Phytochemistry, 1965, Vol. 4, pp. 207 to 228. Pergamon Press Ltd. Printed in England

A REVIEW

STUDIES ON ORGANIC SULPHUR COMPOUNDS AND OTHER LABILE SUBSTANCES IN PLANTS

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(*Received* 12 *September* 1964)

IT IS significant from the points of view of both plant physiology and the nutrition of man and animals that vegetables and fodder plants contain in smaller amounts numerous organic compounds which give characteristic properties to the plants. Even thousands of years ago many vegetables had been found to possess peculiar effects. Plinius stated that onion cures twenty-eight different diseases and cabbage as many as eighty-seven.[1] Garlic was considered as a holy plant in Egypt. Even today a great number of people daily use garlic in Western Europe in the belief that it keeps them well and enables them to live longer. In South-east Europe people consume plenty of onions in the belief that it has a favourable effect on health and prevents different diseases, e.g. tuberculosis. Beliefs play an important role in this, but the few scientific findings made so far indicate that certain physiological effects, e.g. antimicrobial activity, are due to many kinds of vegetables. The special flavour substances, and those irritating the mucous membrane act as stimulants and belong also to the important physiologically effective substances of vegetables. The effect of these kind of substances cannot be measured quantitatively and objectively like vitamins, which are essential nutritional factors. This is probably the reason why nutritional research has not paid more attention to such properties of vegetables.

The importance of this type of substance to the plants themselves, e.g. as resistant factors, is probable although very little is known of its effects. Without adequate knowledge of the chemical nature of these substances it is impossible to investigate the effect of them in plant and animal organisms. Therefore, the first task in the development of this research field is, from both the nutrition researcher's and plant physiologist's point of view, to elucidate the chemical composition of vegetables more thoroughly than before.

Physiological effects can be investigated systematically only with pure substances. In this paper I shall deal principally with organic sulphur compounds isolated in our institute in recent years from onion species (*Allium*), cresses (*Lepidium* and *Tropaeolum*), and cabbage (*Brassica*) and their enzymatic and chemical decomposition products.

It has proved to be a general rule that there occur, in intact plants, physiologically inactive precursors from which active substances, e.g. different flavour substances, mucous membrane- and gland-stimulating compounds, antimicrobial and antithyroid substances etc. are formed through enzymatic reactions. The precursors and enzymes, through which they are decomposed, are separated from each other in plants so that the enzyme reactions can only take place when the plants are crushed, e.g. when vegetables are eaten. Proof has been

[1] E. F. KOHMAN, *Science* **106**, 625 (1947).

presented of the fact that enzymes and substrates appear in different cells of plants.[2] The question, however, demands a more critical examination.

When enzymes in intact plants are destroyed in a suitable way it is possible to isolate the precursors which are present. The method of inactivation must be effective, but it is just as important to avoid chemical decomposition. It has become obvious, in connexion with our investigations, how important the right method of treatment is, when the isolation of primary plant substances is sought, and how easily one can obtain wrong results because of incomplete or unsuitable methods of enzyme inactivation. Literature contains much data concerning substances in plants which in reality are not present at all.

I shall illustrate these difficulties with one example. When looking for the possible anti-fungal factor responsible for the resistance of young rye plants to *Fusarium nivale* we managed, in the middle 1950's, to isolate an active substance from these plants which we found to be 2(3)-benzoxazolinone[3] (III). From maize and wheat plants we isolated 6-methoxy-2(3)-benzoxazolinone[4] (VI). The structure was confirmed by synthesis.[5,6] We considered

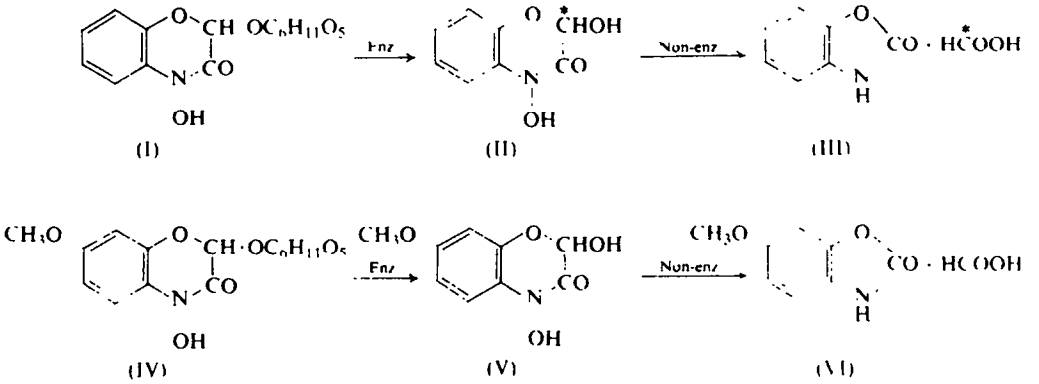


FIG. 1. FORMATION OF 2(3)-BENZOXAZOLINONE III FROM GLUCOSIDE I (RYE PLANT) AND 6-METHOXY-2(3)-BENZOXAZOLINONE VI FROM GLUCOSIDE IV (MAIZE AND WHEAT PLANTS).

these to be the primary anti-fungal substances,[7] until it became evident that, on the contrary, these benzoxazolinones were formed during isolation.[8]

Glucoside I (in rye plants)[9-11] and glucoside IV (in maize and wheat plants)[12,13] proved to be primary plant substances occurring in these plants which are split by glucosidases to give glucose and 2,4-dihydroxy-1,4-benzoxazine-3-one (II) or 8-methoxy-2,4-dihydroxy-1,4-benzoxazine-3-one (V) respectively. In the aglucones the benzene ring is condensed with a heterocyclic 6-membered ring, and in the corresponding benzoxazolinones with a hetero-

[2] L. GUIGNARD, *Comt. Rend.* **111**, 249 (1890).
[3] A. I. VIRTANEN and P. K. HIETALA, *Acta Chem. Scand.* **9**, 1543 (1955).
[4] A. I. VIRTANEN, P. K. HIETALA and Ö. WAHLROOS, *Suomen Kemistilehti B* **29**, 143 (1956).
[5] A. I. VIRTANEN, P. K. HIETALA and Ö. WAHLROOS, *Suomen Kemistilehti B* **29**, 171 (1956).
[6] P. K. HIETALA and Ö. WAHLROOS, *Acta Chem. Scand.* **10**, 1196 (1956).
[7] A. I. VIRTANEN, P. K. HIETALA and Ö. WAHLROOS, *Arch. Biochem Biophys.* **69**, 486 (1957).
[8] A. I. VIRTANEN and Ö. WAHLROOS, *Suomen Kemistilehti B* **31**, 402 (1958)
[9] A. I. VIRTANEN and P. K. HIETALA, *Suomen Kemistilehti B* **32**, 138, 252 (1959).
[10] A. I. VIRTANEN and P. K. HIETALA, *Acta Chem. Scand.* **14**, 499 (1960).
[11] P. K. HIETALA, *Ann. Acad. Sci. Fennicae A II*, **100**, 46 (1960).
[12] Ö. WAHLROOS and A. I. VIRTANEN, *Suomen Kemistilehti B* **32**, 139 (1959).
[13] Ö. WAHLROOS and A. I. VIRTANEN, *Acta Chem. Scand.* **13**, 1725 (1959).

cyclic 5-membered ring. Now it proved that the 6-membered ring undergoes an unusual decomposition to give a 5-membered ring, C-atom 2 being split off as formic acid (Fig. 1). When the aglucone II, in which the C-atom 2 was labelled with ^{14}C, was synthesized,[14] it could be proved that this C-atom was released as formic acid[15] (Fig. 2).

The formation of benzoxazolinone takes place when an aqueous solution is heated. In kinetic studies[16] it was shown that the reaction follows first order kinetics and is dependent on the concentration of the anion of the aglucone. Ethanol accelerates the reaction so much that, should the plant material contain free aglucone, it is difficult to prevent it partially reacting in this way during the extraction with ethanol and its evaporation. The free aglucone is either present to some extent in intact plants or it is rapidly formed enzymatically from the glucoside if the enzyme is not effectively inactivated before crushing. Ethanol, which is generally used for the inactivation of enzymes in plants, causes, in this case, the formation of a secondary reaction product which is not originally present.

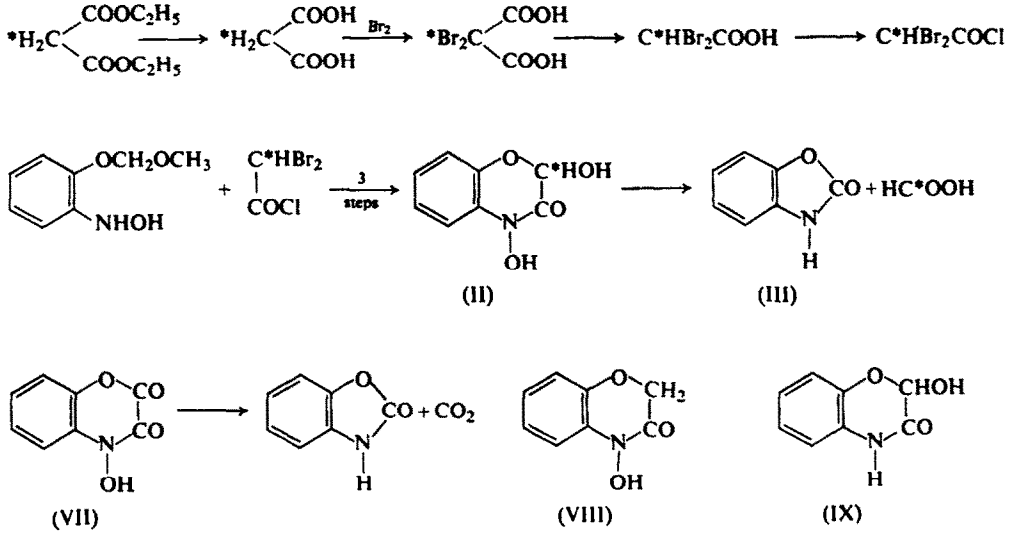


FIG. 2. SYNTHESIS OF AGLUCONE II LABELLED WITH ^{14}C AT C-ATOM 2.

