sequences, which have been found to be devoid or almost devoid of biological activity. In addition to the physical properties and elemental analyses of these peptides, the Table also indicates the level of activity on the guinea-pig ileum compared with that of Eledoisin itself taken as 100. The methods used for the synthesis of these peptides will be described elsewhere in detail[3].

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Zusammenfassung. Die Eigenschaften einer ersten Serie von synthetischen Peptiden, die mit Eledoisin strukturell verwandt sind, wurden beschrieben.

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Synthesis of N-Acetylneuraminic Acid

N-acetylneuraminic acid (NANA) occurs in a wide variety of biological materials, e.g. mucins, colostrum, erythrocytes, gangliosides, gonadotropins, enzymes, bacterial cell walls, etc.[1]. Its biochemical role, as yet largely unknown, is likely to be associated with the electrical charge imparted to the structure to which it is bound. The ever increasing interest in this ubiquitous substance prompted us to evaluate the available synthetic methods for large scale preparation. The synthesis of NANA from N-acetyl-D-glucosamine and oxaloacetic acid at pH 11 in 1–2% yield has been recorded by CORNFORTH et al.[2,3]. After the finding[4–6] that NANA is structurally related to N-acetyl-D-mannosamine and not to N-acetyl-D-glucosamine, BRUG et al.[7] and later CARROLL and CORNFORTH[8] sought to improve this synthesis by employing N-acetyl-D-mannosamine in the condensation reaction. The latter authors thus claimed an 8–10% yield of NANA. Since N-acetyl-D-mannosamine epimerizes rapidly in alkaline medium to an equilibrium with N-acetyl-D-glucosamine the potential yield of NANA in the above condensation obviously depends on the relative rates of the two reactions: (a) epimerization of N-acetyl-D-mannosamine and (b) condensation with oxaloacetic acid. We have followed the rate of the latter reaction spectrophotometrically at 565 mμ[9] by the development of Ehrlich-positive material[1]. Our reaction conditions were somewhat different from those reported by CARROLL and CORNFORTH[8].

To 10 ml of water were added alternately in small portions 5.4 g of oxaloacetic acid and 10 *N* sodium hydroxide in such a manner as to keep the pH between 5 and 10. The temperature was maintained at 10–12°C by external cooling. When all oxaloacetic acid had been added 10 g of N-acetyl-D-mannosamine was added to the mixture, the pH adjusted to 11 and the temperature raised to 25°C, the reaction mixture was stirred until all N-acetyl-D-mannosamine had dissolved and the pH was adjusted to 11 frequently by the addition of small amounts of sodium hydroxide. Spectrophotometric determination of Ehrlich-positive material in samples withdrawn at 15 min intervals indicated that the condensation was essentially complete in approximately 1 h. After dilution with water the reaction mixture was applied directly to 45 ml of Dowex-2 (acetate) in a 1.8 cm dia. column. The column was washed thoroughly with water until the effluent gave a negative direct and indirect Ehrlich test[1]. The products were then eluted with a gradient of pH 6.0 ammonium acetate buffer (0.1–1.5 *M*). The eluate fractions were tested with Ehrlich's reagent. Two distinct peaks were obtained as indicated in the elution diagram (A, Figure). The pooled fractions under each peak were passed through separats Dowex 50 (H-form) columns. The individual effluente were evaporated to a small volume under reduced pressure and then lyophilized to give hygroscopic powders. Paper chromatograms (Schleicher & Schuell ≠589) of the first fraction developed in an *n*-butanol-pyridine-water system (6:4:3) by the descending technique and sprayed with orcinol reagent indicated the presence of NANA-isomer with glucosamine configuration (R_{NANA}:1.5) along with a slightly smaller amount of NANA (R_{NANA}:1.0). The second peak consisted of a main component with an *R* value identical with that of NANA, and a trace of another with the *R* value of NANA isomer. However, all attempts to crystallize this material failed. The ion-exchange separation was further refined by stepwise elution with 0.2 and 0.65 *M* pH 6.0 ammonium acetate buffer (A, Figure). Nonetheless almost equal amounts of the two NANA isomers were still found to be present in the first peak, and the material from the second peak still contained the two components noted above. These contradictory observations can be reconciled by the assumption that the material eluted in the second peak was not NANA, but a mixture of the intermediate dicarboxylic acids (I) formed in the initial step of the condensation of oxaloacetic acid and N-acetyl-D-mannosamine.

This presumed mixture of isomers would then have to possess *R* values on paper chromatograms identical with that of NANA in order to conform to the observed results. The presence of a trace of NANA-isomer shown on paper chromatograms could readily be explained by the occurrence of some decarboxylation in the work-up of this mixture. The two monocarboxylic acids, NANA and its isomer with glucosamine configuration (II) would accordingly be expected to emerge in the first peak, a feature which is

