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ORGANIC THIOCYANATES - GLUCOSINOLATE ENZYMATIC DEGRADATION PRODUCTS OR ARTEFACTS OF THE ISOLATION PROCEDURE?†

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Abstract. *Glucosinolates are abundant in plants of the order Brassicales, and they are degraded by myrosinases into various organic breakdown products: isothiocyanates, thiocyanates, nitriles, etc., depending on their structure, conditions of hydrolysis, the presence of certain protein cofactors. Their most common hydrolysis products are isothiocyanates, while simple nitriles, epithionitriles, and thiocyanates are produced occasionally. Organic thiocyanates are described from a very limited number of Brassicales taxa. Up to now benzyl, (4-hydroxyphenyl)methyl, (4-methoxyphenyl)methyl, 4-methylthiobutyl, and allyl thiocyanates were reported as products of glucosinolates autolysis. The present review summarizes the knowledge on the mechanism of organic thiocyanate formation from the corresponding thioglucosides. The enzymatic formation of organic thiocyanates is believed to be enabled by thiocyanate-forming protein (TFP), but they could be formed* via *metabolic routes that do not involve TFP. All of the reported thiocyanates are produced from stable (carbo)cationic species that allow an isomerization of an isothiocyanate to thiocyanate, and* vice versa*. Although the possibility that thiocyanates can be biosynthesized in plats under certain conditions cannot be dismissed, allyl thiocyanate can be a thermal isomerization artefact of the original isothiocyanate that is formed in the heated zones of the gas chromatograph, while other thiocyanates could form in an aqueous medium* via *heterolytic dissociation to ambident nucleophilic SCN^- and its recapture. One should always be aware of this analytical shortcoming when concluding on the presence and quantity of these specific (iso)thiocyanantes in the analyzed sample*.

Key words: *organic thiocyanate, glucosinolate, thiocyanate-forming protein, isomerization*

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1. INTRODUCTION

Glucosinolates constitute a group of specialized plant metabolites with a characteristic structure and biochemistry. They are sulfur-rich, anionic natural products that, upon hydrolysis by endogenous thioglucosidases, produce several different products (e.g. isothiocyanates, thiocyanates, nitriles). These thioglucosidases, known as myrosinases, co-occur with glucosinolates but are stored separately from glucosinolates in intact plants (Fahey et al. 2001). Upon tissue damage, for example by herbivores, myrosinases come into contact with the glucosinolates and catalyze the formation of the corresponding aglycones, transient species that usually rearrange spontaneously into isothiocyanates. Most glucosinolate-containing genera are clustered within the order Brassicales (or Cruciales). There is now voluminous literature on the glucosinolates of the plant family Brassicaceae (or Cruciferae), which contains more than 3000 species (Al-Shehbaz et al., 2006). Of the many hundreds of cruciferous species investigated, all can synthesize glucosinolates (Kjaer and Olesen, 1976). Curiously, glucosinolates are also known from the genus *Drypetes* of the family Euphorbiaceae, a genus completely unrelated to the other glucosinolate-containing families (Montaut et al., 2017).

The hydrolysis of glucosinolates impacts the taste, flavor, and nutritional value of spices and vegetables derived from plants belonging to the Brassicaceae family, such as mustard, horseradish, cabbage, cauliflower, and broccoli. From a plant perspective, this process is crucial for the function of glucosinolates as chemical plant defenses (Fahey et al., 2001). The glucosinolate hydrolysis products have many different biological activities and are of importance to humans, as well (Halkier and Gershenzon, 2006). In the past decade, many of them have been identified as potent cancer-preventing agents in a wide range of animal models (Conaway et al., 2002). Some of the glucosinolate degradation products have shown significant antibacterial, antinematode, and antifungal properties (Chew, 1988).

