Journal of Chromatography, 210 (1981) 487–504
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,708

GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY OF METHYL ETHERS OF METHYL N-ACETYL-N-METHYL-β-D-NEURAMINATE METHYL GLYCOSIDE*

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(Received February 5th, 1981)

SUMMARY

Partial methylation of methyl N-acetyl-β-D-neuraminate methyl glycoside using methyl iodide and silver oxide gives a mixture of methyl ethers of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside, which was fractionated by chloroform–water partition followed by preparative column chromatography on silica gel. After trimethylsilylation of the fractions, gas–liquid chromatography on OV-101 and mass spectrometry facilitated the identification of 13 methyl ethers.

INTRODUCTION

In glycoproteins and glycolipids, sialic acids are principally present as terminal monosialyl residues of the carbohydrate chains[1]. Furthermore, terminal sialyl-α (2 → 8)-sialyl sequences have been reported for several glycoproteins[2–4] and glycolipids[5–8]. Also, higher (2 → 8)-linked sialyl oligomers have been described[5,9]. Morever, in glycolipids terminal sialyl-α(2 → 4)-sialyl sequences[10,11] and internal (1 → 4)- and (1 → 4)(1 → 8)-linked sialic acid residues have been observed[12–14].

Sialic acids also occur as constituents of homo- and heteropolysaccharides and of oligosaccharides. Terminal N-acetylneuraminyl residues have been found in polysaccharides from streptococcal strains[15,16]. Colominic acid isolated from *Escherichia coli*[17,18] and the polysaccharide from *Neisseria meningitidis* serogroup C[17] consist of linked and (2 → 9)-linked N-acetylneuraminic acid homopolymers, respectively. The heteropolysaccharides from *N. meningitidis* serogroups Y and W-135 contain internal (1 → 4)-linked N-acetylneuraminyl units[17,19].

* Presented in part at the *IX Journées sur la Chimie et la Biochimie des Glucides, Aussois, France, January 12–14, 1981.*

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0021-9673/81/0000–0000/$02.50

The elucidation of the complete structure of carbohydrates and glycoconjugates by methylation analysis, including gas–liquid chromatography (GLC) and mass spectrometry (MS), is facilitated by the availability of series of reference methyl ethers of monosaccharides. In the past, we have prepared a series of partially methylated mannoses and galactoses by under-methylation of the methyl glycosides of α-D-mannose and α-D-galactose, respectively[20–22]. Using the same procedure, Ovodov and Evtushenko[23,24] described the preparation of a series of methyl ethers of methyl β-D-xylopyranoside. The synthesis of partially O-methylated N-acetyl-N-methyl-D-glucosamines was reported by Tai *et al.*[25].

Recently, the linkage analysis of acylneuraminic acids was also incorporated into the methylation procedure. Partially O-methylated derivatives of methyl N-acyl-N-methyl-β-D-neuraminate methyl glycoside (acyl = acetyl or methylated glycolyl) can be obtained by methanolysis (and, if necessary, re-N-acetylation)[26] of permethylated sialocarbohydrates and sialoglycoconjugates. Only a few percent of the corresponding α-D-anomers are present. Several applications have already been reported[4,6–14,17,18]. The methyl ethers formed were analysed as such or as their trimethylsilyl (TMS) and acetyl derivatives. Reference substances were described by Rauvala and Kärkkäinen[6] (three methyl ethers), Bhattacharjee and Jennings[17] (four methyl ethers) and Van Halbeek *et al.*[27] (seven methyl ethers). For a survey of these substances, see the footnote to Table III.

In this paper we describe the preparation of thirty methyl ethers of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside using the previously applied under-methylation procedure[20–22]. Retention times on OV-101 and mass spectrometric data for the TMS derivatives are given.

EXPERIMENTAL

GLC of the various derivatives of methyl N-acetyl-β-D-neuraminate methyl glycoside was carried out on a Girdel Model 30 gas chromatograph (Girdel, Suresnes, France) equipped with a flame-ionization detector and a glass capillary column (80 m × 0.35 mm I.D.) wall-coated with OV-101. The column oven temperature was 215°C and the helium pressure was 0.4 bar.

Mass spectra were recorded on a Riber Mag 10-10 mass spectrometer (Riber, Rueil-Malmaison, France) under the following conditions: electron energy, 70 eV; ion source temperature, 100°C; ionizing current, 200 μA.

Thin-layer chromatography (TLC) was performed on Kieselgel 60 (0.25 mm) (E. Merck, Darmstadt, G.F.R.) using chloroform–methanol (9:1). Spots were revealed with 5% sulphuric acid.

For the trimethylsilylation, the compounds (0.2 mg) were dissolved in pyridine (100 μl) and treated with 100 μl of hexamethyldisilazane and 50 μl of chlorotrimethylsilane for 15 min at room temperature.

Preparation of methyl N-acetyl-β-D-neuraminate methyl glycoside

Methyl N-acetyl-β-D-neuraminate methyl glycoside was prepared by the procedure of Fischer[28] and Matsushima and Miyazaki[29]. A 2.3-g amount of N-acetylneuraminic acid, isolated from the urine of a sialuria patient according to Montreuil *et al.*[30], dried over P_2O_5 for 24 h, was dissolved in 368 ml of anhydrous methanol

containing 4.6 g of Dowex 50-X8 (H^+) (25–50 mesh). The mixture was refluxed for 48 h. After filtration, the solution was concentrated to 10 ml and the methyl N-acetyl-β-D-neuraminate methyl glycoside was precipitated with diethyl ether. The white crystalline material was dried over P_2O_5 and analysed for purity by TLC and by GLC after trimethylsilylation: yield, 30%; $[\alpha]_D^{20} = -34.7°$ (c = 1, methanol).

Partial methylation of methyl N-acetyl-β-D-neuraminate methyl glycoside

To a cooled (0°C), stirred solution of methyl N-acetyl-β-D-neuraminate methyl glycoside (698 mg) in N,N-dimethylformamide (64 ml) (E. Merck) were added, in small portions, methyl iodide (3.2 ml) (Prolabo, Paris, France) and freshly prepared silver oxide[31] (6.4 g). After 23 h at 20°C, the mixture was filtered and the insoluble material was carefully washed on the filter with chloroform. The subsequent extraction procedure was identical with that previously described[20–22]. The organic (587 mg) and aqueous (182 mg) layers obtained were analysed by GLC after trimethylsilylation.

