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IDENTIFICATION OF *O*-ACETYLATED *N*-ACYLNEURAMINIC ACIDS BY MASS SPECTROMETRY

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ABSTRACT

A number of *O*-acetylated *N*-acylneuraminic acids, isolated from submandibular glands of cow and horse and from horse erythrocytes, have been characterized by mass spectrometry On the basis of the typical fragmentation patterns of the pertrimethylsilyl derivatives of the methyl esters of the compounds, they were identified as 4-*O*-acetyl-, 9-*O*-acetyl-, 4,9-di-*O*-acetyl-, and 7,9-di-*O*-acetyl-*N*-acetylneuraminic acid, and 4-*O*-acetyl- and 9-*O*-acetyl-*N*-glycolylneuraminic acid

INTRODUCTION

Sialic acids occur in animal tissues as constituents of glycoproteins and glycolipids In these macromolecules, a variety of *N*- and *O*-acylated sialic acids have been found[1]. The biological significance of the different sialic acids is still a subject for speculation

The *O*-acylated sialic acids are mainly derived from *N*-acetylneuraminic acid[2] (NeuNAc), and to a smaller extent from *N*-glycolylneuraminic acid[3] (NeuNGl) In principle, the *O*-acyl groups in sialic acids can be located at positions 4, 7, 8, and/or 9 The occurrence of mono-*O*-acetyl- and di-*O*-acetyl-*N*-acetylneuraminic acids, as well as mono-*O*-glycolyl-*N*-acetylneuraminic acids, has been demonstrated[1–8] For several of these compounds, analogues exist which stem from *N*-glycolylneuraminic acid as the parent substance[7–10]

The structural determination of sialic acids is rather complicated, only small amounts of pure material can be obtained and the compounds are not very stable Quantitative analysis of the sialic acids and the determination of the number of *O*-acyl groups in these substances are carried out colorimetrically[5,11–13]. Qualitative analysis is performed by thin-layer chromatography (t l c.) of the free sialic acids[5,7]

and gas–liquid chromatography (g l c) of the pertrimethylsilyl (Me_3Si) derivatives[8] So far, the positions of the *O*-acyl groups have been deduced on the basis of the rate of periodate oxidation of the sialic acids[5,7] and of the rate of cleavage by acylneuraminate pyruvate-lyase (EC 4 1 3.3)[7,14].

Recently, we discussed in detail the mass spectra of the Me_3Si 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, and the trideuteriomethyl ester trideuteriomethyl β-glycoside of *N*-acetylneuraminic acid[15]. To gain insight into the various fragmentation patterns, high-resolution mass spectrometry (exact mass measurement) was used, as well as labelled compounds Based on these data, it was suggested that the characteristic mass spectra of these sialic acid derivatives could be of help in the identification of unknown sialic acids. We now report the application of mass spectrometry to the characterization of a number of naturally occurring, *O*-acetylated, *N*-acylated neuraminic acids

RESULTS AND DISCUSSION

For the mass-spectrometric analysis of sialic acids, the carboxyl group is converted into the methyl ester and the hydroxyl functions are protected as Me_3Si ethers Low- and high-resolution mass spectra of these derivatives have been recorded. The results are summarized in Table I

For the identification of the sialic acids **5–8**, **12**, and **13**, a set of specific peaks have been selected from the mass spectra of the previously studied compounds **1–4** and **11** This set has been used to derive the number, type, and/or position of the different substituents in the isolated sialic acids To illustrate the identification procedure, the mass spectrum of **1** is given in Fig 1, together with the typical fragment ions *m/e* 668, 624, 478, 298, 317, 205, and 173. In Scheme 1, the various fragment ions are schematically presented

Fragments *A* and *B* indicate the molecular weight of the sialic acids and thereby the number and type of substituents present. Fragment *A* ($M-CH_3$) is formed by elimination of a methyl group from a trimethylsilyl group. Fragment *B* ($M-COOCH_3$ or $M-COOCD_3$) is formed by elimination of the C-1 part of the molecule In both fragments, the presence of one OAc group (**5** and **6**) causes a negative shift of 30 m u with respect to the corresponding mass values of **1** For one OMe group (**3**), this negative shift amounts to 58 m u , for two OAc groups (**7** and **8**) 60 m u , for one OAc group and one OMe group (**9**) 88 m u , and for two OAc groups and one OMe group (**10**) 118 m u The replacement of an NHAc group in **1** by an NHGl group gives a positive shift of 88 m u (**11**) This shift amounts to 58 m u if an NHGl group, together with one OAc group, is present (**12** and **13**)