The most common glucosinolate autolysis products are isothiocyanates which exhibit marked biological activities in a number of models and are toxic to a variety of organisms, including microorganisms, nematodes, and insects (Chew, 1988; Louda and Mole, 1991; Rask et al. 2000). In addition to isothiocyanates, other hydrolysis products are formed upon tissue damage, including simple nitriles, epithionitriles, and organic thiocyanates (Fahey et al., 2001). The formation of these alternative products depends on the side-chain structure of the parent glucosinolate and it is established that for some of them the presence of specific plant proteins diverts glucosinolate hydrolysis away from isothiocyanates. Proteins that promote simple nitrile and epithionitrile formation have been described (Hasapis and MacLeod, 1982). Three proteins responsible for the formation of nitriles have been identified on a molecular level, namely the epithiospecifier proteins (ESPs) from *Arabidopsis thaliana*, *Brassica napus* var. *oleifera*, and *Brassica oleracea* ssp. *italica*. ESPs all promote the formation of epithionitriles from alkenylglucosinolates, such as allyl glucosinolate, in the presence of myrosinase, without having hydrolytic activity on their own (Burow et al. 2007). Also, the ESPs from *A. thaliana* and *B. oleracea* promote the generation of simple nitriles from aliphatic glucosinolates with saturated side-chains, such as 4-methylsulfinylbutyl glucosinolate (Lambrix et al., 2001; Matusheski et al., 2006).

Little is known about protein factors involved in organic thiocyanate formation. Organic thiocyanates could be formed in a non-enzymatic way, as well. Only a limited number of thiocyanates are known to be breakdown products from glucosinolates (the

ones that form stable cations). This suggests that the transformation in question might not be enzymatic due to this particular selectivity. Correspondingly, there is a viewpoint that isothiocyanates are transformed into thiocyanates during the glucosinolate breakdown process. This isomerization is well-known for certain substrates under specific conditions and will be herein addressed, as well. Thus, in this short review, we wish to summarize the knowledge regarding the formation of organic thiocyanates as products of glucosinolates and draw attention to the possibility that these organic thiocyanates might form artefactually.

2. GLUCOSINOLATE CHEMISTRY

2.1. Glucosinolate structure

The approximately 130 described glucosinolates share a chemical structure consisting of a *β*-D-glucopyranose residue linked *via* a sulfur atom to a (*Z*)-*N*-hydroximinosulfate ester, plus a variable R group (Fig. 1, Table 1), derived from amino acids (Blažević et al., 2020). Glucosinolates could be grouped into several chemical classes based on structural similarities. The most extensively studied glucosinolates are the aliphatic,*ω*-methylthioalkyl, aromatic, and heterocyclic (e.g. indole) glucosinolates (Fahey et al., 2001).

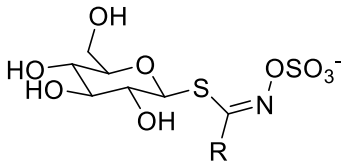


Fig. 1 Chemical structure of glucosinolates (R=variable chain derived from amino acids)

2.2. Glucosinolates degradation

Glucosinolates undergo enzymatic decomposition (Fig. 2) to give a variety of hydrolysis products (isothiocyanates, oxazolidine-2-thiones, thiocyanates, nitriles, epithionitriles), the composition of which depends on pH, metal ion presence, and other protein elements (Bones and Rossiter, 1996). The process begins with myrosinase-catalyzed hydrolysis of the thioglucoside linkage, leading to the formation of glucose and an unstable aglycone.

Table 1 Chemical and common names of selected glucosinolates

Chemical name (side-chain structure)	Common Name
Ethyl	Glucolepidiin
Allyl	Sinigrin
Benzyl	Glucotropaeolin
1*H*-Indol-3-ylmethyl	Glucobrassicin
2(*R*)-2-Hydroxy-3-butenyl	Progoitrin
2(*S*)-2-Hydroxy-3-butenyl	Epiprogoitrin
4-Methylsulfinyl-3-butenyl	Glucoraphenin
3-(Methylsulfinyl)butyl	Glucoraphanin
4-(Methylsulfanyl)butyl	Glucoerucin
4-(Methylsulfonyl)butyl	Glucoerysolin

