Phytochemistry 70 (2009) 833–841

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Review

The thaxtomin phytotoxins: Sources, synthesis, biosynthesis, biotransformation and biological activity

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ARTICLE INFO

Article history:
Received 20 February 2009
Accepted 20 April 2009
Available online 23 May 2009

Keywords:
Phytotoxins
Thaxtomins
Diketopiperazines
Nitrotryptophans
Common scab
Streptomyces scabies
Cellulose synthesis

ABSTRACT

Thaxtomin phytotoxins, first reported in 1989, are cyclic dipeptides (2,5-diketopiperazines) formed from the condensation of 4-nitrotrytophan and phenylalanine groups. Inclusion of a 4-nitroindole moiety that is an essential requirement for their phytotoxicity makes them unique amongst microbial generated metabolites. Individual thaxtomins differ only in the presence or absence of *N*-methyl and hydroxyl groups and their respective substitution sites. The name "thaxtomin" was assigned to these compounds in honor of the eminent American phytopathologist Roland Thaxter who first described the nature of the microorganisms responsible for their production. The great interest in the thaxtomins derives mainly from their established roles as virulence factors in the common scab of potato disease and their apparent ability to inhibit cellulose synthesis in developing plant cells. Surveys of comparable scab-inducing organisms from potato producing regions throughout the world have confirmed the generality of the phytotoxin associated process. Descriptions of the isolation, chemical structure determination, total synthesis, biosynthesis, transformation by microorganisms and biological activity of the thaxtomins are comprehensively reviewed.

Contents

1. Introduction

Common scab of potato (*Solanum tuberosum)* is predominantly attributed to infection by the soil bacterium *Streptomyces scabies* (Lambert and Loria, 1989a) which attacks growing tubers through immature lenticels and wound sites. In response to invasion by the bacterium, suberin (a natural protective layer of the tuber composed of linked fatty acids, fatty alcohols and phenolic compounds) is synthesized at the site of infection (Bernards, 2002 and references therein). The resultant lesions or scabs can range in appearance from small raised cork-like tissue around the lenticels to deep large sunken pits. This bacterium and other scab-causing species can also elicit scab symptoms on other economically important root crops such as beet, carrot, parsnip, radish, sweet potato and turnip (Loria et al., 2006 and references therein). Due to its

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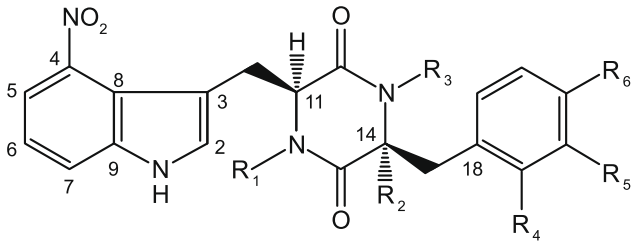
0031-9422/$ - see front matter
doi:10.1016/j.phytochem.2009.04.013



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	Me	OH	Me	H	OH	H
2	Me	OH	Me	OH	H	H
3	Me	H	H	H	H	H
4	Me	OH	Me	H	H	H
5	Me	H	Me	H	H	H
6	Me	OH	H	H	H	H
7	Me	OH	Me	H	H	OH
8	Me	OH	Me	H	OH	OH
9	Me	OH	H	H	OH	H
10	H	OH	Me	H	OH	H
11	H	H	H	H	H	H

Fig. 1. Structural formulae of the thaxtomins.

detrimental effect on appearance, grade and quality, common scab of potato in particular represents a disease of major economic importance in many potato producing areas of the world (Loria et al., 1997; Wilson et al., 1999; Hill and Lazarovits, 2005). Speculation regarding the possible involvement of phytotoxins in the common scab of potato disease has been dated back to 1926 (Fellows, 1926). This speculation was later substantiated by the finding that sterilized extracts of scab lesions from field infected potato tubers would cause the development of scab-like lesions on immature potato tubers (Barker and Lawrence, 1963). In 1989, two phytotoxins directly associated with the potato common scab disease were isolated and characterized (King et al., 1989). The phytotoxins called thaxtomins are cyclic dipeptides (2,5-diketopiperazines) containing 4-nitrotryptophan and phenylalanine residues. They were named "thaxtomins" in honor of Roland Thaxter, the American plant pathologist who first identified pathogenic *Streptomyces* species as the causal organisms of potato common scab (Thaxter, 1891). The inclusion of a 4-nitroindole moiety makes the thaxtomins unique amongst microbial generated metabolites (Winkler and Hertweck, 2005) and this feature is also an essential factor for their phytotoxic activity (King et al., 1989, 1992). Since the first report of the discovery of thaxtomin A (**1**) and B (**4**) in common scab infected potato slices (King et al., 1989), nine other members of this family of compounds have been isolated and characterized (King et al., 1992, 1994; King and Lawrence, 1996). Individual thaxtomins differ only in the presence or absence of *N*-methyl and hydroxyl substituents and their respective substitution sites. The configurations of the two amino acid residues have been established as L by synthetic (King et al., 1989) and X-ray crystallographic (Wagner, 2000) studies. The structures, stereochemistry and numbering system are shown in Fig. 1.