Benzoxazolinone is formed also from diketocompound VII. C-atom 2 is, in this case, split off as carbon dioxide. OH— at N— atom and OH— or O= at C-atom 2 are essential for the reaction. Compounds VIII and IX are not decomposed.[15] The latter compound has the highest anti-fungal activity of the 1,4-benzoxazine derivatives synthesized.[16a]

The absence of methoxybenzoxazolinone has been proved in our laboratory in three varieties of maize.[17,18] This matter became important practically after Beck's research group in Wisconsin came to the conclusion that 6-methoxybenzoxazolinone is a resistance factor which kills the larvae of a dangerous destructive insect of maize, the European maize

[14] E. HONKANEN and A. I. VIRTANEN, *Acta Chem. Scand.* **14**, 504, 1214 (1960).
[15] E. HONKANEN and A. I. VIRTANEN, *Acta Chem. Scand.* **15**, 221 (1961).
[16] J. B. BREDENBERG, E. HONKANEN and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 513 (1962).
[16a] E. HONKANEN and A. I. VIRTANEN, *Acta Chem. Scand.* **14**, 1214 (1960).
[17] A. I. VIRTANEN and Ö. WAHLROOS, *J. Pharm. Sci.* **52**, 713 (1963).
[18] Ö. WAHLROOS and A. I. VIRTANEN, *J. Pharm. Sci.* (In press).

borer (*Pyrausta nubilalis*).[19] As this compound is not found or enzymatically formed in the maize plant it is more probable that the aglucone formed from the glucoside of maize is the true resistance factor.[20] The aglucone occurs in resistant maize species both in free form and bound to glucose.[18]

The glucosides and aglucones described above also have other important practical properties. They react with simazines, the synthetic phytotoxic compounds used as weed killers, as shown by Roth and Knüsli.[21] The relatively high content of the glucoside in young maize plants may partially explain the resistance of these plants to simazine.

The enzymes are, of course, destroyed when vegetables are cooked. On the other hand, different kinds of decomposition of the precursors can take place under the influence of heat. Thus fresh and cooked vegetables differ from each other nutritionally, although the precursors can be decomposed in man and animals so that active substances are formed.

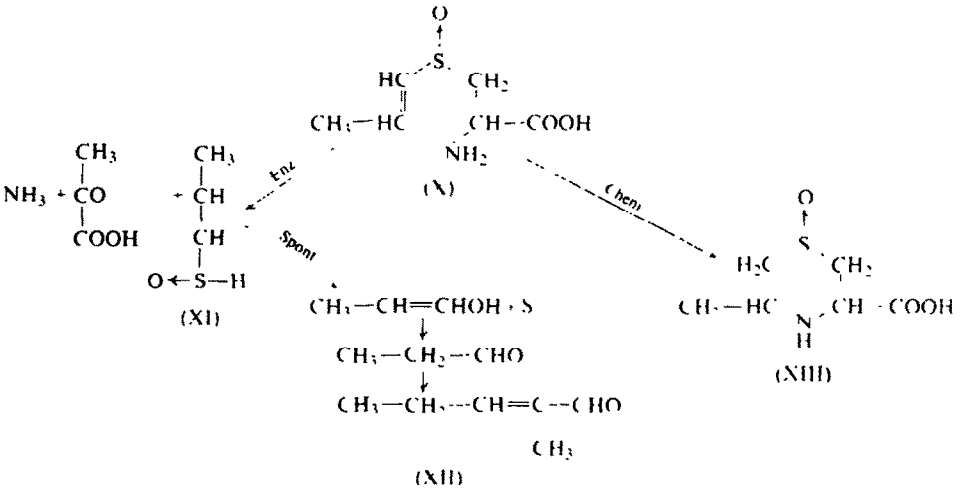


FIG. 3. ENZYMATIC CLEAVAGE AND SPONTANEOUS CYCLIZATION OF THE PRECURSOR OF THE LACHRYMATORY FACTOR.

In recent years we have isolated a large number of new sulphur compounds from *Allium* species. These findings partly explain the different physiological effects attached to these plants. The lachrymatory effect which occurs when the onion (*Allium cepa*) is cut or crushed has surely been observed ever since man started to use this plant. Chemically this phenomenon was first elucidated a couple of years ago when Spåre and Virtanen[22,23] isolated the lachrymatory precursor from the onion. Earlier efforts to determine the chemical nature of the lachrymatory factor had only led to hypotheses about its structure, principally because the substance could not be isolated due to its instability. Our studies on the precursor from undamaged onion, in which the enzymes were destroyed, led to the isolation of a crystalline compound of the formula $C_6H_{11}NSO_3$. The lachrymatory factor was formed in an aqueous

[19] R. S. LOOMIS, S. D. BECK and J. F. STAUFFER, *Plant Physiol.* **32**, 379 (1957).
[20] A. I. VIRTANEN, *Suomen Kemistilehti B* **34**, 29 (1961).
[21] W. ROTH and E. KNUSLI, *Experientia* **17**, 312 (1961).
[22] A. I. VIRTANEN and C.-G. SPÅRE, *Suomen Kemistilehti B* **34**, 72 (1961).
[23] T. MOISIO, C.-G. SPÅRE and A. I. VIRTANEN, *Suomen Kemistilehti B* **35**, 29 (1962).

solution of this substance a few seconds after addition of an enzyme preparation made from onion. Our procedure for the isolation of the precursor, in different phases of the work, depended on this property.

The precursor proved to be (+)-S-(prop-1-enyl)-L-cysteine sulphoxide (X). Upon enzymatic cleavage pyruvic acid and ammonia were formed from the cysteine part of the molecule, analogous to other cysteine sulphoxides, and the lachrymatory factor, propenylsulphenic acid (XI) from the propenyl part of the molecule (Fig. 3).

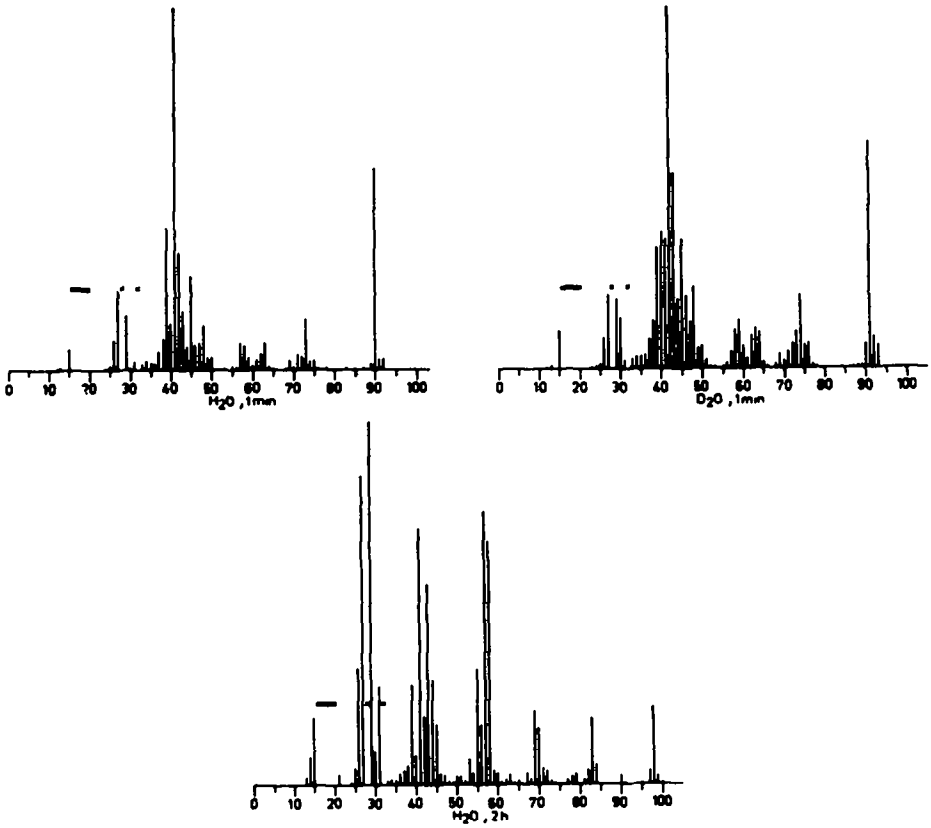


FIG. 4. MASS SPECTRA DETERMINED IN THE SYSTEM: S-(PROP-1-ENYL)-L-CYSTEINE SULPHOXIDE (1 mg) + LYOPHILIZED ONION ENZYME PREPARATION (4 mg) PURIFIED ON SEPHADEX + H_2O OR D_2O (4 DROPS).
Above left: H_2O, time of reaction 1 min; above right: D_2O, 1 min; below: H_2O, 2 h.

The lachrymatory factor decomposes quickly, propionaldehyde being formed and also smaller amounts of 2-methyl-2-pentenal (XII) by aldol condensation.[16] It could thus be assumed that the lachrymatory factor was propenyl sulphenic acid.[22] This assumption was confirmed by a mass spectrometry study.[23,24] Mass spectra of the volatile substances formed, when an enzyme preparation of onion was mixed with the precursor and a small quantity of water or deuterium oxide, are presented in Fig. 4.

From a study of the mass spectra the following conclusions can be made. The molecular

[24] C.-G. SPÅRE and A. I. VIRTANEN, *Acta Chem. Scand.* 15, 641 (1963).

weight of the lachrymatory factor is 90. The small peaks at mass 91 and 92 originate from the same molecule and are normal peaks of isotopes (cf. molecules in which heavier isotopes occur in natural proportion). The relation between the peaks at mass 90, 91 and 92 indicates that an S-atom is present in the molecule. The compound contains no nitrogen, since the mass number of the molecular ion is even. The shift of the peak at mass 90 to mass 91 in D_2O proves the presence of an exchangeable hydrogen atom. The removal of the peak at mass 73 (90 – 17) in water to mass 74 (91 – 17) in D_2O shows that in both cases an OH-fragment is set free. The molecule contains therefore no OH-group (the hydrogen of which would be replaced by deuterium in D_2O). The peak at mass 15 shows that the compound contains a CH_3-group.

All these findings agree with the structural formula given to the lachrymatory factor. Since no aliphatic sulphenic acid was known earlier, the propenyl sulphenic acid identified as the lachrymatory factor is the first representative of this class of compounds. Even it is quite unstable, and therefore it is not easy to investigate what physiological effects other than the lachrymatory action are due to the propenyl sulphenic acid.

Carson and Wong[24a] have recently prepared cis-S-(prop-1-enyl)-L-cysteine, CH_3—CH=CH—S—CH_2—CH(NH_2)—COOH, by a base isomerization of S-allyl-L-cysteine, CH_2=CH—CH_2—S—CH_2—CH(NH_2)—COOH. Hydrogen peroxide converts the former amino acid into a sulphoxide mixture which in the presence of onion enzyme produces a fresh onion aroma with a definite lachrymatory effect. It is thus probable that the sulphoxide preparation contains at least some of the natural lachrymatory precursor.

