UV decomposition of DPN which is in agreement with the findings of Althorpe et al. (1970). It appears, however, that nitramines may also be degraded by UV but at a much slower rate than NAs. The most reliable method for confirmation of nitrosamines continues to be high resolution mass spectral analysis of not only the NO^+ ion but also the molecular (M^+) ion, or complete low-resolution spectra.

Continuing work at this laboratory indicates that the TEA may respond positively to nitramine compounds in general.

SAFETY: *N*-Nitroso compounds must be handled with caution, as many are potent carcinogens.

ACKNOWLEDGMENT

We wish to thank the National Cancer Institute for the loan of the TEA.

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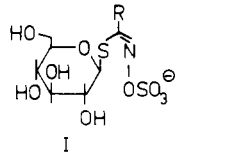
Received for review December 15, 1977. Accepted February 17, 1978. Technical Paper 4728, Oregon Agricultural Experiment Station, Oregon State University. This work was supported in part by Food and Drug Administration Grant No. 1R01 FD-00382.

Studies of Some Nonenzymatic Reactions of Progoitrin

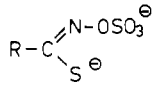
Salo Gronowitz,* Leif Svensson, and Ragnar Ohlson

The hydrolysis of isolated progoitrin (2-hydroxy-3-butenylglucosinolate) at different pHs and in different buffers has been investigated. The rate of disappearance of the glucosinolate was determined, as well as the formed hydrolysis product. No goitrin could be detected. Rapid hydrolysis was observed in the presence of borax and a new product, 5-vinyl-2-oxazolidinone, previously not isolated from progoitrin, was obtained, besides the known hydrolysis product 1-cyano-2-hydroxy-3-butene.

In connection with the development of a process for the preparation of a protein concentrate for human consumption from *Brassica napus* rapeseed (Ohlson, 1972), it became of importance to transform the glucosinolates present in rapeseed, especially progoitrin (Ia) and glu-



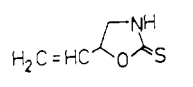
I



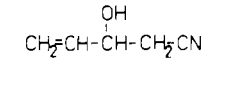
II

a R = CH_2=CH-CH(OH)-CH_2-
b R = CH_2=CH-CH_2-CH_2-

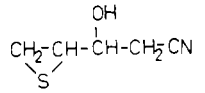
conapin (Ib), to safe degradation products as fast as possible. The glucosinolates appear in the leach water from the protein process. The enzymatic hydrolysis of glucosinolates by myrosinases (thioglucoside glucohydrolases) (Björkman, 1976) gives the aglycon II, which is unstable and by chemical reactions is transformed to isothiocyanates and nitriles. Low pH favors nitrile formation, indicating that nitriles are formed via a S- or N-protonated form of II (van Etten et al., 1966). Nitrile formation could also be promoted by some metal cations, especially Fe(II) ions (Austin et al., 1968; Tookey and Wolff, 1970; Kirk et al., 1971). From Ia, (*R*)-1-cyano-2-hydroxy-3-butene (IV) (Daxenbichler et al., 1966) and



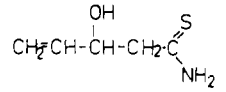
III



IV



V



VI

(*S*)-5-vinyl-2-oxazolidinethione (III) (Astwood et al., 1949) are formed, the latter by further reaction of the isothio-

Division of Organic Chemistry, Chemical Center, University of Lund, S-220 07 Lund 7, Sweden (S.G., L.S.) and Research Laboratory, Karlshamns Oljefabriker, 292 00 Karlshamn, Sweden (R.O.).

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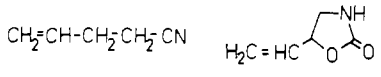
cyanate. Alternative catabolism leads to the formation of the two diastereomers of 1-cyano-2-hydroxy-3-epithiobutane (V) (Daxenbichler et al., 1967; Tookey, 1973). The mechanism for the formation of the cyanoepithioalkanes is not known with certainty, but it is believed that these compounds, as well as thiocyanates, are formed by enzymes which are very labile and hitherto not characterized or identified. A recent discussion of glucosinolate catabolism has appeared (cf. Benn, 1977).

The factors influencing the relative amounts of III and IV from Ia are the same as for other glucosinolates, as it has been found that low temperature and low pH favor for formation of IV over III in enzymatic hydrolysis. However, the pretreatment of the rapeseed meal was also of importance. Thus, only III was obtained when the meal was heated at 120 °C for 1 h and then hydrolyzed by white mustard myrosinase (van Etten et al., 1966).

