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Review

The enzymic and chemically induced decomposition of glucosinolates

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Abstract

While the myrosinase–glucosinolate system has been reviewed in recent years by a number of authors, little attention has been paid to the enzymic and non-enzymic degradation of glucosinolates. Non-enzymic degradation processes are particularly important in the processing of brassica vegetables with respect to both flavour and in the role of glucosinolates as precursors of anticancer compounds in the diet. This review highlights early empirical work on glucosinolate degradation along with more recent aspects related to current research on mechanism of glucosinolate degradation in plants, microbes and animals.

Keywords: Myrosinase (E.C. 3.2.1.147); β-D-thioglucosidase; Epithiospecifier protein; Thermal and chemical degradation; Microbial products; Review

Contents

1. Introduction

Glucosinolates are hydrolysed by the enzyme commonly known as myrosinase (thioglucohydrolase; E.C. 3.2.1.147) to a variety of compounds (isothiocyanates, nitriles, thiocyanates, epithionitriles, oxazolidines), the composition of which depends on pH, metal ions and other protein elements (Fig. 1).

The most common products are isothiocyanates, which arise from a 'Lossen' like rearrangement to give isothiocyanates (Fig. 2).

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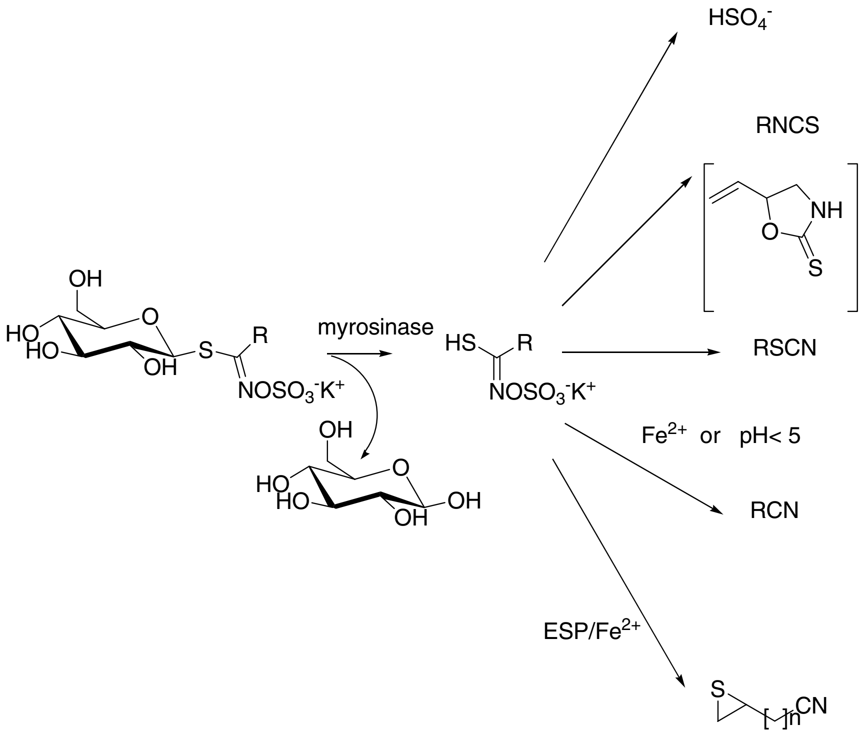

Fig. 1. General diagram showing the main products of enzymic glucosinolate degradation.

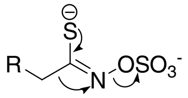

Fig. 2. The 'Lossen' like rearrangement to give isothiocyanates.

Many studies have been carried out on the characterisation of myrosinase which include metal ion dependence, ascorbate activation and inhibitor studies. Some of these topics are dealt with in detail in this review while other aspects of glucosinolates have been more recently reviewed. For an excellent recent review on inhibitors readers are referred to Bourderioux et al. (2005) while the human and animal aspects of glucosinolate metabolism have been reviewed by Holst and Williamson (2004) and more recently by Talalay (2005). The analysis of glucosinolates is covered by Botting et al. (2002) and Kiddle et al. (2001) while biosynthetic aspects have recently been reviewed (Grubb and Abel, 2006; Kliebenstein, 2004; Wittstock and Halkier, 2002). The occurrence of glucosinolates in cruciferous plants is dealt with by Fahey et al. (2002). Throughout the review semi- systematic and IUPAC names for structures and degradation products will be used.

2. Nitrile and epithionitrile formation

The first observation of a nitrile containing sulphur was made by VanEtten et al. (1966). The structures of these sulphur containing nitriles were shown to be epithionitriles (Daxenbichler et al., 1968). The compounds were identified as diastereoisomers of (2*S*)-1-cyano-2-hydroxy-3,4-epithiobutanes ((2*S*)-3-hydroxy-3-(thiiranyl-2-yl)propanenitriles). It was originally thought that nitrile formation was non-enzymic and depended only on ferrous ions and protons. However, it is now accepted that epithiospecifier protein is responsible for nitrile formation although the actual mechanism is still an open question. Epithiospecifier protein uses the unstable thiohydroximate generated by myrosinase to produce nitriles and epithionitriles. ESP was first partially characterised by Tookey (1973) and more recently was purified and characterised by Foo et al. (2000) and Bernardi et al. (2000). ESP is a relatively stable protein which has been shown to be important in plant-insect interactions (Lambrix et al., 2001). The ESP encoding gene has been cloned from *Arabidopsis thaliana* (Lambrix et al., 2001). The reaction requires ferrous ions for activity (de Torres Zabala et al., 2005), which presumably allows the formation of an intermediate between the thiohydroximate and epithiospecifier protein. Insertion of sulphur has been shown (Brocker and Benn, 1983) to occur via a intramolecular reaction by using ^{35}S labelled sulphur. A possible mechanism that can explain both nitrile and thiirane ring formation is shown in Fig. 3.

Recently, it was shown that it was possible to modify the glucosinolate hydrolysis profile by overexpressing ESP in *Arabidopsis thaliana* thereby switching from production/formation of isothiocyanates to nitriles (de Torres Zabala et al., 2005). ESP is an important factor in determining isothiocyanate levels in broccoli as it relatively heat sensitive in relation to myrosinase (Matusheski et al., 2003, 2004).

