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NATURAL SULFUR COMPOUNDS*

This review includes work on the isolation, characterization, and biological testing of sulfur-containing compounds from plants that may be deleterious if consumed in large amounts. Little information indicates that these compounds are so distributed or are concentrated as to be harmful to man when the plants containing them are used in a conventional manner. Possible exceptions arise from circumstantial evidence that certain cruciferous plants such as cabbage, turnips, and rutabagas contribute directly or indirectly to a small fraction of the total incidence of goiter in the world population. Biological effects of the goitrogens responsible for this circumstantial evidence and of other substances discussed herein will be described, quantitatively when possible, to indicate the amount of the natural product required to cause acute or chronic harm. Hopefully, this approach may help to anticipate problems that can arise in new food technology concerned with these natural products.

The goitrogens from certain cruciferous plants are derived from a characteristic group of compounds, referred to in the older literature as thioglucosides. The more definitive name *glucosinolates* is now preferred by most authors to refer specifically to the type of starting material in Reaction 1, since it provides a convenient method of chemically naming related compounds as explained in the next section.

* Literature reviewed to February 1972.

Previous reviews include those related to biological effects of ingestion of these plants,[55,57-59,61,129,131] to the chemistry of the glucosinolates,[27,44,77] to problems in the use of rapeseed meals in animal feeds,[12,15,29,108] and to the transfer of deleterious substances into milk from animals fed crucifers.[135,142]

The unique taste and smell of plants of the *Allium* genus, including onions, garlic, and chives, are caused by sulfur-containing compounds that arise from enzyme action on precursors in the plants. Much of the chemistry of these precursors and their transformations has recently been elucidated, and the chemical nature of some of the compounds that can be formed suggests that harm might result if the derived substances were consumed in large amounts. Previous reviews relate to the chemical identity, odor, and lachrymatory properties of these compounds.[23,136]

GLUCOSINOLATES IN THE CRUCIFERAE

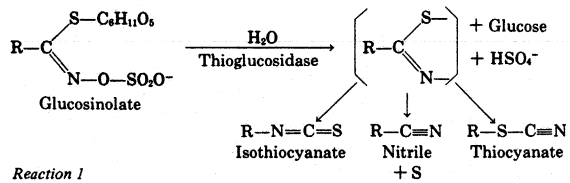
Chemistry of the Glucosinolates and Derived Products

The pungent nature of plants in the Cruciferae such as horseradish and mustard has been known from antiquity. Sinigrin was isolated from black mustard seed in 1840[78] and was identified as the precursor of allyl isothiocyanate, the most common of the mustard oils. At the same time a fraction containing an enzyme called "myrosin" was separated from the seed and shown to cause the breakdown of the sinigrin resulting in mustard oil. Prior to World War I, two additional glucosinolates were identified, sinalbin from white mustard and glucocheirolin from wallflower.[44] Up to the present about 65 different glucosinolates have been isolated from plants of the Cruciferae and related families. Of the 1,500 known species of Cruciferae, 300 have been examined and each has been found to contain from one to seven glucosinolates.[78] One or two of the compounds are usually present in relatively large amounts. The glucosinolate structure[45] (Reaction 1) has been confirmed by synthesis.[46] Use of the generic name glucosinolate with an appropriate prefix added to identify the *R* group provides a nomenclature that clearly defines chemical structures for the large number of glucosinolates that has been found.[43,44]

Reaction 1 shows the products generally formed when glucosinolates are hydrolyzed by thioglucosidase (thioglucoside glucohydrolase, EC 3.2.3.1).[48] Glucose and sulfate ions are always released. The remainder of the glucosinolate molecule, the organic portion of the aglucon, is

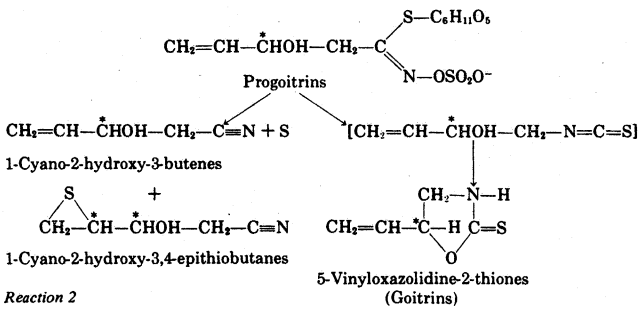
usually converted to thiocyanate, to nitriles and sulfur, or through a Lossen rearrangement to isothiocyanate. Hydrolysis conditions and prior treatment of the plant material determine which of these products predominate, but a mixture of them is often formed.[129]

Table 1 shows the principal glucosinolates in the crucifers that are grown in quantity for food, condiment, or feed. The glucosinolates in which the *R* group is allyl-, 3-butenyl-, 4-pentenyl-, benzyl-, 2-phenylethyl-, or 4-methylthio-3-butenyl- are the sources of the isothiocyanates that are the steam-volatile mustard oils. Although the mustard oils appear to be the main hydrolysis products from the organic aglucon of the glucosinolates, the nitriles may be formed instead.[27,77,89,97,143] An organic thiocyanate has been reported from benzylglucosinolate in *Lepidium* species such as *L. sativum* (garden cress).[51]



