	2	0	3	2.7	2	1	2.8	2	1	2	2.4	2.4	1.2	0.5
	231	3.0	2.3	2.5	2.1	3.5	3.7	1.5	0.7	1.7	0.9	1.5	1.5	0.6	0.6	0.6	0.6	1.4	0.6	0.2	0.2
	205	21	20	20	20	24	3	8	19	23	8.0	0.5	1.0	8.1	1.5	0	22	0.8	8.2	0.4	0.1
	71	0	0	0	0	1	3	3	0	6	7	4	6	10	7	4	8	8	9	15	21
	129	8	6	7	12	10	5	14	8	6	5	4	6	9	9	13	3	4	6	9	0
	259	0.1	0.1	0.1	0.2	0.3	0.7	1.3	0.4	1.1	1.2	0.6	0.9	1.0	1.1	3.7	0.5	1.0	0.2	0.2	-
	317	1.0	0.7	0.7	0.9	1.0	0.3	0.7	1.0	0.2	-	0.1	-	0.4	-	0.2	-	-	-	-	-

were calculated as percentages of the base peak. Only peaks greater than 1.0 % in the lower part of the spectra were taken into account. In the upper, low intensity part of the spectra peaks greater than 0.10 % were considered. The spectra presented in *Tab. 1* include all peaks (except some isotope peaks) greater than 6 % below $m/e = 225$ and greater than 2 % above this mass number. Anomeric derivatives were found to give almost identical spectra. With some species the anomeric configuration was unknown, but all reported data refer to one anomer.

RESULTS AND DISCUSSION

The fragmentation pattern of trimethylsilyl derivatives of aldohexopyranoses and partially methylated aldohexopyranoses

In Tab. 1 the spectra of twenty fully TMS-substituted aldohexoses and partially methylated aldohexoses are given. Five representative spectra of glucose derivatives with 0 to 4 methoxyl groups are reproduced in *Fig. 1*.

Published fragmentation studies using selectively deuteromethylated glucopyranoses (6—8) and gal-

