Insecticidal Cyclodepsipeptides from *Metarhizium anisopliae*

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Bioassay-guided fractionation of the culture broth of *Metarhizium anisopliae* resulted in the isolation of 10 insecticidal cyclodepsipeptides–the destruxins, including two new compounds, destruxin E2 and chlorohydrin. Complete ^{1}H and ^{13}C n.m.r. assignments for the major destruxins, A and B, by various 1D and 2D n.m.r. techniques provided a basis for identification and spectral assignments of other destruxins.

Metarhizium anisopliae, a well known entomopathogenic fungus, is known to produce cyclic depsipeptides—the destruxins.[1] Destruxins are cyclic depsipeptides consisting of five amino acids and an α-hydroxy acid. So far 17 different structurally related destruxins have been isolated from three different sources, and 15 of these are known to be produced by *Metarhizium anisopliae*.[2] Destruxins are insecticidal compounds with a wide range of activity.[3] Recently it was reported that destruxin B is the chlorosis-causing principle of the plant pathogenic fungus, *Alternaria brassicae*, which was also found to produce desmethyldestruxin B and homodestruxin B.[4] Roseotoxin B, the *trans*-3-methylproline anologue of destruxin A, was isolated as the toxic metabolite of *Trichothecium roseum*.[5] Apart from well documented insecticidal activity, destruxins have also been shown to possess immunodepressant activity in insect model systems.[6] This finding is important in view of the fact that cyclosporins, a group of cyclic peptides derived from fungi, are clinically important immunodepressant drugs. Destruxin E was found to exhibit cytostatic and cytotoxic effects on mouse leukemia cells.[7] Destruxins can also activate calcium channels in insect muscles[8] but unlike beauvericin, a cyclodepsipeptide produced by the entomopathogenic fungus *Beauveria bassiana*,[9] destruxins do not seem to possess ionophoric properties.[10] A relationship between the production of destruxins and the pathogenicity of the fungus has been suggested, and the role of the destruxins may be to facilitate establishment of the pathogen on the host.[11] Apparently these insect toxins are the cause of insect mortality after the fungal infection.[12] Total syntheses of destruxin B and protodestruxin have been reported.[13]

During a program of general screening of extracts from various entomogenous fungi, the extract obtained from one of the strains of *Metarhizium anisopliae* (ARSEF No. 1095) showed consistent insecticidal activity when tested against a range of insects. In subsequent bioassay-guided chemical investigations, 10 different destruxins were isolated from the active fractions. A simple protocol was developed for the separation of neutral destruxins by a combination of flash silica gel chromatography and reverse phase h.p.l.c.[14] The structures were assigned by chemical and spectroscopic means. Complete ^{1}H and ^{13}C n.m.r. assignments of the representative destruxins (A and B) were accomplished using different 1D and 2D n.m.r. techniques and this information was subsequently used in the structural elucidation of related compounds and in the complete assignment of their n.m.r. spectra. In this communication we report details of the isolation and characterization of these destruxins, including two new compounds, destruxin E2 and the chlorohydrin.

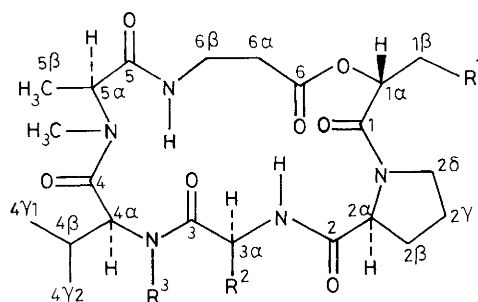
The strain of *Metarhizium anisopliae* used in these studies was originally isolated from an infested *Carpocapsa pomonella* (Lepidoptera: Olethrentidae) from Austria. The fungus was grown in sterilized Czapek-Dox medium with constant shaking and the broth separated by filtration. The aqueous filtrate was acidified and then extracted with methylene dichloride. The extract was found to possess significant insecticidal activity. Subsequent monitoring of the activity was routinely performed using tobacco budworm (*Heliothis virescens*).

Results and Discussion

The filtered culture broth of 10 day old *Metarhizium anisopliae* was extracted with methylene dichloride and the organic layer was washed with dilute aqueous sodium hydrogen carbonate. The solvent was removed and the residue, which showed good insecticidal activity, was subjected to a bioassay-guided fractionation. By a combination of flash chromatography on silica gel and r.p.h.p.l.c. on a C18 column, 10 destruxins were isolated from this extract. The isolated destruxins were identified by physicochemical data, spectral analyses, chemical correlations wherever possible, and comparison with the literature data. The cyclodepsipeptides identified in the present investigation were destruxins A (**1**), A2 (**2**), B (**3**), B2 (**4**), E (**5**), E2 (**6**), C (**7**), C2 (**8**), desmethyldestruxin B (**9**), and the chlorohydrin (**10**) as shown in Figure 1. Compounds E2 and the chlorohydrin are reported for the first time. The nomenclature of the destruxins used in the present report is the same as suggested by Pais *et al.*[2] As discussed below, the complete ^{1}H and ^{13}C n.m.r. assignments of destruxins A (**1**) and B (**3**) were obtained by different high resolution 1D and 2D n.m.r. techniques.

HOM2DJ Resolved Spectrum of Destruxin B.—The HOM2-DJ resolved (*HOM*onuclear 2 *D*imensional *J* resolved)[15] ^{1}H n.m.r. spectrum of destruxin B (**3**) was particularly useful in assigning the multiplicities to resonances in the ^{1}H n.m.r. spectrum. Of particular interest were the regions 0.8—0.98 and 4.5—5.5 p.p.m. As apparent in Figure 2, all six methyl signals could be well resolved. The only methyl triplet (isoleucine-CH_2CH_3) is centred at 0.82 p.p.m. (J 7.7 Hz). The doublet for one methyl at 0.83 p.p.m. J 7.1 Hz) was assigned to isoleucine $> CHCH_3$. The remaining four methyl doublets are centred at 0.86, 0.9, 0.91, and 0.96 p.p.m. (each J 7 Hz). These were assigned to the two pairs of tertiary methyls of α-hydroxy-γ-methylvaleric acid and the valine moieties.

Figure 3 is the enlarged portion of the HOM2DJ resolved spectrum of destruxin B in the range of 4.5—5.3 p.p.m. This region shows the resonances of all the five α-protons of the four α-amino acid residues and one from the α-hydroxy acid residue. The farthest downfield signal at 5.17 p.p.m. is a quartet (J 7.1 Hz) and was assigned to the α-proton of N-methylalanine



Destruxin	R^1	R^2	R^3
A (**1**)	–CH=CH$_2$ (1γ, 1δ)	–CH(CH$_3$)CH$_2$CH$_2$ (3β, 3γ2, 3γ1, 3δ)	CH$_3$
A2 (**2**)	–CH=CH$_2$	–CH(CH$_3$)$_2$ (3β, 3γ1, 3γ2)	CH$_3$
B (**3**)	–CH(CH$_3$)$_2$ (1γ, 1δ1, 1δ2)	–CH(CH$_3$)CH$_2$CH$_3$	CH$_3$
B2 (**4**)	–CH(CH$_3$)$_2$	–CH(CH$_3$)$_2$	CH$_3$
E (**5**)	OCH–CH$_2$ (1γ, 1δ)	–CH(CH$_3$)CH$_2$CH$_3$	CH$_3$
E2 (**6**)	OCH–CH$_2$	–CH(CH$_3$)$_2$	CH$_3$
C (**7**)	–CH(CH$_3$)CH$_2$OH (1γ, 1δ2, 1δ1)	–CH(CH$_3$)CH$_2$CH$_3$	CH$_3$
C2 (**8**)	–CH(CH$_3$)CH$_2$OH	–CH(CH$_3$)$_2$	CH$_3$
DMDB (**9**)	–CH(CH$_3$)$_2$	–CH(CH$_3$)CH$_2$CH$_3$	CH$_3$
CHL (**10**)	–CH(OH)CH$_2$Cl (1γ, 1δ)	–CH(CH$_3$)CH$_2$CH$_3$	CH$_3$
CHL ACE (**11**)	–CH(OCOCH$_3$)CH$_2$Cl	–CH(CH$_3$)CH$_2$CH$_3$	CH$_3$

