PERGAMON

Phytochemistry 59 (2002) 579–596

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Review

The destruxins: synthesis, biosynthesis, biotransformation, and biological activity

M. Soledade C. Pedras*, L. Irina Zaharia, Dale E. Ward

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

Received 5 October 2001; received in revised form 14 December 2001

Abstract

Destruxins, secondary metabolites first reported in 1961, are cyclic hexadepsipeptides composed of an α-hydroxy acid and five amino acid residues. The name "destruxin" is derived from "destructor" from the species *Oospora destructor*, the entomopathogenic fungus from which these metabolites were first isolated. Individual destruxins differ on the hydroxy acid, *N*-methylation, and R group of the amino acid residues; where established, the configurations of the amino acid residues are *S*, and those of the hydroxy acids are *R*. Destruxins exhibit a wide variety of biological activities, but are best known for their insecticidal and phytotoxic activities. The great interest in destruxins derives from their potential role as virulence factors in fungi, whether such microorganisms are useful insect biocontrol agents or detrimental, causing great plant disease epidemics. Reports on isolation, chemical structure determination, total synthesis, transformation by diverse organisms, and biological activity of destruxins and related metabolites are reviewed for the first time.

Contents

1. Introduction 580

2. Chemical structures and producing organisms 580

3. Detection, isolation and structure determination 582

4. Synthesis 583

5. Biosynthesis 587

6. Biotransformation 588
   6.1. In insects 588
   6.2. In plants 589

7. Biological activity 589

8. Concluding remarks 593

References 593

* Corresponding author. Tel.: +1-306-966-4772; fax: +1-306-966-4730.
E-mail address: soledade.pedras@usask.ca (M.S.C. Pedras).

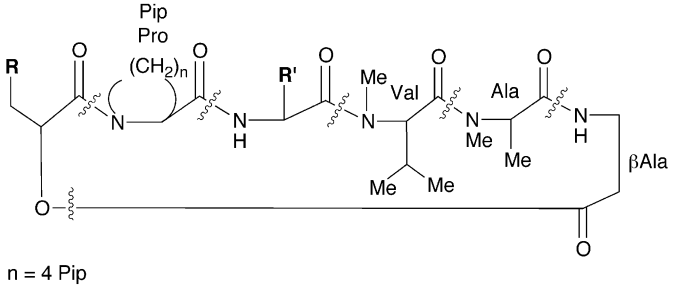
0031-9422/02/$ - see front matter
PII: S0031-9422(02)00016-X

1. Introduction

Since the first report of the discovery of destruxins A (**1**) and B (**10**) by Kodaira (1961), a very large number of studies on this family of compounds have been published. Destruxins are cyclic hexadepsipeptides composed of an α-hydroxy acid and five amino acid residues. The name "destruxin" is derived from "destructor" from the species *Oospora destructor* (Metch.) Delac., the entomopathogenic fungus from which these metabolites were first isolated. Later on, *O. destructor* was re-named *Metarrhizium anisopliae* (Metchnikoff) Sorokin, but as customary in such situations, the compounds' trivial names were retained (Suzuki et al., 1970). Individual destruxins differ on the hydroxy acid, *N*-methylation, and R group of the amino acid residues; where established, the configurations of the amino acid residues are *S*, and those of the hydroxy acids are *R*. The trivial names of destruxins include "destruxin" followed by a single capital which may contain a subscript number, as shown in Fig. 1. Destruxins A–E have the same amino acid sequence but differ in the R group of the hydroxy acid residue. Destruxins where the proline (Pro) residue ($n=3$) is replaced with a pipecolic acid (Pip) residue ($n=4$) were designated by the same letters with the subscript 1, i.e. A_1–E_1, while destruxins with a valine (Val) residue ($R'=CHMe_2$) instead of the isoleucine (Ile) residue ($R'=CHMeCH_2Me$) were designated with subscript 2, i.e. A_2–E_2 (Païs et al., 1981). Although there is no established convention for the use of this nomenclature, for consistency this review will use subscript numbers as proposed by Païs et al. (1981).

2. Chemical structures and producing organisms

The great majority of the destruxins **1**–**35** reported thus far were isolated from cultures of *M. anisopliae,* a pathogen of diverse insect species; however, a few destruxin produced by fungi are plant pathogens, as summarized in Table 1. Destruxin A (**1**) and destruxin B (**10**), first reported as secondary metabolites from *O. destructor* (Kodaira, 1961), were also isolated from fungal cultures of *Trichotecium roseum* TT103 (Tsunoo et al., 1997) and from OS-F68576 (Cai et al., 1998) together with destruxin A_4 (**5**) and homodestruxin B (**15**). Destruxin B (**10**) was also reported to be the major toxin produced by the fungal pathogen of *Brassica* species, *Alternaria brassicae* (Berkeley) Saccardo, both in vitro (Ayer and Peña-Rodriguez, 1987; Bains and Tewari, 1987) and in planta (Buchwaldt and Jensen, 1991; Pedras and Smith, 1997). In addition, isolation of destruxin B (**10**) was reported from liquid cultures of *Ophiosphaerella herpotricha* (Fr.) Walker, which causes spring dead spot on bermuda grass (Venkatasubbaiah et al., 1994). Homodestruxin B (**15**) was first isolated from cultures of *A. brassicae* together with destruxin B (**10**), desmethyldestruxin B (**13**) (Ayer and Peña-Rodriguez, 1987) and destruxin B_2 (**12**) (Buchwaldt and Jensen, 1991), and also detected in leaves of *B. napus* infected with *A. brassicae* (Buchwaldt and Jensen, 1991). Destruxin E diol (**31**), isolated from cultures of *M. anisopliae* (Wahlman and Davidson, 1993), was first reported as a locust metabolic product of destruxin E (**26**) (Cherton et al., 1991). Protodestruxin (**16**) was isolated from a methionine-requiring mutant of *M. anisopliae*, whereas several destruxin chlorohydrins were reported (**7**, **29**, **30**) from the wild type organism. Destruxin E chlorohydrin (**29**) was first thought to result from an artifact of the isolation procedure, either derived from destruxin A (**1**) by addition of hypochlorous acid or from destruxin E (**26**) by epoxide ring opening with HCl (Gupta et al., 1989a). Nonetheless, the formation of **29** was not observed when destruxin E (**26**) was dissolved in concentrated HCl and stored at room temperature for 72 hours (Gupta et al., 1989a). The formation of

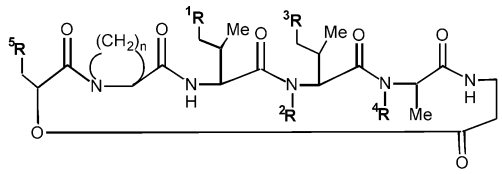


n = 3 Pro; n = 4 Pip

A series	B series	C series	D series	E series
R = –CH=CH$_2$	R = –CHMe$_2$	R = –CHMeCH$_2$OH	R = –CHMeCOOH	R = –CHCH$_2$ (O)
R' = –CH(Me)–CH$_2$Me	R' = –CH(Me)–CH$_2$Me	R' = –CH(Me)–CH$_2$Me	R' = –CH(Me)–CH$_2$Me	R' = –CH(Me)–CH$_2$Me

Fig. 1. Chemical structures and designations of destruxins: A–E ($n=3$, $R'=$–$CHMeCH_2Me$), A_1–E_1 ($n=4$, $R'=$–$CHMeCH_2Me$), A_2–E_2 ($n=3$, $R'=$–$CHMe_2$).

Table 1
Naturally occurring destruxins isolated from *Metarrhizium anisopli*ae (unless stated otherwise) with the general structure:



Destruxin (Dx)	*n*	^{1}R	^{2}R	^{3}R	^{4}R	^{5}R	First isolation
A series							
Dx A (**1**)	3	Me	Me	H	Me	–CH=CH$_2$	Kodaira, 1961
Dx A$_1$ (**2**)	4	Me	Me	H	Me	–CH=CH$_2$	Païs et al., 1981
Dx A$_2$ (**3**)	3	H	Me	H	Me	–CH=CH$_2$	Païs et al., 1981
Dx A$_3$ (**4**)	0[g]	Me	Me	H	Me	–CH=CH$_2$	Wahlman and Davidson, 1993
Dx A$_4$[a] (**5**)	3	Me	Me	Me	Me	–CH=CH$_2$	Krasnoff et al., 1996
Dx A$_5$[a] (**6**)	3[g]	Me	Me	Me	Me	–CH=CH$_2$	Krasnoff et al., 1996
Dx A$_4$[b] chlorohydrin (**7**)	3	Me	Me	Me	Me	–CHOHCH$_2$Cl	Cai et al., 1998
DesmethylDx A (**8**)	3	Me	H	H	Me	–CH=CH$_2$	Wahlman and Davidson, 1993
DihydroDx A (**9**)	3	Me	Me	H	Me	–CH$_2$Me	Jegorov et al., 1992a
B series							
Dx B (**10**)	3	Me	Me	H	Me	–CHMe$_2$	Kodaira, 1961
Dx B$_1$ (**11**)	4	Me	Me	H	Me	–CHMe$_2$	Païs et al., 1981
Dx B$_2$ (**12**)	3	H	Me	H	Me	–CHMe$_2$	Païs et al., 1981
DesmethylDx B (**13**)	3	Me	H	H	Me	–CHMe$_2$	Suzuki et al., 1970
DesmethylDx B$_2$ (**14**)	3	H	H	H	Me	–CHMe$_2$	Chen et al., 1995
HomoDx B[c] (**15**)	3	Me	Me	Me	Me	–CHMe$_2$	Ayer and Peña-Rodriguez, 1987
ProtoDx (**16**)	3	Me	H	H	H	–CHMe$_2$	Suzuki and Tamura, 1972
HydroxyDx B[d] (**17**)	3	Me	Me	H	Me	–COHMe$_2$	Pedras et al., 1999
HydroxyhomoDx B[d] (**18**)	3	Me	Me	Me	Me	–COHMe$_2$	Pedras et al., 1999
β-D-Glucopyranosyl-hydroxyDx B[e] (**19**)	3	Me	Me	H	Me	–CMe$_2$ (O-β-D-glucosyl)	Pedras et al., 2001
C series							
Dx C (**20**)	3	Me	Me	H	Me	–CHMeCH$_2$OH	Suzuki et al., 1970
Dx C$_2$ (**21**)	3	H	Me	H	Me	–CHMeCH$_2$OH	Païs et al., 1981
DesmethylDx C (**22**)	3	Me	H	H	Me	–CHMeCH$_2$OH	Wahlman and Davidson, 1993
D series							
Dx D (**23**)	3	Me	Me	H	Me	–CHMeCOOH	Suzuki et al., 1970
Dx D$_1$ (**24**)	4	Me	Me	H	Me	–CHMeCOOH	Païs et al., 1981
Dx D$_2$ (**25**)	3	H	Me	H	Me	–CHMeCOOH	Païs et al., 1981
E series							
Dx E (**26**)	3	Me	Me	H	Me	–CHCH$_2$ (O)	Païs et al., 1981
Dx E$_1$ (**27**)	4	Me	Me	H	Me	–CHCH$_2$ (O)	Païs et al., 1981
Dx E$_2$ (**28**)	3	H	Me	H	Me	–CHCH$_2$ (O)	Gupta et al., 1989a
Dx E chlorohydrin (**29**)	3	Me	Me	H	Me	–CHOHCH$_2$Cl	Gupta et al., 1989a
Dx E$_2$ chlorohydrin (**30**)	3	H	Me	H	Me	–CHOHCH$_2$Cl	Yeh et al., 1996
Dx E diol (**31**)	3	Me	Me	H	Me	–CHOHCH$_2$OH	Wahlman and Davidson, 1993
Dx E$_1$ diol (**32**)	4	Me	H		Me	–CHOHCH$_2$OH	Jegorov et al., 1998a
F series							
Dx F (**33**)	3	Me	Me	H	Me	–CHOHMe	Wahlman and Davidson, 1993
New series							
PseudoDx A[f] (**34**)	3	Bn	Me	Me	Me	–CHMe$_2$	Che et al., 2001
PseudoDx B[f] (**35**)	3	Bn	Me	H	Me	–CHMe$_2$	Che et al., 2001

[a] Isolated from *Aschersonia* sp.
[b] Isolated from fungal cultures of OS-F68576.

(*continued on next page*)

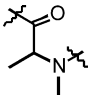
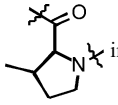
Table 1 (*continued*)

[c] Isolated from *Alternaria brassicae* (Berkeley) Saccardo.

[d] Isolated from *Sinapis alba*.

[e] Isolated from *Brassica napus*.

[f] Isolated from *Nigrosabulum globosum*.