The compound III is also called goitrin due to its strong antithyroidal properties (Greer, 1962). Unfortunately, progoitrin, also after inactivation of myrosinase, shows a goitrogen effect due to the presence of different types of thioglycosidases from certain microorganisms present in the intestinal tract (Greer, 1962; Oginsky et al., 1965). Furthermore, IV (*S* form) is also rather toxic (LD_{50} = 170 mg/kg for mice) (van Etten et al., 1969).

A few investigations have been carried out on the nonenzymatic breakdown of progoitrin and its epimer epiprogoitrin after deactivation of the myrosinases, but mostly without prior isolation of the glucosinolate. On treatment with Fe(II) salts, Ia gave IV and 3-hydroxy-4-pentenethionamide (VI) (Austin et al., 1968; Kirk et al., 1971). At low pH (6 M HCl) 2,4-pentadienic acid has been obtained from epiprogoitrin (Daxenbichler et al., 1965). The minor glucosinolate in *Brassica napus* (Ib) yields 3-butenyl isothiocyanate and 1-cyano-3-butene (VII) upon hydrolysis.



VII VIII

We therefore hoped that by deactivating the myrosinases and using isolated Ia it would be possible to obtain other degradation products, especially if the primary chemical attack could be directed toward other positions than the thioglycosidic bond.

Using essentially the method described by Björkman (1972), a glucosinolate mixture was isolated from peeled, crushed, defatted, and enzyme-inactivated rapeseeds of the variety *Sv. Sinus* supplied by AB Karlshamns Oljefabriker. The hygroscopic glucosinolate mixture consisted of 65–70% of Ia, 15% of Ib, and small amounts of glycobrassicanapin and gluconapoliferin (2-hydroxy-4-pentenylglucosinolate). Quantitative analyses were achieved by silylation using silylated sinigrin as internal standard (Persson, 1974).

We have studied the breakdown of our glucosinolate mixture in distilled water at 100 °C. It can be seen from Figure 1 that Ia and Ib disappear at about the same rate and that after 5 h about 36% of the original amounts are still left.

At room temperature, 52% of Ia was still present after a week. The products identified by GLC–mass spectrometry from these experiments were IV from Ia, which was also isolated, and VII from Ib. No III could be detected. The nitriles, however, were obtained in 60% yields, and the fate of the rest of the glucosinolates is not clear.

Experiments were also carried out in the following buffer solutions: potassium hydrogen phthalate–hydrochloric acid (pH 3), potassium hydrogen phthalate–sodium hydroxide (pH 5), potassium dihydrogen phosphate–sodium hydroxide (pH 7 and 8), sodium bicarbonate–sodium hydroxide (pH 10), and disodium hydrogen phosphate–sodium hydroxide (pH 11). The glucosinolate mixture was hydrolyzed for 1 week at 20, 60, and 80 °C (pH 3, 5, 7, and 11), and the results obtained are summarized in Figure 2. It can be seen that Ia is most stable at pH 7. If the experiments are carried out at 100 °C, (pH 5, 8, and 10), there remain after 30 min at pH 5, 13% of Ia; at pH 8, 58%; and at pH 10, 15%.

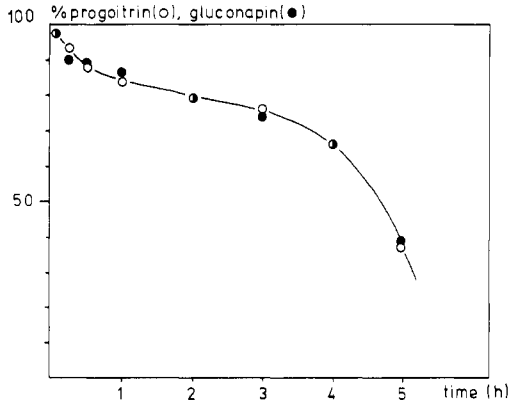


Figure 1. Remaining progoitrin and gluconapin after hydrolysis at 100 °C in distilled water.

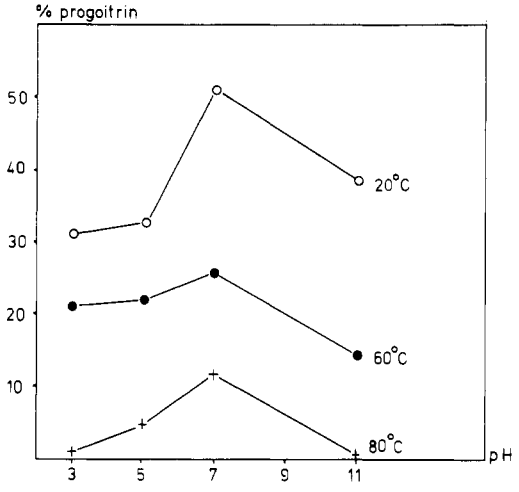


Figure 2. The hydrolysis of progoitrin at different temperatures and pHs.

