Carbohydrate Research, 27 (1973) 5–10

MASS SPECTRA OF ACETYLATED DERIVATIVES OF SIALIC ACIDS

N. K. KOCHETKOV, O. S. CHIZHOV, V. I. KADENTSEV, G. P. SMIRNOVA, AND I. G. ZHUKOVA

N. D. Zelinsky Institute of Organic Chemistry, Academy of Sciences of the U. S. S. R., Moscow (U. S. S. R.)

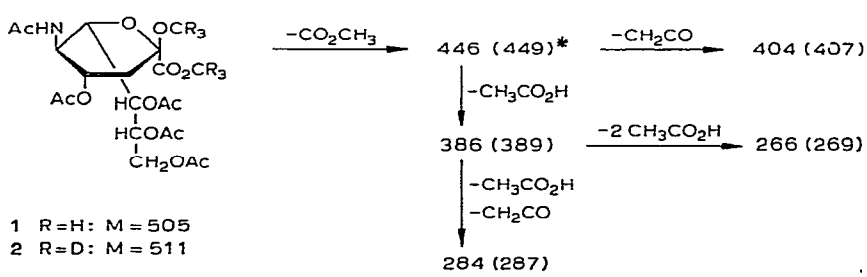
(Received June 26th, 1972; accepted for publication September 5th, 1972)

ABSTRACT

The fragmentation pattern in electron-impact mass spectrometry has been established for the peracetylated methyl ester methyl glycoside derivative of *N*-acetylneuraminic acid. The resulting data allow the interpretation of the mass spectrum of the corresponding derivative of a new sialic acid isolated from the starfish *Distolasterias nipon* which is shown to be 8-*O*-methyl-*N*-acetylneuraminic acid.

INTRODUCTION

For the chemical study of the sialoglycolipids of *Echinodermata*, a convenient and reliable micro-method for the elucidation of the structure of the sialic acids is needed. Mass spectrometry has been successfully applied to various monosaccharides[1–3], but little information is available on sialic acid derivatives[4,5] and no general conclusion can be made about the fragmentation patterns. The peracetylated methyl ester methyl glycoside of *N*-acetylneuraminic acid (**1**) and the trideuteriomethyl analogue **2** have now been used for the investigation of the fragmentation patterns. The peracetylated derivatives were chosen as being more stable and more convenient than trimethylsilyl ethers.



Scheme 1

*The *m/e* values given in brackets correspond to the ions derived from the deuterium-labelled derivative **2**.

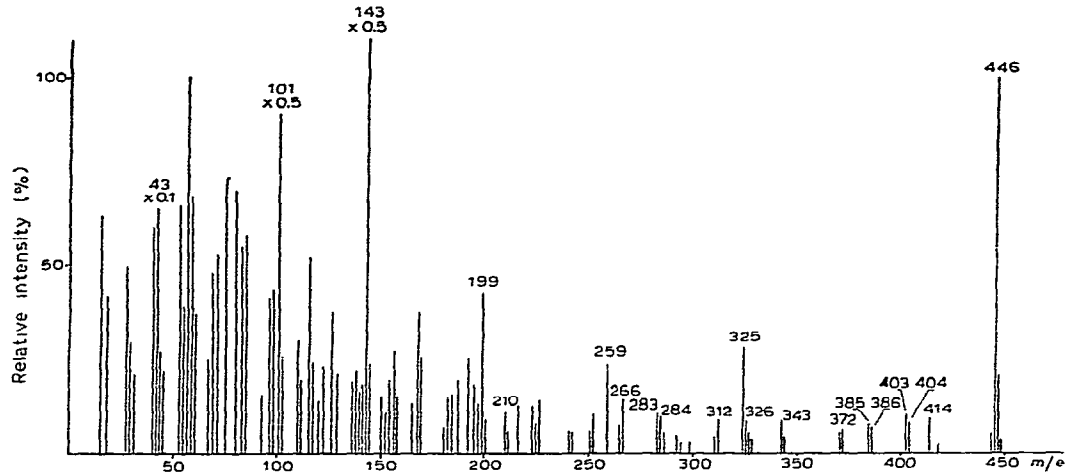


Fig. 1. Mass spectrum of the peracetylated methyl ester methyl glycoside of *N*-acetylneuraminic acid (1).

