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N-Acetyl-9-*O*-L-lactylneuraminic Acid, a New Acylneuraminic Acid from Bovine Submandibular Gland

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The acylneuraminic acid fraction, obtained on mild acid hydrolysis of glycoproteins from bovine submandibular glands, contains approximately 2% *N*-acetyl-9-*O*-L-lactylneuraminic acid. The compound has been isolated and purified by ion-exchange and cellulose column chromatography. The structure has been elucidated using thin-layer chromatography, colorimetry, gas-liquid chromatography/mass spectrometry, periodate oxidation and specific lactate dehydrogenases. An evaluation of the different analytical methods is given.

The occurrence of Ac^7NeuNAc, Ac^9NeuNAc, $Ac_2^{7,9}$NeuNAc and Ac^9NeuNGl besides NeuNAc and NeuNGl as constituents of glycoproteins from bovine submandibular glands has firmly been established [1–3]. In this tissue the amount of acylneuraminic acids with an *O*-acetyl group at C-9 is much higher than that of acylneuraminic acids with an *O*-acetyl group at C-7 or at both C-7 and C-9. There are also indications that a small amount of the acylneuraminic acids from this tissue contains *O*-glycolyl groups [2].

In this report the characterization of a new type of substituted *N*-acetylneuraminic acid from bovine submandibular glands, namely *N*-acetyl-9-*O*-L-lactylneuraminic acid, will be presented.

MATERIALS AND METHODS

Isolation of Acylneuraminic Acids

Glycoproteins from bovine submandibular glands acquired immediately after death of the animals were prepared as described earlier [3]. Acylneuraminic acids were released from 25 g of lyophilized glycoproteins by mild acid hydrolysis (formic acid, pH 2–2.5, 70 °C, 60 min) followed by dialysis. This procedure was repeated twice, on which about 80% of the acylneuraminic acids were split off. The combined dialyzates were concentrated to about 1 l in a rotary evaporator at a water-bath temperature of 35 °C and then lyophilized. The acylneuraminic acids were purified by passage through Dowex 50 (H^+ form), 20–50 mesh, followed by adsorption on Dowex 2-X8 (HCO_2^- form), 50–100 mesh, and elution with 3 l of a gradient of 0–2 M formic acid at 4 °C (column sizes 60 × 4.5 cm). The eluate containing the acylneuraminic acids was lyophilized and fractionated on a cellulose MN 2100 ff (Macherey, Nagel & Co., Düren) column (1.65 m × 3.2 cm) using *n*-butanol/*n*-propanol/water (1/2/1, by vol.) as solvent at 4 °C (fraction volume 10 ml) [2, 3]. In this way 1.2 g of a mixture of acylneuraminic acids was obtained; 40% (0.48 g) of these compounds were *O*-acylated. For further purification and fractionation the *O*-acylated acylneuraminic acids were pooled and rechromatographed on cellulose under the same conditions, yielding 360 mg of *N*-acyl-*O*-acylneuraminic acids. Every other fraction of the eluate was analysed quantitatively by colorimetric methods and qualitatively by one-dimensional and two-dimensional thin-layer chromatography [2, 3].

Quantitative Analyses of Acylneuraminic Acids and O-Acyl Groups

The amount of acylneuraminic acids was determined colorimetrically by the orcinol/Fe^{3+}/HCl reagent [4] and by the periodic acid/thiobarbituric acid

Abbreviations. NeuNAc, *N*-acetylneuraminic acid; NeuNGl, *N*-glycolylneuraminic acid; Ac^7NeuNAc, *N*-acetyl-7-*O*-acetylneuraminic acid; Ac^9NeuNAc, *N*-acetyl-9-*O*-acetylneuraminic acid; $Ac_2^{7,9}$NeuNAc, *N*-acetyl-7,9-di-*O*-acetylneuraminic acid; Ac^9NeuNGl, *N*-glycolyl-9-*O*-acetylneuraminic acid; Lac^9NeuNAc, *N*-acetyl-9-*O*-L-lactylneuraminic acid.

Enzymes. L-Lactate dehydrogenase (EC 1.1.1.27); D-lactate dehydrogenase (EC 1.1.1.28).

reagent [5]. Crystalline NeuNAc and NeuNGl were used as references.

O-Acyl groups of acylneuraminic acids were determined quantitatively with alkaline hydroxylamine and ferric chloride [6]. Ac^9NeuNAc (chromatographically pure) was used as a standard.