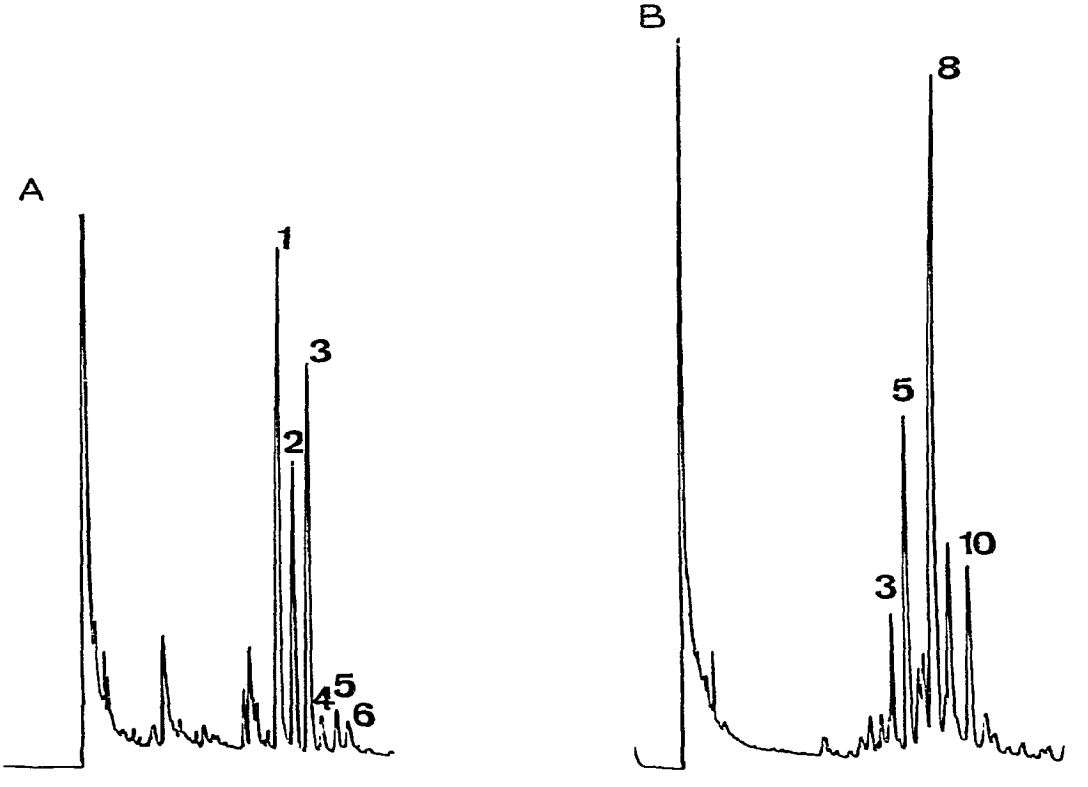


Fig. 1. GLC on an OV-101 glass capillary column of the methyl derivatives of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside after trimethylsilylation, present in the organic layer (A) and in the aqueous layer (B) of the mixture obtained by partial methylation of methyl N-acetyl-β-D-neuraminate methyl glycoside. 1 = Methyl N-acetyl-N-methyl-4,7,8,9-tetra-; 2 = -4,8,9-tri-; 3 = -4,7,9-tri-; 4 = -7,8,9-tri-; 5 = -8,9-di-; 6 = -4,9-di-; 8 = -7,9-di; 10 = -4,7-di-O-methyl-β-D-neuraminate methyl glycosides.

Fractionation of the organic and aqueous layers on a silica gel column and identification of the methyl derivatives

The methyl ethers of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside present in the organic and aqueous layers were fractionated by preparative column chromatography on silica gel as follows: the material of the organic layer (587 mg) and that of the aqueous layer (182 mg), dissolved in chloroform–methanol (9:1) (2 and 1 ml, respectively) were chromatographed on a 100 × 2.5 cm I.D. column of silica gel (Kieselgel 60, 0.063–0.2 mm, 70–230 mesh; E. Merck) using chloroform–methanol (9:1) as eluent. The methylated derivatives in the effluent were located by TLC using chloroform–methanol (9:1); the spots were revealed with 5% sulphuric acid. Each positive fraction obtained was analysed by GLC–MS.

RESULTS AND DISCUSSION

GLC analysis of the methyl derivatives of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside, present in the organic layer (Fig. 1A) and in the aqueous layer (Fig. 1B), showed such complex heterogeneity that it was not possible to identify each methyl ether directly by mass spectrometry. This is in contrast with the results obtained earlier for the methyl ethers of mannose and galactose[20–22]. To resolve this problem, the organic and aqueous layers were further fractionated by preparative column chromatography on silica gel.

Preparative column chromatography of the organic layer provided five fractions, which correspond, according to their order of emergence from the column, to methyl N-acetyl-N-methyl-4,7,8,9-tetra-O-methyl-β-D-neuraminate methyl glycoside (fraction I), methyl N-acetyl-N-methyl-4,7,8,9-tetra- and -4,8,9-tri-O-methyl-β-D-neuraminate methyl glycosides (fraction II), methyl N-acetyl-N-methyl-4,7,8,9-tetra-, -4,8,9-tri- and -4,7,9-tri-O-methyl-β-D-neuraminate methyl glycosides (fraction III), methyl N-acetyl-N-methyl-4,7,9-tri-, -7,8,9-tri-, -4,9-di- and -4,7,8-tri-O-methyl-β-D-neuraminate methyl glycosides (fraction IV) and methyl N-acetyl-N-methyl-4,7,9-tri-, -7,8,9-tri- and -4,7,8-tri-O-methyl-β-D-neuraminate methyl glycosides (fraction V). The assignments in each fraction were made by GLC–MS after trimethylsilylation (see Fig. 2 for GLC). The yields of the fractions are given in Table I.

Preparative column chromatography of the aqueous layer provided eight fractions, which correspond, according to their order of emergence from the column, to methyl N-acetyl-N-methyl-4,7,8,9-tetra-, -4,8,9-tri-, -4,7,9-tri- and -4,9-di-O-methyl-β-D-neuraminate methyl glycosides (fraction I), methyl N-acetyl-N-methyl-4,7,9-tri-, -7,8,9-tri- and -4,9-di-O-methyl-β-D-neuraminate methyl glycosides (fraction II), methyl N-acetyl-N-methyl-4,7,9-tri-, -7,8,9-tri-, -8,9-di-, -4,9-di-, -4,7,8-tri-O-methyl-β-D-neuraminate methyl glycosides (fraction III), methyl N-acetyl-N-methyl-8,9-di-O-methyl-β-D-neuraminate methyl glycoside (fraction IV), methyl N-acetyl-N-methyl-8,9-di-, -7,9-di-, -9-mono-O-methyl-β-D-neuraminate methyl glycosides (fraction V), methyl N-acetyl-N-methyl-7,9-di-, -9-mono-, -7,8-di-O-methyl-β-D-neuraminate methyl glycosides (fraction VI), methyl N-acetyl-N-methyl-7,9-di-, -9-mono-, -4,7-di-, -7,8-di-, -4-mono-O-methyl-β-D-neuraminate methyl glycosides (fraction VII) and methyl N-acetyl-N-methyl-7,9-di-, -9-mono-, -4,7-di-, -7,8-di-, -4-mono-, -7-mono-O-methyl-β-D-neuraminate methyl glycosides and methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside (fraction VIII). For the assignments,

