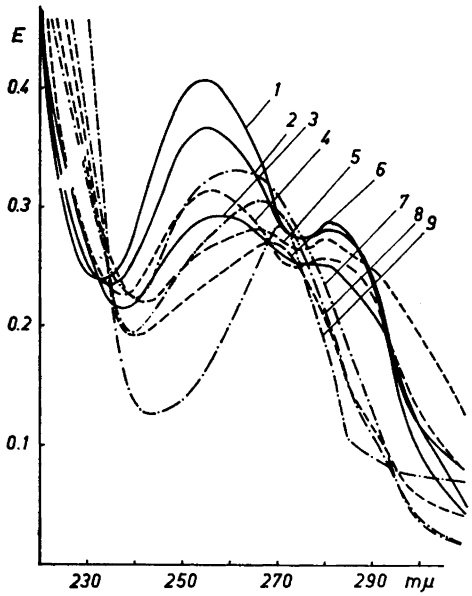

Fig. 2. UV spectra of water solutions of the aglucone of different concentrations heated at 100°C. Aglucone, max. 254 mμ, 282 mμ; BOA max. 270 mμ.

1. 10 mg/ml 5 min, *2.* 15 min, *3.* 60 min, *4.* 300μg/ml 5 min, *5.* 15 min, *6.* 60 min, *7.* 10 μg/ml 5 min, *8.* 15 min, *9.* 60 min.

decreases when the aglucone concentration rises. This is shown by the following test.

The aglucone was heated in a test tube in a boiling water bath at concentrations of 10 mg/ml, 300 μg/ml, and 10 μg/ml for 5 min, 15 min, and 1 h. For the measurement of spectra the first solution was diluted 1:1 000 and the second 1:30, and the most dilute solution was measured as such. Fig. 2 shows the changes occurring in the spectra of the different solutions.

When a relatively short reaction time is used, the formation of BOA can therefore be followed by spectrophotometry only in very dilute solutions of the aglucone.

The highest values found in this laboratory for BOA in rye seedlings have been about 1.1 mg of BOA per g fresh weight[3], corresponding to 2.8 mg of the glucoside if the yield is quantitative. The quantitativeness of the yield depends decisively on how thoroughly the plant material is crushed. A 90 % yield of BOA is more probable when plant material (0.5—1 g) is crushed in a mortar. The hydrolysis of the glucoside in a homogenate of rye seedlings is rapid. For the sake of safety it is best to allow the homogenate to stand at room temperature for 30 min before heating.

The support given by the *Rockefeller Foundation* is gratefully acknowledged.

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3. *cf.* Virtanen, A. I. *Angew. Chem.* **70** (1958) 544.

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The Synthesis of Precursor II of Benzoxazolinone Formed in Rye Plants, and the Enzymic Hydrolysis of Precursor I, the Glucoside

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In earlier papers Virtanen *et al.*[1,2] have presented the structure 2,4-dihydroxy-1,4-benzoxazin-3-one (I) for the aglucone enzymatically formed in crushed rye seedlings. The structure is now confirmed by the synthesis of the aglucone itself and its reduction product (II), which is obtained by reduction with zinc dust in boiling acetic acid[3].

The starting material for the synthesis of the aglucone was *o*-(methoxymethoxy)-nitrobenzene (III), from which the corresponding phenylhydroxylamine derivative (IV) is prepared in the usual way by reduction with zinc dust in a neutral medium. By allowing this compound to react with dichloroacetylchloride, N-(*o*-methoxymethoxyphenyl)-dichloroacethydroxamic acid (V) is obtained. The methoxymethoxy group is then hydrolysed by heating in a dilute methanolic hydrochloric acid solution. Finally the compound formed (VI) is

