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Rapid Screening of Destruxins by Liquid Chromatography/Mass Spectrometry

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A liquid chromatographic/mass spectrometric (LC/MS) method utilizing an atmospheric pressure chemical ionization (APCI) interface and in-source collisionally induced dissociation (CID) was developed for the rapid screening of the cyclic hexadepsipeptides destruxins, produced by the fungi *Metarhizium anisopliae* and *Trichothecium roseum*. The APCI mass spectra are fully consistent with the high-energy CID data but the sequence ions are abundant over the whole mass range. In addition to 22 known destruxins, two new representatives of this family were detected by LC/MS analysis: destruxin Ed_1 and roseotoxin A were isolated and their structures were inferred from MS/MS and NMR data. © **1998 John Wiley & Sons, Ltd.**

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KEYWORDS: destruxins; liquid chromatography; mass spectrometry; atmospheric pressure chemical ionization

INTRODUCTION

Destruxins are cyclic hexadepsipeptides produced by the entomopathogenic fungi *Metarhizium anisopliae*[1–4] and *Aschersonia aleyrodis*,[5] the phytopatogenic fungi *Alternaria brassicae*,[6,7] *Trichothecium roseum*[8–10] and an imperfect fungus,[11] strain D1084. Destruxins exhibit insecticidal,[1–4,12] phytotoxic,[6,7] antiviral,[13] nematocidal[11] and interesting immunomodulating activities.[14] They also influence the calcium balance and phosphorylation processes in cells.[15,16] The general formula of destruxins is Dx = cyclo(–D-HA[1]–L-Pro[2]–L-Ile[3]–L-MeVal[4]–L-MeAla[5]–β-Ala[6]–), where HA represents a D-α-hydroxy acid residue (see Fig. 1 for a representative structure, destruxin A). Similarly to other fungal cyclic peptides, destruxins are most probably produced by extraribosomal multienzymatic synthesis giving rise to the 32 currently described representatives (Table 1).[1–6,8,11,17–20]

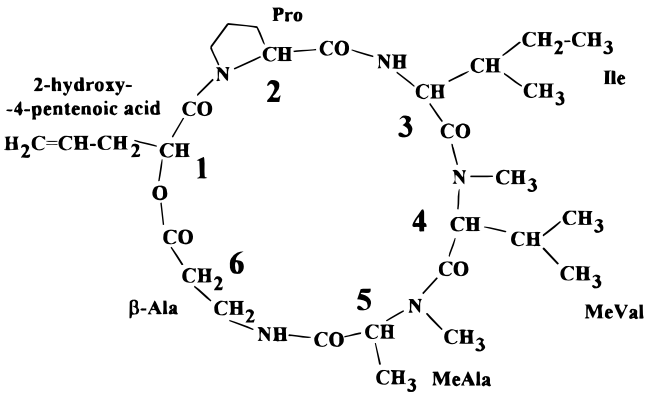

Figure 1. Structure of destruxin A.

Since the production of destruxins by fungi is important for their insecticidal or phytotoxic effects, the screening of their ability to produce these secondary metabolites seems to be a reasonable strategy to identify the more biologically active fungal isolates. Accordingly, several high-performance liquid chromatographic (HPLC) methods permitting the analysis of up to seven destruxins have been described.[21–23] Owing to the absence of any marked chromophore, UV detection of destruxins has been performed by monitoring the end absorption at 210–214 nm. The general problems with all these methods are the presence of interfering substances in various fungal isolates and the co-elution of related destruxins.

In this paper, we report a liquid chromatographic/mass spectrometric (LC/MS) method that permits the separation and identification of the majority of destruxins present in extracts of fungal fermentation broths. A conventional isocratic HPLC method with UV detection at 214 nm was used to set up the elution parameters and to obtain quantitative analytical data based on peak area calculations. Then the LC system was connected to the mass spectrometer via an atmospheric pressure chemical ionization (APCI) interface. Twenty-four destruxins from the fungi *Metarhizium anisopliae* and *Trichothecium roseum* including new members of this group of compounds are described.

