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

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Fragmenteringen är mycket likartad vid fullständigt metylerade aldopentopyranoser och trimetylsilylderivat av partiellt metylerade aldopentopyranoser. En masspektrometrisk undersökning av trimetylsilylderivaten möjliggör därför en enkel bestämning av antalet metoxylgrupper i partiellt metylerade aldopentoser och ett fastläggande av de kolatomer vid vilka dessa grupper är bundna. Referensprov behövs inte för en identifiering. Metoden är av intresse vid strukturstudier av hemicellulosa och andra polysackarider.

A close analogy exists between the fragmentation of fully methylated aldopentopyranoses and trimethylsilyl derivatives of partially methylated aldopentopyranoses. Hence, mass spectrometry of the trimethylsilyl derivatives permits a simple determination of the number of methoxyl groups in partially methylated aldopentoses as well as the carbon atoms to which these groups are linked. Reference substances are not necessary for identification. The method is of interest in studies of the structure of hemicellulose and other polysaccharides.

Vollständig methylierte Aldopentopyranosen und Trimethylsilylderivate von partiell methylierten Aldopentopyranosen zeigen eine weitgehend ähnliche Fragmentierung. Eine massenspektrometrische Untersuchung der Trimethylsilylderivate macht es daher möglich, die Zahl der Methoxylgruppen in partiell methylierten Aldopentosen einfach zu bestimmen und die Lage der Kohlenstoffatome, an welche diese Gruppen gebunden sind, festzustellen. Eine Referenzprobe zur Identifizierung ist nicht erforderlich. Die Methode ist bei Strukturstudien von Hemicellulosen und anderen Polysacchariden von Interesse.

The determination of the position of methoxyl groups in partially methylated sugars is an important problem in investigations of the structure of polysaccharides such as occur in hemicellulose. A generally applicable mass spectrometric method for this purpose has been devised earlier (1—4). It involves trideuteromethylation of the free hydroxyl groups and a study of the shifts of *m/e*-values of important fragment ions for the deuterated compounds compared with fully methylated compounds. As the fragmentation pattern and the origin of important ions have been investigated in detail by mass spectrometry of different deuterated methyl sugars, such a study permits the determination of the positions of the trideuteromethyl substituents. The replacement of methyl substituents by trideuteromethyl substituents has practically no influence upon the peak intensities. This permits calculations of mass spectra tables of deuterated analogs not yet investigated (1, 2), and it is not necessary to have authentic samples for comparison. A further advantage of the method is that the results with one sugar can be used with diastereomeric sugars because of the similarities of the spectra of diastereomers (4, 5). The possibility of using the partially methylated sugars or their acetates for determination of the position of methoxyl groups has also been pointed out (1, 2).

In the present investigation trimethylsilyl (TMS) derivatives of partially methylated aldopentoses were studied by mass spectrometry. The similarity of the fragmentation behavior of fully methylated and fully TMS-substituted derivatives of aldoses suggested that mixed derivatives might show analogous behavior. This was confirmed in striking fashion, thus permitting a determination of the type of substituent at different carbon atoms by a study of the *m/e*-values for characteristic fragment ions in a way corresponding to that used with trideuteromethyl derivatives. Substitution of a methyl group for a trimethylsilyl group in an ion gives a characteristic shift of the *m/e*-value corresponding to the mass difference 58. As will be shown below, a study of such shifts for a few fragment ions offers a very simple and reliable method for the determination of the number and position of methoxyl groups in partially methylated aldopentopyranoses.

TMS-derivatives of sugars can be prepared much more conveniently than the trideuteromethyl derivatives. Very small amounts are needed and the TMS-derivatives are formed quantitatively in a few

Table 1. Peak intensities at 70 eV of characteristic fragment ions in the mass spectra of fully substituted TMS-derivatives of aldopentoses and partially methylated aldopentoses