2. Chemical structures and producing organisms

All but one of the thaxtomins **1**–**11** (Fig. 1) reported so far have been isolated from materials infected with cultures of pathogenic *Streptomyces* species designated as *S. scabies* (Lambert and Loria, 1989a). Thaxtomin A (**1**) (the major metabolite) and thaxtomin B (**4**) were first identified as secondary metabolites in *S. scabies* infected potato slices (King et al., 1989). Four other thaxtomins (compounds **2**, **3**, **5** and **6**) were later isolated from similar sources (King et al., 1992). Examination of the exudates associated with the *in vitro* production of thaxomin A (**1**) by *S. scabies* (Babcock et al., 1993; Loria et al., 1995) subsequently yielded four more thaxtomins (compounds **7**–**10**) (King and Lawrence, 1996). Surveys of comparable scab-inducing organisms from sites throughout North America, Europe, Africa and Asia confirmed the generality of the phytotoxin associated process (King et al., 1991; Loria et al., 1997; Kinkel et al., 1998). It has also been established that at least five other less common *Streptomyces* species: *Streptomyces acidiscabies* (Lambert and Loria, 1989b), *Streptomyces turgidiscabies* (Miyajima et al., 1998), *Streptomyces europaeiscabiei* and *Streptomyces stelliscabiei* (Boucheck-Mechiche et al., 2000) and *Streptomyces niveiscabiei* (Park et al., 2003) are likewise capable of producing thaxtomins (see Table 1 for a representative sampling). Although less widespread than many of the other scab producing organisms, *S. acidiscabies* is more unique in nature. It is unusually operative in acidic soils and when grown *in vitro* generates thaxtomin A (**1**) and a series of naphthoquinone derived pigments that may constitute distinguishing markers (King et al., 1996). Compound **11**, the lone thaxtomin not produced by *S. scabies*, and analogs **3** and **6** were generated in potato and sweet potato slices infected with cultures of the pathogen *Streptomyces ipomoeae* (King et al., 1994). Compound **3**, the major phytotoxin produced by *S. ipomoeae*, was named thaxtomin C.

3. Detection, isolation and structure determination

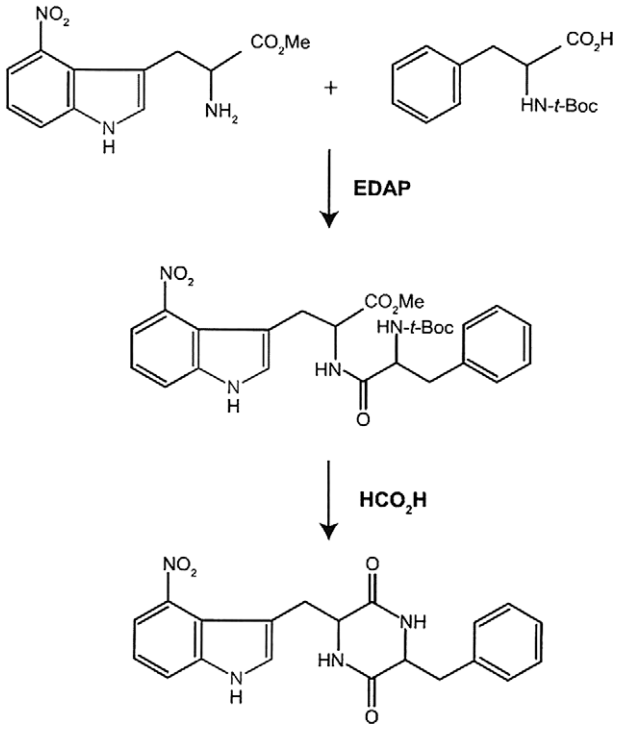
The presence of thaxtomins in extracts of infected materials can be determined on TLC by their appropriate R_f values and bright yellow colour (King et al., 1989). Precise amounts of the thaxtomins can readily be measured by HPLC with UV detection due to their strongly absorbent 4-nitrotryptophan chromophore (King et al., 1992; Kim et al., 1999; Hiltunen et al., 2006). Methodologies utilizing the production of antibodies specific for thaxtomin A (**1**) have also been developed (Jang et al., 2000).

