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SYNTHESIS WITH IMMOBILIZED ENZYME OF THE MOST IMPORTANT SIALIC ACID.

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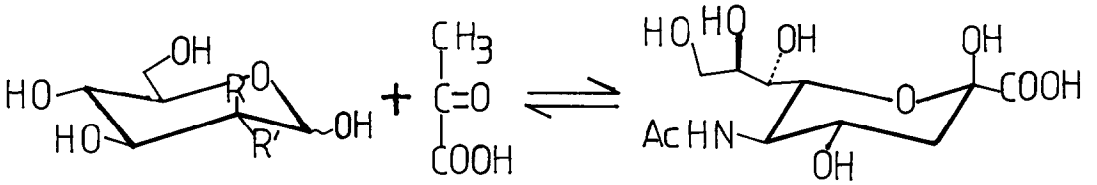
Summary. Starting from a crude mixture of N-acetylmannosamine and N-acetylglucosamine, N-acetylneuraminic acid has been easily synthesized on 2.8 millimoles scale, with one immobilized enzyme.

The sialic acids, peripherical constituents of oligosaccharides and glycoconjugates, are usually obtained from natural materials rather than by synthetic procedures.[1] Our experience in synthesis with immobilized enzymes[2] prompted us to investigate a new preparative access to the most common one, N-acetylneuraminic acid (1).

Acylneuraminate pyruvate-lyase (E.C 4.1.3.3) catalyses the enzymic reversible condensation of pyruvate (4) with N-acetylmannosamine (2) :



2 R=NHAc R'=H
3 R=H R'=NHAc

4 1

This enzyme, considered to be involved in vivo in the catabolic pathway of sialic acids metabolism, has been used previously in vitro to prepare very small amounts of labeled N-acetylneuraminic acid.[3] In this letter we report the synthesis of N-acetylneuraminic acid (1) on a larger scale starting from a crude mixture of N-acetylmannosamine (2) and N-acetylglucosamine (3) with acylneuraminate pyruvate-lyase immobilized on Agarose.

Immobilization. Acylneuraminate pyruvate-lyase (0.5 mg, 5 units, purchased from Sigma) dissolved in 0.5 ml of 0.05 M potassium phosphate buffer, pH 7.2, was stirred at 4°C under N_2 for 14 h with 2 ml of Ultrogel[4] previously activated by BrCN (100 mg per ml of gel) in 4.5 ml of 0.1 M sodium carbonate, pH 8, containing 0.5 M sodium chloride and 0.04 M sodium pyruvate. The gel was washed with ten volumes of M NaCl, ten volumes of bidistilled water and suspended in 0.05 M potassium phosphate buffer, pH 7.2. The immobilization yield was 51%.[5]

Synthesis of N-acetylneuraminic acid. A mixture of N-acetylmannosamine (2) and N-acetylglucosamine (3) in the ratio 50:50 estimated by g.l.c. and NMR[6] (7.2 mmol altogether), sodium pyruvate (4) (40 mmol), mercaptoethanol (0.036 mmol), sodium azide (3.6 mg) were dissolved in 34 ml of 0.05 M potassium phosphate buffer, pH 7.2, and the immobilized enzyme was added (2 ml of gel, 2.7 units). The reaction was allowed to proceed at 37°C with gentle stirring under N_2, until there was no more increase of the quantity of N-acetylneuraminic acid determined by colorimetric method with the periodate resorcinol reagent.[7] The gel was filtered, washed with water and N-acetylneuraminic acid isolated by chromatography on Dowex 1-formate resin, using a gradient of formic acid as eluent.

Results and discussion. The quantity of synthesized N-acetylneuraminic acid did not increase after 4 days. N-acetylneuraminic acid was obtained in 40% isolated yield. It exhibited a 400 MHz ^1H NMR spectrum identical with data reported in the literature.[8] The recovered gel retained about 50% of its enzymatic activity and was used for two further reactions. Altogether 2.8 millimoles of N-acetylneuraminic acid were prepared with as little as 2.7 units of acetylneuraminate pyruvate-lyase.

References and Notes.

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5. Soluble and immobilized enzymatic activities were determined using standard procedure : D. Comb and S. Roseman, Methods in Enzymology, 5, 391 (1962).
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