somewhat similar at late maturity. Trends of sugars in trefoil showed little change from early to later maturity and were much less responsive to environmental conditions. These results seem to fit well with the observations of Smith (*10*) that cyclic trends of carbohydrates readily available as energy to the plant in the roots of trefoil were much less pronounced than in the roots of alfalfa and red clover.

The closest agreement between values for total nonstructural carbohydrates from the two extraction methods occurred for samples harvested at early stages of maturity. In some instances at the early stages, smaller amounts of carbohydrates were extracted by 2% H_2SO_4 than by the enzyme method. The effect, limited to early maturity stages and most pronounced in Ladino clover, may have been due to the destruction of reducing substances in these extracts. In addition, 2% H_2SO_4 may have extracted variable amounts of hemicellulose, depending on the species and maturity stage of the sample. A superficial investigation of some 2% H_2SO_4 extracts by qualitative paper chromatography suggested that pentoses were being extracted. These would be included in carbohydrate determinations as reducing sugars. No attempt was made to determine amounts of these sugars, so the importance of this consideration remains uncertain. The fact that freeze-dried tissue was used must again be stressed since this may be of considerable importance in facilitating a more complete extraction of oligosaccharides, dextrins, or other carbohydrates that otherwise might be occluded within structural material or rendered less extractable in other ways by oven drying.

The interrelationships of percentages of glucose, fructose, and sucrose generally were not consistent with advance in maturity, particularly when the results of one year were compared with those of the other. Trefoil was a notable exception in that percentages of glucose consistently were lower than those of fructose during both years. In Ladino clover, levels of glucose during both years were lower than those of fructose during early stages of maturity with a reversal of that situation during later maturity stages. Levels of sucrose appeared to respond more readily than those of glucose or fructose to seasonal environmental differences and often varied more extremely over the range of maturity stages.

Determinations of total nonstructural carbohydrates and sugar fractions were made on whole-plant samples. The results, therefore, are most meaningful in relation to the use of legume forages as silage or in green feeding. The importance of fermentable carbohydrates in satisfactory silage production usually is emphasized. Stage of maturity may be important. Lanigan and Catchpoole (*6*) noted in laboratory studies with ryegrass and white clover that the proportion of legume could be increased with later maturity stages. The generally higher levels of nonstructural carbohydrates in red and Ladino clovers may be of importance to successful silage-making since they are readily fermentable.

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SEED MEAL HYDROLYSIS

Variation in Enzymatic Degradation Products from the Major Thioglucosides in *Crambe abyssinica* and *Brassica napus* Seed Meals

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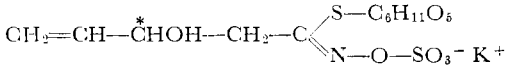
A recent review by Kjaer (*9*), indicated that the thioglucosides of Cruciferae and related plant families generally yield isothiocyanates upon enzymatic hydrolysis. Oxazolidinethiones (goitrins) are formed if the thioglucoside contains a hydroxyl group properly located in the aglycon to facilitate ring closure. References are given concerning the occasional formation of nitrile plus sulfur or thiocyanate instead of the isothiocyanate. Glucose and acid sulfate ion are also released from thioglucosides (*11, 13*), but these products are not often mentioned because they do not vary with the nature of the thioglucoside.

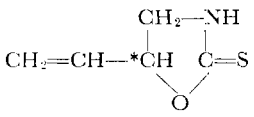
Figure 1 shows the major thioglucosides from *Crambe abyssinica* and *Brassica napus* seeds, and the known products derived from the aglycon (excluding HSO_4^- which is always present). Daxenbichler, VanEtten, and Wolff (*3*) identified the principal thioglucoside of crambe as *epi*-progoitrin (I). (R)-goitrin (II), enzymatically derived from this thioglucoside (*3*), is the enantiomer of (S)-goitrin (II) derived from progoitrin (I) of *B. napus* (*10*). Later Daxenbichler, VanEtten and Wolff (*4*), reported the formation of (S)-1-cyano-2-hydroxy-3-butene (III) when isolated *epi*-progoitrin was hydrolyzed by white mustard myrosinase at pH 3; the (R) enantiomer was formed from progoitrin.