The precursor of the lachrymatory factor is a rather central sulphur compound in onion. From it is formed, by cyclization in weak alkaline solution, a thiazane sulphoxide, 3-methyl-1,4-thiazane-5-carboxylic acid-1-oxide (XIII in Fig. 3), which was isolated by Matikkala and Virtanen[25-28] some years ago from onion and named cycloalliin. At that time nothing was known about the presence of S-(prop-1-enyl)-cysteine sulphoxide. Cycloalliin is the first naturally occurring compound containing the thiazane ring. This compound occurs in the onion in larger amounts than any other sulphur compound and indeed up to 2 per cent of the dry weight of the onion. It could be proved that cycloalliin is an original substance[29] in onion, even if it also can be formed from S-(prop-1-enyl)-cysteine-sulphoxide when amino acids are eluted from exchange resin with ammonia.*

When feeding rats with ^{35}S-labelled cycloalliin it was observed that the main part of it is excreted in the faeces partly unchanged, partly reduced, but a part is absorbed and is excreted in the urine in the same form as in the faeces. Its effect upon the normal metabolism of sulphur in man and animal is not known. It has no antibiotic activity.

The assumption that the thiazane derivative obtained from the algae may have in analogy with cycloalliin, an aliphatic precursor, S-vinyl-cysteine-S-oxide (XIV in Fig. 5), was con-

* It is interesting that Kuriyama, Takagi and Murata[30] have later isolated from a red alga (*Chondria crassicaulis*) and Tominaga and Oka[31] from a brown alga (*Undaria pinnatifida*) a sulphoxide which is structurally "apocycloalliin" (cycloalliin minus the methyl group).

[24a] J. F. CARSON and F. F. WONG, *Chem. & Ind. (Lond.)* 1764 (1963).
[25] A. I. VIRTANEN and E. J. MATIKKALA, *Suomen Kemistilehti B* **29**, 134 (1956).
[26] E. J. MATIKKALA and A. I. VIRTANEN, *Suomen Kemistilehti B* **30**, 219 (1957).
[27] A. I. VIRTANEN and E. J. MATIKKALA, *Suomen Kemistilehti B* **31**, 191 (1958).
[28] A. I. VIRTANEN and E. J. MATIKKALA, *Acta Chem. Scand.* **13**, 623 (1959).
[29] A. I. VIRTANEN and E. J. MATIKKALA, *Suomen Kemistilehti B* **34**, 114 (1961).
[30] M. KURIYAMA, M. TAKAGI and K. MURATA, *Bull. Fac. Fisheries Hokkaido Univ.* **11**, 58 (1960).
[31] F. TOMINAGA and K. OKA, *J. Biochem. (Tokyo)* **54**, 222 (1963).

firmed in our laboratory by synthesis of the precursor.[32] A lachrymatory substance, which according to mass spectral determinations was vinylsulphenic acid (XV), was formed from this sulphoxide by the onion enzyme preparation. Apocycloalliin XVI, which had been isolated by the Japanese researchers from algae, was formed from S-vinyl-cysteine-S-oxide in ammonia solution[32] (Fig. 5). When the synthesis of S-(2-bromoethyl)-cysteine was attempted a small amount of reduced apocycloalliin (XVII) was formed, the chief product being a new S-containing diamino-dicarboxylic acid (XVIII) of djenkolic acid type. It will be interesting to find out whether the algae contain S-vinyl-cysteine-S-oxide and the enzyme

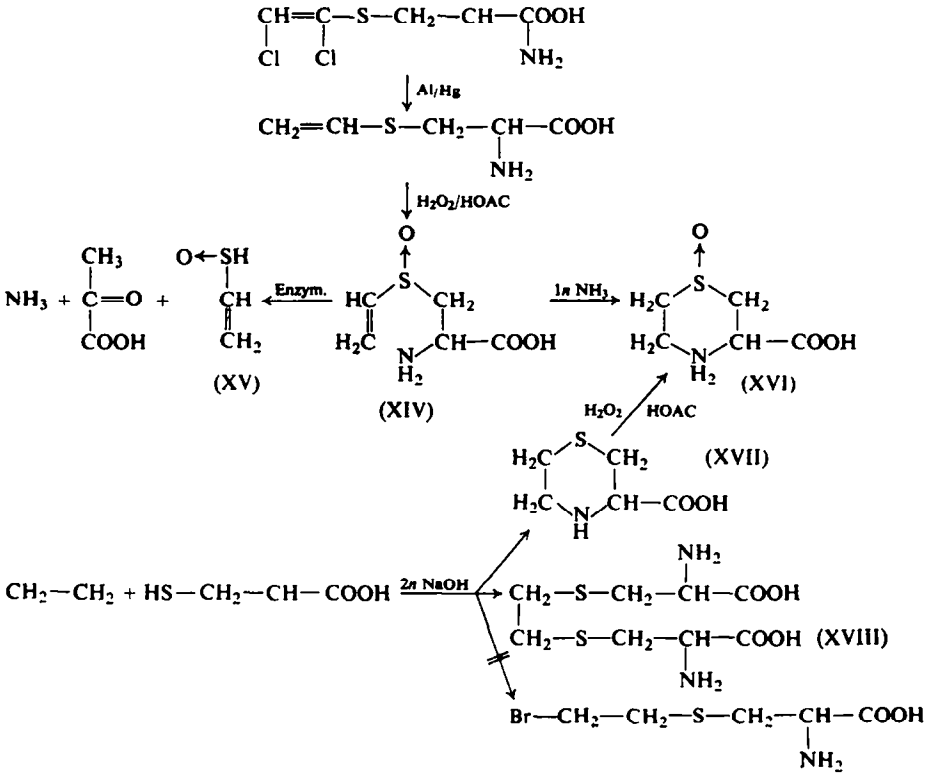


FIG. 5. SYNTHESIS OF S-VINYL-CYSTEINE-S-OXIDE, ITS ENZYMATIC DEGRADATION AND CHEMICAL CYCLIZATION.

which forms the lachrymatory factor. In this case the analogy between onion and the algae species would be astonishing.

Two alkylsulphoxides, S-methylcysteine sulphoxide, and S-propylcysteine sulphoxide, isolated by Matikkala and Virtanen[33] from onion are of importance as precursors of antibiotic substances. These sulphoxides decompose enzymatically so that methyl- and propylthiosulphinates are formed. Earlier Stoll and Seebeck[34] had isolated from garlic allylcysteine sulphoxide (alliin) (XIX in Fig. 6), from which allylthiosulphinate[35] (allicin) (XX)

[32] E. DÄBRITZ and A. I. VIRTANEN, *Acta Chem. Scand.* **18**, 837 (1964).
[33] A. I. VIRTANEN and E. J. MATIKKALA, *Acta Chem. Scand.* **13**, 1898 (1959).
[34] A. STOLL and E. SEEBECK, *Advanc. Enzymol.* **11**, 377 (1951).
[35] C. J. CAVALLITO, J. S. BUCK and C. M. SUTER, *J. Am. Chem. Soc.* **66**, 1952 (1944).

was formed enzymatically. These authors assumed that the reaction occurred in the following way (Fig. 6).

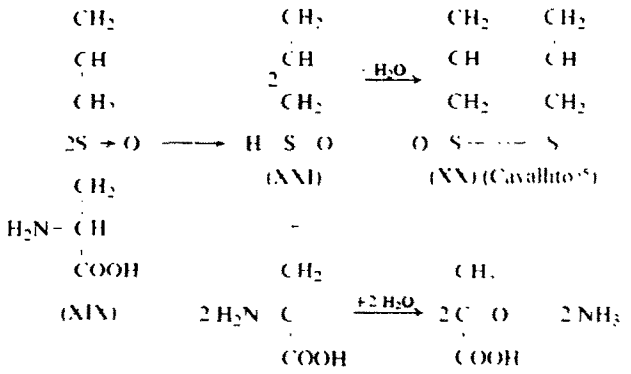


FIG. 6. ENZYMATIC CLEAVAGE OF ALLYLCYSTEINE SULPHOXIDE ACCORDING TO STOLL AND SEEBECK.[34]

Our mass spectral studies did not show any formation of allylsulphenic acid (XXI) or other volatile 3 C-compound during the enzymatic cleavage of S-allylcysteine sulphoxide.[36] It is therefore more probable that allylsylphenic acid is not formed at all as an intermediary product. The formation of allicin from two molecules of S-allylcysteine sulphoxide through

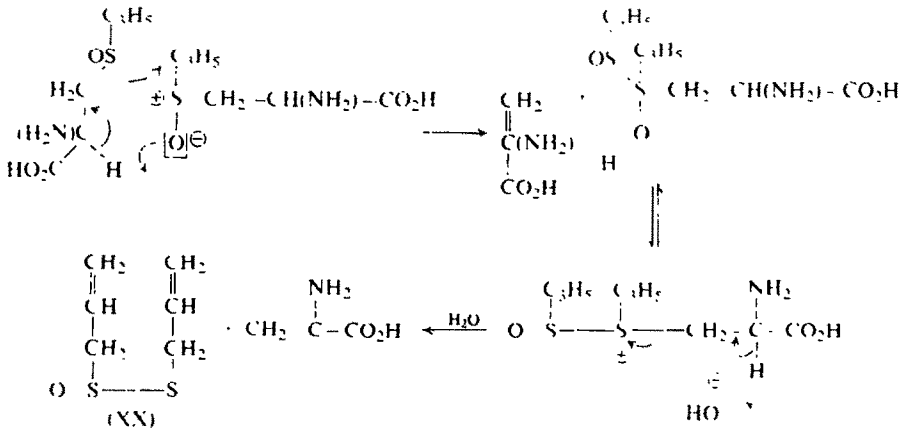


FIG. 7. MECHANISM PROPOSED FOR THE BIOSYNTHESIS OF ALLYLTHIOSULPHINATE FROM ALLYLCYSTEINE SULPHOXIDE.

two electronic shifts and associated β-elimination is to be explained without the formation of two molecules of sulphenic acid (Fig. 7). The different states of the double bonds in S-propenyl-cysteine-S-oxide and S-allyl-cysteine-S-oxide affects obviously the inclination of the S-atoms to unite with each other.

[36] A. I. VIRTANEN, *Suomen Kemistilehti A* 37, 108 (1964).

The antimicrobial effect of allicin is considerable. It has a growth inhibiting effect both on Gram-positive and Gram-negative bacteria (e.g. *Staphylococci, Streptococci, Eberthella typhosa, B. dysenteriae, B. enteritidis* and *Vibrio cholerae*) even in a dilution of about 1:100,000 (Small, Bailey and Cavallito[37]).

