Secondary metabolites isolated from *Colletotrichum* species

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Structural and biosynthetic studies of the metabolites isolated from various *Colletotrichum* species are reviewed. These fungi are destructive post-harvest pathogens on a wide range of plants including cereals, legumes and fruits. The review includes a detailed study of the biological activity of these metabolites and their role in the development of plant diseases. The literature in this field to the present is reviewed and 60 references are cited.

1 Introduction

The genus *Colletotrichum* is a member of the subdivision Deuteromycotinia of the form order Melanconidiales.[1] Species of the anamorphic genus *Colletotrichum* (teleomorph *Glomerella*) are implicated in plant diseases, generally referred to as anthracnoses, which are found throughout the world. The anthracnoses are characterized by oval dark necrotic lesions and spots on leaves and fruit. The various *Colletotrichum* species include some of the most destructive post-harvest pathogens of a wide range of plants including cereals, legumes, fruits and vegetables. Strawberries are a particularly susceptible host, suffering from a variety of *Colletotrichum*-triggered diseases including wilts, rots and anthracnoses.[2,3]

As is the case with other groups of fungal pathogens, the systematic studies of *Colletotrichum* species are at a crossroads. The traditional morphology-based method of characterizing species is now widely recognized as being inadequate. Important studies are in progress with the aim of developing rapid diagnostic tests for *Colletotrichum* species, especially those associated with strawberry diseases.[4,5] This is of particular importance not only because the taxa involved are currently very ill-defined, but also because there may be significant differences in aggression amongst the various pathogens. Moreover legal issues are also involved as *Colletotrichum acutatum*, one of the species implicated, is subject to statutory quarantine regulations (EC Directive 77/93). An essential prerequisite for the

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development of a diagnostic test is the proper characterization of the different species and populations so that the various taxa involved can be properly defined. In this context, the characterization of secondary metabolites isolated from the different species could help to shed light on the identification of species and populations of the genus of phytopathogens; it could also clarify the role that the secondary metabolites play in the infection mechanism. This latter point has been the focus of many studies in the recent literature.

It is well-documented that *Colletotrichum* species produce phytotoxic metabolites which induce symptoms similar to those of the pathogens themselves. Some of the metabolites have therefore been used to screen for resistance and have been shown to play a significant role in pathogenesis.[6–8]

The species of fungi most often studied with regard to their production of toxic metabolites are: *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., a wide-spread pathogen found on strawberries, grapes *etc.*; *Colletotrichum nicotianae*, the causative organism of tobacco anthracnose disease; *Colletotrichum lagenarium*; *Colletotrichum capsici*, a pathogen on peanuts, soyabean, cowpea *etc.*; *Colletotrichum truncatum*; *Colletotrichum fragaria*, found on strawberries; *Colletotrichum dematium* (Pers.) Grove, a pathogen on *Phaseolus vulgaris*. This review deals with the metabolites isolated from these species describing their biological activity and their role in the development of plant disease.

2 Metabolites isolated from *Colletotrichum gloeosporioides*

Due to its ubiquitous nature and the fact that it affects many economically important crops such as strawberries and grapes, *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph *Glomerella cingulata*) has been the subject of much research. This extremely common fungus causes anthracnoses on the fruits, leaves and stems of an extensive range of hosts. A large number of papers have been published on the production of metabolites by this fungus.[7–30]

Early work reported the isolation of the metabolites aspergillomarasmim A and B, **1** and **2**, which had been previously isolated from an *Aspergillus* species.[9,10] Later studies showed that conidia of *C. gloeosporioides* (Penz.) Sacc. f. sp. *jussiaea* germinate poorly when sown in dense conidial concentrations compared to diluted conidia. Aqueous exudates from dense conidial suspensions inhibited the germination of diluted conidia.[11–13] Evaporation of these exudates, extraction with chloroform and crystallization afforded the pure metabolite, gloeosporone **3**.[11] The structure of this metabolite was subsequently revised to **4** using spectroscopic data and X-ray analysis.[12] Various synthetic studies have been reported.[13–19] The structures of the major metabolites of *C. gloeosporioides* are shown in Fig. 1.

