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MASS SPECTROMETRY OF SIALIC ACIDS

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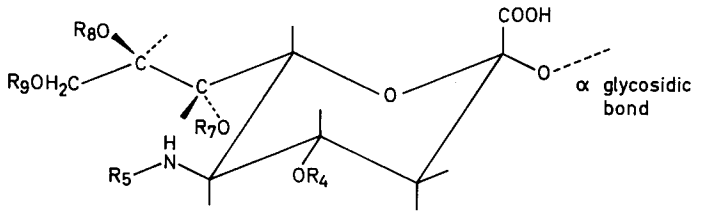
INTRODUCTION

Acyl derivatives of neuraminic acid (5-amino-3,5-dideoxy-D-glycero-D-galactononulosonic acid) are widespread in animals as well as in microorganisms. The amino function is acetylated or glycolylated (Fig. 1) (1). The hydroxyl functions can be acetylated (2), glycolylated (3), lactylated (4,5), methylated (6-8) or sulphated (9). The labile neuraminic acid as such does not occur in nature. In literature the term "sialic acids" is used to comprise the family of naturally occurring neuraminic acid derivatives.

In glycoproteins and glycolipids acylneuraminic acid residues occur generally in terminal positions of the carbohydrate chain, coupled via α-glycosidic linkages (Fig. 2) (1, 10). Sometimes, small oligomeric chains of acylneuraminic acid residues are attached to the carbohydrate backbone of these molecules (Fig. 3) (11, 12). In a number of vital processes sialic acids play a decisive role, e.g. (a) increase in the viscosity of glycoproteins in aqueous solutions, (b) binding and transport of molecules and viruses, (c) effects on surface charge, aggregation and shape of cells, (d) protection of glycoproteins against proteolytic attack, (e) regulation of the life time of cells, and (f) masking of cellular antigens (12).

Sialic acids also occur as constituents of homo- and heteropolysaccharides, isolated from Escherichia coli (colominic acid) and different strains of Neisseria meningitidis (Fig. 4) (13, 14).

O-Acylated N-acylneuraminic acids can be split off from the biopolymers by acidic or enzymatic hydrolysis, and subsequently



R_5	$R_{4,7,8,9}$	
$-\overset{\overset{O}{\|}}{C}-CH_3$	$-H$	
$-\overset{\overset{O}{\|}}{C}-\overset{\overset{OH}{\|}}{CH_2}$	$-\overset{\overset{O}{\|}}{C}-CH_3$	[4,7,9]
	$-\overset{\overset{O}{\|}}{C}-\overset{\overset{OH}{\|}}{CH_2}$	[4]
	$-\overset{\overset{O}{\|}}{C}-\overset{\overset{OH}{\|}}{CH}-CH_3$	[9]
	$-CH_3$	[8]
	$-SO_3H$	[8]

FIG. 1. General structure and survey of naturally occurring sialic acids.

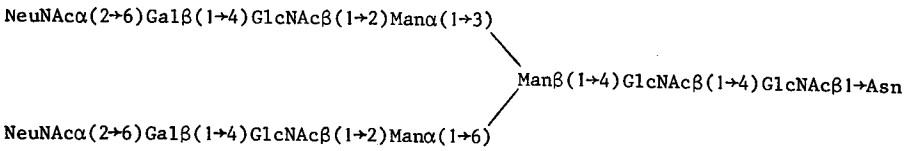


FIG. 2. Structure of a glycopeptide isolated from human serotransferrin . NeuNAc = N-acetylneuraminic acid; Gal = galactose; GlcNAc = N-acetylglucosamine; Man = mannose; Asn = asparagine.

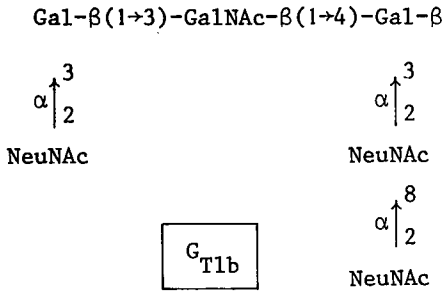


FIG. 3. Structure of a bovine brain ganglioside. NeuNAc = N-acetylneuraminic acid; Gal = galactose; GalNAc = N-acetylgalactosamine; Glc = glucose; Cer = ceramide.

$$[\rightarrow 8)\text{-NeuNAc-}\alpha(2\rightarrow 8)\text{-NeuNAc-}\alpha(2\rightarrow]_n$$

$$[\rightarrow 9)\text{-NeuNAc-}\alpha(2\rightarrow 9)\text{-NeuNAc-}\alpha(2\rightarrow]_n$$

$$[\rightarrow 6)\text{-Glc-}\alpha(1\rightarrow 4)\text{-NeuNAc-}\alpha(2\rightarrow]_n$$

$$[\rightarrow 6)\text{-Gal-}\alpha(1\rightarrow 4)\text{-NeuNAc-}\alpha(2\rightarrow]_n$$

