PERGAMON

Phytochemistry 53 (2000) 59–69

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Biotransformation of the phytoalexin camalexin by the phytopathogen *Rhizoctonia solani*

M. Soledade C. Pedras*, Abdul Q. Khan

Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon SK, Canada S7N 5C9

Received 30 April 1999; received in revised form 13 July 1999

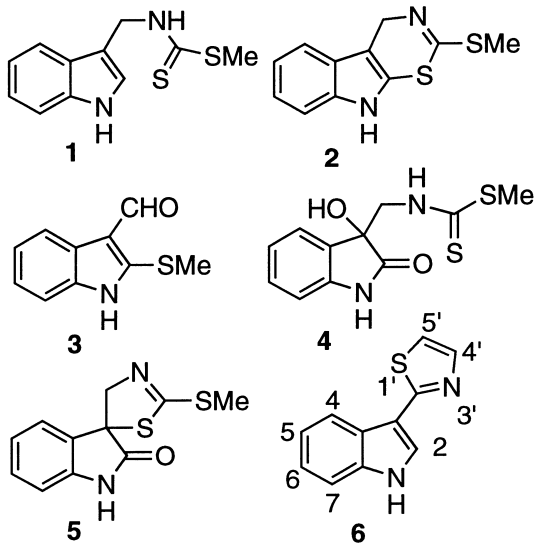
Abstract

The unusual metabolism of the cruciferous phytoalexin camalexin by virulent and weakly virulent isolates of the root rot fungus *Rhizoctonia solani* Kuhn is reported. This biotransformation proceeded via 5-hydroxycamalexin, which was further biotransformed into more polar metabolites. Importantly, the metabolites resulting from transformation of camalexin were significantly less toxic to the pathogen than camalexin. Thus, it was concluded that *R. solani* can detoxify camalexin through oxidation of the indole ring. The chemistry involved in the structure determination of the intermediates of this pathway, their synthesis as well as antifungal activity is described.

Keywords: *Rhizoctonia solani*; Camalexin; Phytoalexin metabolism; Antifungal; Detoxification

1. Introduction

Phytoalexins from crucifers have an indole or related ring, and at least one sulfur atom as a common structural feature (Pedras, Khan, & Taylor, 1997). Despite a close biogenetic relationship, these phytoalexins possess different ring systems and, not surprisingly, different biological activity. For example, brassinin (**1**) and cyclobrassinin (**2**), the first reported cruciferous phytoalexins produced by a variety of *Brassica species* (Takasugi, Katsui & Shirata, 1986), brassicanal A (**3**) (Pedras & Khan, 1996), dioxibrassinin (**4**) (Pedras & Okanga, 1998), spirobrassinin (**5**) (Pedras, 1998), and camalexin (**6**), also a cruciferous phytoalexin produced by *Arabidopsis thaliana* (Tsuji, Jackson, Gage, Hammerschmidt & Somerville, 1992) and *Camelina sativa* (Browne, Conn, Ayer & Tewari, 1991) have significantly different antifungal activities. While brassinin (**1**) and cyclobrassinin (**2**) completely inhibited spore germination of *Phoma lingam* (Tode ex Fr.) (Pedras & Taylor, 1993) (perfect stage *Leptosphaeria maculans* (Desm.) Ces. et de Not.), one of the most damaging cruciferous pathogens, brassicanal A (**3**) dioxibrassinin (**4**), spirobrassinin (**5**) (Pedras, 1998), and camalexin (**6**) (Pedras, Khan & Taylor, 1998) caused significantly lower inhibition at similar concentrations.

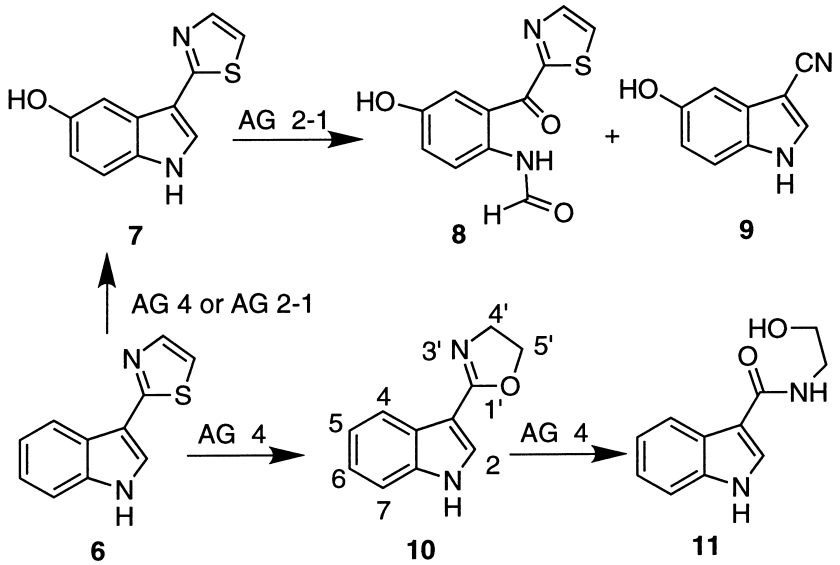


To better understand the role of phytoalexins in the disease resistance of economically valuable brassica

* Corresponding author. Tel.: +1-306-966-4772; fax: +1-306-966-4730.

E-mail address: pedras@sask.usask.ca (M.S.C. Pedras).

0031-9422/00/$ - see front matter
PII: S0031-9422(99)00479-3



Scheme 1. Transformation of camalexin (**6**) by *Rhizoctonia solani*: virulent isolate AG 2-1 and weakly virulent isolate AG 4.

crops (*Cruciferae* family, syn. *Brassicaceae*), e.g. canola (*Brassica napus, B. rapa*), rapeseed (*B. napus, B. rapa*), and mustard (*B. juncea*), we have been investigating the metabolism of diverse cruciferous phytoalexins by agriculturally important pathogens. Although to date only a few examples demonstrate that cruciferous pathogens can detoxify phytoalexins efficiently (Pedras, 1998), multiple examples exist for the phytoalexins of other plant species (Daniel & Purkayastha, 1995; Van Etten, Sandrock, Wasmann, Soby, McCluskey & Wang, 1995; Schafer, 1994; Van Etten, Mathews & Mathews, 1989). We have established that *P. lingam* can rapidly metabolize and detoxify brassinin (**1**) (Pedras & Taylor, 1993), cyclobrassinin (**2**) (Pedras & Okanga, 1998), brassicanal A (**3**) (Pedras & Khan, 1996), and dioxibrassinin (**4**) (Pedras & Okanga, 1998). Conversely, spirobrassinin (**5**) and camalexin (**6**) did not appear to be metabolized or have a significant effect on *P. lingam* mycelial growth (Pedras, 1998). Nonetheless, because the resistance of *C. sativa* to the root rot fungus *Rhizoctonia solani* Kuhn appeared to correlate with the presence of camalexin (**6**) in root tissues (Conn, Browne, Tewari, & Ayer, 1994), we examined the metabolism of camalexin by *R. solani*. Preliminary experiments established that a virulent isolate of *R. solani* (more virulent to *B. napus* than to *C. sativa*, Conn et al., 1994) metabolized camalexin to 5-hydroxycamalexin, which was further transformed into more polar metabolites (Pedras & Khan, 1997). Next, we examined the metabolism of camalexin by a weakly virulent isolate of *R. solani* (weakly virulent to *B. napus* and no infection on *C. sativa*, Conn et al., 1994), as well as the biological activity of the metabolic products. Here we wish to report new aspects of the metabolism of camalexin (**6**) by both virulent and weakly virulent isolates of *R. solani*, as well as the antifungal activity of camalexin (**6**) and its biotransformation products. In addition, we report for the first time the synthesis of the intermediates of camalexin biotransformation pathways, as well as the chemistry involved in camalexin (**6**) oxidation.

