MASS SPECTROMETRY OF OLIGOSACCHARIDE DERIVATIVES

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SUMMARY

A survey is presented of the application of mass spectrometry to pertrimethylsilyl derivatives of oligosaccharides. The following classes are considered : a) containing aldohexoses only, b) containing aldohexoses and aldopentoses, c) containing aldohexoses and 2-acetamido-2-deoxy-aldohexoses, d) containing aldohexoses and 2-ketohexoses and e) containing aldohexoses and sialic acid. Each of these types of oligosaccharides have a characteristic fragmentation pattern. The suitability of the spectral data to establish the position of the glycosidic bond(s) and the monomer sequence is discussed for each of these classes of oligosaccharides.

RESUME

SPECTROMETRIE DE MASSE DE DERIVES OLIGOSACCHARIDIQUES.

Un sommaire a été présenté de l'application de la spectrométrie de masse aux dérivés pertriméthylsilylés des oligosaccharides. Les groupes suivants ont été étudiés : a) composés d'aldohexoses seulement ; b) composés d'aldohexoses et d'aldopentoses ; c) composés d'aldohexoses et d'acétamido-2-déoxy-2-aldohexoses ; d) composés d'aldohexoses et des céto-2-hexoses et e) composés d'aldohexoses et d'acide sialique. Chacun de ces types d'oligosaccharides a un profil de fragmentation caractéristique. L'application des données des spectres pour établir la position des liaison(s) glycosidique(s) et la succession des monomères sont discutées pour chacun des groupes d'oligosaccharides.

The elucidation of the primary structure of a glycoprotein can be divided into the study of the protein chain, the glyco-peptide linkage(s) and the carbohydrate chain(s).

Generally, chemical and enzymatic degradation techniques are applied for the sequence analysis of the amino acids in peptides and proteins. In these studies the sequenator is frequently used, eventually combined with gas-liquid chromatography and mass spectrometry. Sometimes sequence analysis of peptides is carried out by mass spectrometry, using volatile derivatives of the peptides (1, 2). In some special cases this technique has shown to be preferable over the more "classical methods" (3). In 1968 in our laboratory the ethoxycarbonyl group as a blocking group for the amino functions was introduced (4, 5), leading to very volatile derivatives. The application of this group has given good results in the elucidation

of the structure of unknown peptides (6).

The mass spectrometric studies of the different glyco-peptide linkages, as published so far in the literature, are very promising (7-9).

The study of carbohydrates is hampered by the great number of parameters, which define the structure of an oligosaccharide or a polysaccharide :

a. nature and number of the constituting monosaccharides
b. sequence of these units
c. ring size of the monosaccharides
d. position of the glycosidic linkages
e. configuration of these bonds
f. configuration of the anomeric carbon atom of the reducing unit

For the complete elucidation of the structure of saccharides a great number of techniques are described. However, none of the techniques including mass spectrometry allows the determination of all parameters in one run. In literature, the mass spectra of permethyl ethers (10-12), pertrimethylsilyl ethers (13-18) and peracetyl esters (18) are reported. Besides the mentioned derivatives of the sugars, also the corresponding alditol derivatives (19-23) and carbohydrate derivatives with an aromatic group coupled to the reducing unit (24-27) are studied. Recently, a review has been published covering also the literature of monosaccharide derivatives (28).

We have studied a number of oligosaccharide classes in detail by mass spectrometry. To this end we used pertrimethylsilyl derivatives of the free sugars (14-16). In this report we describe the application of this technique to the determination of the glycosidic link and/or the determination of the monomer sequence, in the case that the units differ in molécular weight, for the following classes :

1. disaccharides containing only aldohexoses
2. disaccharides containing an aldohexose and an aldopentose
3. disaccharides containing an aldohexose and a 2-acetamido-2-deoxy-aldohexose
4. disaccharides containing an aldohexose and a 2-ketohexose
5. oligosaccharides containing aldohexoses and 2-ketohexoses
6. trisaccharides containing aldohexoses and a sialic acid