Thin-Layer Chromatography

Thin-layer chromatography of acylneuraminic acids was performed on cellulose MN 300 (Macherey, Nagel & Co., Düren) in the solvent *n*-butanol/*n*-propanol/0.1 M HCl (1/2/1, by vol.) (A) and on silica gel H nach Stahl (Merck AG, Darmstadt) in the solvent *n*-propanol/water (7/3, by vol.) (B) as described before [2, 3].

Thin-layer chromatography of hydroxamates was performed on cellulose MN 300 in the solvent *n*-propanol/10% ammonium carbonate/5 M ammonium hydroxide (6/2/1, by vol.) (C) [2]. The hydroxamate spots were stained by spraying with a 10% $FeCl_3$ solution in water.

Gas-Liquid Chromatography

Gas-liquid chromatography of the methyl esters, trimethylsilyl ethers of acylneuraminic acids [1] was carried out on a Varian aerograph 2740-30-01 gas chromatograph, equipped with a dual-flame ionization detector and glass columns (2.00 m × 4.0 mm) packed with 3.8% SE-30 on Chromosorb W/AW-DMCS, HP, 80–100 mesh. The column-oven temperature was 210 °C and the gas flow rate for N_2 40 ml/min. The retention times (R_{NeuNAc}) are given relative to the pertrimethylsilyl derivative of the methyl ester of NeuNAc.

Mass Spectrometry

Acylneuraminic acids were analysed as the corresponding methyl esters, trimethylsilyl ethers. Gas-liquid chromatography/mass spectrometry was carried out on a Jeol JGC-1100/JMS-07 combination (glass column 2.00 m × 4.0 mm) packed with 3.8% SE-30 on Chromosorb W/AW-DMCS, HP, 80–100 mesh; column-oven temperature 200 °C; ion-source temperature 250 °C; electron energy 75 eV; trap current 300 μA; accelerating voltage 1.5 kV). An AEI MS-902 mass spectrometer (direct inlet system; ion-source temperature 100–120 °C; electron energy 70 eV; trap current 500 μA; accelerating voltage 8 kV) was used for high-resolution mass measurements [1].

Periodate Oxidation

The rate of oxidation of the new acylneuraminic acid by periodate was determined as described before [7]. For this test a sample was taken from fraction 130 (Fig. 1). It was shown by thin-layer chromatography that it did not contain significant amounts of NeuNAc (decomposition product) or of *O*-acetylated acylneuraminic acids.

Identification of Acyl Substituents

The presence of *O*-acyl substituents and the nature of the *N*-acyl group of the acylneuraminic acids, obtained after cellulose column chromatography were determined by two-dimensional thin-layer chromatography on cellulose (solvent A) with intermediary ammonia treatment [2]. The nature of the *O*-acyl substituents was established by thin-layer chromatography on cellulose of the corresponding hydroxamates using solvent C [2]. The hydroxamates of formic acid, acetic acid, glycolic acid, propionic acid, hydracrylic acid and lactic acid were used as reference substances.

Determination of the Configuration of Lactate

For determination of the configuration of the lactate by means of lactate dehydrogenase, the lactyl group was liberated by treatment of the new acylneuraminic acid with 0.05 M NaOH for 1 h at room temperature. After lyophilization the sodium lactate was tested photometrically as substrate for L-lactate dehydrogenase (from porcine muscle; Boehringer Mannheim GmbH) and D-lactate dehydrogenase (from *Lactobacillus leichmannii*; Boehringer Mannheim GmbH) respectively in the presence of NAD^+ and hydrazine [8]. L-Lactate and D-lactate were used as reference compounds.

Determination of Free Lactate in Bovine Submandibular Glands

5 g of freshly prepared submandibular gland were homogenized in 7.5 ml of 0.1 M Tris-HCl buffer, pH 7.4. L-lactate was determined quantitatively by means of L-lactate dehydrogenase [8].