Reaction 1

In some instances the isothiocyanates initially formed undergo further transformations. For example, enzymatic hydrolysis of sinalbin (*p*-hydroxybenzylglucosinolate) from white mustard[77] gives *p*-hydroxybenzyl isothiocyanate, which readily degrades into simpler compounds including thiocyanate ion.[72,73] Similarly, 5-vinyloxazolidine-2-thiones are formed by cyclization of the presumed isothiocyanate intermediates indicated in brackets in Reaction 2. This is a specific example of a general cyclization reaction that occurs for all isothiocyanates in which the



Reaction 2

TABLE 1 Glucosinolates in Familiar Plant Materials from the Cruciferae

Plant	Part	Glucosinolates: Trivial Name	*R* Group	Relative Amount	Reference[a]
FOOD SOURCES					
Brassica oleracea					
Cabbage, kale, brussels sprouts, cauliflower, broccoli, kohlrabi	Head, leaves, bud	Sinigrin	Allyl-[b]	Major	31,66,68
		Glucobrassicin	3-Indolyl-methyl-	Major	52,68
		Gluconapin	3-Butenyl-[b]	Minor	66,68
		Progoitrin	(*R*)-2-Hydroxy-3-butenyl-	Minor	1,68,138
		Neoglucobrassicin	3-(*N*-Methoxy-indolyl)methyl-	Minor	53,68
B. campestris					
Turnips	Roots	Gluconasturtiin	2-Phenylethyl-[b]	Major	68,88
		Progoitrin	(*R*)-2-Hydroxy-3-butenyl-	Minor	6,68
			(*R*)-2-Hydroxy-4-pentenyl-	Minor	123
B. napus					
Rutabaga	Roots	Progoitrin	(*R*)-2-Hydroxy-3-butenyl-	Major	6,68
		Glucobrassicin	3-Indolylmethyl-	Minor	52,68
		Neoglucobrassicin	3-(*N*-Methoxy-indolyl)methyl-	Minor	53,68
Lepidium sativum					
Garden cress	Leaves	Glucotropaeolin	Benzyl-[b]	Major	51
Nasturtium officinale					
Watercress	Leaves	Gluconasturtiin	2-Phenylethyl-[b]	Major	111
Raphanus sativus					
Radish	Roots	–	4-Methylthio-3-butenyl-[b]	Major	49
		Glucobrassicin	3-Indolylmethyl-	Minor	52
CONDIMENT SOURCES					
Armoracia lapathifolia, A. rusticana					
Horseradish	Roots	Sinigrin	Allyl-[b]	Major	120
		Gluconasturtiin	2-Phenylethyl-[b]	Minor	117
B. carinata					
Ethiopian rapeseed	Seed	Sinigrin	Allyl-[b]	Major	47

TABLE 1 (*Continued*)

Plant	Part	Glucosinolates: Trivial Name	*R* Group	Relative Amount	Reference[a]
B. juncea					
Indian or brown mustard	Seed	Sinigrin	Allyl-[b]	Major	47,66
B. nigra					
Black mustard	Seed	Sinigrin	Allyl-[b]	Major	47,66
Sinapis alba					
White mustard	Seed	Sinalbin	*p*-Hydroxybenzyl-	Major	80
S. arvensis					
Charlock	Seed	Sinalbin	*p*-Hydroxybenzyl	Major	47
FEED SOURCES					
B. campestris					
Rape, turnip, turnip rape, Polish rape, rubsen, navette	Seed	Gluconapin	3-Butenyl-[b]	Major	47
		Progoitrin	(*R*)-2-Hydroxy-3-butenyl-	Minor	47
		Glucobrassicanapin	4-Pentenyl-[b]	Minor	47
		Glucoraphanin	4-Methylsulfinylbutyl-	Minor	47
		Glucoalyssin	5-Methylsulfinylpentyl-	Minor	47,113
B. napus					
Rape, Argentine rape	Seed	Progoitrin	(*R*)-2-Hydroxy-3-butenyl-	Major	6,47,79
		Gluconapin	3-Butenyl-[b]	Major	47,81
		Glucobrassicanapin	4-Pentenyl-[b]	Minor	47,79
		Gluconasturtiin	2-Phenylethyl-[b]	Minor	79
		Glucoiberin	3-Methylsulfinylpropyl-	Minor	79,112
		Sinalbin	*p*-Hydroxybenzyl-	Minor	79
Crambe abyssinica					
Crambe, Abyssinian	Seed	*epi*-Progoitrin	(*S*)-2-Hydroxy-3-butenyl-	Major	34,42
		Sinigrin	Allyl-[b]	Minor	39
		Gluconapin	3-Butenyl-[b]	Minor	39
		Gluconasturtiin	2-Phenylethyl-[b]	Minor	39

[a] For the most part, authors who first identified the glucosinolate in the plant material are cited. More details including differences in glucosinolate with variety of *Brassica* are given for the vegetative parts[6,8] and for the seed.[4,7]
[b] The glucosinolate is the source of a volatile mustard oil. For example, allylglucosinolate gives allyl isothiocyanate, a typical pungent compound.

