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MASS SPECTROMETRY OF PERTRIMETHYLSILYL NEURAMINIC ACID DERIVATIVES*

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ABSTRACT

The mass spectra of the trimethylsilyl (TMS) derivatives of the methyl and trideuteriomethyl esters of *N*-acetylneuraminic acid, the methyl ester of *N*-glycolylneuraminic acid, the methyl ester methyl β-glycoside of *N*-acetylneuraminic acid, the trideuteriomethyl ester trideuteriomethyl β-glycoside of *N*-acetylneuraminic acid, and the methyl esters of the (2→3)- and (2→6)-linked isomers of *N*-acetylneuraminic acid–lactose are discussed The characteristic fragmentation patterns of the sialic acid derivatives can be used for the identification of this type of carbohydrate The (2→3)- and (2→6)-linked isomers of *N*-acetylneuraminic acid–lactose can be differentiated

INTRODUCTION

Previously, we reported on the applicability of mass spectrometry of pertrimethylsilyl (TMS) derivatives of several classes of oligosaccharides, namely, aldosyl disaccharides[1], disaccharides consisting of an aldohexose and a 2-acetamido-2-deoxyaldohexose[2], and di- and higher saccharides consisting of aldohexoses and hexuloses[3] The importance of sialic acids in such biopolymers as glycoproteins and glycolipids prompted an extension of these studies to neuraminic acid derivatives Sweeley *et al* have reported on the mass spectrometry of sialoglycosphingolipids[4,5] and the TMS derivative of the methyl ester methyl glycoside of neuraminic acid[6] Recently, Kochetkov *et al* [7] described the mass spectrometry of peracetyl derivatives of some sialic acids We now report on the mass spectrometry of the TMS derivatives of the methyl (**1a**) and trideuteriomethyl (**1b**) esters of *N*-acetylneuraminic acid, the methyl ester of *N*-glycolylneuraminic acid (**2**), the methyl ester methyl β-glycoside of *N*-acetylneuraminic acid (**3a**), the trideuteriomethyl ester trideuteriomethyl β-glycoside of *N*-acetylneuraminic acid (**3b**), and the methyl ester of *N*-acetylneuraminic acid-α-(2→3)- (**4**) and α-(2→6)-lactose (**5**)

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RESULTS AND DISCUSSION

Sialic acids In the mass spectra of **1a**, **1b**, **2**, **3a**, and **3b**, the peak for the molecular ion (M) was very weak The first, detectable fragment ion with reasonably high intensity in the high-mass range was $(M-CH_3)$ Tables I–III contain details of fragment ions given by **1**, **2**, and **3**, based on exact mass measurements and/or relative shifts in mass

TABLE I

INTERPRETATION OF SOME IMPORTANT FRAGMENT IONS, PRESENT IN THE MASS SPECTRA OF THE TMS DERIVATIVES OF THE METHYL (**1a**) AND TRIDEUTERIOMETHYL (**1b**) ESTERS OF *N*-ACETYLNEURAMINIC ACID

