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Review

Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi

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Abstract

To date, the many examples reporting that fungal pathogens can efficiently detoxify phytoalexins provide strong evidence that the pathogenicity and/or virulence of some fungi is linked to their ability to detoxify their hosts' phytoalexins. The pathways used by plant pathogenic fungi to metabolize and detoxify phytoalexins are reviewed. Prospects for application of recent findings are discussed.
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Keywords: Brassicaceae; Cruciferae; Graminaceae; Leguminosae; Solanaceae; Detoxification; Metabolism; Phytoalexins

Contents

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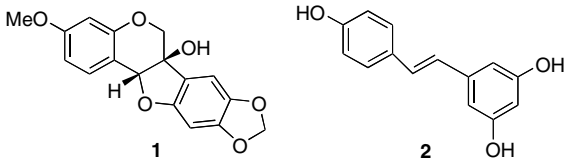
1. Introduction

Many defense mechanisms of plants against pathogenic microorganisms involve the production of secondary metabolites, which may be constitutive, phytoanticipins (VanEtten et al., 1994a,b; Morrissey and Osbourn, 1999), or inducible, phytoalexins (Smith, 1996; Brooks and Watson, 1985; Bailey and Mansfield, 1982). By definition, phytoalexins are low molecular weight antimicrobial compounds biosynthesized de novo by plants in response to diverse forms of stress, including microbial attack. (Bailey and Mansfield, 1982). Phytoalexins were first described by Müller and Börger (1940) during studies on *Phytophthora infestans–Solanum tuberosum* (potato) interactions. Since then, the great variety of phytoalexins isolated from very diverse plants indicate that their chemical structures are usually related within a plant family (Brooks and Watson, 1985). For example, many of the phytoalexins from leguminous plants have an isoflavonoid skeleton, crucifers produce indole alkaloids, cereals produce mostly cyclic hydroxamic acids and diterpenoids, whereas plants of the Solanaceae family produce sesquiterpenoids and polyacetylenes. However, stilbenoid phytoalexins have been isolated from different plant families (Harborne, 1999). Initially, research on phytoalexins dealt mainly with the identification of induced antifungal compounds and correlation with disease resistance. Hence, a large number of studies support the idea that phytoalexins have important roles in the defense of plants against pathogens such as bacteria and fungi. More recently, approaches that use in situ localization and quantification have provided evidence that phytoalexins can accumulate at the right time, concentration, and location to be effective in resistance (Hammerschmidt and Dann, 1999).

The first phytoalexin to be isolated and chemically characterized was (+)-pisatin (**1**) (Cruickshank and Perrin, 1960) from *Pisum sativum* (pea). (+)-Pisatin (**1**) was observed to be less toxic to the pea pathogen *Ascochyta pisi* than to *Monilinia fructicola*, a pathogen that does not attack pea. The toxicity of (+)-pisatin (**1**) to 50 fungal strains representing 45 species showed that only five of these fungi were tolerant of pisatin **1** (less than 50% inhibited by 100 μg/mL), and all five were pathogens of pea (Cruickshank, 1962). Only one of the 45 sensitive strains was a pea pathogen. Although subsequent surveys of the sensitivity of fungi to other phytoalexins and even to (+)-pisatin (**1**) revealed many exceptions to the correlation between tolerance and host range (Smith, 1982; VanEtten et al., 1982), Cruickshank's initial observation established the concept that tolerance to a phytoalexin might be important in pathogenicity. More recent studies on phytoalexin tolerance in pathogenic fungi have shown a clear relationship between virulence and the ability of fungi to detoxify phytoalexins. Examples illustrating tolerance mechanisms and their role as virulence traits in phytopathogenic fungi were recently reviewed (VanEtten et al., 2001).

The importance of phytoalexins as general defense compounds was demonstrated by transforming tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum* Mill.), and alfalfa (*Medicago sativa*) with a stilbene synthase that enabled the transformants to synthesize the grapevine (*Vitis vinifera*) phytoalexin resveratrol (**2**). The transformants showed an increase in the resistance to *Botrytis cinerea*, a fungal pathogen of tobacco (Hain et al., 1993), to *Phytophthora infestans* (Thomzik et al.,

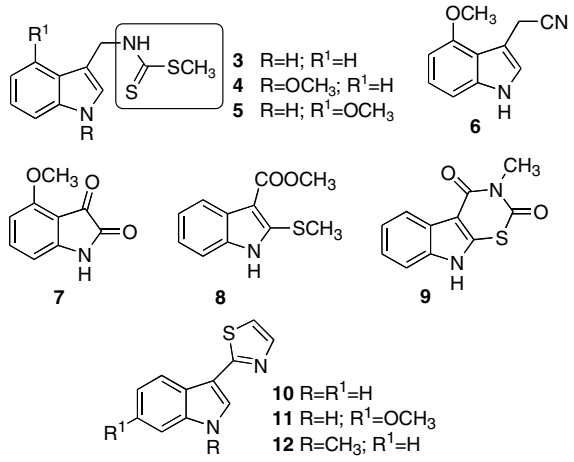
1997), and to *Phoma medicaginis* (Hipskind and Paiva, 2000), respectively. On the other hand, a recent example of transgenic plant tissue with a reduced ability to produce pisatin (**1**) indicated that such tissue was less resistant to fungal infection (Wu and VanEtten, 2004). Transgenic roots that produced the lowest levels of pisatin were more susceptible to isolates of *Nectria haematococca* than the control hairy roots. Nonetheless, since phytoalexins are also toxic to plant cells, they can accumulate in whole plants or cell cultures only transiently, some plant enzymes such as extracellular peroxidases can degrade oxidatively phytoalexins (VanEtten et al., 1982).



Currently, enzymatic detoxification of phytoalexins by phytopathogenic fungi is of great interest due to the potential application of results to understand and control plant pathogens (VanEtten et al., 2001). Additional prospects for increasing plant defenses using phytoalexin related pathways are viable alternatives recently reviewed (Essenberg, 2001). In this article the biochemical reactions used in the metabolism and detoxification of phytoalexins and their analogs by plant pathogenic fungi is reviewed.

2. Metabolism and detoxification of phytoalexins from the family Cruciferae

The first report documenting the isolation of cruciferous phytoalexins suggested the structural uniqueness of these metabolites (Takasugi et al., 1986), possessing an indole ring with substitution at the C-3 position and additional nitrogen and sulfur atoms. A few cruciferous phytoalexins (**3**–**5**) contain a dithiocarbamate group, previously used in synthetic antifungal and herbicidal agrochemicals. Since the last review of the cruciferous phytoalexins (Pedras et al., 2003a,b) four new chemical structures were reported: arvelexin (**6**) isolated from *Thlaspi arvense* (stinkweed) (Pedras et al., 2003a), and isalexin (**7**), brassicanate A (**8**), and rutalexin (**9**) isolated from *Brassica napus*, ssp. *rapifera* (rutabaga) (Pedras et al., 2004b). Most of the plants of the family Cruciferae studied until now include the economically important genus *Brassica*, containing rapeseed and canola (*B. napus*, *B. rapa*), mustards (*B. juncea*, *B. carinata*, *B. nigra*), and various cabbages (*B. oleracea*). Although fewer wild species have been examined, it appears that their phytoalexin profiles are different from those of cultivated species. With the exception of *Thlaspi arvense* (Pedras et al., 2003a) and *Erucastrum gallicum* (Pedras and Ahiahonu, 2004), wild species appear to produce only camalexins **10**–**12**, which were isolated from *Arabidopsis thaliana*, *Arabis lyrate*, *Camelina sativa*, and *Capsella bursa-pastoris* (Pedras et al., 2000). Crucifer phytoalexins, including camalexins (Glawischnig et al., 2004), are biosynthetically derived from tryptophan (Pedras et al., 2003a,b). Considering the most recent studies on secondary metabolic pathways in *A. thaliana*, (Glawischnig et al., 2004), camalexin may become the first crucifer phytoalexin to have its biosynthetic genes unraveled.



Studies of the metabolism of crucifer phytoalexins have been carried out using mainly three economically important cruciferous fungal pathogens: *Phoma lingam* (Tode ex Fr.) Desm. (perfect stage *Leptosphaeria maculans* (Desm.) Ces. et de Not.), *Sclerotinia sclerotiorum* de Bary, and *Rhizoctonia solani* Khun, as summarized in Table 1.

2.1. Brassicanal A

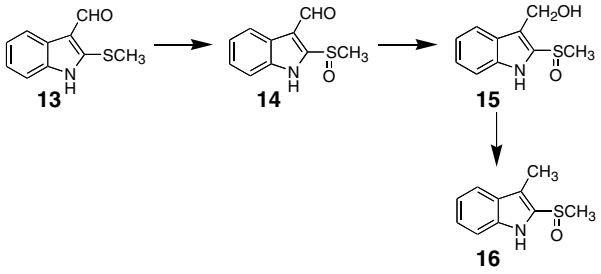
Investigation of the metabolism of the phytoalexin brassicanal A (**13**) by *L. maculans*/*P. lingam* led to the isolation of three metabolites: brassicanal A sulfoxide (**14**), 3-hydroxymethylindolyl-2-methylsulfoxide (**15**) and 3-methylindolyl-2-methyl-sulphoxide (**16**), as shown in Scheme 1 (Pedras and Khan, 1996; Pedras et al., 1997). In the first biotransformation step, the SCH_3 group of brassicanal A (**13**) was oxidized to the corresponding sulfoxide **14**. In subsequent steps the aldehyde group was reduced to alcohol **15** and then further to the 3-methylindole **16**. The biotransformation of brassicanal A (**13**) was shown to be a detoxification, since the antifungal activities of brassicanal A (**13**) and its metabolites indicated that brassicanal A was significantly more inhibitory to *L. maculans*/*P. lingam* than any of the products **14**–**16**.

Table 1
Metabolism of phytoalexins from the family Cruciferae (Brassicaceae) by plant pathogenic fungi

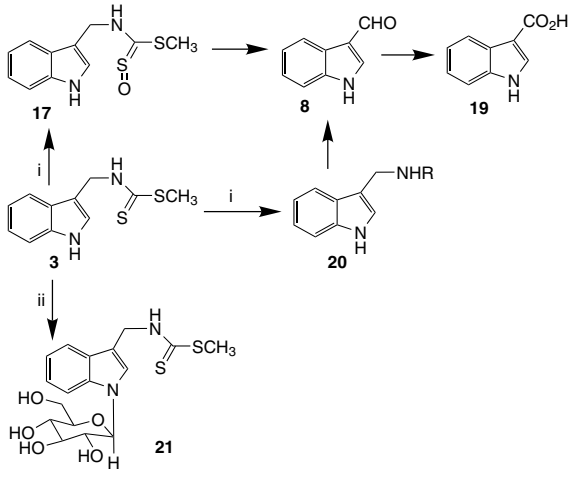
Phytoalexin, plant species	Fungal species	Major products of metabolism
Brassicanal A (**13**), *Brassica rapa, B. oleracea, Raphanus sativus*	*Leptosphaeria maculans* (*Phoma lingam*)	3-Methylindolyl-2-methylsulphoxide (**16**), Scheme 1
Brassilexin (**59**), *B. carinata, B. juncea, B. napus, B. nigra, B. rapa, Sinapis arvensis*	*L. maculans* (*P. lingam*)	Detoxified to undetermined products, Scheme 14
Brassinin (**3**), *B. napus, B. oleracea, B. rapa, R. sativus*	*L. maculans* (*P. lingam*)	Indole-3-carboxaldehyde (**18**), Scheme 2
	Sclerotinia sclerotiorum	1-β-D-Glucopyranosyl brassinin (**21**), Scheme 2
Camalexin (**10**), *Arabidopsis thaliana, Arabis lyrate, Camelina sativa, Capsella bursa-pastoris*	*Rhizoctonia solani*	5-Hydroxycamalexin (**35**), Scheme 7
	S. sclerotiorum	1-β-D-Glucopyranosyl camalexin (**39**), Scheme 7
	L. maculans (*P. lingam*)	Not metabolized
Cyclobrassinin (**57**), *B. carinata, B. juncea, B. napus, B. nigra, B. rapa*	*L. maculans* (*P. lingam*) virulent	Dioxibrassinin (**60**) further detoxified to undetermined products, Scheme 14
	L. maculans (*P. lingam*) aviralent	Brassilexin (**59**), further detoxified to undetermined products, Scheme 14
	R. solani	5-Hydroxybrassicanal A (**61**), Scheme 14
	S. sclerotiorum	1-β-D-Glucopyranosyl cyclobrassinin (63), Scheme 15
Indole-3-acetonitrile (**65**), *B. juncea*	*L. maculans* and *R. solani* (*P. lingam*) Indole-3-acetic acid (**67**), Scheme 16	Indole-3-acetic acid (**67**), Scheme 16
	S. sclerotiorum	Indole-3-acetic acid (**67**), Scheme 16
1-Methoxybrassinin (**4**), *B. napus, B. carinata, B. oleracea*	*S. sclerotiorum*	7-*O*-β-D-Glucopyranosyl 1-methoxybrassinin (**34**, *n* = 1), Scheme 6
6-Methoxy camalexin (**11**), *Capsella bursa-pastoris, C. sativa*	*S. sclerotiorum*	1-β-D-Glucopyranosyl camalexin (39), Scheme 10
1-Methylcamalexin (**12**), *C. bursa-pastoris*	*R. solani*	Indole-3-carbonitrile (**55**), Scheme 13
Spirobrassinin (**69**), *B. napus, B. oleracea, B. rapa, R. sativus*	*L. maculans* (*P. lingam*)	Not metabolized, Scheme 17

2.2. Brassinins and analogs

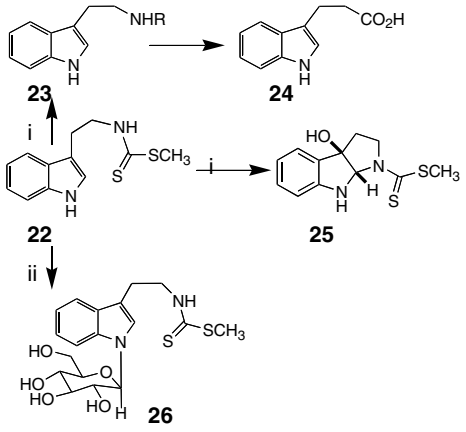
The metabolism and detoxification of the phytoalexin brassinin (**3**) by virulent isolates of *L. maculans*/*P. lingam* led to indole-3-carboxylic acid (**19**) via methyl 3-indolylmethyl dithiocarbamate-S-oxide (**17**) and indole-3-carboxaldehyde (**18**) (Pedras and Taylor, 1991; Pedras et al., 1992; Pedras and Taylor, 1993). However, avirulent isolates of *L. maculans*/*P. lingam* (now considered a different *Leptosphaeria*/*Phoma* species, reviewed in Howlett et al., 2001) transformed brassinin into indole-3-carboxaldehyde (**18**) and indole-3-carboxylic acid (**19**) via indolyl-3-methanamine (**20**, R=H) and N_b-acetyl-3-indolyl-methanamine (**20**, R=Ac). By contrast, the stem rot fungus *S. sclerotiorum* metabolized brassinin (**3**) to 1-β-D-glucopyranosylbrassinin (**21**), a rather different pathway, as shown in Scheme 2 (Pedras et al., 2004a). The antifungal activity of brassinin (**3**) and its metabolites was compared using spore germination and radial mycelial growth assays (Pedras and Taylor, 1993; Pedras et al., 2004a). Results of these assays indicated that the metabolism of brassinin (**3**) by virulent or



Scheme 1. Detoxification of the phytoalexin brassicanal A (**13**) by the fungal pathogen *Leptosphaeria maculans*/*Phoma lingam* (Pedras and Khan, 1996; Pedras et al., 1997).