Fragments *C*, *D*, *E*, *F*, and *G* can be used for the determination of the positions of the different substituents Fragment *C* ($M-CHOR_8CH_2OR_9$) is formed by elimination of the C-8,9 part of the molecule, with localization of the formal charge

TABLE I

CHARACTERISTIC FRAGMENT-IONS *A–G*, USED FOR THE IDENTIFICATION OF THE *O*-ACETYLATED *N*-ACYLNEURAMINIC ACIDS

No.	*Compound*[a]	*Fragments*[b]						
		A	B	C	D	E	F	G
1	NeuNAc	668	624	478	298	317	205	173
2	NeuNAc*	671 (+3)	624 (0)	481 (+3)	301 (+3)	320 (+3)	205 (0)	173 (0)
3	NeuNAc-2-OMe	610 (−58)	566 (−58)	420 (−58)	298 (0)	259 (−58)	205 (0)	173 (0)
4	NeuNAc*-2-OCD_3	616 (−52)	569 (−55)	426 (−52)	301 (+3)	265 (−52)	205 (0)	173 (0)
5	NeuNAc-4-OAc	638 (−30)	594 (−30)	448 (−30)	298 (0)	— (—)	205 (0)	143 (−30)
6	NeuNAc-9-OAc	638 (−30)	594 (−30)	478 (0)	298 (0)	317 (0)	175 (−30)	173 (0)
7	NeuNAc-7,9-di-OAc	608 (−60)	564 (−60)	— (—)	— (—)	317 (0)	175 (−30)	173 (0)
8	NeuNAc-4,9-di-OAc	608 (−60)	564 (−60)	448 (−30)	298 (0)	— (—)	175 (−30)	143 (−30)
9	NeuNAc-9-OAc-2 OMe	580 (−88)	536 (−88)	420 (−58)	298 (0)	259 (−58)	175 (−30)	173 (0)
10	NeuNAc-4,9-di-OAc-2-OMe	550 (−118)	506 (−118)	390 (−88)	298 (0)	— (—)	175 (−30)	143 (−30)
11	NeuNGl	756 (+88)	712 (+88)	566 (+88)	386 (+88)	317 (0)	205 (0)	261 (+88)
12	NeuNGl-4-OAc	726 (+58)	682 (+58)	536 (+58)	386 (+88)	— (—)	205 (0)	231 (+58)
13	NeuNGl-9-OAc	726 (+58)	682 (+58)	566 (+88)	386 (+88)	317 (0)	175 (−30)	261 (+88)

[a]For mass spectrometry, the compounds have been esterified by CH_2N_2, CH_3OH, or CD_3OH, and trimethylsilylated by HMDS/TMCS/pyridine (see Experimental) (*esterified by CD_3OH), NeuNAc = *N*-acetylneuraminic acid, NeuNGl = *N*-glycolylneuraminic acid, NeuNAc-4-OAc = 4-*O*-acetyl-*N*-acetylneuraminic acid, NeuNAc-2-OMe = *N*-acetylneuraminic acid methyl β-glycoside, etc [b]In parenthesis, the increments are given relative to the values of NeuNAc