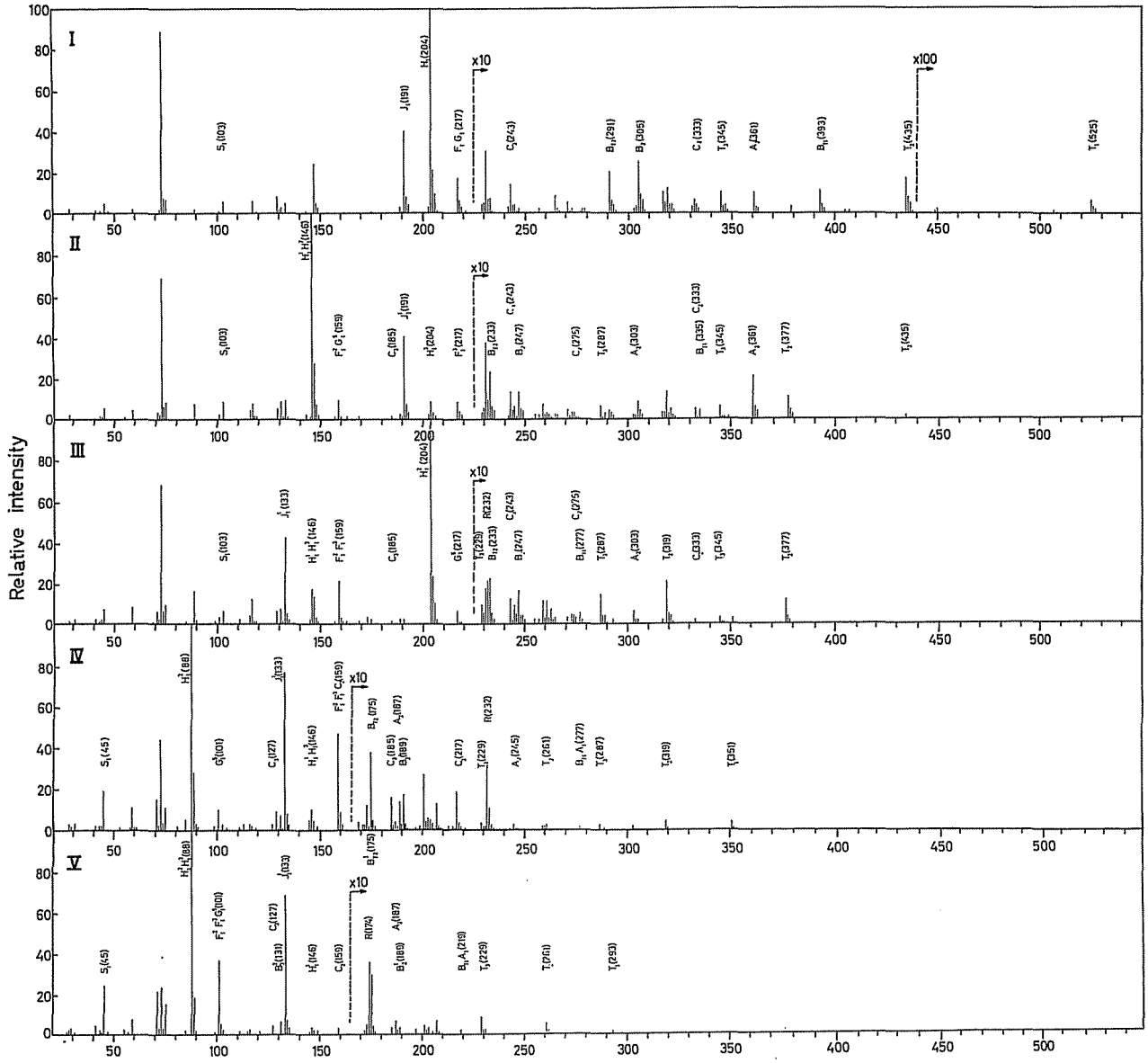

Fig. 1. Mass spectra of fully trimethylsilylated α-D-glucopyranose (I), 2-O-methyl-glucopyranose (II), methyl 4-O-methyl-β-D-glucopyranoside (III), 2,3,6-tri-O-methyl-glucopyranose (IV) and 2,3,4,6-tetra-O-methyl-glucopyranose (V).

actopyranoses (3) make possible a comparison with the fragmentation pattern of fully methylated hexopyranoses. As with the pentoses close analogies exist. It is therefore convenient to apply the fragmentation scheme devised by Kochetkov and Chizhov for fully methylated derivatives (9) to the discussion of the fragmentation of the TMS-derivatives. The denotations of the fragment ions in Fig. 1 and Tab. 1 are made according to this system with a few additions explained below. For fragment ions of interest the peak intensities at the possible mass numbers differing by 58 mass units are given in Tab. 1 with the exception of mass numbers with no peak greater than 0.10 % (m/e = 525 and 451).

The spectra in Tab. 1 of the TMS-derivatives of four aldohexopyranoses and two 2,4-di-O-methyl-aldohexopyranoses show that the difference between diastereomers is very small as expected.

Fig. 2 shows schematically the formation of the most significant ions for a mixed derivative. These ions belong to the first fragmentation series discussed below.

J and F (G) series: The most important initial fragmentation step for ethers of aldopentoses and aldohexoses is considered to be the breakage of the bond between C-1 and C-2 (9, 10). The preferred splitting of this bond is explained by the presence of a total number of three oxygens at C-1 and C-2.