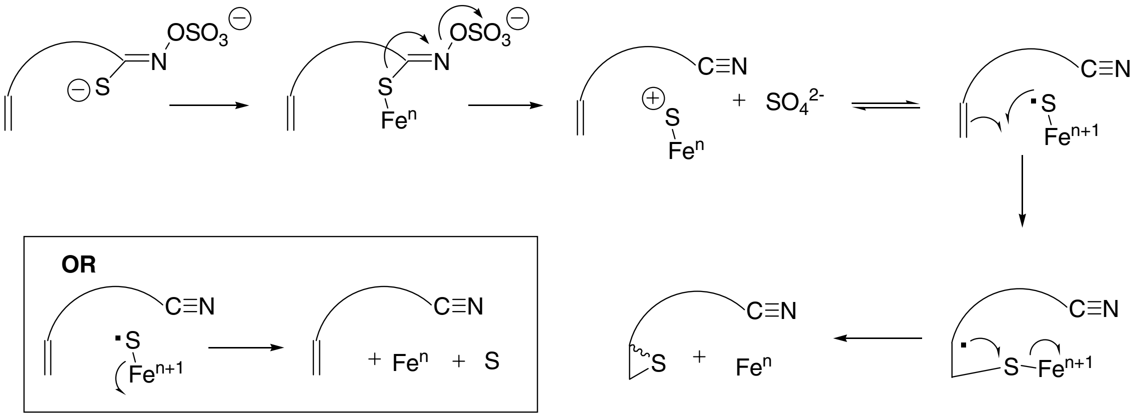

Fig. 3. Proposed mechanism of myrosinase and epithiospecifier protein based formation of cyanoepithioalkanes and nitrile formation (Foo et al., 2000).

Thus a short heat treatment (i.e. brief boiling inactivates ESP) but leaves the myrosinase active therefore enabling generation of isothiocyanates on mastication.

3. Thiocyanate formation

Gmelin and Virtanen (1959) showed that seeds of *Thlaspi arvense* L. gave an garlic oil as well as a mustard oil and showed this was due to the formation of allyl thiocyanate (3-thiocyanatoprop-1-ene). The same authors found that seeds of *Lepidium ruderdale* L. converted benzylglucosinolate (glucotropaolin) into benzylisothiocyanate (1-(thiocyanatomethyl)benzene). A later example of organic thiocyanate production was the formation of 4-methylthiobutylthiocyanate (1-(methylthio)-4-thiocyanatobutane) from the corresponding glucosinolate (Schlueter and Gmelin, 1972). Here seeds of *Eruca sativa* gave the isothiocyanate while leaf material gave the thiocyanate. There are various theories for thiocyanate formation (Gmelin and Virtanen, 1959), such as an enzyme that rearranges the glucosinolate, an isomerase that acts on the isothiocyanate and factors which act on the aglycone to generate thiocyanate. Thiocyanates are thought to arise from glucosinolates that can give rise to a stable carbocation (Luthy and Benn, 1977) such as benzyl-, propenyl- and 4-methylthiobutyl-glucosinolates. Miller (1965) observed that the sinigrin (2-propenylglucosinolate) aglycone was converted to allyl thiocyanate (3-thiocyanatoprop-1-ene) by *T. arvense* L. seed meal. Thus it would appear that thiocyanate formation requires at least two enzymes, a myrosinase and a thiocyanate forming factor. It has been reported (Saarivirta, 1973a,b) that seed powder from *Lepidium sativum* supplemented with ascorbate activates the thiocyanate forming factors while hydrogen sulphide suppresses isothiocyanate production. The formation of allyl thiocyanate (Luthy and Benn, 1977) results in a rearrangement that results in a shift of C1 to C3 (Fig. 4).

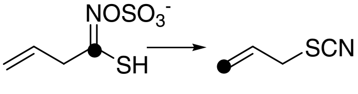

Fig. 4. The rearrangement of carbon in the formation of 3-thiocyanat-

Attempts to purify a ‘thiocyanate-forming factor’ so far have failed (Hasapis and MacLeod, 1982).

4. Indolyl glucosinolate metabolism

Indolylglucosinolates (Fig. 5) give rise to a variety of products on hydrolysis by myrosinase. Thus indol-3-ylmethylglucosinolate (1) gives rise (Agerbirk et al., 1998) to indole-3-carbinol ((1-*H*-indol-3-yl)methanol) (3), indole-3-acetonitrile 2-(1*H*-indol-3-yl)acetonitrile) (5) and 3,3′-diindolylmethane (di(1*H*-indol-3-yl)methane) (4). Indole-3-carbinol (3) is thought to arise from the corresponding unstable isothiocyanate (3-(thiocyanatomethyl)-1*H*-indole) (2) which undergoes solvolysis with water.

Attempts to isolate the isothiocyanate for indol-3-ylmethylglucosinolate have not been successful although the corresponding isothiocyanate for 1-methoxyindol-3-ylmethyl glucosinolate has been isolated by using a low water system (Hanley et al., 1990a,b; Hanley and Parsley, 1990). Attempts to isolate the isothiocyanate for indolylmethylglucosinolate have not been successful. A feature of glucosinolate hydrolysis is the formation of ascorbigens which are formed in the presence of ascorbic acid. For example, the indolyl ascorbigen is shown in Fig. 6.

5. Other degradation products

Rocket salad (*E. sativa*) contains the glucosinolate 4-mercaptobutyl glucosinolate and autolysis of leaf tissue gives rise to the bis isothiocyanate (1,2-bis(4-isothiocyanatobutyl)disulfane) (Fig. 7) which is important as a flavour determinant (Bennett et al., 2002).

White mustard (*Sinapis alba*) contains the glucosinolate 4-hydroxybenzylglucosinolate (sinalbin), which on hydrolysis

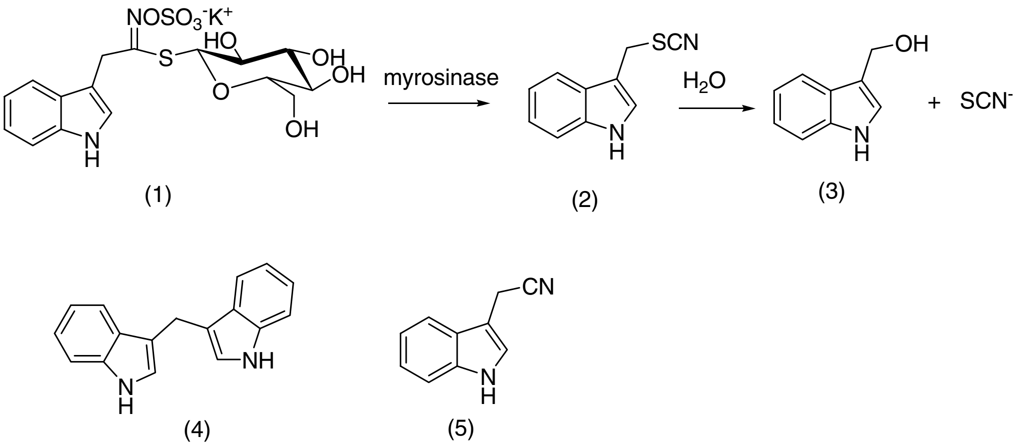

Fig. 5. Products generated from the myrosinase induced hydrolysis of indol-3-ylglucosinolate. (1) Indol-3-ylmethylglucosinolate , (2) 3-(thiocyanatomethyl)-1*H*-indole, (3) (1-*H*-indol-3-yl)methanol, (4) di(1-*H*-indol-3-yl)methane , (5) 2-(1-*H*-indol-3-yl)acetonitrile.

