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Preliminary communication

Structural study of a new sialic acid-containing O-specific polysaccharide of *Salmonella arizonae* O21; formation of anhydro derivatives of neuraminic acid upon treatment with anhydrous hydrogen fluoride

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In our structural studies of *Salmonella arizonae* O-specific polysaccharides (ref 1 and refs cited therein), we analysed a sialic acid-containing O-antigen of *S. arizonae* O21 (*Arizona* 22) by using solvolysis with anhydrous HF[2] as the key approach. The behaviour of sialic acid under these conditions has not been studied hitherto in detail, and we report now the conversion of *N*-acetylneuraminic acid into anhydro derivatives upon HF treatment.

The polysaccharide was obtained by mild acid degradation of the lipopolysaccharide isolated from dry bacterial cells by phenol–water extraction[3] and found conventionally to contain 2-amino-2-deoxy-D-glucose, 2-amino-2,6-dideoxy-L-galactose, and neuraminic acid. Judged by the ^{13}C NMR spectrum, the polysaccharide had a trisaccharide repeating unit which contained one residue of each of the above-mentioned amino sugars, two of them bearing *N*-acetyl groups [Me signal at 23.7 ppm (double intensity) and CO at 175.4 and 175.8 ppm] and the third carrying an *N*-acetimidoyl group (Me at 20.7 ppm, C=NH at 168.0 and 169.7 ppm for *E* and *Z* forms, cf. the published data[4]). One of the monosaccharides was partially *O*-acetylated (Me at 21.7 ppm and CO at 174.8 ppm).

Treatment of the polysaccharide with anhydrous HF[2] (20°C, 2 h) produced the acidic disaccharide **1**, isolated by reversed-phase C18 HPLC in 0.05% CF_3CO_2H. According to the sugar analysis and ^{13}C NMR data, **1** was composed of 2-acetamido-2-deoxyglucose and *N*-acetylneuraminic acid. Therefore, the third component of the repeating unit was 2-acetamidino-2,6-dideoxygalactose.

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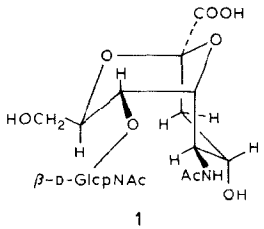
Disaccharide **1** did not react with $NaBH_4$ in water. Methylation[5] of **1** led to compound **2** with M_r 606 ($[M+H]^+$ ion with m/z 607 was observed in the chemical ionisation mass spectrum) which was 46 units less then expected. These results allowed us to conclude that the reducing end of **1** was occupied by a



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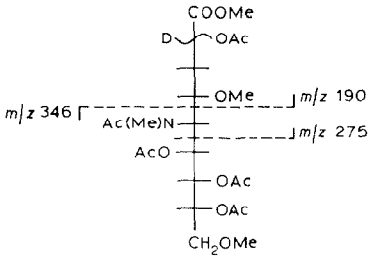

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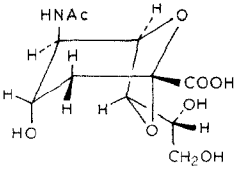

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residue of neuraminic acid in an anhydro form. This conclusion was confirmed by the electron-impact mass spectrum of **2**, which contained the peaks of ions with m/z 260 and 330 corresponding to the glucosamine glycosyl moiety and the anhydroneuraminic acid aglycon, respectively.

Compound **2** was subsequently hydrolysed, reduced with $NaBD_4$, esterified with methanolic HCl, and acetylated with acetic anhydride in pyridine to give the aldonic acid derivative **3** identified by GLC–MS. Location of the OMe group at position 4 and the OAc group at positions 7 and 8 in **3** proved that **1** contained a 7-substituted 2,8-anhydroneuraminic acid or an 8-substituted 2,7-anhydroneuraminic acid.

The ^1H NMR spectrum of **1** was completely assigned with the aid of 2D shift-correlated (COSY) spectroscopy (Table I). The $^3J_{H,H}$ coupling constants were in accord with the 2,8-anhydro derivative of *N*-acetylneuraminic acid having the pyranose ring in the boat conformation and the 1,3-dioxane ring in the chair conformation. The NOEs on H-5 and H-8, observed on preirradiation of H-3*a*, showed the spatial proximity of these protons, which confirmed the structure and the conformation of 2,8-anhydroneuraminic acid as depicted in formula **1**. These data were consistent neither with another conformation of 2,8-anhydroneuraminic acid nor with 2,7-anhydroneuraminic acid (**4**). The latter compound, which was prepared by us as the only product of the action of anhydrous HF on *N*-acetylneuraminic acid and was identified by the ^1H NMR data (Table I, cf. the published data[6]), had quite different ^1H chemical shifts and $^3J_{H,H}$ coupling constants. Glycosylation at position 7 of neuraminic acid was proved independently by the NOE on H-7 resulting from preirradiation of H-1 of the β-GlcNAc residue.

