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MASS SPECTROMETRY OF PERTRIMETHYLSILYL OLIGOSACCHARIDES CONTAINING FRUCTOSE UNITS

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Abstract—Mass spectra of 6 TMS-disaccharides of type aldohexosyl-(1 → *x*)-fructose, in which *x* varies from 1 to 6, were compared and could be divided into two main groups i.e. (1 → 1), (1 → 2) disaccharides and (1 → 3), (1 → 4), (1 → 5), (1 → 6) disaccharides. Within both groups a further differentiation was possible. Also the mass spectra of 2 di-, 6 tri- and 3 tetrasaccharides containing one or more (*x* → 2)-β-D-fructofuranose units (*x* = 1 or 6) were studied. The presence of such a unit gives rise to very abundant ions at *m/e* 437 and/or *m/e* 815. A number of other fragment ions e.g. *m/e* 671, *m/e* 811, *m/e* 1049 and *m/e* 1427 are also of great importance for the characterization of the latter fructosyl oligosaccharides.

INTRODUCTION

FOR TMS-aldosyl oligosaccharides and TMS-disaccharides containing a 2-acetamido-2-deoxy-glycose unit we reported the applicability of mass spectrometry to the determination of the type of glycosidic link and the monomer sequence.[1,2] Because of the importance of fructosyl carbohydrates in Nature, we also investigated the mass spectra of TMS-oligosaccharides with one or more fructose residues. The spectra of the TMS-derivatives of the pyranose, furanose and acyclic forms of D-fructose itself have been studied by several investigators. Curtius *et al.*[3,4] detected the five possible forms of TMS-D-fructose by using a combined GC–MS system and described the value of the intensities of the peaks at *m/e* 204 and *m/e* 217 for differentiating between pyranose and furanose ring forms. They observed further that the presence of an intense peak at *m/e* 437 ($M^{\ddagger}$ minus $^{\bullet}CH_2OTMS$) sets these compounds apart from the aldohexoses. With the aid of some deuterium labelled compounds, Karady *et al.*[5] showed that in the formation of the fragment ion at *m/e* 437 the C_1—C_2 bond is cleaved. In a preliminary communication about the mass spectrometry of fructose containing oligosaccharides[6] we reported some significant mass spectrometric details for this type of carbohydrate. Recently, Binkley *et al.*[7] published data on the mass spectra of oligosaccharides related to sucrose, using peracetyl and pertrimethylsilyl derivatives.

In this paper we discuss in more detail the mass spectra of some pertrimethylsilyl reducing and non-reducing oligosaccharides, in which fructose occurs in pyranose and/or furanose forms.

RESULTS

Disaccharides of the type aldohexopyranosyl-(1 → x)-fructose

Mass spectra of the TMS ethers of the disaccharides I to VI (Table 1) were recorded. In Table 2 significant peaks present above *m/e* 360 are given. With the exception of II, the molecular ion at *m/e* 918 was observed in all cases. The elimination of a

TABLE 1. LIST OF STUDIED OLIGOSACCHARIDES

I	β-D-glucopyranosyl-(1 → 1)-D-fructose
II	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside (= sucrose)[a]
III	α-D-glucopyranosyl-(1 → 3)-D-fructose (= turanose)[b]
IV	β-D-galactopyranosyl-(1 → 4)-D-fructose (= lactulose)[a]
V	α-D-glucopyranosyl-(1 → 5)-D-fructopyranose (= leucrose)
VI	α-D-glucopyranosyl-(1 → 6)-D-fructofuranose (= palatinose)[c]
VII	α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside (= raffinose)[a]
VIII	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(3 → 1)-α-D-glucopyranoside (= melezitose)[a]
IX	α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside (= stachyose)[c]
X	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranoside (= 1-kestose)
XI	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(6 → 2)-β-D-fructofuranoside (= 6-kestose)
XII	β-D-fructofuranosyl-(2 → 6)-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside (= neokestose)
XIII	α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranosyl-(1 → 2)-β-D-fructofuranoside (= nystose)
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

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

$\cdot CH_3$ radical, resulting in the peak at *m/e* 903, was detectable in all spectra. Spectral differences related to the various types of glycosidic linkages are summarized in Tables 3 and 4.

The 1 → 1 and 1 → 2 TMS-aldohexosyl-fructoses are characterized by the presence of a peak at *m/e* 437 with relatively high intensity and a peak at *m/e* 815 with relatively low intensity. For the 1 → 3, 1 → 4, 1 → 5 and 1 → 6 compounds the reverse holds (Table 2). The disaccharides of the last group eliminate a $\cdot CH_2OTMS$ radical from the fructose unit by cleavage of the C_1—C_2 bond, resulting in the intense peak at *m/e* 815. This high intensity has to be explained as a combination of simple cleavage of the linkage and formation of a stable fragment ion. These observations are in agreement with the finding of Karady *et al.*[5] and Curtius *et al.*[3,4] with regard to the intense peak at *m/e* 437 in the TMS-fructoses. The peak at *m/e* 437 in the 1 → 1 disaccharide is formed analogously (Fig. 1). The formation of the ion at *m/e* 437 in the non-reducing 1 → 2 disaccharide is more complicated; a fragmentation mechanism is given in Fig. 2.