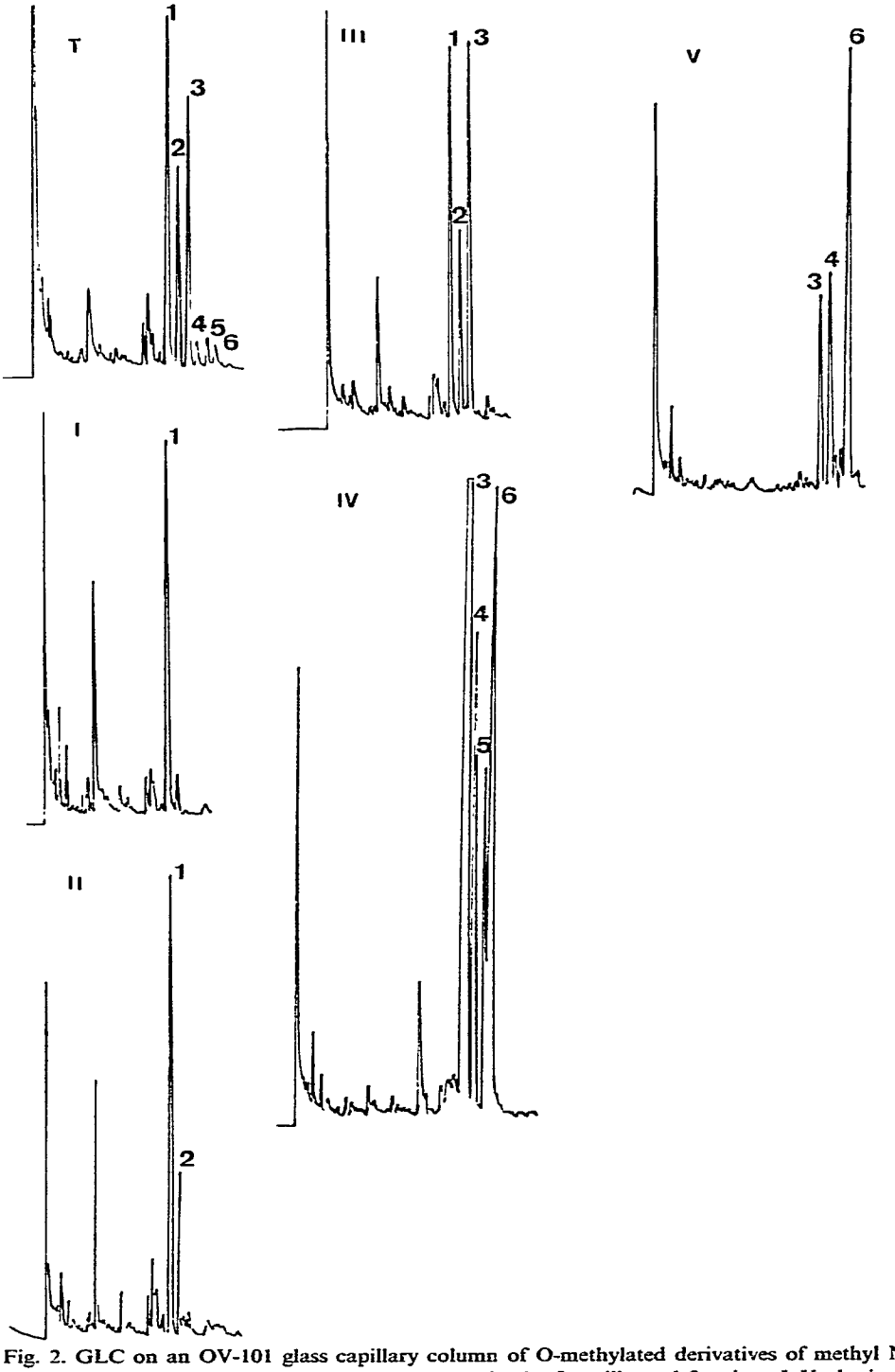


Fig. 2. GLC on an OV-101 glass capillary column of O-methylated derivatives of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside present in the five silica gel fractions I–V obtained from the organic layer (after trimethylsilylation). T = Organic layer; I–V = fraction numbers; 1 = methyl N-acetyl-N-methyl-4,7,8,9-tetra-; 2 = -4,8,9-tri-; 3 = -4,7,9-tri-; 4 = -7,8,9-tri-; 5 = -8,9-di-; 6 = -4,9-di-O-methyl-β-D-neuraminate methyl glycosides.

TABLE I

AMOUNTS OF O-METHYLATED ETHERS OF METHYL N-ACETYL-N-METHYL-β-D-NEURAMINATE METHYL GLYCOSIDE ISOLATED FROM THE ORGANIC LAYER BY SILICA GEL COLUMN CHROMATOGRAPHY

Fraction	*Compound*	*Amount (mg)*	*Yield (%)*
Organic layer		587	–
I	4,7,8,9-Tetra-O-methyl	169	29
II	4,7,8,9-Tetra-O-methyl 4,8,9-Tri-O-methyl	170	29
III	4,7,8,9-Tetra-O-methyl 4,8,9-Tri-O-methyl 4,7,9-Tri-O-methyl	184	31
IV	4,7,9-Tri-O-methyl 7,8,9-Tri-O-methyl 4,7,8-Tri-O-methyl 4,9-Di-O-methyl	46	8
V	4,7,9-Tri-O-methyl 7,8,9-Tri-O-methyl 4,7,8-Tri-O-methyl	5	1

each fraction was analysed by GLC–MS after trimethylsilylation (see Fig. 3 for GLC); the yields of the different fractions are given in Table II.

The GLC retention times of the methyl ethers on OV-101, relative to methyl N-acetyl-N-methyl-4,7,8,9-tetra-O-methyl-β-D-neuraminate methyl glycoside, are presented in Table III.

The interpretation of the mass spectra is based on the data for acylneuraminic acid derivatives reported earlier[27] (Fig. 4). Under conditions of electron impact, the molecule M gives eight specific fragments represented by A–H. The mass spectra and the *m/z* values of the fragment ions A–H of the methylated derivatives of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside and of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside itself (free hydroxyl functions are trimethylsilylated) are presented in Fig. 5 and Table IV, respectively. The primary fragments A ($M-CH_3$) and B ($M-COOCH_3$) allow the classification of the methyl ethers: *m/z* 392 and 348 for the permethyl ether; *m/z* 450 and 406 for the trimethyl ethers; *m/z* 508 and 464 for the dimethyl ethers; and *m/z* 566 and 522 for the monomethyl ethers. In the same way, methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside is characterized by *m/z* 624 and 580.

The other C–H fragments allow to distinguish the different mono-, di- and trimethyl isomers. They give information about the distribution of the methoxy and the trimethylsiloxy substituents over the C-4, -7, -8 and -9 positions (see Table IV).

Fragments C ($M-CHOR_8CH_2OR_9$) and D ($M-CHOR_8CH_2OR_9-CH_3OH-R_4OH$) make it possible to obtain a first impression of the distribution of the substituents.

Fragment E [$M-CHOR_7CHOR_8CH_2OR_9-N(CH_3)HCOCH_3$] indicates directly the substituent at C-4: *m/z* 201, O-methyl group; *m/z* 259, O-TMS group.

