MASS SPECTROMETRY OF PERTRIMETHYLSILYL ALDOSYL OLIGOSACCHARIDES

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Abstract— The mass spectra of 18 trimethylsilyl disaccharides containing only aldohexoses, connected via all possible linkages (1 → 1) to (1 → 6), were compared. These spectra could be divided into three main groups: (1 → 1) disaccharides, (1 → 2), (1 → 3), (1 → 4) disaccharides and (1 → 5), (1 → 6) disaccharides. (1 → 2), (1 → 3) and (1 → 4) disaccharides could be distinguished on the basis of two ratios of peak intensities. (1 → 5) and (1 → 6) disaccharides could not be distinguished. Also, the mass spectra of 4 trimethylsilyl trisaccharides built up from aldohexoses only were recorded. The spectra of three aldopentose containing disaccharides showed that the monosaccharide sequence as well as the position of the glycosidic link could be determined.

INTRODUCTION

As PART of a study on the structure determination of carbohydrate containing biopolymers, we investigated the applicability of mass spectrometry to the structure elucidation of oligosaccharide fragments.

The following parameters define the structure of an oligo- or polysaccharide: (i) nature and number of the constituting monosaccharides, (ii) sequence of these units, (iii) ring size of the monosaccharides, (iv) position of the glycosidic linkages, (v) configuration of these bonds, (vi) configuration of the anomeric carbon atom of the reducing unit. Several approaches have been made to capture some of these parameters by mass spectrometric analysis of oligosaccharide derivatives. The number of monomers in an oligosaccharide can be derived from the mass spectrum. Further, it has been shown that in several oligosaccharides built up from aldohexoses the position of the glycosidic linkages can be established. Chizhov *et al.*[1] demonstrated that disaccharides with (1 → 2), (1 → 4) and (1 → 6) links can be distinguished from each other by mass spectrometry of the methyl ethers. Kochetkov *et al.*[2,3] used trimethylsilyl ethers of oligosaccharides and could determine for some di- and trisaccharides the position of the glycosidic bonds in cases of (1 → 2), (1 → 3), (1 → 4) and (1 → 6) connexion. Kärkkäinen investigated trimethylsilyl ethers[4] and methyl ethers[5] of disaccharide alditols and was capable of establishing (1 → 3), (1 → 4) and (1 → 6) links. Johnson *et al.*[6] described the mass spectra of a few 1-phenylflavazole peracetates of di- to pentasaccharides and could discriminate between (1 → 4) and (1 → 6) glycosidic linkages. Recently, Kärkkäinen published a study concerning the mass spectrometry of permethyl trisaccharides[7] and trisaccharide alditols.[8] In some disaccharides, containing units with different molecular weight, the position of the glycosidic link as well as the sequence of the monomers could be established.[3-5] Vink *et al.*[9] showed that some stereoisomeric aldohexosyl-aldohexoses could be

distinguished by mass spectrometry of their trimethylsilyl derivatives; the configuration of the anomeric C-atom of the reducing unit exhibited a great influence on the fragmentation pattern.

None of the investigations concerning the estimation of the glycosidic link included all possible positions of these bonds in disaccharides. In order to develop a more generally applicable scheme, in which all link possibilities are considered, we studied the mass spectra of the whole series of aldohexosyl-(1 → x)-aldohexoses with x = 1, 2, 3, 4, 5 or 6. Furthermore we investigated how far such a scheme can be extrapolated to disaccharides containing aldopentoses and to aldohexosyl trisaccharides. For this work trimethylsilyl (TMS) derivatives were chosen, because besides their high volatility and suitability for gas-liquid chromatography (Haverkamp et al.[10] and Bhatti et al.[11]) they also lend themselves to NMR spectroscopy for the determination of the configuration of the glycosidic links (Kamerling et al.[12]).

RESULTS
Disaccharides consisting of aldohexoses

Mass spectra of TMS ethers of the disaccharides I to XVIII were recorded (Table 1). The molecular ion at m/e 918 was not detectable in the spectra of I, II, III, VII and VIII; in these cases the first detectable fragment ion was m/e 903 (M^{+}—$^{\cdot}$CH$_3$). In

TABLE 1. LIST OF STUDIED OLIGOSACCHARIDES

I	α-D-glucopyranosyl-(1 → 1)-α-D-glucopyranoside (= α,α-trehalose)[a]
II	β-D-glucopyranosyl-(1 → 1)-β-D-glucopyranoside (= β,β-trehalose)
III	α-D-galactopyranosyl-(1 ·→ 1)-α-D-galactopyranoside (= α,α-galactotrehalose)
IV	β-D-glucopyranosyl-(1 → 2)-D-glucose (= sophorose)
V	α-D-glucopyranosyl-(1 → 2)-D-glucose (= kojibiose)
VI	β-D-glucopyranosyl-(1 → 3)-D-glucose (= laminaribiose)
VII	α-D-mannopyranosyl-(1 → 3)-D-mannose
VIII	α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (= maltose)[a]
IX	β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (= cellobiose)[a]
X	β-D-galactopyranosyl-(1 → 4)-D-glucopyranose (= lactose)[a]
XI	β-D-galactopyranosyl-(1 → 4)-D-altropyranose (= neolactose)
XII	β-D-mannopyranosyl-(1 → 4)-D-mannopyranose (= mannobiose)
XIII	α-D-glucopyranosyl-(1 → 5)-D-glucofuranose (= maniocose)
XIV	β-D-glucopyranosyl-(1 → 6)-D-glucose (= gentiobiose)[a]
XV	α-D-glucopyranosyl-(1 → 6)-D-glucose (= isomaltose)[a]
XVI	α-D-galactopyranosyl-(1 → 6)-D-glucose (= melibiose)[a]
XVII	α-D-mannopyranosyl-(1 → 6)-D-glucose
XVIII	β-D-galactopyranosyl-(1 → 6)-D-galactose
XIX	β-D-glucopyranosyl-(1 → 2)-L-arabinose
XX	β-D-galactopyranosyl-(1 → 3)-D-arabinose
XXI	β-D-xylopyranosyi-(1 → 6)-D-glucose (= primeverose)
XXII	α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (= maltotriose)[a]
XXIII	β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose (= cellotriose)
XXIV	α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 6)-D-glucose (= isomaltotriose)[b]
XXV	α-D-glucopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (= panose)[c]

