Table 1.

°C	η	ηrel
1 550 1 600 1 650 1 700 1 750 1 800	$\begin{array}{c} 2.18 \cdot x + 0.720 \\ 1.55 \cdot x + 0.555 \\ 1.20 \cdot x + 0.480 \\ 0.83 \cdot x + 0.429 \\ 0.62 \cdot x + 0.391 \\ 0.46 \cdot x + 0.375 \end{array}$	$3.02 \cdot x + 1$ $2.79 \cdot x + 1$ $2.33 \cdot x + 1$ $1.94 \cdot x + 1$ $1.59 \cdot x + 1$ $1.23 \cdot x + 1$

 $x = \text{mole fraction of } Ca_3Si_3O_9.$ 

where the relative viscosity is a function of the composition. In the latter set it is seen that the coefficients of  $x_{Ca_sSi_sO_s}$  are smaller for higher temperatures and are a linear function of the temperature (Fig. 2).

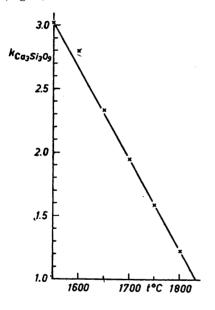


Fig. 2

From the above arguments it can be concluded that very simple relations must exist in the regions of the system under consideration and that it is very probable that essentially only a metasilicate of the composition Ca<sub>3</sub>Si<sub>3</sub>O<sub>9</sub> and an orthosilicate Ca<sub>3</sub>SiO<sub>4</sub> exist in the molten state.

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## Isolation of Sialic Acid from Gangliosides

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s fa 1 back as 1938 one of us 1 called Asttention to the close agreement in certain qualitative reactions between gangliosides and a substance some years earlier isolated from bovine submaxillary mucin \* and later named sialic acid 8. In 1941 Klenk <sup>8</sup> obtained from the methanolysate of gangliosides the monomethoxy derivative of a split product called neuraminic acid which showed the characteristic colour reactions of sialic acid. In 1952 Blix et al.3 pointed out that the neuraminic acid might have been formed during the isolation procedure by deacetylation of sialic acid, which at that time had been with certainty demonstrated as a component of bovine submaxillary mucin only. The unchanged sialic acid from this material contains one N-acetyl and one very labile O-acetyl 2,3 whereas the neuraminic acid has no acetyl group. The difference in elementary composition between the two substances was reasonably in agreement with a deacetylation. This view was further supported by the isolation of methoxy-neuraminic acid from a methanolysate of submaxillary mucin (Klenk and Lauenstein •). Employing a milder procedure Klenk and Faillard 7 later obtained from bovine submaxillary mucin a split product with an elementary composition and an acetyl content corresponding to that calculated for monoacetyl neuraminic acid. This product could by treatment with methanol-HCl be transfor-

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med into methoxyneuraminic acid \*. By the same means we have transformed bovine as well as porcine sialic acid into methoxy-neuraminic acid \*. The close relationship between sialic acids and neuraminic acid is thus well substantiated. Recently Svennerholm announced that he had been able to isolate from gangliosides a crystalline substance which gave the direct Ehrlich reaction of sialic and neuraminic acids, but, in contra-distinction to the latter, a negative ninhydrin reaction.

We have now succeeded in isolating sialic acid from gangliosides by a very simple method. The yield was 20 mg of recrystallized acid from 0.65 g of ganglioside. The X-ray powder photograph of the acid was identical with that of the sialic acid which is obtained from sheep submaxillary mucin as well as from a number of human glycoproteins and from ovomucin 10.

Experimental. The gangliosides were prepared from normal human brains in the main according to Klenk <sup>11</sup>. The substance was however dialysed against water and freed from cations by thorough treatment with a cation

exchange resin. It contained 2.35 % N, 10.4 % hexosamine, and 27 % sialic acid (colorimetrically determined). It was free from phosphorus. 0.65 g of the ganglioside was dissolved in 10 ml of water and dialysed firstly against 0.005 N hydrochloric acid at 4° C for 24 hours, and then against distilled water until free from chlorine. The solution, which had a strongly acid reaction, was placed in a cellophane bag and dialysed against 100 ml of water at 37° C for 24 hours. A few drops of toluene were added to the fluids. The dialysate was freezedried. The dialysis was repeated in the same way on five subsequent days, or as long as appreciable amounts of Bial-positive material appeared in the dialysate. Obviously the acid reaction produced by the ganglioside itself (initially about pH 2.5) was sufficient to split off most of the sialic acid, as the product remaining in the inner fluid at the end of the experiment contained only 7 % of sialic acid. The ease with which the sialic acid was split off indicates that it is attached at least partly as an end group in the ganglioside.