2. Results and discussion

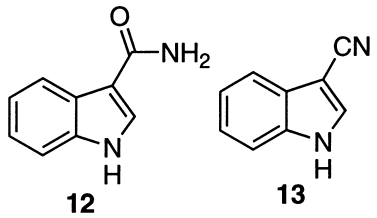
The metabolism of the phytoalexin camalexin (**6**) by virulent and weakly virulent isolates of *R. solani* was investigated. Initial experiments were carried out to determine the minimum inhibitory concentration (5×10^{-4} M) of camalexin to *R. solani*. Subsequently, camalexin (**6**) was incubated with *R. solani* virulent isolate AG 2-1 under normal laboratory light conditions (i.e. 12 h light/12 h dark). Culture samples were withdrawn at different intervals, extracted first with Et_2O, and then acidified and reextracted with Et_2O. Separate cultures containing only the fungus or medium containing only camalexin were incubated under similar conditions and extracted. HPLC analyses of extracts of fungal cultures incubated with camalexin indicated that it was completely metabolized in ca. 72 h. In addition, the HPLC analyses indicated the optimum incubation time for isolation of the putative intermediates/products of camalexin metabolism. Three products **7**–**9** were isolated after incubation of *R. solani* isolate AG 2-1 with camalexin (see Scheme 1). The chemical structure of each metabolite was deduced from comparison of their spectroscopic data with those of camalexin, and confirmed by chemical syntheses.

Relative to camalexin ($C_{11}H_8N_2S$), metabolite **7** con-

Table 1
^{1}H-NMR chemical shifts (ppm) and multiplicities (J in Hz) for compounds **6**–**12** (solvent)

H no.	**6** ($CDCl_3$)	**7** (CD_3OD)	**8** (CD_3OD)	**9** (CD_3OD)	**10** (CD_3OD)	**11** (CD_3OD)	**12** (CD_3OD)
2	8.09	7.90	8.19	7.81	7.83	7.88	7.92
	d (2.8)	*br s*	s	*s*	*s*	*s*	*s*
4	8.13	7.49	7.75	6.97	8.03	8.06	8.09
	m	*d* (2.0)	*d* (2.8)	*d* (1.4)	*m*	*m*	*dd* (1.5, 6.5)
5	7.29	–	–	–	7.10	7.16	7.17
	m				*m*	*m*	*m*
6	7.29	6.77	7.02	6.82	7.10	7.16	7.17
	m	*dd* (2.0, 8.7)	*dd* (2.8, 8.8)	*dd* (1.4, 8.7)	*m*	*m*	*m*
7	7.45	7.30	7.80	7.30	7.42	7.43	7.41
	m	*d* (8.7)	*d* (8.8)	*d* (8.7)	*m*	*m*	*dd* (1.5, 6.5)
4′	7.81	7.73	8.04	–	4.44	3.73	–
	d (3.2)	*d* (3.4)	*d* (3.0)		*t* (9)	*t* (6)	
5′	7.23	7.42	8.10	–	4.00	3.52	–
	d (3.2)	*d* (3.4)	*d* (3.0)		*t* (9)	*t* (6)	

tained an additional oxygen atom ($C_{11}H_8N_2OS$), as determined by HR–EIMS. Comparison of the ^{1}H-NMR spectrum (see Table 1) of **7** with that of camalexin (**6**) indicated the presence of an indolic substituent, since only a three-proton spin system was displayed in addition to the H-2 and two thiazole protons. The ^{13}C-NMR spectrum (Table 2) displayed 11 carbon signals, one of which was characteristic of a deshielded sp^2 C atom (153.3 ppm). These spectroscopic features suggested that metabolite **7** contained an OH group attached to C-5 or C-6. That the OH group was attached to C-5 was deduced from comparison of HMBC and HMQC spectral data with that of camalexin. In addition, methylation of **7** with CH_2N_2 and comparison of the ^{1}H- NMR spectral data of the product with those of 5-methoxycamalexin and 6-methoxycamalexin (Ayer, Peter, Ma & Miao, 1992) indicated that the methyl derivative of **7** was 5-methoxycamalexin. Furthermore, the structural assignment was corroborated by synthesis of 5-hydroxycamalexin (**7**) utilizing a route similar to that followed for camalexin, as shown in Scheme 2 (Furstner & Ernst, 1995).



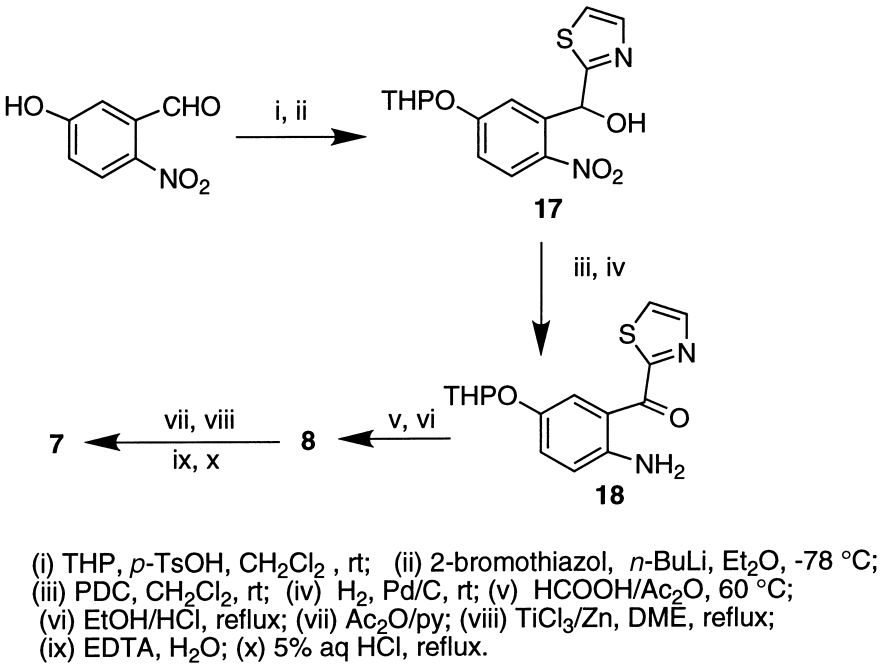
12 **13**

Unlike 5-hydroxycamalexin (**7**), metabolites **8** and **9** did not appear to accumulate to any significant extent in cultures of isolate AG 2-1 incubated with camalexin (**6**), and 5-hydroxycamalexin (**7**) was the main component of the culture extracts, even after six days of incubation. The molecular formula of **8** ($C_{11}H_8N_2O_3S$) obtained by HR–EIMS indicated the presence of two additional oxygen atoms relative to 5-hydroxycamalexin (**7**). While the ^{1}H- NMR spectrum (Table 1) of **8** indicated spin systems similar to those of **7**, its ^{13}C-

Table 2
^{13}C- NMR chemical shifts (ppm) of compounds **6**–**12** (solvent)

C no.	**6**[a] ($CDCl_3$)	**7** (CD_3OD)	**8** (CD_3OD)	**9** (CD_3OD)	**10** (CD_3OD)	**11** (CD_3OD)	**12** (CD_3OD)
2	126.3	127.1	161.9	134.5	130.3	129.3	130.2
3	112.2	111.4	187.1	85.4	104.9	111.9	111.3
3a	124.5	126.6	130.2	131.6	138.1	138.2	138.3
4	120.1	105.1	119.8	103.7	121.9	121.7	121.9
5	123.4[b]	153.3	155.2	154.3	123.5[b]	123.6[b]	123.6[b]
6	121.9[b]	113.6	121.4	115.1	121.8[b]	122.1[b]	122.2[b]
7	111.8	113.7	125.8	114.3	112.8	112.9	112.9
7a	136.6	133.1	124.1	129.8	126.7	127.1	127.3
2′	163.8	166.2	168.8	–	165.1	168.9	–
4′	140.1	142.6	145.9	–	67.9	62.2	–
5′	115.7	116.7	128.4	–	54.6	43.0	–
Other				117.8-C≡N			172.0 C=ONH$_2$

[a] Chemical shifts of camalexin (**6**) change with concentration; reported values are for $c = 10$ mg/ml.
[b] Signals may be interchanged.