m/e[a]		*Formula*	*Fragment*	m/e[b]	
668	(44 2)	$C_{26}H_{58}NO_9Si_5$	$M-CH_3$	671	(32 2)
640	(6 1)	$C_{25}H_{58}NO_8Si_5$	M−Ac	643	(4 7)
624	(27 7)	$C_{25}H_{58}NO_7Si_5$	$M-COOCH_3$	624	(20 9)
593	(11 5)	$C_{24}H_{51}NO_8Si_4$	M−TMSOH	596	(9 5)
580	(5 0)		$M-CH_2OTMS$	583	(4 3)
578	(10 0)	$C_{23}H_{48}NO_8Si_4$	668−TMSOH (m* = 500 1)	581	(7 4)
552	(6 2)	$C_{22}H_{50}NO_7Si_4$	$M-OTMS-CH_2=C=O$	555	(4 5)
534	(5 4)	$C_{22}H_{46}O_7Si_4$	$M-TMSOH-NH_2Ac$	537	(3 4)
			$M-TMSOH-COOCH_3$	534	(0 8)
503	(3 1)		M−TMSOH−TMSOH	506	(2 3)
492	(7 7)		$M-CH_2-C(OH)(COOCH_3)(OTMS)$	492	(5 4)
			$M-COOCH_3-TMSOH-CH_2=C=O$		
490	(14 2)	$C_{20}H_{40}NO_7Si_3$	$M-CH_2OTMS-TMSOH$	493	(14 6)
488	(3 5)		$M-CH_3-TMSOH-TMSOH$	491	(3 4)
478	(80 8)	$C_{19}H_{40}NO_7Si_3$	$M-CHOTMS-CH_2OTMS$	481	(66 2)
462	(3 8)		$M-OTMS-CH_2=C=O-TMSOH$	465	(2 9)
449	(14 6)	$C_{18}H_{39}NO_6Si_3$	$M-CH_2OTMS-CH_2=C=O-OTMS$	452	(14 0)
431	(21 1)	$C_{18}H_{35}O_6Si_3$	$M-CH_2OTMS-TMSOH-NH_2Ac$	434	(17 1)
400	(36 9)	$C_{17}H_{30}NO_6Si_2$	490−TMSOH (m* = 326 5)	403	(29 3)
388	(10 8)	$C_{16}H_{30}NO_6Si_2$	478−TMSOH (m* = 314 9)	391	(8 6)
376	(5 4)	$C_{15}H_{30}NO_6Si_2$	M−CHOTMS−CHOTMS−CH_2OTMS	379	(8 1)
358	(10 0)	$C_{15}H_{28}NO_5Si_2$	$M-CH_2OTMS-TMSOH-TMSOH-CH_2=C=O$	361	(11 7)
330	(13 5)	$C_{14}H_{28}NO_4Si_2$	No reasonable structure can be proposed	333	(12 6)
317	(48 8)	$C_{13}H_{25}O_5Si_2$	$M-CHOTMS-CHOTMS-CH_2OTMS-NH_2Ac$	320	(46 4)
300	(75 8)	$C_{13}H_{26}NO_3Si_2$	$Ac-\overset{+}{N}H=CH-C(OTMS)=CH-CH=CHOTMS$	300	(65 8)
298	(100 0)	$C_{13}H_{20}NO_5Si$	$M-CHOTMS-CH_2OTMS-TMSOH-TMSOH$	301	(100 0)
227	(7 7)	$C_{10}H_{15}O_4Si$	M−CHOTMS−CHOTMS− $CH_2OTMS-NH_2Ac-TMSOH$	230	(9 9)
217	(30 0)	$C_9H_{21}O_2Si_2$	$TMSO-CH=CH-CH=\overset{+}{O}TMS$	217	(32 4)
205	(18 8)	$C_8H_{21}O_2Si_2$	$TMS\overset{+}{O}=CH-CH_2OTMS$	205	(18 7)
204	(18 5)	$C_8H_{20}O_2Si_2$	$TMS\overset{+}{O}=CH-\dot{C}H-OTMS$	204	(18 7)
186	(21 5)	$C_8H_{16}NO_2Si$	$AcNH-CH=CH-CH=\overset{+}{O}TMS$	186	(25 9)
173	(16 2)	$C_7H_{15}NO_2Si$	$AcNH-\dot{C}H-CH=\overset{+}{O}TMS$	173	(21 4)

[a]Compound **1a**; the intensities of the ions, relative to that of *m/e* 298, are given in brackets [b]Compound **1b**, the intensities of the ions, relative to that of *m/e* 301, are given in brackets

The ion at *m/e* 624 for **1a** could be $(M-COOCH_3)$ or $(M-NH_2COCH_3)$, exact mass measurements support the former structure The mass spectrum of **1b** shows a distinct peak at *m/e* 624 $(M-COOCD_3)$ Calculation of the intensities of the isotope peaks of *m/e* 624 did not indicate the presence of a contribution to the peak at *m/e* 627 other than the isotope peak for *m/e* 624 Examination of the mass spectra of **2** and **3** suggests that, in addition to a $COOCH_3$ radical, a NH_2COR_3 ($R_3 = CH_3$ or CH_2OTMS) molecule can be eliminated *via* a McLafferty rearrangement The mass spectrum of **2** shows a relatively intense peak at *m/e* 712

