

Natural Glucosinolates (Thioglucosides) in Foods and Feeds

C. H. VanEtten, M. E. Daxenbichler, and I. A. Wolff

The chemistry of the glucosinolates is reviewed and also the biological effects from ingestion of plants containing these compounds. Isothiocyanates, nitriles, oxazolidinethiones, and thiocyanate ion are formed from glucosinolates in the various Cruciferae plants that are used for food, condiment, and feed. As normally consumed these plants apparently have no deleterious effect because of the low concentration of glucosinolates in the edible parts. Higher concentrations of glucosinolates in

such oilseed meals as rape and crambe limit the amount of the seed meal that can be used in animal feeds. Work at the Northern Laboratory shows that under many conditions of autolysis (endogenous enzyme hydrolysis) of meal from the rapeseed *Brassica napus* and of crambe seed meal, the major glucosinolates form previously unrecognized organic nitriles instead of the 5-vinyloxazolidine-2-thiones (goitrins).

About 4% of the total incidence of nontoxic endemic goiter may be attributed to causes other than iodine deficiency (Greer, 1962b). There is evidence that within this 4%, goitrogens in natural foods may be a contributing factor. Foods from the crucifer plant family, such as cabbage, turnips, and rutabaga, are most often implicated (Greer, 1950, 1957, 1960, 1962b; Roche and Lissitzky, 1960; VanEtten, 1969).

Experimental evidence for a goitrogen in food was obtained by Chesney *et al.* (1928). They reported greatly enlarged thyroids in rabbits when the animals were fed cabbage as a major part of their diet. Later investigators were not always able experimentally to produce the enlarged thyroids. When Hercus and Purves (1936) failed to produce "cabbage goiter," they tried feeding the seed from cabbage and related *Brassica*. The rats fed the *Brassica* seed consistently developed enlarged thyroids, and other investigators were able to repeat the experiment. However, the animals often showed poor growth and organ pathology which indicated that toxic substances other than goitrogens were present in the seed. In later work the effect of feeding processed seed from *Brassica* and related oilseeds has been studied because after extraction of the oil the seed meal has potential use in animal feeds (Bowland *et al.*, 1965; Christian, 1958).

The natural thioglucosides, also called glucosinolates, are likely the source of goitrogens and related deleterious substances found in these plants. This review is concerned with the chemistry of these compounds and their biological effects when foods or feeds containing them or their hydrolysis products are ingested.

Sinigrin, sinalbin, progoitrin, and *epi*-progoitrin are trivial names of some individual glucosinolates. Others have been named by prefixing "gluco" to the botanical name of the plant from which they were first isolated; hence, we have the names gluconapin and glucobrassicinapin. A naming system related to chemical structure by which the thioglucosides are all called glucosinolates was proposed by Ettlinger and Dateo (1961). To the

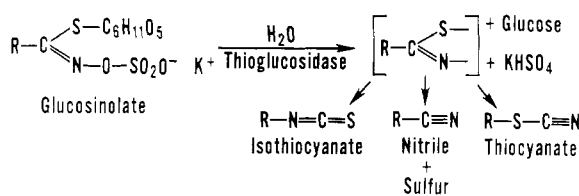


Figure 1. Enzymatic hydrolysis of glucosinolates

glucosinolate is added a prefix that chemically describes the R-group (Figure 1) which distinguishes one glucosinolate ion from another.

Nearly all those plants that are of practical value and contain glucosinolates belong to the Cruciferae family. All the 300 Cruciferae that have been examined for the compounds contain glucosinolates (Ettlinger and Kjaer, 1968; Kjaer, 1960, 1963, 1966). To date there are 50 identified glucosinolates of different chemical structure, many of which have been characterized by Kjaer and coworkers (Ettlinger and Kjaer, 1968). Although only one or two glucosinolates are usually present in relatively large amounts, as many as six of these compounds have been found in a given species.

Glucosinolates are hydrolyzed when the wet, unheated plant material is crushed. The hydrolysis is catalyzed by an enzyme in the plant material. In the early literature this enzyme is called myrosinase or mustard myrosinase. The authors prefer the name thioglucosidase as recommended by the International Union of Biochemistry for an enzyme that hydrolyzes a thioglucosyl bond (Florkin and Stotz, 1965). The separation of a myrosulfatase and a thioglucosidase from a crude mustard myrosinase has been reported by Gaines and Goering (1962), but the separation could not be repeated by Calderon *et al.* (1966). Almost all investigators believe that only one enzyme is involved in the glucosinolate hydrolysis (Ettlinger and Dateo, 1961; Nagashima and Uchiyama, 1959).