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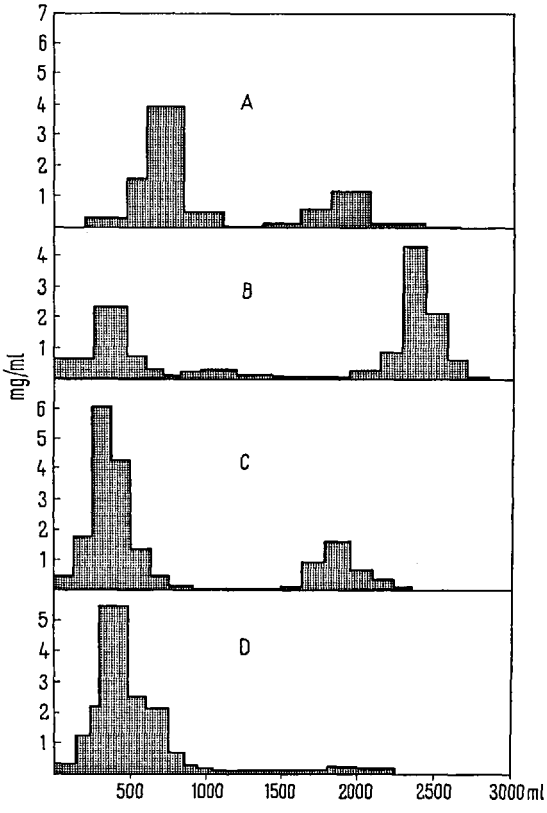
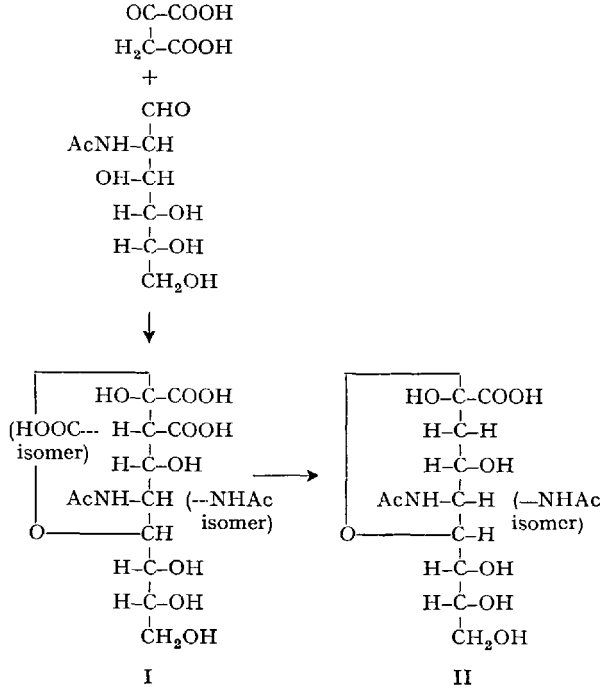
supported by our paper chromatographic evidence. Our hypothesis was further confirmed by titration of the product obtained from the second peak with dilute sodium hydroxide. Based on the molecular weight of (I) 0.4 mmole of this substance consumed 0.88 mmole of sodium hydroxide whereas 0.4 mmole of NANA titrated in the same fashion used 0.39 mmole of sodium hydroxide. Moreover, when a solution of I (from second peak) was kept at pH 11 for 2 h at 45°, approximately equal amounts of NANA and NANA-isomer were obtained whereas at pH 1.5 a more complex mixture of products was observed. Apart from NANA, its isomer, and possibly unchanged (I), two further products with R_{NANA}: 0.4 and R_{NANA}: 2.26 respectively were obtained. These spots were usually observed on chromatograms of acetic or formic acid solutions of NANA which had been concentrated at a temperature above 30° or during a prolonged period of time. An explanation for the retention of unduly large quantities of dicarboxylic acids (I) in our experiments is to be found in the different reaction conditions we employed relative to those reported by CARROLL and CORNFORTH[8]. The dicarboxylic acid fraction accounted for approximately 60% of the total Ehrlich-positive material (B, Figure) when the condensation reaction was terminated in 2 h by acidification with Dowex 50 (H-form) resin, but decreased to about 20% (C, Figure) when the alkaline reaction mixture was allowed to stand at room temperature for 24–48 h, as stipulated by CARROLL and CORNFORTH[8]. When the condensation reaction was carried out at 45° for 2 h, an insignificant amount of dicarboxylic acids (I) survived (D, Figure). The dicarboxylic acids (I) appear to be relatively more stable under very mild acid conditions than in alkaline medium in which decarboxylation to NANA (and isomer) takes place readily. NANA itself is fairly stable under mild alkaline conditions. Reverse aldolization to N-acetyl-D-glucosamine and pyruvic acid, as reported by ZILLIKEN and GLICK[10], was not observed when a solution of NANA was kept at pH 11 and 45° for 2 h or at 25° for 24 h. Relatively mild acid conditions, pH 1.5 at 45° for 2 h, on the other hand, gave rise to decomposition products as pointed out above. Excellent separation of NANA from its isomer with glucosamine configuration was obtained by chromatography on charcoal, as reported by CORNFORTH et al.[2,3].

Runs with 500 g or more of N-acetyl-D-mannosamine may be carried out in accord with the reaction conditions outlined above: (a) Application of the reaction mixture directly to a Dowex-2 (acetate) column. (b) Elution with pH 6 0.2 *M* ammonium acetate buffer. (c) Removal of cations from the eluate by treatment with Dowex 50. (d) Chromatography on charcoal[11].



Elution diagrams of NANA and dicarboxylic acids (I). Reaction mixture: 10 g of N-acetyl-D-mannosamine + 5.4 g oxaloacetic acid. First buffer solution: 0.20 *M* ammonium acetate at pH 6. Second buffer solution: 0.65 *M* ammonium acetate at pH 6, applied when optical density readings following the first peak had reached a minimum. A, Reaction mixture (reaction time 2 h) applied directly to Dowex-2 column. B, Reaction mixture acidified after 2 h and applied to Dowex-2 column. C, as in A, but reaction time 48 h. D, As in A, but reaction temperature raised to 45°C.



Zusammenfassung. Die Synthese der N-Acetylneuraminsäure aus N-Acetylmannosamin und Oxalessigsäure und die Isolierung eines Zwischenproduktes, 2-Keto-3-deoxy-3-carboxy-5-N-acetylnonulosaminsäure wird beschrieben.

H. RINDERKNECHT[12] and T. REBANE

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