FIG. 4. Structures of colominic acid (Escherichia coli) and Neisseria meningitidis polysaccharides.

purified by column chromatography (1). The presence of O-acyl substituents and the nature of the N-acyl group of the acylneuraminic acids can be determined by two-dimensional thin-layer chromatography with intermediary ammonia treatment (3). The number of O--acyl groups in sialic acids can be determined colorimetrically with alkaline hydroxylamine and ferric chloride. To establish the nature of the O-acyl substituents (and also of the N-acyl group), thin-layer chromatography of the corresponding hydroxamates can be applied (3). The positions of the O-acyl groups can be deduced from measurement of the molar periodate uptake of acylneuraminic acids and the rate of the oxidation reaction (15), or by determination of the rate of cleavage by acylneuraminate pyruvate-lyase (3). However, recently it has been found that the results obtained from periodate oxidation studies must be interpreted with great care (15).

Until now linkage analysis of internal sialic acid residues was based mainly on periodate oxidation or Smith degradation (after saponification of possible ester groups), though in some cases ^{13}C magnetic resonance spectroscopy has also been used (13, 14).

During the last five years we have investigated the possibilities of mass spectrometry (m.s.) in combination with gas-liquid chromatography (g.l.c.) for the structural analysis of acylneuraminic acids. By careful studies of the various mass spectra of suitable model substances, we developed a new method of identification of the number, type and position of O-substituents (O-acyl and O-alkyl groups) and of the type of the N-acyl group in acylneuraminic acids. The two main applications of the method are so far: (a) Determination of the number, type and position of the O-acyl groups and the type of the N-acyl group in naturally occurring acylneuraminic acids isolated from different biological materials (2, 4, 5, 16); (b) Determination of the position of the glycosidic linkages of acylneuraminic acids by methylation analysis (17, 18).

In this paper the potency of the mass spectrometric method for the analysis of neuraminic acid derivatives will be summarized (see Table 1).

EXPERIMENTAL

The 70-eV mass spectra were recorded on an AEI MS-902 mass spectrometer (ion source temperature, 100°-120°C; trap current, 500 μA; accelerating voltage, 8 kV) or a Jeol JGC-1100/JMS-07 combination (column material, 3.8% SE-30 on Chromosorb W/AW-DMCS, HP, 80-100 mesh; oven temperature, 200°C; ion source temperature, 250°C; trap current, 300 μA; accelerating voltage, 1.5 kV or 3 kV).

TABLE 1.

NATURALLY OCCURRING SIALIC ACIDS

1. N-acetylneuraminic acid
2. 4-O-acetyl-N-acetylneuraminic acid
3. 7-O-acetyl-N-acetylneuraminic acid
4. 9-O-acetyl-N-acetylneuraminic acid
5. 4,9-di-O-acetyl-N-acetylneuraminic acid
6. 7,9-di-O-acetyl-N-acetylneuraminic acid
7. 9-O-L-lactyl-N-acetylneuraminic acid
8. 8-O-methyl-N-acetylneuraminic acid
9. 2-deoxy-2,3-dehydro-N-acetylneuraminic acid
10. N-glycolylneuraminic acid
11. 4-O-acetyl-N-glycolylneuraminic acid
12. 9-O-acetyl-N-glycolylneuraminic acid

SYNTHETIC SIALIC ACID METHYL ESTERS

13. 2-O-methyl-N-acetylneuraminic acid
14. 8-O-acetyl-2-O-methyl-N-acetylneuraminic acid
15. 9-O-acetyl-2-O-methyl-N-acetylneuraminic acid
16. 4,8-di-O-acetyl-2-O-methyl-N-acetylneuraminic acid
17. 4,9-di-O-acetyl-2-O-methyl-N-acetylneuraminic acid
18. 4,8,9-tri-O-acetyl-2-O-methyl-N-acetylneuraminic acid
19. 2,4,7,8,9-penta-O-methyl-N,N-acetyl,methyl-neuraminic acid
20. 2,4,7,8-tetra-O-methyl-N,N-acetyl,methyl-neuraminic acid
21. 2,4,7,9 -tetra-O-methyl-N,N-acetyl,methyl-neuraminic acid
22. 2,4,8,9-tetra-O-methyl-N,N-acetyl,methyl-neuraminic acid
23. 2,7,8,9-tetra-O-methyl-N,N-acetyl,methyl-neuraminic acid
24. 2,4,7-tri-O-methyl-N,N-acetyl,methyl-neuraminic acid
25. 2,4,9-tri-O-methyl-N,N-acetyl,methyl-neuraminic acid
26. 2,4-di-O-methyl-N,N-acetyl,methyl-neuraminic acid
27. 2,9-di-O-methyl-N,N-acetyl,methyl-neuraminic acid
28. 2-O-methyl-N,N-acetyl,methyl-neuraminic acid

OTHER SYNTHETIC SIALIC ACID DERIVATIVES

29. 4-O-methyl-N-acetylneuraminic acid
30. C_8-N-acetylneuraminic acid
31. C_7-N-acetylneuraminic acid

High-resolution mass measurements were performed with a dynamic resolving power of 10,000 and a scan speed of 16 sec per mass decade, using an AEI MS-902 mass spectrometer connected on-line with a Ferranti Argus 500 computer.