Figure 1. Structure of destruxins (the numbering scheme is given in parentheses)

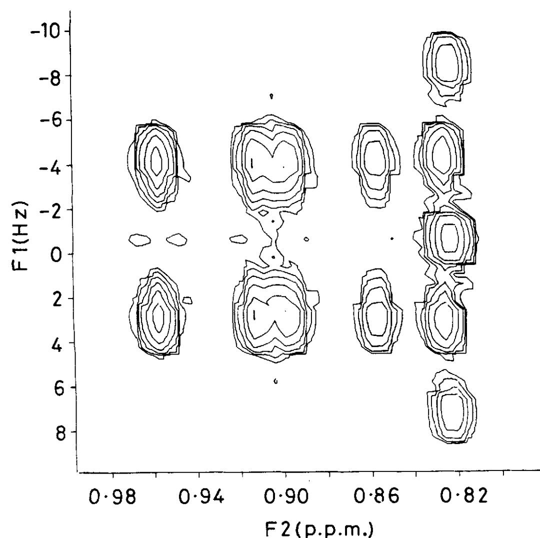


Figure 2. HOM2DJ resolved spectrum of destruxin B (**3**) (0.8—0.98 p.p.m.)

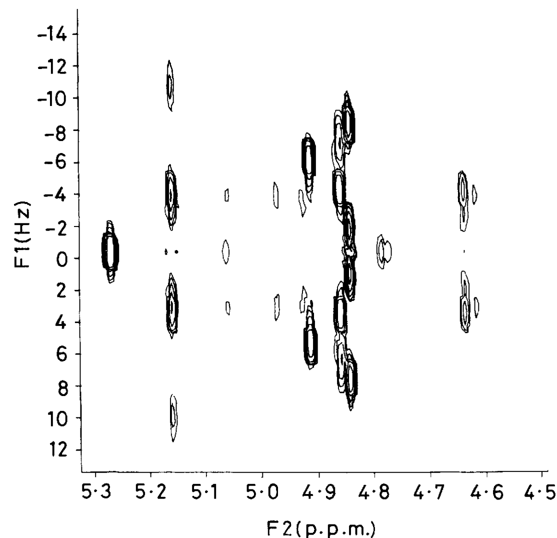


Figure 3. HOM2DJ resolved spectrum of destruxin B (**3**) (4.5—5.3 p.p.m.)

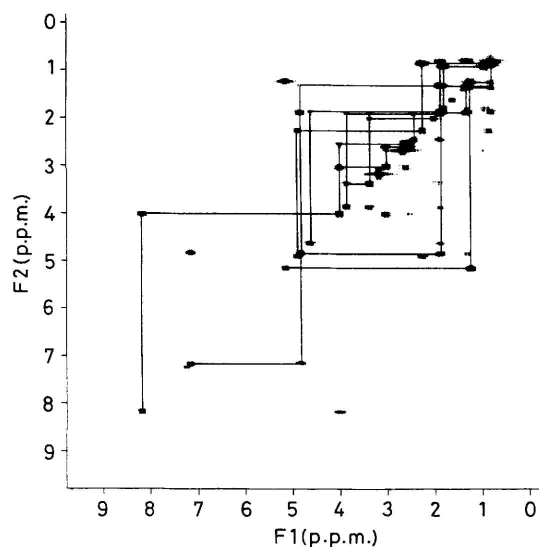


Figure 4. COSYPS of destruxin B (3)

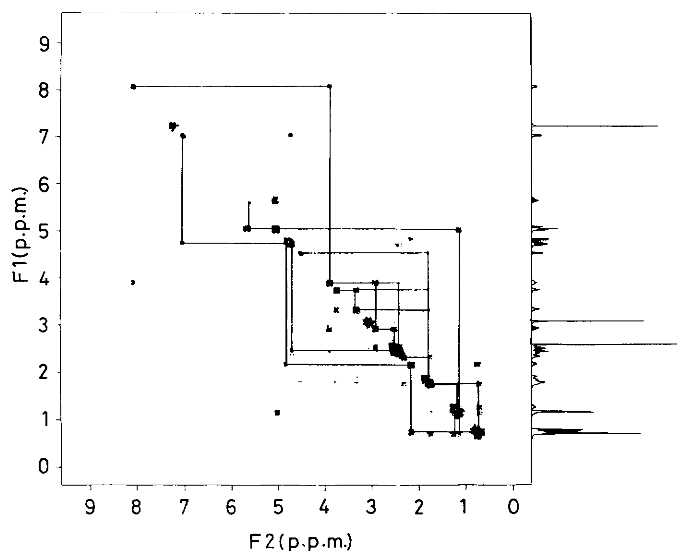


Figure 5. COSYPS of destruxin A (1)

on the basis of its multiplicity. The double doublet at 4.65 p.p.m. was ascribed to the α-proton of proline (*J* 6.2, 1.6 Hz). The appearance of a clear double doublet indicates the nonequivalence of two proline β-CH_2 protons. The only doublet at 4.91 p.p.m. (*J* 11.4 Hz) was assigned to the α-C*H* of *N*-methylvaline. Two double doublets at 4.86 (*J* 6.6, 9.4 Hz) and 4.87 p.p.m. (*J* 2.8, 10.5 Hz) were assigned to the α-protons of isoleucine and α-hydroxy acid residue respectively. The signal at 5.27 p.p.m. is from the contaminant methylene dichloride. These assignments were further substantiated by analysis of the COSYPS (^{1}H-^{1}H correlation) and HETCOR (^{1}H-^{13}C corelation) spectra as described below.

COSYPS.—Figure 4 is the contour plot of the COSYPS (^{1}H-^{1}H *CO*rrelation *S*pectroscop*Y* *P*hase *S*ensitive)[16] of destruxin B (3). The correlations have been marked by the perpendicular lines. The two N*H* resonances at 8.18 (β-alanine) and 7.17 p.p.m. (isoleucine) clearly show correlations with the rest of the spectrum. The signal at 8.18 p.p.m. is correlated to resonance at 4.13 p.p.m. (1 H, β-proton of β-alanine) which in turn shows connectivity to resonance at 3.06 (1 H, β-proton of β-alanine) and 2.58 (1 H, one of the α-methylene protons). The other α-methylene proton of β-alanine resonates at 2.65 p.p.m. Isoleucine N*H* at 7.17 p.p.m. shows direct correlation with the resonance at 4.86 p.p.m. which was assigned to the α-C*H* of the isoleucine, which is also correlated with the β-C*H* of isoleucine at 1.9 p.p.m. The correlation of this proton with γ2-methyl (assigned at 0.83 p.p.m; doublet) is also discernible. The γ1-methylene of isoleucine was assigned at 1.83 p.p.m.; and this resonance shows correlation with the methyl resonance at 0.82 p.p.m. (the only triplet).

The α-C*H* of alanine at 5.17 p.p.m. shows a strong cross-peak for a correlation with the methyl doublet at 1.29 p.p.m. Similarly, the α-C*H* of *N*-methylvaline at 4.91 p.p.m. displays a correlation with the one proton multiplet at 2.3 p.p.m. (β-C*H*) which is then strongly coupled with the methyls (assigned 0.86 and 0.9 p.p.m.). The proline α-C*H* was assigned at 4.65 p.p.m. which shows coupling with the proton at 1.91 p.p.m. (β-C*H*) which in turn is coupled with a signal at 2.53 p.p.m. (β-C*H*). The two γ-protons of proline were assigned at 1.9 and 2.04 p.p.m. (both multiplets). The signal at 1.9 p.p.m. shows connectivity with the signals at 3.4 and 3.89 p.p.m. which were assigned to the two δ-protons of proline, and the interaction between the two signals was evident. The signal at 2.04 p.p.m. also shows connectivity with the δ-proton at 3.4 p.p.m., thus confirming the assignment. Analysis of the HETCOR spectrum as detailed below provided additional support for these assignments. The α-C*H* of the α-hydroxy acid moiety resonated at 4.87 p.p.m. and was correlated to the proton resonances at 1.9 and 1.38 p.p.m. which were assigned to the β-methylene protons of the α-hydroxy acid moiety (geminally coupled nonequivalent protons). The γ-C*H* of the acid was assigned to the resonance at 1.89 p.p.m. which showed correlation with the methyls (assigned at 0.91 and 0.96 p.p.m.).