[g] In destruxin A_3 (**4**) and destruxin A_5 (**6**), the proline residue is replaced by the following residues:  in destruxin A_3 and  in destruxin A_5.

destruxin A_4 chlorohydrin (**7**) during the isolation procedure was excluded (Cai et al., 1998) since extraction in the presence or absence of chlorinated solvents gave equivalent quantities of chlorohydrin. Mutants and new strains of *M. anisopliae* producing larger amounts of destruxins A, B, E, and desmethyldestruxin B were recently isolated (Hsiao and Ko, 2001). Pseudodestruxins A (**34**) and B (**35**) are the most recently reported new destruxins, and are produced by the coprophilous fungus *Nigrosabulum globosum* (Che et al., 2001). The isolation of the destruxin structural analogues bursaphelocides A and B from an imperfect fungus strain D1084 (Kawazu et al., 1993), roseocardin from *Trichothecium roseum* (Tsunoo et al., 1997) and roseotoxin B from *T. roseum* (Engstrom et al., 1975; Springer et al., 1984) was also reported (Fig. 2); however, these compounds were not named destruxins as they were first isolated from fungal species different from *M. anisopliae*.

3. Detection, isolation and structure determination

The presence of destruxins in extracts of fungal cultures can be detected by TLC, using plates with fluorescence indicators or visualized by oxidizing reagents (Kaijiang and Roberts, 1986; Gupta et al., 1989b; Venkatasubbaiah et al., 1994). Destruxins are readily analyzed by HPLC with UV detection at 206–214 nm, due to the absence of strong chromophores in their structure. These toxins were isolated from organic extracts obtained from fungal cultures (EtOAc, CCl_4, CH_2Cl_2) following bioassay-guided fractionation; both silica gel chromatography and reversed-phase HPLC have been utilized (Païs et al., 1981; Ayer and Peña-Rodriguez, 1987; Bains and Tewari, 1987; Samuels et al., 1988a; Gupta et al., 1989a, b; Buchwaldt and Jensen, 1991; Che et al., 2001). The quantification of various destruxins in extracts of fungal cultures of *M. anisopliae* was carried out by HPLC using calibration curves of purified destruxins (Loutelier et al., 1996; Chen et al., 1999). Fast atom bombardment–mass spectrometry (FAB–MS) has been a very useful method for analysis of destruxins in crude fungal extracts (Lange et al., 1991a, 1992a; Loutelier et al., 1995a). More recent methods include LC–MS with atmospheric pressure chemical ionization (Jegorov et al., 1998b), and LC-electrospray ionization time-of-flight spectrometry (Potterat et al., 2000).

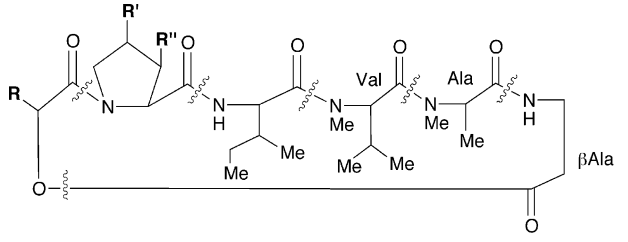


Fig. 2. Chemical structures of naturally occurring destruxin analogues: roseotoxin B (R = –$CH_2CH=CH_2$, R′ = –H, R″ = –Me), bursaphelocide A (R = –$CHMeCH_2Me$, R′ = –H, R″ = –H), bursaphelocide B (R = –$CHMeCH_2Me$, R′ = –Me, R″ = –H), roseocardin (R = –CH_2CHMe_2, R′ = –H, R″ = –Me).

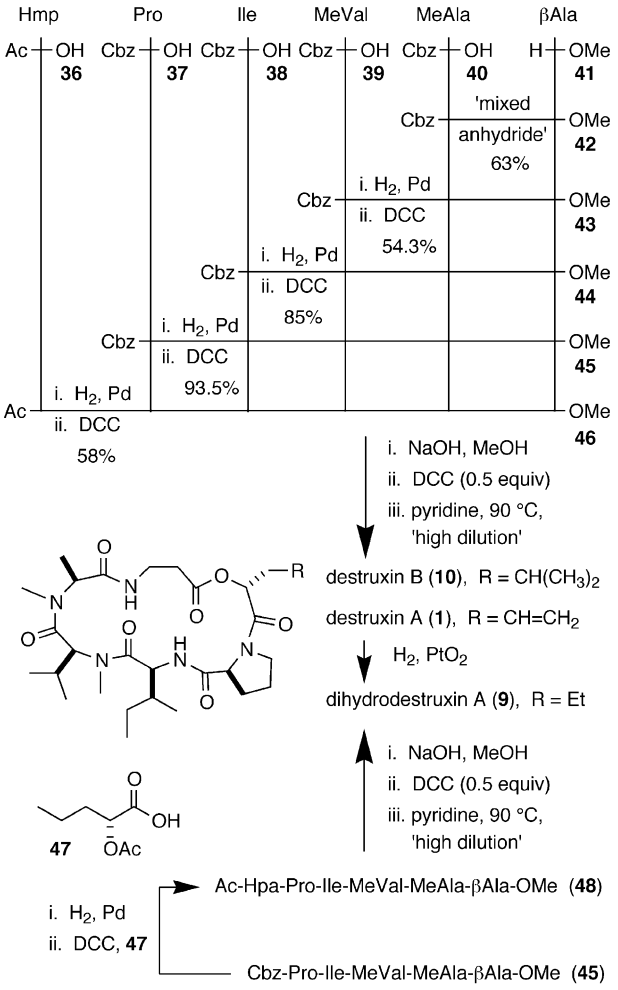
The chemical structures of destruxins A (**1**) (Suzuki et al., 1966) and B (**10**) (Tamura et al., 1964) were established upon acidic hydrolysis and paper chromatography, and confirmed by total synthesis. Interestingly, Suzuki and co-workers confirmed the structure of destruxin A via dihydrodestruxin A (**9**), which was several years later isolated from cultures of *M. anisopliae* (Jegorov et al., 1992a,b). The structures of hydroxydestruxin B (**17**), hydroxyhomodestruxin B (**18**), and β-D-glucopyranosyl hydroxydestruxin B (**19**) were assigned from NMR and MS data and confirmed by total synthesis (Pedras et al., 1999, 2001). In other cases the complete structural assignments of naturally occurring destruxins have been accomplished using MS and NMR spectroscopic techniques. The fragmentation patterns obtained by MS analysis of the linear peptides resulting from hydrolysis of the cyclic depsipeptide ester bond, were widely used to assign the structures of diverse destruxins (Suzuki et al., 1966; Suzuki and Tamura, 1972; Païs et al., 1981; Ayer and Peña-Rodriguez, 1987). However, the MS sequencing of the cyclic molecules using negative-ion FAB have also been reported (Lange et al., 1989, 1991a). Due to the great developments in NMR instrumentation, 2D NMR spectroscopic techniques are now commonly used for sequencing cyclic peptides, thus avoiding chemical hydrolysis and MS degradation (Gupta et al., 1989a; Wahlman and Davidson, 1993; Krasnoff et al., 1996; Cai et al., 1998; Che et al., 2001). As well, NMR techniques were used in conformational studies of protodestruxin (**16**), desmethyldestruxin B (**13**) and destruxin B (**10**) in DMSO-d_6 (Naganawa et al., 1976), and destruxin A (**1**) in $CDCl_3$ (Gupta et al., 1989c), water and CD_3CN (Rees et al., 1996). Results of these studies were in agreement with the crystal structures of destruxin B (**10**) (Steiner and Barnes, 1988) and destruxin A (**1**) (Gupta et al., 1989c). The conformations of destruxins A (**1**)

and B (**10**) were found to be similar; the ester linkage as well as the four peptide bonds were *trans*, while the MeAla-MeVal peptide bond was *cis*. The two hydrogen bonds βAla–NH·····O=C–Ile and Ile–NH·····O=C–β-Ala oriented towards the interior of molecule appear to contribute to the rigid conformation of these destruxins (Steiner and Barnes, 1988; Gupta et al., 1989c). The absolute configuration of the amino acid residues present in the diverse destruxins was confirmed by total synthesis (Suzuki et al., 1966; Kuyama and Tamura, 1965; Lee et al., 1975; Pedras et al., 1999, 2001; Ward et al., 2001), or enzymatic treatment (Engstrom et al., 1975). The absolute configuration of the phenylalanine residue present in pseudodestruxin A (**34**) was recently established by chiral TLC analysis and the complete structure confirmed by single-crystal X-ray diffraction analysis (Che et al., 2001).

4. Synthesis

Relatively large amounts of destruxins are required to investigate their biological activity and biotransformation. Because their isolation from natural sources can be quite laborious, chemical synthesis has provided sufficient material necessary for bioactivity studies, and has confirmed the structures of several naturally occurring destruxins. Cyclic peptides are typically synthesized by coupling intact amino acid and hydroxy acid residues followed by intramolecular cyclization of the resulting linear peptide chain (Izumiya et al., 1979). The order of the residue couplings and the choice of the cyclization site are key aspects to consider in the design of a synthetic route.

Destruxin B (**10**) was the first member of this category to be synthesized (Kuyama and Tamura, 1965). The synthesis was undertaken to corroborate the proposed structure and stereochemical configurations and was accomplished through linear coupling of the constituent amino acid and hydroxy acid residues followed by cyclization, as shown in Scheme 1. Using an unspecified 'mixed anhydride' method, β-alanine methyl ester (H–βAla–OMe; **41**) was coupled with benzyloxycarbonyl (Cbz) protected *N*-methylalanine (Cbz–MeAla–OH; **40**) to give the protected dipeptide Cbz–MeAla–βAla–OMe (**42**). The Cbz protecting group in **42** was removed by hydrogenolysis and the resulting amine was condensed with Cbz–protected *N*-methylvaline (Cbz–MeVal–OH; **39**) using dicyclohexylcarbodiimide (DCC) to obtain the tripeptide **43**. Using the same deprotection/coupling protocol, Cbz-protected isoleucine (Cbz–Ile–OH; **38**), Cbz-protected proline (Cbz–Pro–OH; **37**), and (2*R*)-2-acetoxy-4-methylpentanoic acid (Ac–Hmp–OH; **36**) were sequentially added to **43** to obtain the protected hexadepsipeptide **46** (16% overall yield from **41**). Interestingly, despite the difficulties associated with acylation



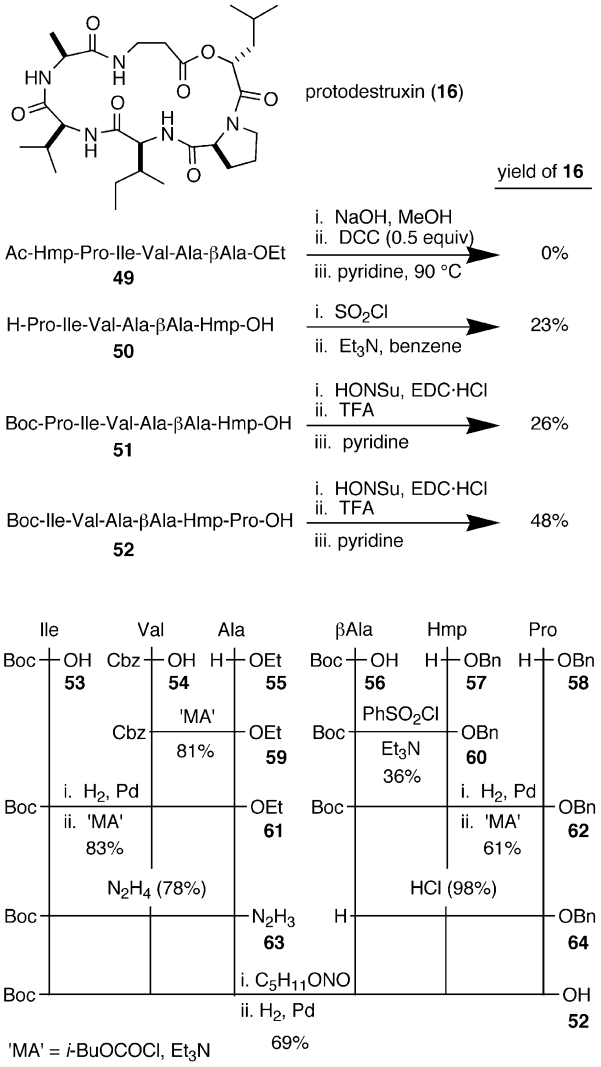
Scheme 1. Synthesis of destruxin B (**10**) (Kuyama and Tamura, 1965) and dihydrodestruxin A (**9**) (Suzuki et al., 1966).

of *N*-methylamino acid residues (Humphrey and Chamberlin, 1997), respectable yields were obtained for these steps (i.e. the formation of **43** and **44**) using traditional DCC mediated condensations. The ester protecting groups in **46** were hydrolyzed and the resulting hydroxy acid converted to the corresponding anhydride (DCC) followed by heating in pyridine solution at high dilution to give destruxin B (**10**) identical with that obtained from natural sources. The yield obtained for the cyclization step was not reported but is likely to be low judging from similar examples (vide infra).