RESULTS AND DISCUSSION

Mass spectrometric identification procedure. To obtain volatile acylneuraminic acid derivatives free carboxyl groups are converted into methyl esters and free hydroxyl groups into trimethylsilyl (TMS) ethers or acetyl (Ac) esters. The derivatized compounds 1 - 31 have been studied by high- and low-resolution mass spectrometry. As typical examples, the spectra of the methyl ester, TMS ether of N-acetylneuraminic acid 1 and the methyl ester of 2,4,7,8,9--penta-O-methyl-N,N-acetyl,methyl-neuraminic acid 19 are given in Figs. 5 and 6, respectively.

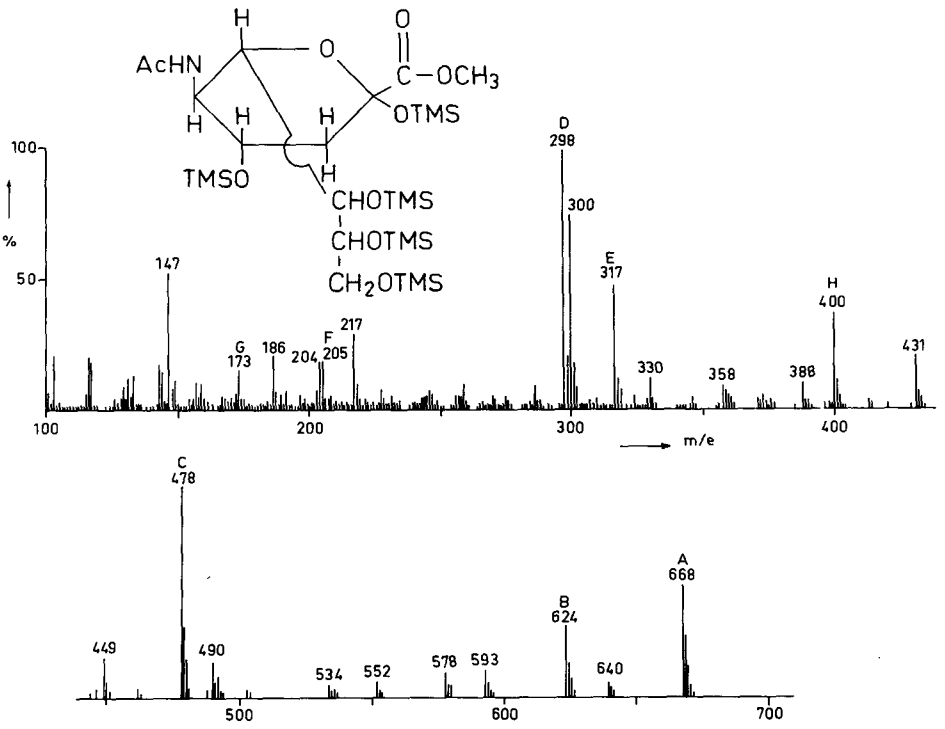


FIG. 5. Mass spectrum of the methyl ester, TMS ether of N-acetylneuraminic acid 1. Only values > m/e 100 and intensities ≥ 2% are given.

