Glucosinolates and Derived Products in Cruciferous Vegetables. Identification of Organic Nitriles from Cabbage

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Autolysis of fresh cabbage at its unadjusted pH (5.6–6.3) gave a preponderance of nitriles instead of the related isothiocyanates and goitrin. These latter compounds were the products when the same cabbage was air-dried prior to autolysis (pH 5.8–6.3). In addition to 1-cyano-2-hydroxy-3-butene and the two forms of 1-cyano-2-hydroxy-3,4-epithiobutane from breakdown of the progoitrin glucosinolate, other nitriles identified in the preparations were 1-cyano-2,3-epithiopropane, 1-cyano-3,4-epithiobutane, 1-cyano-3-methylthiopropane, 1-cyano-3-methylsulfinylpropane, 1-cyano-4-methylthiobutane, 1-cyano-4-methylsulfinylbutane, and phenylethyl cyanide. Gas chromatographic evidence was obtained for the presence of 1-cyano-3-methylsulfinylpropane in commercial preparations of sauerkraut. Under optimum conditions for goitrin formation, six accessions that included red cabbage and Brussels sprouts were hydrolyzed to yield goitrin ranging from 5 to 61 ppm of fresh weight.

The kinds and amounts of organic products derived from hydrolysis of glucosinolates in crucifer plants may vary with conditions of hydrolysis or with treatment during harvest and storage (VanEtten et al., 1969). Such information is of interest because these crops are used for food, and the glucosinolate products are suspect as potential hazards. With the establishment of regulations to be met in the development of improved varieties of vegetables (Senti and Rizek, 1974), the glucosinolate content of new varieties will likely be required.

This paper reports the formation of 10 different nitriles from glucosinolates during the autolysis of edible cabbage, and their identification by GLC and MS data. Also included are estimates of the relative quantities of nitriles and goitrin for several accessions of cabbage as determined by infrared and ultraviolet measurements.

EXPERIMENTAL SECTION

Estimation of Nitriles and Goitrin. Fresh cabbage was purchased from local markets over a 10-month period. A portion from each head was dried overnight at 50 °C in a forced draft oven. Drying in this manner removed 88 to 92% of the weight. A 100-g sample of the same cabbage was not dried but was instead pulped with 150 ml of water in a blender. After standing about 30 min, the mixture was heated to boiling, cooled, and filtered with Celite Filter aid. The residue was extracted two times with 100-ml volumes of water. The combined filtrates were concentrated to 20–25 ml under reduced pressure at 40 °C, and 200 ml of ethanol was added. After filtration, the liquid was again reduced to 20 ml and the concentrate was extracted three times with 60-ml volumes of dichloromethane. The combined dichloromethane extracts were concentrated to an oil and resuspended in about 5 ml of water. Insoluble material was removed by centrifugation and the water phase was reextracted three times with 15-ml volumes of dichloromethane. The combined extracts were dried over sodium sulfate and filtered. The solvent was evaporated under nitrogen, and the residue dried in a desiccator. The weighed material was then redissolved in a small volume of dichloromethane for estimation of the total nonvolatile nitriles by infrared (Daxenbichler et al., 1966). Also, the specific nitriles and goitrin from progoitrin were determined by GLC (Daxenbichler et al., 1970). For confirmation of amount, the goitrin was often also measured by UV absorption at its maximum of 244 nm in alcohol. However, some of the samples gave too much background absorption for estimation by UV.

For the duplicate sample that had been dried at 50 °C, an amount of sample was taken equivalent to 100 g of fresh cabbage. The sample was pulped with water in the blender as described for the fresh material. Cabbage leaves dried in this manner did not require added myrosinase for hydrolysis of the glucosinolates. This is noteworthy because crambe and rape leaves, when air-dried, lost activity for glucosinolate hydrolysis (VanEtten and Daxenbichler, 1971).

