Carbohydrate Research, 59 (1977) 235–239

Note

Mass spectrometry of sialic acids: peracetylated methyl esters of 5-acylamino-3,5-dideoxy-nononic and -heptonic acids

GALINA P. SMIRNOVA, NATALYA V. CHEKAREVA, OLEG S. CHIZHOV, BORIS M. ZOLOTAREV, AND NIKOLAY K. KOCHETKOV

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

(Received December 13th, 1976; accepted for publication, March 11th, 1977)

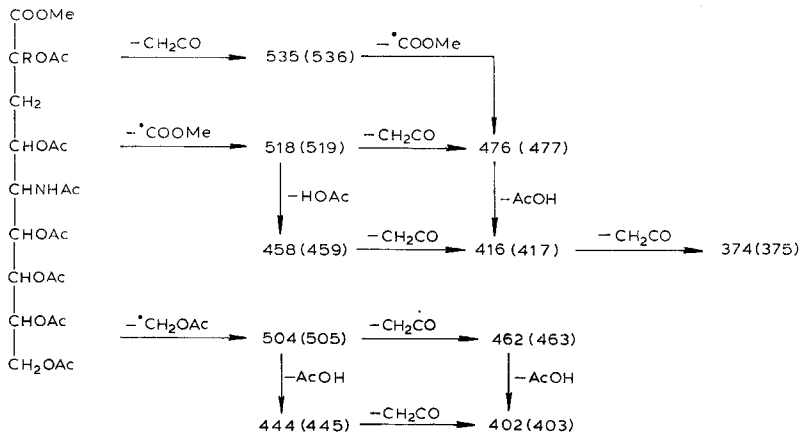
The peracetylated methyl ester methyl glycosides of sialic acids[1], the trimethylsilyl derivatives of the methyl ester methyl glycoside of neuraminic acid[2] or its *N*-acetyl and *N*-glycoloyl derivatives[3], and the Me_3Si derivatives of the methyl esters of sialic acids[3] have been used for the mass-spectrometric analysis of the structures of sialic acids. However, methanolysis of sialic acid-containing preparations may be incomplete or the Me_3Si esters unstable. We now report on the mass spectrometry of the peracetylated methyl esters of 5-acylamino-3,5-dideoxynononic acids prepared after the reduction ($NaBH_4$) of the keto group in sialic acids. These derivatives are stable, readily prepared in high yield, and give mass spectra which can be easily interpreted due to very simple fragmentation patterns.

Methyl esters of 5-acetamido-2,4,6,7,8,9-hexa-*O*-acetyl-3,5-dideoxynononic acid (**1**), its 2-d analogue (**2**), and methyl 5-(acetoxyacetamido)-3,5-dideoxynononate (**3**) have been studied.

The mass spectrum of **1** shows a peak for the $M^{+\bullet}$ ion at *m/e* 577 which indicates the molecular weight of the compound. The $M^{+\bullet}$ ion loses AcO$^\bullet$ or $^\bullet CH_2OAc$ groups to form the ions with *m/e* 518 and 504, respectively. These fragments lose further molecules of acetic acid and ketene (Scheme 1). The $M^{+\bullet}$ ion also loses ketene to form the ion at *m/e* 535 which can lose the methoxycarbonyl group to form the ion at *m/e* 476. Further fragmentation of this ion involves elimination of acetic acid and ketene molecules (Scheme 1). For the deuterium-labelled compound (**2**), the peaks of the $M^{+\bullet}$ ion and of the above-mentioned fragments are shifted by one mass unit towards higher mass (in Scheme 1, these peaks are given in brackets).

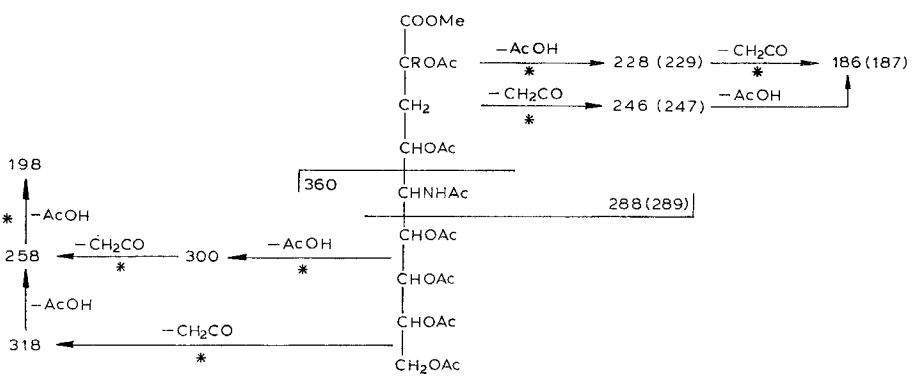
The cleavage of the bonds (C-5–C-6 and C-4–C-5) adjacent to the nitrogen-bearing carbon atom leads to the most characteristic, and most abundant, nitrogen-containing primary fragments at *m/e* 288 (289 for **2**) and 360, which decompose further through the elimination of AcOH and CH_2CO (Scheme 2). The peaks of the C-1/C-5 fragment and of the corresponding secondary ions in the mass spectrum of **1** are shifted by one mass unit to higher mass in that of **2**, whereas the peaks of the C-5/C-9