Ion denotation	*m/e*-value	β-D-arabino-pyranose	α-D-arabino-pyranose	methyl α-D-xylo-pyranoside	2-O-methyl-D-xylopyranose	3-O-methyl-xylopyranose	2,3-di-O-methyl-xylopyranose	2,3,4-tri-O-methyl-xylo-pyranose	2,3,4-tri-O-methyl-lyxo-pyranose
	73	100	100	100	83	78	71	34	37
	147	25	27	40	29	21	4	0.4	0.9
H_1	88	0	0	0	0	0	100	100	100
	146	0	0	10	100	67	10	1.4	2.0
	204	58	68	100	12	1	0		
J_1	75	10	10	10	12	11	17	13	15
	133	6	6	35	11	41	63	74	71
	191	42	48	3	48	4	3		0.1
F_1	101	5	5	8	7	5	29	58	56
	159	0	0	3	33	11	87	2.7	1.9
	217	58	71	65	3.2	100	1	1.0	0.8
K_1	58	0	0	0	0	0	3	11	11
	116	3	3	9	11	7	8	4	6
T_1	249			0.5	0.2	0.2		0.4	
	307	0.7	0.8	0.4	0.2		0.4		
	365			0.2			0.2		
	423		0.2						
B_{T1}	176	0	0	0	0	0	1	0.5	0.5
	219	6	8	8	1.4	9	0		0.1
	277			0.2	0.1	0.2			
	335	0.4	0.4	0.5	0.2	0.7			
	393	0.1	0.1						
B_2	131	4	3	7	19	13	7	6	17
	189	5	5	4	2	2	2	0.1	0.2
	247			1.9	1.8	1.9			
	305	3.3	4.2	2.7	1.7	0.2			
A_1B_{T2}	175	1	0	1	0	0	6	3.4	2.1
	233	0.2	0.2	1.9	1.9	4.3	1.1		
	291	1.8	1.4	0.6	0.1	0.3			
	349			0.9					
A_2	143	2	2	2	2	2	1	0.9	0.4
	201	0	0	5	0	0	0		
	259	1.8	1.7	2.8	4.1	0.6			
C_2	115	0	0	1	1	0	2	25	14
	173	0	0	0	0	2	6	0.2	0.4
	231	1.8	1.7	1.7	2.8	3.4	0.2		
T_2	217	58	71	65	3.2	100	1	1.0	0.8
	275			1.6	1.1	0.6	0.5		
	333	1.9	2.4	1.0	0.5	1.8			

minutes. TMS-derivatives have been used for gas chromatographic analyses of partially methylated aldopentoses (13) and are well suited for mass spectrometric analyses by gas chromatography—mass spectrometry combination instruments. The use of such instruments also makes it possible to analyze mixtures of methylated sugars without previous separations. This is an important advantage for the TMS-derivatives over the trideuteromethyl derivatives. With these it is not possible to investigate mixtures containing more than one methylated sugar from the same parent sugar. On the other hand it is easier to differentiate between different parent sugars when using trideuteromethyl derivatives since the physical and chemical properties of the fully methylated sugar can be used for comparison.

Experimental

The TMS-derivatives were prepared according to Sweeley *et al.* (14). About 1 mg of each sugar was used. The pyridine and excess reagents were removed in a rotating vacuum evaporator at 40°C and the TMS-derivatives dissolved in diethyl ether.

A LKB 9000 gas chromatograph—mass spectrometer was used for the mass spectral measurements. The samples (about 1 μg of the TMS-derivatives) were introduced through the GLC inlet using a column with 1 % SE-30 on Chromosorb P as stationary phase. A column temperature below 150°C was used. The temperature of the molecule separators was 200—210°C. An elevated inlet temperature (250°C) was found to cause changes in the relative intensities of some peaks and should be avoided. The temperature of the ion source was 270°C. The ionizing voltage was 70 eV or 20 eV. The derivatives investigated consisted of one anomer in all cases. However, as can be seen from *Tab. 1* the differences between the spectra of diastereomers are small as is also the case for the fully methylated derivatives (5).

Results and discussion

The fragmentation pattern of trimethylsilyl derivatives of aldopentoses and partially methylated aldopentoses compared to the fragmentation pattern of the fully methylated derivatives

In *Fig. 1* mass spectra at the ionizing voltage 70 eV are reproduced for the following fully trimethylsilylated pentose derivatives:

TMS 2,3,4-tri-O-TMS-β-D-arabinopyranoside (I)
Methyl 2,3,4-tri-O-TMS-α-D-xylopyranoside (II)
TMS 2-O-methyl-3,4-di-O-TMS-D-xylopyranoside (III)
TMS 2,3,4-tri-O-methyl-D-xylopyranoside (IV)

In *Fig. 2* mass spectra at 20 eV for I and II are reproduced. The intensity of the base peak was set to 100 % in all spectra. The peaks in the upper mass range were enlarged by a factor of ten. The spectra of the compounds I—IV will be used as examples in the following discussion of the fragmentation pattern of TMS-derivatives of partially methylated aldopentopyranoses.

As expected, several peaks corresponding to fragment ions typical for TMS-derivatives appeared especially in the low mass range. The peaks at *m/e*=45, 59, 73, 75 and 89 have been shown to be characteristic of the trimethylsiloxyl function (6—8). The peak at *m/e*=73, corresponding to the trimethylsilyl ion, was intense in all spectra at 70 eV. The relative importance was less for IV with only one TMS-group than for the other derivatives. Peaks at *m/e*=103 and 147 have been observed earlier with TMS-derivatives of aldonic acids (9, 10) and other carbohydrates (11, 12). The ion with *m/e*=147 was prominent for I, II and III at 70 eV but was virtually absent in the case of IV. This is explained by the participation of two TMS-groups in the formation of this ion (7). From the spectra of I and II in Fig. 2 it is seen that the formation of the ions mentioned was reduced very strongly when an ionizing voltage of 20 eV was used.