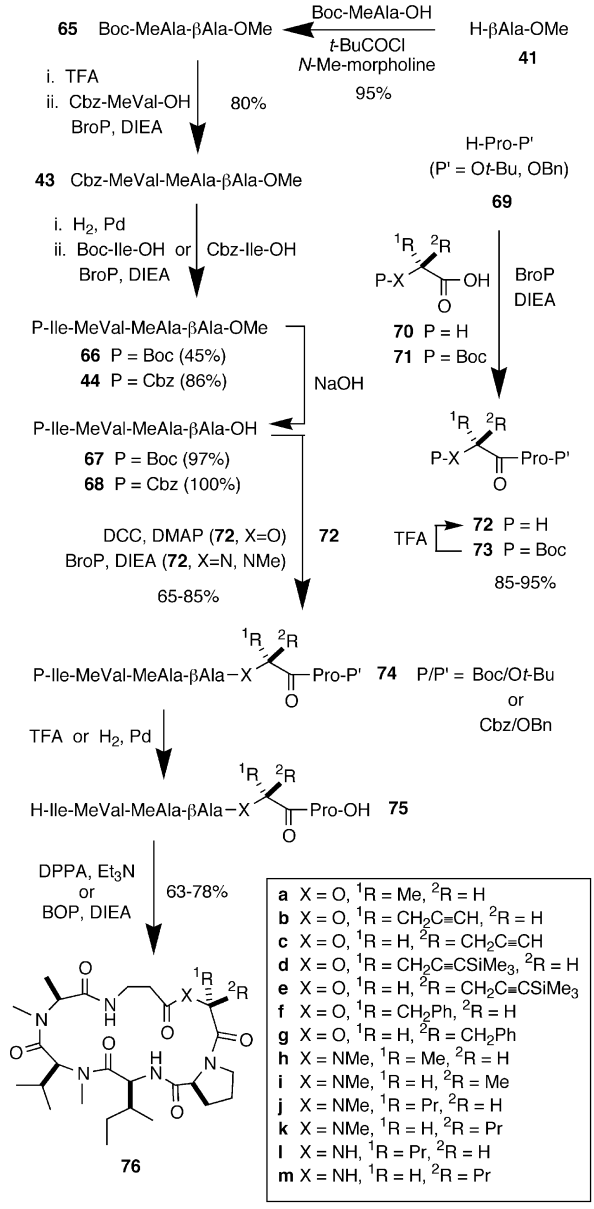
The total synthesis of dihydrodestruxin A (**9**) was reported by the same group (Suzuki et al., 1966) using an analogous approach (Scheme 1). Thus, the pentapeptide **45** was converted to **48** in unspecified yield by hydrogenolysis followed by DCC mediated condensation with (2*R*)-2-acetoxypentanoic acid (Ac–Hpa–OH; **47**). Subjecting **48** to the same procedure used for cyclization of **46** gave **9** in 'rather low yield'. The so obtained **9** was identical to that resulting from hydrogenation of destruxin A (**1**) thereby confirming the structure and stereochemical assignments for both **1** and **9**.

Several approaches to the synthesis of protodestruxin (**16**) were reported (Scheme 2) (Lee et al., 1975; Lee and Izumiya, 1977). In contrast to the above syntheses of **9** and **10**, all attempts at cyclization of **49** by formation of the βAla-Hmp ester linkage failed; however, cyclizations by formation of the amide bond at the Hmp-Pro site (i.e. **50** and **51**) or the Pro-Ile site (i.e. **52**) were successful. The cyclization of **52** using the *N*-hydroxysuccinimide (HONSu) activated ester method was particularly efficacious (48% yield). The authors rationalized the different propensities for cyclization by considering the number of possible intramolecular H-bonds between the amide N–H of one residue and the amide carbonyl 3 residues removed (i.e. the H-bond stabilizing a 3_{10} helix) among the different hexadepsipeptides (1 such H-bond for **52** and **46**; 2 for **50** and **52**; 3 for **49**) and the effect of such helical conformers on the proximity of the terminal groups. The absence of *N*-methylamino acid residues in **16** simplified the synthesis of the requisite linear hexadepsipeptides (**49**–**52**) and these were prepared using standard peptide coupling methods as illustrated for **52** (Scheme 2).

Cavelier et al. have synthesized a variety of destruxin analogues where the hydroxy acid residue in **1** was replaced by various α-hydroxy and amino acids (**76a**–**m**) to establish structure-activity relationships (Scheme 3) (Calmes et al., 1993; Cavelier et al., 1996, 1997, 1998). Noting the results of the protodestruxin (**16**) syntheses (cf. Scheme 2) and the resistance of *C*-terminal proline residues to epimerization during coupling, the Pro-Ile site was selected for cyclization. The requisite linear hexa(depsi)peptides were prepared by condensation of



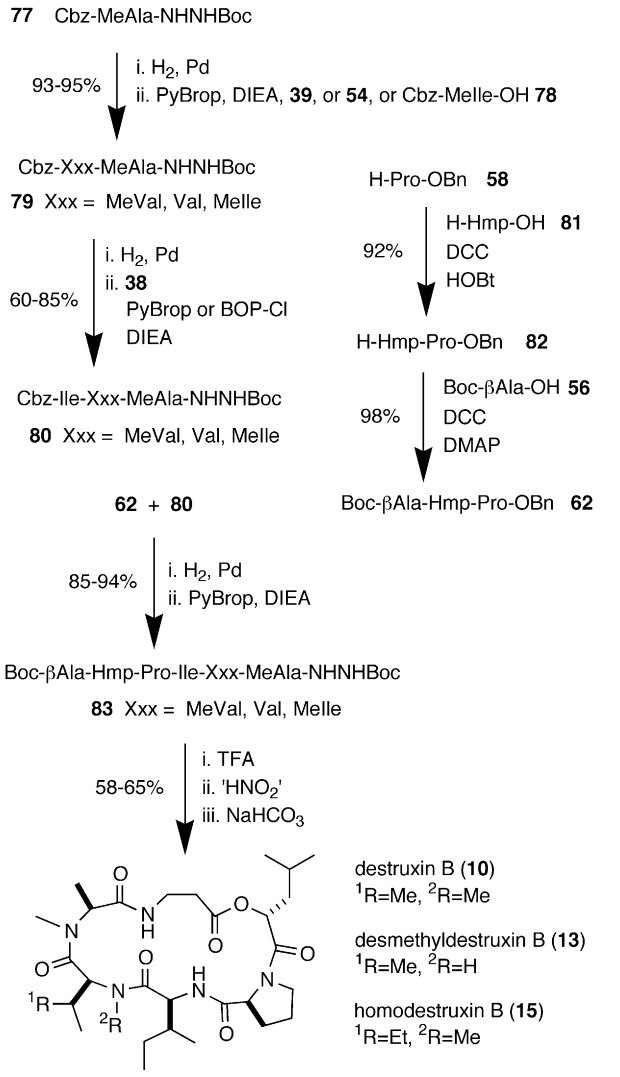
Scheme 2. Synthesis of protodestruxin (**16**) (Lee et al., 1975; Lee and Izumiya, 1977).



Scheme 3. Synthesis of destruxin analogues **76a-m** (Calmes et al., 1993; Cavelier et al., 1996, 1997, 1998).

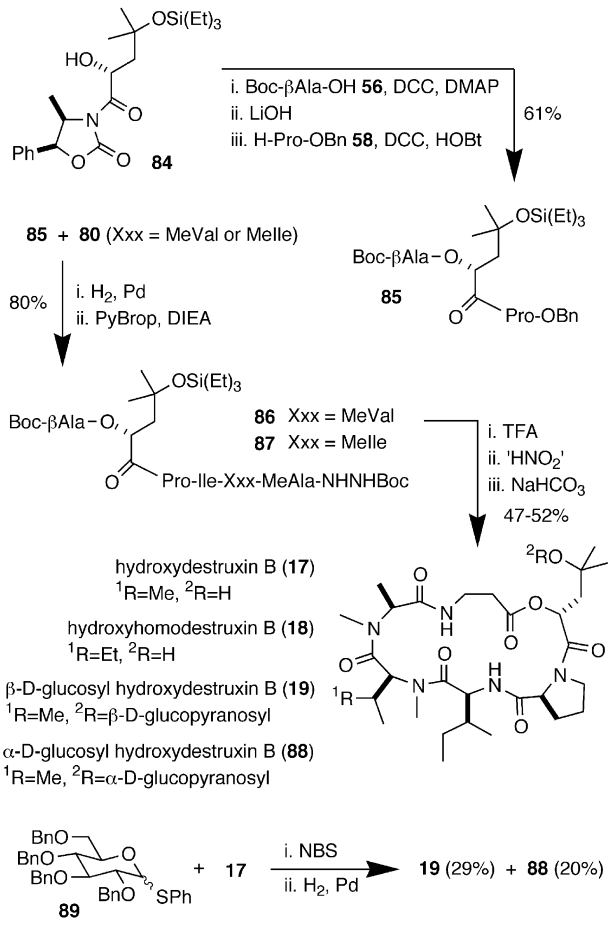
the tetrapeptides **67** or **68** with various di(depsi)peptides (**72a**–**m**). This convergent approach allowed incorporation of the vicinal *N*-methylamino acid residues early in the synthesis and, with the use of modern coupling reagents [e.g. bromotris(dimethylamino)phosphonium hexafluorophosphate (BroP) in the presence of *i*-Pr_2NEt (DIEA)], the synthesis of the tetrapeptide **44** (and **66**) was achieved with greatly improved yield compared to earlier work (cf. Scheme 1). The linear hexa(depsi)peptides **75** were similarly prepared in good yield by coupling of **67** or **68** with **72**. In the majority of cases (**75f**–**m**), the Cbz-protected **68** was coupled to a benzyl ester protected **72** (P' = OBn) to give **74** (P/P′ = Cbz/OBn) which gave **75** upon hydrogenolysis. However, in cases where the side chain was incompatible with hydrogenolysis (e.g. **74b**–**e**), the Boc-protected **67** was coupled to a *t*-butyl ester protected **72** (P′ = O*t*-Bu) yielding **74** (P/P′ = Boc/O*t*-Bu) which was treated with trifluoroacetic acid (TFA) to give **75**. The cyclization of **75a** to **76a** was achieved in a modest 30% yield using diphenylphosphoryl azide (DPPA) as the coupling reagent (Calmes et al., 1993). Subsequently, the cyclization of **75h** was examined with several reagents under various conditions (Cavelier et al., 1996, 1997) and excellent yields of the destruxin analogues **76b**–**m** (63–78%) were obtained from cyclizations of **75b**–**m** using DPPA or benzotriazol-1-yloxytri(dimethylamino)phosphonium hexafluorophosphate (BOP) as the coupling reagent under optimized conditions.

