In Vitro Fungitoxic Activity of Some Glucosinolates and Their Enzyme-Derived Products toward Plant Pathogenic Fungi

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The enzymatic hydrolysis of glucosinolates (GLs), typical compounds of Cruciferae, produces molecules with fungitoxic activity. Eleven GLs and their enzymatic hydrolysis products obtained by myrosinase were tested *in vitro* against *Fusarium culmorum*. Toxicity of hydrolysis products from glucoiberin, glucotropaeolin, sinigrin, and epiprogoitrin were assayed on eight plant pathogenic fungi. The results showed (i) the native GLs showed no fungitoxic activity, whereas their hydrolysis products inhibited fungal growth depending on their chemical structure; (ii) the hydrolysis products from glucoiberin, glucoerucin, glucocheirolin, and glucotropaeolin were the most effective, with 50% inhibition of fungal growth at 0.1 mg/mL; (iii) the fungitoxic activity of hydrolysis products obtained from glucoiberin, glucotropaeolin, sinigrin, and epiprogoitrin was confirmed on eight pathogenic fungi, with different responses depending on their chemical structure; (iv) the most effective hydrolysis products were those from glucoiberin, showing EC_{50} values of 0.05 mg/mL on *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Diaporthe phaseolorum*, and *Pythium irregulare* and a minimum inhibitory concentration varying from 0.1 to 1.2 mg/mL.

Keywords: *Cruciferae; isothiocyanates; Fusarium culmorum; myrosinase*

INTRODUCTION

Reports of biocidal activity of plant extracts have appeared in the literature since the 19th century. Over the past decade several authors have reported that many compounds extracted from wild, cultivated, and medicinal plants show fungitoxic activity (Gourinath and Manoharachary, 1991; Rahalison *et al*., 1993; Yegen *et al*., 1992). The most important classes of these molecules are terpenoids (Elakovich, 1988; Wagner and Flores, 1994), tannins (Laks *et al*., 1988; Scalbert, 1991), phenolics (Lattanzio *et al*., 1994; Weidenbörner *et al*., 1990), peptides (Terras *et al*., 1993), and glucosinolates (GLs) (Chew, 1988; Drobnica *et al*., 1967).

The antifungal properties of GLs and their enzymatic hydrolysis derived products (EHDPs) and their role in plant resistance have also been known for a long time. In 1937, Walker *et al.* observed the antifungal activity of mustard oils; Hooker *et al.*, in 1943, confirmed the antifungal activity of cruciferous plant extracts containing allyl and phenethyl isothiocyanates. More recently, Mithen *et al.* (1986) and Angus *et al.* (1994) showed that some GLs and their EHDPs protected rapeseed from *Laeptosphaeria maculans* and wheat from *Gaeumannomyces graminis*. In addition, Mari *et al.* (1993, 1996) reported EHDPs' activity protected fruit during shelf life against some postharvest pathogenic fungi.

GLs are a structurally homogeneous class of approximately 100 secondary plant metabolites present in high quantities in cruciferous seeds (Kjaer and Skrydstrup, 1987). These thioglucosides have a side chain (R) structure constituted of aliphatic, aromatic, or heteroaromatic residues (Table 1). They are sugar anionic thioesters containing a β-thioglucoside-type bond, hydrolyzed by myrosinase (EC 3.2.3.1) (MYR) to β-D-glucose, hydrogen sulfate ion, and a series of diverse agluconic EHDPs. These can be, for example, isothiocyanates (ITCs), nitriles, thiocyanates, or thiones, depending on the substrate and reaction condition as well as the pH of the catalyzed reaction (Gil and Macleod, 1980; Uda *et al*., 1986a,b) (Figure 1).

The GL−MYR system could theoretically generate hundreds of EHDPs. The system is present at different concentrations in all Cruciferae organs, where it plays an important defensive role. In plant cells GLs are kept separate from endogenous MYR. GLs and the enzyme come into contact as a result of mechanical wounding or pathogen attack. In this case *in situ* production of EHDPs, in addition to D-glucose and a sulfate ion, is quickly elicited (Mathile, 1980) (Figure 1).