R group has an appropriately located hydroxyl substituent. These cyclic products are called goitrins because of their potent goitrogenic activity. Progoitrin from rapeseed (*Brassica napus* or *Brassica campestris*) and *epi*-progoitrin from crambe (*Crambe abyssinica*)* seed are the names given to the precursors, which differ only in stereochemistry at the carbon atom marked with an asterisk. By the glucosinolate method of naming, progoitrin is (*R*)-2-hydroxy-3-butenylglucosinolate, and *epi*-progoitrin is the corresponding (*S*)-compound, where (*R*) and (*S*) designate the stereochemistry at the 2-position by the sequence rule.[20] The nature of this rule is such that compounds in the same stereochemical series may have opposite (*R*)- and (*S*)-designations. Thus progoitrin gives rise to (*S*)-goitrin [(*S*)-5-vinyloxazolidine-2-thione], and *epi*-progoitrin forms (*R*)-goitrin [(*R*)-5-vinyloxazolidine-2-thione].

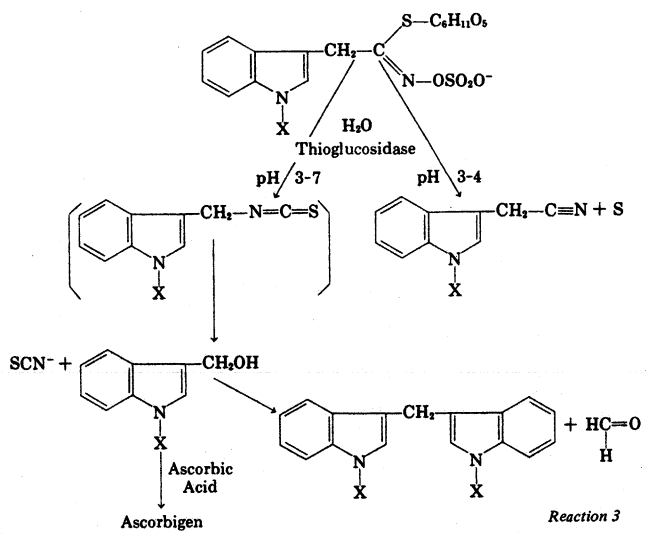
Depending on conditions during the enzymatic reaction and on the prior treatment of the plant material, an unsaturated nitrile (cyano compound) and two diastereomeric epithio nitriles may be formed instead of (*S*)- or (*R*)-goitrin from either progoitrin or *epi*-progoitrin as shown in Reaction 2. The progoitrins and each of the hydrolysis products have been isolated and characterized.[6,21,34-37] Treatment with ferrous iron, which has been proposed as a means of detoxifying rapeseed meal,[146] causes nonenzymatic degradation of the progoitrins to the unsaturated nitriles and 3-hydroxypent-4-enethionamides.[7,8] Besides the goitrins, other 4- or 5-substituted oxazolidine-2-thiones formed in cruciferous plant material have been isolated and characterized, and their parent glucosinolates have been identified.[44]

Glucobrassicin,[52] 3-indolylmethylglucosinolate, starting material in Reaction 3 (X = H), and neoglucobrassicin,[53] 3-(*N*-methoxyindolyl)-methylglucosinolate, starting material in Reaction 3 ($X = OCH_3$), were isolated from cabbage leaves and from rutabaga root. These two glucosinolates form the corresponding nitriles at pH 3 to 4 and presumably the unstable isothiocyanates at pH 3 to 7. The isothiocyanates are believed to be the intermediates that further degrade to thiocyanate ion and 3-hydroxymethylindoles. The indoles may react with ascorbic acid to form a compound called ascorbigen, which is reported to have vitamin C activity.[76,90]

Glucosinolates in Foods, Condiments, and Feeds

Identified glucosinolates from domesticated crucifers are listed in Table 1. Each glucosinolate in a given plant is found in all of the plant parts

*Crambe is a potential new oilseed crop in the United States. It is referred to frequently in this chapter because some of the most definitive chemical studies on sulfur-containing glucosides of Cruciferae have been carried out on this species.