The variation in the kind and amount of aglycon products (other than HSO_4^-) derived from enzymatic hydrolysis of the thioglucosides when the seed meals of crambe and of *Brassica napus* (rapeseed) are autolyzed under various conditions is reported.

Epi-progoitrin (I), the principal thioglucoside of crambe seed, has at least two patterns of degradation in wetted seed meal. Under some conditions, the product is a "cyano" fraction (IV) consisting of (S)-1-cyano-2-hydroxy-3-butene (III) and unknown substances containing sulfur, instead of the expected (R)-goitrin (II). Formation of II is increased by diluting the meal with water, by increasing the temperature, by raising the pH, by dry heating of the seed meal, or by storage of the seed under ambient conditions (compared with cold storage). Under conditions of test, enzyme hydrolysis to form IV only is faster than when II is also formed. Tests with *Brassica napus* (rapeseed) show a similar phenomenon in seed from this related plant.



Thioglucoside, I



Goitrin, II

1-Cyano-2-hydroxy-3-butene, III

Figure 1. Thioglucosides and enzymatic products from the organic portion of the aglycon

The configurations of the chiral-centers (*) are not shown. For configuration according to the rule of Cahn, Ingold, and Prelog (2), see text

Materials and Methods

Seed Source and Meal Preparation. Samples of seed from 50 accessions of *Crambe abyssinica* Hochst ex R. E. Fries were examined. These were grown in 12 states between 1959 and 1964. Total thioglucoside content ranged from 7.8 to 10.3% of the air-dry defatted seed calculated as *epi*-progoitrin potassium salt (*5*). About 90% of the thioglucoside was *epi*-progoitrin (*13*). Some of the original seed from which the plantings were made were of known Polish, Russian, or Swedish seed source; for others, the original seed sources were unknown.

After removal of the pericarp (pod), the seed was ground in a hammermill. The oil was removed by Soxhlet extraction with petroleum ether, boiling range 30° to 60° C., unless otherwise described. The seed meals were routinely reground in a Wiley mill to pass a 60-mesh screen.

For tests with *Brassica napus* (Argentine-type rapeseed), two accessions of seed grown in Montana in 1963 were prepared for testing in the same manner as the crambe.

Methods of Analysis. Tests for extent of thioglucoside hydrolysis and assays of total thioglucosides were made by titration of the free HSO_4^- ion (*13*). *Epi*-progoitrin and progoitrin were measured by conversion to goitrin with white mustard myrosinase (*13*). Goitrin was estimated by measurement of ultraviolet absorbance (*12*). For the estimation of cyano compounds, the absorbance at 4.4 microns was measured (*4*), and results were calculated as 1-cyano-2-hydroxy-3-butene.

Ultraviolet absorbance was measured on a Beckman Model DK-2A spectrophotometer and infrared absorbance on a Perkin-Elmer Model 137 or Model 337. A Burrell Kromo-Tog K5 was used for gas chromatography. Total sulfur was determined by the method of White (*17*) and total nitrogen by micro-Kjeldahl.

Autolysis of Seed Meals. All experiments were carried out with the endogenous enzymes in the intact defatted meal. Except for the experiment in which the effect of pH was studied, all autolyses were in water-meal slurries, which ranged from pH 4.8 to 5.6. No effect was observed that could be assigned to differences of pH within this range. For room temperature autolyses, the desired amounts of water were mixed with the meal and the mixtures allowed to stand 1 hour. Autolyses above room temperature were carried out in a constant temperature bath.

After being autolyzed and heated in a water bath to coagulate protein, meal solids were removed by centrifugation. Supernate and water washes of the solids were combined and made to a standard volume. An aliquot was taken for sulfate titration. The remaining water solution was mixed with twice its volume of absolute ethanol to precipitate protein-like material. The solubles were concentrated on a rotary evaporator to remove ethanol. The aqueous solution was extracted four times with twice its volume of peroxide-free ethyl ether in order to extract quantitatively the goitrin and cyano compounds. After combined ether extracts were dried with anhydrous sodium sulfate, appropriate volumes were taken for the goitrin and cyano compound assays.