EXPERIMENTAL

Fungal strains, cultivation and identification of destruxins

The strain *Metarhizium anisopliae* ARSEF 1095 (Boyce Thompson Institute, Cornell University, Ithaca, NY,

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Table 1. Known natural destruxins[a]

Destruxin	Hydroxy acid[1]	Amino acid constituents AA[2]	AA[3]	AA[4]	AA[5]	AA[6]	MH^+
A	2-Hydroxy-4-pentenoic	Pro	Ile	MeVal	MeAla	β-Ala	578
B	2-Hydroxy-4-methylpentanoic	Pro	Ile	MeVal	MeAla	β-Ala	594
C	2,5-Dihydroxy-4-methylpentanoic	Pro	Ile	MeVal	MeAla	β-Ala	610
D	2-Hydroxy-4-methylpentane-1,5-dioic	Pro	Ile	MeVal	MeAla	β-Ala	624
E	2-Hydroxy-4,5-epoxypentanoic	Pro	Ile	MeVal	MeAla	β-Ala	594
Ed	2,4,5-Trihydroxypentanoic	Pro	Ile	MeVal	MeAla	β-Ala	612
F	2,4-Dihydroxypentanoic	Pro	Ile	MeVal	MeAla	β-Ala	596
A_1	2-Hydroxy-4-pentenoic	Pip	Ile	MeVal	MeAla	β-Ala	592
B_1	2-Hydroxy-4-methylpentanoic	Pip	Ile	MeVal	MeAla	β-Ala	608
D_1	2-Hydroxy-4-methylpentane-1,5-dioic	Pip	Ile	MeVal	MeAla	β-Ala	638
E_1	2-Hydroxy-4,5-epoxypentanoic	Pip	Ile	MeVal	MeAla	β-Ala	608
Ed_1 [b]	2,4,5-Trihydroxypentanoic	Pip	Ile	MeVal	MeAla	β-Ala	626
A_2	2-Hydroxy-4-pentenoic	Pro	Val	MeVal	MeAla	β-Ala	564
B_2	2-Hydroxy-4-methylpentanoic	Pro	Val	MeVal	MeAla	β-Ala	580
C_2	2,5-Dihydroxy-4-methylpentanoic	Pro	Val	MeVal	MeAla	β-Ala	596
D_2	2-Hydroxy-4-methylpentane-1,5-dioic	Pro	Val	MeVal	MeAla	β-Ala	610
E_2	2-Hydroxy-4,5-epoxypentanoic	Pro	Val	MeVal	MeAla	β-Ala	580
Cl	5-Chloro-2,4-dihydroxypentanoic	Pro	Ile	MeVal	MeAla	β-Ala	630[c]
A_3	2-Hydroxy-4-pentenoic	MeAla	Ile	MeVal	MeAla	β-Ala	566
A_4	2-Hydroxy-4-pentenoic	Pro	Ile	MeIle	MeAla	β-Ala	592
A_5	2-Hydroxy-4-pentenoic	3-MePro	Ile	MeIle	MeAla	β-Ala	606
DesmA	2-Hydroxy-4-pentenoic	Pro	Ile	Val	MeAla	β-Ala	564
DesmB	2-Hydroxy-4-methylpentanoic	Pro	Ile	Val	MeAla	β-Ala	580
$DesmB_2$	2-Hydroxy-4-methylpentanoic	Pro	Val	Val	MeAla	β-Ala	566
DesmC	2,5-Dihydroxy-4-methylpentanoic	Pro	Ile	Val	MeAla	β-Ala	596
ProtoB	2-Hydroxy-4-methylpentanoic	Pro	Ile	Val	Ala	β-Ala	566
HomoB	2-Hydroxy-4-methylpentanoic	Pro	Ile	MeIle	MeAla	β-Ala	608
DH-A	2-Hydroxy-4-pentanoic	Pro	Ile	MeVal	MeAla	β-Ala	580
RosA[b]	2-Hydroxy-4-methylpentanoic	3-MePro	Ile	MeVal	MeAla	β-Ala	608
RosB	2-Hydroxy-4-pentenoic	3-MePro	Ile	MeVal	MeAla	β-Ala	592
BursaA	2-Hydroxy-3-methylpentanoic	Pro	Ile	MeVal	MeAla	β-Ala	594
BursaB	2-Hydroxy-3-methylpentanoic	4-MePro	Ile	MeVal	MeAla	β-Ala	608

[a] Abbreviations: AA, amino acid; Bursa, bursaphelocide; Desm, desmethyl; DH-A, dihydrodestruxin A; Homo-B, homodestruxin B; ProtoB, protodestruxin B; Ros, roseotoxin; MeAla, *N*-methylalanine; MeIle, *N*-methylisoleucine; MeVal, *N*-methylvaline; Pip, pipecolic acid; 3-MePro, 3-methylproline;[10] 4-MePro, 4-methylproline.[11] The superscript numbers refer to a particular amino or hydroxy acid position in the cycle.
[b] This work.
[c] Containing ^{35}Cl isotope.