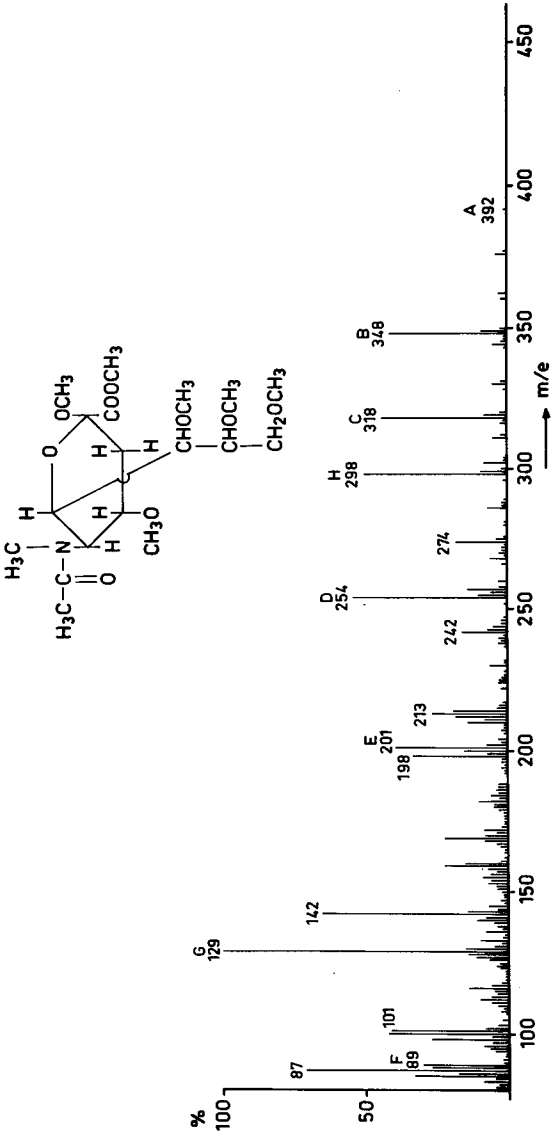
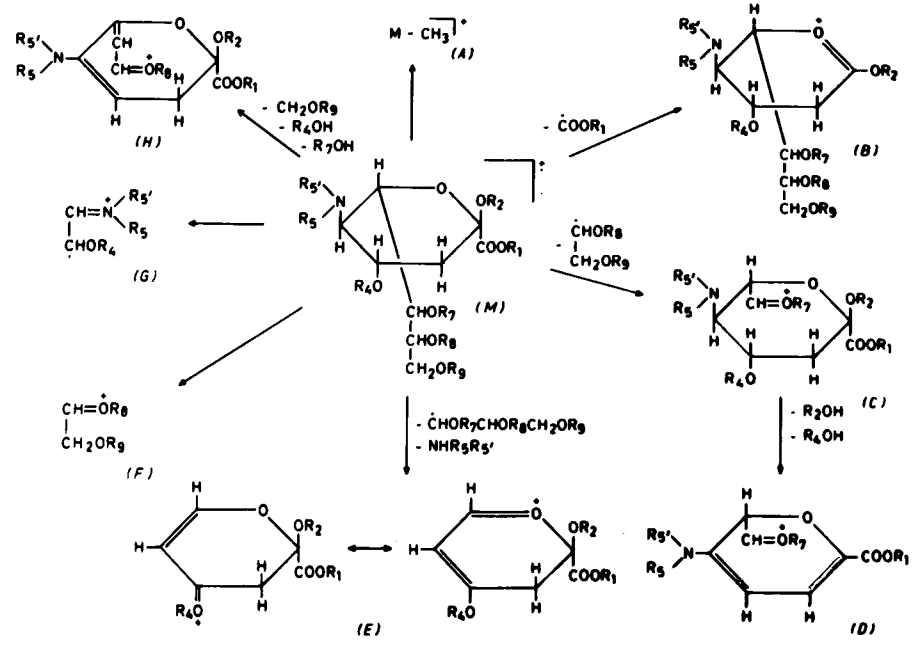


FIG. 6. Mass spectrum of permethylated N-acetylneuraminic acid 19. Only values > m/e 80 and intensities ≥ 1% are given.

The selected fragments A - H, which furnish the information (abundances and m/e values of the ions) necessary to determine the complete structure of the acylneuraminic acids, are shown in Fig. 7. Fragments A and B indicate the molecular weight of the



R_1 = CH_3

R_2 = CH_3 or $Si(CH_3)_3$

R_4, R_7, R_8, R_9 = CH_3, $Si(CH_3)_3$, $COCH_3$ and / or $COCH[OSi(CH_3)_3]CH_3$

R_5 = $COCH_3$ or $COCH_2OSi(CH_3)_3$

R_5' = H or CH_3

FIG. 7. Formation of the selected fragment ions A - H.

sialic acid derivatives and thereby the number and type of substituents. Fragments C - H can be sued for determination of the positions of the different substituents.

Fragment A is formed by elimination of a methyl group from the molecular ion. In trimethylsilylated (O-acylated) N-acyl-neuraminic acid derivatives the methyl group originates from a TMS substituent, whereas in acetylated partially O-methylated

N,N-acetyl,methyl-neuraminic acid derivatives the N,N-acetyl, methyl group is responsible. Trimethylsilylated partially O--methylated N,N-acetyl,methyl-neuraminic acid derivatives give rise to both possibilities, but the elimination from a TMS group strongly dominates.

Fragment B is formed by elimination of the C-1 part of the molecule. Eliminations of $OCOCH_3$ in O-acetylated neuraminic acid derivatives and of NH_2COCH_3 in N-acetylneuraminic acid derivatives, which in principle give rise to the same m/e value as fragment B in the case of $R_1=CH_3$, can be neglected.

Fragment C is formed by elimination of the C-8,9 part, with localization of the charge on position 7. For partially methylated alditol acetates, Björndal et al. (19) have demonstrated that the charge is preferentially located on an ether oxygen instead of on an ester oxygen. Therefore, in general cleavage occurs between two alkoxylated (methoxylated or trimethylsiloxylated) carbon atoms, or between an acyloxylated and an alkoxylated carbon atom, rather than between two acyloxylated carbon atoms; the alkoxyl function carries the positive charge. Therefore fragment C has only significant abundance if C-7 bears an ether group. When at C-7 an acyl group is present, this fragment ion is absent or hardly observable.