The COSYPS of destruxin A (1) (Figure 5) as expected, exhibited a strong cross peak between the olefinic protons of the pentenoic acid moiety which resonate at 5.77 (=C*H*-) and 5.14 and 5.19 p.p.m. (=CH_2). However, in the present experiment, no correlation could be observed between the olefinic methine proton at 5.77 p.p.m. and the β-methylene protons of the pentenoic acid, which were assigned at 2.63 p.p.m. (multiplet) and showed correlation with the α-proton at 4.81 p.p.m. As in the case of destruxin B as described above, β-alanine β-protons appeared at 3.03 and 4.01 p.p.m. (correlated with each other as well as with the α-CH_2 protons at 2.59 and 2.63 p.p.m. respectively). The assignments were further confirmed by the HETCOR analysis. The proton at 4.01 p.p.m. also shows correlation with the N*H* proton at 8.18 p.p.m. The δ-methylene protons of proline appeared as a characteristic pair of multiplets centred at 3.46 and 3.87 p.p.m. showing connectivity with each other as well as with the γ-protons of proline, which were assigned at 1.89 and 2.02 p.p.m. (HETCOR) (geminal and vicinal couplings respectively). The proton at 1.89 p.p.m. was found to be coupled with the signal at 2.47 p.p.m. and these signals were assigned to the β-protons of the proline. The proton at 1.89 p.p.m. also showed correlation with the α-proton which resonated at 4.64 p.p.m.

The α-proton (α-C*H*) of alanine resonated at 5.16 p.p.m. and showed a cross peak with the methyl doublet at 1.28 p.p.m. The valine α-C*H* resonated at 4.93 p.p.m. (doublet) and showed coupling with the multiplet centred at 2.29 p.p.m., which in turn showed correlations with the methyls assigned at 0.9 and 0.86 p.p.m. The isoleucine α-C*H* was assigned at 4.86 p.p.m. The β-C*H* of isoleucine was assigned at 1.89 p.p.m. on the basis of HETCOR and was found to be coupled with γ1-methylene protons at 1.25 and 1.37 p.p.m., which in turn showed the connectivities with the δ-methyl. The β-C*H* at 1.89 p.p.m. also showed correlation with the γ2-methyl, but no correlation could be observed between this proton and the α-proton at 4.86 p.p.m.

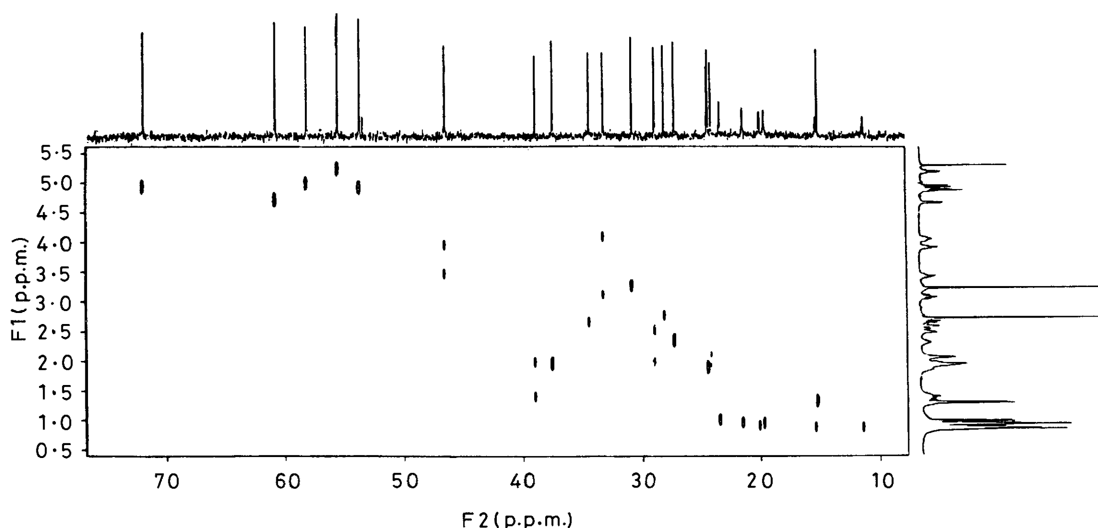


Figure 6. HETCOR of destruxin B (**3**)

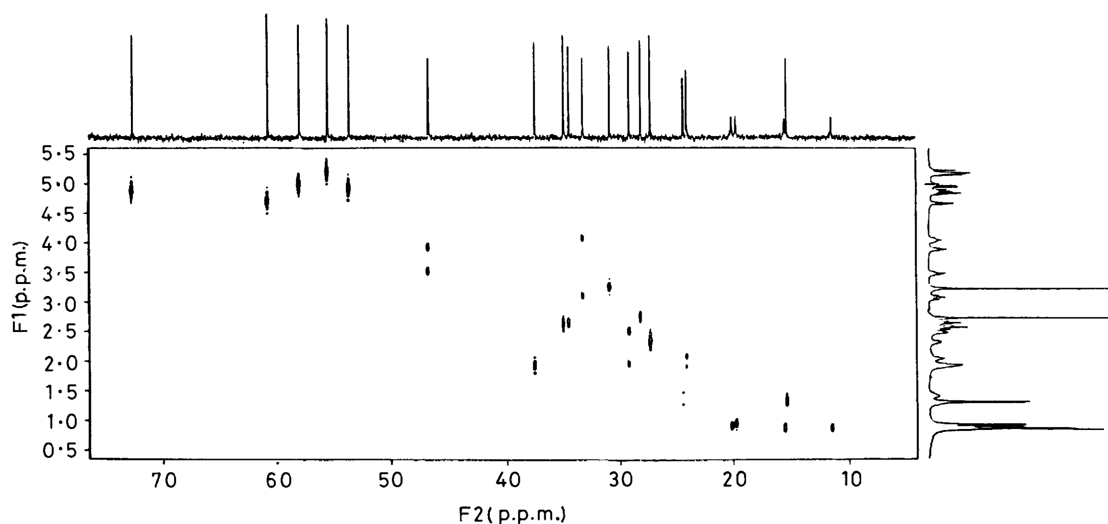


Figure 7. HETCOR of destruxin A (**1**)

The complete ^{1}H n.m.r. assignments for different destruxins have been tabulated in Table 1.

DEPT.—Spectral editing of the ^{13}C n.m.r. spectra of destruxins was achieved by the DEPT (*D*istortionless *E*nhancement by *P*olarization *T*ransfer)[17] analysis in order to confirm multiplicities of the individual signals. DEPT analysis of the 13C n.m.r. spectrum of destruxin B (**3**) revealed the presence of nine methyls, seven methylenes, and eight methines in the molecule. Similarly, the DEPT spectrum of destruxin A (**1**) confirmed the presence of seven methyls, eight methylenes and eight methines. An identical analysis of destruxin A2 (**2**) confirmed the presence of seven methyls, seven methylenes and eight methines in the molecule, and the of the DEPT spectrum of destruxin C (**7**) confirmed the presence of eight methyls, eight methylenes and eight methines in the molecule. The methylenic nature of the resonance at 67.93 p.p.m. in destruxin C readily indicated it to be the hydroxy methylene carbon on the basis of chemical-shift considerations. The knowledge of the multiplicity of each peak in the ^{13}C n.m.r. spectra of these compounds greatly facilitated the ^{13}C signal assignments as described below.