The phytotoxins thaxtomin A (**1**) and B (**4**) were first isolated from the acetone soluble extracts of potato tuber slices infected with cultures of *S. scabies* (King et al., 1989). Purification involved a bioassay-guided normal phase TLC fractionation using aseptically grown potato mini-tubers (Lawrence et al., 1990). The molecular formula of thaxtomin A (**1**) was established from high resolution FAB mass spectra and DEPT ^{13}C NMR spectroscopic analysis. The presence of nitroindol-3-yl and benzyloxy groups was first suggested by a detailed analysis of its electron impact (EI) mass spectroscopic fragmentation pattern. Subsequent analysis of the ^{1}H homonuclear shift correlated (COSY) spectra indicated substitution in the C-4 position of the indol-3-yl nucleus and at the *meta* position of the benzyloxy moiety. Comparative NMR spectroscopic studies with related synthetic compounds (i.e., *N*-methyl-4-nitrotryptophan and *m*-hydroxyphenylacetic acid) then facilitated complete carbon and proton assignments for the aromatic positions. The IR data, which displayed the presence of disubstituted amide bands combined with the unassigned ^{1}H and ^{13}C NMR signals, was rationalized as indicative of a biosynthetically feasible *N*,*N*-dimethyl-2,5-dioxo-3-hydroxypiperazine unit coupling the 4-nitroindol-3-yl and *m*-hydroxybenzyl groups. For the purposes of structural confirmation and assignment of relative stereochemistry (at C-11 and C-14) cyclo-(L-4-nitrotryptophyl-L-phenylalanyl) (**11**) and cyclo-(L-4-nitrotryptophyl-D-phenylalanyl) were prepared (King et al., 1989). Significantly, only the former compound exhibited thaxtomin-like activity. Appropriately it also displayed the

Table 1
Thaxtomin producing pathogen sources.

Streptomyces spp.	Host crop	Location	References
S. scabies	Potato	S. Africa	De Klerk et al. (1996)
S. scabies	Peanut	S. Africa	De Klerk et al. (1996)
S. scabies	Potato	Canada	King et al. (1991)
S. scabies	Potato	China	Liu et al. (2004)
S. scabies	Potato	Egypt	El-Sayed, 2001)
S. scabies	Potato	Finland	Hiltunen et al. (2006)
S. europaeiscabiei	Potato	France	Loria et al. (2006)
S. stelliscabiei	Potato	France	Loria et al. (2006)
S. europaeiscabiei	Potato	Germany	Flores-Gonzalez et al. (2008)
S. scabies	Potato	Japan	Natsume et al. (1996)
S. turgiscabies	Carrot	Japan	Bukhalid et al. (1998)
S. turgiscabies	Potato	Japan	Bukhalid et al. (1998)
S. turgiscabies	Sugar beet	Japan	Bukhalid et al. (1998)
S. turgiscabies	Potato	Korea	Kim et al. (1999)
S. niveiscabiei	Potato	Korea	Park et al. (2003)
S. europaeiscabiei	Potato	Netherlands	Flores-Gonzalez et al. (2008)
S. europaeiscabiei	Potato	Scotland	Flores-Gonzalez et al. (2008)
S. scabies	Potato	USA	Babcock et al. (1993)
S. acidiscabies	Potato	USA	King et al. (1991)
S. ipomoeae	Sweet potato	USA	King et al. (1994)