The main ions of the J and F series, J_1^1 and F_1^2 (see Fig. 2), contain the substituents from C-1 and C-3 (J_1^1) and from C-2 and C-4 (F_1^2). With methylated sugars (10) these very abundant ions are most likely formed by the initial cleavage of the C-1 to C-2 bond and migration of the C-3 substituent to C-1. With the derivatives studied in the present work the J_1^1 and F_1^2 intensities are greater

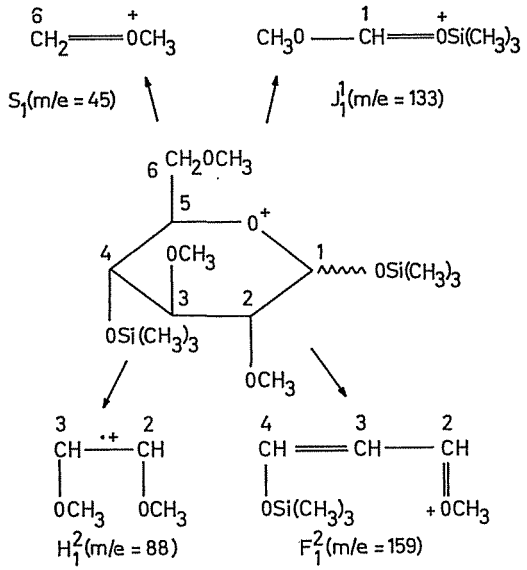

Fig. 2. Schematic representation of the origin of the most important ions of the H, J, F (G) and S series of TMS 2,3,6--tri-O-methyl-4-O-TMS-glucopyranoside (IV).

for all the 3-O-methylsubstituted species than for the other derivatives. For three derivatives with a methoxyl group at C-3 the peak at the J_1^1 mass number is the base peak. A corresponding although less pronounced effect of the substituent at C-3 was observed with the mixed derivatives of aldopentoses studied previously (1). The results indicate that the same fragmentation path is valid with the mixed derivatives as with the fully methylated species and that methoxyl radicals migrate more easily than trimethylsiloxyl radicals.

For all derivatives the J_1^1 peaks are more than three times as intense as any other peak at the J_1 mass numbers [i.e. 75, 133 and 191; cf. Tab. 1] even though ions of different origin contribute to these peaks. For the F_1^2 ions the same holds true for all the 3-O-methylsubstituted derivatives. As can be seen from Tab. 1 the contributions from other F_1 and G_1 ions of importance for each of the G_1^5 (substituents from C-2 and C-3) and F_1^3 (substituents from C-4 and C-6) ions can be estimated to about 5—10 % of the base peak. These contributions are independent of those from F_1^2 ions which are formed in a different fragmentation path. With 2-O-methyl-3-O-TMS-substituted species the F_1^2 peaks are of particularly low intensity and F_1^3 and G_1^5 peaks can give rise to the greatest peak at the F_1 (G_1) mass numbers (the 2,4-di-O-methylsubstituted derivatives in Tab. 1). The contributions from C_2 ions are small compared to those from the F_1 (G_1) ions.

A further observation concerning the J_1 and F_1 ions, as well as several other ions, is that the peak intensities seem to indicate a somewhat greater ion stabilization effect from trimethylsiloxyl groups than from methoxyl groups.

H-series: The H_1 ions consist of two adjacent carbon atoms from the pyranoid ring with their substituents. From all derivatives in Tab. 1 the largest H_1 peak is obtained at the mass number of H_1^2 (C-2 and C-3). Other H_1 peaks indicate that the abundances of the H_1^1 (C-1 and C-2) and H_1^3 (C-3 and C-4) ions range from 2—3 per cent to more than ten per cent of the H_1^2 ion abundance.
For the H_1 mass numbers the peaks with no contributions from the three H_1 ions were less than one per cent of the base peak. The H_1^2 peak constitutes the base peak for most derivatives.

With the hexopyranose as well as with the pentopyranose derivatives (1) the H_1 radical ions split off methyl radicals forming even-electron ions which contribute to the peaks at $m/e = 73$, 131 and 189. The peak at $m/e = 131$ is greater in all instances if the H_1^2 mass number is 146 than if the H_1^2 mass number is 88 or 204. For all derivatives with H_1^2 ions containing one or two TMS-groups this fragmentation is supported by well discernible metastable peaks (1).