From a great number of mass spectra it could be deduced that within a circumscribed class of saccharides the position of the glycosidic link has a pronounced influence on the spectra. Sometimes this influence became clear by the presence of specific peaks in definite saccharides, which were "absent" in other saccharides. If this criterium could not be applied, ratios of peak intensities were used for the assignment of the position of the glycosidic bond. We have demonstrated (17, 29) that a close investigation of peak intensities in the mass spectra of stereoisomeric pertrimethylsilyl mono- and disaccharides shows minor but definite and reproducible differences. For this reason, it is clear that in oligosaccharides also the configuration of the glycosidic link(s) and the type of monomers of the same molecular weight have an effect on the mass spectra, although to a lesser extent than the position of the glycosidic bond. Nevertheless, the utilization of relative peak intensities expressed as a percentage of the base peak or of a sum of peaks is in many cases not characteristic enough. The difference become more pronounced and more reliable when intensity ratios

are compared. In this way it was possible to develope a method for the computer-aided mass spectrometric identification of some stereoisomeric monosaccharides (29). For the discrimination between types of glycosidic bonds, it is necessary to use the intensity ratios, which show large differences for the various linkages, to eliminate the secundary effects mentioned above. Evidently, the ratio ranges will become more significant when more model compounds are investigated. For pertrimethylsilyl aldohexopyranoses and furanoses it has been demonstrated that they can be distinguished on the basis of one intensity ratio, namely 217/204 (30). This feature can not be simply extrapolated to higher oligosaccharides ; the type of glycosidic link and substituents different from OTMS have a distinct influence on the formation of the fragment ions m/e 217 and m/e 204.

GENERAL FEATURES OF THE MASS SPECTRA

The mass spectra of TMS-oligosaccharides show in the high mass range peaks at m/e M (M = molecular weight), m/e (M - CH_3), m/e (M - TMSOH), m/e (M - CH_2OTMS) and/or m/e (M - CH_3 - TMSOH). When the molecular ion is not detectable, at any rate m/e (M - CH_3) and/or m/e (M - CH_2OTMS) are/is present. Therefore the molecular weight of the oligosaccharides can be determined by mass spectrometry.

DETERMINATION OF THE TYPE OF GLYCOSIDIC LINK IN DISACCHARIDES

A - Disaccharides containing only aldohexoses (M = 918). The mass spectra of aldohexopyranosyl-(1 → x)-aldohexoses (x = 1 - 6) were studied. Table I gives the list of disaccharides. In Table II the relative peak intensities of some characteristic peaks are shown. Scheme I presents the conclusions of the results, obtained on the basis of spectra of 18 disaccharides. The mass spectra of the 1 → 1 disaccharides stand completely apart by the presence of m/e 565, m/e 553 and m/e 540. The spectra of the 1 → 5 and 1 → 6 disaccharides show a relatively intense peak at m/e 583. Up to now, by means of mass spectrometry it is impossible to differentiate between both types of glycosidic links. However, by NMR-spectroscopy between these derivatives can be discriminated. It has been found that trimethylsilylation hardly influences the ratio of the different anomers present (31). Furthermore the reducing unit of the 1 → 6 compounds exists very likely in the pyranose form (13). For this reason, using the values of the coupling constants of the anomeric protons of the reducing end, in case of an axial proton on C_2 ($^4C_1$-conformation) of the pyranose ringform, a differentiation is possible (32-34). We have found that pyranose rings show coupling constants of about 0 - 3 and 7 - 8 Hz for the α and β anomer respectively, while furanose rings show coupling constants of about 0 - 3 and 4,5 Hz for the β (trans coupling) and α (cis coupling) anomer respectively. The 1 → 2, 1 → 3 and 1 → 4 disaccharides show highly similar spectra. On the basis of the ratios of the intensities of the peaks at m/e 569 and m/e 539 (569/539) and at m/e 569 and m/e 668 (569/668) a differentiation can be made, as illustrated in Table III. The 1 → 3 disaccharides show a high value for the ratio 569/668, because in this type of saccharides the peak at m/e 668 has a low intensity.

TABLE I - LIST OF STUDIED DISACCHARIDES

I	α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside
II	β-D-glucopyranosyl-(1 → 1)-β-D-glucopyranoside
III	α-D-galactopyranosyl-(1 → 1)-α-D-galactopyranoside
IV	β-D-glucopyranosyl-(1 → 2)-D-glucose
V	α-D-glucopyranosyl-(1 → 2)-D-glucose
VI	β-D-glucopyranosyl-(1 → 3)-D-glucose
VII	α-D-mannopyranosyl-(1 → 3)-D-mannose
VIII	α-D-glucopyranosyl-(1 → 4)-D-glucopyranose
IX	β-D-glucopyranosyl-(1 → 4)-D-glucopyranose
X	β-D-galactopyranosyl-(1 → 4)-D-glucopyranose
XI	β-D-galactopyranosyl-(1 → 4)-D-altropyranose
XII	β-D-mannopyranosyl-(1 → 4)-D-mannopyranose
XIII	α-D-glucopyranosyl-(1 → 5)-D-glucofuranose
XIV	β-D-glucopyranosyl-(1 → 6)-D-glucose
XV	α-D-glucopyranosyl-(1 → 6)-D-glucose
XVI	α-D-galactopyranosyl-(1 → 6)-D-glucose
XVII	α-D-mannopyranosyl-(1 → 6)-D-glucose
XVIII	β-D-galactopyranosyl-(1 → 6)-D-galactose