USA) was used as a standard strain with well defined producing ability.[4,19,24] The strain *Trichothecium roseum* 111 was isolated from an apple in our laboratory. Submerged cultivation (10 days) of the fungus was carried out in conical flasks containing 200 ml of Czapek-Dox medium [sucrose (30 g), $NaNO_3$ (3 g), KH_2PO_4 (1 g), KCl (0.5 g), $FeSO_4$ (0.5 g) in water (1000 ml]; 72 h preculture was used as inoculum. The fermentation broth was filtered and destruxins were extracted with dichloromethane. Crude destruxins were prepared by LC on silica gel (stepwise gradient from 1 to 10% of methanol in chloroform). Destruxins-containing fractions were identified using their positive insecticidal effect on *Galleria melonella* larvae. Standards were prepared by a combination of LC on silica gel and HPLC on a reversed-phase column [methanol–water (3:2, v/v)] as described previously.[19] Identification was based on MS and NMR data.[24]

High-performance liquid chromatography

Extracts of fungal cultures were separated by a quaternary gradient Waters Model 616 HPLC pump directed by a Waters Model 600S controller. A 5 μm Nucleosil ODS reversed-phase column (250 × 2.0 mm i.d.) was used (Macherey-Nagel, Düren, Germany). Isocratic elution with acetonitrile–water (56:44, v/v) was performed at a flow rate of 150 μl min^{-1}. The chromatographic column was linked either to the UV detector set at 214 nm or to the APCI interface of the mass spectrometer.

Mass spectrometry

Positive-ion APCI mass spectra were recorded on a Finnigan MAT 95 double-focusing instrument

(Finnigan MAT, Bremen, Germany) of BE geometry equipped with a Finnigan API ion source. For the APCI measurements, the in-source ion collisional decomposition was induced by increasing the cone voltage by 30 V (compared with conventional ionization conditions). The pure compounds (either isolates or standards) dissolved in mobile phase were continuously infused through a stainless-steel capillary held at 3.0 kV into the ion source via a linear syringe pump at a rate of 30 μl min^{-1} (Harvard Apparatus Model 22). A mixture of polypropylene glycols (average $M_r = 425$) (Aldrich-Chemie, Steinheim, Germany) was used to calibrate the m/z scale of the mass spectrometer.

Data on new destruxins

Destruxin Ed_1. Cyclo(–D-HA^1–L-Pip^2–L-Ile^3–L-$MeVal^4$–L-$MeAla^5$–β-Ala^6–), where HA represents 2,4,5-trihydroxypentanoic acid; $M_r = 625$; white amorphous solid. ^{1}H NMR (Varian VXR-400, 400 MHz, $CDCl_3$, 25 °C), δ (ppm): 0.854 (3 H, t, $J = 7.4$ Hz, H-3δ), 0.880 (3 H, d, $J = 6.6$ Hz, H-4γ_u), 0.914 (3 H, d, $J = 6.6$ Hz, H-4γ_d), 0.922 (3 H, $J = 6.7$ Hz, 3β-CH_3), 1.308 (3 H, d, $J = 6.7$, H-5β), 1.349 (1 H, m, H-3γ_u), 1.465 (1 H, m, H-3γ_d), 1.472 (2 H, m, H-2δ), 1.750 (2 H, m, H-2γ), 1.962 (1 H, m, 1β_u), 2.003 (1 H, m, 2β_u), 2.014 (1 H, m, 2β_d), 2.023 (1 H, m, 3β), 2.064 (1 H, m, 1β_d), 2.323 (1 H, m, 4β), 2.580 (1 H, ddd, $J = 18.7$, 4.6, 2.4 Hz, H-6α_u), 2.656 (1 H, ddd, $J = 18.7$, 10.6, 2.6 Hz, H-6α_d), 2.724 (3 H, s, 5 N-CH_3), 3.017 (1 H, dddd, $J = 13.4$, 10.2, 3.0, 3.0 Hz, H-6β_u), 3.229 (3 H, s, 4 N-CH_3), 3.503 (1 H, dd, $J = 10.9$, 7.1 Hz, H-1δ_u), 3.720 (1 H, dd, $J = 10.9$, 3.2 Hz, H-1δ_d), 3.898 (1 H, m, H-1γ), 3.979 (1 H, m, H-2ε_u), 4.062 (1 H, m, H-6β_d), 4.073 (1 H, m, H-2ε), 4.893 (1 H, d, $J = 11.0$ Hz, H-4α), 4.986 (1 H, dd, $J = 9.2$, 6.1 Hz, H-3α), 5.178 (1 H, q, $J = 6.7$ Hz, H-5α), 5.286 (1 H, m, H-1α), 5.431 (1 H, dd, $J = 8.2$, 4.9, H-2α), 7.077 (1 H, d, $J = 9.2$ Hz, 3 N-H), 8.119 (1 H, d, $J = 9.9$ Hz, 6 N-H). Subscripts u and d denote upfield and downfield resonating diastereotopic methylene or methyl protons.