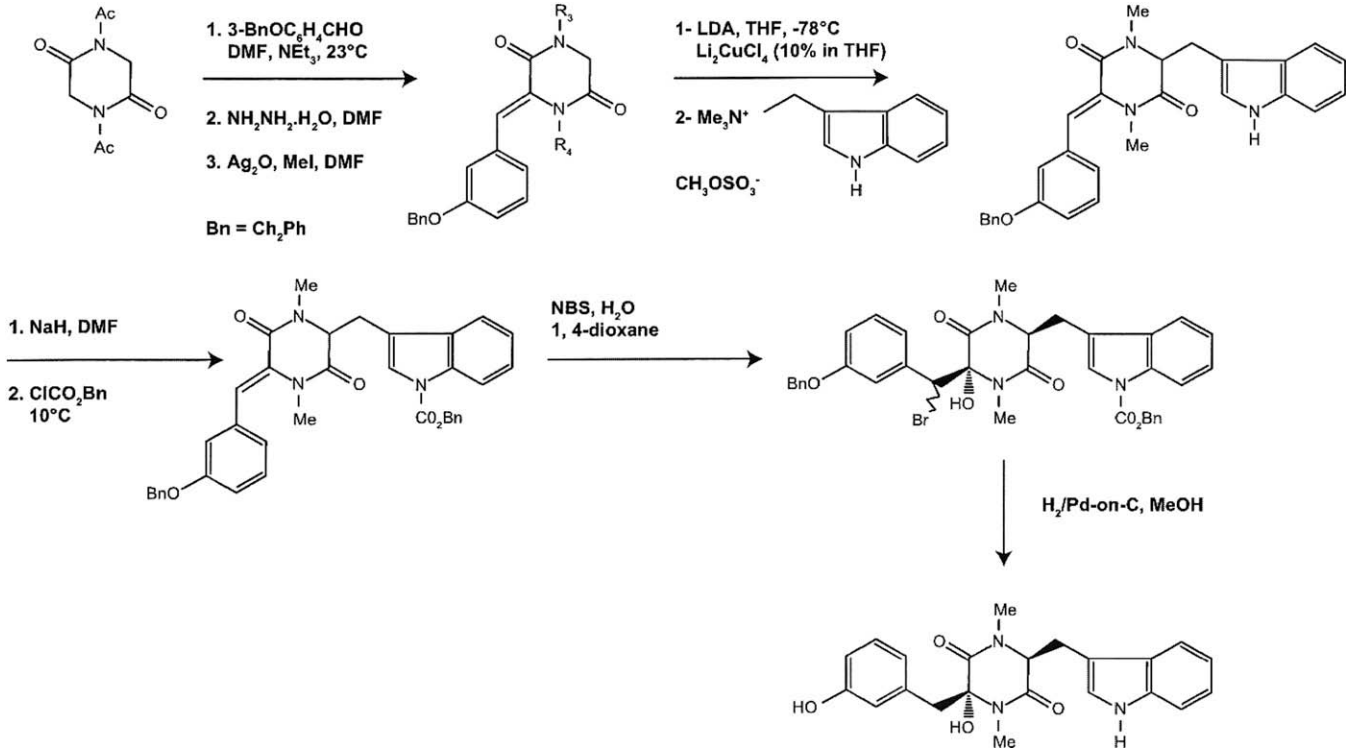
most notably comparable ^{1}H NMR spectrum. Thaxtomin A (**1**) is the most prominent member of the thaxtomins and its proposed structure was also confirmed by X-ray analysis (Wagner, 2000). The structure of thaxtomin B (**4**) as a C-20 deoxy analog of thaxtomin A (**1**) was readily apparent from its mass spectroscopic data and a comparative analysis of its ^{1}H NMR and ^{13}C NMR spectra (King et al., 1989). Mass spectrometry confirmed that compound **2** had the same molecular composition as thaxtomin A (**1**) and its structural assignment as the *o*-benzyloxy isomer was based on ^{1}H COSY spectra and comparative studies with a synthetic sample of *o*-hydroxyphenylacetic acid (King et al., 1992). A comparative analysis of its ^{1}H NMR and mass spectroscopic data established the structure of thaxtomin C (**3**) as 12-*N*-methyl-cyclo-(L-4-nitrotryptophyl-L-phenylalanyl). Assignment of the *N*-methyl substituent was determined on the basis of an observed coupling between H-14 and an adjacent amide proton (King et al., 1992). Compound **5** was characterized as a C-14 deoxy analog of thaxtomin B (**4**) from its mass spectroscopic data and ^{1}H NMR decoupling experiments (King et al., 1992). Compound **6** was formulated as a mono-oxygenated analog of thaxtomin C (**3**) on the basis of its molecular weight. A comparative analysis of its ^{1}H COSY and ^{1}H NMR spectra with that of thaxtomin C (**3**) then established the structure of this compound as 12-*N*-methyl-14-hydroxycyclo-(L-4-nitrotryptophyl-L-phenylalanyl) (**6**). Assignment of an hydroxyl group to the C-14 position was determined on the evident lack of a proton therein. Assignment of the structure for compound **7** as the *p*-benzyloxy isomer of thaxtomin A (**1**) was based on the ^{1}H COSY spectra and comparative studies with a sample of *p*-hydroxyphenylacetic acid (King and Lawrence, 1996). Compound **8**, the most complex member of the thaxtomins, was formulated as a mono-oxygenated derivative of thaxtomin A (**1**) on the basis of its molecular weight. Ultimate assignment of the structure as a 3,4-dihydroxyphenyl analog was based on its ^{1}H COSY spectra and comparative studies with a sample of 3,4-dihydroxyphenylacetic acid (King and Lawrence, 1996). MS and ^{1}H NMR spectroscopic analysis of compounds **9** and **10** indicated that they were de-*N*-methyl analogs of thaxtomin A (**1**). Characterization of compound **9** as the 15-de-*N*-methyl analog relied primarily upon a comparative analysis of its MS and ^{1}H COSY spectra with the 12-*N*-methyl compound **6** described previously. The validity of the preceding assignment was later substantiated on the basis of an observed coupling between H-11 and an adjacent amide proton when a ^{1}H NMR spectrum of the alternate isomer compound **10** was recorded in deuterated dimethyl sulfoxide (King and Lawrence, 1996).



Scheme 1. Synthesis of cyclo-(L-4-nitrotryptophyl-L-phenylalanyl) (**11**).

4. Synthesis

Biosynthetically, the thaxtomins are envisaged as being derived from the combination (diketopiperazine ring formation) of two amino acids: tryptophan and phenylalanine, with the required nitration, hydroxylation and *N*-methylation steps occurring at some point along the way. In line with this approach, the first thaxtomin to be synthesized (Scheme 1) required condensation of a sample of 4-nitrotryptophan methyl ester prepared through a modified malonic ester synthesis of 4-nitrogramine (Endo et al., 1986; King and Calhoun, 2009) with *t*-Boc-L-



Scheme 2. Synthesis of an un-nitrated analog of thaxtomin A (**1**).