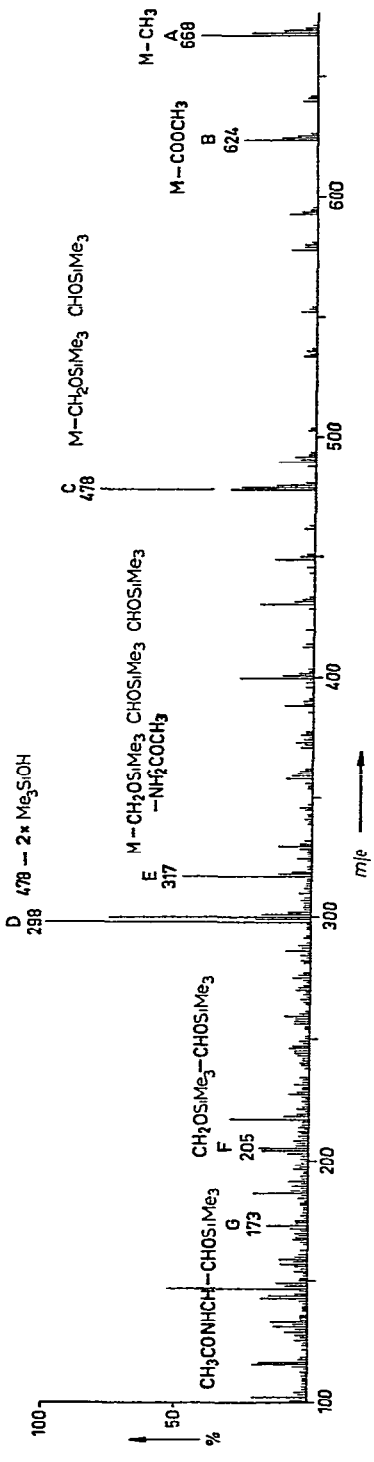
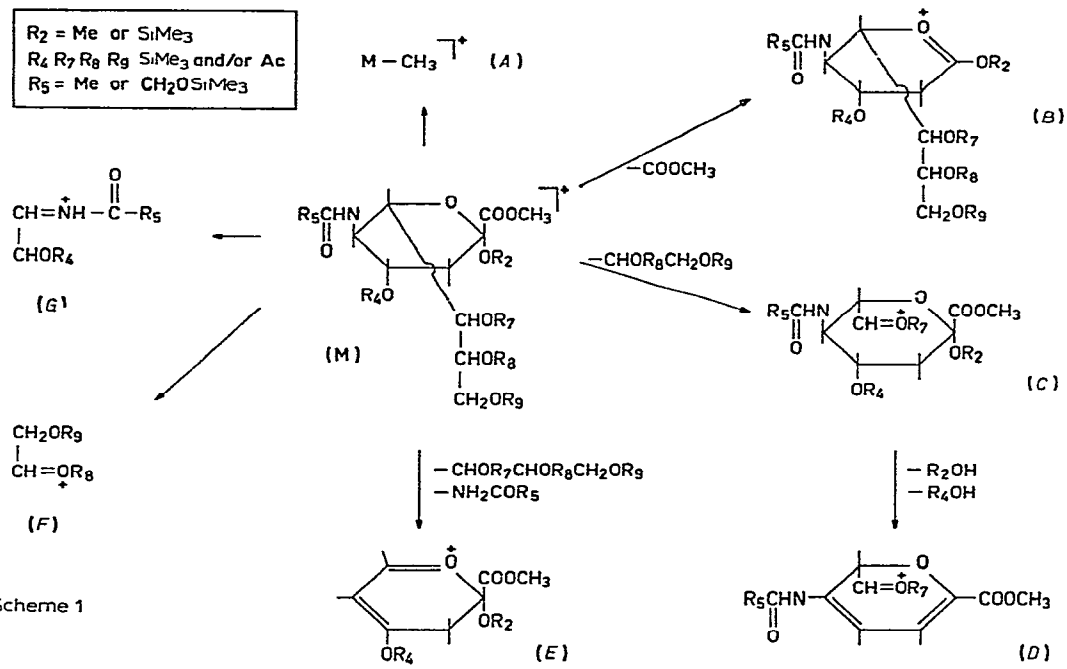


Fig. 1 Mass spectrum of the Me_3Si derivative of the methyl ester of *N*-acetylneuraminic acid. Only values > *m/e* 100 and intensities ≥ 2% are given



Scheme 1

on position 7 For partially methylated alditol acetates, Lindberg and his co-workers[16,17] have demonstrated that the charge is preferentially located on an ether oxygen instead of on an ester oxygen Therefore, cleavage between two methoxylated carbon atoms or between an acetoxylated and a methoxylated carbon atom occurs, rather than cleavage between two acetoxylated carbon atoms, the methoxyl function carries the positive charge In general, permethylated and pertrimethylsilylated carbohydrates give rise to similar fragmentation patterns, allowing extrapolation of the above-mentioned fragmentation rules to Me_3Si derivatives For this reason, the formation of fragment *C* is improbable if an ester group is present at C-7 Fragment *D* ($M-CHOR_8CH_2OR_9-R_2OH-R_4OH$) is formed from fragment *C* by consecutive eliminations[15] of R_2OH and R_4OH It is evident that, for 7-*O*-acylated sialic acids, this fragment ion does not occur if fragment *C* is scarcely formed Elimination of the whole side-chain C-7,8,9 and the substituent at C-5 from the parent ion yields fragment *E* ($M-CHOR_7CHOR_8CH_2OR_9-R_5CONH_2$) Fragment *F* ($CH_2OR_9CHOR_8$) contains the C-8,9 part of the molecule Based on the same fragmentation rules mentioned above, the formation of this fragment ion cannot be expected if C-8 bears an ester group Finally, fragment *G* ($R_5CONHCHCHOR_4$) contains the C-4,5 part of the molecule