Fragment D is formed from fragment C by consecutive eliminations of R_2OH and R_4OH. It is evident that the occurrence of this fragment ion is influenced in the same manner as described for fragment C.

Fragment E is formed by elimination of the whole side-chain C-7,8,9 and the substituent at C-5. This fragment ion is not observed if an O-acetyl group is attached to C-4, illustrating that the transition state in the McLafferty rearrangement is more favored when the substituent at C-4 is an ether group rather than an ester group.

Fragment F contains the C-8,9 part. Based on the same fragmentation rules as mentioned above for fragments C and D, this ion can only readily be formed if an ether group is attached to C-8.

Fragment G consists of the C-4,5 part.

Fragment H is formed by elimination of the C-9 part, followed by elimination of R_4OH and R_7OH. This fragment ion is useful to discriminate between an OTMS group at C-8 or C-9 in trimethylsilylated partially methylated N-acylneuraminic acids. For the identification of trimethylsilylated O-acylated N-acylneuraminic acids and acetylated partially methylated N-acylneuraminic acids,

it is not necessary to take fragment H into consideration, since the occurrence of fragment F is already indicative of an O-acyl group at C-8 or C-9.

Naturally occurring (O-acylated) N-acylneuraminic acids. N-Acetylneuraminic acid 1 and N-glycolylneuraminic acid 10 can be isolated from various biological sources. Glycoproteins from submandibular gland mucins have been extensively investigated and form a rich source of O-acylated N-acylneuraminic acids. Compounds 1, 3, 4, 6, 7, 10 and 12 occur in bovine samples, whereas equine samples contain 1, 2, 5, 10 and 11 (2, 5, 10). Furthermore 1, 4 and 7 have been detected in serum and saliva of normal humans (4). In brain gangliosides of different vertebrates, the occurrence of 1, 4 and 10 has been proven (Haverkamp, Veh, Sander, Schauer, Kamerling and Vliegenthart, to be published).

In Table 2 the relative retention times on g.l.c. as well as

TABLE 2.
G.l.c. and m.s. data of the naturally occurring acylneuraminic acids 1-7 and 10-12, analyzed as the methyl ester, TMS ethers. The R_S-values on 3.8% SE-30 at 210°C are given relative to the methyl ester, TMS ether of N-acetylneuraminic acid 1.

No.^	R_S	A	B	C	D	E	F	G
1	1.00	668	624	478	298	317	205	173
2	1.18	638	594	448	298	-··	205	143
3	1.04	638	594	-··	-··	317	205	173
4	1.13	638	594	478	298	317	175	173
5		608	564	448	298	-··	175	143
6	1.14	608	564	-··	-··	317	175	173
7°	2.55	740	696	478	298	317	277	173
10	1.81	756	712	566	386	317	205	261
11	2.02	726	682	536	386	-··	205	231
12	2.04	726	682	566	386	317	175	261

^The compounds were esterified by CH_2N_2 and trimethylsilylated by hexamethyldisilazane/trimethylchlorosilane/pyridine (2).
°The absolute configuration of the lactyl substituent was determined enzymatically using L-lactate and D-lactate dehydrogenase (5).
·· - means intensity $\leq$ 2% of the base peak in the region > m/e 100.

the m/e values of the fragment ions A - G of the various methyl ester, TMS ethers of the (O-acylated) N-acylneuraminic acids are presented. Compared with the m/e values of the fragment ions of 1, fragments containing one OAc group give rise to a negative shift of 30 m.u., of - 60 m.u. for two OAc groups, etc.. A trimethylsilylated O-lactyl group causes a shift of + 72 m.u.. Replacement of an N-acetyl group by a trimethylsilylated N-glycolyl group gives a shift of + 88 m.u. in those fragment ions containing the substituted amino function.

Fragment G needs some comment. The occurrence of an OAc group at C-4 as in 2 and 5 leads to a shift of - 30 m.u. for this fragment ion (m/e 173 → m/e 143). However in all spectra a peak at m/e 143 having a general formula $C_6H_{11}O_2Si$ (143.0528) is observed. In the mass spectra of 2 and 5 the main contribution to the intensity of m/e 143 stems from fragment G ($C_6H_9NO_3$; 143.0582). For 11, m/e 231 is the analogue of m/e 143 in 2 and 5. By high-resolution mass measurements, this fragment could not be distinguished from other generally occurring fragment ions in sialic acid, which contribute also to the intensity of this peak.