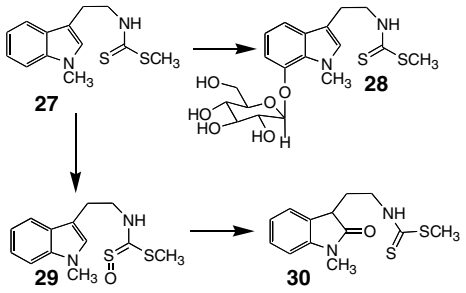
Scheme 2. Detoxification of the phytoalexin brassinin (**3**) by the fungal pathogens (i) *Leptosphaeria maculans*/*Phoma lingam* (Pedras et al., 1992; Pedras and Taylor, 1991; Pedras and Taylor, 1993); (ii) *Sclerotinia sclerotiorum* (Pedras et al., 2004a).



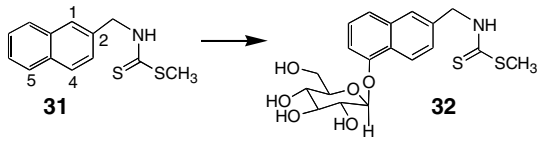
Scheme 3. Detoxification of the phytoalexin analog methyl tryptamine dithiocarbamate (**22**) by the fungal pathogens: (i) *Leptosphaeria maculans*/*Phoma lingam* (Pedras and Okanga, 1998; Pedras and Okanga, 2000); (ii) *Sclerotinia sclerotiorum* (Pedras et al., 2004a).

avirulent isolates of *L. maculans*/*P. lingam* and *S. sclerotiorum* was a detoxification, as brassinin metabolites had no significant antifungal activity. In addition, it was established for the first time that brassinin (**3**) inhibited the biosynthesis of non-selective toxins in *L. maculans*/*P. lingam* (Pedras and Taylor, 1993).

To probe the selectivity of the enzymes involved in the detoxification of brassinin (**3**), the metabolism of several analogs by *L. maculans*/*P. lingam* and *S. sclerotiorum* was investigated (Pedras and Okanga, 1998, 2000; Pedras et al., 2004a). Compounds **23** and **25** were the major metabolites obtained from transformation of **22** by *L. maculans*/*P. lingam* (Scheme 3). There appeared to be two major pathways, one leading to acid **24** via tryptamine (**23**, R=H) and acetyl tryptamine (**23**, R=Ac) and the other leading to dithiocarbamate **25**. The metabolism of dithiocarbamate **22** by virulent isolates of *L. maculans*/*P. lingam* was a detoxification that proceeded slower than that of the naturally occurring phytoalexin **3**. The difference between the fungal metabolism of compounds **3** and **22** was attributed to either higher toxicity of **22**, and/or to the specificity of the enzymes involved in the transformations (Pedras and



Scheme 4. Detoxification of the phytoalexin analog methyl 1-methyltryptamine dithiocarbamate (27) by *Sclerotinia sclerotiorum* (Pedras et al., 2004a).



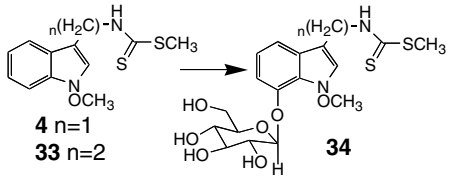
Scheme 5. Detoxification of the phytoalexin analog methyl 2-naphthylmethyl dithiocarbamate (**31**) by *Sclerotinia sclerotiorum* (Pedras et al., 2004a).

Okanga, 2000). In the case of *S. sclerotiorum*, compounds **22**, **27** and **31** were metabolized to their respective glucosides **26**, **28** and **32** as shown in Schemes 3–5 (Pedras et al., 2004a). The rate of detoxification was much slower for these analogs than that observed for brassinin (**3**), probably because these analogs displayed stronger antifungal activity. Compound **27** was however metabolized by *S. sclerotiorum* via two pathways: glucosylation of **22** at C-7 and oxidation to methyl 1-methyl-2-oxotryptamine dithiocarbamate (**30**) via methyl 1-methyltryptamine dithiocarbamate-S-oxide (**29**) as shown in Scheme 4. It is worthy to note that the 2-naphthylmethyl dithiocarbamate **31** was glucosylated at C-5, similar to the 1-protected indole **27**. However, detoxification of 1-naphthylmethyl dithiocarbamate yielded a number of products (Pedras et al., 2004a).

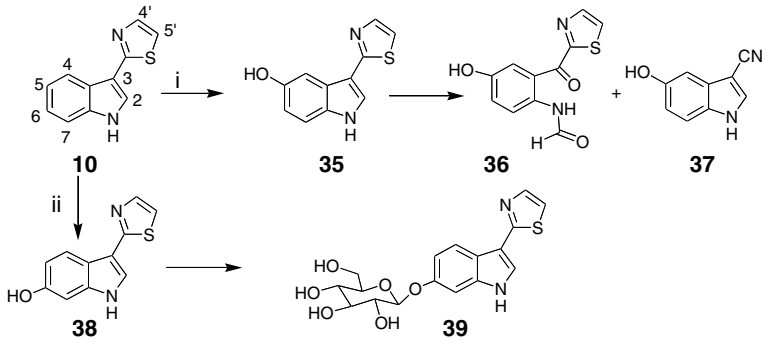
S. sclerotiorum metabolized and detoxified 1-methoxybrassinin (**4**), another brassica phytoalexin to the 1-methoxy-7-(β-*O*-glucopyranosyl brassinin (**34** $n = 1$) which showed significantly lower antifungal activity than **4** (Scheme 6) (Pedras et al., 2004a). Similarly, the analog **33** was metabolized to **34** ($n = 2$) (Scheme 6) (Pedras et al., 2004a).

2.3. Camalexins and analogs

Camalexin (**10**) was metabolized by *R. solani*, a pathogen of *Camelina sativa* (false flax), (Pedras and Khan, 1997, 2000) and *S. sclerotiorum* (Pedras and Ahiahonu, 2002). Incubation of *R solani* with camalexin (**4**) resulted in the detection and isolation of metabolites **35**–**37**, whose structures were deduced from their respective spectroscopic data and confirmed by synthesis (Scheme 7). Camalexin (**10**) was metabolized by *S. sclerotiorum* to **39** via 6-hydroxycamalexin (**38**) (Pedras and



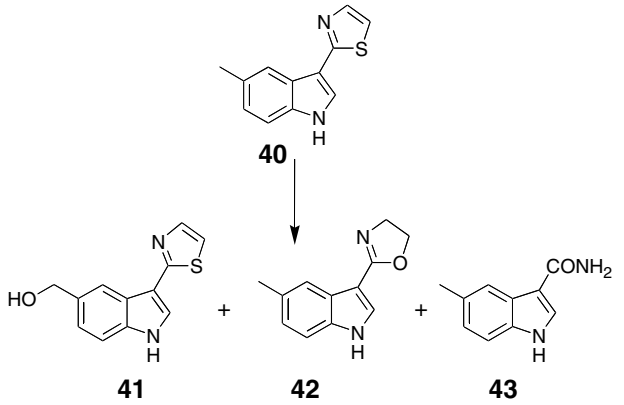
Scheme 6. Detoxification of the phytoalexin 1-methoxybrassinin (**4**) and analog methyl 1-methoxytryptamine dithiocarbamate (**33**) by *Sclerotinia sclerotiorum* (Pedras et al., 2004a).



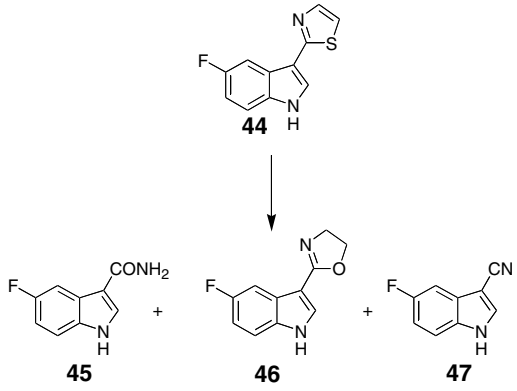
Scheme 7. Detoxification of the phytoalexin camalexin (**10**) by fungal pathogens (i) *Rhizoctonia solani* (Pedras and Khan, 1997); (ii) *Sclerotinia sclerotiorum* (Pedras and Ahiahonu, 2002).

Ahiahonu, 2002). The biotransformation products of camalexin (**10**) by *R. solani* (Pedras and Khan, 1997) and *S. sclerotiorum* (Pedras and Ahiahonu, 2002) were found to be significantly less toxic than camalexin (**10**) itself. Agar plates containing camalexin (**10**) at 5×10^{-4} M inoculated separately with *R. solani* and *S. sclerotiorum* showed no mycelia growth after incubation for one week, whereas metabolites **35** and **39** at identical concentrations showed only a very slight inhibitory effect. This led to the conclusion that the metabolism of camalexin (**10**) by *R. solani* and *S. sclerotiorum* were detoxifications (Pedras and Khan, 1997; Pedras and Ahiahonu, 2002).

To attempt to probe the selectivity of the detoxifying enzymes of *R. solani*, designer camalexins **40** and **44** were synthesized and fed to cultures of *R. solani*. 5-Methylcamalexin (**40**) was transformed via two pathways, one hydroxylation to **41**, and the other to **42** and **43** as shown in Scheme 8 (Pedras and Liu, 2004). 5-Fluorocamalexin (**44**) was metabolized by *R. solani* to metabolites **45**–**47** (Scheme 9). It was determined that 5-hydroxymethylcamalexin (**41**), the major metabolite from **40**, did not inhibit mycelial growth, even at the highest concentration tested. In addition, 5-fluoroindole-3-carbonitrile (**47**), showed partial mycelial growth



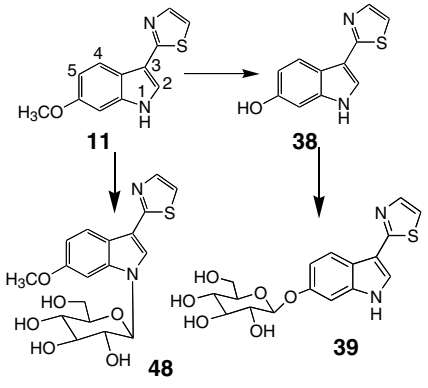
Scheme 8. Detoxification of the camalexin analog 5-methylcamalexin (**40**) by the fungal pathogen *Rhizoctonia solani* (Pedras and Liu, 2004).



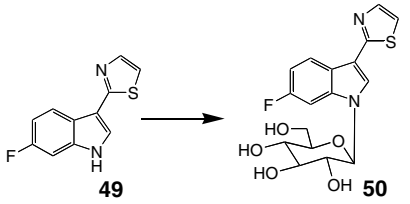
Scheme 9. Detoxification of the camalexin analog 5-fluorocamalexin (**44**) by the fungal pathogen *Rhizoctonia solani* (Pedras and Liu, 2004).

inhibition, but both were substantially less inhibitory than their starting materials. Thus, it was concluded that the transformations of **40** and **44** by *R. solani* are detoxification processes (Pedras and Liu, 2004).

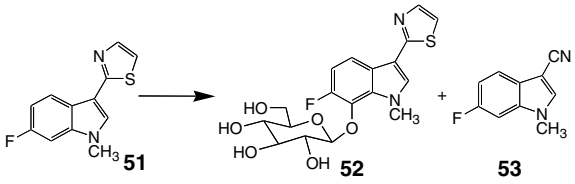
The phytoalexin 6-methoxycamalexin (**11**) was also metabolized by *S. sclerotiorum* to **39** via **38**, as observed in the metabolism of camalexin (**10**) (Schemes 7 and 10). In addition, 6-methoxycamalexin (**11**) was partly converted to the minor metabolite **48**; that is, *S. sclerotio-*



Scheme 10. Detoxification of the phytoalexin 6-methoxycamalexin (**11**) by the fungal pathogen *Sclerotinia sclerotiorum* (Pedras and Ahiahonu, 2002).