The effect of S-methyl- and S-propyl-thiosulphinate is much weaker than that of allicin. The antimicrobial effect of the onion species depends on, besides the activity of the formed thiosulphinate, the quantities of the precursors occurring in the onions. Different kinds of the same onion species can also differ greatly from one other in this respect. Climatic factors and manural treatments effect also the composition of onions. The onion (cultivated in Finland) inhibits, as a homogenate in a 10–15-fold dilution, the growth of *Staphylococcus* for 24 hr. Homogenized garlic has an activity ten to twenty times higher. On the basis of experiments carried out *in vitro* the antibiotic effect of onion and garlic cannot, however, be determined in the animal or human organism, since: (1) the tissue of plants is not completely crushed when it is eaten; (2) no enzymatic reaction occurs at the pH of the stomach; (3) alkylcysteine

TABLE 1. APPROXIMATE CONCENTRATIONS OF THE SULPHUR COMPOUNDS SO FAR ISOLATED FROM THE ONION

Compound	mg/kg fresh wt.
S-Propylcysteine-S-oxide	50
S-Methylcysteine-S-oxide	200
S-Propenylcysteine-S-oxide	40
Cycloalliin*	2500
γ-L-Glutamyl-(+)-S-propenyl-cysteine-S-oxide	1300
γ-L-Glutamyl-S-methylcysteine	50
γ-L-Glutamylmethionine	50
S-(2-Carboxyprop-1-yl)glutathione	330

* A part of the cycloalliin may be formed from S-propenyl-cysteine-S-oxide during elution of amino acids with ammonia from the Amberlite column.

sulphoxides are oxidizing agents and are reduced themselves to the S-form from which no alkyl thiosulphinates can be formed and, finally S-alkylcysteine-S-oxides are reduced by *Coli*-type intestinal bacteria to disulphides (Saarivirta and Virtanen). These have nevertheless an antimicrobial effect against *Staphylococcus* but weaker than that of the alkyl thiosulphinates. Quastel's research group[38] has found out that e.g. ethylsulphide and diethyldisulphide have a considerable antituberculous effect. It is possible that alkyl sulphides represent important antimicrobial factors *in vivo*. Cooked onions may thus also have a certain antimicrobial effect, even though their enzymes have been inactivated.

The sulphoxides and sulphur-containing γ-glutamylpeptides (see below) isolated in our laboratory from the onion bulb cultivated in Finland are shown in Table 1. About 90 per cent of the soluble organic-bound sulphur occurs in these compounds, so that there are no other larger quantities of unknown S-containing substances in the onion. Several unknown sulphur compounds present in smaller quantities can indeed be traced chromatographically

[37] L. V. SMALL, J. H. BAILEY and C. J. CAVALLITO, *J. Am. Chem. Soc.* **69**, 1710 (1947).
[38] H. D. BROWN, A. R. MATZUK, H. J. BECKER, J. P. CONBERE, J. M. CONSTANTIN, M. SOLOTOROVSKY, S. WINSTEN, E. IRONSON and J. H. QUASTEL, *J. Am. Chem. Soc.* **76**, 3860 (1954).

(Fig. 9). This appeared very clearly from the experiments performed by Ettala and Virtanen[39] in which $^{35}SO_4^{2-}$ was injected into onion bulbs. Two-dimensional paper chromatograms prepared 7 and 46 days after the injection are shown in Fig. 8. Twenty-one radioactive spots appeared on the chromatogram after 7 days; after hydrolysis with 1 N-hydrochloric acid the number was reduced to 12.

As mentioned above we have found in our laboratory besides sulphur-containing amino acids numerous γ-glutamylpeptides as typical components in different *Allium* species. In the

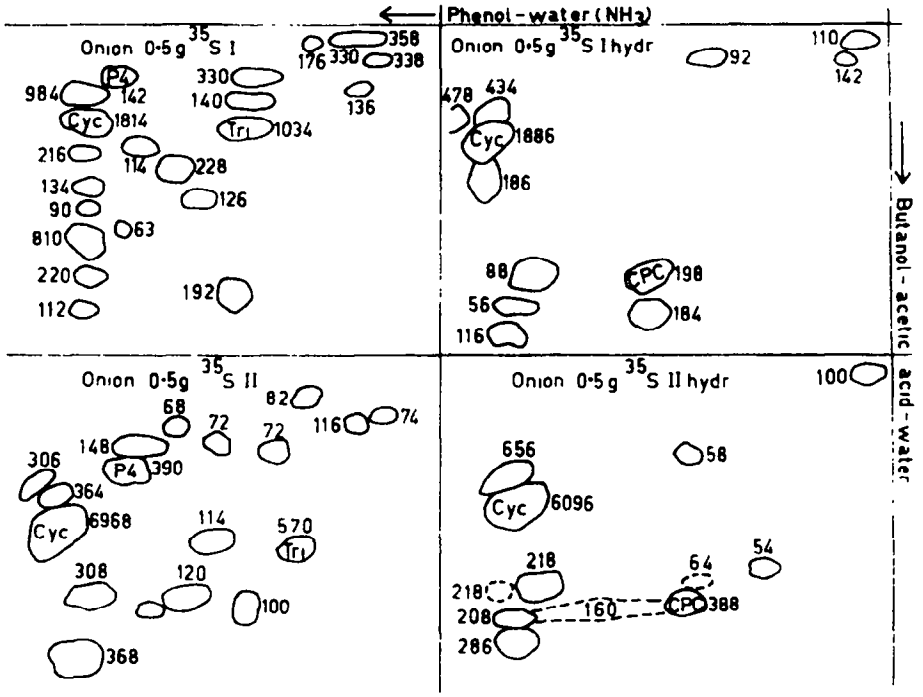


FIG. 8. TWO-DIMENSIONAL CHROMATOGRAMS OF ^{35}S-CONTAINING AMINO ACIDS AND γ-GLUTAMYL PEPTIDES IN ONION.

Onions were placed for 6 days in moist sand and then injected with labelled sulphate. The upper chromatograms were developed 7, and the lower ones 46, days after the injection of labelled sulphate. The chromatograms to the left were developed before, and those to the right after hydrolysis. The spots were drawn on the basis of radiation measurements, and the numbers give the counts/min. Cyc = cycloalliin, P4 = γ-L-glutamyl-(+)-S-(prop-1-enyl)-L-cysteine, Tri = γ-L-glutamyl-S-(β-carboxy-propyl)-L-cysteinyl-glycine, CPC = (-)-S-(β-carboxy-propyl)-L-cysteine.

investigations which have been in progress since the end of the 1950's. Matikkala and Virtanen[40,41] have isolated in pure form and characterized chemically 9 γ-glutamylpeptides from onion bulbs. The isolation of the peptides was performed on a Dowex-1 column using 1-2 N-acetic acid and finally 1 N-hydrochloric acid as eluting solutions (Fig. 9).

Peptides not separated from one another by fractionation with acids were separated on a cellulose column with butanol-acetic acid–water as solvent and isolated in pure form. The

[39] T. ETTALA and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 2061 (1962).
[40] A. I. VIRTANEN and E. J. MATIKKALA, *Suomen Kemistilehti B* **33**, 83 (1960).
[41] A. I. VIRTANEN and E. J. MATIKKALA, *Suomen Kemistilehti B* **34**, 53, 84 (1961).

following peptides have been isolated in crystalline form and characterized chemically (Table 2).

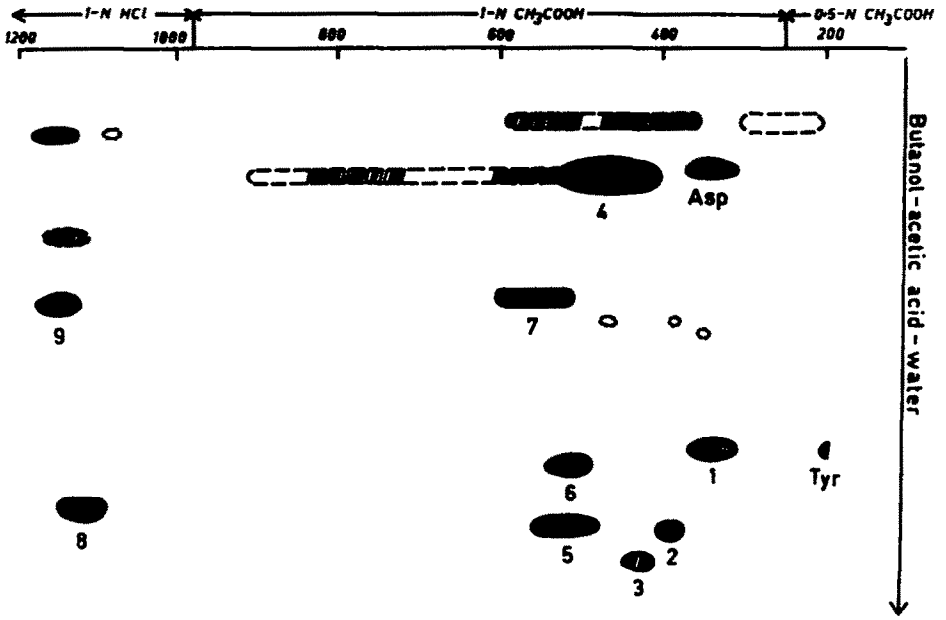


FIG. 9. ELUTION OF γ-GLUTAMYLPEPTIDES OF ONION BULBS FROM A DOWEX-1 COLUMN WITH 1 N-ACETIC ACID AND 1 N-HYDROCHLORIC ACID.

Every second fraction was examined on paper chromatograms. Peptides corresponding to spots provided with numbers have been isolated in pure form and characterized chemically. The unnumbered, weak ones have not yet been characterized.