HETCOR.—Figure 6 shows the contour plot of the HETCOR (^{1}H-^{13}C *HET*ero *COR*relation spectroscopy)[18] spectrum of destruxin B(**3**) in the region 0—77 p.p.m. excluding the carbonyl region. All the correlations are derived from the single bond ^{13}C-^{1}H scalar couplings.[19] The final ^{13}C assignments were mainly done on the basis of the ^{1}H n.m.r. analysis, comparison with the published data for the destruxins and the chemical shift arguments. The most downfield signal in this region at 71.95 p.p.m. (CH from DEPT analysis) correlated with the proton multiplet at 4.87 p.p.m. and was assigned to the α-carbon of the α-hydroxy acid. The signals in the cluster of four methine peaks at 60.74, 58.13, 55.5, and 53. 71 p.p.m. displayed correlations with the proton resonances at 4.65 (m), 4.91 (d), 5.17 (q) and 4.86 (dd) respectively and were assigned to the α-carbons of proline, valine, alanine, and isoleucine respectively.

The other salient feature of the HETCOR spectrum was the clear presence of five methylene carbon signals with geminally coupled protons (nonequivalent protons because of the restricted rotation around the single bonds) as evidenced by the presence of a pair of crosslinking peaks. The signal at 46.5 p.p.m. was assigned to the δ-methylene of proline. This carbon links with the protons resonating at 3.4 and 3.89 p.p.m. (assigned to the δ-methylene protons of proline) thus confirming the ^{1}H assignments in retrospect. The signal at 38.95 p.p.m. connects to the protons at 1.9 and 1.38 p.p.m. and was assigned to the β-methylene of the α-hydroxy-γ-methylvaleric acid moiety. This

observation suggests the magnetic nonequivalence of the two methylene protons, probably because of the conformational stability of the molecule leading to steric crowding around the α-hydroxy acid methyls, thus resulting in restricted rotation of the acid side chain.[20] These assignments were further corroborated by the ^{1}H assignments. The β-carbon of the β-alanine moiety resonated at 33.26 p.p.m. and showed cross correlation peaks with the proton resonances at 4.13 and 3.06 p.p.m. However, the α-carbon of β-alanine at 34.43 p.p.m. only showed a correlation with the proton multiplet centred at 2.65 p.p.m., thus confirming the close chemical shifts of the two methylene protons (assigned at 2.58 and 2.65 p.p.m.).

The β- and γ- carbons of the proline moiety were assigned at 28.93 and 24.13 p.p.m. respectively. The signal at 28.93 p.p.m. was coupled with the proton resonances at 2.53 and 1.91 p.p.m. (assigned to the β-methylene of proline) and the signal at 24.13 p.p.m. showed correlations with the proton resonances at 1.9 and 2.04 p.p.m. (assigned to the γ-methylene of proline). The γ-carbon of the α-hydroxy acid entity was assigned at 24.4 p.p.m. which showed the correlation with the proton signal at 1.89 p.p.m. The two methyls were assigned at 21.47 and 23.41 p.p.m. The β-methine carbon of the isoleucine moiety resonated at 37.48 p.p.m. and showed connectivity with the proton at 1.9 p.p.m. The γ1-methylene of the isoleucine resonated at 24.47

Table 1. Proton n.m.r. assignments of destruxins[a]

Proton	A (**1**)	B (**3**)	C (**7**)	E (**5**)	CHL (**10**)
1α	4.81 (dd, 9,7)	4.87 (dd, 2.8, 10.5)	4.92 (dd, 3.5, 10)	4.91 (dd, 4, 7)	5.09 (dd, 5, 6)
1β	2.63 (m)	1.38, 1.9 (m,m)	2.04 (m)	1.9, 2.02 (m,m)	2.05 (m)
1γ	5.77 (m, 7, 9, 16)	1.89 (m)	1.91 (m)	2.77 (m)	4.05 (m)
1δ1	5.14, 5.19 (br d, 9; br d, 16)	0.91 (d,7)	3.51 (d, 6)	2.51 (m)	3.53, 3.65 (m,m)
1δ2		0.96 (d, 7)	0.99 (d, 7)		
2α	4.64 (br d, 6.9)	4.65 (dd, 6.2, 1.6)	4.64 (br d, 7.5)	4.64 (br d, 5)	4.66 (br d, 6.9)
2β	1.89, 2.47 (m,m)	1.91, 2.53 (m,m)	1.91, 2.48 (m,m)	1.9, 2.59 (m,m)	1.93, 2.55 (m,m)
2γ	1.89, 2.02 (m,m)	1.9, 2.04 (m,m)	1.98, 2.04 (m,m)	1.9, 2.23 (m,m)	1.93, 2.05 (m,m)
2δ	3.46, 3.87 (m,m)	3.4, 3.89 (m,m)	3.47, 3.89 (m,m)	3.59, 3.9 (m,m)	3.65, 3.91 (m,m)
3α	4.86 (dd, 6.5, 9.4)	4.86 (dd, 6.6, 9.4)	4.82 (dd, 4, 9)	4.84 (dd, 4.5, 9)	4.87 (dd, 6.4, 9.1)
3β	1.89 (m)	1.9 (m)	1.99 (m)	1.92 (m)	1.93 (m)
3γ1	1.25, 1.37 (m,m)	1.83 (m)	1.29, 1.38 (m,m)	1.89 (m)	1.2, 1.38 (m,m)
3γ2	0.83 (d, 5.5)	0.83 (d, 7.1)	0.89 (d, 6.5)	0.8 (d, 6)	0.85 (d, 6)
3δ	0.82 (t, 6.5)	0.82 (t, 7.7)	0.86 (t, 6)	0.78 (t, 6)	0.83 (t, 6)
3NH	7.13 (d, 9.4)	7.17 (d, 9.4)	7.16 (d, 9)	7.1 (d, 9)	7.1 (d, 9.1)
4α	4.93 (d, 10.9)	4.91 (d, 11.4)	4.93 (d, 10)	4.93 (d, 10)	4.95 (d,11)
4β	2.29 (m)	2.3 (m)	2.34 (m)	2.23 (m)	2.3 (m)
4γ1	0.86 (d, 6.9)	0.9 (d, 7)	0.94 (d, 7)	0.87 (d, 6.5)	0.9 (d, 6.2)
4γ2	0.9 (d, 5.6)	0.86 (d, 7)	0.91 (d, 7)	0.83 (d, 6.5)	0.87 (d, 6.5)
4NCH$_3$	3.19 (s)	3.2 (s)	3.2 (s)	3.17 (s)	3.2 (s)
5α	5.16 (q, 6.6)	5.17 (q, 7.1)	5.18 (q, 6.5)	5.11 (q, 6)	5.14 (q, 6.8)
5β	1.28 (d, 6.6)	1.29 (d, 7.1)	1.29 (d, 6.5)	1.25 (d, 6)	1.28 (d, 6.8)
5NCH$_3$	2.69 (s)	2.72 (s)	2.7 (s)	2.72 (s)	2.7 (s)
6α	2.59, 2.63 (m,m)	2.58, 2.65 (dd, 4, 13; dd, 5, 13)	2.53, 2.62 (m,m)	2.59, 2.61 (dd, 3.5, 11; m)	2.56, 2.63 (m,m)
6β	3.03, 4.01 (m,m)	3.06, 4.13 (m,m)	3.06, 4.03 (m,m)	3.02, 3.97 (m,m)	3.05, 3.96 (m,m)
6NH	8.18 (d, 9.4)	8.18 (d, 9.2)	8.18 (d, 9)	8.16 (d, 9.5)	8.13 (d, 9.6)

Table 1 (*continued*)