TABLE II

INTERPRETATION OF SOME IMPORTANT FRAGMENT IONS, PRESENT IN THE MASS SPECTRUM OF THE TMS DERIVATIVE OF THE METHYL ESTER OF *N*-GLYCOLYLNEURAMINIC ACID (2)

m/e[a]	*Formula*	*Fragment*
756 (23 5)	$C_{29}H_{66}NO_{10}Si_6$	$M-CH_3$
712 (19 7)	$C_{28}H_{66}NO_8Si_6$	$M-COOCH_3$
681 (4 1)	$C_{27}H_{59}NO_9Si_5$	M−TMSOH
668 (6 7)	$C_{26}H_{58}NO_9Si_5$	$M-CH_2OTMS$
666 (4 3)		756−TMSOH (m* = 586 7)
640 (5 8)	$C_{25}H_{58}NO_8Si_5$	$M-COCH_2OTMS$
624 (1 6)		$M-NH_2COCH_2OTMS$
591 (2 2)		M−TMSOH−TMSOH
580 (5 4)		$M-CH_2-C(OH)(COOCH_3)(OTMS)$
578 (9 0)	$C_{23}H_{48}NO_8Si_4$	$M-CH_2OTMS-TMSOH$
576 (2 2)		$M-CH_3-TMSOH-TMSOH$
566 (54 7)	$C_{22}H_{48}NO_8Si_4$	$M-CHOTMS-CH_2OTMS$
537 (11 0)	$C_{21}H_{47}NO_7Si_4$	$M-COCH_2OTMS-CH_2OTMS$
534 (3 2)		$M-TMSOH-NH_2COCH_2OTMS$
501 (3 2)		M−TMSOH−TMSOH−TMSOH
488 (27 1)	$C_{20}H_{38}NO_7Si_3$	578−TMSOH (m* = 412 0)
476 (6 8)	$C_{19}H_{38}NO_7Si_3$	566−TMSOH (m* = 400 3)
464 (4 2)	$C_{18}H_{38}NO_7Si_3$	$M-CHOTMS-CHOTMS-CH_2OTMS$
431 (10 4)	$C_{18}H_{35}O_6Si_3$	$M-CH_2OTMS-TMSOH-NH_2COCH_2OTMS$
418 (10 1)	$C_{17}H_{36}NO_5Si_3$	No reasonable structure can be proposed
388 (100 0)	$C_{16}H_{34}NO_4Si_3$	$TMSOCH_2CO-\overset{+}{N}H=CH-C(OTMS)=CH-CH=CHOTMS$
386 (58 7)	$C_{16}H_{28}NO_6Si_2$	476−TMSOH (m* = 313 0)
374 (7 1)	$C_{15}H_{28}NO_6Si_2$	$M-CHOTMS-CHOTMS-CH_2OTMS-TMSOH$
317 (26 7)	$C_{13}H_{25}O_5Si_2$	$M-CHOTMS-CHOTMS-CH_2OTMS-NH_2COCH_2OTMS$
274 (23 2)	$C_{11}H_{24}NO_3Si_2$	$TMSOCH_2CONH-CH=CH-CH=\overset{+}{O}TMS$
261 (13 3)	$C_{10}H_{23}NO_3Si_2$	$TMSOCH_2CONH-\dot{C}H-CH=\overset{+}{O}TMS$
227 (8 7)	$C_{10}H_{15}O_4Si$	$M-CHOTMS-CHOTMS-CH_2OTMS-NH_2COCH_2OTMS-TMSOH$
217 (27.4)	$C_9H_{21}O_2Si_2$	$TMSO-CH=CH-CH=\overset{+}{O}TMS$
205 (21 3)	$C_8H_{21}O_2Si_2$	$TMS\overset{+}{O}=CH-CH_2OTMS$
204 (24 2)	$C_8H_{20}O_2Si_2$	$TMS\overset{+}{O}=CH-\dot{C}H-OTMS$

[a]The figures in brackets are the intensities of the ions, relative to that of *m/e* 388

($M-COOCH_3$) and a peak of low intensity at *m/e* 624 ($M-NH_2COCH_2OTMS$) [I(624) I(712) = 1 12] In the mass spectrum of **3a**, a peak at *m/e* 566 ($M-COOCH_3$ and $M-NH_2COCH_3$) is observed, which is mainly shifted to *m/e* 569 in the spectrum of **3b** Calculation of the intensities of the isotope peaks of *m/e* 569 indicated the