The 1 → 1 and 1 → 2 disaccharide can be distinguished with the aid of some peaks as summarized in Table 3.

The 1 → 3, 1 → 4, 1 → 5 and 1 → 6 disaccharides can be discriminated on the basis of peak intensity ratios e.g. *m/e* 347/*m/e* 345, *m/e* 393/*m/e* 361, *m/e* 569/*m/e* 539 and *m/e* 583/*m/e* 582 (Table 4).*

* The derivatives of the saccharides III to VI were contaminated to a small extent with a compound of a higher molecular weight (origin unknown). In the high mass range peaks at *m/e* 875, *m/e* 889, *m/e* 891, *m/e* 963 and *m/e* 990 were detectable with intensities smaller than the molecular ion. This contamination was also present after the application of other silylation procedures. The impurity has only been found in this type of saccharides, irrespective of their origin. Purification by TLC or GLC could not be carried out without disturbing the anomeric equilibrium of the disaccharide.

TABLE 2. SIGNIFICANT PEAKS ABOVE *m/e* 360, EXPRESSED IN THE INTENSITY OF THE PEAK AT *m/e* 361, IN THE MASS SPECTRA OF THE TMS-GLYCOSYL-(1 → *x*)-FRUCTOSES WITH *x* = 1–6.

m/e	Carbohydrates					
	I	II	III	IV	V	VI
918	0·9	—	1·2	4·4	6·4	1·0
903	1·4	0·2	0·9	2·4	1·6	1·7
828	0·9	0·1	1·7	1·4	0·5	1·3
815	1·4	0·05	88	138	150	184
813	1·9	0·05	1·4	3·3	4·4	5·0
801	—	—	1·0	2·7	0·3	1·3
771	0·4	0·02	0·3	1·2	0·8	0·8
725	1·2	0·1	1·2	2·4	3·7	1·3
723	2·2	0·1	1·4	2·6	3·6	1·5
685	9·8	—	0·6	1·0	1·9	0·5
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	—	—	—	—	—
611	3·8	0·1	0·5	2·0	1·9	—
583	0·4	0·05	0·8	—	0·6	1·3
582	0·4	0·03	1·3	—	1·2	1·0
569	11·4	0·03	2·4	2·7	11·3	27·6
565	—	0·1	—	—	0·4	—
553	—	0·2	—	—	—	—
539	17·4	0·1	1·9	6·8	3·0	7·4
525	3·3	0·6	1·2	4·3	1·2	3·5
521	1·4	0·05	0·2	4·0	5·8	0·8
451	9·6	39·7	11·1	15·2	15·4	11·6
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
393	1·4	0·1	2·4	11·8	13·2	10·4
361	100	100	100	100	100	100

— = not detectable

The peaks are not corrected for the isotopic contribution of peaks of lower masses. For the interpretation of the various peaks see Kamerling *et al.*[1] For compound II intensities smaller than 0·1% are given, because in the mass spectrum of this disaccharide the ions above *m/e* 451 have a low abundance.

TABLE 3. PEAK INTENSITIES USED FOR DIFFERENTIATION BETWEEN THE 1 → 1 AND 1 → 2 DISACCHARIDES (*m/e* 361 = 100%).

m/e	*Type of glycosidic linkage*	
	1 → 1	1 → 2
569	11·4%	0·03%[a]
671	1·4%	—
684	16·2%	0·02%[b]

[a] not corrected for the isotopic contribution of *m/e* 565

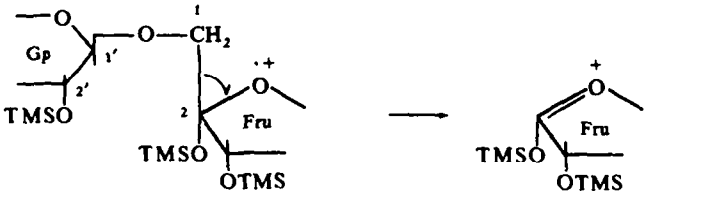
[b] not corrected for the isotopic contribution of *m/e* 680

— = not detectable

TABLE 4. PEAKINTENSITY 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*	0·6	—	0·5	1·3

* Isotopic ratio 583/582 = 0·52 (calculated)



Fru = Fru*f* or Fru*p*

FIG 1. Formation of *m/e* 437 in a 1 → 1 disaccharide

Because of the small number of disaccharides available (one of each bonding type), it is not clear if all differences are only due to varieties in glycosidic links. The constituent aldohexose[8] and the ratio pyranose–furanose–acyclic forms as well as the anomeric configuration may also display a distinct influence.