The occurrence of most of the other peaks in the spectra of I—IV can be explained by analogy with the fragmentation pattern of a fully methylated aldopentopyranose. The mass spectra of the compounds I—IV will be discussed following the fragmentation scheme given by Kochetkov and Chizhov (1). Characteristic features will be compared with those of the thoroughly investigated mass spectrum of methyl 2,3,4-tri-O-methyl-β-arabinopyranoside (V) at 70 eV (1, 2, 3, 4). Ions from I—IV analogous to ions from V will when possible be denoted according to the system of Kochetkov and Chizhov (1). In Fig. 1 the peaks are denoted in this way. In *Fig. 3* the formation of a few important ions for III is outlined. Since the fragmentation analogy in most cases is more marked for the fragmentation paths leading to intense peaks, these are discussed first.

F and G series

The ions of these series are composed of three adjacent carbon atoms together with the substituents from two of them. For V about 80 % of these ions retain the substituents at C-2 and C-4 (F_1^2) and about 10 % the substituents at C-2 and C-3 (G_1^4) and both contribute to the base peak at *m/e*=101 (3, 4). If one methyl group is replaced by a TMS-group the mass number of the ions would become 159 and if both methyl groups are replaced by TMS-groups the mass number would become 217.

For I and II with TMS-substituents at C-2, C-3 and C-4, a peak of very high intensity was obtained at *m/e*=217. Only negligible peaks were recorded at *m/e*=159. For III having a methoxyl at C-2 an intense peak was obtained at *m/e*=159 whereas the peak at *m/e*=217 was small. The spectrum of IV with methoxyls at C-2, C-3 and C-4 exhibited a very intense peak at *m/e*=101 whereas the peaks at *m/e*=159 and 217 were very small. The peaks at *m/e*=101 for I, II and III can be explained as arising from the K series. The relative intensity of the peaks from the F and G series was somewhat lower for I—IV than for V especially for *m/e*=101 and 159 but the main peak was among the four most intense peaks in all spectra. From Fig. 2 it is seen that the relative importance was greater at 20 eV than at 70 eV. These observations support the assumption that the ions are formed in an analogous way and that substituents from the same carbon atoms are retained for TMS-derivatives and for mixed derivatives as for fully methylated sugars.

H-series

The H_1 ions consist of two adjacent carbon atoms with their substituents. Of the possible ions for V the ion H_1^1 (C-1 and C-2) contributes about 10 %, H_1^2 (C-2 and C-3) 75 % and H_1^3 (C-3 and C-4) 15 % to the total intensity of the peak at *m/e*=88 (3). This peak is the second most intense in the spectrum of V. Replacement of one or two methyl groups by TMS-groups would give H_1 ions with *m/e*=146 and *m/e*=204 respectively.

