Phytochemistry, Vol. 20, No. 4, pp. 715–723, 1981.
Printed in Great Britain.

0031-9422/81/040715-09 $02.00/0

DEPSIPEPTIDES FROM *METARHIZIUM ANISOPLIAE*

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(*Revised received* 14 *July* 1980)

Key Word Index—*Metarhizium anisopliae*; Fungi Imperfecti; new cyclodepsipeptides.

Abstract—From the culture medium of a strain of *Metarhizium anisopliae*, 14 depsipeptides have been isolated. Five of them were identified as known destruxins A, B, C, D and desmethyldestruxin B. The structures of the new compounds, named destruxins E, A_1, A_2, B_1, B_2, C_2, D_1, D_2 and E_1, were established mainly from the mass spectral analysis of their corresponding open-chain derivatives.

INTRODUCTION

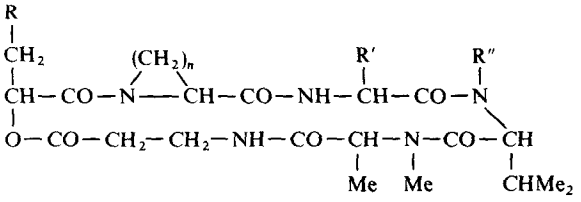
Fungi of the species *Metarhizium anisopliae* are known to cause diseases in several kinds of insects [1]. Toxic substances named destruxins have been isolated from submerged culture media and their structures shown to be **1** (destruxin A), **2** (destruxin B), **3** (destruxin C), **4** (destruxin D) and **5** (desmethyldestruxin B) [1, 2].

Besides the above-mentioned five compounds, we wish to report in this paper the isolation of nine new depsipeptides named destruxins E, A_1, A_2, B_1, B_2, C_2, D_1, D_2 and E_1 from the culture medium of a strain of *M. anisopliae*. Their structures, closely related to those of the known destruxins, were established to be **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13** and **14**, respectively.

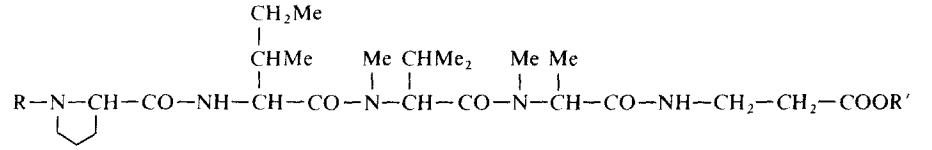
RESULTS AND DISCUSSION

The fungus was cultured on Czapek–Dox medium containing 0.5% peptone in a fermenter for 5 days. The mycelium was separated by centrifugation and the supernatant extracted with methylene chloride. Repeated chromatography of the extract on silica gel afforded pure destruxins A (**1**), B (**2**), E (**6**), A_1 (**7**), B_1 (**9**), B_2 (**10**) and a mixture of destruxins A_2 (**8**) and E_1 (**14**), further separated by reverse-phase HPLC. The more polar products were fractionated into acidic and neutral fractions. The neutral fraction was essentially a mixture of destruxins C (**3**) and C_2 (**11**) which could be separated by reverse-phase HPLC. The acidic fraction contained the carboxylic destruxins D (**4**), D_1 (**12**) and D_2 (**13**). After esterification with diazomethane, the corresponding esters **15**, **16** and **17** were separated by silica-gel chromatography and reverse-phase HPLC.

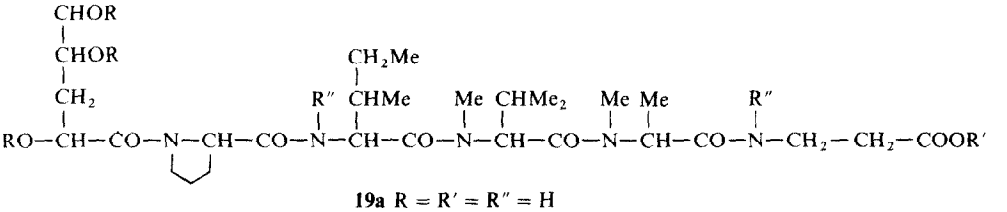
The most abundant depsipeptides in the extract were destruxins A (**1**), B (**2**) and E (**6**). High resolution mass spectrometry of **6** established its molecular formula as $C_{29}H_{47}N_5O_8$; thus, destruxin E which had the same MW as destruxin B differed from the latter in having one carbon less but containing an additional oxygen. Four major peaks were observed in the mass spectrum at *m/e* M-57, M-85, M-142 and M-170, similar to those previously described for destruxins A and B [3] and characteristic of the presence of *N*-methyl-valyl-*N*-methyl-alanyl in the ring structure Alkaline opening of the lactone ring in the usual way afforded two products, one basic (**18a**) and the other neutral (**19a**), which were separated on Amberlite IR 120 resin. **18a** was subjected to successive methylation and acetylation to give **18b**, whose mass spectrum indicated the amino acid sequence shown in Fig. 1. **19a** was also methylated and acetylated affording **19c**. Mass spectral analysis of **19c** suggested the presence of three *O*-acetyl groups on the hydroxy-acid unit. However, the exact structure of **19** was