Efficient syntheses of destruxin B (**10**) and several natural analogues (**13**, **15**, **17**–**19**) including radiolabeled congeners were recently achieved using a highly convergent and versatile strategy (Schemes 4–6) (Ward et al., 1997, 2001; Pedras et al., 1999, 2001). At the outset of this work (Ward et al., 1997), destruxins had been successfully prepared by cyclizations at the βAla-Hmp (Kuyama and Tamura, 1965; Suzuki et al., 1966) and the Pro-Ile sites (Calmes et al., 1993) but with a maximum yield of only 30%. Recognizing that the choice or cyclization site can be crucial for successful synthesis, these researchers considered the MeAla–βAla site because it is the least sterically hindered among the five amide linkages in **10**. The Pro-Ile amide bond in the requisite linear hexadepsipeptide precursor for cyclization (i.e. **83**) presented a logical disconnection for fragment coupling because *C*-terminal proline residues in oligopeptide chains are resistant to isomerization during chain extension. This strategy of condensing two three-residue fragments (e.g. **62** and **80**) is not only maximally convergent but, by locating the more difficult *N*-methylamide linkages in one tripeptide fragment (e.g. **80**), maximizes the overall yield in the longest linear sequence. In addition, this approach seemed to be well suited to the preparation of analogues with a radiolabel in the peptide backbone (vide infra). As anticipated, syntheses of the required tripeptide fragments were

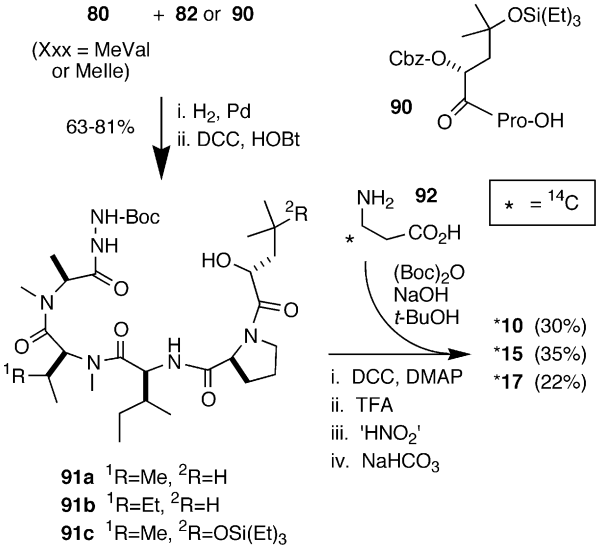


Scheme 4. Synthesis of destruxin B (**10**), desmethyldestruxin B (**13**), and homodestruxin B (**15**) (Ward et al., 1997, 2001).

hampered by facile cyclization of the dipeptide intermediates to dioxopiperazines during chain extension. This problem was addressed by the novel use of a Boc-hydrazide protecting group which not only effectively impeded dipeptide cyclization but served as a latent activating group for the eventual cyclization of the hexadepsipeptides via the azide method. Thus, the desired tripeptide fragments **80** were readily assembled from Cbz-protected *N*-methylalanine Boc-hydrazide (Cbz–MeAla–NHNHBoc; **77**), **38**, and either **39**, **54**, or **78** although the use of 'specialized' coupling reagents such as bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) or bromotri(pyrrolidino)phosphonium hexafluorophosphate (PyBrop) was required to obtain good yields with negligible isomerization (Scheme 4) (Ward et al., 1997, 2001). The tridepsipeptide fragment **62** was prepared in excellent yield (cf. Scheme 2) from the component residues using standard coupling methods [i.e. DCC with 1-hydroxybenzotriazole (HOBt) or 4-dime-



Scheme 5. Synthesis of hydroxydestruxin B (**17**), hydroxyhomodestruxin B (**18**), β-D-glucosyl hydroxydestruxin B (**19**), and α-D-glucosyl hydroxydestruxin B (**88**) (Pedras et al., 1999, 2001; Ward et al., 2001).



Scheme 6. Synthesis of radiolabeled destruxin B (***10**), homodestruxin B (***15**), and hydroxydestruxin B (***17**) (Pedras et al., 1999, 2001; Ward et al., 2001).

thylaminopyridine (DMAP)]. Condensations of **62** with **80** were efficient and the resulting linear hexadepsipeptides **83** were readily cyclized using the azide method to give synthetic destruxins (**10**, **13**, **15**; 58–65%) identical in all respects to those isolated from natural sources.

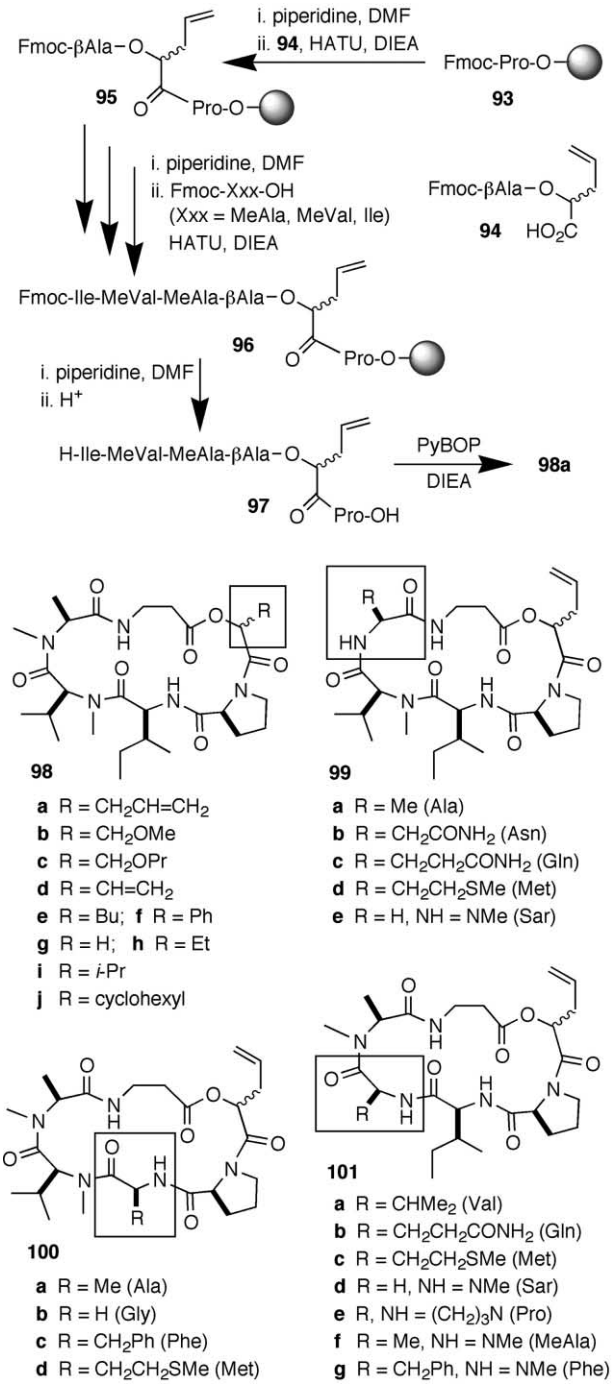
The above synthetic strategy was easily adapted to produce the hydroxylated destruxin analogues **17**–**19** arising from biotransformations of **10** and **15** (Scheme 5) (Pedras et al., 1999, 2001; Ward et al., 2001). These syntheses required incorporation of a (2*R*)-2,4-dihydroxy-4-methylpentanoic acid (Dhmp) residue in place of the (2*R*)-2-hydroxy-4-methylpentanoic acid (Hmp) residue present in **10** and **15**. The tridepsipeptide fragment **85** was assembled from **56**, **58**, and the Dhmp derivative **84** using routine coupling protocols. The hexadepsipeptides **86** and **87**, obtained by coupling **85** with the MeVal and MeIle versions of **80**, were subjected to the above cyclization procedure to give hydroxydestruxin B (**17**) and hydroxyhomodestruxin B (**18**), respectively. The α- and β-D-glucosyl derivatives of **17** (i.e. **88** and **19**, respectively) were prepared by glucosidation with **89** in the presence of *N*-bromosuccinimide (NBS) followed by hydrogenolysis of the benzyl ether protecting groups. The β-anomer **19** was identical with the product isolated from biotransformation of **17**.

Radiolabeled destruxins were required for metabolic studies in cruciferous plants (Pedras et al., 1999, 2001). For this purpose, the above synthetic strategy was modified to allow for the introduction of commercially available [3-^{14}C]-β-alanine (**92**) in the penultimate step. To minimize the handling of radiolabeled compounds, a procedure was developed whereby **92** was converted into its Boc derivative, coupled with **91**, and the resulting hexadepsipeptides cyclized to give the radiolabeled destruxins without isolation of the intermediates (Scheme 6) (Pedras et al., 1999, 2001; Ward et al., 2001). The requisite pentadepsipeptides **91** were prepared by coupling **80** with the didepsipeptide fragments **82** or **90** and, following the above procedure, were converted with good overall yields into radiolabeled destruxin B (***10**), homodestruxin B (***15**) and hydroxydestruxin B (***17**) (specific activities 9–13×10^6 dpm/mg).

A solid phase synthesis of destruxin A (**1**) and several analogues (**98**–**101**) has been reported (Scheme 7) (Ast et al., 2001). The synthesis began with a commercially available solid support (**93**) consisting of 9-fluorenyloxycarbonyl (Fmoc) protected proline attached to a sterically hindered resin (TentaGel–S–Trt). After removal of the Fmoc protecting group (piperidine), the proline residue of **93** was acylated with the synthetic racemic didepsipeptide fragment **94** using *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethaminium hexafluorophosphate *N*-oxide (HATU) as the coupling reagent. Following the same deprotection/coupling protocol, Fmoc protected MeAla, MeVal, and Ile residues were sequentially added to **95** to obtain the

resin linked hexadepsipeptide **96**. All of the coupling steps proceed with excellent conversion (>90%). After removal of the Fmoc group from **96**, the linear hexadepsipeptide **97** was released from the resin by treatment with mild acid and then cyclized in high yield using benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP). The resulting mixture of diastereomers **98a** (arising from use of racemic **94**) could be separated by HPLC, the (*R*)-isomer corresponding to destruxin A (**1**). Using the same methodology, three series of analogues (**99**–**101**) were prepared where either the MeAla, MeVal, or Ile residue of **1** was replaced with various other amino acids; each analogue was obtained as a mixture of diastereomers due to use of racemic **94**. By varying the hydroxy acid residue of **1**, another series of analogues (**76a**–**c** and **98b**–**j**) was prepared using solution methods by adapting the established [4 + 2] fragment coupling route (**76b**/**76c**, **98b**–**f**; cf. Scheme 3) (Cavelier et al., 1996, 1997) or [3 + 3] fragment coupling route (**76a**, **98g**–**j**; cf. Scheme 4) (Ward et al., 1997). Because racemic hydroxy acid residues were incorporated, these analogues (except **98g**) were also obtained as a mixture of diastereomers.

In summary, although only a few of the naturally occurring destruxins (**1**, **9**, **10**, **13**, **15**–**19**) have been prepared by total synthesis to date, the synthetic approaches are sufficiently well developed to conclude that most (if not all) of the destruxins could be prepared by straightforward adaptation of one of the routes described above.



Scheme 7. Synthesis of destruxin A analogues (Ast et al., 2001).

5. Biosynthesis

The biosynthesis of destruxins was studied using isotopically labeled precursors (^{13}C) administered to *M. anisopliae* fungal cultures (Jegorov et al., 1993). Feeding experiments using *L*-[$^{13}CH_3$]methionine showed the incorporation of ^{13}C into the *N*-methyl group of the MeVal and MeAla residues of destruxins. Administration of sodium[2-^{13}C]acetate and sodium [1,2-$^{13}C_2$]acetate to fungal cultures led to the effective incorporation of five intact acetate units, as shown in Fig. 3. No significant incorporation was observed in the Val, Ala or βAla residues or in the hydroxy acid side chain, in destruxin A (**1**), destruxin B (**10**) and destruxin E (**26**) (Jegorov et al., 1993). Also, isolation of protodestruxin B (**16**) from a methionine-requiring mutant of *M. anisopliae* suggested that destruxin B (**10**) and desmethyldestruxin B (**13**) are biosynthesized from protodestruxin (**16**) by *N*-methylation (Suzuki and Tamura, 1972). A

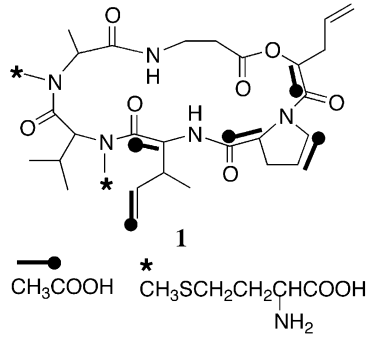


Fig. 3. Labeling pattern observed for the incorporation of [$^{13}CH_3$]methionine, sodium[2-^{13}C]acetate and sodium [1,2–$^{13}C_2$]acetate into destruxin A (**1**) (Jegorov et al., 1993).

gene encoding a peptide synthase was cloned and partially sequenced from *M. anisopliae* (Bailey et al., 1996), but it remains to be established which destruxin(s) are biosynthesized by the peptide synthase. Considering the number of destruxins produced by *M. anisopliae* and the lability of peptide synthases, this might prove to be a challenging task.

6. Biotransformation

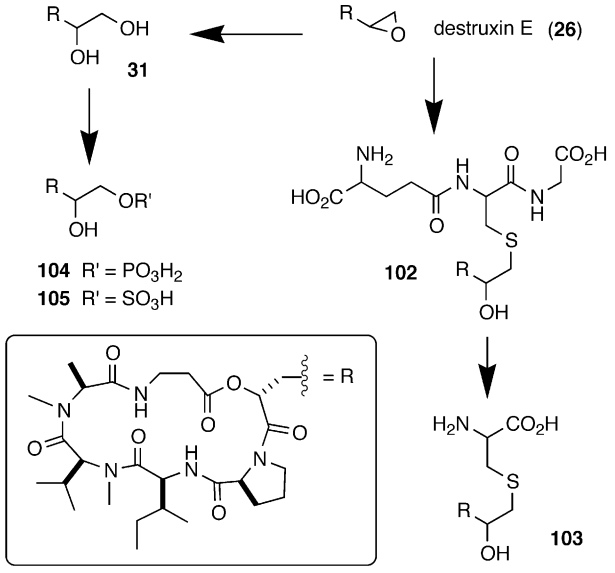
The biotransformation and potential metabolic detoxification of toxins can occur in all living organisms as such enzymatic processes may be essential to their survival. Data accumulated on the biotransformation of destruxins indicate that hosts of the destruxin producing fungi can enzymatically detoxify destruxins and that these conversions appear to prevent potentially irreversible cell damage. Since destruxins are produced by fungal pathogens of insects and plants, it is not surprising to find that detoxification reactions occur in both insects and plants.