RESULTS

Isolation and Chromatographic Data

Rechromatography on cellulose of the *O*-acylated *N*-acylneuraminic acids from bovine submandibular glands resulted in the elution pattern as given in Fig. 1. By using the previously described analytical techniques [2], fractions 128–133 were shown to contain mainly an unknown acylneuraminic acid, while the other peaks could be related to already known acylneuraminic acids such as $Ac_2^{7,9}$NeuNAc,

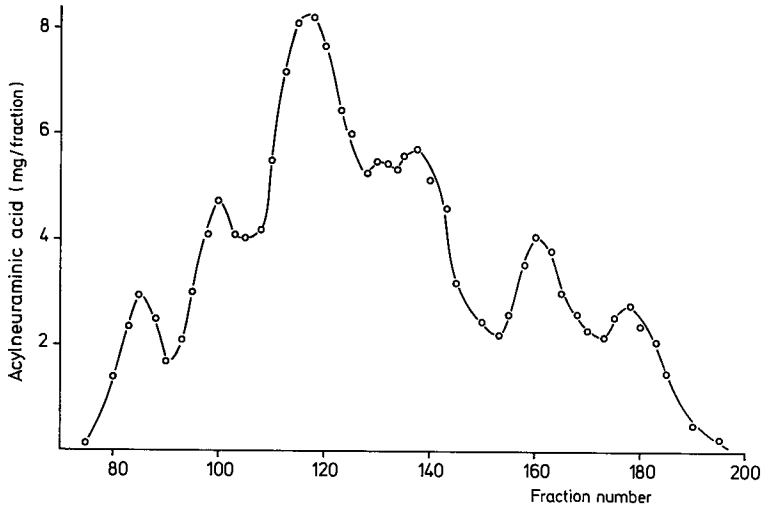

Fig. 1. *Rechromatography of* N-*acyl*-O-*acylneuraminic acids from bovine submandibular gland glycoproteins on cellulose in* n-*butanol*/n-*propanol*/*water (1/2/1, by vol.)*. The acylneuraminic acids were quantitatively determined using the orcinol/Fe^{3+}/HCl reagent. Main acylneuraminic acids of fractions: 75–92, propyl and butyl esters of acylneuraminic acids formed spontaneously during cellulose chromatography; 93–104, $Ac_2^{7,9}$NeuNAc; 105–127, Ac^9NeuNAc and Ac^7NeuNAc; 128–133, Lac^9NeuNAc; 134–152, Ac^9NeuNGl; 153–172, NeuNAc; 173–200, NeuNGl. NeuNAc and NeuNGl are degradation products of the *O*-acylated acylneuraminic acids

Ac^9NeuNAc, Ac^7NeuNAc, Ac^9NeuNGl, NeuNAc and NeuNGl [1–3].

Thin-layer chromatography on cellulose with solvent A gave an R_F value of 0.70 for the unknown compound, which differs from the R_F values of Ac^7NeuNAc, Ac^9NeuNAc and Ac^9NeuNGl. On silica gel with solvent B an R_F value of 0.61 was found, identical to that of these mono-*O*-acetylated *N*-acylneuraminic acids. The various R_F values for the acylneuraminic acids from bovine submandibular gland in both systems are given in Table 1. On gas chromatography a R_{NeuNAc} value of 2.55 was found for the new compound indicating a strong retardation with regard to the above-mentioned acylneuraminic acids (see Table 1).

The amount of the new acylneuraminic acid was estimated to be approximately 20 mg (orcinol/Fe^{3+}/HCl reagent). This corresponds to 1.7% of the total amount of acylneuraminic acids (1.2 g) obtained from the glycoproteins of bovine submandibular gland by mild acid hydrolysis.

Identification

The number and type of *N*-acyl and *O*-acyl substituents in the new acylneuraminic acid were determined by colorimetry and thin-layer chromatography [2]. The presence of 1 *O*-acyl group/mol of the new acylneuraminic acid was demonstrated by the hydroxamate/Fe^{3+} reaction. On cellulose thin-layer chromatography the hydroxamate, obtained from the *O*-acyl group, had the same R_F value (0.65) as the hydroxamate of lactic acid. The R_F values of the hydroxamates of a series of organic acids as reference substances are presented in Table 2. Two-dimensional thin-layer chromatography on cellulose with intermediary ammonia treatment showed the formation of NeuNAc, indicating the presence of a *N*-acetyl group.