fragment and of the related secondary ions have identical *m/e* values in both mass spectra.



1 R = H, $M^{+\bullet}$ = 577
2 R = D, $M^{+\bullet}$ = 578

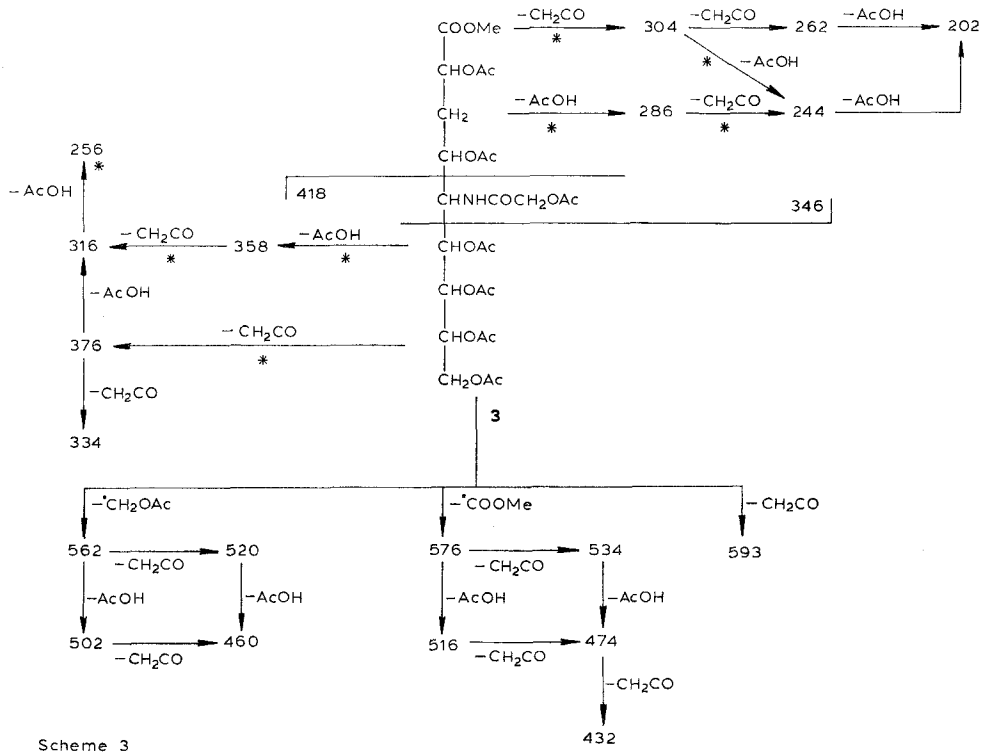
Scheme 1



1 R = H
2 R = D

Scheme 2

The fragmentation of **3** resembles that of **1** and **2**, although all the peaks corresponding to the above-mentioned ions are shifted by 58 mass units towards higher mass, *e.g.*, the $M^{+\bullet}$ peak for **3** is at *m/e* 635, and the C-1/C-5 and C-5/C-9 fragments show intense peaks at *m/e* 346 and 418, respectively (Scheme 3).



Scheme 3

Therefore, *N*-acetyl- and *N*-glycoloyl-neuraminic acids can be detected in a mixture on the basis of the characteristic peaks for the $M^{+}\bullet$ ion and the C-1/C-5 and C-5/C-9 fragments in the mass spectra of the above-mentioned derivatives.

We have applied the method for the analysis of the sialic acid of the sialoglycolipids isolated from the eggs of the sea urchin *Strongylocentrotus intermedius*. The sialoglycolipids from gonads of this sea urchin contain *N*-acetyl- and *N*-glycoloyl-neuraminic acids[4]. It was of interest to compare the contents of sialic acids in sialoglycolipids from gonads and eggs of the same species. The sialoglycolipid from the eggs was isolated[4], and hydrolyzed with 0.05M sulphuric acid. The sialic acids released were reduced with sodium borohydride and the products were subjected in sequence to treatment with diazomethane and acetic anhydride–pyridine (1:1), and then analyzed by mass spectrometry. The spectrum obtained was identical to that of **3** and no peaks characteristic of **1** were observed. Hence, only *N*-glycoloylneuraminic acid is present in the sialoglycolipid from the eggs of *S. intermedius*.