6.1. In insects

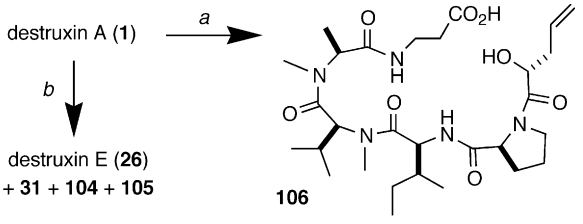
The initial studies of the metabolic transformation of destruxins in insects were driven by the reversibility of the tetanic paralysis observed in *Galleria mellonella* larvae upon destruxin ingestion, which suggested a detoxification process (Roberts, 1966). Numerous analytical techniques were developed and applied to study the biotransformation of destruxins in various insect organs and biological fluids. Metabolism of destruxin E (**26**), known for its high insecticidal activity, was intensively studied in highly resistant insects like locusts (*Locusta migratoria*), as well as host insects like the greater wax moth (*G. mellonella*) which are known to be infected by *M. anisopliae*. The techniques used in monitoring the in vivo metabolism of destruxin E (**26**) included HPLC (Cherton et al., 1991) assisted by FAB–MS (Lange et al., 1991b). Direct HPLC using C_1 and C_4 "wide-pore" packing or on-line clean-up with a guard column, concurrently with FAB–MS were also used (Cherton et al., 1993; Loutelier et al., 1994). Due to the small amounts available, the metabolic products of destruxin E were not isolated and spectroscopically analyzed, but were identified by comparison with authentic samples. Initial studies suggested that, in locusts, destruxin E (**26**) was metabolized to destruxin E diol (**31**) (Cherton et al., 1991). Further studies (Lange et al., 1992b), using conventional mass spectrometry suggested glutathionyl destruxin E (**102**), cysteinyl destruxin E (**103**), phosphorylated (**104**) and sulfated destruxin E (**105**) as additional detoxification products in locust organs. In host insect larvae (*G. mellonella*) it was proposed that detoxification of destruxin E (**26**) (Hubert et al., 1999a) was similar to that occurring in locusts (Scheme 8).

A different trend was observed for metabolism of destruxin A (**1**) in the same two species (Scheme 9); in locusts (Loutelier et al., 1994, 1995b) the biotransformation of destruxin A (**1**) occurred through hydrolysis of the ester bond with ring opening giving **106**, while in *G. mellonella*, **1** was converted to destruxin E (**26**) and its metabolites **31**, **104**, and **105** (Hubert et al., 1999a). Jegorov et al. (1992b) injected larvae of *G. mellonella* with [^{3}H]-dihydrodestruxin A (**9**) and monitored the fate of the labeled toxin in different organs by HPLC and liquid scintillation counting (LSC). It was suggested that metabolism via hydrolysis of the ester bond was possible (determined by HPLC comparison with authentic samples); interestingly, in vitro experiments with the separate organs showed that [^{3}H]-dihydrodestruxin A (**9**) was not metabolized.

The metabolism of two synthetic diastereomers of an acetylenic analog of destruxin A (**76b**, **76c**), differing only in the configuration of the stereogenic carbon of the α-hydroxy acid, was investigated in larvae of *G. mellonella* (Hubert et al., 1999b). This study showed that the biotransformation occurred through hydrolysis



Scheme 8. Detoxification of destruxin E (**26**) in *Locusta migratoria* and *Galleria mellonella* (Cherton et al., 1991; Lange et al., 1992b; Hubert et al., 1999a).

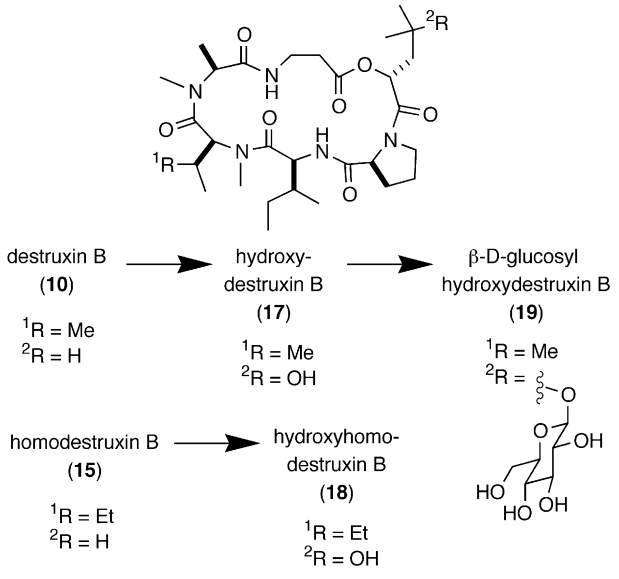


Scheme 9. Detoxification of destruxin A (**1**) in *Locusta migratoria* (a) and *Galleria mellonella* (b) (Loutelier et al., 1994, 1995b; Hubert et al., 1999a; Jegorov et al., 1992b).

of the ester bond and ring opening, similar to the detoxification of destruxin A (**1**). Apparently, the rate of this transformation indicated that the (*S*)-isomer **76c** was transformed more rapidly than the (*R*)-isomer **76b**.

6.2. In plants

The apparent selective phytotoxicity of destruxin B (**10**) and homodestruxin B (**15**) was hypothesized to be due to detoxification reactions occurring in tissues of plant species resistant to *A. brassicae* (Pedras et al., 1999). Thus, the metabolism of ^{14}C-labeled destruxins **10** and **15** in plants susceptible (*B. napus* and *B. juncea*) and resistant (*Sinapis alba*) to *A. brassicae* was investigated (Pedras et al., 1999, 2001). Both destruxins were first transformed to the corresponding hydroxydestruxins **17** and **18** (Scheme 10). The complete transformation of ^{14}C-labeled hydroxydestruxin B was further investigated; the complete detoxification of destruxin B (**10**) in plants resistant to *A. brassicae* appeared to be a two-step process involving sequential hydroxylation and glucosylation. The chemical structure of the non-toxic destruxin B product, β-D-glucopyranosyl hydroxydestruxin B (**19**), was determined by a combination of spectroscopic analyses and chemical synthesis of both the β and α glucosides **19** and **88**, respectively. Interestingly, these hydroxylation and glucosylation reactions occurred in both resistant (*S. alba*) and susceptible (*B. napus* and *B. juncea*) species, but hydroxylation was the rate limiting step in the susceptible species, whereas glucosylation was the rate limiting step in the resistant species. During this investigation (Pedras et al., 2001), it was also established that hydroxydestruxin B (**17**) induced the biosynthesis of phytoalexins in the resistant but not in the susceptible plant species. The authors proposed that *S. alba* can overcome the fungal invader through detoxification of destruxin B coupled with production of phytoalexins. A similar pathway for destruxin B detoxification (Scheme 10) was observed in several cultivars of *S. alba* (resistant), *B. napus* (susceptible), *B. rapa* (susceptible), and *B. juncea* (susceptible); however, the transformation rates in susceptible plants were significantly slower than in resistant plants (Pedras et al., 2001). This detoxification process may be common to all hosts of *A. brassicae*, but it would be important to establish if such rate differences are due to the quantity and/or selectivity of the detoxifying enzymes and if cruciferous weeds have similar detoxification mechanism.



Scheme 10. Metabolism of destruxin B (**10**) and homodestruxin B (**15**) in crucifers (Pedras et al., 1999, 2001).

7. Biological activity

Aside from the well-documented insecticidal (Table 2) and phytotoxic (Table 3) activities, destruxins exhibit a wide variety of biological activities. For example, important cytotoxic effects were observed on L1210 leukemia cells and spleen lymphocytes treated with destruxins A, A_2, B, B_1 and E (Morel et al., 1983). Destruxin E (**26**) appeared to be substantially more potent than destruxins A or B in antiproliferative activity on mammalian neoplastic cells in in vitro assays with P388 murine neoplasms (Morel et al., 1983; Odier et al., 1987, 1992). Destruxin B (**10**) and homodestruxin B (**15**) showed also suppressive effects on the hepatitis B viral surface antigen and were suggested as potential candidates for development of new antihepatitis agents (Sun et al., 1994; Chen et al., 1995, 1997), whereas destruxin-A_4 chlorohydrin (**7**) acted as an inducer of erythropoietin (Cai et al., 1998). Destruxin A (**1**) and destruxin B (**10**) exhibited a positive inotropic effect with negative chronotropy on rat cardiac tissue (Tsunoo et al., 1997; Tsunoo and Kamijo, 1999), whereas **10** was shown to be a specific, dose dependent and reversible inhibitor of vacuolar-type ATPase, which maintains the acidity in the vacuolar organelles (Muroi et al., 1994; Togashi et al., 1997; Bandani et al., 2001).

As shown in Table 2, the insecticidal activity of destruxins was tested on a large variety of insects. Toxins were administered by topical application, forced ingestion, immersion or injection to larvae or adult insects. Apparently, destruxins cause an initial tetanic paralysis, which at lethal doses, lead to insect's death. The tetanic paralysis is attributed to muscle depolarization by direct opening of the Ca^{2+} channels in the membrane (Samuels et al., 1988c). Moreover, it was found (Dumas et al., 1996a) that destruxin E (**26**) induced Ca^{2+} influx and phosphorylation of intracellular proteins in insect cells. Structure-activity relationship studies showed that the presence of the ester bond is essential for biological activity (Cavelier et al., 1996). Also, variation of the side chain of the hydroxy acid residue showed that the presence of a hydrophilic group (e.g. the hydroxyl

Table 2
Insecticidal activity of destruxins

Destruxin (Dx)	Species (reference)	References
Dx A (**1**)	*Bombyx mori*	Kodaira, 1962, Suzuki et al., 1971, Quiot et al., 1985
	Cetonia aurata	Fargues et al., 1985
	Epilachna sparsa	Kodaira, 1962
	Galleria mellonella	Kaijiang and Roberts, 1986, Calmes et al., 1993, Dumas et al., 1994, Loutelier et al., 1996
	Heliothis virescens	Gupta et al., 1989a, Ast et al., 2001
	Musca domestica	Robert and Fargues, 1986
	Oryctes rhinocerus	Fargues et al., 1985
	Phaedon cochleariae	Amiri et al., 1999
	Plutella xylostella	Amiri et al., 1999
Dx A_2 (**3**)	*G. mellonella*	Loutelier et al., 1996
	H. virescens	Gupta et al., 1989a
Dx A_3 (**4**)	*Coptotermes formosanus*	Wahlman and Davidson, 1993
Dx A_4 (**5**)	*Drosophila melanogaster*	Krasnoff et al., 1996
	Rhagoletis pomonella	Krasnoff et al., 1996
Dx A_5 (**6**)	*D. melanogaster*	Krasnoff et al., 1996
	R. pomonella	Krasnoff et al., 1996
DesmethylDx A (**8**)	*C. formosanus*	Wahlman and Davidson, 1993
Dx B (**10**)	*B. mori*	Kodaira, 1962, Suzuki et al., 1971, Quiot et al., 1985
	C. aurata	Fargues et al., 1985
	E. sparsa	Kodaira, 1962
	G. mellonella	Kaijiang and Roberts, 1986, Dumas et al., 1994, Loutelier et al., 1996
	H. virescens	Gupta et al., 1989a
	M. domestica	Robert and Fargues, 1986
	O. rhinocerus	Fargues et al., 1985
	P. cochleariae	Amiri et al., 1999
	P. xylostella	Amiri et al., 1999
Dx B_2 (**12**)	*G. mellonella*	Loutelier et al., 1996
	H. virescens	Gupta et al., 1989a
DesmethylDx B (**13**)	*G. mellonella*	Loutelier et al., 1996
	H. virescens	Gupta et al., 1989a
HomoDx B (**15**)	*D. melanogaster*	Krasnoff et al., 1996
	R. pomonella	Krasnoff et al., 1996
Dx C (**20**)	*G. mellonella*	Loutelier et al., 1996
Dx C_2 (**21**)	*G. mellonella*	Loutelier et al., 1996
DesmethylDx C (**22**)	*C. formosanus*	Wahlman and Davidson, 1993
Dx D (**23**)	*G. mellonella*	Loutelier et al., 1996
Dx E (**26**)	*B. mori*	Quiot et al., 1985
	Brevicoryne brassicae	Robert and Riba, 1989
	C. aurata	Fargues et al., 1985
	Culex pipiens	Vey et al., 1987
	Delia antiqua	Poprawski et al., 1985
	D. melanogaster	Vey et al., 1987
	Empoasca vitis	Poprawski et al., 1994
	G. mellonella	Fargues et al., 1986, Calmes et al., 1993, Dumas et al., 1994, Loutelier et al., 1996
	H. virescens	Gupta et al., 1989a
	M. domestica	Robert and Fargues, 1986, Vey et al., 1987
	Myzus persicae	Robert and Riba, 1989
	O. rhinocerus	Fargues et al., 1985
	P. cochleariae	Amiri et al., 1999