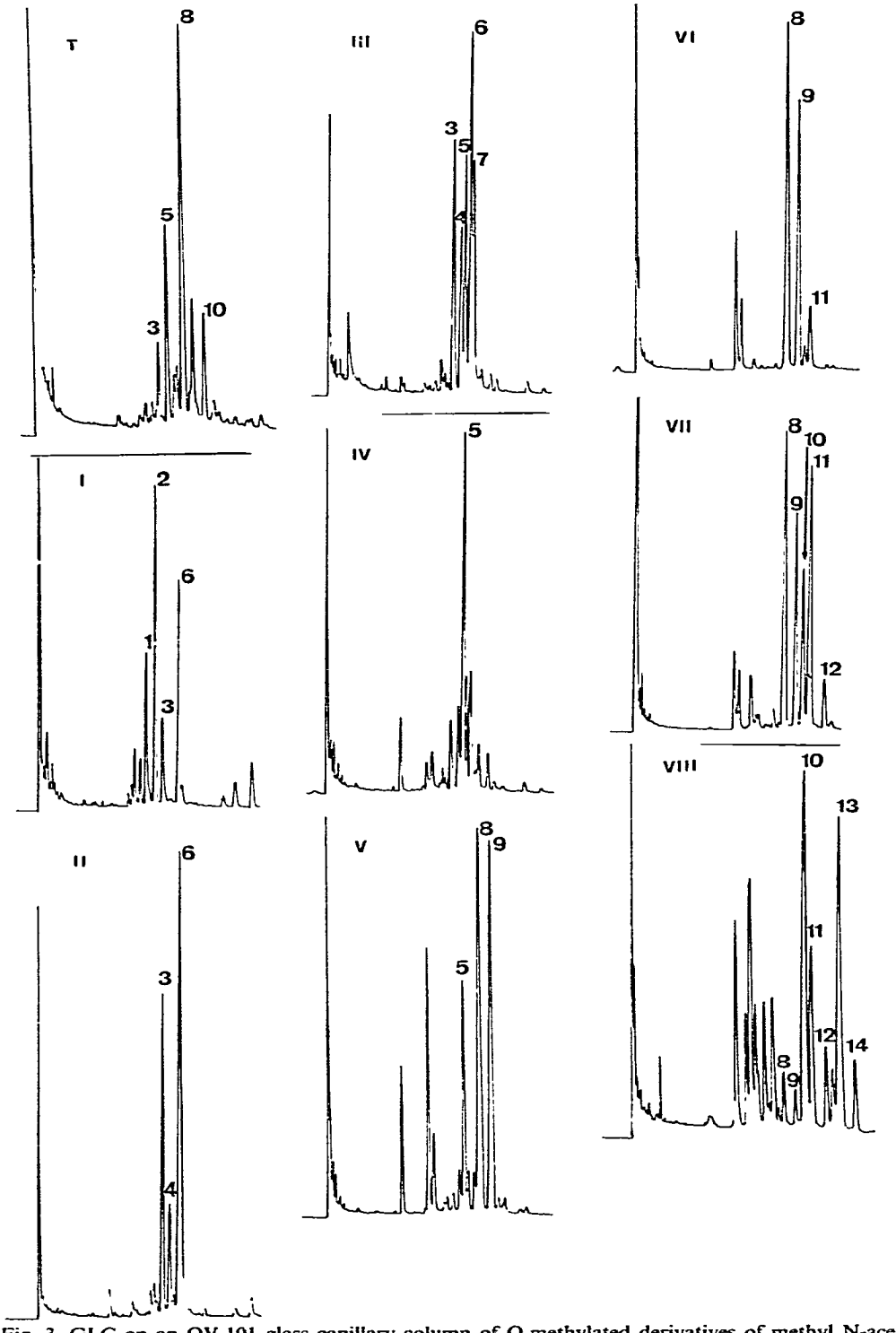


Fig. 3. GLC on an OV-101 glass capillary column of O-methylated derivatives of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside present in the eight silica gel fractions I–VIII obtained from the aqueous layer (after trimethylsilylation). T = Aqueous layer; I–VIII = fraction numbers; 1 = methyl N-acetyl-N-methyl-4,7,8,9-tetra-; 2 = -4,8,9-tri-; 3 = -4,7,9-tri-; 4 = -7,8,9-tri-; 5 = -8,9-di-; 6 = -4,9-di-; 7 = -4,7,8-tri-; 8 = -7,9-di-; 9 = -9-mono-; 10 = -4,7-di-; 11 = -7,8-di-; 12 = -4-mono-; 13 = -7-mono-O-methyl-β-D-neuraminate methyl glycosides; 14 = methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside.

TABLE II

AMOUNTS OF O-METHYLATED ETHERS OF METHYL N-ACETYL-N-METHYL-β-D-NEURAMINATE METHYL GLYCOSIDE ISOLATED FROM THE AQUEOUS LAYER BY SILICA GEL COLUMN CHROMATOGRAPHY

Fraction	*Compound*	*Amount (mg)*	*Yield (%)*
Aqueous layer		182	–
I	4,7,8,9-Tetra-O-methyl 4,8,9-Tri-O-methyl 4,7,9-Tri-O-methyl 4,9-Di-O-methyl	17	9
II	4,7,9-Tri-O-methyl 7,8,9-Tri-O-methyl 4,9-Di-O-methyl	48	26
III	4,7,9-Tri-O-methyl 7,8,9-Tri-O-methyl 4,7,8-Tri-O-methyl 8,9-Di-O-methyl 4,9-Di-O-methyl	19	10
IV	8,9-Di-O-methyl	2	1
V	8,9-Di-O-methyl 7,9-Di-O-methyl 9-Mono-O-methyl	7	4
VI	7,9-Di-O-methyl 7,8-Di-O-methyl 9-Mono-O-methyl	9	5
VII	7,9-Di-O-methyl 4,7-Di-O-methyl 7,8-Di-O-methyl 9-Mono-O-methyl 4-Mono-O-methyl	13	7
VIII	7,9-Di-O-methyl 7,8-Di-O-methyl 4,7-Di-O-methyl 4-Mono-O-methyl 7-Mono-O-methyl 9-Mono-O-methyl NeuN(Ac, Me)	29	16

Fragment F ($CH_2OR_9CHOR_8$) is found at *m/z* 89 when $R_8 = R_9 = CH_3$ and at *m/z* 205 when $R_8 = R_9 = TMS$[32]. The fragment ion *m/z* 147 (F; $C_6H_{15}O_2Si$) occurs when a methoxy and a trimethylsiloxy substituent are present in the mass spectra of trimethylsilyl-carbohydrates having more than one TMS group; in the latter instance, the structure of the *m/z* 147 ion is $(CH_3)_3SiOSi(CH_3)_2(C_5H_{15}OSi_2)$[27].

Fragment G [$CH_3CON(CH_3)CH-CHOR_4$] is found at *m/z* 129 when R_4 is CH_3. Fragment H ($M-CH_2OR_9-R_4OH-R_7OH$) allows to identify the nature of the ether group on C-8. It has an *m/z* value of 298 when R_8 is a methyl group. When R_8 is a TMS group, this fragment is found at *m/z* 356.

TABLE III

RETENTION TIMES ON AN OV-101 GLASS CAPILLARY COLUMN AT 215°C OF O-METHYLATED METHYL N-ACETYL-N-METHYL-β-D-NEURAMINATE METHYL GLYCOSIDES, ANALYSED AS THEIR TRIMETHYLSILYL DERIVATIVES, RELATIVE TO THE METHYL N-ACETYL-N-METHYL-4,7,8,9-TETRA-O-METHYL-β-D-NEURAMINATE METHYL GLYCOSIDE

Compound	*Relative retention time*
1,2,4,7,8,9-O-Me-NeuN(Ac, Me)*,**,***,§	1.00
1,2,4,8,9-O-Me-NeuN(Ac, Me)§	1.06
1,2,4,7,9-O-Me-NeuN(Ac, Me)**,***,§	1.14
1,2,7,8,9-O-Me-NeuN(Ac, Me)**	1.20
1,2,8,9-O-Me-NeuN(Ac, Me)	1.23
1,2,4,9-O-Me-NeuN(Ac, Me)§	1.27
1,2,4,7,8-O-Me-NeuN(Ac, Me)**,§	1.31
1,2,7,9-O-Me-NeuN(Ac, Me)	1.39
1,2,9-O-Me-NeuN(Ac, Me)§	1.50
1,2,4,7-O-Me-NeuN(Ac, Me)	1.52
1,2,7,8-O-Me-NeuN(Ac, Me)	1.60
1,2,4-O-Me-NeuN(Ac, Me)	1.78
1,2,7-O-Me-NeuN(Ac, Me)§	1.91
1,2-O-Me-NeuN(Ac, Me)§	2.05

* 1,2,4,7,8,9-O-Me-NeuN(Ac,Me) = methyl N-acetyl-N-methyl-4,7,8,9-tetra-O-methyl-β-D-neuraminate methyl glycoside, etc.