For the above reasons, GLs and their EHDPs also have been studied as natural plant protective agents and as potential alternative biocidal compounds for postharvest fruit storage (Lazzeri *et al*., 1993; Mari *et al*., 1993, 1996; Mithen *et al*., 1986). This could be of interest especially in view of the more restrictive laws envisaged for the use of some synthetic pesticides in agriculture.

The first aim of this study was to determine the antifungal activity of some GLs and their EHDPs on *Fusarium culmorum*. In addition, we evaluated the activity of four EHDPs against eight plant pathogenic fungi belonging to different taxonomical classes. The ability of these compounds to inhibit fungal growth was determined as a function of EHDPs concentration.

MATERIALS AND METHODS

Glucosinolates. The 11 glucosinolates tested (Table 1) were isolated from some cruciferous seeds according to the method of Thies (1988) with some modifications (Visentin *et al*., 1992). Individual GLs were characterized via ^{1}H NMR, and their purity was checked by using HPLC as described in the *Official Journal of the European Communities* (1990), using a Hewlett-Packard chromatograph Model 1090L equipped with a diode array detector and a 200 × 4.6 mm column HP ODS Hypersil C_{18}, 5 μm. GLs showed a purity range from 75 to 91%, with the exception of rapeseed GLs (RSG), which contained a typical GL mixture (*ca.* 69% progoitrin, 17% gluconapin, and 14% minor GLs such as epiprogoitrin, 4-OH-glucobrassicin, gluconasturtin, and gluconapoleiferin).

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Table 1. Origin and Structure of the Glucosinolates Tested

glucosinolate (common name)	glucosinolate (semisystematic name)	glucosinolate (structure of the side chain R)	abbreviation	MW	species of origin (ripe seeds)
sinigrin	allyl	$CH_2{=}CHCH_2-$	SIN	397.5	*Brassica juncea* cv. Vitasso
gluconapin	3-butenyl	$CH_2{=}CH(CH_2)_2-$	GNA	411.5	*Brassica rapa* cv. Silla
glucotropaeolin	benzyl	$PhCH_2-$	GTL	447.6	*Lepidium sativum*
sinalbin	*p*-hydroxybenzyl	p-$HOPhCH_2-$	SNB	463.6	*Sinapis alba* cv. Maxi
mixture of rapeseed GLs			RSG		*Brassica napus* cv. Jet Neuf
epiprogoitrin	(2*S*)-2-hydroxy-3-butenyl	(2S) $CH_2{=}CHCHOHCH_2-$	E-PRO	427.5	*Crambe abyssinica* cv. Belenzian
glucobarbarin	2-hydroxy-2-phenylethyl	$PhCHOHCH_2-$	GBB	477.5	*Barbarea vulgaris*
glucoerucin	4-methylthiobutyl	$CH_3S(CH_2)_4-$	GER	459.6	*Eruca sativa*
glucocheirolin	3-methylsulfonylpropyl	$CH_3SO_2(CH_2)_3-$	GCH	477.6	*Cheirantus annuus*
glucoraphenin	4-methylsulfinyl-3-butenyl	$CH_3SOCH{=}CH(CH_2)_2-$	GRE	473.6	*Raphanus sativus* cv. Pegletta
glucoiberin	3-methylsulfinylpropyl	$CH_3SO(CH_2)_3-$	GIB	461.6	*Iberis amara*

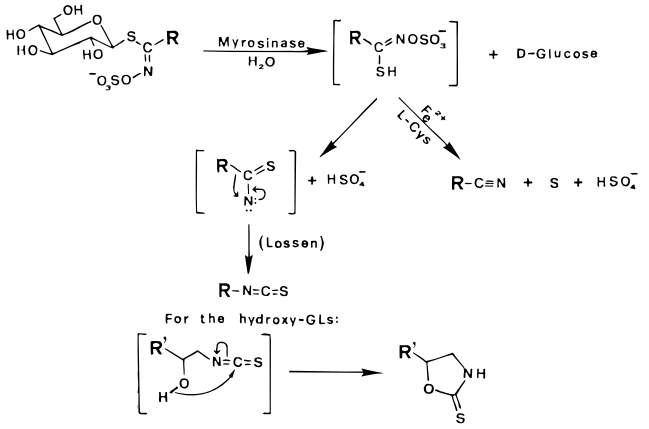

Figure 1. General GL-MYR-catalyzed hydrolysis pathway for the production of isothiocyanates, nitriles, and thiones.