Tsurushima *et al.*[20] isolated, from the same species, three self-inhibitors of conidial germination: (*E*) and (*Z*)-3-ethylidene-1,3-dihydroindol-2-ones **5** and **6**, and (2*R*)-(3-indolyl)propionic acid **7**. These inhibitors reduced the germination of conidia with ED_{50} values of 3, 5 and 100 μg ml^{-1} respectively. Among sixteen species of anthracnose fungi which were tested, compounds **5**–**7** were detected only in *C. gloeosporioides* (Penz.) Sacc. f. sp. *jussiaea* and *C. fragariae*. The activity of the self-inhibitors was found to be greater for germination than for germ tube growth and the effects were most significant against the fungal species that produced them. In addition, two phytotoxic substances were isolated from *Glomerella cingulata* and identified as phenoxyacetic acid and indoleacetic acid. The authors indicated that both substances could be responsible for the tea brown blight caused by the fungus.[21]

The generic name mycosporine has been given to a series of water soluble UV-absorbing fungal metabolites whose structures contain a substituted cyclohexenone linked to an amino acid or the corresponding amino alcohol. These compounds are involved in the conidial production process. Their distribution and biological properties have been reviewed.[22,23] The structures of mycosporine glutamine **8** and mycosporine glutamic acid **9** have been established and their presence demonstrated in two fungi: *Pyronema omphalodes* and *Glomerella cingulata*.[24,25] In addition, mycosporine alanine **10** has shown phytotoxic activity and was isolated from *C. graminicola*.[26] The composition of

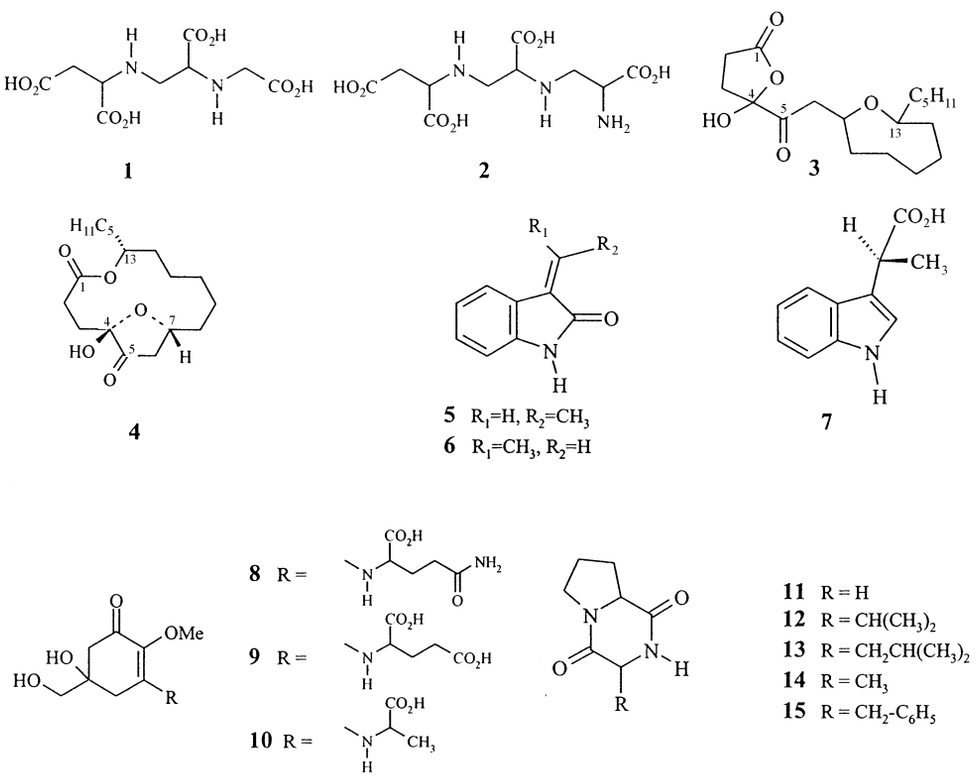


Fig. 1 Metabolites isolated from *Colletotrichum gloeosporioides*.

the mucilaginous spore matrix, and a study on a volatile self-inhibitor of *C. graminicola*, have also been reported.[26b,c]