Scheme 2. Synthesis of 5-hydroxycamalexin (**7**) and 5-hydroxy-2-formamidophenyl-2′-thiazolylketone (**8**).

NMR spectrum (Table 2) displayed two resonances indicative of C=O groups (or equivalents, at 161.9 and 187.1 ppm). Furthermore, while HMQC spectral data suggested that the thiazole ring of metabolite **8** was intact, a carbon signal at (161.9 ppm) attached to a singlet proton (8.19 ppm) indicated that the indole ring had been oxidized. The structure of **8** was ultimately confirmed by synthesis, as shown in Scheme 2 and described in Section 3. The structure of nitrile **9** was readily deduced from analysis of its NMR spectra (Tables 1 and 2) and HR–MS data and was confirmed by synthesis.

To establish the sequence of biotransformation steps, compounds **7**, **8**, and **9** were separately administered to cultures of *R. solani* isolate AG 2-1. The cultures were incubated and samples withdrawn at different intervals, extracted, and analyzed by TLC and HPLC. As expected, **8** and **9** were detected in the cultures incubated with **7** (ca. 24 h; complete metabolism of **7** occurred in nine days). Furthermore, while **8** did not appear to be metabolized, cultures of *R. solani* metabolized **9** to undertermined products (fifteen days). The results of these experiments are summarized in Table 3, and the proposed sequence of reactions that camalexin (**6**) undergoes when incubated with *R. solani* isolate AG 2-1 is shown in Scheme 1.

Next, camalexin (**6**) was incubated with *R. solani* weakly virulent isolate AG 4 under conditions similar to those used for isolate AG 2-1 (12 h light/12 h dark). HPLC analyses of extracts of fungal cultures incubated with camalexin (**6**) indicated that it was completely metabolized in ca. 96 h. Interestingly, while the major product resulting from biotransformation of camalexin (**6**) by weakly virulent isolate AG 4 was also 5-hydro-

Table 3
Products of metabolism of camalexin (**6**) and compounds **7**–**9** by *Rhizoctonia solani* virulent isolate AG 2-1 (12 h light/12 h dark)

Compound added to fungal cultures[a]	Concentration (M) in incubation medium	Products (%)[b] of metabolism (incubation period)
Camalexin (**6**)	5×10^{-4}	No metabolism, complete growth inhibition
	1.2×10^{-4}	Complete biotransformation to **7** (90%) and traces of **8** and **9** in three days[c]
7	1.2×10^{-4}	Complete biotransformation to **8** and **9** (nine days)
8	1.2×10^{-4}	No metabolism or growth inhibition
9	1.2×10^{-4}	Slow biotransformation to undetermined products (15 days)

[a] Compounds were dissolved in DMSO and added to 48-h-old cultures and incubated at 24 ± 2°C.
[b] Percentage of products detected by HPLC analysis.
[c] Under constant light, similar amounts of metabolites are formed in ca. 24 h.

Table 4
Products of metabolism of camalexin (**6**) and compounds **7** and **9**–**13** by *Rhizoctonia solani* weakly virulent isolate AG 4 (12 h light/12 h dark)

Compound added to fungal cultures[a]	Concentration (M) in incubation medium	Products (%)[b] of metabolism (incubation period)
6	1.2×10^{-4}	75% biotransformation to metabolites **7** (ca. 50%), **10** (ca. 20%), **9** + **11**–**13** (ca. 25%), (four days)[c]
7	1.2×10^{-4}	Slow biotransformation to undetermined products (nine days)
9	1.2×10^{-4}	Slow biotransformation to undetermined products (15 days)
10	1.2×10^{-4}	Slow biotransformation to **11** (seven days)
11	1.2×10^{-4}	No metabolism or growth inhibition
12	1.2×10^{-4}	No metabolism or growth inhibition
13	1.2×10^{-4}	Slow biotransformation to **9** (nine days)

[a] Compounds were dissolved in DMSO and added to 48-h-old cultures and incubated at 24 ± 2°C.
[b] Percentage of products detected by HPLC analysis.
[c] Under constant light, similar amounts of metabolites are formed in four days.

xycamalexin (**7**), new metabolites **10**–**13** were isolated. Compound **8** was never detected in cultures of isolate AG 4 incubated with camalexin. The structural assignments of **10**–**13** followed from the analysis of NMR spectroscopic data (Tables 1 and 2) as well as from HR–MS. For example, oxazoline **10** contained oxygen and no sulfur ($C_{11}H_{10}N_2O$), as determined by HR–EIMS. Comparison of the ^{1}H-NMR spectrum (Table 1) of **10** with that of camalexin (**6**) indicated the presence of an intact indole ring and the absence of thiazole protons; two new triplets due to four protons (4.44 and 4.00 ppm) indicated that the thiazole moiety of camalexin (**6**) had been reduced. This hypothesis was also supported by the ^{13}C-NMR spectrum (Table 2) of **10**, which displayed two signals indicative of sp^3 C atoms (67.9 and 54.6 ppm), in addition to nine carbon signals due to sp^2 C atoms. These spectroscopic features suggested that metabolite **10** contained an oxazoline instead of a thiazole moiety. The structures of compounds **10**–**12** were ultimately confirmed by synthesis as described in Section 3, while structure **13** was confirmed by comparison with an authentic sample (obtained from Sigma Aldrich). The structure of oxazoline **10** is of particular interest as the presence of oxygen instead of sulfur suggests that this isolate possesses an enzymatic system not present in isolate AG 2-1.

To establish a possible pathway for the metabolism of camalexin by weakly virulent isolate AG 4, compounds **7**, and **9**–**13** were separately administered to liquid cultures. The cultures were incubated and samples withdrawn at different intervals, extracted, and analyzed by TLC and HPLC. Surprisingly, the results of these analyses indicated that isolate AG 4, unlike isolate AG 2-1, metabolized **7** to products not extractable with organic solvents (complete transformation of **7** occurred in nine days). In addition, isolate AG 4 metabolized **10** to **11** (seven days); however, neither **11** or **12** appeared to be metabolized. The results of these experiments are summarized in Table 4, and the proposed sequence of reactions that camalexin (**6**) undergoes when incubated with *R. solani* isolate AG 4 are shown in Scheme 1. It appears that the major pathway for metabolism of camalexin involves the formation of 5-hydroxycamalexin (**7**); a minor pathway involved transformation to oxazoline **10**, which was further metabolized to **11**. Although it was anticipated that metabolite **11** might be a precursor of **12** and **12** a precursor of **13**, we were unable to confirm this correlation. Our results indicated that **12** and **13** resulted from camalexin degradation by isolate AG 4, but their close precursor(s) could not be established. It might be that the enzyme catalyzing the degradation of **11** requires induction by camalexin (**6**) or that the solubility of **11** prevents its transport into the appropriate cellular site. Metabolites **10**–**13** were not detected in cultures of isolate AG 2-1 incubated with camalexin (**6**).

Additional experiments carried out to determine the effect of light on camalexin (**6**) metabolism, demonstrated that continuous light affected the rate of its metabolism by virulent isolate AG 2-1 and weakly virulent isolate AG 4 differently. Camalexin (**6**) was metabolized by isolate AG 2-1 (liquid culture) in ca. 24 h under continuous light, while in darkness its metabolism occurred over a much longer period (ca. ten days). Unexpectedly, light did not appear to affect to a detectable level the rate of metabolism of camalexin by isolate AG 4 in liquid culture; however, camalexin was more inhibitory in the absence of light to both isolates AG 2-1 and AG 4 grown on solid medium, as shown in Table 5. Furthermore, measurements of the diameter of mycelia showed a similar growth pattern for both fungal isolates in the absence of camalexin (**6**) (Table 5, control plates).