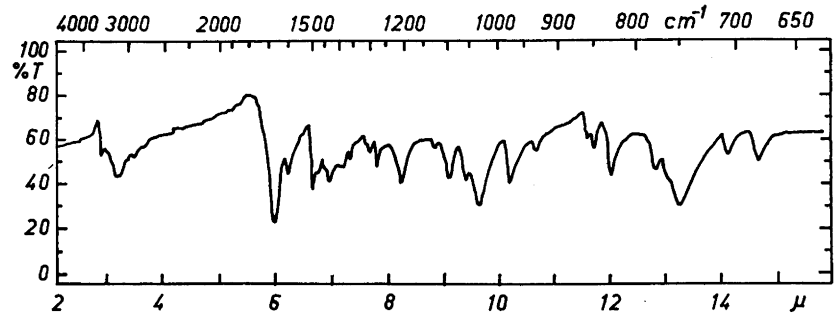


Fig. 1. The IR spectrum of the synthetic and the natural aglucone.

dissolved in a sodium hydroxide solution and allowed to stand for some time at room temperature, whereby hydrolysis and cyclisation to 2,4-dihydroxy-1,4-benzoxazin-3-one(I) occurs.

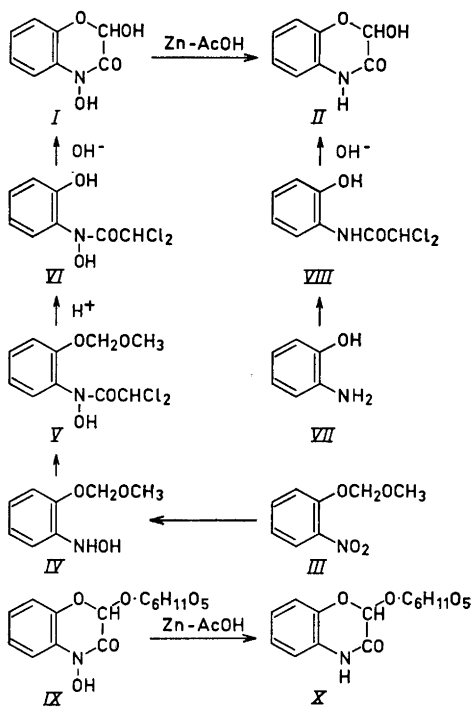
The synthesis of the reduction product [3] is carried out by an analogous procedure. The starting material was now *o*-aminophenol (VII), from which the N-dichloroacetyl derivative (VIII) is prepared. When this compound is dissolved in a dilute sodium hydrogencarbonate solution and boiled for a short time, the 2-hydroxy-1,4-benzoxazin-3-one (II) is formed.

The identity of these compounds with the aglucone and its reduction product were confirmed by the mixed melting points, the UV and the IR spectra. Fig. 1 shows the IR spectrum of the synthetic aglucone. The IR spectrum of the natural aglucone is identical with the spectrum in the figure. The spectrum is in accord with the structure proposed by Virtanen and Hietala for the aglucone.

When the glucoside, also isolated from rye seedlings, was reduced by an analogous procedure as the aglucone, a substance (X) is obtained, which also did not give any colour reaction with ferric chloride. On the basis of the similarity of its UV spectra with that of the reduction product of the aglucone, the structure (X) can be suggested for this compound and the structure (IX) for the glucoside. The attempts to synthesize the compound (X) from the reduction product (II) and acetobromoglucose failed.

All melting points are corrected.

o-(Methoxymethoxy)-nitrobenzene. A mixture of 5.31 g of dry potassium salt of *o*-nitrophenol and 2.5 g of chloromethyl-



methylether [4] in 15 ml of dry benzene was refluxed for one hour. The benzene solution was then washed several times with dilute sodium hydroxide solution and dried over sodium sulphate. The solvent was evaporated and the residue distilled in a vacuum. The yield was 3.9 g (71 %). B. p. 134—135°/7 mm. (Found: N 7.74. Calc. for $C_8H_9NO_4$: N 7.65.)

o-(Methoxymethoxy)-phenylhydroxylamine. A solution of 3.66 g of *o*-(methoxymethoxy)-nitrobenzene and 2.0 g of ammonium chloride in 50 ml of 60 % alcohol was cooled below 10°C. To this solution 2.0 g of zinc dust were added in the course of half an hour with stirring. The solution was filtered and 50 ml of water were added, and then extracted with ether. The ether solution was dried and the solvent evaporated. The residue (3.3 g) was a yellow oil which did not crystallize. It was used without further purification for the following reaction.