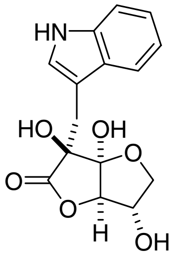

Fig. 6. Indol-3-ylascorbigen.

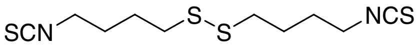

Fig. 7. 1,2-bis(4-isothiocyanatobutyl)disulfane.

by myrosinase gives rise to an unstable isothiocyanate (Kawakishi and Muramatsu, 1966; Kawakishi et al., 1967) and decomposes to *p*-hydroxybenzyl alcohol (4-(hydroxymethyl)phenol). Other compounds identified were di-(*p*-hydroxybenzyl) disulfide (4-((benzyldislfanyl)methyl)-phenol), thiocyanate and under acidic conditions *p*-hydroxybenzylnitrile (2-(4-hydroxyphenyl)acetonitrile).

6. Enzymic mechanism of plant and aphid myrosinase

The characterisation of plant myrosinase has received a great deal of attention over the years in terms of mechanistic and kinetic studies (Bones and Rossiter, 1996). Much of the early work on myrosinase characterisation was carried out in Sweden (Bjorkman and Janson, 1972; Lonnerdal and Janson, 1972) and Japan (Ohtsuru et al., 1973). Myrosinase is an abundant protein in some cruciferous seeds and is easily obtainable by simple ammonium sulphate fractionation followed by ion-exchange chromatography. A feature of plant myrosinase is its activation by ascorbate. Early work had shown that ascorbate created an allosteric effect

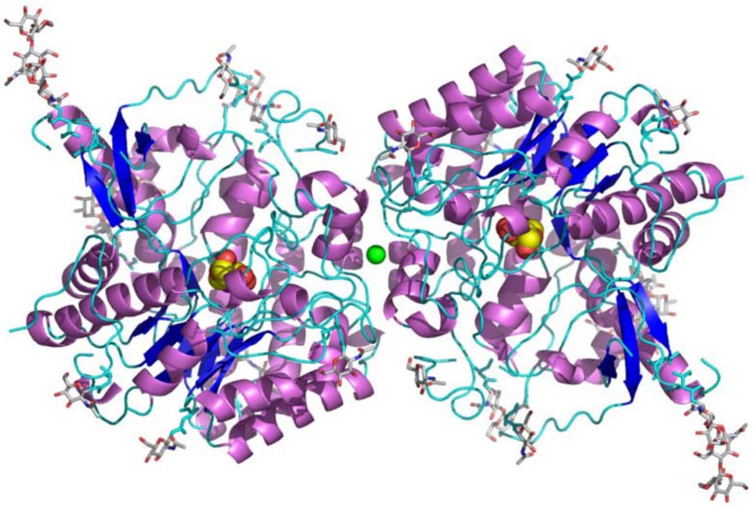

Fig. 8. The overall structure of plant myrosinase showing the dimer held together by a zinc (green) atom, fluoroglucose (yellow) and carbohydrate (sticks) (kindly provided by Wim Burmeister).

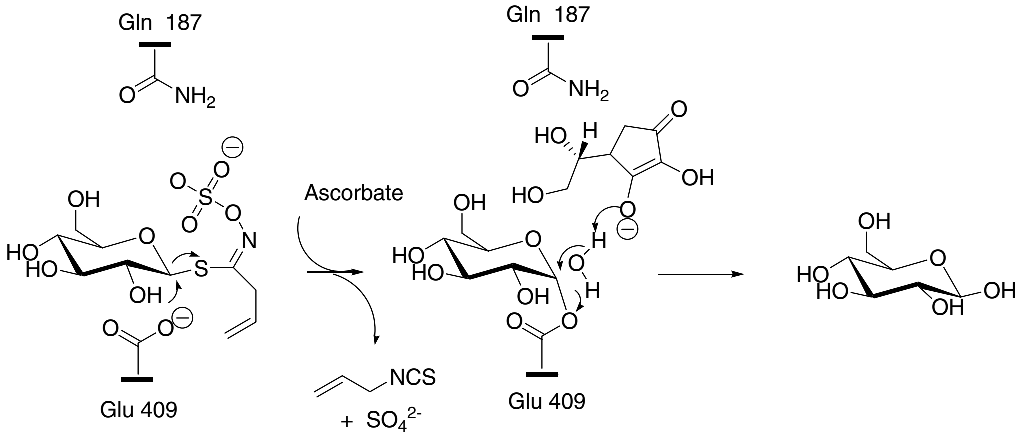

Fig. 9. The ascorbate activated catalysis of glucosinolate hydrolysis by myrosinase.

on the activity of the enzyme (Ohtsuru and Hata, 1979; Ohtsuru et al., 1973). Elegant work by Burmeister and colleagues has enabled the mechanism of myrosinase to be elucidated by X-ray structural analysis of plant myrosinase (Burmeister et al., 1997). This was achieved by using a 2-F-2-deoxybenzylglucosinolate myrosinase inhibitor (Cottaz et al., 1997) which traps the glutamic acid residue (Glu 409). The myrosinase is a dimer linked by a zinc atom and has a characteristic $(\beta/\alpha)_8$-barrel structure (Fig. 8) in common with the glycosyl family I enzymes which act through a mechanism that gives retention of the anomeric configuration.

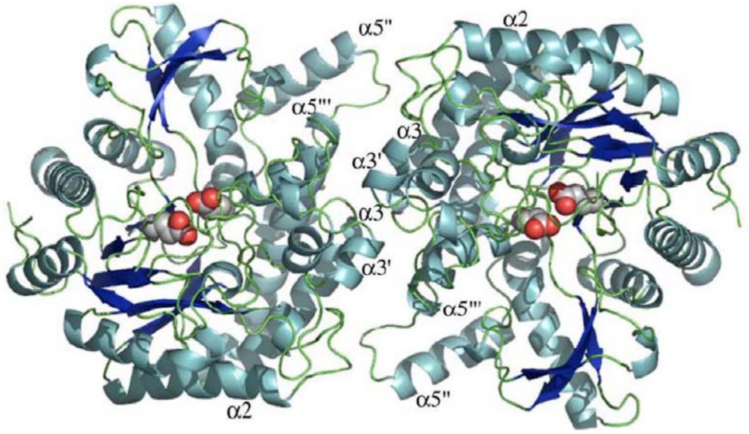

Fig. 10. The structure of aphid myrosinase showing the dimer. The two catalytic glutamic acid residues are shown in red (reproduced from Husebye et al., 2005).