Therefore, the glycosidic linkage of *N*-acetylneuraminic acid, unlike that of di-*N*-acyl derivatives of 5,7-diamino-3,5,7,9-tetradeoxynonulosonic acids[2,4,7], is labile towards anhydrous HF. In the case of substitution at position 7, the cleavage of the linkage resulted in formation of a 2,8-anhydroneuraminic acid which, to our knowledge, has not been described hitherto. The unsubstituted acid, upon HF

TABLE I

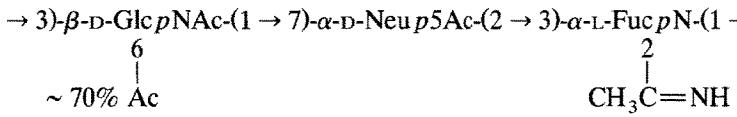
360-MHz ^{1}H NMR data for anhydro derivatives of *N*-acetylneuraminic acid (δ in ppm, $^3J_{H,H}$ in Hz for samples in D_2O at 20°C)

H-3*a* $J_{3a,4}$ $J_{3a,3e}$	H-3*e* $J_{3e,4}$	H-4 $J_{4,5}$	H-5 $J_{5,6}$	H-6 $J_{6,7}$	H-7 $J_{7,8}$	H-8 $J_{8,9b}$	H-9a $J_{8,9a}$	H-9b $J_{9a,9b}$
1								
1.90	2.60	3.76	4.05	4.44	3.76	4.06	3.73	3.59
10.8	6.4	9.7	3.2	6.1	10.4	4.6	2.1	12.2
15.8								
4 [a]								
2.20	2.03	3.95	4.59	3.92	4.47	3.52	3.72	3.55
(2.162)	(1.998)	(3.942)	(4.532)	(3.910)	(4.426)	(3.534)	(3.746)	(3.584)
5.3	< 2	< 2	< 2	< 2	7.7	2.3	6.2	11.5
(5.9)	(0)	(0)	(0)	(0.7)	(8.1)	(2.9)	(5.9)	(11.9)
15.0								
(15.0)								

[a] The published data[6] for the Na-salt of **4** are given in parentheses.

treatment, gave quantitatively the 2,7-anhydro derivative which has been obtained previously as a by-product of acid hydrolysis of *N*-acetylneuraminic acid at pH 2.0[6] or methanolysis in the presence of methanesulfonic acid[8]. One can suggest that the above-mentioned difference in behaviour of the nonulosonic acids towards HF is associated with the ability of neuraminic acid to form anhydro derivatives. The results obtained may be useful in studies of other sialic acid-containing carbohydrates.

Complete structural elucidation of the *S. arizonae* O21 O-specific polysaccharide, which, according to our preliminary data, has the structure **5** related to that of the O-antigen of *S. arizonae* O61[4], will be described elsewhere.



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REFERENCES

1 A.S. Shashkov, E.V. Vinogradov, Y.A. Knirel, N.E. Nifant'ev, N.K. Kochetkov, J. Dabrowski, E.V. Kholodkova, and E.S. Staniskavsky, *Carbohydr. Res.*, 241 (1993) 177–188.

2 Y.A. Knirel, E.V. Vinogradov, and A. Mort, *Adv. Carbohydr. Chem. Biochem.*, 47 (1989) 167–202.

3 O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
4 E.V. Vinogradov, A.S. Shashkov, Y.A. Knirel, N.K. Kochetkov, J. Dabrowski, H. Grosskurth, E.S. Stanislavsky, and E.V. Kholodkova, *Carbohydr. Res.*, 231 (1992) 1–11.
5 I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
6 N. Sugiyama, K. Saito, K. Fujikura, K. Sugai, N. Yamada, M. Goto, C. Ban, E. Hayasaka, and K. Tomita, *Carbohydr. Res.*, 212 (1991) 25–36.
7 Y.A. Knirel and N.K. Kochetkov, *FEMS Microbiol. Rev.*, 46 (1987) 381–384.
8 M.R. Lifely and F.H. Coffee, *Carbohydr. Res.*, 107 (1982) 187–197.