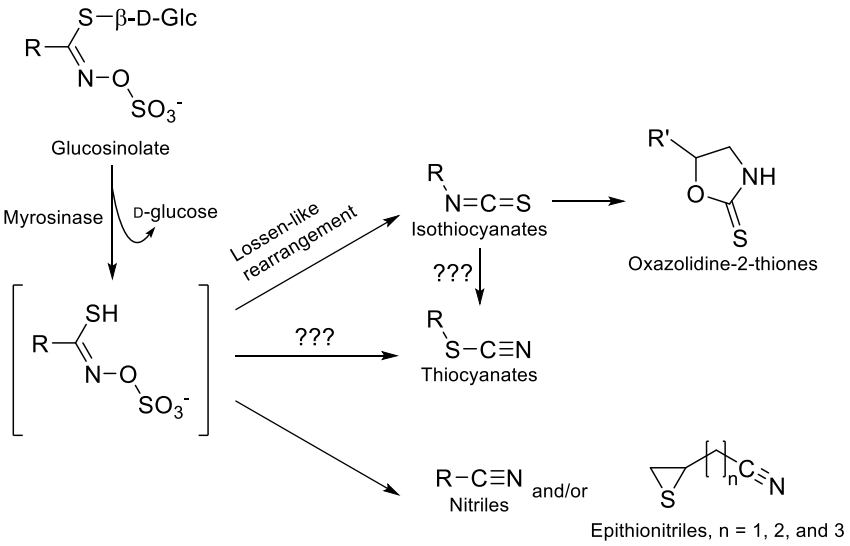


Fig. 2 General diagram showing the main products of enzymatic glucosinolate degradation

2.2.1. Catalytic mechanism of myrosinase activity

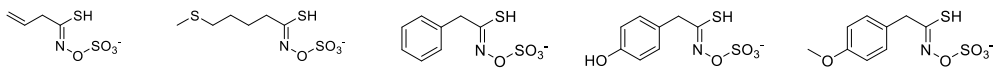
The most common glucosinolate hydrolysis products in many species are isothiocyanates, which are formed from the aglycone by a Lossen-like rearrangement involving the *anti*-stereospecific migration of the side-chain from the oxime carbon to the adjacent nitrogen. Although the most common ones, isothiocyanates are not the only important hydrolysis products. Apart from them, plants produce nitriles and, in certain circumstances, some glucosinolates are capable of forming thiocyanates as well. One of the first reports on the occurrence of glucosinolate hydrolysis products other than isothiocyanates was published in 1959 when benzyl thiocyanate and allyl thiocyanate were identified as the substances that are responsible for the garlic-like odor of *Lepidium ruderale, L. sativum*, and *Thlaspi arvense* (Brassicaceae) (Gmelin and Virtanen, 1959). The compounds were shown to be derived from myrosinase-catalyzed hydrolysis of benzylglucosinolate and allylglucosinolate and suggested to be formed under the influence of an additional protein. In 1961, nitriles were demonstrated to be formed spontaneously when glucosinolates were hydrolyzed by myrosinase at pH<5 (Ettlinger et al., 1961). Only a few years later, evidence was provided that benzyl cyanide (phenylacetonitrile), as well as benzyl thiocyanate, can be formed from benzylglucosinolate after myrosinase-catalyzed hydrolysis at a broader pH range (pH 3.6–8.6) under the influence of a so-far-unknown protein factor or enzyme (Virtanen, 1965).

The formation of an epithionitrile was first discovered in the seed meal of *Crambe abyssinica* (Brassicaceae) but subsequently found in several other species containing alkenylglucosinolates with a terminal double bond (Daxenbichler et al., 1968; Kirk and MacDonald, 1974; Cole, 1976).

2.3. Thiocyanate formation

2.3.1. Thiocynate-forming protein

While the formation of simple nitriles and epithionitriles is widespread within glucosinolate-containing plants, organic thiocyanates are only generated in a very few plant species including *Lepidium ruderale*, *L. sativum*, and *Thlaspi arvense* (Brassicaceae) (Gmelin and Virtanen, 1959). Furthermore, only three types of glucosinolates: allylglucosinolate (**1**), 4-methylthiobutylglucosinolate (**2**), and benzylglucosinolates (**3-5**, Fig. 3), have been reported to form organic thiocyanates. As a common structural feature of these glucosinolates, the ability of their side-chains to form stable (carbo)cations, or more precisely anchimeric assistance, has been suggested as a prerequisite for thiocyanate formation (Luthy and Benn, 1977). After the initial attempts to purify the thiocyanate-forming factor from plant extracts failed (Gil and MacLeod, 1980), the first representative of these proteins, thiocyanate-forming protein (TFP) from *L. sativum*, was identified using a molecular approach some 13 years ago (Burow et al., 2007). The most intriguing feature of the *L. sativum* TFP that Burow and co-workers showed is its substrate and product specificity and the fact that thiocyanate formation by TFP is promoted by the presence of iron. However, the effect of both ferrous and ferric ions in crude preparations of *L. sativum* fruits is most pronounced for nitrile formation while thiocyanate formation is reduced at higher iron levels (Hasapis and MacLeod, 1982).