Autolyses at different pH's were controlled by continuous adiustment of pH by addition of 0.1 *N* HCl or NaOH. The water extracts were neutralized before ether extraction. To determine autolysis rate, the enzyme reaction was stopped by adding boiling water at appropriate time intervals.

Nature of Thioglucoside Aglycon Products

Isolation. An aglycon-products fraction was prepared by room temperature autolysis of crambe meal (1.2 ml. water per gram) followed by extraction of the wet paste with acetone (8 ml. per gram of meal), and then by two additional extractions (4 ml. per gram) (*12*). The combined aqueous-acetone extracts were freed of acetone in a circulating evaporator. The aqueous residue was extracted twice with two volumes of petroleum ether to remove gumlike material and then extracted four times with two volumes of peroxide-free ethyl ether. The combined ether extracts were dried, and the solvent was removed to give the aglycon-products fraction. This fraction from five 500-gram batches of autolyzed crambe meal averaged 1.30% (range 1.27–1.36%) of the weight of the air-dry defatted meal. Analysis gave: sulfur, av. 16.4, range 15.8 to 17.2%; nitrogen, av. 10.7, range 10.6 to 11.2%. Products as per cent of original meal were: cyano compounds calculated as 1-cyano-2-hydroxy-3-butene, av. 0.90, range 0.88 to 0.92%; (R)-goitrin 0.0%. The clear brown liquid had an odor of hydrogen sulfide or mercaptans. At room temperature in the presence of air, the liquid became viscous and eventually solidified in about a week's time. When the liquid was kept in the refrigerator under nitrogen this change was inhibited. With increase in viscosity, the product became less soluble in water and chloroform but remained soluble in acetone. With a further increase in viscosity it became acetone insoluble.

The aglycon-products fraction from 500 grams of meal without autolysis was 0.23% by weight of the starting meal. Products (as per cent of the original meal) were cyano compounds calculated as 1-cyano-2-hydroxy-3-butene, 0.05%; goitrin, 0.0%.

The aglycon-products fraction was also isolated from similar preparations of

autolyzed *B. napus* seed. The fraction (average of two preparations) was equal in weight to 0.95% of the original meal; sulfur content, 17.4%; nitrogen, 9.8%; cyano compounds calculated as 1-cyano-2-hydroxy-3-butene, 0.64%; (S)-goitrin, 0.0%. A preparation from the seed without prior autolysis was only 0.15% by weight of the starting meal.

Identification of 1-Cyano-2-hydroxy-3-butene. One hundred milligrams of the aglycon-products fraction from autolyzed crambe meal dissolved in 0.2 ml. of methanol were applied to five thin-layer chromatographic plates (8 × 8 inch) spread with silica gel. The major components migrated as one band and separated from minor components when the plates were developed with ethyl ether–*n*-hexane (95 to 5). This major band, detected with iodine vapor, was removed and rechromatographed with the same solvent on plates spread with silica gel containing 1% silver nitrate. Under these conditions, the nitrile separated from the remaining substance(s) and migrated to the same position as authentic 1-cyano-2-hydroxy-3-butene (*4*). The nitrile band gave a yield of 13 and 15 mg. from two 100-mg. samples. The infrared spectrum from a film on a sodium chloride plate was identical with that of authentic 1-cyano-2-hydroxy-3-butene. Quantitative infrared analysis by the method of Daxenbichler, Van-Etten, and Wolff (*4*) showed 89% purity. Sulfur content was 0.1%. Gas chromatography on both a polar and a nonpolar column gave elution peaks identical with that of the authentic nitrile.