Reaction 3

that have been examined except glucobrassicin and neoglucobrassicin, which have not been found in the seed. In general, the glucosinolate concentration is higher in the seed than in other parts of the plant. Because of this fact and the economic importance of some species as oilseeds, the glucosinolate content of *Brassica* seed in particular has been determined more accurately than has that of edible parts of the same plants. Estimates on nondefatted turnip seed from 19 varieties ranged from 0.7 to 2.0 % 3-butenylglucosinolate and 0.0 to 0.1% (*R*)-2-hydroxy-3-butenylglucosinolate (progoitrin). Nondefatted white cabbage seed from 19 varieties ranged from 0.1 to 2.6% allylglucosinolate (sinigrin) and 0.0 to 0.7% (*R*)-2-hydroxy-3-butenylglucosinolate.[47] In comparison, fresh edible cabbage contains about 350 ppm allylglucosinolate,[87] traces of three additional mustard oil-forming glucosinolates,[31] at least 0.5 ppm progoitrin,[1] and from 60 to 800 ppm 3-indolylmethylglucosinolate (glucobrassicin).[94] These data show that the edible part of cabbage, used as a food for centuries with no apparent harm, is quite low in glucosinolate content.

A recently developed method for *p*-hydroxybenzylglucosinolate[69] showed defatted white mustard seed meal to contain from 8.5 to 10.1% of the compound. This range of values was obtained from five varieties of the condiment mustard grown in different localities over several years.[70]

The glucosinolates as sources of toxic compounds from rape and crambe seeds have been examined in some detail because the by-product meals from these oilseed crops will have more value in animal feeds if the harmful compounds can be removed or inactivated. Amino acid contents indicate a high nutritional quality for the protein in meals from cruciferous oilseeds,[99] and feeding studies have confirmed this for crambe.[134] Preliminary work has been done on the preparation and testing of protein isolates from rapeseed for use in foods.[3,19,41]

Since protein concentrates and isolates from rapeseed and seeds of related plants may in the future find their way into the food chain, the nature of glucosinolate breakdown products, as revealed by animal studies, merits discussion. Earlier indications that more than one toxic substance can be produced[15,64,74,124] have been substantiated by studies on crambe seed meal.[131,133,134] Experimental conditions were developed for causing glucosinolate hydrolysis to occur in order to give either nitriles or goitrin (Reaction 2). The nitrile mixture has eight times the acute toxicity of goitrin toward mice[131] and causes pathological liver and kidney lesions but not thyroid effects in rats.[134] Goitrin caused mild hyperplastic goiter, with slight reduction in weight gain and mild liver enlargement without pathology in rats. The mode of glucosinolate degradation was in part dependent on whether endogenous enzymes present were destroyed before feeding. The experiments cited show results obtained in extreme cases designed to reveal toxic effects. The experimental materials contained high (8–10%) concentrations of glucosinolate and were fed as major (10%) constituents of the rat diet for 90 days. *No analogous results have been reported or are implied for crucifers eaten as human food under usually encountered circumstances.*

Variables that favor formation of goitrin by thioglucosidase hydrolysis in the seed or seed meal include: dry-heat treatment or long storage of the seed under ambient conditions before enzyme hydrolysis; hydrolysis at high pH; high temperature; and low concentration of the seed meal in the aqueous hydrolysis medium. Nitrile formation is favored by autolysis of the seed meal from untreated, recently harvested seed, wetted to a paste at ambient temperature. More detailed studies on thioglucosidase hydrolysis in crambe meal are given in a recent report.[125] Variables affect the nature of the hydrolysis products from the progoitrins in crambe and in rape leaves in a similar manner.[130]

The total glucosinolate content of a natural product that has not been subjected to thioglucosidase hydrolysis may be estimated by measurement of the glucose or the sulfate ion released after adding a source of

oglucosidase. Such methods have been utilized.[93,110,132] However, ey do not measure the products released from the organic aglucon of e glucosinolate by the hydrolysis. These products from the aglucon e deleterious and may vary depending on the specific glucosinolate, the or treatment of the material, and the conditions of hydrolysis. Conquently, analyses for each specific product are often required. On the ner hand, attempts to quantitatively determine glucosinolates through easurement of products from the organic aglucon moieties can also e misleading results. In early methods of analyses for progoitrin by nversion to goitrin and measurement of the latter by its ultraviolet sorption, the hydrolysis of the glucosinolate was carried out without ntrol of the pH or at pH 4. Under these conditions analytical results re often low because the progoitrin was not exclusively and quantitaely hydrolyzed to goitrin. By dry heating the seed or seed meal before alysis and by carrying out the thioglucosidase hydrolysis at pH 7, ore accurate and higher values have been obtained.[2,129,132] Defatted ed meal from commercial varieties of winter-type rapeseed (*B. napus*) ntained 0.8–2.0% (average 1.7%) of glucosinolates that give volatile ustard oils and 2.6–5.5% (average 4.5%) progoitrin. The same method plied to *B. campestris* (winter-type rapeseed) gave 2.8–3.2% (average 0%) mustard oil-forming glucosinolates and 0.14–0.65% (average 35%) progoitrin.[2] The variability among species and varieties suggests at glucosinolate content may be lowered or removed by the plant neticist.[40] A summer-type variety of *B. napus* called Bronowski has en found that contains less than 1% total glucosinolate in the defatted ed meal.[71]