Table I lists crucifers commonly used as food, as condiments, or as oilseeds, and the glucosinolates they contain (VanEtten, 1969). Either leaves or roots, both of which contain glucosinolates in lower concentrations than the

Table I. Glucosinolates in Domesticated Crucifer Plants

Plant ^a	Glucosinolate(s) ^b Present	Reference
	For Food	
<i>Brassica oleracea</i> Cabbages, kale, brussel sprouts, cauliflower, broccoli, kohlrabi	Sinigrin Glucobrassicin Progoitrin	Jensen <i>et al.</i> (1953) Gmelin and Virtanen (1961) Altamura <i>et al.</i> (1959); Virtanen <i>et al.</i> (1958)
	Gluconapin Neoglucobrassicin	Jensen <i>et al.</i> (1953) Gmelin and Virtanen (1962)
<i>Brassica campestris</i> Turnips	Progoitrin Gluconasturtiin (<i>R</i>)-2-Hydroxy-4- pentenylglucosinolate	Astwood <i>et al.</i> (1949) Lichtenstein <i>et al.</i> (1962) Tapper and MacGibbon (1967)
<i>Brassica napus</i> Rutabaga	Progoitrin Glucobrassicin Neoglucobrassicin	Astwood <i>et al.</i> (1949) Gmelin and Virtanen (1961) Gmelin and Virtanen (1962)
<i>Lepidium sativum</i> Garden crest	Glucotropaeolin	Gmelin and Virtanen (1959)
<i>Raphanus sativus</i> Radish	4-Methylthio-3-butenyl- glucosinolate Glucobrassicin	Friis and Kjaer (1966) Gmelin and Virtanen (1961)
	For Condiments	
<i>Amoracia lapathifolia</i> <i>A. rusticana</i> Horseradish	Sinigrin Gluconasturtiin	Stoll and Seebeck (1948) Stahmann <i>et al.</i> (1943); Kjaer <i>et al.</i> (1953b)
<i>Brassica carinata</i> Ethiopian rapeseed	Sinigrin	Jensen <i>et al.</i> (1953); Ettlinger and Thompson (1962)
<i>B. juncea</i> Indian or brown mustard	Sinigrin	
<i>B. nigra</i> Black mustard	Sinigrin	
<i>Sinapis alba</i> White mustard	Sinalbin	Kjaer and Rubinstein (1954)
<i>Sinapis arvensis</i> Charlock	Sinigrin	Schultz and Gmelin (1953)
	For Feed as Processed Seed Meal	
<i>Brassica campestris</i> Rape, turnip rape, Polish rape, rubsen, naverte	Gluconapin Progoitrin Glucobrassicinapin Glucoalyssin Glucoraphanin	Kjaer <i>et al.</i> (1953a); Ettlinger and Thompson (1962)
<i>Brassica napus</i> Rape, Argentine rape, winter rape	Progoitrin Gluconapin Glucobrassicinapin Gluconasturtiin Glucoiberin Sinalbin	
<i>Crambe abyssinica</i> Crambe, Abyssinian kale	<i>epi</i> -Progoitrin Sinigrin Gluconapin Gluconasturtiin	Daxenbichler <i>et al.</i> (1965) Daxenbichler <i>et al.</i> (1964)

^a Taxonomy of the *Brassica* and related genera has been reviewed by Downey (1965).

^b Organic radicals in the glucosinolates given are as follows: sinigrin, allyl-; glucobrassicin, 3-indolylmethyl-; progoitrin, (*R*)-2-hydroxy-3-butenyl-; gluconapin, 3-butenyl-; neoglucobrassicin, *N*-methoxy-3-indolylmethyl-; gluconasturtiin, 2-phenylethyl-; glucotropaeolin, benzyl-; sinalbin, *p*-hydroxybenzyl-; glucobrassicinapin, 4-pentenyl-; glucoalyssin, 4-methylsulfinylbutyl-; glucoraphanin, 5-methylsulfinylpentyl-; glucoiberin, 3-methylsulfinylpropyl-; *epi*-progoitrin, (*S*)-2-hydroxy-3-butenyl-.

seed, are used for food. Pungency of the condiments is derived from mustard oils (isothiocyanates) which are hydrolysis products of specific glucosinolates. Varieties of *Brassica oleracea*, *B. campestris*, and *B. napus* are sources of forage for livestock (Clements and Wishart, 1956; Morrison, 1959). Grazing cattle and sheep reportedly eat cruciferous plants that grow in the wild (Bachelard and Trikojus, 1960).

STRUCTURE OF NATURAL GLUCOSINOLATES

The structure of all the glucosinolates is that of the prototype shown in Figure 1. This structure was established and later confirmed by synthesis by Ettlinger and Lundeen (1956, 1957). The structure replaces that proposed by Gadamer in 1897, in which the nitrogen was placed between the glucosinolate carbon and the R-group.

Gadamer's structure offered no explanation for the known formation of organic nitriles from the glucosinolates (Challenger, 1959; Kjaer, 1960). On hydrolysis of glucosinolates an intramolecular rearrangement may occur to give isothiocyanates or thiocyanates. Nitriles and sulfur may be formed with no change in the carbon skeleton as first reported by Schmalfluss in 1935 (Challenger, 1959; Kjaer, 1960). Glucose and acid sulfate ion are always formed from the hydrolysis. Formation of isothiocyanates, thiocyanates, and nitriles depends on conditions of hydrolysis and other unidentified variables (Miller, 1966; VanEtten *et al.*, 1966; Virtanen, 1965). Each of the characterized glucosinolates (Ettlinger and Kjaer, 1968) differs from the others only in the nature of the R-group. Many characterizations include the preparation of readily crystallized thiourea derivatives formed by reaction of ammonia with the isothiocyanate (Kjaer, 1960).

Mustard oils, long known for their pungency, are formed by thioglucosidase hydrolysis of their parent glucosinolate (Figure 2). Allyl isothiocyanate mustard oil is formed from sinigrin (allylglucosinolate), the first glucosinolate to be isolated. It is the major glucosinolate in brown and black mustard seeds and in horseradish. Similar isothiocyanates, 3-butenyl and 4-pentenyl, are formed from the glucosinolates gluconapin and glucobrassicinapin which are found in rapeseed (Kjaer and Boe Jensen, 1956; Kjaer *et al.*, 1953a). As will be apparent later, the isothiocyanates and nitriles from some of the glucosinolates are so unstable that they form other products.