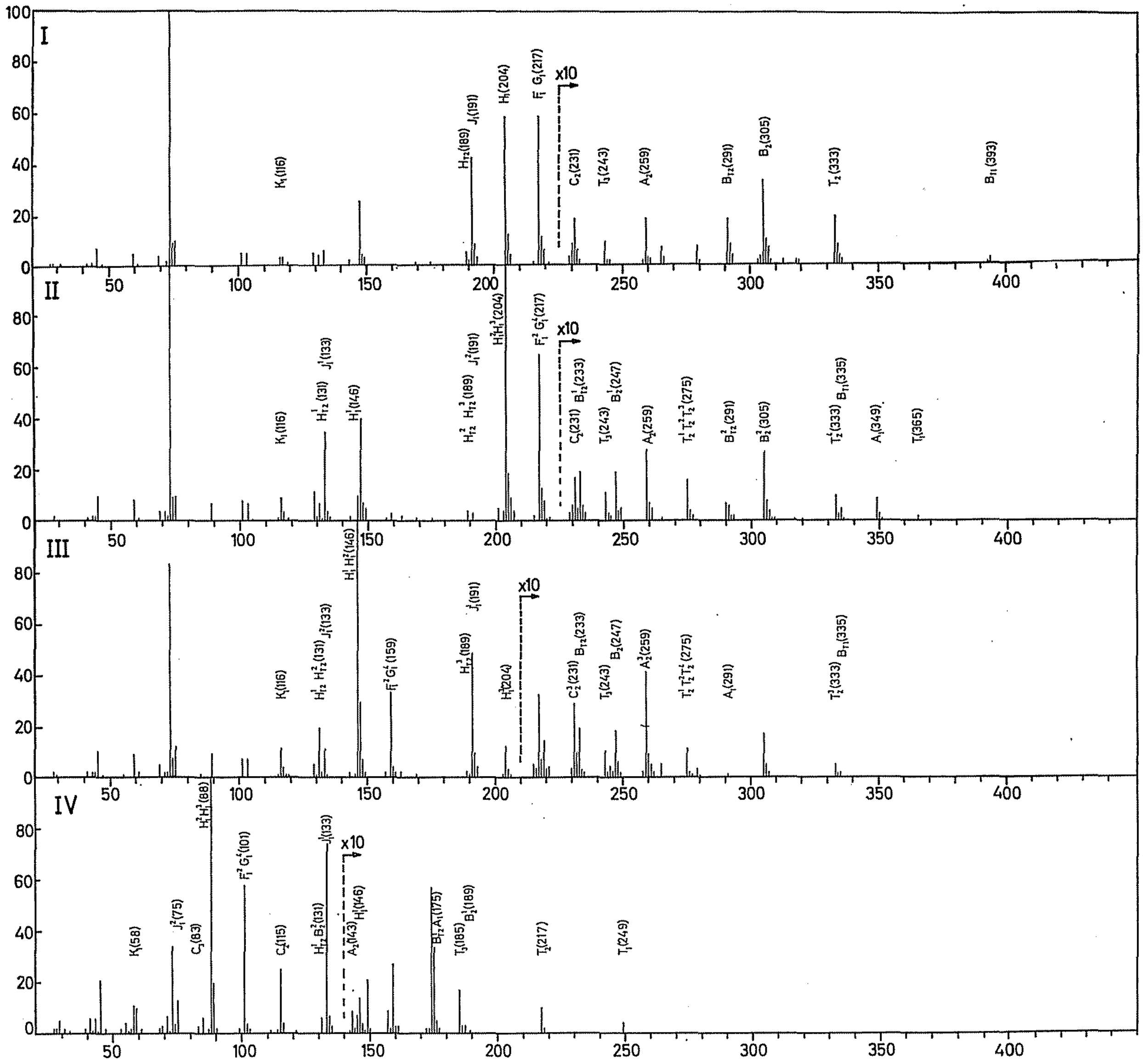

Fig. 1. Mass spectra at 70 eV of fully trimethylsilylated *β*-D-arabinopyranose (I), methyl *α*-D-xylopyranoside (II), 2-O-methyl-D-xylopyranose (III) and 2,3,4-tri-O-methyl-D-xylopyranose (IV).

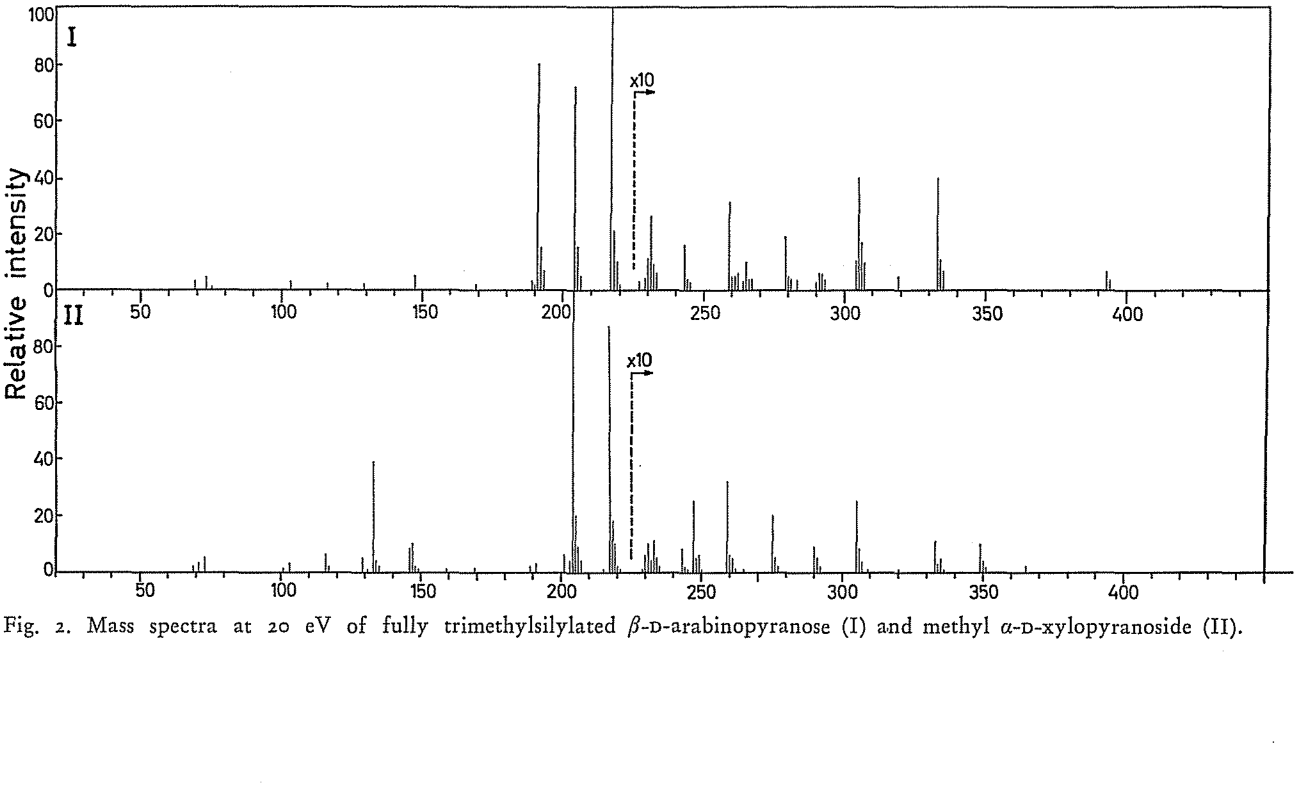

Fig. 2. Mass spectra at 20 eV of fully trimethylsilylated *β*-D-arabinopyranose (I) and methyl *α*-D-xylopyranoside (II).