Table I. Thioglucoside Content and Myrosinase Activity in Crambe Meal after Various Treatments[a]

Experiment No.	Treatment	Total Thioglucoside, %	Myrosinase Activity
1	Meal, defatted at 27° ± 2° C.	9.2	+
2	Defatted meal, dry heated at 116°–119° C., 4 hours[b]	8.6	+
3	Defatted meal, dry heated at 128°–134° C., 3¾ hours[b]	5.4	+
4	Defatted meal, dry heated at 140°–160° C., 12 hours[b]	0.0	−
5	Defatted meal, autoclaved 5 minutes, 121° C.	4.8	−
6	Defatted meal, autoclaved 50 minutes, 121° C.	0.0	−
7	Meal, defatted with boiling carbon tetrachloride	9.3	+
8	Flaked seed containing 38% water, at 27° C. for 3 hours	0.0	+
9	Flaked seed containing 26% water, at 27° C. for 3 hours	1.4	+
10	Flaked seed containing 16% water, at 27° C. for 3 hours	7.7	+
11	Flaked seed (air-dry) containing 7% water	9.0	+

[a] Percentages based on air-dry defatted meal. Myrosinase activity qualitatively tested for by titrating sulfate ion formed from 5–10 mg. *epi*-progoitrin added to wetted sample held at room temperature and pH 4.8–5.6 for 5–10 minutes. Experiments 1 to 7 run on seed from a single typical accession.

[b] Time required to reach the stated temperature (1 to 1½ hours) is not included. Meals were heated in open trays.

Variation in Thioglucoside Aglycon Products

Stability of Myrosinase and Thioglucosides in Crambe Seed Meal. The effects of various combinations of heat and moisture on the thioglucoside content and enzymatic activity of crambe meals are summarized in Table I. This information was useful in selecting conditions for studying the aglycon products. Experiment 2, Table I, shows both thioglucoside stability and the presence of enzyme activity after oven-heating for 4 hours at 116–119° C. In contrast, both myrosinase and the thioglucosides were easily destroyed by autoclaving in contact with steam (Experiments 5 and 6).

Experiment 7 shows that boiling carbon tetrachloride extraction of the oil from air-dry flaked seed does not destroy the myrosinase enzyme. Experiments 8 to 11 show that the minimum moisture content at which thioglucoside hydrolysis occurs at room temperature lies between 7 and 16%.

No loss of thioglucosides or myrosinase activity was observed in various crambe accessions, some of which were stored dry in unheated barns longer than 4 years.

Screening of Seed Meals for (R)-Goitrin Formation. Autolysis of crambe seed meals from 50 accessions carried out in 5 ml. of water per gram of meal at 27° C. showed the following: From 0.7 to 7.3% (R)-goitrin (as per cent *epi*-progoitrin in the meal) was found in 21 accessions. Only a trace or no (R)-goitrin was found in the remaining. Twelve accessions stored more than 3 years under ambient conditions in an unheated barn gave 3.2 to 7.3% (R)-goitrin. Most, but not all, recently harvested seed showed no (R)-goitrin formation under the test conditions. Those seed meals that gave no (R)-goitrin also had no isothiocyanate odor during autolysis although 10% of the thioglucosides should give volatile isothiocyanates (*13*).

Effect of Controlled Variables on Aglycon Products from Crambe. A seed meal that gave no (R)-goitrin upon autolysis at room temperature formed (R)-goitrin in significant amount at a temperature of 45–50° C. (Figure 2). (R)-goitrin formation increased with temperature to 68° C. with a concomitant decrease of the cyano-containing products. Total goitrin at 68° C. was about 75% of that formed by white mustard myrosinase acting on the isolated thioglucosides at an optimum pH of 7.0 to 7.5. Nevertheless, sulfate ion titration showed that hydrolysis was 98% complete at both 4° and 65° C. At 75° C. only 51% of the thioglucosides were hydrolyzed because of heat destruction of the enzyme.