Roseotoxin A. Cyclo(–D-HA^1–L-*trans*-3–$MePro^2$–L-Ile^3–L-$MeVal^4$–L-$MeAla^5$–β-Ala^6–), where HA represents 2-hydroxy-4-methylpentanoic acid; $M_r = 607$; white amorphous solid. ^{1}H NMR (Varian VXR-400, 400 MHz, $CDCl_3$, 25 °C), δ (ppm): 0.854 (3 H, d, $J = 7.0$ Hz, 3β-CH_3), 0.859 (3 H, t, $J = 7.4$ Hz, H-3δ), 0.893 (3 H, d, $J = 6.6$ Hz, H-4γ_u), 0.932 (3 H, d, $J = 6.5$ Hz, H-4γ_d), 0.959 (3 H, d, $J = 6.6$ Hz, H-1δ_u), 1.003 (3 H, d, $J = 6.6$ Hz, H-1δ_d), 1.112 (3 H, d, $J = 7.1$ Hz, 2β-CH_3), 1.306 (3 H, d, $J = 6.8$ Hz, H-5β), 1.334 (1 H, m, H-3γ_u), 1.373 (1 H, m, H-1β_u), 1.421 (1 H, m, H-3γ_d), 1.718 (1 H, m, H-2γ_u), 1.890 (1 H, m, H-1γ), 1.953 (1 H, m, H-3β), 1.972 (1 H, ddd, $J = 14.2$, 10.5, 4.8 Hz, H-1β_d), 2.099 (1 H, m, H-2γ_d), 2.319 (2 H, m, H-2β and H-4β), 2.567 (1 H, ddd, $J = 18.5$, 5.0, 1.8 Hz, H-6α_u), 2.681 (1 H, ddd, $J = 18.5$, 11.3, 2.3 Hz, H-6α_d), 2.730 (3 H, s, 5 N-CH_3), 3.079 (1 H, m, H-6β_u), 3.228 (3 H, s, 4 N-CH_3), 3.565 (1 H, ddd, $J = 9.6$, 9.6, 7.4 Hz, H-2δ_u), 3.864 (1 H, ddd, $J = 9.6$, 9.5, 3.0 Hz, H-2δ_d), 4.052 (1 H, m, H-6β_d), 4.278 (1 H, d, $J = 1.8$ Hz, H-2α), 4.844 (1 H, dd, $J = 9.2$, 6.8 Hz, H-3α), 4.905 (1 H, dd, $J = 10.4$, 3.3 Hz, H-1α), 4.957 (1 H, d, $J = 11.0$ Hz, H-4α), 5.179 (1 H, q, $J = 6.8$ Hz, H-5α), 7.093 (1 H, d, $J = 9.2$ Hz, 3 N-H), 8.257 (1 H, d, $J = 10.0$ Hz, 6 N-H).

RESULTS AND DISCUSSION

Positive-ion APCI was chosen for all LC/MS experiments in view of the production of abundant protonated molecules $[M + H]^+$. In contrast to the APCI spectra, the $[M + Na]^+$ and $[M + K]^+$ ions were the dominant species in the positive-ion electrospray ionization mass spectra. The extreme stability of sodiated molecules of destruxins, which are not amenable to collisionally induced dissociation (CID), was the main reason why the more sensitive electrospray ionization was not employed in further experiments.

A typical reconstructed ion chromatogram from the analysis of *Metarhizium anisopliae* fermentation broth extract is given in Fig. 2. The distinction between about 15 expected destruxins is not possible using a simple isocratic HPLC method with one-dimensional UV detection. Monitoring of protonated molecules of all potential destruxins (Table 1) is also not very helpful without some additional information. Of the complete series of destruxins, only seven can be identified directly owing to their molecular masses (578, A; 606, A_5; 612, Ed; 624, D, 626, Ed_1; 630, chlorhydrin[4] (probably an isolation procedure artefact); and 638, D_1). All other destruxins are grouped into two isobaric pairs (564, A_2, desmethyl A; and 610, C, D_2), four triads (566, A_3, desmethyl B_2, protodestruxin B; 592, A_1, A_4, roseotoxin B; 594, B, E, bursaphelocide A; and 596, F, C_2, desmethyl C), one isobaric group of four (580, B_2, E_2, desmethyl B, dihydrodestruxin A) and finally one group of five (608, B_1, E_1, homodestruxin B, bursaphelocide B, roseotoxin A). However, it should be noted that despite the structural similarity of destruxins, bursaphelocides and roseotoxins, these compounds are produced by different fungi and hence cannot be encountered in one sample simultaneously.