S-series: From Tab. 1 it is seen that there exists a correlation between the substitution at C-6 and the relative intensities of the peaks at $m/e = 45$ and 103. This correlation permits the conclusion that ions with these mass numbers are formed by a cleavage of the C-5 to C-6 bond with retention of the positive charge on the C-6 fragment. These ions will be denoted by S_1.

Most 6-O-TMS-substituted derivatives give rise to a more intense peak at $m/e = 117$ than those containing a methoxyl group at C-6. It is likely that ions of this mass number consist of the ring oxygen, C-5, and C 6 with its TMS-substituent, minus a methyl radical from the TMS-group.

A-series: The A_1 ions are formed by the loss of the C-1 substituent, and the other members of this series by additional elimination of one (A_2) or two (A_3) molecules of methanol and/or trimethylsilanol. Since the base peak for the 3-O-methylsubstituted derivatives is less predominant, the relative intensities of the peaks of most fragmentation series are higher with these derivatives than with other species. A characteristic feature of the fragmentation path leading to the A-series is revealed by high A_2 peak intensities for the 2-O-methyl-3-O-TMS-substituted species. The A_2 peak corresponding to elimination of the C-2 substituent is clearly the largest one at the A_2 mass numbers for these species, whereas a preferred loss of the C-3 substituent has been observed for the fully methylated derivatives (6). These observations also apply to the pentose derivatives investigated earlier (1).

The peaks at mass numbers corresponding to A_1 only were very small and the A_3 peaks were of low intensity compared to other ions in this region of the spectrum.

B-series: The odd-electron B_1 ions consist of carbon atoms 1—4 with substituents. With the mixed methyl-TMS-derivatives even-electron B_{T1} ions are formed which correspond in mass to the subtraction of a methyl radical from the B_1 ions [cf. (1)]. Like the ions of the A-series, the B_{T1} ions are formed in a fragmentation path that competes with those starting with C-1 to C-2 bond cleavage. For most derivatives the B_{T1} ions are more intense for species with the same substituent at C-1 and C-2, than for

the other species. Ions of a different origin often contribute greatly to the *m/e* = 219 peak.

The B_{T2} ions are probably analogous to the B_{T1} ions, but consist only of C-1, C-2 and C-3 (B_{T2}^1) or C-2, C-3 and C-4 (B_{T2}^2) with substituents. The values in Tab. 1 indicate that B_{T2}^1 and B_{T2}^2 ions are formed to approximately the same extent. For derivatives with the same substituent at C-1 and C-4 the positions of these ions coincide and they give rise to the largest peak at the B_{T2} mass numbers.

It has been suggested (10) that the B_2^2 ions are formed in a fragmentation path analogous to that of the F_1^2 ions, but that a migration of a hydrogen atom is involved. The B_2^1 ions contain C-1, C-2 and C-3. Contributions from other ions are obtained at the B_2 mass numbers 131 and 189.

C-series: The peaks at C_2 mass numbers (9) are less important for the determination of the location of the substituents in the mixed derivatives one reason being that some C_2 mass numbers coincide with F_1 mass numbers. The results obtained with the monomethylated pentoses and hexoses with the methoxyl group at C-2 or C-3 indicate that the methoxyl groups are lost more easily than the trimethylsiloxyl groups. Instead of an elimination of the substituent at C-2 or C-3 the substituent at C-4 or C-6 can be lost although to a lesser extent. This is reflected in peaks at the highest possible mass number with all derivatives studied.

The C_3 ions corresponding to the elimination of methanol or trimethylsilanol from C_2 ions possess a higher intensity than the C_2 ions.

T-series: In most spectra the peak with highest mass number which can be recognized is at M-15, corresponding to a loss of a methyl radical from the molecular ion (1). This peak (T_1) is typical of TMS-derivatives and, although of low intensity (often less than 0.10 %) it can be recognized in most cases because of the absence of disturbing neighbouring peaks.