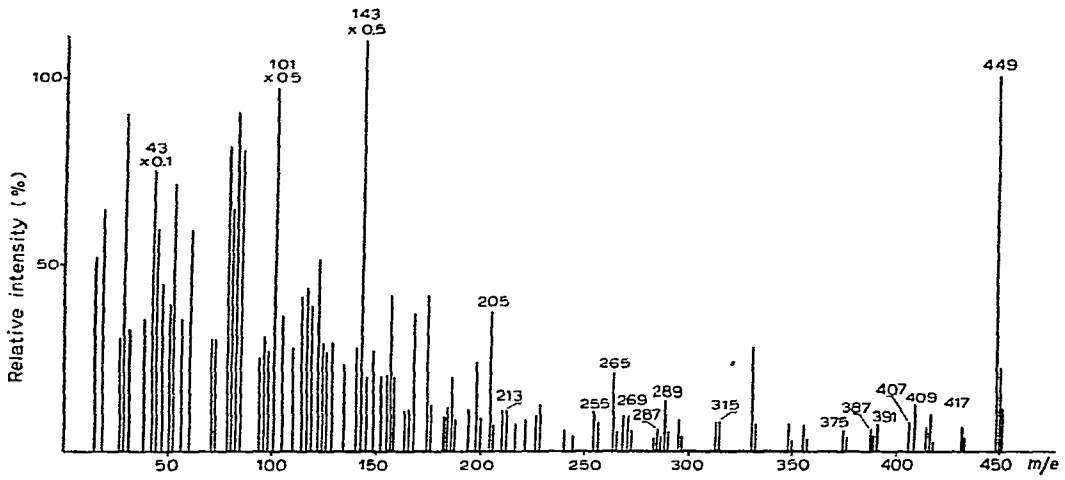


Fig. 2. Mass spectrum of the peracetylated trideuteriomethyl ester trideuteriomethyl glycoside of *N*-acetylneuraminic acid (2).

RESULTS AND DISCUSSION

It is evident from the mass spectrum of **1** (Fig. 1) that there are several main pathways of fragmentation of the M^+ ion. The cleavage of an AcO group gives an ion with *m/e* 446 (M−59) which is very important for calculation of the molecular weight. For the deuterium-labelled compound (**2**), the peak for this fragment is shifted to *m/e* 449 (Fig. 2). This primary fragment loses acetic acid and ketene to form secondary fragments (Scheme 1).

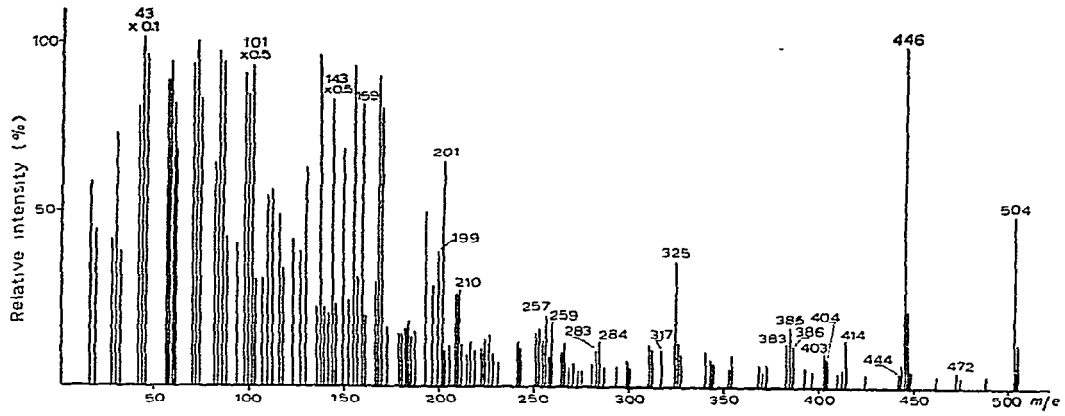


Fig. 3. Mass spectrum of a mixture of peracetylated methyl ester methyl glycosides of *N*-acetyl- and *N*-glycolyl-neuraminic acids.