The assignments of the number and positions of the OAc groups in **5–8, 12,** and **13** is made on the basis of the intensities and *m/e* values of the fragment ions *A* to *G*, and on the following consideration

The presence of an intense peak at *m/e* 175 (fragment *F*), together with the shifts in mass for other fragment-ions in the spectra of the mono-OAc derivatives **6** and **13** and the di-OAc derivatives **7** and **8**, leads to the conclusion that an OAc group is present at C-9. This assignment differs from the results deduced from periodate-oxidation studies The consumption of 0 2–0 3 mole of periodate per mole of sialic acid after 20 min suggests the presence of an OAc group at C-8, as was described earlier[5]. The small uptake of periodate is explained in terms of over-oxidation

To support the mass-spectrometric conclusion of an OAc group at C-9 instead of C-8, the methyl ester of **9** was synthesized by reaction of the mild acylating-reagent *N*-acetylimidazole with the methyl ester methyl *β*-glycoside of *N*-acetylneuraminic acid (molar ratio 1 2 1 0). On the basis of the reactivity of this acylating reagent, it can be expected that the primary hydroxyl group (C-9) will react preferentially The 60-MHz p m r. spectrum of the main reaction product shows the presence of one OAc group besides the NHAc group The mass spectrum of the Me_3Si derivative of this compound gives rise to an intense peak at *m/e* 175 These observations definitely exclude the location of the OAc group at C-8 in **6**, **7**, **8**, and **13**, which were isolated from biological materials As a by-product in this synthesis, the methyl ester of **10** was obtained The consumption of periodate by the synthetic compounds **9** and **10** appeared to be identical to that reported[18] for the isolated sialic acids **6** and **13**.

To obtain independent evidence for the presence of an OAc group in position 9, use has been made of the enzymic cleavage of **6** and **13** by acylneuraminate pyruvate-lyase The resulting *O*-acetyl-*N*-acylmannosamines (*O*-acetyl-2-*N*-acylamino-2-deoxy-D-mannoses) were investigated by g l c–m s after trimethylsilylation, and identified as the pyranoid forms of 6-OAc-*N*-acetylmannosamine and 6-OAc-*N*-glycolylmannosamine, respectively[19]. This finding is in accordance with an OAc group at C-9 in the starting sialic acids It should be noted that an OAc group at C-8 would give 5-OAc-*N*-acylmannosamine, having a furanoid ring

In all spectra, a peak at *m/e* 143 having a molecular formula $C_6H_{11}O_2Si$ (143 0528) was observed In the low-resolution mass spectra of **5**, **8**, and **10**, the main contribution to the intensity of *m/e* 143 stems from fragment *G*, as was proved by high-resolution mass spectrometry ($C_6H_9NO_3$, 143 0582) This confirms the location of an OAc group at C-4 For **12**, *m/e* 231 is the analogue of *m/e* 143 in **5**, **8**, and **10** By high-resolution mass measurements, this fragment could not be distinguished from other fragment ions, which contribute also to the intensity of this peak and frequently occur in other sialic acids

The absence of fragments *C* and *D* in the mass spectrum of **7** points to the location of an OAc group at C-7.

From the various mass-spectra of sialic acids having an AcO-4 group, it can be deduced that the formation of fragment *E* does not take place

The mass spectrum of **1**, presented in Fig 1, shows additional, intense peaks (*m/e* 103, 186, 217, and 300), which were not used for the characterization of the compounds described in Table I.

The fragment ion at *m/e* 103 is present in all of the mass spectra recorded and

has the structure $CH_2=\overset{+}{O}SiMe_3$ From a study of the mass spectra of partially methylated, partially trimethylsilylated alditols, using deuterium labelling, it was concluded that this ion is not characteristic for a primary trimethylsiloxyl group The fragment ion can also be formed as a result of a migration of a hydrogen atom to any charged one-carbon fragment bearing an $OSiMe_3$ group (*cf.* Björndal *et al* [20] and Golovkina *et al* [21]) For these reasons, *m/e* 103 is not suitable for the determination of the substituent at C-9 in sialic acid derivatives.

