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MASS SPECTROMETRY OF 2-ACETAMIDO-2-DEOXY-GLYCOSE CONTAINING DISACCHARIDES

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Abstract—The mass spectra of six trimethylsilyl 2-acetamido-2-deoxy-aldohexosyl-aldohexoses with (1 → 2), (1 → 3), (1 → 4) and (1 → 6) glycosidic linkages were compared. The spectra could be divided in two main groups on the basis of the ratio of the intensities of the peaks at *m/e* 217 and *m/e* 204 (217/204): (1 → 2), (1 → 3) disaccharides and (1 → 4), (1 → 6) disaccharides. (1 → 2) and (1 → 3) disaccharides could be distinguished on the basis of some ratios of peak intensities, (1 → 4) and (1 → 6) disaccharides on the basis of the presence or absence of one intense peak (*m/e* 552). Further, the mass spectrum of an aldohexosyl-(1 → 6)-2-acetamido-2-deoxy-aldohexose is discussed. In all cases the sequence of the monomers could be determined by using the sum of the intensities of two related peaks.

INTRODUCTION

PREVIOUSLY, we reported that the position of the glycosidic link in pertrimethylsilyl-aldohexosyl-aldohexoses can be determined by mass spectrometry.[1] It was found that the non-reducing (1 → 1) disaccharides are characterized by the occurrence of peaks at *m/e* 565, *m/e* 553 and *m/e* 540, which are not detectable in the spectra of the reducing compounds. The (1 → 5) and (1 → 6) linked disaccharides show both a strong peak at *m/e* 583, whereas they can not be distinguished from each other. In the group of the (1 → 2), (1 → 3) and (1 → 4) bonded disaccharides the type of linkage can be assigned on the basis of the ratios of the peak intensities 569/539 and 569/668.

To get a further insight into the applicability of mass spectrometry for structure determination of degradation products of the carbohydrate part of glycoproteins, we investigated the mass spectra of some disaccharides containing a N-acetyl amino sugar unit. Literature data are rather scarce for the latter compounds; Kärkkäinen investigated the pertrimethylsilyl[2] and permethyl[3] derivatives of the disaccharide alditols of β-D-Gal*p*-(1 → 4)-D-GNAc*p* and β-D-GalNAc*p*-(1 → 4)-D-Gal*p*. More data are available about the N-acetyl monosaccharides. Several derivatives of 2-acetamido-2-deoxy-aldohexoses were investigated: methyl ethers (Heyns *et al.*[4] and Kochetkov *et al.*[5]), acetyl esters (Heyns *et al.*[6]) and trimethylsilyl ethers (Kärkkäinen[7] and DeJongh *et al.*[8]). In this study we describe the determination of the sequence of the constituting monomers and of the position of the glycosidic link in disaccharides containing a 2-acetamido-2-deoxy-aldohexose and an aldohexose unit.

RESULTS

In the mass spectra of the TMS-2-acetamido-2-deoxy-aldohexosyl-aldohexoses I to V and TMS-α-D-G*p*-(1 → 6)-D-GNAc VII (Table A) the molecular ion at *m/e* 887 was present. The first detectable ion in the spectrum of TMS-β-D-GalNAc*p*-(1 → 6)-

TABLE A. 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
VII	α-D-glucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-D-glucose

D-Gal VI was *m/e* 872 ($M^{\ddagger}$ minus $\cdot CH_3$). This ion was also abundant in the other spectra. The most relevant peaks and their interpretations are listed in Table B. In comparison to the mass spectra of the TMS-aldohexosyl-aldohexoses,[1] analogous fragments now containing the N-acetyl group are shifted to 31 mass units lower.