TABLE III

INTERPRETATION OF SOME IMPORTANT FRAGMENT IONS, PRESENT IN THE MASS SPECTRA OF THE TMS DERIVATIVES OF THE TRIDEUTERIOMETHYL ESTER TRIDEUTERIOMETHYL GLYCOSIDE OF *N*-ACETYLNEURAMINIC ACID AND OF THE METHYL ESTER METHYL GLYCOSIDE (**3a**)

m/e[a]	*Fragment*	m/e[b]
610 (29 5)	$M-CH_3$	616 (28 3)
594 (1.7)	$M-OCH_3$	597 (1 7)
578 (2 3)	$610-CH_3OH$ (m* = 547 7)	581 (1 7)
566 (22 7)	$M-COOCH_3$	569 (21 9)
	$M-NH_2Ac$	572 (3 0)
535 (13 5)	M−TMSOH	541 (25 3)
522 (3 5)	$M-CH_2OTMS$	528 (3 9)
520 (4 3)	610−TMSOH (m* = 443 3)	526 (4 3)
504 (3 1)	$M-OCH_3$−TMSOH	507 (3 0)
494 (5 7)	M−OTMS−CH_2=C=O	500 (5 2)
490 (2 1)	$M-CH_2OTMS-CH_3OH$	493 (1 9)
488 (1 7)	$M-CH_3$−TMSOH−CH_3OH	491 (1 5)
476 (11 5)	$535-NH_2Ac$ (m* = 423 5)	482 (11 2)
	M−TMSOH−$COOCH_3$	479 (1 5)
462 (2 3)	$M-OCH_3-CH_2$=C=O−TMSOH	465 (6 0)
445 (1 8)	M−TMSOH−TMSOH	451 (3 2)
434 (5 5)	$M-COOCH_3-CH_2$=C=O−TMSOH	437 (6 9)
432 (6 9)	$M-CH_2OTMS$−TMSOH	438 (15 5)
420 (70 5)	M−CHOTMS–CH_2OTMS	426 (80 3)
391 (10 1)	$M-CH_2OTMS-CH_2$=C=O−OTMS	397 (12 0)
388 (8 1)	$420-CH_3OH$ (m* = 358 4)	391 (5 2)
373 (25 2)	$M-CH_2OTMS$−TMSOH−NH_2Ac	379 (30 0)
355 (5 2)	M−TMSOH−TMSOH−TMSOH	361 (8 6)
342 (26 9)	432−TMSOH (m* = 270 8)	348 (30 0)
318 (5 9)	M−CHOTMS–CHOTMS–CH_2OTMS	324 (7 1)
300 (69 8)	$Ac-\overset{+}{N}H=CH-C(OTMS)=CH-CH=CHOTMS$	300 (81 5)
298 (100 0)	M−CHOTMS–$CH_2OTMS-CH_3OH$−TMSOH	301 (100 0)
259 (35 2)	M−CHOTMS–CHOTMS–$CH_2OTMS-NH_2Ac$	265 (39 5)
227 (5 5)	M−CHOTMS–CHOTMS–$CH_2OTMS-NH_2Ac-CH_3OH$	230 (12 9)
217 (24 4)	$TMSO-CH=CH-CH=\overset{+}{O}TMS$	217 (39 1)
205 (13 5)	$TMS\overset{+}{O}=CH-CH_2OTMS$	205 (42 9)
204 (12 6)	$TMS\overset{+}{O}=CH-\dot{C}H-OTMS$	204 (32 8)
186 (21 5)	$AcNH-CH=CH-CH=\overset{+}{O}TMS$	186 (57 1)
173 (8 5)	$AcNH-\dot{C}H-CH=\overset{+}{O}TMS$	173 (20 6)

[a]Compound **3a**, the intensities of the ions, relative to that of *m/e* 298, are given in brackets [b]Compound **3b**, the intensities of the ions, relative to that of *m/e* 301, are given in brackets