[a] J. T. Baker Chemicals N.V.
[b] Pierce Chemical Company
[c] EGA-Chemie KG

TABLE 2. EXPLANATION OF SOME IMPORTANT FRAGMENT IONS, PRESENT IN MOST OF THE SPECTRA OF TMS-ALDOHEXOSYL-ALDOHEXOSES

m/e	measured brutoformula	fragment
918		M^+
903		M^+-CH_3
828		$M^+-TMSOH$
815		M^+-CH_2OTMS
813		$903^+-TMSOH$
771	$C_{30}H_{71}O_9Si_7$	$903^+-TMSOCH_2-CH=O$
739		828^+-OTMS
738		$828^+-TMSOH$
725		$815^+-TMSOH$
723		$813^+-TMSOH$
683	$C_{27}H_{63}O_8Si_6$	$TMSO-CH=C(OTMS)-CH=\overset{+}{O}-Gl$ (reducing end)
668	$C_{27}H_{64}O_7Si_6$	$M^+-TMSOCH_2-CH=O-TMSO-CH=O$
649		$739^+-TMSOH$
633		$723^+-TMSOH$
611	$C_{24}H_{55}O_8Si_5$	$815^+-TMSO-CH=CH-OTMS$
595	$C_{24}H_{55}O_7Si_5$	$Gl-\overset{+}{O}=CH-CH=CH-OTMS$
583		$Gl-O-CH_2-CH=\overset{+}{O}TMS$ $(1\rightarrow6)$ (non-reducing end)
		$Gl-\overset{+}{O}=CH-CH_2OTMS$ $(1\rightarrow5)$ (non-reducing end)
582	$C_{23}H_{54}O_7Si_5$	$[TMSO-CH=CH-O-Gl]^+$
578	$C_{24}H_{54}O_6Si_5$	$668^+-TMSOH$
569	$C_{22}H_{53}O_7Si_5$	$TMSO-CH=\overset{+}{O}-Gl$ (reducing end)
539	$C_{21}H_{51}O_6Si_5$	540^+-H (see text)
525		540^+-CH_3
521	$C_{21}H_{45}O_7Si_4$	$611^+-TMSOH$
467	$C_{18}H_{43}O_6Si_4$	$[Gl-O]^+$
451	$C_{18}H_{43}O_5Si_4$	$[Gl]^+$
435	$C_{17}H_{39}O_5Si_4$	$525^+-TMSOH$
361	$C_{15}H_{33}O_4Si_3$	$451^+-TMSOH$
217	$C_9H_{21}O_2Si_2$	$TMSO-CH=CH-\overset{+}{C}H-OTMS$
204	$C_8H_{20}O_2Si_2$	$TMSO-\overset{+}{C}H-\overset{.}{C}H-OTMS$
191	$C_7H_{19}O_2Si_2$	$TMSO-CH=\overset{+}{O}TMS$

Gl = Glycose unit

general the fragmentations show great similarity, as can be expected for so closely related substances. For the interpretation of a number of fragments, (Table 2), use has been made of the data given by Kochetkov et al.[3] The characteristic differences in the spectra resulting from variations in the position of the glycosidic bond may become evident from Tables 3 and 4 and from Figs 1–6, which represent the spectra of TMS-glucosyl-glucoses with the intermolecular bond in the positions $(1\rightarrow1)$, $(1\rightarrow2)$, $(1\rightarrow3)$, $(1\rightarrow4)$, $(1\rightarrow5)$ and $(1\rightarrow6)$ respectively.

The spectra of the TMS-aldohexosyl-$(1\rightarrow1)$-aldohexoses stand completely apart; by the presence of m/e 565, m/e 553 and m/e 540.