The T_2 ions correspond to loss of a methanol or trimethylsilanol molecule from the T_1 ions. As is the case for the A_2 ions from fully methylated derivatives the loss of the C-3 substituent is favored. With all 3-O-methylsubstituted species the resulting ions give rise to the largest peak at the T_2 mass numbers. A peak with an intensity greater than 0.10 % was recorded at the highest T_2 mass number possible for the derivative in question, indicating that instead of a loss of the C-3 substituent any other substituent can be lost. With the mixed derivatives the mass number is M-47. Among the peaks of appreciable intensity this peak has very often the highest mass number.

The T_3 ions, corresponding to the loss of an additional molecule of methanol or trimethylsilanol, are of lower intensity than the T_2 ions. As indicated in Tab. 1 other ions may contribute to the intensities of the peaks at the lower T_2 and T_3 mass numbers.

R-series: The formation of a rearrangement ion denoted R is suggested by the presence of a peak at *m/e* = 174, 232 or 290 for several derivatives. A prerequisite for the formation of these ions seems to be that C-1 and C-3 have different substituents. The ions evidently contain the substituents from C-2 and C-4. The R peaks are greater than 1 % for all nine derivatives which fulfill the above requirement. Other peaks at R mass numbers are very small if the isotope peaks from *m/e* = 173 and *m/e* = 231 ions are substracted.

Formation of R ions offers an alternative explanation to the M-90 peaks for the methyl xylopyranoside and 3-O-methyl-xylopyranose derivatives (1).

Other fragment ions: With the investigated derivatives the peaks of the two series denoted D and E by Kochetkov and Chizhov (9) are of very low intensity. The E peaks are also often difficult to recognize because they coincide with isotope peaks from more intense T_2 ions.

Among the remaining peaks, the most intense arise from fragmentations characteristic of TMS-derivatives. As expected the intensity of the peak at *m/e* = 73 from the trimethylsilyl ion decreases with an increased number of methoxyl substituents. A substantial part of the ions with *m/e* = 59, 89 and 147 should also be due to ions from the trimethylsiloxyl function (1).

The peaks at mass numbers given in the lower part of Tab. 1 are of little interest from an analytical point of view. Since their origin has not been established with certainty these peaks will not be discussed in this paper.

The use of mass spectra for the determination of the number and position of methoxyl substituents

The mass spectra investigated indicate that there exist close analogies between the fragmentation of methyl- and TMS-derivatives, including the mixed derivatives of aldohexopyranoses. This makes possible an unambiguous determination of the O-methyl-substituted carbon atoms without a need for reference substances.

An identification scheme, valid for all derivatives in Tab. 1, is given below. It involves only peaks of high intensity thus permitting an interpretation of spectra of low intensity. Only peaks at a few mass numbers listed in the upper part of Tab. 1 have to be studied.

Substitution at C-1, C-2 and C-3: The number of methoxyls at C-2 and C-3 is 2, 1 or 0 if the most intense peak at the H_1 mass numbers is obtained at *m/e* = 88, 146 or 204 respectively.

The number of methoxyls at C-1 and C-3 is 2, 1 or 0 if the most intense peak at the J_1 mass numbers is obtained at *m/e* = 75, 133 or 191 respectively.

If *m/e* = 146 and *m/e* = 133 give the most intense peaks within these groups of mass numbers, there exist two possibilities. If the intensity of the *m/e* = 133 peak is more than half that of the base peak there is one methoxyl at C-3, otherwise there are methoxyls both at C-1 and C-2.

Substitution at C-4: The 2-O-methyl-3-O-TMS-substituted derivatives have a methoxyl group at C-4 if the *m/e* = 204 peak is less than 2 % of the base peak, otherwise not.