presence of a small peak at *m/e* 572, besides the isotopic contribution to this peak [I(572) I(569) = 1 17] Similarly, it can be deduced that the peak at *m/e* 534 in the spectrum of **1a** is due mainly to (M−TMSOH−NH_2COCH_3); the spectrum of **1b** contains a small peak at *m/e* 534 and a more intense peak at *m/e* 537 [I(534) I(537) = 1 4] In the spectrum of **2**, a distinct peak at *m/e* 534 (M−TMSOH−NH_2COCH_2-OTMS) is observable, besides a peak of low intensity at *m/e* 622 (M−TMSOH−$COOCH_3$) [I(622) I(534) = 1 7] The spectrum of **3a** shows a peak at *m/e* 476, which is shifted mainly to *m/e* 482 but also partly to *m/e* 479 in the spectrum of **3b** [I(479) I(482) = 1 8]. These data, together with the presence of metastable peaks at 423 5 and 429 4 in the spectra of **3a** and **3b**, respectively, make it clear that (M−TMSOH) eliminates mainly NH_2COCH_3, ($m^* = 423\ 5\quad 535^+ \rightarrow 476^+ + NH_2$-$COCH_3$, $m^* = 429\ 4\quad 541^+ \rightarrow 482^+ + NH_2COCH_3$), in contrast to the molecular ion which eliminates mainly $COOCH_3$ From the different spectra, it can be deduced that (M−$COOCH_3$) is hardly degraded further However, the elimination of NH_2COCH_3 (or NH_2COCH_2OTMS) *via* a McLafferty rearrangement occurs frequently, probably mainly from fragment ions In Fig 1, the formation of (M−[CHOTMS–CHOTMS–CH_2OTMS]−NH_2COR_3−R_2OH) is shown.

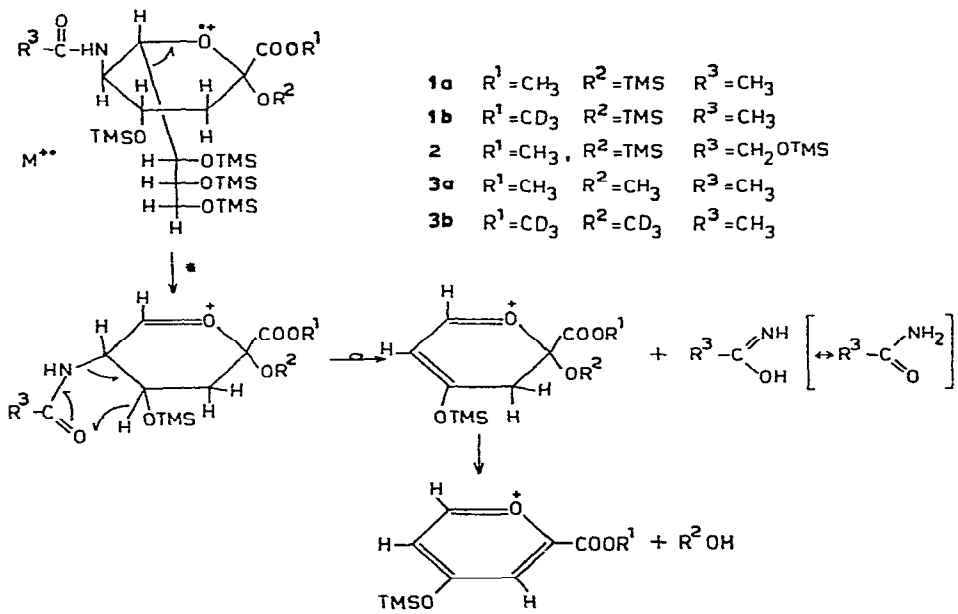

Fig 1 Formation of M−[CHOTMS–CHOTMS–CH_2OTMS]−NH_2COR_3−R_2OH

The neuraminic acid derivatives can eliminate CH_2OTMS (C-9), CHOTMS–CH_2OTMS (C-8,9), and CHOTMS–CHOTMS–CH_2OTMS (C-7,8,9), respectively. Because of the intensities of the peaks for (M−[CHOTMS–CH_2OTMS]) in each spectrum, the second fragmentation pathway appears to have a high preference

The peak at *m/e* 298, which is one of the most intense peaks in the mass spectra of **1a** and **3a**, is shifted 3 m u in the spectra of **1b** and **3b**, and 88 m u. in the spectrum of **2**. In Fig 2, fragmentation schemes are given for the different compounds It can be deduced from the spectra that the C-8,9 fragment is first eliminated from the molecular ion, followed by consecutive eliminations of R_2OH ($R_2 = CH_3$, CD_3, or TMS) in which OR_2 originates from C-2, and TMSOH in which OTMS originates from C-4. Furthermore, it can be concluded that the elimination of the C-9-fragment from M is combined with the elimination of R_2OH ($R_2 = CH_3$, CD_3, or TMS) in which OR_2 originates from C-2, or with a TMSOH elimination in which OTMS originates from C-4 or C-7