phenylalanine to give a dipeptide methyl ester. Its formate salt was then dissolved in 2-butanol/toluene and thermally cyclized (Nitecki et al., 1968) to yield the requisite thaxtomin analog (**11**) (King et al., 1989). The synthesis of thaxtomin C (**3**) was later undertaken via a similar route from *N*-methyl-4-nitrotryptophan methyl ester (King, 1997) in order to provide convenient access to reasonable quantities of the phytotoxin for host-interaction studies. *S. ipomoeae* the major producer of this phytotoxin could not be induced to generate it when grown on any of the usual culture media. Unequivocal evidence for confirmation of the integrity of the synthetic sample was obtained by comparing it (UV, IR, MS, NMR, TLC and biological activity) with an authentic sample of thaxtomin C (**3**). The earlier preparation of a denitrothaxtomin A analog (**12**) required a substantially more involved process (Gelin et al., 1993; Moyroud et al., 1996). The key step was reaction of a lithiated anion of sarcosine anhydride with gramine methosulfate in the presence of a catalytic amount of dilithium tetrachlorocuprate in THF. The remainder of the sequence involved protection of the indole NH, aldol condensation with 3-benzloxybenzaldehyde, bromohydrin formation and catalytic hydrogenation (Scheme 2).

5. Biosynthesis

The opportune finding that pathogenic *Streptomyces* species responsible for potato common scab could be induced to generate the thaxtomin phytotoxins *in vitro* (Babcock et al., 1993; Loria et al., 1995) provided an excellent venue for identifying biosynthetic intermediates. Without discarding the possibility of nitroanthranilic acid precursors (King et al., 1998), the identification of *N*-acetyl-4-nitrotryptophan and *N*-methyl-4-nitrotryptophan residues in the phytotoxin production medium (King and Lawrence, 1995) suggested a pathway in which nitration of the indole group precedes formation of the diketopiperazine ring. *N*-acetyl-4-nitrotryptophan may function as a precursor to *N*-methyl-4-nitrotryptophan because on its addition to isolates of *S. scabies* (in a minimal growth media) substantial de-acetylation was observed (King et al., 2003). It also follows that the presence of *N*-methyl-4-nitrotryptophan in the phytotoxin production extracts implies its probable involvement as an immediate precursor for condensation with phenylalanine to produce 12-*N*-methylcyclo-(L-4-nitrotryptophyl-L-phenylalanyl) (**3**) (thaxtomin C) a logical precursor for the biosynthesis of all but one of the thaxtomins presently identified (see Fig. 2). Evidence for both aspects of this supposition was effectively illustrated in two different ways. First, the progression from thaxtomin C (**3**) via compounds **5** and thaxtomin B (**4**) to thaxtomin A (**1**) was monitored on TLC (Babcock et al., 1993). Then radioactive tryptophan labeled in the C-8 position, when added to isolates of *S. scabies* grown in an oat bran medium, was shown to be effectively incorporated into the thaxtomin A (**1**) produced (Lauzier et al., 2002). The lone thaxtomin missing from the *S. scabies* production sequence, compound **11** is produced only by *S. Ipomoeae* (King et al., 1994). It would logically result from the condensation of an L-4-nitrotryptophan and an L-phenylalanine group.

The finding that pathogenic *Streptomyces species* grown *in vitro* also generated substantial quantities of 5-nitroanthranilic acid during thaxtomin production provided another conundrum regarding the nitration stage. Anthranilic acid is a normal precursor of tryptophan but if 5-nitroanthranilic acid proceeded to the tryptophan stage it would provide a tryptophan, analog nitrated in the C-5 position instead of the requisite C-4. Although the co-generation of 5-nitroanthranilic acid residues could not be linked to any definitive role in the biosynthesis of the thaxtomins the finding has interesting implications for further research into the vital nitration step i.e., it may well take place at an earlier stage than had previously been envisaged.

Molecular genetic studies of *S. acidiscabies* (using a gene disruption approach) have demonstrated a good accord with the proposed production sequence in *S. scabies* (Fig. 2). They indicate