Previously, two structures were published[15] for the peak at *m/e* 186 in *N*-acetylneuraminic acids, namely $Ac-\overset{+}{N}H=CH-CH=CHOSiMe_3$ (C-5,6,7) and $Ac-\overset{+}{N}H=CH-C(OSiMe_3)=CH_2$ (C-3,4,5) It was stated that labelling on C-4 and/or C-7 could discriminate between these alternatives From the mass spectra of **1–10**, it can be concluded that both structures are possible. Moreover, a third possibility, with the structure $\overset{+}{N}H_2=CH-CO-CH_2-CH=CHOSiMe_3$ (C-5,6,7,8,9), cannot be excluded The compounds **5–10** show a peak at *m/e* 186 ($C_8H_{16}NO_2Si$) and a small peak 30 m u lower at *m/e* 156 ($C_7H_{10}NO_3$), corresponding to the replacement of an $OSiMe_3$ group by an OAc group It should be mentioned that, in many cases, another peak at *m/e* 156 was found with molecular formula $C_7H_{14}NOSi$ The origin of this fragment is not clear For the analogue of *m/e* 186 in *N*-glycolylneuraminic acids, *m/e* 274 ($C_{11}H_{24}NO_3Si_2$), the same reasoning holds because in **12** a partial shift of this ion to *m/e* 244 ($C_{10}H_{18}NO_4Si$) is also found These findings make the peaks at *m/e* 186 or *m/e* 274 unsuitable for the characterization of *O*-acylated neuraminic acids The presence of these ions gives information only about the presence of an *N*-acetyl or an *N*-glycolyl group in the sialic acids.

The compounds having $OSiMe_3$ groups at C-7, C-8, and C-9 can eliminate Me_3SiOH from the intact side-chain $CH_2OSiMe_3-CHOSiMe_3-CH=\overset{+}{O}SiMe_3$ (*m/e* 307) In principle, for this elimination (*m/e* 307→*m/e* 217), each Me_3Si group has to be taken into account (*cf* Golovkina *et al* [21]) The mass spectra of the compounds having an OAc group at C-9 and $OSiMe_3$ groups at C-7 and C-8 (**6**, **8–10**, and **13**) show a peak at *m/e* 217, owing to the elimination of HOAc from *m/e* 277 [$CH_2OAc-CHOSiMe_3-CH=\overset{+}{O}SiMe_3$ (*m/e* 277, $C_{11}H_{25}O_4Si_2$)→$CH_2=C(OSiMe_3)-CH=\overset{+}{O}SiMe_3$ (*m/e* 217, $C_9H_{21}O_2Si_2$)+HOAc] It is noteworthy that no measurable elimination of Me_3SiOH takes place, since *m/e* 187 is mainly the isotopic peak of *m/e* 186 In the spectrum of **7**, an intense peak at *m/e* 187 is observable, formed by elimination of HOAc from *m/e* 247 [$CH_2OAc-CHOSiMe_3-CH=\overset{+}{O}Ac$ (*m/e* 247, $C_{10}H_{19}O_5Si$)→$CH_2=C(OSiMe_3)-CH=\overset{+}{O}Ac$ (*m/e* 187; $C_8H_{15}O_3Si$)+HOAc] By high-resolution mass measurements, it was shown that the small peak at *m/e* 157 could not be formed *via* elimination of Me_3SiOH from *m/e* 247 These data are in accordance with those reported by Björndal *et al* [20] for partially methylated alditol acetates For example, the elimination of MeOH occurs in the fragment $CH_2OMe-CHOAc-CH=\overset{+}{O}Me$, giving $CH_2=C(OAc)-CH=\overset{+}{O}Me$, but not in the fragment $CH_2OAc-CHOMe-CH=$

$\overset{+}{O}Me$. In both cases, HOAc can be eliminated It has to be mentioned that, in acylneuraminic acids, the side chain C-7,8,9 can be split off as a charged fragment, even when an OAc group is present at C-7