	R	*n*	R′	R″
1	$CH{=}CH_2$	3	$CHMeCH_2Me$	Me
2	$CHMe_2$	3	$CHMeCH_2Me$	Me
3	$CHMeCH_2OH$	3	$CHMeCH_2Me$	Me
4	CHMeCOOH	3	$CHMeCH_2Me$	Me
5	$CHMe_2$	3	$CHMeCH_2Me$	H
6	$CH(O)CH_2$	3	$CHMeCH_2Me$	Me
7	$CH{=}CH_2$	4	$CHMeCH_2Me$	Me
8	$CH{=}CH_2$	3	$CHMe_2$	Me
9	$CHMe_2$	4	$CHMeCH_2Me$	Me
10	$CHMe_2$	3	$CHMe_2$	Me
11	$CHMeCH_2OH$	3	$CHMe_2$	Me
12	CHMeCOOH	4	$CHMeCH_2Me$	Me
13	CHMeCOOH	3	$CHMe_2$	Me
14	$CH(O)CH_2$	4	$CHMeCH_2Me$	Me
15	CHMeCOOMe	3	$CHMeCH_2Me$	Me
16	CHMeCOOMe	4	$CHMeCH_2Me$	Me
17	CHMeCOOMe	3	$CHMe_2$	Me



18a R = R′ = H
18b R = Ac, R′ = Me



19a R = R′ = R″ = H
19b R = R″ = H, R′ = Me
19c R = Ac, R′ = Me, R″ = H
19d R = R′ = R″ = Me

ascertained from the mass spectrum of the permethylated derivative **19d** (Fig. 2).

On the foregoing basis, destruxin E was assigned structure **6** as **19a** obviously resulted from alkaline opening of both epoxide and lactone rings, and **18a** from a fragmentation reaction giving rise to an α-aldehydic amide **20** which is cleaved in an alkaline medium.

Confirmation of the structure of destruxin E (**6**) came from the comparison of its ^{13}C NMR spectrum (Table 1) with those of destruxins A and B, and also from its correlation with destruxin A (**1**) through the following reaction sequence. **6** was converted with 48% hydrobromic acid in tetrahydrofuran to the bromhydrin **21**, which was acetylated and further treated with zinc powder in aqueous acetic acid to give **1**.

The other depsipeptides found in the extract, destruxin C (**3**), destruxin D (**4**), desmethyldestruxin (**5**) and the new destruxins A_1 (**7**), A_2 (**8**), B_1 (**9**), B_2 (**10**), C_2 (**11**), D_1 (**12**), D_2 (**13**) and E_1 (**14**) were minor components. The new compounds were obtained pure only in very small quantities because of the difficulty in resolving the mixtures. Structure assignment followed from ^1H NMR data (Table 2) and from the mass spectral analysis of both the depsipeptide (or its Me ester in the case of the carboxylic destruxins) and the corresponding linear esters **23–32**, the mass spectra of the latter indicating the amino acid sequence (Table 3). The esters **23–29** were easily prepared by alkaline opening of the lactone ring and subsequent methylation and acetylation. In the case of destruxin E_1, in which the α-hydroxy acid unit bears an epoxide ring, cleavage of the amide bond between the hydroxy acid and the *vic*-amino acid was observed as in destruxin E to afford **29**, but no ring-opening product comparable to **19a** was found. Compounds **30–32** were prepared by alkaline treatment of the carboxylic destruxins esters **15–17**, followed by methylation with diazomethane. In these compounds the terminal lactone ring was obviously a result of internal esterification between the α-hydroxy acid carboxyl and hydroxyl groups, as previously described for destruxin D [2].

The structure thus assigned to the destruxins were confirmed by acid hydrolysis and subsequent amino acid analysis on cellulose TLC (Table 4). Destruxin E_1 (**14**) was further correlated with destruxin A_1 (**7**) through the sequence **11** → **33** → **7** comparable to **6** → **20** → **1**. Destruxin C (**3**) was correlated with both the destruxin D ester **15** by oxidation with Jones reagent followed by esterification with diazomethane [2] and destruxin B

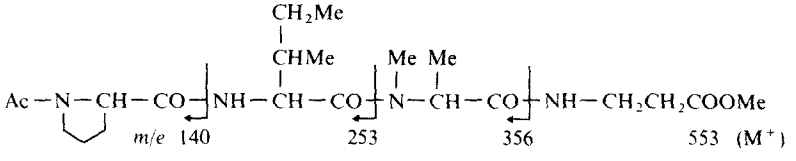


Fig. 1. Mass spectral fragmentation of **18b**.

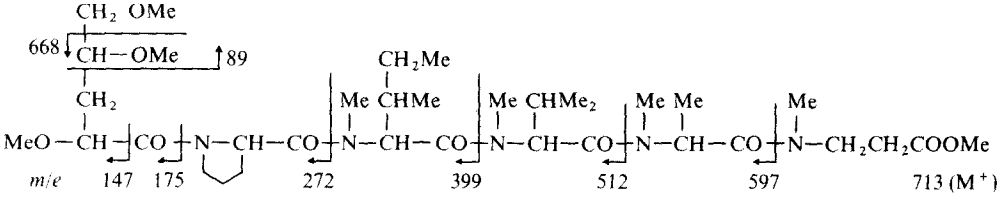
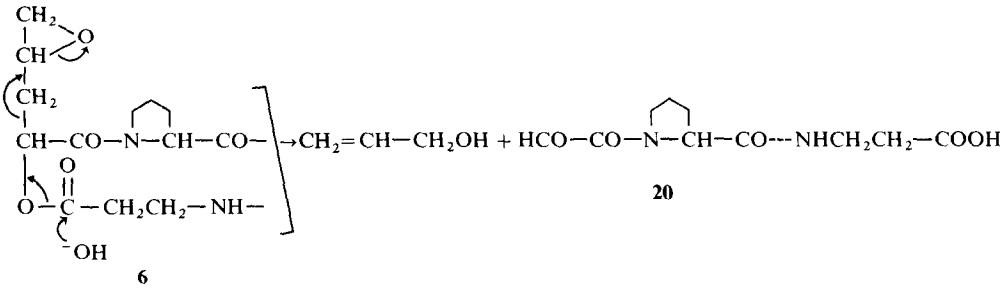


Fig. 2. Mass spectral fragmentation of **19d**.