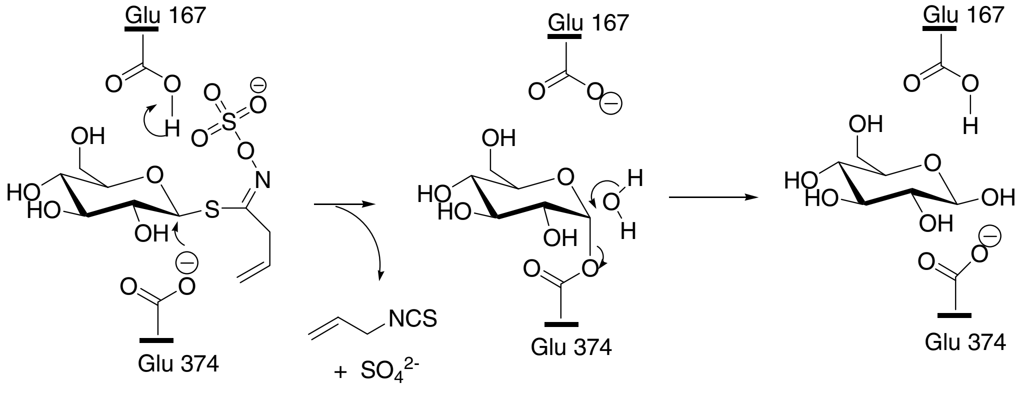

Fig. 11. The catalysis of glucosinolates by aphid myrosinase.

Subsequently the mechanism of ascorbate activation has been worked out where it was shown that ascorbate acts as a catalytic base (Burmeister et al., 2000). The mechanism of the plant myrosinase is shown in Fig. 9.

The potential of plant myrosinase to carry out transglycosylation reactions has been explored (Botti et al., 1995). It was thought that since plant myrosinase accepts a charged substrate, i.e. sulphated then it might undergo transglycosylation reactions with molecules that contain sulphate and phosphate. Several potential glycosyl acceptors were evaluated and included 3-hydroxy-1-propanesulfonate, glycerol-2-phosphate and mercaptoethane sulfonate. However, the plant myrosinase failed to give any transglycosylation products suggesting that the active site does not have the inherent flexibility for this type of process. Interestingly an insect myrosinase has been purified and an X-ray structural determination carried out (Husebye et al., 2005; Jones et al., 2001, 2002). In common with the plant myrosinase the aphid enzyme has the characteristic $(\beta/\alpha)_8$-barrel structure (Fig. 10).

Unlike the plant enzyme the aphid enzyme is not activated by ascorbate and is more like a β-*O*-glucosidase than a plant myrosinase. A paper (Francis et al., 2002) reporting aphid myrosinase to have a dual function acting both as an oxidase and thioglucosidase requires further examination as nothing in the structure of this protein (Husebye et al., 2005) suggests that this is possible. The likely mechanism of the aphid enzyme is shown in Fig. 11.

7. Chemical degradation

Gadamer (1897a,b) was one of the first chemists to investigate the non-enzymic reactions of glucosinolates. The glucosinolate used was 2-propenylglucosinolate and its reactions with various chemicals are shown in Fig. 12.

Renewed interest in glucosinolates led to the isolation of the so-called sulphoraphene ((*E*)-4-isothiocyanato-1-(methylsulfinyl)but-1-ene) (Fig. 13) and the corresponding nitrile from radish seeds (Schmid and Karrer, 1948a).

The parent glucosinolate, 4-(methylsulfinyl) but-3-enylglucosinolate was purified and converted to the silver derivative and decomposed by sodium thiosulphate, which mainly resulted in production of the isothiocyanate with some of the corresponding nitrile. Similarly (Schmid and Karrer, 1948a,b) it was found that the silver derivative of benzylglucosinolate on acid and alkaline hydrolysis gave benzylcyanide (2-phenylacetonitrile) (Fig. 14).

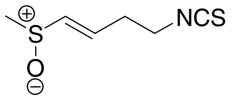

Fig. 13. The structure of ((*E*)-4-isothiocyanato-1-(methylsulfinyl)but-1-ene).

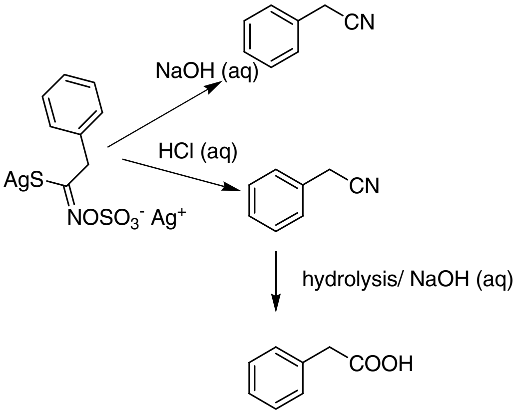

Fig. 14. The degradation of the silver salt of benzylglucosinolate.

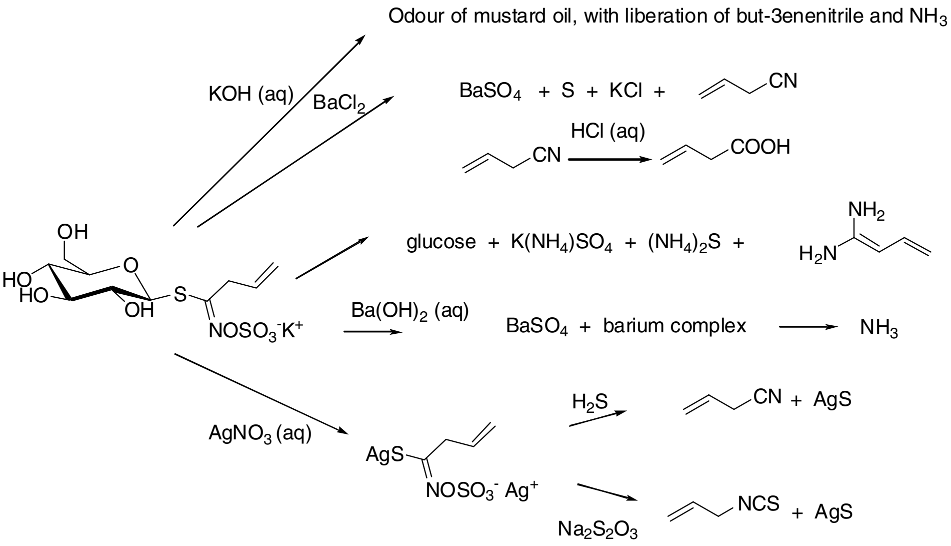

Fig. 12. The chemical degradation of 2-propenylglucosinolate.

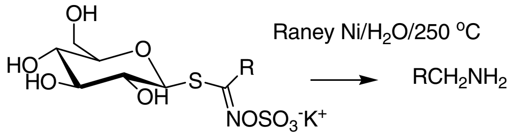

Fig. 15. The reduction of glucosinolates to amines.