The TMS-disaccharides with $(1\rightarrow5)$ or $(1\rightarrow6)$ links can be distinguished from

Table 3. Relative peak intensities of some peaks, expressed with regard to m/e 361 as base peak, which are used for the characterization of the glycosidic links in TMS-aldohexosyl-aldohexoses

	1→1			1→2		1→3		1→4					1→5			1→6		
m/e	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	2·5	2·7	1·5	4·9	7·0
540	1·2ᵃ	0·4ᵃ	0·8ᵃ	2·3	0·8	6·1	8·7	2·6	4·9	9·2	11·7	3·3	1·4	1·4	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	21·0	23·4	42·0	72·4	15·2
583	0·4	0·1	0·4	1·2ᵇ	0·5ᵇ	0·4ᵇ	0·8ᵇ	0·7ᵇ	—	—	—	0·4ᵇ	29·3	20·2	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	0·6	0·9	·	·	—

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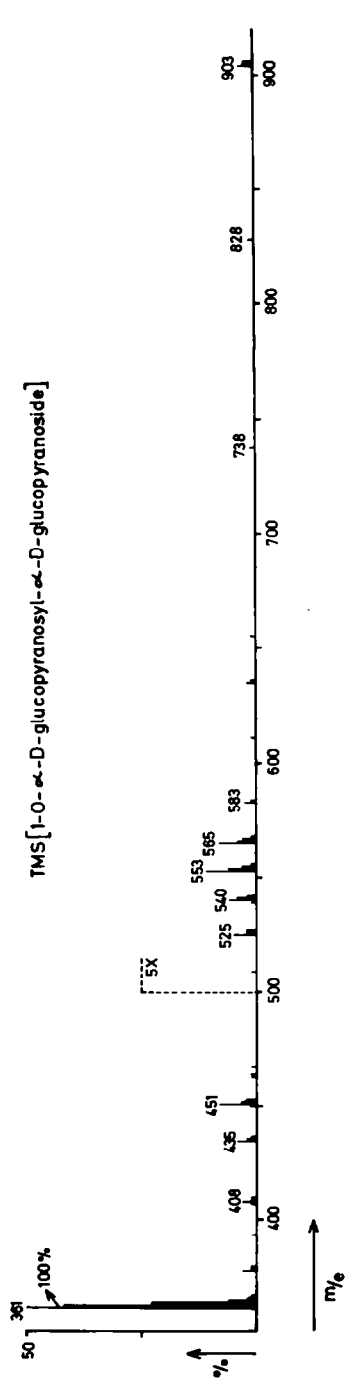ᵇ Isotope peak of m/e 582.

TMS[1-0-α-D-glucopyranosyl-α-D-glucopyranoside]

FIG 1. Mass spectrum of TMS-α,α-trehalose Only values > m/e 361 are given

TMS[2-0-β-D-glucopyranosyl-D-glucose]
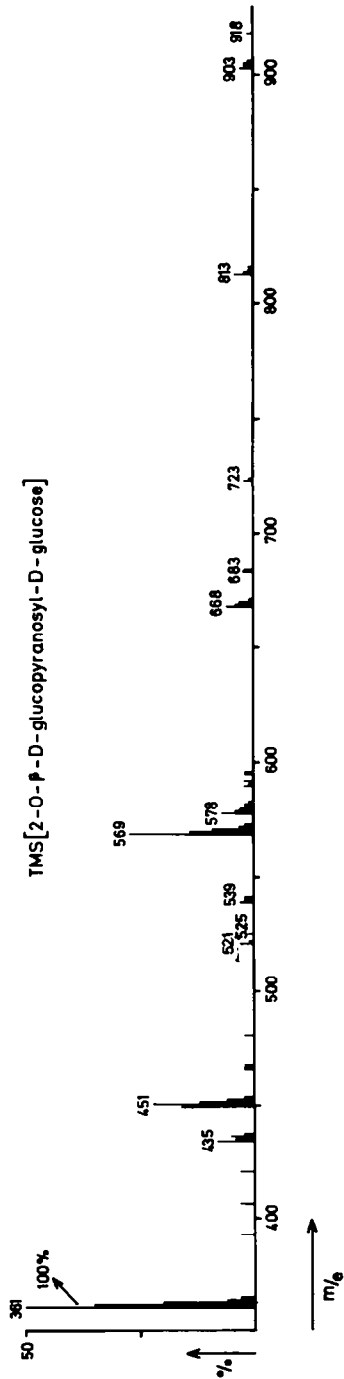
FIG 2. Mass spectrum of TMS-sophorose Only values > m/e 361 are given

TMS [3-O-β-D-glucopyranosyl-D-glucose]
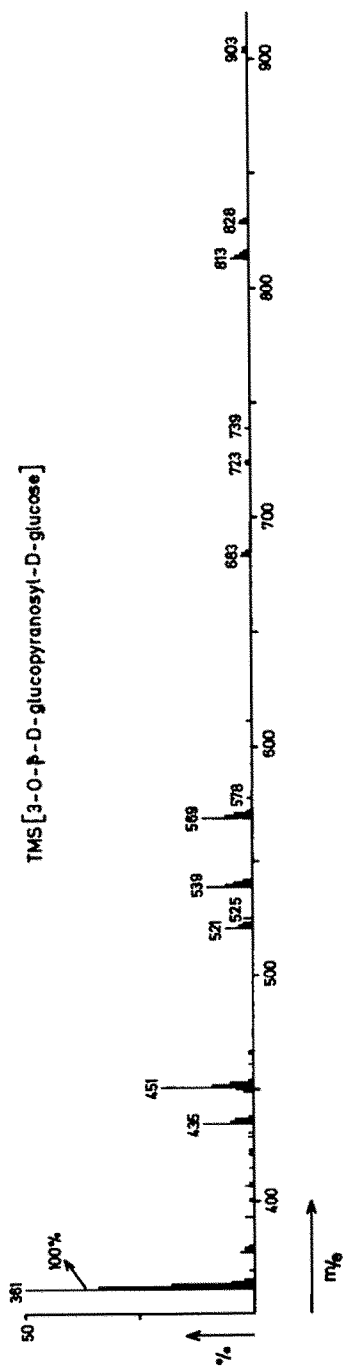
FIG 3. Mass spectrum of TMS-laminaribiose Only values > m/e 361 are given