The monomer sequence can be inferred from the mass spectrum by comparison of the ion abundances, which are mainly formed from the reducing or the non-reducing site of the molecule. It is essential that these ions contain that part of the monomers in which they differ in molecular weight. Preference has to be given to ions which are present in the spectra independent of the type of glycosidic link. The fragment ion at *m/e* 451 (Table B) and its shifted analogue at *m/e* 420 (Table B) occur in all spectra. From the spectra of the disaccharides consisting of an aldopentose and an aldohexose, it was deduced that the fragment ion at *m/e* 451 stems for the greater part from the non-reducing site.[1] By consequence, it is to be expected that the intensity of the peak at *m/e* 451 is smaller than that of the peak at *m/e* 420 when the N-acetyl amino sugar is the non-reducing unit, whereas it should be greater for the reversed sequence. Table D shows that this criterion is not decisive in all cases and gives the wrong answer for compound VII. This difficulty arises by the further fragmentation of the ions at *m/e* 451 and *m/e* 420 which proceeds to a different extent for the various disaccharides. The main fragmentation reaction is the elimination of TMSOH, resulting in ions at *m/e* 361 (metastable peak at m* = 289·0) and *m/e* 330 (metastable peak at m* = 259·3) respectively. Assuming that the peaks at *m/e* 361 and *m/e* 330 originate predominantly from the fragments at *m/e* 451 and *m/e* 420 respectively, comparison of the sum of the intensities of the ions at *m/e* 451 and *m/e* 361 to that of *m/e* 420 and *m/e* 330 is a far better criterion for sequence determinations than that of the intensities of the ions at *m/e* 451 and *m/e* 420 only (Table D).

The reducing position of the N-acetyl amino sugar in VII is also characterized by the absence of the peak at *m/e* 569 in combination with the presence of that at *m/e* 538 (Fig. 5). In the TMS-aldohexosyl-(1 → 6)-aldohexoses, the fragment at *m/e* 583 originates from the non-reducing site. Its absence in VI combined with the presence of the fragment at *m/e* 552 forms an indication for the non-reducing position of the N-acetyl amino sugar (Fig. 4).

The influence of the position of the glycoside bond on the fragmentation pattern is illustrated by Figs 1 to 5. The (1 → 6) link in β-D-Gal NAc*p*-(1 → 6)-D-Gal is characterized by a very strong peak at *m/e* 552. This peak is the analogue of *m/e* 583 in TMS-aldohexosyl-(1 → 6)-aldohexoses. However, in the mass spectrum of α-D-G*p*-(1 → 6)-D-GNAc the expected fragment ion at *m/e* 583 was completely absent. Starting from the fragmentation scheme for the formation of *m/e* 583, suggested by Kochet-

TABLE B. EXPLANATION OF SOME IMPORTANT FRAGMENT IONS, PRESENT IN MOST OF THE MASS SPECTRA OF PERTRIMETHYLSILYL DISACCHARIDES CONTAINING AN ALDOHEXOSE AND A 2-ACETAMIDO-2-DBOXY-ALDOHEXOSE UNIT

m/e	measured brutoformula	fragment
887		M^+
872		M^+ minus CH_3
828		M^+ minus NH_2COCH_3
784		M^+ minus CH_2OTMS
782		872^+ minus TMSOH
694		784^+ minus TMSOH
692		782^+ minus TMSOH
683	$C_{27}H_{61}NO_9Si_5$	M^+ minus TMSO—CH=CH—OTMS
638	$C_{26}H_{60}NO_7Si_5$	M^+ minus OTMS minus CH_2=C=O minus TMSO—CH=O
638		isotope peak of *m/e* 637
637	$C_{26}H_{59}NO_7Si_5$	M^+ minus $TMSOCH_2$—CH=O minus TMSO—CH=O M^+ minus TMSOH minus CH_2=C=O minus TMSO—CH=O
611	$C_{24}H_{55}O_8Si_5$	784^+ minus TMSO—CH=CH—$NHCOCH_3$
583		Gl—O—CH_2—CH=$\overset{+}{O}$TMS (non-reducing end)
569		TMSO—CH=$\overset{+}{O}$—Gl (reducing end)
552		GlNAc—O—(CH_2—CH=OTMS (non-reducing end)
539		Ref. 1
538		TMSO—CH=$\overset{+}{O}$—GlNAc (reducing end)
521		611^+ minus TMSOH
510	$C_{20}H_{48}NO_6Si_4$	509^+ plus H
510		isotope peak of *m/e* 509 and *m/e* 508
509	$C_{20}H_{47}NO_6Si_4$	[tetratrimethylsilyl-2-acetamido-2-deoxy-glycose]$^+$ (ref. 1)
509		isotope peak of *m/e* 508
508	$C_{20}H_{46}NO_6Si_4$	509^+ minus H (ref. 1) NH=CH—CH=$\overset{+}{O}$—Gl
463	$C_{19}H_{45}NO_4Si_4$	see text
451		$[Gl]^+$
420		$[GlNAc]^+$
361		451^+ minus TMSOH
330		420^+ minus TMSOH
217		TMSO—CH=CH—$\overset{+}{C}$H—OTMS
204		TMSO—$\overset{+}{C}$H—$\dot{C}$H—OTMS
186		TMSO—CH=CH—$\overset{+}{C}$H—$NHCOCH_3$
173		TMSO—$\overset{+}{C}$H—$\dot{C}$H—$NHCOCH_3$