The reduction of glucosinolates to amines has been demonstrated (Ettlinger and Lundeen, 1956) by using Raney nickel/water at high temperatures (Fig. 15). Here the amino group is at the carbon atom corresponding to C-0 in glucosinolates, and the products are not the amines proposed to arise from catabolism, in which the amino group is bonded to C-1 of the glucosinolates (Dalgaard et al., 1977; Olsen and Sorensen, 1979, 1980; Sorensen, 1970).The occurrence of naturally occurring amines has been linked to corresponding glucosinolates (Sorensen, 1970).

Acid decomposition (Ettlinger and Lundeen, 1956) of glucosinolates leads to the corresponding carboxylic acid together with hydroxylammonium ion (Fig. 16) and has been used in the identification of new glucosinolates (Olsen and Sorensen, 1979, 1980).

Base hydrolysis of glucosinolates results in the formation of several products. In addition to allyl cyanide (but-3-enenitrile) and ammonia, thioglucose is obtained from 2-propenylglucosinolate with aqueous sodium hydroxide (Fenwick et al., 1981; Friis et al., 1977). Thioglucose has also been reported as a product of the reaction of 2-propenylglucosinolate with potassium methoxide (Schneider, 1912; Schneider and Becker, 1930) while, basic degradation of 4-hydroxybenzylgluosinolate gives thiocyanate, indol-3-ylmethylglucosinolate gives glucose, sulphate, H_2S, thiocyanate, indol-3-ylacetic acid, indol-3-ylacetamide (2-(1*H*-indol-3-yl)acetamide), indol-3-ylmethyl cyanide (2-(1*H*-indol-3-yl)acetic acid), 3-(hydroxymethyl)indole (1-*H*-indol-3-yl)methanol), 3,3′-methylenediindole (di(1*H*-indol-3-yl)methane), indole-3-carbaldehyde 2-(1*H*-indol-3-yl)acetaldehyde, and indole (Fenwick et al., 1981). A general scheme for indolylglucosinolate acid/base degradation is shown in Fig. 17.

The base hydrolysis (Fig. 18) of 2-propenylglucosinolate and benzylglucosinolate with 2 M NaOH has been found to give the corresponding amino acids (Friis et al., 1977).

It is thought that the amino acids are derived from a Neber rearrangement. Mechanistically this takes place by

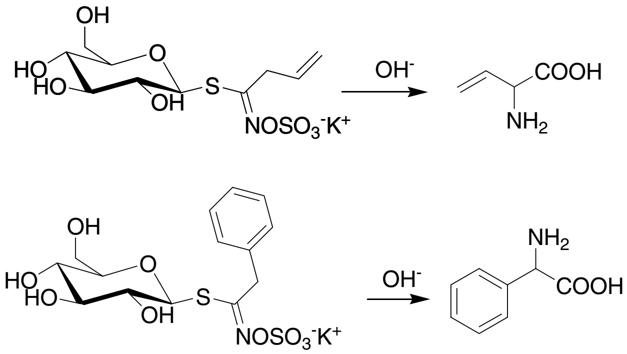

Fig. 18. The formation of amino acids from glucosinolates.

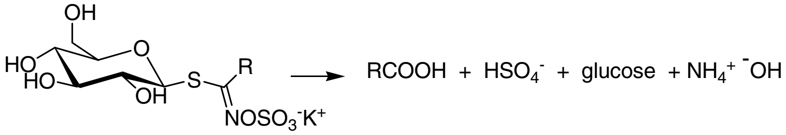

Fig. 16. Acid decomposition of glucosinolates.

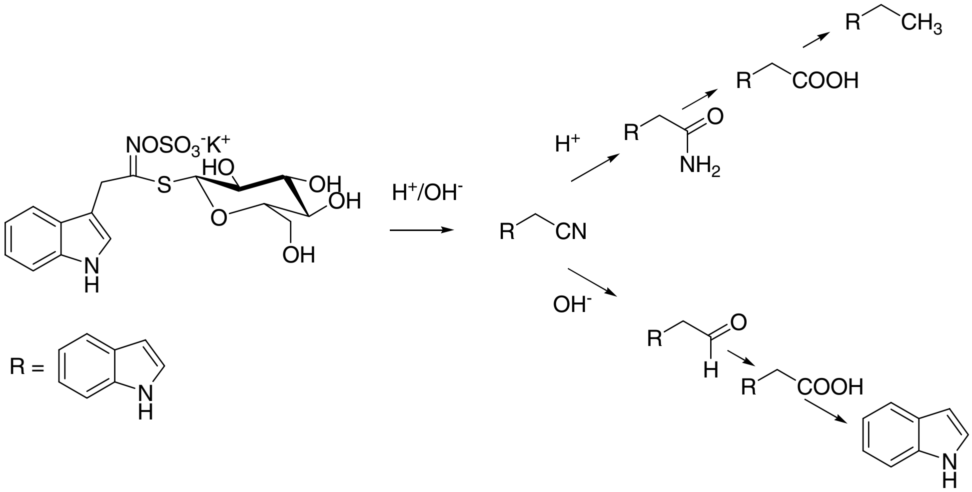

Fig. 17. General acid/base degradation of indol-3-ylglucosinolate.

the loss of a proton from the carbon atom α to the C=N grouping, with subsequent bond formation between the alpha carbon atom and the nitrogen atom with the loss of arene sulphonate ion to give an azirine, and addition of an alcohol molecule to give an alkoxyaziridine (Conley and Ghosh, 1971). Ring opening then takes place by the addition of a second molecule of alcohol, and hydrolysis gives the α-aminoketone (Fig. 19). An analogy of this rearrangement has been made for glucosinolates (Friis et al., 1977).

Depending on the pH of the solution, metallic salts of aglucones can undergo decomposition to the cyanide or isothiocyanate. Miller (1965) found that the silver derivative of the aglucone of 2-propenylglucosinolate could degrade in the presence of iodide ion to give either cyanide or isothiocyanate, depending on the pH of the solution (Fig. 20).

An interesting reaction results from the formation of nitriles with equimolar amounts of ferrous ions (Youngs and Perlin, 1967). Refluxing 2-propenylglucosinolate with equimolar amounts of Fe^{2+} gave the quantitative formation of allyl cyanide. The mechanism for this reaction has not yet been shown, although (Youngs and Perlin, 1967) suggested that the metallic ion complex of 2-propenylglucosinolate, provided a route for a concerted electron transfer (Fig. 21).