TABLE III - RANGES OF THE RATIOS OF THE PEAK INTENSITIES USED FOR DIFFERENTIATION BETWEEN (1 → 2), (1 → 3) AND (1 → 4) GLYCOSIDIC LINKS

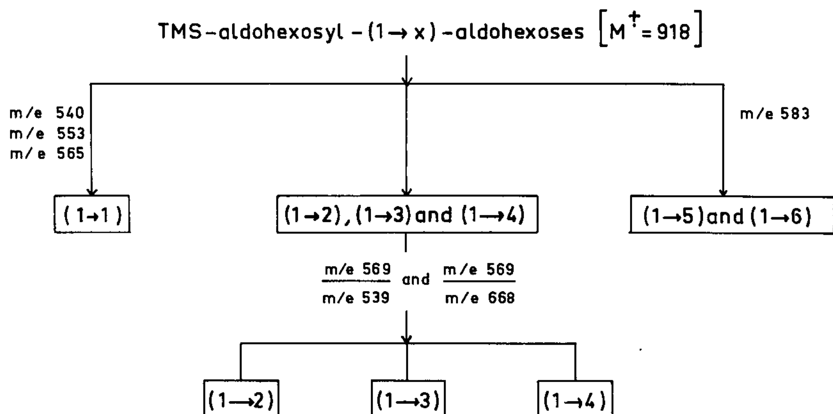
	569/539	569/668
1 → 2	5.1 - 8.1	1.1 - 8.3
1 → 3	1.1 - 1.4	13.6 - 19.6
1 → 4	0.2 - 2.1	0.6 - 7.0

B - Disaccharides containing an aldohexose and a 2-acetamido-2-deoxy-aldohexose (M = 887). The mass spectra of 2-acetamido-2-deoxy-aldohexopyranosyl-(1 → x)-aldohexoses, in which x = 2, 3, 4 or 6 were investigated. Table IV gives the list of disaccharides. Scheme II presents the conclusions of the results which were obtained on the basis of spectra of 6 disaccharides. In Table V the peak intensity ratio values used for the differentiation between the types of glycosidic linkages are shown. The ratio of the intensities of the peaks at m/e 217 and m/e 204 (217/204) differentiates between the 1 → 2 and 1 → 3 disaccharides and the 1 → 4 and 1 → 6 disaccharides. The peaks at m/e 217 and m/e 204 can be interpreted as TMSO-CH=CH-$\overset{+}{C}$H-OTMS and TMSO-$\overset{+}{C}$H-CH-OTMS respectively. Petersson et al. (35) have demonstrated for aldohexopyranoses

TABLE II - RELATIVE PEAK INTENSITIES OF SOME PEAKS, EXPRESSED WITH REGARD TO m/e 361 AS BASE PEAK

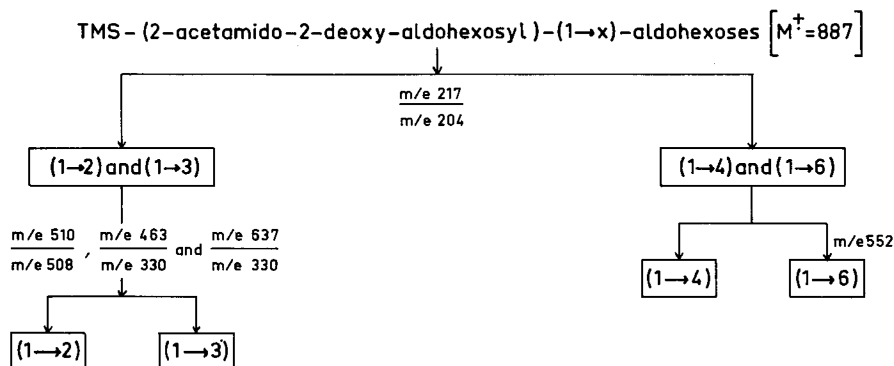
	Type of glycosidic linkage																	
	1 → 1			1 → 2		1 → 3		1 → 4					1 → 5	1 → 6				
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
539	0.2	0.2	0.2	3.3	1.3	10.1	14.4	6.0	7.3	19.0	25.7	7.0	2.5	5.6	2.7	1.5	4.9	7.0
540	1.2(a)	0.4(a)	0.8(a)	2.3	0.8	6.1	8.7	2.6	4.9	9.2	11.7	3.3	1.4	2.8	1.5	0.9	2.7	3.7
553	2.2	0.2	0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
565	1.6	0.2	4.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
569	0.1	0.05	-	26.5	6.7	10.9	20.3	4.6	9.9	11.6	4.4	14.4	28.1	48.6	23.4	42.0	72.4	15.2
583	0.4	0.1	0.4	1.2(b)	0.5(b)	0.4(b)	0.8(b)	0.7(b)	-	-	-	0.4(b)	29.3	37.9	22.2	44.5	55.2	46.6
668	0.1	-	-	6.0	6.2	0.6	1.5	2.4	8.2	8.8	7.9	2.1	0.4	-	0.9	-	-	-