6 20

Table 1. ^{13}C NMR data of destruxins **1**, **2**, **6** and **15**

Carbon	Chemical shifts (δ)*			
	Destruxin A (**1**)	Destruxin B (**2**)	Destruxin D Me ester (**15**)	Destruxin E (**6**)
$\underline{CH_3}CH_2$	11.4	11.4	11.4	11.3
Me NMeAla	15.2	15.3	15.3	15.3
$\underline{CH_3}$—CH Ileu	15.5	15.3	15.3	15.2
$\underline{CH_3}$(COOMe)CH			17.6	
Me Val	19.7	19.7	19.5	19.5
Me Val	20.1	20.1	19.7	20.0
Me acid		21.5		
Me acid		23.4		
NMe	24.1	24.2	24.2	21.1
NMe	24.5	24.5	24.5	24.5
CH_2Pro	27.3	27.3	27.3	27.3
CO—$\underline{CH_2}$ Pro	28.2	28.2	28.2	28.3
CO—$\underline{CH_2}$ βAla †	29.2	29.0	29.3	29.3
CH Val	30.9	30.9	30.9	30.9
CH Ileu	33.3	33.3	33.3	33.3
CH_2 Ileu	34.6	34.5	34.5	34.6
CH_2 acid	35.0	24.5	35.6	33.7
COO$\underline{CH_3}$			33.3	
CH_2NH βAla	37.5	37.5	37.5	37.5
$\underline{CH}$ Me_2 acid		39.1		
CH_2—NH Pro	46.8	46.6	46.6	46.8
CH_2—O				47.2
CH—O				47.9
$\underline{CH}$(Me) COOMe			52.0	
CH—NH	53.8	53.8	53.8	53.7
CH—NH	55.7	55.6	55.6	55.6
CH—NH	58.2	58.2	58.2	58.2
CH—NH	60.9	60.8	61.0	61.0
O$\underline{CH}$—CO	72.9	72.1	71.6	70.8
$\underline{CH}$=CH_2	131.6			
CH=$\underline{CH_2}$	119.7			
CO amide	169.3	170.0	169.1	169.0
CO amide	170.1	170.0	170.1	170.0
CO amide	171.3	171.3	171.3	171.2
CO amide	171.4	171.3	171.5	171.4
CO amide	174.4	173.9	173.8	173.4
CO amide	174.4	174.1	173.8	173.9
CO ester			176.4	

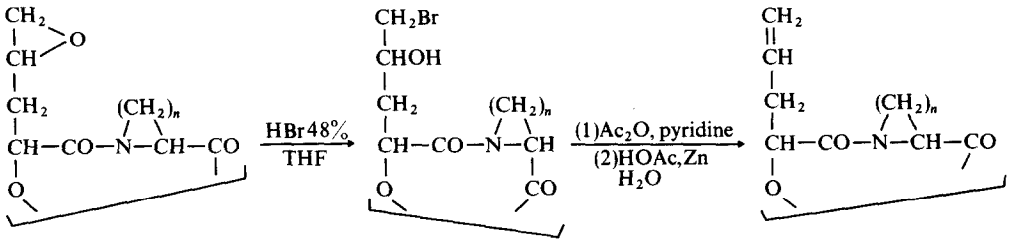
*In $CDCl_3$ at 60 MHz with TMS as internal standard.

† No assignment made for each carbon.

Table 2. ^{1}H NMR data of destruxins **1**–**14** and **15**–**17***

	Chemical shifts (δ)							
Destruxin	Me_2(NMeVal)	Me_n(Ileu or/and Val)	Me or Me_2 acid	Me(Ala) (d, J = 6.5 Hz)	NMe (Ala)	NMe (Val)	CH=CH_2	COOMe
A **1**	← 0.80–0.98 (12H) →			1.30	2.72	3.22	5.78	
B **2**	← 0.80–1.01 (18H) →			1.29	2.71	3.21		
C **3**	← 0.80–0.94 (12H) →		1.02 (d, J = 6.5)	1.30	2.72	3.21		
Desmethyl B **5**	← 0.85–1.01 (18H) →			1.31	2.73			
E **6**	← 0.80–0.98 (12H) →			1.31	2.73	3.22		
A_1 **7**	← 0.79–1.03 (12H) →			1.30	2.72	3.23	5.78	
A_2 **8**	← 0.76–1.00 (18H) →			1.31	2.73	3.23	5.80	
B_1 **9**	← 0.78–1.02 (18H) →			1.30	2.72	3.23		
B_2 **10**	← 0.85–1.02 (18H) →			1.31	2.73	3.23		
C_2 **11**	← 0.80–0.98 (12H) →		1.00 (d, J = 6.5)	1.30	2.72	3.23		
E_1 **14**	← 0.81–0.97 (12H) →			1.30	2.72	3.22		
D ester **15**	← 0.78–0.97 (12H) →		1.25 (d, J = 7)	1.30	2.72	3.22		3.69
D_1 ester **16**	← 0.78–0.97 (12H) →		1.25 (d, J = 7)	1.30	2.72	3.22		3.67
D_2 ester **17**	← 0.84–0.99 (12H) →		1.23 (d, J = 7)	1.31	2.72	3.23		3.67

*In $CDCl_3$ at 250 MHz with TMS as internal standard. Coupling constants in Hz.