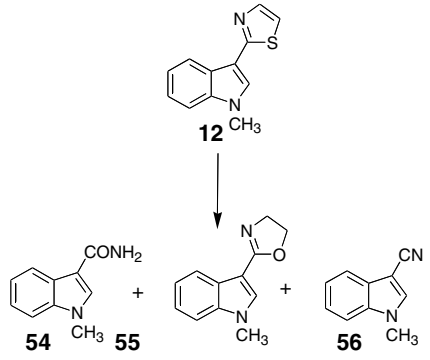


Scheme 11. Detoxification of the phytoalexin analog 6-fluorocamalexin (**49**) by the fungal pathogen *Sclerotinia sclerotiorum* (Pedras and Ahiahonu, 2002).

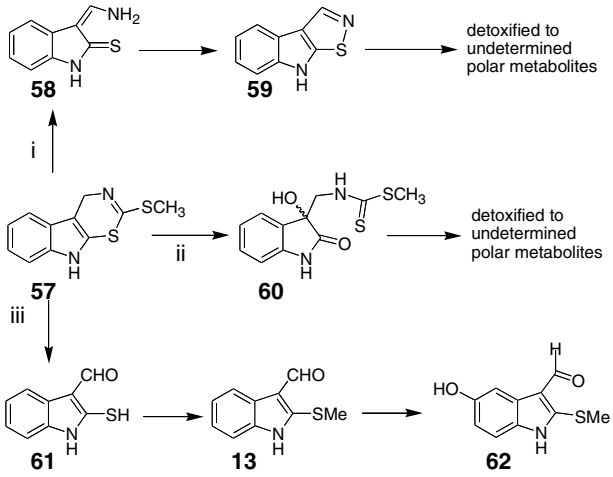


Scheme 12. Detoxification of the phytoalexin analog 6-fluoro-1-methylcamalexin (**51**) by the fungal pathogen *Sclerotinia sclerotiorum* (Pedras and Ahiahonu, 2002).

rum converted 6-methoxycamalexin (**11**) via two pathways, with the major product **39** resulting from demethylation of the methoxy group at C-6, followed by glucosylation. Interestingly, similar to brassinin detoxification, the minor product **48** resulted from direct *N*-glucosylation (Scheme 10). Subsequently, it was anticipated that if the detoxifying enzyme(s) of *S. sclerotiorum* were specific, blocking the C-6 of camalexin would direct glucosylation to N-1, and blocking both N-1 and C-6 sites would slow down if not stop glucosylation. Thus camalexins **49** and **51** were synthesized and separately administered to fungal cultures of *S. sclerotiorum*. Compounds **49** and **51** were metabolized to **50**, **52** and **53** as shown in Schemes 11 and 12 but at significantly slower rates (Pedras and Ahiahonu, 2002). 1-Methylcamalexin (**12**), a phytoalexin from *Capsella bursa-pastoris*, was metabolized by *R. solani* to metabolites **54**–**56** devoid of antifungal activity (Scheme 13).



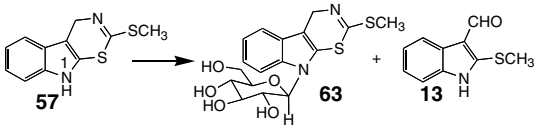
Scheme 13. Detoxification of the phytoalexin 1-methylcamalexin (**12**) by the fungal pathogen *Rhizoctonia solani* (Pedras and Liu, 2004).



Scheme 14. Detoxification of the phytoalexin cyclobrassinin (**57**) by fungal pathogens (i) *Leptosphaeria maculans*/*Phoma lingam* avirulent; (ii) *L. maculans*/*P. lingam* virulent; (iii) *Rhizoctonia solani* (Pedras and Okanga, 1998; Pedras and Okanga, 1999).

2.4. Cyclobrassinin

The metabolism of cyclobrassinin (**57**) by avirulent and virulent isolates (now considered different species) of *L. maculans*/*P. lingam* was strikingly different from that of brassinin (**3**) (Scheme 14) (Pedras and Okanga, 1998, 1999). HPLC analysis of organic extracts of cultures of avirulent isolates incubated with **57** indicated a rapid decrease in the concentration of **57** and the concurrent appearance of two additional constituents. Spectroscopic analysis established that one of the constituents was the known phytoalexin brassilexin (**59**) and the other constituent, a relatively less stable metabolite, a mixture of 3-methylenaminoindolyl-2-thione (**58**) and/or related tautomers. Two days after incubation of the avirulent isolates with cyclobrassinin (**57**) no brassilexin (**59**) or other putative metabolites were detected in any of the cultures or their extracts. Similar experiments carried out with virulent isolates of *L. maculans*/*P. lingam* incubated with cyclobrassinin (**57**) afforded yet another known phytoalexin, dioxibrassinin (**60**). Two days after incubation of a virulent isolate with cyclobrassinin (**57**), no phytoalexins or derivatives were detected in any of the cultures or their extracts. Additional studies with the root rot fungus *R. solani* established that cyclobrassinin (**57**) was metabolized and detoxified via the phytoalexin brassicanal A (**13**), which was further metabolized into non-toxic products (Scheme 14). These interesting results demonstrated that cyclobrassinin (**57**) was detoxified via the phytoalexins brassilexin (**59**), dioxibrassinin (**60**), or brassicanal A (**13**), depending on the fungal species, as shown in Scheme 14 (Pedras and Okanga, 1999). It is worth noting that cyclobrassinin (**57**) was demonstrated to be an in planta

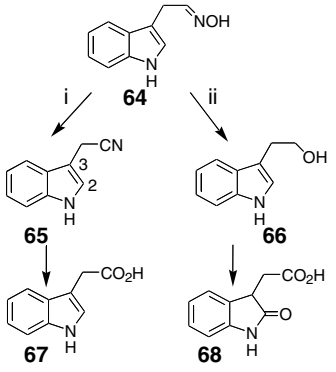


Scheme 15. Detoxification of the phytoalexin cyclobrassinin (**57**) by the fungal pathogen *Sclerotinia sclerotiorum* (Pedras et al., 2004a).

biosynthetic precursor of brassilexin (**59**) (Pedras et al., 1998) and also proposed to be a biosynthetic precursor of brassicanal A (**13**) (Monde et al., 1996). Therefore, it was suggested that some phytopathogens might have acquired a more effective mechanism for overcoming this phytoalexin by adopting biosynthetic pathways operating in planta (Pedras and Okanga, 1999). By contrast, the metabolism of the phytoalexin cyclobrassinin (**57**) by the fungal pathogen *S. sclerotiorum* was similar to that of brassinin (**3**), in that the main product resulted from glucosylation of **57** at N-1, as shown in Scheme 15.

2.5. Indole-3-acetonitrile

Indole-3-acetonitrile (**65**) was determined to be a phytoalexin in *B. juncea* but a phytoanticipin in other *Brassica* species (Pedras et al., 2002). Indole-3-acetaldoxime (**64**) is an immediate precursor of **65** and a precursor of most if not all cruciferous phytoalexins. To determine if fungal pathogens can metabolize **64** and **65**, cultures of three plant pathogens (*L. maculans*/*P. lingam*, *S. sclerotiorum*, and *R. solani*) and an insect pathogen (*Beauveria bassiana*) were used (Pedras and Montaut, 2003). Interestingly, all plant pathogenic species converted **64** via dehydration to indole-3-acetonitrile (**65**), whereas the insect pathogenic species converted **64** via reduction to tryptophol (**66**). Nitrile **65** was further transformed into indole-3-acetic acid (**67**) by the plant pathogens, but the insect pathogen, *B. bassiana*, converted **65** into 2-oxoindole-3-acetic acid (**68**) as shown in Scheme 16. The overall results of these studies demonstrated that all the species were able to metabolize oxime **64**; however, similar to the plant biosynthetic pathway, the plant pathogens metabolized **64** via nitrile **65**, independently of the species. To determine if the biotransformations observed were a detoxification process, the antifungal activity of indole-3-acetaldoxime (**64**), indole-3-acetonitrile (**65**) and acids **67** and **68** were determined. Results of these assays showed that indole-3-acetaldoxime (**64**) was less inhibitory to *P. lingam* and *R. solani* than indole-3-acetonitrile. However, oxime **64** was more inhibitory to *S. sclerotiorum* than nitrile **65** (little effect). Acids **67** and **68** had no detectable inhibitory effect on any of the species at the concentrations tested. Neither indole-3-acetaldoxime (**64**) nor indole-3-ethanol (**66**) showed inhibitory activity against *B. bassiana* (Pedras and Montaut, 2003). Because the biotransformation of **64** by the non-phytopathogen *B. bassiana* yielded indole-3-acetic acid via tryptophol (**66**), it was suggested that the enzymes responsible for these transformations are specific to crucifers and to their pathogens. Due to the central role played by oxime **64** in the biosynthesis of several secondary metabolites of crucifers, it would be of great interest to analyze and compare the fungal enzymes involved in the transformation of **64** with related plant enzymes. Such a study should provide a better understanding of the co-evolution of indole containing secondary metabolites in plants and their pathogens (Pedras and Montaut, 2003) (Scheme 17).



Scheme 16. Metabolism of indole-3-acetaldoxime (**64**) and detoxification of the phytoalexin indole-3-acetonitrile (**65**) by the fungal pathogens: (i) *Leptosphaeria maculans*/*Phoma lingam, Sclerotinia sclerotiorum*, and *Rhizoctonia solani*; (ii) *Beauveria bassiana* (Pedras and Montaut, 2003).

2.6. Miscellaneous

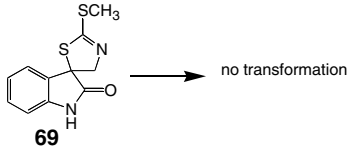
Studies on the biotransformation of the phytoalexins brassilexin (**59**) and dioxibrassinin (**60**) by *L. maculans*/*P. lingam* (Scheme 14) indicated that these phytoalexins were transformed but no biotransformation products were detected or isolated (Pedras and Okanga, 1998, 1999). This work confirmed results of a previous study on the fungal metabolism of brassilexin (**59**) (Rouxel et al., 1995). It is likely that the metabolic product(s) of brassilexin might be very polar and more soluble in the aqueous medium than in organic solvents, thus precluding extraction and detection. Although blackleg fungi could metabolize and detoxify brassinin (**3**), cyclobrassinin (**57**), dioxibrassinin (**60**), brassilexin (**59**) and brassicanal A (**13**), neither camalexin (**10**) nor spirobrassinin (**69**) were metabolized by either virulent or avirulent type isolates of *L. maculans*/*P. lingam* (Pedras et al., 1997; Pedras, 1998).

Table 2
Metabolism of phytoalexins from the family Leguminosae by plant pathogenic fungi

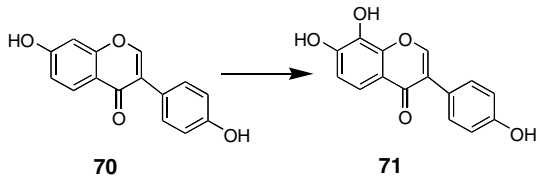
Phytoalexin, plant species	Fungal species	Major products of metabolism
Daidzein (**70**), *Arachis hypogaea*	*Aspergillus saitoi*	8-Hydroxydaidzein (**71**), Scheme 18
Formononetin (**72**), *Arachis hypogaea*	*Fusarium proliferatum*	7-*O*-methylformononetin (**74**)
	F. avenaceum	Calycosin (**74**), Scheme 19
Kievitone (**75**), *Phaseolus vulgaris*	*F. solani*	Kievitone hydrate (**76**), Scheme 20
2,3-Dehydrokievitone (**77**), *Phaseolus vulgaris*	*A. flavus*	Dihydrofurano-isoflavone (**78**)
	Botrytis cinerea	Dihydropyrano-isoflavone (**79**) 2,3-Dehydrokievitone glycol (**80**), Scheme 21
Maackiain (**81**), *Medicago sativa, Cicer arietinum*	*Stemphylium botryosum*	Maackiainisoflavan (**82**)
	N. haematococca	(−)-6a-Hydroxymaackiain (**83**) 1a-Hydroxymaackiain (**84**) Sophorol (**85**), Scheme 22
Medicarpin (**95**), *Arachis hypogaea, Medicago sativa, Cicer arietinum*	*F. proliferatum, B. cinerea*	Demethylmedicarpin (**98**)
	Sclerotinia trifoliorum	6a-Hydroxypterocarpans (**97**)
	S. botryosum	Vestitol (**96**)
	N. haematococca	Vestitone (**102**)
	F. oxysporum	Isoflaven (**100**)
		Demethylvestitol (**99**), Scheme 23
Phaseollidin (**111**), *Phaseolus vulgaris*	*F. solani*	Phaseollidin hydrate (**112**), Scheme 26
Phaseollin (**113**), *Phaseolus vulgaris*	*S. botryosum*	Phaseollin isoflavan (**117**)
	F. solani	1a-Hydroxyphaseollone (**118**)
	Cladosporium herbarum	6a-Hydroxyphaseollin (**114**)
	Collectotrichum	6a,7-Dihydroxyphaseollin (**115**)
	Lindemuthianum	11,1 2-Dihydrodihydroxyphaseollin
	Septoria nodorum	(**116**), Scheme 27
(+)-Pisatin (**1**), *Pisum sativum*	*N. haematococca*	(+)-6a-Hydroxymaackiain (**109**), Scheme 25
	Aschochyta pisi	

3. Metabolism and detoxification of phytoalexins from the family Leguminosae

Most of the legume phytoalexins have an isoflavonoid skeleton derived from mixed acetate–malonate and shikimate pathways. Among these isoflavonoids are isoflavones, isoflavanones, pterocarpans, stilbenes and chalcones (Harborne, 1999; Farooq and Tahara, 1999). Phytopathogenic fungi have been shown to detoxify phytoalexins produced by plants of the Leguminosae family, including pea (*P. sativum*) from which the phytoalexin (+)-pisatin (**1**) was isolated, bean (*Phaseolus vulgaris*), yam bean (*Pachyrrhizus erosus*), alfalfa (*Medicago sativa*), chickpea (*Cicer arietinum*), and groundnut (*Arachis hypogaea*), as summarized in Table 2.