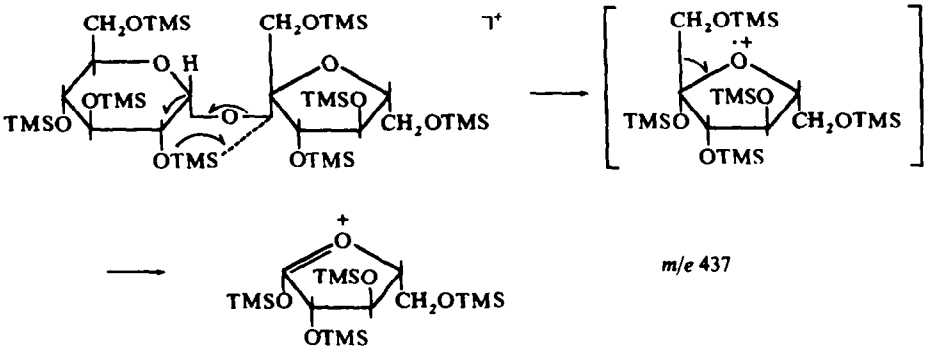


FIG 2. Formation of *m/e* 437 in a 1 → 2 disaccharide

Oligosaccharides with an (x → 2)-β-D-*fructofuranose unit*

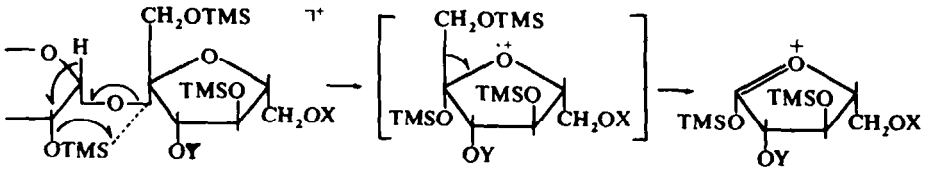
The mass spectra of the TMS ethers of oligosaccharides VII to XVI (Table 1) were recorded. In the spectra of VII and IX the molecular ion M^+ could be observed.

TABLE 5. SIGNIFICANT PEAKS ABOVE *m/e* 360, EXPRESSED IN THE INTENSITY OF THE PEAK AT *m/e* 361, IN THE MASS SPECTRA OF THE TMS-DISACCHARIDES (M = 918), THE TMS-TRISACCHARIDES (M = 1296) AND THE TMS-TETRASACCHARIDES (M = 1674).

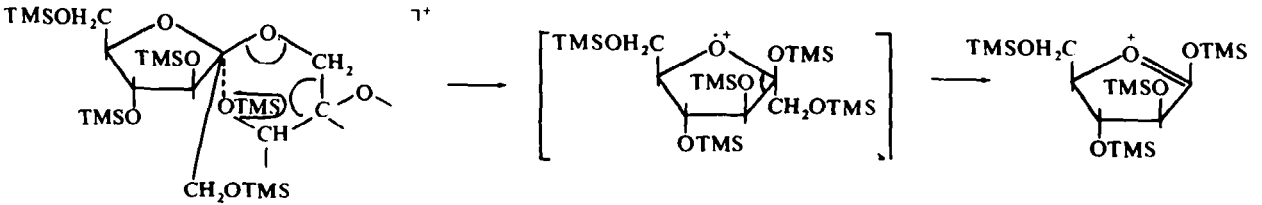
m/e	Carbohydrates									
	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
1674	—	—	0·05	—	—	—	—	—	—	—
1659	—	—	—	—	—	—	0·05	—	—	—
1571	—	—	—	—	—	—	0·6	—	—	0·6
1427	—	—	—	—	—	—	1·6	—	—	5·4
1325	—	—	0·9	—	—	—	—	—	—	—
1296	0·02	—	—	—	—	—	—	—	—	—
1281	0·05	—	—	0·1	0·05	0·05	—	—	—	—
1207	—	—	2·1	—	—	—	0·2	—	—	0·4
1193	0·02	0·1	—	0·3	3·7	1·5	0·6	—	2·8	3·1
1049	—	—	—	0·2	0·5	0·4	5·4	—	7·9	5·4
961	—	—	0·2	—	—	—	0·2	—	—	0·2
947	0·8	0·2	0·6	—	—	—	—	—	—	—
903	0·05	0·2	—	0·1	0·05	-	0·1	0·6	—	0·2
845	0·3	—	—	0·4	—	0·02	0·05	—	—	—
829	0·5	0·6	0·8	4·2	2·1	0·6	7·6	—	2·4	2·3
828	0·6	0·6	0·2	—	0·9	0·02	—	—	—	0·6
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
739	0·3	0·7	0·3	1·5	9·8	0·2	6·1	0·4	10·2	17·8
725	0·1	0·3	0·2	0·3	0·6	0·1	1·3	1·1	1·6	1·1
723	0·3	0·4	0·2	0·8	0·6	0·2	1·3	1·8	1·6	1·5
721	—	0·2	—	1·0	0·1	—	2·5	—	0·3	—
671	—	—	—	8·6	1·0	—	2·5	2·8	7·5	3·1
649	0·4	0·4	0·4	1·9	2·0	0·1	8·0	—	8·3	19·4
639	—	—	0·2	—	1·0	0·02	—	—	—	—
595	0·05	0·4	0·2	0·5	0·3	0·05	0·2	1·2	1·6	1·0
583	1·6	—	1·4	0·1	0·1	0·05	—	4·3	0·5	—
569	0·05	0·05	0·4	—	—	—	—	—	—	—
539	0·6	0·2	0·6	0·3	0·2	0·02	0·1	—	—	—
525	1·2	0·2	1·0	0·4	1·0	1·1	1·0	1·7	0·8	3·1
509	0·05	0·2	0·2	0·6	0·5	0·2	4·1	1·4	3·1	7·7
467	2·3	1·2	1·6	—	0·1	0·8	0·05	1·2	—	—
451	72·8	11·9	85·0	58·3	57·7	110	40·4	67·9	60·6	45·0
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