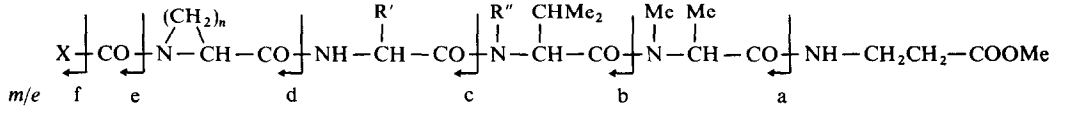
6 $n = 3$ **14** $n = 4$ **21** $n = 3$ **33** $n = 4$ **1** $n = 3$ **7** $n = 4$

Table 3. Mass spectral fragmentation of destruxins **3**, **5**, **7–11** and **14–17** and of the corresponding open-chain derivatives **22–32**

		Principal peaks in the mass spectra of the depsipeptides				Principal peaks in the mass spectra of the open-chain derivatives (see formulae)							
Destruxin	Formulae	M^+	M−57 M−85 M−142 M−170	*m/e* 70*	*m/e* 84†	Formulae	M^+	a	b	c	d	e	f
C	**3**	609	+	+	−	**22**	725	623	538	425	312	215	
Desmethyl B	**5**	579	−	+	−	**23**	653	550	466	367	212	157	129
A_1	**7**	591	+	−	+	**24**	665	562	478	365	252	141	113
A_2	**8**	563	+	+	−	**25**	637	535	450	337	238	141	113
B_1	**9**	607	+	−	+	**26**	681	579	494	381	268	157	113
B_2	**10**	679	+	+	−	**27**	653	551	466	353	254	157	129
C_2	**11**	595	+	+	−	**28**	711	609	524	411	312	215	
E_1	**14**	607	+	+	−	**29**	567	464	380	267	154		
D Me ester	**15**	637	+	−	+	**30**	637	535	450	337	224		99
D_1 Me ester	**16**	651	+	+	−	**31**	651	549	464	351	238		99
D_2 Me ester	**17**	623	+	+	−	**32**	623	521	436	323	224		99

* Ion $CH{=}\overset{+}{N}H$ from proline unit.
$(CH_2)_3$

† Ion $CH{=}\overset{+}{N}H$ from pipecolic acid unit.
$(CH_2)_4$



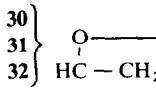
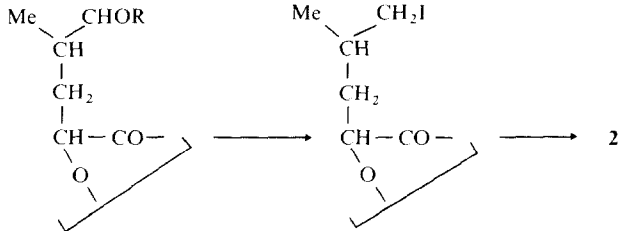
	X	*n*	R′	R″
22	$CH(OAc)CH_2CHMe_2$	3	$CHMeCH_2Me$	H
23	$CH(OAc)CH_2CHMeCH_2OAc$	3	$CHMeCH_2Me$	Me
24	$CH(OAc)CH{=}CH_2$	4	$CHMeCH_2Me$	Me
25	$CH(OAc)CH{=}CH_2$	3	$CHMe_2$	Me
26	$CH(OAc)CH_2CHMe_2$	4	$CHMeCH_2Me$	Me
27	$CH(OAc)CH_2CHMe_2$	3	$CHMe_2$	Me
28	$CH(OAc)CH_2CHMeCH_2OAc$	3	$CHMeCH_2Me$	Me
29	Ac	4	$CHMeCH_2Me$	Me
30		3	$CHMeCH_2Me$	Me
31		4	$CHMeCH_2Me$	Me
32		3	$CHMe_2$	Me

Table 4. Amino acid analysis of destruxins **3**, **5**, **7**–**11**, **14** and **15***

Destruxin	Formulae	Pro (0.15)	Pip (0.22)	Ileu (0.38)	Val (0.27)	NMeVal (0.35)	NMeAla (0.17)	βAla (0.09)†
C	**3**	+	−	+	−	+	+	+
Desmethyl B	**5**	+	−	+	+	−	+	+
A_1	**7**	−	+	+	−	+	+	+
A_2	**8**	+	−	−	+	+	+	+
B_1	**9**	−	+	+	−	+	+	+
B_2	**10**	+	−	−	+	+	+	+
C_2	**11**	+	−	−	+	+	+	+
E_1	**14**	−	+	+	−	+	+	+
D Me ester	**15**	+	−	+	−	+	+	+
D_1 Me ester	**16**	−	+	+	−	+	+	+
D_2 Me ester	**17**	+	−	−	+	+	+	+

* TLC on cellulose (Merck) plates (MeCOEt–pyridine–H_2O–HOAc, 70:15:15:2) [4].
† R_f value.



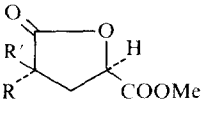
3 R = H
34 R = Ts

35

through the following route. On treatment with *p*-toluene sulfonyl chloride in pyridine, **3** gave the ester **34**. Displacement of the *p*-toluene sulfonyl group with sodium iodide in methylethylketone afforded the iodo derivative **35**, which on catalytic hydrogenation in the presence of Raney nickel gave destruxin B (**2**).

As for the stereochemistry of the destruxins, the *S* configuration has been previously assigned to the asymmetric α-carbon of the amino acids and the *R* configuration to the hydroxy acid α-carbon in destruxins A and B [2]. The correlations established above between destruxins E and A on the one hand, and destruxins C, D and B on the other allow the same configuration assignment for the ring carbons in destruxins C, D and E. These configurations are also very likely for the minor destruxins isolated here.