Myrosinase. Myrosinase was isolated from *Sinapis alba* seeds, according to the method of Palmieri *et al.* (1986). The enzyme had a specific activity of *ca.* 25 units/mg. One myrosinase unit was defined as the amount of enzyme capable of hydrolyzing 1 μmol min^{-1} of sinigrin as reported by Palmieri *et al.* (1982).

Sample Preparation. The samples used in this study were obtained by dissolving GLs in two different buffered solutions: (i) in 50 mM phosphate buffer (pH 6.5) without myrosinase (method A); (ii) in 50 mM phosphate buffer (pH 6.5) with addition of a suitable amount of myrosinase (method B*).* All solutions were sterilized before use with 0.22 μm Millex-GV filters (Millipore, Malsheim, France) and then immersed in a water bath for 3 h at 37 °C. The hydrolysis time was sufficient to hydrolyze >95% of GLs. Some solutions were opaque after hydrolysis due to the hydrophobicity of some EHDPs and were sonicated for 15 min using a Transonic Elma Model 310.

EHDP Analyses. The compositions of the reaction mixtures obtained using the methods reported above were analyzed by gas−liquid chromatography (GLC) and HPLC. The HPLC procedure was the same as that used for GLs, the only difference being that the chromatographic fractions were detected at 200 and 210 nm. The GLC analyses were carried out using a Fison Carlo Erba Model Mega 5330, equipped with a 30 m × 0.32 mm capillary column Restek Rtx 2330. The flow rate of carrier gas (He) was 1.8 mL min^{-1} (split rate 1:80). The flow rates of the auxiliary gases (H_2 and air) were 25 and 300 mL min^{-1}, respectively. The column temperature increased from 40 to 230 °C at 10 °C min^{-1}; the temperature of the injector (split) and detector (FID) was 260 °C. The GBB and GIB partition coefficients were determined following the procedure reported by Nastruzzi *et al.* (1996). The values were calculated as the ratio between the EHDPs concentration in aqueous solution and in organic solvent. All EHDP concentration values (w/v) were repoted as those of the correspondent GL.

Fungitoxic Activity against *F. culmorum*. The fungitoxic activity was determined using 2 mg/mL of all the GLs used in this study and their EHDPs on a monoconidial culture of *F. culmorum* (W. G. Smith) Sacc. isolated in our laboratory from rotten potatoes. This value was chosen on the basis of preliminary trials, taking into account the limit for an economical application of these compounds for fungal disease control. The culture was maintained on potato dextrose agar (PDA) in the dark at about 8 °C and subcultured periodically. The activity of EHDPs was assayed at 2 mg/mL of native GLs, using the poison food technique (Dhiangra and Sinclair, 1986): 3 mL of buffer solution containing a quantity of GL or EHDP was added to 27 mL of cooled (50 °C) molten PDA to obtain the desired concentration and then divided among three Petri plates. A buffer solution (3 mL) containing 0.3 unit of MYR was added to control plates. Plates then were inoculated, three per dose, in the center with a 4 mm diameter disk of fungus taken from the edge of a growing colony. The minor and major radii of each colony were measured when the control colony reached the edge of the Petri plate (5−6 days after inoculation). Samples that showed complete inhibition were monitored for an additional 15 days. The entire colony was measured starting from the center. When a total growth inhibition occurred, the radius did not increase and was considered zero. Sporulation also was assayed every day after growth began. Colonies that showed a reduced growth rate were transferred to PDA to evaluate the growth as compared to colonies transferred from the control. All of the experiments were repeated once and the data expressed as fungal growth inhibition effectiveness index (EI). The values were calculated using the following formula: EI (%) = 100 (diameter control − diameter treatment)/(diameter control). The growth rate for the sample treated with EHDP was expressed as diameter (mm) and subjected to analysis of variance with the Statgraphic Program, version 2.6 (Statistical Graphics Corp., Rockville, MD) and Scott Knott analysis (Gates and Bilbro, 1978). To complete this screening, the EHDPs that determined a total growth inhibition at 2 mg/mL also were assayed at a 0.1 mg/mL concentration, following the procedure described above. The colony growth rate data were subjected to analysis of variance and to the least significant difference (lsd) (P = 0.05) test using the Statgraphic Program, version 2.6.