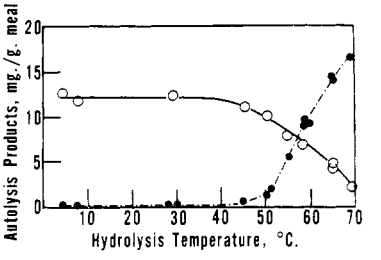


Figure 2. *epi*-Progoitrin hydrolysis products as a function of autolysis temperature

Conditions of autolysis: 3 grams of meal per 15 ml. H_2O. ●——●, (R)-goitrin; ○——○, cyano-containing products as 1-cyano-2-hydroxy-3-butene. The maximum yield of 16.3 mg. (R)-goitrin is equivalent to 5.4% *epi*-progoitrin in the meal. The 12 mg. of 1-cyano-2-hydroxy-3-butene is equivalent to 5.3% *epi*-progoitrin. Total *epi*-progoitrin in the meal: 7.2%

Figure 3 (meal A) shows that increase in pH from about 6 to 11 caused an increase in (R)-goitrin and a decrease in formation of the cyano-containing products. Increase in pH seemed to increase (R)-goitrin formation in a manner similar to an increase in temperature (Figure 2) except below pH 5. Here (R)-goitrin formation increased somewhat with an accompanying decrease in the cyano-containing products.

With a seed meal that gave consider-

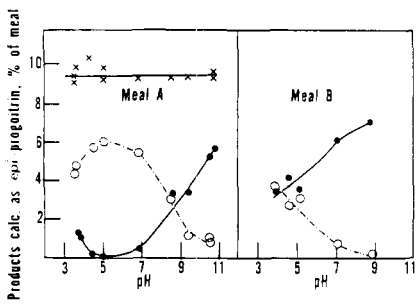


Figure 3. Hydrolysis products from *epi*-progoitrin as a function of pH from defatted meal incubated with water (5 ml. per gram)

For pH control, see *Materials and Methods*. Meal A gives no (R)-goitrin on autolysis in water at room temperature; meal B yields appreciable (R)-goitrin. x——x, total thioglucosides by sulfate titration; ○——○, cyano-containing products; ●——●, (R)-goitrin

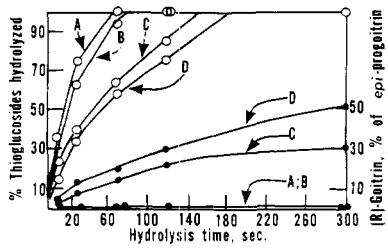


Figure 4. Rate of autolysis of crambe meals with formation of cyano compounds (curves A and B) or with formation of (R)-goitrin (curves C and D) as the principal product

Conditions of autolysis: 250 mg. crambe meal, 1.25 ml. water, pH 4.8–5.6, 25–27° C. ○——○, per cent of total thioglucosides; ●——●, (R)-goitrin

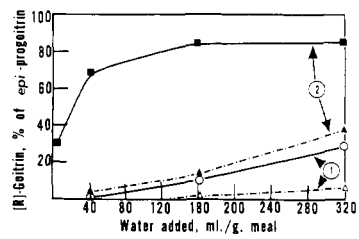


Figure 5. (R)-Goitrin formation as related to crambe meal autolysis in varying amounts of water from seed stored under various conditions

(1) Age of seed, 1 year; △——△, stored at 5° C.; 0——0, stored in unheated barn. (2) Age of seed 4 years, ▲——▲, stored at 5° C.; ■——■, stored in unheated barn. Total *epi*-progoitrin in each sample 8.1 and 7.5%, respectively

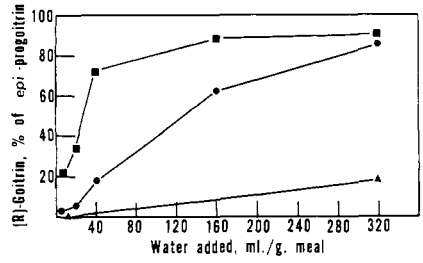


Figure 6. Effect of dry heat on (R)-goitrin formation by autolysis of crambe meal

▲——▲, defatted meal unheated; ●——●, heated to 120° C. 1 1/4 hours; ■——■, heated to 120° C. (1 1/4 hours) and held at that temperature 1 hour

