

Biological activity of glucosinolate derived compounds isolated from seed meal of Brassica crops and evaluated as plant and food protection agents

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Glucosinolates are amino acid derived allelochemicals characteristic of plants of the order Capparales. These compounds are present in seeds of agriculturally common Brassica crops in varying amounts and mixture profiles depending on the plant species. Glucosinolates are hydrolysed by endogenous enzymes (myrosinases; EC 3.2.1.147) and a variety of biologically active products are produced, with different structures depending on the parent glucosinolate and the reaction conditions. Isothiocyanates and their derivatives (e.g. oxazolidine-2-thiones), nitriles, epithionitriles, and the different products derived from indol-3-ylmethylglucosinolates have fungicidal, nematocidal and herbicidal effects and therefore their potential use as biodegradable natural products for crop protection has attracted much attention in the last years. In the present study, we have evaluated the effects of some glucosinolate transformation products on a number of plant pathogenic fungi. Different concentrations of glucosinolate hydrolysis products both individually and in combination were tested. All glucosinolate products were active against the different fungi and active concentrations ranged from 10 μ M to over 10 mM. Glucosinolate products were shown to be specific depending on the fungi tested; therefore the targeted control of plant pathogens through the use of glucosinolates is possible. The use of glucosinolate concentrates or isolates could furthermore contribute to increase the value of the Brassica seed meal.

Introduction

Glucosinolates are a group of allelochemicals with well defined structures (Sørensen, 1990, Sørensen, 2001; Bellostas et al., 2007) characteristic of plants of the order Capparales. More than 140 structurally different glucosinolates are known (Bellostas et al., 2007) and about 10% are present in seeds of agriculturally common Brassica crops, although in varying quantities depending on the species (Bellostas et al., in press). The use of the remaining seed cake after oil extraction has traditionally been limited by the concentration of these compounds (Bellostas et al., this conference), however, the extraction of glucosinolates from seed meal is nowadays possible and it further contributes to an increased quality of the seed meal for feed (Bagger et al., this conference). Glucosinolates are hydrolysed by endogenous enzymes (myrosinases; EC 3.2.1.147) and a number of compounds are produced depending on the parent glucosinolate and the environmental conditions (Sørensen, 1990, Bellostas et al., 2007). Among these compounds, 5-vinyl-oxazolidine-2-thione is known for their antinutritional effects on monogastric animals, whereas isothiocyanates are fungicidal, nematocidal and herbicidal (Brown and Morra, 1997). Isothiocyanates are very reactive compounds that can easily react with nucleophiles present in

the environment, such as free amino groups or thiol groups. Their reaction with free amino groups gives rise to thioureas, whereas reacting with thiol groups produces dithiocarbamates (Figure 1). At acidic pH and in the presence of redox co-factors such as Fe^{2+} , glucosinolate hydrolysis yields also nitriles, epithionitriles and thionamides (Bellostas et al., this conference), which are more hydrophilic and stable than isothiocyanates (Sørensen, 1990). The possibility for using glucosinolates as precursors for environmental friendly biocides therefore exists and it could contribute to increase the value of the Brassica seed meal.