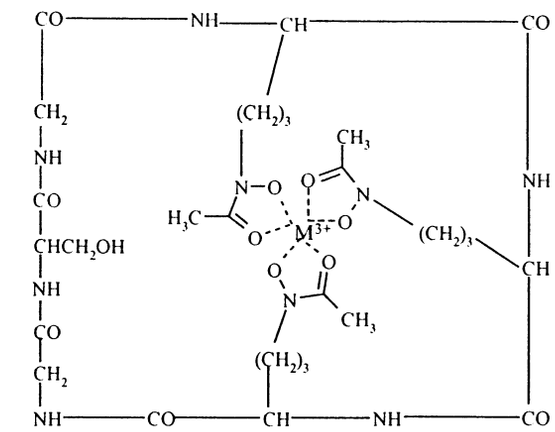
Recent research has indicated that the phytotoxins produced by many phytopathogens such as *Colletotrichum* species, could be of potential bioherbicidal use. In addition to compound **10** other metabolites with phytotoxic activity have been reported from *C. gloeosporioides*. The five diketopiperazines **11**–**15** and the siderophore, ferricrocin **16** (Fig. 2),[28] showed high phytotoxic activity. For example, when tested against cotyledons, compound **16** showed phytotoxic activity from 1×10^{-2} M to $1/64 \times 10^{-2}$ M. This phytotoxicity was enhanced by the removal of iron suggesting that the activity has some relation to chelating activity.

A new antimicrobial metabolite named colletotric acid **17**,[29] was isolated from a liquid culture of *C. gloeosporioides*, an endophytic fungus which had colonized the inside of stems of *Artemisia mongolica*. The structure was determined by spectroscopic methods. Compound **17** inhibited the growth of *Bacillus subtilis, Staphylococcus aureus* and *Sarcina lutea* with minimal inhibitory concentrations (MICs) of 25, 50 and 50 μg ml^{-1} respectively. It also inhibited a culture of the pathogenic fungus, *Helminthosporium sativum* at an MIC of 50 μg ml^{-1}. In a different study, Lu *et al.* obtained some further novel compounds from an unidentified species of *Colletotrichum* which was isolated as an endophytic fungus of *Artemisia annua*.[30] These compounds included 3β,5α-dihydroxy-6β-acetoxyergosta-7,22-diene **18**, 3β,5α-dihydroxy-6β-phenylacetyloxyergosta-7,22-diene **19** and 6-isopropenylindole-3-carboxylic acid **20**. In addition, other known metabolites including indoleacetic acid and several derivatives of ergosterol were reported.[30]

3 Metabolites isolated from *C. nicotianae*

Several biologically active metabolites have been isolated from the phytopathogen *C. nicotianae* which causes anthracnose in tobacco.[1] As in the previous case the biological activity of these compounds is largely responsible for the symptoms of the plant disease.

Tamura and co-workers [31–34,36–39,41] have carried out numerous studies on the metabolites of this fungus (see Fig. 3). As a result,



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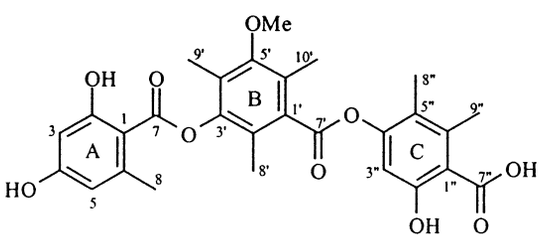

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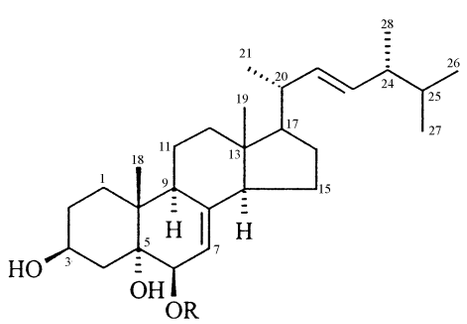

18 R= $COCH_3$
19 R= $COCH_2C_6H_5$

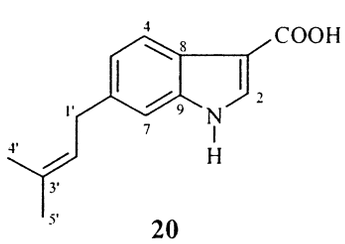

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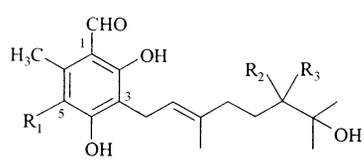
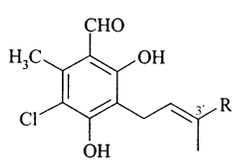
Fig. 2 Metabolites isolated from *Colletotrichum gloeosporioides*.