(a) Only in these cases the fragment at m/e 540 is clearly observable besides the isotope peak of m/e 539. (b) Isotope peak of m/e 582.



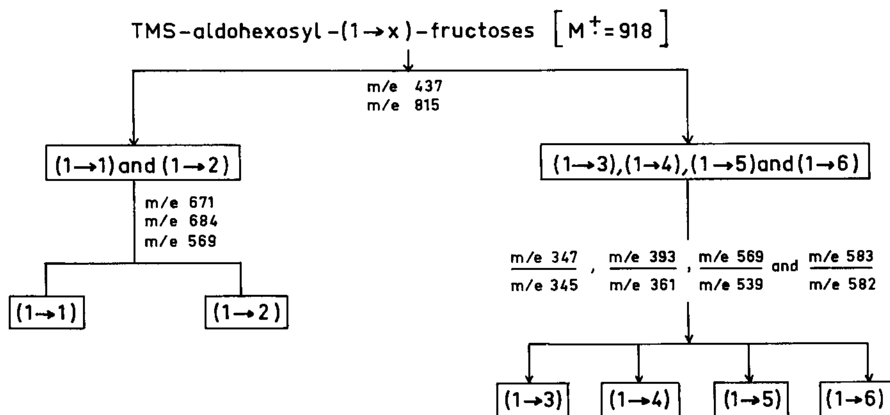
Scheme I

Identification of the type of glycosidic linkage in aldosyl disaccharides



Scheme II

Identification of the type of glycosidic linkage in NAc-disaccharides



Scheme III

Identification of the type of glycosidic linkage in Fru-disaccharides.

TABLE IV - LIST OF STUDIED DISACCHARIDES

I	2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-D-mannose
II	2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 3)-D-galactose
III	2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-D-galactopyranose
IV	2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-D-galactopyranose
V	2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-D-mannopyranose
VI	2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 6)-D-galactose

TABLE V - SOME CHARACTERISTIC RATIOS OF PEAKINTENSITIES

$\frac{m}{e}$ / $\frac{m}{e}$	Type of glycosidic linkage					
	1 → 2	1 → 3		1 → 4		1 → 6
	I	II	III	IV	V	VI
217/204	3.8	4.7	0.4	0.6	0.6	0.7
510/508	19.5	1.5	2.2	2.0	1.7	1.6
463/330	0.04	0.5	0.09	0.1	0.06	0.4
637/330	0.2	0.04	0.8	1.0	0.6	0.05

that the fragment ion at m/e 217 originates mainly from C_2-C_3-C_4 of the monosaccharide unit and m/e 204 mainly from C_2-C_3. Therefore it is clear that the presence of an NHAc group on C_2, as well as the position of the glycosidic link and the ring sizes, will influence the ratio 217/204. The 1 → 6 disaccharides show a peak at m/e 552 of high intensity, which is absent in the 1 → 4 disaccharides. This peak is the analogue of m/e 583, present in the aldohexosyl-(1 → 6)-aldohexoses ; the formation of the last fragment ion is given in figure 1 (13). For the differentiation between the 1 → 2 and 1 → 3 disaccharides the ratios 510/508, 463/330 and 637/330 are used (Table V).