Peptide 9 is a derivative of glutathione and contains S-(2-carboxy-*n*-propyl)-L-cysteine instead of cysteine.[41,42] This peptide is present also in garlic.[43] It is interesting that later

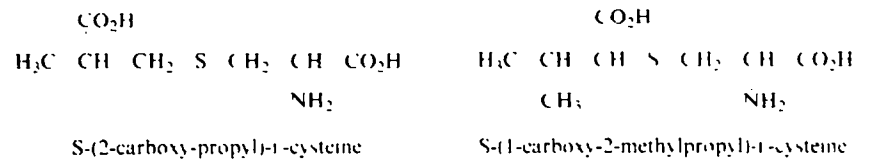
TABLE 2. γ-GLUTAMYLPEPTIDES SO FAR ISOLATED FROM ONION BULBS

1. γ-L-Glutamyl-L-valine
2. γ-L-Glutamyl-L-isoleucine
3. γ-L-Glutamyl-leucine
4. γ-L-Glutamyl-S-(prop-1-enyl)-cysteine-S-oxide
5. Ethyl ester of γ-glutamylpeptide No. 9 (possibly an artifact)
6. γ-L-Glutamyl-L-methionine
7. γ-L-Glutamyl-S-methylcysteine
8. γ-L-Glutamyl-L-phenylalanine
9. γ-L-Glutamyl-S-(2-carboxyprop-1-yl)-cysteinyl-glycine (S-(2-carboxypropyl)glutathione)

Mizuhara and Oomori[44] independently found this amino acid in human urine in both free and bound forms. They state (private communication) that the occurrence of this amino

[42] A. I. VIRTANEN and E. J. MATIKKALA, *Z. Physiol Chem.* **322**, 8 (1960).
[43] T. SUZUKI, M. SUGII and T. KAKIMOTO, *Chem. Pharmacol. Bull. (Tokyo)* **9**, 77 (1961).
[44] S. MIZUHARA and S. OOMORI, *Arch. Biochem. Biophys.* **92**, 53 (1961).

acid in urine cannot be traced back to the consumption of onions. The same investigators[45] have also isolated another closely related amino acid, S-(1-carboxy-2-methylpropyl)-cysteine ("isovalthine") from the urine of patients suffering from arteriosclerosis or myxodema.



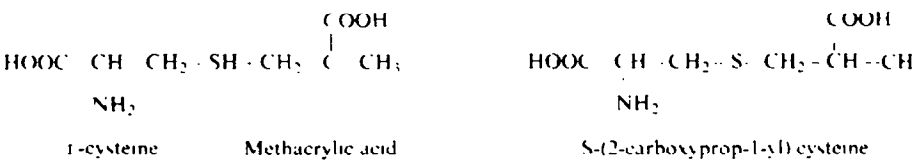
S-(2-carboxy-propyl)-L-cysteine S-(1-carboxy-2-methylpropyl)-L-cysteine

Kuwaki *et al.*[46] have shown that S-(1-carboxy-2-methylpropyl)-cysteine is synthesized in liver homogenate, the precursor being S-(isopropylcarboxymethyl)glutathione.

Some of the γ-glutamylpeptides occurring in the onion are also present in garlic, few are found in one species only. γ-L-glutamyl-S-allyl-L-cysteine, which had not been found in onion, was isolated in our laboratory[47] from garlic and later independently by Suzuki *et al.*[48] As already stated above the sulphoxide of S-allylcysteine is in the free form characteristic of garlic, since the antibiotically active allicin is enzymatically formed from the free amino acid sulphoxide. On the other hand S-propyl-cystein sulphoxide is so far found only in onion,[33] γ-glutamyl-S-propyl-cysteine only in garlic.[49]

The S-atom of alkyl- and alkenylderivatives of cysteine is, in general, present in reduced form in the γ-glutamylpeptides isolated by us, although the corresponding free amino acids occur as sulphoxides. The only exception to the rule is the above-mentioned peptide 4, γ-glutamyl-S-(prop-1-enyl)-cysteine-sulphoxide present in the onion.[40] In the corresponding γ-glutamylpeptide isolated from the seeds of the chive (*Allium schoenoprasum*) the sulphur atom also of this amino acid occurs in reduced form.[50,51] Sugii *et al.*[52] have recently found this amino acid in the free form in garlic. Suzuki *et al.*[43] mention also γ-glutamyl-S-methyl-cystein sulphoxide as a component of garlic but its presence in the undamaged bulb needs further studies. The sulphur-containing γ-glutamylpeptides hitherto isolated from *Allium* species in our laboratory are presented in Table 3.

The addition of the SH-group on to the double bond would explain the formation of many S-carboxyalkylcysteines. Schöberl and Wagner[54] have synthetized S-(2-carboxy-propyl)-cysteine from cysteine and methacrylic acid by heating their aqueous solution.

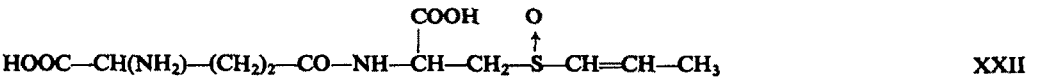


L-cysteine Methacrylic acid S-(2-carboxyprop-1-yl) cysteine

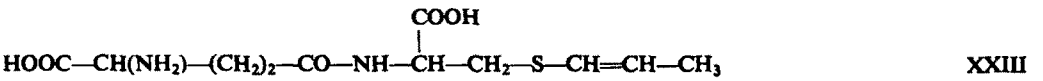
[45] S. OOMORI and S. MIZUHARA, *Biochem. Biophys. Res. Commun.* **3**, 343 (1960).
[46] T. KUWAKI, S. OOMORI and S. MIZUHARA, *Biochem. Biophys. Acta* **78**, 553 (1963).
[47] A. I. VIRTANEN and I. MATTILA, *Suomen Kemistilehti B* **34**, 44 (1961).
[48] T. SUZUKI, M. SUGII and T. KAKIMOTO, *Chem. Pharmacol. Bull.* **10**, 346 (1962).
[49] A. I. VIRTANEN, M. HATANAKA and M. BERLIN, *Suomen Kemistilehti B* **35**, 52 (1962).
[50] A. I. VIRTANEN and E. J. MATIKKALA, *Suomen Kemistilehti B* **35**, 245 (1962).
[51] E. J. MATIKKALA and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 2461 (1962).
[52] M. SUGII, S. NAGASAWA and T. SUZUKI, *Chem. Pharmacol. Bull. (Tokyo)*, In press.
[53] E. J. MATIKKALA and A. I. VIRTANEN, *Acta Chem. Scand.* **17**, 1799 (1963).
[54] A. SCHOBERL and D. WAGNER, *Melliand Textilber.* **41**, 984 (1960).

Suzuki *et al.*[55] consider that glutathione condenses with methacrylic acid or the coenzyme A derivative to form S-(2-carboxypropyl)glutathione in garlic. Methacrylic acid was found to be formed from valine.

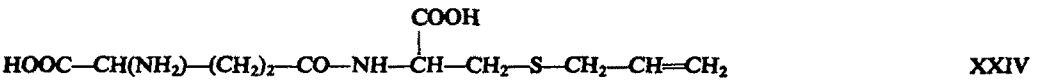
TABLE 3. FORMULAE OF THE SULPHUR CONTAINING γ-GLUTAMYLPEPTIDES ISOLATED FROM BULBS OF ONION, GARLIC AND SEEDS OF CHIVE



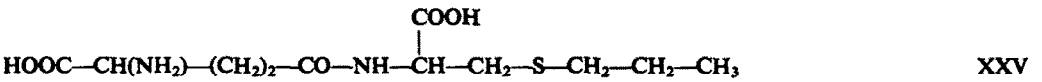
γ-L-Glu-(+)-S-(prop-1-enyl)-L-cysteine sulphoxide (onion) XXII



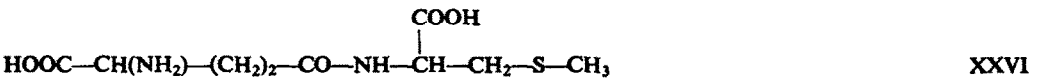
γ-L-Glu-S-(prop-1-enyl)-L-cystein (seeds of chive) XXIII



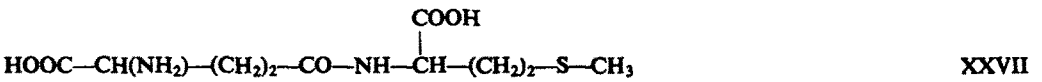
γ-L-Glu-(−)-S-allyl-L-cysteine (garlic) XXIV



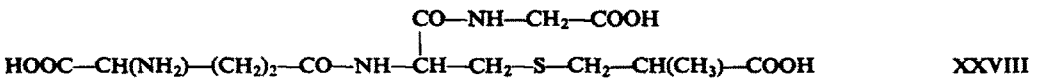
γ-L-Glu-S-propyl-cysteine (garlic) XXV



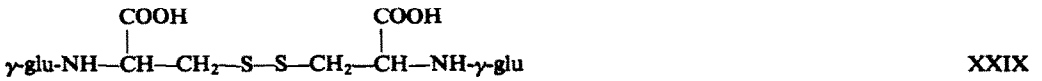
γ-L-Glu-S-methylcysteine (onion and garlic) XXVI



γ-L-Glu-L-methionine (onion and garlic) XXVII

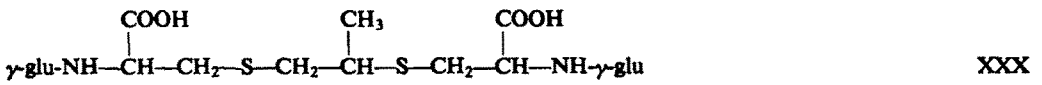


γ-L-Glu-S-(2-carboxypropyl)-L-cysteinyl-glycine (onion and garlic) S-(2-carboxypropyl)glutathione XXVIII



γ-glu-NH—CH(COOH)—CH$_2$—S—S—CH$_2$—CH(COOH)—NH-γ-glu XXIX

N,N′-bis(γ-glu)-cystine (seeds of chive)



γ-glu-NH—CH(COOH)—CH$_2$—S—CH$_2$—CH(CH$_3$)—S—CH$_2$—CH(COOH)—NH-γ-glu XXX

N,N′-bis(γ-glu)-3,3′-(2-methylethylene-1,2-dithio)-dialanine (seeds of chive)

The biosynthesis of the complicated bis-γ-glutamylpeptide, N,N′-bis(γ-glutamyl)-3,3′-(2-methylethylene-1,2-dithio)-dialanine isolated from the seeds of the chive[53] takes place probably analogically from γ-glu.-S-(prop-1-enyl)-cysteine[50] and γ-glu.-cysteine, both present in the seeds (Fig. 10). Peptide XXX could not be synthesized from XXIII and XXIX

[55] T. SUZUKI, M. SUGII and T. KAKIMOTO, *Chem. Pharmacol. Bull.* **10**, 328 (1962).

under the experimental conditions which prevailed when the peptide was isolated from the seeds. It is therefore obviously an original plant substance.