The first additional information contributing to the identification of individual isobaric destruxins is their retention time. The basic series of destruxins is eluted from the reversed-phase column in the sequence D < C < E < A < B, as could be expected from their structure. The members of [Pip^2]Dx series (labeled by subscript 1) and the members of [Val^3]Dx series (subscript 2) are always eluted at slightly longer or shorter retention times when compared with the corresponding parent compounds, respectively. The prediction of the retention times of other analogues is not so easy. It should be noted that roseotoxin B, the only member of the roseotoxin series originally reported from *Trichothecium roseum*,[9,10] is in fact a destruxin A higher homologue. Hence the new roseotoxin described in this work is denoted roseotoxin A, regardless of whether it is the 'true' analogue of destruxin B eluted after roseotoxin B.

Another additional information could be obtained by MS/MS experiments. Under fast atom bombardment (FAB) ionization conditions, the primary protonation in destruxins occurs preferably at the amide nitrogens of *N*-methylalanine and *N*-methylvaline[25–29] and leads to

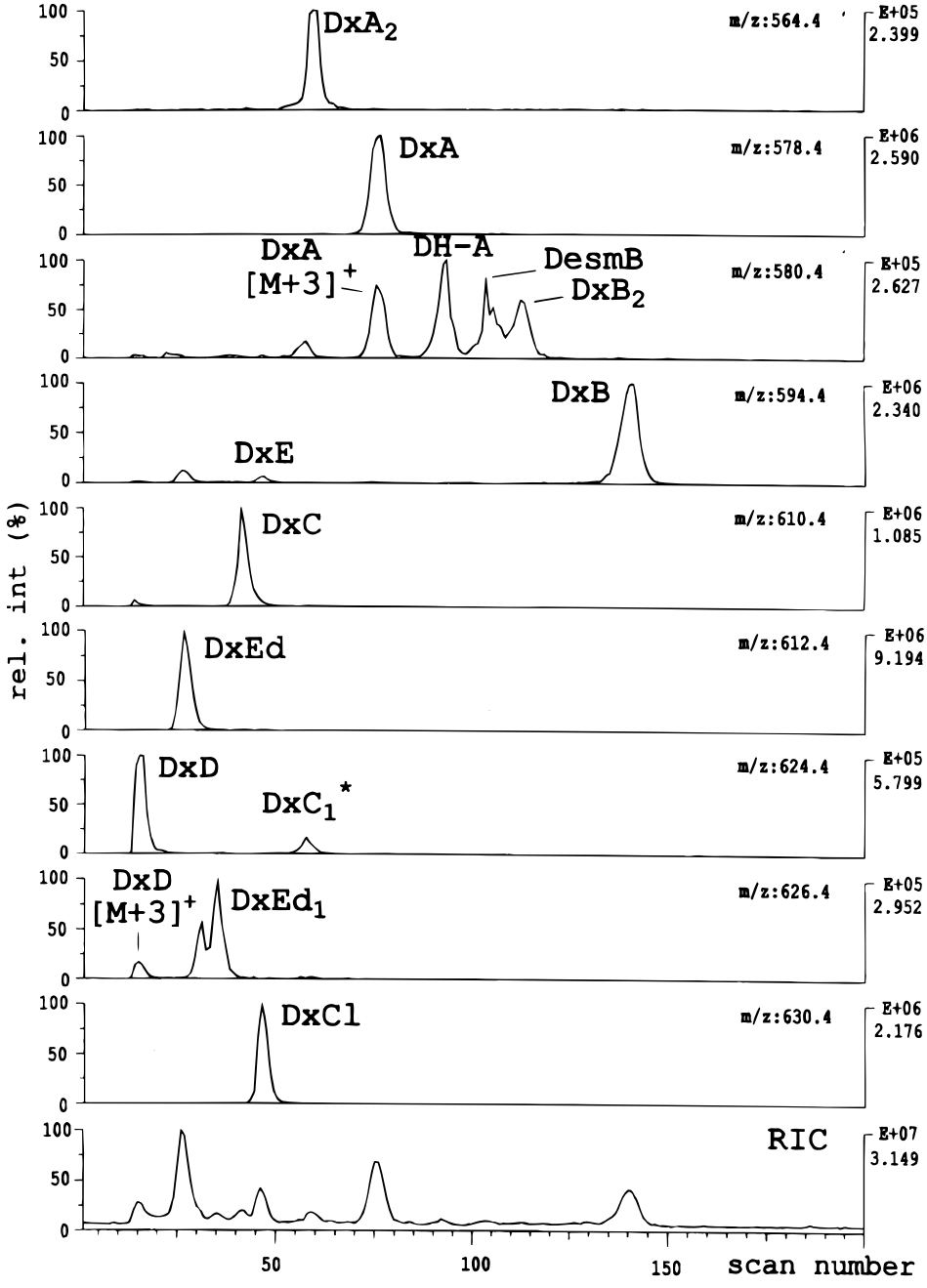

Figure 2. Reconstructed (RIC) and representative selected ion chromatograms, $[M + H]^+$ ions, obtained from the LC/APCI-MS analysis of a mixture of natural destruxins. The $[M + 3]^+$ ion represents the isotopic ion of a given destruxin, which contributes to the protonated molecule trace of a 2u higher destruxin. * DxC_1: preliminary assignment.

ring opening and consequent fragmentations of linear peptide ions, giving rise to the series of acylium (predominantly $^{4-5}b_i$ and some $^{5-6}b_i$) and immonium ($^{4-5}a_i$ and $^{5-6}a_i$) ions as generally encountered in non-polar peptides (for nomenclature see Ref. 30; the superscript defines the pair of amino acids between which the primary splitting takes place).[31] In addition to these series, internal a destruxin-specific fragment ion denoted D^+ was detected.[29]

The ion composition of the in-source CID APCI mass spectra is qualitatively consistent with that observed in the high-energy CID spectra of $[M + H]^+$ ions generated by FAB ionization.[29] The reasonable abundance of sequence ions seems to be a strong advantage of the APCI mass spectra. The presence of corresponding immonium ions provides information on the amino acid composition of a given destruxin (Fig. 3). In high-energy FAB CID mass spectra the abundance of low-mass ions is poor[29] and the immonium ions are entirely missing (Fig. 4).

The most abundant ions found in positive-ion APCI mass spectra of destruxins studied in this work are summarized in Table 2. The relative abundance ratios of dominant ions are similar for all destruxins, which permits a fast orientation in the spectra (Fig. 3). The dominating sequence ions are $^{4-5}b_5$, $^{4-5}a_5$, $^{4-5}b_4$, $^{4-5}a_4$, $^{4-5}b_3$ and the internal fragment D^+. The lower mass ions reflect the sequence of a highly conserved