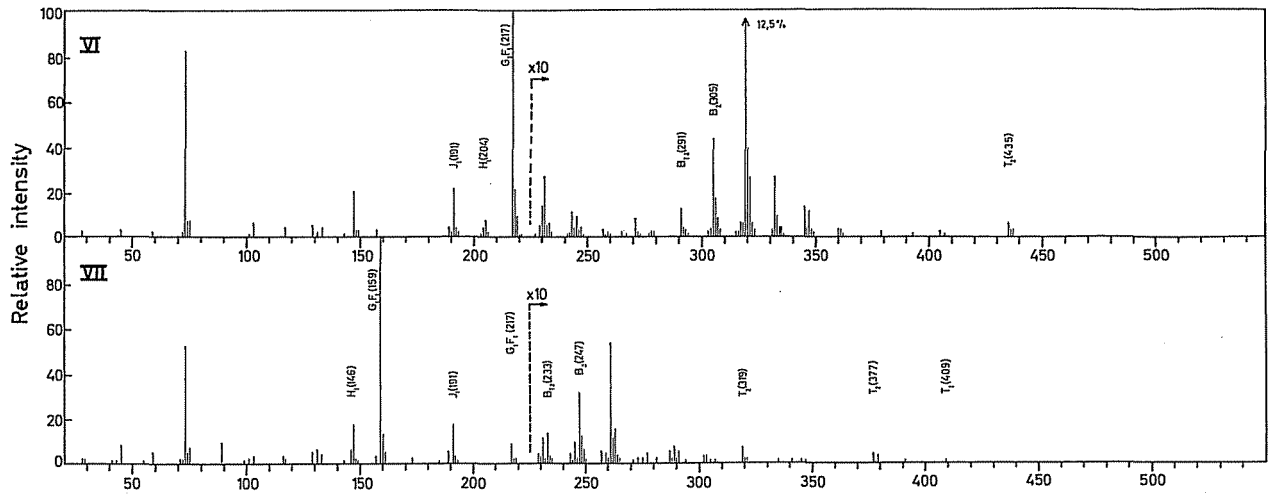

Fig. 3. Mass spectra of fully trimethylsilylated anomers of galactofuranose (VI) and 2,6-di-O-methyl-galactofuranose (VII).

With other compounds (often with 2-O-methyl--3-O-TMS-substituted derivatives as well) the number of methoxyls at C-2 and C-4 is 2, 1 or 0 if the most intense peak at the F_1 (G_1) mass numbers is obtained at m/e = 101, 159 or 217 respectively.

Substitution at C-6: If the intensity of the m/e = = 45 (S_1) peak is greater than the sum of the intensities of the m/e = 103 (S_1) and m/e = 117 peaks, there is a methoxyl group at C-6, otherwise not.

It is believed that this scheme, possibly with slight modifications for other instruments, is generally applicable to the determination of the number and position of the methoxyl groups in aldohexopyranoses. Doubtful results should be checked by a study of peaks from other fragmentation series. As indicated in the previous paragraph there exist numerous possibilities for control, a few of which are pointed out below.

Peaks at H_1 and F_1 (G_1) mass numbers other than those of H_1^2 and F_1^2 often indicate the mass numbers of other important H_1 and F_1 (G_1) ions.

A high intensity of the main J_1 and F_1 peaks indicates 3-O-methylsubstitution.

The major peaks of the A_2 and R mass number series can often be used to check the C-4 substitution.

The total number of methoxyl groups can be determined from the T_1 peak and from the T_2 peak of highest mass number.

APPENDIX I

Characteristic features of furanose forms of the aldohexose derivatives

To make possible a comparison of the characteristic features of pyranose and furanose forms, the spectra of fully TMS-substituted derivatives of galactofuranose (VI) and 2,6-di-O-methyl-galactofuranose (VII) were studied. These spectra are reproduced in *Fig. 3.*

Mass spectra of fully methylated aldohexofuranoses have been published previously (10, 11), and were in part interpreted according to the fragmentation schemes for the pyranose forms. Similarities between the fragmentation of fully TMS-substituted and fully methylated aldopentofuranoses have also been demonstrated (1). Common fragmentation features were therefore anticipated for the corresponding hexose derivatives and mixed hexose derivatives as well.