Effective Doses of Fungitoxic EHDPs on *F. culmorum*. After a preliminary screening on *F. culmorum*, four EHDPs were chosen on the basis of their EI at 2 and 0.1 mg/mL and on the GL structural side chain characteristics. These EHDPs were from sinigrin (SIN), epiprogoitrin (E-PRO), glucotropaeolin (GTL), and glucoiberin (GIB) belonging to four groups of GLs, namely alkenyl, hydroxyalkenyl, benzylic, and thioaliphatic (Table 1). These compounds were tested at six decreasing concentrations from 2 to 0.1 mg/mL of native GLs; four replications were made, and the experiment was repeated once.

Fungitoxic Activity of EHDPs on Some Plant Pathogenic Fungi. The four EHDPs tested on *F. culmorum* also were assayed on eight phytopathogenic fungi, chosen on the basis of their taxonomic class (Agrios, 1988). Six species of the Deuteromycotina subdivision, one of Mastigomycotina and two of Ascomycotina were chosen. Four species were isolated from infected tissues: *Alternaria alternata* (Fr.) Keissler, *Botrytis cinerea* Pers. ex Pers., *Colletotricum coccodes* (Wallh.) Hughes, and *Rhizoctonia solani* Kühn. *Sclerotinia sclerotiorum* (Lib.) de Bary (sclerotial stage) was obtained from the

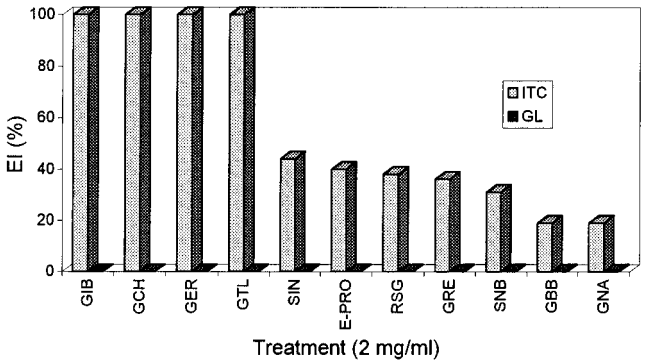

Figure 2. Effectiveness index for *F. culmorum* growth inhibition with 2 mg/mL of GLs and ITCs.

Table 2. Growth of *F. culmorum* on PDA Treated with 2 mg/mL GL-ITC, Determined When the Control Was Completely Grown (84 mm)

ITC derived from	av (mm)	SD	cluster[a]
GNA	68.3	1.1	A
GBB	67.7	3.2	A
SNB	58.0	2.6	B
GRE	53.3	2.1	C
RSG	52.3	1.1	C
E-PRO	50.7	2.5	C
SIN	47.0	1.7	D
GTL	0	0	E
GER	0	0	E
GCH	0	0	E
GIB	0	0	E

[a] Clusters obtained by Scott Knott analysis.

Osservatorio Malattie delle Piante, Bologna, whereas *Diaporthe phaseolorum* var. *caulivora* Athow et Caldwell was from Agra Seed Co. (Massa Lombarda, Ravenna, Italy). *Fusarium oxysporum* f. sp. *lycopersici* r.1 (Sacc.) Snyder et Hansen (ATCC 16417) was obtained from American Type Culture Collection, while *Pythium irregulare* Buisman was isolated in our laboratory and then included in the International Mycological Institute Culture collection (IMI 368281).