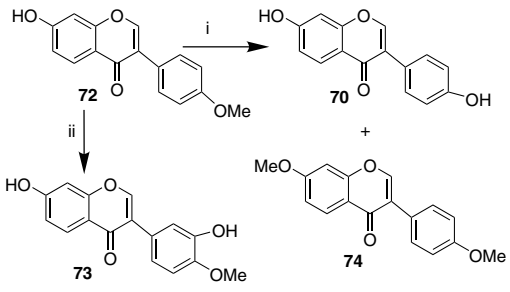
Scheme 17. The phytoalexin spirobrassinin (**69**) is not metabolized by *Leptosphaeria maculans*/*Phoma lingam* (Pedras, 1998).



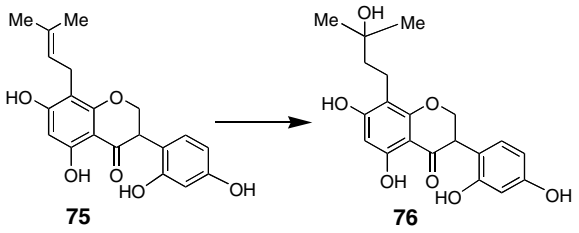
Scheme 18. Detoxification of the groundnut phytoalexin daidzein (**70**) by the fungal pathogen *Aspergillus saitoi* (Esaki et al., 1998).

3.1. Daidzein and formononetin

The phytoalexin daidzein (**70**) (Durango et al., 2002) was metabolized by the fungal pathogen *Aspergillus saitoi* to the potent antioxidant isoflavone 8-hydroxydaidzein (**71**) (Scheme 18) (Esaki et al., 1998). The co-occurring phytoalexin formononetin (**72**) was also



Scheme 19. Detoxification of the groundnut phytoalexin formononetin (**72**) by the fungal pathogens: (i) *Fusarium proliferatum*; (ii) *F. avenaceum* (Weltring et al., 1982).



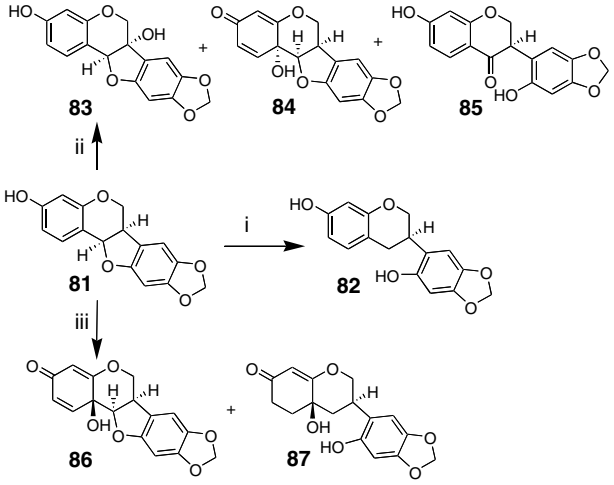
Scheme 20. Detoxification of the bean phytoalexin kievitone (**75**) by fungal pathogen *Fusarium solani* f. sp. *phaseoli* (Smith et al., 1981).

metabolized by the fungal pathogen *Fusarium avenaceum* to calycosin (**73**) and by *F. proliferatum* to the major product 7-*O*-methylformononetin (**74**) and to daidzein (**70**) (Scheme 19) (Weltring et al., 1982).

3.2. Kievitones

The bean phytoalexin kievitone (**75**) was detoxified by *F. solani* f. sp. *phaseoli* to kievitone hydrate **76** (Scheme 20) (Smith et al., 1981). The enzyme responsible for this transformation, kievitone hydratase (KHase) is a highly glucosylated acidic extracellular glycoprotein with MW 158–188 kDa, which was purified from culture broths by ion exchange, affinity chromatography, gel filtration and isoelectric focusing (Kuhn and Smith, 1979; Turbek et al., 1990). The KHase cDNA and gene were cloned (Li et al., 1995) and expressed in *Neurospora crassa* and *Emericella nidulans*. In a survey of different isolates and mutants of *F. solani* the level of KHase activity correlated with the degree of tolerance to kievitone (**75**) and pathogenicity on bean (Weltring, 1991).

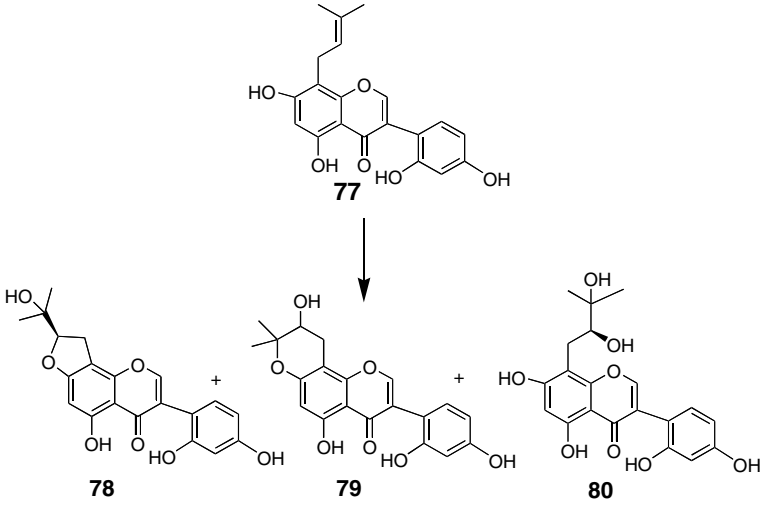
2,3-Dehydrokievitone (**77**) was metabolized by *Aspergillus flavus* and *B. cinerea* to three metabolites namely dihydrofurano-isoflavone (**78**), dihydropyrano-isoflavone (**79**) and 2,3-dehydrokievitone glycol (**80**) as outlined in Scheme 21 (Tahara et al., 1987).



Scheme 22. Detoxification of the alfalfa and chickpea phytoalexin maackiain (**81**) by the fungal pathogens (i) *Stemphylium botryosum* (Higgins, 1975); (ii) *Nectria haematococca* (Denny and VanEtten, 1982); (iii) *Cercospora medicaginis* (Soby et al., 1996).

3.3. Maackiain

Microbial transformation of the phytoalexin maackiain (**81**) to maackiainisoflavan (**82**) was reported using *Stemphylium botryosum* (Higgins, 1975), whereas *N. haematococca* (Denny and VanEtten, 1982) afforded (–)-6a-hydroxymaackiain (**83**), 1a-hydroxydienone **84** and sophorol (**85**), as summarized in Scheme 22. Field isolates of *N. haematococca* were tested for their ability to metabolize maackiain (**81**), tolerance of maackiain, and virulence on chickpea (Lucy et al., 1988). Most



Scheme 21. Detoxification of the bean phytoalexin 2,3-dehydrokievitone (**77**) by the fungal pathogens *Aspergillus flaviis* and *Botrytis cinerea* (Tahara et al., 1987).

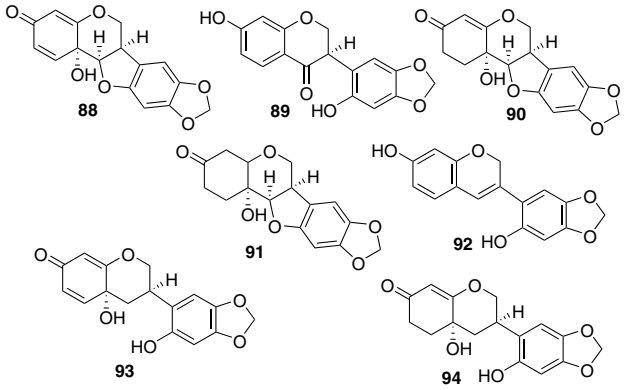
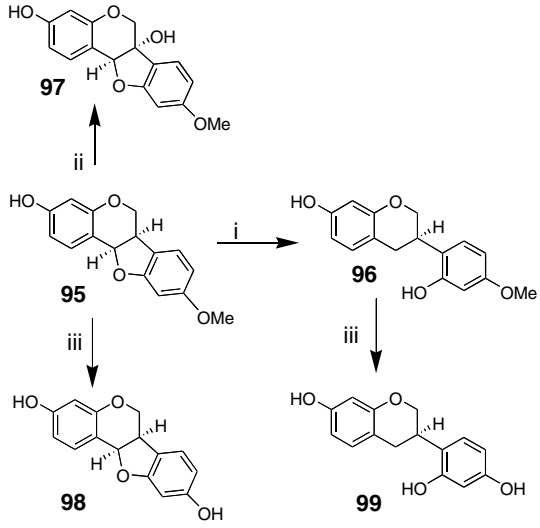


Fig. 1. Metabolites from the detoxification of the alfalfa and chickpea phytoalexin maackiain (**81**) by the fungal pathogen *Ascochyta rabiei* (Lucy et al., 1988; Höhl et al., 1989).



Scheme 23. Detoxification of medicarpin (**95**) by the plant pathogens of alfalfa and chickpea: (i) *Stemphylium botryosum* (Steiner, 1977); (ii) *Botrytis cinerea* (Ingham, 1976) and *Sclerotinia trifoliorum* (Bilton et al., 1976); (iii) *Fusarium proliferatum* (Weltring and Barz, 1980).

of the isolates were quite tolerant of maackiain, as the growth was inhibited by less than 25% at 50 μg/mL. The more sensitive isolates were all low in virulence on chickpea, suggesting that some level of tolerance to maackiain (**81**) was required for high virulence on this plant (Lucy et al., 1988). All isolates that were able to metabolize maackiain (**81**) were tolerant of it, and most isolates lacking this ability were sensitive. However, several maackiain-tolerant isolates failed to metabolize maackiain, and one of these was virulent on chickpea, suggesting that phytoalexin tolerance mechanisms other than detoxification may also be important. Another fungal pathogen *Cercospora medicaginis* detoxified maackiain (**81**) to **86** and **87** (Scheme 22) (Soby et al., 1996). In addition, *N. haematococca* and *S. botryosum* metabolized **81** to compounds **82**–**85** as shown in Scheme 22 (Denny and VanEtten, 1982).

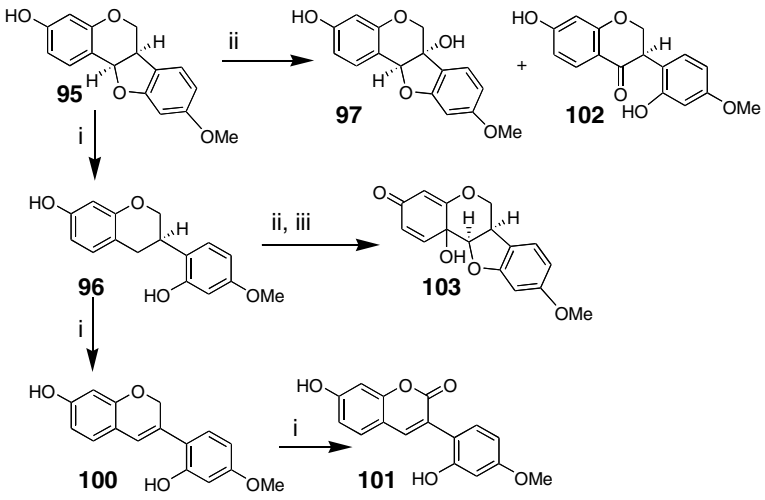
Three different maackiain metabolism genes were identified in *N. haematococca* MP VI (Miao and VanEtten, 1992a). Strains carrying *MAK1* and *MAK2* detoxified maackiain (**81**) via 1a-hydroxylation to 1a-hydroxymaackiain (**84**) whereas strains carrying *MAK3* led to 6a-hydroxylation to yield (–)-6a-hydroxymaackiain (**83**) (Scheme 22). *MAK1* and *MAK2* were unusual in that they often failed to be inherited by progeny. *MAK1* was closely linked to *PDA*6, a new member in a family of genes in *N. haematococca* that encode enzymes for detoxification of pisatin (**1**). Like *MAK1*, *PDA6* was also transmitted irregularly to progeny (Miao and VanEtten, 1992a). The contribution of *MAK1* to the virulence of *N. haematococca* MP VI on chickpea was tested (Enkerli et al., 1998). The *MAK1* gene was disrupted in a highly virulent MAK^{+} isolate or added to a weakly virulent MAK^{-} isolate via transformation. The disruption of *MAK1* decreased virulence to a moderate level, while addition of multiple copies of *MAK1* increased virulence to either a moderate or high level. These data demonstrated that maackiain (**81**) detoxification was a determinant, but not the only determinant of virulence in *N. haematococca* MP VI isolates capable of causing disease on chickpea (Enkerli et al., 1998). *MAK1* is located on a 1.6-Mb conditionally dispensable chromosome. Loss of the *MAK1* chromosome did not reduce virulence towards chickpea further, indicating that no additional genes for virulence are located on the *MAK1* chromosome (Enkerli et al., 1998).

Investigation of the metabolism and detoxification of maackiain (**81**) by 10 strains of *Ascochyta rabiei* (using mycelial preparations and crude protein extract) led to the isolation of seven metabolites **88**–**94** (Fig. 1), which have lower antifungal activity than **81** (Lucy et al., 1988; Höhl et al., 1989).