** Reported by Bhattacharjee and Jennings[17].

*** Reported by Rauvala and Kärkkäinen[6].

§ Reported by Van Halbeek *et al.*[27].

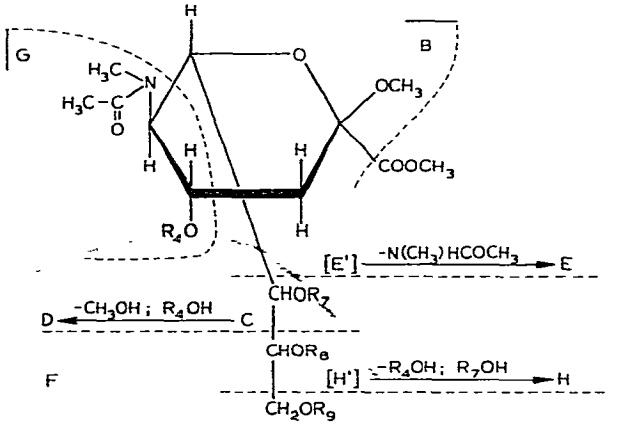


Fig. 4. Characteristic fragment ions A–H used for the mass spectrometric determination of the position of CH_3 and $(CH_3)_3Si$ substituents in the neuraminic acid derivatives. A = $M^{+\cdot} - CH_3$; R = CH_3 or $(CH_3)_3Si$.

CONCLUSIONS

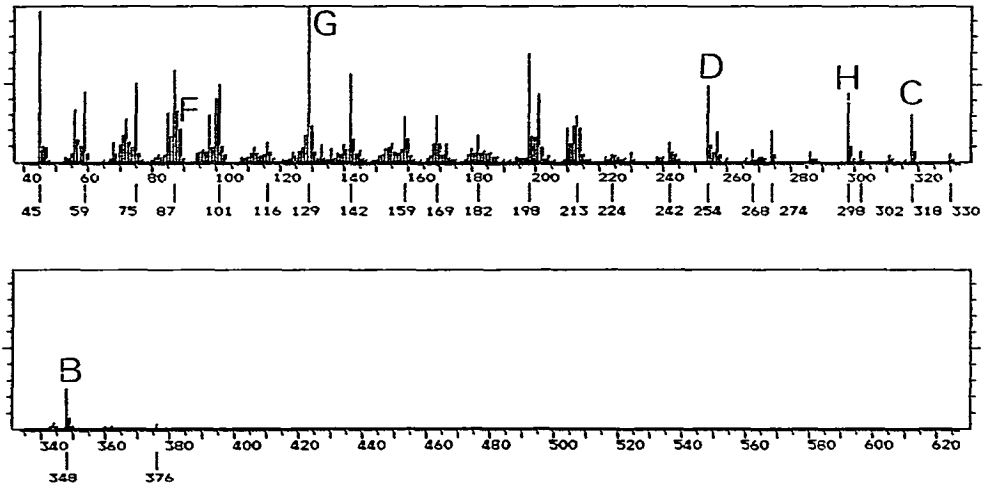
This investigation has shown that the partial methylation of methyl N-acetyl-β-D-neuraminate methyl glycoside using methyl iodide and silver oxide as a catalyst is a

TABLE IV

SPECIFIC FRAGMENTS A–H OF THE O-METHYLATED METHYL N-ACETYL-N-METHYL-β-D-NEURAMINATE METHYL GLYCOSIDES, ANALYSED AS THEIR TRIMETHYLSILYL DERIVATIVES

OCH_3 groups	*m/z*																							
	624	*580*	*566*	*522*	*508*	*464*	*450*	*434*	*406*	*392*	*376*	*356*	*348*	*318*	*312*	*306*	*298*	*259*	*254*	*205*	*201*	*187*	*147*	*129*	*89*
1,2,4,7,8,9-										A			B	C			H		D		E			G	F
1,2,4,7,8-							A		B					C			H		D		E		F	G	
1,2,4,7,9-							A		B			H		C					D		E		F	G	
1,2,4,8,9-							A		B		C				D		H				E			G	F
1,2,7,8,9-							A		B		C						H	E	D			G			F
1,2,4,7-					A	B						H		C					D	F	E			G	
1,2,4,9-					A	B					C	H			D						E		F	G	
1,2,7,8-					A	B					C						H	E	D			G	F		
1,2,7,9-					A	B					C	H						E	D			G	F		
1,2,8,9-					A	B		C							D		H	E				G			F
1,2,4-			A	B							C	H			D					F	E			G	
1,2,7-			A	B							C	H						E	D	F		G			
1,2,9-			A	B				C				H			D			E				G	F		
1,2-	A	B						C				H			D			E		F		G			

methyl-N-acetyl-N-methyl-4,7,8,9-
tetra-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-4,7,8-
tri-O-methyl-D-neuraminate
methyl glycoside

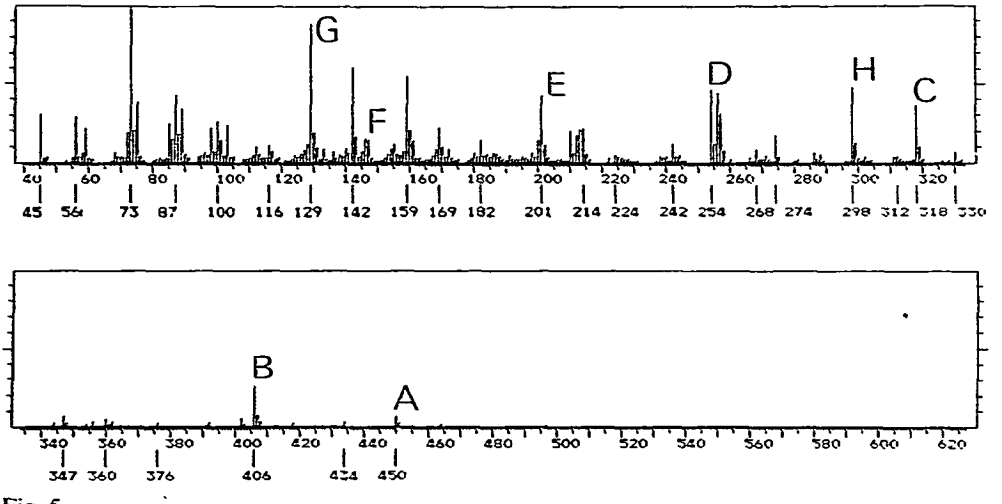
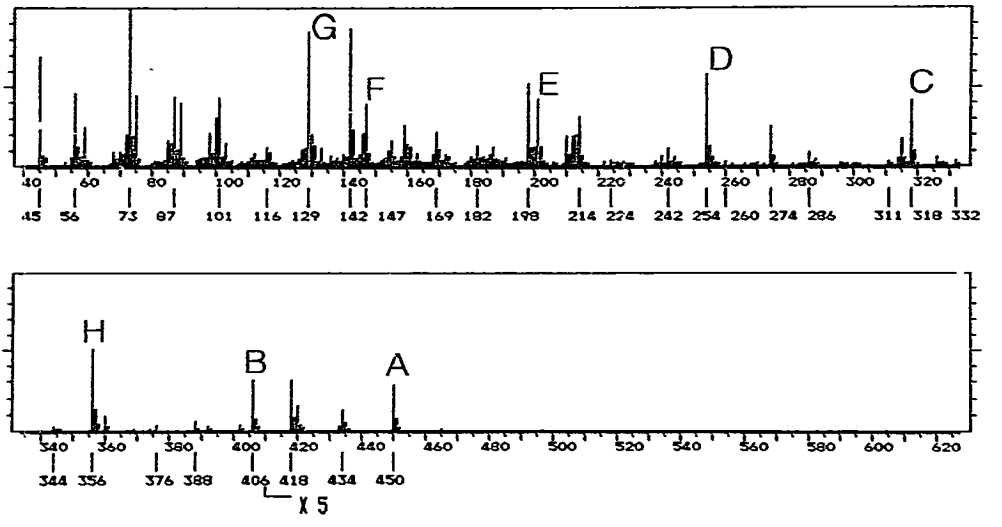