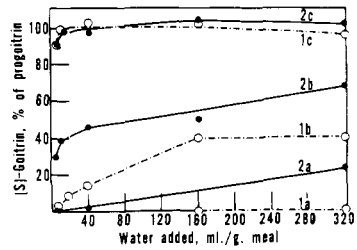


Figure 7. Effect of dry heat on (S)-goitrin formation by autolysis of *Brassica napus* seed meal

○——○, defatted meal accession 1; ●——●, accession 2; 1a, 2a, unheated; 1b, 2b, heated at 100° C. for 1 hour; 1c, 2c, heated at 120° C. for 1 hour. Progoitrin content of air-dry meal 1, 3.3%; meal, 2, 3.4%. Total thioglucoside content of air-dry meal 1, 7.3%; meal 2, 7.2%. In dry heating, about 1/2 hour and 1 hour were required for the meal to reach 100° and 120° C.

able (R)-goitrin on autolysis at pH 5 (Figure 3, meal B), (R)-goitrin increased with rising pH, but the point of equal goitrin and cyano production was found at pH 5 rather than near pH 9 as in meal A.

When the end products were all cyano or related products and contained no (R)-goitrin, the hydrolysis was essentially complete in 60 seconds (Figure 4). In those seeds from which some (R)-goitrin was formed, the rate of hydrolysis was slower. Only 75 to 80% of the hydrolysis was complete in 120 seconds.

(R)-Goitrin formation increased as a given meal sample was diluted with water (Figure 5). Agitation to keep the insoluble meal particles dispersed had no effect on the results. Figure 5 also shows that seed from the same planting, stored in an unheated barn and thus exposed to extremes in temperature, gave more (R)-goitrin under a given condition of autolysis than did the same seed stored at a constant temperature of 5° C.

A sample of air-dry defatted seed meal that gave only a small amount of (R)-goitrin at room temperature autolysis was dry heated. Dry heating the meal at temperatures at which the myrosinase and the thioglucosides were not destroyed (Table I) accelerated the effect caused by long storage of the seed at ambient temperatures (Figure 6).

Effect of Controlled Variables on Aglycon Products from *Brassica napus*. Of the two *Brassica* species which constitute the rapeseed of commerce, *B. napus* contains the greater amount of progoitrin (*16*) and, therefore, more nearly resembles crambe. Examination of two accessions of *B. napus* 1 year after harvest and after dry heating (Figure 7) shows that the same type of autolysis of the thioglucosides in rapeseed occurs as does in crambe. Hydrolysis of the progoitrin in seed meals that had been heated 1 hour at 120° C., gave as the final product essentially 100% (S)-goitrin.

Discussion

The autolysis products from *epi*-progoitrin that may have physiological activity are those derived from the aglycon. These products can be extracted from crambe meal with aqueous-acetone. A weight comparison of the aglycon-products fraction from autolyzed and unautolyzed crambe meal showed that more than 80% of this fraction was formed as a result of endogenous enzyme action. None of the amino acid or sulfate sulfur was carried into the ether extract. Since the products fraction contained an average sulfur content of 16.4%, it must have come from the thioglucosides. This fraction from autolyzed meal contained an average of 69% cyano compounds calculated as 1-cyano-2-hydroxy-3-butene which is a hydrolysis product of *epi*-progoitrin (*4*). At least 13% of the fraction was isolated and identified as 1-cyano-2-hydroxy-3-butene. Identification of the remaining portion of this fraction is the object of current research. When the crambe meal was autolyzed under conditions that give (R)-goitrin, the (R)-goitrin was found in the aglycon-products fraction (*12*). The corresponding fraction from autolysis of rapeseed meals with no formation of (S)-goitrin are similar to those from crambe formed under similar conditions. This fact is evidence that the course of enzyme hydrolysis is similar in the seed from both species.