(*continued on next page*)

Table 2 (*continued*)

Destruxin (Dx)	Species (reference)	References
	P. xylostella	Amiri et al., 1999
	Rhopalosiphum padi	Robert and Riba, 1989
Dx E chlorohydrin (**29**)	*G. mellonella*	Loutelier et al., 1996
	H. virescens	Gupta et al., 1989a
Dx E diol (**31**)	*G. mellonella*	Loutelier et al., 1996
Dx F (**33**)	*Coptotermes formosanus*	Wahlman and Davidson, 1993
ProtoDx (**16**)	*B. mori*	Lee and Izumiya, 1977
	G. mellonella	Loutelier et al., 1996
Mixture of (**1**), (**10**), (**26**)	*Bemisia argentifolii*	Davidson et al., 1996
	Choristoneura fumiferana	Brousseau et al., 1996
Mixture of (**1**), (**3**), (**10**), (**13**), (**26**)	*Manduca sexta*	Kershaw et al., 1999
	Othiorhynchus sulcatus	Kershaw et al., 1999
	Schistocerca gregaria	Kershaw et al., 1999
Mixture of (**1**), (**3**), (**10**), (**13**)	*M. sexta*	Samuels et al., 1988b
Analogue **76a**	*G. mellonella*	Calmes et al., 1993
Analogues **76h-m**	*G. mellonella*	Cavelier et al., 1996
Analogues **76b-g**	*G. mellonella*	Cavelier et al., 1997
Various destruxin derivatives	*G. mellonella*	Dumas et al., 1994
Analogues **76a + 76b** (1:1), **98a**–**98c**, **98e**, **98f**, **98h**, **98j**, **99a**, **99c, 99e**	*H. virescens*	Ast et al., 2001

group in destruxin C (**20**) or destruxin E diol (**31**) or the carboxyl group in destruxin D (**23**)) considerably decreased the insecticidal activity. Compounds with an electron-rich side chain (destruxin A (**1**)) were more effective than those with a fully saturated side chain (destruxin B (**10**)) but overall, destruxin E (**26**) seemed to be the most potent of all (Cavelier et al., 1997). Destruxins, especially destruxin E (**26**) exhibited a cytotoxic effect on insect cells (Quiot et al., 1985; Dumas et al., 1994, 1996b). Destruxin A (**1**), destruxin B (**10**) and destruxin E (**26**) were shown to have antiviral (Quiot et al., 1980, 1985) and immunodepressant (Vey et al., 1985; Huxham et al., 1989) activity in insect cells. Studies for establishing the role of the toxins in the pathogenicity of *M. anisopliae* suggested a correlation between in vitro production of destruxins and fungal virulence (Fargues et al., 1985; Kershaw et al., 1999), with destruxins acting as virulence factors by facilitating the penetration of the pathogen in the host (Samuels et al., 1988b). As shown in Table 2, the destruxin analogues **76a + 76b** (1:1), **98a**–**98c**, **98e**, **98f**, **98h**, **98j**, **99a**, and **99c** were tested on *H. virescens* (Ast et al., 2001) and the analogues **76a**–**76g** were tested on *G. mellonella* (Calmes et al., 1993; Cavalier et al., 1996, 1997).

Destruxins produced by *A. brassicae* were shown to be phytotoxic to a variety of plants (Table 3), manifested by chlorosis and necrotic spots on the leaf surface, symptoms similar to those produced by fungal infection. The phytotoxicity of destruxin B (**10**) was tested in whole plants (Hansen and Earle, 1996; Pedras et al., 2000), excised leaves (Ayer and Peña-Rodriguez, 1987; Bains and Tewari, 1987; Buchwaldt and Green, 1992; Pedras et al., 2000), leaf disks (Tewari and Bains, 1997), and also on seedlings (Tewari and Bains, 1997), pollen grains (Shivanna and Sawhney, 1993; Tewari and Bains, 1997), protoplasts (Tewari and Bains, 1997) and cell cultures (Pedras and Biesenthal, 2000; Pedras et al., 2000, 2001). Ultrastructural studies have shown that destruxin B (**10**) caused tissue damage similar to that observed in plants naturally infected with *A. brassicae* (Agarwal et al., 1997). In phytotoxicity assays homodestruxin B (**15**) (Buchwaldt and Green, 1992; Bains et al., 1993; Pedras et al., 2000), as well as desmethyldestruxin (**13**) (Pedras et al., 2000) and destruxin B_2 (**12**) (Buchwaldt and Green, 1992) were also shown to be active. In cell culture assays homodestruxin B appeared to be more phytotoxic than destruxin B, whereas desmethyldestruxin B showed the lowest phytotoxicity (Pedras et al., 2000). Bains and Tewari (1987) reported that the degree of sensitivity of different *Brassica* species to destruxin B (**10**) correlated with their degree of susceptibility to *A. brassicae*, suggesting that the toxin is host-specific. The selective phytotoxicity of destruxin B (**10**) was inferred by Buchwaldt and Green (1992) who showed that the toxin caused chlorosis and necrosis on 30 species of host and non-host plants. *Brassica* species

Table 3
Phytotoxic activity of destruxins

Destruxin (Dx)/plant tissue	Plant species	Reference
Dx B (***10***)		
Excised leaves	*Avena sativa, B. campestris, B. hirta, B. juncea, B. napus, B. nigra, B. rapa, Cucumis sativus, H. vulgare, Linum usitatissimum, L. esculentum, Secale cereale, T. aestivum, Vigna unguiculata, Zea mays*	Bains and Tewari, 1987
Excised leaves	*B. fruticulosa, B. juncea, B. napus, B. nigra, B. oleracea, B. rapa, B. tournefortii, Camelina sativa, Capsella bursa-pastoris, Capsicum annuum, Chenopodium quinoa, Citrullus lanatus, Diplotaxis erucoides, Eruca sativa, Hirschfeldia incana, Hordeum vulgare, Lycopersicon esculentum, Nicotiana benthamiana, N. tabacum, Phaseolus vulgaris, Raphanus sativus, Sinapis alba, S. arvensis, Sisymbrium officinalis, Solanum tuberosum, Thlaspi arvense, Triticum aestivum*	Buchwaldt and Green, 1992
Excised leaves	*B. napus*	Ayer and Peña-Rodriguez, 1987, Bains et al., 1993
Excised leaves, pollen grains	*B. campestris, B. juncea, B. napus, B. nigra, S. alba*	Shivanna and Sawhney, 1993
Excised leaves, whole plants	*B. oleracea, C. sativa, C. bursa-pastoris, L. esculentum, P. hybrida, S. alba*	Hansen and Earle, 1996
Excised leaves	*B. campestris*	Agarwal et al., 1997
Leaf disks, pollen grains, protoplasts, seedlings	*B. rapa, C. sativa*	Tewari and Bains, 1997
Excised leaves, whole plants, cell cultures	*B. juncea, B. napus, S. alba*	Pedras et al., 2000
DesmethylDx B (***13***)		
Whole plants, cell cultures	*B. juncea, B. napus, S. alba*	Pedras et al., 2000
Dx B_2 (***12***)		
Excised leaves	*B. napus*	Buchwaldt and Green, 1992
HomoDx B (**15**)		
Excised leaves	*B. napus*	Buchwaldt and Green, 1992
Excised leaves	*A. sativa, B. napus, Callistephus chinensis, C. sativa, Chenopodium amaranticolor, C. quinoa, Euphorbia pulcherrima, Helianthus annuus, H. vulgare, L. esculentum, Nicotiana glutinosa, Petunia hybrida, P. vulgaris, S. tuberosum, V. unguiculata, Z. mays*	Bains et al. 1993
Excised leaves, whole plants, cell cultures	*B. juncea, B. napus, S. alba*	Pedras et al., 2000

were most sensitive to the toxin, and sensitivity decreased as the relationship of plant species to *Brassica* species became more distant. The authors suggested destruxin B (**10**) to be a virulence factor, contributing to the aggressiveness of *A. brassicae*, by conditioning the host tissue, and thus, determining the susceptibility of the host (Buchwaldt and Green, 1992). The toxic host-selectivity of destruxin B (**10**) was also suggested by Shivanna and Sawhney (1993), who showed that the response of pollen grains of different cruciferous species was in agreement with their degree of susceptibility/resistance to *A. brassicae* fungus. Thus, leaf assays, together with pollen germination and pollen tube growth assays showed that *B. napus* cv. Westar (blackspot susceptible) was more sensitive to destruxin B (**10**) than *S. alba* cv. Ochre (blackspot resistant) (Shivanna and Sawhney, 1993). This result was also observed in a cell suspension culture bioassays (Pedras et al., 2000) which showed that up to four days of incubation with destruxin B (**10**), the viability of cells of *S. alba* cv. Ochre was substantially higher (83%) than that of *B. napus* cv. Westar cells (52%), in direct correlation with plant disease susceptibility to *A. brassicae*. Pedras et al. (2001) also compared the toxicity of destruxin B (**10**) and hydroxydestruxin B (**17**) using a cell suspension culture assay; the viability of cell cultures of *Alternaria* blackspot susceptible cultivars

of *B. napus* treated with hydroxydestruxin B (**17**) for six days was close to those of untreated cell cultures, whereas the viability of cells treated with destruxin B (**10**) was substantially lower. Similarly, leaf puncture and leaf uptake bioassays carried out with hydroxydestruxin B (**17**) and hydroxyhomodestruxin B (**18**) indicated that the hydroxylated compounds were less phytotoxic than the respective destruxins. Furthermore, bioassays to determine the phytotoxicity of β-D-glucosyl hydroxydestruxin B (**19**) to leaves and cell suspension cultures of Alternaria blackspot resistant and susceptible plants, indicated that **19** had no toxic effect on either leaves or cell suspension cultures. The viability of cell suspension cultures incubated with β-D-glucosyl hydroxydestruxin B (**19**) for 10 days was similar to those of control cultures.

8. Concluding remarks

The great interest in destruxins derives from their diverse and significant biological activity as well as potential role as virulence factors in fungi, whether such microorganisms are useful insect biocontrol agents or detrimental, causing great plant disease epidemics. The broad host-range of *M. anisopliae* and pathogenicity to insects prompted the use of diverse strains as biocontrol agents. For example, *M. anisopliae* has been extensively applied to control spittle bugs on sugar cane, rhinoceros beetle on coconut, and cockchafer on pastures (Gillespie and Claydon, 1989); it was also registered by the US Environmental Protection Agency for control of nuisance flies and cockroaches (Genthner et al., 1998). Nonetheless, the apparent role of destruxins on the virulence of *M. anisopliae* has not been evaluated. Because a destruxin gene was cloned in *M. anisopliae* (Bailey et al., 1996) it should now be possible to generate non-destruxin producing mutants by molecular genetics techniques, which could clarify the role destruxins in the virulence of the pathogen. On the other hand, knowledge of the detoxification pathways used by insects to avoid cell death caused by destruxins, might lead to the development of inhibitors of those detoxifying enzymes that could be used in conjunction with *M. anisopliae* as selective biocontrol agents.

Finally, although there is good evidence indicating the role of destruxins as mediators in the interaction of the phytopathogen *A. brassicae* with crucifers, further work is necessary to fully demonstrate this hypothesis. Thus, it would be most useful to generate mutants of *A. brassicae* lacking destruxin biosynthesis genes to evaluate the role of destruxins in the Alternaria blackspot resistance observed in *S. alba*. Furthermore, isolation of the enzymes responsible for the hydroxylation of destruxin B in both resistant (*S. alba*) and susceptible (*B. napus*, *B. juncea*, and *B. rapa*) plant species would show whether the different detoxification rates are due to the selectivity of the enzymes. If in fact destruxin B detoxification confers a specific disease resistance trait to *S. alba*, it should become possible to transfer this trait to *B. napus* and *B. rapa*. In this context it is worthy to note that metabolism of different destruxins appeared to occur consistently at the hydroxy acid residue, whether the organism was an insect or a plant, yet rather different reactions were observed.

References

Agarwal, A., Garg, G.K., Mishra, D.P., Singh, U.S., 1997. Ultrastructural changes in *Brassica* leaves caused by *Alternaria brassicae* and destruxin B. J. Plant Biochem. Biotechnol. 6, 25–28.

Amiri, B., Ibrahim, L., Butt, T.M., 1999. Antifeedant properties of destruxins and their potential use with the entomogenous fungus *Metarrhizium anisopliae* for improved control of crucifer pests. Biocontr. Sci. Technol. 9, 487–498.