In this connection, we discovered that if the buffer for pH 8 was prepared from sodium tetraborate and sodium hydroxide, an unusually rapid disappearance of the glucosinolates was observed, apparently caused by the tetraborate. We therefore studied the effect of borate at different pHs. It was found that Ia and Ib were completely decomposed within 30 min at 100 °C in the pH interval between 2 and 13.5. The hydrolysis is still fast at room temperature. At pH 5, 9%, at pH 8, 13%, and at pH 11, 3% of the original Ia is left after 30 min at room temperature. Sodium tetraborate was very efficient in increasing the rate of hydrolysis. Even less than 0.5 mol of borate/mole of glucosinolate has a strong effect (Figure 3).

Two compounds were isolated from this experiment, IV and a compound previously not known as a product of the hydrolysis of Ia, namely 5-vinyl-2-oxazolidinone (VIII), which is preferentially formed between pH 8–12 (cf. Figure 4). The structure of VIII followed from its IR, UV, NMR, and mass spectra and was confirmed by synthesis.

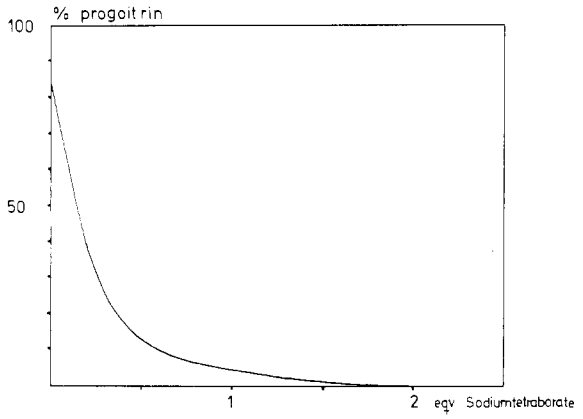


Figure 3. Remaining progoitrin after 30 min at 25 °C as a function of added sodium tetraborate.

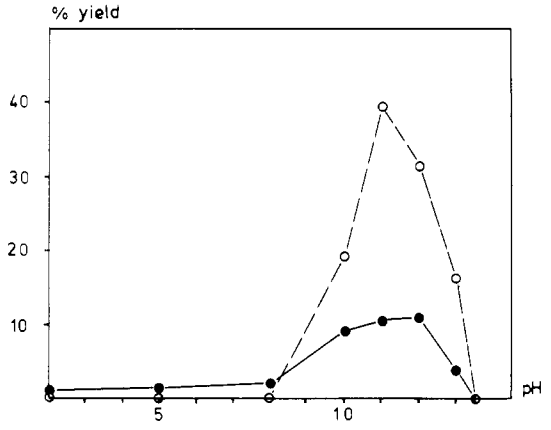


Figure 4. Yield of 1-cyano-2-hydroxy-3-butene (●) and 5-vinyloxazolidinone (○) in the hydrolysis of progoitrin at 100 °C at various pHs in the presence of sodium tetraborate buffer.

Authentic VIII was synthesized by reacting ethyl 3-hydroxy-4-pentenate, obtained through a Reformatsky reaction between acrolein and ethyl bromoacetate, with hydrazine hydrate to the hydrazide. Treatment of the latter with sodium nitrite in hydrochloric acid followed by heating gave the desired compound via a Curtius rearrangement of the intermediate acid azide. The synthesis of VIII by the reaction of butadiene monooxide and cyanuric acid is described in a patent (Little and Pickens, 1964).

The stability of VIII under hydrolytic conditions was also investigated. After 30 min at 100 °C, only 30–40% remained, both at pH 5.8 and 11. The amino alcohol which should be formed could not be found.

Various attempts to detect other water-soluble compounds by GLC, TLC, and other techniques failed.

It has recently been demonstrated (Friis et al., 1977) that treatment of glucosinolates with strong base leads to α-amino acids via a Neber rearrangement. Thus, benzyl glucosinolate yields phenylglycine. Upon treatment of Ia with 2 M sodium hydroxide, a positive ninhydrin test was obtained. However, all attempts to isolate the expected amino acid failed.