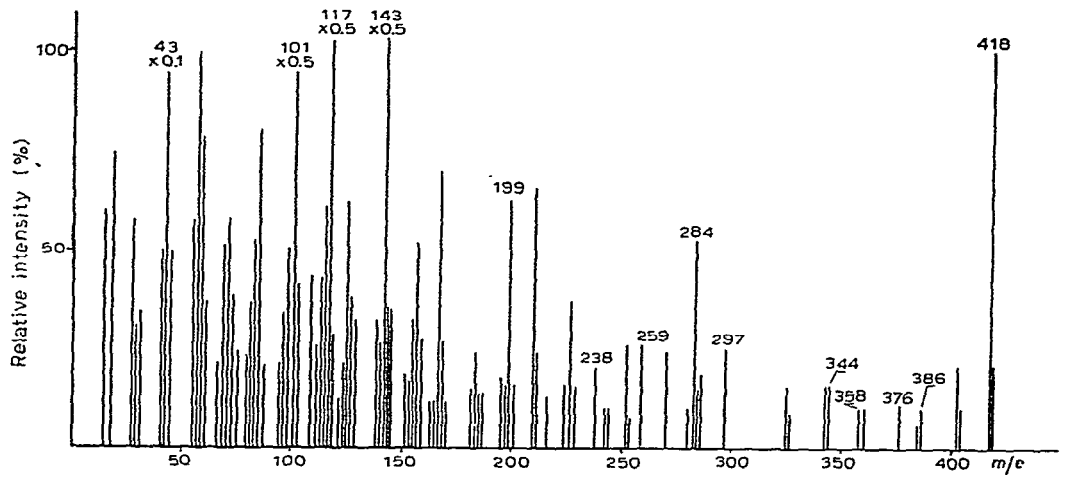


Fig. 4. Mass spectrum of the peracetylated methyl ester methyl glycoside of sialic acid **3**.

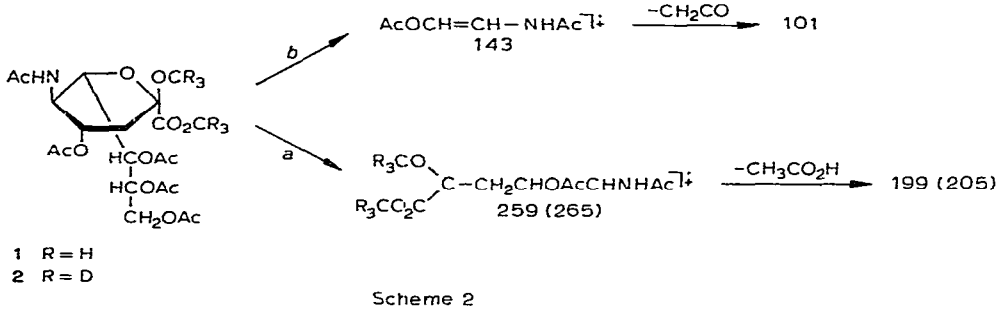
The second pathway of the fragmentation of **1** involves the loss of CH_3O (or CD_3O for **2**) and CH_3COOH to form the fragment of *m/e* 414 (417) with subsequent loss of acetic acid and ketene as follows:

$$414\,(417) \xrightarrow{-CH_2CO} 372\,(375) \xrightarrow{-CH_3CO_2H} 312\,(315) \xrightarrow{-CH_3CO_2H}$$

$$252\,(255) \xrightarrow{-CH_2CO} 210\,(213).$$

Simultaneous fission of the C-5–C-6 and C-2–O-6 bonds leads to an ion with *m/e* 259 (265) which further loses a molecule of acetic acid to form a secondary fragment with *m/e* 199 (205) (Scheme 2*a*). These fragments are very important for