Gl = Glycose unit GlNAc = 2-acetamido-2-deoxy-glycose unit

kov *et al.*,[9] it is still uncertain, whether the absence of this ion has to be explained by a difficult bond cleavage or by a stagnant OTMS migration. An alternative criterion for the (1 → 6) bonded compounds may be the peak at *m/e* 743 (brutoformula $C_{29}H_{69}NO_9Si_6$), which was only observed in the spectra of VI and VII.

To discriminate between the (1 → 2), (1 → 3) and (1 → 4) linkages in the 2-acetamido-2-deoxy-aldohexosyl-aldohexoses no use can be made of the ratios of the peak

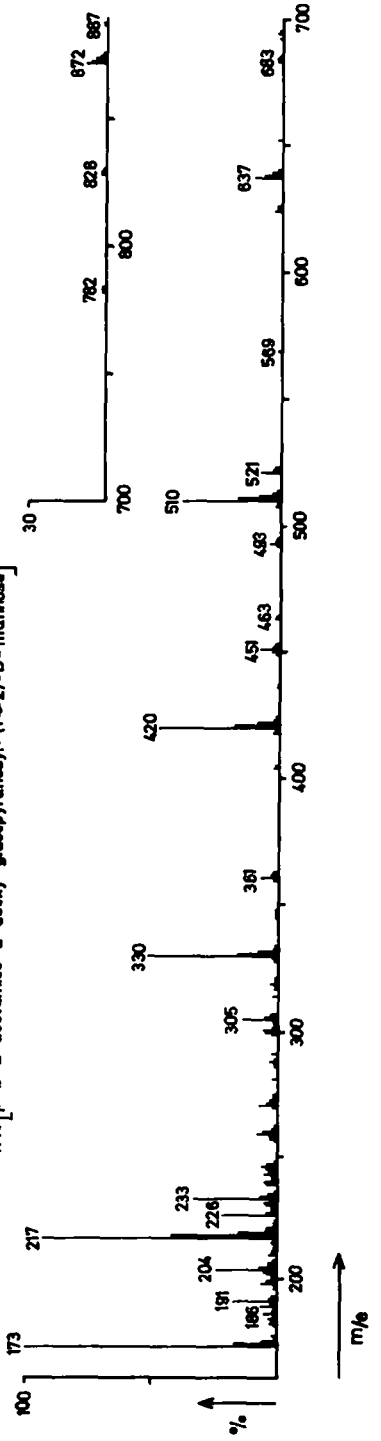

FIG. 1 Mass spectrum of TMS(β-D-GNAcp-(1 → 2)-D-Man). Only values > m/e 173 are given

FIG. 2 Mass spectrum of TMS(β-D-GalNAcp-(1 → 3)-D-Gal). Only values > m/e 173 are given