The significance of the γ-glutamylpeptides in the nitrogen metabolism of man and animals has not yet been elucidated. In *Allium* plants these peptides occur especially in the bulbs and seeds of the onion. We have been able to show experimentally that the peptides disappear when the green leaves grow from the onion bulb. The γ-glutamylpeptides are thus primarily reserve substances which actively take part in nitrogen metabolism in the beginning of the growth. They are, after all, also derivatives of glutamine which again represents both a reserve and an active substance in nitrogen metabolism. We are not yet aware of the way in which the γ-glutamylpeptides are used in the metabolic reactions in the onion bulbs. Enzymatic hydrolysis would seem to be the simplest, but it has not been proved with onion bulbs. Hanes *et al.*,[56] Hird and Springell[56a] have observed that kidney preparation causes a reaction

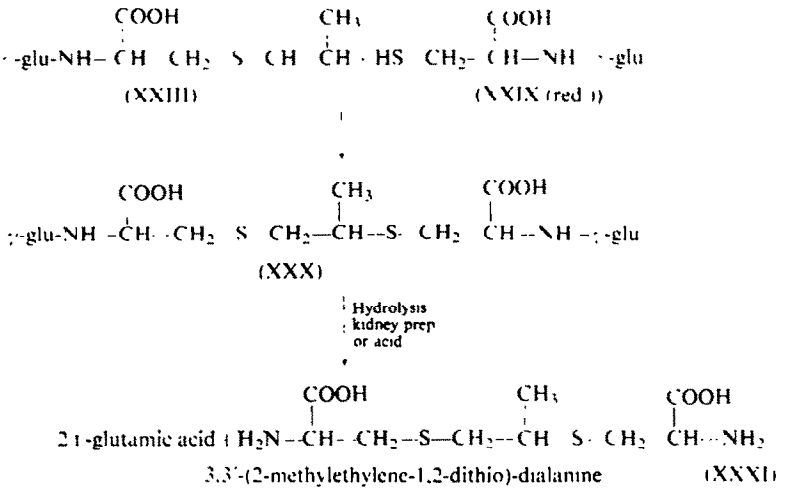


FIG. 10. PROBABLE BIOSYNTHESIS OF DIGLUTAMYLPEPTIDE XXX AND FORMATION OF NEW DIAMINO DICARBOXYLIC ACID XXXI OF DJENCOLIC ACID TYPE BY HYDROLYSIS OF XXX.

between glutathione and alanine as well as many other amino acids whereby the γ-glutamyl group is transferred to the free amino acid and cysteinyl-glycine is freed.

γ-L-glutamyl-L-cysteinyl-glycine + L-alanine
↓ γ-glutamyl-transferase
γ-L-glutamyl-L-alanine + cysteinyl-glycine

The experiments performed in our laboratory have shown that the same reaction takes place between the γ-glutamylpeptides isolated from onion and alanine in the presence of kidney homogenate. At the same time also the hydrolytic decomposition of the γ-glutamyl bond is completed without alanine. Onion leaves, dormant or sprouting bulbs, curiously, do not show any activity of γ-glutamyl transpeptidase nor of γ-glutamylpeptidase.[36,42] Thompson *et al.*[57] have recently found γ-glutamyl transpeptidase in many plants but con-

[56] C. S. HANES, F. J. HIRD and F. A. ISHERWOOD, *Biochem. J.* **51**, 25 (1952).
[56a] F. J. HIRD, and P. H. SPRINGELL, *Biochem. J.* **56**, 417 (1954).
[57] J. F. THOMPSON, D. H. TURNER and R. K. GERING, *Phytochem.* 3, 33 (1964).

firmed the absence of this enzyme in onion. It is therefore still unknown how the numerous γ-glutamylpeptides present in onion bulbs are used in nitrogen metabolism of onion plant. On the other hand, a homogenate from germinating seeds of *Allium* species hydrolyses glutamylpeptides strongly. The γ-peptidase can be readily concentrated using Sephadex filtration (Matikkala and Virtanen[36]).

Mustard oil glucosides from which isothiocynanic acid esters, the so-called mustard oils, are formed enzymatically, are characteristic of plants which belong to the family *Cruciferae* and some other families (e.g. *Tropaeolaceae*, *Resedaceae*). Many of these oils

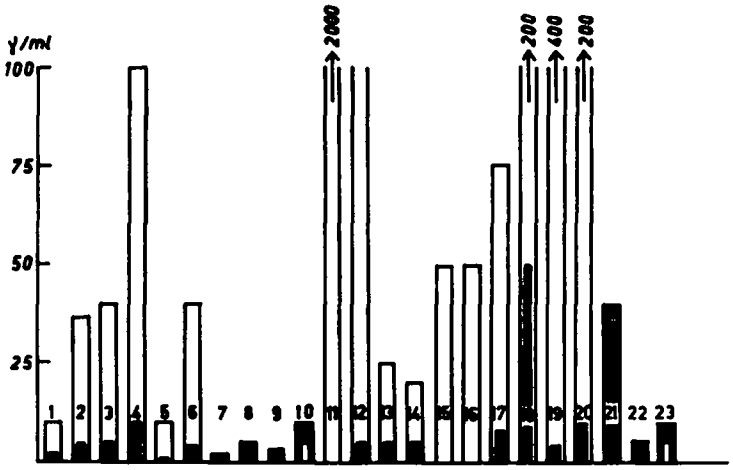


FIG. 11. LOWEST INHIBITION-CONCENTRATIONS (μg/ml) OF DIFFERENT MUSTARD OILS, SOME OF THEIR PRECURSORS, AND OTHER COMPOUNDS.

White area: Activity against *Staphylococcus aureus* 209 Innsbruck.

Black area: Activity against *Penicillium glaucum*.

Ordinate: Minimum concentration required for inhibition (μg/ml).

Isothiocyanates: 1. Methyl-, 2, Ethyl-, 3. Propyl-, 4. Isopropyl-, 5. Allyl-, 6. Phenyl-, 7. Benzyl-, 8. Ethylphenyl-, 9. *m*-Methoxybenzyl-, 10. *p*-Methoxybenzyl-, 11. *p*-Hydroxybenzyl- (Sinalbin), 12. Methylthiopropyl-, 13. Methylthiobutyl-, 14. Methylthiopentyl-, 15. Methylsulfinylpropyl- (Glucoiberin), 16. Methylsulfonyl-, 17. Methylsulfonylbutyl-.

Thiocyanates: 18. Isopropyl-, 19, Benzyl-, Ethylphenyl-.

Other sulphur compounds: 21. Dibenzylcarbothialdin, 22. Na-Benzyldithiocarbamate, 23. Benzylammoniumbenzyldithiocarbamate.

have a pungent odour, but at low concentrations a rather pleasant, appetite-stimulating flavour. Many mustard oils accelerate the blood circulation in the skin and irritate the mucous membranes. They also have an antimicrobial activity against moulds and some also against bacteria. The antimicrobial activity of some isothiocyanates according to the determinations in this laboratory (Saarivirta[58]) is shown in Fig. 11.

Benzyl isothiocyanate (compound 7, Fig. 11) is the most active of all the investigated mustard oils when both antibacterial and antifungal activity are taken into consideration. It inhibits the growth of *Staphylococcus* and *Penicillium glaucum* in a dilution of from 1:500,000 to 1:1,000,000. Its precursor-glucoside, glucotropaeolin is present in both garden

[58] A. I. VIRTANEN, *Angew. Chem. (Int. Ed.)* **1**, 303 (1962).

cress (*Lepidium sativum*) and Indian cress (*Tropaeolum majus*), both used as vegetables in Central Europe. According to the determinations of Winter.[59] 20–30 g fresh leaves of *Tropaeolum* or *Lepidium* eaten at one time would cause a complete inhibition of the growth of *Staphylococcus* and some strains of *Escherichia coli* in the urine for many hours.

After Ettlinger and Lundeen[60] had shown mustard oil glucosides to be hydroxylamine derivatives, they corrected the old structural formula of these compounds given by Cadamer to the following (Fig. 12).

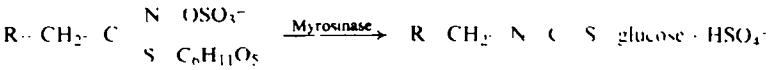


FIG. 12. ENZYMATIC CLEAVAGE OF MUSTARD OIL GLUCOSIDES.

The work of Ettlinger laid the basis for the understanding of the properties of mustard oil glucosides or, according to the new nomenclature of Ettlinger.[61] glucosinolates, especially their enzymatic cleavage which leads through a Lossen rearrangement to the formation of isothiocyanic acid esters, "mustard oils".

However, besides the characteristic isothiocyanates, organic cyanides or nitriles are formed in the cleavage when the pH of the medium is under 5. In some plants the nitrile formation takes place also in neutral and alkaline media. The reaction is in these cases enzymatic, and the cleavage proceeds hereby without Lossen rearrangement (see below). Additionally, thiocyanic acid esters R—CH_2—S—C≡N also are formed in some cases by enzymatic cleavage of mustard oil glucosides (Gmelin and Virtanen[62]). It was first believed that both isothiocyanate and thiocyanate are formed immediately after myrosinase had split off glucose from mustard oil glucosides, a special enzyme being needed for the formation of thiocyanate (Fig. 13).

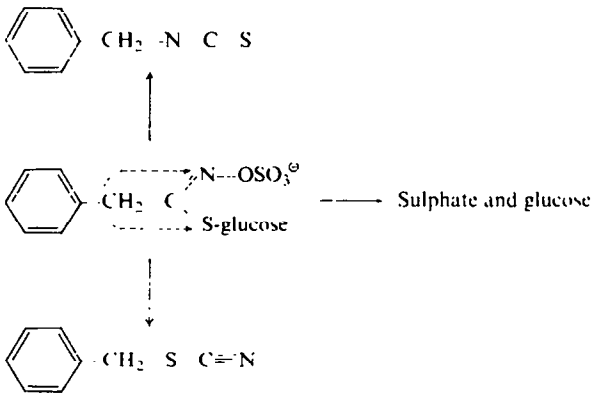


FIG. 13. THE FIRST HYPOTHESIS OF THE FORMATION OF BENZYLISOTHIOCYANATE (BITC) AND BENZYLTHIOCYANATE (BTC) FROM BENZYL MUSTARD OIL GLUCOSIDE (GLUCOTROPAEOLIN IN MOISTENED SEEDPOWDER OF *Lepidium sativum*.

[59] A. G. WINTER, *Hippokrates* **28**, 645 (1957).
[60] M. G. ETTLINGER and A. J. LUNDEEN, *J. Am. Chem. Soc.* **78**, 4172 (1956).
[61] M. G. ETTLINGER, G. P. DATEO, B. W. HARRISON, T. J. MABRY and C. P. THOMPSON, *Proc. Nat. Acad. Sci. U.S.* **47** (1961).
[62] R. GMELIN and A. I. VIRTANEN, *Acta Chem. Scand.* **13**, 1474 (1959).