Fig. 5.

(Continued on p. 498)

methyl-N-acetyl-N-methyl-4,7,9-
tri-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-4,8,9-
tri-O-methyl-D-neuraminate
methyl glycoside

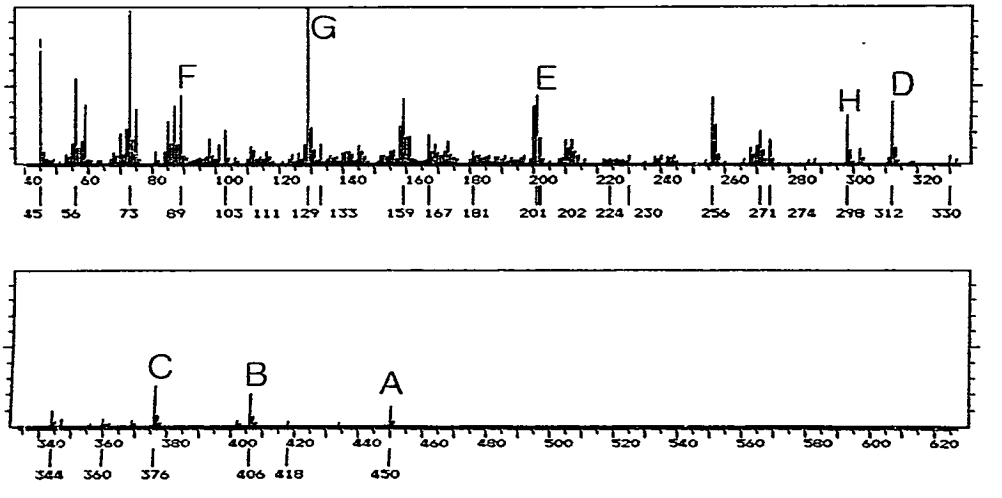
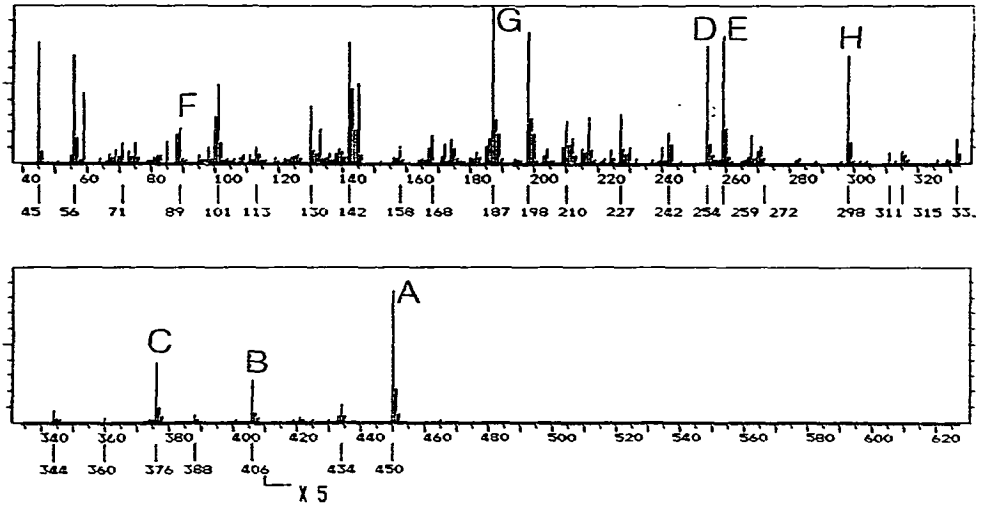


Fig. 5.

methyl-N-acetyl-N-methyl-7,8,9-
tri-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-4,7-
di-O-methyl-D-neuraminate
methyl glycoside

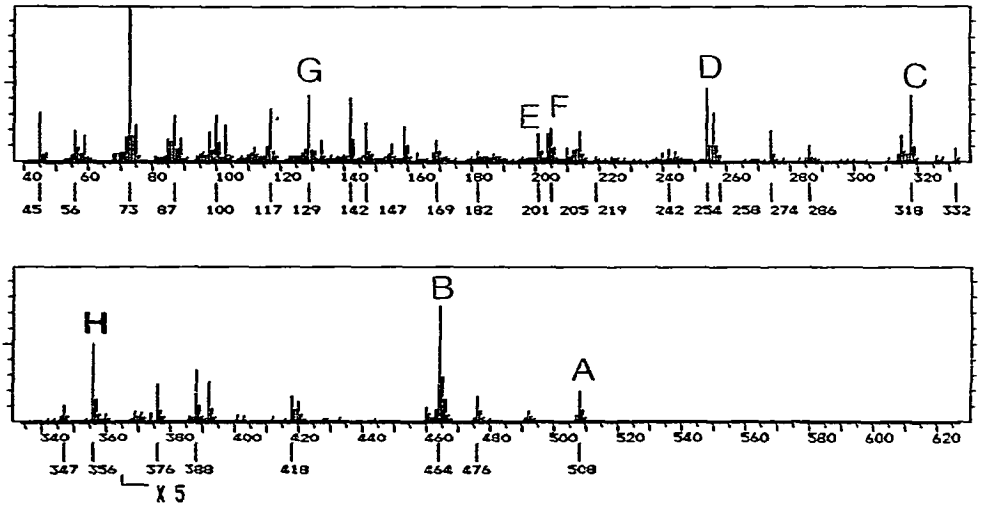
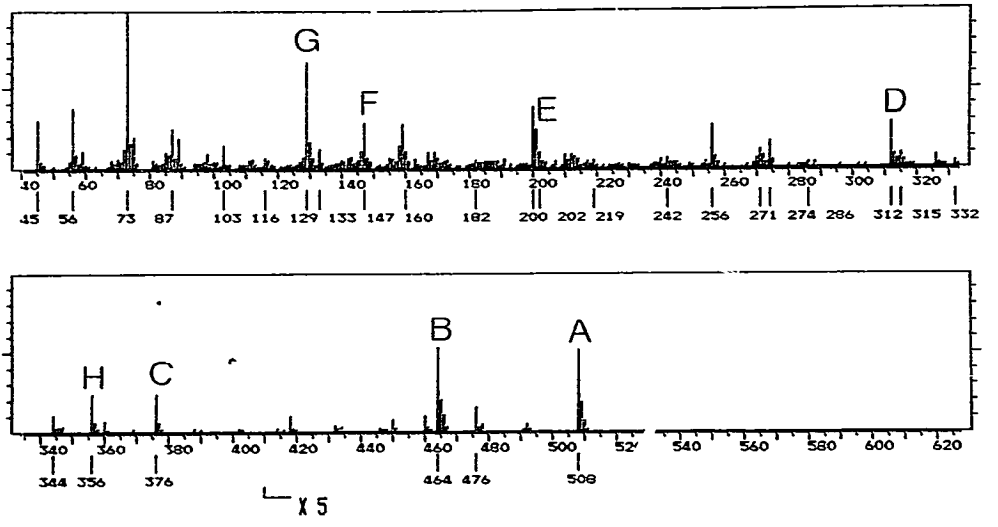


Fig. 5.