Since on several instances we found that *m*-CPBA oxidation of indolic phytoalexins yielded products identical to those resulting from fungal oxidation

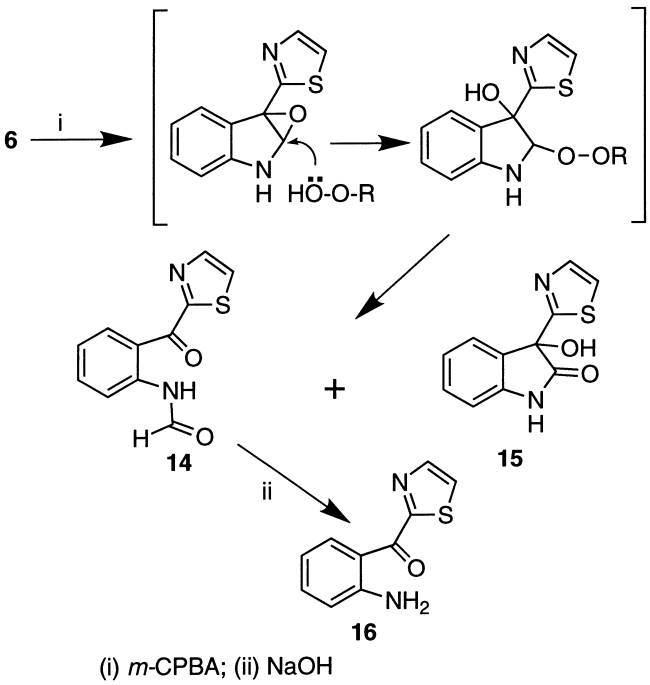
Table 5
Mycelium radial growth of *Rhizctonia solani* virulent isolate AG2-1 and weakly virulnt isolate AG 4 incubated with camalexin (**6**) in constant light and constant darkness

Concentration of camalexin (M)	Isolate AG 2-1 % of inhibition[a] (mycelium diameter mm)		Isolte AG-4 % of inhibition[a] (mycelium diameter mm)[b]	
	Constant light	Constant dark	Constant light	Constant dark
5.0×10^{-4}	100 (5)	100 (5)	100 (5)	100(5)
2.5×10^{-4}	57 (33 ± 1)	66 (26 ± 1)	71 (21 ± 1)	81 (15 ± 1)
1.2×10^{-4}	42 (45 ± 2)	53 (37 ± 2)	28 (56 ± 2)	45 (43 ± 2)
Control[c]	0 (78 ± 1)	0 (78 ± 1)	0 (78 ± 1)	0 (78 ± 1)

[a] The percentage of inhibition was calculated using the formula: % inhibition = 100 − [(growth on treated/growth in control) × 100]; results are the mean of at least three separate experiments (SD ± 0.7).
[b] The size of mycelium plug to initiate cultures was *ca.* 5 mm (described in Section 3).
[c] Control plates contained potato dextrose agar and 1% dimethylsulfoxide and no camalexin.

(Pedras et al., 1997), camalexin (**6**) was oxidized with *m*-CPBA. Contrary to other brassica phytoalexins, the oxidation of camalexin (**6**) proceeded rather slowly yielding two isomeric products **14** and **15** in a 1:1 ratio. The HR–MS analysis of each product ($C_{11}H_8N_2O_2S$) indicated the presence of two additional oxygen atoms relative **6**. The ^{13}C-NMR and HMQC spectroscopic data of **14**, displayed signals that resembled those of metabolite **8**, namely, two C═O groups at 187.4 and 162.2 ppm, and a direct correlation between the latter signal and a singlet proton at 8.32 ppm. In this regard, it is interesting to note that **14** was recently reported as an intermediate in the synthesis of camalexin (**6**) (Furstner & Ernst, 1995). Alkaline hydrolysis of **14** yielded quantitatively the expected product **16** ($C_{10}H_8N_2OS$). The structure of **15** was determined from analysis of its spectroscopic data. Although thiazoles are known to yield N-oxides on treatment with *m*-CPBA (Begtrup & Hanson, 1992) oxidation of camalexin (**6**) occured at the indole moiety to yeild the isomeric products **14** and **15** (see Scheme 3).

The antifungal activity of camalexin (**6**) to *R. solani* was determined utilizing the radial mycelial growth inhibition assay described in Section 3 (see Table 6). The bioactivity of metabolites **7**–**13** was compared with that of camalexin (**6**) ultilizing identical bioassays. After 96 h of incubation, the mycelium of control plates incubated with *R. solani* covered 100% of the agar surface area, while agar plates containing camalexin (**6**) at 5×10^{-4} M showed no mycelial growth, i.e. camalexin (**6**) inhibited completely the growth of isolates AG 2-1 and AG 4. Among metabolites **7**–**13**, only nitrile **13** showed some inhibitory effect towards both isolates. These results demonstrated that the metabolism of camalexin (**6**) leads to products having no significant antifungal activity, thus suggesting this metabolism is a detoxification process. It is clear that *R. solani* can detoxify camalexin (**6**) effectively under normal light conditions, but in darkness this process was significantly slower. Considering that *R. solani* is a root pathogen, it is likely that plants able to biosynthesize camalexin (**6**) in their root tissues will show higher resistance to root diseases. Therefore, camalexin (**6**) might be a useful metabolite to control root disease caused by *R. solani*. In this context it is worthy to note that a higher resistance of *C. sativa* to *R. solani* was attributed to the presence of camalexin (**6**) in root, stem, and leaf tissues (Conn et al., 1994).



Scheme 3. Oxidation of camalexin (**6**) with *m*-chloroperbenzoic acid (*m*-CPBA).

Table 6
Percentage of inhibition of *Rhizoctonia solani* virulent isolate AG 2-1 and weakly virulent isolate AG 4 incubated with camalexin (**6**) and compounds **7**–**13** (after 96h, constant light)

Compound added to fungal cultures	Concentration (M)	*Rhizoctonia solani* isolate AG 4 (% of inhibition)[a]	*Rhizoctonia solani* isolate AG 2-1 (% of inhibition)[a]
Camalexin (**6**)	5.0×10^{-4}	100	100
	2.5×10^{-4}	71	57
	1.2×10^{-4}	28	42
7	5.0×10^{-4}	10	25
	2.5×10^{-4}	0	8
	1.2×10^{-4}	0	4
8	5.0×10^{-4}	0	0
	2.5×10^{-4}	0	0
	1.2×10^{-4}	0	0
9	5.0×10^{-4}	0	5
	2.5×10^{-4}	0	5
	1.2×10^{-4}	0	5
10	5.0×10^{-4}	0	23
	2.5×10^{-4}	0	5
	1.2×10^{-4}	0	0
11	5.0×10^{-4}	0	0
	2.5×10^{-4}	0	0
	1.2×10^{-4}	0	0
12	5.0×10^{-4}	12	0
	2.5×10^{-4}	0	0
	1.2×10^{-4}	0	0
13	5.0×10^{-4}	52	57
	2.5×10^{-4}	23	36
	1.2×10^{-4}	10	16

[a] The percentage of inhibition was calculated using the formula: % inhibition = 100 − [(growth on treated/growth in control) × 100]; results are the mean of at least three separate experiments (SD ± 0.7).

3. Experimental

3.1. General

All chemicals were purchased from Sigma-Aldrich Canada, Oakville, ON. All solvents were HPLC grade and used as such, except for CH_2Cl_2 and $CHCl_3$ which were redistilled. Solvents utilized in syntheses were dried over the following drying agents prior to use: benzene, THF, and EtO_2–Na/benzophenone; CH_2Cl_2–CaH_2. Organic extracts were dried over anhydrous Na_2SO_4 and solvents removed under reduced pressure in a rotary evaporator. Yields reported are based on recovered starting material.