1 **2** **3** **4** **5**

Fig. 3 Chemical structures of glucosinolate aglycone anions which have been reported to form organic thiocyanates upon hydrolysis. **1**: allyl-; **2**: 4-methylthiobutyl-; **3**: benzyl-; **4**: 4-hydroxybenzyl-; **5**: 4-methoxybenzyl glucosinolate residues

2.3.2. Alternative ways of thiocyanate formation

The first theory of thiocyanate formation was proposed by Gmelin and Virtanen (Gmelin and Virtanen, 1959) who suggested an enzyme-induced rearrangement of the glucosinolate itself, but all attempts to isolate this enzyme merely resulted in a preparation with thioglucosidase activity. Later, Saarivirta and Virtanen (1963) proposed that an isomerase acted on the initially formed isothiocyanate and although this was a popular idea for some time, they later abandoned it on the basis of unsuccessful model system experiments. Miller (1965) showed that a model aglycone of allylglucosinolate was converted to allyl thiocyanate by extracts of *Thlaspi arvense* fruits and this led to the suggestion that there was a thiocyanate-forming enzyme that acted on the aglycone (Fig. 2) rather than on the glucosinolate itself or the isothiocyanate. This seems a more reasonable suggestion, but for a long time, all attempts to isolate this or any other, thiocyanate-forming enzyme had failed. It was proposed that the unknown enzyme is very labile, unlike the robust thioglucosidase. An alternative to the above enzyme theories, before the discovery of the first TFP (Bones and Rossiter, 1996), is that thiocyanates are formed by a purely chemical mechanism (but possibly from the aglycone), and this could explain why only a few glucosinolates undergo this

reaction. A common feature of the three glucosinolate types concerned is that they all yield stable cations, R (Fig. l), and this has led to the suggestion of thiocyanate formation *via* an ion-pair mechanism, which would also involve the bidentate nature of the isothiocyanate/ thiocyanate ion. This is an attractive theory, but efforts to substantiate it have not been successful. Thus, there are two broad theories of thiocyanate production, one based on an enzyme or other biological 'factor', and one based on a specific structural feature of thiocyanate-forming glucosinolates. It is relevant to consider some other evidence to evaluate these theories further. Many studies of thiocyanate formation have been based on benzylglucosinolate in various *Lepidium* species. Numerous reports describe the identification of benzyl thiocyanate in extracts of the fruits of *L. sativum*, but it has never been located in extracts of the green leaves of this plant (Burow et al., 2007). On the other hand, it has been readily identified in extracts of both fruits and leaves of *L. ruderale*, *L. virginicum* (Gmelin and Viratanen, 1959), and also *Coronopus didymus* (Wittstock and Burow, 2010). These data provide strong evidence in favor of an enzymatic theory of thiocyanate formation since it would be expected that if the structural feature of glucosinolates was the sole requirement, then benzyl thiocyanate would always be produced, regardless of the plant system or part thereof. The enzyme or biological factor presumably must be absent from *L. sativum* leaves, but present in the fruits, although an inhibitor in the leaves could be the cause of this behavior. Gil and MacLeod (1980) succeeded in obtaining a crude enzyme preparation from *L. sativum* fruits which contained a thiocyanate-forming factor as well as a thioglucosidase, since it converted pure benzylglucosinolate into both thiocyanate and isothiocyanate (as well as nitrile). However, this preparation produced only isothiocyanate and nitrile from pure 2-phenethylglucosinolate. These results, therefore, indicate that there is also a structural requirement within the glucosinolate for thiocyanate formation. Some *in vivo* work support these findings (Gil and MacLeod, 1980). We believe that a combined theory is necessary for the mechanism of thiocyanate formation from glucosinolates, in which an enzyme or some other biological factor is required, but which functions only with glucosinolates that possess specific structural properties (i.e. those capable of forming stable cations).