(Continued on p. 500)

methyl-N-acetyl-N-methyl-4,9-
di-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-7,8-
di-O-methyl-D-neuraminate
methyl glycoside

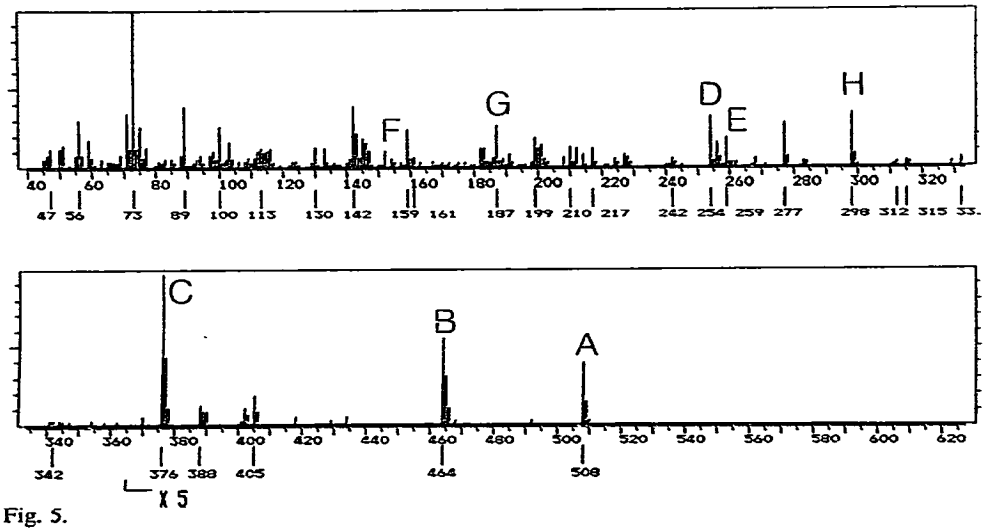
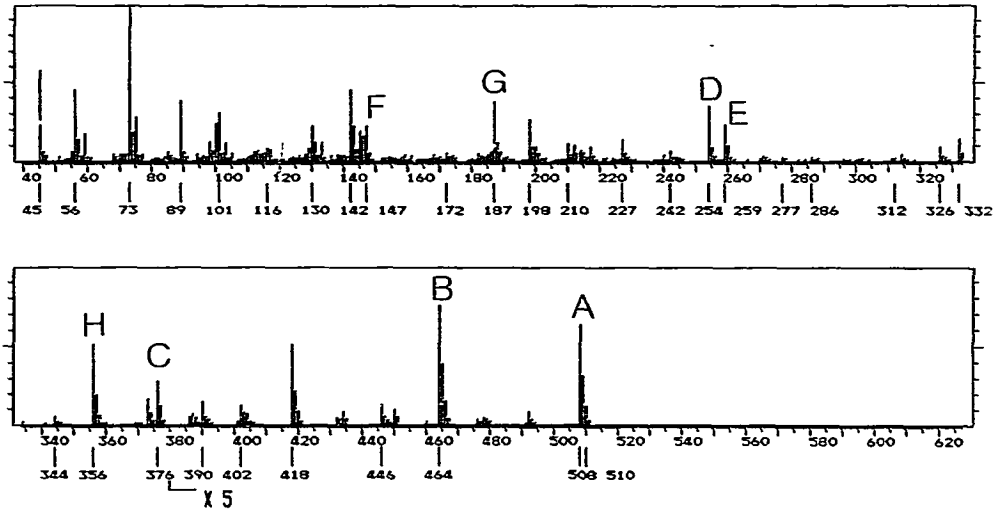


Fig. 5.

methyl-N-acetyl-N-methyl-7,9-
di-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-8,9-
di-O-methyl-D-neuraminate
methyl glycoside

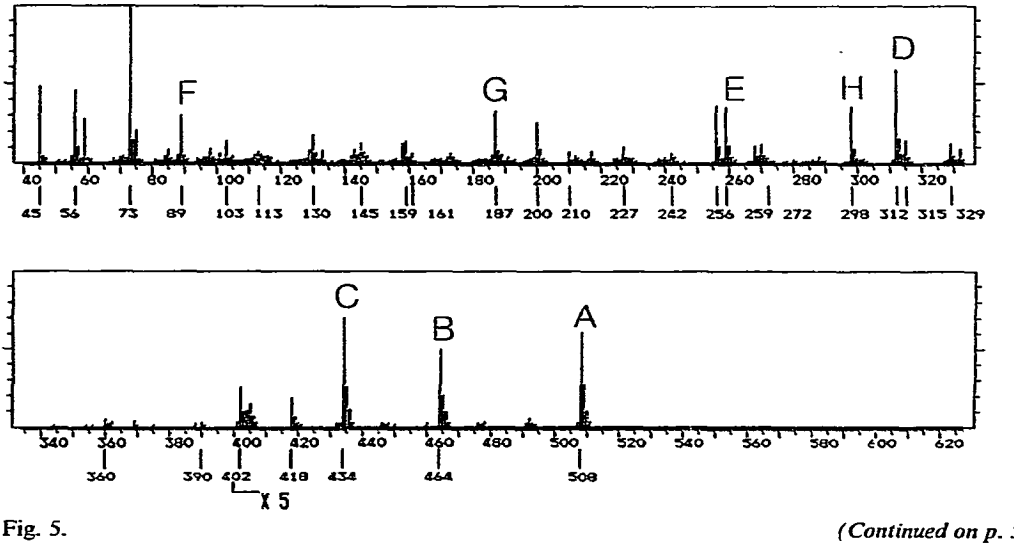
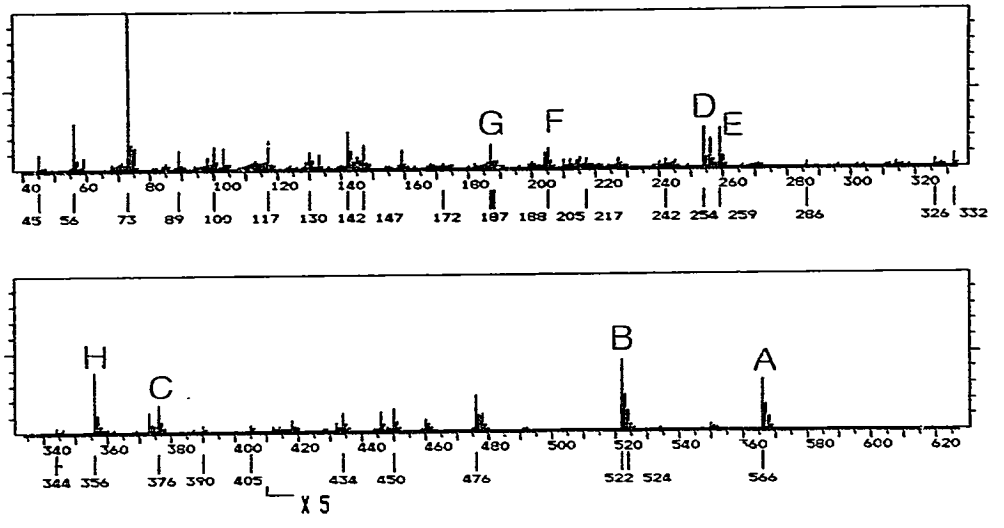


Fig. 5.