C - Disaccharides containing an aldohexose and a 2-ketohexose (M = 918). The mass spectra of the aldohexopyranosyl-(1 → x)-fructoses (x = 1 - 6) were studied. Table VI gives the list of disaccharides, one of each type of linkage. In scheme III the conclusions of the results are summarized. The intensities of the peaks at m/e 437 and m/e 815 were used for the discrimination between the 1 → 1 and 1 → 2 disaccharides and the 1 → 3, 1 → 4, 1 → 5 and 1 → 6 disaccharides as illustrated in Table VII. The 1 → 1 and 1 → 2 disaccharides can be distinguished on the basis of the intensities of the peaks at m/e 569, m/e 671 and m/e 684 (Table VII). The 1 → 3, 1 → 4, 1 → 5 and 1 → 6 disaccharides can be differentiated on the basis of the peak intensity ratios 347/345, 393/361, 569/539 and 583/582 as given in Table VIII.

TABLE VI - LIST OF STUDIED DISACCHARIDES

I	β-D-glucopyranosyl-(1 → 1)-D-fructose
II	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside
III	α-D-glucopyranosyl-(1 → 3)-D-fructose
IV	β-D-galactopyranosyl-(1 → 4)-D-fructose
V	α-D-glucopyranosyl-(1 → 5)-D-fructopyranose
VI	α-D-glucopyranosyl-(1 → 6)-D-fructofuranose

TABLE VII - CHARACTERISTIC PEAKS ABOVE m/e 360, EXPRESSED IN THE INTENSITY OF THE PEAK AT m/e 361

m/e	Carbohydrates					
	I	II	III	IV	V	VI
815	1.4	0.05	88	138	150	184
813	1.9	0.05	1.4	3.3	4.4	5.0
684	16.2	0.02	0.9	1.0	0.4	0.7
683	1.7	0.03	0.5	1.5	0.6	0.8
680	-	0.1	-	-	-	-
671	1.4	-	-	-	-	-
569	11.4	0.03	2.4	2.7	11.3	27.6
565	-	0.1	-	-	0.4	-
437	412	36.8	3.4	4.2	9.2	6.8
435	15.0	1.2	10.0	10.4	4.4	10.3
361	100	100	100	100	100	100

- : not detectable. The peaks are not corrected for the isotopic contribution of peaks of lower masses.

TABLE VIII - PEAK INTENSITY RATIOS USED FOR DIFFERENTIATION BETWEEN THE 1 → 3, 1 → 4, 1 → 5 AND 1 → 6 DISACCHARIDES

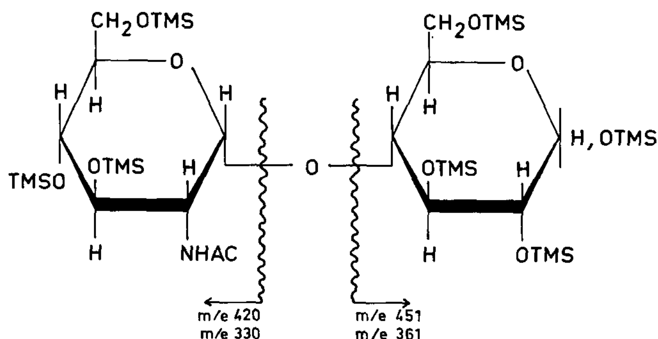
m/e / m/e	Type of glycosidic linkage			
	1 → 3	1 → 4	1 → 5	1 → 6
347/345	1.1	13.8	1.5	0.9
393/361	0.02	0.12	0.13	0.10
569/539	1.3	0.4	3.8	3.7
583/582(a)	0.6	-	0.5	1.3

(a) Isotopic ratio 583/582 = 0.52 (calculated)

DETERMINATION OF THE MONOMER SEQUENCE IN ALDOSYL DISACCHARIDES

The determination of the monomer sequence in disaccharides is possible when both units differ in molecular weight. For this purpose several fragment ions can be used, which are present in the mass spectra of aldohexosyl-aldohexoses and which are shifted in mass if one of the aldohexoses is substituted by another aldose. For instance in the case of 1 → 6 disaccharides the peak at m/e 583 is suitable, because it contains the intact non-reducing unit (Fig. 1). Another example is the peak at m/e 569 which contains the intact reducing unit as is illustrated in figure 2 (13) (m/e 569 is formed only for a very little percentage from the non-reducing site of the molecule). However, the occurrence of these types of fragments in the mass spectra is influenced by the position of the glycosidic link and/or to some extent dependent on the nature of constituting monosaccharides.

