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Neuraminic acid. - IV. Gas-Liquid Chromatography of N-Acetyl- and N-Glycolylneuraminic Acids *

by

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In the preceding decade numerous papers have been published concerning the analytical applications of gas chromatography to the biochemical field due to the extraordinary advantages of this technique. However, carbohydrates and related products have been less studied by this method than other compounds due to their non volatility. This difficulty has been resolved by the preparation of volatile derivatives such as polymethyl ethers, polyacetyl esters and, lately, polytrimethylsilyl ethers (4).

Refering to acylneuraminic acids, SWEELY and colb. (5) have been able to convert the N-acetylneuraminic acid (NANA**) to the 2-O-methyl ketal of methyl neuraminate and have separated this compound from hexoses as the trimethylsilyl derivative. Also BOLTON and colb. have published (1) a paper concerning the study of NANA and oses of glycoproteins by gas-liquid chromatography; the methyl glycosides are acetylated and then sylanizated.

We have tried to study by gas chromatography the behaviour of N-Glycolylneuraminic acid (NGNA) and the eventuel possibility of differentiation of NANA and related compounds by this method.

Material and methods

Products and apparatus. NGNA has been synthetized by FAILLARD and BLOHM. NANA, together with NGNA, has been recrystallized from calf serum (2). NANA (synthetic), glucose, N-acetylglucosamine, hexamethyldisilizane and trimethylchlorosilane were employed as commercially available. Pyridine was redistilled and stored over KOH.

«Argon Chromatograph» from W. G. PYE & LTD was used for this study. The SE-30 column was prepared according to SWEELEY (5). The temperature used was 160° C and the pression of the gas 0.8 kg/cm². The sensitivity of the apparatus was: 3. Samples of 2.5,

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** *Abbreviations:*
NANA = N-acetylneuraminic acid;
NGNA = N-glycolylneuraminic acid;
Hexamet. = Hexamethyldisilizane
Trymetchlor. = Trymethylchlorosilane.

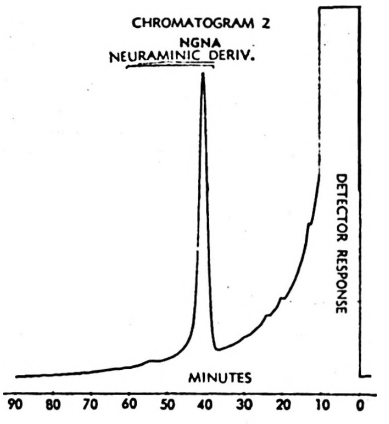
3.7 or 5.0 µl were injected with a Hamilton syringe.

Procedure. In the first series of these experiments the acylneuraminic acid and the glucose were submitted to the action of 1.5 ml of 5 % methanolic HCl, at 75°-80° (or 50°-55°), during 17 hours (or 15 hours, respectively). The resulting products were converted in their trimethylsilyl derivative by addition of pyridine, hexamethyldisilizane and trimethylchlorosilane (1 :0.2 :0.1).

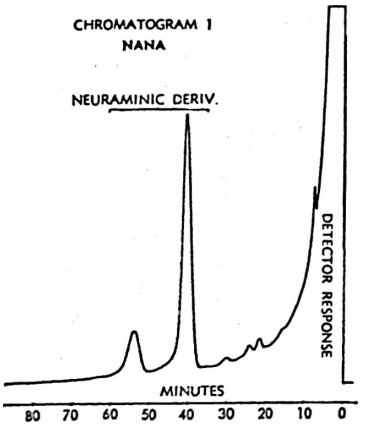
In the second series, the sylanization was directly applied to the assay substances, without methanolic-HCl treatment. 5 mg (or 10) of these products were completely or partially dissolved in 0.5 ml of pyridine; after a shaking of 30 sec, 0.2 ml of hexamethyldisilizane and 0.1 ml of trimethylchlorosilane were added; centrifugation was also made after 15 min, as in the first series, and the upper layer sample was injected into the chromatograph.