2,4-Dihydroxy-1,4-benzoxazin-3-one. To a solution of 3.3 g of the crude *o*-(methoxymethoxy)-phenylhydroxylamine in 50 ml of dry ether 1.45 g of dichloroacetylchloride in 20 ml of ether were gradually added under cooling (0°C). The ether solution was decanted from the dark brown oil and evaporated to dryness. The residue was dissolved in 20 ml of methanol and 2 ml of 2 N hydrochloric acid and refluxed for half an hour. The solvent was evaporated under reduced pressure, and the residue was dissolved in 20 ml of 1 N sodium hydroxide solution, and allowed to stand for 2 h at room temperature. After addition of dilute hydrochloric acid to the acid reaction, the solution was filtered and extracted first several times with benzene (to remove the coloured impurities) and then with ether. The ether solution was dried and the solvent evaporated. The residue was dissolved in a few ml of 0.5 N hydrochloric acid and extracted several times with benzene. The water solution was filtered and then extracted with ether. After drying, the solvent was evaporated and the residue crystallized from a mixture of ether-benzene. The overall yield was 61 mg (3.5 %). M. p. 153–154° C, mixed m. p. 151–152°C with the aglucone (m. p. 151–152°C).

UV spectrum (in ethanol).

Synthetic product: max. 255 mμ, ε = 8 850; max. 282 mμ, ε = 5 880.

Aglucone: max. 254 mμ, ε = 8 500; max. 282 mμ, ε = 5 800.

Reduction of the aglucone: 48 mg of the aglucone were dissolved in 20 ml of glacial acetic acid, and 100 mg of zinc dust were added. The mixture was boiled for 15 min and excess zinc was filtered off. The acetic acid was evaporated under reduced pressure, and the residue was crystallized from an ether-benzene mixture. Yield 25 mg (57 %). M. p. 201–203°C. UV spectrum (in ethanol): Max. 250 mμ, ε = 8 900, max. 282 mμ, ε = 4 350. (Found: C 58.23 H 4.45; O 28.82. Calc. for $C_8H_7NO_3$: C 58.18; H 4.27; O 29.06.)

o-(Dichloroacetamido)-phenol. To a solution of 2.18 g of pure *o*-aminophenol in dry ether 1.5 g of dichloroacetylchloride in ether were added gradually. After standing for one hour at room temperature, the precipitated *o*-aminophenol hydrochloride was filtered off, and the filtrate evaporated to dryness, whereat the product crystallizes. After recrystallization from a mixture of benzene-petroleum ether the m. p. was 134–135°C and the yield 1.7 g (77 %). (Found: N 6.20. Calc. for $C_8H_7Cl_2NO_2$: N 6.37.)

The synthesis of the reduction product. 220 mg of *o*-(dichloroacetamido)-phenol and 10 ml of 0.2 M $NaHCO_3$ solution were boiled for 10 min. The solution was cooled and made slightly acid with hydrochloric acid and extracted with ether. The ether solution was dried, a few ml of benzene were added, and most of the ether was evaporated, upon which the product crystallizes. M. p. 201–203°C. Yield 124 mg (75 %). Mixed m. p. 201–203°C with the reduction product of the aglucone. UV spectrum (in ethanol): Max. 250 mμ, ε = 8 850, max. 282 mμ, ε = 4 400.

Reduction of the glucoside. To a boiling solution of 10 mg of the glucoside in 5 ml of acetic acid 25 mg of zinc dust were added. After boiling for 15 min, the excess zinc was filtered off. The acetic acid was evaporated under reduced pressure, and the residue was dissolved in a few ml of water. The zinc was then precipitated as sulphide. After filtration the solution was evaporated to dryness and the residue was dissolved in absolute alcohol. Benzene was added, and the alcohol was distilled off, upon which the product crystallized. M. p. 158–160°C. (Found: N 4.12. Calc. for $C_{14}H_{17}NO_8$: N 4.28.) UV spectrum (in ethanol): Max. 250 mμ, ε = 7 500, max. 278 mμ, ε = 3 800.