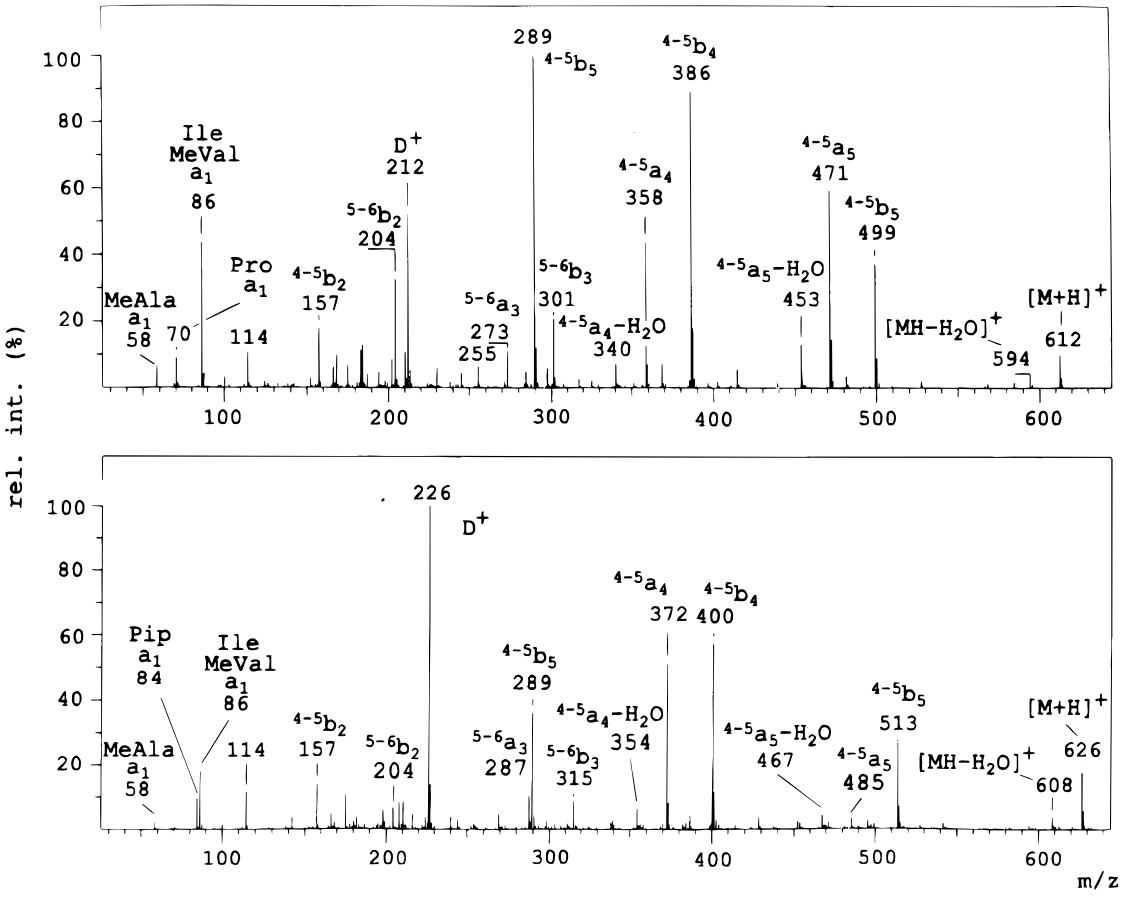

Figure 3. In-source CID LC/APCI mass spectra of destruxins Ed (top) and Ed_1 (bottom).

structural part of destruxins (Table 2). In addition to the series discussed above, the ions $[M + H - H_2O]^+$, $[^{4-5}a_5 - H_2O]^+$, $[^{4-5}a_4 - H_2O]^+$, $[^{4-5}a_3 - H_2O]^+$ and $[D^+ - H_2O]^+$ were