Results

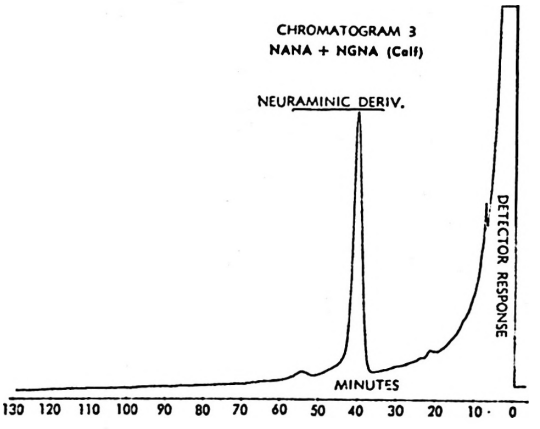
First series. The chromatograms 1, 2, 3 and 4 have in common the existence of a characteristical peak which appears in the above mentioned conditions at 41 minutes approximately. Both NANA and NGNA, independently or together, give the same peak. (The presence of a second peak, at 55 minutes, easily appreciable in the chromatogram 1 is not significative; see Discussion).



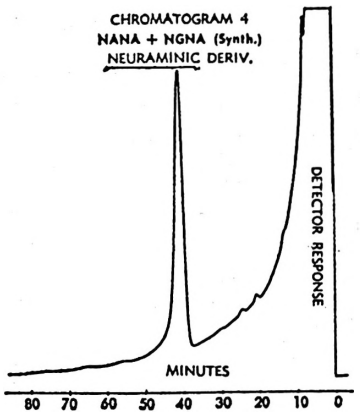
First Series. — CHROMATOGRAM 2. — NGNA, synth., 20 mg. + Methanol-HCl (1.5 ml.), 15 h., 50°-55° C.
Pyridine (1 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 2.5 µl.



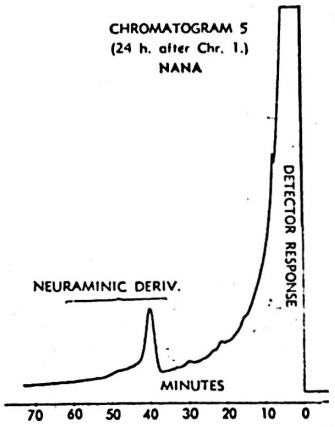
First Series. — CHROMATOGRAM 1. — NANA, synth., 20 mg. + Methanol-HCl (1.5 ml.), 17 h., 75°-80° C.
Pyridine (1 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 2.5 µl.



First Series. — CHROMATOGRAM 3. — NANA + NGNA, both from calf serum, 13 mg. + Methaonl-HCl (1.5 ml.), 15 h., 50°-55° C.
Pyridine (1 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 3.7 µl.



First Series. — CHROMATOGRAM 4. — NANA + NGNA, both synth., 10 + 10 mg. + Methanol-HCl (1.5 ml.), 15 h., 50°-55° C. Pyridine (1 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 2.5 μl.



First Series. — CHROMATOGRAM 5. — (The injection being made 24 hours after the chrom. 1, with the same product and quantity).

The treatment with pyridine and the other reagents causes a decrease of the chief peak 24 hours after storage at 5° (chromatogram 5).

Standard for glucose gives two peaks, corresponding to ist anomeric forms (chromatogram 6).

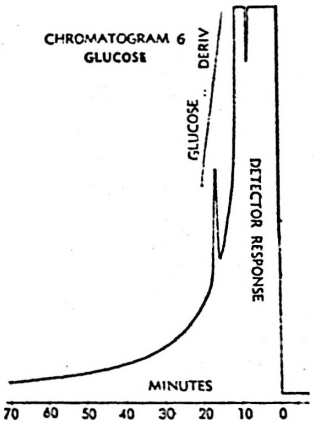
Second series. In the conditions employed in this series, it seems that NANA gives a peak (chromatogram 7), whilst NGNA (chromatogram 8) does not give any.

Standards for glucose and N-acetylglucosamine give two peaks for glucose (chromatogram 9) and a great peak for N-acetylglucosamine (chromatogram 10) at definite positions.