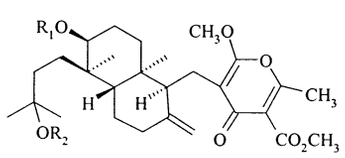


(21) R_1=Cl, R_2=H, R_3=OH
(22) R_1=Cl, R_2=O
(25) R_1=H, R_2=H, R_3=OH
(26) R_1=H, R_2=O

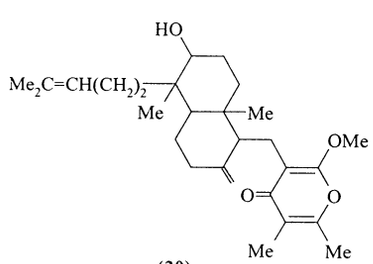


(23) R=CH_3

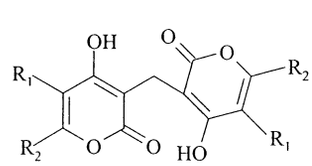
(24) R=



(27) R_1=H, R_2=H
(28) R_1=CHO, R_2=H
(29) R_1=H, R_2=CHO



(30)



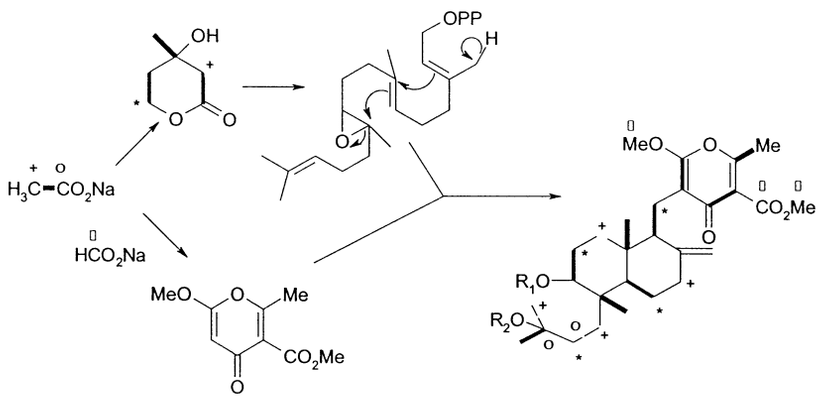
(31) R_1=CH_3, R_2=CH_3

Fig. 3 Metabolites isolated from *Colletotrichum nicotianae*.

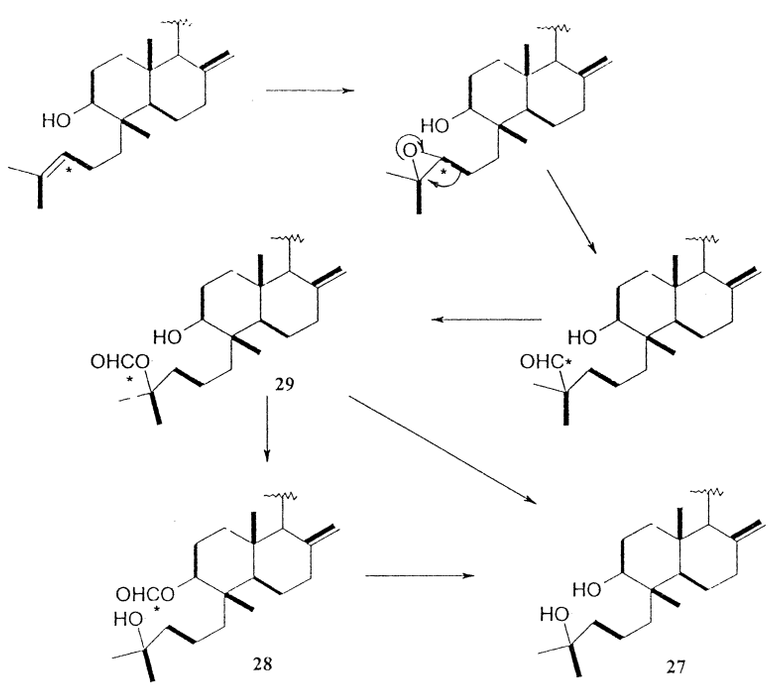
this group has reported the isolation of colletochlorins A–D, **21**–**24**,[31–34] which possess an orsellinaldehyde structure containing a monoprenyl or diprenyl side chain at C-3 together with a chlorine atom at C-5. They have also isolated two orsellinaldehydes with similar structures but without the chlorine atom, namely colletorins A, **25** and C, **26**. These compounds were biologically inactive. However, the research group have isolated the phytotoxic metabolites colletotrichins A, **27**, B, **28** and C, **29**.[34–37] When these were applied to tobacco leaves they induced symptoms similar to those of the tobacco anthracnose caused by the fungus.[38]