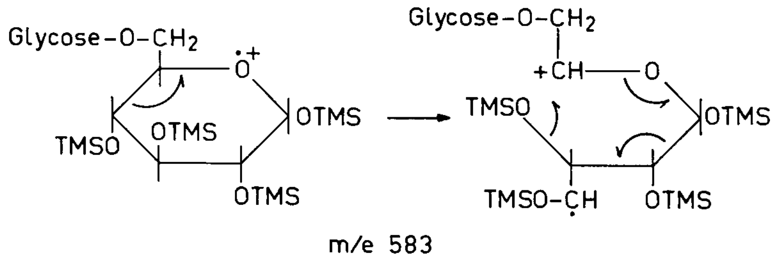
The following method as shown in Scheme IV can be applied irrespective of the type



Scheme IV

$I_{420} + I_{330} > I_{451} + I_{361}$

Principles of sequence determination.



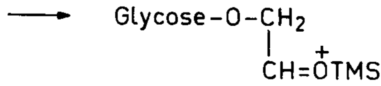
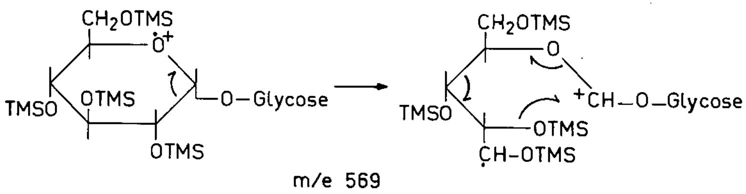


Fig. 1 - Fragmentation pathway of the peak at m/e 583.



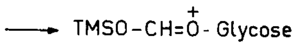
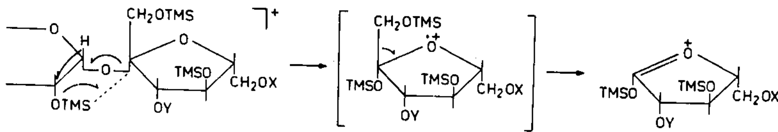


Fig. 2 - Fragmentation pathway of the peak at m/e 569.



II	m/e 437	X=Y=TMS	VIII	m/e 815	X=TMS ;	Y=G*p*
VII	m/e 437	X=Y=TMS	XI	m/e 815	X=Fru*f* ;	Y=TMS
IX	m/e 437	X=Y=TMS	Planteose[8]	m/e 815	X=Gal*p* ;	Y=TMS
XII	m/e 437	X=Y=TMS				

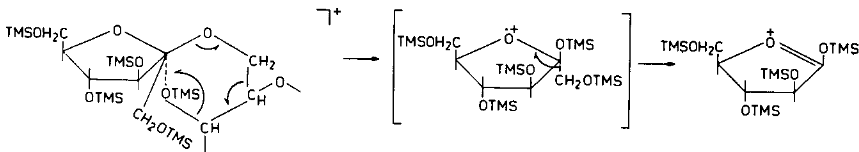
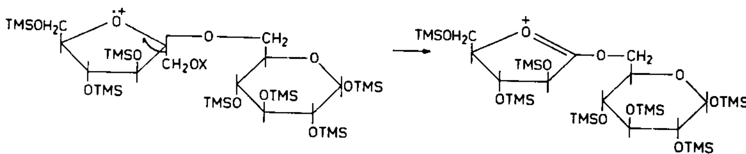


Fig. 3 - Formation of m/e 437 and m/e 815.

m/e 437 in X, XI, XII, XIII, XIV, XV and XVI



XIV	m/e 815	X=TMS
XV	m/e 815	X=Fru*f*
XVI	m/e 815	X=Fru*f*-Fru*f*

of glycosidic link and the constituting aldoses. In an aldohexosyl-aldohexose cleavage of the glycosidic bond on the right or left side of the O atom gives rise to fragment ions of the same mass value (P). This ion eliminates very easily a TMSOH molecule (P-TMSOH), which is the main fragmentation reaction, and we have no indication that the fragment ion m/e (P-TMSOH) is formed along other routes. Replacement of one of the aldohexoses by another aldose yields peaks at P' and P'-TMSOH in addition to the peaks at P and P-TMSOH. It has been demonstrated that in pertrimethylsilyl derivatives of reducing aldohexosyl-aldohexoses the largest contribution to the sum of the intensities of the peaks at P and P-TMSOH stems from the non-reducing site of the molecule. Therefore when I(P) + I(P-TMSOH) > I(P') + I(P'-TMSOH) (I = intensity), the aldohexose is the non-reducing monomer. In the case that I(P) + I(P-TMSOH) < I(P') + I(P'-TMSOH), the aldohexose is the reducing monomer. In Table IX the results are summarized for aldopentoses and 2-acetamido-2-deoxy-aldohexoses replacing one aldohexose unit either at the non-reducing or the reducing end of the aldohexosyl-aldohexoses.