Previously, on the basis of the mass spectra of **1–4** and **11**, a fragmentation scheme was postulated for the formation of *m/e* 300 ($C_{13}H_{26}NO_3Si_2$) in *N*-acetylneuraminic acid and *m/e* 388 ($C_{16}H_{34}NO_4Si_3$) in *N*-glycolylneuraminic acid[15] In this scheme, after cleavage of the C-4/C-5 bond, a Me_3Si rearrangement from O-4 to the ring oxygen was proposed However, the mass spectra of **5** and **12** also show intense peaks at *m/e* 300 and 388, respectively, suggesting that the substituent at C-4 is not present in the resulting fragment-ions An alternative explanation, also in accordance with metastable measurements using the defocusing technique of Barber and Elliott[22], will be discussed now The formation of the fragment ions requires (*a*) cleavage of the C-4/C-5 bond, (*b*) elimination of $HO–C(OR_2)(COOCH_3)–CH_2–CHOR_4$, wherein a H-rearrangement from C-7 to the ring oxygen has occurred, and (*c*) elimination of R_8OH or R_9OH In this way, two stable structures are formed with a conjugated double-bond system $R_5CO–\overset{+}{N}H=CH–CH=C(OR_7)–CH=CHOR_9$ and $R_5CO–\overset{+}{N}H=CH–CH=C(OR_7)–C(OR_8)=CH_2$ These structures are supported by the following facts (*1*) No shift in mass occurs when, in the neuraminic acid derivatives, $COOCH_3$ is replaced by $COOCD_3$, $OSiMe_3$ at C-2 by OMe, and/or $OSiMe_3$ at C-4 by OAc (*2*) The structure contains the substituent at C-5, because replacement of NHAc by $NHCOCH_2OSiMe_3$ causes a shift of 88 m u (*3*) The mass spectra of **6**, **8**, **9**, and **10** show a relatively small peak at *m/e* 300 in comparison with **1**, **3**, and **5** The presence of this ion indicates that the OAc group at C-9 is eliminated Furthermore, in the spectra of **6**, **8**, **9**, and **10**, a small peak at *m/e* 270 ($C_{12}H_{20}NO_4Si$) was detectable The presence of this peak suggests that the substituent at C-8 ($OSiMe_3$) can be eliminated In the spectrum of **7**, *m/e* 300 is absent, only a small peak at *m/e* 270 is observable, indicating the elimination of OAc from C-9 The mass spectrum of **13** shows a reduction in intensity of *m/e* 388 compared with **11**, and the presence of a small peak at *m/e* 358 ($C_{15}H_{28}NO_5Si_2$), similar to the NHAc analogue **6**

CONCLUSIONS

The structures of a number of *O*-acetylated *N*-acylneuraminic acids, isolated from submandibular glands of cow and horse and from horse erythrocytes, have been identified by mass spectrometry The fragmentation patterns of the Me_3Si derivatives of the methyl esters of *O*-acetylated *N*-acylneuraminic acids are quite characteristic Therefore, the types of *N*-acyl groups, as well as the number, type, and position of the *O*-acyl groups, in sialic acids can be determined unequivocally

The demonstration of an OAc group at C-4 in the equine sialic acids **5**, **8**, and **12** is in accordance with earlier periodate-oxidation studies[4 5 7] However, the assignment of an OAc group at C-9 in the bovine sialic acids **6**, **7**, and **13** is not in agreement with these studies, which pointed[5 7] to a location at C-8. Since the presence

of the substituent at C-9 is supported by the investigation of synthetic model compounds and by analysis of the 6-*O*-acetyl-*N*-acylmannosamines obtained by enzymic cleavage, it is important to re-evaluate the periodate oxidation of acylneuraminic acids substituted in the side chain Hitherto, the structure of **8** was unknown On the basis of its resistance towards the action of bacterial neuraminidase (EC 3 2 1 18), it was proposed[5] that one OAc group is located at C-4; the position of the other group had not been determined previously The demonstration of an OAc group at C-7 in **7**, isolated from bovine, submandibular-gland glycoproteins, is in agreement with earlier results achieved by periodate oxidation[4,7]

It is important to know whether the acetyl groups are present in the native state at the positions where they are found after the isolation procedure At present, the occurrence of acyl migrations during the isolation procedure cannot be excluded Model studies with synthetic *O*-acylated *N*-acylneuraminic acids are necessary to furnish more information on this aspect.