3.4. Medicarpin

Studies on the fungal metabolism of the phytoalexin medicarpin (**95**) revealed that alfalfa pathogenic fungi could metabolize it. *Stemphylium botryosum* metabolized **95** to vestitol (**96**) (Steiner, 1977), whereas *B. cinerea* (Ingham, 1976) and *Sclerotinia trifoliorum* (Bilton et al., 1976) yielded 6a-hydroxymedicarpin (**97**) (Scheme 23). In addition, demethylmedicarpin (**98**) and demethylvesitol (**99**) were also isolated when **95** and **96** were incubated with *Fusarium proliferatum* (Weltring and Barz, 1980).

Medicarpin (**95**) was also metabolized by *F. oxysporum* f. sp. *lycopersici* (Weltring et al., 1983) to isoflaven (**100**), 3-arylcoumarin (**101**) via vestitol (**96**). *N. haematococca* transformed medicarpin (**95**) to less toxic



Scheme 24. Detoxification of medicarpin (**95**) by pathogens of alfalfa and chickpea: (i) *Fusarium oxysporum* f. sp. *lycopersici* (Weltring et al., 1983); (ii) *Nectria haematococca*; (iii) *Fusarium solani* f. sp. *phaseoli* (Denny and VanEtten, 1982).

metabolite **97**, vestitone (**102**) and metabolite **103** as shown in Scheme 24 (Denny and VanEtten, 1982).

The study of the metabolism and detoxification of medicarpin (**95**) by four strains of *A. rabiei* (Lucy et al., 1988; Höhl et al., 1989) another chickpea pathogen resulted in the identification of 10 metabolites (**96**, **98**, **99**, **101**, **103**, **104**–**108**), which seemed to be degraded to non-aromatic compounds. These metabolites were less toxic to the fungus than **95**, demonstrating that the metabolism was a detoxification process (Fig. 2).

3.5. Pisatin

The pea (*P. sativum*) phytoalexin (+)-pisatin (**1**) was metabolized by the pea fungal pathogen *N. haematococca* to (+)-6a-hydroxymaackiain (**109**) via demethylation (Scheme 25) (VanEtten et al., 1975; Schäfer et al., 1989). (+)-Pisatin (**1**) was also metabolized by *A. pisi* (Scheme 25) to (+)-6a-hydroxymaackiain (**109**) and metabolite **110** (VanEtten et al., 1989). In addition, it was established that several fungal pea pathogens (*F. oxysporum*, f. sp. *pisi*, *Mycosphaerella pinodes*, *Phoma pinodella* and *Thanatephorus cucumeris*) were substantially more sensitive to (+)-pisatin (**1**) than to (+)-6a-hydroxymaackiain (**109**), confirming that the metabolism was a detoxification process (Desjardins and VanEtten, 1986). The detoxifying enzyme, pisatin demethylase (PDA), which is inducible in *N. haematococca* MP VI, is a microsomal cytochrome P450 monooxygenase (Matthews and VanEtten, 1983; Maloney and VanEtten, 1994). The product of the *PDA* gene was the first member of a new family of cytochrome P450's. Through genetic analysis, six other pisatin demethylase genes have been identified: *PDA2-7* (Mack-

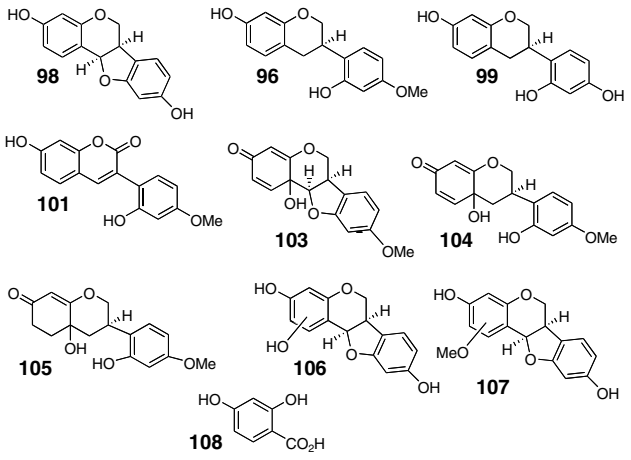
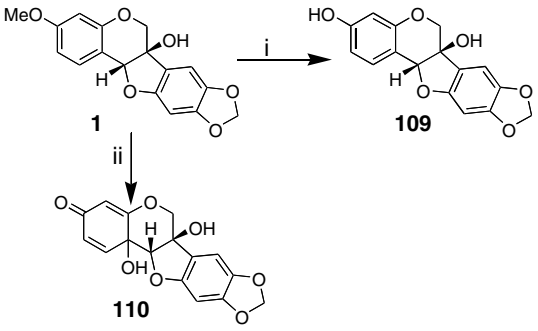


Fig. 2. Metabolites from the detoxification of the alfalfa and chickpea phytoalexin medicarpin (**95**) by the fungal pathogen *Ascochyta rabiei* (Lucy et al., 1988; Höhl et al., 1989).

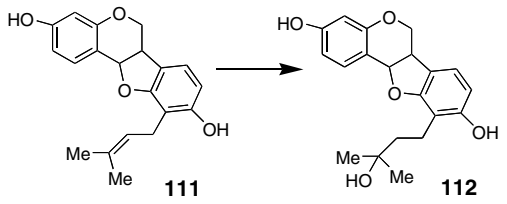


Scheme 25. Detoxification of the pea phytoalexin (+)-pisatin (**1**) by the fungal pathogens: (i) *Ascochytapisi* (VanEtten et al., 1989); (ii) *Nectria haematococca* (VanEtten et al., 1975; Schäfer et al., 1989).

intosh et al., 1989; Funnell et al., 2002). While *PDA1* and *PDA4* confer high levels of demethylase activity, *PDA2*, *PDA3*, *PDA5*, *PDA6*, and *PDA7* confer low rates of demethylation. However, all highly virulent progenies always contained one of *PDA1* or *PDA4* and a combination of *PDA5* and *PDA7* (Funnell et al., 2002). When *PDA1* was used to probe the DNA of other pea pathogens with *PDA* activity, only the DNA of *F. oxysporum* f. sp. *pisi* hybridized (Delserone et al., 1999). A *PDA* homologue from this fungus was cloned and sequenced and its amino acid sequence was 87% identical to the *N. haematococca* MP VI *PDA* gene (VanEtten et al., 2001).

Furthermore, the possibility that *PDA* can confer pathogenicity to fungi not normally pathogenic on pea was investigated (Schäfer et al., 1989). Genes encoding PDA were transformed into and highly expressed in *Cochliobolus heterostrophus*, a fungal pathogen of maize but not of pea, and *Aspergillus nidulans*, a saprophytic fungus, neither of which produces a detectable amount of PDA. Transformants contained as much *PDA* as wild type *N. haematococca*. Recombinant C. *heterostrophus* was, like the wild-type fungus, virulent on maize, but it also caused symptoms on pea, whereas recombinant *A. nidulans* did not affect pea (Schäfer et al., 1989). These important studies indicated that phytoalexins can function in non-specific resistance of plants to microbes and that saprophytes lack genes for basic pathogenicity.

Recent studies have shown that natural variants of pathogens, that either do not detoxify their host's phytoalexin, or are more sensitive, are less virulent (VanEtten et al., 2001). Results with specific mutants of *N. haematococca* have shown that phytoalexin tolerance can be a virulence trait. Early studies with *N. haematococca* demonstrated that isolates pathogenic on pea had the ability to detoxify the pea phytoalexin pisatin (**1**) via demethylation and that most isolates virulent on chickpea had the ability to detoxify the chickpea phytoalexins medicarpin (**95**) and maackiain (**81**) (Lucy et al., 1988; VanEtten et al., 1980). After the genes encoding the enzymes catalyzing these detoxifications were cloned, their role in pathogenicity was determined by mutating these genes.



Scheme 26. Detoxification of the bean phytoalexin phaseollidin (**111**) by the fungal pathogen *Fusarium solani* f. sp. *phaseoli* (Turbek et al., 1992).

In each case, the virulence of the fungus on its respective host was reduced but not eliminated, indicating that phytoalexin detoxification is a virulence trait in this fungus. Importantly, the mutants retained substantial tolerance to these phytoalexins corroborating earlier suggestions that *N. haematococca* had non-degradative mechanisms for tolerance to these phytoalexins (Denny et al., 1987; Denny and VanEtten, 1981).

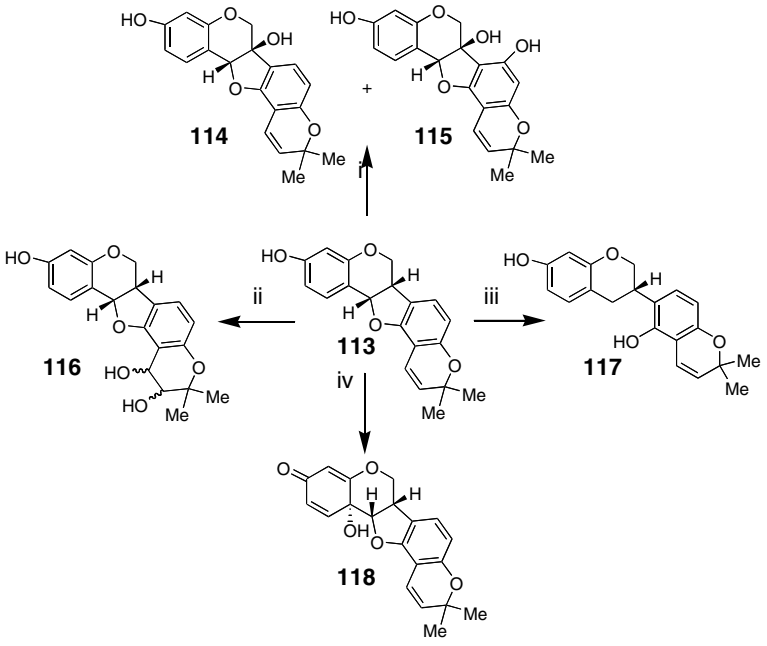
3.6. Phaseollins

Phaseollidin (**111**), a bean phytoalexin, was detoxified by *F. solani* f. sp. *phaseoli* to phaseollidin hydrate **112** (Scheme 26) (Turbek et al., 1992). When the phytoalexins kievitone (**75**) and phaseollidin (**111**) were added to liquid cultures of *F. solani* both compounds were simultaneously detoxified to kievitone hydrate (**76**) and phaseollidin hydrate (**112**), respectively. However, a purified preparation of KHase detoxified kievitone (**75**) but not phaseollidin (**111**), confirming that the hydratases involved in the detoxification of **75** and **111** were different (Miao and VanEtten, 1992b). An additional role in pathogenesis was suggested for phaseollidin hydratase during the infection of bean by *F. solani* f. sp. *phaseoli*. Because phaseollidin (**111**) is the biosynthetic precursor of both phaseollin (**113**) and phaseollin isoflavan (**117**) in bean, its unavailability prevents the biosynthesis of additional phytoalexins in the pathway, potentially reducing the resistance of the host (VanEtten et al., 1995).

The metabolism of the phytoalexin phaseollin (**113**) by *Stemphylium botryosum* yielded the phaseollin isoflavan (**117**) and by *Collectotrichum lindemuthianum* yielded 6a-hydroxyphaseollin (**114**) and 6a,7-dihydroxyphaseollin (**115**) (Higgins et al., 1974). Two pathogens, *F. solani* f. sp. *phaseoli* and *Cladosporium herbarum* (Van den Heuvel et al., 1974) oxidized **113** to 1a-hydroxyphaseollone (**118**). The metabolism of phaseollin (**113**) by *Septoria nodorum* (non-pathogenic to *Phaseolus* species), afforded a mixture of isomeric 11,12-dihydrodihydroxyphaseollin (**116**) (Scheme 27) (Bailey et al., 1977).

4. Metabolism and detoxification of phytoalexins from the family Solanaceae

Phytoalexins from plants of the Solanaceae family are from diverse biogenetic origins. They include the sesquiterpenoids and polyacetylenes derived from the acetate–mevalonate pathways, coumarins and stilbenoids derived from shikimate and acetate pathways, isoflavans and isoflavones derived from mixed acetate–mevalonate and shikimate pathways (Harborne, 1999). Phytoalexins from potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), and pepper (*Capsicum annuum*) are



Scheme 27. Detoxification of the bean phytoalexin phaseollin (**113**) by fungal pathogens (i) *Colletotrichum lindemuthianum* (Higgins et al., 1974); (ii) *Septoria nodorum* (Bailey et al., 1977; (iii) *Stemphylium botryosum*; (iv) *Fusarium solani* f. sp. *phaseoli* and *Cladosporium herbarum* (Van den Heuvel et al., 1974).

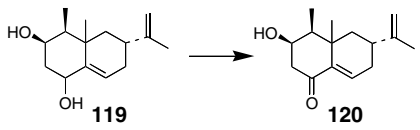
Table 3
Metabolism of phytoalexins from the family Solanaceae by plant pathogenic fungi

Phytoalexin, plant species	Fungal species	Major products of metabolism Scheme number
Capsidiol (**119**), *Capsicum annum*	*Botrytis cinerea Fusarium oxysporum*	Capsenone (**120**) Scheme 28
3-Hydroxylubimin (**130**), *Solanum tuberosum*	*Penicillium chrysogenum*	3-Hydroxy-15-dihydrolubimin (**131**), Scheme 31
Lubimin (**121**), *S. tuberosum*	*Gibberella pulicaris*	2-Dehydrolubimin (**123**)
	P. chrysogenum	15-Dihydrolubimin (**122**) Isolubimin (**124**), Scheme 29 3-Hydroxy-15-dihydrolubimin (**131**), Scheme 30
Rishitin (**132**), *S. tuberosum*	*Gibberella pulicaris*	Epoxyrishitin (**133**) 13-Hydroxyrishitin (**134**) Scheme 32
Wyerone epoxide (**135**), *Vicia faba*	*B. cinerea*	Wyerol epoxide (**136**)
	B. fabae	Dihydrodihydroxywyerol (**137**) Scheme 33

detoxified by various pathogenic fungi as summarized in Table 3.