found in spectra of Ed_x destruxins, as expected from the presence of 2,4,5-trihydroxypentanoic acid in their structure.

Using the combination of retention time and sequence information obtained from the in-source CID

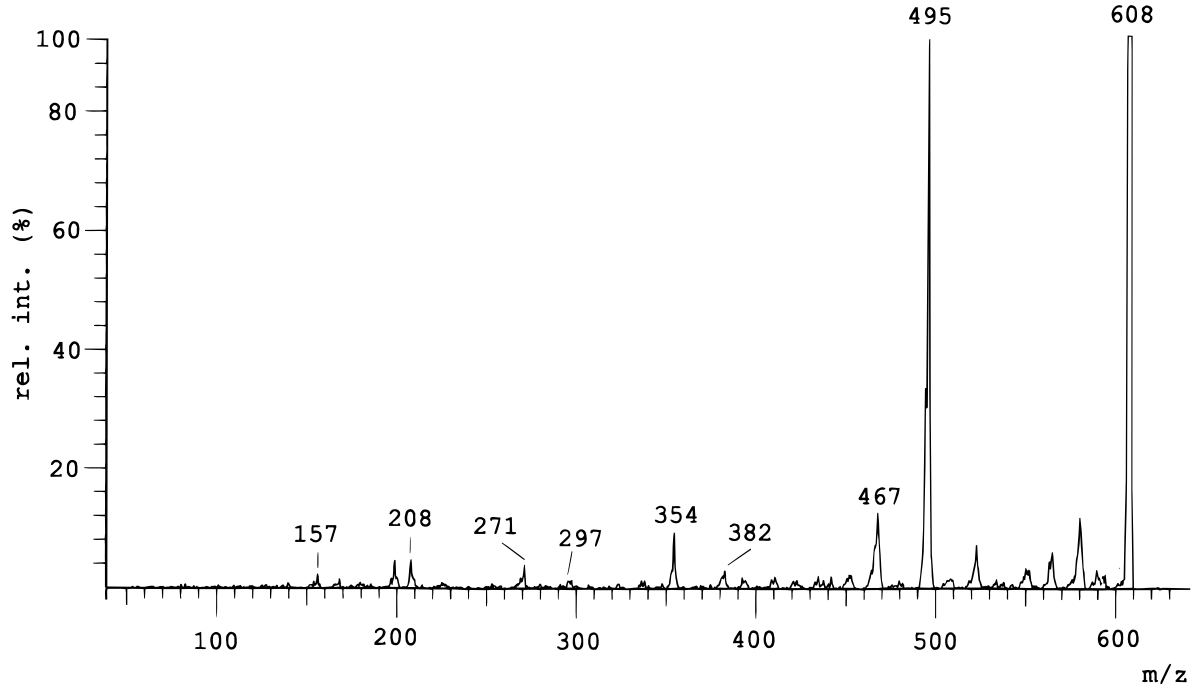

Figure 4. High-energy FAB CID mass spectrum of $[M + H]^+$ ion of roseotoxin A.