Separation and Identification of Nitriles in Autolyzed Cabbage. The GLC method applied was a modification of that by Daxenbichler et al. (1970). This modified method (Daxenbichler and VanEtten, 1976) permits estimation of all the organic isothiocyanates and goitrin from the various glucosinolates found in cabbage. The method was recently applied to determine these constituents in 22 varieties of cabbage (VanEtten et al., 1976). For the separation shown in Figure 1, and to obtain the components listed in Table I, a blend of two cabbage varieties was prepared based on our data of the constituents present. For GLC curve a, the sample was extracted and the glucosinolates were hydrolyzed (VanEtten et al., 1976) to yield quantitative amounts of the appropriate isothiocyanates and goitrin. For the production of nitriles by autolysis, the subject of this report, the same blend of an equivalent amount of cabbage was simply pulped with a small volume of water and the nitriles were extracted into dichloromethane to obtain the comparison data shown in GLC curve b. In the preparation of this sample, heat was not applied to the mixture following autolysis and care was taken during concentration of the dichloromethane extract to minimize loss of volatile components. Although the results are not included in Figure 1, the procedure uses also a separation on a 4-ft column packed with 1% EGSS-X on Gas-Chrom Q in addition to the separation shown on a 6-ft column packed with 3% Apiezon-L on Gas-Chrom Q. A Packard Co. 7400 series gas chromatograph was used for the chromatography. Samples were injected at 40 °C and programmed 40 min to an upper limit and hold temperature of 204 °C. The GLC–MS tandem system used was as described by Spencer et al. (1976), and the GLC in that system employed a 6-ft Apiezon-L column as was used for the initial chromatography.

In the preparations from autolyzed cabbage, one of the major components encountered was the nitrile identified

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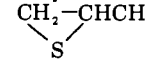
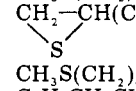
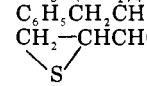
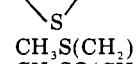
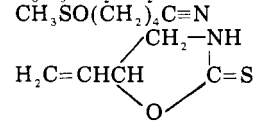
Table I. Glucosinolate Derived Products from Cabbage

Peak[a]	Component	Structure
1	Allyl isothiocyanate	$CH_2=CHCH_2NCS$
2	3-Butenyl isothiocyanate	$CH_2=CH(CH_2)_2NCS$
3 (sh)	1-Cyano-2-hydroxy-3-butene	$CH_2=CHCHOHCH_2C\equiv N$
3	1-Cyano-2,3-epithiopropane	
4	1-Cyano-3-methylthiopropane	$CH_3S(CH_2)_3C\equiv N$
5	1-Cyano-3,4-epithiobutane	
6	1-Cyano-4-methylthiobutane	$CH_3S(CH_2)_4C\equiv N$
7	Phenylethyl cyanide	$C_6H_5CH_2CH_2C\equiv N$
8	*threo*-1-Cyano-2-hydroxy-3,4-epithiobutane	
9	*erythro*-1-Cyano-2-hydroxy-3,4-epithiobutane	
10	3-Methylthiopropyl isothiocyanate	$CH_3S(CH_2)_3NCS$
11	1-Cyano-3-methylsulfinylpropane	$CH_3SO(CH_2)_3C\equiv N$
12	4-Methylthiobutyl isothiocyanate	$CH_3S(CH_2)_4NCS$
13	Phenylethyl isothiocyanate	$C_6H_5CH_2CH_2NCS$
14	1-Cyano-4-methylsulfinylbutane	$CH_3SO(CH_2)_4C\equiv N$
15	5-Vinyloxazolidine-2-thione (goitrin)	
16	3-Methylsulfinylpropyl isothiocyanate	$CH_3SO(CH_2)_3NCS$
17	4-Methylsulfinylbutyl isothiocyanate	$CH_3SO(CH_2)_4NCS$
18	Methyl palmitate[b]	

[a] Refers to peak number for both curves in Figure 1. [b] Added for internal standard.