In order to determine whether the above method is valid for the analysis of mixtures containing C_7-sialic acids, we isolated C_7-*N*-glycoloylneuraminic acid by application, in sequence, of periodate oxidation, borohydride reduction, and mild hydrolysis with acid to egg sialoglycolipid. We have shown that each molecule of the egg sialoglycolipid contained one residue of *N*-glycoloylneuraminic acid in the

terminal position of the oligosaccharide chain and hence can be oxidized by periodate with cleavage of the C-7–C-8–C-9 bonds. The peracetylated methyl 5-(acetoxyacetamido)-3,5-dideoxyheptonate (**4**), prepared in the usual way, gave a mass spectrum containing a peak (*m/e* 491) for the $M^{+\bullet}$ ion which was shifted by 144 mass units towards lower mass but was weak compared to that (*m/e* 635) for the $M^{+\bullet}$ ion of **3**. The peak of the ion at *m/e* 346 (the C-1/C-5 fragment) is the same in the mass spectra of **3** and **4**. The ion at *m/e* 418 for **4** is far less abundant than that for **3**, and apparently it is the $(M^{\bullet} - CH_2OAc)^+$ fragment (the corresponding fragment from **3** has *m/e* 562). However, the mass spectrum exhibits a very intense peak at *m/e* 274 which is absent from the mass spectrum of **3** and corresponds to the C-5/C-7 fragment formed by rupture of the C-4–C-5 bond in **4**. This ion loses acetic acid to form the fragment with *m/e* 214. The metastable peak at *m/e* 167.1 in the mass spectrum of **4** corresponds to the transformation *m/e* 274→214. The C-5/C-7 fragment from the derivative of the C_7-*N*-acetylneuraminic acid shows an intense peak at *m/e* 216 which is absent from the mass spectra of **1**, **3**, and **4**. Thus, the peaks with *m/e* 274 and 216 can be used to indicate the presence of C_7-*N*-glycoloyl- and C_7-*N*-acetyl-neuraminic acids.

Hence, the proposed method of analysis of sialic acids will probably be useful for the study of mixtures containing C_9- and C_7-*N*-acetyl- and *N*-glycoloyl-neuraminic acids. These mixtures are formed frequently when glycolipids or glycoproteins containing several residues of sialic acids are treated with periodate. Analysis of such mixtures provides information on the positions of the residues of sialic acid in the oligosaccharide chains.

If the mixtures contain *O*-acylated sialic acids, it is better to avoid the use of sodium borohydride, because of the possibility of deacylation. Milder reducing agents, *e.g.*, zinc borohydride, sodium cyanoborohydride, and polymethylhydrosiloxane[5], should selectively reduce the keto group of sialic acid, leaving *O*-acyl groups intact. Subsequent acetylation with hexadeuterioacetic anhydride and mass-spectrometric analysis should then give information on the positions of the *O*-acyl groups.

EXPERIMENTAL

C_7-*N*-acetylneuraminic acid was prepared[6] from *N*-acetylneuraminic acid. The isolation of the sialoglycolipid from the eggs of the sea urchin *S. intermedius* and its degradation with periodate have been described elsewhere[4]. The sialic acids from the native sialoglycolipid and the periodate-oxidized and borohydride-reduced sialoglycolipid were isolated after hydrolysis[7] with 0.05M H_2SO_4 at 80° for 1 h.

G.l.c. was performed with a Pye 104 chromatograph; the flow rate of nitrogen was 50 ml/min. Mass spectra were recorded with a CH-6 Varian MAT instrument at 70 eV and an inlet temperature of 200°.

Peracetylated derivatives of methyl 5-acetamido-3,5-dideoxynononate (**1**) *and its 2-d analogue* (**2**), *methyl 5-(acetoxyacetamido)-3,5-dideoxynononate* (**3**), *and the C_7-analogues of* **1** *and* **3**. — A solution of the sialic acid (~1 mg) in water (~1 ml)

was treated with cold, aqueous $NaBH_4$ (4 h, 5°), and then passed through a column of Amberlite IR-120 (H^+) resin, eluted with water, and concentrated to dryness *in vacuo* with the addition of methanol. A solution of the residue in methanol (~1 ml) was treated with excess of ethereal diazomethane at 20° overnight. The colourless solution was concentrated to dryness, the residue was treated with acetic anhydride–pyridine (1:1) for 7 h at 20° or 30 min at 100°, and the mixture was concentrated *in vacuo*. The products gave no colour with the resorcinol reagent[8], and were homogeneous by t.l.c. (silica gel; chloroform–methanol, 49:1.5).