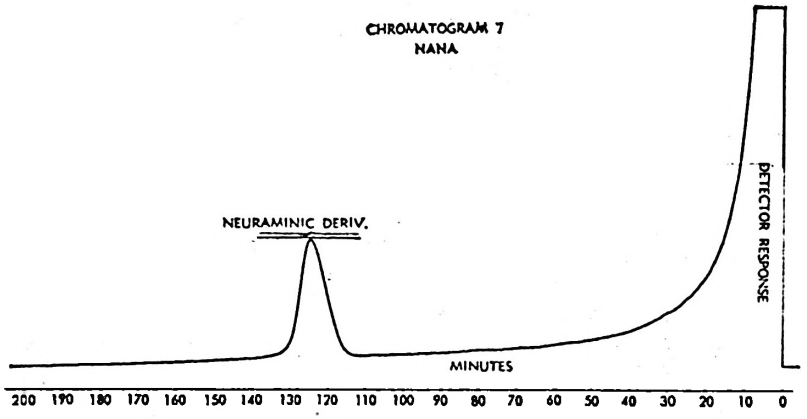
Finally, in the chromatograms 11 and 12, made with NANA + NGNA + glucose + N-acetylglucosamine, one may appreciate the peaks of glucose, N-acetylglucosamine and NANA but none corresponding to NGNA.

Discussion

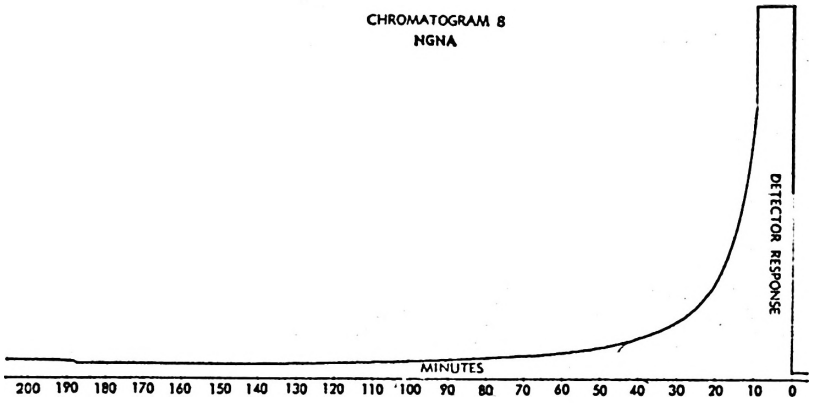
The necessity of transforming neuraminic acid into volatile products for gas chromatography requires some previous treatments. These treatments modify mainly the original molecule. In the case of NANA and NGNA the N-acetyl- and the N-glycolyl- residues, respectively, would be removed from the neuraminic acid component during the action of methanol-HCl treatment indicated in the



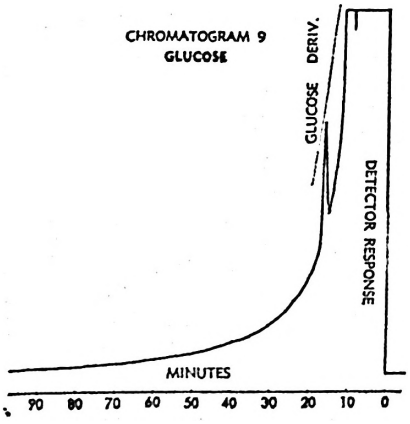
First Series. — CHROMATOGRAM 6. — GLUCOSE, 20 mg. + Methanol-HCl (15 ml.), 15 h., 50°-55° C. Pyridine (1 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 5 μl.



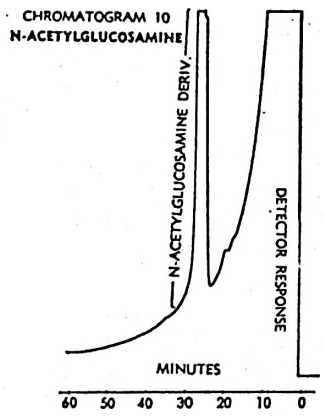
Second Series. — CHROMATOGRAM 7. — NANA, synth., 5 mg.
Pyridine (0.5 ml) + Hexamet. (0.2 ml) + Trymetchlor. (0.1 ml). Injection : 5 µl.