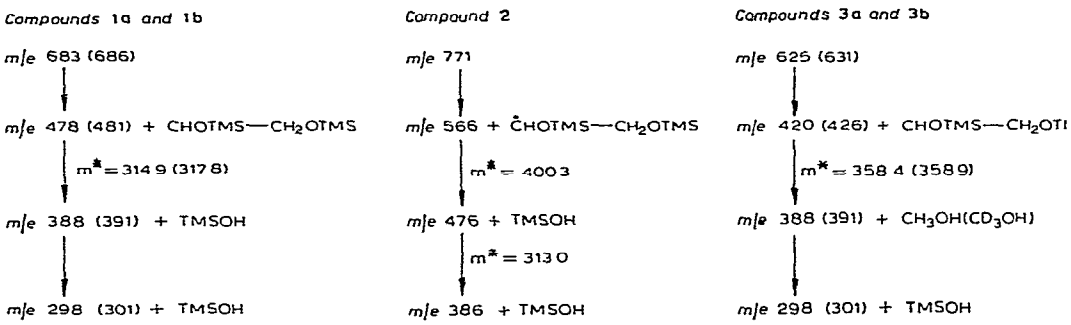

Fig 2 Formation of *m/e* 298 from **1a** and **3a**, *m/e* 301 from **1b** and **3b**, and *m/e* 386 from **2**

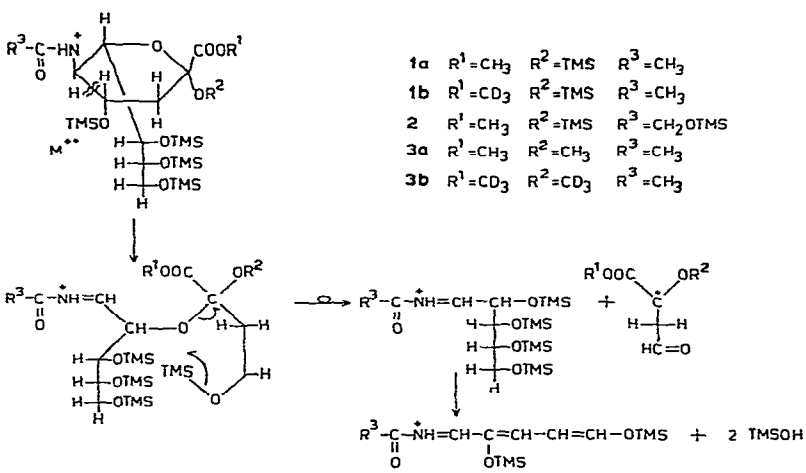

Fig 3. Formation of *m/e* 300 from **1** and **3**, and *m/e* 388 from **2**

The fragment ion at *m/e* 300, which is the second most-intense peak in the spectra of **1a**, **1b**, **3a**, and **3b**, is shifted to *m/e* 388 in the spectrum of **2**. It does not contain the substituents at C-2 ($COOR_1$ with $R_1 = CH_3$ or CD_3, OR_2 with $R_2 = CH_3$,

CD_3, or TMS) From metastable measurements, using a defocusing technique according to Barber and Elliott, these fragment ions are identified as {M−TMSOH−TMSOH−[O=CH–CH_2–C($COOR_1$)OR_2]} A fragmentation scheme is presented in Fig 3

The origin of the peaks at *m/e* 204 and 217 is quite different from that in the spectra of the TMS-aldohexoses[8] The fragment at *m/e* 217 can be formed from the side chain C-7,8,9 by elimination of TMSOH from *m/e* 307 (CH_2OTMS–CHOTMS–CH=$\overset{+}{O}$TMS → TMSOCH=CH–CH=$\overset{+}{O}$TMS + TMSOH) The peak at *m/e* 204 can be formed from the side chain by loss of a CH_2OTMS radical (CH_2OTMS–CHOTMS–CH=$\overset{+}{O}$TMS → TMSO–CH–CH=$\overset{+}{O}$TMS + ·CH_2OTMS) and then it comprises the fragment C-7,8 The peaks at *m/e* 173 and 186 in the mass spectra of **1** and **3** contain the *N*-acetyl group and are shifted 88 m u in the spectrum of **2** to *m/e* 261 and 274, respectively (*N*-glycolyl group) If no migrations take place, the fragment ion at *m/e* 173 contains C-4,5 (Ac–NH–$\dot{C}$H–CH=$\overset{+}{O}$TMS) Fig 4 shows the formation of the fragment *m/e* 186, containing C-5,6,7 (Ac–NH–CH=CH–CH=$\overset{+}{O}$TMS) However, Dawson *et al*[5] suggest that this ion has the structure C-3,4,5 (Ac–NH–$\overset{+}{C}$H–C(OTMS)=CH_2) The formation of this ion is possible only if a hydrogen transfer from C-4 is assumed Labelling on C-4 and C-7 can discriminate between these alternatives