Recent gas–liquid chromatographic methods include those for the alyses of the individual mustard oils[147] and for goitrin and each of the ganic nitriles from progoitrins.[38] These methods permit complete alysis for all the volatile mustard oils and for all the known thioglusidase hydrolysis products from the progoitrin aglucon.

oitrogenic Properties of the Cruciferae

f the widespread prevalence of human goiter, only about 4% may be ue to causes other than that of iodine deficiency.[59] In a detailed study the etiology of endemic goiter, Roche and Lissitzky[107] state that the use may often be interactions of factors that vary from case to case. some areas of the world, cruciferous plants as a part of the diet could e one of the contributing factors. Goiter has been attributed to the nsumption of large amounts of cabbage[75] or of kale that was shown be high in thiocyanate, isothiocyanate, and goitrin.[96]

Experimental goiter due to cruciferous plants was first produced in 1928 by feeding rabbits a ration of high cabbage content,[28] but the experiment could not always be repeated in other laboratories. Later, enlarged thyroids were more consistently obtained in experimental animals when the seed of cabbage or other *Brassica* species was fed.[63] However, with the feeding of the seed, variable poor growth and pathology in body organs other than the thyroid were also observed. From this experimental work it seems plausible that consumption of large amounts of cabbage or related vegetables might cause goiter. In particular, consumption of these vegetables might contribute to the relatively high incidence of the disease in areas of the world where the iodine content of the diet is low.

A brief review of the testing methods for antithyroid activity is necessary in order to more critically evaluate recent pertinent experimental work. Goitrogenic activity was first detected by visual inspection and weight of the thyroid after feeding the test material to experimental animals. Histological examination of the glands gave additional information as to the nature of the agents or the conditions that cause enlargement. Other criteria are growth rate of the test animal, basal metabolism tests, and assays for iodine content of the thyroid and blood. A test developed more recently consists of measuring the reduced radioactive iodine uptake of the thyroid following the feeding of the material under investigation.[118] This procedure has been used with rats,[5] chicks,[126] and humans.[118] Since antithyroid response varies with the species, the test should be used with humans for a more accurate evaluation of a substance for use in food. Advantages of the test are speed and sensitivity. A disadvantage is that the test gives no information on the antithyroid effect of feeding natural products containing goitrogens at low levels over long periods of time. This aspect of the problem requires extended feeding of the test material (usually in a ration of known iodine content) and then examination of the thyroids.[103]

Thiocyanate ion, a product from the enzymatic hydrolysis of the indolylglucosinolates (Reaction 3) and of *p*-hydroxybenzylglucosinolate, causes enlarged thyroids. This fact was first discovered in patients fed the thiocyanate ion to reduce hypertension.[11] Goitrogenic action was attributed to inhibition by the thiocyanate ion of iodine uptake by the thyroid.[127] For this reason the antithyroid effect is most likely to occur when the iodine content of the diet is low.[4] However, based on recent *in vitro* and *in vivo* experiments, higher levels of the thiocyanate ion also inhibit formation of thyroxine and related compounds, even when the iodine supply is not marginal.[62] Analysis of the edible part of fresh cabbage showed thiocyanate ion concentrations ranging from 0.7 to 10.2

mg/100 g.[94] Concentrations were highest in the spring; they varied little with the region where grown, but varied from plant to plant within a field.[95] Ingestion of cabbage by animals or by man caused a rise in thiocyanate ion concentration in the blood and its appearance in the urine.[85] Single feedings of the cabbage to guinea pigs and to rats on a low-iodine ration caused reduced iodine uptake by the thyroid to a greater extent than expected due to the known amount of thiocyanate ion in the plant material.[82,86] A mixture of thiocyanate ion, allyl isothiocyanate, and goitrin was fed to rats on a low-iodine ration at levels equivalent to those in cabbage fed *ad libitum*. Increase in weight and depression of iodine uptake by the thyroid and less synthesis of iodoamino acids occurred about as would be expected to result from the cabbage ration.[84]

It appears that allyl isothiocyanate acts as a goitrogen, because the compound is metabolized to give the thiocyanate ion as one of the products. Doses of 2–4 mg of allyl isothiocyanate mixed with water and fed by stomach tube to rats caused a marked increase in thiocyanate ion in the blood plasma and a decrease in radioactive iodine uptake by the thyroid.[83] This dosage is equivalent to the amount of the compound each rat would get in eating 40 g of cabbage per day. Treating the rats in this manner for 60 days gave histological changes in the thyroid indicative of goiter.[87] The mustard oil cheiroline (3-methylsulfonylpropyl isothiocyanate) when injected into rats also inhibits radioactive iodine uptake by the thyroid.[9] This compound is found in a pasture weed, *Rapistrum rugosum*, that grows in Australia. Since mustard oils are irritating and act as vesicants, they are not likely to be eaten per se, even as a condiment, in large enough amounts to cause malfunction of the thyroid. However, if they were consumed as the glucosinolate over long periods of time and slowly released by the accompanying thioglucosidase or a similar enzyme in the intestinal tract, it is conceivable that they might act as goitrogens through conversion to the thiocyanate ion.