TMS [4-O-β-D-glucopyranosyl-D-glucopyranose]
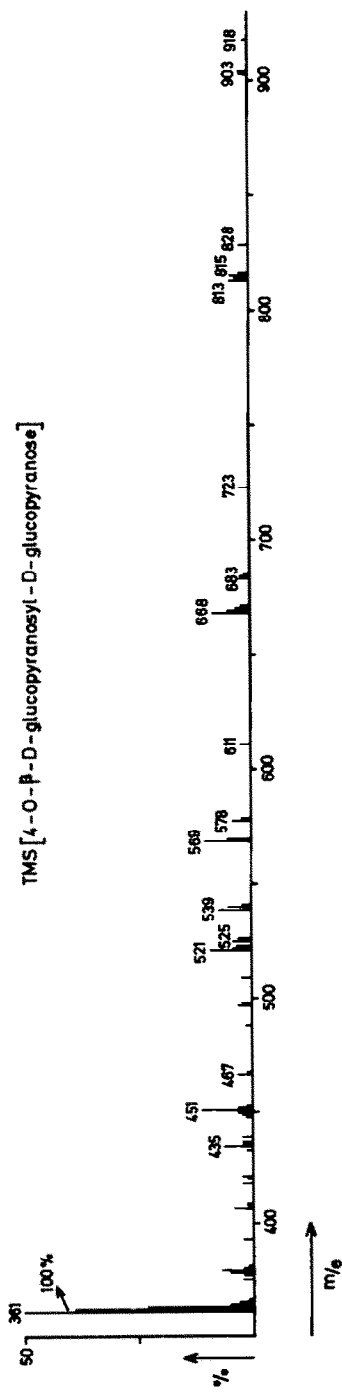
FIG 4. Mass spectrum of TMS-cellobiose Only values > m/e 361 are given

TMS [5-O-α-D-glucopyranosyl-D-glucofuranose]
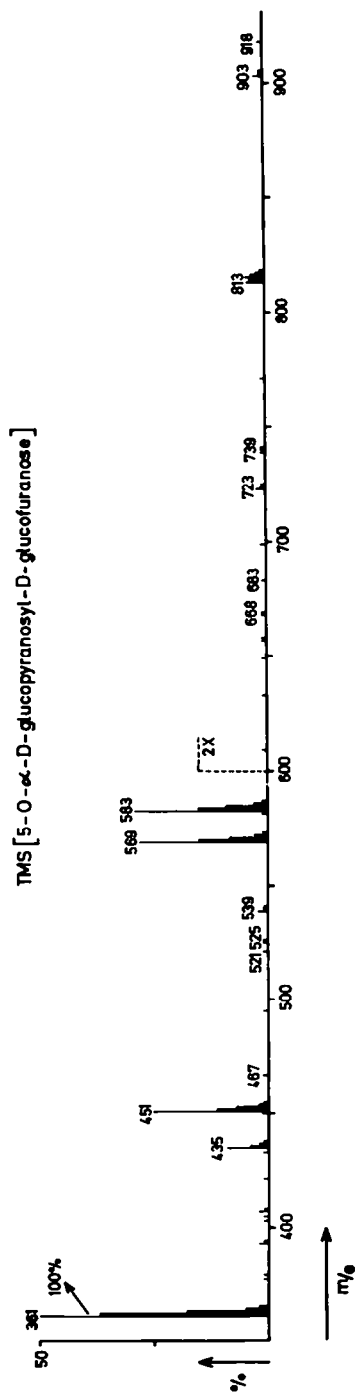
FIG 5. Mass spectrum of TMS-maniocose Only values > m/e 361 are given
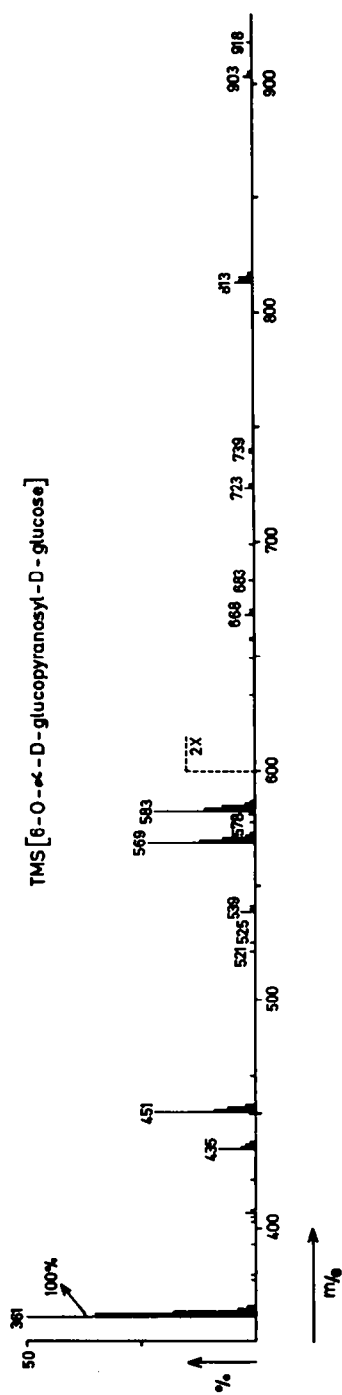
TMS [6-O-α-D-glucopyranosyl-D-glucose]

FIG 6. Mass spectrum of TMS-isomaltose Only values > m/e 361 are given

the remaining disaccharides by the occurrence of a very strong peak at m/e 583. It was impossible to differentiate between these two types of bonding.