CHL ACE (**11**)	A2 (**2**)	B2 (**4**)	C2 (**8**)	E2[b] (**6**)	DMDB (**9**)
5.17 (dd, 5, 6)	4.94 (dd, 4, 10.4)	4.91 (dd, 2.7, 10.4)	4.97 (m)	4.95 (dd, 4, 10.5)	4.88 (dd, 2.5, 10)
2.22, 2.31 (m,m)	2.55 (m)	1.29, 1.88 (m,m)	2.02 (m)	1.93 (m)	1.35, 1.93 (m,m)
4.97 (m)	5.77 (m, 6, 9, 15)	1.88 (m)	1.93 (m)	2.82 (m)	1.8 (m)
3.63, 3.74 (dd, 6, 12; dd, 4.5, 12)	5.18, 5.26 (br d, 9; br d, 15)	0.97 (d, 6.5)	3.52 (d, 6.5)	2.51 (m)	1.01 (d, 7)
2.09 ($COCH_3$, s)		0.93 (d, 6.7)	0.99 (d, 6.6)		0.96 (d, 7.5)
4.66 (br d, 8)	4.68 (br d, 6.9)	4.66 (br d, 6.9)	4.66 (br d, 8.5)	4.69 (br d, 7)	4.2 (dd, 6.5, 9)
1.96, 2.51 (m,m)	2.05, 2.55 (m,m)	1.9, 2.54 (m,m)	1.93, 2.58 (m,m)	1.93, 2.51 (m,m)	1.93, 2.57 (m,m)
1.96, 2.09 (m,m)	1.92, 2.05 (m,m)	1.88, 2.05 (m,m)	1.93, 2.02 (m,m)	1.99, 2.13 (m,m)	1.99 (m)
3.46, 3.89 (m,m)	3.5, 3.91 (m,m)	3.4, 3.9 (m,m)	3.44, 3.89 (m,m)	3.65, 3.84 (m,m)	3.41, 3.89 (m,m)
4.86 (dd, 6, 9.5)	4.84 (dd, 7, 9.2)	4.85 (m)	4.93 (m)	4.98 (dd, 7, 9.5)	4.52 (dd, 7, 9.5)
1.96 (m)	2.16 (m)	2.14 (m)	2.08 (m)	2.37 (m)	1.89 (m)
1.27, 1.38 (m,m)	0.91 (d, 6.2)	0.91 (d, 6.5)	0.94 (d, 6.5)	0.89 (d, 7)	2.05 (m)
0.88 (d, 7.5)	0.89 (d, 6.2)	0.9 (d, 6.5)	0.91 (d, 6.5)	0.87 (d, 7)	0.91 (d, 7)
0.86 (t, 6)					0.88 (t, 6)
7.11 (d, 9.5)	7.2 (d, 9.2)	7.2 (d, 9.1)	7.22 (d, 9.5)	7.18 (d, 9.5)	7.02 (d, 9.5)
4.93 (d, 10.5)	4.91 (d, 10.4)	4.87 (d, 10.4)	4.89 (d, 10)	4.92 (d, 10.5)	4.61 (br d, 8)
2.31 (m)	2.31 (m)	2.3 (m)	2.3 (m)	2.27 (m)	2.44 (m)
0.92 (d,7)	0.91 (d, 6.2)	0.88 (d, 7)	0.89 (d, 7)	0.92 (d, 7)	0.87 (d, 6.5)
0.9 (d, 7)	0.93 (d, 6.2)	0.86 (d, 6.9)	0.84 (d, 7)	0.9 (d, 7)	0.85 (d, 6.5)
3.23 (s)	3.23 (s)	3.21 (s)	3.23 (s)	3.22 (s)	4N*H*, 6.46 (br s)
5.17 (q, 7)	5.17 (q, 7.1)	5.17 (q, 6.4)	5.19 (q, 7)	5.15 (q, 7.5)	5.06 (q, 7.5)
1.31 (d, 7)	1.31 (d, 7.1)	1.28 (d, 6.4)	1.27 (d, 7)	1.3 (d, 7.5)	1.34 (d, 7.5)
2.71 (s)	2.73 (s)	2.71 (s)	2.72 (s)	2.71 (s)	2.73 (s)
2.57, 2.64 (m,m)	2.56, 2.65 (m,m)	2.54, 2.63 (dd, 7.8, 17.8; dd, 5, 17.8)	2.54, 2.62 (m,m)	2.51, 2.63 (m,m)	2.54, 2.66 (m,m)
3.07, 4.05 (m,m)	3.07, 4.01 (m,m)	3.05, 4.01 (m,m)	3.07, 4.02 (m,m)	3.09, 3.98 (m,m)	3.13, 3.99 (m,m)
8.14 (d, 9)	8.13 (d, 9.7)	8.09 (d, 9.1)	8.09 (d, 9.5)	8.1 (d, 9)	8.28 (d, 9)

[a] The data in the parentheses indicate multiplicity followed by the coupling constant (*J*/H2). All the C-methyl signals were assigned tentatively and the individual assignments can be interchanged. Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet; br d, broad doublet; dd, double doublet. [b] The n.m.r. sample had $< 5\%$ contamination of a closely related unknown compound.

p.p.m. and showed correlation with the proton at 1.83 p.p.m. The two methyls were assigned at 15.38 and 11.39 p.p.m. The valine β-methine carbon was assigned at 27.28 p.p.m. and exhibited coupling with the proton resonance at 2.3 p.p.m. The two methyls were assigned at 19.68 and 20.05 p.p.m. The valine *N*-methyl was assigned at 30.87 p.p.m. on the basis of published data for destruxins and chemical-shift consideration. The carbon signal showed correlation with the proton singlet at 3.2 p.p.m. The alanine β-methyl carbon absorbed at 15.28 p.p.m. and showed a correlation peak with the proton doublet at 1.29 p.p.m. The alanine *N*-methyl carbon resonated at 28.13 p.p.m. and was coupled with the proton singlet at 2.72 p.p.m.

Figure 7 shows the contour plot of the HETCOR of destruxin A (**1**). The assignments were done on the basis of similar reasoning as above for destruxin B. The two olefinic carbons,

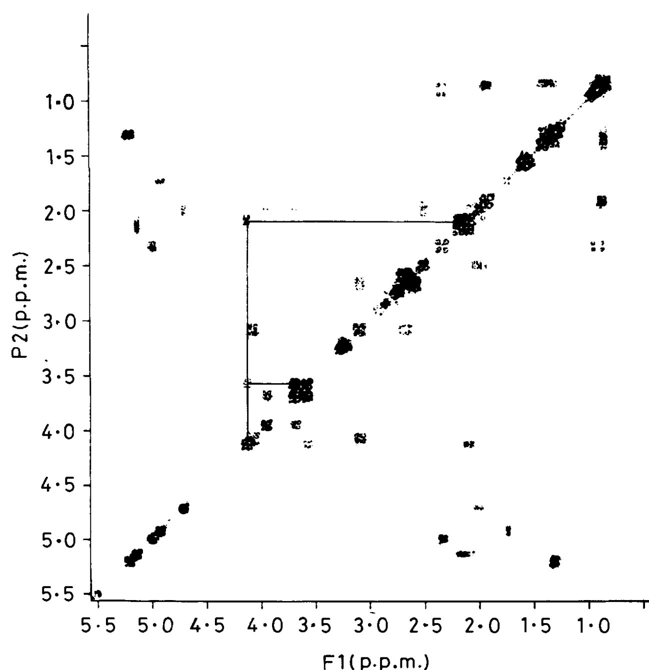


Figure 8. COSYPS of chlorohydrin (**10**)

the methylene, and the methine resonate at 119.51 and 131.4 p.p.m. respectively and are correlated to the proton resonances at 5.14 and 5.19 (doublets) and 5.77 p.p.m. (multiplet). The α-carbon of the α-hydroxypentenoic acid entity resonated at 72.78 p.p.m. and showed coupling with the proton resonance at 4.81 p.p.m. (α-C*H*). The α-carbons of proline, valine, alanine, and isoleucine entities were assigned at 60.8, 58.06, 55.51, and 53.65 p.p.m. respectively and showed correlations with the proton resonances at 4.64, 4.93, 5.16, and 4.86 p.p.m. respectively (α-C*H*s). The δ-carbon of proline resonated at 46.7 p.p.m. and showed connectivity with the proton resonances at 3.46 and 3.87 p.p.m. (δ-methylene protons). The β- and γ-carbons of the proline moiety were assigned at 29.12 and 24.09 p.p.m. respectively and showed correlation with the proton resonances at 1.89 and 2.47 p.p.m. (β-methylene) and 1.89 and 2.02 p.p.m. (γ-methylene) respectively.