Ast, T., Barron, E., Kinne, L., Schmidt, M., Germeroth, L., Simmons, K., Wenschuh, H., 2001. Synthesis and biological evaluation of destruxin A and related analogues. J. Peptide Res. 58, 1–11.

Ayer, W.A., Peña-Rodriguez, L.M., 1987. Metabolites produced by *Alternaria brassicae*, the black spot pathogen of canola. Part 1. The phytotoxic components. J. Nat. Prod. 50, 400–407.

Bailey, A.M., Kershaw, M.J., Hunt, B.A., Paterson, I.C., Charnley, A.K., Reynolds, S.E., Clarkson, J.M., 1996. Cloning and sequence analysis of an intron-containing domain from a peptide synthase-encoding gene of the entomopathogenic fungus *Metarrhizium anisopliae*. Gene 173, 195–197.

Bains, P.S., Tewari, J.P., 1987. Purification, chemical characterization and host-specificity of the toxin produced by *Alternaria brassicae*. Physiol. Mol. Plant Pathol. 30, 259–271.

Bains, P.S., Tewari, J.P., Ayer, W.A., 1993. A note on phytotoxicity of homodestruxin B, a compound produced by *Alternaria brassicae*. Phytoprotection 74, 157–160.

Bandani, A.R., Amiri, B., Butt, T.M., Gordon-Weeks, R., 2001. Effects of efrapeptin and destruxin, metabolites of entomogenous fungi, on the hydrolytic activity of a vacuolar type ATPase identified on the brush border membrane vesicles of *Galleria mellonella* midgut and on plant membrane bound hydrolytic enzymes. Biochim. Biophys. Acta 1510, 367–377.

Brousseau, C., Charpentier, G., Belloncik, S., 1996. Susceptibility of spruce budworm, *Choristoneura fumiferana* clemens, to destruxins, cyclodepsipeptidic mycotoxins of *Metarrhizium anisopliae*. J. Invertebr. Pathol. 68, 180–182.

Buchwaldt, L., Green, H., 1992. Phytotoxicity of destruxin B and its possible role in the pathogenesis of *Alternaria brassicae*. Plant Pathol. 41, 55–63.

Buchwaldt, L., Jensen, J.S., 1991. HPLC purification of destruxins produced by *Alternaria brassicae* in culture and leaves of *Brassica napus*. Phytochemistry 30, 2311–2316.

Cai, P., Smith, D., Katz, B., Pearce, C., Venables, D., Houck, D., 1998. Destruxin-A4 chlorohydrin, a novel destruxin from fungus OS-F68576: isolation, structure determination, and biological activity as an inducer of erythropoietin. J. Nat. Prod. 61, 290–293.

Calmes, M., Cavelier-Frontin, F., Jacquier, R., Mercadier, J.L., Sabil, S., Verducci, J., Quiot, J.M., Vey, A., 1993. Synthesis and biological activity of a destruxin analogue: D-Lac-6 destruxin E. Int. J. Pept. Protein Res. 41, 528–535.

Cavelier, F., Jacquier, R., Mercadier, J.L., Verducci, J., 1996. Destruxin analogues: depsi peptidic bond replacement by amide bond. Tetrahedron 52, 6173–6186.

Cavelier, F., Jacquier, R., Mercadier, J.L., Verducci, J., Traris, M., Vey, A., 1997. Destruxin analogs: variations of the α-hydroxy acid side chain. J. Peptide Res. 50, 94–101.

Cavelier, F., Verducci, J., André, F., Haraux, F., Sigalat, C., Traris, M., Vey, A., 1998. Natural cyclopeptides as leads for novel pesticides: tentoxin and destruxin. Pestic. Sci. 52, 81–89.

Che, Y., Swenson, D.C., Gloer, J.B., Koster, B., Malloch, D., 2001. Pseudodestruxins A and B: new cyclic depsipeptides from the Coprophilous fungus *Nigrosabulum globosum*. J. Nat. Prod. 64, 555–558.

Chen, H.C., Yeh, S.F., Ong, G.T., Wu, S.H., Sun, C.H., Chou, C.H., 1995. The novel desmethyldestruxin B, from *Metarrhizium anisopliae*, that suppresses hepatitis B virus surface antigen production in human hepatoma cells. J. Nat. Prod. 58, 527–531.

Chen, H.C., Chou, C.K., Sun, C.M., Yeh, S.F., 1997. Suppressive effects of destruxin B on hepatitis B virus surface antigen gene expression in human hepatoma cells. Antiviral Res. 34, 137–144.

Chen, J.W., Liu, B.L., Tzeng, Y.M., 1999. Purification and quantification of destruxins A and B from *Metarrhizium anisopliae*. J. Chromatogr. A 830, 115–125.

Cherton, J.C., Lange, C., Mulheim, C., Païs, M., Cassier, P., Vey, A., 1991. Direct in vitro and in vivo monitoring of destruxins metabolism in insects using internal surface reversed-phase high-performance liquid chromatography. I. Behaviour of E destruxin in locusts. J. Chromatogr. Biomed. Appl. 566, 511–524.

Cherton, J.C., Loutelier, J., Lange, C., Cassier, P., 1993. Direct injection onto the HPLC column for monitoring the behaviour of xenobiotics in biological media. II. Behaviour of cymoxanil in fungus and locust and of E-destruxin in locust. Sci. Total Environ. 132, 381–397.

Davidson, E.W., Patron, R.B.R., Lacey, L.A., Frutos, R., Vey, A., Hendrix, D.L., 1996. Activity of natural toxins against the silverleaf white fly, *Bemisia argentifolii*, using a novel feeding bioassay system. Entomol. Experim. Appl. 79, 25–32.

Dumas, C., Robert, P., Païs, M., Vey, A., Quiot, J.M., 1994. Insecticidal and cytotoxic effects of natural and hemisynthetic destruxins. Comp. Biochem. Physiol. 108C, 195–203.

Dumas, C., Matha, V., Quiot, J.M., Vey, A., 1996a. Effects of destruxins, cyclic depsipeptide mycotoxins, on calcium balance and phosphorylation of intracellular proteins in lepidopteran cell lines. Comp. Biochem. Physiol. 114C, 213–219.

Dumas, C., Ravallec, M., Matha, V., Vey, A., 1996b. Comparative study of the cytological aspects of the mode of action of destruxins and other peptidic fungal metabolites on target epithelial cells. J. Invertebr. Pathol. 67, 137–146.

Engstrom, G.W., DeLance, J.V., Richard, J.L., Baetz, A.L., 1975. Purification and characterization of roseotoxin B, a toxic cyclodepsipeptide from *Trichothecium roseum*. J. Agric. Food Chem. 23, 244–253.

Fargues, J., Robert, P.H., Vey, A., 1985. Effect of destruxins A, B, and E on the pathogenesis of *Metarrhizium anisopliae* in larvae of the *Coleoptera scarabaeidae*. Entomophaga 30, 353–364.

Fargues, J., Robert, P.H., Vey, A., Païs, M., 1986. Relative toxicity of destruxin E to the lepidopteran *Galleria mellonella* larvae. C. R. Acad. Sci., Ser. 3 303, 83–86.

Genthner, F.J., Chancy, C.A., Couch, J.A., Foss, S.S., Middaugh, D.P., George, S.E., Warren, M.A., Bantle, J.A., 1998. Toxicity and pathogenicity testing of the insect pest control fungus *Metarrhizium anisopliae*. Arch. Environ. Contam. Toxicol. 35, 317–324.

Gillespie, A.T., Claydon, N., 1989. The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. Pestic. Sci. 27 (2), 203–215.

Gupta, S., Roberts, D.W., Renwick, J.A.A., 1989a. Insecticidal cyclodepsipeptides from *Metarrhizium anisopliae*. J. Chem. Soc. Perkin Trans. 1, 2347–2357.

Gupta, S., Roberts, D.W., Renwick, J.A.A., 1989b. Preparative isolation of destruxins from *Metarrhizium anisopliae* by high performance liquid chromatography. J. Liq. Chromatogr. 12, 383–395.

Gupta, S., Roberts, D.W., Renwick, J.A.A., 1989c. Molecular conformation of destruxin A. Tetrahedron Lett. 30, 4189–4192.

Hansen, L.N., Earle, E.D., 1996. Studies of host specificity and potential use for in vitro selection of destruxin B from *Alternaria brassicae* Berk. (Sacc). Cruciferae Newsletter 112–113.

Hsiao, Y.M., Ko, J.L., 2001. Determination of destruxins, cyclic peptide toxins, produced by different strains of *Metarhizium anisopliae* and their mutants induced by ethyl methane sulfonate and ultraviolet using HPLC method. Toxicon 39, 837–841.

Hubert, M., Cherton, J.C., Vey, A., Lange, C., 1999a. Metabolites of A and E destruxins in *Galleria mellonella* larvae using negative-ion fast-atom bombardment mass spectrometry. Rapid Commun. Mass Spectrom. 13, 179–184.

Hubert, M., Cavelier, F., Verducci, J., Cherton, J.C., Vey, A., Lange, C., 1999b. Behaviour of diastereoisomers of synthetic analogues of destruxin using high performance liquid chromatography and fast-atom bombardment mass spectrometry. Rapid Commun. Mass Spectrom. 13, 860–864.

Humphrey, J.M., Chamberlin, A.R., 1997. Chemical synthesis of natural product peptides: coupling methods for the incorporation of noncoded amino acids into peptides. Chem. Rev. 97, 2243–2266.

Huxham, I.M., Lackie, A.M., McCorkindale, N.J., 1989. Inhibitory effects of cyclodepsipeptides, destruxins, from the fungus *Metarrhizium anisopliae*, on cellular immunity in insects. J. Insect. Physiol. 35, 97–105.

Izumiya, N., Kato, T., Aoyagi, H., Waki, M., Kondo, M., 1979. Synthetic Aspects of Biologically Active Cyclic Peptides. Halsted Press, New York.

Jegorov, A., Matha, V., Sedmera, P., Roberts, D.W., 1992a. Dihydrodestruxin A: a natural destruxin from *Metarrhizium anisopliae*. Phytochemistry 31, 2669–2670.

Jegorov, A., Matha, V., Hradec, H., 1992b. Detoxification of destruxins in *Galleria mellonella* L. larvae. Comp. Biochem. Physiol. 103C, 227–229.

Jegorov, A., Sedmera, P., Matha, V., 1993. Biosynthesis of destruxins. Phytochemistry 33, 1403–1405.

Jegorov, A., Sedmera, P., Havlicek, V., Matha, V., 1998a. Destruxin Ed_1 a cyclopeptide from the fungus *Metarrhizium anisopliae*. Phytochemistry 49, 1815–1817.

Jegorov, A., Havlicek, V., Sedmera, P., 1998b. Rapid screening of destruxins by liquid chromatography–mass spectrometry. J. Mass Spectrom. 33, 274–280.

Kaijiang, L., Roberts, D.W., 1986. The production of destruxins by the entomogenous fungus *Metarrhizium anisopliae* var. *major*. J. Invert. Pathol. 47, 120–122.

Kawazu, K., Murakami, T., Ono, Y., Kanzaki, H., Kobayashi, A., Mikawa, T., Yoshikawa, N., 1993. Isolation and characterization of two novel nematicidal depsipeptides from an imperfect fungus, strain D1084. Biosci. Biotech. Biochem. 57, 98–101.

Kershaw, M.J., Moorhouse, E.R., Bateman, R., Reynolds, S.E., Charnley, A.K., 1999. The role of destruxins in the pathogenicity of *Metarrhizium anisopliae* for three species of insect. J. Invertebr. Pathol. 74, 213–223.

Kodaira, Y., 1961. Toxic substances to insects, produced by *Aspergillus ochraceus* and *Oospora destructor*. Agric. Biol. Chem. 25, 261–262.

Kodaira, Y., 1962. Studies on the new toxic substances to insects, destruxin A and B, produced by *Oospora destructor*. Agric. Biol. Chem. 26, 36–42.

Krasnoff, S.B., Gibson, D.M., Belofsky, G.N., Gloer, K.B., Gloer, J.B., 1996. New destruxins from the entomopathogenic fungus *Aschersonia* sp. J. Nat. Prod. 59, 485–489.

Kuyama, S., Tamura, S., 1965. Total synthesis of destruxin B. Agric. Biol. Chem. 29, 168–169.

Lange, C., Mulheim, C., Le Berre, A., Cherton, J.C., Basselier, J.J., Vey, A., Païs, M., 1989. Sequencing of cyclodepsipeptides (destruxins) and biological behaviour using HPLC and FAB-desorption-tandem MS. Spectrosc. Int. J. 7, 169–184.