4.1. Capsidiol

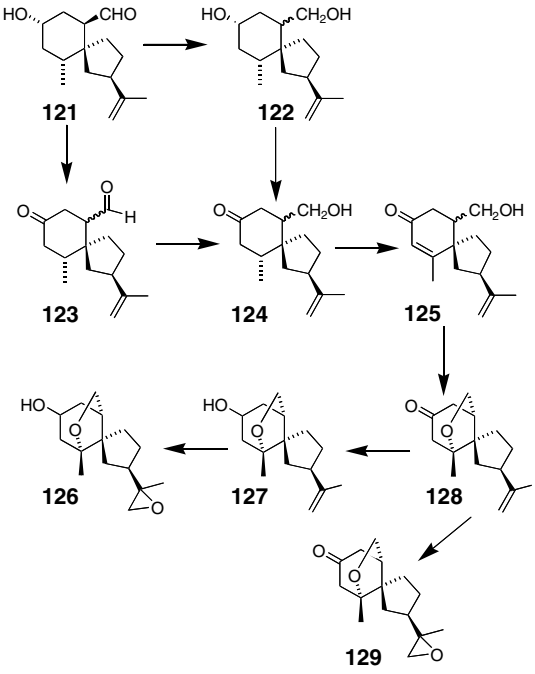
Studies on the metabolism of the pepper phytoalexin capsidiol (**119**) by *B. cinerea* and *F. oxysporum* f. sp. *vasinfectum*, pathogens of pepper, led to the detection and isolation of capsenone (**120**) (Scheme 28) (Ward and Stoessl, 1972). Results of fungitoxicity assays of capsidiol (**119**) and capsenone (**120**) indicated that the biotransformation of capsidiol (**119**) by *B. cinerea* and *F. oxysporum* was a detoxification, as capsenone (**120**) had no significant antifungal activity (Ward and Stoessl, 1972).



Scheme 28. Detoxification of hot pepper phytoalexin capsidiol (**119**) by fungal pathogens *Botrytis cinerea* and *Fusarium oxysporum* f. *vasinfectum* (Ward and Stoessl, 1972).

4.2. Lubimins

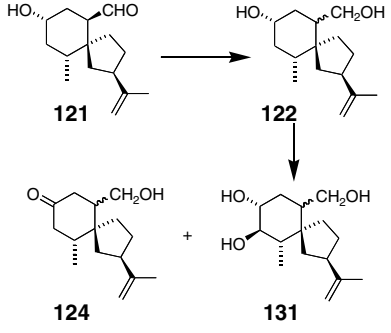
Biotransformation studies with the potato phytoalexin lubimin (**121**) and tolerant and sensitive strains (strains R-7715 and R-583 tolerant, strain R-110 sensitive) of the potato pathogen *Gibberella pulicaris* showed that the metabolism by the lubimin sensitive strain was slow but that of the tolerant strains was fast and complex (Gardner et al., 1988; Desjardins et al., 1989). The metabolism and detoxification of lubimin (**121**) by the tolerant strain R-7715 led to the detection and isola-



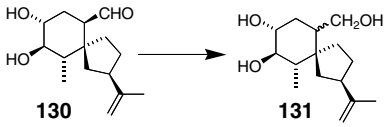
Scheme 29. Detoxification of the potato phytoalexin lubimin (**121**) by the fungal pathogen *Gibberella pulicaris* strain R-7715 (Gardner et al., 1988; Desjardins et al., 1989).

tion of seven metabolites: 2-dehydrolubimin (**123**), 15-dihydrolubimin (**122**), isolubimin (**124**), cyclodehydro isolubimin (**128**), cyclolubimin (**127**), 11,12-epoxycyclodehydrolubimin (**129**) and 11,12-epoxycyclolubimin (**126**), as shown in Scheme 29.

The reaction producing the cyclic ethers appeared to be the detoxifying step because the tricyclic products were not toxic to the fungus (Gardner et al., 1988). The pattern of lubimin (**121**) metabolism in strain R-583 was different as cyclodehydroisolubimin (**128**) was a very minor metabolite, and no lubimin metabolites of any kind were detected in extracts after 48 h incubation. The authors suggested that further metabolism of lubimin (**121**) by this strain may involve formation of water-soluble products by oxygenation or conjugation (Desjardins et al., 1989). Although all naturally occurring strains of *G. pulicaris* possess some ability to metabolize lubimin (**121**), only lubimin tolerant strains were able to rapidly convert it to completely non-toxic products. Furthermore, only strains with a high level of lubimin detoxification in vitro were highly virulent on potato tubers (Desjardins et al., 1989). Lubimin metabolism however is apparently not sufficient to ensure virulence on potato because some strains were not highly virulent, even though they metabolized lubimin in vitro (Desjardins et al., 1989).



Scheme 30. Detoxification of the phytoalexin lubimin (**121**) by the fungal pathogen *Penicillium chrysogenum* (Whitehead et al., 1990).

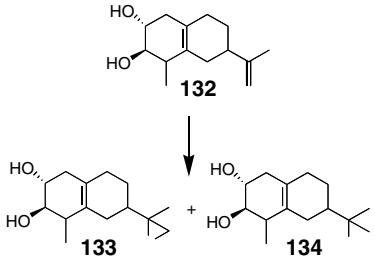


Scheme 31. Detoxification of the phytoalexin 3-hydroxylubimin (**130**) by *Penicillium chrysogenum* (Whitehead et al., 1990).

Penicillium chrysogenum, a pathogen of thorn-apple (*Datura stramonium* L.) metabolized lubimin (**121**) and another potato phytoalexin 3-hydroxylubimin (**130**) to 15-dihydrolubimin (**122**) (Schemes 30 and 31) and 3-hydroxy-15-dihydrolubimin (**131**) (Scheme 30) respectively, both in fruit cavities inoculated with spores of the fungus and in culture (Whitehead et al., 1990). However, the metabolism of **121** and **130** by *P. chrysogenum* occurred at remarkably different rates. Lubimin (**121**) was quantitatively converted to **122** in 3 h whereas 3-hydroxylubimin (**130**) was slowly metabolized to **131** (only 64% in 48 h). The 15-dihydrolubimin (**122**) formed in the fruits by the fungus was further metabolized to both isolubimin (**124**) and 3-hydroxy-15-dihydrolubimin (**131**).

4.3. Rishitin

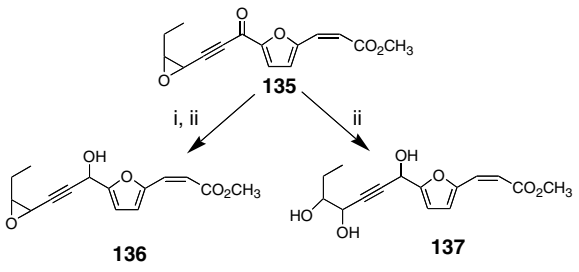
Metabolism of the potato phytoalexin rishitin (**132**) by *G. pulicaris*, a pathogen of potato, resulted in two metabolites: epoxyrishitin (**133**) and 13-hydroxyrishitin



Scheme 32. Detoxification of the potato phytoalexin rishitin (**132**) by fungal pathogen *Gibberella pulicaris* (Gardner et al., 1994).

Table 4
Metabolism of phytoalexins from the family Graminaceae by plant pathogenic fungi

Phytoalexin, plant species	Fungal species	Major products of metabolism (Scheme number)
Avenalumin I (**144**), *Avena sativa*	*Fusarium graminearum*	Undetermined
Avenalumin II (**145**), *A. sativa*	*F. graminearum*	Undetermined
Benzoic acid (**147**), *Malus domestica*	*Monilia fructigena*	4-hydroxybenzoic acid (**148**), Scheme 34
o-Hibiscanone (**149**), *Hibiscus cannabinus*	*Verticillium dahliae*	*o*-Hibiscanone hydroquinone (**150**), Scheme 35
Resveratrol (**2**), *Vitis vinifera, Arachis hypogeae,* and several others, including *Arachis hypogaea* (Leguminosae)	*Botrytis cinerea*	Resveratrol *trans*-dehydrodimer (**151**)



Scheme 33. Detoxification of the broad bean phytoalexin wyerone epoxide (**135**) by the fungal pathogens: (i) *Botrytis cinerea*; (ii) *Botrytis fabae* (Hargreaves et al., 1976).

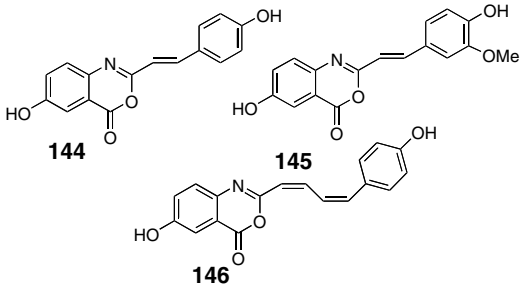


Fig. 4. Cyclic hydroxamic acid phytoalexins from oats (*Avena sativa*): avenalumin I (**144**); avenalumin II (**145**); avenalumin III (**146**) (Mayama, 1983; Gross, 1989).

(**134**) (Scheme 32). Epoxyrishitin (**133**) was found to be less toxic to the fungus than rishitin (**132**) suggesting that the metabolism is a detoxification process (Gardner et al., 1994). The metabolism of rishitin (**132**) by *G. pulicaris* on solid agar medium was usually completed in 24 h (Weltring and Altenburger, 1998). By contrast, incubations in various liquid media and buffers slowed the rate of metabolism of rishitin (**132**) to five days. The structurally related lubimin (**121**) was metabolized completely within 12 h, suggesting that rishitin (**132**) metabolism is under an unusual genetic control requiring growth on a solid surface for efficient metabolism (Weltring and Altenburger, 1998).

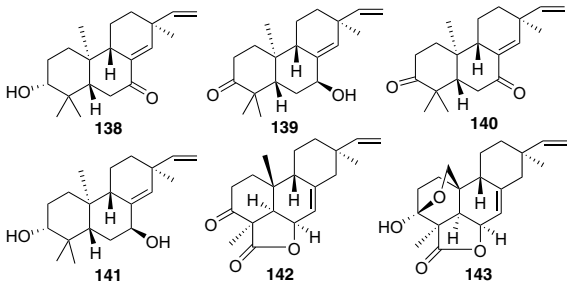
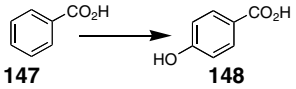


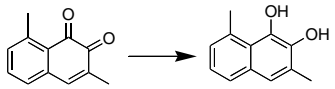
Fig. 3. Diterpenoid phytoalexins from rice (*Oryza sativa*): **138** (oryzalexin A), **139** (oryzalexin B), **140** (oryzalexin C), **141** (oryzalexin D), **142** (momilactone A), **143** (momilactone B) (Akatsuka et al., 1985).

4.4. Wyerone epoxide

The metabolism of the polyacetylenic phytoalexin wyerone epoxide (**135**) by *B. cinerea* (Scheme 33) resulted in wyerol epoxide (**136**) (Hargreaves et al., 1976). Similarly, *B. fabae* metabolized wyerone epoxide (**135**) to wyerol epoxide (**136**) and dihydrodihydroxywyerol (**137**) (Scheme 33). The ED_{50}'s for activity of wyerone epoxide (**135**) against germ tube growth in *B. cinerea* and *B. fabae* were 6.4 and 16.0 μg/mL respectively. The metabolites were less antifungal, but wyerol epoxide (**136**) was more active against *B. fabae* (ED_{50} 38.5 μg/mL) than *B. cinerea* (ED_{50} 583 μg/mL). Dihydrodihydroxywyerol (**137**) did not inhibit germ tube growth at the highest concentration tested (100 μg/mL). These biotransformation results indicated that the metabolism of wyerone epoxide (**135**) by *B.*



Scheme 34. Detoxification of the phytoalexin benzoic acid (**147**) by the apple fungal pathogen *Monilia fructigena* (Bykova et al., 1977).



149 150

Scheme 35. Detoxification of the phytoalexin *o*-Hibiscanone (**149**) by the fungal pathogen *Verticillium dahliae* (Puckhaber et al., 1998).

cinerea and *B. fabae* was a detoxification process (Hargreaves et al., 1976).

5. Metabolism and detoxification of phytoalexins from the family Graminaceae and others

Phytoalexins from the Graminaceae family include the diterpenoids oryzalexin A (**138**), oryzalexin B (**139**), oryzalexin C (**140**), oryzalexin D (**141**), momilactone A (**142**) and momilactone B (**143**) from rice (*Oryza sativa*) (Fig. 3) (Akatsuka et al., 1985) and cyclic hydroxamic acids like avenalumin I (**144**) avenalumin II (**145**) and avenalumin III (**146**) from oats (*Avena sativa*) (Fig. 4) (Mayama, 1983; Gross, 1989). Cyclic hydroxamic acids with the 1,4-benzoxazin-3-one skeleton are found in several grasses including corn, wheat and rye (Yue et al., 1998). The cyclic hydroxamic acids are inhibitory to several fungal plant pathogens including *Helminthosporium tucicum* Passerini, *Septoria nodorum* (Berk.) Berk, *Microdochium nivale* (Fries), and *Fusarium moniliforme* (Yue et al., 1998). Despite the enormous amount of research on oryzalexins, their detoxification by phytopathogenic fungi has not been reported to date (Table 4).

5.1. Avenalumins

The metabolism of the phytoalexins avenalumin I (**144**) and avenalumin II (**145**) by *Fusarium* species indicated that the phytoalexins were transformed but no biotransformation products were detected or isolated. The biotransformation products may be very polar precluding solvent extraction and isolation (Bratfaleanu and Steinhauer, 1994). This degradation occurred at a much faster rate with *F. graminearum* (highly virulent) than with other *Fusarium* species.