The base peak for VI and VII was obtained at the F_1 (G_1) mass numbers. The mass number shift from 217 for VI to 159 for VII suggests that G_1^5, retaining the substituents at C-2 and C-3, is the most abundant F_1 (G_1) ion. The unchanged mass number (191) of the main J_1 peak for VII suggests that, as with the pyranose derivatives, the substituents at C-1 and C-3 are retained. The mass number (146) of the H_1 peak for VII indicates that the H_1 ions contain C-2 with substituent. The most abundant F_1 (G_1), H_1 and J_1 ions are probably formed in the same way as for fully methylated aldopentofuranoses (3).

The relative intensities of the main G_1 (F_1), H_1 and J_1 peaks for VI and VII agree well with those of the fully methylated derivatives, but among the ions of low intensity there are some distinct differences. The A and E series are less important for VI and VII. Peaks at the mass numbers corresponding to ions formed by a cleavage of the bond between C-4 and C-5 with charge retention on either side exhibited rather low intensities. The same holds true for the ions corresponding to a cleavage of the C-5 to C-6 bond. The recognizable peaks of highest mass belonged to the T series. The peaks at m/e = 319 and m/e = 305 for VI were shifted to m/e = 261 and m/e = 247 for VII from which it can be concluded that one of the substituents at C-2 or C-6 is incorporated in these ions.

The spectra of VI and VII indicate that the furanosides of mixed methyl- and TMS-derivatives can be recognized by several characteristic features of their mass spectra. The high intensity of the main H_1 peak for the pyranosides and of the main G_1 (F_1) peak for the furanosides readily permits a distinction between these forms. The possibilities for deductions concerning methoxyl positions are obvious.

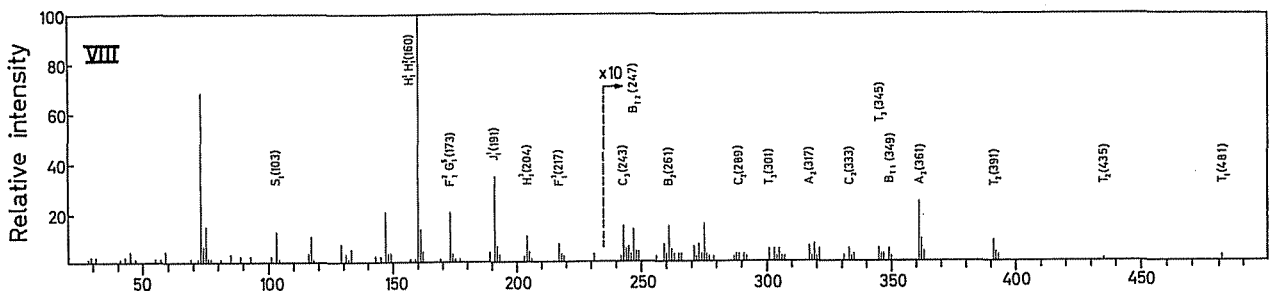

Fig. 4. Mass spectrum of a fully trimethylsilylated anomer of 2-O-ethyl-glucopyranose (VIII).

APPENDIX II

The fragmentation of TMS-derivatives of O-ethylsubstituted hexopyranoses

For purposes of comparison the spectrum of fully TMS-substituted 2-O-ethyl-glucopyranose (VIII) was recorded. The spectrum is reproduced in *Fig. 4* and should be compared with that of the TMS-derivative of 2-O-methyl-glucopyranose given in Fig. 1. Apart from shifts of 14 m.u. to higher mass numbers for ions containing the substituent at C-2, the spectra are very similar and can be interpreted in the same way. Corresponding fragmentation analogies were observed for 2,3-di-O-ethyl-glucopyranose. The potential applications for determination of the position of ethoxyl groups in partially ethylated sugars are evident.