Saarivirta and Virtanen[63, 64, 65] continued to investigate the formation of thiocyanates more closely especially in moistened seedpowder of *Lepidium sativum* and *Tropaeolum majus*, which contain the same mustard oil glucoside, glucotropaeolin (benzylthioglucoside). In the former benzylisothiocyanate, benzylthiocyanate and benzylcyanide are all formed, whereas in the latter only benzylisothiocyanate is produced. The reaction rate, even near 0°, is very high. After 30 sec about 2/3 of the glucoside was already degradated. The ratio of benzylthiocyanate to benzylisothiocyanate was decreased when the temperature was increased from 2° to 37° (reaction time 5 min). Accordingly, a low temperature favours thiocyanate and a higher temperature isothiocyanate formation (Fig. 14).

When the rate of the formation of the different reaction products in moistened seedpowder of *Lepidium sativum* was studied results presented in Fig. 15 were obtained.

From the curves in Fig. 15 it appears that in the moistened seedpowder of *Lepidium*: 1. benzylcyanide and benzylisothiocyanate are formed in the beginning roughly at the

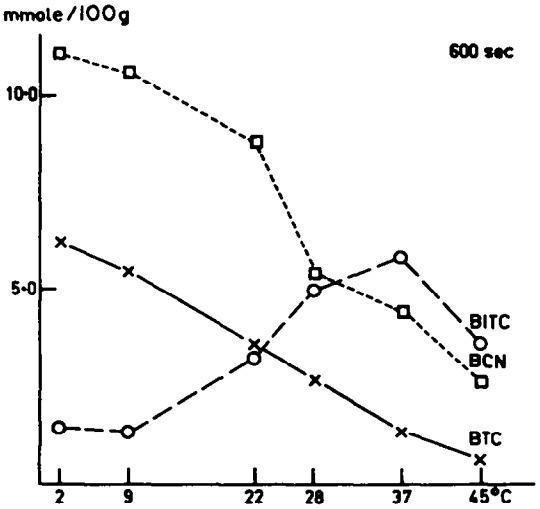


FIG. 14. FORMATION OF BENZYLCYANIDE BCN, BENZYLISOTHIOCYANATE BITC and BTC BENZYLTHIOCYANATE AT DIFFERENT TEMPERATURES IN MOISTENED SEEDPOWDER OF *Lepidium sativum*.

same high rate; 2. benzylthiocyanate is formed at lower rate; 3. benzylisothiocyanate disappears at nearly the same rate as benzylthiocyanate is formed.

The first hypothesis (Fig. 13) according to which isothiocyanate and thiocyanate are formed from the same intermediate after enzymatic splitting of the glucoside, was not in agreement with the finding in point 3. The result is far more in favour of the idea that benzylthiocyanate is formed from benzylisothiocyanate through rearrangement (Fig. 16). The latter is so stable at low temperatures that it cannot be spontaneously decomposed during some minutes so that the decrease of it after 30 sec is strong evidence for this hypothesis. From added benzylisothiocyanate, it is true, no benzylthiocyanate is formed in moistened seedpowder, but it is possible that the practically insoluble isothiocyanate does not come into contact with the enzyme. Further experiments are still needed to clarify the mechanism of thiocyanate formation.

[63] A. I. VIRTANEN and M. SAARIVIRTA, *Suomen Kemistilehti B* 35, 102, 248 (1962).
[64] M. SAARIVIRTA and A. I. VIRTANEN, *Acta Chem. Scand.* (*Suppl.*) 17, 74 (1963).
[65] A. I. VIRTANEN, *Arch. Biochem. Biophys.* (*Suppl.*) 1, 200 (1962).

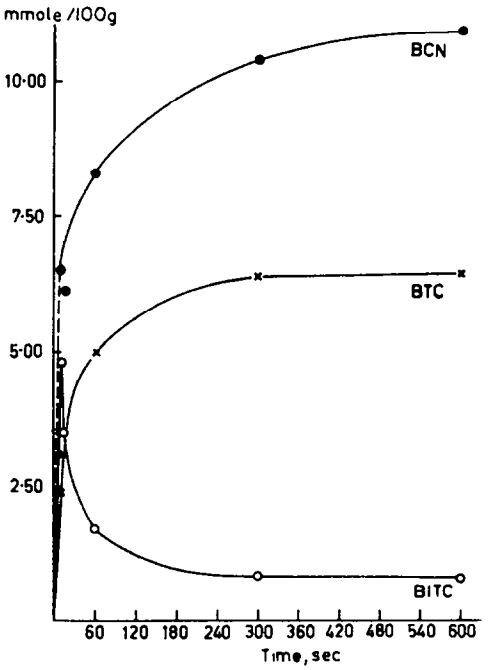


FIG. 15. FORMATION OF BENZYLCYANIDE (BCN), BENZYLISOTHIOCYANATE (BITC) AND BENZYLTHIOCYANATE (BTC) IN MOISTENED SEEDPOWDER OF *Lepidium sativum*.
Reaction temperature about +1°C.

As the formation of benzylthiocyanate does not occur in crushed seeds of *Tropaeolum*, and as also in the seedpowder of *Lepidium* inactivated by heating only benzylisothiocyanate is formed after addition of myrosinase (a raw preparation from the seeds of *Sinapis alba*), the formation of benzylthiocyanate is enzymatic. It has not been possible for the present to

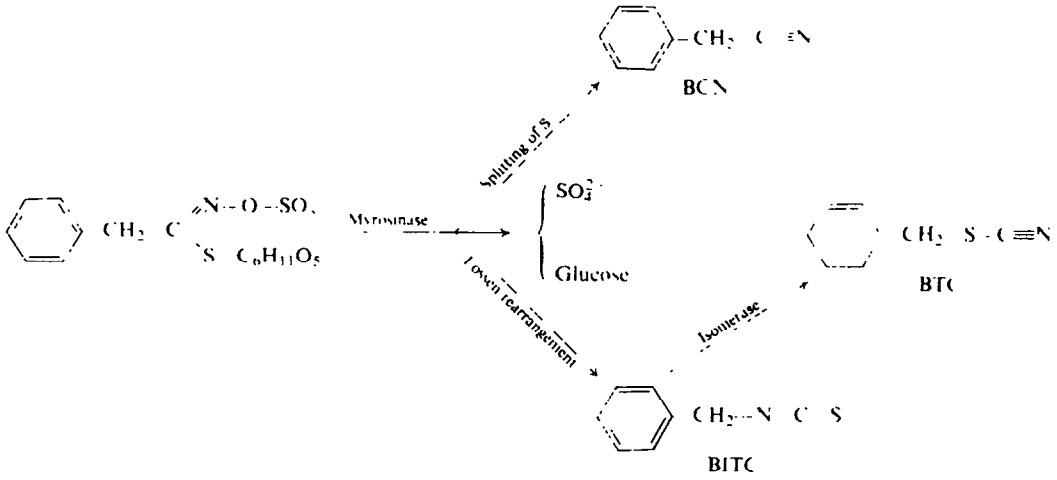


FIG. 16. ENZYMIC SPLITTING OF BENZYL MUSTARD OIL GLUCOSIDE IN MOISTENED SEEDPOWDER OF *Lepidium sativum*.

extract the enzyme from crushed seeds of *Lepidium* into solution. The nature of the enzyme is therefore still unknown. It is probably connected with an energy-giving system. No benzylthiocyanate is formed in the green leaves of *Lepidium sativum* although it is in those of *Lepidium ruderale*.

On the basis of our present experimental evidence the formation of benzylcyanide in the moistened seedpowder of *Lepidium* must also be considered as enzymatic, for this reaction does not occur in crushed seeds of *Tropaeolum* in the wide pH-range 3·6–8·6, in which the experiments have been performed, while benzylcyanide is formed in the seedpowder of *Lepidium* in the whole pH-range, i.e. also in an alkaline medium. The non-enzymatic mode of formation which takes place only in an acid medium from some glucosinolates, for instance glucobrassicin and sinigrin, cannot, therefore account for the formation of BCN in *Lepidium*. Ettlinger *et al.*[61] have investigated more closely the non-enzymatic formation of allyl cyanide from sinigrin.

When eating garden cress as a salad, man receives nearly the same amount of benzylcyanide as benzylisothiocyanate, a fact which was not known earlier.[36] The lethal dose of benzylcyanide for the mouse is 4–5 mg. Man consumes in 30 g garden cress about 10 mg benzylcyanide, an amount which has not yet any provable effect on him.

Thiocyanic acid ester is formed in crushed, moistened seeds not only of *Lepidium* (benzylthiocyanate) but according to Gmelin and Virtanen[62] also of some other crucifers which contain sinigrin allyl glucosinolate.

Cabbage species contain several different mustard oil glucosides. The antimicrobial effect of the cabbage is, however, weak and may be caused by many different substances which are formed enzymatically. Morris and Thompson,[66] Synge and Wood[67] have isolated S-methyl cysteine sulphoxide from cabbage and the latter authors have shown its presence in many other species of crucifer. The content of this compound in cabbage is appreciable[67] and, if methylthiosulphinate were formed from it, as in onion, one could expect a higher antimicrobial activity in crushed cabbage. In accordance with this low activity, the enzyme which splits alkyl cystein sulphoxides was not found in *Cruciferae* plants.[67a] Recently, however, Mazelis[68] has demonstrated the reaction in acetone powder extracts of *Brassica* species but only in alkaline medium the optimum being at pH 10. At pH 7 the activity is negligible. Using gas chromatographical method many authors have found in crushed cabbage besides at least four mustard oils, different sulphides, disulphides and even dimethyltrisulphide. Dimethyl-, dipropyl- and diallyl-sulphide are the same substances which are also formed in crushed *Allium* species as secondary products from thiosulphinates, and therefore it is possible that cabbage contains besides S-methyl sulphoxide also small amounts of S-propyl- and S-allyl sulphoxide which, however, have not yet been isolated from cabbage. Kjaer[69, 69a] and his collaborators have isolated and characterized a great number of new mustard oil glucosides from plants of different families and enlarged our insight into this group of plant substances tremendously. It seems that the radical in these glucosides can belong to very different chemical groups.