The hydrolysis of the glucoside occurs rapidly through an enzyme present in rye seedlings, as previously shown in this laboratory. The enzyme has not been purified or further investigated yet. The glucoside was not hydrolysed by yeast in our experiments. On the other hand, hydrolysis of the glucoside was observed in a homogenate of the small intestine of the rabbit, although a much weaker one than in rye seedlings. A homogenate of rat intestine had much the same effect as that

of the rabbit. From these observations we may conclude that the glucoside can be decomposed in the animal organism without the enzyme present in plants.

The support given by the *Rockefeller Foundation* is gratefully acknowledged.

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The Enzymic Formation of Thiocyanate (SCN^-) from a Precursor(s) in *Brassica* Species

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The chemical nature of the goitrogenic "*Brassica* factors" is still unclear to a great extent. This is partly due to the fact that several active factors may be involved, which are moreover formed only through enzymic reactions when plants are crushed. This has long been known in regard to the formation of the strongly goitrogenic thiooxazolidones occurring in the crushed and moistened seeds of many crucifers [1]. Recently Virtanen *et al.*[2], and Kreula and Kiesvaara [3] found the formation of vinyl-thiooxazolidone also in cabbage, kale, rape, and other fodder plants belonging to the *Cruciferae* family.

The same authors [4] have also demonstrated the transfer of this substance to milk in very small amounts (about 0.05 % of the amount fed). These quantities are so small that they cannot be expected to have any goitrogenic effect in the milk. Kreula and Kiesvaara [4] have given a detailed report on the methods used in these investigations. Somewhat later Altamura *et al.*[5] independently also demonstrated the formation of vinyl-thiooxazolidone in cabbage.

The goitrogenic effect of thiooxazolidones is due to the fact that they inhibit the synthesis of thyroid hormones. This effect cannot be overcome by high doses of iodide.

Another type of goitrogenic substances belonging to the *Brassica* factors has a primary influence on the uptake of iodide by the thyroid gland. This influence can be prevented by increasing the amount of the iodide dose. Many pieces of information given in the literature [6,7] indicate that salts of thiocyanic acid (SCN^-) represent this type in crucifers. Jirousek [8], who has investigated and reviewed the thiocyanate metabolism, found that in the animal organism SCN^- is formed endogenously from cyanides, nitriles, and sulphur-containing compounds, and that both the SCN^- brought exogenously into the organism and that formed endogenously in it have to be taken into consideration as goitrogenic factors. Michajlovskij and Langer [9,10] have performed systematic determinations on the SCN^- content in different vegetables. They found a particularly high SCN^- content in the press juice of different cabbage species (up to 50 mg %). They used the term "präformiertes Rhodanid" for the SCN^- present in food stuffs, obviously in contrast to the SCN^- formed endogenously.

Our investigations concerning the problem whether it is possible to make milk goitrogenic by feeding cows with plants, especially those belonging to the *Cruciferae*, have led to some new findings regarding the *Brassica* factors. These findings are briefly reported in the present paper.

An account was recently given about the formation of organic thiocyanates in some crucifers [11]. Benzyl thiocyanate was found to be formed enzymatically from glucotropaeolin in *Lepidium ruderale* and allyl thiocyanate from sinigrin in *Thlaspi arvense*. It was found in this laboratory that after the injection of benzyl thiocyanate into rats, the SCN^- content has risen considerably in blood serum and in different organs. This finding led us to look for thiocyanate esters also in *Brassica oleracea* species. For the present we have, however, no indications for the formation of such esters in fresh cabbage species. On the other hand, the result of these investigations was the finding that free SCN^- is formed from glucosidic precursors, present in cabbage. The thiocyanate found by Michajlovskij and Langer in the press juice of cabbage is thus not "preformed SCN^-"