Monoconidial cultures of sporulating fungi and hyphal tip cultures of the other fungi were maintained on PDA in the dark at 8 °C and subcultured periodically. The activity was assayed using the above procedures. The incubation time varied from 2 days for *P. irregulare* to 8−10 days for *A. alternata* and *C. coccodes*. The EHDPs from GTL and GIB, which showed high activity in the first screening, were assayed starting from 1 mg/mL, while the ITCs from SIN and E-PRO were started at 2 mg/mL. The concentrations then were decreased according to fungal growth response. The half-diameter (42 ± 5 mm) of control colony, recorded when it reached the edge of the Petri plates (84 mm), was considered the concentration in the amended agar required to inhibit fungal growth by 50% as compared to the control (EC_{50}). Minimum inhibitory concentration (MIC) and EC_{50} were determined at least twice.

RESULTS

Composition of the GLs and Their EHDPs. Chromatographic analyses demonstrated that all samples prepared according to method A contained the GLs in their native form. Those prepared according to method B contained the ITCs as the main products (95−99%). Only in the case of RSG did the reaction products contain 20% of but-3-enyl-ITC and 75% of vinyloxazolidine-2-thione (VOT), derived from spontaneous cyclization of 2-hydroxybut-3-enyl-ITC. Although the enzyme-catalyzed hydrolysis of E-PRO and the corresponding epimer (PRO) produced >90% of VOT, all of the EHDPs prepared with method B have been named ITCs.

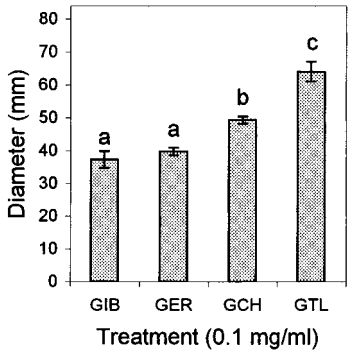

Figure 3. Growth reduction (diameter) of *F. culmorum* on media to which was added 0.1 mg/mL of the most active ITCs, determined when the diameter of control colony was completely grown (84 mm). Bars represent standard deviation. Columns with the same letter are not significantly different ($P \leq 0.05$).

Table 3. Dose Response of *F. culmorum* at Various Concentrations of Four ITCs Derived from Natural GLs

	ITC derived from			
concn (mg/mL)	SIN EI[a] (%)	E-PRO EI (%)	GTL EI (%)	GIB EI (%)
2	44.0 ± 1.2[b]	39.7 + 1.7	100	100
1	25.8 ± 4.9	31.7 ± 0.4	100	100
0.75	26.2 ± 3.6	7.0 ± 0.8	100	100
0.5	23.8 ± 5.4	7.9 ± 0.8	27.0 + 8.6	81.0 ± 1.1
0.25	10.3 ± 1.7	9.1 ± 3.2	27.0 + 0.8	64.7 ± 2.0
0.1	9.1 ± 0.4	4.7 ± 1.4	23.8 ± 2.1	55.7 ± 2.0

[a] Effectiveness index. [b] Mean ± SE for four replications.

Fungitoxic Activity on *F. culmorum*. None of the native GLs assayed at 2 mg/mL affected either growth or sporulation of *F. culmorum*, whereas the ITCs inhibited the fungal growth (Figure 2) without influencing sporulation time. Significant differences resulted ($P \leq 0.01$) depending on the GL employed. In particular, the ITCs from GCH, GER, GIB, GTL, inhibited fungal growth, while the EI for the other seven ITCs ranged from *ca.* 19 to 44%.