EXPERIMENTAL

Isolation of O-*acetyl*-N-*acylneuraminic acids* — Submandibular glands of cow and horse were prepared immediately after death of the animals and cooled in ice The sialoglycoproteins were extracted from the glands by water and subsequently lyophilized[5] Erythrocytes from horse blood, prevented from clotting by 0 2% oxalate, were sedimented at 3,000 *g* within 30 min The erythrocyte membranes were prepared with hypotonic phosphate buffer[23] The sialic acids were released from submandibular-gland glycoproteins or erythrocyte membranes by mild hydrolysis with acid (pH 2 2–2 5, 70°, 1 h) or by neuraminidase, and purified by dialysis and ion-exchange chromatography on Dowex-2 x8 (formate form) resin[5,7] Subsequently, the sialic acids were fractionated by passage through a cellulose column with 1-butanol–1-propanol–water (1 2 1) as solvent[5,7]. After lyophilization, the sialic acids can be stored for several months at −20° without appreciable decomposition

Analysis of O-*acetyl*-N-*acylneuraminic acids* — The quantitative analysis of the sialic acids eluted from the cellulose column was carried out colorimetrically using the orcinol/Fe^{3+}/HCl reagent[11] and the periodic acid/thiobarbituric acid reagent[12] The molar ratio of the *O*-acyl/sialic acid residues was determined by using the hydroxylamine/Fe^{3+} reagent[13] Qualitative analysis of the sialic acids was performed by t l c on cellulose and silica gel in several solvent systems[5] A recently developed, two-dimensional t l c technique with intermediate ammonia or hydroxylamine treatment was also applied This technique enables the rapid identification of both *N*-acyl and *O*-acyl groups[7]; the *N*-acyl groups can be recognized by the nature of the *O*-deacylated sialic acids (NeuNAc or NeuNGl), and the *O*-acyl groups by the type of their hydroxamates The position of the *O*-acyl groups was determined tentatively by the rate of periodic-acid oxidation of the sialic acids, which was followed polarographically[5,7]. The number, type, and position of *O*-acyl groups were determined by mass spectrometry, using the Me_3Si derivatives of the methyl

esters of the isolated sialic acids (this paper). The different, naturally occurring *O*-acylated sialic acids are represented in Table I (**5–8, 12,** and **13**)

Preparation of the methyl esters, trimethylsilyl ethers of O-*acetyl*-N-*acylneuraminic acids* (**5–8, 12,** and **13**) — To 50–100 μg of sialic acid in 0 5 ml of methanol, diazomethane in ether was added until a faint-yellow colour was obtained. The solution was then immediately evaporated, and the residue was dissolved in 1 ml of pyridine Subsequently, 0 2 ml of hexamethyldisilazane (HMDS) and 0 1 ml of chlorotrimethylsilane (TMCS) were added After 2 h at room temperature, 2 ml of chloroform and 2 ml of water were added to the turbid mixture The chloroform layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*

Preparation of model substances (**1–4** *and* **9–11**). — **1–4** and **11** were esterified by methanol or methanol-d_3, as previously described[15] A full description of the synthesis of the methyl esters of **9** and **10** will be given elsewhere[18] The silylation procedure was identical to that mentioned above

Preparation of O-*acetyl*-N-*acylmannosamines* — *O*-Acetyl-*N*-acylneuraminic acids (1–2 μmoles) dissolved in 1 ml of 0 2M potassium phosphate buffer (pH 7 2) were incubated at 37° for 4 h in the presence of 0 3 unit of acylneuraminate pyruvate-lyase (Sigma Chemical Company) The incubation mixtures were rinsed through Dowex-50(H^+) and Dowex-2 x8 (formate form) resins, and the acylmannosamines in the neutral effluent were lyophilized They were analyzed both colorimetrically and by t l c [7]

Mass spectrometry. — The 70-eV mass spectra were recorded on an AEI MS-902 mass spectrometer at an ion-source temperature of 100–120°, a trap current of 500 μA, and an accelerating voltage of 8 kV High-resolution mass measurements were performed with a dynamic resolving power of 10 000 and a scan speed of 16 sec per mass decade by using an AEI 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[24]. For the recording of the 70-eV mass spectra of the acylmannosamines, a Jeol JGC-1100/JMS-07 GC-MS combination was used As column material, 3% of SE-30 on Chromosorb W-AW DMCS (80–100 mesh) was used. The oven temperature was 170°, the ion-source temperature 250°, the accelerating voltage 3 kV, and the ionizing current 300 μA.

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