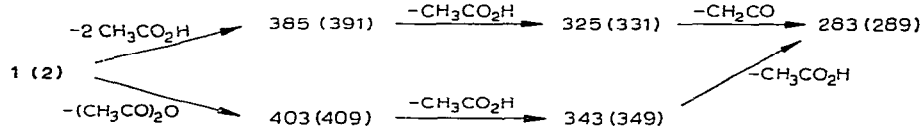
recognition of the substituents at C-4 and C-5, especially the *N*-acyl substituents. This pathway of fragmentation is not known for the derivatives of other monosaccharides.



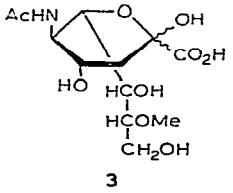
Scheme 2

One of the most-intense signals in the mass spectrum of **1** is the peak with *m/e* 143 corresponding to the cleavage of the C-3–C-4 and C-5–C-6 bonds. The resulting fragment $AcOCH{=}CHNHAc^{\dagger}$ eliminates ketene to give an ion with *m/e* 101 (Scheme 2*b*).

The acetylated derivatives **1** and **2** can also eliminate two molecules of acetic acid to form ions with *m/e* 385 (391) which then lose acetic acid and ketene to form ions with *m/e* 325 (331) and 283 (289), respectively (Scheme 3).



Scheme 3



Finally, the molecular ion can lose $(CH_3CO)_2O$ to form an ion with *m/e* 403 (409), and then one or two molecules of acetic acid to form ions with *m/e* 343 (349) and 283 (289), respectively (Scheme 3).

Mass spectrometry was also used for the analysis of mixtures of *N*-acetyl- and *N*-glycolyl-neuraminic acids which are often formed by hydrolysis of natural sialic acid-containing compounds. The mass spectrum of a mixture of the peracetylated methyl ester methyl glycosides of *N*-acetyl- and *N*-glycolyl-neuraminic acids (~1:1) exhibits all of the above-mentioned peaks from the *N*-acetylneuraminic acid derivative, and the peaks associated with fragmentation of the *N*-glycolylneuraminic acid derivative are shifted by 58 m.u. towards larger mass. The peak at *m/e* 504 formed from the *N*-glycolylneuraminic acid derivative corresponds to the peak at *m/e* 446 formed

from the *N*-acetylneuraminic acid derivative; the peaks at *m/e* 317, 257, 201, and 159 correspond to the peaks at *m/e* 259, 199, 143, and 101 (Fig. 3). Therefore, the presence of *N*-acetyl- and *N*-glycolyl-neuraminic acids in admixture can be deduced by mass spectrometry.

The preceding data were applied to establish the structure of a new sialic acid (3) isolated from sialic acid-containing compounds of the starfish *Distolasterias nipon*[6]. The sialic acid 3 contained no *O*-acetyl groups, and the mass spectrum of the peracetylated methyl ester methyl glycoside exhibited an intense peak for M^+ at *m/e* 418 (*cf.* *m/e* 446 for the *N*-acetylneuraminic acid derivative). The mass difference of 28 m.u. suggests that sialic acid 3 has an *O*-methyl group instead of an *O*-acetyl group. All the secondary fragments formed from the ion with *m/e* 418 and all the fragments formed from the M^+ ion by elimination of 1–3 molecules of acetic acid and ketene are shifted by 28 m.u. towards smaller mass (Fig. 4). In addition, the mass spectrum exhibits a very intense peak at *m/e* 117 which is almost absent from the mass spectra of the *N*-acetyl- and *N*-glycolyl-neuraminic acid derivatives. This peak corresponds to the fragment $CH_2OAc\overset{+}{C}HOCH_3$ which is evidently formed by cleavage of the C-7–C-8 bond. Hence, the *O*-methyl group must be attached to C-8. The cleavage of a C–C bond vicinal to a methoxyl group is well-known in the fragmentation of partially methylated alditol acetates, the charge being located on an ether oxygen[7]. Since ions with *m/e* 259, 199, 143, and 101, which are also present in the mass spectrum of the acetylated derivative of *N*-acetylneuraminic acid, are present in the mass spectrum of the acetylated derivative of 3, the sialic acid from *D. nipon* must have an acetyl group at nitrogen and no substituent at C-4.