There are reports that daily doses of organic nitriles fed to rabbits caused enlargement of the thyroids that was prevented by increasing the iodine content of the ration.[55] It was observed that most of the nitriles ingested were converted to thiocyanate ion during metabolism by the experimental animals. However, the goitrogenic effect of feeding nitriles could not consistently be repeated by other investigators.[55,134]

A polysulfide fraction and "trithiones" (1,2-dithiocyclopent-4-en-3-thiones) isolated from cabbage juice brought about histological changes in the thyroid of rats similar to those caused by feeding excessive amounts of cabbage.[67] Gas chromatographic and mass spectrometric analyses of volatiles from raw cabbage showed, in addition to thiocyanates, small amounts of tentatively identified mono-, di-, and trisulfides of low molecular weight.[10] The methyl mono-, di-, and trisulfides were the major volatile components from cooked cabbage.[89] Large amounts of methyl-, *n*-propyl-, and allyl disulfides have been reported to have antithyroid activity.[33]

The workers who isolated goitrin (*S*-5-vinyloxazolidine-2-thione) from rapeseed demonstrated that the compound had thyroid-inhibiting activity.[6] The (*R*) isomer of goitrin, which is derived from the major crambe glucosinolate, has equal antithyroid activity.[59] When fed to rats, the goitrins cause slightly less hyperplasia of the thyroid than thiourea (a well-known thyroid-inhibiting drug).[22] The goitrins have 2% and 133% of the goitrogenic activity of the antithyroid drug propylthiouracil as measured by the radioactive iodine method with rats and with man, respectively.[59] After goitrin from crambe was fed to rats as 0.23% of the ration for 90 days, body weights of the animals were 85% of those of the controls, and the thyroids were enlarged with lesions typical of mild hyperplastic goiter.[134] When racemic goitrin was fed to chicks as 0.15% of the ration, growth depression and hyperplasia and hypertrophy of the thyroid gland were observed. During the initial feeding, radioiodine uptake of the thyroid was decreased, and the release of iodine by the gland into the bloodstream was increased.[30] After the goitrin was fed over 3 or 4 weeks, these two measurements returned to normal levels, possibly because the thyroid after enlargement could function normally.

Of the antithyroid agents found in the Cruciferae, the goitrins are the most active. They act presumably by inhibiting the incorporation of iodine into organic compounds to form thyroxin and related compounds; they are not counteracted by larger amounts of iodine in the diet as is the case with thiocyanate ion.[61] Of the many foods from plants and animals that were tested by the radioactive iodine uptake method, turnip and rutabaga consistently gave positive results, with rutabaga about twice as active as turnip.[59] A maximum of 0.13 ppm goitrin can be formed from the 0.5 ppm of progoitrin estimated to be in cabbage.[1] This amount of goitrin is probably negligible in practical nutrition. In contrast, some defatted crucifer seed meals contain progoitrin equivalent to 2.7% goitrin.

Feeding Intact Glucosinolates in the Absence of Thioglucosidase

It was first thought that if foods from the Cruciferae were cooked in such a manner that the thioglucosidase enzyme was inactivated without hydrolysis of the glucosinolates, the goitrogenic properties would be lost.[57] After isolation of pure progoitrin[56] the compound was tested for goitrogenic activity without the accompanying enzyme. By feeding the

compound to rats and to man and testing for radioiodine uptake of the thyroid, the progoitrin was found to be active.[60] Goitrin appeared in the blood serum and in the urine. Incubation of progoitrin with rat or human feces gave goitrin as a hydrolysis product. Goitrin formation was prevented by boiling, ultrasonic treatment, or Seitz filtration of the feces. Antibiotics fed with the progoitrin caused a marked decrease in the formation of goitrin. A number of isolated bacteria, including *Paracolobactrum*, commonly found in the mammalian intestinal tract produce thioglucosidase[102] as does the fungus *Aspergillus sydowii* QM 31c.[106] Hydrolysis by the microflora of the intestine as tested was much slower than by the plant thioglucosidase.

Glucosinolate-Derived Substances in Milk

Following the introduction of marrow-stem kale (a crucifer plant) as pasture and hay for dairy cattle in Tasmania, it was reported[32] from a statistical survey that children from the area who consumed the milk developed enlarged thyroids. The suspected milk also caused inhibited thyroid uptake of radioactive iodine following ingestion of test samples. The investigators did not isolate the goitrogens from the milk. They made the hypothesis that antithyroid compounds were being transmitted into the milk in large enough quantity to cause harm. There are reports that animals may develop enlarged thyroids when fed large amounts of cruciferous plants or when grazed on pastures contaminated with cruciferous weeds.[9,104,145] However, surveys made in England[54] and in Holland[128] in areas where crucifer forages are used for dairy cattle gave no indications that the cow's milk was goitrogenic.

