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NEW DESTRUXINS FROM THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM ANISOPLIAE*

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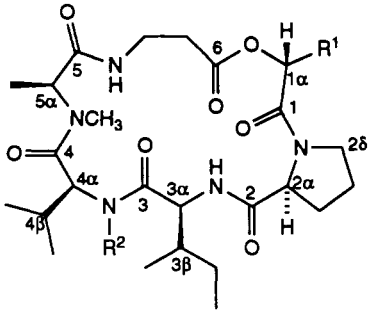
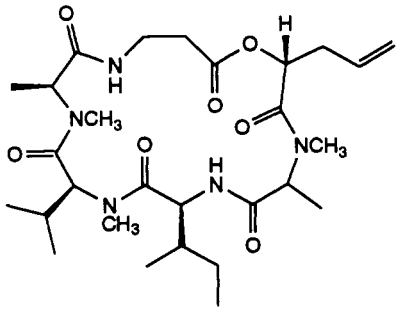
ABSTRACT.—Four new cyclic depsipeptides have been isolated from the entomopathogenic fungus *Metarhizium anisopliae*. The chemical structures of destruxins A3 [**4**] and F [**5**] and desmethyldestruxins A [**6**] and C [**7**] were determined based on nmr and mass spectral data. Destruxin A3 bears an *N*-methylalanine moiety in place of the usual proline amino acid, while destruxin F contains a new 2,4-dihydroxypentanoic acid subunit. Spectral data for destruxin *E* diol [**8**], previously reported as a metabolic product of destruxin E, are also presented.

Ever since the discovery in 1961 (1) that the entomopathogenic fungus *Metarhizium anisopliae* Sorokin (Deuteromycota) produced a family of insecticidal compounds called the destruxins (e.g., destruxins A [**1**], C [**2**], and E [**3**]),



1 R^1=$CH_2CH{=}CH_2$, R^2=Me
2 R^1=$CH_2CH(Me)CH_2OH$, R^2=Me
3 R^1=CH_2-(epoxide), R^2=Me
5 R^1=$CH_2CH(OH)Me$, R^2=Me
6 R^1=$CH_2CH{=}CH_2$, R^2=H
7 R^1=$CH_2CH(Me)CH_2OH$, R^2=H
8 R^1=$CH_2CH(OH)CH_2OH$, R^2=Me



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there has been considerable effort invested in searching for related cyclic depsipeptide compounds with enhanced activity. Of the 19 structurally related destruxins isolated to date (2–5), many exhibit a variety of biological activities (4–6). For example, in addition to acting as neurotoxins in insect model systems (7), the destruxins have shown immunodepressant activity (8); furthermore, destruxins can activate calcium channels in insect muscles (8), and destruxin E, which is the lone member of this group to exhibit topical insecticidal activity, has been found to exhibit cytostatic and cytotoxic effects on mouse leukemia cells (9). In addition, destruxins have been isolated as phytotoxins from plant pathogenic fungi (10,11).

In an effort to find naturally occurring insecticides with activity against the subterranean termite *Coptotermes formosanus*, we have isolated four new cyclic depsipeptides, destruxin A3 [**4**], destruxin F [**5**], desmethyldestruxin A (DMDA) [**6**], and desmethyldestruxin C (DMDC) [**7**], together with ten previously reported destruxins, from culture extracts of the fungus *M. anisopliae*.

RESULTS AND DISCUSSION

Extraction of the culture filtrate with CH_2Cl_2 yielded a crude extract that was composed almost entirely of cyclic depsipeptides, which, on the basis of characteristic ^{1}H-nmr signals, could be identified as destruxins. A combination of Si

TABLE 1. ^{1}H-nmr Chemical Shifts for Compounds **4–8**.[a]