Destruxin D has been described previously [2] as a mixture of diastereoisomers yielding, by acid hydrolysis



36a R = H, R′ = Me
36b R = Me, R′ = H

and subsequent esterification with diazomethane, an equimolar mixture of the lactones **36** arising from the hydroxy acid unit. Acid hydrolysis of depsipeptide **15** also afforded the lactones **36**, which were separated by preparative HPLC, but one isomer was predominant (major: minor, 4:1). As the major lactone treated in the acid hydrolysis conditions gave again a mixture of the two lactones (major: minor 9:1), while the minor lactone remained stable, it is more likely that destruxin D ester **15** is a pure product (as shown also by ^{13}C NMR spectroscopy and HPLC), the minor lactone arising from partial isomerization of the hydroxy acid γ-carbon during hydrolysis. Configuration assignment could not be made for the lactones **36** by ^1H NMR, but one can assume that the major lactone is the less stable isomer, namely the *Z* isomer **36a**. As a consequence the *R* configuration is the most probable for the hydroxy acid γ-carbon in destruxins D and C.

The toxins produced by *M. anisopliae* appear to be more varied than has been previously described. Thus, differences are observed in the peptide chain: destruxins A_1 (**7**), B_1 (**9**), D_1 (**12**) and E_1 (**14**) contain pipecolic acid instead of proline occurring in destruxins A (**1**), B (**2**), C (**3**), D (**4**) and E (**6**), while destruxins A_2 (**8**), B_2 (**10**), C_2 (**11**) and D_2 (**13**) have valine instead of isoleucine which is found in destruxins A, B, C and D. Although all possible products were not isolated, it is observed that any given α-hydroxy acid is associated with three different peptide chains, which differ within the chain by only one amino acid. Furthermore, a new α-hydroxy acid unit was found in destruxins E (**6**) and E_1 (**14**), the peptide chain of which was identical to that of destruxins A, B, C, D and A_1, B_1, C_1, D_1, respectively. The names chosen for the new

destruxins follow directly from the preceding considerations.

The major depsipeptide, destruxin E (**6**), was tested for its insecticidal activity against *Galleria mellonella* larvae and showed a toxicity comparable to that of destruxins A and B ([1]; J. Fargues and P. H. Robert, personal communication). These data, as well as the details of the biological activity of the main depsipeptides described here will be published elsewhere.

EXPERIMENTAL

Mps were uncorr. Optical rotations were measured at 20°. Column chromatography was carried out on Si gel Merck H 60 (30 × wt of product) except for the first separation. HPLC was performed on a Waters Associates apparatus (pump 6000 A, injector U6K, detection by refractive index); unless otherwise stated preparative separations (5 mg/injection) were carried out on two Microbondapak C-18 columns with a flow rate of 1.5–2 ml/min (eluant; MeOH–H_2O, 11:9).

Fungus and culture conditions. The strain used is designated MA 120 in the culture collection of 'Mycothèque INRA', La Minière, 78280 Guyancourt (France). The fungus was grown in submerged culture in Czapek–Dox broth containing 0.5% peptone. A 300 l. capacity fermenter was seeded with a 3-day-old rotary shake culture which had been started by introduction of the spores in 3 × 6 l. flasks containing 2 l. of medium. The fermenter was run at 250 rpm, 300 l. air/min at 25° for 5 days.

Solvent extraction. The mycelium was removed from the culture by centrifugation and the supernatant repeatedly extracted with CH_2Cl_2. Evapn of the solvent *in vacuo* gave 30 g of crude extract.

Isolation of destruxins. The crude extract (10 g) was chromatographed on Si gel Zeozyl 45 (1.5 kg). Elution with CH_2Cl_2 containing increasing concns of MeOH (1–2%) gave 3 fractions, fractions I (2.7 g), II (3.7 g) and III (0.78 g). Further elution with CH_2Cl_2–MeOH (9:1) afforded fraction IV (1.1 g). Fraction I was rechromatographed (hexane–Me_2CO 7:3) yielding the following fractions.

Fraction	Weight	Destruxin (TLC)
I_1	0.033	B_1
I_2	0.055	$B_1 + A_1 + B$
I_3	0.764	B
I_4	0.172	$B + B_2 + A$
I_5	1.20	A
I_6	0.330	$A + B_2 + E_1$

Destruxin B_1 (**9**) (fraction I_1) crystallized from C_6H_6–hexane. Mp 195°, $[\alpha]_D$ − 214° ($CHCl_3$, *c* 1). (Found: C, 61.75; H, 8.69; O, 19.03; N, 11.40. $C_{31}H_{53}O_7N_5$ requires: C, 61.26; H, 8.79; O, 18.43; N, 11.53%).

Destruxin B (**2**) (fraction I_3) crystallized from C_6H_6–hexane. Mp 238°, $[\alpha]_D$ −241° (MeOH, *c* 0.5). Lit. [1]: mp 234°, $[\alpha]_D^{23}$ −228° (MeOH, *c* 0.5).

Destruxin A (**1**) (fraction I_5) crystallized from C_6H_6–hexane. Mp 126–129°, $[\alpha]_D$ −230° (MeOH, *c* 1). Lit. [1]: mp 125°, $[\alpha]_D^{15}$ −225° (MeOH, *c* 0.5).

Destruxin A_1 (**7**). Fraction I_2 was rechromatographed (hexane–Me_2CO, 3:1) to give destruxin A_1 (25 mg) which crystallized from C_6H_6–hexane. Mp 209–210°, $[\alpha]_D$ −226° ($CHCl_3$, *c* 0.7). (Found: C, 60.71; H, 8.41; O, 19.10; N, 11.70. $C_{30}H_{49}O_7N_5$ requires: C, 60.89; H, 8.35; O, 18.93; N, 11.84%).