As a result of the observations made in Tasmania, experimental work was carried out in Finland to ascertain if goitrogens accumulated in milk when the dairy cow was fed known amounts of crucifer forage or goitrogens known to be in the forage. After a single feeding of progoitrin, goitrin, or rapeseed containing progoitrin equal to that found in a daily full feed of kale or rape forage, only about 0.05% of the ingested goitrin was found in the milk at a maximum concentration of 100 μg/liter.[137]

A positive radioactive iodine test for thyroid inhibition in man requires 20 mg of goitrin. The milk from cows fed 30 kg of marrow-stem kale per day contained 5–8 mg thiocyanate ion per liter compared with milk from cows fed no crucifer plants, which contained 2 mg per liter. By feeding thiocyanate ion equal to that in the 30 kg of marrow-stem kale, a level of 18 mg of thiocyanate ion per liter of milk was achieved.[105] A dose of 200–1,000 mg of thiocyanate ion is required to give a positive radioactive iodine test. These experimental results argue that the goitrogens cannot be transferred to the milk in large enough amounts to cause harm. However, later another Finnish investigator reported that feeding of 0.0, 0.1, 0.5, 1.0, and 2.0 μg of goitrin per day to rats for long periods of time caused larger thyroids as the dose of goitrin was increased. The feeding of milk that contained 50–100 μg goitrin per liter to the rats over long periods of time also caused enlarged thyroids.[103] Thus, while the answer to the question "Can endemic goiter in humans be caused by the ingestion of milk from cows eating cruciferous plants?" appears to be negative for practical purposes, it is not unequivocally so.

In the course of some of this work[105] it was observed that with large amounts of thiocyanate ion in the ration, the iodine content of the cow's milk was lowered markedly but increased to normal levels when thiocyanate feeding was stopped. This observation has been made by other investigators.[50,98] Possibly, ingestion of thiocyanate ion by a lactating mammal could cause goiter in her young or in those consuming her milk because of its low iodine content.

Although not considered toxic as consumed by the dairy cow, land cress (*Coronopus didymus*) contains benzylglucosinolate, which is the source of off-flavors in milk that increased during pasteurization. This problem is the subject of a recent review.[142]

Safety of Edible Products from Glucosinolate-Containing Plants

Little information is available on the effect of simple processing of the vegetable *Brassicas*, such as the shredding of cabbage to make slaw, or fermenting to make kraut. Similarly, the maximum amount of condiment seed (i.e., white mustard, table mustard, or horseradish) that may be consumed without harm is not known. In view of the diversity of products from glucosinolates and the chemically active nature of many of these products, it is difficult to evaluate specifically harm that might occur from consumption of these plants under all conceivable circumstances. However, their use as vegetables and condiments as currently practiced causes no known harm and adds nutritional value and variety to the diet.

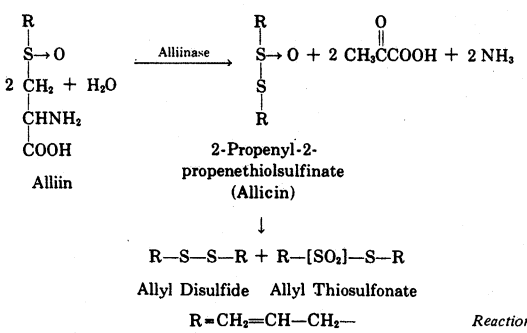
COMPOUNDS FROM ONION, GARLIC, AND CHIVE

Volatile Oils from Allium Species

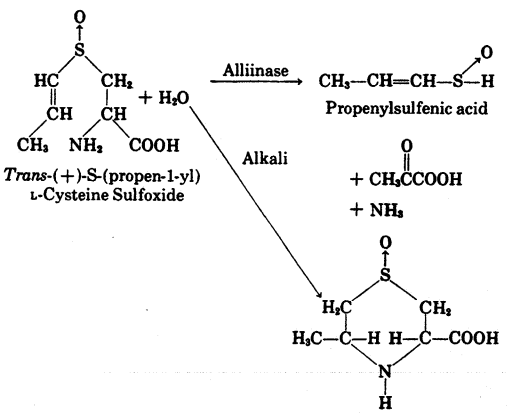
Throughout the history of man, species from the *Allium* genus of the Liliaceae have been used as vegetables, spices, and folk medicines for the cure of various diseases. Of the greatest practical interest are onions (*A.*

cepa L.), garlic (*A. sativum* L.), and chive (*A. schoenoprasum* L.). The volatile compounds from these plants, most of which contain sulfur, appear to be the sources of the flavor, odor, lachrymatory, and antibiotic properties attributed to them. These compounds are the products of enzyme action on precursor substrates stored in the intact plant.