Proton	Compound				
	4	**5**	**6**	**7**	**8**
H-1α	5.01 t (7.4)	5.06 dd (10.6, 2.8)	4.84 dd (7.8, 6.5)	4.95 dd (10.6, 2.8)	5.11 dd (6.9, 4.9)
H-1β	2.55–2.80[b]	2.02 m, 1.93 m	2.62 m, 2.53 m	2.1 m, 1.45 m	2.05 m
H-1γ	5.77 m	4.08 m	5.78 m	1.91 m	3.97 m
H-1δ2	5.23 dd (17.2, 1.4)	1.24 d (6.3)	5.22 dd (17.2, 1.3)	3.55 dd (10.6, 5.8)	3.67 m
	5.18 dd (11.2, 1.4)		5.18 dd (11.2, 1.3)	3.51 dd (10.6, 6.0)	3.48 dd (11.1, 6.8)
H-1δ2				0.98 d (6.8)	
2-NMe	3.03 s				
H-2α	5.38 q (7.1)	4.68 br d (7.8)	4.61 d (6.8)	4.59 br d (4.0)	4.66 br d (6.8)
H-2β	1.37 d (7.1)	2.48 m, 1.95 m	2.42 m, 2.0 m	2.43 m, 2.06 m	2.46 dd (11.6, 6.1), 2.0 m
H-2γ		2.06 m, 1.95 m	2.06 m, 1.94 m	2.2 m, 1.96 m	2.16 m, 1.9 m
H-2δ		3.90 t (9.2), 3.67 m	3.91 t (7.0), 3.48 q (7.0)	3.92 t (8.8), 3.45 m	3.92 t (9.9), 3.69 m
3-NH	7.08 d (8.8)	7.15 d (9.3)	7.01 d (9.2)	6.98 d (9.3)	7.12 d (9.1)
H-3α	4.91 dd (9.1, 5.6)	4.89 dd (9.3, 6.6)	4.52 dd (9.2, 6.3)	4.49 dd (9.3, 6.6)	4.89 dd (9.1, 6.3)
H-3β	1.92 m	1.92 m	1.77 m	1.78 m	1.9 m
H-3γ1	1.4 m, 1.25 m	1.41 m, 1.27 m	1.43 m, 1.21 m	1.45 m, 1.22 m	1.39 m, 1.29 m
H-3γ2	0.82 d (7.5)	0.86 d (7.8)	0.89 d (7.4)	0.91 d (6.8)	0.86 d (6.8)
H-3δ	0.90 t (6.6)	0.85 t (7.6)	0.89 t (5.9)	0.89 t (7.3)	0.85 t (7.6)
4-NH			6.37 d (6.3)	6.40 d (5.8)	
4-NMe	3.21 s	3.22 s			3.22 s
H-4α	4.83 d (11.0)	4.97 d (10.8)	4.24 dd (9.6, 6.3)	4.23 dd (9.8, 6.0)	4.97 d (10.8)
H-4β	2.32 m	2.32 m	2.1 m	2.03 m	2.32 m
H-4γ1	0.92 d (6.4)	0.92 d (6.6)	1.02 d (6.8)	1.02 d (6.6)	0.93 d (6.6)
H-4γ2	0.90 d (7.1)	0.88 d (6.6)	0.90 d (6.5)	0.89 d (6.8)	0.88 d (6.6)
5-NMe	2.72 s	2.71 s	2.72 s	2.73 s	2.71 s
H-5α	5.18 q (6.6)	5.19 q (6.8)	5.04 q (6.8)	5.06 q (6.7)	5.18 q (6.8)
H-5β	1.31 d (6.6)	1.30 d (6.8)	1.33 d (6.8)	1.33 d (6.7)	1.30 d (6.8)
6-NH	8.06 d (9.8)	8.15 dd (9.8, 1.3)	8.27 d (7.3)	8.27 dd (9.1, 2.5)	8.15 dd (10.3, 2.5)
H-6α	2.55–2.80[b]	2.67 ddd (18.4, 11.3, 2.0)	2.68 ddd (18.6, 11.0, 2.0)	2.69 ddd (18.6, 10.6, 2.0)	2.67 ddd (18.4, 11.4, 2.0)
		2.56 br dd (18.4, 3.5)	2.61 m	2.60 ddd (18.6, 6.0, 1.8)	2.57 br dd (18.4, 4.8)
H-6β	4.07 m, 3.01 m	4.04 m, 3.07 br t (11.8)	3.98 m, 3.16 br t (8.4, 1.7)	4.00 m, 3.15 br t (10.8)	4.04 m, 3.07 br t (12.1)

[a]Data were recorded in $CDCl_3$ at 500 MHz.

