

SYNTHESIS OF N-FORMYL- AND N-SUCCINYL-D-NEURAMINIC ACID ON THE SPECIFICITY OF NEURAMINIDASE

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1. Introduction

The sialic acids are important constituents of numerous glycoproteins, glycolipids, and oligosaccharides. Little is known about their functional significance for the biological effect of these complex substances. In many instances removal of sialic acid is accompanied by a loss of biological activity [1].

So far only N-acetylneuraminic acid (lactaminic acid) and N-glykolyneuraminic acid were found in nature together with O-acetylated derivatives. The terminal sialyl moiety seems always to be bound to the next carbohydrate unit by an α -ketosidic bond. Ample evidence has now been accumulated that only this type of linkage is split by the various neuraminidase enzymes.

We were interested in obtaining a sialic acid with two anionic sites in the molecule and chose the succinyl group as N-substituent. Furthermore it seemed worthwhile to prepare a sialic acid with a C₁ residue at the amino group.

In this communication we describe the synthesis of N-formyl and N-succinyl-D-neuraminic acid and of their corresponding benzyl α -ketosides. In addition we wish to report the result of the interaction between *Vibrio cholerae* neuraminidase and the above mentioned ketosides.

It was found that the benzyl-ketoside of N-succinylneuraminic acid is not split at all by the enzyme. This result could be expected, since the N-butyryl group also abolishes the property to serve as substrate [2].

On the other hand the enzyme cleaves the corresponding ketoside of N-formylneuraminic acid. However, compared to the naturally occurring 3-O-N-

acetylneuraminosyl lactose (lactaminyl lactose), which represents a remarkably good substrate for bacterial and virus neuraminidases [3], the reaction proved to be much slower. The enzymatic cleavage of the trisaccharide is not inhibited by the addition of either the free acids or the respective ketosides.

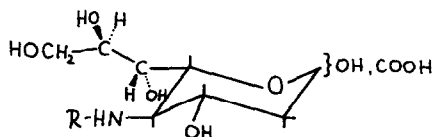
2. Materials and methods

Vibrio cholerae neuraminidase was obtained from Behring Werke, Marburg, 1 ml containing 500 units (producer's specification). The incubation was carried out in 0.05 M acetate buffer, pH 5.5, containing in addition 9 mg/ml NaCl and 1 mg/ml CaCl₂. Thin layer chromatography (TLC) was performed on glass plates (10 × 20 cm) coated with a 300 μ layer of Silica Gel G "Merck". *n*-Butanol/acetic acid/water (5 : 2 : 3, v/v) was the developing solvent. The substances were revealed by charring with H₂SO₄. Melting points were determined with a Tottoli-Büchi apparatus, optical rotations with a Zeiss REPM 12 spectropolarimeter.

3. Results

Both N-formyl- and N-succinyl-D-neuraminic acid were synthesized according to ref. [4]. N-Formyl-D-glucosamine [5] was converted to the 4,6-benzylidene derivative; m.p. 222–225°, $[\alpha]_D^{24} - 12^\circ$ (c, 2.3, in pyridine). Condensation with the potassium salt of di-tert-butyl oxalacetate yielded after hydrogenation, decarboxylation and mild saponification of the lactone crude N-formyl-D-neuraminic acid A which was

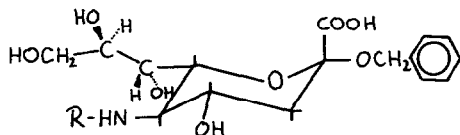
purified by column chromatography on Dowex 1 \times 4 (HCOO^-). Yield after crystallization (H_2O /acetic acid 1 : 10) 23% based on the benzylidene derivative; m.p. 177–179°, $[\alpha]_D^{24} - 31^\circ$ (c, 1.9, in H_2O), $R_{\text{NANA}} 0.95$. Elemental analyses were in good agreement with theoretical values. Infrared spectra show clearly that A contains a free formyl group. A gave a well crystallized chinoxaline derivative. The m.p. was 173–175°, $[\alpha]_D^{24} - 42^\circ$ (c, 1.2, in DMSO).



A: R = -CHO

B: R = -COCH₂CH₂COOH

N-succinyl-D-glucosamine [6] was converted with methanol and Dowex 50 W \times 8 (H^+) to the methyl ester; m.p. 188–192°, $[\alpha]_D^{24} + 40^\circ$ (c, 5.6, in H_2O). Subsequently the ester was transformed to the 4,6-benzylidene derivative; m.p. 223–226°, $[\alpha]_D^{24} + 23^\circ$ (c, 5.5, in pyridine). In an analogous manner to the preparation of A this compound gave N-succinyl-D-neuraminic acid B. Yield after crystallization (H_2O /acetic acid 1 : 10) 25%; m.p. 182–184°, $[\alpha]_D^{24} - 27^\circ$ (c, 5.1, in H_2O), $R_{\text{NANA}} 1.2$; $\text{pK}_1 2.6$, $\text{pK}_2 4.7$. Elemental analyses agreed with theoretical values. For comparison, B was also prepared by succinylation of the methyl β -ketoside of neuraminic acid methylester [7] in dioxan/ H_2O [8] and subsequent hydrolysis of the methoxy groups. Both compounds proved to be identical in m.p., mixed m.p., elemental analyses, titration equivalent, and infrared spectra. The chinoxaline derivative established once more the structure of B; m.p. 177–179°, $[\alpha]_D^{24} - 64^\circ$ (c, 0.8, in DMSO).



C: R = -CHO

D: R = -COCH₂CH₂COOH

The benzyl α -ketosides C and D were prepared essentially according to ref. [2]. The acetochloro derivative of B crystallized (ethyl acetate/ethyl ether/petroleum ether), m.p. 142–143°, as well as the acetylated benzyl ketoside of A (acetone/ H_2O), m.p. 202°. After removing the acetyl groups, amorphous, yet pure C and D were obtained. They produced correct elemental analyses, typical infrared and NMR spectra and showed $[\alpha]_D^{24} - 15^\circ$ (c, 1.8, in H_2O) and $[\alpha]_D^{24} - 10^\circ$ (c, 2.0, in H_2O), respectively.

In the experiments with neuraminidase the incubation mixtures consisted each of 0.4 ml buffer, 0.1 ml enzyme containing 50 units, and 1 mg substrate. Incubation temperature was 37°. The reaction was followed by TLC. After 3 hours all trisaccharide had disappeared whereas ca. 50% of N-formyl-neuraminic acid benzyl ketoside remained even after 5 days. Under the same conditions no cleavage of N-succinyl-neuraminic acid benzyl ketoside could be observed.

References

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