The α-carbon of β-alanine was assigned at 34.93 p.p.m. and the signal showed correlation with the proton resonance at 2.59 p.p.m. (α-methylene). The β-carbon of β-alanine resonated at 33.23 p.p.m. and showed cross peaks with the proton resonances at 4.01 and 3.03 p.p.m. (the β-CH_2 protons). The pentenoic acid β-carbon was assigned the resonance at 34.49 p.p.m. and showed coupling with the proton multiplet at 2.63 p.p.m. The isoleucine β-carbon was assigned the resonance at 37.49 p.p.m. and the corresponding correlation peak revealed that the methine proton resonated at 1.89 p.p.m. The γ1-methylene carbon of isoleucine resonated at 24.4 p.p.m. and the correlation peaks confirmed the corresponding proton resonances at 1.25 and 1.37 p.p.m. (γ1-CH_2 protons). The valine β-methine carbon resonated at 27.27 p.p.m. and showed correlation with the proton resonance at 2.29 p.p.m. (methine proton). The valine *N*-methyl carbon was assigned at 30.85 p.p.m. which showed a cross peak with the singlet proton resonance at 3.19 p.p.m. The alanine β-methyl carbon was assigned at 15.24 p.p.m. which correlated with the doublet proton resonance at 1.28 p.p.m. (CH_3 protons). The alanine *N*-methyl carbon resonated at 28.12 p.p.m. and had a correlation peak to the singlet proton resonance centred at 2.69 p.p.m. (CH_3 protons).

The complete ^{13}C assignments for different destruxins have been listed in Table 2. The tentative assignments for the individual amide and ester carbonyl carbons have been made on the basis of the published data for peptides and for roseotoxin B, and may be interchanged.[5]

Identification of Chlorohydrin.—Chlorohydrin (**10**) was isolated by the combination of the flash chromatography on silica gel followed by preparative h.p.l.c. on a reverse phase C18 column according to the previously published procedure.[14] The crude methylene dichloride-soluble extract from the broth of *Metarhizium anisopliae* was fractionated into acidic, basic, and neutral fractions. The active neutral fraction was then subjected to chromatographic separation monitored by bioassay for insect toxicity. The total yield of the chlorohydrin from the extract was 1.4% as calculated from the weight of the active neutral fraction.[14]

Amino acid analysis of the chlorohydrin (PTH derivative on Waters Pico Tag system) confirmed the presence of proline, isoleucine, and β-alanine in an equimolar ratio. The mass spectrum of the sample showed a molecular ion peak at *m*/*z* 629 and an isotopic peak at *m*/*z* 631 (relative intensities 3:1) which suggested the presence of a chlorine atom in the molecule.[21] The mass spectral fragmentation pattern of destruxins has been reported in literature.[22] The characteristic fragment ions for destruxin containing a *N*-methylalanine-*N*-methylvaline entity are $M-57$, $M-85$, $M-142$, and $M-170$ generated by the sequential loss of fragments as a result of the ring opening and cleavage within the *N*-methylalanine-*N*-methylvaline entity. The mass spectrum of the chlorohydrin showed the presence of peaks at 572 ($M-57$), 544 ($M-85$), 487 ($M-142$), and 459 ($M-170$) and thus confirmed the presence of the *N*-methylalanine-*N*-methylvaline entity in the molecule. All these peaks had corresponding isotopic peaks at 574, 546, 489, and 461 in the ratio of *ca.* 3:1, further substantiating the presence of one chlorine atom in the molecule. The other major peaks were observed at *m*/*z* 601 ($M-CO$, accompanied by the isotopic peak at 603), 580 ($M-CH_2Cl$), 469 (487-H_2O, isotopic peak at 471) and 441 (459 − H_2O, isotopic peak at 443). All these data suggested that the compound was closely related to the known destruxins and the difference probably was in the α-hydroxy acid entity which, as suggested by the mass fragmentation pattern, was a chlorohydrin. Chlorohydrin can easily be derived from destruxin E (by acidolytic opening of the epoxide) or destruxin A (by addition on the exocyclic double bond).

The ^1H and ^{13}C n.m.r. data for the chorohydrin (**10**) are listed in Tables 1 and 2 respectively. Occurrence of two signals at 66.91 and 49.84 p.p.m. in the ^{13}C n.m.r. suggested the presence of the HO-C-C-Cl type entity in the molecule. As evident from the listed data, the rest of the spectrum was comparable to other destruxins. Final confirmation of the structure as the chlorohydrin (**10**) as shown in Figure 1 was obtained by analysis of the ^1H n.m.r. spectrum, which showed the presence of a multiplet integrating for two protons at 3.65 p.p.m. and one proton multiplets centred at 3.53 p.p.m., 3.96 and 4.05 p.p.m. respectively. On the basis of the COSYPS spectrum of the sample (*vide infra*) and chemical shifts comparison with the known destruxins, it could readily be determined that the two mutually coupled signals at 3.96 and 3.05 p.p.m. were due to the β-alanine β-methylene protons while the correlated resonances centred at 3.91 and 3.65 p.p.m. (total integration for two protons) were from the δ-methylene protons of the proline moiety. The only unassigned three proton resonances were the multiplets centred at 4.05, 3.65, and 3.53 p.p.m. The literature value for aliphatic secondary hydroxy methine (C*H*-OH) is 3.9 p.p.m.[23] while CH_3CH_2Cl resonates at 3.57 p.p.m.[24] The proton resonances at 3.65 and 3.53 p.p.m. were assigned to the geminally coupled chloromethylene protons (CH_2Cl) while the one proton multiplet at 4.05 p.p.m. was assigned to the secondary hydroxylic methine proton (C*H*OH). The

Table 2. Carbon n.m.r. assignments of destruxins[a]

Carbon	A[b] (1)	B[b] (3)	C[b] (7)	E (5)	CHL (10)	A2[b] (2)	B2 (4)	C2 (8)	DMDB (9)
1α	72.8	71.95	71.54	70.62	70.37	72.8	71.93	71.56	71.51
1β	34.49	38.95	33.61	33.59	34.54	35.0	38.94	33.67	38.93
1γ	131.4	24.4	31.83	47.84	66.91	131.42	24.38	31.87	24.43
1δ1	119.51	21.47	67.93	47.09	49.84	119.55	21.47	68.01	21.3
1δ2		23.41	16.06				23.4	16.11	23.38
1C=O	173.56	173.6	173.54	173.42	173.47	173.3	173.4	173.24	171.85
2α	60.8	60.74	60.75	60.85	61.02	60.91	60.77	60.84	60.79
2β	29.12	28.93	28.84	29.16	29.15	29.18	30.3	28.87	30.44
2γ	24.09	24.13	24.09	23.97	23.98	24.1	24.09	24.1	24.17
2δ	46.7	46.5	46.47	46.64	46.69	46.8	46.54	46.57	46.61
2C=O	171.07	171.11	171.07	171.0	171.04	171.7	171.2	171.4	171.7
3α	53.65	53.71	53.67	53.59	53.7	54.15	54.16	54.18	55.64
3β	37.49	37.48	37.45	37.37	37.38	30.36	28.11	30.31	38.43
3γ1	24.4	24.47	24.4	24.3	24.36	17.29	17.32	17.31	25.04
3γ2	15.44	15.38	15.39	15.38	15.3	20.15	20.11	20.13	15.2
3δ	11.41	11.39	11.38	11.37	11.4				11.3
3C=O	168.91	169.73	169.58	168.64	169.56	169.1	169.8	169.32	169.71
4α	58.06	58.13	58.07	57.97	58.02	58.19	58.16	58.17	56.25
4β	27.27	27.28	27.25	27.18	27.25	27.44	27.39	27.41	28.5
4γ1	19.66	19.68	19.65	19.58	19.59	19.44	19.38	19.41	19.57
4γ2	20.06	20.05	20.04	19.98	20.01	19.48	19.84	19.83	19.62
4NCH_3	30.85	30.87	30.84	30.82	30.9	30.85	30.83	30.84	
4C=O	170.94	171.0	170.89	170.79	170.7	171.2	170.98	171.02	170.83
5α	55.51	55.5	55.44	55.46	55.5	55.58	55.48	55.48	55.64
5β	15.24	15.28	15.23	15.15	15.2	15.13	15.13	15.12	14.87
5NCH_3	28.12	28.13	28.09	28.09	28.14	28.14	28.9	28.11	29.17
5C=O	169.7	169.78	169.73	169.68	169.76	169.75	169.9	169.72	169.92
6α	34.93	34.43	34.42	34.45	34.91	34.52	34.41	34.44	34.24
6β	33.23	33.26	33.2	33.14	33.17	33.32	33.28	33.26	33.55
6C=O	173.6	173.82	173.65	173.51	173.65	173.69	173.8	173.67	173.36