Mass-spectral data. — Compound **1**: *m/e* 43 (40%), 56 (39), 60 (38), 72 (32), 84 (55), 96 (50), 102 (52), 115 (67), 126 (38), 138 (46), 144 (43), 156 (86), 172 (42), 180 (16), 186 (90), 198 (33), 204 (50), 214 (34), 228 (67), 246 (80), 258 (45), 259 (88), 276 (16), 288 (100), 300 (27), 318 (100), 330 (16), 356 (6), 360 (100), 374 (6), 388 (4), 402 (6), 416 (11), 432 (7), 444 (3), 458 (7), 462 (4), 476 (9), 486 (2), 504 (22), 518 (6), 535 (3), and 577 (2, $M^{+\bullet}$).

Compound **2**: *m/e* 43 (54%), 56 (63), 60 (51), 69 (34), 72 (55), 84 (49), 85 (73), 98 (62), 102 (73), 103 (51), 115 (63), 116 (40), 127 (64), 128 (88), 138 (82), 139 (83), 145 (84), 156 (60), 157 (60), 169 (52), 170 (65), 173 (67), 187 (70), 198 (59), 205 (63), 215 (68), 229 (74), 247 (87), 258 (74), 259 (81), 276 (36), 289 (90), 300 (60), 318 (100), 331 (30), 357 (14), 360 (100), 375 (11), 389 (6), 403 (19), 417 (24), 433 (110), 445 (10), 459 (14), 463 (9), 477 (18), 487 (5), 505 (54), 519 (16), 536 (6), and 578 (10, $M^{+\bullet}$).

Compound **3**: *m/e* 43 (59%), 56 (50), 60 (54), 69 (58), 73 (53), 83 (65), 97 (41), 101 (53), 115 (57), 127 (47), 129 (60), 139 (78), 156 (52), 166 (57), 172 (50), 187 (64), 189 (50), 202 (42), 214 (86), 230 (36), 244 (67), 256 (36), 259 (86), 262 (41), 272 (74), 286 (48), 299 (44), 304 (88), 316 (33), 318 (47), 334 (17), 346 (100), 358 (30), 360 (32), 376 (100), 388 (19), 390 (13), 418 (94), 432 (6), 460 (6), 474 (10), 502 (7), 516 (4), 520 (4), 534 (7), 544 (4), 562 (12), 576 (5), 593 (2), and 635 (2, $M^{+\bullet}$).

Compound **4**: *m/e* 43 (220%), 56 (180), 60 (100), 69 (93), 73 (200), 101 (240), 114 (200), 130 (70), 154 (250), 172 (136), 187 (37), 202 (45), 214 (108), 230 (29), 244 (29), 272 (100), 274 (100), 286 (12), 302 (8), 304 (26), 314 (8), 316 (5), 346 (8), 358 (4), 374 (4), 418 (4), and 491 (2, $M^{+\bullet}$).

REFERENCES

1 N. K. KOCHETKOV, O. S. CHIZHOV, V. I. KADENSEV, G. P. SMIRNOVA, AND I. G. ZHUKOVA, *Carbohydr. Res.*, 27 (1973) 5–10.
2 C. C. SWEELEY AND D. E. VANCE, *Lipid Chromatogr. Anal.*, 1 (1967) 476–486.
3 J. P. KAMERLING, J. F. G. VLIEGENTHART, AND J. VINK, *Carbohydr. Res.*, 33 (1974) 297–306.
4 N. K. KOCHETKOV, I. G. ZHUKOVA, G. P. SMIRNOVA, AND I. S. GLUKHODED, *Biochim. Biophys. Acta*, 326 (1973) 74–83.
5 E. R. H. WALKER, *Chem. Soc. Rev.*, 5 (1976) 23–50.
6 R. K. YU AND R. LEDEEN, *J. Biol. Chem.*, 244 (1969) 1306–1313.
7 L. SVENNERHOLM, *Acta Chem. Scand.*, 12 (1958) 547–554.
8 T. MIETTINEN AND I. T. TAKKI-LUUKKAINEN, *Acta Chem. Scand.*, 13 (1959) 856–858.