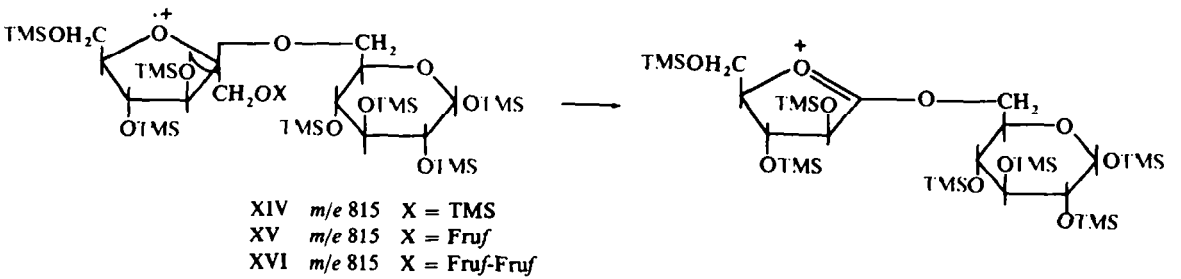
The peaks are not corrected for the isotopic contribution of peaks of lower masses.



II *m/e* 437 X = Y = TMS VIII *m/e* 815 X = TMS; Y = *Gp*
VII *m/e* 437 X = Y = TMS XI *m/e* 815 X = *Fruf*; Y = TMS
IX *m/e* 437 X = Y = TMS Planteose[8] *m/e* 815 X = *Galp*; Y = TMS
XII *m/e* 437 X = Y = TMS



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



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

FIG 3. Formation of *m/e* 437 and *m/e* 815 in fructosyl oligosaccharides

The spectra of X, XI, XII, XIII and XIV showed as the first detectable ion [M^+ minus $^{\cdot}CH_3$]. In the remaining compounds [M^+ minus $^{\cdot}CH_2OTMS$] was the first observed ion. Table 5 summarizes significant peaks present in the various mass spectra.

The presence of an ($x \rightarrow 2$)-(β-D-fructofuranose unit in an oligosaccharide ($x = 1$ or 6) has a very characteristic influence upon the mass spectrum of its TMS-derivative. All spectra except that of VIII (melezitose) show a peak at *m/e* 437 (measured atomic composition $C_{17}H_{41}O_5Si_4$) with relatively high intensity, as observed for II (sucrose). This highly abundant ion is typical of the presence of an ($x \rightarrow 2$)-β-D-fructofuranose unit at one end of the molecule. Compounds VIII and XI show an intense peak at *m/e* 815 (measured atomic composition $C_{32}H_{75}O_{10}Si_7$). These two trisaccharides contain an ($x \rightarrow 2$)-β-D-fructofuranose unit in the middle of the molecule. In Fig. 3 the fragmentation reactions resulting in the intense peaks at *m/e* 437 and/or *m/e* 815 are given for each oligosaccharide. Because of the relatively low intensity of the ion at *m/e* 437 (comparable with that of *m/e* 435) in the mass spectra of the TMS-aldohexosyl-(1 → 6)-aldohexoses (Kamerling *et al.*[1]), it is highly improbable that formation of the fragment ion at *m/e* 437 in compounds XIV, XV and XVI is the result of cleavage of the C_5—C_6 bond in the reducing aldohexose unit. In the mass spectra of compounds XV and XVI, which contain one and two fructose units respectively in the middle of the molecule, an abundant ion at *m/e* 815 was also present, whereas in the spectra of compounds X and XIII it has an intensity comparable with that of *m/e* 813. In compounds X, XIII, XV and XVI C_1 of the inner fructose units is substituted by a hexose unit. The presence of a peak at *m/e* 815 with relatively high intensity in compounds XV and XVI cannot be explained by the supposed mechanism in which an OTMS migration is followed by a $^{\cdot}CH_2OTMS$ elimination (Fig. 3). In the case of trisaccharide XV it is unlikely that from the intermediate, formed after an OTMS migration from the glucose to the middle fructose unit, a $^{\cdot}CH_2OTMS$ radical is eliminated from the other fructose unit, because this elimination cannot be correlated with the mass spectrum of trisaccharide X in which the intensity of *m/e* 815 is nearly identical to that of *m/e* 813 (c.f. Fig. 3).† The same holds for the analogue of *m/e* 815, 378 a.m.u. higher, *m/e* 1193 in XVI. The mass spectrum of disaccharide XIV shows an intense peak at *m/e* 815, corresponding to easy elimination of a $^{\cdot}CH_2OTMS$ radical contrary to disaccharide II. Saccharides X and XIII belong to the sucrose-series (compound II with a 1 → 2 linkage) and saccharides XV and XVI to the β-D-Fru*f*-(2 → 6)-D-G-series (compound XIV with a 6 → 2 linkage). Therefore, the intense peaks at *m/e* 815 in XV and *m/e* 815 together with *m/e* 1193 in XVI can be explained in the same way as for disaccharide XIV (Fig. 3). In the β-D-Fru*f*-(2 → 6)-D-G-series the C_1—C_2 bonds in the fructose units will be cleaved more easily than in the sucrose-series.