Synthetic (partially O-acetylated) N-acetylneuraminic acid methyl ester methyl glycosides. The O-acetylated N-acetylneuraminic acid methyl ester methyl β-glycosides 14 - 18 have been prepared by partial O-acetylation of 13 with N-acetyl-imidazole in pyridine (15, 17). Table 3 presents the relative retention times on g.l.c. as well as the m/e values of the fragment ions A - G of the TMS ethers. Compared with the intensity of peak at m/e 175 (fragment F) in the mass spectra of 15 and 17, this peak is observed in a low intensity in the spectra of 14 and 16. The remarks concerning fragment G, given for the naturally occurring compounds 2 and 5, hold also for 16 - 18.

It has to be noted that the side-chain $CH_2OAc-CHOTMS-CH=\overset{+}{O}TMS$ in 15 and 17 only eliminates HOAc (m/e 217). In 14 and 16 the fragment $CH_2OTMS-CHOAc-CH=\overset{+}{O}TMS$ eliminates HOAc (m/e 217) as well as TMSOH (m/e 187). This observation can be used as an additional criterion for discrimination between 8-OAc and 9-OAc derivatives (see also ref. 2, 20).

Methylation analysis. Methylation analysis is a frequently used method to determine the position of glycosidic bonds in glycoconjugates and polysaccharides. After permethylation the compound is solvolysed and the formed mixture of partially methylated monomers is analysed.

(Partially) O-methylated derivatives of N,N-acetyl,methyl-neuraminic acid methyl ester methyl glycoside 28, obtained by methanolysis and re-N-acetylation of a permethylated sialoglyco-conjugate, can be analysed by g.l.c.-m.s. after trimethylsilylation

TABLE 3.
G.l.c. and m.s. data of the synthetic acylneuraminic acid methyl esters 13 - 18, analysed as the TMS ethers. The R_S-values on 3.8% SE-30 at 210°C are given relative to the methyl ester, TMS ether of N-acetylneuraminic acid 1.

No.^	R_S	A	B	C	D	E	F	G
13	1.00	610	566	420	298	259	205	173
14	1.12	580	536	420	298	259	-"	173
15	1.20	580	536	420	298	259	175	173
16	1.28	550	506	390	298	-°	-"	143
17	1.46	550	506	390	298	-°	175	143
18	1.42	520	476	390	298	-°	-"	143

^The compounds were trimethylsilylated by hexamethyldisilazane/trimethylchlorosilane/pyridine (2).
"See Text.
°-means intensity ⩽ 1% of the base peak in the region > m/e 100.
"The small peak at m/e 145 corresponds with $C_6H_{13}O_2Si$.

or acetylation of the free OH functions. The equilibrium mixture after methanolysis contains predominantly the methyl β-glycoside of each acylneuraminic acid derivative; only a few percent of the corresponding α-anomers are present.

For identification of partially O-methylated derivatives of 28, the reference compounds 19 - 22 and 24 - 28 have been prepared (17 and Fournet, unpublished results). To obtain a perfectly reliable system for the characterization by g.l.c.-m.s., TMS as well as Ac derivatives are investigated. In Table 4 the relative retention times on g.l.c. as well as the m/e values of the selected fragment ions are presented (A -H for TMS derivatives; A - G for Ac derivatives). Owing to the presence of only ether substituents, the mass spectra of the TMS derivatives contain each of the selected fragment ions. The Ac derivatives give mass spectra which, depending on the position of the Ac groups, some selected fragment ions may be absent or hardly observable (see Part I).

The fragment ions F and G deserve further consideration:
Fragment F. — In the Ac derivatives of 21 and 24 - 28 fragment F is almost absent (⩽ 3% of the base peak). To the intensity of the peak at m/e 89 in the TMS derivatives of 22 and 23 two

TABLE 4.
G.l.c. and m.s. data of the methylated N,N-acetyl,methyl--neuraminic acid methyl esters 19 - 28, analysed as the TMS ethers and Ac esters. The R_N-values on 3.8% SE-30 at 220°C are given relative to the methyl ester of 2,4,7,8,9--penta-O-methyl-N,N-acetyl,methyl-neuraminic acid 19.

No.^	R_N	Derivative	A	B	C	D	E	F	G	H
19	1.00		392	348	318	254	201	89	129	298
20	1.30	TMS	450	406	318	254	201	147	129	298
	1.47	Ac	420	376	318	254	201	117	129	
21	1.14	TMS	450	406	318	254	201	147	129	356
	1.25	Ac	420	376	318	254	201	-"	129	
22	1.07	TMS	450	406	376	312	201	89	129	298
	1.08	Ac	420	376	-'	-'	201	89	129	
23"		TMS	450	406	376	254	259	89	187	298
		Ac	420	376	346	254	-'	89	157	
24°	1.55	TMS	508	464	318	254	201	205	129	356
	1.75	Ac	448	404	318	254	201	-"	129	
25	1.27	TMS	508	464	376	312	201	147	129	356
	1.26	Ac	448	404	-'	-'	201	-"	129	
26	1.70	TMS	566	522	376	312	201	205	129	356
	1.70	Ac	476	432	-'	-'	201	-"	129	
27	1.43	TMS	566	522	434	312	259	147	187	356
	1.63	Ac	476	432	-'	-'	-'	-"	157	
28	1.89	TMS	624	580	434	312	259	205	187	356
	2.17	Ac	504	460	-'	-'	-'	-"	157	