The effect of colletotrichin **27** on a diverse array of physiological processes has been examined. Compound **27** caused a rapid loss of membrane integrity. The first ultra-structural damage observable was plasmolysis and disruption of the plasmalemma by an unknown mechanism.[39]

The structure of colletotrichin derivatives consists of a unique norditerpene and a polysubstituted γ-pyrone. The biosynthesis of these compounds was studied using carbon-13 NMR methods. Kimura *et al.*[36,37] have demonstrated the labelling patterns shown in Scheme 1 for the compounds **27**–**29** biosynthesized by *C. nicotianae* from ^{13}C-formate, [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetate as well as [5-^{13}C]-mevalonate. The results were consistent with a polyketide origin for the pyrone unit whilst the terpenoid portion arose *via* geranylgeranyl pyrophosphate. These authors proposed the cyclization of geranylgeranyl pyrophosphate shown in Scheme 2 followed by epoxidation of the terminal double bond, cleavage and finally a Baeyer–Villiger type oxidation to account for the formation of the norditerpene moiety of the colletotrichins. Colletochin **30** (Fig. 3), a metabolite with a structure similar to that of **27** isolated by Kimura *et al.*,[40] is thought to be a precursor of the colletotrichins in *C. nicotianae*. Since compounds with the basic skeleton **21**–**24** have been isolated, the biosynthesis of the pyrone ring was explained by the *ortho*-hydroxylation of an orsellinaldehyde precursor. The oxidative cleavage to an α-pyrone followed a *meta*-pyrocatechase type of fission and the rearrangement of the α-pyrone to the γ-isomer.[34] Interestingly, the α-pyrone colletopyrone, **31** has been isolated from *C. nicotianae* and it is considered to be one of the fungal metabolites that function as a toxic principle.[41] Thus when solutions of compound **31** of different concentrations were placed on young tobacco leaves, brown necrotic spots analogous to those symptomatic of the pathogenic fungus appeared on the leaves.



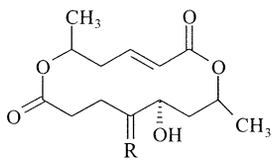
Scheme 1 Incorporation of ^{13}C-labelled formiate, acetate and mevalonate into colletotrichins.



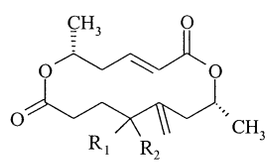
Scheme 2 Formation of the norditerpene moiety in colletotrichins.

4 Metabolites isolated from *C. capsici*

Several papers have dealt with the toxins excreted by *Colletotrichum capsici*,[42,43] although the structures were not established. Preliminary results have indicated that the toxins contain polysaccharides while bioassays have shown that culture filtrates and toxin solutions not only inhibit the root growth of different capsicums, mungbean, pea and cowpea varieties but also that they cause wilting in capsicum seedlings. In addition to colletotrichin **27**,[35] the new metabolites colletodiol **32** and its relatives colletoketol **33**, colletol **34** and colletallol **35**, were isolated from *C. capsici*.[44,45] Both the absolute stereochemistry and biosynthesis of colletodiol **32** have been elucidated.[46,47]



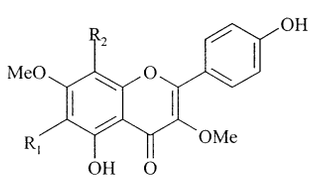
(**32**) R= OH, H
(**33**) R= O
(**34**) R= H_2