[b]Due to severe signal overlap, precise chemical shifts could not be determined for these multiplets.

gel cc and extensive reversed-phase hplc yielded 14 compounds: the new destruxins A3 [**4**], F [**5**], DMDA [**6**], and DMDC [**7**], along with destruxin E diol [**8**], which had only been reported as a locust metabolic product, and previously described destruxins A [**1**], A2, B, B2, DMDB, C [**2**], C2, E [**3**], and the chlorohydrin of E (Chl), which could easily be identified by comparing spectral data to literature values.

^{1}H-nmr spectra of destruxins generally contain characteristic signals for two *N*-Me groups (2.7 and 3.2 ppm), two amide protons (7.1 and 8.2 ppm), and a methyl doublet (ca. 1.3 ppm) that belongs to the alanine side chain. The ^{1}H-nmr spectrum of destruxin A3 [**4**] (Table 1) contains, in addition to signals for a terminal double bond as in destruxin A [**1**], a new *N*-Me singlet (H-2, 3.03 ppm) and an additional methyl doublet at δ 1.37 (H-2β), while lacking the signals normally associated with the proline moiety. The ^{13}C-nmr spectrum (Table 2) provided additional support for a structure which incorporates an *N*-methylalanine amino acid in place of the usual proline group, showing new signals at δ 30.96 and 13.73, which were

TABLE 2. ^{13}C-nmr Chemical Shifts for Compounds **4–8**.[a]

Carbon	Compound				
	4	**5**	**6**	**7**	**8**
C-1α	70.80	71.06	72.41	71.12	70.70
C-1β	34.14	39.29	34.91	33.64	33.83
C-1γ	130.98	63.53	131.47	31.94	66.41
C-1δ1	119.71	23.88	119.43	67.86	67.55
C-1δ2				16.04	
1C=O	173.37	173.58	171.85	171.83	173.98
C-2α	58.49	60.95	60.86	60.90	60.98
C-2β	13.73	29.07	30.46	30.44	29.20
C-2γ		23.98	24.12	24.19	23.95
C-2δ		46.57	46.82	46.65	46.68
2-NMe	30.96				
2C=O	170.10[b]	171.05	171.64	171.69	171.06[b]
C-3α	54.00	53.67	55.59	55.67	53.68
C-3β	37.78	37.40	38.44	38.15	37.33
C-3γ1	24.12	24.39	25.07	25.01	24.38
C-3γ2	15.87	15.38	15.29	15.36	15.38
C-3δ	11.57	11.36	11.37	11.31	11.35
3C=O	169.67	169.74	169.06	169.70	169.84
C-4α	57.79	58.04	56.28	56.32	58.06
C-4β	27.28	27.23	28.48	28.50	27.22
C-4γ1	19.34	19.56	19.53	19.53	19.56
C-4γ2	20.11	19.99	19.67	19.64	19.97
4-NMe	30.89	30.87			30.86
4C=O	170.88[b]	170.80	170.84	170.92	170.90[b]
C-5α	55.41	55.48	55.68	55.66	55.50
C-5β	15.31	15.20	14.81	14.82	15.16
5-NMe	28.08	28.10	29.35	29.14	28.13
5C=O	170.53	169.74	169.68	169.81	169.98
C-6α	35.34	34.50	33.58	34.23	34.48
C-6β	33.28	33.18	34.26	33.57	33.23
6C=O	173.96	173.64	173.16	173.18	173.63

[a]Data were obtained in $CDCl_3$ at 125 MHz. Carbon assignments are based on literature comparisons and HMQC results.