TABLE IX - RELATIVE INTENSITIES OF SOME FRAGMENT IONS, USED FOR THE DETERMINATION OF THE MONOMER SEQUENCE

Disaccharides	I(P)	I(P-TMSOH)	sum	I(P')	I(P'-TMSOH)	sum
D-GNAc-β(1 → 2)-D-Man(a)	8	7	15	48	52	100
D-GalNAc-β(1 → 3)-D-Gal	6	12	18	100	55	155
D-GalNAc-β(1 → 4)-D-Gal (b)	4	8	12	54	34	88
D-GNAc-β(1 → 4)-D-Gal(b)	9	7	16	47	32	79
D-GNAc-β(1 → 4)-D-Man(a)	4	7	11	22	18	40
D-GalNAc-β(1 → 6)-D-Gal(b)	14	9	23	21	18	39
D-G-α(1 → 6)-D-GNAc(a)	2	22	24	5	3	8
D-G-β(1 → 2)-L-Ara	15	100	115	6	11	17
D-Gal-β(1 → 3)-D-Ara	24	100	124	5	17	22
D-Xyl-β(1 → 6)-D-G(c)	9	11	20	42	35	77
L-Ara-α(1 → 6)-D-G(d)	29	40	69	32	100	132
L-Rha-α(1 → 6)-D-G(d)	41	32	73	100	31	131

P = m/e 451 (aldohexose). P' = m/e 420 (2-acetamido-2-deoxy-aldohexose) ; 363 (6-deoxy-aldohexose) ; 349 (aldopentose). (a) m/e 173 = 100%. (b) m/e 204 = 100%. (c) m/e 481 = 100%. (d) Kochetkov et al. (13).

DETERMINATION OF THE POSITION OF β-D-FRUCTOFURANOSES IN OLIGOSACCHARIDES

The mass spectra of the oligosaccharides containing one or more β-D-fructofuranose units were investigated (Table X). In di-, tri- and tetrasaccharides built up from aldohexo-

ses and <u>one</u> (x → 2)-β-D-fructofuranose moiety (x = 1 or 6) the position of the latter monomer in the molecule can be inferred from the intense peak at m/e (437 + A.378 (a)) in which A = 0 or 1. Compounds II (b), VII, IX and XIV show an intense peak at m/e 437 (A = 0) which points to a terminal position of the furanose unit. Melezitose (VIII) and planteose[18] show an intense peak at m/e 815 (A = 1), characteristic for an inner position of fructose. Table XI shows the relative peakintensities of some of the main peaks. The fragmentation pathways are given in figure 3.

TABLE X - LIST OF STUDIED OLIGOSACCHARIDES

VII	α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside
VIII	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(3 → 1)-α-D-glucopyranoside
IX	α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside
X	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranoside
XI	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(6 → 2)-β-D-fructofuranoside
XII	β-D-fructofuranosyl-(2 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside
XIII	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranoside
XIV	β-D-fructofuranosyl-(2 → 6)-D-glucose
XV	β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 6)-D-glucose
XVI	β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl-(2 → 6)-D-glucose

In the mass spectra of oligosaccharides containing <u>more than one</u> (x → 2)-β-D-fructofuranose moiety, the sequence of these 2-ketohexoses can be deduced from the peaks at m/e (671 + B.378) in which B = 0,1 or 2 (see Fig. 4). The absence of m/e 671 in compound XII is in agreement with the arrangement of the two fructose units in this trisaccharide. In all other cases the fructose units are linked to each other. All these compounds show also peaks of high intensity at m/e 437 and/or m/e 815 (see Fig. 3).

<u>TRISACCHARIDES CONTAINING ALDOHEXOSES AND A SIALIC ACID (M = 1439)</u>

The mass spectra of two trisaccharides consisting of N-acetylneuraminic acid and lactose were studied : N-acetylneuraminyl methyl ester-α(2 → 3)-lactose and its 2 → 6 isomer, In view of this investigation also the TMS-derivatives of N-acetylneuraminic acid methyl es-

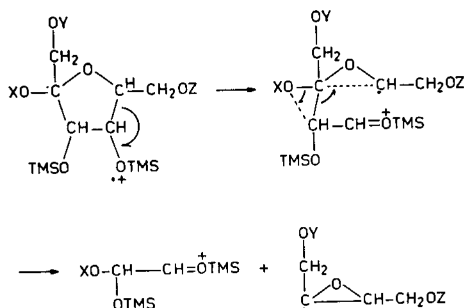
(a) Addition of an extra hexosyl unit in the chain results in a shift of 378 m.u.