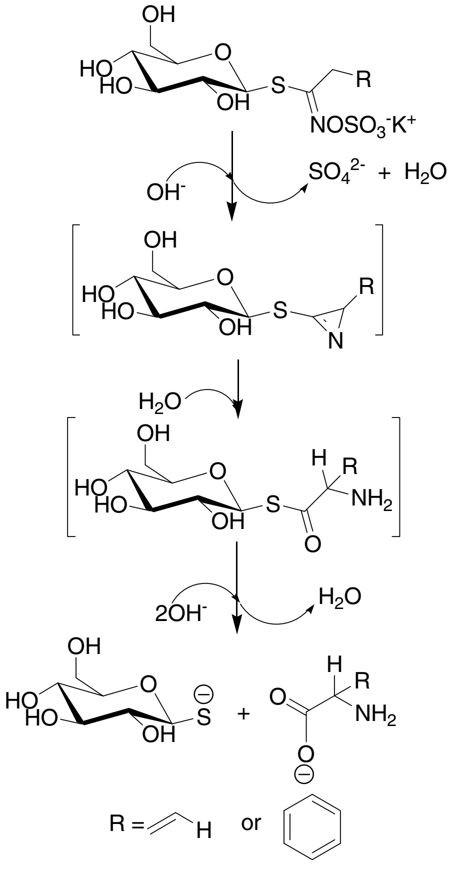

Fig. 19. A Neber like rearrangement of glucosinolates to give amino acids.

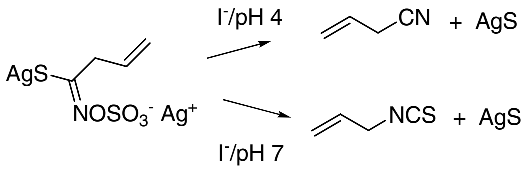

Fig. 20. Hydrolysis of the silver salt of 2-propenylglucosinolate with iodide at different pHs.

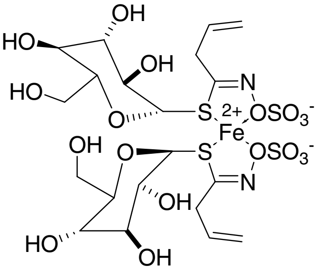

Fig. 21. The ferrous ion complex of 2-propenylglucosinolate.

This proposal was based on the known structure of the stable 1:2 complex between Ni^{2+} and methylthiohydroxamic acid (Youngs and Perlin, 1967). However, complex formation between 2-propenylglucosinolate and Fe^{2+} has never been shown. Glucosinolates with a hydroxyl group in the 2-position of the side chain (e.g. 2-hydroxybut-3-enylglucosinolate) have been shown to give thionamides (Fig. 22) with Fe^{2+}, although an eightfold excess of Fe^{2+} is necessary (Austin et al., 1968a,b) but it seems unlikely that these compounds would be generated during food processing.

(*S*)-2-Hydroxybut-3-enylglucosinolate of *Crambe abyssinica* meal has been found to be decomposed by heating in the presence of bases ($Ca(OH)_2$, Na_2CO_3, NaOH) and metal salts ($FeSO_4$, $Fe(NO_3)_3$, CuC1, $CuSO_4$) with Fe^{2+} and Cu^+ being the most active species. *N*-methylindol-3-ylmethylglucosinolate, studied by Michajlovskij was by itself decomposed by Fe^{2+} (aq) to the nitrile (Michajlovskij, 1968, 1974) while photolysis gave products identical to those obtained by enzymic cleavage (Michajlovskij, 1968).

8. Thermal degradation

Although model systems have been used to study thermal degradation, other factors in the plant matrix (e.g. metal ions) are likely to affect the process and caution is required in the interpretation of data. Given the importance of glucosinolates in terms of their anti-cancer activity surprisingly few thermal degradation studies in relation to food processing have been carried out. It has been (Lanzani et al., 1976, 1978) found that (*R*)-2-hydroxybut-3-enylglucosinolate heated at 100 °C for 3 h resulted in total decomposition. The products obtained were 2,3-pentadienoic acid (35%), S and NH_3 and 50% conversion to 1-aminobutadiene, 1-amino-2-hydroxybut-3-ene, H_2S and CO_2. Grono-

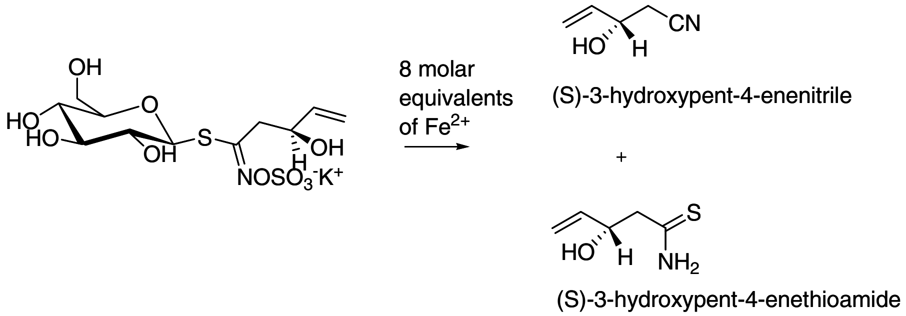

Fig. 22. The formation of thionamides with ferrous ions.

witz et al. (1978) have studied some of the aqueous decomposition reactions of (*R*)-2-hydroxybut-3-enylglucosinolate and but-3-enylglucosinolate with various buffers and the application of heat. After 5 h of refluxing in water, 36% of the original glucosinolates were left and the corresponding nitriles were obtained in a 60% yield. The fate of the remainder of the glucosinolates was not clear. Refluxing at pH 5 and 100 °C caused the decomposition of 87% of (*R*)-2-hydroxybut-3-enylglucosinolate in 30 min. Sodium tetraborate was found to be the most active of the decomposing buffer solutions, giving an unexpected product identified as 5-vinyl-2-oxazolidinone (Fig. 23).

Previous work using a model system has shown that (*R*)-2-hydroxybut-3-enylglucosinolate is relatively unstable as compared to 2-propenylglucosinolate and benzylglucosinolate (MacLeod and Rossiter, 1986). Maheshwari et al. (1980) have systematically studied the heat stability of 2-propenylglucosinolate in aqueous and model systems. This study investigated the effects of conventional and microwave heating on the decomposition of 2-propenylglucosinolate. Here the disappearance of 2-propenylglucosinolate was followed by UV, but the products of decomposition were not identified. They found microwave heating caused more decomposition than conventional heating. When 2-propenylglucosinolate was added to fat-free soya flour, decomposition with microwave heating was less when the moisture content was low. The observation was expected, since the effectiveness of microwave heating is dependent on moisture content.

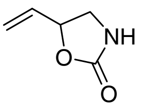

Fig. 23. The structure of 5-vinyl-2-oxazolidinone.

Further studies have been carried out examining the effects of microwave treatment on red cabbage (Verkerk and Dekker, 2004). One interesting feature of this work was the increased concentration of glucosinolate compared to the control. This is presumed to be due to increased extractability of glucosinolates using microwave treatment. The effects of microwave treatment on myrosinase activity was determined and as expected increasing exposure results in a decrease in activity. A recent paper describes the effect of pressure on myrosinase (Van Eylen et al., 2006). Here it was found that myrosinase activity was retained even at 600 MPa.