HYDROLYSIS PRODUCTS FROM GLUCOSINOLATES IN CABBAGE

Barker (1936) observed that the thiocyanate ion may act as a goitrogen in man, apparently by lowering the iodine concentration in the thyroid. This effect can be overcome by increasing the amount of iodine in the diet (Astwood, 1943).

Langer and Michajlovskij (1958) found that cabbage contained thiocyanate ion. Measurements ranged from 0.7 to 10.2 mg. per 100 grams of fresh cabbage leaves. With rats on a low iodine ration, Langer and Stolc (1964) showed that feeding of thiocyanate ion caused enlarged thyroids. However, a greater thyroid enlargement was observed in rats fed cabbage that contained the same levels of thiocyanate. Sinigrin, source of allyl isothiocyanate, is one of the major glucosinolates in cabbage leaves. Daily doses of allyl isothiocyanate were fed to rats by stomach tube at levels approximating their consumption of the mustard oil if they were on a ration of high cabbage content (Langer, 1964; Langer and Stolc, 1965). The rats developed enlarged thyroids. Since thiocyanate ion concentration in the blood and in the urine of the rats increased, thiocyanate formation from the mustard oil was indicated. By these experiments Langer and his

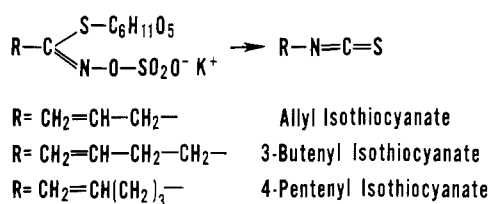


Figure 2. Formation of mustard oils

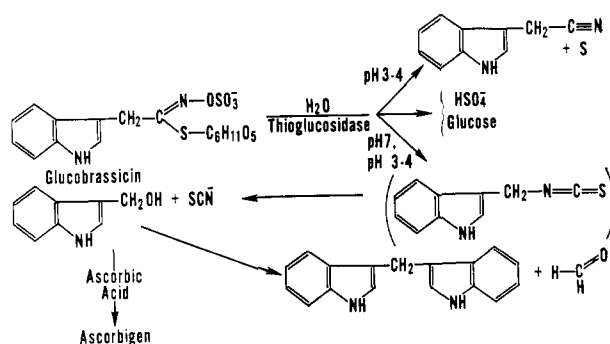


Figure 3. Enzyme-initiated degradation of 3-indoylmethylglucobrassicin

coworkers showed that in the rat on a low iodine ration, goiter was likely caused by the thiocyanate ion and allyl isothiocyanate formed from the glucosinolates in the cabbage eaten.

At about the same time Gmelin and Virtanen (1961) isolated and characterized glucobrassicin (3-indoylmethylglucosinolate) from cabbage leaves. Figure 3 includes the structure for glucobrassicin. This indole-containing glucosinolate hydrolyzes at acidic pH to give a nitrile as one of the products. At acidic and at neutral pH an unstable isothiocyanate apparently formed yields thiocyanate ion and 3-hydroxymethylindole. If ascorbic acid is present, it condenses with the 3-hydroxymethylindole to form a complex molecule known as ascorbigen. Considerable work has been done on its structure (Kiss and Neukom, 1966). Ascorbigen retains vitamin C activity (Kiesvaara and Virtanen, 1962; Matano and Kato, 1967). In the absence of ascorbic acid 3-hydroxymethylindole forms 3,3'-diindolylmethane and formaldehyde.

Later, Gmelin and Virtanen (1962) isolated small amounts of neoglucobrassicin (*N*-methoxy-3-indoylmethylglucosinolate) from cabbage leaves. They found that thioglucosidase hydrolysis of the glucobrassicins yields from 4 to 31 mg. of thiocyanate ion per 100 grams of fresh cabbage leaves. These amounts are about the same as those reported by Langer and Michajlovskij (1958). Cabbage leaves also contain small amounts of progoitrin [(*R*)-2-hydroxy-3-butenylglucosinolate] (Altamura *et al.*, 1959) and 3-butenylglucosinolate (Jensen *et al.*, 1953).

PROGOITRINS AND GOITRINS

If a hydroxyl group is present on the 2-carbon of the organic aglucon part of a glucosinolate, the isothiocyanate presumably formed by hydrolysis cyclizes. An example is progoitrin, which on hydrolysis may give (*S*)-5-vinyl-oxazolidine-2-thione, also called goitrin (Figure 4). Goitrin was isolated and characterized from rape, rutabaga, and cabbage seeds, and from rutabaga root by Astwood *et al.* (1949). Greer (1956, 1962a) later isolated progoitrin from rutabaga seed. The optical configuration of the asymmetric carbon in goitrin from these plant sources is (*S*), as established by Kjaer *et al.* (1959), according to the system of Cahn *et al.* (1956). Later, Daxenbichler *et al.* (1965) isolated the enantiomer (*R*)-5-vinyl-oxazolidine-2-thione, (*R*)-goitrin, from crambe seed. Hence its parent glucosinolate was called *epi*-progoitrin or (*S*)-2-hydroxy-3-butenylglucosinolate. Other oxazolidine-2-