Analytical TLC: (Merck, Kieselgel 60 F_{254}, aluminum sheets) 5 × 2 cm × 0.2 mm; compounds were visualized under UV light and by dipping the plates in a 5% aqueous (w/v) phosphomolybdic acid solution containing 1% (w/v) of ceric sulfate and 4% (v/v) H_2SO_4, followed by heating to 200°C. Preparative TLC: Merck, Kieselgel 60 F_{254}, 20 × 20 cm × 0.25 mm. Flash column chromatography (FCC): silica gel Merck, grade 60, mesh size 230–400, 60 Å. HPLC analysis was carried out with a high performance Hewlett Packard liquid chromatograph equipped with a quaternary pump, automatic injector, and diode array detector (wavelength range 190–600 nm), degasser, and a Hypersil ODS column (5 μm particle size silica, 4.6 i.d. × 200 mm), equipped with an in-line filter. Mobile phase: 75% H_2O-25% CH_3CN to 100% CH_3CN, for 35 min, linear gradient, and a flow rate 1.0 ml/min. The samples to be analyzed by HPLC were dissolved in CH_3CN and filtered through a tight cotton wool plug. NMR spectra were recorded on a Bruker AMX 300 or on a Bruker AMX 500 spectrometer; for ^{1}H-NMR (300 or 500 MHz), δ values were referenced to CD_3OD (CD_2HOD 3.31 ppm), $CDCl_3$ ($CHCl_3$ 7.27 ppm), or CD_3CN (CD_2HCN 1.94 ppm) and for ^{13}C-NMR (75.5 or 125.8 MHz) chemical shifts were referenced to CD_3OD (49.15 ppm), $CDCl_3$ (77.23 ppm), or CD_3CN (1.39, 118.69 ppm). Fourier transform infrared (FTIR) spectra were obtained on a Bio-Rad FTS-40 spectrometer using a diffuse reflectance cell. Mass spectra (MS) were obtained on a VG 70 SE mass spectrometer [high resolution (HR), electron impact (EI) or chemical ionization (CI) with ammonia], employing a solids probe.

3.2. Fungal cultures

Standard isolates of *Rhizoctonia solani*, virulent isolate AG 2-1 and weakly virulent isolate AG 4 utilized in this study were obtained from the AAFC collection (Saskatoon, SK). The fungal isolates were grown on potato dextrose agar (PDA) plates at 24 ± 2°C, under constant light for five days.

3.3. Fungal growth assays

The antifungal activity of compounds **6**–**13** was investigated using the following mycelial radial growth bioassay. A DMSO solution (final concentration ≤ 1%) of the compound to be tested (final concentration of each compound 5.0×10^{-4}, 2.5×10^{-4} and 1.2×10^{-4} M) was added to agar medium at ca. 50°C, mixed quickly and poured onto Petri plates. An agar plug (5 mm diameter) cut from edges of 5-day-old solid cultures of *R. solani* was placed upside down on the center of each plate, the plates were sealed with parafilm, and incubated under constant light at 24 ± 2°C for 96 h. The diameter (in mm) of the mycelial area was measured at 24-h intervals for 96 h. Control plates containing only DMSO were prepared and incubated similarly. Each assay was repeated at least three times.

3.4. Metabolism of camalexin (6)

Liquid shake cultures (100 ml of media in 250 ml Erlenmeyer flasks) were initiated by inoculating potato dextrose broth (PDB) with five agar plugs cut from edges of five-day-old solid cultures. Solutions of camalexin (**6**), synthesized as previously reported (Ayer et al., 1992), (final concentration 1.2×10^{-4} M) in DMSO (final concentration $\leq 1\%$) were added to 48-h-old liquid cultures and to uninoculated media. Cultures were incubated either in 12 h light/12 h dark, constant light, or constant darkness, on a shaker at 130 rpm, at $24 \pm 2^{\circ}C$. Samples (5–10 ml) were withdrawn at different intervals up to two weeks, and were either immediately frozen or were filtered and extracted with Et_2O, followed by *n*-butanol. The extracted broth was acidified to pH 2 with HCl and extracted with Et_2O, followed by neutralization with NaOH and extraction with $CHCl_3$ [containing 1% NH_4OH, (v/v)]. Camalexin (**6**) was stable in uninoculated medium for at least fifteen days.

3.5. Metabolism of compounds 7–13

Solutions of compounds **7**–**13** (final concentration 1.2×10^{-4} M) in DMSO (final concentration of DMSO in PDB was $\leq 1\%$ v/v) were added separately to 48-h-old liquid cultures and to uninoculated media, and samples were withdrawn and analyzed as described for camalexin (**6**). All the compounds were stable in uninoculated medium for at least fifteen days.

3.6. Analysis and isolation of metabolites

Analysis of the organic extracts and biotransformation products was performed with the HPLC system described above. Only the chromatograms of the Et_2O extracts of the neutral broth showed peaks not present in chromatograms of extracts of control cultures; *n*-butanol extracts, as well as Et_2O extracts of acidic and basic broths, were similar to those of control cultures.

The isolation of products resulting from the fungal metabolism of camalexin (**6**) was carried out by prep. TLC (CH_2Cl_2–MeOH, 95:5 v/v). The structures of the products were determined from analyses of the spectroscopic data (NMR, MS, FTIR, UV) of the purified metabolites, and confirmed by synthesis, as described below. The structure of metabolite **13** was confirmed by direct comparison of its spectroscopic data with those of commercially available indole-3-carbonitrile.

3.7. Oxidation of camalexin (6)

m-Chloroperbenzoic acid (200 mg, 1 mmol, in CH_2Cl_2, 2 ml) was added to a stirred solution of camalexin (**6**) (Ayer et al., 1992) (100 mg, 0.5 mmol) in CH_2Cl_2 (3 ml) at room temperature. After 24 h, Me_2S (200 μl) was added to the reaction mixture, the mixture was concentrated in vacuo and the residue was separated by FCC (CH_2Cl_2–MeOH, 98:2 v/v) to yield **14**, (21.7 mg, 22%), **15** (22.7 mg, 23%), and unreacted camalexin (**6**) (15 mg).

3.8. 5-Hydroxycamalexin (7)

A suspension of 5-acetoxy-2-formamidophenyl-2′-thiazolylketone (25 mg, 0.086 mmol) (prepared from 5-hydroxy-2-formamidophenyl-2′-thiazolylketone (**8**), acetic anhydride-pyridine), $TiCl_3$ (423 mg, 0.15 mmol), and Zn dust (20 mg, 0.3 mmol) in DME (3 ml) was refluxed for 1 h under Ar atmosphere (Furstner & Ernst, 1995). The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (5 ml), a solution of EDTA (558 mg) in H_2O (5 ml) was added, and the mixture was vigorously stirred at room temperature for 20 min. The suspension was filtered, the inorganic residue was washed with EtOAc, the organic phases were combined, dried (Na_2SO_4), and concentrated. The residue was separated by prep. TLC (CH_2Cl_2–MeOH, 97:3, v/v, multiple development) to yield 5–acetoxycamalexin (7.5 mg, 34%). 5-Acetoxycamalexin in 5% aqueous HCl was refluxed for 30 min to yield **7** quantitatively (R_f 0.38, CH_2Cl_2–MeOH, 95:5, v/v). ^{1}H-NMR spectral data in Table 1; ^{13}C-NMR spectral data in Table 2; FTIR ν_{max} 3307, 2923, 1581, 1543, 1472, 1444, 1243, 1205 cm^{-1}; EIMS, *m/z* 216.0364 [M^+] (100), (calculated for $C_{11}H_8N_2OS$, 216.0357), 158 (33), 149 (25); CIMS, *m/z* 217 [$M^+ + 1$] (100).

3.9. 5-Hydroxy-2-formamidophenyl-2′-thiazolylketone (8)

A solution of 5-hydroxy-2-nitrobenzaldehyde (250 mg, 1.5 mmol) in 3 ml of CH_2Cl_2–Et_2O (4:1 v/v) was added with stirring to a mixture of 3,4-dihydro-2*H*-pyran (630 mg, 7.5 mmol) and *p*-toluenesulfonic acid monohydrate (2.5 mg, 0.015 mmol) in CH_2Cl_2 (4 ml) dropwise (Boom & Herschied, 1973). The reaction mixture was stirred at room temperature for 4 h, then diluted with CH_2Cl_2 (10 ml), washed with a saturated solution of $NaHCO_3$, and finally with water. The organic phase was dried (Na_2SO_4), concentrated under vacuum, and the residue separated by FCC (CH_2Cl_2–MeOH, 99:1 v/v) to yield 5-(2-tetrahydropyranyloxy)-2-nitrobenzaldehyde (340 mg, 90%). ^{1}H-NMR spectral data (CD_3CN) δ 10.32 (1H, s), 8.14 (1H, *d*, J = 8.9 Hz), 7.36 (2H, *m*), 5.65 (1H, *br s*), 3.75 (1H, *m*), 3.59 (1H, *m*), 1.85–1.65 (6H, *m*); ^{13}C-NMR spectral data (CD_3CN) δ 190.6, 162.8, 144.1, 135.7, 128.5, 121.5, 117.2, 98.0, 63.4, 30.9, 26.0, 19.5; FTIR ν_{max}, 2946, 1697, 1584, 1518, 1338, 1284, 1119 and 1023 cm^{-1};

EIMS, *m/z* 167.0251 [M^+-84] (47), (calculated for $C_7H_5NO_4$, 167.0218); CIMS, *m/z* 269 [$M^+ + 18$] (100).