Destruxin B_2 (**10**). Fraction I_4 was rechromatographed (hexane–Me_2CO, 7:3) to give destruxin B_2 (20 mg) which crystallized from C_6H_6–hexane. Mp 227–229°, $[\alpha]_D$ −234° ($CHCl_3$, *c* 0.5). (Found: C, 59.80; H, 8.46: N, 11.96. $C_{29}H_{49}O_7N_5$ requires: C, 60.08; H, 8.52; N, 12.08%).

Destruxin A_2 (**8**). Fraction I_6 was rechromatographed (hexane–Me_2CO, 7:3) to afford a mixture of destruxin A_2 and E_1 (0.230 g) as shown by MS. This mixture (70 mg) was separated by prep. HPLC. Destruxin A_2 (35 mg) crystallized from C_6H_6–hexane. Mp 161–162°, $[\alpha]_D$ −225° ($CHCl_3$, *c* 1). (Found: C, 59.35; H, 8.02; N, 12.28. $C_{28}H_{45}O_7N_5$ requires: C, 59.66; H, 8.05; N, 12.43%).

Destruxin E_1 (**14**) (15 mg) obtained from fraction I_6 (see destruxin A_2) was amorphous. $[\alpha]_D$ −261° ($CHCl_3$, *c* 0.5).

Destruxin E (**6**). Chromatography of fraction II (CH_2Cl_2–MeOH, 49:1) gave destruxin E (2.53 g) which crystallized from C_6H_6–hexane. Mp 172–173° (dec.), $[\alpha]_D$ −253° ($CHCl_3$, *c* 1). (Found: C, 58.45; H, 7.99; N, 11.75. $C_{29}H_{47}O_8N_5$ requires: C, 58.66; H, 7.98; N, 11.80%). HR MS: M^+ 593.345; requires 593.348.

Desmethyldestruxin B (**5**). Fraction III was chromatographed (hexane–Me_2CO, 3:2) to give desmethyldestruxin B (70 mg), amorphous, $[\alpha]_D$ −200° ($CHCl_3$, *c* 1).

Fraction IV dissolved in CH_2Cl_2 was extracted with satd aq. $NaHCO_3$. The extract was washed, dried and evapd to dryness *in vacuo* to give a mixture F IV_1 (0.43 g). The aq. layer was acidified with N HCl and extracted with CH_2Cl_2 to give a mixture of carboxylic destruxins F IV_2 (0.610 g).

Destruxin C (**3**). Fraction IV_1 was rechromatographed (CH_2Cl_2–MeOH, 19:1) to give a mixture (0.15 g) of destruxins C and C_2, further separated by prep. HPLC. Destruxin C (80 mg) crystallized in C_6H_6–hexane. Mp 219–220°, $[\alpha]_D$ −223° ($CHCl_3$, *c* 1). Lit. [3]: mp 158–162° (perhaps mixture of diastereoisomers).

Dexstruxin C_2 (**11**) (20 mg) was obtained from fraction IV_1 (see destruxin C), amorphous. $[\alpha]_D$ −230° ($CHCl_3$), *c* 0.5).

Destruxin D_1 Me ester (**15**). Fraction IV_2 was methylated with CH_2N_2–Et_2O in the usual way. The resulting Me ester was chromatographed (hexane–Me_2CO, 7:3) to give successively destruxin D_1 Me ester (23 mg), destruxin D Me ester (0.37 g) and a mixture of destruxin D Me ester **16** and destruxin D_2 Me ester **17** (0.12 g). Destruxin D_1 Me ester crystallized from Et_2O–hexane. Mp 166–168°. $[\alpha]_D$ −223° ($CHCl_3$, *c* 0.5). (Found: C, 58.89; H, 8.09; N, 10.45. $C_{32}H_{53}O_9N_5$ requires: C, 58.96; H, 8.20; N, 10.75%).

Destruxin D Me ester (**16**) was obtained from fraction IV_2 (see destruxin D_1 Me ester **15**) crystallized from Et_2O. Mp 225–226°. $[\alpha]_D$ −240° ($CHCl_3$, *c* 1). Lit. [3]: mp 235°.

Destruxin D_2 Me ester **17**. Preparative HPLC of the mixture of destruxin D and D_2 Me ester (35 mg) obtained by chromatography of fraction IV_2 (see destruxin D_1 ester) gave pure destruxin D_2 Me ester (14 mg) which crystallized from Et_2O. Mp 254°. $[\alpha]_D$ −227° ($CHCl_3$; *c* 0.5). (Found: C, 57.67; H, 7.80; N, 11.22. $C_{30}H_{49}O_9N_5$ requires C, 57.76; H, 7.92; N, 11.23%).

Ring-opening of destruxin E, derivatives **18** *and* **19**. To a soln of destruxin E (**6**) (0.110 g) in MeOH (6.4 ml) was added 2 M NaOH (1.6 ml). The mixture was left 2 hr at room temp., neutralized (pH ≃ 6) with N HCl and evapd to dryness *in vacuo*. The residue was mixed with $CHCl_3$–MeOH (17:3). The ppt. (NaCl) was filtered off and the filtrate evapd to give a mixture of **18a** and **19a** (0.1 g) which was dissolved in H_2O (30 ml). This soln was passed through a column containing Amberlite IR 120 resin (5 ml) and evapd under red. pres. to give **19a** (60 mg), which was esterified with CH_2N_2 to afford the crude ester **19b**. Subsequent chromatography (CH_2Cl_2–MeOH, 9:1) gave the pure product (TLC) as an oil (40 mg). The peptide **18a** was eluted from the

resin with 15% NH_4OH and esterified with CH_2N_2 to give a crude ester which was purified by chromatography (CH_2Cl_2–MeOH, 19:1). To a soln of the pure ester (TLC, 22 mg) in MeOH (1 ml) was added Ac_2O (5 μl). The mixture was left 30 min at room temp. and evapd to dryness *in vacuo* to afford **18b** as an oil.