5.2. Benzoic acid

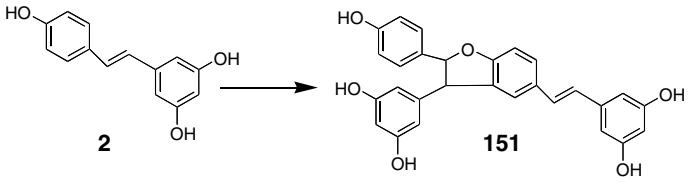
The metabolism of the apple (*Malus domestica*) phytoalexin benzoic acid (**147**), by the apple fungal pathogen *Monilia fructigena* led to the detection and isolation of 4-hydroxybenzoic acid (**148**) (Bykova et al., 1977), which was less toxic to the pathogen than **147**, indicating that the metabolism was a detoxification process (Scheme 34).

5.3. o-Hibiscanone

Kenaf (*Hibiscus cannabinus*) produces the phytoalexin *o*-hibiscanone (**149**) which appears to be strongly inhibitory to *Verticillium dahliae*, an important cotton pathogen. Despite of its bioactivity, *o*-hibiscanone (**149**) is metabolized by *V. dahliae* to the corresponding hydroquinone **150**, which was devoid of antifungal activity (Scheme 35) (Puckhaber et al., 1998).

5.4. Resveratrol

Investigation of the metabolism of the stilbenoid phytoalexin resveratrol (**2**) (Scheme 36) by *B. cinerea* led to the isolation of the metabolite resveratrol *trans*-dehydrodimer (**151**) (Pezet et al., 1991; Breuil et al., 1998; Jeandet et al., 2002). A laccase-like stilbene oxidase produced by *B. cinerea* is responsible for the detoxification of **2** (Pezet et al., 1991). Evidence that stilbenes protect plants against attack by fungal pathogens was obtained from experiments in which expression of resveratrol synthase from grapevine in a number of other plant species increased disease resistance (Jeandet et al., 2002). This resveratrol (**2**) detoxification has also been observed in the conidia of *B. cinerea*. With the use of light microscopy it was observed that approximately 30% of *B. cinerea* treated with semi-lethal concentrations of **2** possessed intracellular brown coloration. This coloration was never observed in the absence of **2** or in conidia treated with **2** together with sulfur dioxide (antioxidant compound



2 151

Scheme 36. Detoxification of the phytoalexin resveratrol (**2**) by the fungal pathogen *Botrytis cinerea* (Pezet et al., 1991; Breuil et al., 1998).

or sodium diethyl dithiocarbamate (inhibitor of laccase action), suggesting that discoloration resulted from the laccase-mediated oxidation of resveratrol (**2**). Further studies using transmission electron microscopy enabled the observation of particular intravacuolar spherical vesicles and of granular material deposits along the tonoplast. These observations are likely to be related to the oxidation of resveratrol (**2**) by an intracellular laccase-like stilbene oxidase of *B. cinerea* (Adrian et al., 1998).

6. Conclusion

The interactions of plants with pathogenic fungi are very complex. As part of the defense mechanisms against pathogen attack plants can synthesize phytoalexins, while fungi may produce enzymes that metabolize and detoxify these plants' chemical defenses. Elucidation of these detoxification mechanisms, followed by isolation and characterization of the enzymes responsible for these processes will, no doubt, lead to a better understanding of the interactions between plants and their pathogens. Particularly where the outcome of the plant–pathogen interaction is detrimental to the plant, an understanding of the detoxification mechanisms employed by the fungus to overcome the plant's defenses could lead to new strategies to prevent plant diseases. For example, it would be possible to design antifungal agents (i.e. paldoxins) selective against a particular plant pathogen (Pedras et al., 2003a,b). Furthermore, optimized mixtures of paldoxins might have the advantage of acting synergistically with the natural disease resistance factors of plants, including their phytoalexins, and be more effective crop protection agents (Pedras, 2004). In addition, cloning of the fungal genes encoding phytoalexin detoxifying enzymes and similar genes in plants could lead to an understanding of co-evolutionary metabolic pathways and novel environmentally safer methods for controlling phytopathogenic fungi.

References

Adrian, M., Rajaei, H., Jeandet, P., Veneau, J., Bessis, R., 1998. Resveratrol oxidation in *Botrytis cinerea* conidia. Phytopathology 88, 472–476.

Akatsuka, T., Kodama, O., Sekido, H., Kono, Y., Takeuchi, S., 1985. Novel phytoalexins (oryzalexins A, B and C) isolated from rice blast leaves infected with *Pyricularia oryzae*. Part I: Isolation, characterization and biological activities of oryzalexins. Agric. Biol. Chem. 49, 1689–1694.

Bailey, J.A., Mansfield, J.W. (Eds.), 1982. Phytoalexins. Blackie and Son, Glasgow, UK, p. 334.

Bailey, J.A., Burden, R.S., Mynett, A., Brown, C., 1977. Metabolism of phaseollin by *Septoria nodorum* and other non-pathogens of *Phaseolus vulgaris*. Phytochemistry 16, 1541–1544.

Bilton, J.N., Dernam, J.R., Smith, I.M., 1976. 6a-Hydroxypterocarpans from red clover. Phytochemistry 15, 1411–1412.

Bratfaleanu, O., Steinhauer, B., 1994. Degradation of avenalumins by fungi in vitro and in vivo. Mededelingen – Facultiet Landbouwkundige en Toegepaste Biologische Wetenschappen 59, 877–884.

Breuil, A., Adrian, M., Pirio, N., Meunier, P., Bessis, R., Jeandet, P., 1998. Metabolism of stilbene phytoalexins by *Botrytis cinerea*. Tetrahedron Lett. 39, 537–540.

Brooks, C.J.W., Watson, D.G., 1985. Phytoalexins. Nat. Prod. Rep., 427–459.

Bykova, T.D., Davydova, M.A., Ozeretskovskaya, O.L., Moiseeva, N.A., 1977. Antifungal substances in apples. Mikologiya i Fitopatologiya 11, 116–122.

Cruickshank, I.A.M., 1962. Studies on phytoalexins, IV. The antimicrobial spectrum of pisatin. Aust. J. Biol. Sci. 15, 147–159.

Cruickshank, I.A.M., Perrin, D.R., 1960. Isolation of a phytoalexin from *Pisum sativum* L. Nature 187, 799–800.

Delserone, L.M., McCluskey, K., Matthews, D.E., VanEtten, H.D., 1999. Pisatin demethylation by fungal pathogens and nonpathogens of pea: association with pisatin tolerance and virulence. Physiol. Mol. Plant Pathol. 55, 317–326.

Denny, T.P., VanEtten, H.D., 1981. Tolerance by *Nectria haematococca* MP VI of the chickpea (*Cicer arietinum*) phytoalexins medicarpin and maackiain. Physiol. Plant. Pathol. 19, 419–437.

Denny, T.P., VanEtten, H.D., 1982. Metabolism of the phytoalexins medicarpin and maackiain by *Fusarium solani*. Phytochemistry 21, 1023–1028.

Denny, T.P., Matthews, P.S., VanEtten, H.D., 1987. A possible mechanism of nondegradative tolerance of pisatin in *Nectria haematococca* MP VI. Physiol. Molec. Plant Pathol. 30, 93–107.

Desjardins, A.E., VanEtten, H.D., 1986. Partial purification of pisatin demethylase, a cytochrome P-450 from the pathogenic fungus *Nectria haematococca*. Arch. Microbiol. 144, 84–90.

Desjardins, A.E., Gardner, H.W., Plattner, R.D., 1989. Detoxification of the potato phytoalexin lubimin by *Gibberella pulicaris*. Phytochemistry 28, 431–437.

Durango, D., Quinones, W., Torres, F., Rosero, Y., Gil, J., Echeverri, F., 2002. Phytoalexin accumulation in Colombian bean varieties and aminosugars as elicitors. Molecules 7, 817–832.

Enkerli, J., Bhatt, G., Covert, S.F., 1998. Maackiain detoxification contributes to the virulence of *Nectria haematococca* MP VI on chickpea. Mol. Plant–Microbe Interact. 11, 317–326.

Esaki, H., Onozaki, H., Morimitsu, Y., Kawakishi, S., Osawa, T., 1998. Potent antioxidative isoflavones isolated from soybeans fermented with *Aspergillus saitoi*. Biosci. Biotechnol. Biochem. 62, 740–746.

Essenberg, M., 2001. Prospects for strengthening plant defenses through phytoalexin engineering. Physiol. Mol. Plant Pathol. 59, 71–81.

Farooq, A., Tahara, S., 1999. Fungal metabolism of flavonoids and related phytoalexins. Curr. Top. Phytochem. 2, 1–33.

Funnell, D.L., Matthews, P.S., VanEtten, H.D., 2002. Identification of new pisatin demethylase genes (*PDA5* and *PDA7*) in *Nectria haematococca* and non-Mendelian segregation of pisatin demethylating ability and virulence on pea due to loss of chromosomal elements. Fungal Gen. Biol. 37, 121–133.

Gardner, H.W., Desjardins, A.E., Weisleder, D., Plattner, R.D., 1988. Biotransformation of the potato phytoalexin, lubimin, by *Gibberella pulicaris*. Identification of major products. Biochim. Biophys. Acta. 966, 347–356.

Gardner, H.W., Desjardins, A.E., McCormick, S.P., Weisleder, D., 1994. Detoxification of the potato phytoalexin rishitin by *Gibberella pulicaris*. Phytochemistry 37, 1001–1005.

Glawischnig, E., Hansen, B.G., Olsen, C.E., Halkier, B.A., 2004. Camalexin is synthesized from indole-3-acetaldoxime, a key