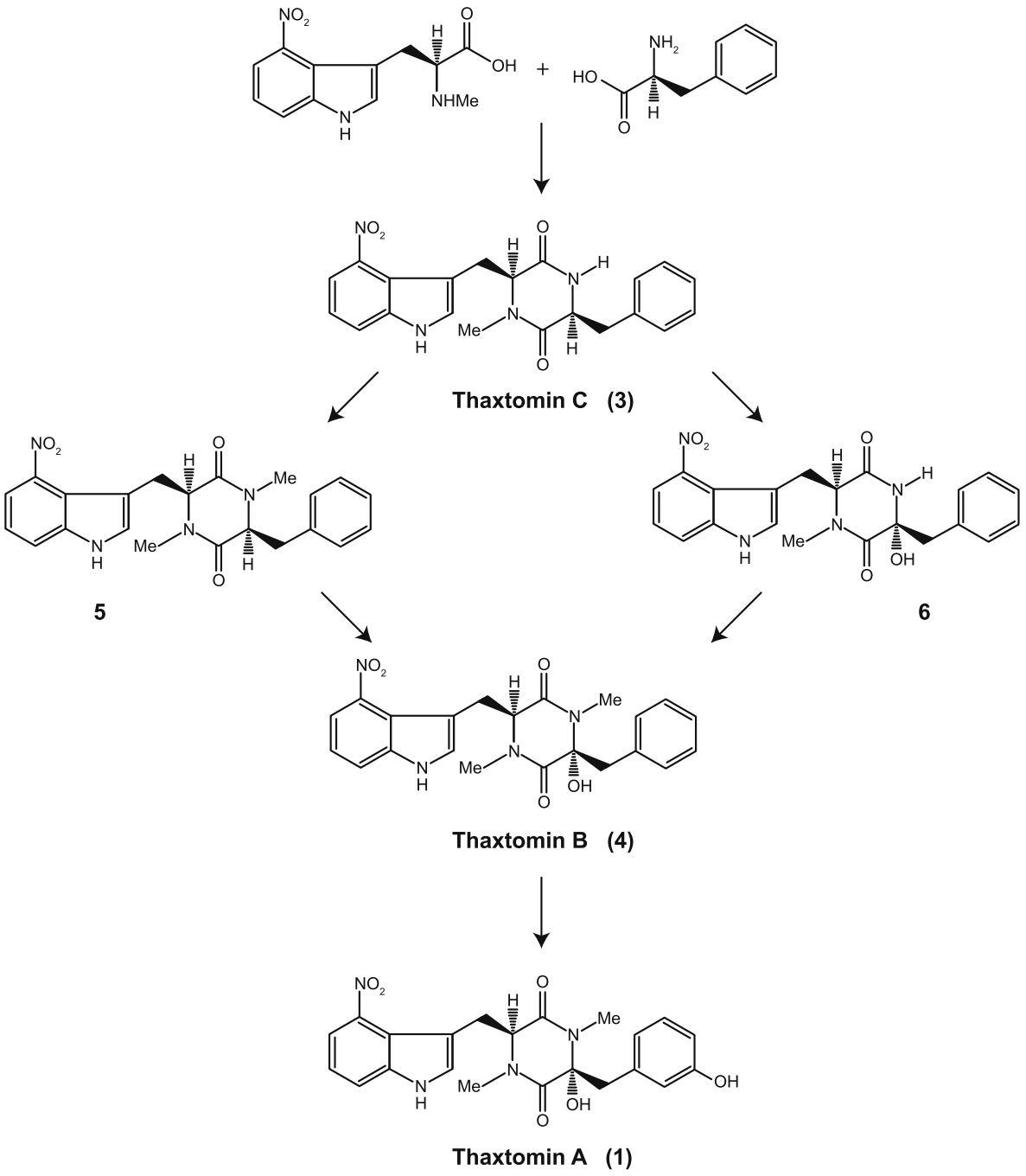


Fig. 2. Proposed biosynthetic sequence for thaxtomin A (**1**).

that the biosynthesis of the thaxtomins involve conserved non-ribosomal peptide synthetases encoded by genes named *TxtA* and *TxtB* (Healy et al., 2000). The *TxtA* and *TxtB* genes are responsible for production of the *N*-methylated cyclic dipeptide backbone of the toxins and a P450 mono-oxygenase named *TxtC* is required for the post-cyclization hydroxylation steps (Healy et al., 2002). Although biosyntheses of the vast majority of aromatic nitro compounds are known to occur through the enzymatic oxidation of primary amines (Winkler and Hertweck, 2005) genetic and isotope labeling studies have indicated that a nitric oxide synthetase (NOS) participates in the thaxtomin nitration process (Kers et al., 2004; Wach et al., 2005). The chemical mechanism of this NOS-mediated nitration is somewhat uncertain because NO is unlikely to react directly with an indole moiety (Hughes, 1999). It has also been found that disruption of the NOS-encoding gene greatly diminishes but does not abolish thaxtomin production (Loria et al., 2008). A related study which revealed that tryptophan is selectively nitrated in the 4-position by a complex of *Deinoccus radiodurans* NOS and Trp-tRNA synthetase (Buddha et al., 2004) may prove a useful guide for further investigations of the nitration step in production of the thaxtomins. In the latter study it was theorized that the *D. radiodurans* NOS may interact with tryptophan in a manner that protects the more reactive sites on the indole ring from being modified. Also of notable interest the thaxtomin biosynthetic genes and other putative virulence genes are carried on a conserved pathogenicity island (PAI) that can mobilize between *Streptomyces* species, sometimes resulting in the emergence of a new pathogenic species (Kers et al., 2005).

With respect to genetic studies of *S. ipomoeae* which produces mainly the less modified thaxtomin C (**3**) one cosmid was found to contain what may be the entire thaxtomin C gene cluster (Grau et al., 2006). Homologs of *TxtA* and *TxtB* genes were organized similarly to those of *S. turgidiscabies*, but no evidence of a *TxtC* homolog was found. Earlier attempts to isolate potential nitrated tryptophan precursors were restricted because pathogenic *S. ipomoeae* isolates could not be induced to generate the requisite phytotoxins when grown on any of the usual growth media (King et al., 1994). The isolation of an un-nitrated thaxtomin C analog associated with *S. ipomoeae* infected sweet potato slices (King et al., 1994), however, suggests the possibility that in this venue the nitration step may well occur in the latter stages of thaxtomin C (**3**) biosynthesis.