The compounds were trimethylsilylated by hexamethyldisilazane/trimethylchlorosilane/pyridine or acetylated by acetic anhydride/pyridine (17).
"Data published by Bhattacharjee and Jennings (21).
°Sample kindly provided by B. Fournet, University of Lille, France.
"See text.
'- means absent or intensity $\leq$ 1% of the base peak in the region m/e > 80.

different ions contribute, namely $CH_2OCH_3-CH=\overset{+}{O}CH_3$ F; $C_4H_9O_2$) and $OTMS^+$ (C_3H_9OSi). The latter fragment always occurs in the mass spectra of TMS carbohydrates. Two different fragment ions contribute to the intensity of the peak at m/e 147 in the TMS derivatives of 25 and 27, namely $CH_2OCH_3-CH=\overset{+}{O}TMS$ (F; $C_6H_{15}O_2Si$) and $TMSO-\overset{+}{S}i(CH_3)_2$ ($C_5H_{15}OSi_2$). The fragment with formula $C_5H_{15}OSi_2$ is generally present in the mass spectra of TMS carbohydrates bearing more than one TMS group. In the TMS derivatives of 24, 26 and 28 the peak at m/e 147 stems only from $C_5H_{15}OSi_2$.

Fragment G. —— This fragment is found at m/e 129 when $R_4 = CH_3$, and is the base peak in the region > m/e 80 (19 and the TMS and Ac derivatives of 20 - 22 and 24 - 26). The mass spectra of TMS carbohydrates always show a peak at m/e 129 with low intensity originating from the fragments $C_5H_9O_2Si$ and $C_6H_{13}OSi$. In the TMS derivatives of 23, 27 and 28 the peak at m/e 129 is of low intensity since R_4 = TMS ($C_5H_9O_2Si$ and $C_6H_{13}OSi$). High--resolution mass spectrometry and comparison of the various mass spectra indicate that the main contribution to the peak at m/e 129 in the TMS derivatives of 20 - 22 and 24 - 26 stems from $C_6H_{11}NO_2$ (fragment G). In the Ac derivatives of 20 - 22, 24 and 25 the peak at m/e 129 consists mainly of fragment G, together with a small contribution of $C_6H_9O_3$. The latter fragment ion originates from C-7,8,9 after elimination of CH_3OH or HOAc as appropriate.

Applications of the identification procedure in the methylation analysis of polysaccharides, glycolipids and glycoproteins have been published recently by Bhattacharjee and Jennings (21), Finne et al. (12) and Haverkamp et al. (18).

Other sialic acid derivatives. In Table 5 the data of some mono-O-methylated and unusual sialic acid derivatives are summarized. The unsaturated sialic acid 9 has been isolated from urine, serum and saliva of man (4, 22). Interestingly, the compound could be identified on guidance of the fragmentation scheme developed for saturated sialic acids. The absence of fragments B and G is connected with the double bond between C-2 and C-3. The scheme was also applied to 30 and 31, being analogues of N-acetylneuraminic acid 1 in which the side-chain is shortened by one or two carbon atoms, respectively (23).

Comments on some additional important fragment ions. $CH_2=\overset{+}{O}R$. — The fragment ion at m/e 103 in the mass spectra of the TMS derivatives of sialic acids ($CH_2=\overset{+}{O}TMS$) is not characteristic for a primary trimethylsiloxyl group, but can also be formed by migration of a hydrogen atom to any charged one-carbon fragment bearing an OTMS group. Therefore, m/e 103 is not suitable for the determination of the substituent at C-9 in sialic acid derivatives. The same holds for the peak at m/e 45 ($CH_2=\overset{+}{O}CH_3$) in the mass spectra of methyl derivatives.

TABLE 5.
G.l.c. and m.s. data of the acylneuraminic acid derivatives 8, 9 and 29 - 31. The R_S-values on 3.8% SE-30 at 210°C for 9 and 190°C for 30 and 31 are given relative to the methyl ester, TMS ether of N-acetylneuraminic acid 1.

No.	R_S	Derivative	A	B	C	D	E	F	G
8°			-^	418	-^	-^	-^	117	143
9	1.09	TMS"	578	-^	388	"298"	227	205	-^
29'		TMS"	610	566	420	298	259	205	115
30+	0.50	TMS"	566	522	478	298	317	"103"	173
31+	0.29	TMS"	464	420			317		173

°Data published by Kochetkov et al. (7) for the peracetylated methyl ester methyl glycoside of 8.
^- means absent.
"The compounds were esterified by CH_2N_2 and trimethylsilylated by hexamethyldisilazane/trimethylchlorosilane/pyridine (2).
'Sample kindly provided by P. Sinaij, University of Orléans, France.
+Samples kindly provided by R.W. Veh, University of Bochum, W. Germany.