The mass spectra of the TMS-disaccharides with $(1 \rightarrow 2)$, $(1 \rightarrow 3)$ and $(1 \rightarrow 4)$ connexions are very similar. Nevertheless it was possible to correlate the type of bonding with 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) (Table 4).

TABLE 4. RANGES OF THE RATIOS OF THE PEAK INTENSITIES USED FOR DIFFERENTIATION BETWEEN $(1 \rightarrow 2)$, $(1 \rightarrow 3)$ AND $(1 \rightarrow 4)$ GLYCOSIDIC LINKS IN TMS-ALDOHEXOSYL-ALDOHEXOSES

	569/539	569/668
$1 \rightarrow 2$ (IV–V)	$\boxed{5 \cdot 1 – 8 \cdot 1}$	$1 \cdot 1 – 8 \cdot 3$
$1 \rightarrow 3$ (VI–VII)	$1 \cdot 1 – 1 \cdot 4$	$\boxed{13 \cdot 6 – 19 \cdot 6}$
$1 \rightarrow 4$ (VIII–XII)	$0 \cdot 2 – 2 \cdot 1$	$0 \cdot 6 – 7 \cdot 0$

Disaccharides consisting of an aldohexose and an aldopentose

In the mass spectra of XIX to XXI (Table 1) the molecular ion at m/e 816 was present. Remarkably, also a $(M-1)^+$ ion was detectable. Other important ions in the high mass range were m/e 801 ($M^{\ddagger}—{}^{\cdot}CH_3$), m/e 726 ($M^{\ddagger}—TMSOH$), m/e 711 ($M^{\ddagger}—{}^{\cdot}CH_3—TMSOH$) and m/e 621 ($M^{\ddagger}—{}^{\cdot}CH_3—2x$ TMSOH). Comparison with the mass spectra of aldohexosyl-aldohexoses shows that the fragmentation patterns are practically identical; as a matter of fact fragments now containing an aldopentose unit are shifted to 102 mass units lower. The shifts of ions, which are derived predominantly from either the reducing or the non-reducing site of the molecule, can be used to establish the monosaccharide sequence.

TABLE 5. RELATIVE INTENSITIES OF PAIRS OF PEAKS, APPLICABLE FOR THE SEQUENCE DETERMINATION IN DISACCHARIDES CONTAINING ONE ALDOHEXOSE AND ONE ALDOPENTOSE UNIT. THE PEAKS OF EACH PAIR DIFFER 102 MASS UNITS

m/e	β-D-Gp-(1 → 2)-L-Ara	β-D-Galp-(1 → 3)-D-Ara	β-D-Xylp-(1 → 6)-D-G
259	10·7% (R)	17·3% (R)	35·3% (NR)
361	100 % (NR)	100 % (NR)	10·7% (R)
349	5·8% (R)	4·6% (R)	41·9% (NR)
451	¹5·3% (NR)	24·0% (NR)	9·3% (R)
467ᵃ	36·5% (R)	35·2% (R)	2·2% (NR)
569	0·6% (NR)	2·9% (NR)	9·4% (R)
581	1·8% (R)	3·3% (R)	0 % (NR)
683	0·2% (NR)	0·3% (NR)	0·3% (R)
481			100 % (NR)
583			0 % (R)

R = reducing end   NR = non-reducing end
ᵃ In Table 2 still another explanation for this fragment is given

In the mass spectrum of β-D-Xylp-(1 → 6)-D-G (XXI) a strong peak is present at m/e 481, which is the analogue of m/e 583 in aldohexosyl-aldohexoses. This observation makes clear that m/e 583 originates from the non-reducing end. By consequence the fragment at m/e 481 in XXI is indicative of the sequence of monomers and also characteristic for the (1 → 6) linkage in aldopentosyl-(1 → 6)-aldohexoses. The rather high intensity of the ion at m/e 569, which stems from the reducing site, in comparison to that of m/e 467 is likewise characteristic for the sequence.

When the aldopentose unit forms the reducing site of the molecule, as is the case in β-D-Gp-(1 → 2)-L-Ara (XIX) and β-D-Galp-(1 → 3)-D-Ara (XX), the intensity of the fragment at m/e 467 is much higher than that of the ion at m/e 569. In a similar way several other ions (Table 5) can be used for sequence determinations.