DISCUSSION

Thus no goitrin was found in the hydrolysis of isolated Ia at different pHs. This can hardly be due to the rapid destruction of III, as it has been found that III, when formed under enzymatic conditions, has a rather long life-time (Michajlovskij et al., 1970). Experiments with 2-oxazolidinethione (Ettlinger, 1950) as a model substance have confirmed this observation. No change in the intensity of the UV maximum at 240 nm of 2-oxazolidinethione could be observed when a 10 mM solution in water was heated at 100 °C for 30 min. This was also the case when 1 equiv of sodium tetraborate was added. It is therefore clear that VIII cannot have been formed by rapid desulfurization of III due to the presence of borate. It seems reasonable, therefore, to explain the absence of goitrin in the nonenzymatic hydrolysis products by rapid isomerization of the *Z* isomer of II to the *E* isomer, which prevents migration of the R group to give the isothiocyanate. *Z* and *E* forms of thiohydroxymates have been studied, and it is known that the latter give nitriles (Davies et al., 1968). It can, however, not be excluded that this hydrolysis does not primarily proceed via II. This could be true for the hydrolysis in the presence of tetraborate. However, there is a possibility that the tetraborate causes the transformation of II to a hydroxamic acid derivative with *Z* configuration, which through a Lossen rearrangement yields isocyanate, which spontaneously undergoes ring closure to VIII.

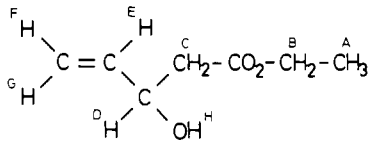
EXPERIMENTAL SECTION

Isolation of Glucosinolates. Following the procedure of Björkman (1972), a yellowish white crystalline mixture of glucosinolates, consisting of 65–70% of progoitrin, 15% of gluconapin, and small amounts of glucobrassicanapin, and gluconapolipherin, was obtained.

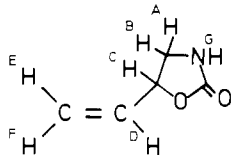
Hydrolysis Experiments. The following buffer solutions were prepared: pH 3, potassium hydrogen phthalate and hydrochloric acid; pH 5, potassium hydrogen phthalate and sodium hydroxide; pH 7, potassium dihydrogen phosphate and sodium hydroxide; pH 8, potassium dihydrogen phosphate and sodium hydroxide; pH 8, sodium tetraborate and hydrochloric acid; pH 10, sodium bicarbonate and sodium hydroxide; pH 11, disodium hydrogen phosphate and sodium hydroxide. The pHs were checked at 25 °C with a pH meter and adjusted to within ±0.05 pH unit.

The hydrolysis experiments were carried out in 7-mL test tubes with grooves. Approximately 15 mg of the glucosinolate mixture was dissolved in 2.5–3 mL of the buffer solution. The solutions were heated in a thermostat for the appropriate time at different temperatures. The solutions were then freeze-dried, ca. 3 mg of sinigrin was added as internal standard and thereafter 1 mL of a silylating agent consisting of hexamethyldisilazane, trimethyl chlorosilane, and pyridine in the proportions of 2:1:10 by volume. The mixtures were heated at 110 °C for 40 min. For the GLC analyses a 1.5-m glass column packed with 3% OV-17 on Chromosorb Q 80/100 in a temperature interval of 195–235 °C (temperature gradient 2 °C/min) was used. The injector temperature was 235 °C, the detector temperature 250 °C, and the carrier gas (helium) rate was 20–25 mL/min. The response for the rapeseed glucosinolates compared to sinigrin was 1, and the sinigrin was estimated to be 90% pure. Some of the results obtained are given in Figures 1–3.

In order to determine the products formed in the various hydrolysis experiments, ca. 15 mg of the glucosinolate mixture in 2.5–3 mL of the buffer solution was heated for various times. After cooling, the solution was extracted five times with 10 mL of chloroform. To the combined chloroform solution 30 μL of a methyl palmitate solution in chloroform (10 g/L) was added as internal standard and the mixture dried over magnesium sulfate. After filtration, the mixture was evaporated to a volume of a few milliliters and analyzed by GLC on a Perkin-Elmer 900 apparatus using a 2.5-m column packed with 10% NPGS on Chromosorb W in a temperature interval of 60–200 °C (tem-