The mass spectra of X (1-kestose) and XIII (nystose) are characterized by the presence of an abundant ion at *m/e* 811 (measured atomic composition $C_{33}H_{75}O_9Si_7$). This fragment ion is formed by elimination of H_2O from *m/e* 829 (measured atomic composition $C_{33}H_{77}O_{10}Si_7$) as indicated by the presence of the metastable peak m* at 793·4. A mechanism is presented in Fig. 4. The peak at *m/e* 811 was also observable

† It cannot be ruled out, that formation of the ion at *m/e* 437 in these trisaccharides proceeds also *via* elimination of a $^{\cdot}CH_2O$-Fru*f* radical from this intermediate.

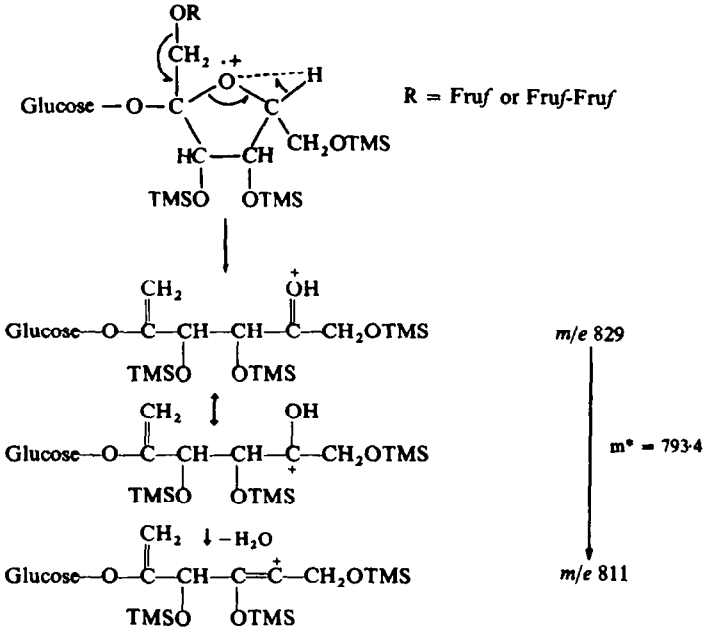
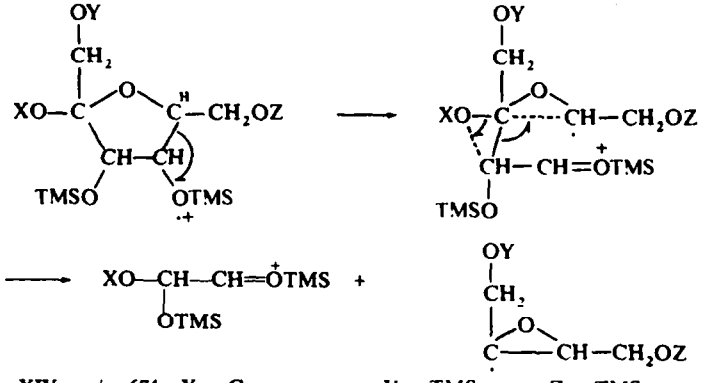


FIG 4. Formation of *m/e* 811 in TMS-1-kestose and TMS-nystose



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 5. Formation of *m/e* 671, *m/e* 1049 and *m/e* 1427 in fructosyl oligosaccharides

in the saccharides VIII, XI, XII, XV and XVI, albeit in lower intensities. For the saccharides VIII, XI and XII this ion must be formed *via* a fragmentation pathway different from the one suggested in Fig. 4.