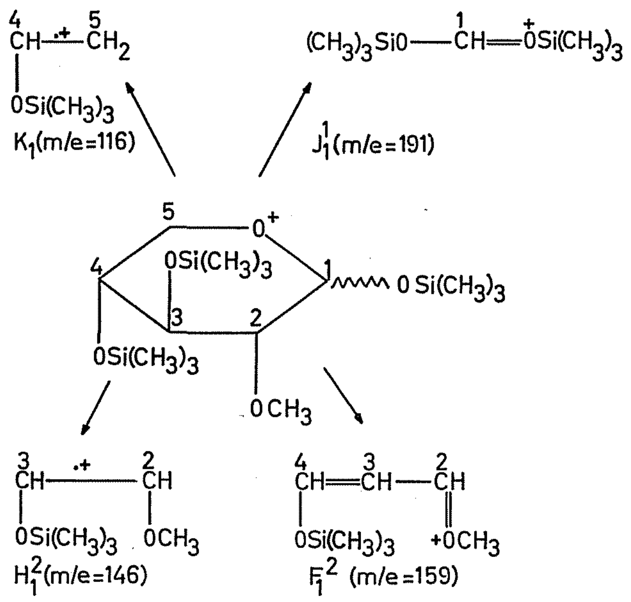

Fig. 3. Schematic representation of the origin of the most important ions of the H, J, F (G) and K series of TMS 2-O-methyl-3,4-di-O-TMS-D-xylopyranoside (III).

In the spectrum of I, with all hydroxyls TMS-substituted, an intense peak was recorded at *m/e*=204 and no peaks were observed at *m/e*=88 and 146. For II the base peak had the mass number 204 which corresponds to the $H_1{}^2$ and $H_1{}^3$ ions. At *m/e*=146, the mass number of the $H_1{}^1$ ion, a peak of 10 % of the intensity of the *m/e*=204 peak was observed. No peak was evident at *m/e*=88. For III the base peak was recorded at *m/e*=146. This is the mass number of $H_1{}^1$ and $H_1{}^2$ for III. At *m/e*=204, which is the mass number of $H_1{}^3$ for III, a peak of 12 % of the intensity of the base peak was recorded. No peak at *m/e*=88 was observed. For IV the peak at *m/e*=88 ($H_1{}^2$ and $H_1{}^3$) was the base peak. Only a small peak was present at *m/e*=146 ($H_1{}^1$) and no peak at *m/e*=204. The comparisons of the peak intensities at *m/e*=88, 146 and 204 for I—IV illustrate the close analogy with the fragmentation of V for the H_1 ions. From the spectra of I—IV it is evident that the relative importance of the H_1 ions is greater for the TMS-derivatives than for V. The relative intensity is somewhat decreased in the spectra at 20 eV.

The H_1 ions are radical ions. By loss of a methyl radical, H_2 ions with an even number of electrons are formed in the case of V. These give rise to a peak at *m/e*=73. The occurrence of this fragmentation for IV was supported by the presence of a metastable peak (m^*=60.5). No evidence was found for the analogous splitting off of TMS-groups from the ions with *m/e*=204 and *m/e*=146. Metastable peaks for I and II (m^*=175) and for III (m^*=118) indicated that these H_1 ions also split off methyl radicals with formation of even electron ions with *m/e*=189 and *m/e*=131. The methyl radicals probably originate from the TMS-groups. These ions are denoted H_{T2} in Fig. 1. They were of rather low intensity compared to the H_1 radical ions in all spectra. Moreover it is likely that ions of a different origin contribute to the peaks at these *m/e*-values.

J-series

The J_1 ions contain one carbon atom from the pyranoid ring together with the substituents from this and from one additional carbon atom. For V most of the J_1 ions contain C-1 and are formed by migration of a methoxyl radical from C-3 to C-1. About 85 % of the ions with *m/e*=75 contain the substituents from C-3 and C-1 ($J_1{}^1$) and 15 % the substituents from C-2 and C-4 ($J_1{}^2$) (3, 4). Exchange of one or two methyl groups for TMS-groups would give two additional possible *m/e*-values for the J_1 ions, *m/e*=133 and *m/e*=191.

The occurrence of intense peaks corresponding to the J_1 ions for TMS-derivatives of monosaccharides (*m/e*=191) and methyl glycosides of monosaccharides (*m/e*=133) has been demonstrated recently (11).

For V the peak at *m/e*=75 is the third most intense peak of the spectrum. Peaks of approximately the same magnitude as the latter appeared at *m/e*=191 for I and III and at *m/e*=133 for II and IV. Compared to these peaks, those at *m/e*=75 and 133 for I and III and at *m/e*=75 and 191 for II and IV were small. Contributions to the peak intensities at *m/e*=75 and *m/e*=133 are also made by ions characteristic of TMS-derivatives of carbohydrates but these are small compared to the peak intensity from the $J_1{}^1$ ion. The peak at *m/e*=191 for II, with about 10 % of the *m/e*=133 peak intensity, corresponds to the mass number of $J_1{}^2$. The related shifts of the *m/e*-values in the spectra of I—IV show that the formation of J_1 ions is analogous to that from V.