The number, type and position of the substituents in the new acylneuraminic acid were further established

Table 1. *Thin-layer and gas-liquid chromatographic data of acylneuraminic acids from bovine submandibular gland glycoproteins*
R_F values on cellulose in solvent A and on silica gel in solvent B; R_{NeuNAc} values on 3.8% SE-30 of the methyl esters, trimethylsilyl ethers of acylneuraminic acids

Compound	R_F value in		R_{NeuNAc}
	system A	system B	
NeuNGl	0.48	0.39	1.81
NeuNAc	0.57	0.39	1.00
Ac^9NeuNGl	0.65	0.61	2.04
Lac^9NeuNAc	0.70	0.61	2.55
Ac^9NeuNAc	0.76	0.61	1.13
Ac^7NeuNAc	0.76	0.61	1.04
$Ac_2^{7,9}$NeuNAc	0.83	0.73	1.14

Table 2. R_F-*values of hydroxamates obtained by thin-layer chromatography on cellulose in solvent C*

Hydroxamate from	R_F
Formic acid	0.50
Acetic acid	0.71
Glycolic acid	0.52
Propionic acid	0.81
Hydracrylic acid	0.60
Lactic acid (and *O*-acyl residue of Lac^9NeuNAc)	0.65

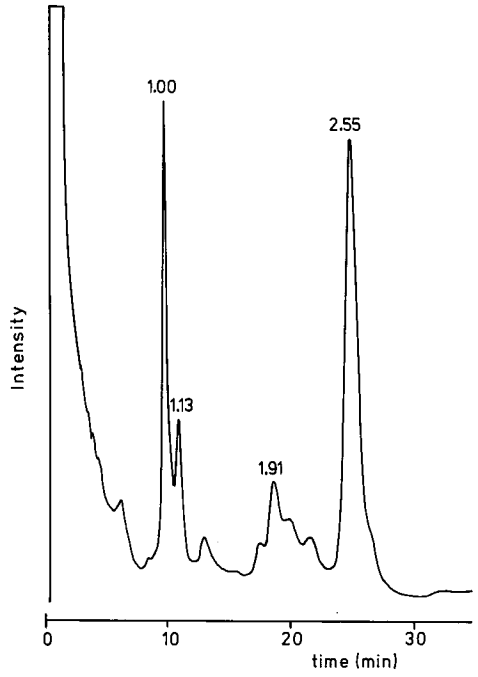

Fig. 2. *Gas-liquid chromatography on 3.8% SE-30 of acylneuraminic acids from fraction 130 of Fig. 1 as their methyl esters pertrimethylsilyl ethers.* Peaks, identified by combined gas-liquid chromatography/mass spectrometry: R_{NeuNAc} 1.00, NeuNAc; R_{NeuNAc} 1.13, Ac^9NeuNAc; R_{NeuNAc} 1.91, undersilylation product of Lac^9NeuNAc; R_{NeuNAc} 2.55, Lac^9NeuNAc

by combined gas-liquid chromatography/mass spectrometry. In Fig. 2 the gas chromatogram on 3.8% SE-30 of the esterified and trimethylsilylated compounds of fraction 130 is given. The most significant peaks in the gas chromatogram are those with R_{NeuNAc} values of 1.00, 1.13, 1.91 and 2.55 respectively. The mass spectrum of the new acylneuraminic acid derivative, having an R_{NeuNAc} value of 2.55, is given in Fig. 3. The interpretation of this spectrum was carried out on guidance of a recently developed mass spectrometric method for the characterization of *O*-acetylated *N*-acylneuraminic acids [1]. In this method a set of specific fragment ions, A up to G, is used, as illustrated in Fig. 4.

The first detectable peak in the high-mass range of the spectrum of the acylneuraminic acid derivative represents $M^{+\cdot}$ minus $^{\cdot}CH_3$ (*m/e* 740, fragment A; $C_{29}H_{62}NO_{11}Si_5$). The CH_3 group is eliminated from a trimethylsilyl group. The peak at *m/e* 696 corresponds with $M^{+\cdot}$ minus $^{\cdot}COOCH_3$ (fragment B; $C_{28}H_{62}NO_9Si_5$). Thus in comparison to the mass spectrum of the trimethylsilyl derivative of the methyl ester of NeuNAc [1, 9], the *m/e* values of the fragments A and B are 72 daltons higher. This is in agreement with the presence of an *N*-acetyl group and the replacement of one trimethylsilyl group by a trimethylsilylated hydroxypropionyl group.