[b]Assignments may be interchanged within a column.

assigned to the amide *N*-methyl and the methyl side chain, respectively. Finally, a high resolution mass measurement of the $[M]^+$ ion (565.3489, Δ +1.1 mmu) confirmed a molecular formula of $C_{28}H_{47}N_5O_7$ for destruxin A3 [**4**], compared to $C_{29}H_{47}N_5O_7$ for destruxin A.

The hrms of destruxin F [**5**] suggested a molecular formula of $C_{29}H_{49}N_5O_8$ (595.3555, Δ +2.6 mmu), which contains two additional hydrogen atoms relative to destruxin E [**3**]. The nmr spectral data matched those of **3**, except for the α-hydroxyacid subunit. Signals assigned to the epoxide ring protons were absent; however, a COSY spectrum clearly showed coupling from a 1-proton multiplet at δ 4.08 (H-1γ), which was not present in the spectrum of **3**, to both a 3-proton methyl doublet at δ 1.24 (H-1δ) and a diastereotopic pair of signals H-1β (2.02 and 1.93 ppm), which further correlated to H-1α (5.06 ppm). These assignments were consistent with a 2,4-dihydroxypentanoic acid moiety, as shown in structure **5**. An HMQC spectrum (12) provided added support for the proposed structure, showing a one-bond correlation between the proton signal at δ 4.08 and an oxygenated carbon at δ 63.53.

The structures of DMDA [**6**] and DMDC [**7**] were deduced by comparing their nmr and mass spectral data to those of destruxins A [**1**] and C [**2**], respectively. In each case, both the ^{1}H- and ^{13}C-nmr spectra were identical, except for the presence of an additional amide proton signal (ca. 6.4 ppm) and the absence of the signals associated with the valine *N*-methyl group (3.2 ppm for ^{1}H and 30.8 ppm for ^{13}C). Hreims measurements for the $[M]^+$ ions of **6** (563.3316, Δ +0.3 mmu) and **7** (595.3610, Δ −2.9 mmu) supported the predicted molecular formulas $C_{28}H_{45}N_5O_7$ and $C_{29}H_{49}N_5O_8$, respectively.

Destruxin E diol [**8**] has never been isolated directly from liquid cultures of *M. anisopliae* but has been observed as an enzymatic hydrolysis product when destruxin E was injected into the locust *Locusta migratoria* (13). To date, only mass spectral data have been reported for compound **8** (14); therefore, we have provided both the ^{1}H- and ^{13}C-nmr chemical shift assignments in Tables 1 and 2, respectively. The nmr spectra closely match those reported for destruxin E [**3**], except for the signals assigned to the α-hydroxy acid substituent. Characteristic signals for compound **8** include the ^{13}C signals at δ 67.55 and 66.41, assigned to C-1γ and C-1δ, respectively, the ^{1}H-nmr multiplet at δ 3.97, assigned to H-1γ, and the multiplets at δ 3.67 and 3.48, assigned to the H-1δ methylene protons.

The family of compounds known as the destruxins consists of a series of cyclic depsipeptides. For each of the six different α-hydroxyacid subunits previously reported, the following structural variations have been found: amino acid 3 may be valine or isoleucine; amino acid 4 may be valine or *N*-methylvaline; amino acid 2 may be proline or pipecolic acid. DMDA [**6**] and DMDC [**7**] follow this trend, providing examples of destruxins A and C with a valine for amino acid 4; however, destruxin A3 [**4**] is the first non-proline/pipecolic acid containing destruxin, and destruxin F [**5**] contains a new α-hydroxyacid moiety. Preliminary assay results indicate that destruxins are toxic to termites; however, complete evaluation is still in progress and will be reported at a later time.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr data were recorded on a GE OMEGA 500 instrument at 500 and 125 MHz operating frequencies, respectively. Chemical shifts are referenced to solvent peaks: δ_H 7.24 (residual $CHCl_3$) and δ_C 77.0 for $CDCl_3$. Ir spectra were obtained using a Perkin-Elmer 1600 FTIR, and the optical rotations were recorded using a Jasco DIP-370 polarimeter and a 10 cm microcell. Mass spectral data were obtained on a VG-70SE mass spectrometer operating in the ei mode.