To a stirred solution of *n*-buthyllithium (0.264 ml, 0.66 mmol in 2.5 ml hexane) was added 2-bromothiazole (153 mg, 0.93 mmol) in Et_2O (1 ml), at −78°C under Ar atmosphere over a period of 1 h (Furstner & Ernst, 1995). The resultant suspension was stirred at −78°C for another 30 min and a solution of 5-(2-tetrahydropyranyloxy)-2-nitrobenzaldehyde (156 mg, 0.62 mmol) in THF (3 ml) was added over a period of 1 h and the mixture was stirred for another 45 min at −78°C. The cooled mixture was poured onto a solution of NH_4Cl (1g/10 ml) and the aqueous layer was extracted with EtOAc. The combined organic phases were washed with brine, dried (Na_2SO_4), and the solvent was removed under vaccum. The crude material was separated by FCC (hexane–acetone, 3:2 v/v) to yield 5-(2-tetrahydropyranyloxy)-2-nitrophenyl-2′-thiazolylmethanol (17, 159 mg, 84%). ^{1}H-NMR spectral data (CD_3CN) δ 8.05 (1H, *d*, *J* = 9 Hz), 7.60 (1H, *d*, *J* = 3 Hz), 7.44 (2H, *m*) 7.10 (1H, *dd*, *J* = 3.0, 9.0 Hz), 6.81 (1H, *d*, *J* = 5.7 Hz, singlet on D_2O exchange), 5.57 (1H, *br s*), 4.83 (1H, *m*), 3.76 (1H, *m*), 3.59 (1H, *m*) and 1.81–1.65 (6H, *m*); ^{13}C-NMR spectral data (CD_3CN) δ 174.2, 162.3, 143.4, 142.4, 141.2, 128.5, 121.1, 116.8, 116.6, 97.6, 69.3, 63.2, 30.8, 25.8 and 19.4; FTIR ν_{max} 3456–3200 (*br*), 2945, 1580, 1515, 1340, 1284 and 1035 cm^{-1}; EIMS, *m/z* 252.0209 [M^+-84] (**23**), (calculated for $C_{10}H_8N_2O_4S$, 252.0204); CIMS *m/z* 337 [$M^+ + 1$] (**25**).

Pyridinium dichromate (PDC, 335 mg, 0.89 mmol) was added with stirring to a solution of 17 (150 mg, 0.44 mmol) in CH_2Cl_2 (6 ml) at room temperature under Ar atmosphere (Furstner & Ernst, 1995). After 3 h the reaction mixture was filtered through a short silica gel pad and the inorganic residue was washed with CH_2Cl_2. The combined organic phases were concentrated under vacuum to yield 5-(2-tetrahydropyranyloxy)-2-nitrophenyl-2′-thiazolylketone (111.3 mg, 74.6%). ^{1}H-NMR (CD_3CN) δ 8.00 (1H, *d*, *J* = 9 Hz), 7.73 (2H, *m*), 7.12 (1H, *dd*, *J* = 2.7, 9.0 Hz), 7.02 (1H, *d*, *J* = 2.7 Hz), 5.44 (1H, *m*), 3.55 (1H, *m*), 3.40 (1H, *m*) and 1.75–1.48 (6H, *m*); ^{13}C-NMR (CD_3CN) δ 186.5, 167.2, 163.2, 146.6, 142.2, 137.7, 129.0, 128.0, 119.5, 117.7, 98.2, 63.5, 30.9, 26.0 and 19.5; FTIR ν_{max} 3110, 2946, 1679, 1580, 1517, 1339, 1233 and 1034 cm^{-1}; EIMS *m/z* 250.0053 [M^+-84] (**14**) (calculated for $C_{10}H_6N_2O_4S$, 250.0048), 217 (27), 204 (14), 191 (40), 159 (14), 130 (15) and 85 (100); CIMS *m/z* 335 [$M^+ + 1$] (15).

5-(2-Tetrahydropyranyloxy)-2-nitrophenyl-2′-thiazolylketone (30 mg, 0.089 mmol) in EtOAc was hydrogenated (H_2 1 atm) over 10% palladium on charcoal (3 mg). After 10 h the orange solution was diluted with EtOAc and filtered through a cotton plug. The filtrate was evaporated under reduced pressure to give 5-(2-tetrahydropyranyloxy)-2-aminophenyl-2′-thiazolyl ketone (18, 26 mg, 96%). ^{1}H-NMR (CD_3CN) δ 8.73 (1H, *d*, *J* = 2.8 Hz), 8.05 (1H, *d*, *J* = 3 Hz), 7.83 (1H, *d*, *J* = 3 Hz), 7.14 (1H, *dd*, *J* = 2.8, 9.0 Hz), 6.75 (1H, *d*, *J* = 9 Hz), 6.48 (2H, *br s*), 5.22 (1H, *m*), 3.89 (1H, *m*), 3.56 (1H, *m*) and 1.86–1.60 (6H, *m*); ^{13}C-NMR (CD_3CN) δ 185.0, 171.3, 150.3, 148.1, 145.7, 128.2, 127.4, 122.4, 119.1, 116.2, 99.5, 63.7, 31.70, 26.4 and 20.6; FTIR ν_{max}, 3463, 3342, 2941, 1580, 1544, 1390, 1253, 1219, 1200 and 1034 cm^{-1}; EIMS *m/z* 304.0876 [M^+] (7) (calculated for $C_{15}H_{16}N_2O_3S$, 304.0881), 220 (100), 192 (76), 191 (33) 166 (5), 148 (15), 136 (6), and 85 (24); CIMS *m/z* 305 [$M^+ + 1$] (100).

A solution of **18** (30 mg, 0.098 mmol) in CH_2Cl_2 was added with stirring to a hot mixture (heated to 60–70°C for 1 h) of acetic anhydride (48 mg, 0.47 mmol) and formic acid (23 mg, 0.5 mmol) (Furstner & Ernst, 1995). The mixture was allowed to cool and quenched with a saturated solution of $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, the combined organic phases were dried (Na_2SO_4) and concentrated under vacuum to yield 5-(2-tetrahydropyranyloxy)-2-formamidophenyl-2′-thiazolylketone (32.0 mg, 98%). ^{1}H-NMR spectral data (CD_3CN) δ 10.0 (1H, *br s*), 8.32 (3H, *m*), 8.11 (1H, *d*, *J* = 3 Hz), 7.96 (1H, *d*, *J* = 2.9 Hz), 7.33 (1H, *dd*, *J* = 2.9, 9.0 Hz), 5.43 (1H, *m*), 3.83 (1H, *m*), 3.55 (1H, *m*) and 1.80–1.60 (6H, *m*); ^{13}C-NMR spectral data (CD_3CN) δ 187.0, 169.1, 161.3, 153.6, 146.4, 134.3, 129.0, 125.9, 124.6, 123.7, 122.7, 98.5, 63.5, 31.3, 26.2 and 20.2; FTIR ν_{max} 3305, 3104, 2943, 1694, 1627, 1584, 1514, 1480, 1384, 1286 and 1035 cm^{-1}; EIMS *m/z* 248.0253 [M^+-84] (95) (calculated for $C_{11}H_8N_2O_3S$, 248.0255), 220 (10), 192 (100), 148 (5), 106 (5) and 85 (71); CIMS *m/z* 333 [$M^+ + 1$] (100).