The lyophilized residues were taken up in methanol, and the extract evaporated in vacuo. The partly crystalline residue was washed with ether, and dissolved in a minimum amount of water. After addition of a few ml of methanol, ether was added in small portions as long as amorphous precipitates were formed. These contained sialic acid together with other material. They were removed by filtration, and not further examined. Petroleum ether was added to the filtrate until a faint opalescence appeared. On standing at room temperature the sialic acid crystallized in needles. The crystalline material was washed with cold methanol, dissolved in a little water and methanol, and recrystallized by addition of ether and petroleum ether. (Found: N 4.40; 4.48. Calc. for C<sub>11</sub>H<sub>19</sub>NO<sub>9</sub>: N 4.53). Quantitative determinations with Bial's and Ehrlich's reagents 12 and paper-chromatographic analysis

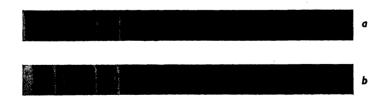


Fig. 1. X-ray powder diagrams of sialic acid isolated from (a) ganglioside, (b) sheep submaxillary mucin (Guinier camera. Cu Ka-radiation).

<sup>\*</sup> Apparently the same substance has been obtained by us from bovine submaxillary mucin which had been dialysed against weak HCl. The substance showed the same X-ray diffraction pattern as the sialic acid from sheep submaxillary mucin 5. This form of sialic acid contains only an N-acetyl. Obviously the labile O-acetyl was also split off during the isolation procedure employed by Klenk and Faillard. As it cannot be excluded that treatment with methanol-HCl at 105° C brings about intramolecular changes of the very unstable sialic acids, the name 'acetyl neuraminic acid' in this connexion is somewhat questionable.

gave identical results for the substance isolated and for sialic acid from sheep submaxillary mucin. (The  $R_F$  value is about 0.4 in the butanol-acetic acid solvent of Partridge.) The X-ray diffraction patterns of the sialic acid from the two sources are shown in Fig. 1.

An acetyl determination made on another ganglioside preparation gave a value corresponding to the content of hexosamine and sialic acid determined colorimetrically. There is thus no reason to believe that the gangliosides in addition to sialic acid contain neuraminic acid as a preformed component.

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Correction and Addendum to "Two Methods for the Isolation of Tracer Amounts of Plutonium" \*

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A ccidentally an error was introduced in the last line of p. 1252, which should read "equation (I) reduces to  $I_1 = f \sigma N \ a_0 \ A_0^{-1} \ \lambda_1 \ T$ ."

In order to correct for the radioactivities of the daughter products formed during the irradiation time T, the constants  $C_i$  in equation (2) should be multiplied by

$$\frac{\lambda_{n} (1-e^{-\lambda_{i}T})}{\lambda_{i} (1-e^{-\lambda_{i}T})}$$

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## 2(3)-Benzoxazolinone, an Anti-Fusarium Factor in Rye Seedlings

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Fusarium nivale has a decisive effect upon the overwintering of rye in snow-covered fields. Generally speaking plant breeders have more and more begun to take the view that certain fungi often have a greater effect on the winter hardiness of winter cereals than frost. The possible anti-fungal factors in different over-wintering plants are therefore of great interest both from the biochemical and the plant breeding point of view. The first results of investigations on this line, in our laboratory, are briefly presented in this paper.

In the experiments "Oiva" variety of rye and a strain of Fusarium nivale from the Division of Plant Disease, Tikkurila, were used. The anti-Fusarium effect of the investigated extracts from rye seedlings and finally that of the isolated pure anti-Fusarium factor was determined using agar cultures in Petri dishes.

No activity could be discovered in ungerminated rye seeds, but after 5 to 6 days of germination in light and at room temperature (seedlings ca. 10 cm high) the anti-Fusarium effect was strong. The active factor could be extracted from homogenized seedlings with ether especially after addition of acid. The substance was unaffected by mild acid hydrolysis (1 N HCl at 108° C), but this was not the case when strong hydrolysis was employed. The substance appeared to decompose in alkaline solution.

The ether extract was evaporated to dryness and was then extracted with water

<sup>\*</sup> Acta Chem. Scand. 9 (1955) 1252.