To distinguish the (1 → 2) from the (1 → 3) glycosidic link in aldohexosyl-aldopentoses the ratio of the intensities of the ions at m/e 467 and m/e 539 can be used (comparable with 569/539 in aldohexosyl-aodohexoses). For the (1 → 2) and (1 → 3) links the ratios amount to 15·3 and 2·2 respectively. For aldohexosyl-aldopentoses the analogue of the ratio 569/668 in aldohexosyl-aldohexoses should be 467/566. By the absence of the fragment at m/e 566, this ratio could not be established. As no (1 → 4) model compound was available, no alternative criteria can be given which are characteristic for this type of bonding. It is noteworthy that the fragment ion at

TABLE 6. PARTIAL MASS SPECTRA OF SOME TMS-TRISACCHARIDES
(m/e 361 = 100%)

m/e	XXII	XXIII	XXIV	XXV
1296	0·2	0·1	0·1	0·2
1281	0·1	0·2	0·2	0·2
1206	0·1	0·1	0·2	0·2
1191	0·2	0·5	0·6	0·7
1061	0·2	0·4	0·1	0·2
1046	0·7	0·2	—	0·7
961	0·2[a]	0·1[a]	1·3	0·7[a]
960	0·3	0·2	0·6	0·7
956	0·1	—	—	0·7
947	0·8	0·6	10·6	7·4
917	1·2	1·2	0·4	2·2
829	1·5	1·0	2·8	1·1
813	0·5	0·5	1·1	1·1
739	0·8	1·1	2·4	1·1
683	0·5	0·5	0·4	0·7
668	0·2	0·2	—	0·3
583	0·6[b]	0·7[b]	10·6	2·6
582	0·8	1·3	0·5	0·7
578	0·2	0·3	—	0·4
569	2·6	3·6	8·3	2·7
539	1·9	5·9	1·5	2·0
451	10·8	11·0	26·4	15·1
435	1·7	2·4	4·0	2·5
361	100	100	100	100

[a] isotope peak of m/e 960
[b] isotope peak of m/e 582

m/e 467 in the aldohexosyl-arabinoses is formed *via* two routes. For the greater part, this ion is the analogue of m/e 569 and for a minor part it is identical to the fragment presented in Table 2.

Trisaccharides consisting of aldohexoses

Mass spectra were recorded of TMS ethers of the trisaccharides XXII to XXV (Table 1). The fragment ions are directly comparable with those formed in TMS-aldohexosyl-aldohexoses. The ions containing the extra hexosyl unit are found 378 mass units higher than in the disaccharides. In Table 6 a series of the most important fragment ions is given.

For the assignment of the two $(1 \rightarrow 6)$ links in α-D-Gp-$(1 \rightarrow 6)$-α-D-Gp-$(1 \rightarrow 6)$-D-G (XXIV) the same fragment ion as in disaccharides *viz.* m/e 583 and its equivalent 378 mass units higher at m/e 961 can be used. The intense peak at m/e 583 in a trisaccharide is typical for the $(1 \rightarrow 6)$ link between the non-reducing unit and the middle sugar unit, and the intense peak at m/e 961 for the $(1 \rightarrow 6)$ link between the middle sugar unit and the reducing unit. In α-D-Gp-$(1 \rightarrow 6)$-α-D-Gp-$(1 \rightarrow 4)$-D-Gp (XXV) the $(1 \rightarrow 6)$ link is characterized by a strong peak at m/e 583.

It is probable that the $(1 \rightarrow 4)$ links in panose (XXV), maltotriose (XXII) and cellotriose (XXIII) can be assigned on the basis of similar ratios of peak-intensities as described for aldohexosyl-aldohexoses. By the lack of model compounds containing $(1 \rightarrow 2)$ and $(1 \rightarrow 3)$ linkages it would be premature to indicate these ratios.

DISCUSSION

It was found that the most characteristic differences in the mass spectra of di-saccharides built up from units of equal molecular weight arise from variations in the position of the glycosidic link. In some cases these differences are rather small, so that careful recording of the spectra under standard conditions is advisable.

In the following, a further comment is given on the fragment ions which are used for the assignment of the glycosidic linkage.

$1 \rightarrow 1$ *linkage.* The mass spectra of the non-reducing disaccharides are unique by the presence of the peaks at m/e 565, m/e 553 and m/e 540. Exact mass measurements revealed the following brutoformulae: m/e 565 = $C_{23}H_{53}O_6Si_5$, m/e 553 = $C_{22}H_{53}O_6Si_5$ and m/e 540 = $C_{21}H_{52}O_6Si_5$. The fragment ion at m/e 540 may be formed as indicated in Fig. 7.

$1 \rightarrow 5$ *and* $1 \rightarrow 6$ *linkages.* It is remarkable that in aldohexosyl-aldohexoses a $(1 \rightarrow 5)$ link could not be distinguished from a $(1 \rightarrow 6)$ link, as the ring size of the
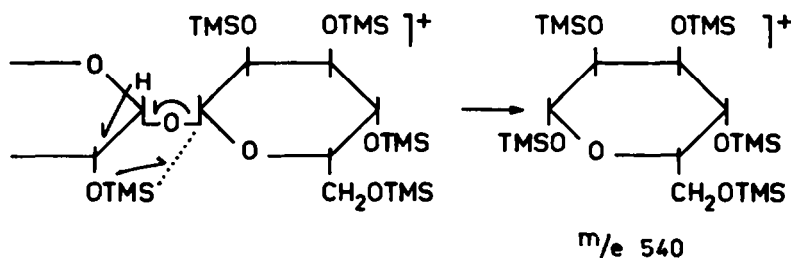