In model studies it was found that the trimethylsilyl ester, ether of 2-hydroxypropionic acid (lactic acid) gives rise to an intense peak at *m/e* 117, which was absent in the case of the trimethylsilyl ester, ether of 3-hydroxypropionic acid (hydracrylic acid) [10]. The splitting between C-1 and C-2, resulting in abundant fragment ions at *m/e* [*M* minus $COOSi(CH_3)_3$], was generally observed for 2-hydroxycarboxylic acids (J. P. Kamerling, unpublished work). The presence of the intense peak at *m/e* 117, which is related for the greater part with $C_5H_{13}OSi$ [$CH_3-CH=\overset{+}{O}Si(CH_3)_3$] and for the minor part with $C_4H_9O_2Si$ (generally present in acylneuraminic acids), classifies the hydroxypropionyl group in the new acylneuraminic acid as a lactyl group and rules out the hydracryl group.

The *m/e* values of the fragments C, D and E, representing $M^{+\cdot}$ minus $^{\cdot}CHOR_8-CH_2OR_9$, $M^{+\cdot}$ minus $^{\cdot}CHOR_8-CH_2OR_9$ minus R_2OH minus R_4OH and $M^{+\cdot}$ minus $^{\cdot}CHOR_7-CHOR_8-CH_2OR_9$ minus R_5CONH_2 respectively, are identical to those found for the trimethylsilyl derivatives of the methyl esters of NeuNAc and Ac^9NeuNAc (*m/e* 478, *m/e* 298 and *m/e* 317 respectively). By consequence R_5 is a CH_3 group and R_2, R_4 and R_7 are trimethylsilyl groups. Furthermore, the trimethylsilylated lactyl group $\{CO-CH[OSi(CH_3)_3]-CH_3\}$ can only be located at C-8 or C-9.

Fragment F ($CH_2OR_9-CH=\overset{+}{O}R_8$), represented by the peak at *m/e* 277 ($C_{11}H_{25}O_4Si_2$), is shifted +72 daltons with respect to the trimethylsilyl derivative of the methyl ester of NeuNAc. This indicates that R_8 is a trimethylsilyl group and R_9 a $CO-CH[OSi(CH_3)_3]-CH_3$ group. Thus the *O*-lactyl group is present at C-9. In case of an *O*-acyl substituent at C-8, the fragment F would not occur [1, 7].

Fragment G ($R_5\overset{+}{C}ONH=CH-\dot{C}HOR_4$) is detected at *m/e* 173, in accordance with the assignments given above for R_5 and R_4, being CH_3 and trimethylsilyl groups respectively.

It has to be noted that the presence of the peak at *m/e* 186 is also indicative for the occurrence of an *N*-acetyl group ($CH_3CO-\overset{+}{N}H=CH-CH=CHOSi(CH_3)_3$ or $CH_3CO-\overset{+}{N}H=CH-C[OSi(CH_3)_3]=CH_2$) [1]. Finally, the presence of the peak at *m/e* 217 $\{CH_2=C[OSi(CH_3)_3]-CH=\overset{+}{O}Si(CH_3)_3\}$ formed by elimination of $CH_3-CH[OSi(CH_3)_3]-COOH$ from the C-7–C-9 part of the molecule, in combination with the absence of *m/e* 289 (elimination of trimethylsilanol from the C-7–C-9 part of the molecule) points also to the presence of the *O*-lactyl group at C-9 [1].

The absolute configuration of the lactyl substituent in Lac^9NeuNAc was determined enzymically using L-lactate and D-lactate dehydrogenase respectively. The ester residue, which was liberated from the acylneuraminic acid by alkaline hydrolysis, was readily oxidized under the influence of L-lactate dehydro-