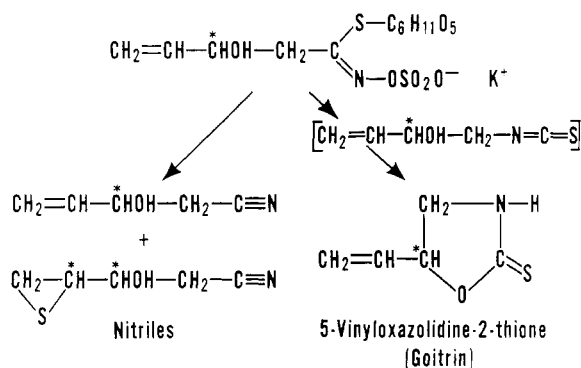


Figure 4. Products from progoitrins formed during meal autolysis

thiones from plants containing glucosinolates have been isolated and characterized (Ettlinger and Kjaer, 1968).

The (R)- and (S)-5-vinyloxazolidine-2-thiones are equally strong antithyroid agents (Greer, 1962b). They act by inhibiting the organic binding of iodine in the thyroid. Their goitrogenic effect is not prevented by the addition of iodine to the diet.

Such antithyroid compounds may be detected by a radioactive iodine test described by Stanley and Astwood (1947). After feeding radioactive iodine to healthy humans, the test material is fed. The inhibition of radioactive iodine uptake by the thyroid as compared with controls is used to estimate the amount of antithyroid activity in the test substance. The method applied to many foodstuffs by Greer and Astwood (1948) consistently gave a positive test for rutabaga root and a weaker positive test for turnip root. Cabbage leaves gave a negative test. Virtues of the method are speed and sensitivity. The test may give false positive readings and may not detect a goitrogen that is active owing to ingestion in small amounts over long periods (Peltola, 1965).

It was first thought that unhydrolyzed progoitrin fed without the active thioglucosidase would not act as a goitrogen. Later, Greer and Deeney (1959) showed that goitrin accumulated in the blood and urine after ingestion of progoitrin without thioglucosidase. An antithyroid effect was also observed. Both Greer (1962b) and Oginsky *et al.* (1965) demonstrated that thioglucosidases from microorganisms that are often found in the digestive tract, such as *Paracolobacterium*, act on progoitrin to yield goitrin.

THE QUESTION OF TRANSFERRING GOITROGENS TO COW'S MILK

Australian workers (Clements and Wishart, 1956) presented evidence in support of the hypothesis that goitrogens might be transmitted by cow's milk in high enough concentrations to cause benign goiter in children who consumed the milk. The evidence was based on a statistical study of school children in Tasmania. Their study showed significantly increased thyroid enlargement in the children as increased amounts of *Brassica* forage were used as feed for dairy cattle in the region. Thyroid enlargement increased even during a period when potassium iodide tablets were given to prevent iodine deficiency. Concentrates of the milk from the dairies were positive for antithyroid substances by the radioactive iodine test. However, the validity of the tests, as administered on the

milk concentrates, has been questioned by Virtanen and coworkers (Virtanen *et al.*, 1963).

Later, Piironen and Virtanen (1963), Vilkki *et al.* (1962), Virtanen (1961, 1965), and Virtanen *et al.* (1958, 1963) conducted extensive experiments to test the hypothesis of Clements and Wishart (1956). They found no evidence for inhibition of the radioactive iodine uptake of the thyroid in man by ingestion of milk from cattle on a high *Brassica* forage ration. Goitrin or thiocyanate ion was fed to cattle at levels equivalent to those in a high *Brassica* forage ration. Goitrin increased to 0.1 mg. and thiocyanate ion to 10 mg. per liter of milk. More than 10 liters of milk would be required to reach the lowest levels that give a positive radioactive iodine test. On the basis of these and other experiments, Virtanen concluded that milk from cattle fed *Brassica* forage contains such low amounts of known goitrogens that human consumption of the milk should not be harmful.

Peltola (1965) demonstrated, however, that the weight of the thyroids of rats increased significantly with increased doses of goitrin fed as 0.0, 0.1, 0.5, 1.0, and 2.0 $\mu\text{g.}$ per day over long periods. Since milk from cattle in endemic goiter areas of Finland contain 50 to 100 $\mu\text{g.}$ of goitrin per liter, the rats could obtain the compound in a milk diet at the level fed as an isolate. These levels of goitrin were not high enough to give a positive radioactive iodine test.

From the somewhat conflicting experimental evidence, the possible transfer of goitrogens through cow's milk to the human diet in large enough amounts to cause benign goiter remains a question not yet completely answered.

ECONOMIC IMPORTANCE OF OILSEEDS FROM THE CRUCIFERAE

According to the U. S. Department of Agriculture (1967), rapeseed ranks sixth in the world as a major oilseed of commerce. World production in 1965 and 1966 averaged 5 million tons per year, an increase of about 2 million tons per year over the annual average production between 1948 and 1953. The Far East, including India, is the major producer. Almost all rapeseed is grown from a number of varieties of *B. campestris* L. and *B. napus* L. After extraction of the oil, the meal can be fed to livestock but in limited amounts because of the goitrogens and other deleterious substances derived from the glucosinolates. If not incorporated into animal feeds, the meal is often used as fertilizer. Under the sponsorship of the U. S. Department of Agriculture (1962), a related plant, *Crambe abyssinica* Hochst. ex R. E. Fries (Abyssinian kale), is being evaluated as a farm crop. Its seed oil has industrial applications because of its high erucic acid content. Other members of the crucifer family also have promise as oilseeds for industrial use. Their successful development will depend in part on obtaining a satisfactory byproduct feed from the seed meal.