Lange, C., Mulheim, C., Vey, A., Païs, M., 1991a. Direct determination of the sequence of destruxins (cyclodepsipeptides) using nega-

tive-ion fast-atom bombardment mass spectrometry. Rapid Commun. Mass. Spectrom. 5, 86–91.
Lange, C., Mulheim, C., Cherton, J.C., Cassier, P., Vey, A., Païs, M., 1991b. Desorption of ions from locust tissues. I. Behaviour of E-destruxin using positive-ion fast atom bombardment mass spectrometry. Rapid Commun. Mass Spectrom. 5, 169–174.
Lange, C., Mulheim, C., Vey, A., Païs, M., 1992a. Sequencing of cyclodepsipeptides (destruxins) using positive fast atom bombardment desorption tandem mass spectrometry. Biol. Mass Spectrom 21, 33–42.
Lange, C., Loutelier, C., Cherton, J.C., Cassier, P., Vey, A., Païs, M., 1992b. Desorption of ions from locust tissues. II. Metabolites of E-destruxin using negative-ion fast atom bombardment mass spectrometry. Rapid Commun. Mass Spectrom 6, 28–31.
Lee, S., Izumiya, N., Suzuki, A., Tamura, S., 1975. Synthesis of a cyclohexadepsipeptide, protodestruxin. Tetrahedron Lett. 16, 883–886.
Lee, S., Izumiya, N., 1977. Cyclic peptides. II. Synthesis of a cyclodepsipeptide, Protodestruxin. Int. J. Peptide Protein Res. 10, 206–218.
Loutelier, C., Lange, C., Cassier, P., Vey, A., Cherton, J.C., 1994. Non-extractive metabolism study of E and A destruxins in the locust, *Locusta migratoria* L. III. Direct high-performance liquid chromatographic analysis and parallel fast atom bombardment mass spectrometric monitoring. J. Chromatogr. B, Biomed. Appl. 656, 281–292.
Loutelier, C., Hubert, M., Marcual, A., Lange, C., Mercadier, J.L., Cavelier, F., Verducci, J., Jacquier, R., 1995a. Gas-phase reactivity of protonated cyclodepsipeptidic toxins: the study of a systematic fragmentation pathway under fast-atom bombardment desorption. Rapid Commun. Mass Spectrom. 9, 1512–1515.
Loutelier, C., Marcual, A., Cassier, P., Cherton, J.C., Lange, C., 1995b. Desorption of ions from locust tissues. III. Study of a metabolite of A-destruxin using fast-atom bombardment linked-scan mass spectrometry. Rapid Commun. Mass Spectrom. 9, 408–412.
Loutelier, C., Cherton, J.C., Lange, C., Traris, M., Vey, A., 1996. Studies on the dynamics of the production of destruxins by *Metarrhizium anisopliae*. Direct high-performance liquid chromatographic and fast atom bombardment mass spectrometric analysis correlated with biological activity tests. J. Chromatogr. A 738, 181–189.
Morel, E., Païs, M., Turpin, M., Guyot, M., 1983. Cytotoxicity of cyclodepsipeptides on murine lymphocytes and on L 1210 leukemia cells. Biomed. Pharmacother. 37, 184–185.
Muroi, M., Shiragami, N., Takatsuki, A., 1994. Destruxin B, a specific and readily reversible inhibitor of vacuolar-type H^+-translocating ATPase. Biochem. Biophys. Res. Commun. 205, 1358–1365.
Naganawa, H., Takita, T., Suzuki, A., Tamura, S., Lee, S., Izumiya, N., 1976. Conformational studies of destruxins, insecticidal cyclodepsipeptides. Agric. Biol. Chem. 40, 2223–2229.
Odier, F., Vago, P., Quiot, J.M., Devauchelle, G., Bureau, J.P., 1987. Cytometric study of the effects of destruxin E on mouse leukemia cells. C. R. Acad. Sci., Ser. 3 305, 575–578.
Odier, F., Vey, A., Bureau, J.P., 1992. In vitro effect of fungal cyclodepsipeptides on leukemic cells: study of destruxins A, B and E. Biol. Cell 74, 267–271.
Païs, M., Das, B.C., Ferron, P., 1981. Depsipeptides from *Metarrhizium anisopliae*. Phytochemistry 20, 715–723.
Pedras, M.S.C., Smith, K.C., 1997. Sinalexin, a phytoalexin from white mustard elicited by destruxin B and *Alternaria brassicae*. Phytochemistry 46, 833–837.
Pedras, M.S.C., Zaharia, I.L., Gai, Y., Smith, K.C., Ward, D.E., 1999. Metabolism of the host-selective toxins destruxin B and homodestruxin B: probing a plant disease resistance trait. Org. Lett. 1, 1655–1658.
Pedras, M.S.C., Biesenthal, C.J., 2000. Vital staining of plant cell suspension cultures: evaluation of the phytotoxic activity of the phytotoxins phomalide and destruxin B. Plant Cell Rep. 19, 1135–1138.
Pedras, M.S.C., Biesenthal, C.J., Zaharia, I.L., 2000. Comparison of the phytotoxic activity of the phytotoxin destruxin B and four natural analogs. Plant Science 156, 185–192.
Pedras, M.S.C., Zaharia, I.L., Gai, Y., Zhou, Y., Ward, D.E., 2001. In planta sequential hydroxylation and glycosylation of a fungal phytotoxin: avoiding cell death and overcoming the fungal invader. Proc. Natl. Acad. Sci. USA 98, 747–752.
Poprawski, T.J., Robert, P.H., Maniania, N.K., 1985. Susceptibility of the onion maggot, *Delia antiqua* (Diptera: Anthomyiidae), to the mycotoxin destruxin E. Can. Entomol. 117, 801–802.
Poprawski, T.J., Robert, P.H., Maniania, N.K., 1994. Contact toxicity of the mycotoxin destruxin E to *Empoasca vitis* (Göthe) (Hom., Cicadellidae). J. Appl. Ent. 117, 135–143.
Potterat, O., Wagner, K., Haag, H., 2000. Liquid chromatography-electrospray time-of-flight mass spectrometry for on-line accurate mass determination and identification of cyclodepsipeptides in a crude extract of the fungus *Metarrhizium anisopliae*. J. Chromatogr. A 872, 85–90.
Quiot, J.M., Vey, A., Vago, C., Païs, M., 1980. Antiviral activity of a mycotoxin. Study of the toxin of the hyphomycete *Metarrhizium anisopliae* (Metsch) Sorok in cell culture. C. R. Séances Acad. Sci. Ser. D 291, 763–766.
Quiot, J.M., Vey, A., Vago, C., 1985. Effects of mycotoxins on invertebrate cells in vitro. Adv. Cell Cult. 4, 199–212.
Rees, N.H., Penfold, D.J., Rowe, M.E., Chowdhry, B.Z., Cole, S.C.J., Samuels, R.I., Turner, D.L., 1996. NMR studies of the conformation of destruxin A in water and in acetonitrile. Magn. Reson. Chem. 34, 237–241.
Robert, P.H., Fargues, J., 1986. Relative oral toxicity of destruxins A, B and E to *Musca domestica* larvae. C. R. Acad. Sci. Ser. 3 303, 641–643.
Robert, P., Riba, G., 1989. Toxic and repulsive effects of spray, 'per os' and systemic applications of destruxin E to aphids. Mycopathologia 108, 179–183.
Roberts, D.W., 1966. Toxins from the entomogenous fungus *Metarrhizium anisopliae*. 2) Symptoms and detection in moribund hosts. J. Invertebr. Pathol. 8, 222.
Samuels, R.I., Charnley, A.K., Reynolds, S.E., 1988a. Application of reversed-phase HPLC in separation and detection of the cyclodepsipeptide toxins produced by the entomopathogenic fungus *Metarrhizium anisopliae*. J. Chromatogr. Sci. 26, 15–19.
Samuels, R.I., Charnley, A.K., Reynolds, S.E., 1988b. The role of destruxins in the pathogenicity of 3 strains of *Metarrhizium anisopliae* for the tobacco hornworm *Manduca Sexta*. Mycopathologia 104, 51–58.
Samuels, R.I., Reynolds, S.E., Charnley, A.K., 1988c. Calcium channel activation of insect muscle by destruxins, insecticidal compounds produced by the entomopathogenic fungus *Metarrhizium anisopliae*. Comp. Biochem. Physiol. 90C, 403–412.
Shivanna, K.R., Sawhney, V.K., 1993. Pollen selection for *Alternaria* resistance in oilseed brassicas: responses of pollen grains and leaves to a toxin of *A. brassicae*. Theor. Appl. Genet. 86, 339–344.
Springer, J.P., Cole, R.J., Dorner, J.W., Cox, R.H., Richard, J.L., Barnes, C.L., van der Helm, D., 1984. Structure and conformation of roseotoxin B. J. Am. Chem. Soc. 106, 2388–2392.
Steiner, J.R., Barnes, C.L., 1988. Crystal and molecular structure of destruxin B. Int. J. Pept. Protein Res. 31, 212–219.
Sun, C.M., Chen, H.C., Yeh, S.F., 1994. Suppressive effects of metabolites from *Alternaria brassicae* on the hepatitis B surface antigen. Planta Med. 60, 87–88.
Suzuki, A., Kuyama, S., Kodaira, Y., Tamura, S., 1966. Structural elucidation of destruxin A. Agric. Biol. Chem. 30, 517–518.
Suzuki, A., Taguchi, H., Tamura, S., 1970. Isolation and structure elucidation of three new insecticidal cyclodepsipeptides, destruxins C and D and desmethyldestruxin B, produced by *Metarrhizium anisopliae*. Agric. Biol. Chem. 34, 813–816.
Suzuki, A., Kawakami, K., Tamura, S., 1971. Detection of destruxins in silkworm larvae infected with *Metarrhizium anisopliae*. Agric. Biol. Chem. 35, 1641–1643.
Suzuki, A., Tamura, S., 1972. Isolation and structure of protodestruxin from *Metarrhizium anisopliae*. Agric. Biol. Chem. 36, 896–898.

Tamura, S., Kuyama, S., Kodaira, Y., Higashikawa, S., 1964. The structure of destruxin B, a toxic metabolite of *Oospora destructor*. Agric. Biol. Chem. 28, 137–138.

Tewari, J.P., Bains, P.S., 1997. Phytotoxins produced by *Alternaria brassicae* and bioassay of destruxin B. In: Upadhyay, R. (Ed.), Toxins in Plant Disease Development and Evolving Biotechnology. Science, Enfield, NH, pp. 21–35.

Togashi, K., Kataoka, T., Nagai, K., 1997. Characterization of a series of vacuolar type H^+-ATPase inhibitors on CTL-mediated cytotoxicity. Immunol. Lett. 55, 139–144.

Tsunoo, A., Kamijo, M., Taketomo, N., Sato, Y., Ajisaka, K., 1997. Roseocardin, a novel cardiotonic cyclodepsipeptide from *Trichothecium roseum* TT103. J. Antibiot. 50, 1007–1013.

Tsunoo, A., Kamijo, M., 1999. Non-cyclic AMP-dependent, positive inotropic cyclodepsipeptides with negative chronotropy. J. Pharmacol. Exp. Ther. 290, 1006–1012.

Venkatasubbaiah, P., Tisserat, N.A., Chilton, W.S., 1994. Metabolites of *Ophiosphaerella herpotricha*, a cause of spring dead spot of bermuda grass. Mycopathol. 128, 155–159.

Vey, A., Quiot, J.M., Vago, C., 1985. Immunodepressive effect of fungal toxins: inhibition of the reaction of multicellular encapsulation by the destruxins. C. R. Acad. Sci. Ser. 3 300, 647–651.

Vey, A., Quiot, J.M., Vago, C., 1987. Mechanisms of insecticidal activity of a mycotoxin, destruxin E, on dipteran pathogen vectors and disseminators. C. R. Acad. Sci. Ser. 3 304, 229–234.

Wahlman, M., Davidson, B.S., 1993. New destruxins from the entomopathogenic fungus *Metarrhizium anisopliae*. J. Nat. Prod. 56, 643–647.

Ward, D.E., Gai, Y., Lazny, R., Pedras, M.S.C., 2001. Probing Host-Selective Phytotoxicity: Synthesis of Natural Analogs of the Host-Selective Toxin Destruxin B. J. Org. Chem. 66, 7832–7840.

Ward, D.E., Lazny, R., Pedras, M.S.C., 1997. Synthesis of the host-selective phytotoxin destruxin B. Avoiding diketopiperazine formation from an *N*-methyl amino acid dipeptide by use of the Boc-hydrazide derivative. Tetrahedron Lett. 38, 339–342.

Yeh, S.F., Pan, W., Ong, G.T., Chiou, A.J., Chuang, C.C., Chiou, S.H., Wu, S.H., 1996. Study of structure–activity correlation in destruxins, a class of cyclodepsipeptides possessing suppressive effect on the generation of hepatitis B virus surface antigen in human hepatoma cells. Biochem. Biophys. Res. Commun. 229, 65–72.