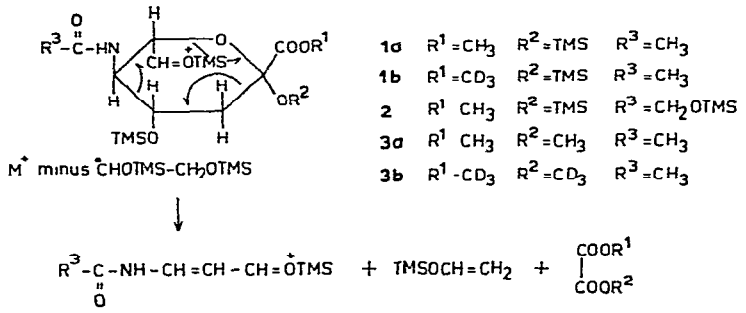

Fig 4 Formation of *m/e* 186 from **1** and **3**, and *m/e* 274 from **2**

Trisaccharides consisting of sialic acid and lactose The mass spectra of the TMS derivatives of the methyl esters of *N*-acetylneuraminic acid-α-(2→3)- (**4**) and α-(2→6)-lactose (**5**) show a great similarity. Some significant peaks are given in Table IV A molecular ion peak (*m/e* 1439) was observed in each spectrum The peaks at *m/e* 1424, 1380, and 1234 are assigned to (M−CH_3), (M−$COOCH_3$ or NH_2-$COCH_3$), and (M−[CH_2OTMS–CHOTMS]), respectively. The mass spectrum of the (2→6)-isomer shows a peak at *m/e* 726 ($C_{29}H_{64}NO_{10}Si_5$), which is the analogue of the peak at *m/e* 583 in TMS-aldohexosyl-(1→6)-aldohexoses[1] The (2→3)-isomer does not yield this fragment ion

TABLE IV

INTENSITIES OF SOME SIGNIFICANT PEAKS IN THE MASS SPECTRA OF THE (2→3)- AND (2→6)-LINKED ISOMERS OF *N*-ACETYLNEURAMINIC ACID METHYL ESTER–LACTOSE IN PERCENTAGES RELATIVE TO THE INTENSITY OF THE PEAK AT *m/e* 204

m/e	*2→3*	*2→6*	m/e	*2→3*	*2→6*
1439	0 6	0 7	504	31 9	13 8
1424	2 1	1 7	451	1 8	1 6
1380	1 8	1 8	361	15 1	7 1
1234	5 8	2 9	300	12 5	14 6
972	4 6	2 5	298	9 5	8 3
726	0 6	3 1	217	64 3	57 8
683	0 6	3 3	204	100 0	100 0
624	7 0	7 9	186	24 2	19 3
595	12 1	8 9	173	12 1	7 5
594	22 4	8 9			

In the spectrum of the (2→6)-isomer, there is also a peak at *m/e* 683 ($C_{27}H_{63}O_8Si_6$) In such aldohexosylaldohexoses as lactose, the main contribution to the peak at *m/e* 683 stems from the reducing end of the molecule[1] and can be attributed to TMSO–CH=C(OTMS)–CH=$\overset{+}{O}$–Glycose In the (2→3)-isomer, C-3 of the galactose residue is attached to the sialic acid residue Therefore, in the latter isomer, this fragment cannot easily be formed Each mass spectrum shows a relatively intense peak at *m/e* 594 ($C_{24}H_{52}NO_8Si_4$) Moreover, in the spectrum of the (2→6)-isomer, a peak at *m/e* 595 ($C_{24}H_{55}O_7Si_5$) was present in addition to the isotope peak of *m/e* 594 The observed peak-intensity ratio 595/594 is 0 54 for the (2→3)-isomer, and 1 00 for the (2→6)-isomer, whereas the calculated ratio is 0 49 The structure of the fragment ion with *m/e* 595 is TMSO–CH=CH–CH=$\overset{+}{O}$–Glycose The attachment of sialic acid at C-3 of the galactose residue in the (2→3)-isomer hinders the formation of this ion