EXPERIMENTAL FEEDING OF RAPE AND CRAMBE SEED MEALS

In early experiments with processed rapeseed meals fed to either laboratory animals or livestock (Bell, 1955), few attempts were made to relate biological response to meal constituents derived from the glucosinolates. Following isolation of goitrin from rapeseed by Astwood *et al.* (1949), the goitrin content of the meals was often estimated and attempts were made to relate biological effect to the

amount present. Estimating goitrin by a method later found to give low results showed that rape varieties from *B. napus* averaged 1.56% progoitrin in the oil-free seed meal and from *B. campestris*, 0.51%. Both species contained other glucosinolates, which formed mustard oils (Wetter and Craig, 1959). This difference probably accounted in part for the marked variation in growth response and thyroid enlargement observed by early workers in which the variety of rapeseed fed was not reported. However, many investigators report evidence for the presence of unidentified toxic substances other than known goitrogens in seed from Cruciferae (Bell, 1955, 1957a, 1957b, 1965; Bell and Baker, 1957; Bell and Williams, 1953; Bowland *et al.*, 1963; Hesketh *et al.*, 1963; Holmes, 1965; Holmes and Roberts, 1963; Kennedy and Purves, 1941; Korsrud and Bell, 1967; Nakaya, 1964; Nakaya and Nakamura, 1963; Tookey *et al.*, 1965; VanEtten *et al.*, 1965, 1969).

The present status of the practical feeding of processed rapeseed meal to livestock is apparent from recommendations made by Robblee (1965) of the Canadian Department of Agriculture. Robblee recommends that rapeseed meal processed under specified conditions be limited to no more than 10% of the ration of any livestock excepting possible 15% in feed for broilers. For breeding swine, levels below 10% are recommended.

Rodent and chick-feeding experiments with crambe seed meal gave biological effects similar to those reported for rapeseed by earlier workers (Hesketh *et al.*, 1963; VanEtten *et al.*, 1965). Various heat treatments of the crambe meal gave some improvement in animal performance (Kirk *et al.*, 1966; Korsrud *et al.*, 1967; Mustakas *et al.*, 1968).

The only nontoxic crambe meals were those aqueous acetones extracted either before or after autolysis of the meal (Tookey *et al.*, 1965; VanEtten *et al.*, 1965, 1969). Analysis of some of these meals showed that they contained no glucosinolates, goitrin, or organic nitriles. This work was the first in which the organic nitrile content of cruciferous seed meals for feeding was estimated.

HYDROLYSIS OF *epi*-PROGOITRIN IN CRAMBE SEED MEAL AND PROGOITRIN IN *B. napus* SEED MEAL

Products formed from the progoitrins in crambe and in the rapeseed *B. napus* during autolysis of the seed meals include either goitrin (5-vinylloxazolidine-2-thione), or the unsaturated nitrile and diastereomeric episulfide-containing nitriles (Figure 4), or a mixture of all these compounds (Daxenbichler *et al.*, 1965, 1967, 1968). In contrast, when the isolated progoitrins are hydrolyzed with a preparation of the thioglucosidase mustard myrosinase, the unsaturated nitrile and goitrin are formed. Under these conditions the formation of the unsaturated nitrile is favored at low pH and of goitrin, at high pH (Daxenbichler *et al.*, 1966). When fresh, unheated crambe seed meal is autolyzed, *epi*-progoitrin usually gives a mixture of the three nitriles—i.e., 1-cyano-2-hydroxy-3-butene and the diastereomeric isomers of 1-cyano-2-hydroxy-3,4-epithiobutane. According to the system of Cahn *et al.* (1956), all have the (S) configuration for the carbon atom with the hydroxyl (Daxenbichler *et al.*, 1968). The epithionitriles are a mixture of isomers with respect to asymmetry about carbon 3 to which the sulfur is attached. It is assumed that episulfide formation occurs through an unstable compound produced by thiogluco-

sidase-catalyzed hydrolysis of the glucosinolate. By controlling conditions of autolysis and analysis, nitriles are found to comprise as much as 1.8% of the autolyzed oil-free meal (Daxenbichler, 1969). Possibly, unidentified toxic compounds are produced in the meal by further reaction of the episulfides with meal constituents or from the *epi*-progoitrin or other glucosinolates present in small amounts. Daxenbichler *et al.* (1967) have also isolated and identified the isomeric nitriles derived from progoitrin by autolysis of rapeseed meal (*B. napus*).

Autolysis conditions and previous history of the seed meal markedly affect the production of either goitrin or nitriles (VanEtten *et al.*, 1966) (Figures 5, 6, 7, and 8). Higher temperature during autolysis is required to obtain goitrin from untreated crambe meal under conditions of test as shown in Figure 5. The response of crambe meal A in Figure 6 is typical of meals prepared from recently harvested seed and given no heat treatment before autolysis. A water-meal slurry without added buffers has a pH near 5. At this pH only nitriles are produced from the *epi*-progoitrin. Response of meal B is typical of crambe meal from seed that has been stored at above refrigerator temperature for several months or has been dry-heated. At pH 5, both nitriles and (R)-goitrin are formed. With both meals A and B autolysis to give goitrin is favored at higher pH. Estimation of total glucosinolates hydrolyzed in meal A shows that the enzyme hydrolysis has gone to completion and that goitrin and the nitriles do not account for all the original glucosinolates.