(b) See Table VI

TABLE XI - SOME SIGNIFICANT PEAKS ABOVE m/e 360, EXPRESSED IN THE INTENSITY OF THE PEAK AT m/e 361, IN THE MASS SPECTRA OF THE TMS-DI-, TRI- AND TETRASACCHARIDES.

m/e	Carbohydrates									
	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1427	-	-	-	-	-	-	1.6	-	-	5.4
1049	-	-	-	0.2	0.5	0.4	5.4	-	7.9	5.4
815	0.5	27.4	0.3	0.5	16.7	0.6	1.3	21.7	13.4	7.7
813	0.3	0.5	0.2	1.6	0.7	0.2	2.5	1.9	2.0	2.3
811	-	0.5	-	2.9	0.3	0.1	5.1	-	0.8	1.2
671	-	-	-	8.6	1.0	-	2.5	2.8	7.5	3.1
437	57.0	0.7	37.1	41.4	18.4	11.9	30.6	114.1	74.8	55.0
435	1.2	1.1	1.3	0.7	1.5	1.8	1.9	7.5	3.1	3.9
361	100	100	100	100	100	100	100	100	100	100

- : not detectable. The peaks are not corrected for the isotopic contribution of peaks of lower masses.



XIV m/e 671 X=Gp ; Y=TMS ; Z=TMS

XV m/e 671 X=Gp ; Y=Fruf ; Z=TMS
m/e 1049 X=Gp-Fruf ; Y=TMS ; Z=TMS

XVI m/e 671 X=Gp ; Y=Fruf-Fruf ; Z=TMS
m/e 1049 X=Gp-Fruf ; Y=Fruf ; Z=TMS
m/e 1427 X=Gp-Fruf-Fruf ; Y=TMS ; Z=TMS

X m/e 671 X=Gp ; Y=Fruf ; Z=TMS
m/e 1049 X=Gp-Fruf ; Y=TMS ; Z=TMS

XI m/e 671 X=Gp ; Y=TMS ; Z=Fruf
m/e 1049 X=Gp-Fruf ; Y=TMS ; Z=TMS

XII m/e 1049 X=Fruf-Gp ; Y=TMS ; Z=TMS

XIII m/e 671 X=Gp ; Y=Fruf-Fruf ; Z=TMS
m/e 1049 X=Gp-Fruf ; Y=Fruf ; Z=TMS
m/e 1427 X=Gp-Fruf-Fruf ; Y=TMS ; Z=TMS

Fig. 4 - Formation of m/e 671, m/e 1049 and m/e 1427

ter, N-glycolylneuraminic acid methyl ester and the α and β methyl glycosides of N-acetylneuraminic acid methyl ester were studied. Comparison of the spectra of the trisaccharides shows some differences in peak intensities. Furthermore in the spectrum of the 2 → 6 isomer a definite peak at m/e 726 (measured brutoformula $C_{29}H_{64}NO_{10}Si_5$) is present, which is the analogue of m/e 583 in aldohexosyl-(1 → 6)-aldohexoses (Fig. 1). The 2 → 3 isomer does hardly give rise to this fragment ion. The spectrum of the 2 → 6 isomer shows also distinct peaks at m/e 595 and m/e 683 (measured brutoformulas $C_{24}H_{55}O_7Si_5$ and $C_{27}H_{63}O_8Si_6$ respectively). The peak at m/e 683 is almost absent in the 2 → 3 isomer, but a definite peak at m/e 684 can be observed. In aldohexosyl-aldohexoses like lactose the main contribution to the formation of m/e 683 stems from the reducing end of the molecule (14) and can be explained as TMSO-CH=C(OTMS)-CH=$\overset{+}{O}$-Gly. The structure of the peak at m/e 595 is : TMSO-CH=CH-CH=$\overset{+}{O}$-Gly[14]. This peak is absent in the spectrum of the 2 → 3 isomer, because of the presence on C_3 of a sugar unit in stead of an OTMS group. Some significant ratios are given in Table XII.

TABLE XII - COMPARISON OF TWO OBSERVED AND CALCULATED PEAK INTENSITY RATIOS

$\frac{m}{e}$ / $\frac{m}{e}$	2 → 3	2 → 6	isotopic ratios
684/683	3.6	0.8	0.62
595/594	0.5	1.0	0.49(a)

(a) m/e 594 = $C_{24}H_{52}NO_8Si_4$

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