A solution of 5-(2-tetrahydropyranyloxy)-2-formamidophenyl-2′-thiazolylketone (10 mg, 0.03 mmol) in ethanol acidified 1.5 ml (EtOH, 10 and 0.1 ml of 0.1 M hydrochloric acid) was refluxed for 3 h (Boom & Herschied, 1973). The reaction mixture was then cooled, concentrated under vacuum and the residue was dissolved in EtOAc. The solution was washed with a saturated solution of $NaHCO_3$, and then with H_2O, dried (Na_2SO_4), and concentrated under vacuum. The crude material was purified by prep. TLC using (CH_2Cl_2–MeOH, 95:5, v/v) to yield 5-hydroxy-2-formamidophenyl-2′-thiazolylketone (**8**, 7 mg, 94%), R_f 0.41 (CH_2Cl_2–MeOH, 95: 5, v/v). ^{1}H-NMR spectral data in Table 1; ^{13}C- NMR in Table 2; FTIR ν_{max} 3314, 3090, 1672, 1522, 1383, 1304, 1234 and 827 cm^{-1}; EIMS *m/z* 248.0254 [M^+] (33) (calculated for $C^{11}H^8N^2O^3S$, 248.0256), 220 (18), 192 (100), 148 (18), 149 (17) and 108 (12); CIMS *m/z* 249 [$M^+ + 1$] (100).

3.10. 5-Hydroxyindole-3-carbonitrile (**9**)

Phosphorus oxychloride (60 mg, 0.40 mmol, freshly distilled) was added with stirring to freshly distilled DMF (0.15 ml) under Ar atmosphere at room temperature (Pedras & Khan, 1996). A solution of 5-hydroxyindole (50 mg, 0.37 mmol) in DMF (0.1 ml) was added dropwise with continuous stirring. The reaction mixture was kept at room temperature for 45 min and then poured onto crushed ice. Aqueous NaOH solution (3.8 g in 20 ml) was added to the reaction mixture (slow addition exothermic reaction!), the reaction mixture was acidified to pH 2 with 10% HCl, and was extracted with EtOAc. The organic phases were combined, dried (Na_2SO_4) and concentrated under vacuum. The residue obtained was separated by FCC (CH_2Cl_2–MeOH, 93:7 v/v) to afford 5-hydroxyindole-3-carboxaldehyde (50 mg, 84% yield). ^{1}H-NMR (CD_3OD) δ 9.79 (1H, *s*), 7.98 (1H, *s*), 7.57 (1H, *d*, J = 2.4 Hz), 7.30 (1H, *d*, J = 8.7 Hz), 6.79 (1H, *dd*, J = 2.4, 8.7 Hz); ^{13}C-NMR (CD_3OD) δ 187.3, 154.9, 139.9, 133.4, 127.0, 119.8, 114.8, 113.8, 107.1; FTIR ν_{max} 3330, 3220, 1628, 1472 and 1200 cm^{-1}; EIMS m/z 161.0473 [M$^+$] (94) (calculated for $C_9H_7NO_2$, 161.0476), 160 (100), 132 (24), 105 (7); CIMS m/z 162 [M$^+$ + 1] (100).

Pyridine (0.01 ml in 0.5 ml of $CHCl_3$) was added to a refluxing mixture of $HONH_2$·HCl (8.6 mg, 0.12 mmol) and 5-hydroxyindole-3-carboxaldehyde (20 mg, 0.12 mmol) in $CHCl_3$–EtOH (7:3, v/v, 3 ml) (Sosnovsky, Krogh & Umhoefer, 1979). After 3 h, SeO_2 (13.7 mg, 0.12 mmol) was added to the reaction mixture and refluxing proceeded for 3 h. The reaction mixture was allowed to cool, anhydrous $MgSO_4$ (2 mg) was added and stirring was continued for 10 min at room temperature. The mixture was then filtered and the solvent was removed under vaccum. The residue was separated by prep. TLC (CH_2Cl_2–MeOH, 93:7 v/v, multiple development) to yield **9** as a white powder (13 mg, 77%, R_f 0.30, CH_2Cl_2–MeOH 95:5, v/v). ^{1}H NMR in Table 1; ^{13}C-NMR spectral data in Table 2; FTIR ν_{max} 3325, 3225, 2220, 1204 cm^{-1}; EIMS m/z 158.0478 [M$^+$] (100) (calculated for $C_9H_6N_2O$, 158.0480) 129 (11), 103 (11); CIMS m/z 176 [M$^+$ + 18] (100).

3.11. 2(3-Indolyl)-2-oxazoline (**10**)

Compound **11** (10 mg, 0.049 mmol) was heated to 170°C with anhydrous zinc acetate (89.7 mg, 0.49 mmol) under Ar atmosphere for 2 h (Ghera & Shoua, 1972). The mixture was diluted with CH_2Cl_2, filtered, washed with brine, dried (Na_2SO_4), and the solvent evaporated under reduced pressure. The crude material was separated by prep. TLC (CH_2Cl_2–MeOH, 95:5 v/v) to yield oxazoline **10** (7.7 mg, 84%, R_f 0.28, CH_2Cl_2–MeOH, 95: 5, v/v). ^{1}H-NMR spectral data in Table 1; ^{13}C-NMR in Table 2; FTIR ν_{max} 3075, 1646, 1451, 1245, 1175 and 1019 cm^{-1}; EIMS m/z 186.0785 [M$^+$] (100) (calculated for $C_{11}H_{10}N_{2O}$, 186.0793), 156 (59), 144 (25), 130 (12), 116 (10), 78 (10); CIMS m/z 187 [M$^+$ + 1] (100).

3.12. N(3-Hydroxyethyl)-indole-3-carboxamide (**11**)

Thionyl chloride (0.74 g, 6.2 mmol) was added to indole-3-carboxylic acid (100 mg, 0.62 mmol) at room temperature (Meyers, Temple, Haidukewych & Mihelich, 1974)[1]. The mixture was stirred for 6 h then kept at room temperature overnight. The reaction mixture was diluted with toluene and the excess of thionyl chloride was removed in vacuo. The residue obtained was dissolved in CH_2Cl_2 (5 ml), cooled to 0°C, and freshly distilled 2-aminoethanol (137 mg 2.2 mmol) was added dropwise. The mixture was stirred for 2 h at room temperature, was poured onto brine solution, and extracted with EtOAc. The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. The residue was separated by FCC (CH_2Cl_2–MeOH, 93:7 v/v) to yield N(3-hydroxyethyl)-indole-3-carboxamide (**11**) (100 mg; 79%, R_f 0.2, CH_2Cl_2–MeOH, 93:7, v/v). ^{1}H-NMR spectral data in Table 1; ^{13}C-NMR spectral data in Table 2; FTIR ν_{max} 3300, 2950, 1610, 1544, 1455, 1318, 1207 and 1061 cm^{-1}; EIMS m/z 204.0902 [M$^+$] (32) (calculated for $C_{11}H_{12}N_2O_2$, 204.0898), 160 (29), 144 (100), 116 (17); CIMS m/z 205 [M$^+$ + 1] (100).

3.13. Indole-3-carboxamide (**12**)

Indole-3-carboxylic acid (100 mg 0.62 mmol) was treated with thionyl chloride as described above. The residue obtained was dissolved in CH_2Cl_2, the solution was cooled to 0°C, and excess of ammonia was added (ammonia gas was condensed at −70°C). The reaction mixture was stirred for 2 h at 0°C, and then 1 h at room temperature, the solvent was evaporated under reduced pressure, and the crude material was separated by prep. TLC (CH_2Cl_2–MeOH, 93:7 v/v, developed twice) to yield indole-3-carboxamide (**12**) (70 mg, 90%, R_f 0.3, CH_2Cl_2–MeOH, 93:7, v/v). ^{1}H-NMR spectral data in Table 1; ^{13}C NMR spectral data in Table 2; FTIR ν_{max} 3382, 3325, 3170, 1647, 1618, 1532, 1453 and 749 cm^{-1}; EIMS m/z 160.0638 [M$^+$] (74) (calculated for $C_9H_8N_2O$, 160.0366), 144 (100), 116 (23); CIMS m/z 161 (M$^+$ + 1) (100).