In a review of work at his laboratory, Virtanen (*15*) reported that the formation of benzylisothiocyanate, thiocyanate, and nitrile occurs when seed from the crucifer *Lepidium sativum* is crushed and moistened. These compounds are hydrolysis products from the thioglucoside glucotropaeolin. Probably these investigators were studying a phenomenon from the seed of *L. sativum* that is similar to the one the authors observed from crambe seed. Points of similarity are: Autolysis at higher temperatures favors the formation of benzylisothiocyanate or (R)-goitrin which may be derived from an intermediate isothiocyanate; heat treatment to destroy the enzymes in *L. sativum* (*14*) or in crambe seed followed by hydrolysis with added crude white mustard myrosinase yields from the respective seeds only benzylisothiocyanate or (R)-goitrin; formation of benzyl-

nitrile or 1-cyano-2-hydroxy-3-butene occurs in either seed when autolysis is carried out in the wetted seed meal with no pH adjustment; and in both seeds the hydrolysis of the thioglucosides to give products other than benzylisothiocyanate or (R)-goitrin is extremely rapid. The reaction products from the crambe thioglucoside differ in that no evidence for the formation of organic thiocyanates has been found.

Mild handling—such as storage of crambe seed under ambient conditions or even dilution of a slurry with water—causes an increase in goitrin formation and a decrease in the formation of the cyano-containing compounds. If nitrile formation is enzymatic, the enzyme must be extremely labile. Other labile substances present in the seed which might react with the initial enzymatic product(s) could also explain this behavior. We have no evidence that either goitrin or the group of cyano compounds is a precursor of the other product. The variation in product with pH is similar to that shown by white mustard myrosinase, although in the latter case the transition from nitrile to goitrin is from pH 3 to pH 7 (*4*). The possible enzymatic formation of nitrile is not to be confused with a two-enzyme system, reported for myrosinase in which a thioglucosidase and sulfatase are separated (*6*). The pathway of enzymatic breakdown of *epi*-progoitrin is not apparent. Clarification of the mechanism is an objective of current research.

Minor differences in autolysis conditions and treatment of the seed before autolysis affect the kind and amount of end products from the thioglucoside aglycon. Each end product may give a different physiological effect when fed to animals. Similar effects would also be produced by seed of different age or drying treatments even when the thioglucosides were not hydrolyzed. If the endogenous enzymes were still present, rapid hydrolysis (see Figure 4) would be expected during the initial stages of digestion by the animal.

In the absence of thioglucoside-hydrolyzing enzymes, progoitrin can be hydrolyzed to give goitrin by microorganisms commonly found in the nonruminant intestinal tract (*7*). It would be interesting to know whether under these conditions of thioglucoside hydrolysis the final products from the aglycon of *epi*-progoitrin and progoitrin consist also of 1-cyano-2-hydroxy-3-butene and other cyano-containing products.

Feeding experiments with rapeseed, for the most part unidentified as to species, show a wide variation in animal response (*1*, *8*). Variation of feeding value of rapeseed meals may be caused by variation in the nature of the thioglucosides present in the particular species of seed, as well as variation in the nature of enzymatic hydrolysis products because of processing conditions.

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RESIDUES IN SPINACH

Conversion of DDT to TDE in Canned Spinach

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OWING TO Federal regulations governing the registration of pesticide chemicals, large quantities of data have been accumulated on the persistence of residues on raw agricultural products. Tolerances established by the Food and Drug Administration also apply to the raw crops. The behavior of pesticide residues during the interval between the harvest of food crops and their arrival on the plate of the consumer has received little attention.

For a significant portion of the fruits and vegetables consumed in the United States, commercial canning operations intervene between harvest and use, and many foods reach the plate of the consumer after processing and storage in enameled or plain-bodied tin cans or in glass jars. The effect of commercial canning operations on pesticide residues has not been studied by modern methods. Some data on the effect of processing on DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] in various fruits and vegetables were obtained during the period between 1947 and 1950 (*1*, *2*, *13*, *14*, *20*). The analytical procedures available to these workers would not distinguish conveniently among the various isomers and analogs of DDT that might be present initially or formed during the processing operation.

The need for information on the behavior of pesticide compounds in all parts of the environment has been recognized,