2.3.2.1. Thiocyanates from isothiocyanates

As indicated previously, the thiocyanate ion possesses ambivalent reactivity, as it can be nucleophilic at its sulfur or its nitrogen extremity. This specificity was used to convert organic thiocyanates into isothiocyanates through simple molecular rearrangements. Allylic thiocyanates are known to easily undergo [3,3]-sigmatropic rearrangement to the corresponding allylic isothiocyanates (Fig. 3). This reaction is also reported as the Billeter–Gerlich rearrangement (Castanheiro et al., 2016).

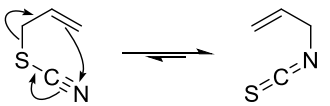


Fig. 4. Isothiocyanate to thiocyanate (and *vice versa*?) isomerization: Billeter–Gerlich rearrangement

The rearrangement of isothiocyanates to thiocyanates and possibly *vice versa* may generally occur by way of several mechanisms with sufficient likelihood according to the nature of the organic residue R (Drobnica et al., 1977). The rearrangement passes through

a cyclic intramolecular transition state, involving an allylic shift, which is especially characteristic for allylic isothiocyanates and thiocyanates isomerization in organic solvents (Smith and Emerson, 1960). In addition to the intramolecular path, there are other routes of isothiocyanate rearrangement. One such route is ionization and recombination of ions. Finally, the analogous homolytic cleavage into alkyl and thiocyanogen radicals followed by their recombination may lead to the same product. The rate of rearrangement of allylic thiocyanates, which is a first-order process, does not significantly depend on solvent polarity. The effects of substituents, solvent, and salt effects on the rate of the reaction seem to indicate that the rearrangement of allylic thiocyanates essentially occurs by way of a nonionic cyclic mechanism, i.e. it is a [3,3]-sigmatropic rearrangement.

The free thiocyanate ion was quantified during a 24 h-incubation period to determine its spontaneous release from 4-(methylthio)butyl isothiocyanate. The concentration of thiocyanate ion increased in a time-dependent manner. Except for 4-(methylthio)butyl thiocyanate, there was no spontaneous degradation product observed among all breakdown products in an aqueous environment in the study of Lee and Kwon (2014). The release of SCN^- from 4-(methylthio)butyl thiocyanate may involve the formation of the *S*-methylthiolanium cation (Benn and Singh, 1986). In addition to the spontaneous thiocyanate ion formation from 4-(methylthio)butyl thiocyanate in an aqueous solution, previously, 3-indolylmethyl and *p*-hydroxybenzyl isothiocyanates were also reported to readily release SCN^- ions in aqueous media. This could also be a formation pathway of thiocyanates in aqueous solutions, i.e. dissociation of organic isothiocyanates to stable cations and recapture of the ambident nucleophilic SCN^- ion, leading to organic thiocyanates.

Isomerization of thiocyanate may be initiated by the presence of a thiocyanate ion (Fava et al., 1965), and heating (Smith and Emerson, 1960), as well as could be photolytically (Lex et al., 2006), or catalytically promoted (metal salts such as cadmium iodide, zinc chloride, strong acids; Fava, 1966).

Dekić (2011) dealt with the synthesis of aryl thiocyanates and cyanides. Thiocyanates (Fig. 5) were obtained in a reaction of differently substituted benzyl chlorides and potassium rhodanide at room temperature in DMF as the solvent, as the products of a faster, kinetically controlled reaction. Contrary to thiocyanates, isothiocyanates are the products of a thermodynamically controlled reaction (C–N bond is stronger than the C–S bond) and are formed by isomerization of thiocyanates at a rate that depends on the nature of the electrophile and the reaction conditions.

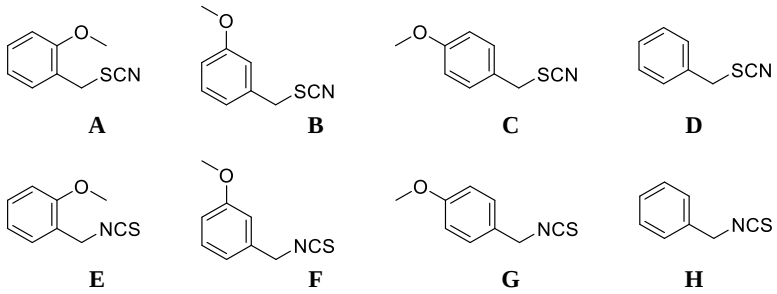


Fig. 5 Chemical structures of (iso)thiocyanates reported by Dekić and co-workers (2017)