K-series

The K_1 ions contain C-4 with its substituent and C-5 and are formed by splittings of the bonds between C-3 and C-4 and between C-5 and the ring oxygen. For V a peak at *m/e*=58 is obtained which is about 10 % of the base peak (3, 4). If the methyl group is replaced by a TMS-group the mass number of the corresponding ion becomes 116.

In the mass spectra of I, II and III significant peaks at *m/e*=116 but not at *m/e*=58 were obtained. In the spectrum of IV a significant peak was obtained at *m/e*=58 whereas the peak at *m/e*=116 was small and can be ascribed to ions of the same structure as the *m/e*=115 ions but containing heavy isotopes of carbon or silicon. These observations strongly indicate that K_1 ions are formed in the same way for I—IV as for V. The relative intensities of the K_1 ions for I—IV were about the same or somewhat lower than for V.

The occurrence of peaks at *m/e*=101 for I, II and III suggests the loss of a methyl radical from the TMS-group in the K_1 radical ions.

A-series

The ions of this and all the following series are of much lower intensity than those of the series discussed so far. The fragmentation analogies are less obvious but in many cases very striking nevertheless.

The A_1 ions are formed by loss of the substituent at C-1. For V the A_2 and A_3 ions are formed by stepwise elimination of one or two substituents respectively from A_1 as methanol. For the TMS-derivatives formation of ions of the A series should also be possible by analogous splitting off of a trimethylsiloxyl group (89 m.u.) at C-1 and elimination of trimethylsilanol (90 m.u.). Peaks at mass numbers corresponding to A_1 were observed for II at $m/e=349$ (M-31), for III at $m/e=291$ (M-89) and for IV at $m/e=175$ (M-89). Peaks at mass numbers corresponding to A_2 were observed for I at $m/e=259$ (M-89-90), for II at $m/e=259$ (M-31-90) and for IV at $m/e=143$ (M-89-32). For III a rather intense peak was obtained at $m/e=259$ (M-89-32) corresponding to A_2^3, whereas no significant peak was observed at $m/e=201$ (M-89-90). This indicates that the elimination of the C-2 substituent predominates for III. For V most of the isomeric A_2 ions have lost the substituents at C-3 or C-4 (3, 4). The intensities of most of the peaks of the A series for I—IV were about the same as for V.

B-series

The B_1 ions are formed by elimination of C-5 and the ring oxygen as formaldehyde (3). A peak with an intensity of about 5 % that of the base peak is obtained for V at $m/e=176$ (M-30). No analogous peaks were observed in the spectra of I—IV. For I, II and III small peaks were observed at *m/e*-values corresponding to M-30-15. Peaks at corresponding mass numbers appeared also for the TMS-derivatives of hexoses and partially methylated hexoses which have been studied so far. These ions are probably formed by the loss of a methyl radical from one of the TMS-groups in the same way as for the ions of the H series. The peaks are denoted B_{T1} in Fig. 1.

The B_2 ions are formed mainly in a way analogous to that of the ions of the F series but with migration of a hydrogen atom instead of a methoxyl radical. The ions contain the substituents at C-2, C-3 and C-4 (B_2^2) or, to a less extent, the substituents at C-1, C-2 and C-3 (B_2^1) and give rise to a peak at $m/e=131$ for V (3). Peaks at mass numbers corresponding to B_2 were observed:

for I at $m/e=305$,
for II at both $m/e=247$ (B_2^1) and $m/e=305$ (B_2^2),
for III at $m/e=247$ and
for IV at both $m/e=189$ (B_2^1) and $m/e=131$ (B_2^2).

The relative intensities for the B_2^1 and B_2^2 ions were similar to those for V. However, an unexpected peak at $m/e=305$ was observed for III.

The B and F fragmentation paths lead to the formation of the resonance stabilized even electron ions B_2 and F_1. For the TMS-derivatives the formation of even electron ions by loss of a methyl radical from a TMS-group is likely. Peaks at mass numbers corresponding to such ions were observed for I—IV and have also been recorded in unpublished investigations of hexoses and partially methylated hexoses. The spectra indicate that for these ions the distribution of the substituents retained is about the same as for the B_2 ions and they are denoted B_{T2} in Fig. 1. Peaks at mass numbers corresponding to B_{T2} ions were obtained for I at $m/e=291$, for II at both $m/e=233$ (B^1_{T2}) and $m/e=291$ (B^2_{T2}), for III at $m/e=233$ and for IV at $m/e=175$ (B_{T2}). The relative intensities were about the same as for the B_2 ions. Ions of different origin probably contribute to the intensity of the B_2 and B_{T2} ions in several cases.