MacLeod et al. (1981) have looked at certain aspects of heat-induced glucosinolate degradation, particularly with regard to the formation of glucosinolate products on gas chromatography columns and injectors. Here, the glucosinolates were degraded using various injection and column temperatures on a gas chromatograph. For example, they found that at a column temperature of 200 °C 2-propenylglucosinolate degraded to give 44% nitrile, 32%

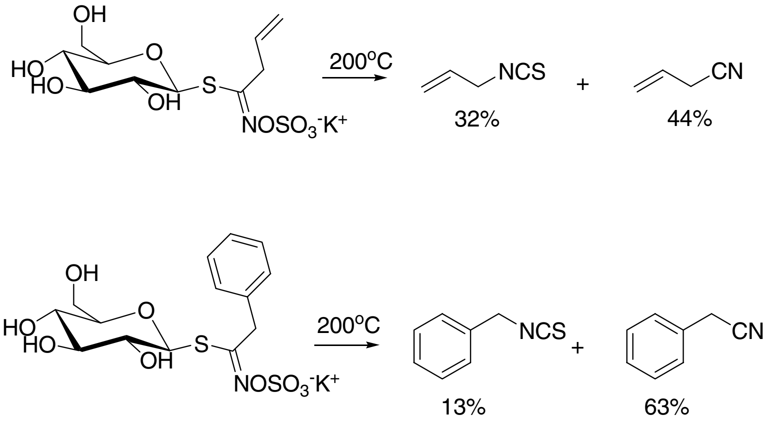

Fig. 24. The thermal degradation of 2-propenyl and benzylglucosinolates at 200 °C in a GC injector.

isothiocyanate, while benzylglucosinolate degraded to give 63% nitrile and 13% isothiocyanate (Fig. 24). This was the first demonstration that isothiocyanate had been produced non-enzymically, other than that obtained by degradation of the silver derivative, but then this compound is effectively equivalent to the enzyme-produced aglucone.

The thermal degradation of indolylglucosinolates has been examined in some detail by a number of researchers (Slominski and Campbell, 1988, 1989a,b). It was observed that heat treatment resulted in substantial decomposition of indole glucosinolates with thiocyanate and indoleacetonitriles as products while autolysis (macerated tissue) gave little indoleacetonitriles but high levels of thiocyanate and carbinols. An interesting degradation product using synthetic indol-3-ylmethylglucosinolate is the 2-(3′-indolylmethyl)glucosinolate (Fig. 25). This was produced using a model system by boiling indol-3-ylmethylglucosinolate for 1 h in pure water (Chevolleau et al., 1997). However, this compound has not yet been identified in cooked vegetables.

The concentrations of individual and total glucosinolates have been measured in four types of Portuguese cabbage and in one hybrid white cabbage before and after cooking (Pereira et al., 2002). About 50% of total glucosinolates were lost during the cooking process. The degradation products of fermented cabbage have been analysed and a variety of products identified such as goitrin, nitriles and epithionitriles (Ciska and Pathak Dorothy, 2004).

The thermal degradation of glucosinolates in red cabbage has been studied in some detail by first inactivating myrosinase (Oerlemans et al., 2006). It was found that the indolyl glucosinolates were more sensitive to heat treatment compared to aliphatic glucosinolates (38% and 8%, respectively). Recently a great deal of attention has been paid to the degradation of 4-methylsulphinylbutylglucosinolate (glucoraphanin) which is found in broccoli. The isothiocyanate, sulphoraphane (1-isothiocyanato-4-(methylsulfinyl)butane)), derived from this glucosinolate has been shown to have anticarcinogenic properties. There has been just one thermal degradation study with this glucosinolate and the substance was shown to be unstable (Jin et al., 1999). These authors found that increasing the temperature of the aqueous solutions increased the rate of formation of degradation products. Compounds identified were dimethyl disulfide, *S*-methyl methylthiosulfinate, *S*-methyl methylthiosulfonate, methyl (methylthio)methyl disulfide, 1,2,4-trithiolane and sulphoraphene, while the main component of the degradation process was *N*,*N*-di(methylsulfinyl)butyl thiourea.

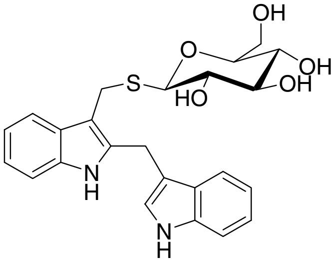

Fig. 25. The structure of 2-(3′-indolylmethyl)glucosinolate.

9. Mammalian intestinal degradation

In recent years, a great deal of attention has been paid to the potential health benefits of glucosinolate degradation products. In essence raw cruciferous vegetables will generate isothiocyanates and nitriles depending on factors such as ESP/ferrous ions while residual glucosinolates in cooked vegetables with thermally inactivated myrosinase are degraded to isothiocyanates by the gut microflora. Very little work has been carried out on the degradative enzymes of the gut microflora, but more recently several groups have identified bacterial strains associated with glucosinolate degradation. The formation of allyl isothiocyanate from 2-propenylglucosinolate in the digestive tract of rats monoassociated with a human colonic strain of *Bacteroides thetaiotaomicron* has been shown and it has been presumed that a bacterial myrosinase is involved (Elfoul et al., 2001).

The degradation of glucosinolates by the human colonic microflora has been studied in some detail in a dynamic in vitro large-intestinal model (Krul et al., 2002). The study was carried out with 2-propenylglucosinolate which was shown to be degraded to allyl isothiocyanate. The degree of conversion depended on the concentration of 2-propenylglucosinolate used and the nature of the inoculum. Since not all the isothiocyanate was accounted for further metabolism was indicated to, as yet, unidentified metabolites.

A detailed analysis of glucosinolate degradation by human digestive microflora has been carried out using ^1H NMR on both 2-propenylglucosinolate and benzylglucosinolate. Using this technique it was shown that the two amines (Fig. 26) were exclusively produced (Combourieu et al., 2001). However, this appears to conflict with the later report where isothiocyanates were observed (Krul et al., 2002).

Recent work (Cheng et al., 2004) with strains of *Bifidobacterium* sp., *B. pseudocatenulatum*, *B. adolescentis*, and *B. longum* showed they were able to digest glucosinolates, 2-propenylglucosinolate and benzylglucosinolate, in vitro. All strains digested both glucosinolates during 24–48 h cultivation, accompanied by a decline in the medium pH from 7.1 to 5.2. The digestion of glucosinolates by a cell-free extract prepared from sonicated cells of *B. adolescentis* increased in the presence of 0.5 mM ascorbic acid suggest-

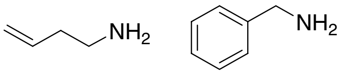

Fig. 26. Amines derived form hydrolysis of 2-propenyl and benzylglucosinolates by human microflora.