FIG 7. Formation of m/e 540 in $(1 \rightarrow 1)$ disaccharides

reducing unit may be different for these components; in the (1 → 5) compound it must be a furanose ring and in the (1 → 6) compound very likely a pyranose ring (Kochetkov et al.[3]). Because in TMS-monosaccharides the intensity of the peak at m/e 217 is smaller than that of m/e 204 for pyranose forms, whereas the reverse holds for the furanose forms (DeJongh et al.[13] and Petersson et al.[14]), it was obvious to look for the intensity of these peaks in disaccharides. In all reducing aldohexosyl-aldohexoses including the (1 → 5) and (1 → 6) bonded components as well as for the trisaccharides, the intensity of the peak at m/e 217 was smaller than that of the peak at m/e 204. Only in the non-reducing (1 → 1) disaccharides was the intensity of m/e 217 greater than that of m/e 204. Evidently, the intensity of these peaks can not be used to differentiate between furanose and pyranose ring forms in aldohexosyl oligosaccharides. Although the fragments at m/e 217 and m/e 204 must arise from the constituting monosaccharides, it is clear that the type of glycosidic link has also a distinct influence on the formation of these ions.

In accordance with the explanation for m/e 583 in (1 → 6) disaccharides, offered by Kochetkov et al.[3] this peak is shifted to m/e 481 in β-D-Xylp-(1 → 6)-D-G (XXI). However, their conclusion that the intense peak at m/e 583 is typical of (1 → 6) linkages has to be modified, as it is also present in (1 → 5) disaccharides. In the latter case the fragment at m/e 583 arises from cleavage of the bond between C_4 and C_5 in the reducing moiety. Surprisingly, in the mass spectra of the three non-reducing disaccharides of the trehalose type the fragment at m/e 583 was also present, albeit in a very low abundance. Exact mass measurements revealed that its brutoformula $C_{23}H_{55}O_7Si_5$ was identical to that of m/e 583 in (1 → 5) and (1 → 6) bonded components. For the peak at m/e 583 in (1 → 1) disaccharides a structure is given in Fig 8.
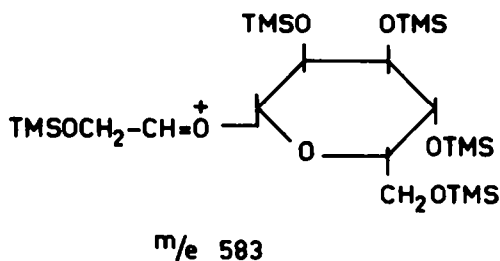

$^m/e$ 583

FIG 8. Structure of m/e 583 in (1 → 1) disaccharides

1 → 2, 1 → 3 and 1 → 4 linkages. The differentiation between the (1 → 2), (1 → 3) and (1 → 4) aldohexosyl-aldohexoses is rather subtle. Kochetkov et al.[3] postulated that this group of components is characterized by a common peak at m/e 683. The (1 → 2) and (1 → 4) linked have furthermore a relatively intense peak at m/e 668, whereas they differ in the intensities of the peak at m/e 569. However, in the present study it was found that m/e 683 was also observable in most of the spectra of (1 → 5) and (1 → 6) linked disaccharides and the peaks at m/e 668 and m/e 578 were also detectable in the spectra of the (1 → 3) linked aldohexosyl-aldohexoses, although their intensities were rather low. Furthermore, the relative intensity of the fragment at m/e 569 varied strongly with the type of the constituent monosaccharides (Vink

et al.[9]) and to a smaller extent with the configuration of the glycosidic link. On the basis of these observations preference was given to the use of ratios of peak intensities over the utilization of relative peak intensities expressed as a percentage of a base peak or of a sum of peaks. The latter data are not characteristic enough.

m/e 683. From our results it can be concluded that the peak at *m/e* 683 in aldo-hexosyl-aldohexoses originates mainly from the reducing site of the molecule, because the peak is present in the spectrum of the xylosyl-glucose while it has for the greater part been shifted to *m/e* 581 in the aldohexosyl-arabinoses. Therefore the assumption of Kochetkov *et al.*[3] that the fragment at *m/e* 683 stems from the non-reducing site holds true only for a small part of the intensity of this peak (Tables 2 and 5).

m/e 668. The brutoformula of the fragment at *m/e* 668 (in (1 → 2) disaccharides) was established by exact mass measurement to be $C_{27}H_{64}O_7Si_6$ (Table 2). By consequence, this ion can not be formed by the elimination of $\cdot CH_3$ from *m/e* 683 $(C_{27}H_{63}O_8Si_6)$ as suggested by Chizhov *et al.*[2] However, the latter explanation can be correct for the very low peak at *m/e* 668 in (1 → 1) disaccharides because in this case the brutoformula turned out to be $C_{26}H_{60}O_8Si_6$.

m/e 569. Besides the way of formation of *m/e* 569 as proposed by Kochetkov *et al.*[3] (Table 2) another route exists, because a small peak at *m/e* 569 was present in the aldohexosyl-arabinoses. A structure for this fragment is given in Fig 9. In some of the non-reducing disaccharides the fragment at *m/e* 569 is also observable, albeit in very low abundance.
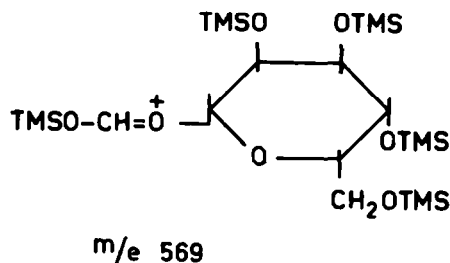