The Scott Knott analysis of growth inhibition (Table 2) divides ITCs into five clusters, with an increasing toxicity from cluster A to E. Moreover, colony growth on PDA transferred from ITC-treated media did not differ significantly from the control (data not shown). All of the ITCs in cluster E (Table 2) showed an appreciable fungistatic activity even at 0.1 mg/mL of the original GLs, with colony growth reductions that varied significantly ($P \leq 0.01$). The lsd test divided the growth rate data into three significantly different groups. GIB- and GER-ITCs were the most active, followed by GCH-ITC, while GTL-ITC was least active (Figure 3).

Four ITCs, two from cluster E (GTL and GIB), one from cluster D (SIN), and one from cluster C (E-PRO), were tested at decreasing concentrations using *F. culmorum*. At 0.1 mg/mL SIN- and E-PRO-ITCs were weakly active (EI < 10%), while GIB- and GTL-ITCs inhibited fungal growth by 56 and 24%, respectively (Table 3). At 0.75 mg/mL, SIN- and E-PRO-ITCs inhibited growth at <30%, whereas GIB- and GTL-ITCs completely inhibited fungal growth. At concentrations <0.75 mg/mL, GIB-ITC displayed an activity with a linear correlation radial growth *vs* dose, while GTL-ITC showed a biphasic trend with the same inhibition until 0.5 mg/mL; beyond this concentration it became completely inhibitory.

Fungitoxic Activity on Other Plant Pathogenic Fungi. The fungitoxic activity of ITCs from E-PRO,

Table 4. Minimum Concentration of Four GL-ITCs (Milligrams per Milliliter) Required to Inhibit Selected Fungal Species

	ITC derived from			
species	GTL	GIB	SIN	E-PRO
F. culmorum	0.55	0.75	≫2	≫2
F. oxysporum	0.55	1.2	≫2	≫2
B. cinerea	0.55	1.2	≫2	≫2
A. alternata	0.55	1.2	≫2	≫2
C. coccodes	0.55	1	≫2	≫2
R. solani	0.9	0.75	>2	≫2
S. sclerotiorum	0.55	0.75	>2	≫2
D. phaseolorum	0.55	0.5	1.5	2
P. irregulare	0.9	0.1	1	2

Table 5. Effective Concentration of Four GL-ITCs (Milligrams per Milliliter) To Inhibit 50% Growth of Selected Fungi

	ITC derived from			
species	GTL	GIB	SIN	E-PRO
F. culmorum	0.52	0.1	>2	>2
F. oxysporum	0.52	0.25	>2	>2
B. cinerea	0.52	0.65	>2	>2
A. alternata	0.52	0.65	>2	2
C. coccodes	0.52	0.65	>2	>2
R. solani	0.75	0.05	0.75	>2
S. sclerotiorum	0.52	0.07	0.75	>2
D. phaseolorum	0.52	0.05	0.5	1.25
P. irregulare	0.75	0.04	0.5	1.5

SIN, GIB, and GTL also was studied using other pathogenic fungi (Tables 4 and 5). E-PRO-ITC showed a MIC at 2 mg/mL on *P. irregulare* and *D. phaseolorum* but at this concentration inhibited the growth of all other fungi by <50%. SIN-ITC showed MICs of 1 and 1.5 mg/mL for *P. irregulare* and *D. phaseolorum*, respectively, while all the other fungi tested required a MIC of > 2 mg/mL. The EC_{50} was 0.5 mg/mL on these fungi, 0.75 mg/mL on *R. solani* and *S. sclerotiorum*, and >2 mg/mL on all other fungi tested. GTL-ITC showed a low selectivity to the fungal species (Tables 4 and 5). The MICs were 0.9 mg/mL for *P. irregulare* and *R. solani* and 0.55 mg/mL for all other fungi. At 0.5 mg/mL, growth inhibition was 30%, with an activation threshold similar to that observed on *F. culmorum*. Above 0.55 GTL-ITC was very inhibitory to all fungi (data not shown). The EC_{50} values for GTL-ITC were not so different from those determined for MIC.