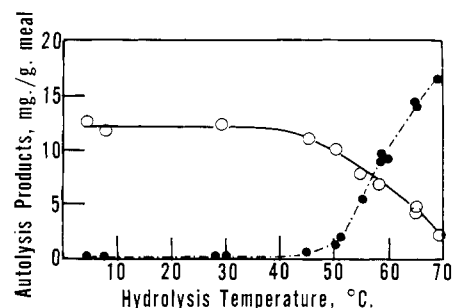


Figure 5. Effect of temperature on hydrolysis of *epi*-progoitrin during crambe meal autolysis

Conditions of autolysis: 3 grams of defatted meal per 15 ml. of H_2O . ●—●, (R)-Goitrin; ○—○, nitrile-containing products calculated as 1-cyano-2-hydroxy-3-butene

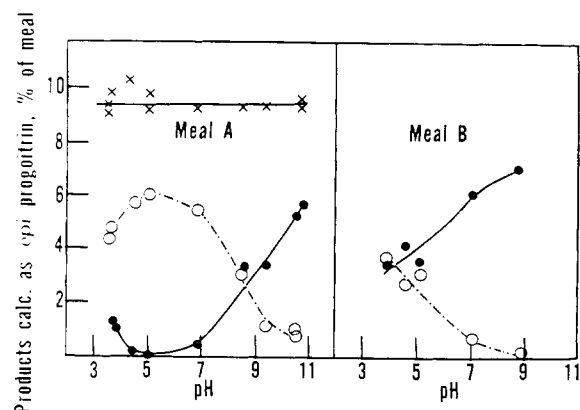


Figure 6. Products formed from *epi*-progoitrin in crambe meal vs. pH during autolysis

●—●, (R)-Goitrin; ○—○, nitrile-containing products, as 1-cyano-2-hydroxy-3-butene; x—x, total glucosinolates hydrolyzed by titration of HSO_4 ion liberated

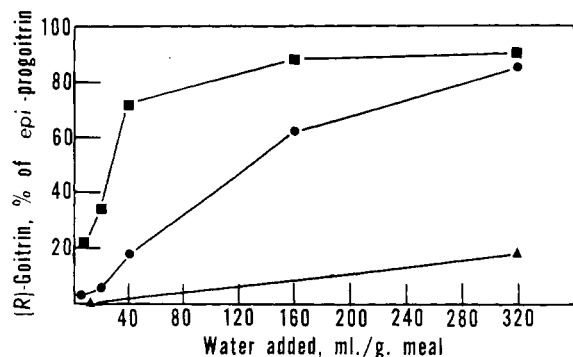


Figure 7. (*R*)-Goitrin formation as related to crambe meal autolysis in varying amounts of water with a meal given dry heat treatments before autolysis

▲—▲, Defatted meal unheated; ●—●, heated to 120° C. in 1-1/4 hours; ■—■, heated to 120° C. in 1-1/4 hours and held at that temperature for 1 hour

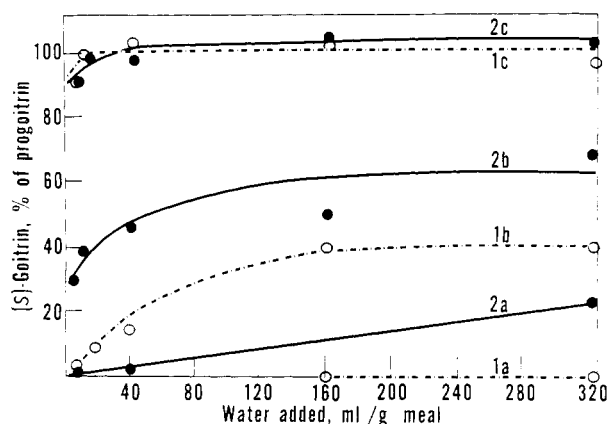


Figure 8. Effect of dry heat on (*S*)-goitrin formation by autolysis of *B. napus* species of rapeseed meal

○—○, 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 meals 1 and 2, 3.3 and 3.4%; total glucosinolate content 7.3 and 7.2%, respectively; calculated as progoitrin potassium salt

Dry heating, if not extensive, does not inactivate the thioglucosidase(s) in seed or seed meal. The effect of dry heating on (*R*)-goitrin production by seed meal autolysis is shown in Figure 7. Figures 7 and 8 also illustrate how goitrin increases as a fixed weight of meal is autolyzed in increasing amounts of water. Figure 8 shows that (*S*)-goitrin formation from progoitrin in rapeseed meal is affected by conditions in a manner similar to that of (*R*)-goitrin from *epi*-progoitrin in crambe seed. Analytical results on rapeseed meals from two accessions of seed with similar histories indicate that the amount of (*S*)-goitrin obtained depends in part on previous treatments or factors not yet identified. However, if dry-heat treatment is extensive (Figure 8, 1c, 2c), (*S*)-goitrin formation is quantitative for both accessions.