19b (10 mg) was acetylated (Ac_2O–pyridine) overnight. Usual work-up afforded **19c** as an oil (10 mg). MS *m/e*: 769 (M^+), 667, 582, 469, 356, 259, 217. Permethylation of **19b** (1 mg) was carried out with MeI (500 μl) in the presence of Na^+ $MeSOCH_2^-$ base (500 μl) according to ref. [5] and gave **19d** as an oil.

Ring-opening of destruxin C (**3**), *dexmethyldestruxin* (**5**) *and destruxins* A_1 (**7**), A_2 (**8**), B_1 (**9**), B_2 (**10**) *and* C_2 (**11**); *derivatives* **22–28**. To a soln of each destruxin (2 mg) in MeOH (0.4 ml) was added 2 M NaOH (0.1 ml). The mixture was left overnight at room temp, acidified with M HCl and extracted with CH_2Cl_2. Evapn of the extracts afforded the open-chain acids (2 mg), which were methylated with CH_2N_2 and further acetylated (Ac_2O–pyridine) in the usual way to give the derivatives **22–28** (2 mg) as oils.

Ring-opening of destruxins D Me *ester* (**15**), D_1 Me *ester* (**16**) *and* D_2 Me *ester* (**17**); *derivatives* **29–31**. To a soln of the destruxin ester (3 mg) in MeOH (0.4 ml) was added 2 M NaOH (0.1 ml). After 1 hr at room temp., the mixture was diluted with MeOH, demineralized with Amberlite IR 120 resin (1 ml) and evapd to dryness. The acids (3 mg) were esterified with CH_2N_2 to give the corresponding esters **29–31**, which were purified by prep. TLC. IR δ_{max}^{film} cm^{-1}: 3300, 1650 and 1530 (CONH); 1780 (>C=O lactone); 1730 (>C=O ester).

Ring-opening of destruxin E_1 (**14**); *derivative* **32**. To a soln of a mixture of destruxin A_2 and E_1 (0.15 g) in MeOH (0.4 ml) was added 2 M NaOH (0.1 ml). The mixture was left overnight at room temp., acidified with M HCl and extracted with CH_2Cl_2. The aq. phase was neutralized with M NaOH and evapd to dryness *in vacuo*. The residue was mixed with $CHCl_3$–MeOH (17:3). The ppt (NaCl) was filtered off and the filtrate evapd to give the crude peptide (21 mg), which was esterified with CH_2N_2. Chromatography (CH_2Cl_2–MeOH, 19:1) afforded a pure product (TLC, 10 mg) which was acetylated (Ac_2O–MeOH) to give **32** (10 mg) as an oil.

Destruxin A (**2**) *from destruxin E* (**6**). *Bromhydrin* **21**: To a stirred soln of destruxin E (60 mg) in THF (5 ml) was added 48% aq. HBr (0.03 ml). Stirring was continued for 5 min at room temp. satd aq. $NaHCO_3$ was added and the mixture extracted with CH_2Cl_2 to furnish the bromhydrin **21** (62 mg) as a colourless glass. MS *m/e*: 673–675 (M^+), 616–618 ($M^+ - 57$), 588–590 ($M^+ - 85$), 511–513 ($M^+ - 142$), 503–505 ($M^+ - 170$). *Destruxin A* (**2**): The bromhydrin (60 mg) was acetylated (Ac_2O–pyridine) to afford the corresponding acetyl bromhydrin (60 mg). To a stirred suspension of Zn powder (100 mg) in HOAc–H_2O (2:1) (3 ml) was added a soln of $CuSO_4 \cdot 5H_2O$ (20 mg) in H_2O (1 ml) and then a soln of the acetyl bromhydrin in HOAc (1.2 ml). The mixture was stirred for 6 hr at room temp., filtered and extracted with CH_2Cl_2. The extract was washed with satd aq. $NaHCO_3$ and evapd to give destruxin A (44 mg) which crystallized from C_6H_6–hexane. It was identical in all respects (mp, $[\alpha]_D$, IR, NMR) to an authentic sample.

Destruxin A_1 *from destruxin* E_1. As described for destruxin E, destruxin E_1 (10 mg) on treatment with 48% aq. HBr in THF (1 ml of a 0.5% soln) gave the bromhydrin **33** (10 mg) which was acetylated and further treated with Zn powder in aq. HOAc (Zn 100 mg, HOAc 1.6 ml, H_2O 0.5 ml, $CuSO_4 \cdot 5OH_2$ 10 mg) to give destruxin A_1 (7 mg) which crystallized from C_6H_6–hexane and was identical in all respects (mp, $[\alpha]_D$, IR, NMR) to an authentic sample.

Destruxin D ester (**16**) *from destruxin C* (**3**). To a stirred soln of destruxin C (30 mg) in Me_2CO (3 ml) was added, in small portions during 20 min, Jones reagent (0.05 ml). Stirring was continued for 10 min. The mixture was then diluted with H_2O, made alkaline with 10% NH_4OH and extracted with Et_2O. The aq. phase was acidified with conc HCl and extracted with CH_2Cl_2. The extract was washed with H_2O, dried and evapd to give a residue (22 mg) which was esterified with CH_2N_2–Et_2O. The crude ester was purified by chromatography (hexane–Me_2CO, 7:3) to yield destruxin D Me ester (17 mg) which crystallized from Et_2O. It was identical in all respects (mp, $[\alpha]_D$, IR, NMR) to an authentic sample.