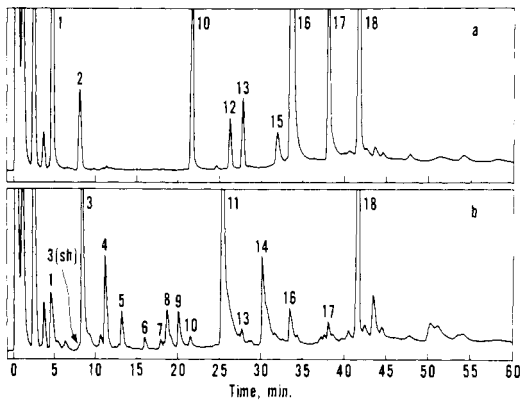


Figure 1. Chromatograms of cabbage glucosinolate products. Peak numbers refer to components listed in Table I. Curve a is from products by hydrolysis to yield isothiocyanates and goitrin. Curve b is from products by autolysis to yield nitriles.

as 1-cyano-3-methylsulfinylpropane. For confirmation of the identity of this compound, a preparation of it was obtained by enzymatic hydrolysis of the glucosinolate precursor. Prior work (unpublished) with glucosinolate preparations from *Lesquerella gordonii* seed meal showed it to be a rich source of this glucosinolate based on critical comparisons with a preparation from *Iberis amara* L. seed meal—the original literature source for the compound (Schultz and Gmelin, 1954). Hot water extraction of the glucosinolate from defatted *L. gordonii* seed meal and subsequent ion-exchange purification (on Ag 1 × 2 Cl^-) gave 3-methylsulfinylpropyl glucosinolate with a minor amount of 3-methylthiopropyl glucosinolate as a contaminant. Subsequent enzymatic hydrolysis of the glucosinolate at pH 7 gave the appropriate 3-methylsulfinylpropyl isothiocyanate, whereas enzymatic hydrolysis of the glucosinolate preparation at pH 3.0 gave the 1-cyano-3-methylsulfinylpropane and a much smaller amount of the corresponding isothiocyanate. The nitrile gave the same GLC retention on two columns as did the component in autolyzed cabbage and gave the appropriate NMR and MS data.

Extraction and Tentative Identification of 1-Cyano-3-methylsulfinylpropane in Sauerkraut. Two commercial and one home preparation of sauerkraut were examined. The variety of cabbage used to make the kraut and its glucosinolate composition were not known. Extraction of the nitriles and examination of the extracts were as follows: a 200-g portion of the preparation, that included a reasonable amount of the juice packaged with the kraut, was extracted by adding 200 ml of water and heating 10 min in a steam bath. The hot mixture was blended a few minutes at high speed, 400 ml of ethanol was added, and the mixture was brought to boiling. After boiling a few minutes, the mixture was cooled, filtered, concentrated to a volume of less than 25 ml, and filtered a second time. The filtrate was twice extracted with 200-ml volumes of hexane and the hexane extracts were discarded. The aqueous solution was adjusted to weakly alkaline pH with NaOH and then extracted three times with 200-ml volumes of dichloromethane. The dichloromethane extracts were combined, dried over anhydrous Na_2SO_4, and concentrated for GLC analysis as described by Daxenbichler and VanEtten (1976).

