

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

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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

$$\frac{\lambda_n (1 - e^{-\lambda_i T})}{\lambda_i (1 - e^{-\lambda_n T})}$$

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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 overwintering 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.

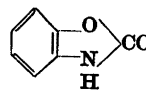
No activity could be discovered in ungerminated rye seeds, but after 5 to 6 days of germination in light and at room temperature (seedlings ca. 10 cm high) the anti-Fusarium effect was strong. The active factor could be extracted from homogenized seedlings with ether especially after addition of acid. 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

to separate lipids and other substances insoluble in water. On a one-dimensional paper chromatogram (solvent: *isopropanol-ammonia* (25 %)-water 8:1:1) the substance travelled near the solvent front. In zones where a strong auxin effect could be shown no anti-Fusarium effect was to be found in the concentrations used. Determinations with pure  $\beta$ -indole-acetic acid and 3-indoleacetonitrile showed that in concentrations in which they appear in plants they have no anti-Fusarium effect.

The extract was dissolved in *isopropanol-water* (80 : 20) and the insoluble part separated by centrifugation. The anti-Fusarium factor was separated from other substances in the extract in a cellulose powder column (9 x 50 cm) using as solvent *isopropanol-water*. When chlorophyll started to come out of the column 14 fractions of 200 ml each were taken. An anti-Fusarium activity could be found in fractions 2 and 3. These fractions were evaporated into dryness *in vacuo* and thoroughly shaken (glass beads added) with water. The water phase was separated. When evaporating the water solution crystals were formed. These crystals dissolved easily in hot water slightly in cold water. After three crystallizations the substance melted at 140° C. (Found: C 61.69; H 3.76; N 10.25 (Kjeldahl digestion with  $\text{KMnO}_4$ ). Calc. for  $\text{C}_7\text{H}_7\text{O}_2\text{N}$ : C 62.20; H 3.73; N 10.36.)

On the basis of its empirical formula and chemical and physical characteristics (*e. g.* aromatic ring, no ninhydrin reaction, no carboxyl group and the absorption spectrum in the ultra violet with a strong maximum at 270  $m\mu$ ) it appeared possible that the compound might be 2(3)-benzoxazolinone. This substance was synthesized from *o*-aminophenol and urea according to the method of Baywater *et al.*<sup>2</sup> M. p. of the synthetic substance 141° C, mixed m. p. with the substance isolated by us 140° C. M. p. of the acetyl derivate of the synthetic substance 91° C, of the isolated substance 92° C, mixed m. p. 92° C. The UV spectra of isolated and synthetic benzoxazolinone were identical. Also on paper chromatograms both substances behaved identically in different solvent systems. The anti-Fusarium factor in rye seedlings was thus shown to be 2(3)-benzoxazolinone. As far as we are aware benzoxazolinone had not hitherto been found in Nature.



In our experiments on an agar medium the benzoxazolinone isolated by us inhibited the growth of *Fusarium nivale* completely in 0.05% and weakly in 0.01% solution. The amount of benzoxazolinone we could isolate in a crystalline form from fresh rye seedlings was 0.01%. The actual content of the substance is, however, obviously higher. 2(3)-benzoxazolinone also inhibits the growth of clover-rot fungus (*Sclerotinia trifoliorum*) in the same concentrations as the growth of *Fusarium nivale*. Red clover contains some factor which inhibits the growth of *Sclerotinia* and *Fusarium*. The inhibitory substance in clover is being investigated.

Investigations on the content of benzoxazolinone in different varieties of rye and wheat are in progress. We are also investigating the mechanism of formation of this substance during germination. In our opinion the elucidation of the structures of natural anti-factors of different fungi causing plant diseases and destroying crops is of considerable importance in connection with plant breeding.

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