The precursors are *S*-substituted cysteine sulfoxides or peptides containing them,[114] of which (+)-*S*-allyl-L-cysteine sulfoxide (alliin)[119] is the major one in garlic, present at about 2.4 mg/g.[121] Products formed from alliin are given in Reaction 4. Allicin (allylthiosulfinate) is a labile compound that has the odor of fresh garlic; it also has antibiotic activity.[26] Compounds have been isolated from onions that are analogous to alliin but have the allyl group (*R* in the starting material in Reaction 4) replaced, respectively, with methyl, propyl, and *trans*-1-propenyl radicals.[24,25,116,140] The (+)-*S*-methyl-L-cysteine sulfoxide has been isolated from other plants, including turnip root, cabbage, kohlrabi, together with mustard leaves, cauliflower flowers, and broccoli stems of the crucifer family.[101,122] All three substituted cysteine sulfoxides form the labile thiosulfinates by action of alliinase.[92] The thiosulfinates slowly decompose by disproportionation to give thiosulfonates and disulfides. The disulfides are volatile and are responsible for part of the characteristic odor of the crushed plants that contain them. The thiosulfinates have antibiotic activity.[115] A series of mono-, di-, and trisulfides with methyl, propyl, isopropyl, 1-propenyl, and allyl groups, combinations of two of these, dimethylthiophenes, hydrogen sulfide, and methyl-, propyl-, and allyl-thiols has been found in fresh onion, dehydrated onion, distilled onion oil,[13,14,16,17] and garlic.[65] It seems likely that these sulfides are derived from substituted cysteine sulfoxides.



Reaction 4



Reaction 5

The major cysteine sulfoxide in onion is *trans*-(+)-*S*-(propen-1-yl)-L-cysteine sulfoxide[24,116] (Reaction 5), present at about 2 mg/g fresh weight.[91] Alliinase hydrolysis gives the very labile propenylsulfenic acid with the structure shown, which is based on mass spectrometry of the compound within 1 min after it was formed.[100] This compound or a closely related substance appears to be the major lachrymator from onion.* The decomposition products from the lachrymator have not been clearly established. When the precursor cysteine sulfoxide is treated with alkali, the compound cycloalliin is obtained.[139] Cycloalliin is inert to enzymes in the onion and apparently makes no contribution to onion flavor. From gas-chromatographic and mass-spectrometric studies, volatiles from chives contain methyl propyl disulfide, propyl disulfide, and propenyl propyl disulfide (*cis* and *trans*), but there is no evidence for propenyl or allyl disulfide.[141]

Evidence for Goitrogenic Properties of Allium *Species*

There is little evidence that the sulfur-containing compounds in the *Allium* species are harmful as consumed in edible products. It has been suggested, however, that the known high occurrence of endemic goiter

*After this manuscript was completed, the labile lachrymatory factor from onion was shown to be thiopropanal *S*-oxide, the structure of which ($CH_3CH_2CH{=}S{=}O$) was confirmed by synthesis.[18]

in the valleys of mountainous areas of Lebanon may be due in part to the large amount of onions in the diet.[109] By the radioactive iodine method, with rats as the test animal, methyl-, *n*-propyl-, and allyl disulfides; allyl sulfide; and allyl alcohol were assayed for antithyroid activity. The disulfides depressed radioiodine uptake by the thyroid when the rats were fed a high level (30–60 μl) of the compounds. The allyl sulfide had little effect. The allyl alcohol was toxic at levels above 25 μl per rat.[33] These results do not imply that any of the vegetables and spices that contain these compounds at relatively low levels are harmful when used in a conventional manner; indeed, there are no direct observations in support of harm occurring in the use of these vegetables and spices. However, it is believed that due consideration should be given to the chemistry and biological effect of these compounds when new ways of processing food materials containing them are developed, especially if the processing could conceivably involve fractionation and concentration of the natural sulfur compounds or deleterious products derived from them.

COMPOUNDS FROM SOURCES OTHER THAN THE CRUCIFERAE AND *ALLIUM*

The antithyroid action of cyanide from cyanogenic glycosides due to cyanide detoxification by conversion to the thiocyanate ion is covered in Chapter 14, p. 303. The sulfur-containing peptides from mushrooms that are toxic are described in Chapter 5, p. 120, the toxic amino acid djenkolic acid in Chapter 21, p. 466.

By a careful restudy, reported in 1949, of the antithyroid activity claimed for ergothionine, it was shown that there is no significant antithyroid activity of the compound in the rat, monkey, or man.[144] No later reports were found in the literature that disagree with this conclusion.

NOTE

According to a report that appeared after completion of this chapter [C.-S. Tang, *Phytochemistry 12*:769 (1973)], the dried latex from the green, immature fruit of papaya (*Carica papaya,* Caricaceae) contains 73 to 116 mg potassium benzylglucosinolate per gram of dried material. The ground dried latex from the unripe papaya is the source of papain, a proteolytic enzyme with important food and medicinal uses. However, no reports have been found that the crude enzyme preparations from this source are harmful.

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