CULTURE CONDITIONS.—*M. anisopliae* (iso-

late ARSEF No. 2162 obtained from the USDA-ARS Plant Protection Research Unit at Cornell University) was cultured in 2-liter flasks containing 1 liter of 3.5% Czapek-Dox broth (Difco) supplemented with 0.5% Bacto peptone (Difco). After 15–19 days on a rotary shaker at 230 rpm, between 22 and 25°, the liquid culture was acidified with HCl, vacuum-filtered, and extracted three times with CH_2Cl_2.

ISOLATION.—The crude extract (1.11 g concentrated), obtained from two 1-liter liquid cultures grown for 19 days, was fractionated by Si gel cc employing a step-wise CH_2Cl_2-to-MeOH solvent gradient to give two mixed fractions and a third fraction that contained destruxin E diol (41.9 mg). The two mixed fractions were separated further by Si gel cc using hexane-Me_2CO (7:3) as a solvent system, and by reversed-phase hplc on a C-18 semi-preparative column (5 μm Rainin Microsorb™, 10 mm × 25 cm, 2.20 ml/min) using MeCN-H_2O (60:40) as the eluent. Fractions 1 and 2, described above, yielded the following compounds after hplc: fraction 1 destruxins A (73.6 mg, Rt 10.8), A2 (8.9 mg, Rt 9.8), B (12.6 mg, Rt 14.6), B2 (2.2 mg, Rt 12.6), E (3.6 mg, Rt 8.6), A3 (1.4 mg, Rt 10.6); fraction 2 destruxins DMDB (6.4 mg, Rt 11.4), C (11.0 mg, Rt 8.0), Chl (7.6 mg, Rt 8.2), DMDA (4.1 mg, Rt 9.2), DMDC (3.8 mg, Rt 7.2).

Investigation of a second batch of *M. anisopliae* (1.35 g crude extract from three 1-liter cultures grown for 15 days), which was subjected to Si gel chromatography followed directly by extensive reversed-phase hplc using the same conditions as outlined above, yielded destruxin F (4.2 mg, Rt 7.0).

Dextruxin A3 [**4**].—Eims *m/z* (rel. int.) 565 (7), 508 (12); hreims see text; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Destruxin F [**5**].—$[\alpha]^{18}D$ −239.6° (*c* = 0.17, $CHCl_3$); ir ν max (film) cm^{-1} 3420, 3384, 2966, 2878, 1729, 1630, 1523, 1449, 1182; eims *m/z* (rel. int.) 596 (1), 538 (3), 510 (2), 453 (2); hreims see text; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Desmethyldestruxin A [**6**].—$[\alpha]^{18}D$ −167.1° (*c* = 0.18, $CHCl_3$); ir ν max (film) cm^{-1} 3384, 2963, 1734, 1640, 1522, 1448, 1377, 1180; eims *m/z* (rel. int.) 563 (22), 506 (42), 407 (8); hreims see text; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Desmethyldestruxin C [**7**].—$[\alpha]^{18}D$ −134.2° (*c* = 0.16, $CHCl_3$); ir ν max (film) cm^{-1} 3423, 2963, 1648, 1523, 1448, 1377, 1180; eims *m/z* (rel. int.) 596 (18), 538 (18), 467 (8); hreims see text; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Destruxin E diol [**8**].—$[\alpha]^{18}D$ −206.6° (*c* = 2.0, $CHCl_3$); ir ν max (film) cm^{-1} 3384, 3017, 1731, 1664, 1630, 1522, 1450, 1215, 1189; eims *m/z* (rel. int.) 611 (1), 554 (3), 526 (2), 469 (2), 385 (2), 324 (7); hreims *m/z* $[M]^+$ 611.3492 (calcd for $C_{29}H_{49}N_5O_9$, 611.3530); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

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