6. Biotransformation

The biotransformation of phytotoxins by plants and other organisms has been an active area of research for many years (Amusa, 2006 and references therein). Plants that can inactivate phytotoxins are of great value in breeding programs and microorganisms that do likewise have good potential as bio-control agents and the provision of resistant genes for generating disease-tolerant crops (Zhang et al., 1999; Pedras et al., 2002). Glucosylation is one of the mechanisms by which toxins can be rendered less toxic (Park et al., 1994; Strobel and Hess, 1997) and this was effectively demonstrated when thaxtomin A (**1**) and thaxtomin B (**4**) were transformed into C-14 linked β-glucosides upon incubation with cultures of *Baccillus mycoides* in an oatmeal broth (King et al., 2000). The conjugated toxins individually produced less than one-fifth of the potato tissue necrosis observed with the parent compounds. Subsequent studies (Acuna et al., 2001) explored the concept of thaxtomin A glucosylation as a possible means of detoxification in potato tubers. However, in those efforts, *p*-hydroxythaxtomin A (**8**) the most polar member of the thaxtomins appears to have been misidentified as a thaxtomin A (**1**) di-glucoside derivative (King et al., 2003). In a more recent study Lazarovits et al. (2004) found that a fungal isolate identified as *Aspergillus niger* van Tiegham var. *niger* was able to transform thaxtomin A (**1**) into a mixture of much less toxic C-14 alkyl ether derivatives (**13, 14**) and C-14 dehydrated analogs (**15–17**) (Fig. 3). The production of unknown thaxtomin A (**1**) derivatives after treatment of *Arabidopsis thaliana* seedlings with ^{3}H-labeled thaxtomin A (**1**), has also been noted, but their identities were not investigated (Scheible et al., 2003).

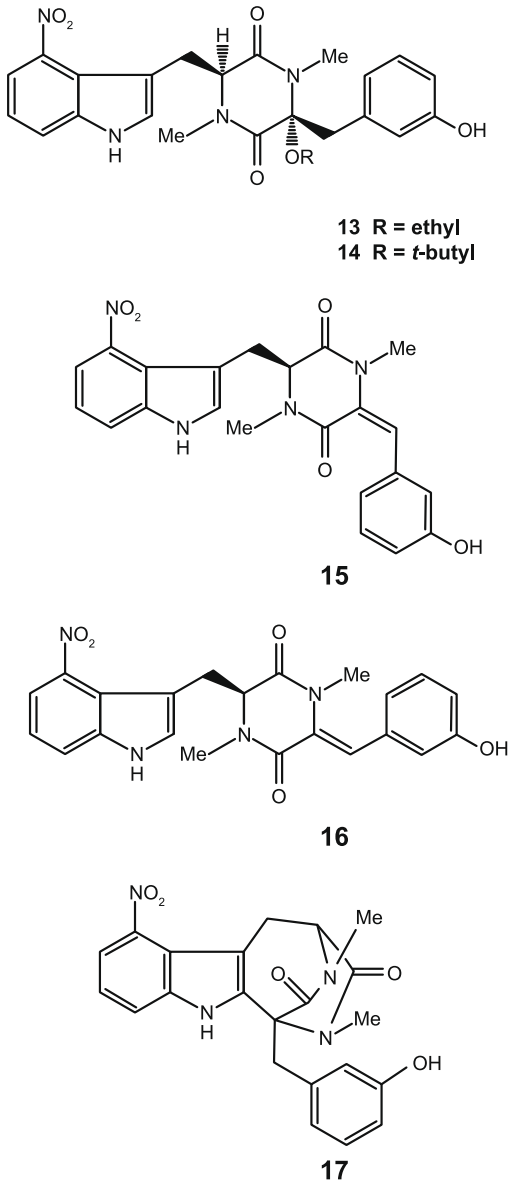


Fig. 3. Biotransformation products from thaxtomin A (**1**).

7. Biological activity

Initial bioactivity studies demonstrated that thaxtomin A (**1**) caused hypertrophy of plant cells at nanomolar amounts and cell death at concentrations similar to those found in scab lesions on field infected potato tubers (Lawrence et al., 1990). Thereafter, investigations were initiated to further understanding of the structural requirements for the thaxtomins observed bioactivity. In the characterization of thaxtomin A (**1**) and B (**4**), it had been demonstrated that the presence of a nitro group in the indole ring of the tryptophan moiety and an L,L configuration of the diketopiperazine ring appeared to be the minimal requirements for phytotoxicity (King et al., 1989). These strict stereochemical requirements were later demonstrated in the loss of bioactivity upon isomerization of the C-14 configuration in thaxtomin A alkyl ethers (Krasnoff et al., 2005) and the *ortho* isomer (**2**) of thaxtomin A (**1**) (King et al., 2003). Also investigated was any prerequisite for the phenyl portion of phenylalanine and how alternate positioning of the nitro group in the indole ring would affect bioactivity. For this purpose, the 2,5-dioxopiperazines, cyclo-(L-5-nitrotryptophyl-L-phenylalanyl), cyclo-(L-6-nitrotryptophyl-L-phenylalanyl), cyclo-(L-7-nitrotryptophyl-L-phenylalanyl) and cyclo-(L-4-nitrotryptophyl-L-leucyl) were prepared from the appropriate nitrotryptophan precursors (King and Calhoun, 2009) as outlined for cyclo-(L-4-nitrotryptophyl-L-phenylalanyl) (**11**) in Scheme 1. Subsequent examination of these analogs for phytotoxicity toward aseptically cultured mini-tubers proved negative in all cases (King et al., 1992). It is thus apparent that the phenyl portion of phenylalanine plays a necessary part in the structural requirements for bioactivity and that the positioning of the nitro group in the indole ring is very site specific. Since bacterial plant pathogens can experience difficulties growing on living plant cells, thaxtomin production may be a requisite for the establishment of infection (Loria et al., 1997). In this context, electron microscopy studies have demonstrated that thaxtomin A causes cell collapse in potato tubers resulting in an electron-dense mass of cell walls and cytoplasm remains (Stein et al., 1995; Goyer et al., 2000). Other studies indicated that very low concentrations (0.4 ppb) of thaxtomin A (**1**) are sufficient to cause a great increase in cell volume in the hypocoyls of onion