The presence or absence of the fragment ions at *m/e* 671, *m/e* 1049 and/or *m/e* 1427 in the saccharides X to XVI are characteristic for the sequence of the fructose units in these compounds. A mechanism for their formation is given in Fig. 5 for each oligosaccharide. Exact mass measurements established that the atomic composition of *m/e* 671 is $C_{26}H_{63}O_8Si_6$ and that of *m/e* 1049 (378 a.m.u. higher) $C_{41}H_{97}O_{13}Si_9$. The absence of *m/e* 671 in TMS-neokestose (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.

DISCUSSION

Formation of the fragment ions at m/e 204 *and* m/e 217

De Jongh *et al.*[9] and Petersson *et al.*[10] have demonstrated that in TMS-aldohexopyranoses the greatest contribution to the formation of the fragment ion at *m/e* 204 ($TMSO—\overset{+}{C}H—\dot{C}H—OTMS$) stems from $C_2—C_3$ and to a smaller extent from $C_3—C_4$, while the fragment at *m/e* 217 ($TMSO—CH{=}CH—\overset{+}{C}H—OTMS$) stems mainly from $C_2—C_3—C_4$. Karady *et al.*[5] have shown that in TMS-2-ketohexopyranoses the ion at *m/e* 204 stems from $C_3—C_4$ and $C_4—C_5$, while *m/e* 217 stems from $C_1—C_2—C_3$ and $C_3—C_4—C_5$. The mass spectra of the TMS-2-ketohexofuranoses published by Curtius *et al.*[3] make clear that in these compounds the peak at *m/e* 204 is hardly formed, just as in the TMS-aldohexofuranoses.[9,10] For the pyranose forms the intensity of the peak at *m/e* 217 is smaller than that of *m/e* 204, while for the furanose forms the reverse holds.

From the mass spectra of compounds II and VI, containing a fructofuranose unit, it can be deduced that the intensity of the peak at *m/e* 217 is greater than that of *m/e* 204 (Table 6). The spectrum of disaccharide V, containing a fructopyranose unit, shows the reverse. On the basis of CMR spectroscopy, Doddrell *et al.*[11] stated that an aqueous solution of turanose contains ± 40% of the fructopyranose form and ± 60% of the fructofuranose form. The mass spectrum of TMS-turanose (III) shows that the peak at *m/e* 217 is more abundant than that of *m/e* 204, suggesting that the fructofuranose form is dominating under the conditions of our experiment.

TABLE 6. PEAKINTENSITY RATIOS OF THE IONS AT *m/e* 217 AND *m/e* 204.

Compound	*f*	*p*	217/204	Compound	*f*	*p*	217/204
I	*	*	0·8	IX	1	3	0·6
II	1	1	7·4	X	2	1	8·1
III	*	*	1·3	XI	2	1	5·9
IV	*	*	0·9	XII	2	1	8·8
V	0	2	0·6	XIII	3	1	10·3
VI	1	1	1·4	XIV	1	1	1·7
VII	1	2	1·3	XV	2	1	2·6
VIII	1	2	1·6	XVI	3	1	3·5

f = number of furanoses present
p = number of pyranoses present
* = unknown

In earlier investigations we have demonstrated[1] that the intensities of the abundant ions at *m/e* 204 and *m/e* 217 in TMS-disaccharides are also influenced by the type of glycosidic link. Furthermore Avigad *et al.*[12] have described the presence of very small amounts of the acyclic form of the reducing fructose unit in the disaccharides I, III, IV and VI. Therefore, the usefulness of the ratios *m/e* 217/*m/e* 204 from the spectra of compounds I and IV for the determination of the pyranose–furanose equilibria is still unclear.

The results deduced from the mass spectra of the remaining compounds support that the presence of an (x → 2)-β-D-fructofuranose unit greatly influences the ratio *m/e* 217/*m/e* 204 (Table 6).

Formation of the fragment ion at m/e 437

The mechanism for the formation of the ion at *m/e* 437 in (x → 2)-β-D-fructofuranose containing oligosaccharides[6] (Figs. 2 and 3) is based on the following: (1) Rearrangements of OTMS groups in TMS-carbohydrates are very common (Kochetkov *et al.*[13] and De Jongh *et al.*[9]). (2) Mass spectra of the TMS-trehaloses show clearly the presence of a fragment ion at *m/e* 540 (Kamerling *et al.*[1]) corresponding to the supposed intermediate. (3) By this mechanism, in which the formation of a TMS-hexose ion is supposed, it is also possible to explain the presence of the ions at *m/e* 525 and *m/e* 435 (540^+ minus $^{\cdot}CH_3$ and 540^+ minus $^{\cdot}CH_3$ minus TMSOH respectively) in TMS-disaccharides.[1] (4) TMS-fructoses in their pyranose and furanose forms eliminate a $^{\cdot}CH_2OTMS$ radical very easily, by cleavage of the C_1—C_2 bond (Karady *et al.*[5] and Curtius *et al.*[3]).