Thus, from the mass-spectral data, the structure of 8-*O*-methyl-*N*-acetylneuraminic acid (3) can be ascribed to the new sialic acid from *D. nipon*. 8-*O*-Methyl-*N*-glycolylneuraminic acid has been isolated by Warren[8] from the starfish *Asterias forbesi*. Structure 3 has been confirmed by alkaline degradation data[6].

EXPERIMENTAL

The methyl glycosides of sialic acid methyl esters were prepared by boiling the sialic acids in methanol in the presence of either Dowex–1(H^+) resin[9] or 0.1M hydrogen chloride[10] (for preparation of the deuterium-labelled derivative, 0.1M HCl in CD_3OD was used) and isolated by column chromatography on Dowex-50 (AcO^-) resin. The products gave no colour in the Warren reaction[11] and were homogeneous by g.l.c. as *O*-trimethylsilyl derivatives (1-m glass column; 5% SE-30 on Chromosorb W, 210°; or 3% OV-17 on Chromosorb G, 197°).

The peracetylated derivatives were obtained by heating the methyl glycosides of sialic acid methyl esters with acetic anhydride–pyridine (1:1) and proved to be homogeneous by t.l.c. on silica gel in chloroform–methanol (46:4) and by g.l.c. (1-m glass column, 10% SE-30 on Chromosorb W, 208°).

Mass spectra were measured with a CH-6 Varian MAT instrument at 70 eV and an inlet temperature of 140°.

REFERENCES

1 N. K. KOCHETKOV AND O. S. CHIZHOV. *Advan. Carbohyd. Chem.*, 21 (1966) 39.
2 D. C. DEJONGH AND S. HANESSIAN, *J. Amer. Chem. Soc.*, 87 (1965) 3744.
3 N. K. KOCHETKOV AND O. S. CHIZHOV, *Methods Carbohyd. Chem.*, 6 (1972) 540.
4 C. C. SWEELEY AND D. E. VANGE, in C. MARINETTI (Ed.), *Lipid Chromatographic Analysis*, Vol. 1, Dekker, New York, 1967, p. 477.
5 R. K. YU AND R. W. LEDEEN, *J. Biol. Chem.*, 244 (1969) 1306.
6 I. G. ZHUKOVA, G. P. SMIRNOVA, T. A. BOGDANOVSKAYA, AND N. K. CHEKAREVA, *Abstr. Papers, 5th All-Union Conference on Chemistry and Biochemistry of Carbohydrates*, "*Nauka*", 1972, p. 65.
7 H. BJÖRNDAL, B. LINDBERG, A. PILOTTI, AND S. SVENSSON, *Carbohyd. Res.*, 15 (1970) 339.
8 L. WARREN, *Biochim. Biophys. Acta*, 83 (1964) 129.
9 R. KUHN, P. LUTZ, AND D. L. MACDONALD, *Chem. Ber.*, 99 (1966) 611.
10 R. K. YU AND R. W. LEDEEN, *J. Lipid Res.*, 11 (1970) 506.
11 L. WARREN, *J. Biol. Chem.*, 233 (1959) 1971.