

Nitrous Acid Deamination of the Methyl Ester Methyl β -Glycoside of Neuraminic Acid

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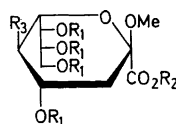
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N-Acylneuraminic acids are common constituents of glycoconjugates. During a study of the quantitative analysis of monosaccharides in glycoconjugates by means of methanolysis and deamination, the identification of the major deamination products of glycosides of neuraminic acid became a matter of considerable importance.¹ In an earlier study,² in which synthetic reference compounds and deamination products were compared chromatographically, the results suggested that the major products formed from glycosides of neuraminic acids were glycosides of 3-deoxy-*D*-glycero-*D*-galactonulosonic acids. We have now elucidated the structure of the major product formed on nitrous acid deamination of methyl 5-amino-3,5-dideoxy-*D*-glycero- β -*D*-galacto-nonulopyranosidonic acid methyl ester (*1*); this glycoside is formed in the aforementioned analytical procedure.¹

A sample of glycoside *1* was subjected to nitrous acid deamination and after work-up the products were transformed into trimethylsilyl ether (TMS) derivatives and analyzed by GLC and MS. One main peak and several minor peaks were obtained on GLC. Mass spectrometry of trimethylsilylated neuraminic acid derivatives has been studied by Kamerling *et al.*³ The mass spectrum of the compound in the main peak showed no molecular ion, but the molecular weight (MW 656) was indicated by ions at *m/e* 597 (*M* - CO₂CH₃) and *m/e* 566 (*M* - TMSOH). These data suggest that the

major deamination product is a methyl ester methyl glycoside of a 3-deoxy-*D*-glycero-nonulopyranosidonic acid. The origin of some pertinent fragments in the MS is depicted in Fig. 1.

Further structural evidence was adduced by acetylating the deamination product of glycoside *1* using acetic anhydride-pyridine and fractionating the product by silica gel chromatography to yield compound *2*. Although only a low yield of compound *2* was obtained, examination of the acetylation mixture by TLC showed only traces of other products. The ¹H NMR data for compound *2* are given in the Experimental part. The interpretation was facilitated by spin decoupling experiments. The coupling constants of H-5 (*J*_{4,5} 10.0 Hz, *J*_{5,6} 10.0 Hz) indicate that the acetoxy group at C-5 occupies an equatorial position. Compound *2* was de-esterified to give compound *3*. As expected the ¹³C NMR spectrum of compound *3* did not reveal any signals at about 52 ppm derived from carbons carrying amino or acetamido functions. The combined evidence presented here demonstrates that the main product of deamination of glycoside *1* is methyl 3-deoxy-*D*-glycero- β -*D*-galacto-nonulopyranosidonic acid methyl ester (*4*). This is in accordance with the results of the deamination of other glycosides carrying equatorial amino groups in analogous positions, *e.g.* methyl 4-amino-4-deoxy- α -*D*-glucopyranoside for which the main product was methyl α -*D*-glucopyranoside.⁴ Presumably, the ring-oxygen atom participates in the deamination reaction to give a cyclic oxonium ion which then reacts with water to give the product with retained configuration.



- 1 R₁=H, R₂=Me, R₃=NH₂
- 2 R₁=Ac, R₂=Me, R₃=OAc
- 3 R₁=H, R₂=H, R₃=OH
- 4 R₁=H, R₂=Me, R₃=OH

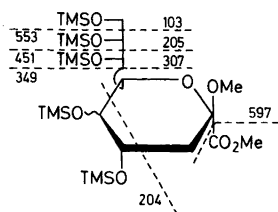


Fig. 1. Some fragments in MS of the TMS derivative of the major deamination product.

Experimental. General. Mass spectra were obtained using a Varian MATCH-7 instrument equipped with a Varian Aerograph 1700 gas chromatograph and an SS 100S computer system. For GLC a Carlo Erba Fractovap 2151 AC instrument equipped with a Hewlett-Packard SP-2100 glass capillary column (25 m \times 0.2 mm) was used. A gas-flow rate of 0.5 ml helium per min was used. Instruments for NMR and other general methods have been described.⁵

Deamination. Sodium nitrite (350 mg) in water (2 ml) was added to a solution of compound *1* (410 mg) in water (8 ml). The mixture was kept at 0 °C for 6 h,

treated with Dowex-50 (H^+), neutralized with Ag_2CO_3 and freeze-dried. Part (200 μg) of the product was trimethylsilylated with trimethylchlorosilane-hexamethyldisilazane-pyridine (1:2:10) for 30 min at room temperature. On GLC the TMS-derivative of compound 4 showed T 1.43 (retention time at 245 °C relative to *myo*-inositol TMS₆). MS [70 eV; (% rel.-int.)]: 73(100), 103(14), 147(27), 204(33), 205(23), 307(4), 329(3), 349(0.4), 361(6), 419(4), 451(2), 553(0.9), 566(0.4), and 597(0.6).

Another part (200 mg) of this material was acetylated with acetic anhydride-pyridine (1:1, 4 ml) at 4 °C for 18 h, water (10 ml) was added and the mixture was treated with Dowex-50(H^+) in order to remove the pyridine, filtered and freeze-dried. The mixture was fractionated on a silica gel column (2.5 × 20 cm) irrigated with ethyl acetate. The main component (2) was obtained as a syrup (37 mg), $[\alpha]_{D}^{25} -6.5^\circ$ ($CHCl_3$, c 1). 1H NMR (200 MHz, $CDCl_3$): δ 1.88 (H-3_{ax}, $J_{3ax,4}$ 11.7 Hz, $J_{3ax,3eq}$ 12.9 Hz), 2.56 (H-3_{eq}, $J_{3eq,4}$ 5.1 Hz), 5.36 (H-4, $J_{4,5}$ 10.0 Hz), 4.94 (H-5, $J_{5,6}$ 10.0 Hz), 4.10 (H-6, $J_{6,7}$ 2.2 Hz), 5.46 (H-7, $J_{7,8}$ 5.4 Hz), 5.34 (H-8, $J_{8,9}$ 6.6 Hz, $J_{8,9}$ 2.4 Hz), 4.20 (H-9, $J_{9,9'}$ 12.6 Hz), 4.76 (H-9') 3.86 (OMe), 3.30 (CO_2Me), 2.03, 2.06, 2.07, 2.12 and 2.16 (5 × OAc).

Compound 2 (33 mg) was treated with sodium methoxide in moist methanol at room temperature overnight. The mixture was neutralized with Dowex-50 (H^+), concentrated to dryness, dissolved in water and freeze-dried to give compound 3 (17 mg) as an amorphous powder $[\alpha]_{D}^{25} -39^\circ$ (H_2O , c 0.3). 1H NMR (99.60 MHz, D_2O , 85 °C) showed, *inter alia*, signals at δ 1.64 (H-3_{ax}, $J_{3ax,4}$ 11.2 Hz, $J_{3ax,3eq}$ 13.0 Hz), 2.32 (H-3_{eq}, $J_{3eq,4}$ 4.8 Hz) and 3.23 (OMe). ^{13}C NMR (25.05 MHz, D_2O): δ 40.6 (C-3), 51.8 (Me), 64.5 (C-9), 68.9 (C-4/7), 70.0 (C-7/4), 71.1 (C-8), 71.9 (C-5/6), 72.7 (C-6/5), 101.9 (C-2) and 170.0 (C-1).

Acknowledgements. We are indebted to Professor Bengt Lindberg for his interest, and to the Swedish Natural Science Research Council and the Sigrid Jusélius Foundation (Finland) for financial support.

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Received September 30, 1980.