(Continued on p. 502)

methyl-N-acetyl-N-methyl-7-
mono-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-4-
mono-O-methyl-D-neuraminate
methyl glycoside

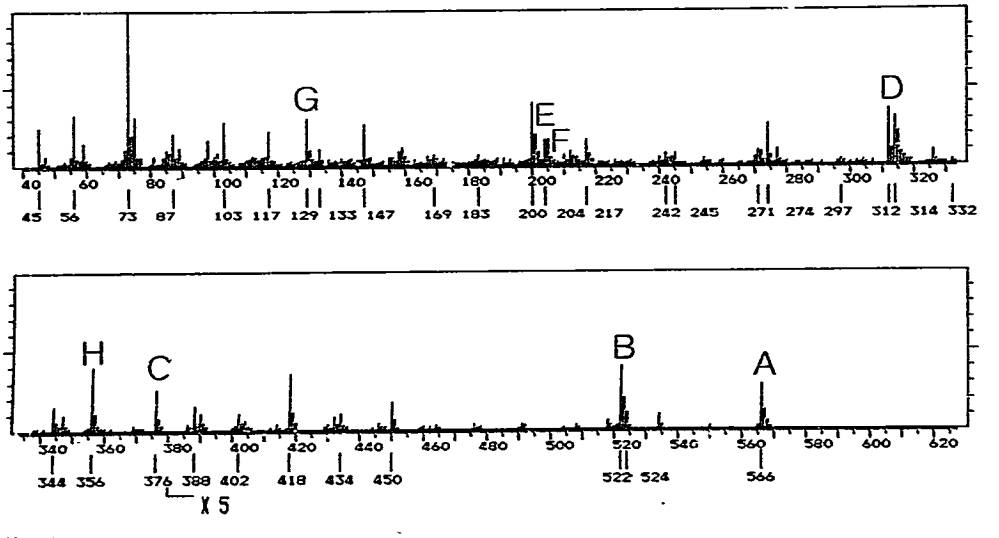
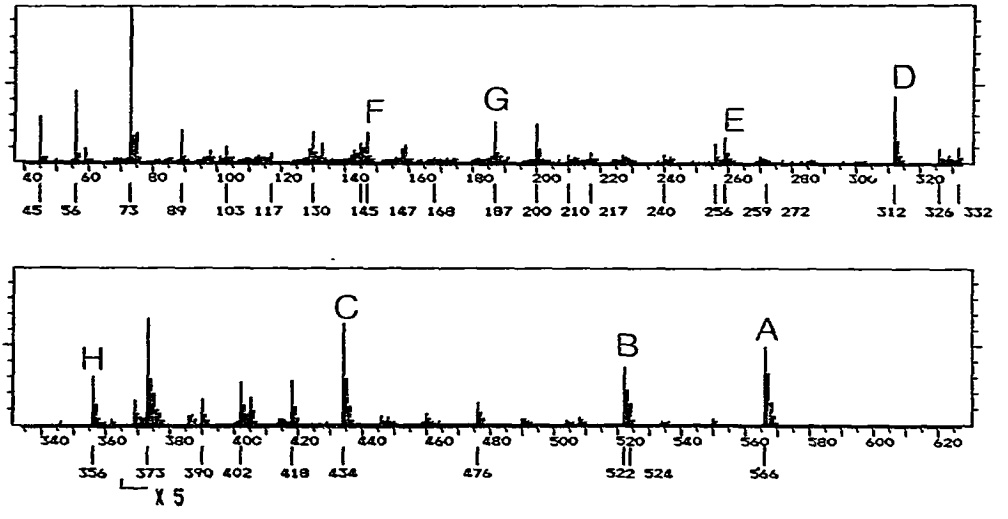


Fig. 5.

methyl-N-acetyl-N-methyl-9-
mono-O-methyl-D-neuraminate
methyl glycoside



methyl-N-acetyl-N-methyl-D-
neuraminate methyl glycoside

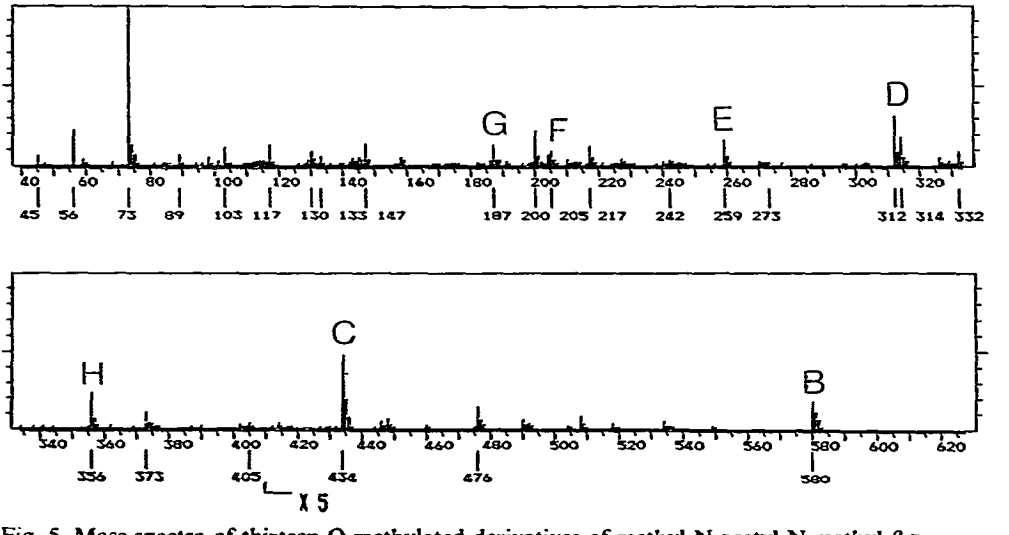


Fig. 5. Mass spectra of thirteen O-methylated derivatives of methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside and methyl N-acetyl-N-methyl-β-D-neuraminate methyl glycoside itself. Free hydroxyl functions are trimethylsilylated.

rapid method for the preparation of all methyl ethers of N-acetyl-N-methyl-β-D-neuraminate methyl glycoside except the 4,8-di- and 8-mono-O-methyl derivatives. This series of model substances including the GLC retention times and the mass spectrometric data will be of great value for further investigations in the field of sialocarbohydrate and sialoglycoconjugate analysis (linkage analysis as well as the analysis of naturally occurring O-acetylated N-acylneuraminic acids).

ACKNOWLEDGEMENTS

This work was supported in part by the Centre National de la Recherche Scientifique (L.A. No. 217) and by the Délégation Générale à la Recherche Scientifique et Technique (Contrat 79-7-0669). The authors are grateful to G. Ricart (mass spectrometer engineer) and J. Celen for skillful assistance.

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