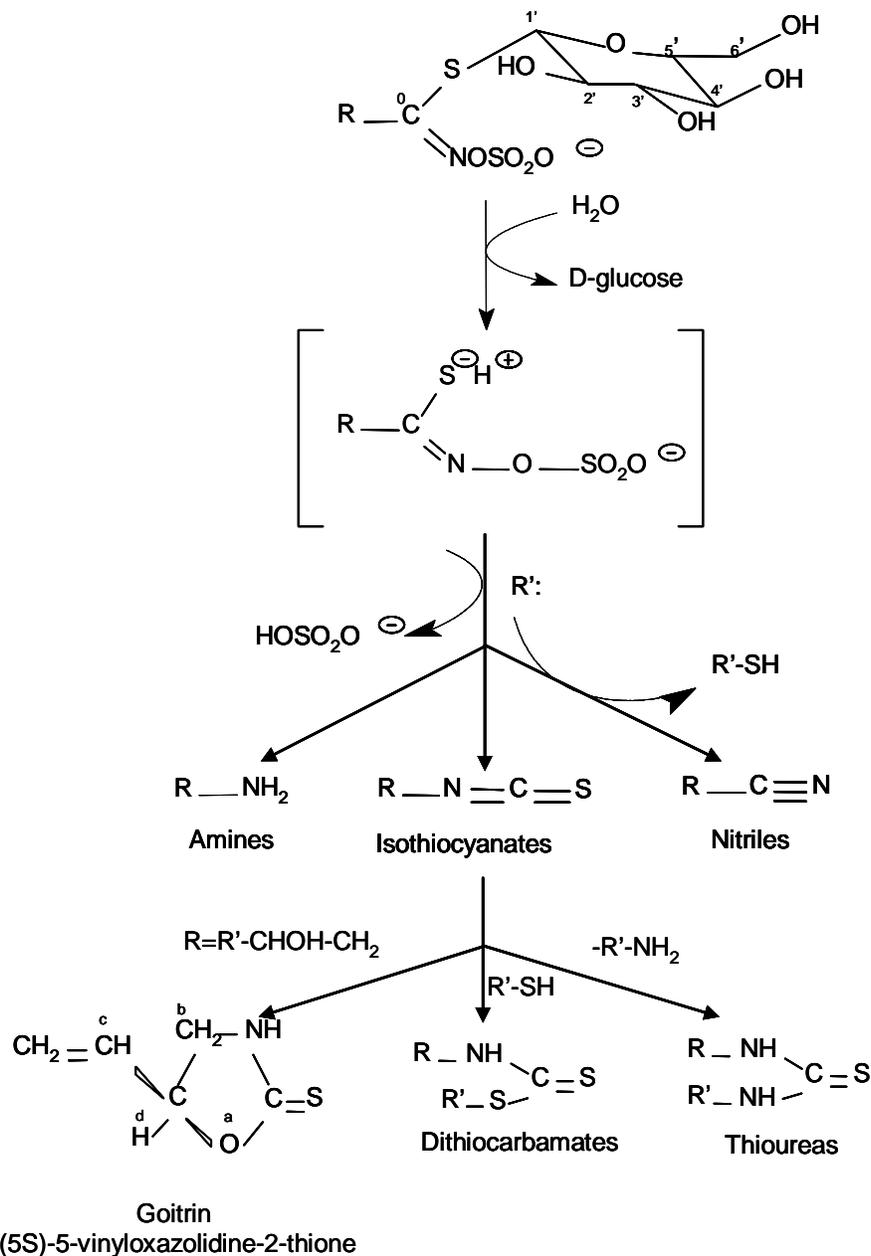


Figure 1. Hydrolysis of glucosinolates by myrosinase gives rise to a number of degradation products. Isothiocyanates are very reactive compounds and their reaction with nucleophiles gives rise to dithiocarbamates (reaction with thiol groups) and thioureas (reaction with free amino groups).

Materials and methods

Glucosinolates and myrosinases were isolated by laboratory standard procedures as well as in pilot plant scale (Bagger et al., this conference). Isothiocyanates and oxazolidine-2-thiones were prepared by hydrolysis of the corresponding parent glucosinolates in 100 mM phosphate buffer pH 6.5. Thioureas were produced by allowing the corresponding isothiocyanates to react with ammonia in an ethanol solution. Concentration and purity of these compounds was determined spectrophotometrically and by capillary electrophoresis. Nitriles were purchased from Sigma-Aldrich (St. Louis, MO). The compounds were diluted in an emulsion of hydrolysed rapeseed oil (Sørensen, 2001) and afterwards added to cooled (50-60°C) PDA medium (0.5 % v:v). Different formulas were developed, including the compounds individually and in combination. *Pseudocercospora herpotricoides*, *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium oxysporum* *Aphanomyces euteiches* var. *pisi*, *Gaeumannomyces graminis* var. *tritici* and *Verticillium dahliae* were the fungi chosen due to their economic importance. Control Petri dishes consisted of the PDA medium with emulsion (0.5% v:v). The tests were performed at the Danish Institute of Agricultural Sciences at Flakkebjerg, at Grønt Center in Holeby and at Inabonos, Spain following their standard methods.