A number of mechanisms have been proposed for this reaction (Smith and Emerson, 1960). To elucidate the transformation between thiocyanates and isothiocyanates, Dekić

(2011) carried out a thermal isomerization of a thiocyanate mixture (Fig. 5 A and C) into isothiocyanates (Fig. 5 E and G). The reaction was carried out by heating the thiocyanate mixture in DMF at reflux temperature, and the reaction course was monitored by GC-MS. After 90 min of reflux, the isomerization into the corresponding isothiocyanates was almost quantitative (Fig. 6).

In a paper published by Slater (1992), the synthesis and gas chromatography of allyl thiocyanate and isothiocyanate were studied. When a sample of commercial allyl isothiocyanate was analyzed by GC, on DB-23 column with the injector at 50 ºC, two main components were detected - allyl isothiocyanate (9.73 min) and allyl thiocyanate followed by allyl disulfide (13.87 min). The GC data indicated 0.5% of the thiocyanate and 99.5% of the isothiocyanate.

The effect of injector temperature on the proportion of isomers was also monitored. The synthetic sample of allyl isothiocyanate was analyzed by splitless injection with the injector temperature varied from 50-200 ºC. About 0.2% of thiocyanate was found when the synthetic allyl isothiocyanate was chromatographed on a DB-23 column with the injector at 125 ºC, increasing to 8.9% when the temperature was raised to 200 ºC. On the other hand, 0.5% of the thiocyanate was shown to isomerize to isothiocyanate at the injector temperature of 50 ºC, while 88.1% isomerized at 200 ºC.

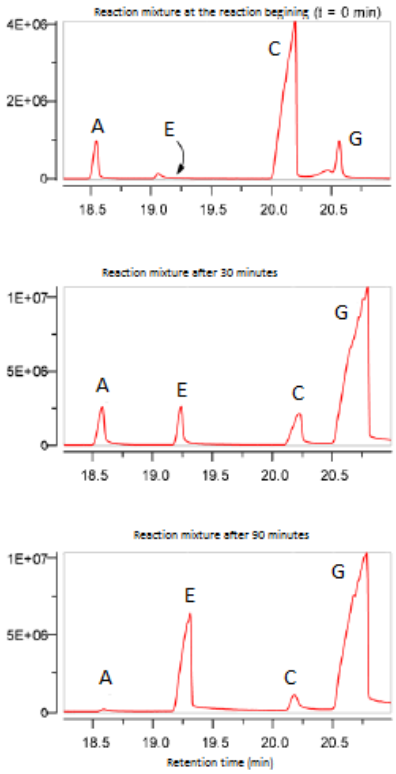


Fig. 6 Gas chromatograms of the thiocyanate (A and C) isomerization into the corresponding isothiocyanate (E and G, respectively; peak designations correspond to the ones given in Figure 5), taken from Dekić (2011).

2.3.3. Thiocyanates from plant sources

Organic thiocyanates have been reported to be formed from only five glucosinolates, sinigrin, glucotropaeolin, glucoerucin, (4-methoxyphenyl)methyl-, and (4-hydroxyphenyl)methyl glucosinolates, upon hydrolysis. The ability of their side-chains to form stable (carbo)cations is a common feature of these glucosinolates (allyl-, benzyl-, and 4-methylthiobutyl-). As a breakdown product of glucoerucin, 4-(methylthio)butyl thiocyanate, was found in a very limited number of plant species, such as *Eruca sativa, Diplotaxis tenuifolia*, and *Alyssum* species (Fahey et al., 2001).