Table 2. Prominent sequence fragment ions observed in the in-source CID APCI mass spectra of destruxins

		Ion type (*m/z*)									
Destruxin	MH^+	$^{4-5}b_5$	$^{4-5}a_5$	$^{4-5}b_4$	$^{4-5}a_4$	$^{5-6}b_3$	$^{4-5}b_3$	$^{5-6}a_3$	D^+	$^{5-6}b_2$	$^{4-5}b_2$
A	578	465	437	352	324	267	255	239	178	170	157
B	594	481	453	368	340	283	271	255	194	186	157
C	610	497	469	384	356	299	287	271	210	202	157
D	624	511	483	398	370	313	301	285	224	216	157
E	594	481	453	368	340	283	271	255	194	186	157
Ed	612	499	471	386	358	301	289	273	212	204	157
A_1	592	479	451	366	338	281	255	253	192	170	157
B_1	608	495	467	382	354	297	271	269	208	186	157
D_1	638	525	497	412	384	327	301	299	238	216	157
E_1	608	495	467	382	354	297	271	269	208	186	157
Ed_1	626	513	485	400	372	315	289	287	226	204	157
A_2	564	451	423	352	324	267	255	239	178	170	157
B_2	580	467	439	368	340	283	271	255	194	186	157
C_2	596	483	455	384	356	299	287	271	210	202	157
D_2	610	497	469	398	370	313	301	285	224	216	157
E_2	580	467	439	368	340	283	271	255	194	186	157
Cl	631	517	489	404	376	337	307	309	230	240	157
DesmA	564	465	437	352	324	267	255	239	178	170	157
DesmB	580	481	453	368	340	283	271	255	194	186	157
$DesmB_2$	566	481	453	368	340	283	271	255	194	186	157
DesmC	596	497	469	384	356	299	287	271	210	202	157
DH-A	580	467	439	354	326	269	257	241	180	172	157
RosA	608	495	467	382	354	297	271	269	208	186	157
RosB	592	479	451	366	338	281	255	253	192	170	157

mass spectra, the discrimination among the members of the closed series of destruxins is relatively simple and enables one to assign the majority of them unambiguously (Figs 2 and 3). All in all, 22 destruxins were characterized in our fermentation broths of *Metarhizium anisopliae*, including destruxins A, B, C, D, E and Ed, their [Pip^2]- and [Val^3]-analogues, dihydrodestruxin A, chlorhydrin and desmethyldestruxin B (Table 2). Since the production of individual destruxins strongly depends on the cultivation conditions,[19] only about 15 of them are typically found in one culture. Among them, destruxin Ed_1 has not previously been described. Destruxin Ed_1 was isolated and its structure, originally deduced from MS/MS data (Fig. 3), was confirmed using 1D and 2D NMR techniques. Details on its isolation and full NMR assignment will be published elsewhere.[32] In addition to the new destruxin Ed_1, another compound having $M_r = 623$ was tentatively assigned to destruxin C_1 (Fig. 2). Some new destruxins were also detected at molecular masses 14 u higher than those of the basic destruxins series, indicating a possible [$MeVal^4$] → [$MeIle^4$] replacement (see the structures of destruxins A_4, A_5 and homodestruxin B, Table 1). However, the trace amounts of these compounds available did not allow their detailed examination.

Three roseotoxins (A, B and C, according to their retention on silica gel) were originally reported from the fungus *Trichothecium roseum*,[8] but apparently only the structure of roseotoxin B was elucidated.[9,10] A brief comparison with the three most abundant destruxins enables one to estimate that roseotoxin B is [3-methyl-Pro^2]DxA (as already proved by an x-ray study[10]), and roseotoxin A is [3-methyl-Pro^2]DxB. Concerning roseotoxin C, its structure is reasonably that of [3-methyl-Pro^2]DxC (assumption based on the relative retention on thin-layer chromatographic plates).[8] Our preliminary LC/MS examination of *Trichothecium roseum* metabolites revealed a series of cyclopeptides closely related to destruxins from *Metarhizium anisopliae* having molecular masses 14 u higher than the parent compounds. The discrimination of isobaric amino acids including pipecolic acid, 3-methylproline and 4-methylproline by the current LC/MS method is not possible. Hence the most abundant new roseotoxin was isolated and its structure determined from MS/MS (Fig. 4) and NMR data (see Experimental) as cyclo(–D-HA^1–L-*trans*-3-$MePro^2$–L-Ile^3–L-$MeVal^4$–L-$MeAla^5$–β-Ala^6–), where HA is 2-hydroxy-4-methylpentanoic acid. The isolation of further roseotoxins present in corresponding fermentation broths is in progress.

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