RESULTS AND DISCUSSION

Since the method of preparation does not retain the volatile allyl and butenyl cyanides, the total nitriles by IR in Table II are from nonvolatile compounds. Comparison of the amount of nitriles derived from progoitrin with the total nitrile amounts produced was evidence early in our investigation that most of the nitriles do not originate from progoitrin. When the hydrolysis was carried out under optimum conditions for goitrin formation (dried material), the amount found ranged from 5 to 61 ppm which is close to the values reported by Josefsson (1967) and VanEtten et al. (1976). No significant changes in the pH of the hydrolysis solutions were observed in the formation of

Table II. Nitriles and Goitrin in Parts per Million from Autolysis of Fresh and Dried Cabbage Leaves and Brussels Sprouts[a]

Vegetable	Condition	pH of autolyzate	Nitriles[b] Total	Nitriles[b] From progoitrin	Goitrin[c]
Brussel sprouts	Fresh	5.9	110	46	9
	Dried	6.0	32	1	61
Cabbage					
Accession 1	Fresh		95	8	4
	Dried	5.3	40	1	8
Accession 2	Fresh	6.3	42	16	3
	Dried	6.1	11	9	32
Accession 3	Fresh	6.3	28	3	5
	Dried	6.1	0	0	11
Accession 4	Fresh	6.2	49	10	0
(Red variety)	Dried	5.9	0	1	17
Accession 5	Fresh	5.6	41	8	0
	Dried	5.8	19	3	5

[a] Calculated on basis of fresh material. [b] Calculated as 1-cyano-2-hydroxy-3-butene. [c] Calculated as 5-vinyloxazolidine-2-thione.

Table III. Nitriles from Cruciferae Plants Reported in Recent Literature

Nitriles	Glucosinolates[a]	Sources	References
3-Indolylacetonitrile	3-Indolylmethyl (glucobrassicin)	*Brassica oleracea*, cabbage leaves	Gmelin and Virtanen (1961)
1-Cyano-2-hydroxy-3-butene; 1-cyano-2-hydroxy-3,4-epithiobutanes	2-Hydroxy-3-butenyl (progoitrins)	*B. napus*, *Crambe abyssinica*, leaves and seed	Daxenbichler et al. (1967, 1968); Van Etten et al. (1966, 1971)
1-Cyano-2,3-epithiopropane	Allyl (sinigrin)	Cruciferae plants	Cole (1975)
1-Cyano-3,4-epithiopentane	Pentenyl (glucobrassicanapin)		
1-Cyano-3,4-epithiobutane	3-Butenyl (gluconapin)	*B. campestris* seed	Kirk and MacDonald (1974)
Phenylacetonitrile	Benzyl (glucotropaeolin)	*Lepidium* plants	Cole (1976); Buttery et al. (1976)
p-Methoxyphenylpropionitrile	*p*-Methoxybenzyl (glucoaubrietin)	*Aubrietia* plants	
2-Phenylpropionitrile	2-Phenylethyl (gluconasturtiin)	*Brassica* plants	
Allyl cyanide	Allyl (sinigrin)	Volatiles from cooked cabbage	Macleod and Macleod (1968)
1-Cyano-3-methylthiopropane	3-Methylthiopropyl (glucoibervirin)	Volatiles from cauliflower	Buttery et al. (1976)
1-Cyano-4-methylthiobutane	4-Methylthiobutyl (glucoerucin)	Volatiles from broccoli	

[a] Glucosinolate source based on structure of nitriles obtained. Trivial name in parentheses.

goitrin or nitriles. Tookey (1973) reported a protein isolated from crambe seed that does not hydrolyze glucosinolates by itself, but when present with thioglucosidase it directs the hydrolysis of epiprogoitrin to nitriles with no pH change.

A comparison of the chromatograms that may be obtained by directing the breakdown of the glucosinolates from cabbage is shown in Figure 1. Curve a shows a chromatogram from the cabbage when glucosinolates were extracted and subsequently hydrolyzed in a manner to produce optimum amounts of the isothiocyanates or goitrin compounds. When the fresh cabbage was autolyzed, simply pulped with water to allow glucosinolate breakdown by an endogenous system, the isothiocyanates and goitrin peaks were essentially absent. Instead, as shown in curve b, other peaks appeared in somewhat proportional amounts elsewhere in the chromatograms. A listing of the components identified in the chromatograms is given in Table I.