(**35**) R_1=H, R_2=OH, R_3=H

5 Metabolites isolated from *C. dematium*

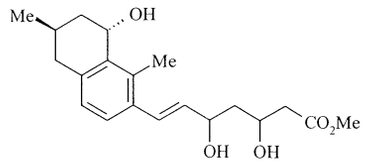
Colletotrichum dematium f. sp. *epilobii*, a specific and indigenous pathogen of fireweed *Epilobium angustifolium* L. subsp. *angustifolium* (family Onagraceae) has been investigated as a biological weed control agent.[48] This pathogen causes large necrotic lesions on the leaves and stem of the infected plant. Two C-6 and C-8 methylflavonols were isolated from ethyl acetate extracts of liquid culture filtrates of the fungus. These filtrates demonstrated a high degree of antimicrobial and phytotoxic activity. However the flavonols 5,4′-dihydroxy-3,7,8-trimethoxy-6*C*-methylflavone **36** and 5,4′-dihydroxy-3,6,7-trimethoxy-8*C*-methylflavone **37**, which were isolated,[48] did not display biological activity. The mycelia of four isolates of *C. dematium* were found to contain 0.8–1.28% cholesterol. The highest percentage of cholesterol was found in the isolate which had a relatively poor growth suggesting that cholesterol retards the growth of the fungus.[49]



(**36**) R_1= CH_3; R_2= OCH_3
(**37**) R_1= O CH_3; R_2= CH_3

6 Metabolites isolated from other *Colletotrichum* species

The soyabean pathogen *C. truncatum* has also been studied. Metabolites isolated from this fungus have been identified as *meso*- and D(−)-butane-2,3-diol, 2-hydroxymethylhexa-2,4-dienol and colletruncoic acid methyl ester **38** which has a polyketide skeleton that is without precedent. The structure was elucidated mainly by NMR methods.[50]



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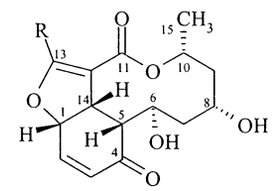
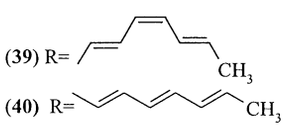
The germination of the conidia of another *Colletotrichum* species, *C. fragariae* depends heavily on the population density with germination inhibited at higher concentrations of conidia in water. This phenomenon is thought to be due to chemical self-inhibitors secreted from the spores of the fungus. Five active principles isolated from acetone extracts of PSA plate cultures of the fungus have been identified as (*E*) and (*Z*)-3-ethylidene-1,3-dihydroindol-2-ones **5** and **6**, (2*R*)-(3-indolyl)-propionic acid **7** and the colletofragarones A1, **39** and A2, **40**.[51] The mixture of **39** and **40** reduced the germination of *C. fragariae* conidia by approximately 50% at a concentration of 20 μg ml^{-1}. These compounds were also detected in the conidial suspension under crowded conditions suggesting that the colletofragarones act as germination self-inhibitors.[52]



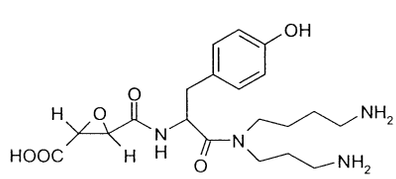


(**39**) R=

(**40**) R=

Other researchers have described the principal constituents of *C. lagenarium* including some nitrogenous metabolites.[53,54] In addition, several studies have been carried out on plant growth regulatory substances isolated from this fungus.[55–57] The antiauxin, 2-pyruvoylaminobenzamide has been isolated and its biological activity on *Avena* coleoptile segments has been examined. The aminobenzamide derivative did not affect the straight growth segments at a concentration of 30 mg l^{-1}. However when applied in combination with indole-3-acetic acid at 1 mg l^{-1}, the compound interfered with the response of the segments to the hormone even at doses as low as 10 mg l^{-1}.

Recently a novel cathepsins B and L inhibitor, WF14861 **41**,[58,59] has been obtained from the mycelium of a fungus strain *Colletotrichum* sp. no. 14861. Compound **41** also showed inhibitory activities against bone-derived crude proteases and other cysteine proteases *in vitro*.[59] A paper on the chemical factors regulating the germination of fungal spores on *Colletotrichum* species has been published.[60]



(**41**)

7 Conclusions

In summary, the literature on the various species of *Colletotrichum* has been reviewed with an emphasis on the secondary metabolites produced by these fungi. The characterization of these metabolites not only facilitates the identification of different species of this genus but also sheds light on the role of these toxins in the infection mechanism of these organisms.

8 Acknowledgements

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