C-series

The C_2 ions are the most important of this series. They consist of the carbon atoms C-2, C-3, C-4 and C-5 together with the substituents from two of these atoms (1, 3), and give rise to a peak at $m/e=115$ for V. Peaks at mass numbers corresponding to C_2 which can be attributed at least partly to ions of this origin were observed for I, II and III at $m/e=231$ and for IV at $m/e=115$. The intensities for I, II and III were lower than for IV and V. A C_3 ion corresponding to the elimination of methanol or trimethylsilanol from C_2 was observed only for IV at $m/e=83$, the same mass number as for V.

D-series

The D_1 ions are of low intensity. In the spectra of I—IV only non-specific peaks of very low intensity were obtained at the relevant *m/e*-values 105, 163 and 221 and no conclusions about fragmentation analogies were possible.

T-series

Indications of the loss of a methyl radical from radical ions with one or more TMS-groups have been given above for the H_1, K_1 and B_1 ions. The molecule ion is also a radical ion and loss of a methyl radical could therefore be anticipated. This phenomenon has been observed earlier with other types of TMS-derivatives (6, 7, 8, 11, 12). Peaks at M-15 and peaks corresponding to elimination of one or two methanol or trimethylsilanol molecules from M-15 were observed both in the spectra of I—IV and in the spectra of TMS-derivatives of hexoses and partially methylated hexoses. The peaks of this series, which have no analogs in the spectrum of V, are denoted in Fig. 1 as T_1, T_2 and T_3 with superscripts as usual. Distinct peaks at mass numbers corresponding to T_1 (M-15) were observed for II at $m/e=365$ and for IV at $m/e=249$. Since in most cases no peak corresponding to the molecule ion could be detected, the T_1 ions very often have the highest mass numbers in the spectra. Peaks at *m/e*-values corresponding to T_2 were obtained for I at $m/e=333$ (M-15-90), for II at both $m/e=275$ (M-15-90) and $m/e=333$ (M-15-32), for III at both $m/e=275$ (M-15-90) and $m/e=333$ (M-15-32) and finally for IV at $m/e=217$ (M-15-32). Peaks which can be ascribed to T_3 ions were recorded at $m/e=243$ (M-15-90-90) for I, at $m/e=243$ (M-15-32-90) for II, at $m/e=243$ (M-15-32-90) for III and at $m/e=185$ (M-15-32-32) for IV.

No peaks were obtained for II and III at $m/e=185$ (M-15-90-90). The ions of the T series were of approximately the same relative intensity as the corresponding ions of the A series.

In the spectra of II and IV rather intense peaks were recorded at $m/e=290$ and $m/e=174$ respectively. These probably arose from an elimination of trimethylsilanol directly from the molecule ion (12).

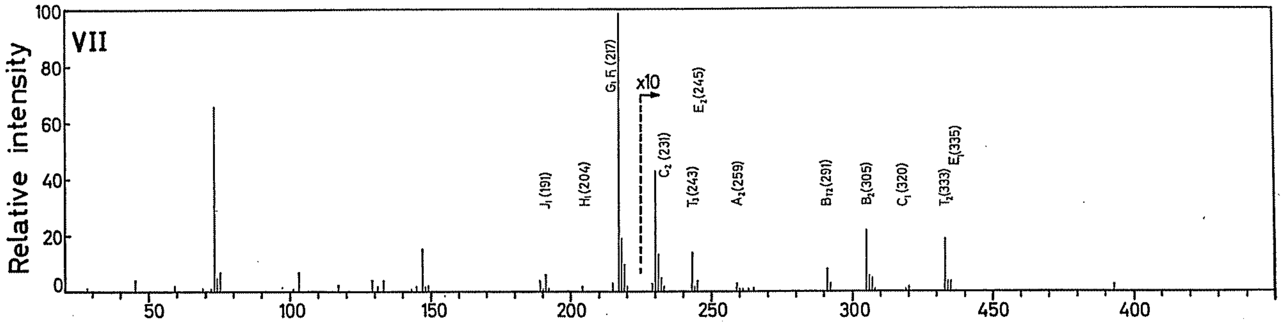

Fig. 4. Mass spectrum at 70 eV of a fully trimethylsilylated arabinofuranose anomer (VII).

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

The close analogy between the fragmentation of fully methylated aldopentopyranoses and TMS-derivatives of partially methylated aldopentopyranoses permits a simple determination of the number and position of methoxyl groups by studies of mass spectra of the TMS-derivatives even if no reference substances are available. The results given above indicate that only a few fragment ions have to be studied. This is confirmed by the results given in Tab. 1 which shows that the position of the methoxyl groups can be derived simply from the main peaks at the possible *m/e*-values for each of the H_1, J_1, F_1 and K_1 ions according to the following scheme.

H_1 Most of the H_1 ions retain the substituents at C-2 and C-3. The number of methoxyls in these positions is 2, 1 or 0 if the most intense H_1 peak is obtained at *m/e*=88, 146 or 204, respectively.