In a paper published by Shinohara and co-workers (1991), headspace volatiles collected from Japanese horseradish (*Wasabi japonica*) at 90 ºC were found to contain allyl isothiocyanate and the corresponding thiocyanate. The authors suggested that the thiocyanate arose from the reversible isomerization of the isothiocyanate on the chromatography column. However, Gilbert and Nursten (1972), who also observed these products in extracts of Japanese, English, and Hungarian horseradish, reported that allyl isothiocyanate, isolated by preparative gas chromatography, did not isomerize when chromatographed. Benzyl thiocyanate was obtained by the autolysis of seeds of *Lepidium* spp. (garden cress) (Gmelin and Viratanen, 1959) and 3-methylthiobutyl thiocyanate from *Eruca sativa* (salad rocket) (Schlutter and Gmelin, 1972). Allyl thiocyanate was identified as the main product of the autolysis of *Thlaspi arvense* (stinkweed, pennycress) (Gmelin and Viratanen, 1959) and was shown (Luthy and Benn, 1977) to be the initial product which subsequently isomerized to allyl isothiocyanate. These results together with the demonstration of a “thiocyanate-forming factor” (Hasapis and MacLeod, 1982), and the observation that allyl isothiocyanate did not isomerize during gas chromatography, suggest that the allyl thiocyanate isolated from horseradish (Gilbert and Nursten, 1972) could be a natural product and not a laboratory artefact.

3. Conclusions and Perspectives

One can conclude that organic thiocyanates are formed from plant material usually by hydrolysis, in an enzymatically catalyzed reaction. They can also be formed during the isolation or analysis process, thus, when glucosinolate breakdown is in question, care must be taken when handling the samples and during their analysis by gas chromatography. However, one should not dismiss the possibility that under certain conditions, the plant can yield organic thiocyanates independently of the glucosinolate metabolism. The detailed mechanisms and conditions of the formation reactions of thiocyanates, as well as the purpose of their formation, remain to be discovered in the future.

Because these isomers have very similar mass spectra and there are scarce literature data on GC retention indices of these compounds, care should be taken when identifying the glucosinolate breakdown products and their conceivable isomers. One should always consider GC co-injection with the autolysates to acquire an unambiguous corroboration of their identities.

Future scientific findings on this topic will be of great importance not only because glucosinolates are found in many edible plants (and one often comes in contact with them and their degradation products) but also because of their pharmacological activities and opportunities for their use as medicinal compounds.

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ORGANSKI TIOCIJANATI – PROIZVODI ENZIMSKE DEGRADACIJE GLUKOZINOLATA ILI ARTEFAKTI?

Glukozinolati su sekundarni metabolti biljaka, najčešće zastupljeni u biljkama reda Brassicales. Zavisno od njihove strukture, uslova hidrolize i prisustva određenih proteinskih kofaktora, dejstvom mirozinaza glukozinolati daju različite degradacione proizvode: izotiocijanate, tiocijanate, nitrile itd. Najčešći proizvodi hidrolize (autolize) su izotiocijanati, dok nekada nastaju nitrili, epitionitrili i tiocijanati. Organski tiocijanati su detektovani u vrlo malom broju taksona reda Brassicales. Do sada su benzil-, (4-hidroksifenil)metil-, (4-metoksifenil)metil-, 4-metiltiobutil- i alil-tiocijanati poznati su kao proizvodi autolize glukozinolata. Ovaj pregledni rad ima za cilj da sažme znanje o mehanizmu stvaranja organskih tiocijanata iz odgovarajućih tioglukozida. Veruje se da je enzimsko formiranje organskih tiocijanata omogućeno specifičnim tiocijanat-formirajućim proteinom (TFP), međutim, pod nekim uslovima, do formiranja tiocijanata može doći na druge načine koji ne uključuju TFP. Nastajanje svih do sada poznatih tiocijanata prirodnog porekla uključuje stabilne (karbo)katjonske vrste koje potencijalno omogućavaju izomerizaciju izotiocijanata u tiocijanat i obrnuto. Iako ne bi trebalo odbaciti mogućnost da pod određenim uslovima biljka može sintetisati tiocijanate, alil-tiocijanat može predstavljati artefakt termičke izomerizacije izotiocijanata, koja se može odvijati u visokotemperaturnim zonama gasnog hromatografa, dok bi drugi tiocijanati mogli da se formiraju u vodenom medijumu putem heterolitičke disocijacije na ambidentatni nukleofil SCN^-, a zatim i njegovog ponovnog vezivanja. Važno je uvek imati na umu ovaj analitički nedostatak prilikom donošenja zaključaka o prisustvu i količini ovih specifičnih (izo)tiocijananata u analiziranim uzorcima.

Ključne reči: *organski tiocijanati, glukozinolati, tiocijanat-formirajući protein, izomerizacija*