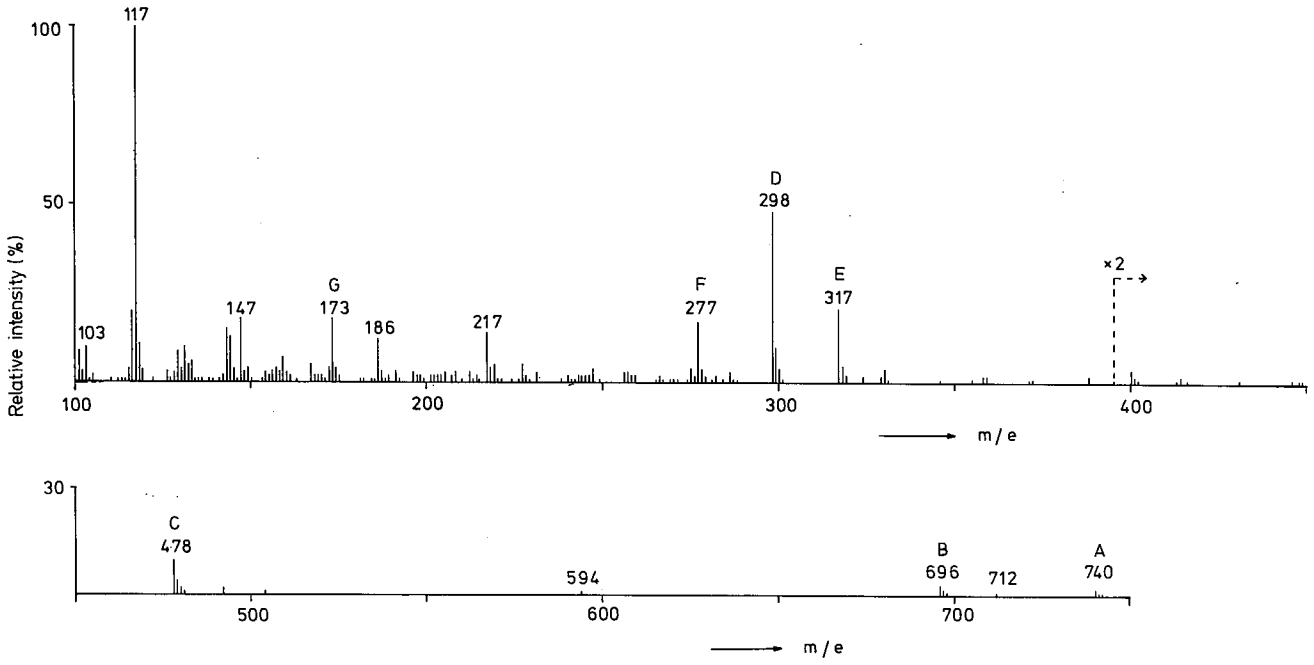

Fig. 3. *Mass spectrum of the trimethylsilyl derivative of the methyl ester of* N-*acetyl-9-*O-L-*lactylneuraminic acid.* Only *m/e* values > 100 are given. The spectrum is taken at the top of the peak with R_{NeuNAc} 2.55 (Fig. 2) on combined gas-liquid chromatography/mass spectrometry

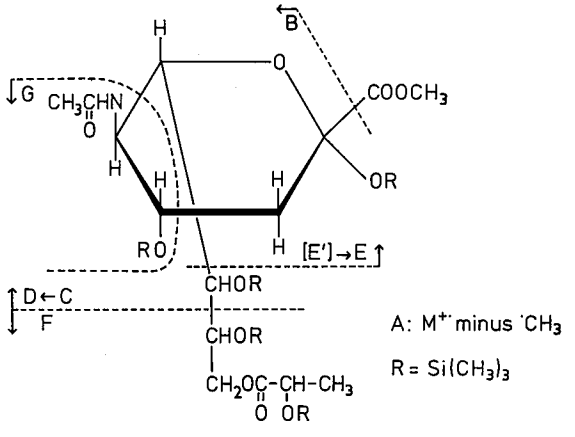

Fig. 4. *Formula of the trimethylsilyl derivative of the methyl ester of* N-*acetyl-9-*O-L-*lactylneuraminic acid showing the characteristic fragmentations observed on mass spectrometry and the nomenclature of the fragments*

genase, whereas it was resistant to the action of D-lactate dehydrogenase. Quantitative estimation showed the presence of 1 mol L-lactic acid/mol neuraminic acid.

The origin of the other peaks in the gas chromatogram of Fig. 2 was also established. The mass spectrum of the compound with R_{NeuNAc} 1.00 corresponds with that of the trimethylsilyl derivative of the methyl ester of NeuNAc [9]. NeuNAc is a degradation product of Lac^{9}NeuNAc. It might be formed on storage and/or during the silylation procedure. In the series of *O*-acetylated *N*-acylneuraminic acids [1] such a de-*O*-acylation during silylation has not been observed so far. The spectrum of the peak at R_{NeuNAc} 1.13 shows that this compound is the trimethylsilyl derivative of the methyl ester of Ac^{9}NeuNAc. Ac^{9}NeuNAc is present as a minor component in the fraction 130. The peak at R_{NeuNAc} 1.91 is an undersilylation product of Lac^{9}NeuNAc in which the hydroxyl group of the lactyl residue is unsubstituted. The mass spectrum of this compound shows at first glance a great similarity with that of the trimethylsilyl derivative of the methyl ester of NeuNAc, because both compounds have the same nominal molecular weight. However, no peaks at *m/e* 204 and *m/e* 300 were observed in the spectrum of the undersilylated compound.