[a] The C-methyl and the carbonyl assignments are tentative and may be interchanged. [b] Multiplicities of the carbon signals for these compounds were confirmed by the spectral editing of their DEPT analyses.

site of the substitution of Cl and OH in the chlorohydrin was finally obtained by acetylation of the sample (pyridine–acetic anhydride). The data from the ^{1}H n.m.r. spectrum of the acetate has been given in Table 1. Analysis of the ^{1}H n.m.r. spectrum of the acetate clearly revealed the downfield shift of one proton from 4.05 to 4.97 p.p.m. This shift of 0.92 p.p.m. suggested the presence of a secondary hydroxy, and thereby confirmed the structure of the chlorohydrin as $CH(OH)CH_2Cl$. In the acetate, the two protons of multiplets centred at 3.65 and 3.53 p.p.m. in the chlorohydrin, resonated as two separate multiplets centred at 3.63 and 3.74 p.p.m. (CH_2Cl protons). The contour plot of the COSYPS of the chlorohydrin is shown in Figure 8. The secondary hydroxy methine proton which resonates at 4.05 p.p.m. clearly shows the correlation (marked by perpendicular lines) with the protons of β-methylene of the acid entity which resonate at 2.05 p.p.m. The secondary hydroxy methine at 4.05 p.p.m. also shows a correlation with one of the methylene protons at 3.53 p.p.m. (CH_2Cl) which in turn is coupled to the geminal methylene proton at 3.65 p.p.m., thus confirming the structure. The chlorohydrin may be an artefact of the isolation procedure as it can be derived by the addition of one molecule of hypochlorous acid to the double bond of destruxin A (**1**). When destruxin E (**5**) was set aside in concentrated HCl at room temperature for 72 h, no formation of the chlorohydrin (**10**) could be observed.

Identification of Destruxin E2.—Destruxin E2 (**6**) was isolated as a minor component from the neutral destruxin fraction with a total yield of 0.4%. Details of the isolation procedure which involved the use of flash chromatography on silica gel followed by reverse phase h.p.l.c. have already been published.[14]

Amino acid analysis of the peptide revealed the presence of β-alanine, proline, and valine in an equimolar ratio. This indicated the presence of free (*N*-demethylated) valine in the molecule (the automated analyzer which compares the retention time of the PTH derivatives of the amino acids from the complete hydrolysate with the standards will not detect the *N*-methylated amino acids). The electron impact mass spectrum of the peptide showed a molecular ion at m/z 579. As discussed above for the chlorohydrin (**10**), the destruxins with an *N*-methylalanine-*N*-methylvaline moiety show a characteristic fragmentation pattern,[22] and the mass spectrum of the peptide showed major fragment ions at m/z 522 ($M-57$), 494 ($M-85$), 437 ($M-142$), and 409 ($M-170$). This indicated the presence of a *N*-methylalanine-*N*-methylvaline entity in the molecule which was also supported by the analysis of the ^{1}H n.m.r. spectrum of the sample. The ^{1}H n.m.r. spectral data for the compound which was eventually identified as destruxin E2 (**6**) have been listed in Table 1. The ^{1}H n.m.r. spectrum of the toxin had close similarities with the spectra of the other common destruxins and clearly showed the presence of *N*-methylalanine (2.71 p.p.m., NCH_3) and *N*-methylvaline (3.22 p.p.m., NCH_3). There was no aliphatic unsaturation in the molecule, and the presence of two amide protons (β-alanine and valine, 8.1 and 7.18 p.p.m. respectively) could be readily established. Four α-proton resonances corresponding to proline, valine, *N*-methylvaline and *N*-methylalanine and one resonance corresponding to the α-proton of α-hydroxy acid were present at 4.69, 4.92, 4.98, 5.15, and 4.95 p.p.m. respectively. Two characteristic pairs of multiplets for δ-methylene of proline and β-methylene of β-alanine protons were present at 3.84 and 3.65 and at 3.98 and 3.09 p.p.m. respectively. Due to the small available sample, a ^{13}C n.m.r. spectrum could not be obtained. Out of all the known

Table 3. Insecticidal activity of destruxins on tobacco Budworm (*Heliothis virescens*)

Destruxin	Concentration (p.p.m.)	%Mortality
A (**1**)	312	100
	155	100
	78	80
	38	67
B (**3**)	268	100
	125	40
	63	47
	30	13
E (**5**)	220	100
	108	100
	93	100
	75	93
A2 (**2**)	250	100
	150	93
	60	73
B2 (**4**)	250	100
	128	73
	75	47
DMDB (**9**)	225	73
	115	67
	50	40
CHL (**10**)	265	53
	150	27
	53	33

destruxins so far, only destruxin B2 (**4**) and desmethyldestruxin B (**9**) have a molecular weight of 579, and a direct comparison with these samples (h.p.l.c. retention upon co-injection) confirmed (**6**) to be a different compound. The presence of a free valine in the molecule and its h.p.l.c. elution profile [identical relationship with destruxin E (**5**) as for destruxin A2 (**2**) with destruxin A (**1**) and for destruxin B2 (**4**) with destruxin B (**3**)] suggested it to be a member of the A2 and B2 series, which differ from destruxins A and B only in that the isoleucine unit in the molecule has been substituted by a valine unit. The data for the compound suggested that it is related to destruxin E (**5**) in which the isoleucine has been replaced by valine, and hence on the basis of earlier nomenclature used for destruxins, the compound was named destruxin E2. Final confirmation of the structure was obtained by direct conversion of the sample into destruxin A2 (**2**) according to the reaction sequence used by Pais *et al.* for the correlation of destruxin E (**5**) to destruxin A (**1**). The peptide upon treatment with hydrobromic acid in tetrahydrofuran afforded a bromohydrin which was acetylated and further treated with zinc metal and cupric sulphate in aqueous acetic acid. The crude mixture so obtained was subjected to analytical h.p.l.c. which showed a peak with a retention time identical with that of destruxin A2 (**2**). The identity of the two was further substantiated by co-injection.

Monitoring of Insecticidal Activity.—The crude extract showed insecticidal activity against tobacco budworm (*Heliothis virescens*, Lepidoptera: Noctuidae), colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera: Chrysomelidae) and southern armyworm (*Spodoptera eridania*, Lepidoptera: Noctuidae). Subsequent monitoring of activity during the fractionation of the crude extract and testing of the purified samples was done using tobacco budworm because of convenience as well as consistency of the test results.

The bioassays were performed by spraying the formulated toxin solutions onto the host plant leaves, which were then infested with the insects. After three days, percent mortalities were calculated as dead insects/total number of insects $\times$ 100. Percent mortality observed in the controls was typically 5—10%. The toxicity data for different compounds are listed in Table 3.