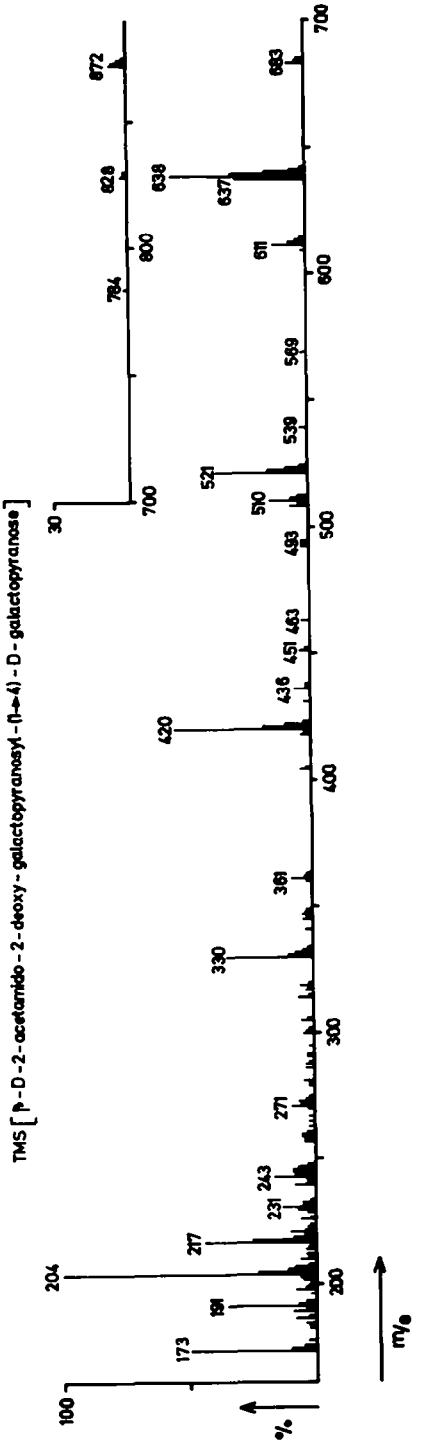

FIG. 3 Mass spectrum of TMS(β-D-GalNAcp-(1 → 4)-D-Galp). Only values > *m/e* 173 are given

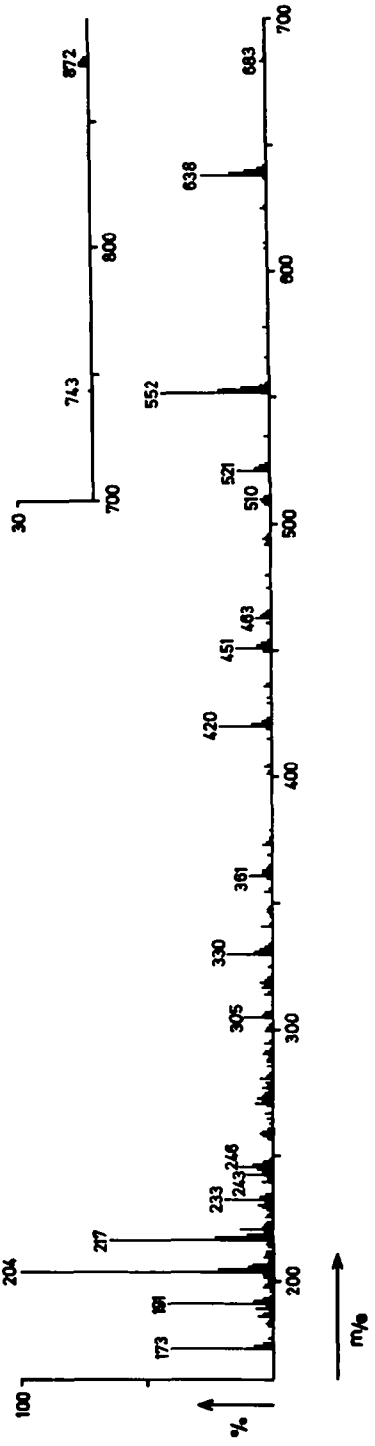

FIG. 4 Mass spectrum of TMS(β-D-GalNAcp-(1 → 6)-D-Gal). Only values > *m/e* 173 are given