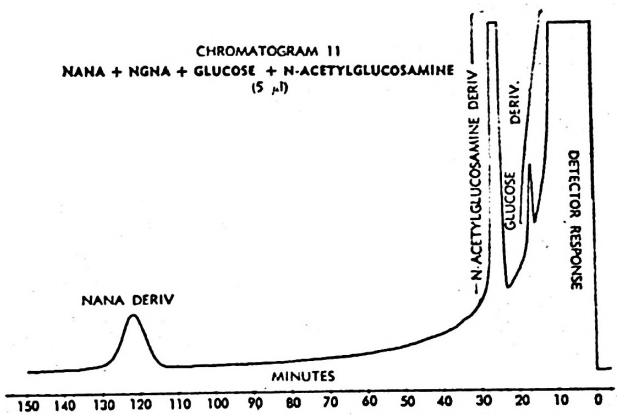
Second Series. — CHROMATOGRAM 8. — NGNA, synth. 5 mg.
Pyridine (0.5 ml) + Hexamet. (0.2 ml) + Trymetchlor. (0.1 ml). Injection : 5 µl.



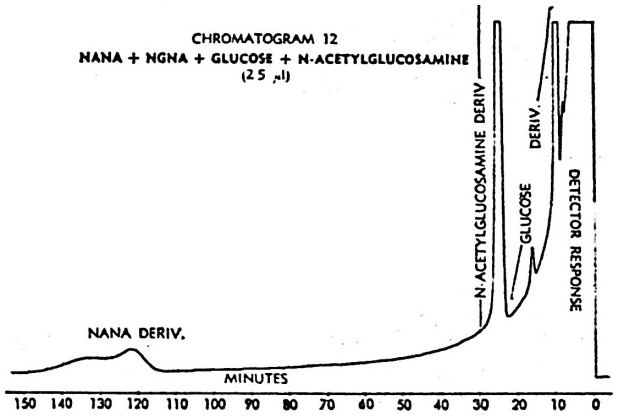
Second Series. — CHROMATOGRAM 9. — GLUCOSE, 10 mg.
Pyridine (0.5 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 5 µl.



Second Series. — CHROMATOGRAM 10. — N-ACETYLGLUCOSAMINE, 10 mg.
Pyridine (0.5 ml.) + Hexamet. (0.2 ml.) + Trymetchlor. (0.1 ml.). Injection : 5 µl.



Second Series. — CHROMATOGRAM 11. — NANA, synth. + NGNA, synth. + GLUCOSE + N-ACETYLGLUCOSAMINE, 5 + 5 + 5 + 5 mg.
Pyridine (0.5 ml) + Hexamet. (0.2 ml) + Trymetchlor. (0.1 ml).
Injection : 5 µl.



Second Series. — CHROMATOGRAM 12.
(The injection being made immediately after chrom. 11 with the same product but with 2.5 µl).

first series work. This removal would explain the identity of the chromatograms corresponding to NANA, NGNA and both mixture. On the other hand, the methoxyneuraminic acid methyl ester resulting after methanol-HCl treatment can probably suffer one anomerization, the two forms of which would be registered as two different peaks, the second easily appreciable when the conditions of temperature and time are stronger, as in chromatogram 1.

In the second series assays, only with sylanization treatment at room temperature, NGNA is not registered on the chromatograms, whilst NANA and other products are. In the interpretation of these results one should remind the pro-

duction of artifacts and the treatment with pyridine should be taken into special consideration. KARKAS and colb. (3) have studied the stability of simple derivatives of neuraminic acid and determined their degradation when heated with glacial acetic acid at 100° or pyridine at the same temperature. However in the conditions used in our work, that risk is smaller or at least seems to be smaller because of the rapidity of treatment.

On the other hand, NGNA can be analysed by gas chromatography following another procedure based on the release of glycolic acid, which is then propilated by propanol (6).

It seems that the gas-liquid procedure described above, according to the first modality, would permit at least the rapid characterization of neuraminic acid, but not the differentiation between NANA and NGNA. The second procedure would exclude NGNA of the analysis.

Summary

The behaviour of N-Glycolylneuraminic acid in comparison with N-Acetylneuraminic and glucose and N-acetylglucosamine has been studied by gas-liquid chromatography. With methanol-HCl treatment, previous to sylanization, both acylneuraminic acids derivatives give the same peak. With sylanization only, N-Glycolylneuraminic acid does not give any characteristical peak.

A brief discussion is made on these facts.

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