EC_{50} values for GIB-ITC are about an order of a magnitude less with the best antifungal activity on *P. irregulare* (0.04 mg/mL), *R. solani* and *D. phaseolorum* (0.05 mg/mL), *S. sclerotiorum* (0.07 mg/mL), and *F. oxysporum* (0.25 mg/mL) (Table 5). Although the MIC for GIB-ITC ranged from 0.1 mg/mL (*P. irregulare*) to 1.2 mg/mL (*F. oxysporum*, *B. cinerea*, and *A. alternata*) (Table 4), the EC_{50} and MIC values for GTL-, SIN-, and E-PRO-ITCs show much less of a distinction.

DISCUSSION

Our data confirm that native GLs do not possess any cytotoxic or fungitoxic activity (Mari *et al*., 1993; Mithen *et al*., 1986; Nastruzzi *et al*., 1996). However, all of our ITCs inhibited fungal growth, *in vitro*, without interference with sporulation. These observations suggest the need for further studies to verify their suitability for direct use in plant protection. In principle, the utilization of ITCs of GLs may be a good compromise between fungitoxic activity toward some phytopathogenic fungi and environmental protection. GLs and their ITCs are natural compounds, present in cabbage, broccoli, Brussels sprouts, cauliflower, and other cruciferous-derived processed products such as sauerkraut and dark and light mustard. In concentrations not dangerous to human and animal health, these compounds also have shown an inhibitory effect against cancer (Lee *et al*., 1989; Manousos *et al*., 1983; Nastruzzi *et al*., 1996; Stoewsand *et al*., 1988; Zhang *et al*., 1992). The results of this study suggest that some ITCs are of potential and practical importance as biocidal compounds against some phytopathogenic fungi. In particular, the ITCs from GIB give a strong and lasting inhibition at relatively low concentrations in the 0.04−0.65 mg/mL range (Table 5).

Table 6. Water−Oil ITC Partition Coefficient Values

glucosinolate	ITC partition coeff	glucosinolate	ITC partition coeff
sinigrin	0.015[a]	glucobarbarin	0.044
gluconapin	0.628[a]	glucoerucin	0.012[a]
glucotropaeolin	0.006[a]	glucocheirolin	0.580[a]
sinalbin	0.468[a]	glucoraphenin	0.405[a]
epiprogoitrin	0.568[a]	glucoiberin	0.680

[a] Determined by Nastruzzi *et al*. (1996).

Although the limited number of GLs and fungi tested does not permit a decisive estimation of the side chain structure/biocidal activity relationship, these data showed that the most active class is the thioaliphatic ITC. They showed high (GIB, GCH, and GER) or intermediate (GRE) biocidal activity, which could be attributed to a side chain containing an extra sulfur atom in different oxidation states. The low fungitoxic activity of GRE-ITC seems to be attributed to the higher oxidation state of the double bond present in its side chain, since the other sulfinyl GL-derived ITCs, such as GIB-ITC, showed the maximum fungitoxic activity. On the other hand, similar middle−low antifungal activities were shown by alkenyl-ITCs obtained from E-PRO, RSG, and SIN, while those determined for ITCs from GNA were significantly lower. The different activities determined for ITCs from SIN and GNA seem to be due to the side chain length, which significantly affects not only the biocidal activity but also their water−oil partition coefficients. In fact, the partition coefficients of the ITCs obtained from these two GLs were 0.015 and 0.628 (Nastruzzi *et al*., 1996) (Table 6).

With regard to the aromatic GLs, the activity of GTL-ITC was higher than those of the other aromatic ITCs tested (from SNB and GBB); presumably this result also is due to the different water−oil partition coefficients and to the final products obtained (*i.e.*, GBB, like E-PRO and RSG, produces essentially the corresponding thione). Nastruzzi *et al*. (1996) report that the ITC from GTL is more hydrophobic than the ITC from SNB, with partition coefficients of 0.006 and 0.468, respectively (Table 6). The partition coefficient is a measure of molecular lipophilicity or hydrophobicity and so it could markedly affect the capability of the molecule to pass through the cell membrane, determining a different final concentration in the cell. This hypothesis could also explain the high biocidal activity differences observed in the aromatic ITCs tested in this study.