Destruxin B (**2**) *from destruxin C* (**3**). *Iodo derivative* **35**. To a stirred soln of destruxin C (40 mg) in pyridine (2 ml) was added *p*-toluene sulfonyl chloride (25 mg); stirring was continued for 60 hr at room temp. The mixture was then diluted with H_2O, washed with M HCl and H_2O. Evapn yielded the crude *p*-toluene sulfonic ester (33 mg) still containing destruxin C ($\simeq$20%) as shown by TLC and ^1H NMR (60 MHz $CDCl_3$; δ 2.40, *s*, $\underline{CH}_3$ C_6H_4; 7.10–7.68, A_2B_2 system, C_6H_4). To a soln of this ester in MeCOEt (1.5 ml) was added NaI (20 mg). The mixture was refluxed for 30 min, diluted with H_2O and extracted with CH_2Cl_2. The extract was washed with a satd soln of $Na_2S_2O_3$ and evapd to give the crude iodo derivative, which was purified by prep. TLC (hexane–Me_2CO, 3:2). Pure **35** (17 mg) crystallized from C_6H_6–hexane, mp 224°, $[\alpha]_D$ −196°. (Found: C, 49.70; H, 6.89. $C_{30}H_{50}O_5N_7I$ requires: C, 50.06; H, 7.00). MS *m/e*: 719 (M^+), 662 ($M^+ - 57$), 634 ($M^+ - 85$), 577 ($M^+ - 142$) and 549 ($M^+ - 170$). *Destruxin B* (**2**): A soln of the iodo derivative **35** (12 mg) in EtOH (5 ml) containing NEt_3 (5 μl) was hydrogenated in the presence of Raney Ni (0.1 g) overnight at atmos. pres. The catalyst was filtered off and the filtrate evapd *in vacuo*. The residue was dissolved in CH_2Cl_2 and this soln was washed with H_2O, dried and evapd to dryness to afford destruxin B (9 mg) which crystallized from C_6H_6–hexane. It was identical in all respects (mp, $[\alpha]_D$, IR, NMR) to an authentic sample.

Acid hydrolysis of destruxins. A soln of the destruxin (3–5 mg) in 6 M HCl–H_2O–HOAc (2:1:1) (1 ml) was refluxed overnight and then evapd to dryness under red. pres. Excess HCl was eliminated by co-evapn with $CHCl_3$–MeOH and the residue was analysed by TLC. Results are summarized in Table 4.

Lactones **36a** *and* **36b**. A soln of destruxin D Me ester **15** (0.3 g) in 6 M HCl–H_2O–HOAc (2:1:1) (30 ml) was refluxed overnight and evapd to dryness as described above for the acid hydrolysis of the other destruxins. The residue was dissolved in H_2O and passed through a column containing Amberlite IR 120 resin (15 ml). Evapn under red. pres. afforded a mixture of crude acids which were methylated with CH_2N_2 to give a crude mixture of the corresponding esters **36a** and **36b**, further purified by prep. TLC (CH_2Cl_2–MeOH, 95.5: 0.5). Analytical HPLC of the purified mixture [30 mg; MS *m/e*: 158 (M^+), 140, 126, 114, 99, 71 (3)] on a microporasil column (flow rate 1.5 ml/min; cyclohexane–EtOAc, 4:1) showed the ratio **36a** (R_t 9 min) to **36b** (R_t 7.2 min) to be 4:1 (peak integration). Prep. HPLC on two microporasil columns (flow rate 2 ml/min, same eluent) yielded pure **36a** (20 mg) and **36b** (4 mg) as oils. *Lactone* **36a**. $[\alpha]_D$ + 15° ($CHCl_3$, *c* 0.5). ^1H NMR (400 MHz, $CDCl_3$): δ 1.31 (3 H, *d*, J = 7 Hz, Me), 1.96 (1 H, *ddd*, $J = J' = 9$, $J'' = 12$ Hz, C$\underline{H}$H), 2.7 (1 H, *qdd*, J = 7, $J' = J'' = 9$ Hz, C$\underline{H}$Me), 2.77 (1 H, *ddd*, J = 9, J' = 7, J'' = 12 Hz, CH$\underline{H}$), 3.82 (3 H, *s*, COOMe), 4.81 (1 H, *dd*, J = 7, J' = 9 Hz, CHCOOMe). *Lactone* **36b**. $[\alpha]_D$ − 17° ($CHCl_3$, *c* = 0.4). ^1H NMR (400 MHz, $CDCl_3$): δ 1.29 (3 H, *d*, J = 7 Hz, Me), 2.20 (1 H, *ddd*, J = 9, J' = 11, J'' = 13 Hz, C$\underline{H}$H), 2.55 (1 H,

ddd, $J = 9$, $J' = 2.2$, $J'' = 13$ Hz, CHH), 2.75 (1 H, *qdd*, $J = 7$, $J' = 9$, $J'' = 11$ Hz, CHMe), 3.80 (3 H, *s*, COOMe), 4.89 (1 H, *dd*, $J = 2.2$, $J' = 9$ Hz, CHCOOMe).

Acid treatment of lactones **36a** *and* **36b**. Each lactone was treated as **15** (**36a** 5 mg or **36b** 2 mg; 6 M HCl–H_2O–HOAc, 2:1:1, 1 ml). Analytical HPLC showed a ratio **36a**:**36b** 9:1 starting from **36a** and of pure **36b** starting from **36b**.

Acknowledgements—We are grateful to Dr. J. Fargues for providing the fungus. We also thank Mr. A. Escaut, Ingenieur CNRS, for the fermenter culture and his help in the isolation of the destruxins.

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