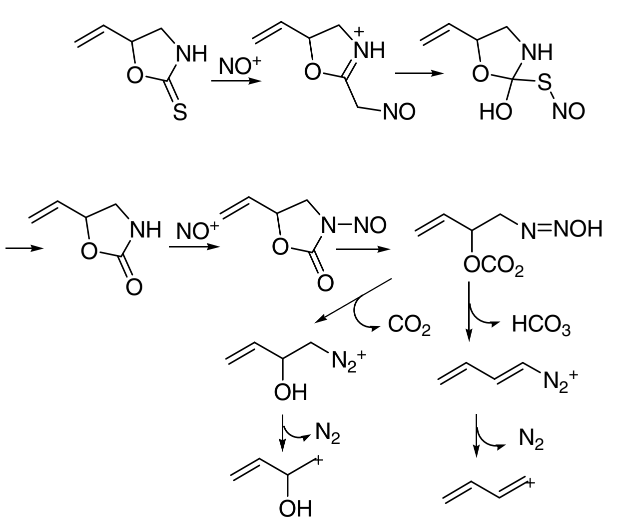

Fig. 27. The putative pathway of nitrosation of 5-vinyloxazoilidine-2-thione and decomposition to give highly electrophilic species.

ing that ascorbate maybe an activator of this bacterial myrosinase. GC–MS analysis of the *bifidobacterial* cultured broth showed that the major products were 3-butenenitrile and 2-phenylacetonitrile, probably due to a decrease in the pH of the media. Allyl isothiocyanate and benzyl isothiocyanate (1-(isothiocyanatomethyl)benzene) were barely detectable in the broth. It was concluded that the three species of *bifidobacteria* could be involved in digestive degradation of glucosinolates in the human intestinal tract (Cheng et al., 2004).

Given that there seems to be an issue regarding the formation of isothiocyanates as opposed to amines, the possibility exists that once isothiocyanates are formed they maybe further metabolised to amines. However, analytical studies have shown that glutathione–isothiocyanate conjugates and other conjugates of the mercapturic acid pathway (Holst and Williamson, 2004) are formed in vivo so it seems unlikely that the in vitro NMR technique used actually shows the complete picture. The isothiocyanate sulphoraphane, from 4-methylsulfinylbutylglucosinolate is a particularly potent anti-cancer compound, which induces phase II detoxification enzymes, which are thought to induce apoptosis in cancer cells. The isothiocyanates are metabolised through the mercapturic acid pathway. This topic has been recently reviewed by Holst and Williamson (2004). The antinutritional properties of glucosinolate degradation are well documented with goitrin and thiocyanate as the main antinutrional factors. Goitrin is derived from the intramolecular cyclisation of 1-isothiocyanato-but-3-en-2-ol to goitrin. Goitrin is an inhibitor of thyroid peroxidase and prevents oxidation of iodide to iodine for subsequent iodination of tyrosine residues in the biosynthesis of the thyroxines T_3 and T_4. Thiocyanate anions act as a competitive inhibitor of iodide and thus prevent iodide uptake by the thyroid. Apart from effects on the thyroid it has been shown that goitrin (Luthy et al., 1984) can undergo nitrosation reactions (Fig. 27) which might have implications for human health where nitrate levels in water are high. The potential negative effects of glucosinolates require further examination as this topic has been scarcely researched in recent years. Other investigations have explored the formation of nitroso compounds from a number of glucosinolates (Tiedink et al., 1991). Both acid and enzyme hydrolysed products were evaluated although in this study only glucobrassicin, and 4-hydroxyglucobrassicin formed *N*-nitroso compounds.

The electrophilic nature of isothiocyanates enables them to react (pH dependant) readily with small peptides, amino acids and probably flavonols to potentially generate new compounds, particularly in cooking processes and this area of research warrants further investigation. The reactions of allyl isothiocyanate with alanine, glycine, and several peptides in model systems have been described (Cejpek et al., 2000) together with the characterisation of adducts such as *N*-allylthiocarbamoyl amino acids and peptides and 3-allyl-2-thiohydantoins originating by cyclization of conjugated allyl isothiocyanate amino acids or by cleavage of conjugated allyl isothiocyanate peptides. Similarly the

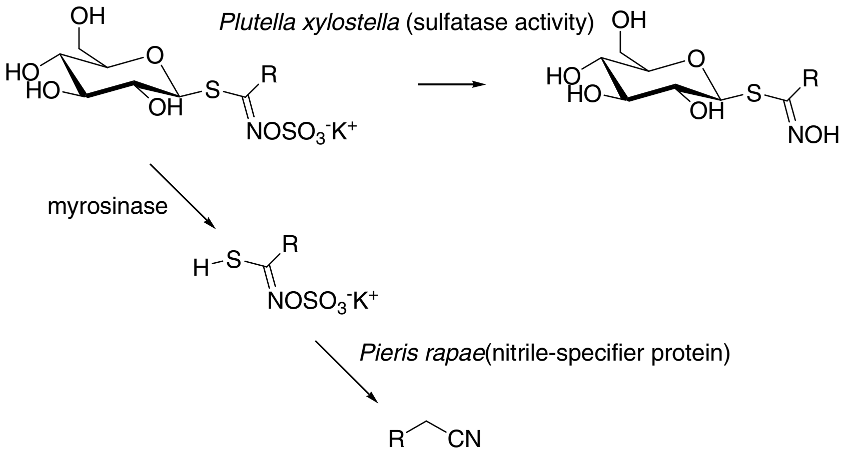

Fig. 28. The metabolism of glucosinolates by *Plutella xylostella* and *Pieris rapae*.

reactions of benzyl isothiocyanate have been well documented (HernandezTriana et al., 1996; Kroll et al., 1994; Kroll and Rawel, 1996; Rawel et al., 1998a,b,c,d).

10. Insect degradation systems

In recent years it is becoming clear that insects have evolved pathways to handle the potentially toxic isothiocyanates derived from glucosinolates. Two such pathways (Fig. 28) have been discovered one of which (Ratzka et al., 2002) is in the specialist cruciferous feeder *Plutella xylostella* which removes the sulphate from the glucosinolate rendering it inactive to myrosinase and the other in the specialist *Pieris rapae* which converts the aglycone to a nitrile via a nitrile-specifier protein which is not related to the plant epithiospecifier protein (Wittstock et al., 2004).

The specialist cruciferous aphids *Brevicoryne brasicae* and *Lipaphis erysimmi* have evolved their own myrosinase–glucosinolate system which appears to have a crucial role in defence against predators (Bridges et al., 2002; Husebye et al., 2005; Jones et al., 2001, 2002). Recent work (Muller and Wittstock, 2005) has shown that the larvae of the sawfly *Athalia rosae* sequester glucosinolates from their various host plants of the Brassicaceae into their hemolymph for defensive purposes. Feeding experiments with [C-14]-labelled glucosinolates gave as yet unidentified products.

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