The component designated as peak 3sh, due to 1-cyano-2-hydroxy-3-butene, gives a well-resolved peak in the separation on the EGSS-X column.

The mustard oil components, and indirectly their respective glucosinolate precursors, shown in curve a, were well known (to us) from our prior work (VanEtten et al., 1976; Daxenbichler and VanEtten, 1976). The nitrile compounds (curve b) were identified mainly by MS data. The three nitriles from the breakdown of progoitrin were readily recognized from our prior work and from the reference compounds we had isolated from crambe and rapeseed meals (Daxenbichler et al., 1968; VanEtten et al., 1966, 1971). The reference compound for 1-cyano-3-methylsulfinylpropane prepared from *L. gordonii* served to identify it as a typical major component of the autolysis preparations. Prominent features in the MS were: 131 (16) C_5H_9ONS molecular ion, 115 (13) C_5H_9NS, 68 (55) C_4H_6N, 67 (22) C_4H_5N, 64 (95) CH_4OS, 63 (31) CH_3OS, 61 (47) C_2H_5S, 47 (38) CH_3S, and 41 (100) C_2H_3N. Features of the NMR spectrum included a singlet at δ 2.60 for the methyl protons and three multiplets for the three different methylene groups. One multiplet was centered at δ 2.82 for the methylene protons adjacent to the sulfoxide group. A second multiplet was centered at δ 2.60 for the methylene protons adjacent to the nitrile group. The third multiplet, due to the middle methylene group, was centered at δ 2.20. Not shown by our work is the configuration of the asymmetric sulfur atom. It seems likely that the absolute configuration of the asymmetric sulfur atom is *R*, since Cheung et al. (1965) have established the *R* configuration for the same sulfur atom in the isothiocyanates and the natural glucosinolates from which they are derived.

MS data in literature reports were in excellent agreement with those for the components identified as 1-cyano-2,3-epithiopropane (Cole, 1975) and 1-cyano-3,4-epithiobutane (Kirk and MacDonald, 1974). The remaining four nitrile compounds identified in our work gave appropriate MS data for the assigned structures and spectra for three of them agreed with MS data reported by Buttery et al. (1976). These spectra and that of the remaining component (no. 14, Table I) were easily interpreted from spectra of the known related compounds and knowledge of the constituent glucosinolate precursors.

Based on GLC, three sauerkraut preparations contained the 1-cyano-3-methylsulfinylpropane in estimated amounts of 4 to 16 ppm of the whole kraut. No GLC evidence for the corresponding isothiocyanate was found. Since no compositional information was available for the cabbages from which the krauts were made, further experiments are being planned in which krauts from cabbages of known glucosinolate content will be studied.

Nitriles previously identified as hydrolysis products from the Cruciferae plants are listed in Table III.

Formation of nitriles instead of the expected isothiocyanates and goitrin from the glucosinolates by simply crushing the cabbage shows that consideration must also be given to the possible physiological effects of these compounds. In view of the long history of raw and cooked cabbage as a food staple without acute toxic effects, there is no immediate cause for alarm. However, more biological testing is warranted to properly evaluate the effects of the glucosinolates and their breakdown products and how food preparation methods affect their presence.