Results and discussion

The glucosinolate hydrolysis products tested showed very different inhibition potential depending on the fungi and the structure of the degradation compounds, both regarding their side chain but also in relation to the type of degradation product (isothiocyanate, nitrile or thiourea). Figure 2 shows an example of how a single fungus (*Pseudocercospora herpotricoides*) was differently inhibited by six types of degradation products, three nitriles (left) and three isothiocyanates (right). The side chains of these compounds correspond to three different parent glucosinolates: sinigrin (prop-2-enylglucosinolate), glucotropaeolin (benzylglucosinolate) and gluconasturtiin (phenethylglucosinolate). Sinigrin is the dominant glucosinolate in Brassica mustards (*B. nigra*, *B. carinata* and *B. juncea*), in which it can be present in concentrations of up to 200 $\mu\text{mol g dm}^{-1}$ (Bellostas et al., in press).

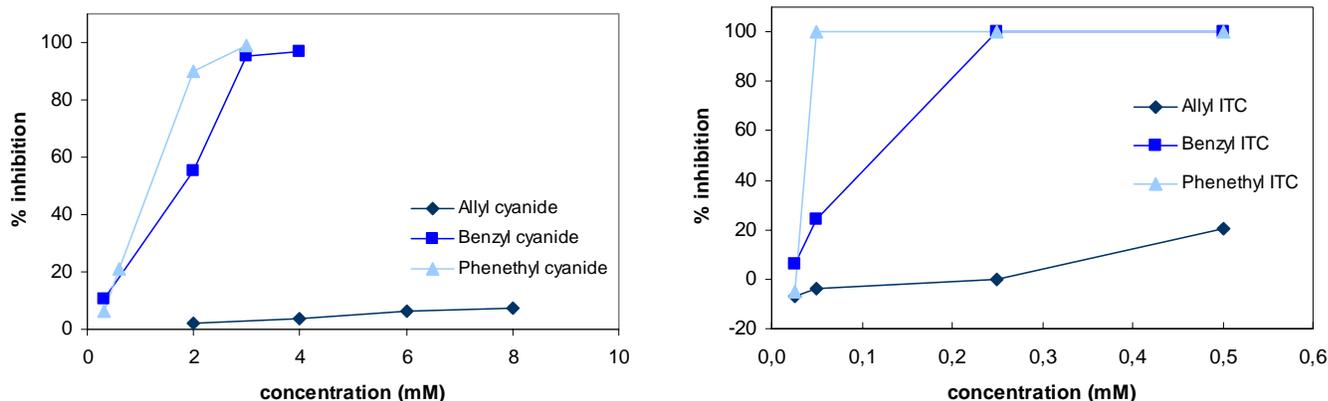
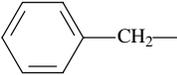
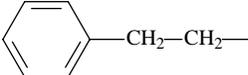


Figure 2. Effect of allyl, benzyl and phenethyl nitriles (left) and isothiocyanates (right) on *Pseudocercospora herpotricoides*, a wheat soil-borne pathogen.

The three nitriles tested showed much lower activity than the isothiocyanates, as the concentration needed to inhibit the growth of the fungus was up to 10 times higher. Both the nitrile and the isothiocyanate derived from the allylglucosinolate showed the lowest inhibition potential of the fungus, whereas the nitrile and isothiocyanate from phenethylglucosinolate showed the highest inhibition potential. Benzyl nitrile and –isothiocyanate showed an intermediate activity, closer to that of phenethylglucosinolate derived products, as the structure of its side chain is very similar to this glucosinolate (Table 1).

Table 1. Semisystematic name and structure of side chain of the three glucosinolates tested against *Pseudocercospora herpotricoides* (Figure 2).

Glucosinolate name	Structure of side chain
Prop-2-enylglucosinolate	$\text{CH}_2=\text{CH}-\text{CH}_2-$
Benzylglucosinolate	
Phenethylglucosinolate	

Conclusions

The glucosinolate hydrolysis products tested showed a high degree of specificity, being very toxic against some targets, while innocuous towards other organisms. This can be explained by the fact that high hydrophobicity of the side chain of a compound can confer it the ability to penetrate membranes. The possibility therefore exists for a selective control of plant pathogens while respecting the non-target organisms. Despite their lower toxicity compared to isothiocyanates, nitriles are generally more soluble in water, which may allow them to persist longer in soils. Nitriles are produced at low pH and in the presence of thiol groups and ferrous ions, which can be quite common in soils, therefore, the role of nitriles in biofumigation should also be considered.

Acknowledgement

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