In our laboratory a new type of mustard oil glucoside, glucobrassicin, which is the first

[66] C. J. Morris and J. F. Thompson, *Chem. & Ind. (Lond.)* 951 (1955).
[67] R. L. Synge and J. C. Wood, *Biochem. J.* **60**, xv (1955); **64**, 252 (1956).
[67a] M. Fujiwara, M. Yoshimura, S. Tsuno and F. Murakami, *J. Biochem., Tokyo* **45**, 141 (1958).
[68] M. Mazelis, *Phytochem.* **2**, 15 (1963).
[69] A. Kjaer, *Organic Sulphur Compounds* (Edited by N. Kharasch) Vol. I, p. 409, Pergamon Press, Oxford (1961).
[69a] A. Kjaer, *Pure Appl. Chem.* **7**, 229 (1963).

indole glucosinolate has drawn special attention because of its important chemical and physiological properties. Gmelin, Saarivirta and Virtanen[70] reported the isolation of this compound as a crystalline tetramethyl ammonium salt for the first time four years ago. Its isolation resulted from our search for the precursor of thiocyanate ion (SCN^-), which is rapidly formed when *Brassica* plants are crushed. Since SCN^- has a goitrogenic effect the problem concerning the antithyroid substances in plants and their eventual transfer to milk has been investigated intensively, and from many sides in our laboratory.[71] The formation of SCN^- in *Brassica* plants has aroused special interest. Langer and Michajlovskij[72] found considerable amounts of SCN^- in cabbage and believed it to be an original component of cabbage. Glucobrassicin, which can be as much as 3 per cent of the dry matter of cabbage

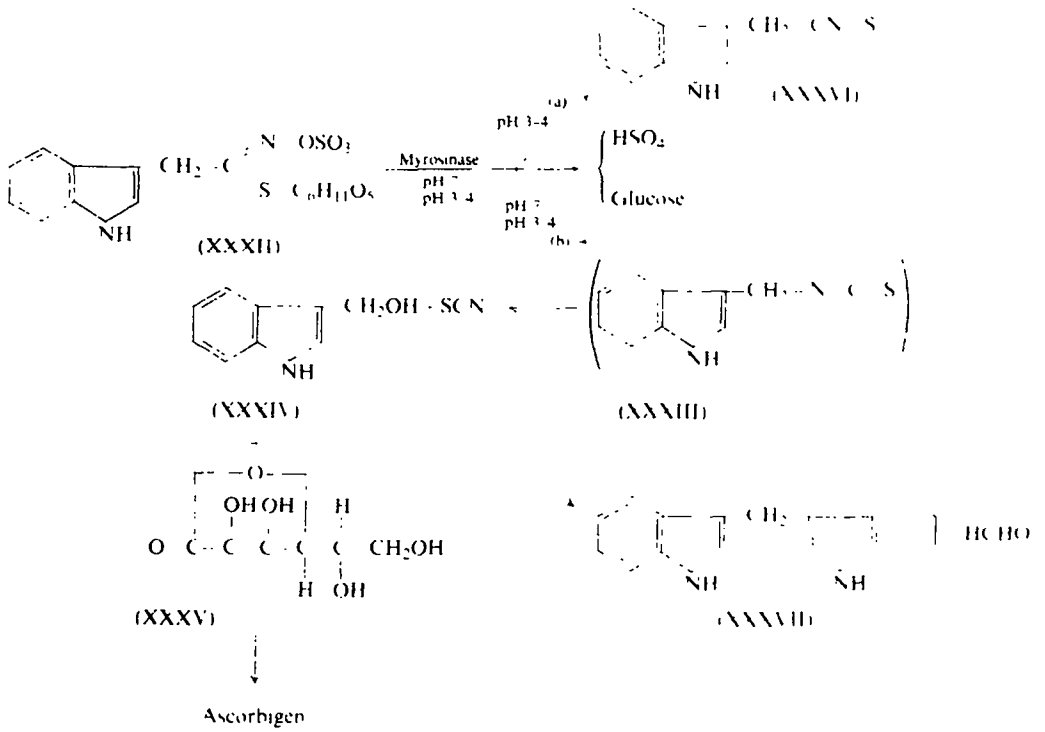


FIG. 17. PRODUCTS FORMED BY THE ENZYMATIC DEGRADATION OF GLUCOBRASSICIN (XXXII).

(Savoy variety), proved to be the precursor of SCN^-, and simultaneously also of some other physiologically important substances. The structure and decomposition products of glucobrassicin (Gmelin and Virtanen)[73] are shown in Fig. 17. SCN^- is formed from glucobrassicin not only enzymatically but also in boiling water; the yield, however, is only 50 per cent of that formed enzymatically.

As is seen from Fig. 17, indole compounds are normal enzymatic splitting products of glucobrassicin. Besides 3,3'-diindolymethane, some other indole compounds, at present

[70] R. GMELIN, M. SAARIVIRTA and A. I. VIRTANEN, *Suomen Kemistilehti B* **33**, 172 (1960).
[71] A. I. VIRTANEN, *Final Report on Investigations on the Alleged Goitrogenic Properties of Milk* pp. 1–226. A collection of papers from Biochemical Institute, Helsinki (1963).
[72] P. LANGER and N. MICHAJLOVSKIJ, *Z. Physiol. Chem.* **312**, 31 (1958).
[73] R. GMELIN and A. I. VIRTANEN, *Ann. Acad. Sci. Fennicae. A II* No. 107 (1961).

characterized, are formed in small amounts from 3-hydroxymethylindole. The formation of indolylacetonitrile (XXXVI) in an acid medium (Fig. 17) explains the mystery of the so-called "bound growth substance" in cabbage. Because the nitrile is formed as a split product of glucobrassicin the strong auxin effect found in acid extracts of cabbage is not derived from "freed" growth hormons. The hypothesis that glucobrassicin functions in *Brassica* plants as "a growth factor-reserve-pool" will remain unproven until its enzymatic splitting in the growing, undamaged plant is verified.

The "bound ascorbic acid" (ascorbigen) which Procházka *et al.*[74] isolated from cabbage and characterized as an indole compound was shown to be formed spontaneously from ascorbic acid and 3-hydroxymethylindole (XXXIV), the split product of glucobrassicin. The pH-optimum of the synthesis is about 4.[74a]

The glucobrassicin content in cabbage and in other *Brassica* plants can be calculated from the amounts of SCN^- formed in the crushed plant. N_1-Methoxy-glucobrassicin,[75] which was isolated from rutabaga and shown to be present in small amounts even in some other *Brassica* species, is also determined at the same time.

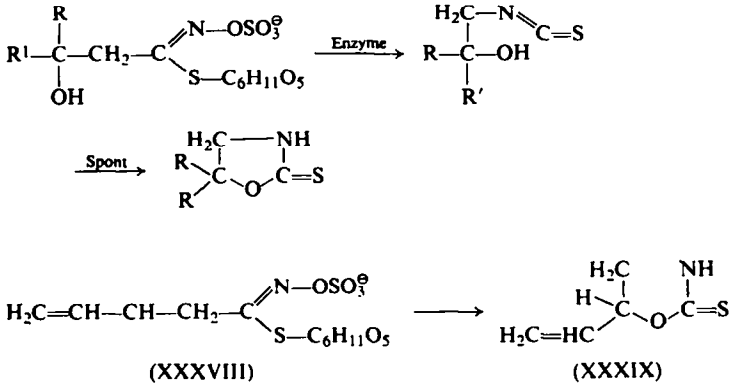


FIG. 18. FORMATION OF THIOOXAZOLIDONES FROM MUSTARD OIL GLUCOSIDES AND OF GOITRIN (XXXIX) FROM PROGOITRIN (XXXVIII)

SCN^- is transferred in such a small amount to cows milk that the amount of thiocyanate ingested from it has no significance in the formation of goitre.[76] In any case cabbage goitre can be prevented by raising the iodine intake.

This is, on the contrary, not the case with *Cruciferae* seed goitre which is caused by L-5-vinyl-2-thiooxazolidone, goitrin (Fig. 18).

Astwood, Greer and Ettlinger[77] isolated this substance which prevents the biosynthesis of thyroid hormones, from crushed moistened *Brassica* seeds. The formation of this factor was not observed in cabbage. Since the development of a quantitative method for the determination of goitrin by Kreula and Kiesvaara,[78] however, it was possible to detect the formation of goitrin and to estimate it quantitatively in crushed cabbage also. In the autumn the amount of goitrin is significant, and can possibly be a factor in the formation of goitre in areas

[74] Z. PROCHÁZKA, V. ŠANDA and F. ŠORM, *Coll. Czech. Comm.* **22**, 654 (1957).
[74a] E. PIIRONEN and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 1286 (1962).
[75] R. GMELIN and A. I. VIRTANEN, *Acta Chem. Scand.* **16**, 1378 (1962).
[76] A. I. VIRTANEN and R. GMELIN, *Acta Chem. Scand.* **14**, 941 (1960).
[77] E. B. ASTWOOD, M. A. GREER and M. G. ETTLINGER, *J. Biol. Chem.* **181**, 121 (1949).
[78] M. KREULA and M. KIESVAARA, *Acta Chem. Scand.* **13**, 1375 (1959).

where several kilos of cabbage are consumed per person per day. Goitron is transferred in such small quantities to milk, at most 0·05 of the amount fed, that it has no significance as a goitrogenic factor in milk. It has not been possible in our experiments with cows to produce goitrogenic milk using any feeding stuff. These experiments were performed to test the claim of some workers of the goitrogenic properties of milk derived from the feeding of cruciferous plants. I refer in this connexion to papers from our laboratory.[71]

The precursor glucoside of goitrin has a hydroxyl group at C-atom 2. The cyclization of this type of mustard oils is a general reaction. The glucoside precursor of goitrin was isolated independently by several investigators (Greer,[79] Kjaer *et al.*.[80] Schultz and Wagner[81]).

The examples of the occurrence of sulphur-containing amino acids, γ-glutamylpeptides, thioglucosides and sulphur-free glucosides in some vegetables and fodder plants as precursors of physiologically effective substances presented in this review strongly emphasize how defective our knowledge of the chemical composition, even of common food plants, still is. Otherwise it is not understandable that, from such generally used vegetables as cabbage and onion, numbers of new substances, some of which are present in amounts as great as a few per cent of the dry matter, could be isolated. The finding that from the same substrate different products can be formed enzymatically in different plant species also demonstrates peculiar differences in enzyme systems. Plant chemistry has a tremendous task ahead to widen our knowledge of this field.

This research has been financed in part by a grant made by the *United States Department of Agriculture, Agricultural Research Service.*

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[80] A. KJAER, R. GMELIN and R. BOE JENSEN, *Acta Chem. Scand.* **10**, 432 (1956).
[81] O. E. SCHULTZ and W. WAGNER, *Arch. Pharm.* **289/61**, 597 (1956).