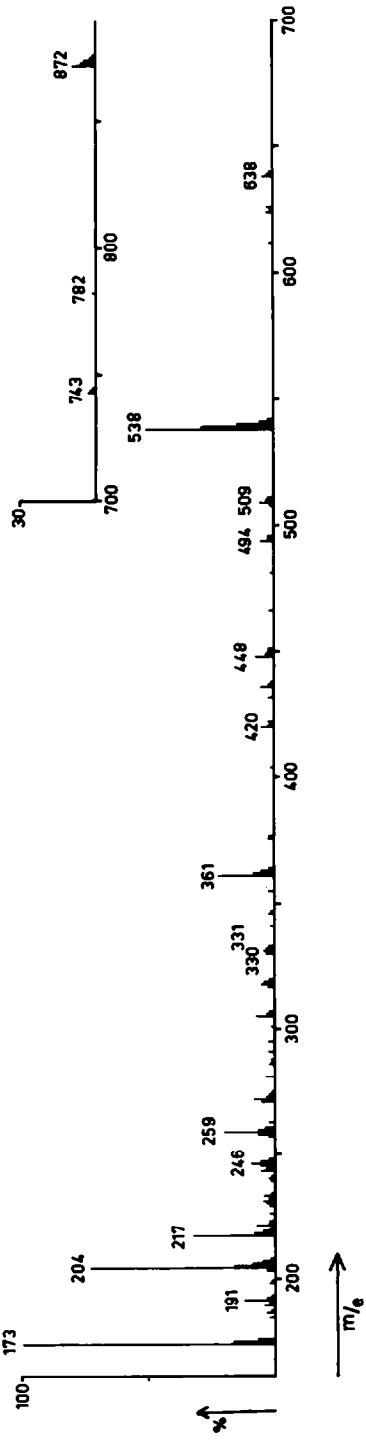

FIG. 5 Mass spectrum of TMS(α-D-Glc*p*-(1 → 6)-D-GNAc). Only values > *m/e* 173 are given

TABLE C. RATIOS OF PEAKINTENSITIES USED IN THIS STUDY

$\frac{m}{e}$ / $\frac{m}{e}$	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
509/508	0·7	1·6	0·6	0·7	0·6	1·2
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

TABLE D. RELATIVE INTENSITIES OF SOME FRAGMENT IONS, USED FOR THE DETERMINATION OF THE MONOMER SEQUENCE

	Type of glycosidic linkage						
	1 → 2	1 → 3	1 → 4			1 → 6	1 → 6
	I*	II‡	III†	IV†	V*	VI†	VII*
330	52	55	34	32	18	18	3
420	48	100	54	47	22	21	5
330 + 420	100	155	88	79	40	39	8
361	7	12	8	7	7	9	22
451	8	6	4	9	4	14	2
361 + 451	15	18	12	16	11	23	24

* *m/e* 173 = 100%
† *m/e* 204 = 100%
‡ *m/e* 420 = 100%

intensities 569/508 and 569/637 (comparable to the ratios 569/539 and 569/668 in aldohexosyl-aldohexoses), because the formation of the fragment at *m/e* 569 appeared to be strongly hindered in these disaccharides.[9] In the (1 → 2) and (1 → 4) compounds the intensity of *m/e* 569 was very low and in the (1 → 3) [and (1 → 6)] compounds this ion was not observable. The splitting of the C1—C2 bond and/or the migration of an OTMS group may be more difficult than in aldohexosyl-aldohexoses (for the fragmentation scheme see Kochetkov *et al.*[9]). An alternative criterion was found in the ratio of the intensities of the peaks at *m/e* 217 and *m/e* 204; for the (1 → 4) and (1 → 6) compounds 217/204 is smaller than unity and for the (1 → 2) and (1 → 3) compounds this ratio is greater than unity (Table C). The (1 → 6) component can be distinguished from the (1 → 4) compounds by the presence of a strong peak at *m/e* 552. For the (1 → 2) and (1 → 3) compounds the ratios of some peak intensities were calculated viz. 510/508, 509/508, 463/330 and 637/330 (Table C), which show significant differences. These ratios may be useful for assignments of the type of glycosidic link, although the constituting monosaccharides may display a distinct influence.[10] The investigation of more reference compounds including those bonded via a (1 → 1) and (1 → 5) link is necessary to estimate which structural parameters determine these ratios.

DISCUSSION

The replacement of an OH function in an aldohexosyl-aldohexose on the 2 or 2' position by an acetamido group gives an alteration of the fragmentation pattern of the TMS-derivatives. As a consequence of the low intensity of the fragment at *m/e* 569 in the (1 → 2), (1 → 3) and (1 → 4) disaccharides I to V and the absence of the ion at *m/e* 583 in compound VII, the rules developed for the determination of the position of the glycosidic bond in aldohexosyl-aldohexoses had to be modified. In the reducing aldohexosyl-aldohexoses the ratio of the intensities of the peaks at *m/e* 217 and *m/e* 204 (217/204) is always < 1.[1] These results show that in the (1 → 2) and (1 → 3) connected disaccharides containing a 2-acetamido-2-deoxy-glycose as non-reducing unit, the ratio 217/204 is > 1, whereas in the (1 → 4) and (1 → 6) linked components this ratio is < 1. It is not possible to correlate this change in ratio with an alteration in the intensity of the ion at *m/e* 204 or that at *m/e* 217 only. Apparently, the formation of these ions is influenced by the position of the glycosidic bond. In 2-acetamido-2-deoxy-aldohexose containing disaccharides, the intensity of the fragment at *m/e* 204 will be lower than in aldohexosyl-aldohexoses, because C2—C3 of the 2-acetamido-2-deoxy-glycose moiety of the molecule does not contribute to the intensity of this ion. In TMS-monosaccharides it has been established that the greatest contribution to the formation of the ion at *m/e* 204 stems from C2—C3.[11]