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LITERATURE CITED

Buttery, R. G., Guadagni, D. G., Ling, L. C., Seifert, R. M., Lipton, W., *J. Agric. Food Chem.* **24**, 829 (1976).
Cheung, K. K., Kjaer, A., Sim, G. A., *Chem. Commun.*, 100 (1965).
Cole, R. A., *Phytochemistry* **14**, 2293 (1975).
Cole, R. A., *Phytochemistry* **15**, 759 (1976).
Daxenbichler, M. E., Spencer, G. F., Kleiman, R., VanEtten, C. H., Wolff, I. A., *Anal. Biochem.* **38**, 374 (1970).
Daxenbichler, M. E., VanEtten, C. H., Northern Regional Research Center, Peoria, Ill., unpublished data, 1976.
Daxenbichler, M. E., VanEtten, C. H., Tallent, W. H., Wolff, I. A., *Can. J. Chem.* **45**, 1971 (1967).
Daxenbichler, M. E., VanEtten, C. H., Wolff, I. A., *Biochemistry* **5**, 692 (1966).
Daxenbichler, M. E., VanEtten, C. H., Wolff, I. A., *Phytochemistry* **7**, 989 (1968).
Gmelin, R., Virtanen, A. I., *S. Kimistilehti B* **34**, 15 (1961).
Josefsson, E., *Phytochemistry* **6**, 1617 (1967).
Kirk, J. T. O., MacDonald, C. G., *Phytochemistry* **13**, 2611 (1974).
Macleod, A. N., Macleod, G., *J. Sci. Food Agric.* **19**, 273 (1968).
Schultz, O. E., Gmelin, R., *Arch. Pharmacol.* **287** (59), 404 (1954).
Senti, F. R., Rizek, R. L., *Crop Sci. Soc. Am. Spec. Publ.* **No. 5**, (1974).
Spencer, G. F., Powell, R. G., Plattner, R. D., *J. Chromatogr.*, in press (1976).
Tookey, H. L., *Can. J. Biochem.* **51**, 1654 (1973).
VanEtten, C. H., Daxenbichler, M. E., *J. Agric. Food Chem.* **19**, 194 (1971).
VanEtten, C. H., Daxenbichler, M. E., Peters, J. E., Tookey, H. L., *J. Agric. Food Chem.* **14**, 426 (1966).
VanEtten, C. H., Daxenbichler, M. E., Williams, P. H., Kwolek, W. F., *J. Agric. Food Chem.* **24**, 452 (1976).
VanEtten, C. H., Daxenbichler, M. E., Wolff, I. A., *J. Agric. Food Chem.* **17**, 483 (1969).

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Cooxidation of β-Carotene by an Isoenzyme of Soybean Lipoxygenase

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Soybean lipoxygenase 1 (SL1) will cooxidize β-carotene while catalyzing the oxidation of linoleic acid (LH). This study established that SL1 will also cooxidize β-carotene when linoleic hydroperoxide (LOOH) is substituted for LH. Reduction of LOOH with sodium borohydride stops the cooxidation of β-carotene, and molecular oxygen is required for the cooxidation. The rate of cooxidation of β-carotene is less with LOOH than with LH. The products of the enzymatic β-carotene oxidation are essentially the same with LOOH as with LH. Rate studies indicate that LH and β-carotene are competitive inhibitors, but LOOH and β-carotene are not competitive.

Interest in the activity of soybean lipoxygenase (SL) on β-carotene ranges from practical applications to basic questions about the mechanism. Wolf (1975) recently reviewed the use of soybean flour as an aid to bleaching wheat flour for bread and pointed out that the process is currently in wide use.

Kies et al. (1969) raised a question about whether soybean lipoxygenase or some other enzyme in soy extracts causes β-carotene oxidation. That question has now been answered with the discovery of isozymes of soybean lipoxygenase (Guss et al., 1967; Christopher et al., 1970, 1972; Verhue and Francke, 1972). Several authors have found that the acid lipoxygenases, SL2 and SL3, are more active catalysts of β-carotene oxidation than the alkaline soybean lipoxygenase, SL1 (Weber et al., 1974; Arens et al., 1973). However, SL1 does catalyze the cooxidation of β-carotene in the presence of linoleic acid (LH). Since SL1 is the predominant isozyme present in soybean flour and since SL1 is much more stable than SL2 or SL3, SL1 may be the important catalyst in wheat flour bleaching by soybean flour.

Early work on the cooxidation of β-carotene by SL and LH established rates of oxidation of β-carotene and LH (Blain et al., 1953; Tookey et al., 1958). The general conclusion from these studies was that an intermediate free

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