FIG 9. Structure of *m/e* 569 resulting from the non-reducing site of the molecule

m/e 539. In the literature no explanation has been presented for the fragment ion at *m/e* 539 (*c.f.* Kärkkäinen[4]). By exact mass measurements the brutoformula was found to be $C_{21}H_{51}O_6Si_5$, corresponding to TMS-aldohexose minus 1H. A structure for this fragment is given in Fig 10. From the spectra of disaccharides containing
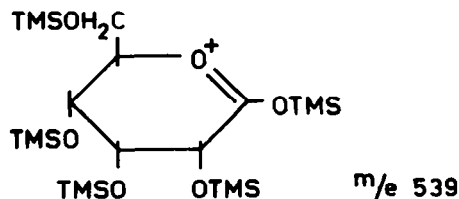

FIG 10. Structure of *m/e* 539

an aldopentose unit it can be deduced that this ion originates for the greater part from the non-reducing unit.

The spectra of all TMS-aldohexosyl-aldohexoses have some important peaks in common, which need further explanation. The formation of the fragment ions at m/e 525, m/e 435, and m/e 393 can be explained starting from the intermediate presented in Fig. 11. The presence of m/e 540 in the non-reducing disaccharides (Fig. 7) supports this proposal. For the formation of the fragment at m/e 451 three routes are available: direct cleavage of the bond on the right or the left side of the O-atom of the glycosidic
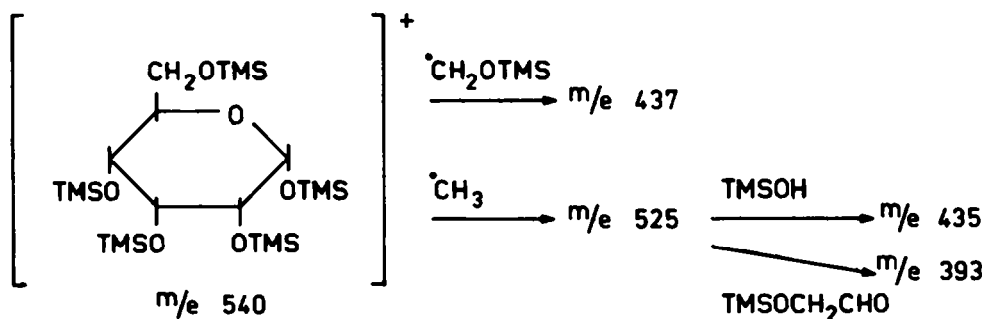
FIG 11. Formation of some fragment ions from m/e 540

linkage, or degradation of the fragment ion at m/e 569 (metastable peak at m* = 358·5). As is evident from Table 5 the ion at m/e 451 in an aldohexosyl-aldohexose is for the greater part formed by the direct cleavage of the bond between the anomeric C-atom of the non-reducing moiety and the glycosidic oxygen. The peak at m/e 361 arises by the elimination of TMSOH from m/e 451. Besides the criteria for the determination of the monosaccharide sequence in aldopentose containing disaccharides mentioned in Table 5, a more generally applicable parameter was found to be the sum of the intensities of the related fragments m/e 451 and m/e 361. This can also be illustrated by the mass spectra of the 2-acetamido-2-deoxy-D-glycose containing oligosaccharides (to be published) and by the mass spectra of α-L-Ara-(1 → 6)-D-G (m/e 361 + m/e 451 < m/e 259 + m/e 349) and α-L-Rha-(1 → 6)-D-G (m/e 361 + m/e 451 < m/e 273 + m/e 363) published by Kochetkov et al.[3]

This study has shown the applicability of mass spectrometry for the determination of the position of the glycosidic bond in lower oligosaccharides. In oligosaccharides consisting of units which differ in molecular weight, determination of the monosaccharide sequence is possible.

EXPERIMENTAL

The trimethylsilyl derivatives were synthesized as follows. An oligosaccharide (0·5 mg) was dissolved in pyridine (1 ml). Subsequently hexamethyldisilazane (HMDS) (0·2 ml) and trimethylchlorosilane (TMCS) (0·1 ml) were added. After two hr at room temperature (disaccharides) and 3 hr at 70° (higher oligosaccharides) hexane (2 ml) and water (2 ml) were added to the turbid mixture. The two layer system was strongly agitated and the water layer was removed. The hexane layer was washed with water (1 ml), dried over anhyd Na_2SO_4 and evaporated in vacuo. The residue was dissolved in hexane (1 ml). When the oligosaccharide did not dissolve easily in pyridine, a very small drop of water was added before the addition of pyridine. In this case 0·6 ml of HMDS and 0·3 ml of TMCS was used. Reducing oligosaccharides were anomerized in water (48 hr, room temperature). 10 μl of this solution was used for mass spectrometry.

Carbohydrates were either commercially available (J. T. Baker Chemicals N.V., Pierce Chemical Company and EGA-Chemie KG) or were gifts from various investigators (acknowledgements).

The 70 eV-mass spectra were recorded on an AEI MS9 mass spectrometer at an ion chamber temperature of 80–100° for the disaccharides and 120–140° for the trisaccharides.

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