The presence of the fragment ion at *m/e* 638 ($C_{26}H_{60}NO_7Si_5$) constitutes another difference from the spectra of the TMS-aldohexosyl-aldohexoses. The intensity of the ion at *m/e* 638 in relation to that at *m/e* 637 may be useful in the determination of the position of the glycosidic bond, as the following regularity in intensities was observed (Figs 1 to 5):

(1 → 2)	*m/e* 638 < *m/e* 637
(1 → 3)	*m/e* 638 ≈ *m/e* 637
(1 → 4)	*m/e* 638 > *m/e* 637
(1 → 6)	*m/e* 638 ≫ *m/e* 637

In none of the cases the intensity of *m/e* 638 was corrected for the isotopic contribution of *m/e* 637 to this intensity.

The fragment at *m/e* 510 (brutoformula $C_{20}H_{48}NO_6Si_4$; 509 plus 1H) was present in all mass spectra although in various intensities (corrected for the isotopic contributions of the peaks at *m/e* 508 and *m/e* 509). The ion at *m/e* 509 (brutoformula $C_{20}H_{47}NO_6Si_4$) occurred definitely in the mass spectra of the compounds II, VI and VII besides the isotope peak of *m/e* 508. The analogue of the fragment at *m/e* 509 in the TMS-aldohexosyl-aldohexoses, *m/e* 540, was only detected in the non-reducing trehaloses,[1] whereas the analogue of *m/e* 510, *m/e* 541, was observable in the aldohexosyl-aldopentose β-D-*Gp*-(1 → 2)-L-Ara.[1]

The following ions were present in all mass spectra of the 2-acetamido-2-deoxy-aldohexosyl-aldohexoses (I to VI):

m/e 463 (brutoformula $C_{19}H_{45}NO_4Si_4$): A structure for this fragment ion, based on exact mass measurement and on the presence of this ion in the (1 → 2), (1 → 3), (1 → 4) as well as the (1 → 6) compounds, is given in Fig. 6. From the reducing moiety a TMSO—CH=O molecule is eliminated,[8] while in the non-reducing end a migration of a TMS group from an O to a N atom is suggested.

$$TMS{-}NH{-}\dot{C}H{-}\overset{+}{C}H{-}O{-}R \qquad m/e\,463$$

R = reducing moiety minus TMSO—CH=O

FIG. 6

m/e 683 (brutoformula $C_{27}H_{61}NO_9Si_5$): Evidently, these compounds eliminate readily TMSO—CH=CH—OTMS. Also from the mass spectra of TMS-2-acetamido-2-deoxy-D-galactose published by Kärkkäinen[7] and DeJongh *et al.*[8] it can be inferred that this elimination takes place, resulting in the fragment ion at *m/e* 305. In the mass spectra of the aldohexosyl-aldohexoses, this reaction was not observed, nor in the spectrum of compound VII. It can be concluded that in the 2-acetamido-2-deoxy-aldohexosyl-aldohexoses the eliminated molecule must originate from C3—C4 of the non-reducing site.

m/e 637 (brutoformula $C_{26}H_{59}NO_7Si_5$): This fragment is the analogue of *m/e* 668 in TMS-aldohexosyl-aldohexoses. The intensity of the peak is low in the (1 → 3) disaccharide, compared with the (1 → 2) or (1 → 4) disaccharides (see also Kamerling *et al.*[1]). A possible second explanation is given in Table B.

In this study we have shown that in the compounds I to VII the sequence of the monomers and the position of the glycosidic bond can be determined. However, extension of the work with other examples of these disaccharides is necessary.

EXPERIMENTAL

The trimethylsilyl derivatives were synthesized as described earlier.[12] The carbohydrates used in this study were gifts from various investigators (see acknowledgements). The 70 eV-mass spectra were recorded at an MS-9 mass spectrometer (AEI) at an ion chamber temperature of 120–140°.

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