The hydrolysis of glucotropaeolin (benzylglucosinolate) in seed from *Lepidium sativum* gives benzyl isothiocyanate, -thiocyanate, and -cyanide depending on the conditions of autolysis as reported by Virtanen (1965). This hydrolysis is similar to that of the progoitrins in crambe and *B. napus*—i.e., nitrile production is rapid compared to that of the isothiocyanate or goitrins. Also, production of the nitrile is favored by low-temperature autolysis of unheated seed. However, the authors found no report

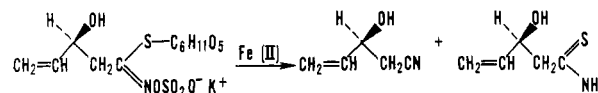


Figure 9. *epi*-Progoitrin degradation by ferrous iron

in the literature that organic thiocyanates are formed in crambe or *B. napus* seed meals.

The nonenzymic degradation of *epi*-progoitrin brought about by ferrous ion is shown in Figure 9 (Austin *et al.*, 1968). Progoitrin in *B. napus* degrades similarly. The nitriles and the newly discovered (*S*)- and (*R*)-3-hydroxy-pent-4-enethionamides are formed in an approximate ratio of 1 to 4 from *epi*-progoitrin in crambe and 1 to 2 from progoitrin in *B. napus*.

In determining progoitrins in seed meals by estimating the goitrins formed by enzymic hydrolysis, pretreatment of the meal must be done and conditions during enzyme hydrolysis must be maintained to give goitrin instead of nitriles. To avoid production of nitriles a boiling water extract is made of the hot, air-dried meal. Under these conditions the enzymes are inactivated without any hydrolysis of the glucosinolates. A suitable aliquot of the extract is buffered to pH 7.0. Mustard thioglucosidase is added and the hydrolysis carried out at 40° to 50° C. Based on the (*R*)-goitrin formed under these conditions, we find that dehulled, defatted, air-dry crambe seed meal contains from 7 to 9% *epi*-progoitrin calculated as 2-hydroxy-3-butenylglucosinolate potassium salt (VanEtten *et al.*, 1965, 1966). Selected defatted, air-dry seed meals from *B. napus* contained from 2 to 4% progoitrin. Similar progoitrin contents have recently been reported by other investigators where the thioglucosidase hydrolysis has been carried out at pH 7.0 on dry-heated meals (Appelqvist and Josefsson, 1965, 1967; Youngs and Wetter, 1967).

As far as is known, HSO_4^- ion forms quantitatively from all glucosinolates regardless of conditions for thioglucosidase hydrolysis. By titrating the HSO_4^- ion liberated, the total glucosinolate (calculated as 2-hydroxy-3-butenylglucosinolate potassium salt) of oil-free, air-dry crambe meal is found to range from 8 to 11% and that of *B. napus* to range from 3 to 8% (VanEtten *et al.*, 1966). The difference between total glucosinolates and progoitrins in the meals is due to glucosinolates that form mustard oils.

RAT FEEDING OF MODIFIED CRAMBE SEED MEALS

By controlled treatments crambe seed meals can be prepared to contain unhydrolyzed *epi*-progoitrin with or without the active thioglucosidase enzyme, with the *epi*-progoitrin hydrolyzed to give a mixture of the nitriles and with the *epi*-progoitrin hydrolyzed to give (*R*)-goitrin. Crambe meals so prepared were fed to weanling rats as 10% of their ration for more than 90 days (VanEtten *et al.*, 1969). Positive controls consisted of animals on the complete ration without supplemental test material and of animals fed 10% crambe seed meal which had been aqueous acetone-extracted and contained no *epi*-progoitrin, (*R*)-goitrin, or nitriles. Isolated *epi*-progoitrin, (*R*)-goitrin, and a preparation containing the nitriles were also fed, each at levels approximating those consumed by the rats fed crambe meals as 10% of their ration. Survival and body weights of the animals were compared. Also the livers, kidneys, and thyroids were examined histologically for lesions and the weights of the organs were recorded

Table II. Weight and Survival of Rats Fed Preparations From Crambe Seed

Test Substance	Amount in Ration, %	Composition of Substance			Wt. % of Controls
		<i>epi</i> -Progoitrin, %	(<i>R</i>)-Goitrin, %	Nitriles, %	
Experiment A ^a					
Defatted meal with active enzymes	10	7.6	0.0	0.0	41 ^b
Meal without active enzymes	10	7.1	0.0	0.0	77
Meal autolyzed to give (<i>R</i>)-goitrin	10	0.0	1.3	0.2	85
Meal autolyzed to give nitriles	10	0.0	0.0	0.8	Died ^c
Meal aqueous acetone extracted	10	0.0	0.0	0.0	105
Isolated <i>epi</i> -progoitrin	0.85	90.0	0.0	0.0	85
Isolated (<i>R</i>)-goitrin	0.23	0.0	100.0	0.0	85
Experiment B					
Defatted meal with active enzymes	10	6.7	0.0	0.0	60
Meal autolyzed to give nitriles ^d	10	0.0	0.0	0.6	61
Nitrile-containing isolate	0.10	0.0	0.0	65.0	17 ^e

^a All crambe meals were from a single preparation of dehulled, hexane-extracted material. Those meals requiring treatment with water were lyophilized to prepare an air-dry material for feeding.

^b One of five animals survived the experiment. However, the first meal shown under Experiment B provided growth 60% that of the controls, with all five animals surviving the test period on a similarly constructed diet.