Cleavage of the glycosidic bond between C-1 of the galactose residue and the glycosidic oxygen atom, with retention of the charge at the galactose residue, leads to the fragment ion at *m/e* 972, and cleavage between C-2 of the neuraminic acid residue and the glycosidic oxygen atom, with retention of the charge at the neuraminic acid residue, leads to *m/e* 594 ($C_{24}H_{52}NO_8Si_4$) and 504 ($C_{21}H_{42}NO_7Si_3$, 594−TMSOH) Splitting of the glycosidic linkage between the glycosidic oxygen atom and C-4 of the glucose residue, with retention of the charge at the glucose residue, gives rise to peaks at *m/e* 451 and 361 ($C_{15}H_{33}O_4Si_3$, 451−TMSOH)

Previously, we postulated that, in oligosaccharides, migration of an OTMS group occurs between saccharide units[1–3]. For a disaccharide, a pertrimethylsilyl monosaccharide is formed as intermediate To explain the peak at *m/e* 624 ($C_{25}H_{58}NO_7Si_5$), migration of an OTMS group to C-2 of the neuraminic acid residue is assumed, resulting in the formation of the TMS derivative of the methyl ester of *N*-acetylneuraminic acid as an intermediate from which a $COOCH_3$ radical is eliminated

In comparison with the spectra of the TMS derivatives of the methyl ester of *N*-acetylneuraminic acid and its methyl glycoside, the intensities of the peaks at *m/e* 300 and 298 in the spectra of the trisaccharides are strongly diminished However, the peaks at *m/e* 204 and 217 are amongst the most intense peaks in the spectra, owing to the presence of the lactose molecule in the trisaccharides The occurrence of the peaks at *m/e* 186 and 173 can be used for the identification of an *N*-acetyl group in the neuraminic acid residue

The characteristic fragmentation patterns of the TMS derivatives of the methyl esters of the *N*-acylneuraminic acids and their methyl glycosides indicate that mass spectrometry may be of great help in the identification of sialic acids. For oligosaccharides containing a sialic acid residue attached to lactose, it has been shown that the (2→3)- and (2→6)-linked isomers can be distinguished

EXPERIMENTAL

N-Acetylneuraminic acid was purchased from Calbiochem, *N*-glycolylneuraminic acid and *N*-acetylneuraminic acid-α-(2→3)-lactose were obtained from Sigma Chemical Company, and *N*-acetylneuraminic acid-α-(2→6)-lactose was a gift of Dr A Gauhe (Heidelberg) *N*-Glycolylneuraminic acid was contaminated with *N*-acetylneuraminic acid Before use, it was purified by preparative chromatography on Whatman No 3MM paper, using butyl acetate–acetic acid–water[9] (3 2 1), detection was effected with Ehrlich's reagent[10] The trideuteriomethyl ester of *N*-acetylneuraminic acid and its trideuteriomethyl glycoside were prepared according to procedures described by Yu and Ledeen[11] The methyl ester of *N*-glycolylneuraminic acid was prepared by the method of Sweeley *et al* [12], which could also be used for the preparation of the methyl ester of *N*-acetylneuraminic acid The methyl esters of the trisaccharides were obtained by the method of Derevitskaya *et al* [13] The pertrimethylsilyl derivatives of the different compounds were synthesised as described earlier[1] If necessary, the TMS derivatives were purified by preparative g l c (Pye 105 gas chromatograph, 10% of OV-17 on Chromosorb W-AW-DMCS, 30–60 mesh)

The 70-eV mass spectra were recorded on A E I MS-9 and MS-902 mass spectrometers at an ion-chamber temperature of 80–100° for the sialic acid, and 120–140° for the trisaccharides containing sialic acid High-resolution, mass measurements were performed at a scan speed of 16 sec per mass decade by using an A E I MS-902 mass spectrometer connected on-line with a Ferranti Argus 500 computer The exact masses measured were converted into element lists as described by Van't Klooster *et al* [14] The molecular formulae omitted from Tables I and II could not be determined because of the low intensity of the associated peaks

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