APPENDIX III

Mass spectral characteristics of dimethylsilyl derivatives

Like TMS-derivatives DMS-derivatives have applications in gas chromatographic analysis of carbohydrates (5). A similar fragmentation for TMS- and DMS-derivatives, with the possibility of gaining further information about the fragmentation paths, was anticipated since the replacement of a methyl group by a hydrogen atom is the only structural difference. In *Fig. 5* the spectra of fully DMS-substituted pyranose derivatives of glucose and 2,3,6-tri-O-methyl-glucose are reproduced. The spectra of the corresponding TMS-derivatives are given in Fig. 1.

A comparison shows that H_1, J_1 and F_1 (G_1) peaks, in close agreement with the corresponding main peaks for TMS-derivatives, are obtained at mass numbers corresponding to the mass difference of 14 between TMS- and DMS-groups. The peaks in the upper part of the spectra are of very low intensity, indicating a more extensive fragmentation for DMS-derivatives. The peak at $m/e = 59$ should correspond to the dimethylsilyl ion. For the glucose derivative the peaks at $m/e = 59 + n \cdot 74$, n = 1, 2, 3, 4, probably arise from silicon polymer ions (12) formed from the DMS-groups. A metastable peak ($m^* = 100.5$) indicates the formation of ions with $m/e = 133$ from H_1 ions ($m/e = 176$).

The results obtained with DMS-derivatives lend support to some of the fragmentation paths discussed above for the TMS-derivatives. On the other hand the application of DMS-derivatives does not offer any advantages from an analytical point of view.

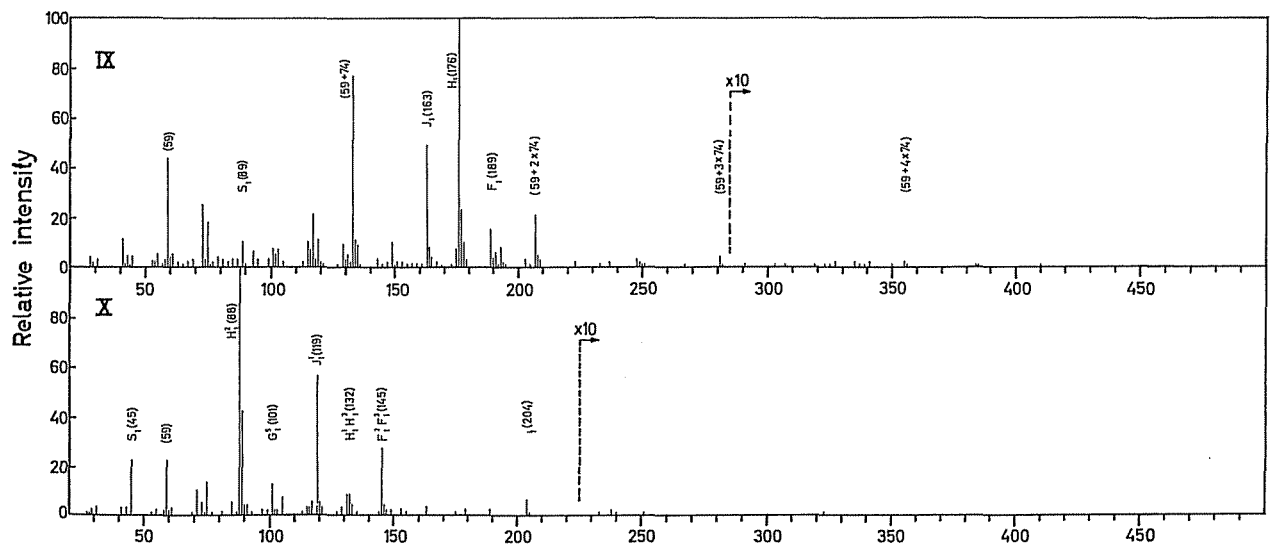

Fig. 5. Mass spectra of fully dimethylsilylated anomers of glucopyranose (IX) and 2,3,6-tri-O-methyl-glucopyranose (X).

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