^c All five animals died within 21 days of the experiment.

^d Meal autolyzed to give nitriles was air-dried at <50° C. before feeding.

^e Three of five animals survived the experiment.

at autopsy. Growth and survival were poor for the rats which were fed the meal with intact *epi*-progoitrin and active enzymes, the meal autolyzed to contain nitriles, and the nitrile fraction (Table II, Experiments A and B). In contrast, all rats fed the following rations survived and weighed from 77 to 85% of the weight of the controls: The meal with intact *epi*-progoitrin and no active enzymes; the meal autolyzed to contain mostly (*R*)-goitrin; isolated (*R*)-goitrin; and the isolated *epi*-progoitrin.

Rats receiving either the isolated (*R*)-goitrin or the (*R*)-goitrin in the meal had mild hyperplastic goiter and some enlargement of the liver with a mild degenerative nonspecific alteration of the liver cells. In contrast, rats receiving either the isolated nitrile fraction or the meal containing nitriles had enlarged livers with bile duct hyperplasia, fibrosis, and megalocytosis of hepatocytes. Their kidneys were also enlarged with renal lesions in the tubular epithelial cells. The thyroids appeared normal. Rats receiving the meal containing *epi*-progoitrin with active enzymes showed pathological lesions in the liver and the kidney similar to lesions in the animals fed nitriles. Some hyperplasia of the thyroid was also observed. Those receiving isolated *epi*-progoitrin or *epi*-progoitrin in the meal without active enzymes had mild lesions in all three organs.

The relative acute toxicities of the nitriles and of (*R*)-goitrin with mice as the test animal are shown in Table III. These data indicate that the nitriles either separately or as a mixture are about eight times as toxic as (*R*)-goitrin.

The episulfide-containing nitriles become less soluble with heat treatment presumably as a result of polymerization. Apparently polymerized episulfide-containing nitriles are less toxic. For example, in Table II, Experiment A, the meal autolyzed to give nitriles and dried by lyophilization contained more nitriles than Experiment B

Table III. Toxicity of Nitriles and (*R*)-Goitrin^a

Compound	Estimated LD ₅₀ , Mg./Kg. Body Wt.
(<i>S</i>)-1-Cyano-2-hydroxy-3-butene	170
(2 <i>S</i>)-1-Cyano-2-hydroxy-3,4-epithiobutane, A	178
(2 <i>S</i>)-1-Cyano-2-hydroxy-3,4-epithiobutane, B	240
Mixture of nitrile-containing compounds from crambe meal	159
(<i>R</i>)-5-Vinylloxazolidine-2-thione	1260-1415

^a Booth and Robbins, 1968.

meal which had been autolyzed to give nitriles and then air-dried at 50° C. Experiment A meal was also more toxic to the rats. This observation suggests that rigorous heating and drying treatments of a crambe meal autolyzed to contain nitriles may give a nontoxic product. Analysis of meals prepared in our laboratory showed that such treatments lowered the amount of nitriles but did not completely remove them. The remaining nitrile may be the unsaturated compound, which does not polymerize.

FUTURE AREAS OF RESEARCH

Since organic nitriles may be formed instead of (*S*)-goitrin from progoitrin in the rapeseed *B. napus*, the biological effects of feeding rapeseed meals containing nitriles should be determined. Do *epi*-progoitrin and progoitrin hydrolyze to give nitriles, as well as goitrins, when acted on by thioglucosidase from microorganisms in the intestinal tract? Do glucosinolates in oilseed meals that form mustard oils also form organic nitriles? How extensive is nitrile formation in the processing of cruciferous oilseed meals? What is the biological effect of

feeding these compounds for long periods? Does the formation of organic nitriles in foods need to be further investigated? These are some of the questions that need to be answered from both a basic and a practical viewpoint. In developing new food or feed processing methods to achieve more economy, better convenience, or esthetic appeal, the food and feed technologist must be certain that none of these deleterious substances are concentrated in the final products.

By amino acid composition (VanEtten *et al.*, 1965) and by protein efficiency tests (VanEtten *et al.*, 1969) cruciferous seed meals are high in crude protein of good nutritional quality. An efficient means of removing the glucosinolates and their hydrolysis products should give a high-quality feed or food. Development of economical methods to recover the protein is a challenge to the feed or food technologists. Water extraction of the glucosinolates from the wet-ground seed, after inactivation of the enzymes, appears promising (Eapen *et al.*, 1969).

We need to know more about the nature of thioglucosidase enzyme system(s) including isolation of the enzyme(s) in pure form. More accurate methods of measuring total glucosinolates and end products from their hydrolysis in plants are required.

The organic aglucon portion of many glucosinolates bears striking resemblance to the carbon skeleton of some of the common amino acids. Radioactively labeled amino acids have been used to demonstrate that they can serve as precursors for glucosinolates, with high levels of incorporation of the tracer atom(s). The investigations on biosynthesis of glucosinolates have been reviewed by Ettlinger and Kjaer (1968). Profitable research in this area includes that of Wetter and Chisholm (1968) and Matsuo and Yamazaki (1966). Plant breeding to eliminate biosynthesis of the glucosinolates also still offers a promising area of research (Youngs, 1965).

As our knowledge of the glucosinolates expands, their widespread occurrence among Cruciferae and related families should permit further development of useful chemotaxonomic relationships.

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