$R_5R_5,\overset{+}{N}=CH-CH=CHOR_7$ and $R_5R_5,\overset{+}{N}=CH-C(OR_4)=CH_2$. — In the mass spectrum of the methyl ester, TMS ether of 1 an intense peak at m/e 186 is present (Fig. 5), which can be explained in two ways, namely $CH_3CO-\overset{+}{N}H=CH-CH=CHOTMS$ (C-5,6,7) and $CH_3CO-\overset{+}{N}H=CH-C(OTMS)=CH_2$ (C-3,4,5). The same holds for the analogues of this peak, m/e 274 in the mass spectrum of the methyl ester, TMS ether of 10 (R_4=R_7=TMS; R_5=$COCH_2OTMS$; R_5'=H) and m/e 142 in the mass spectrum of the methyl ester of 19 (R_4=R_5'=R_7=CH_3; R_5=$COCH_3$) (Fig. 6). The existence of these two fragments was proved by the spectra of those sialic acids, having different substituents at C-4 and C-7. It is difficult to predict which structure prevails. In any case the type of substituent influences the abundance of the fragment ions. Therefore it is evident that the m/e values of these fragments give information only about the type of substituent at C-5 (amino function) in the sialic acids.

$R_5R_5'\overset{+}{N}=CH-CH=C(OR_7)-CH=CHOR_9$ and $R_5R_5'\overset{+}{N}=CH-CH=C(OR_7)-C(OR_8)=CH_2$. — The mass spectrum of the methyl ester, TMS ether of 1 gives rise to an intense peak at m/e 300 (R_5=$COCH_3$; R_5'=H;

R_7=R_8=R_9=TMS) (Fig. 5), which shifts to m/e 388 in the spectrum of the methyl ester, TMS ether of 10 (R_5=$COCH_2OTMS$; R_5'=H; R_7=R_8=R_9=TMS), and to m/e 198 in that of the methyl ester of 19 (R_5=$COCH_3$; R_5'=R_7=R_8=R_9=CH_3) (Fig. 6). In Fig. 8 the formation

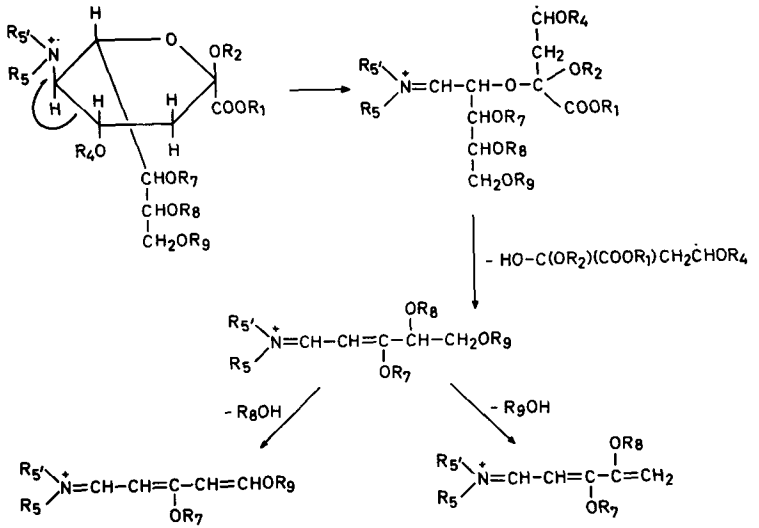


FIG. 8. Formation of the fragment ions with general structures $R_5R_5'\overset{+}{N}$=CH-CH=C(OR_7)-CH=$CHOR_9$ and $R_5R_5'\overset{+}{N}$=CH-CH=C(OR_7)-C(OR_8)=CH_2.

of these fragment ions is presented, based on comparison of the various mass spectra, high resolution mass spectrometry and defocussing metastable measurements (2). In general the elimination of ester or ether substituents at C-8 seems to be preferred. It has to be noted that ester substituents at C-7 and/or C-9 strongly reduce the abundance of these fragment ions.

CONCLUDING REMARKS

The aim of this study was primarily the qualitative identification of sialic acids. The results obtained so far show this mass spectrometric approach is generally applicable to establish the structure of sialic acids. It is important to know whether the O--acyl groups in the isolated naturally occurring sialic acids are present at the same positions as in the native state. Therefore, analysis of mixtures of sialic acids in earlier stages of the isolation procedure will be necessary to establish whether or not

acyl migrations occur during isolation and purification. For this purpose the application of more advanced techniques, such as capillary g.l.c.-m.s. and mass fragmentography, will be of great help. A further advantage of these techniques is that, besides the structure, the relative amounts of sialic acids can also be determined.

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