The mechanism by which ITCs inhibit microrganisms and fungal growth, in particular, is not known. Nevertheless, some hypotheses have been proposed: (i) inactivation of intracellular enzymes by oxidative breakdown of −S−S− bridges (Zsolnai, 1966); (ii) inhibition of metabolic enzymes by thiocyanate radical, indicated as a degradation product of ITCs (Banks *et al*., 1986); and finally, (iii) uncoupler action of oxidative phospho-

rylation suggested from the inhibition of oxygen uptake of three yeast strains by several ITCs (Kojima and Oawa, 1971).

The fungitoxic activities of ITCs were confirmed by treating *F. culmorum* with decreasing doses of four ITCs (GIB, SIN, E-PRO, and GTL) chosen on the basis of their different molecular structures (Table 3). This experiment indicated that the ITCs from E-PRO, SIN, and GIB displayed a growth inhibition activity roughly correlated to dose. On the other hand, the ITC from GTL showed a sudden complete effectiveness at around 0.55 mg/mL. This concentration could represent the activation threshold for this compound to control *F. culmorum* growth. At lower concentrations, in fact, the activity displays a plateau, while beyond this value it rapidly produced total growth inhibition. This behavior could be associated not only with molecular cytotoxicity but also with the capability of these molecules to enter fungal cells, due to physicochemical characteristics including the partition coefficient.

In the case of sulfur side chain EHDPs, such as the ITC from GIB, the linear response up to a concentration of 0.5 mg/mL might indicate a dominant effect of cytotoxicity on cell permeation. This would mean that these molecules can enter the fungus cells more easily, although they have high partition coefficients (0.68 for GIB-ITC and 0.58 for GCH-ITC) (Table 6). Therefore, it appears reasonable to suggest that these molecules can degrade the lipid bilayers of cell membranes. Although this hypothesis appears to be very attractive, it remains to be demonstrated. Further experiments are at present underway in our laboratory for a better understanding of these findings.

This study demonstrates the distinct sensitivity shown by the different classes of plant pathogenic fungi toward each GL-ITC. This sensitivity is linked not only to the intrinsic physicochemical and biological properties of ITCs but also to the taxonomic class of the fungus. In each trial, for instance, *P. irregulare*, Ascomycotina, and *R. solani* were the most sensitive, while other Deuteromycotina were generally more resistant toward this class of molecule.

All MIC and EC_{50} values of GIB-ITC demonstrated a general high biocidal activity for this compound. MIC and EC_{50} values determined on *P. irregulare*, Ascomycotina, and *R. solani* were particularly promising, suggesting a possible practical use of this compound in plant protection (Tables 4 and 5). These growth inhibition doses are comparable to those of some common commercial synthetic fungicides such as dithiocarbamates (Khan and Saxena, 1995; Len *et al.*, 1996). The fungal sensitivity to GIB-ITC varied considerably with the MIC ranging from 1.2 to 0.1 mg/mL (Table 4). GTL-ITC activity, however, did not seem to be related to fungal taxonomic characteristics, since the EC_{50} and MIC values were very similar. Finally, SIN- and E-PRO-ITCs were much less active, showing MIC only at concentrations >1 mg/mL and only against *P. irregulare* and *D. phaseolorum* (Table 4). Nevertheless, further studies on this topic are necessary to evaluate the efficacy of the GL−MYR system in the Cruciferae plant−host interaction mechanism since there are some reports that strongly support this hypothesis (Monde *et al.*, 1991; Soledade *et al.*, 1992).

In conclusion, the fungitoxic activity of some ITCs, in particular of sulfur side chain ITCs, suggests many interesting perspectives for controlling some soil-borne fungal pathogens, *viz. Pythium*, *Rhizoctonia*, *Sclerotinia*, and *Fusarium*. In addition, this study suggests the use of compounds from the Cruciferae as a potentially valid alternative to controlling soil-borne pathogens. A study of the qualitative and quantitative glucosinolate content of several Cruciferae will make it possible to choose and test species with high levels of sulfur side chain GLs against soil-borne pathogenic fungi.

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