branching point between primary and secondary metabolism in *Arabidopsis*. Proc. Nat. Acad. Sci. USA 101, 8245–8250.
Gross, D., 1989. Antimicrobial defense compounds in Gramineae. Zeitsch. Pflanzen. Pflanzen. 96, 535–553.
Hain, R., Reif, H.J., Krause, E., Langebartels, R., Kindl, H., Vornam, B., Wiese, W., Schmelzer, E., Schreier, P.H., Stocker, R.H., Strenzel, K., 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. Nature 361, 153–156.
Hammerschmidt, R., Dann, E.K., 1999. The role of phytoalexins in plant protection. Novartis Foundat. Symp. 223, 175–190.
Harborne, J.B., 1999. The comparative biochemistry of phytoalexin induction in plants. Biochem. System. Ecol. 27, 335–367.
Hargreaves, J.A., Mansfield, J.W., Coxon, D.T., Price, K.R., 1976. Wyerone epoxide as a phytoalexin in *Vicia faba* and its metabolism by *Botrytis cinerea* and *B. fabae* in vitro. Phytochemistry 15, 1119–1121.
Higgins, V.J., 1975. Induced conversion of the phytoalexin maackiain by the alfalfa pathogen *Stemphylium botryosum*. Physiol. Plant Pathol. 6, 5–18.
Higgins, V.J., Stoessl, A., Heath, M.C., 1974. Conversion of phaseollin to phaseollinisoflavan by *Stemphylium botryosum*. Phytopathology 64, 105–107.
Hipskind, J.D., Paiva, N.L., 2000. Constitutive accumulation of a resveratrol-glucoside in transgenic alfalfa increases resistance to *Phoma medicaginis*. Mol. Plant–Microbe Interact. 13, 551–562.
Höhl, B., Arnemann, M., Schwenen, L., Stöckl, D., Bringmann, G., Jansen, J., Barz, W., 1989. Degradation of the pterocarpan phytoalexin (−)-maackiain by *Ascochyta rabiei*. Z. Naturforsch. 44c, 771–776.
Howlett, B.J., Idnurm, A., Pedras, M.S.C., 2001. *Leptosphaeria maculans* the causal agent of blackleg disease of brassicas. Fungal Gen. Biol. 33, 1–14.
Ingham, J.L., 1976a. Induced and constitutive isoflavonoids from stems of chickpeas (*Cicer arietinum* L.) inoculated with spores of *Helminthosporium carbonum* Ullstrup. Phytopatholog. Zeitschrift 87, 353–367.
Ingham, J.L., 1976b. Fungal modification of pterocarpan phytoalexins from *Melilotus alba* and *Trifolium pratense*. Phytochemistry 15, 1489–1495.
Jeandet, P., Douillet-Breuil, A.C., Bessis, R., Debord, S., Sbaghi, M., Adrian, M., 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. J. Agric. Food Chem. 50, 2731–2741.
Kuhn, P.J., Smith, D.A., 1979. Isolation from *Fusarium solani* f. sp. *phaseoli* of an enzymic system responsible for kievitone and phaseollidin detoxification. Physiol. Plant Pathol. 14, 179–190.
Li, D., Chung, K.R., Smith, D.A., Schardl, C.L., 1995. The *Fusarium solani* gene encoding kievitone hydratase, a secreted enzyme that catalyzes detoxification of a bean phytoalexin. Mol. Plant–Microbe Interact. 8, 388–397.
Lucy, M.C., Matthews, H.D., VanEtten, H.D., 1988. Metabolic detoxification of the phytoalexins maackiain and medicarpin by *Nectria haematococca* field isolates: relationship to virulence on chickpea. Physiol. Mol. Plant. Pathol. 33, 187–199.
Mackintosh, S.F., Matthews, D.E., VanEtten, H.D., 1989. Two additional genes for pisatin demethylation and their relationship to the pathogenicity of *Nectria haematococca* on pea. Mol. Plant–Microbe Interact. 2, 354–362.
Maloney, A.P., VanEtten, H.D., 1994. A gene from the fungal plant pathogen *Nectria haematococca* that encodes the phytoalexin-detoxifying enzyme pisatin demethylase defines a new cytochrome P450 family. Mol. General Genetics 243, 506–514.
Matthews, D.E., VanEtten, H.D., 1983. Detoxification of the phytoalexin pisatin by a fungal cytochrome P-450. Arch. Biochem. Biophys. 224, 494–505.
Mayama, S., 1983. The role of avenalumin in the resistance of oats to crown rust. Kagawa Daigaku Nogakubu Kiyo 42, 1–64.
Miao, V.P.W., VanEtten, H.D., 1992a. Genetic analysis of the role of phytoalexin detoxification in virulence of the fungus *Nectria haematococca* on chickpea (*Cicer arietinum*). Appl. Environ. Microbiol. 58, 809–814.
Miao, V.P.W., VanEtten, H.D., 1992b. Three genes for metabolism of the phytoalexin maackiain in the plant pathogen *Nectria haematococca*: meiotic instability and relationship to a new gene for pisatin demethylase. Appl. Environ. Microbiol. 58, 801–808.
Monde, K., Tanaka, A., Takasugi, M., 1996. Trapping experiment with aniline for a biosynthetic intermediate of sulfur containing cruciferous phytoalexins. J. Org. Chem. 61, 9053–9054.
Morrissey, J.P., Osbourn, A.E., 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol. Mol. Biol. Rev. 63, 708–724.
Müller, K.O., Börger, H., 1940. Experimentelle Untersuchungen uber die Phytophthora: Resistenz der Kartoffel. Arb. Biol. Anst. Reichsanst. 23, 189–231.
Pedras, M.S.C., 1998. Metabolism of cruciferous phytoalexins by phytopathogenic fungi: mimicking or overcoming plant defenses?. Recent Res. Dev. Phytochem. 2, 259–267.
Pedras, M.S.C., 2004. Prospects for controlling plant fungal diseases: alternatives based on chemical ecology and biotechnology. Can. J. Chem. 82, 1329–1335.
Pedras, M.S.C., Ahiahonu, P.W.K., 2002. Probing the phytopathogenic stem rot fungus with phytoalexin analogs: unprecedented glucosylation of camalexin and 6-methoxycamalexin. Bioorg. Med. Chem. 10, 3307–3312.
Pedras, M.S.C., Ahiahonu, P.W.K., 2004. Phytotoxin production and phytoalexin elicitation by the phytopathogenic fungus *Sclerotinia sclerotiorum*. J. Chem. Ecol. 30, 2163–2179.
Pedras, M.S.C., Khan, A.Q., 1996. Biotransformation of the brassica phytoalexin brassicanal A by the blackleg fungus. J. Agric. Food Chem. 44, 3403.
Pedras, M.S.C., Khan, A.Q., 1997. Unprecedented detoxification of the cruciferous phytoalexin camalexin by a root phytopathogen. Bioorg. Med. Chem. Lett. 7, 2255–2260.
Pedras, M.S.C., Khan, A.Q., 2000. Biotransformation of the phytoalexin camalexin by the phytopathogen *Rhizoctonia solani*. Phytochemistry 53, 59–69.
Pedras, M.S.C., Liu, J., 2004. Designer phytoalexins: probing camalexin detoxification pathways in the phytopathogen *Rhizoctonia solani*. Org. Biomol. Chem. 2, 1070–1076.
Pedras, M.S.C., Montaut, S., 2003. Probing metabolic pathways in fungal pathogens of crucifers: biotransformation of 4-hydroxyphenylacetaldoxime, and their metabolites. Bioorg. Med. Chem. 11, 3115–3120.
Pedras, M.S.C., Okanga, F.I., 1998. Probing the phytopathogenic blackleg fungus with a phytoalexin homolog. J. Org. Chem. 63, 416–417.
Pedras, M.S.C., Okanga, F.I., 1999. Strategies of cruciferous pathogenic fungi: detoxification of the phytoalexin cyclobrassinin by mimicry. J. Agric. Food Chem. 47, 1196–1202.
Pedras, M.S.C., Okanga, F.I., 2000. Metabolism of analogs of the phytoalexin brassinin by plant pathogenic fungi. Can. J. Chem. 78, 338–346.
Pedras, M.S.C., Taylor, J.L., 1991. Metabolic transformation of the phytoalexin brassinin by the blackleg fungus. J. Org. Chem. 56, 2619–2621.
Pedras, M.S.C., Taylor, J.L., 1993. Metabolism of the phytoalexin brassinin by the blackleg fungus. J. Nat. Prod. 56, 731–738.
Pedras, M.S.C., Borgmann, I., Taylor, J.L., 1992. Biotransformation of brassinin is a detoxification mechanism in the "blackleg" fungus. Phytochemistry 11, 1–7.
Pedras, M.S.C., Khan, A.Q., Smith, K.C., Stettner, S.L., 1997. Preparation, biotransformation, and antifungal activity of methyl benzyldithiocarbamates. Can. J. Chem. 75, 825–828.

Pedras, M.S.C., Khan, A.Q., Taylor, J.L., 1998. The phytoalexin camalexin is not metabolized by *Phoma lingam*, *Alternaria brassicae*, or phytopathogenic bacteria. Plant Sci. 139, 1–8.

Pedras, M.S.C., Okanga, F.I., Khan, A.Q., Zaharia, I.L., 2000. Phytoalexins from crucifers: synthesis, biosynthesis, and biotransformation. Phytochemistry 53, 161–176.

Pedras, M.S.C., Nycholat, C., Montaut, S., Xu, Y., Khan, A.Q., 2002. Chemical defenses of crucifers: elicitation and metabolism of phytoalexins and indole-3-acetonitrile in brown mustard and turnip. Phytochemistry 59, 611–625.

Pedras, M.S.C., Chumala, P.B., Suchy, M., 2003a. Phytoalexins from *Thlaspi arvense*, a wild crucifer resistant to virulent *Leptosphaeria maculans*: structures, syntheses and antifungal activity. Phytochemistry 64, 949–956.

Pedras, M.S.C., Jha, M., Ahiahonu, P.W.K., 2003b. The synthesis and biosynthesis of phytoalexins produced by cruciferous plants. Curr. Org. Chem. 7, 1635–1647.

Pedras, M.S.C., Ahiahonu, P.W.K., Hossain, M., 2004a. Brassinin detoxification by *Sclerotinia sclerotiorum* requires an inducible glucosyl transferase. Phytochemistry 65, 2685–2694.

Pedras, M.S.C., Montaut, S., Suchy, M., 2004b. New phytoalexins from rutabaga: structures, syntheses, biosyntheses, and biological activity. J. Org. Chem. 69, 4471–4476.

Pezet, R., Pont, V., Hoang-Van, K., 1991. Evidence for oxidative detoxification of pterostilbene and resveratrol by a laccase-like stilbene oxidase produced by *Botrytis cinerea*. Physiol. Mol. Plant. Pathol. 39, 441–450.

Puckhaber, L.S., Stipanovic, R.D., Bell, A.A., 1998. Kenaf Phytoalexin: toxicity of *o*-hibiscanone and its hydroquinone to the plant pathogens *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum*. J. Agric. Food Chem. 46, 4744–4747.

Rouxel, T., Kollman, A., Ballesdent, M.H., 1995. Phytoalexins from the crucifers. In: Daniel, M., Purkayastha, R.P. (Eds.), Handbook of Phytoalexin Metabolism and Action. Marcel Dekker, Inc., New York, pp. 229–261.

Schäfer, W., Straney, D., Ciuffetti, L., VanEtten, H.D., 1989. One enzyme makes a fungal pathogen, but not a saprophyte, virulent on a new host plant. Science 246, 247–249.

Smith, D.A., 1982. Toxicity of phytoalexins. In: Bailey, J.A., Mansfield, J.W. (Eds.), Phytoalexins. Blackie & Son, Glasgow, UK, pp. 218–252.

Smith, C.J., 1996. Accumulation of phytoalexins: defense mechanism and stimulus response system. New Phytol. 132, 1–45.

Smith, D.A., Harrer, J.M., Cleveland, T.E., 1981. Simultaneous detoxification of phytoalexins by *Fusarium solani* f. sp. *phaseoli*. Phytopathology 71, 1212–1215.

Soby, S., Caldera, S., Bates, R., VanEtten, H.D., 1996. Detoxification of the phytoalexins maackiain and medicarpin by fungal pathogens of alfalfa. Phytochemistry 41, 759–765.

Steiner, P.W., 1977. Alfalfa phytoalexin degradation by *Stemphylium botryosum* in relation to pathogenicity and virulence. Diss. Abstr. Int. B 38, 5467–5468.

Tahara, S., Misumi, E., Mizutani, J., Ingham, J.L., 1987. Fungal metabolism of the prenylated isoflavone 2,3-dehydrokievitone. Z. Naturforsch. 42c, 1055–1062.

Takasugi, M., Katsui, N., Shirata, A., 1986. Isolation of three novel sulfur-containing phytoalexins from the Chinese cabbage *Brassica campestris* L. ssp. *pekinensis* (Cruciferae). J. Chem. Soc., Chem. Commun., 1077–1078.

Thomzik, J.E., Stenzel, K., Stocker, R., Schreier, P.H., Hain, R., Stahl, D.J., 1997. Synthesis of a grapevine phytoalexin in transgenic tomatoes (*Lycopersicon esculentum* Mill.) conditions resistance against *Phytophthora infestans*. Physiol. Molec. Plant Pathol. 51, 265–278.

Turbek, C.S., Li, D., Choi, G.H., Schardl, C.L., Smith, D.A., 1990. Induction and purification of kievitone hydratase from *Fusarium solani* f. sp. *phaseoli*. Phytochemistry 29, 2841–2846.

Turbek, C.S., Smith, D.A., Schardl, C.L., 1992. An extracellular enzyme from *Fusarium solani* f. sp. *phaseoli*, which catalyzes hydration of the isoflavonoid phytoalexin phaseollidin. FEMS Microbiol. Lett. 94, 187–190.

Van den Heuvel, VanEtten, H.D., Serum, J.W., Coffen, D.L., Williams, T.H., 1974. Identification of 1a-hydroxyphaseollone, a phaseollin metabolite produced by *Fusarium solani*. Phytochemistry 13, 1129–1131.

VanEtten, H.D., Pueppke, S.K., Kelsey, T.C., 1975. 3,6a-Dihydroxy-8,9-methylenedioxy pterocarpan as a metabolite of pisatin produced by *Fusarium solani* f. sp. *pisi*. Phytochemistry 14, 1103–1105.

VanEtten, H.D., Matthews, P.S., Tegtmeier, K.J., Dietert, M.F., Stein, J.I., 1980. The association of pisatin tolerance and demethylation with virulence on pea in *Nectria haematococca*. Physiol. Plant Pathol. 16, 257–268.

VanEtten, H.D., Matthews, D.E., Smith, D.A., 1982. Metabolism of phytoalexins. In: Bailey, J.A., Mansfield, J.W. (Eds.), Phytoalexins. Blackie and Son, Glasgow, UK, pp. 181–217.

VanEtten, H.D., Matthews, D.E., Matthews, P.S., 1989. Phytoalexin detoxification: Importance for pathogenicity and practical implications. Ann. Rev. Phytopathol. 27, 143–164.

VanEtten, H.D., Funnell-Baerg, D., Wasmann, C., McCluskey, K., 1994a. Location of pathogenicity genes on dispensable chromosomes in *Nectria haematococca* MP VI. Antonie van Leeuwenhoek 65, 263–267.

VanEtten, H.D., Mansfield, J.W., Bailey, J.A., Farmer, E.E., 1994b. Two classes of plant antibiotics: phytoalexins versus ''phytoanticipins''. The Plant Cell 6, 1191–1192.

VanEtten, H.D., Sandrock, R.W., Wasmann, C.C., Soby, S.D., McCluskey, K., Wang, P., 1995. Detoxification of phytoanticipins and phytoalexins by phytopathogenic fungi. Can. J. Bot. 73 (1), S518–S525.

VanEtten, H.D., Temporini, E., Wasmann, C., 2001. Phytoalexin (and phytoanticipin) tolerance as a virulence trait: why is it not required by all pathogens?. Physiol. Mol. Plant Pathol. 59, 83–93.

Ward, E.W.B., Stoessl, A., 1972. Postinfectional inhibitors from plants. III. Detoxification of capsidiol, an antifungal compound from peppers. Phytopathology 62, 1186–1187.

Weltring, K.M., 1991. Phytoalexins in the relation between plants and their fungal pathogens. In: Stahl, U., Tudzynski, P. (Eds.), Molecular Biology of Filamentous Fungi. VCH, pp. 111–124.

Weltring, K.M., Altenburger, M., 1998. Metabolism of the phytoalexin rishitin by *Gibberella pulicaris* is highly reduced in liquid culture. Z. Naturforsch. 53c, 806–810.

Weltring, K.M., Barz, W., 1980. Degradation of 3,9-dimethoxypterocarpan and medicarpin by *Fusarium proliferatum*. Z. Naturforsch. C 35C, 399–405.

Weltring, K.M., Mackenbrock, K., Barz, W., 1982. Demethylation, methylation and 3′-hydroxylation of isoflavones by *Fusarium* fungi. Z. Naturforsch. 37c, 570–574.

Weltring, K.M., Barz, W., Dewick, P.M., 1983. Degradation of the phytoalexin medicarpin by *Fusarium oxysporum*. Phytochemistry 22, 2883–2884.

Whitehead, I.M., Atkinson, A.L., Threlfall, D.R., David, R., 1990. Studies on the biosynthesis and metabolism of the phytoalexin lubimin and related compounds in *Datura stramonium* L. Planta 182, 81–88.

Wu, Q., VanEtten, H.D., 2004. Introduction of plant and fungal genes into pea (*Pisum sativum* L.) hairy roots reduces their ability to produce pisatin and affects their response to a fungal pathogen. Mol. Plant–Microbe Interact. 17, 798–804.

Yue, Q., Bacon, C.W., Richardson, M.D., 1998. Biotransformation of 2-benzoxazolinone and 6-methoxybenzoxazolinone by *Fusarium moniliforme*. Phytochemistry 48, 451–454

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