J_1 Most of the J_1 ions retain the substituents from C-1 and C-3. The number of methoxyls in these positions is 2, 1 or 0 if the most intense J_1 peak is obtained at *m/e*=75, 133 or 191, respectively.

F_1 Most of the F_1 ions retain the substituents from C-2 and C-4. The number of methoxyls in these positions is 2, 1 or 0 if the most intense F_1 peak is obtained at *m/e*=101, 159 or 217, respectively.

K_1 The K_1 ions retain the substituent from C-4. The number of methoxyls at C-4 is 1 or 0 if the most intense K_1 peak is obtained at *m/e*=58 or 116, respectively. A study of the K_1 ions should be needed only to distinguish the pentoses with methoxyls at C-1 and C-2 from those with methoxyls at C-3 and C-4.

Many of the other fragment ions in Tab. 1 provide further means of identification. The relative contributions of different H_1, J_1 and F_1 (G_1) ions are about the same for all the derivatives and should agree with the intensity distribution for the peaks at the corresponding *m/e*-values. The B_{T1} and T_1 ions can be used to obtain a check of the total number of methoxyl substituents. However, these ions are often of very low intensity and difficult to distinguish from background and noise peaks in the spectrum. The same holds true for the A_1 ions from which the number of methoxyls at C-2, C-3 and C-4 can be obtained. The B_2 and B_{T2} ions retain the substituents at C-2 and C-3 completely, and the substituents from C-1 and C-4 partly, and the distribution of these ions among different *m/e*-values should agree with the structure. The mass number series is the same for both the A_1 and B_{T2} ions, but the B_{T2} ions usually have a lower mass number and a higher intensity than the A_1 ions for the same derivative. The A_2, C_2 and T_2 ions seem to be less useful for structure determinations since the favored substituent elimination often occurs at different carbon atoms in different compounds.

The spectra of I and II in Fig. 2 indicate that spectra at the ionizing voltage 20 eV can also be used for the determination of the number and position of methoxyl groups.

In an unpublished investigation the mass spectra of the TMS-derivatives of partially methylated aldohexopyranoses were studied. It was found that a close analogy exists between their fragmentation paths and those of the fully methylated aldohexopyranoses and that a simple scheme similar to that given above can be used for determination of the number and position of the methoxyl groups in these sugars as well.

Appendix

Characteristic features of the mass spectrum of the fully TMS-substituted furanose form of an aldopentose

Aldopentofuranose units occur in many polysaccharides and furanose derivatives are therefore of interest in structure determinations by methylation. Mass spectrometry has proved to be very suitable for the differentiation of furanose and pyranose forms of sugars. The fragmentation pattern for fully methylated furanose forms of arabinose (VI) has been investigated by mass spectrometry of deuterium labelled compounds (4, 5).

In order to compare the fragmentation characteristics of TMS-derivatives of furanose forms of pentoses with those of the corresponding methyl derivatives and with those of TMS-derivatives of the pyranose forms, the spectrum of a fully TMS-substituted arabinofuranose anomer (VII) was recorded. The spectrum is reproduced in *Fig. 4*. As expected intense peaks characteristic of TMS-derivatives were recorded at *m/e*=73 and *m/e*=147. The spectrum of the fully methylated derivative (VI) has a predominant base peak at *m/e*=101, corresponding to the F_1 and G_1 ions for the pyranose derivatives. The main contribution comes from the $G_1{}^5$ ions containing the substituents from C-2 and

C-3. For the fully TMS-substituted derivative (VII) a still more predominant base peak was obtained at $m/e=217$ ($101+2\times 58$). The H_1 and J_1 ions give rise to peaks of considerably lower intensity at $m/e=88$ and $m/e=75$ in the spectrum of VI. Low intensity peaks at $m/e=204$ ($88+2\times 58$) and $m/e=191$ ($75+2\times 58$), probably corresponding to H_1 and J_1 ions, were observed for VII.

Peaks at *m/e*-values corresponding to ions of the A and C series were not very significant for VII. On the other hand significant peaks were obtained at *m/e*-values corresponding to B_2 ($m/e=305$) and B_{T2} ($m/e=291$) ions for VII. The B_2 peak at $m/e=131$ is of low intensity for VI. Significant peaks were also obtained at $m/e=333$ and $m/e=243$ for VII corresponding to T_2 and T_3 ions. No significant peaks which can be ascribed to the E series (1, 2, 4) were recorded for VII. A peak of unknown origin was observed at $m/e=230$.

As can be seen from Fig. 4 almost all significant peaks in the spectrum of VII can be explained as arising from fragmentations analogous to those of VI with modifications corresponding to those for the pyranose derivatives. A comparison between the spectrum given in Fig. 4 and those of the fully TMS-substituted pyranose forms shows that the fragmentation pattern is quite different. The furanose spectrum is characterized by a predominant G_1 (F_1) peak whereas the three intense peaks from the H_1, J_1 and F_1 (G_1) series are characteristic of the pyranose spectra. The results indicate that mass spectra of TMS-derivatives permit an easy distinction to be made between furanose and pyranose forms of partially methylated aldopentoses.

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