A δ 1.25 (t) J_{CD} = 6 Hz
B δ 4.14 (q) J_{ED} = 5.3 Hz
C δ 2.52 (d) J_{EG} = 17.3 Hz
D δ 4.54 (q) J_{EF} = 10.0 Hz
E δ 5.93 (2 q) $J_{GF} \sim J_{GD} \sim J_{FD} \sim$ 1.5 Hz
F δ 5.07 (m)
G δ 5.24 (m)
H δ 3.87 (s)



A, B δ 3.24 (t) and 3.67 (t) J_{AB} = 8.5 Hz
$J_{AC} \sim J_{BC}$ = 8.5 Hz
C δ 4.95 (split q) J_{CD} = 6.5 Hz
D δ 5.87 (2q) J_{DE} = 16.8 Hz
E δ 5.32 (2t) J_{DF} = 10.0 Hz
F δ 5.22 (2t) $J_{EF} \sim J_{EC} \sim J_{FC}$ = 1.2 Hz
G δ 6.72 (broad s)

Figure 5. NMR spectral data for ethyl 3-hydroxypentenate (60 MHz, $CDCl_3$) and 5-vinyloxazolidinone (100 MHz, $CDCl_3$).

perature gradient 8 °C/min). The responses for IV and VIII were 0.65 and 0.34, respectively. The results are given in Figure 4. In order to isolate acids or bases, the pH of the aqueous phase was adjusted and then again extracted with ether, but no other compounds could be isolated.

In order to isolate VIII, 5 g of the glucosinolate mixture (containing 70% of progoitrin) was refluxed in 250 mL of borate buffer (pH 10) for 30 min. The solution was cooled and extracted with chloroform. After drying with sodium sulfate and evaporation, the residue was chromatographed on 10 g of silica gel with ether–petroleum ether (3:1) as eluent. After the elution of IV (0.08 g), VIII was obtained in pure form (0.13 g). Both IV and VIII had the same spectral properties as authentic samples.

1-Cyano-3-butene, bp 40 °C (15 mmHg), was obtained in 57% yield. Literature value: 50% yield, bp 136 °C (Podzimková et al., 1969).

Ethyl 3-Hydroxy-4-pentenate. To 40 g (0.60 mol) of zinc powder, which had been washed with dilute sodium hydroxide solution, water, dilute acetic acid, water, ethanol, acetone, and ether and subsequently dried in a vacuum desiccator at 100 °C, 10 mL of a solution of 83.5 g (0.50 mol) of ethyl bromoacetate and 33.6 g (0.60 mol) of acrolein in 80 mL of anhydrous benzene and 20 mL of dry ether was added. The mixture was heated until the reaction started and the remaining solution was added dropwise during 1 h. The mixture was cooled in an ice bath and 200 mL of cold 10% sulfuric acid was added with vigorous stirring. The organic layer was separated and extracted with 5% sulfuric acid until extraction with 10% sodium carbonate caused no precipitation. The organic phase was then extracted once more with 5% sulfuric acid and with water. The combined acid solutions were extracted with ether and the combined organic phases dried with magnesium sulfate and the solvent evaporated in vacuo. Distillation gave 21.6 g (30%) of the title compound, bp 50–54 °C (0.25 mmHg). Its NMR data are summarized in Figure 5.

3-Hydroxy-4-pentenylhydrazide. Ethyl 3-hydroxy-4-pentenate (3.7 g, 0.027 mol) and 1.93 g (0.041 mol) of 99% hydrazine hydrate were dissolved in hot ethanol, enough to form a single phase. The solution was refluxed for 5 h and the ether was evaporated. The residue was used directly in the next step.

5-Vinyl-2-oxazolidinone. The hydrazide was dissolved in a slight excess of 1 M hydrochloric acid, covered with ether, and cooled below 10 °C, 22 g (0.032 mol) of sodium nitrite was then added. The ether layer was separated, dried with magnesium sulfate, diluted with benzene, and heated cautiously until nitrogen evolution had ceased. After evaporation, the product was distilled at 115–116 °C (10^{-2} mmHg) [lit. (Little and Pickens, 1964) 125–130 °C (10^{-1} mmHg)]; yield, 1.1 g (34.7%). Its NMR data are summarized in Figure 5.

2-Oxazolidinethione was prepared according to Ettlinger (1950), in 40% yield, mp 97–98 °C [lit. (Ettlinger, 1950) yield, 43%; mp 98–99 °C].

ACKNOWLEDGMENT

Grants from the Board for Technical Development (STU) and from the Royal Physiographic Society in Lund are gratefully acknowledged.

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Received for review December 12, 1977. Accepted February 28, 1978.