For the explanation of the ions at *m/e* 525 and *m/e* 435, Binkley *et al.*[7] also accepted the formation of a TMS-hexose ion at *m/e* 540. However, they did not use this intermediate for the formation of the fragment ion at *m/e* 437. In this case the authors suggested a mechanism based on migration of the TMS group of C_1 of the fructose unit to the glycosidic O-atom, followed by a $CH_2{=}O$ and a glycosyl radical elimination.

A further consideration of a number of fragment ions

The mass spectra of the 1 → 1 and 1 → 3 disaccharides I and III show a peak at *m/e* 684 with atomic composition $C_{27}H_{64}O_8Si_6$. In Fig. 6 an explanation is given for its formation. This mechanism accounts for the facts that in the case of the 1 → 4, 1 → 5 and 1 → 6 disaccharides this ion is not formed.

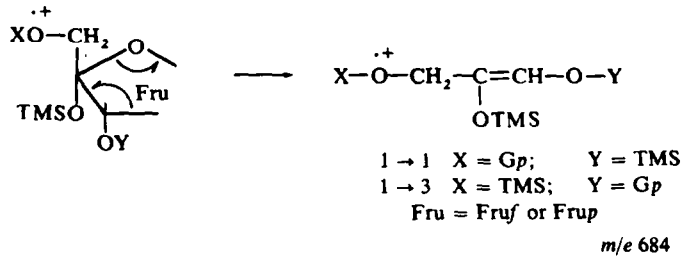


FIG 6. Formation of *m/e* 684 in the 1 → 1 and 1 → 3 disaccharides

In the mass spectrum of the 1 → 5 disaccharide V a peak at *m/e* 685 (measured atomic composition $C_{27}H_{65}O_8Si_6$) of reasonable intensity was observed. The presence of this fragment ion might support the presence of the acyclic form of fructose in this disaccharide (Fig. 7). However, this assumption could not be confirmed by IR experiments (presence of a C=O vibration).

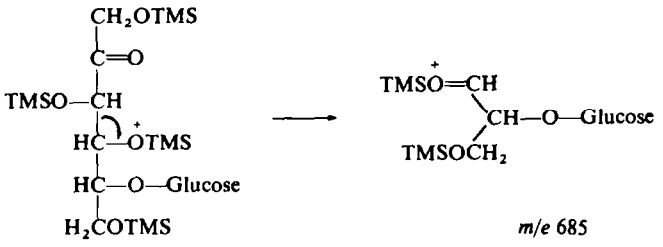


FIG 7. Formation of *m/e* 685 in the 1 → 5 disaccharide

The mass spectrum of the 1 → 1 disaccharide I shows a peak at *m/e* 671, which cannot be explained according to Fig. 5. Karady *et al.*[5] have described a mechanism for the formation of *m/e* 293 in TMS-fructopyranose which can be used for the explanation of the fragment ion at *m/e* 671, 378 a.m.u. higher than *m/e* 293 (Fig. 8).

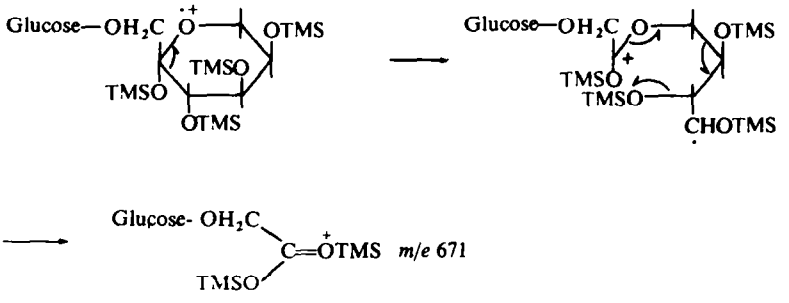


FIG 8. Formation of *m/e* 671 in the 1 → 1 disaccharide

In earlier experiments[1] we established that the atomic composition of the fragment ion at *m/e* 393 in aldohexosyl-aldohexoses is $C_{15}H_{37}O_4Si_4$ (525^+ minus $TMSOCH_2$—CH=O). The same holds for the peak at *m/e* 393 in the 1 → 1 and 1 → 2 disaccharides I and II. However, exact mass measurements of the same peak in the 1 → 3, 1 → 4, 1 → 5 and 1 → 6 disaccharides III to VI gives the atomic composition as $C_{15}H_{33}O_6Si_3$. It was not possible to also detect the peak with atomic composition $C_{15}H_{37}O_4Si_4$. In Fig. 9 an explanation is given for the formation of the peak corresponding to $C_{15}H_{33}O_6Si_3$, partially based on elimination of a $^{\cdot}CH_2OTMS$ radical in these four compounds as described before.

The mass spectrum of TMS-lactulose (IV) shows a peak at *m/e* 347 with a relatively high abundance compared with the peak at *m/e* 345. All other disaccharides investigated up to now (ref. 1 and this study) show peaks at *m/e* 347 and *m/e* 345 of similar intensities. Exact mass measurements established that the atomic composition of the peak at *m/e* 347 is $C_{14}H_{31}O_4Si_3$. The fragment ion can be formed by elimination of TMSOH from *m/e* 437.