Periodate Oxidation

The rate of oxidation of Lac^{9}NeuNAc by periodate was similar to that observed earlier for acylneuraminic acids having 9-*O*-acetyl groups [7]. At room temperature, Lac^{9}NeuNAc consumed 0.1 µmol periodate/µmol neuraminic acid within 10 min and 0.25 µmol within 60 min, respectively. Possible reasons for this retarded oxidation rate of acylneuraminic acids substituted at C-9 (when compared with NeuNAc) were discussed recently [7].

Due to this low oxidation rate the molar absorbance coefficient in the periodic acid/thiobarbituric acid assay of Lac^{9}NeuNAc was only 40% when compared with NeuNAc. This corresponds to the behaviour of Ac^{9}NeuNAc in this colorimetric test [7]. In contrast, the molar absorbance coefficient of

Lac^{9}NeuNAc in the orcinol/Fe^{3+}/HCl assay was identical with that of NeuNAc.

DISCUSSION

The described identification procedure for *N*-acetyl-9-*O*-L-lactylneuraminic acid illustrates the value and limitations of the various methods which are applied to this purpose. The occurrence of *O*-acyl substituents in acylneuraminic acids can easily be established by means of the two-dimensional thin-layer chromatographic method with intermediary ammonia treatment [2]. This method can also give information about the *N*-acyl substituent. The number of *O*-acyl substituents follows from the quantitative hydroxamate/Fe^{3+} reaction. The nature of the *O*-acyl substituents can be established by identification of the corresponding hydroxamates. Similarly, the *N*-acyl substituents can be determined after conversion to their methyl esters by methanolysis [2]. It is difficult to gain conclusive evidence for the position of the *O*-acyl substituents along classical routes [2,3]. Recently it has been demonstrated [7] that the frequently applied periodate oxidation is hindered for the vicinal hydroxyl groups at C-7 and C-8 when an acyl group is located at C-9. Mass spectrometry of the pure compound is a suitable method to determine the positions of the substituents. Concomitantly, information is obtained on the number and nature of the substituents. Application of a gas-liquid chromatography/mass spectrometry combination is obligatory if no pure compounds can be isolated.

Nothing is known about the formation of *N*-acetyl-9-*O*-L-lactylneuraminic acid in the submandibular gland. Although the lactate concentration was found to be relatively high (7.5 mM) in this tissue, it is unlikely that the compound is formed by a non-enzymic process. Incubation during 24 h at 37 °C and pH 7.4 or during 1 h at 70 °C and pH 2 (conditions for acid hydrolysis of glycoproteins) of ^{14}C-labelled NeuNAc in the presence of this lactate concentration did not result in the formation of radioactive Lac^{9}NeuNAc. Work is in progress to elucidate the biosynthesis of *N*-acetyl-9-*O*-L-lactylneuraminic acid.

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REFERENCES

1. Kamerling, J. P., Vliegenthart, J. F. G., Versluis, C. & Schauer, R. (1975) *Carbohydr. Res. 41*, 7–17.
2. Buscher, H.-P., Casals-Stenzel, J. & Schauer, R. (1974) *Eur. J. Biochem. 50*, 71–82.
3. Schauer, R. & Faillard, H. (1968) *Hoppe-Seyler's Z. Physiol. Chem. 349*, 961–968.
4. Böhm, P., Dauber, St. & Baumeister, L. (1954) *Klin. Wochenschr. 32*, 289–292.
5. Warren, L. (1959) *J. Biol. Chem. 234*, 1971–1975.
6. Hestrin, S. (1949) *J. Biol. Chem. 180*, 249–261.
7. Haverkamp, J., Schauer, R., Wember, M., Kamerling, J. P. & Vliegenthart, J. F. G. (1975) *Hoppe-Seyler's Z. Physiol. Chem. 356*, 1575–1583.
8. Hohorst, H. J. in Bergmeyer, H. U. (1970) *Methoden der enzymatischen Analyse II*, pp. 1425–1450, Verlag Chemie, Weinheim.
9. Kamerling, J. P., Vliegenthart, J. F. G. & Vink, J. (1974) *Carbohydr. Res. 33*, 297–306.
10. Petersson, G. (1970) *Tetrahedron, 26*, 3413–3428.

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