Experimental

M.p.s were determined in a sulphuric acid bath and are uncorrected. The separation of the destruxins was achieved by a combination of flash silica gel chromatography followed by reverse phase h.p.l.c. on a C18 column as previously described.[14] U.v. spectra were recorded on a Perkin-Elmer Lambda 5 UV/VIS spectrophotometer in methanol. Amino acid analyses were done on a Waters Pico Tag amino acid analyzer and the amino acids were identified as their phenylthiohydantoin derivatives by comparing their h.p.l.c. retention times with those of the standards. The e.i. mass spectra were recorded on a Hewlett–Packard 5985A mass spectrometer equipped with a direct insertion probe. The n.m.r. spectra were recorded on a Varian XL-400 n.m.r. spectrophotometer with 400 MHz field for the ^{1}H and a 100 MHz field for the ^{13}C resonances. The chemical shifts have been reported in p.p.m. (δ) units downfield from tetramethylsilane which was used as an internal standard. In some cases the solvent (residual $CHCl_3$ in deuteriated chloroform) signal was used for referencing. The n.m.r. spectra were recorded in deuteriated chloroform at ambient temperature. The HOM2DJ resolved, DEPT, COSYPS, and HETCOR spectra were recorded with the standard pulse sequences and programming as supplied by Varian Associates. The parameters used were as given below. The standard abbreviations have been used and are same as published in the Varian Operator's manuals for the XL-series n.m.r. superconducting spectrometer systems.

HOM2DJ resolved: 35 mM solution of the sample in deuteriated chloroform was used to acquire the spectrum. AT = 0.512, NP = 4096, P1 = 31, D1 = 2, PW90 = 15.5, CT = 4, SW2 = 50, N1 = 64.

DEPT: Typically DEPT spectra were recorded with *ca.* 70 mM solutions of destruxins in deuteriated chloroform. AT = 0.752, NP = 11776, CT = 158, PW90 = 11.5, D1 = 2, J = 140, MULT ARRAY, Index (Value) 1 (0.5), 2 (1), 3 (1), 4 (1.5).

COSYPS: COSYPS (COSY Phase sensitive) spectra were recorded with the 35 mM solution of the samples in deuteriated chloroform. AT = 0.128, NP = 1024, P1 = 16, D1 = 2, CT = 70, PW90 = 16, PHASE ARRAY 1, SW2 = 4000, NI = 128.

HETCOR: ~150 mM solution of destruxins in deuteriated chloroform was used for obtaining the HETCOR spectra. AT = 0.102, NP = 4096, PW90 = 11, D1 = 1, CT = 32, SW2 = 4330, N1 = 256, J1CH = 140, JNCH = 0.

Culture of Metarhizium anisopliae.—The strain of *Metarhizium anisopliae* (isolate number ARSEF 1095) used in the present studies was obtained from the collection of entomopathogenic fungi, USDA-ARS, Plant Protection Research Unit, Boyce Thompson Institute at Cornell University, Ithaca. The isolate was originally obtained from parasitized *Carpocapsa pomonella* (Lepidoptera: Olethrentidae) collected in Austria. The fungus was grown in sterilized Czapek-Dox medium enriched with 0.5% bactopeptone. The inoculation was done in 6 $\times$ 1-l Fernbach flasks and the culture was allowed to grow at ambient temperature for 10 days on a rotary shaker (150 r.p.m.). The culture was then filtered under suction (Whatman no. 1) and the filtrate was treated with dilute hydrochloric acid to adjust the pH to 5.5. The aqueous broth was then extracted with methylene dichloride to afford the crude extract. Pure destruxins were isolated from the crude extract by flash chromatography on silica gel followed by preparative r.p.h.p.l.c. on a C18 column according to the procedure reported earlier.[14]

Chlorohydrin.—Chlorohydrin was isolated as a colourless

glassy material. ^{1}H and ^{13}C N.m.r. data for the compound and ^{1}H n.m.r. data for its acetate derivative (all in deuteriated chloroform) have been listed in Tables 1 and 2 respectively; $\lambda_{max.}$(MeOH): 203 nm (log ε 4.19); *m/z* (relative intensity) 629 (15) and 631 (5) (M^+, ^{35}Cl and ^{37}Cl, $C_{29}H_{48}ClN_5O_8$), 603 (7), 601 (16, $M^+ - 28$), 580 (25, $M^+ - CH_2Cl$), 574 (36), 572 (92), $M^+ - 57$), 546 (37), 544 (100, $M^+ - 85$), 536 (16), 528 (14), 526 (24), 518 (12), 516 (38), 508 (19), 489 (41), 487 (78, $M^+ - 142$), 471 (20), 469 (46), 461 (30), 459 (70, $M^+ - 170$), 451 (15), 443 (25), 441 (58), 433 (20), 431 (41), 414 (15), 406 (23), 404 (28), 396 (11), 377 (15), 375 (36), 349 (15), 347 (47), 322 (31), 314 (13), and 307 (12).

Acetylation of Chlorohydrin.—The sample (2 mg) with dry pyridine (200 μl) and acetic anhydride (200 μl) was sealed under dry nitrogen and left at ambient temperature for 24 h. The solvent was then removed under reduced pressure and the residue passed through a small column of silica gel in methylene dichloride. The fractions containing the product were combined and solvent removed to afford an amorphous residue (1.3 mg) which was used for n.m.r. measurements; $\lambda_{max.}$(MeOH) (log ε) 203 nm (4.42).

Destruxin E2.—Destruxin E2 was isolated as one of the minor components and crystallized from benzene–hexane, m.p. 191—192 °C. The h.p.l.c. separation has already been reported.[14] The ^{1}H n.m.r. data (in deuteriated chloroform) of the compound are listed in Table 1; $\lambda_{max.}$(MeOH) 204 nm (log ε 3.77); *m/z* (relative intensity) 579 (24) (M^+, $C_{28}H_{45}N_5O_8$), 564 (2) ($M^+ - 15$), 551 (17) ($M^+ - 28$), 535 (6) ($M^+ - 44$), 522 (96) ($M^+ - 57$), 507 (18) ($M^+ - 72$), 494 (100) ($M^+ - 85$), 479 (15), 478 (16), 466 (17), 451 (15), 437 (73) ($M^+ - 142$), 422 (13), 409 (70) ($M^+ - 170$), 382 (17), 369 (14), 312 (38), 294 (21), and 211 (21).

Formation of Destruxin A2 from Destruxin E2.—The sample (1 mg) in THF (400 μl) was treated with 48% aq. HBr (3 μl) and the solution was sonicated for 10 min at room temperature. Aqueous $NaHCO_3$ (10%) was then added to the mixture and the solution extracted with methylene dichloride to furnish a residue which was then directly acetylated with pyridine–acetic anhydride at room temperature overnight. The solvent was then removed under reduced pressure and the residue redissolved in acetic acid (100 μl). This solution was then added to a stirred suspension of Zn powder (10 mg) acetic acid–water (2:1) (500 μl) and cupric sulphate (2 mg in 500 μl water) and the mixture stirred at room temperature for 8 h. The solution was then filtered and extracted with methylene dichloride (3 × 1 ml) and the solvent evaporated. The residue was dissolved in methanol and passed through a Waters SepPak C18 cartridge. The clear eluate was concentrated and redissolved in acetonitrile (100 μl) and examined by h.p.l.c., which confirmed the presence of a peak with a retention time identical with that of destruxin A2. The h.p.l.c. conditions were the same as for destruxin A2.[14]

Bioassays of Isolated Destruxins.—The isolated cyclodepsipeptides were tested for insecticidal acitivity on tobacco budworm (*Heliothis virescens*). The samples were formulated in acetone–5% Triton X155 (Rhom & Haas), and in case of partially soluble samples, homogenous suspensions were obtained by sonication. In a typical run, the freshly cut discs from garbanzo bean leaves were set in Petri dishes over moist cotton and then sprayed with the toxin solution until run-off occurred. The leaves were immediately infested with 1st instar larvae (typically 10 larvae per disc, four discs for each run) and allowed to stand in a moist chamber at ambient temperature with a 12:12 light-dark cycle. All the tests were run in duplicate and the results reported are averages from at least three runs. After 3 days, percent mortalities were recorded. Percent mortality observed in the controls was typically 5—10%. Bioassay results from tests where control mortality was more than 10% were not included in the final calculations. Some phytotoxicity (yellowing and tissue decay) was also observed on garbanzo leaves when relatively high concentrations of the toxins were tested. The activity data for different compounds tested are listed in Table 3.

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