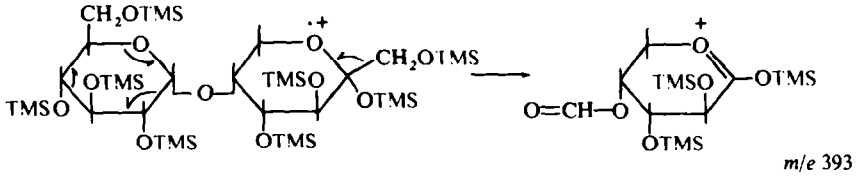


FIG 9. Formation of *m/e* 393 with the brutoformula $C_{15}H_{33}O_6Si_3$ in e.g. a 1 → 5 disaccharide

The mass spectrum of TMS-melezitose (VIII) shows a distinct peak at *m/e* 541 besides *m/e* 539. It can be explained as an [TMS-hexose + 1H] ion. The mass spectra of the disaccharides containing a 2-acetamido-2-deoxy-glycose unit frequently show the analogue of this fragmentation ion, 31 a.m.u. lower (Kamerling *et al.*[2]).

The significance of the peak at m/s 583 in relation to the oligosaccharide structure

Recently it has been shown that the mass spectra of TMS-aldohexosyl-aldohexoses with a 1 → 5 or 1 → 6 linkage have a relatively intense peak at *m/e* 583 (Kochetkov *et al.*[13] and Kamerling *et al.*[1]). The spectra of VII, IX, XII and XIV show a peak at *m/e* 583, in accordance with a 1 → 6 or 2 → 6 linkage in these compounds. A fragmentation pathway for this ion has been published by Kochetkov *et al.*[13] A peak at *m/e* 961 in IX can be correlated with the second 1 → 6 link.[1] However, the mass spectra of compounds XV and XVI, each with a 2 → 6 linkage, did not give rise to peaks at *m/e* 961 and *m/e* 1339 respectively. Compounds VI and XI also show a peak at *m/e* 583, in accordance with the 1 → 6 and 2 → 6 linkages respectively in these saccharides. Furthermore the mass spectrum of the TMS-derivative of α-D-Gp-(1 → 2)-β-D-Fruf-(6 → 1)-α-D-Galp (planteose) shows a peak at *m/e* 583 (Binkley *et al.*[7]). Because of the furanose ringstructure of the fructose unit in palatinose (VI), 6-kestose (XI) and planteose a somewhat different fragmentation pathway must be expected as originally introduced by Kochetkov *et al.*[13] Taking into account that palatinose contains a very small amount of the acyclic form,[12] the formation of *m/e* 583 can also be explained on the basis of this last structure (glucose—O—CH_2—$\overset{+}{C}H$=OTMS).

In the mass spectra of the saccharides II, X and XV a peak at *m/e* 583 of low intensity was observable. A small peak at *m/e* 961 was present in the spectra of the saccharides XIII and XVI. These peaks cannot be correlated with the presence of a *x* → 6 linkage. They must be considered in a similar way to the peak at *m/e* 583 of very low intensity in the mass spectra of the non-reducing 1 → 1 aldohexosyl-aldohexoses.[1]

Comparison of the different intensities of the ions at *m/e* 583 and *m/e* 961[1] (Table 5) lead to the conclusion that for an unknown saccharide it is only allowed to correlate the occurrence of these ions with the presence of a $x \rightarrow 6$ (or $1 \rightarrow 5$) linkage, if the relative intensities of these ions are evidently more abundant than those of the surrounding peaks.

In this study we have shown that the presence of fructose in an oligosaccharide has a typical influence on the fragmentation pattern. On the basis of the mass spectra of the three known kestoses a new method is developed for their differentiation. The very characteristic peaks present in the mass spectra of fructosyl oligosaccharides can be of great help for the elucidation of new unknown oligosaccharides.

EXPERIMENTAL

The trimethylsilyl derivatives were prepared as described earlier.[6] However, by this procedure reducing disaccharides with a free hemiacetal hydrogen-group on C_2 (i.e. the compounds I, III, IV, V and VI) were silylated by only 97%. Further experiments have shown that it is necessary to silylate these compounds for 48 hr at 60° to attain a degree of silylation of more than 99·5%. Recently, Okuda *et al.*[14,15] described the difficult silylation of the hemiacetal hydroxy-group in fructoses. The application of N-trimethylsilyl-imidazole or N,0-bis-trimethylsilyl-trifluoracetamide plus 1% TMCS did not give better results. The carbohydrates were obtained from J. T. Baker Chemicals N.V., Pierce Chemicals Company or EGA-Chemie K.G., or were gifts from several 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, 120–140° for the trisaccharides and about 160° for the tetrasaccharides.

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