(*Allium cepa* L.), in radish seedlings (*Raphanus sativus* L.) and in tobacco suspension cells (*Nicotiana tabacum* L.) (Fry and Loria, 2002). Low concentrations of thaxtomin A (**1**) (4–10 ppb) caused stunting and hypertrophy of roots and shoots of radish seedlings, whereas higher concentrations (20–40 ppb) caused tissue necrosis (Leiner et al., 1996). An eventual major step in elucidating the thaxtomins mode of action resulted from an extensive survey of their herbicidal properties (King et al., 2001). Eight of the 11 more readily available thaxtomins (compounds **1–4**, **6–8** and **11**), when assessed by a variety of standard phytotoxicity tests, elicited symptoms remarkably similar to those caused by known cellulose inhibitors such as dichlobenil and isoxaben (Schneegurt et al., 1994; Delmer and Amor, 1995). Scheible et al. (2003) subsequently obtained three further lines of evidence that helped confirm and extend these observations. Most telling was the finding that thaxtomin A (**1**) specifically inhibited incorporation of ^{14}C-glucose into the cellulosic cell wall fraction of dark-grown wild-type *A. thaliana* seedlings. In a related study, Duval et al. (2005) observed that isoxaben (the previously noted highly specific inhibitor of cellulose biosynthesis) also induced in *A. thaliana* cell suspensions a programmed cell death similar to that induced by thaxtomin A (**1**). More recently, in a related context, it has been demonstrated that thaxtomin production is affected by a transcriptional regulator that responds to the presence of cellobiose the smallest oligomer of cellulose (Wach et al., 2007; Joshi et al., 2007; Johnson et al., 2007). Tegg et al. (2005) in a different approach provided the first evidence that thaxtomin A (**1**) triggers an early signaling cascade, causing rapid and tissue specific changes in net Ca^{2+} and H^+ ion flux profiles that may be crucial in these plant–pathogen interactions. The study also suggests a possible interaction between thaxtomin A (**1**) and plasma membrane receptors as demonstrated from experiments on an auxin sensitive *A. thaliana* mutant.

8. Concluding remarks

The generation of thaxtomins by common scab-causing species from diverse geographic areas of the world and the quantitative relationship established between phytotoxin production and virulence overwhelmingly supports the concept of these toxins as pathogenicity determinants (King et al., 1991; Leiner et al., 1996; Goyer et al., 1998; Kinkel et al., 1998). Consequently, the presence of the *TxtA* gene encoding thaxtomin biosynthesis has become a very reliable pathogenicity determinant in the identification (Wanner, 2004, 2006, 2007; Flores-Gonzalez et al., 2008; St-Onge et al., 2008) and quantification (Wang and Lazarovits, 2004; Qu et al., 2008) of common scab-causing *Streptomyces* spp. The requirement for thaxtomin production by pathogenic *Streptomyces* spp. has also provided another means of screening new potato lines for resistance to common scab i.e., potato seedlings can be selected for resistance to the phytotoxins by tissue culture methodologies (Acuna et al., 1999; Tarn et al., 2004; Hiltunen et al., 2006; Khu et al., 2007). Tissue culture technologies have also been utilized in the selection and isolation of thaxtomin resistant cells of current commercial varieties (Luckman and Wilson, 2000; Wilson, 2004; Wilson et al., 2009). Possibly most important characterization of the thaxtomin phytotoxins as primary agents of scab development has introduced the possibility of control measures based on the restriction or inhibition of their effects. For example, Scheible et al. (2003) has demonstrated the potential for isolation and integration of genes conferring thaxtomin tolerance to genotypes susceptible to pathogenic *Streptomyces* species while Tegg et al. (2008) in a more indirect approach has shown that auxin treatments can significantly ameliorate the toxicity of thaxtomin A. Also of note a patent has recently been filed that proposes to utilize thaxtomins for the purpose of treating and/or controlling algae (Novozymes Biological, 2008).

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