3.13.1. 2-Formamidophenyl-2′-thiazolylketone (**14**)

^{1}H-NMR spectral data (CD_3OD) δ 8.43 (1H, *d*, J = 8 Hz, H-4), 8.32 (1H, *s*, H-2), 8.26 (1H, *d*, J = 8 Hz, H-7), 8.10 (1H, *d*, J = 3 Hz, H-4′), 8.03 (1H, *d*, 3 Hz, H-5′), 7.64 (1H, *m*, H-5/6), and 7.20 (1H, *m*, H-6/5);

^{13}C-NMR spectral data (CD_3OD) δ 187.4 (C-3), 169.0 (C-2′), 162.2 (C-2), 145.9 (C-4′), 139.0 (C-3a), 135.0 (C-5/6), 134.4 (C-4), 128.5 (C-5′), 126.9 (C-7a), 125.0 (C-6/5) and 123.6 (C-7); FTIR ν_{max} 3342, 3080, 1701, 1624, 1604, 1580, 1513, 1448, 1376 and 1271 cm^{-1}; EIMS m/z 232.0306 [M$^+$] (32) (calculated $C_{11}H_8N_2O_2S$, 232.0306), 204 (10), 176 (100), 175 (60), 150 (11), 148 (11), 146 (13), 120 (15); CIMS, mass (relative intensity) = 233 [M$^+$ + 1] (100).

3.13.2. 3-Hydroxy-3-(2′-thiazolyl)-2-oxindole (15)

^{1}H-NMR spectral data, (CD_3OD) δ 7.66 (1H, *d*, J = 3 Hz, H-4′), 7.56 (1H, *d*, J = 3 Hz, H-5′), 7.30 (1H, *m*, H-5/6), 7.19 (1H, *d*, J = 7.4 Hz, H-4), 7.00 (1H, *d*, *m*, H-6/5) and 6.96 (1H, *d*, J = 7.7 Hz, H-7); ^{13}C-NMR spectral data (CD_3OD) δ 178.6 (C-2), 173.8 (C-2′), 143.7 (C-4′), 143.3 (C-7a), 133.2 (C-3), 132.7 (C-3a), 131.3 (C-6/5), 125.8 (C-4), 123.9 (C-5/6), 121.3 (C-5′) and 111.7 (C-7); FTIR ν_{max} 3274, 3090, 1725, 1619, 1470, 1180 and 1056 cm^{-1}; EIMS m/z 232.0298 [M$^+$] (100) (calculated for $C_{11}H_8N_2O_2S$, 232.0306), 176 (32), 175 (32), 149 (27), 69 (22); CIMS m/z 233 [M$^+$ + 1] (100).

*3.13.3. 2-Aminophenyl-2′-thiazolylketone (**16**)*

2-Formamidophenyl-2′-thiazolylketone (**14**, 5 mg) in NaOH–MeOH (5 g/100 ml, 2 ml) was refluxed for 2 h. The solvent was removed under vacuum, the residue dissolved in H_2O, extracted with Et_2O, the aqueous layer neutralized with 5% HCl and extracted with Et_2O, the organic phases combined and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was separated by prep TLC (acetone–hexane, 1:4 v/v, multiple development) to yield 2-aminophenyl-2′-thiazolylketone (**16**) (4.0 mg, 91%). ^{1}H-NMR spectral data (CD_3OD) δ 8.70 (1H, *dd*, J = 1.4, 8.0 Hz, H-4), 8.04 (1H, *d*, J = 3 Hz, H-4′), 7.90 (1H, *d*, J = 3 Hz, H-5′), 7.30 (1H, *m*, H-5/6), 6.80 (1H, *dd*, J = 1.4, 8 Hz, H-7) and 6.60 (1H, *m*, H-6/5); ^{13}C-NMR spectral data (CD_3OD) δ 186.0 (C-3), 171.3 (C-2′), 154.8 (C-3a), 145.2 (C-4′), 136.4 (C-5/6), 135.6 (C-4), 126.6 (C-5′), 118.1 (C-7), 116.5 (C-7a) and 116.2 (C-6/5); FTIR ν_{max} 3468, 3343, 1616, 1578, 1542, 1299, 1256, 1161 and 898 cm^{-1}; EIMS m/z 204. 0351 [M$^+$] (68) (calculated for $C_{10}H_8N_2OS$, 204.0357), 176 (88), 175 (100), 150 (21), 120 (33); CIMS m/z [M$^+$ + 1] (100).

Acknowledgements

Support for the authors' work was obtained in part from the Natural Sciences and Engineering Research Council of Canada and the University of Saskatchewan. We would like to thank P.R. Verma and D. Makenzie, Agriculture and Agri-Food Canada Research Station (AAFC collection), Saskatoon SK, for kindly providing isolates of *Rhizoctonia solani*.

References

Ayer, W. A., Peter, A. C., Ma, Y., & Miao, S. (1992). *Tetrahedron*, *48*, 2919.

Begtrup, M., & Hanson, L. B. L. (1992). *Acta Chemica Scandinavia*, *46*, 372.

Boom, J. H. V., & Herschied, J. D. M. (1973). *Synthesis*, 169.

Browne, L. M., Conn, K. L., Ayer, W. A., & Tewari, J. P. (1991). *Tetrahedron*, *47*, 3909.

Conn, K. L., Browne, L. M., Tewari, J. P., & Ayer, W. A. (1994). *Journal Plant Biochemistry & Biotechnology*, *3*, 125.

Daniel, M., & Purkayastha, R. P. (1995). *Handbook of Phytoalexin metabolism and action* (p. 615). New York, NY: Marcel Dekker.

Furstner, A., & Ernst, A. (1995). *Tetrahedron*, *51*, 773.

Ghera, E., & Shoua, S. J. (1972). *Journal of the Chemical Society* Chemical Communications, 639.

Meyers, A. I., Temple, D. L., Haidukewych, D., & Mihelich, E. D. (1974). *Journal of Organic Chemistry*, *39*, 2787.

Pedras, M. S. C. (1998). *Recent Research Developments in Phytochemistry*, *2*, 259–267.

Pedras, M. S. C., & Khan, A. Q. (1997). *Bioorganic & Medicinal Chemistry Letters*, *7*, 2255.

Pedras, M. S. C., & Khan, A. Q. (1996). *Journal of Agricultural and Food Chemistry*, *44*, 3403.

Pedras, M. S. C., Khan, A. Q., & Taylor, J. L. (1997). *ACS Symposium Series*, *658*, 155.

Pedras, M. S. C., Khan, A. Q., & Taylor, J. L. (1998). *Plant Science*, *138*, 1.

Pedras, M. S. C., & Okanga, F. I. (1998). *Chemical Communications*, 67.

Pedras, M. S. C., & Taylor, J. L. (1993). *Journal of Natural Products*, *56*, 731.

Schafer, W. (1994). *Annual Review Phytopathology*, *32*, 461.

Sosnovsky, G. Krogh, J. A., & Umhoefer, S. G. (1979). *Synthesis*, 722.

Takasugi, M., Katsui, N., & Shirata, A. (1986). *Journal of the Chemical Society, Chemical Communications*, 1077.

Tsuji, J., Jackson, E. P., Gage, D. A., Hammerschmidt, R., & Somerville, S. C. (1992). *Plant Physiology*, *98*, 1304.

Van Etten, H. D., Mathews, D. E., & Mathews, P. S. (1989). *Annual Review Phytopathology*, *27*, 143.

VanEtten, H. D., Sandrock, R. W., Wasmann, C. C., Soby, R. D., McCluskey, K., & Wang, P. (1995). *Canadian Journal Botany*, *73*(1), S518.